PRIVILEGED COMMUNICATION
FOR INVESTIGATIONAL USE ONLY

SWOG

A RANDOMIZED PHASE ii STUDY OF CO-EXPRESSION EXTRAPOLATION (COXEN) with NEOADJUVANT CHEMOTHERAPY FOR LOCALIZED, MUSCLE-INVASIVE BLADDER CANCER

NCT #02177695

STUDY CHAIRS:

Principal Investigator
Thomas W. Flaig, M.D. (Medical Oncology)
University of Colorado, Denver
MS C290, 13001 E. 17th Pl., Room E1342
Aurora, CO 80045
Phone: 303/724-0499
FAX: 303/724-3889
E-mail: thomas.flaig@ucdenver.edu

Siamak Daneshmand, M.D. (Urology)
University of Southern California
Norris Comprehensive Cancer Center
Institute of Urology
1441 Eastlake Avenue, #7416
Los Angeles, CA 90089
Phone: 323/865-3700
FAX: 323/865-0120
E-mail: daneshma@usc.edu

Seth P. Lerner, M.D. (Urology)
Baylor College of Medicine
Scott Department of Urology
6620 Main Street, Suite 1325
1441 Eastlake Avenue
Houston, TX 77030
Phone: 713/798-6841
FAX: 713/798-5553
E-mail: slerner@bcm.edu

M. Scott Lucia, M.D. (Pathology)
University of Colorado, Denver
12801 East 17th Avenue
Pathology/MS 8104
Aurora, CO 80045
Phone: 303/724-3470
FAX: 303/724-1427
E-mail: scott.lucia@ucdenver.edu

AGENTS:

IND-Exempt Agents:
Gemcitabine hydrochloride (NSC-613327)
Cisplatin (NSC-119875)
Methotrexate (NSC-740)
Vinblastine sulfate (NSC-49842)
Doxorubicin (Adriamycin) (NSC-123127)
Filgrastim (G-CSF) (NSC-614629)
Pegfilgrastim (Neulasta) (NSC-725961)

David J. McConkey, Ph.D. (Translational Medicine)
Johns Hopkins Greenberg Bladder Cancer Institute
Brady Urological Institute at Johns Hopkins
600 North Wolfe Street,
Marburg Building / Suite 149
Baltimore, MD 21287-2101
Phone: 410/502-4149
Fax: 410/614-7526
Email: djmcconkey@jhmi.edu

Dan Theodorescu, M.D., Ph.D. (Translational Medicine)
University of Colorado Cancer Center
13001 East 17th Place, MS F434
Aurora, CO 80045
Phone: 303/724-7135
FAX: 303/724-3162
E-mail: dan.theodorescu@ucdenver.edu

Amir Goldkorn, M.D. (Translational Medicine)
University of Southern California
Norris Comprehensive Cancer Center
Los Angeles, CA 90089
Phone: 323/442-7721
E-mail: agoldkor@med.usc.edu

BIOSTATISTICIANS:

Cathy M. Tangen, Dr.P.H. (Biostatistics)
Melissa Plets, M.S.
SWOG Statistics and Data Management Center
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North, M3-C102
P.O. Box 19024
Seattle, WA 98109-1024
Phone: 206/667-4623
Fax: 206/667-4408
E-mail: ctangen@fredhutch.org
E-mail: mplets@fredhutch.org

PATIENT ADVOCATE:

Rick Bangs
Phone: 248/377-4290
E-mail: chezrick@comcast.net

ALLIANCE CHAMPION:

Matthew I. Milowsky, M.D.
University of North Carolina at Chapel Hill
Lineberger Comprehensive Cancer Center
E-mail: matt_milowsky@med.unc.edu

PARTICIPANTS

ALLIANCE/ Alliance for Clinical Trials in Oncology

ECOG-ACRIN/ECOG-ACRIN Cancer Research Group

NRG/NRG Oncology

SWOG/SWOG

NCIC-CTG/NCIC-CTG Trials Group

TABLE OF CONTENTS

TITLE PAGE 1
PARTICIPANTS 2
TABLE OF CONTENTS 3
CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION 5
SCHEMA 7
1.0 OBJECTIVES 8
1.1 Primary Objective 8
1.2 Secondary Objectives 8
2.0 BACKGROUND 8
3.0 DRUG INFORMATION 21
3.1 Gemcitabine hydrochloride (Gemzar®) (NSC-613327) 21
3.2 Cisplatin (CDDP) (Platinol®) (NSC-119875) 23
3.3 Methotrexate (NSC-740) 25
3.4 Vinblastine sulfate (Velban) (NSC-49842) 28
3.5 Doxorubicin (Adriamycin) (NSC-123127) 30
3.6 Filgrastim (r-metHuG-CSF) (Neupogen®) (NSC-614629) 31
3.7 Pegfilgrastim (Neulasta™) 32
4.0 STAGING CRITERIA 34
5.0 ELIGIBILITY CRITERIA 34
5.1 Disease Related Criteria 34
5.2 Prior Therapy Criteria 35
5.3 Clinical/Laboratory Criteria 35
5.4 Specimen Submission Criteria 36
5.5 Regulatory Criteria 37
6.0 STRATIFICATION FACTORS 37
7.0 TREATMENT PLAN 37
7.1 General Considerations 37
7.2 Arm 1: Gemcitabine + Cisplatin (GC) Treatment 38
7.3 Arm 2: Dose Dense Methotrexate, Vinblastine, Doxorubicin, Cisplatin (DDMVAC) Treatment 39
7.4 Timing of Cystectomy After Chemotherapy 39
7.5 Criteria for Removal from Protocol Treatment 40
7.6 Discontinuation of Treatment 40
7.7 Follow-Up Period 40
8.0 TOXICITIES TO BE MONITORED AND DOSE MODIFICATIONS 40
8.1 NCI Common Terminology Criteria for Adverse Events 40
8.2 General Dose Modification Considerations 40
8.3 Chemotherapy Dose Levels 41
8.4 Dose Modifications 42
8.5 Dose Modifications Contacts 45
8.6 8Adverse Event Reporting 45
9.0 STUDY CALENDAR 46
9.1 Arm 1 GC – Study Calendar – **21 day cycles** 46
9.2 Arm 2 DDMVAC – Study Calendar – **14 day cycles** 48
10.0 CRITERIA FOR EVALUATION AND ENDPOINT ANALYSIS 50
10.1 Recurrence criteria 50
10.2 Recurrence-Free Survival 50
10.3 Overall Survival 50
10.4 Performance Status 50
10.5 Pathologic Response 50
11.0 STATISTICAL CONSIDERATIONS 51
11.1 Accrual Goal 51
11.2 Analysis of Primary Endpoint 51
11.3 Analysis of Secondary Endpoints 52
11.4 Data and Safety Monitoring Committee Oversight 53
12.0 DISCIPLINE REVIEW 53

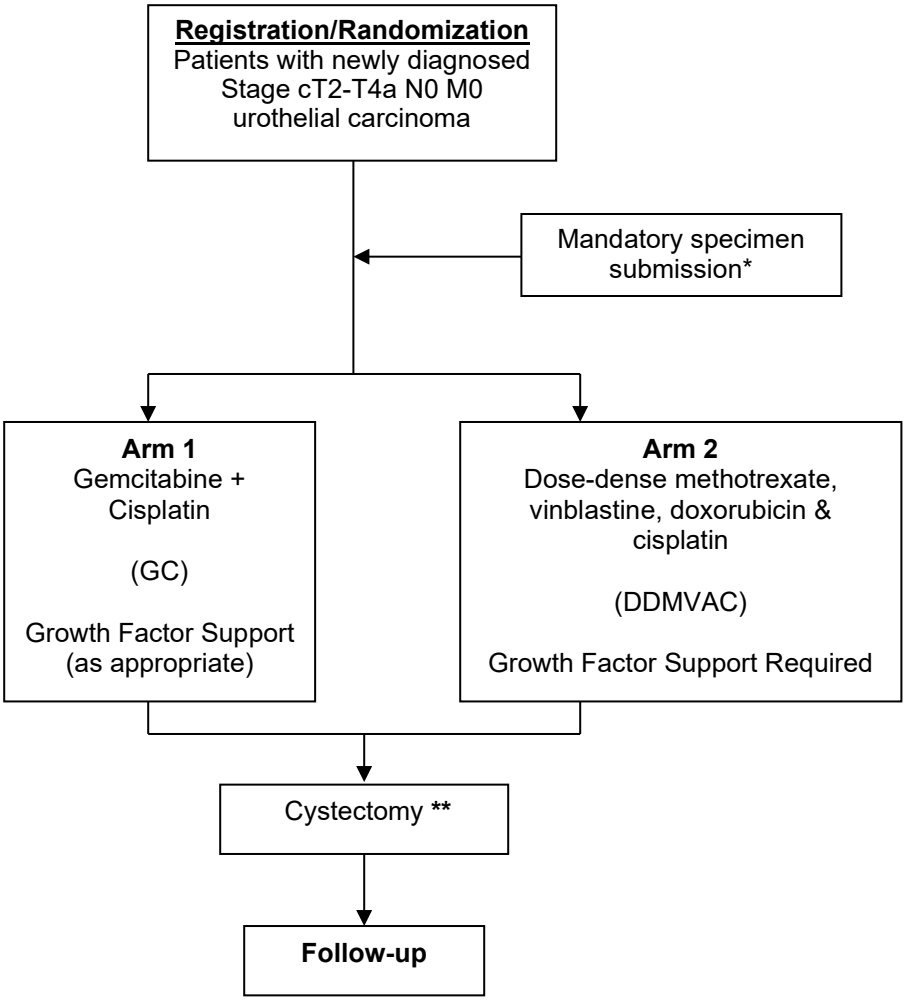
13.0 REGISTRATION GUIDELINES 53
13.1 Registration Timing 53
13.2 Investigator/Site Registration 54
13.3 OPEN Registration Requirements 54
13.4 Registration Procedures 55
13.5 Exceptions to SWOG registration policies will not be permitted. 56
14.0 DATA SUBMISSION SCHEDULE 56
14.1 Data Submission Requirement 56
14.2 Master Forms 56
14.3 Data Submission Procedures 56
14.4 Data Submission Overview and Timepoints 57
15.0 SPECIAL INSTRUCTIONS 59
15.1 Specimens for mandatory correlative studies (submitted to the SWOG Specimen Repository – Solid Tissue, Myeloma and Lymphoma Division, Lab #201*)* are **mandatory** for the patient. 59
15.2 Blood Specimen for upCTC (submitted to the Amir Goldkorn Lab, Lab #181) optional for patient 60
16.0 ETHICAL AND REGULATORY CONSIDERATIONS 62
16.1 Adverse Event Reporting Requirements 63
17.0 BIBLIOGRAPHY 66
18.0 APPENDIX 71
18,1 New York Heart Association Classifications 72
18.2 Pathology Verification of Tissue Submission Form 73
18.3 **Translational Medicine Studies** 74
18.3a microRNA (miRNA) expression profile 75
18.3b **Ultra Pure Circulating Tumor Cells Using Coxen** 77
18.3c **Cisplatin Germline Pharmacogenomics** 85
18.3d **DNA Damage Response Gene Alterations** 89
18.4 Circulating Tumor Cell (CTC) Instructions for Blood Sample Processing, Packing and Shipping 96

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

CONTACT INFORMATION		
For regulatory requirements:	**For patient enrollments:**	**For study data submission:**
The study protocol and all related forms and documents must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Sites must use the current form version and adhere to the instructions and submission schedule outlined in the protocol. CTSU sites should follow procedures outlined in the protocol for Site registration, Patient Enrollment, Adverse Event Reporting, Data Submission (including ancillary studies), and Drug Procurement. Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 866-651-2878 to receive further information and support. Contact the CTSU Regulatory Help Desk at 866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section for instructions on using the OPEN system.	Online Data Submission: This protocol will use Medidata Rave® for electronic data submission. Access Rave® using your active CTEP-IAM userid and password at the following URL: https://login.imedidata.com/selectlogin Other Tools and Reports: Institutions participating through the CTSU continue to have access to other tools and reports available on the SWOG Workbench. Access this by using your active CTEP-IAM userid and password at the following URL: https://crawb.crab.org/TXWB/ctsulogon.aspx
The study protocol and all related forms and documents must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Sites must use the current form version and adhere to the instructions and submission schedule outlined in the protocol. CTSU sites should follow procedures outlined in the protocol for Site registration, Patient Enrollment, Adverse Event Reporting, Data Submission (including ancillary studies), and Drug Procurement.		
For questions unrelated to patient eligibility, treatment, or data submission contact the CTSU Help Desk by phone or e-mail: 888-823-5923 ctsucontact@westat.com		

CONTACT INFORMATION		
For regulatory requirements:	**For patient enrollments:**	**For study data submission:**
All calls and correspondence will be triaged to the appropriate CTSU representative.		
For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website: https://www.ctsu.org		
The CTSU Web site is located at https://www.ctsu.org		

SCHEMA



* **Mandatory** submission of viable cancer tissue and other specimens for gene expression profiling and COXEN score analysis

** Collect and submit residual tumor (*if applicable*). Enrollment in **S1011** should also be considered.

1.0 OBJECTIVES

1.1 Primary Objective

To characterize the relationship of DDMVAC- and GC-specific COXEN scores in terms of pT0 rate at cystectomy in patients treated with neoadjuvant chemotherapy. This will be done in two ways:

- By assessing whether the treatment-specific COXEN score is prognostic of pT0 rate or ≤ pT1 in this patient population and to assess in a preliminary fashion whether the COXEN score is a predictive factor distinguishing between these two chemotherapy regimens.
- By evaluating the correlation between the GC- and the DDMVAC-COXEN score.

1.2 Secondary Objectives

a. To assess, in a hypothesis generating fashion, the ability of COXEN to select for an individual chemotherapy regimen (GC versus DDMVAC).

b. To assess the value of gene expression profiling in predicting overall survival (OS) in bladder cancer patients treated with neoadjuvant chemotherapy.

c. To assess the difference in pT0 rate between the 21-day GC and 14-day DDMVAC arms, regardless of gene expression.

d. To assess the safety and tolerability of 21-day GC and 14-day DDMVAC chemotherapy when given in the neoadjuvant setting for bladder cancer.

2.0 BACKGROUND

It is estimated that there will be 74,690 new cases and 15,580 deaths from bladder cancer in the United States in 2014. *(1)* The majority of cases are non-muscle invasive and are treated by cystoscopic resection, with or without intravesical medical therapy instilled directly into the bladder. For those with muscle-invasive disease, therapy consisting of a radical cystectomy or definitive radiation therapy is indicated. Even with these radical treatments, the mortality from muscle-invasive bladder cancer remains high. Despite the survival advantage associated with its use, neoadjuvant chemotherapy remains poorly utilized in general medical practice in the United States. One explanation for the low utilization of neoadjuvant chemotherapy is the concern that some patients with surgically curable disease will be chemotherapy-resistant and may progress during treatment with neoadjuvant chemotherapy, being rendered incurable. This protocol will examine the utility of gene expression profiling, and more specifically the coexpression extrapolation (COXEN) approach, to predict chemotherapy sensitivity in individual patients undergoing neoadjuvant chemotherapy for bladder cancer.

<u>Chemotherapy for bladder cancer</u>

The mainstay of contemporary, cytotoxic chemotherapy for advanced bladder cancer has been cisplatin-based combination therapy. MVAC chemotherapy was designed in the mid 1980's and came into general use in the 1990's. *(2)* When compared to single-agent cisplatin, MVAC was associated with improved response rates and survival, although the regimen produced notable toxicity, mainly hematologic, gastrointestinal and infectious. *(3,4)* The doublet of gemcitabine and cisplatin was later recognized to also have significant activity against urothelial cancer. In a randomized, Phase III study, 405 subjects with Stage IV transitional cell carcinoma were given MVAC versus GC. *(5)* The response rates between the arms were similar (GC, 49%; MVAC 45%) as was the overall and progression-free survival (PFS). The side effects, especially those related to bone marrow suppression (neutropenic fever 1%, GC; 12%, MVAC), were greater in the MVAC

arm as compared to GC-treated subjects. The long-term results of this study have been reported with a median overall survival (OS) of 14.0 months with GC versus 15.2 months with MVAC (p=0.66). *(6)*

Three versus 4 week GC chemotherapy

In the Phase III trial of GC versus MVAC, the GC was given on a 28 day schedule, the same duration as the traditional MVAC cycle. In regimens utilizing a 28-day cycle of GC, gemcitabine is given on Days 1, 8, and 15, although the Day15 dose is oftentimes delayed or skipped due to bone marrow suppression, notably thrombocytopenia. With this recognition, a 21-day GC regimen, with gemcitabine given on Days 1 and 8 only, was investigated. In one retrospective analysis, 212 subjects with Stage IV transitional cell carcinoma were treated with either 21- or 28-day GC. *(7)* The response rate between the 2 schedules was similar, with an overall response rate of 59.7% with the 3 week and 55.6 % with the 4 week regimen; the complete response rate was 21% in both arms. Treatment compliance was improved with the 21-day schedule; with only 19% requiring dose modification in the 21-day arm, versus 62% in the 28-day group, with 47% in the latter required to omit Day15 gemcitabine. The rate of febrile neutropenia in the 21-day arm of this study was less than 10%. A smaller, single arm study of 21-day GC in 27 bladder cancer patients demonstrated a strong overall response rate of 48% without any Grade 4 toxicity or treatment-related death. *(8)* In addition, a randomized study of 107 patients with mixed cancer types (predominately lung cancer) examined the safety and efficacy of 21- versus 28-day GC. The 3-week schedule was better tolerated, with less Grade 3/4 thrombocytopenia (29.5% versus 5.5%) and less delays or dose reductions (51% versus 19%). Only the lung cancer patients were analyzed for response rate, since they represented the majority of the enrolled subjects, and these rates were similar between the arms.*(9)* In summary, even though the randomized Phase III trial of GC versus MVAC used 28-day GC schedule, subsequent studies have reported improved tolerance, dose-density and preserved efficacy with 21-day GC.

Dose-dense MVAC (DDMVAC)

In a parallel fashion, efforts were undertaken to improve the tolerability and dose-density of MVAC. Early reports of the use of hematologic growth factor support found that dose escalation of MVAC in urothelial carcinoma patients was possible and such an approach was associated with a favorable response rate. *(10,11)* With this emerging information, Sternberg et al pursued a randomized Phase III trial of high-dose intensity MVAC with granulocyte colony-stimulating factor (G-CSF) versus traditional MVAC in 263 advanced urothelial patients. *(12)* There was a complete response rate (CRR) of 21% and an overall response rate (ORR) of 62% with high-dose MVAC, compared to a CRR or 9% and ORR of 50% with standard-dose MVAC; the p value for the CR difference was 0.009. Neutropenic fever was more common with standard-dose MVAC (26% versus 10%), although this may be attributed to the disproportionate use of GCSF on the high-dose (94%) compared to the standard-dose (19%). In an updated report, with 7 years of follow-up, 24.6% were alive in the high-dose, versus 13.2% in the standard-dose MVAC arms. *(13)* While the median survival was similar between the 2 groups (15.1 versus 14.9 months), the overall mortality hazard ratio (HR) did favor high-dose MVAC (0.76; 95% CI, 0.58 to 0.99). Taken together, high-dose MVAC allows for more dose density, with lower toxicity and improved markers of efficacy.

Neoadjuvant chemotherapy for bladder cancer

Shortly after the development of active, cisplatin-based chemotherapy combinations for bladder cancer, the effectiveness of neoadjuvant chemotherapy was investigated. In an early Scandinavian study (NORDIC 1), 325 subjects with T1G3 or T2-T4aNxM0 bladder cancer were randomized to 2 cycles of cisplatin and doxorubicin versus no chemotherapy before cystectomy, with all subjects receiving a short radiation course. *(14)* The 5 year survival was 59% with chemotherapy compared to 51% without (p=0.1), although most of the advantage seems restricted to those with more advanced, local disease; for those with T3/T4a cancer, there was a 15% advantage with the application of chemotherapy. In a follow-up study (NORDIC 2), 317 subjects with T2-T4aNxM0 urothelial carcinoma of the bladder were randomized to 3 cycles of cisplatin and methotrexate versus no neoadjuvant chemotherapy. *(15)* The rate of complete pathologic response (pT0) was higher in the chemotherapy group (26.4%) compared to the non-chemotherapy group (11.5%)

(p=0.001), with the pT0 finding in the control arm likely attributable to complete transurethral resection of the tumor pre-operatively. The 5-year overall survival (OS) was improved in absolute terms by 7%, from 46% to 53%, with neoadjuvant chemotherapy, although this was not statistically significant in this relatively small study. With these and several other small randomized trials available, a meta-analysis of platinum-based neoadjuvant chemotherapy in patients with muscle-invasive bladder cancer was published in 2003. *(16)* This work found a significant survival benefit with neoadjuvant chemotherapy, yielding a HR of 0.87 (95% CI, 0.78 to 0.98). A second meta-analysis on this topic was performed in 2004 and included 8 randomized trials of cisplatin combination neoadjuvant therapy, with a pooled HR for OS of 0.87 (95% CI, 0.78 to 0.96). *(17)* This work also noted that a major pathologic response was associated with OS in 4 trials, supporting the use of pT0 rates as a marker of survival.

Two randomized studies were launched in the late 1980's to specifically assess the utility of MVAC and CMV chemotherapy in the neoadjuvant bladder cancer setting, as these regimens emerged as more effective than single-agent cisplatin. **SWOG 8710** enrolled 317 subjects with T2-T4aN0M0 bladder cancer, randomizing subjects to 3 cycles of standard-dose (28-day) MVAC versus no chemotherapy before cystectomy. *(18)* The median survival was improved in the experimental arm (77 versus 44 months) with a p = 0.06, favoring chemotherapy. The proportion of subjects with a pT0 at the time of surgery was also increased in the MVAC arm, 38% versus 15% (p < 0.001). In an important post hoc analysis of **SWOG 8710**, the significance of mixed histology (pure urothelial carcinoma versus a proportion of urothelial carcinoma) was analyzed. *(19)* Among those with mixed tumors, there was a clear survival advantage (HR 0.46; 95% CI, 0.25 to 0.87), supporting the use of neoadjuvant, cisplatin-based chemotherapy in those with a component of squamous or glandular differentiation in addition to urothelial carcinoma in their tumor.

A second, European-led neoadjuvant study was larger and enrolled 976 subjects with T2 (Grade 3), T3-T4a N0M0 urothelial cancer of the bladder in subjects planning for either cystectomy or definitive external beam radiation therapy. *(20)* Participants were randomized to a 21-day cycle of cisplatin-methotrexate-vinblastine (CMV) chemotherapy for 3 cycles versus no chemotherapy. For the 417 subjects undergoing cystectomy, the pathologic response rate (pT0 at the time of surgery) was 32.5% with chemotherapy versus 12.3% without. An update of the survival results was subsequently published with 8 years of follow-up. *(21)* Combining both the radiation and surgery groups, neoadjuvant chemotherapy was associated with a 16% reduction in the risk of death (HR, 0.84; 95% CI, 0.72 to 0.99) and an improvement in the 10-year survival from 30% to 36%. Considering the surgical cystectomy subjects alone, the reduction in the risk of death was 26% (HR, 0.74; 95% CI, 0.57 to 0.96; p = 0.022).

Neoadjuvant GC chemotherapy

As noted, since the initiation of these Phase III neoadjuvant studies of MVAC and CMV chemotherapy, GC has become a standard approach in advanced urothelial carcinoma, frequently favored over the more toxic MVAC regimen. There is a limited amount of clinical data for the use of GC in the neoadjuvant setting. In one report from investigators at Memorial Sloan-Kettering, the results of 42 bladder cancer patients given 21-day neoadjuvant GC was reported and compared historical controls given MVAC. *(22)* The pT0 proportion was 26% with GC, compared to 28% with the historical MVAC group. They also assessed <pT2 rate, which was 36% with GC, versus 35% with MVAC.

The prognostic importance of the pathologic T staging

pT0 at the time of radical cystectomy is associated with long-term and survival outcomes in bladder cancer patients. In the **SWOG 8710** trial, regardless of treatment arm, the finding of pT0 at the time of surgery correlated with survival. *(23)* In a subsequent analysis, the impact of pT0 versus <pT2 (pT0, pTa, pTis, and pT1) on survival was analyzed. In the chemotherapy arm, pathologic findings of < pT2 was observed in 44%, with 30% having pT0. The pT0 rate is lower in this analysis compared to the original New England Journal of Medicine publication, as patients who did not actually receive any chemotherapy were also included. Those with pT0 had a median OS of 13.6 years, those with pT1/pTis/pTa had a median OS of 10.6 years, while those with pT2 or great

disease at surgery had a median OS of just 3.7 years. *(24)* The impact of pathologic stage on long-term outcomes was also evaluated in 2230 patients with radical cystectomy, but no adjuvant chemotherapy. They found 5.1% had pT0 at the time of surgery; with a median follow-up of 48 months, 10.1% of these pT0 patients had recurrent disease. Of note, the recurrence-free outcomes were similar between the pT0 and pTa/pTis patients (p = 0.557). Taken together, these data suggest the prognostic significance of both pT0 and <pT2 at the time of cystectomy.

Low utilization of neoadjuvant chemotherapy for bladder cancer

Despite the supportive data for neoadjuvant chemotherapy in large, Phase III studies, the integration of pre-operative chemotherapy for bladder cancer into routine management of bladder cancer patients remains stubbornly low. While there are two randomized Phase III studies with positive findings for the use of neoadjuvant chemotherapy with bladder carcinoma, there is no high-level data supporting adjuvant chemotherapy. An analysis of newly diagnosed bladder cancer patients, as capture in the National Cancer Data Base, examined the use of perioperative chemotherapy. *(25)* During the period from 1998-2003, the rate of perioperative chemotherapy use in Stage III (T3N0M0) patients was 11.6%, with only 1.2% receiving neoadjuvant chemotherapy. This showed that while perioperative chemotherapy was uncommon in general, when it was administered, it was almost exclusively in the adjuvant/post-operative setting. A more recent analysis of 2287 patients treated with cystectomy in Canada from 1998-2008, shows a similar trend. *(26)* The overall application of perioperative chemotherapy was low, with only 3.1% of pre-operative and 19.4% of post-operative patients receiving such therapy. A similar trend of low neoadjuvant chemotherapy use in eligible bladder cancer patients has been reported in modern, US single-institutional studies. *(27)*

Predictive biomarkers of chemotherapy for bladder cancer

There are many recent examples of the use of predictive biomarkers for the selection of therapy in cancer patients across several different cancer types. In breast cancer, it has been established that response to trastuzumab is largely limited to those with HER2/neu overexpression and specific national guidelines detail the testing procedure for HER2/neu assessment to determine the appropriateness of trastuzumab therapy. *(28,29,30)* Other examples of predictive biomarkers in oncology include epidermal growth factor receptor mutation in non-small cell lung cancer for gefitinib/erlotinib and the presence of the ALK fusion oncogene for the use of crizotinib. *(31,32,33,34)* Notably, these successes involve biologic agents against specific targets, allowing for the rational identification and validation of a predictive marker. While targeted agents have been integrated into the general oncology care of many cancer patients, there is currently no proven or widely accepted use of molecularly targeted agents in bladder cancer.

The development of predictive markers for cytotoxic agents has been less successful than for the newer targeted agents. One recent Phase III study assessed the usefulness of p53 assessment in the adjuvant bladder cancer setting. *(35)* Past work had suggested both a prognostic and predictive role for p53 in bladder cancer. *(36)* To test this prospectively, patients with pT1-pT2 N0 M0 disease at the time of cystectomy were tested for p53 expression by immunohistochemistry. Those with positive expression were randomized to receive 3 cycles of MVAC versus observation, and all p53 negative subjects were observed without additional chemotherapy prescribed. The 5-year recurrence rate was approximately 20% regardless of p53 status, failing to show a prognostic value of p53 in this setting. Additionally, there was no difference in the recurrence rate of p53-positive patients randomized to chemotherapy versus observation, and a predictive role for p53 in selecting cisplatin-sensitive bladder cancer patients was not supported.

With the importance of cisplatin therapy in bladder cancer, much of the predictive biomarker discovery effort in bladder cancer has focused on markers of platinum sensitivity. The excision repair cross complementing 1 (ERCC1) gene acts in a DNA repair capacity and specifically plays a role in the repair of nucleotide excision – a key mechanism of cisplatin activity. *(37)* In an early assessment of the significance of ERCC1's expression in chemotherapy-treated metastatic bladder cancer, Bellmunt et al assessed mRNA levels of ERCC1 in 57 advanced bladder cancer patients treated with cisplatin-based combination chemotherapy. *(38)* Notably, the median OS was

significantly higher in those with lower ERCC1 levels (25.4 versus 15.4 months) and ERCC1 was an independent predictor of survival. However, there were no significant differences in chemotherapy response rates, based on ERCC1 levels, and it was not possible to distinguish this as a predictive versus prognostic factor. In a retrospective analysis of 108 subjects from the AUO-AB 05/95 adjuvant bladder cancer study treated with cisplatin-combination chemotherapy, ERCC1 expression levels (by RT-PCR) were assessed and correlated with long-term outcomes. *(39)* Low ERCC1 levels (less than the 75th percentile) were associated with an improved progression-free survival (PFS) (HR 0.52; 95% CI, 0.27 to 1.01). Using Cox regression analysis, there was a significant association between ERRC1 levels and PFS (p=0.01). Despite an improvement in the median OS in those with low ERCC1 expression (72.4 versus 33.1 months), this did not reach statistical significance (p = 0.19), although this was a relatively small sample size, without substantial power to detect small to moderate differences. Similar findings of a PFS benefit were reported in a second study in which the immunohistochemistry expression of ERCC1 was retrospectively assessed in 89 patients with advanced urothelial carcinoma treated with cisplatin-based chemotherapy.*(40)* There was not a statistical difference in the overall response rate (p=0.61) or the OS between the low and high-ERCC1 expression groups of this study; however, the PFS was 2.2 months longer in the ERCC1-negative patients and this reach statistical significance. A small study of 22 muscle-invasive bladder cancer patients treated with radiation and cisplatin-based chemotherapy assessed clinical response in relation to ERCC1 immunoreactivity scores. *([41])* For those with negative ERCC1 reactivity, the complete response rate was 86% compared to 25% in those with positive ERCC1 expression. Not all studies have demonstrated a survival or progression advantage with lower ERCC1 levels in cisplatin-treated bladder cancer patients. Matsumura et al assessed ERCC1 via immunostaining in 40 metastatic urothelial carcinoma patients treated with GC, but there was no statistically significant difference, although the median OS was 13.6 versus 17.1 months in the high and low expression groups, respectively. *(42)*

Other markers have been assessed to predict chemotherapy sensitivity in bladder cancer. Notably, the breast cancer susceptibility gene 1 (BRCA1), an important actor in DNA repair, was assessed in the neoadjuvant bladder cancer setting. *(43)* The pretreatment samples of 57 patients undergoing CMV or GC neoadjuvant chemotherapy were assessed for mRNA expression levels of BRCA1. In those with low BRCA1 levels, the pathologic down-staging (< pT2) rate was 66% versus 22% in those with high expression (p=0.01). Additionally, the median OS was 168 months in the low/intermediate versus 34 months in the high-expression group (p=0.002). Of note, Bellmunt et al had examined BRCA1 mRNA levels in their evaluation of 57 advanced cancer patients and found no correlation with survival or chemotherapy response. *(44)* The multi-drug resistance gene, MDR1, was also assessed by reverse transcription polymerase chain reaction (RT-PCR) in the AUO-AB 05/95 adjuvant bladder cohort. MDR1 expression below the 75% percentile was associated with improved OS (HR 0.25; 95% CI, 0.11 to 0.55). *(45)*

While ERCC1 has been directly associated with cellular response mechanism related to repairing the DNA adducts caused by cisplatin, the mechanism of action of other chemotherapy agents is different and may have separate markers of response. Gemcitabine requires specific plasma membrane transporters in order to enter the cells and exert its anti-cancer effect. One of the main transporters in this regard is human equilibrate nucleoside transporter 1 (hENT1) and its immunohistochemistry expression has been associated with prolonged survival in patients with advanced pancreatic adenocarcinoma treated with gemcitabine. *(46,47)* A recent investigation assessed the hENT1 expression by immunostaining in 40 metastatic bladder cancer patients treated with GC chemotherapy. *(48)* The median OS was longer in patients with high hENT1 expression compared to low hENT1 expression (17.3 versus 11.6 months, p=0.003)

<u>COXEN – CO-eXpression ExtrapolatioN</u>
The NCI-60 is an intensively studied group of sixty cell lines from nine common cancer types, which provides a rich dataset for evaluating gene expression profiling of drug sensitivity. These sixty cell lines have been treated with tens of thousands of compounds for which the response data is publically available. *(49)* COXEN represents a “correlation of correlations”, allowing for the

customization of the in silica NCI-60 results and the application of these findings to specific cells or patients. *(50)* The first step in this process is the identification of the gene expression associated with individual drug sensitivity in the NCI-60, which was used for the Gene Expression Model (GEM) development for the single drug COXEN scores. These genes are then compared with the application set (cellular or human samples), identifying concordant expressing genes. Therefore, only those genes which are relevant to one's planned application (e.g. present in both the application set and the NCI-60 set) are included in the final gene expression profile. By comparing the NCI-60 results with the gene expression of the target findings, a correlation coefficient (COXEN coefficient) is derived. In a phrase, COXEN translates the NCI-60 drug sensitivity data to any other cellular or clinical gene expression data set. This general use of predictive gene expression profiling has been employed in other cancer types, such as breast cancer. In that setting, the application of a 21-gene signature to estrogen receptor-positive women being treated with tamoxifen adjuvantly, allows for the predication of the likelihood of cancer recurrence. *(51)*

The COXEN algorithm may be divided into 6 distinct steps *(52)*:

1. Step 1: Experimentally determine the drug's pattern of activity in cells of set 1.
- Step 2: Experimentally measure molecular characteristics of the cells in set 1.
- Step 3: Select a subset of those molecular characteristics that most accurately predicts the drug's activity in cell set 1 (''chemosensitivity signature'' selection).
- Step 4: Experimentally measure the same molecular characteristics of the cells in set 2.
- Step 5: Among the molecular characteristics selected in step 3, identify a subset that shows a strong pattern of coexpression extrapolation between cell sets 1 and 2.
- Step 6: Use a multivariate algorithm to predict the drug's activity in set 2 cells on the basis of the drug's activity pattern in set 1 and the molecular characteristics of set 2 selected in step 5. The output of the multivariate analysis is a COXEN score.

COXEN therefore fundamentally differs from the more standard, a posteriori approach to biomarker discovery in the search for predictive biomarkers. In the traditional approach, the baseline tissue is profiled, the clinical trial subjects are assessed for treatment response, and this is followed by supervised biomarker discovery using these data. The main disadvantage to this approach is that it is not a priori and requires the completion of a clinical trial to generate the gene expression profile. It also does not easily allow for the evaluation of drugs with a low overall response rate, even if a small subset will respond. COXEN allows for the immediate validation of the gene expression profile in human trials, since the signature is derived in the preclinical arena. Additionally, COXEN has the advantage of identifying new drug candidates with an accompanying predictive biomarker (e.g. gene expression profile), providing a means of screening patients to enrich for a responding population. *(53)* COXEN may also shed light on previously unknown mechanisms of drugs actions, based on the genes identified as part of the algorithm. *(54)*

For the COXEN development of the GC and MVAC regimens in bladder cancer, the described approach was followed. The NCI-60, BLA-40, or GDSC-648 cell panels were used in the initial discovery of predictive biomarkers for drug sensitivity. These biomarkers were then filtered based on their co-expression with a human bladder tumor cohort. *(55)* Single drug GEMs were developed by means of principal component regression analysis on the NCI60 panel and finalized via univariate COX proportional hazard regression on two human bladder cancer cohorts. The Lehmann-treated cohort utilized MVEC and was used for the GEM evaluation and final selection. The MVAC-treated Laval cohort (Laval 54) was used for independent validation for methotrexate, vinblastine and adriamycin. For Cisplatin, the entire Laval cohort of 90 was used for independent validation, since all received cisplatin. In contrast, since gemcitabine was not used in the Lehmann cohort, the GEM evaluation and final selection utilized the GC-treated Laval cohort (Laval 36) with independent validation on the BL40 panel. *(56)*

Single drug COXEN scores (S_i) can be calculated by solving the linear regression model:

$$S_i = \alpha + \sum_{k=1}^{K} \beta_k X_{ki}$$

Which is to be interpreted as the single drug score (S_i) = model intercept (α) + summation of probe gene expression data (X_{ki}) multiplied by corresponding coefficients (β_k).

The **COXEN score for combination drugs** is a probability score based on 5-year survival and is derived from a parametric survival regression model of Single Drug COXEN scores. Let M_i, V_i, A_i, C_i, and G_i denote the percentile of COXEN drug response prediction score of i-th patient for each Methotrexate, Vinblastine, Adriamycin, Cisplatin, and Gemcitabine, respectively. Then the COXEN scores P_{MVAC} and P_{GC} of i-th patient for MVAC and GC are calculated as below.

$$P_{MVAC} = e^{-(5\lambda_{MVAC})^{1.091486}}$$
$$P_{GC} = e^{-(5\lambda_{GC})^{0.8961938}}$$

Where

$$\lambda_{MVAC} = e^{-(0.761211+(0.766369\times M_i)+(0.3657074\times V_i)+(0.7634502\times A_i)+(0.8308788\times C_i))}$$
$$\lambda_{GC} = e^{-(-0.2456275+(2.8852677\times G_i)+(1.6766004\times C_i))}.$$

Model training and evaluation for the combination COXEN models were done via parametric survival regression, ROC, and survival analysis on the Lehmann-188 for the MVAC model and 36 GC treated patients of the Laval-90 for the GC model. The MVAC model was independently validated on the 54 MVAC-treated patients of the Laval-90 cohort (Figure 1). A quality control procedure is in place to determine if any samples need to be discarded for technical or quality concerns (Table 1).

Table 1. Quality Control Analysis Steps for Individual MicroArrays Data Sets

1. Inspect array images
   - Examine for "spatial artifacts" (gross problems)
2. Assess a boxplot and histogram plot of log2 (PM) values for each array
   - Looking for consistency across arrays, identifying outliers
3. Proportion present plot analysis
   - Use probe pairs (PM,MM) to test whether gene is "Present" of "Absent"
   - Examine for consistency across arrays, identifying outliers
4. Plot of Hybrid Controls
   - Assess that bioB, bioC, bioD, and cre are present in increasing concentrations on each array
5. QC stats plot
   - The "qc" function returns an object containing scale-factors,% present, average background intensities (values greater than 100 indicates problem) and bioB, bioC, bioD, and creX present calls. It also plots the 3'/5' ratios of actin and GAPDH (3-fold or greater discrepancies between arrays indicates an outlier)
6. RNA Degradation plot
   - It is expected that 5' end will have more degradation than 3' end. Arrays will be examined for consistency, identifying outliers

*If a sample is found to have an outlier in more than 2 of these tests, the sample should be discarded.

Figure 1

A

Biomarker Discovery

Step 1: Define microarray probes whose expression correlates with drug (M,V,A,C,G) sensitivity (IC50)
Datasets: Affy profiled cell lines: NCI60 (GSE5946), BLA40 (GSE5845), GDSC648 (www.cancerrxgene.org)

Step 2: "Humanization" of probes from Step 1 (the "co-expression" step)
Datasets: Affy profiled human bladder tumors (Sanchez-Carbayo N=89, J Clin Oncol. 2006;24(5):778)

Deliverable: ~100-300 probesets for each drug (M,V,A,C,G) whose expression relates to IC50

B

Development of *Single Drug* Gene Expression Models (GEM) and COXEN scores

Step 1: Single Drug GEM development and validation
Datasets: Affy profiled human bladder tumors (Lehmann N=188, Laval N=90, Lancet Oncol. 2011;12(2):137)

	Analysis Type	Methotrexate	Vinblastine	Adriamycin	Cisplatin	Gemcitabine
GEM development	Principal component regression	NCI60 panel	NCI60 panel	NCI60 panel	NCI60 panel	NCI60 panel
GEM evaluation and final selection	Univariate COX proportional hazard regression	Lehmann 188 (MVEC or CM treated)	Lehmann 92 (MVEC treated)	Lehmann 92	Lehman 188	Laval 36 (GC treated)
Independent validation		Laval 54 (MVAC treated)	Laval 54	Laval 54	Laval 90 (MVAC or GC treated)	BLA40 panel
Final number of probes* in GEM with corresponding probe coefficient* data		20 probes	60 probes	40 probes	35 probes	35 probes

Step 2: Single Drug COXEN score calculation is a linear regression model seen below:

Single Drug COXEN score = $\alpha + \sum_{k=1}^{K} \beta_k X_{ki}$ = intercept (α) + summation of probe gene expression data (X_{ki}) multiplied by corresponding coefficients (β_k). * GEM probes and their coefficients needed to compute COXEN Scores are **Fixed**

C

Development of *Combination Drug* COXEN scores

Step 1: Parametric survival regression analysis to obtain coefficients for *each* Single Drug COXEN score

	Analysis	MVAC model	GC model
Model training and evaluation	Parametric survival regression, ROC, and survival analysis	Lehmann 188	Laval 36
Independent validation	ROC and survival analysis	Laval 54	No Dataset Available

Step 2: Combination Drug COXEN score calculation:
The COXEN score for combination drugs is a probability score and derived from a parametric survival regression model of Single Drug COXEN scores. Let M_i, V_i, A_i, C_i, *and* G_i denote the percentile of COXEN drug response prediction score of *i*-th patient for each Methotrexate, Vinblastine, Adriamycin, Cisplatin, and Gemcitabine, respectively. Then the COXEN scores P_{MVAC} and P_{GC} of i-th patient for MVAC and GC are calculated as below.

$$P_{MVAC} = e^{-(5\lambda_{MVAC})^{1.091486}} \quad \text{where} \quad \lambda_{MVAC} = e^{-(0.761211+(0.766369\times M_i)+(0.3657074\times V_i)+(0.7634502\times A_i)+(0.8308788\times C_i))}$$

$$P_{GC} = e^{-(5\lambda_{GC})^{0.8961988}} \quad \text{where} \quad \lambda_{GC} = e^{-(-0.2456275+(2.8852677\times G_i)+(1.6766004\times C_i))}$$

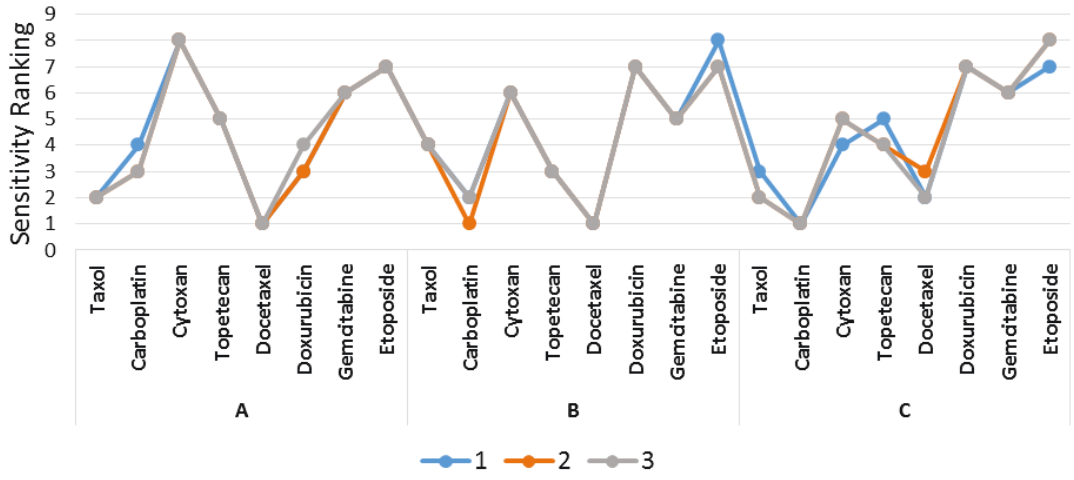
(intercept; coefficient*; coefficient; coefficient; coefficient)

*Coefficients from Survival Regression Model are **Fixed** for Combo COXEN Scores

Since calculating the final combination COXEN score involves ranking the single drug COXEN scores within a population of samples, generating final scores for patients one at a time in a prospective trial setting necessitates the use of a representative baseline cohort. We will use a combined baseline cohort consisting of the Laval90 and Lehmann188 datasets (n=278) with which to rank our new patients single drug scores against. For proper normalization of microarray samples, we will use frozen robust multiarray analysis (fRMA) to process both the baseline cohort and the individual patient sample. *(57)* In contrast to normal RMA, fRMA is able to process single samples because it uses normalization parameters from publicly available microarray databases that are pre-computed and "frozen". This method has been shown to outperform traditional RMA in adjusting for batch effects. After fRMA processing of the baseline cohort and single patient sample, single drug and final combination COXEN scores are generated for the individual patient.

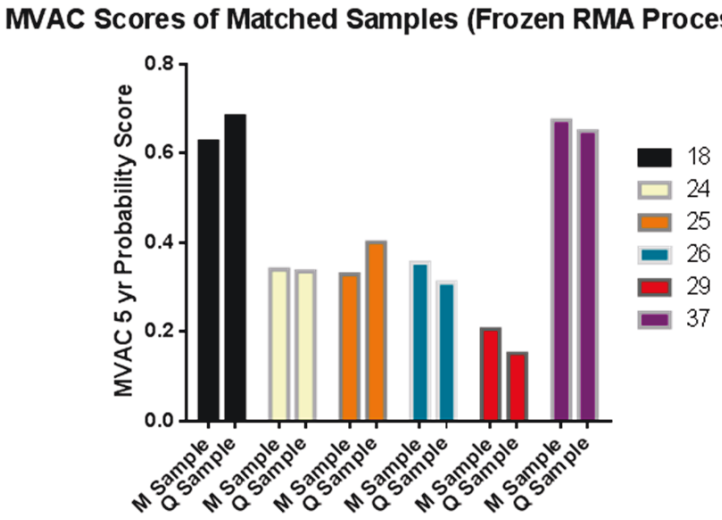
The reproducibility and consistency of COXEN assay processing and prediction has been confirmed from several ongoing COXEN studies. In particular, both pre-analytic and analytic consistency of the COXEN evaluation for ovarian cancer patients has carefully examined. In a study by Jae Lee, PhD at the University of Virginia, three replicated tissue samples (from three different tumor blocks/locations) of each of three ovarian cancer patients were acquired. Each sample was then independently processed for all pre-analytic steps including RNA extraction, labeling, microarray hybridization, and scanning as well as for all analytic steps including microarray data preprocessing, normalization, data quality control, and COXEN computational prediction. He found that the COXEN chemosensitivity predictions across eight chemotherapy options were consistent for all replicates of the three patients in predicting drug sensitivity, providing identical therapeutic recommendations for them. In Figure 2, the COXEN chemo-sensitivity prediction ranks (1-best to 8-worst) of eight standard chemotherapy drugs are shown.

FIGURE 2. Predicted Sensitivity Rankings to Specified Drugs of Triplicate Samples (1-3) from Three (A,B,C) Independent Human Ovarian Tumors



Tests of reproducibility for COXEN score determination have also been performed on bladder cancer samples. From the same clinical tumor, 2 separate biopsy samples from different tumor locations were analyzed in 6 different patients (Figure 3). Their COXEN scores for MVAC were compared between the two samples for each patient. There was strong agreement between the 2 samples for each patient, with the mean prediction error (MPD) = 2.17% and the mean absolute prediction error (MAPD) = 12.43%.

Figure 3. Accuracy Across Tumor Samples



COXEN application to bladder cancer

COXEN-derived gene expression profiles have been assessed in the neoadjuvant and advanced bladder cancer setting. Als et al performed gene expression profiling on 30 urothelial carcinoma patients with locally advanced (T4b or N2-3) or metastatic disease. *(58)* Chemotherapy treatments consisted of MVAC or GC and tumor responses to chemotherapy were scored based on WHO criteria. Using a COXEN combination gene expression profile for MVAC and applied to the MVAC-treated patients (n=14), the COXEN scores were statistically different between the "responders" and "non-responders" (p = 0.033). *(59)* Additionally, the 3 year survival rates were 61% in the COXEN-selected patients versus 16% in those not predicted to respond to chemotherapy by COXEN (p = 0.015) (Figure 4A).

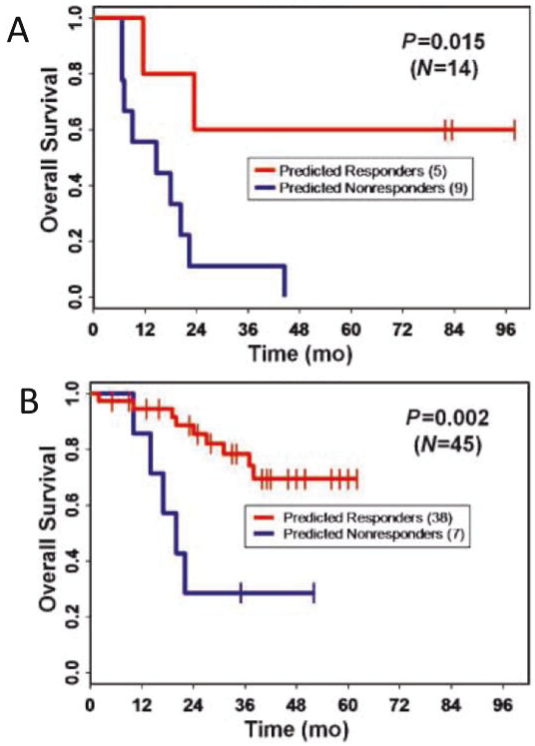


Figure 4 - Survival curves of bladder cancer patient treated with MVAC according to COXEN score. (Cancer Res 2009; 69: 8308)

In a neoadjuvant study conducted in Japan, 45 patients with T2a-T3N0M0 bladder cancer were given 2 cycles of 28-day MVAC prior to cystectomy. *(60)* "Responders" were defined as achieving down-staging to ≤pT1 at the time of cystectomy. Applying the combination gene expression profiling (GEP) for MVAC to this population, there was a statistical difference between the COXEN scores of those with down-staging and those without a pathologic response (p = 0.002). *(61)* In accordance, the 3 year survival rates for COXEN-predicted

responders was 81% versus 33% in the predicted non-responders (p = 0.002), as shown in Figure 4B. Importantly, the COXEN-derived GEP predictions in this cohort were independent of tumor stage, grade, sex and gender (Spearman correlation was -0.14 to 0.07).

Takata et al defined a 15 gene signature, derived in a traditional posteriori approach using a training and validation set, to predict for tumor down-staging with neoadjuvant MVAC. To test the robustness of the Takata gene expression profile versus the COXEN gene expression profile for MVAC in this setting, both were applied to the Als et al independent data set. While the COXEN gene expression profile did separate these patients with respect to OS in a statistically significant manner, the Takata gene expression profile did not, with a p-value of 0.73. *(62)* Combining the results of the 2 bladder cancer studies (Als and Takata) and using combination COXEN-derived gene expression profile for MVAC, with respect to "responders", the gene expression profile had a sensitivity of 83% and specificity of 64% with a positive predictive value of 71% and a negative predictive value of 78%. *(63)* In considering this published data, it is noted that the application of COXEN in this protocol will use a different predictor than used in these studies, converting to a one-at-a-time predictor, to avoid over fitting. Along with this, we will note that the data from Als et al and Taketa et al have the potential to be optimistically biased based on the risk of over fitting and re-substitution.

It is recognized that both MVAC and GC incorporate cisplatin, the most active agent against bladder cancer. As shown in Figure 1, despite the commonality of cisplatin in both regimens, the COXEN GEMs for GC and MVAC are distinct due to the activity of the other agents involved in each regimen. To assess the overall effectiveness and specificity of the COXEN GEMs relative to each regimen, the MVAC and GC COXEN GEMs were both applied to GC and to MVAC-treated patients from the Laval cohort. As shown in Figure 5, in MVAC-treated patients (n=54), the MVAC COXEN GEM (p=0.0019), but not the GC COXEN GEM effectively segregated patient outcomes. The distribution of the 5 year survival of the combined Laval and Lehman cohorts is show in Figure 6. Additionally, a plot of MVAC versus GC COXEN scores performed separately for both the Laval and Lehmann cohorts demonstrate that the scores do not provide identical information, despite the commonality of cisplatin in each regimen (Figure 7).

Figure 5

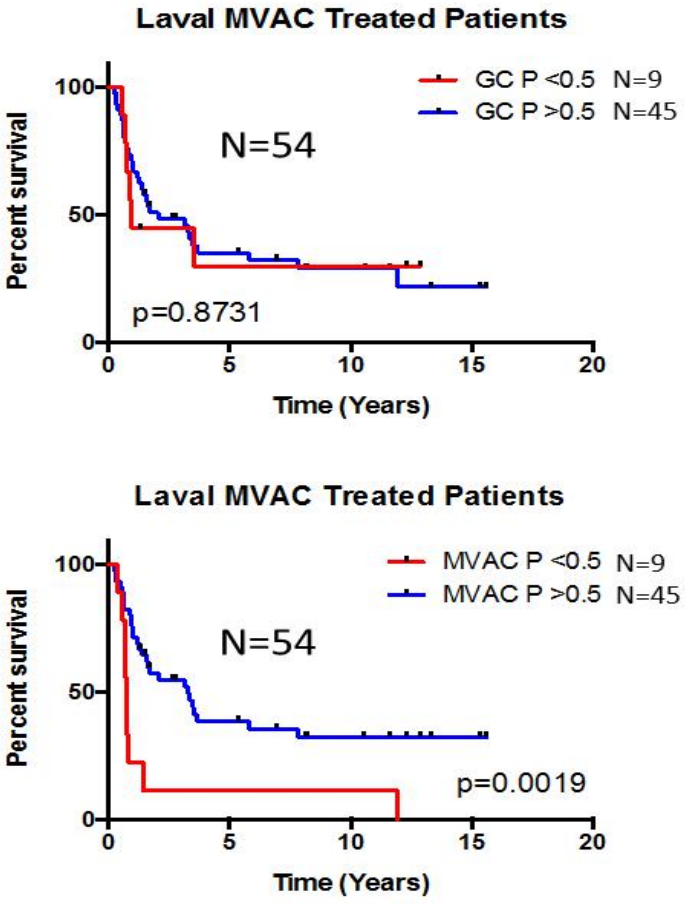


Figure 6

Distribution of 5 Year Survival Probability Combination Scores
for GC and MVAC in Lehmann 188 and Laval 90 Patient Populations

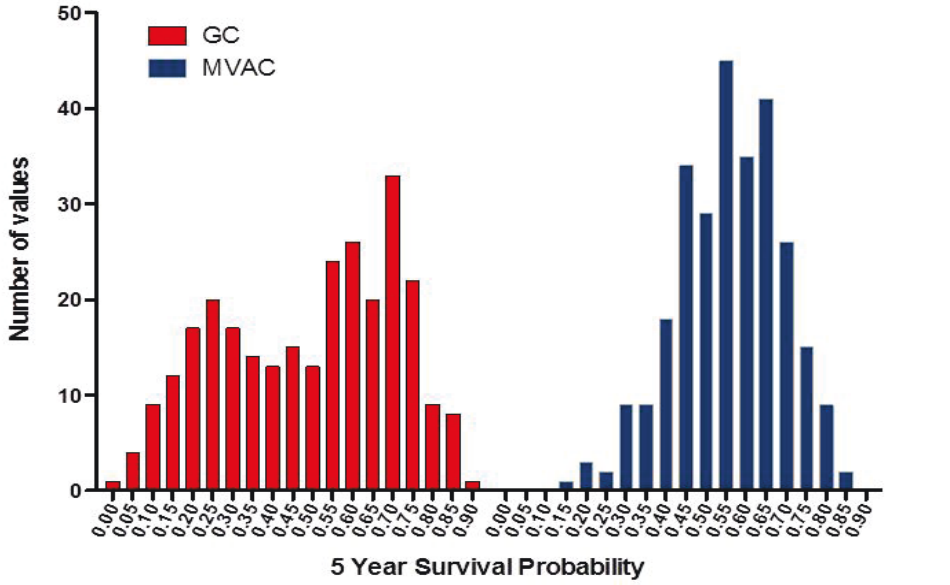
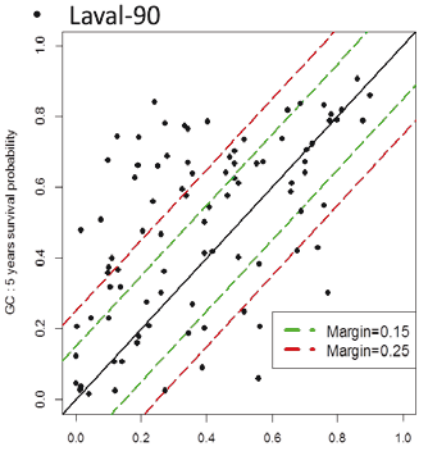


Figure 7

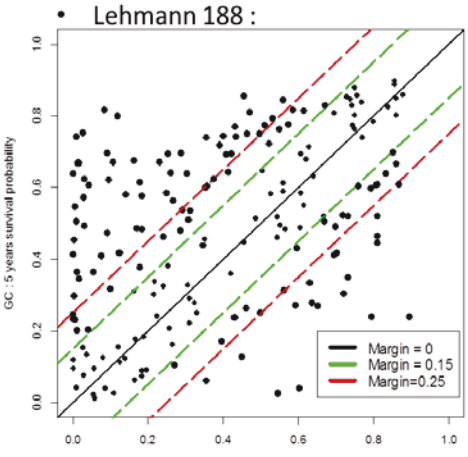
Comparison of MVAC (X-axis) and GC(Y-axis) scores by different margin

- Laval-90



Margin	GC>MVAC	GC < MVAC	GC=MVAC
0	61 (67.78%)	29 (32.22%)	**0 (0%)**
0.15	36 (40%)	13 (14.44%)	**41 (45.56%)**
0.25	22 (24.44%)	7 (7.78%)	**61 (67.78%)**

- Lehmann 188 :



Margin	GC>MVAC	GC < VAC	GC=MVAC
0	116 (61.7%)	72 (38.2%)	**0 (0%)**
0.15	76 (40.4%)	38 (20.2%)	**74 (39.4%)**
0.25	54 (28.7%)	19 (10.1%)	**115 (61.2%)**

Rationale
Despite positive findings in two Phase III studies in support of cisplatin-based, neoadjuvant chemotherapy, the generalize use of such therapy is very low. While these studies demonstrate a survival benefit, it is also clear that some patients are not responsive to chemotherapy. Therefore, it is possible that a chemotherapy-resistant patient may progress to an advanced, unresectable state during neoadjuvant chemotherapy. Such a concern is undoubtedly a factor in the low adoption rate of neoadjuvant chemotherapy for bladder cancer. There are also different chemotherapy agents that may be combined with cisplatin including gemcitabine, methotrexate and doxorubicin. There is currently no accepted method to select the optimal secondary chemotherapy agent(s) to use in a specific patient, in order to maximize the chances of a response, acknowledging that some patients may have a particularly methotrexate-sensitive tumor and would be better served by MVAC rather than GC chemotherapy.

COXEN gene expression profile predicts an individual patient's likelihood of responding to a specific chemotherapy agent or combination of chemotherapy agents. As detailed here, COXEN gene expression profiles have been applied to hundreds of patients, including bladder cancer patients, with strong predictive characteristics. This study will begin by randomizing subjects with localized, muscle-invasive bladder cancer to either GC or DDMVAC chemotherapy. Gene expression, microRNA expression and tissue microarray samples will be collected prior to chemotherapy. Along with the assessment of other biomarkers, the ability of COXEN to correctly predict response, as assess via the pT0 rate, will be tested. In a follow up study, this collected data will serve as the control for a COXEN directed application of neoadjuvant chemotherapy, assessing for an increased response rate with this approach.

The ability to predict chemotherapy response would allow a more rational application of neoadjuvant chemotherapy. First, those not sensitive to any standard chemotherapy agents could proceed directly to surgery, avoiding potentially detrimental delays and toxicity from predicted ineffective chemotherapy. Second, for those found sensitive to chemotherapy, COXEN could direct them to the chemotherapy combination most likely to yield a response, such as GC rather than DDMVAC in someone with a predicted response to gemcitabine.

Inclusion of Women and Minorities:
This study was designed to include women and minorities, but was not designed to measure differences of intervention effects. The anticipated accrual in the ethnicity/race and sex categories is shown in the table below. Both men and women of all races and ethnic groups are eligible for this study.

Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	3	18	21
Not Hispanic or Latino	41	168	209
Total Ethnic	44	186	230
Racial Category			
American Indian or Alaskan Native	0	1	1
Asian	0	2	2
Black or African American	2	9	11
Native Hawaiian or other Pacific Islander	0	1	1
White	42	173	215
Racial Category: Total of all Subjects	44	186	230

3.0 DRUG INFORMATION

For this study, all drugs are commercially available; therefore, Investigator Brochures are not applicable to this/these drug(s). Information about commercial drugs is publicly available in the package insert and other resources.

3.1 Gemcitabine hydrochloride (Gemzar®) (NSC-613327)

a. PHARMACOLOGY

Mechanism of Action: Gemcitabine (2'-Deoxy-2', 2'-difluorocytidine monohydrochloride), like cytarabine, is a nucleoside analog of deoxycytidine. This antimetabolite, a pyrimidine analog inhibiting both DNA and RNA viruses, is cell-cycle-specific in blocking the cells at the G1/S and is retained in human tumor cells for long periods. Studies suggest that gemcitabine is activated by deoxycytidine kinase. Deoxycytidine has been shown to reverse the growth inhibitory activity of gemcitabine.

b. PHARMACOKINETICS

1. Distribution: Gemcitabine plasma protein binding is negligible. The volume of distribution is increased with the infusion length. In a pharmacokinetics study of patients with various solid tumors, the volume of distribution of gemcitabine was 50 L/m^2 following infusions lasting <70 minutes. For long infusions (70 to 285 minutes), the volume of distribution rose to 370 L/ m^2.

2. Metabolism: Gemcitabine is metabolized intracellularly to form active gemcitabine di- and tri-phosphates. The gemcitabine di- and tri-phosphates do not appear to circulate in plasma in measurable amounts. Gemcitabine is metabolized by the liver to form the inactive uracil derivative, 2'-deoxy-2',2'-difluorouridine (dFdU). The inactive metabolite does not appear to accumulate with weekly dosing; however, it is excreted by the kidneys and may accumulate in patients with decreased renal function.

3. Elimination: Following a single 1,000 mg/m^2/30 min [^{14}C]-gemcitabine infusion, 92% to 98% of the dose was recovered within 1 week after gemcitabine administration. Urinary excretion of the parent drug and the dFdU metabolite accounted for 99% of the excreted dose, and less than 1% of the dose was excreted in feces. The renal clearance of gemcitabine is less than 10%; therefore, the parent drug appears to be almost completely metabolized to the inactive dFdU.

   Clearance of gemcitabine is affected by age and gender and is lower in women and the elderly. Differences in either clearance or volume of distribution based on patient characteristics or the duration of infusion result in changes in half-life and plasma concentrations. Studies showed that gemcitabine half-life for short infusions ranged from 42 to 94 minutes, for long infusions it varied from 245 to 638 minutes, depending on age and gender, reflecting a greatly increased volume of distribution with longer infusions. The terminal phase half-life for the active metabolite, gemcitabine triphosphate, in mononuclear cells ranges from 1.7-19.4 hours.

c. ADVERSE EFFECTS

1. Possible Side Effects of gemcitabine: Adverse effects reported in 10% or more of patients receiving gemcitabine monotherapy include myelosuppression (i.e., anemia, leukopenia, neutropenia, thrombocytopenia), proteinuria, hematuria, increased BUN, nausea, vomiting, pain, fever, rash, pruritus, dyspnea, constipation, diarrhea, hemorrhage, peripheral edema, edema, flu-like symptoms, infection, alopecia, stomatitis, somnolence, paresthesias, and increased serum AST, ALT, alkaline phosphatase, and bilirubin concentrations.

   Dose limiting toxicity is bone marrow suppression with mild to moderate granulocytopenia, anemia and thrombocytopenia. There has been no evidence of cumulative WBC or platelet toxicity. Gastrointestinal toxicities include nausea, vomiting, and diarrhea. Gemcitabine should be used with caution in patients with impaired liver function since abnormalities of liver transaminase enzymes have been reported. Mild proteinuria and hematuria have been reported but were not clinically significant and usually not associated with any change in serum creatinine or BUN. A few cases of renal failure of uncertain etiology have been reported. Gemcitabine should be used with caution in patients with impaired renal function. Toxicities associated with allergic reaction include rash, pruritus, desquamation, vesiculation, ulceration, and dyspnea. Bronchospasm has been reported in less than 1% of patients. Twenty percent of patients have also experienced flu-like symptoms such as fever, headache, back pain, chills, myalgia, asthenia, anorexia, cough, rhinitis, malaise, sweating, and insomnia. Other toxicities include edema or peripheral edema in 30% of patients, alopecia, somnolence, diarrhea, constipation, and oral toxicity (soreness and erythema). Pulmonary edema has been a rare occurrence (less than 1%). A few cases of hypotension have been reported, as well as myocardial infarction, congestive heart failure and arrhythmia. However, there is no clear evidence that gemcitabine causes cardiac toxicity.

   Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions.

2. Pregnancy and Lactation: Category D. Gemcitabine may cause fetal harm when administered to a pregnant woman. This agent has produced teratogenic effects in mice and rabbits when administered at a dose of < 2 mg/m^2. Adverse effects included decreased fetal viability, weight and morphologic defects. There is no data on gemcitabine administration during human pregnancy, and it is not currently known if metabolites are excreted in human milk. However, many drugs are excreted in human milk, and there is a potential for adverse effects in nursing infants. Therefore, the use of gemcitabine should be avoided in pregnant or nursing women because of the potential hazard to the fetus or infant.

   Drug Interactions: Per gemcitabine package insert, no formal drug interaction studies have been performed to date. When gemcitabine was administered with carboplatin or paclitaxel there was minimal or no effect on the pharmacokinetics of the studied drugs.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Gemcitabine is commercially available and will not be supplied. Refer to the current FDA-approved package insert.

3.2 Cisplatin (CDDP) (Platinol®) (NSC-119875)

a. PHARMACOLOGY

Mechanism of Action: Cisplatin (cis-diamminedichloroplatinum) is a heavy metal complex containing a central platinum atom surrounded by two chloride atoms and two ammonia molecules in the cis position. It is water soluble and acts as a bifunctional alkylating agent with cell cycle nonspecific characteristics. The intra-strand cross-links, in particular with guanine and cytosine, change DNA conformation and inhibit DNA synthesis leading to the cytotoxic and anti-tumor effects of cisplatin. Although cisplatin seems to act as an alkylating agent, there are data to indicate that its mode and sites of action are different from those of nitrogen mustard and the standard alkylating agents and that cisplatin does not exhibit cross-resistance with other alkylating agents or nitrosoureas.

b. PHARMACOKINETICS

1. Absorption: Following rapid IV injection of cisplatin over up to one hour, peak plasma drug and platinum concentrations occur immediately. When cisplatin is administered by IV infusion over 6 or 24 hours, plasma concentrations of total platinum increase gradually during the infusion and peak immediately following the end of the infusion.

2. Distribution: Following intravenous dosing, cisplatin distributes rapidly into tissues, with highest concentrations in the liver, prostate and kidney. Plasma levels of cisplatin decay in a biphasic mode with an initial half-life of 25 to 49 minutes, and a secondary phase ranging from 58 to 73 hours. This prolonged phase is due to protein binding, which exceeds 90%. Cisplatin penetrates poorly into the CNS.

3. Metabolism: Cisplatin is non-enzymatically transformed to one or more metabolites that are extensively protein bound and have minimal cytotoxic activity. The non-protein bound (unchanged) fraction is cytotoxic.

Elimination: Urinary excretion is incomplete. Following bolus injection or infusion over a dose range of 40-140 mg/m^2 varying in length from 1-24 hours, from 10 to about 40% of the administered platinum is excreted in the urine in 24 hours. Renal clearance of free platinum exceeds the glomerular filtration rate, indicating that cisplatin or other platinum-

containing molecules are actively secreted by the kidneys. Renal clearance of free platinum is nonlinear and variable, and is dependent on dose, urine flow rate, and individual variability in the extent of active secretion and possible tubular reabsorption.

c. ADVERSE EFFECTS

1. Possible Side Effects of cisplatin: Adverse effects reported in 10% or more of patients receiving cisplatin include peripheral neuropathy, nausea, vomiting, diarrhea, myelosuppression, liver enzymes elevation, nephrotoxicity (acute renal failure and chronic renal insufficiency), alopecia, tissue irritation, and ototoxicity.

   Human toxicity includes anorexia, nausea, vomiting, renal toxicity (with an elevation of BUN, creatinine, serum uric acid and impairment of endogenous creatinine clearance, as well as renal tubular damage), ototoxicity (with hearing loss which initially is in the high-frequency range, as well as tinnitus), peripheral neuropathy and hyperuricemia. Much more severe and prolonged toxicity has been observed in patients with abnormal or obstructed urinary excretory tracts. Raynaud's phenomena and digital ischemia has been described. Anaphylactic-like reactions including facial edema, bronchoconstriction, tachycardia and hypotension may occur within minutes of administration. Myelosuppression, often with delayed erythrosuppression, is expected. In the high-dose treatment regimen with osmotic diuresis, the nadir of white cells and platelets occurred regularly at about two weeks with recovery generally at about three weeks after the initiation of therapy. Alopecia, malaise and asthenia have been reported. Rare complications are alopecia, seizures, loss of taste and allergic reactions. Tetany may occur due to hypomagnesemia and/or hypocalcemia. Other electrolyte disturbances may occur. At high doses patients have experienced optic neuritis, papilledema, cerebral blindness, blurred vision, and altered color perception. Patients have also experienced cardiac abnormalities, elevated aspartate aminotransferase and rash. Subsequent courses should not be given until serum creatinine returns to normal if elevated. Audiometric analyses should be monitored, and courses withheld until auditory acuity is within normal limits. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

   Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions.

2. Pregnancy and Lactation: Category D. Cisplatin can cause fetal harm when administered to a pregnant woman. In mice, cisplatin is teratogenic and embryotoxic. This drug has been found to be excreted in human milk and because of the potential for serious adverse reactions in nursing infants, patients receiving cisplatin should not breast feed.

3. Drug Interactions: During cisplatin therapy, plasma levels of anticonvulsant agents may become sub-therapeutic and should be monitored. For complete information refer to the current FDA-approved package insert.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Cisplatin is commercially available and will not be supplied. Refer to the current FDA-approved package insert.

3.3 Methotrexate (NSC-740)

a. PHARMACOLOGY

Mechanism of Action: Methotrexate, an antimetabolite, inhibits dihydrofolic acid reductase. Dihydrofolates must be reduced to tetrahydrofolates by this enzyme before they can be utilized as carriers of 1-carbon groups in the synthesis of purine nucleotides and thymidylate. Therefore, methotrexate interferes with DNA synthesis, repair, and cellular replication. Actively proliferating tissues such as malignant cells, bone marrow, fetal cells, buccal and intestinal mucosa, and cells of the urinary bladder are in general more sensitive to this effect of methotrexate. When cellular proliferation in malignant tissues is greater than in most normal tissues, methotrexate may impair malignant growth without irreversible damage to healthy tissues.

b. PHARMACOKINETICS

1. Absorption: Methotrexate is generally completely absorbed from parenteral routes of injection. After IM injection, peak serum concentrations occur in 30 to 60 minutes.

2. Distribution: After IV administration, the initial volume of distribution is approximately 0.18 L/kg (18% of body weight) and steady-state volume of distribution is approximately 0.4 to 0.8 L/kg (40% to 80% of body weight). Methotrexate competes with reduced folates for active transport across cell membranes by means of a single carrier-mediated active transport process. At serum concentrations greater than 100 mcM, passive diffusion becomes a major pathway by which effective intracellular concentrations can be achieved. Methotrexate in serum is approximately 50% protein bound. Laboratory studies demonstrate that it may be displaced from plasma albumin by various compounds including sulfonamides, salicylates, tetracyclines, chloramphenicol, and phenytoin.

Methotrexate does not penetrate the blood-CSF barrier in therapeutic amounts when given orally or parenterally. High CSF concentrations of the drug may be attained by intrathecal administration.

3. Metabolism: Methotrexate undergoes hepatic and intracellular metabolism to polyglutamated forms which can be converted back to methotrexate by hydrolase enzymes. These polyglutamates act as inhibitors of dihydrofolate reductase and thymidylate synthetase. Small amounts of methotrexate polyglutamates may remain in tissues for extended periods. The retention and prolonged drug action of these active metabolites vary among different cells, tissues and tumors. A small amount of metabolism to 7-hydroxymethotrexate may occur at doses commonly prescribed. Accumulation of this metabolite may become significant at the high doses used in osteogenic sarcoma. The aqueous solubility of 7-hydroxymethotrexate is 3- to 5-fold lower than the parent compound.

4. Elimination: Renal excretion is the primary route of elimination and is dependent upon dosage and route of administration. With IV administration, 80% to 90% of the administered dose is excreted unchanged in the urine within 24 hours. There is limited biliary excretion amounting to 10% or less of the administered dose. Enterohepatic recirculation of methotrexate has been proposed.

   Renal excretion occurs by glomerular filtration and active tubular secretion. Nonlinear elimination due to saturation of renal tubular reabsorption has been observed in psoriatic patients at doses between 7.5 and 30 mg. Impaired renal function, as well as concurrent use of drugs such as weak organic acids that also undergo tubular secretion, can markedly increase methotrexate serum levels. Excellent correlation has been reported between methotrexate clearance and endogenous creatinine clearance.

   Methotrexate clearance rates vary widely and are generally decreased at higher doses. Delayed drug clearance has been identified as 1 of the major factors responsible for methotrexate toxicity. It has been postulated that the toxicity of methotrexate for normal tissues is more dependent upon the duration of exposure to the drug rather than the peak level achieved. When a patient has delayed drug elimination due to compromised renal function, a third-space effusion, or other causes, methotrexate serum concentrations may remain elevated for prolonged periods.

   The terminal half-life reported for methotrexate is approximately 3 to 10 hours for patients receiving treatment for psoriasis, or RA or low-dose antineoplastic therapy (less than 30 mg/m^2). For patients receiving high doses of methotrexate, the terminal half-life is 8 to 15 hours.

c. ADVERSE EFFECTS

1. Possible Side Effects of Methotrexate: Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions. In general, the incidence and severity of acute side effects are related to dose and frequency of administration. The most frequently reported adverse reactions include ulcerative stomatitis, leukopenia, nausea, and abdominal distress.

2. Pregnancy and Lactation: *Category X*. Methotrexate causes embryotoxicity, abortion, and fetal defects in humans. Pregnancy should be avoided if either partner is receiving methotrexate; during and for a

minimum of 3 months after therapy for male patients, and during and for at least 1 ovulatory cycle after therapy for female patients.

Because of the potential for serious adverse reactions from methotrexate in breastfed infants, it is contraindicated in nursing mothers.

3. Drug Interactions: Nonsteroidal anti-inflammatory drugs should not be administered prior to or concomitantly with the high doses of methotrexate, such as used in the treatment of osteosarcoma. Concomitant administration of some NSAIDs with high dose methotrexate therapy has been reported to elevate and prolong serum methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity.

   Caution should be used when NSAIDs and salicylates are administered concomitantly with lower doses of methotrexate. These drugs have been reported to reduce the tubular secretion of methotrexate in an animal model and may enhance its toxicity.

   Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored.

   In the treatment of patients with osteosarcoma, caution must be exercised if high-dose methotrexate is administered in combination with a potentially nephrotoxic chemotherapeutic agent (e.g., cisplatin).

   Methotrexate increases the plasma levels of mercaptopurine. The combination of methotrexate and mercaptopurine may therefore require dose adjustment.

   Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria.

   Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

   The potential for increased hepatotoxicity when methotrexate is administered with other hepatotoxic agents has not been evaluated. However, hepatotoxicity has been reported in such cases. Therefore, patients receiving concomitant therapy with methotrexate and other potential hepatotoxins (e.g., azathioprine, retinoids, sulfasalazine) should be closely monitored for possible increased risk of hepatotoxicity.

   Methotrexate may decrease the clearance of theophylline; theophylline levels should be monitored when used concurrently with methotrexate.

Vitamin preparations containing folic acid or its derivatives may decrease responses to systemically administered methotrexate. Preliminary animal and human studies have shown that small quantities of intravenously administered leucovorin enter the CSF primarily as 5methyltetrahydrofolate and, in humans, remain 1 to 3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration. However, high doses of leucovorin may reduce the efficacy of intrathecally administered methotrexate.

Folate deficiency states may increase methotrexate toxicity. Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression in patients receiving methotrexate, probably by decreased tubular secretion and/or an additive antifolate effect.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Methotrexate is commercially available and will not be supplied. Refer to the current FDA-approved package insert.

3.4 Vinblastine sulfate (Velban) (NSC-49842)

a. PHARMACOLOGY

Mechanism of Action: Vinblastine sulfate is an alkaloidal antineoplastic agent that inhibits microtubule formation in the mitotic spindle resulting in an arrest of dividing cells at the metaphase stage. It may also interfere in cell-energy production in mitosis and nucleic acid synthesis.

b. PHARMACOKINETICS

1. Distribution: Volume of distribution: 70% of body weight. Protein binding: 98 - 99.7%. Vinblastine blinds extensively to tissues. It does not penetrate CNS or other fatty tissues.

Metabolism: Vinblastine sulfate is metabolized by hepatic cytochrome P450 isoenzymes in the CYP 3A subfamily to an active metabolite, deacetylvinblastine.

2. Elimination: After rapid IV administration, a triphasic serum decay pattern followed. The respective half-lives were 3-7 minutes, 1.6 hours, and 24.8 hours. About 13.6 to 23.3% of the administered dose was excreted in urine and 10% in the feces, the remaining activity was not accounted for.

c. ADVERSE EFFECTS

1. Possible Side Effects of vinblastine

Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions.

Commonly reported adverse events include: myelosuppression, diarrhea or constipation, and peripheral neuropathy (e.g. jaw pain).

CAUTION: Vinblastine sulfate injection is for intravenous use only. The intrathecal administration of vinblastine, a vesicant, can results in death. Syringes containing vinblastine sulfate should be labeled, “FATAL IF GIVEN INTRATHECALLY. FOR INTRAVENOUSLY USE ONLY.”

2. Pregnancy and Lactation: *Pregnancy category D* Information on the use of vinblastine sulfate during human pregnancy is very limited. Animal studies suggest that teratogenic effects may occur. Women of childbearing potential should be advised to avoid becoming pregnant.

Aspermia has been reported in man. Animal studies show metaphase arrest and degenerative changes in germ cells.

It is not known whether vinblastine is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions from vinblastine sulfate in nursing infants, a decision should be made whether

3. Drug Interactions:

Refer to the current FDA-approved package insert.

Concurrent oral or intravenous administration of phenytoin and antineoplastic chemotherapy combinations that included vinblastine sulfate has been reported to have reduced blood levels of the anticonvulsant and to have increase seizure activity. The contribution of vinblastine sulfate to this interaction is not certain. Dosing adjustment of phenytoin should be based on serial blood level monitoring.

Caution should be exercised in patients concurrently taking drugs known to inhibit drug metabolism by hepatic cytochrome P450 isoenzymes in the CYP3A subfamily, or in patients with hepatic dysfunction. An earlier onset and/or an increased severity of side effects of vinblastine can occur.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Vinblastine is commercially available and will not be supplied. Refer to current FDA-approved package insert.

3.5 Doxorubicin (Adriamycin) (NSC-123127)

a. PHARMACOLOGY

Mechanism of Action: Doxorubicin is a cytotoxic antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius*. The cytotoxic effect of doxorubicin is related to nucleotide base intercalation and cell membrane lipid binding activities through the interaction with topoisomerase II. This inhibits nucleotide replication and the action of DNA and RNA polymerases.

b. PHARMACOKINETICS

1. Absorption: N/A

2. Distribution: Steady-state volume of distribution: 809-1214 L/m^2; protein binding: 74-76%; doxorubicin does not cross the blood brain barrier.

3. Metabolism: Primarily hepatic

4. Elimination: Half-life for distribution: 5-10 minutes, half-life for elimination of doxorubicin: 1-3 hours & for metabolites: 3-3.5 hours, terminal half-life: 17-48 hours. 40% appears in the bile within 5 days, 5-12% of doxorubicin and metabolites appear in urine.

c. ADVERSE EFFECTS

1. Refer to package insert or manufacturer website for the most complete and up to date information on contraindications, warnings and precautions, and adverse reactions.

   Dose limiting toxicities of doxorubicin include myelosuppression and cardiotoxicity. Other commonly reported adverse events of doxorubicin are nausea, vomiting, mucositis, and alopecia.

2. Pregnancy and Lactation: *Pregnancy Category D*. Doxorubicin can cause fatal harm when administered to a pregnant woman. Teratogenicity and embryotoxicity were seen in animals. Women of childbearing potential should be advised to avoid becoming pregnant.

   Doxorubicin and its major metabolite, doxorubicinol have been detected in the milk of at least one lactating patient. Mothers should be advised to discontinue nursing while receiving doxorubicin.

3. Drug Interactions: Refer to package insert or manufacturer website for the most complete and up to date information.

Due to potential drug interactions, a complete patient medication list, including doxorubicin, should be screened prior to initiation of doxorubicin. Of note, doxorubicin is a substrate for CYP2D6, CYP3A4, and P-glycoprotein and moderately inhibits CYP2B6 and induces P-glycoprotein.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to FDA-approved package insert for drug administration

e. STORAGE & STABILITY

Please refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Doxorubicin is commercially available and will not be supplied. Please refer to the current FDA-approved package insert for additional information.

3.6 Filgrastim (r-metHuG-CSF) (Neupogen®) (NSC-614629)

a. PHARMACOLOGY

Mechanism of Action: Filgrastim stimulates the production, maturation, and activation of neutrophils; filgrastim activates neutrophils to increase both their migration and cytotoxicity.

b. PHARMACOKINETICS

1. Absorption: First-order pharmacokinetic modeling with maximum serum concentration reached within 2 to 8 hours after subcutaneous injection

2. Distribution: Average Vd 150 mL/kg

3. Metabolism: Unknown

4. Elimination: Renal and neutrophil receptor-mediated, elimination half-life is approximately 3.5 hours.

c. ADVERSE EFFECTS

1. Possible Side Effects of Filgrastim: Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions.

Most frequent adverse reactions reported are skeletal pain (> 20%).

2. Pregnancy and Lactation: Category C, filgrastim should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Animal studies have demonstrated adverse effects and fetal loss. Filgrastim has been shown to cross the placenta in humans. There are no adequate and well-controlled studies in pregnant women. Excretion in breast milk unknown/use caution.

3. Drug Interactions: Drug interactions between filgrastim and other drugs have not been fully evaluated. Drugs which may potentiate the release of neutrophils, such as lithium, should be used with caution.

Increased hematopoietic activity of the bone marrow in response to growth factor therapy has been associated with transient positive bone-imaging changes. This should be considered when interpreting bone-imaging results.

Filgrastim should not be administered on the same day with anticancer chemotherapeutic agent(s) with leukocyte suppressive properties.

Filgrastim is contraindicated in patients with hypersensitivity to *E.coli*-derived proteins, filgrastim, or any component of the product.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Filgrastim is commercially available and will not be supplied. Refer to the current FDA-approved package insert.

3.7 Pegfilgrastim (Neulasta™)

a. PHARMACOLOGY

Mechanism of Action: Similar to filgrastim, pegfilgrastim is a colony-stimulating factor that acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end-cell functional activation. Studies on cellular proliferation, receptor binding, and neutrophil function demonstrate that filgrastim and pegfilgrastim have the same mechanism of action.

b. PHARMACOKINETICS

Absorption: Similar to filgrastim, first-order pharmacokinetic modeling is expected with maximum serum concentration reached within 2 to 8 hours after subcutaneous injection.

1. Distribution: Similar to filgrastim, volume of distribution of averaged at 150 mL/kg

2. Metabolism: Unknown

3. Elimination: Neutrophil receptor binding is an important component of the clearance of pegfilgrastim, and serum clearance is directly related to the number of neutrophils. Pegfilgrastim has reduced renal clearance and

prolonged persistence in vivo as compared with filgrastim. The half-life of pegfilgrastim ranges from 15 to 80 hours after subcutaneous injection.

c. ADVERSE EFFECTS

1. Possible Side Effects of Pegfilgrastim: Refer to the current FDA-approved package insert for the most comprehensive and up to date information on adverse reactions. Most frequent adverse reactions are skeletal pain (< 20%).

2. Pregnancy and Lactation: Category C, pegfilgrastim should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Animal studies have demonstrated adverse effects and fetal loss. Pegfilgrastim has been shown to cross the placenta in humans. There are no adequate and well-controlled studies in pregnant women. Excretion in breast milk unknown/use caution.

3. Drug Interactions: Drug interactions between filgrastim and other drugs have not been fully evaluated. Drugs such as lithium may potentiate the release of neutrophils; ensure that patients receiving lithium and pegfilgrastim have more frequent monitoring of neutrophil counts.

   Pegfilgrastim should not be administered on the same day with anticancer chemotherapeutic agent(s) with leukocyte suppressive properties.

   Pegfilgrastim is contraindicated in patients with hypersensitivity to *E.coli*-derived proteins, filgrastim, or any component of the product.

d. DOSING & ADMINISTRATION

1. Dosing – See Section 7.0 Treatment Plan

2. Refer to the current FDA-approved package insert for drug administration.

e. PREPARATION, STORAGE & STABILITY

Refer to the current FDA-approved package insert for storage, stability and special handling information.

f. HOW SUPPLIED

Pegfilgrastim is commercially available and will not be supplied. Refer to the current FDA-approved package insert.

4.0 STAGING CRITERIA

AJCC Seventh Edition, 2010
Patients will be staged according to standard of care methods. All patients will undergo cystoscopy with transurethral resection of the tumor, adequate for staging as muscle-invasive. Consistent with World Health Organization/International Society of Urologic Pathology (WHO/ISUP) – tumors will be classified as either low or high-grade.

Clinical Stage
Primary Tumor (T)

T2 Tumor invades muscularis propria
T3 Tumor invades perivesical tissue
T4a Tumor invades prostatic stroma, uterus, vagina

Regional Lymph Nodes (N)

Regional lymph nodes include both primary and secondary drainage regions. All other nodes above the aortic bifurcation are considered distant lymph nodes.

N0 No lymph node metastasis

Distant Metastasis (M)

M0 No distant metastasis

5.0 ELIGIBILITY CRITERIA

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. Use the spaces provided to confirm a patient's eligibility. For each criterion requiring test results and dates, please record this information on the **S1314** Onstudy Form and submit via Medidata Rave® (see Section 14.0). Any potential eligibility issues should be addressed to the SWOG Statistics and Data Management Center in Seattle at guquestion@crab.org or 206/652-2267 prior to registration.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday 4 weeks later would be considered Day 28. This allows for efficient patient scheduling without exceeding the guidelines. **If Day 28 or 56 falls on a weekend or holiday, the limit may be extended to the next working day.**

5.1 Disease Related Criteria

a. Patients must have histologically proven urothelial carcinoma of the bladder. Those with mixed histology, including a component of urothelial carcinoma, are eligible. Small cell carcinoma, pure adenocarcinoma, and pure squamous cell carcinoma are excluded.

Related to the section outlining disease measurement - the last sentence of that section should be as follows). All disease must be assessed and documented on the Baseline Tumor Assessment Form.

b. Patients must have Stage cT2-T4a N0 M0 disease. Clinical T stage is based on the TURBT sample and imaging studies. Patients must undergo cystoscopy and TURBT as part of the staging procedure (detailed in Section 4.0) within 56 days prior to registration. To exclude non-bulky/low-risk tumors and ensure adequate

tissue for assessment, subjects must have documented muscle invasion with at least one of the following:

1. Disease measuring at least 5 mm on cross-sectional imaging or by endoscopic assessment. Bladder thickening on imaging without definable tumor is not adequate. Pathology verification of ≥ 0.5 cm of viable tumor (longest diameter) from the biopsy sample and represented on the submitted slides is also acceptable.

2. The presence of tumor-associated hydronephrosis.

c. Patients must have staging scans with abdominal/pelvic CT or MRI scan and CT scan or x-ray of the chest within 56 days prior to registration. If alkaline phosphatase is above the treating institution's upper limit of normal (ULN), presence of suspicious bone pain, or if other clinical suspicion, a whole body bone scan is required within 56 days prior to registration.

d. Patients must have a Zubrod performance status of 0 or 1 (see Section 10.4).

e. Patients must be 18 years of age or older.

5.2 Prior Therapy Criteria

a. Patients must not have received previous systemic cytotoxic chemotherapy for urothelial carcinoma.

b. Patients must not have received previous systemic anthracycline (intravesical anthracycline is allowed).

5.3 Clinical/Laboratory Criteria

a. Patients must not have peripheral neuropathy ≥ Grade 2.

b. Patients must not have presence of Class III or IV heart failure, according to New York Heart Association Classifications (see Appendix 18.2), or a known left ventricular ejection fraction of less than 50%. Note: LVEF evaluation by echocardiogram or multi-gated acquisition scan (MUGA) is not required prior to registration.

c. Patients must not have a clinically relevant hearing impairment > Grade 2.

d. Patients must have adequate renal function as evidenced by calculated creatinine clearance ≥ 60 mL/min. The serum creatinine value used in the calculation must have been obtained within 28 days prior to registration. Use the modified Cockcroft-Gault formula below:

$$\text{Calculated creatinine clearance} = \frac{(140 - \text{age}) \times \text{wt (kg)} \times 0.85 \text{ (if female)}}{72 \times [\text{creatinine (mg/dl)}]}$$

e. Patients must have adequate hepatic function as evidenced by total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN) (or ≤ 2.5 x ULN with Gilbert's disease), SGOT (AST) and SGPT (ALT) ≤ 2 x institutional ULN. These results must be obtained within 28 days prior to registration.

f. Patients must have adequate hematologic function as evidenced by absolute neutrophil count (ANC) ≥ 1,500/mcL, hemoglobin ≥ 9 g/dL, and platelets ≥ 100,000/mcL. These results must be obtained within 28 days prior to registration.

g. Patients must not be known to have hypersensitivity to cisplatin, gemcitabine, doxorubicin, vinblastine, methotrexate or filgrastim/pegfilgrastim.

h. Patients must not have any incidence of or uncontrolled medical illness (e.g. active cardiac symptoms, active systemic infection, etc.) that would limit the patient's ability to participate in the protocol.

i. Prestudy history and physical must be obtained within 28 days prior to registration.

j. Patients must not be pregnant or nursing due to the potential teratogenic side effects of the protocol treatment. Women/men of reproductive potential must agree to use an effective contraceptive method during and for 6 months after completing protocol treatment. A negative pregnancy test (either serum or urine) is required within 7 days prior to registration.

A woman is considered to be of "reproductive potential" if she has had menses at any time in the preceding 12 consecutive months. In addition to routine contraceptive methods, "effective contraception" also includes heterosexual celibacy and surgery intended to prevent pregnancy (or with a side-effect of pregnancy prevention) defined as a hysterectomy, bilateral oophorectomy or bilateral tubal ligation. However, if at any point a previously celibate patient chooses to become heterosexually active during the time period for use of contraceptive measures outlined in the protocol, he/she is responsible for beginning contraceptive measures.

k. No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, adequately treated Stage I or II cancer from which the patient is currently in complete remission, or any other cancer from which the patient has been disease free for five years. Patients with localized prostate cancer who are being followed by an active surveillance program are also eligible.

5.4 Specimen Submission Criteria

a. Patients must have tumor tissue from transurethral resection of the bladder tumor (TURBT) available for submission for COXEN testing and must agree to submission of 20 (10 micron) slides of formal-fixed paraffin embedded (FFPE) tissue, with 2 (5 micron) slides at the start and end of the 20 slides, for a total of 22 unstained slides. The diagnostic TURBT sample must have been obtained within 56 days prior to registration. All sections should be placed on "plus" slides, as is the standard procedure in most pathology units.

b. Patients must consent, if residual tumor is present at the time of cystectomy, to the submission of 20 (10 micron) unstained slides with 2 (5 micron) slides at the start and stop of the series (total of 22 unstained slides).

c. Patients must consent to have voided urine (40-50 mL) submitted prior to initiating chemotherapy (pre-treatment) and after chemotherapy prior to surgery (post-treatment).

d. Patients must consent to whole blood (2 x 10 mL) submitted prior to initiating chemotherapy.

e. Patients must agree to participate in the translational medicine studies outlined in Section 18.3.

f. Patients must be offered the opportunity to participate in the ultra pure Circulating Tumor Cells (upCTCs) study.

5.5 Regulatory Criteria

a. Patients or their legally authorized representative must be informed of the investigational nature of this study and must sign and give written informed consent prior to any study-related procedures in accordance with institutional and federal guidelines.

b. As a part of the OPEN registration process (see Section 13.4 for OPEN access instructions) the treating institution's identity is provided in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered in the system.

6.0 STRATIFICATION FACTORS

Patients will be randomized using a dynamic balancing algorithm with stratification based on *(57)*:

a. Clinical stage: T2 vs. T3 or T4a. NOTE: Clinical stage is based on TURBT(s), bimanual exam, and imaging (see Section 5.1b)

b. Performance status: 0 vs. 1

7.0 TREATMENT PLAN

For treatment questions, please contact Dr. Thomas W. Flaig at 303/724-0499 (**S1314**question@swog.org)) or Dr. Siamak Daneshmand at 323/865-3700 (**S1314**question@swog.org). For dosing principles or questions, please consult the SWOG Policy #38 "Dosing Principles for Patients on Clinical Trials" at http://swog.org (then click on "Policies and Manuals" under the "Visitors" menu and choose Policy 38).

7.1 General Considerations

a. Subject will be randomized to either Arm 1 (GC) or Arm 2 (DDMVAC)

b. Subjects randomized to DDMVAC will have a standard of care assessment of their cardiac ejection fraction (via ultrasound or nuclear medicine MUGA assessment) to ensure adequate cardiac function before receiving doxorubicin (according to institutional standards). A cardiac ejection fraction assessment within the last 12 months is acceptable as long as there is no clinical change or new cardiac concerns.

c. Due to the risk of methotrexate accumulation, subjects randomized to DDMVAC must have any moderate to large pleural effusion or ascites drained prior to starting chemotherapy.

d. Patients are allowed to participate in this study, followed by **S1011** (*A Phase III Surgical Trial to Evaluate the Benefit of a Standard Versus an Extended Pelvic Lymphadenectomy Performed At Time of Radial Cystectomy For Muscle Invasive Urothelial Cancer*) as long as all the eligibility criteria for **S1011** are met.

e. While the protocol plan is for 4 cycles of chemotherapy, patients who receive at least three cycles of chemotherapy with cisplatin can go on to protocol cystectomy for evaluation purposes, otherwise patients will be removed from protocol treatment and the patient can receive a cystectomy off protocol.

7.2 Arm 1: Gemcitabine + Cisplatin (GC) Treatment

Treatment should be given in the order listed below:

AGENT	DOSE	ROUTE	DAYS	INTERVAL
Gemcitabine	1,000 mg/m^2	IV (over 1 hour)*	Days 1,8	21 days x 4 cycles
Cisplatin	70 mg/m^2	IV (over 1 hour)*	Day 1	21 days x 4 cycles
In select patients:				
Pegfilgrastim**	6 mg	SubQ**	Day 9	21 days x 4 cycles
Alternative to pegfilgrastim:				
Filgrastim***	5 mcg/kg	SubQ/IV***	Days 9-13** *	21 days x 4 cycles

* While the doses of each drug here are standardized for GC, the duration and timing of administration varies regionally and may be given according to institutional practice.
** One dose given 24-48 hours after completion of chemotherapy for each cycle.
*** The duration of filgrastim administration may be altered based on patient response or institutional practice.

The optional use of growth factor support in Arm 1 should be based on age (notably age 65 years and older), preexisting conditions (neutropenia at baseline, etc), and other patient risk factors for the development of neutropenic fever, pegfilgrastim may be included with the first cycle of this regimen at the treating physician's discretion and institutional practice. Additionally, according to your institutional practice, filgrastim may be used as an alternative to pegfilgrastim (as outlined in Arm 2) in accordance with your local practice.

7.3 Arm 2: Dose Dense Methotrexate, Vinblastine, Doxorubicin, Cisplatin (DDMVAC) Treatment

Treatment should be given in the order listed below:

AGENT	DOSE	ROUTE	DAYS	INTERVAL
Methotrexate	30 mg/m^2	IV (over 3 minutes)*	Day 1	14 days x 4 cycles
Vinblastine	3 mg/m^2	IV (over 3 minutes)*	Day 1 or 2	14 days x 4 cycles
Doxorubicin	30 mg/m^2	IV (over 5 minutes)*	Day 1 or 2	14 days x 4 cycles
Cisplatin	70 mg/m^2	IV (over 1 hour)*	Day 1 or 2	14 days x 4 cycles
Filgrastim	5 mcg/kg	SubQ/IV	Days 3-7**	14 days x 4 cycles
Alternative to filgrastim: Pegfilgrastim***	6 mg	SubQ***	Day 2 or 3	14 days x 4 cycles

* While the doses of each drug here are standardized for DDMVAC, the duration of administration varies regionally and may be given according to institutional guideline.

** On Days 2-6 if all the chemotherapy is given on Day 1. The duration of administration may be altered based on patient response or institutional practice. All patients in Arm 2 must receive either filgrastim or pegfilgrastim.

*** One daily dose given 24-48 hours after completion of chemotherapy for each cycle or per institutional guidelines. All patients in Arm 2 must receive either filgrastim or pegfilgrastim.

a. Use of a PICC line or med-port should be discussed with all patients in Arm 2, as this treatment arm includes vesicant with the risk of extravasation. The timing of chemotherapy after either med port or PICC is at the discretion of the treating investigator.

b. Appropriate pre- and post-hydration for the cisplatin chemotherapy and electrolyte replacement including at least 1 liter of pre-hydration solution will be included according to institutional guidelines. Furosemide, mannitol, and electrolyte supplementation may be included according to local practices as well.

c. With the inclusion of high dose cisplatin, anti-emetic prophylaxis should also be administered according to institutional guidelines and are recommended to include a corticosteroid, 5-HT3, and NK1 receptor antagonist.

7.4 Timing of Cystectomy After Chemotherapy

The cystectomy and lymph node dissection must be performed within **70 days** from the last dose of neoadjuvant chemotherapy with recovery from all associated toxicities to grade 1 or less at the time of surgery. At minimum, a standard lymph node dissection must be performed at the time of the radical cystectomy. All potential node bearing tissue should

be removed within the following boundaries: laterally the genitofemoral nerve; distally Cooper's ligament to include the lymph node of Cloquet; proximally the common iliac bifurcation; medially the bladder to include the tissue medial to the hypogastric artery; and posteriorly the floor of the obturator fossa with circumferential mobilization of the external iliac artery and vein unless contraindicated due to extensive atherosclerotic vascular disease.

7.5 Criteria for Removal from Protocol Treatment

a. Completion of cystectomy

b. Unacceptable toxicity.

c. Inability to complete protocol chemotherapy due to the following reasons:

- A continuous treatment delay for any reason > 3 weeks at any time after starting chemotherapy, a cumulative delay in chemotherapy of 4 weeks total, or if CrCl is < 50mL/min for one week despite best supportive care and holding the chemotherapy.
- More than one dose level reduction for cisplatin or more than two dose level reductions for any of the other chemotherapy agents.
- Less than 3 cycles of cisplatin-containing chemotherapy due to toxicity or maximum dose reduction reached.

d. The patient may withdraw from the study at any time for any reason.

e. Patient is no longer a candidate for cystectomy in the opinion of the physician.

7.6 Discontinuation of Treatment

All reasons for discontinuation of chemotherapy and cystectomy must be documented in the **S1314** Off Chemotherapy Form and **S1314** Cystectomy Status Form.

7.7 Follow-Up Period

All patients will be followed until death or 5 years after registration, whichever occurs first..

8.0 TOXICITIES TO BE MONITORED AND DOSE MODIFICATIONS

8.1 NCI Common Terminology Criteria for Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for toxicity and Serious Adverse Event reporting. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0.

8.2 General Dose Modification Considerations

a. No dose re-escalations are allowed. If multiple dose reductions are considered, take the lowest level.

b. For cisplatin, only one dose level reduction is allowed. For all other chemotherapy agents, two dose level reductions are permitted.

c. Any continuous delay in chemotherapy of more than 3 weeks or cumulative delays of more than 4 weeks will lead to removal from protocol treatment and referral to surgery.

8.3 Chemotherapy Dose Levels

Dose levels for each chemotherapy agent are shown below. Please note that once a drug dose is reduced, there is no re-escalation to a previous dosing level
Cisplatin dose levels

Dose level	CrCl > 59 ml/min	CrCl > 50 ml/min and <60 ml/min
Level 0	70 mg/m^2	35 mg/m^2 on Day 1 and Day 8 for the Gemcitabine/Cisplatin arm and on Days 1 and 2 for the DDMVAC arm
Level -1	50 mg/m^2	25 mg/m^{2}on Day 1 and Day 8 for the Gemcitabine/Cisplatin arm and on Days 1 and 2 for the DDMVAC arm

Gemcitabine dose levels

Dose level	Dose
Level 0	1,000 mg/m^2
Level -1	750 mg/m^2
Level -2	500 mg/m^2

Methotrexate dose levels – myelosuppression and mucositis

Dose Level	Dose
Level 0	30 mg/m^2
Level -1	23 mg/m^2
Level -2	15 mg/m^2

Vinblastine dose levels – myelosuppression, nausea, and mucositis

Dose level	Dose
Level 0	3 mg/m^2
Level -1	2.3 mg/m^2
Level -2	1.5 mg/m^2

Doxorubicin dose level

Dose level	Dose
Level 0	30 mg/m^2
Level -1	23 mg/m^2
Level -2	15 mg/m^2

8.4 Dose Modifications

a. Arm 1 –Gemcitabine and Cisplatin (GC)

1. Hematologic toxicity

Day 1: For ANC < 1,500 on Day 1, delay treatment until the ANC is > 1500. If delay is one week or less, continue at the same dose and consider peg filgrastim with subsequent cycles, if not receiving. If the delay is for more than a week and pegfilgrastim has not been used, include pegfilgrastim in all subsequent cycles. If there is a dose delay of more than one week due to neutropenia despite pegfilgrastim use, reduce cisplatin and gemcitabine by one dose level for this and all subsequent cycles and continue pegfilgrastim.

Day 1: For platelets < 100,000 on Day 1, delay treatment until platelets are > 100,000. If the delay is one week or less, continue at the same dose levels. If the delay is for more than a week, reduce the gemcitabine by one dose level for this and all subsequent cycles.

Day 8: For and ANC <500, hold gemcitabine and reduce both cisplatin and gemcitabine by one dose level for all subsequent cycles.

Day 8: For platelets <50,000, hold gemcitabine and reduce gemcitabine by one does level for all subsequent cycles.

2. Febrile neutropenia

Febrile neutropenia is defined as ANC of < 500 and a temperature of 38.3°C orally (101°F) or a temperature of ≥ 38°C (100.4°F) for more than 1 hour. Add pegfilgrastim if not previously included, continuing at the same dose level. If pegfilgrastim had been utilized, then dose reduce both the gemcitabine and cisplatin by one dose level for all subsequent cycles and continue pegfilgrastim.

3. Kidney dysfunction

Calculate the CrCl on Day 1 of each cycle. If CrCl is < 50 ml/min, hold chemotherapy for up to 1 week. If the CrCl does not improve to ≥ 50 ml/min in 1 week despite hydration and other supportive measures, discontinue the chemotherapy and proceed to surgery. If the CrCl improves to ≥ 50 ml/min within 1 week or less of the scheduled Day 1, proceed with split dose cisplatin administration on Days 1 and 8 of that cycle. If the CrCl improves to ≥ 60 ml/min on Day 1 of subsequent cycles, split dosing of cisplatin is not required for those cycles.

If the CrCl is between 50-60 ml/min on Day 1 of chemotherapy, administer cisplatin in split dosing, without a dose level reduction. Pegfilgrastim or filgrastim will be given on Day 9 or Days 9-13, respectively, when the cisplatin dose is split. With split dosing, if CrCl is < 50 ml/min on Day 8, the cisplatin is to be held for that day.

4. Liver dysfunction

For a total bilirubin of > 1.5 X the upper limit of normal (or 3 X ULN in patients with Gilbert's syndrome), hold therapy until the bilirubin is < 1.6 x ULN (or < 3 X ULN in those with Gilbert's syndrome), and then restart with a one dose level reduction in gemcitabine for this and all remaining cycles.

5. Neurologic toxicity

For Grade 3 neuropathy, hold chemotherapy until this resolves to Grade 1 or 2 and then resume with a one dose level reduction in the cisplatin. For Grade 4 neuropathy, remove the patient from protocol treatment.

6. Gastrointestinal toxicity

For Grade 3 or 4 vomiting, despite maximal antiemetic medical intervention with aprepitant, corticosteroids, and 5HT-3 antagonists (e.g. ondansetron), proceed with a dose reduction for the next cycle in both cisplatin and gemcitabine. If Grade 3 or 4 vomiting occurs despite one dose reduction in chemotherapy and maximal antiemetic therapy, remove the patient from protocol treatment.

7. Other toxicities

For all other, non-specified adverse events at Grade 2, patients should be treated symptomatically. For all other Grade 3 or 4 toxicities (excluding alopecia and skin pigment changes), hold cisplatin and gemcitabine and monitor the patient at least weekly. If toxicity resolves to Grade 1 or completely resolves in ≤ 3 weeks, reduce the cisplatin and gemcitabine by one dose level for this and all subsequent cycles. Any single delay in chemotherapy of more than 3 weeks or cumulative delays of more than 4 weeks will lead to removal from protocol treatment and referral to surgery.

b. Arm 2 – Dose Dense Methotrexate, Vinblastine, Doxorubicin, Cisplatin (DDMVAC)

1. Hematologic toxicity

For ANC < 1500 or platelets < 100,000 on Day 1, delay treatment until the ANC is > 1500 and platelets > 100,000. If delay is one week or less, continue at the same doses. If the delay is for more than a week, reduce methotrexate, vinblastine, doxorubicin, and cisplatin by one dose level for this and all subsequent cycles.

For febrile neutropenia, defined as ANC of < 500 and a temperature of 38.3 C orally (101 F) or a temperature of ≥ 38 C (100.4F) for more than 1 hour, dose reduce all chemotherapy agents by one dose level and consider switching to pegfilgrastim (if previously giving filgrastim).

2. Kidney dysfunction

Calculate the CrCl on Day 1 of each cycle. If CrCl is < 50 ml/min, hold chemotherapy for up to 1 week. If the CrCl does not improve to ≥50 ml/min in 1 week despite hydration and other supportive measures, discontinue

the chemotherapy and proceed to surgery. If the CrCl improves to ≥ 50 ml/min within 1 week or less of the scheduled Day1, proceed with split dose cisplatin administration on Days 1 and 2. A delay of filgrastim/pegfilgrastim until Day 3 should be done in this case with consideration of extending the cycle by one day. If CrCl improves to ≥ 60 ml/min on Day 1 of subsequent cycles, split dosing of cisplatin is not required for those cycles.

If the CrCl is between 50-60 ml/min on Day 1 of chemotherapy, administer cisplatin in split dosing on Days 1 and 2, without a dose level reduction.

3. Liver dysfunction

   For a total bilirubin of > 1.5 X the upper limit of normal (or 3 X ULN in patients with Gilbert's syndrome), hold therapy until the bilirubin is < 1.6 x ULN (or < 3 X ULN in those with Gilbert's syndrome), and then restart with a one dose level reduction in doxorubicin and vinblastine for this and all remaining cycles.

4. Neurologic toxicity

   For Grade 3 neuropathy, hold chemotherapy until this resolves to Grade 1 or 2 and then resume with a one dose level reduction in the cisplatin and vinblastine. For Grade 4 neuropathy, remove the patient from protocol treatment.

5. Gastrointestinal toxicity

   For Grade 3 or 4 vomiting, despite maximal antiemetic medical intervention with aprepitant, corticosteroids, and 5HT-3 antagonists (e.g. ondansetron), proceed with a dose reduction for the next cycle in both cisplatin, methotrexate and doxorubicin. If Grade 3 or 4 vomiting occurs despite one dose reduction in chemotherapy and maximal antiemetic therapy, remove the patient from protocol treatment.

6. Stomatitis

   For Grade 2 toxicity, delay infusion until it resolves to Grade 0 or 1. For Grade 3 or 4 toxicity, delay chemotherapy until it resolves to Grade 0 or 1 and reduce doxorubicin, vinblastine and methotrexate by one dose level on all subsequent cycles.

7. Other toxicities

   For all other, non-specified adverse events at Grade 2, they should be treated symptomatically, as indicated. For all other Grade 3 or 4, hold all chemotherapy and monitor the subject at least weekly. If toxicity resolves to Grade 1 or completely resolves in ≤ 3 weeks, reduce methotrexate, vinblastine, doxorubicin, and cisplatin by one dose level for this and all subsequent cycles. Any single delay in chemotherapy of more than 3 weeks or cumulative delays of more than 4 weeks will lead to removal from protocol treatment and referral to surgery.

c. Filgrastim (G-CSF) Dose Modifications

Dose adjustments for toxicities associated with filgrastim (G-CSF) (bone pain, splenomegaly, abnormalities in uric acid concentrations, LDH and alkaline phosphatase, transient elevations of serum creatinine and aminotransferase activity)

Dose modifications for G-CSF toxicity should only be initiated if symptomatic control of the toxicity fails (i.e., analgesics such as acetaminophen or acetaminophen with codeine for myalgias or bone pain, etc.).

Toxicity Grade	**Dose Adjustment**
Grade 0 - 1	No change
Grade 2	Decrease G-CSF to 3 mcg/kg/d
Grade 3 - 4	Discontinue G-CSF

8.5 Dose Modifications Contacts

For dose modification questions, please contact Dr. Thomas W. Flaig at 303/724-0499 (**S1314**question@swog.org) or Dr. Siamak Daneshmand at 323/865-3700 (**S1314**question@swog.org).

8.6 8Adverse Event Reporting

Toxicities (including suspected reactions) that meet the expedited reporting criteria as outlined in Section 16.0 of the protocol must be reported to the Operations Office, Study Chair and NCI via CTEP-AERS, and to the IRB per local IRB requirements.

9.0 STUDY CALENDAR

9.1 Arm 1 GC – Study Calendar – **21 day cycles**

		(a)									(c)		
PHYSICAL	Pre-study	C1D1	C1D8	C2D1	C2D8	C3D1	C3D8	C4D1	C4D8	Pre-Surgery	Surg	F/U prior to prog	F/U post prog
History and physical (with BSA, BP, and wt)	X	X		X		X		X		X		X(f)	X(g)
Performance status	X	X		X		X		X		X		X(f)	X(g)
Toxicity notation	X			X		X		X		X		X(f)	X(g)
LABORATORY													
CBC	X	X	X	X	X	X	X	X	X	X		X(f)	X(g)
Total bilirubin	X	X		X		X		X		X		X(f)	X(g)
AST and ALT	X	X		X		X		X		X		X(f)	X(g)
Alkaline phosphatase	X(e)	X		X		X		X		X		X(f)	X(g)
Magnesium (h)	X(e)	X		X		X		X		X		X(f)	X(g)
Phosphorus (h)	X(e)	X		X		X		X		X		X(f)	X(g)
Serum creatinine	X(e)	X	X	X	X	X	X	X	X	X		X(f)	X(g)
Electrolytes (sodium, potassium, chloride, bicarbonate (or CO_2)	X(e)	X		X		X		X					
Blood urea nitrogen (BUN)	X(e)	X	X	X	X	X	X	X	X	X			
Glucose	X(e)	X		X		X		X					
Pregnancy test (b)	X												
STAGING													
TURBT and bimanual exam	X												
CORRELATIVE SAMPLES													
20 (10 micron) slides plus 2 (5 micron) slides at start & stop of 20 slide cut from TURBT	X												
20 (10 micron) slides plus 2 (5 micron) slides at start & stop of 20 slide cut from cystectomy											X		

Arm 1 continued on next page. Footnotes for Arm 1 on next page.

9.1 Arm 1 GC – Study Calendar – **21 day cycles** (contd.)

	(a)									(c)			
PHYSICAL	Pre-study	C1D1	C1D8	C2D1	C2D8	C3D1	C3D8	C4D1	C4D8	Pre-Surgery	Surg	F/U prior to prog	F/U post prog
CORRELATIVE SAMPLES (cond.)													
CTC blood samples (j)		X		X									
Biomarker blood samples	X												
Biomarker urine sample	X									X			
RADIOLOGY													
CT or MRI of the abdomen and pelvis	X									X		X(f)	
CT of the chest or 2- view chest X-ray	X									X		X(f)	
Whole body bone scan (d)	X												
TREATMENT (i)													
Cisplatin		X		X		X		X					
Gemcitabine		X	X	X	X	X	X	X	X				

Footnotes for Arm 1
(a) Pre-study lab results may be used if within 3 days prior to Cycle 1, Day 1
(b) For women of childbearing potential; to be done within 7 days prior to registration – see Section 5.3i.
(c) Within **70 days** from the last dose of neoadjuvant chemotherapy (see Section 7.4)
(d) A bone scan should only be performed if there is presence of suspicious bone pain, or other clinical suspicion – see Section 5.1d.
(e) A baseline value should be collected at prestudy; these tests are not required for eligibility purposes.
(f) F/U procedures (prior to progression): CT or MRI of the abdomen & pelvis, CT of the chest or 2-view chest x-ray, and physical exam/history and laboratory assessments-every 3 months for one year, then every 6 months for 2 years, then annually until 5 years from registration.
(g) F/U procedures (post progression): Physical exam/history and laboratory assessments -every 3 months for one year, then every 6 months for 2 years, then annually until 5 years from registration.
(h) Phosphorus and magnesium monitoring are recommended every cycle, but may be omitted at the discretion of the treating medical provider.
(i) In select patients: Pegfilgrastim 6 mg SubQ Day 9 of each cycle. Alternative to pegfilgrastim: Filgrastim 5 mcg/kg SubQ/IV Days 9-13.
(j) 1 EDTA tube and 1 DNA Streck tube (in kit provided) should be collected pre-treatment on C1D1 and C2D1.

Allowable windows
Allowable windows for scheduled procedures and assessments performed every 7-14 days is +/- 1 day, every 21 days is +/- 3 days, every 3-6 months is +/- 7days, and every year is +/- 14 days. The window is to be calculated from the scheduled date of the procedure/assessment Treatment should begin within 14 days of randomization.

9.2 Arm 2 DDMVAC – Study Calendar – **14 day cycles**

		(a)									(c)		
PHYSICAL	Pre-study	C1D1	C1D2	C2D1	C2D2	C3D1	C3D2	C4D1	C4D2	Pre-Surg	Surg	F/U prior to prog	F/U post prog
History and Physical (with BSA, BP, & wt)	X	X		X		X		X		X		X(h)	X(i)
Performance status	X	X		X		X		X		X		X(h)	X(i)
Toxicity notation	X			X		X		X		X		X(h)	X(i)
LABORATORY													
CBC	X	X		X		X		X		X		X(h)	X(i)
Total bilirubin	X	X		X		X		X		X		X(h)	X(i)
AST and ALT	X	X		X		X		X		X		X(h)	X(i)
Alkaline phosphatase	X(g)	X		X		X		X		X		X(h)	X(i)
Magnesium (j)	X(g)	X		X		X		X		X		X(h)	X(i)
Phosphorus (j)	X(g)	X		X		X		X		X		X(h)	X(i)
Serum creatinine	X(g)	X		X		X		X		X		X(h)	X(i)
Electrolytes (sodium, potassium, chloride, bicarbonate (or CO_2)	X(g)	X		X		X		X		X		X(h)	X(i)
Glucose	X(g)	X		X		X		X		X		X(h)	X(i)
Calcium	X(g)	X		X		X		X		X		X(h)	X(i)
Albumin	X(g)	X		X		X		X		X		X(h)	X(i)
Total protein	X(g)	X		X		X		X		X		X(h)	X(i)
Pregnancy test (b)	X												
STAGING													
TURBT and bimanual exam	X												
CORRELATIVE SAMPLES													
20 (10 micron) slides plus 2 (5 micron) slides at start & stop of 20 slide cut from TURBT	X												
20 (10 micron) slides plus 2 (5 micron) slides at start & stop of 20 slide cut from cystectomy											X		
CTC blood samples (k)		X		X									

9.2 Calendar continued on next page. Footnotes for Arm 2 on next page

9.2 Arm 2 DDMVAC – Study Calendar – 14 day cycles (contd.)

		(a)									(c)		
PHYSICAL	Pre-study	C1D1	C1D2	C2D1	C2D2	C3D1	C3D2	C4D1	C4D2	Pre-Surg	Surg	F/U prior to prog	F/U post prog
CORRELATIVE SAMPLES (contd.)													
Biomarker blood samples	X												
Biomarker urine sample	X									X			
RADIOLOGY													
CT or MRI of ABD/pelvis	X									X		X(h)	
CT/chest or 2-view chest X-ray	X									X		X(h)	
Whole body bone scan (d)	X												
Cardiac Ejection Fraction (see Section 5.3b)	X												
TREATMENT (e)													
Methotrexate		X		X		X		X					
Cisplatin		X		X		X		X					
Vinblastine		X		X		X		X					
Doxorubicin		X		X		X		X					
Filgrastim (or Pegfilgrastim) (f)			X		X		X		X				

Footnotes for Arm 2:

(a) Pre-study lab results may be used if within 3 days prior to Cycle 1, Day 1.
(b) For women of childbearing potential; to be done within 7 days prior to registration – see Section 5.3i.
(c) Within **70 days** from the last dose of neoadjuvant chemotherapy (see Section 7.4).
(d) A bone scan should only be performed if there is presence of suspicious bone pain, or other clinical suspicion – see Section 5.1d.
(e) Cisplatin, vinblastine and doxorubicin may be given on Day 2 alternatively with filgrastim starting 24-48 hours after the chemotherapy infusion is finished.
(f) Pegfilgrastim is to be given 24-48 hours after the chemotherapy is completed. Alternatively, filgrastim may be given daily for 5 days starting 24-48 hours after the chemotherapy is complete.
(g) A baseline value should be collected at prestudy; these tests are not required for eligibility purposes.
(h) Follow-up procedures (prior to progression): CT or MRI of the abdomen & pelvis, CT of the chest or 2-view chest x-ray, and physical exam/history and laboratory assessments – every 3 months for one year, then every 6 months for 2 years, then annually until 5 years from registration.
(I) Follow-up procedures (post progression): Physical exam/history and laboratory assessments every 3 months for one year, then every 6 months for 2 years, then annually until 5 years from registration.
(j) Phosphorus and magnesium monitoring are recommended every cycle, but may be omitted at the discretion of the treating medical provider.
(k) 1 EDTA tube and 1 DNA Streck tube (in kit provided) should be collected pre-treatment on C1D1 and C2D1.

Allowable windows

Allowable windows for scheduled procedures and assessments performed every 7-14 days is ± 1 day, q 21 days is ± 3 days, q 3-6 months is ± 7days, and q year is ± 14 days. The window is to be calculated from the scheduled date of the procedure/assessment. Treatment should begin within14 days of randomization.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT ANALYSIS

10.1 Recurrence criteria

Criteria for recurrence will include measurable disease on cross-sectional imaging or plane radiography targeting lung, liver and bone as most common sites for recurrence. Bone scintigraphy may be used according to Good Medical Practice and is recommended with confirmed elevation in the alkaline phosphatase. Local pelvic recurrence may be determined by cross-sectional imaging and/or DRE (and confirmed by biopsy if indicated). PET/CT may be used to confirm suspicious abnormalities on other imaging modalities.

10.2 Recurrence-Free Survival

To be estimated in a subset of patients who received protocol cystectomy. The recurrence-free time interval is from date of cystectomy to date of first documentation of relapse/recurrence or death due to any cause. Patients last known to be alive without report of relapse/recurrence are censored at date of last contact.

10.3 Overall Survival

Overall survival is measured from date of randomization to date of death from any cause. Patients known to be alive are censored at date of last contact.

10.4 Performance Status

Patients will be graded according to the Zubrod Performance Status Scale.

POINT	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g. light housework, office work).
2	Ambulatory and capable of self-care, but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair

10.5 Pathologic Response

Pathologic staging is based on the operative and pathology report from cystectomy at the local site. *(58)* Patients not undergoing a cystectomy will be judged to be a nonresponder.

TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
Ta	Noninvasive papillary carcinoma.
Tis	Carcinoma in situ: "flat tumor."
T1	Tumor invades subepithelial connective tissue.
T2	Tumor invades muscularis propria.
pT2a	Tumor invades superficial muscularis propria (inner half).
pT2b	Tumor invades deep muscularis propria (outer half).

T3 Tumor invades perivesical tissue.
pT3a Microscopically.
pT3b Macroscopically (extravesical mass).
T4 Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall.
T4a Tumor invades prostatic stroma, uterus, vagina.
T4b Tumor invades pelvic wall, abdominal wall.

NX Lymph nodes cannot be assessed.
N0 No lymph node metastasis.
N1 Single regional lymph node metastasis in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node).
N2 Multiple regional lymph node metastases in the true pelvis (hypogastric, obturator, external iliac, or presacral lymph node).
N3 Lymph node metastases to the common iliac lymph nodes.

M0 No distant metastasis.
M1 Distant metastasis

11.0 STATISTICAL CONSIDERATIONS

11.1 Accrual Goal

Two treatment-specific COXEN gene signatures will be evaluated for prognostic and predictive significance using pathologic response to neoadjuvant chemotherapy as the endpoint of interest. We will look at both pathologic complete response (pT0) and pathologic favorable downgrading (≤ pT1 [pT0, pTa, pTis]). We expect to randomize 184 eligible patients with adequate tissue submission for COXEN assessment who received at least 3 cycles of chemotherapy, and are assessable for pT0 status or have clinical progression. To account for ineligibles, inadequate specimens and non-evaluable patients, we will over-accrue by 25% so the total accrual goal is 230 patients. We assume it will take 3.5 years of accrual (71 patients/yr) to reach this goal.

11.2 Analysis of Primary Endpoint

The two established COXEN gene signatures that were developed for each chemotherapy regimen will be evaluated using each patient's tissue from the TURBT, regardless of treatment arm assignment. Thus, each patient will have a DDMVAC-GEP and a GC-GEP. The two GEPs are fully specified from the analysis of prior bladder cohorts predicting 5-year survival (see Section 2.0 of the protocol for details). We know from SWOG's neo-adjuvant chemotherapy trial (**S8710**) that the pT0 rate among all patients randomized to MVAC (assuming those without a cystectomy were not pT0) was 31%, and attaining a pT0 was highly correlated with superior survival. *(64)* We went back to the Laval and Lehmann combined bladder cohorts that were used to both develop the gene signatures and validate them in order to identify the 5-year predicted survival cut-point from the DDMVAC-GEP and GC-GEP for the top 31% of predicted favorable survivors. If a patient in **S1314** has a treatment-specific GEP that provides a 5-year predicted probability greater than or equal to that treatment-specific threshold, we will assume that also predicts for a pT0 at cystectomy. An examination of stage-specific COXEN score "cutoffs" will be performed as part of an exploratory analysis.

Data from the 184 patients (92/arm) will be used to evaluate the association of each GEP with pT0 and secondary downgrading outcomes within each treatment-specific group. This will evaluate the prognostic significance of each treatment-specific GEP within each treatment group. Since this is a Phase II trial and assessments are exploratory, there will

not be adjustment for multiple testing. If there is later found to be no interaction between the treatment-specific GEP and treatment arm, then data from both treatment arms can be combined to evaluate the prognostic association of each GEP with pT0 assessment. By using the continuous distribution of the GEP, we will increase our power to evaluate associations. When applying COXEN to DDMVAC-treated patients in the bladder cancer setting, the COXEN GEP has previously yielded a sensitivity and specificity of 83% and 64%, respectively in a neo-adjuvant MVAC chemotherapy dataset in bladder cancer. *(65)* For modeling considerations, we lack specificity and sensitivity values for the GC GEP in this clinical situation, so we will make the same assumptions for GC that we did for DDMVAC. Based on prior bladder cancer paired GEP data, we do not expect there to be a large overlap of patients who will have both a favorable GC-GEP and a favorable DDMVAC-GEP in terms of predicting 5-year survival and pT0 status.

11.3 Analysis of Secondary Endpoints

The validity of the COXEN score threshold that was identified in a previous bladder cohort will be assessed by evaluating the association of COXEN score (low vs. high) for the outcome of pT0 (Y/N) in a multivariate logistic regression model that also will include age, gender, clinical T-stage, and grade. Applying a one-sided alpha of 0.05 and using 92 patients for each treatment arm comparison, there will be 99% statistical power to detect differences in pT0 rates such as that specified in Table 2 where the absolute difference in the pT0 rate between the predicted responders and non-responders is 50%. There will be 82% power to detect differences in the absolute range of 30% (e.g., 22% vs. 52%).

Table 2:

	Predicted Responder from Treatment-Specific Gene Expression Profiling		Total
Path Response	Yes	No	
pT0	24 (52%)	6 (13%)	33% (n=30)
No pT0	22	40	67% (n=62)
	46	46	N=92 for each treatment arm analysis

Sensitivity=80%, specificity=65%

A similar modeling approach will be used to evaluate the outcome of favorable downgrading as a secondary endpoint. Although statistical power will be low, we are also interested in whether there is any suggestion that the treatment-specific GEP is a predictive factor for pT0 status. That is, can the treatment specific GEP score help to direct which of the two neoadjuvant treatment regimens the patient should receive? Each of the two treatment-specific GEPs will be evaluated in separate analyses. In order to evaluate each GEP's predictive property, all 182 patients will be included in a logistic regression model. For illustrative purposes, we will specify the process for the DDMVAC-specific GEP analysis. Indicators for age group, gender, clinical T-stage, grade, treatment arm, and indicator for favorable DDMVAC-specific GEP score will be placed in the model along with an interaction term of treatment arm x DDMVAC GEP indicator. Testing this interaction is equivalent to testing whether those with a favorable DDMVAC GEP score who receive DDMVAC have greater odds of the pT0 outcome compared to those with a favorable GEP score who were randomized to GC. This differentiates the GEP score as predictive in contrast to prognostic.

If we assume the pT0 rate will be the same for both treatment arms and will be 31%, and we assume that a favorable GEP score (split at 31% for this example in order to illustrate power) has an odds ratio of 1.2 for the GC arm (prognostic strength), and using a two-sided type I error rate, then there will be 60% power to detect a very large interaction term

of 5.5. That would imply that the odds ratio for the favorable DDMVAC- GEP score group compared to unfavorable is 5.5 times greater if the patient gets DDMVAC treatment compared to GC. A similar analysis will also be conducted for the GC-specific GEP, assessing whether it's predictive. Statistical properties will be similar.

Regardless of the predictive properties of either GEP score, being able to identify a subset of patients with poor prognosis based on their GEP score will have a meaningful clinical impact. Our goal is to find a group of patients with chemotherapy resistant disease for whom the risk of progression is so great that they would likely benefit from going directly to surgery and not receiving neoadjuvant therapy. Conversely, identifying groups of patients who have a high probability of benefiting from neoadjuvant chemotherapy will allow physicians to better evaluate the risks of delaying surgery to deliver chemotherapy. If we assume there is no interaction between GEP score and treatment arm, then we will pool all 184 patients together to evaluate the prognostic properties of each GEP with respect to overall survival. We assume median survival (both arms) for those with an unfavorable score will be 40 months, and we expect half the patients will have a favorable GEP score. If we assume 3 years of accrual, an additional 3 years of follow-up, and a two-sided alpha=0.05 then we will have 83% power to detect a hazard ratio of 1.90 between those with an unfavorable GEP score relative to the favorable group. This is comparable to a median OS of 40 months versus 76 months. The absolute level of the survival curves as well as their degree of separation will be important in this setting.

There will be no formal interim analyses since the objective of this trial is not to directly compare the two treatment regimens as both could be considered standard-of-care. The final analysis using the pathologic response endpoint is expected approximately one year after the last patient has been randomized or four years after study activation. Subsequent analyses related to survival will occur 3-4 years after accrual is completed.

11.4 Data and Safety Monitoring Committee Oversight

A Data and Safety Monitoring Committee will oversee the conduct of the study. The Committee consists of four members from outside of the SWOG, 3 SWOG members, 3 non-voting representatives from the National Cancer Institute (NCI), and the Group Statistician (non-voting). The members of this Committee will receive confidential reports every 6 months from the SWOG Statistics and Data Management Center, and will meet at the Group's bi-annual meetings as necessary. The Committee will be responsible for decisions regarding possible termination and/or early reporting of the study.

Note: The specific statistical considerations for the TM studies can be found in Appendix 18.3.

12.0 DISCIPLINE REVIEW

There will be no formal discipline review done in conjunction with this study.

13.0 REGISTRATION GUIDELINES

13.1 Registration Timing

Patients must be registered prior to initiation of treatment (no more than 14 days prior to planned start of treatment).

13.2 Investigator/Site Registration

Prior to the recruitment of a patient for this study, investigators must be registered members of a Cooperative Group. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU Web site (enter credentials at https://www.ctsu.org; then click on the Register tab) or by calling the PMB at 240/276-6575 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each investigator or group of investigators at a clinic site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site by entering credentials at https://www.ctsu.org.

13.3 OPEN Registration Requirements

The individual registering the patient must have completed the appropriate SWOG Registration Worksheet. The completed form must be referred to during the registration but should not be submitted as part of the patient data.

Oncology Patient Enrollment Network (OPEN) will also ask additional questions that are not present on the SWOG Registration Worksheet. The individual registering the patient must be prepared to provide answers to the following questions:

a. Institution CTEP ID

b. Protocol Number

c. Registration Step

d. Treating Investigator

e. Cooperative Group Credit

f. Credit Investigator

g. Patient Initials

h. Patient's Date of Birth

i. Patient SSN (SSN is desired, but optional. Do not enter invalid numbers.)

j. Country of Residence

k. ZIP Code

l. Gender (select one):
- Female Gender
- Male Gender

m. Ethnicity (select one):
- Hispanic or Latino
- Not Hispanic or Latino
- Unknown

n. Method of Payment (select one):
- Private Insurance
- Medicare
- Medicare and Private Insurance
- Medicaid
- Medicaid and Medicare
- Military or Veterans Sponsored NOS
- Military Sponsored (Including Champus & Tricare)
- Veterans Sponsored
- Self Pay (No Insurance)
- No Means of Payment (No Insurance)
- Other
- Unknown

o. Race (select all that apply):
- American Indian or Alaska Native
- Asian
- Black or African American
- Native Hawaiian or other Pacific Islander
- White
- Unknown

13.4 Registration Procedures

a. All site staff (SWOG and CTSU Sites) will use OPEN to enroll patients to this study. OPEN is a web-based application and can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org, or from the OPEN Patient Registration link on the SWOG CRA Workbench.

b. Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes and the affirmation of eligibility on the Registration Worksheet has been signed by the registering investigator or another investigator designate. Site staff should refer to Section 5.0 to verify eligibility.

- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

- The study site is listed as "approved" in the CTSU RSS.

c. Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user ID and password) used for the CTSU members' web site.

- To perform registrations, the site user must have been assigned the 'Registrar' role on the SWOG or CTSU roster:

1. If you are a SWOG member, to perform registrations on SWOG protocols you must have an equivalent 'Registrar' role on the SWOG roster. Role assignments are handled through SWOG.

2. If you are not a SWOG member, to perform registrations on SWOG protocols you must have the role of Registrar on the CTSU roster. Site and/or Data Administrators can manage CTSU roster roles via the new Site Roles maintenance feature under RSS on the CTSU members' web site. This will allow them to assign staff the "Registrar" role.

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

d. Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

13.5 Exceptions to SWOG registration policies will not be permitted.

a. Patients must meet all eligibility requirements.

b. Institutions must be identified as approved for registration.

c. Registrations may not be cancelled.

d. Late registrations (after initiation of treatment) will not be accepted.

14.0 DATA SUBMISSION SCHEDULE

14.1 Data Submission Requirement

Data must be submitted according to the protocol requirements for **ALL** patients registered, whether or not assigned treatment is administered, including patients deemed to be ineligible. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

14.2 Master Forms

Master forms can be found on the protocol abstract page on the SWOG website (www.swog.org) and (with the exception of the sample consent form and the Registration Worksheet) must be submitted on-line via the Web; see Section 14.3a for details.

14.3 Data Submission Procedures

a. SWOG institutions must submit data electronically via the Web using Medidata Rave® at the following url: https://login.imedidata.com/selectlogin

1. If prompted, select the 'CTEP-IAM IdP' link.

2. Enter your valid and active CTEP-IAM userid and password. This is the same account used for the CTSU members' web site and OPEN.

b. You may also access Rave® via the SWOG CRA Workbench. Go to the SWOG web site (http://swog.org) and logon to the Members Area using your SWOG Roster ID Number and password. After you have logged on, click on *Workbenches*, then *CRA Workbench* to access the home page for the CRA Workbench and follow the link to Rave® provided in the left-hand navigation panel

To access the CRA Workbench the following must be done (in order):

1. You are entered into the SWOG Roster and issued a SWOG Roster ID Number,

2. You are associated as an investigator or CRA/RN at the institution where the patient is being treated or followed,

3. Your Web User Administrator has added you as a web user and has given you the appropriate system permissions to view data for that institution.

For assistance with points 1 and 2 call the Operations Office at 210/614-8808. For point 3, contact your local Web User Administrator (refer to the "Who is my Web User Administrator?" function on the swog.org Members logon page).

For difficulties with the CRA Workbench, please email technicalquestion@crab.org.

c. Institutions participating through the Cancer Trials Support Unit (CTSU) please refer to the CTSU Participation Table.

14.4 Data Submission Overview and Timepoints

a. WITHIN 7 DAYS OF REGISTRATION:

Submit the following:

S1314 Onstudy Form

Operative Report from TURBT

Pathology Report from TURBT

Pathology Verification of Tissue Submission Form (see Section 18.4)

Submit radiology reports from all scans performed to assess disease at baseline.

b. AT PRECHEMOTHERAPY (WITHIN 28 DAYS AFTER REGISTRATION), PRIOR TO SURGERY (WITHIN 7 DAYS PRIOR TO SCHEDULED PROCEDURE), AND POST-SURGERY (WITHIN 14 DAYS AFTER SURGERY):

Submit specimens (including pathology report) as outlined in Section 15.1.

NOTE: All cystectomy and cystoprostatectomy specimens should be processed for pathological examination and reported according to the recommended guidelines of the College of American Pathologists: (http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2009/UrinaryBladder_09protocol.pdf).

c. IF PATIENT CONSENTED, WITHIN 24 HOURS OF C1D1 AND C2D1 OF CHEMOTHERAPY

Submit specimens as outlined in Section 15.2

d. WITHIN 7 DAYS AFTER EACH CYCLE OF CHEMOTHERAPY:

Submit the following:

S1314 Treatment Form

S1314 Adverse Event Form

e. WITHIN 14 DAYS OF DISCONTINUATION OF CHEMOTHERAPY:

Submit the following:

S1314 Treatment Form

S1314 Adverse Event Form

S1314 Off Chemotherapy Notice

f. WITHIN 14 DAYS OF COMPLETION OF CYSTECTOMY:

Submit the following:

If patient received protocol cystectomy

S1314 Cystectomy Status Form

S1314 Cystectomy Reporting Form

Operative report from cystectomy

Pathology report from cystectomy

If patient received cystectomy off protocol

S1314 Cystectomy Reporting Form

Operative report from cystectomy

Pathology report from cystectomy

g. WITHIN 14 DAYS OF DECISION TO NOT HAVE PROTOCOL CYSTECTOMY:

Submit the following:

S1314 Cystectomy Status Form

h. WITHIN 14 DAYS OF PROGRESSION/RELAPSE::

If patient was still on protocol chemotherapy

Submit the following:

S1314 Off Chemotherapy Notice

S1314 Treatment Form

S1314 Adverse Event Form

If patient was off chemotherapy

Follow Up Form.

i. EVERY 3 MONTHS FOR FIRST YEAR, EVERY 6 MONTHS FOR SECOND AND THIRD YEARS, THEN ANNUALLY UNTIL 5 YEARS FROM REGISTRATION:

Submit the following:

Follow Up Form

Late Effects Form (if prior to treatment for progression or relapse or a second primary, and prior to non-protocol treatment, the patient experiences any severe [Grade ≥ 3] long term toxicity that has not been previously reported)

j. WITHIN 4 WEEKS OF KNOWLEDGE OF DEATH:

Submit the following:

Notice of Death

S1314 Off Chemotherapy Notice (if the patient was still on chemotherapy)

S1314 Cystectomy Status Form (if not previously submitted)

S1314 Treatment Form (if the patient was still on chemotherapy)

S1314 Adverse Event Form (if the patient was still on chemotherapy)

Follow Up Form (if protocol cystectomy was received)

15.0 SPECIAL INSTRUCTIONS

15.1 Specimens for mandatory correlative studies (submitted to the SWOG Specimen Repository – Solid Tissue, Myeloma and Lymphoma Division, Lab #201*)* are **mandatory** for the patient.

a. Specimen Submission Timepoints

Specimens must be submitted at the following times (see Section 9.0). Additional specimen collection and submission instructions can be accessed on the SWOG Specimen Submission webpage: (http://swog.org/Members/ClinicalTrials/Specimens/STSpecimens.asp).

Pre-chemotherapy (within 28 days after registration):

1. 20 (10 micron) slides plus 2 (5 micron slides) at start and stop of 20 slide cut for a total of 22 unstained slides. All sections should be on "plus" slides.

2. Twenty milliliters (2 x 10 mL) of whole blood (pink/lavender top vacutainer tubes with EDTA)

3. Forty to fifty milliliters (40-50 mL) of voided urine (see Section 15.2 for details)

4. Pathology report (de-identified)

Prior to surgery (within 7 days prior to scheduled procedure):

Forty to fifty milliliters (40-50 mL) of voided urine (see Section 15.2 for details)

Post-surgery (within 14 days after scheduled procedure):

20 (10 micron) slides plus 2 (5 micron) slides at start and stop of 20 slide cut from the cystectomy sample (if applicable) for a total of 22 unstained slides. All sections should be on "plus" slides.

b. Specimen Submission Instructions

Specimen collection and submission instructions can be accessed on the SWOG Specimen Submission webpage: SOLID TUMOR (http://swog.org/Members/ClinicalTrials/Specimens/STSpecimens.asp).

Urine specimen collection instructions: Collect mid-stream of urine in a sterile https://www.facebook.com/urine cup, collecting approximately 40-50 ml. Urine should be shipped at ambient temperature within 24 hours of collection. Do not centrifuge or process urine at your site; the urine processing will take place centrally.

c. Specimen Collection Kits

Specimen collection kits are not being provided for this submission; sites should use institutional supplies.

15.2 Blood Specimen for upCTC (submitted to the Amir Goldkorn Lab, Lab #181) optional for patient

a. With patient's consent specimens must be submitted at the timepoints listed below. Collection instructions are outlined in Section 15.2c and submission instructions are outlined in Section 15.2e and Appendix 18.4.

b. With patient's consent specimens must be submitted at the following times (see Sections 9.1 and 9.2):

1. Peripheral whole blood (8-10 mL) drawn into provided EDTA tube and DNA Streck tube at C1D1 (after registration but prior to treatment) and C2D1. The specimens must be shipped on the same day as collection.

c. Specimen Collection Instructions for upCTC

ONLY COLLECT AND SHIP SAMPLES TO THE LABORATORY MONDAY THROUGH THURSDAY. DO NOT DRAW SAMPLES ON A FRIDAY OR THE DAY BEFORE A HOLIDAY. SAMPLES MUST BE SHIPPED OVERNIGHT ON THE SAME DAY OF COLLECTION.

- Materials required for blood collections is one (1) EDTA Tube and (1) DNA streck tube, VacutainerÒ brand adapter, and needles.
- For each patient, perform a venous puncture using a VacutainerÒ brand adapter and needle and fill the 2 blood collection tubes (minimum blood volume of 8-9 mL for each tube). Alternatively, blood samples may be obtained from a port or other central venous catheter using appropriate access needles and techniques.

- Invert each tube a minimum of eight (8) times to ensure proper mixing of the additives contained in the tube.
- Write the SWOG patient number, visit designation (i.e. C1D1, C2D1) and the date of collection on the tubes.
- The filled tubes must be maintained at ambient (15–30°C) temperature, avoiding extremes of heat and cold, at all times.

d. Specimen Collection and shipping Kits for upCTC

Supplies can be obtained by sending an e-mail to the contacts below. To facilitate the ordering process, please provide your study number on the subject line of your e-mail and your site (e.g. SWOG **S1314** – [your site]).

Contacts for this correlative study (in order of preference):
Gareth Morrison, Ph.D.: garethmo@usc.edu
Yucheng Xu, Ph.D.: yuchengx@usc.edu
Tong Xu, Ph.D.: tongxu@usc.edu

The upCTC Shipping Kit includes 1EDTA tube, 1 DNA Streck tube, 1 return mailing container, 1 pre-addressed return envelope, and shipping instructions.

The hours of operation for the Goldkorn Lab are Monday-Friday, 8:00 AM – 5:00PM PST. Although the lab will attempt to ship supplies out as quickly as possible, orders should be placed approximately one week before supplies are needed.

e. Shipping Instructions

ONLY COLLECT AND SHIP SAMPLES TO THE DESIGNATED STUDY LABORATORY MONDAY THROUGH THURSDAY, DO NOT DRAW SAMPLES ON A FRIDAY OR THE DAY BEFORE A HOLIDAY. SAMPLES MUST BE SHIPPED OVERNIGHT ON THE SAME DAY OF COLLECTION.

1. SWOG Specimen Tracking System (STS)

All specimen submissions for this study must be entered and tracked using the SWOG online Specimen Tracking system. SWOG members may log on the online system via the CRA Workbench. To access the CRA Workbench, go to the SWOG Web site (http://swog.org) and logon to the Members Area. After you have logged on using your SWOG roster ID number and password, click on the *CRA Workbench* link to access the home page for CRA Workbench website. First time non-SWOG users must refer to start-up instructions located at https://gill:crab.org/SpecTrack/.

A copy of the Shipment Packing List produced by the online Specimen Tracking system should be printed and placed in the pocket of the specimen bag if it has one, or in a separate resealable bag.

ALL SPECIMENS MUST BE LOGGED VIA THIS SYSTEM; THERE ARE NO EXCEPTIONS.

To report technical problems with Specimen Tracking, such as database errors or connectivity issues, please send an e-mail to technicalquestion@crab.org. For procedural help with logging and shipping specimens, there is an introduction to the system on the Specimen Tracking main page (http://dnet.crab.org/SpecTrack/Documents/Instructions.pdf); or contact the SWOG Statistics and Data Management Center at 206/652-2267 to be routed to the Data Coordinator for further assistance.

In the online specimen tracking system, the appropriate SWOG laboratory for submission of upCTC testing (1 EDTA tube) is identified as follows:

Lab #181: Goldkorn Laboratory
Phone: 323/442-7722

2. Federal guidelines for the shipment of blood products (NOTE: Return shipping supplies and pre-addressed envelopes will be provided by the Goldkorn Lab):

   a. The tube must be wrapped in an absorbent material.

   b. *The tube must then be placed in an AIRTIGHT container (like a resealable bag).*

   c. *Pack the resealable bag and tube in a Styrofoam shipping container.*

   d. *Pack the Styrofoam shipping container in a cardboard box.*

   e. *Mark the box "Biohazard".*

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31.

Confidentiality

Please note that the information contained in this protocol is considered confidential and should not be used or shared beyond the purposes of completing protocol requirements until or unless additional permission is obtained.

16.1 Adverse Event Reporting Requirements

a. Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. (Directions for routine reporting are provided in Section 14.0.) Additionally, certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following guidelines prescribe expedited adverse event reporting for this protocol. See also Appendix 18.1 for general and background information about expedited reporting.

b. Reporting method

This study requires that expedited adverse event reporting use the Cancer Therapy Evaluation Program Adverse Event Expedited Reporting System (CTEP-AERS). CTEPs guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted to the SWOG Operations Office electronically via the CTEP-AERS web-based application located at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm.

In the rare event when internet connectivity is disrupted an electronic report MUST be submitted immediately upon re-establishment of internet connection.

c. When to report an event in an expedited manner

When the adverse event requires expedited reporting, submit the report within 10 calendar days of learning of the event.

d. Other recipients of adverse event reports

The SWOG Operations Office will forward reports and documentation to the appropriate regulatory agencies and drug companies as required.

Adverse events determined to be reportable to the Institutional Review Board responsible for oversight of the patient must be reported according to local policy and procedures.

e. Expedited reporting for commercial agents

Commercial reporting requirements are provided in Table 16.1. The commercial agents used in Arms 1 and 2 of this study are gemcitabine, cisplatin, methotrexate, vinblastine, doxorubicin, and filgrastim/pegfilgrastim. If there is any question about the reportability of an adverse event or if on-line CTEP-AERS cannot be used, please telephone or email the SAE Program at the Operations Office, 210/614-8808 or adr@swog.org, before preparing the report.

Table 16.1. Expedited reporting requirements for adverse events experienced by patients on study Arms 1 or 2 within 30 days of the last administration of the commercial agents.

ATTRIBUTION	Grade 4		Grade 5[a]	
	Unexpected	Expected	Unexpected	Expected
Unrelated or Unlikely			**CTEP-AERS**	**CTEP-AERS**
Possible, Probable, Definite	**CTEP-AERS**		**CTEP-AERS**	**CTEP-AERS**

CTEP-AERS: Indicates an expedited report is to be submitted via CTEP-AERS within 10 calendar days of learning of the event[b].

[a] This includes all deaths within 30 days of the last dose of treatment with a commercial agent(s), regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent(s) and is attributed (possibly, probably, or definitely) to the agent(s) and is not due to cancer recurrence must be reported according to the instructions above.

[b] Submission of the on-line CTEP-AERS report plus any necessary amendments generally completes the reporting requirements. You may, however, be asked to submit supporting clinical data to the Operations Office in order to complete the evaluation of the event. If requested, the specified data should be sent within 5 calendar days by fax to 210-614-0006.

f. **Reporting Pregnancy, Fetal Death, and Death Neonatal**

1. **Pregnancy** Study participants who become pregnant while on study; that pregnancy should be reported in an expedited manner via CTEP-AERS as **Grade 3 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy)"** under the **Pregnancy, puerperium and perinatal conditions** SOC.

Additionally, the pregnancy outcome for patients on study should be reported via CTEP-AERS at the time the outcome becomes known, accompanied by the same Pregnancy Report Form used for the initial report.

Fetal Death Fetal Death defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation" should be reported expeditiously as **Grade 4 "pregnancy, puerperium and**

perinatal conditions – Other (pregnancy loss)" under the Pregnancy, puerperium and perinatal conditions SOC.

2. **Death Neonatal:** Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention should be reported expeditiously.

   A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration – Other (neonatal loss)"** under the **General disorders and administration** SOC.

   *Fetal death and neonatal death should **NOT** be reported as a Grade 5 event. If reported as* such, the CTEP-AERS interprets this as a death of the patient being treated.

   NOTE: When submitting CTEP-AERS reports for "Pregnancy, "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should also be completed and faxed with any additional medical information to 301-230-0159. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

   The Pregnancy Information Form is available at:
   http://ctep.cancer.gov/protocolDevelopment/adverse_effects/htm

17.0 BIBLIOGRAPHY

1 Siegel R, Ward E, Brawley O, et al. Cancer statistics, 2014: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 64:9-29, 2014.

2 Sternberg CN, Yagoda A, Scher HI, et al. Preliminary results of M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) for transitional cell carcinoma of the urothelium. J Urol 133:403-7, 1985.

3 Logothetis CJ, Dexeus FH, Finn L, et al. A prospective randomized trial comparing MVAC and CISCA chemotherapy for patients with metastatic urothelial tumors. J Clin Oncol 8:1050-5, 1990.

4 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 10:1066-73, 1992.

5 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 18:3068-77, 2008.

6 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 23:4602-8, 2005.

7 Als AB, Sengelov L, Von Der Maase H. Gemcitabine and cisplatin in locally advanced and metastatic bladder cancer; 3- or 4-week schedule? Acta Oncol 47:110-9, 2008.

8 Adamo V, Magno C, Spitaleri G, et al. Phase II study of gemcitabine and cisplatin in patients with advanced or metastatic bladder cancer: long-term follow-up of a 3-week regimen. Oncology 69:391-8, 2005.

9 Soto Parra H, Cavina R, Latteri F, et al. Three-week versus four-week schedule of cisplatin and gemcitabine: results of a randomized phase II study. Ann Oncol 13:1080-6, 2002.

10 Logothetis CJ, Dexeus FH, Sella A, et al. Escalated therapy for refractory urothelial tumors: methotrexate-vinblastine-doxorubicin-cisplatin plus unglycosylated recombinant human granulocyte-macrophage colony-stimulating factor. J Natl Cancer Inst 82:667-72, 1990.

11 Sternberg CN, de Mulder PH, van Oosterom AT, et al. Escalated M-VAC chemotherapy and recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) in patients with advanced urothelial tract tumors. Ann Oncol 4:403-7, 1993.

12 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 Organization for Research and Treatment of Cancer Protocol no. 30924. J Clin Oncol 19:2638-46, 2001.

13 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 tumours. Eur J Cancer 42:50-4, 2006.

14 Malmstrom PU, Rintala E, Wahlqvist R, et al. Five-year followup of a prospective trial of radical cystectomy and neoadjuvant chemotherapy: Nordic Cystectomy Trial I. The Nordic Cooperative Bladder Cancer Study Group. J Urol 155:1903-6, 1996.

15 Sherif A, Rintala E, Mestad O, et al. Neoadjuvant cisplatin-methotrexate chemotherapy for invasive bladder cancer -- Nordic cystectomy trial 2. Scand J Urol Nephrol 36:419-25, 2002.

16 Neoadjuvant chemotherapy in invasive bladder cancer: a systematic review and meta-analysis. Lancet 361:1927-34, 2003.

17 Winquist E, Kirchner TS, Segal R, et al. Neoadjuvant chemotherapy for transitional cell carcinoma of the bladder: a systematic review and meta-analysis. J Urol 171:561-9, 2004.

18 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 349:859-66, 2003.

19 Scosyrev E, Messing EM. Do mixed histological features affect survival benefit from neoadjuvant platinum-based combination chemotherapy in patients with locally advanced bladder cancer? BJU Int 108:700, 2011.

20 Neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: a randomised controlled trial. International collaboration of trialists. Lancet 354(9178):533-40, 1999.

21 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 29:2171-7, 2011.

22 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 113:2471-7, 2008.

23 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 349:859-66, 2003.

24 Sonpavde G, Goldman BH, Speights VO, et al. Quality of pathologic response and surgery correlate with survival for patients with completely resected bladder cancer after neoadjuvant chemotherapy. Cancer 115:4104-9, 2009.

25 David KA, Milowsky MI, Ritchey J, et al. Low incidence of perioperative chemotherapy for stage III bladder cancer 1998 to 2003: a report from the National Cancer Data Base. J Urol 178:451-4, 2007.

26 Yafi FA, Aprikian AG, Chin JL, et al. Contemporary outcomes of 2287 patients with bladder cancer who were treated with radical cystectomy: a Canadian multicentre experience. BJU Int 108(4):539-45, 2011.

27 Raj GV, Karavadia S, Schlomer B, et al. Contemporary use of perioperative cisplatin-based chemotherapy in patients with muscle-invasive bladder cancer. Cancer 117:276-82, 2011.

28 Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353:1673-84, 2005.

29 Perez EA, Romond EH, Suman VJ, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 29:3366-73, 2011.

30 Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 131:18-43, 2007.

31 Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-57, 2009.

32 Fukuoka M, Wu YL, Thongprasert S, et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 29:2866-74, 2011.

33 Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 12:1004-12, 2011.

34 Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 363:1693-703, 2010.

35 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 29:3443-9, 2011.

36 Ferreira CG, Tolis C, Giaccone G. p53 and chemosensitivity. Ann Oncol 10:1011-21, 1999.

37 Gossage L, Madhusudan S. Current status of excision repair cross complementing-group 1 (ERCC1) in cancer. Cancer Treat Rev 33:565-77, 2007.

38 Bellmunt J, Paz-Ares L, Cuello M, et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol 18:522-8, 2007.

39 Hoffmann AC, Wild P, Leicht C, et al. MDR1 and ERCC1 expression predict outcome of patients with locally advanced bladder cancer receiving adjuvant chemotherapy. Neoplasia 12:628-36, 2010.

40 Kim KH, Do IG, Kim HS, et al. Excision repair cross-complementation group 1 (ERCC1) expression in advanced urothelial carcinoma patients receiving cisplatin-based chemotherapy. APMIS 118:941-8, 2010.

41 Kawashima A, Nakayama M, Kakuta Y, et al. Excision repair cross-complementing group 1 may predict the efficacy of chemoradiation therapy for muscle-invasive bladder cancer. Clin Cancer Res 17:2561-9, 2011.

42 Matsumura N, Nakamura Y, Kohjimoto Y, et al. The prognostic significance of human equilibrative nucleoside transporter 1 expression in patients with metastatic bladder cancer treated with gemcitabine-cisplatin-based combination chemotherapy. BJU Int 108:E110-6, 2011.

43 Font A, Taron M, Gago JL, et al. BRCA1 mRNA expression and outcome to neoadjuvant cisplatin-based chemotherapy in bladder cancer. Ann Oncol 22:139-44, 2011.

44 Bellmunt J, Paz-Ares L, Cuello M, et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol 18:522-8, 2007.

45 Hoffmann AC, Wild P, Leicht C, et al. MDR1 and ERCC1 expression predict outcome of patients with locally advanced bladder cancer receiving adjuvant chemotherapy. Neoplasia 12:628-36, 2010.

46 Spratlin J, Sangha R, Glubrecht D, et al. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin Cancer Res 10:6956-61, 2004.

47 Marechal R, Mackey JR, Lai R, et al. Human equilibrative nucleoside transporter 1 and human concentrative nucleoside transporter 3 predict survival after adjuvant gemcitabine therapy in resected pancreatic adenocarcinoma. Clin Cancer Res 15:2913-9, 2009.

48 Matsumura N, Nakamura Y, Kohjimoto Y, et al. The prognostic significance of human equilibrative nucleoside transporter 1 expression in patients with metastatic bladder cancer treated with gemcitabine-cisplatin-based combination chemotherapy. BJU Int 108:E110-6, 2011.

49 Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer 6:813-23, 2006.

50 Smith SC, Baras AS, Lee JK, et al. The COXEN principle: translating signatures of in vitro chemosensitivity into tools for clinical outcome prediction and drug discovery in cancer. Cancer Res 70:1753-8, 2010.

51 Paik S, Tang G, Shak S, et al. A multigene assay to predict recurrence of tamoxifen-treated node-negative breast cancer. N Engl J Med 351:2817-26, 2004.

52 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 U S A 104:13086-91, 2007.

53 Smith SC, Havaleshko DM, Moon K, et al. Use of yeast chemigenomics and COXEN informatics in preclinical evaluation of anticancer agents. Neoplasia 13:72-80, 2011.

54 Nagji AS, Cho SH, Liu Y, et al. Multigene expression-based predictors for sensitivity to Vorinostat and Velcade in non-small cell lung cancer. Mol Cancer Ther 9:2834-43, 2010.

55 Sanchez-Carbayo M, Socci N, Lozano J, et al. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. J Clin Oncol 24:778-89, 2006.

56 Smith S, Baras A, Dancik G, Ru Y, Ding K, Moskaluk C, Fradet Y, Lehmann J, Stöckle M, Hartmann A, Lee J, Theodorescu D. A 20-gene model for molecular nodal staging of bladder cancer: development and prospective assessment. Lancet Oncol 12:137-43, 2011.

57 McCall M, Bolstad B, Irizarry R. Frozen robust multiarray analysis (fRMA). Biostatistics 11:242-53, 2010.

58 Als AB, Dyrskjot L, von der Maase H, et al. Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer. Clin Cancer Res 13:4407-14, 2007.

59 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

60 Takata R, Katagiri T, Kanehira M, et al. Predicting response to methotrexate, vinblastine, doxorubicin, and cisplatin neoadjuvant chemotherapy for bladder cancers through genome-wide gene expression profiling. Clin Cancer Res 11:2625-36, 2005.

61 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

62 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

63 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

64 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 349:859-66, 2003.

65 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 18:3068-77, 2008.

18.0 APPENDIX

18.1 New York Heart Association Classifications

18.2 Pathological Verification of Tissue Submission Form

18.3 Translational Medicine Studies

a. microRNA Expression Profile
b. Ultra Pure Circulating Tumor Cells Using Coxen
c. Cisplatin Germline Pharmacogenomic
d. DNA Damage Response to Gene Alterations

18.4 Circulating Tumor Cell (CTC) Instructions for Blood Sample Processing, Packing and Shipping

18,1 New York Heart Association Classifications

Class	Cardiac Symptoms	Need for Limitations	Physical Ability Additional Rest*	To Work**
I	None	None	None	Full Time
II	Only moderate	Slight or occasional	Usually only slight	Usually full time
III	Defined, with less than ordinary activity	Marked	Usually moderate	Usually part time
IV	May be present even at rest, & any activity increases discomfort	Extreme	Marked	Unable to work

* To control or relieve symptoms, as determined by the patient, rather than as advised by the physician.
** At accustomed occupation or usual tasks.

18.2 Pathological Verification of Tissue Submission Form

SWOG Patient Identifier: _____ Patient Initials: _____ (L, F, M)

Pathology Diagnosis: _____

Specimen Review

Viable Tumor Size in Submitted Slides **(PLEASE CHECK ONLY ONE)**:

☐ Adequate (≥ 0.5 cm of viable tumor-longest diameter)

☐ Inadequate (< 0.5 cm of viable tumor), patient is ineligible

_____ _____
Pathologist Date

NOTE: This form must be signed by pathologist and submitted as source documentation via the Web using Medidata Rave.

Comments:

18.3 Translational Medicine Studies

Objectives

1. To assess microRNA expression profile.
2. To assess Ultra Pure CTC
3. To assess Cisplatin germline pharmacogenomics
4. To assess DNA damage response to gene alterations
5. To assess other translational endpoints via gene expression, tissue microarray, microRNA, SNP and genetic profiling data collected in the neoadjuvant bladder cancer setting. (The pre-chemotherapy TURBT sample and the post-chemotherapy cystectomy samples will be tested for mRNA, genetic, SNP, and microRNA expression. Additionally, blood, urine and tissue for tissue microarray will be obtained.)

Analysis of Translational Medicine (TM) Endpoints

Biologic samples for this study will be collected via the consolidated SWOG Biorepository at Nationwide Children's Hospital Biopathology Center in Columbus, Ohio. Study protocols for non-COXEN studies currently described in Section 18.3a-18.3d. There will be three types of biologic samples, with a prioritization schedule for each, delineated below. Of note, the principle of analyses will be to perform a global evaluation of a specific metric (e.g. RNA), which can be applied to the specific hypothesis being tested in the trial but will also be released after this prescribed assessment to the general research community for use. For example, gene expression will be assessed on all of the TURBT samples, the COXEN scores will be calculated/reported, and then the gene expression data linked to clinical outcomes will be released for general use.

TURBT:
1. RNA (COXEN GEM + microRNA)
2. DNA (Oncogenomic)
3. With additional tissue for future studies as available

Residual tumor at cystectomy:
1. RNA (COXEN GEM, microRNA)
2. DNA (Oncometrics)
3. With additional tissue for future studies as available

Blood sample:
1. DNA (Oncogenomic)
2. Cisplatin pharmacogenomics)
3. With additional samples for future studies as available

18.3a microRNA (miRNA) expression profile

Objective

1. To explore whether there is a miRNA expression profile that correlates with pathologic complete response, a pathologic downstaging or the lack thereof after TURBT and neoadjuvant chemotherapy with either GC or DDMVAC.

Study Design

microRNA (miRNA) expression profile – 20 (10 micron) slides plus 5 micron slides at start and stop of 20 slide cut. The 5 micron slides will be stained with H&E centrally, and a pathologist will use it to mark the tumor area(s). Using the H&E as a template, the tumor areas on the remaining (unstained) slides are manually removed to microcentrifuge tubes for RNA isolation. Total RNA is isolated using the total RNA isolation method in the High Pure miRNA isolation Kit (catalog number 05080576001, Roche Applied Science). A NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and an Agilent 2100 Bioanalyzer are used to measure RNA purity and integrity. Total RNA is used for the synthesis of biotinylated cDNA that is annealed to assay specific oligonucleotides and captured on streptavidin-conjugated paramagnetic particles. After extension and ligation, the ligated oligonucleotides are labeled with fluorescent primers by PCR, and these PCR products are hybridized to Illumina HT12V4 chips. After being washed, the slides are scanned with BeadStation 500X (Illumina, Inc.) and the signal intensities are quantified with GenomeStudio (Illumina, Inc.). Quantile normalization in the Linear Models for Microarray Data (LIMMA) package in the R language environment is used to normalize the data.

Specific hypotheses

- To explore whether there is a miRNA expression profile that correlates with pathologic complete response, pathologic downstaging or the lack thereof after TURBT and neoadjuvant chemotherapy with either GC or DDMVAC.

MicroRNAs play a central role in the regulation of EMT, particularly members of the miR200 family and miR205, and in preliminary analyses of 34 tumors from patients treated with neoadjuvant MVAC at MD Anderson, we have discovered that the miR200 family and miR205 identify subsets of tumors that are enriched for response to therapy (downstaging to ≤ pT1). With our optimization of Ion Torrent's Ampliseq platform for miRNA sequencing from FFPE tissue sections, it has become just as cost-effective and feasible to use micro RNA sequencing to measure the expression of all micro RNAs as it is to perform a more focused quantitative real-time PCR analysis of the expression of specific miRNAs. Furthermore, we recently developed a micro RNA-based PAM classifier that can be used to assign bladder cancers to the 4 known intrinsic subtypes, including "p53-like" tumors that are resistant to neoadjuvant chemotherapy. The focus of our studies will be to prospectively examine the relationship between intrinsic subtype membership as determined by miRNA expression profiling and pathological response within the context of this clinical trial. This work will complement the p53 sequencing studies discussed below.

Whole genome micro RNA expression will be determined by sequencing (Ion Torrent platform). The amount of small RNA in the total RNA sample is quantified with the use of Small RNA and RNA 6000 Nano bioanalyzer chips from Life Technologies. Twenty ng of small RNA is used for library preparation with the Ion Total RNA Seq v2 library preparation kit. The resulting cDNA library is quantified with High Sensitivity DNA bioanalyzer chips from Life Technologies to determine the molar concentration of each library, and to calculate the percentage of library that represents barcoded small RNAs. The cDNA libraries are diluted to the same molar concentration, pooled, and diluted to 100 pM. The samples are then templated and sequenced with an Ion Proton sequencer. The normalized

micro RNA sequencing data will be used to first assign tumors to the intrinsic basal and luminal subtypes using a PAM classifier. A 15-gene micro RNA panel will then be used to divide the basal and luminal tumors based on EMT and fibroblast infiltration as described previously. (1)

Statistical design

General strategies for the analyses of these projects will proceed by the following principles. As this is only a Phase II trial, the aims being addressed are hypothesis-building in nature, and any findings will need to be validated in another bladder cohort. Because both treatment arms involve neoadjuvant chemotherapy including cisplatin, it will not be possible to attribute lack of response as resistance to chemotherapy or particularly cisplatin. These tumors may have poor prognosis and be resistant to any current treatment options. We can only interpret significant factors as being prognostic for pathologic and clinical outcomes.

For most of these hypotheses, it will be reasonable to pool the treatment arms as both neoadjuvant chemotherapy regimens contain cisplatin and relationships of markers with pathologic response, RFS and OS are expected to be similar for DDMVAC and GC-treated patients. The exception to this will be the COXEN GEP analysis where the detailed analysis of the treatment interaction is specified in Section 11.0 of this protocol.

Measures of expression – miRNA expression data and stem, progenitor and differentiation markers will likely be log- transformed before any analysis. Quantile normalization in the Linear Models for Microarray Data (LIMMA) package in the R language environment is used to normalize the data. BRB ArrayTools version 4.2 developed by National Cancer Institute is used to analyze the data. This software uses a two-sample t-test to calculate the significance of the observations with false discovery rate (FDR) (i.e., $P < 0.001$). To predict the class of an independent data set, we developed a model based on Nearest Neighbor Classification (k=1, k=3). The prediction error of a model was estimated using leave one out cross-validation (LOOCV). For LOOCV training set, the entire model building process was repeated, including the gene selection process. We also evaluated whether the cross-validated error rate estimate for a model was significantly less than one would expect from random prediction.

Exploratory analyses will be performed to examine the underlying distributions using box plots, density plots, and scatter plots. For assessment of relationship with pathologic response (both pT0 and <pT1), logistic regression will be conducted upon tens of thousands of values simultaneously, adjusting for the stratification factors from the trial. False discovery rate (FDR) will be used to control the average false positive proportions among selected measures.

18.3b Ultra Pure Circulating Tumor Cells Using Coxen

Objective

To assess whether the COXEN gene expression profiles of ultra-pure circulating tumor cells (upCTCs) predict response (T0) to neoadjuvant chemotherapy at cystectomy.

Hypothesis and Specific Aims

Locally advanced bladder cancer has high recurrence and mortality rates despite surgical intervention. Neoadjuvant chemotherapy prior to resection improves outcomes in some patients but causes unnecessary morbidity and delays surgery in others. **S1314** is a SWOG trial testing the value of COXEN, a gene expression profiling strategy that aims to predict chemotherapy response. Currently, COXEN and other gene expression panels are measured from tumor biopsies, but such tissue is rarely available in advanced metastatic disease. An emerging alternative is enrichment of circulating tumor cells (CTCs) from the blood of patients with advanced disease, allowing minimally invasive, repeated sampling of tumor cells to guide precision care. Our laboratory has developed and implemented multiple techniques for enriching and molecularly characterizing circulating tumor cells (CTCs) in clinical trial settings and founded a CTC Research Core at USC Norris. Most recently, we have incorporated new technologies and methods that for the first time allow not only DNA analysis, but also enrichment of ultra-pure cells (upCTCs) for gene expression profiling without white blood cell (WBC) contamination. The convergence of our upCTC technology with **S1314**/COXEN offers a unique translational science opportunity to test the hypothesis that CTC gene expression can predict chemotherapy response. To test this hypothesis we will collect and enrich upCTCs at two time points (before and after start of chemotherapy) in **S1314** and extract mRNA from upCTCs for the following Specific Aims (Figure 1):

1. We will assess whether the COXEN gene expression profiles of upCTCs predict response (T0) to neoadjuvant chemotherapy at cystectomy.

2. We will test the association between upCTC-derived COXEN gene expression profiles and tumor biopsy-derived COXEN gene expression profiles.

3. We will analyze the change in upCTC-derived COXEN gene expression profile before vs. after chemotherapy.

4. (Exploratory) upCTC-derived DNA will be isolated for targeted sequencing and correlated with COXEN scores and with matched primary tumor DNA (beyond immediate study scope.

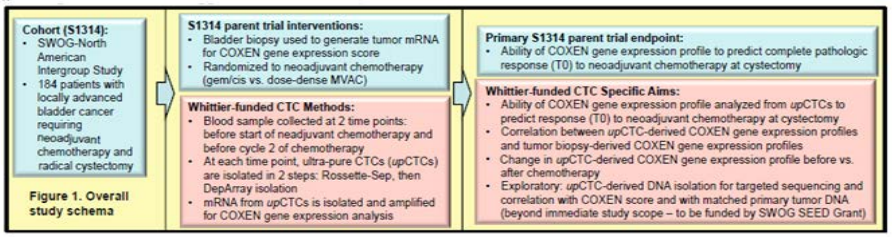


Figure 1. Overall study schema

SIGNIFICANCE AND INNOVATION

- Advances a key area in translational precision cancer care: Expands the role of CTC analysis from its current state (DNA analysis) to the realm of CTC gene expression profiling, enabling new insights into real- time tumor response during and after treatment

Unique trial opportunity: **S1314** allows comparison of CTC and tumor phenotypes before and after therapy, establishing a potential biomarker role for CTC gene expression in precision cancer care

BACKGROUND AND PRELIMINARY DATA

Bladder Cancer: Cancer of the urinary bladder is an aggressive malignancy that will account for 75,000 new cases and 15,000 deaths in the U.S. in 2014. *(2)* Tumors arise in the mucosal epithelium that lines the bladder lumen, and the standard treatment for tumors that invade beyond the mucosa (T2-T4) is radical cystectomy. Despite this aggressive surgical approach, within 2 years approximately half of patients develop recurrent metastatic disease that is invariably lethal. *(3)* Use of neoadjuvant chemotherapy prior to radical cystectomy has been shown to improve overall survival and reduce recurrence rates, in particular when the resected bladder is found to contain no viable tumor (T0) after chemotherapy. *(4)* However, neoadjuvant chemotherapy is effective only in some tumors, while in others it may only create a delay prior to radical cystectomy, perhaps allowing time for spread of disease that otherwise would have been curable with immediate resection. Hence, there is a need for accurate biomarkers that predict who will respond to chemotherapy, and to which regimen.

S1314 and COXEN: **S1314** is a North American Intergroup trial opening nation-wide that is led by the Southwest Oncology Group (SWOG), the NCI-designated cooperative group in which USC plays a key role (**Figure 2**). **S1314** will enroll 184 patients with locally-advanced (T2-T4) bladder cancer who will be randomized to one of two neoadjuvant chemotherapy regimens, followed by radical cystectomy. At enrollment, all patients will undergo tumor biopsy for mRNA extraction. The primary goal of **S1314** is to analyze tumor mRNA using a novel predictive tool: COXEN (CO-eXpression ExtrapolatioN). COXEN begins by using the NCI-60 panel of cancer cell lines and tumor archives to establish unique gene expression profiles highly correlated with response to various chemotherapeutic agents; then, patient tumors are analyzed for presence or absence of those same expression profiles to predict response to various chemotherapy regimens. *(5,6,7,8)* The primary endpoint of **S1314** will be whether the COXEN gene expression profiles generated from tumor biopsies are predictive of response to neoadjuvant therapy, defined as T0 (no viable tumor) at cystectomy.

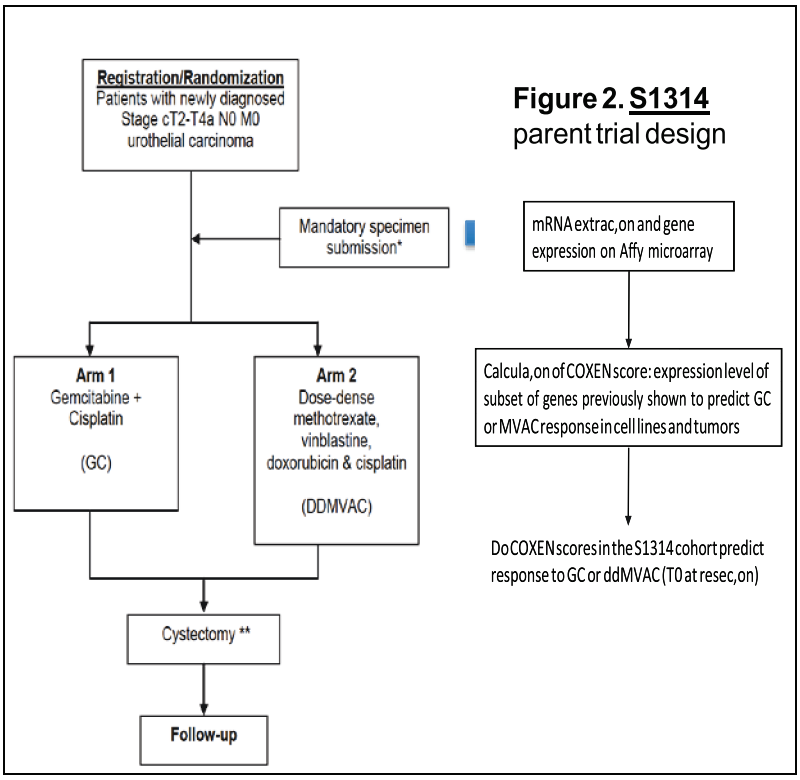


Figure 2. S1314 parent trial design

Circulating tumor cells and the Goldkorn Laboratory and CTC Research Core: New predictive biomarkers such as COXEN – while promising – are limited by the availability of tumor tissues. Often, such tissues are not available after initiation of therapy or when cancer recurs and progresses. Circulating tumor cells (CTCs) are cancer cells shed by tumors into the circulation, where they can be recovered and enriched repeatedly from peripheral blood samples to provide prognostic and predictive information throughout the disease course. *(9)* The Goldkorn Laboratory has developed novel methods for CTC enrichment and analysis, and in addition we have assimilated multiple other commercially available state-of-the-art platforms for CTC recovery, placed in our laboratory by industry partners for collaborative research purposes (**Figure 3**). *(10)*

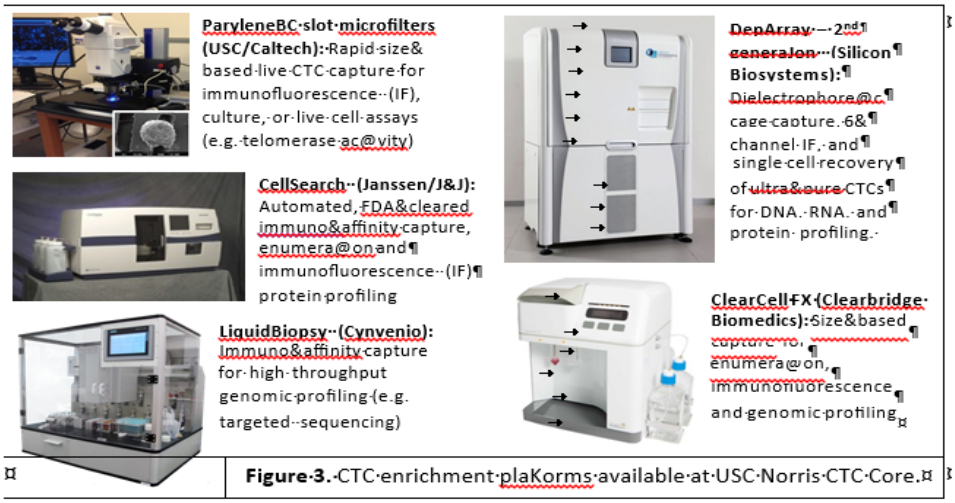


Figure 3. CTC enrichment plaKorms available at USC Norris CTC Core.

Using these capabilities, we have collected thousands of CTC samples in multiple clinical trials in prostate and bladder cancer, including 2 R01- funded translational science projects embedded in phase III SWOG studies where we demonstrated the prognostic value of CTC analysis. (11,12) Although enumeration and DNA analysis of CTCs has advanced rapidly, gene expression profiling has lagged far behind, hampered by the persistence of contaminating background white blood cells (WBC) in enriched CTC samples. Our group has nonetheless pursued the goals of CTC expression profiling using a motorized micromanipulator pipette custom-designed in our laboratory for this purpose. Using this device, we successfully recovered ultra-pure CTCs (upCTCs) with 0 WBC contamination from human and mouse blood samples. mRNA from these rare cells was successfully amplified and analyzed using several new methodologies that we optimized in direct collaboration with the R&D teams of their manufacturers, such as highly-multiplexed (624 gene) qPCR on the Open Array platform (Life Tech), and WTA kits (Nugen) for Affymetrix chip analysis or for RNAseq (see examples in **Figure 4**). Most recently, our laboratory has procured a CTC isolation platform engineered specifically for rapid, robust recovery of ultra-pure cells: The DepArray (Silicon Biosystems – see link) uses dielectrophoretic “cages” to trap and manipulate single cells, allowing selection of ultra-pure cells for recovery based on their immunofluorescent (IF) staining pattern in up to 6 IF channels. (13) Thus, the DEPArray platform offers a much more rapid, robust, and precise capability for recovering upCTCs, which then can be lysed for total RNA recovery, amplification, and analysis as described above.

Figure 4

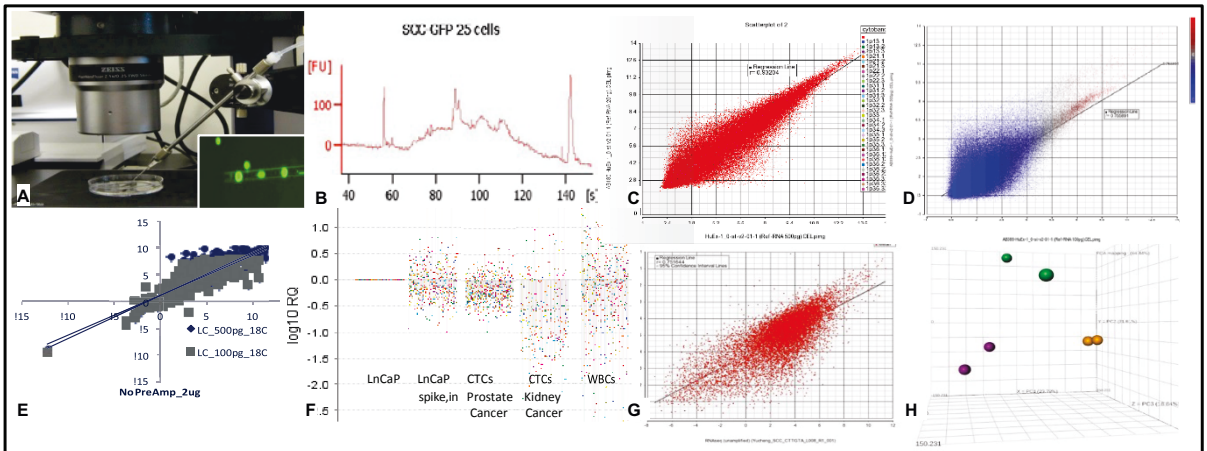


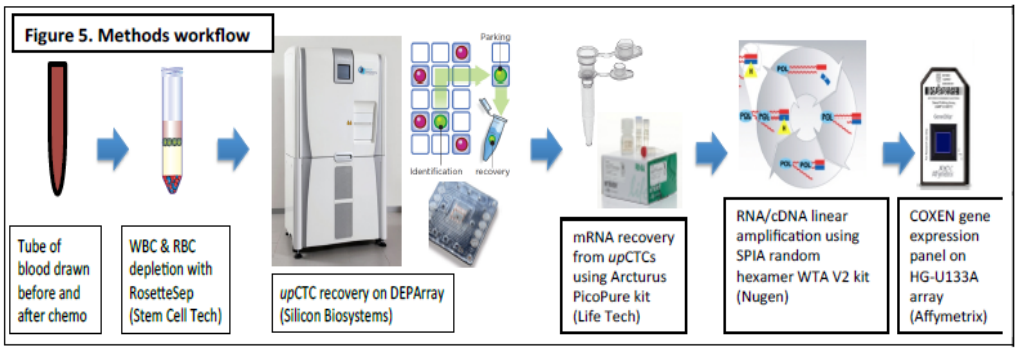
Figure 4. CTC gene expression technologies previously developed in Goldkorn Lab. A. Motorized micropipette apparatus for single CTC pickup and captured cells (inset). **B.** Agilent bioanalyzer curves with appropriate peaks from cDNA amplified from 25 captured cells using Nugen random hexamer SPIA amplification WTA V2 kits. **C-D.** Gene expression plots from Affymetrix HuEX array analyzing total RNA standard amplified from 500pg starting material (x axis) compared with unamplified 20ng starting material (y axis), (C, r=0.93), and from 100pg (x axis) vs. 500pg (y axis), (D, r=0.76). **E.** Multiplexed PCR pre-amplification (LifeTech) from 100pg RNA yields R^2=0.71 using 624 cancer gene Open Array panel. **F.** Open Array 624 gene expression (from L to R): 25 LNCaP cells, 25 LNCaP cells spiked into blood and retrieved by WBC depletion and micropipette, pure CTCs from a patient with prostate cancer, pure CTCs from a patient with kidney cancer, WBC only. Relative quantities (RQs) are normalized to unspiked LNCaP (first column). R^2 =0.87 for unspiked LNCaP vs. spiked and retrieved LNCaP (1st vs. 2nd column). **G.** Random hexamer linear amplification of total RNA (R^2=0.8) using Nugen Ovation kit. **H.** PCA map from RNAseq of duplicate HiSeq2000 gene expression patterns of ultra- pure CTCs (green) compared to primary tumor cells (orange) vs. metastases (purple) from the same mouse hosts.

Methods Rationale:

The existing design of S1314 calls for tumor biopsy and gene expression profiling in the setting of neoadjuvant chemotherapy and radical cystectomy. Hence, it provides the ideal context for parallel CTC gene expression analysis using the new workflow methods our group has developed for upCTC recovery, mRNA isolation, amplification and analysis. The proposed studies will thus enable direct assessment of how well CTC-derived gene expression signatures predict response to therapy, how they compare with matched tumor-derived signatures, and also how those signatures change after the start of chemotherapy.

upCTC isolation, mRNA recovery, and gene expression analysis (**Figure 5**):

At each of two time points – Cycle 1 Day 1 of chemotherapy and Cycle 2 Day 1 of chemotherapy – two tubes (~8ml each) of blood will be drawn and shipped overnight to our laboratory. Upon arrival, blood will be processed using the Rosette-Sep Kit (Stem Cell Technologies) for WBC and RBC depletion. The WBC/RBC-depleted, CTC-enriched sample will be IF-stained for Cytokeratins (CK), Epithelial Cell Adhesion Molecule (EpCAM), 4',6-diamidino-2-phenylindole nuclear stain (DAPI), and CD45 (WBC marker) and loaded onto the DEPArray for *up*CTC isolation (DAPI+CK+EpCAM+CD45-). Isolated *up*CTCs will be further processed for mRNA recovery using the Arcturus PicoPure kit (Life Technologies), followed by linear amplification using random hexamer WTA V2 kits (Nugen) with QC measures (e.g. BioAnalyzer) as done previously in our lab. Amplified cDNA will be loaded onto Affymetrix HG-U133A array (USC CHLA Genomics Core) for gene expression measurement.



Determination of COXEN score for each sample based on Affymetrix array gene expression:

The COXEN workflow was developed and published by Dr. Jay Lee (U Virginia) and studied extensively in bladder cancer by Dr. Dan Gustafson (Colorado State) in collaboration with Drs. Thomas Flaig and Dan Theodorescu (U Colorado, SWOG), who serve as collaborators for this proposal (see letter of support from Dr. Gustafson). *(14,15,16,17)* Briefly, the COXEN algorithm may be divided into 6 distinct steps. The first of these 3 steps ***have already been accomplished in the lead-in studies to S1314*** (**Table 1**), yielding the coefficients and probe sets to be used to calculate single-drug and combination-drug COXEN scores in our study.

- Step 1 (done): Determine drug's activity in discovery/validation bladder cancer cohorts (cell lines, tumors)
- Step 2 (done): Measure gene expression (Affymetrix array) of discovery/validation bladder cancer cohorts
- Step 3 (done): Select a subset of gene probes that most accurately predicts the drug's activity in the discovery/validation bladder cancer cohorts

	Analysis Type	Methotrexate	Vinblastine	Adriamycin	Cisplatin	Gemcitabine
GEM development	Principal component regression	NCI60 panel	NCI60 panel	NCI60 panel	NCI60 panel	NCI60 panel
GEM evaluation and final selection	Univariate COX proportional hazard regression	Lehmann 188 (MVEC or CM treated)	Lehmann 92 (MVEC treated)	Lehmann 92	Lehman 188	Laval 36 (GC treated)
Independent validation		Laval 54 (MVAC treated)	Laval 54	Laval 54	Laval 90 (MVAC or GC treated)	BLA40 panel
Final number of probes* in GEM with corresponding probe coefficient* data		20 probes	60 probes	40 probes	35 probes	35 probes

	Analysis	MVAC model	GC model
Model training and evaluation	Parametric survival regression, ROC, and survival analysis	Lehmann 188	Laval 36
Independent validation	ROC and survival analysis	Laval 54	No Dataset Available

Table 1. Cohorts used to derive gene expression models (GEM) of Affymetrix probe sets associated with single drug response (top) and of survival with multi-drug COXEN scores (bottom).

Using the probe sets and correlation coefficients already derived in Steps 1-3 (Table 1), new COXEN scores can now be calculated in our proposed study for each *up*CTC sample in Steps 4-6 as follows:

- Step 4: Measure gene expression in *up*CTC samples in the new study cohort (**S1314**)
- Step 5: Of the gene probes selected in Step 3, identify a subset that shows a strong pattern of coexpression extrapolation between the discovery/validation bladder cancer cohorts and our new study cohort (**S1314**).
- Step 6: Use a multivariate algorithm to predict single drug and combination drug activity in **S1314** samples on the basis of the drugs' activity patterns in the discovery/validation bladder cancer cohort and the gene probe subset selected in step 5. The output of the multivariate analysis is a COXEN score.

Single drug COXEN scores (S_i) can be calculated by solving the linear regression model:

$$S_i = \alpha + \sum_{k=1}^{K} \beta_k X_{ki}$$

Which is to be interpreted as the single drug score (S_i) = model intercept (α) + summation of probe gene expression data (X_{ki}) multiplied by corresponding coefficients (β_k).

The COXEN score for combination drugs is a probability score based on 5-year survival and is derived from a parametric survival regression model of single drug COXEN scores. Let M_i, V_i, A_i, C_i, and G_i denote the percentile of COXEN drug response prediction score of i-th patient for each Methotrexate, Vinblastine, Adriamycin, Cisplatin, and Gemcitabine, respectively. Then the COXEN scores P_{MVAC} and P_{GC} of i-th patient for MVAC and GC are calculated as below.

$$P_{GC} = e^{-(5\lambda_{GC})^{0.8961928}} \qquad P_{MVAC} = e^{-(5\lambda_{MVAC})^{1.091486}}$$

Where

$$\lambda_{MVAC} = e^{-(0.761211+(0.766369\times M_i)+(0.3657074\times V_i)+(0.7634502\times A_i)+(0.8308788\times C_i))}$$

$$\lambda_{GC} = e^{-(-0.2456275+(2.8852677\times G_i)+(1.6766004\times C_i))}$$

Isolation of *up*CTCs, mRNA recovery and amplification from *up*CTCs, and processing of Affymetrix arrays will be done at USC Norris (Goldkorn Lab and CHLA Genomics Core). The array data then will be analyzed collaboratively with the laboratory of Dr. Daniel Gustafson (see Gustafson letter of support) and with Dr. Cathy Tangen at SWOG Statistics and Data Management Center (see SWOG letter of support) using the algorithms described above. The *up*CTC-derived single drug and combination drug COXEN scores will be analyzed for association with chemotherapy response (T0) and with tumor-derived COXEN gene expression, as well as for changes before and after chemotherapy.

CTC REFERENCES

1 Ochoa A, Choi W, Su X, et al. Specific micro-RNA expression patterns distinguish the basal and luminal subtypes of muscle-invasive bladder cancer. (Oncotarget, in press)

2 Siegel R, Ma J, Zou Z, et al. Cancer statistics 2014. CA Cancer J Clin 64(1):9-29, 2014.

3 Stenzl A, Cowan NC, De Santis M, et al. The updated EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol 55(4):815-25, 2009.

4 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(9):859-66, 2003.

5 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

6 Smith SC, Baras AS, Lee JK, et al. The COXEN principle: translating signatures of in vitro chemosensitivity into tools for clinical outcome prediction and drug discovery in cancer. Cancer Res 70:1753-8, 2010.

7 Takata R, Katagiri T, Kanehira M, et al. Predicting response to methotrexate, vinblastine, doxorubicin, and cisplatin neoadjuvant chemotherapy for bladder cancers through genome-wide gene expression profiling. Clin Cancer Res 11:2625-36, 2005.

8 Smith S, Baras A, Dancik G, et al. A 20-gene model for molecular nodal staging of bladder cancer: development and prospective assessment. Lancet Oncol 12:137-43, 2011.

9 Hu B, Fish H and Goldkorn A. Circulating tumor cells in prostate cancer. Cancers 5(4):1676-90, 2013.

10 Xu T, Lu B, Tai YC and Goldkorn A. A cancer detection platform which measures telomerase activity from live circulating tumor cells captured on microfilter. Cancer Research 70(16): 6420-6426, 2010.

11 Goldkorn A, Ely B, Quinn DI, Tangen CM, et al. Circulating tumor cell (CTC) counts are prognostic of overall survival (OS) in SWOG S0421-docetaxel with or without atrasentan for metastatic castration resistant prostate cancer (mCRPC). J Clinical Oncology 32(11):1136-42, 2014.

12 Goldkorn A, Ely B, Tangen CM, Tai YC, et al. Circulating tumor cell telomerase activity as a prognostic marker for overall survival in metastatic castration resistant prostate cancer: results from SWOG 0421. Int J of Cancer 2014 [Epub Sep 2014]

13 Hodgkinson CL, Morrow CJ, Li Y, et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nature Medicine 20:897-903, 2014.

14 Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. Cancer Res 69:8302-9, 2009.

15 Smith SC, Baras AS, Lee JK, et al. The COXEN principle: translating signatures of in vitro chemosensitivity into tools for clinical outcome prediction and drug discovery in cancer. Cancer Res 70:1753-8, 2010.

16 Takata R, Katagiri T, Kanehira M, et al. Predicting response to methotrexate, vinblastine, doxorubicin, and cisplatin neoadjuvant chemotherapy for bladder cancers through genome-wide gene expression profiling. Clin Cancer Res 11:2625-36, 2005.

17 Smith S, Baras A, Dancik G, et al. A 20-gene model for molecular nodal staging of bladder cancer: development and prospective assessment. Lancet Oncol 12:137-43, 2011.

18.3c **Cisplatin Germline Pharmacogenomics**

Objectives

1. To assess the association of single nucleotide polymorphisms (SNP), selected for cisplatin sensitivity, and complete pathologic response (pT0) in muscle invasive bladder cancer after cisplatin-based neoadjuvant chemotherapy.

2. To assess the association of single nucleotide polymorphisms (SNP), selected for cisplatin sensitivity, and pathologic response (downstaging to <pT2) in muscle invasive bladder cancer after cisplatin-based neoadjuvant chemotherapy.

3. To evaluate these SNP associations, individually and in combination, with RFS and OS.

4. To perform exploratory genome-wide analysis for platinum susceptibility germline polymorphisms associating with pathologic response after neoadjuvant cisplatin-based combination chemotherapy.

5. To explore whether candidate SNPs are associated with chemotherapy-related adverse events in the neoadjuvant setting.

Primary endpoint

Pathologic stage at cystectomy

Secondary endpoints

Recurrence-Free Survival
Overall Survival

Genomic DNA will be extracted using standard protocols by Nationwide (SWOG Biorepository). All DNA samples will be stored until batched genotyping is conducted. Genotyping for the pre-defined SNPs of interest will be performed using commercially available Taqman assays. Secondary genome-wide association analysis will be conducted using the Illumina OmniExpress array (although the investigators reserve the right to use an updated platform if one is available and technically more advantageous).

Background

Cisplatin-based chemotherapy represents the only available standard therapy for locally advanced and metastatic urothelial cancer. Despite the fact that cisplatin-based therapy improves survival in these settings, only 38% of bladder cancer patients achieve a complete pathologic response to cisplatin in the locally advanced setting, and only 50% respond in the metastatic setting. Prediction of which individuals will benefit is currently not possible, but the era of genomics offers a new avenue for this type of pursuit.

The pharmacogenomics of cisplatin has been an area of active interest, however most work has been conducted by searching for somatic alterations in the tumor that confer sensitivity to platinum-based therapy. Some somatic "candidate genes"—genes known to be involved in a drug's pharmacokinetic or pharmacodynamic pathways—have been recently identified (e.g., *ERCC2*, *ATM, RB1*, *FANCC*), but these are typically present only in a small subset of urothelial cancer patients. It is clear that these candidate genes do not entirely explain an individual's platinum susceptibility, meaning that chemotherapeutic sensitivity is likely a multigenic trait. Furthermore, both somatic and germline alterations are likely important, and both probably act in a complementary fashion to determine clinical sensitivity.

Dr. O'Donnell and colleagues previously refined a genome-wide approach that permits the identification of multigenic chemotherapy susceptibility determinants based upon germline, rather than tumor DNA. Using an in vitro model which employs well-genotyped lymphoblastoid cell lines (LCLs) from hundreds of healthy individuals in the International HapMap Project, we treated LCLs with cisplatin to produce "sensitivity phenotypes". Then, genome-wide association (GWAS) was performed to associate specific single nucleotide polymorphisms (SNPs). We identified a number of novel SNPs associated with cisplatin sensitivity in these pre-clinical models. Others have recently identified novel cisplatin-associated SNPs in other cancers (e.g., lung, ovarian). These SNPs represent novel genetic determinants of cisplatin sensitivity, identified across multiple genes or in regions of the genome not previously implicated. We believe that such determinants, if validated, could permit identification of individuals most likely to respond to cisplatin-based chemotherapy.

Statistical Plan

In the primary analysis, we will test 10 candidate SNPs of interest in an attempt to replicate these as platinum susceptibility markers for urothelial cancer. These SNPs have been selected (a) based upon their identification through our above-described cell-based model of platinum chemosensitivity, (b) based upon published demonstration of their potential utility in other cancer types treated with platinums (lung, ovarian, head and neck), and (c) based upon candidates emerging from an ongoing multi-institutional study of germline platinum susceptibility markers currently being conducted by the PI (O'Donnell) in collaboration with colleagues at MSKCC, Fox Chase Cancer Center, and supported through the Alliance for Clinical Trials in Oncology cooperative group.

For assessment of relationship with pathologic response (both pT0 and <pT2), logistic regression will be conducted after adjusting for the stratification factors from the trial (T2 vs. T3, T4a and PS 0 vs. 1), and treatment arm. To increase power, we will evaluate whether an ordinal outcome (pT0 vs. not pT0 but <pT2 vs. no response) could be used to evaluate the association with candidate SNPs.

Best parameterization of SNPs will be evaluated (i.e. co-dominant, recessive, log-additive), but our pre-specified primary analysis will code SNP (Y vs. N). Only genotypes from patients self-identified as Caucasian will be included in the primary (first-pass) analysis to avoid confounding by population stratification. Evaluation for population stratification will be conducted using a combination of self-reported race and ethnicity to serve as a reasonable surrogate to identify genetic ancestry. All SNPs will be evaluated for deviation from Hardy-Weinberg.

The following table assumes that there is usable DNA from 167 eligible and evaluable patients, and it shows the minimally detectable odds ratio for each respective response outcome that can be evaluated with 80% statistical power and a two-sided type I error rate of 0.05. False discovery rate (FDR) will be used to control the average false positive proportions among selected measures and will be implemented for actual reporting of results. Specifically, we will use methods proposed by Benjamini and Hochberg.

There will be minimal statistical power to detect associations of SNPs or mutations with pathologic response when the prevalence of the SNP is low.

Minimally detectable odds ratio for Associations of SNPs with Pathologic Response (assuming 80% statistical power, 2-sided α= 0.05)

Prevalence of SNP in the non-response group

	5%	10%	20%
pT0	4.30	3.10	2.59

<pT2	4.52	3.19	2.55

We anticipate a 33% pathologic complete response rate (pT0) and 52% will have <pT2 path response. If the SNP prevalence is in the range of 20%, then we will have good power to detect odds ratios in the range of 2.5 for pathologic response (pT0 or <pT2).

Cox regression will be used to evaluate associations of SNPs with relapse-free survival and overall survival. With three years of accrual and three additional years of follow-up and median survival of approximately five years, we expect 85 total deaths in both arms to occur on this trial and so power will be low to evaluate moderate sized marker/OS and marker/RFS hazard ratios. For example, splitting a continuous marker at the median and evaluating the association of low vs. high marker status with survival, there will be 88% power to detect a hazard ratio of 2.0 which would be equivalent to detecting a median OS of 3.5 years versus 7.0 years.

For a single SNP that is found to be significantly associated with outcome, we will report the sensitivity and specificity for each factor predicting pathologic response, and RFS and OS status at a specific landmark. For combinations of factors, a logistic regression model will be used to estimate a risk algorithm and the resulting risk score will be used as the prognostic marker. For the modeling of pathologic complete response, we expect approximately 70 pathologic complete responders (of the 167 eligible patients. That would allow roughly 7 predictors in a multivariable logistic regression model.

It is acknowledged that RFS and OS clinical data will likely be available at a future time point (allowing for data maturation), while pathologic staging will be available immediately. Thus, the analysis of the primary endpoint will occur first, with the secondary analyses performed in a second stage at the time(s) when RFS and OS data are released.

Finally, we may use the DNA received to consider other candidate SNPs and/or to conduct a GWAS to investigate other novel candidates. Analyses to identify germline SNPs that associate with clinically important chemotherapy-related adverse events (e.g., vascular thrombotic events [VTEs], nephrotoxicity) will also be conducted, with the opportunity to validate VTE-associated SNPs that will be discovered in the above-mentioned ongoing Alliance project. As next generation sequencing platforms become more cost effective, we will also consider exome or whole-genome sequencing.

Data analysis performed by:
SWOG statistical group.

Specimen Information:

Who will be performing testing?
University of Chicago Genomics Facility (https://fgf.uchicago.edu/)

What types of specimen(s) are to be utilized?
Germline DNA extracted from peripheral blood sample collected as part of the primary study

What amounts of specimen(s) are to be utilized?
Germline DNA at a concentration no lower than 10ng/uL, and a sample volume no less than 40uL.

At what time points during treatment are specimens being utilized from?
One time sample only, obtained anytime during the study

Will any processing of specimens be required by the repository prior to shipment?
n/a

Specify shipping instructions (e.g. room temp, wet or dry ice)
Ship on dry ice for best quality;
Attn: Peter H. O'Donnell M.D./Peter W. Faber Ph.D.
Genomics Facility
Knapp Center for Biomedical Discovery
900 E. 57th St.
Room 1230C
Chicago, IL 60637

Expected timeline of Project completion:
Within 1 year of project completion for the primary endpoint.
*As noted above, the secondary analyses will be dependent on the timing of the availability/release of RFS and OS clinical data.

Disclosure of conflict of interest:
No relevant conflicts of interest

Other considerations (including plan for financial support, grant submission, etc.):
PI (O'Donnell) has internal funding from a University of Chicago Comprehensive Cancer Center Auxiliary Board Award

Pharmacogenomics References:

O'Donnell PH. Bladder cancer pharmacogenomics: recent insights and future perspectives. Pharmacogenomics. 13(14):1553-6, 2012.

O'Donnell PH et al. Clinical Evaluation of Cisplatin Sensitivity of Germline Polymorphisms in Neoadjuvant Chemotherapy for Urothelial Cancer. Clin Genitourin Cancer. 14(6):511-17, 2016.

Chang SS…O'Donnell PH et al., 12th Annual meeting of the Society of Urologic Oncology (SUO) bladder cancer sessions I and II summary report. Urol Oncol. 30(6):944-7, 2012.

Huang RS et al., Platinum sensitivity-related germline polymorphism discovered via a cell-based approach and analysis of its association with outcome in ovarian cancer patients. Clin Cancer Res, 2011.

Wu C et al., Genome-wide interrogation identifies *YAP1* variants associated with survival of small-cell lung cancer patients. Cancer Res, 2010.

18.3d **DNA Damage Response Gene Alterations**

Objectives

1. **Evaluate whether DNA damage response (DDR) gene alterations are associated with response to cisplatin-based neoadjuvant chemotherapy (NAC). We will perform this analysis using two different methodologies:**

   *Correlate deleterious alterations within 9 DDR genes (*ERCC2*,* ERCC5*,* ATM*,* ATR*,* FANCC*,* BRCA1*,* BRCA2*,* RECQL4*, and* RAD51C*) with pathologic downstaging to <pT2N0 after cisplatin-based neoadjuvant chemotherapy (NAC) using the CLIA-certified Next Generation Sequencing (NGS) targeted sequencing panel MSK-IMPACT (Integrated Molecular Profiling of Actionable Cancer Targets).*

   Correlate any alterations in ATM*,* RB1*,* FANCC *or* ERCC2 *with pathologic downstaging to pT0N0 or <pT2N0 after cisplatin-based neoadjuvant chemotherapy (NAC) using the CLIA-certified 592 Gene Next Gen Sequencing panel from Caris Life Sciences (Caris 592).*

2. **Assess whether the presence of one or more mutations as defined in the primary objective is associated with overall survival and metastasis free survival.**

3. **To evaluate whether the mutation – pathologic response rate association varies by chemotherapy treatment arm.**

   Primary endpoint: Pathologic stage at cystectomy using two cutoffs as specified in Section 10 definitions of the S1314 protocol. (1) <pT2N0 vs ≥pT2 or N+ (2) pT0N0 Secondary endpoints: Overall survival, metastasis free survival.

   MSK-IMPACT: DNA will be extracted from SWOG 1314 pre-treatment tumor and germline specimens for genomic DNA sequencing using MSK-IMPACT, a 468-gene exon capture next-generation sequencing (NGS) panel, to determine the association of deleterious alterations within 9 pre-selected genes with pathologic down-staging. Deleterious mutations within this subset of 9 DDR genes will also be evaluated for an association with long-term clinical outcomes, including overall survival and 2-year metastasis-free survival.

   We will perform whole exome sequencing of pre-treatment tumor and germline DNA from patients who exhibit pathologic down-staging at radical cystectomy but whose tumors have no deleterious alterations within the 9 DDR genes detected by MSK-IMPACT to identify novel somatic and/or germline alterations predictive of chemosensitivity. We will subsequently use these results to interrogate the tumors of non-responders in a targeted fashion to confirm that these alterations are enriched in the DDR wild-type responders.

   In addition, we hope to work with the SWOG investigative team to perform an integrated analysis of the identified genomic mutations in conjunction with mRNA and miRNA expression subtypes already planned or proposed.

   Caris 592 Gene Next Gen Sequencing panel (Caris 592):

   Direct sequence analysis is performed on tumor derived DNA. An Agilent custom designed SureSelect XT assay is used to enrich 592 whole-gene targets. The genes and amino acids evaluated in this report can be found at www.carislifesciences.com. All variants reported by this assay are detected with > 99% confidence based on the frequency of the mutation present and the amplicon coverage. This test is not designed to distinguish between germ line inheritance of a variant or acquired somatic mutation. This test has a sensitivity to detect as low as approximately 10 % population of cells containing a mutation with an

analytic sensitivity of 96.9% to detect variants with frequency greater than 5%. This may not detect insertion/deletions events that are larger than 44 bases. This test has not been cleared or approved by the United States Food and Drug Administration (FDA) as such approval is not necessary. All performance characteristics were determined by Caris Life Sciences. Benign and non-coding variants are not included in this report but are available upon request. The versioned reference identifier used for the transcript ID was Feb.2009 (GRCh37/hg19). Total mutational load is calculated using only missense mutations that have not been previously reported as germline alterations. A high mutational load is a potential indicator of immunotherapy response (Le et al., NEJM, 2015; Rizvi et al., Science, 2015; Rosenberg et al., Lancet, 2016; Snyder et al., NEJM, 2014). Microsatellite instability status by NGS (MSI-NGS) is measured by the direct analysis of known microsatellite regions sequenced in the NGS panel. To establish clinical thresholds, MSI-NGS results were compared with results from over 2,000 matching clinical cases analyzed with traditional, PCR-based methods. Genomic variants in the microsatellite loci are detected using the same depth and frequency criteria as used for mutation detection. Only insertions and deletions resulting in a change in the number of tandem repeats are considered in this assay. Some microsatellite regions with known polymorphisms or technical sequencing issues are excluded from the analysis. The total number of microsatellite alterations in each sample are counted and grouped into three categories: High, Equivocal and Stable. MSI-Low results are reported in the Stable category. Equivocal results have a total number of microsatellite alterations in between High and Stable.

Background

Cisplatin-based NAC followed by radical cystectomy (RC) is a standard of care for the curative-intent treatment of MIBC. Down-staging to <pT2 (non-muscle invasive disease) is associated with > 85% survival at 5 years. However, prospective identification of those patients who derive maximal benefit from NAC remains elusive. Published findings by our groups reveal two separate but related mutational patterns predictive of response and survival.

4. **Deleterious alterations in DDR genes identified by whole exome sequencing (WES) and MSK-IMPACT**

    a. Deleterious alterations in DDR genes, most commonly the nucleotide excision repair (NER) gene ERCC2, are present in 23-39% of bladder cancers. (1,2,3)

    b. DDR gene mutations are associated with pathologic down-staging and improved survival in MIBC patients treated with cisplatin-based NAC. (4,5,6)

    c. *ATM/RB1/FANCC/ERCC2*

    d. In the TCGA the rate of ERCC2 mutations was 10%, and the rate of one or more ATM/RB1/FANCC mutations was 38%. Therefore, we estimate that at least 38% of patients in the COXEN study will have one or more of these mutations. (7)

    e. These four specific DDR gene mutations are associated with pathologic down-staging and improved survival in MIBC patients treated with cisplatin-based NAC in both discovery and validation cohorts. (8,9,10)

On the basis of these preliminary data and observations, we hypothesize that two separate but related genomic mutational patterns (1) deleterious DDR gene alterations within 9 genes (MSK-IMPACT) and (2) *ATM/RB1/FANCC/ERCC2* alterations (Caris 592) are

associated with pathologic down-staging after NAC, and that these alterations can identify patients with deep, durable outcomes. We propose to test this hypothesis using pre-treatment samples from SWOG **S1314**. Pre-treatment tumor and germline DNA are required for analysis. This prospective trial provides an ideal opportunity to rigorously validate the association of these mutational patterns with pathologic down-staging in a retrospective-prospective fashion, and also to identify new genomic mediators of NAC sensitivity in those patients without the presence of these gene mutations, since 50-60% of patients who demonstrate down-staging after NAC lack somatic alterations in canonical DDR pathway genes. *(11,12,13)* We will also compare the rates of mutations by treatment arm to delineate differences in frequency and association with response that may be attributable to chemotherapy regimen.

5. **Rationale for separate testing using MSK-IMPACT and Caris 592**

We are requesting separate aliquots of DNA to test two genomic profiling platforms: MSK-IMPACT and Caris 592. While both methods allow for detection of genomic alterations, the methods used, results expected, and clinical access differ between platforms.

MSK-IMPACT is a CLIA certified custom hybridization-capture based assay that sequences all exons and select introns within 468 commonly altered genes across cancer types. The assay utilizes tumor and matched normal sample pairs to interrogate the somatic (tumor-specific) genomic profile to detect single nucleotide variants, short insertions and deletions, copy number aberrations, and structural rearrangements. While the initial discovery that *ERCC2* alterations were predictive for chemo-sensitivity used a more unbiased approach (i.e., whole exome sequencing, WES), the MSK-IMPACT gene panel includes *ERCC2* and additional genes that comprise all critical DNA damage response pathways. Moreover, a targeted panel approach has the advantage of lower DNA input requirements (as low as 50-100 ng for MSK-IMPACT) and greater coverage (higher sensitivity) at reasonable cost compared to WES. The platform has successfully detected genomic alterations within DNA extracted from both frozen and paraffin-embedded tissue from a variety of specimen types, including biopsies, surgical resections, and cytology. The sequencing data from several published projects are available through the publicly accessible cBio portal, while access is provided to collaborators for ongoing projects that have yet to be published. Notably, this platform successfully identified deleterious DDR gene alterations within multiple DDR pathways in patients who responded to neoadjuvant dose dense gemcitabine and cisplatin in a multicenter phase II study and this finding serves as the basis for the 9 pre-defined genes that have been selected for further validation within this proposal (Iyer et al, JCO, in press). Moreover, MSK-IMPACT will be the centralized sequencing platform for a prospective cooperative group trial that will open to accrual in 2018 (A031701) in which patients with muscle-invasive bladder cancer will be molecularly stratified to organ preservation versus definitive local therapy based upon the presence or absence of deleterious alterations within 9 DDR genes within their pre-treatment TUR specimen, including *ERCC2*, *ERCC5*, *ATM*, *ATR*, *FANCC*, *BRCA1*, *BRCA2*, *RECQL4*, and *RAD51C.*

The Caris 592 test is also panel-based but uses a curation process to make calls without incorporating data from the germline. Though the Caris method is slightly different from the FoundationOne testing that was used to discover and validate the *ATM/RB1/FANCC* mutation pattern, it is overall similar in being agnostic to the germline. The Caris method has the distinct advantage over the FoundationOne method of including *ERCC2*, which based on our groups' collaborative efforts, we feel is critical to the accurate assessment of cisplatin sensitivity. When analyzing the samples from reference 2 below using the Broad WES method that, similar to

MSK-IMPACT, deletes germline mutations, the contributions of *RB1*, *ATM* and *FANCC* were lost. However, we have shown that using germline agnostic sequencing approaches, these alterations – most powerfully *RB1* and *ATM* – allow for greater sensitivity and predictive ability. Lastly, it is important to test for these mutations using a method that is both CLIA-certified and commercially available to practicing clinicians outside of MSKCC. The 4-gene panel (*ATM/RB1/FANCC/ERCC2*) is currently being used to assess predictive ability of this panel for complete response allowing for bladder preservation in an ongoing clinical trial at Fox Chase (NCT02710734). The disadvantage is that some aspects of the curation process are proprietary and thus not easily reproducible outside of Caris. Caris has agreed to share and allow for public deposit of all sequencing data/calls as part of this project.

6. **Statistical Considerations for DNA Damage Response gene alterations**

For genes that are common between the two platforms, we will descriptively compare the level of agreement (Yes vs. No) of alteration calls, but no formal test will be employed.

For each platform (MSK-IMPACT and Caris 592), we will conduct similar analytic strategies. Patients found with any alteration in the panel of pre-specified genes will be coded as alteration=Y, otherwise patients will be coded as N. Two by two tables will be constructed which cross-classify alteration with pathologic response (down-staging or pT0 status) vs. no response. Estimates of sensitivity and specificity along with 95% confidence intervals will be provided. Additionally, a logistic regression model will be fit with stratification factors placed in the model as covariates along with an indicator for alteration, and we will model the outcome of path response. The area under the curve will be estimated, and the ROC curve will be generated based on the risk score from the model. Comparisons of categorization will also be made for prediction of path response using the model with only strat factors with strat factors and an indicator for presence of alterations. The incremental improvement between the models will be attributed to the contribution of alteration information. Because the list of genes and methodology is pre-specified, we will employ a p-value of 0.05 as the significance level

In additional exploratory analyses we will examine individual gene alterations and downstaging and pT0 status combined and separately to see if some genes are driving the association with response. We will also evaluate whether having more than one alteration increases the odds of having a path response to platinum-based chemotherapy.

In secondary analyses, we will also adjust for treatment arm and evaluate an alteration by treatment arm interaction term to see if the alteration – path response association varies by treatment arm. The statistical power for this test will be very low, and results will focus on descriptive statistics.

We estimate that 160 patients from **S1314** will be eligible for this TM substudy by being eligible for the clinical trial, having TURBT tissue and blood available, received at least 3 cycles of neoadjuvant chemotherapy or progressed on chemo, and are assessable for pathologic response at time of cystectomy. This number also allows for a small fraction of assay failures. We further assume that the path response rate in those without an alteration is either 15% (top half of table) or 25% (lower half of table). We specify a two-sided alpha of 0.05 and report minimally detectable response rate differences that can be observed with at least 80% power. We are not adjusting for multiple testing because the finite number.

Minimally Detectable Odds Ratios for Gene Alteration and Pathologic Response (downgrading or pT0) n=160 Patients Gene Alteration (Y/N) Frequency				
15%	20%	30%	40%	
Assume 15% path response in those without alteration				
Path response difference (w/o vs. w/)	15% vs. 44%	15% vs. 41%	15% vs. 37%	15% vs. 36%
Assume 25% path response in those without alteration				
Path response difference (w/o vs. w/)	25% vs. 57%	25% vs. 53%	25% vs. 50%	25% vs. 48%

Based on prior MSK-IMPACT analyses that showed that 885 of patients bearing an alteration of interest had a pathologic response and only 15% response for those without an alteration, our sample of 160 patients should provide good power to detect response rates differences similar to those that have been observed previously.

Additional analyses looking at gene alteration and its association with metastasis-free survival and overall survival will use a Cox regression model adjusting for stratification factors, treatment arm and evaluating the hazard ratio for alteration status (Y/N). Patients will be censored at their last contact date. We anticipate these outcome data will be available 1-2 years after the pathologic response data have been analyzed. In order to evaluate sensitivity and specificity we will select a landmark timepoint for events occurring prior to 3 years (Y/N) and we will generate ROC curves and calculate the AUC.

Data analysis performed by:

MSK-IMPACT sequencing calls performed by MSKCC Center for Molecular Oncology and annotated by Dr. Gopa Iyer.
Caris 592 sequencing calls performed by CARIS per their commercial platform.
Raw data and calls made using both methods will be available to investigators within SWOG and publicly deposited as per journal requirements.

- Statistical analysis by S1314 SWOG Statistical Team

Specimen Information:

Who will be performing testing? (Please provide complete laboratory and contact information.)
Gopa Iyer, M.D.
Memorial Sloan Kettering Cancer Center
Mortimer B. Zuckerman Research Center (ZRC)
417 East 68th Street
8th floor—Room ZRC-827
New York, NY 10065
T: 646-888-2649 (Lab)
646-888-4737 (Office)

Caris Life Sciences
Translational Medicine Study Proposal Form (updated 3/14) p. 6 Attachment 1
4610 South 44th Place, Suite 100
Phoenix, Arizona 85040

What types of specimen(s) are to be utilized?

Peripheral blood will be utilized for germline DNA extraction and sequencing.
DNA from tumor tissue (previously extracted through SWOG) will be used for tumor tissue sequencing.

What amounts of specimen(s) are to be utilized?

DNA has already been extracted from the TURBT pre-treatment tumor samples.

- For MSK-IMPACT a minimum of 250 ng of tumor and 200 ng of germline DNA will be required.
- For Caris 592, 250 ng of tumor DNA is preferred. A minimum of 100 ng is required.

All DNA should be shipped in dry ice. At the end of the study, any remaining DNA will be returned to the SWOG biorepository.

At what time points during treatment are specimens being utilized from?

Only pre-treatment TURBT or biopsy and peripheral blood specimens are required.

Will any processing of specimens be required by the repository prior to shipment?

No

Specify shipping instructions
Blood should be shipped in dry ice.
Germline or somatic DNA should be shipped in dry ice.

Expected timeline of Project completion:
1 year

Disclosure of conflict of interest:

MSK patent for predicting platinum sensitivity
FCCC patent for predicting platinum sensitivity

DNA Damage Response Gene Alterations References

1 Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, et al. Somatic ERCC2 Mutations Correlate with Cisplatin Sensitivity in Muscle-Invasive Urothelial Carcinoma. Cancer Discov. 4(10):1140-53, 2014.

2 Plimack ER, Dunbrack RL, Brennan TA, Andrake MD, Zhou Y, Serebriiskii IG, et al. Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. Eur Urol. 68(6):959-67, 2015.

3 Liu D, Plimack ER, Hoffman-Censits J, Garraway LA, Bellmunt J, Van Allen E, et al. Clinical validation of chemotherapy response biomarker ERCC2 in muscle-invasive urothelial bladder carcinoma. JAMA oncology. 2(8):1094-6, 2016.

4 Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, et al. Somatic ERCC2 Mutations Correlate with Cisplatin Sensitivity in Muscle-Invasive Urothelial Carcinoma. Cancer Discov. 4(10):1140-53, 2014.

5 Plimack ER, Dunbrack RL, Brennan TA, Andrake MD, Zhou Y, Serebriiskii IG, et al. Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. Eur Urol. 68(6):959-67, 2015.

6 Liu D, Plimack ER, Hoffman-Censits J, Garraway LA, Bellmunt J, Van Allen E, et al. Clinical validation of chemotherapy response biomarker ERCC2 in muscle-invasive urothelial bladder carcinoma. JAMA oncology. 2(8):1094-6, 2016.

7 Lotan Y, Woldu SL, Sanli O, Black P, Milowsky MI. Modelling cost-effectiveness of a biomarker-based approach to neoadjuvant chemotherapy for muscle-invasive bladder cancer. BJU Int. Epub ahead of print (PMID: 29603871), 2018.

8 Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, et al. Somatic ERCC2 Mutations Correlate with Cisplatin Sensitivity in Muscle-Invasive Urothelial Carcinoma. Cancer Discov. 4(10):1140-53, 2014.

9 Plimack ER, Dunbrack RL, Brennan TA, Andrake MD, Zhou Y, Serebriiskii IG, et al. Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. Eur Urol. 68(6):959-67, 2015.

10 Liu D, Plimack ER, Hoffman-Censits J, Garraway LA, Bellmunt J, Van Allen E, et al. Clinical validation of chemotherapy response biomarker ERCC2 in muscle-invasive urothelial bladder carcinoma. JAMA oncology. 2(8):1094-6, 2016.

11 Van Allen EM, Mouw KW, Kim P, Iyer G, Wagle N, Al-Ahmadie H, et al. Somatic ERCC2 Mutations Correlate with Cisplatin Sensitivity in Muscle-Invasive Urothelial Carcinoma. Cancer Discov. 4(10):1140-53, 2014.

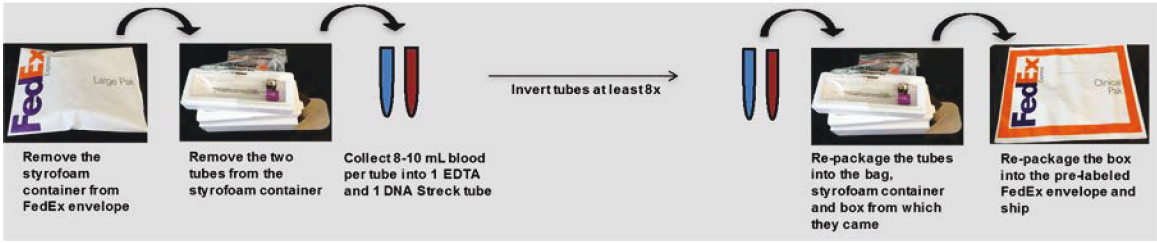
[12] Plimack ER, Dunbrack RL, Brennan TA, Andrake MD, Zhou Y, Serebriiskii IG, et al. Defects in DNA repair genes predict response to neoadjuvant cisplatin-based chemotherapy in muscle-invasive bladder cancer. Eur Urol. 68(6):959-67, 2015

13 Liu D, Plimack ER, Hoffman-Censits J, Garraway LA, Bellmunt J, Van Allen E, et al. Clinical validation of chemotherapy response biomarker ERCC2 in muscle-invasive urothelial bladder carcinoma. JAMA oncology. 2(8):1094-6, 2016.

18.4 Circulating Tumor Cell (CTC) Instructions for Blood Sample Processing, Packing and Shipping

For questions or to order kits, please contact (in order of preference)
Gareth Morrison: garethmo@usc.edu
Yucheng Xu: yuchengx@usc.edu
Tong Xu: tong.xu@med.usc.edu
Amir Goldkorn, MD: agoldkor@med.usc.edu
Telephone: 323.442.7722

Blood Collection



1. Remove the Styrofoam container from the FedEx packaging, and remove the tubes from the container and biohazard bag (retain these for re-packaging).

2. Collect 8-10 mL blood into each tube and label the tubes with subject ID and date.

3. Gently invert the tubes at least 8x.

4. Repackage the 2 tubes into the biohazard bag with absorbent sheet, then into the Styrofoam shipping container and back into its cardboard box.

5. Place the closed box into the pre-labeled FedEx Shipping Package and seal the package.

6. Call FedEx at 800-463-3339 for courier pick-up no later than 3PM Monday-Thursday. *Do not place the shipment in a drop box.*

Federal guidelines for the shipment of blood products (all materials provided in the kit):

1. The tube must be wrapped in an absorbent material.

2. The tube must then be placed in an AIRTIGHT container (like a re-sealable bag).

3. Pack the re-sealable bag and tube in a Styrofoam shipping container.

4. Pack the Styrofoam shipping container in a cardboard box.

5. Mark the box "Biohazard".

Informed Consent Model for **S1314**

*NOTES FOR LOCAL INSTITUTION INFORMED CONSENT AUTHORS:

This model informed consent form has been reviewed by the DCTD/NCI and is the official consent document for this study. Local IRB changes to this document are allowed. (Institutions should attempt to use sections of this document that are in bold type in their entirety.) Editorial changes to these sections may be made as long as they do not change information or intent. If the institutional IRB insists on making deletions or more substantive modifications to the risks or alternatives sections, they may be justified in writing by the investigator and approved by the IRB. Under these circumstances, the revised language, justification and a copy of the IRB minutes must be forwarded to the SWOG Operations Office for approval before a patient may be registered to this study.

Please particularly note that the questions related to banking of specimens for future study are in bolded type and may not be changed in any way without prior approval from the SWOG Operations Office.

Readability Statistics:	
Flesch Reading Ease	61.7 (targeted above 55)
Flesch-Kincaid Grade Level	8.6 (targeted below 8.5)

- Instructions and examples for informed consent authors are in *[italics]*.
- A blank line, _____, indicates that the local investigator should provide the appropriate information before the document is reviewed with the prospective research participant.
- The term "study doctor" has been used throughout the model because the local investigator for a cancer treatment trial is a physician. If this model is used for a trial in which the local investigator is not a physician, another appropriate term should be used instead of "study doctor".
- The dates of protocol updates in the header and in the text of the consent is for reference to this model only and should not be included in the informed consent form given to the prospective research participant.
- The local informed consent must state which parties may inspect the research records. This includes the NCI, the drug manufacturer for investigational studies, any companies or grantors that are providing study support (these will be listed in the protocol's model informed consent form) and SWOG.

  "SWOG" must be listed as one of the parties that may inspect the research records in all protocol consent forms for which patient registration is being credited to SWOG. This includes consent forms for studies where all patients are registered directly through the SWOG Data Operations Office, all intergroup studies for which the registration is being credited to SWOG (whether the registration is through the SWOG Data Operations Office or directly through the other group), as well as consent forms for studies where patients are registered via CTSU and the registration is credited to SWOG.

- When changes to the protocol require revision of the informed consent document, the IRB should have a system that identifies the revised consent document, in order to preclude continued use of the older version and to identify file copies. An appropriate method to identify the current version of the consent is for the IRB to stamp the final copy of the consent document with the approval date. The stamped consent document is then photocopied for use. Other systems of identifying the current version of the consent such as adding a version or approval date are allowed as long as it is possible to determine during an audit that the patient signed the most current version of the consent form.

*NOTES FOR LOCAL INVESTIGATORS:

- The goal of the informed consent process is to provide people with sufficient information for making informed choices. The informed consent form provides a summary of the clinical study and the individual's rights as a research participant. It serves as a starting point for the necessary exchange of information between the investigator and potential research participant. This model for the informed consent form is only one part of the larger process of informed consent. For more information about informed consent, review the "Recommendations for the Development of Informed Consent Documents for Cancer Clinical Trials" prepared by the Comprehensive Working Group on Informed Consent in Cancer Clinical Trials for the National Cancer Institute. The Web site address for this document is http://cancer.gov/clinicaltrials/understanding/simplification-of-informed-consent-docs/
- A blank line, _____, indicates that the local investigator should provide the appropriate information before the document is reviewed with the prospective research participant.
- Suggestion for Local Investigators: An NCI pamphlet explaining clinical trials is available for your patients. The pamphlet is titled: "Taking Part in Cancer Treatment Research Studies". This pamphlet may be ordered on the NCI Web site at *https://cissecure.nci.nih.gov/ncipubs* or call 1-800-4- CANCER (1-800-422-6237) to request a free copy.
- Optional feature for Local Investigators: Reference and attach drug sheets, pharmaceutical information for the public, or other material on risks. Check with your local IRB regarding review of additional materials.

*These notes for authors and investigators are instructional and should not be included in the informed consent form given to the prospective research participant.

Study Title for Study Participants: Testing the use of the co-expression extrapolation (COXEN) program to predict response to usual chemotherapy in patients with localized, muscle invasive bladder cancer

Official Study Title for Internet Search on http://www.ClinicalTrials.gov: S1314, "A Randomized Phase II Study of Co-Expression Extrapolation (COXEN) With Neoadjuvant Chemotherapy for Localized, Muscle-Invasive Bladder Cancer"

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

What is the usual approach to my muscle-invasive bladder cancer? *(updated 5/22/15)*

You are being asked to take part in this study because you have muscle invasive bladder cancer. People who are not in a study are usually treated with neoadjuvant therapy, which involves a combination of chemotherapy drugs followed by surgery. Radiation therapy with chemotherapy or surgery alone are other treatment options. In this study, tests on your bladder biopsy sample will be used to see if it can predict your response to chemotherapy. You will be randomized between a combination of gemcitabine, cisplatin, or a different combination of methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim) which will be used before surgery. These are both standard treatment combinations for bladder cancer and may be given even if you are not in this study.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- **you may choose to have the usual approach described above without being in a study**
- **you may choose to take part in a different study, if one is available**
- **or you may choose not to be treated for cancer but you may want to receive comfort care, also called palliative care, to relieve symptoms. This type of care helps reduce pain, tiredness, appetite problems, and other problems caused by the cancer. It does not treat the cancer directly, but instead tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.**

Why is this study being done?

The primary focus of this study is to see if looking at tumor biomarkers using a program called "coexpression extrapolation or COXEN" may predict your response to chemotherapy before surgery. The COXEN program will not select your therapy, but the type of chemotherapy that you receive will be randomly decided. Information will be gathered and after you complete your participation in the study, researchers will look at this information and outcomes for all study participants to see how COXEN relates to how participants responded to chemotherapy. Other potential tests to predict a patient's response to chemotherapy will also be evaluated. In this study, you will receive the treatment in Arm 1 (gemcitabine and cisplatin) or the treatment in Arm 2 [methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim). There will be about 230 people taking part in this study (115 in each arm).

What are the study groups?

A computer will randomly put you in either of two treatment groups (Arm 1 or Arm 2). This is done because no one knows if one type of chemotherapy is better, the same, or worse than the other type of chemotherapy. Once you are put in one group, you cannot switch to the other group. Neither you nor your doctor can choose which group you will be in.

Patients in Arm 1 will receive the combination chemotherapy gemcitabine, cisplatin, and possibly filgrastim (or pegfilgrastim). All of the drugs will be delivered into your vein with the exception of filgrastim (or pegfilgrastim) which will be given as a shot in the fatty layer of tissue just under the skin. On Day 1, you will receive gemcitabine and cisplatin. On Day 8, you will only receive the gemcitabine, possibly followed by a single dose of pegfilgrastim 24-48 hours after the completion of chemotherapy for each cycle or alternatively filgrastim on Days 9-13. In some cases, you will not receive either filgrastim or pegfilgrastim based on the assessment of your treating medical provider. The drugs will be given to you again every 21 days for a total of 4 cycles. (Each 21-day period is called a "cycle".) You will have a physical exam and blood tests done at the beginning of each cycle. A CT scan or MRI (of the abdomen and pelvis) and a CT scan or x-ray (of the chest) will be done after 4 cycles of treatment.

Patients in Arm 2 will receive the combination chemotherapy methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim). All of the drugs will be delivered into your vein with the exception of filgrastim (or pegfilgrastim) which will be given as a shot in the fatty later of tissue just under the skin. On Day 1, you will receive methotrexate. On Day 1 (or Day 2) you will receive the vinblastine, doxorubicin, and cisplatin. On Days 3-7 (or Days 2-6, if all the chemotherapy was given on Day 1), you will receive the filgrastim; alternatively, you may receive a single dose of pegfilgrastim 24-48 hours after the completion of chemotherapy for each cycle. The drugs will be given to you again every 14 days for a total of 4 cycles. (Each 14-day period is called a "cycle".) You will have a physical exam and blood tests done at the beginning of each cycle. A CT scan or MRI (of the abdomen and pelvis) and a CT scan or x-ray (of the chest) will be done after 4 cycles of treatment.

Patients in both arms will undergo a standard lymph node and bladder removal within 70 days after completing chemotherapy.

How long will I be in this study?

You will receive the study drugs for 4 cycles of treatment or as long as your disease does not get worse and the side effects are not too severe. After you finish the study drugs, your doctor will continue to watch you for side effects and follow your condition for up to five years.

What extra tests and procedures will I have if I take part in this study?

Most of the exams, tests, and procedures you will have are part of the usual approach for your cancer. However, there are some extra exams and tests that you will need to have if you take part in this study.

Small pieces of cancer tissue removed by a previous surgery or biopsy will be collected before you begin the study and tested using the COXEN program. In addition, a small amount of your blood (about 3 teaspoons) and urine (about 8 teaspoons) will be collected before you begin the study for study related tests. Prior to surgery, an additional urine collection (about 8 teaspoons) will be done and saved for additional study. If any cancer remains at the time of surgery, a small sample of cancer tissue will be taken for the study as well. All of these specimens will be sent to a central laboratory for testing. These samples are required in order for you to take part in this study because the research on the samples will be analyzed to look for biologic markers to learn about bladder cancer and also to study ways to predict who will respond to chemotherapy.

Neither you nor your health care plan/insurance carrier will be billed for the collection of the samples that will be used for this study. You and your study doctor will not receive the results of any tests done on your samples.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

- **Lose time at work or home and spend more time in the hospital or doctor's office than usual**
- **Be asked sensitive or private questions which you normally do not discuss**

The drugs used in this study may affect how different parts of your body work such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects.

Here are important points about side effects:

- **The study doctors do not know who will or will not have side effects.**

- **Some side effects may go away soon, some may last a long time, or some may never go away.**
- **Some side effects may interfere with your ability to have children.**
- **Some side effects may be serious and may even result in death.**

Here are important points about how you and the study doctor can make side effects less of a problem:

- **Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.**
- **The study doctor may be able to treat some side effects.**
- **The study doctor may adjust the study drugs to try to reduce side effects.**

The tables below show the most common and the most serious side effects that researchers know about. There might be other side effects that researchers do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Arm 1 - Possible side effects of gemcitabine, cisplatin, and pegfilgrastim may include the following:

COMMON, SOME MAY BE SERIOUS More than 20 out of 100 people receiving gemcitabine, cisplatin, and pegfilgrastim may have:
• **Low white blood cell counts (may make you more likely to get an infection)** • **Low red blood cell counts (may make you feel tired or weak)** • **Low platelet counts (may make you more likely to bruise or bleed)** • **Nausea** • **Vomiting** • **Loss of appetite** • **Diarrhea** • **Fatigue** • **Blood measurements of kidney function (creatinine) and normal elements in the blood including blood sugar (glucose), potassium, magnesium, calcium, and sodium may become abnormal** • **Lightheadedness** • **Headaches** • **Changes in blood pressure** • **Skin irritation at site of drug injection** • **Damage to kidneys** • **Damage to hearing** • **Rash** • **Flu-like illness with fever on day of chemotherapy** • **Electrolytes, which are normal elements measured in the blood, including potassium, magnesium, and sodium may become low and possibly require replacement** • **Bone pain**

OCCASIONAL, SOME MAY BE SERIOUS About 4 to 20 out of 100 people receiving gemcitabine, cisplatin, and pegfilgrastim may have:
• **Sores in mouth and/or throat** • **Alterations in taste** • **Allergic reaction (including flushing, skin rash, changes in blood pressure and/or difficulty breathing)** • **Stomach cramps** • **Hair loss** • **Numbness in the hands and feet** • **Loss of blood supply to the intestines that may require surgery** • **Inflamed pancreas** • **Dizziness and shooting back pain when bending your neck forward** • **Confusion** • **Blurred vision or a sensation of flashing light** • **Mood changes** • **Liver damage and/or failure** • **Seizures** • **Fainting** • **Irregular heartbeat** • **Heart attack** • **Ringing in the ears** • **High frequency hearing loss – This can be permanent**

RARE, AND SERIOUS About 3 or fewer out of 100 people receiving gemcitabine, cisplatin, and pegfilgrastim may have:
• **Cancer of the bone marrow caused by chemotherapy later in life.** • **Seizure**

Arm 2 - Possible side effects of methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim) may include the following:

COMMON, SOME MAY BE SERIOUS More than 20 out of 100 people receiving methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim) may have:
• **Low number of red blood cells (anemia) - This can lead to shortness of breath, weakness, and fatigue.** • **Low number of platelets which help the blood clot – This can lead to unexplained bruising or bleeding.** • **Low number of white blood cells – This can make you more vulnerable to infection.** • **Hair loss** • **Nausea** • **Vomiting** • **Skin irritation at site of drug injection which can be severe** • **Damage to kidneys** • **Damage to hearing** • **Changes in urine color (doxorubicin may discolor your urine for up to 1-2 days but this is harmless)** • **Soreness or painful ulcers of the mouth lasting a couple of days** • **Sensitivity to the sun** • **Blood measurements of kidney function (creatinine) and normal elements in the blood including blood sugar (glucose), potassium, magnesium, calcium, and sodium may become abnormal** • **Electrolytes, which are normal elements measured in the blood, including potassium, magnesium, and sodium may become low and possibly require replacement** • **Sores in mouth and/or throat** • **Bone pain**

OCCASIONAL, SOME MAY BE SERIOUS About 4 to 20 out of 100 people receiving methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim) may have:
• **Allergic reaction** • **Upset bowels – this can lead to either constipation or diarrhea** • **Loss of appetite** • **Weight loss** • **Strange taste - A metallic or bitter taste can occur during treatment. A flavored hard candy or mint will help to disguise this taste.** • **Flu-like symptoms (such as fever, aches, pains, and shivering)** • **Tingling and numbness in the fingers or toes** • **Ringing in the ears** • **High frequency hearing loss – This can be permanent** • **Skin and nail changes** • **Rash or irritation at the injection site** • **Weakness of the heart – This is uncommon and can happen with increasing doses of doxorubicin.**

RARE, AND SERIOUS About 3 or fewer out of 100 people receiving methotrexate, vinblastine, doxorubicin, cisplatin, and filgrastim (or pegfilgrastim) may have:
• **Sore eyes** • **Depression** • **Headaches** • **Jaw pain** • **Seizure** • **Cancer of the bone marrow caused by chemotherapy later in life.** • **Death – In rare cases, death can occur in patients with a severe allergic reaction to the chemotherapy or in patients who develop a life-threatening infection.**

Let your study doctor know of any questions you have about possible side effects. You can ask the study doctor questions about side effects at any time.

Reproductive risks: You should not get pregnant, breastfeed, or father a baby while in this study. The drugs used in this study could be very damaging to an unborn baby. Birth control measures should be continued for at least 6 months after the completion of chemotherapy and until your study doctor indicates it is acceptable to stop. Check with the study doctor about what types of birth control or pregnancy prevention to use while in this study.

What possible benefits can I expect from taking part in this study?

This study is designed to test the COXEN program and its ability to predict responses to chemotherapy. It is not possible to know at this time if the COXEN program will be able to accurately predict responses to chemotherapy. This study will help researchers learn things that will help people in the future.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

The study doctor will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes and the study is no longer in your best interest
- If new information becomes available
- If you do not follow the study rules
- If the study is stopped by the sponsor, IRB or FDA

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the _____ *(insert name of center)* Institutional Review Board at _____ *(insert telephone number). (Note to Local Investigator: Contact information for patient representatives or other individuals at a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can also be listed here.)*

What are the costs of taking part in this study?

You and/or your health plan/insurance company will need to pay for all of the other costs of treating your cancer while in this study, including the cost of managing any side effects. Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

You will not be paid for taking part in this study.

What happens if I am injured or hurt because I took part in this study?

If you are injured or hurt as a result of taking part in this study and need medical treatment, please tell your study doctor. The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance, you would be responsible for any costs.

If you feel this injury was a result of medical error, you keep all your legal rights to receive payment for this even though you are in a study.

Who will see my medical information?

Your privacy is very important to us and the researchers will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, the researchers will do their best to make sure that any information that is released will not identify you. Some of your health information, and information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private, unless required by law to provide information. Some of these organizations are:

- SWOG
- Your local Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the U.S., and similar ones if other countries are involved in the study.
- The Cancer Trials Support Unit (CTSU), a research group sponsored by the National Cancer Institute (NCI) to provide greater access to cancer trials.

[Note to Local Investigators: The NCI has recommended that HIPAA regulations be addressed by the local institution. The regulations may or may not be included in the informed consent form depending on local institutional policy.]

Where can I get more information?

You may visit the NCI Web site at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At

most, the Web site will include a summary of the results. You can search this Web site at any time.

[Note to Informed Consent Authors: the above paragraph complies with the new FDA regulation found at 21 CFR 50.25(c) and must be included verbatim in all informed consent documents. The text in this paragraph cannot be revised.]

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ *(insert name of study doctor[s])* at _____ *(insert telephone number).*

NOTE: Use the following types of format only when the "additional studies" are optional for the patient. When mandatory, the description should be imbedded as a part of the study description in the main consent.

ADDITIONAL STUDIES SECTION:

This part of the consent form is about optional studies that you can choose to take part in. You will not get health benefits from any of these studies. The researchers leading this optional study hope the results will help other people with cancer in the future.

The results will not be added to your medical records, nor will you or your study doctor know the results.

You will not be billed for these optional studies. You can still take part in the main study even if you say 'no' to any or all of these studies. If you sign up for but cannot complete any of the studies for any reason, you can still take part in the main study.

Circle your choice of "yes" or "no" for each of the following studies.

1. Future Contact

I agree to allow my study doctor, or someone approved by my study doctor, to contact me regarding future research involving my participation in this study.

Yes No

2. Optional Blood Collection for Ultra-Pure Circulating Tumor Cells (upCTC)

In an effort to determine if there are some patients who are more or less likely to benefit from the combination of gemcitabine and cisplatin versus the combination of methotrexate, vinblastine, doxorubicin, cisplatin and filgrastim (or pegfilgrastim), two tubes of your blood will be collected

before you start treatment and before Cycle 2. If you agree to participate in this study, your blood will be studied for the presence of cancer cells that are shed by your tumors into your blood stream. DNA and RNA from these circulating tumor cells (CTCs) will be analyzed to better understand which cancers respond to chemotherapy. This blood will also be studied for DNA not found in cells but that is also present in your blood because it has been released from the cancer in your body.

Blood Sample for upCTC:

I agree to participate in the upCTC study and have two additional blood samples collected and submitted.

Yes No

3. Optional Biobanking for Possible Future Studies

Researchers are trying to learn more about cancer, diabetes, and other health problems. Much of this research is done using samples from your tissue, blood, urine, or other fluids. Through these studies, researchers hope to find new ways to prevent, detect, treat, or cure health problems.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

If you choose to take part in the main study, a sample of tissue from your previous biopsy and blood and urine samples will be collected prior to starting on protocol treatment. The researchers ask your permission to store and use your leftover samples from the planned research and health information for medical research. The research that may be done is unknown at this time. Storing samples for future studies is called “biobanking”. The Biobank is being run by SWOG and supported by the National Cancer Institute.

WHAT IS INVOLVED?

If you agree to allow your specimens to be stored and used in future research, here is what will happen next:

1) Your specimens will be collected as described above and will be sent to the Biobank.
2) Your specimens and some related information may be stored in the Biobank, along with samples and information from other people who take part. The specimens will be kept until they are used up.
3) Qualified researchers can submit a request to use the materials stored in the Biobanks. A science committee at the clinical trials organization, and/or the National Cancer Institute, will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.

4) Neither you nor your study doctor will be notified when research will be conducted or given reports or other information about any research that is done using your samples.
5) Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1) The most common risks related to drawing blood from your arm are brief pain and possibly a bruise.
2) There is a risk that someone could get access to the personal information in your medical records or other information researchers have stored about you.
3) There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
4) In some cases, genetic information could be used to make it harder for you to get or keep a job or insurance. There are laws against the misuse of genetic information, but they may not give full protection. There can also be a risk in knowing genetic information. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe that the chance that these things will happen is very small, but they cannot promise that you will avoid any risk.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers, and they will make every effort to protect it. Here are just a few of the steps they will take:

1) When your samples are sent to the researchers, no information identifying you (such as your name) will be sent. Samples will be identified by a unique code only.
2) The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and SWOG staff with access to the list must sign an agreement to keep your identity confidential.
3) Researchers that receive your sample and information from SWOG will not know who you are. Researchers must also sign an agreement that they will not try to find out who you are.
4) Information that identifies you will not be given to anyone, unless required by law.
5) If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part in this study. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. You will not be paid for taking part. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)* who will let the researchers know. Then, any samples that remain in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

If you decide to withdraw your specimens from a SWOG Specimen Repository in the future, a written withdrawal of consent should be submitted through your study doctor to the SWOG Operations Office. Please designate in the written withdrawal whether you would prefer to have the specimens destroyed or returned to the study doctor.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the study doctor, _____, *(insert name of study doctor for main trial)*, at _____ *(insert telephone number of study doctor for main trial)*.

Please circle your answer to show whether or not you would like to take part in each option (*include only applicable questions)*:

SAMPLES FOR FUTURE RESEARCH STUDIES:

My samples and related information may be kept in a Biobank for use in future health research.

YES NO

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies where I circled 'yes'.

Participant's signature_____

Date of signature_____

Specimen Consent Supplemental Sheets

How are Specimens Used for Research?

Where do specimens come from?

A specimen may be from a blood sample or from bone marrow, skin, toenails or other body materials. People who are trained to handle specimens and protect donors' rights make sure that the highest standards of quality control are followed by SWOG. Your doctor does not work for SWOG, but has agreed to help collect specimens from many patients. Many doctors across the country are helping in the same way.

Why do people do research with specimens?

Research with specimens can help to find out more about what causes cancer, how to prevent it, how to treat it, and how to cure it. Research using specimens can also answer other health questions. Some of these include finding the causes of diabetes and heart disease, or finding genetic links to Alzheimer's.

What type of research will be done with my specimen?

Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat or even cure diseases. In the future, some of the research may help to develop new products, such as tests and drugs. Some research looks at diseases that are passed on in families (called genetic research). Research done with your specimen may look for genetic causes and signs of disease.

How do researchers get the specimen?

Researchers from universities, hospitals, and other health organizations conduct research using specimens. They contact SWOG and request samples for their studies. SWOG reviews the way that these studies will be done, and decides if any of the samples can be used. SWOG gets the specimen and information about you from your hospital, and sends the specimen samples and some information about you to the researcher. SWOG will not send your name, address, phone number, social security number or any other identifying information to the researcher.

Will I find out the results of the research using my specimen?

You will not receive the results of research done with your specimen. This is because research can take a long time and must use specimen samples from many people before results are known. Results from research using your specimen may not be ready for many years and will not affect your care right now, but they may be helpful to people like you in the future.

Why do you need information from my health records?

In order to do research with your specimen, researchers may need to know some things about you. (For example: Are you male or female? What is your race or ethnic group? How old are you? Have you ever smoked?) This helps researchers answer questions about diseases. The information that will be given to the researcher may include your age, sex, race, diagnosis, treatments and family history. This information is collected by your hospital from your health record and sent to SWOG. If more information is needed, SWOG will send it to the researcher.

Will my name be attached to the records that are given to the researcher?

No. Your name, address, phone number and anything else that could identify you will be removed before they go to the researcher. The researcher will not know who you are.

How could the records be used in ways that might be harmful to me?

Sometimes, health records have been used against patients and their families. For example, insurance companies may deny a patient insurance or employers may not hire someone with a certain illness (such as AIDS or cancer). The results of genetic research may not apply only to you, but to your family members too. For disease caused by gene changes, the information in one person's health record could be used against family members.

How am I protected?

SWOG is in charge of making sure that information about you is kept private. SWOG will take careful steps to prevent misuse of records. Your name, address, phone number and any other identifying information will be taken off anything associated with your specimen before it is given to the researcher. This would make it very difficult for any research results to be linked to you or your family. Also, people outside the research process will not have access to results about any one person which will help to protect your privacy.

What if I have more questions?

If you have any questions, please talk to your doctor or nurse, or call our research review board at (Insert IRB's Phone Number).