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Reshaping the future of patient care

EA8141

A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Upper Tract Urothelial Carcinoma

STUDY CHAIR: Vitaly Margulis, M.D.
STUDY CO-CHAIR: Jean Hoffman-Censits, M.D.
STUDY STATISTICIAN: Yu-Hui Chen, M.S.
BLADDER SUBCOMMITTEE CHAIR: Noah Hahn, M.D.
GU COMMITTEE CHAIR: Michael Carducci, M.D.
UROLOGY TRANSLATIONAL SCIENCE CO- CHAIR: Edouard Trabulsi, M.D.
TRANSLATIONAL SCIENCE CO-CHAIR: Elizabeth Plimack, M.D.

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STUDY PARTICIPANTS

ALLIANCE / Alliance for Clinical Trials in Oncology
NRG / NRG Oncology
SWOG / SWOG
US Sites Only

NCTN GROUP STUDY CHAMPIONS

SWOG: Guilherme Godoy, M.D., Ajjai Alva,M.D.

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Update #1 – 11/15
Addendum #3 – 6/16
Update #2 – 10/17
Addendum 4

Agents	IND#	NSC#	Supply
Gemcitabine	IND Exempt Study	613327	Commercially Available
Doxorubicin		123127	Commercially Available
Cisplatin		119875	Commercially Available
Carboplatin		241240	Commercially Available
Methotrexate		740	Commercially Available
Vinblastine		49842	Commercially Available
Pegfilgrastim		725961	Commercially Available

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STUDY CHAIR

Vitaly Margulis, M.D
University of Texas Southwestern Medical Center
5339 Harry Hines Blvd
Dallas, TX 75390
Phone: 214-648-0567
Fax: 214-648-8786
vitaly.margulis@utsouthwestern.edu

STUDY CO-CHAIR

Jean Hoffman-Censits, M.D
Thomas Jefferson University
Department of Medical Oncology
1025 Walnut St, Suite 700
Philadelphia, PA 19107
Phone: 215-503-5455
Jean.hoffman-censits@jefferson.edu

STUDY CHAIR LIAISON (SCL)

Allison Beaver, R.N
University of Texas Southwestern Medical Center
5339 Harry Hines Blvd
Dallas, TX 75390
Phone: 214-645-8788
Fax: 214-645-8766
Allison.Beaver@UTSouthwestern.edu

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	**For patient enrollments:**	**Submit study data**
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone – 1-866-651-CTSU Fax – 215-569-0206 Email: CTSURegulatory@ctsu.coccg.org (for submitting regulatory documents only)	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.		
For clinical questions (i.e., patient eligibility or treatment-related) Contact the Study PI of the Coordinating Group.		
For non-clinical questions (i.e., unrelated to patient eligibility, treatment, or data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. 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 > education and resources tab > CTSU Operations Information >CTSU Regulatory and Monitoring Policy		
The CTSU Web site is located at https://www.ctsu.org		

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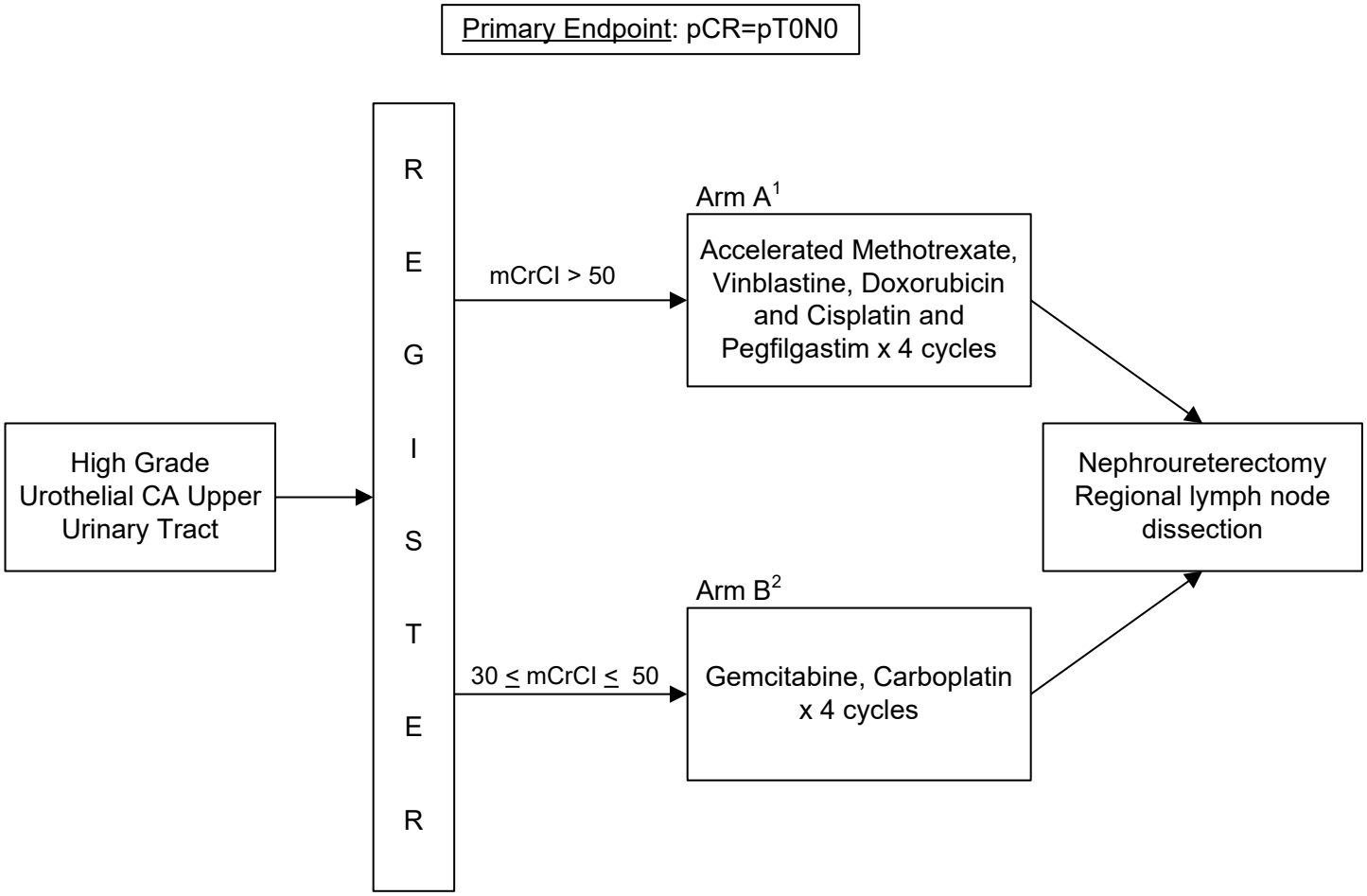
Schema



Accrual Goal= 60
30 Patients per arm

1. Regimen repeated every 14 days for 4 cycles.
2. Regimen repeated every 21 days for 4 cycles.

1. Introduction

1.1 Research Hypothesis

We hypothesize that neoadjuvant systemic chemotherapy in patients with high-grade upper tract urothelial carcinoma prior to extirpative surgery will result in at least a 18% rate of complete pathologic response (pCR = pT0pN0). Ultimately, we assert that neoadjuvant systemic chemotherapy will be safe, well tolerated and result in improved long-term oncologic outcomes for patients with upper tract urothelial carcinoma.

1.2 Background

Upper tract urothelial cancer (UTUC) accounts for roughly 5% of all urothelial cancers (UC), with 11, 000 new cases annually in United States. At presentation, 30% of patients demonstrate invasive and/or locally advanced disease, 30-40% have regional lymph node involvement, and 20% harbor metastatic disease. Systemic recurrence and progression rates after surgery alone for patients with advanced UTUC are high with ranges between 45-60%. 5-year cancer specific survival (CSS) rates for pT2 and pT3 tumors are 73% and 40%, respectively.[1,2] While radical nephro-ureterectomy (RNU) represents the standard therapeutic option for patients with UTUC, there are significant challenges associated with inaccurate clinical staging, high prevalence of occult metastatic disease, poor baseline and worsening post-operative global renal function, and subsequent difficulties with administration of meaningful systemic therapies.

Cisplatin based neoadjuvant chemotherapy (NAC) has shown a therapeutic benefit in patients with bladder UC. Patients who received neoadjuvant therapy had a 5% absolute improvement in CSS, compared to patients treated with cystectomy alone. [3] Patients also tolerated NAC better than adjuvant therapy in bladder cancer studies.[4] Several retrospective studies have shown that the use of NAC prior to RNU has been associated with pathological downstaging.[5] In Matin et al.'s retrospective review of 150 patients who received platinum-based NAC treatment of biopsy proven high grade UTUC, the overall incidence of pT2 or higher disease was significantly lower in the NAC group compared to the control patients (46.5% vs. 65%; p=.043).[5] Moreover, a 14% pathologic complete response rate (pCR) was observed in that retrospective study. Finally, several authors report favorable oncologic outcomes in patients with advanced UTUC treated with pre-surgical systemic chemotherapy, compared to historic controls.[6-8]

Of particular relevance, is the fact that UTUC patients are at an increased risk of baseline or future chronic kidney disease (CKD), rendering many poor candidates for perioperative cisplatin chemotherapy. [9] Several studies have shown 25-52% and 65-78% incidence of CKD before and after RNU. [9,10] Lane et al. evaluated 336 patients who underwent NU for UTUC and found that at baseline, 52% of the cohort were poor cisplatin-based chemotherapy candidates due to impaired renal function. Following RNU, there was a 21% reduction in renal function and RNU rendered 78% of the patient's poor candidates for cisplatin-based therapy. In a sub-group analysis of 144 high-risk patients (T2-T4 NX disease) at the time of RNU, who would be ideal candidates for systemic

treatment, impaired preoperative renal function was observed in nearly 60% of patients. Following RNU, the frequency of renal impairment increased to 78% (p=.009). Based on this alone, 61% of all patients and 49% of high risk patients who could have received chemotherapy preoperatively were unable to receive treatment after RNU.[10] Thus a strong rationale exists to optimize delivery of cisplatin-based regimens to patients with adequate renal function and to define the role of non-cisplatin regimens in the large portion of UTUC patients who are poor candidates for cisplatin therapy.

The target population of this study includes patients with clinically localized high grade UTUC, since histologic grade is one of the few reliable and readily obtainable pathologic staging variables, and correlates well with advanced pathologic stage, and eventual oncologic outcomes.[11-13] Patients will be assigned to a treatment arm and receive a platinum based NAC regimen based on their screening creatinine clearance (CrCl), determined by Cockcroft-Gault calculation or direct 24-hour urine measurement, followed by nephro-ureterectomy and regional lymph node dissection. NAC with accelerated methotrexate, vinblastine, doxorubicin and cisplatin (AMVAC) will be administered to patients with CrCl ≥ 50 mL/min, based on CrCl cut-off with demonstrated safety in modern neoadjuvant cisplatin studies.[3,5] Patients with 30 ≤ CrCl ≤ 50 mL/min, will be offered gemcitabine/carboplatin (GCa), which has demonstrated response in patients with UC and impaired CrCl treated in the metastatic setting.[14-16] As stated above, because of additional nephron loss with up-front surgery, many of UTUC patients with baseline CKD will not qualify for any additional systemic therapy after surgery, thus the rationale for including a non-cisplatin arm for this group.

Pathologic complete response (pCR) is a relevant, generalizable, early end-point for NAC in UC of the bladder and therefore was chosen as the initial primary efficacy endpoint in this proposed UTUC NAC trial.[3] A targeted pCR rate of 18% was chosen based on well characterized results of NAC in UC of the urinary bladder and from limited experience with NAC in UTUC.[3,5,8] Upper tract urothelial cancers are difficult to access for initial staging and resection, thus the expected pCR rate in this trial is lower than the near 40% seen in bladder NAC studies, a rate achieved by NAC and aggressive transurethral debulking. Important additional endpoints of renal functional outcomes (both improvements and declines on therapy), bladder and systemic recurrence-free survival and cancer-specific/overall survival will be assessed.

Finally, the trial design permits utilization of pre-treatment and post-treatment tumor tissue, peripheral blood, and urine specimens to study, among others, biomarkers and molecular and pathways important in resistance and response to chemotherapy.

2. Objectives

2.1 Primary Endpoints

2.1.1 To evaluate the rate of complete pathologic response (pCR = pT0pN0) as assessed by standard pathologic review attained by neoadjuvant systemic chemotherapy and nephroureterectomy

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2.2 Secondary Endpoints

2.2.1 To evaluate the safety of neoadjuvant systemic chemotherapy in patients with upper tract urothelial carcinoma preceding nephroureterectomy

2.2.2 To evaluate distant recurrence-free survival of patients treated with neoadjuvant systemic chemotherapy preceding nephroureterectomy

2.2.3 To evaluate event-free survival of patients treated with neoadjuvant systemic chemotherapy preceding nephroureterectomy

2.2.4 To evaluate bladder cancer-free survival of patients treated with neoadjuvant systemic chemotherapy preceding nephroureterectomy

2.2.5 To evaluate cancer specific survival of patients treated with neoadjuvant systemic chemotherapy preceding nephroureterectomy

2.2.6 To evaluate renal functional outcomes of patients treated with neoadjuvant systemic chemotherapy preceding nephroureterectomy

2.3 Tertiary Endpoints

2.3.1 To collect pre-treatment and post-treatment tumor tissue, peripheral blood mononuclear cell (PBMC), peripheral blood plasma, and urine specimens for potential evaluations of markers of chemotherapy response/resistance

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

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 four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. _____

Patient's Initials (L, F, M) _____

Physician Signature and Date _____

NOTE: All questions regarding eligibility should be directed to the study chair or study chair liaison.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

3.1 Eligibility Criteria

_____ 3.1.1 Patients must be ≥ 18 years of age.

_____ 3.1.2 Patients must have high grade upper tract urothelial carcinoma proven by one of the following:

- Biopsy;
- Urinary cytology with a 3-dimensional upper urinary tract mass on cross-sectional imaging; or
- Urinary cytology and a mass visualized during upper urinary tract endoscopy

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_____ 3.1.3 Patients must have a Creatinine clearance ≥ 30 ml/min within 28 days of registration to be eligible for the study.

This will be determined by Cockroft-Gault calculation and 24-hour urine creatinine clearance measurement. (Please note, renal function, measured by 24-hour urine creatinine clearance in screening and post operatively, are required)

Cockcroft-Gault CrCl = (140-age) * (Wt in kg) * (0.85 if female) / (72 * Cr)

_____ 3.1.4 Patients must have ECOG Performance Status 0 or 1 (see Appendix III).

_____ 3.1.5 Patients must have no evidence of metastatic disease or clinically enlarged lymph nodes on CT or MRI of the abdomen and pelvis and CT chest obtained within 28 days of registration (A negative biopsy is required for lymph nodes ≥ 1 cm in size to confirm lack of involvement). Patients with lymph nodes ≥ 1 cm in whom a biopsy is deemed not feasible are not eligible. Patients with elevated alkaline

phosphatase or suspicious bone pain should also undergo baseline bone scans to evaluate for bone metastasis.

_____ 3.1.6 Patients with any component of small cell carcinoma are not eligible. Other variant histologies are permitted provided the predominant (≥ 50%) subtype is urothelial carcinoma.

_____ 3.1.7 Patients must not have peripheral neuropathy > Grade 2.

_____ 3.1.8 Patients must not have a history of allergy or hypersensitivity to methotrexate, vinblastine, doxorubicin, cisplatin, gemcitabine, carboplatin or filgrastim or pegfilgrastim.

_____ 3.1.9 Patients must have a left ventricular ejection fraction (LVEF) ≥ 50% by (either MUGA or 2-D echocardiogram) within 28 days of registration.

_____ 3.1.10 Patients must have normal organ and marrow function as defined below within 28 days prior to registration:

3.1.10.1 Absolute Neutrophil Count (ANC) ≥ 1500/mm^3

3.1.10.2 Platelets ≥ 100,000/mm^3

3.1.10.3 HgB ≥ 9

3.1.10.4 AST(SGOT)/ALT(SGPT) < 2X institutional ULN

Rev. 10/15 3.1.10.5 Total Bilirubin within institutional normal limits (or < 2.5 X the ULN for patients with Gilbert's disease)

_____ 3.1.11 Patients with concomitant primaries of the bladder/urethra are allowed, as long as these sites are surgically resected and non-invasive cancers (< cT1N0).

_____ 3.1.12 Patients must not have another active second malignancy other than non-melanoma skin cancers and biochemical relapsed prostate cancer. Patients that have completed all necessary therapy and are considered to be at less than 30% risk of relapse are not considered to have an active second malignancy and are eligible for enrollment.

Rev. 10/15 _____ 3.1.13 For patients who have Creatinine Clearance that meets > 50 ml/min they must not have received prior systemic doxorubicin.

_____ 3.1.14 Patients with uncontrolled illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, myocardial infarction in last 3 months, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements are not eligible.

_____ 3.1.15 Patients who are known to have HIV or are on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with cytotoxic chemotherapy. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

Rev. 10/15 _____ 3.1.16 Patients must have no prior radiation therapy to ≥ 25% of the bone marrow for prior systemic anthracycline therapy. Prior intravesical anthracycline therapy for non-muscle invasive urothelial carcinoma of the bladder is permitted.

_____ 3.1.17 Patients may have a history of resectable urothelial cancer (including neoadjuvant chemotherapy) as long as patients meet one of the following:

- pT0, Tis, or T1N0 and have no evidence of disease (NED) for more than 2 years from surgery or chemotherapy;
- pT2-3aN0 and NED for more than 3 years from surgery or chemotherapy; or
- >pT3b, or N+ and NED for more than 5 years from surgery or chemotherapy.

_____ 3.1.18 Women must not be pregnant or breast-feeding due to use of cytotoxic chemotherapy and risk of teratogenic side effects.

All females of childbearing potential must have a blood test or urine study within 2 weeks prior to registration to rule out pregnancy.

A female of childbearing potential is any woman, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Female? _____ (Yes or No)

Date of blood test or urine study: _____

_____ 3.1.19 Women of childbearing potential and sexually active males must use an accepted and effective method of contraception or to abstain from sexual intercourse for the duration of their participation in the study.

_____ _____

Physician Signature Date

OPTIONAL: This signature line is provided for use by institutions wishing to use the eligibility checklist as source documentation.

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4. Registration Procedures

CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillabe PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to 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 members' website by entering

credentials at https://www.ctsu.org. For sites under the CIRB initiative, IRB data will automatically load to RSS.

Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. However, sites must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB (via IRBManager) to indicate their intention to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office for compliance in the RSS.. The Signatory site may be contacted by the CTSU Regulatory Office or asked to complete information verifying the participating institutions on the study. Other site registration requirements (i.e., laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

Downloading Site Registration Documents:

Site registration forms may be downloaded from the [NCI protocol #] protocol page located on the CTSU members' website. Add if a restricted access protocol: Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the (state organization type e.g. P2C, CITN, NCTN Groupname) link to expand, then select trial protocol #[NCI Protocol #]
- Click on the Site Registration Documents link

Requirements For EA8141 Site Registration:

- CTSU IRB Certification (for sites not participating via the NCI CIRB)
- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)

Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-866-651-2878
FAX: (215) 569-0206
E-mail: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

Required Protocol Specific Regulatory Documents

1. CTSU Regulatory Transmittal Form.
2. Copy of IRB Informed Consent Document.

   NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

3. A. CTSU IRB Certification Form.
   Or
   B. Signed HHS OMB No. 0990-0263 (replaces Form 310).
   Or
   C. IRB Approval Letter

   NOTE: The above submissions must include the following details:

   - Indicate all sites approved for the protocol under an assurance number.
   - OHRP assurance number of reviewing IRB
   - Full protocol title and number
   - Version Date
   - Type of review (full board vs. expedited)
   - Date of review.
   - Signature of IRB official

Checking Your Site's Registration Status:

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website.

NOTE: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Patient Enrollment

Patients must not start protocol treatment prior to registration.

Treatment should start within seven working days after registration.

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the LPO or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data *{add if a Rave study: and, upon enrollment, initializes the patient in the Rave database.}.* OPEN 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.

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

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.

4.1 Registration to Treatment

The following information is to be provided at registration.

4.1.1 Protocol Number

4.1.2 Investigator Identification

- Institution and affiliate name
- Investigator's name

4.1.3 Patient Identification

- Patient's initials (first and last)
- Patient's Hospital ID and/or Social Security number
- Patient demographics
  - Gender
  - Birth date (mm/yyyy)
  - Race
  - Ethnicity
  - Nine-digit ZIP code
  - Method of payment
  - Country of residence

4.2 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.

4.3 Classification Factors

Treatment arm will be determined by baseline renal function as determined by Cockroft-Gault calculation or 24-hour urine creatinine clearance measurement.

Treatment will be assigned as follows:

- Arm A: mCrCl > 50
- Arm B: 30 ≤ mCrCl ≤ 50

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4.4 Additional Requirements

4.4.1 Patients must provide a signed and dated, written informed consent form.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office – Boston.

4.4.2 Biospecimens are to be submitted as indicated in Section 10.

4.4.3 Data collection for this study will be done exclusively through the Medidata Rave clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an

active CTEP-IAM account
(check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on either the LPO or participating organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

4.5 Instructions for Patients who Do Not Start Assigned Protocol Treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted through Medidata Rave according to the schedule in the EA8141 Forms Completion Guidelines.

5. Treatment Plan

5.1 Administration Schedule

Patients will receive neoadjuvant therapy according to their measured CrCl. Standardized protocols for administration of AMVAC and GCa will be employed. The doses of each drug are standard, but the infusion duration may be given in accordance with institutional protocol. Central venous access is recommended for patients in the AMVAC arm due to vesicant risk. All chemotherapy calculations should be based on actual body weight. Appropriate pre hydration, electrolyte repletion and antiemetic prophylaxis per institutional guidelines, supportive care guidelines for administration are included in Section 5.4.1. Use of erythroid stimulating agents (ESAs) is prohibited per NCCN guideline recommendation as treatment on this protocol is given with curative intent. Patients must have surgery per Section 5.1.3, within 60 days from completion of chemotherapy.

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NOTE: Weight dependent dose calculations will not require recalculation unless the patient's weight changes by greater than 10% when compared to the weight used for the previous dose calculation.

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5.1.1 Arm A: AMVAC

Cycle length is 14 days.

Methotrexate 30 mg/m^2 IV day 1 over 2-3 minutes

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Vinblastine 3 mg/m^2 IV day 1 infusion

Doxorubicin 30 mg/m^2 IV day 1 slow push

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Cisplatin 70 mg/m^2 IV day 1 infusion over at least 2 hours

(At the discretion of the investigator, cisplatin dose can be divided and given 35 mg/m^2 over day 1 and 2)

Pegfilgrastim 6 mg once 24-48 hours after completion of chemotherapy is required.

NOTE: Auto injector, Neulasta, is permitted for use on this trial.

Regimen repeated every cycle for a total of 4 cycles.

5.1.2 Arm B: GCa

Cycle length is 21 days.

Gemcitabine 1000 mg/m2 IV days 1 and 8 infusion over 30-60 minutes

Carboplatin AUC 5 IV day 1 infusion over 30-60 minutes

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Carboplatin dosing is by the Calvert formula[18]:
Total Dose (mg) = (target AUC) x (CrCl + 25)

Use of prophylactic myeloid growth factor support in the GCa arm at investigator discretion per NCCN guidelines for use of myeloid growth factors.

Regimen repeated every cycle for a total of 4 cycles.

5.1.3 Nephrectomy and retroperitoneal lymph node dissection

Patients with continued lack of radiographic presence of metastatic disease following neoadjuvant chemotherapy will proceed to nephroureterectomy and lymph node dissection. The surgery must take place 21 to 60 days from completion of chemotherapy. Surgical approach (open, laparoscopic, robotic assistance) is left at surgeon's discretion as long as the following criteria are met. The entirety of the affected upper urinary tract is removed (kidney, ureter and cuff of urinary bladder). Regional lymph node dissection is performed, preferably according to template as proposed by Kondo et al (17). Specifically, for the tumor of the right renal pelvis, proximal and/or mid ureter – hilar, paracaval, retro-caval and inter-aortocaval lymph nodes should be removed. For the left-sided renal pelvis, proximal, and/or mid-ureter – hilar and para-aortic lymph nodes should be harvested. Finally, for the distal ureteral tumors, ipsilateral common iliac, external iliac, internal iliac and obturator lymph nodes are removed.

Primary tumor, normal kidney/ureter tissue, and regional lymph nodes will be harvested at the time of surgery and the tissue examined and stored according to SOP for consistent handling of the tissue located in Appendix I.

5.2 Adverse Event Reporting Requirements

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NOTE: For this protocol, the Adverse Event Reporting period includes the time the patient is on chemotherapy and continues through 30 days post- surgery.

5.2.1 Purpose

Adverse event (AE) data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of the patients enrolled, as well as those who will enroll in future studies using similar agents.

- **Routine reporting:** Adverse events are reported in a routine manner at scheduled times during a trial using Medidata Rave.
- **Expedited reporting:** In addition to routine reporting, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The following sections provide information and instructions regarding expedited adverse event reporting.

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5.2.2 Terminology

- **Adverse Event (AE):** Any untoward medical occurrence associated with the use of a drug (or surgery) in humans, whether or not considered drug related. Therefore, an AE can be **ANY** unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product (or surgery), whether or not considered related to the medicinal product (or surgery).

- **Attribution:** An assessment of the relationship between the adverse event and the protocol treatment, using the following categories.

ATTRIBUTION	DESCRIPTION
Unrelated	The AE is *clearly NOT related* to treatment.
Unlikely	The AE is *doubtfully related* to treatment.
Possible	The AE *may be related* to treatment.
Probable	The AE is *likely related* to treatment.
Definite	The AE is *clearly related* to treatment.

- **CTCAE:** The NCI Common Terminology Criteria for Adverse Events provides a descriptive terminology that is to be utilized for AE reporting. A grade (severity) is provided for each AE term.
- **Expectedness:** Expected events are those that have been previously identified as resulting from administration of the agent (or surgery). An adverse event is considered unexpected, for expedited reporting purposes, when either the type of event or the severity of the event is NOT listed in the protocol or drug package insert.

5.2.3 Reporting Procedure

This study requires that expedited adverse event reporting use CTEP's Adverse Event Reporting System (CTEP-AERS). The CTEP's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted electronically to ECOG-ACRIN and the appropriate regulatory agencies via the CTEP-AERS Web-based application located at http://ctep.cancer.gov.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made by telephone to

- the AE Team at ECOG-ACRIN (617-632-3610)
- the FDA (1-800-FDA-1088)

An electronic report MUST be submitted immediately upon re-establishment of internet connection.

Supporting and follow up data: Any supporting or follow up documentation must be uploaded to the Supplemental Data Folder in Medidata Rave within 48-72 hours. In addition, supporting or follow up documentation must be faxed to the FDA (800-332-0178) in the same timeframe.

CTEP Technical Help Desk: For any technical questions or system problems regarding the use of the CTEP-AERS application, please contact the NCI Technical Help Desk at ncictephelp@ctep.nci.nih.gov or by phone at 1-888-283-7457.

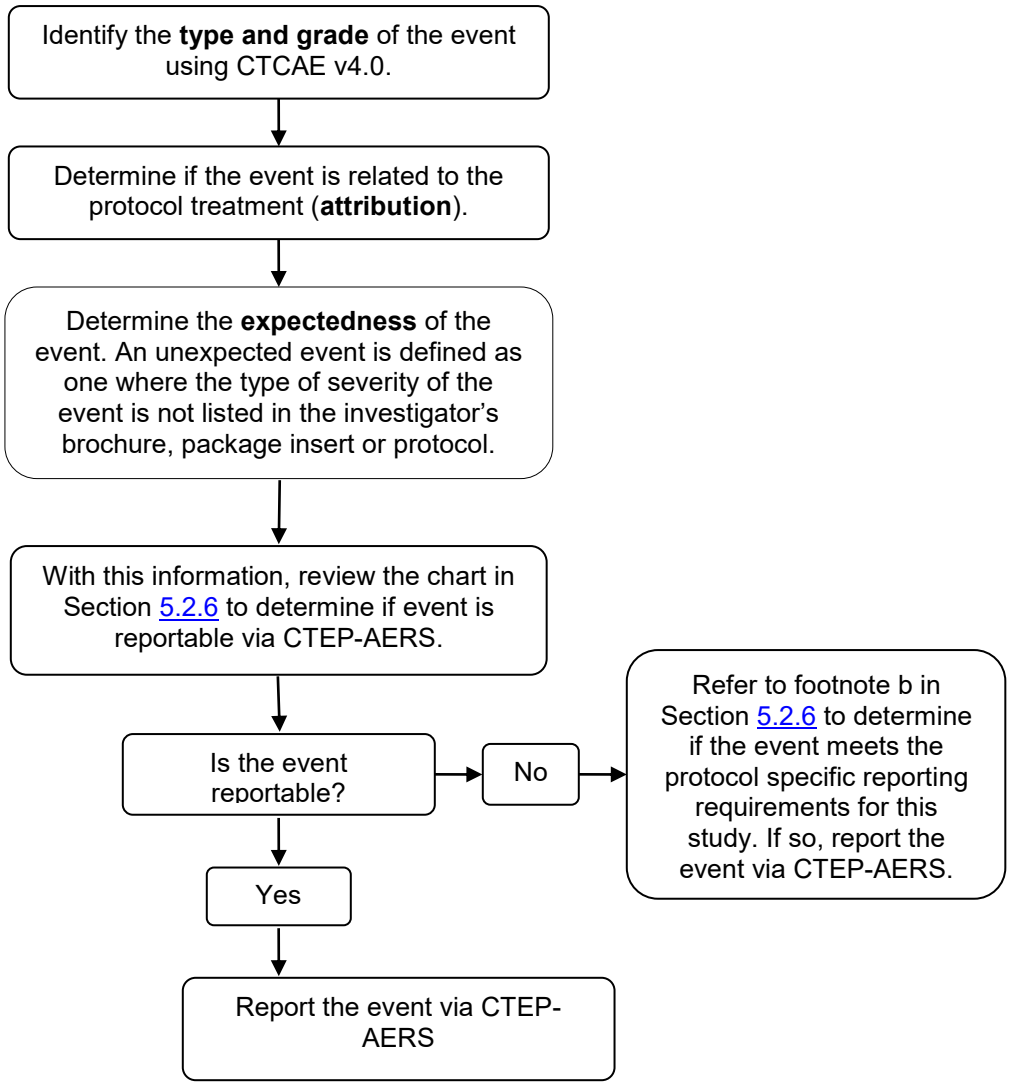
5.2.4 Determination of Reporting Requirements

Many factors determine the reporting requirements of each individual protocol, and which events are reportable in an expeditious manner, including:

- the phase (0, 1, 2, or 3) of the trial
- whether the patient has received an investigational or commercial agent or both
- the seriousness of the event
- the Common Terminology Criteria for Adverse Events (CTCAE) grade
- when the adverse event occurred (within 30 days of the last administration of investigational agent vs. ≥ 30 days after the last administration of investigational agent)
- the relationship to the study treatment (attribution)
- the expectedness of the adverse event

Using these factors, the instructions and tables in the following sections have been customized for protocol EA8141 and outline the specific expedited adverse event reporting requirements for study EA8141.

5.2.5 Steps to determine if an event is to be reported in an expedited manner


Identify the **type and grade** of the event using CTCAE v4.0.
Determine if the event is related to the protocol treatment (**attribution**).
Determine the **expectedness** of the event. An unexpected event is defined as one where the type of severity of the event is not listed in the investigator's brochure, package insert or protocol.
With this information, review the chart in Section 5.2.6 to determine if event is reportable via CTEP-AERS.
Is the event reportable?
No
Refer to footnote b in Section 5.2.6 to determine if the event meets the protocol specific reporting requirements for this study. If so, report the event via CTEP-AERS.
Yes
Report the event via CTEP-AERS

5.2.6 Expedited Reporting Requirements for Arms A and B on protocol EA8141

Commercial Agents: Gemcitabine, Doxorubicin, Methotrexate, Vinblastine, Cisplatin and Carboplatin

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NOTE: For this protocol, the Adverse Event Reporting period includes the time the patient is on chemotherapy and continues through 30 days post- surgery. Please continue to report via CTEP-AERS all adverse events that meet the criteria in the chart below until the patient is 30 days post-surgery.

Expedited reporting requirements for adverse events experienced by patients on arm(s) with commercial agents only – Arms A and B

Attribution	**Grade 4**		**Grade 5[a]**		**ECOG-ACRIN and Protocol-Specific Requirements**
	Unexpected	Expected	Unexpected	Expected	
Unrelated or Unlikely			7 calendar days	7 calendar days	See footnote (b) for special requirements.
Possible, Probable, Definite	7 calendar days		7 calendar days	7 calendar days	

7 Calendar Days: Indicates a full CTEP-AERS report is to be submitted within 7 calendar days of learning of the event.

a This includes all deaths within 30 days of the last dose of treatment regardless of attribution.

NOTE: Any death that occurs > 30 days after the last dose of treatment and is attributed possibly, probably, or definitely to the treatment must be reported within 7 calendar days of learning of the event.

b Protocol-specific expedited reporting requirements: The adverse events listed below also require expedited reporting for this trial:

Serious Events: Any event following treatment that results in *persistent or significant disabilities/incapacities, congenital anomalies, or birth defects* must be reported via CTEP-AERS within 7 calendar days of learning of the event. For instructions on how to specifically report these events via CTEP-AERS, please contact the AEMD Help Desk at aemd@tech-res.com or 301-897-7497. This will need to be discussed on a case-by-case basis.

5.2.7 Other recipients of adverse event reports and supplemental data

Adverse events determined to be reportable via CTEP-AERS must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

5.2.8 Second Primary Cancer Reporting Requirements

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported to ECOG-ACRIN using Medidata Rave

- **A second malignancy is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave.
- **A secondary malignancy is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**
  1. Complete a Second Primary Form in Medidata Rave within 14 days.
  2. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov

     Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy
  3. Upload a copy of the pathology report to ECOG-ACRIN via Medidata Rave.
  4. If the patient has been diagnosed with AML/MDS, upload a copy of the cytogenetics report (if available) to ECOG-ACRIN via Medidata Rave.

NOTE: The ECOG-ACRIN Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one ECOG-ACRIN study, the ECOG-ACRIN Second Primary Form must be submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the ECOG-ACRIN Second Primary Form.

5.3 Dose Modifications

All toxicity grades below are described using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov).

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. Skipped doses will not be made up.

Treatment holds should be < 2 weeks. For treatment hold > 2 weeks, patient should proceed to surgery. The maximum total period of treatment delay should not exceed 4 weeks. Patients with cumulative treatment hold of > 4 weeks should proceed to surgery.

METHOTREXTAE

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

VINBLASTINE

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

CISPLATIN - no dose reductions

Dose Level	Dose	
	CrCl ≥ 60 mL/min	CrCl > 50 ml/min and < 60 ml/min
Level 0	70 mg/m^2	35 mg/m² on days 1 and 2

CARBOPLATIN - no dose reductions

Dose Level	Dose
Level 0	AUC 5

GEMCITABINE

Dose Level	Dose
Level 0	1000 mg/m^2
Level -1	750 mg/m^2
Level -2	500 mg/m^2

AMVAC Treatment arm

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5.3.1 Hematologic toxicity and febrile neutropenia

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, and doxorubicin by one dose level for this and all subsequent cycles. Continue cisplatin at full dose.

For febrile neutropenia, defined as ANC of < 500 and a temperature of 38.3°C orally (101°F) or a temperature of ≥ 38°C (100.4°F) for more than 1 hour, reduce methotrexate, vinblastine, and doxorubicin by one dose level. Continue cisplatin at full dose.

5.3.2 Kidney Dysfunction

Calculate or measure the CrCl on Day 1 of each cycle. If the CrCl is > 50 and < 60 ml/min on Day 1 Cycle 1 of chemotherapy, administer cisplatin in split dosing on Days 1 and 2. If CrCl improves to ≥ 60 ml/min on Day 1 of subsequent cycles, split dosing of cisplatin is not required for those cycles.

Beyond cycle 1 if CrCl is < 50 ml/min, hold chemotherapy for up to 2 weeks. If the CrCl improves to > 50 ml/min within 2 weeks or less of the scheduled Day1, proceed with split dose cisplatin administration on Days 1 and 2 for this and all subsequent cycles. If the CrCl does not improve to > 50 ml/min within 2 weeks despite hydration and other supportive measures, discontinue the chemotherapy and proceed to surgery.

There are no cisplatin dose reductions for kidney dysfunction.

5.3.3 Liver Dysfunction

Methotrexate: Bilirubin >3 to 5 mg/dL **or** transaminases >3 times ULN: reduce by one dose level. Bilirubin >5 mg/dL, avoid until bilirubin < 5 mg/dL. See separate recommendations for bilirubin elevations in Gilbert's disease.

Bilirubin elevations

	1.5 – 3 mg/dL	> 3– 5 mg/dL	> 5 mg/dL
Methotrexate	Dose level 0	Dose level -1	Hold

AST / ALT elevations

	2 – 3x ULN	> 3x ULN
Methotrexate	Dose level 0	Dose level -1

Methotrexate dose reductions for Bilirubin elevations due to Gilbert's Disease

	> 3 – 5 mg/dL)	*> 5 – 8 mg/dL*	*> 8 mg/dL*
Methotrexate	*Dose level 0*	*Dose level -1*	*Hold*

Vinblastine: Bilirubin 1.5 to 3 mg/dL or transaminases 2 to 3x ULN: reduce by two dose levels. Transaminases >3x ULN, Hold. Serum bilirubin >3x ULN, avoid until bilirubin $\leq$ 3x ULN. See separate recommendations for bilirubin elevations in Gilbert's disease.

Bilirubin elevations

	1.5 – 3 mg/dL	> 3 – 5 mg/dL	> 5 mg/dL
Vinblastine	Dose level -2	Hold	Hold

AST / ALT elevations

	2 – 3x ULN	> 3x ULN
Vinblastine	Dose level -2	Hold

Vinblastine dose reductions for Bilirubin elevations due to Gilbert's Disease

	> 3 – 5 mg/dL	*> 5– 8 mg/dL*	*> 8 mg/dL*
Vinblastine	*Dose level 0*	*Dose level -2*	*Hold*

Doxorubicin: Serum bilirubin 1.2 to 3 mg/dL: reduce by two dose levels. Serum bilirubin > 3 mg/dL, avoid until bilirubin < 3 mg/dL.Transaminases 2 to 3x ULN: reduce by one dose level. Transaminases >3 times ULN: reduce by two dose levels. Transaminases >3x ULN, Hold. See separate recommendations for bilirubin elevations in Gilbert's disease.

Bilirubin elevations

	1.2 – 3 mg/dL	>3 – 5 mg/dL	> 5 mg/dL
Doxorubicin	Dose level -2	Hold	Hold

AST / ALT elevations

	2 – 3x ULN	>3x ULN
Doxorubicin	Dose level -1	Dose level -2

Doxorubicin dose reductions for Bilirubin elevations due to Gilbert's Disease

	1.5 – 3 mg/dL (Gilbert's disease 3.1 – 5 mg/dL)	*3.1 – 5 mg/dL (Gilbert's disease 5.1 – 8 mg/dL)*	*> 5 mg/dL (Gilbert's disease > 8 mg/dL)*
Doxorubicin	*Dose level 0*	*Dose level -2*	*Hold*

Cisplatin: Cisplatin undergoes non-enzymatic metabolism and predominantly renal elimination; therefore, dosage adjustment for liver dysfunction is likely not necessary.

5.3.4 Neurologic toxicity

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

5.3.5 Gastrointestinal Toxicity

For Grade 3 or 4 vomiting, despite maximal antiemetic medical intervention with aprepitant, corticosteroids, and 5HT-3 antagonists (e.g.ondansetron), split cisplatin dosing over days 1 and 2, and dose reduce methotrexate, doxorubicin and vinblastine by one dose level following resolution to grade < 1. If Grade 3 or 4 vomiting occurs despite cisplatin split dosing and maximal antiemetic therapy, with dose reduced methotrexate, doxorubicin and vinblastine, remove the patient from protocol treatment.

5.3.6 Mucositis / Stomatitis

For Grade > 2 toxicity, delay infusion until it resolves to Grade < 1. Begin leucovorin 15 mg PO TID until recovery. Reduce methotrexate by one dose level for this and all subsequent cycles.

5.3.7 Other toxicities

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

Gemcitabine and Carboplatin - Treatment arm (Arm B)

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5.3.8 Hematologic toxicity and febrile neutropenia

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 pegfilgrastim with subsequent cycles following day 9, if not receiving. If the delay is for more than a week and pegfilgrastim has not been used, include pegfilgrastim in this and all subsequent cycles following day 9.

If there is a dose delay of more than one week due to neutropenia despite pegfilgrastim use, reduce 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 ANC ≥ 500 and ≤ 999, decrease gemcitabine by two dose levels and include pegfilgrastim this and in all subsequent cycles following day 9, if not receiving.

For ANC < 500, hold gemcitabine and include pegfilgrastim in all subsequent cycles following day 9, if not receiving. If ANC < 500 despite pegfilgrastim use, reduce gemcitabine by one dose level for all subsequent cycles.

Day 8:

For platelets ≥ 75,000 - < 100,000, reduce gemcitabine by one dose level for this and all subsequent doses.

For platelets ≥ 50,000 – < 75,000 reduce gemcitabine by two dose levels for this and all subsequent doses.

For platelets < 50,000, hold gemcitabine and reduce gemcitabine by one dose level for all subsequent doses.

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. If patient recovers in 2 weeks or less, add pegfilgrastim if not previously included, continuing at the same dose level. If pegfilgrastim had been utilized, then reduce gemcitabine by one dose level for all subsequent doses and continue pegfilgrastim. If recovery does not occur in 2 weeks, no further chemotherapy should be given and the patient should proceed per protocol to surgery.

5.3.9 Kidney dysfunction

Calculate the CrCl on Day 1 of each cycle. If CrCl is < 30 ml/min, hold chemotherapy for up to 2 weeks. If the CrCl does not improve to ≥ 30 ml/min in 2 weeks despite hydration and other supportive measures, discontinue the chemotherapy and proceed to surgery.

There are no carboplatin dose reductions for kidney dysfuntion.

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5.3.10 Liver dysfunction

Gemcitabine: For a total bilirubin of > 1.6 (or < 2.5 X ULN in those with Gilbert's syndrome), reduce one dose level.

Carboplatin undergoes minimal hepatic metabolism therefore dosage adjustment may not be needed.

5.3.11 Neurologic toxicity

For Grade 3 neuropathy, hold chemotherapy until this resolves to Grade < 2 and resume at same dose level. For Grade 4 neuropathy, remove the patient from protocol treatment.

5.3.12 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 all subsequent doses of gemcitabine. If Grade 3 or 4 vomiting occurs despite one dose reduction in chemotherapy and maximal antiemetic therapy, remove the patient from protocol treatment.

5.3.13 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 chemotherapy and monitor the patient at least weekly. If toxicity resolves to Grade 1 or completely resolves in ≤ 2 weeks, reduce the gemcitabine by one dose level for this and all subsequent doses. Any single delay in chemotherapy of more than 2 weeks or cumulative delays of > 4 weeks will lead to removal from protocol treatment and referral to surgery.

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5.4 Supportive Care

5.4.1 All supportive measures consistent with optimal patient care will be given throughout the study. Use of erythroid stimulating agents (ESAs) is prohibited per NCCN guideline recommendation as treatment on this protocol is given with curative intent. Transfusion support consistent with routine clinical practice can be utilized.

5.4.2 Suggested supportive care for all patients on the **AMVAC treatment arm (Arm A)** are:

Aprepitant or fosaprepitant	Aprepitant 125 mg po day 1 and 80 mg po days 2 and 3 or bioequivalent Fosaprepitant 150 mg IV day 1 only
Ondansetron Or Palonosetron	Ondansetron 24 mg IV Day 1 8 mg po q8 days 4-14 prn Palonosetron 0.25 mg IV day 1 with Ondansetron 8 mg po q8h PRN days 3-14.
Dexamethasone	12 mg IV Day 1 4 mg PO BID Days 2 and 3
Compazine	10 mg every 6 hrs prn
Salt and Soda Mouth rinse	1 mouthful Swish and spit After every meal or snack, at least 5 times daily.

Proton pump inhibitor	PO Daily or BID for duration of chemotherapy
Benzodiapine (Ativan)	0.5 - 1 mg q 8 hrs prn
Intravenous hydration	Appropriate pre- and post hydration with total of 2 liters of fluid should be administered.
Electrolyte support	Pre-treatment and repletion with potassium and magnesium IV / po per institutional guidelines
Diuresis	Mannitol or furosemide diuresis recommended per institutional guidelines
Myeloid growth factor support	**Neulasta 6 mg sq 24-48 hours following chemotherapy is a REQUIRED component of this regimen**

For patients on the **Gemcitabine Carboplatin arm (Arm B)** of the study, suggested supportive therapies should include hydration and antiemetic prophylaxis per institutional guidelines:

Aprepitant or fosaprepitant	Aprepitant 125 mg po day 1 and 80 mg po days 2 and 3 or bioequivalent Fosaprepitant 150 mg IV day 1 only May reserve in cycle 1 at investigator discretion
Ondansetron Or Palonosetron	Ondansetron 24 mg IV Day 1 8 mg po q8 days 4-14 prn Palonosetron 0.25 mg IV day 1 with Ondansetron 8 mg po q8h PRN days 3-21.
Compazine	10 mg every 6 hrs prn
Dexamethasone	12 mg IV Day 1 4 mg PO BID Days 2 and 3 at investigator discretion
Salt and Soda Mouth rinse	1 mouthful Swish and spit After every meal or snack, at least 5 times daily.
Proton pump inhibitor	PO Daily or BID for duration of chemotherapy at investigator discretion
Benzodiapine (Ativan)	0.5 - 1 mg q 8 hrs prn
Intravenous hydration	Appropriate pre- hydration with at least 500 cc normal saline per institutional guidelines.
Electrolyte support	Pre-treatment and repletion with potassium and magnesium IV / po per institutional guidelines
Myeloid growth factor support	Use of prophylactic myeloid growth factor support at investigator discretion per NCCN guidelines for use of myeloid growth factors

5.5 Duration of Therapy

Patients will receive protocol therapy unless:

5.5.1 Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued. In this event submit forms according to the instructions in the EA8141 Forms Packet.

5.5.2 Patient withdraws consent.

5.5.3 Patient experiences unacceptable toxicity.

5.5.4 Non-protocol therapies are administered.

5.5.5 Patient completes 4 cycles of therapy and surgery per Section 5.3.

5.5.6 Development of clinically evident metastatic disease during chemotherapy. **Note:** patients in whom disease progression is noted on post-chemotherapy preoperative imaging, may stay on study and proceed to surgery at discretion of the surgeon if he/she feels the patient can undergo surgical resection for cure.

5.6 Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed 5 years from the date of registration. Patients will be followed by their urologists or medical oncologists according to standard institutional practices. Systemic recurrence-free survival, bladder recurrence-free survival and overall survival information will be collected every 3 months for 2 years, and every 6 months for 3-5 years.

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6. Measurement of Effect

6.1 Pathologic Complete Response

Pathologic proof of a clinically complete response during pathologic evaluation of nephrectomy/ureterectomy and any identifiable regional lymph nodes.

6.2 Diagnosis of Urothelial Cancer Recurrence

The diagnosis of urothelial cancer recurrence can be made only when the clinical and pathology findings meet one of the following criteria.

- Urothelial carcinoma noted on cytology or biopsy.
- Radiologic evidence on CT or MRI felt to be consistent with recurrence.

Any recurrence of malignant disease should be proven by core needle biopsy whenever possible. At the time of tumor recurrence the investigator should clearly indicate the site of tumor recurrence (e.g. bladder, contralateral upper tract) and whether multiple sites are involved.

Supporting documentation (copies of radiology, pathology, and if relevant, surgery reports) must be submitted using Medidata Rave following diagnosis of urothelial carcinoma recurrence or second primary cancer. Refer to the EA8141 Forms Completion Guidelines for data to be collected. If biopsy material is available, a copy of the pathology report should also accompany the materials submission to the CBPF.

NOTE: Development of urothelial cancer of the bladder and/or contralateral upper urinary tract will not constitute systemic recurrence and will be considered as bladder recurrence.

6.3 Diagnosis of Systemic Recurrence (Distant Recurrence)

The diagnosis of systemic cancer recurrence can be made only when the clinical and pathology findings meet one of the following criteria.

- Carcinoma noted on cytology or biopsy.
- Radiologic evidence on CT or MRI felt to be consistent with recurrence.

Any recurrence of malignant disease should be proven by core needle biopsy whenever possible. At the time of tumor recurrence the investigator should clearly indicate the site of tumor recurrence (i.e. non-urothelial) and whether multiple sites are involved.

Supporting documentation (copies of radiology, pathology, and if relevant, surgery reports) must be submitted using Medidata Rave following diagnosis of systemic carcinoma recurrence or second primary cancer. Refer to the EA8141 Forms Completion Guidelines for data to be collected. If biopsy material is available, a copy of the pathology report should also accompany the materials submission to the CBPF.

NOTE: Development of urothelial cancer of the bladder and/or contralateral upper urinary tract will not constitute systemic recurrence and will be considered as bladder recurrence.

6.4 Distant Recurrence-Free Surviva

The time from the date of surgery to distant recurrence or death from any cause. Patients alive without documented distant recurrence will be censored at the date of last disease assessment.

6.5 Bladder Cancer-Free Survival

The time from the date of surgery to the earliest return of bladder cancer or death from any cause. Patients alive without documented bladder cancer will be censored at the date of last disease assessment.

6.6 Disease Progression

Disease progression will be assessed using RECIST 1.1 (see Section 6.12) in the following settings:

- From registration to surgery for all patients.
- For patients who are not deemed disease-free after surgery.

6.7 Second Primary Cancer

Second primary cancer is defined as any cancer other than localized breast cancer, localized prostate cancer, or non-melanoma skin cancer.

The diagnosis of a second primary cancer must be confirmed histologically whenever possible.

6.8 Local, Regional Recurrence

Local regional recurrence is defined as any pathologic or radiographic evidence of recurrent disease within the patient's primary upper tract urothelial carcinoma surgical field, or in the regional lymph nodes.

6.9 Event-Free Survival

The time from registration to the earliest occurrence of recurrence of any type, disease progression, new invasive primary cancer, or death from any cause.

6.10 Overall Survival

Time from registration to date of death from any cause.

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6.11 Renal Function Endpoints

Renal insufficiency is defined as CrCl < 60 ml/min.

NOTE: Standardized measures of renal function will be compared pre and post combined therapy. Thus all patients will have at least 2 assessments by 24 hour urine for creatinine clearance. One measure will be in screening, and another following completion of chemotherapy and nephroureterectomy, and will be thus timed following NU.

Assessments of renal function will occur with each cycle. Patients must continue to meet CrCl eligibility criteria in their chemotherapy group before proceeding on next cycle. This can be assessed by Cockroft-Gault or 24 hr urine. Thus at the least, patients with have two

24 hour urine assessments, and at most, 6, adding in one for every cycle.

6.12 Antitumor Effect – Solid Tumors

Prior to patient receiving protocol surgery, response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in RECIST.

Patients will also be evaluated according to RECIST criteria in the event that after protocol surgery, patient's resection status is not R0 (e.g., disease is still present after protocol surgery). In the event that this occurs, a new baseline reference point will be established based on the post-surgery scan.

The following general principles must be followed:

1. To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. All baseline evaluations should be performed as closely as possible to the beginning of treatment and **never more than four weeks** before registration.
2. All measurements should be recorded in metric notation by use of a ruler or calipers.
3. The same method of assessment and the same technique must be used to characterize each identified lesion at baseline and during follow-up.

6.12.1 Disease Parameters

Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters.

NOTE: Tumor lesions that are situated in a previously irradiated area are not considered measurable.

Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in **short** axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the **short** axis will be measured and followed.

Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as

non-measurable. Non-measurable also includes lesions that are < 20 mm by chest x-ray.

NOTE: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease

Non-target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow-up.

6.12.2 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before registration.

The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest X-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up must be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations

cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy

The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor Markers

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, Histology

These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

6.12.3 Response Criteria

6.12.3.1 Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR)

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

NOTE: For patients that have disease presence after protocol surgery, the new baseline reference point established on post-protocol surgery scans should be used.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression, See Section 6.12.3.3).

NOTE: For patients that have disease presence after protocol surgery, refer to the smallest sum post-surgery.

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. (Note: a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease)

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 8 weeks.

NOTE: For patients that have disease presence after protocol surgery, stable disease can be assigned as a status if measurements met criteria at least once after surgery at a minimal interval of 8 weeks.

6.12.3.2 Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis)

Non-CR/Non-PD

Persistence of one or more non-target lesion(s).

Progressive Disease (PD)

Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions (see Section 6.12.3.3). *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

When the patient also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest “increase” in the size of one or more non-target lesions is

usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from “trace” to “large”, an increase in nodal disease from “localized” to “widespread”, or an increase sufficient to require a change in therapy.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.12.3.3 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it:

a) increases in size to ≥ 15 mm in the short axis, or
b) there is new pathological confirmation that it is disease (regardless of size).

6.12.3.4 Evaluation of Best Overall Response

Prior to protocol surgery (or for those patients who do not receive protocol surgery), the best overall response is the best response recorded from the start of the treatment until disease progression/recurrence or non-protocol therapy (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria.

After protocol surgery, if patient has presence of disease, the best overall response is the best response recorded from the surgery until disease/progression/recurrence or non-protocol therapy (taking as reference for progressive disease the smallest measurements recorded since the surgery). The patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 6.1: Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions*	Best Overall Response	Remarks
CR	CR	No	CR	
CR	Non-CR/Non-PD***	No	PR	
CR	Not evaluated	No	PR	
PR	Non-PD***/not evaluated	No	PR	
SD	Non-PD***/not evaluated	No	SD	Documented at least once ≥ 8 wks. from study entry (or surgery if patient had disease presence post-protocol surgery)
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD**	Yes or No	PD***	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

*** PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to the Evaluation of Non-Target Lesions – Progressive Disease (Section 6.12.3.2) for further explanation.

NOTE: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

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7. Study Parameters

All required prestudy evaluations must be completed within 28 days of registration unless otherwise specified.

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7.1 Arm A: AMVAC

PHYSICAL	Pre study	Week1 C1	Week 3 C2	Week 5 C3	Week 7 C4	Pre NU	NU	Post NU[9]	Follow-up[11]
History and Physical Exam (Ht/WT included)	X	X	X	X	X			X	X
ECOG performance status	X	X	X	X	X			X	X
Toxicity evaluation	X	X	X	X	X			X	
LABORATORY									
CBC with Differential[1]	X	X	X	X	X	X[7,14]		X	X
Serum or Urine Pregnancy Test[2]	X								
Comprehensive metabolic panel[3]	X	X	X	X	X	X[7,14]		X	X
Creatinine clearance[4]	X	X	X	X	X	X[14]		X[10]	
RADIOLOGIC STUDIES									
Radiographic staging[5]	X					X[14]		X[13]	X
Bone scan[6]	X					X[14]			X
ECHO or MUGA	X								
EKG	X								
TREATMENT									
Methotrexate		X	X	X	X				
Vinblastine		X	X	X	X				
Adriamycin		X	X	X	X				
Cisplatin		X	X	X	X				
Pegfilgrastim or filgrastim		X	X	X	X				
Nephroureterectomy (NU)							X[8]		
Biospecimen Submissions[12]									
FFPE tumor tissue	X						X		
Serum, red top tube	X					X		X	
Peripheral Blood, EDTA	X					X		X	
Plasma, EDTA	X					X		X	
Urine, Fresh Catch	X					X		X	

1. CBCs (with differential and platelet count) which includes WBC, ANC, Platelets, Hgb, and Hct required for protocol therapy must be done ≤ 2 days prior to the treatment cycle. For cycle 1 day1, labs done < 5 days prior will be accepted.
2. For women of childbearing potential. Must be done within 2 weeks prior to enrollment.
3. Should include potassium, magnesium, phosphorus. Electrolyte levels should be monitored during the study and repleted per standard of care. Total bilirubin, AST and ALT should be included. Laboratory assessments must be done ≤ 2 days prior to the treatment cycle. For cycle 1 day 1, labs done < 5 days prior will be accepted.
4. Standardized measures of renal function will be compared pre and post combined therapy. Thus all patients will have at least 2 assessments by 24 hour urine for creatinine clearance. One measure will be in screening, and another following completion of chemotherapy and nephroureterectomy, and will be thus timed following NU.

   The creatinine clearance should be measured by 24-hour urine or calculated by Cocroft-Gault equation prior to each cycle. Patients must continue to meet creatinine clearance eligibility criteria in their arm to continue therapy; 24-hour urine assessment for creatinine clearance can be measured if needed.
5. Radiographic imaging done at baseline should be continued throughout protocol if allowable. Assessments should include contrast enhanced CT chest/abdomen and pelvis, or MRI abdomen and pelvis with gadolinium with a non-contrast CT chest. Non-contrast CT chest/abdomen and pelvis are allowable if gadolinium is contraindicated.
6. Nuclear medicine bone scan should be done for suspicion of bone lesions or elevated alkaline phosphatase.
7. Post chemotherapy CBC with differential, comprehensive metabolic panel should be performed within 14 days prior to and up to one day before nephroureterectomy.
8. Nephroureterectomy and lymph node dissection must be completed 21 to 60 days from completion of chemotherapy.
9. Protocol completion visit 2-6 weeks from date of surgery.
10. 24-hour urine creatinine clearance assessment post therapy +/- 10 days from completion visit.
11. All patients, including those who discontinue protocol therapy early, will be followed according to standard institutional practices (see NCCN guidelines for guidance) until progression/systemic recurrence, even if non-protocol therapy is initiated, and for survival for 5 years from the date of registration. If patients develop a local recurrence (bladder or contralateral upper urinary tract), they must still be followed for response until development of a systemic recurrence. Patients will be followed by their urologists or medical oncologists according to standard institutional practices, but systemic recurrence-free survival, bladder cancer-free survival, cancer specific survival, and overall survival information will be collected every 3 months for 2 years, and every 6 months for 3-5 years.
12. Refer to Section 10 – submission of biospecimens for research, for instructions.
13. Scans must be obtained within 6 months of patient's nephroureterectomy.
14. Scans and labs must be done at least 1 week after the last dose of chemotherapy, but no later than 1 calendar day prior to surgery.

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7.2 Arm B: GCa

PHYSICAL	**Pre study**	**C1D1**	**C1D8**	**C2D1**	**C2D8**	**C3D1**	**C3D8**	**C4D1**	**C4D8**	**Pre NU**	**NU**	**Post NU[9]**	**Follow-up[11]**
History and Physical Exam (Ht/WT included)	X	X		X		X		X				X	X
ECOG performance status	X	X		X		X		X				X	X
Toxicity evalutation	X	X		X		X		X					
LABORATORY													
CBC with Differential[1]	X	X	X	X	X	X	X	X	X	X[7,14]		X	X
Serum or Urine Pregnancy Test[2]	X												
Comprehensive metabolic panel[3]	X	X		X		X		X		X[7,14]		X	X
Creatinine clearance[4]	X	X		X		X		X		X[14]		X[10]	
RADIOLOGIC STUDIES													
Radiographic staging[5]	X									X[14]		X[13]	X
Bone scan[6]	X									X[14]			X
ECHO or MUGA	X												
EKG	X												
TREATMENT													
Carboplatin		X		X		X		X					
Gemcitabine		X	X	X	X	X	X	X	X				
Nephroureterectomy (NU)											X[8]		
Biospecimen Submissions [12]													
FFPE tumor tissue	X										X		
Serum, red top tube	X									X		X	
Peripheral Blood, EDTA	X									X		X	
Plasma, EDTA	X									X		X	
Urine, Fresh Catch	X									X		X	

1. CBCs (with differential and platelet count) which includes WBC, ANC, Platelets, Hgb, and Hct required for protocol therapy must be done ≤ 2 days prior to the treatment cycle. For cycle 1 day1, labs done < 5 days prior will be accepted.
2. For women of childbearing potential. Must be done within 2 weeks prior to enrollment.

3. Should include potassium, magnesium, phosphorus. Electrolyte levels should be monitored during the study and repleted per standard of care. Total bilirubin, AST and ALT should be included. Laboratory assessments must be done ≤ 2 days prior to the treatment cycle. For cycle 1 day1, labs done < 5 days prior will be accepted.
4. Standardized measures of renal function will be compared pre and post combined therapy. Thus all patients will have at least 2 assessments by 24 hour urine for creatinine clearance. One measure will be in screening, and another following completion of chemotherapy and nephroureterectomy, and will be thus timed following NU.

   The creatinine clearance should be measured by 24-hour urine or calculated by Cocroft-Gault equation prior to each cycle. Patients must continue to meet creatinine clearance eligibility criteria in their arm to continue therapy; 24-hour urine assessment for creatinine clearance can be measured if needed.
5. Radiographic imaging done at baseline should be continued throughout protocol if allowable. Assessments should include contrast enhanced CT chest/abdomen and pelvis, or MRI abdomen and pelvis with gadolinium with a non-contrast CT chest. Non-contrast CT chest/abdomen and pelvis are allowable if gadolinium is contraindicated.
6. Nuclear medicine bone scan should be done to for suspicion of bone lesions or elevated alkaline phosphatase.
7. Post chemotherapy CBC with differential, comprehensive metabolic panel should be performed within 14 days prior to and up to one day before nephroureterectomy.
8. Nephroureterectomy and lymph node dissection must be completed 21 to 60 days from completion of chemotherapy.
9. Protocol completion visit 2-6 weeks from date of surgery.
10. 24-hour urine creatinine clearance assessment post therapy +/- 10 days from completion visit.
11. All patients, including those who discontinue protocol therapy early, will be followed according to standard institutional practices (see NCCN guidelines for guidance)for response until progression/systemic recurrence, even if non-protocol therapy is initiated, and for survival for 5 years from the date of registration. If patients develop a local recurrence (bladder or contralateral upper urinary tract), they must still be followed for response until development of a systemic recurrence. Patients will be followed by their urologists or medical oncologists according to standard institutional practices, but systemic recurrence-free survival, bladder recurrence-free survival and overall survival information will be collected every 3 months for 2 years, and every 6 months for 3-5 years.
12. Refer to Section 10 – submission of biospecimens for research, for instructions.
13. Scans must be obtained within 6 months of patient's nephroureterectomy.
14. Scans and labs must be done at least 1 week after the last dose of chemotherapy, but no later than 1 calendar day prior to surgery.

8. Drug Formulation and Procurement

All agents in this protocol are commercially available and guideline endorsed treatment options for patients with invasive urothelial cancer. Drug will be obtained from institutional pharmacy supply.

8.1 Methotrexate

8.1.1 Other Names

Folex, abitrexate, mexate

8.1.2 Classification

antimetabolite and antifolate

8.1.3 Mode of Action

Methotrexate is a folate antimetabolite that inhibits DNA synthesis, repair, and cellular replication. Methotrexate irreversibly binds to and inhibits dihydrofolate reductase, inhibiting the formation of reduced folates, and thymidylate synthetase, resulting in inhibition of purine and thymidylic acid synthesis, thus interfering with DNA synthesis, repair, and cellular replication.

8.1.4 Storage and Stability

Store at room temperature 20° to 25°C (68° to 77°F). Protect from light. Opened vials are stable for 24 hours

8.1.5 Dose Specifics

30 mg/m2

8.1.6 Preparation

I.V.: Solution diluted in D_5W or NS

8.1.7 Route of Administration

IV push over 2-3 minutes

8.1.8 Incompatibilities

Chlorpromazine, gemcitabine, idarubicin, ifosfamide, midazolam, nalbuphine, promethazine, propofol.

8.1.9 Availability

8.1.10 Side Effects

Cardiovascular: Arterial thrombosis, cerebral thrombosis, chest pain, deep vein thrombosis, hypotension, pericardial effusion, pericarditis, plaque erosion (psoriasis), pulmonary embolism, retinal thrombosis, thrombophlebitis, vasculitis

Central nervous system: Dizziness (≤3%), headache (pJIA 1%), abnormal cranial sensation, brain disease, chemical arachnoiditis (intrathecal; acute), chills, cognitive dysfunction (has been reported at low dosage), drowsiness, fatigue, leukoencephalopathy (intravenous administration after craniospinal irradiation or repeated high-dose

therapy; may be chronic), malaise, mood changes (has been reported at low dosage), neurological signs and symptoms (at high dosages; including confusion, hemiparesis, transient blindness, seizures, and coma), severe neurotoxicity (reported with unexpectedly increased frequency among pediatric patients with acute lymphoblastic leukemia who were treated with intermediate-dose intravenous methotrexate), speech disturbance

Dermatologic: Alopecia (≤10%), burning sensation of skin (psoriasis 3% to 10%), skin photosensitivity (3% to 10%), skin rash (≤3%), dermatitis (rheumatoid arthritis 1% to 3%), pruritus (rheumatoid arthritis 1% to 3%), acne vulgaris, dermal ulcer, diaphoresis, ecchymoses, erythema multiforme, erythematous rash, exfoliative dermatitis, furunculosis, hyperpigmentation, hypopigmentation, skin abnormalities related to radiation recall, skin necrosis, Stevens-Johnson syndrome, telangiectasia, toxic epidermal necrolysis, urticaria

Endocrine & metabolic: Decreased libido, decreased serum albumin, diabetes mellitus, gynecomastia, menstrual disease

Gastrointestinal: Diarrhea (≤11%), nausea and vomiting (≤11%), stomatitis (2% to 10%), abdominal distress, anorexia, aphthous stomatitis, enteritis, gastrointestinal hemorrhage, gingivitis, hematemesis, intestinal perforation, melena

Genitourinary: Azotemia, cystitis, defective oogenesis, defective spermatogenesis, dysuria, hematuria, impotence, infertility, oligospermia, pancreatitis, proteinuria, severe renal disease, vaginal discharge

Hematologic & oncologic: Thrombocytopenia (rheumatoid arthritis 3% to 10%; platelet count <100,000/mm3), leukopenia (1% to 3%; WBC <3000/mm3), pancytopenia (rheumatoid arthritis 1% to 3%), agranulocytosis, anemia, aplastic anemia, bone marrow depression (nadir: 7-10 days), decreased hematocrit, eosinophilia, gastric ulcer, hypogammaglobulinemia, lymphadenopathy, lymphoma, lymphoproliferative disorder, neutropenia, non-Hodgkin's lymphoma (in patients receiving low-dose oral methotrexate), tumor lysis syndrome

Hepatic: Increased liver enzymes (14% to 15%), cirrhosis (chronic therapy), hepatic failure, hepatic fibrosis (chronic therapy), hepatitis (acute), hepatotoxicity

Hypersensitivity: Anaphylactoid reaction

Infection: Cryptococcosis, cytomegalovirus disease (including cytomegaloviral pneumonia, sepsis, nocardiosis), herpes simplex infection, herpes zoster, histoplasmosis, infection, pneumonia due to pneumocystis jiroveci, vaccinia (disseminated; following smallpox immunization)

Neuromuscular & skeletal: Arthralgia, myalgia, myelopathy (subacute), osteonecrosis (with radiotherapy), osteoporosis, stress fracture

Ophthalmic: Blurred vision, conjunctivitis, eye pain, visual disturbance

Otic: Tinnitus

Renal: Renal failure

Respiratory: Interstitial pneumonitis (rheumatoid arthritis 1%), chronic obstructive pulmonary disease, cough, epistaxis, pharyngitis, pneumonia, pulmonary alveolitis, pulmonary disease, pulmonary fibrosis, respiratory failure, upper respiratory tract infection

Miscellaneous: Fever, nodule, tissue necrosis

8.1.11 Nursing/Patient Implications

Use with caution in conditions such as renal impairment, peptic ulcer disease, ulcerative colitis, pericardial or pleural effusion, hepatic impairment, or bone marrow suppression. Vesicant.

8.1.12 References

lexicomp

8.2 Drug Name - Vinblastine

8.2.1 Other Names

vinblastine sulfate

8.2.2 Classification

vinka alkaloid

8.2.3 Mode of Action

Binds to tubulin and inhibits microtubule formation, therefore, arresting the cell at metaphase by disrupting the formation of the mitotic spindle; it is specific for the M and S phases. Vinblastine may also interfere with nucleic acid and protein synthesis by blocking glutamic acid utilization.

8.2.4 Storage and Stability

Store intact vials under refrigeration at 2°C to 8°C (36°F to 46°F). Protect from light. Solutions reconstituted in bacteriostatic NS are stable for 28 days under refrigeration.

8.2.5 Dose Specifics

3 mg/m^2

8.2.6 Preparation

Hazardous agent; use appropriate precautions for handling and disposal (NIOSH, 2012). Reconstitute lyophilized powder to a concentration of 1 mg/mL with NS or bacteriostatic NS. For infusion, may dilute in 50 mL NS or D5W; dilution in larger volumes (≥100 mL) of I.V. fluids is not recommended.

8.2.7 Route of Administration

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IV infusion

8.2.8 Incompatibilities

Cefepime, furosemide.

8.2.9 Availability

Commercial supply.

8.2.10 Side Effects

Cardiovascular: Hypertension, Angina, cerebrovascular accident, coronary ischemia, ECG abnormalities, limb ischemia, MI, myocardial ischemia, Raynaud's phenomenon

Central nervous system: Malaise, Depression, dizziness, headache, neurotoxicity (duration: >24 hours), seizure, vertigo

Dermatologic: Alopecia, Dermatitis, photosensitivity (rare), rash, skin blistering

Gastrointestinal: Constipation, Abdominal pain, anorexia, diarrhea, gastrointestinal bleeding, hemorrhagic enterocolitis, ileus, metallic taste, nausea (mild), paralytic ileus, rectal bleeding, stomatitis, toxic megacolon, vomiting (mild)

Endocrine & metabolic: Aspermia, hyperuricemia, SIADH

Genitourinary: Urinary retention, Hemolytic uremic syndrome

Hematologic: Myelosuppression, leukopenia/granulocytopenia (nadir: 5-10 days; recovery: 7-14 days; dose-limiting toxicity), Anemia, thrombocytopenia (recovery within a few days), thrombotic thrombocytopenic purpura

Neuromuscular & skeletal: Bone pain, jaw pain, tumor pain, Deep tendon reflex loss, myalgia, paresthesia, peripheral neuritis, weakness

Ocular: Nystagmus

Otic: Auditory damage, deafness, vestibular damage

Respiratory: Bronchospasm, dyspnea, pharyngitis

8.2.11 Nursing/Patient Implications

Vesicant. intrathecal administration is contraindicated.

8.2.12 References

lexicomp

8.3 <u>Gemcitabine</u>

8.3.1 Other Names

2'-Deoxy-2',2'-difluorocytidine monohydrochloride, Gemzar

8.3.2 Classification

Antimetabolite (nucleoside pyrimidine analogue)

8.3.3 Mode of Action

Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S phase) and also blocking the progression of cells through the G1/S phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

8.3.4 Storage and Stability

Unreconstituted drug vials are stored at controlled room temperature (15ºC to 30ºC, 59ºF to 86ºF). Reconstituted solution should be stored at controlled room temperature and used within 24 hours. Solutions of gemcitabine should not be refrigerated; as crystallization may occur. The unused portion should be discarded.

8.3.5 Dose Specifics

1000 mg/m2 IV over 30-60 minutes on Days 1 and 8 of each cycle. A Cycle is 21 days.

8.3.6 Preparation

Reconstitute the 200 mg vial with 5ml and the 1 gm vial with 25 ml preservative free normal saline to make a solution containing 38 mg/ml. Shake to dissolve. Gemcitabine may be further diluted with NS as per institutional standards.

8.3.7 Route of Administration

IV infusion.

8.3.8 Incompatibilities

No information available.

8.3.9 Availability

Gemcitabine is commercially available in 200 mg and 1 gm vials.

8.3.10 Side Effects

1. Hematologic: Neutropenia, anemia, thrombocytopenia, and leukopenia are reported.
2. Dermatologic: A rash is seen in about 25% of patients and is associated with pruritus in about 10% of patients. The rash is usually mild, not dose-limiting, and responds to local therapy. Desquamation, vesiculation, and ulceration have been reported rarely. Alopecia is usually minimal. Injection-site reactions.
3. Gastrointestinal: Nausea and vomiting are reported in about two-thirds of patients and requires therapy in about 20% of patients. It is rarely dose limiting, and is easily manageable with standard antiemetics. Diarrhea, constipation, mucositis.
4. Hepatic: Abnormalities of hepatic transaminase enzymes occur in two-thirds of patients, but they are usually mild, nonprogressive, and rarely necessitate stopping treatment. However, gemcitabine should be used with caution in patients with impaired hepatic function.
5. Pulmonary: Bronchospasm and/or dyspnea within a few hours of infusion of the drug, cough, rhinitis, pneumonitis.
6. Neurologic: Somnolence, insomnia, paresthesia, pain.
7. Cardiovascular: A few cases of hypotension were reported. Some cases of myocardial infarction, congestive heart failure, and arrhythmias have been reported. Peripheral edema is reported in about 30% of patients. Some cases of facial edema have also been reported. Edema is usually mild to moderate, rarely dose-limiting, sometimes painful, and reversible after stopping gemcitabine treatment.
8. Other: Flu-like symptoms are reported for about 20% of patients. This includes fever, headache, back pain, chills, myalgia, asthenia, and anorexia. Malaise and sweating are reported.

8.3.11 Nursing/Patient Implications

1. If the patient reports burning at the injection site, slow down rate to allow the dose to run in over 1 hour.
2. Rash can be treated with topical therapy or the administration of diphenhydramine prior to administration.
3. Flu-like symptoms can be treated with acetaminophen.

8.4 Doxorubicin

8.4.1 Other Names

Adriamycin R, Rubex R, Adriamycin RDF R, Adriamycin PFS R, hydroxydaunorubicin, hydroxydaunomycin, ADR.

8.4.2 Classification

Anthracycline antibiotic.

8.4.3 Mode of Action

Intercalation between adjoining nucleotide pairs in the DNA helix causes inhibition of DNA and DNA-dependent RNA synthesis. Free radical generation is responsible for cardiac toxicity. Doxorubicin also inhibits topoisomerase II.

8.4.4 Storage and Stability

Rubex or Adriamycin RDF intact vials are stable protected from light at room temperature. Adriamycin PFS vials must be refrigerated. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours under refrigeration. The Adriamycin RDF 150 mg multidose vial is stable after reconstitution for 7 days at room temperature or 15 days if refrigerated and protected from sunlight.

8.4.5 Dose Specifics

The usual dose is 30 mg/m^{2}day 1 slow IV push.

8.4.6 Preparation

Add 5, 10, 25, 50, or 75 ml of preservative-free normal saline to the 10, 20, 50, 100, or 150 mg vial to produce a solution containing 2 mg/ml.

8.4.7 Administration

Intravenously, either as a bolus injection or as a continuous infusion through a central venous line.

8.4.8 Incompatibilities

Physically incompatible with heparin, fluorouracil, aminophylline, cephalothin, dexamethasone, diazepam, hydrocortisone, and furosemide.

8.4.9 Compatibilities

Stable with vincristine in normal saline for five days at room temperature protected from light. Also compatible in solution with cyclophosphamide.

8.4.10 Availability

Commercially available as powder for injection in 10, 20, 50, 100, 150 mg vials, and as 2 mg/ml solution for injection in 10, 20, 50, and 200 mg vials.

8.4.11 Side Effects

1. Hematologic: Leukopenia (dose-limiting), also thrombocytopenia and anemia. Nadir 10-14 days, recovery in 21 days.
2. Dermatologic: Alopecia, usually complete; hyperpigmentation of nailbeds and dermal creases; radiation recall.
3. Gastrointestinal: Nausea and vomiting, sometimes severe; anorexia, diarrhea; mucositis, especially with daily x 3 schedule.
4. Cardiovascular: Arrhythmias, ECG changes; rare sudden death. Congestive heart failure due to cardiomyopathy related to total

cumulative dose; risk is greater with total doses > 550 mg/m^2, mediastinal irradiation pre-existing cardiac disease, advanced age; risk is reduced with weekly or continuous infusion regimens.

5. Other: Red discoloration of urine; fever; anaphylactoid reaction; may enhance cyclophosphamide cystitis or mercaptopurine hepatotoxicity.
6. Local effects: Vesicant if extravasated; flush along vein, facial

8.4.12 Nursing Implications

1. Monitor CBC, platelet counts.
2. Vesicant - do not extravasate. Refer to extravasation protocol if inadvertent infiltration occurs.
3 Advise patient of alopecia. Instruct on how to obtain wig, hairpiece, etc. Hair loss generally occurs 2-4 weeks after injection and is usually complete.
4. Advise patient of red discoloration of urine for 24 hours after administration of the drug.
5. Administer antiemetics as indicated.
6. Assess for stomatitis and treat symptomatically. Generally occurs 7-10 days after injection.
7. Be aware of "Adria" flare - most common reaction consists of an erythematous streak up the vein. It is associated with urticaria and pruritus. Occasionally the use of corticosteroids and/or antihistamines has been useful.
8. Monitor for signs and symptoms of cardiomyopathy. Calculate total cumulative dose with each administration.

NOTE: Please refer to the commercially-available package labeling for more information.

8.4.13 References

Speth PA. Clinical pharmacokinetics of doxorubicin. Clin Pharmacokinetics 15:51-31, 1988.

Von Hoff DD, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91:710-17, 1979.

Lum BL et al. Doxorubicin: Alteration of dose scheduling as a means of reducing cardiotoxicity. Drug Intell Clin Pharm 19:259-64, 1985.
ECOG 2/91

8.5 Cisplatin

8.5.1 Other Names

Cisdiaminedichloroplatinum, Cis-diaminedichloroplatinum (II), diaminedichloroplatinum, cis-platinum, platinum, Platinol, Platinol-AQ, DDP, CDDP, DACP, NSC 119875.

8.5.2 Classification

Alkylating agent

8.5.3 Mode of Action

Inhibits DNA synthesis by forming inter- and intra-strand crosslinks. Other possible mechanisms include chelation of DNA and binding to cell membranes thereby stimulating immune mechanisms.

8.5.4 Storage and Stability

Inhibits DNA synthesis by forming inter- and intra-strand crosslinks. Other possible mechanisms include chelation of DNA and binding to cell membranes thereby stimulating immune mechanisms.

8.5.5 Dose Specifics

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Cisplatin will be given by IV at a dose of 70 mg/m^2 IV day 1 infusion over at least 2 hours of each cycle (each cycle to be repeated every 3 weeks for a maximum of 4 cycles). At the discretion of the investigator, cisplatin dose can be divided and given 35 mg/m^2 over day 1 and 2.

8.5.5.1 Suggested supportive care for all patients on the AMVAC treatment arm (Arm A) are:

Aprepitant or fosaprepitant	Aprepitant 125 mg po day 1 and 80 mg po days 2 and 3 or bioequivalent Fosaprepitant 150 mg IV day 1 only
Ondansetron Or Palonosetron	Ondansetron 24 mg IV Day 1 8 mg po q8 days 4-14 prn Palenosetron 0.25 mg IV day 1 with Ondansetron 8 mg po q8h prn days 3-14.
Dexamethasone	12 mg IV Day 1 4 mg PO BID Days 2 and 3
Compazine	10 mg every 6 hrs prn
Salt and Soda Mouth rinse	1 mouthful Swish and spit After every meal or snack, at least 5 times daily.
Proton pump inhibitor	PO Daily or BID for duration of chemotherapy
Benzodiapine (Ativan)	0.5 - 1 mg q 8 hrs prn
Intravenous hydration	Appropriate pre- and post hydration with total of 2 liters of fluid should be administered.
Electrolyte support	Pre-treatment and repletion with potassium and magnesium IV / po per institutional guidelines
Diruesis	Mannitol or furosemide diuresis recommended per institutional guidelines
Myeloid growth factor support	**Neulasta 6 mg sq 24-48 hours following chemotherapy is a REQUIRED component of this regimen**

8.5.6 Preparation

The desired dose of cisplatin is diluted per institutional standards.

8.5.7 Administration

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Cisplatin solution should be administered over at least 2 hours.

8.5.8 Incompatibilities

Amsacrine, cefepime, gallium nitrate, mesna, piperacillin, sodium bicarbonate, thiotepa. Cisplatin may react with aluminum which is found in some syringe needles or IV sets, forming a black precipitate.

8.5.9 Compatibilities

Admixture: Amphotericin-B, aztreonam, carmustine, cefazolin, cephalothin, droperidol, etoposide, floxuridine, hydroxyzine, ifosphamide, leucovorin, magnesium sulfate, mannitol, potassium chloride. Y-site: Allopurinol, bleomycin chlorpromazine, cimetidine, cyclophosphamide, dexamethasone, diphenhydramine, doxapram, doxorubicin, famotidine, filgrastim, fludarabine, fluorouracil, furosemide, ganciclovir, heparin, hydromorphone, lorazepam, melphalan, methotrexate, methylprednisolone, metoclopramide, mitomycin, morphine, ondansetron, paclitaxel, prochlorperazine, ranitidine, sargramostim, vinblastine, vincristine, vinorelbine.

Consult your pharmacist regarding specific concentrations

8.5.10 Availability

Commercially available as a mg/mL solution in 50 and 100 mg vials.

8.5.11 Side Effects

Renal: A dose-related, cumulative renal tubular injury can occur; adequate hydration and diuresis usually minimize the risk. Salt-wasting nephropathy and/or orthostatic hypotension with hyporeninemic hypoaldosteronism can occur in up to 10% of patients.

Neurologic: A dose-related ototoxicity, manifested by high-frequency hearing loss and tinnitus, occurs in about 30% of patients. Paresthesias, decreased vibratory, position, and touch sensations are less common; particularly at cumulative doses < 400 mg/m^2.

Hematologic: Mild leukopenia and thrombocytopenia occur in 25-30% of patients, but are rarely dose-limiting; anemia is less common. A potentially fatal hemolytic uremic syndrome has been reported.

Gastrointestinal: Severe, dose-limiting nausea and vomiting occur in almost 100% of patients unless adequate antiemetic prophylaxis is given. Even with successful prophylaxis of acute nausea a delayed (72-96 hour) reaction, requiring additional therapy may occur. Anorexia and taste changes may also occur.

Hypersensitivity: Allergic reactions are reported in up to 20% of patients Symptoms include: rash, facial edema, wheezing, hypotension, and tachycardia. Severe anaphylaxis is rare.

Other: Electrolyte wasting (magnesium, potassium and sodium), papilledema, optic neuritis, retrobulbar neuritis, diarrhea, mouth sores, hair loss, dizziness, dehydration, nail changes, fatigue, fluid retention,

chills, lowered white blood cell counts, rash, muscle aches, joint pain, headache, confusion, loss of coordination, difficulty swallowing, indigestion, blood clotting in veins, fainting, seizures, difficulty urinating.

8.5.12 Nursing/Patient Implications

1. Prior to administration, assess as per local standards:
   A. Labs: CBC, platelet count, BUN, creatinine.
   B. Urine output: 100-150 mL/hr for at least 4-6 hours.
   C. Signs of ototoxicity or neurotoxicity.
2. Administer supportive medications:
   A. Antiemetics: 5HT3 antagonists and dexamethasone combinations can usually be given once daily. NOTE: Dexamethasone dose should be reduced by 50% when administered with aprepitant.
   B. Hydration.
   C. Diuretics - may be ordered.
   D. ESAs are allowed as per package guidelines.
3. Observe for signs of allergic reaction.

8.5.13 References

Alberts DS. Carboplatin versus cisplatin in ovarian cancer. Semin Oncol 1995;22 (5 Suppl 12):88-90.

Bonomi P. Platinum/etoposide therapy in non-small cell lung cancer. Oncology 1992;49 (Suppl 1):43-50.

Dabholkar M, Reed E. Cisplatin. Cancer Chemother Biol Response Modifiers 1993;14:86-97.

Fram RJ. Cisplatin and platinum analogues: recent advances. Curr Opin Oncol 1992;4:1073-9.

Garrow GC, Johnson, DH. Treatment of "good risk" metastatic testicular cancer. Semin Oncol 1992;19:159-65.

Markman M. Current status of intraperitoneal therapy for ovarian cancer. Curr Opinion Obstet Gynecol 1993;5:99-104.

Ozols RF, et al. Advanced ovarian cancer. Dose intensity. Ann Oncol 1993; (4 Suppl 4):49-56.

Saxman S. Salvage therapy in recurrent testicular cancer. Semin Oncol 1992;19:143-7.

Wheeler RH, Spencer S. Cisplatin plus radiation therapy. J Infusional Chemother 1995;5:61-6.

8.6 Carboplatin

8.6.1 Chemical Name

Carboplatin (carboplatin for injection or platinum diamine [1,1-cyclobutane-decarbozxylate (2—0,0')-,(SP-4-2)]) is a platinum

compound used as a chemotherapeutic agent. It will be supplied commercially.

8.6.2 Formulation

Carboplatin is available as a sterile lyophilized powder in single-dose vials containing 50 mg, 150 mg, or 450 mg of carboplatin. Each vial contains equal parts by weight of carboplatin and mannitol. Commercial supplies of carboplatin will be used in this trial.

8.6.3 Storage and Stability

Intact vials of carboplatin are stable for the period indicated on the package when stored at room temperature (15-30°C or 59-86°F) and protected from light.

When prepared as described above, carboplatin solutions are stable for 8 hours at room temperature if protected from light. The solution should be discarded after 8 hours since no antibacterial preservative is contained in the formulation.

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8.6.4 Dose Specifics and Administration

All patients on protocol treatment will receive Carboplatin at AUC 5 IV infusion over 30-60 minutes, every 21 days for a total of 4 cycles on Arm B. Carboplatin dosing is by the Calvert formula[18]:
Total Dose (mg) = (target AUC) x (CrCl+ 25). See Section 5.1.2 for information on calculation of carboplatin dose.

NOTE: When calculating dose, CrCl should not exceed 125 mL/min. Thus, maximum carboplatin dose for this protocol is 5 x (125+25), or 750mg.

8.6.5 Preparation

Immediately before use, the contents of a carboplatin vial must be reconstituted with either sterile water for injection, USP, 5% dextrose in water, or 0.9% sodium chloride injection, USP. The following shows the proper diluent volumes to be used to obtain a carboplatin concentration of 10 mg/mL. Carboplatin solution can be further diluted to concentrations as low as 0.5 mg/mL with D5W or 0.9% normal saline.

Vial size	Diluent volume
50 mg	5 mL
150 mg	15 mL
450 mg	45 mL

Carboplatin reacts with aluminum to form a precipitate and cause a loss of potency. Therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

8.6.6 Availability

Carboplatin is commercially available

8.6.7 Adverse Events Associated with Carboplatin

Incidence rates of adverse events associated with carboplatin are provided in the product package insert. Some of the adverse events expected with Carboplatin treatment are listed below.

Hematologic:	Myelosuppression is the major dose-limiting toxicity. Thrombocytopenia, neutropenia, leukopenia, and anemia are common, but typically resolve by Day 28 when carboplatin is given as a single agent.
Allergic Reactions:	Hypersensitivity to carboplatin has been reported in 2% of patients receiving the drug. Symptoms include rash, urticaria, erythema, pruritus, and rarely bronchospasm and hypotension. The reactions can be successfully managed with standard epinephrine, corticosteroid, and antihistamine therapy.
Neurologic:	Peripheral neuropathies have been observed in 4% of patients receiving carboplatin with mild paresthesia being the most common.
Gastrointestinal:	Nausea and vomiting are the most common GI events; both usually resolve within 24 hours and respond to antiemetics. Other GI events include diarrhea, weight loss, constipation, and gastrointestinal pain.
Hepatic Toxicity:	Elevated alkaline phosphatase, total bilirubin, and SGOT have been observed.
Other:	Pain and asthenia are the most common miscellaneous adverse events. Alopecia has been reported in 3% of the patients taking carboplatin.

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8.7 Pegfilgrastim

8.7.1 Other Names

Neulasta

NOTE: Auto injector, Neulasta, is permitted for use on this trial

8.7.2 Classification

Leukocyte Growth Factor

8.7.3 Mode of Action

Pegfilgrastim (Neulasta™) is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol. Both pegfilgrastim and filgrastim are colony-stimulating factors that act on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

8.7.4 Storage and Stability

Pegfilgrastim should be stored refrigerated at 2 to 8°C (36to 46°F); syringes should be kept in their carton to protect from light until time of use. Shaking should be avoided. Before injection, pegfilgrastim maybe allowed to reach room temperature for a maximum of 48 hours but should be protected from light. Pegfilgrastim left at room temperature for more than 48 hours should be discarded. Freezing should be avoided; however, if accidentally frozen, pegfilgrastim should be allowed to thaw in the refrigerator before administration. If frozen a second time, pegfilgrastim should be discarded.

8.7.5 Dose Specifics

No preparation is required for administration of pegfilgrastim. Each subject will receive a fixed dose of 6 mg of pegfilgrastim. The entire contents of the 0.6 mL prefilled syringe should be administered subcutaneously irrespective of the subject's actual weight.

8.7.6 Preparation

No preparation is required for administration of pegfilgrastim. Each subject will receive a fixed dose of 6 mg of pegfilgrastim. The entire contents of the 0.6 mL prefilled syringe should be administered subcutaneously.

8.7.7 Route of Administration

The entire contents of the 0.6 mL prefilled syringe should be administered subcutaneously.

8.7.8 Availability

Pegfilgrastim is commercially available.

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8.8 Neulasta

8.8.1 Generic Name

Pegfilgrastim

8.8.2 Manufacturer

Amgen

8.8.3 Class

Colony Stimulating Factor, Hematopoietic Agent

8.8.4 Mechanism of Action

Stimulate the production, maturation, and activation of neutrophils to increase both their migration and cytotoxicity. Has prolonged duration of effect relative to filgrastim, and a reduced renal clearance.

8.8.5 Pharmacokinetics

- Bioavailability: 85% - 90%
- Tmax: 24 – 72
- $T_{1/2}$: 15-80 hours

8.8.6 Dosage Forms

- Single-use prefilled syringe with needle guard
- Sterile on-body injector*

8.8.7 Available Preparations

- Syringe: 6mg/ 0.06mL
- On-body injector: 6mg/ 0.6mL

8.8.8 Storage/Stability

Refrigerate at 36° to 46°F in the carton to protect from light. Avoid freezing; avoid shaking.

Discard syringes stored at room temperature for more than 48 hours.

8.8.9 Dose specifics

Per protocol

8.8.10 Route of Administration

Subcutaneous Injection

Administer by:

- Health care professional
- On-body injector*

8.8.11 Interchangeable

No

8.8.12 Adverse Events

- MSK: bone pain (25% to 45%)
- Cardiovascular: capillary leak syndrome
- Hematologic: leukocytosis (<1%), sickle cell anemia with crisis
- Renal: glomerulonephritis
- Respiratory: acute respiratory distress syndrome
- Other: spleen rupture

8.8.13 Contraindications

Serious allergic reactions to human G-CSFs, or any component of the product.

8.8.14 Interactions

Lithium – greater than expected increase in white blood cell count.

9. Statistical Considerations

9.1 Study Design/Primary Endpoint

The primary objective of this study is to evaluate the complete pathologic response (pCR = pT0pN0) rate attained by neoadjuvant chemotherapy followed by extirpative surgery in patients with high-grade upper tract urothelial carcinoma. There are two treatment arms, and treatments will be assigned based on the measured creatinine clearance (mCrCl) at baseline. Patients with mCrCl > 50 ml/min will receive AMVAC (accelerated methotrexate, vinblastine, doxorubicin and cisplatin) for 4 cycles followed by nephroureterectomy, while patients with 30 ml/min ≤ mCrCl ≤ 50 ml/min will receive GCa (gemcitabine and carboplatin) for 4 cycles followed by nephroureterectomy. The primary analysis will be performed in eligible patients who start any part of protocol therapy. A sensitivity analysis will be done in eligible patients who receive at least one dose of chemotherapy. There will be no direct comparison between the two treatment arms.

The primary endpoint of this study is pCR rates. Based on prior studies, a pCR rate of 18% would be worthy of further study, while a pCR rate of 4% would not justify further utilization of the treatment. With 28 eligible patients per arm, a treatment will be deemed promising if at least 3 pCRs are observed in a given arm. Using this design, there is a 10% probability of declaring a treatment worthy of further study if the true pCR rate is 4%, and a 90% probability of declaring a treatment worthy of further study if the true pCR rate is 18% by using a one-sided exact binomial test.

Per protocol patients should complete protocol therapy with nephroureterectomy by 60 days following chemotherapy. However, patients who are unable to complete protocol therapy in this timeframe will be evaluable for response if surgery is within 6 months of completion of chemotherapy. Patients who undergo nephroureterectomy beyond 6 months of completion of chemotherapy will be followed for recurrence and other endpoints but not evaluable for pathologic response.

9.2 Secondary Endpoints

The secondary endpoints include recurrence-free survival (RFS), event-free survival (EFS), bladder cancer-free survival, cancer-specific survival and renal functional outcomes.

RFS and bladder cancer-free survival will be evaluated in patients deemed disease free after surgery. RFS is defined as the time from the date of surgery to disease recurrence or death from any cause. Patients alive without documented recurrence will be censored at the date of last disease assessment. Bladder cancer-free survival is defined as the time from the date of surgery to the earlier of a return of bladder cancer or death from any cause. Patients alive without documented bladder cancer will be censored at the date of last disease assessment. EFS is defined as the time from registration to the earlier of recurrence, disease progression, new invasive primary cancer, or death from any cause. The method of Kaplan and Meier will be used to characterize RFS, EFS and bladder cancer-free survival.

Cancer-specific survival is defined as the time from registration to death due to cancer; deaths due to other causes will be counted as competing events. Cancer-specific survival will be analyzed using Gray's method and cumulative incidence estimates will be reported.

Renal function outcomes and safety are also important endpoints of this study. The proportion of patients with renal insufficiency (CrCl < 60 ml/min) post chemotherapy and post nephroureterectomy as well as the proportion of patients with renal function improvement (CrCl < 60 ml/min at baseline and CrCl ≥ 60 ml/min on study) will be reported along with exact binomial confidence intervals. The distribution of changes in renal function post chemotherapy as well as post surgery from baseline will also be reported. The analysis of renal function outcomes will be performed among patients who receive at least one dose of chemotherapy. All patients who receive any part of the treatment will be monitored for toxicity, and the percent of patients with various toxicities will be tabulated.

9.3 Accrual

We propose to accrue 28 eligible patients per arm for a total of 56 eligible patients in this study. With about 7% allowance for ineligibility, additional 4 patients will be accrued for a total of 60 patients. We expect to accrue 2 patients per month. The total accrual will be completed in 2.5 years.

9.4 Safety Monitoring

Interim analyses of toxicity are performed twice yearly for all ECOG-ACRIN studies. Reports of these analyses are sent to the ECOG-ACRIN Principal Investigator or Senior Investigator at the participating institutions. Expedited reporting of certain adverse events is required, as described in Section 5.2.

9.5 Gender and Ethnicity

Based on previous data from E1804 and E4802, the anticipated accrual in subgroups defined by gender and race is:

Ethnic Category	**Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	3	4
Not Hispanic or Latino	13	43	56
Ethnic Category: Total of all subjects	14	46	60

Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	1	0	1
Black or African American	1	3	4
Native Hawaiian or other Pacific Islander	0	0	0
White	12	43	55
Racial Category: Total of all subjects	**14**	**46**	**60**

The accrual targets in individual cells are not large enough for definitive subgroup analyses. Therefore, overall accrual to the study will not be extended to meet individual subgroup accrual targets.

10. Submission of Biospecimens for Research

PATIENT CONSENT: Samples are to be submitted for research studies from patients who answer "Yes" to "*My samples and related information may be kept in a Biobank for use in future health research."*

LABELING: Specimens are to be labeled clearly with the ECOG-ACRIN protocol number "EA8141", patient initials, date and time of collection, and sample type. All sample submissions are to be logged into the ECOG-ACRIN Sample Tracking System (STS). An STS-generated shipping manifest is to accompany all submissions.

Receiving Laboratory: Samples are to be shipped to the **ECOG-ACRIN Central Biospecimen and Pathology Facility (CBPF)** at MD Anderson.

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10.1 Submissions to the CBPF

If these criteria cannot be met, please contact the ECOG-ACRIN CBPF (eacbpf@mdanderson.org) to obtain alternative submission requirements.

10.1.1 Sample Preparation

A. TUMOR TISSUE

The submitting pathologist and clinical research associate should refer to Appendix I (Pathology Submission Guidelines) for guidelines and summary of submission requirements.

Tumor tissue: One representative formalin-fixed paraffin-embedded **primary tumor block** is requested from:

- Pre-trial diagnostic biopsy, should be submitted within four (4) weeks following registration
- Surgery, should be submitted within four (4) weeks following surgery

NOTE: If there is no residual tumor found in the surgical specimen please go into the STS and click on 'Can't Submit' and write in the comment section 'no residual tumor found in specimen.' Please send the pathology report to the CBPF for documentation and note on the cover letter that no block is being submitted as there was no residual tumor.

NOTE: If blocks are unavailable for submission, the following alternative submission is requested per availability: one (1) H&E, twenty (20) unstained slides 4µm thick and two (2) 4mm cores (if enough tissue is available).

Forms and Reports (Submit with all pathology submissions):

1. A copy of the surgical (if appropriate) and pathology reports.
2. Immunologic studies, if available.
3. Other Immunologic and cytologic reports.
4. STS-generated shipping manifest.

B. BLOOD SUBMISSIONS

Blood is to be collected at the following timepoints:

- After registration, prior to start of treatment
- End of chemotherapy, prior to surgery
- 2-6 weeks post-surgery

It is requested that samples be batched at > -70°C and shipped on dry ice on a quarterly basis. If samples must be stored at -20°C, ship on dry ice within one (1) week of draw.

Draw the blood tubes in the following order: no anti-coagulant (red top or SST), potassium EDTA (EDTA, purple top). Note that vacutainer top colors are for BD vacutainers. Verify tube contents prior to the collection of any samples.

Ship Frozen →

1. Serum
   - At each time point specified, draw one (1) 10mL vacutainer (no anti-coagulant)
   - Allow to coagulate at room temperature for 20 minutes
   - Separate by centrifugation at approximately 1200g x 20 minutes
   - Aliquot serum into four cryovials. Discard residual cells
   - Freeze the serum, at > -70°C preferred

Ship Frozen →

2. Plasma, EDTA
   - At each time point specified, draw 10mL plastic EDTA vacutainer
   - Separate by centrifugation at approximately 1200g x 20 minutes
   - Aliquot plasma into four (4) cryovials.

Ship Frozen →

   - Replace the cap on the vacutainer
   - Freeze the plasma and residual cells (WBC + RBC) at > -70°C preferred

Ship Frozen →

3. Whole Blood, EDTA
   - At each time point specified, draw 10mL plastic EDTA vacutainer
   - Freeze at > -70°C preferred

C. URINE SUBMISSIONS

Urine is to be collected at the following time points:

- After registration, prior to start of treatment
- End of chemotherapy, prior to surgery
- 2-6 weeks post-surgery

Ship Frozen →

At each time point specified, collect 20-50mL of urine fresh catch

- It is requested that the urine be aliquoted into four cryovials. However, one large cryovial may be submitted.

- Freeze the urine, > -70ºC preferred, and ship with the blood samples

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10.1.2 Shipping Guidelines

The requested initial diagnostic FFPE tumor tissue materials, reports and forms are to be submitted at ambient within four (4) weeks following patient registration.

Frozen blood samples and frozen urine are to be shipped overnight on dry ice.

The receiving laboratory is not available to receive shipments over holidays or weekends. Therefore, samples are only to be shipped via overnight courier Sunday through Thursdays (excluding a day before a holiday).

Ship using the CBPF's FedEx account using the FedEx on-line Ship Manager.

Access to the shipping account for shipments to the CBPF can only be obtained by logging into fedex.com with an account issued by the CBPF. For security reasons, the account number will no longer be given out in protocols, over the phone, or via email. If your site needs to have an account created, please contact the CBPF by email at eacbpf@mdanderson.org.

Shipping Address:

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN, Room G1.3586
1515 Holcombe Boulevard
Houston, TX 77030
Toll Free Phone: (844) 744-2420 (713-745-4440 Local or International Sites)
Fax: (713) 563-6506
Email: eacbpf@mdanderson.org

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10.2 ECOG-ACRIN Sample Tracking System

All specimens submitted must be entered and tracked using the ECOG-ACRIN Sample Tracking System.

It is **required** that all samples submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (STS). The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the samples required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst.

Important: Please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link:

http://www.ecog.org/general/stsinfo.html Please take a moment to familiarize yourself with the software prior to using the system.

An STS generated shipping manifest should be shipped with all specimen submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu.

Study Specific Notes

If STS is unavailable at time of sample submissions, a completed Generic Specimen Submission Form (#2981) is to be submitted in lieu of the STS shipping manifest. Include site contact information on the form. Notify the laboratory of the shipment by faxing a copy of the completed form to the laboratory the day of shipping. Indicate the appropriate receiving laboratory on the submission form:

- ECOG-ACRIN CBPF

Retroactively enter all specimen collection and shipping information when STS is available.

10.3 Use of Specimens in Research

Specimens submitted from patients who consented to allow their specimens to be used for future research studies will be retained in an NCI-affiliated central repository. Specimens submitted on this trial will be retained at the ECOG-ACRIN Central Biospecimen and Pathology Facility at MD Anderson.

Requests for the use of these specimens will require a correlative science proposal (or a protocol amendment) detailing the scientific hypothesis, research plan, assay methods for use of the biospecimens, and a complete statistical section (with adequate power justification and analysis plan) which would be submitted and reviewed by the cooperative group and CTEP in accordance with the NCI National Clinical Trials Network (NCTN) review process.

Possible future studies include:

- Tumor RNA/DNA analysis
- Circulating free tumor RNA/DNA in plasma
- Circulating free tumor RNA/DNA in urine

Specimens submitted will be processed to maximize their utility for research projects. Tissue processing may include, but not limited to, extraction of DNA and RNA and construction of tissue microarrays (TMAs). DNA, RNA, and plasma (if appropriate) will be isolated from the submitted peripheral blood samples.

Tumor blocks will be available for purposes of individual patient management on specific written request.

If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study. Tissue samples may be available for return upon written request and other samples may be destroyed per protocol of the given lab. Samples may also be anonymized (stripped of all identifiers) and used for instrument calibration or other quality control measures which are not published or linked to the clinical trial.

10.4 Sample Inventory Submission Guidelines

Inventories of all samples submitted from institutions will be tracked via the ECOG-ACRIN STS and receipt and usability verified by the receiving laboratory. Inventories of specimens forwarded and utilized for approved laboratory research studies will be submitted by the investigating laboratories to the ECOG-ACRIN Operations Office – Boston on a monthly basis in an electronic format defined by the ECOG-ACRIN Operations Office – Boston.

11. Electronic Data Capture

Please refer to the **EA8141** Forms Completion Guidelines for the forms submission schedule. Data collection will be performed exclusively in Medidata Rave.

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG-ACRIN Operations Office – Boston to CTEP by electronic means.

12. Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

13. References

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Appendix I

Pathology Submission Guidelines

The following items are included in Appendix I:

1. Guidelines for Submission of Pathology Materials (instructional sheet for Clinical Research Associates [CRAs])
2. Instructional memo to submitting pathologists
3. ECOG-ACRIN Generic Specimen Submission Form (#2981)

Guidelines for Submission of Pathology Materials

EA8141: A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Urothelial Cancer of the Upper Urinary Tract

A. The following materials are to be submitted:

Provide adequate patient identifying information with the submitted pathology materials. At a minimum the following information must be provided with all pathology submissions:

- Patient name (last, first). Initials must be provided at a minimum
- Protocol number EA8141 and protocol-specific ECOG-ACRIN patient ID number
- Site's pathology identification numbers
- Site information, including pathologist and site contact are to be provided on the Submission form and in STS

Prior to Start of Treatment

1. Institutional pathology report, including immunological reports if available
2. STS-generated shipping manifest
3. Tumor tissue block from the diagnostic ureteroscopic or percutaneous biopsy and/or cytology of the primary tumor.

   NOTE: If a block is unavailable for submission, submit the following: one (1) H&E, twenty (20) unstained slides 4µm thick and two (2) 4mm cores (if enough tissue is available).

Surgery

4. Institutional pathology report, including immunological reports if available (if enough tissue is available)
5. Surgical Report
6. STS-generated shipping manifest
7. Tumor tissue block

   NOTE: If a block is unavailable for submission, submit the following: one (1) H&E, twenty (20) unstained slides 4µm thick and two (2) 4mm cores.

B. Mail pathology materials to:

ECOG-ACRIN Central Biorepository and Pathology Facility
MD Anderson Cancer Center
Department of Pathology, Unit 085
Tissue Qualification Laboratory for ECOG-ACRIN
Room G1.3586
1515 Holcombe Boulevard
Houston, TX 77030

If you have any questions concerning the above instructions or if you anticipate any problems in meeting the pathology material submission deadline of one month, contact the Pathology Coordinator at the ECOG-ACRIN Central Biospecimen and Pathology Facility (CBPF) by telephone: (844) 744-2420, by fax: (713) 563-6506, or by email: eacbpf@mdanderson.org.

Reminder: All biospecimen submissions must be logged and tracked in the ECOG-ACRIN Sample Tracking System (STS).

Robert L. Comis, MD, and Mitchell D. Schnall, MD, PhD
Group Co-Chairs

MEMORANDUM

TO: _____
(Submitting Pathologist)

FROM: Stanley Hamilton, M.D., Chair
ECOG-ACRIN Laboratory Science and Pathology Committee

DATE: _____

SUBJECT: Submission of Pathology Materials for EA8141: A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Urothelial Cancer of the Upper Urinary Tract

The patient named on the attached Generic Specimen Material Submission Form (#2981) has been entered onto an ECOG-ACRIN protocol by _____ (ECOG-ACRIN Investigator). This protocol requires the submission of pathology materials for laboratory research studies.

Please complete the Submission Form, keep a copy for your records and return the completed Submission Form, the surgical pathology report(s), the slides and/or blocks and any other required material (see List of Required Material) to the Clinical Research Associate (CRA). The CRA will forward all required pathology material to the ECOG-ACRIN Central Biospecimen and Pathology Facility (CBPF) at MD Anderson.

Blocks and slides submitted for this study will be retained at the ECOG-ACRIN CBPF for future studies. Paraffin blocks will be returned upon written request for purposes of patient management.

Please note: Since blocks are being used for research, in some cases the material may be depleted, and, therefore, the block may not be returned.

If you have any questions regarding this request, please contact the CBPF at (844) 744-2420 or Fax (713) 563-6506.

The ECOG-ACRIN CRA at your institution is:

Name:_____

Address: _____

Phone: _____

Thank you.

ECOG-ACRIN Generic Specimen Submission Form Form No. 2981v3

Institution Instructions: This form is to be completed and submitted with **all specimens** ONLY if the Sample Tracking System (STS) is not available. **Use one form per patient, per time-point.** All specimens shipped to the laboratory must be listed on this form. Enter all dates as MM/DD/YY. Keep a copy for your files. Retroactively log all specimens into STS once the system is available. **Contact the receiving lab to inform them of shipments that will be sent with this form.**

Protocol Number _____ **Patient ID** _____ **Patient Initials** Last _____ First _____

Date Shipped _____ **Courier** _____ **Courier Tracking Number** _____

Shipped To (Laboratory Name) _____ **Date CRA will log into STS** _____

FORMS AND REPORTS: Include all forms and reports as directed per protocol, e.g., pathology, cytogenetics, flow cytometry, patient consult, etc.

Required fields for all samples				Additional fields for tissue submissions				Completed by Receiving Lab
Protocol Specified Timepoint:								
Sample Type (fluid or fresh tissue, include collection tube type)	Quantity	Collection Date and Time 24 HR		Surgical or Sample ID	Anatomic Site	Disease Status (e.g., primary, mets, normal)	Stain or Fixative	Lab ID

Fields to be completed if requested per protocol. Refer to the protocol-specific sample submissions for additional fields that may be required.					
Leukemia/Myeloma Studies:	Diagnosis	Intended Treatment Trial	Peripheral WBC Count (x1000)	Peripheral Blasts %	Lymphocytes %
Study Drug Information:	Therapy Drug Name	Date Drug Administered	Start Time 24 HR	Stop Time 24HR	
Caloric Intake:	Date of Last Caloric Intake		Time of Last Caloric Intake 24HR		

CRA Name _____ **CRA Phone** _____ **CRA Email** _____

Comments

9/12/14

A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Upper Tract Urothelial Carcinoma

Appendix II

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient for enrolling in this trial. The template is intended as a guide and can be downloaded from the web site at http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG-ACRIN and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

[PATIENT NAME] [DATE]

[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in this important research study. Many questions remain unanswered in cancer. With the participation of people like you in clinical trials, we hope to improve treatment and quality of life for those with your type of cancer.

We believe you will receive high quality, complete care. I and my research staff will maintain very close contact with you. This will allow me to provide you with the best care while learning as much as possible to help you and other patients.

On behalf of ***[INSTITUTION]*** and ECOG-ACRIN, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Upper Tract Urothelial Carcinoma

Appendix III

ECOG Performance Status

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

A Prospective Phase II Trial of Neoadjuvant Systemic Chemotherapy Followed by Extirpative Surgery for Patients with High Grade Upper Tract Urothelial Carcinoma

Appendix IV

Lab Study EA8141LGS1

Use of Next Generation Sequencing as a Urinary Biomarker Molecular Correlate for Pathologic Complete Response to Neoadjuvant Chemotherapy in Upper Tract Urothelial Carcinoma

Study PI: Philip H. Abbosh, MD, PhD, Fox Chase Cancer Center

Statistician: Eric Ross, PhD, Fox Chase Cancer Center

Aim:

To define genetic correlates of stage, grade, and pathologic complete response (pCR) after neoadjuvant platinum-based chemotherapy (NAC) in high grade UTUCC using next generation sequencing (NGS) of urinary DNA (uDNA).

Background:

The explosion in the identification of new drug targets in many cancers has been fueled in large part by NGS efforts. However, given the difficulty of access to preoperative tissue and the rarity of the disease, NGS studies of UTUCC has been absent with a few exceptions, thus contributing to the lack of improvements or new therapies in this disease.

Although accurate clinical staging is difficult in bladder cancer, the problem is compounded in UTUCC because of difficulty in accessing and navigating the upper urinary tract. Often only small pieces of tissue from a heterogeneous tumor are available, making pathologic assignment of T-stage or histologic grade (and therefore therapy assignment) unreliable. Biopsy for the identification of high grade UTUCC is specific but not sensitive[1-4]. Urinary biomarkers which associate with grade or stage could lessen some of the diagnostic dilemmas surrounding UTUCC and provide predictive or prognostic biomarkers which are completely lacking in UTUCC as well as MIBC. For example, several genetic alterations are enriched only in non-muscle-invasive bladder cancer (NMIBC) or MIBC but not both[5]. These might be used to more effectively stage high grade T1 tumors if they genetically resemble muscle-invasive tumors. Enhanced clinical T-staging in UTUCC might thus more appropriately triage patients into endoscopic, extirpative, or multidisciplinary treatments. uDNA-based biomarkers would make for a facile, reproducible, and robust diagnostic medium in the management of UTUCC.

In preliminary studies in MIBC, our group has shown that uDNA can be used to identify mutations in key bladder cancer genes, and quantitate their presence or clearance after NAC. Clearance of mutations from uDNA associates with pCR, while persistence of mutations correlates with persistence of disease. Our approach was recently funded by the NIH for further study in MIBC patients (R21CA218976). I now seek to apply the same approach in UTUCC patients treated in EA8141 as proposed in my recently-funded Bladder Cancer Advocacy Network Young Investigator Award 2018. This approach in the future could be used to identify candidate UTUCC patients who achieve pCR after NAC and possibly steer them into organ preservation algorithms and avoid

radical nephroureterectomy. Enumeration of somatic variants in high grade/high stage UTUCCs such as those which are being recruited in EA8141 would also serve to help develop uDNA-based biomarkers to enhance clinical staging both at time of diagnosis (invasive vs noninvasive) and after NAC (pCR vs residual disease). Since several mutated genes in urothelial cancers are targetable with currently available drugs, uDNA might also one day be used for predictive biomarkers of response to these therapies.

I propose to perform targeted NGS of uDNA isolated from urine specimens collected in the EA8141 study to define molecular correlates of T-stage and/or pCR after NAC. These molecular correlates might one day be used in conjunction with traditional clinical staging for precision medicine, precision surgery, or organ preservation approaches to treating UTUCC.

Therefore, I propose to perform targeted exome sequencing of uDNA from patients treated in the EA8141 trial for the purpose of annotation of mutations before and after administration of NAC. Several biomarkers may be evaluable using this type of data including enhanced clinical T-staging, prospective biomarkers of cisplatin response (i.e. '*who will respond to NAC*?'), as well as retrospective biomarkers (i.e. '*who responded to chemotherapy*?'). From a basic research standpoint, this endeavor will provide insight into upper tract biology and chemoresponse. From a clinical/translational standpoint, it will set the stage for personalized medicine algorithms which assign targeted therapies based on susceptibility-conferring mutations, and for personalized surgical algorithms possibly including surgical avoidance in patients who are complete responders at the molecular level.

Rationale:

Layering "genetic staging" onto clinical staging at the time of diagnosis of high grade UTUCC would deepen the clinicians understanding of each patient's disease. In the future, if targeted therapies are to be applied to urothelial cancers, uDNA would be a natural source of companion biomarkers. Additionally, if patients achieve pCR after NAC, then there will be no residual source of tumor to shed variant DNA into the urine. Pre-surgical identification of these complete responders might allow for nephron-preserving approaches to UTUCC treatment. Additionally, follow up uDNA sampling might be used to identify subclinical recurrence and allow for early salvage nephroureterectomy.

Preliminary Data

Method: A panel of 50 MIBC genes was selected for targeted NGS by virtue of being TCGA-nominated drivers[6] or drivers in other cancers that are frequently mutated in MIBC[7] (right). Mutation of at least one panel gene is present in 99% of TCGA MIBC tumors with a median of 4 variants/tumor. Haloplex^{HS} (Agilent)

ARID1A	*CDKN2A*	*ERCC2*	*FOXA1*	*KMT2B*	*PAIP1*	*RXRA*
ATM	*CREBBP*	*ERCC4*	*FOXQ1*	*KMT2C*	*PBRM1*	*SETD2*
BAP1	*CTNNB1*	*FANCA*	*HRAS*	*KMT2D*	*PIK3CA*	*STAG2*
BRCA1	*ELF3*	*FANCC*	*TERT***	*KRAS*	*RAD51*	*TP53*
BRCA2	*EP300*	*FANCD2*	*KDM6A*	*NF1*	*RB1*	*TSC1*
BTG2	*ERBB2*	*FBXW7*	*KLF5*	*NFE2L2*	*RHOA*	*TXNIP*
CCND3	*ERBB3*	*FGFR3*	*KMT2A*	*NRAS*	*RHOB*	*ZFP36L1*
CDKN1A						

TCGA-nominated bladder cancer drivers
**promoter mutations only, no exon coverage

was chosen for sequencing library preparation because it uses single molecule tags, which greatly enhances the sensitivity of variant detection, well below the Taq polymerase error rate[8, 9]. This allows for highly accurate variant calling by "stacking" PCR-duplicates to create consensus sequences. Variants are only called if they are seen in all of the family members (true PCR duplicates) which comprise a consensus sequence because false calls arising from Taq errors will occur in <100% of the family members. Single molecule tags also allow for true depth-of-sequencing by counting the number of unique tags for each fragment or base. This makes variant detection highly accurate even at low variant allele fraction (VAF) which is expected after endoscopic debulking and/or NAC.

Urine DNA (uDNA) was isolated from urine from 6 patients with upper tract urothelial cell carcinoma (UTUCC) before and after AMVAC treatment on Dr. Plimack's trial (NCT01031420). Urine samples from this trial were centrifuged, and the pellets were used for other studies. The urine supernatants (4-8 mL of urine) were used to isolate uDNA. This resulted in an average yield of 3.6 µg of DNA per sample (12 samples; range 540 ng to 8.4 ug). In addition, germline DNA was isolated from white blood cells. These three DNA sources were subjected to sequencing using the 50-gene panel. Median depth of sequencing was 775x for pre-AMVAC samples (range 80 to 1307x), 456x for post-AMVAC samples (range 280-1352x), and 762x for germline (range 166 to 1023x).

	NONRESPONDERS			
	pre	post		**Grade-associated variants**
JG052	18 muts	8/18 seen	ypTa	*ATM, HRAS*
KM051	9 muts	0/9 seen	ypT1	*FGFR3*
LP019	21 muts	6/21 seen	pT3	*HRAS*
PS028	15 muts	8/15 seen	ypT1	*ATM, TP53*
WK030	38 muts	5/38 seen	ypT3	*FGFR3, CDKN1A*
	RESPONDERS			
	pre	post		
WES045	3 muts	0/3 seen	ypT0	*TP53*

Figure 1: uDNA and germline DNA from 6 patients was subjected to targeted sequencing and mutation clearance/persistence status was assigned to each subject as described. Subject-level mutation persistence was identified in 4 of 5 nonresponders. Additionally, mutations associating with high-grade UTUCC are present in 5 of 6 patients with high grade disease.

Pre-AMVAC variants in the uDNA were identified using SureCall. These variants were compared against germline to filter SNPs, sequencing errors, and rare variants that may be associated with clonal. hematopoiesis of indeterminant potential[10]. Using this approach, 106 variants (hereafter called mutations) were identified (median 16.5, range 3 to 38). All six samples had evidence of branched evolutionary tumor growth as described by Gerlinger et al[11, 12]. This manifested as multiple distinct variants in the same gene (*KMT2C*, *KMT2D*, *ARID1A*, etc), often in multiple genes.

Mutation persistence associates with tumor persistence. To determine if mutations persisted or cleared after neoadjuvant chemotherapy, post-AMVAC sequencing libraries were searched using the genomic coordinates of the pre-AMVAC mutations. Under the same calling conditions used for the previously described bladder cancer

Figure 2: Mutation clearance status was correlated with responder status. In analysis of the combined cohorts, the biomarker is strongly associated with responder status (p=0.0037, Fisher's exact test).

UTUCC only

	ypT0	yp>T0
clearance	1	1
persistence	0	4

UTUCC + MIBC

	ypT0	yp>T0
clearance	4	1
persistence	0	10

preliminary data (at least 3 variant reads, at least 30 reads per site, Phred>30, mapping quality>30, strand bias<2:1), each pre-AMVAC mutation clearance or persistence status in the post-AMVAC urine sample was ascribed at the mutation level. Subject-level mutation clearance was assigned only if *all* mutations demonstrated mutation clearance. Persistence of even one pre-AMVAC mutation in the post-AMVAC sample assigns subjects to mutation persistence status. Mutation persistence was identified in 4 of 5 nonresponders (Figure 1). Mutation clearance was identified in the single complete responder and one nonresponder. This translates to an 80% sensitivity for identifying patients with residual disease, and 83.3% accuracy of the biomarker test. In combined analysis of the UTUCC cohort described here and an additional 9 patients with MIBC, mutation persistence status translates to a 91% sensitivity for identifying patients with residual disease and 93% accuracy for the biomarker test. Combined cohort analysis shows that the biomarker strongly associates with responder status (p=0.0037, Fisher's exact test; Figure 2). Cautious optimism is being exercised because of the small cohort size, and additional patients with MIBC are being studied.

Mutations associated with high grade disease are detectable in high grade disease. Additional analysis was performed to determine if mutations associated with grade. All UTUCC in the AMVAC trial were high grade. Mutations in *TP53*, *HRAS*, *ATM*, *CDKN1A*, and deletion of *CDKN2A* are highly specific for high grade UTUCC, whereas *FGFR3* hotspot mutations are present in both high and low grade UTUCC[13, 14]. *FGFR3* mutations, however, are much more common in low grade tumors (>90%) vs high grade tumors (10-15%). Mutations in genes associated with high grade disease were identified in 5 of 6 AMVAC patients' pre-AMVAC urine. An *FGFR3* hotspot mutation was identified in the lone patient without high-grade genetics. This approach may therefore also be useful in assigning grade to disease using urine as a diagnostic medium.

Mutations in tissue are represented in the urine. Twelve MIBC cases where pre-AMVAC urine was available underwent whole exome sequencing of the tissue. In the tissue, 64 mutations in tissue (MT) were identified, and 39 of these 64 mutations (61%) were detected in the pre-AMVAC uDNA (MU). Additional MU were identified that were not called in the tissue. Ten of the 64 MT were subclonal in the tissue; 5 of 10 subclonal MT were identified in uDNA. Two MT undetected in urine had <100x coverage in uDNA deep sequencing. Although no whole exome sequencing has been performed on UTUCC samples to similarly validate the approach, we expect to find a similar diagnostic overlap between MT and MU when we sequence a cohort of UTUCC patients in collaboration with Surena Matin, MD (MD Anderson Cancer Center, Houston). In addition, most UTUCC patients had well-known hotspot mutations in either the *TERT* promoter, *HRAS*, *TP53*, or *FGFR3*, which suggests that some/most of the other identified variants are true variants.

Experimental Plan:

Specimens: The following specimens from patients who consented on EA8141 to allow their specimens to be used for future research will be utilized for these assessments:

- Pre- and post-NAC urine, 4-8 mL
- Germline DNA isolated from EDTA whole blood or PBMCs isolated from whole blood.

No samples are expected to be exhausted.

Specimen Preparation: At the time of analysis, DNA will be isolated from 8mL (or 4mL if 8mL is not available) of urine and from blood cells collected from each patient. uDNA

quantity/quality will be measured OD260/280/230. dsDNA will be quantified using Qubit (Invitrogen).

Sequencing: Germline DNA and pre- and post-chemotherapy uDNA sequencing libraries will be generated using the HaloPlex^{HS} kit. Captured and pooled specimens will be subjected to targeted sequencing on the HiSeq 2500 (Illumina) which is housed within the Fox Chase Cancer Center Genomics Facility. 100 bp paired-end reads will be used to ensure complete coverage of fragments to a depth of 1000x. This depth of coverage was quite adequate to detect mutations in preliminary studies.

Mutation Calling: Agilent provides variant calling software, SureCall, to detangle PCR duplicates which are identified using single molecule tags and call variants from HaloPlex^{HS} sequencing output. Variants will be filtered out if they have <30 reads at the variant base, <3 variant reads, Phred <30, mapping quality <30, or strand bias >2:1 to maintain high quality of the called variants. Variants remaining after de-duplication, quality filters, and SNP removal will be considered true variants. Under these conditions, 500x-coverage will reliably detect a 0.01 VAF allele 87.6% of the time based on Poisson statistics. Similarly, 1000x-coverage will reliably detect a 0.01 VAF allele 99.7% of the time. For reference, the lowest VAF was 0.02 in the preliminary specimens excluding specimen #16pre, which missed two variants as the result of a technical error. Pre- and post-NAC sequences will be filtered against the germline to remove SNPs.

Description of the High Grade UTUCC Genome: With this limited but relevant panel of genes, commonly mutated genes in high grade UTUCC will be identified. Some correlation to chemoresponse may be feasible for candidate DNA repair genes such as *ERCC2* or *ATM*/*FANCC*/*RB1*.

Evaluation of Mutation Clearance: Patients' specimens will be analyzed to determine if mutation clearance or persistence occurred. Test negative and test positive cases will be separated into true responders and true non responders. Fisher's exact test will be used to determine if mutation clearance/persistence identifies complete responders. Statistical analysis will be performed by ECOG/ACRIN statisticians supported by Dr. Abbosh.

Sequence Validation: PCR products spanning NGS-identified variants will be amplified from uDNA using specific primer pairs and Sanger sequenced to confirm specificity of NGS findings for 10% of the variants. If variants are not identified, tumor tissue from nephroureterectomy specimens will be requested form EA8141 for confirmation of variants. Given that many variants identified in the preliminary data were found at low frequencies, amplicon-based NGS approaches may be necessary instead.

Feasibility: The sequencing approach chosen to study urine samples from patients with urothelial cancer of the upper tract or bladder appears to be robust in pilot studies. The main feasibility concern now becomes whether there will be adequate DNA isolated from the urine specimens to accomplish quality sequencing. Several factors may impact DNA yield. In preliminary studies cellular debris was removed prior to freeze-thaw and NGS. In contrast, EA8141 bio-specimen processing and storage protocol does not involve separation of cells and debris from urine supernatant. One freeze-thaw cycle is anticipated to lyse intact cells (both malignant and benign), thus releasing more DNA. This may increase yield, but the impact on urine variant allele frequency (VAFU) may increase or decrease. For instance, if the intact cells are mostly normal urothelial cells or malignant cells, the VAFU may decrease or decrease, respectively. Also, nephrostomy tubes placed to treat urinary obstruction may decrease the amount of shed uDNA if urine from the collecting system is diverted from the bladder (where it will be voided), whereas stents may increase DNA yield by promoting antegrade drainage. Lastly, uDNA used in

generation of preliminary data was previously isolated after 'debulking' by TURBT and still there was adequate template for sequencing. Pre-chemotherapy UTUCC tumors probably retain a greater percentage of initial tumor bulk when compared to MIBC since endoscopic diagnostic or ablative procedures are probably less effective than TURBT.

Sample size and statistical considerations: It is anticipated that 30 patients will have adequate specimens for study. pCR rates for NAC in UTUCC patients is historically 12-33%[15-18]. Therefore, of the 30 patients, I anticipate 24 patients (80%) will have residual disease identified in their nephroureterctomy specimen (the EA8141 investigators expected 18% of patients to achieve pCR). Because a clinician would want to be very sure that a negative test (no residual mutations detected) is highly reassuring that there is no disease present, the statistical analysis is based on the true positive rate (TPR; ie sensitivity). These 24 anticipated patients with residual disease will be used to estimate the TPR for the test (where the test positively identifies residual disease). The alternative hypothesis is 90% TPR, and there is a reasonably high power to detect a difference from the null hypothesis with a type I error of ≤0.1. In reality, the test is likely not clinically useful if sensitivity is <70%.

Sample size	Null True Positive Rate	Alternative True Positive Rate	Exact one-sided type I error	Power
30	0.65	0.90	0.06	97.4
30	0.70	0.90	0.08	92.7
30	0.75	0.90	0.10	82.5
30	0.80	0.90	0.04	41.1

Significance:

The proposal outlined here will provide a genetic roadmap for high-grade UTUCC and its potential biomarkers, which are sorely needed for a disease where staging and grading is so difficult. It will also provide the basis for further studies into the nature of chemosensitivity and resistance in UTUCC. Prospective identification of chemosensitive tumors will lead to improvements in UTUCC patients in several respects: new opportunities might be identified to shuttle putative nonresponders to targeted therapies or immediate surgery; and nephron-sparing approaches might be within reach if chemotherapy reliably results in pCR as detected by mutation clearance; larger studies sequencing the whole exome through the urine could be undertaken to identify putative biomarkers of prospective response such as mutations in *ERCC2*[19] or other DNA repair genes[20].

Conclusion and Future Studies:

Described here is a robust method to characterize the UTUCC genomes of patients undergoing neoadjuvant chemotherapy and perform correlative studies to determine if urine biomarkers enhance clinical staging before or after chemotherapy. Correlations of mutations to long term outcomes and evaluation of predictive and prognostic alterations would be undertaken as follow up increases. Additionally, work will be ongoing in similar analyses on specimens from MIBC patients and might result in improvements to the technology and analysis pipeline prior to initiating studies on UTUCC specimens. Lastly, data will be made publically available after publication to serve as a resource for other scientists or clinicians. Although the sample size is inadequate to establish a clinically useful test, this cohort will be combined with other cohorts which have been committed by collaborators to achieve stronger power. If the accuracy of the test is confirmed, larger prospective studies will be sought to establish the test as an enhancement to clinical staging and potentially use chemotherapy for curative intent.

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