Abbreviated Title: COXEN study

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Title: A Pilot Clinical Trial of Genomic Based Assignment of Therapy in Advanced Urothelial Carcinoma

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Investigational agents: none

Commercial agents: COXEN Drug Index

PRÉCIS

Background:

- Patients presenting de novo with metastatic bladder cancer, or developing visceral metastatic disease after local treatment, are incurable with currently available therapeutic modalities.
- Only a small number of chemotherapeutic agents have been tested and very few have some single agent activity in the treatment of metastatic urothelial carcinoma. However most (>100) FDA approved anticancer agents have yet to be tested in this disease.
- Novel approaches to the development of genomic predictors of chemosensitivity that do not require clinical trials for their identification are urgently needed in order to identify agents that are clinically effective when either repurposed or discovered "de novo" specifically for urothelial carcinoma. Such "repurposing" of an FDA approved anticancer agent in order to advance therapy from one cancer to another would require only minimal clinical development, saving billions of dollars and reducing the time required to reach routine clinical practice.
- Our established extramural-intramural NCI collaboration pulls together significant expertise in biomarker development and clinical trial design in bladder cancer. The innovation of this group lies not only in the novel scientific approaches i.e. Co-eXpression ExtrapolatioN (COXEN) under investigation, but also in the successful creation of a cohesive multi-institutional research collaboration dedicated to improved clinical outcomes in bladder cancer patients.
- COXEN uses molecular profiles as a "Rosetta Stone" for translating drug sensitivities of one set of cancers into predictions for another completely independent set of cell lines or human tumors. The COXEN methodology has been scrutinized and deemed methodologically sound by peer review. The ability of COXEN to predict drug effectiveness in patients a priori, from purely in vitro assays, is unique as no other tool currently either in practice or in development provides similar results.

Objectives:

- To determine the feasibility of using the "Co-eXpression ExtrapolatioN" (COXEN) model in making a real-time treatment decision (within 3 weeks) in patients with advanced urothelial carcinoma.

Eligibility:

- Patients must have a histologically confirmed diagnosis of metastatic, progressive urothelial carcinoma of the bladder, urethra, ureter, or renal pelvis.
- Patients must have progressive metastatic disease defined as new or progressive lesions on cross-sectional imaging.
- Patients must have at least:
    - One measurable site of disease (according to RECIST criteria)
    - Or, appearance of one new bone lesion

- Patients must have been previously treated, as defined by treatment with at least one prior cytotoxic chemotherapy regimen or agent. Patients may have received any number of prior cytotoxic agents.
- Archival tumor tissue must be available for enrollment.
- Tumor amenable to biopsy will be mandatory for this study.
- 18 years of age or older
- ECOG performance status <2 (Karnofsky >60%)

Design:

- This will be a pilot single-arm, open-label study using the COXEN score to select the "best next therapy" from a list of 75 FDA-approved anti-neoplastic drugs, in patients with metastatic bladder cancer who have progressed despite treatment with cytotoxic chemotherapy. Combinations of the listed agents may also be utilized provided that phase 1 data are available.
- The COXEN algorithm requires a multi-step process (pathology, tissue processing, mRNA profiling, bioinformatics, etc.) and is potentially labor intensive and time intensive.
- Given the disease state of patients eligible for this protocol, using this algorithm to select a treatment would only be a worthwhile process to undertake if it can be demonstrated that a very high fraction of patients is likely to obtain the benefit from the procedure.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To determine the feasibility of using the "Co-eXpression ExtrapolatioN" (COXEN) model in making a real-time treatment decision (within 3 weeks) in patients with advanced urothelial carcinoma.

1.1.2 Secondary Objectives

1.1.2.1 To assess the progression-free survival using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma.

1.1.2.2 To assess the response rate using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma.

1.1.2.3 To assess the overall survival using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma.

1.1.2.4 To evaluate toxicity with "best next therapy".

1.1.3 Exploratory Objectives

1.1.3.1 To characterize changes in mutation frequency due either to metastatic progression or initial therapy by exome sequencing FFPE metastatic tumor samples from patients prior to "best next therapy" compared to archival primary tissue.

1.1.3.2 To identify the sensitivity of cellular circulating tumor DNA mutation detection and changes in the mutational landscape as patients undergo COXEN directed treatment.

1.1.3.3 To assess feasibility of PET-MRI in patients with metastatic bladder cancer.

1.1.3.4 To determine sensitivity and specificity of whole body diffusion weighted MRI imaging in detection of distant metastasis.

1.1.3.5 To evaluate role of PET-MRI in evaluation of response to therapy.

1.2 BACKGROUND AND RATIONALE

1.2.1 The Burden and Natural History of Bladder Cancer

Bladder cancer is the fifth most common cancer in the United States, with >70,000 new cases and > 14,000 deaths each year [1]. While the majority of patients present with non-muscle-invasive tumors, approximately 30-40% of patients have muscle-invasive tumors and a few harbor metastatic disease at the time of diagnosis. Surgical resection of the bladder (radical cystectomy) cures approximately 50% of these patients, while the remainder of patients develops lethal metastatic recurrence.

Summary: Patients presenting de novo with metastatic bladder cancer, or developing visceral metastatic disease after local treatment, are incurable with currently available therapeutic modalities.

1.2.2 Chemotherapy for Metastatic Bladder Cancer

The development of the MVAC (methotrexate, vinblastine, doxorubicin, cisplatin) regimen in the 1980's was an advance in the treatment of metastatic bladder cancer, with multiple trials

demonstrating objective response rates of <50% [2-5]. However, durable responses were rare and treatment-related toxicities were problematic. A landmark phase III trial comparing MVAC with gemcitabine plus cisplatin (GC) demonstrated similar overall survival outcomes (~14 months) between the regimens with better tolerability with GC [6] resulting in GC becoming the first-line treatment regimen of choice.

There is currently no standard chemotherapy for patients with metastatic bladder cancer who have progressed on first-line treatment. A variety of cytotoxic agents used in this "second-line" or "salvage" setting have demonstrated response rates of approximately 5-20% [7-14]. A randomized phase III trial of second-line vinflunine versus placebo in patients with metastatic disease failed to demonstrate a statistically significant improvement in survival [15]. These findings can be summarized as follows: 1) only a subset of patients with bladder cancer benefit (i.e. has some level of objective response) from cytotoxic agents; 2) only a small number of the >120 FDA approved anticancer agents have ever been tested and only a few of these have clinically significant single agent activity in urothelial cancer. The latter fact is emblematic of urothelial cancer being one of the most nationally underfunded cancers despite its clinical impact on the American population.

Combination second-line therapy may have more clinical activity then just single-agent therapy. The COXEN waterfall plot reports on the high activity of several agents that may be used in combination if previously tested in at least a phase I study and found to be safe.

Summary: Only a small number of chemotherapeutic agents have been tested and very few have some single agent activity in the treatment of metastatic urothelial carcinoma. However most (>100) FDA approved anticancer agents have yet to be tested in this disease.

1.2.3 Current Genomic Predictors of Chemosensitivity

The therapeutic response to a particular anticancer agent is modulated by multiple signaling pathways. As a result, effective prediction of a given patient's response requires analysis of multiple biomarkers across different gene networks [16]. Multi-gene expression-based prediction strategies have already demonstrated utility in predicting clinical outcomes [17-21]. Some of these tools, such as Oncotype DX™ and Mamma Print™, have become incorporated into the clinical care of patients with breast cancer. Despite the tremendous potential utility of multi-gene predictors, the current development of such predictors of drug sensitivity has critical limitations. Foremost, the majority of these genomic models have been developed using human patient data from clinical trials exploring treatment with a specific regimen. As a result, the predictive models are relevant only to that specific treatment and new models must be developed for each new treatment regimen. Furthermore, as indicated above, since only a small fraction of all FDA approved anticancer agents has been used in bladder cancer, single agent and combination trials would be required in order to generate these biomarkers. This lengthy and costly approach is clearly not feasible and cannot keep pace with the current arsenal of chemotherapeutic or targeted agents in the drug pipeline.

Summary: Novel approaches to the development of genomic predictors of chemosensitivity that do not require clinical trials for their identification are urgently needed in order to identify agents that are clinically effective when either repurposed or discovered "de novo" specifically for urothelial carcinoma. Such "repurposing" of an FDA approved anticancer agent as single agent therapy or in combination in order to advance therapy from one cancer to another would require

only minimal clinical development, saving billions of dollars and reducing the time required to reach routine clinical practice.

1.2.4 UCCC-NCI Bladder Cancer Collaboration

Progress in clinical drug development in advanced bladder cancer is hampered by a relative lack of industry interest and the inability of most single centers to conduct even large Phase II trials in a timely manner. Over the past 3 years, we have formed a collaboration between the University of Colorado Comprehensive Cancer Center (UCCC), the National Cancer Institute (NCI) and the NIH-Clinical Center (CC). This group is comprised of basic, translational and clinical investigators focused on the development of novel therapeutic approaches for the treatment of bladder cancer. In this manner, the group leverages the bladder cancer expertise of its participating researchers and NCI funded infrastructure (UCCC P30, NCI and NIH Clinical Center).

Summary: Our established extramural-intramural NCI collaboration pulls together significant expertise in biomarker development and clinical trial design in bladder cancer. The innovation of this group lies not only in the novel scientific approaches (i.e. COXEN) under investigation, but also in the successful creation of a cohesive multi-institutional research collaboration dedicated to improved clinical outcomes in bladder cancer patients.

1.2.5 COXEN for Individualized Chemosensitivity Prediction

The "Co-eXpression ExtrapolatioN" (COXEN) algorithm utilizes molecular profiles as a "Rosetta Stone" for translating drug sensitivity signatures of one set of cancers into that of another [22]. The COXEN Principle has been validated using the NCI Developmental Therapeutics Program NCI-60 cell lines [23-25]. The NCI-60 panel is comprised of cell lines from diverse human cancers that have been profiled at the DNA, RNA, protein, and functional levels. The molecular characteristics of each of the NCI-60 cell lines and their relationship to patterns of anticancer activity in over 100,000 chemically defined compounds and natural product extracts has been established [23, 26-28]. Unfortunately, it was not feasible to include all important tumor types in the NCI-60 such as bladder cancer. Furthermore, even if other cancer cell types were added to the panel now, all compounds screened in the past 20 years would have to be tested again in the updated panel to gain the full predictive power of the database for all legacy compounds. Several years ago, these limitations raised two practical questions for our research team: Can drug sensitivity data on the NCI-60 panel be extrapolated to predict sensitivity of cell lines not included? More ambitiously, can the screening data be used to obtain predictive power for clinical responses of human cancers? To address these questions, we developed the COXEN algorithm and have subsequently demonstrated accurate predictions of chemotherapy sensitivity in both human bladder cancer cells lines and in bladder cancer patients [22, 29]. COXEN was retrospectively tested in seven independent cohorts of patients with breast (n = 275), bladder (n = 59), and ovarian (n= 143) cancer treated with multiagent chemotherapy, of which 233 patients were from prospectively enrolled clinical trials. In all studies, COXEN effectively stratified tumor response and patient survival independent of established clinical and pathologic tumor variables. In bladder cancer patients treated with neoadjuvant methotrexate, vinblastine, adriamycin, and cisplatin, the 3-year overall survival for those with favorable COXEN gene expression models' score was 81% versus 33% for those with less favorable scores (P = 0.002). COXEN for breast cancer patients treated with 5-fluorouracil, doxorubicin, and cyclophosphamide and ovarian cancer patients treated with platinum-containing regimens also stratified patient survival [5-year overall survival

100% versus 74% (P = 0.05) and 3-year overall survival 68% versus 43% (P = 0.008), respectively]. The COXEN algorithm is currently being studied in a randomized phase II study of muscle-invasive bladder cancer being run by the Southwest Oncology Group (SWOG) (NCT02177695). Muscle-invasive (pT2-4N0) patients are randomized to receive gemcitabine and cisplatin (GC) or methotrexate, vinblastine, adriamycin and cisplatin (MVAC), the patient then undergoes cystectomy and tumor is analyzed by the COXEN and the COXEN score for the GC vs MVAC combination is compared to test whether COXEN predicted a pathologic response for the combination (pT0). This approach has also been intensely scrutinized by well-known statisticians (MD Anderson Statistics Group, K. Baggerly; CTEP-Statistical Group, Lisa Meier-McShane; SWOG-Statistical Group, Cathy Tangen) and evaluated in other tumor types and by other investigators with similarly promising predictive results. This approach remains to be examined in a prospective clinical study.

Summary: COXEN uses molecular profiles as a "Rosetta Stone" for translating drug sensitivities of one set of cancers into predictions for another completely independent set of cell lines or human tumors. The COXEN methodology has been scrutinized and deemed methodologically sound by peer review. The ability of COXEN to predict drug effectiveness in patients a priori, from purely in vitro assays, is unique as no other tool currently either in practice or in development provides similar results.

1.2.6 Preliminary Data

1.2.6.1 Use of COXEN to rank predicted sensitivity of bladder tumors to 75 FDA approved anticancer agents

1.2.6.1.1 Drug Selection

Approximately 120 FDA approved anticancer agents have been tested in vitro by the Developmental Therapeutics Program (DTP) at the National Cancer Institute using the NCI-60 cell line panel (http://dtp.nci.nih.gov). The 75 FDA approved anticancer agents that will be used in Aim 1 (**Table 1**) of this proposal were those drugs that had 1) complete data sets and 2) a variation in response between "sensitive" and "resistant" cell lines that was determined by performing a linear regression on scaled rankings of cell lines vs. scaled negative logGI50 values for each drug following the removing cell lines in the sensitive and resistant groups. This analysis verified that there was a graded drug response within the NCI-60 panel that could be utilized for developing and testing gene expression-based COXEN models.

Table 1. Selected 75 FDA Approved Anticancer Agents

Abiraterone	Cisplatin	Eribulin	Imatinib	Oxaliplatin	Thiotepa
Arsenic Trioxide	Cladribine	Erlotinib	Irinotecan	Paclitaxel	Topotecan
Asparaginase	Clofarabine	Estramustine	Ixabepilone	Pazopanib	Toremifene
Axitinib	Crizotinib	Etoposide	Lapatinib	Pentostatin	Tretinoin
Azacitidine	Cytarabine	Exemestane	Lomustine	Romidepsin	Vandetanib

Bendamustine	Dacarbazine	Floxuridine	Mechlorethamin	Ruxolitinib	Vemurafenib
Bleomycin	Dactinomycin	Fludarabine	Melphalan	Sorafenib	Vinblastine
Bortezomib	Dasatinib	Fluorouracil	Mercaptopurine	Streptozocin	Vincristine
Busulfan	Daunorubicin	Gefitinib	Methotrexate	Sunitinib	Vismodegib
Carboplatin	Decitabine	Gemcitabine	Mitomycin	Tamoxifen	Vorinostat
Carfilzomib	Docetaxel	Hydroxyurea	Mitotane	Temsirolimus	
Carmustine	Doxorubicin	Idarubicin	Mitoxantrone	Teniposide	
Chlorambucil	Epirubicin	Ifosfamide	Nilotinib	Thioguanine	

1.2.6.1.2 COXEN Score Determination and Patient Drug Assignment

Microarray expression data from the GeneChip® Human Genome U133 Plus 2.0 Array will be processed and normalized using frozen robust multiarray analysis (fRMA) [30] that allows for the individual analysis of microarray data and subsequent utilization with other microarray data for data analysis such as the COXEN algorithm [22]. Gene expression models (GEM) for the prediction of chemosensitivity have been constructed for all the 75 FDA approved anticancer agents listed in **Table 1** using the COXEN method [22] with biomarker identification done using drug sensitive and resistant gene expression from the NCI60 cell panel and co-expression done using gene expression microarray data from a bladder tumor cohort [31]. The modeling process is completed in three distinct steps; preprocessing, feature extraction, and finally, model construction and validation. Preprocessing involves the application of frozen robust multiarray analysis (frma) to perform background correction, normalization, and summarization allowing for direct comparison among samples [30]. fRMA was applied using the fRMA package included in the Bioconductor suite in R. Second, for each drug, a set of biomarkers was found by implementing COXEN (coexpression extrapolation) [22] to 189 bladder tumor samples from the Lehmann tumor panel in conjunction with the National Cancer Institute 60 cell anticancer drug screening panel (NCI60). Finally, model building has been approached from two different methods. Using the biomarkers selected by COXEN, a linear discriminant analysis model (LDA) was employed on a smaller subset of genetic markers identified by Misclassification Penalized Posterior Classification (MiPP) to the top twelve most resistant and most sensitive cell lines in the NCI60 for every selected drug. This approach requires a new model to be constructed whenever a new sample is to be predicted and often leads to unstable outcomes. For this reason, a second approach was developed.

The second method employed logistic regression with an L2 penalty using dual optimization included in the Scikit-learn machine learning suite in Python [32]. While MiPP systematically adds features until addition of features does not increase model performance beyond a user defined threshold the L2 penalty allows all COXEN selected features to be included in the model by mathematically weighting features that contribute most to model performance. These weights are controlled via a parameter that was selected by performing cross validation over all possible 0.75/0.25 testing validation splits of the top 12 most resistant and sensitive cell lines in the NCI60. After the L2 parameter was determined via cross-validation, a final model was constructed using

all 12 most resistant and sensitive cell lines. Once complete the final model is stationary, meaning the model is not reconstructed for each new sample allowing robust prediction among new samples. Drug sensitivity and resistance predictions will range from -1 to +1 with -1 representing the most drug-resistant and +1 representing the most drug-sensitive. The resulting data will be displayed for the treating clinician as shown in **Figure 1** with the agents ranked in groups of 5 in descending order from the most sensitive on one of the tumors shown in **Figure 2**. The robust nature of these predictions is shown in **Figure 2** which plots the correlation of predicted sensitivity to these 75 drugs in 2 separate biopsies taken from 3 urothelial bladder tumors. While we will evaluate this intratumoral variation in the proposal by obtaining 2-3 core biopsies of the same site, we do not expect this to metrically alter the predictions.

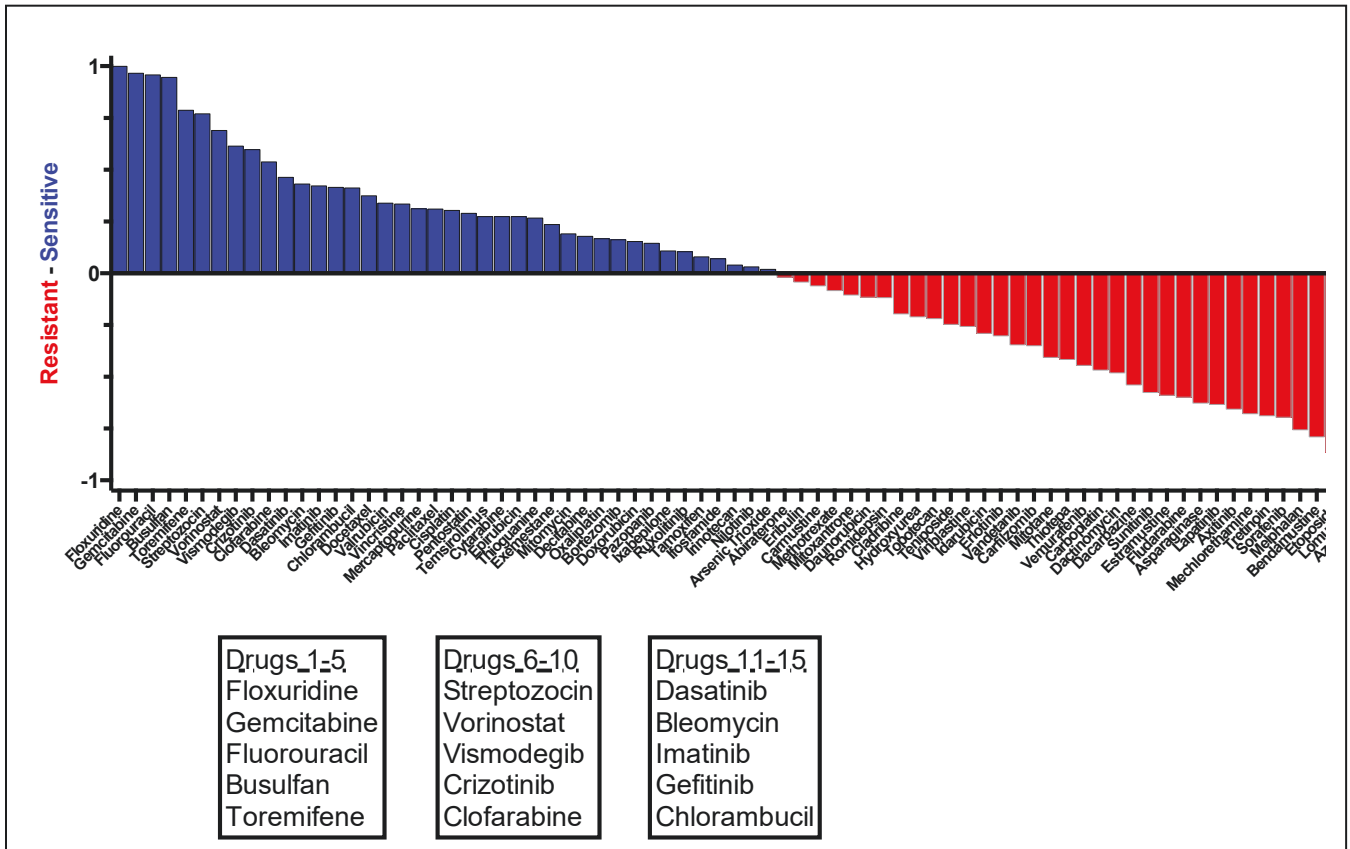

Figure 1: Waterfall-plot of predicted drug sensitivity of a bladder tumor (one of the samples in Figure 2) to the 75 FDA anti-neoplastic drugs based on microarray gene expression and COXEN analysis. COXEN scores are normalized from -1 to +1 with -1 representing the most drug resistant and +1 representing the most drug sensitive. Agents ranked in groups of 5 in descending order from the most sensitive.

1.2.7 Overview and Hypothesis

Multiple chemotherapeutic agents have demonstrated response rates of 5-20% when utilized as second-line chemotherapy in patients with metastatic bladder cancer. However, no chemotherapeutic agents have been shown to improve survival in this setting and therefore, there is no FDA-approved second line drug for patients with metastatic bladder cancer. Furthermore, the ability to select patients most likely to respond to a given anti-neoplastic agent is critical to improving outcomes in this clinical state. Hence, our ultimate objective is to build on the prior

retrospective experience with COXEN and prospectively test the hypothesis that use of tumor COXEN scores to select “best next therapy” for individual patients with chemotherapy-refractory metastatic bladder cancer will provide superior response rates, progression free and overall survival compared to historical controls. However, given the complexity of this approach, before embarking on such a trial, we propose to carry out a pilot trial evaluating feasibility of COXEN assigned “best next therapy”.

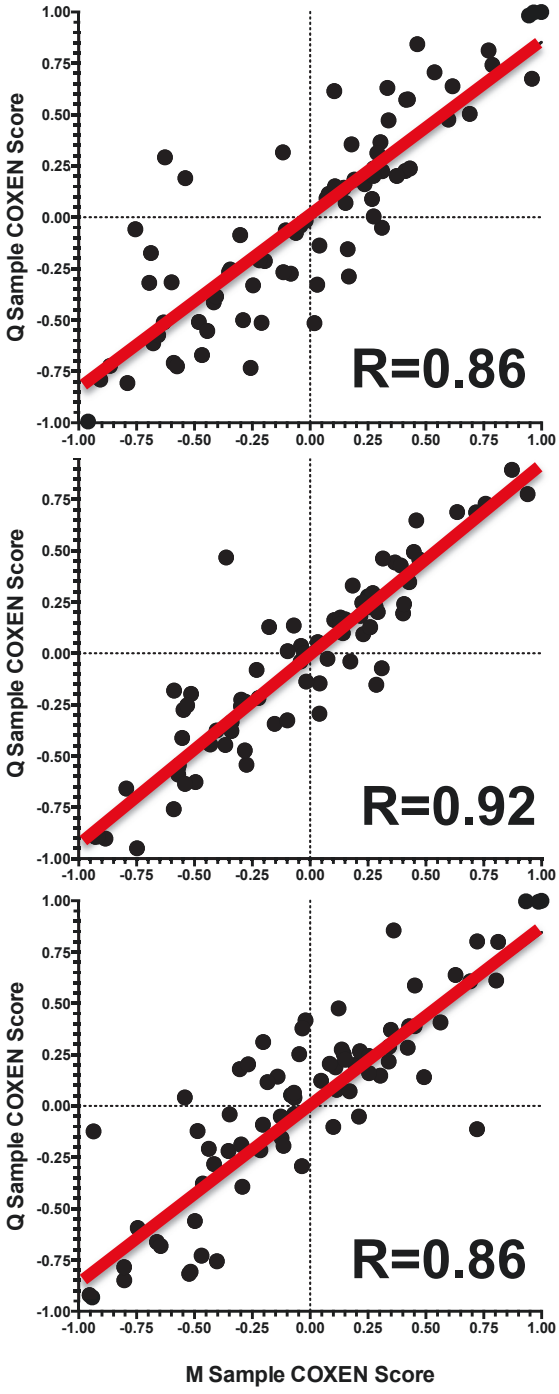

Figure 2: COXEN Scores for 75 FDA anti-neoplastic drugs on two independent core biopsies (M and Q) in tumors from 3 different patients. Correlations coefficient is shown.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have a histologically confirmed diagnosis of metastatic, progressive urothelial carcinoma of the bladder, urethra, ureter, or renal pelvis.

2.1.1.2 Patients must have progressive metastatic disease defined as new or progressive lesions on cross-sectional imaging.

2.1.1.3 Patients must have at least:

2.1.1.3.1 One measurable site of disease (according to RECIST criteria), defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan. See Section **6.3** for the evaluation of measurable disease.

2.1.1.3.2 Or, appearance of one new bone lesion

2.1.1.4 Patients must have been previously treated with at least one prior cytotoxic chemotherapy regimen or agent. Patients may have received any number of prior cytotoxic agents.

2.1.1.5 Archival tumor tissue must be available for enrollment.

2.1.1.6 Tumor amenable to biopsy will be mandatory for this study.

2.1.1.7 Age ≥18 years. ECOG performance status ≤2 (Karnofsky ≥60%, see **Appendix A**).

2.1.1.8 Patients must have normal organ and marrow function as defined below:

– hemoglobin	≥ 9 g/dL
– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,200/mcL
– platelets	≥75,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of normal
– creatinine	1.5 x the normal institutional limits
	OR
– creatinine clearance	≥40 mL/min/1.73 m^2

2.1.1.9 Because many of the therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.10 HIV-positive patients on combination antiretroviral therapy may be eligible if there are no pharmacokinetic interactions with the agents used on the study, stable on CART therapy and CD4 is >200 and viral load is undetectable.

2.1.1.11 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 The patient has received cytotoxic chemotherapy (including investigational cytotoxic chemotherapy) within 3 weeks or biologic agents (e.g., cytokines or antibodies) within 4 weeks prior to study enrollment.

2.1.2.2 Patients who are receiving any investigational agents.

2.1.2.3 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with brain metastases that are stable after ≥ 1 year after primary surgery or radiation will not be excluded.

2.1.2.4 The subject has not recovered to baseline or CTCAE ≤ Grade 1 from toxicity due to all prior therapies except alopecia and other non-clinically significant AEs.

2.1.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.2.6 Patients who are Hepatitis B or C positive.

2.1.2.7 Pregnant women are excluded from this study because the agents used in the study have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these agents, breastfeeding should be discontinued if the mother is treated with these agents. These potential risks may also apply to other agents used in this study.

2.2 SCREENING EVALUATION

Study eligibility is based on meeting all of the study inclusion criteria and none of the exclusion criteria at screening before study treatment administration. Screening evaluations may be performed as part of an NIH Screening protocol. This does not include the baseline correlative studies that will only be performed after the patient has signed the consent form.

The following assessments will be conducted no more than 28 days prior to subjects receiving the first dose of study drug on this protocol:

- History including performance status assessment and medication history
- Physical examination including height, weight, vital signs
- Laboratory assessments: CBC with differential, acute care panel (sodium, potassium, chloride, total CO2 Bicarbonate, creatinine, glucose, urea nitrogen), mineral panel (albumin, calcium total, magnesium total, phosphorus), hepatic panel (alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin), ionized calcium, amylase, lipase, LDH, total protein, GGT, Hepatitis B and C.
- 24-hour urine collection for creatinine clearance if needed
- Urinalysis
- Urine or serum HCG (in women of childbearing potential)
- CT scan of chest, abdomen and pelvis (or all known sites of disease) (MRI may be performed in subjects unable to tolerate contrast for CT).

- CD4 levels and HIV viral load (for patients with known HIV infection on combination antiretroviral therapy)
- Pathology evaluation/confirmation of urothelial carcinoma as documented in a pathology report confirmed by the referring institution's pathology department
- Research biopsy (see section **5.1**). This biopsy may be performed while patients are receiving other therapies, if they have signed this consent and been registered for screening as described in Section **2.3**.

2.3 REGISTRATION PROCEDURES

Registration will be a two-part process as patients are screened on this protocol. Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. To initially register a subject after the participant has signed the consent, complete the top portion the registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) indicating that the patient is being registered for screening and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. Once eligibility is confirmed after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and email the completed registration checklist to the CRO at NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

Subjects that do not meet screening criteria should be removed from the study following the procedure in section **3.5.4**.

2.3.1 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Patients with metastatic, progressive urothelial carcinoma of the bladder, urethra, ureter, or renal pelvis

Arms

Number	Name	Description
1	Arm 1	Treatment regimen as selected by COXEN model

2.3.2 Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1.

2.4 BASELINE AND ON STUDY EVALUATIONS

The baseline assessments do not need to be repeated if they have been performed at screening within the appropriate 16 day timeframe.

- 12-lead ECG
- Vital Signs
- Laboratory Assessments
  - CBC with differential

  Serum chemistries:

  - Hepatic panel (alkaline phosphatase, ALT/GPT, AST/GOT, total bilirubin, direct bilirubin)
  - Acute care panel (sodium, potassium, chloride, total CO2 (Bicarbonate), creatinine, glucose, urea nitrogen)
  - Mineral panel (albumin, calcium total, magnesium total, phosphorus)
  - Ionized calcium
  - Amylase
  - Lipase
  - Lactate dehydrogenase (LDH)
  - Total protein
  - γ-glutamyl transferase (GGT) (baseline only)
- Urinalysis
- TSH (baseline only)
- PT/INR or PTTT
- Urine or serum HCG (in women of childbearing potential) within 48 hours prior to receiving study medication.
- Research blood (see section **5.1**)
- Research FDG-PET MRI (see section **5.1.10**)

On study assessments for other procedures performed during the study are found in the study calendar section **3.4**.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This will be a Pilot single-arm, open-label study using the COXEN score to select the "best next therapy" from a list of 75 FDA anti-neoplastic drugs (**Table 1**), in patients with metastatic bladder cancer who have progressed despite treatment with cytotoxic chemotherapy. Agents on the list may be given as single agent therapy or as combination therapy provided there are at least phase 1 data to support the use of the combination. Eligible patients will be treated with one or a combination of drugs based on the 75-drug COXEN algorithm analysis. Each participant will be given an option to be assigned to a second COXEN-based regimen at the time of disease

progression. The COXEN analysis for retreatment will be performed on the biopsy collected at the time of progression; however, if the patient refuses the biopsy or the recurrent disease is not amenable to biopsy, the baseline COXEN waterfall plot will be used to make the decision. No more than 2 COXEN-based assignments will be allowed on study. The primary objective of this pilot study is to determine if a sufficiently high fraction of patients is able to have results produced by the COXEN algorithm within 3 weeks of initiating the process. The COXEN algorithm requires a multi-step process (pathology, tissue processing, mRNA profiling, bioinformatics etc.) and is potentially labor intensive and time intensive. Given the disease state of patients eligible for this protocol, using this algorithm to select a treatment would only be a worthwhile process to undertake if it can be demonstrated that a very high fraction of patients is likely to obtain the benefit from the procedure. The secondary objectives include: assessment of toxicity, response rate and progression-free survival by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and overall survival of the COXEN selected therapeutic agent(s).

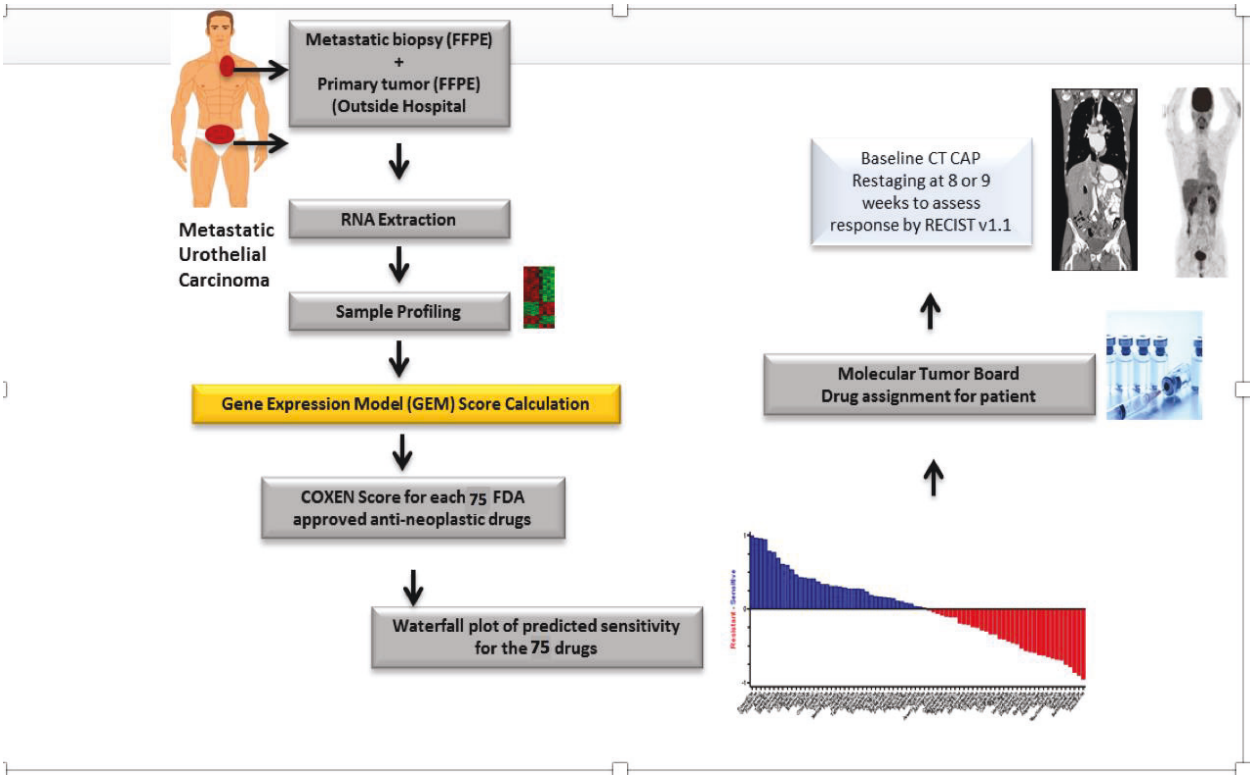

Figure 3

3.1.1 Schema

(See **Figure 3**)

1. Consultation in the medical oncology clinic at the NIH Clinical Center
2. Provide tumor block of the primary tumor
3. Undergo a CT of the chest, abdomen and pelvis (MRI may be performed in subjects unable to tolerate contrast for CT)

5. Mandatory core biopsy of a metastatic site of disease (Sample 2, **Figure 4**). (Note: samples 1 and 2, **Figure 4** will be batched and analyzed together once sample 2 has been collected.)
6. Tissue will be processed and whole genome Affymetrix microarray (Hu133A or similar) will be performed in Dr. Meltzer's CLIA certified lab at the NIH Clinical Center.
7. The genomic data will be transferred from the NIH to University of Colorado Cancer Center (UCCC) as described in section **6.2.1** and COXEN analysis performed.
8. UCCC team will return a rank report with COXEN scores for the 75 anti-neoplastic FDA approved drugs (**Figure 1**).
9. Molecular Tumor Board: Drug selection will be based on COXEN predicted potency and tailored to the patient's case (renal function, allergies prior use etc.) as determined by the advisement from the members of a molecular tumor board.
10. Drug(s) will be ordered from the NIH pharmacy.
11. COXEN tailored treatment will be started within 3 weeks of initial biopsy.
12. Depending on restaging schedule, restaging CT at 8 weeks or 9 weeks (± 1 week).
13. Optional core biopsy of a metastatic site will be scheduled at time of disease progression. COXEN analysis will be performed along with mutational analysis as described in section **5.1.1**.

3.1.2 Pathology

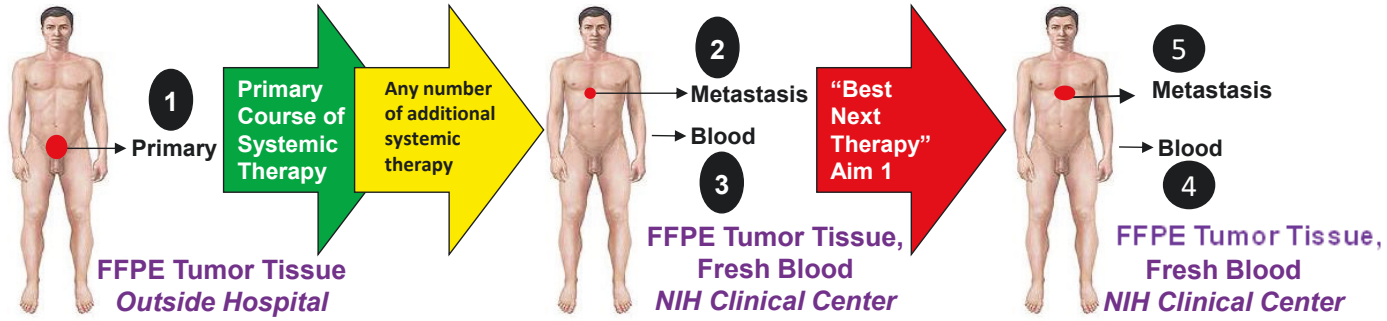

Figure 4: Tissue and blood samples available for gene expression and mutational evaluation in relation to prior or planned treatment

Tumor profiling will be carried out on FFPE tissues from the primary site obtained from the referring hospital (Sample 1, **Figure 4**) and on FFPE biopsy tissue (Samples 2 and 5, **Figure 4**) obtained at the NIH Clinical Center with study enrollment. The RNA extraction and methodology will be as described in our previous work [31]. We will profile the metastasis biopsy and use this data to generate the COXEN Score that will rank the 75 FDA approved anti-neoplastic drugs.

3.1.3 COXEN Score Determination

This will be carried out in two steps.

3.1.3.1 Quality Control Assessment of Microarray Data

Microarray data from individual patient tumors will be generated using the GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA) and will be assessed for quality using the following accepted criteria: 1) inspection of array images and observe for "spatial artifacts" and obvious problems; 2) analysis of box and histogram plots of log2(PM) values for each individual array with comparison for consistency across a set of fixed bladder tumor arrays; 3) proportion present plots will be generated using probe pairs (PM, MM) testing gene "presence" or "absence" and analyzing for consistency compared to a fixed set of bladder tumor arrays; 4) hybridization controls (bioB, bioC, bioD, and cre) will be plotted for verification of increasing concentrations within the array; 5) QC stats plots will be created measuring % present, average background intensity, bioB, bioC, bioD and creX presence, and 3':5' ratios of actin and GAPDH; and 6) evaluation of RNA degradation plots. If an outlier is identified in more than 2 of these tests, the sample will be flagged and the data deemed unreliable for COXEN analysis.

3.1.3.2 COXEN analysis

COXEN analysis will be done by the Cancer Pharmacology Shared Resource for the University of Colorado Cancer Center by Dr. Daniel L. Gustafson and Dr. Ryan J. Hansen as a backup under the supervision of Dr. Dan Theodorescu.

Sample Processing & Reporting:

- Coded gene expression data will be sent to Dr. Gustafson or Hansen at the Cancer Pharmacology Shared Resource with a unique identifier (UI).
- Data will be processed for COXEN analysis with the UI carried and validated at all steps using a defined SOP.
- All data processing and predictive steps will be tracked with the use of a "sample checklist" as outlined in the SOP verifying each step.
- Sample output (drug sensitivity/resistance prediction) will be sent to the proper individual responsible for collecting the data along with an electronic copy of the "sample checklist" using the UI to identify the sample.

Sample Analysis:

1. Receive and log in sample data and begin "sample checklist"
2. Perform QC Analysis for MicroArray Data.
3. Frozen RMA process the gene expression data.
4. Run the COXEN algorithm for prediction of drug sensitivity and resistance.
5. Complete the data analysis and presentation (as specified in the SOP) and deliver.

QC Analysis Steps for Individual MicroArrays

1. Inspect array images
    - Look for "spatial artifacts", obvious problems
2. Boxplot and histogram plot of log2 (PM) values for each array

- Looking for consistency across arrays, identifying outliers

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

***If an outlier is identified in more than 2 of these tests, the sample should be thrown out.**

3.2 DRUG ADMINISTRATION

Agents will be administered per package insert. Please see **Appendix B** for a summary of FDA approved doses. Alternate dosing or frequencies of the listed agents may also be utilized provided that phase 1 data are available. Agents will be administered until whichever occurs first among the following: disease progression or toxicity which requires discontinuation of study drug.

Subjects who have a response to COXEN-based therapy lasting 6 months or greater have the option to be re-assigned to a second COXEN-based agent. If there is not an established combination (with at least phase 1 data for safety), only one drug would be given. No more than 2 COXEN-based assignments will be allowed on study.

3.3 DOSE MODIFICATIONS

Dose modifications will be guided by package inserts and consultation between the investigator and NIH CC Pharmacy.

If a drug must be permanently discontinued due to toxicity, an alternative COXEN based regimen may be selected per PI discretion. Only one change in regimen will be made. At the second occurrence of intolerable toxicity in a patient, the patient must be removed from study therapy.

3.4 STUDY CALENDARS

All drugs have different schedules; we will try (as much as possible) to establish an every 2 week (28 day cycle) or every 3 week (21-day cycles) clinic visits and infusions when appropriate.

Unless otherwise specified, screening evaluations may be performed within 28 days prior to initiation of study therapy, baseline assessments may be performed within 16 days prior to initiation of study therapy, day 1 assessments and beyond may be performed within 1 week prior to indicated time.

3.4.1 For subjects seen every 2 weeks (28 day cycle)

Procedure	Screening/ Baseline	Cycle 1 (28 days)		Subsequent Cycles		Post Therapy Follow-up	
		Day 1	Day 15	Day 1	Day 15	Safety Visit (4-5 weeks post treatment)	Long-term follow up
History and PE	X	X	X	X	X	X	
Vital signs	X	X	X	X	X		
Performance Score	X	X	X	X	X		
Laboratory assessments[1]	X	X	X	X	X	X	
Urinalysis	X						
TSH, PT/INR, PTT	X						
24 hour urine collection if needed	X						
Anti HIV 1/2, HIV viral load, CD4	X						
Urine or serum HCG in women of childbearing potential	X						
Confirmation of diagnosis	X						
Biopsies[2]	X			X			
COXEN assessment	X						

Procedure	Screening/ Baseline	Cycle 1 (28 days)		Subsequent Cycles		Post Therapy Follow-up	
		Day 1	Day 15	Day 1	Day 15	Safety Visit (4-5 weeks post treatment)	Long-term follow up
Correlative studies[3]	X	Refer to Section **5.1** and **Appendix C**					
CT CAP or MRI[4]	X	Every 2 cycles					
FDG-PET MRI[4]	X			C3 only			
EKG	X						
Adverse Events		X→					
Concomitant Medications		X→					
Phone calls to assess survival status[5]							X

[1] Laboratory evaluations include: For screening see section **2.2**; for baseline and on study evaluations, see section **2.4.** For follow up evaluations, see section **3.5.2.1**

[2] Screening/Baseline biopsy is mandatory. Optional biopsy scheduled with restaging scans, but only performed at the first occurrence of progressive disease.

[3] Refer to Section **5.1** and **Appendix C** for further information.

[4] Imaging assessments may be performed within +/- 1 week of indicated time.

[5] Patients will be followed for survival with telephone calls every 2 months until death.

3.4.2 For subjects seen every 3 weeks (21 day cycle)

Procedure	Screening/ Baseline	Cycle 1 (21 days)	Subsequent Cycles	Post Therapy Follow-up	
		Day 1	Day 1	Safety Visit (4 – 5 weeks post rx)	Long-term follow up
History and PE	X	X	X	X	
Vital signs	X	X	X		
Performance Score	X	X	X		
Laboratory assessements[1]	X	X	X	X	
Urinalysis	X				
TSH, PT/INR, PTT	X				
24 hour urine collection if needed	X				
Anti HIV 1/2, HIV viral load, CD4	X				
Urine or serum HCG in women of childbearing potential	X				
Confirmation of diagnosis	X				
Biopsies[2]	X		X		
COXEN assessment	X				
Correlative studies[3]		Refer to Section **5.1** and **Appendix C**			
CT CAP or MRI[4]	X	Every 3 cycles			
FDG-PET MRI[4]	X		C4 only		
EKG	X				
Adverse Events		X →		→	
Concomitant Medications		X →		→	
Phone call to assess survival status[5]					X

[1] Laboratory evaluations include: For screening see section **2.2**; for baseline and on study evaluations, see section **2.4.** For follow up evaluations, see section **3.5.2.1**
[2] Screening/Baseline biopsy is mandatory. Optional biopsy scheduled with restaging scans, but only performed at the first occurrence of progressive disease.
[3] Refer to Section **5.1** and **Appendix C** for further information.
[4] Imaging assessments may be performed within +/- 1 week of indicated time.
[5] Patients will be followed for survival with telephone calls every 2 months until death.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.5.1 Criteria for removal from protocol therapy

- Progressive disease after 2 COXEN based regimens
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in section **3.3**
- Investigator discretion

3.5.2 Follow Up

3.5.2.1 Safety visit

Patients will be asked to return to NIH, 4-5 weeks after receiving the last dose of study medication for a safety assessment that will include a history, physical examination, CBC, acute care panel, mineral panel and LFTS. Those patients that cannot return to the NIH will be contacted by telephone. Patients removed from study therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

3.5.2.2 Long term follow up

Patients will be followed until death. The Investigator or designees will make every possible attempt every 2 months (±7 days) after the final safety visit to contact the patient or family to obtain the survival information of the patient and start date of additional anticancer treatment.

3.5.3 Off-Study Criteria

- Participant requests to be withdrawn from study
- Death

3.5.4 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Supportive care will be administered per NIH CC Pharmacy guidelines.

All concomitant medications will be recorded. Medications will be prohibited if contraindicated in the package insert. Other medications may be administered at the discretion of the investigators with the guidance of the package insert.

5 CORRELATIVE STUDIES

5.1 BIOSPECIMEN COLLECTION

5.1.1 Mutational landscape of bladder cancer before and after initial therapy

5.1.1.1 Overview and Hypothesis

Exome sequencing has opened the door for cost efficient, high-throughput, and accurate mutation calling by limiting the search space to the small fraction of the genome (~2%) that encodes for proteins. With 340 treatment naïve bladder cancer tumors already sequenced, we have a compendium of catalogued mutations for which we can compare new samples. Here we will evaluate the catalogued mutations on exome sequences from treatment naïve FFPE patient samples to both verify if common mutations occur in the patient and also determine if the detection of mutations is different between exome sequenced fresh frozen and FFPE tumor tissue samples. In addition, we will also compare the same patient's treatment naïve FFPE primary tumor samples to an FFPE biopsy taken after initial therapy but before "best next therapy" to test the hypothesis that certain mutations will be found at a higher frequency in treated patients suggesting their possible importance in drug resistance.

5.1.1.2 Approach

5.1.1.2.1 Tissue Samples

Patients will undergo a percutaneous core needle biopsy of the tumor under local anesthesia. These percutaneous biopsies will be performed by interventional radiology (CT scan or ultrasound guidance). If needed, patients will be offered conscious sedation for the biopsy procedure. Sample collection will be performed according to standard operating procedures. 2-3 core needle biopsies will be obtained and 2-32 samples will be formalin fixed and sent to the Laboratory of Pathology in the NCI. The remaining samples will be immediately placed in cryovials, snap frozen on liquid nitrogen or dry ice and transported to Dr. Donald Bottaro's laboratory (See **Appendix C**).

Archival samples will be labeled and reported as sample 1 – formalin fixed paraffin embedded (FFPE) sample; fresh tissue biopsies will be labeled and reported as sample 2 – fresh baseline biopsy or sample 5 – biopsy at time of progression (optional).

Mutation Detection: Here we will perform either directed exome sequencing (DES) from formalin fixed paraffin embedded (FFPE) tissues for 476 mutated genes described above or whole exome sequencing (WES) using Illumina HiSeq machines and other technologies as needed.

Tissues: Tissues from patients will be collected for the specific analysis as described below in detail. Matched normal tissues (Buffy coat/WBC) will also be collected for SNP verification.

5.1.1.2.2 Application of Next Generation Sequencing (NGS) on FFPE Tissues

Recent prior work has demonstrated the feasibility and robustness of FFPE exome sequencing. One study describes massively parallel DNA sequencing that characterizes base substitutions, short insertions and deletions (indels), copy number alterations and selected fusions across 287 cancer-related genes from FFPE clinical specimens [33]. Validation of this approach using pooled cell lines that model key determinants of accuracy, including mutant allele frequency, indel length and amplitude of copy change indicated a test sensitivity of 95-99% across alteration types with

>99% positive predictive value. The ability to carry out reliable exome sequencing in FFPE was confirmed by others [34]. There is less clarity on the issue of whether the same mutations are found in identical tissue samples preserved by FFPE and frozen methodologies for the purposes of discovery but this appears to be the case. A small study compared the validity of whole exome sequencing in one primary fresh-frozen sample, corresponding FFPE prostate cancer sample and matched adjacent normal prostatic tissue. The sequenced reads were mapped and compared and showed comparable (but not identical) exome sequencing results between FFPE and corresponding fresh-frozen cancer tissues in one sample [35].

5.1.1.2.3 DNA extraction from FFPE Tissues

There are several challenges that are present when preparing FFPE versus frozen genomic DNA, all of which can be overcome to provide high quality sequence information. The first step will be to extract the sample from its paraffin embedding. For the FFPE samples from the metastasis biopsy volume of 17.7mm3, we expect between 3.5-5µg of isolated DNA. The second challenge with FFPE samples is the general poor quality of the DNA extracted due to cross-linking of protein to DNA, separating the DNA from paraffin and DNA degradation from the embedding process, which includes time to embedding, temperature, and buffering of the paraffin itself. These issues have been worked out with gel separation techniques and proper buffering of DNA extraction solutions. Standard protocols are now available from peer-reviewed publications such as the two cited above [33, 34]. Furthermore, we have successfully used the FFPE DNA Extraction Kit (Diagenode) and will use this kit for all FFPE gDNA extractions.

5.1.1.2.4 Library Prep and Exome Sequencing

Isolated DNA from FFPE samples will be sent to the Meltzer lab for library preparation and exome sequencing (pmeltzer@mail.nih.gov – 240-760-7376). We will be following the SureSelect Human All Exon Capture Kit (V5, Agilent Technologies) protocols for library preparation, hybridizations, and exome capture. Sequencing will be done using the Illumina HiSeq 2500, providing roughly 46Gbp per lane of a flow cell run in the 2X125 mode. The exome represents roughly 30Mbp, thus by multiplexing 8 samples per lane, we can produce roughly 150X coverage of the exome.

Sequence reads will be aligned using GSNAP [36] to produce .bam files (binarized sequence alignment/map files). We will use the GATK platform [37] to make SNP and indel calls, thus producing .vcf files (variant call format). We will finally use AnnoVar [38] to annotate the variant calls. FFPE samples require two additional corrections to be directly compared. First, we must correct for the allelic fraction to account for the purity or the admixture of the sample. By plotting the frequency of alleles between the two samples, we can model the difference allele frequencies by fitting a simple regression and thus correct of the any bias between samples. Secondly, we must correct for the clonality of the cells within the population. That is, does the mutation occur in all of the cells, thus clonal, or does a mutation arise in a subpopulation of cells, thus being subclonal. We will make this correction using the program ABSOLUTE [39]. After these two corrections have been made we can directly compare the mutations between the FFPE samples.

5.1.1.3 Sample Comparisons

Several analyses will assess relationships between mutation frequency and preservation method of the specimen, tumor heterogeneity, tumor progression and prior treatment.

Frozen vs. FFPE (DES): The 476 mutated genes will be evaluated on the 20 patients enrolled in the study on their treatment naïve tumor sample (original specimen-Specimen 1, **Figure 4**). The frequency of the mutations will be compared in a stage and grade matched fashion (to eliminate the effect of stage/grade on tumor mutation frequency which we know exist [40, 41]) to determine if there are any differences in mutation frequency (i.e. detection) as a function of preservation method and if this is gene/mutation dependent.

Tumor Heterogeneity (WES): Three replicate biopsy samples will be collected (Samples 2 and 5, **Figure 4**) and 3 cores will be taken from the FFPE block (as described by us [31]) of the original specimen (Specimen 1, **Figure 4**). For each specimen source, an analysis will be carried to determine the changes in mutation frequency as a function of tumor heterogeneity.

Tumor Progression and Initial Treatment (WES): We assess the relationship of mutation frequency as a function of progression (primary vs metastasis) and initial treatment. Only one comparison will be made: baseline untreated from the primary tumor -Specimen 1, **Figure 4**) vs. metastatic site biopsy (pre "best next treatment", Specimen 2, **Figure 4** and between pre and post treatment biopsies (Specimens 2 and 5 in **Figure 4** respectively), which will encompass both variables (primary vs metastasis and pre vs post treatment). This is a recognized limitation of the study but it is nevertheless a clinical reality since we know almost all patients that present will NOT have a biopsy of a treatment naïve metastasis, while most will have a treatment naïve tissue specimen from the initial specimen.

5.1.2 Saliva samples for genetic/genomic analysis

Tumor samples will be used as a source of DNA for genetic/genomic studies. Saliva samples will be used as a source of genomic (germline DNA) from the patients to compare with tumor DNA for somatic mutations. Whole genome and whole exome studies may be conducted using the material collected. Studies for the analysis of chromosomal abnormalities may also be performed.

We plan to do genomic analysis of the saliva samples by both whole-genome and whole-exome sequencing. We will confirm recurrent mutations in genes previously identified as being mutated in urothelial carcinoma and we will identify additional altered genes and pathways that have been implicated in this disease and compare the responders and non-responders.

See **Appendix C** for details regarding saliva collection and handling

5.1.3 Mutational landscape of circulating tumor DNA before and after "best next therapy"

5.1.3.1 Overview and Hypothesis

There are currently no peripheral blood-based markers available to provide an early indication of response to systemic therapy in patients with metastatic bladder cancer. We will test the hypothesis that circulating tumor DNA (ctDNA) with cancer specific mutations will be present in all patients before "best next therapy". We will also examine 1) the molecular similarity of the "liquid biopsy" (ctDNA) to the contemporaneous tumor biopsy; 2) The change in mutation profile of the liquid biopsy following the "best next therapy" described in Aim 1 which may provide clues to the mechanisms responsible for the observed clinical response.

5.1.3.2 Sample Collection and Handling

Patients enrolled in the trial will undergo collection of a blood sample for evaluation of changes in mutation profile, as detailed in **Appendix C**.

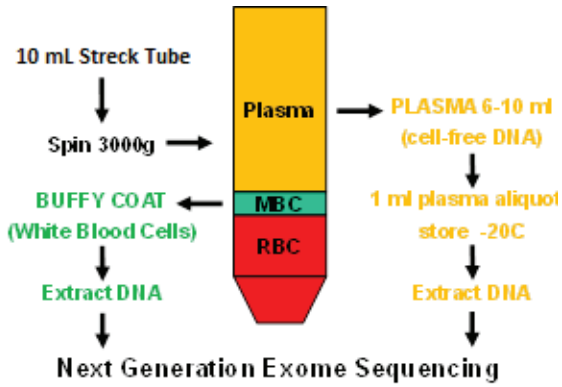

Figure 5: Blood sample processing

5.1.3.3 Approach

The ctDNA will be evaluated using well described approaches that we [42] and other have recently described [43, 44]. The studies will be performed in the laboratory of Dr. Paul Meltzer.

5.1.3.3.1 ctDNA isolation

ctDNA will be extracted using the QIAmp Circulating Nucleic Acid Kit (Qiagen). It is estimated that there is between 1-100ng/ml ctDNA in blood plasma. Given the 6-10ml of plasma samples, we estimate the total ctDNA yield to be 6ng-1µg. To determine if somatic mutations or copy number alterations have arisen in patient tumors, we will also extract gDNA from white blood cells, which can be done using the DNAeasy Blood & Tissue Kit (Qiagen).

5.1.3.3.2 Exome Sequencing

Isolated DNA samples will be sent to the Meltzer Lab for library preparation and exome sequencing. We will be following the SureSelect Human All Exon Capture Kit (V5, Agilent Technologies) protocols for library preparation, hybridizations, and exome capture. Sequencing will be done using the Illumina HiSeq 2500, providing roughly 46Gbp per lane of a flow cell run in the 2X125 mode. We expect to find only a small fraction of the full exome within ctDNA but use the full 30Mbp for calculating coverage. The exome represents roughly 30Mbp, thus by multiplexing 8 samples per lane, we can produce roughly 150X coverage of the exome. To avoid technical differences in sequencing, we will match multiplexed ctDNA with exome white blood cell exomes on the same flow cell. We will follow the same sequence analysis methods as outlined in Aim 2. Sequence reads will be aligned using GSNAP to produce bam files (binarized sequence alignment/map files). We will use the GATK platform to make SNP and indel calls, thus producing vcf files (variant call format). We will finally use AnnoVar to annotate the variant calls.

5.1.3.3.3 Sample Comparisons and Patient Outcome Analyses

Two analyses will assess relationships between mutation frequency and prior treatment and mutation frequency and patient outcome.

Sensitivity of in ctDNA Tumor Mutation to Detect Metastasis (DES): ctDNA will be obtained at baseline. Since all these patients have metastatic disease detectable by imaging, we expect the ctDNA to harbor detectable mutations. This will be carried out on a smaller subset (to enhance the feasibility of detection by using multiple methods) [42] of the 476 mutated genes mentioned above with the addition of any new genes found in Aim 2 as a function of progression and treatment.

Tumor Mutation in ctDNA vs. Mutation in Biopsy (DES): ctDNA and contemporaneous biopsy mutations will be compared (Specimen 2 vs. 3, **Figure 4**). This will be carried out on a smaller subset (to enhance the feasibility of detection by using multiple methods) [42] of the 476 mutated genes mentioned above with the addition of any new genes found in Aim 2 as a function of progression and treatment.

Tumor Mutation vs "best next therapy" (WES): We assess the relationship of mutation frequency as a function of treatment. Only one comparison will be made: baseline pretrial blood (Specimen 3, **Figure 4**) vs. post-treatment (Specimen 4, **Figure 4**). We realize that it is possible that all 20 patients will be treated with a different drug. However, we will still be able to answer the question if some treatments alter the genomic composition of the ctDNA, a question that has not yet been answered in bladder cancer and a stepping stone to larger and more directed studies.

Tumor Mutation vs Patient Outcome (WES): As above, the ctDNA will be obtained at baseline and 8 or 9 weeks post "best next therapy". Cox proportional hazards model will be used to determine the association of overall survival and progression-free survival with various ctDNA findings (presence or absence of detectable mutations, type of mutations/gene families in baseline, type of mutations/gene families in post treatment sample, presence of new mutations as a function of treatment vs no new mutations arising after treatment). For all survival analyses, the time to death due to bladder cancer will be defined as the time between the date when the baseline sample of blood was obtained and the date of death. Survival curves will be compared using log-rank testing. This will be an exploratory analysis based on the limited number of patients that will be enrolled on the study.

5.1.4 Immune Subset Analysis

Peripheral blood mononuclear cells (PBMC) will be assessed using multiparameter flow cytometry for immune subsets including but not necessarily limited to CD8+ T-cells, CD4+Foxp3- T-cells, Tregs, monocyte subsets, MDSC subsets. Assessment will include functional markers, i.e. PD-1, Tim-3, CTLA-4, PD-L1, HLA-DR and/or CD40.

See **Appendix C** for details regarding blood collection and handling.

Members of the Trepel lab will enter the samples in a secure patient database, process the samples for viable cell storage, label each sample with a unique 2D barcode, and viably store the samples. They will prepare the samples for staining, stain and run the samples by multiparametric flow cytometry (MACSQuant, Miltenyi Biotec, Bergisch Gladbach, DE), and analyze the data using FlowJo (FlowJo LLC, Ashland, OR) software.

5.1.5 Peripheral Blood Immune Gene Expression

Peripheral blood immune gene expression will be evaluated by the Trepel Lab using the NanoString nCounter® platform (NanoString Technologies, Seattle, WA). RNA will be isolated using the PAXgene Blood RNA Kit according to the manufacturer's instructions. Peripheral immune gene expression will be evaluated using the PanCancer Immune Profiling Panel of 730

immune genes and 40 control genes at baseline and post-therapy to look for correlates of clinical response with innate or adaptive immunity.

Contact the Trepel Lab, as detailed in Section **5.1.4** and in **Appendix C: Sample Collection for Correlative Studies**.

5.1.6 Pro-inflammatory cytokines

Plasma levels of a comprehensive panel of pro-inflammatry molecules will be tested, including IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α (inflammatory cytokines panel III). Many of the markers can be produced by activated T cells. The analysis will be conducted with MSD V-PLEX technology that provides the best assays for cytokine analysis.

See **Appendix C: Sample Collection for Correlative Studies** for details regarding collection and handling of specimens

For samples collected by outside institutions:

Please notify the lab by email (NCIBloodcore@mail.nih.gov) when the sample is being shipped (Include the Fed Ex tracking number in the email)

Send samples by FedEx Priority Overnight Shipping. The NIH is not open on weekends or government holidays. Be careful not to ship any samples to arrive on a Saturday, Sunday or government holiday. We will send the site a government holiday calendar. If you are not certain whether the government will be open, please send an email to the addresses above at any time and we will respond as quickly as possible.

Ship Monday-Thursday only to:

William Figg Laboratory
9000 Rockville Pike
Bldg. 10/Room 4B11
Bethesda, MD 20892
Phone: 240-760-6180

5.1.6.1 Laboratory test and data analysis

All plasma samples will be transferred to Dr. Liang Cao's laboratory (at Bldg. 37/Rm6134, phone: 301-435-9039) for the analysis of angiogenesis markers and pro-inflammatory cytokines.

5.1.7 Plasma HGF and MET

Plasma will be collected to determine whether urinary hepatocyte growth factor (HGF), urinary soluble MET receptor (sMet), plasma HGF and plasma Met levels are biomarkers of bladder cancer (transitional cell carcinoma; TCC) and/or response to systemic treatment with the combination of cabozantinib and nivolumab. Blood samples for correlatives may be drawn ± 2 days of specified collection time point.

Electrochemiluminescence immunoassays for c-Met and HGF. We use Streptavidin-coated 96-well plates (MSD, Gaithersburg, MD) designed specifically for use in a Meso Scale Discovery Sector 2400 Imager. For both assays, a biotin-tagged, affinity-purified specific capture antibody either R&D BAF 358 or R&D MAB 694 is added to each well and incubated for 1 hour at room temperature (RT) with shaking. Sample wells are then washed with PBS before adding standards

and patient plasma / urine samples; diluted standards are used for c-Met (R&D 358-MT-100) and HGF (R&D 294-HG recombinant human HGF) to generate a curve. Plates are then incubated for 1 hour at RT while shaking. Wells are then washed with PBS before adding detection antibody (R&D AF 276 with MSD Sulfotag for c-Met), (R&D AF-294 labeled with MSD Sulfotag for HGF) for 1 hour at RT with shaking. Finally, plate wells are washed four times with PBS before adding MSD Read Buffer T and then read immediately in a MSD Sector 2400 Imager. All samples and standards are measured in duplicate. Mean values from a standard curve are constructed by plotting signal intensity against standard concentration. A nonlinear regression curve fitting algorithm using GraphPad Prism is used to generate an equation from which sample values for c-Met or HGF concentration are calculated from mean signal intensity values.

- Blood samples should be collected prior to any surgery or drug treatment in the presence of citrate or EDTA as anticoagulant. Assuming 50% of the whole blood volume will be obtained as plasma, the blood volume required for sMet analysis is 1 ml per sample; the blood volume required for HGF analysis is 4 mL.
- Blood samples should be centrifuged within 30 min at 1,300 rcf at room temperature for 10 minutes. Do not use braking to stop the centrifuge. This will give three layers: (from top to bottom) plasma, leucocytes (buffy coat) and erythrocytes.
- Carefully aspirate the supernatant (plasma) at room temperature and transfer to a new centrifuge tube. Take care not to disrupt the cell layer or transfer any cells.
- Inspect the plasma for turbidity. Turbid samples should be centrifuged and the clarified supernatant should be transferred to cryovials and stored at –80°c. Ensure that the cryovials are properly labeled.

Plasma samples will be stored by Dr. Figg's laboratory and processed and analyzed by Dr. Bottaro's laboratory. See **Appendix C: Sample Collection for Correlative Studies** for details regarding collection and handling of specimens.

5.1.8 Circulating tumor cells (CTC)

Peripheral blood will be collected to correlate changes in circulating tumor cells with clinical response. CTCs will be assessed using ferrofluidic enrichment and multi-parameter flow cytometric detection. CTCs are identified by positive expression of epithelial markers and a viability marker and negative expression of hematopoietic markers.
See Section **5.1.4** and **Appendix C: Sample Collection for Correlative Studies** for details regarding collection and handling of specimens.

5.1.9 Circulating tumor cells (Epic Science)

For details regarding collection and handling of blood samples to be sent to Epic Science, see **Appendix C: Sample Collection for Correlative Studies**.

IMPORTANT: The first 5 mL of blood collected from the fresh venipuncture cannot be used for the collection into the Streck tubes due to possibility of contaminating epithelial cells during venipuncture. Please ensure that at least one blood tube of 5 mL or more is collected prior to collection of the CTC sample to avoid adversely affecting the test results.

Prevention of Backflow: Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

5.1.9.1 Specimen Shipment Logistics

All shipments must include requisition forms that contain Patient ID, Collection Date and time, Collection Site (including address), Time Point (if applicable), and the appropriate trial code (Partner Protocol # / Epic Internal ID). Requisition forms for samples being shipped to LabCorp must also contain the appropriate LSN (Accessioning) code listed in the table below. Partner will provide a copy of the requisition form to Epic for review and approval prior to trial initiation. Clinical sites should provide email notification of sample shipment to Epic Sciences on the day of collection, using the applicable email address specified in the table below. The email should contain:

- Trial codes (Partner Protocol # / Epic Internal ID)
- Patient ID
- Collection date and time*
- Time point/Visit
- Tracking information

If possible, include a scanned copy of the completed sample requisition form. Partner will be responsible for all blood collection supplies, shipping materials, and shipping expenses.

* When collection time is not provided, Epic Sciences will assume the sample was collected at 8:00AM (local time) on the date of collection.

Sent notification to: partners@epicsciences.com
Ship to: Epic Science
Attn: (Partner Protocol #/Epic internal ID)
9381 Judicial Dr. Suite 200
San Diego, CA 92121
Phone: 1-858-356-6610

Holiday Outages

Epic Science will promptly notify the study team via email of any holiday closures .

5.1.10 Pharmacogenetic Studies

Blood samples will be collected for pharmacogenetic studies to analyze the genomic DNA and assess genotype of the most relevant drug metabolizing enzymes and transporters (DMET). DNA will be analyzed on a DMET Plus (Affymetrix) genotyping platform that tests for 1,936 genetic variations in 225 drug disposition genes, including 47 CYP (phase I metabolism) genes, 13 non-CYP (phase I metabolism) genes, 78 phase II metabolizing genes (including UGTs), 63 transporters, 4 genes involved in facilitation of drug transporters, 9 genes involved in global regulation of drug metabolizing/transporting proteins, 4 drug binding proteins, and 4 drug targets.

Of specific interest to etoposide are polymorphisms in CYP3A4, ABCB1 (P-glycoprotein), and UGT1A1, all of which are included in the DMET analysis.

See **Appendix C: Sample Collection for Correlative Studies** for details regarding collection and handling of specimens.

5.2 FDG-PET MRI

Whole body Magnetic Resonance Imaging (MRI) combined with diffusion-weighted imaging is becoming an established method in staging of cancers and has been proven to be a feasible method for detection of small foci of metastases (1). In addition, MRI provides superb soft tissue characterization, which is helpful in evaluation of local spread and recurrence of pelvic malignancies – such as bladder cancer - where Computed Tomography (CT) can be limited due to attenuation and beam hardening, caused by bony frame of the pelvis.

Positron Emission Tomography (PET), on the other hand, provides sensitive functional data for N and M staging of malignancies with poor anatomical details and soft tissue characterization. This pitfall of PET imaging led researchers to propose and ultimately develop a combined technique comprising of PET and MRI (2). PET-MRI scanners became available relatively recently, and several applications of this powerful tool have been investigated in central nervous system and body malignancies (3). However, metastatic bladder cancer has not been a focus of evaluation with this technique and combined PET-MRI data for staging of bladder cancer is scarce.

Patients will be scanned at baseline on a combined 3T Siemens Verio MRI –PET system (Erlangen, Germany) in the Laboratory of Diagnostic Radiology Research, Clinical Center. The first scan will be performed at baseline before chemotherapy, followed by a second scan after the first restaging scans (8 or 9 weeks) to assess the treatment response. Patient will arrive at the nuclear medicine department and will receive an injection of radiotracer –^{18}F Fludeoxyglucose (FDG) – via an intravenous catheter. Dose of FDG will be calculated based on patient's body weight. Patient will be encouraged to be hydrated during the uptake phase of 60 mins. Afterwards, patient will be brought to MRI-PET suite to undergo whole body MRI-PET, which will be performed using several predetermined pulse sequences including; diffusion weighted imaging, conventional T1 and T2 weighted sequences, and dynamic post contrast. Patient will receive a single injection of 0.1 mmol/kg of gadolinium-based contrast via a previously placed intravenous line. We expect the entire image acquisition - from neck down to mid thigh - to be accomplished in less than 1 hour.

5.3 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.3.1 Laboratory of Paul Meltzer

All samples will be stored in monitored freezers/refrigerators in the Meltzer laboratory at specified temperatures with alarm systems in place. All samples will be tracked by unique sample identifiers with bar code labeling, using the Labmatrix software and the Genologic Clarity LIMS system. Secure computer database systems (Labmatrix and Clarity LIMS) will be used to track all samples collected on this protocol. The system will contain data that includes, but is not limited to the unique sample identifiers, storage locations and conditions, biologic study results, clinical

information, and corresponding records of all derivatives generated from samples/tissues collected on this protocol. The system will employ mechanisms for restricting users to viewing only the level of data appropriate for each individual user, will provide the capability to audit any data modification, and will be maintained and backed up according to established standards.

5.3.2 Laboratory of Jane Trepel

Samples will be processed immediately by the Trepel laboratory. Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. Specimen labels will indicate: protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

5.3.3 Clinical Pharmacology Program

All samples sent to the Blood Processing Core (BPC) will be bar-coded, with data entered and stored in the LABrador (aka LabSamples) utilized by the CPP. This is a secure program, with access to LABrador limited to defined CPP personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All Figg lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (e.g. delay in sample processing, storage conditions on the ward, etc.).

Bar-coded samples are stored in bar-coded boxes in locked freezers at either -20 C or -80 C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

Sample bar-codes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3.4 Sample Destruction, End of Protocol Procedures

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NIH Intramural IRB as soon as he is made aware of such loss.

If the patient withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

The genetic studies are discussed in detail in sections **5.1.1** and **5.1.2**. They will be performed on tumor tissue as well as blood and will include directed exome sequencing of 476 genes and/or whole exome sequencing.

5.4.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described in section **5.3.1.**

5.4.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. A list of current guidelines is maintained on the CCR intranet:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects who still remain on the study will be contacted at this time with a request to provide a blood sample to

be sent to a CLIA certified laboratory within the NIH or as a send-out test to Mayo Clinic. If the research findings are verified in the CLIA certified lab, the subject will be referred to a genetic healthcare provider within the NIH for the disclosure of the results.

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

C3D and LabMatrix will be used for data collection purposes on this study. All data will be kept secure. The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

As all agents on the study are FDA approved, only the following events will be recorded:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded/linked data in an NIH-funded or approved public repository.

_X_ Coded/linked data in BTRIS

_X_ Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: _dbGaP, clinical trials.gov.

_X_ BTRIS

_X_ Approved outside collaborators* under appropriate individual agreements.

_X_ Publication and/or public presentations.

* Genomic data will be transferred from Dr. Meltzer's lab to UCCC via a password protected CEL file identified by an accession number and sent through Fileman.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 or 9 weeks depending on visit schedule. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [45]. 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 the RECIST criteria.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with any of the agents.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 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:

- By chest x-ray: ≥20 mm
- By CT scan:
    - Scan slice thickness 5 mm or under: as >10 mm
- With calipers on clinical exam: >10 mm

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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.

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 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, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable 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 the beginning of the treatment.

The same method of assessment and the same technique should 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 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 should 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. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [46-48]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [49]. 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.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described

in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 Response Criteria

6.3.4.1 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 of diameters.

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 progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

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

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).

6.3.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (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.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
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.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
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.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.3.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. 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 web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NIH Intramural IRB and Clinical Director Reporting

7.2.1 NIH Intramural IRB and NCI Clinical Director Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem form to the NIH Intramural IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NIH Intramural IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NIH Intramural IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.3 Data and Safety Monitoring Plan

7.3.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the

investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

It would be desirable if a fraction consistent with 90% of patients has a successful drug assignment by COXEN. To evaluate this in a pilot fashion, a total of 20 patients will undergo the processes necessary to undertake the algorithm and the time required to obtain the final treatment assignment will be classified as being less than or equal to 21 days or greater than 21 days. A success would be a result in 21 days or less. With 20 patients, if 16 or greater are able to have a success, the probability that this would occur would be 95.7% if the true probability of a success for a given patient were 90% and it would be 5.1% if it were only 60%. Thus, observing 16 or more out of 20 patients with success would make it much more likely that the process would be associated with 90% success for each patient than 60%. Should only 15 patients be included in this trial, if 12 or greater are able to have a success, then the probability that this would occur would be 94.4% if the true probability of a success for a given patient were 90% and it would be 9.1% if it were only 60%. Thus, while 15 patients would yield useful information, 20 patients as opposed to 15 patients would reduce the probability of accepting the results as positive when they may not be, based on a low true rate of 60% success, from 9.4% to 5.1%.

The secondary objectives for this study are as follows: to assess the progression-free survival using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma; to assess the response rate using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma; to assess the overall survival using treatment suggested by the COXEN model in patients with advanced urothelial carcinoma; to evaluate toxicity with “best next therapy”. The Kaplan Meier method will be used to estimate PFS and OS. Responses will be estimated along with a 95% confidence interval. Toxicity will be evaluated using descriptive methods.

We expect that accrual of 20 patients can be completed within 12 months given our previous rates of accrual for similar patients.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Subjects with a diagnosis of urothelial carcinoma of the bladder, urethra, ureter, or renal pelvis will be eligible for participation in this study. Individuals of any race or ethnic group will be eligible for this study. Eligibility assessment will be based solely on the patient’s medical status. Recruitment of patients onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

9.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups and both genders are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism, immune response or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand

and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

9.1.2 Justification for Exclusions

Pregnant women are excluded due to potentially increased risks of serious side effects from the drugs administered on this study. Many of the therapeutic agents used in this trial are known to be teratogenic.

9.2 Participation Of Children

Individuals under the age of 18 will not be eligible for participation in this study based on the fact that patients under 18 are unlikely to have this disease and there are unknown toxicities in pediatric patients.

9.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **9.4.1**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

9.4 Evaluation of Benefits and Risks/Discomforts

9.4.1 Benefits

The potential benefit to a patient who enters study is a reduction in the bulk of his/her tumor, which may or may not have a favorable impact on symptoms and/or outcome.

9.4.2 Risks

9.4.2.1 Study Agents

The risks of the study are as described in the various package inserts.

9.4.2.2 Biopsy risks

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NCI's Clinical Center in Bethesda, MD. Although

no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

9.4.2.3 Radiation Risks

Some biopsies may be performed with CT guidance. If that is the case, then this research study involves exposure to radiation from up to 2 CT scans with a combined effective dose of 1.6 rem and 2 injections of ^{18}F-FDG of 7 mCi each for 2 FDG-PET MRI scans with a combined effective dose of 0.94 rem for a total dose of 2.5 rem. This is below the guideline of 5.0 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

9.4.2.4 Non-Physical Risks of Genetic Research

9.4.2.4.1 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers. In addition, a Certificate of Confidentiality will be obtained for the study.

9.4.2.4.2 Risk related to possibility that information may be released

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

9.4.2.4.3 Risk to family or relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems.

9.4.2.5 Other Risks

Additional risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

9.5 RISKS/BENEFITS ANALYSIS

Although urothelial cancer is a chemosensitive malignancy with response proportions of over 50% with conventional cytotoxic regimens, the response durations are short and the median survival of patients with metastatic disease is approximately 14 months. There is no FDA-approved second line drug for metastatic urothelial cancer.

Given the efforts to minimize risk with the administration of these agents, this protocol involves greater than minimal risk, but presents the potential for direct benefit to individual subjects. The ongoing risks and benefits of participation for adults who become unable to consent are no different than those described for less vulnerable patients.

9.6 CONSENT PROCESS AND DOCUMENTATION

The principal investigator or a designee will obtain written informed consent from each subject participating in this study after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. Potential hazards of the selected agent(s) will also be provided as an information sheet in conjunction with the consent as it would be impractical to include all 75 agents in the consent. Consent for the optional biopsies on this study will be obtained at the time of the procedure, using the procedure consent. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

If new safety information results in significant changes in the risk/ benefit assessment, the consent form will be reviewed and updated as necessary. All subjects (including those already being treated) will be informed of the new information, be given a copy of the revised form, and be asked to give their consent to continue in the study.

It will be documented in the medical record that informed consent has been obtained.

9.6.1 Telephone Re-consent Procedure

Re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject signature will sign and date the consent. The original informed consent document will be mailed back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator.

10 PHARMACEUTICAL INFORMATION

For this study, all drugs are commercially available; therefore, Investigator Brochures are not applicable to this/these drug(s). Information about commercial drugs is publicly available in the package insert and other resources. Refer to **Appendix B** for the COXEN drug index FDA approved dose and schedule.

All of the agents are not approved for use in urothelial cancer; however, the purpose of the investigation is to determine the feasibility of the use of the COXEN algorithm. The study is exempt from IND application because it meets all of the following requirements: the investigation is not intended to support a new indication for use or any other significant changes to labeling; the drugs are already approved and marketed and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug products.

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12 APPENDICES

12.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

12.2 APPENDIX B: COXEN DRUG INDEX WITH FDA-APPROVED DOSE AND SCHEDULE

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
1	Abiraterone (PO)	N	mCRPC	1000mg QDay w/ prednisone
2	Arsenic Trioxide (IV)	N	APL, relapsed or refractory	Induction: 0.15 mg/kg/day until bone marrow remission, max 60 induction doses Consolidation: 0.15 mg/kg/day starting 3-6 weeks after completion of induction, max 25 consolidation doses
3	Asparaginase, *Escherichia coli* source (IV, IM)	Y	ALL	6000 units/m^2/dose TIW
4	Axitinib (PO)	N	RCC, advanced	Initial: 5mg BID Dose Increases: If tolerance criteria are met (see package insert), dose may be increased to 7mg BID then to 10mg BID
5	Azacitidine (IV, SubQ)	Y	MDS	Initial Cycle: 75mg/m^2/day x 7days Subsequent Cycles: 75 mg/m^2/day x 7 days Q4weeks Dose Increases: may increase to 100mg/m^2/day after 2 cycles
6	Bendamustine (IV)	Y	CLL	100mg/m^2 on days 1 and 2 of a 28-day cycle, max 6 cycles
			NHL	120mg/m^2 on days 1 and 2 of a 21-day cycle, max 8 cycles
7	Bleomycin (IV, IM, SubQ)	Y	Squamous Cell	0.25-0.5units/kg Qweek or BIW Note: Lymphoma patients should be treated with ≤ 2 units for the first two doses
			Lymphomas	
			Testicular	
8	Bortezomib	Y	MM,	1.3mg/m^2 days 1, 4, 8, 11, 22, 25, 29, and 32 of a 42-day cycle x 4 cycles

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
	(IV, SubQ)		first-line	THEN 1.3mg/m^2 days 1, 8, 22 and 29 of a 42-day cycle x 5 cycles w/ melphalan and prednisone
			MM, relapsed	1.3mg/m^2 days 1, 4, 8, and 11 of a 21-day cycle
			Mantle Cell Lymphoma	
9	Busulfan (PO)	N	CML, palliation	60mcg/kg/day or 1.8 mg/m^2/day
	Busulfan (IV)	Y	HSCT conditioning regimen	0.8mg/kg Q6hours x 4 days
10	Carboplatin (IV)	Y	Ovarian, advanced	AUC 4-6 Q4weeks
			Bladder (unlabeled use)	AUC 5 Q3weeks w/gemcitabine and paclitaxel OR AUC 5 Q3weeks w/ gemcitabine OR AUC 6 Q3weeks w/ paclitaxel
11	Carfilzomib (IV)	N	MM, relapsed or refractory	<u>Cycle 1:</u> 20mg/m^2 on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle <u>Subsequent Cycles:</u> 27mg/m^2 on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle
12	Carmustine (IV)	Y	Brain Tumors	150-200mg/m^2 Q6weeks OR 75-100mg/m^2/day x 2 days Q6weeks
			Hodgkin's Lymphoma	
			MM	
			NHL	
13	Chlorambucil (PO)	Y	CLL	0.1mg/kg/day x 3-6 weeks
			NHL	
			Hodgkin's Lymphoma	0.2mg/kg/day x 3-6 weeks

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
14	Cisplatin (IV)	Y	Bladder, advanced	50-70mg/m^2 Q3-4weeks
			Ovarian, metastatic	Single Agent: 100mg/m^2 Q4weeks Combination Therapy: 75-100 mg/m^2 Q4weeks
			Testicular, metastatic	20mg/m^2/day x 5 days Q3weeks
15	Cladribine (IV)	Y	Hairy Cell Leukemia	0.09mg/kg/day CIVI x 7 days x 1 cycle
16	Clofarabine (IV)	N	ALL, adults ≤ 21 years	52mg/m^2/day days 1-5 Q2-6weeks
17	Crizotinib (PO)	N	NSCLC, metastatic, ALK-positive	250mg BID
18	Cytarabine-Conventional (IV)	Y	AML, remission induction	Combination Therapy: 100mg/m^2/day CIVI x 7 days OR 100mg/m^2 Q12hours x 7 days
19	Dacarbazine (IV)	Y	Hodgkin's Disease	Combination Therapy: 375mg/m^2/dose days 1 & 15 Q4weeks
			Melanoma, metastatic	250mg/m^2/dose days 1-5 Q3weeks
20	Dactinomycin (IV)	N	Testicular, metastatic	Combination Therapy: 1000mcg/m^2 on day 1
			Gestational Trophoblastic Neoplasm	Single Agent: 12mcg/kg/day x 5 days Combination Therapy: 500mcg/dose days 1 and 2
			Wilms' Tumor, Ewing's Sarcoma, Rhabdomyo-sarcoma	Combination Therapy: 15mcg/kg/day x 5 days
21	Dasatinib	Y	CML, Ph+	Chronic Phase: 100mg Qday (may increase to 140mg Qday)

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
	(PO)			Accelerated or Blast Phase: 140mg Qday (may increase to 180mg Qday)
			ALL, Ph+	Initial: 140mg Qday Dose Increases: may increase to 180mg Qday
22	Daunorubicin-Conventional (IV)	Y	ALL	Combination Therapy: 45mg/m^2/day x 3 days Q3-4weeks
			AML, induction, adults <60 years	Combination Therapy Cycle 1: 45mg/m^2/day x 3 days Subsequent Cycles: 45mg/m^2/day x 2 days Q3-4weeks
			AML, induction, elderly ≥ 60 years	Combination Therapy Cycle 1: 30mg/m^2/day x 3 days Subsequent Cycles: 30mg/m^2/day x 2 days Q3-4weeks
23	Decitabine (IV)	N	MDS	15mg/m^2 Q8H x 3 days Q6weeks OR 20mg/m^2 Qday x 5 days Q4weeks
24	Docetaxel (IV)	Y	Breast	60-100mg/m^2 Q3weeks
			NSCLC	75mg/m^2 Q3weeks
			Prostate	75mg/m2 Q3weeks w/prednisone
			Gastric Adeno-carcinoma	75mg/m2 Q3weeks w/cisplatin and fluorouracil
			Head and Neck	75mg/m2 Q3weeks w/cisplatin and fluorouracil x 3-4 weeks, followed by RT
			Bladder (unlabeled use)	Single Agent: 100mg/m^2 Q3weeks Combination Therapy: 35mg/m^2 day 1 and 8 Q21 days w/ gemcitabine and cisplatin
25	Doxorubicin-	Y	Breast	60mg/m^2 on day 1 of a 21-day cycle x 4 cycles w/ cyclophosphamide

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
	Conventional (IV)		Metastatic Solid Tumors, Leukemia, or Lymphoma	Single Agent: 60-75mg/m^2 Q21days Combination Therapy: 40-75mg/m^2 Q21-28days
26	Epirubicin (IV)	N	Breast, adjuvant	60mg/m^2 day 1 and 8 Q28days x 6 cycles w/ cyclophosphamide and fluorouracil OR 100mg/m^2 day 1 Q21days x 6 cycles w/ cyclophosphamide and fluorouracil
27	Eribulin (IV)	N	Breast, metastatic	1.4mg/m^2/dose days 1 and 8 Q21days
28	Erlotinib (PO)	Y	NSCLC, first-line EGFR mutations	150mg Qday
			NSCLC, refractory or maintenance	
			Pancreatic	100mg Qday
29	Estramustine (PO)	Y	Prostate, progressive or metastatic	14mg/kg/day (3 to 4 divided doses)
30	Etoposide (IV)	N	SCLC	35mg/m^2/day x 4 days, up to 50mg/m^2/day x 5 days Q3-4weeks
			Testicular	50-100mg/m^2/day x 5 days OR 100mg/m^2/day days 1, 3, and 5 Q3-4weeks
	Etoposide (PO)	Y	SCLC	PO twice IV dose
31	Exemestane (PO)	N	Breast	25mg Qday
32	Floxuridine (intra-arterial)	N	CRC, hepatic mets	0.1-0.6mg/kg/day continuous infusion

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
33	Fludarabine (IV)	Y	CLL	25mg/m^2/day x 5 days Q28days
34	Fluorouracil (IV)	Y	Breast	Initial Course: 12mg/kg Qday x 4 days then 6mg/kg days 6, 8, 10 and 12 Maintenance Therapy: repeat dosage of initial course every 30 days after the last day of previous course OR 10-15mg/kg Qweek
			CRC	
			Pancreatic	
			Gastric	
35	Gefitinib (PO)	N	NSCLC, first-line EGFR mutations	250mg Qday
36	Gemcitabine (IV)	Y	Breast, metastatic	1250mg/m^2 days 1 and 8 Q21days w/ paclitaxel
			NSCLC, locally advanced or metastatic	1000mg/m^2 days 1, 8, and 15 Q28days w/ cisplatin OR 1250mg/m^2 days 1 and 8 Q21days w/cisplatin
			Ovarian, advanced	1000mg/m^2 days 1 and 8 Q21days w/ carboplatin
			Pancreatic, locally advanced or metastatic	1000mg/m^2 Qweek x 7 weeks followed by 1 week rest THEN Qweek x 3 weeks every 4 weeks
			Bladder (unlabeled use)	1000mg/m^2 days 1, 8, and 15 Q28 days w/ cisplatin OR 1000mg/m^2 days 1 and 8 Q21 days w/ carboplatin
37	Hydroxyurea (PO)	Y	CML, resistant	20-30mg/kg Qday
			Solid Tumors (Head and Neck, Melanoma, Ovarian)	Intermittent: 80mg/kg Q3days Continuous: 20-30mg/kg Qday
38	Idarubicin (IV)	Y	AML	Induction: 12mg/m^2/day x 3 days

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
				Consolidation: 10-12mg/m^2/day x 2 days
39	Ifosfamide (IV)	Y	Testicular	1200mg/m^2/day x 5 days Q3weeks, combo therapy & w/mesna
40	Imatinib (PO)	Y	CML, Ph+	Chronic Phase: 400mg Qday, may increase to 600mg Qday Accelerated Phase or Blast Crisis: 600mg Qday, may increase to 400mg BID
			GIST	400mg Qday, may increase to 400mg BID
			ALL, Ph+	600mg Qday
41	Irinotecan (IV)	Y	CRC, metastatic	125mg/m^2 days 1, 8, 15, and 22 of a 6 week cycle OR 350mg/m^2 Q3weeks
42	Ixabepilone (IV)	N	Breast, metastatic or locally advanced	40mg/m^2/dose Q3 weeks, max 88mg
43	Lapatinib (PO)	N	Breast, HER2+	1250mg Qday w/ capecitabine OR 1500mg Qday w/ letrozole
44	Lomustine (PO)	N	Brain Tumors	130mg/m^2 Q6weeks
			Hodgkin's Lymphoma	
45	Mechlor-Ethamine (IV)	Y	Hodgkin's Lymphoma, palliative	0.4mg/kg
46	Melphalan (IV)	Y	MM, palliative	16mg/m^2 Q2weeks x 4 doses then Q4weeks
	Melphalan (PO)	Y	MM, palliative	6mg Qday x 2-3 weeks, followed by 4 weeks rest then 2mg Qday
			Ovarian	0.2mg/kg/day x 5 days Q4-5weeks

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
47	Mercapto-Purine (PO)	Y	ALL	1.5-2.5mg/kg Qday
48	Methotrexate (intrathecal, IV, IM, SubQ)	Y	Numerous Oncology-Related Indications	Intrathecal: 15mg day 1, 8, 15, 22, and 29; 12mg Q2-7days IV (large range of approved doses): 40mg/m^2 Qweek; 12gm/m^2 (max 20gm) weeks 3, 4, 8, and 9
	Methotrexate (PO)	Y	Numerous Oncology-Related Indications	20mg/m^2 Qweek
49	Mitomycin (IV)	Y	Stomach or Pancreas Adeno-carcinoma	20mg/m^2 Q6-8weeks
50	Mitotane (PO)	Y	ACC	2-6gm Qday (divided) then increase incrementally to 9-10gm Qday (divided)
51	Mitoxantrone (IV)	N	ANLL	Induction: 12mg/m^2 Qday x 3 days w/ cytarabine Consolidation (6 weeks after induction): 12mg/m^2 Qday x 2 days, repeat in 4 weeks
			Prostate, advanced, hormone-refractory	12-14mg/m^2 Q3weeks w/ steroids
52	Nilotinib (PO)	Y	CML, Ph+	Newly-Diagnosed in Chronic Phase: 300mg BID Resistant, Chronic or Accelerated Phase: 400mg BID
53	Oxaliplatin (IV)	Y	CRC	85mg/m^2 Q2weeks
54	Paclitaxel (IV)	Y	Ovarian	135-175mg/m^2 Q3weeks OR 50-80mg/m^2 Qweek OR

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
				1.4-4mg/m^2/day CIVI x 14 days Q4weeks
			Breast, metastatic	175-250mg/m^2 Q3weeks OR 50-80mg/m^2 Qweek OR 1.4-4mg/m^2/day CIVI x 14 days Q4weeks
			NSCLC	135mg/m^2 Q3weeks
			AIDS-Related Kaposi's Sarcoma	135mg/m^2 Q3weeks OR 100mg/m^2 Q2weeks
55	Pazopanib (PO)	N	RCC Soft Tissue Sarcoma, advanced refractory	800mg Qday
56	Pentostatin (IV)	Y	Hairy Cell Leukemia	4mg/m^2 Q2weeks
57	Romidepsin (IV)	N	Cutaneous T-cell Lymphoma Peripheral T-cell Lymphoma	14mg/m^2 days 1, 8 and 15 of a 28-day cycle
58	Ruxolitinib (PO)	N	Myelofibrosis	Platelets >200,000/mm^3: 20mg BID Platelets 100,000-200,000/mm^3: 15mg BID Platelets 50,000-<100,000/mm^3: 5mg BID
59	Sorafenib (PO)	Y	HCC RCC, advanced Thyroid, differentiated	400mg BID
60	Streptozocin (IV)	Y	Pancreatic Islet Cell	500mg/m^2/day x 5 days Q6 weeks OR 1000mg/m^2 Qweek

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
61	Sunitinib (PO)	Y	GIST	50mg Qday x 4 weeks Q6weeks
			RCC, advanced	
			PNET, advanced	37.5mg Qday
62	Tamoxifen (PO)	Y	Breast	20mg Qday
63	Temsirolimus (IV)	N	RCC	25mg Qweek
64	Teniposide (IV)	N	ALL, pediatric	165-250 mg/m2 twice weekly or weekly x 8-9 doses.
65	Thioguanine (PO)	Y	AML	2mg/kg Qday
66	Thiotepa (intravesical, IV)	N	Bladder	60mg Qweek x 4 weeks (intravesical)
			Ovarian	0.3-0.4mg/kg Q1-4weeks (IV)
			Breast	
67	Topotecan (IV)	Y	Cervical	0.75mg/m^2/day x 3 days Q21days w/ cisplatin
			Ovarian	1.5mg/m^2/day x 5 days Q21days
			SCLC	
	Topotecan (PO)	N	SCLC	2.3mg/m^2/day x 5 days Q21days
68	Toremifene (PO)	N	Breast, metastatic	60mg Qday
69	Tretinoin (PO)	Y	APL	45mg/m^2/day (divided)

#	Drug Name and route of administration	Formulary Status (Y, yes; N, no)	FDA-Approved Indication	FDA-Approved Dose & Schedule
70	Vandetanib (PO)	N	MTC, locally advanced or metastatic	300mg Qday
71	Vemurafenib (PO)	N	Melanoma, metastatic or unresectable, BRAFV600E mutation	960mg BID
72	Vinblastine (IV)	Y	Hodgkin's, NHL, Testicular, Breast, Kaposi's Sarcoma	Typical dosages: 3.7mg/m^2(initial), up to 5.5mg/m^2 (dose 2), up to 7.4mg/m^2 (dose 3), up to 9.25mg/m^2 (dose 4), and up to 11.1mg/m^2 (dose 5); administered Q7days Usual Range: 5.5-7.4mg/m^2 Q7days
73	Vincristine (IV)	Y	Numerous Oncology-Related Indications	1.4mg/m^2/dose, max 2mg/dose, various frequencies
74	Vismodegib (PO)	N	Basal Cell, metastatic or locally advanced	150mg Qday
75	Vorinostat (PO)	N	Cutaneous T-cell Lymphoma	400mg Qday

12.3 APPENDIX C: SAMPLE COLLECTION FOR CORRELATIVE STUDIES

The date and exact time of each blood draw should be recorded on each tube.

Test description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instructions
Archival Tissue	1-2 Tumor blocks or 25 unstained slides and 2 H&E slides (Sample 1)	Baseline (Pre treatment)	Marissa Mallek, marissa.mallek@nih.gov, 240-760-7498	Tumor blocks will be submitted to Maria Merino for cutting into slides. Unstained slides will be distributed by research nurse to: Laboratory of pathology, Dr. Merino:5 unstained slides and 1 H&E Meltzer's lab for NGS: 10 unstained slides and 1 H&E Trepel's lab for Immune gene signature: 10 unstained slides and 1 H&E
Fresh Tumor Biopsy	2-3 cores (Sample 2 and Sample 5)	Baseline (mandatory) and at progression (optional)	Marissa Mallek, marissa.mallek@nih.gov, Ph: 240-760-7498 Dr. Bottaro, don.bottaro@nih.gov, Ph: 301-402-6499 Rene Costello, rene.costello@nih.gov, Ph: 301-443-6915 to arrange for pick-up	Fresh tumor will be fixed and submitted to Maria Merino for processing. Unstained Slides will be distributed by research nurse to: Meltzer's lab for NGS: 10 unstained slides and 1 H&E Trepel's lab for Immune gene signature: 10 unstained slides and 1 H&E

Test description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instructions
Saliva	3 mL; Oragene saliva collection kit (DNA Genotek)	Baseline (at any time after consent is signed, but preferably at baseline)	Marissa Mallek, marissa.mallek@nih.gov, 24 hours prior to anticipated collection. Call 240-760-7498 for immediate pick-up of sample	Patient should not eat or drink anything 30 minutes prior to collection. After the sample collection, when the top of the device is closed a preservative is released. Remove the top and close the tube with a cap. Store at room temperature.
ctDNA isolation	10 mL of blood; Streck tube (Streck, Omaha, NE) (Sample 3 and Sample 4)	Baseline and C3D1	Meltzer lab, pmeltzer@mail.nih.gov. Email 24 hr prior to collection.	Invert Streck tube 2-4 times, keep at room temperature (15- 30 degrees C) and transferr to the lab for processing .
Immune Subset analysis	8 mL of blood; two BD Vacutainer Cell Preparation Tubes (CPT) citrate	Baseline (just prior to beginning therapy), C2D1 and C3D1	Developmental Therapeutics Branch: Jane Trepel, trepelj@mail.nih.gov Yusuke Tomita, yusuke.tomita@nih.gov Sunmin Lee, lees@pop.nci.gov Mi-Jung leemin@,mail.nih.gov Call 240-760-6330 immediately after blood is drawn	Invert several times and keep at room temp

Test description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instructions
Peripheral Blood Immune Gene Expression	2.5 ml of blood; one PAXgene RNA tube (PreAnalytix)	Baseline (just prior to beginning therapy) and C2D1	Developmental Therapeutics Branch : Phone: 240-760-6330 Jane Trepel, trepelj@mail.nih.gov Yusuke Tomita, yusuke.tomita@nih.gov Sunmin Lee, lees@pop.nci.gov Mi-Jung, leemin@mail.nih.gov	Invert several times and keep at room temp
Pro-Inflammatory Cytokines	10 mL of blood; one EDTA tube	Baseline, C2D1, and C3D1	Figg lab. NCIBloodcore@mail.nih.gov. Contact 24 hr prior or for questions regarding sample processing. Page 102-11964 for sample pick-up. For immediate help, call 240-760-6180 (main blood processing core number) or, 240-760-6190 (main clinical pharmacology lab number).	Invert EDTA tube 2-4 times, place on wet ice and store at 4°C until processing for a maximum of 30-60 min. Upon arrival in the BPC, blood samples will be centrifuged for 5 minutes at 1200 x *g* at 4°C. Plasma will be aliquoted into 2 cryovials and stored at -80°C until the time of analysis.

Test description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instructions
Plasma HGF and MET	4 mL of blood; one EDTA tube	Baseline and day 1 of each treatment cycle	Figg lab. NCIBloodcore@mail.nih.gov. Contact 24 hr prior or for questions regarding sample processing. Page 102-11964 for sample pick-up. For immediate help, call 240-760-6180 (main blood processing core number) or, 240-760-6190 (main clinical pharmacology lab number).	Invert EDTA tube 2-4 times, place on wet ice and store at 4°C in the refrigerator until processing for a maximum of 30-60 min. Upon arrival in the BPC, samples will be centrifuged and the plasma transferred into cryovials for storage at -80 C until the time of analysis. Samples will be barcoded as described in Section **5.3**.
DMET	6 mL of blood; one EDTA tube	Baseline	Figg lab. NCIBloodcore@mail.nih.gov. Contact 24 hr prior or for questions regarding sample processing. Page 102-11964 for sample pick-up. For immediate help, call 240-760-6180 (main blood processing core number) or, 240-760-6190 (main clinical pharmacology lab number).	Invert EDTA tube 2-4 times, place on wet ice and store at 4°C in the refrigerator until processing for a maximum of 30-60 min. The date and exact time of each blood draw should be recorded on the sample tube and on the PK sheet. Upon arrival in the Clinical Pharmacology Lab, samples will be centrifuged and the plasma transferred into cryovials for storage at -80°C until the time of analysis. Samples will be barcoded as described in Section **5.3**

Test description	Amount/Type/ Tube/Handling	Collection Timing	Contact	Special Instructions
Circulating tumor cells (Trepel Lab)	10 ml of blood; Cell save tube	Baseline, (just prior to beginning therapy)and C2D1	Jane Trepel, trepelj@mail.nih.gov Yusuke Tomita, yusuke.tomita@nih.gov Sunmin Lee, lees@pop.nci.gov Call 240-760-6330 for immediate pick-up	Invert several times and place at room temp or refrigerate
Circulating tumor cells (Epic Science)	4-10 mL of blood; Streck Cell-Free DNA BCT	Baseline and First Restaging	Send to Epic Science as detailed in Section **5.1.9.1**	Confirm blood tube is not expired. Collect minimum of 4 mL blood per sample, but a full 10 mL tube of blood should be provided when possible (*see note regarding prevention of backflow in Section **5.1.9**). Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times to prevent clotting. Inadequate or delayed mixing may result in inaccurate test results. Label the tube with subject's identification and date and time of blood draw. Unlabeled blood tubes may not be processed. Keep sample at room temperature and ship on day of collection in shipper with ambient gel packs.