ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB 90601

A RANDOMIZED DOUBLE-BLINDED PHASE III STUDY COMPARING GEMCITABINE, CISPLATIN, AND BEVACIZUMAB TO GEMCITABINE, CISPLATIN, AND PLACEBO IN PATIENTS WITH ADVANCED TRANSITIONAL CELL CARCINOMA

NCI-supplied agent(s): Bevacizumab/Placebo (NSC 704865, IND #113911)

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy / Dose Modifications / Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[X] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other :	

Expedited review is allowed. IRB approval (or disapproval) is required within 90 days. Please follow your IRB of record guidelines.

UPDATES TO PROTOCOL:

Cover Page (page 1):

-Misty Bova-Solem has replaced Nick Jeffries as Data Coordinator. All contact information has been updated.
-Karla Ballman has replaced Susan Halabi as GU Faculty Statistician. All contact information has been updated.
-Ben Sanford has been removed as Staff Statistician.

Cover Page (page 2):

-The study document history table has been removed. This table now appears as a separate document on the protocol landing page, on the member side of the Alliance website.
-Data submission for this study will now be performed using Medidata Rave®, and thus the website link for Medidata Rave has been added to the left side of the table as an additional resource.

CTSU Contact Information (page 3):

The CTSU Contact Information table has been updated with the current CTSU boilerplate language.

Section 5.1 (CTEP Investigator Registration Procedures):
This section has been retitled "CTEP Registration Procedures" and updated with the current CTSU boilerplate language.

Section 5.2 (CTEP Associate Registration Procedures):
This section has been removed as it is now encompassed within Section 5.1

Section 5.3 (CTSU Registration Procedures):
This section has been renumbered as Section 5.2 and updated with the current CTSU boilerplate language. The remaining sections have been renumbered accordingly.

Section 5.3.3 (Submitting Regulatory Documents):
-This section has been renumbered as Section 5.2.3.
-The suite number in the CTSU Regulatory Office mailing address has been changed to 3000.

Section 6.1 (Data Submission):
-Data submission for this study will now be performed using Medidata Rave®; therefore, all text above the table has been replaced with four new paragraphs that outline the new data submission procedures.
-Reference to submitting forms to the Alliance Statistics and Data Center has been removed from the instructions above the table.
-In the second sentence of the "Common Toxicity Criteria" heading under the data submission table, the CTCAE version has been updated from 4.0 to 5.0 for expedited adverse event reporting. The reference to the AER section has been updated from 14.0 to 16.0.

Section 9.12 (Hypersensitivity and infusion reactions):
A sentence has been added to the end of the first paragraph to note that serious adverse events will be reported through CTEP-AERS using CTCAE version 5.0.

Section 11.3 (Bevacizumab):
-The text in the first paragraph under the "Drug Accountability" heading has been replaced with updated PMB language regarding drug accountability.
-In the third paragraph under the "Drug Ordering" heading, the phrase "and active person registration status" has been added to the end of the third sentence. A new sentence has been added after the third sentence to specify CTEP registration requirements for ordering agents. Two new sentences have been added to the end of the third paragraph with instructions for contacting PMB and accessing specific policies and guidelines related to agent management.
-A new section heading titled "Investigator Brochure Availability" has been added after the "Drug Accountability" section in order to be consistent with the current CTEP protocol template.
-The PMB Online Agent Order Processing (OAOP) application website URL has been updated under the "Useful Links and Contacts" heading.

Section 16.0 (Adverse Event Reporting):
-The fourth sentence has been updated to reflect the use of CTCAE version 5.0 for serious AE reporting.
-In the first sentence of the second paragraph, the CTCAE version has been changed from 4.0 to 5.0, and the URL for the website has been updated.
-The CTEP-AERS website URL has been added to the last sentence of the second paragraph.

Section 16.2 (Additional Instructions or Exclusions):
-The eighth bullet point has been replaced with new language regarding the reporting of death due to progressive disease to reflect the change in expedited reporting requirements using CTCAE version 5.0.

-The ninth bullet point has been replaced with two new bullet points regarding the reporting of secondary and second malignancies using CTCAE version 5.0.
-The eleventh bullet point beginning with "The CTEP-AERS expedited reporting system…" has been removed, as it contains redundant and outdated information.
-Two new bullet points have been added with instructions on reporting pregnancy loss and neonatal death using CTCAE version 5.0.

UPDATES TO MODEL CONSENT FORM:

No updates have been made to the model consent form.

A replacement protocol document and model consent form have been issued.
This study remains closed to new patient accrual.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

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CALGB 90601

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NCI-supplied agent(s): Bevacizumab/Placebo (NSC 704865, IND #113911)

ClinicalTrials.gov Identifier: NCT00942331

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Adverse Event Reporting
https://eapps-ctep.nci.nih.gov/ctepaers/

Medidata RAVE® iMedidata Portal

https://login.imedidata.com

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The pharmacogenomic component of this study is conducted as part of the NIH Pharmacogenomics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/pharmacogenomics/research_net.html for details.

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

For regulatory requirements:	**For patient enrollments:**	**For study data submission**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.		
For clinical questions (i.e. patient eligibility or treatment-related) Contact the Study Chair, Protocol Coordinator, and (where applicable) Data Manager. Contact information can be found on cover page of protocol		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website https://www.ctsu.org > education and resources tab > CTSU Operations Information >CTSU Regulatory and Monitoring Policy		
The CTSU Website is located at https://www.ctsu.org.		

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Patient Eligibility

Histologically or cytologically documented metastatic or unresectable transitional cell carcinoma of the urinary tract with metastatic disease or locally advanced disease (T4b, N2, N3, or M1) (see Sec 4.1)

Prior Treatment (see Sec. 4.2):

- No prior combination chemotherapy for metastatic disease
- Radiosensitizing single agent chemotherapy is not considered systemic therapy
- Prior neoadjuvant or adjuvant therapy is permissible, provided the interval from end of therapy to diagnosis of metastatic disease is at least 1 year.
- ≥ 4 weeks since any radiation therapy (including palliative) or major surgery and fully recovered
- ≥ 7 days since any minor surgery such as port placement
- ≥ 4 weeks from any intravesical therapy
- No prior treatment with bevacizumab or other angiogenesis inhibitors.

No known history of brain metastases (brain imaging (MRI/CT) is not required).

No current congestive heart failure (NYHA Class 2 or higher).

Patients with history of hypertension must be well controlled (<150/90).

Patients on full-dose anticoagulants must be on a stable dose of warfarin and have an in-range INR or be on a stable dose of LMW heparin (see Section 4.3.4).

No significant history of bleeding events within 6 months of registration (except for hematuria able to be controlled with endoscopic intervention) (see Section 4.3.5).

No history of gastrointestinal perforation within 12 months of registration

No clinically significant peripheral arterial disease

No arterial thrombotic events within 6 months (see Section 4.3.6).

No serious or non-healing wound, ulcer or bone fracture.

No clinically significant peripheral neuropathy (grade ≥ 2).

Patients that are pregnant or nursing are not eligible (see Section 4.4)

No known hypersensitivity to Chinese hamster ovary cell products (see Section 4.3.9).

ECOG Performance Status: 0-1.

Age ≥ 18.

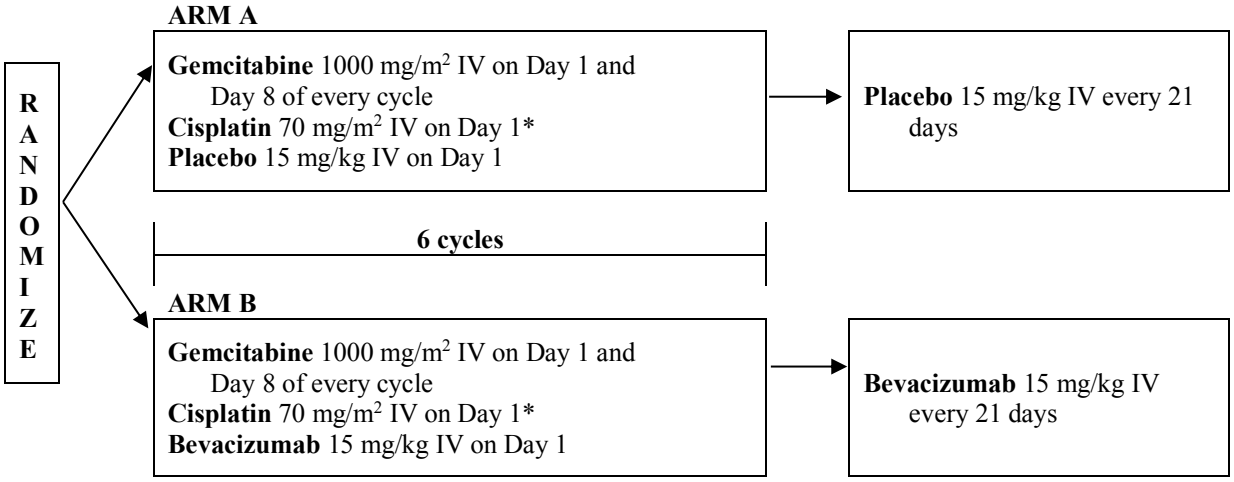
Required Initial Laboratory Values

ANC	≥ 1500/µL
Platelet Count	≥ 100,000/µL
Bilirubin	≤ 1.25 x ULN*
AST	≤ 2.0 x ULN
Calc. or meas.	
Creat. Clearance	≥ 50 mL/min.

UPC ratio < 1, or urine protein ≤ 1+, or 24-hour urine protein ≤ 1 gram *

*See Section 4.6

Schema
1 cycle = 21 days



* For patients with a creatinine clearance of ≥ 50 and < 60 mL/min, cisplatin will be administered at a dose of 35 mg/m^2 on Days 1 and 8 of each cycle, see Section 8.3.

Treatment with gemcitabine and cisplatin should continue for a maximum of 6 cycles. Treatment with bevacizumab/placebo alone will continue until disease progression or unacceptable toxicity.

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Background

Transitional cell carcinoma (TCC) is the fifth most common new cancer reported in the United States, with an incidence of 67,000 new cases per year, and about 13,000 deaths per year.[1] The majority (70%) of TCC are superficial, without invasion of lamina propria or muscle; approximately 30% present with invasive or metastatic disease.[2] Up to 70% of patients with superficial TCC recur, and one-third progress to higher grade or stage. Patients with progression to invasive or metastatic disease have poor survival with current therapies. Survival in patients with metastatic or locally advanced unresectable transitional cell carcinoma (TCC) of the bladder is prolonged with cisplatin-based combination chemotherapy. However, durable complete remissions in patients with advanced disease are rare, and median time to progression (TTP) is short. In an updated report of the randomized trial comparing M-VAC with gemcitabine and cisplatin, survival at five years was 15.3% and 13%, respectively (p=NS).[3] These results confirm that TCC is a chemotherapy-sensitive disease, and that improvements in first-line chemotherapy may yield improved progression-free and overall-term survival with this disease. Addition of novel agents to standard chemotherapy may provide such improvements.

1.2 Gemcitabine and cisplatin combination chemotherapy

Gemcitabine/cisplatin (GC) is the most commonly used first-line chemotherapy regimen in transitional cell carcinoma (TCC). It has largely replaced MVAC as the standard of care on the basis of an international phase III trial that showed similar overall survival with significantly less toxicity.[4] Updated results demonstrated a median TTP on this trial for patients treated with GC of 7.7 months and median overall survival (OS) or 14.0 months.[3] Recently updated results of this clinical trial continue to affirm similar long-term disease outcomes of these two regimens. This study used the 28-day regimen of gemcitabine/cisplatin, with gemcitabine administered on days 1, 8, and 15 at a dose of 1000 mg/m^2.

Gemcitabine and cisplatin dose and schedule: Extensive work has been done in other malignancies regarding the comparability and dose intensity of gemcitabine/cisplatin given on a 21-day and a 28-day schedule. While the 21-day schedule has not been tested in a randomized phase III study in urothelial carcinoma, it has been tested in non-small cell lung carcinoma and found to be similar in efficacy to the 28 day schedule, and associated with a higher dose-intensity for both drugs. Data exist suggesting that the 28-day regimen is associated with significant dose reduction and omission of the day 15 gemcitabine, yielding an average weekly dose of gemcitabine that is inferior to the 21-day regimen. With the 21-day regimen at a dose of 1000 mg/m^2, the gemcitabine average weekly dose is approximately 588-639 mg/m^2/wk, leading to a dose intensity of 88-89%.[5-8] For the four-week regimen using a dose of 1000 mg/m^2, the gemcitabine average weekly dose is 520-600 mg/m^2/wk, leading to a dose intensity of 69-80%. [4,9-14] Thus, more gemcitabine is administered with the three-week regimen due to fewer omissions and dose reductions. In addition, more frequent dosing of cisplatin using the 21-day schedule results in higher cisplatin dose intensity. Cisplatin average weekly dose (based on 70 mg/m^2) ranged from 20.6 – 22.9 mg/m^2/week with the 21-day schedule, and from 16.7-17.8 mg/m^2/week with the 28-day schedule. Cisplatin dose intensity may be important for outcomes in bladder cancer, as a trend towards improved long-term survival (although not median survival), objective response, and progression-free survival was observed in EORTC 30924, which compared dose dense MVAC with conventional MVAC.[15]

In bladder cancer, clinical outcomes with the 21-day regimen in phase II studies were similar to those observed with the 28-day regimen from the previous phase III study. The overall

response rate in urothelial carcinoma with the 21-day regimen is 45-49%, and median survival ranges from 9.1-13.2 months[16-18] compared with a response rate of 41-57% and a median survival range of 10.5 to 14.3 months.[4,12-14] In the Winquist study, there were a majority of patients with intermediate and poor risk features, which may explain the 9.1-month median survival. These data suggest that the 21-day regimen is legitimate to use in this context and compares favorably with the 28-day regimen in TCC. Given the increased dose intensity with the 21-day regimen, similar efficacy with this regimen, and its de facto use as the standard of care, this phase III trial will use this 21-day regimen as the control arm.

1.3 VEGF in Bladder Cancer

The importance of angiogenesis in invasive TCC is well documented. Increased microvessel density has been shown to predict advanced disease and poor prognosis in TCC.[19-23] Preclinical models in bladder cancer suggest that anti-angiogenic therapies may inhibit progression of bladder cancer, and that VEGF is the primary pro-angiogenic mediator of this progression.[20,24-26] Both VEGF mRNA and protein are over-expressed in advanced bladder cancer compared with normal bladder epithelium.[27-29] In addition to its pro-angiogenic properties, recent in vitro experiments also suggest a role for VEGF signaling as an autocrine and paracrine growth factor to directly promote bladder cancer growth.[30] Furthermore, retrospective evaluation of serum VEGF levels in the metastatic setting appears to correlate high levels with poor disease-free survival.[31] Baseline VEGF mRNA expression levels and microvessel density were found to be independent prognostic factors for recurrence and metastasis in 51 patients treated with neoadjuvant MVAC chemotherapy and cystectomy.[32]

In addition to its pro-angiogenic role, elevated levels of VEGF in tumors lead to abnormal microvasculature. Excessive angiogenic factors recruit endothelial and perivascular cells to form tortuous and dilated blood vessels with poor rheological characteristics, leading to abnormal tumor blood flow. Elevated VEGF levels in tumors leads to increased vascular permeability.[33] These changes lead to increased interstitial fluid pressure, which impairs the delivery of chemotherapy to tumor cells due to a decrease in the pressure gradient.[34-36] By reducing VEGF levels, not only are the aberrant tumor-associated blood vessels eliminated, but the microvasculature also appears to be remodeled, leading to more "normal" blood vessel architecture. This leads to improved transvascular drug delivery directly to tumor cells. Anti-VEGF strategies decrease interstitial fluid pressure in tumors and enhance delivery of chemotherapy to tumor cells, resulting in improved and prolonged responses.[34] Preclinical studies have shown that addition of anti-VEGF strategies to TCC chemotherapy leads to improved responses.[37-40] Addition of bevacizumab to chemotherapy has been shown in phase III studies to improve overall survival in lung (22% improvement) and colorectal cancer (30% improvement with 1st-line, and 19% improvement with 2nd-line) using different chemotherapy regimens, and progression-free survival in breast cancer (49% improvement).[41-44]

1.4 Gemcitabine, cisplatin, and bevacizumab data in bladder cancer and other solid tumors

Preliminary data is available from the Hoosier Oncology Group single arm phase II trial of gemcitabine, cisplatin, and bevacizumab (GCB) (personal communication, C. Sweeney). Although the primary endpoint of this study is progression-free survival, objective response data is available for 30 patients. Of the 30 patients who are evaluable for response, 5 patients have experienced complete responses (16.7%), 19 patients have had partial responses (63.3%), five patients with stable disease (16.7%), and 1 with progressive disease (3.3%). 13 patients are not yet evaluable for response. The gemcitabine dose in the study was reduced from 1250 mg/m^2 to 1000 mg/m^2 after seven thromboembolic events were observed in the first 17 patients enrolled (58%). Of the next 26 patients, two patients experienced thromboembolism (8%). Cisplatin-based combination chemotherapy itself is associated with a high rate of

thromboembolic events in urothelial carcinoma patients, although with gemcitabine dose reduction appears to have reduced the risk in this patient population.[45] Bleeding has not been observed to a significant degree in the phase II study. Gemcitabine, carboplatin, and bevacizumab in bladder cancer patients unfit for cisplatin is being tested at Memorial Sloan-Kettering Cancer Center, and the investigators have not observed any significant bleeding or thrombotic episodes (personal communication, D. Bajorin).

Although no complete phase II data in TCC are available for the proposed experimental regimen in TCC, GCB has been tested in a randomized phase III study compared with gemcitabine, cisplatin, and placebo in non-small cell lung carcinoma.[46] In that study, gemcitabine was administered at a dose of 1250 mg/m^2 days 1 and 8, cisplatin at a dose of 80 mg/m^2 day 1 in combination with either bevacizumab 7.5 mg/kg, bevacizumab 15 mg/kg, or placebo every 21 days. Bevacizumab given at both doses showed an improvement in progression-free survival compared to placebo. In addition, objective responses were more frequent in patients treated with bevacizumab. Overall survival data was not available at the time of the report. Arterial thrombotic events were not more frequent with the bevacizumab arms (5% placebo, 2% bevacizumab 7.5 mg/kg, and 3% bevacizumab 15 mg/kg), and venous thrombotic events were similar in both arms (6% placebo, 7% bevacizumab 7.5 mg/kg, and 7% bevacizumab 15 mg/kg).

There are also data available for safety from a randomized phase II trial of GCB vs. GC plus placebo in 95 patients with malignant mesothelioma.[47] Patients in this trial were treated with gemcitabine 1250 mg/m^2 IV days 1 and 8 every 21 days, cisplatin 75 mg/m^2 day 1, and placebo or bevacizumab 15 mg/kg every 21 days. There was no statistically significantly increased risk associated with GCB over GC except for hypertension (28% vs. 6%) and epistaxis (37% [4% grade 3] vs. 6%). No visceral perforations were observed, and thrombotic complications were less than 10% in both arms.

1.5 Impact of pharmacogenomic variation on benefits associated with bevacizumab, gemcitabine, and cisplatin therapy

The regulatory region of *VEGF* contains many transcription factor binding sites and its transcriptional as well as translational regulation appears to be quite complex. Over the past few years, several *VEGF* variants have been identified in the *VEGF* promoter and UTRs, and some of the variants have been associated with altered VEGF levels. In a few retrospective studies, *VEGF* polymorphisms have also been linked to altered disease risk.[48,49] Since bevacizumab directly neutralizes VEGF, it is quite likely that *VEGF* variants that are associated with higher VEGF levels could influence the drug response to such a therapy. One such common variant (936 C>T) in the 3'UTR of the *VEGF* gene has been associated with VEGF plasma levels. Individuals with CC genotype had significantly higher VEGF levels than individuals with CT or TT genotypes, and about 71% of a Caucasian population studied carried the CC genotype.[49]

Gemcitabine pharmacology is quite complex with its various metabolites responsible for a wide array of mechanisms that lead to its cytotoxicity. Briefly, gemcitabine is first transported across the plasma membrane via active nucleoside transporters. It is subsequently activated by deoxycytidine kinase intracellularly to its monophosphate metabolite, and the monophosphate metabolite can be phosphorylated further to di- and tri-phosphate metabolites by deoxycytidine kinase and nucleoside diphospho kinase. Gemcitabine is degraded predominantly by cytidine deaminase to difluorodeoxyuridine. Given the reported variability in gemcitabine pharmacology and clinical response, and emerging information regarding genetic variants in different genes (*CDA, DCK, DCTD, SLC29A1, SLC28A1, SLC29A2)* involved in gemcitabine metabolism, degradation, and transport, it would be prudent to explore the potential role such variants might play in gemcitabine drug response.[50-52]

The pharmacology of cisplatin is less well-defined. However, all platinum agents appear to be influenced by intracellular levels of the glutathione S-transferase (GST) family.[48] This multigene family is a key component of detoxifying pathways and is responsible for conjugation of reactive radicals. The frequency of a deletion in GST M1 (frequency 53%) and a single nucleotide polymorphism in GST P1 (AA frequency 51%) will be evaluated to determine the influence of detoxification genotype on cytotoxicity and outcome.[53] In addition, genetic variations in XRCC1 have been associated with response to platinum agents.[54] The hypothesis is that patients with the XRCC1 Arg/Arg genotype (frequency 41%) will be more resistant to cisplatin therapy.

In addition to specific hypothesis testing for the above candidate genes, this study will also provide the framework for hypothesis generating investigations of genotype and/or haplotype in additional candidate genes of putative importance to gemcitabine, cisplatin, or bevacizumab drug response.

1.6 Rationale

Based on the evidence that angiogenesis and VEGF play an important role in TCC progression, that TCC is a chemosensitive disease, and the pre-clinical data that bevacizumab improves chemotherapy delivery, locally advanced or metastatic TCC represents an opportunity to test anti-VEGF strategies. Testing anti-angiogenic agents in the first-line setting in combination with standard chemotherapy is likely to have the largest impact on TCC outcomes. Inhibition of VEGF using an anti-VEGF monoclonal antibody inhibits the growth of a number of human cancers in nude mice.[55] Bevacizumab, a recombinant humanized murine monoclonal antibody against VEGF, has shown significant clinical activity in other cancer types. It has been successfully combined with gemcitabine and cisplatin in other diseases, and proven safe.

Survival in patients with metastatic or locally advanced unresectable TCC of the bladder is prolonged with cisplatin-based combination chemotherapy. However, durable complete remissions in patients with advanced disease are rare, and median time to progression (TTP) is short. In an updated report of the randomized trial comparing M-VAC with gemcitabine and cisplatin, survival at five years was 15.3% and 13%, respectively (p = NS).[3] These results confirm that TCC is a chemotherapy-sensitive disease, that gemcitabine and cisplatin is an appropriate therapy, and that improvements in first-line chemotherapy may yield improved progression-free and overall-term survival with this disease. Recently reported data from the randomized phase III study of gemcitabine and cisplatin or gemcitabine, cisplatin, and paclitaxel did not show a significant advantage for the triplet combination.[56] As a result, gemcitabine and cisplatin remains a standard of care for patients with advanced TCC. If the addition of bevacizumab improves progression-free and overall survival, it will change the standard of care in this disease.

Reported survival for patients with metastatic TCC treated on chemotherapy trials varies widely. This variation may be explained by pre-treatment disease- and patient-related factors. To define the effect of pre-treatment patient characteristics on clinical outcome, Bajorin conducted a multivariate analysis evaluating eighteen variables in 203 patients treated with MVAC. In this analysis, KPS < 80% and presence of visceral metastases were independently prognostic of survival. The presence of both adverse features was associated with a median survival of 9.3 months; the presence of one adverse feature was associated with a median survival of 13.4 months; and the presence of no adverse prognostic features was associated with a median survival of 33 months.[57] These factors were confirmed in the long term follow-up of the randomized phase III trial of gemcitabine/cisplatin vs. MVAC in metastatic TCC.[3] As a result, because eligibility will be limited to patients with KPS > 80% (ECOG performance status 0-1), randomization will be stratified in this study according to the

presence of visceral metastasis. In addition, patients will be stratified by prior chemotherapy for muscle-invasive TCC as neoadjuvant or adjuvant therapy.

This randomized phase III study will compare standard GC chemotherapy to GCB chemotherapy with overall survival as the primary endpoint. This study will have early stopping rules for futility of the experimental arm to ensure that excessive numbers of patients are not exposed to an obviously inferior regimen. In addition, real-time toxicity monitoring with monthly conference calls will be incorporated into the protocol to monitor for excess toxicity in the GCB arm. Blood will be collected for all patients at baseline, prior to cycle 4, at the end of protocol treatment, and at the time of progression to evaluate the predictive value of VEGF plasma levels in a large study of metastatic TCC patients treated with chemotherapy with or without bevacizumab. Based on the Hoosier Oncology Group data suggesting excessive frequency of thromboembolic events with gemcitabine 1250 mg/m^2 in combination with bevacizumab in TCC, the wide use of gemcitabine 1000 mg/m^2 in bladder cancer on a 3 week schedule, and the improved dose intensity of the 3 week schedule compared to the four week schedule, the dose of gemcitabine will be 1000 mg/m^2 administered on days 1 and 8, with cisplatin 70 mg/m^2 on day 1 every three weeks.

Progression free survival is a secondary endpoint of this study. A non-biased assessment of this endpoint necessitates that this trial be a placebo-controlled, double-blinded study. Previous randomized clinical trials of advanced TCC have treated patients for a maximum of six chemotherapy cycles. Therefore, this trial will allow administration of up to six cycles of chemotherapy. Bevacizumab/placebo as maintenance therapy may be administered until progression.

Patients with creatinine clearance 50 mL/min or greater will be eligible for this study. Split dosing of cisplatin is a reasonable approach for patients with creatinine clearances ≥ 50 and < 60 mL/min.[199, 200] The use of split dosing is associated with no obvious increase in renal dysfunction or other toxicity in phase II studies. Therefore, for those patients with creatinine clearances ≥ 50 and < 60 mL/min, the cisplatin dose will be split to 35 mg/m^2 on day 1 of the cycle, and again on day 8 with pre- and post-hydration according to institutional guidelines. Dosing for patients with a creatinine clearance ≥ 60 mL/min will remain at 70 mg/m^2 on day 1.

There is increasing evidence suggesting that germline polymorphisms related to anticancer drug metabolism, transport, and resistance correlate with drug response. Furthermore, germline polymorphisms related to therapeutic targets and/or therapeutic pathways might also help predict therapeutic outcome.[58,59] Assays for genetic variants will be performed for *VEGF* (Bevacizumab) and *CDA, DCK, DCTD, SLC29A1, SLC28A1, SLC29A2* (gemcitabine) and *GST P1, GST M1, XRCC1* (cisplatin). These candidate genes have been chosen based on their potential influence on activation, degradation, transport disposition or cytotoxicity of gemcitabine, cisplatin, and bevacizumab. Additional genes or variants of interest might also be explored as new information relevant to the study emerges. The primary hypothesis of the pharmacogenomic substudy is that patients with the VEGF CC genotype will receive significantly greater benefit (OS, PFS) from anti-VEGF therapy.

1.7 Study Design

Patients will be randomized in a 1:1 allocation ratio between gemcitabine, cisplatin, and bevacizumab and gemcitabine, cisplatin, and placebo. In the absence of unacceptable toxicity or progression, patients will receive a maximum of 6 cycles of chemotherapy. If patients do not have disease progression after six cycles, then patients will continue on bevacizumab/placebo until disease progression or until patients experience unacceptable toxicity. Study participants will remain blinded at progression with no crossover permitted.

1.8 Inclusion of Women and Minorities

In selecting patients for study in this protocol, we have taken due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations. We expect that the study population will be fully representative of the range of patients seen at Alliance participating institutions, without exclusion as to age, gender, or ethnic background. The agents used in this trial may be teratogenic, especially in the first trimester. Therefore, pregnant women are excluded from participation in this study.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	7	+	14	=	21
Not Hispanic or Latino	139	+	340	=	479
Ethnic Category: Total of all subjects	146	+	354	=	500
Racial Category					
American Indian or Alaskan Native	0	+	7	=	7
Asian	0	+	7	=	7
Black or African American	14	+	21	=	35
Native Hawaiian or other Pacific Islander	0	+	7	=	7
White	132	+	312	=	444
Racial Category: Total of all subjects	146	+	354	=	500

2.0 OBJECTIVES

2.1 Primary Objective

To determine if patients with advanced transitional cell carcinoma treated with bevacizumab, gemcitabine and cisplatin will have increased overall survival when compared to patients treated with gemcitabine, cisplatin, and placebo.

2.2 Secondary Objectives

2.2.1 To compare the progression-free survival of these two regimens in patients with advanced transitional cell carcinoma.

2.2.2 To compare the proportion of patients who experience an objective response on each regimen.

2.2.3 To compare the grade 3 and greater toxicities in patients treated on the two regimens.

[See Section 10.0 correlative sciences and pharmacogenetic substudy objectives.]

3.0 ON-STUDY GUIDELINES

The following guidelines are to assist physicians in selecting patients for whom protocol therapy is safe and appropriate. Physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness which would prevent the patient from giving informed consent

- Medical condition such as uncontrolled infection (including HIV), uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed any necessary therapy and are considered by their physician to be at less than 30% risk of relapse.
- Participants in this study must agree to use adequate contraception for the duration of treatment and for at least three months following the completion of protocol therapy.

4.0 ELIGIBILITY CRITERIA

4.1 Histologic Documentation and Stage

Patients must have histologically or cytologically documented metastatic or unresectable transitional cell (urothelial) carcinoma of the urinary tract (renal pelvis, ureter, bladder, prostate, or urethra), with metastatic or locally advanced disease (T4b, N2, N3, or M1). Patients must not be candidates for potentially curative surgery or radiotherapy.

For patients that have had surgical resection prior to study enrollment, residual or unresected disease (measurable and/or unmeasurable) must be evident on post-surgical scans.

4.2 Prior Treatment for TCC:

- Patients may not have received combination systemic chemotherapy for metastatic disease.
- For the purposes of this study, radiosensitizing single agent chemotherapy is not considered prior systemic therapy.
- Prior neoadjuvant or adjuvant systemic chemotherapy is permissible provided the interval from end of therapy to diagnosis of metastatic disease is at least 1 year.
- ≥ 4 weeks since any prior radiation (including palliative) or major surgery and fully recovered.
- ≥ 7 days since any minor surgery such as port placement
- ≥ 4 weeks since any intravesical therapy
- No prior treatment with bevacizumab or other angiogenesis inhibitors.

4.3 Patient History

4.3.1 No known history of brain metastases: Brain imaging (MRI/CT) is not required.

4.3.2 No current congestive heart failure: New York Heart Association Class II, III or IV.

4.3.3 Patients with history of hypertension must be well controlled (< 150/90) on a regimen of anti-hypertensive therapy.

4.3.4 Patients on full-dose anticoagulants must be on a stable dose of warfarin and have an in-range INR (usually between 2 and 3) or be on a stable dose of LMW heparin. Patients receiving anti-platelet agents are also eligible. In addition, patients who are on daily prophylactic aspirin or anticoagulation for atrial fibrillation are eligible.

4.3.5 No significant history of bleeding events or GI perforation.

- Patients with a history of a significant bleeding episode (e.g. hemoptysis, upper or lower GI bleeding, grade 3 or 4 gross hematuria unable to be controlled by trans-urethral resection of the bladder tumor) within **6 months** of registration are not eligible.

- Patients with a history of GI perforation within **12 months** of registration are not eligible.
- Patients with a history of peritoneal carcinomatosis are not eligible.

4.3.6 No arterial thrombotic events within 6 months of registration, including transient ischemic attack (TIA), cerebrovascular accident (CVA), peripheral arterial thrombus, unstable angina or angina requiring surgical or medical intervention in the past 6 months, or myocardial infarction (MI). Patients with clinically significant peripheral artery disease (i.e., claudication on less than one block) are ineligible.

Patients who have experienced a deep venous thrombosis or pulmonary embolus within the past 6 months must be on stable therapeutic anticoagulation to be enrolled to this study.

4.3.7 No serious or non-healing wound, ulcer or bone fracture.

4.3.8 No sensory or motor peripheral neuropathy ≥ grade 2.

4.3.9 Patients with known hypersensitivity to Chinese hamster ovary cell products or other recombinant human antibodies are not eligible.

4.4 Pregnancy status

Patients that are pregnant or nursing are not eligible. Women of child bearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to registration. This is because DNA alkylating agents are known to be teratogenic, and the effects of gemcitabine, cisplatin, and bevacizumab on a developing fetus at the recommended therapeutic doses are unknown.

For women of child-bearing potential with an elevated beta-HCG that is believed to be related to cancer and not pregnancy, a negative trans-vaginal ultrasound and gynecological examination are required.

Women of child-bearing potential include any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal [defined as amenorrhea ≥ 12 consecutive months; or women on hormone replacement therapy (HRT) with documented serum follicle stimulating hormone (FSH) level > 35mIU/mL]. Even women who are using oral, implanted or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

4.5 Age and performance status

4.5.1 Age ≥ 18

4.5.2 ECOG performance status 0-1 (or KPS ≥ 80)

4.6 Required Initial Laboratory Values (other tests are required; see Section 6.0):

ANC	≥ 1500/μL
Platelet count	≥ 100,000/μL
Calculated*** or measured creatinine clearance	≥ 50 mL/minute
Bilirubin	≤ 1.25 x upper limits of normal**
AST	≤ 2.0 x upper limits of normal

Urine protein to creatinine ratio* < 1.0 or Urine protein ≤ 1+ or 24-hour Urine protein ≤ 1 gram

* See Appendix II for information regarding the calculation of UPC ratio.
** For patients with Gilbert's Disease, ≤ 2.5 X ULN is allowed.
*** Modified Cockroft and Gault formula; see below

Modified Cockcroft and Gault Formula for Estimated Creatinine Clearance (CL_{cr})

For Serum Creatinine Concentration (Sr Cr) in mg/dL:

$$Cl_{cr}\ (mL/min) = \frac{(140 - age)\ (actual\ weight)^{a}}{(72)\ (Sr\ Cr)}$$

a Age in years and weight in kilograms

For females, use 85% of calculated Cl_{cr} value

5.0 REGISTRATION/RANDOMIZATION AND STRATIFICATION

5.1 CTEP Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or TRIAD or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval

Additional information can be found on the CTEP website at < https://ctep.cancer.gov/investigatorResources/default.htm >. For questions, please contact the RCR Help Desk by email at < RCRHelpDesk@nih.gov >.

5.2 CTSU Registration Procedures

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

IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRB Manager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

5.2.1 Downloading Site Registration Documents:

Site registration forms may be downloaded from the CALGB 90601 protocol page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the Alliance link to expand, then select trial protocol CALGB 90601.
- Click on the Site Registration Documents link

5.2.2 Requirements for CALGB 90601 Site Registration:

•CTSU IRB Certification (for sites not participating via the NCI CIRB)

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

5.2.3 Submitting Regulatory Documents:

Submit completed forms along with a copy of your IRB Approval (for sites not participating via the NCI CIRB), Model Informed Consent (for sites not participating via the NCI CIRB), and any other required documentation (see above) to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103
Phone: 1-866-651-2878
Fax: 215-569-0206
E-mail: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

5.2.4 Checking Your Site's Registration Status:

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password

Click on the Regulatory tab at the top of your screen

Click on the Site Registration tab

Enter your 5-character CTEP Institution Code and click on Go

5.3 Registration Requirements

Informed Consent: The patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form is required.

5.4 Patient Registration/Randomization Procedures

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

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

•All eligibility criteria have been met within the protocol stated timeframes.

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

To receive site reimbursement for specific tests and/or bio-specimen submissions, completion dates must be entered in the OPEN Funding screen post registration. Please refer to the protocol specific funding page on the CTSU members' website for additional information. Timely entry of completion dates is recommended as this will trigger site reimbursement.

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

5.5 Registration to companion studies

There are two substudies within CALGB 90601. These substudies must be offered to all patients enrolled on CALGB 90601 (although patients may opt not to participate). The substudies included within CALGB 90601 are:

- Correlative science studies: CALGB 150609 (Sections 10.1-10.3)
- Pharmacogenomic studies: CALGB 60707 (Section 10.4)

If a patient answers "yes" to "I agree that my specimens may be used for the research studies described above." (Question #1) in the Model Consent, s/he has consented to participate in the biomarker studies described in Sections 10.1 through 10.3. The patient should be registered to CALGB 150609 at the same time that s/he is registered to the treatment trial (90601) and samples submitted per Sections 6.2.1 and 6.2.2.

If a patient answers "yes" to "I agree that my blood may be used for the genetic research studies described above" (Question #2) in the Model Consent, s/he has consented to participate in the studies described in Section 10.4. Patients should be registered to CALGB 60707 at the same time that they are registered to 90601. Samples should be submitted per Section 6.2.3.

5.6 Stratification Factors:

5.6.1 Presence of visceral metastases (defined as lung, liver, bone, splenic, or intra-abdominal metastases).

a) no

b) yes

5.6.2 Prior chemotherapy for treatment of TCC, including adjuvant, neoadjuvant, and single agent radiosensitizers.

a) no

b) yes

6.0 DATA AND SAMPLE SUBMISSION

6.1 Data Submission

As of Update #12 to the protocol, this study will use Medidata Rave® for remote data capture (RDC) of all future data collection. All data originally received by the Alliance and Statistics and Data Center (SDC) (either electronically using the "Print and/or Submit to CALGB" button [i.e. Teleform form] or by mail) has been transferred to Medidata Rave ® and can be accessed via the Medidata Rave ® system. If necessary, data originally submitted to the SDC electronically (or by mail) can be amended via the Medidata Rave ® system.

The Rave system can be accessed through the iMedidata portal at https://login.imedidata.com. For additional information regarding account setup or training, please visit the training section of the Alliance website. Forms should be submitted in compliance with the table below, and a copy of the All Forms Packet can be downloaded from the Alliance and CTSU websites.

Site personnel with Rave roles assigned on the appropriate roster may receive a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. Personnel who did not receive an invitation should contact the Alliance Service Center.

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

Data Submission: Submit forms at the following intervals:

Form		Submission Schedule
	Baseline	
C-1760 C-2078 C-816 Report	CALGB 90601 Registration Worksheet CALGB 90601 Eligibility Checklist CALGB 90601 On Study Form CALGB 90601 Charlson Index Form CALGB 90601 Solid Tumor Measurement Form (Baseline) Baseline Scan Reports*	Within two weeks of registration
	Treatment	
C-1764 C-1765	CALGB 90601 Treatment Form CALGB 90601 Adverse Event Form†	Every cycle during protocol therapy.
C-817	CALGB 90601 Solid Tumor Measurement Form (Follow Up)	Every 3 cycles during protocol therapy and after any scan done at physician's discretion.
C-1767 Report	CALGB 90601 Follow Up Form Follow Up Scan Reports*	Every 3 cycles and at the end of protocol treatment.
C-1766	CALGB 90601 Adverse Event Form (Thromboembolic Events)†	Within 24 hrs of knowledge of ≥ grade 3 thromboembolic event.
	Follow-up (after end of protocol treatment)	
C-1767	CALGB 90601 Follow Up Form	Every 3 months until progression, then every 6 months until 7 years after randomization. In addition, when new primary or secondary malignancies occur, and at death.
C-817 Report	CALGB 90601 Solid Tumor Measurement Form (Follow Up) Follow Up Scan Reports*	Submit anytime scans are done until progression or initiation of non-protocol treatment.
	Other	
C-1962	CALGB 90601 Tobacco Use Questionnaire**	Within 2 weeks of registration and after Day 1 of Cycle 4.

* Submit copies of all required reports to confirm eligibility and restaging results.

** For patients who consent to sub-study CALGB 150609.

† **Institutions that do not submit adverse event forms in a timely manner may be denied future registrations to this study (see Section 15.5).**

Common Toxicity Criteria: This study will use the NCI Common Terminology Criteria for Adverse Events version 3.0 for routine toxicity reporting on study forms. However, adverse events reported via CTEP-AERS must use CTCAE version 5.0 (See Section 16.0).

6.2 Sample submission for correlative studies

All participating institutions must ask patients for their consent to participate in the correlative substudies planned for CALGB 90601, although patient participation is optional. Biomarker and pharmacogenomic studies will be performed. Rationale and methods for the scientific components of these studies are described in Section 10.0. For patients who consent to participate, tissue and blood will be collected at the following time points for these studies:

	Within 60 days after registration	Prior to treatment	Prior to chemotherapy on Day 1 of Cycle 4*	At the end of all protocol treatment**	At progression **
Tissue blocks[1]	X				
	Number and volume of tubes to draw				
EDTA plasma[1] (lavender top)		3 x 6 mL	3 x 6 mL	3 x 6 mL	3 x 6 mL
Citrated plasma[1] (light blue top)		4 x 2.7 mL	4 x 2.7 mL	4 x 2.7 mL	4 x 2.7 mL
Serum[1] (red/gray top)		2 x 6 mL	2 x 6 mL	2 x 6 mL	2 x 6 mL
Whole blood[2] (EDTA/lavender top)	1 x 10 mL				

1 Tissue and blood samples to be used for biomarker studies (150609)
2 To be used for pharmacogenomic assays (60707).
* Samples may be collected up to 48 hours prior to chemotherapy.
** Patients for whom the end of treatment occurs within 28 days of documented progression do not need to have samples submitted twice at this time point.

Specimen submission using the Alliance Biospecimen Management System

Use of the Alliance Biospecimen Management System (BioS) is mandatory and all specimens must be logged and shipped via this system.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org.

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

All submitted specimens must be labeled with the protocol number (90601), Alliance patient number, patient's initials and date and type of specimen collected (e.g., serum, whole blood).

A copy of the Shipment Packing Slip produced by BioMS must be printed and placed in the shipment with the specimens.

Instructions for the collection of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

Shipment on Monday through Friday by overnight service to assure receipt is encouraged. If shipping on Friday, FedEx or UPS must be used and the air bill must be marked "For Saturday delivery." Do not ship specimens on Saturdays.

All specimens should be sent to the following address:

Alliance Biorepository
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

6.2.1 Blood samples

For patients who consent to participate, plasma and serum samples will be used for the biomarker analyses described in Sections 10.1 and 10.3.

For EDTA plasma, collect 18 mL of peripheral venous blood in three 6 mL EDTA (lavender-top) tubes prior to the initiation of treatment, then 18 mL prior to chemotherapy on Day 1 of Cycle 4, at the end of treatment, and, if more than 28 days apart, at progression. The tube(s) should be inverted several times to mix the EDTA and refrigerated until shipped on cool pack by overnight mail to the Alliance Biorepository at OSU. The samples should be shipped the same day that the blood is drawn.

For citrated plasma, collect 10.8 mL of peripheral venous blood in four 2.7 mL citrate (light blue-top) tubes prior to the initiation of treatment, then 10.8 mL prior to chemotherapy on Day 1 of Cycle 4, at the end of treatment, and, if more than 28 days apart, at progression. The tube(s) should be inverted several times to mix the citrate and refrigerated until shipped on cool pack by overnight mail to the Alliance Biorepository at OSU. The samples should be shipped the same day that the blood is drawn.

For serum, collect 12 mL of venous blood in two 6 mL serum separator (red/gray-top) tubes prior to the initiation of treatment, then 12 mL prior to chemotherapy on Day 1 of Cycle 4, at the end of treatment, and, if more than 28 days apart, at progression. Gently invert 5 times to mix clot activator with blood. Let blood clot for up to one hour. Observe a dense clot. Centrifuge at 1300g for 10 minutes. The sample should be refrigerated until shipped on cool pack by overnight mail to the Alliance Biorepository at OSU. The sample should be shipped the same day that the blood is drawn.

6.2.2 Submission of paraffin blocks of archived TCC tumors

For patients who consent to participate, tumor blocks will be used for the analyses described in Section 10.2.

Paraffin blocks of tissue obtained from archival TCC tumor specimens from primary and/or metastatic sites should be sent to the Alliance Biorepository at OSU. Please specify the source of the tumor block (primary or metastatic site). Submit one block of tumor tissue and one block of normal tissue.

The Alliance has instituted special considerations for the small percentage (5%) of hospitals whose policy prohibits long-term storage of blocks, and the smaller percentage (4%) of hospitals whose policies prohibit release of any block. If, due to institutional

policy, a block cannot be sent, please call the Alliance Biorepository at OSU at 614-293-7073 to obtain a protocol to cut the sections at your institution.

The goal of the Alliance Biorepository at OSU is to provide investigators with quality histology sections for their research while maintaining the integrity of the tissue. All paraffin blocks that are to be stored at the Alliance Biorepository at OSU will be vacuum packed to prevent oxidation and will be stored at 4º C to minimize degradation of cellular antigens. For these reasons it is preferred that the Alliance Biorepository at OSU bank the block until the study investigator requests thin sections. Please contact the Alliance Biorepository at OSU if additional assurances with your hospital pathology department are required.

6.2.3 Blood submission (for pharmacogenomic studies)

For patients who consent to participate, whole blood samples will be used for the pharmacogenomic studies described in Section 10.4. This sample should be collected prior to the initiation of protocol treatment.

Collect 10 mL of peripheral venous blood in an EDTA (purple-top) tube. The tube should be inverted several times to mix the EDTA and refrigerated until shipped on cool pack by overnight mail to the Alliance Biorepository at OSU. The sample should be shipped the same day that the blood is drawn.

7.0 REQUIRED DATA

Guidelines for Pre-Study Testing

To be completed within 16 DAYS before registration:
- All blood work, EKG, history and physical.

To be completed within 28 DAYS before registration:
- CT Scan of chest/abd/pelvis, **OR** CT chest plus MRI abd/pelvis

To be completed within 42 DAYS before registration:
- Bone scan (or FDG-PET)

	Prior to Registration	Day 1 of each cycle*	Day 8 of each cycle	Post Treatment Follow up**
Tests & Observations				
History and Progress Notes	X	X		X
Physical Examination	X	X		X
Pulse, Blood Pressure	X	X		
Height	X			
Weight/BSA***	X	X		
Performance Status	X	X		
Tumor Measurements	X	A		X
Drug Toxicity Assessment		X		
Laboratory Studies				
CBC, Differential, Platelets	X	X	B	
Serum Creatinine, BUN	X	X	B	
AST, Alk. Phos., Bili, LDH	X	X		
Albumin	X	X		
Serum or Urine HCG	C			
Urinalysis/Dipstick	X	D		
Staging				
Bone Scan	X (1)	E (1)		F (1)
CT Scan of chest/abd/pelvis, **OR** CT chest plus MRI abd/pelvis	X (2)	E (2)		F (2)
Correlative studies†				
Plasma and serum samples	*See Section 6.2.*			
Tissue block/slides and pharmacogenomics samples	*See Section 6.2.*			

* Pre-registration tests, observations and laboratory studies completed within 14 days prior to the first day of treatment need not be repeated. Labs and physical exam may be obtained up to 24 hours prior to protocol therapy for all other cycles.

** At least every 3 months until evidence of progression or relapse for a maximum of 7 years after registration. After progression, patients are to be followed for survival every 6 months until 7 years after registration.

*** Drug dosages need not be changed unless the calculated dose changes by ≥ 10%.

† For those patients who consent to participate in one or both companion substudies (see Section 6.2)

A Within 2 days prior to each gemcitabine/cisplatin treatment if accessible to physical examination.

B Only required for patients receiving gemcitabine and/or cisplatin

C For women of childbearing potential (see Section 4.4)

D Required only for patients who have not discontinued bevacizumab/placebo. All patients receiving bevacizumab/placebo will have a urinalysis or urine dipstick performed within 48 hours prior to every bevacizumab/placebo dose; if urine protein is ≥ 2+, 24-hour urine collection or UPC ratio will be required (see Section 9.11).

E Every 3 cycles (beginning prior to Cycle 4) until evidence of progression, relapse or initiation of non-protocol therapy. Scans may be done up to 7 days prior to beginning a cycle. Confirmatory scans should also be obtained at least 4 weeks following documentation of objective response (see Section 13.0). **Response assessment should include assessment of all sites of disease and use the same imaging method as was used at baseline.**

F Staging scans must be repeated after the end of protocol treatment unless performed within the prior 4 weeks. Thereafter, every 3 months until disease progression or initiation of non-protocol therapy for a maximum of 7 years following registration.

1 FDG-PET scans may substitute for bone scans at baseline evaluation. Follow up bone scan or FDG-PET scans are only necessary for patients with bone metastases as the only site of evaluable for metastatic disease and are optional for other patients.

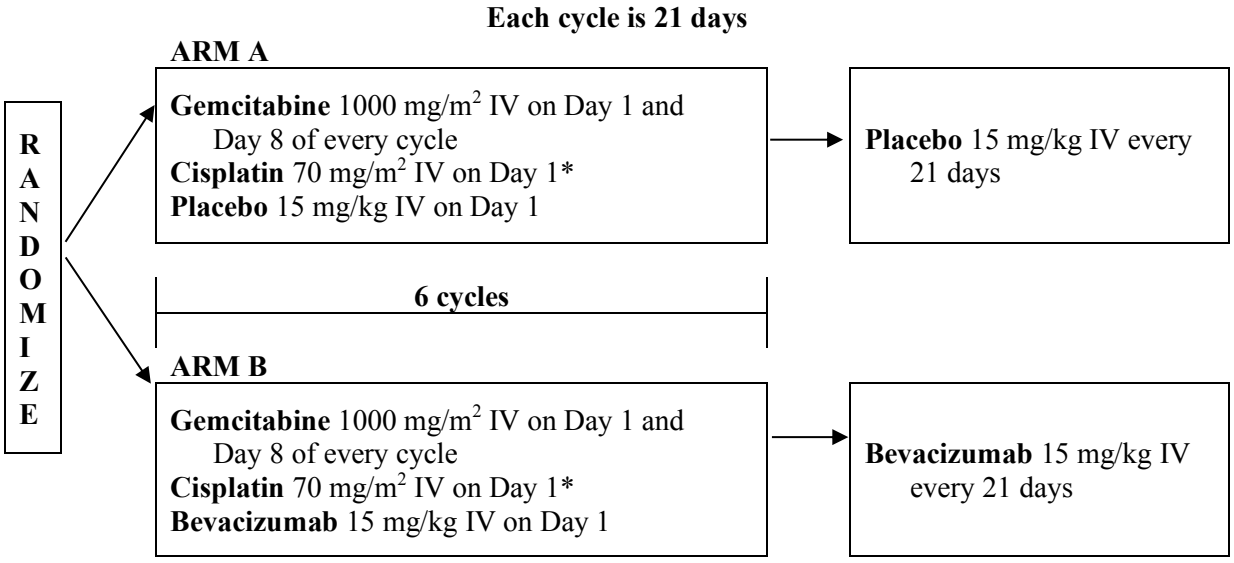
2 Diagnostic CT performed with both IV and oral contrast, and the CT acquired with 5 mm or less slice thickness.

8.0 TREATMENT PLAN

Protocol treatment is to begin within 14 days of randomization. Questions regarding treatment should be directed to the Alliance Study Chair.

This is a randomized, double-blind trial. Initial blinded, patient-specific clinical supplies of bevacizumab/ placebo will be requested by the Alliance Statistics and Data Center at the time of randomization and should arrive at the clinical site within approximately seven to ten days of randomization (see Section 11.3).

It is acceptable for individual chemotherapy doses to be delivered ≤ a 24-hour (business day) window before and after the protocol-defined date for Day 1 of a new cycle. For example, if the treatment due date is a Friday, the window for treatment includes the preceding Thursday through the following Monday. In addition, patients are permitted to have a new cycle of chemotherapy delayed up to 7 days for major life events (e.g., serious illness in a family member, major holiday, vacation that cannot be rescheduled) without this being considered a protocol violation. Documentation to justify this delay should be provided.



*Patients whose creatinine clearance is ≥ 50 and < 60 mL/min will receive a divided dose, see Section 8.3 below.

8.1 Duration of treatment

Treatment with gemcitabine and cisplatin should continue for a maximum of 6 cycles. Treatment with bevacizumab/placebo alone will continue as maintenance therapy until disease progression or unacceptable toxicity. Gemcitabine, cisplatin, and bevacizumab need not be given in the order listed below.

8.2 Gemcitabine

1000 mg/m^2 IV on Days 1 and 8, every 21 days.

8.3 Cisplatin

Patients whose creatinine clearance is ≥ 60 mL/min will receive cisplatin at a dose of 70 mg/m^2 IV per institutional guidelines on Day 1 every 21 days. At least 1 liter of normal saline will be given intravenously for hydration prior to cisplatin. Patients may receive additional intravenous hydration, mannitol, electrolytes, or furosemide according to institutional practice.

At the beginning of any cycle**, patients whose creatinine clearance is ≥ 50 and < 60 mL/min** will be treated with cisplatin 35 mg/m^2 on Day 1 and with cisplatin 35 mg/m^2 on Day 8 of the cycle. At least 1 liter of normal saline will be given intravenously for hydration prior to cisplatin. Patients may receive additional intravenous hydration, mannitol, electrolytes, or furosemide according to institutional practice.

If at the beginning of a subsequent cycle, creatinine clearance recovers to ≥ 60 mL/min, patients may resume full dose cisplatin on Day 1 of the cycle (with any applicable dose modifications per Section 9.0).

8.4 Bevacizumab/Placebo

Bevacizumab/placebo 15 mg/kg IV is to be administered every 21 days. The initial dose is to be given over 90 minutes, second dose over 60 minutes, and all subsequent doses over 30 minutes if prior infusions are tolerated without infusion-associated adverse events.

See Section 9.13 for instructions regarding patients who require surgery.

8.5 Cisplatin-induced emesis

For prophylaxis of cisplatin-induced emesis, a 5-HT3 antagonist and a corticosteroid are recommended. NK1 receptor antagonists such as aprepitant are allowed per institutional practices.

8.6 Aspirin

81 mg daily p.o. should be considered at the discretion of the treating physician for all patients who are not already receiving daily aspirin and are at risk for arterial thromboembolic events (age ≥ 65, history of arterial thrombotic events). Aspirin should be considered because of the increased risk of arterial thromboembolic events from bevacizumab. Patients who cannot tolerate aspirin or in whom it is contraindicated should not receive it.

9.0 Dose Modifications and Management of Toxicity

Skipped doses are not made up. If Day 1 treatment cannot be administered, initiation of all protocol treatment for that cycle should be delayed.

Dose Levels for Gemcitabine

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

Dose Levels for Cisplatin

Dose Level	Cisplatin	
	CrCl ≥ 60 mL/min	**CrCl ≥ 50 and < 60 mL/min***
Level 0	70 mg/m^2	35 mg/m^2 on Day 1, and 35 mg/m^2 on Day 8 of cycle
Level -1	50 mg/m^2	25 mg/m^2 on Day 1, and 25 mg/m^2 on Day 8 of cycle
Level -2	36 mg/m^2	18 mg/m^2 on Day 1, and 18 mg/m^2 on Day 8 of cycle

Gemcitabine and **cisplatin** doses may be modified separately based on individual toxicity according to the rules outlined below. There is no dose reduction below level -2 for gemcitabine or cisplatin. If dose reduction below level -2 is required, gemcitabine and/or cisplatin should be discontinued.

* **Divided dose cisplatin:** If at the beginning of any cycle, a patient's measured or calculated creatinine clearance is ≥ 50 mL/min and < 60 mL/min, administer cisplatin at the appropriate dose level, but at a divided dose to be given on Days 1 and 8 of the cycle.

Bevacizumab/placebo dose is always 15 mg/kg. Bevacizumab/placebo may be skipped or discontinued as described below, but the dose is not reduced. If 2 sequential doses of bevacizumab/placebo are skipped due to toxicity, bevacizumab/placebo should be permanently discontinued. If bevacizumab/placebo is discontinued, gemcitabine and cisplatin should be continued for a total of up to 6 cycles, or until disease progression or unacceptable toxicity.

9.1 Hematologic Toxicity

9.1.1 Gemcitabine and cisplatin dose guidelines for Day 1 hematologic toxicity

For ANC < 1500 or platelets < 100,000 on Day 1, delay all protocol treatment, including bevacizumab/placebo, and repeat CBC weekly. Resume treatment with ANC improves to ≥ 1500 and platelets improve to ≥ 100,000.

- If treatment was delayed for 1 week, resume treatment at the previous doses of gemcitabine and cisplatin.
- If treatment was delayed for more than one week and less than six weeks, reduce gemcitabine and cisplatin by one dose level for this and all subsequent cycles.

For delays of 6 weeks or greater, discontinue gemcitabine and cisplatin. If gemcitabine and cisplatin are discontinued for hematologic toxicity, treatment with bevacizumab/placebo should be continued until disease progression or unacceptable toxicity.

9.1.2 Dose modifications for Day 8 hematologic toxicity

For ANC 500-999 or platelets 50,000-74,999, decrease gemcitabine by one dose level for this and all subsequent doses. For patients receiving split dose cisplatin during this cycle, also decrease cisplatin by one dose level for this and all subsequent doses.

For ANC < 500 or platelets < 50,000, skip gemcitabine and decrease gemcitabine by one dose level for all subsequent doses. For patients on split dose cisplatin during this cycle, also skip cisplatin and decrease cisplatin by one dose level for this and all subsequent doses.

9.1.3 Dose modifications for hematologic toxicity outside of Day 1 and/or Day 8

For patients with platelets < 50,000 with clinically significant bleeding (grade 2 or greater) at any time, dose reduce gemcitabine one dose level for next and all subsequent doses.

9.1.4 Febrile neutropenia: For febrile neutropenia (defined as temperature ≥ 38.5° C [101° F] sustained for more than one hour concomitant with ANC < 500/mm^3), reduce gemcitabine and cisplatin by one dose level for this and subsequent cycles.

9.1.5 Dose modifications during bevacizumab/placebo maintenance therapy

For ANC < 500 or platelets < 25,000, hold bevacizumab/placebo treatment. Resume treatment when ANC improves to ≥ 1500 and platelets improve to ≥ 100,000. If treatment is held greater than 6 weeks for hematologic toxicity, discontinue bevacizumab/placebo.

9.2 Hepatic Dysfunction

9.2.1 For bilirubin > 1.5 x ULN (or > 3 x ULN for patients with Gilbert's syndrome), delay treatment until bilirubin ≤ 1.5 x ULN (< 3 x ULN for patients with Gilbert's syndrome), then resume with one dose level reduction of gemcitabine and at the previous dose of cisplatin and bevacizumab.

9.2.2 If bilirubin > 1.5 x ULN despite two gemcitabine dose reductions, gemcitabine should be discontinued but cisplatin and bevacizumab/placebo may be continued. For patients with Gilbert's syndrome, if bilirubin > 3 x ULN despite two gemcitabine dose reductions, gemcitabine and cisplatin should be discontinued; treatment with bevacizumab/placebo may be continued.

9.3 Neurotoxicity

9.3.1 For grade 3 sensory or motor neuropathy, skip cisplatin until the toxicity resolves to ≤ grade 2 and then resume therapy with one dose level reduction of cisplatin on Day 1 of the next scheduled cycle. If cisplatin is skipped for two consecutive cycles, discontinue cisplatin. Treatment with gemcitabine and bevacizumab/placebo may continue.

9.3.2 For grade 4 sensory or motor neuropathy, skip all therapy until resolution to ≤ grade 2, discontinue cisplatin; resume gemcitabine and bevacizumab/placebo at the previous dose.

9.4 Dose modifications for gastrointestinal toxicity

For grade 3 or 4 nausea or vomiting despite maximal antiemetic therapy (including 5HT-3 antagonist, corticosteroids, and aprepitant), discontinue cisplatin. Continue gemcitabine and bevacizumab/placebo at the previous dose when symptoms resolve to ≤ grade 1.

9.5 Dose modifications for Day 1 and Day 8 kidney function

9.5.1 For creatinine clearance < 50 mL/min (measured or calculated) on Day 1 of a cycle, delay all treatment until creatinine clearance improves to ≥ 50 mL/min.

- If creatinine clearance improves to ≥ 60 mL/min within 1 week, resume with one dose level reduction for cisplatin for all subsequent doses, and the previous dose of gemcitabine and bevacizumab/placebo.
- If creatinine clearance improves to ≥ 50 and < 60 mL/min within 1 week, resume with one dose level reduction of cisplatin for this and all subsequent cycles. Administer cisplatin as a split dose on Days 1 and 8 of this cycle. Resume gemcitabine and bevacizumab/placebo at the previous dose.
- If creatinine clearance does not improve to ≥ 50 mL/min within 1 week, skip cisplatin for this cycle only. Treat with gemcitabine and bevacizumab/placebo at the previous dose. For the next cycle, if creatinine clearance is still < 50 mL/min, discontinue cisplatin. Continue gemcitabine and bevacizumab/placebo.

9.5.2 For creatinine clearance < 50 mL/min (measured or calculated) on Day 8 of a cycle, skip cisplatin for this day (applies only if split-dose cisplatin is indicated for this cycle). Refer to the above dose modifications for Day 1 dosing for the next cycle.

9.6 Redact

9.7 Redact

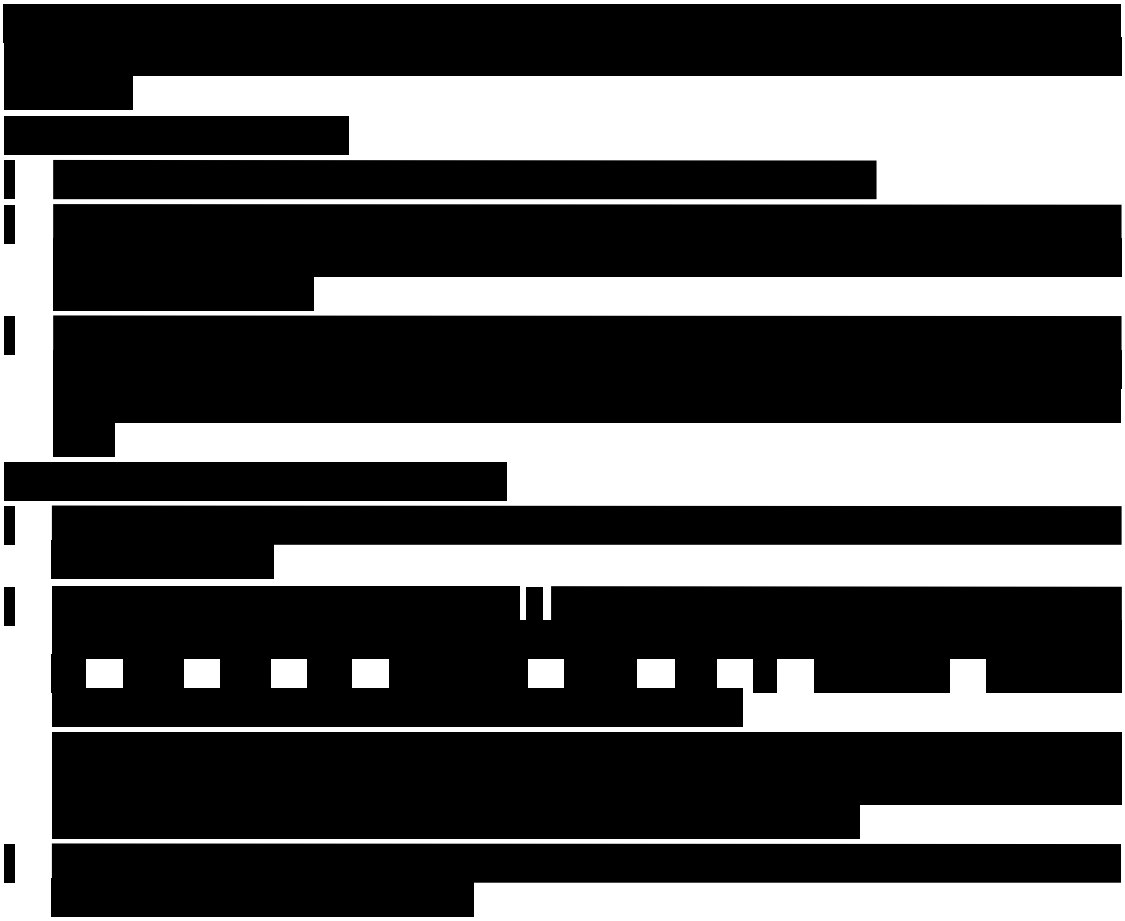
9.8 Redact

9.8.2 Redact

9.9 Redact

9.10 Redact

9.11 Redact



9.12 Hypersensitivity and infusion reactions

Note that the NCI CTCAE defines hypersensitivity reactions differently from infusion reactions: "Cytokine release syndromes/acute infusion reactions are different from allergic/hypersensitivity reactions, although some of the manifestations are common to both AEs. An acute infusion reaction may occur with an agent that causes cytokine release (e.g., monoclonal antibodies or other biological agents). Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion." See the "Syndromes" section of the CTCAE version 3 for a complete list of signs and symptoms of "Cytokine release syndrome/acute infusion reaction;" and see the "Allergy/Immunology" section for a description of hypersensitivity. **Note:** Serious adverse events will be reported through CTEP-AERS using CTCAE version 5.0.

9.12.1 Dose modifications for hypersensitivity reactions (for all agents)

- **For grade 1 hypersensitivity reactions** (transient rash, drug fever < 38°C): Decrease the infusion rate by 50% until symptoms resolve, then resume at the initial planned rate.
- **For grade 2 hypersensitivity reactions** (urticaria, drug fever ≥ 38°C and/or asymptomatic bronchospasm): Stop infusion. Administer H_1 and/or H_2 blockers, and/or steroids according to institutional policy. Restart the infusion when symptoms resolve and pretreat before all subsequent doses. Treat according to institutional policy.

- **For grade 3 or grade 4 hypersensitivity reactions:** Stop the infusion. Discontinue all protocol treatment and notify the study chair.

9.12.2 Bevacizumab/placebo dose modifications for infusion reactions

The initial bevacizumab/placebo dose should be administered over a minimum of 90 minutes. If no adverse reactions occur, the second dose should be administered over a minimum of 60 minutes. Again, if no adverse reactions occur, the third and subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, subsequent infusions should be administered over the shortest period that is well-tolerated. Patients may receive premedication with diphenhydramine 25 to 50 mg intravenously or orally 30 minutes prior to bevacizumab/placebo if they have previously experienced mild infusion reactions. Acetaminophen premedication may also be used.

9.13 Surgery

For patients who require surgery while on study, it is recommended that bevacizumab/placebo be discontinued for at least 60 days prior to surgery whenever possible. For minor surgery such as port placement at least seven days is needed between the insertion and treatment with bevacizumab/placebo. Re-initiation of protocol therapy should be discussed with the Alliance Study Chair.

9.14 Fatigue

For persistent fatigue interfering with activities of daily living (grade 2/3) during treatment with gemcitabine, cisplatin, and bevacizumab/placebo, any or all of these agents may be held for up to 3 weeks at the discretion of the treating physician.

For persistent fatigue interfering with activities of daily living (grade 2/3) during treatment bevacizumab/placebo alone, bevacizumab/placebo may be held for up to 6 weeks at the discretion of the treating physician.

9.15 Other non-hematologic toxicities

For all other treatment-related ≥ grade 3 non-hematologic toxicities not described above, hold all protocol treatment and monitor toxicity at least weekly. If toxicity resolves to ≤ grade 1 within 6 weeks, treatment may be resumed, with gemcitabine and cisplatin at one lower dose level, and with bevacizumab/placebo at the previous dose.

9.16 Dose Modification for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, **all dosing is to be determined solely by the patient's actual weight without any modification unless explicitly described in the protocol**. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. **Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.** Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on Alliance protocols.

10.0 CORRELATIVE SCIENCE SUBSTUDIES

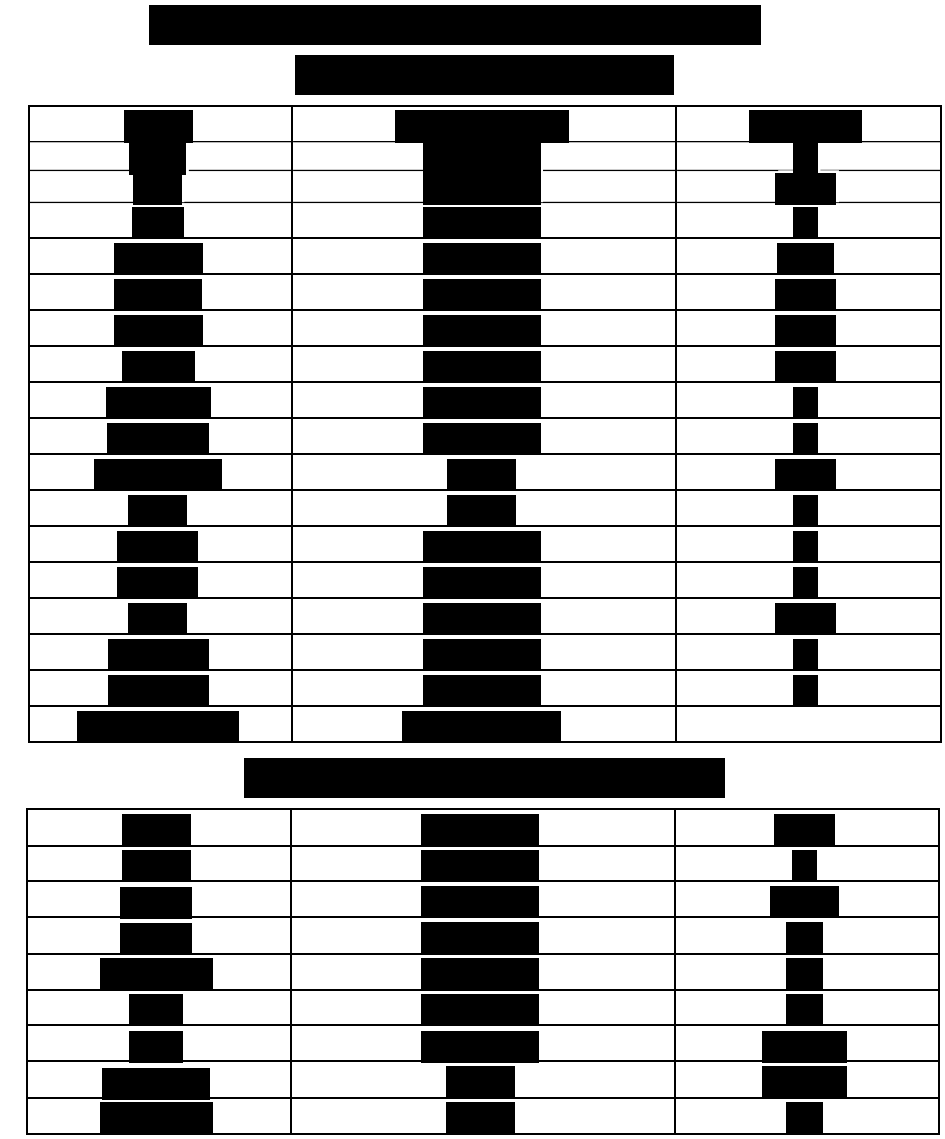
There are four components of the correlative science substudies for CALGB 90601 and all patients are encouraged to participate. The first part is comprised of serum-based biomarker studies and is described in Section 10.1. The second part is comprised of tissue-based biomarker studies and is described in Section 10.2. The third part is comprised of an evaluation of the effect of tobacco on bladder cancer outcomes. Taken together, these three sections make up the companion study

150609. The fourth part, pharmacogenomic studies in patients with advanced transitional cell cancer, is described in Section 10.4 and makes up the companion study, 60707.

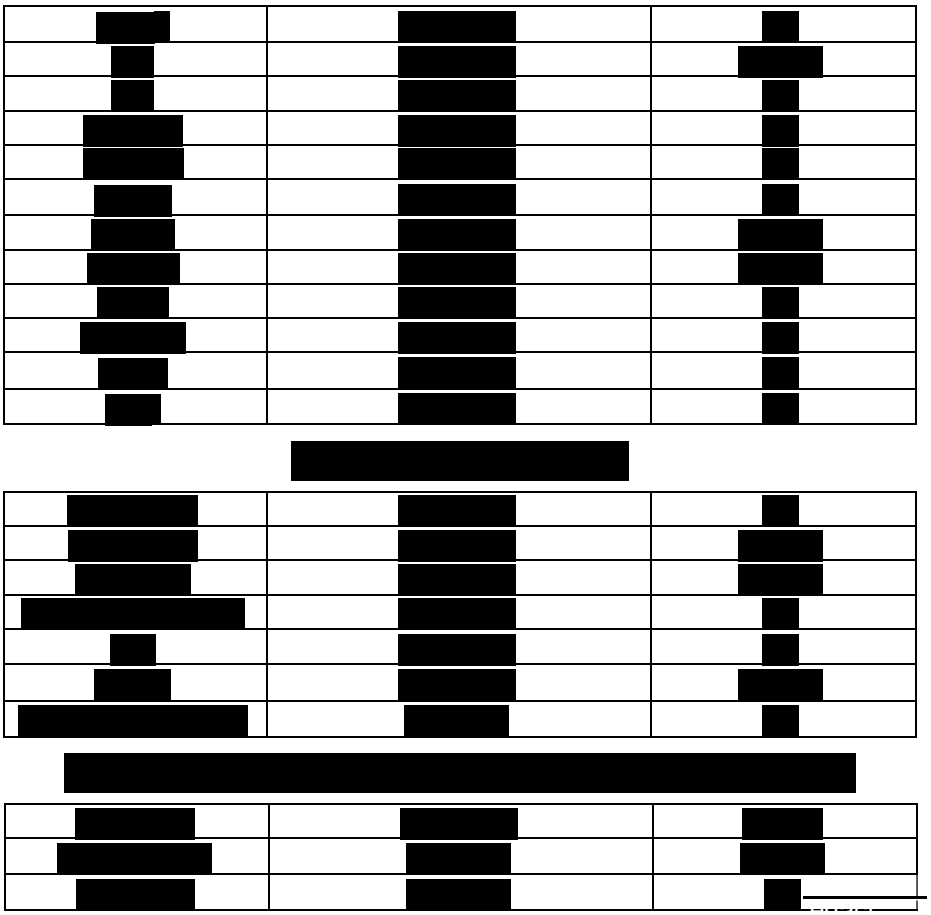
10.1 Redact

- Redact

Redact



C. Redact



10.1.2 Primary objective:

To determine if serum-based biomarkers reflecting tumor angiogenesis are prognostic biomarkers of overall survival for patients with advanced transitional cell carcinoma receiving gemcitabine/cisplatin/bevacizumab or gemcitabine and cisplatin.

Secondary objectives:

1) To determine if patients with advanced TCC and elevated baseline levels of VEGF will have worse prognoses than patients with low baseline VEGF levels.
2) To determine if elevated baseline levels of VEGF will predict for better response to bevacizumab/cisplatin/gemcitabine in patients with advanced TCC. Additionally, to determine if elevated levels of urinary VEGF and/or soluble plasma VEGFR2 on treatment will correlate with resistance to therapy and disease progression in patients with advanced TCC treated with bevacizumab/ cisplatin/gemcitabine.
3) To determine if levels of non-VEGF angiogenic factors at baseline and during treatment will correlate with primary and acquired resistance to bevacizumab, respectively.
4) To determine if proteomic profiling will help define novel markers of disease biology and can help predict for response to bevacizumab.

10.1.3 ELISA and coagulation assay methods

Both EDTA and citrated plasma samples are required to meet the assay specifications for the various biomarkers. EDTA plasma will be collected in a standard 13 x 75 mm (5 mL purple top) tube. Three 5 mL EDTA tubes of blood should yield approximately 9 mL plasma after processing per time point. One 5 mL tube of citrated plasma will be collected for coagulation profiling. One 5 mL tube of serum (red top or SST) will be collected for proteomic profiling. One additional 5 mL tube of citrated plasma and one additional 5 mL tube of serum will be archived for further potential exploratory analyses related to predicting efficacy or toxicity of gemcitabine and bevacizumab or a better understanding of transitional cell cancer biology. In total, nine tubes of blood (40.8 mL) will be drawn at the time of registration, prior to Cycle 4, at the time of discontinuation of protocol therapy, and at progression (3 x 6 mL EDTA plasma, 4 x 2.7 mL citrate plasma, and 2 x 6 mL serum).

Samples will be analyzed at Duke University using either SearchLight technology, commercially available ELISA assays (R&D Systems, Biosource), or ELISAs developed within our laboratory. Analysis of cytokine levels in urine will be normalized to creatinine.

The CV's of the ELISAs are typically 20% or less with sensitivities in the picogram range and a 3-4 log dynamic range. The CV's of the multiplex arrays are approximately 20%, depending on the particular assay. In order to validate each specific plate design, we will run samples spiked with known quantities of purified growth factors in parallel with study samples to verify the performance characteristics of the assays. Known amounts of cytokines will be added into buffer. The mean recovery for each cytokine will be ascertained. Any study samples that fall outside the linear portion of the standard curve will be retested. Samples that read below the limit of detection will be retested as neat plasma to confirm the initial results. Samples that read above the linear portion of the standard curve will be serially diluted and retested to obtain accurate measurements. Any analyte that does not meet the aforementioned criteria will result in the sample being re-evaluated.

Thrombin/AT complexes, serum albumin, serum LDH, and urine creatinine determinations will be performed by the clinical laboratories at Duke University Medical Center.

10.2 Tissue-based chemotherapy resistance biomarkers

10.2.1 Background

Chemotherapy resistance continues to be a significant cause of morbidity and mortality in advanced bladder cancer. While many mechanisms of resistance have been suggested, there are no validated markers predictive for response to chemotherapy. However, biomarkers reflecting DNA repair mechanisms have now been found to have predictive significance for patient outcome following adjuvant cisplatin-based therapy for resected lung cancer [133] and functional resistance to cisplatin can also be achieved through re-establishing effective homologous recombination to repair double-strand break (DSB).[134, 135] In this large, multi-institutional study, we propose to determine the predictive strength of markers of DNA repair mechanisms for patients with advanced transitional cell carcinoma treated with gemcitabine/cisplatin or gemcitabine/cisplatin/ bevacizumab.

There is a significant literature focused on molecular determinants of chemotherapy sensitivity (primarily cisplatin and gemcitabine) in bladder cancer. Cisplatin resistance in

bladder cancer has been linked to multiple molecular features including altered expression of bcl-2,[136, 137] p53,[138] p73,[139] inositol 1,4,5-trisphosphate receptor type 1[140] to name a few of the most common. In contrast, p-Glycoprotein has generally been found to have low expression and transporters are thought to play less of a role.[141] However, as yet no mechanism based biomarker has had sufficient predictive strength to warrant clinical use.

Increasing evidence suggests that the status of DNA repair mechanisms, specifically nucleotide excision repair (NER) and homologous recombination (HR), impact a cancer cell's sensitivity to agents causing interstrand cross links, DNA adducts, and double-stranded breaks (DSB). For cisplatin based therapy, low levels of protein expression of excision repair cross complementation 1 (ERCC1) have been associated with improved survival following adjuvant therapy in lung cancer.[133] In patients with advanced lung cancer treated with the combination of gemcitabine and cisplatin, low expression of both excision repair cross complementation 1 (ERCC1) and ribonucleotide reductase M1 (RRM1) were both found to be associated with median survival time.[142] In addition, two papers have recently demonstrated that the status of BRCA2, which plays a functional role in homologous recombination, is important to sensitivity to cisplatin and PARP.[134, 135] Specifically, BRCA2 -/- (BRCA2 null and, as a result, HR deficient) ovarian cell lines and cultured tumor cells selected for resistance to PARP and cisplatin treatment were found to genetically restore BRCA2 function and restore HR. A potential biomarker for intact HR is nuclear Rad51 staining, as immunofluorescent detection of Rad51-containing foci in the nuclei of cells suggests intact HR.[143] Thus, there is strong evidence that a tumor's ability to repair interstrand cross links through NER and HR may strongly impact overall tumor sensitivity to cisplatin-based therapy and biomarkers for DNA repair mechanisms may anticipate clinical response to therapy.

Preliminary investigations in bladder cancer suggest that DNA repair mechanisms may impact clinical response to chemotherapy. With respect to DNA repair mechanisms, hMLH-1 and hMLH-2 have been found to have high expression in bladder cancer.[139] In advanced bladder cancer, low mRNA expression of ERCC1 has already been found to be associated with improved survival (25.4 mo v. 15.4 m, P=0.03) in a small number of patients treated with chemotherapy (n = 57) [144] and other mRNA levels of other DNA repair genes such as RRM1, BRCA1, and caveolin-1 had a trend toward significance but did not meet statistical threshold in this small study.[144]

Importantly, while the interaction between cisplatin and DNA repair mechanisms may be the primary biology driving clinical benefit from the combination of gemcitabine and cisplatin, expression of a few of the same proteins have been associated with gemcitabine response. RRM1 overexpression has been associated with resistance to gemcitabine [145] and caveolin-1 expression is also associated with poor outcomes with gemcitabine-based chemotherapy.[146]

Here, formalin-fixed paraffin embedded primary tumors from patients enrolled on CALGB 90601 will be requested and stored at the Alliance Biorepository at OSU. Tissue microarrays will be created and biomarkers reflecting the status of DNA repair in these primary tumors will be assessed for their predictive strength when patients are treated with cisplatin/gemcitabine or cisplatin/ gemcitabine/bevacizumab. Specific markers to be assessed include ERCC1, Rad51, RRM1, BRCA1, BRCA2, and caveolin-1. Specifically, A) expression of ERCC1, Rad51, RRM1, BRCA1, BRCA2, and caveolin-1, B) immunofluorescence will be used to determine the nuclear pattern of Rad51, and C) germ-line polymorphisms in ERCC1 (118C/T and C8092A) will be assessed for their association with outcome in patients with advanced TCC.

The hypotheses of the tissue based analysis are:

- **Low levels of ERCC1 expression by immunohistochemistry are associated with improved progression free or overall survival.** Building upon results from lung cancer and preliminary studies in bladder cancer, we will definitively determine if low ERCC1 levels, as measured by IHC, are associated with improved clinical outcome following cisplatin based therapy. Low ERCC1 levels suggest impaired nucleotide excision repair which is the first step in the repair of interstrand cross links. As this is the major mechanism by which cisplatin exerts its cytotoxic impact, poor DNA repair capability is likely to result in increased cellular apoptosis in response to cisplatin. While we will be determining ERCC1 levels in primary tumor blocks and treating metastatic disease, the compelling preliminary data from lung cancer establishing an association between low ERCC1 expression in primary lung cancers and finding a survival benefit following adjuvant chemotherapy, suggests that primary tumor expression of ERCC1 is relevant to metastatic disease.
- **Expression of alternative markers of DNA repair (Rad51, RRM1, BRCA1, BRCA2, and caveolin-1) are associated with improved progression free or overall survival.** While ERCC1 is the most widely used and validated biomarker reflecting a cell's capacity for DNA repair, variation in Rad51, RRMI, BRCA1, BRCA2, and caveolin-1 may also reflect DNA repair and provide prognostic or predictive biomarkers. Each of these markers have been found to be expressed in or associated with bladder cancer. Rad51 has variable expression across multiple bladder cancer cell lines [147] but its association with prognosis or response to chemotherapy remains unknown. RRM1 expression correlates with increased survival in patients with advanced bladder cancer.[144] BRCA1 gene hypermethylated has been shown to help with diagnosis when detected in the urine sediment [148] and IHC for both BRCA1, BRCA2, and Rad51 has been shown to be predictive for response to radiation therapy in breast cancer.[149] Finally, expression of Caveolin-1 staining has been found to be present in bladder cancer tumors and correlates with tumor grade.[150] These proteins will be assessed for expression and correlated with progression free survival and overall survival.
- **Nuclear Rad51 staining patterns by immunofluorescence is associated with progression free or overall survival.** While expression level of Rad51 may provide sufficient information with respect to cisplatin sensitivity, the specific pattern of Rad51 expression in the nucleus is a more accurate marker of intact homologous recombination. Immunofluorescence for Rad51 performed on the TMA will be assessed and each tumor will be annotated for the presence or absence of Rad51-containing nuclear foci. The progression free and overall survival of individuals with tumors having Rad51-containing nuclear foci present will be compared to those without the nuclear foci.

10.2.2 Primary objective

To determine if low levels of ERCC1 expression is prognostic of overall survival in patients with advanced transitional cell carcinoma treated with gemcitabine/cisplatin and/or gemcitabine/cisplatin/bevacizumab.

Secondary objectives

1) To determine if low levels of ERCC1expression by immunohistochemistry is associated with improved progression free survival.
2) To determine if expression of alternative markers of DNA repair (Rad51, RRM1, BRCA1, BRCA2, and caveolin-1) are associated with improved progression free or overall survival
3) To determine if nuclear Rad51 staining patterns by immunofluorescence is associated with progression free or overall survival.

10.2.3 Methods for Tissue Protein Analysis

Outcome-linked paraffin-embedded tissues from patients on randomized treatment trials constitute a valuable but consumable resource for studies of tumor biology and treatment response. In order to make maximal use of the tissue resources provided by this study, tissue microarrays (TMAs) containing tumor from all participants who have their paraffin blocks sent to the Alliance Biorepository at Ohio State University will be constructed to facilitate targeted protein analysis using immunohistochemistry. TMAs will be constructed at the Alliance Biorepository at Ohio State University. Briefly, H&E stained slides will be reviewed, and areas marked from which the donor cores should be taken. In general, areas measuring at least 7 mm in diameter are preferred in order to facilitate the harvesting of at least 6 cores of tissue (3 in the primary TMA block and 3 in the duplicate). Arrays are constructed with 3 additional cores of normal corresponding tissue at one end for orientation. The TMA's will be stored at the Alliance Biorepository at OSU and sent to investigators for the analysis of specific markers. These TMAs can be used for both immunohistochemistry and immunofluorescence.

Biomarker immunostaining of the TMA's will be performed using a Ventana automated immunostainer, which not only increases the rate at which novel immunohistochemistry and in situ hybridization markers can be optimized for staining TMAs, but also can match the staining methods used in large hospital diagnostic laboratories, facilitating translation of validated markers into clinical use for diagnosis or prediction. Image capture and scoring will be facilitated by automated digital imaging systems (Bacus Lab Inc Slide Scanner (BLISS)), which accelerates the rate at which biomarkers can be scored, provides a secure image archive linked to secure molecular and clinical database, permits on-line publication of results, and yields high quality images suitable for quantitative analyses. Cores will be visualized with a Web based image display at 20X magnification. To assist with diagnostic interpretation in NHT tissues, H&E and CK5/6 stained replicate array slides will be used. In untreated tissue cores, nuclear grade will be assigned to each specimen on the TMA. Subjective visual scoring and objective computer assisted scoring systems will be applied to each stained slide and an automated intranet analysis system that displays images, scoring results as well as clinical features of each patient will be used to facilitate analysis.

Biomarker evaluation: The staining intensity of malignant tissue will be evaluated and scored by a minimum of two pathologists, as well as automated quantitative image analysis by pro-plus image software (MediaCybernetics, San Diego, Ca). For scoring of the majority of markers, including ERCC1, Rad51, RRM1, BRCA1, BRCA2, and caveolin-1, specimens will be graded from 0 to +3 intensity representing the range from no staining to heavy staining, as previously reported.[133, 149-167] The overall percentage of cancer cells showing staining (0-100%) will also be reflected in the score as follows:

0: No reactivity

1+: Weak reactivity, with 0-10% of tumor cells showing positive staining.

2+: Moderate activity, with 10-50% of tumor cells showing positive staining.

3+: Strong reactivity, with > 50% of tumor cells showing positive staining.

Immunofluorescence of nuclear Rad51 will be performed on the TMAs as previously described [135] and scored as absent (0) or present (1). The presence of any Rad51-containing nuclear foci suggests intact homologous recombination thus making this dichotomous grading appropriate.

10.3 Evaluation of somatic mutations and copy number changes associated with treatment response

10.3.1 Background

Cisplatin-based combination chemotherapy represents standard therapy for patients with metastatic transitional cell carcinoma (1, 2). While response rates to chemotherapy are high in this disease, durable remissions remain rare, in only 9-20% of patients (3, 4). The lack of a predictive biomarker for benefit from cisplatin-based chemotherapy has limited the use of this relatively toxic approach in the urological community; only a minority of patients actually receives cisplatin chemotherapy.(5) Prospective identification of those patients most likely to benefit has not been possible, as no predictive biomarker has been identified. However, cisplatin-based therapy can be curative in those 9-20% of patients, and beyond clinical prognostic factors (6) there is no way to identify them in advance.

Cisplatin administration leads to inter- and intra-strand DNA adducts, resulting in DNA cross-linkage and cell death. Cisplatin-induced DNA damage repair (DDR) occurs primarily through the nucleotide excision repair (NER) pathway(7), with additional repair via homologous recombination.(8) NER occurs initially through two sub-pathways (global repair and transcription-coupled repair), followed by downstream convergence in a common pathway. The NER pathway is comprised of multiple genes, including ERCC1-5. ERCC2 is a DNA helicase within the transcription factor IIH (TFIIH) complex that opens DNA around a damaged lesion to allow excision repair.(9) Many TFIIH genes are implicated in recessive inherited DDR disorders such as xeroderma pigmentosum (XP), Cockayne syndrome, and trichothiodystrophy. Whole exome sequencing (WES) of muscle invasive TCGA specimens identified recurrent somatic mutations in ERCC2 in 12% of tumors.(10)

Multiple studies have implicated germline NER single nucleotide polymorphisms (SNPs) as modulators of cisplatin-based chemotherapeutic efficacy in cancer patients,(11, 12) although prospective assessment of either germline SNPs(13) or expression changes(14) in NER genes have not confirmed these findings. In vitro experiments have previously demonstrated that cell lines with deficiencies in the transcription-coupled NER sub-pathway, including members of the TFIIH complex, have enhanced sensitivity to cisplatin.(15, 16) However, no previous work has rigorously evaluated the NER pathway for somatic alterations in tumors.

Preliminary work has identified ERCC2 somatic mutations as predicting exquisite sensitivity to neoadjuvant cisplatin-based chemotherapy (MVAC or gemcitabine/cisplatin) in patients with muscle invasive transitional cell carcinoma.(17) Patients with pathological complete response (pT0 or pTis; n=25) vs. no-response or progression (n=25) in the cystectomy specimen underwent whole exome sequencing of DNA extracted from untreated transurethral resection specimens (complete responders) or post-treatment cystectomy with matched germline samples. ERCC2 missense mutations in 36% of patients with CRs (ERCC2 [XPD] n=9). Strikingly, none of the platinum-resistant tumors harbored these mutations. Since the somatic ERCC2 mutation prevalence in The Cancer Genome Atlas bladder cancer cohort was 12%, the mutation

frequency in CRs (35%) was dramatically higher than expected in unselected patients ($q<0.001$). Six of 9 ERCC2 mutations in cisplatin-sensitive patients lay within consensus helicase domains required for ERCC2 function. One additional mutation is adjacent to a helicase domain. The 3D structure of ERCC2 indicates that the extreme sensitivity mutations line the DNA interaction cleft; these regions are conserved from Archaea to humans suggesting a highly conserved function.

While ERCC2 may be closely linked with response to cisplatin-based therapy, only a minority of responders possess these mutations. Somatic mutations in other DNA damage response genes have been reported to have an association with response to cisplatin-based therapy.(18) Whether these mutations impact overall survival is not clear.

The association between other somatic mutations and survival in metastatic transitional cell carcinoma has not been fully elucidated in a large prospectively collected cohort. TCGA bladder cancer cohort remains immature, and many patients have not developed metastatic disease. Therefore, evaluating whether other somatic mutations are associated with good or poor outcomes in metastatic disease remains a relevant question.

The availability of high-throughput next-generation sequencing platforms that are able to handle small quantities of DNA from formalin-fixed paraffin embedded tissues has allowed large-scale assessment of multiple genes at reasonable cost. IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) (19) is a 340 gene next-generation bait-capture sequencing assay designed to capture and sequence all protein-coding exons and select introns of 300 cancer-associated genes to 500-1000x coverage, and includes ERCC2 plus other DNA repair genes.

10.3.2 Rationale

Based on these findings in muscle invasive patients, we hypothesize that somatic mutations in ERCC2 are associated with more durable responses in patients with metastatic transitional cell carcinoma treated with cisplatin-based chemotherapy. Conversely, we hypothesize that lack of an ERCC2 somatic mutation is associated with a lower likelihood of a durable response to chemotherapy. As patients on both arms of this trial are treated with gemcitabine and cisplatin chemotherapy, this patient population is ideal to test the hypothesis that ERCC2 somatic mutation is associated with enhanced clinical benefit to cisplatin-based combination therapy. While the magnitude of benefit associated with an ERCC2 mutation may differ between the bevacizumab and placebo arms, we hypothesize that ERCC2 mutations will predict improved survival in both arms. We will also determine the impact of ERCC2 mutation on objective response and progression-free survival. Finally, we will explore the role of other somatic mutations on overall survival.

Recent data suggests that both immunotherapy (e.g. MPDL3280a)(20) and targeted agents (e.g. anti-FGFR3)(21) may find wider use in advanced UC. Predictors of response to these agents (e.g. PD-L1 immunohistochemistry, FGFR3 mutation and fusion) are being explored, and ultimately patients may be selected for therapy based on the presence of a molecular biomarker. Similarly, the emerging evidence cited above suggests that identification of patients who will derive maximum benefit from cisplatin-based chemotherapy will allow rational selection of patients for this potentially curative therapy. If somatic ERCC2 mutation predicts overall survival with cisplatin-based combination chemotherapy, then “standard” cisplatin-based therapy may become a biomarker-driven therapy, and patients who may not derive significant benefit may be directed towards other therapeutic approaches which appear to hold promise.

10.3.3 Objectives

Primary objective

Determine whether somatic ERCC2 mutation predicts overall survival in patients treated with cisplatin-based therapies.

Secondary objectives

Determine whether ERCC2 somatic mutation is a predictive biomarker of objective response and progression-free survival

Exploratory objectives

Determine whether somatic mutations in other cancer-related genes predict overall survival, progression-free survival, and objective response to cisplatin-based chemotherapy. .

10.3.4 Methods

DNA will be extracted from both germline blood specimens and tumor blocks. IMPACT sequencing will be performed at MSKCC. Sequence reads will be aligned using the Burrows-Wheeler Aligner and post-processed using the Genome Analysis Toolkit following standard best practices.(22, 23) Variants, sequence coverage, and copy number alterations in tumor samples will be determined using additional algorithms developed at the Broad Institute.(23) Tumor and matching germline DNA are analyzed simultaneously. In over 1000 tumor-normal pairs, IMPACT has achieved >250x coverage of >98% of targeted exons. A further analysis performed in matched frozen and FFPE samples found a ≥ 97% concordance with no excess of false positives within FFPE material.(24) The exon capture approach requires as little as 15ng input DNA allowing analysis of metastatic fine needle and core biopsy specimens. High coverage depth allows mutant allele detection even in the setting of stromal admixture. Optimization ensures complete coverage of key genes compared to commercial whole exome capture. Currently, IMPACT gene list includes ERCC2 and many additional DNA-damage response genes. If superior sequencing technology is available at the time of analysis that provides similar or improved information, it may be substituted for IMPACT sequencing.

10.3.5 Power Computation and Data Analysis

The primary endpoint for this CS analysis is overall survival. It is expected that 80% of patients enrolled on this study to have germline and tumor DNA available. Based on muscle invasive bladder TCGA cohort, the prevalence of ERCC2 is expected to be 12%. Since this represents a metastatic cohort, it is not certain what the prevalence will be in this group of patients. The null hypothesis is that the hazard ratio=1 for survival. Table 1 provides the detectable hazard ratio assuming a two-sided type I error rate of 0.05, power of 0.80, event rates of 0.89, and ERCC2 somatic mutation prevalence of 0.05-0.15. The power computations are based on the assumption that the OS endpoint follows an exponential distribution and 400 patients have available DNA.

Table 1. Detectable hazard ratio under a range of conditions and assuming a two-sided type I error rate =0.05, and 0.80 power.

Prevalence of *ERCC2* somatic mutation	Hazard Ratio
0.05	1.98

0.10	1.64
0.12	1.58
0.15	1.52

In addition, power calculations are based on testing treatment by ERCC2 somatic mutation interaction is provided in Table 2. The null hypothesis is that there is no treatment-ECCR2 somatic mutation interaction. The hypothesized median OS time in the treatment trial are 13.8 and 18.68 months for the gemcitabine/cisplatin and the gemcitabine/cisplatin/bevacizumab arms, respectively. No discrepancy in OS distribution is expected for the gemcitabine and cisplatin arm (i.e the hazard rate =0.0502 in both the mutated and unmutated groups). It is assumed that the OS distribution follows an exponential distribution, an accrual rate of 400 patients over 36-months period, and 36 months post-accrual follow-up. Adequate power will be detected for only large interaction terms. Table 2 presents the power for testing the null hypothesis of no treatment effect by ERCC2 somatic mutation interaction using a two-sided type I error rate $\alpha = 0.05$ and assuming ERCC2 prevalence of 0.12. Let $\Delta 1$ and $\Delta 2$ be the hazard ratios for treatment effect within ERCC2 mutated and wild-type patients, respectively.

Table 2.

Prevalence of ECCR2 Mutation	Δ_1	Δ_2	Power
0.12	1.0	2.5	0.81
	1.0	2.4	0.77
	1.0	2.3	0.73
	1.1	2.5	0.70
	1.1	2.4	0.67
	1.1	2.2	0.57

10.3.6 Data Analysis:

The proportional hazards (PH) model will be used for assessing the prognostic value of ERCC2 mutation in predicting PFS and OS adjusting for the stratification factors. Furthermore, the Kaplan-Meier method will be used to estimate PFS and OS by ERCC2 mutation status. In addition, the PH model will be used to assess the predictive importance of ERCC2 status in predicting PFS and OS adjusting on treatment arm, ECCR2 status and treatment-ERCC2 interaction with and without the stratification factors. The chi-square test will be used to compare the two mutations groups by the complete response status. The Logistic regression model will be utilized to assess the prognostic important of the ERCC2 mutation in predicting the probability of having a complete response, adjusting for treatment arm, and stratification factors. Estimates of the hazards ratios for PFS and OS or Odds Ratio of CR will be presented separately within each treatment arm if there is a suggestion that there is an ERCC2 mutation-treatment arm interaction.

Additionally, IMPACT will identify co-mutation patterns in metastatic UC, as the genotype of metastatic urothelial carcinoma has not been thoroughly investigated. Their impact on outcomes (OS, ORR, PFS) will be evaluated as above purely in an exploratory hypothesis generating fashion. Finally, additional genes beyond those in the IMPACT

panel may be tested if new genes are identified that may be relevant to urothelial carcinoma biology by spiking in a customized bait panel.

10.4 Pharmacogenomic companion study

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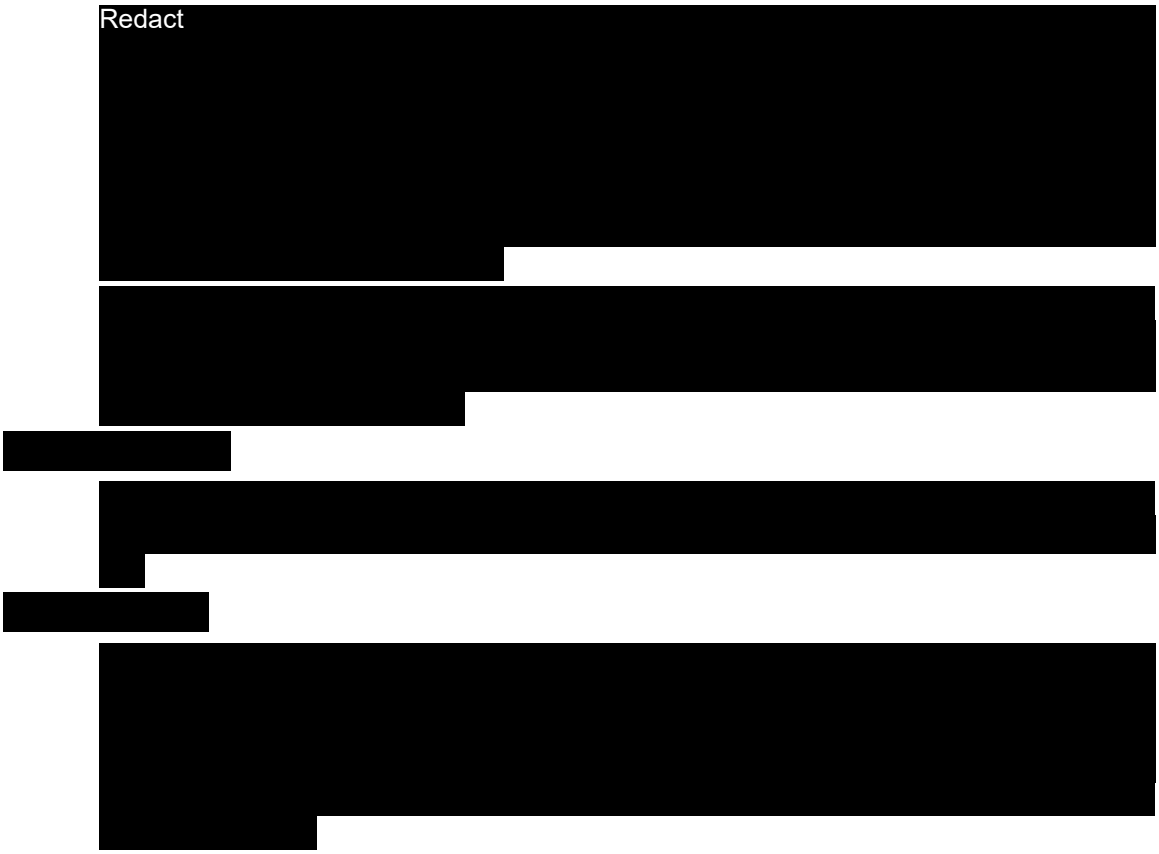


10.5 Evaluation of the Relationship between Intrinsic Subtype Membership and Treatment Response

10.5.1 Background

Bladder cancers are clinically heterogeneous with different patterns of progression and response to conventional and targeted therapies. Several recent, large-scale genomics projects were completed that provided new insight into potential molecular mechanisms that control this heterogeneity. Whole genome mRNA expression profiling revealed that muscle invasive bladder cancers (MIBCs) can be grouped into intrinsic basal and luminal subtypes that share similarities with the corresponding subtypes of breast cancer and display distinct clinical characteristics (201-204). Like their breast cancer counterparts, basal MIBCs were intrinsically aggressive – they were enriched with epithelial-to-mesenchymal (EMT) and stem cell biomarkers and were associated with advanced stage and metastatic disease at presentation and shorter disease-specific and overall survival in the absence of chemotherapy. However, a subset of basal MIBCs, characterized by enrichment with a gene expression signature indicative of T and B cell infiltration, were sensitive to neoadjuvant cisplatin-based combination chemotherapy (NAC) (202), and as a consequence, NAC produced the greatest clinical benefit in patients with basal tumors (205). Conversely, tumors that belonged to the "p53-like" luminal MIBC intrinsic subtype were resistant to NAC (202,205). These observations are consistent with parallel studies in breast cancer, where NAC produced the largest impact in patients with basal-like cancers and much less (if any) benefit in patients with luminal A cancers (206).

The gene expression patterns that characterize the intrinsic basal and luminal MIBC subtypes contain features that suggest that they will be sensitive to distinct panels of targeted agents (207). In addition to being enriched with immune checkpoint biomarkers,

basal MIBCs contain an active HIF-1α gene expression signature (202) and micro RNAs that are known to be direct targets of HIF-1 (A. Ochoa et al, manuscript under review). Therefore, it is possible that HIF-1 pathway-targeting agents, including inhibitors of vascular endothelial growth factor (VEGF) or its receptors, will have clinical activity in basal MIBCs. Consistent with this idea, patients with basal tumors who were enrolled in a recently completed Phase II clinical trial of dose-dense MVAC (DDMVAC) plus bevacizumab (Avastin) had unusually good clinical outcomes when compared with patients with basal tumors who were treated with neoadjuvant DDMVAC alone (205).

The MD Anderson SPORE in Bladder Cancer has established a Genomics Core, located in the Department of Urology, and led by Woonyoung Choi, PhD. The Core's priority has been to develop methods for generating high quality mRNA expression profiling data from archival FFPE tissues, focusing on macrodissected material from unstained slides. The Core has performed quality control experiments on two new RNAseq platforms (Illumina's TrueSeq RNA Access and Ion Torrent's Ampliseq) and has concluded that they both generate very high quality data. However, the Ampliseq platform requires less RNA (10 ng versus 20-100 ng for TrueSeq) and can produce high quality data from RNA that would fail the Illumina quality control standards (i.e., less than 30% of the RNA fragments are greater than 200 bp in length).

10.5.2 Rationale

Basal bladder cancers are enriched with an active HIF-1 gene expression signature, and patients with basal tumors had the best clinical outcomes in a completed Phase II clinical trial of DDMVAC plus Avastin. Therefore, we predict that therapy with GC plus Avastin produced more clinical benefit in the patients enrolled in Alliance 90601 whose tumors belonged to the basal intrinsic subtype than it did in patients with luminal tumors or in patients with basal tumors treated with GC alone. Conversely, we predict that the patients whose tumors belonged to the p53-like subtype had the worst clinical outcomes in both arms of the trial because of intrinsic chemoresistance and absence of the HIF-1 gene signature. Finally, we predict that tumor intrinsic subtype membership will correlate with the presence of specific DNA alterations identified in 10.3, consistent with previous studies in other tumor cohorts.

10.5.3 Primary objective

To determine whether patients with p53-like luminal tumors had the worst clinical outcomes (i.e., shorter PFS and/or OS).

10.5.4 Secondary objectives

To determine whether combination therapy with GC plus Avastin produced the most clinical benefit (i.e., longer PFS and/or OS) in patients with basal bladder cancers.

To determine whether patients treated with GC alone whose basal tumors contained an immune infiltration signature had better outcomes than did patients whose basal tumors did not.

To correlate intrinsic subtype membership with the presence of mutations and CNVs associated with chemo-sensitivity and/or resistance (in conjunction with 10.3)

To correlate intrinsic subtype membership with the presence of mutations and CNVs (i.e., in RB1, FGFR3, PPARG, RXR, FOXA1, GATA3, etc) that were enriched in basal or luminal tumors in previous studies

10.5.5 Methods

Ion Torrent's Ampliseq platform will be used to generate whole transcriptome RNA expression data from all available tumors. Total RNA will be purified from macrodissected 10 μm unstained slides (5 slides) using Roche HiPure FFPE miRNA Isolation kits (using the total RNA isolation method and a marked H&E-stained slide as a template. RNA purity and integrity will be measured using a Nanodrop instrument (Thermo Fisher) and a Bioanalyzer (Agilent Technologies, Inc), and the fraction of RNA fragments that are greater than 200 bp in length will be quantified. Ten nanograms of total RNA will be used for library and template preparation according to the manufacturer's instructions (Ion Torrent, Thermo Fisher) and will be sequenced on an Ion Proton sequencer. Normalized read count data will be generated using the AmpliSeq RNA plug-in in the Torrent Suite software package that is provided with the Ion Proton sequencer. These data will be used to assign the tumors to intrinsic subtypes using a one nearest neighbor (oneNN) classifier developed by Woonyoung Choi (202). Subtype assignments will be compared to those generated using an independent classifier (BASE47) developed by William Kim, MD, PhD (University of North Carolina). The RNAseq data will be uploaded to GEO and released to the public once the manuscript describing the results is has been accepted for publication. Residual total RNA will be returned to the Alliance tissue bank. Notably, changes to the methods for any of the above procedures may be adapted depending upon the most recent, generally accepted protocols.

Power Computation and Data Analysis

It is hypothesized that p-53 like basal bladder cancers have worst prognosis than patients without the basal signatures. The primary goal for this correlative science component is to test whether p-53 like subtype bladder patients have a worse prognosis than non-basal subtype. The primary endpoint for this analysis is overall survival. It is assumed that 33% (n=67) of the patients are expected to have p-53-like tumors and 67.7% of the patients would have either basal or luminal tumors. There are 200 patients with tumor specimen available and it is expected that 89% (178 events) of the patients would experience an event. With 178 deaths, the log-rank test has 80% power to detect a hazard ratio of 1.56 assuming a two-sided type I error rate of 0.05 and that OS follows an exponential distribution.

Data Analysis

The log-rank statistic will be used to test if the p-53 like tumors have worst OS compared to the non-basal tumors. In addition, the proportional hazards (PH) model will be used to estimate the treatment effect of bevacizumab in p53-like tumors, luminal and basal tumors. Furthermore, the Kaplan-Meier method will be used to estimate PFS and OS by the three subtypes. Finally, in an exploratory data the proportional hazards (PH) model will be used for testing for the intrinsic subtype (p53-like, luminal, basal) treatment arm interaction terms in models of PFS and OS

Finally, the data will be used to validate the classifiers that assign subtype developed by Choi (202), BASE47 developed by Damrauer (University of North Carolina) (203).

11.0 DRUG FORMULATION, AVAILABILITY AND PREPARATION

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with un preserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

The total administered dose of study drug may be rounded up or down within a range of 5% of the actual calculated dose. Drug dosages need not be changed unless the calculated dose changes by at least 10%.

11.1 Gemcitabine (2'deoxy-2',2'-difluorocytidine; dFDC; difluorodeoxycytidine; gemcitabine hydrochloride; Gemzar®)

Please refer to the FDA-approved package insert for gemcitabine for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Gemcitabine is a nucleoside analogue in the pyrimidine antimetabolite class which is S-phase specific. Its phosphorylated product is incorporated into DNA and interferes with DNA synthesis. Gemcitabine also exhibits self-potentiation by causing an enzymatically-mediated reduction in the intracellular nucleotide pool.

Availability

Gemcitabine is commercially supplied as a powder for reconstitution in 200 and 1 gram vials.

Storage and Stability

Intact vials containing sterile powder are stored at room temperature. When prepared as directed, reconstituted vials are reportedly stable for 35 days at room temperature and protected from light. Further diluted solutions of gemcitabine are stable for up to 7 days at room temperature when protected from light. However, the manufacturer recommends that solutions be used within 24 hours. The diluted solution should be clear and colorless to light straw-colored solution.

Preparation

Reconstitute the 200 mg vial with 5 mL 0.9% NaCl and the 1 g vial with 25 mL 0.9% NaCl. The resulting solution is approximately 38 mg/mL, but the concentration varies. It is suggested that when the desired dose is less than the entire vial, the entire volume be drawn up into a syringe in order to determine the actual concentration. Then the desired amount should be measured and diluted in 0.9% NaCl for infusion.

Administration

In this study, gemcitabine will be given intravenously over 30 minutes in an appropriate volume of 0.9% NaCl.

Toxicities

Common toxicities include a flu-like syndrome manifested by fever, fatigue, myalgias, headache, and cough. Myelosuppression is the usual dose limiting toxicity. Infusions longer than 1 hour as planned in this study are associated with increased myelosuppression. Mild elevations in hepatic transaminase levels occur in as many as two-thirds of patients but are reversible. Dyspnea occurs in 10-23% and is occasionally associated with a drug-induced pneumonitis. More often, dyspnea is likely associated with the underlying malignancy. Nausea, vomiting and anorexia are common, but usually of mild to moderate severity;

stomatitis and diarrhea or constipation occur less often. Proteinuria and hematuria are usually asymptomatic though frequent. A serious hemolytic-uremic syndrome is, however, rare (<1%). Paresthesias and peripheral neuropathies occur in 2-10%. Allergic reactions including bronchospasm occur infrequently (4%). Minimal alopecia (15%) and macular or maculaopapular rashes have also been reported.

11.2 Cisplatin

Please refer to the FDA-approved package insert for cisplatin for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Cisplatin is a platinum-containing heavy metal complex which acts as an alkylating agent. Cisplatin inhibits DNA synthesis by the formation of interstrand and intra-strand DNA crosslinkages, denaturation of the DNA double helix, and covalent binding to DNA bases.

Availability

Cisplatin is commercially available as a 1 mg/mL concentration aqueous injection in multidose vials of 50 mL, 100 mL, and 200 mL.

Storage and stability

Intact vials should be stored at room temperature and be protected from light. Solutions diluted in 0.9% or 0.45% NaCl to a concentration of 0.05-2mg/mL are stable for up to 72 hours at room temperature and protected from light.

Administration

Cisplatin is to be administered as an intravenous infusion according to institutional practice. Patients should receive intravenous hydration with at least 1 L of NaCl prior to cisplatin. Needles, syringes, catheters, or IV administration sets containing aluminum parts should not be used, as contact with cisplatin yields a black precipitate.

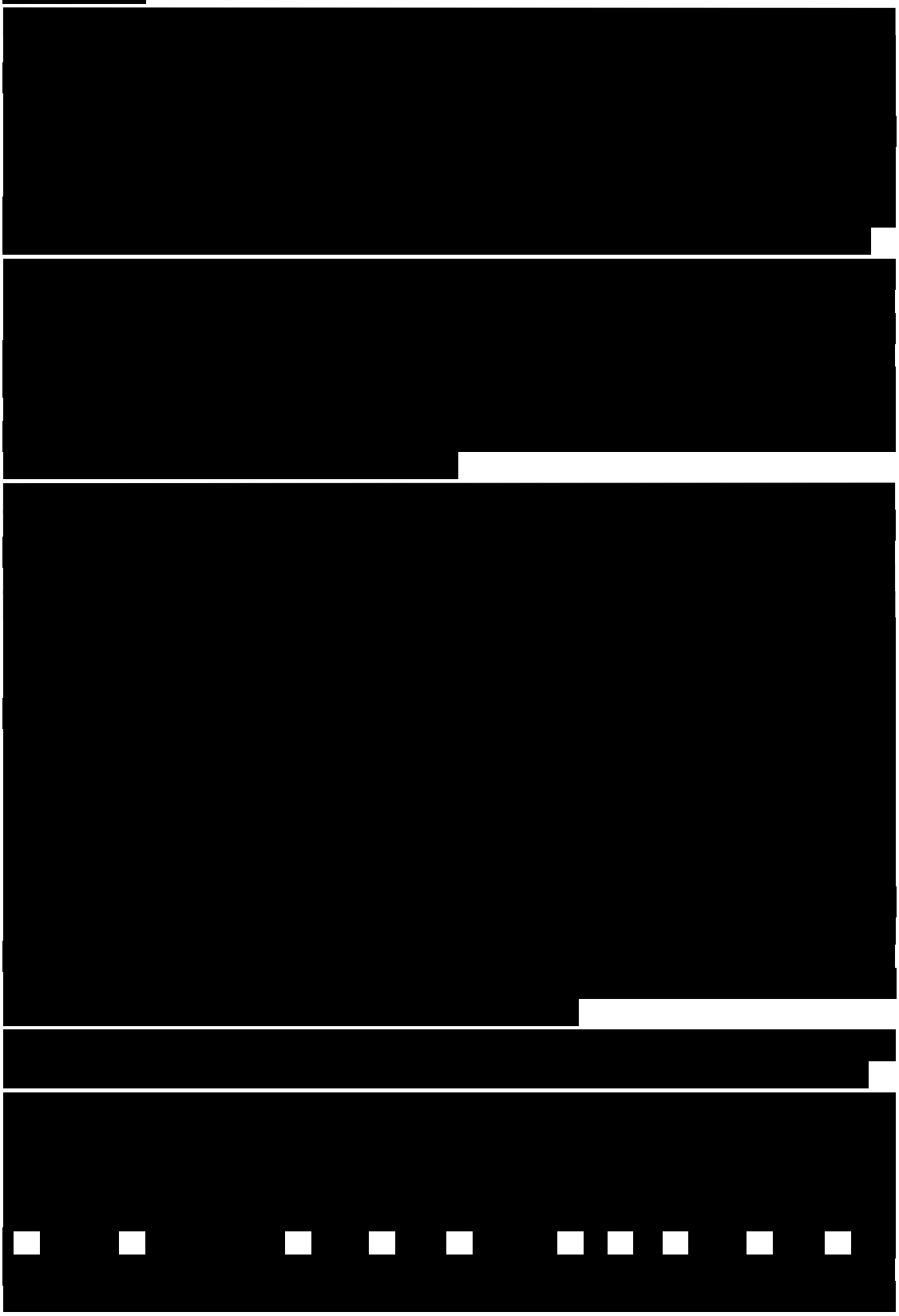
Toxicity

Common toxicities (> 10%) include nausea and vomiting which can occur within 24 hours and/or may be delayed up to 1 week following cisplatin, and are almost universal in the absence of effective antiemetic agents. Renal impairment is also not uncommon, but can be minimized with aggressive hydration. In addition to increases in BUN and creatinine, renal tubular damage leads to renal sodium, water, magnesium and potassium wasting, causing hypovolemia, hypomagnesemia and hypokalemia. Nephrotoxicity is worse in the presence of an obstructed urinary tract. Ototoxicity occurs in 10-30% and mainly consists of high frequency hearing loss (above the range of speech tones), which is largely irreversible. Neurotoxicity is a dose- and duration-dependent axonal degenerative process which is clinically manifested as irreversible peripheral neuropathy. Leukopenia and thrombocytopenia are mild and typically reverse in 3 weeks. A slowly progressive anemia is often noted with continued cisplatin therapy. Rarely, an anaphylactic reaction can be seen within a few minutes of administering cisplatin. Other rare toxicities (<1%) include mild alopecia, local phlebitis at the injection site, arrhythmias, optic neuritis and papilledema.

11.3 Redact


Redact

Redact



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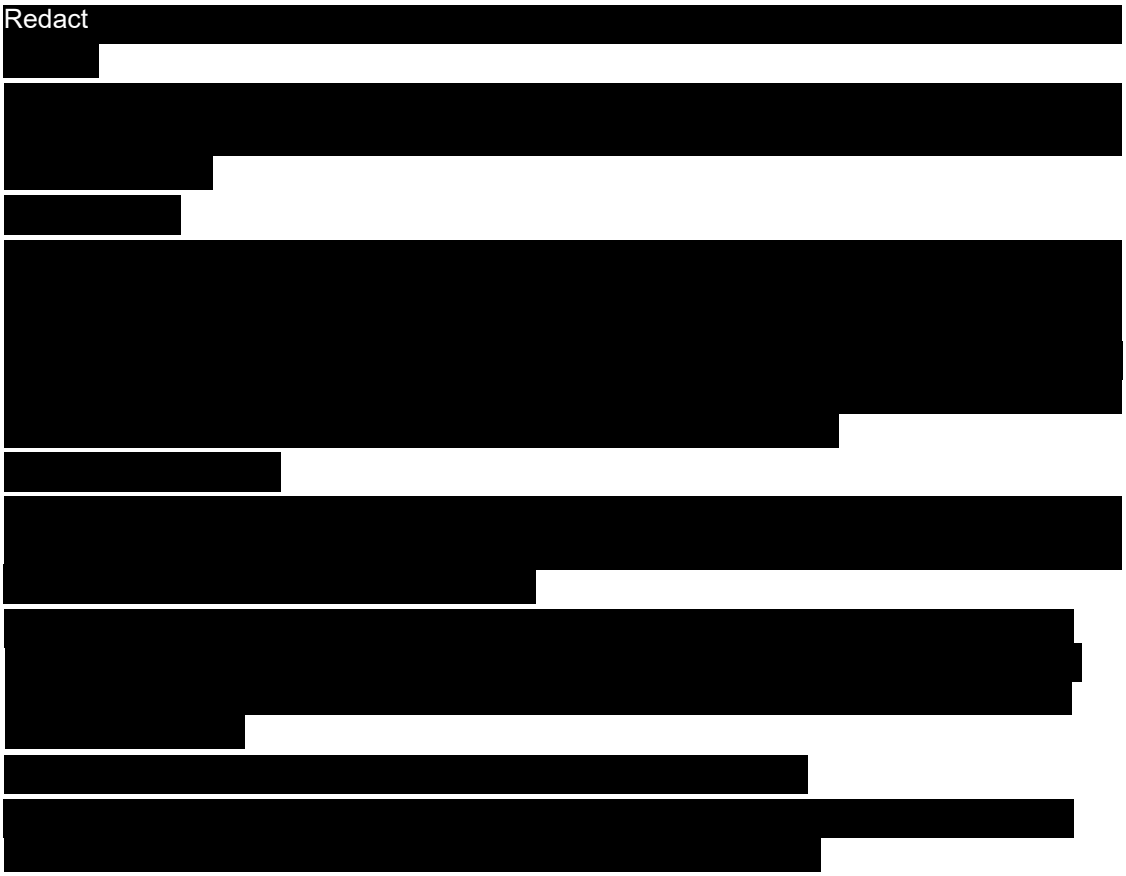
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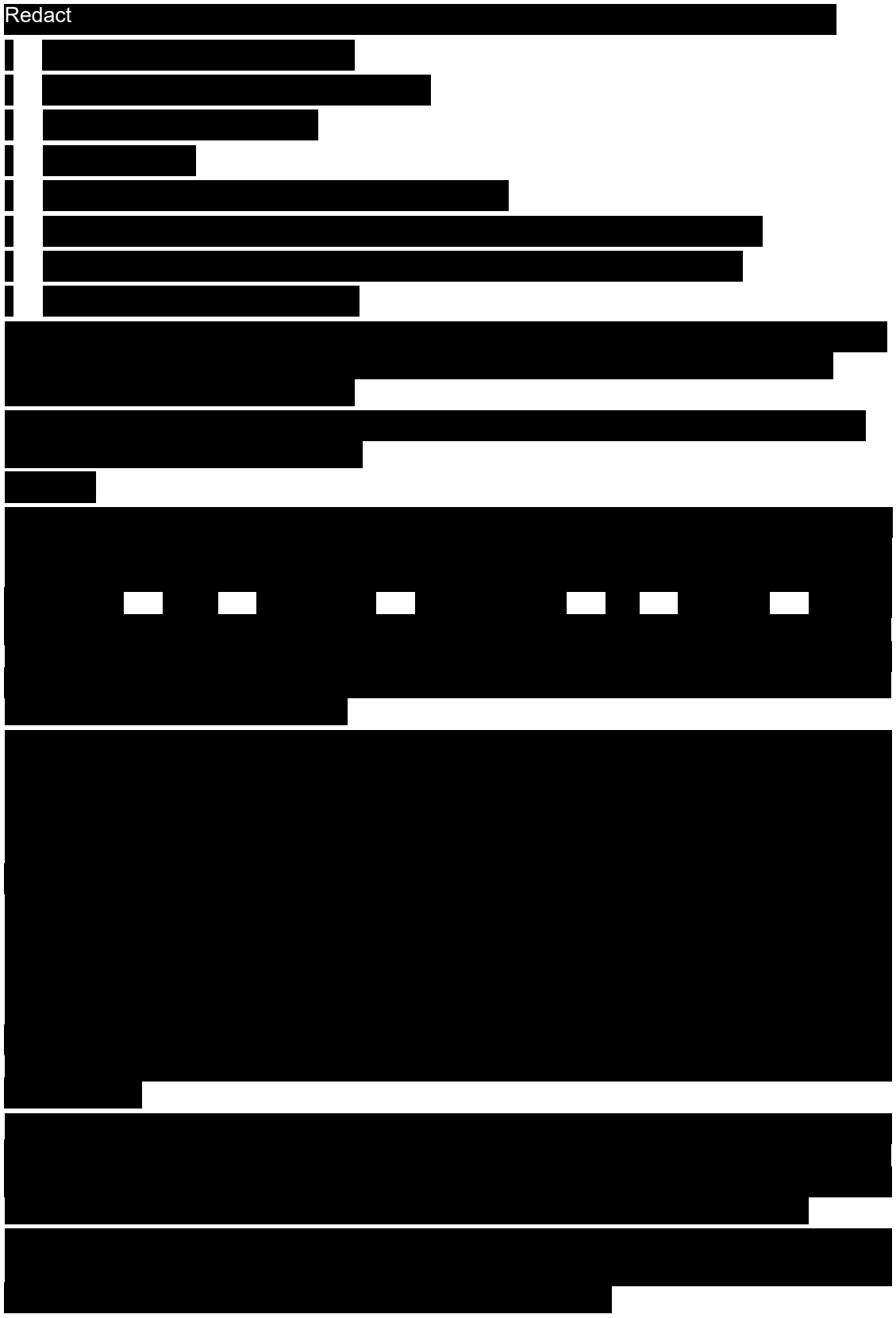
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12.0 ANCILLARY THERAPY

12.1 Supportive care

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on the CALGB Remarks Addenda (C-260).

12.2 Palliative radiation

Palliative radiation therapy may not be administered while the patient is on study treatment. A symptomatic lesion or one which may produce disability (e.g., unstable femur) may be irradiated before study initiation, provided other measurable or evaluable disease is present and radiation therapy is completed ≥ 4 weeks before start of therapy. All eligibility criteria for progression must still be met. Any other indications for radiotherapy after protocol treatment has begun will constitute disease progression, and the patient will stop protocol treatment (see **Section 13.1.3**).

12.3 CALGB 90601 Policy Concerning the Use of Growth Factors

12.3.1 Epoetin (EPO)

The use of erythropoietic stimulating agents (epoetin alfa and darbepoetin) is discouraged in this trial due to the risk of thrombotic events associated with these medications. If an investigator chooses to use these medications, the guidelines of the package insert and the warnings and limitations associated with those agents should be followed carefully.

12.3.2 Filgrastim (G-CSF), pegfilgrastim, and sargramostim (GM-CSF)

1. Filgrastim/pegfilgrastim and sargramostim should not be used to avoid dose reductions or delays.
2. Filgrastim, pegfilgrastim, or sargramostim, may be used for secondary prophylaxis following an episode of febrile neutropenia, according to the ASCO guidelines.
3. If pegfilgrastim is used in CALGB 90601, it should be administered on Day 9 of the cycle.
4. For the treatment of febrile neutropenia, the use of CSF's should not be routinely instituted as an adjunct to appropriate antibiotic therapy. However, the use of CSF's may be indicated in patients who have prognostic factors that are predictive of clinical deterioration such as pneumonia, hypotension, multi-organ dysfunction (sepsis syndrome) or fungal infection, as per the ASCO guidelines. Investigators should therefore use their own discretion in using the CSF's in this setting. The use of CSF (filgrastim/pegfilgrastim or sargramostim) must be documented and reported on the CALGB C-260 Remarks Addenda
5. If filgrastim/pegfilgrastim or sargramostim are used, they must be obtained from commercial sources.

12.3.3 Oprelvekin (IL-11, Neumega®)

The use of oprelvekin for patients enrolled in this study is discouraged.

13.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE

For the purposes of this study, patients with measurable and non-measurable lesions should be reevaluated with appropriate imaging studies every 9 weeks (every three cycles) during chemotherapy and treatment with bevacizumab/placebo alone, then every 3 months until progression. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

While all areas of malignant disease will be monitored, patients will be categorized into having either measurable disease or non-measurable disease.

13.1 Measurable Disease/Target Lesions

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques (CT, MRI, x-ray) or as ≥ 10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

All measurable lesions (up to a maximum of 10) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions, including small lesions and bone metastasis, will be identified as non-target lesions. (See Section 13.2. for response criteria for non-target lesions.)

13.1.1 Complete Response (CR): Disappearance of all target lesions. Changes in tumor measurement must be confirmed by repeat studies (see Section 13.1.5).

13.1.2 Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD. Moreover, performance status must be stable or improved at the time that a PR is determined. Changes in tumor measurements must be confirmed by repeat studies (see Section 13.1.5).

13.1.3 Progression (PD) is defined if <u>any</u> of the criteria below are met:

- At least a 20% increase in the sum of the LDs of target lesions (taking as a reference the smallest sum LD recorded since the treatment started) or the appearance of one or more new lesions.
- Development of an indication for radiation therapy while on treatment.

13.1.4 Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as a reference the smallest sum LD since the treatment started.

13.1.5 Confirmation Measurement and Duration of Response for Measurable Disease/Target Lesions

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be at least 4 weeks after the criteria for response are first met. For this study, restaging is scheduled to take place every 9 weeks (every 3 cycles).

13.1.6 Duration of Overall Response for Measurable Disease/Target lesion

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

13.2 Non-target Lesions

All other lesions (or sites of disease) not included in the "target disease" definition should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent."

13.2.1 Complete Response (CR): Disappearance of all non-target lesions.

13.2.2 Non-complete response (non-CR)/Non-progression (non-PD): Persistence of one or more non-target lesion.

13.2.3 Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions.

13.3 Cytology and Histology

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

These techniques can be used to differentiate between PR and CR in rare cases (for example, 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.

13.4 Evaluation of Best Overall Response

The best overall 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). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Notes:

- 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" on the C-1764, CALGB 90601 Treatment Form under "other." Every effort should be made to document the objective progression even after discontinuation of treatment.
- **Early progression/Deaths**: Those patients who progress or die within 3 weeks of starting therapy as a result of an event unrelated to their tumor or to treatment (e.g. motor vehicle accident) will be deemed inevaluable.

- **Loss to Follow Up:** Patients in whom inadequate data on response or toxicity results from loss of contact with the patient, will be deemed inevaluable.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

14.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

14.1 Duration of Treatment

14.1.1 Complete Response: Patients may discontinue therapy after achievement of a complete response in all response categories at the discretion of the treating physician. Bevacizumab/placebo may be continued until disease progression or unacceptable toxicity. For patients who discontinue protocol treatment, all further treatment will be at the physician's discretion.

14.1.2 PR or SD: Gemcitabine and cisplatin treatment is not to exceed 6 cycles. Continue treatment at the highest tolerable dose until the appearance of disease progression or unacceptable toxicity per Section 9.0. Bevacizumab/placebo may be continued until disease progression or unacceptable toxicity.

14.1.3 Disease Progression: Patients should receive a minimum of three cycles of therapy. Patients that have disease progression after 3 cycles of therapy based on measurable disease (see Section 13.1.3) or non-measurable disease (see Section 13.2.2) should be removed from protocol therapy. All sites of disease progression should be recorded.

Patients will be followed for survival for up to 7 years after randomization. This study has been designed with no crossover permitted. Patient unblinding will occur only in cases of emergency (see Section 11.3).

In the case that a patient has rapid clinical disease progression at any time during protocol therapy, s/he may be removed from protocol treatment by the treating physician only after discussion with the study chair. Document details, including tumor measurements, on CALGB Form C-660. Patients will be followed for secondary malignancies and survival.

14.2 Extraordinary Medical Circumstances

If, at any time, the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy on the C-260 CALGB Remarks Addenda.
- Follow the patient for secondary malignancies, disease response, and survival for a minimum of 7 years following registration.

14.3 Crossover

There is no crossover in this study. After disease progression (or termination of protocol therapy), bevacizumab/placebo should be discontinued and will no longer be provided. Further treatment is at the discretion of the patient and the treating physician.

15.0 STATISTICAL CONSIDERATIONS

15.1 Endpoints

The primary endpoint is overall survival (OS). OS will be measured from date of randomization to date of death due to any cause.

Secondary endpoints will be progression-free survival (PFS), objective response rate (defined as confirmed complete and partial responses), and toxicity. PFS will be measured from the date of randomization to date of progression or death due to any cause, whichever occurs first. Progression and response will be defined using the RECIST criteria.

15.2 Stratification

Randomization will be stratified on a) the number of negative prognostic features (0, 1) based on the presence or absence of visceral metastases; and b) prior chemotherapy (no, yes).

15.3 Power Considerations

This is a randomized, double-blind phase III trial in which 500 patients will be randomized with equal probability to one of two possible treatment regimens: gemcitabine, cisplatin, and placebo or gemcitabine, cisplatin, and bevacizumab.

The following calculations assume a monthly accrual rate of almost 14 patients patients/month accrued over a 36-month period, and will be followed for 36-months after study closure and a two-sided alpha level of 0.05. Survival time is assumed to follow an exponential distribution. With 454 deaths, the power to detect a hazard ratio of 0.74 or a 26% decrease in hazard rate (equivalent to an increase in median OS from 13.8 months to about 18.63 months in the gemcitabine, cisplatin, and placebo treatment group or gemcitabine, cisplatin, and bevacizumab group, respectively) is 87%.

15.4 Interim Analysis

Efficacy (overall survival) analyses will be conducted on semiannual basis to coincide with the semiannual meetings of the Alliance Data and Safety Monitoring Board (DSMB). Under the alternative hypothesis, four hundred forty-five events (deaths) are expected at the end of the follow-up period. The first interim analysis for OS will be performed at about 28% of the full information (approximately 24 months after study activation). Other interim analyses will be performed at 41% of the full information (at approximately 30 months), at 55% (at about 36 months), at 68% of the total information (at about 42 months), at 78% (at about 48 months), at 86% (54 months), at 91% (at about 60 months) and at 100% (at about 72 months after study activation). To help insure complete data on which to base the interim analyses, institutions will be asked to submit survival status on their patients on a semi-annual basis.

A group sequential design by O'Brien and Fleming will be used to stop the trial early to reject the null hypothesis.[189] Assuming a one-sided type I error rate = 0.025 and the above percent information available at each look, the z-score boundaries for stopping for superiority for (OS) are: 3.96, 3.27, 2.83, 2.54, 2.37, 2.26, 2.20 and 2.10. The z-score boundaries for stopping for futility for OS under the alternative hypothesis at a fixed type I rate = 0.0025 are: –1.13, –0.78, –0.46, –0.20, 0.01, 0.13, 0.21 and 2.10. Should any boundary be crossed, accrual to the study

will be stopped. These rules have a negligible impact on the type I and II error rates of this trial.[190]

		Boundaries for Interim Analysis	
Interim Analysis	**Percent Information (number of deaths)**	**For Superiority**	**For Futility**
1	28% (125)	3.96	-1.13
2	41% (182)	3.27	-0.78
3	55% (244)	2.83	-0.46
4	68% (302)	2.54	-0.20
5	78% (347)	2.37	-0.012
6	86% (382)	2.26	0.13
7	91% (405)	2.20	0.21
8 (Final)	100% (445)	2.10	2.10

Table 2

In addition, this trial incorporates a phase II PFS-based decision rule. The phase II rule will be implemented as follows: after the first 115 PFS events are observed the proportional hazards model estimate of the PFS hazard ratio will be computed and reported to the Alliance DSMB. The analysis based on 115 PFS events will take place at approximately 18 months after study activation. This is halfway through the study accrual, assuming that the accrual rate is as projected. As a result, it is unlikely that the trial will be accrued before the interim PFS analysis. If the observed hazard ratio is less than 1.2 the DSMB should recommend closing the study. To help insure complete data on which to base the interim analysis on the PFS endpoint, events (progression) should be faxed to the Alliance Statistics and Data Center within one week of institution's assessment of the patient's event. Institutions with patients enrolled on this phase III study that do not submit PFS in a timely manner may be denied future registration to the study. Furthermore, the progression endpoint will be reviewed for completeness of data in the database at about 50% of accrual. The trial will remain open if the number of progression events is at 115. If the number of progression events is lower than the target of 115, the trial will be suspended to further accrual until the database is updated to permit the futility analysis to be performed.

15.5 Toxicity Monitoring

Monthly conference calls (including the Study Chair, Committee Chair, study statisticians, data coordinator, protocol coordinator, and Alliance Executive Officer) will be held to monitor the first 120 patients for 4 cycles for early stopping due to unacceptable treatment-related events. Unacceptable toxicity will be defined as: treatment-related death; grade 4 febrile neutropenia; any treatment-related irreversible grade 3 or 4 toxicity excluding nausea and vomiting (irreversible toxicity will be defined as grade 3 or greater toxicity that persists at grade 3 or higher for more than one cycle of treatment); any treatment-related grade 3 or higher arterial thrombotic event; grade 3 or higher CNS hemorrhage; treatment-related grade 3 or higher hemorrhage other than hematuria; or grade 3 or higher gastrointestinal perforation. Grade 3 uncomplicated deep venous thrombosis which does not require invasive or thrombolytic intervention will not be considered an irreversible adverse event. Institutions will be asked to submit electronically or fax toxicity forms after each cycle of treatment (every 21 days). Institutions with patients enrolled on this phase III study that do not submit toxicity in a timely manner may be denied future registration to the study.

Furthermore, the study will be monitored for deaths, particularly among the first 60 patients randomized to Arm B. If the observed proportion of treatment-related deaths exceeds 10% by at least one standard error, accrual will be immediately suspended to the trial.

It is assumed that the incidence of unacceptable toxicity in patients treated with gemcitabine and cisplatin arm is 18%. If at any scheduled time of analysis the lower boundary of a one-sided 90% confidence interval for the difference in unacceptable toxicity exceeds 10%, accrual to the trial will be immediately suspended. The trial will remained closed until the review of all toxicity data is completed and a decision is made about whether it is safe to resume accrual. This decision will be made by consensus of the study team, the Alliance DSMB and CTEP.

15.6 Data Analysis

An intent-to-treat approach will be used in this phase III study to analyze OS. Patients who withdraw consent or withdraw from the study due to toxicity will continue to be followed for survival, even if they begin another therapy. The Kaplan-Meier product-limit estimator will be used to estimate the OS, and PFS.[191] The stratified log-rank statistic will be the primary analysis to compare the two treatment arms on OS with the stratification factors: presence of visceral metastases (no, yes) and prior chemotherapy (no, yes).[192] In addition, the proportional hazards model will be used to assess the importance of the treatment arm adjusting on patient characteristics, stratification variables and other important covariates in predicting OS.[193]

PFS will be measured from the date of randomization to date of progression or death due to any cause, whichever occurs first. Progression will be defined using the RECIST criteria. Data for patients without disease progression or death at the time of analysis will be censored at the time of the last tumor assessment (or, if no tumor assessments were performed after the baseline visit, at the time of randomization plus 1 day). Data for patients who receive non-protocol-specified anti-cancer therapy prior to experiencing documented disease progression will also be censored at the time of the last tumor assessment prior to receiving the non-protocol-specified therapy. The primary analysis of PFS will be a two-sided stratified log-rank test comparing Arm A and Arm B. The stratification factors will consist of the two stratification factors used for patient randomization: prior nephrectomy (yes vs. no) and Motzer score (0 vs. 1–2 vs. 3+). Results from unstratified log-rank tests will also be provided. Kaplan-Meier methodology will be used to estimate median PFS for each treatment arm.

Furthermore, the Cochran-Mantel-Haenszel test will be used to compare the two arms on the proportion of patients who experience an objective response (defined as either a confirmed CR or a PR) adjusting on the stratification factors [presence of visceral disease (no, yes) and prior chemotherapy (no, yes)]. In addition, the Fisher exact test will be used to compare the two treatment arms on the proportion of patients with unacceptable treatment related grade 3 or higher toxicity.

15.7 Accrual and Follow-up

Based on previous data from patients enrolled on CALGB 90102, the projected accrual rate is 2.6 patients per month. However, the accrual is anticipated to be higher as the study would also be opened through the CTSU. Assuming an accrual rate of about 14 patients/month, accrual is expected to be completed in about 36 months within study activation. This rate is reasonable as we have obtained commitments to participate in this trial from ECOG, SWOG, and other institutions through the CTSU. All patients will be followed for a maximum period of 7 years after randomization.

Statistical considerations for correlative sciences studies

15.8 Serum and tissue-based biomarker studies

15.8.1 Power Computations

The target sample size for this study is 500 patients with metastatic transitional cell carcinoma. Power computations are computed based on the primary objectives (10.1.2 and 10.2.2) and are presented assuming that 80% (n = 400) of the samples will be available. Because we are testing two primary hypotheses (10.1.2 and 10.2.2), each hypothesis will be tested with a significance level of 0.025. The plasma levels will be dichotomized at the median level and patients will be classified as having either low (below or equal the median) or high (above the median) levels. With 400 samples, the log-rank statistic has 80% power to detect a HR=1.42. The power computations are based on the following assumptions: the survival distribution time follows an exponential distribution, an accrual rate of about 12 patients/month, 36-months accrual period, 36 months post-accrual follow-up, a two-sided significance level of 0.025 and the median survival time among patients with high VEGF levels is 13.8 months.

In addition, power computations for testing treatment by marker interaction are provided in the Table 3, below. Adequate power will be detected for only very large interactions terms. The table below presents the power for testing the null of no treatment by marker interaction using a two-sided level of significance of $\alpha = 0.05$ and assuming positive prevalence of 0.30 and 0.50 in the markers. The assumed median OS for is 13.8 for the gemcitabine and cisplatin only arm. No discrepancy in OS distributions of the markers is expected for this treatment arm. If there is a suggestion that there are treatment by marker interactions, then the estimates of HR and 95% CI will be presented separately within each treatment group.

Prevalence of Positive Marker	Δ_{12}	Δ_{22}	Power
0.50	1.1	2.2	0.90
	1.1	2.1	0.86
	1.1	2.0	0.80
	1.2	2.2	0.80
	1.2	2.1	0.74
	1.2	2.0	0.67
0.30	1.1	2.2	0.84
	1.1	2.1	0.79
	1.1	2.0	0.73
	1.2	2.35	0.82
	1.2	2.3	0.79
	1.2	2.2	0.74

Table 3

Quality Control: A sample of 40 (10% of 400) specimens will be used to assess the reproducibility of the VEGF plasma assays. Inter-batch and intra-batch variation (based on duplicate samples) will be estimated by splitting the 40 specimens into two batches. If the between correlation coefficient between the VEGF replicates is at least 0.8, then the assay will be considered "reproducible." However, if the correlation coefficient is less than 0.80, the study team will discuss how to proceed with these assays.

Recommendations may include modifying the assay, or training the laboratory personnel who are performing these assays. The reproducibility rates will be reported at the end of the study.

15.8.2 Data Analysis

The Kaplan-Meier product-limit method will be used to estimate the survival distribution by the plasma VEGF dichotomized at the median level. We will use the log-rank test statistic to compare the low and high VEGF levels. Furthermore, the proportional hazards regression model will be used to test if plasma VEGF levels are prognostic factors of overall survival adjusting for the baseline covariates and other known prognostic factors. Because of the multiplicity of analysis, we will use the Bonferroni correction to adjust on the type I error rate. A type I error rate of 0.025 will be used for the primary analyses based on survival time by plasma VEGF level dichotomized below the median. For all secondary data analysis, a type I error rate = 0.05 will be used.

15.8.3 TMA Evaluation

Potential biomarkers will be identified based on their associations with PFS or OS in both and in either arm of the trial controlling for the number of comparisons using the method of Jung et al.[194] In addition, the proportional hazard models will be used to test if certain markers will predict PFS or OS. Furthermore, the proportional hazards model will be used to explore if treatment arm, gene expression and gene expression-arm interaction terms, adjusting for stratification variables and other important clinical variables, even though this study is not powered to detect except large interaction terms. Furthermore, the proportional hazards model will be used to explore if 118C/T and C8092A polymorphisms adjusting on treatment and polymorphisms-arm interaction will predict clinical outcomes (PFS and OS).

We plan to perform a secondary, subgroup analysis of the association between markers of DNA repair mechanisms and outcome in primary tumors and metastatic tumors to determine if there are large differences in the degree of association. The data will not be combined if these analyses show statistical differences of p-value < 0.10. Estimates of the hazard ratio and 95% CI for the hazard ratio will be reported within each group.

Unsupervised clustering will be used to identify clusters that may be associated with survival. The log-rank test will be used to quantify discrepancy in survival profiles among the clusters. In addition, we will use a method proposed by Li and Luan.[195]

For scoring of the majority of markers, including ERCC1, Rad51, RRMI, BRCA1, BRCA2, and caveolin-1, specimens will be graded from 0 to 3+ intensity representing the range from no staining to heavy staining, as previously reported (3-18). The overall percentage of cancer cells showing staining (0-100%) will also be reflected in the score as follows:

0: No reactivity

1+: Weak reactivity, with 0-10% of tumor cells showing positive staining.

2+: Moderate activity, with 10-50% of tumor cells showing positive staining.

3+: Strong reactivity, with > 50% of tumor cells showing positive staining.

All comparisons of staining intensities and percentages will be made at 200X magnification.

For Immunofluorescence of Rad51, each tumor will be scored as having the Rad51-containing nuclear foci absent (0) or present (1).

Furthermore, unconditional logistic regression model will used to test if markers will predict response and resistance to bevacizumab/cisplatin/ gemcitabine.

15.9 Statistical considerations for the evaluation of the effect of tobacco on bladder cancer outcomes

15.9.1 Endpoints

Primary: Overall survival (by smoking status, as determined by patient report). Overall survival was chosen as the primary endpoint rather than cancer-specific survival, despite the widely recognized deleterious effects of tobacco use on non-cancer related causes of death, such as cardiovascular disease, because of the difficulty in definitively establishing whether the cause of death is cancer-related.

Secondary: Correlation between smoking status by patient report and VEGF levels. Variables with possible effects on VEGF levels include obesity, renal disease, hypertension, diabetes, and cardiovascular disease.[196] In accordance, data will be collected on the following: height and weight (to determine BMI), estimated creatinine clearance using the Cockcroft-Gault equation, blood pressure, history of diabetes and cardiovascular disease, cardiovascular events during the course of the study, chronic infections, and infections during the course of the study.

Secondary: Association between patient-reported tobacco use and serum cotinine levels. Cotinine levels have been validated as an accurate method of assessing tobacco usage. To assess the accuracy of obtaining tobacco usage information by patient survey, the association between smoking status as determined by cotinine level and smoking status by patient report will be determined.

15.9.2 Power Computations

The primary objective is to determine if tobacco use is associated with overall-survival (OS). Overall survival is defined from the date of randomization until date of death due to any cause. The smoking prevalence in the US in men in 2007 was 23.9%. Smoking prevalence for the entire population (men and women) varies by major metropolitan area and is as low as 15% in California and as high as 23% in other cities with Alliance sites, such as Chicago and Raleigh-Durham.[197] The prevalence of current tobacco use in patients with bladder cancer in a case control study in Maine, New Hampshire, and Vermont was 32%, whereas the smoking prevalence for an unselected population in these states ranges from 18-20%.[198]

Tobacco use will be determined based on the cotinine levels among patients with available serum samples. A patient with serum cotinine levels greater than 3.08 ng/mL will be considered a tobacco user. We neither know the prevalence of tobacco users nor the number of available serum samples in patients who will be randomized to CALGB 90601. We anticipate that this correlative science component will be activated in the next year. We project that the remaining number of patients to be randomized to CALGB 90601 is 350. We anticipate that 86% of the 350 patients who will be randomized to CALGB 90601 will consent to provide serum based on our experience from other Alliance GU correlative sciences studies. Table 1 presents the minimum hazard ratio (HR) detectable assuming event (death) rates of 0.80-0.90, tobacco use prevalence of 0.15-0.35, a two-sided type I error rate of 0.05, 80% power for the log-rank statistic and the OS endpoint follows an exponential distribution.

	Event rate among 300 patients who will have consented to give serum		
Prevalence of tobacco use	**80%**	**85%**	**90%**
0.15	1.66	1.63	1.61
0.20	1.57	1.55	1.53
0.25	1.52	1.50	1.48
0.30	1.48	1.47	1.45
0.35	1.46	1.44	1.43

Table 4

Minimum detectable HR under a range of prevalence of tobacco use rates and death rates assuming a two-sided type I error rate = 0.05 and 80% power.

15.9.3 Data analysis

The Kaplan-Meier[191] product limit method and the log-rank statistic will be used to estimate and to test the OS distribution by tobacco use, respectively. Moreover, the proportional hazards model[193] will be used to assess the prognostic importance of tobacco use in predicting OS adjusting on treatment arm, baseline characteristics and stratification factors (presence of visceral metastases and prior chemotherapy). In addition, exploratory analyses will be performed where the incidence of infection, as well as other factors that can affect VEGF levels, such as, creatinine clearance, BMI, blood pressure, and the presence of cardiovascular disease will be considered as potential predictors of overall survival. Furthermore, the proportional hazards model will be used to test for treatment, tobacco use, and treatment by tobacco use interaction. Estimates of HR and 95% CI will be presented separately within each treatment arm if there is a suggestion that there is a treatment-tobacco use interaction. For objective 2, analysis of variance will be used to test differences in VEGF levels due to smoking if the cotinine levels follow a normal distribution. Otherwise, the Kruskal-Wallis rank sum statistic will be used to test for difference in VEGF levels by smoking. The kappa statistic to estimate the concordance between patient reported smoking status and tobacco use as measured by the serum cotinine level will be used.

15.10 Pharmacogenomic analysis

The primary objective of the pharmacogenetic portion of this study is the genotype (CT/TT versus CC) and treatment (GC versus GCB) interaction in predicting OS. This analysis will be carried out within the framework of a two-way multiplicative log-linear Cox model whose canonical hazard function can be presented as $\lambda[z|t]= \lambda_0[t]\exp[\beta_1 Z_1+\beta_2 Z_2+\beta_{12} Z_1 Z_2]$ ($Z_1=0$ if CC/TT and 1 otherwise, $Z_2=0$ if GC and 1 otherwise).

For notational simplicity, we will denote individuals with CT or TT genotypes by group 1 and individuals with CC genotypes as group 2 and the two treatment arms by A (GC) and B (GCB). Out of the 500 patients accrued to the study, we expect to have about 400 (roughly 80%) available for pharmacogenomic sampling. The putative prevalence rates for groups 1 and 2 are $p_1 = 0.29$ and $p_2 = 0.71$ respectively (Krippl et al, 2003). The expected cell counts are provided in Table 5.

n_{A1}	n_{A2}	n_{B1}	n_{B2}
58	142	58	142

Table 5

The power of the Wald test for testing the hypothesis H_0: $\beta_{12}=0$, at a two-sided level of significance of $\alpha = 0.05$, is presented for a number of examples in Table 5 (results are based on 10000 simulations for each case). Needless to say, given the relatively small sample size, only very large interactions will be detectable with adequate power. The assumed/hypothesized median OS for the treatment arms are M_A=13.8 and M_B=18.68 months respectively. On arm A (GC) no discrepancy in the OS profiles of the genotypes is expected (i.e., $\lambda_{1A}= \lambda_{2A}$ = log[2]/13.8 assuming exponential survival). For group 1, Δ_{1B} denotes the improvement, compared to treatment A (GC), in survival (hazard ratio) for receiving treatment B (GCB).

λ_{1A}	λ_{2A}	Δ_{1B}	Δ_{2B}	**Power**
0.0502	0.0502	1.1	2.1	0.84
0.0502	0.0502	1.1	2.0	0.75
0.0502	0.0502	1.1	1.9	0.65
0.0502	0.0502	1.2	2.1	0.68
0.0502	0.0502	1.2	2.0	0.61
0.0502	0.0502	1.2	1.9	0.52

Table 6

Secondary Objectives: We will also look at the above questions in the context of other genotypes in additional candidate genes of putative importance. We will also carry other exploratory analyses based on other clinical endpoints (e.g., PFS, tumor response and toxicity). The association between genes implicated in gemcitabine metabolism, transporter and clinical phenotype (systemic toxicity, response, and survival) will be performed.

16.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Investigators are required to notify the Investigational Drug Branch, the Study Chair, and their Institutional Review Board if a patient has a reportable adverse event. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for serious AE reporting beginning April 1, 2018.

All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). All reactions determined to be "reportable" in an expedited manner must be reported using the CTEP Adverse Event Reporting System (CTEP-AERS), accessed via the CTEP website, https://eapps-ctep.nci.nih.gov/ctepaers.

In the rare occurrence when Internet connectivity is lost, an AE report may be submitted using CTEP's Adverse Event Expedited Report – Single Agent or Multiple Agent paper template (available at http://ctep.cancer.gov) and faxed to 301-230-0159. A 24-hour notification is to be made to CTEP by telephone at 301-897-7497, **only** when Internet connectivity is disrupted. Once Internet connectivity is restored, an AE report submitted on a paper template or a 24-hour

notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

The reporting of adverse events described in the table below is in addition to and does not supplant the reporting of adverse events on study-specific adverse event forms (see Section 6.1 for required forms).

Please note: Adverse event reporting on the CALGB 90601 study forms uses CTCAE version 3.0. However, adverse events that require reporting via CTEP-AERS utilize CTCAE version 5.0.

16.1 CALGB 90601 Reporting Requirements:

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: CTEP-AERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of Treatment

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unexpected	Expected	with Hospitali-zation	without Hospitali-zation	with Hospitali-zation	without Hospitali-zation	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hrs; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

•Expedited AE reporting timelines defined:

- "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
- "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

16.2 Additional instructions or exclusions

Additional instructions or exclusions from CTEP-AERS expedited reporting requirements for phase 2 and 3 trials utilizing an agent under a CTEP-IND:

- CALGB 90601 uses a drug under a CTEP IND. The reporting requirements for investigational agents under a CTEP IND should be followed for all agents (either treatment arm) in this trial.
- For the purposes of expedited adverse event reporting, the CAEPR (which includes expected adverse events) for bevacizumab may be found in Section 16.3, below. Expected adverse events for gemcitabine and cisplatin may be found in Sections 11.1 and 11.2, and the package inserts. Note: The ASAEL column of the CAEPR has been replaced with the specific protocol exceptions to expedited reporting (SPEER) list. This list now includes 'expected' severity grades in addition to event terms.
- A discussion of the adverse events associated with the agents used in this trial can be found in Section 11.0 (Drug Formulation, Availability and Preparation).
- Grade 3/4 myelosuppression and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- Grade 3/4 nausea or vomiting, or grade 3/4 neurotoxicity and hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- Grade 3/4 hematuria or hospitalization resulting from such do not require CTEP-AERS, but should be submitted as part of study results.
- When evaluating hypertension, consider the description of severity grade 3 relative to the last AE reporting period. That is, for "more than one drug or more intensive therapy than previously used," "previously" should be considered the last reporting period. A regimen more intensive than a previous reporting period need only prompt expedited reporting the first time that it is used. If BP is stable on the more intensive regimen, do not continue to report grade 3 HTN via CTEP-AERS.
- Death due to progressive disease should be reported as Grade 5 "Disease progression" in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.
- Secondary malignancy: A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

  CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. In CTCAE version 5.0, three options are available to describe the event:

  -Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])

  -Myelodysplastic syndrome (MDS)

  -Treatment-related secondary malignancy

  Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

- Second malignancy: A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.
- All adverse events reported via CTEP-AERS (i.e., serious adverse events) should also be forwarded to your local IRB.
- The reporting of adverse events described in the table above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see Section 6.1 for required forms).
- In CTCAE v5.0, pregnancy loss is defined as "Death in utero," and any pregnancy loss should be reported expeditiously as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC. A pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC as currently CTEP-AERS recognizes this event as a patient death.
- A neonatal death should be reported expeditiously as Grade 4, "Death neonatal" under the General Disorders and Administration SOC.

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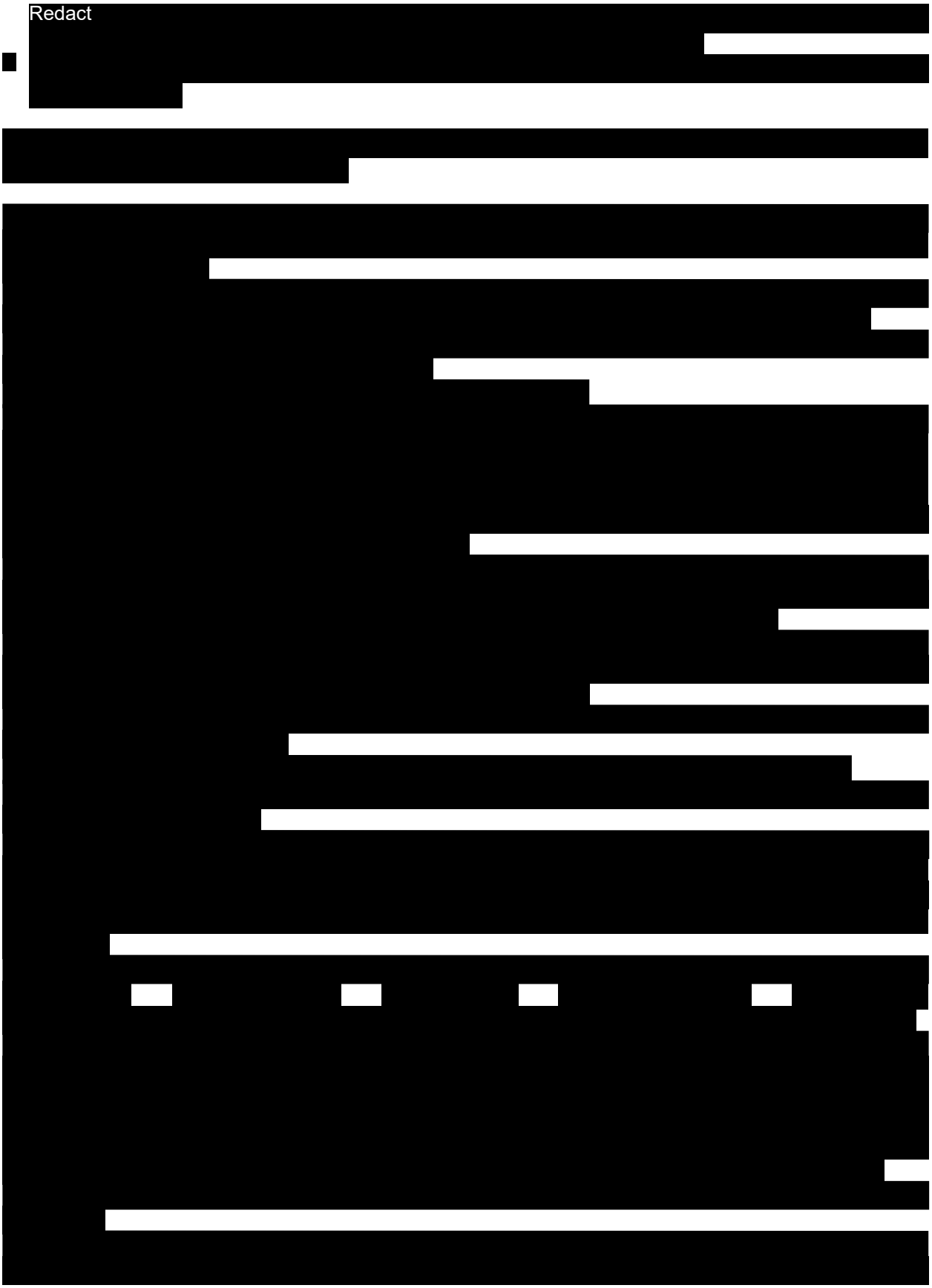

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17.0 REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer Statistics, 2007. CA Cancer J Clin 2007;57(1):43-66.
2. Cookson MS, Herr HW, Zhang ZF, Soloway S, Sogani PC, Fair WR. The treated natural history of high risk superficial bladder cancer: 15- year outcome. J Urol 1997;158(1):62-7.
3. von der Maase H, Sengelov L, Roberts JT, et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J Clin Oncol 2005;23(21):4602-8.
4. Von Der Maase H, Hansen SW, Roberts JT, et al. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 2000;18(17):3068-77.
5. Kunikane H, Kurita Y, Watanabe K, et al: A study of the combination of gemcitabine hydrochloride (LY188011) and cisplatin in non-small-cell lung cancer: 3-week schedule. Int J Clin Oncol 6:284-90, 2001
6. Rinaldi M, Crino L, Scagliotti GV, et al: A three-week schedule of gemcitabine-cisplatin in advanced non-small-cell lung cancer with two different cisplatin dose levels: A phase II randomized trial. Ann Oncol 11:1295-1300, 2000
7. Soto Parra H, Cavina R, Latteri F, et al: Three-week versus four-week schedule of cisplatin and gemcitabine: results of a randomized phase II study. Ann Oncol 13:1080-6, 2002
8. Als AB, Sengelov L, Von Der Maase H: Gemcitabine and cisplatin in locally advanced and metastatic bladder cancer; 3- or 4-week schedule? Acta Oncol 47:110-9, 2008
9. Soto Parra H, Cavina R, Latteri F, et al. Three-week versus four-week schedule of cisplatin and gemcitabine: results of a randomized phase II study. Ann Oncol 2002;13(7):1080-6.
10. Nowak AK, Byrne MJ, Williamson R, et al. A multicentre phase II study of cisplatin and gemcitabine for malignant mesothelioma. Br J Cancer 2002;87(5):491-6.
11. Crino L, Scagliotti GV, Ricci S, et al. Gemcitabine and cisplatin versus mitomycin, ifosfamide, and cisplatin in advanced non-small-cell lung cancer: A randomized phase III study of the Italian Lung Cancer Project. J Clin Oncol 1999;17(11):3522-30.
12. Moore MJ, Winquist EW, Murray N, et al. Gemcitabine Plus Cisplatin, an Active Regimen in Advanced Urothelial Cancer: A Phase II Trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 1999;17(9):2876-81.
13. Lorusso V, Manzione L, De Vita F, Antimi M, Selvaggi FP, De Lena M. Gemcitabine plus cisplatin for advanced transitional cell carcinoma of the urinary tract: a phase II multicenter trial. J Urol 2000;164(1):53-6.
14. Kaufman D, Raghavan D, Carducci M, et al. Phase II trial of gemcitabine plus cisplatin in patients with metastatic urothelial cancer. J Clin Oncol 2000;18(9):1921-7.
15. Sternberg CN, de Mulder PH, Schornagel JH, et al. Randomized phase III trial of high-dose-intensity methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) chemotherapy and recombinant human granulocyte colony-stimulating factor versus classic MVAC in advanced urothelial tract tumors: European Organization for Research and Treatment of Cancer Protocol no. 30924. J Clin Oncol 2001;19(10):2638-46.

16. Adamo V, Magno C, Spitaleri G, et al. Phase II study of gemcitabine and cisplatin in patients with advanced or metastatic bladder cancer: long-term follow-up of a 3-week regimen. Oncology 2005;69(5):391-8.

17. Dogliotti L, Carteni G, Siena S, et al. Gemcitabine plus Cisplatin versus Gemcitabine plus Carboplatin as First-Line Chemotherapy in Advanced Transitional Cell Carcinoma of the Urothelium: Results of a Randomized Phase 2 Trial. Eur Urol 2006.

18. Winquist E, Wilson JJ, Dorreen M, Wong R, Jonker D, Iscoe N. A 3-week gemcitabine-cisplatin regimen for metastatic urothelial cancer. Can J Urol 2004;11(6):2445-9.

19. Philp EA, Stephenson TJ, Reed MW. Prognostic significance of angiogenesis in transitional cell carcinoma of the human urinary bladder. Br J Urol 1996;77(3):352-7.

20. Dickinson AJ, Fox SB, Persad RA, Hollyer J, Sibley GN, Harris AL. Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinomas. Br J Urol 1994;74(6):762-6.

21. Jaeger TM, Weidner N, Chew K, et al. Tumor angiogenesis correlates with lymph node metastases in invasive bladder cancer. J Urol 1995;154(1):69-71.

22. Bochner BH, Cote RJ, Weidner N, et al. Angiogenesis in bladder cancer: relationship between microvessel density and tumor prognosis. J Natl Cancer Inst 1995;87(21):1603-12.

23. Chaudhary R, Bromley M, Clarke NW, et al. Prognostic relevance of micro-vessel density in cancer of the urinary bladder. Anticancer Res 1999;19(4C):3479-84.

24. Dinney CP, Bielenberg DR, Perrotte P, et al. Inhibition of basic fibroblast growth factor expression, angiogenesis, and growth of human bladder carcinoma in mice by systemic interferon-alpha administration. Cancer Res 1998;58(4):808-14.

25. Tanaka Y, Kawamata H, Fujimoto K, Oyasu R. Angiogenesis inhibitor TNP-470 suppresses tumorigenesis in rat urinary bladder. J Urol 1997;157(2):683-8.

26. Campbell SC, Volpert OV, Ivanovich M, Bouck NP. Molecular mediators of angiogenesis in bladder cancer. Cancer Res 1998;58(6):1298-304.

27. O'Brien T, Cranston D, Fuggle S, Bicknell R, Harris AL. Different angiogenic pathways characterize superficial and invasive bladder cancer. Cancer Res 1995;55(3):510-3.

28. Crew JP, Fuggle S, Bicknell R, Cranston DW, de Benedetti A, Harris AL. Eukaryotic initiation factor-4E in superficial and muscle invasive bladder cancer and its correlation with vascular endothelial growth factor expression and tumour progression. Br J Cancer 2000;82(1):161-6.

29. Yang CC, Chu KC, Yeh WM. The expression of vascular endothelial growth factor in transitional cell carcinoma of urinary bladder is correlated with cancer progression. Urol Oncol 2004;22(1):1-6.

30. Wu W, Shu X, Hovsepyan H, Mosteller RD, Broek D. VEGF receptor expression and signaling in human bladder tumors. Oncogene 2003;22(22):3361-70.

31. Bernardini S, Fauconnet S, Chabannes E, Henry PC, Adessi G, Bittard H. Serum levels of vascular endothelial growth factor as a prognostic factor in bladder cancer. J Urol 2001;166(4):1275-9.

32. Inoue K, Slaton JW, Karashima T, et al. The prognostic value of angiogenesis factor expression for predicting recurrence and metastasis of bladder cancer after neoadjuvant chemotherapy and radical cystectomy. Clin Cancer Res 2000;6(12):4866-73.

33. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146(5):1029-39.

34. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004;10(2):145-7.

35. Gutmann R, Leunig M, Feyh J, et al. Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. Cancer Res 1992;52(7):1993-5.

36. Boucher Y, Kirkwood JM, Opacic D, Desantis M, Jain RK. Interstitial hypertension in superficial metastatic melanomas in humans. Cancer Res 1991;51(24):6691-4.

37. Teicher BA, Holden SA, Ara G, et al. Influence of an anti-angiogenic treatment on 9L gliosarcoma: oxygenation and response to cytotoxic therapy. Int J Cancer 1995;61(5):732-7.

38. Sweeney CJ, Miller KD, Sissons SE, et al. The antiangiogenic property of docetaxel is synergistic with a recombinant humanized monoclonal antibody against vascular endothelial growth factor or 2-methoxyestradiol but antagonized by endothelial growth factors. Cancer Res 2001;61(8):3369-72.

39. Shishido T, Yasoshima T, Denno R, Mukaiya M, Sato N, Hirata K. Inhibition of liver metastasis of human pancreatic carcinoma by angiogenesis inhibitor TNP-470 in combination with cisplatin. Jpn J Cancer Res 1998;89(9):963-9.

40. Hu L, Hofmann J, Holash J, Yancopoulos GD, Sood AK, Jaffe RB. Vascular endothelial growth factor trap combined with paclitaxel strikingly inhibits tumor and ascites, prolonging survival in a human ovarian cancer model. Clin Cancer Res 2005;11(19 Pt 1):6966-71.

41. Sandler AB, Gray R, Brahmer J, et al. Randomized phase II/III trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC #704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): An Eastern Cooperative Oncology Group (ECOG) Trial - E4599. J Clin Oncol (Meeting Abstracts) 2005;23(16_suppl):LBA4-.

42. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for Metastatic Colorectal Cancer. N Engl J Med 2004;350(23):2335-42.

43. Giantonio BJ, Catalano PJ, Meropol NJ, et al. High-dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: Results from the Eastern Cooperative Oncology Group (ECOG) study E3200. J Clin Oncol (Meeting Abstracts) 2005;23(16_suppl):2-.

44. Miller KD, Wang M, Gralow J, et a. Gralow J, et al. E2100: A randomised phase III trial of paclitaxel versus paclitaxel plus bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer. . Presented at 2005 ASCO Annual Meeting.

45. Czaykowski PM, Moore MJ, Tannock IF. High risk of vascular events in patients with urothelial transitional cell carcinoma treated with cisplatin based chemotherapy. J Urol 1998;160(6 Pt 1):2021-4.

46. Reck M, von Pawel J, Zatloukal P, et al. Phase III Trial of Cisplatin Plus Gemcitabine With Either Placebo or Bevacizumab As First-Line Therapy for Nonsquamous Non-Small-Cell Lung Cancer: AVAiL. J Clin Oncol 2009:JCO.2007.14.5466.

47. Kindler HL, Karrison T, Lu C, et al. A multicenter, double-blind, placebo-controlled randomized phase II trial of gemcitabine/cisplatin (GC) plus bevacizumab (B) or placebo in patients (pts) with malignant mesothelioma (MM). J Clin Oncol (Meeting Abstracts) 2005;23(16_suppl):7019-.

48. Kim EJ, Jeong P, Quan C, et al. Genotypes of TNF-alpha, VEGF, hOGG1, GSTM1, and GSTT1: useful determinants for clinical outcome of bladder cancer. Urology 2005;65(1):70-5.

49. Krippl P, Langsenlehner U, Renner W, et al. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer 2003;106(4):468-71.

50. Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL. Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 2004;4(5):307-14.

51. Osato DH, Huang CC, Kawamoto M, et al. Functional characterization in yeast of genetic variants in the human equilibrative nucleoside transporter, ENT1. Pharmacogenetics 2003;13(5):297-301.

52. Yue L, Saikawa Y, Ota K, et al. A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 2003;13(1):29-38.

53. Chen YK, Lin LM. Evaluation of glutathione S-transferase activity in human buccal epithelial dysplasias and squamous cell carcinomas. Int J Oral Maxillofac Surg 1997;26(3):205-9.

54. Stoehlmacher J, Ghaderi V, Iobal S, et al. A polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. Anticancer Res 2001;21(4B):3075-9.

55. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18(1):4-25.

56. Bellmunt J, von der Maase H, Mead GM, et al. Randomized phase III study comparing paclitaxel/cisplatin/gemcitabine (PCG) and gemcitabine/cisplatin (GC) in patients with locally advanced (LA) or metastatic (M) urothelial cancer without prior systemic therapy; EORTC 30987/Intergroup Study. J Clin Oncol (Meeting Abstracts) 2007; 25: LBA5030-.

57. Bajorin DF, Dodd PM, Mazumdar M, et al. Long-term survival in metastatic transitional-cell carcinoma and prognostic factors predicting outcome of therapy. J Clin Oncol 1999;17(10):3173-81.

58. Evans WE, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. N Engl J Med 2003;348(6):538-49.

59. Nagasubramanian R, Innocenti F, Ratain MJ. Pharmacogenetics in cancer treatment. Annu Rev Med 2003;54:437-52.

63. Chodak, G.W., et al., Angiogenic activity as a marker of neoplastic and preneoplastic lesions of the human bladder. Ann Surg, 1980. 192(6): p. 762-71.

64. Chodak, G.W., C.J. Scheiner, and B.R. Zetter, Urine from patients with transitional-cell carcinoma stimulates migration of capillary endothelial cells. N Engl J Med, 1981. 305(15): p. 869-74.

65. Bochner, B.H., et al., Angiogenesis in bladder cancer: relationship between microvessel density and tumor prognosis. J Natl Cancer Inst, 1995. 87(21): p. 1603-12.

66. Dickinson, A.J., et al., Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinomas. Br J Urol, 1994. 74(6): p. 762-6.

67. Jaeger, T.M., et al., Tumor angiogenesis correlates with lymph node metastases in invasive bladder cancer. J Urol, 1995. 154(1): p. 69-71.

68. Crew, J.P., et al., Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. Cancer Res, 1997. 57(23): p. 5281-5.

69. Mizutani, Y., Y. Okada, and O. Yoshida, Expression of platelet-derived endothelial cell growth factor in bladder carcinoma. Cancer, 1997. 79(6): p. 1190-4.

70. O'Brien, T., et al., Two mechanisms of basic fibroblast growth factor-induced angiogenesis in bladder cancer. Cancer Res, 1997. 57(1): p. 136-40.

71. Grossfeld, G.D., et al., Thrombospondin-1 expression in bladder cancer: association with p53 alterations, tumor angiogenesis, and tumor progression. J Natl Cancer Inst, 1997. 89(3): p. 219-27.

72. George, D.J., et al., Prognostic significance of plasma vascular endothelial growth factor levels in patients with hormone-refractory prostate cancer treated on Cancer and Leukemia Group B 9480. Clin Cancer Res, 2001. 7(7): p. 1932-6.

73. Miyashita, M., et al., Serum levels of vascular endothelial growth factor, basic fibroblast growth factor and endostatin in human metastatic liver tumors. Hepatogastroenterology, 2003. 50(50): p. 308-9.

74. Verstovsek, S., et al., Plasma hepatocyte growth factor is a prognostic factor in patients with acute myeloid leukemia but not in patients with myelodysplastic syndrome. Leukemia, 2001. 15(8): p. 1165-70.

75. Maemura, M., et al., Serum concentration of hepatocyte growth factor in patients with metastatic breast cancer. Cancer Lett, 1998. 126(2): p. 215-20.

76. Chen, C.N., et al., The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. Cancer Lett, 2004. 213(1): p. 73-82.

77. Akbulut, H., et al., Prognostic role of serum vascular endothelial growth factor, basic fibroblast growth factor and nitric oxide in patients with colorectal carcinoma. Cytokine, 2002. 20(4): p. 184-90.

78. Beecken, W.D., et al., Reduced plasma levels of coagulation factors in relation to prostate cancer. Prostate, 2002. 53(2): p. 160-7.

79. Chao, Y., et al., Prognostic significance of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin in patients with resectable hepatocellular carcinoma after surgery. Ann Surg Oncol, 2003. 10(4): p. 355-62.

80. Miyashita, M., et al., Response to preoperative chemotherapy affects prognosis in esophageal cancer. Dis Esophagus, 2003. 16(2): p. 99-101.

81. Tamura, M. and Y. Ohta, Serum vascular endothelial growth factor-C level in patients with primary nonsmall cell lung carcinoma: a possible diagnostic tool for lymph node metastasis. Cancer, 2003. 98(6): p. 1217-22.

82. Tang, R.F., et al., Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. Pancreas, 2001. 22(3): p. 285-92.

83. Kummel, S., et al., Changes in the circulating plasma levels of VEGF and VEGF-D after adjuvant chemotherapy in patients with breast cancer and 1 to 3 positive lymph nodes. Anticancer Res, 2006. 26(2C): p. 1719-26.

84. Hanrahan, V., et al., The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. J Pathol, 2003. 200(2): p. 183-94.

85. Ahmad, S.A., et al., The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer. Cancer Res, 2001. 61(4): p. 1255-9.

86. Ellis, L.M., et al., Angiopoietins and their role in colon cancer angiogenesis. Oncology (Williston Park), 2002. 16(4 Suppl 3): p. 31-5.

87. Ebert, M., et al., Induction of platelet-derived growth factor A and B chains and over-expression of their receptors in human pancreatic cancer. Int J Cancer, 1995. 62(5): p. 529-35.

88. Myers, C., et al., Sustained expression of homeobox D10 inhibits angiogenesis. Am J Pathol, 2002. 161(6): p. 2099-109.

89. Kobayashi, M., et al., Localization of thymidine phosphorylase in advanced gastric and colorectal cancer. J Mol Histol, 2004. 35(1): p. 69-74.

90. Takahashi, Y., L.M. Ellis, and M. Mai, The angiogenic switch of human colon cancer occurs simultaneous to initiation of invasion. Oncol Rep, 2003. 10(1): p. 9-13.

91. Karna, E., et al., Serum and tissue level of insulin-like growth factor-I (IGF-I) and IGF-I binding proteins as an index of pancreatitis and pancreatic cancer. Int J Exp Pathol, 2002. 83(5): p. 239-45.

92. Zhang, L.J., et al., [Expression of placental growth factor and pigment epithelium-derived factor in non-small cell lung cancer and the prognostic significance thereof]. Zhonghua Yi Xue Za Zhi, 2005. 85(47): p. 3328-31.

93. Ren, J.G., C. Jie, and C. Talbot, How PEDF prevents angiogenesis: a hypothesized pathway. Med Hypotheses, 2005. 64(1): p. 74-8.

94. Karayiannakis, A.J., et al., Serum vascular endothelial growth factor levels in pancreatic cancer patients correlate with advanced and metastatic disease and poor prognosis. Cancer Lett, 2003. 194(1): p. 119-24.

95. Bok, R.A., et al., Vascular endothelial growth factor and basic fibroblast growth factor urine levels as predictors of outcome in hormone-refractory prostate cancer patients: a cancer and leukemia group B study. Cancer Res, 2001. 61(6): p. 2533-6.

96. Koshiba, T., et al., Detection of matrix metalloproteinase activity in human pancreatic cancer. Surg Today, 1997. 27(4): p. 302-4.

97. Koshiba, T., et al., Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. Cancer, 1998. 82(4): p. 642-50.

98. Senzaki, H., et al., Circulating matrix metalloproteinases and their inhibitors in patients with Kawasaki disease. Circulation, 2001. 104(8): p. 860-3.

99. McGuire, T.R., et al., Elevated transforming growth factor beta levels in the plasma of cytokine-treated cancer patients and normal allogeneic stem cell donors. Cytotherapy, 2001. 3(5): p. 361-4.

100. Friess, H., et al., Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology, 1993. 105(6): p. 1846-56.

101. Coppola, D., et al., Analysis of p53, p21WAF1, and TGF-beta1 in human ductal adenocarcinoma of the pancreas: TGF-beta1 protein expression predicts longer survival. Am J Clin Pathol, 1998. 110(1): p. 16-23.

102. von Bernstorff, W., et al., Systemic and local immunosuppression in pancreatic cancer patients. Clin Cancer Res, 2001. 7(3 Suppl): p. 925s-932s.

103. Chakraborty, G., et al., The multifaceted roles of osteopontin in cell signaling, tumor progression and angiogenesis. Curr Mol Med, 2006. 6(8): p. 819-30.

104. Straume, O. and L.A. Akslen, Expresson of vascular endothelial growth factor, its receptors (FLT-1, KDR) and TSP-1 related to microvessel density and patient outcome in vertical growth phase melanomas. Am J Pathol, 2001. 159(1): p. 223-35.

105. de Fraipont, F., et al., Thrombospondins and tumor angiogenesis. Trends Mol Med, 2001. 7(9): p. 401-7.

106. Kawakami, T., et al., Interleukin 10 expression is correlated with thrombospondin expression and decreased vascular involvement in colon cancer. Int J Oncol, 2001. 18(3): p. 487-91.

107. Fukai, Y., et al., Reduced expression of transforming growth factor-beta receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. Int J Cancer, 2003. 104(2): p. 161-6.

108. Sherief, M.H., et al., Matrix metalloproteinase activity in urine of patients with renal cell carcinoma leads to degradation of extracellular matrix proteins: possible use as a screening assay. J Urol, 2003. 169(4): p. 1530-4.

109. Monier, F., et al., Urinary release of 72 and 92 kDa gelatinases, TIMPs, N-GAL and conventional prognostic factors in urothelial carcinomas. Eur Urol, 2002. 42(4): p. 356-63.

110. Roy, R., et al., ADAM 12 cleaves extracellular matrix proteins and correlates with cancer status and stage. J Biol Chem, 2004. 279(49): p. 51323-30.

111. Li, A., M.L. Varney, and R.K. Singh, Constitutive expression of growth regulated oncogene (gro) in human colon carcinoma cells with different metastatic potential and its role in regulating their metastatic phenotype. Clin Exp Metastasis, 2004. 21(7): p. 571-9.

112. Salgado, R., et al., Arterio-venous gradients of IL-6, plasma and serum VEGF and D-dimers in human cancer. Br J Cancer, 2002. 87(12): p. 1437-44.

113. Salgado, R., et al., Platelet number and interleukin-6 correlate with VEGF but not with bFGF serum levels of advanced cancer patients. Br J Cancer, 1999. 80(5-6): p. 892-7.

114. Zaman, K., et al., Monitoring multiple angiogenesis-related molecules in the blood of cancer patients shows a correlation between VEGF-A and MMP-9 levels before treatment and divergent changes after surgical vs. conservative therapy. Int J Cancer, 2006. 118(3): p. 755-64.

115. Peter, K., et al., Circulating vascular cell adhesion molecule-1 correlates with the extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule-1, E-selectin, P-selectin, and thrombomodulin. Arterioscler Thromb Vasc Biol, 1997. 17(3): p. 505-12.

116. Kuenen, B.C., et al., Dose-finding and pharmacokinetic study of cisplatin, gemcitabine, and SU5416 in patients with solid tumors. J Clin Oncol, 2002. 20(6): p. 1657-67.

117. Orimo, A., et al., Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell, 2005. 121(3): p. 335-48.

118. Alexiou, D., et al., Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. Eur J Cancer, 2001. 37(18): p. 2392-7.

119. Alexiou, D., et al., Clinical significance of serum levels of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in gastric cancer patients. Am J Gastroenterol, 2003. 98(2): p. 478-85.

120. Salcedo, R., et al., Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood, 2000. 96(1): p. 34-40.

121. Kimura, T., et al., Clinical significance of MUC1 and E-cadherin expression, cellular proliferation, and angiogenesis at the deepest invasive portion of colorectal cancer. Int J Oncol, 2000. 16(1): p. 55-64.

122. Alexandrakis, M.G., et al., Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis. Am J Clin Oncol, 2003. 26(2): p. 135-40.

123. Wobser, M., et al., Complete remission of liver metastasis of pancreatic cancer under vaccination with a HLA-A2 restricted peptide derived from the universal tumor antigen survivin. Cancer Immunol Immunother, 2006. 55(10): p. 1294-8.

124. Hansen, S., et al., Independent prognostic value of angiogenesis and the level of plasminogen activator inhibitor type 1 in breast cancer patients. Br J Cancer, 2003. 88(1): p. 102-8.

125. Leik, C.E., et al., Effect of pharmacologic plasminogen activator inhibitor-1 inhibition on cell motility and tumor angiogenesis. J Thromb Haemost, 2006. 4(12): p. 2710-5.

126. Blackwell, K., et al., Circulating D-dimer levels are better predictors of overall survival and disease progression than carcinoembryonic antigen levels in patients with metastatic colorectal carcinoma. Cancer, 2004. 101(1): p. 77-82.

127. Garber, M.E., et al., Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci U S A, 2001. 98(24): p. 13784-9.

128. Kouloubinis, A., et al., ProANP and NT-proBNP levels to prospectively assess cardiac function in breast cancer patients treated with cardiotoxic chemotherapy. Int J Cardiol, 2007.

129. Kuropkat, C., et al., Tumor marker potential of serum matrix metalloproteinases in patients with head and neck cancer. Anticancer Res, 2002. 22(4): p. 2221-7.

130. Gasparini, G., et al., Angiogenic inhibitors: a new therapeutic strategy in oncology. Nat Clin Pract Oncol, 2005. 2(11): p. 562-77.

131. Hurwitz, H., et al., Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med, 2004. 350(23): p. 2335-42.

132. Kohne, C.H., et al., Clinical determinants of survival in patients with 5-fluorouracil-based treatment for metastatic colorectal cancer: results of a multivariate analysis of 3825 patients. Ann Oncol, 2002. 13(2): p. 308-17.

133. Olaussen, K.A., et al., DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med, 2006. 355(10): p. 983-91.

134. Edwards, S.L., et al., Resistance to therapy caused by intragenic deletion in BRCA2. Nature, 2008. 451(7182): p. 1111-5.

135. Sakai, W., et al., Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature, 2008. 451(7182): p. 1116-20.

136. Miyake, H., et al., Overexpression of Bcl-2 in bladder cancer cells inhibits apoptosis induced by cisplatin and adenoviral-mediated p53 gene transfer. Oncogene, 1998. 16(7): p. 933-43.

137. Cho, H.J., et al., Upregulation of Bcl-2 is associated with cisplatin-resistance via inhibition of Bax translocation in human bladder cancer cells. Cancer Lett, 2006. 237(1): p. 56-66.

138. Chresta, C.M., E.L. Arriola, and J.A. Hickman, Apoptosis and cancer chemotherapy. Behring Inst Mitt, 1996(97): p. 232-40.

139. Ono, Y., et al., Loss of p73 induction in a cisplatin-resistant bladder cancer cell line. Mol Urol, 2001. 5(1): p. 25-30.

140. Langowski, J.L., et al., IL-23 promotes tumour incidence and growth. Nature, 2006. 442(7101): p. 461-5.

141. Schuldes, H., et al., Cytostatic sensitivity and MDR in bladder carcinoma cells: implications for tumor therapy. Int J Clin Pharmacol Ther, 2000. 38(4): p. 204-8.

142. Ceppi, P., et al., ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. Ann Oncol, 2006. 17(12): p. 1818-25.

143. Livingston, D.M. and D.P. Silver, Crossing over to drug resistance. Nature, 2008. 451: p. 1066-67.

144. Bellmunt, J., et al., Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. Ann Oncol, 2007. 18(3): p. 522-8.

145. Rosell, R., et al., Gene expression as a predictive marker of outcome in stage IIB-IIIA-IIIB non-small cell lung cancer after induction gemcitabine-based chemotherapy followed by resectional surgery. Clin Cancer Res, 2004. 10(12 Pt 2): p. 4215s-4219s.

146. Ho, C.C., et al., Caveolin-1 expression is significantly associated with drug resistance and poor prognosis in advanced non-small cell lung cancer patients treated with gemcitabine-based chemotherapy. Lung Cancer, 2008. 59(1): p. 105-10.

147. Natrajan, R., et al., High-resolution deletion mapping of 15q13.2-q21.1 in transitional cell carcinoma of the bladder. Cancer Res, 2003. 63(22): p. 7657-62.

148. Yu, J., et al., A novel set of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer. Clin Cancer Res, 2007. 13(24): p. 7296-304.

149. Soderlund, K., et al., The BRCA1/BRCA2/Rad51 complex is a prognostic and predictive factor in early breast cancer. Radiother Oncol, 2007. 84(3): p. 242-51.

150. Fong, A., et al., Expression of caveolin-1 and caveolin-2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation. Am J Clin Pathol, 2003. 120(1): p. 93-100.

151. Liu, X., et al., Ribonucleotide reductase subunits M2 and p53R2 are potential biomarkers for metastasis of colon cancer. Clin Colorectal Cancer, 2007. 6(5): p. 374-81.

152. Budendorf, L., et al., Hormone therapy failure in human prostate cancer: Analysis by complementary DNA and tissue arrays. JNCI, 1999. 91: p. 1758-64.

153. Chen, C.D., et al., Molecular determinants of resistance to antiandrogen therapy. Nat Med, 2004. 10(1): p. 33-9.

154. Craft, N., et al., A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Med, 1999. 5(3): p. 280-5.

155. Ettinger, S.L., et al., Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence. Cancer Res, 2004. 64(6): p. 2212-21.

156. Garrido, C., et al., HSP27 and HSP70: potentially oncogenic apoptosis inhibitors. Cell Cycle, 2003. 2(6): p. 579-84.

157. Gimenez-Bonafe, P., et al., YB-1 is upregulated during prostate cancer tumor progression and increases P-glycoprotein activity. Prostate, 2004. 59(3): p. 337-49.

158. Kiyama, S., et al., Castration-induced increases in insulin-like growth factor-binding protein 2 promotes proliferation of androgen-independent human prostate LNCaP tumors. Cancer Res, 2003. 63(13): p. 3575-84.

159. Knudsen, B.S., et al., Regulation of hepatocyte activator inhibitor-1 expression by androgen and oncogenic transformation in the prostate. Am J Pathol, 2005. 167(1): p. 255-66.

160. Krueckl, S.L., et al., Increased insulin-like growth factor I receptor expression and signaling are components of androgen-independent progression in a lineage-derived prostate cancer progression model. Cancer Res, 2004. 64(23): p. 8620-9.

161. Liu, Y., et al., Inhibition of HER-2/neu kinase impairs androgen receptor recruitment to the androgen responsive enhancer. Cancer Res, 2005. 65(8): p. 3404-9.

162. Mohler, J.L., et al., Identification of differentially expressed genes associated with androgen-independent growth of prostate cancer. Prostate, 2002. 51(4): p. 247-55.

163. Rocchi, P., et al., Heat shock protein 27 increases after androgen ablation and plays a cytoprotective role in hormone-refractory prostate cancer. Cancer Res, 2004. 64(18): p. 6595-602.

164. Titus, M.A., et al., Steroid 5alpha-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res, 2005. 11(12): p. 4365-71.

165. Wang, S., et al., Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell, 2003. 4(3): p. 209-21.

166. Yamanaka, K., et al., A novel antisense oligonucleotide inhibiting several anti-apoptotic Bcl-2 family members induces apoptosis and enhances chemosensitivity in androgen-independent human prostate cancer PC3 cells. Mol Cancer Ther, In press.

167. Yang, L., et al., Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun, 2003. 305(3): p. 462-9.

168. Gritz, E.R., C. Dresler, and L. Sarna, Smoking, the missing drug interaction in clinical trials: ignoring the obvious. Cancer Epidemiol Biomarkers Prev, 2005. 14(10): p. 2287-93.

169. Brennan, P., et al., Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. Int J Cancer, 2000. 86(2): p. 289-94.

170. Bernardini, S., et al., Serum levels of vascular endothelial growth factor as a prognostic factor in bladder cancer. J Urol, 2001. 166(4): p. 1275-9.

171. Inoue, K., et al., The prognostic value of angiogenesis factor expression for predicting recurrence and metastasis of bladder cancer after neoadjuvant chemotherapy and radical cystectomy. Clin Cancer Res, 2000. 6(12): p. 4866-73.

172. Inoue, K., et al., Treatment of human metastatic transitional cell carcinoma of the bladder in a murine model with the anti-vascular endothelial growth factor receptor monoclonal antibody DC101 and paclitaxel. Clin Cancer Res, 2000. 6(7): p. 2635-43.

173. Hahn, N.M., et al., A multicenter phase II study of cisplatin (C), gemcitabine (G), and bevacizumab (B) as first-line chemotherapy for metastatic urothelial carcinoma (UC): Hoosier Oncology Group GU-0475. Proc. Am. Soc. Clin Oncol, 2009. 27(15s): p. Abstr 5018.

174. Roberts, J.T., et al., Long-term survival results of a randomized trial comparing gemcitabine/cisplatin and methotrexate/vinblastine/doxorubicin/cisplatin in patients with locally advanced and metastatic bladder cancer. Ann Oncol, 2006. 17 Suppl 5: p. v118-22.

175. Hanahan, D., Folkman J., Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell, 1996. 86(3): p. 353-64.

176. Heeschen, C., Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, et al., Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nature Medicine, 2001. 7(7): p. 833-9.

177. Heeschen, C., Weis M, Aicher A, Dimmeler S, Cooke JP., A novel angiogenic pathway mediated by non-neuronal nicotine acetylcholine receptors. J Clin Invest., 2002. 110(4): p. 527-36.

178. Heeschen, C., Weis M, Cooke JP, Nicotine promotes arteriogenesis. J Am Coll Cardiol., 2003. 41(3): p. 497-8.

179. Zhu, B., Heeschen C, Sievers RE, Karliner JS, Parmley WW, Glantz SA, Cooke JP., Second-hand smoke stimulates tumor angiogenesis and growth. Cancer Cell., 2003. 4(3): p. 191-6.

180. Hukkanen, J., Jacob P 3rd, Benowitz NL., Metabolism and disposition kinetics of nicotine. Pharmacol. Rev., 2005. 77(3): p. 159-69.

181. Matt, G.E., et al., Measuring secondhand smoke exposure in babies: the reliability and validity of mother reports in a sample of low-income families. Health Psychol, 2000. 19(3): p. 232-41.

182. Glasgow, R.E., et al., Developing a brief measure of smoking in the home: description and preliminary evaluation. Addict Behav, 1998. 23(4): p. 567-71.

183. Jefferis, B.J., et al., Changes in environmental tobacco smoke (ETS) exposure over a 20-year period: cross-sectional and longitudinal analyses. Addiction, 2009. 104(3): p. 496-503.

184. Benowitz, N., Jacob P 3rd., Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Oharmacol Ther., 1994. 56(5): p. 483-93.

185. Gritz, E., Carr CR, Rapkin D, Abemayor E, Chang LJ, Wong WK, et al., Predictors of long-term smoking cessation in head and neck cancer patients. Cancer Epidemiol Biomarkers Prev, 1993. 2(3): p. 261-70.

186. Benowitz, N.L., et al., Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol, 2009. 169(2): p. 236-48.

187. Determination of the Nicotine Metabolites Cotinine and Trans-3'-Hydroxycotinine in Biologic fluids of Smokers and Non-Smokers using Liquid Chromatography - Tandem Mass Spectrometry: Biomarkers for Tobacco Smoke Exposure and for Phenotyping Cytochrome P450 2A6 Activity. Jacob P 3rd, Yu L, Duan M , Ramos L, Benowitz NL, Manuscript in Preparation.

188. Bernert JT, Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/ atmospheric pressure ionization tandem mass spectrometry. Clin Chem 1997; 43:2281-2291.

189. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics 1979;35(3):549-56.

190. Freidlin B, Korn EL, George SL. Data monitoring committees and interim monitoring guidelines. Control Clin Trials 1999;20(5):395-407.

191. Kaplan EL, Meier P. Non-paramenteric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.

192. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Cancer 1977;35(1):1-39.

193. Cox DR. Regression models and life tables (with discussion). Journal of the Royal Statistical Society 1972;B74:187-220.

194. Jung SH, Owzar K, George SL. A multiple testing procedure to associate gene expression levels with survival. Stat Med 24(20):3077-88, 2005.

195. Li H, Luan Y. Kernel Cox regression models for linking gene expression profiles to censored survival data. Pac Symp Biocomput:65-76, 2003.

196. Stumpf, C., Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, et al., Elevated VEGF-plasma levels in young patients with mild essential hypertension. Eur J Clin Invest., 2009. 39(1): p. 31-6.

197. Kilmer, G., et al., Surveillance of certain health behaviors and conditions among states and selected local areas--Behavioral Risk Factor Surveillance System (BRFSS), United States, 2006. MMWR Surveill Summ, 2008. 57(7): p. 1-188.

198. Baris, D., et al., A case-control study of smoking and bladder cancer risk: emergent patterns over time. J Natl Cancer Inst, 2009. 101(22): p. 1553-61.

199. Hussain SA, Stocken DD, Riley P, Palmer DH, Peake DR, Geh JI, Spooner D, James ND: A phase I//II study of gemcitabine and fractionated cisplatin in an outpatient setting using a 21-day schedule in patients with advanced and metastatic bladder cancer. Br J Cancer 91:844-849, 2004

200. Hussain SA, Palmer DH, Swinson DE, Riley P, Wills A, Brown C, Draycott C, El-Modir A, Peake DR, Rea DW, Chetiyawardana AD, Cullen MH: A phase II clinical trial of gemcitabine and split dose cisplatin in advanced non-small cell lung cancer in an outpatient setting. Oncol Rep 20:233-7, 2008

201. Cancer Genome Atlas Research N. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014;507(7492):315-22

202. Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell. 2014;25(2):152-65.

203. Damrauer JS, Hoadley KA, Chism DD, Fan C, Tiganelli CJ, Wobker SE, et al. Intrinsic subtypes of high-grade bladder cancer reflect the hallmarks of breast cancer biology. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(8):3110-5

204. Rebouissou S, Bernard-Pierrot I, de Reynies A, Lepage ML, Krucker C, Chapeaublanc E, et al. EGFR as a potential therapeutic target for a subset of muscle-invasive bladder cancers presenting a basal-like phenotype. Science translational medicine. 2014;6(244):244ra91

205. McConkey DJ, Choi W, Shen Y, Lee IL, Porten S, Matin SF, et al. A Prognostic Gene Expression Signature in the Molecular Classification of Chemotherapy-naive Urothelial Cancer is Predictive of Clinical Outcomes from Neoadjuvant Chemotherapy: A Phase 2 Trial of Dose-dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin with Bevacizumab in Urothelial Cancer. European urology. 2015.

206. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast cancer research : BCR. 2010;12(5):R68.

207. McConkey DJ, Choi W, Ochoa A, Siefker-Radtke A, Czerniak B, Dinney CP. Therapeutic Opportunities in the Intrinsic Subtypes of Muscle-Invasive Bladder Cancer. Hematology/oncology clinics of North America. 2015;29(2):377-94.

APPENDIX I

Clinical Trials Agreement

The bevacizumab and placebo supplied by CTEP, DCTD, NCI used in this protocol are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between Genentech Inc. (hereinafter referred to as "Collaborator" and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (at http://ctep.cancer.gov/industry) contained within the terms of award, apply to the use of bevacizumab in this study:

1. Bevacizumab/placebo may not be used for any purpose outside the scope of this protocol, nor can it be transferred or licensed to any party not participating in the clinical study. Collaborator's data for bevacizumab are confidential and proprietary to Collaborator and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol in which there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):
    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for phase III studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for the clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group Office for immediate delivery to Collaborator for advisory review and comment prior to submission for publication. Collaborator will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator's intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release.

Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
NCI Shady Grove
Room 5W-520, MSC 9740
9609 Medical Ctr. Dr.
Bethesda, MD 20892-9740
FAX 240-276-7894
Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator. No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

APPENDIX II

UPC (Urine Protein to Creatinine) Ratio

The UPC (urine protein to creatinine) ratio directly correlates with the grams of protein found in a 24 hr urine. The UPC ratio can be used in place of a 24-hour urine.

Procedure for Obtaining a Urine Protein/Creatinine Ratio:

1. Obtain at least 4 mL of a random urine sample in a sterile container (does not have to be a 24-hour urine sample).
2. Determine protein concentration (mg/dL).
3. Determine creatinine concentration (mg/dL).
4. Divide #2 by #3 above:

UPC Ratio =

$$\frac{\text{Protein Concentration (mg/dL)}}{\text{Creatinine Concentration (mg/dL)}}$$