THE UNIVERSITY OF KANSAS
CANCER CENTER

Investigator Initiated Trial

IV vitamin C with chemotherapy for cisplatin ineligible bladder cancer patients: A forgotten group

SPONSOR - INVESTIGATOR

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Protocol Number:	IIT-2019-IVC-CarboGem
NCT Number:	NCT04046094
Study Drug(s):	Intravenous ascorbic acid/vitamin C (Investigational) Carboplatin (Standard of Care) – commercially available Gemcitabine (Standard of Care) – commercially available
Initial Version:	Version 1.0 - 05-29-2019
Amendments	Version 2.0 - 02-19-2020

LIST OF COLLABORATORS

John Taylor, III, MD, MS – Principal Investigator
Rahul Parikh, MD, PhD – Co-Investigator
Jeanne Drisko, MD – Co-Investigator
Ben Woolbright, PhD – Co-Investigator
Qi Chen, PhD – Co-Investigator
Jeffrey Thompson, PhD – Co-Investigator / Biostatistician
Katie Glavin – Coordinator
Ashley Shores – Project Director

STATEMENT OF COMPLIANCE / PROTOCOL AGREEMENT

The trial will be conducted in accordance with International Council on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the [specify NIH Institute or Center (IC) [Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the funding agency and documented approval from the Institutional Review Board (IRB), and the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, if applicable, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form(s) must be obtained before any participant is consented. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form(s) will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Protocol Number	**IIT-2019-IVC-CarboGem**
Protocol Title	IV vitamin C with chemotherapy for cisplatin ineligible bladder cancer patients: A forgotten group
Protocol Version and Date	**Version 1.0 - 05-29-2019**
Sponsor/Investigator Name	**John Taylor, III, MD, MS**
Site Number	001
Site Name	University of Kansas Cancer Center

Sponsor/Investigator Signature Date

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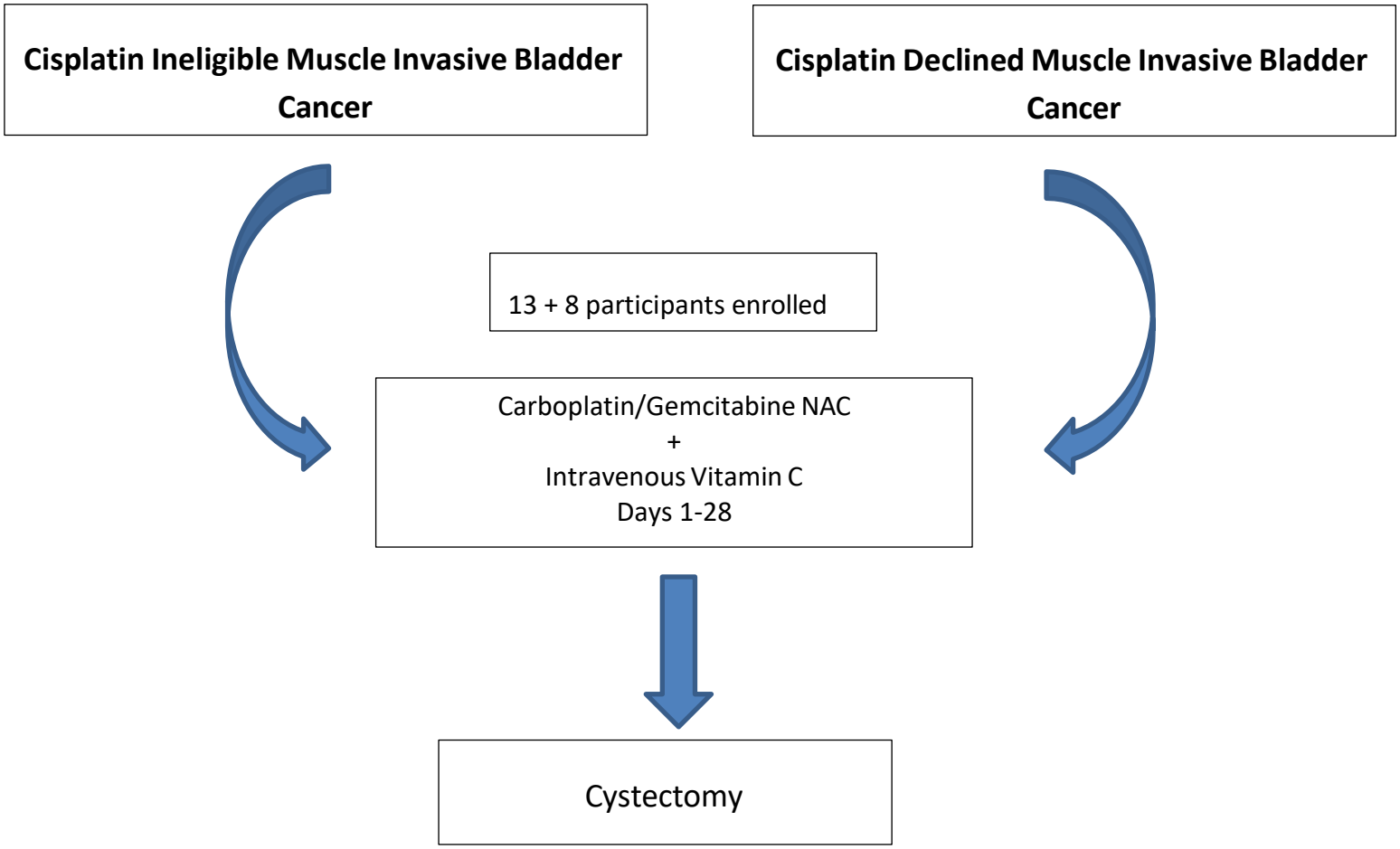
2 SCHEMATIC OF STUDY DESIGN



3 PROTOCOL SUMMARY

Title	IV vitamin C with chemotherapy for cisplatin ineligible bladder cancer patients: A forgotten group
Protocol Number	IIT-2019-IVC-CarboGem
Phase	Clinical study Phase 1b/2
Design	Single-arm, Simon 2-stage design, "window of opportunity" trial enrolling patients with cisplatin-ineligible MIBC and patients with MIBC who decline cisplatin-based NAC
Study Duration	2 years
Study Center(s)	Single-center
Objectives	**Primary:** To assess pathologic downstaging rate in MIBC cisplatin-ineligible patients when IVC is added to a gemcitabine/carboplatin NAC regimen. **Secondary:** Quality of life, DFS and DSS. **Exploratory:** Biomarkers for Cell death, IVC related biomarkers and cellular outcome biomarkers will be evaluated in tumor samples at initial resection and radical cystectomy. Adding IVC to a gemcitabine/carboplatin NAC regimen will significantly improve pathological downstaging in cisplatin-ineligible/declined MIBC patients undergoing cystectomy. We will also study molecular mediators for cellular outcome and ascorbate specific mechanism, and quality of life (QOL) to gather preliminary information for a larger multi-center, randomized, two-arm trial.

Number of Participants	13 – 21. Projected Accrual Rate: 1 or more per month. The Department of Urology performs more than 100 cystectomies per year of which approximately 30% will be cisplatin ineligible or will decline cisplatin-based NAC. We fully expect to recruit at least 1 participant per month to reach our first stage enrollment goal. Simon's two-stage minimax[34] will be used. Pathologic downstaging to non-invasive disease <pT2 is reported as approximately 15% with cystectomy alone. Therefore, the null hypothesis; that the true response rate of downstaging is 0.15, will be tested against a one-sided alternative. Typically, with effective NAC we might expect 30% of subjects to respond with downstaging to non-invasive disease. Therefore, we will use 0.30 as our desirable rate of response. In the first stage of the trial, 13 subjects will be accrued. If there are 1 or fewer responses in these 13 subjects, the study will be stopped. Otherwise, 8 additional subjects will be accrued for a total of 21.
Diagnosis and Main Inclusion Criteria	Patients with newly diagnosed cisplatin-ineligible MIBC or ones who decline cisplatin-based NAC
Study Product(s), Dose, Route, Regimen	Intravenous ascorbic acid/ vitamin C. A dose escalation regimen will be initiated at a single dose of 25 g, administered intravenously 2 to 3 times per week X 4 weeks.
Duration of Administration	4 weeks
Reference Therapy	None
Interim Monitoring	We will evaluate efficacy and toxicity after accrual of 13 subjects and completion of the stage 1 portion of the 2-stage design. We anticipate this will be approximately 6 – 12 months after study beginning. The study accrual and safety will be evaluated at monthly GU-DWG meetings. The DSMC of the KUCC is responsible for monitoring participant safety for this trial.

Statistical Methodology For Primary Objective	Outcome metrics will include pathologic downstaging rates at cystectomy, QOL measurements, DFS and DSS. Simon's two-stage minimax[34] will be used. Pathologic downstaging to non-invasive disease <pT2 is reported as approximately 15% with cystectomy alone. Therefore, the null hypothesis that the true response rate of downstaging is 0.15 will be tested against a one-sided alternative. Typically, with effective NAC we might expect 30% of subjects to respond with downstaging to non-invasive disease. Therefore, we will use 0.30 as our desirable rate of response. In the first stage of the trial, 13 subjects will be accrued. If there are 1 or fewer responses in these 13 subjects, the study will be stopped. Otherwise, 8 additional subjects will be accrued for a total of 21. The null hypothesis will be rejected if 5 or more responses are observed in 21 subjects, which we will take as evidence that this treatment should be studied further. This design yields a type I error rate of .21 and power of 79% when the true response rate is 0.30.
Correlative Studies / Sample Banking For Future Research	Samples to include tumor tissue, blood and urine will be collected at the time of entry into and during the study as well as at the time of surgery under the IRB approved KUMC Bladder Cancer Longitudinal Biorepository for Development of Novel Therapeutics/Biomarkers (PI: John Taylor III, MD, MS, IRB#: STUDY00141546, Appendix C).
Stopping Rules	If > 20% subjects have Grade 3 or 4 toxicity probably related or directly related to IV Vitamin C, we will hold the study for analysis of toxicity. Based on a Simon 2-Stage Design, toxicity and efficacy will be evaluated after the first 13 subjects are enrolled on this study, prior to enrolling additional subjects.

4 INTRODUCTION: BACKGROUND INFORMATION AND RATIONALE

Background Information

Bladder cancer (BCa) is the fifth most common solid tumor in the United States and has high rates of mortality in patients with muscle invasive disease (MIBC). Standard of care (SOC) treatment for MIBC is cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy, as NAC has been shown to both downstage tumors and improve recurrence free survival (RFS) and disease specific survival (DSS). Unfortunately, a large portion of MIBC patients are cisplatin ineligible and other treatment options are limited. Additionally, a small group of patients will decline SOC NAC due to concerns for adverse affects due to cisplatin (hearing loss, peripheral neuropathy, cardiotoxicity, renal toxicity). While an alternate regimen of gemcitabine and carboplatin has been used, it is only minimally effective and there remains no accepted SOC. New efficacious therapeutics are sorely needed for these patients.

Previous data from our group indicates intravenous Vitamin C (IVC) is an effective chemotherapeutic agent and yields superior pharmacokinetics with >100-fold increases in plasma concentrations . Studies in multiple cancer models have demonstrated that IVC is safe, synergistically improves carboplatin[1] and gemcitabine [2, 3] based chemotherapy, has mechanistic plausibility, and affects several cancer tissue markers. BCa is sensitive to high dose VC *in vitro* and *in vivo*. Based on results of prospective and retrospective studies, IVC may potentiate chemotherapeutic effects, improve quality of life, and reduce adverse events in patients receiving chemotherapy.

Study Agent(s) / Treatment(s)

1. *Recent studies reveal the pharmacokinetics of intravenous ascorbate/vitamin C (IVC) to be different than that of oral vitamin C.* Oral ascorbate had been shown non-effective in treating cancer patients. It is now recognized that with oral ascorbate, plasma concentrations are tightly controlled by mechanisms of intestinal absorption and renal excretion [16, 17] to a plateau of 70~80 µM. When ascorbate is administered IV, the "tight control" mechanisms are bypassed, and millimolar (mM) concentrations are achieved and maintained for hours until renal excretion reestablishes equilibrium. Peak ascorbate concentrations, as high as 25–30 mM, are safely achieved.[10, 14, 18] In patients with normal glucose-6-phosphate dehydrogenase (G6PD) activity and normal renal function, adverse events and toxicities are minimal even with IV doses as high as 1.5 g/kg, which is equivalent to 105 grams for a 70 kg person.[15, 18] These results motivated us to re-examine the role of ascorbate in cancer treatment.

2. *Mechanistic and translational studies support the use of IVC in cancer treatment.* At pharmacologic concentrations easily achievable with IV administration, ascorbate preferentially induced cytotoxicity to cancer cells versus normal cells.[8-13, 19-22] Our data in 48 cancer cell lines and 5 normal cells indicate that BCa is one of the most sensitive cancers to ascorbate treatment (**Fig. 1**).[10] Ascorbate (2-20 mM) at only 1-2 hours exposure, decreases survival in tested BCa cell lines, but does not induce death in human peripheral white blood cells, fibroblasts, and epithelial cells.[10] The ascorbate-induced cancer cell death is mediated by H_2O_2 as addition of catalase to the cell culture media completely protected cancer cells from ascorbate-induced cell death.[8-10]

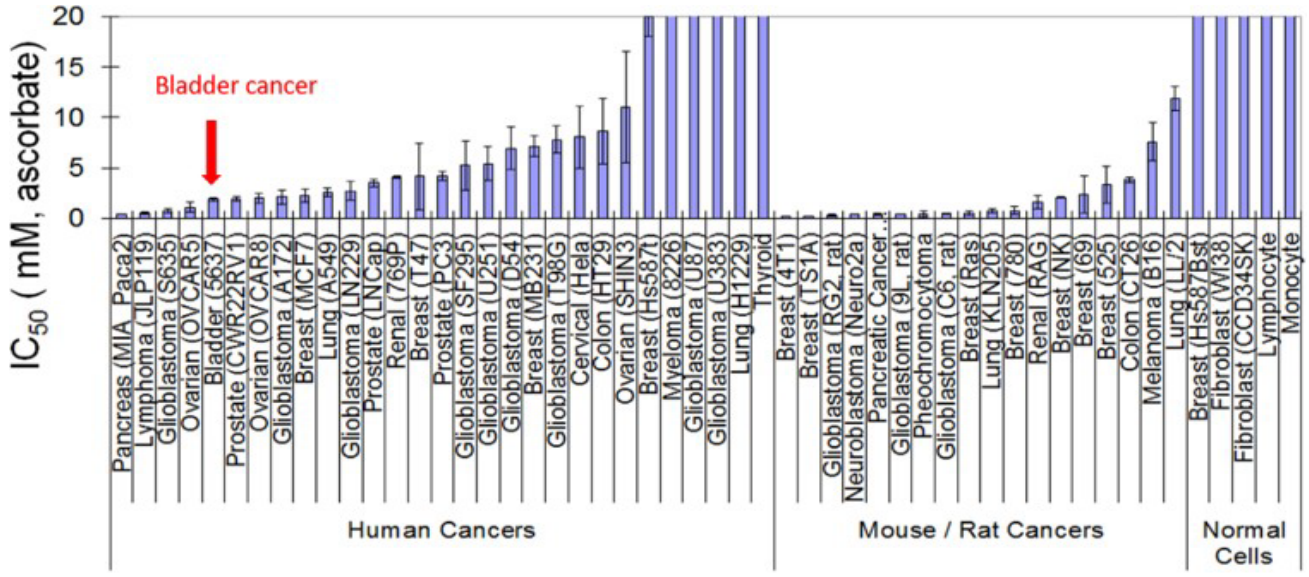


Fig 1. IC_{50}s of ascorbate in 48 cancer cell lines and 5 normal cells. Cells were exposed to 0-20 mM ascorbate for 1-2 h to mimic clinical conditions with IV use. Cell survival was evaluated at 24 h by MTT assay. (***Chen et al, PNAS 2005 and 2008)***

The chemistry is well-known for the formation of H_2O_2 from ascorbate in the presence of transition metals such as iron (Fe^{2+}).[23] To investigate whether the formation of H_2O_2 by ascorbate is physiologically relevant, we conducted *in vivo* studies in rodents. Rats and tumor bearing mice were given high doses of ascorbate (0.25-4 g/kg body weight) either by intravenous injection (IV), intraperitoneal injection (IP), or oral gavage. Data confirmed our hypothesis and showed that only IV or IP administration achieved ascorbate concentrations in millimolar ranges *in vivo* (**Fig. 2**).

H_2O_2 triggers generation of downstream reactive oxygen species (ROS) and induces cell death. Because blood has a rich reducing system to prevent H_2O_2 accumulation, these ROS induced by ascorbate are not detected in blood stream (**Fig. 2**).[8, 9] Therefore, IVC acts as a pro-drug to deliver oxidative stress to tissues, without ROS formation in the blood. This central mechanism of ascorbate-induced cytotoxicity, generated by our group, now have been confirmed by laboratories around the world.[11-13, 19-22, 24-26]

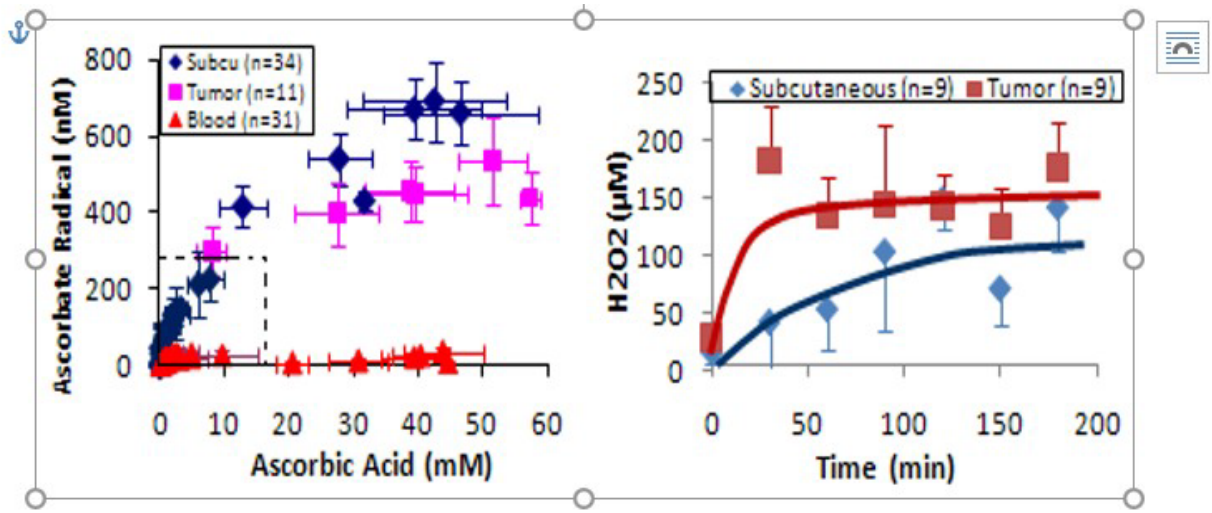


Fig. 2. Ascorbate radical and H_2O_2 formation in rodents received high-dose parenteral ascorbate. Ascorbate was given by IP injection. Data in the dashed box were with 0.25 and 0.5 g/kg in rats, and outside the dashed box were 4 g/kg in tumor bearing mice. Ascorbate radical (left panel) was detected with electron paramagnetic resonance (EPR). H_2O_2 (right panel) was detected over time using a synthesized fluorescent probe peroxanthone (PX1) in fluids obtained by in situ microdialysis of tissues. ***(Chen et al. PNAS 2007, 2008)***

Ascorbate generates H_2O_2 at both tumor sites and in normal tissues, but apparently exerts cytotoxicity specifically towards tumor cells and spares the normal cells. The selectivity is suggested by recent studies using multiple tumor models to be related to tumor cells' abnormal energy metabolism and iron metabolism.[1, 9, 12, 13] First, H_2O_2 generated by ascorbate damages DNA[1], and therefore activates polyADP-ribose polymerase (PARP) for repair. Activated PARP catabolizes NAD+, thereby depleting substrate for NADH formation and consequent ATP synthesis.[1, 9] ROS produced by H_2O_2 can disrupt intracellular iron metabolism, and thereby selectively sensitize cancer cells to ascorbate through pro-oxidant chemistry involving redox-active labile iron and enhanced H_2O_2 formation.[13] As a result, cancer cells susceptible to ascorbate have elevated levels of redox-active labile iron and increased levels of transferrin receptor (Trf) compared to normal cells.[13] Ascorbate also selectively kills SVCT2 (sodium-dependent vitamin C transporter 2) high-expressing cancer stem cells in hepatocellular carcinoma in theory due to increased uptake of ascorbate by SVCT2.[27] Our survey of the TCGA database indicates upregulation of both Trf and SVCT in BCa tissues versus normal tissues. We will further investigate these mechanisms of cellular susceptibility.

A number of animal tumor models have been tested and high dose parental ascorbate either alone or combined with standard chemotherapies showed promising tumor inhibitory effects. In a metastatic ovarian cancer model, we have shown that 4 g/kg daily IP injection of ascorbate (equivalent to ~1.3 g/kg by IV,

clinically relevant, [9, 10]) enhanced treatment effects of carboplatin and paclitaxel.[1] Ascorbate (Asc) alone reduced tumor burden relative to control group.

Adding ascorbate to Cp and Pax reduced tumor weight by 94%, and completely abrogated ascites formation, an effect significantly better than Cp+Pax (**Fig. 3A, B**). In pancreatic cancer models, our data showed that 4 g/kg IP ascorbate potentiated gemcitabine effects (**Fig. 3C**).[2, 3]

FIGURE 3

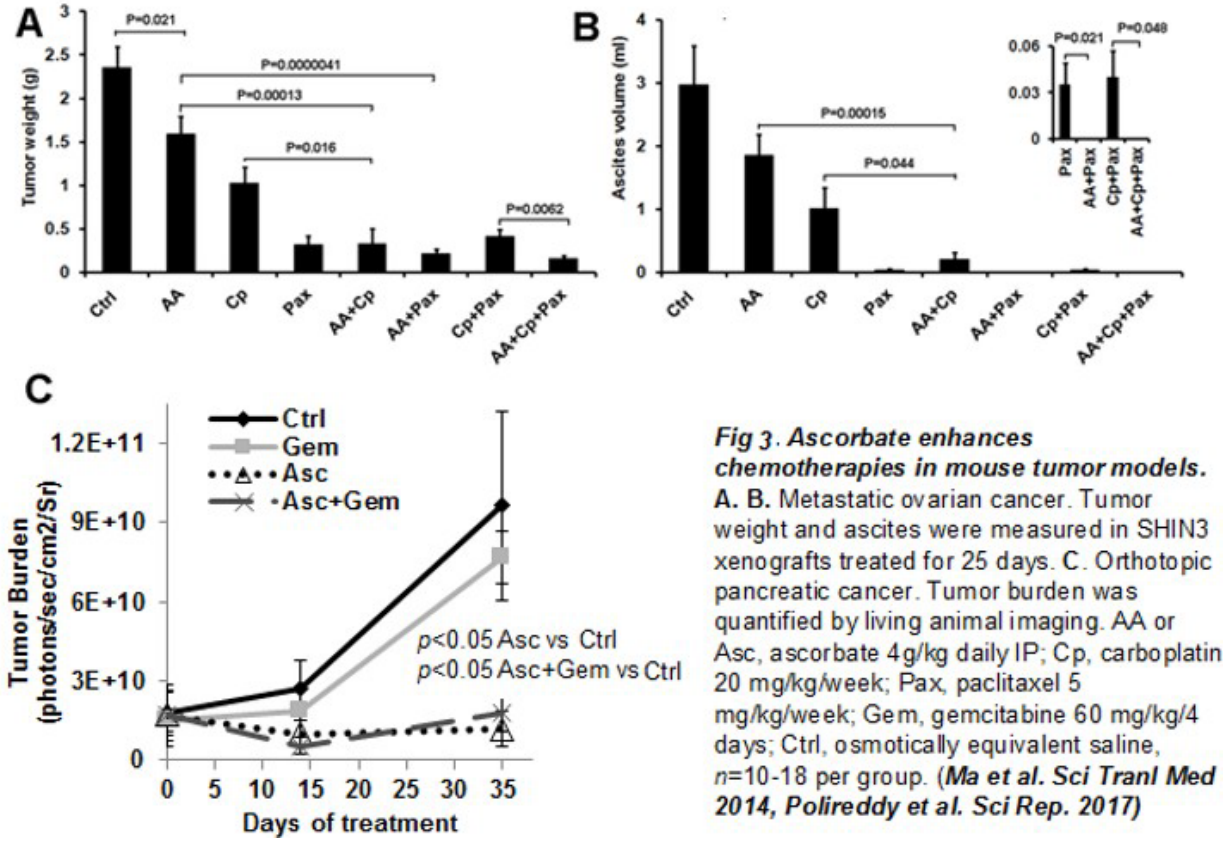


Fig 3. Ascorbate enhances chemotherapies in mouse tumor models. **A. B.** Metastatic ovarian cancer. Tumor weight and ascites were measured in SHIN3 xenografts treated for 25 days. **C.** Orthotopic pancreatic cancer. Tumor burden was quantified by living animal imaging. AA or Asc, ascorbate 4g/kg daily IP; Cp, carboplatin 20 mg/kg/week; Pax, paclitaxel 5 mg/kg/week; Gem, gemcitabine 60 mg/kg/4 days; Ctrl, osmotically equivalent saline, *n*=10-18 per group. (***Ma et al. Sci Tranl Med 2014, Polireddy et al. Sci Rep. 2017)***

None of these combined treatments nor ascorbate alone influenced body weight or induced any pathological changes in liver, kidney or spleen. *In vitro* combination index also showed strong synergy between ascorbate combined with either Cp, Pax, or gemcitabine.[1, 28]

3. Phase I clinical data in multiple types of cancers showed feasibility, tolerability and potential efficacy

More than a dozen of early phase trials in multiple types of cancers have shown safety, feasibility, and potential efficacy of IVC. A dose finding trial [18] in advanced oncological patients showed that IVC at the dose of 1.5 g/kg 3X weekly was safe and free of important toxicity. This dose sustains plasma concentrations >10 mM for >4 hours, which is sufficient to induce death in many cancer cells. Two phase I trials in patients with advanced pancreatic cancers [29, 30] showed the same good tolerance, and had unexpected stable disease and prolonged survival when combining IVC with gemcitabine or gemcitabine+erlotinib. Pilot trials with non-small-cell lung cancer (NSCLC) and glioblastoma multiforme (GBM) showed again that IVC is safe and well

tolerated when combine with standard chemo or radiation therapies and that IVC is promising in improving patient survival.[13] Our Phase I/IIa trial in ovarian cancer patient adding IVC to standard paclitaxel/carboplatin therapy showed that IVC was very well tolerated.[1] It substantially decreased grade 1 and 2 toxicities, and did not increase the rate of grade 3 or 4 toxicities in all categories of toxicity evaluated. The median progress free survival time (PFS) was prolonged (25.5 months with IVC versus 16.75 months without IVC). Similar results were obtained in our other phase I trial in pancreatic cancer patients: IVC was well tolerated, did not influence gemcitabine pharmacokinetic parameters, and tended to prolong median survival. Many cases including ours have been reported that IVC showed tumor inhibitory effects.[28, 31-33]

4.1 Other Agent(s) / Treatment(s)

As an acceptable alternate to standard of care cisplatin, participants will receive a single cycle of carboplatin (AUC 5 on day 1) and gemcitabine 1000 mg/m2 on days 1 and 8 of a 21-day cycle with cystectomy scheduled at termination of the intervention.

4.2 Rationale

Compelling data from studies *in vitro*, *in vivo* and in patients show that pharmacologic concentrations of ascorbate have potential as a therapeutic agent for cancer by exerting oxidative damage selectively to cancer cells.[1, 8-13] High dose intravenous administration of ascorbate (IVC) can provide concentrations sufficiently high to kill cancer cells.[10, 14] IVC does not have cumulative toxicity, has been shown to work synergistically with standard chemotherapies including gemcitabine and carboplatin, and reduces toxicities that accompany chemotherapy.[1] IVC has wide-spread off-label use in cancer patients.[15] If clinically adopted, IVC could enhance anti-tumor effects, reduce chemotherapy-associated toxicities, and improve QOL.

A number of phase II clinical trials are ongoing or in planning to detect benefits of IVC in multiple types of cancers, including prostate, lung, kidney, colon, ovarian, liver, and lymphoma. To our knowledge, IVC has not been clinically tested in BCa, and of more importance in treatment of cisplatin-ineligible MIBC patients.. Given the compelling data in multiple cancer models, and lack of treatment options for cisplatin ineligible MIBC, it is worthwhile to test the low-toxic potentially life-saving treatment of IVC combination therapy.

This pilot study aims to fill gaps currently present in treatment of this understudied patient population, and will further explore multiple mechanism-related molecules that have the potential to be developed as true biomarkers for treatment response after IVC treatment.

This study will use the BCa tumor bank established by Dr. John Taylor (PI). This is an IRB approved, annotated, longitudinal tumor bank of patient samples from initial point of entry to the KU system for up to 2 years post-treatment. This tumor bank allows us access to patient tissue throughout the study giving us a broad view of the patient response to treatment.

Our **primary hypothesis** is that adding IVC to a gemcitabine/carboplatin NAC regimen will significantly improve pathological downstaging in cisplatin-ineligible MIBC patients, as well as those who decline cisplatin based NAC, undergoing cystectomy. We will also study molecular mediators for cellular outcome and ascorbate specific mechanism, and quality of life (QOL) to gather preliminary information for a larger multi-center, randomized, two-arm trial.

An expanded trial would utilize The Albert Institute for Bladder Cancer Research, a non-profit headed by Dr. Taylor, providing cooperative agreement for trials among leading BCa institutions. Given the expertise and reputation of our research team, a multicentered trial would be attractive to our partner institutions. With the specificity of the patient population and the limited number of trials open nationally in this space, we believe the multicentered trial would be competitive, timely, effective, and likely to have substantial impact on the field and on clinical management of MIBC.

4.3 Study Risk / Benefit Ratio

The potential benefit of this study is judged to outweigh risk; therefore, the risk/benefit ratio is in favor of benefit.

4.3.1 Study Known Potential Risks

The research in this study poses greater than minimal risk but is likely to yield generalizable knowledge about the participant disorder or condition.

4.3.2 Study Known Potential Benefits

Potential benefits include the possibility of increased response and increased overall survival for participants enrolling / registering on this study. In addition, participation in this trial may benefit future patients by advancing generalizable knowledge of treatments for patients with MIBC.

5 STUDY DESIGN AND METHODS

This is a single arm, Simon design, “window of opportunity” trial, enrolling patients with cisplatin ineligible MIBC, or those who decline cisplatin base NAC.

Assess rates of pathologic downstaging and quality of life in MIBC cisplatin-ineligible/declined patients when IVC is added to gemcitabine-carboplatin NAC.
We have hypothesized adding IVC to carbo/gem NAC will enhance pathological downstaging and improve QOL. The patients eligible for this study (cisplatin ineligible or declined with MIBC) typically proceed straight to cystectomy within 6 weeks of first appointment.

In this study, participants will receive a single cycle of an accepted SOC alternate, gemcitabine/carboplatin, along with IVC and then proceed to cystectomy.

Outcome metrics will include pathologic downstaging rates at cystectomy, QOL measurements, DFS and DSS.

QOL by patient-reported outcomes will be evaluated using the FACT-BI questionnaire. All four subscales will be used to evaluate physical, social, emotional, and functional well-being. A higher score represents better QOL.

Participants will complete these at enrollment, just prior to cystectomy, and then post-cystectomy.

Adverse events will be evaluated by the NCI Common Terminology Criteria for Adverse Events (CTCAE 5.0), and scored from 1-5. .

Evaluate mechanism-related molecules that have the potential to reflect/predict responses to IVC treatment when added to NAC.

1) *Assess Mediators of Cancer Cells' Responses to IVC.* As described in Preliminary Data, upregulated TfR and SVCT2 expression have been proposed to increase cancer cells susceptibility to ascorbate treatment, by increasing cellular liable iron pool and ascorbate uptake.[13,27] We will collect tumor specimens from all participants at initial resection and at cystectomy via the IRB approved BCa tumor bank. For measurement of TfR and SVCT1/2 expression levels, qRT-PCR will be used for detecting mRNA expression levels in snap frozen tumor tissues. Immunofluorescence or immunohistochemistry assay will be utilized using OCT embedded frozen tissues. The expression levels will be given numerical H scores (0-300) by pathologist review, defined as sum of staining intensity (0, 1, 2, 3) multiplied by percentage of positive stained cells. The results from those who respond to IVC treatment will be compared to non-responders.

2) *Assess Mediators of Cellular Outcome and/or Validate IVC-specific Anti-tumorigenic Mechanisms*. Initially we will evaluate cell death and cellular proliferation as defined mechanisms of the anti-cancer activity of ascorbate in complex with carboplatin/gemcitabine in our pre-cystectomy samples versus the post-treatment RC samples[1]. We will use TUNEL staining as a measure of cell death and Ki67 as a measure of cell proliferation. Our study found ascorbate treatment robustly enhanced α-tubulin acetylation selectively in cancer cells versus normal cells[2], presumably because of H_2O_2 formation. Ascorbate decreases NAD+, and NAD+ is an essential co-factor for the activity of the tubulin deacetylase Sirt-2. Inhibition in Sirt-2 activity elevates acetylation in α-tubulin. Increased acetylation in α-tubulin disrupts microtubule dynamics by over-stabilizing polymerized tubulin, and therefore inhibited mitosis, and mobility of cancer cells.[2] We will detect the levels of α-tubulin acetylation by immunofluorescence or immunohistochemistry and H scores will be given. This could serve as an indicator for an ascorbate-specific mechanism in our trial.

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 OBJECTIVE(S)

Primary Objective

To assess pathologic downstaging rate in MIBC cisplatin-ineligible patients when IVC is added to a gemcitabine/carboplatin NAC regimen.

Secondary Objective(s)

1. To assess Quality of life.
2. To measure Disease Free Survival (DFS).
3. To measure Disease Specific Survival (DSS).

Exploratory Objective(s)

Biomarkers for Cell death, IVC related biomarkers and cellular outcome biomarkers will be evaluated in tumor samples at initial resection and radical cystectomy.

6.2 ENDPOINTS AND MEASURE(S)

6.2.1 Primary **Endpoint**

What is being measured	Measurement time frame	How will change be evaluated or measured
Post treatment pathological staging	4 to 6 weeks after first IVC infusion	Pre and Post treatment specimen pathology results evaluated per American Joint Committee on Cancer (AJCC) staging guidelines

6.2.2 Secondary **Endpoint(s)**

What is being measured	Measurement time frame	How will change be evaluated or measured
Overall change in patient-reported quality of life outcomes	6 to 8 weeks	Fact-Bladder (FACT-BI) Quality of life questionnaire
Disease free survival rate (DFS) among participants	Up to 3 years	Medical Record
Overall survival rate among participants	Up to 5 years	Medical Record

6.2.3 Exploratory **Endpoint(s)**

What is being measured	Measurement time frame	How will change be evaluated or measured
Cellular outcome markers	Initial resection and at cystectomy	TUNEL staining Ki67
IVC uptake - Levels of SLC23A2 (SVCT2, vitamin C tissue receptor).		
IVC specific mechanism markers – Acetylated tubulin levels and transferrin receptor levels.		
Cell death.		
Cell proliferation.		

7 STUDY ENROLLMENT / REGISTRATION AND WITHDRAWAL

7.1 Participant Inclusion Criteria

Males and females of all races and ethnicities are eligible to participate in this study and must meet **all** of the inclusion criteria listed below to participate in this study.

MRN		**INITIALS**	
Verified	**Criteria**		
	Ability of participant OR Legally Authorized Representative (LAR) to understand this study, and participant or LAR willingness to sign a written informed consent		
	Age ≥ 18 years	Age:	
	ECOG Performance Status = 0 -- 2 (*Appendix A*).	PS:	
	Diagnosis/disease status Cisplatin-ineligible or declined muscle invasive bladder cancer. Cisplatin ineligibility will be defined based on Galsky criteria: • CTCAE ver. 5.0 Grade 2 or greater peripheral neuropathy. • CTCAE ver. 5.0 Grade 2 or greater hearing loss. • Creatinine clearance estimated or calculated < 60 ml/min.	Type: DX Date:	
	Patients must have adequate organ function at enrollment, defined as follows:		
		Result	Date
	Absolute neutrophil count ≥ 1,500/µL	µL	
	Platelets ≥ 100,000/µL	µL	
	Hemoglobin ≥ 9 g/dL	dL	
	Creatinine clearance (estimated or calculated) ≥ 30 ml/min		
	Total bilirubin ≤ 2.0 mg/dl		
	AST(SGOT) ≤ 2.5 X institutional upper limit of normal		
	ALT(SGPT) ≤ 2.5 X institutional upper limit of normal		
	Normal Glucose-6-phosphate dehydrogenase (G6PD) status		
	Women of child-bearing potential and men with partners of child-bearing potential must agree to practice sexual abstinence, or to use the forms of contraception listed below, prior to study entry, for the duration of study participation, and for **WOMEN: 6 months after EOT, MEN: 3 months after EOT** following completion of therapy. If a woman becomes pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.		

MRN	INITIALS

Verified	Criteria
A woman of child-bearing potential is any female (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria: *(Please check the appropriate response and mark the other response as N/A).*	
	Has not undergone a hysterectomy or bilateral oophorectomy;
	Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months) and is < 45 years of age.
	Men of child-bearing potential must not donate sperm while on this study and for **3 months** after their last study treatment.
Acceptable birth control methods include: *(Please check the appropriate response and mark the other responses as N/A).*	
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:	
	Oral
	Intravaginal
	Transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation:	
	Oral
	Injectable
	Implantable
Other Methods:	
	Intrauterine device (IUD)
	Intrauterine hormone-releasing system (IUS)
	Bilateral tubal occlusion
	Vasectomized partner
	Sexual abstinence, if this is the preferred and usual lifestyle of the participant

7.2 Participant Exclusion Criteria

Participants meeting **any** of the exclusion criteria listed below at screening will be excluded from study participation.

Absent	Criteria
	Patient simultaneously enrolled in any therapeutic clinical trial
	Current or anticipated use of other investigational agents while participating in this study
	Psychiatric illness/social situations that would limit compliance with study requirements
	Pregnant or breast feeding. There is a potential for congenital abnormalities and for this regimen to harm breast feeding infants.
	Women of childbearing age expecting to conceive children while receiving study treatment and for **3 months** after the last dose of study treatment
	Other study specific criteria

MRN	**Initials**

Absent	**Criteria**
	Histology of pure adenocarcinoma, pure squamous cell carcinoma, or pure small cell carcinoma in the TURBT sample
	Prior systemic chemotherapy (prior intravesical therapy is allowed) for bladder cancer and/ or prior radiation therapy to the urinary bladder with curative intent for bladder cancer.
	Uncontrolled intercurrent illness, including but not limited to, ongoing or active infection, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, interstitial lung disease, serious chronic gastrointestinal conditions associated with diarrhea,
	Current consumption of tobacco products, patients may be asked to quit for 2–3 weeks prior to enrollment
	If tobacco use is suspected at any point during the trial, continine level will be obtained
	History of G6PD deficiency
	History of oxalate renal calculi

Eligibility Confirmed by	**Print Name**	**Signature**	**Date**
Clinical Research Coordinator			
Clinical Research Coordinator Double Check			
Treating Physician			

Once eligibility has been confirmed, use the link below to register your participant.
Please allow up to 24 business hours for the registration process.

https://redcap.kumc.edu/surveys/?s=TTLWNND9TP

8 PARTICIPANT REGISTRATION PROCEDURES

General Guidelines
Eligible participants will be registered through the KUCC Clinical Research Office central registration process. Registration must occur prior to the initiation of therapy, with treatment assignment provided by KUCC. Any participant not registered to the protocol before treatment begins will be considered ineligible and study treatment will be denied.

The completed source documentation for eligibility verification and registration must be kept in the participant binder for monitoring purposes and documentation.

Issues that would cause treatment delays should be discussed with the Sponsor - Investigator. If a participant does not receive protocol therapy following registration, notify the KU Cancer Center Project Director or designee and update participant's status in the CRIS system.

Registration Process
The Coordinating Center (KUCC) is accessible for registration Monday through Friday from 8:00 AM to 5:00 PM Central Time. Please allow up to 24 hours for completion of registration.

The registration procedures are as follows:

1. Obtain written informed consent from the patient prior to the performance of any study related procedures or assessments. Tests required at screening and performed as part of customary care prior to signing consent, are allowed IF those tests were performed within the timeframe listed in the section of this protocol with title *STUDY PROCEDURES AND SCHEDULE*.
2. Complete appropriate baseline demographic information in CRIS. Print, complete and obtain appropriate signatures for the inclusion/exclusion criteria to document eligibility. Maintain completed documents in participant's research record. Participants must meet all eligibility criteria to be eligible for registration.
3. Use the hyperlink at the bottom of the eligibility criteria, complete information and submit to initiate the registration process.
4. Email confirmation of registration will be sent to the person initiating the registration. Registration confirmation should be maintained as part of the participant's research record.

8.1 Participant Withdrawal Or Termination

Reasons For Withdrawal Or Termination

Participants can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator. The reason(s) for discontinuation will be documented and may include:

- Participant voluntarily withdraws from treatment (follow-up permitted);
- Participant withdraws consent (termination of treatment and follow-up);
- Participant is unable to comply with protocol requirements;
- Participant demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);
- Participant experiences toxicity that makes continuation in the protocol unsafe;

- Inter-current illness that prevents further administration of treatment
- Treating physician judges continuation on the study would not be in the participant's best interest;
- Participant becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;
- Lost to follow-up.

Participant Replacement

Since this is small single arm study, participants who do not complete study treatment or undergo definitive surgery will be replaced. All participants will be evaluated for toxicity.

9 MEASUREMENT OF EFFECT

9.1 Solid Tumor

Antitumor Effect

Definitions
Evaluable for toxicity. All participants will be evaluable for toxicity from the time of signing of consent.

Evaluable for objective response. Only those participants who have disease present at baseline, and have received one cycle of therapy, and have had their disease re-evaluated at definitive surgery will be considered evaluable for response. These participants will have their response classified according to the definitions stated below.

Disease Parameters

Methods for Evaluation of Disease:
We will evaluate the patients for response after definitive surgery (radical cystectomy). Pathologic downstaging to non-invasive disease <pT2 via pathological inspection of cystectomy specimen will be recorded after enrolling 13 subjects and based on these results, an additional 8 subjects may be enrolled by a Simon 2-stage design.

Evaluation of Overall Response:
The number of subjects who have downstaging of their tumor to non-invasive disease (<ypT2 disease) will be evaluated after surgery.

In addition, we will evaluate the complete response rate defined as patients with no evidence of residual disease at radical cystectomy (ypT0).

Disease-Free Survival (DFS)

DFS will be defined as time from cystectomy to disease recurrence or death resulting from disease. Patients alive without disease progression at the time of analysis were censored at the date of last disease assessment.

Overall Survival (OS)
Overall survival is defined as the duration of time from start of treatment to death. Participants who do not experience death during the evaluation period, or are lost to follow-up, will be censored on the date they were last known to be alive.

10 STUDY AGENT(S) / THERAPY / DEVICE

10.1 ASCORBIC ACID / VITAMIN C

ASCORBIC ACID/VITAMIN C is investigational.

IV DRUG
PHARMACOLOGY -
Please refer to current Package Insert.

PHARMACOKINETICS

Please refer to current Package Insert.

ADVERSE EFFECTS

There are no contraindications to the administration of ascorbic acid except for high-dose infusion (over 15 grams) with enzyme G6PD deficiency, which results in hemolysis when high-dose intravenous ascorbic acid is given. As part of the requirement for enrollment, study participants will have their G6PD status screened and if below normal activity, they will not be allowed to participate.

Oxalate renal calculi have been theorized to occur in susceptible individuals after infusions of high-dose ascorbate. As part of the Inclusion/Exclusion criteria, patients with a history of oxalate renal calculi will not be allowed to enroll in the study. This information will be collected at screening. In addition, there will be close monitoring of the renal function by urinalysis and blood tests. This occurrence has not been identified in our prior clinical trials and clinic experience.

Shifts in serum calcium levels have been theorized to occur from intravenous ascorbic acid and manifests as shaking or shivering. This was not found in our pharmacokinetic study (Unpublished data).

Theoretical concerns that have not been reported or not seen by the Principal Investigator or her colleagues include pH abnormalities, hypoglycemia, electrolyte disturbances, cardiac arrhythmias, and uric acid nephropathy. We will evaluate metabolic profiles and clinical status as part of the routine monitoring of the study participants.

It is always possible that phlebitis could result from venipuncture or intravenous line placement. It is also possible that phlebitis and cellulites could result from infiltration of the study drug fluid outside of the vein. This will be routinely monitored by the staff of the consortium sites. This is considered only a slight risk.

Transient mild soreness may occur at the site of intramuscular or subcutaneous injection.

It is possible that a study participant could experience an idiosyncratic reaction to the study drug that could result in a hypersensitivity reaction or potentially anaphylaxis. Patients' vital signs and status are monitored as part of routine care.

General Precautions
Too-rapid intravenous injection is to be avoided. Too-rapid intravenous administration of the solution may cause temporary faintness or dizziness.

Laboratory Tests
Diabetics taking more than 500 mg of ascorbic acid daily, may obtain false reading of the urinary glucose test. No exogenous ascorbic acid should be ingested for 48 to 72 hours before amine dependent stool occult blood tests are conducted because possible false negative results may occur.

Pregnancy and Lactation:

Usage in Pregnancy
Pregnancy Category C
Animal reproduction studies have not been conducted with Ascorbic Acid Injection. It is also not known whether Ascorbic Acid Injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Ascorbic Acid Injection should be given to a pregnant woman only if clearly needed.

Nursing Mothers
Caution should be exercised when Ascorbic Acid Injection is administered to a nursing woman.

Drug Interactions:

Limited evidence suggests that ascorbic acid may influence the intensity and duration of action of bishydroxycoumarin.

Permitted and Excluded Concomitant Medications.

Supportive Medications

Supportive medication/class of drug:	Usage:
Concomitant medications or treatments (e.g., acetaminophen or diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care, except for those medications identified as "prohibited," as listed above	To be administered as prescribed at the discretion of the site specific Investigator

Best supportive care (including antibiotics, nutritional support, correction of metabolic disorders, optimal symptom control, and pain management [including palliative radiotherapy to non-target lesions, etc])	Should be used, when necessary, for all patients
Inactivated viruses, such as those in the influenza vaccine	Permitted

Excluded concomitant medications
Prohibited Concomitant Medications

Prohibited medication/class of drug:	**Usage:**
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly while the patient is on study treatment
Limited evidence suggests that ascorbic acid may influence the intensity and duration of action of bishydroxycoumarin.	Appropriate monitoring for participants who continue anticoagulation therapy with bishydroxycoumarin.
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Other than paclitaxel/carboplatin chemotherapy included in this trial, no other chemotherapy will be given concomitantly while the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [e.g., insulin for diabetes] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [e.g., by local surgery or radiotherapy])
Diabetics should not undergo glucose monitoring by glucometer fingerstick method within 4 hours of receiving intravenous ascorbic acid. False positive elevations of glucose readings result because of testing method error.	All glucose monitoring 4 hours post intravenous ascorbic acid must be done by phlebotomy and routine laboratory testing. **All decisions about insulin dosing post IV vitamin C infusion must be based on laboratory metabolic testing and not on glucometer fingerstick method as hypoglycemia may result from false reading.**

DOSING & ADMINISTRATION

See Method of Administration below, sections with titles TREATMENT PLAN and DESCRIPTION OF PROCEDURES.

HOW SUPPLIED

Ascorbic acid/vitamin C will be supplied as 500 mg/ml in a 50 mL vial (total 25 grams/vial).

The sterile dispensing vials contain per mL: Ascorbic Acid 500 mg, Disodium Edetate 0.25mg in Water for Injection q.s. pH (range 5.5-7.0) , adjusted with Sodium Bicarbonate and Sodium Hydroxide. Contains no preservatives.

STORAGE, PREPARATION & STABILITY

Storage

Investigational product vials are stored at 2°C to 8°C (36°F to 46°F) and must not be frozen. Drug product should be kept in secondary packaging until use to prevent excessive light exposure.

Preparation and Stability

The dose of intravenous vitamin C for administration must be prepared by the Investigator's or site's designated INVESTIGATIONAL PHARMACY using usual technique. Total time from needle puncture of the Ascorbic acid/Vitamin C vial to the start of administration should not exceed:

- 2 hours at 2°C to 8°C (36°F to 46°F).
- 2 hours at room temperature.

Infusion solution must be allowed to equilibrate to room temperature prior to commencement of administration.

For 25gm dose: remove 50mL from a 500mL bag of Lactated Ringers. Inject 25gm (50mL or 1 bottle) of ascorbic acid and 2mL of MgCl into the bag.

For the 50gm dose: Remove 100mL from a 1L bag of Sterile Water for Injection. Inject 50gm (100mL or 2 bottles) of ascorbic and 2mL of MgCl into the bag.

For the 75gm dose: Remove 150mL from a 1L bag of Sterile Water for Injection. Inject 75gm (150mL or 3 bottles) of ascorbic acid and 2ml of MgCl into the bag.

For 100gm dose: Remove 200mL from a 1L bag of Sterile Water for Injection. Inject 100gm (200mL or 4 bottles) of ascorbic acid and 2mL of MgCl into the bag.

For 125gm dose: Remove 250mL from a 1L bag of Sterile Water for Injection. Inject 125gm (250mL or 5 bottles) of ascorbic acid and 2mL of MgCl into the bag.

DOSE ESCALATION/PREPARATION TABLE						
Na Ascorbic Acid (calculated using 500mg/mL ascorbic acid)	**Osmolarity calculated in Sterile Water**			**Osmolarity calculated in Ringer's Lactate**		
	250 mL	**500 mL**	**1000 mL**	**250 mL**	**500 mL**	**1000 mL**
25 grams	**1212**	**606**	**303**	**1433**	**857**	**568**
50 grams	**2400**	**1200**	**600**	**2565**	**1423**	**851**
75 grams	**3588**	**1794**	**897**	**3697**	**1989**	**1134**
100 grams	**4776**	**2388**	**1194**	**4829**	**2555**	**1427**
125 grams	**XXX**	**XXX**	**1191**	**XXX**	**XXX**	**XXX**

- All mixing and dispensing will occur at the site's Investigational Pharmacy or oncologist's infusion center where logs will be kept of product lot numbers and dispensing volumes, dates and times.
- Product will be ordered from supplier by the University of Kansas study team and shipped to dispensing sites via Federal Express in temperature-controlled containers.
- All records and necessary documentation of product delivery and condition will be kept in the study log in Investigational Pharmacy or dispensing site.
- After delivery of product to the site, the vials will be kept in a dark secure location and refrigerated at 2˚ to 8˚ C until use.
- Mixing in the appropriate carrier fluid must not occur more than 2 hours prior to infusion to prevent degradation of the ascorbic acid and delivered to the site immediately upon mixing.
- The MgCl prevents vascular spasm and discomfort during infusion.
- Standard IV tubing is used and the infusion is delivered by infusion pump so that the IV vitamin C solution is delivered at 0.5 grams/minute. For example, 75 grams of vitamin C solution will be delivered over 150 minutes.
- In the event of opened study material not used or expired material, the ascorbic acid will be disposed of in Investigational Pharmacy at each site according to procedure. Records of discarded material will be kept in the Investigational Pharmacy study log.

Route of Administration

IV infusion. Do not administer as an IV push or bolus injection.

Method of Administration

The IV line will be flushed with a volume of IV diluent equal to the priming volume of the infusion set used after the contents of the IV bag are fully administered, or complete the infusion according to institutional policy to ensure the full dose is administered and document if the line was not flushed.

Ascorbic Acid/vitamin C at escalating doses will be administered at room temperature (approximately 25°C) by controlled infusion into a peripheral or central vein.

Starting dose of vitamin C will begin at 25 grams and may be escalated to 125 grams based on plasma vitamin C level.

One dose of 25 grams will be administered, then the Dose Escalation/Preparation Table outlining dose escalations (above) will be used. Following preparation of vitamin C for infusion, the entire contents of the IV bag should be administered as an IV infusion at a rate of 0.5 grams/minute (i.e. 50 grams = 100 minutes), using a volumetric pump with a 0.2-1.2 micron in-line filter at the protocol specified dose. It is not to be administered as an IV push or bolus injection.

Dose escalation will continue until a plasama level of at least 350 mg/dL is reached but will not exceed 125 grams of vitamin C.

In the event that there are interruptions during infusion, the **total** allowed time should not exceed **2 hours** for the 25g and 50 g doses.

Do not co-administer other drugs through the same infusion line.

If either preparation time or infusion time exceeds the time limits a new dose must be prepared from new vials. Injectable ascorbate does not contain preservatives, and any unused portion must be discarded.

DRUG ORDERING AND ACCOUNTABILITY

Drug Ordering

Ascorbic Acid/Vitamin C will be ordered through vendor.

Ordering will be managed by KUMC study team.

Drug Handling and Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from vendor.

Drug return and/or disposition instruction

Per institutional standard procedures.

10.2 CARBOPLATIN DRUG INFORMATION

Carboplatin is considered standard of care in this setting.
Please refer to current Package Insert.

10.3 GEMCITABINE DRUG INFORMATION

Gemcitabine is considered standard of care in this setting.

Please refer to current Package Insert.

11 TREATMENT PLAN

Participants will receive a single cycle of an accepted SOC alternate, gemcitabine/carboplatin, along with IVC and then proceed to cystectomy.

IVC administration is established by dose escalation regimen[1, 2] initiated at a single dose of 25 g and titrated up to a target peak plasma concentration of 350 to 400 mg/dL (~20 mM), monitored by HPLC detection.[1] Dose of vitamin C can be increased up to but not more than 125 grams to reach target plasma level. Once the therapeutic dose is established, the participants will continue to receive IVC 2 to 3 times per week at the Westwood Urologic Oncology treatment clinics during the 28-day window leading into cystectomy.

If target plasma level is not reached with dose escalation, surreptitious tabocco use will be evaluated by continine blood level. If positive for tobacco use, participant will be removed from trial and replaced.

Participants will receive a single cycle of carboplatin (AUC 5 on day 1) and gemcitabine 1000 mg/m2 on days 1 and 8 of a 21-day cycle with cystectomy scheduled at termination of the intervention. Thus, there is no delay in surgery based on the proposed NAC regimen.

QOL by patient-reported outcomes will be evaluated using the FACT-BI questionnaire. All four subscales will be used to evaluate physical, social, emotional, and functional well-being. A higher score represents better QOL. Participants will complete these at enrollment, just prior to cystectomy, and then post-cystectomy.

Outcome metrics will include pathologic downstaging rates at cystectomy, QOL measurements, DFS and DSS.

11.1.1 Duration Of Therapy

Participants will be enrolled and treated for one 28 day cycle (21 days for Gem/Carbo, 28 days for IVC.). Treatment will occur during the 4 to 6 weeks after diagnosis and before standard of care surgery.

Study Agent Accountability Procedures / Participant Compliance

Study drug accountability per Institutional standard operating procedures.

11.1.2 Nursing Staff Duty Implications

NOTE for nursing staff:
Intravenous vitamin C administration may be infused through surgically implanted indwelling venous catheters that are commonly placed during oncology care. If an indwelling catheter is not available, peripheral venous access may be used. Access and deaccess must be undertaken via standard of care policies of the sub-site institutions.

Infusion administered with infusion pumps to assure 0.5 gram/ minute infusion rate (i.e. 75 grams = 150 minutes).

Diabetics should not undergo glucose monitoring by glucometer fingerstick method within 4 hours of receiving intravenous ascorbic acid. False positive elevations of glucose readings result because of testing method error. All glucose monitoring 4 hours post intravenous ascorbic acid must be done by phlebotomy and routine laboratory testing.

All decisions about insulin dosing post IV vitamin C infusion must be based on laboratory metabolic testing and not on glucometer fingerstick method as hypoglycemia may result from false reading with subsequent insulin administration.

Participant Access To Study Agent At Study Closure

Participant can have access to IV vitamin C at study closure. Note: Participants may incur out-of-pocket costs (IV vitamin C off-study may not be covered by participant insurance).

Potential referral for infusion site trained in IV C administration can be found at

http://www.acam.org/ or *http://www.faim.org/international-college-of-integrative-medicine*

11.1.3 Dose Adjustments/Modifications/Delays

Any participant who receives treatment on this protocol will be evaluable for toxicity. Each participant will be assessed for the development of toxicity according to the Schedule of Events table. CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting and toxicity assessment. 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 web site:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Information and links regarding CTCAE version 5.0 can be found at the CTEP website:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

1. Adverse events, whether volunteered by the study participant, discovered by the investigators during questioning, or detected by physical examination, laboratory tests, or other means will be collected and

recorded. An adverse event is any undesirable sign, symptom, or medical condition occurring after starting the study drugs even if the event is not considered to be related to study drugs. Pre-existing medical conditions will be considered adverse events if they worsen during study protocol. Events will be recorded from the time the consent is signed until 4 weeks after the study protocol is discontinued.
2. Patients experiencing Grade 4 neutropenia, Grade 3 thrombocytopenia, or Grade 2 peripheral neuropathy that do not recover will have treatment protocol discontinued.
3. Colony-stimulating factors will be prescribed at the discretion of the treating oncologist.
4. Although intravenous ascorbic acid in the doses administered in this study has been shown not to be toxic, to ensure that these therapies are non-toxic, liver enzymes and creatinine will be monitored during the treatment phase.
5. Idiosyncratic intolerance of intravenous ascorbic acid such as acute hypersensitivity will be recorded.
6. Significant adverse events related to the study drug will necessitate removal from the protocol. The study participants will be monitored until the AE resolves.

SURGICAL TREATMENT PLAN

Patients will undergo a radical cystectomy with lymph node dissection as is standard of care for MIBC. This is not part of this study or the study experimental procedures.

12 GUIDELINES FOR MANAGEMENT OF POTENTIAL ADVERSE EVENTS

Participants will be monitored for AEs from the time of signing informed consent through their last follow-up visit (90 days after the date of the last dose of study drug treatment.) Participants will be instructed to notify their physician immediately at the onset of any AE. Seriousness, severity grade, and relationship to study treatment of AEs will be determined by the investigator. AE severity will be graded by the investigator in accordance with CTCAE v.5.0.

INTRAVENOUS ASCORBIC ACID / VITAMIN C

Guidelines for management of potential adverse events for intravenous Vitamin C are not needed.

Previous studies and clinical experience have shown no toxicities to the dose of IV Vit C to be used in this study.

Adverse events will be collected in the usual manner using CTCAE v 5.0. Dose modifications will not be made after the dose for IV C has been established. There have been no dose limiting toxicities identified from our previous clinical trials, the clinical trial experience of others, or our extensive clinical experience administering IV C. Previous studies and clinical experience have shown no toxicities referable to the doses of IV C to be used in this study.

CARBOPLATIN

Bone marrow suppression (leukopenia, neutropenia, and thrombocytopenia) is dose-dependent and is also the dose-limiting toxicity. Peripheral blood counts should be frequently monitored during carboplatin injection treatment and, when appropriate, until recovery is achieved. Median nadir occurs at day 21 in patients receiving single agent carboplatin. In general, single intermittent courses of carboplatin should not be repeated until leukocyte, neutrophil, and platelet counts have recovered.

Since anemia is cumulative, transfusions may be needed during treatment with carboplatin, particularly in patients receiving prolonged therapy.

Bone marrow suppression is increased in patients who have received prior therapy, especially regimens including cisplatin. Marrow suppression is also increased in patients with impaired kidney function. Initial carboplatin injection dosages in these patients should be appropriately reduced and blood counts should be carefully monitored between courses. The use of carboplatin in combination with other bone marrow suppressing therapies must be carefully managed with respect to dosage and timing in order to minimize additive effects.

Carboplatin has limited nephrotoxic potential, but concomitant treatment with aminoglycosides has resulted in increased renal and/or audiologic toxicity, and caution must be exercised when a patient receives both drugs. Clinically significant hearing loss has been reported to occur in pediatric patients when carboplatin was administered at higher than recommended doses in combination with other ototoxic agents.

Carboplatin can induce emesis, which can be more severe in patients previously receiving emetogenic therapy. The incidence and intensity of emesis have been reduced by using premedication with antiemetics. Although no conclusive efficacy data exist with the following schedules of carboplatin, lengthening the duration of single intravenous administration to 24 hours or dividing the total dose over five consecutive daily pulse doses has resulted in reduced emesis.

Although peripheral neurotoxicity is infrequent, its incidence is increased in patients older than 65 years and in patients previously treated with cisplatin. Pre-existing cisplatin-induced neurotoxicity does not worsen in about 70% of the patients receiving carboplatin as secondary treatment.

Loss of vision, which can be complete for light and colors, has been reported after the use of carboplatin with doses higher than those recommended in the package insert. Vision appears to recover totally or to a significant extent within weeks of stopping these high doses.

As in the case of other platinum-coordination compounds, allergic reactions to carboplatin have been reported. These may occur within minutes of administration and should be managed with appropriate supportive therapy. There is increased risk of allergic reactions including anaphylaxis in patients previously exposed to platinum therapy

GEMCITABINE

Prophylaxis against sepsis: At the occurrence of fever >38.5°C (regardless of neutrophil count), the subjects should contact their physician and begin treatment with oral antibiotics per institutional guidelines (e.g., ciprofloxacin 500 mg PO twice per day or levofloxacin 500 mg PO once a day). It is advised that all subjects should be given a prescription for the appropriate antibiotics so they can immediately begin treatment with the first appearance of the fever.

Colony stimulation factors: These should be given according to institutional guidelines.

Interstitial pneumonitis: This can be seen with either Abraxane or GEM or the combination. Study medications should be immediately discontinued. If an infection etiology is ruled out, corticosteroids should be initiated.

Posterior reversible encephalopathy syndrome (PRES): GEM should be permanently discontinued and supportive measures implemented, including blood pressure control and anti-seizure therapy, if PRES develops during therapy.

Capillary leak syndrome: GEM should be discontinued and supportive measures implemented if capillary leak syndrome develops during therapy.

13 CHILD-BEARING POTENTIAL / PREGNANCY

Women, Men, and Pregnancy

Because the effect of the study drug(s) is considered possibly teratogenic and has potential risks to the fetus, pregnant females will not be included in the study. However, no female of childbearing potential will be excluded from the study. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with study drug(s), breastfeeding should be discontinued if the mother is treated with study drug(s). An effective form of contraception of the woman's choice will be required at all times during study. Female participants should not get pregnant or breastfeed while in this study and for **6 months** after the last dose of study medication. Male participants should not father a baby or donate sperm while in this study and for **3 months** after the last dose of the study medication. A pregnancy test will be performed on enrollment/registration women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. If a woman becomes pregnant or suspects she is pregnant while participating in this study or if her male partner is a participant in this study, the treating physician should be informed immediately.

The effects of the study drug(s), on the developing human fetus are unknown.

A woman of child-bearing potential is any female (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:
Has not undergone a hysterectomy or bilateral oophorectomy;
or

Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months)

NOTE: Acceptable forms of birth control are listed below and must be documented in the participant's chart:

- Sexual Abstinence OR
  - One Barrier method (cervical cap with spermicide plus male condom; diaphragm with spermicide plus male condom)

PLUS

  - Hormonal method (oral contraceptives, implants, or injections) or an intrauterine device (e.g., Copper-T).

Women of child-bearing potential and men with partners of child-bearing potential must agree to practice sexual abstinence, or to use two forms of adequate contraception (hormonal AND barrier method of birth control) prior to study entry, for the duration of study participation, and for **6 months (for women)** and **3 months (for men)** following completion of therapy.

- If a woman becomes pregnant or suspects she is pregnant (missed or late menstrual period) while participating in this study, she should inform her treating physician immediately.

- If the partner of a man becomes pregnant or suspects she is pregnant (missed or late menstrual period) while he is participating in this study, he should inform his treating physician immediately.

- Men of child-bearing potential must not father a child or donate sperm while on this study and for **3 months** after their last study treatment.

- Pregnancy must be reported along the same timelines as a serious adverse event.

14 STUDY PROCEDURES AND SCHEDULE

14.1 DESCRIPTION OF PROCEDURES / ASSESSMENTS

Please refer to the Schedule of Events Table(s) for summarized timelines of procedures.

14.1.1 Screening/Enrollment/Baseline

If the tests required at screening were performed as part of standard of care prior to signing consent for this study, the results from those tests are allowed in this study if the tests were completed within the timeframe listed below.

All screening procedures must be performed within **28 Days** prior to registration unless otherwise stated.

SCREENING VISIT

- **Informed Consent**

- **Medical history**
  - Complete medical (including child-bearing status), surgical and oncology history are obtained at screening. Any changes from time of signing consent (e.g. worsening severity or abnormal findings) are considered to be adverse events (AEs).

- **Demographics**
  - Demographic profile will include date of birth, gender, race, and zip code.

- **Review participant eligibility criteria**
  - Review of eligibility criteria to ensure participant qualification for study entry.

- **Review previous and concomitant medications**
  - All prior medication taken by the participant within 4 weeks before starting the study is to be recorded. Concomitant medications taken by the participant during the study are to be recorded up until 30-days after last study dose. If a reportable adverse event (see section with title *Adverse Events*) occurs within 30 days after last study dose, recording of concomitant medications should continue until resolution of the adverse event.

- **Physical exam**
  - Exam will include vital signs, height and weight – height to be measured at screening/baseline only
  - Note: Vital signs include temperature, pulse, SpO2, blood pressure

- **Performance status - ECOG**
  - Performance status evaluated prior to study entry and at specified study visits.

- **Adverse event assessment - CTCAE v5.0**
  - Baseline assessment via medical history, for determining later adverse events.

- **Urinalysis**

Urinalysis Tests[a]	
Bilirubin	pH
Blood	Protein
Glucose	Specific gravity
Ketones	Colour and appearance

[a] Microscopy should be used as appropriate to investigate white blood cells and use the high-power field for red blood cells if clinically indicated.

- **Hematology**
  - Hematology to include hemoglobin (Hgb), platelets, total white blood cell count (WBC), and differential.

- **Serum chemistries**
  - Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

- **Additional chemistries:**
  - Glucose-6-Phosphate Dehydrogenase (G6PD) at baseline only

- **ECG**
  - Standard 12-lead electro-cardiogram will be performed at screening only (ECG to be repeated at later study visits per PI discretion/if clinically indicated

- **Urine Pregnancy test (for females of child bearing potential)** only done at screening

- **Tumor Biopsies, Blood and Urine Sample Collection [REQUIRED for participants]**

Tumor tissue, blood and urine will be collected under the current, IRB approved, bladder cancer tumor bank (Appendix C). Tissue evaluated under this study will be pre-treatment transurethral resection (TURBT) and post-treatment cystectomy specimens obtained from the bladder cancer biorepository (PI: John Taylor, III, MD, MS).

Archival tissue will be accepted for pre-treatment specimens if TURBT was performed outside of KUMC.

14.1.2 Procedures During Treatment

All participants will receive a single cycle of standard of care chemotherapy with Carboplatin AUC5 on day 1 and Gemcitabine 1000 mg/m2 on days 1 and 8 of a 21 day cycle.
ONE TREATMENT CYCLE for IVC = 28 DAYS TO INCLUDE INITIAL 1 WEEK FOR IV C DOSE ESCALATION + 21 DAYS FOR CHEMOTHERAPY TREATMENT

Week 1 Day 1 IV Vitamin C dosing
NOTE: - Screening procedures performed within 72 hours of Cycle 1 Day 1 (C1D1) do not need to be repeated

- Physical exam, including weight, vital signs (temperature, pulse, SpO2, blood pressure)
- Review previous and concomitant medications
- Baseline FACT-Bl QOL instrument
- CBC (WINDOW: + / - 7 DAYS) - Tests as listed in the table in SCREENING VISIT, above.
- Serum chemistries (WINDOW: + / - 7 DAYS) - Tests as listed in the table in SCREENING VISIT, above.
- ECOG

- Urinalysis - Tests as listed in the table in SCREENING VISIT, above
- Adverse Events Review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
- IV Carboplatin Infusion
- IV Gemcitabine Infusion

Monitoring of intravenous vitamin C dose administration - On the **first infusion day**, participants will be monitored and vital signs collected/recorded prior to and after infusion described below.

- BP and pulse will be collected from participants before and after each infusion at the following times (based on a 60-minute infusion):
- Prior to the beginning of the infusion (measured once from approximately 30 minutes before up to 0 minutes [i.e., the beginning of the infusion])
- At the end of the infusion(±5 minutes)
- If the infusion takes longer than 60 minutes, then BP and pulse measurements should follow the principles as described above or be taken more frequently if clinically indicated. A 1-hour observation period is recommended after the first infusion of vitamin C.
- IV C **up to 3 days/week** while participanting in dose escalation phase of the trial.

Infusion schedule for escalating doses of vitamin C/ascorbic acid

- Percutaneous intravenous catheters will be placed in all study participants for venous access approximately 30 minutes prior to infusion. If indwelling ports are available, they will be accessed according to standard protocol.
- Infusion materials will be prepared by Investigational Pharmacy and delivered to the clinic responsible for infusion by standard procedure.
- All infusions will be administered by infusion pump at a rate of 0.5 grams/minute (i.e. 50 grams = 100 minutes).

Week 1 Ascorbic Acid Dose Escalation

1st Infusion schedule for escalating doses of vitamin C/ascorbic acid

- *First Infusion of ascorbate*: The starting infusion is one dose of 25 grams of Ascorbic acid in ringer's lactate administered at 0.5 grams per minute (see instructions for mixing requirements). The first infusion will be administered preferably on Monday. No plasma sampling on day 1 for ascorbate levels after 25-gram dose.
- CARBOPLATIN / GEMCITABINE IV INFUSION schedule, this will be standard of care dosing with adjustments made per discretion of treating oncologist to be administered in Westwood treatment area/ clinic as outlined above.

NOTE: During the IV VIT C dose escalation phase, Cycle 1 chemotherapy may be started and can be given ***after*** an IV C dose or on alternate day. There is no inhibition of chemotherapy when IV C is given prior to the chemotherapy and can be used as the fluid loading dose if desired. In fact, there is an additive effect when IV C and carboplatin and gemcitabine are combined.

Week 1 Day 3

- Review previous and concomitant medications
- Adverse events review

- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
- **Monitoring of subsequent infusions -** BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated
- IV **up to 3 days/week for the first week**dose escalation phase trial.

2nd Infusion schedule for escalating doses of vitamin C/ascorbic acid

- Percutaneous intravenous catheters will be placed in all study participants for venous access approximately 30 minutes prior to infusion. If indwelling ports are available, they will be accessed according to standard protocol.
- Infusion materials will be prepared by Investigational Pharmacy or prepared in oncologist's infusion center at the participating sites and delivered to the clinic responsible for infusion by standard procedure.
- All infusions will be administered by infusion pump at a rate of 0.5 grams/minute (i.e. 50 grams = 100 minutes).

Week 1 Day 3 Ascorbic Acid Dose Escalation

- *Second infusion of ascorbate:* The second infusion will not be given less than 24 hours apart and preferably on Wednesday. The second dose of ascorbic acid is scheduled to be 50 grams administered at 0.5 gram per minute in the Westwood treatment area of investigators. **Plasma sampling for ascorbate level is mandatory at this dose level. Please note further dose escalation may not be necessary if 2nd dose given reaches targeted plasma level of 350-450 mg/dL.**
- Sampling of plasma for ascorbic acid levels – Blood (10 mL in dark green top tube with sodium heparin) drawn immediately upon the completion of IVC infusion in the dose escalation phase, and immediately put on ice and transferred to the research laboratory and centrifuged at 4ºC at 1500 × g for 10 min. Plasma is taken and stored at -80ºC until analysis by HPLC for detection of ascorbate.
- Plasma remains frozen at -80˚C and placed on dry ice
- Attention: Qi Chen, PhD
- The sample processing and HPLC analysis are performed according to a method established by Levine et al. Plasma is diluted 5~1000 times in 90% methanol with 1 mM EDTA, vortex, and then centrifuged at 4ºC at 20,000 × g for 15 min. Supernatant is analyzed by Waters e2695 HPLC (Waters, Milford, MA) with electrochemical detection (ESA, Chelmsford, MA). The column used is an ODS-DABS Ul Trasphere, 5µ, 4.6 mm × 25 mm (Beckman Coulter, Fullerton, CA). Mobile phase is run at a flow rate of 1 mL/min, containing 30% methanol, 0.05 M sodium phosphate monobasic, 0.05 M sodium acetate, 189 µM dodecyltrimethyl-ammonium chloride, and 36.6 µM tetraoctylammonium bromide. The Empower II software (Waters, MA) is used for instrument control and data analysis.

Week 1 Day 5

- Review previous and concomitant medications
- Adverse events review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS

- **Monitoring of dose escalation infusions -** BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated
- IV C **up to 3 days/week for the first week** only while participant is on dose escalation phase trial.

3rd Infusion schedule for escalating doses of vitamin C/ascorbic acid

- Percutaneous intravenous catheters will be placed in all study participants for venous access approximately 30 minutes prior to infusion. If indwelling ports are available, they will be accessed according to standard protocol.
- Infusion materials will be prepared by Investigational Pharmacy or prepared in oncologist's infusion center at the participating sites and delivered to the clinic responsible for infusion by standard procedure.
- All infusions will be administered by infusion pump at a rate of 0.5 grams/minute (i.e. 50 grams = 100 minutes).

Week 1 Day 5 Ascorbic Acid Dose Escalation

- **Please note this dose <u>may not be necessary</u>** if 2nd dose given reaches targeted plasma level of 350-450 mg/dL. *Third infusion of ascorbate:* The final dose of the week will be 75 grams of ascorbic acid given at 0.5 gm/minute. <u>Plasma sampling for ascorbate level is mandatory at this dose level.</u>

Ascorbic acid plasma sample preparation:

- Sampling of plasma for ascorbic acid levels – Blood (10 mL in dark green top tube with sodium heparin) drawn <u>immediately</u> upon the completion of IVC infusion in the dose escalation phase, and immediately put on ice and transferred to the research laboratory and centrifuged at 4ºC at 1500 × g for 10 min. Plasma is taken and stored at -80ºC until analysis by HPLC for detection of ascorbate.
- Plasma remains frozen at -80˚C and placed on dry ice
- <u>Attention: Qi Chen, PhD</u>
- The sample processing and HPLC analysis are performed according to a method established by Levine et al (61,62). Plasma is diluted 5~1000 times in 90% methanol with 1 mM EDTA, vortex, and then centrifuged at 4ºC at 20,000 × g for 15 min. Supernatant is analyzed by Waters e2695 HPLC (Waters, Milford, MA) with electrochemical detection (ESA, Chelmsford, MA). The column used is an ODS-DABS Ul Trasphere, 5µ, 4.6 mm × 25 mm (Beckman Coulter, Fullerton, CA). Mobile phase is run at a flow rate of 1 mL/min, containing 30% methanol, 0.05 M sodium phosphate monobasic, 0.05 M sodium acetate, 189 µM dodecyltrimethyl-ammonium chloride, and 36.6 µM tetraoctylammonium bromide. The Empower II software (Waters, MA) is used for instrument control and data analysis.

Week 2 Day 8

- Review previous and concomitant medications
- Adverse events review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
    - **Monitoring of subsequent infusions -** BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be

carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated

- IV C continues 2 days/week while participant is on trial.
- If necessary, dose escalation may continue until plasma vitamin C level reaches targeted level of 350 – 450 mg/dL up to 125 grams of IV vitamin C.
- If dose escalation fails to reach targeted plasma vitamin C level, it will be necessary to consider surreptitious tobacco use and a cotinine level will need to be drawn to confirm or refute. If participant is found to have findings consistent with tobacco use, the participant will be removed from trial.
- IV Gemcitabine Infusion

Week 2 Day 10

- Review previous and concomitant medications
- Adverse events review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
    - **Monitoring of subsequent infusions -** BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated
- IV C continues 2 days/week while participant is on trial.

Week 2 Day 12

- Review previous and concomitant medications
- Adverse events review

Week 3 Days 15-19

- Review previous and concomitant medications
- Adverse events review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
    - **Monitoring of subsequent infusions -** BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated
- IV C continues 2 days/week while participant is on trial.

Week 4 Pre-Operative Period

- Physical exam, including weight, vital signs (temperature, pulse, SpO2, blood pressure)
- Review previous and concomitant medications
- CBC (WINDOW: + / - 7 DAYS)
    - Tests as listed in the table in SCREENING VISIT, above.
- Serum chemistries (WINDOW: + / - 7 DAYS)
    - Tests as listed in the table in SCREENING VISIT, above.
- ECOG
- Adverse Events Review
- Administer FACT-Bl QOL instrument

Week 4 Days 22-26

- Review previous and concomitant medications

- Adverse events review
- IV VITAMIN C INFUSION (WINDOW: + / - 3 DAYS)
- Monitoring of subsequent infusions - BP, pulse and other vital signs should be measured, collected/recorded prior to the start of the infusion. Patients should be carefully monitored and BP and other vital signs should be measured post infusion as clinically indicated
- IV C continues 2 days/week while participant is on trial.

Termination of Treatment Phase to be scheduled for surgery

14.1.3 End Of Treatment / Early Study Termination / Participant Study Withdrawal Visit

- Review previous and concomitant medications
- Hematology (WINDOW: + / - 7 DAYS) - Tests as listed in SCREENING VISIT, above.
- Serum chemistries: (WINDOW: + / - 7 DAYS) - .Tests as listed in SCREENING VISIT, above.
- Adverse Events review
- **Blood and Urine Sample Collection [REQUIRED for participants]**under current IRB approved bladder cancer tumor bank (Appendix C)
- **Final Biopsy** (WINDOW: + / - 7 DAYS) **[REQUIRED for participants].** Post-treatment tumor tissue collection to be done during SOC surgery under current IRB approved bladder cancer tumor bank (Appendix C)

14.1.4 Post-EOT/Surgery Follow-Up Visit(s)

EOT visit will occur during SOC post-surgical office follow-up at 42 days (approximately 6 weeks) post-operatively

- Physical exam, vital signs
- Review previous and concomitant medications
- Adverse events review
- FACT-Bl questionnaire

14.1.5 Pregnancy Follow-Up

3 months after EOT for women
6 months after EOT for men

- Phone call to check pregnancy status

14.1.6 Long Term Follow-Up

1, 3 and 5 years after EOT

- Visit or phone call to check Disease Free Survival (DFS).
- Visit or phone call to check Disease Specific Survival (DSS).

SCHEDULE OF EVENTS TABLE

Cycles = 28 days (21 days for Chemotherapy [Gemcitabine and Carboplatin] 28 days for IVC)		**Week 1**	**Week 2**	**Week 3**	**Week 4**	**EOT / Day of Surgery**	**Post-Surgery / EOT Follow-Up**	**Pregnancy Follow Up**	**Long Term Follow Up**
Protocol Activity	**Eligibility Screening and Baseline -28 to 1**	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**		**42 days (approximately 6 weeks) after surgery/ EOT) per SOC**	**Women: 6 months after EOT Men: 3 months after EOT (+/- 7 days)**	**1, 3 and 5 years after EOT**
Informed Consent	X								
Medical History	X								
Demographics	X								
Review Eligibility Criteria	X								
Review Concomitant Medications	X	With infusions				X	X		
Physical Exam (Height at screening only)	X	With infusions					X		
Weight	X				X				

Cycles = 28 days (21 days for Chemotherapy [Gemcitabine and Carboplatin] 28 days for IVC)		**Week 1**	**Week 2**	**Week 3**	**Week 4**	**EOT / Day of Surgery**	**Post-Surgery / EOT Follow-Up**	**Pregnancy Follow Up**	**Long Term Follow Up**
Protocol Activity	**Eligibility Screening and Baseline -28 to 1**	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**		**42 days (approximately 6 weeks) after surgery/ EOT) per SOC**	**Women: 6 months after EOT Men: 3 months after EOT (+/- 7 days)**	**1, 3 and 5 years after EOT**
ECOG Performance Status	X				X				
Adverse Event Assessment	X	With infusions				X	X		
FACT-BL		X Day 1			X		X		
CBC with Diff	X	X Day 1			X	X			
CMP	X	X Day 1			X	X			
Plasma sampling for		During 2nd and 3rd IVC							

Cycles = 28 days (21 days for Chemotherapy [Gemcitabine and Carboplatin] 28 days for IVC)		**Week 1**	**Week 2**	**Week 3**	**Week 4**	**EOT / Day of Surgery**	**Post-Surgery / EOT Follow-Up**	**Pregnancy Follow Up**	**Long Term Follow Up**
Protocol Activity	**Eligibility Screening and Baseline -28 to 1**	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**		**42 days (approximately 6 weeks) after surgery/ EOT) per SOC**	**Women: 6 months after EOT Men: 3 months after EOT (+/- 7 days)**	**1, 3 and 5 years after EOT**
ascorbate level assessment		infusion – note 3rd may not be necessary							
G6PD	X								
Urinalysis	X	X Day 1							
Serum Pregnancy[1]	X								
12 Lead ECG	X								
IV Ascorbic Acid Infusion		Week 1: dose escalation up to 3 doses, Days 1, 3, 5	Weeks 2-4 - maximum of 2 to 3 doses / week if dose escalation is indicated; if no further dose escalation needed then 2 doses / week						

Cycles = 28 days (21 days for Chemotherapy [Gemcitabine and Carboplatin] 28 days for IVC)		**Week 1**	**Week 2**	**Week 3**	**Week 4**	**EOT / Day of Surgery**	**Post-Surgery / EOT Follow-Up**	**Pregnancy Follow Up**	**Long Term Follow Up**
Protocol Activity	**Eligibility Screening and Baseline -28 to 1**	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**		**42 days (approximately 6 weeks) after surgery/ EOT) per SOC**	**Women: 6 months after EOT Men: 3 months after EOT (+/- 7 days)**	**1, 3 and 5 years after EOT**
Carboplatin Infusion		X Day 1							
Gemcitabine Infusion		X Day 1	X Day 8						
Urine Sample Collection REQUIRED for participants	X					X			
Blood Sample Collection REQUIRED for participants	X					X			
Tumor tissue Collection REQUIRED for participants	X					X			

Cycles = 28 days (21 days for Chemotherapy [Gemcitabine and Carboplatin] 28 days for IVC)		**Week 1**	**Week 2**	**Week 3**	**Week 4**	**EOT / Day of Surgery**	**Post-Surgery / EOT Follow-Up**	**Pregnancy Follow Up**	**Long Term Follow Up**
Protocol Activity	**Eligibility Screening and Baseline -28 to 1**	**Days 1-7**	**Days 8-14**	**Days 15-21**	**Days 22-28**		**42 days (approximately 6 weeks) after surgery/ EOT) per SOC**	**Women: 6 months after EOT Men: 3 months after EOT (+/- 7 days)**	**1, 3 and 5 years after EOT**
Phone Call to check pregnancy status								X	
Phone Call or visit to check health status									X

CORRELATIVE / EXPLORATORY STUDIES

Sample collection is REQUIRED for ALL participants.

Abstract

BACKGROUND AND SIGNIFICANCE

We will perform correlative studies to evaluate cell death and apoptosis in response to a single cycle of NAC with Vitamin C. Our underlying hypothesis is that Vitamin C will potentiate the action of tumor cell death. We will also study biomarkers related to the mechanism of action for Vitamin C as outlined below.

Objectives and Endpoints (For Correlative / Exploratory Studies Only)

Exploratory Objective(s)

Biomarkers for Cell death, IVC related biomarkers and cellular outcome biomarkers will be evaluated in tumor samples at initial resection and radical cystectomy.

Exploratory Endpoint(s)
Tumor samples will be collected at initial resection and at cystectomy for evaluation of generally used cellular outcome markers, as well as markers for IVC specific mechanism, via the IRB approved BCa tumor bank (Taylor PI). Acetylated tubulin levels and transferrin receptor levels in tumor samples will be detected as IVC related markers. SLC23A2 (SVCT2, vitamin C tissue receptor) will be assessed for drug uptake. We will use TUNEL staining as a measure of cell death and Ki67 as a measure of cell proliferation. This aim will generate information for future studies in reflective and predictive biomarkers in a larger clinical trial.

Sample Collection Instructions (For Correlative / Exploratory Studies Only)

Samples to include tumor tissue, blood and urine will be collected at the time of entry into the study as well as at the time of surgery under the IRB approved KUMC Bladder Cancer Longitudinal Biorepository for Development of Novel Therapeutics/Biomarkers (PI: John Taylor III, MD, MS, IRB#: STUDY00141546, Appendix C). Samples will be stored in Wahl Hall East 3019.

15 STATISTICAL CONSIDERATIONS

15.1 Sample Size Justification

Simon's two-stage minimax[34] will be used. Pathologic downstaging to non-invasive disease <pT2 is reported as approximately 15% with cystectomy alone. Therefore, the null hypothesis that the true response rate of downstaging is 0.15 will be tested against a one-sided alternative. Typically, with effective NAC (for cisplatin eligible cases) we might expect 30% of subjects to respond with downstaging to non-invasive disease. Although we do not know exactly what to expect with this regimen, we will therefore use 0.30 as our

desirable rate of response. In the first stage of the trial, 13 subjects will be accrued. If there are 1 or fewer responses in these 13 subjects, the study will be stopped for futility. In other words, 2 or more responses will be required to move to the second stage. In the first stage, we are looking for evidence of futility. If we do not have early evidence of futility. 8 additional subjects will be accrued for a total of 21. The null hypothesis will be rejected if 5 or more responses are observed in 21 subjects, which we will take as evidence that this treatment should be studied further. This design yields a type I error rate of .21 and power of 79% when the true response rate is 0.30. We feel this increased type I error rate is warranted by the pilot nature of this study, given that success of this study will be used to justify a larger study and estimate its power. With this design, the probability of early termination if the null hypothesis is true is approximately 0.39. The Department of Urology performs ~100 cystectomies per year with an anticipated 30-40 patients being either cisplatin ineligible or decline cisplatin based NAC. We do not foresee recruitment problems and fully expect to fulfill enrollment goals in the timeframe of the trial. However, due to funding considerations, only the first stage will be completed during the 1 year timeframe. Then, we anticipate further funding will be available to complete the analyses described in this protocol.

15.2 Description Of Statistical Methods

15.2.1 General Approach

Simon's two-stage minimax[34] design will be used. For this design, we defined a response as downstaging to non-invasive disease. During the first year, only 13 subjects for the first stage will be accrued. Then, we anticipate further funding will be available to complete the analyses described in this protocol. Assuming a successful first stage (2 or more responses), we will accrue a total of 21 subjects. Descriptive statistics will be calculated and provided for all data collected (I.e. demographic and study specific measures). These will include the mean, median, standard deviation, and histograms for all quantitative values, and frequency tables for categorical values. Additionally, the informed consent rate wand adverse event rates will be calculated and 95% confidence intervals generated to provide valuable information for any future study.

Analysis Of Primary Objective

The null hypothesis of a response rate of just 0.15 will be considered rejected if 5 or more responses are observed, albeit with a type I error rate of 0.21. However, this type I error rate is justified as the primary purpose of this study is to gather preliminary data for a larger, randomized trial, as well as getting some indication as to whether or not such a trial seems warranted. The response rate will also be calculated and a 95% confidence interval generated.

Analysis Of Secondary Objective(s)

QOL, as measured by FACT-BI will be reported using summary statistics (mean, median, standard deviation) for each time point (at enrollment, just prior to cystectomy, and post-cystectomy). DFS will be evaluated at 1 and 3 years and DSS will be evaluated at 5 years using the Kaplan-Meier method. Survival curves and median survival time will be reported.

Analysis Of Exploratory Objective(s)

Assess Mediators of Cancer Cells' Responses to IVC. As described in Preliminary Data, upregulated TfR and SVCT2 expression have been proposed to increase cancer cells susceptibility to ascorbate treatment, by increasing cellular liable iron pool and ascorbate uptake.[13,27] We will collect tumor specimens from all participants at initial resection and at cystectomy via the IRB approved BCa tumor bank. For measurement of TfR and SVCT1/2 expression levels, qRT-PCR will be used for detecting mRNA expression levels in snap frozen tumor tissues. Immunofluorescence or immunohistochemistry assay will be utilized using OCT embedded frozen tissues. The expression levels will be given numerical H scores (0-300) by pathologist review, defined as sum of staining intensity (0, 1, 2, 3) multiplied by percentage of positive stained cells. The results from those who respond to IVC treatment will be compared to non-responders.

15.3 STUDY STOPPING RULES

Based on a Simon 2-Stage Design, toxicity and efficacy will be evaluated after the first 13 subjects are enrolled on this study, prior to enrolling additional subjects.
If > 20% subjects have Grade 3 or 4 toxicity probably related or directly related to iv Vitamin C, we will hold the study for analysis of toxicity.

16 ASSESSMENT OF SAFETY

16.1 Specification Of Safety Parameters

Analyses will be performed for all participants having received at least one dose of study drug / one administration of therapy.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting and toxicity assessment. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

Information and links regarding CTCAE version 5.0 can be found at the CTEP website:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site:

https://view.officeapps.live.com/op/view.aspx?src=https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5.0.xlsx

The investigators in this study will use this document for assessing and reporting of adverse events.

16.1.1 Definition Of Adverse Events (AE)

Text below in italics is verbatim from "Guidance for Industry and Investigators. Safety Reporting Requirements for INDs and BA/BE Studies", issued December 2012 by U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. The guidance may be retrieved from:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf?source=govdelivery

Adverse Event [21 CFR 312.32(a)]
An adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Suspected Adverse Reaction [21 CFR 312.32(a)]
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Suspected adverse reactions are the subset of all adverse events for which there is a reasonable possibility that the drug caused the event. Inherent in this definition, and in the requirement to report suspected adverse reactions, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the drug actually caused the adverse event.

Unexpected [21 CFR 312.32(a)]
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application… "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the Particular drug under investigation.

This definition relies entirely on a listing of the adverse events or suspected adverse reactions in the investigator brochure…as the basis for determining whether newly acquired information generated from clinical trials or reported from other sources is unexpected. This means that events not listed for the Particular drug under investigation in the investigator brochure are considered "unexpected" and those listed are considered "expected." When new adverse event information is received, it is the sponsor's responsibility to determine whether the event is "unexpected" for safety reporting purposes.

Serious [21 CFR 312.32(a)]
An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Life-threatening
An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death

16.1.2 Relationship To Study Agent

Factors to be considered in assessing the relationship of the adverse event to study drug include:

- The temporal sequence from study drug administration: The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on discontinuation (de-challenge), recurrence on reintroduction (re-challenge): participant's response after drug discontinuation (de-challenge) or participants response after study drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the participant may have.
- Concomitant medication or treatment: The other drugs the participant is taking or the treatment the participant receives should be examined to determine whether any of them may be suspected to cause the event in question.
- The pharmacology and pharmacokinetics of the study drug: The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the test drug(s), coupled with the individual participant's pharmacodynamics should be considered.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment.

Attribution will be assigned as follows:

Unrelated – The AE is clearly **NOT** related to the study treatment.
Unlikely – The AE is **doubtfully related** to the study treatment.
Possible – The AE **may be related** to the study treatment.
Probable – The AE is **likely related** to the study treatment.
Definite – The AE is **clearly related** to the study treatment.

16.2 Reporting Procedures

Adverse Event Reporting

Information for adverse events, whether reported by the participant, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed and reported in the study Case Report Form (CRF) as described in the following sections.

ALL adverse events experienced by participants will be collected and reported as follows:

- Adverse events will be collected and reported] from the time of signing consent, during screening, then from Day 1 of study treatment and/or procedure, throughout the study, and up to and including **day 30** after the last dose of study drug and/or last study procedure.

- SERIOUS adverse events that meet the definition(s) of a serious adverse event will be collected and reported from Day 1 of study treatment and/or procedure, and up to and including **day 30** after the last dose of study drug and/or last study procedure.

Participants who experience an ongoing adverse event related to a study procedure and/or study medication beyond 30 days will continue to be contacted by a member of the study team until the event is resolved, stabilized, or determined to be irreversible by the Sponsor -investigator.

Study participants should also be instructed to report any new serious post-study event(s) that might reasonably be related to participation in this study.

Medical conditions/diseases, or cancer related symptoms present before starting study treatment are considered adverse events only if they worsen after initiation of study drug.

Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, or require therapy. In this case they will be recorded on the Adverse Events CRF, along with the associated signs, symptoms or diagnosis.

16.2.1 Recording Adverse Events And Documentation In *CRIS*

All **expected** and **unexpected** adverse events and serious adverse events occurring after the participant has signed the informed consent will be reported as described previously in this document, and must be fully recorded in the participant's case record form.

All AEs and SAEs regardless of causality must be entered in the KU implementation of Velos eResearch; at KU, called the Comprehensive Research Information System (CRIS). Unexpected and expected adverse events must be entered within 5 days and include: new unexpected adverse events; worsening baseline conditions; clinically significant laboratory findings; disease-related signs and symptoms that were not present at baseline, and any event of findings that the Investigator feels is clinically significant.

Documentation must be supported by an entry in the participant's file. A laboratory test abnormality considered clinically significant(e.g., causing the participant to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event). Each event should be described in detail along with start and stop dates, severity, expectedness, relationship to investigational product, action taken and outcome.

16.2.2 Serious Adverse Event Reporting

For serious adverse events, the clinical research site will follow local IRB policies and procedures

All SAEs regardless of causality must be entered into CRIS within 24 hours. Entering the event into CRIS will send an automatic email to the KUCC Regulatory team.

Follow-up source documentation is required within 5 days.

Send all supporting documents with a cover sheet to:

KUCC DSMC
Email: *kucc-dsmc@kumc.edu*

16.2.3 Summary Of Expedited Serious Adverse Event Reporting

	Relationship to Study Drug	**IRB**	**CRIS (KU DSMC and PI)**	**FUNDER**
Unexpected SAE/AESI	Related	Per local IRB reporting policy	24 hrs	24 hrs
Unexpected SAE/AESI	Not-related	Per local IRB reporting policy	24 hrs	24 hrs
Expected SAE/AESI	Related	Per local IRB reporting policy	24 hrs	24 hrs
Expected SAE/AESI	Not-related	Per local IRB reporting policy	24 hrs	24 hrs

Submitting IND Safety Reports To FDA

The University of Kansas Cancer Center Regulatory Affairs Department is delegated by the Sponsor-Investigator to report any IND safety report any suspected adverse reaction that is both serious and unexpected. Before submitting this report, the sponsor-investigator needs to ensure that the event meets all three of the definitions contained in the requirement:

- Suspected adverse reaction
- Serious
- Unexpected

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The following reports require expedited reporting.

- unexpected fatal or life-threatening adverse experiences associated with the use of the drug are to be reported by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32©(2)]
- any adverse experience associated with the use of the drug that is both serious and unexpected is to be reported in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]

16.2.4 Reporting Of Pregnancy

Any pregnancy occurring on study must be reported as an adverse event.

In the unlikely event pregnancy occurs, pregnancy outcomes will **not** be followed.

17 DATA AND SAFETY AUDITING AND MONITORING

DSMC Oversight And Monitoring Plan

The multidisciplinary KUCC Data and Safety Monitoring Committee (DSMC) is charged with overseeing the monitoring of participant safety, conduct and scientific progress of research protocols, and the validity and integrity of the data for clinical trials. The KUCC DSMC has the authority to require amendments, suspend, or terminate any research activities that fall within its jurisdiction, and can institute other appropriate actions as needed to protect participant safety.

The study will be audited by the DSMC at appropriate intervals, no less than those assigned by the KUCC Protocol Review and Monitoring Committee (PRMC), to assure compliance to GCP and to assess the data quality and study integrity.

This trial will also be monitored by CTO Clinical Site Managers, which will occur as described in a separate monitoring plan for the trial.

The investigator and staff are expected to cooperate and provide all relevant study documentation in detail at each site visit on request for review. The study monitor will have direct access to source data for data verification. Data verification will be conducted by comparing the data entered into the CRFs with source data

Data Management

Web-based eCRFs will be used to collect patient data in this study. All eCRFs and resulting data will be developed and maintained in a manner consistent with currently available regulations and guidance pertinent to the use of computerized systems in clinical trials. All Sponsor, Sponsor designees, and study-site users of the eCRF system will be trained prior to the use of the system. A complete review of source documentation and safety data will be conducted at each monitoring visit for verification that all information recorded in the eCRF accurately reflects the data recorded in the subject's source documents.

Safety Review And Oversight Requirements

Serious Adverse Event

Serious adverse events that require expedited reporting will be reviewed by the DSMC Chair or designee who will determine if immediate action is required. If determined to be necessary by the DSMC, all participating sites will be notified of the event and any resulting action within one working day of this determination.

Review Of Serious Adverse Event Rates

Once per month, serious adverse event rates will be monitored by the DSMC Coordinator. If any study site has had 2 or more of the same SAE reported within one month, or more than 6 of the same SAE in 6 months, the DSMC will review summaries of SAEs, and discuss events in detail with the PI. The DSMC chair or designee determines whether further action is required. The sponsor-investigator, in collaboration with the DSMC Coordinator ensure that collaborating investigators and IRBs for all participating sites are notified of any resulting action.

Study Safety And Progress

An overall assessment of toxicities as described in the protocol is reviewed at DSMC meetings. This review enables DSMC committee members to assess whether significant risks are occurring that would warrant study suspension/closure or protocol amendment.

The DSMC is an autonomous committee. However, its actions are communicated to other committees engaged in oversight of clinical research at KUCC. The PI is responsible for forwarding all DSMC letters, including those recommending continuation of the study, to the IRB and PRMC. DSMC recommendations for modifications to the trial are forwarded to the Deputy Director of KUCC. The PI is notified of this recommendation, and is expected to alert all collaborating investigators about the DSMC action. At this time the PI may appeal the Committee’s decision to the Deputy Director of KUCC or their designee. The Deputy Director of the KUCC or their designee will notify the PI if he/she concurs with the DSMC’s recommendation, including suspension or closure.

18 REGULATORY CONSIDERATIONS

18.1 Protocol Review And Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The sponsor- Investigator will disseminate protocol amendment information to all study team members.
All decisions of the IRB concerning the conduct of the study must be made in writing.

19 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

19.1 Ethical Standard

Ethics and Good Clinical Practice (GCP)
This study is to be conducted according to the following considerations, which represent good and sound research practice:

1. State laws
2. ICH Consolidated Good Clinical Practice: Guidelines (E6)
3. US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

With attention to the following specific regulations:

a. Title 21 Part 50 – Protection of Human Subjects
b. Title 21 Part 56 – Institutional Review Boards
c. Title 21 Part 312 – Investigational New Drug Application Responsibilities of Sponsors and Investigators

4. Institutional research policies and procedures:

19.2 Informed Consent Process

Informed Consent
All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a

copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

20 DATA HANDLING AND RECORD KEEPING

20.1 Data Collection And Management Responsibilities

Case report forms (CRFs) will be completed for each participant enrolled and registered on this study. All CRFs will be customized per this study, in order to emphasize completeness and accuracy. The medical chart and any other clinical worksheets, procedural reports, etc. will be the source documentation of data captured into the study database.

20.2 Study Records Retention

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified. Original source documents supporting entries in the case report forms include but are not limited to hospital records and clinic charts, laboratory and pharmacy records, ECG, signed ICFs, participant diaries and pathology reports. All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

20.3 Protocol Deviations

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. All deviations must be reported to the IRB according to the local reporting policy.

21 APPENDICES

Appendix A: Performance Status

ECOG Performance Status

Developed by the Eastern Cooperative Oncology Group, Robert L. Comis, MD, Group Chair.*

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5:649-655.

Appendix B: Quality Of Life Questionnaire(s)

The FACT-BL questionnaire will be maintained in this study as a separate, stand alone document.

Appendix C: Bladder Cancer Longitudinal Biorepository Protocol – IRB Approved HSC # STUDY00141546

Investigator Initiated Trial
Bladder Cancer Longitudinal Biorepository for Development of Novel Therapeutics/Biomarkers

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Protocol Number: GUB-BCR-001
Study Type: Registry
Initial Version: August 9, 2017

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LIST OF ABBREVIATIONS

SID	Study Identification
KUMC	University of Kansas Medical Center
U	Urine
S	Serum

T Tissue
M Month
HSC# Human Subjects Committee #

1.0 STUDY SUMMARY

1.1 AIM AND HYPOTHESIS

To develop a comprehensive tumor bank that includes a fully annotated biorepository with longitudinal data points/samples to allow for more robust research into targetable pathways involved in tumorigenesis, biomarker discovery and potential for novel therapeutics to enhance patient outcomes. This is a non-treatment study. It represents a longitudinal convenience sample.

BACKGROUND AND RATIONALE

2.1 DISEASE BACKGROUND

Study Significance: Bladder cancer is the 3rd most common solid tumor in men and the 8th in women (1). There will be an estimated 76,960 new cases and 16,390 deaths in 2017. The reported 5-year survival rates for localized and metastatic bladder cancer are 69% and 6%, respectively (2). Treatment advances resulting in significant changes in outcomes have been absent over the past several decades. In the same time, advances in our ability to critically assess the molecular and genetic nature of human disease have exploded. This has resulted in significant improvement in the stratification, management and outcomes for many other cancers, most notably breast. The majority of recent advances in cancer care have resulted from molecular/genetic evaluation of large biorepository based tissue specimens. Findings from a comparatively small cooperative data set have led to significant advances in the understanding of the molecular events associated with bladder cancer tumorigenesis as well as identification of lead candidate pathways for targetable molecules (3).

2.2 RATIONALE

Critical evaluation of biorepository specimens have led to improved understanding of molecular alterations and genetic changes found in cancer, including bladder. Even so many of these studies are limited due to 1) the relatively large number of samples necessary to give meaningful results 2) the multi-institution nature of the studies suggesting high variability in tissue handling/preservation 3) the use of paraffin embedded samples limiting the use of several research techniques 4) the variable amount of tumor tissue required in the sample to be deemed adequate and the lack of longitudinal data for recurrent disease such as bladder cancer. This protocol is being designed to overcome the shortcomings of the large datasets that have been evaluated to date. The additional resources of blood and urine are also a tremendous and novel resource.

3.0 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVES

The goal of this project is to develop a robust repository of patient samples representing diagnosis, treatment and long term outcome in order to study tissue characteristics and how they are related to

tumor development, recurrence and survival. These form critical leads for the development of novel biomarkers of disease outcomes, as well as potential for targetable pathways/molecules for novel drug/therapy development

4.0 PATIENT ELIGIBILITY

4.1 INCLUSION CRITERIA

1. Patients must be 18 years of age and older
2. Patients who present to clinic with presumed bladder cancer or have a diagnosis of bladder cancer are eligible to participate
3. Patients can participate multiple times in tumor bank throughout the duration of their care
4. Patients can participate in any additional research studies during the patients' participation within this protocol.

4.2 EXCLUSION CRITERIA

1. Patients who are under 18 years old are not allowed to participate
2. Patients who do not have presumed bladder cancer will not be eligible

4.3 WITHDRAWL/TERMINATION CRITERIA

Patients may withdraw from the study at any time. If patient cancels permission to use their specimens or health information the research team will stop collecting any additional information about them moving forward, and any of the subjects' identifiable specimens will be destroyed. The research team may use and share information that was gathered before they received the patients withdraw. The entire study may be discontinued for any reasons without the patients consent by the investigator conducting the study.

5.0 STUDY PROCEDURES

5.1 SCREENING/BASELINE PROCEDURES

1.1.1 PATIENTS WILL PRESENT TO CLINICS FOR TREATMENT OF BLADDER CANCER. PATIENTS WILL BE IDENTIFIED BY CLINICAL CARE TEAM AS AN ELIGIBLE CANDIDATE FOR STUDY PARTICIPATION. THE STUDY PARTICIPANT WILL BE PRESENTED WITH THE STUDY DETAILS AND GIVEN OPPORTUNITY TO ASK QUESTIONS. IF PATIENT DESIRES TO PARTICIPATE IN REGISTRY THE CONSENTING PROCESS WILL BE COMPLETED AT THAT TIME BY A MEMBER OF THE RESEARCH TEAM. PATIENTS WILL BE ASSIGNED A STUDY ID GENERATED BY THE STUDY TEAM TO BE USED TO LINK THE PATIENT WITH THEIR BIOLOGICAL SPECIMENS AND DATA. THE PATIENT WILL NEED TO CONSENT TO THE COLLECTION OF ALL THREE TYPES OF SPECIMENS

(BLOOD, URINE AND TISSUE). ALL TIME POINTS WILL BE FROM TIME OF CONSENTING. PLEASE SEE STUDY CALENDAR BELOW TO DESCRIBE TIME POINTS AND COLLECTION.

Patients' demographics and eligibility criteria will be reviewed prior to study participation to ensure patients qualify for study entry.

5.2 STUDY IDENTIFICATION

Patient study identification (SID) will be created by study team at the time the patient consenting and is enrolled into the study. SID will be the HSC# followed by number in chronological order. For example: HSC#-000X-9999.

The samples collected for each patient will include their SID along with the type of collection and number X (Serum (SX), Urine (UX), and Tissue (TX)) followed by the time point, along with the month (MX) of collection. Time points will include month (M), diagnosis (D) , 0, 3, 6, 9, 12, 18, 24. i.e. such as HSC#-0001-S1-M6. This refers to patient 1 serum collection at month 6.

5.3 STUDY PROCEDURES

After the patient is consented to participate and a SID is assigned, the patient will have a screening visit. The diagnosis sample collection will include blood, urine and tissue (only tissue if applicable). Tissue will be collected as available. Patients will continue to be seen in clinic per routine clinical standard of care. During patients, routine follow up appointments additional samples will be collected per the study calendar. Tissue will be collect at any time during patients care and is not necessary affiliated with a designated time point.

The sample collection will include blood, urine and tissue (as collected). Blood collection will included approximately 5 tablespoons of blood. Blood collection will be collected by a phlebotomist, or during routine lab draws. This patient population can sometimes have troubles with voiding and therefore an adequate urine sample might not be able to be collected. A maximum of 50mL of urine will be collected and a minimum of 8mL. Urine volumes will vary. Tissue will be collected per standard of care, adequate amount will be provided to pathology for clinical diagnosis and remaining tissue will be provided to the study for sample collection. Tissue samples can be collected from standard of care treatments such as biopsies, surgeries, or any procedure that may result in removing tissue from the patient. Tissue amounts will vary for each patient collection. Specimens will be collected in a biological specimen banking fashion. Specimens thus collected will be candidates for any and all forms of subsequent testing.

Patients will have tissue removed per standard of care at required time points for patient's standard of care treatment. No tissue will be removed from the patient solely for research purposes. Blood and Urine specimens will be provided from the patient for research purposes at specified time points listed above in the study calendar. All patient specimen collection and processing will not be billed to the patient. If patient is already having a blood draw due to standard of care, additional blood will be collected at that time. Insurance will cover the patient's standard of care lab draw and tissue acquisition.

See Laboratory Section for Sample Collection Details

5.4 SCHEDULE OF EVENTS

Visits:	Screening (0)	Diagnosis (D)	3 Months (M3)	6 Months (M6)	9 Months (M9)	12 Months (M12)	18 Months (M18)	24 Months (M24)	Recurrence**
Informed Consent	X								
Inclusion/Exclusions	X								
Demographics	X								
Blood Collection		X	X	X	X	X	X	X	X
Urine Collection		X	X	X	X	X	X	X	X
Tissue Collection		A	A	A	A	A	A	A	A

Footnotes: All visits can be ±2 weeks
A = Tissue collected at any time point during study participation will be collected for the tumor bank. Remaining tissue after clinical care and clinical diagnosis will be collected
D = Diagnosis: The timepoint diagnosis can be at any point a patient has their initial diagnosis for bladder cancer or any recurrance.
Recurrance**: Each time a patient has a new bladder cancer recurrance the study calendar starts over at the Diagnosis (D) timepoint
Screening (0): Screening occurs at the time patient is consented. This can happen anytime prior (up to day of) patient being diagnosis (initial or recurrence), where tissue is being collected. Some patients may begin participation after initial bladder cancer diagnosis.

6.0 LABORATORY

1.1.2 6.1 LABORATORY ALL SAMPLES PROVIDED FROM PATIENT FOR RESEARCH PURPOSES WILL BE LABELED WITH STUDY IDENTIFIERS PROVIDED AT TIME OF CONSENTING AND INFORMATION WILL BE COLLECTED IN A DE-IDENTIFIED FASHION. WE WILL BE UTILIZING VELOS(CRIS) DATABASE TO STORE THE NAME AND MRN OF PATIENTS TO LINK THE PATIENT TO THE STUDY IDENTIFIERS, AND SPECIMENS WILL BE LABELED WITH STUDY ID. SPECIMENS WILL BE STORED IN A LOCKED LABORATORY IN -80 DEGREE FREEZERS UNTIL SPECIMENS ARE UTILIZED OR DESTROYED. SPECIMENS WILL BE KEPT FOR RESEARCH PURPOSES FOR APPROXIMATELY 20 YEARS. ONLY TRAINED AND QUALIFIED PERSONNEL OF THE RESEARCH TEAM WILL HAVE ACCESS TO THE STUDY RELATED INFORMATION AND REDCAP DATABASE. DATA FOR STUDY WILL BE KEPT IN A PASSWORD PROTECTED REDCAP DATABASE. SAMPLES WILL BE COLLECTED DURING ROUTINE CLINICAL VISITS. ALL RESEARCH SAMPLES WILL BE COLLECTED AND LABELED WITH A STUDY ID, AND SPECIMEN COLLECTION INFORMATION SUCH AS: DATE OF COLLECTION, TYPE OF COLLECTION, HSC/STUDY; INITIALS, DOB, TIME POINT, AND ANY ADDITIONAL INFORMATION AT PI/TREATING PHYSICIAN DISCRETION. STORAGE FOR ALL SAMPLES WILL BE FOR UP TO 20 YEARS.

ALL SPECIMENS WILL BE BEHIND LOCKED DOORS AND ONLY SPECIFIED STUDY TEAM MEMBERS WILL HAVE ACCESS TO SPECIMENS.

1.1.3

Information will only be shared with the trained and approved research team and staff. Samples will be used for appropriate research studies. It is not possible to anticipate the scope of future research but will likely fall under general categories of pathway/biomarker/novel therapeutic discovery.

Once samples are obtained and stored in the biospecimen repository, samples can be sent to internal and external qualified collaborators and will contain no identifiable information. Qualified collaborators will be approved by the principal investigator and vetting through the research manager. Samples from the biorepository will not be unnecessarily depleted, and KUMC faculty and staff will get priority over outside collaborators.
Clinical manager and principal investigator are responsible for training and ensuring samples are tracked and that PHI or any identifiers are not sent with tissue when requested from the biospecimen repository. Qualified researchers can submit a request to use the stored samples. A committee will review each request. There will also be an ethics review to ensure that the study is necessary and proper. Researchers will not be given your name or any other information that could identify you

Clinical manager and principal investigator will make the decision regarding the necessary steps should any samples be returned to the biospecimen repository.

6.2 SERUM COLLECTION

Serum collection will be collected during routine clinic visits. After collection, lab kits will be provided to the lab with the appropriate label and identifiers. Invert tube 5 times rapidly to thoroughly mix sample. Allow blood to clot by leaving tube upright for 30 minutes at room temperature prior beginning the processing of the blood. Centrifuge sample for 5 minutes at 1500 rpm at 4 degrees Celsius. Aliquot 200µL serum into tubes using a pipette into 1.5mL microcentrifuge tubes. Blood tubes will be labeled with the patient study ID. Patient study ID are assigned by HSC#- (000X-9999): Following the Study ID will be the sample type and time point. The sample type and number (Serum (S), Urine (U), Tissue (T)). Time points will include month (M), D (diagnosis), TR (treatment), 0, 3, 6, 9, 12, 18, 24. i.e. HSC# 0001 S1 3M would be patient 1, serum #1, 3 months sample. Samples will be stored in the patient specific freezer box The box will be labeled with patient study ID, and HSC# and type of sample. Remove buffy coat by gently removing the white layer (blood and platelets) with a pipette tip. Store in one individual microcentrifuge tube that is appropriately labeled. Store all tubes at -80C freezer located in the lab.

6.3 URINE COLLECTION

Urine Collection will be collected during routine clinic visits. Urine samples will be provided in a urine collection cup. Urine volumes collected will be between 8-50mL. Remove the cap from the urine specimen collection. Aliquot 1mL samples into microcentrifuge cryotubes. Tubes will be labeled as state above in patient identification before aliquoting. Sample will be stored in the patient specific freezer box – The box should be labeled with patient study ID and URINE. Store all tubes at -80C freezer located in the lab

6.4 TISSUE COLLECTION

Tumor specimens will be collected during routine clinical care. Tissue will be sent to Pathology for clinical care/diagnosis. Remaining tissue will be sent to the lab for storing and process. Tumor samples will be stored OCT embedding fluid in an embedding block and stored at -80C freezer. Place tissue in embedding block and immerse with OCT compound. Samples should be labeled with Patient SID: HSC#(000X-9999): Followed by the sample Type (Tissue (T), followed by procedure type (i.e. TURBT, PATH) and date of acquisition.

7.0 REGULATORY REQUIREMENTS

7.1 PROTOCOL REVIEW AND AMENDMENTS

The protocol, the proposed informed consent and all forms of participant information related to the study and any other necessary documents must be submitted, reviewed and approved by the University of Kansas Medical Center (KUMC) properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The Principal Investigator will disseminate protocol amendment information to all participating investigators. All decisions of the IRB concerning the conduct of the study must be made in writing. A yearly continuing review will also be submitted to the IRB.

7.2 Informed Consent

Any patient who is present a consent form that describes this study will be provided the most up to date consent form. The purpose of the consent form is provide sufficient information to the study participant in order to make an informed decision about their participation in the study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

Subjects will have time to ask any questions and will be given the opportunity to review the consent form in the absence of members of the study team prior to signing. Our team assures the informed consent process is an ongoing exchange of information between our research team and the study participants throughout the course of the research study. Patients who are interested in learning about participation in our trial will be introduced to a member of the study team who will then verbally explain the study in detail. Our process ensures respect for persons through provision of thoughtful consent for voluntary participation. Information will be presented in a manner that will enable the participant to voluntarily decide whether or not to participate as a research subject. Our procedures used in obtaining informed consent will educate the subject population in terms that they can understand. Therefore, our informed consent language and its documentation are written in "lay language" and presented in a way that facilitates understanding. It will be explained to the subjects that the study is voluntary and that they may discontinue at any time without prejudice. Subjects will have the opportunity to ask questions before signing the consent form. Subjects who agree to participate will be consented according to our

institution's guidelines and in a private, closed-door setting. The consent document will be revised when deficiencies are noted or when additional information will improve the consent process. Informed consent will be documented by the use of a written, HSC-approved consent form and signed and dated by the subject or the subject's legally authorized representative. A signed copy will be given to the person signing the form. Consent forms, and all other study documentation, will be retained in accordance with the KUMC Research Records Retention Policy. This policy states, in part, that research records are to be retained by the investigator for a minimum of fifteen years.

Participation in the study is entirely voluntary. Subjects will have time to ask any questions and will be given the opportunity to review the consent form in the absence of members of the study team prior to signing (further details above).

Since potential subjects will be identified by their clinical care team during the course of routine care, the treating provider and care team will already have determined whether the subject is able to give informed consent prior to screening for participation.

7.3 ETHICS AND GOOD CLINICAL PRACTIVE (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice. All personnel will have completed all required GCP training. The study conduct will follow the ICH E6 Good Clinical Practice Guidelines. Along with the US Code of Federal Regulations (CFR). The conduct will follow all state, federal, local, and institutional laws, regulations, and policies.

It is understood that deviations from the protocol should be avoided, except with necessary to eliminate an immediate hazard to the research participant. In the instance of a deviation, it must be reported to the IRB according to the local reporting policy.
Out of window deviations will not be report to the IRB as they are not considered a risk/hazard to the patient.
This protocol is a registry and will not report deaths as serious adverse events (SAEs).

8.0 PATIENT PROCEDURES

8.1 PATIENT REGISTRATION

Patient registration will occur at the time of consenting. Patients will be assigned a SID at time for consenting. Patient SID will be added to Velos(CRIS).

Patient demographics will be added to the redcap. All completed source documents for patient eligibility verification and registration will be kept in a study binder for monitoring and documentation purposes. Consenting process will also be documented.

9.0 STUDY MANAGEMENT

9.1 INVESTIGATOR FILESAND RETENTION OF DOCUMENTS

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified. Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinic charts, laboratory, ECG, signed ICFs, participant diaries, pathology reports and any other related documents. All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

9.2 CASE REPORT FORMS

Case report forms will be completed for each subject enrolled. All data will be entered and stored in a KUMC RedCap database. Only trained and approved personnel will have access to this database. Any source documents or additional research related items will be stored behind locked doors or on the R Drive within the study folder.

9.3 DATA MANAGEMENT AND SECURITY

The PI and research team will be responsible for handling the data and will allow study personnel to have access to study data within the KUMC REDCap database as necessary. Study data will be stored in a secure KUMC REDCap database. Permission will only be granted to trained and qualified study team members. Human subjects will be identifiable directly within the secure Velos(CRIS) system and information will be kept in REDCap database (excluding MRN# and Names). Study team will be utilizing velos to link the patient with the study ID. This is a locked and secure database and only persons granted permission/access will be able to view this system. Data will be stored in Velos, RedCap, R Drive and within the Research Urology Offices.

Patient information from the medical record will be stored in a REDCap database outside of the medical record. Study team will abide by all HIPAA privacy laws and will do everything they can to ensure confidentiality of patients participation and information about sample collection, processing, storage, data, etc.

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22 LITERATURE REFERENCES

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