SYLVESTER COMPREHENSIVE CANCER CENTER
UNIVERSITY OF MIAMI HEALTH SYSTEM

MILLER SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

IRB # 20130896

A Phase II Study Of Chemoradiation For Bladder Preservation In Patients With Muscle Invasive Bladder Carcinoma After Complete Response To Neoadjuvant Chemotherapy.

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OTHER CONTACT INFORMATION

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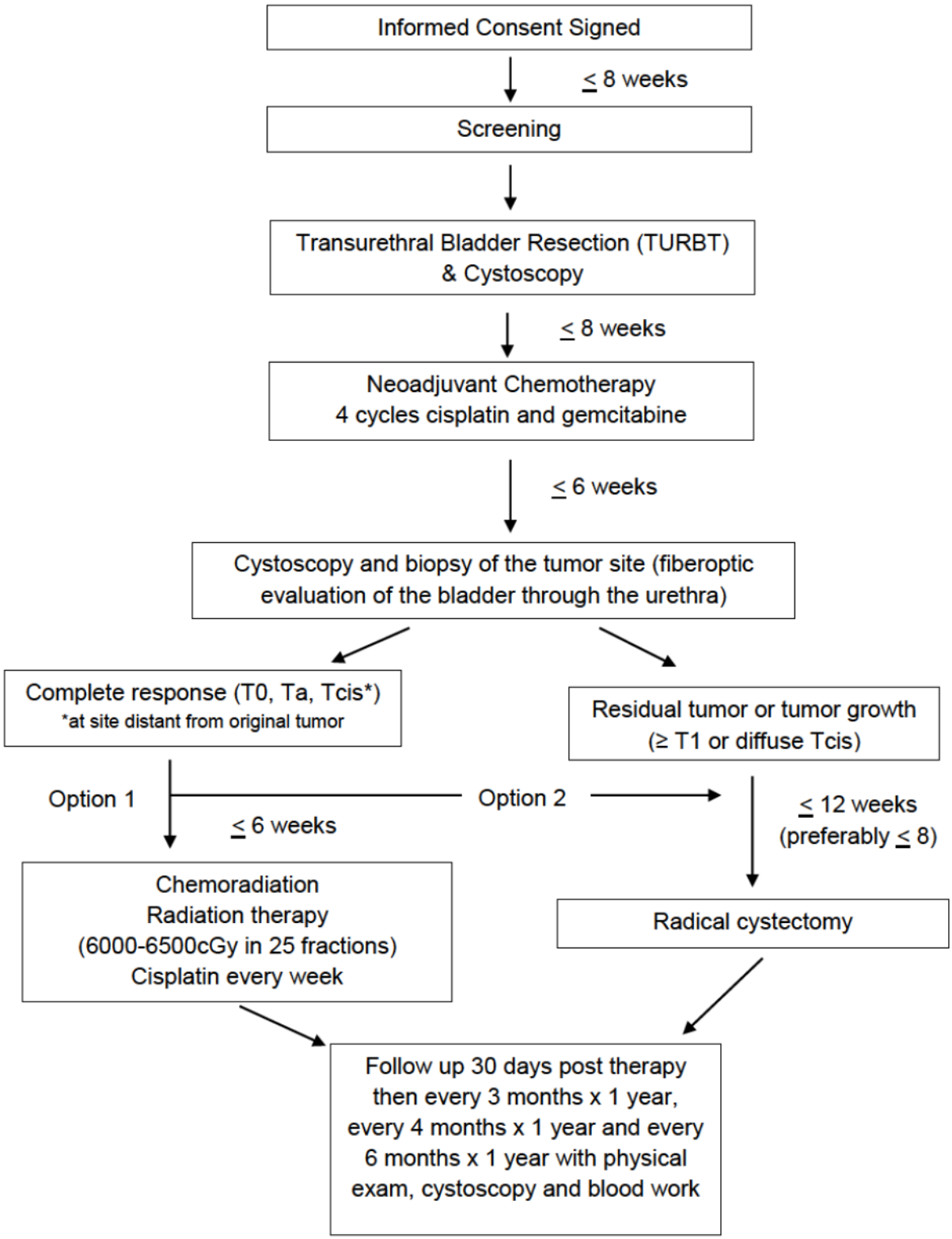
SCHEMA:


Informed Consent Signed
≤ 8 weeks
Screening
Transurethral Bladder Resection (TURBT) & Cystoscopy
≤ 8 weeks
Neoadjuvant Chemotherapy
4 cycles cisplatin and gemcitabine
≤ 6 weeks
Cystoscopy and biopsy of the tumor site (fiberoptic evaluation of the bladder through the urethra)
Complete response (T0, Ta, Tcis*)
*at site distant from original tumor
Residual tumor or tumor growth (≥ T1 or diffuse Tcis)
Option 1
≤ 6 weeks
Option 2
≤ 12 weeks (preferably ≤ 8)
Chemoradiation
Radiation therapy
(6000-6500cGy in 25 fractions)
Cisplatin every week
Radical cystectomy
Follow up 30 days post therapy then every 3 months x 1 year, every 4 months x 1 year and every 6 months x 1 year with physical exam, cystoscopy and blood work

HYPOTHESIS:

Bladder preservation in patients with complete response after neoadjuvant chemotherapy will lead to equivalent or superior relapse free rates compared to cystectomy rates from historical controls.

1.0 BACKGROUND/RATIONALE

Radical Cystectomy for definitive management of muscle invasive bladder cancer:

Radical cystectomy is considered the current standard of care for treatment of medically operable patients with muscle invasive bladder carcinoma. In absence of randomized studies comparing bladder preservation to cystectomy, review of the largest radical cystectomy series provides comparison to bladder preservation approaches. In the largest published series by Stein, et al, 1054 patients showed 5 year recurrence free and overall survival of 68% and 66% respectively (1). Another large single institution series of 507 patients who underwent radical cystectomy alone with no neoadjuvant radiochemotherapy showed 5 year recurrence free and overall survival rates of 73% and 62%, respectively, in those with node negative organ confined disease (2). These and other series provide an estimate of approximately 45-65% long term survival with this approach (3) while producing significant morbidity and changes to quality of life due to the absence of intact bladder. Therefore, alternate approaches including bladder preservation are greatly needed.

Neoadjuvant chemotherapy in muscle invasive bladder cancer:

There is randomized evidence showing an absolute survival benefit with neoadjuvant chemotherapy prior to cystectomy in patients with muscle invasive bladder cancer. Pooled Nordic collaborative group analysis of 620 patients involved either neoadjuvant platinum with adriamycin or methotrexate. Overall survival was 56% at five years in patients receiving chemotherapy versus 48% in those with cystectomy alone. Data from eight randomized controlled trials using cisplatin based combination chemotherapy demonstrated an improved absolute overall survival benefit from 50% to 56.6% (4). A recent meta-analysis of 11 randomized trials including 3005 patients assessing neoadjuvant chemotherapy showed that platinum-based combination chemotherapy was associated with a significant (5%) overall survival benefit, and a 9% increase in disease free survival at 5 years (5).

While a subset of patients undergoing neoadjuvant chemotherapy demonstrates a complete clinical response, there are no clinical or molecular biomarkers capable of predicting this outcome. There is need for these markers as those with complete response (CR) post-neoadjuvant chemotherapy demonstrate improved outcome after definitive management. In a recent study of 317 patients with muscle invasive disease, 38 percent of patients that received neoadjuvant MVAC chemotherapy were pathologically free of cancer (pT0) at the time of surgery. By contrast, only 15 percent of patients who received cystectomy alone were free of disease at the time of surgery ($p<0.001$). The median survival among patients assigned to surgery alone was 46 months, as compared with 77 months among patients assigned to combination therapy ($P=0.06$ by a two-sided stratified log-rank test). At five years, 85 percent of the patients with a pT0 surgical specimen were alive (6). Supporting data from Sternberg showed 51% of patients had a CR or superficial disease after 3 cycles of MVAC. These patients also had a dramatically improved 5 year overall survival of 71% versus 29%. This data highlights the significance of CR to neoadjuvant chemotherapy, and supports further diagnostic tools to predict response to this treatment (7).

Chemoradiation for Bladder Preservation:

The efficacy of neoadjuvant chemoradiotherapy to select patients with muscle invasive bladder cancer for bladder preservation has been established. Initial TURBT with maximal safe resection is required as incomplete resection portends an unfavorable prognosis (8,9). Neoadjuvant chemoradiotherapy is then delivered with subsequent evaluation by cystoscopy for response. Those patients with CR to neoadjuvant therapy proceed to completion radical chemoradiation and those without CR are advised to undergo radical cystectomy (10-12). Neoadjuvant regimens using cisplatin and 5FU have achieved 67% CR prior to consolidation chemoradiation, and 66% three year overall survival with an intact bladder, and 83% actuarial OS after radical bladder preservation (13). Other regimens that used paclitaxel within the neoadjuvant and consolidation phases with BID fractionation regimen achieved post-neoadjuvant CR of 81% and a 3 and 5 year overall survival rate of 67% and 56%, respectively. Five year DSS was 71%. 47% survived with intact bladder at 5 years (14). This approach has been evaluated in a larger cohort through RTOG 0233 with BID fractionated radiotherapy and a comparison between cisplatin + 5FU and paclitaxel + Cisplatin. Highest CR rate shown was 87% using cisplatin/paclitaxel and 40.3Gy (at 1.5/1.6Gy per fraction daily). These patients had a 4 year OS rate with intact bladder of 73%,

demonstrating equivalent to improved OS rates when compared to large surgical series (15). The overall MGH experience, reported in 2012 demonstrated 72% CR with neoadjuvant therapy and a 5 year DSS and OS of 64%, and 52%, respectively. The most recent pooled analysis of bladder preservation series by Mak et al reviewed 468 patients with MIBC enrolled in six RTOG bladder preservation protocols. 5 year DSS was 79% in patients with CR versus 56% for those without CR (16). These data demonstrate chemoradiation for bladder preservation is a feasible approach in appropriately selected patients and that this treatment portends equivalent survival to large surgical series.

Neoadjuvant chemotherapy alone followed by bladder preservation:

Neoadjuvant chemotherapy alone followed by bladder preservation approach has been investigated recently with encouraging results. (Apatero et al 2012. Urology 80:1056-1062.) In non-randomized data from 2 bladder sparing approaches in T2-4 disease, 41 patients were treated with three cycles of neoadjuvant MCV followed by 60Gy alone in those with CR. 39pts were subsequently treated with weekly cisplatin concurrent with RT to 64.8Gy. Five year cumulative OS was 73% and cancer specific survival was 82%. 83% of all patients kept their bladders intact. CR was higher in the chemoradiation arm, however, the neoadjuvant CT arm did not receive CT concurrently with RT definitively so these groups could not be compared directly for rate of CR. While there was no benefit in survival or rate of metastases in the neoadjuvant chemotherapy arm versus concurrent chemoradiation arm, bladder preservation with either schema achieved equivalent to improved 5 year OS rates to modern surgical series(17). Recent single institution data delivering neoadjuvant cisplatin plus gemcitabine followed by concurrent chemoradiotherapy for muscle invasive bladder cancer also suggests this approach is feasible. 78% (32/43) achieved CR at the time of cystoscopic evaluation after three cycles of cisplatin/gemcitabine alone. OS was 61% at 36 months after concurrent radical chemoradiation with 65Gy and weekly cisplatinum (18). Finally, retrospective analysis of 94 patients with T1-T2aN0M0 bladder TCC treated with neoadjuvant chemotherapy followed by radical radiation showed 84% bladder preservation rates with survival comparable to modern cystectomy series (19). This data suggests patients with complete response after neoadjuvant chemotherapy may select patients most suitable for bladder preservation. This warrants further validation in the setting of a clinical trial.

Patient selection for radical chemoradiation for bladder preservation:

Correct patient selection for bladder preservation is paramount for achieving durable response. Prognostic factors for survival after treatment with radiotherapy

with or without chemotherapy include grade, T stage, tumor bulk, hydronephrosis, age, T category, serum creatinine, lymphatic invasion, and extent of TURBT (20-23). Advanced T category, large tumor size, the presence of an extravesical mass and hydronephrosis have all been associated with incomplete response to radiation or local disease recurrence. The benefit of definitive radiotherapy with bladder preservation is likely limited in patients with pretreatment bladder function is compromised and in those with high risk of acute or long term treatment complications. Those with irritable bladder symptoms at presentation secondary to outflow obstruction, chronic infection, multiple prior transurethral resections, or prior intravesical chemotherapy may have permanent impairment of bladder function after radiation that reduces or negates the effect of organ preservation. The ideal candidate for curative bladder preservation therefore is one with a small solitary tumor up to 5cm with no associated carcinoma *in situ* (CIS), no lymph node metastases and a normal functioning bladder. The patient must also be committed to long term bladder surveillance and be willing to have further treatment for new disease (24-26). These data support bladder preservation after complete response by neoadjuvant chemotherapy as those with low bulk have the best prognosis with a chemoradiation approach. This supports our current trial design of selecting those with complete response for bladder preservation.

2.0 OBJECTIVES

2.1 Primary objectives

To evaluate the rate of failure free survival with intact bladder (FFSIB) at two years in patients undergoing bladder preservation.

2.2 Secondary objectives

1. To estimate the two year rate of failure free survival (FFS). This will include locoregional recurrence, and distant metastases.
2. To estimate the rate of acute and late grade 2 or higher (CTCAE v4.0) treatment related GU, GI and hematologic toxicity of bladder preservation.
3. To evaluate the quality-of-life at baseline, after neoadjuvant therapy and after definitive management using the SF-12 and EPIC questionnaires.
4. To estimate the overall survival (OS) in patients undergoing bladder preservation.

2.3 Exploratory analyses:

1. To evaluate known predictive and prognostic biomarkers for complete response to neoadjuvant chemotherapy and bladder preservation. Blood, urine and tumor tissue will be collected pre- and post-neoadjuvant

chemotherapy, post-cystectomy or chemoradiation, and at any time point of distant metastases.

2. To perform exploratory molecular analysis to identify new predictive and prognostic biomarkers for response to neoadjuvant chemotherapy, and bladder preservation. Blood, urine and tumor tissue for muscle invasive bladder cancer. Blood, urine and tumor tissue will be collected pre and post-neoadjuvant chemotherapy, post-cystectomy or chemoradiation, and at any time point of distant metastases.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Pathologically proven diagnosis of primary carcinoma of the bladder(transitional cell cancer). Must be operable patients with muscularis propria invasion and AJCC clinical stages T2-4a, N0 or N+, M0. Patients with prostatic urethra involvement with transitional cell cancer (TCC) are eligible if it is completely resected and the patient has no evidence of stromal invasion of the prostate.

3.1.2 Patients must be able to tolerate systemic chemotherapy combined with pelvic radiation therapy and radical cystectomy

3.1.3 Zubrod Performance Status of ≤1.

3.1.4 Age ≥18.

3.1.5 CBC/differential obtained no more than 8 weeks prior to enrollment on study, with adequate bone marrow function defined as follows:

- WBC ≥ 4000/ml
- Absolute neutrophil count (ANC) ≥1,800 cells/mm
- Platelets ≥100,000 cells/mm
- Hemoglobin ≥ 10.0 mg/dl (Note: the use of transfusion or other intervention to achieve this level is acceptable)

3.1.6 Serum bilirubin of 2.0mg or less;

3.1.7 Serum creatinine of 1.5mg or less; creatinine clearance of 60ml/min or greater no more than 8 weeks prior to enrollment (Note: calculated creatinine clearance is permissible, using Cockcroft-Gault formula. If the creatinine clearance is greater than 60ml/min, then a serum creatinine of up to 1.8mg is allowable at the discretion of the principal investigator.)

Note: Prechemotherapy laboratory investigations and ECOG evaluation must meet inclusion criteria irrespective of where they were drawn,

retroactive, prior to cycle 1 of cisplatin/gemcitabine. Inclusion criteria from these initial investigations will be used for evaluation of enrollment eligibility

3.1.8 Patients must be willing and able to provide study-specific informed consent prior to study entry

3.2 Exclusion Criteria

3.2.1 Tumor related untreated active hydronephrosis
3.2.2 Evidence of distant metastases.
3.2.3 Diffuse bladder carcinoma *in situ* (CIS) not able to be encompassed in a boost radiotherapy volume.
3.2.4 Previous systemic chemotherapy (for any cancer) or pelvic radiation therapy
3.2.5 A prior or concurrent malignancy of any other site or histology unless the patient has been disease free for greater than or equal to five years except for non-melanoma skin cancer and/or stage T1a prostate cancer or carcinoma *in situ* of the uterine cervix
3.2.6 Patients that are not candidates for radical cystectomy (T4b disease are considered unresectable)
3.2.7 Pregnancy or women of childbearing potential [not post-menopausal or permanently sterilized (e.g. tubal occlusion, hysterectomy, bilateral salpingectomy)] and men who are sexually active and not willing/able to use medically acceptable forms of contraception; this exclusion is necessary because the treatment involved in this study may be significantly teratogenic
3.2.8 Severe active co-morbidity:
- Unstable angina and/or congestive heart failure requiring hospitalization within the last 6 months
- Transmural myocardial infarction within the last 6 months
- Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration
- Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of enrollment
- History of hepatic insufficiency resulting in clinical jaundice and/or coagulation defects (Note: laboratory tests for liver function and

coagulation parameters are not required for enrollment into this protocol)

- Known diagnosis of Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition (Note: HIV testing is not required for enrollment into this protocol). The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive.
- As determined by the investigator or principal investigator

3.3 Enrollment

Study entry, as used in this protocol, will be defined as a subject signing informed consent. Study enrollment, as used in this protocol, will be defined as the investigator's confirmation of the subject's eligibility by signing an eligibility checklist.

As per UM/SCCC Clinical Research Services policy, subject eligibility must also be reviewed by a CRS director or designee. The investigator or study coordinator will provide the following to a CRS representative:

1) Completed and signed protocol-specific eligibility checklist;
2) All pages of the original signed informed consent forms (ICFs), including HIPAA Form B.
3) Relevant source document such as: subject medical history and physical exam, admission or discharge notes, diagnostic reports, pathologic confirmation of diagnosis, and relevant subject-specific written communication.

Emergency Enrollment
If an emergency enrollment takes place after business hours, the items listed in section 3.3 above must be submitted by the next business day.

Cancellation Guidelines
If a subject does not receive protocol therapy, the subject may withdraw. Contact the CRS representative, or e-mail the information including the reasons for withdrawal within 10 working days.

4.0 TREATMENT PLAN

4.1 Transurethral Resection of the Bladder Tumor (TURBT) & Cystoscopy (Evaluation 1)

The following will be performed by the participating urologist:

- cystoscopic evaluation
- bimanual examination under anesthesia,
- as thorough as possible a transurethral resection (TUR) of the bladder tumor,
- and a biopsy of the prostatic urethra including both mucosa and stroma using a resection loop.

Endoscopic evaluation must include cystoscopy with tumor mapping on the initial cystoscopic report and transurethral resection (TUR) of the tumor as thoroughly as is judged safely possible. Tumor specimens will be stored in liquid nitrogen. No medications delivered as part of pre or intraoperative standard of care therapy during any TURBT, or during radical cystectomy, when performed, will be reported

4.2 Neoadjuvant chemotherapy

All patients will receive the neoadjuvant course of chemotherapy. This regimen will begin within 8 weeks following the TURBT and cystoscopic evaluation by the urologic surgeon. Ideally, treatment should start within 8 weeks and on a Monday.

The recommended neoadjuvant chemotherapy regimen consists of gemcitabine and cisplatin given on a 21-day cycle. A cycle is defined as 2 consecutive weeks of treatment followed by a week of rest. Patients will receive four cycles of neoadjuvant chemotherapy as per standard of care. Week 1 (cisplatin and gemcitabine) of chemotherapy can be given as outpatient or inpatient, at the investigator's discretion. Week 2 (gemcitabine) will be given as outpatient. Doses of chemotherapy as per standard of care will be as follows:

Cisplatin (70 mg/m^2) will be administered as a sixty-minute infusion (+/- 10 minutes) on day 1 (outpatient regimen) or day 2 (inpatient regimen) of each 21-day cycle as per standard of care.

Gemcitabine (1000 mg/m^2) will be administered intravenously over 30-60 minutes (preferably 30 minutes) on Days 1 and 8 of each 21-day cycle as per standard of care.

Calculation of the body surface area of the patient will be done according to the subject's height and weight at initiation of the neoadjuvant chemotherapy. The BSA will only be recalculated in the event that the patient's weight changes by $\geq$10%.

Supportive care can include blood transfusions and administration of antiemetics and analgesics as per standard of care. IV hydration before and after cisplatin will be given as per standard of care. The use of erythropoiesis stimulating agents is not allowed (no wash-out period is needed). The use of GCSF (filgrastim) is allowed after cycle 1 of therapy, at the discretion of the treating investigator. Patients will be permitted to sign and enroll on this trial prior to, during, or after cycle one of neoadjuvant cisplatin/gemcitabine chemotherapy, but not once they have commenced cycle 2. Cycle one can be initiated and delivered either at the University of Miami, or at an outside hospital, provided that once they have enrolled, all further treatment on this protocol is delivered at the University of Miami by a physicians listed as Co-Investigators on this trial.

4.3 Re-evaluation After Post-Neoadjuvant Chemotherapy - (Evaluation 2)

This evaluation will take place ≤ 6 weeks following the completion of the neoadjuvant chemotherapy. Evaluation will include:

- urine cytology,
- cystoscopy,
- tumor site transurethral biopsy,
- and bimanual examination after biopsy
- and biopsy of TURBT site

Operative reports and pathology reports from the TUR specimens will be reviewed. In particular, the surgeon should comment on tumor burden in the bladder and prostatic urethra and the overall clinical stage at the conclusion of the resection, as well as the bimanual examination. Pathology report should include the gross and microscopic description of tumor location, tumor grade, and tumor stage using T-classification of the TNM staging system. Specifically, pathology reports should include a description of the depth of tumor invasion and document the T stage to confirm those patients with T0, Tcis, Ta disease that are eligible for bladder preservation.

Patients with a complete response to the neoadjuvant chemotherapy after TURBT will receive the option for receiving consolidation chemoradiation in place of radical cystectomy. All patients with node positive disease at diagnosis must have PET confirmed CR of node positive disease after chemotherapy to be offered bladder preservation. Patients with a partial response in primary or lymph nodes after the neoadjuvant regimen will receive

radical cystectomy within 12 weeks after cystoscopic re-evaluation (ideally by 8 weeks if feasible).

Target localization for radiotherapy boost:

Lipiodol injection will be performed at the time of cystoscopy, for those subjects who are eligible for bladder preservation. A flexible cystoscope with a 6 French working channel should be used. Through the working channel a 200cm flexible needle with a diameter of 0.6 cm and a needle length of 6mm should be inserted. The needle will be filled with lipiodol and inserted under direct vision from the flexible scope. Lipiodol should be injected until a small deposit (.25cc) into the bladder wall 5-10 mm away from the border of the primary tumor resection site. This injection should then be repeated to a total of up to 10 injections in a circumferential pattern around the primary tumor or resection site. Excess lipiodol should be removed from the bladder by irrigation. Fluoroscopy will be used to verify that the lipiodol remains in the bladder wall.

4.4 ChemoRadiation Therapy:

Chemoradiation should commence within 6 weeks after cystoscopic re-evaluation.

4.4.1 Intensity Modulated Radiation Therapy (IMRT/VMAT)

4.4.1.1 Dose Specifications:

All patients will receive 25 daily fractions (5 weeks) of radiation therapy for 5 days a week (Monday to Friday) except on weekends or holidays, when remaining fractions will be added to the end of treatment. Radiation therapy will be started within 6 weeks following the neoadjuvant chemotherapy. The overall schema is for IMRT based radiation to the entire bladder, prostate (in men) and the pelvic lymph nodes. The pelvic lymph nodes will receive 45 Gy in 25 fractions at 1.8 Gy per fraction. The whole bladder and prostate will receive 50 Gy in 25 fractions at 2.0 Gy per fraction. The tumor boost area will be defined as the area of bladder involved by tumor based on TURBT, CT imaging, and lipiodol injection at time of cystoscopy, and will receive 60-65 Gy in 25 fractions at 2.4-2.6 Gy per day. Final boost dose will be determined at the discretion of the treating physician based on normal tissue exposure and volume.

4.4.1.2 Technical Factors:

Linear accelerators with a beam energy of ≥6 MV must be used.

4.4.1.3 Localization, Simulation and Immobilization:

A 1.5-2.0 mm slice planning CT must be obtained 1-2 weeks after cystoscopy and lipiodol injection to ensure the bladder recovers from possible edema and to allow excess agent to wash out. The patient should be placed in the supine position with arms folded across chest with ankle supports. Rectum should be as empty as possible, and patients should be instructed to have a partially full bladder. A recommended approach is to void one hour before simulation and drink 250cc of water to better reproduce bladder size for daily treatment; but, in our experience, with feedback, patients manage how best to prepare for partial bladder filling. A pelvic immobilization device is recommended such as a vacloc device. IV and oral contrast is required. Daily cone beam with soft tissue and lipiodol contrast injection matching is required for daily target set up and verification. Daily online CBCT guidance will be employed for boost localization.

4.4.1.4 Treatment Planning/Target Volumes:

CTV1 will constitute the entire bladder, prostate (in men) and proximal urethra (in women). The CTV2 is the regional pelvic lymph nodes which should include the internal and external iliac, obturator and presacral vessels to no higher than L5-S1 (mid-SI joint, S2-S3, is acceptable). PTV1 will encompass a 5-10mm uniform expansion of CTV1. PTV2 will constitute a 5mm-10mm uniform expansion of CTV2. CTV3 will include the area of bladder involved by tumor based on TURBT, palpable disease and/or CT/MRI staging investigations as well as lipiodol injection at time of cystoscopy. PTV3 will be a 5mm-10mm expansion from CTV3.

4.4.1.5 Critical Structures:

A DVH for the rectum, bladder and both femoral heads should be submitted. The following DVH criteria should be achieved. The small contours should start 2 cm above the most superior vessel contour. <300cc of small bowel should receive a dose greater than 45Gy. V60cc small bowel should be <2cc. The anorectum is defined on CT from the anus (at the level of the ischial tuberosities for a length of 15cm or to

the rectosigmoid flexure). 50% of the anorectum should receive less than 55Gy. Each femoral head should receive a maximum dose of ≤ 45Gy.

4.4.1.6 Compliance Criteria:

Specified Radiation Dose (Critical Structures): **Variation:** Planned dose is within 10% of the specified protocol dose. **Deviation:** Planned dose deviates by more than 10% from the specified protocol dose.

Minimum Isodose Coverage (Applies to each CTV independently): Generally the minimum dose to any target should be 95% of the prescription dose to that target. To address the single pixel calculation anomalies the D99% is used as the dose specifier. **Per protocol:** D99% > 95%. Dose covering 99% of the volume of any target volume is no less than 95% of the prescribed dose. **Variation:** D99% <95% but D99% >90%. Dose covering 99% of the volume of any target volume is no less than 90% of the prescribed dose. **Deviation:** D99% <90%. Target structures coverage falls below 90% of the prescribed dose.

Maximum Dose (Applies to each CTV independently): The maximum dose to any target should be less than 107% of that target's prescribed dose. **Per protocol:** 107% <0.12cc. Less than 0.12 cc of the CTV receives a dose exceeding 107% of the prescribed dose. **Variation:** V107% >0.12 cc but this dose does not exceed 110% of this dose. **Deviation:** The maximum dose to the 0.12 cc volume dose exceeds 110% of the prescribed dose.

Elapsed Days: **Per protocol:** No more than 3 break days. **Variation:** 4-7 break days. **Deviation:** 8 or more break days

4.4.2 Concurrent chemotherapy

Weekly cisplatin will be used as a radiation sensitizer during chemoradiation as per standard of care. Calculation of the body surface area of the patient will be done according to the subject's height and weight at initiation of the concurrent chemotherapy. The BSA will only be recalculated in the event that the patient's weight changes by 10%. Cisplatin (35 mg/m^2) will be administered as a 60 minute infusion (+/- 10 minutes) starting on day 1 of radiation, and on days 8,15,22,29. Premedication and hydration will be performed as per standard of care.

4.4.3 Treatment Interruption

Treatment (both radiotherapy and chemotherapy) should be delayed if any treatment-related grade 3 toxicity is encountered. This includes grade 3 acute colitis, cystitis, or any other grade 3 infield (radiation related) toxicity during any treatment week. Delay should continue until toxicity is reduced to grade 2 or lower. If the delay is greater than 3 weeks, the patient will be considered intolerant to the protocol therapy and appropriate off protocol therapy will be given. For dose modifications see section 6.0.

4.5 Radical Cystectomy

Operable patients who have pT1 or worse tumor response after initial TUR and neoadjuvant chemotherapy will have radical cystectomy within12 weeks following the post-neoadjuvant response evaluation (ideally at approximately 8 weeks if feasible). In the male, radical cystectomy will include en bloc resection of the bladder, prostate, seminal vesicles, and intramural ureters as well as associated peritoneum and perivesical fat. In the female, radical cystectomy will include resection of the bladder along with the intramural ureters, perivesical fat and peritoneum associated with the bladder. Depending on the local extent of the tumor and urinary diversion choice of the patient, a resection of the urethra, anterior and lateral walls of the vagina, uterus, fallopian tubes and ovaries may also be required. Orthotopic diversion, continent cutaneous diversion, and incontinent cutaneous diversions are permissible after neoadjuvant chemotherapy, as jointly determined by the urologist and patient.

When feasible, total lymphadenectomy should be performed. The dissection should include resection of nodal tissue from the bifurcation of the common iliac vessels to the inguinal ligament and from the genitofemoral nerve to the hypogastric artery.

Operative reports and pathology reports from cystectomy specimens should be submitted. The pathology report should include the gross and microscopic description of tumor location, tumor grade, and tumor stage using the TNM staging system. Pathology reports should also include description of the depth of tumor invasion, involvement of other organs or pelvic structures, summary of the margin status and the location and total number of lymph nodes resected an involved with carcinoma.

4.6 Post-Consolidation Endoscopic Evaluations

The first post-treatment evaluation will be 30 days +/- 14 days within the end of chemoradiation, surgery or at progression. Subsequent cystoscopic evaluation will be every three months in the first year, every four months in the second year, and every six months in the third year (all evaluations to occur +/- 14 days). Each evaluation will include serum, plasma, whole blood, urine cytology. Biopsy of the original tumor site and any suspicious areas will be performed in all patients that undergo bladder preservation. If after two re-evaluations in which the tumor site re-biopsies have been negative and the urologist observes nothing suspicious, cystoscopy and cytology without biopsy is permitted. The post-neoadjuvant evaluation may be considered the first of these two negative re-evaluations. Regular cystoscopic follow up will allow additional therapy such as transurethral surgery, intravesical chemotherapy or cystectomy to be initiated at the earliest prompt opportunity, if relapse occurs in those who have retained their bladder.

5.0 CLINICAL, LABORATORY AND RADIOLOGIC EVALUATIONS

See Appendix I

6.0 DOSING DELAYS/DOSE MODIFICATIONS

Dose Modification for Chemotherapy (gemcitabine, cisplatin)
Dose modifications to chemotherapy apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose. A maximum of 2 dose reductions for each agent is allowed per patient. After 2 dose reductions, patients should discontinue treatment.

6.1 Dose Modification within a Cycle

If the start of an adjuvant cycle is delayed, re-evaluate weekly to initiate the cycle. If day 8 of the cycle is held, do not make up the gemcitabine dose for day 8. The next adjuvant treatment cycle will start according to schedule after a re-evaluation of patient's toxicities.

Gemcitabine dose adjustments: Dose adjustments within a cycle for gemcitabine will be made following the guidelines shown below based on weekly absolute neutrophil count (ANC) and platelet counts taken before infusions (see Appendix I) and on clinical assessment of non-hematologic toxicities.

Hematologic Toxicities

			Percent of Full Dose
ANC (x 10^9/L)		**Platelets (x 10^9/L)**	**Gemcitabine**
≥ 1.0	and	≥ 75	100% dose
0.5 - 0.99	or	50- 74	50% dose
< 0.5	or	< 50	HOLD dose

Treatment Related Non-hematologic Toxicities

	Percent of Full Dose
CTCAE Version 4.02	**Gemcitabine**
Grade 1-2	100% dose
Grade 3	50% or hold dose*
Grade 4	HOLD dose*

*This decision will depend upon the type of non-hematologic toxicity seen and which course is medically most sound in the judgment of the treating physician.

Cisplatin dose adjustments: If creatinine clearance is below 60ml/min prior to the start of a cycle, cisplatin should not be administered.

Hematologic Toxicities

ANC (x 10^9/L)	**Platelet Count**			
	≥ 150K	**100 - 149K**	**75 - 99K**	**<75 - 50K**
≥ **1.4**	100% dose	100% dose	HOLD dose	HOLD dose
1.2 - 1.39	100% dose	75% dose	HOLD dose	HOLD dose
< 1.2	HOLD dose	HOLD dose	HOLD dose	HOLD dose

Treatment Related Non-hematologic Toxicities

	Percent of Full Dose
CTCAE Version 4.02	**Cisplatin**
Grade 1-2	Physician discretion*
Grade 3 nausea and vomiting (without appropriate anti-emetic support)	75% of full dose
Grade 3-4 ototoxicity	Physician discretion*
Grade 3-4 neurotoxicity	Physician discretion*
Creatinine clearance < 60ml/min	Not administered

*This decision will depend upon the type of non-hematologic toxicity seen and which course is medically most sound in the judgment of the treating physician.

6.2 Dose Modification for Subsequent Cycles

The following guidelines should be followed:

- Doses of gemcitabine cannot be escalated above the starting dose.
- Absolute neutrophil count must be greater than 1.2 x 109/L, and platelet count must be greater than 100x109/L to proceed with the next cycle.
- If day 8 is held for a grade 3 or grade 4 hematologic toxicity, subsequent cycles will be dose reduced by 25% and the reduction will be maintained for the duration of treatment.

6.3 Hematologic Toxicity

Patients who sustain either febrile neutropenia or Grade 4 thrombocytopenia, or Grade 3 thrombocytopenia associated with new-onset gross hematuria or other clinical evidence of bleeding should be dosed at 50% of the starting dose of gemcitabine delivered in the previous cycle, the latter to apply to each gemcitabine dose administered during that cycle. Subsequent dose escalation of gemcitabine only by 50% (e.g., from 500 mg/m2 to 750 mg/m2) will be allowed in subsequent cycles provided the patient tolerates the initial dose of adjustment.

Dose Modifications of Cisplatin for Nephrotoxicity during neoadjuvant and consolidation chemoradiotherapy are listed in the table below:

Please note: If serum creatinine is out of range, but CrCl is in range, 100% can be given if in the judgment of the treating physician. If cisplatin is held, re-evaluate day 1 each week to restart.

Day 1 Level	Dose
CrCl >60 ml/min or serum creatinine ≤ 1.5 mg	100%
serum creatinine > 1.33 x baseline	75%
serum creatinine >1.5 x baseline	Hold cisplatin

Dose Modifications of Cisplation for Myelosuppression during neoadjuvant and consolidation chemoradiotherapy are as listed in the table below:

% Calculated Dose

ANC (x 10^9/L)	Platelet Count			
	≥ 150K	**100 - 149K**	**75 - 99K**	**<75 - 50K**
≥ 1.4	100% dose	100% dose	HOLD dose	HOLD dose
1.2 - 1.39	100% dose	75% dose	HOLD dose	HOLD dose
< 1.2	HOLD dose	HOLD dose	HOLD dose	HOLD dose

Modification of Cisplatin for Peripheral Neurotoxicity during neoadjuvant and consolidation chemoradiotherapy ≥ Grade 3: Omit cisplatin.

7.0 AGENT FORMULATION AND PROCUREMENT

7.1 Drug Name: Cisplatin (Platinol®)

Cisplatin is being given as per standard of care. For storage, handling and administration recommendations refer to manufacturers' current package insert.

7.2 Drug Name: Gemcitabine

Gemcitabine is being given as per standard of care. For storage, handling and administration recommendations refer to manufacturers' current package insert.

8.0 CORRELATIVE/SPECIAL STUDIES

8.1 Molecular markers in Bladder Cancer

Selection of patients that will have a favorable response to both neoadjuvant cisplatin based chemotherapy and radical chemoradiation will greatly improve patient stratification for treatment by allowing those who may not respond to neoadjuvant therapy the opportunity for immediate surgery without delay. Similarly, those with favorable response profiles will be provided with the opportunity for bladder preservation with equivalent outcomes. A number of molecular biomarkers have shown potential for predicting outcome to neoadjuvant chemotherapy and radical chemoradiation. We will utilize pre-treatment biopsies to evaluate these markers and for exploratory analyses to discover additional molecular candidates that will predict outcome to therapy.

Recent data from Chakravarti et al showed on multivariate analysis that from a panel of known molecular markers in bladder cancer, only Her-2 expression by immunohistochemistry correlated to reduced CR after chemoradiation (27). Both Bcl2 and p53 expression have been correlated to poor outcome after radiotherapy in retrospective series (28). However, this data is conflicting (29). Overexpression of bcl-2 and normal pRb expression was also correlated to poorer response to neoadjuvant radiation in an independent series (30).

ERCC1 expression has been shown to predict for response to cisplatin-based chemotherapy in non-small cell lung cancer (31). Similar analysis has been performed in bladder cancer. While ERCC1 has been shown to correlate to response to cisplatin and to outcome with primary chemoradiation in muscle invasive bladder cancer (32,33), this data is preliminary and requires further

investigation in a prospective setting (34).

We will investigate the role of Her2, Bcl2, p53, pRb and ERCC1 expression in both prediction to neoadjuvant chemotherapy and prognosis for patients undergoing bladder preservation.

While published data on next generation sequencing of bladder tumors is limited, select studies highlight the potential for this approach both for identification of novel predictive markers as well as for improving targeted individualized therapy. TSC1 mutation has recently been correlated to a complete response and over two year remission after treatment with everolimus. This suggested mTORC1-directed therapy could be effective in patients with tumors harboring TSC1 somatic mutations, and demonstrates the power of NGS platforms in translational settings to identify novel biomarkers for response in bladder cancer (35).

As we will collect fresh frozen tissue from primary TURBT site pre and post-neoadjuvant chemotherapy and from radical cystectomy, our aim is to elaborate both novel data and candidate biomarkers from prior retrospective data outlined above using next generation sequencing platforms which will produce global high-density evaluation of the genome, transcriptome and the methylome. We will attempt to identify candidate biomarkers for complete response to neoadjuvant chemotherapy to develop a predictive model for selecting appropriate patients for bladder preservation. Further, molecular studies will be conducted on tissue obtained from local failures or distant metastatic disease to identify predictive and prognostic markers for aggressivity and micrometastatic disease, and to discern new potential targets for systemic agents.

Rationale for acquiring biopsies of metastatic recurrence for molecular analysis:

Long term survival for metastatic disease remains low and chemotherapy in this setting remains essentially palliative. Advances in the understanding of the molecular composition of metastatic disease will allow identification of new molecular targets for this disease. Current molecular targeted agents in this setting are limited and include common gene alterations not specific to bladder cancer. These include EGFR inhibitors Gefitinib, erlotinib and cetuximab, erb2 inhibitor trastuzumab, sorafenib targeting receptor tyrosine kinases and a number of VEGF inhibitors including bevacizumab, and sunitinib. Currently these agents are being evaluated in multiple phase I/II clinical trials and

preliminary data shows encouraging results. The ability to test metastatic lesions for these and other novel alterations will potentially aid in the treatment of this disease. (36,37).

8.2 Tissue Specimen Submission

Primary tumor tissue from pretreatment TURBT as well as post-neoadjuvant chemotherapy TUR will be obtained. Blood samples at pre-treatment TURBT and post-neoadjuvant chemotherapy and post-radical treatment evaluations will also be obtained. Radical cystectomy tissue, blood and urine will be obtained and banked for future molecular analysis. Tissue and blood from patients with post-treatment recurrence evaluations will be obtained for molecular studies. In addition, tissue from distant metastatic disease will be obtained and stored for molecular studies. Attempt will be made to collect blood from patients at the time of biopsy for metastatic disease. In every instance, there will be attempt to obtain FFPE blocks and fresh frozen tissue.

Storage Conditions:

Store frozen specimens at -80C (-70C to -90C) in either freezer or liquid nitrogen vapor phase.

Specimen Collection Summary: For each patient consented to participate in this study the following tissue for diagnosis and molecular analysis will be obtained.

1. H&E stained slides of primary tumor from pre-chemotherapy TUR for diagnosis, and a pathology report documenting patient name and/or other identifying information.
2. A paraffin –embedded tissue block of the primary tumor at time of TUR.
3. 1 H&E and 1 fresh frozen biopsy of primary tumor from pre-chemotherapy TUR.
4. 1 H&E and 1 fresh frozen biopsy of primary tumor location from post-chemotherapy TUR.
5. 1 H&E and 1 fresh frozen biopsy at the region of the primary tumor location post-consolidation chemoradiotherapy or cystectomy.
6. Whole blood, plasma, urine.
    a. Serum: 5-10mL of whole blood in 1 red-top tube and centrifuge
        i. Pre-treatment (Evaluation 1)
        ii. Post-neoadjuvant chemotherapy (Evaluation 2)
        iii. Post-consolidation chemoradiotherapy or cystectomy
        iv. At one year follow-up
        v. At the time of relapse

b. Plasma: 5-10mL of anticoagulated whole blood in EDTA tube (purple/lavender top) and centrifuge
   i. Pre-treatment (Evaluation 1)
   ii. Post-neoadjuvant chemotherapy (Evaluation 2)
   iii. Post-consolidation chemoradiotherapy or cystectomy
   iv. At one year follow up
   v. At the time of relapse
c. 10-20mL clean-catch urine
   i. Pre-treatment (Evaluation 1)
   ii. Post-neoadjuvant chemotherapy (Evaluation 2)
   iii. Post-consolidation chemoradiotherapy or cystectomy
   iv. At one year follow-up
   v. At the time of relapse

8.3 Viable Tumor Cell Banking and Cell Line Derivation for Chemotherapeutic Testing

Standard chemotherapy agents are suboptimal, as are current methods of bladder preservation using chemotherapy and radiotherapy. Central to bladder cancer management is effective chemotherapy. The cell culture and testing methods proposed would allow for the identification of drugs that are much more effective at killing bladder cancer, which given the high recurrence and metastases rates would translate into an immediate opportunity to improve clinical outcomes.

As a proof-of-principle, our collaborator, Dr. Ince has established a cell line from a bladder cancer pelvic lymph node metastasis. 160 oncology chemotherapy agents were then tested on this cell line and tumor response was ranked. The results were highly actionable because there was minimal or no response to 151 drugs, including all of the mainline chemotherapies, even in the uM-mM range. In dramatic contrast, 100% cell kill was achieved in the nM range for 9 drugs. Notably, three of these drugs target protein turnover (the proteasome and hsp90) and 4 drugs target the PI3K/mTOR pathway. Identification of seven drugs, among the nine, that target just two pathways is highly significant and indicates that this approach can be used to pre-screen patients to develop personalized therapy

Using this novel technology, we plan to establish cell lines from the patients enrolled on this trial. Drug testing will be carried out in pre-treatment, post-neoadjuvant and metastatic cell lines from each patient, providing unique insight into why patients fail first line chemotherapy, how the tumor response characteristics change, and what agents hold the most promise for the treatment of metastases that develop. Each of these samples will also have mutation and gene expression analyses performed, to determine the underlying mechanisms behind the chemotherapy response profiles.

[redacted]

[redacted]

[redacted]

9.0 MEASUREMENT OF EFFECT

Examination under anesthesia with cystoscopy and biopsy of previously positive tumor site(s) will be performed within 6 weeks following completion neoadjuvant

chemotherapy. Patients will be graded as complete response when all biopsies are negative for any tumor at the site(s) of the pretreatment tumor(s). If severe dysplasia or Ta tumor or carcinoma in situ is documented by mucosal biopsy at a distant site from original tumor this will not prevent the patient from being graded as a complete response and the patient will still undergo definitive surgery or bladder preservation.

Distant Metastases:

This will be defined as first appearance of disease in a non-regional lymph node, solid organ or bone. This can be identified on routine follow-up CT scan or bone scan or any study performed to work up a specific patient complaint. Additional radiographic studies or biopsies may be performed at the treating physician's discretion. Radiographic evidence of metastases is sufficient for evaluation of this endpoint.

Defining Bladder Response:

Complete Response (CR or a pT0 response):

Requires the absence of any tumor in the tumor-site biopsy. For a primary tumor response following consolidation, a urine cytology specimen that is not positive is also required.

Partial Response (PR):

Requires that all response criteria of a CR except that the urine cytology remains positive or CIS is seen in the biopsy.

No response (NR):

Requires the continued presence of the tumor (T≥1) in the tumor site biopsy specimen.

Progression:

Requires the increase of 50% or more in the largest diameter of the endoscopically appreciable tumor in the tumor-site biopsy specimen, the development of new bladder tumors, or the development of metastatic disease.

10.0 ADVERSE EVENT REPORTING

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for

adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). Additionally, if assistance is needed, CTEP has a web-based dictionary and index to the CTC/CTCAE that provides help for classifying and locating terms.

10.1 Definitions

10.1.1 ***Adverse events (AEs):*** Any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, medical treatment or procedure, causality. whether or not considered related to the drug, medical treatment or procedure. An adverse event can arise from any use and from any route of administration, formulation, or dose including an overdose. This includes any newly occurring event or a previous condition that has increased in severity or frequency since initiation of a drug, medical treatment, or procedure.

10.1.2 A ***serious adverse event*** **(**SAE) is i any adverse event (experience) occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, 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.

The definition of serious adverse event (experience) also includes ***important medical events***. Medical and scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or **may require intervention** to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

10.1.3 **Expected events** are those that have been previously identified as resulting from administration of the agent.

10.1.4 An adverse event is considered ***unexpected*** when either the type of event or the severity of the event is *not* listed in the investigator's brochure, or , drug/device package insert.

10.1.5 The definition of ***related*** is that there is a reasonable possibility that the drug caused the adverse experience.

10.1.6 ***Commercial agents*** are those agents not provided under an IND but obtained instead from a commercial source.

10.2 Reporting of Adverse Events

10.2.1 Baseline Adverse Events

Adverse events will be considered baseline adverse events or medical history as noted from the time of informed consent until the time of Evaluation 1 (TURBT and cystoscopy).

Any worsening of the patient's clinical condition while the patient is on study will be considered to be an adverse event unless it is within the normal range of disease fluctuation for that patient.

10.2.2 Recording Abnormal Findings

In any clinical assessment, a value outside the normal or reference range (such as a clinical laboratory, vital sign, or ECG) will not be recorded or assessed as an adverse event unless that value is considered to be of clinical significance by the investigator. A value of clinical significance is one that leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered to be a clinically significant change from baseline by the investigator.

For this study all grade 2 and higher (by CTCAE v4.0) adverse events will be captured.

10.2.3 Recording Signs and Symptoms

Sign, symptoms, or procedures resulting from an underlying clinical diagnosis should be documented as one comprehensive adverse event. If no underlying clinical diagnosis can be identified, each sign and symptom should be recorded as a separate independent event.

However, a new or worsening event resulting from an underlying clinical diagnosis or a reaction to concurrent medications should be documented as a separate independent adverse event unless it is within the normal range of fluctuation for that patient.

10.2.4 Recording Grade Changes

Adverse events will be recorded at the maximum grade/severity experienced for the duration of the event. Should one particular AE

warrant further investigation, additional details may be collected at the discretion of the Principal Investigator.

10.2.5 Reporting Period

At the discretion of the PI, adverse event collection will be discontinued at the 2 year post treatment follow-up visit or when the subject initiates any new therapy that would confound the accurate collection of adverse events.

10.2.6 IRB Reporting

All adverse events that are serious adverse events **and** are unexpected **and** are related or possibly related IRB within ten (10) working days of being made known to the Principal Investigator. Events that are more frequent than anticipated or more severe than expected must be reported to the IRB within ten (10) working days of being made known to the Principal Investigator.

All **unanticipated** deaths or life-threatening problems suspected as being a direct outcome or possibly an outcome of the study intervention must be reported to the IRB within 24 hours of being made known to the Principal Investigator.

For all SAE's, the investigator is obligated to pursue and provide follow-up reporting information until the event has resolved or until an acceptable medical endpoint has been reached or the patient is lost to follow-up.

11.0 CRITERIA FOR DISCONTINUATION OF THERAPY

Subjects will be taken off treatment for the following reasons:

1) Progression of disease.
2) Intolerance to therapy or intercurrent illness that prevents further administration of treatment.
3) Patient initiated discontinuation or refusal to follow protocol.
4) A delay in protocol treatment of more than 3 weeks due to any event.
5) An unacceptable adverse event requiring discontinuation of treatment*

*Early discontinuation of therapy will occur if a patient experiences grade 3 or higher acute colitis, cystitis, or any other grade 3 in-field (radiation-related) toxicity during any treatment week, treatment (both radiation and chemotherapy) should be delayed until the toxicity subsides to the grade 2 level. If the delay is greater than 3 weeks, then the patient should be considered intolerant of protocol therapy and appropriate off-protocol therapy given.

End of treatment evaluations will be done at the discretion of the investigator.

12.0 CRITERIA FOR DISCONTINUATION OF STUDY PARTICIPATION

1) Subject withdraws consent
2) Subject death

13.0 DATA REPORTING

Data must be submitted for ALL patients entered into the study or enrolled into the study. If using hard copies of CRF's, study center personnel will complete individual CRF's in black ink. All corrections to entered data will be made by drawing a single line through the information to be corrected without obscuring it. All corrections will be initialed, dated and explained, if necessary. **Do not use "white-out" or obscuring correction tape.** A CRF is required for every patient who received any amount of study treatment. The investigator will ensure that the CRF's are accurate, complete, legible and timely. Separate source records are required to support all CRF entries.

14.0 STATISTICAL CONSIDERATIONS

14.1 Overview

This nonrandomized noncomparative phase II study will investigate efficacy of definitive chemoradiation therapy in muscle invasive bladder cancer patients who achieve favorable response from neoadjuvant chemotherapy. This study will enroll 68 patients receiving neoadjuvant chemotherapy in order to obtain 35 favorable response patients undergoing definitive chemoradiation (Group 1) which is the main focus of this study. The majority of the remaining patients will have radical cystectomy by patient choice or physician recommendation (Group 2).

Response to neoadjuvant chemotherapy will be evaluated by cystoscopy. A patient achieving favorable response from neoadjuvant chemotherapy will be recommended definitive chemoradiation, while a patient achieving non-favorable response will be recommended radical cystectomy. However, a patient who achieves favorable response may choose to have radical cystectomy.

Sample size justification of 35 patients in group 1 is provided in Section 14.3. In addition, early stopping guideline, based on Bayesian posterior probabilities, for monitoring toxicity on a continuous basis throughout the course of the trial is included in Section 14.5.

14.2 Definitions and endpoints

Analysis set

Primary analysis set: All study-eligible patients who complete ***definitive chemoradiation (Group 1)*** after completion of neoadjuvant chemotherapy will be considered evaluable for the purpose of analyzing data on safety, toxicity, failure-free survival and overall survival.

Secondary analysis set: All study-eligible patients who receive a partial course of chemoradiation or do not receive definitive chemoradiation after neoadjuvant chemotherapy **(Group 2)** will be considered evaluable for the purpose of analyzing data on failure-free survival and overall survival. This set includes the following:

- Patients who receive ***radical cystectomy*** after completion of neoadjuvant chemotherapy.
- Patients who had progression (locoregional or metastatic disease) during neoadjuvant chemotherapy.
- Patients who do not receive a complete course of chemoradiation

Exclusions

Patients who are enrolled on study but do not receive any protocol-related therapy (i.e. TURBT) will be excluded from all analyses. Reasons for such exclusions will be characterized such as consent withdrawn or eligibility subsequently not confirmed.

Endpoints

The primary endpoint is failure-free survival with intact bladder (FFSIB) at 2 years and it will be evaluated in primary analysis set of patients who complete definitive chemoradiation.

The secondary endpoints include:

- failure-free survival (FFS) at 2 years;
- overall survival;
- acute and late grade 2 or higher GU, GI, and hematologic toxicities; and
- quality-of-life assessment.

The endpoint for correlative study is genomic analysis to be evaluated in both analysis sets.

These endpoints are defined as follows:

FFSIB is defined by the absence of any failures (locoregional, distant metastasis, and death) and bladder preservation (no radical cystectomy for any causes) after definitive chemoradiation.

FFSIB is defined as the time elapsed from the start of neoadjuvant chemotherapy to the date of documented failure events or radical cystectomy. For failure-free patients (without failure events and no radical cystectomy), FFSIB will be censored at the last date of documented failure-free bladder preservation (FFBP) status.

Overall survival (OS) is defined as the time elapsed from the start of neoadjuvant chemotherapy until death. Surviving patients (including patients lost to follow up) will be censored at the date of last contact.

Toxicity by CTCAE v4.0 will be summarized. Acute toxicity rate is defined as the proportion of patients experiencing grade 2 or higher toxicity during or within 3 months of treatment completion. Late toxicity rate is defined as the proportion of patients experiencing grade 2 or higher toxicity occurring >3 months of definitive chemoradiation completion.

Quality-of-life (QoL) will be assessed by SF-12 and EPIC at pre-neoadjuvant chemotherapy (baseline), post-neoadjuvant chemotherapy, 3 month follow-up visit after treatment (definitive chemoradiation / radical cystectomy), and 9 month follow-up visit after treatment.

14.3 Patient enrollment, follow-up, and sample size justification

Total of 68 patients, 17 patients per year, will be enrolled over a 4 year period. We expect that around 60% of patient (40 patients) will show favorable response from neoadjuvant chemotherapy. Among 40 patients, 35 patients (around 90%) will follow the physician's treatment recommendation and will received definitive chemoradiation. To gather data on recurrence and survival, all 68 patients will be under follow-up for a minimum of 2 years unless they withdraw consent or die within that time.

The main study endpoint failure-free bladder preservation survival (FFBPS) will be evaluated in the primary analysis set of 35 patients receiving definitive chemoradiation. Sample size of 35 evaluable patients will provide reasonable precision in estimating FFBPS, as measured by the width of the 95% confidence interval for estimating 2-year FFBPS rate. It is expected that definitive chemoradiation will show 2-year FFBPS rate of 80% as compared with the current standard of care of approximately 60% in this patient population.

Table 14.1 gives examples of study findings that would provide evidence in favor of further consideration of the proposed treatment (definitive

chemoradiation) following favorable response to neoadjuvant chemotherapy.

Table 14.1 Possible study finding with 35 evaluable patients

Number of FFBP patients at 2 year	2-year FFBPS rate	95% confidence Interval (%)[#]	
26	74.3%	59.8	88.8
27	77.1%	63.2	91.1
28	80.0%	66.7	93.3

[#] Based on Peto's approximation for the standard error assuming no censoring.

Taking the middle row as an example, if 27 out of 35 patients do not experience any failure event and all 35 study patients have been followed for at least 2 year, we will report an estimated 2-year FFBPS of 77.1% with a corresponding 95% CI of 63.2% to 91.1%. This finding can be interpreted with high confidence (97.5%) the possibility that the 2-year FFBPS is better than 63.2%. The first and third rows of the table illustrate other possible study findings that could be considered favorable.

14.4 Statistical analysis

The primary endpoint is failure-free bladder preservation survival (FFBPS) at 2 years and it will be evaluated in primary analysis set of 35 patients receiving definitive chemoradiation. The secondary endpoints include failure-free survival (FFS) at 2 years; overall survival; acute and late grade 2 or higher GU, GI, and hematologic toxicities; and quality-of-life assessment. These will be evaluated in the primary analysis set. Failure-free survival, overall survival, and quality-of-life will be evaluated in the secondary analysis set. The endpoint of correlative study will be evaluated in both analysis sets comprising 68 total patients. Baseline characteristics will be summarized using descriptive statistics: counts, percentages, ranges, median, mean, and standard deviation as appropriate. This will include demographics (age, sex, and race/ethnicity), performance status, and disease characteristics such as stage and grade, separately for both analysis sets.

Toxicities will be tabulated by type, grade, duration, and attribution to study treatment. We will also report the distribution of worst grade toxicities in the study patients. Separate summaries will be provided for acute toxicity defined as toxicity occurring during or within 3 month of treatment completion, and for late toxicity defined as toxicity occurring more than 3 months after treatment completion.

Locoregional and distant metastasis failure rate will be evaluated by the method of cumulative incidence to allow for competing risks. Point

estimates with corresponding 95% confidence intervals for the cumulative incidence of locoregional and distant metastasis failure will be given for selected times, such as 1, 2, and 3 years following start of study treatment. Kaplan-Meier method will be used to estimate failure-free and overall survival. Point estimates with corresponding 95% confidence intervals for the proportion of recurrence-free and surviving patients will be provided for selected times, such as 1, 2, and 3 years. Median time to recurrence and median survival, if attained, will be reported. Cox proportional hazards regression will be used to determine whether FFS and OS are associated with baseline and disease characteristics.

QoL score will be tabulated in accordance with established scoring methods. Descriptive summaries of scores at baseline and each subsequent follow up will include median and range, mean and standard deviation. We will use changes in these scores (computed by subtracting baseline from subsequent corresponding score), which categorize improvement, no change, or deterioration for criteria for clinically meaningful differences. We will also compare mean score over time using mixed model.

The exploratory analysis component of this study aims to identify predictive biomarkers to distinguish favorable response and non-favorable response patients to neoadjuvant chemotherapy. In addition, depending on number of events, analysis will be conducted to identify predictive biomarkers of locoregional and distant metastasis failure; and acute and late toxicities. Tissue samples are obtained from cystoscopic evaluation after neoadjuvant chemotherapy. Fresh frozen tissue samples will be obtained prior to chemotherapy, post-chemotherapy and from radical cystectomy and will be subjected to next generation DNA and RNA sequencing. Univariate logistic regression or univariate Cox regression will be used to screen for potential genetic predictors (p-value<0.01). Random forest machine learning algorithm will be used to develop a genomic classifier to distinguish favorable response from non-favorable response patients. For locoregional / distant metastasis failures and acute/late toxicities we will use same approach to identify genomic classifier.

14.5 Interim monitoring

The Research Team will continuously monitor study accruals, toxicities, and response to treatment. The UM/Sylvester Comprehensive Cancer Center's Data and Safety Monitoring Committee (DSMC) will monitor this protocol according to the Cancer Center's DSM Plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study. DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event data, and periodic review of response. The DSMC also reviews reports from internal audits of protocol compliance and

data integrity conducted by the University of Miami, Office of Research Compliance Assessment. The guidelines appearing in this section are offered for DSMC consideration in assessing adverse events and response to study treatment.

Early stopping guidelines

Criteria for early stopping of the study will be identification of 30% of patients or greater G3 toxicity and/or 10% or greater G4 toxicity based on CTCAE v4.0 criteria.

Only subjects who have initiated chemoradiation will be subject to early stopping guidelines based on adverse events.

We propose the following guidelines for the DSMC in its review of accumulating data on toxicity and response. The proposed guidelines were developed using Bayesian methods, which can be applied at any stage of enrollment without advance specification of the number of interim analyses to be performed, or the number of patients evaluable for toxicity, or response, at the time such assessments are made (38,39).

Under the Bayesian method, we assign a prior probability (level of belief at the start of the trial) to a range of possible values for the true toxicity rate, and likewise for the true response rate. As data on treated patients become available, each of these probability distributions is revised and the resulting posterior probability becomes the basis for recommending either early termination or continuation of the study. Details are given in the remainder of this section.

Early stopping due to toxicity

If a treatment-related (possible, probable, or definite) death occurs, enrollment will be suspended and continuation of the study will be reassessed by the DSMC.

If two patients out of first five treated patients experiences treatment-related acute grade 4 or higher toxicity then the study will be terminated. It is based on Bayesian stopping rule that treatment-related acute grade 4 or higher toxicity rate should not exceed 10% with a posterior probability of 90% or higher.

For purposes of safety monitoring, we define an unacceptable toxicity to be any treatment-related grade 3 or higher acute toxicity, where acute refers to the period of time from start of study treatment to three months following completion or discontinuation of treatment. Unacceptable toxicity is expected to occur in no more than 10% of study patients. Early stopping (suspension and possibly termination) will be considered if there is

evidence that the proportion of patients experiencing unacceptable toxicity exceeds 30%. Specifically, we suggest as a guideline for early termination a posterior probability of 90% or higher that the rate of unacceptable toxicity exceeds 30%.

The table below shows specific instances where this guideline is met, thus suggesting early termination due to evidence of excessive toxicity.

Table 14.2 Stopping rule for toxicity

Number of patients with grade 3 or higher acute toxicity among 35 patients[#]	Total patients evaluated	Observed toxicity rate
3	3 to 3	≥ 100%
4	4 to 6	≥ 67%
5	7 to 8	≥ 63%
6	9 to 11	≥ 55%
7	12 to 13	≥ 54%
8	14 to 16	≥ 50%
9	17 to 19	≥ 47%
10	20 to 22	≥ 45%
11	23 to 24	≥ 46%
12	25 to 27	≥ 44%
13	28 to 30	≥ 43%
14	31 to 33	≥ 42%
15	34 to 35	≥ 43%

[#] Acute toxicity refers to episodes occurring during treatment or within 3 months following completion or discontinuation of study treatment.

For example, if 4 patients have been assessed for toxicity, the second row of the above table indicates that early stopping should be considered if 4 (44%) of these patients have experienced grade 3 or higher toxicity during or within one month of completing study treatment.

Posterior probabilities used to derive the preceding table are calculated under a prior beta distribution with parameters $\beta_1 = 0.2$ and $\beta_2 = 1.8$, which corresponds to an expected rate of 10% based on very limited information, roughly equivalent to having studied two patients. This prior distribution implies also an a priori chance of only 11.8% that the rate of unacceptable

toxicity is 30% or greater.

Early stopping due to lack of efficacy
We do not intend to stop the study early if the proposed treatment shows lack of efficacy of primary endpoint in order to assess recurrence-free and overall survivals.

15.0 INVESTIGATOR'S RESPONSIBILITIES

15.1 Investigator Responsibility/Performance

The investigator will ensure that this study is conducted in accordance with all regulations governing the protection of human subjects.

The investigator will ensure that all work and services described in or associated with this protocol will be conducted in accordance with the investigational plan, applicable regulations, and the highest standards of medical and clinical research practice.

15.2 Confidentiality

The investigator must ensure that each subject's anonymity will be maintained and each subject's identity will be protected from unauthorized parties. A number will be assigned to each subject upon study enrollment and the number and the subject's initials will be used to identify the subject for the duration of the study. The investigator will maintain all documents related to this study in strict confidence.

15.3 Informed Consent and Permission to Use Protected Health Information

It is the responsibility of the investigator to obtain written informed consent from each subject participating in this study after adequate explanation, in lay language, of the methods, objectives, anticipated benefits, and potential hazards of the study. The investigator must also explain that the subject is completely free to refuse to enter the study or to discontinue participation at any time (for any reason) and receive alternative conventional therapy as indicated. Prior to study participation, each subject will sign an IRB approved informed consent form and receive a copy of same (and information leaflet, if appropriate). For subjects not qualified or able to give legal consent, consent must be obtained from a parent, legal guardian, or custodian.

The investigator or designee **must** explain to the subject before enrollment into the study that for evaluation of study results, the subject's protected health information obtained during the study may be shared with the study sponsor, regulatory agencies, and the IRB. It is the investigator's (or designee's) responsibility to obtain permission to use protected health

information per HIPAA from each subject, or if appropriate, the subjects' parent or legal guardian.

15.4 Source Documentation and Investigator Files

The investigator must maintain adequate and accurate records to fully document the conduct of the study and to ensure that study data can be subsequently verified. These documents should be classified into two separate categories: (1) investigator study file and (2) subject clinical source documents that corroborate data collected on the CRF's. Subject clinical source documents would include hospital/clinic patient records; physician's and nurse's notes; appointment book; original laboratory, ECG, EEG, radiology, pathology, and special assessment reports; pharmacy dispensing records; subject diaries; signed informed consent forms; and consultant letters. When the CRF or any form is used as the source document, this must be clearly stated in the investigator study file.

Minimally, the following be documented in source documents:

- Medical history/physical condition and diagnosis of the subject before involvement in the study sufficient to verify protocol enrollment criteria
- Verification that written informed consent was obtained
- Progress notes for each subject visit
- Documentation of treatment
- Laboratory test results
- Adverse events (action taken and resolution)
- Condition and response of subject upon completion of or early termination from the study

15.5 Non-Protocol Research

No investigative procedures other than those described in this protocol will be undertaken on the enrolled subjects without the agreement of the IRB.

15.6 Ethics

The investigator agrees to conduct the study in compliance with the protocol, current good clinical practices, and all applicable (local, FDA) regulatory guidelines and standard of ethics.

15.7 Essential documents for the conduct of a clinical trial

Essential documents are those documents with individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced.

16.0 DATA AND SAFETY MONITORING

The Sylvester Comprehensive Cancer Center (SCCC) Data and Safety Monitoring Committee (DSMC) will monitor this clinical trial according to the Cancer Center's DSM Plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study.

DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event data, and periodic review of toxicity and feasibility. The guidelines appearing in Section 10.0 are offered for DSMC consideration in assessing adverse events. In addition, the DSMC will review reports from all audits, site visits, or study reviews pertaining to this clinical trial and take appropriate action.

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APPENDIX I: STUDY CALENDER

Enrollment

Event	Screening	Maximal TURBT (Evaluation 1)	Neoadjuvant Chemotherapy[l]		Post Neoadjuvant (Evaluation 2)[m]	Chemo-radiation[n]	End of Treatment[j]	Follow Up[k]
Study Day	**≤ 8 weeks prior to enrollment**		**Cycle 1-4 Day 1 (- 3 days)**	**Cycle 1-4 Day 8 (- 1 day)**	**≤ 28 days**		**30 days post treatment, cystectomy or at progression**	
Informed Consent[a]	X							
Medical History, Physical Examination, ECOG Performance Status, Weight[b]	X		X		X	Weekly	X	X
Vital Signs[c]	X		X		X	Weekly	X	X
Pregnancy test[d]	X		As Clinically Indicated					
Serum Chemistry[e]	X		X	X	X	Weekly	X	X
CBC w/ differential and platelet count[e]	X		X	X	X	Weekly	X	X
Tumor assessment by imaging studies[f]	X				X		X	X
Cystoscopy[g]		X			X		X	X
Biopsy[g]		X			X		X	X[k]
Urine tests[h]	X				X		X	X
Material for Molecular Analysis[i]		X			X		X	X
AUA/IPSS	X		X[o]		X	Weekly	X	X
EPIC-SF-12	X				X	Last week	X	X
Monitor Adverse Events		X	Throughout the "Treatment Phase" Period				X	X[p]

[a] Must be obtained prior to any study specific procedures

[b] Recalculate BSA every cycle only if body weight changed >10%. Height is to be obtained at screening only. Pre-chemotherapy ECOG performance status will be used to measure eligibility for enrollment irrespective of the institution at which it was performed.

[c] Blood pressure, pulse, temperature and respiratory rate

[d] For women of child bearing potential only (WoCBP)

[e] To include CBC and differential, serum creatinine, blood urea nitrogen, ALT, AST, alkaline phosphatase, total bilirubin, serum electrolytes (e.g. sodium, potassium, chloride, bicarbonate, calcium), glucose, total protein, albumin. Pre-chemotherapy laboratory investigations will be used to assess eligibility for all patients enrolled including those enrolled after cycle 1 of neoadjuvant chemotherapy

[f] Tumor assessment to be performed at baseline (within 8 weeks prior to treatment initiation). During treatment tumor assessments can be done within 7 days of corresponding cycle. For subjects who are taken off study for reasons other than disease progression, tumor assessment is to be performed at the discretion of the investigator. Imaging studies include CT chest, abdomen, and pelvis. Bone scan at the discretion of the investigator as clinically indicated. PET scan is required for N+ disease at screening and at post-neoadjuvant evaluation. In N0 disease PET scans will be performed at the discretion of the treating oncologist.

[g] Cystoscopy includes bimanual exam under anesthesia (EUA), TUR and biopsy of prostatic urethra and any site of disease. Cystoscopy at screening must include TURBT and biopsy at the base of the resected tumor site for molecular analysis.

[h] To include random urine chemistry, creatinine, total protein & cystoscopy at baseline. All other visits will only include urine cytology.

[i] Serum/plasma/whole blood/urine and tumor tissue will be collected as described in Section 8. All sample types in Section 8 must be collected prior and post-neoadjuvant chemotherapy. Tissue will only be collected after completion of radical therapy if there is evidence of residual disease, or progression on follow up if there is local, locoregional or distant disease. These specimens will be collected at the discretion of the treating oncologist.

[j] End of treatment visit should be performed within 30 days (+/- 14 days) of the end of chemoradiation, surgery, or at the time of progression or early discontinuation of therapy for any other reason.

[k] Follow up schedule consists of every 3 months (+/- 14 days) x 1 year, then every 4 months (+/- 14 days) x 1 year, then every 6 months (+/- 1 month) x 1 year, and then annually (+/- 1 month) as per standard of care and the discretion of the treating oncologist. Each evaluation within the study period of three years will include serum, plasma, whole blood, urine cytology, biopsy of the original tumor site and any suspicious areas, and bimanual examination. If after two re-evaluations in which the tumor site re-biopsies have been negative and the urologist observes nothing suspicious, cystoscopy and cytology without biopsy is permitted.

[l] To start within 12 weeks of screening.

[m] If patient has only a partial response at this evaluation, they will proceed to radical cystectomy within 12 weeks of completing neoadjuvant chemotherapy. After radical cystectomy, they will follow the schedule “at progression/end of treatment”.

[n] To begin within 6 weeks of post-neoadjuvant chemotherapy evaluation. Patients will have pre-chemotherapy IV hydration, blood work and anti-emetic treatment as per standard of care at the discretion of the medical oncologist. Patients will be seen in weekly review by radiation oncology for history and physical exam.

[o] To be done within one week before start of cycle 1 day 1

[p] At the discretion of the PI, adverse event collection will be discontinued at the 2 year post treatment follow-up visit or when the subject initiates any new therapy that would confound the accurate collection of adverse events.

APPENDIX II

ECOG/WHO/Zubrod	Karnofsky	Activity
Zubrod 0	Karnofsky 90 – 100	Normally active
Zubrod 1	Karnofsky 70 – 80	Symptoms but ambulatory where strenuous physical activity is restricted
Zubrod 2	Karnofsky 50 – 60	In bed less than 50 percent of the time. Capable of self care.
Zubrod 3	Karnofsky 30 - 40	In bed more than 50 percent of the time. Capable of only limited self-care.
Zubrod 4	Karnofsky 10 – 20	Completely disabled and bedridden
Death		

APPENDIX III

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