Official Protocol Title: Evaluating Local and Regional Immune Responses to Intravesical BCG administration to Patients with Invasive Bladder Cancer

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TABLE of Contents

STUDY SCHEMA.....3
1.0 OBJECTIVES.....4
2.0 BACKGROUND 4
3.0 DRUG INFORMATION 5
4.0 STAGING CRITERIA.....10
5.0 ELIGIBILITY CRITERIA 11
6.0 STUDY PLAN…….....11
7.0 STUDY CALENDAR 15
8.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS 15
9.0 STATISTICAL CONSIDERATIONS..... 15
10.0 REGISTRATION GUIDELINES 16
11.0 ETHICAL AND REGULATORY CONSIDERATIONS..... 16
12.0 DATA SAFETY MONITORING 16
REFERENCES……..20

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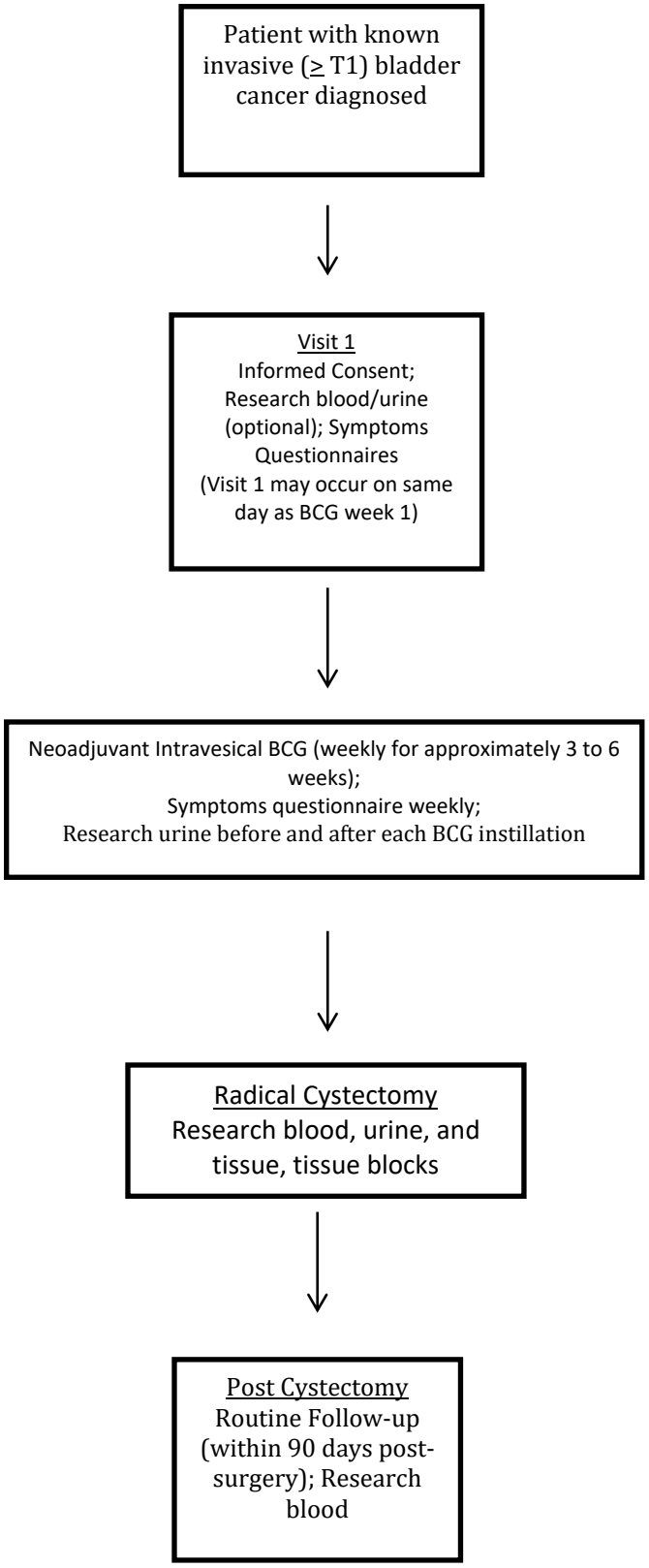
Study Schema



1.0 Objectives

1.1 The primary objectives of the study are to determine:

a. the ability of intravesical BCG immunotherapy to enhance tumor-specific immunity as measured by autologous T cells proliferation following co-culture with autologous monocyte-derived dendritic cells pulsed with autologous tumor lysate

1.2 Secondary objectives of the study are to determine:

a. the immune effects of BCG in the tumor microenvironment and tumor-draining lymph nodes
b. the extent to which BCG is processed and presented by tumors cells and/or antigen-presenting cells
c. the ability of BCG to elicit tumor necrosis/apoptosis in invasive disease
d. the pathologic stage of disease following BCG treatment
e. to determine if immune changes elicited by BCG in patients with bladder cancer will provide an opportunity for benefit from mTOR inhibition in tumor milieu

2.0 Background

2.1 Overview

Urothelial carcinoma of the bladder (UCB) accounted for 70,530 new cases of cancer and 14,680 cancer-related deaths in the United States during 2010[1]. Of these new cases, approximately 25% presented as invasive tumors involving the muscular wall of the bladder. Transurethral resection of bladder tumor (TURBT) is an endoscopic procedure that allows for histopathologic assessment and disease staging. TURBT is the primary diagnostic procedure for new and recurrent bladder tumors. TURBT can also be therapeutic for small or non-muscle invasive tumors. However, when muscle-invasive disease is detected, the standard of care involves bladder removal or radical cystectomy (RC) and urinary diversion.

Despite aggressive surgical treatment with bladder removal, up to 50% of patients undergoing RC will experience disease recurrence[2,3]. Chemotherapy given both before and after surgery has been investigated to improve survival for these patients[4-10]. Neoadjuvant chemotherapy renders only a modest improvement in survival- approximately 5-7% absolute benefit in 5-year recurrence free survival[4,11]. Nevertheless neoadjuvant chemotherapy has not been widely adopted due to concerns of toxicity for a modest benefit[11]. In addition, many consider that giving adjuvant chemotherapy for only those patients with high risk pathologic features is a better alternative than treating all patients with neoadjuvant chemotherapy[11]. Current research efforts, therefore, are aimed towards identifying less toxic regimens for use in the neoadjuvant setting. Such effective therapies, especially using those agents with low to moderate toxicity that are easily administered, would be a significant advance in the treatment of invasive bladder cancer. The development of novel, effective therapies, remains an area of unmet need.

2.2 Pre-surgical trial

Pre-surgical drug testing is an excellent venue in which paired tumor tissue can be evaluated before and after exposure to a drug to address target specificity, drug delivery, physiologic effects on tumor growth and apoptosis, and correlation of biomarkers with clinical activity[12-15]. Pre-surgical trials have been conducted in radical cystectomy populations were patients were given drugs for 4-7 weeks prior to surgery[12,13]. This approach has been acceptable given the evidence that delaying cystectomy for more than 3 months is not associated with a worse clinical outcome[16-18]. In our protocol, we will seek to characterize the immune effects of intravesical administration in patients undergoing radical cystectomy to help elucidate the anticancer immune mechanisms of BCG in patients with bladder cancer.

2.3 BCG immunotherapy

BCG immunotherapy is the mainstay treatment for high-risk non-muscle invasive bladder cancer. BCG specifically, and Mycobacteria generally, have long been known to be potent immune stimulators but the mechanism of action of BCG in cancer remains incompletely understood. BCG reduces bladder cancer relapse by 50%[19-21] and without it, the risk for cancer relapse is extremely

high (45-80% in non-muscle invasive disease)[22-24]. In animal models, it has been established that BCG could be effective to treat bladder cancer through immune mechanisms mediating anti-cancer immunity against other cancers, including T cells[25], NK cells[26] and IFN-γ[27]. Nevertheless, many mechanistic details are lacking, notably to what extent BCG generates BCG-specific and tumor-specific immunity and whether BCG affects tumor tolerance. Recently, it was demonstrated that BCG efficacy is mediated, at least in part, through stimulation and recruitment of T cells into peri-tumoral draining lymph nodes[28]. This finding has important implications for the utilization of BCG as discussed below.

Currently BCG is principally used to manage non-muscle invasive bladder cancer and has not been employed to treat muscle-invasive disease, since muscle-invasive disease requires bladder removal. However, to date, BCG is the most immunostimulant utilized in bladder cancer there is increasing evidence that BCG could improve tumor-specific immunity[28,29]. If BCG can improve tumor-specific immunity, then it could be used as an adjuvant therapy in the management of bladder cancers of all stages, including muscle-invasive disease. For example, BCG given to patients with muscle-invasive disease prior to cystectomy could improve tumor-specific immunity and enable patients to improve tumor clearance in cases of nodal or distant metastasis. By increasing tumor-specific immunity and memory T cell responses, BCG could also enable sustained long-term protection from recurrent disease.

2.4 Rapamycin and immune modulation.

mTOR is a protein kinase that regulates cell growth, proliferation, and other key elements of cell survival. Rapamycin (sirolimus) has long been used in combination with immunosuppressive agents to prevent organ allograft rejection. However, there is no published human study of normal subjects that shows that rapamycin alone is immune suppressive[30]. In fact, recent publications specifically looking at rapamycin immune effects demonstrate that rapamycin boosts immunity to infections[31-33], accelerates memory $CD8^+$ T cell differentiation[33], and improves antigen-specific immunity[33]. Further, rapamycin fed to mice prolongs lifespan[34], which is inconsistent with significant immunosuppression. Finally, immune modulating properties of mTOR inhibition include effects on T cells, interferon (IFN)-γ and other immune mediators critical to anti-cancer immune defenses[33,35-38]. In preclinical studies, we found rapamycin to improve immune responses and improve the activity of BCG. In secondary objectives, we aim to clarify the effect of BCG in the tumor milieu to determine mTOR inhibition could be beneficial. Specifically, we aim to measure the quantity of regulatory immune activity including PD-1 expression and Treg cells which could negatively influence BCG's activity. If these populations are elevated, mTOR inhibition may be beneficial, as we have observed rapamycin to decrease PD-1 T cells.

3.0 Drug Information (TICE® BCG)

3.1 Description: TICE® BCG for intravesical use, is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis. The TICE® strain was developed at the University of Illinois from a strain originated at the Pasteur Institute. The medium in which the BCG organism is grown for preparation of the freeze-dried cake is composed of the following ingredients: glycearin, asparagine, citric acid, potassium phosphate, magnesium sulfate, and iron ammonium citrate. The final preparation prior to freeze drying also contains lactose. The freeze-dried BCG preparation is delivered in glass vials, each containing 1 to 8 x 108 colony forming units (CFU) of TICE® BCG which is equivalent to approximately 50 mg wet weight. Determination of in-vitro potency is achieved through colony counts derived from a serial dilution assay. A single dose consists of 1 reconstituted vial (see DOSAGE AND ADMINISTRATION). For intravesical use the entire vial is reconstituted with sterile saline. TICE® BCG is viable upon reconstitution. No preservatives have been added.

3.2 Mechanism of Action: TICE® BCG induces a granulomatous reaction at the local site of administration. Intravesical TICE® BCG has been used as a therapy for, and prophylaxis against, recurrent tumors in patients with carcinoma *in situ* (CIS) of the urinary bladder, and to prevent recurrence of Stage TaT1 papillary tumors of the bladder at high risk of recurrence. The precise mechanism of action is unknown but is thought to be related to immune stimulation and subsequent immune-mediated cytotoxicity.

3.3 Toxicology**:** Symptoms of bladder irritability, related to the inflammatory response induced, are reported in approximately 60% of patients receiving TICE® BCG. The symptoms typically begin 4–6 hours after instillation and last 24–72 hours. The irritative side effects are usually seen following the third instillation, and tend to increase in severity after each administration.

3.4 Clinical Studies: To evaluate the efficacy of intravesical administration of TICE® BCG in the treatment of carcinoma in situ, patients were identified who had been treated with TICE® BCG under six different Investigational New Drug (IND) applications in which the most important shared aspect was the use of an induction plus maintenance schedule. Patients received TICE® BCG (50 mg; 1 to 8 x 10^8 CFU) intravesically, once weekly for at least 6 weeks and once monthly thereafter for up to 12 months. A longer maintenance was given in some cases. The study population consisted of 153 patients, 132 males, 19 females, and 2 unidentified as to gender. Thirty patients lacking baseline documentation of CIS and four patients lost to follow-up were not evaluable for treatment response. Therefore, 119 patients were available for efficacy evaluation. The mean age was 69 years (range: 38–97 years). There were two categories of clinical response: (1) Complete Histological Response (CR), defined as complete resolution of carcinoma in situ documented by cystoscopy and cytology, with or without biopsy; and (2) Complete Clinical Response Without Cytology (CRNC), defined as an apparent complete disappearance of tumor upon cystoscopy. The results of a 1987 analysis of the evaluable patients are shown in Table I.

TABLE I: THE RESPONSE OF PATIENTS WITH CIS BLADDER CANCER IN SIX IND STUDIES

	Entered	Evaluable	CR	CRNC	Overall Response
No. (%)Of Patients	153	119 (78%)	54 (46%)	36 (30%)	90 (76%)

A 1989 update of these data is presented in Table II. The median duration of follow-up was 47 months.

TABLE II: FOLLOW-UP RESPONSE OF PATIENTS WITH CIS BLADDER CANCER IN SIX IND STUDIES

	1989 Status of 90 Responders (CR or CRNC)			
	1987/CR	1987/CRNC	1987 Response	
Response	n = 54	n = 36	n = 90	Percent
CR	30	15	45	50
CRNC	0	0	0	0
Unrelated Deaths	6	6	12	13
Failure	18	15	33	37

There was no significant difference in response rates between patients with or without prior intravesical chemotherapy. The median duration of response, calculated from the Kaplan-Meier curve as median time to recurrence, is estimated at 4 years or greater. The incidence of cystectomy for 90 patients who achieved a complete response (CR or CRNC) was 11%. The median time to cystectomy in patients who achieved a complete response (CR or CRNC) exceeded 74 months. The efficacy of intravesical TICE® BCG in preventing the recurrence of a TaT1 bladder cancer after complete transurethral resection of all papillary tumors was evaluated in two open-label randomized phase III clinical trials. Initial diagnosis of patients included in the studies was determined by cystoscopic biopsies. One was conducted by the Southwestern Oncology Group (SWOG) in patients at high risk of recurrence[39]. High risk was defined as two occurrences of tumor within 56 weeks, any stage T1 tumor, or three or more tumors presenting simultaneously. The second study was conducted at the Nijmegen University Hospital; Nijmegen, The Netherlands. In this study patients were not selected for high risk of recurrence. In both studies treatment was initiated between 1 and 2 weeks after TUR. In the SWOG trial (study 8795) patients were randomized to TICE® BCG or mitomycin C (MMC)[39]. Both drugs were given intravesically weekly for 6 weeks, at 8 and 12 weeks, and then monthly for a total treatment duration of 1 year. Cystoscopy and urinary cytology were performed every 3 months for 2 years. Patients with progressive disease or residual or recurrent disease at or after the 6 month follow-up were removed from the study and were classified as treatment failures.

A total of 469 patients was entered into the study: 237 to the TICE® BCG arm and 232 to the MMC arm. Twenty-two patients were subsequently found to be ineligible, and 66 patients had concurrent CIS, and were analyzed separately. Four patients were lost to follow-up, leaving 191 evaluable patients in the TICE® BCG arm and 186 in the MMC arm. Of the patients, 84% were male and 16% were female. The average age of these patients was 65 years old. The Kaplan-Meier estimates of 2 year disease-free survival are shown in Table III. The difference in disease-free survival time between the two groups was statistically significant by the log rank test (p=0.03). The 95% confidence interval of the difference in 2 year disease-free survival was 12% ± 10%. No statistically significant differences between the groups were noted in time to tumor progression, tumor invasion, or overall survival.

TABLE III: RESULTS OF SWOG STUDY 8795

	TICE® BCG Arm N = 191	MMC Arm N = 186
Estimated Disease-Free Survival at 2 years 95% Confidence Interval (CI)	57% (50%, 65%)	45% (38%, 53%)

In the Nijmegen study, the efficacy of three treatments was compared: TICE substrain BCG, Rijksinstituut voor Volksgezondheid en Milieuhygiene substrain BCG (BCG-RIVM), and MMC. TICE® BCG and BCG-RIVM were given intravesically weekly for 6 weeks. In contrast to the SWOG study, maintenance BCG was not given. Mitomycin C was given intravesically weekly for 4 weeks and then monthly for a total duration of treatment of 6 months. Cystoscopy and urinary cytology were performed every 3 months until recurrence. A total of 469 patients was enrolled and randomized. Thirty-two patients were not evaluable, 17 were ineligible, 15 were withdrawn before treatment, and 50 had concurrent CIS and were analyzed separately, leaving 387 evaluable patients: 117 in the TICE® BCG arm, 134 in the BCG-RIVM arm, and 136 in the MMC arm. Twenty-eight patients (24%) in the TICE® BCG arm, 32 patients (24%) in the BCG-RIVM arm and 24 patients (18%) in the MMC arm had TaG1 tumors. The median duration of follow-up was 22 months (range 3–54 months). The Kaplan-Meier estimates of 2 year disease-free survival are shown in Table IV. The differences in disease-free survival among the three arms were not statistically significant by the log-rank test (p=0.08).

TABLE IV: RESULTS OF NIJMEGEN STUDY

	TICE® BCG Arm N = 117	BCG-RIVM Arm N = 134	MMC Arm N = 136
Estimated Disease-Free Survival at 2 years 95% Confidence Interval (CI)	53% (44%, 64%)	62% (53%, 72%)	64% (55%, 74%)

In both the SWOG 8795 study and the Nijmegen study, acute toxicity was more common, and usually more severe, with TICE® BCG than with MMC (see ADVERSE REACTIONS).

3.5 Warnings: BCG LIVE (TICE® BCG) is not a vaccine for the prevention of cancer. BCG Vaccine, U.S.P., not BCG LIVE (TICE® BCG), should be used for the prevention of tuberculosis. For vaccination use, refer to BCG Vaccine, U.S.P. prescribing information. TICE® BCG is an infectious agent. Physicians using this product should be familiar with the literature on the prevention and treatment of BCG-related complications, and should be prepared in such emergencies to contact an infectious disease specialist with experience in treating the infectious complications of intravesical BCG. The treatment of the infectious complications of BCG requires long-term, multiple-drug antibiotic therapy. Special culture media are required for mycobacteria, and physicians administering intravesical BCG or those caring for these patients should have these media readily available.

3.5.1 Instillation of TICE® BCG with an actively bleeding mucosa may promote systemic BCG infection. Treatment should be postponed for at least one week following transurethral resection, biopsy, traumatic catheterization, or gross hematuria.

3.5.2 Deaths have been reported as a result of systemic BCG infection and sepsis.2,3 Patients should be monitored for the presence of symptoms and signs of toxicity after each intravesical treatment. Febrile episodes with flu-like symptoms lasting more than 72 hours, fever ≥103°F, systemic manifestations increasing in intensity with repeated instillations, or persistent abnormalities of liver function tests suggest systemic BCG infection and may require antituberculous therapy. Local symptoms (prostatitis, epididymitis, orchitis) lasting more than 2–3 days may also suggest active infection.

3.5.3 The use of TICE® BCG may cause tuberculin sensitivity. Since this is a valuable aid in the diagnosis of tuberculosis, it is advisable to determine the tuberculin reactivity by PPD skin testing before treatment.

3.5.4 Intravesical instillations of BCG should be postponed during treatment with antibiotics, since antimicrobial therapy may interfere with the effectiveness of TICE® BCG (see PRECAUTIONS). TICE® BCG should not be used in individuals with concurrent infections.
Small bladder capacity has been associated with increased risk of severe local reactions and should be considered in deciding to use TICE® BCG therapy.

3.6 Management of Serious BCG Complications: Acute, localized irritative toxicities of TICE® BCG may be accompanied by systemic manifestations, consistent with a “flu-like” syndrome. Systemic adverse

effects of 1–2 days' duration such as malaise, fever, and chills often reflect hypersensitivity reactions. However, **symptoms such as fever of ≥38.5°C (101.3°F), or acute localized inflammation such as epididymitis, prostatitis, or orchitis persisting longer than 2–3 days suggest active infection, and evaluation for serious infectious complication should be considered.**

3.6.1 In patients who develop persistent fever or experience an acute febrile illness consistent with BCG infection, two or more anti-mycobacterial agents should be administered while diagnostic evaluation, including cultures, is conducted and BCG treatment should be discontinued. Negative cultures do not necessarily rule out infection. Physicians using this product should be familiar with the literature on prevention, diagnosis, and treatment of BCG-related complications and, when appropriate, should consult an infectious disease specialist or other physician with experience in the diagnosis and treatment of mycobacterial infections. TICE® BCG is sensitive to the most commonly used antituberculous agents (isoniazid, rifampin and ethambutol). TICE® BCG is not sensitive to pyrazinamide.

3.7 PRECAUTIONS:

3.7.1 TICE® BCG contains live mycobacteria and should be prepared and handled using aseptic technique (see **Preparation of Agent** subsection of DOSAGE AND ADMINISTRATION). BCG infections have been reported in health care workers preparing BCG for administration. Needle stick injuries should be avoided during the handling and mixing of TICE® BCG. Nosocomial infections have been reported in patients receiving parenteral drugs which were prepared in areas in which BCG was prepared.4

3.7.2 BCG is capable of dissemination when administered by intravesical route and serious reactions, including fatal infections, have been reported in patients receiving intravesical BCG.3 Care should be taken not to traumatize the urinary tract or to introduce contaminants into the urinary system. Seven to 14 days should elapse before TICE® BCG is administered following TUR, biopsy, or traumatic catheterization.

3.7.3 TICE® BCG should be administered with caution to persons in groups at high risk for HIV infection

3.8 Drug Interactions

3.8.1 Drug combinations containing immunosuppressants and/or bone marrow depressants and/or radiation interfere with the development of the immune response and should not be used in combination with TICE® BCG. Antimicrobial therapy for other infections may interfere with the effectiveness of TICE® BCG. There are no data to suggest that the acute, local urinary tract toxicity common with BCG is due to mycobacterial infection and antituberculosis drugs (e.g., isoniazid) should not be used to prevent or treat the local, irritative toxicities of TICE® BCG.

3.8.2 Carcinogenesis, Mutagenesis, Impairment of Fertility: TICE® BCG has not been evaluated for its carcinogenic, mutagenic potentials or impairment of fertility.

3.9 Pregnancy

3.9.1 Teratogenic Effects – Pregnancy Category C: Animal reproduction studies have not been conducted with TICE® BCG. It is also not known whether TICE® BCG can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. TICE® BCG should not be given to a pregnant woman except when clearly needed. Women should be advised not to become pregnant while on therapy.

3.10 Nursing Mothers: It is not known whether TICE® BCG is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions from TICE® BCG in nursing infants, it is advisable to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

3.11 Pediatric Use: Safety and effectiveness of TICE® BCG for the treatment of superficial bladder cancer in pediatric patients have not been established.

3.12 Geriatric Use: Of the total number of subjects in clinical studies of TICE® BCG, the average age was 66 years old. No overall difference in safety or effectiveness was observed between older and younger subjects. Other reported clinical experience has not identified differences in response between elderly and younger patients, but greater sensitivity of some older individuals to BCG cannot be ruled out.

3.13 ADVERSE REACTIONS

Symptoms of bladder irritability, related to the inflammatory response induced, are reported in approximately 60% of patients receiving TICE® BCG. The symptoms typically begin 4–6 hours after instillation and last 24–72 hours. The irritative side effects are usually seen following the third instillation, and tend to increase in severity after each administration.

The irritative bladder adverse effects can usually be managed symptomatically with products such as

pyridium, propantheline bromide, oxybutynin chloride and acetaminophen. The mechanism of action of the irritative side effects has not been firmly established, but is most consistent with an immunological mechanism.3 There is no evidence that dose reduction or antituberculous drug therapy can prevent or lessen the irritative toxicity of TICE® BCG. “Flu-like” symptoms (malaise, fever, and chills) which may accompany the localized, irritative toxicities often reflect hypersensitivity reactions which can be treated symptomatically. Antihistamines have also been used.5 Adverse reactions to TICE® BCG tend to be progressive in frequency and severity with subsequent instillation. Delay or postponement of subsequent treatment may or may not reduce the severity of a reaction during subsequent instillation. Although uncommon, serious infectious complications of intravesical BCG have been reported.2,3,6 The most serious infectious complication of BCG is disseminated sepsis with associated mortality. In addition, *M. bovis* infections have been reported in lung, liver, bone, bone marrow, kidney, regional lymph nodes, and prostate in patients who have received intravesical BCG. Some male genitourinary tract infections (orchitis/epididymitis) have been resistant to multiple drug antituberculous therapy and required orchiectomy. **If a patient develops persistent fever or experiences an acute febrile illness consistent with BCG infection, BCG treatment should be discontinued and the patient immediately evaluated and treated for systemic infection (see WARNINGS).** The local and systemic adverse reactions reported in a review of 674 patients with

TABLE V: SUMMARY OF ADVERSE EFFECTS SEEN IN 674 PATIENTS WITH SUPERFICIAL BLADDER CANCER, INCLUDING 153 WITH CARCINOMA *IN SITU*

	Percent of Patients			Percent of Patients	
Adverse Event	N	Overall (Grade ≥3)	Adverse Event	N	Overall (Grade ≥3)
Dysuria	401	60% (11%)	Arthritis/Myalgia	18	3% (<1%)
Urinary Frequency	272	40% (7%)	Headache/Dizziness	16	2% (0)
Flu-Like Syndrome	224	33% (9%)	Urinary Incontinence	16	2% (0)
Hematuria	175	26% (7%)	Anorexia/Weight Loss	15	2% (<1%)
Fever	134	20% (8%)	Urinary Debris	15	2% (<1%)
Malaise/Fatigue	50	7% (0)	Allergy	14	2% (<1%)
Cystitis	40	6% (2%)	Cardiac (Unclassified)	13	2% (1%)
Urgency	39	6% (1%)	Genital Inflammation/		
Nocturia	30	5% (1%)	Abscess	12	2% (<1%)
Cramps/Pain	27	4% (1%)	Respiratory (Unclassified)	11	2% (<1%)
Rigors	22	3% (1%)	Urinary Tract Infection	10	2% (1%)
Nausea/Vomiting	20	3% (<1%)	Abdominal Pain	10	2% (1%)

TABLE VI: MOST COMMON ADVERSE REACTIONS IN SWOG STUDY 8795*

	Study Arm			
	TICE® BCG (N = 222)		MMC (N = 220)	
Adverse Event	All Grades	Grade ≥3	All Grades	Grade ≥3
Dysuria	115 (52%)	6 (3%)	77 (35%)	5 (2%)
Urgency/Frequency	112 (50%)	5 (2%)	63 (29%)	7 (3%)
Hematuria	85 (38%)	6 (3%)	56 (25%)	5 (2%)
Flu-Like Symptoms	54 (24%)	1 (<1%)	29 (13%)	0
Fever	37 (17%)	1 (<1%)	7 (3%)	0
Pain (Not Specified)	37 (17%)	4 (2%)	22 (10%)	1 (<1%)
Hemorrhagic Cystitis	19 (9%)	3 (1%)	10 (5%)	0
Chills	19 (9%)	0	2 (1%)	0
Bladder Cramps	18 (8%)	0	9 (4%)	0
Nausea	16 (7%)	0	12 (5%)	0
Incontinence	8 (4%)	0	3 (1%)	0
Myalgia/Arthralgia	7 (3%)	0	0	0
Diaphoresis	7 (3%)	0	1 (<1%)	0
Rash	6 (3%)	1 (<1%)	16 (7%)	2 (1%)

3.14 **OVERDOSAGE** Overdosage occurs if more than one vial of TICE® BCG is administered per instillation. If overdosage occurs, the patient should be closely monitored for signs of active local or systemic BCG infection. For acute local or systemic reactions suggesting active infection, an infectious disease specialist experienced in BCG complications should be consulted.

3.15 DOSAGE AND ADMINISTRATION

The dose for the intravesical treatment of carcinoma *in situ* and for the prophylaxis of recurrent papillary tumors consists of one vial of TICE® BCG suspended in 50 ml preservative-free saline.

3.16 Preparation of Agent

The preparation of the TICE® BCG suspension should be done using aseptic technique. To avoid cross-contamination, parenteral drugs should not be prepared in areas where BCG has been prepared. A separate area for the preparation of the TICE® BCG suspension is recommended. All equipment, supplies and receptacles in contact with TICE® BCG should be handled and disposed of as biohazardous. The pharmacist or individual responsible for mixing the agent should wear gloves and take precautions to avoid contact of BCG with broken skin. If preparation cannot be performed in a biocontainment hood, then a mask and gown should be worn to avoid inhalation of BCG organisms and inadvertent exposure to broken skin.

Option-1 (Using Syringe Method)

Draw 1 ml of sterile, preservative-free saline (0.9% Sodium Chloride Injection U.S.P.) at 4–25°C, into a small syringe (e.g., 3 ml) and add to one vial of TICE® BCG to resuspend. Gently swirl the vial until a homogenous suspension is obtained. Avoid forceful agitation which may cause clumping of the mycobacteria. Dispense the cloudy TICE® BCG suspension into the top end of a catheter-tip syringe which contains 49 ml of saline diluent, bringing the total volume to 50 ml. To mix, gently rotate the syringe.

Option-2 (Using Reconstitution Accessories)

Reconstitution Accessories may be provided with each TICE® BCG product order. Please refer to the Instructions For Use provided with the accessories for a full description of the product reconstitution procedures using these accessories.

The reconstituted TICE® BCG should be kept refrigerated (2–8°C), protected from exposure to direct sunlight, and used within 2 hours. Unused solution should be discarded after 2 hours.

Note: DO NOT filter the contents of the TICE® BCG vial. Precautions should be taken to avoid exposing the TICE® BCG to direct sunlight. Bacteriostatic solutions must be avoided. In addition, use only sterile preservative-free saline, 0.9% Sodium Chloride Injection U.S.P. as diluent.

3.17 HOW SUPPLIED

TICE® BCG is supplied in a box of one vial of TICE® BCG. Each vial contains 1 to 8 x 108 CFU, which is equivalent to approximately 50 mg (wet weight), as lyophilized (freeze-dried) powder. NDC 0052-0602-02.

3.18 STORAGE

The intact vials of TICE® BCG should be stored refrigerated, at 2–8°C (36–46°F).

This agent contains live bacteria and should be protected from **direct** sunlight. The product should not be used after the expiration date printed on the label.

4.0 Staging Criteria

Bladder cancer
Primary Tumor (T)

Clinical Stage

cT1	Invades subepithelial connective tissue (lamina propria)
cT2	Muscle-invasive
cT3	Residual mass on exam under anesthesia following TURBT
cT4	Clinically fixed disease on exam under anesthesia

Pathologic Stage

pT1	Invades subepithelial connective tissue (lamina propria)
pT2	Muscle-invasive
pT3	Invades perivesicle fat
pT4	Invades adjacent organs (prostate, vagina, uterus, bone, etc…)

Histiologic subtypes

Urothelial cell carcinoma (Transitional cell carcinoma)
Squamous cell carcinoma
Adenocarcinoma or glandular carcinoma

Sarcomatoid carcinoma
Micropapillary carcinoma

Histiologic grade

High-grade Poorly- or un-differentiated

5.0 Eligibility Criteria

Inclusion Criteria:
5.1 Have suspected or known invasive (≥T1) bladder cancer
5.2 Not be in an immunosuppressed state (e.g. HIV +, use of chronic steroids (>1 month))
5.3 Be able to give informed consent
5.4 Be age 18 or older

Exclusion Criteria:
5.1.1 Have non-invasive (<T1) bladder cancer
5.1.2 Is in an immunosuppressed state (e.g. HIV +, use of chronic steroids (>1 month))
5.1.3 Unable to give informed consent
5.1.4 < 18 or older

6.0 Study Plan

6.0 Study Design
This is a one-arm, open label, interventional study. For the purposes of enrollment "study procedure" refers to intravesical BCG instillation. See below for a description of study procedure.

6.1 Recruitment

Participants will be recruited through the urology outpatient clinics at the Medical Arts and Research Center (MARC). Attending physicians in the Department of Urology, and selected other personnel who also serve as research staff for this study, will identify prospective study participants. To eliminate selection bias, any patient who presents with invasive bladder cancer and meets the inclusion/exclusion criteria will be given an option to participate in the study. The inclusion of participants or refusal to participate by patients eligible for this study will in no way compromise the quality of health care they will receive.

Study eligibility will be determined by investigators and study staff with legitimate access to patient PHI due to their clinical role and/or as members of the research team. Patients who meet the eligibility criteria will be approached by one of the investigators or other members of the study staff to determine whether they are willing to participate in the study. Prior to study entry, the study staff will explain to each potential subject the research objectives, risks and benefits of study participation, alternative treatments available, and the subjects' rights and responsibilities. If the patient agrees to participate, informed consent will be obtained. Research blood and urine may be obtained-OPTIONAL (details in 6.2). Baseline symptoms questionnaires will be administered.

Patients will be approached in a private consult room located within the GU clinic at the MARC. Usual privacy policy practices will be followed. The study procedures and objectives will be described to them and they will be given a copy of the consent document. Participants will also be given contact information for someone they can call with any questions that may arise.

6.2 Visit 1

Routine medical history and demographics and physical exam will be performed during the visit (standard care).

6.2.1 Research blood and urine collection & handling OPTIONAL

Research blood and urine may be collected after signing informed consent (OPTIONAL). Approximately 50 mL or 3.5 tablespoons of blood will be taken from a peripheral vein or an indwelling catheter if present and functional specifically for research studies. Study staff will then provide a urine specimen cup and self-catch instruction to the participant. The participant will be able to do this in the privacy of the clinic restroom.

If collected, research blood and urine specimens will be transported to our lab in the STRF in approved containers by qualified personnel. Additional blood will be collected as needed for initial eligibility screening. Urine will be processed by centrifugation and the supernatant will be stored separately from the pellet. Both blood and urine samples are de-identified in the lab per HIPAA protocol.

Baseline symptoms questionnaires will also be administered.

6.3 Neoadjuvant Intravesical BCG Instillation

Prior to undergoing routine radical cystectomy (standard care), participant will receive 3 to 6 weeks of neoadjuvant intravestical BCG instillation weekly as part of the research study procedure. The instillation will be scheduled roughly one week apart pending on the clinic's and participant's schedules, and also participant's reaction to the BCG instillation. BCG Week 1 may occur on the same day as Visit 1.

Intravesical BCG will be given as the intervention measure for treatment of bladder cancer. In brief, following sterile preparation, a Foley catheter is introduced into the bladder thru the urethral meatus. The BCG is instilled through gravity drainage and held in the bladder for approximately 2 hours. Instructions are provided to the patient regarding the proper disposal of the BCG. The duration of BCG treatment is allowed to fluctuate between 3-6 weeks for two reasons: (1) This allows for flexibility in scheduling participants for radical cystectomy as the time from enrollment to cystectomy would vary based on surgeon and patient preferences and on operative room availability. In this manner, the surgery date can be established first and then duration of treatment would be based on amount of time available between enrollment and cystectomy date. In our experience, this "time period" is usually 4-6 weeks; (2) The number of BCG treatments needed to elicit expected immune responses is not known. In fact the current paradigm of 6 weekly treatments has little scientific justification but was based on a rather arbitrary dosing schedule; (3) Allowing for multiple durations of therapy, could provide us with the opportunity to explore differences in immune responses across different dosing duration regimens.

6.3.1 Research Urine Collection and Handling Pre and Post-BCG Instillation (research only)

We will collect a urine sample pre-BCG treatment. We will also collect urine samples post-BCG instillations, specifically the second void, which typically occur 4-6 hours post Intravesical BCG treatment. Participants will be given the following options for collection of urine sample post intravesical BCG treatments:

a) Participant may remain in the clinic for urine collection
-OR-
b) Participant may leave the clinic with a urine sample collection cup and biohazard bag. Participant will be given specific instruction to collect second void post intravesical BCG therapy, and return the sample to the clinic.

Study team member will work with participant on how post BCG therapy urine will be collected and returned to the team. Post-BCG urine collection will take place at weekly during the 3-6 weeks of intravesical BCG treatments. Urine will be processed by centrifugation and the supernatant will be stored separately from the pellet.

6.3.2 Dose Modification and Toxicity

BCG at a dose of 1 to 8 x 10^8 is given intravesically for 2 hours, irrespective of weight or age. This dose was chosen because previous experience and standard care with BCG.

Adjustments (1/2 strength or ¼ strength) are allowed for participants demonstrating poor toleration of treatment as is done in the course of usual standard care.

*Note: Due to the nationwide shortage of BCG, the entire vial (1 full dose) may not be used for the treatment. Patients may elect to receive BCG treatments at standard of care physician.

BCG treatment may be held by the treating physician for clinical reasons such as active urinary tract infection or gross bleeding from the bladder as per standard care. Regardless of dose or amount of BCG given, patients will be allowed to continue with study procedures.

6.3.3 Monitoring during BCG Instillation

At each visit (including baseline) symptom questionnaires will be administered prior to any procedures. The symptom questionnaires will assess irritative LUTS present. If BCG Grade 3 bladder toxicity occurs it will result in postponing combined therapy by 1 week, provided that the patient's symptoms resolved.

Participant-reported side effect questionnaires will be administered prior to each weekly instillation to ascertain the previous instillation's effects. Patients will be monitored in the clinic during instillation of BCG.

Adverse event (AE) monitoring will be assessed at each clinic visit, during BCG treatments, and on the day of cystectomy. Safety will be assessed by a urologist with training in administration of intravesical BCG.

Dose-Limiting Toxicity (DLT) - Dose-limiting toxicity (DLT) is defined as any grade 3 or 4 systemic toxicities or evidence of grade 4 bladder toxicity using the National Cancer Institute common toxicity criteria.

6.4 Research Blood, urine, & tissue collection (research only) at the time of Radical Cystectomy (standard care)

Research blood and urine will be collected at radical cystectomy.. Approximately 50 mL or 3.5 tablespoons of blood will be taken from a peripheral vein or an indwelling catheter if present and functional specifically for research studies. The handling method can be found in Section 6.2.1. Voided urine or urine from an indwelling catheter will be collected.

At the time of radical cystectomy, a section of tumor tissue, normal adjacent bladder tissue, and adjacent lymphatic tissue will be taken for immune studies. A small section of the tissue from the bladder (approximately 5 mm x 5 mm x 5 mm) will be taken. Most patients with muscle invasive tumors have large tumors so taking a small section does not compromise pathologic assessment of stage. This research tissue would be excess tumor and would not compromise the ability of the pathologist to stage or grade the tumor, and poses no additional meaningful risk to the patient. To ensure this we clarify that the tumor tissue taken for research represents a small (10% of the tumor volume or less) component of the tumor. If sufficient excess tumor is not available for research purposes, then no research bladder tissue would be removed. We have experience with such post-treatment tumor acquisition for research in bladder cancer studies here at UTHSCSA. For benign adjacent bladder mucosa, we will also take a small section (approximately 5 mm x 5 mm x 5 mm) of tissue. Normally for lymph node dissections, we typically remove 20-60 lymph nodes during a cystectomy. We will isolate several (2-8) lymph nodes to be used for research purposes. The lymph node will removed and then surgically split in half. One half will be sent for pathologic processing and the other half will be used for research. In this way, we do not compromise the ability to pathologically examine the lymph nodes.

We will also obtain the tissue blocks from cystectomy on all subjects from pathology where surgery was done.

6.5 Post-Radical Cystectomy Follow-up Visit (standard care)

As per routine care, participants will return to clinic approximately within 90 days following surgery for a follow-up visit. During this visit, research blood will be collected.

Approximately 50 mL or 3.5 tablespoons of blood will be taken from a peripheral vein or an indwelling catheter if present and functional specifically for research studies. The research blood specimens will be transported to our lab in the STRF in approved containers by qualified personnel. The blood sample will be de-identified in the lab per HIPAA protocol.

7.0 Study Calendar

Study Component/Procedures	Visit 1	BCG Week 1 (may be same day as Visit 1)	BCG Week 2	BCG Week 3	BCG Week 4[a]	BCG Week 5[a]	BCG Week 6[a]	Cystectomy	Post Cystectomy (~within 90 days post cystectomy)
Informed Consent	R								
Medical History, Demographics, Physical Exam	S								
Assessment of AEs		R	R	R	R	R	R		
Urinary symptoms questionnaires	R	R	R	R	R	R	R		
Blood Collection	R*							R	R
Urine Collection (pre and post treatment urine collected on BCG weeks)	R*	R	R	R	R	R	R	R	
Tissue collection								R	
Tissue blocks from cystectomy procedure								R	

"R" indicates performed only for research purposes and "S" indicates performed for standard care practice
a- Additional BCG treatments beyond week 3 will be given selectively to participants based on time interval between study enrollment and scheduled cystectomy date
* Optional blood and urine collection

8.0 Criteria for Evaluation and Endpoint Definitions

8.1 The primary end-point is taken as difference in tumor-specific immune response (comparing before and after treatment with BCG). Tumor-specific immune response will be measured using a standard T cell proliferation assay. T cell response to autologous tumor will be measured using a paired analysis by utilizing PBMCs collected before and after BCG therapy. Autologous monocyte-derived dendritic cells (moDCs) and autologous T cells will be co-cultured following pulsation of moDCs with whole tumor lysates.

8.2 Secondary endpoints are exploratory and will not be considered for determining sample size. Secondary endpoints include (1) description of tumor infiltrating lymphocyte populations following BCG (examining 3 weeks of treatment versus 4,5, or 6 weeks as available); (2) determination of the presence/absence of BCG in lymph nodes following treatment; (3) examining extent of necrosis/apoptosis in tumor following BCG treatment; (4) pathologic assessment of tumor stage following BCG treatment; (5) characterization of systemic immune populations before treatment, at time of cystectomy,

9.0 Statistical Considerations

9.1 Sample Size Determination

This is a one-arm open label study of the tumor-specific immune effect of BCG in participants undergoing radical cystectomy for bladder cancer. The sample size calculation is based on one-sided testing on the proportion of subjects who experience an increase in T cell proliferation (≥100% increase in proliferative index during the proliferation assay) in post treatment assessment compared to pretreatment assessment with power=90% and target alpha=5%. The null proportion responding is taken to be 0.10 and the desired proportion responding is specified as greater than or equal to 0.50. The estimated sample size, under these assumptions, is 10. In order to achieve this number

we would need to consent 25 participants to account for completion rate and assay variability.

Sample size calculations were run using Stata 10.1 (College Station, Texas).

Data analysis
Descriptive graphs will be developed to describe immune subpopulations and immunodynamic end points on paired samples on an intraindividual basis. For all other immune analyses, comparisons between groups will be conducted using the nonparametric Wilcoxon rank-sum test and comparison within groups will be conducted using the Wilcoxon signed-rank test. Correlation will be analyzed with the Spearman rank test.

10.0 Registration Guidelines

Participants will be registered on to IDEAS.

11.0 Ethical and regulatory

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

12.0 Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan (DSMP) is required for all an individual protocols conducted at CTRC. All protocols conducted at CTRC are covered under the auspices of the CTRC Institutional Data Safety Monitoring Plan.

The CTRC Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the CTRC Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
- determining level of risk (Priority of Audit Level Score – PALS) ,
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the CTRC Quality Assurance Division.

Monitoring Safety:
Due to the low risk associated with participation in this protocol, the Principal Investigator will perform primary assessment of adverse events, adverse event trends and treatment effects on this study. The PI will conduct independent review and report findings yearly to the CTRC Data Safety Monitoring Committee (DSMC) and the UTHSCSA IRB.

Baseline events and adverse events will be captured using the CTRC Master Adverse Events Document for each patient using CTCAE V. (specify version you will use in this protocol) for the grading and attribution of adverse events. Usage of the CTRC Master Adverse Events Document centrally documents:

- the event and grades the seriousness of it,
- if the event was a change from baseline,
- determines the relationship between the event and study intervention,
- if the event was part of the normal disease process, and
- what actions were taken as a result of the event.

Safety Definitions:
For this study, the following safety definitions will be applicable:

Adverse Event Definition: An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research For this study, all adverse events will be documented starting with specify here when you want to start collecting adverse event information, generally this would be with the first dose of the study drug and ending 30 days after the last dose of study drug is received.

Serious Adverse Event Definition: is any adverse event that:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as "anticipated" constitutes serious non-compliance);
B. definitely related or probably related to participation in the research; and
C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Reporting Requirements

For this study, all Master Adverse Events Documents collected on participants for this protocol will be reviewed by the Principal Investigator at each study visit/as they occur to determine if a serious safety problem has emerged that result in a change or early termination of a protocol such as:

- dose modification,
- suspending enrollment due to safety or efficacy, or
- termination of the study due to a significant change in risks or benefits.

The PI will use clinical judgment to make dose reduction and dose delay in BCG as per standard of care. Since these adjustments are very patient-specific, this will be done on a case-by-case basis.

As per the CTRC DSMP, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to all members of the research team Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance (DQA) who will promptly notify the sponsor and the UTHSCSA IRB.

The PI will review the Master Adverse Events documents to determine the significance of the reported events and will file the Investigator Initiated Study Quarterly DSMC Report Form on a yearly basis with the CTRC DSMC. The Investigator Initiated Study Quarterly DSMC Report Form includes information on adverse events, current dose levels, number of participants enrolled, significant toxicities per the protocol,

patient status (morbidity and mortality), dose adjustments with observed response, and any interim findings. Any trend consisting of three or more of the same event will be reported to the CTRC DSMP for independent review outside of the quarterly reporting cycle, which begins three months following protocol start up. Conflict of interest is avoided by the independent review of the CTRC DSMC and by ongoing independent review of adverse events trends by the Director of Quality Assurance.

All SAE and UPRISO's will be reported following CTRC and UTHSCSA institutional guidelines.

UTHSCSA SAE/UPRISO REPORTING REQUIREMENTS		
Type Event	Report to	Timeframe
All AE, SAE and UPIRSO	Regulatory Affairs and DQA	Same as other notification timeframes except for SAE/AE which should be reported on Monday for the prior week of PI being made aware
SAE	Clinical Trial Sponsor	within 24 hours of PI being made aware
AE/SAE	UTHSCSA IRB	Annually
UPIRSO - all	Clinical Trial Sponsor	within 24 hours of the PI determining a UPIRSO exists
UPIRSO - life threatening	UTHSCSA IRB	within 48 hours of the PI determining a UPIRSO exists
UPIRSO - non-life threatening	UTHSCSA IRB	within 7 days of the PI determining a UPIRSO exists

Include the following adverse event reporting requirement tables for NCI Trials supported by Grant or Contract Funding:

TABLE A: Expedited Reporting for Phase I Studies (including hospitalization* to NCI Investigational Drug Branch (IDB)

UNEXPECTED EVENT		**EXPECTED EVENT**	
GRADES 2 - 3 Attribution of Possible, Probable or Definite	**GRADES 4 - 5 Regardless of Attribution**	**GRADES 1 – 3**	**GRADES 4 - 5 Regardless of Attribution**
Grade 2 - Expedited report within 10 working days **Grade 3** - Report by phone to IDB within 24 hrs. Expedited report to follow within 10 working days. **(Grade 1** - Adverse Event Expedited Reporting NOT required.)	Report by phone to IDB within 24 hrs. Expedited report to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.	Adverse Event Expedited Reporting NOT required.	Report by phone to IDB within 24 hrs. Expedited report to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 10 working days.

* For Hospitalization Only — Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of requirements for Phase of study, expected or unexpected and attribution.

Expedited reporting may not be appropriate for specific expected adverse events for certain later Phase II and Phase III protocols. In those situations the adverse events that will not have expedited reporting must be specified in the text of the approved protocol. An expected Grade 3 event that is definitely related to the investigational agent is only to be reported if the patient is hospitalized using the generic reporting criteria. For instance, in a trial of an investigational agent where Grade 3 diarrhea requiring hospitalization is expected, only diarrhea requiring ICU care (Grade 4) might be designated for expedited reporting.

Serious adverse events on NCI sponsored trials utilizing a commercially available agent (with no IND's involved) will additionally be reported via the FDA's Medwatch program.

Assuring Compliance with Protocol and Data Accuracy
As with all studies conducted at CTRC, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. (Please write in here any specific monitoring functions such as source verification etc that are either conducted by the sponsor's monitor or newly implemented CTRC monitoring plan –for example, source verification of data will be performed routinely at each visit/as they occur). Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the CTRC DSMC.

DSMB #2 (for Solid Tumors)
Kevin Kelly, MD
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