GEMINI: An Open-Label, Single-Arm, Phase II Study of Intraoperative GEMcitabine INtravesical Instillation in Patients Undergoing Radical Nephroureterectomy for Upper Tract Urothelial Carcinoma

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
RNU	Radical Nephroureterectomy
UTUC	Upper Tract Urothelial Carcinoma
CT	Computed Tomography
ECOG	Eastern Cooperative Oncology Group
eGFR	Estimated Glomerular Filtration Rate
LG	Low Grade
HG	High Grade
NAC	Neoadjuvant Chemotherapy
UC	Urothelial Carcinoma
NMIBC	Non-Muscle-Invasive Bladder Cancer
TURBT	Transurethral Resection of Bladder Tumor
BCG	Bacillus Calmette-Guérin
UUT	Upper Urinary Tract
AJCC	American Joint Committee on Cancer

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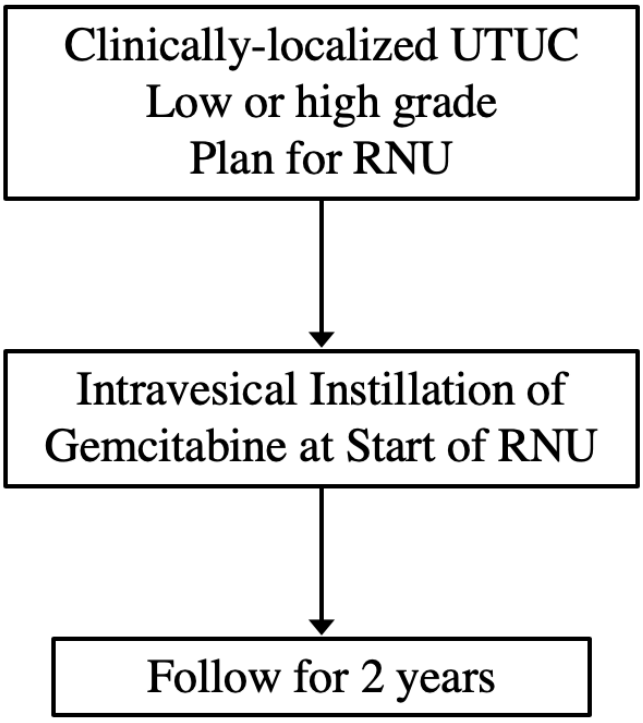
Study Summary

Title	**GEMINI**: An Open-Label, Single-Arm, Phase II Study of Intraoperative **Gem**citabine **In**travesical **I**nstillation in Patients Undergoing Radical Nephroureterectomy for Upper Tract Urothelial Carcinoma
Running Title	GEMINI: A Phase II Trial of Gemcitabine Intravesical Instillation for Nephroureterectomy
Phase	Phase II
Methodology	Open-Label, Single-Arm
Overall Study Duration	5 years
Subject Participation Duration	Minimum 1 year (2-year maximum)
Single or Multi-Site	Multi-center; 2 Mayo sites: Rochester, Florida, Additional External Sites
Objectives	Primary Objective: • To assess the efficacy of a single intraoperative intravesical instillation of gemcitabine at time of radical nephroureterectomy (RNU) to prevent intravesical recurrence of urothelial carcinoma (UC) at 1 year in patients with clinically localized upper tract urothelial carcinoma (UTUC) Secondary Objectives: • To assess time to recurrence for entire duration of follow-up • To assess adverse events Exploratory Objectives: • To stratify intravesical UC recurrence free survival by tumor grade, neoadjuvant chemotherapy, tumor stage, ureteral tumor location, and history of bladder cancer • To assess incidence and time to development of muscle invasive bladder cancer
Number of Subjects	90
Diagnosis and Main Inclusion Criteria	Newly diagnosed, clinically localized UTUC; Candidate for RNU; ECOG 0-2; No prior intravesical UC within 12 months
Study Product, Dose, Route, Regimen	Intravesical instillation of 2 g Gemcitabine in 100 ml of 0.9% Sodium Chloride solution
Duration of Administration	Single intraoperative intravesical instillation of gemcitabine for at least 1 hour at time of RNU

Statistical Methodology	Based on prior literature, the historical event rate for no treatment is expected to be 30% with an estimated 40% relative reduction in event rate in the Gemcitabine arm. A sample size of 81 patients provides at least 80% power to detect this difference, based on a one-sided exact test of proportions with alpha level 0.05. To account for loss-to-follow up and dropout due to death, accrual will be increased by 10%, yielding a final accrual goal of 90 patients. Follow-up will require cystoscopy and urine cytology at 3, 6, and 12 months with mandated histologic confirmation of recurrences.

Study Schema



1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures. The described study will be conducted in compliance with the protocol, Good Clinical Practices standards and associated Federal regulations, and all applicable institutional research requirements.

1.1 Background

Overview

Intravesical recurrence of urothelial carcinoma following radical nephroureterectomy for upper tract urothelial carcinoma is common and contributes considerable morbidity and cost. Two prior randomized controlled trials have demonstrated that perioperative instillation of intravesical Mitomycin C and Pirarubicin significantly decrease the incidence of subsequent intravesical recurrence for these patients. However, utilization of these agents has not been embraced by urologists, in part due to concerns for local toxicity. Intravesical gemcitabine is a promising agent for use in this setting given demonstrated efficacy and low toxicity in a recent randomized controlled trial for non-muscle invasive bladder cancer.[1]

Upper Tract Urothelial Carcinoma

There are an estimated 549,393 new cases of bladder cancer diagnosed per year worldwide, of which 81,190 cases are diagnosed in the United States.[2,3] Bladder cancer is the sixth most common malignancy in the United States.[2] Urothelial carcinoma (UC) is the most common histology of bladder cancer and can affect the entire urothelial lining which includes the kidney, ureter, bladder, and urethra. Upper tract urothelial carcinoma (UTUC) refers to UC of the kidney and ureter. Accounting for 10% of all UC cases, this results in more than 55,000 and 8,000 new cases of UTUC per year worldwide and in the United States, respectively.[2,3] Although endoscopic approaches are utilized in select cases, the standard primary treatment for clinically localized UTUC is radical nephroureterectomy (RNU).[4] This surgery involves resection of the kidney, ureter, and bladder cuff surrounding the ureteral orifice. Mirroring management for UC of the bladder, current guidelines state that patients undergoing RNU may benefit from adjuvant and neoadjuvant chemotherapy.[4,5] There is also emerging literature regarding the benefit of checkpoint inhibitors for these patients.[6]

Intravesical Recurrence after RNU

Approximately 30-50% of patients experience intravesical recurrence of UC after RNU for UTUC.[7–9] There are numerous identified risk factors for intravesical recurrences, including increasing age, ureteral tumor location, prior bladder cancer, increasing tumor stage, concomitant carcinoma in situ, and lymph node involvement.[10] Gender has shown disparate associations with the risk of intravesical recurrence in meta-analyses.[10,11]

In addition to the risks of disease progression and cancer-specific mortality, intravesical recurrences add patient-level morbidity, as treatment is typically with transurethral resection under anesthesia.[12] Surveillance for and treatment of these recurrences also add significantly to cost of management for this disease.[13]

Mechanisms for Intravesical Recurrence

There are two presumed mechanisms for intravesical recurrence after RNU: (1) oligoclonal recurrence from the "field change" nature of UC that is associated with increased malignant potential of the entire urothelium, and (2) monoclonal recurrence from seeding of disrupted tumor cells that subsequently implant in the bladder.

The "field change theory" presumes a malignant potential of the entire urothelial tract, primarily due to carcinogenic exposure. This oligoclonal model supports independent synchronous and metachronous urothelial tumor development throughout the urinary tract. Therefore, if the urothelium of the upper tract is predisposed to developing and subsequently develops urothelial carcinoma, the urothelium of the lower urinary tract and bladder may also independently be susceptible to developing urothelial carcinoma in the future as well.

Meanwhile, the "tumor seeding theory" is that seeding of malignant cells occurs from the upper urinary tract (UUT) tumor implanting into the bladder. This monoclonal model for tumor dissemination is favored for several reasons. First, there is animal and clinical data for primary bladder cancer supporting that urothelial injury predisposes intravesical recurrence specifically in those injured sites.[14,15] Second, the majority of intravesical recurrences arise within 1 year from RNU, suggesting there is a temporal link of recurrences to manipulation of the UUT tumor during surgery. Third, while the incidence of intravesical recurrence after RNU for UTUC is 30-50%, the incidence of UTUC in the setting of primary bladder urothelial carcinoma is less than 5%.[8] The low incidence of ascending tumor recurrence likely occurs secondary to lack of urinary reflux from the bladder to the ureter and kidney. This pattern is further reflected by low rates of contralateral UUT recurrence in only 2-6% of patients with UTUC.[16] Finally and most convincingly, there have been several genetic studies showing that intravesical recurrences are of monoclonal origin with similar genetic make-up to UTUC, consistent with tumor seeding.[17,18] In fact, a recent analysis of clonal relatedness using somatic mutation data in 29 paired tumor samples from the UUT and bladder in patients with intravesical recurrence after RNU for UTUC revealed that 100% of samples shared clonal origin.[19]

Perioperative Intravesical Therapy as a Strategy to Reduce Intravesical Recurrence

Several strategies have been evaluated in reducing intravesical recurrence after RNU. Surgical techniques include avoiding entry into the urinary tract, removal of the kidney and ureter en bloc with a bladder cuff, and early clipping the ureter prior to extensive manipulation of the kidney to avoid seeding of tumor cells down the ureter and into the bladder. However, none of these techniques have been evaluated prospectively.

An effective strategy to reduce intravesical recurrence is the perioperative instillation of intravesical chemotherapy. This has been reported at various times from completion of RNU to

catheter removal. Catheter removal generally occurs after 10-14 days to allow for bladder healing. Several institutional reports evaluated perioperative instillation of a variety of intravesical chemotherapy agents after RNU, including BCG, anthracyclines (Epirubicin, Pirarubicin Doxorubicin), and Mitomycin C +/- cytosine arabinoside.[20–22] The mechanism of perioperative intravesical therapy presumably targets the "tumor seeding theory".

Two prospective randomized controlled trials have evaluated the use of perioperative intravesical chemotherapy to reduce intravesical recurrence after RNU. The first was the ODMIT-C trial, published in 2011— a multi-center trial in the United Kingdom which randomized 284 patients to a single postoperative dose of Mitomycin C at time of catheter removal versus standard care following RNU for UTUC.[23] This trial showed an 11% absolute risk reduction in 1 year intravesical recurrence and a 40% relative risk reduction in 1 year intravesical recurrence using per protocol analysis. This trial excluded patients with prior history of bladder cancer. A second randomized phase II trial was published in 2012 from Japan, evaluating Pirarubicin.[24] A total of 77 patients were randomized to receive a single instillation of Pirarubicin within 48 hours after RNU versus saline. The results showed significantly lower rate of 2-year intravesical recurrence in the Pirarubicin vs control group (16.9% vs 42.2%). A meta-analysis including the ODMIT-C and Pirarubicin assessed the effects of a single-dose intravesical chemotherapy instillation after RNU for UTUC, estimated that instillation resulted in a hazard ratio of 0.51 (95% CI 0.32 – 0.82) for recurrence and approximately 127 (95% CI 182 to 44) fewer recurrences after 12 months, relative to no instillation.[25]

There are currently ongoing trials for Pirarubicin (NCT02923557, 200 patients), Docetaxel (NCT03209206, 84 patients), and Gemcitabine (NCT03062059, 134 patients) in this setting.

Table 1. Randomized Placebo-Controlled Trials of Intravesical Chemotherapy for RNU

Reference	Country	Total Patients	Agent	Timing	Anticipated Completion	1-year recurrence; Drug vs placebo
O'Brien et al	UK	284	Mitomycin	Post-op; 10-14 days	Completed	16% vs 27%
Shintaku et al	Japan	77	Pirarubicin	Post-op; < 48 hours	Completed	16.9% vs 31.8%
NCT02923557	China	200	Pirarubicin	Post-op; < 24 hours	2021	N/A
NCT03209206	Korea	84	Docetaxel	Post-op; < 48 hours	2022	N/A
NCT03062059	Korea	134	Gemcitabine	Intra-op	2022	N/A

Timing of Perioperative Intravesical Therapy

The majority of data for perioperative intravesical chemotherapy describes its use at a delayed interval from completion of RNU. For example, the ODMIT-C trial required instillation of Mitomycin C at time of decatheterization which occurred at least 1 week following RNU.[23] The rationale for this time-frame was the concern for risk from extravasation of Mitomycin C from the bladder cuff repair, whether that be intraoperative or postoperatively prior to complete bladder healing. Exact timing of instillation may be unimportant to reduce recurrence based on the "field change theory". However, timing may be crucial based on the "tumor seeding theory", since implantation may have already occurred prior to a delayed instillation. For this reason, the Pirarubicin trial elected to perform instillation within 48 hours of RNU, which may have resulted in their relatively larger effect size.[24] Unfortunately, the specific timing of instillation was not reported.[24]

Based on the seeding theory, the earliest possible instillation of intravesical chemotherapy would theoretically result in the greatest reduction of recurrences. This is heavily supported in the bladder cancer literature, for which most perioperative intravesical chemotherapy is administered within 24 hours of transurethral resection of bladder tumor, with some data advocating for immediate instillation following resection.[26,27] Data is emerging that describes the use of intravesical chemotherapy intraoperatively during RNU. The first publication in 2015 described instillation of Mitomycin C or Adriamycin for 51 patients at the beginning of the surgical procedure.[28] The catheter was clamped for up to two hours and unclamped to drain the bladder well before the bladder cuff excision was performed.[28] There were no adverse events secondary to Mitomycin reported in this study.[28] A second publication in 2018 confirmed the safety of this technique, comparing instillation of Mitomycin C intraoperatively versus postoperatively on post-operative day 1 or later.[29] Supporting the theory of tumor cell seeding and implantation, patients who received immediate intravesical chemotherapy had a lower 1-year rate of intravesical recurrence (16% versus 33%).[29] There is an ongoing prospective single arm Phase II trial assessing the safety and efficacy of intravesical Mitomycin C administered intraoperatively at the start of RNU (NCT03658304).

A 2014 survey assessing practice patterns of Society of Urologic Oncology urologists revealed that, of those who instilled perioperative intravesical chemotherapy, 33% did so intraoperatively, 7% within 3 days of RNU, 37% between 4-7 days of RNU, 20% between 8-14 days of RNU, and 3% at 14 days after RNU.[30] A 2019 poll conducted on Twitter targeting UTUC experts similarly showed that 26% and 24% of 170 respondents favor instillation at the beginning of RNU and immediately after bladder closure, respectively.

Poor Adoption of Perioperative Intravesical Therapy

The European Association of Urology (EAU) and National Comprehensive Cancer Network (NCCN) guidelines recommend instillation of a single perioperative instillation of intravesical chemotherapy following RNU.[4]

Due to the relatively severe side effect profile of anthracycline class agents, Mitomycin C is the primary perioperative intravesical therapy utilized in the United States. The 2014 survey administered to Society of Urologic Oncology urologists demonstrated that of those who administered intravesical chemotherapy at time of RNU, 88% utilize Mitomycin C. However, only 51% of respondents reported utilizing intravesical chemotherapy, most commonly due to perceived lack of data supporting use, personal preference, and office infrastructure.[30] Given the low national usage of perioperative intravesical chemotherapy for primary bladder urothelial carcinoma (0.33%-28%), the true national rate of utilization after RNU is likely substantially lower.[31,32]

Changing Intravesical Therapy Paradigm of Gemcitabine for Urothelial Carcinoma of the Bladder

There is a high incidence of intravesical recurrence following transurethral resection of bladder tumor, estimated between 30-80% within 5 years. Since urothelial carcinoma of the bladder is far more common than UTUC, strategies to reduce intravesical recurrence have been evaluated for

several decades. Numerous randomized clinical trials and meta-analyses have shown that perioperative instillation of intravesical chemotherapy reduces disease recurrence and progression.[33–36] Previously investigated agents primarily include anthracyclines, Thiotepa, and Mitomycin C, although other classes of agents have also been evaluated. The most commonly utilized agent in the United States is Mitomycin C.[37] To reduce recurrences, the EAU and AUA guidelines for non-muscle invasive bladder cancer recommend a single postoperative instillation of intravesical chemotherapy within 24 hours of transurethral resection of bladder cancer, in patients with suspected known low- or intermediate-risk bladder cancer.[38,39]

However, there has been poor adherence to guidelines recommending use of Mitomycin C following transurethral resection of bladder cancer. A survey in 2012 reported that 63% of urologists used Mitomycin C routinely.[37] Another national sample of United States urologists from 2012 reported a lower 28% use of postoperative chemotherapy.[31] Meanwhile, a study of MEDSTAT claims showed a dramatically lower national rate of 0.33%.[32] Commonly cited reasons for not utilizing postoperative chemotherapy include absolute contraindications such as suspected bladder perforation and increased suspicion of high-grade urothelial carcinoma. Other reasons include concerns for pharmacy logistics and of toxicity of Mitomycin C.[31] The most common reported toxicities of Mitomycin C range from low grade (dysuria, transient hematuria) to high grade (prolonged chemical cystitis, dystrophic bladder calcifications, perivesical inflammation and fibrosis).[40,41] There are in fact reports of patients requiring cystectomy due to dystrophic bladder changes after Mitomycin C instillation, even if the primary malignancy was eradicated. Drug availability, pharmacy logistics, and expense are also considerations.[42,43] These concerns share similarity to those for administration of perioperative intravesical chemotherapy following RNU.

In an effort to limit toxicity while maintaining reduction in intravesical recurrences, a randomized trial of intravesical gemcitabine versus saline following resection of bladder tumor was performed and published recently in JAMA in May 2018.[1] This trial showed a 12% absolute reduction in 4 year intravesical recurrences in the gemcitabine versus placebo groups. Strikingly, there was an almost identical rate of adverse events between the intervention and control arms. There is currently major adoption of this agent post-transurethral resection of bladder tumor in many centers across the United States.

1.2 Investigational Agent

Gemcitabine hydrochloride

2'-Deoxy-2', 2'-difluorocytidine monohydrochloride (Gemcitabine hydrochloride or Gemcitabine) is a pyrimidine analog of deoxycytidine that kills cells by interfering with DNA synthesis. This analog also disrupts DNA replication and cell division through the G1/S-phase boundary. After this nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands, which eventually results in the initiation of apoptotic cell death.

Gemcitabine is manufactured as a white to off-white lyophilized powder with a molecular weight of 299.66. It is commercially available from multiple distributors in various formulations including a liquid 2g/52.6 mL single use vial or in 1 g single-dose vials, which can be reconstituted in 0.9% Sodium Chloride solution and further diluted.

1.3 Preclinical Data

There is extensive clinical experience with intravesical Gemcitabine for bladder tumors, from numerous early Phase I trials beginning in 2002-2004, followed by more recent Phase II and III studies. Prior to these human trials, animal studies confirmed that relatively high doses of intravesical Gemcitabine could be safe as well as efficacious in treating orthotopic bladder cancer models. Cozzi et al. first studied escalating doses of intravesical Gemcitabine from 100mg to 3500mg dissolved in 50ml 0.9% Sodium Chloride solution in beagle dogs.[44] Doses higher than 350mg were associated with increased systemic absorption and toxicity. Witjies et al. similarly demonstrated the safety of a 6-week instillation course of 350mg Gemcitabine in a pig model, observing minimal bladder wall inflammatory changes.[45] Matera et al. administered the human equivalent of 1575mg/m2 and 3150 mg/m2 intravesical Gemcitabine to rabbits for 5 or 8 weeks.[46] The lower dose regimen showed no clinical toxicity or drug absorption although the higher dose was poorly tolerated.[46] Nativ et al. compared 6 instillations of escalating doses of 0.5mg, 2.5mg, and 10mg intravesical Gemcitabine to 0.9% Sodium Chloride solution in a mouse bladder tumor model and found anti-tumor activity in the Gemcitabine groups.[47] Brocks et al. used instillations of 250ug or 500ug intravesical Gemcitabine in another mouse tumor model, which showed good tolerability of the agent and decreased tumor growth compared to controls.[48] These studies paved the way for human trials of intravesical Gemcitabine for urothelial carcinoma of the bladder.

1.4 Clinical Data to Date

Intravenous Gemcitabine for Solid Malignancies

Intravenous gemcitabine has been utilized in the treatment of metastatic disease across numerous solid tumor types, including pancreatic, biliary, breast, and non-small-cell lung cancer.[49] Efficacy of gemcitabine for advanced UC has been shown since its use in single agent regimens in the 1980s.[50] Regimens subsequently incorporated additional agents to comprise “doublet” and “triplet” regimens that largely included cisplatin, carboplatin, and paclitaxel.[51,52] The contemporary gemcitabine and cisplatin regimen was evaluated in a Phase III trial in 2000 that showed comparable survival but decreased toxicity when compared to the historical standard of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) in patients with locally advanced and metastatic UC .[53] More recently, dose-dense gemcitabine plus cisplatin has been shown to potentially reduce toxicity in the neoadjuvant regiments for muscle invasive bladder cancer.[54] Overall, gemcitabine has been shown to have activity for muscle-invasive, locally advanced, and metastatic urothelial cancer.

Phase I Studies for Intravesical Gemcitabine for NMIBC

Intravesical gemcitabine was first explored for NMIBC in several Phase I trials in the early 1990s.[55–62] Dalbagni et al performed a dose escalation study assessing 500mg, 1000mg, 1500mg, and 2000mg intravesical gemcitabine for 18 patients with NMIBC, with detectable plasma concentrations only found in two patients receiving 2000mg.[55] The instillation regimen in this study was fairly intensive, and involved biweekly instillations for a total of three weeks.[55] Laufer et al also assessed dosages of 500mg, 1000mg, 1500mg, and 2000mg intravesical gemcitabine in

six weekly instillations at 2-4 weeks following TURBT.[56] Plasma concentrations of gemcitabine or its metabolite 2',2',-difluorodeoxyuridine (dFdU) were measured in patients receiving 1500g and 2000g gemcitabine.[56] Two similar studies assessed six weekly instillations and measured low levels of plasma concentration of gemcitabine, finding a maximum plasma concentration of dFdU of 4.19 umol/l.[57,58] By contrast, intravenous administration of 1000mg/m2 gemcitabine, which is well below the standard therapeutic dose administered in men and women, resulted in a peak plasma concentration of 100umol/l.[59]

Several studies also demonstrated the safety of intravesical gemcitabine in the setting of disrupted bladder mucosa and TURBT.[60] Palou et al. assessed 10 patients with intravesical instillation of either 1,500mg or 2,000mg gemcitabine in 100ml NS within 3 hours of complete TURBT.[61] There was low local toxicity apart from Grade 1 bladder spasms and mild hypogastric discomfort. Furthermore, there was no notable systemic toxicity with minimal detectable serum gemcitabine (1.8ug/ml). Buettner et al. performed a multi-center 24 patient study with intravesical instillation of 2,000mg/100ml gemcitabine within 1 hour of complete TURBT, and observed no toxicities greater than Grade 2.[62] The initial monocenter dose escalation phase of this trial assessed 500-2,000 mg gemcitabine diluted in either 50ml or 100ml NS. Interestingly, the only dose causing irritative bladder symptoms was 1,000mg/50ml, while higher doses in 100ml NS caused no adverse effects. While the primary aim of these studies was to assess pharmacokinetics and dose-limiting toxicities for intravesical gemcitabine, promising efficacy was also demonstrated, laying groundwork for subsequent larger observational and Phase II comparative studies.

Phase II Studies for Intravesical Gemcitabine for NMIBC

Several small Phase II studies assessed the ablative ability of gemcitabine for "marker lesions" that were left unresected.[63–66] Gontero et al studied 39 mostly pre-treated patients who first underwent complete resection of all but one marker tumor and subsequently received 2,000mg/50ml intravesical gemcitabine weekly for 6 weeks.[65] The complete response rate was 56%, with a median follow-up not reported.[65] Maffezini et al performed a similar study in 28 patients with 4 weeks of instillation of intravesical gemcitabine followed by resection of residual lesions, finding a complete response rate of 46%.[66]

Other Phase II studies assessed intravesical gemcitabine in more standard clinical setting of completely resected low risk NMIBC.[60,67] Bendary et al assessed 80 patients who underwent TURBT for Ta-T1 UC who were randomized to receive either 6 weekly instillations of either intravesical gemcitabine or BCG.[67] While there was no statistically significant difference in rates of 18-month complete response between groups, gemcitabine resulted in significantly lower rates of lower urinary tract symptoms than BCG.[67]

Phase II Studies for Intravesical Gemcitabine for High Risk and BCG-Unresponsive NMIBC

The impressive safety and efficacy of gemcitabine for low and intermediate risk NMIBC led to evaluation in high risk NMIBC as well, particularly in the salvage setting for BCG-unresponsive disease.[68–74] Bartoletti et al reported a multicenter Phase II trial of 116 intermediate and high risk NMIBC who received 6 weekly instillations of intravesical gemcitabine following TURBT,

noting 75% 1-year disease free survival.[71] Porena et al performed a randomized controlled trial of 64 patients with high risk NMIBC and demonstrated increased recurrences but decreased toxicity in patients who received gemcitabine versus BCG, respectively.[72]

Di Lorenzo and colleagues compared gemcitabine versus additional BCG for patients with NMIBC who previously failed a single course of BCG.[73] The gemcitabine arm yielded developed significant fewer recurrences compared to those receiving BCG, and toxicity was similar between arms.[73] A single arm SWOG S0353 study was reported by Skinner et al in 2013 evaluation of intravesical gemcitabine for patients with NMIBC who failed two prior courses of BCG.[74] For 47 evaluable patients, of whom 89% had high risk disease, there was 21% 2-year recurrence-free survival.[74] While this trial was limited by inclusion of patient with heterogenous T-stages and variable use of maintenance gemcitabine regimens, it provided support that intravesical gemcitabine may have efficacy for high risk BCG-unresponsive NMIBC, in addition to low and intermediate risk disease.

Phase III Studies for Intravesical Gemcitabine for NMIBC

Three Phase III studies have reported to date on intravesical gemcitabine for NMIBC.[1,75,76] Two trials predominantly included patients with low to intermediate risk NMIBC; the third trial assessed patients with BCG-unresponsive disease.

Böhle et al evaluated 248 patients with clinical evidence of papillary NMIBC who underwent TURBT.[75] Patients were randomized to a single intravesical instillation of gemcitabine versus placebo followed by at least 20 hours of post-instillation irrigation with NS. Among 109 evaluable patients, no significant difference in recurrence free survival was observed between groups. However, limitations of this trial included a short 30-40 minute duration of instillation, and the extended duration of post-instillation NS irrigation. Notably, there was minimal toxicity observed with gemcitabine versus placebo.

Addeo assessed 120 patients with NMIBC previously treated with BCG or Epirubicin who were randomized to salvage intravesical regimens of either 6-weeks of gemcitabine or 4-weeks of Mitomycin C, with single monthly maintenance treatments for one year in those who responded.[76] Of 109 assessable patients, with median follow-up of 36 months, those receiving gemcitabine enjoyed improved recurrence free survival compared to those receiving Mitomycin C (72% versus 61%, respectively, $p<0.01$). There were also significantly lower overall adverse events in patients receiving gemcitabine versus Mitomycin C (39% vs 72%, respectively, $p=0.02$).

Messing et al published a large randomized controlled trial in 2018.[1] Herein, 406 patients were accrued with suspected low grade NMIBC. Patients were randomized to immediate post-TURBT intravesical instillation of gemcitabine versus placebo. Notably, patients who received gemcitabine enjoyed a 12% increase in 4-year recurrence free survival compared to those who received placebo (47% versus 35%, respectively, $p<0.001$). Within the pathologically confirmed low grade NMIBC subset, patients who received gemcitabine had a dramatically lower rate of estimated 4-year recurrence versus placebo (34% versus 54%, respectively, $p=0.001$). There were no Grade 4-5 adverse events for patients who received gemcitabine. Remarkably, there was

an almost identical distribution of Grade 1, 2, and 3 adverse events in patients who received gemcitabine versus placebo. This pivotal trial unequivocally demonstrated the efficacy and minimal toxicity of a single post-TURBT instillation of gemcitabine in reducing intravesical recurrences within a cohort of patients with mixed high and low grade NMIBC.

Peri-operative Intravesical Gemcitabine during RNU for UTUC

To date, there are no published reports of utilizing intravesical gemcitabine during RNU. There is currently one ongoing Phase II trial in Korea that is accruing patients to assess intravesical gemcitabine versus placebo for UTUC (NCT03062059). However, this trial's accrual goal is only 134 patients and is therefore unlikely to be adequately powered based on our estimations. Following publication of the Messing trial in 2018, off-label use of intravesical gemcitabine at time of RNU for UTUC has been increasingly adopted at many major academic centers, despite no published evidence to support its use in this setting. (Unpublished, Twitter poll)

The likely benefits of *intraoperative instillation* of intravesical *gemcitabine* as a chemotherapeutic agent in the *setting of RNU for UTUC* include:

1. Established efficacy in preventing recurrence across a spectrum of NMIBC grade and risk categories
2. Improved efficacy with intraoperative instillation (compared to postoperative instillation)
3. Very low toxicity profile (compared to Mitomycin C)
4. No need for urine alkalization (compared to Mitomycin C)
5. Improved speed and ease of preparation by pharmacy (compared to Mitomycin C)
6. Relatively cheap cost (compared to Mitomycin C)

Summary of Rationale for Intravesical Gemcitabine during RNU for UTUC

In totality, the three Phase III trials of intravesical gemcitabine for primary UC show strong evidence that this agent has efficacy in preventing both low and high-risk NMIBC.[1,75,76] There is now an extensive body of evidence demonstrating the safety and low rate of local or systemic toxicity from intravesical administration of gemcitabine for NMIBC.[1,55–58,60–76] Given the (1) established efficacy but suboptimal side effect profile and utilization of intravesical Mitomycin and Pirarubicin for RNU, (2) demonstrated efficacy and rapid adoption of intravesical gemcitabine in the corollary setting of post-TURBT NMIBC, (3) extensive aforementioned evidence of safety intravesical gemcitabine for NMIBC, and (4) increasing off-label use of intravesical gemcitabine for RNU, we feel it is appropriate to proceed to a single-arm Phase II trial to evaluate this agent. The efficacy of gemcitabine will be evaluated versus historical controls, as the baseline rate of intravesical recurrence has been established. If the safety of gemcitabine can be demonstrated, it may be readily adopted in this setting. A randomized Phase III trial requires over 400 patients and thus is not feasible at this time.

1.5 Dose Rationale

The safe and efficacious dosage of intravesical gemcitabine for prevention of intravesical recurrence of UC has been established by large Phase III trials for NMIBC.[1,75,76] We will utilize the same dosage as these trials: 2,000mg diluted in NS for a total volume of 100ml.[1] This agent

will be administered via foley catheter in a single intravesical instillation, similar to prior trials of chemotherapeutic agents for RNU for UTUC.[23,24] Several retrospective series have established the safety of intraoperative instillation of Mitomycin C, as well as potentially improved efficacy over postoperative instillation.[28,29] Thus, we will plan for a single intraoperative instillation of gemcitabine at start of RNU in this trial.

1.6 Risks and Benefits

Potential risks of the study investigational agent include toxicity associated with systemic absorption, allergic response to gemcitabine, cystitis, urinary tract infection, and diminished healing of bladder cuff closure. The recent Messing trial utilized intravesical gemcitabine at an identical dose and route of administration as this trial, and all of the aforementioned risks except for healing of bladder cuff closure (which could not be evaluated) were demonstrated to be equivalent to in the setting of post-TURBT instillation. Instillation at start of RNU as planned for this trial is a lower risk than post-TURBT instillation in the Messing trial due to lack of bladder mucosal disruption and subsequent lymphovenous systemic absorption. With respect to impaired healing of bladder cuff closure, this was not observed in two retrospective reports of intravesical instillation of Mitomycin C during RNU.[28,29] The benefits of the study investigational agent are that it may reduce recurrence relative, with a low toxicity profile, to no treatment. The investigational agent will result in reduced cost versus other alternative agents. There may be oncologic benefit from intraoperative instillation. Should this trial demonstrate these mentioned results, application of this knowledge will result in decreased morbidity and cost in the management of UTUC for all patients.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to determine the efficacy of a single intraoperative intravesical instillation of gemcitabine at time of RNU for clinically localized UTUC in preventing intravesical recurrence of UC at one year.

2.2 Secondary Objectives

a. To assess time to recurrence for entire duration of follow-up

b. To assess the qualitative and quantitative toxicities

2.3 Exploratory Objectives

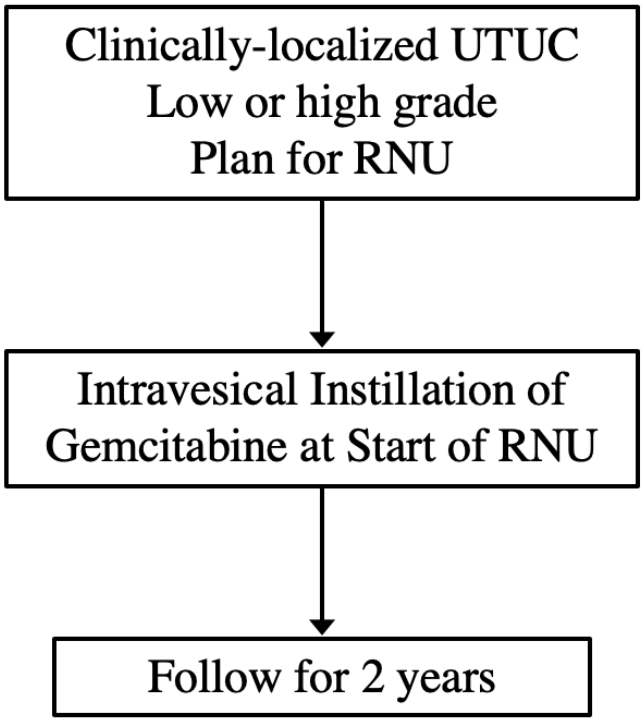
c. To stratify intravesical UC recurrence free survival by tumor grade, neoadjuvant chemotherapy, tumor stage, ureteral tumor location, and history of bladder cancer

d. To assess incidence and time to development of MIBC

3 Study Design

3.1 General Description

This study is an open-label, single-arm, multi-site (within Mayo Clinic) Phase II trial of the safety and efficacy of intraoperative intravesical instillation of gemcitabine at time of RNU for UTUC. Subjects will be screened at outpatient visit for discussion of nephroureterectomy and interested qualified subjects will be offered participation in this trial and consented. Patients will receive intraoperative intravesical instillation of gemcitabine at the beginning of RNU for at least 1 hour. The instilled fluid will be drained prior to resection of the bladder cuff. Patients will undergo routine standard of care postoperative surveillance including cystoscopy at 3, 6, 12, 18, and 24 months. Subjects will be followed for two years or until trial termination. The primary endpoint is 1-year intravesical UC recurrence free survival. Safety and adverse event endpoints will be evaluated. Treatment of recurrences will be according to standard clinical protocols.



3.2 Number of Subjects

Estimated final sample size/accrual goal: 90 patients

Anticipated overall accrual over 3 years is 47 patients from Mayo Clinic Rochester, and 13 from Mayo Clinic Florida, and 30 from all external sites.

3.3 Duration of Participation

Patients will be followed at a maximum of two years or until the trial is terminated. Follow-up will consist of standard of care cystoscopy at 3, 6, 12, 18, and 24 months.

3.4 Primary Study Endpoint

The primary endpoint of this study is 1-year intravesical UC RFS.

- **RFS** will be assessed by cystoscopy and urine cytology at 3, 6, 12, 18, and 24 months. Histologic proof of disease recurrence with biopsy will be mandatory although visual evidence of disease will be noted. Intravesical low or high-grade UC will be considered recurrence. In the event of positive cytology (not atypical or suspicious) but negative cystoscopic assessment, malignancy in the contralateral upper tract and urethra should be ruled out. If cytology remains the only positive assessment, a repeat second cytology should be performed within the next 4 weeks. If the repeat second cytology returns negative, the patient will be considered to be free of disease. If repeat second cytology returns positive, the patient will be considered to have high-grade disease recurrence. Patients without recurrence will be censored at the date of last cystoscopy. Patients who die without any evidence of disease recurrence will be censored at time of death.

3.5 Secondary Study Endpoints

Key Secondary Endpoints

Key secondary endpoints for this study include time to recurrence and adverse event evaluation.

Exploratory Endpoints

We will stratify RFS by tumor grade, neoadjuvant chemotherapy, tumor stage, ureteral tumor location, and history of bladder cancer. Other exploratory endpoints that will be assessed include incidence and time to development of MIBC, and overall survival.

- **MIBC** will be assessed by T2 UC on final pathology of TURBT specimen or T2 UC on final pathology of radical cystectomy specimen.
- **Time to MIBC** will be defined as the time from date of RNU to date of histologic proof of T2 UC.
- **Time to death** will be defined as the time from date of RNU to date of death.

3.6 Primary Safety Endpoints

Safety will be assessed by several methods. First, adverse events will be collected at all times including scheduled time points at time of surgery and each follow-up visit. Adverse events will be categorized by grade and further distinguished as serious adverse events. Furthermore, they will be designated by each site as not related, unlikely, possible, probably, and definitely related to treatment adverse events. These will be confirmed by the PI. Second, lab tests will be performed. Third, physical exams will be performed and vital signs will be assessed. These will occur at time of RNU and at scheduled follow-up times.

The primary specific safety concerns are local bladder toxicity and systemic toxicity. Considering local bladder toxicity, specific symptoms to be assessed include hematuria, dysuria, bladder spasms, urinary frequency and urinary incontinence. Furthermore, intraoperative and postoperative complications will be assessed, specifically with regard to systemic symptoms as well as impaired healing of bladder closure or urine leak.

There is extensive existing Phase I-III data documenting limited systemic absorption of intravesical gemcitabine, ranging from 0.52% - 5.52%.[1,55–58,60–76] Furthermore, systemic adverse events were not observed in the large Phase III Messing trial.[1] To evaluate systemic toxicity, we will monitor for known systemic adverse events of intravenous gemcitabine. These primarily include myelosuppression resulting in anemia, thrombocytopenia, neutropenia.[77] Other important known systemic toxicities from intravenous gemcitabine includes pulmonary toxicity, hepatic toxicity, and Hemolytic uremic syndrome toxicity. Less severe side effects from intravenous gemcitabine include fever, nausea and vomiting, and rash.

We will assess for these systemic effects at several time points. Intra-operative systemic AEs will be monitored by the investigator and recorded in the intra-operative CRF. Post-operative, at least one CBC during the peri-operative hospitalization for RNU will be assessed. If any cytopenia is noted, a CBC will be repeated at least on a weekly basis until blood counts reach pre-treatment levels. We will also perform clinical monitoring (vital signs and physical exam) and LFT measurement during the peri-operative hospitalization to assess for pulmonary and hepatic toxicity. Regarding Hemolytic uremic syndrome toxicity, the diagnosis of hemolytic uremic syndrome will be considered if the patient develops anemia with evidence of microangiopathic hemolysis as indicated by elevation of bilirubin, severe thrombocytopenia, and/or evidence of renal failure.

3.7 Identification of Source Data

The following source data will be recorded on paper documentation by clinical research coordinators and recorded on online data capture forms:

- Demographic information
- Medical and surgical history
- Tumor characteristics
- Vital signs
- Laboratory results
- Intra-operative variables
- Post-operative variables
- Oncologic Follow-up
- Adverse Events/Serious Adverse Events

The following source data will not be directly collected in online data capture forms, but will be captured in supportive documentation (study source documents, EMR):

- Clinic visit notes including history and physical exam, plan
- Imaging records and clinical significance of observations
- Pathology records and clinical significance of observations

- Operative reports

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Both males and females age 18 years or older
- Clinical diagnosis of localized (clinical AJCC stage Ta-T4N0M0) low- and high-grade UC of the renal pelvis and/or ureter
- Plan to undergo RNU
- Adequate bone marrow, renal and hepatic function
    - Creatinine < 2.2 mg/dL (194 mmol/L)
    - Adequate hematologic function (hemoglobin > 9 g/dL; white blood cell count ≥ 3000/μL; platelet count >75,000/μL and <500,000/μL)
    - Serum bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels below 2 times the institution's upper limits of normal
- Easter Cooperative Oncology Group (ECOG) performance status score 0 - 2
- Suitable candidate for surgery at the discretion of the investigator
- Patient must be capable of giving appropriate approved informed consent or have an appropriate representative available to do
- Patient with a prior malignancy allowed if adequately treated > 3 years ago with no current evidence of disease
- Women of childbearing potential (WOCBP) must have a negative pregnancy urine test within 28 days of registration, and be using an adequate method of contraception to avoid pregnancy prior to and for at least 6 months after gemcitabine instillation to minimize the risk of pregnancy
- Male patient who has a partner that is a WOCBP must agree to use physician-approved contraceptive methods (e.g., abstinence, condoms, vasectomy) and should avoid conceiving children prior to and for 6 months following gemcitabine instillation

4.2 Exclusion Criteria

- Pure non-urothelial histology; urothelial carcinoma with differentiation allowed
- Evidence of nodal or distant metastases; enlarged retroperitoneal lymph nodes > 2cm or histologically positive lymph nodes
- History of UC of the bladder within 12 months preceding RNU, or receipt of intravesical therapy within 6 months
- History of or current prostatic urethral, urethral, or contralateral upper tract UC
- History of radical cystectomy or partial cystectomy
- Planned radical cystectomy at time of RNU
- Symptomatic urinary tract infection of bacterial cystitis (once satisfactorily treated, patients can enter the study)
- Patient with any current malignancy except for basal or squamous cell skin cancers, noninvasive cancer of the cervix, or any other cancer deemed to be of low-risk for

progression or patient morbidity during the trial period (i.e. Gleason 6 prostate cancer, renal mass < 3 cm)
- Women who are pregnant or breastfeeding
- Prisoners or subjects who are involuntarily incarcerated
- Inability for adequate follow-up, including concerns for patient compliance or geographic proximity

4.3 Subject Recruitment, Enrollment and Screening

Subjects will be screened and identified for potential recruitment for the study in the outpatient clinic environment by the PI and Co-investigators. The inclusion and exclusion criteria will be discussed with the patient in detail as well as the rationale, the potential benefits, and potential risks associated with the study. The recruitment, enrollment, and screening procedures will occur in the outpatient clinic setting. If subjects want to proceed with the study, informed consent will be obtained by the study coordinator or investigator. The study coordinator will keep the signed informed consent and a physical copy of the informed consent will be given to the patient. If the patient would like additional time to consider the study, they may sign the consent form at a later date, prior to the day of surgery, with the PI, co-investigator, or study coordinator, as long as this allows sufficient time for Pharmacy to prepare the drug.

Evaluation and documentation of inclusion and exclusion criteria will be performed by the PI and Co-investigators after informed consent is obtained.

Non-English speakers will not be specifically targeted for this study. For non-English speaking patients who request it, an interpreter will be used. Advertisements, social media, and letters will not be used to recruit patients to this study. Patients will not be paid for participation.

See Section 6.1 for screening procedures. See Section 11 for details regarding informed consent.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Patients will be advised that they may voluntarily withdraw from the study at any time, for any reason, and it will not affect their medical care or pose adverse safety considerations. Patients who withdraw prior to receipt of treatment will be excluded and replaced. However, in these cases, appropriate attempts will be made by the investigators to determine the reason for withdrawal from the study and to document this reason in the medical record and study files. Patients who receive treatment and subsequently withdraw will not be replaced.

In select circumstances, patients will be intra-operatively withdrawn from the study prior to receipt of intravesical gemcitabine. The surgical team may decide, per their discretion, to intra-operatively to withdraw a subject from the study in the following situations:

- Gross hematuria upon catheter insertion
- Concern for active urinary tract infection

- Need for close urine output such that the catheter cannot be clamped
- Evidence of grossly metastatic disease or intraoperative complication resulting in aborted operation
- Documentation of the reason for withdrawal will be retained in the study files.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

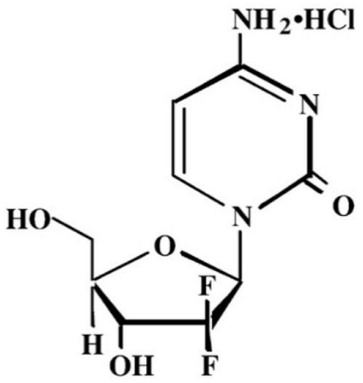
Patients that withdraw prior to RNU or are withdrawn by the surgical team prior to administration of intravesical gemcitabine will not undergo any new or follow-up data collection for this study. Data previously collected for subjects that withdraw consent will continue to be used in the study. Patients who received intravesical gemcitabine and subsequently withdraw from the study will not undergo any new or follow-up data collection for this study. The last known status of patients who withdraw after receiving intravesical gemcitabine will be reported with the study results.

All attempts to locate patients lost to follow-up will also be documented. After three documented attempts (i.e. phone call, patient portal message, and/or mail letter) are made without any response, no further attempts will be made.

5 Study Drug

5.1 Description

Gemcitabine hydrochloride (brand name – Gemzar® or generic – Gemcitabine), is also called 2'-Deoxy-2', 2'-difluorocytidine monohydrochloride (beta-isomer). This is a white to off-white or translucent water-soluble solid with a molecular weight of 299.66. The empirical formula for gemcitabine HCl is $C_9H_{11}F_2N_3O_4 \cdot HCl$. The structural formula is below.



The anti-cancer mechanism of gemcitabine is to act as a nucleoside analog antimetabolite. Gemcitabine is a pyrimidine analog that inhibits both DNA and RNA synthesis. This pyrimidine analog is cell-cycle-specific in blocking the cells at the G1/S and is retained in human tumor cells for long periods. Studies suggest that gemcitabine is activated by deoxycytidine kinase and metabolized intracellularly to form active gemcitabine di- and tri-phosphates. These phosphorylated forms of gemcitabine are incorporated into both DNA and RNA. After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the

growing DNA strands which eventually results in the initiation of apoptotic cell death. Additionally, gemcitabine diphosphate inhibits ribonucleotide reductase which impairs an important tumor nucleotide enzymatic pathway. The gemcitabine di- and tri-phosphates do not appear to circulate in plasma in measurable amounts. Additional metabolites have not been identified in either plasma or urine. The compound is metabolized principally by the liver to form an inactive uridine derivative (dFdU or 2'-deoxy-2',2'-difluorouridine).

Due to its wide use over a long time period for other malignancies, there is considerable existing pharmacokinetic knowledge regarding gemcitabine.[78] Once administered intravenously, the plasma protein binding of gemcitabine is negligible. Gemcitabine is hydrophilic and must be transported across the cell membrane. While the majority of gemcitabine is inactivated in the liver to dFdU (>90%), a lesser amount is inactivated in the blood to dFdU, with less than 10% of unchanged plasma gemcitabine undergoing renal filtration. After administration of a single dose of intravenous gemcitabine, urinary excretion of dFdU (99%) accounted for the majority of the excreted dose.[79] Therefore, the parent drug appears to be almost completely metabolized to the inactive dFdU. There is rapid clearance of plasma gemcitabine, with more than 75% of gemcitabine metabolized to dFdU and excreted in the urine within 24 hours.[79] The half-life of gemcitabine ranges from 11 to 26 minutes for patients receiving single dose infusions (1g/m2 to 2,500 mg/m^2) of 1.1 hours or less. Clearance is affected by gender after normalization for BSA. The clearance obtained for the female patient for all studies was 46.2 L/hr/m^2 and the male's was 66.8 L/hr/m^2.

Considering intravesical pharmacokinetics and systemic absorption, Laufer et al assessed plasma gemcitabine levels before intravesical instillation of gemcitabine and 15, 30, 60, 90, and 120 minutes after the instillation.[56] Gemcitabine was observed in plasma of the 4 patients treated with 40 mg/mL in 50 mL in 0.9% Sodium Chloride solution, but not in the patients treated with 20 mg/mL in 100 mL of 0.9% Sodium Chloride. Peak concentrations in this and several other studies were below 1 μg/mL, which were significantly lower than the 10 to 30 μg/mL observed after a single intravenous (IV) dose of 1,000 mg/m^2 (Lilly 2002). Plasma gemcitabine concentrations declined rapidly, even during the 120-minute dwell time, and no gemcitabine was detectable in plasma beyond 60 minutes after the instillation of the drug. DFdU was also not detected in patients treated with 500 or 1,000 mg of gemcitabine. In the 1,500 mg and 2,000 mg groups, plasma concentrations of dFdU increased progressively during the first 60 to 90 minutes of dwell time, after which they remained constant during the observation period. The authors estimated that based on their PK results and the 120-minute dwell time, the predicted amounts of gemcitabine absorbed from the bladder ranged from 10 to 110 mg, corresponding to 0.52% to 5.52% of the total gemcitabine dose instilled. No dFdU was measured in voided urine, and 61% to 100% of the gemcitabine was accounted for in the voided urine. The authors also studied the in vitro decomposition at 37°C with gemcitabine incubated with three control urine samples. They found no reduction in the concentration of gemcitabine, nor was any production of dFdU observed. Minimal systemic absorption based on measured serum gemcitabine levels following intravesical gemcitabine administration was confirmed by other six Phase I studies. [55,57,58,61,62]

Human Toxicology: Phase I clinical experiences with intravesical gemcitabine have been reported in seven studies where dose ranges of 500 to 2,000 mg at concentration of 20 - 40 mg/ml with 1 to 2 hours of indwelling time were used. Four of these studies were performed in

patients who had intact bladder mucosa. The most common side effects reported were urinary frequency and hematuria. At 2,000 mg dose level, Grade 3 urinary frequency and local irritation were the most common complaints.[55,56,58] Grade 3 thrombocytopenia and neutropenia without infection was reported in one out of six patients.[55] The remaining Phase I studies were performed in patients immediately after, within three hours after, and up to 24 hours after transurethral resection.[61,62] No additional adverse events were noted in these patients. A few cases of renal failure of uncertain etiology have been reported with intravenous gemcitabine administration. While on study, one patient who received prior mitomycin developed hemolytic uremic syndrome requiring dialysis. The relationship of this event to intravenous gemcitabine is not known.

Given known fetal and infant risks when gemcitabine is administered to pregnant or lactating women, patients who are pregnant or lactating will be excluded from this study. Gemcitabine has produced teratogenic effects in mice and rabbits when administered at a dose of < 2 mg/m2. Adverse effects included decreased fetal viability, weight and morphologic defects. There is no data on gemcitabine administration during human pregnancy, and it is not currently known if metabolites are excreted in human milk. However, many drugs are excreted in human milk, and there is a potential for adverse effects in nursing infants. Therefore, the use of gemcitabine should be avoided in pregnant or nursing women because of the potential hazard to the fetus or infant.

5.2 Treatment Regimen

Gemcitabine will be prepared as described in Section 5.3, with 2 grams gemcitabine diluted in 0.9% Sodium Chloride to a total volume of 100mL. This treatment will be administered via intravesical route at the start of RNU. The treatment will be instilled via the foley catheter using a syringe and the foley catheter will be capped. The dwell time will be at least 1 hour and recommended to include time beyond clipping of the ureter. After this period of time the intravesical gemcitabine will be drained from the bladder. Irrigation with 0.9% Sodium Chloride can optionally be used to rinse the bladder, but is not mandated.

Given that there is a single instillation of drug in this study, there will be no dose modifications. Duration of retention of gemcitabine can be shortened by the local investigator if medically indicated (e.g. bleeding, concerns for bladder perforation, etc.), which will be recorded in the CRF.

5.3 Preparation and Administration of Study Drug

Preparation of gemcitabine will be performed by the research pharmacist. Depending on whether the liquid or powder formulation is available to each site, Gemcitabine will be formulated appropriately to ultimately yield 2g gemcitabine diluted in 0.9% Sodium Chloride to a total volume of 100mL, as per prior major studies.[1] While the following protocols are for the most commonly used formulations of gemcitabine, sites may utilize other formulations of gemcitabine and perform local preparation protocols so long as the final volume of each agent is 100mL and the final concentration of gemcitabine is 2g/100mL.

For liquid formulations such as 2g/52.6mL single use vials, the general preparation protocol is as follows. A 100mL bag of 0.9% Sodium Chloride will be used and 52.6mL will be wasted from this using a syringe and needle. Afterwards 52.6mL will be withdrawn from the single use vial and injected into the bag of 0.9% Sodium Chloride to yield 2g gemcitabine diluted in 0.9% Sodium Chloride to a total volume of 100mL.

For powder formulations such as the 1g single use vials, the general preparation protocol is as follows. The drug will be reconstituted with 25 mL of 0.9% Sodium Chloride injected into the 1 gram vial. The vial will be shaken to dissolve. This dilution yields a gemcitabine concentration of 38 mg/mL which includes accounting for the displacement volume of the lyophilized powder (1.3 mL for the 1 gram vial). The total volume upon reconstitution for a single vial will be 26.3 mL. Withdrawal of 26.3 mL of the vial contents will provide 1 gram of gemcitabine. The procedure will be repeated with a second 1 gram vial to reach the 2 gram dose for treatment. The total volume upon reconstitution for two vials will now be approximately 52.6mL. A 100mL bag of 0.9% Sodium Chloride will be used and 52.6mL will be wasted from this using a syringe and needle. Afterwards 52.6mL of the reconstituted gemcitabine will be injected into the bag of 0.9% Sodium Chloride to yield 2g gemcitabine diluted in 0.9% Sodium Chloride to a total volume of 100mL.

The diluted gemcitabine solution should be a clear, colorless to light straw-colored solution. The solution should be inspected visually for particulate matter and discoloration. If particulate matter or discoloration is found, do not administer. Gemcitabine solutions are stable for 24 hours at controlled room temperature 20° to 25°C (68° to 77°F). Solutions of gemcitabine should not be refrigerated, as crystallization may occur.

The treatment will be formulated within 24 hours of use. After formulation, the treatment will be kept in a secure location within the pharmacy with limited access to prevent unintended or unauthorized use of the drug. Prior to the start of RNU, the drug will be sent to the operating room pharmacy and then the operating room. An inventory tracking system will be used to document drug prep time, lot number, and additional pharmacy prep information.

Handling Precautions: Institutional hazardous drug policies must be followed. Gemcitabine is a toxic material which could cause skin and eye irritation. Ingestion or inhalation exposure of sufficient quantities could result in decreased white and red blood cells, hypospermatogenesis, gastrointestinal disturbances, and other signs of toxicity. The compound was positive in one of three tests for mutagenicity. Laboratory animal studies indicate that compounds in this therapeutic class may be reproductive toxins and may induce fetal malformations. Contact or inhalation should be avoided. The urine and solution drained after the foley catheter is unclamped should be discarded as hazardous waste according to local, state and federal policies.

See Section 6.2 for details of drug administration.

5.4 Subject Compliance Monitoring

Given that all treatments given to study subjects in this trial are under anesthesia during RNU, subject compliance monitoring is not applicable as an issue for this study.

5.5 Prior and Concomitant Therapy

There are no prohibited concomitant or prior therapies and no drug wash out periods for this trial.

There will not be required concomitant therapies administered for this trial. In the event of local adverse events, standard local protocols for post-operative oral analgesics, anticholinergics, antispasmodic medications will be permitted, such as phenazopyridine, oxybutynin, and tolterodine. Utilization of medications in the outpatient setting for adverse event treatment will be recorded in the CRF. All prescribed medications in the outpatient setting will be reviewed and recorded in the subject files prior to surgery.

5.6 Packaging

A total of 100mL of 0.9% Sodium Chloride with gemcitabine will be packaged in a single 100mL bag. There will be a label on the bag that this agent is to be used for the IRB number of the study. The label will read: “IRB number: Gemcitabine 2g in 100mL 0.9% Sodium Chloride.” The label will include the statement: "Caution: New Drug--Limited by Federal law to investigational use."

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

Gemcitabine will be kept on inventory by the research pharmacy for each site that participates in this trial. At Mayo Clinic gemcitabine will be held in an investigational refrigerator for inventory management. Each site may use their favored supplier and formulation when ordering gemcitabine, since gemcitabine already is held on inventory at most centers for multiple FDA-approved indications, as well as already commonly utilized in off-label fashion for intravesical instillation for patients with UTUC and primary bladder cancer.

Regarding receipt of drug supplies, standard local pharmacy protocol should be followed, including inventory, filling out a receipt log, counting and verification of drug within each shipment, and logging of discrepancies, damaged, or unusable drug within each shipment.

5.7.2 Storage

Depending on formulation, gemcitabine should be stored appropriately as per package insert. There are no lighting specifications for storage. Special handling, contact precautions, and disposal procedures will be used during handling of the treatments at this time as outlined in Section 5.4 and by the Occupational Safety and Health Administration (http://www.osha.gov/SLTC/hazardousdrugs/index.html).

For the powder formulation, gemcitabine is supplied as a lyophilized powder in sterile vials containing 1,000 mg of gemcitabine as the hydrochloride salt (expressed as the free base), mannitol, and sodium acetate. The lyophilized product should be stored below 30°C. Once

reconstituted, this formulation of gemcitabine should be kept at controlled room temperature (20°C to 25°C, with excursions permitted between 15°C and 30°C), and should not be refrigerated as crystallization can occur.

For the liquid formulation, the single use vial is stored at 2°C to 8°C.

5.7.3 Dispensing of Study Drug

The drug will be dispensed to each subject as described in Sections 5.3 and 5.6. Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged onto local inventory systems such as Vestigo®.

5.7.4 Return or Destruction of Study Drug

Gemcitabine that will not be used will be discarded per local institutional departmental guidelines for cytotoxic waste destruction. Drug destroyed on site will be documented as per standard local departmental protocol.

6 Study Procedures

6.1 Visit 1; Pre-study; Registration

Registration for this study must occur prior to treatment, at approved institutions, and for patients who meet all eligibility requirements. Patients will be allowed to enroll along in any number of other clinical trials including but not limited to perioperative chemotherapy, immunotherapy, or other systemic therapies.

Potential study subjects will be identified by study staff, have their medical history reviewed, vitals taken, and a physical examination performed. The patient's weight and performance status will be recorded. Screening review of eligibility criteria and determination of the patient's eligibility based on the aforementioned information will be performed. The patient will then be provided ample time to review the informed consent document, discuss the study procedures and contents of the informed consent document, and have all questions answered. If patients are agreeable to registration to the study, informed consent must be obtained either at that time or a future date prior to RNU. All patients with signed informed consent must be entered and registered into the REDCap database to generate a trial number.

The following procedures will be required:

- Baseline CBC, BMP, and LFTs will be obtained. If this bloodwork was performed within 28 days of registration at an outside facility, these outside results may be used to satisfy this requirement with copies retained in study files.
- Baseline vital signs will be obtained: blood pressure, heart rate, temperature
- Histologic proof of upper tract urothelial carcinoma will not be required.

- Urinalysis will be obtained within 28 days of RNU. In event of symptomatic UTI within 28 days of RNU, a urine culture should be obtained and the patient treated with full course of antibiotics, with RNU and instillation will be postponed until resolution. In the case of asymptomatic bacteriuria, the use of prophylactic antibiotics and postponement of the treatment is left to the discretion of the PI.
- A CT Urogram with delayed imaging allowing visualization of the ureters and cystoscopy will be required within 6 months of registration. If history of bladder cancer, a cystoscopy within 3 months of registration is required. If delayed CT imaging is not possible then retrograde pyelograms may be performed during cystoscopy to evaluate for ureteral filling defects. If these were more performed within 6 months of registration, these will be ordered and scheduled within that time frame. In the event that a CT scan with contrast is prohibited by renal function, an MRI with contrast of the abdomen and pelvis should be performed instead.
- The study coordinator will confirm the contact information of the outside laboratory facility at the time of the screening visit and will request outside laboratory, imaging, and pathology results if not already available in the institutional medical record. However, all study eligibility requirements; including a review of current medical conditions and medications as well as written informed consent must be documented by study staff and reviewed for acceptability by an Investigator prior to enrolling the subject into the study.
- Documentation of any subjects who were consented but subsequently failed the screening requirements, including the reason for screen failure, will be retained in study files. A screening/enrollment log will be maintained.
- Women of childbearing potential (WOCBP) must have a negative pregnancy urine test within 28 days of registration, and be using an adequate method of contraception to avoid pregnancy prior to and for at least 6 months after gemcitabine instillation to minimize the risk of pregnancy. All females are considered WOCBP unless they have at least 12 months since the last menstrual bleeding, or are without a uterus and/or both ovaries, or has been surgically sterile for at least 6 months prior to registration. Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy.
- Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (e.g., abstinence, condoms, vasectomy) and should avoid conceiving children prior to and for 6 months following gemcitabine instillation.

If patients are enrolled to this trial and subsequently receive neoadjuvant therapy (chemotherapy, immunotherapy, or other systemic therapy) repeat laboratory (CBC, BMP, and LFTs) and imaging (CT and cystoscopy) is recommended prior to RNU.

6.2 Visit 2; Peri-operative; RNU

RNU will be performed in a standard fashion, including removal of the kidney, ureter, and bladder cuff. Bladder cuff removal will be mandatory and is considered standard of care. Open, laparoscopic, and robotic approaches will be permitted. It will be recommended that the ureter be clipped distal to suspected location of tumor prior to manipulation or mobilization of the kidney and upper tract. If a ureteral stent is present this may be removed, or left in situ with a clip placed

on the stented ureter. It will be recommended that instillation of the intravesical treatment be performed at the start of RNU, for at least 1 hour, and preferably at least 30 minutes following clipping of the ureter. The performance of lymphadenectomy will be left to investigator discretion.

At the time of RNU, the intravesical instillation of gemcitabine will be performed. During hospitalization laboratory work and imaging will be obtained as needed by the surgical team but will not be mandated by this study protocol. Patient reported toxicity and adverse events will be noted but not formally collected during the peri-operative hospitalization. The patients' catheter may be removed at any time per discretion of the surgical team, as soon as 1 day following RNU and up to 14 days following RNU if no clinical concern for a urine leak. Utilization of a cystogram prior to de-catheterization will be at the discretion of each surgical team, and will not be required. Utilization of antibiotics around time of de-catheterization will be encouraged but not required.

6.3 Visit 3; Week 2 (+/- 1 week)

At the first post-operative visit at Week 2 (7-21 days) following RNU, history and physical exam, vital signs, adverse event evaluation, and laboratory testing will be obtained including CBC, BMP, and LFTs.

Adverse event evaluation is encouraged to be performed at time of decatherization, should it fall within the 7-21 day window. If Grade 3 or greater adverse events are present at this evaluation, an additional repeat assessment of adverse events should be performed at Week 4.

Laboratory tests may be performed more often at the discretion of the treating investigator. If cytopenias or liver function test abnormalities are observed, patients should be followed weekly until these laboratory test abnormalities have resolved.

6.4 Additional Possible Visit; Week 4 (+/- 1 week)

At this visit, which is performed if needed based on Visit 3, history and physical exam and vital signs will be performed. Adverse event evaluation is performed only if Grade 3 or greater adverse events are present on Visit 3. Repeat laboratory tests may be performed at the discretion of the treating investigator.

6.5 Visit 4; Month 3 (+/- 1 month)

The 3-month visit will consist of routine standard of care surveillance following RNU. This includes history and physical exam, vital signs, adverse event evaluation, cystoscopy with urine cytology, and an assessment of disease recurrence. If suspicion for disease recurrence is noted on cystoscopy or urine cytology, histologic proof will be required with bladder biopsy or transurethral resection of bladder tumor.

6.6 Visit 5; Month 6 (+/- 1 month)

The 6-month visit consists of the same required studies as the 3-month visit, including history and physical exam, vital signs, adverse event evaluation, cystoscopy with urine cytology, assessment of disease recurrence, and histologic proof of disease recurrence, if present.

6.7 Visit 6; Month 12 (+/- 1 month)

The 12-month visit consists of the same required studies as the 3-month visit, including history and physical exam, adverse event evaluation, cystoscopy with urine cytology, assessment of disease recurrence, and histologic proof of disease recurrence, if present. In addition, CT with IV contrast to assess for metastatic disease will be encouraged, but not required at this time.

6.8 Visit 7; Month 18 (+/- 1 month)

The 18-month visit consists of the same required studies as the 3-month visit, including history and physical exam, vital signs, adverse event evaluation, cystoscopy with urine cytology, assessment of disease recurrence, and histologic proof of disease recurrence, if present.

6.9 Visit 8; Month 24 (+/- 1 month)

The 24-month visit consists of the same required studies as the 3-month visit, including history and physical exam, vital signs, adverse event evaluation, cystoscopy with urine cytology, assessment of disease recurrence, and histologic proof of disease recurrence, if present. In addition, CT with IV contrast to assess for metastatic disease will be encouraged, but not required at this time.

6.10 Other assessments at each visit

At 3-month follow-up and subsequent follow-up visits, adjunctive urine testing may be obtained per investigator discretion but is not mandated per this trial. Patients deemed at increased risk for systemic progression may have increased frequency of CT imaging per investigator discretion. Other oncologic outcomes that will be assessed at all time points include development of muscle-invasive bladder cancer, progression, cancer specific survival, and overall survival, as described in Section 3.5.

6.11 Unscheduled visits

An unscheduled visit may be performed at any time during the trial if deemed necessary by the investigator, for assessment of safety, or at the patient's request. The date, reason, and procedures performed at the unscheduled visit, and additional information should be recorded in the patient's file and the CRF.

6.12 Outside visits

While the above visits are encouraged to occur at study sites, it will be acceptable for documentation to be obtained from outside sites, an assessment for disease recurrence may be made based on outside cystoscopy, cytology, and clinical notes. If assessments are being

performed at outside sites, the study coordinator will call the patient to perform adverse event evaluation and document the discussion in a communication note

Schedule of Events									
REQUIRED TIMEPOINTS	PRE-STUDY	PERI-OP	Wk 2	Wk 4	Mo 3	Mo 6	Mo 12	Mo 18	Mo 24
	Visit 1	Visit 2	Visit 3	*Optional*	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
PHYSICAL									
History and Physical Exam	X		X		X	X	X	X	X
Adverse Event Evaluation			X	X[g]	X	X	X	X	X
Concomitant Medication Review	X		X	X	X	X	X	X	X
Weight and Performance Status	X						X		
Disease Assessment	X[d]				X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X
LABORATORY									
CBC[a]	X[d]		X[f]						
BMP[b]	X[d]		X[f]						
LFTs[c]	X[d]		X[f]						
Urinalysis	X[d]								
Pregnancy Test	X[d,j]								
IMAGING									
CT with IV Contrast	X[e]						X[i]		X[i]
Cystoscopy	X[e]				X[h]	X[h]	X[h]	X[h]	X[h]
TREATMENT									
Intravesical Gemcitabine		X							

a: Complete Blood Count including hemoglobin, white blood cell and platelet count
b: Basic Metabolic Panel including creatinine, sodium, potassium, chloride, glucose
c: Liver Function Tests including SGOT (AST), SGPT (ALT), alkaline phosphatase, total bilirubin
d: To be performed within 28 days of registration
e: To be performed within 6 months before registration
f: CBC and LFTs must be obtained 7-14 days post-instillation. Lab tests may be performed more often at the discretion of the treating investigator. If cytopenias or liver function test abnormalities are observed, patients should be followed weekly until these laboratory test abnormalities have resolved.
g: This evaluation only performed if Grade 3 or greater AE noted at Week 2 evaluation.
h: Urine cytology should be performed. Histologic confirmation of recurrence required.
i: Encouraged but optional
j: Only required in women of child-bearing potential. All females are considered WOCBP unless they have at least 12 months since the last menstrual bleeding, or are without a uterus and/or both ovaries, or has been surgically sterile for at least 6 months prior to registration

7 Statistical Plan

7.1 Sample Size Determination

The sample size was determined as follows. A recent meta-analysis of patients who underwent RNU for UTUC found an intravesical recurrence rate of 29% at median follow-up 22 months.[80]

The recent trial by Messing et al assessing intravesical gemcitabine for urothelial carcinoma of the bladder found 4 year intravesical recurrence rates of 35% for gemcitabine versus 47% for placebo, or a 12% 4-year absolute risk reduction.[1] The ODMIT-C trial was powered assuming a relative reduction in event rate of 50%, and found a relative reduction of intravesical recurrence using Mitomycin C of 40%. Thus, based on this prior literature, we want to demonstrate a 40% relative difference in 1-year recurrence, assuming a baseline recurrence rate of 30% with no treatment and thus, recurrence rate for gemcitabine of 18%. This corresponds both with the 12% 4-year absolute risk reduction with Gemcitabine seen in the Messing trial as well as the 40% 1-year relative risk reduction seen in the ODMIT-C trial.

A sample size of 81 patients provides at least 80% power to detect a 40% relative reduction in 1-year UC intravesical recurrence free survival (absolute rate = 18%) compared to a historic reference rate of 30%, based on a one-sided exact test of proportions with alpha level 0.05.

Given the timing of drug administration, ineligible patients (determined during surgery) and dropout are expected to be very low. To account for loss-to-follow up and dropout due to death, we plan to enroll an additional 10% of patients, with a final accrual goal of 90 patients.

Mayo Clinic Rochester performs approximately 40-60 RNUs per year. Once inclusion/exclusion criteria are applied, and accounting for patient participation, the anticipated accrual would be 25 patients a year from Rochester. An additional 5 patients per year may be accrued each from Mayo Clinic Arizona and Florida. Therefore, a total of 64 patients are expected to be accrued from Rochester and 13 from Arizona and 13 from Florida. We anticipate completing accrual within 3 years, with an additional 1-2 years to complete follow-up and analysis.

7.2 Statistical Methods

Descriptive Statistics

Descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Continuous data will be summarized as mean (standard deviation), median (25^{th}-75^{th}) percentiles, and range as appropriate. Categorical data will be summarized as percentage. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated.

Adverse events categorized below will be summarized and organized by organ system, with the number and percent of patients experiencing the adverse event at least once and the number of patients exposed.

- All adverse events graded by severity
- Adverse events not related, unlikely, possible, probably, or definitely related to treatment
- Serious adverse events

Primary Hypothesis and Endpoint

We hypothesize that a single intraoperative intravesical instillation of gemcitabine at time of RNU for clinically localized UTUC will result in 40% relative improvement in 1-year intravesical UC RFS compared to historical controls.

The primary endpoint of 1-year intravesical UC RFS will be evaluated utilizing a one-sided exact test of proportions with alpha level 0.05.

Safety Endpoint

Adverse events will be described and analyzed qualitatively. AEs will be grouped into categories and numerically described.

Exploratory Endpoints

We will also assess 2-year intravesical UC RFS, incidence of MIBC, time to MIBC, and time to death using appropriate methods. Since administrative censoring is likely for some individuals prior to 2 year assessment, overall time-to-recurrence will be assessed through 2 year follow up using survival analysis methods. Subjects with recurrence observed between scheduled visits (unscheduled visit) will have time of event set to the next scheduled visit and all scheduled observations will be analyzed based on the scheduled visit date assuming right censored data for dropout or administrative censoring at 2 years. Kaplan-Meier estimates will describe estimated event rates at 2 years. Time to MIBC and time to death will be analyzed similarly.

In addition, stratified analysis will be performed to assess the effect of tumor grade, neoadjuvant chemotherapy, tumor stage, ureteral tumor location, and history of bladder cancer.

Interim Analysis

No interim analysis will be performed for this study.

Handling of Missing Data

We anticipate little dropout or missing data for the primary endpoint as assessments are part of standard care in this patient population. Complete case data will be evaluated the primary endpoint. Secondary endpoints are assessed using time-to-event data and will use available data, censoring at loss to follow up.

Multiplicity

A single primary endpoint is specified *a priori*. Secondary endpoints will be assessed without adjustment for multiple comparisons.

7.3 Subject Population(s) for Analysis

The full analysis dataset for efficacy and safety outcomes includes patients meeting inclusion/exclusion criteria, providing consent, and with initiation of intravesical instillation as described in Section 6.2. Patients with protocol violations during instillation will continue to be monitored and evaluated for safety and efficacy. A secondary analysis of efficacy outcomes will include patients treated per protocol.

8 Safety and Adverse Events

8.1 Definitions

This study will utilize NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.3 for both data collection and reporting of all toxicities and for serious adverse events. A copy of the CTCAE Version 4.3 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.3.

Additional criteria for safety and adverse events are defined and outlined below.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, monitoring reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event (AE)
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event (SAE)
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization

- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the PI may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
The study treatment follow-up period is defined as the duration of time from initiation of study procedures to the end of the study treatment follow-up. Adverse events must be reported during the study treatment follow-up period.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the PI until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the PI should instruct each subject to report, to the PI, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if they reflect organ toxicity from drug or treatment effect and requires repeat testing. For patients with post-operative abnormalities in laboratory testing including CBC, BMP, and LFTs, repeat testing may be performed at the discretion of the treating investigator. If cytopenias or liver function test abnormalities are observed, patients should be followed weekly until these laboratory test abnormalities have resolved. Since only one administration of the drug is given, dose alterations and scheduling changes are not applicable.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in

this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. This may begin with a standard non-leading question, such as "How have you felt since the last visit?" In addition, and adverse event signs of symptoms that are observed will be documented. If an adverse event worsens in intensity, it should be recorded as a new adverse event. If an adverse event gets milder in intensity, it continues as the first report until the patient is recovered.

Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the electronic CRF. These will be recorded in a single adverse event document locally. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The PI will evaluate the event and determine the necessary follow-up and reporting required to the IRB. The PI will make record of the date the adverse event report is reviewed.

8.3.1 PI reporting: notifying the Mayo IRB

The PI will report to the Mayo (central) IRB any unanticipated problems involving risks to subjects (UPIRTSOs) and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures. Any serious adverse event (SAE) which the PI has determined to be a UPIRTSO will be reported to the Mayo IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event.

Given that this is a multi-site clinical trial, the PI will report unanticipated problems and serious adverse events to participating investigators in addition to the IRB.

For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB. The information will be reported to the IRB in a de-identified manner.

Information collected on the adverse event online report form:

- Subject's name
- Medical record number
- Disease/histology (if applicable)
- The date the adverse event occurred
- Description of the adverse event
- Relationship of the adverse event to the research (drug, procedure, or intervention) *
- If the adverse event was expected
- The severity of the adverse event**
- If any intervention was necessary
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution

*** Relationship Index**
The relationship of an AE to the drug (gemcitabine) is a clinical decision by the PI based on all available information at the time of the completion of the CRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject' s clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other

drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

**** Severity Index**
The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient's daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.4 Stopping Rules

Study stopping rules are outlined in the Data and Safety Monitoring Plan, Section 8.5.1.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see Section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Data and Safety Monitoring Plan

A Data and Safety Monitoring Plan will be in place in a separate document. The PI will review confidential safety reports every 6 months from the central study statistician. The PI will be responsible for decisions regarding possible termination and/or early reporting of the study.

Regular study monitoring will include an assessment of accrual, adverse events and study outcome (see attached Data and Safety Monitoring Plan). Planned study monitoring will occur bi-annually and as needed. NCI guidelines for study monitoring will be followed, recognizing that it often takes approximately 6 months for regulatory approvals at the institutions before an accrual rate can accurately be assessed.

Regarding accrual, at the start of year 3 (at the latest), if accrual is below 50% of projected, the trial will be assessed for an amendment to reflect actual accrual, with implications on study relevance and feasibility to be discussed with study PI and other investigators.

Regarding adverse events, a concerning frequency of SAEs will trigger consideration of possible termination and/or early reporting of the study by the PI.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

Data will be captured at each participating site by qualified study staff who will perform primary data collection from source-document reviews to electronic case report forms (eCRF) via REDCap. REDCap is endorsed by Mayo Clinic's Clinical Trial Management System (CTMS) as described below. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained. Data will be entered for this study utilizing one or a combination of the following methods:

1. Data may be captured electronically, without use of paper.

2. Data may be transcribed from the Electronic Medical Record (EMR-an electronic source that must be available for review) into the REDCap system, without use of paper.
3. Data may be captured on paper (considered source documentation) and transcribed into the REDCap system, BUT paper documentation must be retained and available for review.

REDCap

The Mayo Clinic Center for Clinical and Translational Science (CCaTS) offers a web-based, self-managed, customizable data management solution to investigators and study teams. This solution, Research Electronic Data Capture (REDCap), supports data collection via data collection instruments and surveys.

REDCap is a secure, web-based application designed to support data capture for research studies, providing:1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

REDCap was created in 2004 at Vanderbilt University. It is a secure web platform for building and managing online databases and surveys. REDCap's streamlined process for rapidly creating and designing projects offers a vast array of tools that can be tailored to virtually any data collection strategy.

REDCap is suitable for research projects with low- to medium-complexity data collection requirements and is available at no charge to all Mayo investigators. The REDCap Consortium, a vast support network of collaborators, is composed of thousands of active institutional partners in over one hundred countries who utilize and support REDCap in various ways.

REDCap is HIPAA-compliant with built-in user right controls and audit trails for data security and tracking.

REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the RIC. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap provides secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

9.4 Records Retention

The PI will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. Subject-specific data and Case Report Forms will be inputted electronically and maintained by the central study statistician. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The PI will retain the specified records and reports for the longer of the following specifications;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. For Mayo Clinic sites only; as outlined in the Mayo Clinic Research Policy Manual – "Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

Periodic study monitoring will be provided by staff from the Mayo Clinic Office of Research Regulatory Support. Monitoring may include but will not necessarily be limited to review of the study regulatory documents, source data and database entries throughout the duration of the study to help ensure the completeness, validity and integrity of the data. Original signed informed consent forms will be reviewed. Written monitoring reports with findings and recommended and suggested corrective actions will be provided to the PI, who will subsequently provide them to the IRB at time of annual continuing review.

Either of the co-PIs, assisted by delegated study staff, will also monitor study conduct and documents. Co-PI and study staff meetings will be held monthly, during which time correspondane will also take place with external site co-investigators.

A written Data and Safety Monitoring Plan is a separate document from this protocol (Section 15).

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the PI, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

10.3 Protocol Deviations

A protocol deviation is a departure from the study protocol and/or study related documents. The departure may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. All-important deviations related to study inclusion or exclusion criteria, conduct of the trial, patient managements or patient assessment should be addressed in study source documents and reported to the PI. Protocol deviations must be submitted to the local or central IRB per guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Protocol deviations are not allowed. If a subject's eligibility is in question, please contact the PI.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and institutional research policies and procedures.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject’s legally authorized representative, and the individual obtaining the 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.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the PI before commencement of this study.

12 Study Finances

12.1 Funding Source

Mayo clinic patient accrual, and central statistical and regulatory support will be funded internally by Mayo Clinic. Non-Mayo Clinic sites will locally fund their own accrual of subjects and regulatory support. Additional funding from other internal and external funding mechanisms will be applied for, and if awarded, will primarily be applied for sites unable to provide local internal funding.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study PI prior to participation in this study.

12.3 Subject Stipends or Payments

There will not be any subject stipend/payment in this study.

13 Publication Plan

The primary responsibility for the publication of results will be the PI. Information from the study will not be passed on to third parties unless first approved by the PI. The study will be registered to ClinicalTrials.gov prior to subject recruitment and the results will be reported once final analysis is completed. In the event of early study termination, the results will be reported within 12 months of study termination. Additional secondary analyses of the data will be published at the discretion of the PI.

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15 Attachments

1. DSMP
2. IND Exemption