Clinical Study Protocol: BC001

Study Title: A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-Rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Study number: BC001

Study identifier: *nab*-Rapamycin Study 1

Study Phase: 1, 2

Product name: ABI-009, *nab*-rapamycin, albumin-bound rapamycin nanoparticles

IND number: 120719

Indication: Bladder cancer

ClinicalTrials.gov Identifier: NCT02009332

Principal Investigator(s): James M. McKiernan MD
[redacted]

Sponsor: AADi, LLC

Sponsor Contact: [redacted]

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Original Protocol:	January 31, 2013
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Confidentiality Statement

SYNOPSIS

Sponsor: AADi, LLC

Name of Finished Product:

ABI-009, *nab*-rapamycin, nanoparticle albumin-bound rapamycin

Name of Active ingredient: Rapamycin

Study Title:

A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-Rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Study Number: BC001

Study Phase: 1, 2

Primary Objective(s):

In phase 1, to determine the safety, toxicity, and preliminary efficacy profiles of intravesically administered *nab*-rapamycin at the maximum deliverable dose (MDD). In phase 2, to evaluate the utility (potential for clinical efficacy) and safety of *nab*-rapamycin in combination with gemcitabine in the treatment of BCG refractory or recurrent nonmuscle-invasive transitional cell carcinoma (TCC) of the bladder as measured by complete response rate (defined as negative bladder biopsy at 6 weeks post-treatment).

Secondary Objective(s):

In phase 1 and 2, to assess molecular correlates for response to intravesical *nab*-rapamycin therapy using blood, urine, and tissue bank assembled from patients in this study. The molecular markers analyzed will include but not be limited to p53, p63, Stathmin, Tau, ribosomal s6 kinase, Ki67, and secreted protein, acidic and rich in cysteine (SPARC). In phase 2, to further evaluate the efficacy, including event-free survival (EFS), safety, and toxicity profile of intravesically administered *nab*-rapamycin.

Study Design:

This study is a combined phase 1 and phase 2 multi-institution program to determine the safety and tolerability profile of intravesical *nab*-rapamycin in human patients with nonmuscle-invasive TCC of the urinary bladder that have previously demonstrated resistance to standard intravesical treatment.

In the phase 1 dose escalation portion of the study, up to 30 patients will be enrolled, 3 per cohort, using the 3+3 dose escalation rule: 100 mg/week, 200 mg/week, 300 mg/week, and 400 mg/week for 6 weeks of treatment. There will be an additional cohort to evaluate the safety of 2x/weekly dosing if no significant safety issues are found in cohort 1: cohort 2b – 100 mg 2x/week (total weekly dose of 200 mg).

In phase 2, up to 29 patients will receive intravesical *nab*-rapamycin and gemcitabine using the Simon 2-stage design: initially, there will be only 10 patients enrolled with a rejection rule that only if there are 2 or more positive responses will the study proceed to further enrollment of the next 19 patients.

Study Population:

In phase 1, up to 30 patients will be enrolled, 3 per cohort, using the 3+3 dose escalation rule. In phase 2, up to 29 patients will be enrolled.

Diagnosis and Main Criteria for Inclusion:

Patients, ≥18 years of age, with ECOG 0, 1, or 2, must have a diagnosis of nonmuscle-invasive TCC of the urinary bladder confirmed at the study institution. This will include stage Ta, T1, and Tis, and exclude all patients with muscle invasion (≥T2). All grossly visible disease must be fully resected within 6 weeks prior to enrollment and pathologic stage will be confirmed at the institution where the patient is enrolled. Patients must have either recurrent or refractory disease after receiving BCG therapy. Refractory disease is defined as failure to achieve tumor-free status by 6 months of initiation of adequate BCG therapy. Recurrent disease is defined as reappearance of disease after achieving a tumor-free status by 6 months of initiation of adequate BCG therapy. Adequate therapy is considered a minimum of 6 weekly doses of BCG followed by a second course of therapy in which patient received a minimum of 3 doses.

Test Product, Dose, and Mode of Administration:

In the phase 1 dose escalation portion of the study, *nab*-rapamycin will be given 100 mg/week, 100 mg 2x/week (total weekly dose 200 mg), 300 mg/week, 200 mg 2x/week (total weekly dose 400 mg), and 400 mg/week for 6 weeks of treatment. Patients will receive instillations of *nab*-rapamycin in saline diluent initially at an approximate pH of 5 to 7.5. Patients will be instructed to avoid excessive fluid intake starting the day before treatment. Patients will receive intravesical *nab*-rapamycin by sterile urethral catheterization once or twice weekly for 6 weeks, dependent upon the dosing schedule. Prior to instillation, the bladder will be emptied via the sterile urethral catheter. The patient will be instructed to keep the drug in the bladder for 2 hours before voiding.

The phase 2 portion of the study will be initiated with *nab*-rapamycin at 200 mg/week as determined by phase 1 and 2000 mg/week of gemcitabine. Patients will receive intravesical instillations of *nab*-rapamycin followed by gemcitabine in saline diluent at an approximate pH of 5.0-7.5. *nab*-Rapamycin will be instilled first and the patient will be instructed to keep the drug in the bladder for one hour before voiding. Upon voiding, gemcitabine will then be instilled and the patient will be asked to retain the drug for an additional hour before voiding. Mode of administration will be as described above in phase 1.

Reference Therapy, Dose, and Mode of Administration: N/A

Duration of Treatment:

Patient will continue to receive maintenance instillations until there is a positive cystoscopic biopsy or until a maximum of 1 year from the start of therapy.

Rapamycin Blood Levels:

Rapamycin concentrations in the blood of patients will be measured at pre-treatment (just before instillation) and at 2 hours post-instillation (just before voiding) to determine if there is any systemic absorption from the bladder.

Efficacy Assessments:

A complete response is defined as a cancer-negative cystoscopic biopsy at the 6-week post-treatment assessment. No response will be defined as cystoscopic biopsy positive for cancer.

Event-free survival (EFS) is defined as time from initiation of therapy to tumor recurrence or progression documented by biopsy, or death of any cause. For patients who are lost to follow-up or withdraw from the study, or if study termination occurs before recurrence, progression or death, the EFS will be censored at last disease assessment (date of last cystoscopic biopsy). For patients who start new anticancer treatment (chemotherapy, immunotherapy, or radiotherapy) or undergo a cystectomy, the EFS will be censored at the last disease assessment (date of last biopsy, cystoscopy) before the start of the new anticancer therapy or before the individual undergoes cystectomy.

Safety Assessments:

Safety and tolerability will be monitored through reporting of adverse events (AEs) and serious adverse events (SAEs), laboratory abnormalities, incidence of patients experiencing dose modifications, and/or premature discontinuation of study drug.

Systemic dose-limiting toxicity (DLT) is defined as any grade systemic toxicity using the National Cancer Institute (NCI) Common Toxicity Criteria (CTCAE) version 4.0. Local dose limiting toxicity is defined as grade 3 or 4 bladder toxicity using the NCI CTCAE version 4.0.

Statistical Methods:

The phase 1 segment of the study will have a dose-escalation scheme that will enroll a maximum of 30 patients. Dose escalation will follow the 3+3 rule to establish the MDD.

Once the MDD has been established, the phase 2 segment of the study will be initiated, using the Simon 2-stage design. In stage 1, there will be only 10 patients enrolled with a rejection rule that only if there are 2 or more positive responses will the study proceed to further enrollment of the next 19 patients (stage 2). If 1 or fewer responds in the first stage, we will terminate the study for lack of efficacy. If more than 5 patients respond overall, we will consider the agent promising.

The Null Hypothesis is that there will be no difference between the current response rates to BCG therapy of 10% [1-3] and the response rate to intravesical *nab*-rapamycin with gemcitabine treatment after 6 weeks of therapy in the Phase 2 trial. The Alternative Hypothesis will be that there will be a ≥30% response rate to intravesical *nab*-rapamycin with gemcitabine treatment after 6 weeks of weekly therapy. The overall rejection rule is that if there are 5 or greater responses to treatment out of the entire enrollment, we will reject our Null Hypothesis and accept our Alternate Hypothesis, that there is a significant difference between the response rates of standard BCG therapy and intravesical *nab*-rapamycin with gemcitabine treatment.

The expected sample size for the phase 2 study Simon 2-stage design is 15 patients with a maximum of 29 patients. Based on our operating characteristic of 5% type I error and 20% type II error, the number of patients that is expected to be enrolled will be 15 on average with a maximum total of 29 in order to sufficiently power the study. The probability

of correctly concluding the therapy is worthy of further study is 80% if the true underlying response rate is 30%. The probability of incorrectly concluding the therapy is promising is 5% if the true response rate is approximately 10%.

Correlative studies:

Six weeks after the last treatment, the patients will undergo a cystoscopy and biopsy. Tumor tissue samples will be stored for evaluation of molecular correlative studies to predict response to therapy.

Date of Original Protocol: January 31, 2013

TABLE OF CONTENT

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALT	Alanine aminotransferase (SGPT)
AST	Aspartate aminotransferase (SGOT)
AUC	Area under the curve
BCI	Bladder cancer index
BMP	Basic metabolic panel
β-hCG	β-subunit of human chorionic gonadotropin
CBC	Complete blood count
CFR	Code of federal regulations
CMP	Comprehensive metabolic panel
CRF	Case report form
CRO	Contract research organization
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting toxicity
DHHS	Department of Health and Human Services
ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiogram
EFS	Event-free survival
FDA	Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
NMIBC	Nonmuscle-invasive bladder cancer
MDD	Maximum deliverable dose
NCI	National Cancer Institute

SAE	Serious adverse event
SGOT	Serum glutamic oxaloacetic transaminase (AST)
SGPT	Serum glutamic pyruvic transaminase (ALT)
SPARC	Secreted protein, acidic and rich in cysteine
TCC	Transitional Cell Carcinoma
TURBT	Transurethral resection of bladder tumor
ULN	Upper limit of normal

1. OBJECTIVES AND ENDPOINTS

1.1. Study Objectives

In a combined phase 1 and phase 2 study to determine the safety, toxicity, and efficacy of intravesically administered albumin-bound rapamycin nanoparticles (*nab*-rapamycin, ABI-009, AADi, LLC, Pacific Palisades, CA) in patients with transitional cell carcinoma (TCC) of the urinary bladder that has recurred after or is refractory to standard intravesical Bacille Calmette-Guérin (BCG) therapy.

1.1.1. Phase 1 – Primary Objectives

The primary objectives of the phase 1 study are to determine the MDD and local and systemic DLT of intravesically administered *nab*-rapamycin.

1.1.2. Phase 1 – Secondary Objectives

The secondary objectives are to analyze blood, urine, and tissue from patients in this study to assess rapamycin blood levels and molecular correlates.

1.1.3. Phase 2 – Primary Objectives

The primary objective of this study is to evaluate the utility (potential for clinical efficacy) of *nab*-rapamycin in combination with gemcitabine in the treatment of BCG refractory or recurrent nonmuscle-invasive TCC of the bladder.

1.1.4. Phase 2 – Secondary Objectives

The secondary objectives are to further evaluate the safety and toxicity profile of intravesically administered *nab*-rapamycin with gemcitabine and to assess rapamycin blood levels and molecular correlates.

1.2. Study Endpoints

1.2.1. Phase 1 – Primary Endpoints

The primary endpoints of the phase 1 portion of the study are the MDD, DLT, and safety / toxicity profile of intravesically administered *nab*-rapamycin.

Systemic DLT will be defined as any grade systemic toxicity using the NCI CTCAE version 4.0. Local dose limiting toxicity will be defined as grade 3 or 4 bladder toxicity using the NCI CTCAE version 4.0.

1.2.2. Phase 1 – Secondary Endpoints

The secondary endpoints of the phase 1 portion are to determine rapamycin blood levels and analyze molecular correlates in tissue. The molecular markers analyzed will include but not be limited to p53, p63, Stathmin, Tau, ribosomal s6 kinase, Ki67, and SPARC.

1.2.3. Phase 2 – Primary Efficacy Endpoints

The primary efficacy endpoint of this study is to evaluate the utility of *nab*-rapamycin in combination with gemcitabine as measured by response rate in the treatment of BCG refractory or recurrent nonmuscle-invasive TCC.

Response rate will be measured and documented at the 6-week post-treatment assessment, including cystoscopy with biopsy. A complete response is defined as a

cancer-negative biopsy at the 6-week post-treatment cystoscopy. No response will be defined as positive cystoscopic biopsy.

1.2.4. Phase 2 – Secondary Efficacy Endpoints

The secondary efficacy endpoints include event-free survival (EFS), EFS rates at 1 and 2 years, disease recurrence at 1 and 2 years, disease progression at 1 and 2 years, overall survival, and time to cystectomy. Urine cytology will also be assessed as a secondary marker for disease recurrence.

An event is defined as tumor recurrence, tumor progression to muscle invasive bladder cancer or death, whichever occurs first. Tumor recurrence must be documented by bladder biopsy.

1.2.5. Phase 2 – Safety Endpoints

The safety endpoints of the phase 2 portion of the study include treatment-emergent and treatment-related AEs.

Safety and tolerability will be monitored through reporting of AEs and serious adverse events (SAEs), laboratory abnormalities, and incidence of patients experiencing dose modifications, dose interruptions, and/or premature discontinuation of study drug.

1.3. Pharmacokinetic Analyses and Correlative Studies

Blood levels of rapamycin will be analyzed in both phase 1 and 2 of the study. Plasma levels of gemcitabine and its metabolite dFdU will be analyzed in phase 2 of the study,

This clinical study offers an excellent opportunity to assemble a blood, urine, and tissue bank for further interrogation regarding the molecular correlative markers that may predict sensitivity and resistance to intravesical *nab*-rapamycin therapy in TCC of the bladder. There is not expected to be any formal impact on decision-making within this study based on molecular markers and patients will, of course, have the opportunity to refuse to participate in tissue banking and still participate in the clinical study.

The molecular markers analyzed will include but not be limited to p53, p63, Stathmin, Tau, ribosomal s6 kinase, Ki67, and SPARC.

2. BACKGROUND and RATIONALE

2.1. Nonmuscle-invasive Bladder Cancer

In 2011, it was estimated that 69,250 new cases of bladder cancer were diagnosed in the United States and 14,990 people died from the disease. Bladder cancer is the fourth leading cause of cancer in men and the ninth leading cause of cancer in women in the United States [4]. Nonmuscle-invasive bladder cancer (NMIBC) accounts for 70 to 80 percent of these cases. Bladder cancer has the highest recurrence rate of any malignancy. Although NMIBC is a relatively benign disease (Tis, carcinoma in situ [CIS], Ta, or T1), it recurs in 50-70% of patients, of which 10-20% will eventually progress to high-grade muscle invasive disease [5]. Furthermore, the disease is also characterized by having a large pool of patients who have been previously diagnosed and are still undergoing treatment for unresolved tumors; more than 1 million patients in the United States and Europe are estimated to be affected by the disease [6, 7].

The gold standard first-line treatment for NMIBC is intravesical Bacillus Calmette-Guerin (BCG) therapy, which elicits a nonspecific local immune response. However, up to 50% of patients treated with intravesical therapy for high-risk NMIBC or CIS will recur [8]. Several chemotherapeutic agents have been explored in the second-line setting, but there is no accepted and/or efficacious standard second-line regimen in the US. Additionally, none of the available chemotherapeutic agents, including gemcitabine, docetaxel, and valrubicin, that are currently explored in clinical trials are targeted therapeutics [clinicaltrials.gov].

Because of the lack of targeted therapeutic options, patients who fail to respond to standard intravesical therapies are usually left with radical cystectomy as the only potential cure. However, this surgery carries significant morbidity and mortality. Furthermore, many patients are not appropriate surgical candidates for this procedure and some refuse surgery out of fear and concern for their future quality of life without a normal, functioning bladder. Because of the high disease recurrence and potential morbidity from surgery, the cost per bladder cancer patient is among the highest of all cancers, ranging from US$96,000 to US$187,000 [9]. Thus, the development of an effective molecularly targeted intravesical therapy is highly desirable.

This study is a combined phase 1/2 clinical study to assess the safety, toxicity, and efficacy of local intravesical administration of *nab*-rapamycin to treat patients with BCG-refractory or recurrent NMIBC. This proposal will present a unique opportunity to develop the first targeted molecular therapy for intravesical treatment of bladder cancer. It has been demonstrated at Columbia University that the mTOR pathway plays an important role in bladder cancer development. Additionally, it has been previously established that the nanoparticle albumin-bound, *nab*® platform can successfully deliver hydrophobic drugs such as paclitaxel intravesically to bladder cancer patients [10]. Therefore, we believe that intravesical *nab*-rapamycin, a combination of an effective therapeutic agent and a proven drug delivery platform, will offer a promising avenue for the treatment of bladder cancer.

2.2. mTOR and Rapamycin in Bladder Cancer

The inhibition of the mTOR signaling pathway is a promising therapy for bladder cancer [11]. mTOR is a serine/threonine-specific protein kinase, downstream of the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) pathway, and a key regulator of cell survival, proliferation, stress, and metabolism. Additionally, mTOR is involved in regulating angiogenesis by controlling endothelial and smooth muscle cell proliferation via the hypoxia-inducible factor-1α and vascular endothelial growth factor [12]. mTOR pathway dysregulation has been found in many human carcinomas and mTOR inhibition produced substantial inhibitory effects on tumor progression [13-15]. Specifically, high levels (74%) of phosphorylated mTOR expression were found in human bladder cancer tissue array, and phosphorylated mTOR intensity was associated with reduced survival [16]. In another study, p-mTOR expression was increased in 65/203 (32.0%) of patient urothelial carcinoma specimens cancer vs. non-tumor bladder tissue, and treatment of rapamycin showed activity against a panel of human urothelial carcinoma cell lines, including UMUC3 [17]. Researchers at Columbia University developed an engineered mouse model with targeted deletion of 2 key tumor suppressor genes, p53 and PTEN, in

the bladder epithelium. These deletions result in the development of invasive bladder tumors that share histologic and molecular features of the human disease and display metastases as are prevalent in human bladder cancer patients [11]. Subsequently they have demonstrated that mTOR expression increases as a function of the disease stage in progression from superficial disease to invasive bladder cancer, as evident by activation of pS6-kinase (image below), which was activated in 54 of 70 cases (77%) of T2 muscle-invasive bladder tumors [9].

Research at Columbia University has also demonstrated that intravesical delivery of rapamycin (dissolved in a surfactant/solvent mixture) directly into the bladder lumen was highly effective for suppressing the progression of CIS lesions, and that the intravesical treatment was more effective than systemically given rapamycin.

Preclinical studies evaluated the local versus systemic delivery of rapamycin for effects on the progression of CIS lesions in a mouse model [9]. mTOR inhibition produced substantial inhibitory effects on tumor progression. More important, local delivery of rapamycin (via intravesical administration) was significantly more effective than systemic administration in blocking the growth of and progression of NMIBC. Intravesical rapamycin administration completely inhibited mTOR activation in the bladder lumen as demonstrated by the reduced expression of pS6 level, while it only very modestly inhibited mTOR pathway activity in peripheral tissues, such as the pancreas and liver, indicating that it had low absorption into systemic tissue.

Together these suggest the potential for fewer systemic side effects associated with intravesical rapamycin.

In summary, results from this preclinical study demonstrate the importance of mTOR activation in bladder cancer tumorigenesis, and support the use of an intravesical mTOR inhibitor for high-risk patients with NMIBC cancer to prevent or delay its disease progression.

2.3. Rapamycin, Rapalogs

Rapamycin is a crystalline powder with the empirical formula C51H79NO13 and a molecular weight of 914.17. Systemic absorption of chemotherapeutic drugs through the bladder wall is negligible for compounds that have a molecular weight greater than 300 daltons [18]. Current intravesical chemotherapeutic agents, mitomycin C and doxorubicin, have molecular weights of 334 and 580 daltons, respectively [19], indicating that a rapamycin formulation that is suitable for intravesical delivery should have no or minimal systemic absorption.

Rapamycin is a protein kinase inhibitor that is used for immunosuppression in transplant patients and is under investigation as a cancer treatment. Rapamycin inhibits the mammalian target of rapamycin (mTOR), regulatory protein kinase in cancer that recognizes high stress levels, including depleted nutrient levels and states of hypoxia.

Rapamycin is an oral drug and is therefore not suitable for intravesical administration. Marketed rapamycin analogs (temsirolimus and everolimus) are approved for renal cell carcinoma, but neither of them is developed or suitable for intravesical administration. Temsirolimus, a prodrug of rapamycin, requires conversion by CYP3A, the expression of which is very low in bladder cancer [20] and in the bladder, and is therefore not expected

to be effective for intravesical therapy. Everolimus and ridaforolimus (not yet approved, in development for sarcoma) are oral preparations. Given systemically, they are primarily eliminated via hepatic clearance and therefore result in negligible drug levels in the bladder. None of these rapalogs is highly water soluble, and therefore would require surfactants and solvents in an intravesical formulation, which could potentially cause irritation, local inflammation, and potential reduction of drug efficacy due to micellar sequestration. Indeed, previously intravesical therapies with therapeutics that required a surfactant have failed, for example in the case of cremophor-based paclitaxel (Taxol), and, therefore, improved formulations are needed [21].

2.4. *nab*-Rapamycin

Although rapamycin is an efficacious mTOR inhibitor, it has low oral bioavailability, poor solubility, and dose-limiting intestinal toxicity. The novel nanoparticle albumin-bound rapamycin (*nab*-rapamycin) is freely dispersible in saline and is suitable for intravenous and intravesical administration.

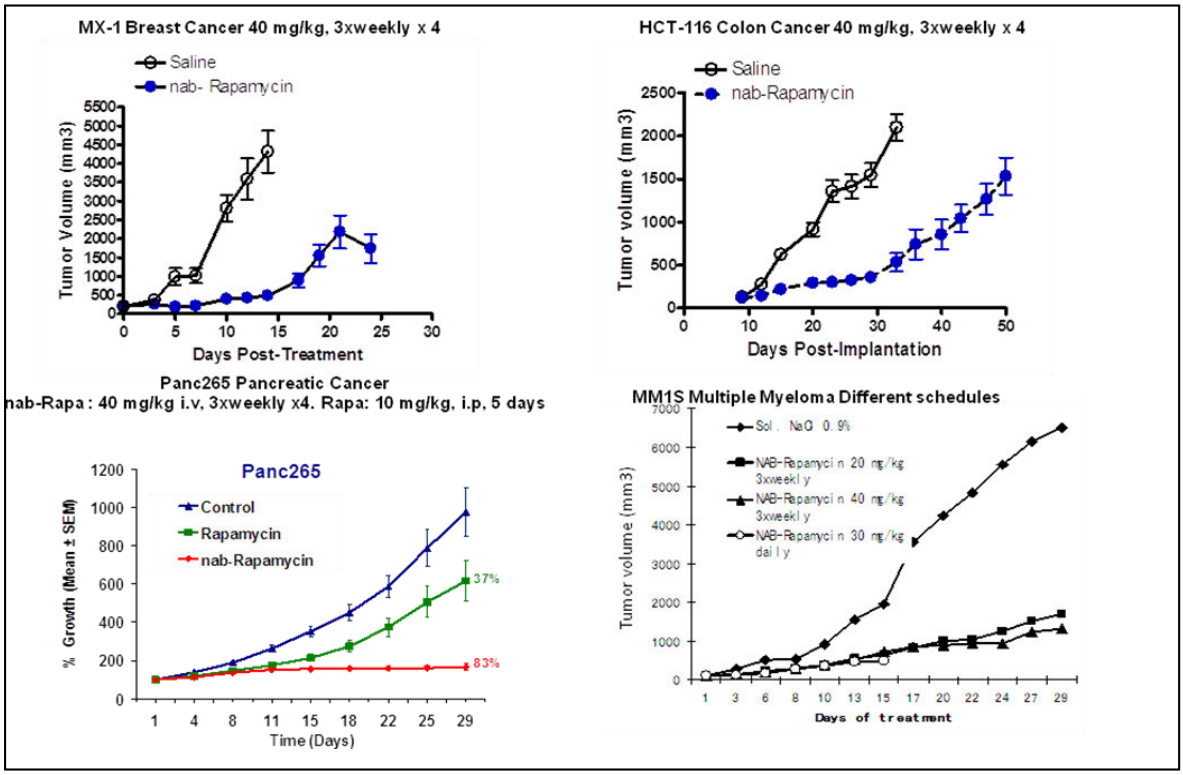
The novel nanoparticle albumin-bound or *nab*® technology (Abraxis BioScience, a wholly-owned subsidiary of Celgene Corporation) when applied to hydrophobic molecules, such as paclitaxel (*nab*-paclitaxel; Abraxane®), has led to improved drug delivery, safety, and efficacy in various solid tumors compared with the conventional paclitaxel formulation. This suggests that the *nab* formulation of rapamycin may also produce similar advantages over the standard rapamycin.

The *nab* technology likely enhances tumor penetration and accumulation via the albumin receptor-mediated (gp60) endothelial transcytosis. Albumin is highly soluble, has long plasma half-life, broad binding affinity, and accumulates in tumors, making it an ideal candidate for drug delivery [22, 23]. Albumin circulating in the bloodstream can interact with gp60 to initiate caveolae-mediated transcytosis to reach tumor cells [24-26]. Indeed, we have seen in our laboratory that *nab*-paclitaxel transcytosis across the epithelial monolayer was dependent on caveolae formation [27]. In accordance with these observations, at equal doses, *nab*-paclitaxel showed greater selectivity to tumors compared with solvent-based paclitaxel, which is likely attributed to the biologically active ingredient albumin and lack of solvent [28]. Additionally, it is hypothesized that extracellular matrix albumin-binding proteins, such as SPARC, may further enhance therapeutic accumulation of albumin-bound-therapeutics in tumor cells. SPARC is known to be overexpressed in bladder cancer [29].

2.4.1. Preclinical Studies with *nab*-Rapamycin

Preclinical primary pharmacology studies in vivo demonstrated significant antitumor activity of *nab*-rapamycin as a single agent administered intravenously at 40 mg/kg, 3 times weekly for 4 weeks, across different tumor xenograft models in nude mice (see Figure 1 below), including breast, colorectal, multiple myeloma, and pancreatic cancer. This dose level correlates to approximately 120 mg/m^2 in human. These findings are consistent with published information on rapamycin as an mTOR inhibitor and the role of mTOR in tumor growth [30-33]. In addition, recent preclinical study has demonstrated that combination of *nab*-rapamycin with the Akt inhibitor perifosine induced synergistic antitumor activity in multiple myeloma [34].

Figure 1: Antitumor Activity of *nab*-Rapamycin in Tumor Xenografts



Importantly, a preclinical study was conducted to test the antitumor activity of *nab*-rapamycin as a single agent or in combination with other chemotherapeutic agents against the UMUC3 human bladder cancer cell line. UMUC3 cells (10 x10^6 cells in 0.1 ml PBS with 20% Matrigel) were injected subcutaneously into the flank of female athymic mice, and mice were randomized once the average tumor volumes reach approximately 100 mm^3, and were treated until study end with *nab*-rapamycin (3 mg/kg, twice weekly, IV via tail vein), mitomycin (0.5 mg/kg, twice weekly, IP), cisplatin (3 mg/kg, twice weekly, IP), gemcitabine (30 mg/kg, twice weekly, IP), valrubicin (20 mg/kg, twice weekly, IP), and docetaxel (3 mg/kg, twice weekly, IP) either as single agent or in combination (*nab*-rapamycin plus chemotherapeutic agent). Animals were monitored for tumor volume and body weight.

nab-Rapamycin as a single agent or in combination with other chemotherapeutic agents were well tolerated overall, with no significant body weight loss in any group (Figure 2). *nab*-Rapamycin as a single agent or in combination with chemotherapeutic agents currently in clinical use to treat NMIBC demonstrated significant antitumor activity and significantly prolonged animal survival compared with saline control (Figure 2). Single agent gemcitabine showed only modest effects in tumor growth inhibition and animal survival, none of which were significantly improved over control.

None of the *nab*-rapamycin combination groups showed significant improvement of antitumor activity when compared with *nab*-rapamycin alone due to strong antitumor activity of *nab*-rapamycin as a single agent. However *nab*-rapamycin/gemcitabine combination showed a numerical trend towards enhanced antitumor activity versus *nab*-

rapamycin alone. Importantly, out of all combinations, only *nab*-rapamycin/gemcitabine demonstrated substantially longer survival compared with *nab*-rapamycin alone (median survival: 48 vs 33 days), with more animals surviving till the study end (Treatment Day 50: 3/8 vs 1/8) (Figure 3).

In contrast, when *nab*-rapamycin combination groups were compared with the corresponding chemotherapeutic agents alone, only *nab*-rapamycin/gemcitabine combination showed a significant improvement of antitumor activity. Correspondingly, *nab*-rapamycin/gemcitabine combination also demonstrated a significantly longer animal survival over gemcitabine alone (median survival: 48 vs 20 days).

Figure 2: Antitumor Activity and Body Weight Following Single Agent and Combination Treatments

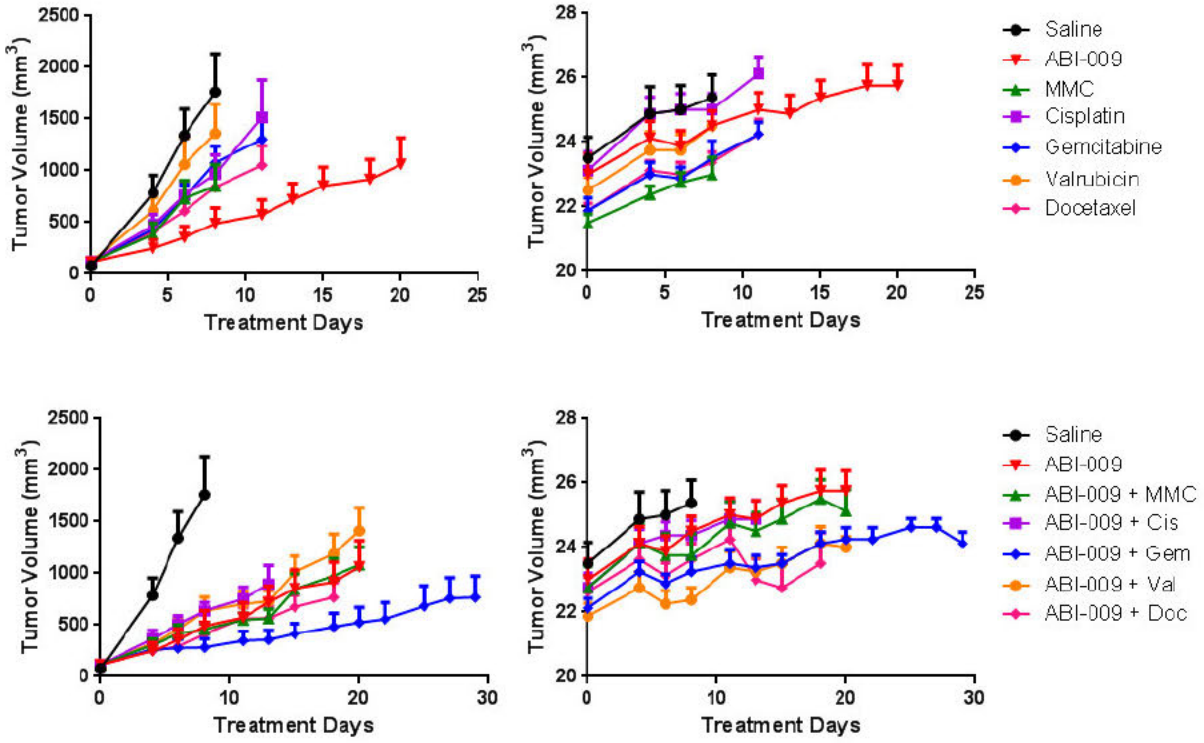
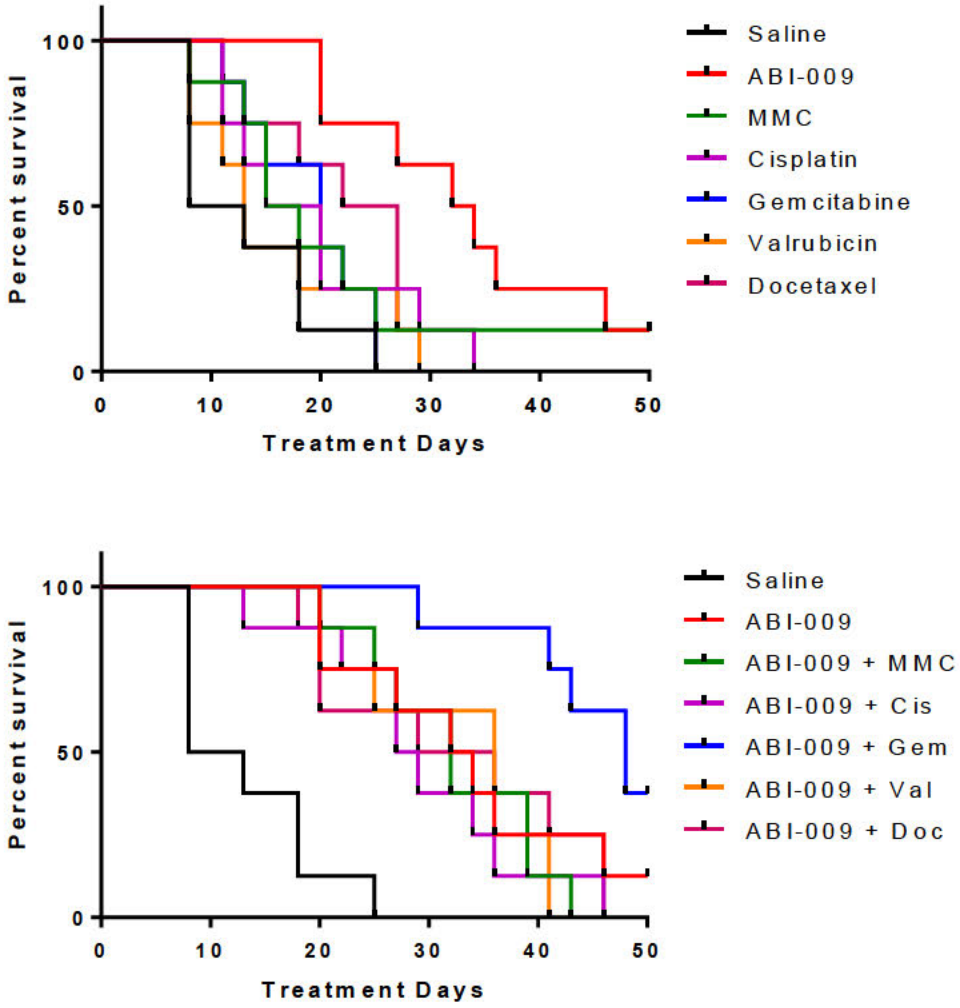


Figure 3: Animal Survival Following Single Agent and Combination Treatments



In conclusion, *nab*-rapamycin administered IV as a single agent or in combination with other chemotherapies was well tolerated with no significant body weight loss. The combination study demonstrated that *nab*-rapamycin/gemcitabine combination was the best combination option in the UMUC3 bladder cancer xenograft model, with better antitumor activity than either *nab*-rapamycin (not significant) or gemcitabine (significant) as a single agent. Importantly, animal survival in the *nab*-rapamycin/gemcitabine group was prolonged compared with either *nab*-rapamycin or gemcitabine as a single agent. Clearly, *nab*-rapamycin/gemcitabine was the best option among all combinations tested in this study, with the addition of *nab*-rapamycin bolstering the efficacy of gemcitabine, a current chemotherapy used to treat recurrent/refractory NMIBC following BCG treatment.

Preclinical pharmacokinetic (PK) studies in rats showed that intravenously administered *nab*-rapamycin exhibited linear PK with respect to dose and large volume of distribution (Vz), due to efficient tissue extraction of rapamycin from the central blood compartment [35]. Immediately after dosing, tissue rapamycin level was 3-5 fold higher than that of blood, indicating efficient extraction. The terminal half life of *nab*-rapamycin was long in

rats, ranging from 13.4 - 25.8 hours and resulted in significant blood level at 48 hours (~10 ng/ml) and 120 hours (>1 ng/ml). Consistent with literature of rapamycin [9], excretion of *nab*-rapamycin was primarily through the fecal route (68.57 - 69.99%) with minimum contribution from the renal route (7.73 - 8.84%).

The safety and toxicity of *nab*-rapamycin were evaluated in a series of preclinical studies. A CNS safety pharmacology study showed no significant demonstrable behavioral changes even at the highest dose level of 90 mg/kg in rat, which is equivalent to 540 mg/m^2 in human [36]. In the HERG study for the assessment of QT prolongation in vitro, *nab*-rapamycin demonstrated a low potency HERG channel blocker potential effect with an IC50 61.3 □M or 56 mg/L (data on file).

In rabbits, single dose intravenous administration of *nab*-rapamycin (5 to 60 mg/kg) resulted in dose-related observable signs of toxicity with all dose levels showing a reduction in food and water consumption with edema and bloating (data on file). All rabbits receiving 5, 10, or 20 mg/kg were reported normal by the end of the 14-day observation period. Signs of toxicity at high dose of 60 mg/kg continued for the course of the study (0-14 days).

In rats, toxicity as assessed in Good Laboratory Practices (GLP) toxicology studies was characterized by involvement of the male reproductive organs, a target organ previously associated with rapamycin exposure. *nab*-Rapamycin was well tolerated at nominal dose levels up to 90 mg/kg/dose (73 mg/kg = 440 mg/m^2 actual dose administered) with mortality occurring at the higher doses of 120 and 180 mg/kg. Toxicities observed at nominal doses of 120 mg/kg and 180 mg/kg were consistent with heart failure and consequential pulmonary congestion and edema [36].

Additionally, rapamycin was determined to be not mutagenic in Salmonella strains TA98 and TA100 both in the presence and absence of S9 metabolic activation, at concentrations tested (5, 10, 50, 100 μM) and *nab*-rapamycin did not cause hemolysis at a 1:1 dilution in whole blood (data on file).

Local delivery of *nab*-rapamycin has been demonstrated to be safe and well tolerated in a porcine femoral artery model of balloon angioplasty injury [37]. Periadventitial injection of a single dose of 500 μg *nab*-rapamycin with an endovascular microinfusion catheter resulted in a perivascular concentration of *nab*-rapamycin 1500-times higher than serum concentration and rapamycin persisted in the tissue for at least 8 days. Luminal stenosis, medial fibrosis and media cell proliferation were significantly reduced by *nab*-rapamycin treatment. There were significantly fewer adventitial leukocytes at 3 days but no difference at 28 days. There was no difference in endothelial coverage or regeneration by Factor VII and H&E staining at 3, 8 or 28 days.

For intravescial delivery of *nab*-rapamycin, minimal or no systemic absorption through the bladder wall is expected due to the nature and size of *nab*-rapamycin nanoparticles and the relatively large molecular weight of rapamycin (~900 g/mol). Serum rapamycin levels are therefore expected to be significantly lower than those resulted from intravenous administration in the preclinical PK and toxicity studies.

2.4.2. Clinical Studies with *nab*-Rapamycin

A phase 1 study conducted at MD Anderson Cancer Center (FDA IND #74,610, filed Dec 27, 2007), showed that *nab*-rapamycin was well tolerated with evidence of responses and stable disease in various solid tumors including renal cell carcinoma and bladder cancer, both of which typically express mTOR [38]. Twenty-six patients were treated with 45, 56.25, 100, 125, 150 mg/m^2 per week for 3 weeks, followed by a week of rest (28-day cycle) *nab*-rapamycin administered intravenously. The maximum tolerated dose was established at 100 mg/m^2 after 4 DLTs were observed: 2 cases at 150 mg/m^2 (elevated transaminase and thrombocytopenia) and 2 at 125 mg/m^2 (suicidal ideations and hypophosphatemia). Stomatitis and mucositis are common AEs observed with the rapalogs; however, with *nab*-rapamycin, only 2 cases of grade 1 stomatitis occurred at 100 mg/m^2 and 1 case of grade 1 mucositis occurred at 45 mg/m^2. Specifically, DLTs such as mucositis/stomatitis that have been observed with other mTOR inhibitors were not dose-limiting with *nab*-rapamycin: all mucositis events were grade 1/2.

Of 19 patients evaluable for efficacy with best overall tumor response assessments, which included assessment of target, nontarget, and new lesions across all cycles, 1 patient in the 45 mg/m^2 (95 mg actual rapamycin dose) cohort diagnosed with adenocarcinoma of the kidney and with bone and intrathoracic metastases had a confirmed partial response. The target lesion of this patient was reduced by 35.1% and the duration of response lasted 183 days. Two (11%) patients (at doses 45 and 125 mg/m^2, with actual rapamycin doses of 88 mg and 193 mg, respectively) had an overall tumor evaluation of stable disease (confirmed): 1 patient with mesothelioma had stable disease for 365 days and 1 patient with a neuroendocrine tumor in the left axillary node had SD for 238 days. Eight patients had stable disease that could not be confirmed either due to absence of follow-up tumor evaluation after the first stable disease, or due to progression after the first stable disease. It was expected that patients with tumors that have activation of the PI3K/AKT/mTOR pathway may benefit most from *nab*-rapamycin treatment. Indeed, the patient with overall tumor evaluation of partial response had kidney adenocarcinoma and the 2 patients with the longest clinical benefit had mesothelioma or neuroendocrine tumor; in all 3 cancer types mTOR over activity has been described. *nab*-Rapamycin produced a fairly dose proportional increase of Cmax and AUC across the dose range tested, and it significantly inhibited mTOR targets S6K and 4EBP1.

With excellent systemic safety at doses of 100 mg/m^2 (167-223 mg actual rapamycin dose) given weekly IV, we believe that intravesical therapy with *nab*-rapamycin should result in minimal systemic rapamycin exposure, and is of significant therapeutic avenue to investigate in humans with BCG refractory or recurrent NMIBC.

2.4.3. Prior Clinical Experience in NMIBC with Intravesical *nab*-Paclitaxel

Research at Columbia University showed in the first clinical phase 1 study in patients with BCG-refractory bladder cancer that intravesical *nab*-paclitaxel exhibited minimal toxicity and systemic absorption with promising efficacy [10]. Specifically, in 18 patients with BCG-refractory high-grade NMIBC receiving 150 to 500 mg *nab*-paclitaxel, no grade 2 or higher toxicities occurred, with only a few cases of grade 1 adverse events (dysuria, hematuria, and frequent urination), and 500 mg was deemed the MDD. Twenty-eight percent patients had complete response in this study. In addition, paclitaxel (molecular weight 854 daltons) levels in blood after intravesical administration were not detectable

except in 1 patient who exhibited a level of 16 ng/ml, which is over 1000 fold lower than levels reached in systemic therapy. Given the excellent safety profile and promising efficacy of the cytotoxic agent paclitaxel in a *nab*® product (*nab*-paclitaxel) delivered intravesically to patients with NMIBC, the 100-400 mg intravesical *nab*-rapamycin (a cytostatic agent) proposed for use in this study is likely to be safe in this patient population.

2.4.4. A Summary of Justification for Use of *nab*-Rapamycin in NMIBC

Based on the background information provided above, it is likely that *nab*-rapamycin will be safe and potentially effective in the NMIBC patient population. The main points for this justification as summarized below:

- *nab*-Rapamycin has already been studied in patients with solid tumors by systemic IV administration at doses ranging from 45-150 mg/m^2 and its safety with systemic exposure has been established at 100 mg/m^2 (approximately 180 mg systemic rapamycin dose) given weekly 3 weeks out of 4 until disease progression (CA401 study).
- Systemic absorption of rapamycin (a cytostatic) from the bladder is highly unlikely due to the molecular size of rapamycin (~900 daltons), its hydrophobicity and the nanoparticle nature of *nab*-rapamycin, therefore no serious safety concerns are expected. Furthermore the safety profile of intravesical *nab*-paclitaxel (a cytotoxic) in this population has already been studied in which only grade 1 local bladder related adverse events were observed.
- Oral rapamycin and other oral and IV rapalogs are not suitable for local instillation in the bladder due to nonavailability of suitable formulations for administration into the bladder, poor drug levels in the bladder with oral administration, or nonavailability of enzymes in the bladder required to convert the prodrug form into the active rapamycin form.
- Preclinical models of bladder cancer that mimic the human disease show very promising activity of rapamycin in these models that occurs through suppression of mTOR.
- *nab*-Rapamycin demonstrated strong antitumor activity against human bladder cancer cell line UMUC3 as a single agent, and the activity was further improved in combination with gemcitabine.
- Preclinical systemic toxicology of *nab*-rapamycin is well studied and a local tolerance study of *nab*-rapamycin in porcine femoral artery injury model showed lower inflammation scores compared to the control.
- A dose higher than 400 mg in this study is not expected to produce additional benefit due to potential saturation of the mTOR target. It is to be noted that responses in the solid tumor study were seen well below the MTD and at the lowest administered dose of 45 mg/m^2 (73-96 mg actual rapamycin dose).

- Results from the Phase 1 part of this study showed that intravesical *nab*-rapamycin was safe in NMIBC patients with no DLT and no severe drug-related adverse events.
- Intravesical gemcitabine treatment is well tolerated in multiple phase 1, 2, and 3 clinical studies, with most toxicities being dysuria or frequency.
- The good individual safety profiles of intravesical *nab*-rapamycin and gemcitabine indicate that the combination of *nab*-rapamycin and gemcitabine is unlikely to cause cumulative toxicities.

2.5 Justification for Adding Gemcitabine in the Phase 2

Gemcitabine inhibits DNA synthesis and is widely used to treat systemic bladder cancer [39]. More importantly, previous studies have demonstrated the activity of intravesical gemcitabine for the treatment of patients with NMIBC that recurred after BCG treatment. In a prospective, randomized trial comparing intravesical gemcitabine with mitomycin C (MMC) in patients who had progressed or failed BCG therapy [40], the rates of recurrence (28% vs 39%) and progression (11% vs 18%) were lower with gemcitabine but did not reach statistical significance. The overall incidence of adverse events was significantly less with gemcitabine (38.8% vs 72.2%, P = 0.02). A large multi-center study (S0353) also demonstrated the feasibility of intravesical delivery of the drug in patients who had failed two or more courses of BCG. Gemcitabine was given at dose of 2000 mg/week for 6 weeks, then monthly for up to 12 months [41]. Of the 47 evaluable patients, 21 (45%) were disease free at 3-month evaluation, and 13 of those were continuously disease-free at the 12 months (28% of all evaluable patients). Other phase 1 and 2 studies have shown that intravesical gemcitabine was tolerable with minimal systemic absorption and metabolite difluorodeoxyuridine plasma levels below 7.5 μM [42]. Thus, although myelosuppresion is a potential side effect of intravenous gemcitabine, intravesical gemcitabine has a more favorable toxicity profile, which includes urinary traction infection and maculopapillary rash. There is also precedence for the use of gemcitabine in combination with another intravesical chemotherapy. In a study with BCG refractory NMIBC patients, the sequential administration of intravesical gemcitabine (1000 mg) followed by mitomycin C (40 mg) weekly for 6 weeks was well tolerated with only grade 1 or 2 toxicities observed [43].

The rationale for selecting gemcitabine specifically for this multidrug regimen derives from work done with animal models. In a preclinical study with athymic mice bearing human UMUC3 bladder cancer xenografts, *nab*-rapamycin and chemotherapeutic agents currently in clinical use to treat NMIBC (mitomycin, cisplatin, gemcitabine, valrubicin, and docetaxel) were administered either alone or as *nab*-rapamycin based combination regimens. *nab*-Rapamycin as a single agent or in combination with other chemotherapeutic agents were overall well tolerated with no significant body weight loss in any group. *nab*-Rapamycin as a single agent demonstrated significant antitumor activity compared with saline control as well as significantly prolonged animal survival, whereas all other single agent groups showed relatively modest antitumor effect. When tested in combination, the *nab*-rapamycin/gemcitabine combination demonstrated the best antitumor efficacy. Among all *nab*-rapamycin combination groups, only the *nab*-rapamycin/gemcitabine combination demonstrated a significant improvement in tumor

growth inhibition and a significantly longer animal survival compared with the corresponding chemotherapeutic agents alone. It was also the only group that showed better tumor growth inhibition and substantially longer survival compared with *nab*-rapamycin alone. These results suggest that *nab*-rapamycin bolsters the efficacy of gemcitabine, a current chemotherapy approved for the treatment of recurrent/refractory NMIBC following BCG treatment. Furthermore, they strongly substantiate the selection of a *nab*-rapamycin/gemcitabine combination for this Phase 2 clinical study of recurrent/refractory NMIBC.

The feasibility and rationale for the combination treatment of intravesical *nab*-rapamycin and gemcitabine are based on individual safety profiles of these two agents and the demonstrated combination activity in a preclinical tumor model. Intravesical gemcitabine may cause irritation to the bladder and reduce the dwelling time for subsequent intravesical drug treatment, therefore, we will administer intravesical *nab*-rapamycin first before gemcitabine.

3. STUDY POPULATION

3.1. Inclusion Criteria

1. Patients must have a diagnosis of transitional cell carcinoma (TCC) of the urinary bladder confirmed at the study institution. The patient must have demonstrated nonmuscle-invasive bladder cancer refractory or recurrent to standard intravesical therapy. Refractory disease is defined as failure to achieve tumor-free status by 6 months of initiation of adequate BCG therapy. Recurrent disease is defined as reappearance of disease after achieving a tumor-free status by 6 months of initiation of adequate BCG therapy. Adequate BCG therapy includes at least 6 weeks induction plus 3 additional doses of either induction or maintenance. Patients with a history of other intravesical agents (except *nab*-rapamycin or gemcitabine) in addition to standard BCG will also be allowed to enroll. All grossly visible disease must be fully resected and pathologic stage will be confirmed at the institution where the patient is enrolled. This will include stage Ta, T1, Tis and exclude all patients with muscle invasion (T2).
    a. For phase 1, patients with multifocal low-grade Ta histology will be eligible for participation
    b. For phase 2, individuals with Ta disease only must have documentation of high-grade histology
    c. For phase 2, prior intravesical treatment with *nab*-rapamycin or gemcitabine is not allowed
2. Age >18 and must be able to read, understand, and sign informed consent
3. Performance Status: ECOG 0, 1, and 2 (See Appendix III)
4. Hematologic inclusion within 2 weeks of start of treatment
    a. Absolute neutrophil count >1,500/mm^3
    b. Hemoglobin >9.0 g/dl

c. Platelet count >100,000/mm^3

5. Hepatic inclusion within 2 weeks of entry
   a. Total bilirubin must be within normal limits.
   b. Adequate renal function with serum creatinine ≤2.5 mg/dL
   c. Aspartate transaminase (AST) and alanine transaminase (ALT) ≤2.5 x ULN for the institution, alkaline phosphatase ≤ 2.5 x ULN for the institution, unless bone metastasis is present in the absence of liver metastasis
6. Women of childbearing potential must have a negative pregnancy test.
7. All patients of childbearing potential must be willing to consent to using effective contraception, ie, intrauterine device, birth control pills, depo-provera, and condoms while on treatment and for 3 months after their participation in the study ends.

3.2. Exclusion Criteria

1. Any other malignancy diagnosed within 1 year of study entry (except basal or squamous cell skin cancers or noninvasive cancer of the cervix) is excluded
2. Concurrent treatment with any chemotherapeutic agent
3. Women who are pregnant or lactating
4. History of vesicoureteral reflux or an indwelling urinary stent
5. Participation in any other research protocol involving administration of an investigational agent within 1 month prior to study entry
6. History of radiation to the pelvis
7. History of interstitial lung disease and/or pneumonitis
8. Evidence of metastatic disease

4. STUDY DESIGN AND PLAN

4.1. Overall Study Design

This study will be designed as a combined phase 1 and phase 2 to determine the safety and tolerability profile of intravesical *nab*-rapamycin in human patients with high-risk nonmuscle-invasive TCC of the urinary bladder that have previously demonstrated resistance to standard intravesical treatment.

The nonrandomized phase 1 study will be designed to test if this novel treatment regimen has an appropriate safety and toxicity profile to be further investigated to afford patients a greater likelihood of complete response than the 20% response noted with currently available second line intravesical chemotherapy agents.

TCC refractory or recurrent patients will be enrolled in the phase 1 portion of the study and these patients will receive 1 to 2 weekly instillations of *nab*-rapamycin in saline diluent initially at an approximate pH of 5 to 8. Patients will be instructed to avoid excessive fluid

intake starting the day before treatment. Patients will receive intravesical *nab*-rapamycin by sterile urethral catheterization once or twice weekly for 6 weeks. Prior to instillation, the bladder will be emptied via the sterile urethral catheter. The patient will be instructed to keep the drug in the bladder for 2 hours before voiding. Six weeks after the last treatment, the patient will undergo assessment, including cystoscopy and biopsy.

Following the completion of the phase 1 portion of the study and appropriate analyses of the results have been performed establishing the MDD, the phase 2 portion will be initiated at 200 mg/week for *nab*-rapamycin and a fixed dose of 2000 mg/week of gemcitabine. The purpose of the phase 2 portion of the study will be to both establish the efficacy of the intravesical treatment of *nab*-rapamycin in combination with gemcitabine for patients with BCG refractory or recurrent TCC of the bladder as well as to further elucidate the safety and toxicity profiles of the MDD. Patients will receive weekly instillations of *nab*-rapamycin followed by gemcitabine in 100 mL saline diluent initially at an approximate pH between 5.0-7.5. Patients will be instructed to avoid excessive fluid intake starting the day before treatment. Patients will receive intravesical *nab*-rapamycin and gemcitabine by sterile urethral catheterization once weekly for 6 weeks. Before instillation, the bladder will be emptied via sterile urethral catheter. *nab*-Rapamycin will be instilled first and the patient will be instructed to keep the drug in the bladder for one hour before voiding. Upon voiding, gemcitabine will then be instilled and the patient will be asked to retain the drug for an additional hour before voiding. Finally, 6 weeks after the last treatment, the patient will undergo assessment, including cystoscopy and biopsy.

If a patient in phase 1 or 2 has a complete response after 6 weeks of instillations, the patient will receive additional monthly maintenance instillations. If the patient was a complete responder of phase 1, that patient will receive maintenance instillations at the maximum dose that particular patient received. If the patient was a complete responder in phase 2, that patient will receive maintenance instillations of *nab*-rapamycin and gemcitabine. Cystoscopic examinations will be performed every 3 months, and the patient will continue to receive monthly maintenance instillations until there is a positive cystoscopic biopsy (disease progression), or until a maximum of 1 year from their first treatment. Patients will continue to be monitored for local and systemic toxicities throughout the duration of maintenance therapy.

As it has been shown that additional maintenance therapy can increase the duration of response to intravesical agents, all patients treated in phase 1 and 2 will be given monthly maintenance instillations throughout the duration of their recurrence-free status for up to 1 year of therapy. This addition will not affect our original hypothesis since the maintenance treatment will occur after the primary endpoint for the study is determined.

This combined phase 1 and phase 2 study would significantly contribute to the body of literature that exists examining molecular determinants of response to rapamycin and molecular markers of high progression risk in TCC. Multiple genes and chromosomal locations have been linked to progression and lack of response in TCC including p53, RB, p21, p16 and p27 and loss of chromosome 9,11, and 17 [44, 45]. Aside from predicting a more aggressive and deadly natural history, certain genetic aberrations provide mechanisms for poor response to therapy. For instance, p53 is a cell cycle regulator that allows a damaged cell to arrest in the G1 phase of the cell cycle and either to repair itself of progress along an apoptotic pathway. In addition, the SPARC

extracellular glycoprotein, which is a key step in the uptake of *nab*-rapamycin into the cell, has been shown to be over-expressed in certain cancers including bladder cancer, thereby increasing *nab*-rapamycin's efficacy [46]. The molecular markers analyzed in this study will include but not be limited to p53, p63, Stathmin, Tau, ribosomal s6 kinase, Ki67, and SPARC.

4.2. Summary of Evaluation

Table 1 details the study evaluations and treatment schedule for both phase 1 and 2.

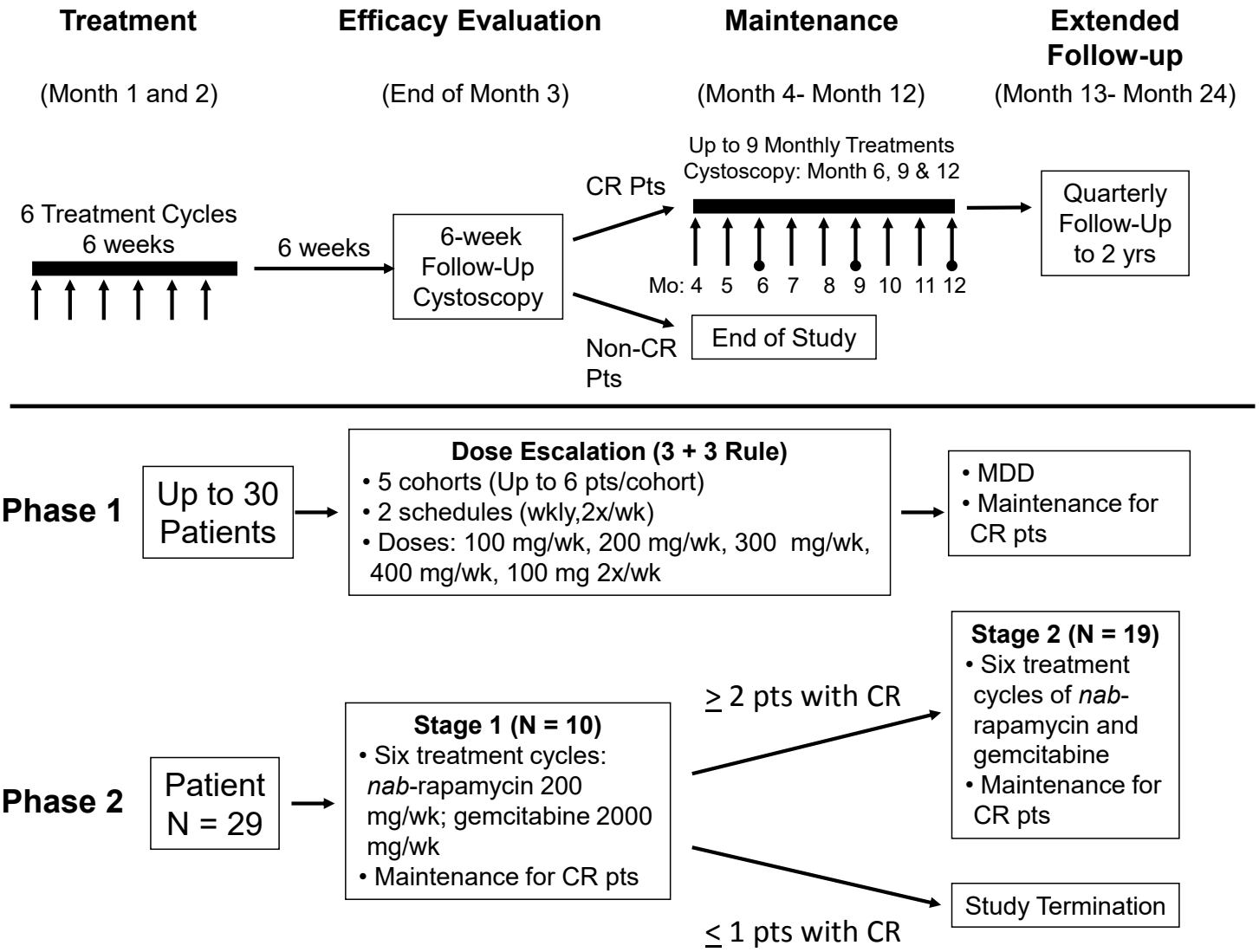
Table 1: Study Evaluation and Treatment Schedule

Parameter	Pre-study	Treatment Day[a]	Every Other Week[b]	EOS	6-week Follow-Up	Monthly Maintenance Treatment Day (mo)									Follow up
						4	5	6	7	8	9	10	11	12	Every 3 mo
History	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Intravesical *nab*-rapamycin (and gemcitabine for Phase 2) administration [c]		X		X		X	X	X	X	X	X	X	X	X	
Urine Cytology [d]	X				X			X			X			X	X
Cystoscopy [e]	X				X			X			X			X	X
TURBT/Biopsy [e]	X				X			X			X			X	X
Pathology	X				X			X			X			X	X
Performance status (ECOG)	X			X	X										
CBC, differential, platelet count [f]	X	X		X	X	X	X	X	X	X	X	X	X	X	
Clinical chemistry panel [f]	X	X		X	X	X	X	X	X	X	X	X	X	X	
Electrolytes (Na, K, Cl, CO_2)	X		X	X	X	X	X	X	X	X	X	X	X	X	
Coagulation Profile	X		X	X	X	X	X	X	X	X	X	X	X	X	
Urine pH [g]	X	X		X	X	X	X	X	X	X	X	X	X	X	
Serum HCG [h]	X														
Chest x-ray [i]	X				X										
Abdominal CT	X														
Serum cholesterol and triglycerides	X		X		X										
EKG [g]	X			X	X										
Serum rapamycin levels [j]		X		X											
Plasma gemcitabine and dFdU levels (for phase 2) [k]		X		X											
BCI QoL Questionnaire	X				X										
Record Serious AE		X		X	X	X	X	X	X	X	X	X	X	X	X

[a] Weekly or 2x weekly.
[b] Even numbered visits.
[c] The phase 2 portion of the study will be conducted with *nab*-rapamycin at 200 mg/week as determined by phase 1 and 2000 mg/week of gemcitabine. *nab*-Rapamycin will be instilled first and the patient will be instructed to keep the drug in the bladder for one hour before voiding. Upon voiding, gemcitabine will then be instilled and the patient will be asked to retain the drug for an additional hour before voiding.
[d] Aside from specific time points in the Schedule of Assessments, cytology can be at any time if symptoms are present and is in the best interest of the patients in the opinion of the investigator. All urine cytology is read by Local Pathology Laboratory.
[e] 6-week, 3-month, and 6-month follow-ups will include cystoscopy and biopsy for all patients not undergoing cystectomy. Biopsy at 6-week follow up is mandatory. Cystoscopy during maintenance therapy is repeated every 3 months (ie, at 6, 9, and 12 months). Biopsy at other time points will only be performed if indicated based on either positive cytology or cystoscopy.
[f] Blood samples for CBC, platelets, chemistry panel, etc should be taken just prior to instillation and include creatinine, serum bilirubin, glucose, alkaline phosphatase, ALT, AST.
[g] Urine pH includes testing of urine pH, urine analysis, and urine culture during visits.
[h] Pre-treatment EKG and HCG will be only drawn prior to initiation of phase 1 if patients enrolled in phase 2 within 3 months of cessation of trial 1.
[i] For participants who are eligible for maintenance therapy, a chest x-ray will be done during maintenance only at the 6 month follow-up visit.
[j] For phase 1, blood samples for rapamycin levels will be measured at pre-treatment (just before instillation) and at 2 hours post-treatment (just before voiding). For phase 2, blood samples for rapamycin levels will be measured at pre-treatment (just before instillation) and just before voiding of gemcitabine. Pretreatment blood samples for rapamycin levels are taken together with samples for CBC, chemistry panels, etc.
[k] For phase 2, blood samples for gemcitabine and dFdU levels will be measured at pre-treatment (just before instillation) and just before voiding of gemcitabine.
Abbreviations: AE, adverse event; ALT, aminotransferase; AST, aspartate aminotransferase; BCI, bladder cancer index; CBC, complete blood count; CT, computed tomography; ECOG, Eastern Cooperative Oncology Group; EKG, electrocardiogram; EOS, end of study; HCG, human chorionic gonadotropin; QoL quality of Life

Figure 4 shows the details of the study evaluations and treatment schedule.

Figure 4: Study Evaluations and Treatment Schema for Both Phase 1 and 2



4.2.1. Prestudy Evaluation

Patients will be seen and evaluated by the principle investigator. Potential candidates will have a history and physical examination performed by the principle investigator in order to make sure they meet the proper inclusion and exclusion criteria. Weight, vital signs, and ECOG performance status will be checked by a member of the medical staff. Patients will provide a urine sample, which will be sent for urine analysis to check the pH value. Patients are required to undergo an initial cystoscopic examination performed by one of the study investigators. If not already performed, all grossly visible tumor will be resected prior to enrollment. Patient will give a blood sample, which will be sent for a complete blood count (CBC), basic metabolic panel (BMP), hepatic function panel, and coagulation profile (prothrombin time, partial thromboplastin time, and international normalized ratio). In female patients of reproductive age, a serum β-hCG value will be checked to rule out evidence of pregnancy. Patients are also required to have a chest X-ray and abdominal CT scan within 3 months, and an electrocardiogram (EKG) within 30 days of treatment. If these tests have not been performed within the specified timelines, they will be performed prior to study enrollment.

4.2.2. Week of Instillation and Treatment Day

Before treatment, a member of the treatment staff will evaluate the patient's CBC and clinical chemistry panel. The patient must have a hemoglobin level >9.0 g/dL, an absolute neutrophil count >1,500/mm^3, a platelet count >100,000/mm^3, AST and ALT <2.5 x ULN, alkaline phosphatase <2.5 x ULN and creatinine <2.5 mg/dL in order to undergo treatment.

During the treatment day, each patient will have an updated history taken by the principle investigator. At each visit, every patient will be asked specific questions to monitor for local bladder toxicity as defined by the NCI CTCAE version 4.0. For example, when monitoring for grade 3 or 4 hematuria, the member of the treatment staff will ask about persistent gross hematuria and/or blood clots. In order to monitor for grade 3 or 4 toxicity bladder spasm, patients will be asked if their symptoms are severe enough to require a narcotic. In order to monitor for grade 3 or 4 toxicity urinary frequency and urgency, patients will be asked if they are urinating hourly or more with frequency or experiencing urgency than prior to beginning treatment. If questioning reveals any grade 3 or 4 toxicity as defined by the NCI CTCAE version 4.0, the patient will be considered to have local toxicity and will be removed from the study and treated appropriately. Questioning to monitor for grade 2 local toxicity will also be performed and the patient will be treated at the discretion of the principle investigator or drug instillation will be postponed 1 week at the discretion of the investigator, provided that resolution of symptoms to a maximum of grade 1 occurs.

At every visit, all study patients will have vitals performed, including weight, blood pressure, and pulse, and an update of their medical history will be obtained. Patients will provide a urine sample, which will be checked by dipstick for pH and then sent to the laboratory for urine analysis. If urine pH is not between 5.0-7.5 and thus outside the range of *nab*-rapamycin (and gemcitabine in phase 2) solubility, the dose will be held. If urine pH is found to be below this range, the oral alkalinizing agent potassium citrate will be prescribed and the treatment will be administered 1week later pending a normalized urine

pH. If the urine pH is greater than 8, oral intake of citrus juices and increased fluid intake will be encouraged and the dose will be administered 1 week later pending a normalized urine pH.

Patients will be administered *nab*-rapamycin (and gemcitabine in phase 2) treatment via sterile urethral catheter. Patients will retain the study drug within their bladder for 2 hours and then void in a standard fashion. No special precautions are required regarding disposal of the study drug after voiding in a normal toilet.

A blood sample will be drawn for CBC, chemistry panel, and rapamycin levels at every visit, just before treatment. Two hours after the treatment is initiated (immediately before voiding), the patient's blood will be drawn for serum rapamycin levels, after which they will conclude the treatment visit. On even-numbered instillations, patients will have basic metabolic panel, hepatic functional panel, lipid panel, and coagulation profile checked. If there is any evidence of grade 3 or 4 systemic toxicity, according to these lab values, the patient will be immediately removed from the study and be deemed to have systemic toxicity.

Additionally, at the initial intake visit will receive the Bladder Cancer Index (BCI), a disease-specific quality of life questionnaire specifically designed and validated for patients with NMIBC [47]. The BCI addresses health outcomes for these patients including urinary, bowel, and sexual bother and function. Patients will additionally be administered the BCI at their follow-up cystoscopy (approximately 3 months after their initial treatment cycle ends).

4.2.3. Week 6 instillation

At the end of the 6-week treatment periods, study patients will have an updated history taken by the medical staff. Blood will be drawn and a CBC, comprehensive metabolic panel, and coagulation profile will be ordered before the visit. The medical staff will perform a physical examination and check the patient's weight, vital signs, and ECOG performance status. Similar to previous treatment days, the patient will receive the appropriate dose of intravesical *nab*-rapamycin (with gemcitabine in phase 2), have a urine sample checked by dipstick for pH value and have blood drawn for rapamycin. The patient will also have an EKG at this visit.

4.2.4. Six-week Follow-up

All study patients will undergo an updated history and perform a physical examination. Weight, vital signs, and ECOG performance status will be obtained by the medical staff. Patients will have blood drawn and a CBC, BMP, hepatic function panel, cholesterol, triglycerides, and coagulation profile will be obtained. Patients will provide a urine sample for pH value and will have an EKG, and chest X-ray. The BCI quality of life questionnaire will also be administered at this point.

4.2.5. Six-week and Quarterly Response Assessments

All study patients will undergo response evaluation after a minimum of 6 weeks following the last treatment. This will consist of urinary cytology, examination under anesthesia and cystoscopy with bladder biopsy or tumor resection. This will serve as the primary endpoint for response for the clinical studies. A complete response will be defined as a negative

biopsy. No response will be defined as positive cystoscopic biopsy. Another end-point will be event-free survival defined as time from the last treatment until biopsy stage is beyond that of the original stage pre-treatment. Cystoscopy with biopsy at 6-week follow up is mandatory. At other time points, biopsy will be performed only if indicated based on either positive cytology or cystoscopy.

Only patients who achieve a complete response at the 6-week assessment will move to monthly maintenance therapy. All patients in phase 1 and 2 who are treated with additional maintenance therapy after a complete response will undergo additional evaluations at 6, 9, and 12 months during the maintenance therapy and every 3 months during the 2-year follow up or until a positive biopsy occurs. A durable complete response will be defined as consistently negative cytology, cystoscopy or biopsies.

Follow-up CT scans will be performed at the discretion of the study investigators as an adjunct to quarterly cystoscopic assessments.

4.3. Study Drug Dosage

4.3.1. Dose Modifications

In the event of a systemic or local DLT, no dose modification will be permitted and the patient will be removed from study.

In the event of grade 2 bladder toxicity, *nab*-rapamycin (and gemcitabine in phase 2) instillation will be postponed 1 week, provided that resolution of symptoms to a maximum of grade 1 occurs.

Any patient that misses more than 1 dose or has more than 1 dose postponed, will be considered to have experienced a DLT and be removed from the study.

In the 2x/week schedule, if continuous accumulation of rapamycin blood level is seen for 3 continuous weeks (ie, rapamycin level not back to baseline prior to next dose), the next dose will be delayed by 1 week and the patient will be moved to the corresponding 1x/week schedule for the remaining 3 weekly treatments.

4.4. Correlative Studies

4.4.1. Tumor Biopsies

Six weeks after the last treatment, the patients will undergo a cystoscopy and biopsy. Response rates will be determined by cystoscopic biopsy at 6 weeks following treatment. Tumor tissue samples will be stored for evaluation of molecular correlative studies to predict response to therapy. Tissue samples will be stored in the Tumor Bank at Columbia University until used for future analysis of molecular markers. Testing for markers may be performed off-site, but any remaining tissue will be returned to the Tumor Bank for potential future testing. Other researchers will not have access to tissue samples unless given permission by Dr. McKiernan, the Primary Investigator.

4.4.2. Pharmacokinetic Sampling Method and Rationale

During the phase 1 clinical study, blood samples will be taken just prior to treatment and at 2 hours after *nab*-rapamycin treatment is initiated (i.e., just before voiding), collected in EDTA-treated tubes, and stored at -80°C until analysis. During the phase 2 study, blood samples will be taken just prior to treatment and just before voiding of gemcitabine. The

tissue samples, if collected, will be collected into 15 mL cryovials, flash frozen on dry ice, and stored at -80ºC until analysis.

Rapamycin levels in serum and in bladder tissue samples will be measured by liquid chromatography/mass spectrometry (LC/MS) at BASI in West Lafayette, IN. Rapamycin concentrations in the target bladder tissue will be correlated with clinical efficacy data, and pharmacodynamic biomarkers to help establish effective biological dose.

Due to the nature and size of the nanoparticles and the relatively large molecular weight of rapamycin (~900 g/mol), very low level or no rapamycin is expected to be detected in serum, indicating minimal or no systemic absorption through the bladder wall. No correlation between *nab*-rapamycin dosage and serum rapamycin levels is expected to be observed.

Because of effective tissue penetration, intravesical *nab*-rapamycin treatment is expected to result in high drug concentrations in the target bladder tissue, which could decrease significantly over time as determined by the tissue samples collected up to 3 or 7 days later. It is possible that after a certain *nab*-rapamycin dose, the rapamycin concentration in bladder tissue would be dose-dependent, but above that dose, the rapamycin concentration would reach a plateau, indicating saturation of drug absorption.

For the phase 2 study, plasma levels of gemcitabine and its metabolite dFdU will be analyzed.

4.5. Concomitant Medications and Nondrug Therapies

For the phase 1 portion, *nab*-rapamycin will be the only chemotherapy drug given. In phase 2, *nab*-rapamycin will be administered before gemcitabine. No other therapeutics to treat NMIBC are allowed. Supportive care per the institution's normal standard of care including concomitant medications can be provided at the Investigator's discretion.

4.6. Withdrawal/Premature Discontinuation

A patient may voluntarily discontinue study participation at any time. At the Investigator's discretion, patients may be discontinued from the study at any time after discussion with the study PI and sponsor. In the event of premature discontinuation, the patient should return to the study site as soon as feasible to have the end of study assessments performed and the appropriate follow-up evaluations should occur. Patients must be withdrawn from this study if any of the following occurs:

- Progressive disease.
- Systemic or local DLT
- Patient declines to continue therapy.
- Initiation of other anticancer therapy.
- In the Investigator's judgment, it is in the patient's best interest to discontinue the study.

Patients who are withdrawn from this study secondary to a laboratory toxicity or AE should be followed by the Investigator as outlined in Section 7.

5. STATISTICAL METHODS

5.1. Study Design and Sample Size

The phase 1 segment of the study will have a dose-escalation scheme that will enroll up to 30 patients. Dose escalation will follow the 3+3 rule to establish the MDD.

Based on ongoing experiences with *nab*-rapamycin and prior experience with dose escalations for intravesical Abraxane, in this phase 1 segment of the study, patients will be enrolled to 4 weekly dosing cohorts, 3 per cohort: cohort 1 – 100 mg/week, cohort 2 – 200, mg/week, cohort 3 – 300 mg/week, and cohort 4 – 400 mg/week for 6 weeks of treatment (

Table 2). If no significant safety issues are found in cohort 1, there will be a 2x/weekly dosing cohort (cohort 2b – 100 mg 2x/week, total weekly dose of 200 mg) enrolled in parallel and evaluated independently from the weekly dosing cohorts for safety and preliminary efficacy.

Table 2: Dosing Schema of Intravesical *nab*-Rapamycin

Regimen	Concentration (Dose Level)	Intravesical Instillation	Cycles	Interval [a]	Total Weekly Dose
1	1.25 mg/mL (100 mg)	80 mL	6	1x / week	100 mg
2	2.5 mg/mL (200 mg)	80 mL	6	1x/week	200 mg
2b [b]	1.25 mg/mL (100 mg)	80 mL	6	2x / week	200 mg
3	3.75 mg/mL (300 mg)	80 mL	6	1x / week	300 mg
4	5.00 mg/mL (400 mg)	80 mL	6	1x / week	400 mg

[a] Patients receiving 1x/week treatment, the treatment window is +/- 1 days of scheduled day. Patients receiving 2x/week treatment will receive their 2nd treatment on day 4 (+/- 1 day) of that week
[b] Cohort 2b (100 mg 2x / week) will accrue patients at any time after cohort 1 safety is established (i.e. no patient develops DLT for the first 3 weekly instillations) and can occur in parallel with cohorts 2, 3, or 4.

Dosages can be escalated if 3 to 6 patients have undergone the first 3 instillations of the medication without experiencing a DLT, as follows. Initially 3 patients will be treated. If none develops DLT following the third weekly instillation, the dose can be escalated. If only 1 of the first 3 patients develops DLT, then an additional 3 patients will be treated at that dose. If the fourth, fifth, and sixth patients do not develop DLT, the dose can be escalated; however, the escalation can occur only after all patients in the cohort complete their 6 weekly doses. Otherwise the prior dose will be defined as the MDD. At any dose level, if 2 or more cases develop DLT, the prior dose will be defined as the MDD once 6 patients have been treated at this level with less than 2 patients experiencing a DLT. The following

Table 3 gives the operating characteristics of this scheme.

Table 3: Operating Characteristics of Dose Escalation

True rate of DLT (%)	Probability of Escalation (%)
10	91
20	71
30	49
40	31

50	17
60	8

The primary objective of the phase 2 segment of this study is to evaluate the utility (potential for clinical efficacy) of *nab*-rapamycin in combination with gemcitabine in the treatment of BCG refractory or recurrent NMIBC as measured by rate of complete responders. Once the MDD has been properly established, only then the nonrandomized phase 2 segment of the study will be initiated, using the Simon 2-stage design. In stage 1, there will be only 10 patients enrolled with a rejection rule that only if there are 2 or more positive responses will the study proceed to further enrollment of the next 19 patients (stage 2). If 1 or fewer respond in the first stage, we will terminate the study for lack of efficacy. If more than 5 patients respond overall, we will consider the agent promising.

The Null Hypothesis is that there will be no difference between the current response rates to BCG therapy of 10% [1-3] and the response rate to intravesical *nab*-rapamycin in combination with gemcitabine treatment after 6 weeks of therapy. The Alternative Hypothesis will be that there will be a ≥30% response rate to intravesical *nab*-rapamycin in combination with gemcitabine treatment after 6 weeks of weekly therapy. The overall rejection rule is that if there are 5 or greater responses to treatment out of the entire enrollment, we will reject our Null Hypothesis and accept our Alternate Hypothesis, that there is a significant difference between the response rates of standard BCG therapy and intravesical *nab*-rapamycin in combination with gemcitabine treatment.

The expected sample size for the phase 2 study Simon 2-stage design is 15 patients with a maximum of 29 patients. Based on our operating characteristic of 5% type I error and 20% type II error, the number of patients that is expected to be enrolled will be 15 on average with a maximum total of 29 in order to sufficiently power the study. The probability of correctly concluding the therapy is worthy of further study is 80% if the true underlying response rate is 30%. The probability of incorrectly concluding the therapy is promising is 5% if the true response rate is approximately 10%.

5.1.1. Adverse Events and Toxicities

Dose-limiting systemic toxicity will be defined any grade 3 or 4 systemic toxicity using the NCI CTCAE version 4.0. If a patient exhibits any evidence of systemic toxicity, the patient will be removed from the study and treated appropriately.

Dose-limiting local toxicity will be defined as any grade 3 or 4 hematuria, dysuria, urinary retention, urinary frequency/urgency, or bladder spasms using the NCI CTCAE version 4.0. If a patient exhibits local toxicity, the patient will be removed from the study and treated appropriately.

Patients who experience a systemic or local DLT will be included in determination of the MDD.

5.2. Patient Population

5.2.1. Analysis Population

The treated population, which includes all enrolled patients who receive at least 1 dose of study drug, will be the primary population used for all safety/tolerability and efficacy analyses.

5.2.2. Patient Characteristics

Patient characteristics including demographics, disease duration and severity at baseline, and relevant medical history will be summarized for the purpose of characterizing the patient population. Descriptive statistics including mean, standard deviation, frequency distributions, etc., as appropriate, will be presented.

5.2.3. Patient Disposition

Patient disposition, including the number of patients enrolled, treated, and reasons for discontinuation from the study will be summarized.

5.2.4. Prior and Concomitant Medications

All concomitant medications and prior medications taken within 30 days of first study drug administration will be coded to therapeutic drug classes and generic drug names using the World Health Organization Drug Classification. The incidence of prior and concomitant medication usage will be summarized by therapeutic drug class and generic drug names.

5.3. Safety Analyses

Safety and tolerability will be monitored through reporting of AEs and SAEs, laboratory abnormalities, incidence of patients experiencing dose modifications, and/or premature discontinuation of study drug.

Systemic DLT is defined as any grade systemic toxicity using the NCI CTCAE version 4.0. Local dose limiting toxicity is defined as grade 3 or 4 bladder toxicity using the NCI CTCAE 4.0.

5.4. Efficacy Analyses

Six weeks after the last dose, patients will undergo a cystoscopy and biopsy. If a patient has a complete response, the patient will receive additional monthly maintenance instillations at the dose that particular patient received. Cystoscopic examinations will be every 3 months, and the patient will receive therapy until disease progression for a maximum of 1 year from the start of therapy.

> A complete response is defined as a cancer-negative biopsy on cystoscopy. No response is defined as positive cystoscopic biopsy.

Another end-point is event-free survival defined as tumor recurrence, tumor progression to muscle invasive bladder cancer or death, whichever occurs first. Urine cytology will also be assessed as a secondary endpoint.

5.4.1. Biomarker and Pharmacokinetic Analyses

This clinical study offers an excellent opportunity to assemble blood, urine, and tissue samples for further interrogation regarding the molecular correlative markers that may predict sensitivity and resistance to intravesical *nab*-rapamycin therapy in TCC. There is not expected to be any formal impact on decision making within this study based on

molecular markers and patients will, of course, have the opportunity to refuse tissue samples and still participate in the clinical study.

The molecular markers analyzed will include but not be limited to p53, p63, Stathmin, Tau, ribosomal s6 kinase, Ki67, and SPARC.

Rapamycin blood levels will be monitored for systemic absorption. Elimination rate constant, half-life, volume of distribution, C_{max}, t_{max}, AUC_{inf}, and clearance will be summarized.

6. STUDY DRUG MANAGEMENT AND ADMINISTRATION

6.1. *nab*-Rapamycin

nab-Rapamycin is a cytostatic therapeutic agent, but should be handled as a cytotoxic anticancer drug. As with other potentially toxic compounds, caution should be exercised in handling *nab*-rapamycin. The use of gloves is recommended. If *nab*-rapamycin (lyophilized cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. If *nab*-rapamycin contacts mucous membranes, the membranes should be flushed thoroughly with water.

6.1.1. *nab*-Rapamycin Packaging, Labeling, and Storage

nab-Rapamycin will be supplied by the Sponsor in single-use vials as lyophilized product. Each single-use vial will contain 100 mg rapamycin and human albumin as a stabilizer. Each vial will be labeled according to country-specific regulatory requirements for labeling of investigational products. For each treatment, the required dose of *nab*-rapamycin will be will be administered in a fixed volume of 80 ml in 0.9% sodium chloride.

Unopened vials of *nab*-rapamycin should be stored in a refrigerator (2°-8°C; 36°-46°F) in original cartons to protect from light. Reconstituted *nab*-rapamycin may be stored for up to 4 hours at 2-8°C (36°- 46°F), followed by 4 hours at room temperature (<25°C) in the IV bag. Both unopened vials of *nab*-rapamycin and reconstituted *nab*-rapamycin should be stored in an area free of environmental extremes and must be accessible only to study personnel.

Temperature records for *nab*-rapamycin must be made available to Sponsor nominated Contract Research Organization (CRO) monitoring teams for verification of proper study drug storage.

Only completely unused study drug vials should be retained by the site until a representative from Sponsor-nominated CRO has completed an inventory. Partially used and completely used vials should be destroyed according to the site's guidelines, and their disposition should be recorded on the Investigational Drug Accountability Record Form.

The Investigator, or designee, shall record the dispensing of study drug to patients and any remaining study drug after dosing in a study drug accountability record. The study drug record will be made available to AADi or authorized AADi-designated monitoring personnel for the purpose of accounting for the study drug supply. Inspections of the study drug supply for inventory purposes and assurance of proper storage will be conducted as

necessary. Any significant discrepancy will be recorded and reported to AADi or their designee and a plan for resolution will be documented.

6.1.2. *nab*-Rapamycin Preparation and Administration

nab-Rapamycin is supplied as a sterile, lyophilized powder and should be reconstituted by appropriate study personnel before use. Each vial of *nab*-rapamycin is reconstituted with 20 mL of sterile 0.9% Sodium Chloride for Injection, USP to form a white-to-yellow translucent suspension containing 5 mg/mL of rapamycin as albumin-bound nanoparticles.

Calculate

Instillations cannot be done immediately following transurethral resection of bladder tumor (TURBT). Investigators must wait a minimum of 10 days before dosing subjects after a TURBT, and/or until any bladder wall integrity issues have resolved.

1. Determine the total dose (in mg) to be administered to the patient:

Total Dose (mg)

2. Calculate the total number of vials required by:

Total Number of Vials = Total dose (mg) / 100 (mg/vial)

Round up the number of vials to be reconstituted to the next higher whole number if a fractional number of vials is obtained by the above formula (eg, if the total number of vials = 1.05 or 1.5, then 2 vials would be reconstituted).

3. Using sterile technique, prepare the vials for reconstitution.

4. Swab the rubber stoppers with alcohol.

5. Reconstitute each *nab*-rapamycin vial by using a 50-cc or 60-cc sterile syringe to inject 20 mL of 0.9% Sodium Chloride Injection, USP into each vial over a period of not less than 1 minute (Note: Change the syringes after reconstituting every 3 vials).

- Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
- **Slowly** inject the 20 mL of 0.9% Sodium Chloride Injection, USP over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the **INSIDE WALL OF THE VIAL.**
- **DO NOT INJECT** the 0.9% Sodium Chloride Injection, USP directly onto the lyophilized cake as this will result in foaming.
- Once the injection is complete, allow the vial to sit for a **minimum of 5 minutes** to ensure proper wetting of the lyophilized cake/powder.
- **Gently** swirl and/or invert the vial **slowly** for at least **2 minutes** until complete dissolution of any cake/powder occurs. **Avoid** generation of foam.
- If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.
- Each mL of reconstituted product will contain 5 mg of rapamycin.

6. Calculate the exact total dosing volume (to the nearest mL) of 5 mg/mL suspension required for the patient:

Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

7. The reconstituted sample should be a white-to-yellow translucent suspension without visible particulates. If unsuspended powder is visible, the vial should be **gently** inverted again to ensure complete resuspension, prior to use.

8. Using a new, sterile 50-cc or 60-cc syringe, withdraw the reconstituted 5mg/ml *nab*-rapamycin solution from the vial(s). Do not remove the rubber stopper from the *nab*-rapamycin vials as this can compromise the sterility of the drug preparation.

9. Inject the contents of the reconstituted *nab*-rapamycin vials into an empty sterile, standard PVC IV bag using an injection port. Inject perpendicularly into the center of the injection port to avoid dislodging plastic material into the IV bag. Repeat steps 9 and 10 until the patient's entire required dose (total mg) is injected into the IV bag.

10. Add additional 0.9% sterile saline into the bag to make up the final dosing volume to 80 ml by using the table below for phase 1 (Table 4) and phase 2 (Table 5):

Table 4: Dosing Volume Preparation for Phase 1 Dosing

Total Weekly Dose (Schedule / Week)	Number of Vials Reconstituted with 20 mL Saline at each Treatment	Amount of 5 mg/mL nab-Rapamycin Introduced into Empty IV Bag	Additional Saline Added to IV Bag to Make up Total Volume to 80mL	Final Volume for Intravesical Instillation	Administered Concentration of Rapamycin (Total Dose)
100 mg (100 mg 1X weekly)	1	20 mL	60 mL	80 mL	1.25 mg/mL (100 mg)
200 mg (200 mg 1X weekly)	2	40 mL	40 mL	80 mL	2.50 mg/mL (200 mg)
200 mg (100 mg 2X weekly)	1	20 mL	60 mL	80 mL	1.25 mg/mL (100 mg)
300 mg (300 mg/1X weekly)	3	60 mL	20 mL	80 mL	3.75 mg/mL (300 mg)
400 mg (400 mg 1X weekly)	4	80 mL	-	80 mL	5.00 mg/mL (400 mg)

Table 5: Dosing Volume Preparation for Phase 2 Dosing

Total Weekly Dose (Schedule / Week)	Number of Vials Reconstituted with 20 mL Saline at each Treatment	Amount of 5 mg/mL nab-Rapamycin Introduced into Empty IV Bag	Additional Saline Added to IV Bag to Make up Total Volume to 80mL	Final Volume for Intravesical Instillation	Administered Concentration of Rapamycin (Total Dose)
200 mg (200 mg 1X weekly)	2	40 mL	40 mL	80 mL	2.50 mg/mL (200 mg)

11. Discard any excess solution left over in the vials in accordance with standard operating procedures for cytotoxic drugs.

12. Administer the reconstituted *nab*-rapamycin suspension from the IV bag intravesically via transurethral catheter.

13. Reconstituted *nab*-rapamycin suspension should be used immediately, but may be stored for up to 4 hours at 2-8°C (36°- 46°F), followed by 4 hours at room temperature (<25°C).

Stability

Unopened vials of *nab*-rapamycin are stable until the date indicated on the package when stored between 2-8°C (36°- 46°F), in the original package. Reconstituted *nab*-rapamycin should be used immediately, but may be refrigerated at 2°C to 8°C (36°C to 46°F) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion. Neither freezing nor refrigeration adversely affects the stability of the product. Some settling of the reconstituted suspension may occur. Ensure complete resuspension by mild agitation before use. Discard the reconstituted suspension if precipitates are observed. The suspension for infusion prepared as recommended in an infusion bag is stable at up to 4 hours at 2-8°C (36°- 46°F), followed by 4 hours at room temperature (<25°C) at ambient lighting conditions.

Storage

Store the vials in original cartons refrigerated at 2°C to 8°C (36°C to 46°F). Retain in the original package to protect from bright light.

Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published [48, 49]. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

6.1.3. Receipt and Return of *nab*-Rapamycin

Upon receipt of the study drug supplies, the Investigator or designee will conduct an inventory and sign both copies of the study drug receipt and forward 1 copy to the address indicated on the form. One copy of the receipt and the packing slip must be retained in the Investigator's regulatory file records.

A representative from Sponsor or designee will inspect the study drug inventory, Drug Accountability Record form(s), and will arrange for the disposition of any remaining unused study drug. No study drug may be returned to AADi without the representative from AADi or other designated personnel first inspecting the study drug inventory and accountability documentation.

6.1.4. Toxicities of *nab*-Rapamycin

This study is the initial study of *nab*-rapamycin, a formulation of rapamycin, administered intravesically. No unexpected toxicities not already known for rapamycin were identified

in the nonclinical or clinical toxicity studies. Please see the Rapamune® Package Insert (0) for more details on the known precautions, warnings, and adverse reactions of rapamycin.

In a clinical study with IV *nab*-rapamycin infusion in 26 patients with various advanced solid tumors, FDA IND #74,610, filed Dec 27, 2007), *nab*-rapamycin was well tolerated. Four DLTs were observed in the study: 2 cases at 150 mg/m^2 (elevated transaminase and thrombocytopenia) and 2 at 125 mg/m^2 (suicidal ideations and hypophosphatemia). While stomatitis and mucositis are common DLTs with rapamycin and rapalogs, all stomatitis/mucositis events were grade 1 and 2 (none dose-limiting). At the maximum tolerated dose of 100 mg/m^2 *nab*-rapamycin only 2 cases of grade 1 stomatitis occurred and 1 case of grade 1 mucositis occurred at 45 mg/m^2. Additionally, the most frequent grade 3 treatment-related adverse events in ≥5% of all treated patients were thrombocytopenia (3 patients), anemia (2), hypophosphatemia (2), and constipation (2). There was 1 case of grade 4 thrombocytopenia.

6.2. Gemcitabine

6.2.1. Gemcitabine Preparation and Administration

Gemcitabine is a commonly used pharmaceutical formulation and its preparation will be according to routine institutional pharmacy protocols. After voiding of *nab*-rapamycin, Gemcitabine (2000 mg in 100 mL saline diluent) will be intravesically administered into bladder, and patients will be asked to retain the drug for one hour before voiding.

6.2.2. Toxicities of Gemcitabine

Although myelosuppression is a serious side effect when gemcitabine is used intravenously, it has a very favorable toxicity profile when administered intravesically.

Most frequent toxicities for intravesical gemcitabine include dysuria, frequency, and neutropenia [41], other toxicities include hematuria, irritative bladder, bladder ulcer, nausea, anemia, thrombocytopenia, fever, dizziness, and pain [42]. Both systemic and local toxicities generally were not higher than grade 2.

7. ADVERSE EVENT MANAGEMENT GUIDELINES

All serious, related, unlabeled, (unexpected) AEs will be reported to the FDA as required by 21 CFR 312.32, to the clinical trials site's Institutional Review Board, as well as to the Data Safety and Monitoring Committee at the clinical trial site.

7.1. Definition of Adverse Events

An AE is defined as the development of an untoward medical occurrence, undesirable medical condition, recurrence or deterioration of a pre-existing medical condition subsequent to exposure of a pharmaceutical product or treatment. An AE is additionally defined as occurring at any dose, independent of perceived causal relationship to the product. Adverse events may or may not be formal medical diagnoses, and can also include signs, symptoms or abnormal laboratory findings. Common examples include nausea, chest pain, tachycardia, enlarged liver, or EKG abnormalities.

The definition of an AE is independent to a perceived causal relationship to the drug. Causality is a separate assessment that is performed for AEs. Causality assessment to a study drug or regimen will be a medical judgment based made in consideration of the following factors: temporal relationship of the AE to study drug exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions.

Any CTCAE grade 3 or 4, or any clinically significant grade 1 or 2 hematology or biochemistry laboratory values not solely considered a result of disease progression will be considered an AE.

7.2. Definition of Serious Adverse Events

A SAE is defined as any AE that results in death, is immediately life-threatening, requires inpatient hospitalization (at least a 24-hour), prolongs existing hospitalization, results in persistent or significant disability/ incapacity, or is a congenital anomaly/birth defect. Additionally, an SAE also includes any "important medical event" that may not have the immediate outcome of being life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent such outcomes.

Medical and scientific judgment will be exercised in deciding whether an AE is an "important medical event," and would therefore meet SAE criteria. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse.

There will be an acknowledged distinction between serious and severe AE. Assessment of seriousness will be made solely by the serious criteria listed above. Severity of AEs will be graded according to the NCI CTCAE v4.0. Therefore, serious events will not be automatically considered severe. For example, a stroke that results in only a limited degree of disability may be considered a mild (not severe) stroke, but it would still meet serious criteria and thus, be captured as an SAE. Similarly, severe events may not always be serious. An example would be an episode of severe, transient nausea, which persists for several hours. This would be classified as a "severe" episode of nausea, but if it did not require treatment, intervention, or somehow meet other serious criteria, it would not be considered an SAE.

In the clinical study setting, AEs are most often subcategorized as either SERIOUS or NONSERIOUS. This distinction is critical, as SERIOUS AEs require additional documentation that is both time-sensitive and detailed.

7.3. Timeframe for Considering an Event to be Reportable AE or SAE

All AEs (serious and nonserious) will be documented. Any experience or condition that is identified from the signing of the informed consent through the 30-day follow-up period must be captured as an AE. Information collected will include a description of the event, date of onset and resolution, assessment of SAE criteria, any action taken (eg, changes to study treatment), final outcome, and the investigator’s assessment of causality (ie, the relationship to the study treatment).

7.4. Lack of Efficacy is Not Considered and AE or SAE

"Lack of efficacy" or treatment nonresponse for an unproven therapy will not generally be considered an AE. If there is deterioration in the underlying condition for which the study regimen is designed to treat, there may be uncertainty as to whether this is an AE. In such a case, the investigating physician must judge the treatment as a possible contributor to the deterioration. Unless local governing regulations require otherwise, such deterioration will be considered to be an issue of treatment efficacy and not an AE. This situation constitutes an exception to the general rule that AEs are initially identified regardless of perceived causality attribution. Adverse events that are unequivocally due to progression of disease should be recorded as “progressive disease” rather than as AEs. However, the development of an additional disease will be regarded as an AE. For example, if a patient taking an experimental drug to treat underlying breast cancer develops a second primary cancer of nonmetastatic origin, this would be considered a unique AE.

7.5. Patient Reporting of AEs and SAEs

Patients are to be encouraged to call the site to report any unexpected symptoms or problems they encounter between office visits. These events should be considered in the same fashion as if they had been reported at a scheduled office visit. At each scheduled office visit, after the patient has had an opportunity to spontaneously mention any problems, the Investigator should inquire about AEs by asking the following standard questions:

• Have you had any (other) medical problems since your last clinic visit?

• Have you taken any new prescribed or over-the-counter medicines or herbal/vitamin preparations, other than those given to you in this study, since your last visit/assessment?

• Have any new procedures been performed since your last study visit?

7.6. Investigator Reporting of AEs and SAEs

The Investigator or designee must completely and promptly record each AE in the source documentation and in the appropriate CRF, regardless of relationship to study drug as determined by the Investigator. The Investigator must assess AE/SAE causality for any patients treated at his/her site and for any patients treated under the direct care of his/her sub-Investigators. The Investigator should attempt, if possible, to establish a diagnosis based on the patient’s signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the Investigator should report the diagnosis, not the symptoms, as the AE.

Clinically significant laboratory abnormalities present at the baseline visit will be recorded as pretreatment signs and symptoms.

Adverse events and SAEs should be reported on the appropriate case report forms. In addition, all SAEs must be reported promptly to AADi after the Investigator recognizes/classifies the event as a SAE. The specific reporting time frame depends on the type of SAE. For life-threatening or fatal events, the Investigator must report initial information on the SAE within 1 business day of becoming aware of the event, preferably by fax or phone or email; at a minimum, a description of the event and the investigator’s judgment of causality must be provided at the time of the initial report. If an SAE is

reported by phone or by e-mail, the Investigator must fax a completed SAE report form to AADi within 1 business day. For an event that is not life-threatening or fatal, the Investigator must fax a completed SAE report form within 2 business days after he/she recognizes/classifies the event as an SAE.

All serious, related, and unexpected AEs will be reported and documented on Form FDA 3500 A (Med Watch Form) and forwarded to the FDA in accordance with 21 CFR 312.32 and with 21 CFR 314.80. A copy of the MedWatch that has been submitted to FDA will also be forwarded directly to SAE Reporting, AADi, LLC.

These reports may be sent by FAX or E-MAIL to:
SAE Reporting
AADi, LLC

Tel: 310-309-9036
Fax: 424-252-9026
E-mail: sae@aadimed.com

FAX transmission will include the following on the provided study SAE REPORT, fax cover form (Appendix II):

Study#: BC001

Study Title: A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Name of Principle Investigator: Dr. James M. McKiernan, MD

7.7. Additional Investigator Responsibilities on Follow-up for SAEs

The Investigator and supporting personnel responsible for patient care should institute any supplemental investigations of SAEs based on their clinical judgment of likely causative factors. This may include extra clinical laboratory tests, physical examinations, or consulting an appropriate specialist. AADi may also request the Investigator to conduct supplemental assessments. The results of any additional assessments conducted must be reported to AADi. If a patient dies during participation in the study, a death certificate is required. If an autopsy is performed, a copy of the report must be submitted to AADi. If during the follow-up period for a SAE a patient dies due to another event unrelated to the SAE being followed, the event causing the death will be reported as a separate SAE.

All AEs (serious and nonserious) that are unresolved or unimproved since initial presentation will be followed until resolution or improvement of the AE. With respect to AEs that are abnormal laboratory or investigational results, an unresolved or persistent CTCAE grade 3 or 4 laboratory abnormality at study completion or withdrawal will be followed until the laboratory abnormality has either returned to a comparable baseline value, or is judged to have a severity of grade 2 or better.

If, in the investigator's medical judgment, any AE noted in the study is not likely to resolve or improve, the investigator will document that opinion in the patient's medical record.

Should the patient become lost to follow-up or further information is otherwise unattainable, the investigator will note this in the patient's medical record.

For 30 days subsequent to study completion or withdrawal, new onset AEs will be captured. Follow up of these events will follow the same procedure as described above for AEs observed during the study period.

7.8. Criteria for Removal from Study

- Progressive disease
- Systemic or local DLT
- Patient declines to continue therapy
- Initiation of another anticancer therapy
- In the Investigator's judgment, it is in the patient's best interest to discontinue the study

7.9. Institutional Review Board Notification of SAEs

The Investigator is responsible for promptly notifying the IRB of all SAEs, including any follow-up information, occurring at his/her site and any SAE regulatory reports and Investigational New Drug Safety Reports that he/she receives from AADi.

7.10. Sponsor Notification of Poststudy SAEs

For 30 days subsequent to study completion or withdrawal, new onset AEs will be captured. Follow up of these events will follow the same procedure as described above for AEs observed during the study period.

7.11. Deaths

ANY deaths occurring within the study period or within 30 days after discontinuing protocol treatment will be captured without exception, regardless of perceived causal relationship to study drug. A single AE term will be selected, the event will be considered an SAE, and "death" will serve as the positive criteria applied to the seriousness assessment. If the cause of death is unknown, then "death" will be captured as the sole AE term. Whenever possible the underlying cause, not the specific mechanism, of death will be selected as the SAE term, unless the investigator feels the specific mechanism of death is clinically relevant or offers significance that might otherwise be lost if not captured.

8. ETHICAL, LEGAL, and ADMINISTRATIVE ASPECTS

8.1. Ethics

8.1.1. Institutional Review Board or Independent Ethics Committee

Before study initiation, this protocol and informed consent form will be submitted for review and approval to the IRBs charged with oversight for the clinical sites. In addition, any form of proposed advertising and advertising text for patient recruitment must be reviewed and approved by AADi prior to submission to the IRB. The Investigator will

forward to AADi or other sponsor-nominated designee a copy of the IRB's approval of this protocol, any amendments, informed consent form, and any modifications to the informed consent, based on the FDA regulations set forth in 21 CFR 56, as well as those of the applicable regulatory bodies in all other participating countries outside of the U.S.

In addition, the Investigator will be responsible for forwarding to AADi or sponsor-nominated designee a description of the IRB board members (including profession and affiliation) or a United States Department of Health and Human Services (DHHS) General Assurance number and expiration date. If neither of these is available, the chairperson must submit a statement indicating that the members of the board responsible for the review meet FDA and other appropriate regulatory requirements. In addition, the labeling for all approved study drugs should be submitted to the IRB for informational purposes.

Clinical supplies will not be shipped to the clinical site until IRB approval is obtained for the protocol. Any existing amendments, informed consent, and photocopies of the approved documents must be received by AADi or other sponsor-nominated designee prior to drug shipment.

8.1.2. Ethical Conduct of the Study

This study will be conducted in compliance with the protocol, Good Clinical Practice, Guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, and in full compliance with the World Medical Association Declaration of Helsinki and its most recent amendments.

8.1.3. Informed Consent

Written informed consent of the patient to participate in the study must be obtained and documented by the Investigator in accordance with the FDA regulations set forth in 21 CFR 50 as well as the applicable regulatory bodies in all other participating countries outside the United States.

The Investigator must provide the patient with a copy of the informed consent form in a language understandable to the patient. Written consent should be obtained before any protocol-required procedures are performed, including any procedure not part of normal patient care (eg, withdrawal of current medications).

Changes made by a participating site to the recommended informed consent must be forwarded to AADi (or to the sponsor-nominated Project Manager) for approval prior to submission to the corresponding IRB. A copy of the signed informed consent will be given to the patient or their legal representative and the original must be retained in the Investigator's study records.

8.1.4. Data Safety and Monitoring

This treatment regimen uses a chemotherapeutic agent with known toxicity and antitumor activity profiles. Because cancer is a life-threatening disease, treatments that result in grade 3 and 4 toxicities are considered to have an acceptable risk profile. SAEs will be reported to the Sponsor immediately and reviewed as they are received. Any unacceptable toxicities or severe toxicities that occur more frequently than expected will be discussed by the Chief Medical Officer or designee and the Investigator who will decide jointly whether the study should be modified, interrupted, or stopped.

8.2. Disclosure of the Data

8.2.1. Confidentiality

The Investigator and any other study personnel involved in this study shall not disclose, or use for any purposes (other than for the performance of this study), any data, records, or other information (hereinafter collectively "information") disclosed to the Investigator or other study personnel. Such information shall remain the confidential and proprietary property of AADi, and shall be disclosed only to the Investigator or other designated study personnel. The obligation of non-disclosure shall not apply to the following:

- relevant disclosure to potential study participants for the purpose of obtaining informed consent;
- information after such time that it is or becomes publicly available through no fault of the Investigator or other study personnel; and,
- information after such time that it is disclosed to the Investigator by a third party entitled to disclose such information.

If the study site is a 'covered site' under the definitions of the Health Insurance Portability and Accountability Act (HIPAA), the Investigator will ensure that the patient consents to the use of data by AADi and its designees for the purposes of regulatory submissions, study publications, and drug approval.

8.2.2. Publication

Data from any individual center must not be published or presented until the complete multicenter study has been published or presented in full. Any subsequent publications should refer to the published multicenter findings.

The Investigator(s) shall have the right, consistent with academic standards and with due regard to the protection of AADi's confidential information and intellectual property, to publish or present the results of work performed in accordance with the study; provided that any proposed publication or presentation is first reviewed and approved in writing by AADi. AADi shall complete its review within 60 days after receipt of the proposed publication or presentation. Upon AADi's request, proposed publication or presentation will be delayed up to 60 additional days to enable AADi to secure adequate intellectual property protection of property of AADi that would be affected by such proposed publication or presentation. If AADi believes in good faith that any proposed publication or presentation contains any confidential information and/or intellectual property, AADi shall have the right to remove references to any such confidential information and/or intellectual property.

8.3. Investigator Documentation

8.3.1. Form FDA 1572

The Investigator must provide AADi with a fully executed Form FDA 1572. Any updates must be provided via a new fully executed Form FDA 1572.

8.3.2. Curriculum Vitae

The Investigator must provide AADi with his/her current signed and dated curriculum vitae and a current signed and dated curriculum vitae for each sub-Investigator listed on Form FDA 1572. Current signed and dated curriculum vitae is defined as updated within 2 years of study start up.

8.3.3. Financial Disclosures

The Investigator and sub-Investigator(s) must complete a Clinical Investigator Financial Certification/Disclosure Statement to report financial interests and arrangements that may be of concern to FDA per 21 CFR 54.

8.3.4. Laboratory Certification and Normal Ranges

The Investigator will indicate on the Form FDA 1572 the name and location of any local and central laboratories that will be used for laboratory assessments. The Investigator will provide a copy of all clinical laboratory certifications, certification numbers, dates of certifications, lab accreditations, and a list of the normal ranges for all laboratory tests for all facilities listed. Updated versions of these documents must be provided to AADi or sponsor-nominated designee as appropriate. In the event the clinical laboratory is changed during the study, AADi will be promptly notified, and the Form FDA 1572 will be updated. Appropriate documentation will be submitted to AADi to verify the certification of the new laboratory.

8.4. Records Retention

In accordance with applicable regulatory requirements, following closure of the study, the Investigator will maintain a copy of all site study records in a safe and secure location. AADi will inform the Investigator of the time period for retaining these records in order to comply with applicable regulatory requirements. AADi reserves the right to terminate the study for refusal of the Investigator and/or investigational site to comply with any requirements stated in this study protocol.

8.5. Protocol Violations/Deviations

Apart from the regulatory requirements, it is vital to the success of the study that the Investigator adheres to the details of the protocol and thus holds to a minimum the number of cases that may be later classified as “incomplete,” “unusable,” or “not evaluable.”

9. TERMINATION of STUDY

AADi reserves the right to discontinue this study at any time.

10. INVESTIGATOR’S PROTOCOL AGREEMENT

The Investigator must sign the Investigator’s Protocol Agreement. The original must be kept on file at AADi or with a sponsor-nominated designee and the Investigator must retain a copy. The completed Investigator’s Protocol Agreement signifies agreement to comply with all procedures outlined by this protocol by the Investigator. An Investigator’s Protocol Agreement must be signed if and when a protocol amendment is issued by AADi.

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12. APPENDICES

Appendix I. Rapamune® Package Insert – Adverse Reactions

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the label.

- Increased susceptibility to infection, lymphoma, and malignancy [see *Boxed Warning*, *Warnings and Precautions (5.1)*]
- Excess mortality, graft loss, and hepatic artery thrombosis in liver transplant patients [see *Boxed Warning*, *Warnings and Precautions (5.2)*]

12

- Bronchial anastomotic dehiscence in lung transplant patients [see *Boxed Warning*, *Warnings and Precautions (5.3)*]
- Hypersensitivity reactions [see *Warnings and Precautions (5.4)*]
- Exfoliative dermatitis [see *Warnings and Precautions (5.4)*]
- Angioedema [see *Warnings and Precautions (5.5)*]
- Fluid Accumulation and Wound Healing [see *Warnings and Precautions (5.6)*]
- Hypertriglyceridemia, hypercholesterolemia [see *Warnings and Precautions (5.7)*]
- Decline in renal function in long-term combination of cyclosporine with Rapamune [see *Warnings and Precautions (5.8)*]
- Proteinuria [see *Warnings and Precautions (5.9)*]
- Interstitial lung disease [see *Warnings and Precautions (5.11)*]
- Increased risk of calcineurin inhibitor-induced hemolytic uremic syndrome/thrombotic thrombocytopenic purpura/thrombotic microangiopathy (HUS/TTP/TMA) [see *Warnings and Precautions (5.13)*].

The most common (≥ 30%) adverse reactions observed with Rapamune in clinical studies are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, constipation, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia.

The following adverse reactions resulted in a rate of discontinuation of > 5% in clinical trials: creatinine increased, hypertriglyceridemia, and thrombotic thrombocytopenic purpura (TTP).

Appendix II. Adverse Events Fax Cover Sheet

Fax

To:	SAE Reporting AADi, LLC	From:	
Email:	sae@aadimed.com		
Phone:	310-309-9036	Date:	
Fax:	424-252-9026	Pages:	
Re:	Adverse Event Notification	CC:	

☐ *Urgent* ☐ *For Review* ☐ *Please Comment* ☐ *Please Reply* ☐ *Please Recycle*

Comments:
Study#: BC001
Study Title: A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Name of Principle Investigator: Dr. James M. McKiernan, MD

Confirmed: This report has been submitted to FDA.

Appendix III. ECOG Performance Status

ECOG PERFORMANCE STATUS [50]*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work, office work
2	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self care. Totally confined to bed or chair
5	Dead

* As published in Oken et al., Am J Clin Oncol, 1982:

Appendix IV. Sponsor Signatures

Study Title: A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-Rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Study Number: BC001

Amendment Date: April 20, 2016

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ Date: _____

[redacted]

[redacted]

[redacted]

Appendix V. Investigator's Protocol Agreement

Study Title: A Combined Phase 1 and Phase 2 Study of Albumin-bound Rapamycin Nanoparticles (*nab*-Rapamycin, ABI-009) in the Treatment of BCG Refractory or Recurrent Nonmuscle-invasive Transitional Cell Bladder Cancer

Study Number: BC001

Amendment Date: April 20, 2016

I confirm that my staff and I have carefully read and understand this protocol. I/we agree to comply with the procedures and terms of the study specified herein. In particular, I/we have agreed to:

• abide by all obligations stated on Form FDA 1572 and on other document(s) required by local regulatory authority.

• retain records and documents related to this study for at least 7 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or at least 7 years have elapsed since the formal discontinuation of clinical development of the investigational product.

• comply with Good Clinical Practice (GCP) and all applicable regulatory requirements.

• maintain confidentiality and assure security of AADi, LLC confidential documents.

• obtain Institutional Review Board (IRB) approval of the protocol, any amendments to the protocol, and periodic re-approval as required, and to keep the IRB informed of adverse events and periodically report the status of the study to them.

• not implement any deviations from or changes to the protocol without agreement from the sponsor and prior review and written approval from the IRB, except where necessary to eliminate an immediate hazard to the patients or for administrative aspects of the study (where permitted by all applicable regulatory requirements).

• assure that each patient enrolled into the study has read, understands, and has signed the Informed Consent.

• ensure that I and all persons assisting me with the study are adequately informed and trained about the investigational drug and of their study-related duties and functions as described in the protocol.

• make prompt reports of serious adverse events (SAEs) and deaths (within 1 business day of learning of the death and 2 business days of learning of the SAE) to AADi.

• assure access by AADi monitors, and/or FDA to original source documents.

• prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated in the investigation.

• arrange for the transfer of appropriate data from case histories to case report forms for the collection and transmission of data to the Sponsor.

• cooperate fully with any study-related GCP audit as performed by AADi quality assurance group specified by the sponsor.

• abide by the stipulations in the Disclosure of Data section and the manuscript preparation/authorship guidelines established at the outset of the study.

_____ _____

INVESTIGATOR'S SIGNATURE DATE

James M. McKiernan, MD

[redacted]

_____ _____

INVESTIGATOR'S SIGNATURE DATE

[redacted]