Phase I/II Study of Everolimus and Intravesical Gemcitabine in BCG-Refractory Primary or Secondary Carcinoma In Situ of the Bladder

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0PROTOCOL SUMMARY AND/OR SCHEMA

This is a Phase I/II trial of Everolimus in combination with intravesical gemcitabine in patients with BCG-refractory TisN0M0.

Phase I: We will evaluate 3 dose levels of continuous Everolimus (Everolimus dose levels: *Dose level 1*: 5 mg every other day, *Dose level 2*: 5 mg daily, and *Dose level 3:* 10 mg daily) in combination with a fixed dose of intravesical gemcitabine at a dose of 2000 mg twice weekly for 3 weeks, followed by a week of rest from gemcitabine—this constitutes one 28-day cycle—and a second course of intravesical gemcitabine followed by a week of rest from gemcitabine. Everolimus will be continued for 12 months in the patients who achieve a CR.

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Agent	Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				Cycles 3-12 (±7 days)
	1	2	3	4*	5	6	7	8*	
Gemcitabine	X	X	X		X	X	X		
Everolimus	X	X	X	X	X	X	X	X	→

*One week of rest from intravesical gemcitabine. There is no week of rest from Everolimus, which is taken continuously.

Phase II: Patients will receive continuous Everolimus administered at the dose determined from the Phase I part of the study and intravesical gemcitabine at a dose of 2000 mg twice weekly for 3 weeks followed by a week of rest from gemcitabine—this constitutes one 28-day cycle—and a second course of intravesical gemcitabine followed by a week of rest from gemcitabine. Everolimus will be continued for 12 months in the patients who achieve a CR. Patients demonstrating a CR (by cystoscopy and cytology) will be observed with serial cystoscopies every 3 months.

Agent	Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				Cycles 3-12 (±7 days)
	1	2	3	4*	5	6	7	8*	
Gemcitabine	X	X	X		X	X	X		
Everolimus	X	X	X	X	X	X	X	X	→

*One week of rest from intravesical gemcitabine. There is no week of rest from Everolimus, which is taken continuously.

2.0OBJECTIVES AND SCIENTIFIC AIMS

Primary

The primary endpoints are:

a.) Phase I - to establish the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) of Everolimus given in conjunction with intravesical gemcitabine.

b.) Phase II - to determine the proportion of patients who are free of disease at 1 year following start of therapy.

Secondary

The secondary endpoints are:

a.) To determine the CR rate (by cytology and cystoscopy) in patients receiving Everolimus in combination with intravesical gemcitabine.

b.) To determine the survival of patients treated with Everolimus in combination with intravesical gemcitabine.

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c.) To evaluate for activated mTOR pathway markers as well as PTEN status and AKT activation in all pretreatment specimens and analysis of posttreatment specimens when available.

3.0 BACKGROUND AND RATIONALE

Everolimus is a novel oral derivative of rapamycin.

Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and has obtained marketing authorization (Certican®) for prophylaxis of rejection in renal and cardiac transplantation in a number of countries, including the majority of the European Union. Everolimus has been in development for patients with various malignancies since 2002.

Everolimus is being investigated as an anticancer agent based on its potential to act:

- Directly on the tumor cells by inhibiting tumor cell growth and proliferation
- Indirectly by inhibiting angiogenesis, leading to reduced tumor vascularity (via potent inhibition of tumor cell HIF-1 activity, vascular endothelial growth factor (VEGF) production, and VEGF-induced proliferation of endothelial cells). The role of angiogenesis in the maintenance of solid tumor growth is well established, and the mammalian target of rapamycin (mTOR) pathway has been implicated in the regulation of tumor production of proangiogenic factors as well as modulation of VEGFR signaling in endothelial cells.

At weekly and daily schedules and at various doses explored, Everolimus is generally well tolerated. The most frequent adverse events (rash, mucositis, fatigue, and headache) associated with Everolimus therapy are manageable. Noninfectious pneumonitis has been reported with mTOR inhibitors, but is commonly low grade and reversible.

3.1 mTOR Pathway and Mechanism of Action

At the cellular and molecular levels, Everolimus acts as a signal transduction inhibitor. Everolimus selectively inhibits mTOR, a key and highly conservative serine-threonine kinase, which is present in all cells and is a central regulator of protein synthesis and ultimately cell growth, cell proliferation, angiogenesis, and cell survival. mTOR is the only currently known target of Everolimus.(1)

mTOR is downstream of the PI3K/AKT pathway, a pathway known to be dysregulated in a wide spectrum of human cancers (e.g., through loss/mutation of the PTEN negative regulator, through PI3K mutation/amplification, through AKT/PKB overexpression/overactivation, and through modulation of tuberous sclerosis complex 1 and 2 (TSC1/TSC2) tumor suppressors). In addition, activation of the PI3K/AKT/mTOR pathway is frequently a characteristic of worsening prognosis through increased aggressiveness, resistance to treatment, and progression.

The main known functions of mTOR include the following (1-2):

- mTOR functions as a sensor of mitogens, growth factors, and energy and nutrient levels, facilitating cell-cycle progression from G1 to S phase in appropriate growth conditions.
- The PI3K/mTOR pathway itself is frequently activated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.
- Through the inactivation of the eukaryotic translation initiation factor (elf)4E-binding proteins (4E-BP1) and activation of the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates the translation of important messages, including those encoding the HIF-1 proteins, c-myc, ornithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.
- The activation of the mTOR pathway leads to the increased production of proangiogenic factors (i.e., VEGF) in tumors and to tumor, endothelial, and smooth muscle cell growth and proliferation.
- The regulation of mTOR signaling is complex and involves positive regulators, such as AKT that phosphorylate and inactivate negative regulators such as the TSC1/TSC2.

mTOR is represented by 2 structurally and functionally distinct multiprotein signaling complexes, mTOR complex 1 (mTORC1, rapamycin sensitive) and mTOR complex 2 (mTORC2, rapamycin insensitive).(3)

mTORC1 is mainly activated via the PI3 kinase pathway through AKT (also known as PKB, protein kinase B) and the TSC1/TSC2.(2) Activated AKT phosphorylates TSC2, which lead to the dissociation of the TSC1/TSC2 complex, thus inhibiting the ability of TSC2 to act as a GTPase-activating protein. This allows Rheb, a small G-protein, to remain in a GTP-bound state and to activate mTORC1. AKT can also activate mTORC1 by phosphorylation of proline-rich AKT substrate of 40 kilodaltons (PRAS40), thereby relieving the PRAS40-mediated inhibition of mTORC1.(4-5)

mTORC2 is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling.(4) It has been suggested that mTORC2 phosphorylates and activates a different pool of AKT that is not upstream of mTORC1. PH domain leucine-rich repeat protein phosphatase (PHLPP) plays a role of a negative regulator. mTORC2 is rapamycin insensitive and is required for the organization of the actin cytoskeleton.(3)

mTORC1-mediated signaling is subject to modulation by the macrocyclic lactone rapamycin and its derivatives, such as Everolimus. Once these agents bind to the 12 kDa cytosolic FK506-binding protein 12 (FKBP12), an immunophilin, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTORC1 and inhibit phosphorylation of mTOR substrates. As a consequence, downstream signaling events involved in regulation of the G1 to S-phase transition are inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity.(6) As many cancers are characterized by dysregulation of G1 transit (e.g., overexpression of cyclin or cyclin-dependent kinases), inhibition of mTOR becomes an intriguing target for inducing cytostasis.(2)

3.2 Preclinical Studies

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Preclinical investigations have demonstrated that Everolimus is a potent inhibitor of the proliferation of a range of human tumor cell lines in vitro, with IC_{50}s ranging from sub/low nanomolar (nM) to µM concentrations, concentrations capable of being reached in patients at the doses used in clinical trials.

Everolimus was shown to have activity in human tumor cell lines originating from lung, breast, prostate, colon, kidney, melanoma, and glioblastoma. Everolimus was also shown to have activity in human pancreatic neuroendocrine cells, where induction of apoptosis was reported,(7) as well as in acute myeloid leukemia cells,(8) adult T-cell leukemia cells,(9) diffuse large B-cell lymphoma cells (DLBCL),(10) pancreatic tumor cells,(11) ovarian cancer cells,(12-13) and hepatocellular carcinoma cells.(14)

As a single agent, Everolimus inhibited proliferation in 3 mantle cell lymphoma cell lines (Jeko1, SP49, and NCEB1) approximately 40% to 65% compared to control cells. This was associated with G1 cell-cycle arrest and reduced phosphorylation of the mTOR downstream target, 4E-BP1.(15)

In a clonogenic assay using cells from 81 patient-derived tumor xenografts never cultured in vitro (11 human tumor types, with 3 to 24 tumors each: bladder, colon, gastric, non-small cell lung cancer (NSCLC) [adeno, squamous epithelium, and large cell], small cell lung cancer (SCLC), breast, ovarian, pancreatic, renal, melanoma, and pleuramesothelioma), Everolimus inhibited colony formation in a concentration-dependent manner. In addition, normal hematopoietic stem cells were insensitive to Everolimus, with an IC_{50} about 15-fold higher than the tumor lines.

Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS), with particular potency against VEGF-induced proliferation. The inhibition of endothelial proliferation and antiangiogenic activity of Everolimus was confirmed in vivo, as Everolimus selectively inhibited VEGF-dependent angiogenic response. Mice with primary and metastatic tumors treated with Everolimus showed a significant reduction in blood vessel density when compared to controls at well tolerated doses. Additionally, activity in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of Everolimus -treated tumors (murine melanoma) provided evidence of in vivo effects of angiogenesis.

Everolimus also inhibits tumor growth in vivo in xenografted, syngeneic, and orthotopic animal models, residing longer in tumor tissue than in plasma and demonstrating high tumor penetration in a rat pancreatic tumor model. These effects occurred within the dose range of 2.5 to 10 mg/kg p.o. daily. Typically, the antitumor activity of Everolimus monotherapy was that of reducing tumor growth rates rather than producing regressions or stable disease.

Everolimus, administered p.o., was a potent inhibitor of tumor growth and well tolerated in:

- an s.c. mouse xenograft model, established from a variety of tumor cell lines of diverse histotypes (NSCLC, pancreatic, colon, melanoma, epidermoid), including a P-gp170 overexpressing multidrug-resistant tumor line
- a series of low-passage tumor xenografts established directly from human tumor material, maintained only in vivo and considered highly predictive of therapeutic outcome in patients. These included breast (5 lines), colorectal (9 lines), gastric (3 lines), lung (22 lines, including

adenocarcinomas, epidermoid cell, large cell and small cell histotypes), melanoma (6 lines), ovarian (4 lines), pancreatic (3 lines), and renal (6 lines).

- in 2 syngeneic models (CA20948 rat pancreatic, B16/Bl6 mouse orthotopic melanoma)

Taken together, these data indicate the broad antiproliferative potential of Everolimus.

It is not clear which molecular determinants predict responsiveness of tumor cells to Everolimus. Molecular analysis has revealed that relative sensitivity to Everolimus in vitro correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein. PTEN status alone may not be predictive of Everolimus relative in vitro sensitivity; however, in some cases (i.e., GBM) there is also a correlation with PTEN status.

In preclinical models, the administration of Everolimus is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (pS6) and p4E-BP1, and occasionally with an increase in phosphorylation AKT (pAKT).

3.2.1 Preclinical Safety

In safety pharmacologic studies, Everolimus was devoid of relevant effects on vital functions, including the cardiovascular, respiratory, and nervous systems. Everolimus had no influence on QT interval prolongation. Furthermore, Everolimus showed no antigenic potential. Although Everolimus passes the blood-brain barrier, there was no indication of relevant changes in the behavior of rodents, even after single oral doses up to 2000 mg/kg or after repeated administration of up to 40 mg/kg/day. Based on these findings, the potential of Everolimus to affect vital functions in patients is considered to be low.

Everolimus is considered to have no genotoxicity or carcinogenicity potential. All significant adverse events observed in preclinical toxicology studies with Everolimus in mice, rats, monkeys, and minipigs were consistent with its anticipated pharmacologic action as an antiproliferative and immunosuppressant, and were, at least in part, reversible after a 2- or 4-week recovery period, with the exception of the changes in male reproductive organs, most notably testes. Ocular effects (lenticular disorders) observed in rats were not observed in any other species and are considered to be a species-specific disorder.

3.3 Clinical Experience

3.3.1 *Everolimus* Pharmacokinetics

Everolimus is rapidly absorbed with a median t_{max} of 1 to 2 hours. The bioavailability of the drug is believed to be 11% or greater. The area under the curve $(AUC)_{0-\tau}$ is dose-proportional over the dose range between 5 mg and 70 mg in the weekly regimen and 5 mg and 10 mg in the daily regimen. C_{max} is dose-proportional between 5 mg and 10 mg for both the weekly and daily regimens. At doses of 20 mg/week and higher, the increase in C_{max} is less than dose-proportional. The coefficient of variation between patients is approximately 50%.

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Trough levels (24 hours postdose) correlate well with AUC_{0-T} at steady state during daily administration.

In whole blood, at a daily dose of 10 mg, about 20% of Everolimus is confined in plasma, with 26% being unbound. The remaining 80% is sequestered in blood cells.

Everolimus is extensively metabolized in the liver and eliminated in the bile. Major metabolites are inactive. Elimination half-life is approximately 30 hours. The clearance of Everolimus is approximately halved in patients with mild-moderate hepatic impairment (Child-Pugh Class A or B), while renal impairment has little or no impact on the pharmacokinetics of Everolimus.

Age, weight, and sex in the adult population do not affect the pharmacokinetics of Everolimus to a clinically relevant extent. The clearance of Everolimus is reduced in children. Pharmacokinetic characteristics are not notably different between Caucasian and Japanese subjects, whereas in Black patients, population pharmacokinetic studies have shown an average 20% higher clearance.

A high-fat meal altered the absorption of Everolimus, with a 1.3-hour delay in t_{max}, a 60% reduction in C_{max}, and a 16% reduction in AUC.

Everolimus is a substrate of cytochrome P450 3A4 isoenzyme (CYP3A4) and a substrate and moderate inhibitor of the multidrug efflux pump P-glycoprotein (P-gp, MDR1, or ABCB1). Hence, its metabolism is sensitive to drugs that modify these enzymes (substrates, inducers, or inhibitors of these enzymes). Competitive inhibition could occur when Everolimus is combined with drugs that are also CYP3A4 or P-gp substrates.

3.3.2 *Everolimus* Pharmacodynamic Studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition of the biomarker p70S6 kinase 1 [S6K1] in peripheral blood mononuclear cells (PBMC) suggests that 5 mg to 10 mg daily should be an adequate dose to produce a high degree of sustained target inhibition ([Study C2101] / [Study 2102]).(16) Furthermore, molecular pharmacodynamic (MPD) studies, using immunohistochemistry (IHC) in biopsied tumor tissue, assessed the degree of inhibition and its duration for pS6, p4E-BP1, and pAKT expression with the daily and weekly dosing. There was high inhibition of the downstream markers S6K1 and 4E-BP1 at 5mg/day, which was complete at 10 mg/day, and preliminary results suggest an increase in pAKT expression, with maximal effect at 10 mg daily ([Study C2107]).(17)

3.3.3 Clinical Experience with *Everolimus*

Since 1996, Everolimus has been investigated as a component of multidrug immunosuppression in solid organ transplantation. On July 8, 2003, the European Union approved the use of Everolimus, under the trade name Certican®, as prophylaxis of organ rejection in adult patients receiving an allogeneic renal or cardiac transplant. The most frequent adverse drug reactions in this setting are highly specific to the transplant context. However, certain events are generalizable, most notably myelosuppression, skin disorders, and increases in blood lipid levels.

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In March 2009, the United States Food and Drug Administration (FDA) approved Everolimus for the treatment of advanced renal cell carcinoma (RCC) in patients with advanced RCC after treatment with sunitinib or sorafenib has failed.

Everolimus has been in development for patients with cancer since 2002. As of August 31, 2008, approximately 4000 patients with various malignancies have been treated in Novartis-sponsored or non-Novartis-sponsored studies, and in 3 healthy volunteer clinical studies. Overall, Novartis sponsored a total of 28 studies of Everolimus administered either as single agent (n=13) or in combination with other antitumor agents (n=15). Ongoing or completed investigator-sponsored studies also enrolled over 1000 patients globally.

Eight single-agent, Novartis-sponsored trials have been or are being conducted in various advanced malignancies. Five Phase I studies evaluated several escalating doses with either weekly or daily administration (Studies C2101/02, C2106, C2107, C1101) of Everolimus, with the objective to identify an optimal regimen and dosage based on safety, pharmacokinetics, and knowledge of the drug's molecular effects on various tumors. The 10 mg/day and 50 mg to 70 mg/week dosages were proposed for further studies when using Everolimus as a single agent and as a target maximum dose in combination studies. In addition, 2 Phase I studies, one conducted in prostate cancer (Study C2106) and the other in Japanese patients with advanced cancers (Study C1101), evaluated the safety and the molecular changes in tumor associated with the administration of Everolimus.

Two Phase II monotherapy studies were designed to evaluate the safety and efficacy of a single dose of 10 mg administered daily, including Study C2235 in advanced NSCLC (n=81) and Study C2239 in advanced pancreatic neuroendocrine tumors (n=160).

A Phase III, randomized, double-blind, placebo-controlled study in patients with metastatic RCC who progressed on a VEGFR TK inhibitor (TKI) demonstrated that everolimus, administered daily at an oral dose of 10 mg, provides positive clinical benefit.(18) Median progression-free survival (PFS) was prolonged from 1.87 months for patients receiving placebo to 4.01 months for everolimus-treated patients, assessed by central independent review blinded to clinical data (hazard ratio, 0.30; 95% CI, 0.22–0.40; p<0·0001).

Updated results presented at the American Society of Clinical Oncology 2009 Genitourinary Cancers Symposium (19) demonstrated everolimus as having even greater superiority to placebo in the primary endpoint of PFS. Median PFS was prolonged from 1.9 months for patients receiving placebo to 4.9 months for everolimus-treated patients, assessed by central independent review blinded to clinical data (hazard ratio, 0.33; 95% CI, 0.25–0.43; p<0.001).

Overall, the most frequent adverse effects suspected to be related to Everolimus have been stomatitis, rash, anemia, fatigue, asthenia, diarrhea, anorexia, nausea, hypercholesterolemia, mucosal inflammation, vomiting, hypertriglyceridemia, cough, peripheral edema, dry skin, epistaxis, pruritus, and dyspnea. The most common grade 3 or 4 adverse reactions suspected to be related to treatment were anemia, infections, hyperglycemia, stomatitis, fatigue, lymphopenia, hypercholesterolemia, pneumonitis, and elevated gamma-glutamyltransferase concentrations.

Noninfectious low-grade (grade 1/2) pneumonitis has led to the development of treatment guidelines for the disorder. The primary dose-limiting toxicity (DLT) has been severe (grade 3) stomatitis and,

occasionally, fatigue, hyperglycemia, and neutropenia. Further detailed information regarding Everolimus clinical development, safety, and efficacy is provided in the [Investigator's Brochure].

The trial is looking at an active agent, namely gemcitabine in combination with a novel agent with pre-clinical data suggesting that urothelial cancer represents a rationale target for m-TOR inhibition. A trial at Memorial Sloan Kettering Cancer Center (MSKCC) (protocol 08-123) of Everolimus in patients with metastatic transitional cell carcinoma of the urothelium that has progressed after first-line therapy with a 2-month progression free survival endpoint is ongoing.

At MSKCC, we performed an immunohistochemical (IHC) study to assess whether mTOR pathway markers are expressed in high-stage bladder urothelial carcinomas. The study was carried out on a tissue microarray of 92 cases of invasive urothelial carcinoma of the bladder (≥ pT2) using antibodies against phospho-S6 and phospho-4E BP1, both markers for an activated mTOR pathway. Staining was graded as 0 to 3+ (0=0-5%; 1+=6-25%; 2+=26-50%; and 3+=>50% tumor cells positive) for phsopho-S6 (cytoplasmic) and phospho-4E BP1 (cytoplasmic and/or nuclear). Presence of at least two evaluable cores from each tumor was required for inclusion in the final evaluation. The results of immunoreactivity in tumor cells demonstrated that mTOR pathway markers are overexpressed in invasive urothelial carcinoma of the bladder with correlation of expression seen within molecules of the mTOR pathway using Spearmans correlation coefficient (rho=0.411) (see table below).(34) This suggests that m-TOR targeted therapies may have activity in patients with urothelial carcinoma.

Tumor cell immunoreactivity

Antibody (# cases)	Grade 0 (%)	Grade 1+ (%)	Grade 2+ (%)	Grade 3+ (%)
p-S6 (85)	45 (53)	11 (13)	9 (11)	20 (23)
p-4E BP1 (84)	25 (30)	10 (12)	17 (20)	32 (38)

3.4 Gemcitabine

Gemcitabine (2',2'-difluoro-2' deoxycytidine; Gemzar, Eli Lilly and Co, Indianapolis, IN) is a novel deoxycytidine analog with a broad spectrum of antitumor activity. Gemcitabine has a molecular weight of 299.66, and after intracellular activation, the active metabolite is incorporated into DNA, resulting in inhibition of further DNA synthesis. Gemcitabine may also inhibit ribonucleotide reductase and cytidine deaminase as part of its cytotoxic activity.(20) Since its approval in 1996 by the FDA for the treatment of pancreatic cancer,(20-21) gemcitabine has been found to have effective antitumor activity against a broad spectrum of tumor types, including breast, ovarian, non-small cell lung, and bladder. Studies in patients with metastatic transitional cell carcinoma (TCC) of the bladder have reported overall response rates to single-agent gemcitabine that ranged from 22.5% in previously treated patients to 28% in those who were not previously treated.(22-24) These studies also reported that single-agent gemcitabine therapy was well tolerated and that the incidence of systemic side effects was low. A multicenter, randomized, Phase III study in patients with

unresectable and metastatic bladder cancer demonstrated that a regimen consisting of gemcitabine plus cisplatin had a better tolerability and toxicity profile than the standard regimen of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC), although overall survival was similar in both treatment groups.(25) On the basis of its excellent clinical activity, patient tolerability, and chemical characteristics, gemcitabine represents a logical candidate for intravesical therapy.

3.4.1 Preclinical Studies with Intravesical Gemcitabine

We studied the toxicology and pharmacokinetics of gemcitabine delivered intravesically in dogs to provide the basis for subsequent clinical studies.

Methods: *Toxicology* (n=6): The animals (in groups of 2) received 100 mg, 350 mg, or 1 g of gemcitabine intravesically on alternate days 3 times/week for 4 weeks. The animals were observed for signs of toxicity; gemcitabine levels and peripheral blood counts were taken 3 times weekly. Tetrahydrouridine (THU) was added to prevent ex vivo degradation of gemcitabine by cytidine deaminase. One dog from each group was euthanized at day 1 after the last dose and the other dog from each group at day 14. Full necropsy was performed in all animals. Urine specimens with and without THU were examined to exclude any degradation of the drug. *Pharmacokinetics* (n=5): Intravesical gemcitabine was given at 100 mg (n=2), 350 mg (equivalent to 1000mg/m2 human dose) (n=2), and 3.5 g (n=1). Intravenous gemcitabine was given at 350 mg (n=2). Serum samples were drawn at 30 minutes and at 1, 2, 4, and 8 hours.

Results: *Toxicology*: 100 mg and 350 mg were well tolerated, with no demonstrable side effects. At 350 mg, the white cell count nadir was 2.9 and 3.1 (x103µl) and the platelet count dropped to 97 and 63 (x103/µl). Necropsy revealed normal bone marrow cellularity, normal bladder histology, and no other abnormalities. At 1000 mg, signs of severe toxicity were seen; these dogs received only 3 doses. One dog died at day 3 following cessation of treatment and 1 was euthanized at day 5 due to febrile neutropenia. The necropsy was consistent with hemorrhagic cystitis and gastrointestinal toxicity. *Pharmacokinetics:* At 100 mg and 350 mg, significant systemic absorption of gemcitabine was seen, with peak serum levels observed at 1 hour and with detectable levels up to 8 hours following intravesical gemcitabine. For a given dose, the AUC for intravesical administration is approximately half that of the intravenous infusion. As the AUC is a better predictor of toxicity, it is concluded that the equivalent systemic dose, if given intravesically, would be half as toxic.

3.4.2 Clinical Experience with Intravesical Gemcitabine

We conducted a Phase I study of gemcitabine administered intravesically in 18 patients with TCC of the bladder refractory to Bacille Calmette-Guérin (BCG), and the results showed excellent tolerability to intravesical gemcitabine given twice a week.(26) The patients included 14 men and 4 women (4:1 male/female ratio), with a median age of 74 years (range, 37–86 years). Stages of bladder cancer included refractory 0is in 12 patients and recurrent T1 carcinoma with or without 0is in 6 patients. Patients were previously treated with 1 to 4 courses of BCG, and 5 patients had been previously treated with intravesical chemotherapy, including mitomycin, valrubicin, thiotepa, and Adriamycin.

Four intravesical dose levels of gemcitabine were evaluated: 500 mg, 1000 mg, 1500 mg, and 2000 mg. Of the 18 eligible patients, 3 were enrolled at dose level I (500 mg), 6 at dose level II (1000 mg), 3 at dose level III (1500 mg), and 6 at dose level IV (2000 mg). Intravesical gemcitabine was administered twice weekly in 2 courses of 3 weeks each, with 1 week of rest between courses, for a total of 12 instillations. At the end of 8 weeks, patients were evaluated for response. Patients who demonstrated CR (negative posttreatment cytology and cystoscopy) underwent close surveillance with serial cystoscopies at 3-month intervals. If recurrence was detected, 2 additional courses (3 weeks each, separated by 1 week) of intravesical gemcitabine could be considered, followed by re-evaluation. For patients who did not achieve initial CR or who suffered a relapse, a radical cystectomy was recommended.

Toxicity: Gemcitabine as an intravesical agent was well tolerated. The most commonly observed toxicities were as follows: At dose level I (500 mg; n=3), 1 patient had grade 1 nausea, and there was no urothelial toxicity. At dose level II (1000 mg; n=6), 4 patients experienced grade 2 urinary frequency (more than 2 times normal frequency or urination) and 1 experienced grade 3 urinary frequency (more than hourly urination with urgency); intermittent gross hematuria without clots was present in 3 patients, 2 patients experienced grade 1 nausea and fatigue, and 1 patient had a skin reaction that resembled a grade 3 hand-foot syndrome. At dose level III (1500 mg; n=3), minimal urinary symptoms were experienced by all 3 patients, but no systemic symptoms were observed. At dose level IV (2000 mg; n=6), urinary symptoms were minimal; grade 3 thrombocytopenia and neutropenia was seen in 1 patient, without infectious complications, but no grade 4 systemic toxicities were observed. DLT was not reached; 2000 mg was the highest soluble dose in 100 mL of sodium chloride.

Pharmacokinetics: Levels of gemcitabine in the serum were evaluated at all 4 dose levels after the first, third, seventh, and ninth instillations. Serum levels of gemcitabine were detected at dose level IV (2000 mg) in 2 patients, including the patient who experienced grade 3 thrombocytopenia and neutropenia, but were undetectable at the first 3 dose levels (500, 1000, and 1500 mg).

Based on the results of our Phase I study, we subsequently conducted a Phase II trial in patients with superficial TCC of the bladder who were refractory or intolerant to BCG therapy and who had refused cystectomy.(27) The patients included 22 men and 8 women (7:3 male/female ratio), with a median age of 70 years (range, 43–89 years). Stages of bladder cancer immediately before intravesical instillation included 0is in 23 patients, T1 in 3 patients, and incompletely resected high-grade Ta in patients. Of 30 patients, 27 were refractory to BCG and 3 were intolerant to BCG.

Intravesical gemcitabine at a dose of 2000 mg was administered twice weekly in 2 courses of 3 weeks each, with 1 week of rest between courses, for a total of 12 instillations. At the end of 8 weeks, patients were evaluated for response. Patients who demonstrated CR (cytology and cystoscopy) underwent close surveillance with urine cytology and serial cystoscopies at 3- to 4-month intervals. For patients who did not achieve initial CR, a radical cystectomy was recommended. The median follow-up for all patients was 19 months (range, 0 to 35 months). Of the 30 patients treated with intravesical gemcitabine, 15 (50%; 95% CI, 32%–68%) had CR; however, only 21% (95% CI, 0%–43%) of these patients were free of recurrence at 1 year. Therefore, of the total 30 patients treated with intravesical gemcitabine, only 10% (95% CI, 0%–21%) were free of recurrence at 1 year. Of the 15 patients who achieved CR, 14 were available for recurrence and
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progression analysis: 12 had tumor recurrence, with a median recurrence-free survival time of 3.6 months (95% CI, 2.9–11.0 months), and 2 had no tumor recurrence at 23 and 29 months, respectively. Overall, 2 patients had progression to a higher stage while receiving gemcitabine therapy. The median follow-up for patients without progression or cystectomy was 19 months (range, 2 to 35 months). Of the 30 patients, 11 (37%) underwent a cystectomy after treatment with gemcitabine; 4 of these 11 patients had CR to the gemcitabine therapy.

Toxicity: Gemcitabine as an intravesical agent was well tolerated in this Phase II study, with a reported toxicity similar to that of our Phase I study: grade 2 dysuria (occasional pain or difficulty urinating) in 3 patients, grade 3 dysuria (continuous difficulty urinating characterized by pain and frequency) in 6 patients, a rash on the glans penis in 1 patient, and cellulitis of the leg (requiring intravenous antibiotics) in 1 patient who was on immunosuppression therapy for a renal transplant.

The efficacy, safety, and pharmacokinetics of intravesical gemcitabine have been evaluated in 2 other Phase I studies, albeit using a less intensive weekly schedule. Laufer et al studied the weekly administration of gemcitabine in 15 patients who had recurrent TCC after intravesical therapy.(28) Levels of gemcitabine in the serum were low in patients receiving 40 mg/mL, and they were undetectable at concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL. Levels of the metabolite dFdU (difluorodeoxyuridine), however, were detectable in plasma of patients receiving gemcitabine at concentrations of 15 mg/mL or higher, which implies that the amount of gemcitabine absorbed at lower doses is minimal. Laufer and colleagues found that intravesical gemcitabine was well tolerated and had minimal toxicity, and 9 of the 13 patients available for assessment were free of recurrence at 12 weeks. In their Phase I study, De Berardinis et al found that at concentrations of 40 mg/mL gemcitabine was systemically undetectable, but they were able to detect the inactive metabolite dFdU in plasma.(29)

In our experience, substantial CR can be achieved initially in patients receiving intravesical gemcitabine, but the 1-year durable CR was only 10% as the majority of patients underwent a relapse within 12 months, which supports the concept of combining intravesical gemcitabine with other agents to improve the durability of CR.

3.5 Carcinoma In Situ (0is)

3.5.1 Natural History of Bladder Cancer

More than 70,000 new cases of bladder cancer are diagnosed each year in the United States.(30) Seventy to 80% of bladder tumors are non-muscle invasive tumors (stage Ta, Tis, T1), 25% are muscle invasive (stage T2, T3), and 5% are metastatic. Sixty to 70% of non-muscle invasive tumors recur, while 20% to 30% progress to a higher stage or grade.(31)

Superficial or non-muscle invasive urothelial bladder cancer (UBC) includes stages Ta, T1, and Tis (carcinoma in situ – 0is), with frequencies of 60%, 30%, and 10%, respectively. The term “non-muscle invasive bladder cancer” embraces a spectrum of tumors with varying degrees of clinical behavior. The low-grade Ta lesions commonly recur but rarely progress to muscle invasion. On the other hand, T1 tumors represent a heterogeneous population, including some of which behave in an indolent fashion and typically respond to conservative therapy, as well as others that behave more

aggressively and progress to muscle invasion and even metastatic disease. At present, we lack the ability to predict the clinical behavior of T1 tumors prospectively.

3.5.2 Recurrence

Recurrence rate after transurethral resection (TUR) ranges from 30% to 90%, depending on size, site, multifocality, the presence of 0is, grade, and prior history of bladder cancer. The high recurrence rate and the unpredictability of the progression pattern of bladder cancer have led to the widespread use of intravesical therapy as a supplement to TUR. Non-muscle invasive UBC lends itself to intravesical therapy owing to direct contact of the chemotherapeutic agent with the bladder mucosa and tumor. Furthermore, some agents can be used at high doses, with minimal systemic side effects due to minimal absorption.

In a prospective longitudinal study, the National Bladder Cancer Collaborative Group A (NBCCGA) identified a group of patients with superficial TCC treated solely by TUR and identified the factors implicated in tumor recurrence. In this study, size itself did not seem to be a factor unless it was greater than 5 cm. An increased recurrence rate occurred in patients with multiple tumors, Grade 3 disease, concomitant 0is, lamina propria invasion, and positive cytology.(32)

3.5.3 Progression

Progression is defined as the development of muscle invasion or metastasis and occurs in 20% to 30% of patients who present with superficial disease. The NBCCGA identified tumor grade, lamina propria invasion, and associated 0is as indicators of progression. Two percent of patients with grade 1 tumors progressed compared to 11% with Grade 2 and 45% with Grade 3, with the majority progressing within 24 months of diagnosis.(32) Four percent of patients with Ta lesions progressed versus 30% with T1 tumors. Eight percent of tumors without mucosal abnormalities progressed versus 33% with moderate to severe dysplasia. In a compilation of data, Herr et al reported a 29% progression for T1 tumors and 40% for T1 G3. (33) It is clear that the poor prognosis of superficial TCC is predominantly due to patients with T1 disease. The high recurrence rate and the unpredictability of the progression pattern have led to the investigation of new approaches.

3.5.4 Current Concepts in Intravesical Therapy

Superficial bladder cancer lends itself to intravesical therapy due to the direct contact between the chemotherapeutic agent and the tumor. Furthermore, very high doses of some agents could be used with minimal systemic side effects due to the lack of absorption (e.g., mitomycin is given at a dose of 40 mg weekly for 8 weeks). This led to the widespread use of intravesical therapy after resecting the papillary tumors endoscopically or documenting the presence of diffuse 0is. BCG, thiotepa, mitomycin C, and doxorubicin have been used as intravesical agents. The highest tumor-free success rate has been reported after intravesical BCG therapy. Multicenter studies have shown that thiotepa and doxorubicin are inferior to BCG in preventing tumor recurrences. In studies comparing mitomycin and BCG, initial tumor-free response rates are comparable for the 2 agents, with some studies suggesting an advantage for BCG therapy. In a randomized trial comparing BCG to no Amended: 1/11/11

intravesical therapy, 38% of patients treated with BCG progressed at 10 years versus 63% for the control group. There was no significant difference between groups with regard to stage. Progression was defined as muscle invasion or metastatic disease. The median survival times for both groups have not been reached. Ten-year survival in BCG was 75% compared with 55% in the control group. Patients with an initial incomplete response to BCG have a poor outcome. The overall risk of progression is 95% at 5 years for 0is patients with an incomplete response but only 19% in those with an initial CR. Alternative therapies are needed for patients who fail BCG. Mitomycin C, with a 15% response when given intravesically, is the most active chemotherapeutic agent. Despite this level of activity, mitomycin C has neither delayed progression nor preserving the bladder. Thus, more active agents need to be identified.

BCG is associated with toxicity in the form of local complications, such as cystitis in 90% of patients, requiring interruption of treatment in 3.6%, hematuria in 9%, epididymitis in 0.4%, contracted bladder in 0.2%, granulomatous prostatitis in 0.9%, renal abscess in 0.1 %, and ureteral obstruction in 0.3%. Systemic complications represent the main concern in BCG-treated patients. Fever up to 38.5°C is frequently observed, pneumonia and hepatitis have been reported in 0.9% of patients, and sepsis in 0.4%.

Everolimus selectively inhibits mTOR (mammalian target of rapamycin), a serine-threonine kinase, which is present in all cells and a central regulator of protein synthesis and ultimately cell growth, proliferation, angiogenesis and survival.(1) mTOR is downstream of PI3K (phosphatidyl-inositol-3-kinase)/AKT, a pathway known to be dysregulated in a wide spectrum of human cancers through various potential mechanisms including loss/mutation of the PTEN (phosphatase and tensin homolog deleted on Chromosome 10) negative regulator, PI3K mutation/amplification, AKT/PKB overexpression/overactivation, and/or modulation of TSC1/TSC2 tumor suppressors. In addition, activation of the PI3K/AKT/mTOR pathway is frequently associated with increased aggressiveness, resistance to treatment and progression. Previous work has demonstrated the over-expression of activated mTOR pathway markers including phospho-S6 and phospho-4E BP1 in invasive urothelial carcinoma specimens.(34) Preclinical studies have demonstrated that that mutational activation of the PI3K pathway through loss of PTEN or activation of the serine/threonine kinase, AKT, makes tumor cells more sensitive to the anti-tumor effects of mTOR inhibitors.(35-38) These findings have provided the rationale to explore the anti-tumor activity of mTOR inhibitors in patients with PTEN-deficient tumors with a proof-of-concept phase 1 neoadjuvant trial of rapamycin in patients with recurrent glioblastoma whose tumors lacked PTEN expression demonstrating anticancer activity. (39)

3.6 Rationale for this Study

Rationale for this study includes the following:

1) Standard management for patients with high-risk superficial disease who fail BCG therapy is removal of the bladder (cystectomy). There is a paucity of active agents for patients who fail intravesical BCG, none of which eliminates the need for cystectomy.

2) Patients wish to avoid a cystectomy because cystectomy generally requires an external collecting device for urine, which is associated with poor self-image and quality of life. Surgery results in impotence in men due to the need to remove the prostate as part of the surgical procedure.

3) The favorable activity of gemcitabine, including CR, when given systemically in metastatic urothelial carcinoma and transient CR when used intravesically for non-muscle-invasive UBC.

4) The acceptable clinical toxicity profile of gemcitabine when given intravesically.

5) The need to increase the durability of the response to intravesical chemotherapy.

6) This proposed study targets patients with stage 0is UBC who have failed or have relapsed after intravesical BCG, the standard treatment for this stage of disease. Unfortunately, standard therapy is frequently ineffective in the setting of persistent or recurrent disease. Persistent or recurrent 0is after BCG is an ominous sign. Despite the poor prognosis, some patients refuse cystectomy because of quality of life concerns and request alternative treatment. The standard of care for patients who failed or did not respond to BCG has not changed in over 25 years and new approaches are needed for this patient population.

7) Intravesical valrubicin is the only agent approved by the FDA. It is a modestly active agent in this setting, with a 21% CR rate reported in a Phase II study, and a 1-year durable CR of 13%. It is infrequently used in the urologic community because of the significant local toxicity and short durability of the response.

8) We have shown that gemcitabine given twice weekly was associated with minimal bladder irritation and tolerable myelosuppression. We proceeded with a Phase II study, documenting a CR of 50%. However, the 1-year durable CR was only 10%, suggesting a need for maintenance therapy.

9) There is a clear need for novel agents in BCG-refractory 0is. Furthermore, an active agent that can be administered systemically has the added advantage of potentially treating upper tract disease, which occurs in 20% of this patient population.

10) The mTOR inhibitor Everolimus has been shown to inhibit protein synthesis and growth of bladder cancer cells in vitro. Everolimus is efficacious in treating bladder cancer cells in vivo. mTOR inhibitors have also shown synergism with gemcitabine in pancreatic cell lines, supporting the concept of combination therapy using Everolimus with intravesical gemcitabine.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

Patient population

Sample size: In Phase I, a minimum of 2 and a maximum of 18 patients will be accrued. In Phase II, a minimum of 33 and a maximum of 45 patients will be accrued (6 can be carried over from the MTD cohort of Phase I).

Time required for accrual of all patients: 24 months (estimate)

Average accrual rate per month: 1-2 patients

Sites: Single institution (MSKCC)

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Recruitment plan: All MSKCC patients with stage TisN0M0 TCC of the bladder who have failed or relapsed after intravesical BCG will be offered the protocol. Both sexes and all racial and ethnic groups will be eligible to participate in this clinical trial, provided all the other eligibility criteria are met. The surgeons will meet weekly with the involved medical oncologists to discuss study patients.

Phase I: 3 dose levels of continuous Everolimus (Everolimus dose levels: *Dose level 1*: 5 mg every other day, *Dose level 2*: 5 mg daily, and *Dose level 3:* 10 mg daily) in combination with a fixed dose of intravesical gemcitabine at a dose of 2000 mg twice weekly for 3 weeks, followed by a week of rest from gemcitabine—this is the first 28-day cycle—and a second course of intravesical gemcitabine, also followed by a week of rest from gemcitabine—the second 28-day cycle—will be evaluated using the DLT rules outlined below:

Table 4.1-1

Number of subjects with DLT	Action
0 of 3 subjects	Escalate to next highest dose level and enroll up to 3 subjects
1 of 3 subjects	Enroll up to 3 more subjects at the same dose level
1 of 6 subjects	Escalate to next highest dose level and enroll up to 3 more subjects
2 or 3 subjects in the first 3 subjects	De-escalate to lower dose level and enroll up to 6 subjects
≥ 2 of 6 subjects	De-escalate to lower dose level and enroll up to 6 subjects

If 2 of the first 2 subjects have DLT, the enrollment of a third subject at the same dose level is not necessary. Similarly, if 2 of 4 subjects or 2 of 5 subjects have DLT, it is not necessary to enroll subsequent subjects at the same dose level. All the Everolimus doses will be given in addition to intravesical Gemcitabine at the dose of 2,000mg in 100cc twice weekly.

There will be no planned dose level below dose level 1. If ≥ 2 DLTs occur at dose level 1, then the study will be terminated.

There will be no planned dose level beyond dose level 3. If no DLTs occur at dose level 3, the Phase II portion of the trial will be initiated.

The MTD is the dose level at which no more than 1 out of 6 patients experience DLT.
If ≥ 2 subjects at the first dose level experience DLT, then the study will be terminated and the combination of Everolimus at a dose of 5 mg every other day with intravesical gemcitabine at a dose of 2000 mg twice weekly will be considered unsafe in this population.

Patients will be observed for DLTs through the 3rd cycle of treatment (the first three 28-day cycles) prior to dose escalation for the next dose level for the next cohort of patients. No intrapatient dose escalation will occur. DLTs will be defined as follows, using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0:

1) Any evidence of grade 3 or 4 toxicity under Renal and Urinary Disorders in CTCAE version 4.0.
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2) Febrile neutropenia (absolute neutrophil count (ANC)) < 1000/μL plus oral temperature to 38.5°C)
3) Grade 4 neutropenia (ANC <500/μL) for ≥ 7 days
4) Other grade 4 hematologic toxicity
5) Other nonhematologic grade 3 or 4 treatment-related toxicity, excluding nausea/vomiting, rash, untreated hyperlipidemia or grade 3 fatigue lasting ≤ 7 days.
6) Any nonhematologic toxicity requiring treatment delay for > 7 days during cycle 1.

Phase II: Patients will receive continuous Everolimus administered at the dose determined from the Phase I part of the study and intravesical gemcitabine at a dose of 2000 mg twice weekly for 3 weeks followed by a week of rest from gemcitabine—this is the first 28-day cycle—and a second course of intravesical gemcitabine followed by a week of rest from gemcitabine—this is the second 28-day cycle. Everolimus will be continued for 12 months in the patients who achieve a CR. Patients demonstrating a CR (by cystoscopy and cytology) will be observed with serial cystoscopies every 3 months.

4.1.2 Proposed Correlative Studies

1) Analysis of pretreatment tumor tissue specimens in all patients by immunohistochemistry markers of activated mTOR pathway phsopho-S6 and phospho-4E BP1 as well as PTEN, .phospho-AKT and PRAS 40, a downstream substrate of AKT. The total number of unstained slides required for this is 10 unstained slides or 1 paraffin-embedded block. This will not require research biopsies.

The staining will be performed at the Core Lab of MSKCC's Department of Pathology. The stains will be evaluated by the GU pathologist on this protocol (Hikmat Al-Ahmadie) and the staining will be graded as 0 to 3+ (0 = 0-5%; 1+ = 6-25%; and 3+ = >50% tumor cells positive).

2) Pretreatment tumor samples will be evaluated for PTEN and PIK3CA mutational status using bidirectional full length sequencing. This assay will be performed by either Sanger sequencing and/or the Sequenom technique at the DNA Sequencing Core Facility at MSKCC. This assay will require 2 curls of 10 microns of the formalin-fixed paraffin-embedded tumor tissue sections.

4.2Intervention

Everolimus administration

The study drug Everolimus will be self-administered on an outpatient basis. The investigator will instruct the patient to take the study drug exactly as specified in the protocol. Patients will be required to maintain a pill diary (Appendix 1, Appendix 2, and Appendix 3). Everolimus will start on Cycle 1, Day 1 of intravesical therapy and will be taken continuously.

Phase I: Everolimus will be administered as follows:
Dose level 1: 5 mg every other day
Dose level 2: 5 mg daily

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Dose level 3: 10 mg daily

Phase II: Everolimus will be administered at the dose determined in Phase I.

Gemcitabine administration

- Patients will receive a dose of 2000 mg of intravesical gemcitabine twice a week (72–96 hours between doses) for 3 weeks for a total of 6 treatments in course 1. After 1 week of rest from intravesical gemcitabine, course 2 will be administered, provided the treating physician notes acceptable patient tolerance. The total number of intravesical instillations will be 12.
- All intravesical chemotherapy will be administered to the patient in the Urology Clinic on an outpatient basis and kept in the bladder for 1 hour.

5.0THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Everolimus

Everolimus is a novel oral derivative of rapamycin. Everolimus will be administered orally continuously from study day 1 until progression of disease or unacceptable toxicity. Patients will be instructed to take Everolimus in the morning, at the same time each day.

Everolimus should be taken by the patient in a fasting state or with no more than a light fat-free meal. Dietary habits around the time of Everolimus intake should be as consistent as possible throughout the study.

If vomiting occurs, no attempt should be made to replace the vomited dose.

5.1.1 Drug Dispensing/Administration

Administration will be performed on an outpatient basis. Everolimus will be dispensed as tablets at the beginning of each treatment cycle. In case of dose modification, patients will be asked to return all of their previously dispensed medication to the clinic and they will be dispensed new-strength tablets.

All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

Medication labels will comply with US legal requirements and will be printed in English. They will supply no information about the patient. The storage conditions for study drug will be described on the medication label.

Everolimus will be provided by Novartis. Everolimus is formulated as tablets for oral administration of 5mg strength. Tablets are blister-packed under aluminum foil in units of 10 tablets, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive.

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5.1.2 Storage and Stability

Current stability data permit a shelf life of either 36 months (for 5mg tablet variants based on solid dispersion dried by evaporation/drying oven) or 24 months (for 2.5mg, 5 mg and 10 mg tablet variants based on solid dispersion dried by paddle dryer), assuming correct storage below 30°C in the original double-sided aluminium blister packaging and protected from light and moisture.

5.1.3 Source of Drug

Novartis, Inc. will supply Everolimus free of charge.

5.1.4 Drug Accountability

All study drug supplies must be kept in a locked room with limited access. The study drug must not be used outside the context of this protocol. Under no circumstances should the investigator or other site personnel supply study drug to other investigators, patients, or clinic, or allow supplies to be used other than directed by this protocol without prior authorization from Novartis.

The pharmacist will maintain a complete drug accountability record for each tablet strength with lot numbers of each drug received, including the number of bottles dispensed to each patient, the dates drug was dispensed, and the daily dose of Everolimus the patient received. The prescribed dose should also be recorded in the patient's medical records.

At the conclusion of the study, all unused Everolimus tablets will be returned to Novartis for destruction.

5.1.5 Toxicity

Adverse events most frequently observed with Everolimus are rash, stomatitis/oral mucositis, fatigue, headache, anorexia, nausea, vomiting, and diarrhea. Infections have not been notably frequent or severe. Non-infectious pneumonitis has also been observed. The majority of these AEs have been of mild to moderate severity (CTC grade 1-2). Overall, the most frequently observed laboratory abnormalities include reduced blood counts, hyperglycemia and hyperlipidemia mostly reported as hypercholesterolemia and/or hypertriglyceridemia.

Other less common adverse events include respiratory, thoracic and mediastinal disorders such as dyspnea, pleural effusions, cough, hemoptysis and acute respiratory failure; general disorders such as fever, general health deterioration, creatinine increase, hypercalcemia and chest pain; musculoskeletal and connective tissue disorders such as back pain, pathologic fractures and bone pain; and psychiatric disorders such as confusional state.

5.2 Gemcitabine (GEMZAR®)

5.2.1 Investigational Drug Description

Gemcitabine is a pyrimidine analogue of deoxycytidine in which the deoxyribose moiety contains 2 fluorine atoms at the 2'position. The drug acts as an inhibitor to ribonucleotide reductase and

inhibition of DNA synthesis may result in perturbations of deoxynucleotide pools and interference with DNA chain elongation. The drug is cell-cycle specific and blocks cells in the G1/S interface. Cytotoxicity is schedule dependent and increases with increasing duration of exposure. The drug is rapidly eliminated from plasma, owing mainly to deamination. Renal clearance of drug is less than 10% of parent drug.

5.2.2 Availability
The drug is supplied as either a 200 mg or 1 gram lyophilized powder in a 50mL sterile single vial for reconstitution.

5.2.3 Administration
The drug is administered via a freely running intravenous catheter per institutional guidelines.

5.2.4 Toxicity
Toxicities include nausea, vomiting, alopecia, stomatitis, anorexia, fatigue, elevations of hepatic transaminases, rash, flu-like symptoms, edema, constipation, paresthesias, hypersensitivity reactions, phlebitis, proteinuria, hematuria, reversible myelosuppression, rarely interstitial pneumonitis and ARDS and rarely kidney damage.

6.0CRITERIA FOR SUBJECT ELIGIBILITY

6.1Subject Inclusion Criteria

- Patients must have BCG refractory TisN0M0. BCG-refractory disease is defined as:
  - Patient positive for Cis after 2 consecutive BCG installations
  - OR, patient had a BCG response and failure within 6 months
  - OR, Cis on maintenance BCG
- Pathologic confirmation of urothelial carcinoma by the Department of Pathology at MSKCC
- Karnofsky Performance Status (KPS) ≥70%
- Age ≥ 18 years
- Adequate bone marrow function as shown by:
  - ANC ≥ 1.5 x 10^9/L
  - Platelets ≥ 100 x 10^9/L
  - Hb >9 g/dL
- Adequate liver function as shown by:
  - serum bilirubin ≤ 1.5 x ULN (upper limit of normal)
  - ALT and AST ≤ 2.5x ULN
- International normalized ratio (INR) ≤1.5 x ULN. (Anticoagulation is allowed if target INR ≤ 1.5 x ULN on a stable dose of warfarin or on a stable dose of low molecular weight (LMW) heparin for >2 weeks)
- Adequate renal function: serum creatinine ≤ 1.5 x ULN

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- Fasting serum cholesterol ≤300 mg/dL OR ≤7.75 mmol/L AND fasting triglycerides ≤ 2.5 x ULN. NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid-lowering medication. After lipid-lowering therapy, patients must meet the same criteria, i.e. a fasting serum cholesterol ≤ 300 mg/dL OR ≤ 7.75 mmol/L AND fasting triglycerides ≤ 2.5 x ULN, to be eligible for study treatment.
- Pre-treatment tumor tissue (minimum 10 slides) or 1 paraffin-embedded block available for analysis of m-TOR pathway markers.
- Testing for hepatitis B viral load and serological markers (HBV-DNA, HBsAg, HBsAb, and HBcAb) for the following patients:
  - All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal, or Greece
  - Patients with any of the following risk factors:
  - Known or suspected past hepatitis B infection
  - Blood transfusion(s) prior to 1990
  - Current or prior IV drug users
  - Current or prior dialysis
  - Household contact with hepatitis B infected person(s)
  - Current or prior high-risk sexual activity
  - Body piercing or tattoos
  - Mother known to have hepatitis B
  - History suggestive of hepatitis B infection, e.g. dark urine, jaundice, or right upper quadrant pain
  - Additional patients at the discretion of the investigator
- Testing for hepatitis C infection (using quantitative RNA-PCR) for patients with any of the following risk factors:
  - Known or suspected past hepatitis C infection (including patients with past interferon “curative” treatment)
  - Blood transfusion(s) prior to 1990
  - Current or prior IV drug users
  - Household contact of hepatitis C infected person(s)
  - Current or prior high-risk sexual activity
  - Body piercing or tattoos
  - Additional patients at the discretion of the investigator

6.2 Subject Exclusion Criteria

- Patients currently receiving anticancer therapies or who have received anticancer therapies within 4 weeks of the start of study drug (including chemotherapy, radiation therapy, antibody based therapy, etc.)
- Patients, who have had a major surgery or significant traumatic injury within 4 weeks of start of study drug, patients who have not recovered from the side effects of any major surgery (defined as requiring general anesthesia) or patients that may require major surgery during the course of the study
- Prior treatment with any investigational drug within the preceding 4 weeks
- Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Topical or inhaled corticosteroids are allowed.
- Patients should not receive immunization with attenuated live vaccines within 1 week of study entry or during study period
- Other malignancies within the past 3 years, except for adequately treated carcinoma of the cervix, basal or squamous cell carcinomas of the skin, or adenocarcinoma of the prostate that has been surgically treated with a post-treatment PSA that is non-detectable.
- Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as:
    - symptomatic congestive heart failure of New York Heart Association Class III or IV
    - unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction within 6 months of start of study drug, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
- Severely impaired lung function as defined by spirometry and diffusing capacity of lung for carbon monoxide (DLCO) that is 50% of the normal predicted value and/or 0_2 saturation that is 88% or less at rest on room air
- Uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN
- Active (acute or chronic) or uncontrolled severe infections, including urinary tract infections
- Liver disease such as cirrhosis, chronic active hepatitis, or chronic persistent hepatitis
- A known history of HIV seropositivity
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of Everolimus (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small-bowel resection)
- Patients with an active, bleeding diathesis
- Female patients who are pregnant or breast feeding. Women of childbearing potential must have a negative serum pregnancy test within 7 days prior to administration of Everolimus.
- Adults of reproductive potential who are not using effective birth control methods. If barrier contraceptives are being used, these must be continued throughout the trial by both sexes. Hormonal contraceptives are not acceptable as a sole method of contraception. Patients who have received prior treatment with an mTOR inhibitor (sirolimus, temsirolimus, Everolimus)

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- Patients with a known hypersensitivity to Everolimus or other rapamycins (sirolimus, temsirolimus) or to its excipients
- History of noncompliance to medical regimens
- Patients unwilling to or unable to comply with the protocol
- Prior radiation to the pelvis

7.0RECRUITMENT PLAN

Patients will be recruited from the outpatient clinics of the Urology and Genitourinary Oncology Services at Memorial Sloan-Kettering Cancer Center (MSKCC). Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or the research team. If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study. The patient's initial conversation with the investigator/research staff and portions of the patient's MSKCC medical records will be used to confirm that the patient is eligible for study participation.

8.0PRETREATMENT EVALUATION

Within 7 days of starting treatment:

- **Pregnancy test**: Adequate contraception must be used while on study and for 8 weeks after last dose of study drug

Within 14 days of starting treatment:

- **Hematology**
  - Complete Blood Count including: hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential.
  - PT (INR) evaluation will be included for baseline evaluations.
- **Blood chemistry**
  - Comprehensive Metabolic Panel including: sodium, potassium, chloride, bicarbonate, calcium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase,
  - Uric acid,
  - Phosphorus,
  - Serum lipid profile (triglycerides, total cholesterol, HDL, and LDL).
  - **Because accurate serum glucose and lipid measurements are required, patients should be fasting at the time of the blood sampling.**

- **Urinalysis**
  - Standard urinalysis. This must be supplemented with laboratory quantification of any potentially relevant abnormalities.
- **Vital signs**
  - Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature, and weight. Blood pressure, pulse, and respiration rate should be measured on patients after at least 3 minutes in the sitting position.
- **History and Physical examination**
  - Physical examination will be performed, which must comprise a total body examination (general appearance, skin, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and basic nervous system).
  - Significant findings made after the start of study drug that meet the definition of an Adverse Event must be recorded.
- **Performance status**
  - Karnofsky Performance Status (KPS) ≥70%

Within 30 days of starting treatment:

- **Chest X-Ray**
- **Pulmonary function tests**
- **Cystoscopy**
- **Transurethral Resection**
- **ECG:**A standard 12-lead ECG is to be performed during screening and significant findings must be recorded.
- **HBV testing**
  - Hepatitis B testing (HBV-DNA, HBsAg, HBsAb, and HBcAb) for all patients who meet the hepatitis B risk criteria outlined in Section 6.1.
  - HBV DNA monitoring should be done depending on the results from serologic markers and viral load as listed in Table 9.3-3.
- **HCV testing**
  - Hepatitis C testing (Quantitative RNS-PCR) for all patients who meet the hepatitis C risk criteria outlined in Section 6.1.
  - Follow-up testing will be performed, as per the visit schedule, only if the patient has a history or is positive at baseline, or both.

9.0TREATMENT/INTERVENTION PLAN

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9.1 Phase I Schedule and Dose Administration

Table 9.1-1 Treatment Drug Dose and Schedule

Dose level	Everolimus Dose and schedule	Gemcitabine Dose and Schedule
1 (starting dose)	5 mg every other day	2000 mg twice weekly
2	5 mg daily	2000 mg twice weekly
3	10mg daily	2000 mg twice weekly

All chemotherapy will be administered in the outpatient chemotherapy unit.

9.1.1 Dose Escalation Scheme

3 dose levels of continuous Everolimus (Everolimus dose levels: *Dose level 1*: 5 mg every other day, *Dose level 2*: 5 mg daily, and *Dose level 3:* 10 mg daily) in combination with a fixed dose of intravesical gemcitabine at a dose of 2000 mg twice weekly for 3 weeks followed by a week of rest from gemcitabine (there is no rest week from Everolimus), and a second course of intravesical gemcitabine followed by a week of rest from gemcitabine will be evaluated using the DLT rules outlined below:

Number of subjects with DLT	Action
0 of 3 subjects	Escalate to next highest dose level and enroll up to 3 subjects
1 of 3 subjects	Enroll up to 3 more subjects at the same dose level
1 of 6 subjects	Escalate to next highest dose level and enroll up to 3 more subjects
2 or 3 subjects in the first 3 subjects	De-escalate to lower dose level and enroll up to 6 subjects
≥ 2 of 6 subjects	De-escalate to lower dose level and enroll up to 6 subjects

If 2 of the first 2 subjects have DLT, the enrollment of a third subject at the same dose level is not necessary. Similarly, if 2 of 4 subjects or 2 of 5 subjects have DLT, it is not necessary to enroll subsequent subjects at the same dose level.

There will be no planned dose level below dose level 1. If ≥ 2 DLTs occur at dose level 1, then the study will be terminated.

There will be no planned dose level beyond dose level 3. If no DLTs occur at dose level 3, the Phase II portion of the trial will be initiated.

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Patients will be assessed for DLTs up the completion of the 3rd cycle of treatment.

9.1.2 Maximum Tolerated Dose

The MTD is the dose level at which no more than 1 out of 6 patients experience DLT.
If ≥ 2 subjects at the first dose level experience DLT, then the study will be terminated and the combination of Everolimus at a dose of 5 mg every other day with intravesical gemcitabine at a dose of 2000 mg twice weekly will be considered unsafe in this population.

Patients will be observed for DLTs through one cycle of treatment (4 weeks) prior to dose escalation for the next dose level. No intrapatient dose escalation will occur. DLTs will be defined as follows, using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0:

1) Any evidence of grade 3 or 4 toxicity under Renal and Urinary Disorders in CTCAE version 4.0.
2) Febrile neutropenia (absolute neutrophil count (ANC)) < 1000/μL plus oral temperature to 8.5°C)
3) Grade 4 neutropenia (ANC <500/μL) for ≥ 7 days
4) Other grade 4 hematologic toxicity
5) Other nonhematologic grade 3 or 4 treatment-related toxicity, excluding nausea/vomiting, rash, untreated hyperlipidemia or grade 3 fatigue lasting ≤ 7 days.
6) Any nonhematologic toxicity requiring treatment delay for > 7 days during cycle 1.

9.2 Phase II Dose Administration

The dose level of Everolimus for the Phase II Study will be based on the MTD from Phase I part of the study, administered continuously, and intravesical gemcitabine will be administered at a dose of 2000 mg twice weekly for 3 weeks, followed by a week of rest and a second course of intravesical gemcitabine, followed by a week of rest (there is no rest week for Everolimus, only for gemcitabine). Everolimus will be continued for 12 months in the patients who achieve a CR after two courses of gemcitabine. Patients demonstrating a CR (by cystoscopy and cytology) will be observed with serial cystoscopies every 3 months.

All chemotherapy will be administered in the outpatient chemotherapy unit.**9.2.1 Interruption or Discontinuation of Treatment During Phase II**

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments of Everolimus, but not of gemcitabine, are permitted. If administration of Everolimus must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Section 9.1. Toxicity will be assessed using the NIH-NCI CTCAE, version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf).

If at any point a patient develops any grade 3 toxicity suspected to be related to gemcitabine, gemcitabine may be delayed for up to 14 days while Everolimus will continue to be taken continuously. If at any point a patient develops any grade 4 toxicity suspected to be related to

gemcitabine, the patient will be taken off study. If a dose delay of gemcitabine greater than 14 is

Nonhematological toxicity	

required, the patient will be taken off study.

Patients who are removed from study due to withdrawal of consent, lost to follow-up, or administrative problems should not be considered unevaluable and will be replaced.

9.3 Phase II Dose Modifications

There will be no dose modifications for gemcitabine, only for Everolimus.

Table 9.3-1 Phase II Everolimus Dose Modifications

Grade 2 (except pneumonitis – refer to Section 4.2)	If the toxicity is tolerable to the patient, maintain the same dose. If the toxicity is intolerable to patient, interrupt Everolimus until recovery to grade ≤1. Then reintroduce Everolimus at same dose. If event returns to grade 2, then interrupt Everolimus until recovery to grade ≤1. Then reintroduce Everolimus at the lower dose level.
Grade 3 (except hyperlipidemia*) (except pneumonitis – refer to Section 4.2)	Interrupt Everolimus until recovery to grade ≤1. Then reintroduce Everolimus at the lower dose level. For pneumonitis, consider the use of a short course of corticosteroids.
Grade 4	Discontinue Everolimus.
Hematological toxicity	
Grade 2 Thrombocytopenia (platelets <75, ≥50x10^9/L)	Interrupt Everolimus until recovery to grade ≤1 (>75 x10^9/L). Then reintroduce Everolimus at initial dose. If thrombocytopenia returns to grade 2, interrupt Everolimus until recovery to grade ≤1. Then reintroduce Everolimus at the lower dose level.
Grade 3 Thrombocytopenia (platelets <50, ≥ 25 x10^9/L)	Interrupt Everolimus until recovery to grade ≤1 (platelets ≥ 75 x10^9/L). Then resume Everolimus at one dose level lower. If grade 3 thrombocytopenia recurs, discontinue Everolimus
Grade 4 Thrombocytopenia (platelets <25 x10^9/L)	Discontinue Everolimus.
Grade 3 Neutropenia (neutrophils <1, ≥0.5 x10^9/L)	Interrupt Everolimus until recovery to grade ≤1 (neutrophils ≥1.5 x 10^9/L). Then resume Everolimus at the initial dose. If ANC returns to grade 3, hold Everolimus until the ANC ≥1.5 x 10^9/L. Then resume Everolimus dosing at the lower dose level. Discontinue patient from study therapy for a third episode of grade 3 neutropenia.
Grade 4 Neutropenia (neutrophils <0.5 x10^9/L)	Interrupt Everolimus until recovery to grade ≤1 (neutrophils ≥1.5 x 10^9/L). Then resume Everolimus at the lower dose level. If grade 3 or grade 4 neutropenia occurs despite this dose reduction, discontinue Everolimus.
Grade 3 febrile neutropenia (not life-threatening)	Interrupt Everolimus until resolution of fever and neutropenia to grade ≤1. Hold further Everolimus until the ANC ≥1,500/mm^3 and fever has resolved. Then resume Everolimus at the lower dose level. If febrile neutropenia recurs, discontinue Everolimus.
Grade 4 febrile neutropenia (life-threatening)	Discontinue Everolimus.
Any hematological or nonhematological toxicity requiring interruption for ≥ 3 weeks	Discontinue Everolimus

*Grade 3 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies

All interruptions or changes to study drug administration must be recorded.

9.3.1 Monitoring of Everolimus Suspected Toxicities

Amended: 1/11/11

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to Everolimus must be followed at least weekly until the adverse event or abnormal laboratory resolves or returns to grade 1. If a patient requires a dose delay of >21 days from the intended day of the next scheduled dose, then the patient must be discontinued from the study.

9.3.2 Management of Stomatitis/Oral Mucositis/Mouth Ulcers

Stomatitis/oral mucositis/mouth ulcers due to Everolimus should be treated using local supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with Everolimus as mouth ulcers, rather than mucositis or stomatitis. If your examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. Please follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers:

1. For mild toxicity (grade 1), use conservative measures such as **non-alcoholic mouth wash or salt water (0.9%) mouth wash** several times a day until resolution.
2. For more severe toxicity, (such as grade 2, in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3, in which case patients cannot maintain adequate oral alimentation), the suggested treatments are **topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol)** with or without **topical corticosteroids,** such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).
3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.
4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of Everolimus metabolism, thereby leading to higher Everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

Note: Stomatitis/oral mucositis should be appropriately graded using the functional grading given on the NCI-CTCAE, version 4.0.

9.3.3 Management of Hyperlipidemia and Hyperglycemia

Treatment of hyperlipidemia should take into account the pretreatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Grade 2 hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or grade 2 hypertriglyceridemia (>2.5 x ULN) should be treated with a 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitor (e.g., atorvastatin, pravastatin) or appropriate lipid-lowering medication, in addition to diet. Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase inhibitors.

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Note: Concomitant therapy with fibrates and an HMG-CoA reductase inhibitor is associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatine kinase (CPK) levels and myoglobinuria, acute renal failure, and, sometimes, death. The risk versus benefit of using this therapy should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia.

Grade 3 hyperglycemia has been observed in patients receiving Everolimus therapy. In many cases in study Everolimus C2222, the affected patients had an abnormal fasting glucose at baseline. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Based on this finding, it is suggested that optimal glucose control should be achieved before starting a patient on Everolimus and should be monitored during Everolimus therapy.

9.3.4 Management of non-infectious pneumonitis

Both asymptomatic radiological changes (grade 1) and symptomatic noninfectious pneumonitis (grade 2 = not interfering with activities of daily living, or grade 3 = interfering with activities of daily living and oxygen indicated) have been noted in patients receiving Everolimus therapy. Noninfectious pneumonitis has been associated with Everolimus and other mTOR inhibitors.(40) In order to monitor for asymptomatic (grade 1) pulmonary infiltrates, a chest X-ray is required if a CT scan of chest is not used for bimonthly disease evaluations. Additional chest CT scans may be performed, when clinically necessary. If noninfectious pneumonitis develops, a consultation with a pulmonologist should be considered. If the patient develops grade 3 pneumonitis, treatment with Everolimus should be interrupted and the patient should be treated as medically indicated (short course corticosteroids, oxygen, etc.).

Management of noninfectious pneumonitis suspected to be associated with Everolimus and dose modifications instructions are provided below.

Table 9.3-2 Management of Non-Infectious Pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	Everolimus Dose Adjustment
Grade 1	CT scans with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every 2 cycles until return to baseline.	No specific therapy is required	Administer 100% of Everolimus dose.
Grade 2	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent cycle until return to baseline. Consider bronchoscopy *	Symptomatic only. Prescribe corticosteroids if cough is troublesome.	Reduce Everolimus dose until recovery to ≤ grade 1. Everolimus may also be interrupted if symptoms are troublesome. Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 within 3 weeks.
Grade 3	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent cycle until return to baseline. Bronchoscopy is recommended *	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Hold Everolimus until recovery to ≤ grade 1. May restart protocol treatment within 2 weeks at a reduced dose (by one level) if evidence of clinical benefit. Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 within 2 weeks.
Grade 4	CT scan with lung windows and required pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent cycle until return to baseline. Bronchoscopy is recommended *.	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Discontinue Everolimus.

*A bronchoscopy with biopsy and/or bronchoalveolar lavage is recommended.

Management of hepatitis reactivation

9.3.5 Management of Hepatitis B

Table 9.3-3 provides details of monitoring and prophylactic therapy according to the baseline results of viral load and serologic markers testing.

Table 9.3-3 Action to be taken for Positive Baseline Hepatitis B Results

Test	Result	Result	Result	Result	Result
HBV-DNA	+	+ or -	-	-	-
HBsAg	+ or -	+	-	-	-
HBs Ab	+ or -	+ or -	**+** and no prior HBV vaccination	+ or -	- or + with prior HBV vaccination
HBc Ab	+ or -	+ or -	+ or -	**+**	-
Recommendation	Prophylaxis treatment should be started 1-2 weeks prior to		No prophylaxis		No specific action

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Test	Result	Result	Result	Result	Result
	first dose of study drug Monitor HBV-DNA approximately every 4-8 weeks		Monitor HBV-DNA approximately every 3-4 weeks		

In cancer patients with hepatitis B, whether carriers or in chronic state, use of antivirals during anticancer therapy has been shown to reduce the risk of hepatitis B virus (HBV) reactivation and associated HBV morbidity and mortality.(41)

Antiviral prophylaxis therapy should continue for at least 4 weeks after last dose of study drug.

For hepatitis B reactivation, definition, and management guidelines, see Table 9.3-4 Guidelines for management of hepatitis B.

Table 9.3-4 Guidelines for Management of Hepatitis B

HBV reactivation (with or without clinical signs and symptoms)*	
For patients with baseline results: Positive HBV-DNA OR positive HBsAg --- reactivation is defined as: [Increase of 1 log in HBV-DNA relative to baseline HBV-DNA value OR new appearance of measurable HBV-DNA] AND ALT elevation x 5 ULN	Treat: Start a second antiviral AND Interrupt study drug administration until resolution: • ≤ grade 1 ALT (or baseline ALT, if > grade 1) and • ≤ baseline HBV-DNA levels If resolution occurs within ≤ 28 days study drug should be re-started at one dose lower, if available. (see Table 9.1-1 – Treatment Drug Dose and Schedule) If the patient is already receiving the lowest dose of study drug according to the protocol, the patient should restart at the same dose after resolution. Both antiviral therapies should continue at least 4 weeks after last dose of study drug. If resolution occurs > 28 days Patients should discontinue study drug but continue both antiviral therapies at least 4 weeks after last dose of study drug.
For patients with baseline results: Negative HBV-DNA and HBsAg AND [Positive HBs Ab (with no prior history of vaccination against HBV), OR positive HBc Ab] --- reactivation is defined as: New appearance of measurable HBV-DNA	Treat : Start first antiviral medication AND Interrupt study drug administration until resolution: • ≤ baseline HBV-DNA levels If resolution occurs within ≤ 28 days study drug should be re-started at one dose lower, if available. (see Table 9.1-1 – Treatment Drug Dose and Schedule) If the patient is already receiving the lowest dose of study drug according to the protocol, the patient should restart at the same dose after resolution. Antiviral therapy should continue at least 4 weeks after last dose of study drug. If resolution occurs > 28 days Patients should discontinue study drug but continue antiviral therapy at least 4 weeks after last dose of study drug.

* All reactivations of hepatitis B are to be recorded as grade 3 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation), unless considered life threatening by the investigator; in which case they should be recorded as grade 4 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation). Date of viral reactivation is the date on which both DNA and ALT criteria were met (e.g. for a patient who was HBV-DNA positive on 01-JAN-10 and whose ALT reached ≥ 5 × ULN on 01-APR-10, the date of viral reactivation is 01-APR-10).

9.3.6 Monitoring for Hepatitis C

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The following two categories of patients should be monitored every 4–8 weeks for HCV reactivation:

- Patients with detectable HCV RNA-PCR test at baseline.
- Patients known to have a history of HCV infection, despite a negative viral load test at baseline (including those that were treated and are considered 'cured')

For definition of hepatitis C reactivation and the management guidelines, see Table 9.3-5. Guidelines for management of hepatitis C.

Table 9.3-5 Guidelines for management of hepatitis C

HCV reactivation*	
For patients with baseline results: Detectable HCV-RNA, reactivation is defined as: ALT elevation x 5 ULN	Discontinue study drug
For patients with baseline results: Knowledge of past hepatitis C infection with no detectable HCV-RNA, reactivation is defined as: New appearance of detectable HCV-RNA	Discontinue study drug

* All reactivations of hepatitis C are to be recorded as grade 3 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation), unless considered life threatening by the investigator, in which case they should be recorded as grade 4 (CTCAE v 3.0 Metabolic Laboratory/Other: Viral Re-activation).

9.4 Concomitant therapy

Patients will be instructed not to take any additional medications (including over-the-counter products) during the course of the study without prior consultation with the investigator. At each visit, the investigator will ask the patient about any new medications he/she is or has taken after the start of the study drug.

All Concomitant medications/Significant non-drug therapies taken ≤30 days prior to start and after start of study drug, including physical therapy and blood transfusions, should be recorded.

The following restrictions apply during the entire duration of the study:

- No other investigational therapy should be given to patients.
- No anticancer agents other than the study medication should be given to patients. If such agents are required for a patient, then the patient must first be withdrawn from the study.
- Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-gp should be avoided
- Seville orange, star fruit, grapefruit, and their juices affect P450 and P-gp activity. Concomitant use should be avoided
- Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or P-gp inhibitors should be used with caution. If patient requires co-administration of moderate CYP3A4

inhibitors or P-gp inhibitors, reduce the dose of Everolimus to half the currently used dose. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued, the Everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/P-gp inhibitor.

- Avoid the use of strong CYP3A4 inducers.
- No chronic treatment with systemic steroids or other immunosuppressive agents (at a dose equivalent or greater than 20 mg prednisone per day). Topical or inhaled corticosteroids are allowed.
- Everolimus may affect the response to vaccinations, making the response to the vaccination less effective. Live vaccines should be avoided while a patient is treated with Everolimus.
- Oral anticoagulants such as warfarin are CYP2C9 substrates and, as such, no interaction with Everolimus is expected. However, drug-drug interaction studies between macrolide antibiotics and warfarin have produced mixed outcomes and the disparity in these findings has led to the conclusion that multiple factors may alter the clearance of warfarin. The co-administration of Everolimus and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week's treatment).

A comprehensive list of cytochrome P450 isoenzymes and CYP3A4 inhibitors, inducers, and substrates can be found at http://medicine.iupui.edu/flockhart. This website is continually revised and should be checked frequently for updates.

Table 9.4-1 Clinically Relevant Drug Interaction: Substrates, Inducers, and Inhibitors of Isoenzyme CYP3A4.

<table>
<tr><td colspan="2">SUBSTRATES</td></tr>
<tr><td>Antibiotics:
Clarithromycin, erythromycin, telithromycin</td><td>Calcium channel blockers:
Amlodipine, diltiazem, felodipine, lercanidipine, nifedipine, nisoldipine, nitrendipine, verapamil</td></tr>
<tr><td>Anti-arrhythmics:
Quinidine</td><td>HMG CoA reductase inhibitors:
Cerivastatin, lovastatin, simvastatin</td></tr>
<tr><td>Benzodiazepines:
Alprazolam, diazepam, midazolam, triazolam</td><td>Steroid 6beta-OH:
estradiol, hydrocortisone, progesterone, testosterone</td></tr>
<tr><td>Immune modulators:
Cyclosporine, tacrolimus (FK506)</td><td rowspan="4">Miscellaneous:
Alfentanil, aprepitant, aripirazole, buspirone, cafergot, caffeine, cilostazol, cocaine, codeine-N-demethylation, dapsone, dexamethasone, dextromethorphan, docetaxel domperidone, eplerenone, fentanyl, finasteride, Gleevec/imatinib, haloperidol, irinotecan, LAAM, lidocaine, methadone, nateglinide, ondansetron, pimozide, propranolol, quetiapine, quinine, risperidone, salmeterol, sildenafil, sirolimus, sorafenib, sunitinib, tamoxifen, taxol, terfenadine, torisel, trazodone, vincristine, zaleplon, ziprasidone, zolpidem</td></tr>
<tr><td>HIV Antivirals:
Indinavir, nelfinavir, ritonavir, saquinavir</td></tr>
<tr><td>Prokinetic:
Cisapride</td></tr>
<tr><td>Antihistamines:
Astemizole, chlorpheniramine, terfenadine</td></tr>
<tr><td colspan="2">INDUCERS</td></tr>
<tr><td colspan="2">Barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John’s wort, troglitazone, efavirenz, nevirapine</td></tr>
<tr><td colspan="2">INHIBITORS</td></tr>
<tr><td colspan="2">Strong inhibitors:
indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin,
Posaconazole (Krishna et al, 2009)

Moderate inhibitors:
aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil,

Weak inhibitors:
Cimetidine,
Seville orange (Malhotra et al, 2001)</td></tr>
</table>

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Unclassified as per the Indiana University DDI listing:

Ciprofloxacin, delaviridine, troleandamycin, mibefradil, amiodarone, chloramphenicol, diethyldithiocarbamate, fluvoxamine, starfruit, gestodene, imatinib, mifepristone, norfloxacin, norfluoxetine, voriconazole*

Based on http://medicine.iupui.edu/clinpharm/ddis/table.asp as of December 01, 2009

*Voriconazole (unclassified as per the Indiana University DDI table) Strong inhibitor according to the following reference: (http://www.nature.com/clpt/journal/v80/n5/pdf/clpt2006438a.pdf)

Table 9.4-2 Clinically Relevant Drug Interactions Mediated by P-gP

PgP Substrates	PgP Inhibitors in vivo	PgP Inducers
digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel	amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, elacridar, erythromycin, felodipine, (GF120918), itraconazole, ketocoanzole, lopinavir, (LY335979), mibefradil, nifedipine, nitrendipine, (PSC833), quinidine, ranolazine, ritonavir, talinolol, valspodar, verapamil	rifampin, St John's wort

Reference:

Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Dec. 2, 2009, which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies, the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table."

NOTES:http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072101.pdf

This list of clinically relevant drug interactions is updated as of December 02, 2009

10.0EVALUATION DURING TREATMENT/INTERVENTION

Amended: 1/11/11

Phase I Visit Schedule																							*continued on next page*
	Screening	Pre-Treatment Evaluations			Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				28-Day Cycles (±7 days)										
		Within 30 Days	Within 14 Days	Within 7 Days	1	2	3	4[1]	5	6	7	8[1]	3	4	5	6	7	8	9	10	11	12	
Informed Consent	X																						
ECG	X																						
Pulmonary Function Tests		X																					
Urine Culture		X																					
Urine Cytology		X											X			X			X			X	
Cystoscopy		X											X			X			X			X	
Transurethral Resection (TUR)[4]		X																					
Chest X-Ray		X											X			X			X			X	
HBV-DNA, HBsAg, HBs Ab, HBs Ab, HCV-RNA-PCR[2]		X																					
HBV DNA, HCV RNA-PCR[3]		X			X			X					X										
Karnofsky Performance Status			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urinalysis			X																				
Complete History			X																				
Physical Exam			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

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Phase I Visit Schedule (Cont.)																						
	Screening	Pre-Treatment Evaluations			Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				28-Day Cycles (±7 days)									
		Within 30 Days	Within 14 Days	Within 7 Days	1	2	3	4[1]	5	6	7	8[1]	3	4	5	6	7	8	9	10	11	12
Vital Signs			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complete Blood Count (hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential)[5]			X				X				X		X	X	X	X	X	X	X	X	X	X
Comprehensive Panel (sodium, potassium, chloride, bicarbonate, calcium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase)[5]			X				X				X		X	X	X	X	X	X	X	X	X	X
Uric Acid			X				X				X		X	X	X	X	X	X	X	X	X	X
Phosphorus			X				X				X		X	X	X	X	X	X	X	X	X	X
Serum Lipid Profile (triglycerides, total cholesterol, HDL, and LDL)			X				X				X		X	X	X	X	X	X	X	X	X	X
Serum Pregnancy Test				X																		
Collection of Tissue Specimen				X																		
Toxicity Assessment						X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gemcitabine					X	X	X		X	X	X											
Everolimus					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Amended: 1/11/11

[1]1 week of rest from gemcitabine. There is no week of rest from Everolimus, which is taken continuously.

[2]All patients should be screened for hepatitis risk factors and any past illnesses of hepatitis B and hepatitis C. It is highly recommended that patients positive HBV-DNA or HBsAg are treated prophylactically with an antiviral for 1-2 weeks prior to receiving study drug (see Section 3.2.7). The antiviral treatment should continue throughout the entire study period and for at least 4 weeks after the last dose of study drug. Patients with viral hepatitis C risk factors should be screened for HCV RNA-PCR.

[3] Patients on antiviral prophylaxis treatment or positive HBV antibodies should be tested for HBV-DNA according to study visit schedule. Patients with positive HCV-RNA PCR or a history of past infection, even if treated and considered 'cured' – should be followed by HCV-RNA PCR according to visit schedule.

[4]During follow-up, if a suspicious or positive cystoscopy/cytology is found, a TUR will be performed no later than 4 weeks.

[5]All blood work will be done monthly.

Phase II Visit Schedule *continued on next page*

	Screening	Pre-Treatment Evaluations			Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				28-Day Cycles (±7 days)												
		Within 30 Days	Within 14 Days	Within 7 Days	1	2	3	4[1]	5	6	7	8[1]	3	4	5	6	7	8	9	10	11	12	15	18	21	24
Informed Consent	X																								
ECG	X																								
Pulmonary Function Tests		X																							
Urine Culture		X																							
Urine Cytology		X											X			X			X			X	X	X	X	X
Cystoscopy		X											X			X			X			X	X	X	X	X
Transurethral Resection (TUR)[4]		X																							
Chest X-Ray		X											X			X			X			X	X	X	X	X
HBV-DNA, HBsAg, HBs Ab, HBs Ab, HCV-RNA-PCR[2]		X																							
HBV DNA, HCV RNA-PCR[3]		X			X			X					X												
Karnofsky Performance Status			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis			X																						
Complete History			X																						
Physical Exam			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Phase II Visit Schedule (Cont.)

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	Screening	Pre-Treatment Evaluations			Cycle 1 (Weeks ±2 days)				Cycle 2 (Weeks ±2 days)				28-Day Cycles (±7 days)												
		Within 30 Days	Within 14 Days	Within 7 Days	1	2	3	4[1]	5	6	7	8[1]	3	4	5	6	7	8	9	10	11	12	15	18	21	24
Vital Signs			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complete Blood Count (hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential)[5]			X				X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Panel (sodium, potassium, chloride, bicarbonate, calcium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase)[5]			X				X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Uric Acid			X				X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phosphorus			X				X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Lipid Profile (triglycerides, total cholesterol, HDL, and LDL)			X				X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum Pregnancy Test				X																						
Collection of Tissue Specimen				X																						
Toxicity Assessment						X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gemcitabine					X	X	X		X	X	X															
Everolimus					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				

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[1]1 week of rest from gemcitabine. There is no week of rest from Everolimus, which is taken continuously.

[2]All patients should be screened for hepatitis risk factors and any past illnesses of hepatitis B and hepatitis C. It is highly recommended that patients positive HBV-DNA or HBsAg are treated prophylactically with an antiviral for 1-2 weeks prior to receiving study drug (see Section 3.2.7). The antiviral treatment should continue throughout the entire study period and for at least 4 weeks after the last dose of study drug. Patients with viral hepatitis C risk factors should be screened for HCV RNA-PCR.

[3] Patients on antiviral prophylaxis treatment or positive HBV antibodies should be tested for HBV-DNA according to study visit schedule. Patients with positive HCV-RNA PCR or a history of past infection, even if treated and considered 'cured' – should be followed by HCV-RNA PCR according to visit schedule.

[4]During follow-up, if a suspicious or positive cystoscopy/cytology is found, a TUR will be performed no later than 4 weeks.

[5]All blood work will be done monthly the first 12 months, then every three months for one year.

11.0TOXICITIES/SIDE EFFECTS

Known Undesirable Side Effects of Everolimus

Overall, the most frequent adverse effects suspected to be related to Everolimus have been stomatitis, rash, anemia, fatigue, asthenia, diarrhea, anorexia, nausea, hypercholesterolemia, mucosal inflammation, vomiting, hypertriglyceridemia, cough, peripheral edema, dry skin, epistaxis, pruritus, and dyspnea. The most common grade 3 or 4 adverse reactions suspected to be related to treatment were anemia, infections, hyperglycemia, stomatitis, fatigue, lymphopenia, hypercholesterolemia, pneumonitis, and elevated gamma-glutamyltransferase concentrations.

The principal DLT in Phase 1 trials has been grade 3 stomatitis.

Hyperlipidemia was reported as a serious adverse reaction. It is a recognized side effect of rapamycins. Use of lipid-lowering drugs should be associated with dietary recommendations. Monitoring of blood lipid levels requires patients to be fasting so that this aspect must be verified when interpreting results.

Hyperglycemia was reported as a serious adverse reaction. Similarly, the fasting state of patients should be verified when interpreting results.

Pneumonitis is a recognized adverse effect of rapamycins (sirolimus, temsirolimus, and everolimus). Numerous case reports in the literature suggest that rapamycin-associated pneumonitis is relatively unaggressive, limited in extent, and reversible upon drug discontinuation. The term 'pneumonitis' is used here to describe noninfectious, nonmalignant infiltration in the lungs, which is evident radiologically. More precise diagnosis should follow histocytological examination following lung biopsy, generally during bronchoscopy that may or may not be symptomatic.

In oncology studies with Everolimus, severe pneumonitis suspected as drug-related has been reported as a serious adverse event on 13 occasions and additionally in the following associated preferred terms, including acute respiratory distress syndrome (n=2), alveolitis (n=1) and allergic alveolitis (n=1), interstitial lung disease (n=10), lung infiltration (n=23), cryptogenic organizing pneumonia, lung consolidation, pulmonary alveolar hemorrhage, pulmonary toxicity, and pulmonary
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fibrosis (n=1, each). One fatal case of drug-related pneumonitis was reported for a patient with metastatic infiltrating ductal carcinoma of the breast treated with 10 mg/day, which developed approximately 2 months after starting Everolimus. Cytologies for both the pleural and pericardial fluids were positive for malignancy. The death was considered possibly related to the underlying late stage tumor and study drug. Additionally, 1 patient treated with 10 mg/day died due to severe acute respiratory distress syndrome and septic shock. Thoracic computed tomography (CT) scan demonstrated condensation in the majority of the left lower lobe and frosted glass appearance in the left upper lobe, lingula, and right lung.

Along with the cases of noninfectious pneumonitis, serious opportunistic infections have also been reported in cancer patients treated with Everolimus: mycobacterium, aspergillus, and fatal candidal sepsis, and fatal pneumocystis carinii, in particular. Because Everolimus, as other rapamycins, inhibits proliferation of activated lymphocytes and reduces neutrophil counts, treatment with Everolimus must be considered as predisposing patients to the risk of infection. This risk will be higher in patients severely immunocompromised because of their underlying disease and/or co-medications. Outcome may be fatal in case of serious infections.

A reduction in blood cell counts is frequent when Everolimus therapy is initiated. Without clinical significance and infrequently, anemia and thrombocytopenia have been reported. In heavily pretreated patients with aggressive lymphoma, the incidence of grade 3 anemia, neutropenia, and thrombocytopenia was reported to be 11%, 16%, and 30%, respectively. Serious, suspected drug-related hemorrhages have been exceptional. Nevertheless, Everolimus should be considered as predisposing patients to hemorrhage, potentially fatal, should they develop severe drug-related thrombocytopenia.

Discrete, reversible changes in liver enzymes have been found to occur in numerous patients during treatment with Everolimus in oncology clinical studies and in a study in rheumatoid arthritis. In oncology studies, these changes may be evident only in patients without severe underlying morbidity. The increase in aspartate transaminase (AST) and alanine transaminase (ALT) generally appears after 4 weeks of treatment. In all but a few cases it does not exceed grade 1 (≤2.5 x ULN). Similarly, mild increases in alkaline phosphatases can coexist. Spontaneous corrections or intermittent correction with continued treatment can occur. Serum bilirubin is not increased. In studies of patients with advanced cancers, clinically relevant changes in liver enzymes have been invariably associated with the presence of liver metastases and/or progression of the underlying cancer.

Renal failure has been reported in 5 suspected cases to date. One patient with no alternative explanation made a complete recovery following study drug adjustment and no treatment/therapy for the event. The rest of the patients had concurrent morbidities, which might have contributed to the reported events.

Hypophosphatemia, hypomagnesemia, hyponatremia, and hypocalcemia have been reported as serious adverse reactions. Electrolytes should be monitored in patients treated with Everolimus.

More detailed information regarding Everolimus reported suspected toxicities and individual cases is provided in the [Investigator's Brochure].

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12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Complete response (CR) is defined as no evidence of disease (by cytology and cystoscopy).

Progression is defined as the development of invasion or metastasis. If the patient develops a recurrence, they will be considered having failed treatment. Patients will also be considered having failed treatment if there is no response to treatment after two cycles.

Patients will receive cystoscopy and cytology every 3 months. If a suspicious or positive cystoscopy or cytology is found, then a TUR will be performed no later than 4 weeks from the suspicious/positive test to determine presence or absence of disease. For purposes of the primary endpoint, patients demonstrating a CR at 11 months will undergo cystoscopy and cytology (and if suspicious, then a TUR) between 11 and 13 months.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient develops progressive disease he/she will be taken off study and referred for alternative therapy.

If at any time the patient develops unacceptable toxicity he/she will be removed from study.

If at any point a patient develops any grade 4 toxicity suspected to be related to gemcitabine, the patient will be taken off study.

If a patient requires a dose delay from Everolimus of >21 days from the intended day of the next scheduled dose, then the patient must be discontinued from the study.

If the patient requires a dose delay from gemcitabine of >14 days, the patient will be taken off study.

If at anytime the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

Patients who are removed from study due to withdrawal of consent, lost to follow-up, or administrative problems should not be considered unevaluable and will be replaced.

14.0 BIOSTATISTICS

Basis for sample size

Phase I: Patients will be accrued in the standard 3+3 fashion such that the MTD is the dose level at which no more than 1 out of 6 patients experience DLT. This portion will accrue between 2 and 18 patients.

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Phase II: The purpose of this phase of the study is to assess the efficacy of gemcitabine plus Everolimus in patients with BCG-refractory disease. The primary endpoint will be the proportion of patients who are disease-free at 1 year following start of therapy.

Historically, only about 10% of patients in this setting will be disease-free 1 year following therapy, therefore a 1-year disease-free rate of 10% would be considered not promising, while a 30% rate would be considered promising. We have chosen a single-stage design and set the type I (falsely accepting a non-promising therapy) and type II error (falsely rejecting a promising therapy) rates at 0.05 and 0.10, respectively. This portion of the study will enroll 33 patients (6 can be carried over from the MTD cohort of the phase I portion). If there are 7 or more who are disease-free at 1 year, the treatment will be declared effective and worthy of further testing.

In order to confirm the safety of the regimen, patients will continuously be monitored for excessive toxicity and early stopping rules for toxicity will be employed. Unacceptable toxicity is defined as any grade 3 or higher local toxicity including hematuria, urgency, and frequency. Stopping rules and the corresponding power calculations are provided in the table below.

Table 14-1

Number of toxicities needed to stop the study	True probability of toxicity in the populations	Probability of study completion (based on projection)
1 in the first 7 patients	0.05	0.91
2 in the first 16 patients	0.11	0.54
3 in the first 28 patients	0.20	0.11
4 in all 33 patients		

Secondary endpoints

a) To determine the CR rate (by cytology and cystoscopy) in patients receiving Everolimus in combination with intravesical gemcitabine. We will calculate the proportion of patients who demonstrate CR at any time within the year following start of therapy, with a 95% confidence interval. With 33 patients, we will be able to estimate this proportion within +/- 18%.

b) To determine the survival of patients treated with Everolimus in combination with intravesical gemcitabine. Overall survival following start of therapy will be estimated using Kaplan-Meier methods.

c) To evaluate for activated mTOR pathway markers as well as PTEN status and AKT activation in all pretreatment and analysis of posttreatment specimens when available. These analyses will be descriptive in nature and will be used to generate hypotheses for future studies. mTOR and PTEN status will be summarized descriptively, overall and separately for those with and without a CR.

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Patients removed from protocol therapy for reasons other than disease progression, death, or toxicity prior to one year will not be evaluable for the primary end-point and will be replaced. However, these patients may still be evaluable for the secondary end-points.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain written informed consent, by following procedures defined in section entitled Informed Consent Procedures.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the informed consent form, the completed signature page of the Research Authorization and a completed Eligibility Checklist must be faxed to PPR.

During the registration process registering individuals will be required to answer specific eligibility questions and provide the following information:

Registering Individual [Last, First Name]
Research Authorization [Date]
MSKCC IRB Protocol#
Attending of Record (if applicable) [Last, First Name]
Consenting Professional [Last, First Name]
Informed Consent Date
Participant's Full Name [Last, First Name]
Participant MRN

15.2 Randomization

Not applicable

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

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The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Monthly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits will be conducted by the study team at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials," which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees, *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A

Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. The report will be forwarded to the FDA by the Institutional SAE Manager through the IND Office.

17.2.1 Serious Adverse Event (SAE) Reporting to Novartis

The Principal Investigator has the obligation to report all serious adverse events to the IRB, and Novartis Pharmaceuticals Clinical Safety and Epidemiology Department (CS&E).

All events must be reported, by Fax (888-299-4565) to Novartis Pharmaceuticals CS&E Department within 24 hours of learning of its occurrence. This includes serious, related, labeled

(expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Novartis study drug (or therapy) is suspected.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

Any pregnancy that occurs during study participation should be reported. To ensure patient safety, each pregnancy must also be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and newborn complications.

18.0INFORMED CONSENT PROCEDURES

Patients will be required to sign a statement of informed consent which meets the requirements of code of Federal Regulations (Federal Register Vol. 46, No. 17, Jan. 27, 1981, part 50) and the IRB of this center.

Consent Process: The consenting professional will review the rationale for the treatment program with the patient. The discussion will review the alternatives available, including supportive care as appropriate, the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. The provisions available to ensure medical intervention should an adverse event occur will also be reviewed. Whether the risks are reasonable in relation to the anticipated benefits, incentives, costs that will or may be incurred as a result of participation in the study, and efforts to maintain the confidentiality of research participants will also be discussed.

At the time of registration, the eligibility checklist will be reviewed, verified, and signed.
MSKCC Physicians Eligible to Register Patients: Final informed consent may be obtained by the consenting professionals listed on the protocol face sheet.

Documentation of Informed Consent: The informed consent will be signed by the patient and consenting professional in triplicate. A signed original copy of the consent form will be given to the
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patient. The second original will be placed in the medical record, and the third original will be kept in the research file.

18.1 Research Authorization

Procedures for obtaining Research Authorization: Before any protocol-specific procedures are carried out, investigators and/or designated staff will fully explain the details of the protocol, study procedures, and the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must sign the Research Authorization component of the informed consent form. The Research Authorization requires a separate set of signatures from the patient. The original signed documents will become part of the patient's medical record, and each patient will receive a copy of the signed documents.

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20.0 APPENDICES

Appendix 1: Everolimus (RAD001) Continuous Cycle Schedule Treatment Log (5mg every other day)

Patient Name:_____	Patient MRN:_____

Protocol 10-165: Phase I/II Study of Everolimus and Intravesical Gemcitabine in BCG-Refractory Primary or Secondary Carcinoma In Situ of the Bladder

EVEROLIMUS (RAD001) **CONTINUOUS CYCLE** SCHEDULE TREATMENT LOG

Place a check in the box next to the date you take this medication. Please bring this sheet to your study doctor's office on each visit					**Cycle: _____**
Date	**Day**	**Time**	**Treatment**	**Dose**	**Questions for Study Doctor/Nurse**
	1		**Everolimus ☐**	**5 mg**	
	2				
	3		**Everolimus ☐**		
	4				
	5		**Everolimus ☐**		
	6				
	7		**Everolimus ☐**		
	8				
	9		**Everolimus ☐**		
	10				
	11		**Everolimus ☐**		
	12				
	13		**Everolimus ☐**		
	14				
	15		**Everolimus ☐**		
	16				
	17		**Everolimus ☐**		
	18				
	19		**Everolimus ☐**		
	20				

Amended: 1/11/11

	21		**Everolimus ☐**		
	22				
	23		**Everolimus ☐**		
	24				
	25		**Everolimus ☐**		
	26				
	27		**Everolimus ☐**		
	28				
MD/RN Signature:_____		Date:_____		Tablets Remaining:_____	
Comments:_____					

Appendix 2: Everolimus (RAD001) Continuous Cycle Schedule Treatment Log (5 mg every day)

Patient Name_____			Patient MRN:_____		
Protocol 10-165: Phase I/II Study of Everolimus and Intravesical Gemcitabine in BCG-Refractory Primary or Secondary Carcinoma In Situ of the Bladder					
EVEROLIMUS (RAD001) **CONTINUOUS CYCLE** SCHEDULE TREATMENT LOG					
Place a check in the box next to the date you take this medication. Please bring this sheet to your study doctor's office on each visit					**Cycle: _____**
Date	**Day**	**Time**	**Treatment**	**Dose**	**Questions for Study Doctor/Nurse**
	1		**Everolimus ☐**	**5 mg**	
	2		**Everolimus ☐**		
	3		**Everolimus ☐**		
	4		**Everolimus ☐**		
	5		**Everolimus ☐**		
	6		**Everolimus ☐**		
	7		**Everolimus ☐**		

Amended: 1/11/11

	8		Everolimus ☐		
	9		Everolimus ☐		
	10		Everolimus ☐		
	11		Everolimus ☐		
	12		Everolimus ☐		
	13		Everolimus ☐		
	14		Everolimus ☐		
	15		Everolimus ☐		
	16		Everolimus ☐		
	17		Everolimus ☐		
	18		Everolimus ☐		
	19		Everolimus ☐		
	20		Everolimus ☐		
	21		Everolimus ☐		
	22		Everolimus ☐		
	23		Everolimus ☐		
	24		Everolimus ☐		
	25		Everolimus ☐		
	26		Everolimus ☐		
	27		Everolimus ☐		
	28		Everolimus ☐		
MD/RN Signature:_____			Date_____		Tablets Remaining_____
Comments:_____					

Appendix 3: Everolimus (RAD001) Continuous Cycle Schedule Treatment Log (10 mg every day)

Amended: 1/11/11

Patient Name:_____	Patient MRN:_____

Protocol 10-165: Phase I/II Study of Everolimus and Intravesical Gemcitabine in BCG-Refractory Primary or Secondary Carcinoma In Situ of the Bladder

EVEROLIMUS (RAD001) **CONTINUOUS CYCLE** SCHEDULE TREATMENT LOG

Place a check in the box next to the date you take this medication. Please bring this sheet to your study doctor's office on each visit

Cycle: _____

Date	Day	Time	Treatment	Dose	Questions for Study Doctor/Nurse
	1		Everolimus ☐	10 mg	
	2		Everolimus ☐		
	3		Everolimus ☐		
	4		Everolimus ☐		
	5		Everolimus ☐		
	6		Everolimus ☐		
	7		Everolimus ☐		
	8		Everolimus ☐		
	9		Everolimus ☐		
	10		Everolimus ☐		
	11		Everolimus ☐		
	12		Everolimus ☐		
	13		Everolimus ☐		
	14		Everolimus ☐		
	15		Everolimus ☐		
	16		Everolimus ☐		
	17		Everolimus ☐		
	18		Everolimus ☐		
	19		Everolimus ☐		
	20		Everolimus ☐		

Amended: 1/11/11

	21		**Everolimus** ☐		
	22		**Everolimus** ☐		
	23		**Everolimus** ☐		
	24		**Everolimus** ☐		
	25		**Everolimus** ☐		
	26		**Everolimus** ☐		
	27		**Everolimus** ☐		
	28		**Everolimus** ☐		
MD/RN Signature:_____			Date_____		Tablets Remaining_____
Comments:_____					