PROTOCOL 08-313

Closed to New Accrual

Closure Effective Date: 12/09/13
No new subjects may be enrolled in the study as described above.
Any questions regarding this closure should be directed to the study's Principal Investigator

A Phase II Biomarker Trial of Everolimus in Patients with Advanced Renal Cell Carcinoma

RAD001

Principal Investigator:	David McDermott, MD
Version Date:	October 03, 2014
Co-Investigators:	James Mier MD, Toni Choueiri MD ; Dana-Farber / Harvard Cancer Center
	Daniel George MD / Duke University Medical Center
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List of abbreviations

4E-BP1	4E-binding protein
ADR	Adverse Drug Reaction
AE	adverse event
ALT/SGPT	alanine aminotransferase/glutamic pyruvic transaminase/Serum glutamic-pyruvic transaminase
AST/SGOT	aspartate aminotransferase/glutamic oxaloacetic transaminase/Serum glutamic-oxaloacetic transaminase
ATC	Anatomical Therapeutic Chemical classification system
AUC	Area under the plasma-concentration time curve
BAC	Bronchoalveolar carcinoma
Cmax	Maximum plasma concentration
CR	Clinical research
CRF	Case report/Record form
CRO	Contract Research Organization
CT	Computer tomography
CTC	Common toxicity criteria
CV	Coefficient of Variation
CYP3A4	CytochromeP450 3A4 isoenzyme
DLT	Dose limiting toxicity
ECG	Electrocardiogram
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
eIF-4E	Eucariotic Initiation Factor 4E
EPR	Early progression rate
FDG-PET	Fluorine-18-2-fluoro-Deoxy-D-Glucose Positron Emission Tomography
FKBP-12	FK506-binding protein 12
GF	Growth factor
HDL	High-density lypoproteins
HER	Human Epidermal Receptor
HUVECS	human umbilical endothelial cells
IC50	Inhibitory concentration at 50%
IEC	Independent Ethics Committee
IGF1-R	Insulin-like Growth Factor 1 Receptor
IHC	Immunohistochemistry
INN	International Non-proprietary Name
INR	International Normal Ratio
IRB	Institutional Review Board

LC-MS	liquid chromatography method with mass spectrometry
LDL	Low-density lypoproteins
LLOQ	Lower limit of quantification
MAPK	Mitogen Activated Protein Kinase
mRNA	messenger Ribonucleic acid
mTOR	mammalian Target of Rapamycin
NIH/NCI	National Institutes of Health/National Cancer Institute
nM	nano-molar
NSCLC	Non-small cell lung cancer
OS	overall survival
P-AKT	phosphor-AKT
PD	Pharmacodynamics
PET	Proton emission tomography
PFS	progression free survival
P-gp	P-glycoprotein
PI3K	Phosphoinositide 3-kinase
PK	Pharmacokinetics
PK/PD model	Pharmacokinetic/pharmacodynamic model
PT/PTT	prothrombin time
PTEN	Phosphatase and Tensin homolog deleted on chromosome 10
RBC	red blood cell count
REB	Research Ethics Board
RR	response rate
S6K1	S6 kinase 1
SAE	serious adverse event
SCLC	Small cell lung cancer
STAT3	Signal Transducer and Activator of Transcription 3
TK	Tyrosine kinase
TSC2	Tuberous Sclerosis Complex 2
TUNNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin Nick End Labeling
ULN	upper limit of normal
VEGF	Vascular Endothelial Growth Factor
WBC	total white blood cell count
WHO	World Health Organization

1 Introduction

1.1 Renal Cell Carcinoma

Renal cell carcinoma (RCC) is the eigth most common cancer with approximately 54,000 new cases and 13,000 deaths estimated in 2008 (1). The incidence of RCC has been rising each year in the United States since 1970, with a 3:2 prevalence in men over women and a median age of diagnosis of 60 years (1). While many patients present with surgically resectable disease, approximately 20-30% present with metastatic disease and 20-40% of patients undergoing nephrectomy for localized disease will develop metastases (2). Traditionally, treatment for metastatic renal cell carcinoma has been limited. Standard chemotherapeutic agents have been largely ineffective and cytokine-based treatments with interleukin-2 (IL-2) or interferon-α (IFN) benefit a small proportion of patients. Objective response rates to IL-2 range from 10-20% with 5-10% of these patients having durable responses (3-5). Additionally, the toxicity of high-dose IL-2 has limited its widespread use. Low dose IFN has produced response rates in the 10% range with several studies showing a modest survival benefit (6, 7). There has thus been significant interest in developing more effective therapies for this group of patients.

Many advances have been made in our understanding of the molecular biology of RCC. Since 1997, renal epithelial tumors have been classified into subtypes (clear cell, papillary type I, papillary type II, chromophobe, and oncocytoma), each with distinct morphology, genetic features, and cell of origin (8, 9). Clear cell or conventional type renal cancer accounts for 65% to 75 % of renal epithelial tumors. It is now known that 75% to 80% of sporadic clear cell renal cancer cases possess bi-allelic somatic alterations in the Von Hippel Lindau (VHL) gene either via deletion, mutational inactivation, or silencing by hypermethylation (10, 11). The VHL gene encodes a tumor suppressor protein that under normoxic conditions forms a complex that binds to Hypoxia Inducible Factor (HIF)-1α and -2α in the presence of oxygen and targets them for proteasomal degradation. Loss of VHL function results in inappropriate accumulation of both HIF-1α and HIF-2α and the subsequent activation of their target genes, including vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor-alpha (TGF-α), erythropoietin, GLUT-1, and CXCR4 (12-15). This accumulation of HIF-regulated proteins is believed to play a critical role in RCC tumorigenesis and RCC-mediated angiogenesis. VEGF has been well established as having a central role in endothelial cell proliferation and survival (16). PDGF is believed to play a role in endothelial stabilization. Disruption of PDGF signaling in perivascular cells has been shown to potentially enhance an anti-angiogenic response triggered by VEGFR inhibition (17). The TGF-α/EGFR pathway has been shown to be activated and critical for tumor progression in VHL-/- RCC tumor lines (18). Thus, VHL dysfunction causing the inappropriate accumulation of HIF-1α and HIF-2α results in a cascade of signaling events favorable for RCC tumorigenesis and proliferation. Given its central role in RCC development, the VHL-HIF pathway has emerged as perhaps the preeminent target in RCC therapy and several agents targeting elements of this pathway have demonstrated substantial clinical efficacy.

1.2 VEGF-Targeted Therapies

Recently, two multi-targeted kinase inhibitors (TKIs) have been approved by FDA for the therapy of metastatic RCC. Sorafenib (BAY 43-9006, Nexavar) is an orally administered TKI originally discovered through a screen to discover inhibitors of Raf-kinase (19). In addition to potent activity against both mutant and wild-type b-raf and c-raf, it was subsequently discovered that sorafenib also had inhibitory activity against a broad group of receptor tyrosine kinases including VEGFR2, PDGFR, c-KIT, p38α, and flt3 (20). Sorafenib gained FDA approval based on a randomized, placebo-controlled trial of sorafenib conducted in patients with advanced RCC who had failed one prior therapy (21). Overall, 903 patients were enrolled and randomized to placebo (n=452) versus sorafenib (n=451). The primary end-point was overall survival with secondary end-points including progression free survival, response rate, and safety. While the final analysis for overall survival has not been completed, the median progression free survival was found to be significantly longer in patients who received sorafenib versus placebo (24 weeks vs. 12 weeks; $p<0.000001$). The objective response rate was only 2% on final analysis. Drug-related adverse events (all grades) for sorafenib versus placebo included rash (34% vs. 13%), diarrhea (33% vs. 10%), hand-foot skin reaction (27% vs. 5%), fatigue (26% vs. 23%) and hypertension (11% vs. 1%). Any grade 3 / 4 events were reported in 30% of patients on sorafenib versus 22% patients on placebo. Based on the promising prolongation of progression free survival and tolerability, sorafenib was approved by the FDA for therapy of patients with metastatic or unresectable RCC on December 20, 2005.

Sunitinib (SU11248, Sutent) is an oral multi-targeted tyrosine kinase inhibitor of VEGFR, PDGFR and c-Kit (22). Sutent has demonstrated promising efficacy in two independent single-agent phase II trials in patients with advanced RCC (23, 24). Sixty-three and 106 previously treated patients were enrolled in the two trials, respectively, with a primary end-point of response rate. The overall response rate for the combined trials was approximately 40% with a median PFS of 8.2 months. Based on these findings, sunitinib received FDA approval for therapy of advanced RCC on January 26, 2006. Sunitinib was also studied in the first-line setting in a randomized phase III trial comparing sunitinib to IFN-α (25). Overall, 750 patients were enrolled and randomized with 375 per treatment arm. The objective response rate was 31% for sunitinib versus 6% for IFN-α ($p<0.001$) and the median PFS was 11 months for sunitinib versus 5 months for IFN-α, corresponding to a hazard ratio of 0.42 (95% confidence interval, 0.32 to 0.54; $P<0.001$). At the time of analysis, the median overall survival had not yet been met in either group.

Both sorafenib and sunitinib represent promising advancement in the therapy of metastatic RCC. However, there are limitations to both agents. While both drugs have been shown to significantly delay time to progression, objective responses have rarely been complete or maintained off therapy and all patients will eventually experience disease progression while on therapy. It is possible that there may be limitations to what can be achieved therapeutically with agents that target the tumor endothelium and have primarily antiangiogenic effects. More robust responses may require agents which directly target oncogenic signaling pathways

in RCC tumor cells. Fortunately, such agents are now emerging and demonstrating efficacy in RCC.

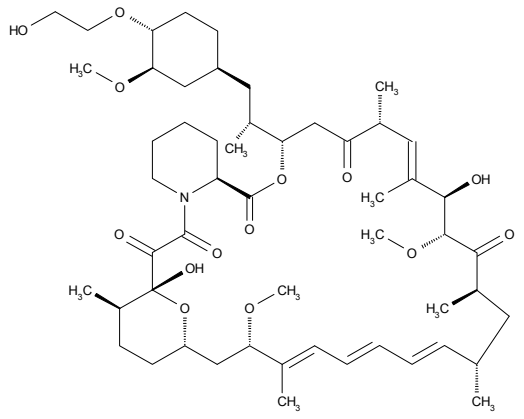
1.3 RAD001 (everolimus)

RAD001 (everolimus) is a novel derivative of rapamycin. RAD001 has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. Since 2003, RAD001 is approved in Europe (trade name: Certican®) via the Mutual Recognition Procedure (MRP) for the prevention of organ rejection in patients with renal and cardiac transplantation. Certican® is also approved in Australia, South Africa, the Middle East, Central and South America, the Caribbean and some Asian countries.

RAD001 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 VEGF production and VEGF-induced proliferation of endothelial cells)

Figure 1 Chemical structure of RAD001



1.3.1 mTOR pathway and cancer

At the cellular and molecular level, RAD001 acts as a signal transduction inhibitor. It selectively inhibits mTOR (mammalian target of rapamycin), a key protein kinase which regulates cell growth, proliferation and survival. The mTOR is mainly activated via the PI3 kinase pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in PTEN, a negative regulator of PI3 kinase, may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development (26).

The main known functions of mTOR include the following (27):

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels, facilitating cell-cycle progression from G1-S phase in appropriate growth conditions.

- The PI-3K (mTOR) pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors. PI3-kinase mutations have been reported in the primary tumor in 10-20% of human colorectal cancers (28, 29).
- The loss of PTEN protein, either through gene deletion or functional silencing (promoter hypermethylation), is reported in approximately 60% of primary human colorectal cancers (30).
- The mTOR pathway is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation.
- Through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates protein translation, including the HIF-1 proteins. Inhibition of mTOR is expected to lead to decreased expression of HIF-1.

1.3.2 Preclinical studies

RAD001 inhibits the proliferation of a range of human tumor cell lines *in-vitro* including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. IC50s range from sub/low nM to µM. RAD001 also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS) *in vitro*, with particular potency against VEGF-induced proliferation suggesting that RAD001 may also act as an anti-angiogenic agent. The anti-angiogenic activity of RAD001 was confirmed *in vivo*. RAD001 selectively inhibited VEGF-dependent angiogenic response at well tolerated doses. Mice with primary and metastatic tumors treated with RAD001 showed a significant reduction in blood vessel density when compared to controls.

The potential of RAD001 as an anti-cancer agent was shown in rodent models. RAD001 is orally bioavailable, residing longer in tumor tissue than in plasma in an s.c mouse xenograft model, and demonstrating high tumor penetration in a rat pancreatic tumor model. The pharmacokinetic profile of RAD001 indicates sufficient tumor penetration, above that needed to inhibit the proliferation of endothelial cells and tumor cell lines deemed sensitive to RAD001 *in vitro*.

RAD001 administered daily p.o. was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and "relatively resistant" *in vitro*. In general, RAD001 was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of RAD001-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

It is not clear which molecular determinants predict responsiveness of tumor cells to RAD001. Molecular analysis has revealed that relative sensitivity to RAD001 *in vitro* correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein; in some cases (i.e., glioblastoma) there is also a correlation with PTEN status.

In vivo studies investigating the anti-tumor activity of RAD001 in experimental animal tumor models showed that RAD001 monotherapy typically reduced tumor cell growth rates rather than produced regressions or stable disease. These effects occurred within the dose range of 2.5 mg to 10 mg/kg, p.o. once a day.

In preclinical models, the administration of RAD001 is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated (p)-S6 (p-S6) and p-4E-BP1, and occasionally with an increase in phosphorylated AKT, a protein upstream of mTOR signaling pathway. Study CRAD001A2107 explored MPD (molecular pharmacodynamic) changes in tumor at different doses and schedules of RAD001 (weekly 20 mg, 50 mg and 70 mg or daily 5 mg and 10 mg).

All significant adverse events observed in toxicology studies with RAD001 in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant and 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.

1.3.3 Clinical experience

1.3.3.1 RAD001 pharmacokinetics

The pharmacokinetic characteristics of RAD001 have been extensively investigated in the context of the drug's development as an immunosuppressant in solid organ transplantation where RAD001 was administered twice daily as a part of an immunosuppressant, multi-drug regimen consistently including cyclosporin A and glucocorticoids. Recent Phase I studies provide steady-state pharmacokinetics for both the weekly and daily schedules at varying dose levels in patients with advanced cancers.

RAD001 is rapidly absorbed after oral administration, with a median time to peak blood levels (t_{max}) of 1-2 hours postdose. The extent of absorption is estimated at above 11%. The area under the blood concentration-time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration C_{max} appears to plateau at dose levels higher than 20 mg. The terminal half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation (CV) of approximately 50%. A high-fat meal altered the absorption of RAD001 with 1.3 hour delay in $t_{max,}$ a 60% reduction in C_{max} and a 16% reduction in AUC. In whole blood, approximately 80% of RAD001 is contained in red blood cells. Of the fraction of drug contained in plasma, 74% is protein-bound. The apparent distribution volume (Vz/F) after a single dose was 4.7 L/kg. RAD001 is eliminated by metabolism, mainly by hydroxylation, then excreted into the feces >80%.

RAD001 is mainly metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. RAD001 is also a substrate of P-glycoprotein (P-gp). Therefore, absorption and subsequent elimination of systematically absorbed RAD001 may be influenced by medicinal products that interact with CYP3A4 and/or P-glycoprotein. *In vitro* studies showed that RAD001 is a competitive inhibitor of CYP3A4 and of CYP2D6 substrates, potentially increasing the concentrations of medicinal products eliminated by these enzymes. In two phase III clinical trials in patients following kidney transplantation, strong inhibitors of

CYP3A4 (azoles, antifungals, cyclosporine, erythromycin) have been shown to reduce the clearance of RAD001 therapy thereby increasing RAD001 blood levels. Similarly, Rifampin, a strong inducer of CYP3A4, increases the clearance of RAD001 thereby reducing RAD001 blood levels. Caution should be exercised when co-administering RAD001 with CYP3A4 inhibitors or inducers.

Pharmacokinetic drug to drug interactions with cancer agents are being evaluated in ongoing phase Ib studies. Based on currently available results, gemcitabine (study 2101 part 2) and paclitaxel (study 2104) did not alter RAD001 pharmacokinetics to a clinically relevant extent whereas imatinib notably increased RAD001 exposure with a mean increase in AUC by a multiple of 3.7 for RAD001 administered weekly and two-fold for RAD001 administered daily (study 2206). Exposure to RAD001 in the presence of letrozole did not exceed that in monotherapy (study 2108). Co-administration of RAD001 did not influence pharmacokinetics of gemcitabine, imatinib or letrozole. Exposure to paclitaxel in the presence of RAD001 was slightly decreased (average by 23%).

RAD001 pharmacokinetics in transplant patients was investigated in special populations such as subjects with hepatic or renal impairment, various ethnic groups and pediatric renal transplant patients. In subjects with mild–moderate hepatic impairment, mean AUC to RAD001 is increased by 3-fold whilst renal impairment does not affect the pharmacokinetics of RAD001. Age, weight (both over the adult range) and gender do not affect the pharmacokinetics of RAD001 to a clinically relevant extent. Also, pharmacokinetics does not alter in Japanese or Asian patients whereas black patients have 21% higher clearance compared to non-blacks. In children, the apparent clearance of RAD001 increases linearly with body surface. The clearance per square meter of body surface area is 12-fold higher compared with adult patients.

The pharmacokinetic parameters derived for RAD001 given weekly are summarized in Table 1-1.

Table 1-1 Steady-state RAD001 pharmacokinetics (weekly dosing)

Parameter	**5 mg**	**10 mg**	**20 mg**	**30 mg**	**50 mg**	**70 mg**
N	4	4	2	5	5	6
Tmax ()	1 (1-2)	1 (1)	1 (1)	2 (1-2)	1 (1-2)	1 (1)
C_{max} (ng/mL)	32 ± 15	69 ± 8	94 ± 0	88 ± 20	163 ± 63	174 ± 49
C_{max}/Dose Ng/mL/mg	6.5 ± 3.1	6.9 ± 0.8	4.7 ± 0.0	2.9 ± 0.7	3.3 ± 1.2	2.5 ± 0.7
AUC ^{ss} (ng-h/mL)	283 ± 48	573 ± 258	1001 ± 301	1798 ± 827	2621 ± 633	3615 ± 1497
AUC ^{ss}/Dose (ng-h/mL/mg)	57 ± 10	57 ± 27	50 ± 15	60 ± 28	52 ± 13	52 ± 21
$T_{1/2}$ (h)	26 ± 3	38 ± 14	31 ± 12	37 ± 6	27 ± 7	26 ± 2

Values are median (range) for tmax and mean ± standard deviation for all others.
Reference: RAD001 Investigator's Brochure 2005

Cmax was achieved by 1 to 2 hours post dose. While Cmax rose in roughly dose proportional manner from 5 to 20 mg/week, it appeared to increase less than proportionally at higher doses.

Pharmacodynamic studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition in a peripheral biomarker (S6 kinase inhibition in peripheral blood mononuclear cells) suggests that 5-10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition. Furthermore, molecular pharmacodynamic (MPD) studies using IHC in biopsied tumor tissue assessed the degree of inhibition and its duration (for p-S6, p-4E-BP1 and p-Akt expression) with the daily and weekly dosing. The pathologist was blinded for the biopsy sequence. There was almost complete inhibition of p-S6 at all doses and schedules studied (p=0.001). Preliminary results suggest a dose-related decrease in p-4E-BP1 and increase in p-Akt expression with maximal effect at 10 mg daily and ≥ 50 mg weekly. The study results are provided in Table 1-1.

Table 1-2 p-S6, p-4E-BP1 and p-Akt expression at various doses of RAD001

Dose of RAD001	p-S6 inhibition (mean %)	p-4E-BP1 inhibition (mean %)	p-Akt activation (mean %)
Daily 5 mg (n=3)	100.0	48.0	22.2
Daily 10 mg (n=6)	92.5	58.2	45.5
Weekly 20 mg (n=5)	96.7	5.9	32.7
Weekly ≥ 50 mg (n=6)	100.0	63.8	63.1
Reference: RAD001 Investigator's Brochure 2005			

1.3.3.2 Phase I and II oncology studies

Data are available from phase I clinical studies of RAD001 given as a single agent to 147 patients with advanced solid tumors. Such studies included various doses and schedules (weekly dosing, range 5-70 mg and daily dosing 5-10 mg). Approximately, 46% of patients reported rash or erythema and 40% of the patients presented with stomatitis/mucositis. The most frequent adverse events suspected to be drug-related observed in three studies using RAD001 as a single agent are listed in Table 1-1.

Table 1-3 Adverse events suspected to be drug-related in ≥10% of patients with advanced cancers reported in Phase I RAD001 monotherapy studies (C2101, C2102 and 2107)

	Weekly			Daily		
	5-30 mg n=30	50 mg n=18	70 mg n=38	5mg n=16	10 mg n=45	Total n=147
No. Pts with AEs						
Any event	23 (1)	17 (2)	38 (10)	14 (1)	43 (14)	135 (28)
By event						
- Rash	5	8	18	10	27 (1)	68 (1)
- Stomatitis/mucositis	6	8 (2)	16 (2)	6 (1)	23 (3)	59 (8)
- Fatigue	8	7 (1)	14 (1)	1	17 (1)	47 (3)
- Nausea	5	4	8	2	18 (1)	37 (1)
- Anorexia	1	6	10	3	15	35
- Diarrhea	1	7	7	-	9	24
- Vomiting	4	5	5	-	10	24
- Headache	7	4	6	6	4	20
- Pruritus	2	1	6	3	4	16
- Infections[1]	1	3	3 (1)	1	6 (2)	14 (3)
- Constipation	-	1	2	2	9	14

The numbers of patients (by dose level and dose schedule) who have reported grade ≥3[1] toxicities is given in brackets.

[1]. events included in brackets reached no more than grade 3 severity

[2] Infections noted as drug-related included:

Herpes simplex: 5 pts (1 at 50 mg/wk; 1 at 5mg/d; 3 at 10 mg/d)
Oral candidiasis: 5 pts (1 at 50 mg/wk; 3 at 70 mg/wk, 1 at 10 mg/d)
Pneumonia (gr3) 1 pt (10 mg/d)
Pustular rash 1 pt (20 mg/wk)
Rhinitis 2 pts (50 mg/wk)
URT Infection 1 pt (50 mg/wk
Urinary Tract Infect 1 pt (50 mg/wk)

Reduced blood cell counts at the initiation of treatment are frequent but remain mostly within the normal range or limited to grade 1 although a grade 3 neutropenia was a DLT in one patient as was a grade 3 thrombocytopenia in a patient receiving RAD001 with letrozole where pharmacodynamic interaction is unlikely. This suggest that some patients may be particularly sensitive to the myelosuppressive effect of RAD001 making it necessary to monitor carefully blood cell counts at initiation of treatment.

Metabolic changes (hyperlipidemia and hyperglycemia) may be observed during treatment with RAD001. Both events may be medically managed. Hyperlipidemia has been reported as an ADR in 10% of patients although review of the laboratory values suggests that as many as a quarter of patients develop grade 1-2 hyperlipidemia on treatment, mostly hypercholesterolemia. Hyperglycemia has been reported as an adverse event in 7% of patients. Grade 3 hyperglycemia has been observed, especially in diabetics receiving RAD001 treatment. Therefore, patients with diabetes should have their blood glucose monitored carefully and their medications adjusted, as needed, to maintain adequate control of their blood glucose levels.

In Novartis-sponsored clinical trials, symptomatic non-infectious pneumonitis has been reported as a serious adverse event in less than 1% of patients out of approximately 1000 cancer patients treated with RAD001 as of April 30, 2006. This adverse event has been noted in the Investigators' Brochure. Corticosteroids were often administered to the patients with symptomatic pneumonitis.

Novartis has recently received reports of low grade non-infectious pneumonitis in cancer patients treated with RAD001. Most of these reports involve patients with no respiratory symptoms (CTC grade 1 pneumonitis: radiographic findings only) or mild severity (CTC grade 2: symptomatic, not interfering activities of daily living), and were from two investigator-sponsored (private IND) trials, as follows:

- In a study of patients with advanced renal cell carcinoma receiving 10 p.o. mg/day, 15/20 patients reviewed by an independent radiologist were noted to have lung infiltrates consistent with pneumonitis on routine chest CT scans performed to follow the patients' thoracic metastases.
- In a study of patients with advanced breast cancer, 7/18 patients treated with RAD001 10 mg/d and 2/16 patients treated with RAD001 70 mg/week, had findings consistent with pneumonitis. In this study, two patients, one on the daily RAD arm and one on the weekly RAD001 arm, developed severe (grade 3) pneumonitis which resolved after RAD001 was discontinued.

In both studies, most patients had radiological changes with mild or no symptoms and have continued RAD001 treatment without developing symptoms. The reason for an increased rate of reported low-grade pneumonitis among oncology patients in these studies is unclear. Both studies included serial chest CT scans allowing prolonged, detailed evaluation of the lung parenchyma; the dosage and drug exposure in these phase 2 trials is generally longer than in the phase 1 experience. In addition, the dosage of RAD001 used in the treatment of cancer patients is substantially higher than that given routinely in the organ transplant setting. Everolimus (RAD001) is approved at a daily dose of 0.75 mg twice a day guided by therapeutic drug monitoring (3-8 ng/ml) in combination with cyclosporine microemulsion in many regions of the world for renal and cardiac transplantation. In phase 3 trials investigating everolimus in renal and cardiac transplantation, the overall reported rate of pneumonitis ranged from 0.0 to 1.4%. The spontaneous reporting rate for pneumonitis following exposure to commercially available everolimus in transplantation is very low (0.08% or 84.4 events/100,000 patient-years). Refer to the latest version of the RAD001 Investigator's Brochure and safety letters (Investigator Notifications) for the most up to date information available.

Everolimus has demonstrated promising activity in advanced renal cell carcinoma. Results from a phase 2 trial of everolimus in previously treated patients with advanced RCC were recently presented (31). Overall, 41 patients with metastatic RCC were enrolled and 37 were evaluable for response and toxicity. 12 patients (32%) showed an objective response and the median survival was over 11.5 months. Based on these promising results, everolimus was studied in a large, randomized, phase 3 trial versus placebo in patients with metastatic RCC who had failed VEGF-targeted TKI (32). Overall, 410 patients were randomized in a 2:1 fashion to receive either everolimus at a dose of 10mg/day or best supportive care (272 pts everolimus, 138 pts placebo). Everolimus resulted in a statistically and clinically significant

improvement in PFS over placebo with a favorable safety profile (PFS 4.0 vs. 1.9 months, p<0.0001). The most common grade 3 or 4 adverse events reported for everolimus were stomatitis (36% grade 3, 4% grade 4), anemia (28% grade 3, 7% grade 4), and asthenia (28% grade 3, 2% grade 4). Everolimus is now being studied in other settings and in combination with other molecularly targeted agents.

1.4 Predictors Biomarkers of Response to mTOR Inhibitors

Concurrent with the development of molecularly targeted therapies in RCC has been the pursuit of biomarkers which might predict for response to these same therapies. With many agents with distinct molecular targets currently available and even more on the horizon, identification of predictive biomarkers of response to these agents is a high priority for directing the most appropriate therapies to individual patients. Unlike VEGF-targeted therapies which putatively target non-malignantly transformed tumor endothelial cells, the clinical benefit of mTOR inhibitors in RCC may be mediated through direct effects on tumor cells. Therefore, mTOR inhibitors may be uniquely suited for the development of tumor-based predictive molecular biomarkers of response. One possible candidate biomarker is the pre-treatment activation status of the PI3-K/Akt/mTOR signaling pathway. The hypothesis that this basal activity of the targeted pathway might predict for sensitivity to molecularly targeted agents is based on similar examples in other malignancies. The link between response to the MEK inhibitor CI-1040 and the presence of an activating B-RAF mutation in melanoma is one example (33).

Recently, pathologic surrogates of pre-treatment mTOR pathway activation were investigated in tumor specimens collected from a subset of patients treated with temsirolimus as part of a randomized phase 2 trial (34, 35). Twenty pre-treatment tumor specimens from patients with metastatic RCC treated with temsirolimus were stained for expression of phospho-S6 (pS6) (Ser 235/236) ribosomal protein, a substrate downstream of mTOR. At the same time, 19 tumor specimens were stained for phospho-Akt (pAkt) (Ser 473), a substrate upstream of mTOR. Higher pS6 expression was significantly correlated with objective response to temsirolimus (p=0.02) while there was a trend towards a significant correlation of pAkt with objective response (P=0.07). No patient without high expression of either pAkt or pS6 experienced an objective response. Although this study has a small sample size, the findings serve as proof of principle that these surrogates of PI3-K/Akt pathway activation (in particular, S6 phosphorylation) are sufficiently stable in formalin-fixed tissue to yield reproducible data that may be predictive of response to molecularly targeted agents that block the PI3-K/Akt pathway.

Despite the findings in this small retrospective analysis, there have been many concerns raised regarding the reliability of phosphoproteins as surrogates of signaling pathway activation in tumor specimens. In particular, while there appears to be consensus that phospho-S6 is a particularly stable epitope, phospho-Akt appears quite variable due to rapid dephosphorylation (36). As such, there are ongoing efforts to identify more reliable surrogates of Akt activation. Akt activation in the nucleus induces the translocation of the FOXO transcription factors into the cytoplasm (37). As the detection of the intracellular location of FOXO is not dependent

upon a phospho-protein, it is possible that this would be a particularly stable marker of Akt activation in tumor specimens. Akt also phosphorylates multiple other downstream substrates which may be more stable than phospho-Akt itself. For example, PRAS40 (proline rich Akt substrate of 40 kd) is known to be directly phosphorylated by Akt and has recently been shown to potentially regulate mTOR activity by binding to raptor and competing with mTOR for binding to its substrates S6Kinase and 4EBP (38). Preliminary work in paraffin-embedded tissue specimens suggests that phospho-PRAS40 may be a more stable epitope than phospho-Akt (Ser 473) and therefore may serve as a more reliable biomarker of Akt activation.

Although the mTOR is traditionally thought to be activated primarily by signaling through the PI3K/Akt pathway, alternate mechanisms of mTOR activation independent of Akt have recently been described. For example, the tumor suppressor Promyelocytic Leukemia (PML) protein has recently been shown to play a critical role in the inhibition of mTOR and activation of FOXO transcription factors (39, 40). Better known for its causative role in Acute Promyelocytic Leukemia, where its function is altered as a result of the (15;17) translocation and fusion with RARα (41), PML has more recently been shown to play a role in epithelial malignancies (e.g. breast, lung and prostate cancer) in which its function is frequently lost (42, 43). Loss of PML function would be expected to result in both the constitutive activation of mTOR independent of Akt and the nuclear activation of Akt independent of PI3-K signaling. Recent data has suggested that the expression and intranuclear distribution of PML is frequently altered in RCC (unpublished observations). Similar to the loss of PML, activation of the MAP-Kinase (MAP-K) pathway may also result in the activation of mTOR independent of PI3-K/Akt signaling. It has recently been shown that MAP-K regulated kinase Erk is capable of phosphorylating TSC2 at a specific residue (Ser^{664}), resulting in inhibition of its GAP activity and subsequent downstream activation of mTOR (44). It is possible that activation of mTOR through either of these pathways, PML loss or Erk activity, may represent oncogenic constitutive mTOR activation which is particularly sensitive to mTOR inhibition. The determination of the frequency and predictive value of these signaling events with respect to response to mTOR inhibitors is of great interest.

Finally, there may be molecular characteristics within individual tumors which determine its susceptibility to the beneficial effects of mTOR inhibition, which are presumably at least in part mediated through the achievement of G1 phase cell cycle arrest. mTOR mediates its control of translation by phosphorylating 4EBP, causing it to disassociate from eurkaryotic translation initiation factor 4E (eIF4E). eIF4E binds to the 5',7-methylguanosine cap structure of mRNAs, allowing them to associate with the eIF4F translation initiation complex, which then enables translation by scanning the 5' UTR for the translation initiation codon. It has recently become clear that individual mRNA vary widely in the ease with which they can be translated dependent upon the length and structure of their 5' UTR (such as CG rich, high-structured 5' UTR) (45). mRNA which are the most difficult to translate are therefore those which are most highly effected by the availability of eIF4E and include several well known growth and survival factors such as cyclin D1, c-myc, VEGF, and survivin. The availability of eIF4E is dependent upon several factors such as activation of the ras and PI3-Kinase/Akt pathways. However, eIF4E can also be overexpressed independent of the activity of these pathways in several tumor types (46). Overexpression of eIF4E beyond the availability of 4EBP is believed to potentially impart resistance to the growth inhibitory effects of mTOR

inhibition. While the existence of such overexpression of eIF4E in RCC remains to be investigated, such baseline variability may be predictive of response to mTOR inhibitors.

In addition to the levels of individual protein markers, the transcriptional expression profile of an individual RCC tumor may also be predictive of activation of the mTOR pathway and predict response to targeted mTOR inhibitors such as everolimus. The Nevins laboratory at Duke University's Institute for Genome Sciences & Policy has significant experience in performing these microarrays and analyzing the resulting data. In our laboratory, microarray data from tumor cell lines have been used to generate genomic signatures to predict response to cytotoxic chemotherapy agents (48).

Expression analysis of transcripts in peripheral blood mononuclear cells (PBMC) of patients with RCC have also proven informative. In a prior study, 184 gene transcripts were found to be differentially expressed in PBMC of RCC patients compared to normal control patients (51). In a separate study, PBMC expression signatures from RCC patients prior to treatment with temsirolimus were correlated with patient outcomes, including overall survival (52). Expression analysis of PBMC from patients enrolled in this study will evaluated with the goal of developing a signature that can predict response to mTOR inhibitor therapy. During the study, PBMC will be collected at the beginning of every other cycle of therapy, and the data will be analyzed for changes in the PBMC expression signature during treatment with everolimus.

1.5 Rationale for Study

Clinical experience with temsirolimus and everolimus has shown that only a subset of patients with advanced RCC derive substantial clinical benefit from mTOR inhibitors (31, 44). The particular benefit of temsirolimus in patients with high-risk MSKCC prognostic features and in non-clear cell histology suggests that this subset of patients may be distinct from those responding to VEGF-targeted therapies (47). The discovery of predictive biomarkers would allow the direction of mTOR inhibitors to those patients most likely to benefit and potentially identify those who should receive VEGF-targeted agents instead.

It has been shown in a small retrospective study that certain markers of mTOR activation (e.g. Akt or S6 phosphorylation) may predict response to temsirolimus (35). This study has several clear limitations which include small sample size, the retrospective nature of the analysis, and the use of mostly primary tumor (nephrectomy) specimens for biomarker determination. The last issue may be particularly concerning as it is not clear if the expression of the biomarkers of interest will be the same in the primary lesion (sometimes resected several years prior to therapy) as in the metastatic lesions which are presumably being treated. We therefore propose to validate these previously identified predictive biomarkers prospectively in metastatic lesions from patients treated with everolimus in a phase II trial. This approach appears feasible as everolimus has demonstrated a robust objective response rate in prior phase II trials in advanced RCC, facilitating the correlation of biomarker expression with response.

In addition, there appear to baseline variations in RCC that may modulate the response to mTOR inhibitors. Both loss of PML function and Erk-mediated phosphorylation of TSC2 are Akt-independent pathways of constitutive mTOR activation which may predict for a particularly robust response to mTOR inhibition. Conversely, overexpression of eIF4E may predict for resistance to mTOR inhibition. This phase II trial is an ideal setting to explore the frequency of these signaling events in metastatic RCC lesions as well as their predictive value with respect to response to mTOR inhibitors.

Microarray expression analysis of the tumor samples that will be obtained during this study will be paired with individual patient response data, and used to develop a genomic expression pattern that could predict response to everolimus, thus rapidly and reproducibly identifying a subgroup of patients with renal cell carcinoma who are more likely to benefit from therapy with this agent (48, 50). This signature could then be validated in future studies with additional tumor samples. In addition, microarray data will be used to determine if gene products corresponding to potential biomarker proteins such as Erk, mTOR and S6 are overexpressed in these tumors.

2 Study objectives

Primary

1. To prospectively validate the expression of phospho-Akt and phospho-S6 as predictive biomarkers of responsiveness to everolimus as determined by progression-free survival in patients with advanced RCC using progression free survival as defined in sections 3.4.2.5, and 8.0.

Secondary

1. To estimate the progression-free survival in patients with advanced RCC treated with everolimus as defined in section 3.4.2.5 and 8.0.

2. To determine the objective response rate to everolimus in patients with advanced RCC as defined in section 3.4.2.2 and 8.0.

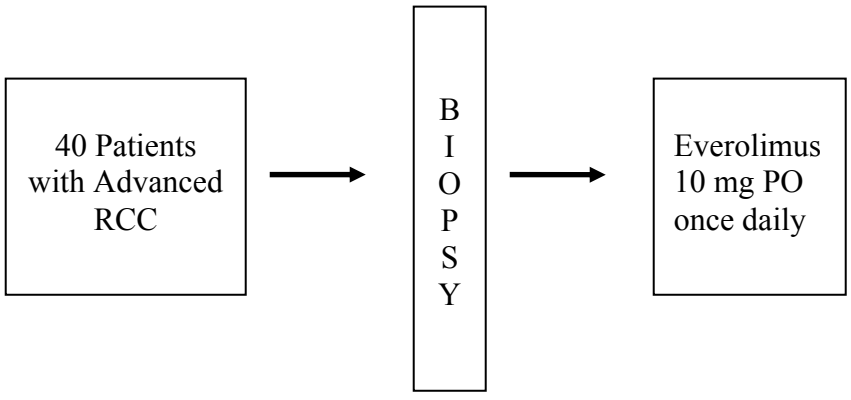
3. To assess in an exploratory fashion the predictive value of PML, phospho-TSC (S664), phospho-PRAS40, eIF4E, and FOXO expression with respect to response to everolimus.

4. To develop a genomic predictor of response to everolimus based on microarray analysis of pretreatment biopsy samples.

5. To develop a peripheral blood cell signature to predict response to everolimus.

3 Investigational plan

3.1 Overall study design



This will be an open-label, single-arm phase II trial of everolimus in patients with advanced RCC. The primary objective of the protocol will be to prospectively validate pS6 and pAkt expression as predictive biomarkers of responsiveness to everolimus using progression free survival. Forty patients total will be enrolled in the trial. Expected enrollment is 2-3 patients per months over 13-20 months. Patients may have had prior VEGF-targeted therapy (i.e. sorafenib, sunitinib, bevacizumab, or pazopanib) or prior cytokine-based therapy (i.e. IL-2). All patients will be required to undergo core needle biopsy or metastectomy of an accessible metastatic lesion prior to beginning therapy. Enrollment, in general, will therefore be limited to patients who have safely accessible metastatic disease. Safely accessible metastatic disease will be defined to include those lesions which are palpable with no overlying viscera and are at least 2cm in size (longest dimension). Given the paucity of subcutaneous lesions in RCC, lesions which are felt safe to biopsy in the opinion of the investigator will also be allowed. These lesions will include pleural-based tumors, peripheral liver lesions, kidney lesions and bone lesions with exophytic soft tissue component. As with palpable lesions, these other lesions should be at least 2cm in size (longest dimension) with no overlying viscera. In addition to patients with safely accessible metastatic disease, patients who have undergone clinically indicated biopsy or surgical resection of metastatic lesions prior to enrollment will also be eligible provided that: 1) They were off any systemic therapy for at least 7 days prior to the biopsy or surgery; 2) They have not received any other systemic therapy since the biopsy or surgical resection; and 3) The tissue has been both paraffin-preserved and frozen as described in section 6.1. A subset of patients will be asked to undergo an on-treatment biopsy between days 7-14. However, this will not be a requirement for participation in the protocol. For those patients who have undergone nephrectomy, the enrolling site will also be asked to submit tumor blocks or at least ten unstained paraffin-embedded slides of the primary tumor for analysis.

All patients will begin everolimus at 10mg once daily. One cycle will be defined as 28 days. Physician and laboratory assessments will occur every 28 days (+/- 2 days). Disease assessment will occur every 2 cycles (q8 weeks +/- 1 week) by either CT or MRI. Patients will continue on therapy until disease progression, intolerable toxicity, or they withdraw consent. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28–42 days) after the decision to discontinue treatment (see Table 3-4 for details of off-study visit). Patients will be followed for a total period of two years following registration.

Dose selection for RAD001

In phase 1 clinical studies of RAD001 as a monotherapy agent in oncology patients, the side-effect profile is essentially mild to moderate adverse events with a low frequency of DLT at the daily dose of 10 mg/d (see Table 1-3).

Based on the PK/PD model, a daily dose of 10mg RAD001 is assumed to provide a persistently high degree of target inhibition in the tumor [Investigators' Brochure-Section 5.3.1.1]. In addition, preliminary data from phase 1 studies, in which changes in molecular characteristics of tumor induced by treatment with RAD001 at the doses of 5 and 10 mg/d were investigated, confirm the pharmacodynamic activity predicted previously by PK/PD modeling [Table 1-2]. Therefore, a dose of 10 mg/d should ensure adequate drug target

inhibition for most patients, taking into consideration the known inter-patient variability in drug levels (CV of approx 50%).

Tumor Sampling

All patients who have not had prior biopsy or metastectomy will undergo either a core needle biopsy or metastectomy of an accessible metastatic lesion prior to beginning therapy. Lesions for core needle biopsy will be limited to subcutaneous and cutaneous lesions, or those otherwise felt by the investigator to be safely accessible as defined above. Core biopsies must be performed with at least a 20 gauge or larger (in size) biopsy needle. Core biopsies can be performed under CT or ultrasound guidance by a radiologist. Four biopsies will be taken of the same metastatic lesion and processed as described in the Correlative Science (Section 6.0).

3.2 Study population

3.2.1 Patient population

A total of 40 patients with metastatic RCC will be enrolled on the study. All patients will have ECOG performance status 0-1. There will be no limit to number of prior therapies, but patients cannot have received prior mTOR inhibitors.

3.2.2 Inclusion and exclusion criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

Inclusion criteria

- Patients must have at least one site of disease which in the opinion of the investigator is safely accessible by CT guided biopsy or metastectomy. Safely accessible metastatic disease will be defined to include those lesions which are palpable with no overlying viscera and are at least 2cm in size (longest dimension). Given the paucity of subcutaneous lesions in RCC, lesions which are felt safe to biopsy in the opinion of the investigator will also be allowed. These lesions will include pleural-based tumors, peripheral liver lesions, kidney lesions and bone lesions with exophytic soft tissue component. As with palpable lesions, these other lesions should be at least 2cm in size (longest dimension) with no overlying viscera.
- In addition to patients with safely accessible metastatic disease, patients who have undergone clinically indicated biopsy or surgical resection of metastatic lesions prior to enrollment will also be eligible provided that: A) They were off systemic therapy at least 7 days prior to the biopsy or surgical resection; B) They have not received any other

systemic therapy since the biopsy or surgical resection; C) the tissue specimens meet the following criteria: 1) All biopsies must be core needle biopsies; specimens obtained by fine needle aspiration are unacceptable. 2) At least one portion of the specimen must be preserved in paraffin. 3) At least one portion of the specimen must be flash frozen in liquid nitrogen.

- Patients must have at least one measurable site of disease, other than the biopsy site, according to RECIST criteria that has not been previously irradiated. If the patient has had previous radiation to the marker lesion(s), there must be evidence of progression since the radiation
- Patients will be required to have metastatic renal cell carcinoma with histologic confirmation by the treating center of either the primary or a metastatic lesion. Non-clear cell histologies will be allowed.
- Age ≥ 18 years
- Minimum of four weeks since any major surgery, completion of radiation, or completion of all prior systemic anticancer therapy (adequately recovered from the acute toxicities of any prior therapy).
- ECOG performance status ≤ 1
- 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
  - INR < 1.3
  - ALT and AST ≤ 2.5x ULN (≤ 5x ULN in patients with liver metastases)
- Adequate renal function: serum creatinine ≤ 2.0 x ULN
- 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.
- Life expectancy greater than 6 months
- Signed informed consent
- As recent studies have shown that treatment with everolimus can result in reactivation of hepatitis B, all patients will undergo screening for hepatitis B infection, either chronic or resolved*. Please see study calendar for screening studies.
- PLEASE NOTE: All patients must be off prior therapy at least 7 days prior to undergoing tumor biopsy on protocol.

* ALL PATIENTS IN ACTIVE TREATMENT WHO ALREADY ENROLLED WILL ALSO BE SCREENED FOR HEPATITIS B INFECTION.

Exclusion criteria

- Prior treatment with any investigational drug within the preceding 4 weeks
- Chronic treatment with systemic steroids or another immunosuppressive agent

- Patients should not receive immunization with attenuated live vaccines within one week of study entry or during study period.
- Uncontrolled brain or leptomeningeal metastases, including patients who continue to require glucocorticoids for brain or leptomeningeal metastases. Treated brain metastases will be allowed. Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection performed within 3 months prior to Day 1 will be excluded.
- Other malignancies within the past 3 years except for adequately treated carcinoma of the cervix or basal or squamous cell carcinomas of the skin.
- Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as:
    - unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤ 6 months prior to first study treatment, serious uncontrolled cardiac arrhythmia
    - severely impaired lung function
    - any active (acute or chronic) or uncontrolled infection/ disorders.
    - nonmalignant medical illnesses that are uncontrolled or whose control may be jeopardized by the treatment with the study therapy
    - liver disease such as cirrhosis, chronic active hepatitis or chronic persistent hepatitis
- Uncontrolled diabetes mellitus as defined by fasting serum glucose >1.5 X ULN
- A known history of HIV seropositivity. Inhibitors of mTOR have been known to have immunosuppressive effects making its safety in the population of patients questionable.
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of RAD001 (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection)
- Patients with an active, bleeding diathesis or on systemic anticoagulation (i.e. warfarin, heparin, lovenox, etc). Aspirin is permitted.
- Women who are pregnant or breast feeding, or women/men able to conceive and unwilling to practice an effective method of birth control. (Women of childbearing potential must have a negative urine or serum pregnancy test within 7 days prior to administration of RAD001). Oral, implantable, or injectable contraceptives may be affected by cytochrome P450 interactions, and are therefore not considered effective for this study.
- Patients who have received prior treatment with an mTOR inhibitor (e.g. sirolimus, temsirolimus, everolimus).
- Patients with a known hypersensitivity to RAD001 (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

3.2.3 Interruption, modification, or discontinuation of treatment

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If administration of RAD001 must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Table 3-1. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 3.0 (CTCAEv3.0, (http://ctep.cancer.gov/forms/CTCAEv3.pdf)).

All patients will start therapy at 10mg orally once daily. Patients will be permitted to have up to two reductions in therapy prior to discontinuing therapy. These dose levels will be defined as:

Dose Level I: 10mg PO once daily
Dose Level II: 5mg PO once daily
Dose Level III: 5mg PO QOD

Toxicities which are intolerable at dose level III will require discontinuation of study medication. Please see Table 3-1 for directions on holding of study drug. Missed or vomited doses will not be made up.

3.2.4 Patients with history of resolved or inactive Hepatitis B infection

Recent findings suggest that treatment of patients who been previously diagnosed with resolved or active Hepatitis B with everolimus may trigger reactivation of Hepatitis B. Therefore, all patients with a known history of resolved or active Hepatitis B should be treated with lamivudine for at least 14 days prior to beginning therapy on trial and continue treatment throughout duration of study drug treatment. Patients with normal renal function should be treated with lamivudine 100mg PO once daily. Patients with impaired renal function should be treated according to creatinine clearance as follows: CrCl 30-49 mL/min, 100 mg first dose then 50 mg once daily; CrCl 15-29 mL/min, 100 mg first dose then 25 mg once daily; CrCl 5-14 mL/min, 35 mg first dose, then 15 mg once daily; CrCl less than 5 mL/min, 35 mg first dose then 10 mg once daily. Please see attached package insert for potential toxicities associated with lamivudine administration. Lamivudine will be charged to patients insurance company. If not covered by insurance, cost of lamivudine will be covered by the sponsor.

Table 3-1 Criteria for dose-modification in case of suspected RAD001 toxicity and re-initiation of RAD001 treatment

Toxicity	Actions
Non-hematological toxicity	
Grade 2 (except pneumonitis – refer to Table 3-2)	**If the toxicity is tolerable to the patient, maintain the same dose. If the toxicity is intolerable to patient, interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at same dose. If event returns to grade 2, then interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level.**
Grade 3 (except pneumonitis – refer to Table 3-2)	**Interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level. For pneumonitis consider the use of a short course of corticosteroids.**
Grade 4	**Discontinue RAD001.**
Hematological toxicity	
Grade 2 Thrombocytopenia (platelets <75, ≥ 50x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (>75 x10^9/L). Then reintroduce RAD001 at initial dose. If thrombocytopenia again returns to grade 2, interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level.**
Grade 3 Thrombocytopenia (platelets <50, ≥ 25 x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (platelets ≥ 75 x10^9/L). Then resume RAD001 at one dose level lower. If grade 3 thrombocytopenia recurs, discontinue RAD001.**
Grade 4 Thrombocytopenia (platelets < 25 x10^9/L)	**Discontinue RAD001.**
Grade 3 Neutropenia (neutrophils <1, ≥0.5 x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (neutrophils ≥ 1.5 x 10^9/L). Then resume RAD001 at the initial dose. If ANC again returns to Grade 3, hold RAD001 until the ANC ≥ 1.5 x 10^9/L. Then resume RAD001 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 RAD001 until recovery to grade ≤ 1 (neutrophils ≥ 1.5 x 10^9/L). Then resume RAD001 at the lower dose level. If grade 3 or grade 4 neutropenia occurs despite this dose reduction, discontinue RAD001.**
Grade 3 febrile neutropenia (not life-threatening)	**Interrupt RAD001 until resolution of fever and neutropenia to grade ≤ 1. Hold further RAD001 until the ANC ≥ 1,500/mm^3 and fever has resolved. Then resume RAD001 at the lower dose level. If febrile neutropenia recurs, discontinue RAD001.**
Grade 4 febrile neutropenia (life-threatening)	**Discontinue RAD001.**

Any hematological or non-hematological toxicity requiring interruption for ≥ 3 weeks	**Discontinue RAD001**

*Grade 2 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies.

3.2.5 Monitoring of RAD001 suspected toxicities

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to RAD001 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.

3.2.6 Known Undesirable Side Effects of RAD001

Adverse events most frequently observed with RAD001 are rash, stomatitis/oral mucositis, fatigue, headache, anorexia, nausea, vomiting, diarrhea and infections. Overall, the most frequently observed laboratory abnormalities include neutropenia, thrombocytopenia, hypercholesterolemia, and/or hypertriglyceridemia. The majority of these AEs have been of mild to moderate severity (CTCAE 3.0 grade 1-2).

Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to RAD001 should be treated using local supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with RAD001 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 (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 RAD001 metabolism, therefore leading to higher RAD001 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.

Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment 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 upper normal limit) should be treated with a statin or appropriate lipid-lowering medication, in addition to diet. Atorvastatin and pravastatin have been shown to have no PK interactions with everolimus and are the preferred statins for this protocol. 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.

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 RAD001 therapy. In many cases the affected patients had an abnormal fasting glucose at baseline. Based on this finding, we suggest that optimal glucose control should be achieved before starting a patient on RAD001 and should be monitored during RAD001 therapy.

Management of non-infectious pneumonitis

Both asymptomatic radiological changes and symptomatic non-infectious pneumonitis have been noted in patients receiving RAD001 therapy. Non-infectious pneumonitis has been associated with RAD001 and other mTOR inhibitors (33). 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 bi-monthly disease evaluations. Additional chest X-rays/CT scans may be done, when clinically necessary. If non-infectious pneumonitis develops, consultation with a pulmonologist should be considered. Management of non-infectious pneumonitis suspected to be associated with RAD001 and dose modifications instructions are provided in Tables 3-1 and 3-2.

Table 3-2 Management of non-infectious pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	RAD001 Dose Adjustment
Grade 1	**CT scans with lung windows.**	**No specific therapy is required**	**Administer 100% of RAD001 dose.**
Grade 2	**CT scan with lung windows. Consider pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to within normal limits. Consider a bronchoscopy.**	**Symptomatic only. Rule out infection. Prescribe corticosteroids if cough is troublesome.**	**Reduce RAD001 dose until recovery to ≤ grade 1. RAD001 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 includes: spirometry, DLCO, and room air O_2 saturation at rest.; Repeat each subsequent Cycle until return to within normal limits. Bronchoscopy is recommended.**	**Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.**	**Hold treatment until recovery to ≤ grade 1. May restart protocol treatment within 3 weeks at a reduced dose (by one level) if evidence of clinical benefit.**
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 within normal limits. Bronchoscopy is recommended.**	**Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.**	**Discontinue treatment.**

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

It will be documented whether or not each patient completed the clinical study. If for any patient either study treatment or observations were discontinued the reason will be recorded. Reasons that a patient may discontinue participation in a clinical study are considered to constitute one of the following:

1. adverse event(s)
2. abnormal laboratory value(s)
3. abnormal test procedure result(s)
4. disease progression
5. protocol violation
6. subject withdrew consent
7. lost to follow-up
8. administrative problems

9. death

3.3 Treatments

3.3.1 Investigational therapy

The investigational therapy used in the course of this study is RAD001. Study medication will be administered by the patients themselves. During the study, RAD001 will be administered orally as once daily dose of 10mg continuously from study day 1 until progression of disease or unacceptable toxicity.

Medication labels will comply with US legal requirements and 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.

RAD001 will be provided by Novartis. RAD001 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.

RAD001 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 RAD001 intake should be as consistent as possible throughout the study.

3.3.2 Concomitant therapy

Patients must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator. All medications taken within 30 days of screening should be recorded. If concomitant therapy must be added or changed, the reason and name of the drug/therapy should be recorded.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (e.g. antiemetics +/- steroids), with the following exceptions:

- no other investigational therapy should be given to patients
- no chronic treatment with systemic steroids or another immunosuppressive agent
- no anticancer agents other than the study medications administered as part of this study protocol should be given to patients. If such agents are required for a patient then the patient must first be withdrawn from the study.
- leukocyte growth factors (e.g.G-CSF and GM-CSF) are not to be administered systematically but may be prescribed by the investigator for severe neutropenia if this is thought to be appropriate.
- no live vaccines should be administered to patient due to immunosuppressant potential of RAD001.

- drugs or substances known to be inhibitors or inducers of the isoenzyme CYP3A should be avoided in association with RAD001 as these can alter metabolism. Strong inhibitors or inducers of the isoenzyme CYP3A should not be administered as systemic therapy (See Table 3-3). Grapefruit juice should be avoided while on study.

The investigator should instruct the patient to notify the study staff about any new medications he/she takes after the start of the study drug. All medications (other than study drug/s) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts treatment with study drug/s must be recorded.

Table 3-3 Clinically relevant drug interaction: substrates, inducers and inhibitors of isoenzyme CYP3A.

Substrates (competitive inhibition)	
Antibiotics[1]: clarithromycin* erythromycin telithromycin* Anti-arrhythmics: quinidine Benzodiazepines: alprazolam diazepam midazolam triazolam Immune Modulators: cyclosporine tacrolimus (FK506) HIV Protease Inhibitors: indinavir* ritonavir* saquinavir* Prokinetic: cisapride Antihistamines: astemizole chlorpheniramine	Calcium Channel Blockers: amlodipine diltiazem felodipine nifedipine nisoldipine nitrendipine verapamil HMG CoA Reductase Inhibitors[2]: atorvastatin cerivastatin lovastatin simvastatin Miscellaneous: aprepitant buspirone haloperidol methadone pimozide quinine sildenafil tamoxifen trazodone vincristine
Inducers	
Carbamazepine Phenobarbital Phenytoin* Rifabutin*	Rifampin* St John's wort Troglitazone
Inhibitors	
Amiodarone Cimetidine Clarithromycin Delaviridine Diltiazem Erythromycin Fluvoxamine* Grapefruit juice Sevilla orange	Indinavir Itraconazole* Ketoconazole* Voriconazole* Posaconazole* Mibefradil Nefazodone* Nelfinavir* Troleandomycin Verapamil

Based on: Ingelman-Sundberg M, Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms, Naunyn Schmiedebergs Arch Pharmacol. 2004 Jan;369(1):89-104. and [http://www.medicine.iupui.edu/flockhart/clinlist.htm as of July 13, 2006]

* asterisk denotes strong inhibition/ induction

Please note:

- strong inhibitor implies that it can cause ≥5-fold increase in AUC or ≥80% decrease in clearance of sensitive CYP substrates
- moderate inhibitor implies that it can cause 2 to 5-fold increase in AUC values or 50-80% decrease in clearance of sensitive CYP substrates.

  (Distinction is not always categorical as interaction can vary according to conditions).

1. Macrolide antibiotics: Azithromycin is not a CYP3A substrate. It may therefore be employed where

antibiotherapy with a macrolide is desirable in a patient being treated with RAD001

2. Statins: Atorvastatin and pravastatin may be associated with RAD001, since a PK interaction study has shown that there is no relevant PK interaction.

3.3.3 Treatment compliance

Records of study medication used, dosages administered, and intervals between visits will be recorded during the study. Drug accountability will be determined and noted by pill count and patients will be asked to return all unused study medication at the beginning of each cycle.

3.4 Visit schedule and assessments

3.4.1 Visit schedule

Table 3-4 Evaluation and visit schedule

	Table 3-4: Study Evaluation Calender														
	Pre-Study[h]	Wk 1 (+/- 2 days)	Wk 2	Wk 3	Wk 4	Wk 5 (+/- 2 days)	Wk 6	Wk 7	Wk 8	Wk 9 (+/- 2 days)	Wk 10	Wk 11	Wk 12	Wk 13 (+/- 2 days)	Off Study[g]
Informed consent	X														
Demographics	X														
Medical history	X	X				X				X				Q4wks	
Physical Exam	X	X				X				X				Q4wks	X
ECG	X														
Vital Signs	X	X				X				X				Q4wks	X
Performance status	X	X				X				X				Q4wks	X
CBC w/diff, plts	X	X				X				X				Q4wks	
Serum chemistry[a]	X	X				X				X				Q4wks	
PT and INR	X	X				X				X				Q4wks	
Urinanalysis	X	X				X				X				Q4wks	
Hepatitis B Screening[I]	X														
Serum pregnancy[b]	X														
Study Blood[c]	X	X[d]				X								Q8wks	
Tumor Sampling	X[e]	X[e,f]													
Adverse event evaluation	X	X ----------													X
Tumor measurements	X	Tumor measurements are repeated every 8 weeks (+/- one week). Documentation (radiological) must be provided for patients removed from study for progressive disease.													X
Radiologic evaluation	X	Radiologic measurements should be performed every 8 weeks. If CT of chest is not done than recommend CXR q 8 wks													X

a. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, sodium, potassium, total protein, SGOT [AST], SGPT [ALT], uric acid, magnesium, fasting (6 hours) serum cholesterol, fasting (6 hours) serum triglycerides.
b. Within 72 hours prior to initiation of therapy for women of childbearing potential only.
c. Submit one 7cc CPT tube
d. Pre-study blood can be submitted either during screening or on Day 1.
e. Either a core needle biopsy or metastectomy of an accessible metastatic lesion prior to beginning therapy.
f. Second biopsy optional
g. Off study visit should be between 28-42 days from day of discontinuation from the study.
h. All pre-study evaluations must be performed ≤ 28 days prior to the start of treatment.
I. Hepatitis screening labs should include: HepB surface Ab, HepB surface antigen, HepB Core Ab. All active patients currently enrolled on this study will also need to be tested for hepatitis B infection.

3.4.2 Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for response until progression and for survival for 2 years from the date of registration. Following the off-study visit, the study team will update survival data every 6 months for up to three years.

Patients who have an ongoing everolimus-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible. Otherwise, there will no specific follow-up visits or studies following the off-study visit.

3.4.3 Efficacy assessments

Tumor measurements will occur every 8 weeks (+/- 1 week) on study by either CT or MRI. RECIST criteria will be used to classify response.

Patients who prematurely exit the study should have the required evaluations performed at the time study drug is discontinued and before initiation of any other treatment. Physical examination, performance status (PS) determinations, and all laboratory tests should be repeated on the last day of study if these have not been evaluated within 14 days, inclusive, before discontinuation. Repeat measurements or evaluations of all tumor sites should be performed if these have not been evaluated within 30 days, inclusive, before discontinuation.

3.4.3.1 Solid Tumor Response Criteria (RECIST)

Malignant Disease Evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All baseline evaluations should be

performed as closely as possible prior to registration and **never more than four weeks** before registration.

The term evaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy. At baseline, tumor lesions will be characterized as either measurable or non-measurable.

Measurable

Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as > 20 mm (2.0 cm) with conventional techniques or as > 10 mm (1.0 cm) with **spiral** CT scan.

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Non-Measurable

All other lesions, including small lesions [longest diameter < 20 mm (2.0 cm) with conventional techniques or < 10 mm (1.0 cm) with **spiral** CT scan] and truly non-measurable lesions.

Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

3.4.3.2 Definition of Response

3.4.3.2.1 Target Lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs. Target lesions should be selected on the basis of their size (those with the longest diameters) and their suitability for accurate repeated measurements

The sum of the longest diameters of all target lesions will be calculated at baseline and reported as the bas*eline sum longest diameter*. The *sum longest diameter* will be used to characterize the objective tumor response. For lesions measurable in 2 or 3 dimensions, always report the longest diameter at the time of each assessment.

Complete Response (CR)

The disappearance of all target lesions. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Partial Response (PR)
At least a 30% decrease in the sum of the longest diameters of target lesions, taking as reference the *baseline sum longest diameter*. To be assigned a status of partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Progressive Disease (PD)
At least a 20% increase in the sum of the longest diameters of target lesions, taking as reference the *smallest sum longest diameter* recorded since the baseline measurements, or the appearance of one or more new lesion(s).

Stable Disease (SD)
Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval.

3.4.3.2.2 Non-Target Lesions

All other lesions or sites of disease not meeting definition of target lesions will be classified as non-target lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Complete Response (CR)
The disappearance of all nontarget lesions and normalization of tumor marker levels, if applicable. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

Incomplete Response/Stable Disease (SD)
The persistence of one or more nontarget lesion(s) and/**or** the maintenance of tumor marker levels above the normal limits. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval at a minimum interval of 8 weeks.

Progressive Disease (PD)
Appearance of one or more new lesion(s) and/or unequivocal progression of existing non-target lesions.

3.4.3.3 Symptomatic Deterioration

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having symptomatic deterioration.

3.4.3.4 Evaluation of Patient's Best Overall Response

The best overall response is the best response recorded from registration until disease progression/recurrence, taking as reference for progressive disease the smallest measurements recorded since registration. The table below provides overall responses for all possible combinations of tumor responses in target and nontarget lesions, with or without new lesions.

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Overall Response for all Possible Combinations of Tumor Response

Target Lesions	Nontarget Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

Documentation of Response
The time between initiation of therapy and first documentation of PR or CR.

Confirmation of Response
To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

3.4.3.5 Duration of Response

Duration of overall response – the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the first date that progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

Duration of Complete Response
The period measured from the time measurement criteria are met for complete response until the first date that progressive disease is objectively documented.

Duration of Stable Disease
The period measured from registration on to the study until the criteria for disease progression is met, taking as reference the smallest measurements recorded since registration. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of eight weeks.

Survival
Survival will be measured from the date of registration on to the study.

Progression Free Survival
Progression-free survival will be measured from the date of registration on to the study until the first date that progressive disease is objectively documented or death from any cause, whichever occurs first.

3.4.3.6 Methods of Measurement

Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality must be used throughout the study to measure disease.

CT and MRI
CT and magnetic resonance imaging (MRI) are the best currently available and most reproducible methods for measuring target lesions. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed by use of a 5 mm contiguous reconstruction algorithm. This specification applies to tumors of the chest, abdomen, and pelvis, while head and neck tumors, and those of the extremities require specific procedures.

3.4.4 Safety assessments

Please see the DF/HCC Multi-Center Data and Safety Monitoring Plan in Appendix C. Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology, blood chemistry and urine values, regular measurement of vital signs and the performance of physical examinations. See multi-center Data Safety Monitoring Plan (Appendix C) for more details.

These assessments should be performed within ±2 days of the scheduled day of assessment except for adverse events that will be evaluated continuously through the study. Safety and tolerability will be assessed according to the NIH/NCI CTCAE version 3.0 http://ctep.cancer.gov/forms/CTCAEv3.pdf.

3.4.4.1 Adverse events

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. the severity grade (grade 1: mild; grade 2: moderate; grade 3: severe; grade 4: life threatening; grade 5:fatal)
2. attribution: the determination of whether an adverse event is related to a medical treatment or procedure

   Attribution categories:
   Definite: The adverse event is clearly related to the investigational agent(s).
   Probable: The adverse event is likely related to the investigational agent(s).
   Possible: The adverse event may be related to the investigational agent(s).
   Unlikely: The adverse event is doubtfully related to the investigational agent(s).
   Unrelated: The adverse event is clearly NOT related to the investigational agent(s)
3. its duration (start and end dates or if continuing at final exam)
4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)

5. whether it constitutes a serious adverse event (SAE)

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the Investigators' Brochure or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

3.4.4.2 Serious adverse events

Information about all serious adverse events will be collected and recorded. To ensure patient safety each serious adverse event must also be reported to the overall principal investigator within 24 hours of learning of its occurrence. The overall principal investigator will then report the event to Novartis and to the Office for Human Research Studies (OHRS). A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

3.4.5 Reporting of Adverse Events

The DFCI Institutional Review Board (IRB) requires the following events to be reported:

- Grade 2 (moderate) and Grade 3 (serious) Events -- Only events that are Unexpected and Possibly, Probably or Definitely Related/Associated with the Intervention.
- ALL Grade 4 (life-threatening) events.
- ALL Grade 5 (Fatal) Events -- When subject is enrolled and actively participating in the trial *or* when event occurs within 30 days of the last study intervention.

Full written SAE report must be submitted to the Office for Human Research Studies (OHRS) as soon as possible, but no later than 10 working days from notification of event. DF/HCC institutions should electronically submit SAE reports via DFCI SAE reporting forms directly to the OHRS. For participating institutions outside the DF/HCC, the overall principal investigator must be notified electronically of any events meeting the above requirements, even if they are not required to be reported by their local IRB. All events should also be reported to the local IRB as per their respective requirements.

- If subject is in Long-Term Follow Up, death is reported at continuing review.

3.4.6 Instructions for rapid notification of serious adverse events

The overall principal investigator has the obligation to report all serious adverse events to the FDA, DFCI IRB, and Novartis Pharmaceuticals Clinical Safety and Epidemiology Department (CS&E).

All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form). This form can be downloaded at:
http://www.fda.gov/medwatch/REPORT/Mfg.htm[fordrugs/biologics]

All events must be reported electronically to the overall principal investigator within 24 hours of learning of its occurrence. The overall principal investigator will notify Novartis Pharmaceuticals CS&E Department by FAX (888-299-4565) also 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.

3.4.6.1 Pregnancies

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.

3.4.6.2 Laboratory evaluations

Hematology

Hematology must include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. PT (INR) evaluation will be included for baseline evaluations.

Blood chemistry

Blood chemistry must include sodium, potassium, chloride, bicarbonate, calcium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, phosphorus, magnesium, LDH, fasting serum triglycerides, and fasting total cholesterol, hepatitis B screening (HepB surface Ab, HepB surface antigen, HepB Core Ab).

Because accurate serum glucose and lipid measurements are required, patients should be fasting at least 6 hours at the time of the blood sampling.

ALL PATIENTS IN ACTIVE TREATMENT WHO ALREADY ENROLLED AND PATIENTS BEING SCREENED FOR THIS STUDY WILL BE TESTED FOR HEPATITIS B INFECTION.

Urinalysis

Standard urinalysis assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. This must be supplemented with laboratory quantification of any potentially relevant abnormalities.

3.4.6.3 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

3.4.6.4 Physical examination

Physical examination will be performed which must comprise a total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and basic nervous system).

Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded.

3.4.6.5 ECG

A standard 12 lead ECG is to be performed during screening and significant findings must be recorded.

3.4.6.6 Performance status

Performance status will be assessed using the ECOG scale.

3.4.7 Drug levels and pharmacokinetic assessments

None will be made

4 Protocol amendments, or changes in study conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed by Novartis and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. A copy of the written approval of the IRB must be provided to Novartis. Examples of amendments requiring such approval are:

1. increases in drug dose or duration of exposure of subjects,
2. significant changes in the study design (e.g. addition or deletion of a control group),
3. increases in the number of invasive procedures,
4. addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include:

1. changes in the staff used to monitor trials
2. minor changes in the packaging or labeling of study drug.

5 Registration Procedures

5.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

5.2 Registration Process for DF/HCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. If a participant must be registered during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

   exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

5.3 General Guidelines for other Participating Institutions

Eligible participants will be entered on study centrally at the BIDMC by the Study Coordinator (Bryan Marion). All sites should call the Study Coordinator at [617-632-9271] to verify treatment availability. The required eligibility checklist can be found in Appendix D.

Following registration, participants should begin protocol treatment within 72 hours or as soon as possible. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. The Study Coordinator should be notified of participant status changes as soon as possible.

Except in very unusual circumstances, each participating institution will order the study agent(s) directly from the supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the supplier.

Any other questions regarding the protocol can be addressed to the overall Principal Investigator: David F. McDermott, MD (617-632-9250; dmcdermo@bidmc.harvard.edu).

5.4 Registration Process for Other Participating Institutions

To register a participant, the following documents should be completed by the research nurse or data manager and faxed to (617-632-9260) or emailed to the Study Coordinator at (bmarion@bidmc.harvard.edu):

- Copy of required laboratory tests
- Signed study consent form
- HIPAA authorization form
- Other appropriate forms (e.g., Eligibility Screening Worksheet, Registration form)

The research nurse or data manager at the participating site will then call (617-632-9271) or email the Study Coordinator at (bmarion@bidmc.harvard.edu) to verify eligibility. To complete the registration process, the Coordinator will:

- Register the participant on the study with QACT
- Fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site

- Call the research nurse or data manager at the participating site and verbally confirm registration

6 Correlative Studies

Correlative studies appended to this clinical protocol include those proposed in tissue specimens and blood collected serially during treatment.

6.1 Studies proposed in tissue

All patients who have not had prior biopsy of a metastatic lesion or metastectomy will undergo either a core needle biopsy or metastectomy of an accessible metastatic lesion prior to beginning everolimus. A subset of patients will be asked to undergo an on-treatment biopsy of the same metastatic lesion between days 7-14 on-study medication. This second biopsy will not be a requirement for participation in the protocol. These on-treatment biopsies will handled and analyzed identically to the pre-treatment biopsies (on-protocol) and will provide an opportunity to assess biologic target inhibition (i.e. suppression of S6 phosphorylation) and pharmacodynamic effects (i.e. changes in eIF4E phosphorylation and Cyclin D1 levels) of drug treatment. We anticipate 6-10 patients will agree to undergo an on-treatment biopsy. At teach time point, four core-needle biopsies will be taken of the same metastatic lesion. One will be immediately placed in formalin, one will be promptly frozen in liquid nitrogen, and two will be placed in OCT and frozen. Samples frozen in liquid nitrogen will ultimately be shattered and the powder dissolved equally into protein lysis buffer and trizol for RNA isolation (followed by RNAeasy cleanup, Qiagen). Samples stored in OCT will be ultimately analyzed via microarray analysis. If for some reason only one core biopsy can be obtained, preference will be given to formalin fixation for immunohistochemical studies. In cases of metastectomy, the specimen will be divided into thirds. One piece will be placed in formalin and processed as decribed below. Another piece will be stored in OCT and the final piece will be frozen separately in liquid nitrogen and stored at -80ºC.

For patients who have undergone clinically indicated biopsy or surgical resection of metastatic lesions prior to enrollment, these specimens must meet the following criteria for these patients to be eligible: A) All biopsies must be core needle biopsies; specimens obtained by fine needle aspiration are unacceptable. B) At least one portion of the specimen must be preserved in paraffin. C) At least one portion must be flash frozen in liquid nitrogen.

Formalin fixed (or paraffin embedded) specimen should be shipped by overnight courier at room temperature to the DF/HCC RCC SPORE Pathology Core using the shipping form in Appendix A. Frozen specimens should be shipped on dry ice also the DF/HCC SPORE Pathology CORE using the form in Appendix A. Frozen specimens can also be stored at -80ºC and batch shipped.

For patients who have undergone nephrectomy, the enrolling site will be asked to obtain and submit the tumor block or at least 10 unstained paraffin-embedded slides of the primary

tumor. These are not required for enrollment on the study and can be submitted at any point during study enrollment using the form in Appendix A.

6.1.1 Phospho-Akt and Phospho-S6 Expression

5μm thick sections will be prepared from formalin-fixed tumor specimens and stained with hematoxylin and eosin. Sections will then be dewaxed, soaked in alcohol, and after microwave treatment in antigen unmasking solution for 10 minutes and then incubated in 3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase. Sections will next be incubated with the appropriate either the Phospho-Akt (Ser^{473}) or Phospho-S6 Ribosomal Protein ($Ser^{235/236}$) antibodies from Cell Signaling Technology, Beverly, MA. Following this, detection of expression will be performed using DAKO EnVision™+ System horseradish peroxidase detection kit (Dako, Carpinteria). Semi-quantitative assessment of antibody staining will be performed by a single pathologist blinded to the clinicopathologic variables. Expression of phospho-S6 will be scored based on a composite of staining intensity (graded 0=1+, 1=2+, 2=2-3+, and 3=3+) and percentage of tumor cells staining positive (1=1-29%, 2=30-69%, 3=70-100%). Patients will then be classified as low (product of intensity and staining percentage score = 0-1), intermediate (2-3), and high (4-9) expressors of pS6. Phospho-Akt expression will be similarly scored based on a composite of intensity (graded 0=1+, 1=1-2+, and 2= 2+ or 2-3+) and percentage of tumor cells positive (1=1-29%, 2=30-69%, 3=70-100%). Patients will then be classified as low (product of intensity and staining percentage score = 0), intermediate (1-2), and high (3-6) expressors of pAkt. The expression of the markers will then be correlated with response to everolimus as determined by PFS. Detection of phospho-Akt and phospho-S6 by IHC will be verified by western blot from protein lysates. Western blots will be performed once at the end of the study when all the biopsy specimens have been obtained and immunohistochemistry has been performed. IHC studies will be performed with the guidance of the Pathology core of the DF/HCC RCC SPORE. The same stains will also be performed in nephrectomy specimens collected. The expression of the substrates in the metastatic and primary tumors will be compared.

6.1.2 Analysis of other biomarkers

Slides will be prepared as above from formalin fixed samples. PML expression will be analyzed by IHC using a commercially available antibody for PML (PGM3, Santa Cruz). For the purpose of the correlative studies outlined below, PML expression/distribution will be categorized as absent, normal (particulate distribution), or increased (diffuse distribution). Phospho-TSC2 (Ser^{664}) expression will be assayed both by IHC using an antibody provided by Dr. Piers Pandolfi. For the purpose of correlative studies, phospho-TSC2 distribution will be categorized as low or high on IHC based on a composite of staining intensity and percentage of tumor cells positive for stain. Phospho-PRAS40, eIF4E, and Cyclin D1 expression will be analyzed by IHC using commercially available antibodies (Cell Signaling, Beverly, MA). Expression will be scored as high or low based on a composite of staining intensity and percentage of tumor cells positive. Localization of FOXO transcription factors will be assessed by IHC using a commercially available antibody (Cell Signaling). FOXO will be classified as predominantly nuclear or cytoplasmic. Expression of PML, phospho-TSC,

eIF4E, Cyclin D1, and phospho-PRAS will be verified by western blot from protein lystates. Expression of these biomarkers thus classified will then be correlated to response to everolimus.

6.1.3 Gene and micro-RNA expression studies

Variations in gene expression patterns may provide insights into mechanisms of response/resistance to mTOR inhibitors. For this reason, depending upon the availability of funding, transcriptional profiling using cDNA microarrays from RNA isolated from frozen specimens will be performed. While these cDNA microarrays may provide part of the picture, it is becoming increasingly clear that post-transcriptional regulation by micro-RNA may play an important role in the ultimate expression of gene products at the translational level. For this reason, also depending upon funding, micro-RNA expression profiling will be performed using RNA isolated from frozen specimens.

In cases of on-study biopsy, three core biopses will be flash-frozen in liquid nitrogen and stored at -80ºC. Samples will be de-identified of any patient data and will be listed by sample number only. The code document linking the sample number to each individual patient will be stored in a locked cabinet in the principal investigator's office. Two of the three core needle frozen biopsies (or one-third of the metastectomy specimen) will be shipped in an insulated container on dry ice via commercial overnight delivery to:

Ranjit Goudar, MD
Nevins Laboratory
CIEMAS Building, Room 2133
101 Science Drive
Durham, NC 27708
919-684-8712

Total RNA will be isolated from frozen tumors using Qiagen Rneasy Mini Kit according to manufacturer's recommendations (Qiagen, Valencia, CA) using approximately 1-2mg of wet tissue from each sample. Total RNA will then be utilized for both cDNA microarrays as well as microRNA arrays. 50ng of total RNA from the qRT-PCR experiments above will be amplified. There will be two rounds of amplification and Biotin-labeled aRNA is generated at the second in vitro transcription step. 15μg of biotin-labeled aRNA is combined with the hybridization mix and loaded on to the Affymetrix Human Genome U133A GeneChip (Affymetrix, Santa Clara, CA). After hybridization, the GeneChip will be washed, stained with Stepavidin/phycoerythrin conjugate and biotinylated antibody, and scanned according to the manufacturer's recommendations. Total RNA will also be sent to Exiqon Inc (Woburn, MA) for micro-RNA array analysis using the miRCURY™ LNA Array.

6.2 Studies to performed on blood

Blood samples will be collected pre-study (or cycle 1 day 1) and day 1 of every other cycle starting with C2 (i.e. C2D1, C4D1, etc). One 7ml CPT tubes should be collected at each time point and submitted by overnight courier as instructed in Appendix B (include shipping form). The peripheral blood will be used in correlative studies investigating the angiogenic effects of treatment as well as surrogates of targeted pathway activation.

6.2.1 Angiogenesis

RCC is known to be a highly vascular tumor type. While mTOR inhibitors are believed to target the process of angiogenesis, the degree to which this effect may correlate with or contribute to tumor responses is unknown. Therefore, studies investigating markers of angiogenesis in the blood before and during therapy are proposed.

Plasma levels of circulating pro-angiogenic cytokines, VEGF, bFGF, TGF-α, and TGF-β will be measured in all patients at the selected time points. These cytokines are chosen because they are prominent pro-angiogenic molecules that are secreted by renal cancer cells.

In addition to more standard ELISA assays, circulating cytokines will be investigated using xMAP technology from Luminex Corporation (Austin, TX) in the Angiogenesis Monitoring Core Lab of the DF/HCC Renal Spore. This is a novel bioassay system in which microspheres are made with internal dyes (red/infrared) and coated with a reagent specific to a certain bioassay (i.e. for circulating VEGF). The internal dye mix is specific for the particular assay of interest and allows its identification based on its laser excitation pattern. The reporter dye from the microsphere coating then allows for the detection and quantification of the protein of interest. The Luminex technology allows for the rapid analysis of very small quantities of serum for simulataneous measurement of up to 100 different proteins of interest with sensitivity comparable to standard ELISA. The access to such technology will allow the rapid exploration of more novel angiogenic cytokines such as SDF-1 and Angiopoietin-like 4 in the context of these antiangiogenic therapies.

6.2.2 HIF activity

Hypoxia-driven increase in HIF activity may be a mechanism of resistance to VEGF-targeted therapies. As the extent to which increased HIF activity is present and effected by the everolimus is unknown, potential surrogates of HIF activity will be measured from serum at the selected time points. Levels of serum erythropoietin and carbonic anhydrase IX will be measured and correlated with response to everolimus.

6.2.3 Peripheral blood cell signature

RNA will be isolated from PBMCs isolated from CPT tubes drawn pre-study. PBMCs will be isolated as described in Appendix D, frozen viably in freezing media (95% Fetal Calf Serum, 5% DMSO), and stored at -80°C or in liquid nitrogen. RNA will be isolated from PBMCs using Trizol® reagent. Briefly, frozen PBMCs will thawed, pelleted, washed once in PBS and pelleted again. Cells will be lysed in 1ml of Trizol® and incubated at room temperature for 5 minutes. 200µl of chloroform will be added to each sample, which will then be shaken vigorously for 10-15 seconds, incubated at room temperature for 5 minutes, and centrifuged at no more than 12,000 G for 15 minutes at 2 to 8°C. Following centrifugation, the mixture will separate into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The aqueous (clear) phase will be transferred to a separate tube and RNA precipitated by the addition of 0.5ml of isopropyl alcohol. After addition of isopropyl alcohol, samples will be incubated at room temperature for 10 minutes, then centrifuged at no more than 12,000 G for 15 minutes at 2 to 8°C. The resulting pellets will be washed once with 70% ethanol, air-dried, dissolved in RNase-free water, and stored at -80°C. At the conclusion of the study, RNA thus isolated should be batch shipped to for analysis:

Ranjit Goudar, MD
Nevins Laboratory
CIEMAS Building, Room 2133
101 Science Drive
Durham, NC 27708
919-684-8712

50ng of total RNA will be utilized for microarray expression analysis. The RNA is amplified twice, and biotin-labeled aRNA is generated at the second in vitro transcription step. 15µg of biotin-labeled aRNA is combined with the hybridization mix and loaded on to the Affymetrix Human Genome U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA). After hybridization, the GeneChip will be washed, stained with streptavidin/phycoerythrin conjugate and biotinylated antibody, and scanned according to the manufacturer's recommendations. Expression data will be analyzed using published protocols (49, 50).

7 Data and safety management

7.1 Data collection

Investigators or their designee must enter the information required by the protocol onto electronic Case Report Forms (eCRFs). The QACT will collect, manage, and monitor for this study.

7.2 Data and safety management

Please see the full DF/HCC Multi-Center Data and Safety Monitoring Plan in Appendix C. This Phase II trial will be managed through the DF/HCC review and monitoring systems and therefore are subject to the Institutional Data and Safety Monitoring Plan (DSMP) that has been approved by NCI. This plan outlines the extensive Scientific Review Process, Institutional Review Board's (IRB) review and monitoring of Adverse Events, and Internal Auditing. A Data and Safety Monitoring Committee (DSMC) reviews patient safety data for high risk Phase II trials**.** The risk level is determined with the help of the disease programs and the committee. The DSMC has representatives from different modalities, disease programs and institutions. The DSMB meetings occur semi-annually and the DSMC meetings occur quarterly for high-risk protocols. The committees will notify the Principal Investigator of safety issues that need to be addressed. In the case of any unexpected safety problems, the PI and/or the DSMB or DSMC have the option to call an unscheduled meeting. A summary of the DSMB and DSMC meetings is shared with the Clinical Investigations Policy and Oversight Committee (CLINPOC) and the IRB. Details of the review are maintained in the Quality Assurance Office for Clinical Trials' files.

8 Statistical methods

This is a single-arm phase II study. Forty patients will be enrolled; expected enrollment is 2-3 patients per month over 13-20 months. Patients will be followed for up to two years.

The biomarkers of primary interest are pAkt and pS6. Expression of phospho-S6 and phospho-Akt will be classified as low, intermediate, or high based on a composite score of staining intensity and percentage of tumor cells staining positive as previously described for temsirolimus [35]. Based on prior analysis for temsirolimus we assume that approximately 50% of patients will have tumors with high pS6 expression and/or high pAkt and therefore be classified in the favorable group. We assume 4 months median progression-free survival (PFS) in this cohort of patients. The primary endpoint will be considered met if the median PFS of favorable versus unfavorable group is $\geq$ 4 months.

When 26 events have been observed, a hazard ratio of 3.0 (2m vs. 6m median PFS) for biomarker low/intermediate (unfavorable) vs. high (favorable) groups would be detectable with 80% power (two-sided α=0.05) [George and Desu, 1974].

Kaplan-Meier estimates of PFS distribution according to biomarker groups will be calculated, and Cox modeling used to estimate HRs and compare PFS between biomarker groups. PML, pTSC2, pPRAS40, and FOXO localization will be analyzed similarly, though the expected proportions of absent/normal/increased PML or low/high pTSC2 are uncertain a priori.

The tumor biopsy expression data will be analyzed to create a genomic predictor of everolimus response. The genes whose level of expression are statistically significantly different between everolimus responders and nonresponders will be used to generate a metagene summary model.[48] Leave-one-out cross-validation will be performed to strengthen

the metagene model. Binary classification tree analysis will be used to predict a probability that a given subject will respond to everolimus.[50] This signature can then be validated in an external data set.

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10 Procedures and instructions

i. Publication of results

Any formal presentation or publication of data from this trial may be published after review and comment by Novartis and prior to any outside submission. Novartis must receive copies of any intended communication in advance of publication (at least fifteen working days for presentational materials and abstracts and thirty working days for manuscripts). These requirements acknowledge Novartis' responsibility to provide peer input regarding the scientific content and conclusions of such publications or presentations. Principal Investigation/Institution shall have the final authority to determine the scope and content of its publications, provided such authority shall be exercised with reasonable regard for the interests of Novartis and, in accord with the trial contract and shall not permit disclosure of Novartis confidential or proprietary information.

ii. Disclosure and confidentiality

The investigator agrees to keep all information provided by Novartis in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided by Novartis (investigators' brochures and other material) will be stored appropriately to ensure their confidentiality. The information provided by Novartis to the investigator may not be disclosed to others without direct written authorization from Novartis, except to the extent necessary to obtain informed consent from patients who wish to participate in the trial.

iii. Discontinuation of study

Novartis reserves the right to discontinue any study under the conditions specified in the clinical trial agreement.

b. Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice, as described in Novartis standard operating procedures and:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki and amendments, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

i. Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation. Any amendments to the protocol, other than administrative ones, must be reviewed by Novartis approved by this committee.

ii. Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval.

iii. Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki. Copies of the Declaration of Helsinki and amendments will be provided upon request or can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

Appendix A

Phase II RAD001 Biomarker Trial RCC
TISSUE SPECIMEN SHIPMENT FORM

Instructions: Send overnight Sunday through Thursday to:

DF/HCC Renal Cancer SPORE Tissue Bank
c/o Sabina Signoretti, M.D. Department of Pathology
Brigham and Women's Hospital
Thorn Building 504A
75 Francis Street
Boston, MA 02115
617-525-7438 (Michael Collins)

PROTOCOL:
Case No.: 00__ __ __

01 - BIDMC
02 – DFCI
03 - Duke

Subject Initials __ __ __ (F,M,L) Site #:_____

Contact Name: _____

Contact Phone: _____

Date of Shipment: _____

Shipping Conditions: _____
(indicate dry ice, room temp, etc)

Sample Sent: _____
(indicate total amount: i.e. one core biospy)

Surgical Path Report Enclosed: **YES** **NO**

Nephrectomy Specimen **YES** **NO**

Fomalin Fixed Biospy: **YES** **NO**

Frozen Tissue Biopsy: **YES** **NO**

Shipping Notes:
Please fax form in advance to 617-264-5169 ATTN: Sabina Signoretti, M.D.
Please include a copy of the form in the shipping container.

BWH Staff Only:
Specimen Received by: _____
Date/Time Received: _____

Appendix B

Phase II RAD001 Biomarker Trial RCC
BLOOD SPECIMEN SHIPMENT FORM

Instructions: Send overnight Sunday through Thursday to:

DF/HCC Renal SPORE Immune Monitoring Core
c/o David Panka, PhD
330 Brookline Avenue; RW 571
Boston, MA 02215
617-667-0428

PROTOCOL:
Case No.: 00__ __ __

01 - BIDMC
02 – DFCI
03 - Duke

Subject Initials __ __ __ (F,M,L) Site #:_____

Contact Name: _____

Contact Phone: _____

Date of Shipment: _____

Shipping Conditions: Room Temperature_____

Sample Sent: One CPT tube

YES **NO**

Shipping Notes:
Please fax form in advance to 617 632-9260, ATTN: Bryan Marion
Please include a copy of the form in the shipping container.

For IMC Staff Only:
Specimen Received By: _____

Date/Time Received: _____

APPENDIX C

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

Protocol #: 08-313

Table of Contents

1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for a DF/HCC Multi-Center research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center (DF/HCC) Multi-center protocol will comply with Federal regulations (21 CFR Part 11); Good Clinical Practice (GCP) Guidelines; and Health Insurance Portability and Accountability Act (HIPAA) requirements in accordance with the CTEP Multi-center Guidelines.

1.2 Multi-Center Data and Safety Monitoring Plan Components

The Multi-Center Data and Safety Monitoring Plan includes the following components:

DF/HCC Multi-center Protocol: One or more outside institutions collaborating with Dana-Farber/Harvard Cancer Center on a research protocol where DF/HCC is the Lead Institution.

Lead Institution: The BIDMC will be the Lead Institution and will be responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and the FDA.

DF/HCC Contract Principal Investigator: Dr. David McDermott located at the BIDMC will be charged with the responsibility of the administration of the DF/HCC Project.

Protocol Chair: The Protocol Chair is the Principal Investigator (Dr. David McDermott). Dr. McDermott will be the single liaison with any regulatory agencies the FDA.

Participating Institution: A participating institution is an institution that desires to collaborate with DF/HCC and commits to accruing participants to a DF/HCC protocol. The participating institution acknowledges the Protocol Chair as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The BIDMC is the Coordinating Center for the DF/HCC Multi-center Protocol. The Coordinating Center will provide the administrative support to the Protocol Chair in order that he/she may fulfill the responsibilities outlined in the DSMP and as specified in applicable regulatory guidelines (ie. CTEP Multi-Center Guidelines). In addition to the Lead Institution, the Quality Assurance Office for Clinical Trials (QACT) provides support services to assist the Protocol

Chair.

2.0 GENERAL ROLES AND RESPONSIBILITIES

In accordance with the CTEP Multi-center Guidelines, the Protocol Chair (Dr. David McDermott), Coordinating Center (BIDMC), and the Participating Institutions will all agree to the general responsibilities as follows (specific procedures for these general responsibilities are detailed in the DSMP):

2.1 Protocol Chair (DF/HCC Principal Investigator)

Dr. Cho will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan to:

1) Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
2) Submit the Multi-Center Data and Safety Monitoring Plan as an inclusion to the protocol.
3) Assure all participating institutions are using the correct version of the protocol.
4) Monitor progress and overall conduct of the study at all participating institutions.
5) Ensure all DFCI IRB, DF/HCC and FDA reporting requirements are met.
6) Review data and maintain timely submission of data for study analysis.
7) Act as the single liaison with the FDA (sponsor-investigator IND trials).
8) Identify participating institutions and obtain accrual commitments. Dr. Cho will also submit a protocol attachment labeled as "Participating Investigators" to the DFCI IRB and FDA that provides the names and contact information for all participating institutions that perform the function of recruiting, enrolling, and treating participants for the protocol. The Coordinating Center (BIDMC) must be designated on the title page. Revisions to the list will be submitted to the DFCI IRB and FDA as an administrative protocol amendment to reflect changes in staff and assignment of responsibility as they occur.

2.2 Coordinating Center (BIDMC)

The Coordinating Center is the DF/HCC Lead Institution's (BIDMC) study team. The BIDMC will ensure that all participating sites within the Multi-Center Protocol demonstrate their intent and capability of complying with Federal Regulations, GCPs and Health Insurance Portability and Accountability Act (HIPAA) requirements. To assist the Protocol Chair in meeting his/her responsibilities as required by the DSMP, the BIDMC's study team or designee will assume the following general responsibilities:

- Assist in protocol review.
- Maintain copies of Institutional Review Board (IRB) approvals.
- Maintain FDA correspondence.
- Maintain updated roster of participants.

- Verify eligibility.
- Verify response.
- Collect data on protocol specific CRFs.
- Prepare all submitted data for review by the Protocol Chair.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to Protocol Chair for timely review.
- Distribute external Serious Adverse Event safety reports.
- Monitor and audit Participating Institutions either by on-site inspection of selected participant records and/or with submitted source documents and research records submitted to the Lead Institution.

In addition to the Lead Institution, the DF/HCC Quality Assurance Office for Clinical Trials provides the following support services to assist the Protocol Chair:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide Central Participant Registration.
- Confirm eligibility and consent.
- Provide auditing services (funding and QACT approval required).

2.3 Participating Institution

The Participating Institution(s) will be identified on the title page for each protocol. In addition, each participating institution will provide to the Lead Institution or designee a list of the key personnel assigned to the role for oversight of data management at their site. All sites must have office space, office equipment, and internet access that meet HIPAA standards.
The general responsibilities for each participating institution are as follows:

- Commit to accrual to the Lead Institution's (DF/HCC) protocol.
- Submit protocol and/or amendments to their local IRB.
- Update Coordinating Center (Lead Institution or designee) with research staff changes on a timely basis.
- Register participants through the QACT.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center (Lead Institution or designee).
- Submit Serious Adverse Event reports directly to the Coordinating Center (Lead Institution or designee).
- Submit deviations and violations to the Coordinating Center (Lead Institution or designee).

- For protocols using investigational agents, the participating institution will order their own investigational agents regardless of the supplier (i.e. NCI, pharmaceutical company).

3.0 DF/HCC QUALITY ASSURANCE OFFICE FOR CLINICAL TRIALS

The DF/HCC QACT is a unit that has been developed to collect, manage, and monitor data for DF/HCC trials. The DF/HCC QACT is located administratively in the office of the Senior Vice President for Clinical Research, at Dana-Farber Cancer Institute. The QACT uses DF/HCC computerized institutional databases for participant registrations and for the management of trial data as well as a set of quality assurance programs designed to monitor DF/HCC trials.

3.1 Organizational Structure

The DF/HCC Quality Assurance Office for Clinical Trials administrative structure consists of:

DF/HCC Quality Assurance Officer for Clinical Trials: Oversees the functions of the DF/HCC QACT.

QACT Assistant Director for Data: Provides direct oversight to the QACT Data Analysts assigned to CRF design, data collection and computerization for DF/HCC trials.

The DF/HCC QACT Data Analysts will be assigned on a protocol by protocol basis. Each protocol's data analyst is responsible for database management, data entry, data quality assurance, and protocol specific correspondence related to the collection and quality assurance of data.

QACT Assistant Director for Monitoring: Provides direct oversight to the QACT Protocol Registrars and Clinical Research Auditors.

The DF/HCC Protocol Registrars are responsible for the confirmation of each participant's eligibility and consent prior to protocol registration.

If funded and QACT approved, the DF/HCC Clinical Research Auditors may assist the Lead Institution in their auditing responsibilities for multi-center trials. The QACT auditor is responsible for systematically evaluating participant safety, protocol compliance, institutional SOPs, ICH GCP and Federal regulation compliance, data accuracy and investigational drug handling to assure a high standard of quality for DF/HCC trials.

4.0 PROTOCOL DEVELOPMENT

4.1 Activation of a Protocol

Dr. Cho is responsible for the coordination, development, and approval of the protocol as well as its subsequent amendments, and reporting SAEs, violations and deviations per DFCI IRB guidelines and FDA. Further, Dr. Cho will be the single liaison with the FDA.

To meet these requirements, the Protocol Chair will be responsible for the following minimum standards:

- Inclusion of the DF/HCC Multi-Center Data and Safety Monitoring Plan in the protocol as an appendix.
- Identify participating institutions and obtain accrual commitments. The Protocol Chair will also submit a protocol attachment labeled as "Participating Investigators" to the DFCI IRB and FDA, that provides the names and contact information for all participating institutions that perform the function of recruiting, enrolling, and treating participants for the protocol. The Coordinating Center (BIDMC) must be designated on the title page.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the Protocol Chair.
- Ensure that there is only one version of the Protocol and that all Participating Institutions use the correct version.
- Oversee the development of data collection forms (case report forms) that are of common format for use at all the Participating Institutions.

4.2 Coordinating Center Support Function

The BIDMC's study staff or designee will provide administrative and clerical support to the Protocol Chair for the development and distribution of the protocol.

The tasks to be performed by the BIDMC's study staff or designee include:

- Review of the protocol and consent to check for logistics, spelling, and consistency. Provide the Protocol Chair a list of queries related to any inconsistencies.
- Provide necessary administrative sections, including paragraphs related to registration logistics, data management schedules, and multi-center guidelines.
- Maintenance of contact list of all participating institutions in the DF/HCC

Multi-center Protocol and the distribution of updates to the sites as needed.

- Derivation of the study calendar, if applicable.
- Assistance in preparation and maintenance of case report forms.
- Maintain and document communication with all participating institutions.

4.3 Training at DF/HCC Sites and Participating Institutions

Study initiation training will be conducted by the BIDMC Research Coordinator or designee by teleconference.

5.0 PROTOCOL MANAGEMENT

The Coordinating Center (BIDMC) is responsible for assuring that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the BIDMC or designee must maintain copies of all IRB approvals, for each participating institution.

5.1 Protocol Distribution

The Coordinating Center (BIDMC) will distribute the final approved protocol and any subsequent amended protocols to all Participating Institutions.

5.2 Protocol Revisions and Closures

The participating institutions will receive phone, fax, mail or e-mail notification of protocol revisions from the Lead Institution or designee. It is the individual participating institution's responsibility to notify its IRB of these revisions.

5.2.1 Non life-threatening revisions

Participating institutions will receive written notification of protocol revisions regarding non life-threatening events from the Lead Institution or designee. Non-life-threatening protocol revisions should be implemented within 90 business days from receipt of the notification.

5.2.2 Revisions for life-threatening Causes

Participating institutions will receive telephone notification from the Lead Institution or designee concerning protocol revisions required to protect lives with follow-up by fax, mail or e-mail. Life-threatening protocol revisions will be implemented immediately.

5.2.3 Protocol Closures and Temporary Holds

Participating institutions will receive fax, e-mail, or phone notification of protocol closures and temporary holds, with follow-up by mail from the Lead Institution or designee. Closures and holds will be effective immediately. In addition, the Lead Institution or designee will update the Participating institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

5.3 Informed Consent Requirements

The Principal Investigator (PI) at each participating site will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. It is DF/HCC policy that Nurses and Fellows cannot obtain consent to greater than minimal risk trials

5.4 IRB Documentation

The following must be on file with the BIDMC prior to participant registration:

- Activation Letter of the institution's IRB **(**An Expedited IRB first approval is NOT acceptable)
- IRB approval for all amendments

It is the individual institution's responsibility to notify its IRB of protocol revisions. Participating institutions will have 90 days from receipt to provide the BIDMC their IRB approval for Major Amendments* to a protocol.

*** DF/HCC defines a Major Amendment** as: A substantive change in the study which may increase or decrease the risk to study participants. Major revisions require full IRB approval. The following criteria are examples of revisions to a protocol that are considered to be major amendments:

- Change of eligibility (inclusion/exclusion) criteria
- Change in design of protocol
- Change in statistical section
- Change in sample size/accrual (e.g., doubling the sample size)
- Change in informed consent
- Change of estimated dropout rate
- Change of treatment or intervention
- Change of device
- Change in primary objective evaluation process

5.5 IRB Re-Approval

Annual IRB re-approval from the Participating institution is required in order to register participants onto a protocol. There is no grace period for annual re-approvals.

Protocol registrations will not be completed if a re-approval letter is not received by the DF/HCC Lead Institution or designee from the Participating Institutions on or before the anniversary of the previous approval date.

5.6 Participant Confidentiality and Authorization Statement

The Health Insurance Portability and Accountability Act of 1996 contains, as one of its six major components, the requirement to create privacy standards for health care information that is used or disclosed in the course of treatment, payment or health care operations. The original Privacy Rule, as it has come to be known, was published in December 2000. The Final Rule was published on August 14, 2002, which has modified the privacy rule in significant ways vis-à-vis research.

In order for covered entities to use or disclose protected health information during the course of a DF/HCC Multi-Center Protocol the study participant must sign an Authorization. This Authorization may or may not be separate from the Informed Consent. The DF/HCC Multi-Center Protocol, with the approval from the DFCI IRB will provide an Informed Consent template, which covered entities (DF/HCC Multi-Center Protocol participating institutions) must use.

The DF/HCC Multi-Center Protocol will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per National Cancer Institute requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

5.7 Participant Registration

To register a participant, the following documents should be completed by the DF/HCC Multi-Center Protocol participating site and faxed to or e-mailed to the Lead Institution or designee (Study Coordinator Bryan Marion):

- Copy of required tests (baseline CBC, baseline chemistries, pathology report, baseline radiographic study reports)
- Signed informed consent form
- HIPAA authorization form (if separate from the informed consent document)
- Eligibility Screening Checklist

The research DF/HCC Multi-center Protocol participating site will then call or e-mail the BIDMC to verify eligibility. To complete the registration process, the Lead Institution or designee will:

- Register the participant on the study with the DF/HCC Quality Assurance Office for Clinical Trials (QACT)
- Fax or e-mail the participant case number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration

5.8 DF/HCC Multi-center Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and written on all data and QACT correspondence for the participant.

5.9 DF/HCC Multi-center Protocol Registration Policy

5.9.1 Initiation of Therapy: Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the participant's Registration Confirmation memo from the DF/HCC QACT. Therapy must be initiated per protocol guidelines. The Protocol Chair and DFCI IRB must be notified of any exceptions to this policy**.**

5.9.2 Eligibility Exceptions: The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

5.9.3 Verification of Registration: A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one working day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

5.9.4 Confidentiality: All documents, investigative reports, or information relating to the participant are strictly confidential. Any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Lead Institution or designee must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number and protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification.

5.10 Schedule of Data Submission

The DF/HCC QACT will develop electronic case report forms, (CRF/eCRFs) for use with the DF/HCC Multi-Center Protocol. These forms are designed to collect data for each study. DF/HCC QACT will require the forms to be submitted as follows:

COMMON FORMS & REPORTS

- Eligibility Checklist, (Informed Consent/ Participant Authorization for the Release of Personal Health Information)
- On-study Form
- Baseline Assessment Form (Baseline disease assessment/measurement)
- Treatment Forms
- Adverse Event Forms
- Response Assessment Form (Follow-up disease assessment/Measurement)
- Off-Treatment/Off Study Form
- Follow-Up/Survival Forms

Note: It is necessary to send only ONE copy of all paper Case Report Forms.

5.10.1 Eligibility Checklist

Purpose - Outlines protocol-specific eligibility criteria and includes the following:

Participant Demographics (address, zip code, sex, race, ethnicity, initials, date of birth)

9) Parameters for eligibility
10) Parameters for exclusion
11) Parameters for stratifications

Schedule for Submission - Completed prior to participant registration. The Informed Consent/ Participant Authorization for the Release of Personal Health Information should be submitted with the Eligibility Checklist at the time of registration.

5.10.2 On-study Form

Purpose - documents the following items:

- Demographic data
- Prior therapy
- Past medical and surgical history
- Description of participant's physical status at protocol registration
- Disease site specific data

Schedule for Submission - Submitted to DF/HCC QACT within 14 days after registration.

5.10.3 Baseline Assessment Form

Purpose – Documents objective and subjective disease status as defined by the protocol**.** Records all pertinent radiographic and laboratory measurements of disease utilized in determining response evaluations.

Schedule of Submission – Submitted within 14 days after registration.

5.10.4 Treatment Form

Purpose - Records the following information related to the time the participant receives protocol treatment:

- Participant, Protocol, Affiliate information
- Protocol treatment and supportive therapy per treatment cycle
- Protocol specific laboratory values per treatment cycle
- All medications other than protocol chemotherapy agents used to treat concomitant diagnoses, if applicable

Schedule for Submission – Submitted within 10 days after the last day of the cycle.

5.10.5 Adverse Event Report Form

Purpose – Documents adverse events that occur while the participant is receiving treatment and for up to 30 days after the last dose of treatment. All adverse events are to be graded by number using the toxicity grading scale required by the protocol. *This form is not for IRB submission, but for recording the AE in the research database.*

Schedule for Submission – Submitted within 10 days after the last day of the cycle.

5.10.6 Response Assessment Form

Purpose – Documents objective and subjective response as defined by the protocol. Records all pertinent radiographic and laboratory measurements of disease utilized in determining response evaluations.

Schedule of Submission – Submitted within 10 days after the completion of the cycle required for response evaluation.

5.10.7 Off Treatment/Off Study Form

Purpose - The Off Treatment/Off Study Form is submitted when the participant is
removed from the study or has completed all protocol treatment. Note: If the participant dies while on protocol**,** the Off Study Form is the last form submitted.

Schedule of Submission – Submitted within 14 days after completing treatment or taken off study for any reason.

5.10.8 Follow up / Survival Form

Purpose - Summarizes participant status at a given point in time after being removed from treatment.

Schedule of Submission – Submitted within 14 days after the protocol defined follow up visit date or call.

5.11 Data Form Review

When data forms arrive at the DF/HCC QACT, they are reviewed for:

Timeliness:
Did the form arrive on time as specified in the protocol?

Completeness:
Is all the information provided as required per protocol?

Participant Eligibility:
Does the participant meet the eligibility requirements for the study based on the demographic data, lab values and measurements provided?

Stratification:
Are the stratification parameters consistent with what was given at the time of registration?

Protocol Treatment Compliance:
Are the body surface area (BSA) and drug dosage calculations correct? The dose must be within 10% of the calculated protocol dose.

Adverse Events (Toxicities):

Did the participant experience adverse events (toxicities or side effects) associated with the treatment? Was the treatment delayed due to the adverse event? What was the most severe degree of toxicity experienced by the participant?

Notations concerning adverse events will address relationship to protocol treatment for each adverse event grade. All adverse events encountered during the study will be evaluated according to the NCI Common Toxicity Criteria assigned to the protocol and all adverse events must be noted on the participant's Adverse Event (Toxicity) Forms.

Response:
Did the participant achieve a response? What level of response did they achieve? On what date did the participant achieve the response and how was the response determined?

Response criteria are defined in the protocol. A tumor assessment must be performed prior to the start of treatment and while the participant is on treatment as specified by the protocol.

Objective responses must have documentation such as physical measurements, x-rays, scans, or laboratory tests.

A subjective response is one that is perceived by the participant, such as reduction in pain, or improved appetite.

5.12 Missing and Deficient Memorandum

Data submissions are monitored for timeliness and completeness of submission. Participating institutions are notified of their data submission delinquencies in accordance with the following policies and procedures:

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written query from the DF/HCC QACT Data Analyst. Responses to the query should be completed and returned within 14 days. Responses may be returned on the written query or on an amended case report form. In both instances the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the participating institution will receive a Missing Form Report from the DF/HCC QACT noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of three times a year.

6.0 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol.

Participating sites should order their own agent regardless of the supplier.

If the agent is commercially available, check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB. If the agent is investigational, ensure that the pharmacy will be able to receive and store the agent. The local IRB should be kept informed of who will supply the agent (i.e., NCI or a pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion.

7.0 SAFETY ASSESSMENTS AND TOXICITY MONITORING

All participants receiving investigational agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported to the investigator by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria assigned to the protocol (CTCAE Version 3.0) and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Protocol Chair and Institutional Review Board (IRB).

Additional safety assessments and toxicity monitoring are outlined in the protocol.

7.1 Serious Adverse Events

A serious adverse event (SAE) is any adverse drug experience at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions in a participant who has never had seizure activity in the past that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

Unless otherwise specified in the protocol, the study will utilize the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 for Toxicity and Adverse Event reporting. A copy of the CTC or CTCAE Criteria can be downloaded from the CTEP home page (http://ctep.info.nih.gov).

7.2 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Serious Adverse Events (SAEs) will be followed as is delineated in the protocol.

In addition, the Participating Institutions must report the serious adverse events to the Protocol Chair and the Coordinating Center (BIDMC) at the time SAEs are submitted.

The Lead Institution will maintain documentation of all Adverse Event Reporting and be responsible for communicating all SAEs to all Participating sites.

7.3 Guidelines for Processing IND Safety Reports

The U.S. Food and Drug Administration (FDA) regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any serious and unexpected adverse experiences that are possibly related to the investigational agent.

IND Safety Reports: Investigators will be sent a copy of expedited adverse events which have been sent to the FDA. The investigators are to file a copy with their protocol file and send a copy to their IRB according to their local IRB's policies and procedures.

8.0 PROTOCOL VIOLATIONS AND DEVIATIONS

Neither the FDA nor the ICH GCP guidelines define the terms "protocol violation" or "protocol deviation." All DF/HCC Protocol Chairs must adhere to those policies set by the DFCI IRB, the definitions for protocol violation and deviation as described by the DFCI IRB will be applied for reporting purposes for all institutions participating in the DF/HCC Multi-center Protocol.

8.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol.

Protocol Violation: Any protocol deviation that was not prospectively approved by the IRB prior to its initiation or implementation.

8.2 Reporting Procedures

The Protocol Chair: is responsible for ensuring that clear documentation is available in the medical record to describe all protocol deviations and violations.

The Protocol Chair will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from DFCI IRB. The Participating institution must submit the deviation request to the Protocol Chair and Lead Institution or designee, who will submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation should be submitted to the participating site's own IRB, per its policy.

Protocol violations occurring at a participating institution will be submitted to that site's own IRB. A copy of the participating institution's IRB report and determination will be forwarded to the DF/HCC Lead Institution or designee by mail, facsimile, or via e-mail within 10 business days after the original submission.

Coordinating Center: Upon receipt of the violation/deviation report from the participating institution, the BIDMC will submit the report to the Protocol Chair for review. Subsequently, the participating institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

9.0 QUALITY ASSURANCE

The quality assurance process for a clinical trial research study requires verification of protocol

compliance and data accuracy. As the Coordinating Center, the BIDMC with the aid of the QACT provides quality assurance oversight for the DF/HCC Multi-center Protocol.

9.1 Ongoing Monitoring of Protocol Compliance

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion. The BIDMC and if applicable QACT Data Analysts assigned to the Protocol will perform the ongoing protocol compliance monitoring with the support of the participating institution's Coordinators, the Principal Investigators, and the Protocol Chair.

9.2 Evaluation of Participating Institution Performance

9.2.1 Eligibility Checklist: Eligibility criteria are checked on a protocol-specific eligibility checklist and faxed to the DF/HCC QACT prior to registration on protocol. The checklist and informed consent document are reviewed by a DF/HCC QACT Protocol Registrar before the participant can be registered on a protocol. The DF/HCC QACT cannot make exceptions to the eligibility requirements.

9.2.5 Accrual of Eligible Participants: Annual accrual rates for eligible participants enrolled onto therapeutic clinical trials is calculated for each institution. Institutions are expected to maintain the minimum annual average accrual as defined by the protocol grant or contract.

9.3 On-Site Auditing

9.3.1 NCI & DF/HCC Sponsored Trials

For all DF/HCC sponsored protocols:

The participating institutions may be required to submit subject source documents to the BIDMC for monitoring. Each participating institution will be subject to on-site monitoring conducted by the QACT. The first on-site audit will be conducted following the enrollment of the third participant at each participating institution. Further on-site audits will be determined as necessary by the results of this initial audit. The BIDMC or designee will also require source documentation for review on 1-2 patients at each participating institution every 6 months. A report of these reviews will be filed in a separate monitoring binder at the lead institution (BIDMC).

9.3.2 Participating Institution

It is the participating institution's responsibility to notify the BIDMC or designee of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve the DF/HCC Multi-Center Protocol. The All institutions will forward a copy of

final audit and/or re-audit reports and corrective action plans (if applicable) to the BIDMC Research Coordinator (Bryan Marion) within 12 weeks after the audit date.

9.3.3 Coordinating Center (BIDMC)

The Protocol Chair (Dr. Cho) will review all DF/HCC Multi-Center Protocol Final Audit reports and corrective action plans if applicable. The Lead Institution or designee must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Subcommittee. Based upon the audit assessments the DF/HCC Audit Subcommittee could accept or conditionally accept the audit rating and final report. Conditional approval could require the Protocol Chair to implement recommendations or require further follow-up. For unacceptable audits, the Audit Subcommittee would forward the final audit report and corrective action plan to the Clinical Investigations Policy and Oversight Committee and the DFCI IRB as applicable.

9.4 Sub-Standard Performance

The Protocol Chair, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the DF/HCC Multi-Center Protocol.

9.4.1 Corrective Actions

Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, and adherence to protocol requirements will be recommended for a six- month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Institutions that fail to demonstrate significant improvement will be considered by the Protocol Chair for revocation of participation.

9.5 Scheduled Conference Calls

Conference calls will be held once monthly involving the study staff from all DF/HCC and participating institutions to discuss protocol progress, accrual, side effects, and relevant regulatory issues.

Appendix D

Collection of blood for both cytokine and for PBMC analysis will be performed as described below:

Approximately 7 cc of venous blood will be collected into a BD Vacutainer CPT™ tube with Sodium Citrate (Becton Dickinson product #362761). The tube should be gently inverted several times to ensure mixing with the anticoagulant. Within 24 hours the CPT tube should be centrifuged at room temperature in a horizontal rotor for 25 minutes at 1600g. After centrifugation, the mononuclear cells will be visible in a whitish layer just under the plasma (see figure below).

Remove 2-3 mls of plasma (top layer) and aliquot into 3 tubes (1 ml cryotubes). Then remove mononuclear layer (1.5 ml) and put into cryotube #1. Then transfer half of mononuclear cells (750 microliters) to cryotube #2. Add 750 microliters of freezing media (RPMI-1640 media with 20% dimethyl sulfoxide) to PBMC cryotubes (#1 and #2). All cryotubes (plasma and PBMC containing tubes) should immediately be placed in a –80 degree freezer. All samples should be labeled with the following:

Patient Initials, ID number
Protocol number
Collection date
Cells or plasma
Institution

Samples should be stored at -70° to -80°C.

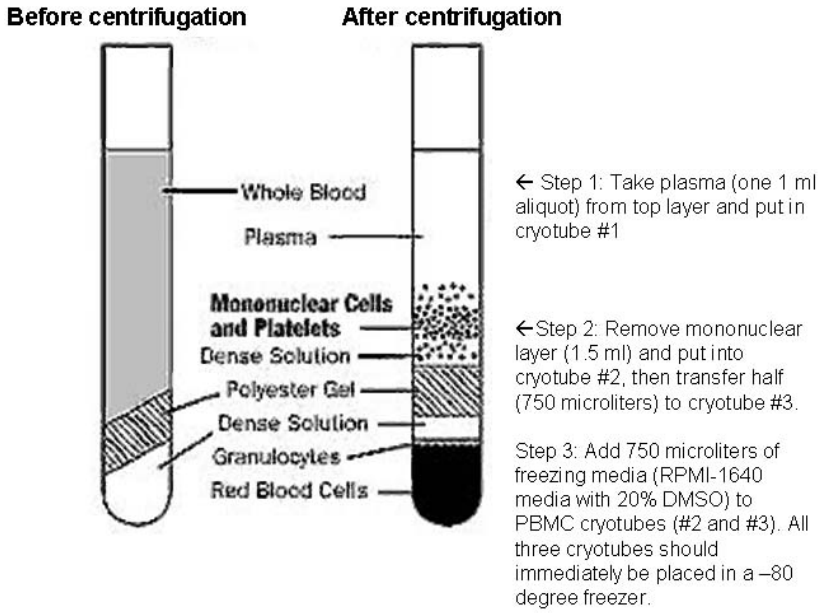


Figure: The preparation of plasma and mononuclear cells using a CPT tube.

Study Participant
Self-Administration
Study Drug Diary

Dana-Farber/Harvard Cancer Center

Participant Identifier: _____

Protocol # **08-313**

Your MD_____ Phone_____

Your RN_____ Phone_____

STUDY DRUG INSTRUCTIONS:

Study Drug: RAD001

How Much: Your dose is:_____

How Often: You will take your medication everolimus (tablets) by mouth, once a day followed by a big glass of water

When: You should take the drug about the same time each day

SPECIAL INSTRUCTIONS:

1. Missed or vomited doses will not be made up.
2. Please keep drug in original container.
3. You may either take everolimus on an empty stomach or after a low fat meal. Taking everolimus after large fatty meals is not advised because it will lower the amount of drug your body absorbs. Some examples of a low fat meal include: cereal with fat free milk, muffin/bagel with fat free spread, fruit salad, etc.

Bring any unused study drug, all empty containers, and this diary to the next clinic visit.

DOSING LOG

CYCLE:	**RAD001** For each dose take: Please indicate the date, time, amount taken and any comments.			
	Date	**Amount Taken**		**Comments**
		AM dose	**PM dose**	
Ex:	6/1/09	8am - 1	7:30pm - 1	Vomited PM dose
Day 1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Symptom	Start Date	End Date	Severity

OTHER MEDICATIONS TAKEN

If you take a daily medication (prescribed or otherwise), please use one line per drug and indicate the start and stop dates under the "Date(s) Taken" section (i.e., 6/2/09-6/5/09).

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials _____ Date _____

FOR OFFICE USE Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

Study Participant Signature: _____ Date _____

Study Staff Signature: _____ Date _____

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use EPIVIR safely and effectively. See full prescribing information for EPIVIR.

EPIVIR (lamivudine) Tablets and Oral Solution
Initial U.S. Approval: 1995

WARNING: LACTIC ACIDOSIS, POSTTREATMENT EXACERBATIONS OF HEPATITIS B IN CO-INFECTED PATIENTS, DIFFERENT FORMULATIONS OF EPIVIR

See full prescribing information for complete boxed warning

- **Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues. Suspend treatment if clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity occur. (5.1)**
- **Severe acute exacerbations of hepatitis B have been reported in patients who are co-infected with hepatitis B virus (HBV) and human immunodeficiency virus (HIV-1) and have discontinued EPIVIR. Monitor hepatic function closely in these patients and, if appropriate, initiate anti-hepatitis B treatment. (5.2)**
- **Patients with HIV-1 infection should receive only dosage forms of EPIVIR appropriate for treatment of HIV-1. (5.2)**

----------RECENT MAJOR CHANGES ----------

Dosage and Administration, Pediatric Patients (2.2) February 2008

----------INDICATIONS AND USAGE----------

EPIVIR is a nucleoside analogue reverse transcriptase inhibitor indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. Limitation of Use: The dosage of this product is for HIV-1 and not for HBV. (1)

---------- DOSAGE AND ADMINISTRATION ----------

- Adults and adolescents >16 years of age: 300 mg daily, administered as either 150 mg twice daily or 300 mg once daily. (2.1)
- Pediatric patients 3 months up to 16 years of age: Dosage should be based on body weight. (2.2)
- Patients With Renal Impairment: Doses of EPIVIR must be adjusted in accordance with renal function. (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------

- Tablets: 300 mg (3)
- Tablets: Scored 150 mg (3)
- Oral Solution: 10 mg/mL (3)

----------CONTRAINDICATIONS----------

EPIVIR Tablets and Oral Solution are contraindicated in patients with previously demonstrated clinically significant hypersensitivity (e.g., anaphylaxis) to any of the components of the products. (4)

---------- WARNINGS AND PRECAUTIONS ----------

- Lactic acidosis and severe hepatomegaly with steatosis: Reported with the use of nucleoside analogues. Suspend treatment if clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatoxicity occur. (5.1)
- Severe acute exacerbations of hepatitis: Reported in patients who are co-infected with hepatitis B virus and HIV-1 and discontinued EPIVIR. Monitor hepatic function closely in these patients and, if appropriate, initiate anti-hepatitis B treatment. (5.2)
- Patients with HIV-1 infection should receive only dosage forms of EPIVIR appropriate for treatment of HIV-1. (5.2)
- Co-infected HIV-1/HBV Patients: Emergence of lamivudine-resistant HBV variants associated with lamivudine-containing antiretroviral regimens has been reported. (5.2)
- Emtricitabine should not be administered concomitantly with lamivudine-containing products. (5.3)
- Hepatic decompensation (some fatal) has occurred in HIV-1/HCV co-infected patients receiving interferon and ribavirin-based regimens. Monitor for treatment-associated toxicities. Discontinue EPIVIR as medically appropriate and consider dose reduction or discontinuation of interferon alfa, ribavirin, or both. (5.4)
- Pancreatitis: Use with caution in pediatric patients with a history of pancreatitis or other significant risk factors for pancreatitis. Discontinue treatment as clinically appropriate. (5.5)
- Immune reconstitution syndrome (5.6) and redistribution/accumulation of body fat (5.7) have been reported in patients treated with combination antiretroviral therapy.

---------- ADVERSE REACTIONS ----------

- The most common reported adverse reactions (incidence ≥15%) in adults were headache, nausea, malaise and fatigue, nasal signs and symptoms, diarrhea, and cough. (6.1)
- The most common reported adverse reactions (incidence ≥15%) in pediatric patients were fever and cough. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------

Zalcitabine is not recommended for use in combination with EPIVIR. (7.2)

---------- USE IN SPECIFIC POPULATIONS ----------

- Pregnancy: Physicians are encouraged to register patients in the Antiretroviral Pregnancy Registry by calling 1-800-258-4263. (8.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: April 2009
EPV:2PI

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: RISK OF LACTIC ACIDOSIS, EXACERBATIONS OF HEPATITIS B IN CO-INFECTED PATIENTS UPON DISCONTINUATION OF EPIVIR®, DIFFERENT FORMULATIONS OF EPIVIR.

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination, including lamivudine and other antiretrovirals. Suspend treatment if clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity occur *[see Warnings and Precautions (5.1)]*.

Severe acute exacerbations of hepatitis B have been reported in patients who are co-infected with hepatitis B virus (HBV) and human immunodeficiency virus (HIV-1) and have discontinued EPIVIR. Hepatic function should be monitored closely with both clinical and laboratory follow-up for at least several months in patients who discontinue EPIVIR and are co-infected with HIV-1 and HBV. If appropriate, initiation of anti-hepatitis B therapy may be warranted *[see Warnings and Precautions (5.2)]*.

EPIVIR Tablets and Oral Solution (used to treat HIV-1 infection) contain a higher dose of the active ingredient (lamivudine) than EPIVIR-HBV® Tablets and Oral Solution (used to treat chronic HBV infection). Patients with HIV-1 infection should receive only dosage forms appropriate for treatment of HIV-1 *[see Warnings and Precautions (5.2)]*.

1 INDICATIONS AND USAGE

EPIVIR is a nucleoside analogue indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection. Limitation of use: The dosage of this product is for HIV-1 and not for HBV.

2 DOSAGE AND ADMINISTRATION

2.1 Adults and Adolescents >16 years of age

The recommended oral dose of EPIVIR in HIV-1-infected adults and adolescents >16 years of age is 300 mg daily, administered as either 150 mg twice daily or 300 mg once daily, in combination with other antiretroviral agents. If lamivudine is administered to a patient infected with HIV-1 and HBV, the dosage indicated for HIV-1 therapy should be used as part of an appropriate combination regimen *[see Warnings and Precautions (5.2)]*.

2.2 Pediatric Patients

The recommended oral dose of EPIVIR Oral Solution in HIV-1-infected pediatric patients 3 months to 16 years of age is 4 mg/kg twice daily (up to a maximum of 150 mg twice a day), administered in combination with other antiretroviral agents.

EPIVIR is also available as a scored tablet for HIV-1-infected pediatric patients who weigh ≥14 kg and for whom a solid dosage form is appropriate. Before prescribing EPIVIR

Tablets, children should be assessed for the ability to swallow tablets. If a child is unable to reliably swallow EPIVIR Tablets, the oral solution formulation should be prescribed. The recommended oral dosage of EPIVIR Tablets for HIV-1-infected pediatric patients is presented in Table 1.

Table 1. Dosing Recommendations for EPIVIR Tablets in Pediatric Patients

Weight (kg)	Dosage Regimen Using Scored 150-mg Tablet		Total Daily Dose
	AM Dose	PM Dose	
14 to 21	½ tablet (75 mg)	½ tablet (75 mg)	150 mg
>21 to <30	½ tablet (75 mg)	1 tablet (150 mg)	225 mg
≥30	1 tablet (150 mg)	1 tablet (150 mg)	300 mg

2.3 Patients With Renal Impairment

Dosing of EPIVIR is adjusted in accordance with renal function. Dosage adjustments are listed in Table 2 *[see Clinical Pharmacology (12.3)]*.

Table 2. Adjustment of Dosage of EPIVIR in Adults and Adolescents (≥30 kg) in Accordance With Creatinine Clearance

Creatinine Clearance (mL/min)	Recommended Dosage of EPIVIR
≥50	150 mg twice daily or 300 mg once daily
30-49	150 mg once daily
15-29	150 mg first dose, then 100 mg once daily
5-14	150 mg first dose, then 50 mg once daily
<5	50 mg first dose, then 25 mg once daily

No additional dosing of EPIVIR is required after routine (4-hour) hemodialysis or peritoneal dialysis.

Although there are insufficient data to recommend a specific dose adjustment of EPIVIR in pediatric patients with renal impairment, a reduction in the dose and/or an increase in the dosing interval should be considered.

3 DOSAGE FORMS AND STRENGTHS

- **EPIVIR Scored Tablets**

150 mg, are white, diamond-shaped, scored, film-coated tablets debossed with “GX CJ7” on both sides.

- **EPIVIR Tablets**

300 mg, are gray, modified diamond-shaped, film-coated tablets engraved with “GX EJ7” on one side and plain on the reverse side.

- **EPIVIR Oral Solution**

A clear, colorless to pale yellow, strawberry-banana flavored liquid, containing 10 mg of lamivudine per 1 mL.

4 CONTRAINDICATIONS

EPIVIR Tablets and Oral Solution are contraindicated in patients with previously demonstrated clinically significant hypersensitivity (e.g., anaphylaxis) to any of the components of the products.

5 WARNINGS AND PRECAUTIONS

5.1 Lactic Acidosis/Severe Hepatomegaly With Steatosis

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination, including lamivudine and other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors. Particular caution should be exercised when administering EPIVIR to any patient with known risk factors for liver disease; however, cases also have been reported in patients with no known risk factors. Treatment with EPIVIR should be suspended in any patient who develops clinical or laboratory findings suggestive of lactic acidosis or pronounced hepatotoxicity (which may include hepatomegaly and steatosis even in the absence of marked transaminase elevations).

5.2 Patients With HIV-1 and Hepatitis B Virus Co-infection

Posttreatment Exacerbations of Hepatitis: In clinical trials in non-HIV-1-infected patients treated with lamivudine for chronic hepatitis B, clinical and laboratory evidence of exacerbations of hepatitis have occurred after discontinuation of lamivudine. These exacerbations have been detected primarily by serum ALT elevations in addition to re-emergence of HBV DNA. Although most events appear to have been self-limited, fatalities have been reported in some cases. Similar events have been reported from postmarketing experience after changes from lamivudine-containing HIV-1 treatment regimens to non-lamivudine-containing regimens in patients infected with both HIV-1 and HBV. The causal relationship to discontinuation of lamivudine treatment is unknown. Patients should be closely monitored with both clinical and laboratory follow-up for at least several months after stopping treatment. There is insufficient evidence to determine whether re-initiation of lamivudine alters the course of posttreatment exacerbations of hepatitis.

Important Differences Among Lamivudine-Containing Products: EPIVIR Tablets and Oral Solution contain a higher dose of the same active ingredient (lamivudine) than EPIVIR-HBV Tablets and EPIVIR-HBV Oral Solution. EPIVIR-HBV was developed for patients with chronic hepatitis B. The formulation and dosage of lamivudine in EPIVIR-HBV are not appropriate for patients co-infected with HIV-1 and HBV. Safety and efficacy of lamivudine have not been established for treatment of chronic hepatitis B in patients co-infected with HIV-1 and HBV. If treatment with EPIVIR-HBV is prescribed for chronic hepatitis B for a patient with unrecognized or untreated HIV-1 infection, rapid emergence of HIV-1 resistance is likely to result because of the subtherapeutic dose and the inappropriateness of monotherapy HIV-1

treatment. If a decision is made to administer lamivudine to patients co-infected with HIV-1 and HBV, EPIVIR Tablets, EPIVIR Oral Solution, COMBIVIR® (lamivudine/zidovudine) Tablets, EPZICOM® (abacavir sulfate and lamivudine) Tablets, or TRIZIVIR® (abacavir sulfate, lamivudine, and zidovudine) Tablets should be used as part of an appropriate combination regimen.

Emergence of Lamivudine-Resistant HBV: In non–HIV-1-infected patients treated with lamivudine for chronic hepatitis B, emergence of lamivudine-resistant HBV has been detected and has been associated with diminished treatment response (see full prescribing information for EPIVIR-HBV for additional information). Emergence of hepatitis B virus variants associated with resistance to lamivudine has also been reported in HIV-1-infected patients who have received lamivudine-containing antiretroviral regimens in the presence of concurrent infection with hepatitis B virus.

5.3 Use With Other Lamivudine- and Emtricitabine-Containing Products

EPIVIR should not be administered concomitantly with other lamivudine-containing products including EPIVIR-HBV Tablets, EPIVIR Oral Solution, COMBIVIR (lamivudine/zidovudine) Tablets, EPZICOM (abacavir sulfate and lamivudine) Tablets, or TRIZIVIR (abacavir sulfate, lamivudine, and zidovudine) or emtricitabine-containing products, including ATRIPLA® (efavirenz, emtricitabine, and tenofovir), EMTRIVA® (emtricitabine), or TRUVADA® (emtricitabine and tenofovir).

5.4 Use With Interferon- and Ribavirin-Based Regimens

In vitro studies have shown ribavirin can reduce the phosphorylation of pyrimidine nucleoside analogues such as lamivudine. Although no evidence of a pharmacokinetic or pharmacodynamic interaction (e.g., loss of HIV-1/HCV virologic suppression) was seen when ribavirin was coadministered with lamivudine in HIV-1/HCV co-infected patients *[see Clinical Pharmacology (12.3)]*, hepatic decompensation (some fatal) has occurred in HIV-1/HCV co-infected patients receiving combination antiretroviral therapy for HIV-1 and interferon alfa with or without ribavirin. Patients receiving interferon alfa with or without ribavirin and EPIVIR should be closely monitored for treatment-associated toxicities, especially hepatic decompensation. Discontinuation of EPIVIR should be considered as medically appropriate. Dose reduction or discontinuation of interferon alfa, ribavirin, or both should also be considered if worsening clinical toxicities are observed, including hepatic decompensation (e.g., Child-Pugh >6). See the complete prescribing information for interferon and ribavirin.

5.5 Pancreatitis

In pediatric patients with a history of prior antiretroviral nucleoside exposure, a history of pancreatitis, or other significant risk factors for the development of pancreatitis, EPIVIR should be used with caution. Treatment with EPIVIR should be stopped immediately if clinical signs, symptoms, or laboratory abnormalities suggestive of pancreatitis occur *[see Adverse Reactions (6.1)]*.

5.6 Immune Reconstitution Syndrome

Immune reconstitution syndrome has been reported in patients treated with combination

antiretroviral therapy, including EPIVIR. During the initial phase of combination antiretroviral treatment, patients whose immune system responds may develop an inflammatory response to indolent or residual opportunistic infections (such as *Mycobacterium avium* infection, cytomegalovirus, *Pneumocystis jirovecii* pneumonia [PCP], or tuberculosis), which may necessitate further evaluation and treatment.

5.7 Fat Redistribution

Redistribution/accumulation of body fat including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance" have been observed in patients receiving antiretroviral therapy. The mechanism and long-term consequences of these events are currently unknown. A causal relationship has not been established.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

The following adverse reactions are discussed in greater detail in other sections of the labeling:

- Lactic acidosis and severe hepatomegaly with steatosis *[see Boxed Warning, Warnings and Precautions (5.1)]*.
- Severe acute exacerbations of hepatitis B *[see Boxed Warning, Warnings and Precautions (5.2)]*.
- Hepatic decompensation in patients co-infected with HIV-1 and Hepatitis C *[see Warnings and Precautions (5.4)]*.
- Pancreatitis *[see Warnings and Precautions (5.5)]*.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Adults - Clinical Trials in HIV-1: The safety profile of EPIVIR in adults is primarily based on 3,568 HIV-1-infected patients in 7 clinical trials.

The most common adverse reactions are headache, nausea, malaise, fatigue, nasal signs and symptoms, diarrhea and cough.

Selected clinical adverse reactions in ≥5% of patients during therapy with EPIVIR 150 mg twice daily plus RETROVIR® 200 mg 3 times daily for up to 24 weeks are listed in Table 3.

Table 3. Selected Clinical Adverse Reactions (≥5% Frequency) in Four Controlled Clinical Trials (NUCA3001, NUCA3002, NUCB3001, NUCB3002)

Adverse Reaction	EPIVIR 150 mg Twice Daily plus RETROVIR (n = 251)	RETROVIR[a] (n = 230)
Body as a Whole		
Headache	35%	27%
Malaise & fatigue	27%	23%
Fever or chills	10%	12%
Digestive		
Nausea	33%	29%
Diarrhea	18%	22%
Nausea & vomiting	13%	12%
Anorexia and/or decreased appetite	10%	7%
Abdominal pain	9%	11%
Abdominal cramps	6%	3%
Dyspepsia	5%	5%
Nervous System		
Neuropathy	12%	10%
Insomnia & other sleep disorders	11%	7%
Dizziness	10%	4%
Depressive disorders	9%	4%
Respiratory		
Nasal signs & symptoms	20%	11%
Cough	18%	13%
Skin		
Skin rashes	9%	6%
Musculoskeletal		
Musculoskeletal pain	12%	10%
Myalgia	8%	6%
Arthralgia	5%	5%

[a] Either zidovudine monotherapy or zidovudine in combination with zalcitabine.

Pancreatitis: Pancreatitis was observed in 9 out of 2,613 adult patients (0.3%) who received EPIVIR in controlled clinical trials EPV20001, NUCA3001, NUCB3001, NUCA3002, NUCB3002, and NUCB3007 *[see Warnings and Precautions (5.5)]*.

EPIVIR 300 mg Once Daily: The types and frequencies of clinical adverse reactions reported in patients receiving EPIVIR 300 mg once daily or EPIVIR 150 mg twice daily (in 3-drug combination regimens in EPV20001 and EPV40001) for 48 weeks were similar.

Selected laboratory abnormalities observed during therapy are summarized in Table 4.

Table 4. Frequencies of Selected Grade 3-4 Laboratory Abnormalities in Adults in Four 24-Week Surrogate Endpoint Studies (NUCA3001, NUCA3002, NUCB3001, NUCB3002) and a Clinical Endpoint Study (NUCB3007)

	24-Week Surrogate Endpoint Studies[a]		Clinical Endpoint Study[a]	
Test (Threshold Level)	EPIVIR plus RETROVIR	RETROVIR[b]	EPIVIR plus Current Therapy	Placebo plus Current Therapy[c]
Absolute neutrophil count (<750/mm^3)	7.2%	5.4%	15%	13%
Hemoglobin (<8.0 g/dL)	2.9%	1.8%	2.2%	3.4%
Platelets (<50,000/mm^3)	0.4%	1.3%	2.8%	3.8%
ALT (>5.0 x ULN)	3.7%	3.6%	3.8%	1.9%
AST (>5.0 x ULN)	1.7%	1.8%	4.0%	2.1%
Bilirubin (>2.5 x ULN)	0.8%	0.4%	ND	ND
Amylase (>2.0 x ULN)	4.2%	1.5%	2.2%	1.1%

[a] The median duration on study was 12 months.

[b] Either zidovudine monotherapy or zidovudine in combination with zalcitabine.

[c] Current therapy was either zidovudine, zidovudine plus didanosine, or zidovudine plus zalcitabine.

ULN = Upper limit of normal.

ND = Not done.

The frequencies of selected laboratory abnormalities reported in patients receiving EPIVIR 300 mg once daily or EPIVIR 150 mg twice daily (in 3-drug combination regimens in EPV20001 and EPV40001) were similar.

Pediatric Patients – Clinical Trials in HIV-1: EPIVIR Oral Solution has been studied in 638 pediatric patients 3 months to 18 years of age in 3 clinical trials.

Selected clinical adverse reactions and physical findings with a ≥5% frequency during therapy with EPIVIR 4 mg/kg twice daily plus RETROVIR 160 mg/m^2 3 times daily in therapy-naive (≤56 days of antiretroviral therapy) pediatric patients are listed in Table 5.

Table 5. Selected Clinical Adverse Reactions and Physical Findings (≥5% Frequency) in Pediatric Patients in Study ACTG300

Adverse Reaction	EPIVIR plus RETROVIR (n = 236)	Didanosine (n = 235)
Body as a Whole		
Fever	25%	32%
Digestive		
Hepatomegaly	11%	11%
Nausea & vomiting	8%	7%
Diarrhea	8%	6%
Stomatitis	6%	12%
Splenomegaly	5%	8%
Respiratory		
Cough	15%	18%
Abnormal breath sounds/wheezing	7%	9%
Ear, Nose, and Throat		
Signs or symptoms of ears[a]	7%	6%
Nasal discharge or congestion	8%	11%
Other		
Skin rashes	12%	14%
Lymphadenopathy	9%	11%

[a] Includes pain, discharge, erythema, or swelling of an ear.

Pancreatitis: Pancreatitis, which has been fatal in some cases, has been observed in antiretroviral nucleoside-experienced pediatric patients receiving EPIVIR alone or in combination with other antiretroviral agents. In an open-label dose-escalation study (NUCA2002), 14 patients (14%) developed pancreatitis while receiving monotherapy with EPIVIR. Three of these patients died of complications of pancreatitis. In a second open-label study (NUCA2005), 12 patients (18%) developed pancreatitis. In Study ACTG300, pancreatitis was not observed in 236 patients randomized to EPIVIR plus RETROVIR. Pancreatitis was observed in 1 patient in this study who received open-label EPIVIR in combination with RETROVIR and ritonavir following discontinuation of didanosine monotherapy *[see Warnings and Precautions (5.5)]*.

Paresthesias and Peripheral Neuropathies: Paresthesias and peripheral neuropathies were reported in 15 patients (15%) in Study NUCA2002, 6 patients (9%) in Study NUCA2005, and 2 patients (<1%) in Study ACTG300.

Selected laboratory abnormalities experienced by therapy-naive (≤56 days of antiretroviral therapy) pediatric patients are listed in Table 6.

Table 6. Frequencies of Selected Grade 3-4 Laboratory Abnormalities in Pediatric Patients in Study ACTG300

Test (Threshold Level)	EPIVIR plus RETROVIR	Didanosine
Absolute neutrophil count (<400/mm^3)	8%	3%
Hemoglobin (<7.0 g/dL)	4%	2%
Platelets (<50,000/mm^3)	1%	3%
ALT (>10 x ULN)	1%	3%
AST (>10 x ULN)	2%	4%
Lipase (>2.5 x ULN)	3%	3%
Total Amylase (>2.5 x ULN)	3%	3%

ULN = Upper limit of normal.

Neonates - Clinical Trials in HIV-1: Limited short-term safety information is available from 2 small, uncontrolled studies in South Africa in neonates receiving lamivudine with or without zidovudine for the first week of life following maternal treatment starting at Week 38 or 36 of gestation *[see Clinical Pharmacology (12.3)]*. Selected adverse reactions reported in these neonates included increased liver function tests, anemia, diarrhea, electrolyte disturbances, hypoglycemia, jaundice and hepatomegaly, rash, respiratory infections, and sepsis; 3 neonates died (1 from gastroenteritis with acidosis and convulsions, 1 from traumatic injury, and 1 from unknown causes). Two other nonfatal gastroenteritis or diarrhea cases were reported, including 1 with convulsions; 1 infant had transient renal insufficiency associated with dehydration. The absence of control groups limits assessments of causality, but it should be assumed that perinatally exposed infants may be at risk for adverse reactions comparable to those reported in pediatric and adult HIV-1-infected patients treated with lamivudine-containing combination regimens. Long-term effects of in utero and infant lamivudine exposure are not known.

6.2 Postmarketing Experience

In addition to adverse reactions reported from clinical trials, the following adverse reactions have been reported during postmarketing use of EPIVIR. Because these reactions are reported voluntarily from a population of unknown size, estimates of frequency cannot be made. These reactions have been chosen for inclusion due to a combination of their seriousness, frequency of reporting, or potential causal connection to lamivudine.

Body as a Whole: Redistribution/accumulation of body fat *[see Warnings and Precautions (5.7)]*.

Endocrine and Metabolic: Hyperglycemia.

General: Weakness.

Hemic and Lymphatic: Anemia (including pure red cell aplasia and severe anemias progressing on therapy).

Hepatic and Pancreatic: Lactic acidosis and hepatic steatosis, posttreatment exacerbation of hepatitis B *[see Boxed Warning, Warnings and Precautions (5.1, 5.2)]*.

Hypersensitivity: Anaphylaxis, urticaria.
Musculoskeletal: Muscle weakness, CPK elevation, rhabdomyolysis.
Skin: Alopecia, pruritus.

7 DRUG INTERACTIONS

Lamivudine is predominantly eliminated in the urine by active organic cationic secretion. The possibility of interactions with other drugs administered concurrently should be considered, particularly when their main route of elimination is active renal secretion via the organic cationic transport system (e.g., trimethoprim). No data are available regarding interactions with other drugs that have renal clearance mechanisms similar to that of lamivudine.

7.1 Interferon- and Ribavirin-Based Regimens

Although no evidence of a pharmacokinetic or pharmacodynamic interaction (e.g., loss of HIV-1/HCV virologic suppression) was seen when ribavirin was coadministered with lamivudine in HIV-1/HCV co-infected patients, hepatic decompensation (some fatal) has occurred in HIV-1/HCV co-infected patients receiving combination antiretroviral therapy for HIV-1 and interferon alfa with or without ribavirin *[see Warnings and Precautions (5.4), Clinical Pharmacology (12.3)]*.

7.2 Zalcitabine

Lamivudine and zalcitabine may inhibit the intracellular phosphorylation of one another. Therefore, use of lamivudine in combination with zalcitabine is not recommended.

7.3 Trimethoprim/Sulfamethoxazole (TMP/SMX)

No change in dose of either drug is recommended. There is no information regarding the effect on lamivudine pharmacokinetics of higher doses of TMP/SMX such as those used to treat PCP.

7.4 Drugs with No Observed Interactions With EPIVIR

A drug interaction study showed no clinically significant interaction between EPIVIR and zidovudine.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C. There are no adequate and well-controlled studies of EPIVIR in pregnant women. Animal reproduction studies in rats and rabbits revealed no evidence of teratogenicity. Increased early embryolethality occurred in rabbits at exposure levels similar to those in humans. EPIVIR should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Lamivudine pharmacokinetics were studied in pregnant women during 2 clinical studies conducted in South Africa. The study assessed pharmacokinetics in: 16 women at 36 weeks gestation using 150 mg lamivudine twice daily with zidovudine, 10 women at 38 weeks gestation using 150 mg lamivudine twice daily with zidovudine, and 10 women at 38 weeks gestation using lamivudine 300 mg twice daily without other antiretrovirals. These studies were not designed or powered to provide efficacy information. Lamivudine pharmacokinetics in pregnant

women were similar to those seen in non-pregnant adults and in postpartum women. Lamivudine concentrations were generally similar in maternal, neonatal, and umbilical cord serum samples. In a subset of subjects, lamivudine amniotic fluid specimens were collected following natural rupture of membranes. Amniotic fluid concentrations of lamivudine were typically 2 times greater than maternal serum levels and ranged from 1.2 to 2.5 mcg/mL (150 mg twice daily) and 2.1 to 5.2 mcg/mL (300 mg twice daily). It is not known whether risks of adverse events associated with lamivudine are altered in pregnant women compared with other HIV-1-infected patients.

Animal reproduction studies performed at oral doses up to 130 and 60 times the adult dose in rats and rabbits, respectively, revealed no evidence of teratogenicity due to lamivudine. Increased early embryolethality occurred in rabbits at exposure levels similar to those in humans. However, there was no indication of this effect in rats at exposure levels up to 35 times those in humans. Based on animal studies, lamivudine crosses the placenta and is transferred to the fetus *[see Nonclinical Toxicology (13.2)]*.

Antiretroviral Pregnancy Registry: To monitor maternal-fetal outcomes of pregnant women exposed to lamivudine, a Pregnancy Registry has been established. Physicians are encouraged to register patients by calling 1-800-258-4263.

8.3 Nursing Mothers

The Centers for Disease Control and Prevention recommend that HIV-1-infected mothers in the United States not breastfeed their infants to avoid risking postnatal transmission of HIV-1 infection. Because of the potential for serious adverse reactions in nursing infants and HIV-1 transmission, mothers should be instructed not to breastfeed if they are receiving lamivudine.

Lamivudine is excreted into human milk. Samples of breast milk obtained from 20 mothers receiving lamivudine monotherapy (300 mg twice daily) or combination therapy (150 mg lamivudine twice daily and 300 mg zidovudine twice daily) had measurable concentrations of lamivudine.

8.4 Pediatric Use

The safety and effectiveness of twice-daily EPIVIR in combination with other antiretroviral agents have been established in pediatric patients 3 months and older *[see Adverse Reactions (6.1), Clinical Pharmacology (12.3), Clinical Studies (14.2)]*.

8.5 Geriatric Use

Clinical studies of EPIVIR did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy. In particular, because lamivudine is substantially excreted by the kidney and elderly patients are more likely to have decreased renal function, renal function should be monitored and dosage adjustments should be made accordingly *[see Dosage and Administration (2.3), Clinical Pharmacology (12.3)]*.

8.6 Patients With Impaired Renal Function

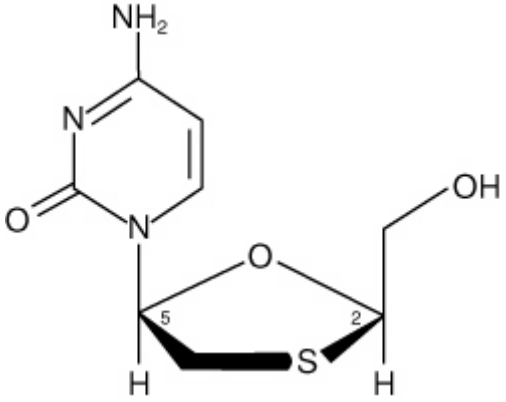
Reduction of the dosage of EPIVIR is recommended for patients with impaired renal function *[see Dosage and Administration (2.3), Clinical Pharmacology (12.3)]*.

10 OVERDOSAGE

There is no known antidote for EPIVIR. One case of an adult ingesting 6 g of EPIVIR was reported; there were no clinical signs or symptoms noted and hematologic tests remained normal. Two cases of pediatric overdose were reported in Study ACTG300. One case involved a single dose of 7 mg/kg of EPIVIR; the second case involved use of 5 mg/kg of EPIVIR twice daily for 30 days. There were no clinical signs or symptoms noted in either case. Because a negligible amount of lamivudine was removed via (4-hour) hemodialysis, continuous ambulatory peritoneal dialysis, and automated peritoneal dialysis, it is not known if continuous hemodialysis would provide clinical benefit in a lamivudine overdose event. If overdose occurs, the patient should be monitored, and standard supportive treatment applied as required.

11 DESCRIPTION

EPIVIR (also known as 3TC) is a brand name for lamivudine, a synthetic nucleoside analogue with activity against HIV-1 and HBV. The chemical name of lamivudine is (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (-)enantiomer of a dideoxy analogue of cytidine. Lamivudine has also been referred to as (-)2′,3′-dideoxy, 3′-thiacytidine. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3. It has the following structural formula:



Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in water at 20°C.

EPIVIR Tablets are for oral administration. Each scored 150-mg film-coated tablet contains 150 mg of lamivudine and the inactive ingredients hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide.

Each 300-mg film-coated tablet contains 300 mg of lamivudine and the inactive ingredients black iron oxide, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate, and titanium dioxide.

EPIVIR Oral Solution is for oral administration. One milliliter (1 mL) of EPIVIR Oral

Solution contains 10 mg of lamivudine (10 mg/mL) in an aqueous solution and the inactive ingredients artificial strawberry and banana flavors, citric acid (anhydrous), methylparaben, propylene glycol, propylparaben, sodium citrate (dihydrate), and sucrose (200 mg).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Lamivudine is an antiviral agent *[see Clinical Pharmacology (12.4)]*.

12.3 Pharmacokinetics

Pharmacokinetics in Adults: The pharmacokinetic properties of lamivudine have been studied in asymptomatic, HIV-1-infected adult patients after administration of single intravenous (IV) doses ranging from 0.25 to 8 mg/kg, as well as single and multiple (twice-daily regimen) oral doses ranging from 0.25 to 10 mg/kg.

The pharmacokinetic properties of lamivudine have also been studied as single and multiple oral doses ranging from 5 mg to 600 mg/day administered to HBV-infected patients.

The steady-state pharmacokinetic properties of the EPIVIR 300-mg tablet once daily for 7 days compared with the EPIVIR 150-mg tablet twice daily for 7 days were assessed in a crossover study in 60 healthy volunteers. EPIVIR 300 mg once daily resulted in lamivudine exposures that were similar to EPIVIR 150 mg twice daily with respect to plasma $AUC_{24,ss}$; however, $C_{max,ss}$ was 66% higher and the trough value was 53% lower compared with the 150-mg twice-daily regimen. Intracellular lamivudine triphosphate exposures in peripheral blood mononuclear cells were also similar with respect to $AUC_{24,ss}$ and $C_{max24,ss}$; however, trough values were lower compared with the 150-mg twice-daily regimen. Inter-subject variability was greater for intracellular lamivudine triphosphate concentrations versus lamivudine plasma trough concentrations. The clinical significance of observed differences for both plasma lamivudine concentrations and intracellular lamivudine triphosphate concentrations is not known.

Absorption and Bioavailability: Lamivudine was rapidly absorbed after oral administration in HIV-1-infected patients. Absolute bioavailability in 12 adult patients was 86% ± 16% (mean ± SD) for the 150-mg tablet and 87% ± 13% for the oral solution. After oral administration of 2 mg/kg twice a day to 9 adults with HIV-1, the peak serum lamivudine concentration (C_{max}) was 1.5 ± 0.5 mcg/mL (mean ± SD). The area under the plasma concentration versus time curve (AUC) and C_{max} increased in proportion to oral dose over the range from 0.25 to 10 mg/kg.

The accumulation ratio of lamivudine in HIV-1-positive asymptomatic adults with normal renal function was 1.50 following 15 days of oral administration of 2 mg/kg twice daily.

Effects of Food on Oral Absorption: An investigational 25-mg dosage form of lamivudine was administered orally to 12 asymptomatic, HIV-1-infected patients on 2 occasions, once in the fasted state and once with food (1,099 kcal; 75 grams fat, 34 grams protein, 72 grams carbohydrate). Absorption of lamivudine was slower in the fed state (T_{max}: 3.2 ± 1.3 hours) compared with the fasted state (T_{max}: 0.9 ± 0.3 hours); C_{max} in the fed state was 40% ± 23% (mean ± SD) lower than in the fasted state. There was no significant difference in systemic

exposure (AUC∞) in the fed and fasted states; therefore, EPIVIR Tablets and Oral Solution may be administered with or without food.

Distribution: The apparent volume of distribution after IV administration of lamivudine to 20 patients was 1.3 ± 0.4 L/kg, suggesting that lamivudine distributes into extravascular spaces. Volume of distribution was independent of dose and did not correlate with body weight.

Binding of lamivudine to human plasma proteins is low (<36%). In vitro studies showed that over the concentration range of 0.1 to 100 mcg/mL, the amount of lamivudine associated with erythrocytes ranged from 53% to 57% and was independent of concentration.

Metabolism: Metabolism of lamivudine is a minor route of elimination. In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. Within 12 hours after a single oral dose of lamivudine in 6 HIV-1-infected adults, 5.2% ± 1.4% (mean ± SD) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Serum concentrations of this metabolite have not been determined.

Elimination: The majority of lamivudine is eliminated unchanged in urine by active organic cationic secretion. In 9 healthy subjects given a single 300-mg oral dose of lamivudine, renal clearance was 199.7 ± 56.9 mL/min (mean ± SD). In 20 HIV-1-infected patients given a single IV dose, renal clearance was 280.4 ± 75.2 mL/min (mean ± SD), representing 71% ± 16% (mean ± SD) of total clearance of lamivudine.

In most single-dose studies in HIV-1-infected patients, HBV-infected patients, or healthy subjects with serum sampling for 24 hours after dosing, the observed mean elimination half-life ($t_{½}$) ranged from 5 to 7 hours. In HIV-1-infected patients, total clearance was 398.5 ± 69.1 mL/min (mean ± SD). Oral clearance and elimination half-life were independent of dose and body weight over an oral dosing range of 0.25 to 10 mg/kg.

Special Populations: *Renal Impairment:* The pharmacokinetic properties of lamivudine have been determined in a small group of HIV-1-infected adults with impaired renal function (Table 7).

Table 7. Pharmacokinetic Parameters (Mean ± SD) After a Single 300-mg Oral Dose of Lamivudine in 3 Groups of Adults With Varying Degrees of Renal Function

	Creatinine Clearance Criterion (Number of Subjects)		
Parameter	>60 mL/min (n = 6)	10-30 mL/min (n = 4)	<10 mL/min (n = 6)
Creatinine clearance (mL/min)	111 ± 14	28 ± 8	6 ± 2
C_{max} (mcg/mL)	2.6 ± 0.5	3.6 ± 0.8	5.8 ± 1.2
AUC∞ (mcg•hr/mL)	11.0 ± 1.7	48.0 ± 19	157 ± 74
Cl/F (mL/min)	464 ± 76	114 ± 34	36 ± 11

Exposure (AUC∞), C_{max}, and half-life increased with diminishing renal function (as

expressed by creatinine clearance). Apparent total oral clearance (Cl/F) of lamivudine decreased as creatinine clearance decreased. T_{max} was not significantly affected by renal function. Based on these observations, it is recommended that the dosage of lamivudine be modified in patients with renal impairment *[see Dosage and Administration (2.3)]*.

Based on a study in otherwise healthy subjects with impaired renal function, hemodialysis increased lamivudine clearance from a mean of 64 to 88 mL/min; however, the length of time of hemodialysis (4 hours) was insufficient to significantly alter mean lamivudine exposure after a single-dose administration. Continuous ambulatory peritoneal dialysis and automated peritoneal dialysis have negligible effects on lamivudine clearance. Therefore, it is recommended, following correction of dose for creatinine clearance, that no additional dose modification be made after routine hemodialysis or peritoneal dialysis.

It is not known whether lamivudine can be removed by continuous (24-hour) hemodialysis.

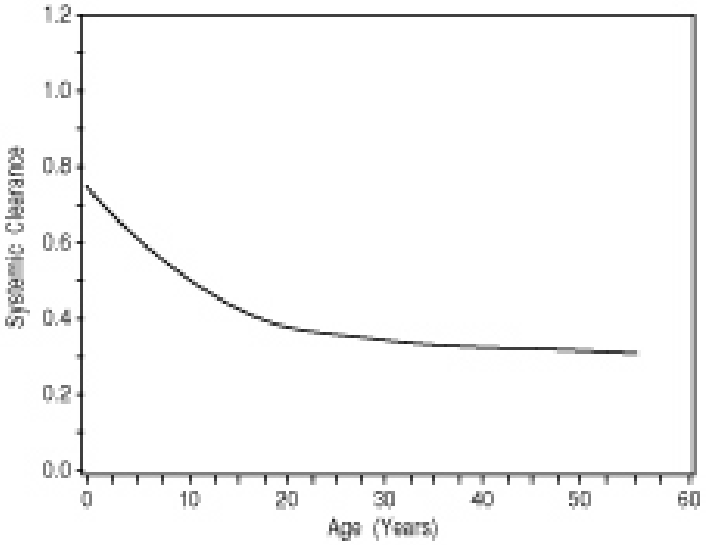
The effects of renal impairment on lamivudine pharmacokinetics in pediatric patients are not known.

Hepatic Impairment: The pharmacokinetic properties of lamivudine have been determined in adults with impaired hepatic function. Pharmacokinetic parameters were not altered by diminishing hepatic function; therefore, no dose adjustment for lamivudine is required for patients with impaired hepatic function. Safety and efficacy of lamivudine have not been established in the presence of decompensated liver disease.

Pediatric Patients: In Study NUCA2002, pharmacokinetic properties of lamivudine were assessed in a subset of 57 HIV-1-infected pediatric patients (age range: 4.8 months to 16 years, weight range: 5 to 66 kg) after oral and IV administration of 1, 2, 4, 8, 12, and 20 mg/kg/day. In the 9 infants and children (range: 5 months to 12 years of age) receiving oral solution 4 mg/kg twice daily (the usual recommended pediatric dose), absolute bioavailability was 66% ± 26% (mean ± SD), which was less than the 86% ± 16% (mean ± SD) observed in adults. The mechanism for the diminished absolute bioavailability of lamivudine in infants and children is unknown.

Systemic clearance decreased with increasing age in pediatric patients, as shown in Figure 1.

Figure 1. Systemic Clearance (L/hr•kg) of Lamivudine in Relation to Age



After oral administration of lamivudine 4 mg/kg twice daily to 11 pediatric patients ranging from 4 months to 14 years of age, C_{max} was 1.1 ± 0.6 mcg/mL and half-life was 2.0 ± 0.6 hours. (In adults with similar blood sampling, the half-life was 3.7 ± 1 hours.) Total exposure to lamivudine, as reflected by mean AUC values, was comparable between pediatric patients receiving an 8-mg/kg/day dose and adults receiving a 4-mg/kg/day dose.

Distribution of lamivudine into cerebrospinal fluid (CSF) was assessed in 38 pediatric patients after multiple oral dosing with lamivudine. CSF samples were collected between 2 and 4 hours postdose. At the dose of 8 mg/kg/day, CSF lamivudine concentrations in 8 patients ranged from 5.6% to 30.9% (mean ± SD of 14.2% ± 7.9%) of the concentration in a simultaneous serum sample, with CSF lamivudine concentrations ranging from 0.04 to 0.3 mcg/mL.

Limited, uncontrolled pharmacokinetic and safety data are available from administration of lamivudine (and zidovudine) to 36 infants up to 1 week of age in 2 studies in South Africa. In these studies, lamivudine clearance was substantially reduced in 1-week-old neonates relative to pediatric patients (>3 months of age) studied previously. There is insufficient information to establish the time course of changes in clearance between the immediate neonatal period and the age-ranges >3 months old *[see Adverse Reactions (6.1)]*.

Geriatric Patients: The pharmacokinetics of lamivudine after administration of EPIVIR to patients over 65 years of age have not been studied *[see Use in Specific Populations (8.5)]*.

Gender: There are no significant gender differences in lamivudine pharmacokinetics.

Race: There are no significant racial differences in lamivudine pharmacokinetics.

Drug Interactions: *Interferon Alfa:* There was no significant pharmacokinetic interaction between lamivudine and interferon alfa in a study of 19 healthy male subjects *[see Warnings and Precautions (5.4)]*.

Ribavirin: In vitro data indicate ribavirin reduces phosphorylation of lamivudine, stavudine, and zidovudine. However, no pharmacokinetic (e.g., plasma concentrations or intracellular triphosphorylated active metabolite concentrations) or pharmacodynamic (e.g., loss of HIV-1/HCV virologic suppression) interaction was observed when ribavirin and lamivudine (n = 18), stavudine (n = 10), or zidovudine (n = 6) were coadministered as part of a multi-drug regimen to HIV-1/HCV co-infected patients *[see Warnings and Precautions (5.4)]*.

Trimethoprim/Sulfamethoxazole: Lamivudine and TMP/SMX were coadministered to 14 HIV-1-positive patients in a single-center, open-label, randomized, crossover study. Each patient received treatment with a single 300-mg dose of lamivudine and TMP 160 mg/SMX 800 mg once a day for 5 days with concomitant administration of lamivudine 300 mg with the fifth dose in a crossover design. Coadministration of TMP/SMX with lamivudine resulted in an increase of 43% ± 23% (mean ± SD) in lamivudine AUC∞, a decrease of 29% ± 13% in lamivudine oral clearance, and a decrease of 30% ± 36% in lamivudine renal clearance. The pharmacokinetic properties of TMP and SMX were not altered by coadministration with lamivudine *[see Drug Interactions (7.3)]*.

Zidovudine: No clinically significant alterations in lamivudine or zidovudine pharmacokinetics were observed in 12 asymptomatic HIV-1-infected adult patients given a single dose of zidovudine (200 mg) in combination with multiple doses of lamivudine (300 mg q 12 hr) *[see Drug Interactions (7.4)]*.

12.4 Microbiology

Mechanism of Action: Intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP is the inhibition of HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue into viral DNA. 3TC-TP is a weak inhibitor of mammalian DNA polymerases α, β, and γ.

Antiviral Activity: The antiviral activity of lamivudine against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes) using standard susceptibility assays. EC_{50} values (50% effective concentrations) were in the range of 0.003 to 15 μM (1 μM = 0.23 mcg/mL). HIV-1 from therapy-naive subjects with no amino acid substitutions associated with resistance gave median EC_{50} values of 0.429 μM (range: 0.200 to 2.007 μM) from Virco (n = 92 baseline samples from COLA40263) and 2.35 μM (1.37 to 3.68 μM) from Monogram Biosciences (n = 135 baseline samples from ESS30009). The EC_{50} values of lamivudine against different HIV-1 clades (A-G) ranged from 0.001 to 0.120 μM, and against HIV-2 isolates from 0.003 to 0.120 μM in peripheral blood mononuclear cells. Ribavirin (50 μM) decreased the anti-HIV-1 activity of lamivudine by 3.5 fold in MT-4 cells. In HIV-1-infected MT-4 cells, lamivudine in combination with zidovudine at various ratios exhibited synergistic antiretroviral activity. Please see the full prescribing information for EPIVIR-HBV for information regarding the inhibitory activity of lamivudine against HBV.

Resistance: Lamivudine-resistant variants of HIV-1 have been selected in cell culture. Genotypic analysis showed that the resistance was due to a specific amino acid substitution in

the HIV-1 reverse transcriptase at codon 184 changing the methionine to either isoleucine or valine (M184V/I).

HIV-1 strains resistant to both lamivudine and zidovudine have been isolated from patients. Susceptibility of clinical isolates to lamivudine and zidovudine was monitored in controlled clinical trials. In patients receiving lamivudine monotherapy or combination therapy with lamivudine plus zidovudine, HIV-1 isolates from most patients became phenotypically and genotypically resistant to lamivudine within 12 weeks. In some patients harboring zidovudine-resistant virus at baseline, phenotypic sensitivity to zidovudine was restored by 12 weeks of treatment with lamivudine and zidovudine. Combination therapy with lamivudine plus zidovudine delayed the emergence of mutations conferring resistance to zidovudine.

Lamivudine-resistant HBV isolates develop substitutions (rtM204V/I) in the YMDD motif of the catalytic domain of the viral reverse transcriptase. rtM204V/I substitutions are frequently accompanied by other substitutions (rtV173L, rtL180M) which enhance the level of lamivudine resistance or act as compensatory mutations improving replication efficiency. Other substitutions detected in lamivudine-resistant HBV isolates include: rtL80I and rtA181T. Similar HBV mutants have been reported in HIV-1-infected patients who received lamivudine-containing antiretroviral regimens in the presence of concurrent infection with hepatitis B virus *[see Warnings and Precautions (5.2)]*.

Cross-Resistance: Lamivudine-resistant HIV-1 mutants were cross-resistant to didanosine (ddI) and zalcitabine (ddC). In some patients treated with zidovudine plus didanosine or zalcitabine, isolates resistant to multiple reverse transcriptase inhibitors, including lamivudine, have emerged.

Genotypic and Phenotypic Analysis of On-Therapy HIV-1 Isolates From Patients With Virologic Failure: *Study EPV20001:* Fifty-three of 554 (10%) patients enrolled in EPV20001 were identified as virological failures (plasma HIV-1 RNA level ≥400 copies/mL) by Week 48. Twenty-eight patients were randomized to the lamivudine once-daily treatment group and 25 to the lamivudine twice-daily treatment group. The median baseline plasma HIV-1 RNA levels of patients in the lamivudine once-daily group and lamivudine twice-daily group were 4.9 $\log_{10}$ copies/mL and 4.6 $\log_{10}$ copies/mL, respectively.

Genotypic analysis of on-therapy isolates from 22 patients identified as virologic failures in the lamivudine once-daily group showed that isolates from 0/22 patients contained treatment-emergent amino acid substitutions associated with zidovudine resistance (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E), isolates from 10/22 patients contained treatment-emergent amino acid substitutions associated with efavirenz resistance (L100I, K101E, K103N, V108I, or Y181C), and isolates from 8/22 patients contained a treatment-emergent lamivudine resistance-associated substitution (M184I or M184V).

Genotypic analysis of on-therapy isolates from patients (n = 22) in the lamivudine twice-daily treatment group showed that isolates from 1/22 patients contained treatment-emergent zidovudine resistance substitutions, isolates from 7/22 contained treatment-emergent efavirenz resistance substitutions, and isolates from 5/22 contained

treatment-emergent lamivudine resistance substitutions.

Phenotypic analysis of baseline-matched on-therapy HIV-1 isolates from patients (n = 13) receiving lamivudine once daily showed that isolates from 12/13 patients were susceptible to zidovudine; isolates from 8/13 patients exhibited a 25- to 295-fold decrease in susceptibility to efavirenz, and isolates from 7/13 patients showed an 85- to 299-fold decrease in susceptibility to lamivudine.

Phenotypic analysis of baseline-matched on-therapy HIV-1 isolates from patients (n = 13) receiving lamivudine twice daily showed that isolates from all 13 patients were susceptible to zidovudine; isolates from 3/13 patients exhibited a 21- to 342-fold decrease in susceptibility to efavirenz, and isolates from 4/13 patients exhibited a 29- to 159-fold decrease in susceptibility to lamivudine.

Study EPV40001: Fifty patients received zidovudine 300 mg twice daily plus abacavir 300 mg twice daily plus lamivudine 300 mg once daily and 50 patients received zidovudine 300 mg plus abacavir 300 mg plus lamivudine 150 mg all twice daily. The median baseline plasma HIV-1 RNA levels for patients in the 2 groups were 4.79 $\log_{10}$ copies/mL and 4.83 $\log_{10}$ copies/mL, respectively. Fourteen of 50 patients in the lamivudine once-daily treatment group and 9 of 50 patients in the lamivudine twice-daily group were identified as virologic failures.

Genotypic analysis of on-therapy HIV-1 isolates from patients (n = 9) in the lamivudine once-daily treatment group showed that isolates from 6 patients had an abacavir and/or lamivudine resistance-associated substitution M184V alone. On-therapy isolates from patients (n = 6) receiving lamivudine twice daily showed that isolates from 2 patients had M184V alone, and isolates from 2 patients harbored the M184V substitution in combination with zidovudine resistance-associated amino acid substitutions.

Phenotypic analysis of on-therapy isolates from patients (n = 6) receiving lamivudine once daily showed that HIV-1 isolates from 4 patients exhibited a 32- to 53-fold decrease in susceptibility to lamivudine. HIV-1 isolates from these 6 patients were susceptible to zidovudine.

Phenotypic analysis of on-therapy isolates from patients (n = 4) receiving lamivudine twice daily showed that HIV-1 isolates from 1 patient exhibited a 45-fold decrease in susceptibility to lamivudine and a 4.5-fold decrease in susceptibility to zidovudine.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies with lamivudine in mice and rats showed no evidence of carcinogenic potential at exposures up to 10 times (mice) and 58 times (rats) those observed in humans at the recommended therapeutic dose for HIV-1 infection. Lamivudine was not active in a microbial mutagenicity screen or an in vitro cell transformation assay, but showed weak in vitro mutagenic activity in a cytogenetic assay using cultured human lymphocytes and in the mouse lymphoma assay. However, lamivudine showed no evidence of in vivo genotoxic activity in the rat at oral doses of up to 2,000 mg/kg, producing plasma levels of 35 to 45 times those in

humans at the recommended dose for HIV-1 infection. In a study of reproductive performance, lamivudine administered to rats at doses up to 4,000 mg/kg/day, producing plasma levels 47 to 70 times those in humans, revealed no evidence of impaired fertility and no effect on the survival, growth, and development to weaning of the offspring.

13.2 Reproductive Toxicology Studies

Reproduction studies have been performed in rats and rabbits at orally administered doses up to 4,000 mg/kg/day and 1,000 mg/kg/day, respectively, producing plasma levels up to approximately 35 times that for the adult HIV dose. No evidence of teratogenicity due to lamivudine was observed. Evidence of early embryolethality was seen in the rabbit at exposure levels similar to those observed in humans, but there was no indication of this effect in the rat at exposure levels up to 35 times those in humans. Studies in pregnant rats and rabbits showed that lamivudine is transferred to the fetus through the placenta.

14 CLINICAL STUDIES

The use of EPIVIR is based on the results of clinical studies in HIV-1-infected patients in combination regimens with other antiretroviral agents. Information from trials with clinical endpoints or a combination of CD4+ cell counts and HIV-1 RNA measurements is included below as documentation of the contribution of lamivudine to a combination regimen in controlled trials.

14.1 Adults

Clinical Endpoint Study: NUCB3007 (CAESAR) was a multi-center, double-blind, placebo-controlled study comparing continued current therapy (zidovudine alone [62% of patients] or zidovudine with didanosine or zalcitabine [38% of patients]) to the addition of EPIVIR or EPIVIR plus an investigational non-nucleoside reverse transcriptase inhibitor (NNRTI), randomized 1:2:1. A total of 1,816 HIV-1-infected adults with 25 to 250 CD4+ cells/mm^3 (median = 122 cells/mm^3) at baseline were enrolled: median age was 36 years, 87% were male, 84% were nucleoside-experienced, and 16% were therapy-naive. The median duration on study was 12 months. Results are summarized in Table 8.

Table 8. Number of Patients (%) With at Least One HIV-1 Disease Progression Event or Death

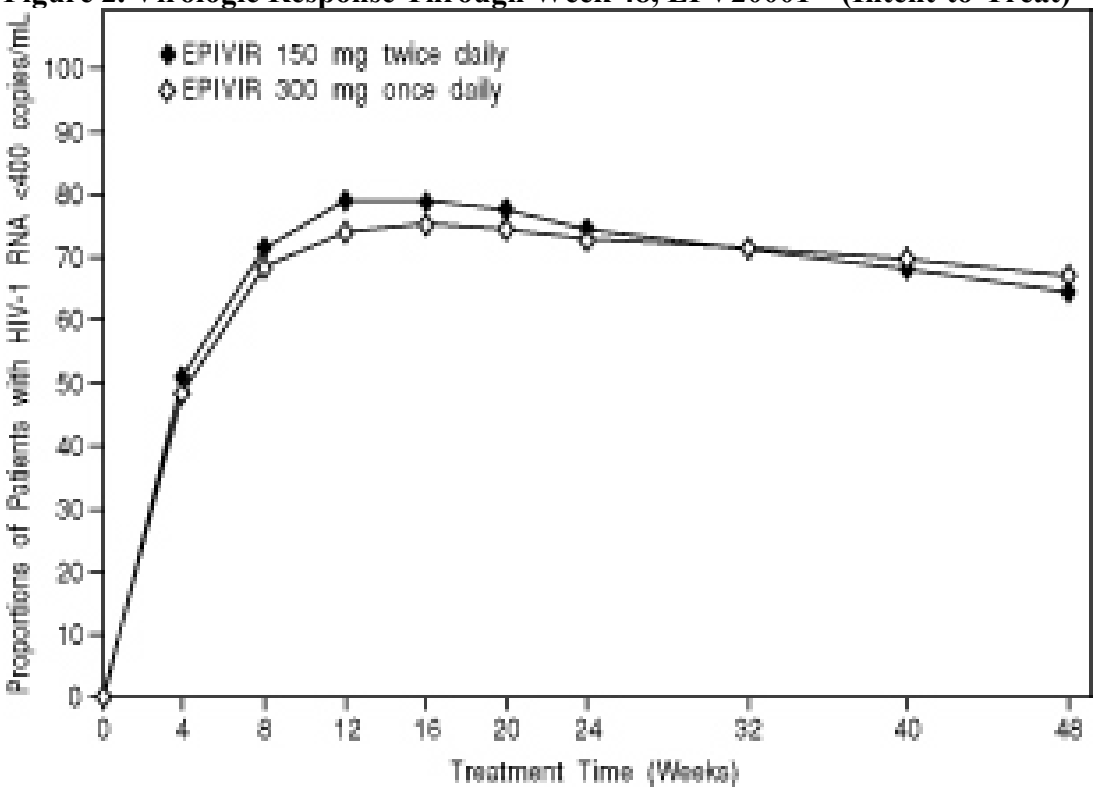
Endpoint	Current Therapy (n = 460)	EPIVIR plus Current Therapy (n = 896)	EPIVIR plus an NNRTI[a] plus Current Therapy (n = 460)
HIV-1 progression or death	90 (19.6%)	86 (9.6%)	41 (8.9%)
Death	27 (5.9%)	23 (2.6%)	14 (3.0%)

[a] An investigational non-nucleoside reverse transcriptase inhibitor not approved in the United States.

Surrogate Endpoint Studies: *Dual Nucleoside Analogue Studies:* Principal clinical trials in the initial development of lamivudine compared lamivudine/zidovudine combinations with zidovudine monotherapy or with zidovudine plus zalcitabine. These studies demonstrated the antiviral effect of lamivudine in a 2-drug combination. More recent uses of lamivudine in treatment of HIV-1 infection incorporate it into multiple-drug regimens containing at least 3 antiretroviral drugs for enhanced viral suppression.

Dose Regimen Comparison Surrogate Endpoint Studies in Therapy-Naive Adults: EPV20001 was a multi-center, double-blind, controlled study in which patients were randomized 1:1 to receive EPIVIR 300 mg once daily or EPIVIR 150 mg twice daily, in combination with zidovudine 300 mg twice daily and efavirenz 600 mg once daily. A total of 554 antiretroviral treatment-naive HIV-1-infected adults enrolled: male (79%), Caucasian (50%), median age of 35 years, baseline CD4+ cell counts of 69 to 1,089 cells/mm^3 (median = 362 cells/mm^3), and median baseline plasma HIV-1 RNA of 4.66 $\log_{10}$ copies/mL. Outcomes of treatment through 48 weeks are summarized in Figure 2 and Table 9.

Figure 2. Virologic Response Through Week 48, EPV20001[ab] (Intent-to-Treat)



[a] Roche AMPLICOR HIV-1 MONITOR.

[b] Responders at each visit are patients who had achieved and maintained HIV-1 RNA <400 copies/mL without discontinuation by that visit.

Table 9. Outcomes of Randomized Treatment Through 48 Weeks (Intent-to-Treat)

Outcome	EPIVIR 300 mg Once Daily plus RETROVIR plus Efavirenz (n = 278)	EPIVIR 150 mg Twice Daily plus RETROVIR plus Efavirenz (n = 276)
Responder[a]	67%	65%
Virologic failure[b]	8%	8%
Discontinued due to clinical progression	<1%	0%
Discontinued due to adverse events	6%	12%
Discontinued due to other reasons[c]	18%	14%

[a] Achieved confirmed plasma HIV-1 RNA <400 copies/mL and maintained through 48 weeks.

[b] Achieved suppression but rebounded by Week 48, discontinued due to virologic failure, insufficient viral response according to the investigator, or never suppressed through Week 48.

[c] Includes consent withdrawn, lost to follow-up, protocol violation, data outside the study-defined schedule, and randomized but never initiated treatment.

The proportions of patients with HIV-1 RNA <50 copies/mL (via Roche Ultrasensitive assay) through Week 48 were 61% for patients receiving EPIVIR 300 mg once daily and 63% for patients receiving EPIVIR 150 mg twice daily. Median increases in CD4+ cell counts were 144 cells/mm^3 at Week 48 in patients receiving EPIVIR 300 mg once daily and 146 cells/mm^3 for patients receiving EPIVIR 150 mg twice daily.

A small, randomized, open-label pilot study, EPV40001, was conducted in Thailand. A total of 159 treatment-naive adult patients (male 32%, Asian 100%, median age 30 years, baseline median CD4+ cell count 380 cells/mm^3, median plasma HIV-1 RNA 4.8 $\log_{10}$ copies/mL) were enrolled. Two of the treatment arms in this study provided a comparison between lamivudine 300 mg once daily (n = 54) and lamivudine 150 mg twice daily (n = 52), each in combination with zidovudine 300 mg twice daily and abacavir 300 mg twice daily. In intent-to-treat analyses of 48-week data, the proportions of patients with HIV-1 RNA below 400 copies/mL were 61% (33/54) in the group randomized to once-daily lamivudine and 75% (39/52) in the group randomized to receive all 3 drugs twice daily; the proportions with HIV-1 RNA below 50 copies/mL were 54% (29/54) in the once-daily lamivudine group and 67% (35/52) in the all-twice-daily group; and the median increases in CD4+ cell counts were 166 cells/mm^3 in the once-daily lamivudine group and 216 cells/mm^3 in the all-twice-daily group.

14.2 Pediatric Patients

Clinical Endpoint Study: ACTG300 was a multi-center, randomized, double-blind study that provided for comparison of EPIVIR plus RETROVIR (zidovudine) with didanosine monotherapy. A total of 471 symptomatic, HIV-1-infected therapy-naive (≤56 days of

antiretroviral therapy) pediatric patients were enrolled in these 2 treatment arms. The median age was 2.7 years (range: 6 weeks to 14 years), 58% were female, and 86% were non-Caucasian. The mean baseline CD4+ cell count was 868 cells/mm^3 (mean: 1,060 cells/mm^3 and range: 0 to 4,650 cells/mm^3 for patients ≤5 years of age; mean: 419 cells/mm^3 and range: 0 to 1,555 cells/mm^3 for patients >5 years of age) and the mean baseline plasma HIV-1 RNA was 5.0 $\log_{10}$ copies/mL. The median duration on study was 10.1 months for the patients receiving EPIVIR plus RETROVIR and 9.2 months for patients receiving didanosine monotherapy. Results are summarized in Table 10.

Table 10. Number of Patients (%) Reaching a Primary Clinical Endpoint (Disease Progression or Death)

Endpoint	EPIVIR plus RETROVIR (n = 236)	Didanosine (n = 235)
HIV-1 disease progression or death (total)	15 (6.4%)	37 (15.7%)
Physical growth failure	7 (3.0%)	6 (2.6%)
Central nervous system deterioration	4 (1.7%)	12 (5.1%)
CDC Clinical Category C	2 (0.8%)	8 (3.4%)
Death	2 (0.8%)	11 (4.7%)

16 HOW SUPPLIED/STORAGE AND HANDLING

EPIVIR Scored Tablets, 150 mg

White, diamond-shaped, scored, film-coated tablets debossed with “GX CJ7” on both sides.

Bottle of 60 tablets (NDC 0173-0470-01) with child-resistant closure.

EPIVIR Tablets, 300 mg

Gray, modified diamond-shaped, film-coated tablets engraved with “GX EJ7” on one side and plain on the reverse side.

Bottle of 30 tablets (NDC 0173-0714-00) with child-resistant closure.

Recommended Storage:

Store EPIVIR Tablets at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature].

EPIVIR Oral Solution, 10 mg/mL

A clear, colorless to pale yellow, strawberry-banana-flavored liquid, contains 10 mg of lamivudine in each 1 mL.

Plastic bottle of 240 mL (NDC 0173-0471-00) with child-resistant closure. This product does not require reconstitution.

Recommended Storage:

Store in tightly closed bottles at 25°C (77°F) [see USP Controlled Room Temperature].

17 PATIENT COUNSELING INFORMATION

17.1 Advice for the Patient

Information About Therapy With EPIVIR: EPIVIR is not a cure for HIV-1 infection and patients may continue to experience illnesses associated with HIV-1 infection, including opportunistic infections. Patients should remain under the care of a physician when using EPIVIR. Patients should be advised that the use of EPIVIR has not been shown to reduce the risk of transmission of HIV-1 to others through sexual contact or blood contamination.

Patients should be advised that the long-term effects of EPIVIR are unknown at this time.

Patients should be advised of the importance of taking EPIVIR with combination therapy on a regular dosing schedule and to avoid missing doses.

EPIVIR should not be coadministered with drugs containing lamivudine or emtricitabine, including COMBIVIR (lamivudine/zidovudine) Tablets, EPZICOM (abacavir sulfate and lamivudine) Tablets, TRIZIVIR (abacavir sulfate, lamivudine, and zidovudine), ATRIPLA (efavirenz, emtricitabine, and tenofovir), EMTRIVA (emtricitabine) or TRUVADA (emtricitabine and tenofovir) *[see Warnings and Precautions (5.3)]*.

Redistribution/Accumulation of Body Fat: Patients should be informed that redistribution or accumulation of body fat may occur in patients receiving antiretroviral therapy, including EPIVIR, and that the cause and long-term health effects of these conditions are not known at this time *[see Warnings and Precautions (5.7)]*.

Differences in Formulations of EPIVIR: Patients should be advised that EPIVIR Tablets and Oral Solution contain a higher dose of the same active ingredient (lamivudine) as EPIVIR-HBV Tablets and Oral Solution. If a decision is made to include lamivudine in the HIV-1 treatment regimen of a patient co-infected with HIV-1 and HBV, the formulation and dosage of lamivudine in EPIVIR (not EPIVIR-HBV) should be used *[see Warnings and Precautions (5.2)]*.

Co-infection With HIV-1 and HBV: Patients co-infected with HIV-1 and HBV should be informed that deterioration of liver disease has occurred in some cases when treatment with lamivudine was discontinued. Patients should be advised to discuss any changes in regimen with their physician *[see Warnings and Precautions (5.2)]*.

Risk of Pancreatitis: Parents or guardians should be advised to monitor pediatric patients for signs and symptoms of pancreatitis *[see Warnings and Precautions (5.5)]*.

Sucrose Content of EPIVIR Oral Solution: Diabetic patients should be advised that each 15-mL dose of EPIVIR Oral Solution contains 3 grams of sucrose *[see Description (11)]*.

GlaxoSmithKline
Research Triangle Park, NC 27709

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