A phase II trial using RAD001 for patients with radioiodine refractory thyroid cancer

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Author(s):

Jochen H. Lorch, M.D., M.S.
Dana Farber Cancer Institute
450 Brookline Avenue
Boston MA 02215
Phone: (617) 632 3090
Email: Jochen_Lorch@dfci.harvard.edu

Site Principal Investigator(s) :

Lori Wirth, M.D.
Massachusetts General Hospital
Center for Head and Neck Cancers
55 Fruit Street, Yawkey Building
Boston, MA 02114
Phone: 617-724-4000
Email: lwirth@partners.org

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Naifa Busaidy, M.D.

University of Texas-MD Anderson Cancer Center

1515 Holcombe Blvd, Unit 1461

Houston, TX 77054

Phone: 713-792-2841

Email: nbusaidy@mdanderson.org

Krzysztof Misiukiewicz, MD

Mount Sinai School of Medicine
One Gustave L.Levy Place,

P.O Box 1079,

New York, NY-10029

Phone: 212-241-6772

Email: krzystof.misiukiewicz@mssm.edu

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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
HBV	Hepatitis B virus
HBcAb	Hepatitis B core antibodies
HBs Ab	Hepatitis B surface antibodies
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
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 RAD001 (everolimus)

RAD001 (everolimus) is a novel oral derivative of rapamycin.

RAD001 has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and has obtained marketing authorization (Certican®) for prophylaxis of rejection in renal and cardiac transplantation in a number of countries, including the majority of the European Union. RAD001 has been in development for patients with various malignancies since 2002. RAD001 is being investigated as an anticancer agent based on its potential to act:

i. Directly on the tumor cells by inhibiting tumor cell growth and proliferation

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

1.1.1 mTOR pathway and mechanism of action

At cellular and molecular level RAD001 acts as a signal transduction inhibitor. RAD001 selectively inhibits mTOR (mammalian target of rapamycin), a key and a highly conservative serine-threonine kinase, which is present in all cells and is a central regulator of protein synthesis and ultimately cell growth, cell proliferation, angiogenesis and cell survival. mTOR is the only currently known target of RAD001[1].

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

The main known functions of mTOR include the following [1, 2]:

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

The PI3K-mTOR pathway itself is frequently activated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.

Through inactivating eukaryotic initiation factor 4E binding proteins (4E-BP1) and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates translation of important

massages, including those encoding the HIF-1 proteins, c-myc, ornithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.

The activation of mTOR pathway leads to the increased production of pro-angiogenic factors (i.e., VEGF) in tumors and to tumor, endothelial and smooth muscle cell growth and proliferation.

The regulation of mTOR signaling is complex and involves positive regulators, such as AKT that phosphorylate and inactivate negative regulators such as the Tuberous Sclerosis Complex (TSC1/TSC2).

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

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

mTORC2 (mTOR complex 2) is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling[4]. It has been suggested that mTORC2 phosphorylates and activates a different pool of AKT, that is not upstream of mTORC1. PHLPP phospatase plays a role of a negative regulator. mTORC2 is rapamycin insensitive and is required for the organization of the actin cytoskeleton [3].

mTORC1-mediated signaling is subject to modulation by the macrocyclic lactone rapamycin and its derivatives, such as RAD001. Once these agents bind to the 12 kDa cytosolic FK506-binding protein immunophilin FKBP12, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTORC1 and inhibit phosphorylation of mTOR substrates. As a consequence, downstream signaling events involved in regulation of the G1 to S-phase transition are inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity [6]. As many cancers are characterized by dysregulation of G1 transit (for example, overexpression of cyclin or cyclin-dependent kinases), inhibition of mTOR becomes an intriguing target for inducing cytostasis [2].

1.1.2 Preclinical studies

Pre-clinical investigations have demonstrated that RAD001 is a potent inhibitor of the proliferation of a range of human tumor cell lines *in-vitro* with IC50s ranging from sub/low nM to μM concentrations, concentrations capable of being reached in patients at the doses used in clinical trials.

RAD001 was shown to have activity in many human tumor cell lines originating from lung, breast, prostate, colon, kidney, melanoma and glioblastoma and neuroendocrine tumor cells. Recent data suggests anti-tumor activity also in thyroid cancer[7]. This study suggests that the

antiproliferative effects of mTOR inhibition may be due to the inhibition PI3 kinase activation and the down-regulation of cyclin D1 and D3.

RAD001 has shown activity in human pancreatic neuroendocrine cells, where induction of apoptosis was reported [8]. Other tumor types in which activity was observed include acute myeloid leukemia cells [9], adult T-cell leukemia cells [10], diffuse large B cell lymphoma cells (DLBCL; [11], pancreatic tumor cells [12], ovarian cancer cells [13] and hepatocellular carcinoma cells [14].

In a clonogenic assay using cells derived from 81 patient-derived tumor xenografts never cultured *in vitro* (11 human tumor types with 3 to 24 tumors each: bladder, colon, gastric, NSCLC [adeno, squamous epithelium and large cell], SCLC, breast, ovary, pancreatic, renal, melanoma, and pleuramesothelioma), RAD001 inhibited colony formation in a concentration-dependent manner. In addition, normal hematopoetic stem cells were insensitive to RAD001, with an IC50 about 15 fold higher than the tumor lines.

RAD001 also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS), with particular potency against VEGF-induced proliferation. This is particularly important in the context of thyroid cancer since thyroid cancer appear to rely to a high degree on angiogenesis and high expression of VEGF has been linked to aggressive tumor growth and poor prognosis[15]. Furthermore, tyrosine kinase inhibitors such as Sorafenib, AMG706 and axitinib which predominantly block VEGF-receptor activation have been successfully used in this disease[16-18]. The inhibition of endothelial proliferation and antiangiogenic activity of RAD001 was confirmed *in vivo* , as RAD001 selectively inhibited VEGF-dependent angiogenic response. Mice with primary and metastatic tumors treated with RAD001 showed a significant reduction in blood vessel density when compared to controls at well tolerated doses. Additionally, activity in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of RAD001-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

RAD001 also inhibits tumor growth *in-vivo* in xenografted, syngeneic and orthotopic animal models, residing longer in tumor tissue than in plasma and demonstrating high tumor penetration in a rat pancreatic tumor model. These effects occurred within the dose range of 2.5 to 10 mg/kg p.o. daily. Typically, the antitumor activity of RAD001 monotherapy was that of reduction of tumor growth rates rather than producing regressions or stable disease.

RAD001, administered p.o., was a potent inhibitor of tumor growth and well tolerated in:

- s.c. mouse xenograft model, established from a variety of tumor cell lines of diverse histotypes (NSCLC, pancreatic, colon, melanoma, epidermoid), including a Pgp170 overexpressing multi-drug resistant tumor line
- in a series of low-passage tumor xenografts established directly from human tumor material, maintained only *in vivo* and considered highly predictive of therapeutic outcome in patients. These included breast (5 lines), colorectal (9 lines), gastric (3 lines), lung (22 lines including adenocarcinomas, epidermoid cell, large cell and small cell histotypes), melanoma (6 lines), ovarian (4 lines), pancreatic (3 lines) and renal (6 lines)

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

Taken together, these data indicate the broad antiproliferative potential of RAD001 which includes thyroid cancer.

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. PTEN status alone may not be predictive of RAD001 relative *in vitro* sensitivity, however in some cases (i.e., GBM) there is also a correlation with PTEN status.

In preclinical models, the administration of RAD001 is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (pS6) and p4E-BP1, and occasionally with an increase in phosphorylation AKT (pAKT).

Pre-clinical safety

In safety pharmacology studies, RAD001 was devoid of relevant effects on vital functions including the cardiovascular, respiratory and nervous systems. RAD001 had no influence on QT interval prolongation. Furthermore, RAD001 showed no antigenic potential. Although RAD001 passes the blood-brain barrier, there was no indication of relevant changes in the behavior of rodents, even after single oral doses up to 2000mg/kg or after repeated administration at up to 40 mg/kg/day. Based on these findings, the potential of RAD001 to affect vital functions in patients is considered to be low.

RAD001 is considered to have no genotoxicity or carcinogenicity potential. All significant adverse events observed in preclinical toxicology studies with RAD001 in mice, rats, monkeys and minipigs were consistent with its anticipated pharmacologic action as an antiproliferative 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. Ocular effects (lenticular disorders) observed in rats were not observed in any other species and are considered to be a species-specific disorder.

More pre-clinical information is provided in the Investigator's Brochure.

1.1.3 Clinical experience

1.1.3.1 RAD001 Pharmacokinetics

RAD001 is rapidly absorbed with a median t_{max} of 1-2 hours. The bioavailability of the drug is believed to be 11% or greater. The $AUC_{0-\tau}$ is dose-proportional over the dose range between 5 to 70 mg in the weekly regimen and 5 and 10 mg in the daily regimen. C_{max} is dose-proportional between 5 and 10 mg for both the weekly and daily regimens. At doses of 20 mg/week and higher, the increase in C_{max} is less than dose-proportional. The coefficient of variation between patients is approximately 50%.

Trough levels (24 hour post-dose) correlate well with $AUC_{0-\tau}$ at steady-state during daily administration.

In whole blood, at a daily dose of 10 mg, about 20% of RAD001 is confined in plasma with 26% being unbound. The remaining 80% is sequestered in blood cells.

RAD001 is extensively metabolized in the liver and eliminated in the bile. Major metabolites are inactive. Elimination half-life is approximately 30 hours. The clearance of RAD001 is approximately halved in patients with mild-moderate hepatic impairment (Child-Pugh Class A or B), while renal impairment has little or no impact on the pharmacokinetics of RAD001.

Age, weight and gender in the adult population do not affect the pharmacokinetics of RAD001 to a clinically relevant extent. The clearance of RAD001 is reduced in children.

Pharmacokinetic characteristics are not notably different between Caucasian and Japanese subjects, whereas in Black patients pharmacokinetic studies have shown an average 20% higher clearance.

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.

RAD001 is a substrate of CYP3A4 and a substrate and a moderate inhibitor of the multi-drug efflux pump P-glycoprotein (P-gP, MDR1, ABCB1). Hence, its metabolism is sensitive to drugs which modify these enzymes (substrates, inducers, or inhibitors of these enzymes). Competitive inhibition could occur when RAD001 is combined with drugs which are also CYP3A4 or P-glycoprotein substrates

Table 3-3a (Section 3.3.3) lists examples of clinically relevant CYP3A inhibitors and inducers.

Please refer to Section 3.3.3 for more information on the concomitant use of CYP3A4 inhibitors/inducers and other medications.

More information on RAD001 pharmacokinetics is provided in the Investigator's Brochure.

1.1.3.2 RAD001 Pharmacodynamic studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition of the biomarker p70S6 kinase 1 [S6K1] in peripheral blood mononuclear cells [PBMC]) 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 immunocytochemistry (IHC) in biopsied tumor tissue, assessed the degree of inhibition and its duration for pS6, p4E-BP1 and pAKT expression with the daily and weekly dosing. There was high inhibition of the downstream markers S6K1 and 4E-BP1 at 5mg/day, which was complete at 10 mg/day, while preliminary results suggest increase in pAKT expression with maximal effect at 10 mg daily [19]

More information is provided in the Investigator's Brochure.

1.1.3.3 Clinical experience with RAD001

RAD001 has been investigated as a component of multi-drug immunosuppression in solid organ transplantation since 1996 and was approved for the indication of prophylaxis of organ rejection in adult patients receiving an allogeneic renal or cardiac transplant on 8 Jul 2003 by

the European Union under the trade name of Certican®. The most frequent adverse drug reactions in this context are highly specific to the transplant context. However, certain events are generalizable, most notably myelosuppression, skin disorders and increases in blood lipid levels.

RAD001 has been in development for patients with cancer since 2002. Approximately 2586 patients with various malignancies have been treated in either Novartis sponsored or non-Novartis sponsored clinical studies as of 31 Aug 2007. Overall, Novartis sponsored a total of 22 studies of RAD001 administered either as single-agent (n=848), or in combination with other anti-tumor agents (n=663). Ongoing or completed Investigator sponsored studies also enrolled over 1000 patients globally.

Eight single-agent Novartis sponsored trials have or are being conducted in various advanced malignancies. Five Phase I studies evaluated several escalating doses with either weekly or daily administration (Studies C2101/02, C2106, C2107, C1101) of RAD001 with the objective to identify an optimal regimen and dosage, based on safety, pharmacokinetics and knowledge of the drug's molecular effects on various tumors. The 10 mg/day and 50-70 mg/week dosages were proposed for further studies, when using RAD001 as a single agent, and as a target maximum dose in combination studies. In addition the Phase I studies, conducted in prostate cancer (Study C2106) and in Japanese patients with advanced cancers (Study C1101), evaluated the safety and the molecular changes in tumor, associated with the administration of RAD001.

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

A Phase III study (Study C2240) is ongoing and designed to demonstrate the safety and efficacy of RAD001 at an oral dose of 10 mg versus matching placebo in patients with metastatic carcinoma of the kidney, whose disease has progressed despite prior VEGF-R tyrosine kinase inhibitor therapy. Over 400 patients have been enrolled in this prospective, randomized, multicenter study which remains blinded as of the cut-off date.

This will be the first study to investigate the effects of RAD001 in thyroid cancer.

Overall, the most frequent mild-moderate Grade 1/2 adverse effects have been rash, stomatitis, fatigue, neutropenia and to a lesser extent gastrointestinal disorders (nausea, anorexia, diarrhea, vomiting), and headache. The primary DLT has been severe (Grade 3) stomatitis, and occasionally fatigue, hyperglycemia, and neutropenia. Reduced blood counts, hyperlipidemia (mainly hypercholesterolemia) and hyperglycemia are relatively frequent laboratory findings. Infections have not been notably frequent or severe. Non-infectious low-Grade (Grade 1/2) pneumonitis has led to development of treatment guidelines for the disorder (Table 3-2a). Preliminary indications of anti-tumor activity are encouraging.

For more information on known undesirable effects of RAD001 refer to Section 3.2.5.

Further detailed information regarding RAD001 clinical development, safety and efficacy is provided in the Investigator's Brochure.

Rationale for this study:

Thyroid cancer is the most common endocrine cancer and affects approximately 7 per 100,000 people in the western hemisphere. Over 33000 new thyroid cancer cases are diagnosed annually in the US alone and it appears that the incidence is rising [20, 21]. Approximately 90% of these are well differentiated cancers, 5% to 9% medullary tumors, and 1% anaplastic tumors. The well-differentiated tumors include papillary, follicular and Hurthle cell types with the papillary subtype predominating. The primary treatment modality for most patients with thyroid cancer is surgery followed by thyroid-stimulating hormone (TSH) suppression and possibly Iodine 131. In cases in which the tumor has spread to distant sites and has become refractory to radioiodine or is not amenable to curative surgery, expected survival declines rapidly. In this setting, no standard therapy exists. In the past, chemotherapy was and sometimes still is attempted but overall traditional chemotherapy is considered ineffective and too toxic. Among classical chemotherapy agents, doxorubicin has been studied most extensively, both as a single agent and in combination. Ahuja et al reviewed 17 trials and almost 250 patients with thyroid cancer who were treated with single agent doxorubicin and showed a composite response rate of 38%[22]. The response rate to doxorubicin monotherapy was further broken down to differentiated 38%, undifferentiated 22%, medullary 42% and Hurthle cell 33%. Based on this data, the doxorubicin received FDA approval in this disease. Because of the short progression-free survival of 2 months, a median survival of 8 months and the toxicity of this drug, it is rarely used[23]. Doxorubicin has also been used as the backbone of combination therapy with many different agents including bleomycin, cisplatin, vincristine, melphalan and paclitaxel. Despite reasonable response rates, the median survival in these mostly small trials appeared not to be increased compared to doxorubicin alone and therefore none of these combinations are used routinely in this disease[24, 25].

Recently, progress has been made using small molecule tyrosine kinase inhibitors that inhibit VEGF, raf, c-kit and RET such as Sorafenib, Axitinib or Motesanib (AMG706) [17, 26, 27] . Sorafenib was used in 30 patients with differentiated, medullary and anaplastic disease with documented disease progression. A partial response (PR) by RECIST criteria was achieved in 23%, 53 % had stable disease. Progression free survival was 79 weeks for all patients and 84 weeks for patients with differentiated thyroid cancer with no differences between papillary and follicular cancer. Axitinib was tested in a similar group of 60 patients although in this trial documented disease progression was not an entry criteria. The objective response rate was the primary endpoint. A partial response by RECIST criteria was achieved in 30% and stable disease was found in 38% in an intent to treat analysis and 4% had disease progression. PFS was 18 months and thus remarkably similar to that seen in the Sorafenib trial. Among the nine patients in this trial who had received chemotherapy prior to treatment with Axitinib, a PR was achieved in five subjects. Motesanib (AMG706) was tested in 93 patients and the results were recently published. Among 93 patients with documented disease progression within the last 6 months prior to study entry, 14% had an objective response and stable disease was achieved in 67%. PFS was 40 weeks. In some patients comorbidities such as uncontrolled hypertension and gastrointestinal side effects make the use of these drugs difficult and

ultimately, the vast majority of these patients becomes refractory to these drugs and their disease will progress. Furthermore, side effects which include fatigue, GI symptoms ranging from abdominal pain to stomach perforations and co-morbidities such as uncontrolled hypertension make this class of drugs a poor choice for some patients. In these cases, no established treatment alternatives exist and new therapies are urgently needed.
Currently there are no major drug trials other than with tyrosine kinase inhibitors under way in this disease. It is therefore important to expand treatment options and provide patients with alternative treatment options that go beyond tyrosine kinase inhibitors either as first or second line treatment.

The activity or RAD001 in thyroid cancer has not been tested in clinical trials. However, data form the RADIANT-1 trial in neuroendocrine tumors (NET) of the pancreas, are very similar to differentiated and medullary thyroid cancer on a molecular level were recently presented[28]. In the trial, 115 patients with metastatic pancreatic NET and documented disease progression on chemotherapy were given either daily RAD001 combined with monthly Sandostatin LAR Depot or daily RAD001 alone. Progression-free survival was 9.3 and 12.9 months and the fifteen month survival was 52.6 and 90.3%. Treatment was generally well-tolerated and most adverse events were mild to moderate in severity. The most frequent adverse events were diarrhea (48%) stomatitis (47%) rash (44%) fatigue (42%) nausea (41%) pyrexia (32%) vomiting (30%) headache (27%) asthenia (27%) peripheral edema (28%) and abdominal pain (27%). 4% of subjects had a grade 4 adverse event possibly related to trial therapy. Grade 1/2 pneumonitis were reported in 4% of subjects and no Grade 3/4 were reported.

Preclinical studies in thyroid cancer cells suggest that RAD001 may have activity in thyroid cancer. Several targets of mTOR have been found to be dysregulated in thyroid cancer, such as the cell cycle stimulators c-Myc and cyclin-D1[29] and the cell cycle inhibitor p27kip1 (p27), suggesting a potential role for mTOR in thyroid cancer progression[30]. mTOR is under control by another important kinase, Akt, which is elevated in several types of cancer, including anaplastic, papillary, and follicular thyroid cancer[31], and may contribute to the dysregulation of mTOR activity. Taken together, these data suggest that mTOR is an appropriate target for thyroid cancer therapy.

Based on the molecular mechanism outlined above and the response rates with mTOR inhibitors in other neuroendocrine cancer types, we propose to study RAD001 as first line therapy in patients who are diagnosed with radioiodine non-avid thyroid cancer or who have failed treatment with a small molecule tyrosine kinase inhibitor (TKI) such as sorafenib, axitinib or AMG706[16, 18, 32].

The primary endpoint in this study is progression-free survival (PFS). A minimum PFS of 6 months in this population with documented disease progression within the last 6 months prior to treatment start in at least 2 patients in the first cohort of patients is needed to assess antitumor activity in this disease. 6 months is considerably shorter than the median PFS reported in the trials with Sorafenib, Axitinib and Motesanib. However, given the palliative nature of treatment with any of these substances, close follow-up to detect disease progression

early and the lack of treatment alternatives to these tyrosine kinase inhibitors makes the use as second- but also as first line agent justifiable. Patients who progress will receive further therapy at the discretion of the investigator and can include off label use of multi-tyrosine kinase inhibitors, traditional chemotherapy or participation in a phase I trial.

2 Study objectives

Rationale

To date, there is no established therapy option for patients with Radioactive Iodine (RAI) refractory thyroid cancer who have progressed on oral tyrosine kinase inhibitors or who are not candidates for treatment with these drugs. This study seeks to obtain first information about the effectiveness of RAD001 in thyroid cancer that has shown evidence for disease progression as first or second line therapy. If promising activity is detected, this will be the basis for larger subsequent studies.

Primary end point:

- Progression free survival

Secondary

Secondary endpoints are:

- Objective response rate (complete and partial responses by modified RECIST criteria)
- Survival
- Symptom improvement (medullary thyroid cancer only)

3 Investigational plan

3.1 Overall study design

This is a non-randomized phase II study with RAD001 as first or second line treatment in patients who are either newly diagnosed with radioiodine refractory thyroid cancer or have progressed after chemotherapy with doxorubicine or a small molecule tyrosine kinase inhibitor such as Sorafenib and who have documented disease progression within 6 months.

Similar to prior studies in patients with thyroid cancer, all histologic thyroid cancer types will be included in this trial. While there may be differences in response to treatment between tumor types, this trial will provide an overview about responsiveness to therapy in this rare disease.

Our trial will follow a 2 step design according to Fleming´s procedure[33]. In the first step, 18 patients with differentiated thyroid cancer (excluding medullary or anaplastic thyroid cancer) and documented disease progression within 6 months will be enrolled. Then a PFS of 6 months or more will be required in at least two patients in order to proceed with the enrollment of 15 more patients for the study to complete its accrual of 33 patients. The time interval of 6 months is chosen because documented disease progression within 6 months is a

requirement for enrollment in this trial. Imaging studies will be obtained every 2 months and if no progression can be demonstrated at the 6 month time point while on therapy, anti-tumor activity is likely. If there is only one or no case of PFS of at least 6 months in the first cohort, the drug combination will be considered ineffective and the trial will be terminated.

In addition, two separate exploratory cohorts will be included in the trial but will not be part of the statistical analysis of the 2 step study. These cohorts include up to 10 patients with medullary thyroid cancer and up to 7 patients with anaplastic thyroid cancer, respectively. These cohorts will be evaluated by descriptive statistics and will provide an initial measure of efficacy in this disease in which no approved and effective treatment options exist. This brings the total number of subjects in this trial to 50.

The patients will be followed for a maximum duration of 24 months. Please see Section 3.4.1.3 for an outline of data that will be collected post 24 months for patient continuing on treatment without progression of disease.

3.2 Study population

3.2.1 Patient population

This is a non-randomized phase II study with RAD001 as first or second line treatment in patients who are either newly diagnosed with radioiodine refractory thyroid cancer or have progressed after chemotherapy with doxorubicine or a small molecule tyrosine kinase inhibitor such as Sorafenib and who have documented disease progression within 6 months during the year prior to protocol treatment with RAD001. The patient must not have had any treatment between the date of progression and the start date of RAD001 treatment, unless discussed with the PI. In selected cases therapy with RAD001 as third or fourth line therapy will be allowed for example if the patient had received sequential therapy with tyrosine kinase inhibitors and chemotherapy that does not contain mTOR inhibitors as part of the regimen.

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

- Histologically confirmed locally advanced or metastatic thyroid cancer, excluding thyroid lymphomas not amenable to or refractory to surgical resection, external beam radiotherapy, radioiodine or other local therapies.
- Medullary thyroid cancer with documented evidence of disease progression by modified RECIST[34] within 6 months during the year prior to study day 1 (see Appendix A) or symptomatic disease (ie, medullary thyroid cancer-related diarrhea and /or flushing episodes) at the time of screening in the absence of documented disease progressionThe patient must not have had any treatment between the date of progression and the start date of RAD001 treatment, unless discussed with the PI.
- Differentiated thyroid cancer (papillary, follicular with subvariants such as Hurthle cell thyroid cancer) with documented evidence of disease progression by modified RECIST within 6 months during the year prior to study day 1 (details see Appendix A). The patient must not have had any treatment between the date of progression and the start date of RAD001 treatment, unless discussed with the PI.
- Anaplastic thyroid cancer with disease progression with documented disease progression by modified RECIST within 6 months during the year prior to study day 1.The patient must not have had any treatment between the date of progression and the start date of RAD001 treatment, unless discussed with the PI.
- Patients must have at least one measurable site of disease 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
- Age $\geq$ 18 years
- ECOG performance status $\leq$ 2
- Adequate bone marrow function as shown by: ANC $\geq$ 1.5 x 10^9/L, Platelets $\geq$ 100 x 10^9/L, Hb >9 g/dL
- Adequate liver function as shown by:
- serum bilirubin $\leq$ 1.5 x ULN
- INR < 1.3 (or < 3 on anticoagulants)
- ALT and AST $\leq$ 2.5x ULN ($\leq$ 5x ULN in patients with liver metastases)
- Adequate renal function: serum creatinine $\leq$ 1.5 x ULN
- Fasting serum cholesterol $\leq$300 mg/dL OR $\leq$7.75 mmol/L AND fasting triglycerides $\leq$ 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.
- Signed informed consent

Exclusion criteria

- Patients receiving anticancer therapies within last 2 weeks or who have received radiation therapy within 3 weeks of study day 1
- Patients, who have had a major surgery or significant traumatic injury within 4 weeks of start of study drug, patients who have not recovered from the side effects of any

major surgery (defined as requiring general anesthesia) or patients that may require major surgery during the course of the study

- Prior treatment with any investigational drug within the preceding 3 weeks
- Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Topical or inhaled corticosteroids are allowed.
- Patients should not receive immunization with attenuated live vaccines within 2 weeks of study entry or during study period
- Patients who have brain or leptomeningeal metastases that can not be controlled with radiotherapy or radioiodine treatment including patients who require glucocorticoids for brain or leptomeningeal metastases.
- Other malignancies within the past 3 years except for adequately treated carcinoma of the cervix or basal or squamous cell carcinomas of the skin. Exceptions may be made by the PI for other low risk cancer diagnosis within the last 3 years.
- Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as:
    - Symptomatic congestive heart failure of New York heart Association Class III or IV
    - unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction within 6 months of start of study drug, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease
    - severely impaired lung function
    - uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN
    - active (acute or chronic) or uncontrolled severe infections
    - liver disease such as cirrhosis, chronic active hepatitis, chronic persistent hepatitis
    - positive screening hepatitis C test or history of HCV infection including those with a negative viral load test at baseline
- A known history of HIV seropositivity
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of RAD001 (e.g., ulcerative disease, uncontrolled nausea, vomiting, malabsorption syndrome or small bowel resection)
- Patients with an active, bleeding diathesis
- Female patients who are pregnant or breast feeding, or adults of reproductive potential who are not using effective birth control methods. If barrier contraceptives are being used, these must be continued throughout the trial by both sexes. Hormonal contraceptives are not acceptable as a sole method of contraception. (Women of childbearing potential must have a negative urine or serum pregnancy test within 14 days prior to administration of RAD001)
- Patients who have received prior treatment with an mTOR inhibitor (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 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. Patients may be re-escalated back to the previous dose if he/she is stable, his/her symptoms are related to disease and not treatment, and you receive the PI's permission. 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)).

Table 3.0 RAD001 dose level modification guidelines

Dose level	Dose and schedule
0 (starting dose)	**10 mg po daily**
-1	**5 mg po daily**
-2	**5 mg po every other day**

If patients are unable to tolerate dose level -2, the patient will come off study.

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 3-2a)	**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 hyperlipidemia*) (except pneumonitis – refer to Table 3-2a)	**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 (except hyperlipidemia*)	**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 3 or higher hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies (see Sec. 3.2.6.2).

3.2.4 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.

3.2.5 Known Side Effects of RAD001

Adverse events most frequently observed with RAD001 are rash, stomatitis/oral mucositis, fatigue, headache, anorexia, nausea, vomiting, diarrhea. Infections have not been notably frequent or severe. Non-infectious pneumonitis has also been observed. The majority of these AEs have been of mild to moderate severity (CTC grade 1-2). Overall, the most frequently observed laboratory abnormalities include reduced blood counts, hyperlipidemia mostly reported as hypercholesterolemia and/or hypertriglyceridemia.

3.2.5a The principal DLT in Phase 1 trials has been Grade 3 stomatitis. For guidance on management of stomatitis refer to Section 3.2.6.1

3.2.5b Hyperlipidemia was reported as a serious adverse reaction. It is a recognized side-effect of rapamycins. Use of lipid-lowering drugs should be associated with dietary recommendations. Monitoring of blood lipid levels requires patients to be fasting so that this aspect must be verified when interpreting results. For guidance on management of hyperlipidemia refer to Section 3.2.6.2.

3.2.5c Hyperglycemia was reported as a serious adverse reaction. Similarly, the fasting state of patients should be verified when interpreting results. For guidance on management of hyperglycemia refer to Section 3.2.6.2.

3.2.5d Pneumonitis is a recognized adverse effect of rapamycins (sirolimus, temsirolimus, and everolimus). Numerous case reports in the literature suggest that rapamycin-associated pneumonitis is relatively unaggressive, limited in extent, and reversible upon drug discontinuation. The term ‘pneumonitis’ is used here to describe non-infectious, non-malignant infiltration in the lungs which is evident radiologically. More precise diagnosis should follow histocytological examination following lung biopsy, generally during bronchoscopy which may or may not be symptomatic. Advice on the management of pneumonitis has been provided in Table 3-2a.

In oncology studies with RAD001, severe pneumonitis suspected as drug-related has been reported as a serious adverse event on 13 occasions and additionally in the following associated preferred terms including acute respiratory distress syndrome (n=2), alveolitis (n=1) and allergic alveolitis (n=1), interstitial lung disease (n=10), lung infiltration (n=23), cryptogenic organizing pneumonia, lung consolidation, pulmonary alvealoar haemorrhage, pulmonary toxicity and pulmonary fibrosis (n=1, each). One fatal case of drug-related

pneumonitis was reported for a patient with metastatic infiltrating ductal carcinoma of the breast treated with 10 mg/day, which developed approximately two months after starting RAD001. Cytology for both the pleural and pericardial fluids were positive for malignancy. The death was considered possibly related to the underlying late stage tumor and study drug. Additionally, one patient treated with 10 mg/day died due to severe acute respiratory distress syndrome and septic shock. Thoracic CT scan demonstrated condensation in the majority of the left lower lobe and frosted glass appearance in the left upper lobe, lingula, and right lung.

Along with the cases of non-infectious pneumonitis, serious opportunistic infections have also been reported in cancer patients treated with RAD001: mycobactrium, aspergillus, and fatal candidal sepsis, and fatal pneumocystis carnii in particular. Because RAD001, as other rapamycins, inhibits proliferation of activated lymphocytes and reduces neutrophil counts, treatment with RAD001 must be considered as predisposing patients to the risk of infection. This risk will be higher in patients severely immunocompromised because of their underlying disease and/or co-medications. Outcome may be fatal in case of serious infections.

3.2.5e Pericardial effusions, pleural effusions, and weight loss have been reported as serious adverse events with suspected causality. Due to the imprecision of causality assessments it should not be assumed that all of these events are indeed the result of therapy with everolimus since confounding factors are present relating mainly to complications of the underlying disease and to the use of concomitant medications.

3.2.5f A reduction in blood cell counts is frequent when RAD001 therapy is initiated. Without clinical significance and infrequently, anemia and thrombocytopenia have been reported. In heavily pretreated patients with aggressive lymphoma, the incidence of grade 3 anemia, neutropenia, and thrombocytopenia was reported to be 11%, 16%, and 30%, respectively. Serious, suspected drug-related hemorrhages have been exceptional. Nevertheless, RAD001 should be considered as predisposing patients to hemorrhage, potentially fatal, should they develop severe drug-related thrombocytopenia.

3.2.5g Discrete, reversible changes in liver enzymes have been found to occur in numerous patients during treatment with RAD001 in oncology clinical studies, and in a study in rheumatoid arthritis. In oncology studies, these changes may be evident only in patients without severe underlying morbidity. The increase in transaminase's (AST and ALT) generally appears after 4 weeks of treatment. In all but a few cases it does not exceed Grade 1 ($\leq$ 2.5 x ULN). Similarly, mild increases in alkaline phosphatases can coexist. Spontaneous corrections or intermittent correction with continued treatment can occur. Serum bilirubin is not increased. In studies of patients with advanced cancers, clinically relevant changes in liver enzymes have been invariably associated with the presence of liver metastases and/or progression of the underlying cancer.

3.2.5h Renal failure has been reported in five suspected cases to date. One patient with no alternative explanation made a complete recovery following study drug adjustment and no treatment/therapy for the event. The rest or the patients had concurrent morbidities, which might have contributed to the reported events.

3.2.5i Hypophosphatemia, hypomagnesemia, hyponatremia and hypocalcemia have been reported as serious adverse reactions. Electrolytes should be monitored in patients treated with RAD001.

Table 3-1 provides general recommendations for the management of patients, with suspected drug toxicities while on treatment with RAD001 as single-agent therapy.

More detailed information regarding RAD001 reported suspected toxicities and individual cases is provided in the [Investigator's Brochure].

3.2.5j Reactivation of Hepatitis B (HBV) has been observed in sporadic cases with patients in this setting with everolimus. Use of antivirals during anti-cancer therapy has been shown to reduce the risk of Hepatitis B virus reactivation and associated morbidity and mortality. A detailed assessment of Hepatitis B/C medical history and risk factors must be done for all patients at screening, with testing performed prior to the first dose of everolimus.

Patients who test positive for a past or active Hepatitis B infection should be given prophylactic administration. If the patient has a positive Hepatitis B test result, they should be treated with Hepatitis B anti-viral lamivudine 100 mg prior to start of RAD001 therapy and throughout the treatment period with RAD001. Some associated toxicities to the lamivudine may include headache, diarrhea, and rash. If the patient needs to be treated with lamivudine, the cost of the treatment will be charged to the patient's insurance. For guidance on management of Hepatitis B and C reactivation, refer to Section 3.2.6.4.

3.2.6 Management of Side Effects of RAD001

3.2.6.1 Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to RAD001 should be treated using local supportive care.

When described, the disorder has been identified as inflammation or ulcers in the mouth. If exam reveals mouth ulcers, rather than a more general inflammation of the mouth, please classify the adverse event as 'mouth ulcers'. If inflammation is limited to the mouth without ulcers please use the term 'stomatitis' rather than the less specific term 'mucositis'.

If the disorder is elsewhere than the mouth please describe the location as well as any specific procedures carried out for exploration (e.g. endoscopy).

Please use the Grading according to the NIH-NCI Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAEv3.0; http://ctep.cancer.gov/forms/CTCAEv3.pdf).

Grade 1: minimal symptoms; normal diet

Grade 2: symptomatic but can eat -and swallow modified diet

Grade 3: symptomatic and unable to adequately aliment or hydrate orally

Grade 4: symptoms associated with life-threatening consequences

Recommendations

For mild toxicity (Grade 1), use conservative measures such as non-alcoholic mouth wash, decadron mouthrinse, or salt water (0.9%) mouth wash several times a day until resolution. For more severe toxicity (Grade 2 or 3), 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% (e.g. Kenalog in Orabase®).

Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

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.

3.2.6.2 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 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or grade 2 or higher hypertriglyceridemia (>2.5 x upper normal limit) should be treated with a statin or appropriate lipid-lowering medication, in addition to diet. Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase inhibitors.

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 in [Study RAD001C2222] the affected patients had an abnormal fasting glucose at baseline. Based on this finding, it is recommended that optimal glucose control is achieved before starting a patient on RAD001. Study patients should have their glucose levels monitored during RAD001 therapy.

3.2.6.3 Management of non-infectious pneumonitis

Both asymptomatic radiological changes (grade 1) and symptomatic non-infectious pneumonitis (grade 2 = not interfering with activities of daily living or grade 3 = interfering with activities of daily living and oxygen indicated) have been noted in patients receiving RAD001 therapy. Non-infectious pneumonitis has been associated with RAD001 and other mTOR inhibitors (Atkins 2004). 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 CT scans may be performed, when clinically necessary. If non-infectious pneumonitis develops, a consultation with a pulmonologist should be considered. If the patient develops grade 3 pneumonitis, treatment with RAD001 should be interrupted and the patient should be treated as medically indicated (short course corticosteroids, oxygen, etc).

Management of non-infectious pneumonitis suspected to be associated with RAD001 and dose modifications instructions are provided in Table 3-2a and Table 3-0, respectively.

Table 3-2a Management of non-infectious pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	RAD001 Dose Adjustment
Grade 1	**CT scans with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every 2 Cycles until return to baseline.**	**No specific therapy is required**	**Administer 100% of RAD001 dose.**
Grade 2	**CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to baseline**. Consider bronchoscopy ***	**Symptomatic only. Prescribe corticosteroids if cough is troublesome.**	**Reduce 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 including: spirometry, DLCO, and room air O_2 saturation at rest.; Repeat each subsequent Cycle until return to baseline**. Bronchoscopy is recommended** *	**Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.**	**Hold 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. Patients will be withdrawn from the study if they fail to recover to ≤ Grade 1 within 3 weeks.**
Grade 4	**CT scan with lung windows and required pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to baseline**. Bronchoscopy is recommended *.**	**Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.**	**Discontinue treatment.**

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

****Pulmonary function tests are dependent on a variety of parameter such as patient's age, gender, height etc. Normal references are provided when a patient undergoes PFT testing based on his or her individual characteristics. These are usually based on normal values published by John L. Hankinson, Robert O. Crapo and Robert L. Jensen et al CHEST November 2003 vol. 124 no. 5 1805-1811.**

3.2.6.4 Management of Hepatitis B or C Reactivation

Monitoring and prophylactic treatment for hepatitis B reactivation

Table 3-2 provides details of monitoring and prophylactic therapy according to the baseline results of viral load and serologic markers testing.

Table 3-2 Action to be taken for positive baseline hepatitis B results

Test	Result	Result	Result	Result	Result
HBV-DNA	+	+ or -	-	-	-
HBsAg	+ or -	+	-	-	-
HBs Ab	+ or -	+ or -	+ and no prior HBV vaccination	+ or -	- or + with prior HBV vaccination
HBc Ab	+ or -	+ or -	+ or -	+	-
Recommendation	Prophylaxis treatment should be started 1-2 weeks prior to first dose of study drug. Monitor HBV-DNA approximately every 6 weeks		No prophylaxis. Monitor HBV-DNA approximately every 3 weeks		No specific action

Antiviral prophylaxis therapy should continue for at least 4 weeks after last dose of study drug. For patients who have already received study drug prior to the approval of the amendment, the same process should be followed at the patient's next visit. The first HBV-DNA result would be regarded as baseline. For hepatitis B reactivation, definition and management guidelines, see Table 3-3 Guidelines for management of hepatitis B.

Table 3-3 Guidelines for management of hepatitis B

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

against HBV), OR positive HBc Ab] --- reactivation is defined as: New appearance of measurable HBV-DNA	study drug according to the protocol, the patient should restart at the same dose after resolution. Antiviral therapy should continue at least 4 weeks after last dose of study drug. If resolution occurs > 28 days, patients should discontinue study drug but continue antiviral therapy at least 4 weeks after last dose of study drug.

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

Screening for Hepatitis C

Patients with a positive Hepatitis C test or known to have a history of HCV infection including those with a negative viral load test at baseline are excluded from the study.

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:

adverse event(s)
abnormal laboratory value(s)
abnormal test procedure result(s)
disease progression
protocol violation
subject withdrew consent
lost to follow-up
administrative problems
death

3.3 Treatments

3.3.1 RAD001 Administration

The study drug RAD001 will be self-administered (by the patients themselves). The investigator will instruct the patient to take the study drug exactly as specified in the protocol. RAD001 will be administered orally as once daily dose of *10 mg (one 10mg tablet or two 5mg tablets)* continuously from study day 1 until progression of disease or unacceptable toxicity. Patients will be instructed to take RAD001 in the morning, at the same time each day.

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. Tablets should be swallowed whole with a glass of water. The tablets must not be chewed or crushed and grapefruit or citrus juices should be avoided. For patients unable to swallow tablets, everolimus tablet (s) should be dispersed completely in a glass of water (containing approximately 30mL) by gently stirring, immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse should be completely swallowed to ensure that the entire dose is administered.

A dose of RAD001 is considered missed if the patient did not take RAD001 within 6 hours of the scheduled time. If vomiting occurs, no attempt should be made to replace the vomited dose. All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

Medication labels will comply with US legal requirements and 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 and 10 mg strength. Tablets are blister-packed under aluminum foil, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive. Patients will be given a two-months supply.

3.3.2 Treatment assignment

This is a non-randomized trial. In the first step of the trial, 18 patients with differentiated thyroid cancer will be enrolled and if responses are seen, the study will proceed to the full enrollment of 50 patients.

3.3.3 Concomitant therapy

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

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

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

- No other investigational therapy should be given to patients.
- No anticancer agents other than the study medication should be given to patients. If such agents are required for a patient then the patient must first be withdrawn from the study.
- Growth factors (e.g.G-CSF, GM-CSF, erythropoietin, platelets growth factors etc.) are not to be administered prophylactically but may be prescribed by the investigator for rescue from severe hematologic events, if this is thought to be appropriate.

Concurrent administration of RAD001 and strong CYP3A4 inhibitors (such as ketoconazole, itraconazole, ritonavir) and inducers (such as rifampin, rifabutin) should be avoided.

Provided there is no alternative treatment available, patients should be closely monitored for potential toxicities.

Concurrent administration of RAD001 and moderate CYP3A4 inhibitors (such as erythromycin, fluconazole, calcium channel blockers, benzodiazepines) and moderate CYP3A4 inducers (e.g. carbamazepine, phenobarbital, phenytoin) should also be avoided if possible, or used subject to caution (e.g. increased frequency of safety monitoring, temporary interruption of RAD001).

Competitive inhibition could occur when RAD001 is combined with drugs which are also CYP3A4 substrates. Therefore caution should be exercised in such cases.

Co-administration with substrates, inducers, or inhibitors of P-glycoprotein should be avoided, if possible, or used subject to caution (e.g. increased frequency of safety monitoring, temporary interruption of RAD001).

Grapefruit,grapefruit juice and pomegranate juice affect cytochrome P450 and P-glycoprotein activity and should therefore be avoided.

- In addition, patients should avoid Seville oranges and star fruit, as well as the juice of these fruits, which are potent CYP3A4-inhibitors.
- No chronic treatment with systemic steroids or other immunosuppressive agents while taking RAD001. Topical or inhaled corticosteroids are allowed.
- In case of severe mucositis, systemic treatment with oral steroids for up to one week is allowed. RAD001 is held while on oral steroids.
- RAD001 may affect the response to vaccinations making the response to the vaccination less effective. Live vaccines should be avoided while a patient is treated with RAD001.

Oral anticoagulants such as warfarin are CYP2C9 substrates and, as such, no interaction with RAD001 is expected. However, drug-drug interaction studies between macrolide antibiotics and warfarin have produced mixed outcomes and the disparity in these findings has led to the conclusion that multiple factors may alter the clearance of warfarin. The coadministration of RAD001 and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week's treatment).

Examples are provided in Table 3-3a. A comprehensive list of cytochrome P450 isoenzymes and CYP3A4 inhibitors, inducers, and substrates can be found at http://medicine.iupui.edu/flockhart. This website is continually revised and should be checked frequently for updates.

Table 3-3a Examples of 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 chlorpheniramine32	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).

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

3.3.4 Treatment compliance

Records of study medication used, dosages administered, and intervals between visits will be recorded during the study. Drug accountability will be noted and patients will be asked to return all unused study medication.

3.4 Visit schedule and assessments

3.4.1 Visit schedule

The visit schedule is outlined in Table 3.4a below.

General/safety assessments	Study entry (within 14 days of day 1 unless otherwise noted))	Day1[2]	At office visit every 4 weeks[1]	Every 8 weeks for max. 24 months[1]
Informed consent	X (within 28 days of day1)			
Medical history, height and vital signs	X		X	
Inclusion/exclusion criteria	X			
ECOG Performance status	X	X	X	
QOL questionnaire (EQ-5D)		X	X	
MTC-related symptoms questionnaire (MTC subjects only)		X	X	
Physical examination and weight	X	X	X	
ECG	X			
AE's and con-medications/treatments	X	X	X	
Laboratory assessments				
Pregnancy test for WOCBP (female subjects <50)	X			
Coagulation tests	X			
Hepatitis B tests (Hep B Core, Hep B Surf AB, Hep B Surf AG, HBV DNA)[3]	X (within 30 days of Day 1)		X (only HBV DNA if positive at baseline)	
Hepatitis C test (HCV RNA PCR)[3]	X (within 30 days of Day 1)			
Hematology and blood chemistry	X		X	
Urinalysis	X			
Calcitonin and CEA if elevated at baseline (only MTC subjects)	X		X (if elevated at baseline)	
Thyroglobulin and anti-thyroglobulin antibodies (only DTC subjects)	X		X (if elevated at baseline)[4]	
Free T4 and TSH	X		X	
Radiological assessments				
CT/MRI neck, chest, abdomen[5]	X (within 30 days of day 1)			X
Whole body bone scan (only MTC, FTC, HCTC subjects)	X (within 30 days of day 1)			
First dose of Rad001		X[2]		
Rad001 dispensed/returned				X

- [1]+/- one week depending on appointment availability due to holidays or unforeseen events
- [2]First dose of RAD001 may start the morning of Study Day 2.

- [3]All patients should be screened for hepatitis risk factors and any past illnesses of hepatitis B and hepatitis C infection. It is highly recommended that patients positive HBV-DNA or HBsAg are treated prophylactically with an antiviral (i.e. Lamivudine) for 1-2 weeks prior to receiving study drug (see Section 3.2.6.4). The antiviral treatment should continue throughout the entire study period and for at least 4 weeks after the last dose of everolimus. Patients on antiviral prohpylaxis treatment or positive HBV antibodies should be tested for HBV-DNA on Cycle 1 Day 1 and Day 1 of all subsequent cycles (every 28 days) to monitor for re-activation. If re-activation is confirmed, everolimus must be interrupted or discontinued according to the guidance in Table 3-3. Patients with viral hepatitis C risk factors should be screened for HCV RNA-PCR. Patients with positive HCV RNA-PCR results at screening and/or a history of past infection (even if test at baseline is negative) will be ineligible for the study.
- [4]**Thyroglobulin and thyroA are both drawn at monthly visits only if AT BASELINE thyroA lab value is negative AND** thyroglobulin value is elevated.
- [5] CT/MRI scans are to be perfomed every 2 months until first progression or death through 24 months from study entry. Post 24 months, if a patient has already stopped study treatment, but still alive without first progression, follow for first progression (CT/MRI scans every 3 months) and survival through 5 years from study entry. Post 24 months, If patient is scheduled to continue treatment, follow for first progression (CT/MRI scans every 3 months) and survival through 5 years from study entry; If treatment is stopped post 24 months but prior to 5 years from study entry for reasons other than progression, follow for first progression and survival through 5 years from study entry; if treatment is stopped post 24 months but prior to 5 years from study entry due to first progression, follow until death or 5 years from study entry, whichever is first.;

3.4.1.1 Screening

All subjects or their legally acceptable representative and the person who conducted the informed consent discussion must personally sign and date the consent form before any study-specific screening procedures are performed. Procedures that have been performed before consent was obtained as part of routine care are not considered study-specific procedures.

Medical history information to be collected during screening must date back to the original diagnosis of thyroid cancer. If a subject is referred to the study center, a copy of all applicable reports and histological or cytological evidence, confirming the diagnosis must be provided to the study center before enrollment. Copies of radiographic images confirming disease progression (ie, within 6 months during the year prior to study day 1) by modified RECIST should be provided to the study center. To document disease progression by modified RECIST, radiographic evidence must consist of 2 radiographic imaging studies that were both obtained within 6 months during the year prior to study day 1. The two radiographic imaging studies cannot be more than 6 months apart. Also, the patient must not have had any treatment between the date of progression and the start date of RAD001 treatment, unless discussed with the PI.

All subjects who have signed the IRB approved consent form must be registered as a screened subject. At the time of registration, the subject will receive a unique 5 digit subject identification number. This number will be used to identify the subject throughout the study and must be used on all study documentation related to that subject. The subject identification number must remain constant throughout the entire study.

All screening tests and procedures must be performed and the results available within a maximum of 14 days before study day 1 unless otherwise noted.

Subjects who do not meet eligibility criteria within the 14 day screening period will not be eligible for enrollment. However, subjects may be re-screened up to 2 additional times at the discretion of the investigator. The subject must be re-consented if more than 28 days have elapsed since the date of last informed consent.

The following screening assessments must be performed:

Review of inclusion and exclusion criteria

Medical history review, documentation of diagnosis and previous treatment.

Physical examination, ECOG performance status, blood pressure, respiratory rate, resting pulse, temperature, height and weight.

A 12 lead ECG

CT scans (contrast-enhanced or MRI scan (contrast-enhanced or PET CT scans of areas with known disease and that are of acceptable quality as baseline study within 30 days prior to study day 1.

Whole body bone scan for subjects with medullary, follicular or Hurthle cell thyroid cancer.

Laboratory assessment consisting of

- Hematology
- Chemistry
- Urinalysis
- Coagulation tests
- beta HCG pregnancy test for WOCBP
- Hepatitis B and C Screening tests

3.4.1.1.1 Registering

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.

For institutions outside of DF/HCC: While in the pre-screening stage, contact the DFCI clinical research coordinator/team to inform them of the potential screening patient. This will allow the DFCI team to monitor and maintain the patient accrual limits for each of the

cancer diagnosis categories. For more information, please refer to Appendix D for registration guidelines.

3.4.1.2 Treatment period procedures

The first does of RAD001 may be administered on the same day as enrollment but no more than 10 business days after enrollment. The treatment period begins on the first day of treatment with RAD001. The time points of study visits are outlined in table 3.4a.

If a subject is unable to come into the clinic for a required study visit or if unforeseen events occur, the visit procedures may be performed within a one week time frame.

The following assessments will be performed at each clinic visit according to table 3.4a throughout the treatment period:

Physical examination,

ECOG performance status,

blood pressure, respiratory rate, resting pulse, temperature, and weight.

Patient reported outcomes questionnaire (EQ-5D), see appendix

Medullary thyroid cancer-related symptoms questionnaire, see appendix

Laboratory evaluations are done at screening and monthly and need not be repeated on day 1.

CT scans (contrast-enhanced or MRI scan (contrast-enhanced or PET CT scans of areas with known disease and that are of acceptable quality every 8 weeks with a 5 business-day time window in case unforeseen events occur. Evaluation according to RECIST criteria compared to previous scan.

Documentation of all adverse events will occur at each clinic visit and at any other time point when necessary.

Survival status will be recorded for all patients until death or 5 years from study entry

Subjects who complete 24 months of therapy without progression may continue treatment with RAD001 and will have the following assessed:

- Physical exam and labs at the discretion of the physician approximately every 4-6 weeks. Deviation from this schedule must first be approved by the Principal Investigator.
- Disease Status per RECIST approximately every 3 months
- Serious Adverse Events (SAEs) per section 3.4.3.2
- Drug accountability

3.4.1.3 End of treatment period procedures

Patients removed from study treatment for reasons other than progression of disease should continue to be followed for first disease progression per RECIST. See footnote #5 in 3.4.1 for details.

All patients who terminate treatment with RAD001 should be evaluated in the outpatient clinic within 5 business days after the last dose of RAD001 and again 4 weeks after their last dose of RAD001 (plus or minus one week depending on appointment availability due to holidays or unforeseen events). In certain instances, if a patient is unable to return to clinic every effort should be made to follow-up with a phone call to the patient or their provider.

This evaluation will include:

Physical examination,

ECOG performance status,

blood pressure, respiratory rate, resting pulse, temperature, and weight.

Patient reported outcomes questionnaire (EQ-5D), see appendix

Medullary thyroid cancer-related symptoms questionnaire, see appendix

Laboratory evaluations are also performed according to table 3.4a. The necessary components of the laboratory tests are outlined below. Documentation of all adverse events

3.4.1.4 Study procedures

3.4.1.4.1 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.

ANC will be determined by this equation:

$$(\%\text{Neutrophils} + \%\text{Bands})\ (\text{WBC Count} \times 10^{1}) = \text{ANC}^{1}$$

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, serum lipid profile (triglycerides, total cholesterol, HDL and LDL).

[1] WBC Count is in K/UL units.

Because accurate serum glucose and lipid m easurements are required, patients should be fasting at the time of the blood sampling.

Tumor markers which were elevated prior to treatment start with RAD001 will be assessed every month or whenever clinically indicated. Patients with differentiated thyroid cancer will have thyroglobulin and thyroglobulin antibody measured every month and patients with medullary thyroid cancer will have CEA and calcitonin levels evaluated every month (see table 3.4a)

The Hepatitis B and C screening tests (Hep B Core, Hep B Surface antibody, Hep B Surface antigen, HCV RNA-PCR) need to be performed at baseline. HBV DNA follow-up testing should be done according to table 3.4a and section 3.2.6 "Management of Hepatitis B or C Reactivation," depending on results from baseline.

Urinalysis

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

Vital signs

Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.

Physical examination

Physical examination will be performed monthly and must comprise a total body examination. Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded.

ECG

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

Performance status

Performance status will be assessed according to the ECOG performance status scale at the screening visit and monthly thereafter (see schedule of assessment, appendix B).

Special tests

Special tests may be added in a future amendment.

Drug levels and pharmacokinetic assessments

No drug levels will be assessed.

3.4.2 Efficacy assessments

Progression-free survival will be measured based on the appearance of disease progression on imaging based on RECIST criteria (see Appendix A). This can be on imaging studies that are part of the regular study schedule or based on studies which were obtained based on the appearance of symptoms.

3.4.3 Safety assessments

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.

These assessments should be performed within 1 week 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 CTC http://ctep.cancer.gov/forms/CTCAEv3.pdf.

3.4.3.1 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:

- the severity grade (mild, moderate, severe) or (grade 1-4)
- its relationship to the study drug(s) (suspected/not suspected)
- its duration (start and end dates or if continuing at final exam)
- 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)
- 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. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed.

3.4.3.2 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 Novartis within 24 hours of learning of its occurrence. 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
- 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
- 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

3.4.4 Novartis and FDA instructions for rapid notification of serious adverse events

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

Reporting to the FDA

The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

All events meeting the criteria of an INDSR will be submitted to the appropriate division center via mail using Form FDA 3500A (Mandatory Reporting Form for investigational agents) in compliance with FDA reporting timelines. Forms are available at http://www.fda.gov/medwatch/getforms.htm.

Reporting to Novartis

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

3.4.5 IRB Instructions for notification of serious adverse events

For DF/HCC Sites:
The DFCI IRB SAE Reporting Form must be used to report SAEs experienced by DF/HCC participants enrolled in a DF/HCC study including any serious adverse events on DF/HCC led Multi-Center trials where the event occurs at a non-DF/HCC site. Full written SAE report must be submitted to OHRS as soon as possible, but no later than **10 working days** from notification of event. For DF/HCC participant centers, reports must be submitted via OHRS Submit, and the lead site must be notified prior to submission to OHRS.

The DFCI IRB requires the following events be reported:

- **Grade 2 (moderate) and Grade 3 (severe) Events** – Only events that are Unexpected and Possibly, Probably or Definitely Related/Associated with the Intervention.

- **ALL Grade 4 (life threatening or disabling) 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.

Follow Up SAE Reports (DF/HCC participating centers only):
When submitting follow up reports to previously reported SAEs, **attach a copy of the original report and any prior IRB determinations to the follow up report**. This gives the reviewer all the information required to conduct a thorough review and eliminates questions that might otherwise be raised.

For Non-DF/HCC Sites:

For institutions outside of DF/HCC, please report grade 2 or higher AEs considered unexpected and possibly, probably, or definitely related/associated with the intervention to DFCI Study Team (Dr. Lorch: Principal Investigator) for reporting to the DFCI IRB. All outside institutions are also required to report events to their IRB per individual institutional guidelines.

3.4.5.1 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.

4 Protocol amendments, or changes in study conduct

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed, and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

For Non-DF/HCC Sites:

For institutions outside of DF/HCC, any protocol or amendment changes need to be first approved by Dr. Lorch.

5 Correlative Studies

For correlative studies, patients will be asked for the permission to obtain and store either a tumor tissue block from a previous biopsy and/or undergo sequential biopsies.

If tumor tissue blocks are unavailable, then up to forty unstained slides should be obtained. These will be kept in the clinical research lab in a secure location.

They will be used for immune histochemistry (IHC) studies and possible analysis of genetic mutations in the future. IHC staining for the expression of AKT, PTEN, PI3K and others will be performed when funding becomes available. Analysis of mutations such as B-raf may also be performed depending on funding in the future. Patients may decide against participating but will be encouraged to give permission for using their tumor tissue.

Patients with easily accessible tumors such as skin metastases, will be asked to undergo sequential biopsies before and during treatment with RAD001. This will be performed in collaboration with Dr Daniel Ruan from the Department of Surgery at BWH at his outpatient office. Obtaining biopsies will be optional for patients and will only be pursued if they can be obtained with a minimal risk to the patient. Biopsies will be evaluated in the lab of Dr Ruan for the presence of markers of autophagy. It will also be analyzed for tyrosine kinase phosphorylation status in collaboration with Dr. Jinyan Du at the Broad Institute, Harvard University.

For Non-DF/HCC Sites:

Institutions outside of DF/HCC will also be participating in the correlative studies. They will send their participants' archival tissue with the submission form to Dr. Jochen Lorch and his study team. Before any tissue is sent, the external sites need to contact the DFCI Study Team to coordinate the shipment. For specimen preparation, submission and shipping guidelines, please see Appendix E.

6 Data management

6.1 Data collection

Investigators must record the information required by the protocol.

6.1.1 Data Safety

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

7 Statistical methods

7.1 Statistical methods

This study is intended to provide initial efficacy data on RAD001 in patients with RAI refractory differentiated thyroid cancer and evidence for progressive disease. The design follows the two step method by Fleming et al[33]. In the first step, 18 patients with differentiated thyroid cancer (excluding medullary and anaplastic thyroid cancer) and documented disease progression within 6 months will be enrolled. Then a PFS of 6 months or more will be required in at least two patients in order to proceed with the enrollment of 15 more patients for the study to complete its full accrual of the differentiated thyroid cancer part of the trial of 33 patients. The time interval of 6 months is chosen because documented disease progression within 6 months is a requirement for enrollment in this trial. Imaging studies will obtained every 2 months and if no progression can be demonstrated at the 6 month time point while on therapy, anti-tumor activity is likely. If there is only one or no case alive and progression-free at 6 months in the first cohort, the drug combination will be considered ineffective and the trial will be terminated. If at least 2 subjects meet the criteria, the study will proceed to its full accrual of patients with differentiated thyroid cancer of 33 patients. Patients who drop out before the 6 month binary evaluation will be counted as failures. We will declare the trial a

success after observing 7 or more patients alive and progression-free at 6 months. The study will have alpha = 0.092 and power =0.970, assuming a 6 month PFS of 0.12 for the null and a PFS of 0.35 as the alternative hypothesis.

This trial will also include 2 separate exploratory cohorts for patients with medullary and anaplastic thyroid cancer to detect a possible signal in these rare diseases in which no established treatment alternatives exist. They are not included in the main analysis since there are important differences compared to differentiated thyroid cancer. Medullary thyroid cancer originates from parafollicular C-cells which are embryologically and molecularly distinct from the rest of the thyroid gland. Anaplastic thyroid cancer is also distinct from differentiated thyroid cancer with a high rate of distant metastasis and a poor prognosis. Ten patients with medullary thyroid cancer and seven patients with anaplastic thyroid cancer will be treated on this protocol and descriptive statistics will be used for analysis of these patient groups. If no signal is detected, no conclusions can be drawn. However, if there are a few responses (i.e. one or more), a case for a larger, multi-institutional effort to analyze this fully could be made.

Since we expect accrual of a relatively large number of medullary thyroid cancer cases based on the patient population that we see at DFCI, we wanted to make sure that the main group for which this trial is designed - papillary and follicular thyroid cancer- are sufficiently represented in the group of 33 patients of the primary analysis.

Thus the grand total of subjects on this trial will be 50.

We will use the Kaplan-Meier survival method and the Cox model to analyze PFS and the confidence intervals will be estimated.

For the categorical outcomes, logistic regression or chi-square test will be used. For the survival outcomes, the Kaplan-Meier method and the Cox regression model will be used.

8 References

1. Boulay A, Lane HA. The mammalian target of rapamycin kinase and tumor growth inhibition. Recent Results Cancer Res 2007;172:99-124.

2. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004;4:335-48.

3. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006;124:471-84.

4. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129:1261-74.

5. Wang L, Harris TE, Roth RA, Lawrence JC, Jr. PRAS40 regulates mTORC1 kinase activity by functioning as a direct inhibitor of substrate binding. J Biol Chem 2007;282:20036-44.

6. Witzig TE, Geyer SM, Ghobrial I, et al. Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. J Clin Oncol 2005;23:5347-56.

7. Yeager N, Brewer C, Cai KQ, Xu XX, Di Cristofano A. Mammalian target of rapamycin is the key effector of phosphatidylinositol-3-OH-initiated proliferative signals in the thyroid follicular epithelium. Cancer Res 2008;68:444-9.

8. Zitzmann K, De Toni EN, Brand S, et al. The novel mTOR inhibitor RAD001 (everolimus) induces antiproliferative effects in human pancreatic neuroendocrine tumor cells. Neuroendocrinology 2007;85:54-60.

9. Zeng Z, Sarbassov dos D, Samudio IJ, et al. Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. Blood 2007;109:3509-12.

10. Ikezoe T, Nishioka C, Bandobashi K, et al. Longitudinal inhibition of PI3K/Akt/mTOR signaling by LY294002 and rapamycin induces growth arrest of adult T-cell leukemia cells. Leuk Res 2007;31:673-82.

11. Wanner K, Hipp S, Oelsner M, et al. Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. Br J Haematol 2006;134:475-84.

12. Tuncyurek P, Mayer JM, Klug F, et al. Everolimus and mycophenolate mofetil sensitize human pancreatic cancer cells to gemcitabine in vitro: a novel adjunct to standard chemotherapy? Eur Surg Res 2007;39:380-7.

13. Treeck O, Wackwitz B, Haus U, Ortmann O. Effects of a combined treatment with mTOR inhibitor RAD001 and tamoxifen in vitro on growth and apoptosis of human cancer cells. Gynecol Oncol 2006;102:292-9.

14. Sieghart W, Fuereder T, Schmid K, et al. Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation. Transplantation 2007;83:425-32.

15. Kilicarslan AB, Ogus M, Arici C, Pestereli HE, Cakir M, Karpuzoglu G. Clinical importance of vascular endothelial growth factor (VEGF) for papillary thyroid carcinomas. APMIS 2003;111:439-43.

16. Gupta V, Puttaswamy K, Lassoued W, et al. Sorafenib targets BRAF and VEGFR in metastatic thyroid carcinoma. J Clin Oncol (Meeting Abstracts) 2007;25:6019-.

17. Cohen EE, Rosen LS, Vokes EE, et al. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. J Clin Oncol 2008;26:4708-13.

18. Cohen EE, Vokes EE, Rosen LS, et al. A phase II study of axitinib (AG-013736 [AG]) in patients (pts) with advanced thyroid cancers. J Clin Oncol (Meeting Abstracts) 2007;25:6008-.

19. Tabernero J, Rojo F, Calvo E, et al. Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. J Clin Oncol 2008;26:1603-10.

20. Hodgson NC, Button J, Solorzano CC. Thyroid Cancer: Is the Incidence Still Increasing? Ann Surg Oncol 2004;11:1093-7.

21. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43-66.

22. Ahuja S, Ernst H. Chemotherapy of thyroid carcinoma. J Endocrinol Invest 1987;10:303-10.

23. Shimaoka K, Schoenfeld DA, DeWys WD, Creech RH, DeConti R. A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. Cancer 1985;56:2155-60.

24. Harada T, Nishikawa Y, Suzuki T, Ito K, Baba S. Bleomycin beneficial for thyroid cancer. Aerosp Med 1972;43:330.

25. Ain KB, Egorin MJ, DeSimone PA. Treatment of anaplastic thyroid carcinoma with paclitaxel: phase 2 trial using ninety-six-hour infusion. Collaborative Anaplastic Thyroid Cancer Health Intervention Trials (CATCHIT) Group. Thyroid 2000;10:587-94.

26. Gupta-Abramson V, Troxel AB, Nellore A, et al. Phase II trial of sorafenib in advanced thyroid cancer. J Clin Oncol 2008;26:4714-9.

27. Sherman SI, Wirth LJ, Droz JP, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. N Engl J Med 2008;359:31-42.

28. Moreno A, Akcakanat A, Munsell MF, Soni A, Yao JC, Meric-Bernstam F. Antitumor activity of rapamycin and octreotide as single agents or in combination in neuroendocrine tumors. Endocr Relat Cancer 2008;15:257-66.

29. Wang S, Lloyd RV, Hutzler MJ, Safran MS, Patwardhan NA, Khan A. The Role of Cell Cycle Regulatory Protein, Cyclin D1, in the Progression of Thyroid Cancer. Mod Pathol;13:882-7.

30. Erickson LA, Jin L, Wollan PC, Thompson GB, van Heerden J, RV L. Expression of p27kip1 and Ki-67 in benign and malignant thyroid tumors. Mod Pathol 1998;Feb;11(2)::169-74.

31. Ringel MD, Hayre N, Saito J, et al. Overexpression and Overactivation of Akt in Thyroid Carcinoma. Cancer Res 2001;61:6105-11.

32. Sherman SI, Schlumberger MJ, Droz J, et al. Initial results from a phase II trial of motesanib diphosphate (AMG 706) in patients with differentiated thyroid cancer (DTC). J Clin Oncol (Meeting Abstracts) 2007;25:6017-.

33. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. Biometrics 1982;38:143-51.

34. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205-16.

9 Procedures and instructions

9.1.1 Publication of results

The results should be made public within 24 months of the end of data collection. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of data collection. Outside institutions planning publications must send 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). Principal Investigation/Institution shall have the final authority to determine the scope and content of the publications.

9.1.2 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.

9.1.3 Discontinuation of study

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

9.2 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:

ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.

US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

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.

9.2.1 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.

9.2.2 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.

9.2.3 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.

Summary of Major Changes, Protocol Amendment Dated January 28th, 2014

Protocol Section	Change
Study Objectives	The secondary endpoint of overall survival will now be collected for 5 years from study entry, updated from 1 year.
Visit Schedule and Assessments	Footnote #5 describes the imaging schedule. The schedule is unchanged; this update is to add clarity to how the imaging schedule

	changes after subjects have been on trial for >24 months.
Treatment Period Procedures	This section now describes all study procedures rather than just those occurring throughout the first 24 months on trial. Procedures for subjects on trial for >24 months were previously located in the "End of Treatment Period Procedures" section
Adverse Events	Protocol now reflects that all serious events are reported to the sponsor, regardless of whether their relatedness to study drug.
Throughout the Protocol	Minor administrative changes

Appendix A

Modified RECIST criteria (adapted from Therasse et al [34].

RECIST: Response Evaluation Criteria in Solid Tumors

Eligibility: To be eligible, subjects must have at least one uni-dimensionally measurable lesion.

Measurable lesion: To be considered measurable, lesions must be equal or greater than 20mm in at least one dimension using conventional techniques (CT or MRI scan) or 10 – 16mm with spiral CT scan.

Lesions identified on physical exam and clinically measured by the investigator will automatically be considered non-target lesions. Lesion in a radiation therapy port will not be considered target lesions.

Non-measurable lesions

Non-measurable lesions are all other lesions including small lesions9 longest diameter less that 20mm with conventional techniques or equal or more that 10-16mm with spiral CT scan. Other examples of non-measurable lesions include bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, lymphangitis cutis/pulmonis, cystic lesions and also abdominal masses that are not confirmed and followed by imaging techniques. Additionally, the following will be considered non-measurable for this study: Non-measurable skin lesions, peritoneal carcinomatosis, military lesions, irradiated lesions and groups of lesions that are small and numerous.

Method

All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up evaluations.

Conventional CT and MRI will be performed with contiguous cuts of 10mm or less in slice thickness and spiral CT will be performed by use of up to an 8mm contiguous reconstruction algorithm. Standard whole body bone scans will be utilized to follow the non-measurable bony lesions for progression.

Measurement of lesions on chest x-ray will not be performed. CT of the chest is required for lesion measurement.

When the primary endpoint of the study is objective response evaluation, ultrasound should not be used to measure tumor lesions that are not clinically easily accessible. It may be used as a possible alternative to clinical measurements for superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. Ultrasound might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Tumor markers will not be used to assess objective response for the primary study endpoint.

Baseline documentation of target and non-target lesions.

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total representative of all involved organs should be identified as target lesions and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline for sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurement of these lesions is not required but the presence or absence of each should be noted through follow-up evaluations.

Response criteria	Evaluation of target lesions
Complete response	Disappearance of all target lesions
Partial response	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
Stable disease (SD)	Neither sufficient shrinkage to qualify for a partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum LD since the treatment started

	Evaluation of non-target lesions
Complete response	Disappearance of all non-target lesions
Incomplete response/stable disease	Persistence of one or more non-target lesion(s)
Progressive disease	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. A clear progression of non-target lesions only is exceptional. In such circumstances, the opinion of the investigator will prevail for the purpose of deciding whether to continue treatment. Bone scans will be utilized to follow subjects at risk for bony disease progression. Because of the difficulties in interpreting changes in the intensity or size of bone lesions on bone scans, progressive disease in bone is defined as the appearance of one or more new lesions determined by the investigator to be compatible with metastases. In questionable cases, plain radiographic films or other imaging may be performed to confirm or refute the clinical suspicion.

Evaluation of Response

Response is recorded each time a CT is performed. The smallest measurements since treatment started are taken as reference for progressive disease. In general, the subject's response assignment will depend on the achievement of both target lesions and non-target lesions.

Target lesions	Non-target lesions	New lesions	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

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at the time should be classified as having symptomatic deterioration. Every effort should be made to document the objective progression even after discontinuation of treatment in the long term follow-up portion of the study.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Response confirmation

To be assigned a status of PR or complete response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met.

Duration of Overall Response

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

Duration of Stable Disease

SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

Appendix B

Drug Diary

Appendix C

Questionnaire

Appendix D

DF/HCC Multi-center Data and Safety Monitoring Plan

Appendix E

Correlative Studies Shipping Guidelines and Submission Form

Participant Name:_____ Record #:_____Cycle #:_____

RAD001

1.) RAD001 should be taken: **Participant Signature:**_____**Date:**____/____/____

- · 1 time every other day in the morning, at approximately the same time every day when taken
- · On an empty stomach or with no more than a light fat-free meal.

2.) Please record each dose in the calendar below, indicating what **time** the dose was taken.

3.) List any noticeable side effect, reason for missed dose, or other pertinent information in the "Notes" column.

4.) If you vomit after taking RAD001 or miss a RAD001 dose, do not repeat or make up that dose. Take your next scheduled dose on the following day. If you do not take RAD001 within 6 hours of the scheduled time this is considered a missed dose. Please record the missed dose in your diary below.

5.) Please do not drink or eat grapefruit juice, pomegranate juice, star fruit, and Seville oranges while taking this medication.

6.) Please return this diary, all empty bottles and unused pills at the end of each cycle.

Day #	Date	Time	Dose of Drug	Notes (Side effects, reason for missed dose, etc.)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
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16				
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21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				

Participant Name:_____ Record #:_____Cycle #:_____

RAD001

1.) RAD001 should be taken: **Participant Signature:**_____**Date:**____/____/____

- 1 time per day in the morning, at approximately the same time every day
- On an empty stomach or with no more than a light fat-free meal.

2.) Please record each dose in the calendar below, indicating what **time** the dose was taken.

3.) List any noticeable side effect, reason for missed dose, or other pertinent information in the "Notes" column.

4.) If you vomit after taking RAD001 or miss a RAD001 dose, do not repeat or make up that dose. Take your next scheduled dose on the following day. If you do not take RAD001 within 6 hours of the scheduled time this is considered a missed dose. Please record the missed dose in your diary below.

5.) Please do not drink or eat grapefruit juice, pomegranate juice, star fruit, and Seville oranges while taking this medication.

6.) Please return this diary, all empty bottles and unused pills at the end of each cycle.

Day #	Date	Time	Dose of Drug	Notes (Side effects, reason for missed dose, etc.)
1				
2				
3				
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ORIGINAL

Health Questionnaire

(English version for the US)

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today

ORIGINAL

Date: ☐☐ / ☐☐ / ☐☐
(month) (day) (year)

Participant's Initials: _____

Participant's Study #: ☐☐☐☐

PLEASE USE A BLACK INK PEN

Study Name: _____

_____

Protocol #: _____

PI: _____

M. D. Anderson Symptom Inventory - Thyroid (MDASI-Thy)

Part I. How severe are your symptoms?

People with cancer frequently have symptoms that are caused by their disease or by their treatment. We ask you to rate how severe the following symptoms have been in the last 24 hours. Please rate each of these symptoms from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be).

CORE Items	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
1. Your **pain** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
2. Your **fatigue (tiredness)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
3. Your **nausea** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
4. Your **disturbed sleep** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
5. Your feeling of being **distressed (upset)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
6. Your **shortness of breath** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
7. Your problem with **remembering things** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
8. Your problem with **lack of appetite** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
9. Your feeling **drowsy (sleepy)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
10. Your having a **dry mouth** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
11. Your feeling **sad** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
12. Your **vomiting** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
13. Your **numbness or tingling** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Date: ☐☐ / ☐☐ / ☐☐
(month) (day) (year)

Participant's Initials: _____

Participant's Study #: ☐☐☐☐

Study Name: _____

_____

Protocol #: _____

PI: _____

PLEASE USE A BLACK INK PEN

THYROID-Specific Symptoms	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
14. Your **hoarseness** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
15. Your **problem with feeling hot** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
16. Your **problem with racing heartbeat** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
17. Your **problem with feeling cold** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
18. Your **difficulty swallowing** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
19. Your **diarrhea** or **loose stools** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Part II. How have your symptoms interfered with your life?

Symptoms frequently interfere with how we feel and function. How much have your symptoms interfered with the following items in the last 24 hours:

	Did not Interfere 0	1	2	3	4	5	6	7	8	9	Interfered Completely 10
20. **General activity?**	○	○	○	○	○	○	○	○	○	○	○
21. **Mood?**	○	○	○	○	○	○	○	○	○	○	○
22. **Work (including work around the house)?**	○	○	○	○	○	○	○	○	○	○	○
23. **Relations with other people?**	○	○	○	○	○	○	○	○	○	○	○
24. **Walking?**	○	○	○	○	○	○	○	○	○	○	○
25. **Enjoyment of life?**	○	○	○	○	○	○	○	○	○	○	○

DFCI IRB Protocol #: *09-049*

APPENDIX *D*

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

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. *Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates are not viewed as outside sites in this definition.*

Lead Institution: One of the Dana-Farber/Harvard Cancer Center sites (DFCI, MGH, BIDMC, CH, BWH) 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 applicable regulatory guidelines (FDA, etc.).

DF/HCC Contract Principal Investigator: Investigator located at the Lead Institution who will be charged with the responsibility of the administration of the DF/HCC Project. This most often will be the Protocol Chair, but occasionally this may be the overall grant or contract holder, as applicable.

Protocol Chair: The Protocol Chair is the Principal Investigator for the DF/HCC protocol submitted as the Lead Institution. For applicable protocols, the Protocol Chair will be the single liaison with any regulatory agencies (i.e. FDA, etc.).

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 Lead Institution 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. 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 (DF/HCC Principal Investigator), Coordinating Center (Lead Institution or designee), and the Participating Institutions will all agree to the general responsibilities as follows (specific procedures for these general responsibilities are detailed in the DSMP):

2.1.1 Protocol Chair (DF/HCC Principal Investigator)

The Protocol Chair, Jochen Lorch MD, MSc, will accept responsibility for all aspects of the Multi-Center Data and Safety Monitoring Plan to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Submit the Multi-Center Data and Safety Monitoring Plan as an inclusion to the protocol.
- Assure all participating institutions are using the correct version of the protocol.

- Monitor progress and overall conduct of the study at all participating institutions.
- Ensure all DFCI IRB, DF/HCC and other applicable (i.e. FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (sponsor-investigator IND trials), as applicable.
- Identify participating institutions and obtain accrual commitments . The title page must include 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 (Lead Institution or designee) must be designated on the title page.

2.2 Coordinating Center (Lead Institution)

The Coordinating Center is the DF/HCC Lead Institution's study team or designee (i.e Medical Monitor, Clinical Research Organization). The DF/HCC Lead Institution Dana-Farber Cancer Institute 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 DF/HCC Lead Institution's study team or designee will assume the following general responsibilities:

- Assist in protocol review.
- Maintain copies of Institutional Review Board (IRB) approvals from all participating institutions.
- Maintain FDA correspondence, as applicable.
- 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 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 to local IRB and directly to the Coordinating Center (Lead Institution or designee). Submit deviations and violations to local IRB and 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. pharmaceutical company).

3.0 DF/HCC QUALITY ASSURANCE OFFICE FOR CLINICAL TRIALS

The DF/HCC QACT is a unit that has been developed to computerize, 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

The Protocol Chair 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 if applicable FDA Guidelines. Further, the Protocol Chair will be the single liaison with the FDA as applicable.

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 title page must include 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 (Lead Institution or designee) 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 DF/HCC Lead Institution'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 DF/HCC Lead Institution'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. Conduct regular communications with all participating sites (conference call, emails, etc) Maintain documentation of all communications.

5.0 PROTOCOL MANAGEMENT

The Coordinating Center (DFCI) is responsible for assuring that each Participating Institution in the DF/HCC Multi-center Protocol has the appropriate assurance on file with the Office of Human Research Protection (OHRP). Additionally, the Lead Institution or designee must maintain copies of all IRB approvals, for each participating institution.

5.1 Protocol Distribution

The Coordinating Center (DFCI) 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.

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 IRB approved and implemented within 90 days from receipt of the notification.

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 followed by IRB request for approval

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 DF/HCC Lead Institution or designee prior to participant registration:

- Approval 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 DF/HCC Lead Institution or designee 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 and Randomization

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 research manager at the Lead Institution:

- Copy of required laboratory tests (coagulation test, hematology and blood chemistry, urinalysis, free T4, TSH, calcitonin/CEA (only MTC subjects), thyroglobulin/anti-thyroglobulin antibodies (only DTC subjects), and pregnancy test)
- Signed informed consent form
- HIPAA authorization form (if separate from the informed consent document)
- Other appropriate forms (e.g., Eligibility Screening Worksheet/Registration form, source documents confirming eligibility (pathology reports, radiology reports showing progression within6 months per RECIST criteria, treatment summary forms))

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

- Verify the patient meets all eligibility criteria through source documentation provided by external site.
- 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, 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.

Please fax all registrations to the research manager, Brian Davis, at the Lead Institution. The fax number is 617-582-7876. If you have any questions, please call 617-632-2503.

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 D F/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, Dose Levels, and Arm Designation: 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 develops a set of either paper or electronic case report forms, (CRF/eCRFs) for use with the DF/HCC Multi-Center Protocol. QACT provides a web based training for eCRF users. These forms are designed to collect data for each study. The schedule for submission of case report forms to the DF/HCC QACT is generally specified in each protocol. When not specified in the protocol, the DF/HCC QACT will require the forms to be submitted as follows:

- For on treatment and follow-up forms: Enter data into eCRF system within 10 days of

visit date.

- For baseline and day 1 required data: Enter data into eCRF system within 2 weeks after registration.

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

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)

1) Parameters for eligibility
2) Parameters for exclusion
3) Parameters for stratifications

If a time frame is not specified in the protocol, tests must be completed as follows:

- Lab tests required for eligibility must be completed within 14 days prior to study enrollment by the QACT.
- For protocols requiring measurable disease, lab baseline measurements must be completed within 14 days prior to study enrollment by the QACT. Examples: flow cytometry, HLA typing, fluid cytology, tumor markers and hormones (CEA, CA-27-29, CA-125).
- Non-lab tests required for eligibility must be performed within 30 days prior to study entry. Example: radiological scans
- For bone marrow transplant (BMT) protocols and non-protocol treatment plans, eligibility tests must be completed within 42 days prior to enrollment by the QACT. The extended period of time is allowed to facilitate insurance approval while ensuring participant safety.

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
- Toxicities or adverse events experienced since last visit (based on monthly visit cycle)

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

5.10.5Adverse 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:

<u>Incomplete or Questionable Data</u>

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 generally specified in the protocol.

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

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 (Novartis) 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 (CTCAE v3.0) assigned to the protocol 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 will be 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.

Novartis guidelines on serious adverse events

Information about all serious adverse events will be collected and recorded. 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

7.2 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Serious Adverse Events (SAEs) will be followed as is delineated in Section 3 of the protocol:

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

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 business hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g.,

participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone or email to:

DF/HCC Overall Principal Investigator: Jochen Lorch, MD, MS
Telephone: 617-632-3090
Email: Jochen_Lorch@dfci.harvard.edu

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation. The Lead Institution will maintain documentation of all Adverse Event Reporting and be responsible for communicating all SAEs to all Participating sites.

Novartis and FDA instructions for rapid notification of serious adverse events

The principal investigator has the obligation to report all serious adverse events to the FDA, 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). 3500A forms should be faxed to the FDA at 301-796-9845. For any questions, you can contact the FDA at 301-796-4058.

All events must be reported, by FAX (888-299-4565), to Novartis Pharmaceuticals CS &E Department within 24 hours of learning of its occurrence. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

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

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

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

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. In compliance with these FDA regulations, the Protocol Chair is responsible for reviewing all IND Safety Reports and forwarding the IND Safety Reports to the Participating Institutions. The investigator's 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 Exception: Any protocol deviation that relates to the eligibility criteria, e.g., enrollment of a subject who does not meet all inclusion/exclusion criteria.

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 exceptions, 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 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 institutional policy.

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.

All protocol violations must be sent to the DF/HCC Lead Institution Protocol Chair or designee in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the participating institution, the DF/HCC Lead Institution or designee 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 DF/HCC Lead Institution or designee 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 Lead Institution or designee 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

9.3.1.2 DF/HCC Sponsored Trials

Sites are expected to notify DFCI of all deviations and violations from the required data set based on the DFCI OHRS guidelines. The site is expected to comply with DF/HCC Lead Institution regulatory submission guidelines and should include IRB documents, CV's, case report forms, and all institutional licenses and documents. We will virtually monitor the external site monthly for source verification of case report forms and all regulatory documents. Some of these documents will include eligibility source documents (radiology reports proving progression, lab values, performance status, pathology reports, etc), prior treatment reports, bimonthly adverse event (toxicity) reports, laboratory hematology and chemistry reports (baseline, bimonthly, and end of treatment), serious adverse events supplementary documents, tumor measurement reports (baseline and bimonthly), and documentation of progression (if applicable).

The participating institution may be subject to on-site monitoring conducted by the DF/HCC Lead Institution or designee. Sites will be monitored for compliance to the data collection guidelines found in the protocol under the section titled, "3.4 Visit Schedule and Assessments." Participating sites should have source documentation for all data collected.

9.3.2 Participating Institution: It is the participating institution's responsibility to notify the DF/HCC Lead Institution or designee of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve the DF/HCC Multi-Center Protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the DF/HCC Lead Institution or designee within 12 weeks after the audit date.

9.3.3 Coordinating Center (Lead Institution or designee): The Protocol Chair 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 Committee. Based upon the audit assessments the DF/HCC Audit Committee 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 Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

9.4 Sub-Standard Performance

The Protocol Chair and DFCI IRB are 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.

Appendix E: Correlative Studies Shipping Guidelines and Submission Form

Submission of specimens

1. **Paraffin-embedded tissue blocks:** In general, this is the preferred specimen and is especially important to submit in cases where tissues have been in formalin for a significant time. Prolonged fixation (>2 weeks) may interfere with some immunohistochemical and molecular diagnostic assays.
2. **Wet tissue:** If available, we also highly recommend that unprocessed tissues in 10% neutral buffered formalin be submitted in addition to paraffin blocks.
3. **Unstained slides**: Although not optimal, it may be possible to utilize unstained sections cut at 3-5 microns (40 slides per block) if paraffin blocks are unavailable.
4. **Fresh-frozen tissue:** (sent separately on dry ice)

Obtaining Biopisies

For formalin fixed biopsy: Immediately place biopsy into biopsy bag and then into the histocassette. Place histocassette into formalin (completely submerged). 24 hours± 2 hours later, transfer histocassette containing the biopsy fixed in formalin and ship in vial with 30ml 70% ethanol. Formalin-fixed tissues should be thin (~1/4 inch) and fixed in 9 parts 10% buffered formalin to 1 part tissue.

For fresh frozen tumor biopsy: Only in cases where a reasonable size biopsy (e.g. excisional biopsy, 5 mm punch, or 14G) could be collected for formalin fixation, a fresh frozen biopsy can be collected. Place tissue in cyrotube and let slide to bottom. Transfer tube into liquid nitrogen, Remove tube from liquid nitrogen with pair of tweezers and transport to freezer in liquid nitrogen or dry ice, and place in tube in a freezer of -70°C or below.

General Guidelines for Shipping Pathology Specimens

Packaging and Shipping Guidelines	
Room Temperature	**Frozen (dry-ice)**
Formalin-fixed wet tissues. Formalin-fixed paraffin-embedded blocks.* Glass slides with sections from paraffin-embedded blocks.	Fresh frozen tissue.

Formalin Fixed Tissues:

• To avoid shipping large quantities of formalin, you may fix an appropriately sized sample for 24 hours, then ship with a smaller quantity of formalin; **please indicate that the sample has been fully fixed prior to shipping.**
• Avoid putting large samples in narrow-mouthed containers; once these tissues fix, they can be difficult to retrieve.
• **DO NOT** ship formalin-fixed tissues and cytology or hematology slides in the same container. Formalin fumes can compromise these results.

• Formalin is a noxious volatile and toxic chemical and should be shipped with appropriate precautions.
• Submit specimens in leak-proof containers.
• Surround this container with enough absorbent material to absorb any possible leakage.
• Containers and absorbent material should then be enclosed in a second leak proof container (e.g., sealed plastic bag).
• Samples can then be enclosed in a sturdy and sealed container (cardboard box, styrofoam, plastic) for shipping.
• Enclose the completed submission form in a separate sealed plastic bag, and place them between the inner sample container and the outer shipping container.
• **A completed submission form must accompany the sample.**
• **Please clearly label each container with the tissue, number of sample (i.e. slides, blocks, etc), and patient initials and ID number.**

Unfixed Fresh Tissue (Only large specimens that are impractical for formalin fixation should be submitted fresh.)**:**

• Arrange for a delivery time to our facility, Monday through Friday only by emailing Dr. Lorch or calling the study manager at 617-632-2503.
• Call our study team at (617) 582-8039 when the specimen is on its way to our facility.
• Double-bag the specimen in leak proof Bio-Hazard bags**.**
• Pack the specimen with sufficient, sealed ice packs in another, leak proof bag.
• Samples can then be enclosed in a sturdy and sealed container (cardboard box, styrofoam, plastic) for shipping.
• Enclose the completed submission form in a separate sealed plastic bag, and place them between the inner sample container and the outer shipping container.
• **A completed submission form must accompany the sample.**
• **Please clearly label each container with the tissue, number of sample (i.e. slides, blocks, etc), and patient initials and ID number.**

Shipping Considerations:
• U.S. Federal holidays should be taken into consideration before mailing the packages. Exceptions can be made on urgent cases with prior approval.
• Specific regulations for packaging, labeling, and shipping may be found at http://www.cdc.gov/ncidod/srp/specimens/shipping-packing.html, and at http://www.cdc.gov/od/ohs/biosfty/shipdir.htm
• Please provide us with the shipper's package tracking number(s) on the submission form. During the warmer months (June-Aug), in order to prevent the melting of paraffin-embedded tissue blocks during transit, it is advisable to ship the block(s) with a frozen gel ice-pack.
• When shipping frozen specimens from long distances, it is best to use a combination of dry-ice and frozen gel ice-packs. The gel ice-packs will remain frozen for a day or two after the dry-ice has dissipated.

Required supporting electronic or hard-copy documentation:

1. Copy of the preliminary or final pathology report

2. Copy of any pertinent laboratory reports (including rapid antigen, culture, and PCR test results)
3. Submission form including the full name, title, complete mailing address, phone and fax numbers of the submitter.

Shipping Address:

C/O Tyler Haddad
Dr. Jochen Lorch
Dana-Farber Cancer Institute
450 Brookline Avenue, LG 1B
Boston, MA 02215

Contact Information:

Principal Investigator: Dr. Jochen Lorch, Jochen_Lorch@dfci.harvard.edu, 617-632-3090

Clinical Research Nurses: Pamela Rothe, RN, 617-632-6817

Clinical Research Manager: Farzana Masood, Farzana_Masood@dfci.harvard.edu,
Phone: 617-632-6725, Fax: 617-582-7876

Clinical Research Coordinator: Tyler Haddad, Tyler Haddad@dfci.harvard.edu,
Phone: 617-582-7323, Fax: 617-582-7876

Clinical Scientists: Dr. Daniel Ruan, Department of Surgery, BWH,
Druan@partners.org, 617-732-6830

Dr. Jinyan Du, Broad Institute, Harvard University,
Jinyan_Du@dfci.harvard.edu, 617-632-6688

Correlative Studies Submission Form

***MAIL SPECIMENS TO ADDRESS BELOW:**

C/O Tyler Haddad
Dr. Jochen Lorch
Dana-Farber Cancer Institute
450 Brookline Avenue, LG 1B
Boston, MA 02215
(P) 617-582-8039
(F) 617-582-7876

****Please clearly label each container with the tissue, patient ID number, and date of birth.**

****Please include the preliminary or final pathology report and any other pertinent laboratory reports with this submission form.**

SENDER'S INFORMATION

DATE :_____
Sender's Name:_____
Title:_____
Doctor's Name:_____
Address:_____
City:_____State_____Zip_____
Phone#:_____Fax #:_____
E-mail Address:_____

PATIENT INFORMATION

Initials and ID#:_____
Type of Sample Sent (formalin fixed, fresh frozen, etc):_____
Number of Sample sent (ie # of slides etc):_____
Anatomic Location of lesion:_____
Tissue
Submitted:_____Date of
Biopsy (if applicable):_____

SHIPPING INFORMATION

Date Sent:_____
Shipping Company (Fedex, UPS, etc):_____
Mode of Shipping (Next day Air, etc):_____
Tracking Number:_____

If you have any questions, please contact Margaret Suda at 617-582-8039.