AMC
AIDS ASSOCIATED MALIGNANCY CLINICAL TRIALS CONSORTIUM

AMC PROTOCOL #042

A Phase II Trial of Imatinib Mesylate (GLEEVEC) in Patients with HIV Related Kaposi's Sarcoma

A Multi-Center Trial of the AIDS Malignancy Consortium

Sponsored by:

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AMC PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site _____, agree to conduct and follow this protocol: **AMC Protocol #042 - A Phase II Trial of Imatinib Mesylate (GLEEVEC) in Patients with HIV Related Kaposi's Sarcoma (Version 7.0, 07/24/2007),** as written according to AMC, NCI and FDA guidelines. I understand that no deviations from the above protocol may be made without written permission from the Protocol Chair (s).

_____ _____

Signature Date (mm/dd/yyyy)

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PROTOCOL ROSTER

AMC # 042

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This trial is open to all AMC sites for subject accrual.

SCHEMA

TITLE:	A Phase II Trial of Imatinib Mesylate (Gleevec®) in Patients with HIV Related Kaposi's Sarcoma.
DESIGN:	Open-label, Phase II Study.
SAMPLE SIZE:	A maximum of 30 subjects will be enrolled.
POPULATION:	HIV infected subjects with biopsy proven Kaposi's sarcoma (KS) involving skin, lymph nodes, oral cavity, GI tract and/or lungs. GI and pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy.
REGIMEN:	Patients will be taking Gleevec 400 mg orally once a day.
DURATION:	Patients will continue on study protocol as long as their KS is stable or responding for up to 6 months. If a patient has been on study for at least 3 months with stable disease and without any toxicity, their dose may be increased to 600 mg/day. Patients who are responding or have stable disease at 6 months may continue on study for an additional 6 months. Protocol treatment will be discontinued if the patient develops tumor progression, unacceptable toxicity or develops one of the protocol defined reasons for treatment discontinuation.
EXPECTED ACCRUAL:	A maximum of 30 subjects over 12 months.
PRIMARY ENDPOINTS:	Evaluation of clinical response.
SECONDARY ENDPOINTS:	Evaluation of inhibition of PDGF-R as determined by immunohistochemistry. Evaluation of cytokine profiles pre and post imatinib therapy. Evaluation of pharmacokinetic profile of imatinib and antiretrovirals in HIV patients. Evaluation of mechanisms of primary and secondary resistance to imatinib therapy. Evaluation of viral transcription profile of KSHV.

1.0 BACKGROUND/RATIONALE

Kaposi's Sarcoma (KS) is a disease of multi-focal vascular proliferation, which predominantly involves the skin, but can also involve the visceral organs. AIDS related KS is associated with co-infection with the HIV virus and Kaposi's Sarcoma herpes virus (KSHV) (or Human Herpes virus-8).[1] Histologically, these lesions appear as clusters of spindle-shaped cells (KS spindle cells), vessels and a variable inflammatory infiltrate. These tumors rely on a number of cytokines for growth including interleukin-1, interleukin-6, vascular endothelial growth factor (VEGF), basic-fibroblast growth factor (b-FGF), stem cell factor (SCF) and platelet derived growth factor (PDGF), which act by autocrine and paracrine mechanisms.[2-8]

Several studies have suggested a role for PDGF and c-kit receptors in KS. When KS spindle cells are cultured they express both α and β PDGF receptors.[9] Cultured KS spindle cells growth arrests when placed in PDGF-depleted media, and this arrest can be mitigated by the addition of recombinant PDGF.[10] Some of the unanswered questions for these studies are whether the cultured cells reflect the KS spindle cells behavior in vivo and what the source of the PDGF in vivo is. Indeed only β PDGF-R appears to be expressed in tumor specimens as assayed by in situ hybridization and immunohistochemistry.[6] PDGF is not expressed by these spindle cells, but rather by a distinct subpopulation of cells in the KS lesion.[6]

Once the role of KHSV was elucidated, model systems that use dermal microvasculature endothelial cells (DMVEC) were developed to study the effect of KSHV infection on gene expression. KSHV infection of DMVECs results in a 5-fold up regulation of the c-kit receptor[8] and results in a 4-fold change in levels of β PDGF-R transcripts while α PDGF-R levels remain unchanged as assayed by cDNA array (unpublished data, AV Moses, pers. comm.). In the prior study it was also demonstrated that KSHV-infected endothelial cell cultures proliferate in response to the ligand of c-kit, SCF, which is basally expressed by cells in culture.[8] Additionally, PDGF had been shown to induce the expression of VEGF by cultured KS spindle cells.[7] It is possible that PDGF-R and c-kit are involved in two critical pathways in KS development; induction of growth of KS spindle cells and induction of angiogenesis through VEGF. This potential role of PDGF-R and c-kit in the development of KS makes inhibition of these receptors an attractive therapeutic target.

1.1 Gleevec®

1.1.1 General Information

Imatinib is a signal transduction inhibitor (STI) approved by the United States Food and Drug Administration (FDA) for use in patients with newly diagnosed chronic myeloid leukemia (CML) or CML in blast crisis, accelerated phase, or in chronic phase after failure of interferon-alfa therapy.[11] In preclinical studies, Gleevec was found to be a potent inhibitor of BCR-ABL as well as PDGF-R and the c-Kit receptor. Since its approval, imatinib has been shown to be beneficial

in gastrointestinal stromal tumor (GIST), which is dependent on c-Kit as well as dermatofibrosarcoma protuberns and hypereosinophilic syndrome, which are dependent on the PDGF pathway.[12,13] The availability of imatinib, which inhibits both the PDGF and c-kit receptors, allows us to test clinical utility of inhibiting these receptors to treat KS.

Imatinib is highly bioavailable as an oral formulation. The pharmacokinetics (PK) of Gleevec has been evaluated in healthy subjects and in population pharmacokinetic studies in over 900 patients. Imatinib is well absorbed after oral administration with C_{max} achieved within 2-4 hours post-dose. Mean absolute bioavailability is 98%. Following oral administration in healthy volunteers, the elimination half-lives of imatinib and its major active metabolite, the N-desmethyl derivative, were approximately 18 and 40 hours, respectively. Mean imatinib AUC increased proportionally with increasing dose in the range 25–1000 mg. There were no significant changes in the PK of imatinib on repeated dosing, and accumulation is 1.5-2.5 fold at steady state when Gleevec is dosed once daily. At clinically relevant concentrations of imatinib, binding to plasma proteins is approximately 95% in vitro, mostly to albumin and α1-acid glycoprotein. CYP3A4 is the major enzyme responsible for metabolism of imatinib. Other cytochrome P450 enzymes, such as CYP1A2, CYP2D6, CYP2C9, and CYP2C19, play a minor role in its metabolism.

1.1.2 Safety Data

Chronic Myeloid Leukemia

The majority of Gleevec-treated patients experienced adverse events (AEs) at some time. Most events were of mild to moderate grade, but drug was discontinued for AEs in 2% of patients in chronic phase, 3% in accelerated phase and 5% in blast crisis. The most frequently reported drug-related AEs were nausea, vomiting, diarrhea, edema, and muscle cramps (see Table 1 for newly diagnosed CML patients and Table 2 for other CML patients). Edema was most frequently periorbital or in lower limbs and was managed with diuretics, other supportive measures, or by reducing the dose of Gleevec. The frequency of severe superficial edema was 0.9-5%.

Table 1

Adverse Experiences Reported in Newly Diagnosed CML Clinical Trial (>10% of All Patients)

Preferred term[(1)]	All Grades		CTC Grades 3/4	
	Gleevec N=551 (%)	IFN+Ara-C N=533 (%)	Gleevec N=551 (%)	IFN+Ara-C N=533 (%)
Fluid retention	54.1	10.1	0.9	0.9
- Superficial edema	53.2	8.8	0.9	0.4
- Other fluid retention events	3.4	1.5	0	0.6
Nausea	42.5	60.8	0.4	5.1
Muscle cramps	35.4	9.9	1.1	0.2
Musculoskeletal pain	33.6	40.5	2.7	7.7
Rash	31.9	25.0	2.0	2.1
Fatigue	30.7	64.7	1.1	24.0
Diarrhea	30.3	40.9	1.3	3.2
Headache	28.5	41.8	0.4	3.2
Joint pain	26.7	38.3	2.2	6.8
Abdominal pain	23.4	22.9	2.0	3.6
Myalgia	20.9	38.6	1.5	8.1
Nasopharyngitis	19.2	7.7	0	0.2
Hemorrhage	18.9	19.9	0.7	1.3
Dyspepsia	15.1	9.0	0	0.8
Vomiting	14.7	26.6	0.9	3.4
Pharyngolaryngeal pain	14.2	11.4	0.2	0
Dizziness	13.2	23.1	0.5	3.4
Cough	12.5	21.6	0.2	0.6
Upper respiratory tract infection	12.5	7.9	0.2	0.4
Pyrexia	11.8	38.6	0.5	2.8
Weight increased	11.6	1.5	0.7	0.2
Insomnia	11.4	18.4	0	2.3

(1) All AEs occurring in ≥ 10% of patients are listed regardless of suspected relationship to treatment.

Table 2

Adverse Experiences in ≥ 10% of Patients During Phase II Leukemia Studies

Preferred Term (% of Patients)	Myeloid Blast Crisis n= 260		Accelerated Phase n=235		Chronic Phase n=532	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Nausea	70	4	71	5	60	2
Fluid retention	71	12	73	6	66	3
Superficial edemas	67	5	71	4	64	2
Other fluid retention events	22	8	10	3	7	2
Muscle cramps	27	0.8	42	0.4	55	1
Diarrhea	42	4	55	4	43	2
Vomiting	54	4	56	3	32	1
Hemorrhage	52	19	44	9	22	2
CNS hemorrhage	7	5	2	0.9	1	1
Gastrointestinal hemorrhage	8	3	5	3	2	0.4
Musculoskeletal pain	43	9	46	9	35	2
Skin rash	35	5	44	4	42	3
Headache	27	5	30	2	34	0.2
Fatigue	29	3	41	4	40	1
Arthralgia	25	4	31	6	36	1
Dyspepsia	11	0	21	0	24	0
Myalgia	8	0	22	2	25	0.2
Weight increased	5	0.8	14	3	30	5
Pyrexia	41	7	39	8	17	1
Abdominal pain	31	6	33	3	29	0.6
Cough	14	0.8	26	0.9	17	0
Dyspnea	14	4	20	7	9	0.6
Anorexia	14	2	17	2	6	0
Constipation	15	2	15	0.9	6	0.2
Nasopharingitis	8	0	16	0	18	0.2
Night sweats	12	0.8	14	1	10	0.2
Pruritus	8	1	13	0.9	12	0.8
Epistaxis	13	3	13	0	5	0.2
Hypokalemia	13	4	8	2	5	0.2
Petechiae	10	2	5	0.9	1	0
Pneumonia	12	6	8	6	3	0.8
Weakness	12	3	9	3	7	0.2
Upper respiratory tract infection	3	0	9	0.4	15	0
Dizziness	11	0.4	12	0	13	0.2
Insomnia	10	0	13	0	13	0.2
Sore throat	8	0	11	0	11	0
Ecchymosis	11	0.4	6	0.9	2	0
Rigors	10	0	11	0.4	8	0
Asthenia	5	2	11	2	6	0
Influenza	0.8	0.4	6	0	10	0.2

With the exception of grade1/2 edema (but not grade 3/4), there were no obvious differences in the incidence of AEs in patients treated at 400 and 600 mg. However, the interpretation of a dose relationship is confounded by the fact that many patients were dose-escalated over time and in all studies AEs were analyzed according to starting dose. Patients aged ≥65 years also experienced a higher incidence of edema.

Less than 50% of patients required dose reduction at any time. Temporary treatment interruptions were required in 25-40% of patients. Despite these dose changes, the overall median dose-intensity (expressed in mg/day) in each study remained close to the initially planned dose.

Cardiotoxicity

Gleevec has been shown to be cardiotoxic in humans in an examination of ten individuals who developed significant left ventricular dysfunction, thus, congestive heart failure (CHF), during their course of therapy. All ten individuals with CML had normal left ventricular function before Gleevec therapy was instituted (ejection fraction 56±7%). Following treatment (mean of 7.2±5.4 months [range 1-14 months]), patients had a significantly lower ejection fraction of 25±8% ($P < 0.001$) and presented with a significant volume overload and symptoms corresponding to a New York Heart Association (NYHA) class 3-4 heart failure.[21] Myocardial biopsies performed on two of the patients who had no history of coronary disease showed prominent membrane whorls in the myocytes, which has been reported to be characteristic of toxin-induced myopathies.

In patients treated with Gleevec on NCI-sponsored trials, the rate of high grade left ventricular systolic function was low (approximately 0.2%), based on AE data available from 1223 patients. However, when edema was reported on the basis of a cardiac cause, the rate of Grade 3/4 edema considered likely related to Gleevec was 1.3% among the 1223 patients. Other high grade cardiac AEs considered less likely related to Gleevec included cardiac ischemia/infarction (0.08%), hypertension (1%), and hypotension (0.7%).

Nonclinical data support the evidence of Gleevec induced cardiotoxicity both in vitro and in vivo. When healthy mice were treated to evaluate the cardiotoxicity of Gleevec, transmission electron micrographs of samples from the hearts of three drug-treated mice showed results similar to those seen in studies of individuals on Gleevec, including membrane whorls in the sarcoplasmic reticulum, and in or immediately adjacent to mitochodria. Treatment of mice with 200 mg/kg/day led to a significant deterioration in contractile function ($P < 0.003$) and moderate left ventricular dilation after 3-4 weeks of treatment. This mimics the effect that Gleevec treatment has shown in human subjects.

1.1.3 Clinically Important Adverse Events

Because of their relative frequency and clinical significance, several AEs occurring during the Phase II leukemia trials were analyzed in greater detail, as summarized below.

1.1.3.1 Edema and Fluid Retention

Superficial edema was one of the most frequently reported AEs but was rarely severe (2-5% Grade 3/4). The most frequently involved sites included the periorbital region, face and lower limbs. The edema usually appeared within

the first 2 months of treatment. A minority of patients with severe edema required dose reduction.

A minority (<5%) of patients developed central collections of fluid (described collectively using the term 'fluid retention') including one or more of the following: CHF, pleural effusion, ascites, pericardial effusion and pulmonary edema. It is unclear if the mechanisms underlying peripheral edema and fluid retention are the same. Fluid retention was usually managed with diuretics and/or dose reduction, or with temporary treatment interruption.

1.1.3.2 Gastrointestinal and CNS Hemorrhages

In toxicology studies, imatinib was shown to be a local irritant, and for that reason, the pattern of GI tract hemorrhages was carefully reviewed. These events were rare, and severe (Grade 3/4) cases were reported only in advanced CML patients. GI hemorrhages occurred in 3-5% of patients with advanced CML, and were considered drug-related in 0-2% and severe in <1.5%. Contributing factors such as severe thrombopenia, the use of concomitant NSAIDS and a history of gastric ulcer were present in most, but not all cases. Upper GI ulceration (esophageal, gastric or duodenal) or gastritis/duodenitis were visualized at gastroscopy in approximately 50% of patients with GI bleeding. Less than 1% of patients discontinued therapy as a result of these events.

1.1.3.3 Cerebral hemorrhages (including subdural hematomas and hygromas) were also reported almost exclusively in advanced CML patients (1-7%). The majority occurred in the context of rapidly progressive disease, with concomitant thrombocytopenia.

1.1.3.4 Skin rash

An erythematous, pruritic, maculopapular skin rash, most prominent over the forearms and trunk but occasionally present also on the face, was reported in up to 44% of patients treated with imatinib. Onset was generally within the first month of therapy. Eosinophilia was rarely seen and skin biopsies showed the typical appearances of a toxic drug reaction with a mixed infiltration of cells. Occasional patients reported pruritus without accompanying rash. In most cases, the rash was mild, easily manageable with antihistamines and/or topical steroids, and gradually subsided without interrupting therapy. However, a troublesome skin rash was the most frequent reason (in

<1% of patients overall) for the permanent discontinuation of therapy. Approximately 3% of patients developed severe skin rashes, some with an exfoliative component. Re-challenge was usually but not always positive.

1.1.3.5 Hypophosphatemia/Vitamin D deficiency

Hypophosphatemia and low levels vitamin D have been reported to develop in patients taking imatinib and other tyrosine kinase inhibitors.[22] Imatinib may interfere with osteoclast function directly by inhibiting PDGF receptor β on osteoclasts or indirectly by inhibiting the activation of C-FMS receptor. These abnormalities were treated with Vitamin D and phosphate supplementation and did not require reducing the dose of imatinib. Additionally, they did not persist with the withdrawal of imatinib.

1.1.3.6 Cardiac Toxicity

Kerkela et al. recently reported 10 patients who had severe CHF.[21] Cardiac biopsies showed mitochondrial changes consistent with drug toxicity and both clinical and pathologic effects could be replicated in mice. The risk of severe cardiac toxicity has been estimated to be approximately 1%.

1.1.3.7 Gastrointestinal Stomal Tumor (GIST)

Treatment with Gleevec was generally well tolerated, although nearly every patient experienced minor AEs. The most frequently reported AEs were edema, nausea, diarrhea, musculoskeletal pain, fatigue, rash, headache, and abdominal pain. Most events were of mild to moderate severity. Superficial edema, most frequently periorbital or lower limb edema was managed with diuretics, other supportive measures, or by reducing the dose of Gleevec. Severe (CTC grade 3/4) superficial edema was observed in two patients, including face edema in one patient. No major differences were seen in the severity of AEs between the 400 mg or 600 mg treatment groups, although overall incidence of AEs was somewhat higher in the 600 mg treatment group. AEs with a suspected relationship to therapy occurring in greater than 10 % of patients in any group are presented in Table 3.

Table 3

Adverse Events with Suspected Relationship to Therapy in GIST (≥ 10 % in any Group)

	All Grades			Grade 3/4		
Preferred Terms Percentage Of Patients	**400 mg n = 73**	**600 mg n = 74**	**All Doses N = 147**	**400 mg n = 73**	**600 mg n = 74**	**All Doses N = 147**
Any AE	97	99	98	21	22	21
Edema/fluid retention	71	77	74	1	1	1
Periorbital edema	45	50	48	0	0	0
Edema lower limb	26	15	20	0	0	0
Face edema	8	12	10	1	0	1
Edema	7	14	10	0	0	0
Eyelid edema	7	8	8	0	0	0
Nausea	51	54	52	1	1	1
Diarrhea	40	50	45	1	3	2
Myalgia/musculoskeletal pain	37	42	40	0	0	0
Fatigue	30	39	35	0	0	0
Dermatitis/rash	25	37	31	3	3	3
Headache	19	32	26	0	0	0
Abdominal pain	26	26	26	1	0	1
Flatulence	19	24	22	0	0	0
Vomiting	14	12	13	0	1	1
Any hemorrhage	11	14	12	4	5	5
Tumor hemorrhage	1	4	3	1	4	3
Upper GI bleed/perforation	4	3	3	4	1	3
Dyspepsia	10	12	11	0	0	0
Lacrimation increased	7	12	10	0	0	0
Anemia	6	12	9	1	3	2
Loose stools	7	10	8	0	0	0
Taste disturbance	3	14	8	0	0	0

There was no hyperuricemia or evidence of tumor lysis syndrome, even in patients with very rapid decreases in tumor volume. The most medically significant AEs were gastrointestinal or intra-abdominal hemorrhage in patients with large bulky tumors, which occurred in approximately 5 % of patients.

1.1.4 Preclinical Studies

As part of continuing preclinical studies on imatinib, Novartis completed the in-life part and the necropsy of 2–year oral (in feed) carcinogenicity study in rats at doses of 15, 30 and 60 mg/kg. An increased incidence of palpable masses in the urogenital region led to an accelerated histopathological analysis of these organs. Target organs for neoplastic changes were kidneys in both sexes, urinary bladder and preputial gland in males, and clitoral glands in females.

A statistically significant increase in renal benign and malignant tumors and the urinary bladder benign tumors were noted only at 60 mg/kg/day, representing approximately 1.74x to 4.14x human daily exposure (based on AUC) to imatinib at the dose of 400 mg/day. In addition, a statistically significant increase in benign and malignant tumors of the preputial/clitoral gland was noted at 30 and 60 mg/kg/day, representing approximately 0.5x to 1.7x human daily exposure (based on AUC) to imatinib at the dose of 400 mg/day.

1.1.5 Summary of Pilot Trial Data

Based on the in vitro data supporting the role of c-kit and PDGF-R activation in KS, a pilot trial at Beth Israel Deaconess Medical Center in Boston, MA was initiated. Ten patients who were on stable doses of highly active anti-retroviral therapy (HAART) and who had failed first line KS therapy have been enrolled. The primary goal of the trial was to confirm the expression of c-Kit and PDGF-R in KS lesions and to determine the activation status of the c-Kit and PDGF receptors in KS at baseline and after 4 weeks of treatment with imatinib. The secondary goal was to collect data on the tolerability and clinical efficacy of imatinib in HIV–related KS. Patients were treated with imatinib 300 mg orally, twice daily with dose reduction permitted for toxicity. At baseline and at 4 weeks, patients had tumor measurements, a 4 mm punch biopsy of a representative lesion and a plasma sample. Patients with a response at 4 weeks were allowed to continue therapy until disease progression. AIDS Clinical Trial Group (ACTG) criteria were used for clinical response. Five of 10 patients had at least a partial response (PR) by clinical criteria after only 4 weeks of imatinib therapy. Three of 10 patients had stable disease. The median duration of therapy was 7 weeks (range 4 to 26 weeks). The majority of patients required dose reduction from 600 mg daily to 400 mg daily after 4 weeks of therapy. One patient had Grade 4 neutropenia that recurred after dose reduction. Five patients had diarrhea (three exhibited Grade 3, and one Grade 4) that resolved with drug withdrawal. Two of these five patients with diarrhea had diarrhea at baseline. Diarrhea returned with drug re-challenge, but was manageable with over-the-counter anti-diarrhea medications. No significant change in CD4 counts or viral load was noted during therapy.

We hypothesize that the high rate of GI toxicity that was observed may be related to a PK interaction between imatinib and HAART. Imatinib is a substrate for the cytochrome p450 isoenzymes, cyp3A4 and cyp2D6. All of the protease inhibitors (PIs) in clinical use at the time of this study, which includes, amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir are cyp3A4 inhibitors with ritonavir inhibiting cyp2D6 to a lesser extent. Diarrhea is a side effect common to all of the PIs. The other class of antiretrovirals shown to affect the

p450 system is the non-nucleoside reverse transcriptase inhibitors. Delavirdine is a cyp3A inhibitor while nevirapine is a cyp3A inducer, and efavirenz is a mixed cyp3A inducer/inhibitor. No relationship between any specific antiretroviral regimen and the incidence of diarrhea was observed in the pilot study.

Plasma samples were analyzed to determine levels of imatinib and its major active metabolite, CGP-74588 in the laboratory of Dr. Merrill Egorin. At steady state the average imatinib concentration was 1,342 ng/mg (range 0 to 2,846 ng/mL) and CGP-74588 was 733 ng/mL (range 459 to 1,046 ng/mL) (n=9). These levels are low compared to CML patients on the same dose schedule, 2,939 ng/mg (range 2,009-3,497 ng/ml) and 733 ng/mL (range 459-1,046 ng/mL). Plasma concentrations of the antiretrovirals were not measured in the pilot study. Although this data suggest there is not inhibition of imatinib metabolism, the imatinib concentrations may not be representative of the steady state concentration. The samples were a random sample from Day 28, and by that time, all four patients with diarrhea had developed symptoms. The presence of the diarrhea could have affected the absorption of imatinib as well as patient adherence to the therapy. Because of the high potential of drug interactions in the HIV population the pharmacokinetics of imatinib in these patients still needs to be established.

We examined tumor biopsies using immunohistochemistry (IHC) with phosphospecific antibodies to determine the activation status of the PDGF and c-kit pathways. A majority of protein kinases require phosphorylation of a serine/theronine or a tyrosine in the activation loop to allow kinase activity. Phosphospecific antibodies have been raised to a number of these sites and are useful as surrogate markers of activity. We used phosphospecific antibodies to PDGF-R and c-Kit as well as their downstream effectors, ERK1/ERK2 and AKT. Antibodies were used anti-c-Kit (Dako); anti-PDGF-R, anti-phospho-β–PDGF-R (tyrosine 751), anti-phospho-AKT (serine 473) and anti-phospho-ERK (threonine 202/tyrosine 204) (Cell Signaling, Beverly, MA). We have not yet found an anti-phospho-c-kit antibody that works reliably for IHC. Six patients had evaluable baseline IHC. All six patients demonstrated constitutive expression of PDGF-R and c-Kit receptors. Four patients had biopsies at baseline and 4 weeks that were evaluable by IHC. Three of four patients demonstrated activation of PDGF-R and ERK, a downstream effector of PDGF-R and c-Kit, at baseline with inhibition of both pathways after treatment with imatinib. We also examine the activation status of AKT, a component of the PI3K pathway and downstream target of PDGF-R and c-kit. Activation of AKT was not seen at baseline or after treatment in any patients. VEGF, which has been shown to be downstream of PDGF-R

activation in culture KS spindle cells, was measured pre and post treatment but no significant changes in VEGF concentrations were seen.

1.1.6 Dose Selection

In the pilot trial, events requiring dose reductions were four cases of diarrhea and one case of neutropenia. After dose reduction the majority of patients with diarrhea had their symptoms controlled with over the counter medications. A dose of 400 mg a day has been shown to induce a 82% hematologic response rate in CML and a 57% response rate in GISTs.[11,12] Once we began to aggressively manage the diarrhea with medication and dose reduction to 400 mg per day, it no longer was a dose limiting toxicity. Based on our clinical experience, the majority of KS patients will tolerate 400 mg a day.

1.2 Biologic/Correlative Studies

1.2.1 Expression/Activation (phosphorylation) imatinib kinase targets and signaling intermediates.

We will determine by immunohistochemistry the expression and phosphorylation status of PDGF-R in biopsies from baseline and 8 days. In our initial study, we did not anticipate the degree of pathologic regression after 4 weeks of therapy. The marked decrease in KS spindle cells after 4 weeks of therapy not only resulted in the qualitative decrease in the phospho-PDGF-R staining but also resulted in a quantitative decrease in the cell population that was immunoreactive initially. In order to more accurately quantitate the effect of imatinib on the phosphorylation state of our targets we will examine biopsies at an earlier time point. We decided to biopsy patients at 8 days because the $t_{1/2}$ of imatinib is ~18 hours and the $t_{1/2}$ of it metabolite, CGP-74588, is ~36 hours and patients should reach steady state concentration after seven days. We have validated phosphospecific antibodies by immunohistochemistry for all of these determinants except c-Kit receptor.

1.2.2 Early Markers of Response

Two to 3 months are often required to determine if a KS patient is responding to a therapeutic agent. If early markers of response could be established that would allow physicians to determine with a degree of confidence which patients were going to respond, then patients who were not going to respond would be free to pursue other treatment options.

Because of the importance of cytokines, angiogenic factors and growth factors produced in KS, a number of recent KS therapeutic trials examined biologic endpoints to determine if they correlated with clinical response. All the studies to date have looked at isolated biologic endpoints based on a priori predictions regarding KS biology and/or the mechanism of action of the drug being studied. In a trial of oral 9-*cis* retinoic acid in KS, soluble Interleukin-6 receptor (sIL6R) was significantly lower at baseline in responders and IL-6 levels increased in responders.[14] In a study of COL-3, a matrix metalloproteinase inhibitor, VEGF levels were shown to decrease in responders while b-FGF remained unchanged.[15] These reports suggest concentrations of cytokines/growth factors at baseline or changes in the concentrations after being on therapy may be used as predictors of response for KS therapies.

Because of the critical role cytokines/chemokines play in KS development, we hypothesize that these growth factors would be useful biologic endpoints that may be predictive of response. In previous studies investigators had chosen two or three biological endpoints to examine. We will perform a proteomics-based screen for biologic endpoints. The reason for this choice is two-fold: 1) This method allows us to simultaneously examine the majority cytokines and chemokines that are implicated in KS development and, 2) We have limited knowledge of which growth factors may be downstream of the PDGF-R and c-kit pathways in KS.
The PerkinElmer Cytokine Chip is designed to analyze the following cytokines from a sample in a multiplexed fashion:

Table 4. Cytokines detected by PerkinElmer Cytokine Chip

IL-1 alpha	IL-2s Ra	IL-8	IL-17	MCP-1	sTNF RI
IL-1 beta	IL-3	IL-10	EGF	MCP-2	sTNF RII
IL-1ra	IL-4	IL-12p40	ENA-78	MCP-3	TARC
IL-1R4	IL-5	IL-12p70	FGF-basic	MIG	TNF alpha
IL-1s RI	IL-6	IL-13	G-CSF	MIP-1 alpha	TNF beta
IL-1s RII	IL-6s R	IL-15	GM-CSF	MIP-1 beta	VEGF
IL-2	IL-7	IL-16	IFN gamma	MIP-3 alpha	

Included in the kit is a mixture of all cytokines to be used for generating four point standard curves. These are used to estimate the concentration of each cytokine from the samples. After running the assay, slides were imaged using a ScanArray™ Confocal Laser Scanner (PerkinElmer, Cambridge, MA). After imaging, the resulting tif files were quantitated using QuantArray software (PerkinElmer, Cambridge, MA). The data was then exported as an Excel 2000 worksheet and these are collated into a single workbook. An analysis macro was run that subtracts the local background signal from the

signal of each spot. This method adjusts for any differences in background that may occur across an array. The replicate spots are then averaged and tested for outliers using the ESD method (extreme studentized deviate; N=4; Critical Z=1.48). The standards were analyzed by point-to-point linear regression and concentrations of experimental samples were estimated from these standards.

We will examine the cytokine profiles of patients at baseline, 7 days and 28 days to see if there is any correlation between baseline concentrations and clinical response. As noted the standards on the cytokine chip are analyzed by point-to-point linear regression and concentrations of experimental samples are estimated from these standards four-point curve. Thus, the chip should be considered a semi-quantitative assay and all cytokines/chemokines that appear to be potential predictors will be confirmed by ELISA.

1.2.2.1 Receptor Mutation Analysis

Mutations of c-kit and PDGF-R have been shown to play a role in tumorigenesis and imatinib resistance. Activating mutations of c-Kit have been reported in mastocytosis, acute myleogenous leukemia, GISTs, myeloproliferative disorders (MPD) and sinonasal lymphomas.[16] In vitro studies suggest activating mutations in the kinase domain of c-Kit are resistant to inhibition by imatinib. Additionally, activating mutations in the kinase domain of PDGF-R α have been reported in GISTs.[17] We will correlate the presence or absence of c-Kit and/or PDGF-R mutations with clinical response to imatinib.

As the experience with imatinib therapy for CML increased, it became clear that some patients who initially responded later relapsed. Studies demonstrated multiple mechanisms of resistance including over-expression of BCR/ABL transcripts, acquisition of new chromosomal abnormalities and mutation of the kinase domain. In one study, mutations of the kinase domain appeared to be the mechanism of resistance in 23 of 43 cases. At the time of discontinuation, we will biopsy a lesion that is clearly growing or has not responded and sequence PDGF-R and c-Kit.

If any novel mutations are found in either of these cases, we will determine in vitro whether they are activating mutations and/or resistant to imatinib.

1.2.2.2 Mechanisms of Toxicity

By reducing the dose of imatinib to 400 mg per day, the incidence of AEs should be decreased, but not eliminated.

We hypothesize three potential mechanisms for the increased toxicity of imatinib: 1) antiretrovirals inhibit the metabolism of imatinib resulting in higher concentrations of imatinib compared to those seen in CML and GIST patients; 2) imatinib inhibits the metabolism of antiretrovirals resulting in higher concentrations of antiretrovirals; 3) the increased incidence of diarrhea is related to response of occult GI KS lesions. Our investigations into the effect of HAART on imatinib in the pilot study were inconclusive. Because of the potential inhibition of imatinib by antiretrovirals, we will formally study the pharmacokinetics of imatinib and antiretrovirals during the first 2 weeks of therapy in the first 12 patients. Because this study will have a limited number of patients, and these patients are taking a variety of antiretroviral combinations, we have chosen not to measure the concentrations of antiretrovirals unless we note increased toxicity with normal imatinib levels in this study. If we do see an increase in the plasma level of imatinib, we will attempt to correlate this with specific antiretrovirals or antiretroviral classes. If we find plasma imatinib concentrations to be elevated, we will be able to determine a dose that provides comparable concentrations to those seen in other patient populations, such as CML.

Interestingly, the diarrhea usually occurred 2 to 3 weeks after the initiation of therapy, and its appearance coincided with the onset of clinical response in some patients. This observation led to a third potential explanation of the diarrhea. The patients who had diarrhea may have had occult GI involvement of their KS and the diarrhea was related to regression of the GI lesions. The standard screening for GI involvement of KS is to test the stool for the presence of occult blood. All of the patients enrolled to date have tested negative and were presumed not to have GI involvement. Regressing KS lesions could potentially have a direct irritant effect on the bowel or could produce cytokines locally that lead to diarrhea. To determine if there is GI involvement of KS, we will offer a colonoscopy to all patients who have diarrhea that is not controlled by OTC medication. Some might argue that we should screen all patients with a colonoscopy prior to enrollment, but we feel this would be unjustified at this point since a colonoscopy is not without risk and diarrhea does not occur in all patients. From a practical standpoint, the results of the colonoscopy would have little effect on the conduct of current trial since we would withhold therapy until the

diarrhea abated and then restart at a reduced dose. However, if the diarrhea is correlated with regression of GI lesion, one could justify starting at the same dose level once the diarrhea had abated in future trials.

1.2.2.3 Transcription profile of KSHV. Changes in gene expression are a fundamental hallmark of cancer progression and invaluable tool of cancer stages. In the case of KS, KSHV/HHV-8 has been identified as the etiological agent and this assay is designed to identify KSHV genes that might change in response to therapy. We will use reverse-transcription (RT) coupled to amplification using polymerase chain reaction (PCR) to measure the mRNA levels of all KSHV/HHV-8 mRNAs in the tumor. This assay is a research test only and should not be used to make clinical decisions. The purpose of including this assay as part of this trial is to determine its usefulness as a prognostic marker of disease.

1.3 Study Design

The proposed study design will provide clinical response data for imatinib in AIDS-related KS. Additionally the study will allow for investigation of mechanisms of resistance, mechanisms of toxicity as well as potentially developing predictors of response.

Patients will continue on study protocol for 6 months. Treatment will be extended 6 months if the patient has met the criteria for a response or has stable disease. At the end of the 12-month study period, further treatment will be at the discretion of the Investigator. Protocol treatment will be discontinued if the subject develops tumor progression, unacceptable toxicity or develops one of the protocol-defined reasons for treatment discontinuation. In the event of toxicity, patients will have their dose reduced (see Section 5.4-5.6).

Clinical and laboratory evaluations will be performed prior to the initiation of study medications and at Days 1, 8, 15, 29 and every 28 days thereafter.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 Evaluation of clinical response.

2.2 Secondary Objectives

2.2.1 Evaluation of inhibition of PDGF receptor as determined by immunohistochemistry.

2.2.2 Evaluation of cytokine profiles pre and post imatinib therapy.

2.2.3 Evaluation of pharmacokinetic profile of imatinib and antiretrovirals in HIV patients.

2.2.4 Evaluation of mechanisms of primary and secondary resistance to imatinib therapy.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Biopsy proven KS involving the skin, lymph nodes, oral cavity, GI tract and/or lungs. GI and pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy. At least five measurable, previously non-radiated, cutaneous lesions must be present, which can be used as indicator lesions. Additionally, patient will need three lesions greater or equal to 5 x 5 mm that are accessible for 4 mm punch biopsy.

3.1.2 Serologic documentation of HIV infection at any time prior to study entry, as evidenced by positive ELISA, positive Western Blot, or other federally approved licensed HIV test.

3.1.3 Karnofsky performance status $\geq$ 60% (Appendix IV).

3.1.4 Female patients of child-bearing potential must have a negative pregnancy test within 72 hours before initiation of study drug dosing. Post menopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential. Male and female patients of reproductive potential must agree to employ an effective barrier method of birth control throughout the study and for up to 3 months following discontinuation of study drug.

3.1.5 The following lab parameters within 21 days prior to study entry:

- Hemoglobin > 8.0 gm/dL.

- Absolute neutrophil count > 1000 cells/$mm^{3.}$
- Platelet count > 75,000/$mm^{3.}$
- Serum creatinine < 1.5 mg/dL or a measured creatinine clearance of > 60 mL/min.
- Total bilirubin should be normal. If, however, the elevated bilirubin is felt to be secondary to indinavir or atazavinir therapy, then subjects will be allowed on protocol if total bilirubin < 3.5 mg/dL, provided that the direct bilirubin is normal.
- AST (SGOT) and ALT (SGPT) < 2.5 times the ULN.

3.1.6 Life expectancy of 3 months or more.

3.1.7 Age of 18 or older.

3.1.8 Ability and willingness to give informed consent.

3.1.9 Subjects must, in the opinion of the Investigator, be capable of complying with this protocol.

3.1.10 No previous imatinib therapy.

3.1.11 Antiretroviral therapy is required for patients except those who have exhausted all available treatment options.

3.2 Exclusion Criteria

3.2.1 Concurrent active opportunistic infection (OI).

3.2.2 Patient is ≤ 5 years free of another primary malignancy except*:* if the other primary malignancy is not currently clinically significant or requiring active intervention, or if other primary malignancy is a basal cell skin cancer or a cervical carcinoma in situ. Existence of any other malignant disease is not allowed.

3.2.3 Acute treatment for an infection or other serious medical illness within 14 days prior to study entry.

3.2.4 Patients may not have had anti-neoplastic treatment for KS (including chemotherapy, radiation therapy, biological therapy, or investigational therapy) within 4 weeks (6 weeks for nitrosourea or mitomycin-C) of study entry.

3.2.5 Previous local therapy of any KS indicator lesion within 60 days, unless the lesion has progressed since treatment. Because of the possibility of tattooing, and the difficulty in ascertaining clinically what is active KS versus residual pigment post treatment, any prior

local treatment to the indicator lesions regardless of the elapsed time should not be allowed unless there is evidence of clear-cut progression of said lesion.

3.2.6 Patient with Grade III/IV cardiac problems as defined by the New York Heart Association Criteria (e.g., CHF, myocardial infarction within 6 months of study entry) (See Appendix XIV).

3.2.7 Female patients who are pregnant or breast-feeding.

3.2.8 Patient has another severe and/or life-threatening medical disease.

3.2.9 Patient has an acute or known chronic liver disease (e.g., chronic active hepatitis, cirrhosis).Patients with known Hepatitis C infection, but with documentation of no or minimal fibrosis on liver biopsy may be enrolled.

3.2.10 Patient has had a major surgery within 2 weeks prior to study entry.

3.2.11 Drug-specific exclusion criteria: Coumadin® and systemic corticosteroid treatment, other than replacement doses.

3.2.12 Subjects must not have received granulocyte colony stimulating factor within 2 weeks of study entry.

3.3 Enrollment Procedures

This study will be available for enrollment at all AMC sites. Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered with the AMC Operations Center before they may enroll patients.

After it has been determined that the patient is eligible and an informed consent has been signed by the patient, the patient must be registered on-line via the AMC AdvantageEDC℠ Internet Data Entry System. Enrollment and data collection will occur via the AMC Internet Data Entry System.

The participating site will ensure the patient meets all eligibility criteria prior to completing the protocol-specific eligibility checklist. Patients will be enrolled on-line via the AMC Internet Data Entry System no more than one week prior to the initiation of treatment (enrollment one day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted a system generated confirmation email will be sent to the enroller upon successful completion of the patient enrollment. If the on-line system is inaccessible, the site should notify the AMC Operations Center (via email at amcpm@emmes.com, or by phone at 301-251-1161) for further instructions.

4.0 CLINICAL AND LABORATORY EVALUATIONS

All signs, symptoms, HIV-related and AIDS-defining events (refer to Appendices II and III), death and toxicities must be documented. All signs, symptoms and laboratory results ≥ Grade 2 that are felt to be clinically significant or drug-related, and all HIV-related and AIDS-defining events and deaths must be recorded on the case report forms (CRFs).

All prescription medications taken within 14 days prior to study entry and during the intervals between each visit must be recorded on CRFs. The duration of all anti-HIV medications and all OI treatment and/or prophylaxis medications at the time of enrollment must be recorded in the CRF. All non-prescription medications must be recorded in the clinic record.

4.1 Eligibility/Screening

4.1.1 Biopsy diagnostic of KS at any time prior to study entry.

4.1.2 Documentation of HIV infection at any time prior to study entry.

4.1.3 Chest X-ray to rule out pulmonary KS (must be done within 4 weeks of study entry). Pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy. Patients with a positive chest x-ray or minimal symptoms suggestive of pulmonary disease will have a chest CT performed at entry.

4.1.4 A medical history within 21 days of study entry to include the following information:

- Previous HIV-related and non-HIV related diagnoses.
- Complete prior anti-HIV therapy, immune based therapy and prior anti-tumor therapy, including start dates of current anti-HIV therapy.
- All prescription medications taken within the preceding 2 weeks.
- A signs and symptoms assessment within 2 weeks prior to study entry, including history of weight change.
- Complete physical exam including the following: vital signs, height, weight, tumor assessment, and Karnofsky performance (Appendix IV) status.

4.1.5 Laboratory studies must be obtained within 21 days (unless noted otherwise) prior to study entry, and will include the following:

- Complete blood count with differential and platelets.

- Serum chemistries: liver enzymes (SGOT, SGPT, alkaline phosphatase), BUN, creatinine, electrolytes, amylase, bilirubin [direct and indirect]), phosphate, and 25-hydroxyvitamin D.
- Urine phosphate, creatinine for calculation of fractional excretion of phosphate (FE_{PO4}). Calculate the FE_{PO4} using the formula [Ur_{PO4} x P_{Cr} x 100] ÷ [P_{PO4} x Ur_{Cr}]. Ur_{PO4}= Urine phosphate; Ur_{Cr} = Urine creatinine, P_{PO4}=Plasma Phosphate P_{Cr} = Plasma creatinine.

4.1.5.1 If FE_{PO4} > 5% or serum phosphate is low, then 25-hydroxyvitamin D should be drawn at next visit.

- For women of child bearing potential, a serum beta HCG pregnancy test within 72 hours of study entry.
- CD4 count within 4 weeks prior to study entry.
- HIV-1 plasma RNA within 4 weeks prior to study entry (to be evaluated at local institution).
- Stool tested for occult blood.

4.1.6 KS Tumor assessments may be performed on day 1 prior to receiving study medication, but may be performed no earlier than within 2 weeks prior to initiating treatment. Tumor measurements should include the following:

- Identify marker lesions: Select five bi-dimensionally measurable marker lesions for assessing changes in lesion dimension. Select the largest lesions with clearly defined margins. If possible, marker lesions will be photographed. Additionally, the patient will need three lesions greater or equal to 5 x 5 mm that are accessible for 4 mm punch biopsy.
- For patients with < 50 total skin and oral lesions, all lesions must be evaluated for changes in number and characteristics. For patients with ≥ 50 total skin and oral lesions, choose three representative areas, if possible, for evaluating change in lesion numbers and characteristics (preferably an area with ≥ 5 lesions).

NOTE: A representative area is a single extremity (the back, chest, or face) that has lesions similar in characteristics, i.e., nodularity, size, color, and number, to those found on other parts of the body. A representative area does not need to be the area with the largest number of lesions, but should contain lesions that are truly representative of those throughout the remainder of the body.

4.1.7 Staging Criteria: KS staging will be based on the modified ACTG Oncology Committee Staging Criteria (see Appendix V).

4.1.8 Specimens for the AIDS and Cancer Specimen Resource (ACSR) to be collected within 28 days prior to study entry (see Appendix VIII for specimen handling), if consent obtained (see Appendix VII).

4.2 Evaluations During Treatment

Evaluations may occur up to 7 days before or after the end of the study visit indicated. Each cycle will be 28 days. Evaluations will continue as outlined below. Patients on the 6-month extension arm will also follow the outline below.

4.2.1 Clinical assessment on Days 1, 8, 15, 29 and then every 28 days thereafter, to include an assessment of the following, at every visit (unless otherwise specified):

4.2.1.1 KS tumor assessment at baseline (to be performed on Day 1 prior to receiving study medication, but no earlier than 2 weeks prior to initiating treatment) Day 29, Day 56 and every 2 months thereafter (see Section 4.1.6).

4.2.1.2 A complete physical exam including: vital signs, weight, Karnofsky performance status (Appendix IV) and toxicity evaluation (Days 8, 15, 29 and then every 28 days thereafter).

4.2.1.3 Signs and Symptoms Review.

4.2.1.4 Complete blood count with differential and platelets.

4.2.1.5 Serum chemistries: liver enzymes (SGOT, SGPT, alkaline phosphatase), BUN, creatinine, electrolytes, amylase, bilirubin (direct and indirect, if total is elevated) and phosphate.

4.2.1.6 Urine phosphate and urine creatinine for calculation of fractional excretion of phosphate (FE_{PO4}). [Fractional excretion of phosphate=(spot urinary phosphate/serum phosphate)/(spot urinary creatinine/serum creatinine) × 100].

4.2.1.6.1 If $FE_{PO4} > 5\%$ or serum phosphate is low then 25-hydroxyvitamin D should be drawn at next visit or within 7 days (whichever is sooner).

4.2.1.7 For women of childbearing potential: serum β-HCG at any time pregnancy is suspected.

4.2.1.8 All HIV-related and AIDS-defining events (Appendix III) and concomitant medications.

4.2.2 CD4 count at baseline, Day 29 and every 3 months thereafter.

4.2.3 HIV-1 plasma RNA at baseline, Day 29 and every 3 months thereafter (to be evaluated at local institution).

4.2.4 HHV-8 Viral Load- Day 1 and Day 15 of cycle 1, and on Day 1 of cycles 3, 5, 7, 9, 11 (see Appendix XIII).

4.2.5 HHV-8 Viral Gene Expression- Day 1 and Day 15 of cycle 1, and on Day 1 of cycles 3, 5, 7, 9, 11 (see Appendix XIII).

4.2.6 Biologic Endpoints - Biopsies will be performed at baseline (two), at Day 8 and at time of progression. One biopsy will be performed at baseline and at time of progression or treatment discontinuation for receptor mutation analysis. The DNA from these biopsies will be used to assess exons 12 and 18 in PDGDR β, and exons 11 and 17 in c-kit for mutations that may activate the target receptors or confer resistance to imatinib. If mutations are now found in these exons, we will sequence other exons in which activating mutations have been described. Additionally, RNA from these biopsies will be stored for sequencing cDNAs and accessing expression of downstream targets. These biopsies will be fresh biopsies stored in RNAlator storage media (see Appendix X). Two more biopsies will be performed at baseline and on Day 8 for receptor phosphorylation. These biopsies will assess inhibition of PDGF receptor and will be performed on each specimen. These biopsies will be fixed in 10% formalin (see Appendix X). Serum and plasma specimens for cytokine profiles will be taken at baseline, Day 8 and Day 29 **(**see Appendix XI). DNA and RNA from PBMCs in these specimens will be stored. The DNA will be used to determine if PDGF-R and c-kit mutations are present in circulating PBMCs, which are infected with KSHV or are restricted to the KS lesions. The RNA will be used for accessing the effect of PDGF-R and c-kit inhibition on the expression of cytokines and other potential downstream targets.

4.2.7 Pharmacokinetics - The first 12 patients will have PK sampling performed on Days 1 and 15. Samples will be collected on both days on the following schedule: 0 (immediately prior to patient taking meds for that day), 0.5, 1, 2, 3, 4, 8 and 24 (immediately prior to the next day's dose) hours (see Appendix XII)**.** These data will be compared with historical PK data acquired at the same time points in normal volunteers.

4.3 Evaluations At The Time Of Treatment Discontinuation

At the time of treatment discontinuation all evaluations should be completed as soon as possible (see Appendix I, Schedule of Events). The following should be performed upon discontinuation of study drug:

4.3.1 Complete blood count with differential and platelets.

4.3.2 Serum chemistries: liver enzymes (SGOT, SGPT, alkaline phosphatase), BUN, creatinine, electrolytes, bilirubin (direct and indirect) and phosphate.

4.3.2.1 Urine phosphate and creatinine for calculation of fractional excretion of phosphate (FE_{PO4}).[Fractional excretion of phosphate=(spot urinary phosphate/serum phosphate)/(spot urinary creatinine/serum creatinine) × 100].

4.3.3 CD4 count and HIV plasma RNA viral load.

4.3.4 HHV-8 Viral Load (see Appendix XIII).

4.3.5 A complete physical exam including: vitals signs, weight, tumor assessment, Karnofsky performance status (Appendix IV), and toxicity evaluation. At the time of discontinuation, a lesion that is clearly growing or has not responded will be biopsied for receptor mutations. If possible, the lesion should be greater or equal to 5 x 5 mm. Biopsy should be performed with a 4 mm punch biopsy.

4.4 Final Evaluations/Off Drug, Off Study

AEs must be reported if the AE began any time within 8 weeks of receiving the study treatment. Additionally, if a site learns of any incidence of death, cancer or fetal anomaly, WHICH IS DRUG RELATED, at any time after the study is closed, the site should notify Novartis. This information may provide additional insight into the safety of Gleevec.

5.0 STUDY TREATMENT

5.1 Drug Regimens, Administration And Duration

Novartis will supply Gleevec as 100 mg tablets packaged in bottles. Medication labels will comply with the legal requirements of the U.S. and will be printed in English. The storage conditions for Gleevec will be described on the medication label. Bottles must be stored in a safe, secure location.

Gleevec is a local irritant and must be taken in a sitting position with a large (250 mL) glass of water. Direction of use on medication label: Take as directed with a large glass of water.

5.2 Drug Orders, Transfers, Returns, And Accountability

Gleevec will be provided by Novartis for the 12-month study period.

Questions about drug orders, transfers, returns, or accountability should be addressed to BIDMC IND Pharmacy, Keith Belken at 617-667-4249, Monday through Friday between 9:00 am and 4:30 pm Eastern Time.

5.2.1 Drug Orders

Once a patient has been registered with the AMC Operations Center, the enrolling site will fax a clinical drug request for that patient to the Beth Israel Deaconess Medical Center IND pharmacy. If the order is received prior to 1:00 pm Eastern Time, it will be processed by IND pharmacy and shipped that day; otherwise, drug orders will be shipped the following business day. The shipments will be sent via FedEx for next business day delivery. Thus, if a patient is registered on Wednesday, the order will be processed on Thursday and shipped on Friday for delivery on Monday. The initial request will be for a 4-week supply of Gleevec. Sites will be sent enough drug for 30 days to cover shipping time if the patient continues on the trial. Sites may reorder additional Gleevec by completing an NCI Clinical Drug Request form and faxing it to the BIDMC IND pharmacy at 617-667-4248. The NCI Clinical Drug Request form is available on the NCI home page (http://ctep.info.nih.gov) or by calling the PMB at 301-496-5725. The patient ID number (e.g. "042-999-999") and the patient initials (e.g., "LFM") should be entered in the *Patient or Special Code* field. All drug orders will be shipped to the pharmacy of the institution where the patient is enrolled.

5.2.2 Drug Transfers

Drug may not be transferred from one patient to another patient, from one center to another center, or from one protocol to another protocol.

5.2.3 Drug Returns

All unused drug supplies should be destroyed on site and the amount recorded.

5.2.4 Drug Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the receipt and disposition of all drugs received using the NCI Investigational Agent Accountability Record available on the CTEP home page (http://nci.cancer.gov) or by calling the PMB at 301-496-5725.

5.3 Dosing

Patients will be instructed to take Gleevec 400 mg/day (a total of 4 tablets). Patients who have been on study for at least 3 months without any toxicities may have their dose increased to 600 mg/day.

5.4 Dose Modifications For Non-Hematological Toxicity

Further continuation of Gleevec treatment after any interruptions lasting longer than 2 weeks is not recommended.

5.4.1 Grade 2

If the patient experiences a new Grade 2 non-hematologic toxicity, with the exception of hypophosphatemia, study drug must be withheld until the toxicity has resolved to ≤ Grade 1. Gleevec may then be resumed at the same daily dose. If the Grade 2 toxicity recurs, Gleevec must be withheld until the toxicity has resolved to ≤ Grade 1, and the daily dose must be reduced by 100 mg if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If the Grade 2 toxicity recurs, Gleevec treatment will be stopped.

For Grade 2 hypophosphatemia the patient should be started on Neutra-Phos 250 mg by mouth four times a day.

Additionally, if a patient has a 25-hydroxy D that is > 30 ng/mL, no Vitamin D supplementation is needed. If less than 20 ng/mL, give Ergocalciferol D2: 50,000 IU weekly x 8 weeks and then maintain with daily OTC Vitamin D3 cholecalciferol 1,000 IU daily. If the level is between 20 ng/mL and 30 ng/mL, calculate the additional Vitamin D the patient will need to add to their current intake by the formula: 30 - (current level) x 100 = # IUs of OTC cholecalciferol to boost up stores to optimum level of 30ng/mL (to increase by 1 ng/mL add 100 IU of Vitamin D3). For example if current level = 25 ng/mL, the patient will need to add 500 IU to their current intake. With

completely optimum Vitamin D levels, patients may be less susceptible to hypophosphatemia.

If the Grade 2 hypophosphatemia recurs in spite of supplementation, Gleevec must be withheld until the toxicity has resolved to ≤ Grade 1, and the daily dose must be reduced by 100 mg if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If the Grade 2 hypophosphatemia recurs after dose reduction, Gleevec treatment will be stopped.

5.4.2 Grade 3/4

If the patient experiences Grade 3/4 toxicity, with the exception of hypophosphatemia, Gleevec must be withheld until the toxicity has resolved to ≤ Grade 1 or baseline, and the daily dose must be reduced by 100 mg if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If the Grade 3/4 toxicity recurs, Gleevec treatment will be stopped.

For Grade 3/4 hypophosphatemia the patient should be started on Neutra-Phos 250mg by mouth four times a day and Gleevec should be held until resolved to Grade $\underline{\leq}$ 1 and then restarted at previous dose.

Additionally, if a patient has a 25-hydroxy D that is > 30 ng/mL, no Vitamin D supplementation is needed. If less than 20 ng/mL, give Ergocalciferol D2: 50,000 IU weekly x 8 weeks and then maintain with daily OTC Vitamin D3 cholecalciferol 1,000 IU daily. If the level is between 20ng/mL and 30ng/mL, calculate the additional Vitamin D the patient will need to add to their current intake by the formula: 30 - (current level) x 100 = # IUs of OTC cholecalciferol to boost up stores to optimum level of 30ng/mL (to increase by 1 ng/mL, add 100 IU of Vitamin D3). For example if current level = 25ng/mL, the patient will need to add 500 IU to their current intake. With completely optimum Vitamin D levels, patients may be less susceptible to hypophosphatemia.

If Grade 3/4 hypophosphatemia recurs in spite of dose reduction, Gleevec must be withheld until the toxicity has resolved to ≤ Grade 1 or baseline, and the daily dose must be reduced by 100 mg if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If the Grade 3/4 hypophosphatemia recurs after dose reduction, Gleevec treatment will be stopped.

Summary of Dose Modifications for Non-Hematologic Toxicity Excluding Hypophosphatemia

OCCURRENCE	NEW GRADE 2	GRADE 3/4
1st	Hold until ≤ Grade 1, then resume at same dose	Hold until ≤ Grade 1 or baseline, then reduce to 300 mg/day if at 400 mg dose level, or to 400 mg/day if at 600 mg dose level.
2nd	Hold until ≤ Grade 1 then reduce to 300 mg/day if at 400 mg dose level, or to 400 mg/day if at 600 mg dose level.	Stop treatment
3rd	Stop treatment	N/A

5.5 Dose Modifications For Hematological Toxicity

5.5.1 Grade 1/2

No dose interruptions or reductions will be performed for Grade 1/2 hematological toxicity.

5.5.2 Grade 3/4

If the patient experiences a Grade 3/4 hematological toxicity, defined as an ANC < 1 x 10^9/L, or a platelet count < 50 x 10^9/L, Gleevec must be withheld until the toxicity has resolved to ≤ Grade 2. ANC will take precedence over a WBC count in determining the degree of neutropenia (e.g., doses should not be interrupted for a patient with a WBC < 2.0 x 10^9 /L, but would be for an ANC < 1 x 10^9/L). If the toxicity resolves to ≤ Grade 2 within 2 weeks, Gleevec treatment may be resumed at the same dose. If the Grade 3/4 toxicity recurs, Gleevec must be withheld. If the toxicity resolves to ≤ Grade 2 within 2 weeks, Gleevec may be recommenced with the daily dose reduced by 100 mg if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If the Grade 3/4 toxicity persists for longer than 2 weeks, Gleevec will be stopped. If the Grade 3/4 toxicity recurs after a dose reduction to 300 mg/day, Gleevec treatment will be stopped. Patients may receive G-CSF while Gleevec is held as clinically indicated.

No dose reductions will be performed for Grade 1-4 anemia. If the patient develops anemia, he/she may be transfused or receive Erythopoietin at the discretion of the Investigator.

Summary of Dose Modifications for Hematologic Toxicity

OCCURRENCE	GRADE 3/4
1st	Hold until ≤ Grade 2 within 2 weeks, then resume at same dose. If ≤ Grade 2 not reached within 2 weeks, then stop treatment.
2nd-Dose >300 mg	Hold until ≤ Grade 2 within 2 weeks, then resume treatment at 300 mg/day if at 400 mg dose level, or by 200 mg if at 600 mg dose level. If ≤ Grade 2 not reached in 2 weeks, then stop treatment.
2nd – Dose = 300 mg Dose at 2nd occurrence	Stop treatment.

5.6 Concomitant Therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed, provided their use is documented in the patient records and on the appropriate CRF. The administration of any other therapies intended to treat the primary condition, including chemotherapy and biologic agents, is NOT permitted. Similarly, the use of other concurrent investigational drugs is not allowed.

Because of the inherent risk of either reduced activity or enhanced toxicity of the concomitant medication and/or Gleevec, drugs known to interact with the same CYP450 isoenzymes (2D6 and 3A4) as Gleevec should be used with caution. Patients using concomitant medications known to be metabolized by these cytochrome p450 enzymes will not be excluded from the study. However, the patients must be carefully monitored for potentiation of toxicity due to individual concomitant medication. Special care has to be given to the concomitant use of acetaminophen (e.g., Tylenol® or Percocet®, paracetamol Panadol®, etc.) with Gleevec. Any use of concomitant medication must be captured in the concomitant medication CRF. (See Appendix XV for complete list).

5.7 Permitted Medications

5.7.1 Prophylactic use of loperamide (e.g., Imodium®, with suggested dosing as start: 4mg po x 1, then 2 mg po after each loose stool, max 16 mg/day) is recommended for patients experiencing Grade 1 or 2 diarrhea, before dose interruption.

5.7.2 Chemoprophylaxis for *Pneumocystis carinii* pneumonia is highly recommended for all subjects with a CD4 count of $\leq$ 200.

5.7.3 Topical and/or oral antifungal agents are permitted except oral itraconazole and ketoconazole. (Topical agents should not be applied to study lesions).

5.7.4 Chemoprophylaxis for *Mycobacterium avium* complex (MAC) is required for patients whose CD4 cell counts < 50 cells/mm^3, preferably with azithromycin 1.2 g orally once a week.

5.7.5 Treatment, maintenance or chemoprophylaxis with approved agents for OI as clinically indicated.

5.7.6 All antibiotics as clinically indicated except for prohibited medications.

5.7.7 Antiretroviral therapy is permitted and strongly encouraged for any subject with a detectable viral load. Patient should be receiving an optimal and stable regimen of HAART for a minimum of 12 weeks prior to entry.

5.7.8 Erythropoietin is permitted at the discretion of the Investigator.

5.7.9 Granulocyte colony stimulating factors are permitted as outlined in Sections 3.2.12 and 5.6.

5.7.10 Regularly prescribed medications such as antipyretics, analgesics, allergy medications, antidepressants, sleep medications, oral contraceptives, megestrol acetate, testosterone or any other medications are permitted except for prohibited medications (see Section 5.8.1.4 – 5.8.1.7).

5.7.11 Prophylactic anti-emetics should be withheld until the patient has experienced Grade 1 nausea or vomiting.

5.7.12 If patient has weight gain greater than 5 pounds above baseline that is felt to be due to fluid retention, patient may be started on diuretics at the Investigator's discretion.

5.7.13 Alternative therapies such as vitamins, acupuncture and visualization techniques will be permitted. Subjects should report the use of these therapies and they will be recorded.

5.8 Prohibited Medications

5.8.1 Investigational drugs, with the exception of antiretroviral agents that are being obtained through an expanded access protocol.

5.8.2 Systemic cytotoxic chemotherapy or any other treatment specifically prescribed to treat KS (e.g., radiation, etc.).

5.8.3 Since warfarin is metabolized through the CYP450 system, no therapeutic anticoagulation with warfarin (e.g. Coumadin) will be permitted in patients participating in this study. As an alternative, therapeutic anticoagulation may be accomplished using low-molecular weight heparin (e.g., Lovenox®) or heparin. A mini-dose coumadin derivative (equivalent to 1 mg QD Coumadin) is permitted for prophylaxis of central venous catheter thrombosis, at the discretion of the treating physician. In general, the use of Coumadin is discouraged on this protocol.

5.8.4 The routine use of systemic corticosteroid therapy, other than replacement doses, is not permitted.

5.9 Prohibited Foods

5.9.1 Grapefruit juice.

5.10 Treatment Compliance

Records of study medication used, dosages administered, and intervals between visits will be kept during the study. Drug accountability will be noted and at the completion of the trial. Patients will be asked to return all unused medication at the monthly visits.

Patients will also be given a diary to carry home and will be instructed to record each time study drug is administered. The patient will be asked to bring diary at each clinic visit.

Patients will be asked to weigh themselves twice a week and will be asked to report to the study team any increase in weight greater than 5 pounds above baseline. If weight gain is felt to be due to fluid retention, patient may be started on diuretics at the Investigator's discretion.

6.0 REPORTING OF ADVERSE EVENTS

This study will utilize the CTCAE version 3.0 for Common Terminology Criteria for Adverse Event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. The documents "NCI Guidelines: Adverse Event Reporting Requirements for NCI Investigational Agents" (sections 2 and 3) clearly outline reporting criteria.

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

6.1 Classification Of Adverse Events By Severity & Relationship To Study Drug Administration

6.1.1 Adverse Event

Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

6.1.2 Life-Threatening Adverse Event

Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

6.1.3 Serious Adverse Event (SAE)

Any adverse event occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Please note for **hospitalization** – All hospitalizations (or prolongation of existing hospitalization) for medical events equivalent to CTCAE Grade 3, 4, 5 must be reported regardless of the requirements for Phase of study, expected or unexpected, and attribution. For example, do not report an admission for pharmacokinetic sampling, but do report an admission for a myocardial infarction.

6.1.4 Toxicity

Toxicity is a term NOT clearly defined by regulatory organizations. Toxicity has been described as an adverse event that has an attribution

of possibly, probably or definitely related to investigational treatment. To minimize confusion the NCI would recommend that the term toxicity NOT be utilized for adverse event reporting purposes. The CTCAE continues to use the term 'toxicity' because of familiarity.

6.1.5 Unexpected Adverse Event

Any adverse event that is not listed in the NCI Agent Specific Expected Adverse Event List. This list is updated electronically in real time.

6.1.6 Adverse Event Expedited Reporting System (AdEERS) (formerly known as Adverse Drug Reaction Reporting).

An electronic system for expedited submission of adverse event reports.

6.1.7 Attribution

The determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories:

Definite – The AE *is clearly related* to the investigational agent(s).

Probable – The AE *is likely related* to the investigational agent(s).

Possible – The AE *may be related* to the investigational agent(s).

Unlikely – The AE *is doubtfully related* to the investigational agent(s).

Unrelated – The AE *is clearly NOT related* to the investigational agent(s).

6.2 Adverse Event Reporting To Novartis

Novartis will need to be notified within 24 hours of any SAE regardless of the presumed relationship to Gleevec.

Instructions for rapid notification of SAEs.

6.2.1 Reporting Responsibility

Each SAE (but not pregnancies) must be reported by the Investigator to Novartis within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related. Follow-up information about a previously reported SAE must also be reported to Novartis within 24 hours of receiving it. If the SAE has not been previously documented (new occurrence) and it is thought to be related to study drug (or therapy), the Medical Safety Expert of the Clinical Safety & Epidemiology (CS&E) Department may contact the Investigator to

obtain further information. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this SAE has been reported.

6.2.2 Reporting Procedures

The Investigator must complete the FDA MedWatch 3500 form and Novartis SAE coversheet (see Appendix XVII) in English, assess the relationship to study treatment and send the initial completed MedWatch form and Novartis SAE coversheet by fax 1.888.299.4565 within 24 hours to the local Novartis Clinical Safety & Epidemiology (CS&E) Department. The Investigator must then ensure that the form and coversheet are accurately and **fully** completed with follow-up information and fax those to Novartis CS&E Department within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other SAEs. The original and the duplicate copies of the FDA MedWatch form, Novartis SAE coversheet, and the fax confirmation sheet must be kept with the CRFs at the study site.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The MedWatch form, Novartis SAE coversheet, and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

6.3 Adverse Events With Commercial Agents

The AE should be reported on the FDA Form 3500 MedWatch (available from the FDA website at www.fda.gov/medwatch). The following AEs should be reported:

- All life-threatening events (Grade 4), which may be due to drug administration.
- All fatal events (Grade 5) while on study (or within 30 days of treatment)
- First occurrence of any previously unknown clinical event (regardless of Grade).

The completed form should be forwarded to:

AMC Operations Center
The EMMES Corporation
401 N. Washington St., Suite 700
Rockville, MD 20850
Fax: (240) 238-2842

All AEs, regardless of severity, and whether or not ascribed to study drug administration, will be recorded in the appropriate section of the CRF. Patients withdrawn from the study due to AEs will be followed by the Investigator until the outcome is determined and, when appropriate, additional written reports and documentation will be provided.

7.0 CRITERIA FOR TREATMENT DISCONTINUATION

- Study medication will be interrupted or permanently discontinued for treatment related toxicities as outlined in Sections 5.4-5.6.

- Disease progression.

- Subjects who become pregnant or breast-feed or who require systemic chemotherapy for the treatment of a malignancy other than KS.

- Subjects who require treatment with medications disallowed as outlined in Sections 5.6.1.4–5.6.1.7.

- Subjects who are noncompliant with respect to taking drugs, keeping appointments or having tests required for the evaluation of drug safety and efficacy.

- Subjects have the right to withdraw from the study at any time for any reason.

- The Investigator has the right to remove subjects from the study for clinical reasons which he/she believes are life-threatening to the subject even if such reasons do not fall into the toxicity classifications discussed above.

8.0 POST-TREATMENT EVALUATION AND EVALUATION AT EARLY DISCONTINUATION OF THERAPY

Thirty days after discontinuation, a follow-up visit will include: **a complete physical exam including: vitals signs, weight, tumor assessment, Karnofsky performance status (Appendix IV), and toxicity evaluation.**

- At a subject's final study visit, the off-study form will be completed. Permanent discontinuation of drug will be documented.

- Subjects who withdraw for toxicity reasons should be followed until the toxicity resolves/returns to baseline, or at least 1 month, whichever is later. In addition, subjects who go off study for reasons other than toxicity should be followed for at least 1 month after discontinuing drug.

9.0 EVALUATION OF RESPONSE

9.1 Definition of Response

9.1.1 Complete response (CR) is defined as the absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks. In patients whose pigmented (brown or tan) macular skin lesions persist after apparent CR, biopsy of at least one representative lesion is required in order to document the absence of malignant cells. In patients known to have had visceral disease, an assessment at restaging with appropriate endoscopic or radiographic procedures should be made.

9.1.2 Clinical CR is defined as the absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks; AND

- For patients with pigmented (brown or tan) macular skin lesions persisting after apparent CR, biopsy of a representative lesion documenting the absence of KS cells is not required; AND
- For patients known to have had visceral disease, restaging with appropriate endoscopic or radiographic procedures is contraindicated or otherwise not performed.

9.1.3 Partial Response (PR) is defined as no new lesions (skin or oral), or no new visceral sites of involvement (or the appearance or worsening of tumor-associated edema or effusions); AND

- A 50% or greater decrease in the number of all previously existing lesions lasting for at least 4 weeks; OR
- Complete flattening of at least 50% of all previously raised lesions (i.e., 50% of all previously nodular or plaque-like lesion become macules); OR
- A 50% decrease in the sum of the products of the largest perpendicular diameters of the marker lesions.

NOTE – Patients with residual tumor-associated edema or effusion who otherwise meet the criteria for CR will be classified as having a PR.

9.1.4 Stable disease is defined as any response not meeting the criteria for CR, PR, or progressive disease.

9.1.5 Progressive disease is defined as follows:

- For patients with <50 cutaneous lesions ≥ 25% increase in the sum of perpendicular diameters of the indicator lesions.

- OR ≥ 25% increase in the total lesion count, or a minimum of 5 new lesions, whichever is greater,
- OR ≥ 25% increase in the number of raised lesions (minimum of 5 new raised lesions if there are very few raised lesions, for example ≤ 8), whichever is greater.

Note: There are body sites where disease is particularly difficult to evaluate, and a few new lesions may be counted in spite of the fact that a patient is not actually progressing. For example, lesions of the foot, particularly those which are flat, are difficult to evaluate because their intensity may be variable based on how much edema is present, how much the person walked the day before, how long their feet have been in a dependent position prior to the physical exam, etc.

- For patients with ≥50 cutaneous lesions:
- ≥ 25% increase in the sum of the perpendicular diameters of the indicator lesions.
- OR ≥ 25% increase in the total number of lesions in the prospectively defined anatomic sites containing representative numbers of lesions,
- OR a total of 5 new lesions in anatomic sites which were previously documented as having no evidence of cutaneous disease on the whole body diagram,
- OR (25% increase in the number of raised lesions. Photographic documentation of "gross" or significant progression, particularly in areas that were not being followed, will be of particular value.

In order to classify a response as PR, the patient must have at least a PR in the cutaneous or noncutaneous sites of disease, and no evidence of progression as defined in the above criteria. In order to classify a response as a CR, the patient must have a CR in both the cutaneous (if applicable) and noncutaneous (if applicable) sites of disease and no evidence of progression as defined by the above criteria.

Noncutaneous Progression

Progressive disease includes new visceral sites of involvement or progression of visceral disease or the development of new or increasing tumor-associated edema or effusion lasting at least 1 week, which interferes with the patient's normal activities. Progressive visceral disease, for measurable and evaluable disease, should be based on RECIST criteria.

9.1.6 Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.

9.1.7 Time to response is defined as time from the first dose of Gleevec until documentation of first response.

9.1.8 Time to progression is defined as time from initiation of Gleevec to documentation of first progression.

9.1.9 Response duration is defined as the time from first documentation of response to documentation of first progression.

10.0 RECORDS TO BE KEPT

CRFs will be provided for each subject via the AMC AdvantageEDC^{SM} Internet Data Entry System upon enrollment. Subjects must not be identified by name on any study documents. Data will be recorded on the CRFs using the unique subject identification number assigned at registration. Sample CRFs will be available on the AMC Operations Center website.

11.0 ROLE OF DATA MANAGEMENT

11.1 CRF Instructions

Instructions concerning the recording of study data on CRFs will be provided by the Operations Center.

11.2 Data Quality

It is the responsibility of the AMC Operations Center to assure the quality of data for the study. This role extends from protocol development to generation of the final study database.

12.0 STATISTICAL CONSIDERATIONS

12.1 Sample Size Estimation

The response rate (CR + PR) to imatinib in the pilot study is 50% to date. It is well known that some patients have regression of their KS after beginning HAART therapy.[18] In order to minimize the effect of HAART being a potential confounding factor in response rates, recent studies have required patients on HAART to be on stable regimens for 12 weeks prior to initiation of treatment. Recent results from two placebo-controlled studies suggest as many 20% of responses in KS clinical trials may be delayed responses to HAART alone.[19, Walmsley, 1999 #136] Simon's two-stage design will be used to test the null hypothesis that the response rate (CR + PR) if 20% against the alternative that it is 50% at the one-sided 0.05 significance level with power of 0.90. Ten patients will be enrolled in the first stage. If no more than two patients respond, then the study will be stopped. If at least three patients respond, then the study will enroll up to a total of 22 patients. If no more than seven patients respond, the drug will be considered ineffective. If at least eight patients respond, the null hypothesis will be rejected in favor of the alternative hypothesis. The probability of early termination of the study is 0.68.

In our original estimate, we assumed a drop out rate of 10-15% based on our experience in the pilot study and calculated a sample size of 25 patients. However, we have had a higher drop out rate. Consequently, based on a drop out rate of 20-30%, we plan to roll a total of 30 patients to have 22 patients evaluable for response.

In the pilot study, we found that decreasing the dose of imatinib by 33% (600 mg/day to 400 mg/day) dramatically decreased the incidence of AEs. This dose reduction should reduce the steady state concentration of imatinib and it's metabolite by 1 standard deviation of steady state plasma concentration, assuming constant clearance. This observation suggests a change in the plasma concentration imatinib and/or its metabolite of 1 standard deviation would be significant in our patient population. Based on this assumption, a PK sampling of 12 patients will provide a power of 88% to detect a 1 standard deviation change in the steady state level of imatinib and its metabolite with a type I error of 0.05 using a two-sided paired t-test.

12.2 Statistical Analysis Plan

Summary statistics will be used to describe the study population (such as ranges, medians of age, gender, baseline performance characteristics). The binomial and its exact 95% confidence interval will be used to estimate clinical response rate. A two-sided one-group chi-square test will be performed to detect a 50% response rate. Due to a small sample size, exact logistic regression may be used to evaluate the effect of baseline characteristics and other covariates with the clinical response. To evaluate inhibition of PDGF receptor, we will

correlate the presence or absence of c-kit and or PDGF-R mutations with clinical response to imatinib by performing chi-square tests or Fisher's exact tests. The cytokine/chemokine profile at baseline, 1 week and 4 weeks after of imatinib therapy will be analyzed using the proteomics approach. The concentration of each cytokine from the samples will be estimated. Friedman's test will be used to evaluate changes between time points with respect to cytokine levels. If a significant difference is detected, the Wilcoxon rank sum test will be used for pairwise comparisons. Exact logistic regression analysis will be used to detect any correlation between baseline concentrations and clinical responses. PK parameters including AUC, C_{max} and elimination half-life of imatinib will be estimated in the first 12 patients. PK parameters including AUC, C_{max} and elimination half-life of selected antiretrovirals may be estimated in selected patients. Summary statistics will be used to describe PK results. The incidence of specific AEs will be summarized by severity grade. The binomial proportion and its 95% confidence interval will be used to estimate the proportion of patients who experience an AE of severity Grade 3 or higher. The proportions of primary and secondary resistance to imatinib therapy will be estimated. Chi-square tests or Fisher's exact tests will be used to evaluate associations between c-kit and PDGF-R mutations with resistance to imatinib therapy.

Cluster analyses are planned for the real-time QPCR data. To evaluate the associations between single genes and response, categorical data analysis methods are proposed. If feasible, a multivariate model relating gene expression to response will be performed.

12.3 AMC Policy for Monitoring of Phase I and Phase II Trials

This protocol will follow the AMC's policy for data monitoring (see Appendix XVI, Data Safety and Monitoring Plan).

13.0 ETHICAL AND REGULATORY CONSIDERATIONS

(See Appendix IV, Informed Consent)

13.1 Informed Consent

The principles of informed consent described in Food and Drug Administration (FDA) regulations (21 CFR part 50) must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The sponsor must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before patient enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the sponsor. Records of all study review and approval documents must be kept on file by the Investigator and are subject to FDA inspection during or after completion of the study. AEs must be reported to the IRB. The IRB should receive notification of completion of the study and final report within 3 months of study completion and termination. The Investigator will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

13.2 Changes To The Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by Novartis, CTEP and the Investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB/IEC/REB. A copy of the written approval of the IRB/IEC/REB must be sent to Novartis. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC/REB approval but the IRB/IEC/REB of each center must be kept informed of such administrative changes.

13.3 Women And Minorities

This is a study being conducted by the NCI-sponsored AIDS Malignancy Consortium (AMC). As part of their contractual obligations, each participating site within the AMC, and the AMC as a whole, is required to assure that the participation of women and minority subjects reflects the percentage representation of these populations in their geographic region and, for the AMC, the United States as a whole. As such, it is expected that the representation of subjects on this trial will reflect the constitution of the respective populations.

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APPENDIX I: SCHEDULE OF EVENTS

Assessment	Eligibility/ Screening	Baseline	D 1	D 2	D 8	D 15	D 16	D 29 (and then every 28 days thereafter)	D 56 (and every two months thereafter)	Every 3 months thereafter	Treatment Discontinuation
Clinical Studies											
Informed Consent	X										
KS Documentation	X[1]										
HIV Documentation	X[1]										
Medical History	X[9]										
Physical Exam: Vital signs, height, weight, KPS	X[9]		X		X	X		X			X
Signs and Symptoms Review	X[8]		X		X	X		X			
KS Tumor Assessment			X[10]					X[7]	X[7]		X
KS Staging	X[9]										
Concomitant Medication Review	X[9]		X		X	X		X			
Adverse Events					X	X		X			X
Chest x-ray	X[2]										
Laboratory Studies											
CBC with differential and platelets	X[9]		X		X	X		X			X
Serum Chemistries[3]	X[9]		X		X	X		X			X
Urine Phosphate and Creatinine	X[9]		X		X	X		X			X
CD4 count		X[5]						X[13]		X	X
Serum beta HCG pregnancy test	X[4]										
HIV plasma RNA		X[5]						X[13]		X	X
HHV-8 Viral Load			X			X[14]					X
Stool Guaiac	X[9]										
Serum/plasma for cytokine profile		X			X			X[12]			
Pharmacokinetics[6]			X	X		X	X				
ACSR Donation		X									
Biopsy for receptor phosphorylation		X			X						
Biopsy for mutation receptor		X[11]									X

1. Anytime prior to study entry.
2. Done within 4 weeks of study entry, if positive x-ray, a chest CT will be performed
3. Serum Chemistries: Liver enzymes (SGOT, SGPT, alkaline phosphatase), BUN, creatinine, electrolytes, amylase, bilirubin (direct and indirect) and phosphate. 25-Hydroxyvitamin D is measured at baseline.
4. To be done within 72 hours of study entry and anytime pregnancy is suspected
5. To be done within 4 weeks of study entry.
6. Collect on the first 12 patients: 0 (immediately prior to the patient taking meds for that day), 0.5, 1, 2, 3, 4, 8, and 24 (immediately prior to the next day's dose) hours.
7. To be done at baseline, D29, D56 and every 2 months thereafter.
8. Within 2 weeks prior to study entry.
9. To be obtained within 21 days prior to study entry.
10. To be performed on Day 1 prior to receiving study medication but may not be performed earlier than within 2 weeks of initiating treatment.
11. To be performed at baseline and time of progression or treatment discontinuation.
12. Day 29 only.
13. Day 29 and every 3 months thereafter.
14. To be collected on Day 1 and Day 15 of cycle 1 and on Day 1 of cycles 3, 5, 7, 9, and 11.

APPENDIX II: DEFINITION OF AIDS INDICATOR CONDITIONS

- Aspergillosis, invasive *
- *Bartonella henselae* infection, disseminated (bacillary angiomatosis, peliosis hepatis)*
- Candidiasis of bronchi, trachea, or lungs *
- Candidiasis, esophageal *
- Cervical cancer, invasive *
- Coccidioidomycosis, disseminated or extrapulmonary *
- Cryptococcosis, extrapulmonary *
- Cryptosporidiosis, chronic intestinal (> 1 month's duration) *
- Cytomegalovirus disease, invasive *
- Cytomegalovirus retinitis *
- Encephalopathy, HIV-related *
- Herpes simplex: chronic ulcer(s) (> 1 month's duration), bronchitis, pneumonitis, or esophagitis *
- Histoplasmosis, disseminated or extrapulmonary *
- Isosporiasis, chronic intestinal (> 1 month's duration) *
- Kaposi's sarcoma (progression to visceral disease)
- Lymphoma, Burkitt's (or equivalent term) *
- Lymphoma, immunoblastic (or equivalent term) *
- Lymphoma, primary, of brain *
- Microsporidiosis, diarrhea > 1 month or disseminated *
- *Mycobacterium avium* complex or *M. kansasii*, disseminated or extrapulmonary *
- *Mycobacterium tuberculosis*, any site (pulmonary[1] or extrapulmonary) *
- *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary *
- Nocardiosis, pulmonary, brain or disseminated *
- *Pneumocystis carinii* pneumonia (new or recurrent diagnosis)
- Progressive multifocal leukoencephalopathy *
- *Salmonella* septicemia, recurrent *
- Toxoplasmosis of brain *
- Wasting syndrome due to HIV *

* New Diagnosis

APPENDIX III: CRITERIA FOR ACCEPTABLE AIDS DEFINING EVENTS

1. Microbiologically- or histologically-confirmed AIDS-related opportunistic infection;

   OR

2. Presumptive diagnosis of CMV retinitis, confirmed by an ophthalmologist, or of CNS toxoplasmosis or CNS lymphoma by accepted imaging technique;

   OR

3. Biopsy-proven AIDS-related malignancy. For the purposes of the protocol, only the development of visceral disease will constitute a KS endpoint;

   OR

4. Physician-documented HIV-1 Cognitive Motor Complex (dementia) or HIV wasting syndrome;

   OR

5. Presumptive diagnosis of PCP as determined by the following:
   - Compatible symptomatology (e.g., fever, cough, shortness of breath).
   - Compatible chest x-ray.
   - $pO_2 < 85$.
   - Absence of other pathogens on sputum examination.
   - Negative blood cultures.
   - Response to appropriate anti-Pneumocystis therapy.

NOTE: For the purposes of this protocol, a primary study endpoint will be defined as:
- A new AIDS defining event, OR
- Recurrent PCP, OR
- Death.

APPENDIX IV: KARNOFSKY PERFORMANCE SCALE

Description	Percent	Karnofsky Performance Scale
Able to carry on normal activity; no special care needed	100	Normal: no complaints: no evidence of disease
	90	Able to carry on normal activity; minor signs or symptoms of disease
	80	Normal activity with effort: some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs: a varying amount of assistance is needed	70	Cares for self, unable to carry on normal activity or to do active work
	60	Requires occasional assistance but is able to care for most of needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care: disease may be progressing rapidly	40	Disabled: requires special care and assistance
	30	Severely disabled: hospitalization is indicated although death is not imminent
	20	Very sick: hospitalization necessary; active supportive treatment is necessary
	10	Moribund: fatal processes progressing rapidly
	0	Dead

APPENDIX V: RECOMMENDED STAGING CLASSIFICATION FOR KAPOSI'S SARCOMA

	GOOD RISK ($_0$) (All of the following)	POOR RISK ($_1$) (Any of the following)
Tumor (T)	- Confined to skin and/or lymph nodes and/or minimal oral disease[1]	- Tumor-associated edema or ulceration - Extensive oral KS - Gastrointestinal KS - KS in other nonnodal viscera
Immune system (I)	- CD4 cells ≥ 200/μL	- CD4 cells < 200/μL
Systemic illness (S)	- No history of OI or thrush - No "B" symptoms[2] - Performance status ≥ 70 (Karnofsky)	- History of OI and/or thrush - "B" symptoms present - Performance status < 70 - Other HIV-related illness (e.g., neurological disease, lymphoma)

T_0 = tumor confined to skin, lymph nodes and/or minimal oral disease.
T_1 = any tumor falling under the "Poor Risk" criteria.
S_0 = no history of OI or thrush, no "B" symptoms, and Karnofsky Performance status ≥ 70.
S_1 = any "Poor Risk" systemic illness signs and symptoms.
NOTE: Staging criteria taken from: Krown SE, Metroka C, Wernz JC. Kaposi's sarcoma in the acquired immunodeficiency syndrome: A proposal for uniform evaluation, response, and staging criteria. J Clin Oncol 1989;7:1201-1207. This criteria was adopted by the ACTG Oncology Committee.

[1]Minimal oral disease is nonnodular KS confined to the palate.

[2]"B" symptoms are unexplained fever, night sweats, > 10% involuntary weight loss, or diarrhea persisting more than 2 weeks.

APPENDIX VI: MODEL INFORMED CONSENT

Phase II Trial of Imatinib Mesylate (Gleevec) in Patients with HIV-Related Kaposi's Sarcoma

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your friends and family.

You are being asked to take part in this study because you have Kaposi's sarcoma (KS), the most common cancer seen in patients with the acquired immunodeficiency syndrome (AIDS).

WHY IS THIS STUDY BEING DONE?
The purpose of this study is to find out what effects (good and bad) Gleevec has on you and your Kaposi's sarcoma skin tumors.

This research is being done because treatments for Kaposi's sarcoma do not cure the disease and better treatments are needed. Gleevec (also known as imatinib) has been approved by the Food and Drug Administration (FDA) for use in the treatment of chronic myelogenous leukemia (cancer of the blood). Gleevec is experimental for the treatment of KS, and its effectiveness in treating KS is unknown. In laboratory studies, it has been found to stop tumor growth by interfering with signals from proteins that are necessary for cancer to grow and spread.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
Approximately 30 patients will participate in this study at multiple medical centers.

WHAT IS INVOLVED IN THE STUDY?
If you agree to participate and join in this study, the following will occur:

1. Eligibility Phase

   You will need to have the following exams, tests, or procedures done to find out if you are eligible and can participate in the study.

   - You will have blood (a maximum of 5 tablespoons) drawn for laboratory testing. Some of this blood will be used to measure antibodies to HIV infection. We will also check your stool for blood.
   - If you are a woman of childbearing potential, a pregnancy test will also be done.
   - A medical history and a physical examination will be completed and will include a review of all medications that you are taking and an examination of your KS lesions.
   - You will have a chest x-ray and may be required to have a chest CT scan, which is a computerized series of X-rays.

2. Treatment Phase

- If you are a good candidate for treatment based upon these evaluations, you will begin treatment with Gleevec. Two biopsies (removal of small tissue samples) of your KS lesions will be required before you begin treatment. The area of the skin where the sample will be removed will be numbed with a local anesthetic. Small tissue samples will then be removed by cutting the tumor tissue.
- On the day you begin treatment, you will have a physical examination including measurement of your weight and vital signs (temperature, pulse, respirations and blood pressure). Blood (a maximum of 5 tablespoons) will be drawn for laboratory testing to monitor your kidney, liver, and bone marrow function. The study doctor will examine your KS lesions and may take up to 12 photographs of the lesions to document their appearance. Additional photographs will be taken during the course of the study to document any changes in appearance. At no time will your entire face appear, and any distinguishing features will be removed from the photo so that you cannot be identified. Only your initials and subject number will be used to identify the photo.
- On the first and fifteenth day of your treatment you may have blood drawn to assess the level of Gleevec in your blood. On these days you will have additional blood drawn to determine if there is any interaction between your HIV medications and Gleevec. A small tube or "IV" may be placed in a vein in your arm so that the blood can be drawn for testing without additional "needlesticks". We will also measure your HHV-8 viral load. This test measures the amount of the HHV-8 virus, which is a virus associated with Kaposi's sarcoma. This blood test will also be performed on the first and fifteenth day of your treatment, every 2 months, and when you finish treatment. 4-5 teaspoons of blood will be needed for each blood test.
- You will be given a bottle containing Gleevec tablets and instructed to take 4 tablets (a total of 400 mg) of Gleevec once a day by mouth. To minimize throat irritation, you should take the tablets with food and a large glass of water (8 ounces) while in a sitting position. Drinking grapefruit juice during treatment is discouraged because it may interfere with the effectiveness of Gleevec.
- Your dose may be increased or decreased by your doctor. You will continue to receive the drug as long as you do not develop unacceptable side effects, become pregnant, show significant worsening of your KS or require systemic therapy for the treatment of a malignancy other than KS.
- You will be instructed to bring all empty and/or partially filled study medication bottles to each monthly visit.
- You will need to return to the study center for a visit after 1 week, 2 weeks, and 1 month of treatment and then once a month for the next 5 to 12 months. At each study visit, you will be asked about signs and/or symptoms and about any other medications that you are taking. Taking certain medications, including other experimental drugs, are not permitted while participating in this research study.
- At your study visits, you will have a physical examination including measurement of your vital signs and weight. Blood (a maximum of 5 tablespoons) will also be drawn

for laboratory tests including blood counts (which tell your doctor how well your bone marrow is functioning), chemistries (which tell your doctor how well your liver and kidneys are functioning) and cytokine levels (which tell your doctor the how Gleevec is affecting your KS). After 1 week a biopsy of a KS lesion will be necessary. This will allow us to determine the effects of Gleevec on the various aspects of your tumor.

- Evaluation of your disease will occur at your visit after months 1 and 2 of treatment and then after every 2 months of treatment. During these evaluations, your doctor will visually inspect your lesions for any change in the number of lesions present as well as any change in their appearance. Up to 12 photographs of your lesions will be taken to document their appearance. If one of your lesions disappears, your doctor may ask you for your permission to biopsy the area of skin where the lesion was previously located.
- You will be asked to weigh yourself twice a week at home and report to the study doctor any rapid body weight increase of more than 5 pounds. If you do not have access to a scale, you should discuss options with your physician.

3. Discontinuation of Treatment

You will initially be treated with Gleevec for 6 months. If at the end of 6 months your condition has remained stable or has improved, you may receive treatment with Gleevec for another 6 months. Once you have completed the study treatment, you will be asked to return to the study center for a final visit. You will have a physical examination including measurement of your vital signs and weight. You will be asked about any signs or symptoms you have experienced and any other medications you are taking. A blood (a maximum of 5 tablespoons) sample will be drawn for laboratory testing. A biopsy of a KS lesion may be necessary at the time of discontinuation.

4. Early Withdrawal from the Study

The Investigator may take you off the study if you are not tolerating the study medications or if you do not comply with the study guidelines. You may withdraw from the study at any time, and this will not affect any future care or treatment for your disease. Your treatment may be stopped by the drug supplier, should the protocol be changed or closed, or if they believe that continuation in your case may not be beneficial. This may include discontinuing your participation in this study without your consent. At the time of withdrawal, you will be asked to undergo the same evaluations you would have had during the discontinuation of treatment visit, as described above.

5. Standard procedures being done because you are in this study include:

- A chest x-ray if you are a woman who could become pregnant, a pregnancy test will be done before treatment.

6. Non standard procedures being done because you are in this study include:

- Punch biopsies of two KS lesions on your skin before you start treatment and after 1 week and at the end of study treatment. There will be a total of 4 biopsies during the course of this study. A punch biopsy involves removal of a small piece of one of your tumors. These biopsies will be done to find out whether treatment has any effect on the cells in your Kaposi's sarcoma.
- A blood test to measure the level of Gleevec in your blood. This blood test will be performed on the day you begin treatment, and after 2 weeks of treatment. 4-5 teaspoons of blood will be needed for eight separate blood draws to measure Gleevec in your blood. This will be done on Day 1 and Day 15 (hour 0, 0.5, 1, 2, 3, 4, 8 and 24 hours).

If one of your KS tumors goes away, your doctor may ask your permission to biopsy the area of skin where the lesion used to be. A pathologist will examine the biopsy to see if there are any tumor cells left.

You will be asked to donate tissues and fluids to the AIDS and Cancer Specimen Resource (ACSR). If you agree, your permission will be obtained in a separate consent form. If you don't agree, it will not prevent you from taking part in this study.

HOW LONG WILL I BE IN THE STUDY?
We think you will be in the study for up to 1 year.
The researcher may decide to take you off this study if:

- Your condition worsens or your doctor believes that further treatment would be of no benefit to you or would be dangerous;
- Your doctors see side effects they consider dangerous;
- You are unable to consistently take the study medication or frequently miss appointments or miss tests required to see if the treatment is safe and effective and to see how the treatment works;
- You need treatment with other drugs that are not allowed in combination with Gleevec;
- You become pregnant.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

WHAT ARE THE RISKS OF THE STUDY?
While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict. Other drugs will be given to make side effects less serious and uncomfortable. Many side effects go away shortly after the drug is stopped, but in some cases side effects can be serious or long-lasting or permanent.

1. Risks and side effects related to the drug we are studying include:
   Very Likely

- Headache
- Vomiting
- Indigestion
- Eczema
- Fever
- Pain in the muscles, joints, limbs and bones, and joint swelling and edema (fluid retention) of the limbs, face and around the eyes
- Nausea
- Diarrhea
- Dermatitis
- Rash
- Muscle spasms and cramps
- edema (fluid retention) of the limbs, face and around the eyes

Less Likely but Serious

- Anorexia (uncontrolled lack of appetite and weight loss)
- Depression
- Taste disturbance
- Inflammation of the abdomen, liver, spleen, stomach, intestines, colon, voluntary muscles, heart tissue, and kidneys
- Increased tear production
- Dizziness
- Abdominal pain or distension
- Dry mouth
- Dry skin
- Petechia (purplish red spots on the skin)
- Unusual hair loss or thinning
- Fatigue
- Increased blood pressure
- Psychotic reactions
- Numbness of the hands or feet
- Wearing down of the eye nerve.
- Flatulence (gas in the digestive tract)
- Inflammation and possible fluid accumulation in the space around the lungs (pleurisy)
- Inflammation of the eyes (conjunctivitis)
- Night sweats
- Itching
- Cough
- Nasopharyngitis (inflammation of the nasal passages)
- Hearing loss
- Weakness
- Increased blood sugar
- Insomnia (sleeping difficulties)

- Bone decay and low blood sugar that could lead to coma
- Reduced vision
- Shock
- Mood swings
- Increased muscle tension
- Increased weight
- Nose bleeds
- Constipation
- Reddening of the skin
- Shortness of breath
- Pneumonia
- Decreased renal (kidney) phosphates and blood phosphate level

2. Abnormal laboratory test values observed in some patients required temporarily interrupting the study drug, or reducing the dose. The laboratory abnormalities included elevated liver or renal (kidney) function values, low platelet, white blood cell or red blood cell counts and disturbances in electrolyte (chemicals found in the blood, e.g. sodium, potassium, magnesium, calcium) balance. Lowering of your white blood cell count could lead to an increased risk of infection and slower healing. Lowering of your platelet count could lead to an increased risk of bleeding. Lowering of your red blood cells can lead to fatigue. If you should develop a fever when your white blood count is low, you may need to be hospitalized to receive treatment. Transfusions may be required to counteract the effects of low platelet or red blood cell counts. Elevated liver function values can indicate liver damage. Your laboratory values will be monitored closely, and the dose of your medication will be adjusted if your blood tests are abnormal. Based upon experience from previous clinical trials, it is expected that these effects may be reversed by decreasing the dose of the drug, or by temporarily stopping the study drug.

3. Gleevec is often associated with edema (fluid retention) and occasionally the fluid retention can be serious. Some patients have reported a rapid gain in body weight. Other patients have developed pleural effusions (fluid around lungs) and/or ascites (swollen abdomen), or pulmonary edema (fluid in the lungs), or pericardial effusions (fluid in the sac that surrounds the heart), all of which occasionally may be serious. A minority have developed signs of congestive heart failure, a premature heartbeat, and a sac forming in the wall of the artery. Therefore, you are asked to closely monitor your body weight twice a week and report to your doctor any rapid increase greater than 5 pounds. If this happens, a check-up including physical examination, blood tests and X-rays will be performed as required by your condition. One patient taking Gleevec who had no known history of liver problems has died due to liver failure while in a clinical study. The patient was also taking acetaminophen (Tylenol®), also known as paracetamol in European countries. You should not take more than 650 mg of acetaminophen every 4 hours. If you take more than one dose of acetaminophen, you should notify the study doctors. Kidney failure as well as multi-organ failure has been noted. Degeneration of the brain, liver, retina, and skeletal muscle cells have been noted as well as the bursting of a patient's spleen and pus found in the folds of the liver.

   Because some over the counter and prescription medications can reduce the effectiveness or enhance the side effects of Gleevec and/or Gleevec can increase the side effects or lessen the

effectiveness of some medications, it is recommended that you review all over-the-counter, health food supplements and prescription medications that you are taking, with your physician prior to taking them. In addition, you must not drink grapefruit juice while taking Gleevec.

4. Upper gastrointestinal bleeding, brain hemorrhages (bleeding in the brain), bleeding in the eye socket (anterior chamber hemorrhage) and inflammation of the capillaries have been reported in a minority of patients. One patient with a history of heart problems experienced chest pain while taking the study drug. Some patients have experienced blood in their urine, which if severe caused death. Another patient experienced abnormal growth of nervous system cells (neuroendocrine tumor). It has been reported that one patient developed Parkinson's disease, a slowly progressive disease of the nervous system that causes your body to experience uncontrollable spasms. One patient, on another study, developed a fistula (an abnormal pathway or channel) in his esophagus near a surgical site.

5. Congestive Heart Failure (CHF) is a rare, but serious adverse event that may develop while you are taking the study medication, Gleevec. CHF is characterized by a decrease in left ventricular systolic function (thickening of the membranes in your heart so that it is unable to adequately pump blood throughout the body). Ten patients with chronic myelogenous leukemia developed severe CHF while being treated with Gleevec. Prior to taking Gleevec, all patients had normal left ventricular function (no CHF). These effects on the heart have also been seen in mice treated with Gleevec.

   In NCI-sponsored clinical trials, other cardiac events have occurred that are likely related to Gleevec. These include cardiac ischemia/infarction, hypertension, and hypotension.

   Some of the symptoms listed above have led to death in a few patients. You will be closely monitored for any side effects and should report any changes in the way you feel to your doctor. You will be kept fully informed of any events that occur during the course of the trial that might affect your safety.

   Study medication must be kept in a secure place, out of the reach of children. Accidental ingestion of Gleevec may cause harmful effects, and the study doctor must be notified immediately if anyone other than you takes Gleevec by mistake.

6. The risk of renal (kidney) phosphate loss and hypophosphatemia (decreased blood phosphate levels) and low levels of vitamin D have been reported in patients taking Gleevec. These abnormalities were treated with Vitamin D and phosphate supplements.

7. Reproductive Risks

   Because Gleevec may affect an unborn baby, you should not become pregnant or father a baby while on this study. Women who are pregnant or nursing a child may NOT participate in this trial. Women who are either pregnant or breast-feeding will not be allowed to start or continue in the study. An effective form of contraception should be used by men who could father a child as well as women who could become pregnant. A barrier method of

contraception (a condom or diaphragm) should be used while taking this medication, and for 3 months afterwards. If you are a woman who could become pregnant, or a man who could father a child, ask about counseling and more information about preventing pregnancy. You should not breast-feed a baby while on this study. If you think you may have become pregnant during the clinical trial, contact your doctor immediately.

8. In non-human animal studies, imatinib has been found to cause cancer of the bladder, kidneys and ureters. At this time, it is uncertain whether imatinib can cause cancer in humans.

For more information about risks and side effects, ask the researcher.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?
If you agree to take part in this study, there may or may not be direct medical benefit to you. A possible benefit of treatment with Gleevec is that your KS will get smaller or go away. We hope what is learned from this study will help other patients with KS in the future.

WHAT OTHER OPTIONS ARE THERE?
Instead of being in this study, you have these options:

- Chemotherapy
- Interferon Therapy
- Radiation Therapy
- Local Treatment such as freezing of lesions, topical Panretin gel, or injection of lesions with chemotherapy
- Other experimental treatments if they are available to you
- No therapy at this time, with care to help you feel more comfortable.

Please talk to your regular doctor about these and other options.

WHAT ABOUT CONFIDENTIALITY?
Efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as trained staff at (Institution Name), and trained staff from the AIDS Malignancy Clinical Trials Consortium (AMC), the National Cancer Institute (NCI), AMC Operations Center (Data Coordinators) the Food and Drug Administration (FDA), and qualified representatives of Novartis.

WHAT ARE THE COSTS?
Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems.
You will not be billed for the cost of research studies. This includes:

- Biopsies before and during treatment that will be done to find out how Gleevec might be working on the tumor cells in your KS lesions and blood tests to measure the HIV viral load.
- Novartis Pharmaceuticals will provide you with Gleevec free of charge while you are being treated on this study.

You or your insurance company will be charged for tests considered part of the routine care of your disease. This includes:

- Any blood tests, x-rays, scans or biopsies needed to confirm whether you can participate in this study.
- Chest x-rays required by the study.
- Any hospitalizations.
- The costs of any medicines needed to control side effects from your treatment.
- Doctor's fees.
- Blood tests done to check for side effects of treatment and the effects of treatment on HIV and your immune function.
- Other x-rays and scans needed to evaluate your condition during your participation in this study.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

You will receive no payment for taking part in this study.

WHAT ARE MY RIGHTS AS A PARTICIPANT?
Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled.

We will tell you about new information that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?
For questions about the study or a research-related injury, contact the researcher.

_____(NAME{S}) at _____ (TELEPHONE NUMBER).

For questions about your rights as a research participant, contact the _____ (NAME OF CENTER) Institutional Review Board (which is a group of people who review the research to protect your rights) at _____ (TELEPHONE NUMBER). A non-physician whom you may call for information about the consent process, research patient's rights, or research-related injury is _____ (TELEPHONE NUMBER).

WHERE CAN I GET MORE INFORMATION?
You may call the NCI's Cancer Information Service at 1 800 4 CANCER (1 800 422 6237) or TTY: 1 800 332 8615

Visit the NCI's Web sites:

- CancerTrials: comprehensive clinical trials information

  http://cancertrials.nci.nih.gov.
- CancerNet: accurate cancer information including PDQ

  http://cancernet.nci.nih.gov.

You will get a copy of this form. You may also request a copy of the protocol (full study plan).

SIGNATURE

I agree to take part in this study.

Participant _____ Date _____

APPENDIX VII: ACSR INFORMED CONSENT

INFORMED CONSENT FORM
RESEARCH STUDY
AIDS AND CANCER SPECIMEN RESOURCE (ACSR)

A. INTRODUCTION

You are being asked to donate tissue for research. Before you decide to be a part of this research study, you need to understand the risks and benefits so that you can make an informed decision. This is known as informed consent.

This consent form provides information about the research study, which has been explained to you. Once you understand the study and the tests it requires, you will be asked to sign this form if you want to take part in the study. Your decision to take part in the study is voluntary. This means that you are free to choose if you will take part in the study.

B. PURPOSE

The National Cancer Institute has set up a Bank for tissues and biological fluids from HIV-positive and HIV-negative individuals in order to have specimens available for scientists studying malignancies associated with HIV disease. Individuals who have had biopsies to determine a malignancy are being asked for permission to take some of the tissue for the Bank. Only tissue in excess of that required for decision making will be given to the Bank. If it turns out that your physician needs more of your tissue for additional studies, the Bank will release all of your tissue back to your doctor. No additional tissues will be taken from your body for the Bank.

In addition, you are requested to donate some of your blood to the Bank so that scientists will also be able to look for any deviation in these body fluids that may explain the malignancy.

C. PROCEDURES

You are being asked for consent to place some of the biopsy material in the ACSR. If you agree to allow the ACSR to have some of your tissue, we would also like to:

1. Confidentially obtain some clinical information from your medical records that could be useful to research investigators. The report of the information retrieved from your medical record that is given to research investigators will not have your name, or include any information, which could personally identify you.

2. Obtain some blood for the Bank. Up to twenty (20) milliliters of blood will be obtained at your next visit to your physician.

If during the course of treatment by your physician, it is necessary to perform any of the following procedures for diagnostic reasons, you will be asked, at that time, to consent to having a portion of that specimen sent to the Bank. These requests will not require you to make any additional visits to your doctor or have any additional specimens taken just for the Bank. The Bank will only receive part of your specimen, and only what is in excess. No additional materials will be removed for the purposes of the Bank alone. Samples of interest would include, (but are not limited to):

- Spinal fluid.
- Airway washes.
- Fluid around lungs and intestines.
- Additional biopsy material.

You will not be asked to fill out any forms for any of these specimens.

D. POSSIBLE RISKS

There is a possibility of a bruise and slight pain at the time the blood samples are taken. There is also the possibility of fainting and infection at the site of the blood draw.

E. POSSIBLE BENEFITS

It may be that there will be no direct benefit to you by consenting to allow the Bank to have portions of your biopsies and biological fluids. However, there may be possible benefits to medical knowledge and HIV-infected individuals in the future.

F. COSTS

There will not be any additional costs to you for consenting to participate in the AIDS and Cancer Specimen Bank.

G. PAYMENT FOR INJURY OR HARM

As the lists of risks shows, taking part in this research study may result in injury or harm to you. If you require immediate medical care, you should go to an emergency room. Otherwise, the doctor in charge of the study will take care of you or help you get the care you need. You will be sent a bill for whatever medical care you receive. All or part of your bill may be paid by the sponsor of the research study (according to its agreement with the AIDS Associated Malignancies Clinical Trials Consortium), or by your health insurance. ***(Institution)*** will not pay for the care. Likewise, ***(Institution)*** will not pay you for pain, worry, lost income, or non-medical care costs that might occur from taking part in this research study.

H. PRIVACY

Your hospital medical records will be confidentially reviewed to obtain clinical information that could be useful to research investigators. However, the report of this information will not have your name or social security number anywhere on the report, so you will not be easily identified. The results of this research study will be given to the AIDS Associated Malignancies Clinical Trials Consortium (AMC), the National Cancer Institute, EMMES Data Coordinators,, and may be asked for by the Department of Health and Human Services. In addition, the Committee on Human Research of ***(Institution)*** may see your records. Except for these people, records from this study will be kept private unless you authorize their release or release is required by law (i.e. court subpoena). Any publications of this study will not use your name, identify you personally, or include any information which could personally identify you.

I. QUESTIONS

If you have any questions about this research study, you should contact Dr. ***(_____)*** at ***(Phone Number)*** (day) or ***(Phone Number)*** (night), or the person in charge of the study, ***(_____)***, the study coordinator, at ***(Phone Number)***. If you have any questions about your rights as a research subject, you should call ***(IRB Representative)***, in ***(Institution)*** Office of Human Research at (_____). ***(IRB Representative)*** is your representative and is not employed by the individuals conducting the study.

J. SIGNATURES

By signing this consent form you are agreeing that the study has been explained to you, that you understand the study, and that all your questions have been answered. You are signing that you agree to take part in this study. You will be given a copy of this consent form. By signing this form, you will not give up any of your legal rights.

_____ _____

Patient Date

_____ _____

Person obtaining consent Date

_____ _____

Witness Date

APPENDIX VIII: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) - SPECIMEN PREPARATION & SHIPPING INSTRUCTIONS

A. COLLECTION

Consent patient for ACSR donation. Collect **20 cc** of whole blood in ACD tubes.

B. SHIPPING

To ship bloods, place the tubes into a canister of a STP-100 SAF-T-PAK shipper wrapping each tube in bubble wrap and using the absorbent paper at the bottom of the canister. Mailers for shipping donations will be supplied by the ACSR upon request. Please allow a few days for receipt of the mailers. Each sample tube should be labeled using a sharpie pen with the following information:

- Protocol #: AMC 042
- 9 digit Patient #
- Date and time of collection
- Specimen type, i.e., **WB**=Whole Blood, **P**=Plasma, **S**=Serum
- Specimen purpose: Donation

Place the lid on the canister and place it inside of the ambient SAF-T-PAK shipper. **Fold and pack ACSR form inside shipping box.** Seal the ambient shipper with cellophane shipping tape. Label the ambient shipper with the "Infectious substance" diamond shaped label. On one side, in black marker write “BIOLOGIC SUBSTANCE, CATEGORY B”, your name or name of responsible person, date of collection and phone number of the person responsible for the package.

B.1 Specimen Shipment

Specimens are accepted **MONDAY** through **THURSDAY**. Specimens are not accepted on Friday.:

All **BLOOD (whole blood, plasma, serum)** specimens should be shipped by overnight express at room temperature to:

ACSR Blood Receiving Laboratory
Johns Hopkins University
1650 Orleans Street, CRB-384
Baltimore, MD 21231-1000
TEL: (410) 955-8721
FAX: (443) 287-3217

All **TISSUE** specimens should be shipped by overnight express to:

Dr. Sylvia Silver
George Washington University Medical Center
2300 I Street, NW, Room 507
Washington, DC 20037
Phone: (202) 994-1444
Fax: (202) 994-5056

Please use the AMC Operations Center FedEx account # to ship: [redacted]

B.2 Instructions for Specimens Collected on Friday

PREPARATION OF PLASMA AND MONONUCLEAR CELLS

It is preferable that separation occurs as soon as possible. If necessary, whole blood in acid citrate dextrose (yellow top tube) can be held at room temperature for no more than 24 hours.

Materials:
Lymphocyte Separation Medium (LSM Solution, Ficoll-Hypaque - sterile)
15 ml conical centrifuge tubes (sterile)
PBS (sterile)
1, 5 ml and 10 ml serologic pipettes (sterile)
NUNC tubes
Alcohol-saturated, control rate freezer container
DMSO freezing media:

- 50% Cryoprotective Medium, Cambrex (catalog no.: 12-132A)
- 50% Heat Inactivated Fetal Bovine Serum

1. Preparation of Plasma Samples
   a. The 7 ml tube of whole blood in acid citrate dextrose should be rotated gently 2 or 3 times before being centrifuged. Do not transfer before centrifugation.
   b. The cells are separated by centrifugation at 500 g for 10 minutes.
   c. 0.5ml aliquots of plasma are removed and put into 1.5 ml screw top tubes and transferred to liquid nitrogen storage.

2. Peripheral Blood Mononuclear Cell Separation and Freezing
   a. The cells and plasma remaining from the previous step are transferred into a 15 ml conical tube, capped and re-suspended by gently tapping the bottom of the tube.
   b. Sterile PBS should be added to the suspended cells until the final volume is 8 ml; invert to mix.

c. The 8 ml whole blood-PBS mixture should be carefully overlaid onto 4 ml of room temperature LSM or Ficoll-Hypaque solution in a sterile 15 ml conical tube. A sharp interface should exist between the LSM and the whole blood mixture. (If the layer of LSM gets mixed with the blood-PBS, the tube should be gently rotated to mix the blood, PBS, and LSM, and transfer to a 50 ml sterile conical tube. An equal volume of PBS is added, and the cells are separated at 600 g for 15 minutes. After removal of LSM-PBS supernatant, return to Step b).
d. The 15 ml conical tube for 30 minutes at 900 g at room temperature. The mononuclear leukocytes (principally lymphocytes and monocytes) will band at plasma/LSM interface.
e. The fluffy white layer just below the plasma layer should be aspirated off, along with approximately half of the LSM layer under it, and transferred to an appropriately labeled 15 ml sterile conical centrifuge tube. Mix by gentle rotation.
f. Washed twice in sterile PBS - centrifuge at 500 g for 10 minutes.
g. Cell pellet should be mixed well with a gentle finger-tapping action.
h. Using a 1 ml pipette, the *DMSO freezing mixture should be added drop wise to the cell pellet suspension. Gently finger-tap between drops. If the cell pellet is small, only 1 ml of freezing media is added (and only one aliquot of cells is frozen). If the cell pellet is large, up to 4 ml of freezing media can be added in a drop wise fashion. (Cell densities of 1 - 10 million PBMC/ml are best for cryopreservation. If a hemocytometer is available, the optimal concentration is 5 million PBMC/ml).

   <u>*Important-Do not put the DMSO containing media on the cell button all at once.</u>

3. Freeze the cell suspension in 0.5 ml aliquots in sterile NUNC vials by placing the NUNC tubes in a room temperature, alcohol saturated, control rate freezer container and store in the -80°C freezer overnight. Transfer the cell suspension into the liquid nitrogen temperature freezer for long-term storage the next working day.

   PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING

   Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package

   Please Note: The shipper will be mailed back to the AMC site.

   The STP-100 SAF-T-PAK shipper is a complete kit w/all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc).

B.3 Record of Specimens

This study will track specimens via GlobalTrace[SM], a component of the AMC AdvantageEDC[SM] system. The GlobalTrace shipment manifest must accompany all specimen shipments.

APPENDIX IX: PUNCH BIOPSY

EXPRESSION/ACTIVATION (PHOSPHORYLATION) IMATINIB TARGETS AND SIGNALING INTERMEDIATES

A. METHODOLOGY

A.1 Biopsies will be performed with a 4 mm punch biopsy at baseline and Day 8 of treatment. Fixed tissue samples in formalin will be received from trial sites. They will be embedded in paraffin blocks with an orientation that allows for sectioning perpendicular to the skin surface. Staining of slides will be batched in order that pre and post sections are stained at the same time to control for variability. Two ribbons of four to five tissue sections 5 um thick will be placed one below the other on ProbeOn Plus slides (Fischer). The two ribbons will be treated identically except the top ribbon will receive the primary antibody and the bottom ribbon, which is serving as a negative control, will not. Slides will be stained using the following antibodies: LANA, anti-PDGF-R and anti-phospho-PDGF-R (Tyrosine 783) (Cell Signaling, Beverly, MA). H&E stains will also be performed on all pre and post specimens.

A.2 Within 48 hours of sectioning, slides will de-paraffinized in xylene and ethanol. Antigen retrieval will be performed by boiling the slides for 10 minutes in pH 6 citrate buffer followed by 10 minutes in 3% H_2O_2. The slides for phospho–PDGF-R require 15 minutes in 1mM EDTA, pH 8.0 prior to H_2O_2. The slides are then incubated overnight with the primary antibody at a 1/100 dilution for anti-PDGF-R, a 1/100 dilution for anti-LANA, and a 1/50 dilution anti-phospho-PDGFR at 40C. The slides are then incubated for 30 minutes with 100 µL of biotinylated anti-rabbit secondary antibody, then for 30 minutes with ABC avidin/biotin (Vector Labs, Burlingame, CA) with washes in after each incubation. Slides were incubated in ethanol and xylene prior to mounting coverslips.

A.3 All slides will be assigned a random numerical identifier and will be presented in a blinded fashion to two pathology reviewers (Drs. Tahan and Dahiya). Based on review of the slides from the pilot study, it was determined nearly all of the KS spindle cells stained uniformly positive with minimal heterogeneity, and after treatment their immunoreactivity decreased uniformly. Staining intensity of basal keratinocytes is reduced after treatment with imatinib, precluding using them as an internal standard for assessment of lesional cell staining intensity in post-treatment samples. Immunoreactivity of lesional KS cells will thus be scored on a scale of 0 to 5+ by two pathologists, with 0 being the negative control staining of basal keratinocytes and 5+ being equal to the staining intensity of basal keratinocytes in pretreatment samples, and a final score for each sample based on the average of 4 sections.

A.4 Analysis: Immunohistochemical methods are amenable to statistical assessment. For instance, even using an activation specific antibody, Weber and his colleagues have been able to statistically correlate erk1/erk2 activation with tumor grade and stage of prostate cancer [52]. In this study we will classify the activation of PDGF-R as low (score of 0, 1+ or 2+) or high (score of 3+, 4+ or 5+). The breakpoint for this grouping is based on our observations of degree of inhibition in the pilot trial. Biopsy pairs that score as high at baseline and low at seven days will be considered PDGF-R inhibited, and those that score as high at baseline and high at seven days will be considered PDGF-R not inhibited. Pairs that score low at baseline will be omitted from the analysis. For the purposes of Aims 2 and 3, clinical response is defined as partial or complete response and non-response will be classified as stable disease or disease progression. Our a priori hypothesis is that inhibition of PDGF-R at 7 days correlates with clinical response. A chi 2 test will be used to test whether phosphorylation of PDGF-R (low or high) at 7 days predicted clinical response.

B. SPECIMEN COLLECTION

Biopsies should be obtained by standard punch technique, using a 4 mm punch. The biopsy should be, if possible, completely within the margins of the lesions. Selection of lesions for biopsy is described in Section 4.1.6.

The specimen should then be placed in 10% neutral buffered formalin and labeled as follows:

- Protocol #: AMC 042
- 9 digit Patient #
- Patient initials
- Study Period: Baseline or Day 8
- Date and time of collection
- Purpose: Phosphorylation

C. SPECIMEN SHIPPING

Specimens are accepted **MONDAY** through **THURSDAY**. (Because overnight shipping is required for these samples, the only specimens to come to Dr. Koon's lab on Mondays will be those from Beth Israel itself. For sites other than Beth Israel, visits involving these labs should be scheduled for Monday through Wednesday and samples shipped overnight for receipt in Dr. Koon's lab Tuesday through Thursday and if for some reason sites need to ship on Thursday or Friday, please contact Dr. Koon). UNLESS OTHER SHIPPING ARRANGEMENTS HAVE BEEN MADE IN ADVANCE WITH THE LAB, ALL SPECIMENS should be shipped within 24 hours of collection.

Samples should be stored at room temperature and shipped within 24 hours of collection to:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

Use FED-EX "Dangerous Goods" airway bills for shipping. FED-EX account for the shipment: [redacted]. **It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.** Call FED-EX at1-800-463-3339 and press 0. Ask for customer service "dangerous goods" department. A FED-EX representative will assist in the specific wording required on the airway bills for pick-up and delivery of "Dangerous Goods". Place the completed airway bill marked **"Priority Overnight"** and the typed "Shippers Declaration for Dangerous Goods" on top of the shipper box inside of a plastic FED-EX pouch.

***PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING.

Please Note: The shipper will be mailed back to the AMC site.
The STP-100 SAF-T-PAK shipper (VWR Cat # 11217-163) is a complete kit w/ all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc). There is a refurbishment kit w/ extra bubble- wrap, absorbent material (STP102) (VWR Cat # 11217-166) enough for 15 mailings.

D. RECORD OF SPECIMENS

A copy of the specimen log at the end of Appendix X will be provided with the specimen collection pack which will be completed and accompany the sample when shipped.

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC^{SM} system. The GlobalTrace shipment manifest must accompany all specimen shipments.

APPENDIX X: PUNCH BIOPSY

RECEPTOR MUTATION ANALYSIS

A. METHODOLOGY

Each patient will have a 4 mm punch biopsy performed at baseline and at the time of progression or treatment discontinuation of a representative lesion. RNA and genomic DNA will be isolated from samples. PCR primers for the genomic DNA were designed to begin in the flanking introns and cover the entire exon being studied The PCR products will be gel purified and will then be subcloned into PCR4blunt-TOPO vector. The ligated vectors for each exon will then be used to transform competent E. Coli XL1 blue and the bacteria will be plated and grown overnight. Single colonies will be picked and then mini-prepped. The DNA from the mini-preps will be sequenced. Because the malignant cells of KS will only be a portion of the biopsy, the heterogeneity of the tumor must be taken into account before we can declare a negative result. Based on our previous studies we estimate that approximately 50% of a 4 mm punch biopsy is composed of KS spindle cells which express PDGF-R β and c-kit. If only one allele of the KS spindle cell has a mutation and the ratio of normal to mutant cells is one to one, only one out of four exons carry the mutation. To calculate the number of sequences to perform on each exon to assure a mutation was not missed, we first decided level of 95% confidence level was acceptable. The number of assays required is the number of times the negative result (normal sequence) must happen to be less that or equal to 5% (100% -95%). Mathematically this can solved as a probability problem.

$(p\ \{\text{normal sequence}\})^n < 0.05$ where n is number of assays and p(normal sequence) = 0.75

This transforms to $(0.75)^n < 0.05 \rightarrow n \log(0.75) < \log(0.05) \rightarrow n(-0.125) = -1.301$
$n = 10.41 \rightarrow 11$

Thus to achieve a 95% confidence of not missing a mutation each exon will be sequenced 11 times. If a positive mutant clone is discovered, it is possible that the mutation is an error introduced by PCR. We are using PFU polymerase, which has a lower mistake rate than Taq polymerase to reduce the incidence of mistakes. Because PFU still has a mistake rate of 1 in 10,000 nucleotides all mutants will have to be confirmed by two more clones from different PCR reactions to be considered a positive result. If mutations are present, we will determine if they correlate with non-response.

B. SPECIMEN COLLECTION

Biopsies should be obtained by standard punch technique, using a 4 mm punch. The biopsy should be, if possible, completely within the margins of the lesions. Selection of lesions for biopsy is described in Section 3.1.1. The biopsy will be placed in an RNAlater

tissue protect tube (these tubes can be obtained by contacting Dr. Koon's lab) and labeled as follows:

- Protocol #: AMC 042
- 9 digit Patient #
- Patient initials
- Study Period: Baseline, progression or discontinuation
- Date and time of collection
- Purpose: Mutation receptor

C. SPECIMEN SHIPPING

Specimens are accepted **MONDAY** through **THURSDAY**. (Because overnight shipping is required for these samples, the only specimens to come to Dr. Koon's lab on Mondays will be those from Beth Israel itself. For sites other than Beth Israel, visits involving these labs should be scheduled for Monday through Wednesday and samples shipped overnight for receipt in Dr. Koon's lab Tuesday through Thursday and if for some reason sites need to ship on Thursday or Friday, please contact Dr. Koon). UNLESS OTHER SHIPPING ARRANGEMENTS HAVE BEEN MADE IN ADVANCE WITH THE LAB, ALL SPECIMENS should be shipped within 24 hours of collection.

Samples should be stored at room temperature and shipped within 24 hours of collection to:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

Use FED-EX "Dangerous Goods" airway bills for shipping. FED-EX account for the shipment: [redacted]. **It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored.** Call FED-EX at1-800-463-3339 and press 0. Ask for customer service "dangerous goods" department. A FED-EX representative will assist in the specific wording required on the airway bills for pick-up and delivery of "Dangerous Goods". Place the completed airway bill marked **"Priority Overnight"** and the typed "Shippers Declaration for Dangerous Goods" on top of the shipper box inside of a plastic FED-EX pouch.

***PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING.

Please Note: The shipper will be mailed back to the AMC site.
The STP-100 SAF-T-PAK shipper (VWR Cat # 11217-163) is a complete kit w/ all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc). There is a refurbishment kit w/ extra bubble-wrap, absorbent material (STP102) (VWR Cat # 11217-166) enough for 15 mailings.

D. RECORD OF SPECIMENS

A copy of the specimen log at the end of Appendix X will be provided with the specimen collection pack which will be completed and accompany the sample when shipped.

This study will track specimens via GlobalTrace[SM], a component of the AMC AdvantageEDC[SM] system. The GlobalTrace shipment manifest must accompany all specimen shipments.

AMC PROTOCOL 042

A Phase II Trial of Imatinib Mesylate in Patients with HIV Related Kaposi's Sarcoma

Biopsy Log - for Baseline, DAY, 8 and Progression

PI: _____

SITE OR INSTITUTION NAME: _____

PATIENT ID: _____

Contact Name& Tel.: _____

Cycle: _____ Day: _____

Biopsies

TIMEPOINT	DATE	TIME	Initials	COMMENTS
Baseline				Phosphorylation - formalin
				Mutation Assay – RNAlater
Day 8				Phosphorylation - formalin
Progression				Mutation Assay – RNAlater

Initials	Signature

Shipping Address:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

APPENDIX XI: CYTOKINE PROFILES ANALYSIS

A. METHODOLOGY

In this study we will examine the patient cytokine profiles at baseline, Day 8 and Day 29. Plasma samples will be assayed using the PerkinElmer cytokine chip as described in the preliminary data. As noted the standards on the cytokine chip are analyzed by point-to-point linear regression and concentrations of experimental samples are estimated from these standard four-point curves. Thus the chip should be considered a semi-quantitative assay and all cytokines/chemokines that appear to be potential predictors will be confirmed by ELISA.

The concentrations of cytokines that are found to be potential predictors by single variant analysis will be determined using commercially available ELISAs. R&D Systems (Minneapolis, MN) produces colorimetric sandwich ELISAs that correspond to the cytokines and chemokines on the cytokine chip. Depending on the ELISA, the intra-assay precision has a CV of 3 to 14 percent and the inter-assay precision has a CV of 7 to 14 percent. The concentrations determined by ELISA will then be analyzed by single variant and multivariate analysis to determine if there is any correlation between concentration at baseline or changes in concentrations after treatment and clinical response.

B. SPECIMEN COLLECTION

Serum (red top) and plasma (green top) specimens should be collected at baseline, 8 days and 29 days (approximately 5ml). Centrifuged at 2,400 xg for 15 minutes. Plasma and serum should be aliquoted into 2 nunc tubes which will be provided with labels by the Koon lab in the specimen collection packs. Ship one tube of each to the Koon lab and keep other at the site in – 70 degrees and cover labels with freezer tape. Please label with appropriate red or green top label. The specimens should be labeled as follows:

- Protocol #: AMC 042
- 9 digit Patient #
- Patient initials
- Study Period: Baseline, day 8 or day 29
- Date and time of collection
- Purpose: Cytokines

C. SPECIMEN SHIPPING

Specimens are accepted **MONDAY** through **THURSDAY**. (Because overnight shipping is required for these samples, the only specimens to come to Dr. Koon's lab on Mondays will be those from Beth Israel itself. For sites other than Beth Israel, visits involving these labs should be scheduled for Monday through Wednesday and samples shipped overnight

for receipt in Dr. Koon's lab Tuesday through Thursday and if for some reason sites need to ship on Thursday or Friday, please contact Dr. Koon). UNLESS OTHER SHIPPING ARRANGEMENTS HAVE BEEN MADE IN ADVANCE WITH THE LAB, ALL SPECIMENS should be shipped within 24 hours of collection.

Samples should be stored in a –70C freezer and shipped on dry ice within 24 hours of collection to:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

Use FED-EX "Dangerous Goods" airway bills for shipping. FED-EX account for the shipment: [redacted]. It is only to be used for billing shipment of specimens to the lab where the sample is processed and/or stored. Call FED-EX at1-800-463-3339 and press 0. Ask for customer service "dangerous goods" department. A FED-EX representative will assist in the specific wording required on the airway bills for pick-up and delivery of "Dangerous Goods". Place the completed airway bill marked "Priority Overnight" and the typed "Shippers Declaration for Dangerous Goods" on top of the shipper box inside of a plastic FED-EX pouch. ***PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING.

Please Note: The shipper will be mailed back to the AMC site.
The STP-100 SAF-T-PAK shipper (VWR Cat # 11217-163) is a complete kit w/ all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc). There is a refurbishment kit w/ extra bubble- wrap, absorbent material (STP102) (VWR Cat # 11217-166) enough for 15 mailings.

D. RECORD OF SPECIMENS

A copy of the specimen log at the end of Appendix XI will be provided with the specimen collection pack which will be completed and accompany the sample when shipped.

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC^{SM} system. The GlobalTrace shipment manifest must accompany all specimen shipments.

AMC PROTOCOL 042

A Phase II Trial of Imatinib Mesylate in Patients with HIV Related Kaposi's Sarcoma

CYTOKINE PROFILES for Baseline, DAY, 8 and DAY 29

PI: _____

SITE OR INSTITUTION NAME: _____

PATIENT ID: _____

Contact Name& Tel.: _____

Cycle: _____ Day: _____

CYTOKINE PROFILES

TIMEPOINT	DATE	TIME	Initials	COMMENTS
Baseline				
Day 8				
Day 29				

Samples will be collected in one heparinized tube (green top) and one red top tube. Centrifuged at 2,400 xg for 15 minutes. Plasma should be aliquoted into 2 nunc tubes, ship one to Henry Koon keep other at site in – 70 degrees and cover labels with freezer tape. Please label with appropriate red or green top label.

Initials	Signature

Shipping Address:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

APPENDIX XII: PHARMACOKINETIC ANALYSIS

A. METHODOLOGY

Blood samples will be collected for pharmacokinetic analysis on Days 1 and 15 as detailed in the protocol. Plasma concentrations of imatinib and its major active metabolite, CGP-74588, will be determined with a previously described and validated liquid chromatographic-mass spectrometric assay.[20]

B. SPECIMEN COLLECTION

Samples will be collected in heparinized tubes (green top) and centrifuged at 2,400 xg for 5 minutes. Plasma will be aliquoted into 2 nunc tubes, which will be provided with labels by the Koon lab in specimen collection packs. Ship one tube to the Koon lab and keep other at the site in – 70 degrees and cover labels with freezer tape. Please label with appropriate label. The specimens should be labeled as follows:

- Protocol #: AMC 042
- 9 digit Patient #
- Patient initials
- Study Period: Day 1 or Day 8
- Date and time of collection
- Purpose: PK

C. SPECIMEN SHIPPING

Specimens are accepted **MONDAY** through **THURSDAY**. (Because overnight shipping is required for these samples, the only specimens to come to Dr. Koon's lab on Mondays will be those from Beth Israel itself. For sites other than Beth Israel, visits involving these labs should be scheduled for Monday through Wednesday and samples shipped overnight for receipt in Dr. Koon's lab Tuesday through Thursday and if for some reason sites need to ship on Thursday or Friday, please contact Dr. Koon). UNLESS OTHER SHIPPING ARRANGEMENTS HAVE BEEN MADE IN ADVANCE WITH THE LAB, ALL SPECIMENS should be shipped within 24 hours of collection.

Samples should be stored in a –70C freezer and shipped on dry ice within 24 hours of collection to:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

Use FedEx account number [redacted] to ship all PK samples.

Please Note: The shipper will be mailed back to the AMC site.
The STP-100 SAF-T-PAK shipper (VWR Cat # 11217-163) is a complete kit w/ all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc). There is a refurbishment kit w/ extra bubble-wrap, absorbent material (STP102) (VWR Cat # 11217-166) enough for 15 mailings.

D. RECORD OF SPECIMENS

A copy of the specimen log at the end of Appendix XII will be provided with the specimen collection pack which will be completed and accompany the sample when shipped.

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC^{SM} system. The GlobalTrace shipment manifest must accompany all specimen shipments.

AMC PROTOCOL 042

A Phase II Trial of Imatinib Mesylate in Patients with HIV Related Kaposi's Sarcoma

PHARMACOKINETIC SAMPLE COLLECTION LOG for Days 1 and 15

PI: _____

SITE OR INSTITUTION NAME: _____

PATIENT ID: _____

Contact Name& Tel.: _____

Cycle: _____ Day: _____

1. PHARMACOKINETIC SAMPLE COLLECTION (PLASMA)

Day 1 or Day 15 (Please circle one)

TIMEPOINT	DATE	TIME	Initials	COMMENTS
Pre-dose 0 hrs.				
Dosing – Gleevec 400mg po				
30 minutes				
I Hour				
2 Hours				
3 Hours				
4 Hours				
8 Hours				
24 Hours Pre-dose				

2. SPECIMEN COLLECTION INSTRUCTION

Samples will be collected in heparnized tubes (green top)(6cc on ice) and centrifuged at 2,400 xg for 15 minutes. Plasma should be aliquoted into 2 nunc tubes, ship one to Henry Koon keep other at site in – 70 degrees and cover labels with freezer tape.

Shipping Address:

Henry B. Koon, M.D.
Case Western Reserve University
2103 Cornell Road / WRB 2204
Cleveland, OH 44106
Tel: (216) 368-1175
Fax: (216) 368-1166

APPENDIX XIII: HHV-8 VIRAL LOAD AND GENE EXPRESSION

A. DESCRIPTION OF ASSAY

This assay is used to quantify the burden of HHV-8 (Human Herpesvirus Type 8, or Kaposi's sarcoma associated virus, KSHV) present in circulating peripheral blood mononuclear cells (PBMC). Cells are separated from whole blood by Ficoll centrifugation, DNA extracted from purified PBMC by column extraction, and the number of HHV-8 copies determined using a quantitative competitive DNA PCR assay. Results are expressed in numbers of copies per million PBMC. The limit of detection for this assay depends upon the amount of DNA available, but is generally $\leq$50 copies/10^6 PBMC. Changes $\geq$ 1.7-fold are unlikely to be due to assay variation, but HHV-8 burden does not appear to be as constant as HIV viral load. While some preliminary data indicates that untreated individuals with an average HHV-8 burden of $\geq$100 copies/10^6 cells may be at greater risk of progression, this assay is a research test only, and should not be used to make clinical decisions. The purpose of including this assay as part of this trial is to determine its usefulness as a prognostic marker.

A.1 Changes in gene expression are a fundamental hallmark of cancer progression and invaluable tool of cancer stages. In the case of KS, KSHV/HHV-8 has been identified as the etiological agent and this assay is designed to identify KSHV genes that might change in response to therapy. We will use reverse-transcription (RT) coupled to amplification using polymerase chain reaction (PCR) to measure the mRNA levels of all KSHV/HHV-8 mRNAs in the tumor. This assay is a research test only and should not be used to make clinical decisions. The purpose of including this assay as part of this trial is to determine its usefulness as a prognostic marker.

A.2 Reverse-transcription (RT) coupled to amplification using polymerase chain reaction (PCR) is widely recognized as the most sensitive method to detect the presence of specific RNAs. We will use real-time quantitative RT-PCR. This assay measures the amount of PCR product based on intercalation of a fluorescent dye. Fluorescence is recorded at each cycle. So-called Ct-values indicate the cycle at which the fluorescence crosses a particular threshold (5 times standard deviation (SD) of the non-template control (NTC)). Hence, Ct-values indicate the abundance of a given mRNA on a log scale. A low Ct value represents a highly abundant target mRNA (Heid, Genome Res. 6:968-94 (1996). We have experience doing these assays at the University of North Carolina Lineberger Cancer Center.

A.3 Cells are separated from whole blood by Ficoll centrifugation. Total RNA will be isolated using RNAzol (Tel-Test, Inc., Friendswood, Texas) according to the supplier's protocol and reverse-transcribed using Mo-MuLV reverse transcriptase and 120 pmol random hexanucleotide primers (Applied Biosystems Inc., Foster

City, CA). After incubation at 42°C for 35 min, the reaction was stopped by heating to 95°C for 5 min, the cDNA pool was diluted, and the resulting sample analyzed by real-time quantitative PCR using a dedicated MJR Opticon2 machine and universal cycle conditions. We will use commercial SYBRgreen-based PCR (SYBR® Green, Applied Biosystems Inc., Foster City, CA) as a uniform detection method.

B. GENERAL

To ship HHV-8 specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The Ambinder lab recommends the use of the SAF-T-PAK STP 210 diagnostic cardboard shipper. These shippers may be ordered at the SAF-T-PAK website www.saftpak.com. The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

NOTE: Specimens MUST BE SHIPPED Mondays through Thursdays as PRIORITY OVERNIGHT. Specimens are NOT ACCEPTED ON SATURDAYS in the Ambinder Lab.

C. SPECIMEN PREPARATION

C.1 Draw three 10 cc (ml) green top (heparin) tubes from study patient.
With a black, water resistant, sharpie pen, label each specimen with the following information:

- Protocol #: AMC 042
- 9 digit Patient #
- Patient initials
- Study Period-Day 1, Day 15, cycle 3, cycle 5, cycle 7,cycle 9, cycle 11 or Treatment Discontinuation
- Date and time of collection
- Specimen type- "Whole Blood"
- Specimen purpose: "HHV-8 PCR"

C.2 Seal the tops of the three 10 cc heparin green tops with parafilm.

D. PACKAGING AND FED-EX FORMS

a. Place the three sealed tubes into bubble wrap (provided in STP-210 kit).
b. Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
c. Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and “self-seal”.
d. Place poly-bag containing tubes into the white TYVEK bag and seal.

e. Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
f. Affix the FED-EX airbill on blank side of the shipper making sure that it is marked "FED-EX PRIORITY OVERNIGHT".
g. Mark "OTHER" in the airbill under "Packaging".
h. Under airbill section "special Handling" indicate "YES-SHIPPERS DECLARATION NOT REQUIRED".
i. Enter FED-EX account # [redacted]
j. Place "From/To" information onto areas provided on the shipper.

Shipping Address is:
Ambinder Lab
Johns Hopkins Oncology
1650 Orleans Street, CRB-384
Baltimore, MD 21231-1000
Tel: (410) 955-8721
Fax: (443) 287-3217

k. Make certain that shipper is already either pre-labeled with 'UN#3373' stamp, or make a paper label with 'UN#3373" and affix it to the shipper.
l. Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in ml (so three 10 cc tubes is 30 ml) and affix to the shipper.
m. Affix airbill to shipper so that the 'UN' and 'VOLUME' labels are visible.
n. RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.
o. Place the box in the FedEx pickup area at your site or call to request a package pickup.

Please Note: The shippers will be mailed back to each AMC site.

E. RECORD OF SPECIMENS

This study will track specimens via GlobalTrace[SM], a component of the AMC AdvantageEDC[SM] system. The GlobalTrace shipment manifest must accompany all specimen shipments.

APPENDIX XIV: NEW YORK HEART ASSOCIATION CRITERIA

A. CLASS I (MILD)

No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).

B. CLASS II (MILD)

Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.

C. CLASS III (MODERATE)

Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.

D. CLASS IV (SEVERE)

Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

APPENDIX XV: DRUGS METABOLIZED BY CYP450 ISOENZYMES 2D6 AND 3A4

CYP2D6	
Substrates	
Amitriptyline (hydroxylation)	Methamphetamine
Amphetamine	Metoclopramide
Betaxolol	Metoprolol
Bisoprolol	Mexitetine
Brofaromine	Mianserin
Buturolol	Meperidine
Bupropion	Methadone Mirtazapine (hydroxylation)
Captopril	Molindone
Carvedilol	Morphine
Cevimeline	Nortriptyline (hydroxylation)
Chlorpheniramine	Olanzapine (minor, hydroxymethylation)
Chlorpromazine	Ondansetron
Cinnarizine	Orphenadrine
Clomipramine (hydroxylation)	Oxycodone
Clozapine (minor pathway)	Papaverine
Codeine (hydroxylation, o-demelhylation)	Paroxetine (minor pathway)
	Penbutolol
Cyclobenzaprine (hydroxylation)	Pentazocine
Cyclophosphamide	Perhexiline
Debrisoquin	Perphenazine
Delavirdine	Phenformin
Desipramine	Pindolol
Dexfenfluramine	Promethazine
Dextromethorphan (o-demethylation)	Propafenone
Dihydrocodeine	Propranolol
Diphenhydramine	Quetiapine
Dolasetron	Remoxipride
Donepezil	Risperidone
Doxepin	Ritonavir (minor)
Encainide	Ropivacaine
Fenlluramine	Selegiline
Flecainide	Sertindole
Fluosetine (minor pathway)	Sertratine (minor pathway)
Fluphenazine	Sparteine
Haiofantrine	Tamoxifen
Haioperidol (minor pathway)	Thioridazine
Hydrocodone	Tiagabine
Hydrocortisone	Timolol
Hydroxyamphetamine	Tolterodine
lmipramine (hydroxylation)	Tramadol

CYP2D6	
Labetalol Loratadine Maprotiline m-Chlorophenylpiperazine (m-CPP)	Trazodone Trimipramine Tropisetron Venlafaxine (o-desmethylation) Yohimbine
Inhibitors	
Amiodarone Celecoxib Chloroquine Chlorpromazine Cimelidine Citalopram Clomipramine Codeine Deiavirdine Desipramine Dextropropoxyphene Diltiazem Doxorubicin Entacapone (high dose) Fluoxetine Fluphenazine Fluvoxamine Haloperidol Labetalol Lobeline Lomustine	Methadone Mibefradil Moclobemide Nortluoxeline Paroxetine Perphenazine Propafenone Quinacrine Quinidine Ranitidine Risperidone (weak) Ritonavir Sertindole Sertraline (weak) Thioridazine Vaiprolc acid Venlafaxine (weak) Vinblastine Vincristine Vinorelbine Yohimbine

CYP3A3/4	
Substrates	
Acetaminophen	Chlorpromazine
Aifentanil	Cimetidine
Alosetron	Cisapride
Alprazolam	Citałopram
Amiodarone	Clarithromycin
Amitriptyline (minor)	Clindamycin
Amlodipine	Clomipramine
Anastrozole	Clonazepam
Androsterone	Clozapine
Antipyrine	Cocaine
Astemizole	Codeine (demethylation)
Atorvastatin	Cortisol
Benzphetamine	Cortisone
Bepridil	Cyclobenzaprine (demethylation)
Bexarotene	Cyclophosphamide
Bromazepam	Cyclosporine
Bromocriptine	Dapsone
Budesonide	Dehydroepiandrostendione
Bupropion (minor)	Delavirdine
Buspirone	Desmethyldiazepam
Busutfan	Dexamethasone
Caffeine	Dextromethorphan (minor, N-demethylation)
Cannabinoids	
Carbamazepine	Diazepam (minor; hydroxylation, N-demethylation)
Cevimeline	
Cerivastatin	
Digitoxin	Nefazodone
Diltiazem	Nelfinavir
Disopyramide	Nevirapine
Docetaxel	Nicardipine
Dolasetron	Nifedipine
Donepezil	Niludipine
Doxorubicin	Nimodipine
Doxycycline	Nisoldipine
Dronabinol	Nitrendipine
Enalapril	Omeprazole (sulfonation)
Erylhromycin	Ondansetron
Estradiol	Oral contraceptives
Ethinyl estradiol	Orphenadrine
Ethosuximide	Paclitaxel
Etoposide	Pantoprazole
Exemestene	Pimozide
Dofetilide (minor)	Pioglitazone

CYP3A3/4	
Felodipine	Pravastatin
Fentanyl	Prednisone
Fexotenadine	Progesterone
Finaxteride	Proguanil
Fluoxetine	Propafenone
Flutamide	Quercetin
Glyburide	Quetiapine
Granisetron	Quinidine
Halofantrine	Quinine
Hydrocortixone	Repaglinide
Hydroxyarginine	Retinoic acid
Lfosfamide	Rifampin
Lmipramine	Risperidone
Lndinavir	Ritonavir
Lsradipine	Salmeterol
Ltraconazole	Saquinavir
Ketoconazole	Sertindole
Lansoprazole (minor)	Sertraline
Letrozole	Sibutramine
Levobupivicaine	Sildenafil citrate
Lidocaine	Simvastatin
Loratadine	Sirolimus
Losartan	Sufentanil
Lovastatin	Tacrolimus
Methadone	Tamoxifen
Mibefradil	Temazepam
Miconazole	Teniposide
Midazolam	Terfenadine
Mifepristone	Testosterone
Mirtazapine (N-demethylation)	Tetrahydrocannabinol
Montelukast	Theophylline
Navelbine	Tiagabine
	Tolterodine
Toremifene	Vincristine
Trazodone	Warfarin (R-warfarin)
Tretinoin	Yohimbine
Triazolam	Zaleplon (minor pathway)
Troglitazone	Zatoestron
Troleandomycin	Zileuton
Venlafaxine (N-demethylation)	Ziprasidone
Verapamil	Zolpidem
Vinblastine	Zonisamide

CYP3A3/4	
Inducers	
Carbamazepine	Phenytoin
Dexamethasone	Primidone
Ethosuximide	Progesterone
Glucocorticoids	Rifabutin
Griseofulvin	Rifampin
Nafcillin	Rofecoxib (mild)
Nelfinavir	St John's wort
Nevirapine	Sulfadimidine
Oxcarbazepine	Sulfinpyrazone
Phenobarbital	Troglitazone
Phenylbutazone	
Inhibitors	
Amiodarone	Ketoconazole
Anastrozole	Metronidazole
Azithromycin	Mibefradil
Cannabinoids	Miconazole (moderate)
Cimetidine	Nefazodone
Clarithromycin	Nelfinavir
Clotrimazole	Nevirapine
Cyclosporine	Norfloxacin
Danazol	Norfluoxetine
Delavirdine	Omeprazole (weak)
Dexamethasone	Oxiconazole
Diethyldithiocarbamate	Paroxetine (weak)
Diltiazem	Propoxyphene
Dirithromycin	Quinidine
Disulfiram	Quinine
Entacapone (high dose)	Quinupristin and dalfopristin
Erythromycin	Ranitidine
Ethinyl estradiol	Ritonavir
Fluconazole (weak)	Saquinavir
Fluoxetine	Sertindole
Fluvoxamine	Sertraline
Gestodene	Troglitazone
Grapefruit juice	Troleandomycin
lndinavir	Valproic acid (weak)
lsoniazid	Verapamil
ltraconazole	Zafirlukast
	Zileuton

Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In : Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 8th ed. Hudson, OH; LexiComp Inc. 2000: 1364-1371

APPENDIX XVI: DATA SAFETY AND MONITORING PLAN

Monitoring The Progress Of Trials And The Safety Of Participants

All AMC protocols follow the Cancer Therapy Evaluation Program (CTEP) guidelines for reporting of adverse events. All adverse events that meet the expedited reporting requirements of the National Cancer Institute (NCI) are reported to the Investigational Drug Branch (IDB) of the NCI via the Adverse Event Expedited Reporting System (AdEERS) web application. All expedited adverse event reports are also required to be submitted to the local Institutional Review Board (IRB) of the reporting institution. If NCI holds the IND or no IND is required for a study, the AMC sites report serious adverse events directly to the AMC Operations and Data Management Center via AdEERS. In some instances, the AMC sites may report serious adverse events directly to the commercial sponsor holding the IND who will then in turn report to the AMC Operations and Data Management Center. However, it is preferred that sites report all serious adverse events via AdEERS with the Operations and Data Management Center forwarding a copy of the report to the sponsor.

The AMC Operations and Data Management Center provides a listing of serious adverse events to the Protocol Chair and Co-chair(s) for review on a regular basis. The AMC Operations and Data Management Center compiles these events in a tabular format and posts them on the password-protected section of the AMC Web site. The AMC Web site is accessible to all AMC investigators, co-investigators, and their staff. Email notification that this information is available on the web site will be sent to all site PIs. It is the responsibility of each site to provide this information to their respective IRBs, if required by their IRB. For blinded studies, the serious adverse events are reviewed and tabulated without treatment assignment.

Accrual summaries for each AMC trial are updated nightly on the password-protected section of the AMC Web page. The progress of each AMC trial is reviewed regularly by the Protocol Chair and also by the appropriate disease-oriented Working Group during scheduled conference calls. For phase I dose escalation trials, dose escalation (or dose de-escalation) is based on the rules in the protocol and the Protocol Chair and Group Statistician determine whether these criteria have been met. For phase II trials, stopping the trial for toxicity or efficacy, or suspending enrollment pending observation of responses in a multi-stage phase II trial, is based on meeting criteria stated in the protocol, and the Protocol Chair and Group Statistician determine whether these criteria have been met.

For phase III trials, the AMC has formed an independent Data Safety and Monitoring Committee (DSMC). Voting members of the DSMC are physicians, statisticians, and a patient advocate. All voting members are from outside the AMC. Non-voting members are the NCI scientific project officers and an NCI statistician. The AMC Data Safety and Monitoring Committee reviews AMC phase III studies in accordance with the National Cancer Institute's Policy for Data Safety and Monitoring. Confidential reports of all phase III trials are prepared by the AMC Group Statistician with support from the AMC Operations and Data Management Center. A written report containing the current status of each trial monitored, and when appropriate, any toxicity and outcome data, are sent to DSMC members by the AMC Operations and Data Management

Center allowing sufficient time for DSMC members to review the report prior to the meeting. This report addresses specific toxicity concerns as well as concerns about the conduct of the trial. The report may contain recommendations for consideration by the DSMC concerning whether to close the trial, report the results, or continue accrual or follow-up.

The results of each DSMC meeting are summarized in a formal report sent by the DSMC Chair to the Group Chair and Operations and Data Management Center within 1 week of the meeting. The DSMC report contains recommendations on whether to close each study reviewed, whether to report the results, and whether to continue accrual or follow-up. A primary recommendation (e.g., continue with no change; recommended or required modification; stop) must be included in the document. The Group Chair is then responsible for notifying the Protocol Chair and relevant Disease-oriented Working Group Chair before the recommendations of the DSMC are carried out. In the unlikely event that the Protocol Chair does not concur with the DSMC, then the NCI Division Director or designee must be informed of the reason for the disagreement. The Study Chair, relevant Disease-oriented Working Group Chair, Group Chair, DSMC Chair and NCI Division Director or designee will be responsible for reaching a mutually acceptable decision about the study. CTEP approval of a formal amendment will be required prior to any implementation of a change to the study.

Following a DSMC meeting, a summary of the serious adverse events reported to the DSMC is posted to the AMC Web page. It is each site's responsibility for conveying this information to its IRB.

Plans for Assuring Compliance with Requirements Regarding the Reporting of Adverse Events (AE).

For trials monitored by the NCI's Clinical Data Update System (CDUS), adverse event information is transmitted electronically to NCI on a quarterly basis. For trials monitored by NCI's Clinical Trials Monitoring Service (CTMS), adverse event information is transmitted electronically to NCI every two weeks.

Plans For Assuring That Any Action Resulting In A Temporary Or Permanent Suspension Of An NCI-Funded Clinical Trial Is Reported To The NCI Grant Program Director Responsible For The Grant

In the event that termination of the trial or major modification to the protocol is under consideration, the Protocol Chair will convene the AMC Data Coordinator and Disease-oriented Working Group Chair by conference call to discuss the options. For phase I and II trials, the Protocol Chair also has the option of asking the AMC DSMC to review the study. The AMC Operations and Data Management Center will inform the CTEP Protocol Information Office (PIO) when studies are temporarily or permanently closed. The Cancer Treatment and Evaluation Program (CTEP) of the National Cancer Institute (NCI) must approve all protocol amendments prior to distributing to the AMC sites.

Plans for Assuring Data Accuracy and Protocol Compliance

All study data for AMC clinical trials are entered directly by AMC site staff into AdvantageEDC^{SM} (a web-based data entry and enrollment system). During data entry, the system performs validation checks on many fields and performs consistency checks between select fields. Range checks are placed on each field to eliminate entry of out-of-range values. Edit check programs are run on the database on a set schedule to identify and resolve inconsistencies between forms or data collected at different points in time. AMC Operations and Data Management Center staff routinely interacts with site staff to resolve any data problems.

In accordance with NCI guidelines, the AMC Operations and Data Management Center conducts monitoring visits at the AMC sites to evaluate compliance with regulatory issues, and to review data for specific cases by checking source documents. These reports are sent to the site Principle Investigator and to the NCI. In the event that major violations are identified, sites are asked to provide a plan to correct deficiencies within 30 days. If needed, a repeat site visit is conducted. In the event that a site does not correct deficiencies in a pre-determined time frame, the AMC Executive Committee has the option of taking action against the site. Possible actions include, but are not limited to, suspending enrollment of new patients to AMC trials until deficiencies are corrected; recommending a decrease in funding to the site; and requiring specific training for site investigators or staff members.

APPENDIX XVII: NOVARTIS SAE COVERSHEET

SINA ID (internal use)

SAE REPORT – IIRP

Please fax to CS&E within 24 hours

Fax: 1-888-299-4565

(If you encounter problems with the fax transmission please call 1 (800) 882-6577)

CONTACT INFORMATION

From	Number of pages* (including fax cover sheet)
	*Please remember to include a copy of the MedWatch form
Phone Number	**Fax Number**

STUDY INFORMATION (please print)

Investigator Name		Trial Drug
Study/ Protocol Number		**Patient ID/ Number**
Novartis #	IRB #	

CASUALITY

Serious Adverse Event	Relationship to Study Drug	
	□ Possibly suspected	□ Not Suspected
	□ Possibly suspected	□ Not Suspected
	□ Possibly suspected	□ Not Suspected
	□ Possibly suspected	□ Not Suspected
	□ Possibly suspected	□ Not Suspected

WAS THIS REPORT SENT TO THE FDA? □ NO □ YES

Date _____/_____/_____
(day) (month) (year) use letter abv

_____ _____
INVESTIGATOR SIGNATURE Date