A Phase II Trial of Protease Inhibitor, Nelfinavir (NFV), Given with Definitive, Concurrent Chemoradiotherapy (CTRT) in Patients with Locally-Advanced, Human Papilloma Virus (HPV) negative, Squamous Cell Carcinoma of the Head and Neck

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Study Product:

Nelfinavir

^{18}F-FMISO

Protocol Number:

817878

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List of Abbreviations

Chemoradiotherapy:	CTRT
Computed Tomography:	CT
Head and neck squamous cell carcinoma	HNSCC
Nelfinavir:	NFV
Human Papilloma Virus:	HPV
Positron Emission Tomography	PET
Tumor to Muscle Ratio	TM

Study Summary

Title	A Phase II Trial of Protease Inhibitor, Nelfinavir (NFV), Given with Definitive, Concurrent Chemoradiotherapy (CTRT) in Patients with Locally-Advanced, Human Papilloma Virus (HPV) negative, Squamous Cell Carcinoma of the Head and Neck
Short Title	Definitive Nelfinavir with Chemoradiation for Head and Neck Cancer
Protocol Number	817878
Phase	Phase II
Methodology	Open Label
Study Duration	5 years
Study Center(s)	University of Pennsylvania; Philadelphia VA Medical Center
Objectives	To determine locoregional control in patients with locally advanced, HPV-negative head and neck cancer treated with definitive NFV + CTRT.
Number of Subjects	28
Diagnosis and Main Inclusion Criteria	Stage III, IVa, or IVb squamous cell carcinoma of the oral cavity, oropharynx, larynx, or hypopharynx with ECOG performance status 0-2
Study Product, Dose, Route, Regimen	Nelfinavir (Viracept®) 1250 mg By mouth two times a day Medicine should be taken with food, preferably high in fat. ^{18}F-FMISO 5-10 mCi Intravenous bolus injection
Duration of administration	Treatment to start 7-14 days prior to radiation and continued throughout radiation and standard chemotherapy.
Reference therapy	Combination of standard chemotherapy and radiation therapy
Statistical Methodology	This is a single arm, phase II study of nelfinavir (1250 mg given po bid) and concurrent chemoradiotherapy in locally advanced head and neck cancer. Locoregional control will be compared to published historical results. There will be a total of 28 patients enrolled on the study.

1 Introduction

This is a Phase II trial of definitive chemoradiotherapy (CTRT) given with the protease inhibitor, Nelfinavir (NFV), in patients with locally advanced head and neck cancer. Eligible patients will receive a "lead-in" period of Nelfinavir (1250 mg po bid) for 7-14 days prior to initiation of CTRT. Nelfinavir will then be given concurrently with platinum-based chemotherapy and radiation therapy (planned total dose of 70 Gy over 7 weeks).

All patients will also undergo the following:

1) ^{18}F-FMISO PET/CT: this scan assesses tumor hypoxia. Patients will undergo 2 separate ^{18}F-FMISO PET/CT scans: the first at baseline to determine baseline tumor hypoxia, the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor oxygenation secondary to NFV). Six patients will receive 3 separate ^{18}F-FMISO PET/CT, the first at baseline to determine baseline tumor hypoxia, the second also at baseline to determine reliability of the scan for test-retest purposes, the third after a 7-14-day lead-in of NFV.
2) ^{18}F-FDG PET/CT: this scan assesses glucose uptake. Patient will undergo 2 separate ^{18}F-FDG PET/CT scans: the first at baseline as part of routine clinical care, for purposes of radiation treatment planning and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor glucose uptake secondary to NFV).

The primary objective of the study will be to determine whether NFV, given with definitive CTRT, will improve locoregional control in locally advanced head and neck cancer.

Secondary objectives of this study include:
- To determine the effect of NFV on hypoxia, as assessed by ^{18}F-FMISO PET/CT.
- To determine the effect of NFV on glucose metabolism, as assessed by ^{18}F-FDG PET/CT, and possible associations between hypoxia and glucose metabolism.

1.1 Background

Radiation therapy is a commonly used treatment for cancer. Despite the use of sophisticated, state-of-the-art techniques, local-regional control is often not achieved in many cases, leading to recurrence. A case in point is locally advanced head and neck squamous cell carcinoma (HNSCC) cancer, which has a 5-year locoregional control rate of 33.7% following combined chemoradiation (1). In 2012 there were over 50,000 new cases of HNSCC with approximately 11,500 deaths (2). While the overall prognosis of HNSCC appears to be improving, this may be due to changing demographics with a higher percentage of cases associated with HPV, which appears to have a better prognosis than HPV-negative HNSCC (3).

Previous studies of hypoxic radiosensitizers have shown promise. In the TROG 02.02 phase III trial of definitive head and neck chemoradiation +/- tirapazamine, there was a trend favoring the tirapazamine arm for improved locoregional control in patients who were HPV-negative (5). For patients identified to have hypoxic disease via F-MISO PET scanning who did not receive tirapazamine, locoregional control was 38% at a median followup of 3.6 yrs, versus 95% in similar patients who received tirapazamine (6).

One approach for increasing the therapeutic window of radiotherapy is to combine radiation therapy with biological agents. Over the past few years, we have focused on agents that can modulate the tumor microenvironment to improve radiation response (7, 8). One particular

agent, nelfinavir, has been widely used in HIV patients with well-characterized pharmacokinetics and low toxicity profile. NFV inhibits the PI3K/Akt/mTOR pathway, which we believe increases the intrinsic radiosensitivity of cells (9-11). However, the effects of NFV on the in vivo radiation response appear to be greater than those seen in vitro, which led us to investigate whether the drug might have effects on the tumor microenvironment (TME) that could improve the radiation response. Indeed, we and others have found that NFV treatment of mice bearing tumor xenografts leads to improved oxygenation (10, 12). This is potentially clinically important because hypoxia is a factor associated with poor prognosis in many cancers, including HNSCC (13-15). Hypoxic cells require higher doses of radiation compared to oxic cells to achieve the same level of killing due to the requirement of oxygen to elicit maximal DNA damage through the oxidation of DNA and other target radicals (16). This leads us to one of our hypotheses that a major mechanism by which NFV improves the radiation response in vivo is by decreasing tumor hypoxia.

Determinants & Mechanisms of Nelfinavir Response

A phase I/II trial at Penn has been conducted in which NFV was combined with cisplatin-based (as with this protocol) chemotherapy and radiation for stage IIIA/B non-small cell lung cancer (17). The phase I trial showed that NFV given at a dose of 1250 mg (which was found to be the MTD) orally twice a day was well tolerated by these patients treated with chemoradiation. There were no dose limiting toxicities on this 16 patient trial, and no patients required dose attenuation of chemotherapy or nelfinavir. This trial did not test the idea than NFV can modulate tumor hypoxia or include hypoxia imaging. There are several methods that are available to measure hypoxia in human tumors including Eppendorf needle electrode and 2-nitroimidazole binding (18). 2-nitroimidazole drugs are reduced and form adducts with protein thiols in hypoxic cells (19).

1-H-1-(3-[18F] fluoro-2-hydroxypropyl)-2-nitroimidazole also known as [^{18}F]fluoromisonidazole or ^{18}F-FMISO is a positron emitting radiopharmaceutical that has been studied in *vivo* in humans for measurement of regional hypoxia in a number of tumor types with positron emission tomography (PET/CT). Grierson et al described synthesis methods that yielded high specific activity^{18}F-FMISO for use in PET imaging studies [Grierson 1989]. Fluoromisonidazole is a congener of misonidazole, which is an electron affinic drug with selective binding in cells with reduced pO_2 [Chapman 1981, 1983, Franko 1984]. An ideal hypoxia-imaging agent should distribute independently of blood flow, which is best achieved when the partition coefficient of the tracer is close to unity. Under these circumstances, imaging data can be acquired at a time when the intracellular tracer distribution has equilibrated with the tracer in plasma near the cells. ^{18}F-FMISO is an azomycin-based hypoxic cell sensitizer, because [^{18}F]FMISO partitions nearly equally between octanol and water, normoxic tissues have T/B (tissue/blood) pixel values of almost 1.0. The hypoxic part of a tumor can be characterized by the maximum T/B value or by the total number of pixels with T/B greater than some threshold. These pixels are summed and multiplied by the known mL/pixel to calculate hypoxic volume, (HV). Rasey et al characterized the binding characteristics of ^{18}F-FMISO in cells *in vitro* at various oxygen levels and in animal studies of tumors. Results demonstrated the oxygen dependent binding of radiolabeled fluoromisonidazole *in vitro* and *in vivo* and supported the use of ^{18}F-FMISO as a quantitative hypoxia probe using the measurement of retention of ^{18}F-FMISO in tumor vs blood and normal tissue to calculate hypoxic fraction in tumors [Rasey 1989]. ^{18}F-FMISO PET/CT provides a valuable non-invasive method to measure viable hypoxic cells in human malignancies, which may aid in better understanding mechanisms of resistance and response to treatments, such as radiation or anti-angiogenesis therapies.

The injectable dose of ^{18}F-FMISO for this study will be ≤ 10mCi (with an expected range of 5- 10 mCi at the time of injection) which has been shown to generate high quality PET/CT images with acceptable radiation dose to subjects, the drug mass injected for each study with be ≤ 15 µg.

In the current protocol we focus on the central hypothesis that NFV will result in a decrease in tumor hypoxia in head and neck tumors, and that this alteration in hypoxia plays a role in its ability to radiosensitize, leading to improved treatment outcomes. We also hypothesize that: 1) the effect of NFV on hypoxia can be assessed by ^{18}F-EF5 or by ^{18}F-FMISO,(the initial 15 subjects have been completed using ^{18}F-EF-5) and 2) changes in hypoxia will correlate with changes in glucose metabolism (decrease in glucose uptake), as assessed by ^{18}F-FDG PET/CT scanning.

Summary

We propose a clinical trial of the HIV protease inhibitor, nelfinavir, and concurrent, definitive chemoradiation in patients with locally advanced, HPV-negative head and neck cancer. This is an ideal population because of their poor prognosis with current therapy and the fact that HNSCC tumors are known to be hypoxic. Furthermore, activation of the EGFR/PI3K pathway is common in HNSCC, identified in 74% of cases in one report (30), so these tumors should respond to NFV. Assessment of tumor oxygenation response to NFV will be made using ^{18}F-FMISO PET/CT scanning and correlation of hypoxia with glucose metabolism will be assessed via ^{18}F-FDG PET/CT scanning. If the combination of NFV and radiation is found to be effective in this group of patients, it could then be extended to patients with other cancers.

2 Study Objectives

2.1 Primary Objective

To determine locoregional control for patients with locally advanced, HPV-negative head and neck cancer, treated with definitive NFV + CTRT and compare it to published historical results.

2.2 Secondary Objectives

2.2.1 To determine the effect of NFV on hypoxia as assessed by ^{18}F-FMISO PET/CT scanning.

2.2.2 To determine whether glucose metabolism (as assessed by ^{18}F-FDG PET/CT) is affected by NFV, and whether this corresponds to a decrease in ^{18}F-FMISO uptake.

3 Study Design

3.1 General Design

Twenty-eight subjects will be enrolled on this clinical trial investigating the efficacy of definitive NFV + CTRT for locally advanced head and neck cancer. Patients will also receive two ^{18}F-FMISO PET/CT studies: the first at baseline to determine baseline tumor hypoxia, the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor oxygenation secondary to NFV). . Additionally, patients will undergo 2 separate ^{18}F-FDG PET/CT scans: the first at baseline as part of routine clinical care, for purposes of radiation treatment planning and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor glucose uptake secondary to NFV). The research ^{18}F-FMISO PET/CT and ^{18}F-FDG PET/CT studies are **mandatory**, in order to participate on this clinical trial. Only under circumstances where the PI, Alexander Lin, deems the patient is unable to comply with the PET/CT requirements will its completion be excused and allow the patient to remain on the study.

3.2 Primary Study Endpoints

The intent of this protocol is to determine the efficacy of NFV + CTRT for locally advanced, HPV-negative head and neck cancer. Therefore, the primary endpoint will be locoregional control. However, secondary objectives will be (1) to determine the effect of NFV on hypoxia as assessed by ^{18}F-FMISO PET/CT imaging (the initial 15 subjects were imaged with ^{18}F-EF5) and (2) determine whether glucose metabolism (as assessed by ^{18}F-FDG PET/CT) is affected by NFV, and whether this corresponds to a decrease in ^{18}F-FMISO uptake. NFV responders have a better treatment outcome than NFV non-responders will be tested by comparing PFS between these groups. The definition of "NFV responder" requires greater knowledge about the distribution and variance of the baseline T:M ratio. For example, a decrease in T:M ratio to <1.25 or percent decrease from baseline of >50% may be reasonable definitions.

3.3 Secondary Study Endpoints

^{18}F-FMISO PET/CT

Patients will receive two hypoxia PET/CT scans with either ^{18}F-FMISO or ^{18}F-EF5 (initial 15 subjects were completed using EF-5, subsequent subjects will be imaged with FMISO): the first at baseline to determine baseline tumor hypoxia, and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor oxygenation secondary to NFV). Tumor regions of interest (ROIs), corresponding to the primary tumor site and pathologic lymph nodes will be drawn with the help of CT images. Tumor to muscle ratios (TMR) will be determined for each ROI. A TMR of $\geq$ 1.3 will be considered significant for hypoxia, and a reasonable starting point in which the outcome of the addition of NFV to CTRT can be evaluated. The ability of NFV to reduce tumor hypoxia will be tested by comparing pre and post-NFV hypoxia (i.e., ^{18}F-FMISO uptake expressed as a TMR) by Student's paired t-test.

^{18}F-FDG PET/CT

Patients will undergo 2 separate ^{18}F-FDG PET/CT scans: the first at baseline as part of routine clinical care, for purposes of radiation treatment planning and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor glucose uptake secondary to NFV). That

change in hypoxia will correlate with change in glucose uptake will be tested by Pearson's correlation.

That baseline hypoxia and glucose metabolism are predictive of treatment outcome will be tested by Cox regression analysis.

NCI Common toxicity grades will be employed.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

4.1.1 Patients ≥ 18 years old.

4.1.2 Histologically confirmed diagnosis of squamous cell carcinoma of the oral cavity, oropharynx, larynx, or hypopharynx AJCC 7th edition stages III, IVa, or IVb, p16-negative on immunohistochemistry (p16 testing to be performed only on oropharynx cancers, as it has not been found to be prognostic or standard of care outside of cancers of the oropharynx.

4.1.3 Determined by the treating physician to be a candidate for organ preserving, concurrent standard chemotherapy and radiation therapy to the head and neck with definitive intent.

4.1.4 ECOG Performance Status 0-2

4.1.5 Patients must sign an informed consent document that indicates they are aware of the investigational nature of the treatment in this protocol as well as the potential risks and benefits.

4.1.6 Ability to understand and the willingness to sign a written informed consent.

4.2 Exclusion Criteria *(All laboratory studies must be completed 1 month prior to signing consent)*

4.2.1 Prior radiation therapy (defined as more than 5 fractions of head and neck radiotherapy, less than 10 Gy total prior radiation) to the head and neck

4.2.2 Prior chemotherapy within the past 5 years

4.2.3 Previous therapy with a human immunodeficiency virus (HIV) protease inhibitor

4.2.4 Females who are pregnant at the time of screening will not be eligible for this study, urine pregnancy test will be performed at screening and prior to injection of FMISO in women of child-bearing potential.

4.2.5 Patients with known HIV disease. These patients have a high probability of treatment with anti-retroviral therapy which may interact with the nelfinavir.

4.2.6 Absolute Neutrophil Count ≤ 1500 per mm^3

4.2.7 Platelet count ≤ 100,000 per mm^3

4.2.8 Serum creatinine > 1.5 times the upper limit of normal

4.2.9 Serum AST or ALT > 2 times the upper limit of normal

4.2.10 Serum bilirubin > 1.2 mg/dl

4.2.11 Weight loss of > 10% over the past 6 months which is due to tumor wasting syndrome

4.2.12 Distant metastases

4.2.13 Patients receiving the following drugs that are contraindicated with NFV will be excluded.

Antiarrhythmics: amiodarone, quinidine	CONTRAINDICATED due to potential for serious and/or life threatening reactions such as cardiac arrhythmias
Antimycobacterial: rifampin	May lead to loss of virologic response and possible resistance to VIRACEPT or other coadministered antiretroviral agents.
Ergot Derivatives: dihydroergotamine, ergonovine, ergotamine, methylergonovine	CONTRAINDICATED due to potential for serious and/or life threatening reactions such as acute ergot toxicity characterized by peripheral vasospasm and ischemia of the extremities and other tissues.

Herbal Products: St. John's wort (hypericum perforatum)	May lead to loss of virologic response and possible resistance to VIRACEPT or other coadministered antiretroviral agents.
HMG-CoA Reductase Inhibitors: lovastatin, simvastatin	Potential for serious reactions such as risk of myopathy including rhabdomyolysis.
Neuroleptic: pimozide	CONTRAINDICATED due to potential for serious and/or life threatening reactions such as cardiac arrhythmias.
Proton Pump Inhibitors	Omeprazole decreases the plasma concentrations of nelfinavir. Concomitant use of proton pump inhibitors and VIRACEPT may lead to a loss of virologic response and development of resistance. **Patients that have been off of proton pump inhibitors for 14 days before enrollment will be allowed to participate.**
Sedative/Hypnotics: midazolam, triazolam	CONTRAINDICATED due to potential for serious and/or life threatening reactions such as prolonged or increased sedation or respiratory depression.

4.2.15 Patients receiving the following drugs will be clinically evaluated by the PI as to whether dosage/medication can and should be changed to permit patient on study:

Anti-Convulsants: carbamazepine, Phenobarbital	↓ nelfinavir	May decrease nelfinavir plasma concentrations. VIRACEPT may not be effective due to decreased nelfinavir plasma concentrations in patients taking these agents concomitantly.
Anti-Convulsant: phenytoin	↓ phenytoin	Phenytoin plasma/serum concentrations should be monitored; phenytoin dose may require adjustment to compensate for altered phenytoin concentration.
Anti-Mycobacterial: rifabutin	↑ rifabutin ↓ nelfinavir (750 mg TID) ↔ nelfinavir (1250 mg BID)	CONTRAINDICATED due to potential for serious and/or life threatening reactions such as acute ergot toxicity characterized by peripheral vasospasm and ischemia of the extremities and other tissues.
PDE5 Inhibitors: sildenafil, vardenafil, tadalafil	↑ PDE5 inhibitors	Concomitant use of PDE5 inhibitors and VIRACEPT should be undertaken with caution. If concomitant use of PDE5 inhibitors and VIRACEPT is required, sildenafil at a single dose not exceeding 25 mg in 48 hours, vardenafil at a single dose not exceeding 2.5 mg in 72 hours, or tadalafil at a single dose not exceeding 10 mg dose in 72 hours, is recommended.

HMG-CoA Reductase Inhibitors: atorvastatin, rosuvastatin	↑ atorvastatin ↑ rosuvastatin	Use lowest possible dose of atorvastatin or rosuvastatin with careful monitoring, or consider other HMG-CoA reductase inhibitors such as pravastatin or fluvastatin in combination with VIRACEPT.
Immuno-suppressants: cyclosporine, tacrolimus, sirolimus	↑ immuno-suppressants	Plasma concentrations may be increased by VIRACEPT.
Narcotic Analgesic: methadone	↓ methadone	Dosage of methadone may need to be increased when coadministered with VIRACEPT.
Oral Contraceptive: ethinyl estradiol	↓ ethinyl estradiol	Alternative or additional contraceptive measures should be used when oral contraceptives and VIRACEPT are coadministered.
Macrolide Antibiotic: azithromycin	↑ azithromycin	Dose adjustment of azithromycin is not recommended, but close monitoring for known side effects such as liver enzyme abnormalities and hearing impairment is warranted.
Inhaled/nasal steroid: fluticasone	↑ fluticasone	Concomitant use of fluticasone propionate and VIRACEPT may increase plasma concentrations of fluticasone propionate. Use with caution. Consider alternatives to fluticasone propionate, particularly for long-term use.
Antidepressant: trazodone	↑ trazodone	Concomitant use of trazodone and VIRACEPT may increase plasma concentrations of trazodone. Adverse events of nausea, dizziness, hypotension and syncope have been observed following coadministration of trazodone and nelfinavir. If trazodone is used with a CYP3A4 inhibitor such as VIRACEPT, the combination should be used with caution and a lower dose of trazodone should be considered.

4.2.16 Women of childbearing potential who have a positive result on screening urine pregnancy test.

4.2.17 Subjects with moderate-severe renal disease.

4.2.18 History of allergic reactions attributed to Flagyl (metronidazole), which has a chemical structure similar to FMISO.

4.2.19 Uncontrolled intercurrent illness including, but not limited to symptomatic congestive heart failure, unstable angina pectoris, or psychiatric

illness/social situations that would limit compliance with study requirements.

4.3 Inclusion of Women and Minorities

The University of Pennsylvania Medical Center serves the metropolitan Philadelphia area, the surrounding suburban counties, southern New Jersey, and northern Delaware.
Inclusion of minorities: The University of Pennsylvania Cancer Center reports that minorities accounted for 14% of all patients (adult and pediatric) enrolled on therapeutic clinical trials. Furthermore, it is estimated that approximately 30% of cancer patients admitted to the Hospital of the University of Pennsylvania are minorities (source: University of Pennsylvania Cancer Center Grant). Female and male patients of all ethnic groups will be eligible for treatment in these protocols. Protocol accrual will be reviewed annually to include a determination of minority representation. An attempt will be made to enroll patients with larynx cancer in a distribution that matches the frequency of incident larynx cancer in the general population. The minority accrual estimates for our trial are based upon the total number of patients, the incidence rates of head and neck larynx cancer, and estimates in the literature regarding minority accrual in clinical trials. A study by Tejeda and Brawley (31) found that the accrual of American cancer patients to NCI-sponsored treatment trials paralleled the incident disease burden among minorities. A report by Sikora et al. (32) indicates the distribution of incident head and neck squamous cell carcinoma to be 81.8%, 21.1%, 11.9%, 5.2%, 3.7%, 0.3%, and 0.8% among whites, all minorities, blacks, Asians, Hispanics, others and unknowns, respectively. The Philadelphia metropolitan area does not have a large American Indian or Alaskan population; therefore, no patients are estimated for this group.

Inclusion of women: Recruitment of patients will be through the University of Pennsylvania Cancer Center, Department of Radiation Oncology. All patients meeting study requirements will be approached for participation without discrimination. According to Cancer Facts and Figures (2011) from the American Cancer Society (33), new cases of larynx cancer were divided approximately 4:1 among male and female patients. Based on these and the above minority data, we estimate the accrual distributions for this trial shown below:

Ethnic Category	Males	Females	Total
Hispanic or Latino	1	0	1
Not Hispanic or Latino	21	6	27
Ethnic Category Total of All Subjects	22	6	28
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian or other Pacific Islander	0	0	0
Black or African American	3	1	4
White	18	5	24
Racial Categories: Total of All Subjects	22	6	28

Proposed Outreach Programs

The University of Pennsylvania Cancer Center has developed a number of minority outreach strategies. These include development of relationships with local community organizations, presentations or distribution of materials to local groups regarding trials, advertisements in minority newspapers and magazines, and presentations to professional organizations. If under-accrual of minority subjects is determined to be a problem, we will employ these methods to improve accrual. We will also make an effort to educate radiotherapists, surgeons, and medical oncologists who are working within our network and who serve minority populations about this trial.

4.4 Subject Recruitment and Screening

Subjects will be recruited from the departments of Medical Oncology and Radiation Oncology. The consent process can be completed in person or via Telemedicine per University of Pennsylvania Health Systems telemedicine policy and procedures.

4.5 Clinical Evaluation and Staging Criteria

- History and Physical Examination
- Laboratory studies: CBC with differential, Comprehensive metabolic panel, pregnancy tests for women of child bearing age or potential.
- Staging studies are to include a chest x-ray, chest CT, or FDG PET scan to evaluate for systemic metastases, performed within 12 weeks prior to study entry.

4.6 Off-Study Criteria

- Extraordinary Medical Circumstances. If at any time the constraints of this protocol are detrimental to the subject's health, the subject will be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.

- Subject's refusal to continue treatment. In this event, the reasons for withdrawal will be documented.

- Any patient who experiences any Grade III or higher toxicity related to ^{18}F-FMISO and not related to the underlying disease.

- Patients will be considered "off-study" at the completion of study procedures. This will occur at the completion of 3 month follow up PET/CT.

- Patients may be taken off study at any time at the discretion of the Principal Investigator

- Every effort will be made to follow subjects off study for toxicity and survival.

4.7 Study Duration

With an estimated accrual of 7-8 subjects per year, it is anticipated that accrual will continue for approximately 4 years. Patients will then be followed an additional 6 months prior to final statistical analysis.

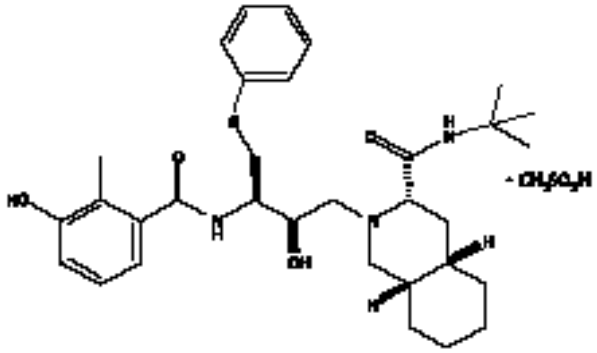
5 Study Drugs

5.1 Description

Nelfinavir

There are ample data available regarding the use of NFV in humans (34). The following is an excerpt of the key features.

VIRACEPT® (nelfinavir mesylate) is an inhibitor of the human immunodeficiency virus (HIV) protease. VIRACEPT Tablets are available for oral administration as a light blue, capsule-shaped tablet with a clear film coating in 250 mg strength (as NFV free base) and as a white oval tablet with a clear film coating in 625 mg strength (as NFV free base). Each tablet contains the following common inactive ingredients: calcium silicate, crospovidone, magnesium stearate, hypromellose, and triacetin. In addition, the 250 mg tablet contains FD&C blue #2 powder and the 625 mg tablet contains colloidal silicon dioxide. VIRACEPT Oral Powder is available for oral administration in a 50 mg/g strength (as NFV free base) in bottles. The oral powder also contains the following inactive ingredients: microcrystalline cellulose, maltodextrin, dibasic potassium phosphate, crospovidone, hypromellose, aspartame, sucrose palmitate, and natural and artificial flavor. The chemical name for nelfinavir mesylate is [3*S*-[2(2*S**, 3*S**), 3a,4ab,8ab]]-*N*-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinoline carboxamide mono-methanesulfonate (salt) and the molecular weight is 663.90 (567.79 as the free base). Nelfinavir mesylate has the following structural formula:



Nelfinavir mesylate is a white to off-white amorphous powder, slightly soluble in water at pH 4 and freely soluble in methanol, ethanol, 2-propanol and propylene glycol.

Pharmacokinetics

The pharmacokinetic properties of NFV were evaluated in healthy volunteers and HIV-infected patients; no substantial differences were observed between the two groups.

Absorption: Pharmacokinetic parameters of NFV (area under the plasma concentration-time curve during a 24-hour period at steady-state [AUC24], peak plasma concentrations [Cmax], morning

and evening trough concentrations [Ctrough]) from a pharmacokinetic study in HIV-positive patients after multiple dosing with 1250 mg (five 250 mg tablets) twice daily (BID) for 28 days (10 patients) and 750 mg (three 250 mg tablets) three times daily (TID) for 28 days (11 patients) are summarized in Table 1.

Table 1
Summary of a Pharmacokinetic Study in HIV-positive Patients with Multiple Dosing of 1250 mg BID for 28 days and 750 mg TID for 28 days

Regimen	AUC_{24} mg•h/L	C_{max} mg/L	C_{trough} Morning mg/L	C_{trough} Afternoon or Evening mg/L
1250 mg BID	52.8 ± 15.7	4.0 ± 0.8	2.2 ± 1.3	0.7 ± 0.4
750 mg TID	43.6 ± 17.8	3.0 ± 1.6	1.4 ± 0.6	1.0 ± 0.5

data are mean ± SD

The difference between morning and afternoon or evening trough concentrations for the TID and BID regimens was also observed in healthy volunteers who were dosed at precisely 8- or 12-hour intervals. In healthy volunteers receiving a single 1250 mg dose, the 625 mg tablet was not bioequivalent to the 250 mg tablet formulation. Under fasted conditions (n=27), the AUC and Cmax were 34% and 24% higher, respectively, for the 625 mg tablets. In a relative bioavailability assessment under fed conditions (n=28), the AUC was 24% higher for the 625 mg tablet; the Cmax was comparable for both formulations. In healthy volunteers receiving a single 750 mg dose under fed conditions, NFV concentrations were similar following administration of the 250 mg tablet and oral powder.

Effect of Food on Oral Absorption: Food increases NFV exposure and decreases NFV pharmacokinetic variability relative to the fasted state. In one study, healthy volunteers received a single dose of 1250 mg of VIRACEPT 250 mg tablets (5 tablets) under fasted or fed conditions (three different meals). In a second study, healthy volunteers received single doses of 1250 mg VIRACEPT (5 x 250 mg tablets) under fasted or fed conditions (two different fat content meals). The results from the two studies are summarized in Table 2 and Table 3, respectively.

Table 2
Increase in AUC, C_{max} and T_{max} for Nelfinavir in Fed State Relative to Fasted State Following 1250 mg VIRACEPT (5 x 250 mg tablets)

Number of Kcal	% Fat	Number of subjects	AUC fold increase	C_{max} fold increase	Increase in T_{max} (hr)
125	20	n=21	2.2	2.0	1.00
500	20	n=22	3.1	2.3	2.00
1000	50	n=23	5.2	3.3	2.00

Table 3
Increase in Nelfinavir AUC, C_{max} and T_{max} in Fed Low Fat (20%) versus High Fat (50%) State Relative to Fasted State Following 1250 mg VIRACEPT (5 x 250 mg tablets)

Number of Kcal	% Fat	Number of subjects	AUC fold increase	C_{max} fold increase	Increase in T_{max} (hr)
500	20	n=22	3.1	2.5	1.8
500	50	n=22	5.1	3.8	2.1

NFV exposure can be increased by increasing the calorie or fat content in meals taken with VIRACEPT. A food effect study has not been conducted with the 625 mg tablet. However, based on a cross-study comparison (n=26 fed vs. n=26 fasted) following single dose administration of NFV 1250 mg, the magnitude of the food effect for the 625 mg NFV tablet appears comparable to that of the 250 mg tablets. VIRACEPT should be taken with a meal.

Distribution: The apparent volume of distribution following oral administration of NFV was 2-7 L/kg. NFV in serum is extensively protein-bound (>98%).

Metabolism: Unchanged NFV comprised 82-86% of the total plasma radioactivity after a single oral 750 mg dose of 14C-NFV. *In vitro*, multiple cytochrome P-450 enzymes including CYP3A and CYP2C19 are responsible for metabolism of NFV. One major and several minor oxidative metabolites were found in plasma. The major oxidative metabolite has *in vitro* antiviral activity comparable to the parent drug.

Elimination: The terminal half-life in plasma was typically 3.5 to 5 hours. The majority (87%) of an oral 750 mg dose containing 14C-NFV was recovered in the feces; fecal radioactivity consisted of numerous oxidative metabolites (78%) and unchanged NFV (22%). Only 1-2% of the dose was recovered in urine, of which unchanged NFV was the major component.

Special Populations

Hepatic Insufficiency: The multi-dose pharmacokinetics of NFV have not been studied in HIV-positive patients with hepatic insufficiency.

Renal Insufficiency: The pharmacokinetics of NFV have not been studied in patients with renal insufficiency; however, less than 2% of NFV is excreted in the urine, so the impact of renal impairment on NFV elimination should be minimal.

Gender and Race: No significant pharmacokinetic differences have been detected between males and females. Pharmacokinetic differences due to race have not been evaluated.

[^{18}F]FMISO

Description

1-H-1-(3-[18F]fluoro-2-hydroxypropyl)-2-nitroimidazole also known as [^{18}F]fluoromisonidazole or [^{18}F]FMISO is a positron emitting radiopharmaceutical that has been studied in *vivo* in humans for measurement of regional hypoxia in a number of tumor types with positron emission tomography (PET/CT). Grierson et al described synthesis methods that yielded high specific activity ^{18}F-FMISO for use in PET imaging studies [Grierson 1989]. Fluoromisonidazole is a congener of misonidazole which is an electron affinic drug with selective binding in cells with reduced pO_2 [Chapman 1981, 1983, Franko 1984]. An ideal hypoxia-imaging agent should distribute independently of blood flow, which is best achieved when the partition coefficient of the tracer is close to unity. Under these circumstances, imaging can be done at a time when the intracellular tracer distribution has equilibrated with the tracer in plasma near the cells. ^{18}F-FMISO is an azomycin-based hypoxic cell sensitizer that has a nearly ideal partition coefficient and, when reduced by hypoxia, binds covalently to cellular molecules at rates that are inversely proportional to intracellular oxygen concentration, rather than by any downstream biochemical interactions. Rasey et al characterized the binding characteristics of ^{18}F-FMISO in cells *in vitro* at various oxygen levels and in animal studies of tumors. Results demonstrated the oxygen dependent binding of radiolabeled fluoromisonidazole *in vitro* and *in vivo* and supported the use of ^{18}F-FMISO as a quantitative hypoxia probe using the measurement of retention of ^{18}F-FMISO in tumor vs blood and normal tissue to calculate hypoxic fraction in tumors[Rasey 1989]. The drug is a clear solution that is provided at room temperature in a sealed, sterile, pyrogen-free vial

and has an expiration time of 6 hours. The injectable dose of ^{18}F-FMISO will be ≤ 10 mCi with an expected range of 5-10 mCi at the time of injection. In the dose of ^{18}F-FMISO, only a small fraction of the FMISO molecules are radioactive. There is no evidence that nonradioactive and radioactive FMISO molecules display different biochemical behavior.

Preparation of Study Drug (^{18}F-FMISO)

The manufacturing of ^{18}F-FMISO will occur in the Cyclotron Facility of the Department of Radiology at the University of Pennsylvania. This facility manufactures USP compliant radio-labeled compounds for human use on a daily basis. The drug manufacturing will be fully documented and controlled by a set of Standard Operating Procedures (SOPs) prepared and maintained by the University of Pennsylvania Cyclotron.

Study Drug Administration: ^{18}F-FMISO

The ^{18}F-FMISO dose will be drawn and activity measured in the dose calibrator, in the imaging facility. The dose will be administered by bolus injection to the patient under the direct supervision of a Nuclear Medicine Authorized User. The injected dose of ^{18}F-FMISO for this study will be ≤ 10 mCi (approximate range for most studies is anticipated to be 5-10 mCi). A lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User, complete imaging data could be generated. In the dose of ^{18}F-FMISO, only a small fraction of the FMISO molecules are radioactive. ^{18}F-FMISO is administered to subjects by intravenous injection. The injection and/or imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain.

5.2 Treatment Regimen

All subjects will begin taking daily oral nelfinavir, at a dose of 1250 mg bid, 7 to 14 days prior to the start of CTRT. At any point during the study, if a patient cannot tolerate swallowing NFV tablets or require PEG for medication administration, then the tablet can be crushed and dissolved in water for consumption orally or via PEG. Directions for dissolving NFV are as follows:

- Place Nelfinavir tablet(s) in small amount of water.
- Once dissolved, mix the cloudy liquid well, and consume it immediately.
- The glass should be rinsed with water and the rinse swallowed to ensure the entire dose is consumed.

Nelfinavir will be continued during the complete course of concurrent CTRT. The 7-14 day window of pre-CTRT nelfinavir is included to allow for imaging with [^{18}F]-FMISO PET/CT and [^{18}F]-FDG PET/CT evaluation to assess for changes in tumor oxygenation and glucose uptake

secondary to NFV. Subjects will be asked to maintain a drug diary to assess compliance with administration of nelfinavir.

5.3 Method for Assigning Subjects to Treatment Groups

The subjects will be identified for this study based on their inclusion/exclusion criteria. Since this is a phase II study, there is no assignment to treatment groups and all subjects are treated as per protocol.

5.4 Preparation and Administration of Study Drug

Nelfinavir will be available in Investigational Drug Services (IDS) at the Hospital of the University of Pennsylvania. When a subject is enrolled, the drug will be obtained from IDS and dispensed in the Department of Radiation Oncology. It is stored at room temperature.

Preparation and administration of [^{18}F]-FMISO is described in the CMC section.

5.5 Subject Compliance Monitoring

Subject medication compliance will be monitored via 2 mechanisms: 1) a medication diary for Nelfinavir. Subjects will be asked to complete and return the diary each week of their treatment. 2) via Epic med reconciliation (MD attestation of med reconciliation is recorded and tracked within Epic) at the patient's weekly visit with their treatment MD during the course of radiotherapy. If necessary, the subject will be counseled on medication issues. Patients who discontinue the use of Nelfinavir due to side effects will still be followed as a study subject as per the intent to treat analysis.

5.6 Prior and Concomitant Therapy

Any prior therapy is permitted with the exception of radiation therapy to the head and neck (defined as more than 5 fractions and more than 10 Gy) and any standard chemotherapy within the last 5 years.

5.7 Packaging

IDS will package the Nelfinavir specific to each subject in 30 day cycles.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

Nelfinavir will be bought and stored as needed by Investigational Drug Services.

[^{18}F]-FMISO will be synthesized at the cyclotron facility of the University of Pennsylvania and delivered to Department of Radiation Oncology in the Perelman Center for Advanced Medicine by a trained Cyclotron team member in single dose vials according to the standard procedures outlined by the Cyclotron Facility. Once the drug has been delivered to the

Department of Radiation Oncology, all standard hospital procedures will apply for handling, processing, and destruction of any residual amounts if applicable. As [^{18}F]-FMISO is a short-lived radiotracer with a half-life of approximately 110 minutes it will be synthesized for same day use, [^{18}F]-FMISO will not be stored. The remaining activity in the vials that exists in the lab will be stored only until the activity decays to undetectable level. All vials will be disposed of according to the standard operating procedure set forth by the hospital.

5.8.2 Dispensing of Study Drug

Nelfinavir will be dispensed by the research coordinator while the subject is in clinic.

The [^{18}F]FMISO dose will be drawn and accurately measured in the dose calibrator in the imaging facility and administered by bolus injection to the patient under the direct supervision of a Nuclear Medicine Authorized User. The injectable dose of [^{18}F]FMISO for most studies will be ≤ 10 mCi (expected range at injection 5 – 10 mCi), a lesser dose may be injected if, in the opinion of a Nuclear Medicine Authorized User complete imaging data could be generated. In the dose of [^{18}F]FMISO, only some fractions of the FMISO molecules are radioactive. [^{18}F]-FMISO is administered to subjects by intravenous injection of ≤ 10 mL. The injection will be followed by a saline flush. The injection or imaging procedure will be terminated in any patient who exhibits anaphylaxis, significant dyspnea or chest pain.

5.8.3 Return or Destruction of Study Drug

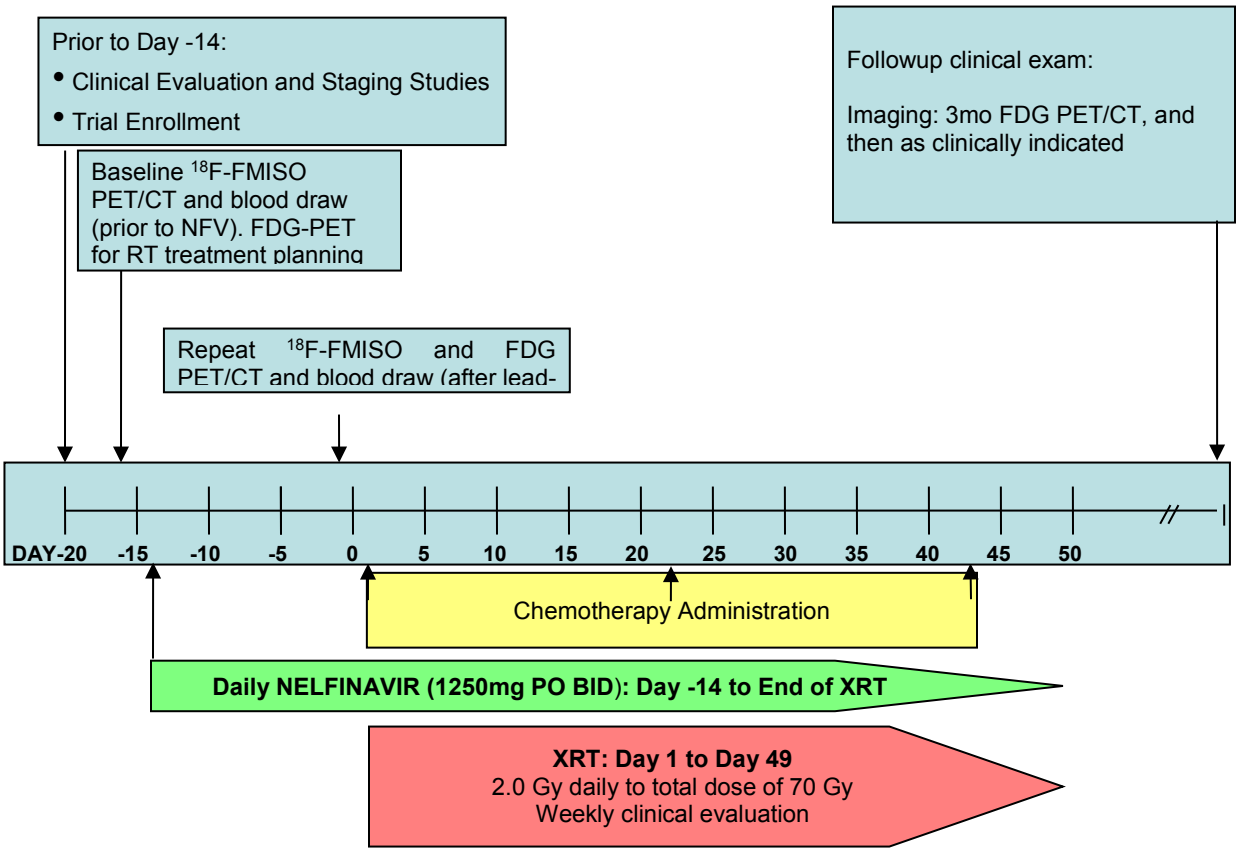
Nelfinavir: At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented by Investigational Drug Services.

[^{18}F]-FMISO: If the entire study drug dose is administered, the radioactive syringe will be disposed of into the radioactive waste in the Department of Radiation Oncology in the Perelman Center for Advanced Medicine. As the radioactive half-life of ^{18}F is 1.83 hours, we anticipate that by the end of a working day, the radioactive syringe can be safely disposed of in the standard biologic waste. In the event that the study drug is not administered or only partially administered, the radioactive syringe, vial and the contents will be disposed of in the radioactive waste in the Department of Radiation Oncology in the Perelman Center for Advanced Medicine. It will be subsequently disposed of in the standard biologic waste upon sufficient decay of its radioactivity.

6 Study Procedures

6.1 General study design

Twenty-eight subjects will be enrolled on this clinical trial investigating the efficacy of definitive NFV + CTRT for locally advanced head and neck cancer. Patients will also receive two ^{18}F-FMISO PET/CT studies: the first at baseline to determine baseline tumor hypoxia and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor oxygenation secondary to NFV). Patients will undergo 2 separate ^{18}F-FDG PET/CT scans: the first at baseline as part of routine clinical care, for purposes of radiation treatment planning and the second after a 7-14 day lead-in of NFV prior to CTRT (to determine change in tumor glucose uptake secondary to NFV). Additionally, blood and serum (two tubes, approximately 15 ml) will be collected for tumor biomarker analysis twice: once at baseline, and again after completion lead-in NFV, prior to CTRT.



Study Schema

6.2 Nelfinavir Administration

All subjects will begin taking daily oral nelfinavir, at a dose of 1250 mg bid, 7 to 14 days prior to the start of CTRT. Nelfinavir will be continued during the complete course of concurrent CTRT. The 7-14 day window of pre-CTRT nelfinavir is included to allow for imaging with ^{18}F-FMISO and ^{18}F-FDG PET/CT to assess for changes in tumor oxygenation and glucose uptake secondary to NFV. Subjects will be asked to maintain a drug diary to assess compliance with administration of nelfinavir. Subjects will also undergo weekly med reconciliation of compliance with taking NFV during their scheduled visit with their treating MD during radiotherapy (MD attestation of med reconciliation is recorded and tracked within EPIC).

6.3 Chemotherapy Administration

Standard cisplatin chemotherapy consisting of cisplatin will be administered as concurrent therapy with radiation. Cisplatin 100 mg/m2 will be administered on days 1, 22, and 43, with pretreatment and post treatment hydration and a polyantiemetic regimen. Patients may also be treated with either weekly cisplatin or weekly cetuximab, which is another FDA-approved drug shown to be an effective radiosensitizer in the treatment of head and neck cancer (36, 37). Patients who are receiving cetuximab may receive a loading dose, as per standard of care, prior to their completion of their repeat imaging, if deemed necessary by the treating physician. The treating medical oncologist may make adjustments to the chemotherapy regimen and/or dosing, as they deem medically appropriate. Development of severe dysphagia, dehydration, orthostasis, or other unforeseen grade 4 toxicity is potential grounds for discontinuation of treatment if therapy is delayed longer than 1 week to permit recovery. Should a holiday or hospital closing interfere with the above detailed chemotherapy administration schedule, the medical oncologist will adjust each subjects administration as they see medically appropriate.

6.4 Radiation Therapy

Radiation therapy in all subjects will begin 7 to 14 days after the initiation of nelfinavir.

Simulation for treatment is required. PET/CT-based treatment planning is required. Treatment will be delivered via intensity-modulated photon or pencil-beam proton radiotherapy. All fields will be treated every session. Interruptions in therapy should be discussed with the principal investigator but will be instituted at the discretion of the attending radiation oncologist.

The gross tumor volume, clinical treatment volume, and planning treatment volume are defined according to ICRU 50. Dose calculation should be performed using inhomogeneity corrections to account for differences in tissue density across the head and neck region. The total dose to gross disease will be 70 Gy. This will be administered in 2.0 Gy daily fractions. Regional lymph node regions may be treated, as dictated by the clinical situation. High-risk elective nodal regions would receive anywhere from 59.5 to 63 Gy, administered in 1.7-1.8 Gy daily fractions, while low-risk elective nodal regions would receive anywhere from 56 to 59.5 Gy, administered in 1.6- 1.7 Gy daily fractions. Treatment will be continuous, 5 days per week for 7 consecutive weeks.

Normal tissue doses: Dose-volume histograms should be performed for organs at risk, such as the spinal cord, brainstem, optic nerves and chiasm, pharyngeal constrictors, and mandible. The

maximal spinal cord dose should not exceed 45 Gy. The maximal dose to the brainstem, optic nerves and chiasm, should not exceed 54 Gy. The mean dose to the pharyngeal constrictors should not exceed 50 Gy. The maximal dose to the mandible (not included in the planning treatment volume) should not exceed 70 Gy.

6.5 ^{18}F-FMISO PET/CT Imaging

Two ^{18}F-FMISO PET/CT imaging scans to assess tumor oxygen level in response to treatment with Nelfinavir will be performed. Pregnancy tests will be performed prior to each scan. The first ^{18}F-FMISO PET/CT will occur shortly after enrollment, prior to administration of any therapy. The second will occur after a 7-14 day period of NFV alone.

The ^{18}F-FMISO PET/CT study will be conducted at the Imaging Services Center located in the Department of Radiation Oncology, Perelman Center for Advanced Medicine, University of Pennsylvania Medical Center. Subjects will receive ^{18}F-FMISO intravenously over a period of approximately a minute. Subjects will then lie on a table under the PET scanning machine for the procedure. The following is a timetable for the ^{18}F-FMISO PET/CT scan.

TIME (APPROXIMATELY)	SCHEDULE OF EVENTS
30 minutes before injection	Arrival at nuclear medicine facility, intravenous line placed: weight taken
0 minutes	Intravenous injection of [F18]-FMISO (5-10 mCi)
10 min prior to imaging	Empty Bladder
2-3 hours after injection	[F18]-FMISO PET/CT scan of the head and neck
20 min of imaging	Study Visit Complete

6.6 FDG PET/CT Imaging

Two ^{18}F-FDG PET/CT imaging scans will be performed. The first is part of routine clinical care, and for purposes of radiation treatment planning, the second is for research purposes, in order to determine the effect of NFV on glucose uptake.

The ^{18}F-FDG PET/CT study will be conducted at the Imaging Services Center located in the Department of Radiation Oncology, Perelman Center for Advanced Medicine, University of Pennsylvania Medical Center. S

6.7 Laboratory Monitoring

Blood and serum (one tube) will be collected twice for research biomarker, correlative studies. The first will be collected at baseline, prior to initiation of NFV, and the second after completion

of lead-in NFV. All subjects on study will be monitored with complete blood counts with differential and comprehensive metabolic panel at the discretion of the treating physicians. All other laboratory tests will be performed as clinically indicated.

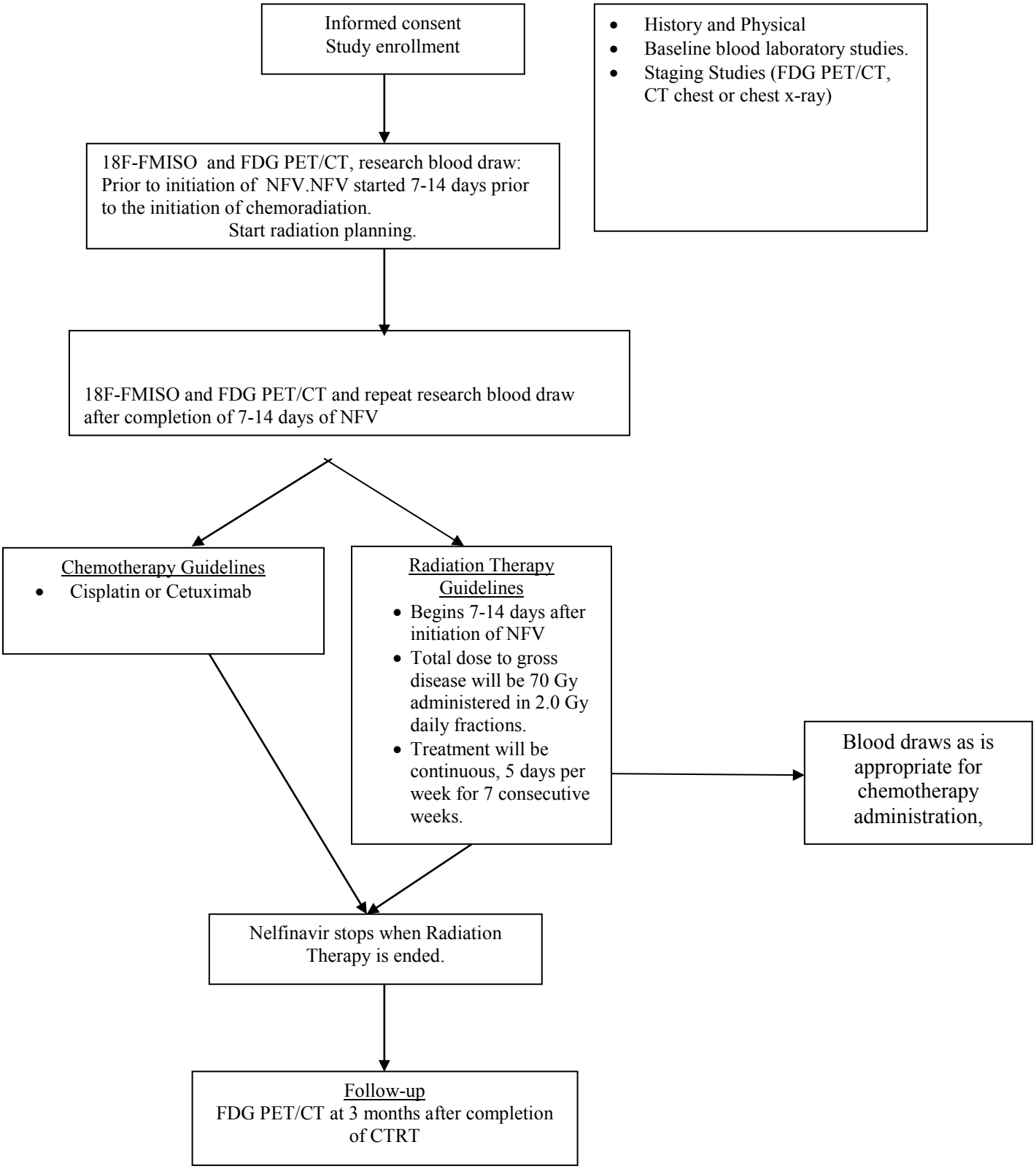
6.8 Study Visit Schedule

6.8.1 After the subject consents and is deemed eligible for the study, the study drug (NFV) will be dispensed and a baseline blood/serum draw (one tube) for research laboratory studies will be taken. Subjects will then be seen weekly during radiotherapy in the outpatient clinics to assess treatment-related toxicities. The following will be performed: (1) history and targeted physical examination and (2) laboratory evaluations as considered appropriate by study physicians.

6.8.2 FDG PET-CT scan will be performed at three months after the completion of radiotherapy as standard of care, in order to assess response to treatment, and for restaging purposes.

6.8.3 After discontinuation of nelfinavir therapy, follow-up including laboratory tests and radiographic studies will be scheduled as per standard of care. Toxicities will be evaluated up to the first standard of care follow-up visit after completion radiation, after which we will then follow for survival.

6.9 Study Visit Flowchart



7 Statistical Plan

A 28 patient phase II trial of NFV and concurrent chemo/RT with measurements of hypoxia by ^{18}EF-5 or FMISO PET imaging and serum biomarkers will be performed. Locoregional control is the primary endpoint, defined from first day of chemo/RT+NFV to local or regional progression. Distant progression and patients free of locoregional control will be censored. The "pre and post-NFV" measurements mentioned below, refer to samples obtained before and after a 7-14 day run-in period of NFV prior to chemo/RT. These pre/post-NFV changes are evaluated as "early change" predictors of treatment outcome. Hypotheses tested by statistical analyses are:

NFV given with chemo/RT will improve locoregional control (primary objective) will be tested by comparing our locoregional control to a historical result by log rank test with adequate power as described below. In a randomized pilot study by Rischin (JCO 24:2098-2104, 2006) in head and neck cancer, patients with hypoxic tumors who received concurrent chemoradiotherapy had a 3-year locoregiona8 control rate of 30%, while patients who received the hypoxia-activing pro-drug tirapazamine and concurrent chemoradiotherapy had a 3-year locoregional rate of 95%. Although the difference in local control was stunning, the sample sizes of these groups was very modest. Therefore, we have powered our study conservatively, in order to detect a more modest treatment effect.

Starting with this amendment, a total of 28 patients will be enrolled over 4 years, assuming a rate of 7 patients per year. To date, only 2 larynx cancer patient had been enrolled on this trial, so 4 years of accrual will be needed to meet the target sample size. We will then follow patients for an additional 6 months. There will be 85% power to detect a 30% absolute increase in 3-year locoregional control from a 40% rate for standard concurrent chemoradiotherapy to 70% for Nelfinavir and concurrent chemoradiotherapy, using a 1-sided log rank test at a 5% type I error rate. Power calculated with SWOG software (http://www.swogstat.org/stat/public/one_nonparametric_survival.htm).

NFV will reduce tumor hypoxia will be tested by comparing pre and post-NFV hypoxia (i.e., ^{18}EF-5 uptake expressed as a tumor to muscle (TMR) ratio) by Student's paired t-test. Transformation of the ratio may be necessary in order to meet the normality assumption of the t-test. Assuming an effect size (δ), in units of Std Dev of differences ($\delta = |\mu d|/\sigma d$), of $1/2\sigma d$, with 28 patients enrolled there is 82% power to detect the difference between pre/post-NFV values using a 1-sided paired t-test at a stricter 5% type I error, used to control false positive conclusions in our correlative analyses.

Baseline hypoxia, and glucose uptake are predictive of treatment outcome will be tested by an exploratory analysis using Cox regression. Each variable will be tested in a univariate survival model to assess association with locoregional control. The hazard ratio, 95% confidence interval and statistical significance will be determined for each variable. Exploratory analyses will also evaluate differences between Nelfinavir responders and nonresponders. The definition of "NFV responder" requires greater knowledge about the distribution and variance of the baseline TMR. For example, a decrease in TMR to <1.25 or percent decrease from baseline of >50% may be reasonable definitions. This exploratory analysis yields estimates of treatment outcomes within response groups.

7.1 Dose modifications of Nelfinavir and Chemotherapy during Radiotherapy

7.1.1 Hematologic toxicities

Because Grade III hematologic toxicity occurs in approximately 50% of patients receiving combined chemoradiotherapy alone for head and neck cancer and no hematologic toxicity was seen in phase I/II trials of nelfinavir in the setting of HIV patients (34), the dose modifications for hematologic toxicity will be reduction in standard chemotherapy dose. , as per the treating medical oncologist. Hematologic toxicities are expected and will not be followed as part of this trial. They will be managed by the treating medical oncologist.

7.1.2 Non-hematologic toxicities

Modifications in dose, frequency, and regimen of chemotherapy will be made for non-hematologic toxicity (such as hearing loss or rise in creatinine to > 1.5x normal value). Nelfinavir will be held if during treatment, grade 4 mucositis, dysphagia, or odynophagia develops.

Regardless of cause, nelfinavir must be immediately held if during treatment, Grade $\geq$ III hepatic function impairment develops as characterized by:

- Total bilirubin > 3x ULN (upper limit of normal of local lab; corresponds to approx 2.7 mg/dl or 45.9 micromol/l) OR
- AST/ALT > 5x ULN

AST, ALT, and total bilirubin must be verified no later than one week after the first assessment, and weekly thereafter until resolution to grade $\leq$ I.

Dose administration must be held until recovery to grade $\leq$ I for all three hepatic parameters, at which time dosing of nelfinavir can resume.

7.2 Correlative Laboratory Studies-Molecular Markers

All patients will have research blood samples drawn for biomarker analysis (prior to NFV therapy and again after lead-in).

8 Safety and Adverse Events

8.1 FMISO IND Investigator Reporting

Notifying the Radiology IND Support Office:

The Principal Investigator or designee will seek information from the subject in reference to any adverse events specific to imaging procedures or injection of the Investigational Agent that may have occurred, by specific questioning and, as appropriate, by examination.

Only events surrounding PET/CT study procedures and the Investigational Radiopharmaceutical Agent outlined in this protocol apply for reporting for the IND. Any treatment related events outlined in this protocol or otherwise will not

be collected or reported as part the imaging protocol and should be processed per the protocol and institutional policies.

It is the responsibility of the Principal Investigator to determine the grade, attribution and expectedness of the event.

Once an event surrounding study procedures and/or the Investigational Agent has been identified the PI should be contacted immediately to complete the AE & SAE Case Repot Form.

- For the events in correlation to the Investigation Agent the event should be recorded on the AE & SAE Case Repot Form if:

  - Record all events regardless grade, attribution or expectedness if the event occurred within 24 hours after the injection of FMISO.

- For the events in correlation to study procedures, the event should be recorded on the AE & SAE Case Repot Form if:

  - Record all events regardless grade, attribution or expectedness.

A copy of the completed AE & SAE Case Repot Form should be sent to the Radiology IND Support Office within 24 hours of knowledge for review by the IND Authorized Sponsor Representative.

The completed AE & SAE Case Repot Form should be placed in the subject's chart and a copy placed in the Regulatory Binder (this is recommended for the preparation of the FDA IND Annual Report and IRB Continuing Review).

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the investigational agent or study procedure is not the cause of the event.

Significant new information on ongoing adverse events should be provided promptly to the Radiology IND Support Office.

Notifying the Penn IRB

The Penn IRB requires Principal Investigators to submit IRB required reports within 10 working days from the time the investigator becomes aware of the event. The event must meet the following requirements:

- Report events in correlation to the Investigational Agent if:
  - The event occurred within 24 hours after the injection of FMISO.

- The event is grade 3 or higher, unexpected and the event is possibly, probably or definitely related to the Investigational Agent.

➢ Report events in correlation to study procedures if:

- The event is grade 3 or higher, unexpected and the event is possibly, probably or definitely related to a study procedure.

If the event involved a death and indicates that participants or others are at risk of increased harm, investigators should report within 3 days.

Follow instructions for report completion on the IRB website.

All other events will be reported at the time of Continuing Review.

Copies of each report and documentation of IRB notification and receipt will be sent to the Radiology IND Support Office and kept in the Clinical Investigator's study file.

Notifying the Cancer Center DSMC (Cancer Research Studies Only)

AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for reporting to the DSMC:

➢ Report events in correlation to the Investigational Agent within 10 working days of knowledge if:

- The event occurred within 24 hours after the injection of FMISO.
- All grade 3 or higher events regardless of attribution or expectedness.

➢ Report events in correlation to study procedures within 10 working days of knowledge if:

- All grade 3 or higher events regardless of attribution or expectedness.

If the event involved an unexpected death and indicates that participant or others are at risk of increased harm, investigators should report within 2 working days.
All other deaths within 30 days of knowledge. Deaths of subjects greater than 90 days from the last study treatment/intervention are not reportable unless a longer time frame is specified in the protocol.

Follow instructions for report completion on the Cancer Center website.

Copies of each report and documentation of Cancer Center DSMC notification and receipt will be sent to the Radiology IND Support Office and kept in the Clinical Investigator's study file.

Sponsor Reporting

Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND Safety Reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

Events in correlation to the Investigational Agent:

Report within 7 calendar days

- The event occurred within 24 hours after the injection of FMISO.
- Associated with the use of the study drug.
- Unexpected.
- Fatal or life-threatening.

Report within 15 calendar days

- The event occurred within 24 hours after the injection of FMISO.
- Associated with the use of the study drug.
- Unexpected.
- Serious, but not fatal or life-threatening.

-OR-

- A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

- Suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Notifying Participating Investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Additional Sponsor Reporting Requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Other IRB Reportable Events:

- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency, for example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- A breach of confidentiality.
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.
- **Exceptions:** A study exception is a one time, intentional action or process that departs from the IRB and CTSRMC approved study protocol, intended for one occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, advance documented IRB and DSMC approval is required. For this in-house study with a sponsor monitor, approval will be obtained from the study sponsor (IND Support Office) prior to submitting the exception request to the IRB and DSMC.
- **Deviations:** A study design deviation is a one time, unintentional action or process that departs from the IRB and CTSRMC approved study protocol,

involving one incident and identified retrospectively, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation will be reported to the DSMC and IND Support Office within 5 business days and the IRB within 10 business days.

8.2 ^{18}F-FMISO and FDG PET/CT

Pregnancy Clause

The effects of ^{18}F-FMISO on unborn children, children who are breast-feeding and the health of the mother are unknown. For this reason, subjects who are pregnant must inform the investigator(s) and understand that she will not be included in the study. Women of childbearing age will have a pregnancy test before making a decision about participating in the study. The cost of the exam is part of regular cancer care and may be done even they do not join the study.

IV Contrast Risks & Procedures Clause

There is a risk that multiple needle-sticks will be necessary in order to ensure proper placement of the Intravenous line. There is a small risk of infection at the site the catheter is placed or there may be a small amount of pain or bruising associated with the placement of the Intravenous catheter.

8.3 Nelfinavir Reporting

Notifying the Penn IRB

The Penn IRB requires Principal Investigators to submit IRB required reports within 10 working days from the time the investigator becomes aware of the event. The event must meet the following requirements:

Report events in correlation to Nelfinavir if the event is grade 3 or higher, unexpected and the event is possibly, probably or definitely related to Nelfinavir.

Report events in correlation to study procedures if the event is grade 3 or higher, unexpected and the event is possibly, probably or definitely related to a study procedure.

If the event involved a death and indicates that participants or others are at risk of increased harm, investigators should report within 3 days.

All other events will be reported at the time of Continuing Review.

Notifying the Cancer Center DSMC (Cancer Research Studies Only)
AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for reporting to the DSMC:

Report events in correlation to Nelfinavir within 10 working days of knowledge for all grade 3 or higher events regardless of attribution or expectedness.

Report events in correlation to study procedures within 10 working days of knowledge of all grade 3 or higher events regardless of attribution or expectedness.

If the event involved an unexpected death and indicates that participant or others are at risk of increased harm, investigators should report within 2 working days.

All other deaths within 30 days of knowledge. Deaths of subjects greater than 90 days from the last study treatment/intervention are not reportable unless a longer time frame is specified in the protocol.

8.3.1 Pregnancy Clause

Because of the effects of Nelfinavir, there could be serious harm to unborn children or children who are breast-feeding. These effects could also harm the mother. It is also possible that harmful side effects that are not yet known could happen to both the mother and unborn or breast-feeding child. If you are currently pregnant, it is important that you inform the investigator(s) because you will not be able participate in the study. If you are able to become pregnant, you will be given a urine pregnancy test before entry into the study. You are asked to use a medically accepted method of birth control (such as condoms, vasectomy, abstinence) while you participate in the study. You should not become pregnant while you are taking this drug. If you do become pregnant, you must discontinue the drug, tell the investigator and consult an obstetrician or maternal-fetal specialist.

If you are a man, you are advised to use a means of birth control (such as condoms, abstinence, vasectomy) while you are taking part in this study because the effect of treatment on your sperm or upon the development of an unborn child is not known. You should not father any children while on this study.

9 Data Handling and Record Keeping

9.1 Records

All patients must have a signed Informed Consent Form and an On-study (confirmation of eligibility) form filled out and signed by a participating investigator prior to entering the study.

Confidential research charts will be kept in locked cabinets at each participating institution. Subjects will be assigned a study number at the time of study enrollment. This study number and not the subject's name will be used on all case report forms.

HIPAA Compliance:

Patients will be asked to read and sign a consent form that includes confidentiality information acknowledging the uses and disclosures of protected health information (PHI) in this study as required by The Health Insurance Portability and Accountability Act (HIPAA). PHI will not be shared with any outside institution except as required by law. Any reporting of the results of this study will be done only with de-identified patient data. Confidentiality will be protected as outlined below.

- Each subject will sign a study informed consent and a study-specific HIPAA authorization form prior to surgery.
- Each subject will be assigned a study number. All research-related material (to include specimens for research) will be labeled with the subject study number and the subject's initials.
- A list of the subject names with the associated subject numbers will be maintained in a locked cabinet and computer by the principal investigator and study coordinator.
- All research subject records will be kept in a study chart.
- An electronic database will be maintained. No subject names will be used in this database. Study numbers will be used. Only data which constitutes a limited data set (as defined by the University of Pennsylvania Health System in the HIPAA Privacy Education website) will be used.

9.2 Data Entry

All patients must have a signed Informed Consent Form and an On-study (confirmation of eligibility) form filled out and signed by a participating investigator prior to entering the study.

A case report form will be used to standardize data keeping and allow entry to a computerized data base.

9.3 Flow of Subject Data

Study entry: Each patient will be evaluated for this study based upon the inclusion and exclusion criteria. If a patient is found to be eligible for the study, an eligibility checklist will be completed after the patient has signed an informed consent form. The patient will be assigned a study number. A list of the subjects with the assigned study numbers will be kept on a confidential computer file in the Department of Radiation Oncology, University of Pennsylvania.

Treatment: After study enrollment, the subject will undergo treatment planning for chemoradiotherapy as per standard clinical care. The subject will begin oral nelfinavir 7-14 days prior to the initiation of radiotherapy. The principal or a co-investigator will fill out a radiotherapy prescription form as per routine clinical care. The investigator will also complete a

prescription form for nelfinavir. The study coordinator will double-check the nelfinavir prescription.

Imaging: Copies of all imaging for these studies will be maintained by the Department of Radiology, Division of Nuclear Medicine, at the University of Pennsylvania.

Follow-up: The clinical status and laboratory results of the subjects will be recorded on a flow sheet and on the CRF. Toxicity forms will be maintained for each subject and will be evaluated up to the first standard of care follow-up visit after completion of radiation. The principal investigator will verify the grades and attribution of the toxicities for each subject. Subjects will be followed for survival for the remainder of the follow-up.

10 Data and Safety Monitoring Plan

10.1 Overview

The University of Pennsylvania Cancer Center (UPCC) has a formal plan for Data Safety and Monitoring of Clinical Trials. The clinical trial, "A Phase II Trial of Protease Inhibitor, Nelfinavir, Given with Definitive, Concurrent Chemoradiotherapy in Patients with Locally-Advanced, Human Papilloma Virus negative, Squamous Cell Carcinoma of the Head and Neck" is a trial that is subject to oversight of the UPCC through the Data and Safety Monitoring Committee (DSMC). DSMC role is to ensure that the rights and well-being of all subjects are protected and that subjects are treated in full compliance with the study treatment and parameters specified in the protocol. The DSMC is responsible for overseeing the process of monitoring of studies and the conduct of audits. The investigators on this study are responsible for the continuous, close monitoring of subjects enrolled on this trial.

10.2 Study Audits

This study will be audited by the Department of Compliance and Monitoring on behalf of the ACC Data and safety monitoring Committee every six months from the first subject enrolled and every six months thereafter for the life of the study. The principal investigator will be notified in advance of the selection of their protocol for review. Three randomly selected patients or 10% of the total accrual, whichever is higher will be audited. A written report is provided to the principal investigator following this audit. Any rating less than satisfactory would warrant a repeat full review at the time of the next scheduled audit or sooner, depending upon the extent of the deficiencies found. Substantial protocol deviations will be reported to the Director of the Cancer Center and the Associate Director for Clinical Research for consideration of appropriate administrative action, such as suspending accrual to the protocol.

10.3 Study Monitoring

This trial will be monitored by the Principal Investigator and the research coordinator.

10.4 ***Study Monitoring for IND 139443 - Radiology IND Support Office***

The transformation of the PET/CT from the radiotracer EF5 to FMISO will change the oversight of the PET/CT radiotracer IND. FDA IND 139443 (FMISO) is managed by the Department of Radiology IND Support Office (INDSO). The INDSO will accept all monitoring and auditing of the study as defined in the protocol and as carried out as so far in the study. The Principle Investigator or designee will promptly forward all reports related to monitoring and/or auditing to the INDSO for review. The INDSO and Authorized Sponsor Representative will review reports and assess the need to schedule monitoring visits by the INDSO as needed.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through the National Institutes of Health, via grant 1R01CA174976-01A1.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the University conflict of interest policy.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be

published or passed on to any third party without the consent of the study sponsor (PI). Any investigator involved with this study is obligated to provide the sponsor (PI) with complete test results and all data derived from the study.

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