A Pilot Trial Assessing the Feasibility of Delivering Topical MTS-01 to Reduce Dermatitis in Patients Receiving Intensity Modulated Radiation with Concurrent 5-Fluoruracil and Mitomycin-C for Stage I-III Carcinoma of the Anal Canal

Abbreviated Title: MTS-01 to prevent radiation dermatitis

Principal Investigator: *Deborah Citrin, MD ROB/CCR/NCI

Lead Associate Investigator: *Thomas Uldrick, MD, MS HAMB/CCR/NCI

Associate Investigators:

*Kevin Camphausen, MD	ROB/CCR/NCI
*Aradhana Kaushal, MD	ROB/CCR/NCI
*Tim Greten, MD	MOB/CCR/NCI
*Austin Duffy, MD	MOB/CCR/NCI
* DeeDee Smart, MD, PhD,	ROB/CCR/NCI
Luz, Giordano, PA	ROB/CCR/NCI
Sharon Smith, RN, MS	ROB/CCR/NCI
Debbie McNally, RN	ROB/CCR/NCI
Frank Maldarelli, MD PhD	DRP/CCR/NCI
Robert Yarchoan, MD	HAMB/CCR/NCI
Joana Shih, PhD,	BRB/DCTD/NCI
Kathleen Wyvill, RN	HAMB/CCR/NCI
Karen Aleman, RN	HAMB/CCR/NCI
Heidi Kong, MD	DB/CCR/NCI
Judith Starling	CC Pharmacy

*** Clinical Investigators who may consent patients**

Drug Manufacturer: Mitos Pharmaceuticals, Inc.
Three San Joaquin Plaza
Newport Beach, CA 92660
IND Sponsor: Deborah Citrin, M.D.
IND number: 112272

PRECIS

Background:

- Patients with non-metastatic carcinoma of the anal canal are treated with concurrent mitomycin C (MMC), 5-fluorourcil (5-FU), and radiotherapy (RT) in the curative setting in an attempt to preserve the anal sphincter.
- Radiation dermatitis is a uniform complication of this therapy which frequently results in treatment delay due to pain and discomfort. High grade dermatitis may also become super infected in the setting of decreased blood counts from chemotherapy and diarrhea from radiation proctitis, further delaying therapy. Approaches that decrease toxicity may be particularly important in patients infected with HIV.
- MTS-01 (tempol, *4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl*) is a piperidine nitroxide known to act as a chemical radioprotector with selective protection of normal versus tumor tissue.
- Tempol gel (tempol 70 mg/mL plus water, ethanol, and hydroxypropyl cellulose) has been evaluated as a topical radioprotector in pilot trials that included a variety of sites.

Objectives:

- Primary Objective: To determine the safety and tolerability of topical MTS-01 on a daily basis prior to irradiation in the groin and gluteal cleft of patients receiving combined therapy with MMC, 5-FU, and RT for carcinoma of the anal canal.
- Secondary Objectives will include evaluation of the following endpoints in a preliminary fashion:
  - To describe the rates and severity of skin toxicity in patients treated with this regimen
  - To describe the need for toxicity related treatment breaks with this regimen
  - To describe the opiate requirements in patients treated with this regimen
  - To describe 12-month progression-free survival, disease-free survival, and overall survival in patients treated with concurrent chemotherapy, radiation therapy, and MTS-01
  - Evaluate the effects of antiretroviral therapy, 5-fluorouracil, mitomycin C, and radiation on low level persistent HIV viremia and HIV genetic diversity during therapy and recovery
  - To evaluate the feasibility of collecting HIV RNA and mononuclear cells from rectal associated lymphoid tissue for correlative studies
  - Collect and store anal cytology and core needle biopsies of tumor for future HPV and tumor based analyses

Eligibility:

- Age >18 years.
- ECOG performance status ≤ 2.
- Histologically confirmed carcinoma of the anal canal without evidence of distant metastases
- No contraindications to definitive chemoradiotherapy for carcinoma of the anal canal

Design:

This is a pilot trial of topical MTS-01 in patients receiving MMC, 5-FU, and IMRT for definitive management of carcinoma of the anal canal. Fifteen patients will be enrolled. MMC will be delivered at a dose of 10mg/m^2 on days 1 and 29. 5-FU will be delivered as 1000mg/m^2/day as 96 hour continuous infusion beginning on day 1 and 29. RT will be delivered to a total dose of

50-54 Gy based on tumor characteristics. Tempol gel will be applied to the bilateral groins and the gluteal cleft, avoiding a 3 cm radius from the anal verge, immediately prior to each fraction of RT. RTOG grading will be used to evaluate skin toxicity in both the groin and gluteal cleft weekly during treatment and at 4 weeks, 3 months and 6 months after completion of treatment. The duration of treatment, number of treatment breaks, opiate requirements, and level of pain will be evaluated weekly during treatment and at 4 weeks and 3 months after the completion of treatment. Disease control will be assessed at 4 weeks, 3 months, 6 months, 9 months, and 12 months of follow-up.

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1 INTRODUCTION

1.1 Study Objectives:

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    - To describe the rates and severity of skin toxicity in patients treated with this regimen
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    - To evaluate the feasibility of collecting HIV RNA and mononuclear cells from rectal associated lymphoid tissue for correlative studies
    - Collect and store anal cytology and core needle biopsies of tumor for future HPV and tumor based analyses

1.2 Background and Rationale:

1.2.1 Carcinoma of the Anal Canal

Anal carcinoma had an estimated incidence of 5,290 cases with 710 deaths in the United States in 2009.[1] Although anal cancer is considered rare, there is an increasing incidence which may be related to a higher prevalence of risk factors such as Human Papilloma Virus Infection (HPV) and Human Immunodeficiency Virus (HIV) infection. The annual incidence of anal cancer in patients with HIV in the US is much higher than that of the general population, and has been increasing since 1996, when combination antiretroviral therapy (cART) that could effectively suppress HIV replication was first widely used. Recent estimates of incidence in patients with HIV range from 10 - 128 per 100,000, 2000-2005 point estimates range from 70-80 per 100,000 in large studies linking US Cancer- and HIV registries (Figure 1.) [2-4]. The increasing incidence is thought to be due to chronic immunosupression in the setting of high rates of co-infection with HPV, and in the absence of data to show the efficacy of screening and treating premalignant lesions, lack of comprehensive anal cancer prevention programs.

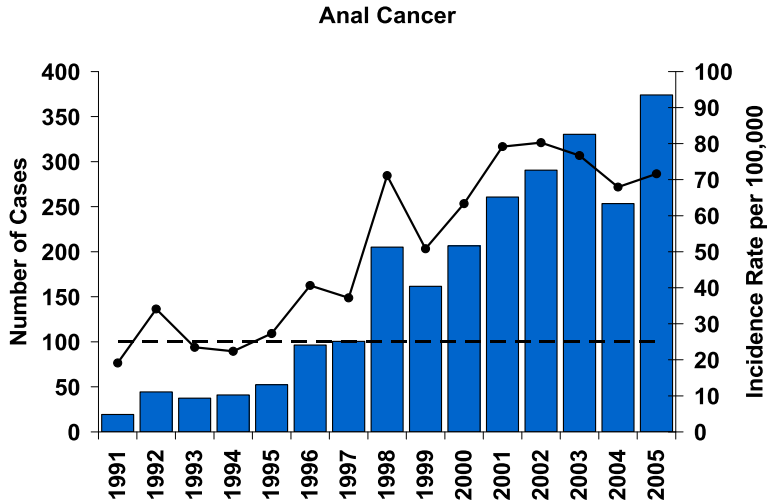
Anal carcinomas were historically treated with abdominoperineal resection (APR). APR resulted in local control rates of 40-70% but also resulted in significant morbidity, primarily due to loss of sphincter function. Nigro first reported the use of chemotherapy combined with radiation for the treatment of anal cancer.[5] Since that time combined chemotherapy and radiation have been evaluated in numerous randomized trials and consistently yield long term colostomy free survival rates of 70-75% and disease free survival rates of approximately 60-75%.[6-8] The current standard therapy includes radiation to the pelvis, primary tumor, and inguinal lymph nodes delivered concurrently with 5-Fluorouracil and Mitotmycin-C. (NCCN)

The ability to preserve the anal sphincter with combined chemoradiation approaches comes at the cost of high rates of acute toxicity. In the RTOG 87-04 study which included Mitomycin-C, 5-FU, and standard radiotherapy, Grade 4-5 acute toxicity occurred in 23% of patients. IN RTOG 98-11 which also included a similar treatment arm, 87% of patients experienced grade 3-4 acute toxicity.[8] These toxicities include rectal toxicity (proctitis), urinary bladder toxicity (cystitis), hematologic toxicity, and dermatitis. These toxicities frequently lead to treatment breaks which prolong and potentially decrease the efficacy of therapy.[9-12]

Intensity modulated radiation therapy (IMRT) has been investigated as a means to reduce the toxicity of chemoradiotherapy approaches for patients with anal carcinoma. IMRT allows the delivery of more conformal radiation treatment plans that can preferentially spare normal tissue from high doses of radiation. A number of studies evaluating the efficacy of IMRT in this setting have been published to date. Local control with IMRT techniques are comparable to those obtained with conformal or 2-Dimensional approaches while toxicities are less frequent or less severe.[13-16]

Skin toxicity is one of the major toxicities observed in patients treated with chemoradiation for anal carcinoma. The perineal, perianal, genital, and inguinal regions are at highest risk of skin breakdown and radiation dermatitis because of the numerous skin folds that result in tangential radiation and higher skin dose and the inherent moisture in the area. In RTOG 98-11 Grade 3-4 acute skin toxicity occurred in 48% of patients treated with the current standard regimen of radiation, 5-FU, and Mitomycin-C. With IMRT techniques, grade 3 toxicity has been reported at rates of 0-35% while grade 2 toxicity occurs nearly uniformly.[14, 15]

Figure 1. Burden Of Anal Cancer Among People Living With AIDS In The U.S.
Shields, M et.al. Confidential/Unpublished Data



1.2.2 Rationale For Inclusion Of Patients With HIV-Infection

The standard of care for stages I-III anal cancer is concurrent chemoradiation, and the current practice is to generally treat HIV-infected patients with standard regimens. In HIV-uninfected patients with anal cancer, a phase III randomized controlled study, RTOG 98-11, demonstrated

that 5-year local-regional control is superior with concurrent 5-fluorouracil, mitomycin-C and radiotherapy when compared to 5-fluorouracil, cisplatin and radiotherapy. Given concern of hematologic toxicities associated mitomycin-C based chemoradiation in patients with HIV, especially patients with CD4 < 200 cells/mL, cisplatin based regimens have been advocated by some practitioners. The AIDS Malignancy Consortium is evaluating concurrent 5-fluorouracil, cisplatin and cetuximab and radiation in patients with local or local regional HIV-associated anal cancer, and results from this study may inform future management. However, there is evidence that with the availability of cART, outcomes in patients with HIV receiving concurrent chemoradiation, including mitomycin-C based therapy, appear to be comparable to that of the general population[17, 18]. The most common severe toxicity observed in one retrospective study of 21 patients receiving conformational RT with concurrent mitomycin C and 5-FU was dermatotoxicity. [17] Anal cancer is now the second most common non-AIDS defining malignancy, and HIV-infected patients comprise a large proportion of patients with anal cancer. Thus it is appropriate to include HIV-infected patients. Moreover, the NCI is encouraging inclusion of HIV-infected patients on clinical trials unless there is a clear reason not to do so. Patients with HIV and anal cancer appear to tolerate standard regimens as long as they have a good performance status, and dose reduced therapy appears to be associated with worse local control[19]. Interventions that limit toxicity are particularly desirable in this patient population.

1.2.3 Preclinical Data With MTS-01

MTS-01 (Tempo1: 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a nitroxide oxygen radical scavenger that has been formulated as a topical gel. MTS-01 has been shown to protect against radiation-induced skin toxicity, specifically alopecia, in nonclinical studies.[20, 21] The nitroxides are a class of stable free radical compounds that have *in vitro* anti-oxidant activity, protecting mammalian cells against hydrogen peroxide, superoxide, and t-butyl hydroperoxide cytotoxicity.[22-25] One water-soluble nitroxide, Tempol, has been shown to protect mammalian cells against aerobic radiation cytotoxicity.[26] The possible mechanisms of Tempol radioprotection include oxidation of reduced transition metals, superoxide dismutase (SOD)-like activity, and scavenging of oxy- and carbon-based free radicals.[26]

Tempol radioprotection of normal tissue has been demonstrated *in vivo*.[27] The maximally tolerated dose, whole blood pharmacology, and radioprotective properties of MTS-01 were previously evaluated in C3H mice. The maximally tolerated dose of Tempol delivered via intraperitoneal injection was 275 mg/kg. Following intraperitoneal administration of 250 mg/kg Tempol, peak concentrations of ~600 µg/mL were observed in whole blood 5 - 10 minutes post injection. Administration of Tempol 5 to 10 minutes prior to whole body radiation provided radioprotection. The $LD_{50/30}$ dose (the dose of radiation which caused 50% lethality 30 days after radiation) for Tempol-treated mice was 9.97 versus 7.84 Gy for saline-treated mice. Tempol provided a dose modification factor of 1.3 at the $LD_{50/30}$ dose level. These studies were extended to evaluate the nitroxides in a murine tumor model.[28] Tempol did not decrease tumor control with radiation of the RIF-1 tumor in C3H mice. The protection of normal tissues without protection of the RIF- I tumor may be the result of differential bioreduction of nitroxide in tumor versus normal tissues.

Two *in vivo* studies in guinea pigs have demonstrated the potential of topically applied Tempol to prevent hair loss when applied prior to irradiation.[20, 21] Tempol solution (70 mg/mL in

70% ethanol) or control solution were applied to partially depilated sites on the dorsal trunk of Hartley guinea pigs.[20] Ten minutes later the test articles were removed, and the treated areas were sequentially x-irradiated at 3.75 Gy/min (total radiation: 25 to 30 Gy). Radiation-induced alopecia (hair density) was visually scored over the next 18 weeks. Sites pretreated with Tempol had substantially greater hair density beginning 7 weeks post-irradiation and continuing through the final observation time point (i.e., 18 weeks).

Cuscela et al (1996) studied the radioprotective effects against hair loss of topically applied Tempol in a fractionated radiation model in guinea pigs.[21] A 2 mL solution containing 70 mg/mL Tempol in 70% ethanol was topically applied to the skin surface of one side; 70% ethanol was applied to the contralateral (control) side 10 minutes before irradiation. Fractionated external beam radiation (7 Gy) was delivered daily for 8 fractions over 10 days via a 4 MeV linear accelerator. Gradual hair loss was detected on both untreated and Tempol-treated areas. However, over 4 to 11 weeks post-irradiation, the hair loss was significantly less in the Tempol-treated radiation areas. Histological evaluation of the Tempol-treated skin showed no appreciable decrease in the number of hair follicles, whereas the control (untreated) skin showed a marked decrease in the number of hair follicles and poor development of the remaining follicles.

One major concern in the development of topical radioprotectors is the possibility of systemic absorption leading to the possibility of systemic toxicity or tumor radioprotection.[29] The available pharmacokinetic data in animals indicate that topically applied Tempol has little systemic bioavailability. Tempol was not detected in blood or brain tissue 10 or 15 minutes after topical application in guinea pigs but was detected in Tempol-treated skin specimens.[20, 21] In pigs treated with topical Tempol for 10 days over a 2-week period, Tempol was detected in whole blood in varying amounts. Maximal systemic exposure appeared to be limited to approximately 14 μM (i.e., 2 μg/mL).

Nonclinical repeated dose toxicity studies have been conducted in miniature swine to determine the repeated dose toxicity of topically administered Tempol (unpublished). Tempol was applied to the skin for 10 out of 14 days. The maximum soluble concentration of Tempol (i.e., 70 mg/mL) was used. In the first study, 100 mL of 70 mg/mL Tempol was applied to 10 and 12% of the skin surface area to 2 pigs, respectively. There was no skin erythema or edema observed. There were no significant gross or microscopic findings at necropsy. Skin histopathology demonstrated a small number of dermal eosinophils and a lymphohistiocytic infiltrate. In the second study, 68 mL of 70 mg/mL Tempol was applied to 7% of the skin surface in 2 pigs. There were no significant gross or microscopic findings at necropsy. Skin histopathology demonstrated dermal eosinophils, increased dermal vascularity, and lymphohistiocytic infiltrate. In summary, there appear to be no serious cutaneous or systemic toxicities associated with the topical application of Tempol to pig skin.

In a separate study conducted at Dow Pharmaceuticals, Tempol was evaluated for absorption following serial applications to skin strips *in vitro*. This study evaluated the effect of multiple applications of the moderately gelled 7% Tempol ethanol / water formulation on the *in vitro* percutaneous absorption of Tempol. Four application regimens were performed: (1) a single application of Tempol, (2) two applications of Tempol, (3) three applications of Tempol, and (4) one application of Tempol followed by one application of vehicle formulation. Each application of formulation had a 30-minute duration of exposure to the skin surface. Then the skin was wiped with two dry cotton swabs after each application. Upon completion of the final application and skin wiping, the stratum corneum was removed from the skin. All samples of stratum

corneum along with the remaining skin (viable epidermis / dermis), receptor fluid, and skin surface wipes collected during the study were shipped to Periannan Kuppusamy, Ph.D. at Ohio State University for analysis of Tempol content. Tempol content was reported as "Normal", "Oxidized", and "Reduced" by the analytical laboratory.

The amount of reduced Tempol penetrating the skin into the receptor fluid was very low and ranged from 0 μg/1.77 cm2 to 0.62 μg/1.77 cm2 of skin following a 30-minute duration of skin exposure. The second application of Tempol and additional 30-minute exposure to the skin surface resulted in a cumulative range from 2.94 μg/1.77 cm2 to 3.80 μg/1.77 cm2 of skin. The vehicle application did not increase the amount of reduced Tempol penetrating the skin. The third application of Tempol and additional 30 minute exposure to the skin surface resulted in a cumulative amount of 8.8 μg reduced Tempol/1.77 cm2 of skin. The highest viable epidermis/dermis levels were seen with two applications of Tempol, 184.1 μg reduced Tempol/1.77 cm2 of skin. This study shows that two applications of the moderately gelled ethanol / water formulation will achieve higher deposition and penetration levels of Tempol than a single application. This study suggests that additional applications beyond two will not appreciably increase Tempol skin levels.

1.2.4 Clinical Experience With MTS-01

MTS-01 has been evaluated in two trials. The first clinical experience with MTS-01 was an open-label study in patients undergoing whole brain irradiation for metastatic disease (IND #66064 entitled "A Phase 2 Study of the Safety, Pharmacokinetics and Preliminary Efficacy of MTS-01 for the Prevention of Alopecia in Patients Undergoing Whole Brain Radiotherapy").[30] Patients received approximately 100 ml of 70 mg/ml Tempol in alcohol and hydroxycellulose applied uniformly to the scalp for 15 minutes daily prior to radiation for up to two weeks. Eleven of 12 patients enrolled in the study completed the protocol. Hair retention was seen in three of five evaluable patients. Tempol was not detectable in the blood in 50% of patients with mean Tempol levels varying from 0.4-3.1 uM per/L. There were no drug-associated safety concerns. Asymptomatic hypoglycemia was observed in 3 patients (2 grade 2, 1 grade 1). Grade 1forehead redness was observed in one patient, grade 1 dry scalp was observed in one patient, and grade 1 tingling of the scalp was observed in one patient. There was 1 non-treatment-related death; a female patient died from heparin-induced thrombocytopenia 2 weeks after receiving MTS-01. One patient withdrew from the study after only 2 Tempol treatments so no assessment could be made.

A second pilot study was initiated to study the safety, pharmacokinetics, and preliminary efficacy of MTS-01 for the prevention of acute skin toxicity induced by radiotherapy. This study was a single arm, open label study in patients undergoing radiotherapy for five to seven weeks. Forty-one patients were treated with the study drug to a mean dose of 0.14 g/cm^2 target area. The target area varied across patients due to the different types of cancer treated (mean 232cm^2, range 95-800 cm^2). Ninety percent of the patients enrolled completed the treatment with MTS-01. Two patients withdrew consent and two patients dropped out due to adverse events. Efficacy was assessed with the Radiation Therapy Oncology Group (RTOG) Acute Radiation Morbidity Scoring Schema. The investigators scored skin reaction with this scale at baseline, at the end of each week of treatment, and at four weeks after completion of treatment. Patients had a mean score of 0.1 (range 0-1) at baseline. During weeks five through 7 of the study approximately 1/3 of subjects were scored as 2. At four weeks follow up, the majority of these

reactions had resolved to grade 0-1. Because there was no control group and the rates of toxicity vary by indication, no firm conclusions can be drawn regarding the efficacy of MTS-01 in this setting, although the levels of skin toxicity observed in this high risk population suggest efficacy.

There were a total of 492 adverse events experienced by patients on this trial. Of these, 32 (7%) were identified by the investigators as being possibly, probably, or definitely related to the study drug. Two of these events were life threatening (neutropenia, pulmonary embolism) but were felt to be unrelated to the study drug. There were three serious adverse events, one mentioned above as life threatening (pulmonary embolism, deep vein thrombosis, skin rash). The patient who developed skin rash was a 73 year old woman with grade 1 erythema at baseline who developed a severe rash/desquamation with burning and pruritis that the investigator recorded as probably related to the study drug. MTS-01 was discontinued at day 27 in the patient. The other two serious events were not considered to be related to the study drug.

The most commonly reported events were gastrointestinal (nausea, diarrhea), constitutional (fatigue), dermatological (pruritus, skin pain, rash). These events were reported in over 50% of subjects. Additional commonly reported adverse events were neurological (dizziness), metabolic (hyperglycemia, hypokalemia), infection, cough, anemia, muscle weakness, and edema, which were reported in at least 20% of subjects. These events are consistent with the nature of the study population (cancer patients receiving radiotherapy), and were reported as 'Unrelated' to study drug in nearly all cases.

There were four skin events that were reported as 'Related' to study (2 Pruritis/itching, 2 Pain/Skin) that were all reported as Mild by the investigator. Eleven events were reported as 'Probably' related to study drug were skin events as well (7 Pruritis/itching, 2 Pain/Skin, 2 Rash/Desquamation) with a range of reported severities (4 Mild, 5 Moderate, 2 Severe). Since subjects were all being treated for skin toxicity due to radiation therapy, it is difficult to assess whether these events are related to the study drug or to the radiotherapy.

Multiple subjects had laboratory values outside of the normal ranges. However, the patients in this study were undergoing radiation treatment for cancer and were expected to have abnormal laboratory values frequently. All of the laboratory values falling out of the normal range that were determined by the principal investigators as being clinically significant were logged by the investigators on the adverse event forms. Each of these were reported as "unrelated to study drug" by the investigator. Systemic levels of study drug were measured prior to treatment, 1 hour after, and 24 hours after topical application for the first, twelfth, and final day of treatment. For all subjects, all time points, and all days, the systemic levels of study drug were below the limit of detection of the assay.

1.2.5 Summary of Rationale

In summary, local skin toxicity is a common complication following chemotherapy and radiotherapy for anal carcinoma. Even with modern techniques grade 3 toxicity has been reported in up to 35% of patients and 2 toxicity occurs nearly uniformly.[14, 15] This toxicity causes significant pain and discomfort and interferes with the delivery of therapy, potentially leading to lower disease control if treatment breaks are required. The results of several nonclinical efficacy studies demonstrate a scientific rationale for the use of topically applied MTS-01 for the prevention of radiation-induced skin toxicity. The bioavailability of topically applied Tempol is limited, which is favorable for a site-specific application to the skin area

exposed to radiotherapy. Finally, ongoing clinical experience with the compound in patients undergoing irradiation at a variety of sites demonstrate Tempol to be a safe and well tolerated topical agent.

1.2.6 Rationale For Translational Endpoints

The nature of HIV persistence during antiretroviral therapy is uncertain, and the additional effects of cytotoxic chemotherapy have not been extensively investigated. Although persistent viremia is detectable during combination antiretroviral therapy, it is not known whether virus is the product of complete cycles of replication or is the product of long lived cells with integrated proviruses. The distinction between these two possibilities is critical for understanding HIV pathogenesis and for designing strategies for HIV eradication. For instance, if persistent viremia is the result of complete ongoing cycles of replication, then current antiretroviral therapy, which targets active infection, requires improvement[31, 32]. In contrast if persistent viremia is derived from long lived cells with integrated proviruses, then current antiretroviral therapy is maximally suppressive, and alternative strategies are necessary to eliminate virus infection[33, 34]. Several approaches have been useful in determining the nature of HIV viremia on therapy. First, we have developed **sensitive real time RT-PCR assay for HIV with a limit of sensitivity of approximately 0.3 copies/ml plasma**[35]. Analysis of viremia during standard and intensive antiretroviral therapy has yielded useful insights on the source of persistent viremia. Second, techniques to **amplify genetic sequences from low-level viremia have enabled detailed phylogenetic analysis of HIV genetic diversity and variation**[36].

We will gain new insights in the present studies by analyzing HIV viremia prior to, during, and following chemotherapy. If HIV persistent viremia is derived from long lived cells capable of expansion, then cytotoxic chemotherapy may decrease the levels of persistent viremia during treatment with mitomycin C and 5-fluorouracil. In addition, genetic analysis of persistent viremia should identify detectable changes in the circulating HIV population. If we find no effect of chemotherapy on the levels of viremia or population genetic characteristics, then persistent viremia may be the result of long lived cells which do not undergo frequent cell division, or of low level active replication. Increases in viremia during therapy may be the result of activated replication, perhaps as the result of inflammatory response or of active replication.

Analysis of levels of viremia and population genetics will help distinguish these possibilities, as new active cycles of replication may result in ongoing accumulation of new mutations or shifts in population structure.

In addition, we will similarly investigate the feasibility of measuring HIV viral load in rectal associated lymph tissue (see Section 3.4.5) obtained from snag biopsies using sensitive RT-PCR. If HIV is measurable, we will likewise evaluate the population genetics of HIV in this rectal associated lymphoid tissue using the same genetic sequence amplification methodology we have previously used in serum samples to derive a detailed phylogenetic analysis, and to explore in a preliminary fashion whether HIV genetic diversity differs between rectal associated lymphoid tissue and serum. These studies may yield new insights into the nature of HIV replication and the sources of genetic diversity. These studies are considered exploratory in nature, and results will not be used to make clinical decisions and will not be communicated to study subjects,

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

- Histologically proven, invasive primary squamous, basaloid, or cloacogenic carcinoma of the anal canal, stage T1-4, N0-3
- No previous therapy for anal cancer.
- Age ≥18 years
- ECOG performance status ≤ 2
- Adequate bone marrow, renal, and hepatic function defined as
  - Absolute neutrophil count ≥ 1,000 cells/mm3
  - Platelet count ≥ 100,000/mm3
  - Hemoglobin ≥ 8mg/dL
  - Creatinine clearance > 60 mL/min using Cockroft-Gault formula
  - Bilirubin ≤ 1.5 X ULN unless, during screening, the patient is receiving protease inhibitor therapy (i.e. indinavir, ritonavir, nelfinavir, and atazanavir) known to be associated with increased bilirubin: in this case total bilirubin ≤7.5 mg/dl and the direct fraction is ≤ 0.7 mg/dl.
  - WBC ≥ 3,000/μL
  - ALT/AST ≤ 3 times the upper limit of normal
  - International normalized ratio (INR) ≤ 1.5
- Patients of childbearing potential must be willing to use a medically effective means of birth control for the duration of treatment and six weeks after treatment.
- Patients must be willing and able to provide informed consent

2.1.2 Exclusion Criteria

- Contraindications to radiotherapy such as a history of prior radiotherapy to the pelvis or a history of inflammatory bowel disease
- Prior malignancy except:
  - non-melanoma skin cancer
  - controlled Kaposi's Sarcoma (no chemotherapy for KS for 3 months, and no expected need for chemotherapy for the 12-month period of the study)
  - other malignancies with disease free period of at least 3 years
- Presence of metastatic disease (M1)
- Co-morbidity that in the estimation of the principal investigator would make the patient unable to tolerate treatment
- Pregnant or lactating females
- HIV positive patients with CD4 < 100 cells/mL AND ECOG PS > 2.
- Dermatitis in the anticipated radiation treatment portal.

2.2 Screening Evaluation

2.2.1 Clinical Evaluation

- A complete history and physical examination will be completed by the PI or an associate investigator. This will include a detailed history with attention to signs, and symptoms caused by the cancer, pain, opiate requirements, determination of ECOG performance status. In patients with HIV, a detailed history that includes date of HIV diagnosis, nadir CD4 count, current CD4 count and HIV viral load, history of opportunistic infections, and medication history
- Vital signs: Including height, weight, and body surface area.

2.2.2 Laboratory Evaluation

Pre- treatment blood tests should be performed within one week of study entry unless otherwise noted.

- Hematology: complete blood count, differential, platelet count, PT, PTT, INR
- Chemistries: LDH, SGOT, SGPT, alkaline phosphatase, bilirubin (total and direct), BUN, serum creatinine, calcium, magnesium, phosphorus, uric acid, albumin.
- Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab., RPR (within 3 weeks of study entry)
- Hepatitis C antibody, unless previously documented as positive (within 3 weeks of study entry)
- Urinalysis
- Urine Pregnancy Test: required for females of childbearing potential
- HIV- ELISA unless documented HIV-infected (within 3 weeks of study entry)
- Lymphocyte subset TBNK. Simultaneous CBC and automated differential must be drawn.
- HIV viral-load in patients infected with HIV

2.2.3 Radiographic Evaluation

- CT of the chest, abdomen, and pelvis with oral and intravenous contrast unless contraindications to contrast exist. Outside study will be considered adequate if obtained within three weeks of study entry. If scans were obtained from another institution, copies should be produced and maintained on file with the PI.
- PET of the torso. Outside study will be considered adequate if obtained within three weeks of study entry. If scans were obtained from another institution, copies should be produced and maintained on file with the PI.

2.2.4 Baseline Evaluation of Primary Tumor and surrounding Mucosa

- Cytology scrapings, core needle biopsy, and flexible sigmoidoscopy will be performed to evaluate the primary tumor and adjacent rectal mucosa (see section 3.4)
- Tumor sampling for HPV analysis and tumor banking will be performed. (detailed in Sections 3.4.2, 3.4.3.)

- When feasible, 15-20 snag biopsies from the distal rectum will be collected to evaluate feasibility of HIV-1 quantification and processing of lymphocytes for correlative studies. (detailed in Section 3.4.4.)
- Photography
- Assessment of RTOG acute skin toxicity

2.2.5 Pathologic Review

Diagnostic biopsies performed at the NIH will be evaluated by the Laboratory of Pathology. Outside reports confirming malignancy will be also acceptable for study entry. NIH Laboratory of Pathology review of pathologic material will be completed in all patients enrolled.

2.3 Registration Procedures

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 Study Design:

This is a Pilot trial of topical MTS-01 in patients receiving MMC, 5-FU, and IMRT for definitive management of carcinoma of the anal canal. MMC and 5FU will be delivered in the MOB clinic or day hospital. MMC will be delivered at a dose of 10mg/m^2 (maximum 20 mg) on days 1 and 29. 5-FU will be delivered as 1000mg/m^2/day as 96 hour continuous infusion beginning on day 1 and 29. RT will be delivered to a total dose of 50-54 Gy based on tumor characteristics. MTS-01 will be applied to the bilateral groins and the gluteal cleft, avoiding a 3 cm radius from the anal verge, immediately prior to each fraction of RT. MTS-01 will be applied by trained personnel in the ROB clinic. RTOG grading will be used to evaluate skin toxicity in both the groin and gluteal cleft weekly during treatment and at 4 weeks, 3 months and 6 months after completion of treatment. Toxicity in the perianal skin where MTS-01 is not applied will not be graded. In addition, two control sites will be marked and will be graded using RTOG skin toxicity criteria. One site will be in the treatment area and will receive radiation only- MTS-01 will not be applied. The second control site will be outside of the treatment field (umbilicus) and will be treated with MTS-01 only. Photography for permanent documentation will occur prior to treatment, weekly on treatment, and at each follow up. Photography will be performed by trained personnel in the ROB clinic. This will allow additional blinded scoring of toxicity for comparison. The duration of treatment, number of treatment breaks, opiate requirements, and level of pain will be evaluated with the Brief Inventory of Pain (Appendix IV) weekly during treatment and at 4 weeks and 3 months after the completion of treatment. Disease control will be assessed at 4 weeks, 3 months, 6 months, 9 months, and 12 months of follow-up.

3.2 Study Drug Administration

3.2.1 MTS-01

Prior to the first treatment, the area to be treated in the gluteal cleft and inguinal regions will be marked with permanent marker (site T). The area of each region will be estimated with bidirectional measurements. The area of the composite volume will be obtained by adding the calculated areas. A trained radiation oncology nurse, associate investigator, or a designee will administer MTS-01 topically. Health care providers will wear gloves while applying MTS-01. The gel preparation may be flammable; precautions should be taken to keep away from fire or flame or machinery and equipment that might cause sparking and hence, an explosion.

Before application, the area for Tempol administration should be marked and measured in at least 2 dimensions so that the area of the application can be calculated. Up to 100 mL of 70 mg/mL MTS-01 will be applied uniformly to the patient's targeted skin area 15 - 30 minutes prior to each fraction of external beam radiation. Care will be taken to make sure that the marked and targeted area is fully covered. MTS-01 will be administered daily prior to each fraction of radiation and will be washed off gently with warm soapy water within one hour after the completion of the daily radiation fraction. Care should be taken not to irritate the skin unnecessarily. Target markings on the skin should be refreshed as needed to accurately maintain radiation exposure areas. Usually permanent marks are used to set up the patient.

The study drug will be applied using a ***folded gauze pad or syringe*** to permit easy retrieval of the study product from the container. The test material will be evenly spread over the skin area targeted for radiotherapy. Additional material will be retrieved from the container and applied to the designated area until the volume of test article necessary to cover the treated area is used. The total amount of Tempol used for each radiotherapy treatment must be recorded on the Case Report Form (CRF). ***The application of MTS-01 to the skin should be allowed to dry enough to keep MTS-01 in place prior to radiation. Do not allow it to dry completely.***

The entire application procedure, should take approximately 15 minutes. The trained personnel will visually confirm that the skin is sufficiently dry before proceeding with positioning of the patient for radiotherapy. Radiation will be delivered within 15 - 30 minutes of drying of the MTS-01 application. MTS-01 application will continue for as long as the affected skin is receiving radiation or until criteria for dose modification are met.

In addition, a small area of skin in the left inguinal area will remain untreated with MTS-01 (site C1). This area will be selected based on the radiation treatment plan. This area will measure approximately 2x2 cm and will be marked with permanent marker for daily reproducibility during radiation treatment. This area will serve as a dermatitis control area as it is unlikely to be treatment limiting or result in treatment breaks. Toxicity scoring of this area will be performed simultaneously. A second area just superior to the umbilicus will be treated daily with MTS-01 only (site C2). This area will measure approximately 2 x 2 cm and will be marked with permanent marker for daily reproducibility during radiation treatment. It will serve as an area outside the radiation field which can be used in the event that dermatitis is observed and there is not clarity if it is caused by radiation or Tempol.

3.2.2 5-Fluoruracil (5-FU) and Mitomycin C

Concurrent chemoradiation will be administered in the MOB clinic or day hospital using the chemotherapy dosing used in RTOG-98-11, and reported by Ajani, et. al. This regimen is considered standard of care by the National Comprehensive Cancer Network (NCCN). Patients will require a central catheter for chemotherapy administration (typically a PICC line). Anti-emetic prophylaxis will be administered as outlined in Section 3.6.1.

Agent	Dose	Route	Schedule
Mitomycin-C	10 mg/m2 Do not exceed 20 mg per cycle	*Intravenous Bolus through central venous access*	On Day 1 and Day 29
5-FU	1000 mg/m^2/day Days 1-4, following Mitomycin C	*96-hour Continuous Intravenous Infusion*	In 5% Dextrose or 0.5 NS daily for 96 hours (M-F) continuously starting Day 1; cycle is repeated on Day 29.

3.2.2.1 On Days 1 and 29 (within 48 hours of initiation of chemotherapy), obtain Chemistry 20, and correct any electrolytic, renal, or volume abnormalities. These should have returned to CTCAE grade 1 or baseline (if baseline higher than CTCAE grade 1) prior to chemotherapy being administered on Day 29

3.2.2.2 On Days 1 and 29 (within 48 hours of initiation of chemotherapy), CBC with differential should be obtained. 5-FU and Mitomycin-C should be held for an absolute neutrophil count (ANC) < 1000 /μL or a platelet count less than 100,000 /μL.

3.2.2.3 Monitoring for clinical signs of dihydropyrimidine dehydrogenase (DPD) deficiency will be performed during weekly evaluations. Obtain CBC for any non-hematologic toxicity (including sepsis, fever, and bleeding) other than rash. If a grade 4 toxicity occurs during the 96-hour infusion of 5-FU, the 5-FU must immediately and permanently be discontinued for that cycle. In a study of patients experiencing severe toxicity from 5-FU, a correlation was shown between the sum of all the toxicity grades for mucositis, neutropenia, thrombocytopenia, diarrhea, and neurotoxicity (always graded 4 as neurotoxicity is a strong predictor for DPD deficiency) and measured DPD levels. Patients with any neurotoxicity or several concurrent grade 4 toxicities should not receive further 5-FU without a DPD assay. Responses to modifications of 5-FU dosage are unpredictable due to genetic polymorphism in DPD. If, in the investigator's opinion it would *not* be in the best interest of the patient to continue with 5-FU after a grade 4 adverse event because of the risk of repeated severe toxicity, further 5-FU should not be administered. Assessment of the patient should be based on the patient's *overall* condition, including consideration of each individual toxicity encountered. Consideration must include the duration of the toxicity in addition to its *severity.* However, there is no absolutely safe level, and all patients require careful individual assessment. Women are more likely to have DPD deficiency than men. A thorough

discussion should be conducted with the patient as to the possible risks and benefit of receiving further 5-FU.

3.3 Dose Modification for Toxicities:

3.3.1 Modification of MTS-01

No dose modifications are allowed. See section 3.9.1 for description of criteria for MTS-01 discontinuation.

3.3.2 Modifications in 5-Fluorouracil

For diarrhea, mucositis, stomotitis, thrombocytopenia, or ANC grade 3 or grade 4 that persist until day 29 after administration of 5-FU on days 1-4,: Reduce 5-FU dose by 50% to 500mg/m^2/day for days 29-32.
5-FU will be discontinued if there is a suspicion of DPD deficiency, as dose adjustment requirements in this setting are unpredictable. See Section 3.2.2.3.

3.3.3 Discontinuation of Mitomycin C

Mitomycin C will be discontinued in patients diagnosed with thrombotic microangiopathy after the first dose. Continuation of 5-FU without mitomycin C will be based on the patients overall condition, and determined by the Principal Investigator.

Dose reductions on Day 29 will be made if there is severe thrombocytopenia after the first dose. Dose reductions will be based on nadir platelet counts Days 7-28.

Nadir after prior dose	***Dose of mitomycin-C on Day 29***
25,000-74,999 platelets/mL	7 mg/m^2 to maximum of 14 mg
<25,000 platelets/mL	5 mg/m^2 to maximum of 10 mg

3.3.4 Dose Delays

Day 29 chemotherapy may be deferred for up to 14 days if necessary for recovery of neutrophils or platelets, or based on timing considerations related to breaks in administration of IMRT due to toxicities.

3.4 Correlative Studies for Research/Pharmacokinetic Studies:

Correlative studies will include blood sampling, biopsies (optional) of rectal mucosa and anal tumor, and anal cytology. Optional biopsies performed at baseline will include a maximum of 2 anal cancer snag biopsies, and up to 30 rectal mucosal snag biopsies. Additionally, at 1 year, and additional rectal mucosal snag biopsies (up to 30) as well as anal cytology will be obtained. Correlative studies within this protocol are outlined below. Both HIV infected and HIV uninfected subjects may also be co-enrolled on 01-C-0038.

3.4.1 PBMC Sampling

PBMC will be sampled from peripheral blood to evaluate the changes in immune cell subsets after treatment with radiation and chemotherapy.

3.4.1.1 Collection, processing and shipping of specimens to be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621)

3.4.1.2 Requirements: 2 green top tubes will be collected for PBMC storage. 2 red top tubes will be collected for storage of serum for future studies of immunologic parameters

Time points: At baseline, week 5, 4-week follow-up visit and month 12

3.4.2 Evaluation of Persistent HIV Viremia During Therapy (HIV+ only)

HIV viremia will be assessed in HIV positive patients in peripheral blood to evaluate the changes in HIV viremia after treatment with radiation and chemotherapy.

3.4.2.1 Collection, processing and shipping of specimens to be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621)

3.4.2.2 Plasma requirements- 7-10 ml plasma per time point (dark lavender EDTA tube)

3.4.2.3 Time points

- Days 1 prior to therapy, 8, 15, 22, 29, 36 (all +/- 1 day weekly during therapy)
- Follow up visits through month 12.

3.4.2.4 Samples will be sent to:

NCI DRP core lab
NCI-Frederick
Building 535, c/o Mary Kearney
Frederick, MD
Phone 301:846-6796

3.4.3 Optional Anal Cancer Biopsy (Patient need not be HIV+)

Optional anal cancer biopsies will be performed with the intent of molecular characterization and submission to the HPV-related cancer project which is a part of the HIV Tumor Molecular Characterization Project. (H+TMPC) See http://cgap.nci.nih.gov/Cancer_Types

3.4.3.1 Collection, processing and transport of specimens to be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621)

3.4.3.2 Patients agreeing to biopsy will be consented specifically for the use of tissue for

molecular characterization. See Appendix V

3.4.3.3 Biospies will generally be performed in concert with rectal mucosal sampling (during the same procedure, see section 3.4.5). A section of tumor will be submitted to pathology for review. A person will be ready to transport tissue to the Laboratory of Pathology for processing (see Appendix III).

3.4.3.4 1 or 2 pinch biopsies (approximately 3 mm each) may be collected for the HIV+ Tumor Characterization Project. (Patients need not be HIV +). In patients who are participating in this portion of the study and additional 10 mL of blood in an ACD tube will be collected and used as a non-tumoral control.

3.4.3.5 Biopsies will be snap frozen in the Laboratory of Pathology (within 20 minutes after tissue is obtained from the patient) then transported and stored in the Laboratory of Robert Yarchoan, HAMB/CCR. (See Appendix III for specific procedures for handling biopsy samples)

3.4.4 Optional Anal Cancer Cytology (Patient need not be HIV+)

Anal cancer cytology will be performed to assess the validity of anal cytology and to determine HPV positivity and subtypes in this population.

3.4.4.1 Specimen collection and processing should be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621). Specimens will be collected at baseline, and month 12.

3.4.4.2 Procedure for obtaining optional anal cytology:

- Anal cytology will be collected by HAMB investigators.
- The subject should undress from the waist down, and either bend over the exam table or lay on the side in the fetal position. The examiner should use one hand to spread the buttocks and expose the anoderm. A moistened Dacron swab will then be inserted as far as is comfortable into the anus, a minimum of 2-3 inches. If there is difficulty inserting the swab, the subject should also retract their buttocks. With pressure on the distal end of the swab rotate it gently and slowly in a circular fashion as it is withdrawn over 15 to 30 seconds. Do not retract the buttocks when the swab is close to the verge to ensure that it is sampled as well.
- Immediately immerse the swab in a liquid-cytology vial *agitating vigorously over 10 to 15 seconds* to disperse the cells.
- Divide the transport media in half:
  - Samples to be sent to AIDS Monitoring Laboratory
  - Science Applications International Corporation (SAIC), NCI-Frederick facility, Fort Detrick
  - One sample to be stored at -70 degrees C.
  - One sample to be sent to for HPV DNA commercial genotyping of high-risk HPV subtypes. (Quest HC2 assay, Test 9662A)

3.4.5 Optional Rectal Mucosal Snag Biopsies (Patient need not be HIV+)

We will evaluate feasibility of evaluating rectal associated lymphoid tissue requirements (see section 3.4.3.) for HIV viral genetic analysis and/or viral RNA testing [32].

3.4.5.1 Specimen collection and processing should be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621), as well as the Laboratory of Dr. Irini Sereti (Building 10, Room 11B03).

3.4.5.2 When anatomically feasible, patients will undergo routine flexible sigmoidoscopy during the initial patient evaluation. Endoscopic evaluation will be performed by Dr. Michael Yao, in Building 10, 3SW-N under moderate conscious sedation

- 15-30 snag biopsies will be randomly obtained from distal rectal mucosa. This technique has been shown to be well tolerated with minimal morbidity.[37]
- Initial biospecimen processing will include the following:
  - In groups of 5, the biopsies will be weighed, placed in 500 μL of medium containing RPMI (Mediatech, Herndon, VA) with 10% heat –inactivated fetal bovine serum.
  - One sample will be sent to the Laboratory of Clinical Pathology for histology
  - Single cell suspension will be extracted from groups of 10-20 samples after tissue digestion and processing, and cells will be processed by flow cytometry (Laboratory of Irini Sereti: Building 10, 11B03, Bethesda, MD). A blood sample (2 green top tubes of 6ml each) will also be obtained for comparative flow results in peripheral blood. The blood draw can be done the same day as the biopsy (prior to the procedure) or in a previous visit within a 2-week range and will also be processed in Dr. Sereti's laboratory.
  - 1 Sample, to be evaluated for HIV RNA (see Section 3.4.1.) will be sent to:
    NCI DRP core lab
    NCI-Frederick
    Building 535, c/o Mary Kearney
    Frederick, MD
    Phone 301:846-6796

3.4.5.3 Follow up flexmoid sigmoidoscopy will be performed at the 12 month follow up visit, with 15-30 rectal mucosal biopsies to be obtained and processed as outlined above.

3.4.5.4 Unused biospecimens, including but not limited to biopsies in RPMI, and/or cell suspension (if any) will be sent via messenger to:

AIDS Monitoring Laboratory
Science Applications International Corporation (SAIC)
NCI-Frederick facility, Fort Detrick

3.5 Study Calendar:

Procedure	Screening/ Baseline	Treatment (week) 1	2	3	4	5	6	Post Therapy Follow-up Wks 1,2,4	3, 6, 9, 12 Mos	
History and PE	X				X			X	X	
Vital signs	X	X	X	X	X	X	X	X	X	
ECOG Performance Score	X								X	
Pain/ opiod assessment	X	X	X	X	X	X	X	X	X	
Labs										
CBC c diff, PLTs, PT, PTT, INR	X	X	X	X	X	X	X	X	X	
LDH, SGOT, SGPT,	X	X	X	X	X	X	X	X	X	
alk phos, bilirubin (total and direct), BUN, Serum creatinine, Ca, Mg, phos, uric acid, albumin	X	X	X	X	X	X	X	X	X	
Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab., RPR Hepatitis C antibody	X									
Urinalysis/ urine Pregnancy Test required for females of childbearing potential	X									
HIV- ELISA unless documented HIV-infected	X									
**Lymphocyte subset TBNK. Simultaneous CBC and automated differential must be drawn.*	X					X		X	X	
If HIV Infected:										
**HIV viral-load*	X							X	X	
**PBMC and Serum storage*[1]	X					X		X (wk4)	X (mo 12)	
**HIV viral studies*[2]	XXX	X	X	X	X	X	X	X	X	
Research Specimens										
**Anal biopsy*[3]	X									
w/10 ml blood in ACD	X									
**Anal cytology*[4]	X								X (mo 12)	
**Endoscopy w/ rectal mucosal snag biopsies (lymphoid tissue*[5]	X								X (mo 12)	
Radiological Assessments[6]										
CT CAP	X							X(wk4)	X	
PET	X								X[7]	
RTOG toxicity [8]	X	X	X	X	X	X	X	X	X	
Photography treatment area[9]	X	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X		
Response Evaluation[10]								X	X	
MTS-01[11]		**X**	**X**	**X**	**X**	**X**	**X**			
Radiotherapy		**X**	**X**	**X**	**X**	**X**	**X**			
5-FU/MMC		**X**				**X**				

[1] *See 3.4.1 2green top tubes will be collected for PBMC storage. 2 red top tubes will be collected for storage of serum*
[2] *See 3.4.2. Plasma requirements 7-10 ml*
[3] *See 3.4.2*
[4] *See 3.4.3*
[5] *See 3.4.4*
[6] *See 2.2.3, 5.2*
[7] *PET CT Month 3 of follow up only*
[8] *Acute toxicity scoring schemata baseline through 4 weeks follow-up. Acute toxicity scoring during treatment at each site- T, C1 and C2 (see 3.2.1) Late toxicity scoring at 3 through 12 months follow-up (site T only)*
[9] *Photography of treatment area*
[10] *See 5.2*
[11] *See 3.2.1 Includes application to radiation treatment site (site T)with exclusion of control site in left inguinal area (C1), and application to test site at umbilicus (C2).*

***Collection, processing and shipping of specimens to be coordinated with a HAMB clinical research nurse, Kathleen Wyvill (301-435-5622) or Karen Aleman (301-435-5621)**

3.6 Concurrent Therapies:

3.6.1 Chemotherapy-Induced Nausea and Vomiting Prophylaxis

5-FU combined with mitomycin C is associated with a moderate risk of emesis. The recommended prophylaxis is a serotonin receptor ($5\text{-}HT_3$) antagonist and dexamethasone prior to mitocmycin C (Days 1 and 29). The recommended drugs are granisetron 2 mg orally and dexamethasone 8 mg intravenously, although alternative agents are acceptable.
Patients may be prescribed an oral anti-emetic for use as needed on Days 1-4 and 29-32.
Acceptable additional agents include (but are not limited to)

- 5-HT3 receptor antagonists: palonosetron, granisetron
- D2 receptor antagonists: metoclopramide, prochloroperazine
- Benzodiazepines: lorazepam, alprazolam

3.6.2 Anti-retroviral Therapy

Patients will receive antiretroviral therapy if it is indicated. Combination therapy will be generally based on Department of Health and Human Services Guidelines for treatment of HIV infection, available at http://www.aidsinfo.nih.gov/guidelines/. However, patients may have extenuating circumstances requiring deviation from these guidelines. Protease inhibitor based regimens are permitted during chemoradiation. However, given the radiation sensitizing effects of protease inhibitors[38-42], patients on a protease inhibitor based regimen may be switched at time of initial evaluation at the discretion of the Principal Investigator if this is deemed feasible, and not detrimental to control of HIV viremia.

HAMB physician investigators will review antiretroviral therapy prior to commencing chemotherapy. Referring physicians may otherwise manage this component of patient care. HIV infected patients not receiving antiretroviral therapy at time of enrollment may be managed by HAMB physician investigators until a primary HIV physician can be identified. Every attempt will be made to establish an outside physician for management of HIV care and to manage delivery of antiretroviral therapy long term.

3.6.3 Pneumocystis jiroveci Prophylaxis

All HIV-infected patients, regardless of baseline CD4 cells count should receive Sulfamethoxazole/Trimethoprim (Bactrim DS, TMP 160 mg) PO 3x/week. Alternatives include but are not limited Bactrim DS PO daily, Dapsone 100 mg PO daily, and monthly aerosolized pentamidine.

3.6.4 MAC Prophylaxis

MAC prophylaxis will be considered for all HIV-infected patients with a historic CD4 nadir less than 75 cells/mm^3, or for patients whose CD4 cells fall below this level while on study. Recommend azithromycin 1200 mg once weekly, but other agents are acceptable.

3.6.5 Topical agents

Topical agents may be applied as per standard clinical practice. These agents will not be applied each treatment day prior to MTS-01 application but may be applied following treatment. Examples of topical agents typically delivered during the course of radiation therapy include Biafine cream, aquaphor, and 1% hydrocortisone cream. In general, treatment will start with Biafine and may progress to aquaphor and Domeboro's soaks if moist desquamation develops. Biafine and aquaphor may be applied with a chilled vigilon dressing for symptomatic relief. Hydrocortisone cream will only be used if patients develop folliculitis. Following completion of treatment, agents such as Silvidine may be prescribed to areas of moist desquamation unless contraindicated.

3.7 Surgical Guidelines:

N/A

3.8 Radiation Therapy Guidelines:

3.8.1 Simulation

A custom immobilization device will be used to minimize setup variability. Oral contrast will be delivered 30 minutes to 1 hour prior to simulation. A radioopaque anal marker will be placed at the anal verge at the time of simulation. Simulation will be performed with the patient prone or supine with arms at the level of or above the head. CT images will be taken with a thickness of $\leq$ 5 mm for treatment planning. All tissues to be irradiated must be included in the CT scan.

3.8.2 Treatment volumes

3.8.2.1 The gross tumor volume (GTV) is defined as all gross disease determined from CT (and MRI and PET if performed), clinical examination, endoscopic findings, and biopsy

- GTVA includes the gross primary anal tumor volumes
- GTVN50.4 includes the involved nodal regions (by clinical examination, biopsy, and/or imaging) containing disease measuring $\leq$ 3.0 cm in greatest dimension
- GTVN54 includes all nodal disease measuring greater than 3.0 cm in greatest dimension

3.8.2.2 The clinical target volume (CTV) is defined as the GTV plus areas considered to contain potential microscopic disease.

- CTVA includes GTVA, the anal canal, and a 2.5 cm expansion. Volumes may be adjusted to remove bone or air.
- CTV45 includes the uninvolved nodal regions with a 1 cm expansion. Volumes may be adjusted to remove, bone, air, genitourinary structures, and muscles)
- CTV50 includes nodal regions with involved nodes < 3 cm with a 1 cm expansion. Volumes may be adjusted to remove, bone, air, genitourinary structures, and muscles)
- CTV54 includes nodal regions with involved nodes measuring > 3cm with a 1 cm expansion. Volumes may be adjusted to remove, bone, air, genitourinary structures, and muscles).

3.8.2.3 The planning target volume (PTV) will provide a margin around CTV to account for

treatment set up error and organ motion. A minimum margin of 1 cm in all dimensions is required. A nodal PTV should not overlap with PTVA (PTVA takes precedence). PTV should be automatically subtracted from the skin surface with a margin of 5 mm.

3.8.2.4 Definition of nodal regions

- Mesorectal (includes peri-rectal and presacral)
- Inguinal (left and right)
- External iliac (left and right)
- Internal iliac (left and right)
- These volumes will be based on the RTOG consensus for rectal and anal cancer planning

3.8.2.5 Normal structures to be contoured

Femoral heads, bladder, external genitalia, iliac crest, small bowel, large bowel (outside CTV), perianal skin.

3.8.3 Treatment Planning

3.8.3.1 Doses for T1-T2 N0 disease

- PTVA: 50.4 Gy in 28 fractions (1.8 Gy per fraction)
- PTV42: 42 Gy in 28 fractions (1.5 Gy per fraction). Will include all nodal regions.

3.8.3.2 Doses for T3 N0 disease

- PTVA: 54 Gy in 30 fractions (1.8 Gy per fraction)
- PTV45: 45 Gy in 30 fractions (1.5 Gy per fraction). Will include all nodal regions.

3.8.3.3 Doses for N+ disease

- PTVA: 54 Gy in 30 fractions (1.8 Gy per fraction)
- PTV45: 45 Gy in 30 fractions (1.5 Gy per fraction). Will include only uninvolved nodal regions.
- PTV50: 50.4 Gy in 30 fractions (1.68 Gy per fraction). Will include all nodal regions containing involved nodes < 3 cm.
- PTV54: 54 Gy in 30 fractions (1.8 Gy per fraction). Will include all nodal regions containing involved nodes > 3cm.

3.8.3.4 Heterogeneity corrections should be used for treatment planning.

3.8.3.5 Dose goals

- No more than 5% of any PTV will receive < 90% of the prescribed dose
- No more than 2% of the PTV will receive < 80% of the prescribed dose
- No more than 2% of PTVA will receive >115% of the prescription dose.

3.8.3.6 Dose constraint goals

- Small bowel: <200 cc above 30 Gy, <150 cc above 35 Gy, <20 cc above 45 Gy, none above 50 Gy
- Femoral heads: <50% above 30 Gy, <35% above 40 Gy, <5% above 44 Gy
- Iliac crests: <50% above 30 Gy, <35% above 40 Gy, <5% above 50 Gy
- External genitalia:<50% above 20 Gy, <35% above 30 Gy, <5% above 40 Gy
- Bladder: <50% above 35 Gy, <35% above 40 Gy, <5% above 50 Gy
- Large bowel: <200 cc above 30 Gy, <150 cc above 35 Gy, <20 cc above 45 Gy

3.8.4 Treatment delivery

- Treatment will be delivered once daily, Monday through Friday, with the exception of federal holidays. Breaks in treatment should be minimized.
- Megavoltage equipment capable of delivering intensity modulated radiation therapy will be used.

3.8.5 Treatment breaks

- A rest period of < 7 days will be allowed for grade 4 skin reactions.
- Radiation therapy will be held for the following indications if clinically appropriate:
  - Platelets < 50,000/mm3
  - ANC <500/mm3
  - Grade 3 diarrhea(≥7 stools per day over baseline)
  - Grade 3 vomiting
  - Localized or generalized infection secondary to moist desquamation

3.9 Criteria for Removal from Protocol Therapy and Off Study Criteria

3.9.1 Criteria for removal from protocol therapy (NOTE: chemotherapy and radiotherapy will continue as tolerated):

- Significant deviation from protocol therapy (i.e., inability to tolerate MTS-01)
- Moist desquamation within the MTS-01 treated areas
- Symptoms attributable to an allergic reaction to MTS-01
- Other Grade 3 or 4 toxicity attributable to MTS-01
- Development of an intercurrent illness, which in the opinion of the Principal Investigator would prevent completion of therapy.
- Patient non-compliance
- Patient refusal to continue receiving protocol therapy

3.9.2 Off-study Criteria

- Withdrawal of consent
- Completion of 12 months of follow-up
- Unwilling to return to NIH for follow-up visits
- Disease recurrence
- Death

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://camp.nci.nih.gov/ccr/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 SUPPORTIVE CARE

4.1 Pain Control

Pain will be managed symptomatically with non-opiate and opiate analgesics as deemed clinically appropriate.

4.2 Diarrhea

Diarrhea must be managed symptomatically. IV hydration and use of loperamide, as well as close observation, are recommended for diarrhea if clinically indicated. If control takes longer than 2 days, medical evaluation including relevant diagnostic procedures, alternative treatment, and possible investigation of DPD deficiency should be considered. In patients with grade 3 diarrhea evaluation for C. difficile colitis should be considered. Patients with neutropenia and diarrhea should be considered for empiric use of prophylactic antibiotics such as oral quinolones.

4.3 Opportunistic infections

Subjects who develop opportunistic infections, including but not limited to pneumocystis jiroveci pneumonia, mycobacterial diseases, cytomegalovirus (CMV), and fungal infections will be treated using standard regimens. All opportunistic infections will be discussed with the Principal Investigator. Consultation with the Infectious Disease Service is mandatory for subjects diagnosed with mycobacterium tuberculosis.

4.4 Anemia

If subject develops symptomatic anemia, or if the hemoglobin falls below 8 mg/dl transfusion may be considered. Appropriate evaluation for etiology of the anemia should be initiated. Erythropoietic growth factors will not be used.

4.5 Thrombocytopenia

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50,000/mm3. Mitomycin C in combination with 5-FU will not be administered unless the platelets are ≥ 100, 000/mL,

4.6 Neutropenia and Neutropenic Fever

Patients who develop febrile neutropenia will be hospitalized and treated with intravenous antibiotics. The use of filgrastim will not be routinely used. Filgrastim will not be used at the same time as radiation therapy, but may be used 96 hours before radiation therapy or immediately after the last radiation fraction to maintain an absolute neutrophil count > 500 / mm3.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection, Patient Confidentiality and Data Disclosure

- Clinical data and acquired images will be recorded in a NCI CCR database (C3D).

- Clinical data collection for participants receiving radiation therapy will include: demographic information, pathologic diagnosis, clinical stage, history including prior and concurrent therapies, CT/ PET reports, lab reports, vital signs, pain assessment, need for opiate analgesia, photograph of treatment area, treatment breaks, dose of radiation delivered, and toxicity assessment.
- The results of any procedures and/or tests will be included in the patients' hospital chart and/or research records as appropriate. Data from these records may be used in research and scientific publications.
- Data generated from correlative samples will be maintained on databases on secure computers maintained by HAMB research nurses. Unique identifiers (no personal info) are assigned to samples prior to release of samples to other research laboratories. Data from participating laboratories will be batched and reported to HAMB every six months. HAMB will be responsible for reporting this data to the principal investigator every six months. Data from these studies may be used in research and scientific publications.
- Data collected may be used anonymously, for publications not originally specified, concerning the natural history of disease processes and long term effects of treatment. The data may also be used as the basis for entirely new protocols.
- Patient medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. All reports and communications relating to patients in this study will identify each patient only by their initials and patient identification number. Medical information resulting from a patient's participation in this study may be given to the patient's personal physician or to the appropriate medical personnel responsible for the patient's welfare, only with appropriate written authorization from the patient. Data generated as a result of this study are to be available for inspection on request by FDA or other government regulatory agency auditors, the Sponsor (or designee), and the Institutional Review Board (IRB/IEC).
- The information developed in this clinical study will be used by the Sponsor in the clinical development of the study drug and therefore may be disclosed by the Sponsor as required to other clinical investigators, to other pharmaceutical companies, to the FDA and to other government agencies.
- Any information, inventions, or discoveries (whether patentable or not), innovations, suggestions, ideas, and reports, made or developed by the Investigator(s) as a result of conducting this study shall be promptly disclosed to the Sponsor and shall be the sole property of the Sponsor. The Investigator agrees, upon the Sponsor's request and at the Sponsor's expense to execute such documents and to take such other actions, as the Sponsor deems necessary or appropriate to obtain patents in the Sponsor's name covering any of the foregoing.
- The results of this study will be published as a collaboration of all investigators under the direction of the Sponsor. Results will not be published without prior review of all of the Study data by each investigator and approval by the Sponsor.

5.2 Response Criteria

5.2.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response at 4 weeks after completion of therapy and at 3, 6, 9, and 12 months. In addition to a baseline scan, confirmatory scans should also be obtained at 12 weeks (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

5.2.2 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with MTS-01.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

5.2.3 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. As this study evaluates on patients with T2-4, N0-3, target lesions will include only the primary tumor and local lymph nodes, up to a maximum of 5 lesions in total, which should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), but in addition should be those that lend themselves to reproducible repeated measurements. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

5.2.4 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET FDG-PET will be performed at baseline and three months after completing concurrent chemoradiation. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate data obtained from FDG-PET scanning to complement CT scanning in assessment of disease response or progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of possible progressive disease (PD) based on a new lesion. In equivocal cases of FDG-avidity on follow up, a biopsy of the effected region may be performed, and if no evidence of anal cell carcinoma is noted, this will not be considered PD.
- FD-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image. However, it should be noted that in patients with HIV, lymph nodes commonly have FDG avidity due to reactive

processes, especially in the setting of HIV viremia.[43] Therefore, a 'positive' PET finding must also be isolated to a suspicious lymph node or mass, and not occur within the setting of diffuse nodal adenopathy.

5.2.5 Response Criteria

5.2.5.1 Evaluation of Target Lesions

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD):	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

5.2.5.2 Evaluation of Non-Target Lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD:	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

5.2.5.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

5.2.5.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Progression-Free Survival (PFS)
PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Disease-Free Survival
DFS is defined as the duration of time from start of treatment to time of progression.

Overall Survival (OS)
OS is defined as the duration of time from start of treatment to time of death.

5.3 Toxicity Criteria

5.3.1 CTCAE v4.0

- The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

5.3.2 RTOG Morbidity Scoring Schema

- EORTC/RTOG Skin toxicity will be measured at baseline, weekly during treatment, and at 4 weeks, 3 months, 6 months, 9 months, and 12 months after completion of therapy with the RTOG Morbidity Scoring Schema.
- The RTOG Acute Radiation Morbidity Scoring Schema will be used at baseline through week 4 of follow-up (http://www.rtog.org/members/toxicity/acute.html).
- The Late RTOG Morbidity Scoring Schema will be used at Months 3-12 of follow-up. (http://www.rtog.org/members/toxicity/late.html)

5.3.3 Photography of treatment area

Photographs of the area in which the Tempol is applied will be obtained pre-treatment, weekly during treatment, and at each protocol follow-up time point. These photographs will be taken with a digital camera in the Radiation Oncology Branch. Patients will be photographed specifically at both inguinal areas and the gluteal cleft. Every effort will be made to have these images obtained at a similar distance (approximately two feet) and similar lighting conditions (ie. Fluorescent lighting) with a disposable ruler in the image field for scale. Images will be stored on the ROB clinical research folder on the ROB group drive and will be password protected. Images will be identified only by subject number and timing (ie. Patient 1 week 1 of therapy, Patient 1 week 3 of follow-up). An ROB physician (not the PI) that has been minimally involved in the care of these patients will review the images for each patient after the completion of therapy and follow up to score the RTOG toxicity in a blinded fashion. This will allow a blinded comparison to the treating physician's evaluation.

5.4 Sample Storage, Tracking, and Disposition

It is understood that per the NCI policy regarding the Requirements for the Research Use of Stored Human Specimens and Data, prospective NIH IRB approval and continuing IRB oversight must be obtained for research involving identified or coded samples or data where investigators can identify the source. This policy applies to research protocols where the remaining research activities are limited to data analysis and to the subsequent research use of specimens or data previously collected under a now terminated protocol. The following guidelines describe how these principles apply to this specific protocol.

5.4.1 AIDS Monitoring Laboratory

Many samples, including blood and cytology, will be processed and stored in the AIDS Monitoring Laboratory (AML) run by Science Applications International Corporation (SAIC) in the NCI-Frederick facility located with Fort Detrick. The samples are stored under code, and the information linking these unique codes to the patients is kept on the AML database. The laboratory informatics system conforms to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. All laboratory staff is trained to adhere to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. Computers used to access inventory systems require username and password for login. The laboratory database is housed in a secure, protected environment and backups are performed routinely. Access to specimen information, clinical data, and stored specimens is limited to approved laboratory staff and the investigator in charge of the study (or individuals authorized by the investigator). Clinical testing of all samples will be one in

accordance to the protocol. The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The HAMB research nurses on the study will be in charge of tracking this information for the protocol team. Any questions regarding samples stored in the AML may be directed to Thomas Uldrick, Kathleen Wyvill, Karen Aleman, or Robert Yarchoan.

5.4.2 Tracking Samples

The protocol team, generally research nurses from the HAMB, will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. A research nurse from the HAMB will be in charge of tracking information on coding for samples stored in the AML, or processed by collaborating laboratories (see section 5.4.3 below) for the protocol team.

5.4.3 Samples for Planned Collaborations

5.4.3.1 HIV Viral Studies: Specimens will be sent to the Laboratory NCI DRP core lab, NCI-Frederick, Building 535, c/o Mary Kearney Frederick, MD, Phone 301:846-6796. The samples sent are coded by the protocol research team and have no patient identifiers. They are logged in and kept in a locked facility. They are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

5.4.3.2 Frozen Core Needle Biopsies: Specimen will be delivered on ice to the Laboratory of Pathology, where they will be snap-frozen. Specimens will be transported to the Laboratory of Robert Yarchoan (Building 10, Room 5A29), where they will be stored in a locked facility, and identifiable using the Clinical Center patient identification. Any samples that are retrieved from storage for use in future studies will be coded by the protocol research team and will have no patient identifiers.

5.4.3.3 Optional Rectal Mucosal Snag Biopsies

- One sample will be sent to the Laboratory of Clinical Pathology for histology, marked with Clinical Center patient identifying data
- Samples for flow cytometry and HIV studies will be coded by the HAMB research team, with labels containing no identifying patient information, then sent, respectively, to the Laboratory of Irini Sereti: Building 10, 11B07A, Bethesda, MD), or the NCI DRP core lab:

  NCI-Frederick
  Building 535, c/o Mary Kearney
  Frederick, MD
  Phone 301:846-6796

5.4.4 Routine Samples

Many routine samples and a sample of the biopsy specimens are sent to the Clinical Pathology or Pathology Departments of the NIH Clinical Center. These samples will be handled according to the procedures of these departments.

5.4.5 Unused, Lost or Destroyed Samples

At the termination of the study, if patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples will be transferred to that study unless the patients request that this not occur. Also, if patients have co-enrolled on other studies approved by the NCI IRB that call for maintaining the samples, then they will be maintained on those protocols. Otherwise, the unused samples will be destroyed.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss. If the patient withdraws consent the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI

6 STATSTICAL SECTION

This is a pilot study with the primary endpoint of determining the safety and tolerability of delivering MTS-01 gel daily in groin and gluteal cleft of patients receiving combined therapy with MMC, 5-FU, and RT for carcinoma of the anal canal. Although Tempol gel is expected to reduce dermatologic toxicity when given concurrently with irradiation, there have been reports of rash. Data from studies including IMRT with concurrent mitomycin-C and 5-FU have reported grade 3 dermatologic toxicity at rates of 0-38% while grade 2 toxicity occurs nearly uniformly.[14, 15] These rates are based on prospective non-randomized data. Specifically, we will estimate the rate of Grade 3 or greater dermatologic toxicity in the areas of skin treated with Tempol, by describing the observed frequency with 80% confidence intervals.

For example, within this pilot study of 15 patients, if the true toxicity rate is 20%, the expected exact 80% confidence interval would be (0.07, 0.33). If the true toxicity level is 30%, then the expected exact 80% confidence interval would be (0.16, 0.50).

Although not the primary endpoint, for the regimen to be considered successful at reducing toxicity, we would expect a lower than 30% rate of grade 3 dermatitis and less than a 100% rate of grade 2 dermatitis. The use of control sites (C1 and C2) will also a better attribution of any observed toxicity (for example attribution of skin toxicity to the MTS-01 versus radiation toxicity) and observed benefit (difference in skin toxicity in radiated areas where MTS-01 is applied or omitted). Comparisons of toxicity at control sites in relation to sites radiated and treated with MTS-01 will be reported with descriptive statistics.

The estimated rate of enrollment is 8 patients per year with an estimated accrual time of two years.

Secondary objectives will be evaluated in a descriptive fashion and include additional description of the rates and severity (as graded by CTCAE) of skin toxicity in patients treated with this regimen, a description of the proportion of patients requiring treatment breaks for treatment related toxicities with this regimen, and a description of the opiate requirements in patients treated with this regimen. We will also describe response rates as well as progression-free and overall survival at 12 months.

Additional correlative assays are planned that will be performed with the intent of descriptive analyses. These include evaluation of the effects of antiretroviral therapy, 5-fluorouracil, mitomycin C, and radiation on low-level persistent HIV viremia and HIV genetic diversity during therapy and recovery and evaluation of the feasibility of collecting HIV RNA and mononuclear cells from rectal associated lymphoid tissue for correlative studies.

Every attempt will be made to enroll patients with diverse racial/ethnic backgrounds and of both genders. No racial or ethnic groups are excluded.

7 HUMAN SUBJECTS PROTECTIONS

7.1 Rationale for Subject Selection:

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. This study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer.) Participants should realize that there is no guarantee of benefit to them from participation in this trial. The results of this trial may benefit future cancer patients. To date, there is no information that suggests that differences in skin absorption, drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully. Inclusion of Women and Minorities: This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met.

Pregnant women are excluded from this study because the effects of MTS-01 on the developing fetus is unknown and because radiation is a known teratogen. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MTS-01, 5-FU, and MMC, breastfeeding should be discontinued if the mother is treated on this protocol. Participants with unstable or serious medical conditions such as uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements are excluded due to the possibility that the underlying condition may obscure the attribution of effect and adverse events and may limit study compliance.

7.2 Participation of Children:

This study includes patients 18 years of age and older. Because insufficient dosing or adverse event data are currently available on the use of MTS-01 in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials. Studies will be performed in patients <18 years of age when it is appropriate to do so.

7.3 Evaluation of Benefits and Risks/Discomforts:

Participants will receive treatment with 5-FU, MMC, and radiotherapy to the pelvis and anal carcinoma. It is not expected that the risks of this therapy will be increased with the use of topical MTS-01 delivered concurrently as a radiation protector. The risks of radiotherapy to the pelvis and anal canal include dermatitis, proctitis, cystitis, lowered blood counts, and fatigue. The risks of 5-FU include lowered blood counts, infection, mucositis, diarrhea, nausea and uncommonly neurotoxicity. The risks of MMC include lowered blood counts, nausea, severe skin damage if not administered correctly and rarely severe kidney damage with associated low platelets (hemolytic uremic syndrome). Patients may experience local skin reactions from MTS-01 application but it is anticipated that the severity of radiation dermatitis in this area will be reduced. With application of a topical radioprotector there is concern about protection of tumor from irradiation. Based on the available pharmacokinetic data from animal studies and humans, this is considered unlikely. The protocol therapy could allow treatment to be delivered without treatment breaks and with decreased side effects. In addition to the risks of protocol therapy, participants may undergo optional biopsies. The risks of biopsy of an anal canal lesion or rectal mucosa include minor bleeding, pain, and infection. Biopsies will only be performed if they are considered minimal risk.

7.4 Risks/Benefits Analysis:

MTS-01 is considered to have an acceptable safety margin when studied in the topical setting. The known discomforts of MTS-01 application include pruritis and burning, which are also known discomforts of radiation exposure. In this study using MTS-01, a conservative approach has been chosen to minimize the likelihood of tumor protection or subclinical disease protection. A 3 cm margin of uninvolved perianal skin will not receive treatment with MTS-01. It is possible that MTS-01 will protect the skin from radiation. This could result in decreased need for treatment breaks and increased comfort during radiation.

In summary the potential risk: benefit ratio for a Pilot study is considered to be acceptable in light of potential benefits, the initial patient eligibility criteria, and proposed monitoring of patients.

Optional biopsies may also result in a risk to the patient. The risks of biopsy include bleeding, infection, pain, and site specific side effects. Biopsies will only be performed if they are felt to be of minimal risk and if the patient consents. While encouraged, biopsies are not mandatory for participation in this study.

7.5 Consent and Assent Process and Documentation:

7.5.1 The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the treatment subject. A signed, informed consent document will be obtained from the subjects by one of the physician investigators.
7.5.2 Consent forms: The original signed informed consent documents will be kept with patient medical records. Central Registration will also retain a copy of the informed consent document. A copy of their own signed informed consent documents will also be given to study participants.
7.5.3 Central Registration will ascertain the date of IRB approval before registering the first subject.
7.5.4 The treatment subject informed consent forms contain all elements required for consent. In addition, the Principal Investigator, or their designee will obtain oral consent and will be available to answer all patient questions.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

8.1 Definitions

8.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section 5.1

AEs that will be specifically excluded from reporting include:

1. Asymptomatic hyperuricemia of any grade related to advanced HIV and/or its therapy
   Any grade lymphopenia (because this is a frequent occurrence in HIV disease), unless it represents an 80% decrease from entry value, a decrease of at least 50 cells/mm3, and occurs in a patient whose HIV disease is well controlled with <10,000 virions/ml.
   Hyperbilirubinemia associated with protease inhibitor therapy for HIV
   Hypophosphatemia caused by disease and/or HAART medications such as Tenofovir.
2. Opportunistic infections related to HIV

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

8.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

8.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

8.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

8.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

8.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

8.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 NCI-IRB Adverse Event Reporting

8.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations

- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

8.2.1.1 Certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting. The following AEs must be reported through the routine reporting mechanism:

1. Asymptomatic hyperuricemia of any grade related to advanced HIV and/or its therapy
2. Any grade lymphopenia (because this is a frequent occurrence in HIV disease), unless it represents an 80% decrease from entry value, a decrease of at least 50 cells/mm3, and occurs in a patient whose HIV disease is well controlled with <10,000 virions/ml.
3. Hyperbilirubinemia associated with protease inhibitor therapy for HIV
4. Hypophosphatemia caused by disease and/or HAART medications such as Tenofovir.
5. Expected non-dermatologic side effects of mitomycin and 5-FU with radiotherapy
6. Opportunistic infections related to HIV

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

For reporting of adverse events at time of continuing review, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol ***since the previous continuing review and in aggregate****. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk: benefit determination. Please sort the events by the system organ class and by grade. The summary report is based on the following guidance: any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk: benefit of study participants in the narrative.*

Please use following table for reporting adverse events at time of CR:

System Organ Class	CTCAE Term	Grade	# of Events since last CR	Total # of Events	Attribution to Research	Serious?	Unexpected?

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

8.2.3 NCI IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

8.3 IND Sponsor Reporting Criteria

An investigator must **immediately** report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

8.4 FDA Reporting Criteria (Refer to 21 CFR 312.32)

8.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any:

- suspected adverse reaction that is both serious and unexpected
- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

8.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study IND holder will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

8.4.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer

SAEs, including death due to any cause, which occur during the treatment and within the first 30 days after treatment with the study drug, whether or not related to the administration of study drug, must be reported immediately (within 24 hours or one business day, of learning of the event) to the Sponsor and Mitos Pharmaceuticals. The manufacturer has agreed to accept the iRIS SAE report as CRF in addition to C3D.
The Sponsor will notify all concerned Investigators, Mitos Pharmaceuticals, and regulatory authorities of all adverse drug reactions that are both serious and unexpected, for which a causal relationship between the study drug and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. MITOS Pharmaceuticals: Mitos Pharmaceuticals, Inc., Three San Joaquin Plaza, Suite 200, Newport Beach, CA 92660.

8.5 Data and Safety Monitoring Plan

8.5.1 Principal Investigator/Research Team

Patient data will be collected in a timely manner and reviewed by a physician Associate Investigator and/or the Principal Investigator, Deborah Citrin, for toxicity. Any toxicity ≥ Grade 3 will be reviewed by the Principal Investigator. In the event that an unacceptable toxicity occurs, the IRB and Mitos Pharmaceuticals will be informed and appropriate measures, including notification of SAE to Mitos Pharmaceuticals and the NCI IRB as outlined in Section 8, will be taken. The Principal Investigator will do the monitoring of this pilot study in an ongoing manner, and a Data and Safety Monitoring Board will not be used.

The clinical research team will meet on a regular basis weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8.5.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, base on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

9 PHARMCEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

9.1 MTS-01

9.1.1 Source: Mitos, IND 112272

9.1.2 Toxicity

The toxicity reported for topical application of MTS-01 is summarized. Previous studies have reported few non-dermatologic toxicities thought to be related to MTS-01 including thrombocytopenia (suspected relationship, rare). Dermatologic events reported include rash, pruritis, and desquamation. Since subjects were all being treated for skin toxicity due to radiation therapy, it is difficult to assess whether these events are related to the study drug or to the radiotherapy. In data from 6 healthy volunteers, one subject experienced an event of application site erythema which was considered to be a mild, probably-related adverse event. The erythema resolved within an hour of onset without intervention.

9.1.3 Formulation and preparation

The drug product is MTS-01, which is a topical gel formulation of Tempol. The chemical name for Tempol is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl. The MTS-01 drug product is a topical gel containing 70 mg/mL of Tempol plus water, ethanol, and hydroxypropyl cellulose. The Sponsor will provide the drug product to the clinical site. Each container of MTS-01 will be labeled with the drug name, the caution statement, sponsor address, and contents.

9.1.4 Stability and storage

MTS-01 is stable at room temperature.

9.1.5 Administration procedures

A trained Radiation oncology nurse, associate investigator, or designee will administer MTS-01 topically. Health care providers will wear gloves while applying MTS-01. The gel preparation may be flammable; precautions should be taken to keep away from fire or flame or machinery and equipment that might cause sparking and hence, an explosion.

Before application, the area for Tempol administration should be marked and measured in at least 2 dimensions so that the area of the application can be calculated. Up to 100 mL of 70 mg/mL MTS-01 will be applied uniformly to the patient's targeted skin area 15 - 30 minutes prior to each fraction of external beam radiation. Care will be taken to make sure that the marked and targeted area is fully covered. MTS-01 will be administered daily prior to each fraction of radiation and will be washed off gently with warm soapy water within one hour after the completion of the daily radiation fraction. Care should be taken not to irritate the skin unnecessarily. Target markings on the skin should be refreshed as needed to accurately maintain radiation exposure areas. Usually permanent marks are used to set up the patient.

The study drug will be applied using a ***folded gauze pad or syringe*** to permit easy retrieval of the study product from the container. The test material will be evenly spread over the skin area targeted for radiotherapy. Additional material will be retrieved from the container and applied to the designated area until the volume of test article necessary to cover the treated area is used. The total amount of Tempol used for each radiotherapy treatment must be recorded on the Case Report Form (CRF). ***The application of Tempol to the skin should be allowed to dry enough to keep Tempol in place prior to radiation. Do not allow it to dry completely.***

The entire application procedure, should take approximately 15 minutes. The trained therapist or nurse will visually confirm that the skin is sufficiently dry before proceeding with positioning of the patient for radiotherapy. Radiation will be delivered within 15 - 30 minutes of drying of the MTS-01 application. MTS-01 application will continue for as long as the affected skin is receiving radiation or until criteria for dose modification are met.

9.1.6 Incompatibilities

No incompatibilities have been described. The chemical name for Tempol is 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl. Othersynonyms or research terms have been used previously to refer to Tempol. These terms include NSC-142784, 142784-J/6, hydroxy-tempo, 4-OH Tempo, and TMPN. The chemical structure of Tempol is provided in Figure 3.1. Tempol is a light orange, crystalline solid that is soluble in aqueous solutions. The molecular formula is $C_9H_{18}NO_2$, and the molecular weight is 172.28. In *in vitro* binding assays with 152 substrates to assess receptor and enzyme binding, Tempol showed no significant binding to any assayed substrate.

9.2 5-Fluorouracil

9.2.1 Source

5-FU is commercially available. The Clinical Center Pharmacy will obtain 5-FU from commercial sources.

9.2.2 Pharmacology

5-FU is a fluorinated pyrimidine antimetabolite. 5-FU resembles the natural uracil molecule in structure, except that a fluorine atom has been substituted for a hydrogen atom in the 5 position. T½ is 8-13 minutes. It is distributed in all body water by passive diffusion and crosses the

placenta. There is evidence that the metabolism of fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid. 5-FU generates fluorinated nucleotides (FdUMP, FUTP), which interfere with the synthesis of DNA and to a lesser extent inhibit the formation of ribonucleic division and growth by incorporation into RNA. The effect of fluorouracil may be to create a thymidine deficiency which provides unbalanced growth and death of the cell. Catabolism is via hepatic and extrahepatic routes via DPD to dehydrofluorouracil (DHFU), which is subsequently metabolized to fluoro-β-alanine (FBAL). Excreted as respiratory CO2, only 5% being excreted unchanged in the urine. Administration should be avoided in the presence of hepatic dysfunction.

9.2.3 Toxicity

Immediate side effects include mild nausea and vomiting (common), lacrimation, conjunctivitis, angina, arrhythmia, radiation recall and anaphylaxis (rare). Early onset effects include stomatitis and esophagopharyngitis (which may lead to sloughing and ulceration), diarrhea with cramping and/or bleeding, and anorexia. Leukopenia usually follows every course of adequate therapy with fluorouracil. The lowest white blood cell counts are commonly observed between the 9th and 14th days after the first dose, although uncommonly the maximal depression may be delayed for as long as 20 days. By the 30th day the count has usually returned to the normal range. Megaloblastosis may occur. Alopecia (usually mild) and dermatitis may be seen. The dermatitis most often seen is a pruritic maculopapular rash usually appearing on the extremities and less frequently on the trunk. Other side effects include myocardial ischemia, lethargy, malaise, headache, allergic reactions, neurotoxicity (disorientation, confusion, euphoria, dizziness, incoordination-acute cerebellar syndrome, encephalopathy), visual changes, photosensitivity (eyes and skin), nail changes including loss of nails, skin thickening, cracking, dryness or sloughing, biliary sclerosis, or acalculous cholecystitis. Hand foot syndrome (palmar plantar erythrodysesthesia) is more (palmar plantar erythrodysesthesia) is more common with continuous infusion. Late effects may include tear duct fibrosis and neurotoxicity.

9.2.4 Formulation and preparation

5-FU is a marketed drug available as 50 mg/ml in 10, 50 or 100 ml vials as a colorless to faint yellow preservative free aqueous solution, with pH adjusted to approximately 9.0 with sodium hydroxide. Compatible with NS, D5W. Administration of 5-FU should be only by the intravenous route taking care to avoid extravasation.

9.2.5 Stability and storage

Although 5-FU solution may discolor slightly during storage, the potency and safety are not adversely affected. Store at room temperature (49°-86°F) and protect from light. If a precipitate occurs due to exposure to low temperatures, resolubilize by heating to 140°F (60oC) with vigorous shaking; allow to cool to body temperature before using. Discard after 8 hours once vial is opened. Discard if yellow coloration is marked since this indicates decomposition. Stable in D5W for 30 days in plastic syringes at room temperatures. In portable infusion pump reservoirs 5-FU is stable for up to 7 days but precipitation may occur at low temperatures.

9.2.6 Administration procedures

Administration by continuous infusion for 96 hours (in 1 L normal saline or ambulatory infusion pump) which has the best therapeutic index. Each ambulatory bag will be prepared with 2000 mg/m^2 over 48 hours (2 bags total). To bags containing 1000 mg/m^2 to be infused over 24 hours may be used in lieu of 2000 mg/m^2 . Vein pigmentation and thrombophlebitis may be seen distal to infusion sites. A central venous access device is required. 5-FU infusion is compatible with heparin and leucovorin but not with intravenous ondansetron.

9.2.7 Incompatibilities

Incompatible with aldesleukin, amphotericin B cholesteryl sulfate complex, cytarabine, diazepam, droperidol, filgrastim, ondansetron, topotecan, vinorelbine

9.3 Mitomycin C

9.3.1 Source

Mitomycin C is commercially available. The Clinical Center Pharmacy will obtain Mitomycin-C from commercial sources.

9.3.2 Pharmacology

Mitomycin-C is an antibiotic with alkylating agent properties cross-linking guanine and cytosine, which selectively inhibits the synthesis of deoxyribonucleic acid (DNA) and degrades preformed DNA. This results in nuclear lysis and formation of giant cells. At high concentrations of the drug, cellular RNA and protein synthesis are also suppressed. Non-phase specific, but has maximum effects in late G1 and S.

In humans, mitomycin, which acts as a pro-drug is rapidly cleared from the serum after intravenous administration. It is distributed in kidneys, muscles, heart, lungs, intestines and ascites; and enters breast milk. Time required to reduce the serum concentration by 50% after a 30 mg bolus injection is 17 minutes. After injection of 30 mg, 20 mg, or 10 mg i.v., the maximal serum concentrations were 2.4 mcg/ml, 1.7 mcg/ml, and 0.52 mcg/ml, respectively. Clearance is affected primarily by metabolism in the liver, but metabolism occurs in other tissues as well. The rate of clearance is inversely proportional to the maximal serum concentration likely due to saturation of the degradative pathways.

Approximately 10% of a dose of mitomycin-C is excreted unchanged in the urine. Since metabolic pathways are saturated at relatively low doses, the percent of a dose excreted in urine increases with increasing dose. Small amounts are also found in the bile and feces. Administer with caution in the presence of impaired liver or renal function.

9.3.3 Toxicity

Immediate effects may include vein irritation, mild nausea and vomiting (1-2 hours) and bronchospasm (4-12 hours).
Early toxicities include thrombocytopenia and leukopenia which are cumulative. Nadir occurs at 24-28 days, with recovery at 42-56 days. Deaths due to septicemia have been reported.

Stomatitis is frequent but mild, alopecia is rare. Rashes are rare and blue bands may be seen in the nails. Mitomycin may also cause dyspnea with cough and radiographic evidence of pulmonary infiltrate. A few cases of adult respiratory distress syndrome (interstitial pneumonitis) have been reported; also fever, anorexia, fatigue, blurred vision, amenorrhea, edema, thrombophlebitis, hematemesis, elevated LFTs or diarrhea may occur. CNS effects may include syncope and rarely acute encephalopathy. Delayed toxicities (weeks to months) include chronic pulmonary fibrosis, erythema and/or ulceration occurring either at or distant from the injection site, rising creatinine, microangiopathic hemolytic anemia, and renal failure. Note: Mitomycin is a vesicant and cellulitis, necrosis, ulceration and consequent sloughing of tissue may result if the drug is extravasated during injection.

9.3.4 Formulation and preparation

Each vial contains either mitomycin-C 5 mg and mannitol 10 mg or mitomycin-C 20 mg and mannitol 40 mg. To administer, add sterile water for injection, 10 ml or 40 ml respectively to a concentration of 0.5 mg per ml. Shake to dissolve. If product does not dissolve immediately, allow to stand at room temperature or shake vial under warm tap water for 2 minutes to assist dissolution. Reconstituted mitomycin is a clear blue or purple solution. If reconstituted to higher concentrations (1-2 mg/ml) avoid low temperatures because crystals may form.

9.3.5 Stability and storage

1) Unreconstituted: Mitomycin-C is stable for the lot life indicated on the package. Store at room temperature. Avoid excessive heat (over 40°C) and keep away from light.
2) Reconstituted Mitomycin-C is stable for 14 days refrigerated or 7 days at room temperature (up to 30oC). Higher concentrations than 0.5 mg/ml may degrade and precipitate if stored.
3) Compatible with D5W, NS and Ringer's lactate Diluted in various i.v. fluids at room temperature, to a concentration of 20 to 40 micrograms per ml:

IV Fluid	Stability
5% Dextrose Injection	3 hours
0.9% Sodium Chloride Injection	12 hours
Sodium Lactate Injection	24 hours

9.3.6 Administration procedures

Mitomycin-C should be given intravenously only through a central catheter, using care to avoid extravasation. Give as slow push through sidearm of free flowing i.v. at 1.5 mg/3 ml per minute. Alternatively, may be mixed in 50-100 ml minibag over 10-30 minutes.

9.3.7 Incompatibilities

Incompatible with aztreonam, cefepime, etoposide phosphate, filgrastim, gemcitabine, piperacillin/tazobactam, sargramostim, topotecan, vinorelbine

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11 APPENDICES

APPENDIX I : RTOG Acute Radiation Morbidity Scoring Criteria

Name _____MR# _____

ACUTE SKIN TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Skin	None	Follicular, faint or dull erythema/ epilation/dry desquamation/ decreased sweating	Tender or bright erythema, patchy moist desquamation/ moderate edema	Confluent, moist desquamation other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis	Death directly due to radiation toxicity

GRADE FOR TREATED AREAS (T)

Grade for LEFT GROIN (inguinal region) _____

Grade for RIGHT GROIN (inguinal region) _____

Grade for GLUTEAL CLEFT _____

GRADE FOR CONTROL AREAS (C1 AND C2)

Grade for irradiated region NOT treated with MTS-01 in left inguinal region (C1)

_____

Grade for area treated with MTS-01 alone near umbilicus (C2)

_____

PHYSICIAN _____ DATE _____

APPENDIX II: ROTG Late Radiation Morbidity Scoring Criteria

Name _____MR# _____

LATE SKIN/ SUBCUTANEOUS TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Skin	None	Slight atrophy Pigmentation change Some hair loss	Patch atrophy; Moderate telangiectasia; Total hair loss	Marked atrophy; Gross telangiectasia	Ulceration	Death directly due to radiation toxicity
Subcutaneous tissue	None	Slight induration (fibrosis) and loss of subcutaneous fat	Moderate fibrosis but asymptomatic Slight field contracture <10% linear reduction	Severe induration and loss of subcutaneous tissue Field contracture >10% linear measurement	Necrosis	Death directly due to radiation toxicity

GRADE FOR TREATED AREAS ONLY (T)
Skin

Grade for LEFT GROIN _____

Grade for RIGHT GROIN _____

Grade for GLUTEAL CLEFT _____

Subcutaneous tissue

Grade for LEFT GROIN _____

Grade for RIGHT GROIN _____

Grade for GLUTEAL CLEFT _____

APPENDIX III: Processing Tissue for Molecular Characterization of Anal Cancer

I. INTRODUCTION:

A. SCOPE AND PURPOSE:
1. To establish a procedure for tissue processing and storage for the NCI's Office of Cancer Genomics (OCG) and Office of HIV and AIDS Malignancies (OHAM) Tumor Characterization Project, which aims at generating high quality data on anal cancer's genome and transcriptome using second-generation sequencing technology.
2. This protocol applies to anal cancer tissue collected prospectively by Investigators in the HIV and AIDS Malignancy Branch (HAMB, CCR).
3. Any deviation from this protocol should be noted in the lab notebook, indicating nature of deviation, times and which samples were affected. This information should be given within 48 hours to an HAMB clinical Investigator (Dr. Thomas Uldrick, uldrickts@mail.nih.gov or Dr. Robert Yarchoan yarchoan@helix.nih.gov).

B. SAFETY PRECAUTIONS:
1. Individuals processing biospecimens must wear personal protective equipment (PPE) such as lab coats and gloves.
2. Liquid nitrogen is extremely cold and can cause 'burns'. Wear gloves that are specially made to withstand liquid nitrogen, eye protection (preferably Face Shield) and a lab coat to protect skin from splashes and spills. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
3. Acute overexposure to formaldehyde solutions and/or vapors causes severe eye, skin, and respiratory tract irritation.

C. EQUIPMENT AND MATERIALS:
1. Personal protective equipment (PPE) to include heavy duty gloves, eye protection (preferably Face Shield), lab coat and closed-toe shoes
2. Plastic cassette mold(s) for Formalin fixation.
3. Cryovials (2mL vials from ChartBiomed, Part Number 10778828)
4. Freezer resistant labels with project-assigned ID (obtained from HAMB Research Team)
5. Dewar thermo-flask
6. Isopentane
7. Liquid Nitrogen
8. Formalin (10% solution)
9. Fine point Cryomarker (such as Nalge Nunc Cryomarker Black #6313-0020)
10. **MARK ALL CONTAINERS WITH THE LABELS CARRYING PATIENT PROJECTASSIGNED ID OBTAINED PRIOR TO SURGERY**.

II. PROCEDURE:
A. 1-2 16 gauge core needle biopsies should be processed as follows:
1. Wearing sterile gloves cut the core into 2 sections as described below using a

sterile scalpel.

2. Place tissue into various containers as follows:
   i. **24-HOUR FORMALIN FIXATION**: Submit a 1 mm representative tissue piece for processing and diagnosis in the Laboratory of Pathology.
   ii. **FREEZING TISSUE**: Select remaining representative pieces of tissue for freezing. Freeze as many pieces as possible. Freeze the tissues as described below (Do not freeze the tissue with Freon):
      a. Perform snap freezing of fresh tissue ASAP
         i. It is generally accepted that for the best tissue preservation snap freezing should take place within 20 minutes after tissue is obtained from patient.
         ii. Do not perform snap freezing with bare hands. Wear gloves at all times.
      b. Set Up Freezing Station
         i. Fill a small 100 ml metal beaker about 1/4 full with isopentane (2-methylbutane, certified grade).
         ii. Fill the Dewar thermo-flask about 1/3 full with liquid nitrogen.
         iii. Use extreme caution when dispensing liquid nitrogen
      c. Label Cryovial As Needed
         i. Use a cryomarker for labelling.
         ii. Label cryovial with freezer-resistant labels obtained prior to surgery.
      d. Freezing Tissue in Cryovial
         i. Cut a 1 cm by 3 cm strip of histowrap
         ii. Put the tissue on the histowrap strip
         iii. Place the histowrap strip containing tissue into a labelled cryovial,using a pair of forceps.
         iv. Screw on the cap **tightly** or else isopentane will seep into the vial during freezing and create a liquid in the vial upon thawing.
         v. Lower the 100 ml metal beaker containing isopentane half-wayinto the liquid nitrogen for cooling. The liquid nitrogen will boil as the beaker is lowered, when the isopentane is reaching its freezing point the tone of the boiling will increase for 2-3 seconds.
         vi. Lift the beaker out of the liquid nitrogen once you see beads of solid isopentane at the bottom of the beaker (about 2 minutes).
         vii. Use long forceps to hold the cryovial down into the cooled isopentane. Hold for at least 1 minute.
         viii. Use the long forceps to take out the cryovial/ frozen tissue.
         ix. Store Frozen tissue vial(s) in Liquid Nitrogen Storage Tanks.
         x. **THE FROZEN SPECIMENS SHOULD BE KEPT FROZEN ON DRY ICE AT ALL TIMES DURING TRANSPORT TO AND FROM STORAGE TANKS**.

B. Make a gross report of the sample using the dictation template below.

C. Any questions regarding this protocol should be directed to the HAMB Investigators, Dr. Thomas Uldrick or Dr, Robert Yarchoan.

ANAL CANCER STUDY GROSS DICTATION TEMPLATE

History:

The patient is a XX year old M/F, diagnosed with HIV (if HIV+) in YEAR, current HIV viral load is XXXX, CD4 count is XXX, diagnosed with STAGE X anal cancer (MONTH/YEAR)

Source/Gross:

The specimen is received (**fresh vs. fixed**) in (**# containers**), each labeled with the project-assigned ID "#" and designated "#." The specimen consists of (**gross to include number of fragments, size, appearance, etc.**)

Specimens submitted are:

Fixed in formalin for 24 hours – (**size, # of pieces in each block, and cassette designation**)

Snap Frozen – (size and # of blocks**)**

Appendix IV: Brief Pain Inventory

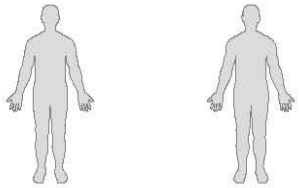
Brief Pain Inventory

Date: _____ Time: _____

Name: _____

1) Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?
1. Yes 2. No

2) On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3) Please rate your pain by circling the one number that best describes your pain at its **worst** in the past 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain — *Pain as bad as you can imagine*

4) Please rate your pain by circling the one number that best describes your pain at its **least** in the last 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain — *Pain as bad as you can imagine*

5) Please rate your pain by circling the one number that best describes your pain on **average**.

0 1 2 3 4 5 6 7 8 9 10
No pain — *Pain as bad as you can imagine*

6) Please rate your pain by circling the one number that tells how much pain you have **right now**.

0 1 2 3 4 5 6 7 8 9 10
No pain — *Pain as bad as you can imagine*

7) What treatments or medications are you receiving for your pain?

_____
_____
_____
_____

8) In the past 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0% 10 20 30 40 50 60 70 80 90 100%
No relief — *Complete relief*

9) Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General activity
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

B. Mood
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

C. Walking ability
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

D. Normal work (includes both work outside the home and housework)
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

E. Relations with other people
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

F. Sleep
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

G. Enjoyment of life
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

H. Ability to concentrate
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

I. Appetite
0 1 2 3 4 5 6 7 8 9 10
Does not interfere — *Completely interferes*

APPENDIX V: TISSUE COLLECTION FOR THE HPV-RELATED CANCERS / HIV+ TUMOR MOLECULAR CHARACTERIZATION PROJECT

V.1 Objectives

To obtain appropriate specimens and patient consent for the use of their samples and associated annotation in order to perform genetic and proteomic analyses. The genetic analyses will be performed by **HPV-Related Cancer Project, part of the HIV+ Tumor Molecular Characterization Project**, a network of collaborating institutions sponsored by the National Cancer Institute (NCI).

V.2 Rationale

The purpose of the **HPV-Related Cancer Project** is to discover somatic genetic changes associated with cancer to lead to better ways to prevent, detect, and treat cancer. The data generated may be used to study other diseases as well.

The **HPV-Related Cancer Project** is designed to identify most of the somatic genetic changes that can cause cancer in people. Therefore, the **HPV-Related Cancer Project** would like to study the genetic material from the retrospective collections of cancer tissue, in which statistically powerful sample numbers have already been collected; as well as prospectively collected cases. The genetic material (DNA, RNA) from the cancer tissue will be compared to germline genetic material from the normal tissue and/or blood to find the differences that exist. By combining the genetic information with clinical information it may be possible to identify the genetic changes that are associated with a particular type of cancer. This same process of analysis will be repeated with many (hundreds of) samples for this research project. By studying many **HPV-Related Cancers** in this way, the **HPV-Related Cancer Project** expects to identify most of the genetic changes associated with this kind of cancer. Since the **HPV-Related Cancer Project** may also combine genetic information with information from medical records, such as the tissue pathology and responses of different cancers to different treatments, this project could lead to more knowledge about why certain cancers respond differently to treatments. With such knowledge, future treatments potentially could become customized to a patient's unique genetic make-up.

V.3 Description of the Research

V.3.1 Eligibility of Subjects

All subjects (both HIV-infected and HIV uninfected) enrolled in "A Pilot Trial Assessing the Feasibility of Delivering Topical MTS-01 to Reduce Dermatitis in Patients Receiving Intensity Modulated Radiation with Concurrent 5-Fluoruracil and Mitomycin-C for Stage I-III Carcinoma of the Anal Canal" are eligible for the HPV-Related Cancer Project

V.3.2 Collection of Samples and Medical Information

Patients with a diagnosis of anal cancer that consent to participation in the **HPV-Related Cancer Project** will have tumor tissue collected through snag biopsies of the primary tumor, as well as collection and storage of matching blood. Up to 15 patients will have tissue collected within this protocol, and these samples will be pooled with samples from other investigators for analysis.

Sample amount requirement specifications:

- Approximately 3 mm^3 mg of solid tumor
- 10 ml blood collected in ACD (or enough to obtain 50 ug of germline DNA)

Specifically, this consent to have tissue collected for the **HPV-Related Cancer Project** allows for:

- Permission to obtain solid tumor tissues donated by the patient.
- Permission to collect a sample by drawing about 4 tablespoons of blood from a vein.
- Permission to collect information from the patient medical records, including age, ethnic background, diagnosis, disease history, medical treatments, and response to treatments.

V.3.3 Coding of Tissue Samples and Medical Information

Tissues, blood sample, and medical information will be labeled with a code.
Only the PI, Lead Associated AI, and Research Coordinators will have the information that matches the code to traditionally-used identifying information, such as the patient name, address, phone number, medical record number, or social security number. (See Section 5.4.3.2.) The PI and Lead AI will keep the information that matches the code to this traditionally used identifying information in a safeguarded database. Only authorized people, who have specifically agreed to protect patient identity, will have access to this database. All materials conveyed to the **HPV-Related Cancer Project** will be labeled with a project-assigned ID, removing traditionally used identifying information, such as your name, address, phone number, or social security number. All other researchers and personnel, including those who will be working with the patient samples and medical information, will not have access to any of the traditionally used identifying information about the patient.

V.3.4 Storage and Release of Samples and Medical Information

Samples will be stored in the Laboratory of Dr. Robert Yarchoan, and released to the **HPV-Related Cancer Project** in batches. Coded tissue samples will be sent to an NCI-sponsored storage facility, designated for the **HPV-Related Cancer Project**. The samples will be processed there and portions of the molecular analyses extracted from samples then will be sent to different types of laboratories as part of this project. The remaining portions of the samples may be stored in the Laboratory of Dr. Robert Yarchoan or at an **HPV-Related Cancer Project** storage facility under this protocol. At the end of this protocol, remaining samples will be disposed of, or if patients have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples will be transferred to that study unless the patients request that this not occur. (See Section 5.4.5.)

Coded clinical data will be sent for reformatting into data structures compliant with standards developed by the NCI cancer Biomedical Informatics Grid (caBIG) standards. That data will then be deposited into a central **HPV-Related Cancer Project** project database, where it will be integrated with the molecular profiling data generated with the DNA and RNA. Only data stripped of identifiers, in compliance with the definition specified in the HIPAA Limited Data Set definition, will be sent from subjects enrolled in this study.

V.4 Research Plan and Methods

As part of the **HPV-Related Cancer Project, the** Genome Sciences Center at the British Columbia Cancer Agency will perform 30X genome sequencing of 100 cases of paired tumor and germline DNA, along with transcriptome sequencing of the HIV+ tumors. These platforms allow discovery of mutations both in coding and non-coding genomic regions, gene expression and genomic alterations (including translocations, insertions and deletions). Comparing tumors of cancer patients both with and without HIV-infection will provide insight into the potential function of this virus in certain cancers.

V.4.1 Data

- Information (data) from analyses of the coded samples and the coded medical information will be put into databases along with information from the other research participants. These databases will be accessible by the Internet.
- Coded medical information and information from more detailed analyses of the coded samples will be put in a controlled-access database. The information in this database will be available only to researchers and institutions who have received approval from an NIH Data Access Committee after certifying their adherence to patient data protection policies for the project.
- Anonymous information from the analyses will be put in a completely public database, available to anyone on the Internet.
- ***Traditionally used identifying information about the patient, such as name, address, telephone number, or social security number, will NOT be put into either the public or controlled-access databases for this project.***

V.5 Recontact

In the future, we may want to obtain additional samples or follow-up information about the patient health or medical care. Should this be needed, and if the patient consents to future contact, this protocol permits a researcher to contact the patient to ask whether the patient would be interested in participating in this additional research.

V.6 Financial Compensation/Costs

Patients will not be paid to participate in this project. Tissue samples and the medical information will be used only for research purposes and will not be sold. It is possible that some of the research conducted using the samples or information eventually will lead to the

development of new diagnostic tests, new drugs or other commercial products. Should this occur, the patient will not receive any part of the profits generated from such products.

The patient will not incur any expenses from participating in this project.

The chance that the patient will be physically injured as a result of participating in this project is very small. However, if the patient is physically injured as a result of participating in this project, emergency medical treatment for the patient's research-related injury will be provided to the patient at no cost.

V.7 Potential Benefits of Participating in the Project

The patient should not expect to personally benefit from this research. The main reason the patient may want to participate is to help researchers and health professionals around the world to better understand the causes of cancer and other diseases so that they can find better ways to prevent, detect, treat, and cure such illnesses. We hope that the participant will feel good knowing that they may be helping future patients with cancer or with other diseases.

V.7.1 Potential Physical Risks

- If a blood sample is NOT taken, there are no physical risks associated with this project.
- If a blood sample is taken, there are very few physical risks. Possible side effects from drawing the blood sample include mild pain, bleeding, bruising, and infection at the site of the needle insertion. Fainting or light-headedness can sometimes occur, but usually last only a few minutes.

V.7.2 Potential Psychological or Social Risks Associated with Loss of Privacy

Patient privacy is very important to us and we will use many safety measures to protect their privacy. However, in spite of all of the safety measure that we will use, we cannot guarantee that the identity of the patient will never become known. Although the genetic information is unique to the patient, he/she does share some genetic information with their children, parents, brothers, sisters, and other relatives. Consequently, it may be possible that genetic information from them could be used to help identify the patient. Similarly, it may be possible that genetic information from the patient could be used to help identify the relatives.

While neither the public nor the controlled-access databases developed for this project will contain information that is traditionally used to identify the patient, such as the patient name, address, telephone number, or social security number, people may develop ways in the future that would allow someone to link the patient genetic or medical information in **HPV-Related Cancer Project** databases back to the patient. For example, someone could compare information in our databases with information from the patient (or a relative) in another database and be able to identify the patient (or the patient's relative). It also is possible that there could be violations to the security of the computer systems used to store the codes linking patient's genetic and medical information to the patient.

Since some genetic variations can help to predict the future health problems of the patient and the patient's relatives, this information might be of interest to employers, health providers, insurance companies, and others. Patterns of genetic variation also can be used by law enforcement agencies to identify a person or his/her relatives. Therefore, the patient genetic information potentially could be used in ways that could cause the patient or his/her family distress, such as by revealing that the patient (or a relative) carries a genetic disease or by leading to the denial of employment or insurance for the patient (or a relative).

There also may be other privacy risks that we have not foreseen. While we believe that the risks to the patient and his/her family are very low, we are unable to tell exactly what all of the risks are. There are some state laws that protect against genetic discrimination by employers or insurance companies, but there is no federal law yet that prohibits such discrimination. We believe that the benefits of learning more about cancer and other diseases outweigh these potential risks.

V.8 Confidentiality

We will make every attempt to protect the patient's confidentiality and to make sure that the patient's personal identity does not become known. The signed consent form will be stored in a locked file that will be accessible only to a very small number of authorized people involved in this project. We will carefully follow the coding, storage, and release plan explained in the *Description of the Research* section on pages 1-3 of this document.
To help us protect the confidentiality of the patient's information, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, we cannot be forced to disclose information that may identify the patient, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. We will use this Certificate to resist any demands for information that would identify the patient, with the following exceptions:

- The Certificate cannot be used to resist a request for the patient information from the United States Government when the information is to be used for auditing or evaluation of federally funded projects or for information that must be disclosed to meet the requirements of the federal Food and Drug Administration (FDA).
- The Certificate does not prevent the patient or a member of patient's family from voluntarily releasing information about the patient or patient's involvement in this research. Also, if the patient have given written consent to an insurer, employer, or other person to receive research information, then we may not use the Certificate to withhold that information.

V.9 Project Results

Scientists at the Genome Sciences Center at the British Columbia Cancer Agency will upload data into the project database using coded samples, and will not report findings to study participants. The Principal Investigator or Lead Associate Investigator from this study will review reported genetic mutations within 3 months from the time they are entered into the project database. Individual results from this research project will generally not be given back to the patient or put into the patient's medical records. However, if a germline mutation that

meets criteria for reporting as an incidental finding based on the NHLBI 2004 Recommendations[44] is identified by the Principal Investigator or Lead Associate Investigator, this may be reported to the patient if the patient consents to receiving such genetic information.

NHLBI Criteria for returning individual genetic results:

(1) "The risk for the disease should be significant, i.e. relative risk>2.0. Variants with greater penetrance or associated with younger age of onset should receive priority." Note: "Genetic test results should not be reported to study participants and their physicians as clinically valid tests unless the test(s) was performed in a CLIA certified laboratory. If the test was performed in a non-CLIA certified laboratory, a CLIA certified laboratory should be sought to confirm results by redrawing a sample and performing the test within the CLIA certified laboratory. Results reported by a research laboratory should be identified as 'research' results."
(2) "The disease should have important health implications, i.e. fatal or substantial morbidity or should have significant reproductive implications" and
(3) "Proven therapeutic or preventive interventions should be available."

For examples of genetic tests that should be reported and a list of CLIA Certified tests at the time of publication, see: http://www.nhlbi.nih.gov/meetings/workshops/gene-results.htm

In such an instance, the abnormal test would be confirmed in a CLIA certified lab, and this finding will be discussed with the patient with the assistance of a genetic counselor.

If research from this project is published in professional journals, there will be no traditionally used identifying information, such as the patient's name, address, telephone number, or social security number, included in the publications.

V.10 Voluntary Participation

The choice to participate in this research by consenting the use the patient's donated tissues and medical information for the **HPV-Related Cancer Project** is completely up to the patient. **No matter what the patient decides to do, his/her decision will not affect their medical care**.

V.11 Withdrawal from the Project

Once coded DNA and RNA samples have been distributed to the participating research centers, and once the molecular analysis and patient information have been transferred to the central project databases, it will not be possible to destroy those data or samples.

Unused tissue samples will be destroyed or returned to **Principal Investigator** per request, and codes linking the sample identifiers back to patient identities at **the Center for Cancer Research** will be destroyed.