Local Protocol #: 13-H&N-24

TITLE: A PHASE II STUDY OF DOCETAXEL, CARBOPLATIN WITH AND WITHOUT LOW DOSE RADIATION AS INDUCTION THERAPY IN LOCALLY ADVANCED HEAD AND NECK CANCER

Coordinating Center: Markey Cancer Center

Principal Investigator:

NAME	Susanne M. Arnold, MD
DEPARTMENT/DIVISION	Internal Medicine/Medical Oncology
OFFICE ADDRESS	Cc457, Markey Cancer Center; 0893
EMAIL ADDRESS	Smarno0@uky.edu
PHONE	859-323-8043

Co-Investigators:

NAME	Mahesh Kudrimoti, MD
DEPARTMENT/DIVISION	Radiation Medicine
OFFICE ADDRESS	N13 A B Chandler Medical Center 40536-0293
PHONE	859-323-6486
EMAIL ADDRESS	Mkudr0@uky.edu
NAME	Stacey Slone, PhD
DEPARTMENT/DIVISION	Division of Cancer Biostatistics
OFFICE ADDRESS	800 Rose Street, Roach Building, CC437
PHONE	859-323--1723
EMAIL ADDRESS	Stacey.slone@uky.edu
NAME	Tadahide Izumi, PhD
DEPARTMENT/DIVISION	Graduate Center for Toxicology
OFFICE ADDRESS	336 Health Sciences Research Building 40536-0305
PHONE	859-257-6472
EMAIL ADDRESS	t.izumi@uky.edu
NAME	David Orren, PhD
DEPARTMENT/DIVISION	Graduate Center for Toxicology
OFFICE ADDRESS	356 Health Sciences Research Building 40536-0305
PHONE	859-323-3612
EMAIL ADDRESS	david.orren@uky.edu

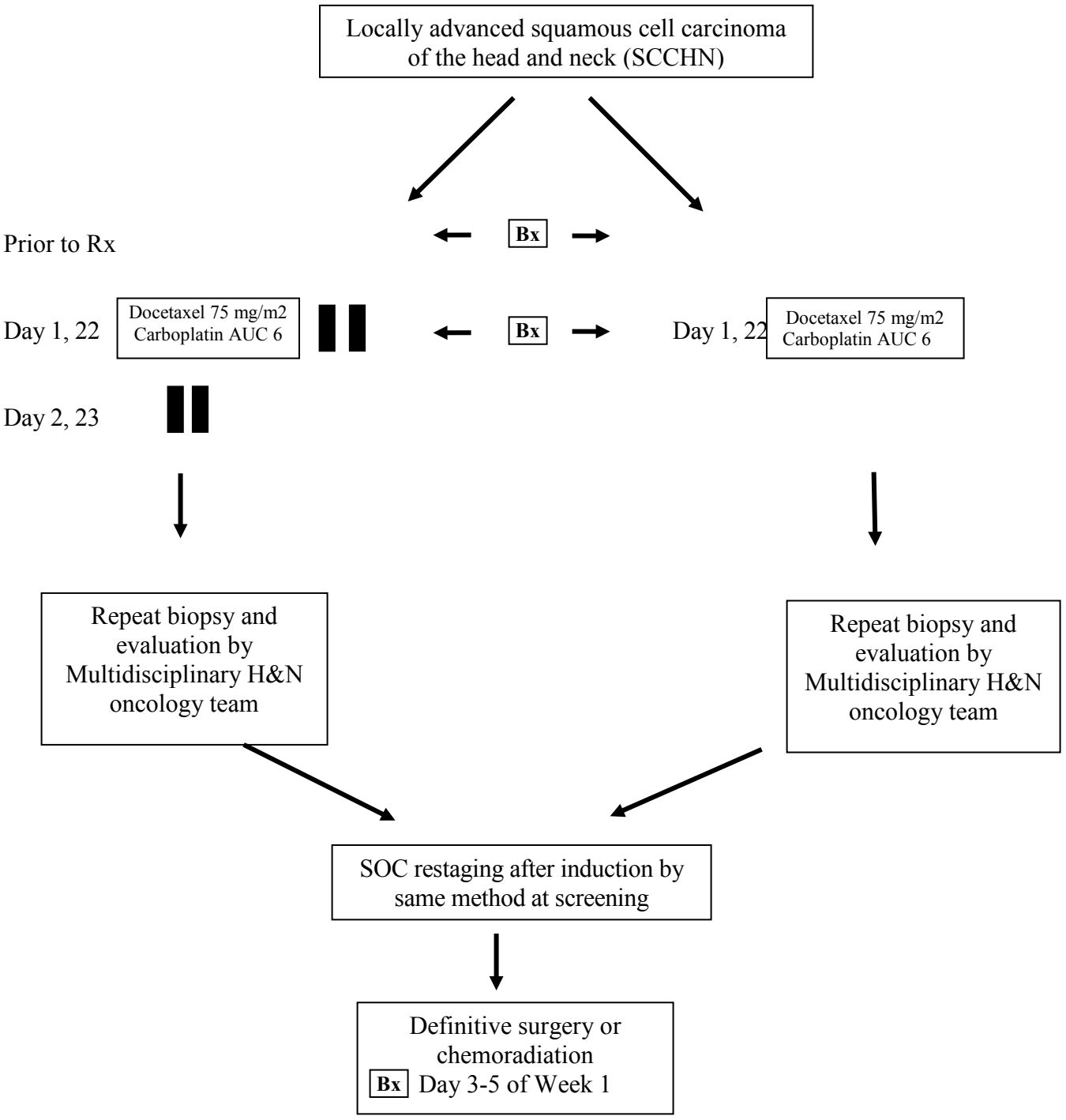
Study Coordinator:
Cynthia A. Leedham, PhD
MCC-CRO
CC140 Pavilion C
Telephone: 859-257-4470
Fax: 859-257-4550

Responsible Data Manager:
Jennifer Land
Cancer Research Informatics
2365 Harrodsburg Rd, Ste A230
Telephone 859-323-9964
fax: 859-257-4177

Commercially Supplied Agent(s): Docetaxel and Carboplatin

Protocol Type / Version # / Version Date: Revised / Version 8.0 / March 1, 2018

SCHEMA: Randomized Phase II study



= 80 cGy of RT, given BID

Bx = In-port radiation biopsy

TABLE OF CONTENTS

1. OBJECTIVES

The primary hypothesis of this study is that hyper-radiosensitivity (HRS) seen at extremely low doses of radiation (80 cGy) can be exploited to enhance the effect of chemotherapy, and that this effect differs from the cellular effect of higher, standard fractions of radiation used in traditional radiation treatment paradigms.

1.1 Primary Objectives

Primary:

1.1 To assess the primary site complete response rate of patients treated with 2 cycles of induction Docetaxel and Carboplatin with low dose fractionated radiation therapy (LDFRT) compared to those treated with chemotherapy alone.

Secondary:

1.2 To assess overall response rate of patients to 2 cycles of induction Docetaxel and Carboplatin with or without LDFRT

1.3 To assess the toxicity and quality of life (QOL) of LDFRT with chemotherapy compared to chemotherapy alone in this patient population

1.4 To assess the 3-year overall survival of patients treated with this regimen.

1.5 To assess measures of DNA repair (gamma-H2AX levels, PARP activation) and DNA repair kinetics (Comet assay) in serial mucosal cell samples obtained from the irradiated region of the head and neck (i.e. buccal, tongue, pharyngeal wall, etc.) of subjects on this trial. These will be collected:

1.5.1 Prior to therapy

1.5.2 During induction therapy and LDFRT

1.5.3 During the first week of full-dose radiation in both groups.

The outcomes of the proposed research will provide direct validation of the hypothesis that LDFRT improves response rates of patients with SCCHN to induction chemotherapy, through a different mechanism than standard fractions of radiation.

2. BACKGROUND

2.1 Squamous cell carcinoma of the head and neck (SCCHN)

Squamous cell cancer of the head and neck (SCCHN) comprise 5% of all cancers, with 40,000 new cases diagnosed annually.[1] The age-adjusted incidence of squamous cell carcinoma of the head and neck (SCCHN) is 13.07 per 100,000 individuals in Kentucky, placing Kentucky third in incidence rate in the United States.[1] Treatment of locally advanced SCCHN consists of aggressive regimens of chemotherapy and radiation with an approximate 5-year survival of 50%.[2] There are substantial opportunities to improve survival in this disease, and one paradigm that has received extensive study is induction therapy, given prior to definitive treatment with radiation or chemoradiation.[3] This has shown a survival advantage in SCCHN in recent years,[4-7] but with the potential for increased toxicity which has kept this promising therapy from wide adoption by clinical

oncologists.

In addition, it is difficult to tease out the effect of induction because its pairing with concomitant chemotherapy and radiation in all of these studies, with response rate (RR) as a surrogate for that effect The synergy between radiation and chemotherapy is well established in vitro and in the field of SCCHN treatment.[8,9] and has become the standard of care for locally advanced SCCHN since initial studies in the 1980's. In general, standard doses of radiation cause single- and double-strand DNA damage, which accumulates unrepaired and cause global cellular catastrophe and rapid cell death. Radiation recruits more cells into active cell cycle, which, in theory, allows a higher percentage of cells to be susceptible to chemotherapy agents. The addition of chemotherapy to radiation, in turn, is thought to alter the intrinsic radioresistance of tumor cells. By killing a percentage of cancer cells, chemotherapy also allows reoxygenation of previously hypoxic areas thus enhancing radiotherapy.

Because low doses of radiation (1-100 cGy) do not cause such global cellular injury, the initial slope of the radiation cell-survival curve was presumed to be an ineffective dose range for human tumor therapy. However, Joiner and colleagues were the first to describe techniques to adequately study low dose radiation and demonstrated an initial phase of hypersensitivity to radiation at doses less than 100 cGy (Figure 1).[10,11] Termed hyper-radiosensitivity (HRS), this phenomenon was postulated to be exploitable over 20 years ago.

This proposal investigates the role of low-dose fractionated radiation (LDFRT) combined with chemotherapy as induction therapy in a phase II randomized clinical trial in locally advanced squamous cell carcinoma of the head and neck. Correlative studies will evaluate the level of DNA repair and DNA repair kinetics in subjects prior to and after exposure to low-dose (<100 cGy) and standard dose (> 180 cGy) fractions of radiation throughout the course of this treatment. This evaluation of DNA damage will help to elucidate the mechanism of cell death that occurs when cells are exposed to radiation doses of less than 100 cGy. The outcomes of the proposed research will provide direct validation of the clinical hypothesis that LDFRT improves response rates in patients with SCCHN to induction chemotherapy, and will evaluate and elucidate the level of DNA damage and repair kinetics in response to different doses of radiation. This phase II trial will provide the evidence needed to test this treatment paradigm in a phase III trial at the national level and will provide preliminary data for independent funding to study the molecular effects of low-dose radiation.

2.2 Preclinical Studies using LDFRT

Until recently, the initial slope of the radiation cell-survival curve (doses of 0-100 cGy) was presumed to be an ineffective dose range for human tumor therapy. However, as techniques to adequately study low dose radiation have improved, quite the opposite effect has been described. Joiner revolutionized thinking about low doses of radiation (<100 cGy) by demonstrating an initial phase of hypersensitivity to radiation using doses from 0 to 50 cGy.[10,11] In work from the University of Kentucky, Ahmed and colleagues[12-14] have expanded our understanding of this synergy by combining LDFRT and various chemotherapeutic agents. Using very low doses of radiation in combination with chemotherapy, they have demonstrated enhanced cell death compared to chemotherapy or radiation alone. More importantly, in cell culture, pro-apoptotic pathways were enhanced without the induction of pro-survival pathways; both in p53 mutant and wild-type cell lines. Significantly, LDFRT avoids the development of one type of radiation resistance (the upregulation of pro-survival pathways) seen with higher dose radiation. This may provide one way to overcome radiation resistance, a major cause of treatment failure in SCCHN, while still enhancing cell death.

2.3 Clinical Studies using LDFRT

These pre-clinical studies suggest a unique synergistic effect between LDFRT and taxane-based

chemotherapy. While Joiner and colleagues were the first to describe the potential of LDRFT as a therapeutic modality, our investigative team has explored the synergy of LDFRT and various chemotherapeutic agents in vitro.[12,14] We found that chemotherapy followed by LDFRT enhanced cell death compared to chemotherapy alone. Based on this preclinical work, we designed a strategy to capitalize on this synergy, using LDFRT as a chemotherapy potentiator in the setting of induction chemotherapy in SCCHN.[13] Our novel induction strategy was successfully translated into two phase II clinical trials,[13,15] and showed less toxicity than other induction chemotherapy regimens and similar efficacy. Using four to eight fractions of 50 to 80 cGy of LDFRT in combination with chemotherapy provided excellent response rates (85%) and favorable long-term survival compared to historical controls.[15] Our initial trials were the first in humans using this approach, but lacked a comparator arm, which is needed to accurately examine the true effect of LDRFT with chemotherapy. This randomized phase II trial design, seeks to prove the hypothesis that LDFRT can enhance the effect of induction chemotherapy in SCCHN, through the phenomenon of HRS that exists at very low doses of radiation.

The application of HRS and LDFRT to clinical cancer treatment was pioneered by our group, who published the first human trials of this novel paradigm. Potentiating the effect of chemotherapy with the lowest doses of radiation had never been shown to be effective in humans prior to our studies. In addition, work by University of Kentucky investigators and colleagues at other institutions has advanced the field of knowledge of LDFRT's a) interactions with a variety of chemotherapies,[12-15] b) mechanisms of cellular injury, and c) role in phase I and II studies in other tumor types.[16]

2.4 Rationale for Docetaxel and Carboplatin

Docetaxel is an inhibitor of microtubule function and is well established as a radiosensitizer of cancer cell lines in vitro.[17,18] Combination therapies utilizing Carboplatin and Docetaxel have become the standard for induction regimens in SCCHN.[6,7,19] Taxanes induce G2/M arrest, the most radiosensitive phase of the cell cycle, through inhibition of microtubule function,[17,18,20] within two hours after administration and this effect peaks between 8 and 12 hours.[21,22] Multiple in-vitro studies in head and neck cancer cell lines show supra-additive effect of taxanes and radiation when cells were exposed to taxanes prior to irradiation.[17] In vitro data with Carboplatin also indicates an additive effect when given prior to irradiation using various cell lines.[23] Additionally, the pharmacokinetics of carboplatin is not altered by pretreatment with taxanes at standard doses. The combination of Docetaxel and Carboplatin was chosen because of the proof of its efficacy in induction therapy in head and neck cancer, as well as the ability to complete chemotherapy infusion in a timely manner to allow for two fractions of LDFRT to be delivered on Day 1. We propose to expand our understanding of LDFRT and chemotherapy in a randomized phase II study of Docetaxel and Carboplatin with LDFRT as induction therapy in patients with bulky Stage II, Stage III and IV H&N cancer.

2.5 Correlative Studies

In preclinical studies, low dose radiation is thought to exert its influence in the early G2/M checkpoint through the damage response of G2-phase cells. Radiation induces DNA double strand breaks with cellular response including phosphorylation of ATM, followed by activation of downstream targets with gamma-H2AX operating as a damage sensor [24]. In preclinical studies, Krueger and colleagues [25] demonstrated that low-dose hyper-radiosensitivity caused a predictable increase in gamma-H2AX within 1-2 hours after each low dose fraction, but this has not been studied in humans to date. To determine the effect of low-dose radiation, we will measure the time dependent expression of gamma-H2AX in serial mucosal sampling from the irradiated mucosa of these patients. Tissues will be collected pre-therapy and after the first dose of induction chemotherapy and LDFRT, as well as within the first week of definitive, full-dose radiation, as listed in section 5.4. In addition, assessment of DNA repair kinetics through the use of Comet assays

will allow an estimation of DNA repair when human cells are exposed to low doses and full doses of radiation. Planned investigation will include the analysis of markers of DNA damage: including immunohistochemical staining for PARP (single-strand breaks) gamma-H2AX (double-strand breaks) and DNA repair kinetics (Comet assay) analyzed at multiple time points to assess radiosensitization of cancer cells by different doses of ionizing radiation. Correlative endpoints will provide intra-patient assessment of response to DNA damage by low-dose and standard dose radiation, by direct evaluation within the radiation port. This avenue of study is unique and sampling at different time points will help define the different cellular responses to low-dose (<100 cGy) radiation and standard dose radiation (≥180 cGy).

2.6 Rationale

The primary hypothesis of this study is that hyper-radiosensitivity (HRS) seen at low doses of radiation (80 cGy) can be exploited to potentiate the effect of chemotherapy, and that this hrs results in DNA damage response that is distinct from the DNA damage response caused by traditional doses of radiation (> 180 cGy) used in cancer treatment.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed locally advanced head and neck cancer of squamous type stage III, IVA and IV B (larynx, oral cavity, oropharynx and hypopharynx or unknown primary isolated to the head and neck region) and select Stage II tumors of the BOT (size more than 3 cm), who are appropriate for potentially curative therapy with chemoradiotherapy or surgical resection, as determined by the Multidisciplinary Head and Neck Cancer Clinical Care and Research Team.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease. All disease must be assessed within 28 days prior to registration.

3.1.3 ECOG performance status of 0, 1 or 2 (see Appendix A).

3.1.4 No prior chemotherapy for the current locally advanced SCCHN. Prior radiation or chemotherapy for a previous head and neck cancer is allowed provided full dose radiation can be delivered to the current treatment and provided the patient remains in remission for greater than 3 years from prior diagnosis.

3.1.5 Age ≥18 years.

3.1.6 Life expectancy of greater than 3 months

3.1.7 Patients must have normal organ and marrow function measured within 14 days of registration as defined below:

– absolute neutrophil count	≥ 1,000/mcL
– platelets	≥ 100,000/mcL
– total bilirubin	≤ institutional upper limit of normal
– AST(SGOT	≤ 1.5 × institutional upper limit of normal

– Alkaline phosphatase	≤ 2.5 × institutional upper limit of normal
– creatinine	within normal institutional limits

OR

– Creatinine clearance	≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.8 The effects of Docetaxel, Carboplatin, and LDFRT are known teratogens. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, throughout the duration of active treatment and for 4 months after completion of chemotherapy and radiation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of active study treatment, and for 4 months after completion of chemotherapy and radiation (both induction and definitive) administration.

3.1.9 ity to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior chemotherapy for the current SCCHN will not be allowed. Patients with second primary cancers of the head and neck who remain in remission for 3 years from the prior diagnosis are eligible for this study, provided they may receive full dose radiation to the current SCCHN cancer.

3.2.2 Patients who are receiving any other investigational agents.

3.2.3 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Carboplatin or Docetaxel.

3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because Docetaxel, Carboplatin, and radiation are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Docetaxel, Carboplatin and radiation, breastfeeding should be discontinued if the mother is treated with these agents and/or radiation. These potential risks may also apply to other agents used in this study.

3.2.7 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with Docetaxel, Carboplatin and radiation. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.8 No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, *in situ* cervical cancer, adequately treated previous Stage I or II cancer from which the patient is currently in complete remission or other cancer from which the patient has been disease-free for 3 years.

3.2.9 Patients with nasopharynx or salivary gland primary site will be excluded from this trial.

3.2.10 Patients with distant metastatic disease (M1c) will not be eligible for this study.

3.2.11 Patients with grade II or greater peripheral neuropathy will be excluded from study.

Note: In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28. If Day 28 falls on a weekend or holiday, the test or measurement may be performed on the next working day

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. The required table below shows estimated accrual targets of the anticipated patient population based on the catchment area of the MCC and are not a limitation to accrual by category.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	2	=	3
Not Hispanic or Latino	32	+	37	=	69
Ethnic Category: Total of all subjects	33	+	39	=	72
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	2	+	2	=	4
Black or African American	5	+	6	=	11
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	26	+	31	=	57
Racial Category: Total of all subjects	33	+	39	=	72

4. REGISTRATION PROCEDURES

4.1 Protocol Review and Monitoring Committee and Institutional Review Board Review

Before implementing this study, the protocol, the proposed informed consent form, and other information to subjects must be reviewed by the Markey Cancer Center's Protocol Review and Monitoring Committee and the University of Kentucky Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be maintained in the Markey Cancer Center Clinical Research and Data Management Shared Resource Facility (MCC CRDM SRF) regulatory binder. Any amendments to the protocol, other than administrative ones, must be reviewed and approved by the PRMC and the UK IRB.

4.2 Enrollment Guidelines

Eligible patients will be identified by the principal investigator and co-investigators of this study. Potentially eligible patients will be screened in the University of Kentucky Markey Cancer Center clinics by the investigators, study personnel, and the Principal Investigator (PI). Upon obtaining proper consent, patients will be enrolled into the study.

4.3 Informed Consent

The goal of the informed consent *process* is to provide people with sufficient information so they can make informed choices about whether to begin or continue participation in clinical research. The process involves a dynamic and continuing exchange of information between the research team and the participant throughout the research experience. It includes discussion of the study's purpose, research procedures, risks, and potential benefits, and the voluntary nature of participation. The informed consent *document* provides a summary of the clinical study and the individual's rights as a research participant. The document acts as a starting point for the necessary exchange of information between the investigator and potential research participant. Also, research participants and their families may use the consent document as an information resource and reference throughout participation in the trial. The informed consent *document* is often considered the foundation of the informed consent process; it does not, however, represent the entirety of the process. Nor is the informed consent document a risk-management tool for the investigator and/or institution.

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

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

4.4 Compliance with Laws and Regulations

The study will be conducted in accordance with U.S. Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), the Declaration of Helsinki, any applicable local health authority, and Institutional Review Board (IRB) requirements. The PI or designee will be responsible for obtaining continuing and not less than annual IRB re-approval throughout the duration of the study. Copies of the Investigator's annual report to the IRB and copies of the IRB continuance of approval must maintained by the MCC CRDM SRF. The PI or designee is also responsible for notifying the Data and Safety Monitoring Committee of the MCC and the UK IRB of any significant adverse events that are serious and/or unexpected, as per SOP's of those entities. DSMC will review all adverse events of this IIT as per its SOP.

5. TREATMENT PLAN

5.1 Pre-treatment Studies

Prior to any study-required tests, subjects must first provide written informed consent to participate in this study. Within 6 weeks, all patients will undergo a direct laryngoscopy (DL) or indirect laryngoscopy (IDL) (if the primary tumor is easily and completely evaluable by IDL). Within 4 weeks of registration, all patients will undergo a history and physical exam, ECOG performance status evaluation,, a CT or MRI scan of the involved area of the head and neck and a CT of the Chest or chest X-ray. Within 2 weeks of registration, all patients will undergo a complete blood count with differential and platelets, and serum chemistries (including sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, BUN, creatinine, AST, alkaline phosphatase, and total bilirubin).

5.2 Agent Administration

Treatment will be administered on an outpatient or inpatient basis. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Carboplatin and Docetaxel are FDA approved for this indication and will be administered as below on Day 1 and 22. Radiation will be given on Days 1, 2, 22, and 23 of chemotherapy. The first fraction will be given within 1 hour after completion of Carboplatin while the remaining fractions of each cycle will have at least a 3-hr interfraction interval.

Induction Regimen Description					
Agent	**Premedications / Precautions**	**Dose (solution and volume)**	**Route**	**Schedule**	**Cycle Length**
Docetaxel	Dexamethasone 8 mg BID PO or IV Day prior, day of and day after Docetaxel	75 mg/m2 (250 cc NS)	IV over 60 minutes per institutional guidelines	Day 1	every 21 days
Carboplatin	Ondansetron 8 mg PO or IV	AUC = 6 (250 cc NS) GFR calculation by Cockcroft-Gault equation; AUC by Calvert formula:	IV over 30 minutes after completion of Docetaxel per institutional guidelines	Day 1	
Low dose fractionated	None	80 cGy	BID with at least 3 hours between doses	Days 1 and 2	

radiation			(total of four fractions per cycle)		
**Anti-emetic regimen per NCCN and institutional guidelines					

5.3 Chemotherapy Premedication and Dosing:

All chemotherapy will use actual body weight and will be administered as an i.v. infusion. Docetaxel will be given at a dose of 75 mg/m2 intravenously over one hour on Days 1 and 22. Docetaxel will be dosed to the nearest 5mg using a standard body surface area chart. Carboplatin infusion will follow Docetaxel and will be given at a dose of Area under the Curve of 6 (using Cockcroft-Gault calculation) intravenously over one hour on Days 1 and 22.

To avoid allergic reactions associated with Docetaxel, the following premedications are recommended:

- Dexamethasone 8mg PO BID 1 day prior to, day of and day after chemotherapy. If a patient fails to premedicate on the day prior to chemotherapy, then give dexamethasone 12mg IV

Other anti-emetics are per institutional standard and at the discretion of the treating physician.

5.4 Pre- and Post-Induction Treatment Tissue sampling

Prior to chemotherapy and radiation and as outlined below, patients will undergo buccal swab sampling in the mouth. Wherever possible this sampling will be in the area of the radiation delivery. See Appendix D for sampling instructions

Buccal sampling:
Subjects will first rinse their mouth with water. The mucosa will be swabbed with a cytology brush (which is then discarded), subjects will rinse again and the same area will be scraped gently with a wooden spatula 10 times. Specimens are fixed onto uncharged slides and spray fixed. A second cytology brush is then applied to the area and placed in media for storage as per sample collection instructions (see correlative section 9.0 and Appendix D). These samples will be collected with a mandatory pre-treatment sample and a minimum of 3 samples during induction, and a minimum of 3 samples during definitive radiation as described in Appendix D.

5.5 Post-Induction Reassessment

Following two cycles of induction therapy and at least 2 weeks after the last LDFRT dose, subjects will have reassessment by radiographic and clinical evaluation (DL or IDL) to assess primary site and complete RR. A panendoscopy will only be performed, if the primary tumor is not easily and completely assessable by indirect laryngoscopy. The assessment of tumor response will be made by the otolaryngologist or oncologist performing the procedure. In the case of apparent progressive disease, biopsies of the primary tumor area will be taken. This is considered part of routine care of patients undergoing neoadjuvant therapy. If a subject has biopsy or surgical removal, those tissues will also be collected for correlative study (although no surgical removal is planned in these patients). Following induction, all subjects will undergo definitive radiation and chemotherapy or surgery, as per standard of care, as pre-determined by a multidisciplinary review of cases. Subjects must have recovered from all study related (possible, probable or definite) toxicities to $\leq$ grade 2 (CTCAE v4.0) as determined by the treating physician prior to initiation of definite treatment. Definite therapy should occur on or after Day 43 and prior to Day 64 from the start of induction (Day 1). Definitive radiation will incorporate the dose of radiation used during induction into the final dose calculation, with dose adjustments of definitive radiation made at the discretion of the treating radiation oncologist. Definitive

surgery will incorporate standard of care surgical removal.

5.6 Dosage and fields Low-Dose Fractionated Radiation (LDFRT)

Doses of 80 cGy per fraction will be administered (to a total of 640 cGy for the entire induction scheme). The patient will be treated with shaped fields encompassing gross disease only (including the primary and gross nodal disease) with a maximum 2cm margin. The spinal cord will be excluded from the radiation field and CT based treatment planning will be used as needed and as appropriate.

5.7 Dosage and fields for Standard Fraction Radiation post-Induction

Following induction, dosage and fields for definitive standard fractionation will be determined by the treating radiation oncologist. The accepted standard total dose of radiation used for definitive therapy ranges from 66-72Gy when once-daily RT is used at 1.8-2.0Gy/fraction and from 74.4-81.6Gy when twice-daily RT is used at 1.2Gy/fraction. In calculating the planned total dose of radiation to be used for subsequent, definitive therapy the radiation oncologist will incorporate the induction dose used into the final calculation for a maximum total dose of approximately 74Gy if once-daily fractionation is used or approximately 82.4Gy if twice-daily fractionation is used. Since the 640 cGy used in the induction regimen is delivered at a very low dose/fraction (i.e. 80cGy), it is expected that the incorporation of this dose into the calculated total doses should not be associated with any significant increase in late radiation damage/effect, as previously described in our non-randomized phase II trials.

Management and dose modifications associated with the above adverse events are outlined in Section 6. Performance status will be evaluated based on the ECOG Performance Status Criteria (Appendix A). Toxicities will be graded as per section 7.0.

5.8 General Concomitant Medication and Supportive Care Guidelines

The use of concomitant bisphosphanates, and other supportive medications determined necessary for the health of subjects are allowed, but concurrent cancer therapies other than the treatment regimen of this trial are not allowed. Caution should be taken when administering supportive medications that are metabolized through the cytochrome P450 system, although this study does not specifically exclude agents known to affect or with the potential to affect selected CYP450 is enzymes.

5.9 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for 2 cycles or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5.10 Duration of Follow Up

Patients will be followed by study team until resolution of side effects from induction therapy to less than or equal to grade 1 toxicity (excluding previously existing conditions, which must return to baseline) AND until the patient has completed their definitive chemotherapy and radiation or definitive surgery with follow-up scans (per institutional guidelines be usually performed within 8 weeks of last dose of radiation). Patients will be followed for 3 years after completion of the study by the Kentucky Cancer Registry which is required by law to follow all cancer patients diagnosed in Kentucky for their lifetime from the time of diagnosis until death. KCR routinely records overall survival, as well as time of first recurrence and sites of recurrence, therefore, is uniquely qualified to follow these patients long-term. At the point three years from completion of final subject, and at interim analysis, the KCR will search the database for survival, both overall and progression-free.

5.11 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.9 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Modifications

Cycle 2 of therapy may be administered provided the subject meets all of the following criteria within 2 days of Cycle 2 Day 1:

- ANC ≥ 1,000/μL.
- Platelet count ≥ 75,000/μL.
- total bilirubin ≤institutional upper limit of normal
- AST(SGOT ≤ 1.5 × institutional upper limit of normal
- Alkaline phosphatase ≤ 2.5 × institutional upper limit of normal
- All other Grade 2, 3 or 4 non-hematological toxicities listed in section 6.3 have resolved.
- Serum creatinine ≤ 1.5 x ULN OR measured or calculated creatinine clearance ≥ 60 mL/min using the Cockcroft-Gault.

Assessment for dose modification will be conducted prior to each dose. Dose escalation will not be allowed during this study. Chemotherapy doses may be reduced or held for hematological and non-hematological effects. **If chemotherapy is held, radiotherapy will be held and will restart on the same day as chemotherapy based on the parameters for alteration due to toxicity listed below.** Radiation doses are not decreased. Treatment may be delayed no more than three weeks to allow recovery from toxicity. A patient will be allowed a maximum of two dose reductions. Dose adjustments will be made according to the following guidelines:

Dose Modification Table		
Modification Episode	Carboplatin (AUC)	Docetaxel (mg/m2)
0	6	75
-1	5	65

-2	4	55

6.2 Hematologic Toxicity

The following table outlines the dose reduction guidelines for docetaxel and carboplatin for thrombocytopenia and neutropenia that occurs at any point during the prior cycle.

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	No change	No Change	Hold until ≤ grade 2, then reduce Carboplatin and Docetaxel by 1 dose level	Hold until ≤ grade 2, then reduce Carboplatin and Docetaxel by 1 dose level
Febrile Neutropenia	No change	No Change	Hold until ≤ grade 2, then reduce Carboplatin and Docetaxel by 1 dose level	Hold until ≤ grade 2, then reduce Carboplatin and Docetaxel by 1 dose level
Thrombocytopenia	No change	Hold until ≤ grade 1, then resume at same dose of Carboplatin and Docetaxel	Hold until < grade 1, then reduce Carboplatin and Docetaxel by 1 dose	Hold until ≤ grade 1, then reduce Carboplatin and Docetaxel by 1 dose level

6.3 Non-hematologic Toxicity

The following table outlines the dose reduction guidelines for docetaxel and carboplatin for non-hematologic toxicities that occur at any point during the prior cycle.

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Mucositis	No change	No change	No change	Hold until ≤ grade 2, then reduce Docetaxel and Carboplatin by 1 dose level
Motor and Sensory Neuropathy	No change	No change	Hold until≤ grade 2, then reduce Docetaxel 1 dose level	Off study
Arthralgias Myalgias	No Change	No Change	Hold until≤ grade 2, then reduce Docetaxel by 1 dose level	Off study
Elevated Bilirubin	Hold until≤ IULN, then reduce Docetaxel by 1 dose level	Hold until≤ IULN, then reduce Docetaxel by 1 dose level	Hold until≤ IULN, then reduce Docetaxel by 1 dose level	Off Study
Elevated AST or ALT	No Change	Hold until≤ 1.5 x ULN, then reduce Docetaxel by 1 dose level	Hold until≤ 1.5 x ULN, then reduce Docetaxel by 1 dose level	Off study
Elevated serum creatinine	No Change	Hold until ≤ grade 1, then resume at same dose of Carboplatin	Hold until ≤ grade 1, then reduce Carboplatin by 1 dose level	Off Study

Nausea and/or Vomiting	No change	No Change	No Change	Hold until≤ grade 2, then reduce Docetaxel and Carboplatin by 1 dose level

For any grade 3 or 4 toxicity not mentioned above (excluding alopecia), and considered possibly, probably or definitely related to treatment, treatment should be withheld until resolution to grade ≤ 2. Treatment should resume at a one dose level reduction. For grade 1 or 2 toxicities not mentioned above, no dose reductions will occur. Chemotherapy will be discontinued if irreversible, symptomatic cardiac arrhythmia/dysfunction occurs.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via Medwatch form 3500A) **in addition** to routine reporting.

7.1 Adverse Events and Potential Risks

7.1.1 Adverse Event List for Docetaxel

Incidence rates of all adverse events associated with Docetaxel are provided in the product package insert. Side effects include:

Cardiac: arrhythmias, pericardial effusions.
Hematologic: dose-related neutropenia, leukopenia, thrombocytopenia, anemia, hypoglycemia, hypernatremia.
Gastrointestinal: nausea and vomiting, diarrhea, oral mucositis, pancreatitis, esophagitis.
Neurologic: reversible dysthesias or paresthesias, peripheral neuropathy, mild or moderate lethargy or somnolence, headache, seizures.
Hypersensitivity: hypersensitivity (local or general skin rash, flushing, pruritus, drug-fever, chills and rigors, low back pain), severe anaphylactoid reactions (flushing with hypo- or hypertension, with or without dyspnea).
Dermatologic: alopecia, desquamation following localized pruriginous maculopapular eruption, skin erythema with edema, extravasation reaction (erythema, swelling, tenderness, pustules), reversible peripheral phlebitis, nail changes.
Hepatic: increased transaminase, alkaline phosphatase, bilirubin; hepatic failure; hepatic drug reaction.
Pulmonary: dyspnea with restrictive pulmonary syndrome, pleural effusions.
Other: asthenia, dysgeusia, anorexia, conjunctivitis, arthralgia, muscle aches, myopathy, peripheral edema, fluid retention syndrome, ascites.

7.1.2 Adverse Event List for Carboplatin

Incidence rates of all adverse events associated with Carboplatin are provided in the product package insert. Side effects include:

Hematologic: Thrombocytopenia (dose limiting), neutropenia, leukopenia, anemia.
GI: Nausea and vomiting (frequent but less severe than with cisplatin), treatable with appropriate antiemetic prophylaxis. Anorexia, diarrhea, and constipation have also been reported.
Dermatologic: Rash, urticaria. Rarer reactions include alopecia, mucositis, and hypersensitivity

reactions.
Hepatic: Abnormal liver function tests, usually reversible with standard doses.
Neurologic: Rarely peripheral neuropathy is seen. May be more common in patients greater than 65 years of age. May also be cumulative, especially in patients with prior cisplatin treatment. Ototoxicity (rare).
Renal: Elevations in serum creatinine, BUN; electrolyte loss (Mg, K, Na, Ca).
Miscellaneous: Pain, asthenia, flu-like syndrome.

7.1.3 Adverse Event List for Low-dose Radiation

Reversible skin changes and mucositis are expected side effects of radiotherapy. Combined modality therapy increases the risk for acute toxicities, but with low doses of radiation (640 cGy total) minimal toxicity is expected, as reported in our prior studies (14-16). The total dose of radiation given as definitive therapy after completion of induction will take into account this initial dose of radiation, and will be at the discretion of the attending radiation oncologist.

7.1.4 Other Adverse Event List for Low-dose Radiation

There is estimated to be a 1% risk per year of second primary cancers in this population of patients, which is unrelated to treatment.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version4.0. A copy of the CTCAE version4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs should be reported using a Medwatch form only if required by the reporting chart below.
  - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 For MCC Investigator-Initiated Trials (IITs), investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form. This applies to the following categories:

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

- **ALL Grade 4 (life threatening or disabling) Events** – Unless expected AND specifically listed in protocol as not requiring reporting.
- **ALL Grade 5 (fatal) Events** regardless of study phase or attribution

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

7.3.2 The following table outlines the required forms and reporting structure for clinical trials.

Study type	Expedited reporting to MCC	Expedited reporting to External Agency	Non-expedited AE	Form	IRB
IIT by MCC investigator of commercially available agent (non-IND and non-IDE)	• Grade 3 – Unexpected AE PLUS Possibly, Probably or Definitely Related • ALL Grade 4 unless expected AND listed in protocol as not requiring reporting. • ALL Grade 5 (fatal) Events	FDA: Suspected AE that is serious and Unanticipated (not listed in IDB or consent)	OnCore and DSMC reporting only	Voluntary Medwatch 3500 for Serious and unanticipated OnCore for all AEs, including SAEs	

7.3.3 **MCC Expedited Reporting Guidelines for MCC IITs**

Investigators within MCC will report SAEs directly to the MCC DSMC per the MCC DSMC SOP and the University of Kentucky IRB reporting policy.

Attribution	MCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 24 business hours of learning of the event.					

7.4 Expedited Reporting to External Agencies

The Overall PI will comply with the policies of all external funding agencies and the UK IRB regarding

expedited reporting, as per the UK IRB's SOP: http://www.research.uky.edu/ori/SOPs_Policies/C4-0150-Mandated_Reporting_to_External_Agencies_SOP.pdf.

7.4.1 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.4.2 Expedited Reporting to Hospital Risk Management

Participating investigators will report to the UK Office of Risk Management any participant safety reports or sentinel events that require reporting according to institutional policy.

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the OnCore case report forms. The exception to this is that all grade 1 and 2 lab AEs that are not clinically significant, are not required to be recorded in OnCore or reported to DSMC but will be recorded as source documentation **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the commercial agents administered in this study can be found in Section 7.1.

8.1 Commercial Agent: Docetaxel

(Please refer to the package insert for further information)

8.1.1 Other Names
Taxotere, RP 56976, NSC #628503.

8.1.2 Classification

Anti-microtubule agent.

8.1.3 Mode of Action

Docetaxel, a semisynthetic analog of taxol, promotes the assembly of tubulin and inhibits microtubule depolymerization. Bundles of microtubules accumulate and interfere with cell division.

8.1.4 Storage and Stability

Docetaxel infusion solution, if stored between 2 and 25ºC (36 and 77ºF) is stable for 4 hours. Fully prepared docetaxel infusion solution (in either 0.9% Sodium Chloride solution or 5% Dextrose solution) should be used within 4 hours (including the administration time). Store between 2 and 25°C (36 and 77ºF). Retain in

the original package to protect from bright light. Freezing does not adversely affect the product.

8.1.5 Dose Specifics

Docetaxel will be administered as a 60 minute intravenous infusion. Dose will be calculated based on the patient's actual body weight.

8.1.6 Preparation

Docetaxel is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing docetaxel solutions. The use of gloves is recommended.

If docetaxel concentrate, initial diluted solution, or final dilution for infusion should come into contact with the skin, immediately and thoroughly wash with soap and water. If docetaxel concentrate, initial diluted solution, or final dilution for infusion should come into contact with mucosa, immediately and thoroughly wash with water.

Docetaxel for Injection Concentrate requires two dilutions prior to administration. Please follow the preparation instructions provided below. **NOTE:** Both the docetaxel for Injection Concentrate and the diluent vials contain an overfill.

A. Preparation of the Initial Diluted Solution

1. Gather the appropriate number of vials of docetaxel for Injection Concentrate and diluent (13% Ethanol in Water for Injection). If the vials were refrigerated, allow them to stand at room temperature for approximately 5 minutes.

2. Aseptically withdraw the contents of the appropriate diluent vial into a syringe and transfer it to the appropriate vial of docetaxel for Injection Concentrate. **If the procedure is followed as described, an initial diluted solution of 10mg docetaxel/mL will result.**

3. Mix the initial diluted solution by repeated inversions for at least 45 seconds to assure full mixture of the concentrate and diluent. Do not shake.

4. The initial diluted docetaxel solution (10 mg docetaxel/mL) should be clear; however, there may be some foam on top of the solution due to the polysorbate 80. Allow the solution to stand for a few minutes to allow any foam to dissipate. It is not required that all foam dissipate prior to continuing the preparation process. The initial diluted solution may be used immediately or stored either in the refrigerator or at room temperature for a maximum of 8 hours.

B. Preparation of the Final Dilution for Infusion

1. Aseptically withdraw the required amount of initial diluted docetaxel solution (10mg docetaxel/mL) with a calibrated syringe and inject into an infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 to 0.74mg/mL. Thoroughly mix the infusion by manual rotation.

2. As with all parenteral products, docetaxel should be inspected visually for particulate matter or discoloration prior to administration whenever the solution and container permit. If the docetaxel for Injection, initial diluted solution, or final dilution for infusion is not clear or appears to have precipitation,

these should be discarded.

The final docetaxel dilution for infusion should be administered intravenously as per protocol under ambient room temperature and lighting conditions.
Contact of the docetaxel concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP (di-2-ethylhexyl phthalate), which may be leached from PVC infusion bags or sets, the final docetaxel dilution for infusion should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

8.1.7 Route of Administration

Docetaxel will be administered as a 60 minute infusion in saline or D5W through an administration set that does not contain phthalate plasticizers along the fluid pathway that is connected to the patient's vascular access catheter.

8.1.8 Incompatibilities

Contact of the undiluted concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion should be avoided. Diluted docetaxel solution should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets. (See Sec. 8.2.6.b).

The metabolism of docetaxel may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3A4, such as cyclosporine, terfenadine, ketoconazole, erythromycin, and troleandomycin. Caution should be exercised with these drugs when treating patients receiving docetaxel as there is a potential for a significant interaction.

8.1.9 Availability

Docetaxel (Taxotere®) is commercially available.
Docetaxel for Injection Concentrate is supplied in a single-dose vial as a sterile, pyrogen-free, non-aqueous, viscous solution with an accompanying sterile, non-pyrogenic, diluent (13% ethanol in Water for Injection) vial. The following strengths are available: 80 mg, 20 mg.

8.2 Commercial Agent: Carboplatin

(Please refer to the package insert for further information)

8.2.1 Other Names

CBDCA, Paraplatin, JM-8, NSC-241240

8.2.2 Classification

Second-generation tetravalent organic platinum compound

8.2.3 Mode of Action

Like cisplatin, carboplatin binds to DNA, thereby inhibiting DNA synthesis, in a cell cycle nonspecific manner. Carboplatin must first undergo activation to produce antineoplastic activity. Bidentate carboxylate ligands of carboplatin are displaced by water forming (aquation) positively charged platinum complexes which bind to nucleophilic sites in DNA, such as the O-6 position on guanine. Carboplatin produces predominantly interstrand DNA crosslinks rather than DNA-protein crosslinks. Intrastrand crosslinks result from the formation of adducts between the activated platinum complexes of the drug and the N-7 atom (not exclusively) atom on guanine to produce 1, 2 intrastrand links between adjacent guanine molecules, between neighboring guanine and adenosine molecules, or between neighboring guanine molecules. Interstrand cross-linking within the DNA helix also occurs. Platinum adducts may inhibit DNA replication, transcription and ultimately cell division.

8.2.4 Storage and Stability

Intact vials are stored at room temperature protected from light. The reconstituted solution is stable for at least 24 hours. When further diluted in glass or polyvinyl plastic to a concentration of 10mg/mL with normal saline or 5% dextrose carboplatin is stable for 8 hours at 25 degrees C. Stability with further dilution to 0.5mg/mL has been reported for up to 8 hours. Other stability data indicate that carboplatin is stable for up to 24 hours and may be refrigerated; however, the manufacturer recommends that reconstituted solutions be discarded after 8 hours due to the lack of preservative in drug formulation.

8.2.5 Dose Specifics

Carboplatin will be given by IV at an area under the curve (AUC) dose of 6. Routine premedication should include at least a 5-HT antagonist and dexamethasone. The dose of carboplatin based on target AUC is calculated using the Calvert equation:
Dose (total mg) = Target AUC X (GFR + 25). The patient's creatinine clearance (GFR) in mL/minute is calculated by the Cockcroft Gault equation.
NOTE: When using the Calvert equation, GFR should not exceed 125 mL/min. Thus, the maximum carboplatin dose is 6 x (125 + 25), or 900 mg.

8.2.6 Preparation

Add 5, 15, or 45 mL sterile water, normal saline, or 5% dextrose to the 50, 150, or 450 mg vial, respectively. The resulting solution contains 10 mg/mL. The desired dose is further diluted, usually in 5% dextrose.

8.2.7 Administration

Infuse over 30 minutes

8.2.8 Incompatibilities

Aluminum displaces platinum from the carboplatin molecule, resulting in the formation of a black precipitate and loss of potency. Carboplatin solutions should not be prepared or administered with needles, syringes, catheters, or IV administration sets containing aluminum parts that might be in contact with the drug.

8.2.9 Drug Interactions

Concomitant myelosuppressive drugs or radiation therapy may potentiate the hematologic toxicity of carboplatin.
Concomitant nephrotoxic drugs may potentiate the nephrotoxicity of carboplatin, particularly when carboplatin is given in high-dose chemotherapy regimens.

8.2.10 Compatibilities

Carboplatin (0.3 mg/mL) is chemically compatible in normal saline or 5% dextrose for 24 hours at room temperature.

8.2.11 Availability

Carboplatin is commercially available as a lyophilized powder in 50, 150, or 450 mg vials.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

It is well established that radiation doses ≥ 180 cGy causes DNA damage in the form of double-strand and single-strand breaks,[8,9] which accumulate unrepaired and cause global cellular catastrophe and rapid cell death.[8,26] In contrast, DNA damage caused by LDFRT is proposed to differ from higher-dose radiation through enhancement of, pro-apoptotic pathways without induction of pro-survival pathways.[12,14] We propose to explore whether these in vitro findings are replicated in humans, through the study of the effect of LDFRT and chemotherapy on human samples obtained during this clinical trial. This will be accomplished by evaluating intra-patient variation in DNA damage responses to different doses of radiation via: a) markers of DNA damage responses, PARP (DNA single-strand breaks) and γ-H2AX (DNA double-strand breaks), and b) DNA repair kinetics measured by Comet assay. The γ-H2AX foci formation is the most frequently used marker for cellular DNA damage response to ionizing radiation. However, the benefit of using the extent of γH2AX foci as a measure of biodosimetry (or dose-dependent radiation-induced DNA damage) during radiation therapy has only recently been described.[27,28] PARP (poly(ADP-ribose) polymerase) is a marker more specific to DNA single-strand breaks, and PARP activity has been linked to both beneficial (DNA repair) and harmful (inflammation caused by PARP over-activation) pathophysiologic effects. Therefore, monitoring both biomarkers will provide us a unique capability to assess key responses to varying doses of radiation in patients.

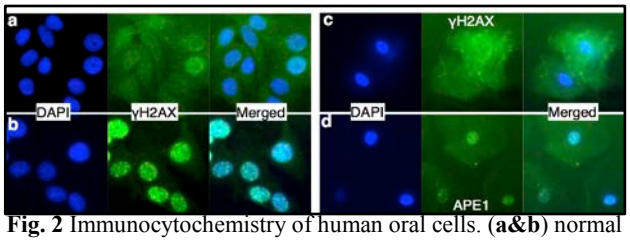


Fig. 2 Immunocytochemistry of human oral cells. (**a&b**) normal oral keratinocyte (OKF4) stained with γH2AX (green) without (**a**) or with (**b**) 50 μM H_2O_2 30 min treatment. Note the γH2AX foci that is intensified in nuclei in (**b**) compared to (**a**) and (**c**).(**c&d**) intact human buccal cells stained with (**c**) anti-γH2AX or (**d**) anti-APE1 antibody.

Using buccal swab sampling [29] [30,31] of the involved-field of radiation to study DNA repair, is novel. In Fig. 2, we assessed the immunostaining procedure with intact buccal cells collected from normal volunteers (Fig. 2a&b). As expected, γ-H2AX foci were absent (Fig. 2a) from unexposed buccal cells and normal oral keratinocytes OKF4 (Fig. 2c, compare to cells exposed to H_2O_2 in

Fig. 2d). However, buccal cells successfully stained with anti-APE1 antibody, which detected APE1 localized in nuclei, demonstrating that the proposed procedure is feasible in human cells.

(c) Approach

Sample collection:

Subjects will rinse their mouth, and the mucosa will be swabbed with a cytology brush, subjects will rinse again and the same area will be swabbed gently with a wooden spatula 10 times. Cytology brush will be placed in media and transported to research lab, and spatula specimens are fixed onto uncharged slides and spray fixed. A second cytology brush is then applied to the area and placed in media for storage and/or subsequent processing for Comet assays (see above). Following collection, fixed slides are then stable until staining. Following collection, fixed slides are then stable until staining. Prior to staining, slides are placed in 95% alcohol for removal of the coating fixative. To enable multiple immunostaining experiment on a glass plates, the buccal spread will be separated by hydrophobic (wax) pen. The samples will be blocked in 1% BSA solution for 1 h, and then antibodies for γ-H2AX and poly(ADP-ribose) will be applied and incubated for 2 h. After washing with 1% BSA, secondary antibodies conjugated to AlexaFluora488 will be applied and incubated for 1 h. If necessary, a positive control (e.g., APE1 antibody) for the staining will be used. The samples will then be washed in 1% BSA, stained with DAPI solution, then mounted on anti-fading reagent and sandwiched on coverslips. Cells will be analyzed using an inverted fluorescence microscope in Izumi lab with x40 objective. Cells will be analyzed using an inverted fluorescence microscope in Izumi lab with x40 objective. For counting foci numbers, an analytical software Image-J (NIH) will be used.

Sampling schedule for buccal collection (minimum of 3 times points needed for kinetics of radiation)		
Pre-treatment	Induction Day 1 (both arms) & 2 (in LDFRT arm)	Definitive Radiation week 1
1 sample	Sample after each dose of LDFRT (4 samples) or during chemotherapy in non-LDFRT arm (2 samples)	Sample after each dose of radiation (3-4 samples)

In preclinical studies, Krueger and colleagues[25] demonstrated that low-dose HRS caused a predictable increase in γ-H2AX within 1-2 hours after each low dose fraction. To determine the effect of LDFRT and full-dose RT, we will measure the time dependent expression of γ-H2AX as well as direct DNA damage in serial mucosal sampling from the irradiated mucosa of these patients. We expect that LDFRT-mediated DNA damage will increase the expression of γ-H2AX, but not to the extent seen in full-dose radiation. Preliminary experiments have shown the feasibility of using γ-H2AX antibody for damage specific foci detection (Fig. 2a&b), including immunostaining procedure for buccal cell staining from normal human volunteers (Fig. 2a&b). APE1, an essential DNA repair protein, was detected predominantly in the nuclei of the buccal cells when stained with an APE1 antibody (Fig. 2d), indicating our immunostaining protocol can effectively probe for damage/repair proteins. In this setting, no γH2AX foci were detected because the specimen was unirradiated. The result in Fig. 2c is still informative as it sets the background intensity of γH2AX, and we will elucidate how the signal changes after LDFRT and full-dose radiation.

Comet assays will determine both damage induction on samples collected immediately after radiation and repair kinetics on samples collected serially from the same subjects during LDFRT and full-dose radiation. After collection or upon removal from storage, buccal cells are washed in PBS, collected by centrifugation, resuspended in low-melting agarose solution, and then immobilized on slides. Immobilized cells are lysed and resulting nucleoids (nuclear DNA spheres) are subject to electrophoresis. Subsequently, DNA in these samples is stained with SYBR Gold and visualized by fluorescence microscopy to assess DNA damage,

evidenced by migration of DNA away from the nucleoid (head) to form a comet-like tail. DNA damage levels are quantified from signal intensity in comet tails relative to the head, from ≥50 cells per sample. Electrophoresis under neutral conditions is used to measure radiation-induced DNA double-strand breaks while alkaline conditions reveal the sum of abasic sites, single- and double-strand breaks. Fig. 3 shows alkaline Comet images of buccal cells untreated or treated ex vivo with H2O2; note the prominent tail produced by H2O2 treatment that induces types of damage similar to that of radiation. Our correlative studies (by Orren/Izumi) will be compared to the clinical assessment of treatment to assess response to treatment, time to progression and survival. These correlations will be exploratory in nature.

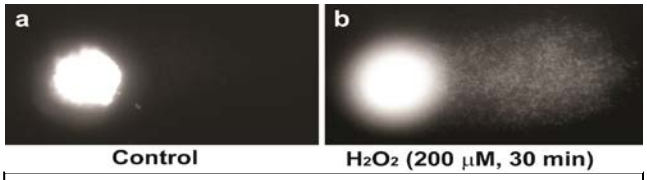


Figure 3. Representative Comet images on freshly collected buccal cells, either untreated (a) or treated ex vivo with H2O2 (b) as indicated.

9.2 Correlative Studies (Tumor Biopsy and Radiation Port Images)

Subjects who have previously collected tumor tissue will be asked to give permission for correlative studies on these biopsies. Participants will not have to undergo any additional biopsies of their tumor to be on this study, only buccal mucosal samples as described in section of the protocol. De-identified images from radiation treatment planning will also be available to investigators for correlation with DNA repair findings and location of the buccal swab in relation to radiation fields. No identifiers will be given to investigators.

10. STUDY CALENDAR

Within 6 weeks, all patients will undergo a direct laryngoscopy (DL) or indirect laryngoscopy (IDL) (if the primary tumor is easily and completely evaluable by IDL). Within 4 weeks of registration, all patients will undergo a history and physical exam, ECOG performance status evaluation, a CT or MRI scan of the involved area of the head and neck and a CT of the Chest or chest X-ray. Within 2 weeks of registration, all patients will undergo a complete blood count with differential and platelets, and serum chemistries. Once informed consent and enrollment have been completed, subjects will be randomized to one of two treatment arms: a) chemotherapy alone or b) chemotherapy plus LDFRT. Treatment will be administered on an outpatient or inpatient basis.

Evaluation	Pre-Study	Day 1	Day 2	Day 22	Day 23	Day 38-50(c)	Week 1 of definitive RT/CT (d)	4-6 weeks post definitive Radiation or surgery	STUDY COMPLETE	LTFU by KCR at interim and at 3 years
H&P* PS assessment	X			X		X		X		
Vital signs and height and weight	X			X						
CBC w/ differential	X			X		X				
Electrolytes (a)	X			X		X				
b-HCG (b)	X									
Chest X-ray or CT scan Chest	X							X		
MRI or CT scan of Neck (e)	X					X		X		
Panendoscopy or Indirect laryngoscopy	X					X				
Mucosal sampling (f)	X	X	X				X			
QOL form	X					X				
Chemotherapy		X		X						
80 cGy RT		X X	X X	X X	X X					
OS and PFS survival assessment										X

*: Day 22 H&P should be within 2 days of chemotherapy cycle 2
a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: Serum pregnancy test (women of childbearing potential).
c: Off-study evaluation.
d: Definite radiation should occur on or after Day 43 and prior to Day 64 from initiation of induction (Day 1). Definitive surgery should occur on or after Day 43.
e: Using same form of radiographic assessment pre-treatment and post-induction
f: Pre dose (1 sample), day 1 and 2 post radiation, cycle 1 induction (4 samples in LDFRT arm 2 samples in non-LDFRT arm) and Day 1-5 full dose radiation (3-4 samples)

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be evaluated for response to induction after 2 cycles of therapy.

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) (34). 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.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Docetaxel, Carboplatin, and LDFRT.

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.

11.1.2 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 or as ≥10 mm with CT scan, MRI, or 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. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. 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. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should 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.

11.1.3 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 to 6 weeks before the beginning of the treatment (see study calendar).

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.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

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. Furthermore, the availability of MRI is variable globally. 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. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

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 later 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.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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 While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment

of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-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.

11.1.4 Response Criteria

11.1.4.1 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.

11.1.4.2 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).

11.1.4.3 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.

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) 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.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Progression-Free Survival

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

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

This study will require data submission and reporting via the OnCore Database, which is the official database of the Markey Cancer Center Clinical Research and Data Management Shared Resource Facility (CRDM SRF). Instructions for submitting data is listed in Study-Specific Data Management Plans created by CRDM SRF staff.

12.1.2 Responsibility for Data Submission

Study staff is responsible for submitting study data and/or data forms to OnCore as per the Markey Cancer Center CRDM SRF SOP's. This trial will be monitored by the MCC Data and Safety Monitoring Committee (DSMC) on a schedule determined by the Protocol Review and Monitoring Committee at the initial PRMC review. The CRDM SRF staff is responsible for compiling and submitting data for all participants and for providing the data to the Principal Investigator for review.

13. STATISTICAL CONSIDERATIONS

13.1 Experimental Design

This is a randomized phase II trial of chemotherapy with or without low-dose radiation in locally advanced SCCHN.

13.2 Accrual and Power Considerations

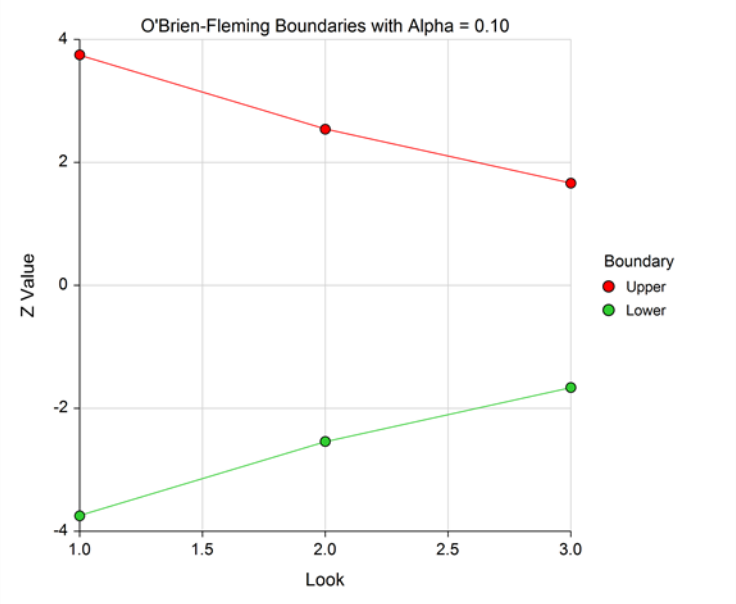
The primary endpoint is primary site CR rate, which in our previous study was 60% using LDFRT + chemotherapy. We will assume that the control arm of this trial (chemotherapy alone) will provide only a 30% primary site CR rate. Using a binomial test of two independent proportions with 2 planned interim analyses (after 25% and 50% of total patient accrual) with O'Brien-Fleming stopping boundaries for futility, 34 subjects per group are needed for this phase II based on a two-sided type I error rate of at most 10% and 80% power. Conservatively allowing 5% drop out or loss to follow up, we plan to enroll 72 total patients. All sample size calculations with corresponding interim analysis rules were conducted using PASS 11.0.[32]

13.3 Interim Analyses

Using a binomial test of two independent proportions with 2 planned interim analyses (after 25% and 50% of total patient accrual) with O'Brien-Fleming stopping boundaries for futility, 34 subjects per group are needed for this phase II based on a two-side type I error rate of at most 10% and 80% power. The O'Brien-Fleming boundaries and alpha spending details are listed in the table and figure below.

13.4 Details for Interim Analyses with O'Brien-Fleming Boundaries

Look	% of Accruals	Lower Boundary	Upper Boundary	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	25%	-3.750	3.750	0.00018	0.00018	0.00018	0.006	0.006
2	50%	-2.540	2.540	0.011	0.011	0.011	0.219	0.225
3	100%	-1.662	1.662	0.096	0.089	0.100	0.582	0.807



13.5 Analysis Plan

Following study completion, the percentage of subjects achieving primary site CR in each group will be estimated along with exact 90% binomial confidence intervals. A binomial test or Fisher's exact test will also be employed to test for a difference in primary site CR rates between treatment and control groups, with p-values < 0.10 considered significant.. All correlative endpoints and secondary endpoints are exploratory

in nature and will be primarily descriptive; however, strategies will include safety incidence tables, differences in QOL scores from baseline to completion of treatment between groups using 2-sample t-test or Wilcoxon Rank Sum tests depending on parametric assumptions and, and Kaplan-Meier curves for overall survival with corresponding log rank tests for comparisons between groups.

13.6 Data Management

All data will be stored in the OnCore Data Management System of the Markey Cancer Center. Case report forms will record all study endpoints and data will be accessed in a secure manner using this password protected, encrypted software. The study statistician, along with staff from the Biostatistics Shared Resource Facility (BSRF), will work closely with the study PI and the CRDM SRF staff on the development of eCRFs for the study. Instructions for data entry will be listed in Study-Specific Data Management Plan created by CRDM SRF staff in conjunction with the study statistician. The OnCore database is housed on secure servers maintained by the Cancer Research Informatics Shared Resource Facility (CRISRF) of Markey Cancer Center. The database is backed up daily. Data will be accessed by the study statistician via OnCore on a regularly scheduled basis to perform statistical programming to assess data quality control, study recruitment and to generate interim reports and analyses. In collaboration with the study team, procedures and timelines will be developed for data quality control, resolution of data queries, interim reporting, and final data analysis.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with Docetaxel, Carboplatin, and LDFRT.

13.7.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

14. REFERENCES

1. CINA+. (2013). Cancer Incidence Rates in North America., 2006-2010
2. Pignon J-P, Maître Al, Maillard E, et al: Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 93 randomised trials and 17,346 patients. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 92:4-14, 2009
3. Vokes EE: Induction Chemotherapy for Head and Neck Cancer: Recent Data. The Oncologist 15:3-7, 2010
4. Haddad R, O'Neill A, Rabinowits G, et al: Induction chemotherapy followed by concurrent chemoradiotherapy (sequential chemoradiotherapy) versus concurrent chemoradiotherapy alone in locally advanced head and neck cancer (PARADIGM): a randomised phase 3 trial. The Lancet Oncology 14:257-264, 2013
5. Posner MR, Haddad RI, Wirth L, et al: Induction chemotherapy in locally advanced squamous cell cancer of the head and neck: Evolution of the sequential treatment approach. Seminars in oncology 31:778-785, 2004
6. Vermorken JB, Remenar E, van Herpen C, et al: Cisplatin, Fluorouracil, and Docetaxel in Unresectable Head and Neck Cancer. New England Journal of Medicine 357:1695-1704, 2007
7. Hitt R, López-Pousa A, Martínez-Trufero J, et al: Phase III Study Comparing Cisplatin Plus Fluorouracil to Paclitaxel, Cisplatin, and Fluorouracil Induction Chemotherapy Followed by Chemoradiotherapy in Locally Advanced Head and Neck Cancer. Journal of Clinical Oncology 23:8636-8645, 2005
8. RL S: Biochemical pharmacology of chemotherapeutic drugs used as radiation enhancers. . Semin Oncol 19:2-7, 1992
9. Milas L MM, Mason KA.: Combination of taxanes with radiation: preclinical studies. Semin Radiat Oncol 9(2 Suppl 1):12-26., 1999
10. Marples B JM: The response of Chinese hampster V79 cells to low radiation doses. Evidence of enhanced sensitization of the whole cell population. Radiat Res 133:41-51, 1993
11. Joiner MC, Marples B, Lambin P, et al: Low-dose hypersensitivity: current status and possible mechanisms. International journal of radiation oncology, biology, physics 49:379-389, 2001
12. Dey S, Spring PM, Arnold S, et al: Low-Dose Fractionated Radiation Potentiates the Effects of Paclitaxel in Wild-type and Mutant p53 Head and Neck Tumor Cell Lines. Clinical Cancer Research 9:1557-1565, 2003
13. Arnold SM, Regine WF, Ahmed MM, et al: Low-dose fractionated radiation as a chemopotentiator of neoadjuvant paclitaxel and carboplatin for locally advanced squamous cell carcinoma of the head and neck: results of a new treatment paradigm. International journal of radiation oncology, biology, physics 58:1411-1417, 2004
14. Chendil D OR, Alcock RA, Patel N, Mayhew C, Mohiuddin M, Gallicchio VS, Ahmed MM.: Low dose fractionated radiation enhances the radiosensitization effect of paclitaxel in colorectal tumor cells with mutant p53. Cancer 89:1893-1900, 2000
15. Gleason J KM, Van Meter E, Mohiuddin M, Regine WF, Valentino J, Kenady D, Arnold SM. : Low-dose fractionated radiation with induction chemotherapy for locally advanced head and neck cancer: 5 year results of a prospective phase II trial. International Journal of Radiation Oncology Biology Physics 81:S502-S503, 2012
16. Regine WF, Hanna N, Garofalo MC, et al: Low-Dose Radiotherapy as a Chemopotentiator of Gemcitabine in Tumors of the Pancreas or Small Bowel: A Phase I Study Exploring a New Treatment Paradigm. International journal of radiation oncology, biology, physics 68:172-177, 2007
17. Creane M SC, Mothersill C. : Effect of docetaxel (Taxotere) on expression of radiation-induced lethal mutations in human cell lines. . Int J Radiat Biol 75:725-730, 1999
18. Dunphy F BJaDT: Paclitaxel and carboplatin in head and neck cancer. . Semin Oncol

24:S19-27, 1997

19. Posner MR, Hershock DM, Blajman CR, et al: Cisplatin and Fluorouracil Alone or with Docetaxel in Head and Neck Cancer. New England Journal of Medicine 357:1705-1715, 2007
20. Hennequin C GN, Favaudon V: Interaction of ionizing radiation with Paclitaxel (Taxol) and docetaxel (Taxotere) in HeLa and SQ20B cells. . Cancer Res 56:1842-1850, 1996
21. Chougule PB AM, Akerley W, et al. : Chemoradiotherapy for advanced inoperable head and neck cancer: a phase II study. . Semin Radiat Oncol 9:58-63, 1999
22. Zanelli GD QM, Robiuex I, et al: Paclitaxel as a radiosensitizer: A proposed schedule of administration based on in-vitro data and pharmcokinetic calculations. Eur J Cancer . 33:486-492, 1997
23. S. SKM: Interaction of platinum drugs with clinically relevant x-ray doses in mammalian cells: a comparison of Carboplatin, carboplatin, iproplatin, and tetraplatin. Int J Radiat Oncol Biol Phys 20:221-225, 1991
24. Löbrich M JP: Harmonising the response to DSBs: a new string in the ATM bow. . DNA Repair (Amst). 4:749-759, 2005
25. Krueger SA, Wilson GD, Piasentin E, et al: The Effects of G2-Phase Enrichment and Checkpoint Abrogation on Low-Dose Hyper-Radiosensitivity. International journal of radiation oncology, biology, physics 77:1509-1517, 2010
26. Chua MLK, Rothkamm K: Biomarkers of Radiation Exposure: Can They Predict Normal Tissue Radiosensitivity? Clinical Oncology 25:610-616, 2013
27. Gonzalez J, Roch-Lefevre S, Mandina T, et al: Induction of gamma-H2AX foci in human exfoliated buccal cells after in vitro exposure to ionising radiation. Int J Radiat Biol 86:752 - 759, 2010
28. Yoon AJ, Shen J, Wu H-C, et al: Expression of Activated Checkpoint Kinase 2 and Histone 2AX in Exfoliative Oral Cells after Exposure to Ionizing Radiation. Radiation Research 171:771-775, 2009
29. Loprevite M, Tiseo M, Chiaramondia M, et al: Buccal Mucosa Cells as In vivo Model to Evaluate Gefitinib Activity in Patients with Advanced Non–Small Cell Lung Cancer. Clinical Cancer Research 13:6518-6526, 2007
30. Szeto YT, Benzie, I.F., Collins, A.R., Choi, S.W., Cheng, C.Y., Yow, C.M. and Tse, M.M: A buccal cell model comet assay: development and evaluation for human biomonitoring and nutritional studies. Mutation Research 578:371-381, 2005
31. Izumi and Orren Personal Communication, 2013
32. Hintze J: PASS 11. NCSS, LLC. Kaysville, Utah, USA www.ncss.com., 2011

APPENDIX A. PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B. STAGING FOR HEAD AND NECK CANCER

Please see www.nccn.org for complete staging system for H&N cancer

APPENDIX C. FACT-H&N V4.
Below is a list of statements that other people with your illness have said are important**.**

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my					

	illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	<u>EMOTIONAL WELL-BEING</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	<u>FUNCTIONAL WELL-BEING</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4

GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.

	<u>ADDITIONAL CONCERNS</u>	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
H&N1	I am able to eat the foods that I like	0	1	2	3	4
H&N2	My mouth is dry	0	1	2	3	4
H&N3	I have trouble breathing	0	1	2	3	4
H&N4	My voice has its usual quality and strength	0	1	2	3	4
H&N5	I am able to eat as much food as I want	0	1	2	3	4
H&N6	I am unhappy with how my face and neck look	0	1	2	3	4
H&N7	I can swallow naturally and easily	0	1	2	3	4
H&N8	I smoke cigarettes or other tobacco products	0	1	2	3	4

H&N9	I drink alcohol (e.g. beer, wine, etc.)	0	1	2	3	4
H&N 10	I am able to communicate with others	0	1	2	3	4
H&N 11	I can eat solid foods	0	1	2	3	4
H&N 12	I have pain in my mouth, throat or neck	0	1	2	3	4

APPENDIX D. BIOASSAY PROCEDURES

Buccal Swab Procedure

Use positively charged slides (ex. Snowcoat Leica 3800299) white with frosted end.

1. Rinse mouth
2. Using PurFybr cytology brushes, twirl brush against inner cheek or oropharynx as near as possible to radiation port, gently but firmly to dislodge cellular materials. Save brush #. Transfer to 15 ml centrifuge tubes containing 2 ml ice-cold Phosphate-Buffered Saline (PBS) solution and using sharp scissors, cut (just enough of) the shaft of the brush and re-cap the tube. Refrigerate tube in preparation for transport.
3. Scrape buccal mucosa 10 times firmly, but gently in same region, with wooden spatula
4. Smear the cells onto slide 1, then gently spread with slide 2
5. Immediately spray fix with Fisher Scientific* PROTOCOL* Cytologic Fixative - 50mL (245688)[1]
6. Allow slide to dry (can leave like this) and then label slides with subject ID and sample letter once slide has dried (i.e. "001-B"), label conical tube with same label number
7. Place slides in slide box and transport to cc419 for pickup by Izumi lab
8. Using a second PurFybr cytology brush, twirl brush in same region of the mouth gently but firmly to dislodge cellular materials.
9. Transfer 2nd brush to 15 ml centrifuge tubes containing 2 ml ice-cold Phosphate-Buffered Saline (PBS) solution and Using sharp scissors, cut (just enough of) the shaft of the brush and re-cap the tube. Keep tubes on ice for transport to Orren lab. Call contact number in Orren lab, and if no answer, leave in refrigerator in cc419 for pickup.

1 Coating fixatives are substitutes for wet fixatives. They are either aerosols applied by spraying the cellular samples or a liquid base, which is dropped onto the slide. They are composed of an alcohol base, which fixes the cells and wax like substance, which forms a thin protective coating over the cells e.g. Carbowax (Polyethylene Glycol) fixative. Most of these agents have a dual action in that they fix the cells and, when dry, form a thin protective coating over the smear. The coating fixative should be applied immediately on fresh smears. The distance from which the slides are sprayed 10 to 12 inches (25-30 cm) is the optimum distance. Prior to staining, the slides have to be kept for 15 minutes in 95% alcohol for removal of the coating fixative.

Time Points for the study:

Buccal Sampling Schedule		
Treatment	Day	Buccal sample
Pre-treatment	Pre-treatment	Prior to all radiation/chemotherapy
Induction Therapy (minimum 3 samples)	Cycle 1 Day 1*	After LDFRT fraction 1
		After LDFRT fraction 2
	Cycle 1 Day 2**	Pre-treatment
		After LDFRT fraction 3
Definitive Radiation (minimum 3 samples)	Definitive Radiation Week 1 Days 1-5***	Pretreatment Day 1
		After radiation fraction Day 1
		After Cisplatin Day 1
		After radiation fraction (preferably Day 4 or 5)

* non-LDFRT arm will have two samples obtained during chemotherapy
** non-LDFRT arm will not have sampling on Day 2
*** surgical patients will not undergo definitive radiation sampling