LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

RESEARCH PROTOCOL

STUDY NUMBER(S):	LCCC1612
PROTOCOL(S) TITLE:	P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers
SPONSOR:	Lineberger Comprehensive Cancer Center
AMENDMENT NUMBER:	Amendment 15
VERSION DATE:	18August2023

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Protocol Amendment #15

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES:
_X_ Editorial, administrative changes
_X_ Scientific changes
__ Therapy changes
__ Eligibility Changes

Rationale for amendment: The purpose of this amendment is to remove patient reported assessments and updated protocol reporting requirements.

Editorial, administrative changes:

Throughout	Grammatical and mechanical edits made throughout
Throughout	Protocol PI updated to Dr. Wendell Yarbrough, additional co-investigators and study personnel removed from protocol.

Scientific changes:

Section 2.2.3 and 3.2.3	Secondary objective and endpoint comparing head and neck quality of life before, during and after CRT removed.
Section 2.2.4 and 3.2.4	Secondary objective and endpoint comparing speech and swallowing function before and after CRT removed.
Section 5.11	All patient assessments removed, including Quality of Life and Patient Reported Outcomes as well as Speech and Swallow evaluations.
Section 5.12	Languaged related to QOLs, PROs and the EAT-10 assessment relative to study duration removed.
Section 6.2	Language relative to reporting updated to add justication lack of trial related adverse event reporting. Language for affiliate sites and UNC removed
Section 7.2	References to QOLs, PROs, and speech and swallow function removed from statistical analysis section.
Appendix	QOL, PRO and Speech and Swallow assessments removed.
Appendix	Section 10.2 added, Time and Events table updated to capture changes to protocol study design.

THE ATTACHED VERSION DATED August 18, 2023 INCORPORATES THE ABOVE REVISION

PROTOCOL AMENDMENT #14

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Scientific changes

- Target accrual increased to 250 (Sections 5.1 and 7.1)

PROTOCOL AMENDMENT #13

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Scientific changes

- As of the date of this protocol, no blood specimens will be obtained beyond Week 4 of treatment. (Section 5.5)

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

PROTOCOL AMENDMENT #12

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Add [redacted] as a Co-Investigator (cover pages)

Scientific changes

- Target accrual increased to 200 (Sections 5.1 and 7.1)
- Blood collection time points revised (Section 5.5, Time and Events table [Appendix])

Eligibility changes

- Current active smokers excluded (Sections 4.2, Eligibility Checklist [Appendix])

THE ATTACHED VERSION DATED 01/07/2020 INCORPORATES THE ABOVE REVISIONS

ATTACH TO THE FRONT OF EVERY COPY OF PROTOCOL

PROTOCOL AMENDMENT #11

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Add [redacted], MD as a Co-Investigator (cover pages)

Scientific changes

- Target accrual increased to 180 (Sections 5.1 and 7.1)

THE ATTACHED VERSION DATED 10/09/2019 INCORPORATES THE ABOVE REVISIONS

ATTACH TO THE FRONT OF EVERY COPY OF PROTOCOL

PROTOCOL AMENDMENT #10

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Scientific changes

- Number of blood tubes to be collected at each time point for UNC-CH patients increased to two (Section 5.5)

PROTOCOL AMENDMENT #9

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Clarification regarding potential additional post-treatment blood draws (Section 5.5)

Scientific changes

- Target accrual increased to 160 (Sections 5.1 and 7.1)

PROTOCOL AMENDMENT #8

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Co-investigator list updated (Cover pages)

Scientific changes

- Details on cfHPV-DNA analyses modified as follows (Sections 5.1 and 5.5, Time and Events table):
  - Blood draws reduced to 2 time points during treatment (vs. 6)
  - Two tubes to be drawn from UNC-CH patients at baseline (vs. 1)
  - Additional tubes may be drawn post-treatment at PI's discretion
- Flexibility in timing allowed for post-treatment chest xrays and thyroid labs (Time and Events table)
- AE reporting requirements limited to within 30 days following cessation of treatment (Section 6.2)
- Patient assessments modified as follows (Sections 5.11.1 and 5.12, Time and Events table):
  - Through year 5 post-treatment only
  - Discontinued upon cancer recurrence
- Total target accrual increased from 120 to 140 (Sections 5.1 and 7.1)

PROTOCOL AMENDMENT #7

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Scientific changes

- Modified to indicate that, in addition to patients with >10 pack years smoking history, those with an extensive smokeless tobacco history may also receive genetic testing of their tumor (based on the PI's assessment) to determine receipt of de-intensifed treatment (sections 1.8, 5.1, and 5.4).

PROTOCOL AMENDMENT #6

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

___ Editorial, administrative changes
___ Scientific changes (IRB approval)
X Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Therapy changes

- Treatment plan revised such that for patients with T0-2 N0-1 disease, >10 pack years smoking history, chemotherapy will be at the discretion of the treating physician (section 5.7).

PROTOCOL AMENDMENT #5

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Addition of Blumberg (UNC) (cover pages)
- Edit SOP reference number (section 6.2)

Scientific changes

- FISH analysis of CCND1 by the UNC Cytogentics Laboratory has been eliminated (sections 1.8, 5.1, 5.4)
- Revise number of slides needed for genetic testing (section 5.4)
- Expanded window of post-treatment Modified Barium Swallow (section 5.11.2, Time and Events table)
- Clarification of time point for first post-treatment thyroid function testing (Time and Events table)

PROTOCOL AMENDMENT #4

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
X Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Co-investigator changes: addition of [redacted] (UFPTI), removal of [redacted] (UNC), [redacted] (UNC), N[redacted] (UNC), [redacted] (UNC), [redacted] (UNC), [redacted] (Rex) (cover pages)
- New site: University of Florida Proton Therapy Institute (cover pages, sections 1.1 and 8.4)
- Revision/clarification of post-treatment blood draws and shipping timeframe (section 5.5)
- Allow for discretion of timing of post-treatment laryngoscopy (Appendix - Time and Events table)
- Clarification of staging guidelines for eligibility (AJCC 7th edition) (section 4.1.2)

Scientific changes

- Increase in target accrual to 120 (section 7.1)

Therapy changes

- Addition of proton radiotherapy as allowable RT (section 5.6)

PROTOCOL AMENDMENT #3

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
___ Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Addition of three UFL co-investigators: Silver, Gopalan, and Dang; spelling correction for Dziegielewski (cover pages)
- Modification to registration procedures to clarify the requirements that patients be registered with UNC prior to enrollment on the study and that the UNC study coordinator confirm eligibility criteria prior to treatment start (section 8.3)

PROTOCOL AMENDMENT #2

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
___ Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Clarification that HPV and/**or** p16 testing to be performed, and this testing may be performed at outside institutions (section 5.3, Time and Events Table)
- Clarification that sites may send tumor block for genetic assessment (section 5.4)
- Details added regarding blood samples for cfHPV-DAN, including shipping address (section 5.5)
- [redacted] and [redacted] moved from Co-Investigators to Co-Principal Investigators (cover pages)
- Delete [redacted] as Co-Investigator, and add [redacted] and [redacted] (cover pages)
- Clarification of AE reporting (section 6.2)
- Clarification of timing for pre-tx MBS (sections 3.2.4, 5.11.2, Time and Events Table)
- Clarification of timing for first post-CRT chest x-ray (Time and Events Table)

Scientific changes

- Revision to data analysis plan to allow for additional analyses of study data (section 7.2)

PROTOCOL AMENDMENT #1

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

AMENDMENT INCORPORATES (check all that apply):

X Editorial, administrative changes
X Scientific changes (IRB approval)
___ Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY:

Editorial, administrative changes

- Clarification of audit process (section 9.4), obligations of investigators (section 9.8), and record retention (section 9.7)
- Section 6.2 edited to include extra chemotherapy dose for those subjects with a p53 mutation
- Clarification of blood collection and shipping process by other participating sites (section 6.0)
- Tumor panel specimen requirements removed from Appendix and pertinent information added to section 5.4
- Schema in Section 5.1 relabeled as Figure 2

Scientific changes

- Clarification of time points for QOL and Swallowing assessments (sections 3.2.3, 3.2.4, 6.6.1, 6.6.2)
- Correction of dose constraints (section 6.1.4)
- Revisions to Time and Events Table (appendix)

Eligibility changes

- Revisions to eligibility criteria modifying time frame of select inclusion critieria (prior to treatment rather than prior to registration) and adding exclusion critieria (systemic lupus, psoriatic arthritis) (section 4.0 and appendix)

LCCC1612

P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

CONFIDENTIALITY AND INVESTIGATOR STATEMENT

The information contained in this protocol are confidential and proprietary information of Lineberger Comprehensive Cancer Center, and except as may be required by federal, state, or local laws or regulation, may not be disclosed to others without prior written permission of Lineberger Comprehensive Cancer Center.

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the regulations stated in the Federal Code of Regulations for Good Clinical Practices and International Conference on Harmonization guidelines and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Lineberger Comprehensive Cancer Center or specified designees. I will discuss the material with them to ensure that they are fully informed about the study.

Principal Investigator Name (printed) Signature

_____ _____

Date

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

LCCC 1612: P53 mutational status and circulating free HPV DNA for the management of HPV-associated Oropharyngeal Squamous Cell Cancers

Principal Investigator
Wendell Yarbrough, MD
Professor
Department of Otolaryngology
101 Manning Drive, Campus Box 7070
Chapel Hill, NC 27599-7070
(919) 843-7091 (phone)
Email: dell@med.unc.edu

Biostatistician
Xianming Tan, PhD
Assistant Professor
Department of Biostatistics

Sponsor: Lineberger Comprehensive Cancer Center

Version Date: 8/18/2023

TABLE OF CONTENTS

Contents

1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

The proposed study is a follow-up study to LCCC 1120 and 1413. We have observed an excellent pathological complete response rate (LCCC 1120) and progression free survival (PFS) (LCCC 1413) in patients with HPV positive and/or p16 positive low-risk oropharyngeal squamous cell carcinoma (OPSCC) who received de-intensified chemoradiotherapy (CRT). We have shown that de-intensification is efficacious in these two phase II studies. We are leaders in the genomic classification of head and neck cancer, and investigators at LCCC [redacted]) have shown that HPV-associated oropharyngeal cancer has a unique genetic profile. A major question is whether we can de-intensify in patients with HPV-associated oropharyngeal cancer who have smoking histories. The aim of the current study is to build upon our two phase II studies and the genomic profiling studies. Our hypothesis is that genomic profiling of patients' tumors (specifically for p53 mutations) will help in triaging patients to de-intensification versus standard of care. We will enroll patients with HPV-associated OPSCC regardless of smoking history and will assess p53 mutational status in patients with a smoking history. We will use the same de-intensification chemoradiotherapy regimen we have already evaluated in LCCC 1120 and 1413 in patients with HPV-associated OPSCC who have a minimal smoking history and in patients with a smoking history but with wild-type p53. If patients with a smoking history have mutated p53 they will not receive de-intensified chemoradiotherapy, but instead will receive standard doses. Our primary objective will be 2 year PFS. In LCCC 1413 the primary null hypothesis was 2 year PFS of 87%. We hypothesize, that by using genomics in the patients with a signicant smoking history we will better select those who we can safely de-intensify and that the 2 year PFS will improve to 93%. We will also prospectively assess circulating free HPV DNA (cf-HPV-DNA) from blood samples. This study will be multi-institutional, with patients also enrolling at the University of Florida, Gainesville, Florida; the University of Florida Proton Therapy Insitute, Jacksonville, FL; and Rex Cancer Center of Raleigh, Raleigh, North Carolina.

1.2 Standard of care CRT for OPSCC

The standard treatment regimen for HPV-associated oropharyngeal or unknown primary squamous cell carcinoma of the head and neck is 70 Gy, 2 Gy per day, 35 fractions, over 7 weeks with 2 to 3 doses of cisplatin 100mg/m^2. Most institutions perform a PET/CT at 10 to 16 weeks after CRT to assess response. Depending on the clinical response, patients may have to have a biopsy of the primary site and/or a neck dissection after treatment. Typically patients with a negative PET/CT scan are observed (i.e. no surgery). Standard CRT is associated with significant acute toxicity with most patients experiencing grade 3 and 4 acute toxicity during treatment. Furthermore, approximately 20% of patients will have Grade 3-4 long-term morbidity related to their definitive CRT.

1.3 HPV related OPSCC

The incidence of OPSCC is increasing and is thought to be secondary to HPV infection of the oropharyngeal mucosa[1-8]. Evidence is accumulating that suggests that HPV-

positive HNSCC may be a distinct clinical and biological entity. The affected individuals are more likely to be white men, younger than 60 years of age, unmarried, and have a minimal history of alcohol or tobacco use[3,9-30]. HPV-positive HNSCC has a higher response rate to neoadjuvant chemotherapy and in general has a better prognosis to therapy as compared to HPV-negative HNSCC[10,13,18,19,22]. Biologically, the HPV oncoproteins E6 and E7 inactivate the tumor suppressor proteins p53 and pRb, respectively. Inactivation of pRB by E7 leads to upregulation of the p16 tumor suppressor protein. Thus, overexpression of p16 may be regarded as a biomarker for HPV-positive HNSCC[18,29]. Furthermore, HPV-positive HNSCC is more likely to be associated with a wild-type p53, unlike tobacco-induced HNSCC[11]. Because of the observed improved prognosis and distinct molecular profile, some have suggested that HPV-positive tumors may be more sensitive to CRT. The standard CRT regimen for most HNSCC is 70 Gy with 3 cycles of concurrent cisplatin at 100mg/m2[31]. Less intensive chemotherapy and/or radiation may be just as effective in HPV-related oropharyngeal HNSCC and reduce the severity of acute toxicity and long-term morbidity associated with CRT.

1.4 Prospective Clinical Data on HPV positive HNSCC

Numerous retrospective studies have shown that patients with HPV-positive HNSCC have a significantly better prognosis than patients with HPV-negative HNSCC[5,25,29]. These preliminary data have been verified in analyses of prospective clinical trials[10,13,18]. Fakhry et al. evaluated the HPV status of 96 patients with laryngeal or oropharyngeal SCC from an Eastern Cooperative Oncology Group (ECOG) phase II trial. Patients with HPV positive tumors had higher response rates after induction chemotherapy (82% vs. 55%, $p = 0.01$) and CRT (84% vs. 57%, $p = 0.007$) as compared to those with HPV-negative tumors. The 2 year overall survival was also improved - 95% (HPV positive) vs. 62% (HPV negative). Lassen et al. analyzed the Danish Head and Neck Cancer Group (DAHANCA) 5 trial and observed an improved local-regional control, disease specific survival, and overall survival in patients whose tumors were p16 positive (a surrogate marker for HPV) versus those who were p16 negative[18].

The RTOG 0129 prospective randomized trial also validated HPV positivity as being a strong, independent prognostic factor for overall and progression free survival[10]. RTOG 0129 compared accelerated fractionation radiotherapy with standard fractionation, both with concurrent cisplatin (2 cycles in the former and 3 cycles in the latter). There was no statistical difference in overall or progression free survival between the two arms. Post-hoc analysis of HPV status and outcomes in the OPSCC subset showed a significant improvement in OS (82.4%, vs. 57.1% at 3 year; $P<0.001$) and PFS in the HPV positive tumors. After accounting for other prognostic factors, HPV positivity conferred a 58% reduction in the risk of death (hazard ratio, 0.42; 95% CI, 0.27 to 0.66). Locoregional control was improved in the HPV-positive tumors; however, distant metastatic control and the rate of second malignancies were similar for both HPV-positive and negative OPSCC subgroups. Through recursive portioning analysis this OPSCC cohort was stratified into low, intermediate, and high risk groups for death (3 year OS of 93%, 70.8%, and 46.2% respectively). The factors used for stratification, in order of importance, were: HPV status, pack-years of tobacco smoking, tumor stage, and nodal stage. To be categorized as having low risk of death, a patient must have a HPV-positive

tumor and either have ≤ 10 pack years of tobacco smoking and any N stage or >10 pack years of tobacco smoking and N0-N2a nodal stage.

O'Sullivan et al. conducted a large retrospective study of 899 OPSCCs patients treated at the Princess Margaret Hospital in Toronto, Canada[32]. HPV status was ascertained in 505 (56%): 382 HPV positive and 123 HPV negative. All patients were treated with radiation alone or CRT from 2001 to 2009, with a median follow-up of 3.9 years. Recursive portioning analysis segregated HPV-positive patients into low (T1-3 N0-N2c) and high (T4 or N3) distant metastasis risk: 93% vs. 76% respectively. Furthermore, smoking > 10 pack years reduced overall survival but did not impact recurrence free survival (local, regional, and distant recurrences) in HPV-positive patients. This suggests that other associated tobacco-related disease may be the reason for decreased survival in HPV-positive smokers vs. HPV-positive non-smokers. Also, the recurrence free survival was excellent (>90%) in low-risk HPV-positive patients with T1-2, N0-1 disease treated with RT alone vs. CRT. Thus the addition of chemotherapy to radiation in this very favorable low-stage subset may not be necessary.

An unanswered question is whether we can safely de-intensify in patients with HPV-associated OPSCC who have significant smoking histories (> 10 pack years). The proposed study will help to answer this question.

1.5 UNC De-intensification chemoradiotherapy regimen

We have completed two phase II studies evaluating the efficacy of a de-intensified chemoradiotherapy regimen in favorable risk oropharyngeal squamous cell carcinoma. In LCCC 1120 we treated 45 patients with de-intensified chemoradiotherapy. Eligible patients had HPV-positive and/or p16-positive OPSCC, T0 – T3, N0 – N2c, M0, and ≤ 10 pack years of smoking. Patients received 60 Gy of Intensity Modulated Radiotherapy (IMRT) with concurrent weekly intravenous cisplatin (30 mg/m^2). Diagnostic imaging (CT and/or MRI) was obtained 4 to 8 weeks after completion of CRT to assess response. All patients had surgical resection of any clinically apparent residual primary tumor or biopsy of the primary site if there was no evidence of residual tumor and underwent a neck dissection to encompass at least those nodal level(s) that were positive pre-treatment, within 4 to 14 weeks after CRT. The primary endpoint of LCCC 1120 was the rate of pCR. 45 patients enrolled and 43 were evaluable for the primary endpoint. The observed pCR rate was 86% (37/43), which is excellent and within the expected result for a positive outcome. We further observed lower feeding tube use (with no permenant feeding tubes) and favorable patient reported outcomes related to dry mouth and dysphagia (the two most common chronic toxicities) as compared to standard dose chemoradiotherapy[33].

In our second phase II study, we evaluated the same de-intensified regimen with the following modifications:

1) We did not require surgical evaluation after de-intensified CRT. Instead, we peformed 12 week post-treatment PET/CT and peformed post CRT neck dissection only in cases where the ~12 week PET-CT was positive for residual adenopathy.

2) We omitted chemotherapy in the most favorable subgroups (p16/HPV positive, T0-2, N0-1, ≤ 10 pack years) based on exisiting data of treating these patients with RT alone[32,34].
3) We allowed enrollment of patients with moderate but remote smoking histories (smoking history of ≤ 30 pack-years with ≥5 years of abstinence from smoking). This was based on data from Princess Margaret Hospital which suggest that moderate tobacco use did not affect cancer control rates in HPV-positive patients[32].

4) We allowed weekly chemotherapy regimens other than cisplatin. Cisplatin 30mg/m^2 was the preferred regimen, but if per medical oncologist evaluation, cisplatin was deemed untolerable (e.g. pre-existing hearing loss, elevated creatinine, neuropathy) other weekly regimens were allowed (cetuximab and carbotaxol).

5) The primary objective was changed to 2 year PFS.

72/80 patients have enrolled. We expect LCCC 1413 to complete accrual by the time the current study is opened. Three patients have had cancer recurrence (median followup 6.9 months, range 1.3 – 18.7 months): two patients had distant recurrences (lung and bone) and one had a local recurrence in the base of tongue.

Together, LCCC 1120 and 1413 (n=115 patients, total) have shown that de-intensified chemoradiotherapy is efficacious and has a favorable toxicity profile as compared to standard dose chemoradiotherapy.

1.6 Genomic characterization of HPV-associated and Tobacco-associated Oropharyngeal cancer

HPV- associated OPSCC has a different genomic profile as compared to HPV-negative, tobacco associated OPSCC[35]. Seminal work done by Dr. [redacted]s using the tumor cancer genome atlas has shown that HPV-negative tobacco associated OPSCC characteristically has p53 mutations and CCND1 amplifications. These genetic alterations are rarely (if ever) seen in HPV-associated OPSCC, especially those with minimal smoking histories. Dr [redacted] et al. have further characterized the tumor genomic profiles in patients with HPV- associated OPSCC with significant smoking histories (> 10 pack years). They observed that not all HPV-associated OPSCC are the same on the genetic level, especially in those with > 10 pack year smoking history [redacted] et al. used next-generation sequencing to analyze genetic mutations in 66 HPV-positive oropharyngeal cancer patients, who were also categorized into groups based on smoking history. They found that disease-free and overall survival were better in the less than 10-pack year group, and there were differences in mutations between the two groups based on smoking history. Mutations in genes such as TP53, CDKN2A, KRAS and NOTCH1 almost exclusively observed in the > 10 pack-year group while HLA-A mutations were almost exclusively in the lighter smoking group. These data provide a molecular criteria based

on genomic mutations to help better stratify risk/prognosis and potentially to guide treatment selection, namely whether de-intensification should be used.

The proposed study aims to build upon the work of [redacted] et al. and [redacted] et al. by evaluating whether genomic profiling can be used to better select patients for de-intensification. By better selecting patients (using genomic profiling, see below) for de-intensification, we hypothesize that we can further improve the 2 year PFS to 93% for patients with HPV- associated OPSCC regardless of smoking history.

1.7 Circulating free HPV DNA (cfHPV-DNA)

Circulating cell free plasma DNA (cfDNA) can be a sensitive biomarker of tumor burden that is accessible by simple phlebotomy. Circulating free HPV DNA is detectable (via polymerase chain reaction) in patients with HPV-associated OPSCC and a highly specific circulating biomarker for HPV positive HNSCC. Limited data on a small number of patients have shown that cfHPV-DNA levels become largely undetectable post-CRT in most patients, and that cfHPV-DNA levels increase at the time of recurrence, thus suggesting that cfHPV-DNA may be prognostic[35-37]. UNC-LCCC has the unique ability to evaluate cfHPV-DNA using digital droplet PCR (ddPCR) technology. Dr. Gaorav Gupta's lab has developed a ddPCR assay for HPV subtype 16 that is exquisitely sensitive and specific in laboratory tests, and has successfully detected cfHPV-DNA in 2 out of 2 patients that have been analyzed to date (Figure 1). In this study we will prospectively collect blood samples pre-treatment, during, and after treatment to evaluate the kinetics of cfHPV-DNA in all patients. We are particularly interested in the kinetics during treatment as it may be used to guide further studies: e.g. to help guide when to stop radiation or, if levels do not become undetectable during some point of treatment, one may change chemotherapy or continue with adjuvant chemotherapy.

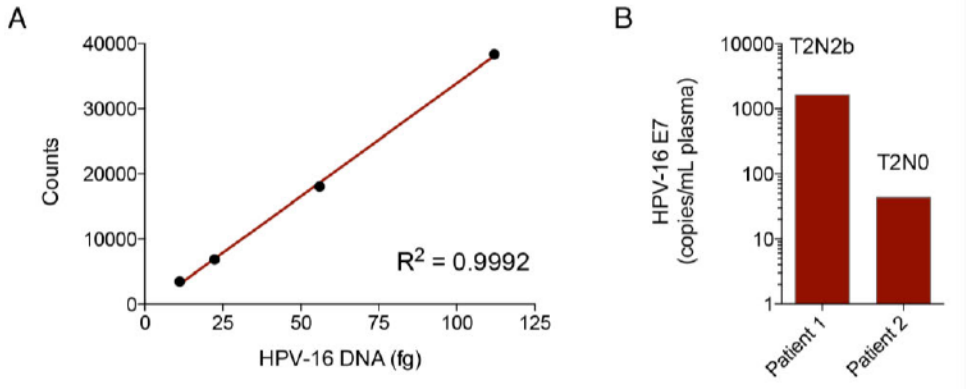

Figure 1 A) Linearity over a broad range of HPV-16 DNA using a ddPCR assay designed to the E7 gene. **B)** Baseline levels of cfHPV-DNA in 2 patients with HPV+ HNSCC, and tumor stage indicated.

1.8 Rationale

The proposed study will build upon on our seminal prior phase II and genomic characterization studies by leveraging unique clinical/translational strengths at UNC-LCCC: cancer genomics and cfHPV-DNA. Our over-arching hypothesis is that the

genomic characterization of OPSCC tumor specimens from patients with > 10 pack year smoking history can be used to help decide whether de-intensification would be efficacious.

Because of the positive results of our first two de-intensification studies (LCCC 1120, LCCC 1413) and the emerging genomic risk stratisfication data from [redacted]s et al. and [redacted] et al., we are proposing conducting a similar study with the major modification of:

1. Tumor Genetics: Tumor genetics will be assessed in all patients with > 10 pack year smoking history, and select patients with extensive smokeless tobacco history, based on the PI's assessment. The UNC Molecular Genetics Laboratory will perform a mutation panel interrogating selected regions of 26 genes (p53 mutation is the most common to this patient population) using next-generation sequencing. P53 mutational status will be used to assess eligibility of patients with >10 pack years smoking history.

2. Circulating free HPV DNA: In this study we will prospectively collect blood samples pre-treatment, during, and after treatment to evaluate the kinetics of cfHPV-DNA in all patients.

3. Primary endpoint: Our hypothesis is that by using genomic profiling in the patients with HPV-associated OPSCC and > 10 pack year smoking history that we will be better able to select those patients with HPV-associated OPSCC who would safely benefit from de-intensification. The primary endpoint is PFS, however by using genomics to select the most favorable patients with a > 10 pack year smoking history, we estimate the PFS to be 93% (for the entire population, regardless of smoking), instead of the 87% as has been reported in previous studies[10] and our prior studies.

2.0 STUDY OBJECTIVES

2.1 Primary Objective:

To evaluate whether genomic based risk-stratification can be used in deciding whether to de-intensify in patients with HPV-associated OPSCC with > 10 pack years smoking history. Hypothesis: Patients with HPV-associated OPSCC, > 10 pack years smoking history, and non-mutated p53 will have similar 2 year PFS as patients with < 10 pack years smoking history.

2.2 Secondary Objectives

To prospectively assess if the changes in plasma circulating free HPV DNA during and after treatment are associated with clinical outcomes in patients with HPV-associated OPSCC. Hypothesis: Changes in levels of plasma circulating free HPV DNA during and after treatment will correlate with cancer control rates.

To assess the 2 year clinical outcomes of local control (LC), regional control (RC), local-regional control (LRC), distant metastasis free survival (DMFS), and overall survival (OS).

3.0 ENDPOINTS

3.1 Primary Endpoint

Patients with HPV-positive and/or p16-positive OPSCC will receive de-intensified CRT. The 2 year PFS will be evaluated. Our null hypothesis (H_0) is that the PFS rate is 93% and our alternate hypothesis (H_1) is that the PFS rate is 86%.

3.2 Secondary Endpoints

Circulating free HPV-DNA: Kinetic of cfHPV-DNA copy numbers during and after treatment.

Clinical Outcomes: Kaplan Meier estimates of LC, RC, LRC, DMFS, and OS will be calculated.

4.0 PATIENT ELIGIBILITY

4.1 Inclusion Criteria

4.1.1 ≥ 18 years of age (no upper age limit)

4.1.2 T0-3, N0 to N2c, M0 squamous cell carcinoma of the oropharynx (AJCC 7th edition)

4.1.3 Biopsy proven squamous cell carcinoma that is HPV and/or p16 positive

4.1.4 Radiologic confirmation of the absence of hematogenous metastasis within 12 weeks prior to treatment; at a minimum, chest x-ray is required. CT imaging of the chest or PET/CT is acceptable.

4.1.5 ECOG Performance Status 0-1

4.1.6 CBC/differential obtained within 8 weeks prior to treatment, with adequate bone marrow function defined as follows:

4.1.6.1 Platelets ≥ 100,000 cells/mm3

4.1.6.2 Hemoglobin ≥ 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hgb ≥ 8.0 g/dl is acceptable.)

4.1.7 Adequate renal and hepatic function within 4 weeks prior to treatment, defined as follows:

4.1.7.1 Serum creatinine < 2.0 mg/dl

4.1.7.2 Total bilirubin < 2 x the institutional ULN

4.1.7.3 AST or ALT < 3 x the institutional ULN.

Note that physician attestation of patient having no known history of liver disease can take the place of bilirubin and AST/ALT labs.

4.1.8 Negative pregnancy test within 2 weeks prior to treatment for women of childbearing potential

4.1.9 Women of childbearing potential and male participants who are sexually active must practice adequate contraception during treatment and for 6 weeks following treatment.

4.1.10 Patients must be deemed able to comply with the treatment plan and follow-up schedule.

4.1.11 Patients must provide study specific informed consent prior to study entry

4.2 Exclusion Criteria

4.2.1 Prior history of radiation therapy to the head and neck

4.2.2 Prior history of head and neck cancer.

4.2.3 Unresectable disease (e.g. immobile node on physical exam, nodal disease that radiographgically involves the carotid arteries, nerves)

4.2.4 Currently taking Disease Modifying Rheumatoid Drugs (DMRDs)

4.2.5 Severe, active co-morbidity, defined as follows:

4.2.5.1 Unstable angina and/or congestive heart failure requiring hospitalization within the last 6 months

4.2.5.2 Transmural myocardial infarction within the last 6 months

4.2.5.3 Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration

4.2.5.4 Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration

4.2.5.5 Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects; Note, however, coagulation parameters are not required for entry into this protocol.

4.2.5.6 Pre-existing ≥ grade 2 neuropathy

4.2.5.7 Prior organ transplant

4.2.5.8 Systemic lupus

4.2.5.9 Psoriatic arthritis.

4.2.6 Known HIV positive.

HIV positive patients are known to have worse clinical outcomes especially for local, regional, and distant cancer control. This poorer prognosis is thought to be secondary to a compromised immune system. Thus, de-escalation of radiation and chemotherapy is not justifiable.

4.2.7 Current active smoker, defined as active cigarette smoking within the 6 months prior to enrollment.

5.0 STUDY PLAN

5.1 Schema

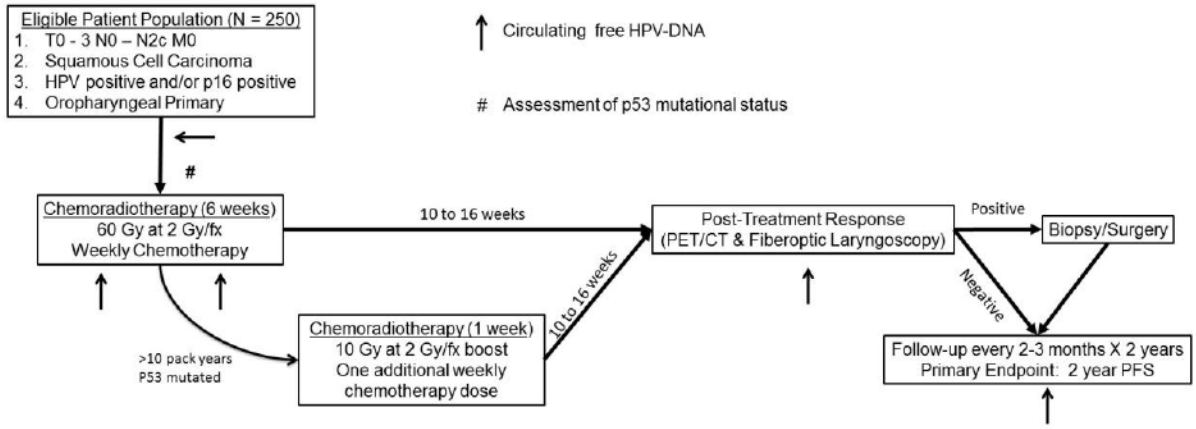

Figure 2: Schema

Figure 2: The primary objective of this study is to assess the 2 year PFS of HPV-associated OPSCC after genetic risk based de-intensified CRT. All patients with ≤ 10 pack years smoking history will receive de-intensified CRT. Patients with > 10 pack years smoking history, and select patients with extensive smokeless tobacco history (based on the PI's assessment), will have genetic assessment of their tumors (pre-treatment archival tissue or biopsies), and if p53 is mutated, they will receive an additional 10 Gy for a total radiation dose of 70 Gy (which is the historical, standard) and one additional weekly chemotherapy dose. Those with > 10 pack year smoking history and without the p53 mutation will receive the de-intensified regimen. Circulating free HPV DNA will be assessed pre-treatment, during treatment, and post-treatment at months 3, 6, 12, 18, and 24 (See Section 5.5 for details on blood collection time points).

5.2 Pretreatment Evaluations: All patients will have standard of care evaluation and staging procedures.

5.2.1 Complete history and physical exam including weight and performance status.

5.2.2 Completion of panendoscopy with directed biopsies and bilateral tonsillectomies if the primary is unknown is strongly recommended, but not required.

5.2.3 Completion of the following radiological studies:

at least contrasted neck CT and chest x-ray. Chest CT, PET/CT and/or MRI are optional studies. CT of the neck should have IV contrast unless contraindicated (allergy or adverse reaction or renal issues). PET/CT is satisfactory and can be done instead of or in addition to contrasted neck CT and chest x-ray. Pretreatment ultrasound evaluation with possible FNA is permissible to stage the neck prior to treatment.

5.2.4 Complete dental evaluation is recommended.

5.2.5 Completion of the following laboratory studies: CBC, serum chemistries, liver function tests, and pregnancy test if female.

5.3 HPV and/or p16 testing

HPV and/or p16 testing will be performed via fluorescence in-situ hybridization and immunohistochemistry, respectively. Methods and techniques for these tests have been established by the UNCH, UF, and Rex Cancer Center of Raleigh Departments of Pathology and these biomarkers are already routinely examined for all head and neck tumor specimens obtained at these institutions. This testing may be performed on FNA tissue obtained from a neck node. Results of HPV and/or P16 testing performed at institutions outside of the study site are acceptable.

5.4 Genetic Assessment

All patients with > 10 pack years smoking history, and select patients with extensive smokeless tobacco history (based on the PI's assessment), will have their tumor assessed (pre-treatment archival tissue/biopsy) for p53 mutational status. The UNC Molecular Genetics Laboratory will perform a mutation panel interrogating selected regions of 26 genes using next-generation sequencing (TruSight Tumor Panel®, Appendix). Genes assessed are listed in the appendix. The turn around time for these tests is approximately two weeks. Only p53 mutational status will be used to risk stratify. The other 25 analyzed genes (TruSight Tumor Panel®) will not be used to decide on whether a patient with > 10 pack years will receive de-intensified CRT. We will archive that data and analyze it retrospectively. Other participating centers will overnight ship tissue samples to UNC for genetic assessment as follows: Ten (10) unstained sections from formalin-fixed, paraffin-embedded tissue on plain, uncoated glass slides (4 microns thick); an H&E stained slide to allow pathologist documentation that sufficient tumor is present (if tissue exhibits less than 20% tumor, specimens are enriched for tumor by manual macro-dissection to allow better assay sensitivity); and a copy of the surgical pathology report to assist in block selection, to document anatomic site, and to confirm histopathologic diagnosis of carcinoma. Alternatively, sites may send archival tissue in a formalin fixed paraffin embedded block (FFPE) in place of the slides.

We will be assessing a narrower number of genetic alterations in this proposal, as compared to the published/presented works of Dr. [redacted]. This decision was made out of necessity to ensure the feasibility (i.e., two week turn around time) of incorporating genomic information to prospectively guide treatment. Thus we are limited to evaluating a select number of genetic alterations, and are focusing the design of our study on p53 mutational status. Other mutational events may be useful in risk stratification, however we hypothesize that the central role of p53 in regulating the cell cycle and mitigating radiation and chemotherapy sensitivity make it the ideal biomarker to use in our proposal.

5.5 Circulating free HPV-DNA (cfHPV-DNA)

Previous versions of this protocol have detailed various requirements regarding blood specimen collection time points as the trial has evolved. These have included blood collection at pretreatment, weekly during treatment, and up to 5 post-treatment time points. As of May 8, 2020, blood specimens will only be collected at pre-treatment and Week 4 of treatment. One tube will be drawn at each time point. Circulating plasma DNA will be extracted and cfHPV-DNA will be quantified using our ddPCR assay using the Biorad QX-200 ([redacted] Gupta's lab). Additional exploratory analyses on plasma cfDNA samples may be performed in study co-investigator laboratories. Additional samples may be obtained at the PI's discretion. If necessary, each additional draw will be one Streck tube. UNC will provide Streck BCT tubes to other participating centers for blood collection. After sample is drawn, tube should be inverted 10 times to mix the preservative. Sites will overnight ship blood samples within one business day to UNC in FedEx boxes without ice for assessment (for samples collected on Friday, shipping on Monday is fine). If necessary for batch shipment, sites may wait to ship blood samples, but must ship within one week (5 business days) of the date the earliest sample was drawn.

Specimens will be retained indefinitely unless a specific participant chooses to withdraw from the study. Should a patient withdraw from this study, any specimens that have been collected, and that contain information that can be used to identify the participant will be destroyed. Any specimens that have been stripped of identifying information cannot be linked back to the participant, and therefore will not be destroyed.

Tissue and blood samples should be sent overnight (or by UNC courier) to [redacted] at the following address: Department of Radiation Oncology; UNC Cancer Hospital; 101 Manning Drive, CB# 7512; Chapel Hill, NC 27599-7512. Please notify [redacted]n by phone [redacted] or email ([redacted] when samples are shipped.

5.6 Radiation Therapy

All patients will receive Intensity Modulated Radiotherapy Treatments (IMRT) or proton radiotherapy. All patients will be initially planned to 60 Gy at 2 Gy/fx, 30 fx, once a day. The turn around time for genetic testing of pre-treatment tissue (archival or biopsy) is approximately two weeks. It is expected that during the first couple of weeks fo the initial 60 Gy plan that the genetic analysis will be completed for the patients with > 10 pack years. Should one of these patients have a p53 mutation an additional 2nd radiation plan ("boost plan") of 10 Gy at 2 Gy/fx will be done. Thus the total dose for patients with HPV-associated OPSCC, > 10 pack years, and p53 mutated will be 70 Gy, the standard dose (two radiaton plans, sequential boost).

5.6.1 CT simulation

CT simulation will be obtained with IV contrast for treatment planning purposes for all patients. For patients receiving proton therapy, a non-contrast CT simulation will also be performed. The head and neck area will be immobilized with an aquaplast mask. Patients will be positioned in the neck extended position.

5.6.2 Target and Organ at Risk Volumes:

5.6.2.1 Gross Tumor Volume (GTV): is defined as all known gross disease determined on the CT simulation scan.

5.6.2.2 High Risk Clinical Target Volumes (CTV-HR): is defined as the GTV plus a non-uniformly expanded 5 to 10 mm to account for high risk areas of microscopic spread. For situations where the primary tumor was removed with the biopsy (e.g. tonsillectomy) and the primary tumor cannot be seen on radiographic imaging the biopsy site will be included in the CTV-HR. For patients with an unknown primary (T0) the ipsilateral oropharynx (base of tongue, tonsil, soft palate) will be included in the CTV-HR volume.

5.6.2.3 Standard Risk Clinical Target Volume (CTV-SR): is defined as the elective nodal regions. The consensus guidelines for the node negative and node positive necks published by Gregoire et al. will be used as a guide to define the CTV-SR[36,37]. For the situation of the unknown primary (i.e. T0), the nasopharynx will be included in the CTV-SR. The following guidelines will be used in delineating the CTV-SR.

5.6.2.3.1 *Node positive hemi-neck (ipsilateral or contralateral to the primary site)[36]:* The following elective nodal regions should be included in the CTV-SR: Levels Ib-V and retropharyngeal. The cranial extent to the base of skull of Level II and retropharyngeal nodes should be electively irradiated.

5.6.2.3.2 *Ipsilateral node negative hemi-neck[37]:* The following elective nodal regions should be included in the CTV-SR: Levels II –IV and retropharyngeal area. The cranial extent to the base of skull of Level II and retropharyngeal nodes should be electively irradiated.

5.6.2.3.3 *Contralateral node negative hemi-neck[37]:* The contralateral parotid may be spared by omitting irradiation of the cranial portion of Level II and retropharyngeal region, defined as the Level II, retropharyngeal region above the transverse process of the C1 vertebrae and/or where the posterior belly of the digastric muscle crosses over the jugular vein. Contralateral neck irradiation may be completely omitted for well lateralized tonsil cancers, defined as having no invasion of the base of tongue, and minimal invasion of the soft palate (i.e. > 1 cm from the uvula)[38].

5.6.2.3.4 *Unknown Primary:* The above elective nodal irradiation guidelines will be used. Furthermore the nasopharynx will be included in the CTV-SR.

5.6.2.4 Planning Target Volumes (PTV): To account for daily setup errors, the CTV-HR will be expanded uniformly by 3 mm to create a High Risk Planning Target Volume (PTV-HR). The CTV-SR will be expanded uniformly by 3 mm to create a Standard Risk Planning Target Volume (PTV-SR).

5.6.2.5 Organs at Risk (OAR): The following normal tissues will be segmented on CT simulation scan: spinal cord, brainstem, parotids, cochleae, and larynx.

5.6.2.6 Planning Risk Volumes (PRV): OAR(s) will be uniformly expanded 3mm to create individual Planning Risk Volumes (PRV).

Dose Specification: Dose painting IMRT, passive-scattered proton therapy, or intensity modulated proton radiotherapy (IMPT) will be used and all doses will be specified to the PTV. The PTV-HR and PTV-SR will be treated to the following respective total doses: 60 Gy and 50 Gy. The dose per fraction to the PTV-HR and PTV-SR will be 2 Gy per day and 1.67 Gy per day respectively for IMRT and IMPT. Because the passive scattered proton technique is not conducive to a dose-painting strategy, both the PTV-HR and PTV-SR will receive 2 Gy per day using a sequential boost. The total number of fractions will be 30. All fields will be treated once a day Monday through Friday. All patients will be initially planned to 60 Gy at 2 Gy/fx, 30 fx, once a day. The turn around time for genetic testing of pre-treatment tissue (archival or biopsy) is approximately two weeks. It is expected that during the first couple of weeks of the initial 60 Gy plan that the genetic analysis will be completed for the patients with > 10 pack years. Should one of these patients have a p53 mutation an additional 2nd radiation plan ("boost plan") of 10 Gy at 2 Gy/fx will be made for the PTV-HR. Thus the total dose for patients with HPV-associated OPSCC, > 10 pack years, and p53 mutation will be 70 Gy (two radiaton plans, sequential boost).

5.6.3 IMRT/Proton Treatment Planning

PTV's and PRV will be included in the radiotherapy optimization. Dose objectives will be chosen for the radiotherapy optimization based on previous institutional experience. Dose painting will be used to create one radiotherapy plan for IMRT and IMPT. The PTV-SR contours will encompass the PTV-HR contours. The dose to the PTV-SR plan will be 50 Gy at 1.67 Gy per daily fraction in 30 fractions. The PTV-HR will be treated to 60 Gy at 2 Gy per daily fraction in 30 fractions. For passive-scattered proton plans, a sequential boost technique will be allowed, and the dose to the PTV-SR will be 50 Gy st 2 Gy per daily fraction, and the PTV-HR will receive an additional 10 Gy (cumulative dose: 60 Gy) at 2 Gy per daily fraction. For those patients requiring a boost plan, the PTV-HR will receive an additional 10 Gy at 2 Gy per daily fraction in 10 fractions. IMRT/Proton radiotherapy to treat the entire neck is preferred, however a matched low anterior neck field photon technique may be used only if it does not result in significant dose heterogeneity for the PTV-HR. Proton therapy will be specified in Gy(RBE) (photon equivalents) to account for the inherent radiobiological differences between proton and photon therapies; thus, the treatments should be considered biologically equivalent.

5.6.4 Dose Constraints:

These apply to both patients who receive a total dose of 60 Gy or 70 Gy.

- PTV-HR and PTV-SR
  - 100% of the prescription should cover 95% of the PTV
  - No more than 1% of the PTV should receive ≥ 110% of the prescribed dose

- No more than 1% of the PTV should receive ≤ 93% of the prescribed dose

- Non-target Tissue
  - No more than 1% of the tissue outside the PTV should receive ≥ 110% of the prescribed dose
- PRV
  - Spinal Cord: 0.1cc ≤ 50 Gy
  - Brainstem: 0.1cc ≤ 54 Gy
  - Parotid: Mean dose < 26 Gy and/or 50% < 30 Gy
  - Cochlea: Mean dose < 45 Gy
  - Larynx: Mean dose < 41 Gy and/or 60 Gy to < 20%

PTV coverage should not be compromised to meet the dose constraints of the parotid, cochlea, or larynx. Sparing of these structures is left at the discretion of the treating radiation oncologists. The dose constraints for the spinal cord and brainstem must be satisfied. This may be done at the cost of altering the PTV.

5.6.5 Treatment Verification

Weekly orthogonal films or cone beam CT's should be performed to verify patient setup (at least).

5.6.6 Radiation Treatment Breaks

Ideally, treatment breaks, if necessary, should not exceed 5 treatment days at a time and 10 treatment days total. Treatment breaks should be allowed only for resolution of severe acute toxicity and/or for intercurrent illness and not for social or logistical reasons.

5.7 Chemotherapy

In our initial study, LCCC 1120, all patients were started on a regimen of cisplatin 30mg/m^2 given intravenously over 60 minutes weekly during IMRT; 6 total doses for a total of 180 mg/m^2. However, if cisplatin was not tolerated, it was permissible to switch to alternative, acceptable weekly chemotherapy regimens such as cetuximab, carboplatin, or carboplatin/taxol. For the proposed study, cisplatin is the preferred mandated first choice chemotherapy, however alternative weekly regimens are permissible. Typical reasons for a patient not being able to receive cisplatin include renal insufficiency and history of hearing loss. Justification for not using cisplatin must be documented. Analysis of a prospective national registry, Longitudinal Oncology Registry of Head and Neck Carcinoma (LORHAN), showed that the three most commonly prescribed concurrent (with radiation) regimens (in order) are single agent cisplatin (51%), single-agent cetuximab (21%), and carboplatin plus paclitaxel (7%). Single agent carboplatin was infrequently used (3%) [redacted] et al. Cancer 2011;117:1679–86.). The acceptable weekly chemotherapy regimens that may be used on this study are the following:

- Cisplatin 30 to 40 mg/m^2 (preferred, first choice)
- Cetuximab 250mg/m^2 (preferred, second choice)
- Carboplatin AUC 1.5 and paclitaxel 45 mg/m^2 (preferred, third choice)
- Carboplatin AUC 3 (preferred, fourth choice)

Chemotherapy will be given intravenously weekly during radiotherapy. 6 total doses will be given. It is preferred that the doses be administered on days 1, 8, 15, 22, 29, and 36;

however, this is not mandatory. Patients with the p53 mutation will receive one additional weekly chemotherapy dose. ***Chemotherapy will not be given to patients with T0-2 N0-1 disease, ≤ 10 pack years smoking history . For patients with T0-2 N0-1 disease, >10 pack years smoking history, whether or not the patient receives chemotherapy will be at the discretion of the treating physician.***

5.7.1 Dose Modifications for chemotherapy

Because of the low weekly dosage of chemotherapy, we anticipate few dose modifications secondary to acute toxicities. Dose modifications are allowed and will be directed by the treating medical oncologist according to his/her discretion on an individual patient basis.

5.7.2 Changing Chemotherapy Regimens

In the event that cisplatin is held for 1 week and the patient is still deemed unable to receive further weekly cisplatin (because of cisplatin-related toxicity), patient will be switched to another protocol-acceptable weekly chemotherapy regimen (cetuximab, carboplatin/taxol, or carboplatin).

Other changes may be done on a case-by-case basis depending on the standard of practice of the treating medical oncologist.

5.8 Post-Chemoradiotherapy Assessment of Clinical Response

PET/CT will be performed 10 to 16 weeks (optimally at week 12) after CRT to assess response. All patients will be evaluated via clinical exam and fiberoptic laryngoscopy by the radiation oncologist and head and neck surgeon (optional) around the same time as the PET/CT scan. Note that the fiberoptic laryngoscopy need only be done once, at either 6 or 12 weeks post-CRT, with additional laryngoscopy procedures performed at the discretion of the physician. Decisions for surgical evaluation will be based on the results of the PET/CT and clinical exam at that time. Other optional imaging studies may be performed (e.g. CT scan 4 to 6 weeks after completion of CRT).

5.9 Surgery after CRT

Patients with a positive PET/CT scan will undergo surgical evaluation at the discretion of the surgeon. This may include biopsies and/or oncological resections of the primary tumor and lymph node metastases. The type of surgical procedure will be left to the discretion of the surgeon however the goal will be to remove any suspected residual tumor with a negative resection margin while maintaining organ preservation. Patients with a negative PET/CT scan will be observed.

5.10 Other Therapy

5.10.1 Permitted Supportive Therapy

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol

5.10.1.1 Antiemetics

Prophylactic antiemetics and supportive therapy for nausea and vomiting are permitted and highly recommended in patients participating in this study. These interventions should be made according to institutional guidelines.

5.10.1.2 Nutritional Supplementation

Close monitoring of patients' volume status and body weight is strongly recommended. Nutritional supplementation through a nasogastric or gastrostomy feeding tube should be considered in patients who are unable to maintain hydration or experience more than 10% loss of body weight due to mucositis.

5.10.2 Non-permitted Supportive Therapy

5.10.2.1 Hematopoietic Growth Factors

Hematopoietic growth factors are not permitted during radiation therapy. Growth factors are only permitted if administered after radiation therapy has been completed. Erythropoiesis stimulating agents are not permitted.

5.10.3 Other Supportive Care Clinical Studies

Patients will be allowed to participate in other supportive care clinical studies that do not interfere with the treatment plan of the current study.

5.11 Duration of Study

The primary endpoint of this study is to evaluate the 2 year PFS rate after de-intensified CRT in HPV-positive and/or p16 positive OPSCC. Patient participation concludes after 2 years of follow-up after completion of CRT. However, patients will continue long-term routine follow-up as is our institutional policy.

5.12 Duration of Follow Up

After completion of treatment patients will be followed according to our institutional standard practice: clinical evaluations every 2 to 3 months for 2 years, every 6 months for 3 additional years, then yearly thereafter. As part of routine surveillance patients will receive chest imaging every 6 months for 2 years then yearly thereafter and thyroid function studies will be checked every 6 months for 2 years then yearly thereafter.

Our routine standard practice for all of our head and neck cancer patients is to encourage post-treatment dental follow-up and care. This study does not increase the need for post-treatment dental care or increase the risk of dental complication. In fact it may decrease the risk/incidence of post-treatment dental complications because the radiation dose is being reduced to 60 Gy. Thus we will continue to practice our routine post-treatment dental care recommendations.

See the Time and Events Table in the Appendix for a summary of the patient assessments/procedures and time periods.

6.0 ADVERSE EVENTS

6.1 Definition

An adverse experience is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse experience or event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

In this study, toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.2 Reporting

Patients will be receiving radiation, chemotherapy, and surgical treatments within the accepted standard of care. We expect patients to experience the known toxicities that are associated with these standard treatments, but only at a lower rate and severity than patients treated with accepted radiation and chemotherapy dosing. Since treatments modalities are the same (radiation and chemotherapy), but doses of radiation and chemotherapy are reduced through this deintensification protocol, no adverse events will be attributable to the trial treatment. In the RTOG 0129 study, based on the same deintensification treatment, the overall rate of grade 3 or 4 acute toxic events was approximately 80% and the late grade 3 or 4 toxic events was approximately 25% of those seen in control patients treated with accepted radiation and chemotherapy doses. Because patients on this trial receive de-intensified chemotherapy and radiation by reducing the total dose of radiation to 60 Gy and administering no chemotherapy or Cisplatin 30mg/m2 weekly the acute and late toxicities can only be decreased in trial patients compared to historical norms.

This de-escalation trial decreases radiation and chemotherapy doses compared to accepted dosing for head and neck cancer; therefore, adverse events will not be reported since there is no chance that adverse events are trial related.

6.3 Treatment of Adverse Event

Patients who develop any adverse event while on study will receive standard of care treatment. Radiation, cisplatin, and surgery are already standard of care treatments for these patients. Standard of care treatment for known potential adverse events from these treatments are established.

7.0 STATISTICAL CONSIDERATIONS

7.1 Study Design/Study Endpoints

The main objective of this Phase-II trial is to estimate the two-year progression-free survival probability (PFSP) in this group of patients treated with de-escalated radiotherapy. The planned total sample size is 80 patients. (This number was increased to 120 as of July 7, 2017, 140 as of February 7, 2019, 160 as of May 29, 2019, 180 as of October 9, 2019, 200 as of January 7, 2020, and 250 as of June 17, 2020. Please see details below.) The recruitment phase is expected to last 48 monthsDrop out is expected to be about 10%, based on a previous trial (LCCC 1120), hence we expect a total of 225 patients to complete the study.

The statistical aim is phrased as a hypothesis test with the null hypothesis being that the two-year PFSP is 0.93 and the alternative hypothesis being that the two-year PFSP is 0.86 (or less). A one sample binomial test will be used to assess this hypothesis. If the null hypothesis is rejected the conclusion will be that the de-escalated treatment is inferior to the standard treatment. If the null hypothesis is not rejected the conclusion will be that the de-escalated treatment is at least as good as the standard RT.

We give power calculations for two sample sizes. For a sample size of n=73 (patients who complete the study), the null hypothesis will be rejected if 11 or more patients fail. Failure is defined as not reaching the two-year time point progression-free. Defining P(Reject H0) to be the probability of rejecting the null hypothesis, the performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.03
0.86	0.58
0.80	0.94
0.75	0.99

For a sample size of n=80 patients, the null hypothesis will be rejected if 12 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.02
0.86	0.57
0.80	0.94
0.75	0.99

For n=73 to 78, the critical point is 11 failures. For n=79 and 80, the critical point is 12 failures. With these critical points, type-I error ranges from 0.023 to 0.045 depending on sample size.

Sample size and power re-calculation: On July 7, 2017, the PI, noticing the higher than expected patient accrual rate, decided to increase sample size to 120. This should result in

about 108 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=108 patients, the null hypothesis will be rejected if 13 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.04
0.86	0.76
0.80	0.99
0.75	1.00

For n=100 to 101, the critical point is 12 failures. For n=102 to 108, the critical point is 13 failures. With these critical points, type-I error ranges from 0.026 to 0.050 depending on sample size.

Sample size and power re-calculation: On February 7, 2019, the PI, noticing the higher than expected patient accrual rate, decided to increase sample size to 140. This should result in about 126 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=126 patients, the null hypothesis will be rejected if 13 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.03
0.86	0.79
0.80	0.99
0.75	1.00

For n=120 to 123, the critical point is 14 failures. For n=124 to 128, the critical point is 15 failures. With these critical points, type-I error ranges from 0.030 to 0.050 depending on sample size.

Sample size and power re-calculation: On May 29, 2019, the PI, waiting for the opening of LCCC 1912 and noticing the completion of accruing planned 140 patients, decided to increase sample size to 160. This should result in about 144 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=144 patients, the null hypothesis will be rejected if 16 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.045
0.86	0.870
0.80	0.99

0.75	1.00

For n=140 to 143, the critical point is also 16 failures. With these critical points, type-I error ranges from 0.036 to 0.043 depending on sample size.

Sample size and power re-calculation: On October 9, 2019, the PI, waiting for the opening of LCCC 1912 and noticing the completion of accruing planned 160 patients, decided to increase sample size to 180. This should result in about 162 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=162 patients, the null hypothesis will be rejected if 18 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.003
0.86	0.45
0.80	0.96
0.75	1.00

For n=159 to 161, the critical point is also 18 failures. With these critical points, type-I error ranges from 0.030 to 0.035 depending on sample size.

Sample size and power re-calculation: On January 7, 2020, the PI, waiting for the opening of LCCC 1912 and noticing the completion of accruing planned 180 patients, decided to increase sample size to 200. This should result in about 180 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=180 patients, the null hypothesis will be rejected if 19 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.048
0.86	0.93
0.80	0.99
0.75	1.00

For n=177 to 179, the critical point is also 19 failures. With these critical points, type-I error ranges from 0.042 to 0.048 depending on sample size.

Sample size and power re-calculation: On June 17, 2020, the PI, waiting for the opening of LCCC 1912 and noticing the completion of accruing planned 200 patients, decided to increase sample size to 250. This should result in about 225 patients to complete the study, after accounting for drop-out with an expected drop-out rate of 10%.

For a sample size of n=225 patients, the null hypothesis will be rejected if 23 or more patients fail. The performance of the test is summarized in the following table.

True 2-year PFSP	P(Reject H0)
0.93	0.045
0.86	0.96
0.80	0.99
0.75	1.00

For n=220 to 224, the critical point is also 23 failures. With these critical points, type-I error ranges from 0.036 to 0.045 depending on sample size.

7.2 Data Analysis Plan

The main hypothesis test will be performed as described above. Subgroup analyses to compare PFS between patients with ≤ 10 pack years vs patients with > 10 pack years (but with no p53 mutation) smoking history will be conducted using log-rank test. Additionally, a Kaplan-Meier curve will be estimated for PFS, local control, regional control, local-regional control, distant metastasis free survival and overall survival. Circulating free HPV DNA before, during, and after CRT will be compared, using the paired t-test or McNemar's test as appropriate. As an exploratory analysis, joint modeling of longitudinal cfHPV-DNA and time-to-progression will be conducted to examine the association between time-varying cfHPV-DNA and disease progression. We will also perform exploratory analyses of the other genetic alterations that are assessed by the TruSight Tumor Panel® and florescent in-situ hybridization studies.

In addition to the above, other exploratory analyses utilizing study-related data may be performed. These analyses will be consistent with the overarching aims of the study.

7.3 Safety Meetings

The principal investigator will provide continuous monitoring of patient safety in this study with periodic reporting to the Data Safety Monitoring Committee (DSMC).

The principal investigator will submit summaries, together with formatted reports, to the DSMC for review. The reports will be reviewed at the time of the appointed meeting schedule established by this committee. Following review, the DSMC will report its recommendations, together with the principal investigator report, to the Oncology PRC. These reports will be reviewed by the PRC at the time of the study's annual IRB renewal. When warranted, the PRC and/or DSMC will have the prerogative to request additional information.

8.0 STUDY MANAGEMENT

8.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment into this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

8.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- A copy of the IRB-approved consent form
- Executed clinical research contract

8.3 Registration Procedures

All patients must be registered with the Study Coordinator at the Department of Radiation Oncology at the University of North Carolina before enrollment in the study. Signed consent forms should be sent to the UNC Coordinator for this purpose. Additional patient information may be requested from the UNC Coordinator to complete registration. Eligibility criteria must be confirmed with the UNC Study Coordinator before the start of treatment.

8.4 Data Management and Monitoring/Auditing

The University of North Carolina will serve as the coordinating center for this trial. All data will be collected, entered, and maintained in secured servers in the Departments of Radiation Oncology at the University of North Carolina, the University of Florida, the University of Florida Proton Therapy Institute, and Rex Cancer Center of Raleigh by the Study Coordinators. De-identified, password protected data from patients enrolled at the University of Florida, the University of Florida Proton Therapy Institute, and Rex Cancer Center of Raleigh will be electronically submitted to the Department of Radiation Oncology at the University of North Carolina via a secured computer server. The data will be pooled at UNC where personnel there will coordinate and manage data for quality

control assurance and integrity. Data analysis will take place at UNC as well. As an investigator initiated study, this trial will be audited by UNC's Office of Clinical Trials (OCT) Clnical Trials Quality Assurance (CTQA) Program annually.

8.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

8.5.1 Emergency Modifications

UNC and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC or their respective institution's IRB/IEC approval/favorable opinion.

For Institutions Relying on UNC's IRB:
For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

For Institutions Relying on Their Own IRB:
For Affiliate investigators relying on their own institution's IRB, as soon as possible after the modification has been made, the implemented deviation or change and the reasons for it should be submitted to:

- To UNC Principal Investigator for agreement
- The Affiliate institution's IRB for review and approval. (Once IRB's response is received, this should be forwarded to the UNC Study Coordinator).

8.5.2 Single Patient/Subject Exceptions

For Institutions Relying on UNC's IRB:
Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the UNC IRB.

For Institutions Relying on Their Own IRB:
Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the participating institution's IRB, per its policy. Please forward the IRB response to the UNC Study Coordinator by facsimile or via email within 10 business days after the original submission.

8.5.3 Other Protocol Deviations/Violations

All other planned deviations from the protocol must have prior approval by the Principal Investigator and the UNC IRB. According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs without prior approval from the Principal Investigator, please follow the guidelines below:

For Institutions Relying on UNC's IRB:
Protocol Deviations: UNC personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

For Institutions Relying on Their Own IRB:
In addition to adhering to the policies regarding protocol compliance set forth by your institution's IRB, the following is also required:

Protocol Deviations: Affiliate personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Any protocol violation that occurs must be reported to your IRB per institutional policies and reported to the UNC Study Coordinator within 5 days. UNC-CH will determine if the violation affects the safety of the patient and integrity of the data. Once your institution's IRB response is received, please forward to the UNC Study Coordinator.

8.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

For Institutions Relying on UNC's IRB:
The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

For Institutions Relying on Their Own IRB:
Investigators must submit the UNC IRB approved amendment to their institution's IRB for approval. For multi-center studies, any affiliate site must submit their informed consent revisions to the UNC Study Coordinator prior to submission to their IRB.

8.7 Record Retention

Study documentation includes all databases, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

8.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and submitted as requested by UNC. Original

records will be made available to the Principal Investigator upon request to permit verification of proper entry of data.

9.0 REFERENCES

1. Canto M, Devesa S. Oral cavity and pharynx cancer incidence rates in the United States, 1975-1998. *Oral Oncol.* 2002;38:610-617.

2. Chaturvedi A, Engels E, Anderson W, al. e. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol.* 2008;26:612-619.

3. D'Souza G, kreimer A, Viscidi R, al. e. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med.* 2007;356:1944-1956.

4. Frisch M, Hjalgrim H, Jaeger A, al. e. Changing patterns of tonsillar squamous cell carcinoma in the United States. *Cancer Causes Control.* 2000;11:489-495.

5. Gillison M, Koch W, Capone R, al. e. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst.* 2000;92:709-720.

6. Schantz S, Yu G. Head and neck cancer incidence trends in young Americans, 1973-1997, with a special analysis for tongue cancer. *Arch Otolaryngol Head Neck Surg.* 2002;128:268-274.

7. Shiboski C, Schmidt B, Jordan R. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. *Cancer.* 2005;103:1843-1849.

8. Shiboski C, Shiboski S, S Silverman J. Trends in oral cancer rates in the United States, 1973-1996. *Community Dent oral Epidemiol.* 2000;28(249-56).

9. Albers A, Visus C, Tsukishiro T, al. e. Alterations in the T-cell receptor variable beta gene-restricted profile of CD8+ T lymphocytes in the peripheral circulation of patients with squamous cell carcinoma of the head and neck. *Clin Cancer Res.* 2006;12:2394-2403.

10. Ang K, Harris J, Wheeler R, al. e. Human paillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* 2010;363:24-35.

11. Braakhuis B, Snijders P, Keune W, al. e. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst.* 2004;96:998-1006.

12. DeWeese T, Walsh J, Dillehay L, al. e. Human papillomavirus E7 and E7 oncoproteins alter cell cycle progression but not radiosensitivity of carcinoma cells treated with low-dose-rate radition. *Int J Radiat Oncol Biol Phys.* 1997;37:145-154.

13. Fakhry C, Westra W, Li S, al. e. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst.* 2008;100:261-269.

14. Gupta A, Lee J, Wilke W, al. e. Radiation response in two HPV-infected head-and-neck cancer cell lines in comparison to a non-HPV-infected cell line and relationship to signaling through AKT. *Int J Radiat Oncol Biol Phys.* 2009;74:928-933.

15. Hoffmann T, Sonkoly E, Hauser U, al. e. Alterations in the p53 pathway and their association with radio- and chemosensitivity in head and neck squamous cell carcinoma. *Oral Oncol.* 2008;44:1100-1109.

16. Klussmann J, Mooren J, Lehnen M, al. e. Genetic signatures of HPV-related and unrelated oropharyngeal carcinoma and their prognostic implications. *Clin Cancer Res.* 2009;15:1779-1786.

17. Kumar B, Cordell K, Lee J, al. e. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol.* 2008;26:3128-3137.

18. Lassen P, Eriksen J, Hamilton-Dutoit S, al. e. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. *J clin Oncol.* 2009;27:1992-1998.

19. Licitra L, Perrone F, Bossi P, al. e. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. *J Clin Oncol.* 2006;24(5630-6).

20. Lohavanichbutr P, Houck J, Fan W, al. e. Genomewide gene expression profiles of HPV-positive and HPV-negative oropharyngeal cancer: potential implications for treatment choices. *Arch Otolaryngol Head Neck Surg.* 2009;135:180-188.

21. Martinez I, Wang J, Hobson K, al. e. Identification of differentially expressed genes in HPV-positive and HPV-negative oropharyngeal squamous cell carcinomas. *Eur J Cancer.* 2007;43:415-432.

22. Mork J, Lie A, Glattre E, al. e. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2001;344:1125-1131.

23. Padilla L, Leung B, Carson L. Evidence of an association between human papillomavirus and impaired chemotherapy-induced apoptosis in cervical cancer cells. *Gynecol Oncol.* 2002;85:59-66.

24. Saxena A, Yashar C, Taylor D, al. e. Cellular response to chemotherapy and radiation in cervical cancer. *Am J Obstet Gynecol.* 2005;192:1399-1403.

25. Schwartz S, Yueh B, McDougall J, al. e. Human paillomavirus infection and survival in oral squamous cell cancer: a population-based study. *Otolaryngol Head Neck Surg.* 2001;125:1-9.

26. Smeets S, Braakhuis B, Abbas S, al. e. Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. *Oncogene.* 2006;25:2558-2564.

27. Spanos W, Nowicki P, Lee D, al. e. Immune response during therapy with cisplatin or radiation for human papillomavirus-related head and neck cancer. *Arch Otolaryngol Head Neck Surg.* 2009;135(1137-46).

28. Tseng C, Trimble C, Zeng Q, al. e. Low-dose radiation enhances therapeutic HPV DNA vaccination in tumor-bearing hosts. *Cancer Immunol Immunother.* 2009;58:737-748.

29. Weinberger P, Yu Z, Haffty B, al. e. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol.* 2006;24:736-747.

30. Westra W, Taube J, Poeta M, al. e. Inverse relationship between human papillomavirus-16 infection and disruptive p53 gene mutations in squamous cell carcinoma of the head and neck. *Clin Cancer Res.* 2008;14:366-369.

31. Network NCC. *NCCN Clinical Practice Guidelines in Oncology.* 2011.

32. O'Sullivan B, Huang SH, Siu LL, et al. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* Feb 10 2013;31(5):543-550.

33. Chera BS, Amdur RJ, Tepper J, et al. Phase 2 Trial of De-intensified Chemoradiation Therapy for Favorable-Risk Human Papillomavirus-Associated Oropharyngeal

Squamous Cell Carcinoma. *International journal of radiation oncology, biology, physics.* Dec 1 2015;93(5):976-985.

34. Garden AS, Asper JA, Morrison WH, et al. Is concurrent chemoradiation the treatment of choice for all patients with Stage III or IV head and neck carcinoma? *Cancer.* Mar 15 2004;100(6):1171-1178.

35. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature.* Jan 29 2015;517(7536):576-582.

36. Gregoire V, Eisbruch A, Hamoir M, Levendag P. Proposal for the delineation of the nodal CTV in the node-positive and the post-operative neck. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology.* Apr 2006;79(1):15-20.

37. Gregoire V, Levendag P, Ang KK, et al. CT-based delineation of lymph node levels and related CTVs in the node-negative neck: DAHANCA, EORTC, GORTEC, NCIC,RTOG consensus guidelines. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology.* Dec 2003;69(3):227-236.

38. O'Sullivan B, Warde P, Grice B, et al. The benefits and pitfalls of ipsilateral radiotherapy in carcinoma of the tonsillar region. *International journal of radiation oncology, biology, physics.* Oct 1 2001;51(2):332-343.

39. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *Journal of the National Cancer Institute.* Mar 3 1993;85(5):365-376.

40. Bjordal K, Hammerlid E, Ahlner-Elmqvist M, et al. Quality of life in head and neck cancer patients: validation of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-H&N35. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* Mar 1999;17(3):1008-1019.

41. Rosenbek JC, Robbins JA, Roecker EB, Coyle JL, Wood JL. A penetration-aspiration scale. *Dysphagia.* Spring 1996;11(2):93-98.

42. Belafsky PC, Mouadeb DA, Rees CJ, et al. Validity and reliability of the Eating Assessment Tool (EAT-10). *The Annals of otology, rhinology, and laryngology.* Dec 2008;117(12):919-924.

43. Chera BS, Vargas C, Morris CG, et al. Dosimetric study of pelvic proton radiotherapy for high-risk prostate cancer. *International journal of radiation oncology, biology, physics.* Nov 15 2009;75(4):994-1002.

44. Pignon J, Maitre Al, Maillard E, al. e. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. *Radiother Oncol.* 2009;92(4-14).

10.0 APPENDIX

10.1 Eligibility Checklist

LCCC 1612 - P53 MUTATIONAL STATUS AND CIRCULATING FREE HPV DNA FOR THE MANAGEMENT OF HPV-ASSOCIATED OROPHARYNGEAL SQUAMOUS CELL CANCERS

STUDY ELIGIBILITY CHECKLIST

Subject: _____
MRN: _____
Study ID #: _____

Inclusion Criteria:

- ☐ (Y) ≥ 18 years of age
- ☐ (Y) T 0-3, N0 to N2c, M0 squamous cell carcinoma of the oropharynx
- ☐ (Y) Biopsy proven squamous cell carcinoma that is HPV and/or p16 positive
- ☐ (Y) Radiologic confirmation of the absence of hematogenous metastasis within 12 weeks prior to treatment; at a minimum, chest x-ray is required. CT imaging of the chest or PET/CT is acceptable.
- ☐ (Y) ECOG Performance Status 0-1
- ☐ (Y) CBC/differential obtained within 8 weeks prior to treatment, with adequate bone marrow function defined as follows:
  Platelets ≥ 100,000 cells/mm3 **Value:** _____
  Hemoglobin ≥ 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hgb ≥ 8.0 g/dl is acceptable.) **Value:** _____
- ☐ (Y) Adequate renal and hepatic function within 4 weeks prior to treatment, defined as follows:
  Serum creatinine < 2.0 mg/dl **Value:** _____
  Total bilirubin < 2 x the institutional ULN **Value:** _____
  AST or ALT < 3 x the institutional ULN **Value:** _____
  Note that physician attestation of patient having no known history of liver disease can take the place of bilirubin and AST/ALT labs. Please note that here.
- ☐ (Y) Negative pregnancy test within 2 weeks prior to treatment for women of childbearing potential
- ☐ (Y) Women of childbearing potential and male participants who are sexually active must practice adequate contraception during treatment and for 6 weeks following treatment.
- ☐ (Y) Able to comply with the treatment plan and follow-up schedule
- ☐ (Y) Study-specific informed consent provided prior to study entry

Exclusion Criteria:

- ☐ (N) Prior history of radiation therapy to the head and neck
- ☐ (N) Prior history of head and neck cancer
- ☐ (N) Unresectable disease (e.g. immobile node on physical exam, nodal disease that radiographgically involves the carotid arteries, nerves)
- ☐ (N) Currently taking Disease Modifying Rheumatoid Drugs (DMRDs)
- ☐ (N) Unstable angina and/or congestive heart failure requiring hospitalization within last 6 months
- ☐ (N) Transmural myocardial infarction within last 6 months
- ☐ (N) Acute bacterial or fungal infection requiring intravenous antibiotics at time of registration
- ☐ (N) Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration
- ☐ (N) Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects
- ☐ (N) Pre-existing $\geq$ grade 2 neuropathy
- ☐ (N) Prior organ transplant
- ☐ (N) Systemic lupus
- ☐ (N) Psoriatic arthritis
- ☐ (N) Known HIV Positive
- ☐ (N) Current active smoker (active cigarette smoking within the 6 months prior to enrollment)

_____ _____

Attending Physician Signature | Date

10.2 Time and Events Table

Study Assessments	Prior to CRT	Weekly During CRT	Weeks 3 and 6 of CRT	6-16 Weeks After CRT	10-16 Weeks After CRT	3, 6, 12, 18 and 24 Months After CRT	Every 2-3 Months for 2yrs after CRT, every 6 months for 3yrs then yearly[1]	Every 6 months for 2yrs after CRT, then yearly
Clinical Evaluation	×	×			×		×	
Panendoscopy (if primary unknown)[2]	×							
Contrasted neck CT (or PET/CT)	×							
Chest X-Ray	×							×[3]
Dental Evaluation[2]	×							
Labs: CB, serum chemistries, liver function tests, pregnancy (F)	×							
HPV and/or p16 testing	×							
Genetic assessment (if > 10 pack year smoking history)	×							
Circulating free HPV-DNA (blood sample)	×	×[4,8]				×[5,8]		
NCI-CTCAE (physician)	×		×		×			×
Fiberoptic Laryngoscopy	×			×			×[6]	
PET/CT					×			
Thyroid Function								×[7]

Footnotes:

1. Exact timing of follow-up visits is flexible based on physican's standard of care
2. Strongly recommended, but not required
3. First post-CRT chest x-ray to be done 6 months after post-CRT PET/CT; Flexibility in timing is allowed based on scheduling of patient's appointments

4. Week 4 and 6 only
5. Additional post-treatment samples at PI's discretion
6. At provider's discretion
7. First one may be done at same time as first post-CRT chest x-ray; flexibility in timing is allowed based on scheduling of patient's appointments
8. See Section 5.5 for details on time points based on enrollment date.