Use of a Proliferation Saturation Index to Determine Personalized Radiotherapy Fractionation for Patients with HPV+ Oropharyngeal Cancers

NCT03656133

Version 2.3

July 9, 2021

Use of a Proliferation Saturation Index to Determine Personalized Radiotherapy Fractionation for Patients with HPV+ Oropharyngeal Cancers

Protocol Identifying Number:

MCC# 19579

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Funded by: Department of Radiation Oncology
Moffitt Cancer Center

Version Number: v.2.3

09 July 2021

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LIST OF ABBREVIATIONS

AE	Adverse Event
CBCT	Cone Beam Computed Tomography
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computed Tomography
CTV	Clinical Target Volume
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FFR	Federal Financial Report
GARD	Genomic-Adjusted Radiation Dose
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GTV	Gross Tumor Volume
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Investigational Review Board
ISO	International Organization for Standardization
LQ	Linear Quadrant
LRC	Locoregional Control
LSMEAN	Least Squares Means
MRI	Magnetic Resonance Imaging
MSDS	Material Safety Data Sheet
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PET	Positron Emission Tomography
PI	Principal Investigator
PTV	Planning Target Volume
QA	Quality Assurance
QC	Quality Control
RSI	Radiosensitivity Index
RT	Radiation Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
UP	Unanticipated Problem

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the <NIH IC> Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed of their obligation to meet the above commitments.

Principal Investigator: _____
Print/Type Name

Signed:_____Date:_____

STUDY SUMMARY

Title: **Use of a Proliferation Saturation Index to Determine Personalized Radiotherapy Fractionation for Patients with HPV+ Oropharyngeal Cancers.**

Précis: **We will use a mathematical model for each HPV+ oropharyngeal cancer patient's tumor growth characteristics to select radiotherapy fractionation. We believe it will most likely to result in a rapid response by week 4 of treatment.**

Objectives:

Primary Objective:

We hypothesize that by using individual patient proliferation saturation index (PSI) to select radiotherapy fractionation (conventional fractionation or hyperfractionation) to improve the likelihood of a rapid response (defined as ≥ 32% reduction in volume at 4 weeks).

Secondary Objectives:

- Rate of complete response by CT at 2 months or PET/CT at 3 months following completion of therapy.
- Correlate response and outcome to pre-treatment and during treatment radiomics features on PET/CT/MRI.
- Correlate response and outcome to mathematical models of tumor growth and death dynamics pre-treatment and during treatment.

Exploratory Objectives:

- Identify potential biomarkers of response to adaptive radiation therapy based on evaluation of tumor immune microenvironment and detection of HPV DNA in oral gargles and plasma.

Endpoint

Primary Endpoint: For patients with HPV+ squamous cancer of the oropharynx, fractionation of radiation will be individualized based on their PSI. Response will be measured after 4 weeks of RT as assessed by CT or MRI, as described in section 10.0. Primary objective is to increase the rate of response of ≥ 32% at 4 weeks to 63% of patients, above the expected 49%.

Population:	Total sample size of 60 patients, which will include men and women ≥ 18 years with pathologically (histologically or cytologically) proven diagnosis of HPV+ squamous cell carcinoma of the oropharynx and no evidence of distant metastases. This is a single center study
Phase:	2
Number of Sites enrolling participants:	1

Description of Study Agent :	Proliferation Saturation Index – a measure of the carrying capacity of the patient for the tumor burden. We will use this to select radiotherapy fractionation.
Study Duration:	**~30 months**
Participant Duration:	**~33 months**

SCHEMATIC OF STUDY DESIGN

PATIENT POPULATION
HPV+ Squamous cell carcinoma of the Oropharynx; AJCC 8th edition T0-T2, N0-N1
(include only 5 or less involved lymph nodes)

↓

REGISTRATION

↓

Pre-treatment imaging with CT with contrast at least 1 week apart to calculatethe Proliferation Saturation Index (PSI)

↓

Based on PSI, patient will be assigned to:
1. Standard Fractionation at 2 Gy once daily
2. Hyperfractionation at 1.2 Gy twice daily (≥ 6 hours apart)

↓

Assessment at week 4
Percentage response by CT and/or MRI.

↓

Radiotherapy completed to standard dose

1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

With increasing understanding of the complexity of tumor heterogeneity, it would be reasonable to hypothesize that cancer therapy should be tailored to individual patients, which is the central principle underlying precision medicine. Currently in radiation medicine, decisions for total dose and fractionation are based empirically on clinical data, albeit often from randomized trials. Current efforts in radiation oncology to personalize radiation therapy mainly address adapting the target volume based on response. However, there have been no trials attempting to individualize total dose and fractionation. Here we provide one of the first clinical studies to deliver personalized radiation fractionation dose therapy for cancer.

2.2 PROLIFERATION SATURATION INDEX

Tumors are composites of proliferating and growth arrested cells. Overall radiation response depends on their respective proportions at irradiation. In multi-compartment mathematical models that distinguish between proliferating and growth-arrested cells, we have shown that proliferation and oxygenation status-dependent radiation response can be simulated on the cellular level[1]. Tumor growth in vivo can be approximated by logistic dynamics. Initial exponential growth at low cell densities when most cells have access to ample resources decelerates when cells at the core of the tumor become growth-arrested, mainly due to limited space and exhausted intratumoral nutrient supply as resources are consumed by cells closer to the tumor surface. This established the notion of a tumor carrying capacity (K) as the maximum tumor volume that can be supported by a given environment[2]. The tumor carrying capacity is patient-specific, depending on the established oxygen and nutrients supply through tissue vascularization, removal of metabolic waste products, and evasion of immune surveillance. Hence, the tumor volume-to-carrying capacity ratio (V/K) describes the saturation in overall tumor cell proliferation as the tumor approaches its carrying capacity, which we call a Proliferation Saturation Index (PSI). Logistic growth is modeled as $\frac{dV}{dt} = \frac{\ln 2}{T_{POT}} V(1 - PSI)$ (eqn. 1), where V is the tumor volume at time t, dV/dt is the change of tumor volume over time TPOT is the intrinsic potential doubling time.

Radiation response is modeled as instantaneous volume changes $V_{postIR} = V - \gamma_d V(1 - PSI)$ (eqn. 2)

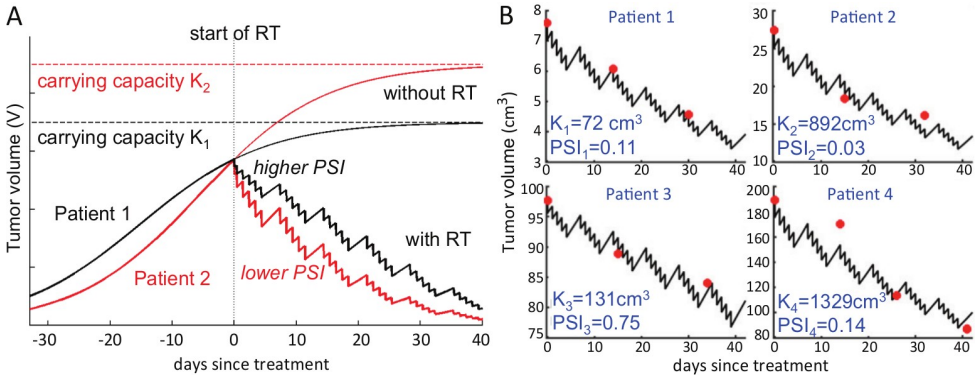

Figure 1. A) Two patients with identical tumor volume but different PSI at treatment begin (day 0) exhibit different reduction in tumor volume after fractionated RT. **B**) Logistic tumor growth and radiation response model predicted curves (*eqns. 1+2*; solid black lines) to four lung cancer patientsdata (red circles[30]) with growth rate $ln2/T_{POT}$=0.045 and radiation-induced death γ_{2Gy}=0.084, and patient-specific carrying capacities K_i. PSI_i: Proliferation Saturation Index for patient P_i at first treatment (t=0).

at irradiation moments, where $\gamma_d = 1 - e^{-(\alpha d + \beta d^2)}$ represents radiation-induced death after dose d following the linear-quadratic model. It follows from (eqns. 1+2) that larger PSI reflect a low proliferating cell fraction and, thus, treatment refractory tumors, whereas tumors with low PSI are more proliferative and radiosensitive. As such, two patients that present with similar tumor volume could have a different tumor growth history and thus a different PSI, which results in different responses to the same radiotherapy protocol (Fig. 1A). In a preliminary study of non-small cell lung cancer and head and neck cancer patients we have confirmed that PSI varies greatly between patients (0.26±0.33; [0.03-0.75]) and correlates inversely with radiotherapy response[2]. A patient-specific pretreatment PSI was sufficient to fit individual patient response data (r^2=0.98; Fig. 1B).

2.3 RADIOTHERAPY IN HEAD AND NECK CANCER

Radiotherapy as definitive treatment for oropharyngeal cancer is a standard of care[3]. Based on multiple clinical trials, standard doses are in the range of 60 – 70 Gy at 2 Gy given once daily or 60 – 81.6 Gy given twice daily[4,5]. However, to date the selection of fractionation has been empiric in nature. We hypothesize that head and neck cancer represents an ideal opportunity to establish a paradigm of individualized radiotherapy dosing based on mathematical models.

We are specifically selecting HPV+ oropharyngeal cancer patients because at an early stage (AJCC 8th edition stage T1-2 N0-1 with limited lymph node involvement), radiotherapy alone is a standard of care, reducing the variables in question. For example, RTOG H0022 treated patients with up to radiographic T1-2 N0-1 (AJCC 7th edition T1-2 N0-2a) oropharyngeal tumors with radiotherapy alone, with locoregional control rates of >90%[6]. Additionally, multiple retrospective reports from large institutions such as MD Anderson and Princess Margaret demonstrate >90% locoregional control rates for oropharyngeal cancer patients with T1-2 N0-2b (AJCC 7th edition).[7,8]

However, RTOG 1016, which treated patients with locoregionally advanced HPV+ oropharyngeal cancer with 70 Gy of radiotherapy and either cisplatin or cetuximab was recently published. This demonstrated the superiority of cisplatin over cetuximab in combination with radiotherapy.[9] RTOG 1016 included overlapping patient population that is eligible for the current trial, specifically patients with a single lymph node > 3 cm or multiple lymph nodes in ipisilateral neck. Examination of Forrest

plots from the trial suggests that patients with a > 10 pack year history of cigarette smoking were at higher risk and had a greater benefit from cisplatin. We therefore will exclude patients who are locoregionally advanced with a significant smoking history.

Additionally, recent review of Moffitt Cancer Center internal data suggests that patients with greater than 5 positive lymph nodes are at a higher risk of regional failure, with locoregional control at 3 years of 94.9% for 1-5 lymph nodes vs 80.1% for > 5 lymph nodes (p = 0.001). By restricting patients to T1-2 N0-1 with 5 or less lymph nodes and a smoking history of 10 pack years or less, 3-year locoregional control of patients treated with radiation alone at Moffitt Cancer Center was 94.7% (n=81) compared to 95.9% with concurrent radiotherapy and systemic therapy (n=98) (personal communication J. Caudell).

In a retrospective analysis of 44 patients, decrease in nodal size of ≥ 40% was associated with a locoregional control (LRC) of 100% at 2 years, compared with 78% for a decrease of < 40%[10]. Retrospective review of a Moffitt cohort (n=49) suggests that a cut point of 32% reduction resulted in 100% LRC and DFS (Figure 2A&B)[11]. Of note, half of these patients were treated with radiation alone.

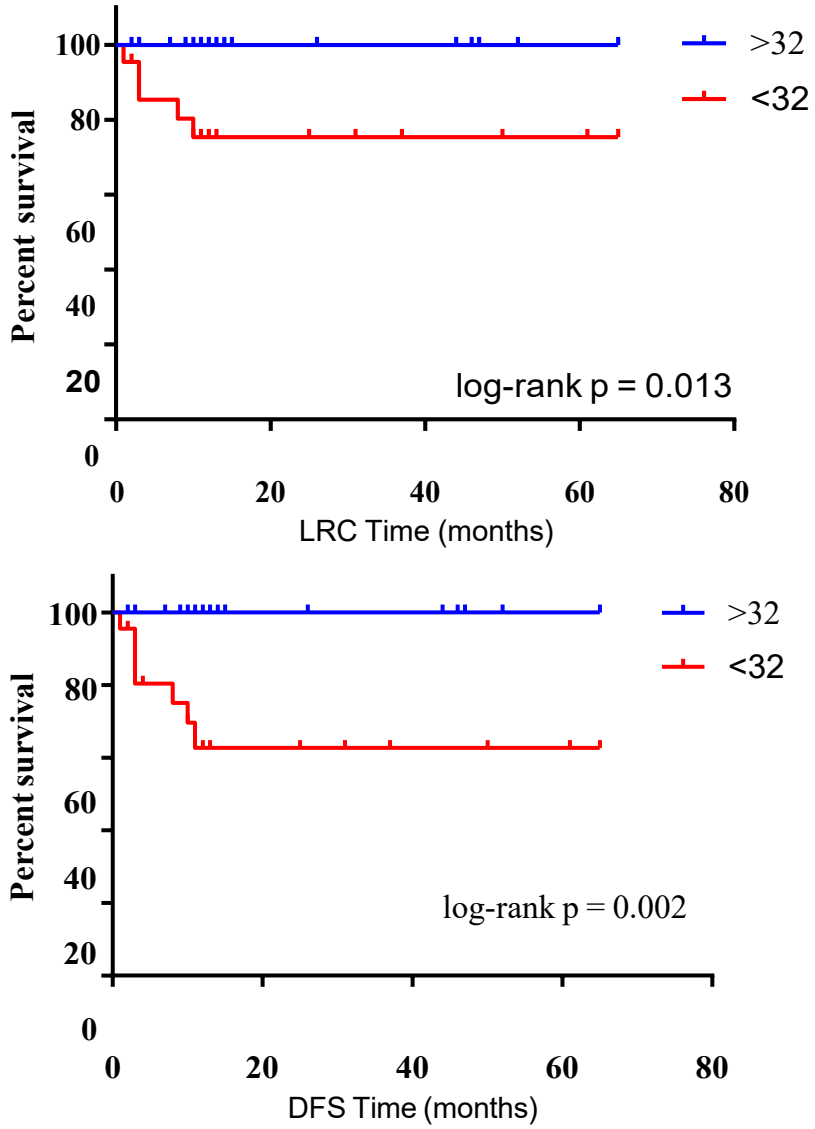

Figure 2: A) Locoregional Control and B) Disease Free Survival by response at 4 weeks

In the mathematical modeling of radiation treatment response, there is a suggestion of a more robust treatment response by hyperfractionation when the PSI is > 0.75 (Figure 3).

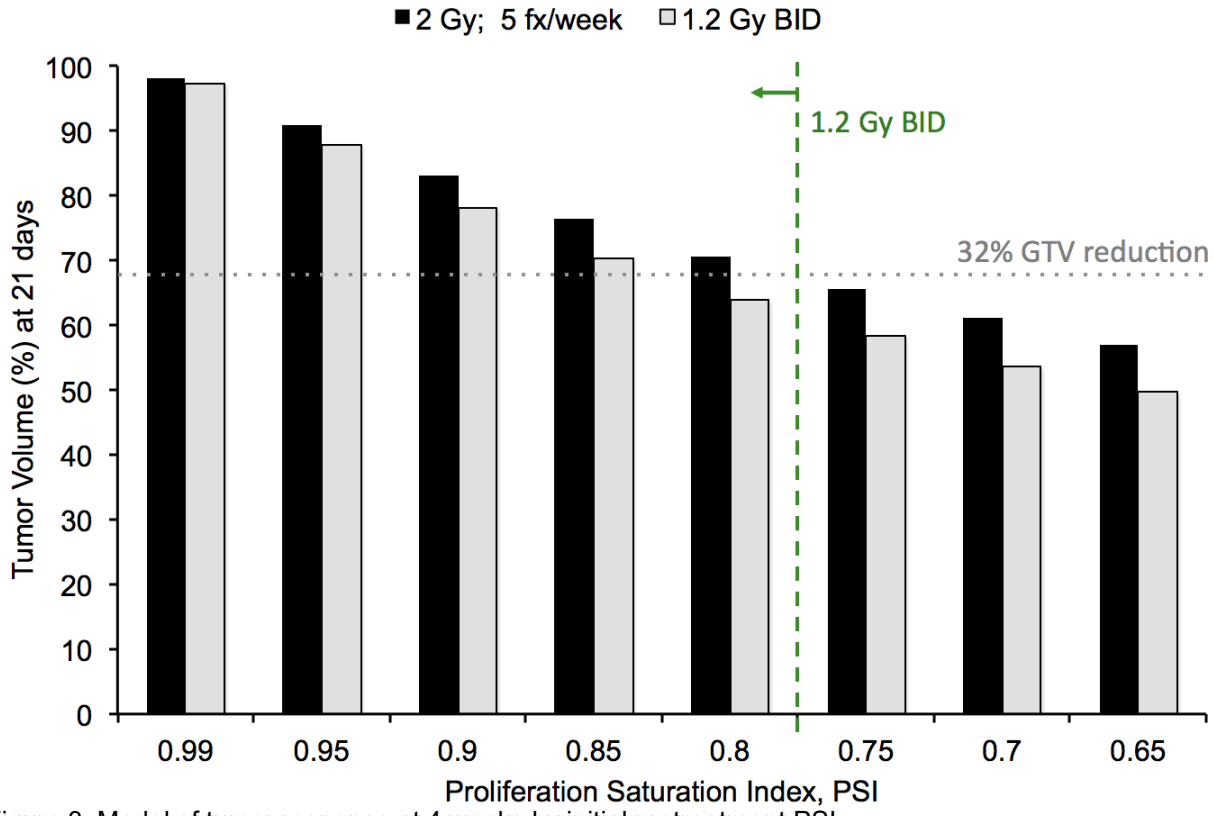

Figure 3: Model of tumor response at 4 weeks by initial pretreatment PSI.

We therefore hypothesize that by personalizing the fractionation, we will increase the number of patients achieving a 32% reduction by week 4.

2.4 RATIONALE FOR FRACTIONATION SCHEMES USED IN THIS STUDY

All fractionation schemes are selected are standards of care in H&N cancer. For example, RTOG 7913 established that the locoregional control rates between these two total dose and fractionation schemes were equivalent[12].

2.5 RATIONALE FOR BIOMARKER STUDIES

2.5.1 EVALUATION OF TUMOR IMMUNE MICROENVIRONMENT (TIM)

The interplay between the tumor and its TIM is critical in tumor formation, metastasis, and clinical outcomes including radiation therapy response[13,14]. Characterization of lymphoid and myeloid immune cells has shown to be prognostic in HNSCC[15], but their association with radiation therapy benefits has not been clearly evaluated. Significance of the TIM in context of the PSI in HNSCC is entirely unknown. The findings from this innovative and highly interdisciplinary project will have a significant clinical impact in improving the selection of patients who will gain the most benefit from receiving PSI based radiation therapy, offering further biological insight to the mechanisms of resistance, and subsequently developing optimal combination therapies for immune modulating agents.

2.5.2 EVALUATION OF HPV DNA VIRAL LOAD IN ORAL GARGLES AND PLASMA

Detection of HPV DNA in oral gargles and plasma after the completion of treatments has shown to associate with recurrence[16,17]. In a study by Ahn, et al, HPV-16 E6 and E7 DNA were determined from plasma and/or saliva samples obtained before and after treatments in 93 patients[16]. Patients with detectable HPV DNA in the posttreatment saliva and either saliva or plasma had reduced overall survival. However, significance of persistent HPV DNA detection in saliva and/or plasma in context of the PSI in HNSCC is entirely unknown. Evaluation of HPV DNA as a predictive biomarker of PSI-based radiation therapy warrants further studies.

2.6 POTENTIAL RISKS AND BENEFITS

2.6. KNOWN POTENTIAL

As all interventions are considered standards of care, no additional potential risks would be incurred.

2.6.2 KNOWN POTENTIAL BENEFITS

By selecting the most ideal fractionation scheme, we may be able to improve the 4 week response rate which has been associated with improved locoregional control.

3 OBJECTIVES AND PURPOSE

3.1 PRIMARY OBJECTIVE

For patients with HPV+ squamous cancer of the oropharynx, fractionation of radiation will be individualized based on their PSI. Response will be measured after 20 days of RT as assessed by CT or MRI, as described in section 10.0. Primary objective is to increase the rate of response of ≥ 32% at 20 days to 63% of patients, above the expected 49%[11].

3.2 SECONDARY OBJECTIVES

- Rate of complete response by CT at 2 months or PET/CT at 3 months following completion of therapy.
- Correlate response and outcome to pre-treatment and during treatment radiomics features on PET/CT/MRI.
- Correlate response and outcome to mathematical models of tumor growth and death dynamics pre-treatment and during treatment.
- Correlate RSI/GARD with mid-treatment and post-treatment response.
- Correlate clearance of HPV in serum/oral gargle specimens with mid-treatment and post-treatment response.

3.3 EXPLORATORY OBJECTIVES

- Identify potential biomarkers of response to adaptive radiation therapy based on evaluation of tumor immune microenvironment and detection of HPV DNA in oral gargles and plasma.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

$$\mathrm{PSI} = \frac{V_{diagnosis} \times e^{\lambda \Delta t} - V_{treatment}}{V_{diagnosis} \times (e^{\lambda \Delta t} - 1)}$$

- PSI will be calculated according the above formula, where Vdiagnosis is the volume of the tumor at first imaging and Vtreatment is the volume of the tumor at second pretreatment imaging, t is time, and λ is doubling time. Patients with a PSI ≤ 0.75 will be assigned to conventional fractionation group, and PSI > 0.75 will be assigned to the hyperfractionation group (Figure 3).
- Each site of disease (primary and each lymph node) will be independently contoured and PSI calculated for each. PSI of the lymph nodes will take priority over primary site. PSI of the largest lymph node will take priority over smaller lymph nodes.
- Based on PSI, patients will be assigned to one of two cohorts:
- *PSI ≤ 0.75: Conventional fractionation – 2 Gy once daily, Monday through Friday.*
- **Should patients be assigned to conventional fractionation, and do not meet the predicted >32% reduction AND they are not receiving concurrent chemotherapy, their treatment will be accelerated via a 6th fraction per week, given as a BID dose once during the week or on a Saturday[18].
- PSI > 0.75: Hyperfractionation – 1.2 Gy twice daily, at least 6 hours apart, Monday through Friday.

4.2 STUDY ENDPOINTS

For the purposes of this study, patients should be evaluated for on-treatment response after 4 weeks, and re-evaluated at 2 or 3 months post RT (the selection of post-RT timepoint for imaging is at physician discretion (either is standard of care; eg if CT is negative a 2 months, no further imaging is required. If disease still present at 2 month CT, a 3 month PET will be obtained, or simply a 3 month PET).

4.2.1 On Treatment response at 4 weeks during RT

Week 4 CT and/or MRI will be loaded into the treatment planning system or other software for volumetric evaluation of tumor size. This will be compared against CT simulation or week 1 CT/CBCT and/or MRI, whichever is larger. The percent response will be calculated.

4.2.2 Post Treatment response at 2-3 months after RT

A complete response will be defined as resolution of all disease in the head and neck by CT at 2 months or PET/CT at 3 months. On CT scan, nodes less than 1 cm in short axis without evidence of necrosis will be defined as negative. On PET/CT, resolution of hypermetabolism to maximum SUV of 3 or less will be defined as negative, even with evidence of a node larger than 1 cm in short axis on CT scan. CT scans or PET scans which do not meet these criteria will be reviewed at the Moffitt H&N tumor board for determination of response.

5 STUDY ENROLLMENT AND WITHDRAWAL

Men and women of all races and ethnic groups are eligible for this trial.

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Provision of signed and dated informed consent form
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Male or female, aged ≥ 18 years
- Pathologically (histologically or cytologically) proven diagnosis of p16+ or HPV+ squamous cell carcinoma (including the histological variants papillary squamous cell carcinoma and basaloid squamous cell carcinoma) of the oropharynx; cytologic diagnosis from a cervical lymph node is sufficient. Patients with unknown primary (T0) should be both p16 and HPV positive. Clinical evidence should be documented, and may consist of pathology, palpation, imaging, or endoscopic evaluation, and should be sufficient to estimate the size of the primary (for T stage).
- AJCC 8th edition staging T0-2 N0-1 M0 (N1 includes clinical tumor involvement in only 1-5 lymph nodes) Patients with any smoking history are included if N0 or a single involved node size ≤3cm. However, if the number of involved lymph node is ≥2 or any lymph node size >3 cm, only patients with ≤ 10 pack years of smoking is eligible.
- Patients must have clinically or radiographically evident measurable disease at the primary site or at nodal stations.
- CT or MRI performed at least 1 week apart. This can consist of diagnostic imaging and radiation therapy planning imaging.
- No evidence of distant metastases
- ECOG Performance Status 0 to 2

5.2 PARTICIPANT EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- Age < 18
- Positive urine pregnancy test
- Evidence of distant metastases
- Patients with >10 pack years of smoking is excluded if the number of involved lymph node is ≥2 or any lymph node size >3 cm.
- Gross total excision of both primary and nodal disease; this includes tonsillectomy,local excision of primary site, and nodal excision that remove all clinically and radiographically evident disease

- Prior radiotherapy to the region of the study cancer that would result in overlap of radiation therapy fields
- Patients with a medical condition or social situation that at the discretion of the PI would preclude them from completion of the trial

5.3 PARTICIPANT WITHDRAWAL OR TERMINATION

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study in the following circumstances:

- If any clinically adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interests of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

5.4 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigators andregulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed.

6 STUDY AGENT

Not applicable.

6.1 DOSING AND ADMINISTRATION

Based on PSI, patients will be assigned to one of two cohorts:

1. Conventional fractionation – 2 Gy once daily, Monday through Friday.

	All disease ≤ 2 cm	Any site of disease > 2 cm but ≤ 4 cm	Any site of disease > 4 cm
Gross Disease	60 Gy / 30 fractions	66 Gy / 33 fractions	70 Gy / 35 fractions
Elective Nodes	41.4 Gy / 23 fractions	41.4 Gy / 23 fractions	41.4 Gy / 23 fractions

**Should patients be assigned to conventional fractionation, and do not meet the predicted >32% reduction AND they are not receiving concurrent chemotherapy, their treatment will be accelerated via a 6th fraction per week (as per standard of care which is one twice daily fraction per week, > 6 hours apart OR on the weekend).

2. Hyperfractionation – 1.2 Gy twice daily, at least 6 hours apart, Monday through Friday.

	All disease ≤ 2 cm	Any site of disease > 2 cm but ≤ 4 cm	Any site of disease > 4 cm
Gross Disease	60 Gy / 50 fractions	64.8 Gy / 54 fractions	69.6 Gy / 58 fractions
Elective Nodes	41.4 / 36 fractions	41.4 / 36 fractions	41.4 / 36 fractions

6.2 TREATMENT PLANNING/TARGET VOLUMES

The treatment plan used for each patient will be based on an analysis of the volumetric dose, including dose-volume histogram analyses of the planning target volume (PTV) and critical normal structures.

Gross tumor volume (GTV) represents the region judged to contain gross primary tumor or involved node(s) based on clinical and endoscopic examinations, CT scan, MRI, and PET-CT.

Clinical target volumes (CTV) are defined and contoured in relation to the targets that they are intended to encompass and the dose they are intended to receive. For gross targets (GTVs), the CTVs are defined by 3D isotropic expansions of 0.5 cm that should then be limited by potential barriers to tumor spread such as air cavities, external contours, and bony or fascial planes through which tumor spread is not possible or apparent.

Elective clinical target volume consists of elective nodal basins at risk depending on size and location of the primary site and grossly involved nodes. In general this consists of levels 2-4 and potentially retrostyloid and retropharyngeal ipsilaterally, and levels 2-3 contralaterally. If tonsil primary without involvement (or <1 cm involvement) of base of tongue or soft palate, unilateral elective neck radiotherapy may be delivered. This will be at the discretion of the treating radiation oncologist.

PTV represents an additional margin around the GTV to compensate for the variability of treatment set up and internal organ motion. The CTVs will be expanded symmetrically 2.5 to 3 mm to generate the PTV.

Detailed Organs at Risk Specifications

SpinalCord: The cord begins at the foramen magnum. Superior to this is brainstem and inferior to this is cord. The inferior border of the spinal cord volume will be defined at approximately the carina (ie, 2-3 cm below the lowest slice level that has PTV on it). The spinal cord shall be defined based on the treatment planning CT scan.

SpinalCord_PRV05: Planning Risk Volume (PRV) spinal cord defined as SpinalCord + 5 mm in all directions.

BrainStem: The inferior most portion of the brainstem is at the cranial-cervical junction where it meets the spinal cord. For the purposes of this study, the superior most portion of the brainstem is approximately at the level of the top of the posterior clinoid. The brainstem shall be defined based on the treatment planning CT scan.

BrainStem_PRV03: Planning Risk Volume (PRV) brainstem defined as Brainstem + 3 mm in all directions.

Parotid_R/L: Parotid glands will be defined in their entirety (superficial and deep lobes) based on the treatment planning CT scan.

Pharynx: This will be defined as superior/middle/inferior constrictor muscles. This extends from the superior constrictor region (level of the inferior pterygoid plates) to the cricopharyngeal inlet (level of the posterior cricoid cartilage).

Esophagus_UP: This will be defined as the cervical or superior (S) esophagus, a tubular structure that starts at the bottom of pharynx (cricopharyngeal inlet) and extends to the thoracic inlet.

Larynx: This will be defined as the glottic and supraglottic larynx, including the tip of the epiglottis, the epiglottis, the aryepiglottic folds, arytenoids, false cords, and true cords, up to but not including the medial border of the thyroid cartilage, and including the cricoid cartilage to the inferior edge of the arytenoid cartilage, but not the hypopharynx.

OralCavity: The oral cavity will be defined as a composite structure consisting of the lips, oral tongue/floor of mouth, buccal mucosa, and superiorly the palate, and inferiorly to the plane containing the tip of the mandible.

Submandibula_R/L: Submandibular glands will be defined in their entirety based on treatment planning CT scan.

Mandible: This includes the entire bony structure of the mandible from TMJ through the symphysis.

Thyroid: Thyroid gland should be contoured in its entirety based on treatment planning CT scan.

The density-corrected dose distributions shall be calculated, and the dose prescription will be based on a dose distribution corrected for heterogeneities.

Dosimetry: Intensity-modulated RT, volumetric modulated arc therapy, or tomotherapy planning is acceptable. Typically, ≥ 7 beams or ≥ 2 arcs of radiation will be used.

Prescription Isodose Surface Coverage: The prescription isodose surface will be chosen such that ≥ 95% of the PTV is conformally covered by the dose and 100% of the PTV would be covered with the isodose surface that is represented by 95% of the prescription dose. 100% of the GTV should be covered by 100% of the prescription dose.

High Dose Spillage: Any dose ≥ 105% of the prescription dose should occur primarily within the PTV itself and not within the normal tissues outside the PTV. Therefore, the cumulative volume of all tissue outside the PTV receiving a dose > 105% of the prescription dose should be no more than 0.03 cc of the PTV volume.

Prioritization for Intensity-Modulated RT Planning:

1. Spinal cord
2. Brainstem
3. Gross target PTV
4. Elective PTV
5. Organs at Risk
    a. Parotid gland contralateral to primary tumor site
    b. Larynx
    c. Pharynx
    d. Contralateral submandibular gland
    e. Oral Cavity/Lips
    g. Esophagus
    h. Parotid gland ipsilateral to primary tumor site
    i. Mandible
6. Unspecified tissue outside the targets

6.3 DOSES TO NORMAL STRUCTURE

Dose limitations to normal structures are described below. For the critical structures of spinal cord and brainstem, these are mandatory. For other structures, recommended limits are provided, but the doses delivered should always be as low as reasonably achievable without compromising doses to PTVs.

- **Spinal cord:** The planning risk volume (PRV) (Spinal cord + 0.5 cm) for spinal cord should not exceed 48 Gy to any volume in excess of 0.03 cm^3 (approximately 3 mm x 3 mm x 3 mm).
- **Brain stem:** The PRV for (brainstem + 0.3 cm) brainstem should not exceed 50 Gy to any volume in excess of 0.03 cm^3 (approximately 3 mm x 3 mm x 3 mm). In treatment planning, the brain stem_03 should be given less priority than the spinal cord, but more than the critical structures listed below.
- **Oral cavity/Lips:** Reduce the dose as much as possible. The mean dose should be <32 Gy for the oral cavity. Efforts should also be made to avoid hot spots (> 60 Gy) within the non-involved oral cavity.
- **Parotid glands:** The treatment planning goal will be for this individual parotid gland to receive a mean dose of < 26 Gy, but efforts should be made to reduce this further if possible without compromising dose to PTVs.

- **Contralateral submandibular gland:** If contralateral level Ib is not a target, aim to reduce mean contralateral submandibular gland to < 39 Gy.
- **Pharynx:** Reduce the dose as much as possible. Mean pharynx dose recommended to be <50 Gy.
- **Esophagus:** Reduce the dose as much as possible; recommended (not mandatory) treatment goal: mean dose < 30 Gy.
- **Larynx:** Reduce the dose as much as possible. The larynx mean dose is recommended to be ≤ 35 Gy if whole-neck *i*ntensity-modulated RT is used.
- **Mandible:** Reduce the dose as much as possible. Hot spots within the mandible should be avoided. It is recommended that maximum dose within the mandible be < 105% of prescription dose.
- **Unspecified tissue outside the targets (Non-PTV):** No more than 1 cm^3 of unspecified tissue outside the targets can receive ≥ 105% of the prescription dose.

6.4 TREATMENT BREAKS

Treatment breaks must be clearly indicated in the treatment record along with the reason(s) for the treatment break(s). Treatment breaks, if necessary, should not exceed 3 treatment days at a time and 5 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. Any treatment break(s) exceeding 2 treatment days for reasons other than toxicity/illness will be considered a protocol deviation.

6.5 DEVICE-SPECIFIC CONSIDERATIONS

Photon beams of ≥ 6MV are required with treatment distance ≥ 80 cm source to axis distance for isocentric techniques.

Immobilization: Treatment should include use of a thermoplastic head and shoulder mask.

Planning CT scan: CT scan thickness should be ≤ 0.3 cm. If possible, planning CT scan should be done with contrast. MRI and/or PET/CT should be fused to the planning CT scan.

Localization: Isocenter or reference point port localization images or volumetric imaging study on the linear accelerator couch should be obtained at each treatment on the treatment unit immediately before treatment to ensure proper alignment of the geometric center (i.e., isocenter) of the simulated fields.

6.5 DRUG THERAPY

There will be no concurrent chemotherapy. Other drug therapy (e.g. lidocaine rinses, skin creams, narcotics) as clinically indicated for the management of radiotherapy side effects as per standard of care.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

$$\mathrm{PSI} = \frac{V_{diagnosis} \times e^{\lambda \Delta t} - V_{treatment}}{V_{diagnosis} \times \left(e^{\lambda \Delta t} - 1\right)}$$

PSI will be calculated according the above formula, where Vdiagnosis is the volume of the tumor at first imaging and Vtreatment is the volume of the tumor at second pretreatment imaging, t is time, and λ is doubling time. Each site of disease will be contoured individually, and PSI calculated. PSI of lymph nodes will have priority over primary site. PSI of the largest lymph node will have priority over smaller lymph nodes. Patients with a PSI ≤ 0.75 will be assigned to conventional fractionation group, and PSI > 0.75 will be assigned to the hyperfractionation group (Figure 3).

- Medical history within 90 days of start of treatment, including history, focused physical exam of the head and neck, assessment of performance status, and weight.

- Imaging will consist of pre-treatment CT and/or MRI, with at least 2 exams one week apart. Pre-treatment PET/CT while ideal, is not required. On first day of RT (or within 5 days of starting RT), CT simulation will be repeated. During week 4 of radiotherapy (~fraction 20 for conventionally fractionated patients or ~fraction 40 for hyperfractionation patients, CT simulation and MRI will be repeated. At ~8 weeks after treatment is complete CT of neck or ~12 weeks PET/CT will be performed for response assessment.

7.2 CORRELATIVE STUDY PROCEDURES/EVALUATIONS

The following samples will be sent to the Moffitt Cancer Center Tissue Core for processing and storage for the following analyses in the Chung Laboratory. Detailed sample collection and processing are described in Lab Manual.

7.2.1 EVALUATION OF TUMOR IMMUNE MICROENVIRONMENT (TIM)

An innovative methodology, multiplex immunohistochemical (mIHC) staining, developed in the Chung Laboratory allows the analysis of an array of markers from limited sample specimens. The evaluation of a complex cellular microenvironment is best accomplished using high mIHC, which enables simultaneous detection and measurement of multiple protein antigens on the same tissue section allowing accurate classification of cells as well as enumeration and quantification of protein signal. This is accomplished by using sequential steps of antigen retrieval followed by primary antibody application, and then antibody stripping. The process is repeated using different primary antibodies for each antibody target. The result is that up to 18 protein targets can be visualized along with the nuclear stain. This complex staining can then be analyzed by imaging systems such as the the Aperio FL scanning system. Image analysis software such as Cell Profiler can then be applied to enumerate cells and quantify signal. Complex analysis can determine cellular densities and also measure co-localized targets. The multiplexing will encompass evaluation of antigen presenting cells, T cells, natural killer cells, B cells, and tumor cells using 30 immune cell markers and EGFR. The goal is to link a comprehensive clinical characterization and immune microenvironment assessment and to correlate these with radiation treatment response and survival.

7.2.2 EVALUATION OF HPV DNA VIRAL LOAD IN ORAL GARGLES AND PLASMA

HPV genotype and viral load will be determined on oral gargle and plasma specimens collected before, during, and after treatments using the most updated assay at the time of analyses.

7.2.3 TUMOR SAMPLE SUBMISSION

Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples will be collected at pre-treatment (MANDATORY). The FFPE tumor could be any archived tissue obtained before starting radiation therapy. The tumor tissue samples will be submitted to the Moffitt Tissue Core for cataloging and storage until the analyses at the Christine Chung's laboratory.

An FFPE tumor block or 10, 4 micron unstained slides should be submitted with the submission form. A Pathology Report and one hematoxylin and eosin-stained slide documenting that the submitted block or slides contain tumor should also be submitted with the tissue. The report and hematoxylin and eosin-stained slide must include the protocol number and patient's case number. The patient's name and/or other identifying information should be removed from the report. The surgical pathology numbers and information must NOT be removed from the report. If the subject has multiple archival samples available, samples from various settings may be requested.

7.2.4 ORAL GARGLE SAMPLE SUBMISSION AND PROCESSING

Collection Notification Form (CNF) for each oral gargle and each blood sample will be email to Tissue Core Acquisition and Tissue Core SPL groups 24 hours before collection.

Collection sites:
Moffitt Cancer Center

Oral gargle specimens will be collected using 15 ml of a locally available mouthwash.

Clinical events:

1. Pre-therapy
2. During treatment at 1 weeks (+/- 3 days)
3. During treatment at 2 weeks (+/- 3 days)
4. During treatment at 3 weeks (+/- 3 days)
5. During treatment at 4 weeks (+/- 3 days)
6. End of the treatment (+/- 3 days)
7. 3 months after the treatment (+/- 14 days)
8. 6 months after the treatment (+/- 14 days)
9. 12 months after the treatment (+/- 14 days)
10. 18 months after the treatment (+/- 14 days)
11. 24 months after the treatment (+/- 14 days)
12. Time of disease progression if sooner than 24 months
13. End of study if the patient is off the study before 24 months with any reasons.

Procedures: The oral gargle samples should be processed within 24 h from collection.
Oral gargle processing: Refer to Lab Manual.

7.2.5 BLOOD SAMPLE SUBMISSION AND PROCESSING

Collection Notification Form (CNF) for each blood sample will be email to Tissue Core Acquisition and Tissue Core SPL groups 24 hours before collection.

Collection sites:
Moffitt Cancer Center

1x 10 ml Lavender EDTA tube of blood and 1x 10ml LBgard blood tube will be collected from each clinical event.

Clinical events:

1. Pre-therapy
2. During treatment at 2 weeks (+/- 3 days)
3. During treatment at 4 weeks (+/- 3 days)
4. End of the treatment (+/- 3 days)
5. 3 months after the treatment (+/- 14 days)
6. 6 months after the treatment (+/- 14 days)
7. 12 months after the treatment (+/- 14 days)
8. 18 months after the treatment (+/- 14 days)
9. 24 months after the treatment (+/- 14 days)
10. Time of disease progression if sooner than 24 months
11. End of study if the patient is off the study before 24 months with any reasons.

Procedures: The blood samples should be processed within 2 h from collection.

Plasma processing: Refer to Lab Manual.

Specimen collection summary for correlative studies

Specimens taken from patient:	Collected when:	Submitted as:
A paraffin-embeddedtissue of archival tissue taken before initiation of treatment	Pre-treatment	Paraffin-embedded tissue block, or 10,4 micron unstained slides (minimum of 10 unstained slides) Refer to Lab Manual for processing and storage
ORAL GARGLE: Collected using 15 ml of a locally available mouthwash.	1. Pre-therapy 2. During treatment at 1 weeks (+/- 3days) 3. During treatment at 2 weeks (+/- 3days) 4. During treatment at 3 weeks (+/- 3days) 5. During treatment at 4 weeks (+/- 3days) 6. End of the treatment (+/- 3 days) 7. 3 months after the treatment (+/- 14 days) 8. 6 months after the treatment (+/- 14 days) 9. 12 months after the treatment (+/- 14days) 10. 18 months after the treatment (+/- 14days) 11. 24 months after the treatment (+/- 14days) 12. Time of disease progression if sooner than 24 months 13. End of study if the patient is off the study before 24 months with anyreasons.	Refer to Lab Manual for Processing and storage
PLASMA: 1x 10 mL of anticoagulated wholeblood in EDTA tube (purple/ lavender top).	1. Pre-therapy 2. During treatment at 2 weeks (+/- 3days) 3. During treatment at 4 weeks (+/- 3days)	Refer to Lab Manual for processing and storage

Specimens taken from patient:	**Collected when:**	**Submitted as:**
1x 10 mL of anticoagulated wholeblood in LBgard blood tube.	4. End of the treatment (+/- 3 days) 5. 3 months after the treatment (+/- 14days) 6. 6 months after the treatment (+/- 14days) 7. 12 months after the treatment (+/- 14days) 8. 18 months after the treatment (+/- 14days) 9. 24 months after the treatment (+/- 14days) 10. Time of disease progression if sooner than 24 months 11. End of study if the patient is off the study before 24 months with anyreasons.	Refer to Lab Manual for Processing and storage
PBMC: 1x10 mL of nticoagulated whole blood in EDTA tube (purple/ lavender top).	1. Pre-therapy 2. During treatment at 2 weeks (+/- 3days) 3. During treatment at 4 weeks (+/- 3days) 4. End of the treatment (+/- 3 days) 5. 3 months after the treatment (+/- 14days) 6. 6 months after the treatment (+/- 14days) 7. 12 months after the treatment (+/- 14days) 8. 18 months after the treatment (+/- 14days) 9. 24 months after the treatment (+/- 14days) 10. Time of disease progression if sooner than 24 months 11. End of study if the patient is off the study before 24 months with anyreasons.	Refer to Lab Manual for Processing and storage

7.3 STUDY SCHEDULE

		On Treatment			
Assessments	**Pre-Study (within 90 d. of Tx start)**	**First day, prior to RT (within5 days prior)**	**Once-a-WeekDuring RT**	**Week 4 During RT**	**End of Tx (+/- 3 days)**
Evaluations					
Rad Onc Eval H&P, ECOG, Weight	X		X		
Imaging					
CT# or MRI of primary and neck [a,b]	X[h]	X		X	
PET/CT	X[c]				
Labs					
Urine PregnancyTest (only Females of Child bearing potential)	X				
Tumor Tissue for RSI	X				
Oral Gargle for HPV[e]	X		X	X	X
Plasma for HPV[f]	X		X	X	X

	From End of Treatment							
Assessments	**4 Wks +/- 14 days**	**2-3 mo +/- 14 days[d]**	**6 mo +/- 14 days**	**12 mo +/- 14 days**	**18 mo +/- 14 days**	**24 mo +/- 14 days**	**Time of disease progression if sooner than 24 mos[g]**	**End of study if the patient is off the study before 24 mos with any reasons[g]**
Evaluations								
Rad Onc Eval H&P, ECOG, Weight	X	X	X	X				

Assessments	4 Wks +/- 14 days	2-3 mo +/- 14 days[d]	6 mo +/- 14 days	12 mo +/- 14 days	18 mo +/- 14 days	24 mo +/- 14 days	Time of disease progression if sooner than 24 mos[g]	End of study if the patient is off the study before 24 mos with any reasons[g]
Imaging								
CT# or MRI of primary and neck [a,b]		X[c]						
PET/CT		X[c]						
Labs								
Urine PregnancyTest (only Females of Child bearing potential)								
Tumor Tissue for RSI								
Oral Gargle for HPV[e]		X	X	X	X	X	X	X
Plasma for HPV[f]		X	X	X	X	X	X	X

[a]Interim MRI or CT for response assessment.
[b]CT at day 1 and week 4 may be done using Department of Radiation Oncology CT Simulation[c]At physician discretion. Pre-treatment PET/CT can be up to 180 days prior to CT simulation. [d]A CT at 2 months and/or PET/CT at 3 months may be done at physician discretion.
[e]Oral Gargle for HPV to be collected at pre-therapy, during treatment at week 1, week 2, week 3, week 4, end of treatment, 3 months post-RT, 6 months post-RT, 12 months post-RT, 18 months post-RT, 24 months post-RT, time of diseaseprogression (if sooner than 24 months), and end of study (if patient discontinues the study with any reason sooner than 24 months). See 7.2.4/ 7.2.5 for collection windows.
[f]Blood samples to be collected at pre-therapy, during treatment at week 2 and week 4, end of treatment, 3 months post-RT, 6 months post-RT, 12 months post-RT, 18 months post-RT, 24 months post-RT, time of diseaseprogression (if sooner than 24 months), and end of study (if patient discontinues the study with any reason sooner than 24 months). See 7.2.4/ 7.2.5 for collection windows.
[g]Following this visit, patients are considered off the trial and are followed as per standard of care.
[h]Pre-treatment CT or MRI for PSI calculation can be up to 180 days prior to CT simulation.

7.4 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable

7.5 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable

7.6 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1. DEFINITION OF ADVERSE EVENTS

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS

Adverse events and suspected adverse reactions are considered "serious" if, in the view of either the investigator or sponsor, they result in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS

OHRP considers unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2. SEVERITY OF

All AEs will be graded using the CTCAE 5 criteria. For AEs not included in the protocol-defined grading system, the following guidelines will be used to describe severity.

- ***Mild*** *– Events require minimal or no treatment and do not interfere with the participant's daily activities.*
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.]>

8.2.2 RELATIONSHIP TO STUDY AGENT

The clinician's assessment of an AE's relationship to the study agent (drug, biologic, device) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

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

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews with study participants presenting for medical care, or upon review by a study monitor. All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of event. All AEs occurring during the study must be documented appropriately, regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of AEs will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution orstabilization are achieved.

All Grade 3 or greater AEs will be monitored until resolution or, if the AE is determined to be chronic, a cause is identified. If an AE is considered potentially related to study treatment and remains ongoing at the conclusion of the study, the event will be followed until resolution, stabilization, or initiation of treatment that confounds the ability to assess the event. RT-related AEs and SAEs will be collected at follow-up contact 90 days from the end of treatment provided no new treatments have been initiated that could confound the ability to assess the event.

8.4 REPORTING PROCEDURES

8.4. ADVERSE EVENT

All Grade 3 or greater Adverse Events possibly, probably, or definitely related to radiotherapy up to the primary outcome time point (4 weeks) **MUST** be reported in routine study data submissions. Data will be captured in Oncore, Moffitt Cancer Center's clinical trials database. Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

Any clinical adverse event or abnormal laboratory test value that meets the definition of serious noted below and occurs in a patient during the course of the study, irrespective of the treatment received by the patient, must be reported within 24 hours of the investigator becoming aware of the occurrence. Serious adverse event definition and reporting requirements will be in accordance with the International Conference of Harmonisation (ICH) Guideline for Clinical Safety Data Managements.

Definitions and Standards for Expedited Reporting, Topic E2A:
An SAE is defined as any untoward medical occurrence that at any dose:

1. Results in death
2. Is immediately life-threatening (i.e., in the opinion of the investigator, the AE places the patient at immediate risk of death; it does not include a reaction that, had it occurred in a more severe form, might have caused death)
3. Requires inpatient hospitalization or results in prolongation of an existing hospitalization
4. Results in persistent or significant disability or incapacity
5. Is a congenital anomaly or birth defect
6. Is an important medical event that may not be immediately life-threatening, result in death, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, it jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

Any SAE, whether or not considered to be related to study treatment, will be reported within 24 hours of the investigator becoming aware of the event. It will be recorded on both the SAE form and the CRF AE page. Additional SAE information including medications or other therapeutic measures

used to treat the event, action taken with the study drug due to the event, and the outcome / resolution of the event will be recorded on the SAE form. Forms for reporting SAEs will beprovided to the study sites.

Conversely, some hospitalizations, particularly those that are the result of elective or previously scheduled surgery for preexisting conditions, which have not worsened after initiation of treatment, will not be classified as SAEs. For example, an admission for a drainage of obstructed bile duct would not be classified as an SAE. Pre-specified study hospitalizations for observation are not considered SAEs. All overdoses, whether or not resulting in an AE, require reporting within 24 hours.

All Grade 3 and greater AEs and SAEs that occur after the first treatment with RT and through 4 weeks of treatment will require reporting by the investigational site. SAEs that meet the criteria for expedited reporting to the FDA will be reported in accordance with US regulations governing safety reporting (Title 21 of the Code of Federal Regulations [CFR] 312.32 and 312.33). Reporting of SAEs by the investigator to his or her Institutional Review Board (IRB) will be done in accordance with the standard operating procedures and policies of the IRB. Adequate documentation must be maintained showing that the IRB was properly notified.

In case of an SAE, the investigator will electronically report to the University of South Florida Institutional Review Board via website http://www.research.usf.edu/cs/.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the DCC/study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the DCC/study sponsor within 1 day of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 7 days of the investigator becoming aware of the problem.

8.5 STUDY HALTING RULES

Not Applicable

8.6 SAFETY OVERSIGHT

The Protocol Monitoring Committee (PMC) monitors its assigned ongoing research protocols for adverse event reporting, data and safety monitoring, and internal audit findings. The PMC,

upon review of any agenda item, may approve the study for continuation, require revisions, or suspend or close a protocol. This study will be reviewed by the PMC for data and safety monitoring. The principal investigator will enter into Oncore all adverse events and deviations as they occur for review by the PMC.

The Principal Investigator is ultimately responsible for every aspect of the design, conduct, and actions of all members of the research team. This includes final analysis of the protocol.

9 CLINICAL MONITORING

This study will be monitored internally by Moffitt Cancer Center using the OnCore reporting system. Data will be captured in OnCore, Moffitt's electronic Clinical Trials Database. For each participant enrolled, the electronic CRF must be completed by the assigned data manager or other authorized study staff. Any paper forms should be typed or filled out indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. This also applies to records for those patients who fail to complete the study. If a patient stops dosing or terminates from the study, the dates and reasons must be noted on the CRF. If a patient terminates from the study because of a DLT, thorough efforts should be made to clearly document the outcome. The Case Report Forms will be reviewed by Moffitt's Internal Monitors periodically throughout the conduct of the trial. The monitoring will include source data verification, utilizing research patients' medical records. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the protocol monitoring committee.

Each Scientific Review Committee conducts a formal internal peer review of all clinical protocols and general scientific oversight of interventional clinical research. Protocols are reviewed for scientific merit, adequate study design, safety, availability of targeted study population, and feasibility of timely completion of all proposed research projects to be conducted by its assigned programs at the Cancer Center. Each SRC is responsible for evaluating the risk/benefit assessment and corresponding data and safety monitoring plan as part of the scientific review and approval process.

No modifications will be made to the protocol without the agreement of the investigators. Changes that significantly affect the safety of the patients, the scope of the investigation, or the scientific quality of the study will require Scientific Review Committee and Institutional Review Board approval before implementation, except where the modification is necessary to eliminate apparent immediate hazard to human subjects. Any departures from the protocol must be fully documented in the case report form and the source documentation.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

Demographic information such as age and race will be tabulated. Descriptive statistics, including means, standard deviations, and ranges for continuous parameters, as well as percents and frequencies for categorical parameters, will be presented.

The effectiveness of PSI to select fractionation schema will be assessed by percent response at the 4th week of treatment. One-sided 95% confidence interval for the percent response at the 4th week of treatment will be also reported. A proportion difference test will be performed to test a difference of complete response rate from a target of 49%.

As for secondary endpoints, response will be correlation with pre-treatment and during treatment radiomics features on PET/CT/MRI using two sample t-tests and univariate/multivariate logistic regression analysis as appropriate. Correlation of response and outcomes to the mathematical models of tumor growth and death dynamics pre-treatment and during treatment will be performedusing logistic regression analysis.

10.2 DESCRIPTION OF STATISTICAL METHODS

10.2.1 GENERAL APPROACH

This protocol is a single arm, phase II design with a primary endpoint of 63% of patients achieving a ≥ 32% reduction in tumor volume by week 4. This study will test the null hypothesis that the true response rate is equal to or less than a historical control of 49%[11] versus the alternative hypothesis that it is greater than 49%.

Based on Fleming's 2-stage group sequential design, a total of 60 subjects will provide approximately 79% power with a type I error rate of 0.1 to detect a true response rate of 63%. The study will enroll subjects in two stages, with 49 subjects in the first stage. One interim efficacy/futility analysis will be conducted when the first 49 subjects have completed the study treatment and response evaluation. If the number of responsive patients is equal to or less than 27, the trial will be stopped for futility. If the number of responsive patients is equal to or greater than 30, the trial will be stopped for efficacy. Otherwise, the trial will continue to the second stage by enrolling 11 additional subjects.

At the final analysis with the total 60 subjects at the second stage, if there are equal to or more than 35 responsive patients, it will be concluded that the null hypothesis is rejected and the study treatment will be promising.

10.2.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The effectiveness of PSI to select fractionation schema will be assessed by percent response at the 4th week of treatment. One-sided 95% confidence interval for the percent response at the 4th week of treatment will be also reported. A proportion difference test will be performed to test a difference of complete response rate from a target of 49%.

10.2.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

- Assess complete response by CT at 2 months or PET/CT at 3 months following completion of therapy. This will be compared against historical controls.
- Response correlation with pre-treatment and during treatment radiomics features on PET/CT/MRI:
  Radiomics features based on CT, MRI, PET-CT, noted during various imaging scans (pre-treatment, at 4 week of RT, and follow-up scan) will be correlated with the overall oncological response and outcome

  Response correlation at 4 weeks and post treatment imaging with clearance of HPV from serum or oral gargle, and RSI/GARD.

- Correlate response and outcome to mathematical models of tumor growth and death dynamics pre-treatment and during treatment:
  Mathematical models will be generated based on the pattern of tumor growth and response as depicted by tumor volume measurement on various scans. These mathematical models will be validated by correlating with the overall response and oncological outcome

10.2.4 Interim Efficacy/Futility Analysis

One interim efficacy/futility analysis will be conducted when the first 49 subjects have completed evaluation of response to the study treatment. If the number of responsive patients is equal to or less than 27, the trial will be stopped for futility. If the number of responsive patients is equal to or greater than 30, the trial will be stopped for efficacy. Otherwise, the trial will continue to the second stage by enrolling 11 additional subjects.

10.2.5 Interim Exploratory Data Analysis

When the first 33 subjects (55% of the total planned sample size of 60) will complete the study treatment, an early interim exploratory data analysis will be performed for data on HPV DNA viral load in oral gargle and plasma samples, demographic variables, and basic biometric screening results. This exploratory analysis is a pilot proof-of-concept study to evaluate the feasibility and consistency of HPV DNA detection in salivary and plasma samples. No analysis of primary and secondary safety and efficacy endpoints will be performed in this exploratory data analysis to keep trial integrity.

- Data for the exploratory analysis will be summarized overall using descriptive statistics. Continuous data will be summarized with number of patients (n), mean, median, minimum, maximum, and standard deviation. Categorical data such as gender, race, and ethnicity will be summarized using frequency counts and percentages.
- Cross-tabulation of descriptive statistics of demographic variables and biometric screening results with dichotomized HPV DNA viral load results (presence/absence of HPV DNA) will be made with Chi-square test and two-sample *t*-test as appropriate.
- Pearson's correlation coefficient, Spearman's rank correlation coefficient, and Kappa statistics will be calculated along with p-values as summary statistics for the consistency of HPV viral loads and detection results in oral gargle samples and plasma samples.

10.3 SAMPLE SIZE

60 patients total. Total accrual time is expected to be 30 months.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator is responsible for ensuring the source data are accurate, legible, contemporaneous, original, and attributable.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Monitoring will be performed regularly to verify data is accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with the currently approved protocol/amendments, Good Clinical Practice (GCP), and applicable regulatory requirements.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will beprotected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Coordinating Center. This will not include the participants' contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Coordinating Center.

13.5 FUTURE USE OF STORED SPECIMENS

Data collected for this study will be analyzed and stored at the Moffitt Cancer Center.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and

timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported inthe eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into <specify name of data capture system>, a 21 CFR Part 11-compliant data capture system provided by Moffitt Cancer Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the site study staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1-3
5.1 Quality Assurance and Quality Control section 5.1.1
5.2 Noncompliance, sections 5.20.1-2

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 10working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to protocol monitoring committee. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

14.3 PUBLICATION AND DATA SHARING POLICY

The study of these patients and results are considered private and confidential. The progress and results of this study will not be presented without approval by the principal investigator.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

Not applicable.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the study sponsor has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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APPENDIX

Version	Date	Significant Revisions
2.0	2/09/2018	Updated inclusion criteria Variable dosing based on size Added exploratory endpoint examining serum/oral gargleHPV pre-treatment, during, and after treatment
2.1	11/04/2019	Updated Inclusion criteria Updated calendar: Pre-treatment PET/CT or MRI can be up to 180 days prior to CT simulation
2.2	05/29/2020	7.2.1 Evaluation of HPV DNA viral load in oral gargles and plasma HPV genotype and viral load will be determined on oral gargle and plasma specimens collected before, during, and after treatments using the most updated assay at the time of analyses.
2.3	07/09/2021	Updated oral gargle and blood sample time points. Updated co-investigator list by removing Drs. Harrison and Trotti. Added interim primary endpoint analysis and interim exploratory data analyses.