Study Title: **A Pilot Phase II Study Evaluating the Role of Stabilized Chlorine Dioxide on Mucositis for Patients Undergoing Head and Neck Radiotherapy**

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Investigator Agreement

I have read, understand and will adhere to the protocol as written, that any changes to the protocol will be approved by the sponsor or sponsor-investigator and the IRB, except changes to eliminate an immediate hazard to study subjects.

I agree to conduct this study in accordance with the current International Conference on Harmonization (ICH) guidance, the Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, FDA regulations, local IRB and legal requirements.

_____ _____

Signature Date (MM/DD/YY)

___Jared R. Robbins, MD____

Name of Principal Investigator

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1. INTRODUCTION / SYNOPSIS

Oral mucositis (OM) is an extremely common and often therapy limiting toxicity in patients receiving radiation therapy (RT) for head and neck cancer (HNC) (1). The pathobiology of OM is complex and some evidence suggests that changes within the oral microbiome during RT may play a contributing role (2). Chlorine dioxide is a known broad spectrum antiseptic against bacterial, fungal, and viral pathogens, and has been shown effective in the treatment of drinking water (3). Stabilized chlorine dioxide based rinsing products are now commercially available (CloSYS®) for the treatment of halitosis and oral hygiene use (4). It is believed that regulating the oral microbiome during RT could perhaps mitigate the incidence, onset, and duration of OM (5). No studies to date have evaluated the role of chlorine dioxide based oral rinses in preventing and treating OM associated with RT for HNC patients.

1.1 Phase

This is a Phase II, single center, prospective, randomized, double-blind, placebo-controlled study assessing the effects of stabilized chlorine dioxide oral rinse versus placebo on incidence, time to onset, duration, and patient reported outcomes on radiation therapy related OM, RT interruption, and changes in oral microbiome and cytokine composition.

1.2 Indication

Stabilized chlorine dioxide oral rinse for the treatment of RT related OM.

1.3 Endpoints

Primary:

- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the incidence of severe OM as measured by the World Health Organization (WHO) scale (≥WHO Grade 3)

Grade	Description
0 (none)	None
I (mild)	Oral soreness, erythema
II (moderate)	Oral erythema, ulcers, solid diet tolerated
III (severe)	Oral ulcers, liquid diet only
IV (life-threatening)	Oral alimentation impossible

References:

WHO: http://www.who.int/en/

Secondary:

- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the time to onset of severe OM (≥WHO Grade 3)
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the duration of severe OM (≥WHO Grade 3)
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect in patient reported outcomes as measured by the Oral Mucositis Weekly Questionnaire (OMWQ) (6) and Patient Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on rates of RT interruption

Exploratory:

- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the oral microbiome
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on salivary TNFα, IL-1β, and IL-6 levels

1.4 Patient Population

Eligible subjects must be able to understand and sign the study specific subject consent form. They must be ≥18 years of age, have a Karnofsky Performance Status Scale (KPS)of ≥60 with confirmed histologic or cytologic diagnosis of HNC (stage I-IV) for which they are receiving definitive or post-operative treatment with either RT alone or in combination with systemic therapy. Subjects may not have previous allergies to chlorine dioxide products and must fulfill the inclusion and exclusion criteria found in Section 6. Approximately 24 subjects will be randomized in a 1:1 ratio to stabilized chlorine dioxide rinse and placebo rinse. Twenty evaluable subjects (10 in each group) are needed in this pilot study to meet 81% statistical power (assuming a one-sided alpha of 0.05). We plan to recruit an additional 4 subjects to account for a 20% drop out/study non-compliance rate to ensure we have 20 evaluable subjects.

2. STUDY DESIGN

2.1 Phase

This is a Phase II, single center, prospective, randomized, double-blind, placebo-controlled study. Approximately 24 subjects will be randomized in a 1:1 ratio to stabilized chlorine dioxide oral rinse and placebo oral rinse.

2.2 Number of centers

One

This study will be performed by The University of Arizona Cancer Center Radiation Oncology Department at both the Banner University North Medical Campus Tucson (BUMC-N) and Orange Grove Clinic (OGC).

2.3 Number of subjects

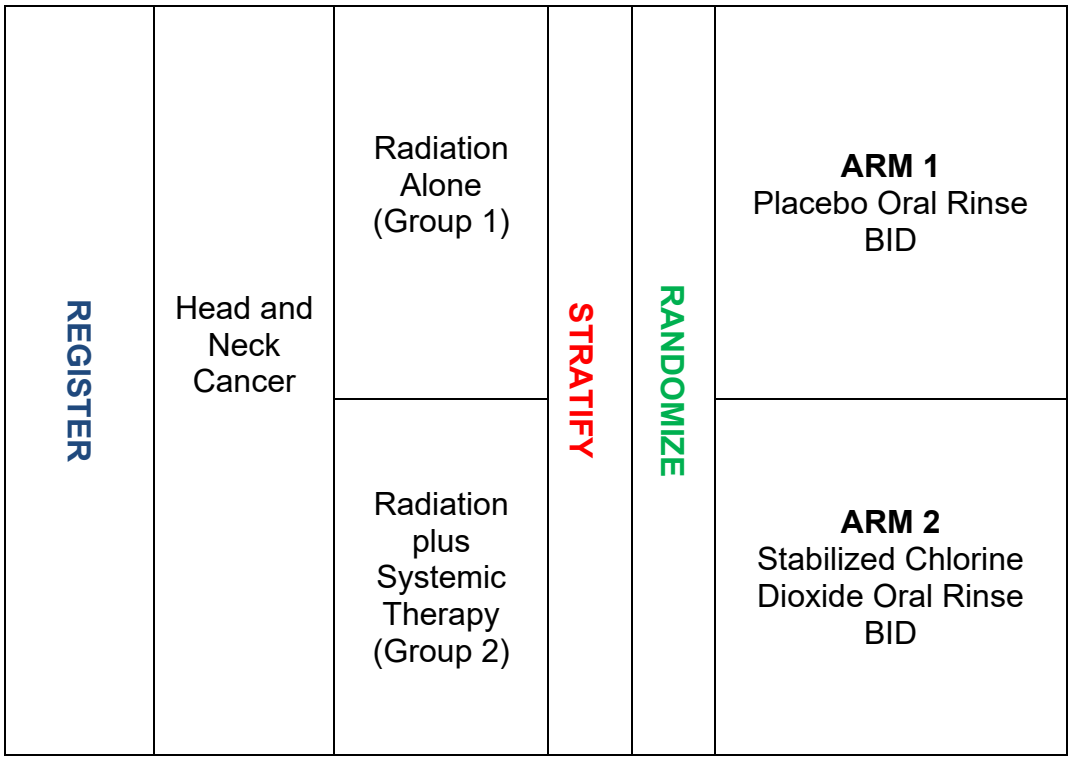
Approximately 24 will be recruited for this pilot study. Twenty evaluable subjects are needed to meet 81% statistical power (assuming a one-sided alpha of 0.05). We plan to recruit 4 additional subjects to account for a 20% drop out/study non-compliance rate.

2.4 The subject participation time period

Subjects will be enrolled in the study for approximately 3 months, including screening time, treatment time, and follow-up at 30 days post RT.

Subjects enrolled in this study are undergoing treatment at BUMC-N or OGC as part of standard of care for the treatment of HNC. Study subjects will be treated with either stabilized chlorine dioxide oral rinse or placebo oral rinse twice daily during RT. Typical RT schedule is 6 to 7 weeks in length with treatments administered Monday through Friday. Subjects will begin the oral rinse (stabilized chlorine dioxide or placebo) on the morning of RT day 1. Whole unstimulated salivary collections, OM scoring, and validated OMWQ and PRO-CTCAE patient surveys will be completed at baseline (pre-RT), during weeks 2, 4, and 6 of RT during their planned on-treatment visits (OTVs), and at their 1-month post-RT follow-up appointment. The number of days and cause of RT treatment interruption will also be tracked.

SCHEMA



3. OBJECTIVES

3.1 Primary Objective

- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the incidence of severe OM as measured by the World Health Organization (WHO) scale (≥WHO Grade 3)

3.2 Secondary Objectives

- To evaluate and compare ClōSYS® UnflavoredOral Rinse effect on the time to onset of severe OM (≥WHO Grade 3)
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the duration of severe OM (≥WHO Grade 3)
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect in patient reported outcomes as measured by the Oral Mucositis Weekly Questionnaire (OMWQ) (6) and PRO-CTCAE
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on rates of RT interruption
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on the oral microbiome
- To evaluate and compare ClōSYS® Unflavored Oral Rinse effect on salivary TNFα, IL-1β, and IL-6 levels

4. BACKGROUND AND RATIONALE

4.1 Disease/Condition

It is estimated that 61,760 new cases of head and neck cancer (HNC) will be diagnosed in the United States in 2016 (7). The majority of HNC patients receive radiation therapy (RT) as part of their care and nearly all are expected to develop treatment related oral mucositis (OM) (1). This often therapy limiting toxicity presents a challenge to practitioners and patients as it is known to reduce both quality of life and prognosis (8)(9). The pathobiology of OM is complex and involves alterations in mucosal barrier integrity, epithelial cell kinetics, extracellular matrix composition, vascular integrity, inflammatory mediators, and the oral microbiome (10)(11). In cases of high grade OM, infection and ulcerations may lead to a breach in the mucosal barrier, sepsis, and even death.

Few effective agents are available today for the prevention, delay, or reduction of RT related OM. Palifermin, a recombinant human keratinocyte growth factor, was demonstrated to improve the incidence, onset, and duration of high grade (WHO 3-4) OM in two independent phase III randomized clinical trials for patients receiving definitive and post-operative HNC RT, however both studies showed no impact on patient reported pain scores, RT compliance, or disease related outcomes (12)(13). Though FDA approved, the results of these studies have called into question the role of palifermin for OM, as it is a difficult drug to administer with weekly IV infusions during RT.

4.2 Investigational Product, Intervention, Device

Chlorine dioxide is a known antimicrobial disinfectant which has been shown to be effective in the treatment of drinking water and is FDA approved for this indication among others (3). Clinically, chlorine dioxide has also been proven efficacious in the treatment of halitosis, which is thought to be related to its effect on decreasing the amount of volatile sulfur compounds and their associated bacterial organisms within the oral cavity (4). Commercially available products including ClōSYS® Unflavored Oral Rinse containing 0.1% (w/v) stabilized chlorine dioxide, are widely available for personal oral hygiene use.

Evidence exists that RT related OM is associated with a shift in the oral microbiome toward opportunistic species as resultant from alterations in salivary production and composition (2). It is believed that regulating the microbiome could eliminate, delay, or reduce the effects of OM as demonstrated by a meta-analysis of clinical trials evaluating various antimicrobials, none which included chlorine dioxide based products (5). In addition to its broad spectrum antiseptic properties (bacterial, fungal, and viral), chlorine dioxide products are alcohol-free, sulfate-free, well-tolerated, available in rinse form, and possess anti-inflammatory properties which are critical to the prevention of OM (14).

We propose that the addition of twice daily usage of ClōSYS® Unflavored Oral Rinse to standard oral hygiene care during RT for HNC will decrease OM incidence, increase time to onset, decrease duration, and improve patient reported outcomes related to OM. Additionally, we hypothesize that ClōSYS® Unflavored Oral Rinse will reduce OM in patients receiving RT for HNC by mechanisms via changes in microbiome and inflammatory cytokine compositions. Lastly, we believe that decreased OM will potentially lead to less RT interruption. We hope that the results of this study will provide preliminary data for future phase III clinical trial testing.

4.3 Preclinical Experience

The safety of chlorine dioxide and its metabolites (chlorite and chlorate) have been studied in animals extensively. Initial evaluations of high dose chlorine dioxide based products have shown adverse hematological, thyroid, and reproductive effects. In a review by Lyman Condie, these animal studies are highlighted in monkey, rat, and mouse experiments, which have shown that at high doses of exposure (>10mg/L or >10mg/kg/d) changes in several key physiologic endpoints have been clearly documented (15). Some studies have shown that chlorine dioxide and its metabolites (chlorite and chlorate) can cause oxidation of hemoglobin into methemoglobin and a subsequent reduction in red cell count by virtue of hemolytic anemia. Other studies have shown decreases in rat T4 levels leading to functional hypothyroidism. Further studies have shown sperm head abnormalities in mice exposed to high levels of chlorine dioxide for 5 days. To our knowledge, no animal studies have specifically evaluated chlorine dioxide in its potential protective effects on OM.

4.4 Clinical Experience

In humans, the over ingestion of sodium chlorate (a similar compound to chlorine dioxide) based herbicides revealed a minimal lethal dose of 220 mg/kg of sodium chlorate in adult humans according to one study (16). Another study in humans showed that chlorine dioxide and its metabolites (chlorite and chlorate) are well tolerated at doses of 2.5 mg or less (<0.04 mg/kg assuming 70 kg average man)

when ingested daily for 12 weeks, except potentially in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency (17). A prospective epidemiological study in humans exposed to chlorine dioxide (0.25 – 1.1 mg/L) after ingesting drinking water for 12 consecutive weeks showed no adverse effects when compared to a control group ingesting water without chlorine dioxide (18).

There are no data we are aware of evaluating the safety and efficacy of ClōSYS® Unflavored Oral Rinse for the prevention, delay, or reduction in RT related OM in patients being treated for HNC. As mentioned above, a meta-analysis of other similar orally available, topically delivered antimicrobials and anti-inflammatory agents have shown equipoise for mitigating RT induced OM (5). ClōSYS® Unflavored Oral Rinse is an FDA approved, commercially available product which is approved for 15 ml twice daily (30 second) rinse applications (19)(20). Two clinical trials of 15 patients each have demonstrated that chlorine dioxide rinsing is safe and effective in the treatment of halitosis and reduction of plaque build-up, while none reporting toxicities associated with the rinse (4)(21).

5. INVESTIGATIONAL PRODUCT, INTERVENTION, DEVICE

5.1 Investigational Product, Intervention or Device

ClōSYS® Unflavored Oral Rinse containing 0.1% (w/v) stabilized chlorine dioxide used twice daily with 15 mL per rinse for 30 seconds

5.2 Investigational product, intervention or device supply

The IP (ClōSYS® Unflavored Oral Rinse) is a commercially available, over-the-counter, product which is currently FDA approved for the treatment of halitosis and general oral hygiene care. The following products will be supplied by Rowpar Pharmaceuticals Inc. specifically for the purpose of this study in 16 oz. white, opaque bottles, along with measuring cups, requiring no additional preparation. ClōSYS® Unflavored Oral Rinse or Placebo Oral Rinse will be labelled in such a way that they are not identifiable by subjects or investigators.

a. ClōSYS® Unflavored Oral Rinse (also referred as ClōSYS® Unflavored Alcohol-Free Oral Rinse).
b. Unflavored Placebo Oral Rinse. The study placebo will be identical in appearance to the IP and without stabilized chlorine dioxide.

5.3 Investigational product, Intervention or Device Accountability

Because the IP (ClōSYS® Unflavored Oral Rinse) and Placebo Oral Rinse will be supplied directly to the Banner UMC Research (BUMC-N) pharmacy for use in this study, the receipt of shipment, dispensing and return or destruction records will be tracked using the Investigational Agent Accountability Record or other applicable and similar record. Once a signed order for IP is received by pharmacy, the IP will be given to the research staff to dispense to the study participant. Participants will be asked to keep a rinse diary, which will include date, time, and length of rinsing. All containers will be returned by the patient

and then taken to pharmacy by the research staff. The amount of rinse solution taken will be documented by taking the initial volume of rinse given to the patient and subtracting any oral rinse not used by measuring the returned residual volume of solution. The unused remaining rinse solution will then be destroyed in accordance with the BUMC Pharmacy policy. We plan to distribute and collect rinse solution bottles at weekly intervals.

5.4 Storage

IP (ClōSYS® Unflavored Oral Rinse) and placebo will be stored at room temperature per manufacturer recommendations in the BUMC-N pharmacy with limited access to personnel.

5.5 Preparation

Once an order is written by the treating doctor and received by pharmacy, the pharmacist or technician will dispense the randomized, blinded study drug. A member of the research staff will pick up the prescription bottle and will give the bottle directly to the subject.

5.6 Handling

There is no special handling required for the IP.

6.SUBJECT ELIGIBILITY

Investigators will maintain an electronic subject log (in the UACC OnCore system and/or Redcap) of all potential (i.e. consented) study subjects, which will include as applicable (demographics, informed consent, eligibility, treatment assignment, on treatment, off treatment, follow up and off study dates).

6.1 Inclusion Criteria

a. Able to provide properly obtained written informed consent
b. Age 18 years or older
c. Pathologically-confirmed diagnosis of head and neck malignancy (stage I-IV)
d. Planned to receive high dose RT ≥50 Gy to visualizable oral cavity and/or oropharyngeal mucosa, with or without administration of concurrent systemic therapy.
e. Karnofsky Performance Status of ≥ 60, within 90 days of registration
f. Baseline hematocrit > 20 within 90 days of registration to the study
g. Willingness to complete OMWQ and PRO-CTCAE forms at each designated time point along with oral rinse diary
h. Life expectancy ≥ 3 months
i. Willing to tolerate oral rinsing for 30 second intervals
j. Negative serum pregnancy test in females of childbearing age
k. Must be willing to use an effective form of birth control if female and of childbearing potential

6.2 Exclusion Criteria

a) Known hypersensitivity to chlorine dioxide products
b) Chlorine dioxide product usage within the past 7 days prior to registration for this study
c) Utilization of any antibiotic medications (orally topical or systemic) within past 7 days prior to registration for this study
d) Utilization of supraphysiological corticosteroid medication (>10 mg oral prednisone or equivalent dose) for chronic indication
e) Sjogrens disease
f) Medically documented glucose-6-phosphate dehydrogenase (G6PD) deficiency
g) Planned daily RT of less than 5 weeks duration
h) Known history of HIV or AIDS
i) Current pregnancy

6.3 Enrollment

The source of subjects is the patient population at the Banner University Medical Group/University of Arizona Cancer Center. Patients who are scheduled as part of their routine care to consult with the radiation oncologist for definitive treatment of HNC (stage I-IV) will be provided with information regarding this study.

Before subjects may be entered into the study, a copy of the written institutional review board (IRB) approval of the protocol, informed consent form (ICF), and all other applicable subject information and/or recruitment material will be on file at the University of Arizona. All efforts will be made to consent the subject at an already scheduled appointment with the radiation oncologist. It is possible they would need to come in for an additional appointment. This could be either at their initial consult visit or during their CT simulation appointment. Subjects will have the opportunity to take their time in making a decision to join the study and will be encouraged to take the Informed Consent Form home and discuss it with whomever they would like. The treating physician will explain to the subjects the risks and benefits. They will be informed that their participation is voluntary, and lack of participation will not affect the subject's relationship with the treating staff or our facility. Subjects will be consented by either the PI, or the Research Staff of the Radiation Oncology department. The written informed consent document will be signed and personally dated by the subject and completed to a fully executed informed consent document and processed per the University of Arizona standard operating procedures.

Subjects will also be made aware that should they consent, they can withdraw their consent at any time. When new information becomes available, it will be given to the patient as soon as possible, either at their next visit, or if that is more than 30 days away, by phone call. Once an approved revised consent form is submitted and approved by the IRB, the subject will be asked to sign the new consent and the subject will be notified of the change to the consent form. The AZCC Verification of Consent form will be completed at the time the subject is consented. This form includes information pertaining to who is present at

consenting, that the consent form was reviewed with the subject, the subject understood the consent form, and the date and the time of the consent. Also documented will be the version of the Subject Consent form and the HIPAA form.

Subjects will be registered by the University of Arizona Research Pharmacy. Once subjects complete the screening process for this study and are deemed eligible, required information will be given to the pharmacy staff to register the subject in a stratification/randomization system designed by biostatistician Denise Roe, DrPH. Each subject will be identified by a unique sequential subject identification number. This number will be used to identify the subject throughout the clinical study and will be used on all applicable study documentation related to that subject. The subject identification number will remain constant throughout the study. The sponsor will be providing study IP with blinded labeling and only they will know which label corresponds with active agent or placebo.

7.STUDY PLAN

7.1 IP/treatment/intervention/device regimen

Subjects will be stratified by whether or not they will be receiving concurrent systemic therapy and then randomly assigned to receive either ClōSYS® Unflavored Oral Rinse twice daily or placebo oral rinse twice daily from the start of RT to their 1-month RT follow-up appointment. Assignment of IP will be done in a double-blinded fashion. Both active rinse and placebo rinse will be formulated to look identical in appearance.

Subjects will receive verbal and written oral hygiene instructions and 11 bottles of either (16 oz. each) of ClōSYS® Unflavored Oral Rinse or placebo oral rinse. Subjects will also receive measuring cups for dispensing the rinse (15 ml per dose) and a diary for recording usage in order to track their rinsing adherence, as well as notating any subjective effects possibly attributable to the rinse.

Treatment with IP will start on the morning of day 1 of RT and subjects will be advised to use the IP (preferably after meals) at approximately 8 a.m. ± 3 hours and 8 p.m. ± 3 hours, with 15 mL per rinse for 30 seconds during each session. They will be instructed to take nothing by mouth for 20 minutes following their rinse to allow it to take effect. They will note in their patient's log the date, time, and duration of rinsing. Subjects will be instructed to continue with other standard oral hygiene practices we offer to our RT patients, including alkaline saline rinses, honey, miracle mouthwash, etc.

Baseline tobacco history including type of tobacco, number of pack-years, duration of smoking, and quit date if applicable will be recorded. This will be captured using the Tobacco Assessment Questionnaire (see section 27). Baseline dental evaluation counting number of teeth and generally graded condition (excellent, good, fair, poor) will be recorded. Baseline weight, BMI, and history of recent (3 months) weight loss will be recorded. Nutritional intake status (solids and liquids, liquids only, no oral intake) and utilization of enteral versus

parenteral feeding will be noted. HPV status of malignancy will be recorded. One week up until registration, concomitant medications will be collected.

Subjects will be seen once per week per standard of care for on-treatment visits (OTVs). Radiation treatments may be 6 or 7 weeks at the discretion of the treating physician. Vital signs and weight measurement along with grading of common head and neck side effects per common terminology criteria for adverse events (CTCAE v5.0) will be performed. CTCAE items will include generalized weakness, skin reaction, weight loss, dry mouth, sore throat, dysphagia, anorexia, dysgeusia, voice alteration, and neck edema. Physical examination will include focused evaluation of the head and neck region, with particular attention paid to documenting skin reaction and oral mucositis (OM).

At baseline (for example during CT-simulation), during weekly OTVs, and at their 1-month post-RT follow-up appointment, physician grading of OM will occur via intraoral examination utilizing the World Health Organization (WHO) Scale, as described in the table below:

Grade	Description
0 (none)	None
I (mild)	Oral soreness, erythema
II (moderate)	Oral erythema, ulcers, solid diet tolerated
III (severe)	Oral ulcers, liquid diet only
IV (life-threatening)	Oral alimentation impossible

References:

WHO: http://www.who.int/en/

At baseline (for example during CT-simulation), during weekly OTVs, and at their 1-month post-RT follow-up appointment, intraoral photo-documentation will be taken and uploaded into patient specific Cerner electronic medical record charts utilizing the Cerner Camera Capture application via handheld mobile devices. These will be filed with study identifier only and not the subject name.

Patient reported assessment of OM will be evaluated at baseline (for example during CT-simulation), at OTVs during weeks 2, 4, and 6 of RT, and at their 1-month post-RT follow-up appointment, with the OMWQ (6) and PRO-CTCAE. The OMWQ questionnaire contains 9 items with the total ranging from 0 to 54.

Whole Unstimulated Saliva (WUS) will be collected at baseline (prior to first treatment), at OTVs during weeks 2, 4, and 6 of RT, and at the 1-month post-RT follow-up appointment. Further information on WUS collection and analyses is provided in Appendix A.

Microbiome samples will also be collected at baseline (prior to first treatment), at OTVs during weeks 2, 4, and 6 of RT, and at the 1-month post-RT follow-up appointment, immediately following collection of patient WUS. Further information on microbiome collection and analyses is provided in Appendix B.

7.2 Pre-medications

Not applicable

7.3 Rescue Medications

Not applicable

7.4 Excluded medications/treatments

Orally topical or systemic antibiotic agents (such as chlorhexidine, nystatin, poviodine-iodine, clotrimazole, clindamycin, benzydamine, etc) are not allowed and are not to be used within 7 days prior to registration. Directed antibiotics prescribed during or after RT for suspected infection related to treatment (ie oral thrush, neck abscess, cellulitis, etc) are allowed but will be documented appropriately.

8.REQUIREMENTS FOR TREATMENT

8.1 Standard dose/treatment/device use

The standard dose of ClōSYS® Unflavored Oral Rinse for the prevention and treatment of plaque build-up and halitosis is 15 ml (containing 0.1% [w/v] stabilized chlorine dioxide) for 30 seconds twice daily, which equates to a total of 30 mg of chlorine dioxide exposure over the oral mucosa. This dosage is the FDA approved and manufacture recommended amount (19)(20). Two randomized trials for the treatment of halitosis also utilized a similar dosage and found no significant adverse effects when compared against a placebo rinse (4)(21).

8.2 Dose IP/intervention/device modification

Dose modifications will be considered at weekly OTVs during patient evaluations, should the patient report bothersome effects thought to be associated with use of the rinse. Dose reduction will occur by eliminating the PM rinse for one week followed by further assessment at the following OTV (approximately 7 days later). Should the patient continue to describe bothersome effects thought likely due to the rinse, the patient will be taken off the rinse at the discretion of the treating physician following discussion with the patient.

8.3 IP treatment/intervention/devise delays

Subjects will begin oral rinsing the morning of day 1 of RT. The IP will be used twice per day continuously to the evening prior to their 1-month post-RT follow-up visit. If RT interruptions occur while taking the IP, subjects should continue IP per protocol, and additional IP may need to be given to the patient to complete the

schedule of treatment. All subjects will take a full continuous course of IP unless instructed by the PI to discontinue because of unacceptable toxicity as deemed appropriate by the PI. Subjects will be advised to rinse with the IP (preferably following meals) at 8 am ± 3 hours and 8 pm ± 3 hours.

If a subject misses a scheduled dose of IP within the recommended window, they should skip the missed dose and resume treatment at the next scheduled time. Treatment course will not be extended beyond the 1-month post-RT follow-up to compensate for missed doses. Subjects will stop treatment on the evening prior to their 1-month post-RT follow-up appointment with their Radiation Oncologist and return any remaining unused IP as described in Section 5.

If more than 7 consecutive days are missed of rinsing with the IP, the patient will be taken off study.

8.4 Definition of a Dose Limiting Toxicity (DLT)

Subjects will continue on IP twice daily from day 1 of RT to the evening prior to their 1-month post-RT follow-up appointment with their Radiation Oncologist or unless instructed to stop IP as described elsewhere. If the subject meets the criteria listed above to be taken off the study, they will discontinue IP, but all assessments will continue to be collected. There are no known pre-specified DLTs associated with the IP.

9.STUDY PROCEDURES

9.1 Screening and Baseline

Potential subjects will enter the screening period of the study after a completely executed informed consent has been obtained. See Section 26 (Study Schedule) for full list of baseline evaluations.

9.2 Registration/Randomization

All regulatory requirements must be in place prior to subject registrations.

Single center study subject identification be assigned sequentially. Screen failure and subjects who withdraw prior to registration will have –“SF” or “WD” added to the end of the subject ID.

The stratification/randomization procedure utilized will be created and will employ a system of prepared envelopes and blocks of random numbers.

9.3 On IP treatment/Intervention/Device

Registered subjects will be treated with either ClōSYS® Unflavored Oral Rinse (0.1% w/v) at 15 ml per rinse for 30 seconds each twice daily or placebo oral

rinse at 15 ml per rinse for 30 seconds each twice daily, from the morning of day 1 of RT to the evening prior to their 1-month post-RT follow-up appointment with their Radiation Oncologist. Subjects will be instructed to place the rinse in their mouth, continuously swish and gargle the solution, and spit the rinse out after 30 seconds, without swallowing the rinse. It will be requested that they do the rinse at 8am (+/- 3 hours) and 8pm (+/- 3 hours) each day that they are receiving radiation treatments. In addition, they will be requested to do the rinse twice per day on weekends even though they do not receive radiation treatment on the weekends. Once starting the IP subjects will be asked to complete a rinse diary with the time, date, and duration of each rinse taken. They will also be asked to complete a patient observation form listing any side effects and/or problems with using the rinse that they may encounter.

See Section 26 (Study Schedule) for weekly evaluations while on IP.

9.4 End of IP treatment/Intervention/Device

Subjects will complete IP the evening prior to their 1-month post-RT follow-up appointment with their Radiation Oncologist.

9.5 Follow up

Subjects will be seen for one month [+21 days] follow-up visit after the end of RT. During these visits, routine vitals, review of systems, physical examination, and adverse event documentation will be performed. See treatment plan calendar. Additionally, any remaining unused IP, rinse diary, and observation forms will be collected. Patients will complete their final OMWQ and PRO-CTCAE, have OM scored by their treating physician, and have their final WUS and microbiome samples collected.

See Section 26 (Study Schedule) for weekly evaluations while on IP.

9.6 Early Treatment Termination

Subjects that terminate IP treatment prior to completing the planned treatment portion of the protocol will still be seen as per the study schedule and have an early termination visit at their 1-month post-RT follow-up appointment with their Radiation Oncologist. However, it the subject decides to withdraw from the study, no further information will be collected.

9.7 Off Study

Subjects will be considered off study after their 1-month post-RT follow-up appointment with their Radiation Oncologist.

10.PHARMACOKINETIC STUDIES

Not applicable.

11.DATA AND SAFETY MONITORING PLAN

11.1. Identification of the DSMB obligated for oversight responsibilities:

The University of Arizona Cancer Center Data (UACC) and Data Safety Monitoring Board (DSMB) will provide ongoing oversight for this trial. UACC will act as the DSMB of record. The Clinical Trials Office (CTO) QA/QC team, the UACC DSMB's monitoring arm, will ensure operational integrity to study operations through regular monitoring visits. This study has been assigned institutionally as a Low Risk level by the UACC DSMB.

11.2. Identification of the entity obligated for routine monitoring duties:

Routine monitoring will be provided by the University of Arizona Cancer Center Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements.

The investigator will perform real time monitoring by reviewing UACC QA/QC periodic reports within 2 weeks of receipt. The PI and the study team will review data monthly to ensure patient safety and integrity of data.

All visits will be conducted according to the FDA principles described in the Code of Federal Regulations and following pertinent Guidance.

11.3. Monitoring progress and data review process:

Routine monitoring of subject data will be conducted at least quarterly by the UACC QA/QC Program.

The first routine monitoring visit will include at a minimum:

- Informed consent – 50% of cases enrolled;
- Subject eligibility – 10% of cases, up to two subjects;
- Data review – 10% of cases, up to two subjects.

All subsequent monitoring visits will consist of randomly selected subject cases based on current enrollment and include continuing review of previously selected cases, as applicable.

A monitoring visit report and follow-up letter will be completed approximately two weeks after the routine monitoring visit; a copy will be maintained in the study file. The monitor will request additional source documentation, clarification, information, or corrections to the CRF and/or regulatory records from the Clinical Research Coordinator (CRC) or other applicable staff responsible for the study and resolution of queries/findings. Documentation of such a request will be maintained with a copy of the monitor's visit report for follow-up at the next monitoring visit. Electronic records will be available in the institutional database or provided by the QA/QC Program staff.

The Principal Investigator will ensure the accuracy, completeness, legibility, and timeliness of the data reported in the Case Report Form (CRF), or other acceptable data formats. Source documentation supporting the study data should indicate the subject's participation in the trial and should document the dates and details of study procedures, adverse events, and patient status.

Case report forms, including but not limited to the inclusion/exclusion criteria form, adverse event and serious adverse event forms, and rinse compliance forms will be completed via the institution database, Redcap. Trials using paper CRFs will have the data entered with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All subject forms and study files will be stored in a secure area limited to authorized staff.

Note: Routine monitoring of regulatory documents will be conducted at least annually by the UACC QA/QC Program.

The Radiation Oncology Staff will generate Case Report Forms (CRFs) for this study. Entries made in the CRF must be either verifiable against source documents, or have been directly entered into the CRF, in which case the entry in the CRF will be considered as the source data. The source data parameter to be verified and the identification of the source document must be documented. Corrections to CRFs and source data will be made only by authorized members of the study staff, clearly entered, initialed, and dated. The Investigator will sign a final CRF to indicate that, to his/her knowledge, they are complete and accurate. If further changes are made after this, the Investigator will be made aware of the corrections and his/her approval will be documented by re-signing.

As well, the study may be audited by regulatory agencies at any time. applicable regulatory agencies, and/or applicable ethical review boards with direct access to the original source documents.

The following topics are planned to be reviewed during each visit. Additional subject matter as appropriate will also be reviewed.

Initiation Visit: The initiation visit will be conducted soon after initiation of the Study.

1. Protocol
2. Credentials of key personnel
3. Facility readiness
4. Study products storage
5. Approval by the appropriate IRB
6. All study forms, including the informed-consent form and case report forms
7. Eligibility criteria
8. Randomization of subjects

Mid-study Visit: The mid-study visit will be scheduled after enrollment of about 60% of the total number of subjects.

1. Any change in key personnel
2. Workflow of the study

3. Informed consent form for subjects enrolled after the initiation visit
4. Eligibility criteria for subjects enrolled after the initiation visit
5. Protocol violations and deviations, if any
6. Any changes in the facility
7. Budget and payments
8. Subject dropouts
9. Adverse events reporting, if any

Closeout Visit: The closeout visit will be conducted after completion the Study.

1. Any change in key personnel
2. Informed consent form for subjects enrolled after the mid-study visit
3. Eligibility criteria for subjects enrolled after the mid-study visit
4. Protocol violations and deviations, if any
5. Any changes in the facility
6. Subject dropouts
7. Case report forms for all subjects
8. Corrections of data by authorized personnel
9. Adverse events reporting, if any
10. Completeness of all site source documents
11. Final accountability of all IP

11.4 Process to implement study closure when significant risks or benefits are identified:

If a Common Terminology Criteria for Adverse Events (CTCAE Version 5.0) grade 5 toxicity occurs that is more likely than not, directly related to the IP or placebo rinse, the study will be discontinued.

11.5 Description of adverse events and reporting procedures:

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

Adverse events will be recorded on the UACC adverse events record form and reviewed by the Principal Investigator. For this study we will focus on collecting the AEs that may be related to the investigational product and/or radiation therapy/chemotherapy with specific focus on generalized weakness, skin reaction, weight loss, dry mouth, sore throat, dysphagia, anorexia, dysgeusia, voice alteration, and neck edema.

All adverse events will be classified using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 and will address:

- Grade

- Relationship to study drug (not related, unlikely, possible, probable, definitely)
- Relationship to radiation and/or chemotherapy (not related, unlikely, possible, probable, definitely)
- Causality other than study drug (disease related, concomitant medication related, intercurrent illness, other)
- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, held, dose reduced, discontinued, medication given)

SERIOUS ADVERSE EVENTS
A serious adverse event (SAE) is any untoward medical occurrence that at any dose:
1) Results in death;
2) Is life-threatening;
3) Requires in-patient hospitalization or prolongation of an existing hospital stay;
4) Results in disability persistent or significant disability/incapacity, or:
5) Is a congenital anomaly/birth defect.

Note: A SAE may also be an important medical event, in the view of the investigator that requires medical or surgical intervention to prevent one of the outcomes listed above.

All serious adverse events, regardless of attribution, and any deaths will be reported within 24 hours of notification of the event to the sponsor and, if applicable, any collaborating entity. All serious adverse events and any deaths will be reported to the DSMB and to the University of Arizona Human Subjects Protection Program per the guidelines set forth in University of Arizona Cancer Center Data and Safety Monitoring Board Charter, Table 5: Adverse Event Reporting.

All submitted serious adverse events will be processed by the DSMB Coordinator monthly for initial trend analysis and then reviewed by the DSMB Chair. The assigned QA/QC Monitor will review the SAE reporting process to confirm reporting requirements are met.

11.6 Plan for assuring data accuracy and protocol compliance:
Routine study activity and safety information will be reported to the DSMB on a quarterly basis, or more frequently if requested. These reports will include:
- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies.

Data, safety and study progress will be reported to:

- Human Subjects Protection Program (IRB) at least annually;
- Sponsor (if applicable) at least every six months.

11.7 Identification of the sponsor or funding agency, as applicable:
The PI will immediately notify, in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study. A copy of this correspondence will also be forwarded to the DSMB and the Scientific Review Committee (SRC).

12. ADDITIONAL SAFETY REPORTING REQUIREMENTS

Serious adverse events will be reported to the Data Safety Monitoring Board as well as the institutional IRB within 24 hours of notification of the event to the PI. Serious adverse events will be reported using the FDA MedWatch form to inform the DSMB, and Report information that is Potentially Problematic to the institutional IRB.

13. QUALITY ASSURANCE MEASURES

Per the UACC DSMB Charter, Internal *Ad Hoc* audits may be performed on any UACC clinical trial if identified for audit, the audit will be conducted by an identified audit team per the UACC DSMB Charter. A QA/QC representative will coordinate the audit team functions and a written audit report will be provided to the principal investigator and the DSMB.

14. RECIST CRITERIA

Not applicable

15. REMOVAL OF SUBJECTS

Subjects have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution. If this occurs, the investigator, or designee, is to discuss with the subject the safe and appropriate processes for discontinuation from the investigational product, intervention or device.

Subjects that wish to discontinue active IP treatment/intervention/device may elect to continue with the other protocol required assessments. The investigator should discuss with the subject the options for continuation of the study schedule of assessments (i.e. blood work, scans, physical exams, diaries) and collection of data, including endpoints and adverse events.

The investigator or designee must document the change in status of the subject's participation in the study and as applicable, the level of follow up that is agreed to

by the subject (i.e. agrees to follow up exams, adverse event review, phone contact, but not to further treatment and/or procedures).

Subject withdrawal of consent for a study indicates that the subject does not wish to receive further protocol required therapies or procedures, and the subject does not wish to, or is unable to continue further study participation. Subject data only up to the time when consent is withdrawn will be included in the analysis of the study.

16. STATISTICAL CONSIDERATIONS

The primary statistical analysis will compare the proportion of patients who develop severe OM (≥WHO Grade 3) in patients randomized to ClōSYS Oral Rinse versus placebo. Analysis will be performed by estimating the proportion with exact 95% binomial confidence interval for each group separately. Statistical testing will use a chi-square test. As an alternative, Fisher's Exact Test will be used if the minimum expected value is < 5.

The secondary statistical endpoint of time to onset of severe OM will be tabulated and the median week to onset will be computed for each group. Comparison will be performed using a Wilcoxon Rank Sum Test (as the values are unlikely to be normally distributed). Duration of severe OM (number of weeks) will be analyzed similarly. The proportion of patients in each group with RT interruption will be tabulated and compared using a chi-square test.

The longitudinal measurements of the OMWQ and PRO-CTCAE, microbiome components and pro-inflammatory cytokines will be compared between groups using a linear mixed-effects model to account for the correlation among the measurements within an individual.

To justify the proposed sample size, we assume that 9/10 (90%) of the placebo control patients will experience severe OM within the 6-week period. Ten patients per group will allow us to detect a decrease to 40% (4/10) in the patients randomized to ClōSYS® Unflavored Oral Rinse with 81% statistical power (assuming a one-sided alpha of 0.05).

17. ANALYSIS

17.1 Safety Analysis

Given the pilot nature of this study and limited number of participants, there will be no formal planned interim safety analysis unless an SAE is documented and thought more likely than not directly related to the IP. We will conduct a safety analysis if deemed necessary upon review of the SAE by the DSMB. Otherwise safety analysis will be performed upon the conclusion of the study.

17.2 Efficacy Analysis

Efficacy analysis will be conducted at the completion of the study.

17.3 Interim Analysis

None planned.

18. REGULATORY OBLIGATIONS

18.1 Informed Consent

Before a subject's participation in the clinical study, the PI or identified designee is responsible for obtaining written informed consent from the subject after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol specified procedures, investigational product, intervention or device are administered or initiated.

18.2 Institutional Review Board

A copy of the protocol, proposed ICF, and all other applicable subject information will be submitted to the IRB for written approval. A copy of the written approval of the protocol and ICF must be on file at the institution before recruitment of subjects into the study.

The investigator is responsible for obtaining IRB approval/renewal at least annually throughout the duration of the study. Copies of the investigator's reports and the IRB continuance of approval must be on file at the institution.

The investigator must submit study information to the IRB as required by all applicable guidelines and requirements. The investigator will obtain IRB approval for subsequent protocol amendments; except changes to eliminate an immediate hazard to study subjects, and changes to the informed consent document from the IRB prior to implementation.

The investigator will notify the IRB of deviations from the protocol or serious adverse events occurring at the site and other serious adverse event reports occurring at or received from participating centers as applicable for multi-center trials following the IRB policies and procedures.

19. ADMINISTRATIVE PROCEDURES

19.1 Investigator Responsibilities

The PI will conduct this study in accordance with the current International Conference on Harmonization (ICH) guidance, the Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, FDA regulations, local IRB and legal requirements.

19.2 Data and Safety Monitoring Board Protocol Review

Initial DSMB protocol review will be conducted prior to SRC and IRB submissions.

Any protocol revision or amendment that includes a potential change to any section of data and safety monitoring plan must be reviewed and approved by the DSMB ***prior to*** **the protocol amendment submission to the IRB**.

19.3 Multicenter Trials

Not applicable. This is a single center trial.

20. SUBJECT CONFIDENTIALITY

The principal investigator will ensure that the subject's confidentiality is maintained in compliance with Federal regulations, the International Conference on Harmonization (ICH), and Good Clinical Practice (GCP) Guidelines.

Oversight entities and/or regulatory authorities will be permitted direct access to review the subject's original medical records, electronic medical records or certified copies for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

21. STUDY DOCUMENTATION AND ARCHIVE

The investigator will maintain a list of appropriately qualified persons to whom he/she has delegated study duties. All persons authorized to make entries and/or corrections on CRFs will be included on the Delegation of Responsibilities Form.

Source documents, data, and records from which the subject's CRF data are obtained include, but are not limited to, hospital records, clinical/office/research charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. Source data will include information necessary for the reconstruction and evaluation of the trial.

The principal investigator or sponsor-investigator is responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation as required per ICH Guidelines. This can be accomplished by the PI, through the site's standard operating procedures and/or the institutions infrastructure.

The investigator will follow ICH Good Clinical Practice Guidelines and the Code of Federal Regulations for records and record retention.

22. DATA

Applicable data as specified as required in the protocol will be reported/submitted in the case report form (CRF). Data reported in the case report forms that are derived from source documents must be consistent with the source documents or the discrepancies must be explained**.** In addition, data will be entered into

RedCap which is currently being used by many BUMC-T departments for their study data bases.

Additional procedures and assessments may be performed as the institution's standard of care; however, these data should remain in the medical records and should not be provided as part of the clinical study data unless it pertains to a serious adverse event.

The investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational product/intervention/device, or employed as a control in the investigation.

At the end of the study, the original samples will be destroyed. Remaining extracted DNA samples will be preserved for further sequencing. All remaining biospecimens will be destroyed after 6 years.

23. PROTOCOL DEVIATIONS

The investigator will conduct the study in conformance with this protocol, generally accepted standards of Good Clinical Practice and all applicable federal, state and local laws, rules, and regulations.

Approvals or waivers for protocol deviations will be obtained from the IRB ***prior to*** occurring, except changes to eliminate an immediate hazard to study subjects. If immediate verbal approval is obtained, it will be documented by the research staff obtaining the approval and followed by a written protocol deviation form per the site standard operating procedures. The sponsor or the sponsor-investigator will sign the Protocol Deviation (Waiver) Approval Form or other similar document. The original will be filed in the regulatory binder and a copy will be placed in the subject's research file.

24. KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

25. COMMON TOXICITY CRITERIA (CTCAE)

CTCAE version 5.0

Expedited Reporting Requirements for Adverse Events

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312) NOTE: Investigators MUST immediately report to the sponsor ANY Serious Adverse Events, whether or not they are considered related to the investigational agents(s) intervention (21 CFR 312.64) An adverse event is considered serious if it results in ANY of that following outcomes: 1. Death 2. A life-threatening adverse event 3. An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours 4. A persistent or significant incapacity or substantial disruptive of the ability to conduct normal life functions 5. A congenital anomaly birth defect 6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition (FDA 21 CFR 312.32, ICH E2A and ICH E6).
Toxicity will be scored using CTCAE Version 5.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 5.0 can be downloaded from the CTEP homepage (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 5.0. The investigator must evaluate each adverse experience for its relationship to the test drug/treatment and for its seriousness.

26. STUDY SCHEDULE

Test or Procedure	Baseline (Prior to first treatment)	Week 1 (±1 day)	Week 2 (±1 day)	Week 3 (±1 day)	Week 4 (±1 day)	Week 5 (±1 day)	Week 6 (±1 day)	Week 7 (if applicable) (±1 day)	1-month post-RT follow-up visit (+21 days)	
Informed consent	X									
Medical history, BMI and tobacco history 1	X									
Concurrent meds 2	X		X		X		X		X	
Physical Exam including dental eval and photos 3	X	X	X	X	X	X	X	X	X	
Hct 4	X								X	
Malignancy info 5	X									
Registration & Randomization	X									
OMWQ and PRO CTCAE 6	X		X		X		X		X	
WUS and Microbiome Collection 7	X		X		X		X		X	
Dispense IP/placebo	X	X	X	X	X	X	X	X		
Adverse Event Check 8		X	X	X	X	X	X	X	X	
Rinse Diary and Observational Form 9	X	X	X	X	X	X	X	X	X	

Test or Procedure	Baseline (Prior to first treatment)	Week 1 (±1 day)	Week 2 (±1 day)	Week 3 (±1 day)	Week 4 (±1 day)	Week 5 (±1 day)	Week 6 (±1 day)	Week 7 (if applicable) (±1 day)	1-month post-RT follow-up visit (+21 days)	
Collect Unused IP/placebo 10		X	X	X	X	X	X	X	X	
Documentation of RT treatment interruptions									X	

1= Complete medical history will include demographics and will be performed within 90 days of registration. Tobacco history will be collected using the Tobacco Assessment Questionnaire.
2= Review medications including usage of any chlorine dioxide, antimicrobials, or corticosteroids within last 7 days prior to registration. Additionally, will review usage of honey, baking soda and salt rinses, magic mouthwash, viscous lidocaine, doxepin solution, chlorhexidine, and other antimicrobials, corticosteroids, anti-inflammatories, etc during study period. Only medications related to either cancer therapy or treatment of a related adverse events will be collected. No other concomitant medications will be tracked.
3= All PEs to include vitals (BP, pulse, temperature, weight), KPS scoring, and OM grading per WHO scoring criteria (see section 7.1)
4= Hematocrit within 90 days of study registration
5= Confirmation of cytologic or histologic evidence of HNC and documentation of stage
6= Oral Mucositis Weekly Questionnaire and Patient Reported Outcomes Common Terminology Criteria for Adverse Events
7= Whole Unstimulated Salivary and Microbiome Collection (see section 7.1)
8= Common Terminology Criteria for Adverse Events (CTCAE) v.5 will be used for grading of generalized weakness, skin reaction, weight loss, dry mouth, sore throat, dysphagia, anorexia, dysgeusia, voice alteration, and neck edema
9= Instruct patients on rinse diary to include time, date, and duration of rinses and to note any potential effects thought related to rinse on observation form. Will dispense rinse diary and observation form at baseline visit (prior to the start of week 1 of RT). Will collect and replace both the rinse diary and observation form each week during OTVs
10= Collect and record by volume amount of unused IP/product

27. Tobacco Assessment Questionnaire

TOBACCO **ASSESSMENT – BASELINE**

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

☐ Yes
☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

☐ Yes In total, for about how many years?_____ If less than 1, write "1."
☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
☐ No

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __ (MM/DD/YYYY)

Investigator Name (please print) _____

28. OMWQ-HN and PRO-CTCAE

Study ID: _____ **Date of Completion:** _____

Type of Visit: _____

OMWQ-HN Questionnaire

Please complete this questionnaire based on how you have felt in the PAST WEEK:

1. On a scale of 1 to 7, with 1 indicating very poor and 7 indicates excellent, how would you rate the following:

   Overall Health: 1 2 3 4 5 6 7

   Overall Quality of Life: 1 2 3 4 5 6 7

2. On a scale of 0 to 4, with 0 being no soreness and 4 being extreme soreness:

   How much mouth and throat soreness did you experience: 0 1 2 3 4

If you answered 0, you may skip the remaining questions on this form

If you answered 1, 2, 3 or 4, on the last question please complete the following:

3. On a scale of 0 to 4, with 0 indicating no limitations and 4 indicating you are unable to do the following activities in the **past week**:

   Sleeping: 0 1 2 3 4

   Swallowing: 0 1 2 3 4

   Drinking: 0 1 2 3 4

   Eating: 0 1 2 3 4

   Talking: 0 1 2 3 4

   Brushing your teeth: 0 1 2 3 4

4. On a scale of 0 to 10, with 0 indicating no pain or soreness and 10 indicating the worst pain or soreness imaginable, please rate the following for the **past week**:

   Overall mouth and throat soreness: 0 1 2 3 4 5 6 7 8 9 10

   Mouth pain: 0 1 2 3 4 5 6 7 8 9 10

   Throat pain: 0 1 2 3 4 5 6 7 8 9 10

   Completed by: _____ Date: _____/_____/_____

NCI PRO-CTCAE™ ITEMS

Item Library Version 1.0
English
Form created on 31 October 2018

As individuals go through treatment for their cancer they sometimes experience different symptoms and side effects. For each question, please check or mark an ☒ in the one box that best describes your experiences over the past 7 days...

1.	In the last 7 days, what was the SEVERITY of your DRY MOUTH at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

2.	In the last 7 days, what was the SEVERITY of your PROBLEMS WITH TASTING FOOD OR DRINK at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

3.	In the last 7 days, what was the SEVERITY of your DECREASED APPETITE at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did DECREASED APPETITE INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

4.	In the last 7 days, how OFTEN did you have NAUSEA?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your NAUSEA at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

5.	In the last 7 days, how OFTEN did you have VOMITING?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your VOMITING at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

6.	In the last 7 days, what was the SEVERITY of your FATIGUE, TIREDNESS, OR LACK OF ENERGY at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did FATIGUE, TIREDNESS, OR LACK OF ENERGY INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

NCI PRO-CTCAE™ ITEMS

Item Library Version 1.0
English
Form created on 31 October 2018

7.	In the last 7 days, how OFTEN did you feel ANXIETY?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your ANXIETY at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did ANXIETY INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

8.	In the last 7 days, how OFTEN did you FEEL THAT NOTHING COULD CHEER YOU UP?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your FEELINGS THAT NOTHING COULD CHEER YOU UP at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did FEELING THAT NOTHING COULD CHEER YOU UP INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

9.	In the last 7 days, how OFTEN did you have SAD OR UNHAPPY FEELINGS?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your SAD OR UNHAPPY FEELINGS at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did SAD OR UNHAPPY FEELINGS INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

NCI PRO-CTCAE™ ITEMS

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English
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Do you have any other symptoms that you wish to report?	
○ Yes	○ No

Please list any other symptoms:

1.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
2.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
3.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
4.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
5.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

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30. APPENDICES

30.1 Appendix A: Whole Unstimulated Saliva (WUS) Collection and Analyses Standard Operating Procedures

To collect WUS, each patient will be asked to refrain from eating, drinking, smoking, and oral hygiene procedures for at least 1 h before saliva collection. Patient will be asked to rinse with plain water prior to resting for 5 minutes with head tilted forward and no swallowing. Pooled saliva in the floor of mouth will then be expectorated every 60 seconds into a dedicated collection device (SpeciMAX™ Saliva Collection; Thermo Fisher) for a total of 5 minutes, as previously described(1-3). Protease inhibitor cocktail has to be added immediately after sample collection to minimize protein degradation. WUS samples will be individually placed in labeled containers with the patient's unique sequential subject identification number and week of collection. These samples will be transferred to the Microbiome Core on the same day, aliquoted and stored at -80°C in a secure location until utilized for salivary cytokine analysis. The samples will be destroyed after analyses are complete. Remaining extracted DNA samples will be preserved for further sequencing. Six years after the samples are collected all remaining biospecimens will be destroyed.

WUS samples will be centrifuged at at 2600x g for 15 min to remove cell pellets and debris, and supernatant divided into 100 μL aliquots to reduce freeze thaw cycles. Multiplexed quantification of pro-inflammatory cytokines IL-1β, IL-6, IL-8/CXCL8, and TNFα will be conducted with customized xMAP Human High Sensitivity T Cell MAGNETIC Bead Panel HSTCMAG-28SK-04 (Sigma-Millipore) using MagPix instrument (Luminex) in the Microbiome Core laboratory. Three serial dilutions of each sample will be made in duplicate and xMAP assays will be performed per the manufacturer's protocol. In cases where severe contamination of WUS samples with blood is observed, samples will be cleared with HemogloBind™ (Biotech Support Group catalog H0145-05), according to the manufacturer's protocol.

Reference:

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30.2 Appendix B: Microbiome Collection and Analyses Standard Operating Procedures

Microbiome collection will be performed by gently swabbing over areas of visible mucositis, or areas of high dose radiation where mucositis would be most likely to present, utilizing disposable medical sterile swabs (OMNIgen ORAL OMR-110, DNA Genotek). Microbiome samples will be individually placed in labeled containers with the patient's unique sequential subject identification number and week of collection. These samples can be stored at room temperature for at least one month and then will be stored in a secure location at the Microbiome Core, at -80°C until utilized for microbiome analyses. An aliquot of WUS collected as described in Appendix A will be used for microbiome analysis. The samples will be destroyed after analyses are complete. Remaining extracted DNA samples will be preserved for further sequencing. Six years after the samples are collected all remaining biospecimens will be destroyed.

The analyses will be performed as per institutional technique:

DNA isolation and the V4 of the 16S rRNA gene amplicon generation:

DNA is isolated from oral swabs using the DNeasy PowerSoli Pro Kit (Qiagen, USA) with the bead beating step following manufacturer's protocol to ensure isolation of DNA from gram positive and gram negative organisms including difficult to isolate species such as those in the genus Bifidobacterium. The hypervariable V4 region of the 16S rRNA gene will be amplified from each sample using barcoded reverse primers (806R) and common forward primer (515F). Both reverse and forward primes are extended with the sequencing primer pads, linkers, and Illumina adapters (Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal. 2012;6(8):1621-4), and with MyFi™ Mix (Bioline Meridian, Cat No. BIO-25050). The PCR will be performed on LightCycler 96 (Roche) in the final volume of 40μL. Amplicons will be quantified using Quant-It PicoGreen dsDNA Assay kit (ThermoFisher Scientific, Cat No. P7589), according to the manufacturer's protocol. Equal amount of amplified DNA (240 ng) from each sample will be pooled and cleaned using UltraClean PCR Clean-Up Kit (MoBio, Cat No. 12500).

16S rRNA gene sequencing:

Pooled amplicons were diluted and denatured with 0.2N NaOH to the final DNA concentration in the library of 7.5-8.0pM. The library was sequenced at the Microbiome Core at the Steele Children's Research Center, University of Arizona, using MiSeq platform (Illumina) and custom primers (Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal. 2012;6(8):1621-4). Due to the limited sequence diversity among 16S rRNA amplicons, 5% of the PhiX Sequencing Control V3 (Illumina, Cat No. FC-110-3001) made from phiX174, will be used to spike the library to increase diversity. Paired-end sequencing, 150 cycles each (PE150) will be used to sequence the amplified fragments.

Sequence analysis:

The raw sequencing data will be demultiplexed using the *idemp* script (https://github.com/yhwu/idemp). Filtering, dereplication, chimera identification, and merging of paired-end reads will be performed with *dada2* R package (Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13(7):581-3). The Amplicon Sequence Variants (ASVs) taxonomy will be assigned using the Ribosomal Database Project (RDP) classifier (Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7) against SILVA database release 138 (or newer if available, Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590-6).
Taxonomic richness and evenness (Shannon and Simpson indices) will be calculated and statistical significance within in each experiment was calculated using Kruskal-Wallis rank sum test followed by Dunn's multiple comparison test with Bonferroni correction (dunn.test R package). Differences in microbial communities will evaluated using non-metric multidimensional scaling (NMDS) ordination analysis on Bray-Curtis distances followed by permutational multivariate analysis of variance (PERMANOVA) to analyze the contribution of different metadata variables to microbial communities composition dissimilarities. Also, to investigate and visualize the association between metadata variables and their effect on the species distribution pattern, redundancy analysis will used in *vegan* R package. The obtained results will be visualized with a *ggplot2* (ver 3.3.2) package and with *heatplus* (ver. 3.11) R package. Changes in microbiome will be correlated with collected bio-medical variables using *stats* R package and visualized using *cor* package.