Pilot Study of Mesenchymal Stromal Cells in Patients with Xerostomia after Radiation Therapy for Head and Neck Cancer

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Protocol Number: UW20025
IRB Protocol Number *2020-1290*
Principal Investigator: Randall Kimple MD, PhD

IND #: 23026
IND Sponsor: Jacques Gallipeau MD

Protocol Version History

Protocol Version	Version Date	Summary of Revisions Made	Rationale
1.0	06/22/2020	Initial version	
2.0	09/08/2020		Modifications made in response to FDA review and IND Application
3.0	10/26/2020		Modifications made in response to HS-IRB review of initial IRB application
4.0	10/7/2021	Change to “Pilot” Study	
6.0	5/2/2022	Change to protocol	Modification made after patient #1 injection for study calendar, updated needle gauge from 23 to 22
7.0	3/27/2023	Change to protocol	A second injection will be offered for injection into each subject’s contralateral submandibular gland
8.0	9/28/2023	Change to protocol	FDA requested changes

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1.0 STATEMENT OF COMPLIANCE

I confirm that I have read this protocol. I will comply with the IRB-approved protocol, and applicable regulations, guidelines, laws, and institutional policies.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitment.

Name	**Signature**	**Date**
Randall Kimple MD, PhD Principal investigator	_____	

2.0 LIST OF ABBREVIATIONS

AE	Adverse Event
BM	Bone marrow
BMI	Body mass index
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CRF	Case Report Form
CRT	Chemoradiation therapy
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management Software
DLT	Dose limiting toxiticy
DSMC	Data & Safety Monitoring Committee
DSMP	Data & Safety Monitoring Plan
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
ELISA	Enzyme-linked immunosorbent assay
EMR	Electronic medical record
EpCAM	Epithelial cellular adhesion molecule
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GDNF	Glial cell line-derived neurotrophic factor
HIPAA	Health Insurance Portability and Accountability Act
HNC	Head and neck cancer
IB	Investigator's Brochure
ICTR	Institute for Clinical and Translational Research
IMP	Investigational Medicinal Product
IND	Investigational New Drug Application
IRB	Institutional Review Board
MAD	Maximal Administered Dose
MDADI	MD Anderson Dysphagia Index
MHC	Major histocompatibility complex
MNC	Mononuclear cells
MOP	Manual of Procedures
MSC	Mesenchymal Stem (or Stromal) Cells
MTD	Maximal Tolerated Dose
NCI	National Cancer Institute
NED	No Evidence of Disease
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OnCore	Online Collaborative Research Environment
PACT	Program for Advanced Cell Therapy
PHI	Protected Health Information
phOL	Pooled human platelet lysate
QoL	Quality-of-life
PI	Principal Investigator
RT	Radiation therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SMC	Safety Monitoring Committee
SMP	Study Monitoring Plan
UP	Unanticipated Problem
US	Ultrasound
UWCCC	University of Wisconsin Carbone Cancer Center
VAS	Visual analogue scale
XeQOL	University of Michigan Xerostomia Quality of Life

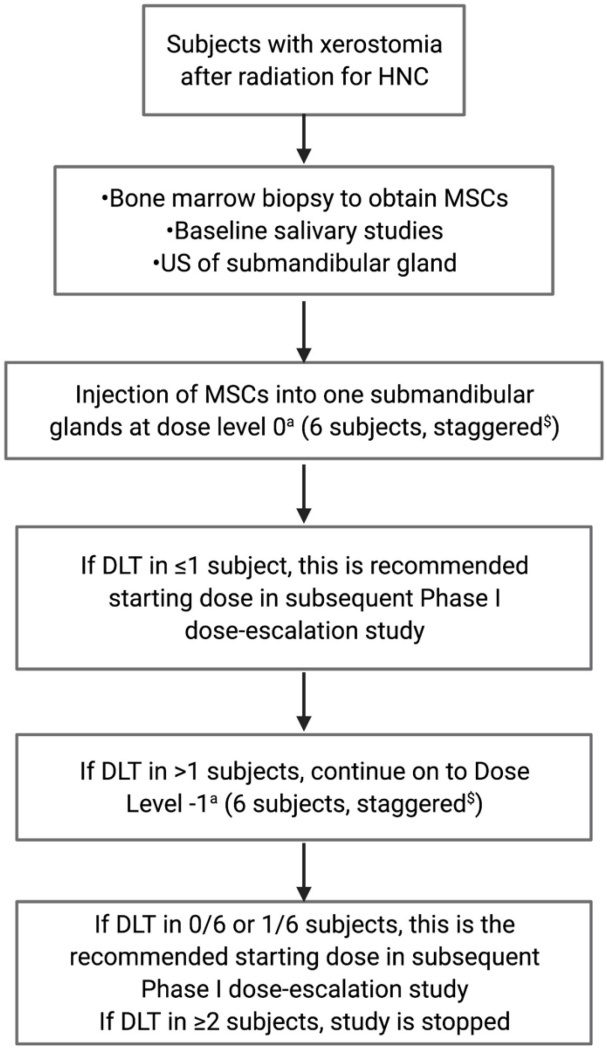
3.0 STUDY SUMMARY

3.1 Synopsis

Full Title	Pilot Study of Mesenchymal Stromal Cells in Patients with Xerostomia after Radiation Therapy for Head and Neck Cancer
Short Title	Mesenchymal stromal cells (MSCs) for xerostomia
Protocol Number	UW 20025
Number of Sites	This is a single-site study conducted at the University of Wisconsin
ClinicalTrials.gov Identifier & Summary	To be submitted to clinicaltrials.gov following FDA and UW SMPH regulatory approvals.
Phase	Pilot
Main Inclusion Criteria	• History of histological diagnosis of head and neck cancer (HNC) that was treated with radiation therapy and clinically or radiologically NED • Xerostomia, defined as patient reported salivary function (pre-treatment) ≤ 80% of healthy (pre-radiation) • ≥ 18 years of age, ≤ 90 years of age. • Patients ≥ 2 years from completion of radiation therapy for HNC • Karnofsky ≥ 70, patient eligible for bone marrow aspirate with wakeful anesthesia • Willing and able to give informed consent • Radiographically confirmed submandibular gland(s)
Main Exclusion Criteria	• Patients with one submandibular gland • Salivary gland disease (i.e., sialolithiasis)
Objective(s)	<u>Primary Objective</u> • To evaluate the safety and tolerability of MSCs for subjects with xerostomia after radiation therapy for HNC. <u>Secondary Objectives</u> • To evaluate the efficacy of MSCs for treatment of xerostomia and salivary hypofunction via quality-of-life (QoL) questionnaires, salivary amount, and salivary compositional analysis. • To assess the imaging characteristics in HNC patients after MSC injection using ultrasound. • To assess the feasibility of a future Phase 1 dose-escalation study.
Endpoints	<u>Primary Endpoint</u> • Recommended starting dose of MSCs for a Phase I dose-escalation study, determined by the proportion of subjects experiencing dose-limiting toxicity (DLT), where DLT is defined as: submandibular pain > 5 on a standard 10-point pain scale of 0-10 at 1-month after MSC injection OR any serious AE OR any of the selected toxicities listed in section 9.7 within one-month post-injection. <u>Secondary Endpoints</u>

	• Salivary function and subjective dry mouth, determined by mean overall and individual salivary amounts, compositional analysis, and QoL xerostomia survey scores at 1, 3, 6, 12, and 24 months. • Shear wave velocity with acoustic radiation force impulse, measured by ultrasound (US) of submandibular glands at 3, 6, and 12 months • Feasibility for a future Phase 1 dose-escalation study will be based on drop-out rate.
Study Design	This is a single-center pilot study designed to determine the safety and tolerability of autologous bone marrow-derived MSCs in patients with xerostomia after undergoing radiation therapy for HNC.
IND Number	23026
Study Intervention	Adults with xerostomia after radiation therapy for HNC will undergo one-time injection of 10 (8 – 12) x 10^6 MSCs into one submandibular gland. A second injection of 10 (8 – 12) x 10^6 MSCs will be offered for injection into each subject's contralateral submandibular gland as no DLT's were noted in any of the study participants (sub study).
Total Number of Subjects	A total of 6-12 subjects will be enrolled from 1 study site.
Study Population	Male and females aged 18 to 90 years with xerostomia 2 years after undergoing radiation therapy for HNC.
Statistical Methodology	The pilot trial with 12 patients total enrolled will allow us to estimate 95% CI of adverse events (e.g. 1 of 6 patients results in a 95% CI of 0-64%) . All patients will be analyzed for adverse events.
Estimated Subject Duration	The duration of the study for each subject is approximately 24 months.
Estimated Enrollment Period & Study Duration	Study enrollment and follow-up will occur over 6 months with the total expected duration of the trial to be 30 months.

3.2 Schematic of Study Design



Dose Level[a]	MSC IMP[b,c]
-1	5×10^6 injected into submandibular gland on Day 1
0	10×10^6 injected into one submandibular gland on Day 1

[a] Dosing to start at level 0
[b] Variance of ± 20% cell dose allowable
[c] Subjects at dose level 0 must have two submandibular glands and will have only one submandibular gland injected
[$] Staggered enrollment for subjects, with a minimum of 14 days between MSC injection in a subject and MSC injection in a subsequent subject, during which time there are no SAEs and no AEs ≥ grade 3 that are probably or definitely attributable to MSC injection

4.0 KEY ROLES

The following is a list of all key personnel and roles:

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Data and Safety Monitoring Committee	UWCCC Carbone Cancer Center Data and Safety Monitoring Committee Email: DSMC@uwcarbone.wisc.edu

5.0 INTRODUCTION

5.1 Radiation-Induced Xerostomia and Salivary Glands Background

Xerostomia, or dry mouth, is a common side effect of head neck radiation. Older, 3D conformal radiation results in long-term xerostomia in up to 80% of patients. The use of intensity modulated radiation allows the sparing of salivary glands but still results in xerostomia in > 40% of patients[1]. Patients with radiation-induced xerostomia can develop dental caries, impaired swallowing ability, difficulty speaking, and diminished taste. These associated symptoms can have a major negative impact on the overall quality of life. Current treatment options for radiation-induced xerostomia are generally supportive in nature. Patients are encouraged to increase water consumption, consume specially prepared food, utilize salivary substitutes or attempt to stimulate salivary production through the use of parasympathomimetic drugs, organic acids, chewing gum, or sugar-free mints[2-5]. Most of these supportive interventions do not reverse xerostomia and are palliative in intent.

Salivary glands are composed of two types of epithelial cells. The first type is acinar cells which comprise approximately 75% of the glands, secrete exocrine proteins, and are the site of water movement[6]. The second is duct cells which are water impermeable and NaCl absorbing[6]. Radiation results in the death of most acinar cells. Adult stem cells are the ultimate source for replenishment of salivary gland tissue, including acinar cells necessary for saliva production. The ductal compartment in the salivary gland has been identified as the location harboring rare ductal epithelial cellular adhesion molecule (EpCAM)+ cells which express nuclear b-catenin, indicating active Wnt signaling as a key driver of adult salivary gland stem cells, allowing extensive expansion and enabling restoration of function[7]. An important role for glial cell line-derived neurotrophic factor (GDNF)-driven salivary stem cell function has also been defined[8].

5.2 Current Standard of Care

Current treatment options for radiation-induced xerostomia are generally supportive in nature. Patients are encouraged to increase water consumption, consume specially prepared food, utilize salivary substitutes or attempt to stimulate salivary production through the use of parasympathomimetic drugs, organic acids, chewing gum, or sugar-free mints[2-5]. Most of these supportive interventions do not reverse xerostomia and are palliative in intent. All interventions including gum and salivary substitutions will be allowed while on trial.

5.3 Mesenchymal Stem Cells

Marrow-derived mesenchymal stem cells (i.e., mesenchymal stromal cells, MSCs) are a viable cell-based therapy for xerostomia. MSCs are a cellular product that can be derived from bone marrow and propagated *ex vivo* using established, clinically applicable methods[9]. According to the International Society of Cell Therapy guidelines, MSCs must be plastic-adherent when maintained in standard culture conditions, express major histocompatibility complex (MHC) class I molecules as well as surface CD105, CD90, CD73 and CD44, lack expression of hematopoietic markers CD45, CD34, CD11b, and CD19, and retain the ability to differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*[10,11]. MSCs are also characterized by a unique transcriptome[12].

MSCs have an ability to promote tissue healing in a variety of injurious settings[13]. At one time, it was thought that this was attributable to their stem-progenitor-like character – that is, MSC-derived cells might directly repopulate damaged organs. However, the small number of MSCs recovered at tissue target sites contradicts this hypothesis and instead supports the alternative hypothesis that MSCs

limit cell death secondary to tissue inflammation and providence of tissue promoting morphogens[13]. Thus the healing effects are more likely from the MSC secretome, which has broad immunomodulatory and trophic activity[14,15]. In the gut, MSCs deploy niche cell properties with the ability to sustain LGR5+ epithelial stem cells via expression of Wnts, FGFs and R-spondins[16]. Akin to intestinal stem cells, the self-renewal ability of salivary gland stem cells is dependent on extrinsic niche signals that could be provided by exogenous administration of MSCs.

After expansion in the laboratory, MSCs are cryopreserved until clinically needed. This cryopreservation and thawing can result in cellular injury or senescence[17,18]. Senescent MSCs have less effective immunomodulation[17-19]. Stimulating the MSCs with interferon gamma prior to cryopreservation has been shown to prevent this immune dysfunctionality[18-20].

A review of preclinical studies on MSCs for xerostomia found five preclinical studies using mouse models, with 32%-200% increases in salivary flow rate after implantation of MSCs[21]. Three studies examined the effects of MSCs on radiation-induced xerostomia, with two of those studies using murine MSCs obtained from bone marrow[21]. The Coppes Lab has used salivary stem cells from non-irradiated HNC patients that are transplanted into immunodeficient irradiated mice to improve murine salivary function. There remain questions about the relevance of this work due to the atrophic nature of irradiated salivary glands in HNC patients[22]. Another group has found that after allogenic transplantation of bone marrow-derived stem cells there was an increase in saliva production, promotion of regenerative activity, and direct differentiation of donor stem cells into salivary epithelial cells[22,23]. We are currently undertaking FDA IND enabling studies to demonstrate the viability of MSCs taken from patients who have undergone radiation ± chemotherapy.

The first human clinical trial (MESRIX) of autologous adipose-derived MSCs to treat radiation-induced xerostomia had the results published early 2018[24]. In this Danish single-center, phase I/II, randomized, placebo-controlled, double-blinded clinical trial, 30 patients were randomized using a 1:1 ratio and received injected adipose-derived MSC or placebo to the submandibular glands. The dosing was based on prior murine studies and ranged from 2.2×10^6 to 4.5×10^7. No adverse events were detected. Unstimulated whole salivary flow rates significantly increased in the MSC-arm at one (33%; p=0.048) and four months (50%; p=0.003), but not in the placebo-arm, compared to baseline. The MSC-arm also had symptom scores significantly decreased on the xerostomia and VAS questionnaires, and significantly improved salivary gland functions of inorganic element secretion and absorption. This study did not examine the use of interferon gamma-stimulated MSCs. These data strongly support the feasibility and benefit of interferon gamma stimulated MSC autotransplantation for treating radiation therapy (RT)-induced xerostomia. Based on a search of clinicaltrials.gov in January 2020 there are currently no ongoing trials of MSCs for xerostomia in the US.

5.4 Rationale

MSCs have been repeatedly studied in subjects with malignancies without showing increased incidences of cancer[25,26]. Autologous MSCs have an immediate and obvious advantage to allogeneic cells since these are not susceptible to immune rejection, may lead to prolonged clinical effect, and can be repeatedly administered. Adipose and marrow derived MSCs share many functionalities, with marrow MSCs having a favorable bias towards niche functionalities considering the pivotal role in maintaining hematopoietic stem cells throughout life. Additionally, research shows a lower chance of an adverse event with a bone marrow (BM) biopsy as compared to liposuction to obtain adipose tissue - a 0.07% incidence of adverse events related to bone marrow biopsies, as compared to 1.36-8.6% adverse events related to liposuction[27-29]. Multiple preclinical studies have shown an increase in murine saliva production after MSC transplantation. The MESRIX trial studied adipose-derived MSCs

without characterizing their secretome profile and excluded patients with severe salivary gland hypofunction.

This trial will be a first-in-human clinical trial of bone marrow-derived, interferon gamma-activated MSCs for the treatment of radiation-induced xerostomia. In this pilot study, we will demonstrate the feasibility and safety of these MSCs and determine the recommended starting dose of interferon gamma stimulated MSCs for a Phase I dose-escalation study. We propose that autotransplantation of marrow-derived mesenchymal stromal cells (MSCs) in salivary glands post-RT or post-chemoradiation therapy (CRT) may provide an innovative remedy to treat xerostomia and restore quality of life.

5.5 Rationale for Injection of Second Submandibular Gland

We have achieved our primary objective of evaluating the safety and tolerability of injection of interferon gama stimulated MSCs into the submandibular gland. No patients have had a DLT within one month of injection. Indeed, only 3 patients reported pain (pain scale rating 1/10) with all 3 paitents having resolution of their pain within 4 days. There were no serious adverse events. Now that the safety of this dose level of MSCs has been shown, injection of the contralateral submandibular gland at the same dose level will be undertaken. We are opening a phase I clinical trial in 2023 in which patients undergo bilateral submandibular gland injection with interferon gamma stimulated MSCs. In order to provide the most benefit for patients, bilateral injection is recommended, as this will allow for the regeneration of both submandibular glands, which provide the majority of unstimulated saliva.

Additionally, the UW PACT has demonstrated stability of the cryopreserved MSC IMP after storage (see Appendix 19.4 for stability data). Each patient's MSC IMP was cryopreserved, thawed, culture-rescued, and formulated for injection in the already conducted pilot clinical study. The MSC IMP utilized for the second injection is composed of MSCs that were manufactured and cryopreserved at the same time as the MSCs for the first injection.

Each patient has cryopreserved autologous MSCs sufficient to allow for injection of the contralateral submandibular gland at dose level 0. We will utilize the cryopreserved MSCs for the contralateral gland injection, removing the need for another bone marrow aspiration and culturing of cells. The MSC IMP for the second injection will be identical to the first injection with the exception that these MSCs have been in cryopreservation longer.

6.0 THIS AMENDMENT WILL BE FORWARDED TO THE FDA FOR REVIEW PRIOR TO IMPLEMENTATION. STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the safety and tolerability of MSCs for subjects with xerostomia after radiation for HNC.	Recommended starting dose of MSCs for a Phase I dose-escalation study, determined by the proportion of subjects experiencing dose-limiting toxicity (DLT), where DLT is defined as: submandibular pain > 5 on a standard 10-point pain scale of 0-10 at 1-month after MSC injection OR any serious AE OR any of the selected toxicities listed in section 9.7 within one-month post-injection.
Secondary	

• To evaluate the efficacy of MSCs for treatment of xerostomia and salivary hypofunction.	• Salivary function and subjective dry mouth, determined by mean overall and individual salivary amounts, compositional analysis, and QoL xerostomia survey scores at 1, 3, 6, 12, and 24 months.
• To assess the imaging characteristics of submandibular gland after MSC injection.	• Shear wave velocity with acoustic radiation force impulse, measured by ultrasound (US) of submandibular glands at 3, 6, and 12 months.
• To assess the feasibility of a future Phase 1 dose-escalation study.	• Feasibility for a future Phase 1 dose-escalation study will be based on drop-out rate.

7.0 STUDY DESIGN

7.1 General Design

This single-center, open-label, non-randomized, non-placebo controlled, single-group assignment, pilot study is designed to determine the safety and tolerability of autologous bone marrow-derived MSCs in patients with xerostomia after undergoing radiation therapy for HNC. A total of 6- subjects will be enrolled at UW-Madison for unilateral gland injection (dose level 0). Subject accrual will occur over 0.5 years with the total duration of the trial expected to be approximately 2.5 years.

Following the completion of screening/baseline procedures, and written consent, eligible subjects will undergo bone marrow aspirate in order to obtain MSCs. MSCs will be prepared as described in Section 9.2.

The MSC IMP (dose level 0) will be injected into one submandibular gland under local anesthesia, in the gland that received the lowest radiation dose. Patients with only one submandibular gland will be ineligible. All subjects will be called by a study coordinator 3 days (+/- 2 days) after injection to assess pain and will have a phone visit with a physician 1 week (+/- 2 days) after injection during which the investigator will assess pain and ask about the area of injection regarding redness and/or swelling. All subjects will complete a pain diary with daily entries over the first month to record the occurrence and severity of pain using a 0-10 visual analog scale and occurrence and severity of other adverse events (e.g., redness, swelling, warmth, tenderness, rash, pruritis, nausea, vomiting, fatigue). Patients will also keep a log of all pain medications taken including both narcotic and non-narcotic medications (e.g. ibuprofen, acetaminophen, etc.) for the first month. Subjects will complete 5 follow-up visits over the course of 24 months – at 1, 3, 6, 12, and 24 months following the intervention. Salivary collection for analysis as well as QoL surveys will be obtained at these visits.

Additional Gland Injection: The 6 subjects will be offered optional contralateral submandibular gland MSC injection at dose level 0 upon completion. The primary objective (1 month of follow-up after injection of MSCs) without any DLTs has now occurred.

Subjects will also be offered injection of MSCs into the contralateral submandibular gland upon completion of the trial's primary objective (1 month of follow-up after injection of MSCs without any DLTs). The MSC IMP (dose level 0, from previously obtained bone marrow aspirate and culture) will be injected into the contralateral submandibular gland under local anesthesia. Secondary objectives will remain unchanged. Each subject will undergo salivary collection and completion of QoL forms just prior to second injection of MSC IMP. All subjects will complete follow up timepoints per the main study at 12 months (+/- 3 months) post second injection to analyze the benefit of the second injection by assessing secondary objectives including salivary amount and salivary QoL. This 12 month timepoint will allow for assement of durable improvement, while not prolonging the overall study duration. Additionally, subjects will be monitored for AEs post-injection. All subjects will be called by a

study coordinator or physican 3 days (+/- 2 days) and 7 days (+/- 2 days) after injection to assess pain, redness, or other AEs. Subjects will also be seen 30 days (+/- 10 days), 90 days (+/- 14 days), 6 months (+/- 28 days), 12 months (+/- 3 months) and 24 months (+/- 3 months) after second MSC injection for safety evaluation and blood collection to assess for AEs.

7.2 End of Study Definition

A subject is considered to have completed the study if they have completed all visits described in the study including the last visit listed in Section 10.1: Study Calendar.

8.0 SUBJECT SELECTION

8.1 Inclusion & Exclusion Criteria

Eligibility will be determined by inclusion and exclusion criteria below and confirmed by medical record review as necessary.

Inclusion Criteria
1. Willing to provide informed consent.
2. Willing to comply with all study procedures and be available for the duration of the study.
3. Histological diagnosis of HNC and ≥ 2 years from completion of radiation treatment for HNC (±surgery, ±chemotherapy), either clinically or radiologically NED, as assessed by ENT or Radiation Oncologist within 28 days of study registration
4. Individuals at least 18 years of age and no older than 90 years of age.
5. Xerostomia defined as ≤ 80% of baseline (pre-radiation) salivary function per patient estimate
6. Karnofsky performance status ≥ 70, patient eligible for bone marrow aspirate with wakeful anesthesia.
7. Radiographically confirmed bilateral submandibular glands
8. Females of childbearing potential must agree to have a negative urine or serum pregnancy test within 7 days prior to bone marrow biopsy. A female of child-bearing potential is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria: • Has not undergone a hysterectomy or bilateral oophorectomy; or • Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).
9. Women of childbearing potential in sexual relationships with men must have used an acceptable method of contraception[§] for 30 days prior to study registration and agree to use an acceptable method of contraception[§] until 4 weeks after completing study treatment. Males must agree to avoid impregnation of women during and for four weeks after completing study treatment through use of an acceptable method of contraception. [§]Note: Includes, but is not limited to, barrier with additional spermicidal foam or jelly, intrauterine device, hormonal contraception (started at least 30 days prior to study enrollment), intercourse with men who underwent vasectomy.
10. Laboratory values: Hgb ≥ 9 g/dL (5.58 mmol/L) Platelets ≥ 100,000/μL ANC ≥ 1000/μL PT/INR and PTT within normal limits based on age/sex Serum creatinine within normal limits based on age/sex

Exclusion Criteria
1. History of sialolithiasis

2.	Patients with one submandibular gland
3.	History of autoimmune diseases affecting salivary glands, including Sjögren's syndrome, lupus, scleroderma, type I diabetes, sarcoidosis, and amyloidosis
4.	Chronic graft versus host disease
5.	Untreated oral candidiasis
6.	Use of anti-cholinergic medications (e.g. atropine, iptratropium, oxybutynin, scopolamine, solifenacin, tiotropium, etc…) while enrolled on study
7.	Malignancy within the past 2 years, except adequately treated stage I lung cancer, low risk prostate cancer that has been treated or is undergoing active surveillance, adequately treated non-melanoma skin cancer, adequately treated DCIS, or adequately treated stage I cervical cancer
8.	Expected life expectancy $\leq$ 6 months.
9.	Lidocaine allergy
10.	Use of investigational drugs, biologics, or devices within 30 days prior to enrollment.
11.	Women who are pregnant, lactating or planning on becoming pregnant during the study.
12.	Not suitable for study participation due to other reasons at the discretion of the investigators.

8.2 Study Populations

Individuals from populations who are under-represented in clinical research (e.g., racial and ethnic minorities, women, individuals from rural/frontier communities, older individuals) will be enrolled with a goal of ensuring that all eligible patients are given the opportunity to participate in novel clinical trials and that research findings can be generalizable to the entire population.

Pregnant women, those who lack consent capacity, the mentally ill, prisoners, cognitively impaired persons, children, and employees will not be included in this research study.

8.3 Lifestyle Considerations

During this study, subjects are asked to:

- Refrain from consumption of any food, beverages, gum, saliva substitutes, or smoking for 1 hour prior to each salivary collection. Patients will be called the day prior to study visits to remind them about these restrictions.

8.4 Subject Identification

Identification in Clinical Practice

Potential subjects may be identified during routine visits to Radiation Oncology and Otolarygology clinics. A member of the clinical team will inform potential subjects of the research opportunity and explain the study. Potential subjects will be pre-screened through medical record review. Information collected from potential subjects who are not enrolled will be destroyed. Potential subjects who meet all pre-screening criteria will be invited for a formal consent and baseline visit. If the potential subject is interested in learning more, an IRB approved consent will be provided and discussed with the potential subject.

Self Identification

Potential subjects may self-identify as potential subjects after reading online information about the study (clinical trials.gov). Potential subjects who self-identified and contact the UWCCC be referred to a member of the clinical trials team. The clinical team member will further discuss the research opportunity and complete a pre-screening. Potential subjects who meet all pre-screening criteria will be invited for a formal consent and baseline visit. If the potential subject is interested in learning more, an IRB approved consent will be provided and discussed with the potential subject.

Electronic Medical Record Query

Potential subjects may also be identified via EMR query using diagnostic codes for head and neck cancer and radiation therapy and other eligibility criteria. The research team will conduct further prescreening of individual records to further refine the list of potential subjects.

Department Database

The head and neck cancer database (HS IRB #2015-1216), described under a separate protocol may be used to identify eligible subjects.

8.5 Subject Recruitment

A total of 6 subjects with bilateral submandibular glands for unilateral gland injection will be recruited from UW-Madison. Potential subjects will be recruited from clinical practice at UWCCC. If the potential subject is agreeable, they will be provided contact information for the study team or the research team will initiate contact.

8.6 Remuneration and Retention Strategies

Many visits will occur in concert with standard of care followup visits for patients. Patients will be contacted 2 weeks prior to each visit to be reminded of the upcoming appointment and the next appointment will be made before the patient leaves the clinic.

8.7 Early Termination and Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

The Principal Investigator (PI) may discontinue or withdraw a subject from the study for the following reasons at his/her discretion:

- Pregnancy
- Subject non-compliance with study requirements (e.g., study intervention non-compliance)
- If any clinical 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 interest of the subject
- If the subject is no longer an appropriate candidate for participation
- There is evidence of progressive disease or unacceptable toxicity
- Subject unable to receive MSC injection, either due to subject choice or investigator decision based on MSC release guidelines

Subjects who sign the informed consent form but do not receive the the MSC injections will be replaced. Subjects who sign the informed consent form and receive the the MSC injections, then subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

The following actions will be taken if a subject withdraws, or fails to return for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within 2 weeks and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address. These contact attempts shall be documented in the subject's study file.
- If the subject continues to be unreachable, they will be considered to be lost to follow-up.

If a subject is prematurely withdrawn from the study for any reason, the investigator will under all circumstances try to perform all evaluations described for the early termination visit

9.0 STUDY TREATMENT

9.1 Description of the Investigational Medicinal Product (IMP)

Drug Substance:

Autologous bone marrow-derived MSCs at a concentration of $10x10^6$ cells/mL

Drug Product (IMP):

Suspension of autologous bone marrow-derived MSCs suspended in a solution of Plasmalyte A containing 0.5% human serum albumin. Cells will be administered at one dose level, Dose 0, with a possible lower dose if the DLT criterion is reached. For each cell dosage we have set a 20% acceptable deviation. Dose levels and acceptable ranges (in parentheses) are as follows:

- Dose -1: 5 (4 – 6) $x10^6$ MSCs (into one submandibular gland; to be used only if Dose level 0 is not tolerated)
- Dose 0: 10 (8 – 12) x 10^6 MSCs (into one submandibular gland)

If the MSC IMP is not administered to the patient due to a failure of the MSCs to expand to achieve the required dose/ or the MSC IMP does not pass QA testing, the subject will be withdrawn from study, and followed for 1 month post the aspiration procedure to ensure AE/SAEs are appropriately recorded. These subjects will be replaced in the study by the recruitment of another subject.

Route of administration: injection into one submandibular gland (must have 2 glands present). The gland selected will be the gland that received the lower radiation dose. The contralateral gland of each interested subject will be injected with autologous MSC IMP at Dose level 0 following completion of the primary objective with no DLTs reported.

9.2 Preparation of the IMP

Up to 30 cc of bone marrow will be aspirated from the posterior superior iliac crest of each subject under aseptic conditions and local anesthesia by a qualified UW medical personnel. The bone marrow aspirate will then be hand delivered to the UW PACT GMP Cell Manufacturing Facility. UW PACT staff member will document receipt of the bone marrow aspirate and commence the processing according to the standard operating procedure. Mononuclear cells (MNCs) will be isolated by using Ficoll-Paque PREMIUM 1.073 g/mL medium (GE Healthcare) and density gradient centrifugation. Cells will be washed in CTS™ DPBS (GIBCO®) and re-suspended in culture medium supplemented with Gentamicin, USP at 5 µg/mL. The culture medium will consist of MSC NutriStem® XF Medium (Biological Industries), 5% PLTMax® human platelet lysate (Mill Creek Life Sciences), 1% CTS™ GlutaMAX™ I (GIBCO®), and Heparin Sodium, USP at 2 U/mL. The MNCs will then be placed into tissue culture flasks at 100,000 – 200,000 cells/cm^2 density. After 72 hours in 5% CO_2/37˚C humidified incubator, culture medium will be aspirated, non-adherent cells will be washed off with CTS™ DPBS (GIBCO®). Adherent cells will be returned into the incubator with fresh culture medium for additional 4-7 days. MSCs will be harvested using CTS™ TrypLE™ Select Enzyme (GIBCO®) and re-seeded into tissue culture flasks at 3,000 cells/cm^2 density for further expansion. Cells will reach approximately 70% confluence after 3 days in culture. Additional passage may be necessary in order to generate clinically relevant number of MSCs. Once desired number of cells and confluence is achieved, MSCs will be subjected to cytokine licensing by replacing spent medium with fresh culture medium supplemented with recombinant human IFN-gamma GMP Protein (R&D

Systems) at 1200 IU/mL. One flask will receive no IFN-gamma and serve a negative control. MSCs will augment their immunomodulatory properties after 24 hours of exposure to IFN-gamma. Stimulated MSCs will be analyzed via flow cytometry for upregulation of PD-L1, MHC I, MHC II, IDO, and Icam markers, which are indicative of the immunosuppressive phenotype. In addition, cell identity will be confirmed using Miltenyi Biotec MSC Phenotyping Kit. MSCs will be expected to stain positive for CD73, CD90, CD105 and negative for CD14, CD19, CD34, CD45 markers. IFN-gamma stimulated MSCs will then be cryopreserved at 5,000 cells/mL in 20% Plasma-Lyte A (Baxter), 20% Human Serum Albumin (FLEXBUMIN, Shire), 10% dimethyl sulfoxid (CryoMACS® DMSO Miltenyi Biotec). Cryovials will be stored in a vapor phase of LN_2 storage dewar within UW PACT GMP Cell Manufacturing Facility. In preparation for injection, vials will be thawed using ThawSTAR automated cell thawing system (BioLife Solutions). Cells will be washed twice in the MSC culture medium and seeded into flasks at 25,000 cells/cm^2 for 16 hours of culture-rescue, at which point cells have been shown to fully restore their immunomodulatory properties. MSCs will be harvested and re-suspended at 10 x 10^6 cells/mL in Plasma-Lyte A containing 0.5% human serum albumin. The suspension is then transferred into appropriately sized syringe. One milliliter of the suspension will be dispensed for the quality control testing. If the remaining volume in the syringe exceeds the pre-determined cell dose, the surplus will be allocated to the retention sample. The IMP will be released upon meeting the following specifications: viability >70%, endotoxin < 5 EU/mL, stat gram stain negative, dose level within 20% deviation of the target dose. Mycoplasma test results and 14 day sterility culture results will not be available on the day of injection.

9.3 Packaging, Labeling and Storage of the IMP

Packaging

The autologous bone marrow-derived IFN-gamma stimulated MSC product will be delivered in a 10 mL Becton Dickinson Luer-Lok™ Syringe(s) sterile, single use (Product Number 302995). This product is sourced from hospital inventory and deployed within the University of Wisconsin Hospital and Clinics.

Labeling

Labeling of IMP is compliant with FDA regulations and includes the patient's unique identifiers (medical record number, date of birth), product name, batch number, date and time of manufacture, expiration date and time, identification of the processing lab.

Storage

The IMP is intended for direct administration after preparation. The IMP containing autologous bone marrow-derived MSCs re-suspended at a concentration of 10x10^6 cells/mL in a solution of Plasma-Lyte A containing 0.5% human serum albumin has been shown to maintain viability >70% for 24 hours at room temperature. However, to maintain highest viability the product will be injected as soon as clinically possible. Until injection, the IMP will be stored at room temperature.

Packaging, labeling and storage of the IMP will follow UW PACT SOPs developed and qualified according to cGMP standards.

9.4 Transport of IMP

The IMP will be transported from the GMP Cell Manufacturing Facility to the clinical site (ENT clinic) in a qualified temperature controlled cooler at 20-25˚C according to the UW PACT SOP.

9.5 IMP Accountability

The PI is responsible for maintaining accurate accountability records throughout the study. The administration of the IMP will be documented in the patient's medical file in a procedure note.

9.6 Retention samples, if available, will be cryopreserved and stored in a vapor phase of LN_2 storage dewar within the GMP Cell Manufacturing Facility. Retention samples will be kept for a minimum of 2 years. Retention samples may then be discarded according to applicable regulations for disposal of biological products. The PACT GMP Cell Manufaucturing facility has assessed each patient's MSC IMP to ensure the cryopreserved product is stable and appropriate for second injection (See Appendix 19.4 for stability data). The MSC IMP administered for the second injection will undergo the same quality control testing detailed in section 9.2 prior to injection. Administration of IMP

MSCs will be supplied in one single use 10 mL Becton Dickinson Luer-Lok™ Syringe sterile, single use (Product Number 302995) at the pre-specified dosage levels for each gland to be injected. No preparation is required. Administeration by Dr. Glazer will occur as follows:

- Subjects will be placed in a semi-recumbent position
- Injection of 2mL of 1% lidocaine with 1:100,000 epinephrine into the submandibular gland area.
- Visually inspect the syringe for particulate matter and/or discoloration prior to administration. If either of these conditions exist, do not administer
- Gently shake the prefilled syringe
- Identify each submandibular gland with US and cleanse the injection site with chlorhexidine.
- Insert 22 G x 1 ½" needle (Becton Dickinson's Precisionglide™ hypodermic needle [Product Number 305194]) at a 90-degree angle to the skin and inject all of the contents of 1 syringe into a submandibular gland under US guidance at a rate of ≤2 mL/min. This rate and needle gauge was chosen in part, due to the work published by Mamidi and colleagues, demonstrating that repeatedly passing mesenchymal stem cells (MSC) through substantially smaller gauge needles (24, 25, and 26G) has no impact on MSC morphology, attachment, viability, phenotype, differentiation potential, and in vivo migration abilities[30]. Do not inject the IMP subcutaneously or intravenously. Care should be taken to avoid administering the injection into or near blood vessels and nerves.
    - Ultrasound guidance will be used to 1) ensure intraglandular (sub-capsular) injection into the submandibular gland and, 2) avoid intravascular injection into branches of facial and lingual vessels.
- Monitor the subject for at least 30 minutes post administration.

9.7 Dose-Reduction Schedule

The cohort of 6 participants will have staggered enrollment**.** A minimum interval of 14 days will occur between MSC injection of a subject and MSC injection in the subsequent subject, during which time there must be no SAEs and no AEs ≥ grade 3 that are probably or definitely attributable to MSC injection. The decision to proceed with enrollment of a subsequent subject within a dose cohort will be made by the PI based on the criteria stated above.

For purposes of possible dose reduction from Dose 0 to Dose -1, dose limiting toxicity is defined as a submandibular pain score > 5 (on a standard 0-10 pain scle) at one-month post-injection OR any serious AE probably or definitely caused by study treatment at any time within one-month post-injection OR any of the toxicities below within 1 month of injection:

- Grade 4 neutropenia > 5 days
- Grade 4 thrombocytopenia, or grade 3 thrombocytopenia with hemorrhage
- Grade 3-4 non-hematological toxicity **except**:
    - Grade 3 nausea, vomiting, and diarrhea lasting < 72 hrs in the absence of maximal medical therapy
    - Grade 4 vomiting and diarrhea lasting < 72 hrs in the absence of maximal medical therapy
    - Grade 3 fatigue < 5 days
    - Grade 3 hypertension in the absence of maximal medical therapy

- Grade 3 rash < 5 days is included as an exception
- Grade 3 metabolic abnormalities such as hypokalemia, hypomagnesemia, hypocalcemia, and hypophosphatemia should only be excepted from the DLT definition if they recover to Grade 1 or less within 48 hours.
- Grade 3 fatigue that persists < 7 days.
- Grade 3 rash that resolves to ≤ Grade 1 within 3 weeks.
- Grade 3 or 4 elevation in serum amylase and/or lipase that are not associated with clinical or radiographic evidence of pancreatitis.
- ≥ Grade 3 electrolyte abnormality that lasts < 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical intervention.
- Grade 3 nausea or vomiting that lasts < 48 hours, and resolves to ≤ Grade 1 either spontaneously or with conventional medical intervention.
- Grade 3 immune related AE (irAE) that resolves to ≤ Grade 1 or to baseline with immunosuppressive therapy within 3 weeks.

Additionally, patient enrollment and treatment will be temporarily halted if any of the following occur, pending review by the UWCCC DSMC and FDA if:

- Any death, irrespective of attribution by the investigator within 30 days of MSC injection
- Any (CTCAE v. 5) of grade 4 or higher
- Any hypersensitivity reaction grade 3 or higher at any time
- Any grade 3 SAE in the first 3 subjects
- Any 2 grade 3 or higher of the same SAE at any time during the study
- 5 or more grade 3 or higher SAEs that are at least possibly related at any time during the study

Dose reduction schedule:

Dose level 0 (10 x 10^6 MSCs into one submandibular gland) given to 6 patients:

- If 0 or 1/6 patients experience DLT at dose level 0, then the dose escalation portion of a subsequent planned study will begin at dose level 0 (10 (8-12) x 10^6 MSCs into both submandibular glands) and the study will be completed.
- If 2/6 patients experience DLT at dose level 0, then decrease dose to dose level -1 (5 x 10^6 MSCs) and continue with unilateral injection at dose level -1.

If needed, Dose level -1 (5 x 10^6 MSCs) given to 3 patients

- If 0/3 or 1/3 patients experience DLT at this dose level 1 then recruit additional 3 patients at this dose.
    - If 2/3 patients experience DLT, then treatment is considered not tolerable and the study will be halted.
- If 0/6 or 1/6 patients experience DLT at this dose level, then the dose escalation portion of a subsequent planned study will begin at dose level -1 (5 (4-6) x 10^6 MSCs into both submandibular glands).
    - If 2/6 patients experience DLT, then treatment is considered not tolerable and the study will be halted.

Part 1 Dose-Reduction Table.

Dose Level	MSC IMP
-1	5 (4-6) x 10^6 injected into one submandibular gland on Day 1
0	10 (8-12) x 10^6 injected into one submandibular gland on Day 1

9.8 Supportive Care

Optimal patient care is to be given to all subjects. The subjects will be followed by their head and neck oncology team and dentist during the study.

Pain:
Pain should be managed with over-the-counter analgesics if possible. Recommend scheduling acetaminophen 650 mg q 6 hours (maximum < 4,000 mg) or ibuprofen 400 mg q 6 hours (if no contraindications such as decreased renal function). Should pain persist at level 6/10 or greater, opioid pain medications may be prescribed.

Swelling:
Subjects with treatment-related swelling should first try supportive measures such as cold packs and ibuprofen 400 mg q 6 hours (if no contraindications such as decreased renal function). Should the swelling persist, subjects should present to UW clinic for evaluation by medical provider.

9.9 Dose Delays/Modifications

Not applicable to this single administration protocol.

9.10 Study Procedural Intervention(s) Description

Pre-Intervention Bone Marrow Biopsy for MSC Collection

Each subject will undergo a bone marrow (BM) aspirate in order to obtain MSCs. Subjects will undergo BM aspiration for the collection of approximately 30 mL of BM aspirate in the UW Oncology Clinic by an experienced practitioner. Female subjects will have a urine pregnancy test which must be negative to participate. An IV will be placed for conscious sedation, if needed, and for blood collection following the aspiration procedure. If conscious sedation is chosen by the patient, IV sedative will be administered by a trained nurse who will monitor the subject throughout the procedure and recovery from sedation. For the BM collection procedure, the subject will be placed in a prone or side-lying position and the area over iliac crest will be anesthetized with a local anesthetic. The skin will be scrubbed with chlorhexadine and draped with sterile towels/drapes. Using sterile technique, the physician will insert a harvesting needle and aspirate BM into 2-4 10cc syringes, previously rinsed with 3000 units of heparin, collecting 5-10 ml at a time. After the first syringe is aspirated, the beveled needle may be turned 90 degree for three more turns and the aspiration repeated after each turn. Each syringe filled with bone marrow will be injected into a tube that has been prepared with Na Heparin. The collection tube will be labeled with a unique hMSC bank ID number. When all BM has been collected the harvesting needle will be removed and a dressing will be placed over the aspiration site. If subjects have received intravenous (IV) sedation, they will be observed for at least 1 hour or until fully recovered. In addition, subjects will receive a phone call from the research study coordinator within 3 +/- 2 days to review any potential side effects that might be attributable to the bone marrow aspiration.

MSC IMP Administration

The injection of the MSC IMP into the subject's submandibular gland(s) will be done by an experienced Otolaryngology physician under ultrasound guidance in the ENT clinic. Should one submandibular gland be surgically absent, then MSCs will be injected into only the present gland. (Note that patients with only one submandibular gland may not enroll in Part 1, dose level 0). Subjects will be in a semi-recumbent position and the skin over the submandibular gland(s) will be cleansed with chlorhexidine. The area over the submandibular gland will be anesthetized with an injection of 2 mL of 1% lidocaine with 1:100,000 epinephrine. Using sterile technique with US

guidance to ensure avoidance of intravascular injection into branches of the facial and lingual vessels, the submandibular glands will be injected with 0.5-2 mL of MSCs (depending on dosing level) via a 22 gauge, 1 ½ inch needle. The needle will then be removed and a dressing will be placed over the site.

9.11 Method for Assigning to Treatment Groups

Enrollment will be on a rolling basis per DLT schedule. Patients with only one submandibular gland will not be enrolled into this pilot trial.

9.12 Unblinding Procedures

Not applicable.

9.13 Study Intervention Compliance

Pain diary for the first 1 month and QoL surveys, salivary analysis, and submandibular gland US for follow-up visits at 1, 3, 6, 12, and 24 months.

9.14 Concomitant Therapy

Permitted Concomitant Therapy

Any supportive care will be allowed as concomitant therapy including, but not limited to, salivary substitutes and stimulatory agents. During the screening and all follow-up visits, data on the use of concomitant therapies will be collected. These therapies may affect the study endpoints, as they may improve QoL or other study measures. However, as subjects will likely have been previously using this, stopping the medications would likely bias the results. Gathering information on the therapies they are using prior to the intervention will allow us to analyze the possible effects the concomitant therapies may have on our endpoints.

Patients without chronic pain will be instructed to refrain from narcotic pain medication for 3 days prior to their scheduled pain assessment. Patients with chronic pain will be instructed not to increase their baseline narcotic pain medication for 3 days prior to scheduled pain assessment.

Prohibited Concomitant Therapy

None

10.0 STUDY VISITS AND PROCEDURES

10.1 Study Calendar

The procedures performed at each study visit are listed in the table below.

Procedure/Visit	Screening	Baseline	Obtain MSCs[2]	MSC IMP Injection	3-day phone call[9]	1-week phone call[9]	Follow-up visit				
							1-month	3-month	6-month	12-month	24-month
Visit window Day ± days	**- 42 ± 14**	**- 42 ± 14**	**- 28 ± 14**	**0**	**3 ± 2**	**7 ± 2**	**30 ± 10**	**90 ± 14**	**180 ± 21**	**365 ± 21**	**730 ± 28**
Informed Consent	X										
Review Eligibility Criteria	X										
Demographics	X										
Review Concomitant Medications	X				X	X	X	X	X	X	X
Dental Evaluation[5]		X									
Obtain Medical History		X									
Physical Exam		X					X	X	X	X	X
Vital Signs[1]		X		X[3]			X				
Bone Marrow Aspiration			X								
MSC IMP Injection				X							
AE Assessment Including Pain			X	X[4]	X	X	X	X	X	X	X
Assessment of QoL, Salivary Collection		X					X	X	X	X	X
Ultrasound of Salivary Glands		X						X	X	X	
Collection of Pain Diary[6]							X				
Blood (for banking)		X					X	X	X	X	X
Complete blood count, PT/INR and PTT, Creatinine		X					X[8]	X[8]			
Pregnancy test (urine or serum)			X[7]	X[7]							

[1] – Vitals include body weight, pulse rate, body temperature, blood pressure, and respiration rate. Height collected only at baseline.

[2] – Subjects will be contacted via phone 3 days +/- 2 days post bone marrow aspiration to assess for toxicities from aspiration by study coordinator

[3] – Vitals to be obtained pre injection and 30 minutes +/- 10 minutes post injection. Weight only obtained pre injection.

[4] – Subjects will be asked to verbally rate their pain 30 minutes post MSC administration.

[5] – Dental examination with x-rays within 6 months of enrollment by patient report

[6] – Patients without chronic pain will be instructed to refrain from narcotic pain medication for 3 days prior to their scheduled pain assessment. Patients with chronic pain will be instructed not to increase their baseline narcotic pain medication for 3 days prior to scheduled pain assessment.

[7]—Within 7 days prior to MSC collection and confirmation of appropriate contraception prior to injection.
[8]—CBC only during follow up
[9]-Subjects will be contacted via phone 3 days (+/- 2 days) and 7 days +/- 2 days post MSC IMP Injection to assess for toxicities from injection by study coordinator or physician.

Sub study calendar

Informed Consent	Screen/Injection of MSC's	Phone call (3 days +/- 2 days)[1]	Phone call (7 days +/- 2 days)[1]	30 day visit (+/-10 days)	90 days (+/- 14 days	6 months (+/- 28 days)	12 months (+/- 3 months	24 months (+/- 3 months
Review Eligibility Criteria	x							
Review Concomitant Medications	x							
Vital Signs[2]	x							
Physical	X			X	X	X	X	X
MSC IMP Injection (contralateral neck)	X							
AE Assessment Including Pain	X[3]	X	X	X	X	X	X	X
Assessment of QoL, Salivary Collection	X			X	X	X	X	
Collection of Pain Diary				X				
Complete blood count	X			X[4]	X[4]			

[1]Subjects will be contacted via phone 3 and 7 days +/- 2 days post MSC IMP Injection to assess for toxicities from injection by study coordinator or physician
[2]Vitals to be obtained pre injection and 30 minutes +/- 10 minutes post injection. Weight only obtained pre injection.
[3] – Subjects will be asked to verbally rate their pain 30 minutes post MSC administration. Patients without chronic pain will be instructed to refrain from narcotic pain medication for 3 days prior to their scheduled pain assessment. Patients with chronic pain will be instructed not to increase their baseline narcotic pain medication for 3 days prior to scheduled pain assessment
[4]CBC only during follow up

10.2 Screening and Enrollment

The Screening and study visits and procedures are described in detail below.

Informed Consent

The informed consent process will be conducted following all federal and institutional regulations relating to informed consent. Informed consent will be obtained prior to conducting any research-related activities.

The informed consent process will be performed as follows:

- The research coordinator will review the informed consent form and discuss the study in detail with the potential research subject.
- The research coordinator will explain the study, its risks and benefits, what would be required of the research subject, and alternatives to participation.
- The research subject will be given the opportunity to take the informed consent form home so that they may discuss it with family members, friends, clergy or others when possible.
- The subject will have the opportunity to ask questions and have all questions answered by the The research coordinator and/or PI.
- The informed consent document must be signed and dated by the research subject and by the study team member performing consent procedures.
- The research coordinator will review the informed consent document to ensure that all fields that require a response are complete (i.e., checkbox marked yes or no, etc.) as applicable.
- The research subject will be given a copy of the signed and dated informed consent form.

Alternatively remote consenting may occur as described below:

- The participant must receive a copy of the ICF (e.g., in person, via mail, fax, email, or a Part 11 compliant manner will be used to document consent) in advance of discussion.
- The study coordinator discusses the study with the potential participant via telephone or video conferencing.
- The study coordinator must implement a method to ensure the identity of the participant (e.g., verification of state identification or other identifying documents or use of personal questions such as date of birth or spell first and last name).
- If the potential participant agrees to participation, they must sign and date the ICF and return it to the study coordinator via email using a scanner or taking a picture of the signature page or using a Part 11 compliant manner to document consent.
- The study coordinator will document the receipt of the signed ICF and the informed consent process in the Electronic Medical Record (Healthlink).
- The study coordinator must then email HIS-ScanningCorrec@uwhealth.org a PDF of the participant's signed ICF to be uploaded in Healthlink. As soon as the consent note is written in Healthlink and consent form has been emailed for scanning, screening procedures may begin.
- No additional signatures are required.

Enrollment/Registration

A research subject will be defined as "enrolled" in the study when they meet the following criteria:

- The subject has been consented by study staff.
- The subject has completed all screening procedures.
- The PI has verified subject eligibility by reviewing inclusion and exclusion criteria
- The subject has been enrolled by the protocol staff in the UWCCC ONCORE database. The following information will be recorded:
    - Protocol number
    - Patient's name and initials
    - Patient's medical record number
    - Patient demographic data

Screening

- Information from SOC clinic visits may be used if within study window. No research only procedures will occur prior to obtaining written informed consent.
- All screening procedures must be performed prior to study enrollment/registration.
- Record data on disease characteristics (e.g., site, stage) and treatment characteristics (e.g., radiation dose distributions)

- Review current medications being taken (SOC)
- Obtain written informed consent

10.3 On-Study/Follow-up Visits

After subjects have been enrolled, the On-Study/Follow-up visit and the procedures performed at each visit are described in detail below.

Baseline (may occur over 1-2 visits)

- Physical exam including weight and height and med history (SOC)
- Vital signs (SOC)
- Ensure patient has undergone dental evaluation with radiographs to exclude dental/oral diseases that could cause treatable xerostomia within 6 months of enrollment
- Salivary collection (research)
- QoL assessment (research)
- Collection of blood sample for banking purposes (research)
- US of salivary glands (research)
- Review of RT DICOM file of head and neck radiation plan (research)
- Complete blood count PT/INR, PTT and creatinine (research)

Bone Marrow Aspiration for MSC Collection

- Bone marrow aspiration

MSC IMP Administration

- Review current medications being taken
- Vital signs
- MSC injection (see section 9.6)
- Patients without chronic pain will be instructed to refrain from narcotic pain medication for 3 days prior to their scheduled pain assessment. Patients with chronic pain will be instructed not to increase their baseline narcotic pain medication for 3 days prior to scheduled pain assessment.

Assessments ***after*** MSC IMP administration

- Acute toxicity: vital signs (pre and 30 minutes +/- 10 minutes post) and visual inspection of injection site **after** the administration of IMP: 30 minutes post-injection and monitoring of adverse events possibly related to acute toxicity including verbal pain assessment.

Three Day Telephone Call (3 days ± 2 days)

- Adverse event assessment

One Week Telephone Call (7 days ± 2 days)

- Adverse event assessment
- Obtain description of injection area

One Month Follow-up Visit (30 days ± 10 days)

- Review current medications being taken
- Physical exam including weight
- Vital signs
- Salivary collection
- QoL assessment
- Adverse event assessment
- Collection of blood sample for banking purposes

- Collection of pain diary
- Complete blood count,

Three Month Follow-up Visit (90 days ± 14 days)

- Review current medications being taken
- Physical exam including weight
- Salivary collection
- US of salivary glands
- QoL assessment
- Adverse event assessment
- Collection of blood sample for banking purposes
- Complete blood count

Six Month Follow-up Visit (180 days ± 21 days)

- Review current medications being taken
- Physical exam including weight
- Salivary collection
- US of salivary glands
- QoL assessment
- Adverse event assessment
- Collection of blood sample for banking purposes

Twelve Month Follow-up Visit (365 days ± 21 days)

- Review current medications being taken
- Physical exam including weight
- Salivary collection
- US of salivary glands
- QoL assessment
- Adverse event assessment
- Collection of blood sample for banking purposes

Twenty Four Month Follow-up Visit (730 days ± 56 days)

- Review current medications being taken
- Physical exam including weight
- Salivary collection
- QoL assessment
- Adverse event assessment
- Collection of blood sample for banking purposes

Screening/MSC IMP Administration in Contralateral Gland

- Physical exam including weight
- Salivary Collection
- QoL assessment
- MSC injection (see section 9.6)
- Vital signs

Three Day Telephone Call (3 days ± 2 days)

- Adverse event assessment

Seven Day Telephone Call After Contralateral Injection (7 days ± 2 days)

- Adverse event assessment

Thirty Day Follow-up Visit After Contralateral Injection (30 days ± 10 days)

- Physical exam including weight
- Adverse event assessment
- Complete Blood Count
- Salivary Collection
- QoL assessment

Ninety Day Follow-up Visit After Contralateral Injection (90 days ± 14 days)

- Physical exam including weight
- Adverse event assessment
- Complete Blood Count
- Salivary Collection
- QoL assessment

Six Month Follow-up Visit (180 days +/- 28 days)

- Physical exam including weight
- Salivary collection
- QoL assessment
- Adverse event assessment

Twelve Month Follow-up Visit After Contralateral Injection (12 months ± 3 months)

- Physical exam including weight
- Adverse event assessment
- Salivary collection
- QoL assessment

Twenty Four Month Follow-up Visit (730 days +/- 3 months)

- Physical exam including weight
- Adverse event assessment

Note: This twenty four month visit will be the subject's final study visit.

10.4 Unscheduled Visits

Notes from unscheduled visits for pain or swelling will be reviewed by the PI to assess for study-related AE/SAE. Whenever appropriate unscheduled visits will be with a study team member.

10.5 Early Termination/Withdrawal Visit

If a patient prematurely discontinues the study for any reason after administration of the MSC IMP, all assessments planned for the final follow-up visit (24 months) should be performed. The reason for premature discontinuation will be documented.

11.0 COLLECTION AND PROCESSING OF PATIENT REPORTED OUTCOMES, BIOSPECMENS, AND IMAGING

11.1 Pain Measurement

Subjects will be asked to rate their pain on a scale from 0 -10 (0=not at all to 10=worst pain imaginable) by refering to a provided visual analog pain scale. The pain scale of 0-10 has been validated to allow for the discrimination of clinically meaningful differences in pain[31,32]. They will be asked to rate their pain at baseline (screening), 30 minutes after the MSC IMP administration, 3 days after administration, one week after administration, 30 days after, and 90 days after MSC administration. Subjects will additionally keep a diary with daily entries to record occurrence and severity of pain and occurrence and severity of other adverse events (redness, swelling, warmth, tenderness, rash, pruritis, nausea, vomiting, fatigue etc.) for the first month after MSC injection. Subjects will also keep a log of all pain medications used during the first 30 days, including narcotic and non-narcotic medications.

11.2 Quality of Life Assessments

Quality of life will be assessed using three validated tools: the University of Michigan Xerostomia Related Quality of Life (XeQOL) scale[33], the MD Anderson Dysphagia Index (MDADI)[34], and the VAS xerostomia questionnaire[35].

The University of Michigan Xerostomia related quality of life scale (XeQOL) is a validated patient-reported 15-item assessment scale with 4 domains: physical functioning, pain/discomfort, personal/psychologic functioning, and social functioning (Appendix 19.1). The score is the average of all responses of all domains and can range from 0 to 4. Higher scores indicate increased xerostomia burden. This scale has high reproducibility and sensitivity.

The MDADI is a 20-item questionnaire designed for evaluating the impact of dysphagia on the QOL of patients with head and neck cancer. The questionnaire and scoring instructions are in Appendix 19.2.

The VAS xerostomia questionnaire is an 8-item questionnaire that provides a validated measure of the perception of dry mouth. Subjects will be asked to mark their responses to each item by placing a vertical line on the 100-mm horizontal scale. The questionnaire is in Appendix 19.3.

Subjects will complete the XeQoL, MDADI, and VAS at the screening (baseline) visit and at 3- and 6-month follow-up visits. Subjects in the dose-escalation cohort will also complete these QoL questionnaires at their one-month follow-up visit.

11.3 Salivary Flow Rate Assessments

Whole saliva production rates (sialometry) will be measured under unstimulated and stimulated saliva collection conditions, as previously described[36,37]. Participants will refrain from eating, drinking, and,

smoking for one hour prior to collection. Unstimulated saliva production will be measured first by the passive drool method over a 5-minute time frame. Participants will allow saliva to pool in the mouth and, then, with head tilted forward, gently guide saliva into a saliva collection aid attached to a cryovial. Stimulated saliva production will be measured by having participants chew inert gum base to the pace of a metronome (70 beats per minute) while expectorating saliva (without swallowing) into the saliva collection aid for 5 minutes total[38]. Cryovials will be weighed before and after collection and the difference in weight (g) will represent the amount of saliva produced in 5 minutes for each condition. Subjects will complete the salivary flow rate assessments at the screening (baseline) visit and at 1-, 3-, 6-, 12-, and 24-month follow-up visits.

11.4 Salivary Composition Analyses

Salivary composition analyses will focus on qualitative aspects of saliva previously found to change following radiation treatment: salivary pH[39,40], total protein concentration (mg/mL)[41,42], amylase (mU/mL)[39,43], and mucins (MUC5B, mU/mL)[43,44]. Given that MSCs will be injected into one of the submandibular glands which contain both serous and mucous acinar secretory cells, we will measure products of both types of cells (both= total protein and pH; serous= amylase; mucous= mucins). Salivary pH will be measured using a pH meter (Orion 9810BN, Thermo Scientific). Enzyme-linked immunosorbent assays (ELISAs) will be used to quantify total protein, amylase, and mucin levels. Saliva composition will be assessed on whole saliva samples collected from subjects at each timepoint salivary flow rate is assessed (see Section 11.3). Saliva will be processed, stored at -80C, and analyzed in batch at a later date.

11.5 Salivary Biobanking

The remaining saliva will be biobanked in the -80 freezer in the Kimple lab in a secure location. Future clinical studies on the biobanked may include investigations in the microbiome or cytokines.

11.6 Ultrasound Imaging Assessment

Salivary gland size will be measured by ultrasound[45]. Ultrasound imaging is a non-ionizing, noninvasive imaging modality that can be quickly obtained in a standard exam room. Three-dimensional (3D) Bmode data sets will be acquired for gland volume measurements and characertization of the gland.

The lower outline of the gland will be used to define the distal (relative to the ultrasound probe) end of the gland. This assessment is necessary to provide a 3D volume. In a series of six patients imaged as part of a pilot study, determination of 3D gland volume was possible in all patients. Rarely the lower, or distal aspect of the gland can be difficult to visualize, particularly after radiation [45]. Under this circumstance, 2D measurements will be employed to estimate gland volume for the study patient. The salivary gland volumes will be used to determine if gland volume increases after MSC administration. Additionally, the stiffness of the salivary glands will be measured by shear wave velocity. Acoustic radiation force impulse imaging is a type of ultrasound technique employed to measure elasticity and fibrosis of salivary glands via shear wave velocity. Stiffness, or fibrosis, will be assessed at baseline and after MSC injection. Acoustic radiation force impulse imaging is used in multiple salivary gland conditions including Sjogren's syndrome to measure fibrosis [46,47]. The evaluators will be blinded to the time point of evaluation (pre- or post- MSC injection).

Ultrasound assessment of salivary glands will be done at the baseline, 3-month, 6-month, and 12-month follow-up visits.

11.7 Biobanking of Blood Sample

Biobanking of participant blood will occur at a baseline visit or the day of the bone marrow aspiration, and at the 1 (for those seen at 1 month), 3, 6, 12, and 24 month visits. Approximately 3 mL of blood will be placed into an EDTA tube which will be transported to the Kimple lab, in WIMR where it will be stored in a secure freezer in a secure location. Future clinical studies on the biobanked blood may include measuring inflammatory cytokines or other immune markers.

12.0 DATA COLLECTION, HANDLING, SHARING, AND RECORD KEEPING

12.1 Data Collection

Data Collection Forms

Standardized data collection forms (e.g., source documents, case report forms, standardized assessment forms, etc.) are used to ensure data collected are consistent and compliant with the protocol and IRB application.

Data collection is the responsibility of study team members under the supervision of the Principal Investigator (PI). The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the recorded and reported data.

All data collection forms must be completed in a legible manner; any missing data will be explained. Data entry errors will be corrected with a single line through the incorrect entry and the correct data is entered above/near the correction. All changes will be initialed and dated.

Data Capture Methods

This study will utilize 2 different web-based platforms, Oncore (Forte Research) for study registration and tracking and REDCap for clinical trial data collection. Both systems are secure web platforms that are compliant with Good Clinical Practices and Federal Rules and Regulations. UW Department of Human Oncology Coordinators will coordinate and manage data collection and entry

Clinical data (including AEs, concomitant medications, and solicited events data) and clinical laboratory data will be entered into the following REDCap. Clinical data will be entered directly from the source documents.

Shadow research charts with original consent forms and documents specifically created for this study will be maintained in the Department of Human Oncology until the study is terminated. The records will then be sent to Wisconsin State Records Archiving facility for long term storage (10 years) and re-archived as needed.

12.2 Confidentiality and Privacy

Subject confidentiality and privacy are 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 subjects. 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.

All research activities will be conducted in as private a setting as possible.

All study staff engaged in the conduct of this project have completed training on the protection of human subjects and the Health Insurance Portability and Accountability (HIPAA) Privacy Rule. In addition, all key personnel (i.e., Principal Investigator, individuals involved in identifying/recruiting subjects, obtaining informed consent, or interacting and intervening with subjects) have undergone Good Clinical Practice (GCP) training.

Information about study subjects will be kept confidential and managed according to HIPAA requirements. All subjects will sign a combined informed consent and HIPAA authorization form that includes specific privacy and confidentiality rights. Study data will be maintained per federal, state, and institutional data policies.

The investigator(s) will ensure that the identities of subjects are protected by using coded subject information. The log of subject identifying information that links subjects to their study-specific identification number will be maintained by the investigator. The log and all study records will be maintained in locked rooms and access will be limited to essential study personnel. The log linking subject code to identifiers will be stored separately from study data. The PI will have access to the log linking code to identifiers, as will authorized study personnel. Electronic study records/files will be stored on a department server and accessed via networked computers that are password-protected with access provided only to authorized study personnel.

Authorized representatives of the following groups may need to review this research as part of their responsibilities to protect research subjects: representatives of the IRB, UWCCC DSMC, UWCCC, authorized personnel of the UW PACT, Other groups at the University of Wisconsin tasked with Research Oversight and compliance, and federal oversight agencies, such as the Food and Drug Administration (FDA).

Records Retention
It is the investigator's responsibility to retain study essential documents for a minimum period of 7 years following completion of the study per UW-Madison institutional policy, or at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, whichever comes last.

12.3 Retention for Future Research: Data & Biospecimen Banking

Purpose of Storage

The purpose of collecting and storing biospecimens is to establish a biobank for use by UW-Madison researchers to use who are investigating the causes, diagnosis, prevention and treatment of cancer.

Data and/or Biospecimens Being Stored

Blood in a 3 mL standard EDTA tube will be collected from each participant on the day of bone marrow biopsy, and at the 1(for those seen at 1 month), 3, 6, 12, and 24 month follow-ups. This blood will be labeled with a unique subject ID code. All tubes of blood will then be stored in a secure freezer in the secured Kimple lab in WIMR.

Saliva will be collected from each participant at the pre-specified timepoints as detailed previously. The saliva will be used for clinical endpoints as detailed previously, any remaining saliva will be stored in an Eppendorf tube in a secure freezer in the secured Kimple lab in WIMR.

Location of Storage

Blood and saliva tubes labelled with coded subject ID number, date, and time point, will be stored in the Kimple Laboratory. Data associated with the blood and saliva tubes is on a UW password-protected server.

Duration of Storage

Biospecimens will be stored until they are used up in their entirety.

Access to Data, Images, Recordings and/or Biospecimens and Security Measures

UW researchers who are doing biomedical research may apply for access to coded data and blood (no patient identifiers on tube).

Procedures to Release Data or Biospecimens

Researchers must have local IRB approval for their study which will include study of the biospecimens and accompanying data.

Process for Returning Results

There are no plans to disclose results of future testing to research subjects.

Process for Tracking Subject Consent and Authorization

In the consent form for this study, subjects are informed of an optional aspect of the study that involves donating blood for future biomedical research. Subjects are made aware they do not have to donate biospecimens to the Biobank to participate in the study. Subjects must opt into the biobanking aspect of the study by checking a box and initialing a line next to the box.

12.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or investigational plan requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. Protocol deviations will be assessed for their impact on safety, study operations, and data integrity. Appropriate corrective and preventive actions will be implemented if warranted.

It is the responsibility of the Principal Investigator/site investigator/study staff to use continuous vigilance to identify and report deviations. The Principal Investigator is responsible for assessing whether the deviation constitutes noncompliance as defined by the reviewing IRB and if so, reporting it within the required time frame(s) to the UWCCC and the IRB as applicable

12.5 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations: This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

13.0 STUDY ANALYSIS

Any deviations from the planned analyses will be described and justified in the final integrated study report. Statistical programming and analyses will be performed using SAS® (SAS Institute Inc., Cary NC), version 9.4 or higher. A formal statistical analysis plan (SAP) will be developed before the database lock. It will include detailed descriptions of summaries and mock-ups of tables, listings, and figures to be included in the clinical study report.

13.1 General Considerations

The statistical analyses in this study will be exploratory since the study is not powered to address any pre-defined statements but to generate valid hypotheses on safety/tolerability, feasibility, and efficacy

issues. A formal sample size calculation was therefore not done. Thus, all resulting p-values and confidence intervals are to be interpreted in the exploratory sense, only.

Data will be appropriately summarized and analyzed using tabulation and graphs for demographic and baseline characteristics, safety and efficacy observations and measurements. Standard descriptive summary statistics (i.e., n, arithmetic mean, standard deviation, median, lower/upper quartiles, and minimum/maximum values) will be calculated for continuous variables. Categorical data will be presented in frequency tables using counts and percentages. For all data collected during the study, listings of the individual raw data will be provided. Individual patient data listings will be presented per parameter.

The main analysis will be performed after the 24-month Follow-up Visit, i.e., when all patients have either completed the visit, are lost to follow-up or have died within this period.

13.2 Determination of Sample Size

Patients in this pilot trial will undergo unilateral gland injection per FDA request. Associated 95% confidence intervals will be provided for the toxicity rate. To describe changes in quality of life or salivary function/composition between baseline and post-injection timepoints, separate one-way ANOVA models will be fit to the data for each measured outcome. After fitting the models to the data, comparisons between the timepoints will be calculated using Tukey's method. Due to the short time frame of intervention, we anticipate that adherence to the intervention will be high (>90% of patients will complete all mandatory study components) and that rates of follow-up will be similarly high (i.e., low rates of drop outs or lost to follow-up).

Interim analysis: In this pilot study, there is not a planned interim analysis.

Sample Size: Six to twelve patients will be accrued, depending on toxicity encountered. If 0/6 or 1/6 participants experience a DLT at Dose Level 0, sample size would be six. If 2/6 participants experience a DLT at Dose Level 0, another cohort would be enrolled at Dose Level -1. Sample size would then be up to 12 patients, unless 2 or more patients at Dose Level -1 experience a DLT, at which point the study would be stopped.

Length of follow-up: Patients are observed for DLT after MSC injection, with a follow-up of 4 weeks after injection. Secondary outcomes will be assessed at 24 months (end of study for patients). DLT is defined as: pain > 5 on a standard pain scale of 0-10 at 1-month after MSC injection OR any serious AE within one-month post-injection (probably or definitely caused by study treatment) or other toxicities described in section 9.7.

13.3 Analysis Sets

Eligibility, Protocol Deviations

Enrolling an ineligible participant is a protocol violation that is reportable to the IRB. If Protocol Deviations are identified, the PI will determine whether the protocol should be modified (to prevent further incidents from occurring) and whether the participant should be withdrawn due to safety or other concerns. Enrollment in another clinical study possibly interfering with the endpoints of the present study will be considered a protocol violation.

Each patient's allocation to the different analysis sets will be identified prior to database lock. Significant deviations from the protocol will lead to the exclusion of a subject from the per-protocol analysis set.

Listings will be prepared to show the characteristics of all subjects for the different analysis sets and a summary will be given on the number of subjects per analysis set. A detailed description of the criteria used for data sort out will be provided before start of the evaluations.

Definitions

The statistical evaluation will be based on separate, hierarchically organized analysis sets as defined below:

- Intention to Treat (ITT) analysis set: The safety analysis set will consist of all patients enrolled.
- Safety analysis set: The full analysis set will consist of all patients who received the injection of the IMP.
- Per-protocol analysis set: The per-protocol analysis set will consist of all patients of the ITT analysis set for whom no major protocol violations have been recorded.

Demographic and baseline characteristics will be analyzed for all three analysis sets. General safety data will be evaluated using the safety analysis set. Efficacy parameters will be evaluated using the full analysis set, the per-protocol analysis set, and based on dose of IMP received.

13.4 Demographics and Baseline Characteristics

Demographics (age, sex, racial, ethnic group based on standard NIH categories) and baseline characteristics will be summarized by means of descriptive statistics for continuous data or frequency tables for categorical data. Demographic data and other baseline characteristics will be analyzed for all three-analysis sets.

13.5 Treatment and Study Compliance

The intervention in this study is a one-time injection that involves a procedure performed by study personnel so that there is limited concern with adherence to the intervention. The primary endpoint is based upon short-term outcomes and thus study compliance is expected to be good. Patients will be called by study coordinator to remind them of all study visits. Secondary endpoints are assessed up to 24 months after the intervention. To maximize study compliance, patients will receive regular reminders of study visits and all visits will be scheduled up to 1 year in advance.

The number of patients who complete each study visit and assessment will be provided in a CONSORT diagram at the time of publication.

13.6 Handling of Missing Data

Guidelines promulgated in the National Research Council report on handling of missing data will be followed[48,49].

13.7 Analyses of the Primary Endpoints

The primary objective of the study is to evaluate the safety and tolerability of MSCs for subjects with xerostomia after radiation for HNC.

The primary endpoint is the recommended starting dose of MSCs for a Phase I dose-escalation study, which will be determined by the proportion of subjects experiencing DLT, where DLT is defined as:

pain > 5 on a standard 10-point pain scale of 0-10 at 1-month after MSC injection OR any serious AE OR any of the selected toxicities listed in section 9.7, within one-month post-injection.

Adverse events will be processed in the statistical analysis after classification into standardized medical terminology using the NCI Common Terminology Criteria for Adverse Events, Version 5.0 (CTCAE v5.0) grouped by grade/severity, attribution/relatedness and expectedness.

All adverse events data will be listed in the individual patient data listings, including all information documented on the adverse event form. Separate listings will be provided likewise for serious adverse events, adverse events in subjects who died, and for adverse events leading to discontinuation of the study. Concomitant medication documented in relation to the occurrence of a serious adverse event after MSC administration on SAE forms will be listed separately as 'Concomitant medication as documented on SAE forms after MSC administration'.

Frequency tabulations of the number and percentage of patients with a pain rating of less than 6 on a VAS of 0-10 will be presented and displayed graphically with two-sided 95% confidence intervals using the Wilson score method.

Standard descriptive summary statistics (i.e., n, arithmetic mean, standard deviation, median, lower/upper quartiles, and minimum/maximum values) will be calculated for continuous variables. Categorical data will be presented in frequency tables using counts and percentages. Graphical presentation will be given by means of box and whisker plots and bar charts, as appropriate.

13.8 Analyses of the Secondary Endpoints

Analyses of the secondary endpoints focus on efficacy of the IMP in the treatment of xerostomia and hyposalivation. All inferential analyses for the secondary outcome variables will be interpreted in the exploratory sense, only.

Patient-reported outcome measures will be evaluated by comparing the changes in salivary QOL from baseline to each follow-up visit and by assessing changes over time, within-group comparisons will be performed with the Wilcoxon signed-rank test.

Salivary flow rate analysis will be analyzed in a manner described by Gronhoj et al. 2018[24]: by comparing the changes from baseline to each follow-up visit and changes over time, within-group comparisons will be performed with the Wilcoxon signed-rank test.

Salivary composition analyses will focus on qualitative aspects of saliva previously found to change following radiation treatment: salivary pH[39,40], total protein concentration (mg/mL)[41,42], amylase (mU/mL)[39,43], and mucins (MUC5B, mU/mL)[43,44]. Given that MSCs will be injected into one of the submandibular glands which contain both serous and mucous acinar secretory cells, we will measure products of both types of cells (both= total protein and pH; serous= amylase; mucous= mucins). Salivary pH will be measured using a pH meter (Orion 9810BN, Thermo Scientific). Enzyme-linked immunosorbent assays (ELISAs) will be used to quantify total protein, amylase, and mucin levels. Saliva composition will be assessed on whole saliva samples collected from subjects at the screening visit and the in-person follow-up visits.

To assess the imaging characteristics of submandibular gland after MSC injection, salivary gland size will be measured by ultrasound[45]. Ultrasound imaging is a non-ionizing, noninvasive imaging modality that can be quickly obtained in a standard exam room. Three-dimensional (3D) Bmode data sets will be acquired for tumor volume measurements. If post-RT submandibular glands suppress ultrasound waves to prevent the visualization of the lower outline of the gland, a 2D estimate of gland size will be made.

Standard descriptive summary statistics (i.e., n, arithmetic mean, standard deviation, median, lower/upper quartiles, and minimum/maximum values) will be calculated for continuous variables. Categorical data will be presented in frequency tables using counts and percentages. Graphical presentation will be given by means of box and whisker plots and bar charts, as appropriate.

Results from physical examinations will be summarized for each scheduled study visit by means of a frequency table.

Briefly, assessment of feasibility for a future Phase I dose-escalation study will be accomplished by analyzing the drop-out rate and reasons for drop-out, time from patient inclusion to administration of the IMP and successful production of MSC IMP on an intent to treat basis.

13.9 Interim Analyses

There is no interim analysis for this pilot study.

14.0 RISK/BENEFIT ASSESSMENT

14.1 Potential Benefits to the Subjects

The potential benefits to research subjects associated with this study include the possible improvement of radiation-induced xerostomia and hyposalivation. If this study is able to demonstrate the safety, tolerability, and possible therapeutic benefit of MSCs in radiation-induced xerostomia and hyposalivation, the community of HNC patients who undergo radiation would benefit by the availability of a treatment option. The MESRIX trial has demonstrated an improvement in salivary production and quality of life after MSC injection[24].

14.2 Known Potential Risks

Known Procedural Risks

- Injection of MSCs: The risks of MSC injection include pain or discomfort within the salivary gland; pain or discomfort at the site of injection; possible bruising and swelling around the injection site; rarely an infection, potentially diminished function of the submandibular gland, damage to small nerves that are located near the submandibular gland – this risk will be minimized by injection with ultrasound guidance.
- Bone Marrow Aspiration: Potential risks from bone marrow aspiration include bruising and mild transient pain around the needle site. There is a small risk of infection around the bone marrow access site that could start locally, but spread systemically. There is also a small risk of severe bleeding around the aspiration site or internally. If sedation is performed there is a small risk of slowed breathing during the procedure and nausea, headache, or feeling drowsy after the procedure.
- Blood Draw: The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

Known Interventional Risks

At present there has not been a defined dose limiting toxicity of MSCs and to date no serious adverse events have been reported following the infusion of either autologous or allogeneic MSCs. Of the toxicities noted so far in MSCs trials a minor (i.e. <10%) portion of patients reported a rash following infusion which can be directly linked to the cryopreservative (DMSO) used in most MSC trial where the cell dose is thawed at the bedside and immediately infused without removal of majority of

DMSO. In our trial the cells will be delivered after being thawed in media and culture rescued for 16 hours to ensure removal of DMSO and thus we do not expect to see any of these types of reactions.

Despite the strong safety profile MSC infusion has shown to date there is the slim possibility that interferon-gamma activated MSCs could result in certain systemic (acute and delayed) reactions. As such each subject will be evaluated and monitored before during and following injection of MSCs during the study.

Systemic reaction is any untoward medical hypersensitivity-like event other than injection site reaction, occurring during or after MSCs infusion administration that can be at least possibly attributed to the MSC infusion. Systemic reaction is further classified as acute and delayed based on timing and presentation of symptoms typical for hypersensitivity reactions. Acute and delayed reactions (within the first 7 days) to MSCs will be reported according to the judgment of the Investigator, based on the typical clinical features:

- Hypotension
- Urticaria
- Flushing
- Facial or hand edema
- Throat tightness
- Oral cavity or lip edema
- Headache
- Shortness of breath

14.3 Risk/Benefit Analysis

The potential procedural risks and the minimal interventional risk are outweighed by the likely benefit of improvement of radiation-induced xerostomia. The procedural risks will be minimized by the use of a skilled practitioner injecting all MSCs under ultrasound guidance. The subjects will be observed for 30 minutes after MSC injection to ensure no acute systemic reaction has occurred.

There have been no SAEs or DLTs occurring within 1 month of MSC injection (primary objective), supporting the safety and low risk of MSC injection into a submandibular gland. Additionally, the cryopreserved MSCs that will be utilized for the second injection are identical to the MSCs used for the first injection. The stability of the cryopreserved MSCs has been validated by the PACT GMP facility. MSCs for the second injection will undergo the same quality control process and will have the same release criteria as the MSCs for the initial injection. There is a very small risk that the crypreserved MSC IMP used for the second injection is not as effective as the MSC IMP used for the first injection. Additionally, the second injection should carry with it the exact same, very small, risk for infection, bleeding, etc. detailed above. There is no known increased risk in the second injection of MSC IMP as compared to the first injection. The likely benefit of an improvement in radiation-induced xerostomia is increased when MSCs are injected into both submandibular glands to allow for optimal tissue recovery. Injecting both submandibular glands has the proposed benefit of allowing both salivary glands to recover and repopulate, leading to reduced xerostomia and improved quality of life.

15.0 ADVERSE EVENTS REPORTING REQUIREMENTS

15.1 Overview of Adverse Event Reporting

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future

studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care. The first step is to identify the event using the Common Toxicity Criteria for Adverse Events (CTCAE). The severity of the event should then be graded using the CTCAE criteria. Next, it must be determined if the adverse event is related to the study treatment (attribution). If so, determine whether the adverse event is expected or unexpected. Using this information and the adverse event reporting section of the protocol, it can be determined whether an adverse event should be reported in an expedited report or a routine report.

15.2 Common Terminology Criteria for Critical Adverse Events (CTCAE)

The CTCAE document provides descriptive terminology for adverse event reporting. A grading (severity) scale is provided for each adverse event term. This study will utilize version 5.0 of the CTCAE of the National Cancer Institute for toxicity and performance reporting. A copy of the CTCAE version 5.0 can be downloaded from the NCI website at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

15.3 Definition of Adverse Event

An *adverse event* (AE) is defined as any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

15.4 Grading the Severity of Adverse Events

Guidance for grading each type of event is found in the CTCAE. Assessment of severity is frequently subjective and medical judgment should be used to compare the reported adverse events to similar types of events observed in clinical practice. **It is important to recognize that severity is not equivalent to event seriousness**; severity is a measure of intensity; thus, a severe event may not necessarily be classified as a serious event. Definitions of the grading categories to assess severity of adverse events are as follows:

- ***Mild*** *(grade 1)*: the event causes a sign or symptom barely noticeable to the patient without disruption of normal daily activities
- ***Moderate*** *(grade 2)*: the event causes sufficient discomfort to interfere with normal daily activities and may require treatment of symptoms
- ***Severe*** *(grade 3)*: an event of sufficient severity to cause the patient severe discomfort and leaving the patient unable to perform normal daily activities; Symptoms may be resistant to conventional symptomatic treatment.
- ***Life threatening*** *(grade 4):* the patient was at risk of death at the time of the event.
- ***Fatal*** *(grade 5):* the event caused death.

15.5 Causality/Attribution

The causality, or attribution, of AEs refers to the relationship of the AE to the experimental intervention (IMP). The investigator, using the following points, makes the assessment of whether there is a reasonable possibility of a causal relationship:

- Timing of the event between administration of IMP and the onset of adverse event
- Dose of IMP and evidence, if any, of overdose

- A known or expected response pattern to the suspected IMP based on previous experience
- Physiological effects/pharmacological action of the IMP
- Adverse events known to occur with the IMP belonging to the same or a similar class
- Specific genetic predisposition of the patient

Based on the aforementioned points, the PI assigns attribution of the adverse event to the study treatment. Attribution categories are as follows:

- Definite: The AE is clearly related to the study treatment
- Probably: 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

15.6 Seriousness of Adverse Event

Adverse events that meet one or more of the following are classified as serious:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours)
- Results in persistent or significant disability or incapacity
- Produces a congenital abnormality or birth defect
- a medical event, based on appropriate medical judgment, that is believed to jeopardize the patient and/or requires medical or surgical intervention to prevent one of the outcomes defining a SAE. For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that may not result in hospitalization.

15.7 Determination of Expectedness

Expected events are those that have been previously identified as resulting from administration of the intervention. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is **not** listed in:

- Current known adverse events listed in this protocol
- Investigator Brochure (for agents provided under an IND)

15.8 Recording Adverse Events

- All adverse events regardless of attribuition will be recorded from the time of the research blood draw through the 1 month post MSC injection visit.
- Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event, unless the condition existed prior to the patient's MSC IMP injection.
- If an AE is determined to be caused by the IMP (definitely related) or associated with the IMP (probably related or possibly related), and is a CTCAE v5.0 grade 3 or higher, it will be reported regardless of the timeframe of the occurrence.

15.9 Baseline Adverse Events

An expedited adverse event report will NOT be submitted if a patient is entered on this study with a preexisting condition (e.g., elevated laboratory value). If the preexisting condition worsens in severity,

the investigator will reassess the event to determine attribution and whether the event requires reporting. If the adverse event resolves and then recurs, the investigator will reassess the event to determine if the event will be reported.

15.10 Expedited Reporting

Refer to Reporting Table in Section 16.4.

15.11 Required Reporting

Reporting requirements to the UW Health Sciences Institutional Review Board (HS-IRB)

The UW HS-IRB website will be referenced for real-time guidance regarding current reporting requirements.

https://irb.wisc.edu/wp-content/uploads/sites/2/sites/2/2021/11/HRP-103-INVESTIGATOR-MANUAL_2021-10-29.pdf. *Pregnancies will be reported to the UWCCC DSMC, IRB. Additionally, Dr. Galipeau, in his role as Sponsor, will report pregnancies to the FDA as required.*

Reporting requirements to the FDA

Any unexpected fatal or unexpected life-threatening event possibly due to the investigational agent, will be reported to the FDA by telephone or fax as soon as possible but no later than 7 calendar days after notification of the event and followed by a written safety report as complete as possible within 8 additional calendar days (i.e., full report 15 calendar days total after notification of event).

Unexpected, non-fatal and non-life-threatening SAEs that are considered due to or possibly due to the study intervention, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the Annual Review Report along with non-serious AEs.

15.12 Follow-up of Adverse Events

Only grade 3 or 4 Adverse Events (AEs) that are thought to be at least possibly related to the study will be followed until the event has resolved or, in the case of a clinically significant abnormal laboratory value, it has returned to baseline or stabilized at an acceptable level according to the PI.

- There is a satisfactory explanation for the changes observed, or
- The patient is lost to follow-up or off study

16.0 DATA AND SAFETY MONITORING PLAN

16.1 Oversight and Monitoring Plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A complete IND Study Monitoring Plan is located in the electronic records component of the Regulatory Binder for this IND. The IND Study Monitoring Plan describes the strategies, responsibilities, and quality management activities in place to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality and integrity of the resulting data, in compliance with applicable laws, regulations, policies, and guidance. Quality management activities include both internal and external quality management processes used throught the study, including but not limited to staff training, standardized

procedures, methods for data collection, study and data monitoring, and routine team meetings to review the study progress and isolate any complicance issues and/or trends. A summary of UWCCC DSMC activities is as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which the UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMS elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports) described in Section II of this document.
- Notifies the protocol PI of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the protocol Principal Investigator.
- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.
- The decision to proceed with enrollment of a subject into the next higher dose group will be made by the study PI and the statistician after review of the AEs within the dose cohort
- The DSMC will have oversight of the attribution of SAEs throughout the duration of the protocol.

16.2 Monitoring and Reporting Guidelines

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the PRMC. All protocols (including Intervention Trials, Non-Intervention Trials, Behavioral and Nutritional Studies, and trials conducted under a Training Grant) are evaluated by the PRMC at the time of committee review. Based on UWCCC PRMC review, monitoring requirements for this trials are as follows:

Intensive Monitoring

Protocols subject to intensive monitoring generally include UW Institutional Phase I and Institutional Trials of any phase involving recombinant DNA/gene transfer. These protocols undergo continuous review of data and subject safety at weekly Disease Oriented Team (DOT) meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOT meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a quarterly basis by the study team for review by the DSMC.

16.3 Review and Oversight Requirements

- Serious Adverse Event: Report **Within 24 Hours**
  - Serious Adverse Events requiring reporting within 24 hours must also be reported to the UWCCC Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day.

- The OnCore SAE Details Report must be submitted along with other report materials as appropriate (The DSMC Chair will review the information and determine if immediate action is required.
- Within 7 calendar days, all available subsequent SAE documentation must be submitted electronically along with a 24-hour follow-up SAE Details Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.
- The Principal Investigator notifies all investigators involved with the study at the UWCCC, the HS-IRB, and funding agencies and provides documentation of these notifications to the DSMC.
- The PI reviews the event in conjunction with the UWCCC Sponsor Investigator Determination Form to determine whether the SAE requires reporting to the FDA.

- Serious Adverse Event: Report **Within 15 Days**
  - Serious Adverse Events requiring reporting within 15 days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu.
  - The OnCore SAE Details Report must be submitted along with other report materials as appropriate (FDA MedWatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.
  - The Principal Investigator notifies all investigators involved with the study at the UWCCC, the HS-IRB, and funding agencies and provides documentation of these notifications to the DSMC.
  - The PI reviews the event in conjunction with the UWCCC Sponsor Investigator Determination Form to determine whether the SAE requires reporting to the FDA and other participating investigators.
  - If a patient experiences any serious and unexpected adverse effect that could be attributable to receipt of a cellular product that has failed a sterility test, then a report of this information will be submitted to the FDA, IRB, DSMC and participating investigators.

- Report to FDA **Within 30 Calendar Days**
  - In the case that a patient receives a product that results in a positive sterility test, a report will be submitted to the FDA within 30 calendar days after initial receipt of the positive sterility results. The report will include information regarding the sterility failure, results of an investigation and any cause if determined, and any corrective or preventative actions.

- Principal Investigator and Sponsor Responsibilities for SAE Review
  - Dr. Randall Kimple reviews all reports of serious adverse events occurring on the study and makes a determination of 1) attribution and 2) expectedness in the context of this study. Dr. Jacques Galipeau, MD FRCP(C), UW Professor in Oncology, Assistant Dean for Therapeutics Discovery and Development, and Director of the Program for Advanced Cell Therapy, holds the IND for this study and therefore assumes responsibility of the study sponsor in accordance with FDA 21 CFR 312.32. He is responsible for reporting SAEs to the FDA.
  - SAE with suspected causality to study treatment and deemed unexpected are reported as IND Safety Reports by the Sponsor to the FDA and all participating investigators on the study within 15 calendar days.
  - All fatal or life-threatening SAE that are unexpected and have suspected causality to the study treatment will be reported by Sponsor to the FDA and all participating investigators on the study within 7 calendar days.

- Study Progress Review
  - Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly; semi-annually; or annually). The PSR provides a cumulative report of SAEs, as well as instances of noncompliance, protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting.
  - Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external DSMC or DSMC reports, and any other pertinent study-related information.
  - In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the PI of the DSMC findings and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

16.4 Expedited Reporting of Serious Adverse Events

- Depending on the nature, severity, and attribution of the serious adverse event, an SAE report will be phoned in, submitted in writing, or both according to the Table below.
  -
  - Reporting of expedited and final SAE reports will go to:
  - 1)UWCCC DSMC via email at: *saenotify@uwcarbone.wisc.edu*
  - 2) Dr. Jacques Galipeau, MD FRCP(C), UW Professor in Oncology, Assistant Dean for Therapeutics Discovery and Development, and Director of the Program for Advanced Cell Therapy, via email at: jgalipeau@medicine.wisc.edu_____

Reporting to the UW IRB will be per IRB guidelines.

Determine the reporting time line for the SAE in question by using Table 3 below.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1,2].

Table 4. FDA Reporting Requirements.

FDA Reporting Requirements for Serious Adverse Events (21 CRF Part 312)
NOTE: Investigators **MUST** immediately report to the UWCCC DSMC and any other parties outlined in the protocol ANY Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). An adverse event is considered serious if it results in ANY of the following outcomes: 1) Death. 2) A life-threatening adverse event (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

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 disruption 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 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 (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events within 30 days of IMP injection that meet the above criteria **MUST** be immediately reported to the UWCCC within the timeframes detailed in the table below:

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in hospitalization ≥ 24 hrs	15 Calendar Days	24 Hour; 7 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- **24-Hour; 7 Calendar Days** – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 7 calendar days of the initial 24-hour report.
- **15 Calendar Days** – A complete expedited report on the AE must be submitted within 15 calendar days of learning of the AE

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of probable or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 7 calendar days for:

- All Grade 3, 4, and 5 SAEs

Expedited 15 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

16.5 Study Stopping Rules

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB, FDA, and will provide the reason(s) for the termination or suspension.

Patient enrollment and treatment will be temporarily halted if the following rules occur, pending review by the UWCCC DSMC, and FDA if:

- Any death, irrespective of attribution by the investigator
- Any (CTCAE v5.0) of grade 4 or higher
- Any hypersensitivity reaction grade 3 or higher at any time
- Any grade 3 SAE in the first 3 subjects
- Any 2 grade 3 or higher of the same SAE at any time during the study
- 5 or more grade 3 or higher SAEs that are at least possibly related at any time during the study

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

- Determination of unexpected, significant, or unacceptable risk to subjects
- Data that are not sufficiently complete and/or evaluable

If the study is suspended, it will not be reopened without prior FDA approval and without protocol modifications if recommended by the FDA or UWCCC DMSC. If the study is suspended, all subjects will still be followed as specified in the protocol.

Any change to the study stopping rules must first be reviewed by the FDA.

17.0 STUDY FEASIBILITY

17.1 Economic Burden to Subjects

Subjects will not have to pay for study procedures. The subject will not be billed by the healthcare system or their health insurance company for any costs related to a study procedure.

17.2 Facilities and Locations

All activities of the trial will be conducted at the UW Carbone Cancer Center, UW Hospital, Wisconsin Institute for Medical Research (WIMR) or UW Otoloaryngology Clinic.

17.3 Feasibility of Recruiting the Required Number of Subjects

The Department of Radiation Oncology sees 2-20 eligible subjects/week for follow-up. Less than five percent of the eligible subjects seen would need to be recruited in order to achieve the required number of clinical trial subjects.

17.4 Principal Investigator Considerations

Time Devoted to Conducting the Research

Dr. Kimple has allocated a sufficient amount of time to be devoted to this clinical trial.

Process for Informing Study Teams

All research staff interacting with subjects will have completed HIPAA and human subjects training. Research staff will have been trained in salivary collection procedures. A board-certified otolaryngology physician (Dr. Glazer) from the Division of Otolaryngology-Head & Neck Surgery in the Department of Surgery at UW-Madison will administer the MSC IMP to subjects. Dr. Galzer has allocated a sufficient amount of time to be devoted to this clinical trial.

17.5 Availability of Medical or Psychological Resources

Not applicable.

18.0 REFERENCES

1. Marta GN, Silva V, de Andrade Carvalho H, et al. Intensity-modulated radiation therapy for head and neck cancer: systematic review and meta-analysis. *Radiother Oncol*. Jan 2014;110(1):9-15. doi:10.1016/j.radonc.2013.11.010
2. Visvanathan V, Nix P. Managing the patient presenting with xerostomia: a review. *Int J Clin Pract*. Feb 2010;64(3):404-7. doi:10.1111/j.1742-1241.2009.02132.x
3. Gornitsky M, Shenouda G, Sultanem K, et al. Double-blind randomized, placebo-controlled study of pilocarpine to salvage salivary gland function during radiotherapy of patients with head and neck cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. Jul 2004;98(1):45-52. doi:10.1016/s1079210404002859
4. Aliko A, Alushi A, Tafaj A, Isufi R. Evaluation of the clinical efficacy of Biotene Oral Balance in patients with secondary Sjogren's syndrome: a pilot study. *Rheumatol Int*. Sep 2012;32(9):2877-81. doi:10.1007/s00296-011-2085-6
5. Shahdad SA, Taylor C, Barclay SC, Steen IN, Preshaw PM. A double-blind, crossover study of Biotene Oralbalance and BioXtra systems as salivary substitutes in patients with post-radiotherapy xerostomia. *Eur J Cancer Care (Engl)*. Sep 2005;14(4):319-26. doi:10.1111/j.1365-2354.2005.00587.x
6. Baum BJ. Principles of saliva secretion. *Ann N Y Acad Sci*. Sep 20 1993;694:17-23.
7. Maimets M, Rocchi C, Bron R, et al. Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals. *Stem Cell Reports*. Jan 12 2016;6(1):150-62. doi:10.1016/j.stemcr.2015.11.009
8. Xiao N, Lin Y, Cao H, et al. Neurotrophic factor GDNF promotes survival of salivary stem cells. *J Clin Invest*. Aug 2014;124(8):3364-77. doi:10.1172/jci74096
9. Sensebe L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. *Hum Gene Ther*. Jan 2011;22(1):19-26. doi:10.1089/hum.2010.197
10. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-7. doi:10.1080/14653240600855905
11. Horwitz EM, Le Blanc K, Dominici M, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*. 2005;7(5):393-5. doi:10.1080/14653240500319234
12. Ren J, Jin P, Sabatino M, et al. Global transcriptome analysis of human bone marrow stromal cells (BMSC) reveals proliferative, mobile and interactive cells that produce abundant extracellular matrix proteins, some of which may affect BMSC potency. *Cytotherapy*. Jul 2011;13(6):661-74. doi:10.3109/14653249.2010.548379
13. Romieu-Mourez R, Coutu DL, Galipeau J. The immune plasticity of mesenchymal stromal cells from mice and men: concordances and discrepancies. *Front Biosci (Elite Ed)*. Jan 1 2012;4:824-37.
14. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. *Nat Rev Immunol*. Apr 25 2012;12(5):383-96. doi:10.1038/nri3209
15. Burt RK, Loh Y, Pearce W, et al. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. *Jama*. Feb 27 2008;299(8):925-36. doi:10.1001/jama.299.8.925
16. Aoki R, Shoshkes-Carmel M, Gao N, et al. Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. *Cell Mol Gastroenterol Hepatol*. Feb 1 2016;2(2):175-188. doi:10.1016/j.jcmgh.2015.12.004
17. Galipeau J, Sensebe L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell*. Jun 1 2018;22(6):824-833. doi:10.1016/j.stem.2018.05.004

18. Chinnadurai R, Copland IB, Garcia MA, et al. Cryopreserved Mesenchymal Stromal Cells Are Susceptible to T-Cell Mediated Apoptosis Which Is Partly Rescued by IFNgamma Licensing. *Stem Cells*. Sep 2016;34(9):2429-42. doi:10.1002/stem.2415
19. Chinnadurai R, Rajan D, Ng S, et al. Immune dysfunctionality of replicative senescent mesenchymal stromal cells is corrected by IFNgamma priming. *Blood Adv*. Apr 25 2017;1(11):628-643. doi:10.1182/bloodadvances.2017006205
20. Kim DS, Jang IK, Lee MW, et al. Enhanced Immunosuppressive Properties of Human Mesenchymal Stem Cells Primed by Interferon-gamma. *EBioMedicine*. Feb 2018;28:261-273. doi:10.1016/j.ebiom.2018.01.002
21. Jensen DH, Oliveri RS, Trojahn Kolle SF, et al. Mesenchymal stem cell therapy for salivary gland dysfunction and xerostomia: a systematic review of preclinical studies. *Oral Surg Oral Med Oral Pathol Oral Radiol*. Mar 2014;117(3):335-342.e1. doi:10.1016/j.oooo.2013.11.496
22. Sumita Y, Liu Y, Khalili S, et al. Bone marrow-derived cells rescue salivary gland function in mice with head and neck irradiation. *Int J Biochem Cell Biol*. Jan 2011;43(1):80-7. doi:10.1016/j.biocel.2010.09.023
23. Tran SD, Sumita Y, Khalili S. Bone marrow-derived cells: A potential approach for the treatment of xerostomia. *Int J Biochem Cell Biol*. Jan 2011;43(1):5-9. doi:10.1016/j.biocel.2010.10.010
24. Gronhoj C, Jensen DH, Vester-Glowinski P, et al. Safety and Efficacy of Mesenchymal Stem Cells for Radiation-Induced Xerostomia: A Randomized, Placebo-Controlled Phase 1/2 Trial (MESRIX). *Int J Radiat Oncol Biol Phys*. Jul 1 2018;101(3):581-592. doi:10.1016/j.ijrobp.2018.02.034
25. Prockop DJ, Brenner M, Fibbe WE, et al. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy*. Sep 2010;12(5):576-8. doi:10.3109/14653249.2010.507330
26. Casiraghi F, Remuzzi G, Abbate M, Perico N. Multipotent mesenchymal stromal cell therapy and risk of malignancies. *Stem Cell Rev*. Feb 2013;9(1):65-79. doi:10.1007/s12015-011-9345-4
27. Bain BJ. Morbidity associated with bone marrow aspiration and trephine biopsy - a review of UK data for 2004. *Haematologica*. Sep 2006;91(9):1293-4.
28. Kim YH, Cha SM, Naidu S, Hwang WJ. Analysis of postoperative complications for superficial liposuction: a review of 2398 cases. *Plast Reconstr Surg*. Feb 2011;127(2):863-71. doi:10.1097/PRS.0b013e318200afbf
29. Roustaei N, Masoumi Lari SJ, Chalian M, Chalian H, Bakhshandeh H. Safety of ultrasound-assisted liposuction: a survey of 660 operations. *Aesthetic Plast Surg*. Mar 2009;33(2):213-8. doi:10.1007/s00266-008-9293-9
30. Mamidi MK, Singh G, Husin JM, et al. Impact of passing mesenchymal stem cells through smaller bore size needles for subsequent use in patients for clinical or cosmetic indications. *J Transl Med*. Nov 21 2012;10:229. doi:10.1186/1479-5876-10-229
31. Hjermstad MJ, Fayers PM, Haugen DF, et al. Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. *J Pain Symptom Manage*. Jun 2011;41(6):1073-93. doi:10.1016/j.jpainsymman.2010.08.016
32. Jensen MP, Turner JA, Romano JM. What is the maximum number of levels needed in pain intensity measurement? *Pain*. Sep 1994;58(3):387-92. doi:10.1016/0304-3959(94)90133-3
33. Henson BS, Inglehart MR, Eisbruch A, Ship JA. Preserved salivary output and xerostomia-related quality of life in head and neck cancer patients receiving parotid-sparing radiotherapy. *Oral Oncol*. Jan 2001;37(1):84-93.
34. Chen AY, Frankowski R, Bishop-Leone J, et al. The development and validation of a dysphagia-specific quality-of-life questionnaire for patients with head and neck cancer: the M. D. Anderson dysphagia inventory. *Arch Otolaryngol Head Neck Surg*. Jul 2001;127(7):870-6.
35. Pai S, Ghezzi EM, Ship JA. Development of a Visual Analogue Scale questionnaire for subjective assessment of salivary dysfunction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. Mar 2001;91(3):311-6. doi:10.1067/moe.2001.111551
36. Navazesh M, Kumar SK. Measuring salivary flow: challenges and opportunities. *J Am Dent Assoc*. May 2008;139 Suppl:35s-40s.
37. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci*. Sep 20 1993;694:72-7.

38. Kaae JK, Stenfeldt L, Eriksen JG. Xerostomia after Radiotherapy for Oral and Oropharyngeal Cancer: Increasing Salivary Flow with Tasteless Sugar-free Chewing Gum. *Front Oncol*. 2016;6:111. doi:10.3389/fonc.2016.00111
39. Chitra S, Shyamala Devi CS. Effects of radiation and alpha-tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer. *Indian J Dent Res*. Jul-Sep 2008;19(3):213-8.
40. Sim C, Soong YL, Pang E, et al. Xerostomia, salivary characteristics and gland volumes following intensity-modulated radiotherapy for nasopharyngeal carcinoma: a two-year follow up. *Aust Dent J*. Jun 2018;63(2):217-223. doi:10.1111/adj.12608
41. Richards TM, Hurley T, Grove L, et al. The effect of parotid gland-sparing intensity-modulated radiotherapy on salivary composition, flow rate and xerostomia measures. *Oral Dis*. Oct 2017;23(7):990-1000. doi:10.1111/odi.12686
42. Valdez IH, Atkinson JC, Ship JA, Fox PC. Major salivary gland function in patients with radiation-induced xerostomia: flow rates and sialochemistry. *Int J Radiat Oncol Biol Phys*. Jan 1993;25(1):41-7.
43. Almstahl A, Wikstrom M, Groenink J. Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins. *Oral Microbiol Immunol*. Dec 2001;16(6):345-52.
44. Dijkema T, Terhaard CH, Roesink JM, et al. MUC5B levels in submandibular gland saliva of patients treated with radiotherapy for head-and-neck cancer: a pilot study. *Radiat Oncol*. Jun 15 2012;7:91. doi:10.1186/1748-717x-7-91
45. Bialek EJ, Jakubowski W, Zajkowski P, Szopinski KT, Osmolski A. US of the major salivary glands: anatomy and spatial relationships, pathologic conditions, and pitfalls. *Radiographics*. May-Jun 2006;26(3):745-63. doi:10.1148/rg.263055024
46. Cindil E, Oktar SO, Akkan K, et al. Ultrasound elastography in assessment of salivary glands involvement in primary Sjogren's syndrome. *Clin Imaging*. Jul - Aug 2018;50:229-234. doi:10.1016/j.clinimag.2018.04.011
47. Zhang S, Zhu J, Zhang X, He J, Li J. Assessment of the Stiffness of Major Salivary Glands in Primary Sjogren's Syndrome through Quantitative Acoustic Radiation Force Impulse Imaging. *Ultrasound Med Biol*. Mar 2016;42(3):645-53. doi:10.1016/j.ultrasmedbio.2015.11.009
48. National Research Council Panel on Handling Missing Data in Clinical T. *The Prevention and Treatment of Missing Data in Clinical Trials*. National Academies Press (US)

49. Little RJ, D'Agostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. *N Engl J Med*. Oct 4 2012;367(14):1355-60. doi:10.1056/NEJMsr1203730

19.0 APPENDICES

19.1 XeQoL

These questions are concerned with your oral health and how it affects your life. Please answer the questions by checking the box that describes best how true each statement has been for you during **the past 7 days**:

1. My mouth/throat dryness limits the kinds or amounts of food I eat.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

2. My mouth/throat dryness causes discomfort.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

3. My mouth/throat dryness causes a lot of worry or concern.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

4. My mouth/throat dryness keeps me from socializing (going out).

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

5. My mouth/throat dryness makes me uncomfortable when eating in front of other people.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

6. My mouth/throat dryness makes me uncomfortable speaking in front of other people.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

7. My mouth/throat dryness makes me nervous.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

8. My mouth/throat dryness makes me concerned about the looks of my teeth and mouth.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

9. My mouth/throat dryness keeps me from enjoying life.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

10. My mouth/throat dryness interferes with my daily activities.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

11. My mouth/throat dryness interferes with my intimate relationships.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

12. My mouth/throat dryness has a bad effect on tasting food.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

13. My mouth/throat dryness reduces my general happiness with life.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

14. My mouth/throat dryness affects all aspects of my life.

❑ Not at all ❑ A little ❑ Somewhat ❑ Quite a bit ❑ Very much

15. If you were to spend the rest of your life with your mouth/throat dryness just the way it is now, how would you feel about this?

❑ Delighted ❑ Mostly satisfied ❑ Mixed: equally satisfied/dissatisfied ❑ Mostly dissatisfied ❑ Terrible

Scoring: Each of the 15 items receive a score of 0 to 4. Items 1-14 are scored as: 0=not at all; 1=a little; 2=somewhat;3=quite a bit;4=very much. Item 15 is scored as: 0=delighted; 1=mostly satisfied; 2=mixed: equally saistifed/dissatisfied; 3= mostly dissatisfied; 4=terrible. Physical functioning is based upon responses to items Nos.1, 6, 10, 12. Pain/discomfort issues are based upon responses to items Nos. 2, 3, 7, 9. Personal/psychological functioning is based upon responses to items Nos. 8, 13, 14, 15. Social functioning is based upon responses to items Nos. 4, 5, 11. Each XeQoLS domain score is calculated by

averaging the values of all the respective items for that individual domain (maximum score per scale: 4). A total average XeQoLS score can also be calculated, if overall XeQoL is of interest.

Source: Henson BS, Inglehart MR, Eisbruch A, Ship JA. Preserved salivary output and xerostomia-related quality of life in head and neck cancer patients receiving parotid-sparing radiotherapy. Oral Oncology 2001;37(1):84-93.

19.2 MDADI Questionnaire

The M.D. Anderson Dysphagia Inventory

This questionnaire asks for your views about your swallowing ability. This information will help us understand how you feel about swallowing.

The following statements have been made by people who have problems with their swallowing. Some of the statements may apply to you.

Please read each statement and circle the response which best reflects your experience in the past week.

My swallowing ability limits my day-to-day activities.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E2. I am embarrassed by my eating habits.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

F1. People have difficulty cooking for me.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P2. Swallowing is more difficult at the end of the day.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E7. I do not feel self-conscious when I eat.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E4. I am upset by my swallowing problem.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P6. Swallowing takes great effort.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E5. I do not go out because of my swallowing problem.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

F5. My swallowing difficulty has caused me to lose income.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P7. It takes me longer to eat because of my swallowing problem.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P3. People ask me, "Why can't you eat that?"

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E3. Other people are irritated by my eating problem.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P8. I cough when I try to drink liquids.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

F3. My swallowing problems limit my social and personal life.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

F2. I feel free to go out to eat with my friends, neighbors, and relatives.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P5. I limit my food intake because of my swallowing difficulty.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P1. I cannot maintain my weight because of my swallowing problems.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

E6. I have low self-esteem because of my swallowing problems.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

P4. I feel that I am swallowing a huge amount of food.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

F4. I feel excluded because of my eating habits.

Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree

Thank you for completing this questionnaire!

Source: Chen AY, Frankowski R, Bishop-Leone J, et al. The development and validation of a dysphagia-specific quality-of-life questionnaire for patients with head and neck cancer: the M. D. Anderson dysphagia inventory. Arch Otolaryngol Head Neck Surg. 2001;127:870–876. [PubMed]

Scoring

Two scores are obtained: a Global Score and a Composite Score.

All questions except for E7 and F2:
- Strongly Agree = 1 point
- Agree = 2 points
- No Opinion = 3 points
- Disagree = 4 points
- Strongly Disagree = 5 point

E7 and F2:
- Strongly Agree = 5 points
- Agree = 4 points
- No Opinion = 3 points
- Disagree = 2 points
- Strongly Disagree = 1 point

Global Score: first question (not numbered)
- Global Score ranges from 1 (extremely low functioning) to 5 (high functioning)

Composite Score: 19 numbered questions
- Calculate total points: add scores for the 19 questions
- Calculate the mean point score: Divide total points by 19
- Calculate final score: Multiply the mean by 20

Composite Score ranges from 20 (extremely low functioning) to 100 (high functioning)

19.3 VAS Xerostomia Questionnaire

1. **Rate the difficulty you experience in speaking due to dryness (DIFSPK)**

   Not difficult at all — Very difficult

2. **Rate the difficulty you experience in swallowing due to dryness (DIFSWL)**

   Not difficult at all — Very difficult

3. **Rate how much saliva is in your mouth (SALMOU)**

   A lot — None

4. **Rate the dryness of your mouth (DRYMOU)**

   Not dry at all — Very dry

5. **Rate the dryness of your throat (DRYTHR)**

   Not dry at all — Very dry

6. **Rate the dryness of your lips (DRYLIP)**

   Not dry at all — Very dry

7. **Rate the dryness of your tongue (DRYTNG)**

   Not dry at all — Very dry

8. **Rate the level of your thirst (LVLTHR)**

   Not thirsty at all — Very thirsty

19.4 Stability Documentation

Test	Criteria	Specifications	T=0 (09FEB2022)	T=1 (29JUN2022)	T=2 (11JAN2023)
Purity	%CD45^{+}	≤ 5%	0%	1%	0%
Identity	%CD105^{+}	≥ 95%	100%	100%	100%
	%CD90^{+}	≥ 95%	100%	100%	99%
	%CD73^{+}	≥ 95%	100%	100%	100%
Potency	Fold increase in MFI after IFNɣ stimulation	≥ 2 for MHC I	5	4	4
		≥ 2 for MHC II	11	11	10
		≥ 2 for ICAM	70	67	50
		≥ 2 for IDO	17	14	13
		≥ 2 for PD-L1	4	4	4
Viability	% viable cells	≥70%	96%	97%	98%