Protocol Title: A Randomized Within-subject, Double-blind, Placebo-controlled Study of Dexamethasone Irrigation of the Parotid Glands in Primary Sjögren's Syndrome Subjects

Abbreviated Title: Dexamethasone Parotid Irrigation

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Total requested accrual:
Approximately 20 female subjects with primary Sjögren's syndrome will be screened, to randomize and treat 16 subjects.

Project Uses Ionizing Radiation ☐ No ☒ Yes (attach *RSC/RDSC documentation)*
- ☐ Medically-indicated only
- ☒ Research-related only
- ☐ Both

IND/IDE ☒ No ☐Yes *(attach FDA documentation)*
Drug/Device/#_____
Sponsor: _____

Durable Power of Attorney ☒ No ☐ Yes

Multi-institutional Project ☒ No ☐ Yes
Institution_____ FWA #_____
Date of IRB approval_____ *(attach IRB documentation)*

Data and Safety Monitoring Board ☐ No ☒ Yes

Technology Transfer Agreement ☒ No ☐ Yes
Agreement type and number _____
Expiration Date_____

Confidential Disclosure Agreement ☒ No ☐Yes

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: 8.7

Précis

Salivary gland dysfunction is one of the major manifestations of Sjögren's syndrome (SS). Although inflammation is thought to play an important role in the exocrinopathy, the correlation between glandular dysfunction and inflammation is limited. Systemic anti-inflammatory therapies tested to date, such as tumor necrosis factor antagonists, have not been effective treatments for SS salivary hypofunction, raising doubts about inflammation being the sole cause of salivary gland dysfunction. However, none of these trials tested whether an anti-inflammatory effect was achieved in glandular tissues.

Studies by Izumi et al found that a limited course of low-dose topical corticosteroid applied to the parotid glands resulted in sustained improvement in saliva production.[1,2] Unfortunately, these studies did not examine the mechanistic effects of corticosteroids on the major salivary glands. A plausible assumption is that corticosteroids improved salivary gland function by reducing inflammation, although other or associated mechanisms, such as an improved transcellular ion transport in epithelial cells cannot be ruled out. This study aims to study the efficacy of low-dose topical corticosteroid (dexamethasone) irrigation of the parotid gland in reducing salivary dysfunction in subjects with SS, and also to evaluate the effects of treatment on inflammation and other possible mechanistic processes.

Primary Objective

- To determine whether irrigation of the parotid gland with low-dose topical dexamethasone improves parotid salivary gland flow in SS subjects.

Secondary Objectives

- To perform mechanistic studies to determine the mechanisms of action of low-dose topical corticosteroid irrigation of the parotid gland.
- To assess biomarkers of inflammation and salivary gland dysfunction in SS subjects treated with low-dose topical corticosteroid irrigation of the parotid glands.
- To assess localized safety of dexamethasone irrigation of the parotid gland, as compared with placebo.

Study Population

The study will enroll up to 20 adult females with primary SS in order to randomize and treat 16 subjects. Key enrollment criterion include a focus score of ≥1 on minor salivary gland biopsy in the previous 7 years and measurable stimulated bilateral parotid salivary flow (≥0.01 mL/min per gland). Subjects will be recruited from protocol 84-D-0056, conducted at the National Institutes of Health (NIH).

Design

This will be a single-site, randomized-within-subject, double-blind, placebo-controlled, phase 2 pilot study in which all subjects receive both active drug (dexamethasone) and placebo (normal saline), thereby acting as their own controls. The study design is doubly-repeated measures; within a subject, measures are repeated in both time and treatment (i.e., one side of mouth receives dexamethasone while the other receives placebo.) After baseline assessment of salivary flow and other measurements of salivary function, subjects will be randomly assigned, in a double-blind fashion, to dexamethasone irrigation of one parotid gland and normal saline irrigation of the other parotid gland. They will undergo a total of 2 treatment sessions, 4 weeks apart (Days 0 and 28). Post-treatment assessments of salivary flow, dry mouth symptoms, and adverse events (AEs) will be performed at specified intervals.

Outcome Measures

Primary Endpoint

- Change in salivary flow from Day 0 to Day 56.

Secondary Endpoints

- Change in focus score on parotid biopsy from Stage II Screening to Day 56.
- Change in salivary flow from Day 0 to study Days 14, 28, 42, and 56.
- Changes in assessments on the Patient Dry Mouth Questionnaire from Day 0 to study Days 14, 28, 42, and 56.
- Changes in assessments on the Sjögren's Disease Activity Index from Day 0 to study Days 14, 28, 42, and 56.

- Changes in other assessments of salivary function from baseline to study Day 56, including technetium scan of the salivary glands.
- Changes in laboratory measures of inflammation.
- Frequency of AEs related to treatment; AE location (body site, right or left), will be recorded and evaluated, as applicable.

Exploratory endpoints

- Changes in mechanistic endpoints from baseline to study Days 14, 28, 42, and 56.

Table of Contents

List of Abbreviations

ADC	apparent diffusion coefficient
AE	adverse event
CBC	complete blood count
CFTR	cystic fibrosis transmembrane conductance regulator
CROMS	Clinical Research Operations and Management Support
CRP	C-reactive protein
DSMC	Data and Safety Monitoring Committee
eCRF	electronic case report form
EDC	electronic data capture
ESR	erythrocyte sedimentation rate
GEE	generalized estimating equations
Ig	immunoglobulin
IND	Investigational New Drug
IRB	Institutional Review Board
mCi	millicurie
MRI	magnetic resonance imaging
MSG	minor salivary gland
MPTB	Molecular Physiology and Therapeutics Branch
NIDCR	National Institute of Dental and Craniofacial Research
NIH	National Institutes of Health
NSAID	nonsteroidal anti-inflammatory drug
PEP	Primary Efficacy Population
SAE	serious adverse event
SD	standard deviation
SEP	Secondary Efficacy Population
SOP	standard operating procedure
SS	Sjögren's syndrome

1 INTRODUCTION

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by exocrinopathy, especially of the salivary and lacrimal glands, which is mediated by lymphocytic infiltration. This lymphocytic infiltration is thought to cause impaired glandular secretory function. Sjögren's syndrome is a slowly progressing disease that primarily affects middle-aged women (female:male ratio of 9:1).

Dry eyes, dry mouth (xerostomia), and fatigue are the most common symptoms of SS. Extra-glandular manifestations are fairly common; for example, gastric involvement has been reported in 55% of patients with primary SS.[3] Major salivary gland enlargement is also very frequent. Reduced salivary gland output leads to a significant increase in the incidence of dental caries, oral mucosal lesions, recurrent fungal infections, difficulty swallowing, and increased susceptibility to gastroesophageal reflux.

Although the prevalence of SS in the United States has been estimated to 1 to 2 million persons, studies have shown that it may be much higher due to the difficulty in diagnosis. The American-European consensus classification criteria published in 2002 are now widely accepted as the standard for diagnosing SS.[4]

Secondary SS usually arises in the presence of another autoimmune disorder, such as rheumatoid arthritis, systemic lupus erythematosus, and polymyositis, whereas primary SS develops in the absence of other connective tissue diseases.

1.1 Systemic and Other Treatments

To date, there is no established therapy to treat the cause of SS, with treatment being directed toward alleviation of symptoms. A variety of medications commonly used in other autoimmune inflammatory diseases have been tested in patients with primary SS, including methotrexate,[5] cyclosporine,[6] infliximab,[7] etanercept,[8] and oral interferon alpha-2.[9] None of these treatments has showed any efficacy in increasing salivary flow.

Relief of oral dryness is achieved by lifestyle modifications such as the use of humidifiers, artificial saliva, and saliva secretory stimulants (e.g., pilocarpine and Evoxac®). Pilocarpine and Evoxac are both muscarinic receptor M3 agonists. Muscarinic receptor activation has been

shown to increase the secretory activity of exocrine glands such as salivary and sweat glands, and also to increase smooth muscle contraction in other organs such as the gastrointestinal tract.[10] However, secretory stimulants are effective in only a small fraction of patients. The muscarinic agonists have undesired affects on a wide spectrum of organs, resulting in side effects such as sweating, headache, nausea, rhinorrhea, and diarrhea. Thus, a large percentage of the patients do not tolerate these medications for xerostomia relief.

Often the presence of extra-glandular symptoms and complications dictate the necessity for other treatments for SS, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and Cox-2 inhibitors for arthralgias and myalgias[11]; hydroxychloroquine for musculoskeletal symptoms[12]; and systemic corticosteroids and pulse cyclophosphamide for severe vasculitis, diffuse interstitial pneumonitis, glomerulonephritis, and neurologic disease.[13,14,15]

Biologics, such as rituximab, have also been tested for the treatment of the oral symptoms of SS. The studies of rituximab have been inconclusive, and its effect on salivary glands is only transient, lasting up to only 3 months.[16] Multiple administrations of this biologic would be required, increasing the possibility of serious adverse events (SAEs). The rate of adverse events (AEs) associated with rituximab is not insignificant either.[17]

The disappointing experience with systemic immunosuppressive and immunomodulating therapies in SS, along with the limited and mainly symptom-targeted therapies for salivary hypofunction, emphasizes the need for a more effective local therapy for xerostomia that specifically targets the salivary glands. An important goal of studies evaluating such therapies is obtaining information about the mechanism of functional improvement within the target organ. One limitation of previous clinical trials of systemic therapies for SS is the possibility that the study drugs had no anti-inflammatory effect on the glandular tissues. Initial clinical studies of new local therapies for xerostomia in patients with SS should evaluate not only improvement in salivary flow, but also data on the mechanism of action and the relationship to clinically important outcomes. Such an approach would confirm the biologic rationale of a particular intervention and allow the design of better and more sophisticated interventions to evaluate clinical efficacy.

Corticosteroids are members of the adrenal hormone family, which are secreted under the control of the hypothalamic-anterior pituitary-adrenocortical axis and include glucocorticoids, mineralocorticoids, and corticotrophins. Glucocorticoids are considered mainly anti-inflammatory agents but have a large number of other functions, such as stabilization of vessel integrity, effects on the water reabsorption and electrolyte concentrations in the kidneys, and regulation of responses to stress.

Because corticosteroids are anti-inflammatory, they have been considered for systemic therapy to prevent the chronic and destructive inflammation of SS within the salivary glands.[18] The only in-depth study examining the effect of systemic corticosteroids on salivary function in SS demonstrated that 6 months of treatment with 30 mg prednisone every other day did not improve either histological or functional parameters of the salivary and lacrimal glands.[18]

A more recent study by Izumi et al. found that a limited course of low-dose topical corticosteroid resulted in sustained improvement in saliva production.[1] The investigators examined the effect of intraductal prednisolone on salivary flow in 31 subjects with SS (24 with primary SS and 7 with secondary SS). They claimed that "corticosteroid irrigation significantly increased the salivary flow rate in patients with Sjögren's syndrome." Study subjects were divided into 3 groups based on MRI findings: early, intermediate, and advanced. They underwent saline irrigation of both parotid glands for 2 to 3 months (1 irrigation every 2 weeks), followed by 4 weekly corticosteroid irrigations of both glands. For the initial saline irrigations, 1 mL of saline was retained in each parotid gland for a period of 2 minutes. During the treatment period, 1 mL of prednisolone sodium succinate at a concentration of 2 mg/mL was used to irrigate each gland, with a retention period of 2 minutes. Whole salivary flow was measured before and after each irrigation with saline and prednisolone, and for a follow-up period of up to 35 months, even though only the parotid glands were irrigated. The duration of effect of steroid irrigation on salivary flow improvement was defined as the time point at which 2 consecutive decreases in salivary flow occurred after the end of prednisolone irrigations. Some of the subjects were treated with a second round of corticosteroid irrigations, most likely because of clinical indications.

In the Izumi et al. study, the overall percentage of subjects with any recognized improvement in symptomatic xerostomia was 64% (18/28 subjects). The mean (standard deviation [SD])

sustained period of salivary flow improvement compared with baseline flow was 8.4 months (3.5 months). Twelve responders who received more than 2 series of corticosteroid irrigations showed no refractoriness to the steroids; after repeated irrigations, their net increase in salivary flow did not diminish when compared with the initial treatment. Eleven of the 12 subjects had net salivary flow increases equal to or more than the initial irrigation. An equally important finding was that prolonged saline irrigation (19 to 35 months) did not significantly increase salivary flow rates.

No adverse reactions due to catheter-administered irrigations were noted in the Izumi et al. study. A follow-up study by the investigators confirmed their earlier results regarding the efficacy of parotid corticosteroid irrigation in subjects with SS.[2] Unfortunately, these studies with local corticosteroids did not examine the mechanism of action on the major salivary glands. A plausible assumption is that corticosteroids improved salivary gland function by reducing inflammation, but other mechanisms cannot be ruled out. A recent publication by Ko et al.[19] explored mechanisms by which corticosteroids improved pancreatic exocrine function and histopathology in autoimmune pancreatitis. The investigators showed that corticosteroids decreased inflammation, restored digestive enzyme secretion through regeneration of acinar cells, and restored HCO_3^- secretion by correcting cystic fibrosis transmembrane conductance regulator (CFTR) localization to the apical membrane (CFTR is known to play a central role in pancreatic duct HCO_3^- secretion). Similar mechanisms may play a role in restoring salivary gland function in SS.

1.2 **Current Study**

The proposed clinical study aims to evaluate intraductal irrigation of the parotid glands with low-dose dexamethasone as a treatment for primary SS. The study population will consist of subjects in Protocols 84-D-0056 and 99-D-0070 who were diagnosed with primary SS according to the American-European Consensus Group Sjögren's Syndrome Classification Criteria (Appendix 24.8). They will be adult female subjects with primary SS, a focus score of ≥1 on minor salivary gland (MSG) biopsy. Minor salivary gland biopsy results up to seven years prior to enrollment will be considered.

Each subject will have both parotid glands irrigated—one with dexamethasone and the other with saline. Because the potency of dexamethasone is approximately 4 to 5 times greater than prednisolone, it may have more sustained effects and thereby decrease the number of needed irrigations. The study will have a single-group, single-arm design in which each subject receives active treatment in one parotid gland and placebo in the other. Subjects will be evaluated for improvement in salivary flow from baseline, as well as changes in the parotid glands by magnetic resonance imaging (MRI), technetium scan, and biopsy.

1.3 **Laboratory Research Studies**

Determination of presumed molecular mechanism of action of dexamethasone in the salivary glands of patients with SS

It is firmly established that fluid secretion is impaired in SS, which is primarily an inflammatory disease. Although knowledge of the molecular mechanism of fluid secretion and its regulation has markedly increased over the last 10 years, little is known about why fluid secretion is impaired in SS. Several factors contributed to this paucity of information.

One factor is the lack of reasonable animal models and the very limited availability of human tissue. An additional significant factor is that, to date, this problem has not been addressed in a systematic way or by teams of investigators who have the required clinical experience, expertise in cell signaling and ion transport, and access to a good supply of tissue from SS patients.

Fluid secretion is an osmotic process driven by transcellular ion transport and the function of water channels. Studies over the last 20 years have established the molecular identity of the major transporters and their regulation. A $[Ca^{2+}]_i$ increase in acinar cells activates basolateral membrane Ca^{2+}-activated K^+ channels KCa3.1 (and perhaps the luminal KCa1.1) and the luminal Ca^{2+}-activated Cl^- channel Anoctamyin (TMEM16A). This results in KCl efflux, an associated luminal water efflux through Aquaporin 5, and thus cell shrinkage.

To maintain luminal electroneutrality, Na^+ flows though the junctional complex from the basolateral to the luminal sides. The end result is NaCl and fluid secretion and K^+ and Cl^- loss from the cytosol and cell shrinkage. Cell shrinkage reduces $[Ca^{2+}]_i$ to inhibit the K^+ and Cl^- channels and activates the basolateral membrane Na^+-K^+-$2Cl^-$ co-transporter NKCC1 to restore cellular ionic content and water. A subsequent increase in Ca^{2+} (Ca^{2+} oscillations) starts the

cycle all over again. NKCC1 is responsible for about 70% of the recovery of cytosolic ionic content, with the remaining mediated by the parallel functions of the basolateral Na^+/H^+ exchanger NHE1 and the $Cl^-/HCO3^-$ exchanger AE2.

The NaCl-rich fluid that enters the duct is modified, with the most important modification being the exchange of Cl^- for $HCO3^-$. $HCO3^-$ is essential in maintaining the mucins and other macromolecules in the saliva in a soluble form. Ductal $HCO3^-$ secretion entails $HCO3^-$ entry across the basolateral membrane that is mediated by the Na^+-$2HCO3^-$ transporter NBCe1B. $HCO3^-$ exit is a complicated process involving the function of at least CFTR and the luminal $Cl^-/HCO3^-$ exchangers Slc26a6 and Slc26a4, which regulate the function of each other. This activity is triggered by cAMP-mediated activation of CFTR that cycles the Cl^- across the luminal membrane and activates the SLC26 transporters. Hence, CFTR supplies the SLC26 transporters with Cl^- and allows Cl^- exchange with $HCO3^-$ by the SLC26 transporters. The salivary gland duct also absorbs the Na^+, in a process mediated by the 3 subunits ENaC, and exchanges it with K^+, in a process that may be mediated by KCa1.1. Ductal function is severely compromised by inflammatory mediators, and in the human pancreas, it has recently been shown that treatment of the inflammatory condition restores ductal function, in particular $HCO3^-$ secretion.

To determine the molecular mechanism of action of dexamethasone in this study, the following investigations are planned:

1. Characterize the fluid secretory mechanism after irrigation with corticosteroids, through assays of activities at different levels. Immunofluorescence will be used to assay localization and expression levels of all key acinar and ductal transporters. This will be followed by studies on the tissue level (sealed duct and gland section), then the cellular level (monitoring activity in single cells), and finally single-protein levels (activity of single channels).
2. Identify changes in proteins involved in calcium signaling, such as Stim1. In vitro and in vivo studies with salivary gland cell lines and primary cells will be run in parallel to examine the effect of dexamethasone on the epithelial cells.

Identification of saliva-derived biomarkers of inflammation and salivary gland dysfunction that can be tested in a future larger study

While many biomarkers of salivary gland inflammation have been proposed, no biomarker has been identified that is strongly associated with SS salivary gland disease activity. This study proposes to isolate exosomal microRNAs and other non-coding small RNAs to be tested for biomarkers. The investigators have shown that they can isolate exosomes and their nucleic acid content from parotid saliva of SS patients and healthy volunteers. Saliva collected during this study will be used for isolation of exosomes, and the nucleic acid content of those exosomes will be correlated with the inflammatory status of the glands. The investigators are planning to use real-time quantitative polymerase chain reaction assays, microRNA microarrays, and/or deep sequencing for the identification of those microRNAs.

The identification of salivary biomarkers, which have a strong enough correlation with tissue inflammation, will serve two objectives:

- Use of salivary biomarkers for replacement of biopsy.
- Dynamic monitoring of inflammation for interventional studies.

If the studies show only an increase in salivary flow without a decrease in inflammation, the discovered microRNAs will serve as functional biomarkers and also be used for functional mechanistic studies of microRNAs affecting salivary flow.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to determine whether irrigation of the parotid gland with low-dose topical dexamethasone improves parotid salivary gland flow in SS subjects.

2.2 Secondary Objectives

Secondary objectives of the study include the following:

- To perform mechanistic studies to determine the mechanisms of action of low-dose topical corticosteroid irrigation of the parotid gland.

- To assess biomarkers of inflammation and salivary gland dysfunction in SS subjects treated with low-dose topical corticosteroid irrigation of the parotid glands.
- To assess localized safety of dexamethasone irrigation of the parotid gland, as compared with placebo.

3 SUBJECTS

3.1 Description of Study Populations

The study will enroll up to 20 adult female subjects with primary SS, a focus score of ≥1 on minor salivary gland (MSG) biopsy in the previous 7 years, and measurable bilateral parotid salivary flow (≥0.01 mL/min per gland) to randomize and treat 16 subjects.

3.2 Inclusion Criteria

1. Female gender and age 18 and greater..
2. Diagnosed with primary SS in Protocol 84-D-0056.
3. Stimulated salivary flow of at least 0.01 mL/min from each parotid gland, using the standard operating procedure (SOP) for the National Institute of Dental and Craniofacial Research (NIDCR) Molecular Physiology and Therapeutics Branch (MPTB) Sjögren's Syndrome Clinic (Appendix 24.6).
4. Minor salivary gland biopsy with a focus score of ≥1 obtained 0 to 7 years prior to study enrollment. Biopsies from outside of the National Institutes of Health (NIH) must be reviewed by the NIH Pathology Department. An MSG biopsy will be required for the following situations:
    a. The last biopsy was obtained before the use of rituximab.
    b. The last biopsy was obtained before the use of immunosuppressants, biologics, or disease-modifying antirheumatic drugs for more than 3 months.
    c. The last biopsy was obtained before the use of systemic corticosteroids (for more than 2 weeks or for shorter periods at doses of more than 0.5 mg/kg) or local parotid

corticosteroids. The use of topical or intra-articular/periarticular corticosteroids will not require a repeat biopsy.

5. For women of childbearing potential, use of, or willingness to use, an effective method of birth control during the study. Effective methods include abstinence, history of hysterectomy, tubal ligation, intrauterine device, licensed hormonal methods, condoms, diaphragm, and cervical cap.
6. Ability to provide written informed consent prior to entry in the study.

3.3 Exclusion Criteria

1. History of lymphoma.
2. History of mycosis, aspergillosis, or other deep fungal infection of the parotid gland.
3. History of salivary gland malignancy (primary or metastatic to the salivary gland).
4. History of secondary Sjögren's syndrome.
5. Parotid infection that does not resolve at least 4 weeks before the start of Screening.
6. Any active viral infection that does not resolve by the start of Screening.
7. Pregnancy or lactation.
8. Use of biologics within 3 months of the start of Screening.
9. Any experimental therapy within 3 months before the start of Screening.
10. Use of immunosuppressants such as methotrexate, leflunomide, azathioprine, cyclophosphamide, systemic cyclosporine, or systemic corticosteroids within 3 months prior to the start of the Screening.
11. Use of inhaled corticosteroids within 3 months prior to the start of Screening.
12. Use of antimalarials and regular use of NSAIDs unless the dose has been stable (or decreased) for at least 2 months.
13. Inability to discontinue the use of saliva stimulants such as pilocarpine and cevimeline for 24 hours before each study visit.
14. Parotid intraductal irrigation or instillation with steroids within the past year.

15. Use of rituximab within 6 months prior to the start of Screening.
16. Allergy to steroids or technetium, or any components of the formulations.
17. Current use of warfarin or heparin.
18. History of bleeding disorder.
19. Both severe atrophy and fibrosis of the MSG noted on the pathology report of the MSG biopsy.
20. Inability to comply with protocol procedures and the number of required visits.
21. Inability to cannulate one or both parotid glands.
22. Parotid fill volume <0.5 mL in one or both parotid glands.
23. Significant concurrent medical condition or other circumstances that, in the opinion of the principal investigator, could affect the subject's ability to tolerate or complete the study.
24. Unable to understand written English for completion of study questionnaires.

The eligibility checklist is included in Appendix 24.3.

4 STUDY DESIGN AND METHODS

4.1 Study Overview

This will be a single-site, randomized-within-subject, placebo-controlled, double-blind phase 2 pilot study in which all subjects receive both active drug (dexamethasone) and placebo (normal saline), thereby acting as their own controls. Subjects are required to enter this study from SS protocol 84-D-0056. Subjects will have one parotid gland treated with dexamethasone irrigation and the other gland treated with normal saline irrigation. The study design is doubly-repeated measures; within a subject, measures are repeated in both time and treatment (i.e., one side of mouth receives placebo while the other receives dexamethasone).

Subjects will be required to attend a minimum of 7 out-patient clinic visits for study assessments and procedures. The maximum duration of on-treatment study participation for each subject is

expected to be up to approximately 11 weeks, with an approximate Screening interval of up to 3 months and an additional Safety Follow-up period of approximately 15 days.

4.2 **Recruitment**

Subjects will be recruited from the subjects enrolled in Sjögren's Clinic Protocols and other sources, including physician referrals, self-referrals, Patient Recruitment Public Liaison, and www.clinicaltrials.gov. Recruitment will include flyers for patients and physicians as well as dear doctor letters. Flyers will be distributed to the Sjögren's syndrome network at John's Hopkins, the Sjögren's Syndrome Foundation, attendees at the annual American College of Rheumatology (ACR) meeting and to other local physicians and dentists. Recruitment efforts will also include posts on the NIH Facebook and Twitter accounts. Patients not already enrolled in 84-D-0056 will be required to enroll to confirm the diagnosis of primary Sjögren's syndrome. Estimated recruitment time will be up to two years.

4.3 **Screening**

Subjects who have been diagnosed with primary SS in Protocol 84-D-0056 will be eligible for participation. Subject consent will be obtained before any study activities or procedures. Screening will be conducted up to 12 weeks before Day 0 in two stages (Stage I & Stage II) to minimize procedures for subjects who do not meet initial salivary flow criteria. Multiple visits will be needed to complete the screening portion of the protocol.

Stage I screening assessments will be done within 6 weeks prior to Stage II and will include vital signs, medical and medication history, review of concomitant medications and evaluation of salivary flow. If salivary flow meets eligibility criteria the following additional Stage I procedures will be done: physical examination, pregnancy testing, routine clinical laboratory tests, and review of previous MSG biopsy. If the subject continues to meet eligibility for study participation according to the preceding assessments, she will undergo parotid saline irrigation and fill volume measurement. If an MSG biopsy has not been performed within 7 years of screening and an MSG biopsy needs to be performed, the saline irrigation and fill volume measurement will occur after the results of the MSG biopsy are obtained, as the final assessment of Screening Stage I.

Screening Stage II will be done within 6 weeks prior to Day 0 and will include vital signs, administration of the dry mouth questionnaire (Appendix 24.7), Sjogren's Disease Activity Index (Appendix 24.9), laboratory blood assessment for baseline values, magnetic resonance imaging, and technetium scan, and a parotid core needle biopsy. For the Screening Stage II, the pregnancy test must be within 24 hours before the technetium and MRI scans (for subjects of childbearing potential). A CBC with differential and platelets, PT, and PTT will be done within 7 days before each of the ultrasound-guided core needle parotid biopsies. All study visit procedures are detailed below in section 4.4 as well as a description of the approximate time to complete each procedure.

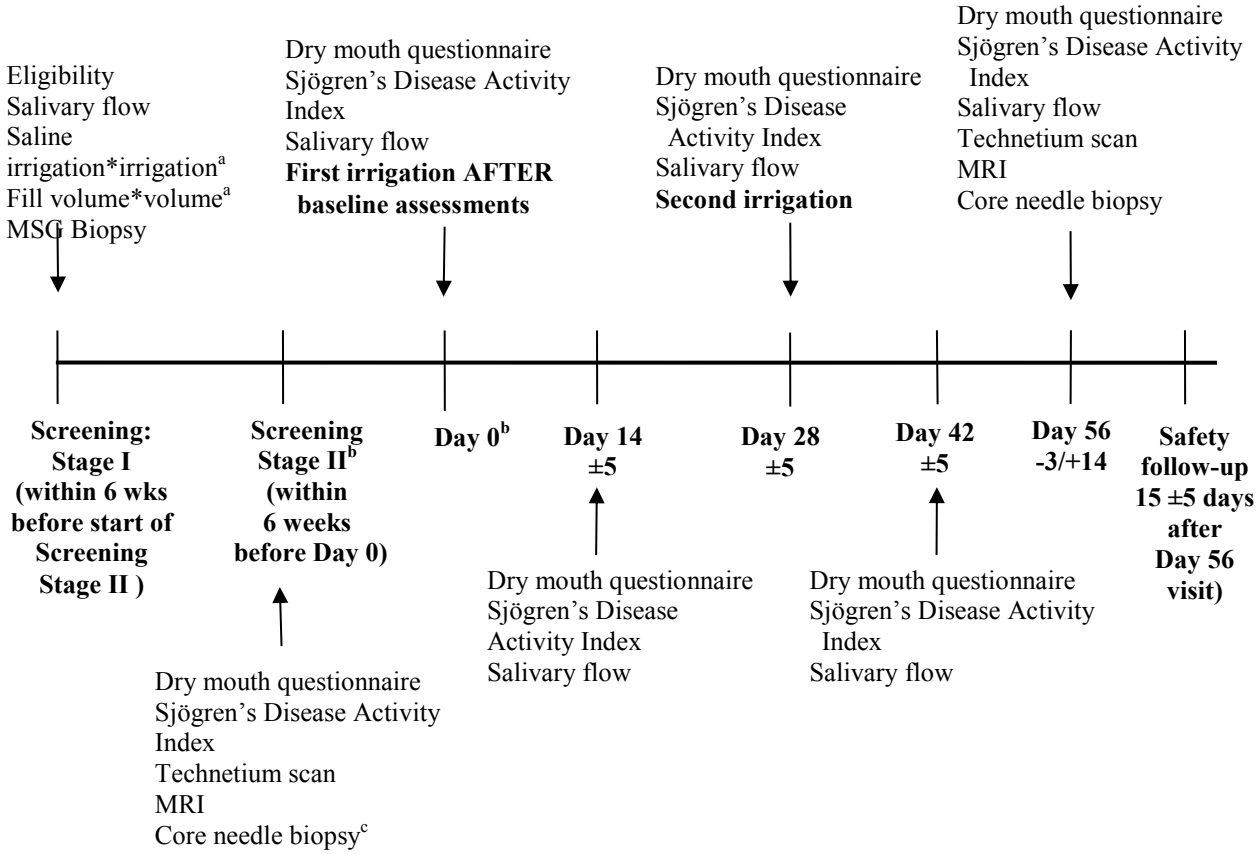
We will accept results of imaging procedures done elsewhere if completed within 3 months of enrollment (screen-stage 1 visit).

Subjects who randomize and receive one study treatment (Day 0) and take an excluded concomitant medication (immunosupressants) may re-screen. They will not have screening MRI, Technetium scan, or parotid biopsy procedures repeated.

4.4 **Study Procedures**

The study scheme depicted in Figure 4-1 presents the study schedule of events. All procedures described are for the purposes of participation in this study and considered research..

Figure 4-1 Study Scheme



[a] Conducted as last assessment of Stage I, only for otherwise eligible subjects

[b] Subjects progress to Stage II then to Day 0, based on their qualification as determined by the assessments conducted during the prior Screening stage.

[c] Conducted as final assessment in Stage II, only for otherwise eligible subjects

Table 4-1 Schedule of Events

			On-treatment Visits					
	Screening							
Procedure	**Stage I (within 6 weeks before Stage II)**	**Stage II[a] (within 6 weeks before Day 0)**	**Day 0[a]**	**Day 14 ± 5 days**	**Day 28 ± 5 days**	**Day 42 ± 5 days**	**Day 56 -3/+14 days**	**Safety Follow-up[b] (15 ± 5 days after Day 56 Visit)**
Consent	X							
Inclusion/exclusion criteria	X							
Relevant medical history/current medical conditions	X							X[15]
Vital signs[1]	X	X	X	X	X	X	X	X[15]
Physical examination	X		X		X		X	X[15]
Urine or serum pregnancy test[2]	X	X	X		X		X	
Patient Dry Mouth Questionnaire		X	X[3]	X	X[3]	X	X	
Sjögren's Disease Activity Index		X	X[3]	X	X[3]	X	X	
Saliva collection (salivary flow measurement)	X[4]		X[4]	X	X[4]	X	X	
Adverse events evaluation	X	X	X	X	X	X	X	X[14]
Concomitant medications	X	X	X	X	X	X	X	X[14]
Randomization			X					
Saline irrigation of parotid glands & determination of fill volume	X[10]							
Randomized irrigation treatment[5] of parotid glands			X		X			
Ultrasound-guided core needle biopsy of right parotid gland[6]		X[11]					X	
Routine clinical laboratory tests[7]	X	X	X		X		X	X[15]
Blood for immunologic tests[8]			X		X		X	
Blood for dexamethasone concentration[9]			X		X			
Minor salivary gland biopsy[10]	X							
Magnetic resonance imaging		X					X	
Technetium scan of salivary glands		X					X	

Research blood			X[13]		X[13]		X	

a Subjects progress to Stage II then to Day 0, based on their qualification as determined by the assessments conducted during the prior Screening stage.

b Telephone contact or clinic visit.

1 Pulse rate, respiratory rate, systolic and diastolic blood pressures, and temperature.

2 For Stage II screening and Day 56, pregnancy test must be performed within 24 hours before technetium and MRI scans for subjects of childbearing potential. For Days 0 and 28, pregnancy test must be performed before irrigation treatment.

3 On Days 0 and 28, may be done 2 days before visit or prior to saliva collection.

4 Performed before irrigation treatments at Stage I screening and on Days 0 and 28.

5 Dexamethasone in one parotid gland and normal saline in the other parotid gland.

6 Baseline ultrasound-guided core needle biopsy will be performed at least 14 days before Day 0. Follow-up biopsy will be performed after the technetium and magnetic resonance imaging scans on Day 56.

7 Complete blood count with differential and platelets, Chem 14, erythrocyte sedimentation rate, C-reactive protein, and amylase. Prothrombin time and partial thromboplastin time will be obtained at Stage II screening and Day 56. The completed blood count with differential and platelets, prothrombin time, and partial thromboplastin time must be done with 7 days before ultrasound-guided core needle biopsy of the parotid. Clinical laboratory tests will be performed at the Safety Follow-up only if clinically indicated.

8 Antinuclear antibodies, antibodies to extractable nuclear antigens, rheumatoid factor, C3 complement, C4 complement, immunoglobulin (Ig)A, IgM, and IgG.

9 Two samples will be obtained: one prior to randomized irrigation treatment of the parotid glands (baseline) and the other 75 (± 15) minutes after irrigation treatment.

10 Performed as final step of Stage I, when subject has otherwise qualified for the study.

11 Performed as final step of Stage II, when subject has otherwise qualified for the study.

12 Performed if a minor salivary gland biopsy is needed based on criteria in Section 3.2. Must be conducted and evaluated prior to any other procedures and activities associated with Stage II screening.

13 On Days 0 and 28, obtained prior to irrigation treatment.

14 Assessed via telephone. Clinic visit scheduled, if deemed necessary based on medical need or per subject request.

15 Performed only if clinic visit deemed necessary per telephone interview.

4.4.1 **Vital Signs Measurement**

Vital signs measurements will include pulse rate, systolic and diastolic blood pressure, respiratory rate, and temperature. Vital signs will be done at Screening Stage I and Stage II, Day 0, 14, 28, 42, 56 and follow-up (if done in clinic). Vital sign measurements take about 10 minutes to complete.

4.4.2 **Medical history and examination**

A medical and medication history will be reviewed at Screening Stage I and an oral and physical exam will be done at Screening Stage I, Day 0, 28, 56 and Safety follow-up (if clinically indicated) to assess overall physical health. The exam will be targeted toward the assessment of Sjögren's and not include a full gynecological exam. These procedures take up to an hour to complete.

4.4.3 **Collection of Saliva/Measurement of Salivary Flow Rate**

Saliva will be collected separately from both parotid glands at the following time points: Screening Stage I and Days 0, 14, 28, 42, and 56. The collection will take place before any saline or treatment irrigation (Screening and Days 0 and 28). The saliva collection procedure is noninvasive and requires no anesthesia. A Teflon collection cup will be placed over the parotid duct orifice and held in place by slight negative pressure. Parotid saliva, either at rest or following a physiologic gustatory stimulus with 2% citric acid, will be allowed to flow freely via plastic tubing connected to the collection cup. The Salivary Flow Collection Procedure is provided in Appendix 24.6. This procedures takes up to 30 minutes to complete.

Saliva samples will be used for the following: 1) identification of changes in proteins involved in calcium signaling, such as Stim1. In vitro and in vivo studies with salivary gland cell lines and primary cells will be run in parallel to examine the effect of dexamethasone on the epithelial cells, 2) exosomal microRNA biomarkers of inflammation. Saliva collected during the study will be used for isolation of exosomes, and the nucleic acid content of the exosomes will be correlated with the inflammatory status of the glands. Real-time quantitative polymerase chain reaction assays, microRNA microarrays, and/or deep sequencing will be used for identifying microRNAs.

4.4.4 Patient Dry Mouth Questionnaire

Subjects will be asked to complete a questionnaire about their dry mouth symptoms (see Appendix 24.7) once during Screening Stage II and on Days 0, 14, 28, 42, and 56. Completing the questionnaire can take up to 15 minutes.

4.4.5 Sjögren's Disease Activity Index

The clinician will use the Sjögren's Disease Activity Index to assess disease activity in each subject (see Appendix 24.9) once during Screening Stage IIand on Days 0, 14, 28, 42, and 56.). This index review takes up to 30 minutes for the clinician to complete.

4.4.6 Parotid Fill Volume Determination

Most subjects are expected to have a fill volume of less than 1 mL per parotid gland. To make certain that the subjects can be cannulated and determine whether the parotid fill volume is less than 1 mL, each subject will undergo cannulation and fill volume assessment at Stage I screening as follows:

1. The parotid duct will be gradually dilated with increasing probe diameter sizes starting at 0000.
2. After the dilations, a soft catheter will be placed over the 000 probe and inserted into the duct.
3. The probe will be slowly removed, and a syringe filled with 1.5 mL saline will be attached on the catheter and the saline slowly injected.
4. The injection will be terminated when the fill volume is completely injected, up to 1 mL.

This assessment takes up to 20 minutes to complete.

4.4.7 Collection of Laboratory Tests

4.4.7.1 Phlebotomy and Urine Tests

- Participants will have a 30mL blood sample drawn through phlebotomy and provide a urine specimen. The laboratory at the NIH Clinical Center will be the central laboratory for all routine laboratory parameters (hematology, chemistry, and urinalysis). Routine laboratory tests will be collected at Screening Stage I, Screening Stage II, and on Days 0, 28, and 56, and, if clinically indicated for procedures at the Safety Follow-up assessment. The following hematology, chemistry, and urine parameters will be assayed. Complete blood count with differential and platelets.
- Inflammatory markers: erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).
- Clotting parameters: PT, PTT (required 7 days before each parotid biopsy)

- Chem panel: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, chloride, carbon dioxide (bicarbonate), creatinine, glucose, potassium, sodium, total bilirubin, and total protein.
- Serum amylase.
- Pregnancy test (urine or serum) (24 hours prior to technetium, MRI or parotid gland biopsy)

Other laboratory tests of immunologic function will be collected on Days 0, 28, and 56.

Immunologic assessments will include:

- antinuclear antibodies, extractable nuclear antigens
- rheumatoid factor
- C3 complement, C4 complement
- immunoglobulin (Ig)A, IgG, and IgM.A

In addition, on Days 0 and 28, blood samples will be drawn for measurement of plasmaconcentrations of dexamethasone prior to randomized irrigation treatment at Days 0 and 28 to obtain a pre-irrigation baseline measure and post-irrigation at 75 (±15) minutes after irrigation. Laboratory assessment collections can take up to 20 minutes to complete.

Blood will be collected at Day 0, Day 28, and Day 56, 24 mL total, to be used for these research tests: The serum and peripheral blood mononuclear cells will be isolated and stored under appropriate conditions. To determine the molecular mechanism of action of dexamethasone in this study, the following investigations are planned: Characterization of the fluid secretion mechanism after irrigation with corticosteroids, through assays of activities at the following levels: single protein (single-channel recording); subcellular; cellular (monitoring organellar and single-cell current, Ca2+, and pH); and tissue (use of volume changes as reported of fluid secretion by acinar cells and the micro-dissected sealed-duct system to measure all ductal secretory and signaling functions). Immunofluorescence will also be used to assay localization and expression levels of all key acinar and ductal transporters.

4.4.8 **Minor Salivary Gland Biopsy**

Some subjects may require an MSG biopsy during Stage I screening to confirm eligibility if their last MSG biopsy was >7 years at the time of screening. Medication to numb the lower inside lip

will be administered and a small incision will be made to extract salivary glands. The area will be closed with stitches. This procedure can take up to 30 minutes to complete.

4.4.9 **Ultrasound-guided Parotid Core Needle Biopsy**

Ultrasound-guided core needle biopsy of the right parotid gland will be obtained at Screening Stage II and Day 56 to evaluate inflammation (including focus score). Using ultrasound, the parotid gland will be visualized, a small incision will be made at the base of the jaw and a needle will be guided into the parotid gland to retrieve a biopsy tissue sample. Two cores will be submitted in a fixative to pathology to be processed for regular histological analysis. The remaining cores will either be placed in cell culture medium or be snap-frozen for further processing. This procedure can take up to 45 minutes to complete. If the baseline measurements are not informative for the study as deemed by the PI, comparable readings at Day 56 will not be performed.

4.4.10 **Technetium Scan**

During Screening Stage II and on Day 56, subjects will have a dynamic technetium scan of the salivary glands to assess gland function. For this scan, they will receive 7 millicuries (mCi) technetium 99m (^{99m}Tc) pertechnetate intravenously, followed immediately by imaging over the head and neck region with a gamma scintillation camera. Subjects will be imaged for 60 minutes, followed by several 2- to 5-minute spot views. At 40 minutes post-injection, they will have 1 mL citric acid solution administered orally. A urine or serum pregnancy test must be done within 24 hours prior to the procedure as applicable. This procedure can take up to 2.5 hours to complete. If the baseline measurements are not informative for the study as deemed by the PI, comparable readings at Day 56 will not be performed.

4.4.11 **Magnetic Resonance Imaging**

Magnetic resonance imaging of the parotid glands will be performed once during Screening Stage II and again on Day 56. Measurements will be checked for changes during the study, which will be compared with changes in inflammatory and functional parameters of the parotid glands. The MRI scans will be performed in the Diagnostic Radiology Department at the NIH

Clinical Center. If the subject is unable to have an MRI for clinical safety reasons, she may still participate in all other aspects of this protocol. . If the baseline measurements are not informative for the study as deemed by the PI, comparable readings at Day 56 will not be performed.

A 3 Tesla magnet will be used for the MRI scans, which will include the following pulse sequences:

1. Axial T1-weighted with fat suppression
2. Axial T1-weighted without fat suppression
3. Axial T2-weighted with fat suppression (short T1 inversion recovery)
4. Diffusion-weighted imaging with apparent diffusion coefficient (ADC) maps
5. Repeat of techniques #1 and # 2 after intravenous administration of contrast

The signal intensity of the parotid glands will be assessed on the T1- and the T2-weighted scans before and after contrast. To normalize the signal intensity values of the glands, the values will be compared with the signal intensity values of a normal structure not affected by the treatment, i.e., the masseter muscle. In addition, the values of diffusivity in the ADC maps will be measured. A urine or serum pregnancy test must be done within 24 hours prior to the procedure , as applicable. This procedure can take up to 2 hours to complete.

4.4.12 **Parotid Treatment Irrigation of the parotid duct (dexamethasone or saline)**
Treatment irrigation will follow saliva collection on Days 0 and 28. Subjects will be randomized to receive dexamethasone irrigation in either the left or right parotid gland, with placebo (normal saline) administered in the opposite gland. Both the subject and the assessor of salivary flow will be blinded as to which parotid gland receives which treatment. Randomization of parotid glands within subjects will be done by the Pharmaceutical Development Service Department of the Pharmacy at the NIH. The person assessing salivary flow will not be involved in performing the irrigation.

Information on the dexamethasone formulation used in the study is provided in the dexamethasone package insert in Appendix 24.5. The Clinical Center Intravenous Admixture Unit at the NIH will prepare vials containing placebo or dexamethasone. The placebo vial will contain 0.9% sodium chloride for injection. The dexamethasone vial will contain 2 mg of dexamethasone sodium phosphate for injection diluted with 0.9% sodium chloride for injection. Solution will be drawn from the vial into a 1 mL syringe. The volume of each irrigation syringe will be determined by the parotid gland filling volume and specified in the medication order. The total volume will be between 0.5 and 1 mL. All vials will be labeled with the following information:

- Dexamethasone or saline.
- Subject name.
- Subject study number.
- Date dispensed.
- The words “Right parotid” or “Left parotid”.
- Dose (2 mg).
- Volume.

Dexamethasone and saline placebo will be dispensed by designated Pharmacy Department staff according to their SOPs. The Pharmacy Department staff will document receipt and dispensation of study drug on drug accountability logs. The dispensation records will identify the name, lot number, and expiration date of the drug, as well as the identification number of subject and the quantity of drug dispensed to each subject. Accidental or intentional destruction of study drug will also be documented. All records will be available for inspection by the clinical trial monitor. Unused study drug will be discarded in the clinic post-procedure per standard NIH drug destruction policy.

The duct system of each gland will be irrigated with the assigned treatment solution (dexamethasone 2 mg or normal saline). For subjects with a fill volume ≥1 mL, Dexamethasone (2 mg/mL) 1mL will be administered. For subjects with a fill volume <1 mL, the concentration of dexamethasone will be adjusted to administer a total dose of 2 mg. Irrigation will be performed by inserting a 1-mm-caliber catheter filled with the appropriate solution 1 to 2 cm into Stensen’s duct. For subjects with a fill volume ≥1 mL, a 5-mL syringe will be filled with the

dexamethasone or saline solution before insertion; the catheter will be connected to the syringe and the solution slowly instilled into to parotid. The catheter will be left in place for 2 minutes of irrigation and then removed. High-speed suction will be used to collect the solution as it runs out. For subjects with a fill volume <1 mL, the dexamethasone or saline will be dispensed in a 1-mL syringe. A urine or serum pregnancy test must be done within 24 hours prior to irrigation, as applicable. This procedure takes up to 30 minutes to complete.

4.4.13 **Description of blood collected solely for research**

Blood will be collected at Day 0 and Day 28 and Day 56, 24 mL total, to be used for future studies. The serum and peripheral blood mononuclear cells will be isolated and stored under appropriate conditions.

To determine the molecular mechanism of action of dexamethasone in this study, the following investigations are planned:

1. Characterization of the fluid secretion mechanism after irrigation with corticosteroids, through assays of activities at the following levels: single protein (single-channel recording); subcellular; cellular (monitoring organellar and single-cell current, Ca^{2+}, and pH); and tissue (use of volume changes as reported of fluid secretion by acinar cells and the micro-dissected sealed-duct system to measure all ductal secretory and signaling functions). Immunofluorescence will also be used to assay localization and expression levels of all key acinar and ductal transporters.

2. Identification of changes in proteins involved in calcium signaling, such as Stim1. In vitro and in vivo studies with salivary gland cell lines and primary cells will be run in parallel to examine the effect of dexamethasone on the epithelial cells.

3. Exosomal microRNA biomarkers of inflammation. Saliva collected during the study will be used for isolation of exosomes, and the nucleic acid content of the exosomes will be correlated with the inflammatory status of the glands. Real-time quantitative polymerase chain reaction assays, microRNA microarrays, and/or deep sequencing will be used for identifying microRNAs.

4.4.14 **Safety Follow-up**

All subjects will be contacted by telephone for a Safety Follow-up, approximately 15 (±5) days after the last on-treatment visit (Day 56 Visit). A clinic visit may be conducted if deemed medically necessary or at the subject's request. Routine laboratory tests will be drawn only if clinically indicated. This contact will complete subject participation in the study.

4.4.15 **End of Participation**

During the study, subjects will continue receiving regular medical care from their primary physicians. The investigators will follow any AEs reported. Following completion of the study, subjects will continue medical care with their primary physicians. Results of technetium scans, MRIs, core needle biopsies, and laboratory tests will be provided to the subjects and/or their primary care physicians upon request. Subjects will be notified of their parotid gland treatment assignment only after data analysis has been completed and the investigators have been unblinded.

5 **STORAGE OF DATA AND SAMPLES**

5.1 **Data**

Study staff will complete electronic case report forms (eCRFs) via a web-based electronic data capture (EDC) system that is compliant with Part 11 Title 21 of the Code of Federal Regulations. The investigators will retain all study-related records for at least 2 years after discontinuation of the study.

5.2 **Samples**

Samples collected for the protocol will be used for data analysis. Any remaining samples will be stored in the MTPB locked freezers according to subject consent sample designation. Samples of subjects who do not consent to future research use of samples will be destroyed at the end of the study. Approval of the IRB will be obtained prior to any research use of stored samples beyond the scope of this protocol. Only study investigators and participating research personnel will have access to the samples. The principal investigator will report to the IRB the loss or destruction of samples collected under this protocol.

6 ADDITIONAL CONSIDERATIONS

6.1 Research with Investigational Drugs or Devices

Review of the federal regulation, Title 21 (Food and Drugs) part 312 (Investigational New Drug Application [IND]), subpart A – General Provisions 312.2 (Applicability) was conducted, and it was determined that this protocol met all the exemptions for IND submission as described in 21 CFR 312.2(b)(1). It was determined that the route of study drug administration, dosage level, and use in the defined protocol patient population would not significantly increase the risk or decrease the acceptability of the risk associated with study drug use as planned in the protocol. Safety information on dexamethasone can be found in the package insert provided in Appendix 24.5. Dexamethasone irrigation is used by specialists for xerostomia, sialadenitis treatment or to decrease inflammation during oral surgical procedures.

6.2 Concomitant Medication Contingencies

During the period of the study, subjects will not be allowed to use the following medications: human or murine antibodies, rituximab, warfarin, heparin, immunosuppressants (e.g., methotrexate, leflunomide, azathioprine, cyclophosphamide, systemic cyclosporine, systemic corticosteroids, and intra-articular corticosteroids), or any experimental drug. If it is necessary to prescribe one of these medications, the subject may participate in subsequent treatments, without repeating baseline MRI, NM Scan or Parotid Biopsy, after a washout of 3 months for immunosuppressants Additionally, subjects must refrain from the use of salivary stimulants within 24 hours prior to each study visit. If a subject enters the study on a stable dose of antimalarial drug or NSAID, the dose should not be increased during the study. All effort should be made to maintain stable dosing of other concomitant medications, including saliva substitutes.

7 RISKS AND DISCOMFORTS

7.1 Assessments/Procedures with No Risks

The following assessments/procedures are deemed to have no risks and to cause no noteworthy discomfort: vital signs measurements, history and physical exam, urine collection, patient dry mouth questionnaire, and Sjögren's Disease Activity Index.

7.2 Collection of Saliva/Measurement of Salivary Flow Rate

There is no known risk associated with this procedure. Subjects may experience discomfort due to increased dryness caused by withholding salivary stimulants for 24 hours prior to saliva

collection. There may be mild discomfort from positioning of mouth during collection. Citric acid (lemon juice) solution used to stimulate salivary flow may cause an unpleasant taste and mild discomfort in the salivary glands if the parotid duct is blocked and saliva cannot be expressed.

7.3 **Parotid Fill Volume Determination and Saline Irrigation of Parotid Gland**

Parotid fill volume will be measured before irrigations. Each subject will undergo 3 irrigations of each parotid gland, the first of which will use saline only. This procedure will involve placement of a catheter within Stensen's duct and filling of the duct with the appropriate solution, either dexamethasone or normal saline. The procedure may cause minor trauma to the parotid ducts and/or infection or inflammation of the parotid gland (sialadenitis). Other rare risks include perforation and/or scarring of the parotid duct(s) and damage to the facial nerve secondary to inflammation, infection, or scarring.

7.4 **Intraductal Dexamethasone**

Dexamethasone sodium phosphate is a synthetic adrenocorticosteroid indicated for a variety of disorders associated with inflammation. Like other glucocorticoids, it can cause profound and varied metabolic effects, as well as modify the body's immune responses to diverse stimuli. At equipotent anti-inflammatory doses, dexamethasone almost completely lacks the sodium-retaining property of hydrocortisone and closely related derivatives of hydrocortisone. Dexamethasone is contraindicated in persons with systemic fungal infections and in persons with hypersensitivity to any component of the formulation.

In this study, dexamethasone will be used topically in small doses within the parotid ducts; therefore, systemic exposure to the drug is expected to be extremely low. The dose (2 mg given twice 4 weeks apart) is within the range approved for intra-articular/intralesional use (0.2 to 6 mg administered once every 3 to 5 days to once every 2 to 3 weeks). Possible risks from local exposure in the mouth include irritation of the oral mucosa, temporary unpleasant taste, and increased risk of infection, allergic reaction, and pain. The risks associated with prolonged systemic exposure to dexamethasone (hypertension, diabetes, osteoporosis, glaucoma, increased risk of systemic infection, and adrenal insufficiency) are very unlikely to occur with this dosing regimen.

7.5 **Ultrasound-guided Parotid Gland Core Needle Biopsy**

Subjects will undergo a core needle biopsy of the right parotid gland during the Screening Period and on Day 56. Ultrasound guidance will be used to localize the biopsy site within the gland, and local anesthetic will be administered to minimize pain during the procedure.

Several recent publications have described ultrasound-guided core needle biopsy of the parotid gland as a low-risk alternative to open surgical biopsy.[20,21,22] Available published data indicate that the procedure is relatively simple and safe to perform and yields sufficient tissue for accurate pathologic diagnosis. The diagnostic accuracy (mainly for tumors) in 319 patients was 96% to 100%, with only 5 (1.6%) reported complications. Four of these complications were hematomas, most of which did not require any intervention. One patient with metastatic squamous cell carcinoma developed a parotid fistula after biopsy. The main risk of an open biopsy of the parotid is injury to the facial nerve (controlling motor function on the ipsilateral face); however, no such complication has been reported with ultrasound-guided core needle biopsy. In the investigators' experience with 24 subjects undergoing ultrasound-guided core needle biopsy of the parotid, no SAEs were reported. One subject had an infected hematoma that responded promptly to antibiotics. The most common complaint was mild to moderate pain or discomfort at the site of the biopsy, which responded to simple analgesics. Other possible risks with the core needle biopsy are allergic reactions to the local anesthetic, bleeding at the biopsy site, and local infection; however, such events are rare.

Subjects in this study will be screened for a history of bleeding disorder and have a CBC with differential and platelets, PT, and PTT before each core needle biopsy. Any discomfort, pain, or swelling at the biopsy site will be treated with acetaminophen or more potent analgesics as needed. If facial nerve injury should occur, the subject will be referred to an appropriate subspecialist for care to minimize the damage. Subjects will be asked to sign a separate surgical consent form prior to the biopsy procedure.

7.6 **Technetium Scan and Radiation**

Subjects will undergo technetium scans of the salivary glands. In this procedure, they will receive an intravenous injection of 7 mCi of ^{99m}technetium pertechnetate, which emits low-energy (approximately 140 kev) gamma rays. The effective radiation dose from the 2 scans will be 0.65 rem, which is below the maximum limit of 5 rem (0.05 rem) per year allowed for

research subjects by the NIH Radiation Safety Committee. Subjects will not be allowed to eat or drink anything except water and medications for 4 hours before the scan. If they have childbearing potential, they will be required to have a negative pregnancy test before the scan. The intravenous injection for the scan may cause discomfort or bruising at the needle site and, rarely, an infection may occur. Allergic reactions to technetium pertechnetate are also possible, but rare.

7.7 **Minor Salivary Gland Biopsy**

Some subjects may require an MSG biopsy if one has not been completed within the past 5 years during Stage I Screening to confirm eligibility (Section 3.2). The biopsy will be performed according to NIDCR SOPs. Local anesthetic will be administered to minimize pain during the procedure. Biopsy of the MSG may cause discomfort, pain, and swelling at the site (usually lower lip) for several days following the procedure; these symptoms can be effectively treated with acetaminophen or more potent analgesics as needed. Subjects may also experience transient paresthesias in the region of the biopsy. Other possible risks are allergic reactions to the local anesthetic and bleeding and infection at the site of biopsy; however, such events are rare. The subject will be asked to sign a separate surgical consent form prior to the procedure.

7.8 **Magnetic Resonance Imaging**

During the study, subjects will undergo MRI scans of the parotid glands. These scans will be performed at the NIH Clinical Center Diagnostic Radiology Department under the direction of a radiologist. If not contraindicated, contrast agent will be used during the MRIs, which may be conducted with or without sedation. For each MRI scan, the subject will have to lie in the scanner for approximately 30 minutes, and will have to lie still for up to 10 minutes at a time.

Persons are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye, of which they may be unaware. Subjects will be screened for these conditions before having the scan, and those determined to have any will not be allowed to undergo MRI without

approval of the radiologist. Each subject will be asked to remove all magnetic objects (e.g., watches, coins, jewelry, and credit cards) before entering the MRI scan room.

It is not known whether MRI is completely safe for a developing fetus. All subjects of childbearing potential will have a urine or serum pregnancy test performed within 24 hours before each MRI scan. The MRI scan will not be performed if the pregnancy test is positive.

Subjects who fear confined spaces may become anxious during the MRI. If sedation is necessary, oral diazepam or another benzodiazepine recommended by the radiologist will be offered prior to the study (all screening and consents will be administered before the subject receives any sedation). Subjects with back problems may also experience back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in persons who already have hearing loss. Subjects will be fitted with hearing protection during the scan. All subjects will be asked to complete an MRI screening form for each scan. There are no known long-term risks associated with MRI scans.

As part of the MRI scans, it is planned that subjects receive an IV injection of contrast agent containing gadolinium. The risks of an intravenous catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling. These events will be treated if they occur. Symptoms from the contrast infusion are usually mild and may include coldness in the arm during the injection, a metallic taste, headache, and nausea. Possible mild allergic reactions include rash, sweating, and itching. In an extremely small number of patients, more severe symptoms have been reported, including shortness of breath, wheezing, urticaria, and hypotension. Persons with renal failure are also at risk for a serious reaction to gadolinium contrast called nephrogenic systemic fibrosis, which has resulted in a very small number of deaths. Subjects will be asked about past allergic reactions to MRI contrast agents and impaired renal function prior to undergoing MRI. Serum renal function test results will also be available to the principal investigator and radiologist at the time of the MRI. The contrast agent will be omitted during the scan if hypersensitivity to gadolinium containing contrast agents is suspected, or if the subject is thought to be at increased risk for nephrogenic systemic fibrosis (glomerular filtration rate <60 mL/min).

7.9 **Blood Collection**

Blood will be drawn from subjects. Approximately 16.5 mL of blood will be drawn during Stage I and Stage II screening; approximately 30 mL on Days 0 and 28; and approximately 24 mL on Day 56. The total volume of blood drawn during the entire study will be approximately 154.5 mL. If clinically indicated, some subjects may have blood drawn at the Safety Follow-up.

Subjects may experience discomfort, bleeding, or bruising at the venipuncture site, which should resolve with time. There is also a small risk of fainting. Pressure will be applied to the venipuncture site for several minutes after needle removal to prevent bruising or bleeding. Infection at the site of needle insertion may also occur but is rare with the use of sterile disposable needles and aseptic technique. The amount of blood drawn for research purposes will be kept within the NIH guidelines for adults, not exceeding 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period.

7.10 **Risk of stopping salivary stimulants**

Subjects will be required to suspend the use of salivary stimulants within 24 hours prior to each study visit, and this suspension could cause discomfort from dryness of the mouth.

7.11 **Unexpected Adverse Events**

Unforeseen adverse effects are a risk with every new drug treatment, including new routes of administration for approved drugs. The risks of participating in this study are reasonable relative to the potential health benefits and generalizable medical knowledge that may be obtained.

8 **SUBJECT SAFETY MONITORING**

All subjects will be monitored from the time of informed consent. Study procedures will be performed and monitored by licensed/certified NIH personnel. At each study visit, subjects will be evaluated for AEs and will be followed until resolution or stabilization of any AEs. All AEs will be graded according to a descriptive severity scale based on the National Institute of Allergy and Infectious Diseases (NIAID), Division of Acquired Immunodeficiency Syndrome Table for Grading the Severity of Adverse Events. The scale has been slightly modified for this protocol (Appendix 24.4). Pregnancy will be monitored throughout the study and subjects study participation will be stopped in the event of pregnancy. Emergency unblinding of a subject's

parotid gland randomization assignment will be allowed if the information is needed to treat a serious adverse event.

Serious adverse events will be reported to CROMS Product Safety group; each event will be reviewed by a CROMS medical monitor. The site's medically responsible Investigator will determine follow-up actions based on event intensity and whether it was expected and at least possibly related to study drug.

Subjects will be informed that they may withdraw or be excluded from the study at any time. The following conditions will require the discontinuation of study drug:

- Pregnancy
- New onset of a condition that requires treatment with any prohibited agent (Sections 3.3 and 6.2).
- Inability of study personnel to cannulate one or both parotid glands.
- Noncompliance with study procedures.
- Adverse event that would substantially increase the risk of continued participation.
- Investigator discretion.
- Violation of eligibility criteria.

Subjects who stop study treatment after receiving 1 of the two doses will be asked to complete the Day 56 assessments and the Safety Follow-up, excluding core needle biopsy of the parotid. Subjects who qualify for the study and who, prior to the first dose of study medication, elect to not receive treatment, will have the following assessments:

- Evaluation of AEs.
- Vital sign measurements (pulse rate, systolic and diastolic blood pressure, respiratory rate, and temperature).
- Laboratory tests if clinically indicated.

No additional data will be collected from subjects who withdraw completely from the study.

9 OUTCOME MEASURES

9.1 Primary Outcome Measure

The primary endpoint of the study is the change in salivary flow from Day 0 to Day 56.

9.2 Secondary Outcome Measures

Secondary endpoints include the following:

- Change in focus score on parotid biopsy from Stage II screening to Day 56.
- Changes in salivary flow from Day 0 to study Days 14, 28, 42, and 56.
- Changes in assessments on the Patient Dry Mouth Questionnaire from Day 0 to study Days 14, 28, 42, and 56.
- Changes in assessments on the Sjögren's Disease Activity Index from Day 0 to study Days 14, 28, 42, and 56.
- Changes in other assessments of salivary function from baseline to study Day 56, including technetium scan of the salivary glands.
- Changes in laboratory measures of inflammation.
- Frequency of AEs related to treatment; location of the AE (body site, right or left), will be recorded and evaluated, as applicable.

Exploratory endpoints will be the changes in mechanistic endpoints from baseline to study Days 14, 28, 42, and 56.

10 STATISTICAL ANALYSIS

10.1 Analysis of Data/Study Outcomes

10.1.1 *Safety Population*

The Safety Population will consist of all enrolled subjects receiving any parotid saline irrigation used to determine parotid filling volume during Stage I screening.

10.1.2 *Primary Efficacy Population*

The Primary Efficacy Population (PEP) will consist of all enrolled subjects who receive all parotid irrigations on study Days 0 and 28, have the same treatment applied to the same parotid

(regardless of random assignment) at both visits, and have values for the primary endpoint (i.e., change in salivary flow from Day 0 to Day 56). The PEP will be used in the primary analysis.

10.1.3 *Secondary Efficacy Population*

The Secondary Efficacy Population (SEP) will consist of all enrolled subjects who receive at least one on-treatment set of parotid irrigations and have at least one salivary flow assessment after study Day 0. The SEP will be used in some secondary analyses.

10.1.4 *Demographic and Baseline Characteristics*

Demographic and baseline characteristics will be summarized in tables. Continuous demographic and baseline variables will be summarized as means, medians, SDs, minimum values, and maximum values. Categorical demographic (e.g., race) and baseline variables will be summarized as frequencies and percents. Baseline variables pertaining to the parotid gland will be summarized by their assigned treatment. Differences between parotid glands by side of the body (right or left) and assigned treatment will be estimated with 90% confidence intervals.

10.1.5 *Primary Efficacy Analysis*

The primary analysis will compare the change from Day 0 in salivary flow in the dexamethasone-irrigated parotid glands (active treatment) with the saline-irrigated parotid glands (placebo treatment) at study Day 56. The treatment effects on the change from Day 0 will be analyzed using repeated measures with change from Day 0 to Day 56 as the dependent variable with random effects for subjects. Day 0 salivary flow will be included as a covariate to adjust for any possible effects of baseline salivary flow on the degree to which salivary flow can change from Day 0. Statistical inferences for treatment effects on salivary flow will be 1-sided (α=0.05) and test for increased salivary flow in the dexamethasone-irrigated parotid glands compared with the saline-irrigated parotid glands. The estimate of the treatment effect will be reported with the associated 1-way confidence interval. The primary analysis will be performed on the PEP.

10.1.6 *Secondary Analyses of Salivary Flow*

Secondary analysis of salivary flow will test for increased salivary flow in the dexamethasone-irrigated parotids compared with the saline-irrigated parotids with respect to change from Day 0 at each of the remaining study days (study Days 14, 28, and 42).

The treatment effects on the change from Day 0 will be analyzed as doubly-repeated measures with change from Day 0 as the dependent variable, random effects for subjects, and repeated effects for treatment and post-Day 0 study day. Day 0 salivary flow will be included as a covariate to adjust for any possible effects of baseline salivary flow on the degree to which salivary flow can change from Day 0. Statistical inferences for treatment effects on salivary flow will be 1-sided (α=0.05) and test for increased salivary flow in the dexamethasone-irrigated parotid glands compared with the saline-irrigated parotid glands. Contrast statements within doubly-repeated measures design will be used to compare the treatment effects on the change in salivary flow from Day 0 at study Days 14, 28, and 42. If significant treatment effects are found at any of the study days, then additional secondary analyses will test for pair-wise differences in change from Day 0 salivary flow between the study days. These tests for pair-wise differences in salivary flow between study days will use 2-sided tests since it is desirable to test for both negative and positive differences (e.g., it is of interest to test whether change in salivary flow from Day 0 to Day 42 is greater than or less than the change in salivary from Day 0 to Day 28). These secondary analyses will be performed on the SEP, with any endpoints following the second on-treatment irrigation set to missing, if the second on-treatment irrigation was discordant with the first treatment irrigation.

The Patient Dry Mouth Questionnaire and the Sjögren's Disease Activity Index will be summarized for each question and at each visit using frequencies and percents. Questions pertaining to symptoms of SS (e.g., difficulty chewing food, changes in rate of tooth decay, mouth dryness, etc.) will be displayed graphically over time as frequencies for binomial and categorical outcomes, and as medians for ordinal outcomes. Binomial- and ordinal-scaled symptoms of SS will be further summarized by visit as change from baseline (Day 0) using shift tables. Shifts from baseline in frequencies or ordinal values that appear substantial will be further analyzed using the McNemar's test. If the data appears rich enough, further longitudinal analysis may be performed using generalized estimating equations (GEE). Any p-values reported from the McNemar's test or GEE analyses will be considered exploratory.

10.1.7 *Exploratory Analyses of Mechanistic and Inflammatory Biomarkers*

Mechanistic and inflammatory endpoints will be studied to gain insight into the molecular mechanism of action of the dexamethasone treatments and to generate hypotheses for future

research. The mechanistic outcome measures may include, but will not be limited to, single-protein single-channel recordings, organellar and single-cell current (Ca^{+2}, and pH), fluid secretion by acinar cells, and ductal secretory and signaling functions. These mechanistic endpoints will be summarized by study day and as change from baseline. Summary statistics employed may include means, geometric means, minimum and maximum values, standard deviations, 90% confidence limits, medians, and frequencies as appropriate for the measure. Log or other transformations may be used if they improve the distributional properties of the outcome measure.

Saliva-derived biomarkers of inflammation and salivary gland dysfunction may be identified from the exosomal microRNAs and non-coding small RNAs. The frequency of the presence/absence of these biomarkers in the saliva both before and after treatment will be reported. Concentrations of biomarkers above the threshold of detection will be summarized using the summary statistics listed above. Biomarkers for inflammation other than non-coding RNAs might be measured in saliva, but those biomarkers will be determined by findings in the biopsies.

Finally, mechanistic endpoints and biomarkers (including changes from baseline) that appear promising will be tested for association with inflammation, salivary flow, or other measures of salivary gland dysfunction. Cross-sectional analyses may include measures of correlation such as Kendall's tau, Spearman rank correlation, and polyserial correlation, as appropriate for the measure. Biomarkers or outcomes that show evidence of correlation with salivary flow or salivary gland dysfunction may be further analyzed longitudinally using mixed-models analysis. These analyses are considered exploratory and hypothesis generating and, as such, will not be corrected for multiple comparisons.

10.1.8 *Criteria for Significance*

The hypothesis test and confidence interval of treatment effects for the primary analysis will be one-sided and test for a positive treatment effect. Hypothesis tests and confidence intervals of secondary analyses will be either 1- or 2-sided as described above. All statistical tests will use an $\alpha = 0.05$.

10.1.9 *Interim Analysis*

No interim analysis will be performed.

10.2 Power Analysis

The primary endpoint is the change in salivary flow (Day 56 – Day 0) following either saline (placebo) or dexamethasone irrigation. Because each subject will simultaneously receive both irrigation treatments over several time points, the experimental design is a doubly-repeated-measures design with one repeated effect for treatment and another repeated effect for study day, and random effects for subject. The expected treatment effect of the dexamethasone irrigation over the saline (placebo) irrigation is an improvement in salivary flow of at least 40%.

The power and sample size computations assume the final primary analysis will be doubly-repeated-measures analysis, including an unstructured variance and random effects for subject to account for the non-independence between glands within a subject. The "unstructured variance" allows the variances (SDs) of the change in salivary flow for each treatment to differ from each other. The power analysis was performed in SAS using the methodology described by Kononoff and Hanford.[23]

The power analysis is based upon the variance parameter estimates in Table 10-1.

Table 10-1 Assumptions for Power Analysis

Parameter	Estimate
1. Standard deviation of the change in salivary flow for the saline-irrigated parotid gland	0.0168
2. Standard deviation of the change in salivary flow for the dexamethasone-irrigated parotid gland	0.0906
3. Within subject correlation between parameters 1 and 2 above	0.15

The SDs of the change in salivary flow for the placebo and dexamethasone irrigations were estimated from data published by Izumi et al.[1] the mean measures and SDs of change in total salivary flow during 2 minutes were converted to flow from a single parotid gland during 1 minute by multiplying by 0.1625 (0.65 for the proportion of total flow from the parotid glands x 0.5 for a single parotid gland x 0.5 for 1 minute). This conversion assumes a very high correlation between total salivary flow and parotid salivary flow.

The above power analysis at 80% power determined that 16 patients would be required to detect a one-sided 40% increase in dexamethasone-irrigated parotid glands compared with the saline-irrigated parotid glands with respect to change in salivary flow from Day 0.

Approximately 20 subjects will be screened to ensure that 16 subjects will be randomized and treated with dexamethasone irrigation. Subjects who discontinue from the study before the Stage I screening parotid irrigation will be replaced. Subjects who do not receive the first irrigation treatment and at least 1 post-treatment salivary flow assessment will also be replaced.

11 HUMAN SUBJECTS PROTECTION

11.1 Subject Selection

Selection of subjects will not be limited by race or ethnicity. Because SS occurs most frequently in adult females, males and children will be excluded from the study. This inclusion strategy is important in a small study like this to insure as homogeneous a population as possible to stabilize the variance. Also, recruitment may be difficult in males and children because it is uncommon. Having a very small set of males or children would make it difficult or even impossible to statistically adjust for their unique contribution to the data.

11.2 Justification for Exclusion of Children

Because SS occurs most frequently in adults, with less than 145 total cases of primary SS reported internationally[24], children will be excluded from the study to reduce variability of results.

11.3 Justification for Exclusion of Non-English speaking Subjects

Subjects that are not able to understand written English will not be included in the study. Subjects will be asked to complete several validated data questionnaires that are not translated and validated in their native languages.

11.4 Justification for Exclusion of Other Vulnerable Subjects

Pregnant women will be excluded from the study because dexamethasone should not be used during pregnancy because the potential benefit does not justify the potential fetal risks.

11.5 Justification of Sensitive Procedures

Although placebo will be used in the study, each subject will also receive active treatment. The placebo treatment will serve as a comparator in evaluating subject response to dexamethasone

treatment. Withholding salivary stimulants will be necessary 24 hour prior to saliva collections to determine baseline salivary flow.

11.6 **Safeguards for Vulnerable Populations**

All subjects of childbearing potential will be required to use an effective method of contraception during the study. A pregnancy test will also be performed before any procedures requiring radiation and prior to any study treatment. Women who are lactating will be excluded from the study because dexamethasone, which is secreted in breast milk, could suppress growth or endogenous corticosteroid production in the breastfed child, or cause other unwanted effects.

11.7 **Qualifications of Investigators**

The qualifications and roles of all investigators are shown in Table 11-1. Investigators obtaining consent are identified with an *.

Table 11-1 Investigator Qualifications and Roles

Investigator Name, Degree	Qualifications/Role
Ilias Alevizos, DMD*	Dental clinician (oral pathologist); responsible for saliva collections, treatment administration, oral examinations, protocol delegation of responsibilities, and clinical and laboratory aspects, oversight of all aspects of the study and obtaining informed consent.
Indu Ambudkar, PhD	Laboratory scientist; will perform multiple laboratory assays on saliva, serum, and tissue samples from subjects.
Lolita Bebris, RN*	Research nurse specialist with 11 years of research experience inclusive of auto-immune diseases and 4.5 years of experience with Sjögren's syndrome research. Responsible for subject screening and recruitment, coordinating study visits, adverse event assessment, and obtaining informed consent.
Richard Chang, MD	Physician, board certified in nuclear medicine; will function as consultant for reading of protocol-required nuclear medicine studies and test interpretations.
Clara Chen, MD	Physician, board certified in nuclear medicine; will function as consultant for reading of protocol-required nuclear medicine studies and test interpretations.
Anna Cotrim, DDS, PhD	Dental clinician (oral pathologist); responsible for specimen saliva collections and assisting with treatment administration and oral examinations.
Alan Baer, MD	Physician, Board-certified Rheumatologist, responsible for physical assessments, adverse event assessments, ordering and interpreting tests, prescribing treatments, regulatory reporting, evaluation and interpretation of test results. Will not obtain consent.
Margaret Grisius, DDS	Dental clinician (oral pathologist); responsible for saliva collections, treatment administration, and oral examinations.
Donna Kelly	Patient Care Coordinator has >14 years of Sjögren's Syndrome research experience in processing and collection of samples (tissue, saliva, serum, PBMC and plasma isolation). Biological lab tech has >10 years experience tracking and processing samples. Trained in the processing of DNA samples. May perform serum assays. Performs data entry. Will not consent patients.
Shmuel Muallem, PhD	Laboratory scientist; will perform multiple laboratory assays on saliva, serum, and tissue samples from subjects.
David Kleiner, MD	Board-certified pathologist; will evaluate clinical tissue samples and consult on pathology and tissue protocol samples.
Nicholas Patronas, MD	Physician, board certified in nuclear medicine; will function as consultant for reading of protocol-required nuclear medicine studies and test interpretations.

Sarfaraz Hasni, MD	Physician, board-certified rheumatologist; responsible for the physical assessments, adverse event assessments, ordering and interpreting tests, prescribing treatments, regulatory reporting, evaluation and interpretation of test results.
Eileen Pelayo, RN	Research nurse specialist with >15 years of research experience, with one year of research experience inclusive of autoimmune diseases. Responsible for subject screening and recruitment, coordinating study visits, and adverse event assessment and obtaining informed consent.
Tammy Yokum, RN*	Research nurse specialist with 22 years of research experience inclusive of auto-immune diseases and 3 years of Sjögren's Syndrome research experience. Responsible for subject screening and recruitment, coordinating study visits, adverse event assessment, and obtaining informed consent.

12 ANTICIPATED BENEFIT

Subjects participating in this study may benefit from improved salivary function, which is important in the daily activities of chewing, swallowing, and speaking and is vital to good dental and oral health.

13 CLASSIFICATION OF RISK

This study is classified as research involving more than minimal risk with the prospect of individual benefit. The risk is justified given the lack of effective treatments for many patients with xerostomia due to primary SS.

14 CONSENT DOCUMENTS AND PROCESS

14.1 Designation of Those Obtaining Consent

The study investigators designated as able to obtain consent in Table 11-1, will obtain informed consent.

14.2 Consent Procedures

Investigators qualified and designated in Table 11-1 of the protocol will obtain informed consent. Written, informed consent will be obtained before any screening or study procedures are initiated. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to participation.

14.3 **Consent Documents**

14.3.1 ***Designation of Those Obtaining Consent***

Study investigators designated as able to obtain consent above will obtain informed consent.

14.3.2 ***Consent Procedures***

For inclusion in the study, each subject will be required to sign the patient population consent form. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. The original consent forms will become part of the permanent medical record, and copies will be provided to the subjects. A copy of the consent form can be found in Section 25. The fact that informed consent was obtained prior to the initiation of study procedures will be documented in the subject's medical or research record.

14.3.3 ***Consent Documentation***

The consent form has all the required elements

15 **DATA AND SAFETY MONITORING**

15.1 **Data and Safety Monitor**

The principal investigator will be responsible for monitoring the conduct of the study and verifying adherence to the protocol. The NIDCR Data and Safety Monitoring Committee (DSMC) will have safety oversight responsibilities for the study. The committee will include members with expertise in a broad range of areas, including human subjects' protection, research ethics, clinical trial implementation, biostatistics, and rheumatology.

15.2 **Data and Safety Monitoring Plan**

Approximately annually, the DSMC will review data related to enrollment progress, study implementation, subject safety, and protocol violations. The DSMC will also consider current information from other sources on the biology of the disease and the subject population under study. Based on these reviews, the DSMC will make recommendations to the principal investigator and the NIDCR Clinical Director concerning the continuation, modification, or termination of the study. The DSMC will also meet ad hoc if relevant issues arise that require committee review. The roles and responsibilities of committee members and meeting procedures will be formally described in a charter.

15.3 Criteria for Stopping the Study or Suspending Enrollment or Procedures

The DSMC chair will be alerted if any of the following situations occur:

- An unexpected fatal or life-threatening event assessed as related to the use of study drug.
- Three or more subjects with similar severe AEs assessed as related to the use of study drug.
- Three or more subjects with SAEs assessed as related to the use of study drug.
- Any occurrence of facial nerve paralysis related to a study procedure.
- Three or more occurrences of bacterial parotid infection requiring antibiotic administration, assessed as related to a study procedure or study drug.

The principal investigator will be responsible for monitoring the accruing safety data related to suspension guidelines and for alerting the DSMC chair when a criterion is met. The DSMC chair will be alerted by email within 7 calendar days of determination that a criterion has been met. The DSMC will issue a recommendation on study continuation to the NIDCR Clinical Director after reviewing data related to the suspension guideline. If the study is stopped, subjects will receive conventional care for any study-related AEs and continue to be followed for clinical and safety outcomes. Otherwise, the study will continue per the DSMC recommendations. The principal investigator and the Clinical Director will provide the recommendations of the DSMC to the IRB.

16 QUALITY ASSURANCE

16.1 Quality Assurance Monitor

The principal investigator will be responsible for monitoring the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all study data. The principal investigator is required to keep accurate records to ensure that the conduct of the study is fully documented.

Staff from CROMS at Rho, Inc. will conduct the clinical monitoring activities and provide reports of the findings. The purposes of the clinical monitoring activities are to ensure that the rights of human subjects are protected, the study is implemented in accordance with the protocol, and the integrity of study data is maintained. Some monitoring activities will be performed

remotely (e.g., review of regulatory documents), while others will take place on site (e.g., verification of study databases against source documentation). The principal investigator will receive copies of the final monitoring reports.

16.2 **Quality Assurance Plan**

Clinical monitoring for this study will be based on a clinical monitoring plan developed by the CROMS at Rho, Inc. in collaboration with the NIDCR Clinical Director and the principal investigator. The clinical monitoring plan will specify the frequency, procedures, and levels of monitoring activities and the frequency of reporting.

The clinical monitor from CROMS will verify that all study subjects have provided informed consent and that subject data agree with the source documents. The monitor will also check that study procedures are compliant with the protocol.

The components of the data quality control and assurance program are 1) real-time detection and correction of errors within the EDC system, 2) verification of key outcome measurements during clinical monitoring visits, and 3) periodic data review by the CROMS. At the time of data entry, the EDC system alerts the user to missing, out-of-range, and inconsistent values and provides the user the opportunity to correct errors in real time. In accordance with federal regulations, the system records all elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry). During periodic clinical monitoring visits, monitors will verify key outcome measurements by comparing data in the EDC system to source documentation. The frequency of monitoring visits and the number of records and data fields audited are described in the study Clinical Monitoring Plan. Data managers at the CROMS will generate periodic summary reports for the study staff to review.

Data fields in the EDC that are the initial source of the data record (i.e., no source documentation is maintained) are the telephone Safety Follow-up and Sjögren's Disease Activity Index eCRF. Further details of measures for quality assurance of the study database are provided in the Data Management Plan.

17 **ADVERSE EVENT, PROTOCOL DEVIATIONS AND UNANTICIPATED PROBLEM REPORTING**

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing on the Problem Report Form not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

18 ALTERNATIVE THERAPIES

Current standard-of-care therapies for xerostomia due to primary SS are salivary stimulants and topical oral moisturizers. Salivary stimulants may not be used in this protocol within 24 hours prior to saliva collection.

Patients with internal organ manifestations of SS may be treated with systemic corticosteroids or immunosuppressive drugs. During the study, subjects will not be allowed to use human or murine antibodies, rituximab, or immunosuppressants.

19 CONFIDENTIALITY

Research Data and Investigator RecordsThe subject's name will appear only on the consent form and medical record, both of which will be kept separate from collected study data. A unique coded study number will be assigned to each subject for data collection and will be used on the electronic CRF and in the clinical database. The number will not contain any personal information (dates, age) to further ensure protection. A subject code log that links the names to identification numbers will be securely maintained at the study site. Charts will be kept in locked cabinets or rooms, and computer research databases will be stored on only NIH

computers, which are password protected and encrypted per standard NIH policy. Only members of the study staff will have access to study samples and data.

Study data will be transmitted to the Clinical Research Operations and Management Support (CROMS) via a secure, encrypted Internet connection and stored on a secure server. De-identified results will be posted on www.clinicaltrials.gov.

19.1 Stored Samples

Research samples collected from subjects consenting to this protocol will be stored in freezers belonging to the MPTB. All of these freezers are located in Building 10, Room 1N121, on the NIH Bethesda campus. All samples will be coded and will not have subject identifiers. The codes for identifiers will be stored in either research charts or NIH computers.

19.2 Special Precautions

Only members of the study staff will have access to study data and samples.

20 CONFLICT OF INTEREST

20.1 Distribution of NIH Guidelines

The NIH guidelines on conflict of interest have been distributed to all investigators.

20.2 Conflict of Interest

There are no conflicts of interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions.

The PI will seek prospective and continuing NIH IRB review and approval for research collaborations in which coded samples (for which the investigators maintain the key) are sent to non-NIH investigator(s). She will identify the names of the collaborating researchers and their affiliated institutions.

20.3 Role of a Commercial Company or Sponsor

Not applicable.

21 TECHNOLOGY TRANSFER

No technology transfer or confidential disclosure agreements are involved in this study.

There are no current tech transfer agreements.

22 RESEARCH AND TRAVEL COMPENSATION

Subjects will be reimbursed $100 for each ultrasound-guided parotid core needle biopsy for the inconvenience of undergoing the biopsy. Reimbursement of travel and subsistence will be consistent with NIH guidelines. In this study, subjects will not be reimbursed for travel or subsistence costs related to study participation.

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24 APPENDICES

24.1 **Protocol Signature Page**

24.2 **Definitions of Unanticipated Problems and Adverse Events**

24.3 **Eligibility Checklist**

24.4 **Rating Scales**

24.5 **Dexamethasone Package Insert**

24.6 **Salivary Flow Collection Procedure**

24.7 **Subject Questionnaires**

24.8 **American-European Consensus Sjögren's Syndrome Classification Criteria**

24.9 **Sjögren's Disease Activity Index**

25 CONSENT FORMS

Adult Subject Consent attached