CLINICAL RESEARCH PROJECT

Project # 07-H-0005
Drug: Dexamethasone Oral Rinse
IND: exempt

Date: February 3, 2021

Title: Pilot Study of Topical Dexamethasone 0.01% Solution for Prevention of Oral Chronic Graft versus Host Disease

Other Identifying Words: oral chronic GVHD, topical dexamethasone solution, tissue markers, salivary proteomics, quality of life

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Subjects of Study:	Number	Sex	Age-range
	Up to 82	Either	≥ 12 years

Off-Site Project?	No
Multi-Institutional Project?	No
DSMB Involvement:	Yes
Tech Transfer?	Yes

PRÉCIS

Prevention of oral chronic graft versus host disease (GVHD) by topical agents is an attractive strategy because it would potentially avoid the adverse effects associated with systemic immunosuppression. Topically administered dexamethasone solution is a commonly used agent for the prophylaxis[1] of oral inflammatory conditions including GVHD. However, the efficacy and systemic effects of topically administered dexamethasone solution are unknown. We therefore propose this trial designed to evaluate the efficacy and safety of topical dexamethasone solution for prevention of oral chronic GVHD in stem cell transplant recipients.

This pilot phase II study will follow a randomized, double-blind, placebo controlled, parallel group design. Consenting subjects who have undergone hematopoietic stem cell transplantation at the NIH Clinical Center and the surrounding transplant clinics will be randomized 50/50 to receive dexamethasone 0.01% solution or placebo as an oral rinse for 3 months starting 90-100 days post-transplant. Subjects will be evaluated monthly after the start of intervention. Diagnostic and research evaluations will include a complete oral examination, oral mucosal biopsy prior to the beginning of the intervention (day -7) and at the time of development of oral chronic GVHD or at the completion of intervention in the absence of clinical GVHD. We will measure serum dexamethasone levels and perform short cosyntropin (ACTH stimulation) test at the end of the 3 months of intervention or onset of clinically significant GVHD.

The primary objective of the study is to evaluate the safety and efficacy of topical dexamethasone 0.01% solution used as an oral rinse for prevention of oral chronic GVHD. Our primary endpoint will be the proportion of subjects that develop clinically significant (severity score 3 or higher) oral chronic GVHD after three months.

Secondary objectives will include the impact of oral chronic GVHD on the quality of life, characterization of the changes in tissue and salivary biomarkers associated with development of oral graft versus host disease, and measures of the effects of topical dexamethasone on hypothalamo-pituitary-adrenal axis. Secondary outcomes will include oral cavity specific quality of life as measured by OHIP-14 questionnaire, oral discomfort levels, improvement in general quality of life scores, and severity of oral chronic GVHD as measured by the site-specific GVHD scoring system.

[1] The term prophylaxis, preventive treatment and treatment are used interchangeably. Treatment using oral dexamethasone will always mean prophylactic treatment.

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1. OBJECTIVES

1.1 Primary Objective

To evaluate the safety and efficacy of topical dexamethasone 0.01% solution used as an oral rinse for prevention of oral chronic graft versus host disease (GVHD).

1.2 Secondary Objectives

1.2.1 To assess the incidence, risk factors and clinical characteristics of oral chronic GVHD.
1.2.2 To evaluate the impact of oral chronic GVHD on quality of life.
1.2.3 To evaluate the degree of systemic absorption of topical dexamethasone and its effect on adrenal cortical function.
1.2.4 To evaluate changes in salivary and plasma proteome associated with oral chronic GVHD.
1.2.5 To evaluate tissue kinetics and gene expression patterns of immune cells associated with the development of chronic oral GVHD and prophylaxis with topical dexamethasone.
1.2.6 To assess the predictive value of subclinical tissue changes on development of chronic oral and systemic GVHD and prognostic significance of these changes in terms of graft versus tumor effect.

2. BACKGROUND

2.1 Graft Versus Host Disease

In recent years, allogeneic stem cell transplantation has been used increasingly for malignant disorders, including solid tumors [1, 2]. GVHD is a major complication and a leading cause of morbidity and mortality in recipients of allogeneic stem cell transplantation. GVHD results from immunologic attack by the donor immune cells on the tissues and organs of the recipient. Skin, gastrointestinal tract, and liver are the most commonly targeted organs. The incidence and severity of GVHD can vary depending on the degree of mismatch of major histocompatibility antigens of the donor and host, age of donor and recipient, source of stem cells, and the type of the preparative regimen. With development of non-myeloablative conditioning regimens, the early post-transplant mortality has dropped with more patients developing GVHD. Given the undersupply of related donors, the use of unrelated and partially matched unrelated donors is likely to expand. As our population ages, the use of both older donors and recipients will become more frequent [3]. These factors are likely to increase the incidence of GVHD in the coming years.

Traditionally, GVHD was classified arbitrarily as acute (occurring within 100 days of transplant) and chronic (occurring 100 days or more after transplant) [4]. Overall, up to seventy percent of patients who survive more than 100 days after transplantation will develop the chronic form of GVHD with the majority manifesting in the first year [5-8]. Chronic GVHD has many features of known autoimmune diseases, such as lupus, lichen planus, and scleroderma, and can have a varied clinical presentation. Mononuclear cellular infiltrates can be found in many areas of the graft recipient, including the liver, skin, oral mucosa, and salivary glands.

In order to provide prognostic and treatment guidelines, chronic GVHD has been classified as limited and extensive depending on the number of the organs involved and the severity of the disease [3]. Limited chronic GVHD was defined as isolated skin involvement less than 50% with or without liver dysfunction and did not require systemic immunosuppression. In contrast, extensive chronic GVHD had evidence of skin, mouth, eyes, liver or other target organ involvement. A more recent classification based on several

risk factors appears to better stratify this complex patient population [9]. This complication of transplant can persist for months to years, and requires long-term management by multiple disciplines.

2.2 Oral Involvement in Chronic GVHD

Oral chronic GVHD is common and is a major cause of morbidity and loss of quality of life in long-term survivors[10, 11]. In a recent randomized trial of peripheral blood stem cell (PBSC) vs. bone marrow stem cells (BMSC) transplants, oral mucosal changes were the most common manifestation of chronic GVHD in BMSC recipients and the second most common (after skin) in PBSC transplants. Overall incidence was around 85% [5]. Oral manifestations of the chronic GVHD include lichenoid changes, mucosal atrophy and ulcerations, taste disturbances, and salivary gland hypofunction. Oral pain and food sensitivity are common and not limited to patients with ulcerations [12, 13]. Oral discomfort was shown to be associated with decrease in food intake and weight loss in this group of subjects [14]. No studies have evaluated the impact of oral chronic GVHD on quality of life after stem cell transplantation.

There have been several reports of increased incidence of oral squamous cell carcinoma (SCCA) after hematopoietic stem cell transplantation (HSCT) [15, 16]. Oral chronic GVHD was an independent risk factor for SCCA after HSCT. Most of the cases described occurred in patients treated with myeloablative conditioning. It is unknown whether early or preventive treatment of oral chronic GVHD can decrease incidence of subsequent SCCA.

The incidence of chronic GVHD in the placebo group was originally estimated as 0.6 based on the published literature. The reported incidence varies from 38 to 83% depending on the type of stem cell transplantation (as shown in the table below). Peripheral blood stem cell (PBSC) transplantation had a higher incidence of chronic oral GVHD compared to bone marrow transplantation. At our institute, we use peripheral blood stem cell as a stem cell source. However there are different protocols utilizing T-cell depleted peripheral stem cell transplantation which provides similar T cell dose (10-H-0154) or even a smaller T cell (06-H-0248, 12-H-0028) dose as the ones used in bone marrow grafts. Furthermore, most literature reported the incidence of chronic oral GVHD irrespective of its severity. Busca et al. (Haematologica 2005; 90: 567) reported up to 62.5% of chronic oral GVHD were graded as moderate to severe (equivalent to grade 3 or higher). Therefore, the incidence of clinically significant (grade 3 or higher) oral chronic GVHD at day 90 after randomization, a primary endpoint of this study would be potentially around 40%, much lower than 60%.

Author	Journal	N	Study population	Incidence of chronic oral GVHD
Flowers	Blood 2002; 100: 415	95	Adult bone marrow or PBSC	Bone marrow 70% PBSC 83%
Busca	Haematologica 2005; 90: 567	104	Adult bone marrow	All grade oral GVHD 38% (62.5% had moderate to severe chronic oral GVHD)
Treistner	Biol Blood Marrow Transplant 2005; 11: 721	49	Pediatric bone marrow	45%
Pavletic	Am J Hematol 2005; 78: 265	87	Adult bone marrow or PBSC	Bone marrow 53% PBSC 70%

2.3 GVHD Prophylaxis

Currently, all subjects undergoing stem cell transplantation receive some form of acute GVHD prophylaxis with systemic immunosuppressive agents, most commonly cyclosporine with methotrexate. The duration of this regimen is based on past studies where the majority of severe GVHD occurred in the first 100 days post transplant. Therefore, subjects typically are maintained on immunosuppression for the first 100 days which in the absence of clinical GVHD is thereafter tapered. Although widely accepted, this arbitrary time point for initiation of immunosuppression withdrawal may not be ideal for all subjects as some subjects may benefit from earlier withdrawal of immunosuppression to boost the anti-tumor effect of the graft. Conversely, subjects with impending GVHD may benefit from longer duration of prophylaxis.

Attempts to prevent chronic GVHD by systemic immunosuppression have been largely unsuccessful [17, 18]. There are no human studies to our knowledge that have evaluated the possibility of topical or local modalities for chronic GVHD prevention. If proven effective, topical or local therapies could be very useful for chronic GVHD prevention given their general lack of systemic side effects.

2.4 GVHD Therapy

2.4.1 Systemic immunosuppressive agents

The treatment for GVHD consists of various systemic immunosuppressive agents, most commonly systemic corticosteroids, cyclosporine, tacrolimus, mycophenolate, and sirolimus. Long-term immunosuppressive treatment for GVHD has a variety of serious side effects including life-threatening infections, aseptic bone necrosis, hypertension, and secondary diabetes [3, 19].

Systemic corticosteroids
Corticosteroids are currently the most commonly used systemic treatment of both acute and chronic GVHD. Although systemic corticosteroids were ineffective for prevention of GVHD and were associated with an increased number of complications like infections and aseptic bone necrosis, the preventive potential of topical therapy has not been investigated. Immunosuppressive and anti-inflammatory effects of corticosteroids are mediated through many mechanisms, including immune cell apoptosis. Corticosteroids have been shown to prevent development of dermal dendritic cells in vitro [20]. Additionally, topical corticosteroids induced apoptosis in the murine epidermal Langerhans cells [21]. The critical role of dendritic cells in the development of GVHD has been outlined in several studies. One study demonstrated the potential of GVHD prevention via depletion of host antigen presenting cells in a murine model [22]. In another animal study, local depletion of Langerhans cells in the skin prevented GVHD.

2.4.2 Topical corticosteroids

Systemic treatment can be supplemented by topical administration of corticosteroids or tacrolimus. Dexamethasone is a readily available corticosteroid used topically and systemically in a wide variety of conditions. It is the only corticosteroid commercially available in the generic solution form for internal use. Dexamethasone does not cross-react in plasma cortisol assay. Dexamethasone solution or elixir is intended for systemic use, but is widely used topically by oral medicine practitioners for the treatment of oral ulcerative conditions due to convenience of administration and lack of irritation from a vehicle. Dexamethasone in the rinse form has been used for the treatment of oral manifestations of GVHD [23]. There are few side effects associated with oral topical corticosteroid use, the most common being oral candidiasis. Since most patients after stem cell transplantation receive antifungal agents preventively, the incidence of this complication is likely to be low.
Corticosteroids have been shown to possess several properties that make them attractive candidates for topical use with the goal of oral GVHD prevention. For example, they interfere with dendritic cell maturation, but not migration [24]. Keeping dendritic cells in an immature state could be advantageous in

the peripheral tissues, because the immature dendritic cells have been shown to play a key role in induction of tolerance [25]. Dexamethasone has been shown to induce immunosuppressive IL-10 producing dendritic cells which may potentially induce tolerance to peripheral tissue antigens [26].

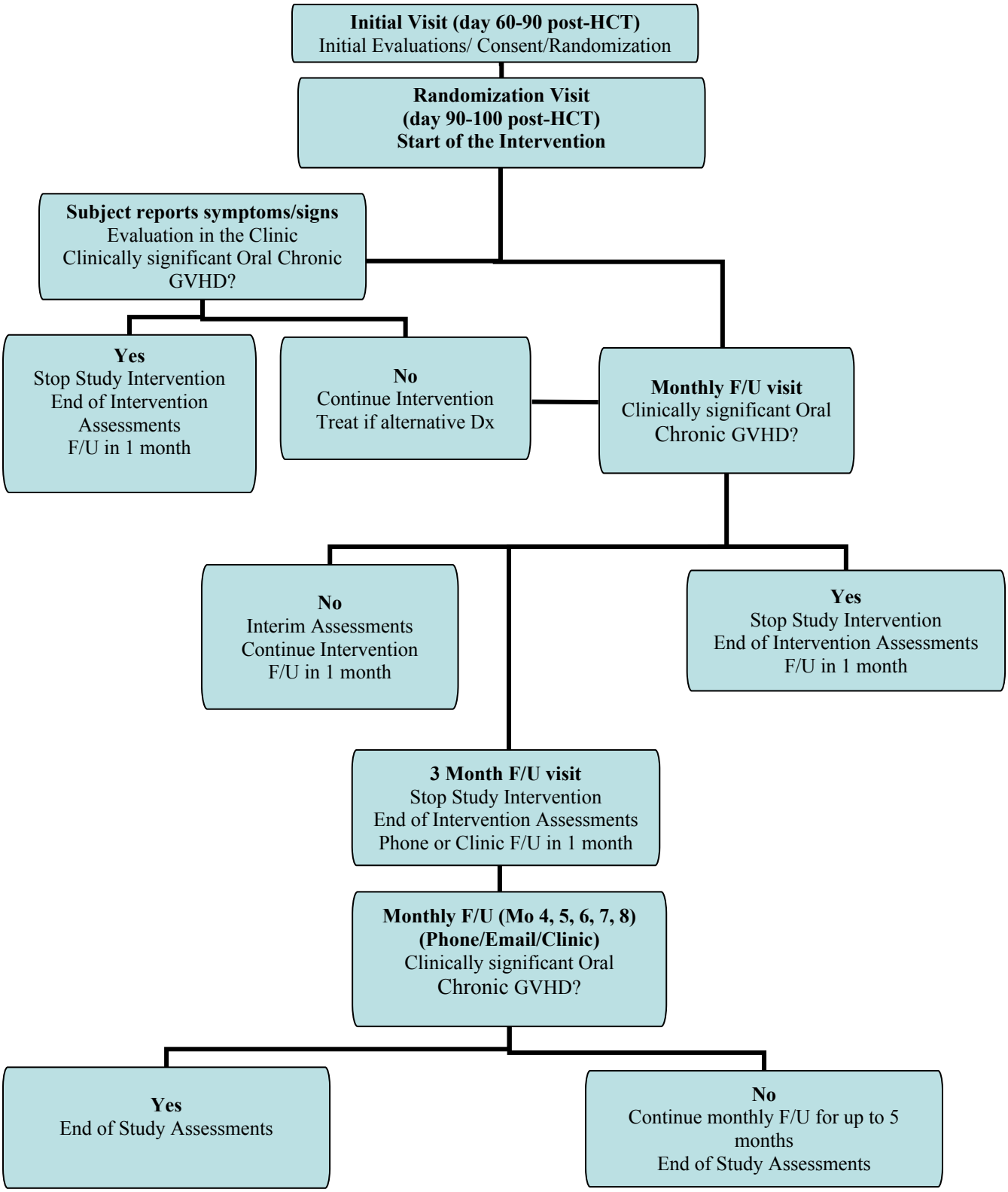
2.5 Scientific and Clinical Justification

Prevention of oral GVHD by topical agents is an attractive strategy because it would potentially avoid the adverse effects associated with systemic immunosuppression. Most importantly, using immunosuppressive and tolerizing agents locally would concentrate the effects in the area where they are beneficial, as GVHD occurs in response to recognition of peripheral antigens by activated donor T-cells. Conversely, use of systemic agents for the same purpose would interfere not only with the undesirable GVH response, but also with immune responses directed against the malignancy (i.e. the graft vs. tumor effect), which is the principal mechanism through which reduced intensity transplants achieve sustained disease remission.

There are no studies of dexamethasone absorption when used topically in the oral cavity and expectorated. The systemic effects of topically administered dexamethasone solution have not been investigated. Since this is a commonly used agent for the treatment of oral inflammatory conditions including GVHD, knowledge of the degree of systemic action would be valuable. If the absorption is significant, some degree of adrenal suppression is likely. This may have implications in many situations when systemic effects of steroids are undesirable.

We therefore propose a trial of topical dexamethasone solution for prevention of chronic oral GVHD in stem cell transplant recipients ages 12 and older. Additionally, this study will provide valuable insights into the cellular and molecular alterations associated with the development of chronic GVHD and treatment with topical corticosteroids. This will lead to better understanding of the pathogenesis of chronic GVHD and potentially development of new therapeutic interventions.

3. STUDY DESIGN



This is a pilot Phase II, randomized, double blind, prospective, parallel group study. Consenting subjects between 90 and 100 days post hematopoietic stem cell transplantation and without evidence of oral chronic

GVHD will be randomized 50/50 to dexamethasone 0.01% or placebo oral rinse. Subjects will be evaluated at monthly intervals during the 3 months of active prophylaxis and 6 months after the intervention period is completed. Development of clinically significant oral chronic GVHD will serve as the primary endpoint and be defined in this study as objective score of 3 or higher on Lichen Planus Severity Scale (Appendix C).

4. ELIGIBILITY ASSESSMENT

4.1 Inclusion Criteria

4.1.1 History of allogeneic hematopoietic stem cell transplantation within 60-90 days of enrollment.
4.1.2 Age 12 or older.
4.1.3 Ability to rinse and expectorate study medication rather than swallow it.
4.1.4 Ability and willingness to come to Clinical Center for follow-up appointments and at the time of development of symptoms/signs suggestive of oral GVHD.

4.2 Exclusion Criteria

4.2.1 Clinically significant oral chronic GVHD at the time of the screening.
4.2.2 Active viral or fungal infection involving oral cavity not resolving by day 90.
4.2.3 Platelet count < 20,000/ml at the time of the screening appointment.
4.2.4 Life expectancy less than 4 months at the time of enrollment.
4.2.5 Documented hypersensitivity to dexamethasone.
4.2.6 Pregnancy or lactation.
4.2.7 Inability to understand the investigational nature of the study.
4.2.8 Inability to provide informed consent.

4.3 Subject Registration and Prophylactic Treatment Randomization

Subjects will be recruited from NIH Clinical Center and the surrounding transplant clinics. After confirmation of eligibility and execution of an informed consent, the Principal Investigator or the research nurse will notify the pharmacy of the subject's enrollment and the pharmacy will randomize the subject to therapy. Both investigators and subjects will be blinded to the group assignment until the completion of the study. Assignment of treatment will be done using a block randomization scheme with the assignment probability remaining fixed over the course of the trial. Construction of the randomization schedule is done by pharmacy using a table of random numbers. Subjects will be stratified by the type of conditioning (myeloablative vs. non-myeloablative) to ensure comparable groups.

4.4 Recruitment

Recruitment activities with surrounding transplant clinics will include electronic physician to physician letter distribution, and will include a copy of the recruitment flyer.

5. TREATMENT PLAN

5.1 Study Drug Administration

The study drug or placebo will be dispensed to the subject by the NIH Clinical Center Pharmacy according to the randomization schedule. Treatment assignment will be unknown to subjects, clinical staff and study investigators in order to preserve the blind. Subjects will be instructed to rinse oral cavity 3 times daily for 2 minutes with 10 mL and expectorate. Subjects will also be instructed to avoid eating, drinking, or rinsing

the oral cavity for 30 minutes after administration.

5.1.1 Dexamethasone

Commercially available dexamethasone 0.01% (0.5mg/5 mL) solution will be repackaged, and labeled by NIH Clinical Center Pharmacy under USP <795> guidelines and FDA 503a regulations for the purpose of this study and to allow blinding to subject, clinical staff, and investigators. The study drug supply will be dispensed by the NIH Clinical Center Pharmacy. In case of accidental ingestion, this would be equivalent to about 20 mg of prednisone/day if all 3 doses were swallowed.

5.1.2 Placebo

The placebo rinse (identical to dexamethasone solution in appearance and taste) will also be compounded, labeled, and dispensed by the NIH Clinical Center Pharmacy under USP <795> guidelines and FDA 503a regulations. The placebo rinse will be administered in the same fashion as the dexamethasone rinse.

5.2 Permitted Concomitant Medications

5.2.1 Topical antifungals (clotrimazole)

Topical corticosteroid treatment is associated with few potential complications. The most common is candidal overgrowth. Subjects will be placed on topical antifungal prophylaxis with clotrimazole 10 mg oral troches 2 times daily for the duration of the intervention to decrease the candidal overgrowth. In subjects unable to tolerate the medication, nystatin swish and spit will be prescribed.

5.2.2 Post transplant medications

There will be no prohibited interventions for this study except topical agents for oral GVHD. All interventions and medications dictated by the subject's primary transplant protocol and clinical necessity will be permitted. Subjects will be queried at interim assessments regarding topical and systemic analgesic use. Factors that can potentially confound the results (such as systemic steroids and other immunosuppressants) will be tracked and taken into consideration at the time of the final statistical analysis.

5.3 Development of Oral Chronic GVHD

In the event of reaching the primary endpoint (development of clinically significant oral chronic GVHD), the subject will be asked to participate in end of study assessments and then will go off study and treatment options for treating the oral chronic GVHD will be per the transplant team.

5.4 Management of Complications

5.41 Oral candidiasis

If oral candidiasis develops despite clotrimazole prophylaxis, standard treatment will be administered.

5.4.2 Adrenal suppression

Asymptomatic subjects without clinical signs of adrenal insufficiency and abnormal ACTH and/or cortisol test results at the end of the intervention period may be observed without steroid replacement and retested in 1 month. In case of development of symptoms suggestive of adrenal suppression (such as abdominal pain, nausea and vomiting, fatigue and hypotension) subjects will then be placed on the standard maintenance replacement regimen of the corticosteroids such prednisone 5-7.5 mg once daily and reevaluated in 1 month

by ACTH testing. All subjects with the abnormal ACTH test results will be advised of the symptoms and signs of clinically significant adrenal suppression and will be given stress doses of hydrocortisone injections for emergencies and a wrist bracelet.

Other complications

Other complications attributable to the medications or procedures used in this study such as allergic reactions will be managed in accordance with the currently accepted standards of care.

6. CLINICAL MONITORING AND ASSESSMENTS

6.1 Pre-study Evaluation and Assessments

6.1.1 Eligibility assessment

Subjects will be screened for eligibility between days 60 and 90 post transplant using the following assessments which are done under the subject's parent transplant protocol or the NHLBI screening protocol 1997-H-0041:

- Review of medical history
- Review of concomitant meds
- Blood pressure
- Complete oral examination
- Complete blood count (CBC with diff) –done concurrently with post transplant monitoring
- Consent if eligible and randomization

6.1.2 Baseline assessments

- Serum chemistry (Acute Care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LD) panel) done concurrently with post transplant monitoring
- Pregnancy test (females of child bearing potential)
- Serum cortisol level (results blinded to investigators)
- The 0-10 Scale for Oral Pain (see Appendix A)
- The 0-10 Scale for Oral Xerostomia (see Appendix A)
- GVHD Severity Scale (see Appendix B)
- Lichen Planus (Oral GVHD) Severity Scale (see Appendix C)
- Disease specific quality of life - OHIP-14 scale (ages 18 and older only-Appendix D)
- Oral photographs (Optional)
- Oral lesion documentation
- Buccal mucosal less than approximately 6mm punch biopsy (ages 18 and older only) (optional)
- Research blood sample collection
- Research saliva sample collection
- Growth chart (length for age, weight for age percentiles and body mass index for age percentiles, pre-pubertal children) (if a hand x-ray is available on parent transplant protocol, the results will be reviewed)

6.2 On Study Drug Interim Assessments (1 month and 2 month phone contact)

The NIH research team will contact the subject by clinical appointment, telephone or email at 1 month and 2 months (+/- 2 weeks) after initiation of study drug to inquire about adverse effects and the use of concurrent medications. Laboratory assessments done as part of their post transplant monitoring will also be reviewed when available. Subjects will be asked to return to clinic when clinically indicated and the following questionnaires will be completed at the time of contact.

- Concomitant meds (immunosuppressive, antimicrobial and analgesics)
- The 0-10 Scale for Oral Pain (see Appendix A)
- The 0-10 Scale for Oral Xerostomia (see Appendix A)
- Patient-assessed Global Scale (perception of changes in oral condition)

6.3 End of Treatment Monitoring and Assessments (3 months)

Subjects will be evaluated at the NIH at 3 months (+/- 7 days) after starting study medication with the following assessments:

- Interim assessment
- Blood pressure
- Concomitant meds (immunosuppressive, antimicrobial and analgesics)
- The 0-10 Scale for Oral Pain (see Appendix A)
- The 0-10 Scale for Oral Xerostomia (see Appendix A)
- Patient-assessed Global Scale (perception of changes in oral condition-Appendix A)
- GVHD Severity Scale (see Appendix B)
- Lichen Planus (oral GVHD) Severity Scale (see Appendix C)
- Disease specific QOL- OHIP-14 scale (ages 18 and older only) (Appendix D)
- Complete blood count (CBC with diff) – done concurrently with post transplant monitoring
- Serum chemistry (Acute Care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen), Mineral (Phosphorus, Magnesium, Albumin, and Calcium), Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin), and Other (Total Protein, CK, Uric Acid, and LD) panel) – done concurrently with post transplant monitoring
- Serum cortisol level (results blinded to investigators)
- ACTH stimulation test (Optional)
- Serum dexamethasone level (results blinded to the investigators)
- Buccal mucosal less than approximately 6mm punch biopsy ages 18 and older only (optional)
- Oral photographs (Optional)
- Oral lesion documentation
- Research blood sample collection
- Saliva sample collection
- Growth chart (length for age, weight for age percentiles and body mass index for age percentiles, pre-pubertal children) (if hand x-ray is available on parent transplant protocol, the results will be reviewed)

6.4 Follow-up Remote Assessment

If available, after completion of study drug, subjects will be contacted by clinic appointment, phone or email monthly +/- 2 weeks for 5 months: Months 4, 5, 6, 7, and 8. Subjects will be asked to report any symptoms or signs consistent with oral chronic GVHD. In addition, the monthly follow-up remote visits will be used to confirm the absence of oral chronic GVHD symptoms (oral discomfort and xerostomia) and collect the information about the current use of medications. If subject reports symptoms or signs consistent with oral

chronic GVHD, the following will be performed at the time of contact:

- Concomitant meds (immunosuppressive, antimicrobial and analgesics)
- The 0-10 Scale for Oral Pain (see Appendix A)
- The 0-10 Scale for Oral Xerostomia (see Appendix A)
- Patient-assessed Global Scale (perception of changes in oral condition)

6.5 End of Study Assessments (6 months after initiation of oral dexamethasone or diagnosed with oral chronic **GVHD)**

After completion of 6 months of follow up, or in the event of symptoms or signs suggestive of oral chronic GVHD, or in case of premature withdrawal from the study and discontinuation of study drug, subjects will return to the Clinical Center for the following end of study assessments:

- Interim assessment including use of immunosuppressive, antimicrobial and analgesic medications
- The 0-10 Scale for Oral pain (see Appendix A)
- The 0-10 Scale for Oral Xerostomia (see Appendix A)
- GVHD Severity Scale (see Appendix B)
- Lichen Planus (Oral GVHD) Severity Scale (see Appendix C)
- Disease specific quality of life - OHIP-14 scale (ages 18 and older only-Appendix D)
- Patient-assessed Global Scale (perception of changes in oral condition)
- Buccal mucosal less than approximately 6mm punch biopsy (18 years or older and if clinically indicated e.g. oral chronic GVHD is suspected)
- Oral photographs (optional)
- Oral lesion documentation
- Research blood sample collection
- Saliva sample collection
- Blood pressure

6.6 Compliance

Subject adherence to the trial interventions will be estimated by measuring the dexamethasone levels at time of analysis (the end of study assessment). Subject adherence to the trial interventions will be estimated by self-reporting compliance and the refill of study medication.

6.7 Data and Records Management

6.7.1 Records to be kept

Each study subject will be assigned a study number and individual record. Individual study records will be held in PIs office. Subject ID number will be used in all computer database records and data analysis procedures for the reasons of confidentiality.

6.7.2 Data management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded in DIR's

Clinical Data System (CDS) or the Laboratory of Cardiac Energetics (LCE) database. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., study-specific identifying number (SSPIN) generated by CDS or other unique code, or minimum PII required for subject identification.

Study data will be stored on the P drive, the Hematology Branch, secure, limited access drive.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. At the completion of the study the data will be analyzed by the study statistician.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Publication policy: Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH Intramural IRB review and approval or an exemption from the NIH Office of Human Subjects Research (OHSR).

6.7.3 Quality assurance

Quality assurance will be provided through ongoing review of records for data quality, completeness of consent forms, and completion of study procedures. Monthly meeting of the investigators on the protocol will be scheduled to ensure continued compliance with the study objectives.

6.7.4 Subject confidentiality

All laboratory specimens, evaluation forms, reports, photographs, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in the subject's medical record. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, NHLBI, FDA, or the DSMB.

6.7.5 Publications and research findings

Publication of the results of this trial will be governed by the policies and procedures of NIH. Any presentation, abstract, or manuscript will be made available for review by NHLBI/NCI prior to submission.

7. ANCILLARY EXPLORATORY LABORATORY RESEARCH STUDIES

7.1 Collecting, Tracking and Disposition of Samples

Sample collection: During the course of participating on this study (screening appointment, follow-up visits, end of study visit, and at the time of development of chronic oral chronic GVHD) blood and saliva will be collected for correlative laboratory research studies. Oral mucosal tissue collection may be performed at baseline, completion of intervention and/or development of oral chronic GVHD, and end of study (6 months after completion of the intervention/12 months post-transplant) and will be collected in the presence of clinical GVHD. Half of the tissue collected will be sent to the department of pathology for

routine H&E staining and the other half will be snap frozen in liquid nitrogen and stored at -80°C for research use.

Intended use: These research specimens will not be read by a pathologist or used for diagnostic purposes. Studies will not be used in assessing the primary endpoint, but are undertaken for descriptive or exploratory ancillary research.

Storage: Research samples will be stored with identifiers in the secure laboratory of Richard Childs, M.D.

Tracking: Samples will be ordered and tracked through the CRIS research screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. In order to ensure proper tracking across several labs, the progress note detailing the consent process will also detail the laboratory to which the sample was initially sent. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

End of study procedures: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

7.2 Technology Transfer

This protocol has no associated patents, CRADAs or CTAs.

Material Transfer Agreements

Between NHLBI and Jerry Bouquot D.D.S. Maxillofacial Center, Morgantown, WV 26508. The collaborator will receive de-identified electronically scanned images of oral biopsy slides and review the pathology findings.
Between NHLBI and Sook-Bin Woo, D.M.D, M.M.Sc, Harvard School of Dental Medicine, Boston, MA. The collaborator will receive de-identified electronically scanned images of oral biopsy slides and review the pathology findings.

Between NHLBI and John Basile, D.D.S., University of Maryland, School of Dentistry, Baltimore, MD 21201. The collaborator will receive de-identified electronically scanned images of oral biopsy slides and review the pathology findings.

Between NHLBI and Ashley Clark, D.D.S., University of Texas Health School of Dentistry, Houston, TX 77054. The collaborator will receive de-identified electronically scanned images of oral biopsy slides and review the pathology findings.

7.3 Tissue Studies of Pathogenesis of Oral Chronic GVHD

7.3.1 Immune cell kinetics in the oral tissues

Microscopic examination of the H&E stained tissue sections will be performed in the Laboratory of Pathology. Oral chronic GVHD grade will be determined based on the following criteria:

1. Number/percentage of the dyskeratotic cells
2. Number/percentage of the intraepithelial lymphocytes
3. Degree of the basal cell layer destruction
4. Degree of the subepithelial lymphocytic infiltrate

Immune cell infiltrate will be characterized by immunohistochemistry and laser scanning cytometry using appropriate markers for T-cell subsets (CD3, CD4, CD8), NK cells (CD16, CD56) and dendritic cell subsets (CD1a, CD11c, CD123, CD205/DEC-205, CD207/Langerin, CD209/DC-SIGN, BDCA-2, 4). Apoptosis will be assessed by immunohistochemical staining of the cells for apoptotic markers (e.g. caspase 3). Laser scanning cytometry allows precise quantitative evaluation of different cell populations in the tissue sections by analogy with flow-cytometry and has been used successfully to characterize immune cell infiltrates [27].

We are particularly interested in the role of peripheral tissue dendritic cells in chronic GVHD. The role of dendritic cells in induction of peripheral tolerance has received considerable attention in the recent literature. For example, plasmacytoid dendritic cells (pDCs) have been suggested to drive Th2 responses and promote induction of T-regulatory cells [28, 29]. The role of plasmacytoid dendritic cells in chronic GVHD is unclear. Studies of association of peripheral blood pDCs with cGVHD rendered conflicting results [30, 31]. pDCs have been reported to be present in small numbers in the normal skin and increase in certain inflammatory conditions [32]. The relative numbers and function of different dendritic cell subsets in the tissues in chronic GVHD setting has not been investigated.

Corticosteroids have been shown to interfere with dendritic cell maturation [24]. Keeping dendritic cells in an immature state could be advantageous in the peripheral tissues, because the immature DCs have been shown to play a key role in induction of tolerance [25]. Dexamethasone has been shown to induce IL-10 producing DCs which may potentially induce tolerance to peripheral tissue antigens [26].

Additionally, we will assess the degree of chimerism of keratinocytes, antigen-presenting cells and lymphocytes in tissue sections. Data from the transplantation literature suggests that transplanted donor cells are capable of stable integration into the recipient tissues [33, 34]. Integration of donor cells into the peripheral tissues has the potential to affect the antigenic repertoire. It is unclear to what degree such "in-situ" chimerism could affect the development of tolerance or conversely – graft versus host disease. We hypothesize that increased donor chimerism of peripheral tissues could facilitate establishment of tolerance to recipient tissue antigens by analogy with organ transplants, where increased population of donor organs by recipient cells is thought to be partially responsible for long-term tolerance to transplanted organs. Adult mesenchymal stem cells have been demonstrated to migrate to the sites of tissue damage and integrate into the tissues [35]. Mesenchymal stem cells have been shown to possess immunomodulating and tolerogenic properties and may play a role in tolerance induction in peripheral tissues of the recipient [36] . Although, studies have shown that mesenchymal stem cells in the bone marrow of the transplant recipients are of the host origin, it is possible that the infused donor mesenchymal stem cells populate peripheral tissues and change their phenotype. We plan to investigate three types of cell populations in oral tissues, namely keratinocytes, antigen presenting/Langerhans cells, and lymphocytes for degree of chimerism and correlate the findings with the clinical and histopathological severity of GVHD. Briefly, tissue sections will be stained by immunohistochemistry for appropriate cell markers and the pure cell populations will be dissected by laser capture microdissection. After isolation of DNA, we will perform PCR for short tandem repeats (STR) of the selected locus and analyze the fluorescent PCR products using capillary sequencer. This method has an advantage of high sensitivity and is independent of donor-recipient gender mismatch[37].

7.3.2 Gene expression profiles (because oral biopsies are optional, samples will not be available for all subjects)

Biopsied oral mucosal tissue will be sectioned and part prepared for the gene array analysis. Six (6) μm thick cryostat frozen sections will be prepared and stored at -80^{o}C. Prior to RNA extraction, the keratinocyte layer will be separated from the underlying layers by laser capture microdissection. Additionally, a 2 mm thick layer of the underlying tissue will also be dissected. This will allow us to take into account different tissue thickness in the oral mucosal biopsies and enrich for keratinocytes and immune cells. The two layers will be assayed separately. We will use focused, pathway specific arrays (Superarray, Superarray Bioscience Corporation, Frederick, MD) for gene array studies. Specifically, arrays focused on apoptosis, dendritic cells/antigen presentation, cytokines, and chemokines/adhesion molecules will be used. The use of smaller, more focused arrays decreases the statistical problems associated with multiple testing and simplifies data analysis. The most interesting and significant findings will be validated by PCR and protein based assays, such as immunohistochemistry and ELISA. These samples will not have direct subject identifiers.

7.3.3 Histopathologic diagnosis of oral cGVHD

In the setting of subclinical tissue changes including absence of inflammation or lichenoid hyperkeratosis, positive histopathologic findings (lichenoid interface lymphocytes with exocytosis and variable apoptosis), have been noted in the baseline oral buccal mucosal biopsies in this study. This phenomenon has been noted in the literature [38-40], however, the diagnostic and prognostic value of this finding has not been reported. This sub-study will use all available baseline oral biopsies from 07-H-0005 to address this question.

7.3.3.1 Re-evaluation of the baseline histopathologic findings of patients in protocol 07-H-0005

Scanned, de-identified images of the H&E slides will be sent to four pathologists with extensive experience in evaluating oral GVHD to individually evaluate all baseline oral biopsies from 07-H-0005. No identifiable patient or clinical information will be shared with the pathologists beyond the general time post-transplant (approximately 90 days) and the reason for the biopsy (protocol baseline). All files will be mailed on a compact disc to the individual pathologist, to be viewed using the Aperio digital pathology viewer software and scored using a standard matrix (Appendix F).

This data will be collected from the pathologists and will be used to calculate

- Inter-rater reliability of the pathologists for oral cGVHD diagnosis
- Incidence of cGVHD diagnostic features in the absence of clinical signs, along with the individual histopathologic features most common in the oral mucosa in the early post-transplant period

This pathology data will then be combined with clinical data, including pre-transplant conditioning regimen, chemotherapy and irradiation history, and any incidence of acute or chronic GVHD to determine the clinical significance and the negative and positive predictive value of this histopathologic finding.

7.4 Salivary and Plasma Proteomics

We hypothesize differential expression of salivary and plasma proteins before and after GVHD development. GVHD specific biomarkers would be potentially useful for predictive and prognostic purposes. It may also provide insight into the biology of the disease as manifested in the oral cavity. We will also compare the salivary protein expression profiles to those from other autoimmune conditions with similar presentation such as Sjogren's syndrome.

We will use surface enhanced laser desorption/ionization – time of flight (SELDI-TOF) mass spectrometry (MS) to investigate screen for differences in protein expression in whole saliva. SELDI-TOF MS Protein Chip technology has been used extensively for biomarker mining and proteomic pattern determination on various body fluids including plasma, urine, and saliva [41, 42]. It uses various affinity surfaces (protein chips) to selectively bind the proteins of interest from the sample. The absorbed proteins are then ionized by a laser shot and the resulting ions accelerate over a certain distance in the mass spectrometer. The time of flight of each ion can be measured and is inversely proportional to the mass of the protein ion. This information in the form of flight times and ion current intensity can be collected, displayed graphically in the form of intensity peaks, and later analyzed by bioinformatic software. Expression differences detected by this high throughput method can be validated using traditional antibody based assays.

Additionally, we will use 2-dimensional difference in gel electrophoresis (2D-DIGE) to further evaluate the salivary and plasma protein expression before and after development of GVHD. This method is complementary to SELDI-TOF in that it has better accuracy for higher molecular weight proteins [43]. Differentially expressed protein spots on a gel can be excised and protein identity determined by mass spectrometric sequencing. The results of the proteomic studies will be used for hypothesis generation for future investigations.

7.5 Oral Bacterial Colonization

We will evaluate the oral bacterial flora changes associated with development of chronic GVHD by broad range bacterial 16S rDNA PCR. This sensitive methodology allows simultaneous testing of multiple, including non-cultivatable, bacterial strains and has been used for microbiological surveillance in various mucosal sites [44]. The important role that bacteria play in maintenance of local homeostasis has been demonstrated in several conditions in which disease phenotype is associated with profound shift from "health-associated" to "disease-associated" flora [45]. The pathogenesis of other conditions, such Crohn's disease has been linked to loss of tolerance to normally harmless commensal bacteria with inappropriate inflammatory response [46]. Within the GI tract, dendritic cells constantly sample the antigens from the lumen of the gut and present them to naïve T-cells in the Peyer's patches and mesenteric lymph nodes inducing tolerance to commensal bacteria and immune responses to pathogens [47]. It is possible that a similar mechanism operates in oral tissues.

8. BIOSTATISTICAL CONSIDERATIONS

8.1 Hypothesis

8.1.1 Primary hypothesis

Our primary hypothesis is that dexamethasone oral rinse 0.5 mg/5 ml administered 3 times a day for 2 minutes will decrease the incidence of clinically significant oral chronic GVHD (primary endpoint) over the three month treatment period.

8.1.2 Secondary hypotheses

Our secondary hypothesis is that the dexamethasone group will have significantly lower oral GVHD scores as measured by the GVHD Severity Scale and The Lichen Planus Severity Scale (Appendix B and C). We also hypothesize that prophylactic use of topical dexamethasone will improve oral health specific and general quality of life (QOL) as measured by the corresponding QOL instruments.

8.2 Sample Size and Accrual

Using the current overall proportion of clinically significant oral chronic GVHD of 0.25, we note that with 36 patients per arm we would have 80% power to detect the same difference of 30% (15% versus 45%) when testing the difference of proportions at a significance level of 0.05. With amendment R (version date 5-6-2016), we thus propose to reduce the required sample size to 72 subjects. We aim to achieve this new accrual goal within two years and we plan to reassess the futility of protocol if we cannot enroll the expected number of subject.

Drop-outs: In order to maintain appropriate statistical power, subjects who prematurely go off study (i.e. are non-compliant with study requirements or develop oral GVHD in the period between baseline assessments and first dose of study drug) will be replaced. To account for a possible drop-out rate of up to 10%, up to 82 subjects may be accrued. Safety data on drop outs will be included in the safety analysis.

8.3 Primary Endpoint

The primary objective of this study is to evaluate the safety and efficacy of topical dexamethasone 0.01% solution used as an oral rinse for prevention of oral chronic graft versus host disease. The primary endpoint is the proportion of subjects that develop clinically significant (severity score 3 or higher) oral chronic GVHD by three months post dexamethaxone administration. For the purposes of this study, oral chronic GVHD with the objective score of 3 or higher on a 6-point oral GVHD scale (Appendix C) will be considered clinically significant. An intention to treat analysis will be conducted as the primary analysis.

8.4 Secondary Endpoints

8.4.1 Scale for Oral Pain, xerostomia

Oral discomfort and xerostomia will be measured by 0-10 scale (Appendix A). We will use this scale to evaluate changes in oral pain and xerostomia over the course of treatment at end of study.

8.4.2 GVHD *S*everity *S*cale

Few objective scales assessing severity of oral chronic GVHD in a standardized way have been published. One of the recently developed systems [48] (Appendix B) has been shown to have good reproducibility and assesses the oral cavity by site (e.g. labial mucosa, dorsal tongue, gingiva) and lesion type (lichenoid, erythematous, ulcerative). Scoring will be performed by investigators trained in assessment of oral cavity lesions. The results will be correlated with scores from the symptom-oriented instruments and the global scale. We will also use a simplified 6 point scale developed for oral lichen planus – a condition that closely resembles oral GVHD [49] (Appendix C). For the purposes of this study we will consider a grade of 3 or higher a clinically significant oral chronic GVHD.

8.4.3 Patient-assessed Global Scale (perception of changes in oral condition)

We will also assess subjects' perceptions of the changes in the oral condition on a 5 item scale (Much worse, A little worse, same, a little better, much better). The data will be correlated with the results from the numerical scoring system to assess internal validity.

8.4.4 Oral Health Impact Profile–14

Oral Health Impact Profile–14 (OHIP-14, Appendix D) is a 14 item questionnaire developed to assess the state of oral health as perceived by a patient [50, 51]. Increased use of patient centered outcome measures has been advocated in the recent years. Patient centered outcome measures complement objective measures

to provide a more complete picture of the impact of disease and treatment. OHIP-14 has been used in oral disease studies including oral lichen planus (a dermatological condition with manifestation similar to chronic oral GVHD). It has been shown to have high validity and reliability, and to be sensitive to treatment effects. This scale has not been well studied in children; therefore it will be limited to adults.

8.4.5 Dexamethasone levels

Systemic absorption of dexamethasone oral solution will be evaluated by measuring plasma dexamethasone levels at the 3 month follow-up appointment. Dexamethasone levels will be blinded to the investigators until the end of the study.

8.4.6 Assessment of adrenocortical function (cortisol levels)

Subjects will be evaluated for possible hypothalamic-pituitary axis (HPA) axis suppression with the following measures:

1. Morning cortisol levels will be determined at baseline, monthly follow-ups (done at NIH), and at completion of the interventional phase (3 months after the last dose of study medication). The results will be blinded to the investigators until the end of the study. At the end of the study, in subjects with morning cortisol levels between 19 mcg/dL and 3 mcg/dL no further adrenal function testing will be performed.

2. In subjects with the symptoms of suspected adrenal insufficiency, a Short Cosynthropin Test (SCT) will be performed to determine if the subject has HPA axis suppression. In brief: venous blood will be collected before administration of 250 mcg of cosynthropin (synthetic ACTH) around 8 AM to 12 noon, and at 30 and 60 minutes after. The expected rise in cortisol levels is to at least 18 mcg/dL (24). In cases of minimally abnormal SCT (stimulated cortisol values of 13-17 mcg/dL), the test will be repeated the next day, as it has been demonstrated that the result is often normal with a repeat test. This test has been shown to be sensitive and specific for evaluation of subclinical adrenal suppression [52, 53]. In the unlikely event of significant adrenal suppression as assessed by repeat ACTH stimulation test, Clinical Center Endocrinology Service will be consulted. Pharmacy will be requested to unblind therapy to the endocrinologist so that appropriate therapy can be initiated and the endocrinologist can appropriately attribute the relationship of the event to study drug administration.

3. The confounding effect of systemic corticosteroids will be taken into account at the time of analysis. We expect to have a subgroup of subjects who will not receive systemic steroids. This subgroup will be used to evaluate the effects of topical dexamethasone on adrenal secretion.

8.5 Data Analyses

This study will be analyzed according to the "intent to treat" principle, that is, all randomized subjects will be included. Subjects not evaluated for endpoints will be considered to have reached the primary endpoint. Every effort will be made to reach and evaluate the subjects at the monthly intervals and at the end of the 3 month intervention period. The differences in proportions of significant oral chronic GVHD will be assessed by a chi-squared test (with continuity correction). Treatment group differences in the severity scores, pain and xerostomia scores, and quality of life scores (OHIP-14) will be assessed using the Wilcoxon test. Adjustments will be made for age and other covariates of interest, such as systemic immunosuppression. Adverse events will be tabulated by severity and treatment assignment. For pediatric subjects, analyses will include growth assessment (growth chart [length for age, weight for age percentiles

and body mass index] for age percentiles) and bone age and the associated z-scores (the number of standard deviations the child's measures [weight, height etc] differ from the population mean).

The intervention period will be 3 months. Should the subject reach the primary endpoint (development of clinically significant oral chronic GVHD), the subject will be asked to participate in end of study assessments and then will go off study. Subjects who did not develop oral chronic GVHD in the intervention phase of the study will be followed for up to 6 months to document late development of oral chronic GVHD. All subjects will be evaluated after 6 months after completion of the intervention phase of the study in the absence of clinical oral GVHD. Final statistical analysis will be performed once all subjects in the sample have completed the study and all records have been collected. We will use stratified analyses to account for effects of systemic immunosuppressive agents and other prognostic factors.

In the event of a protocol violation, the aggregate data will be analyzed with and without those subjects involved in the incident.

8.6 Interim Analysis

There will be no interim analysis due to the short and preliminary nature of this study.

8.7 Stopping Rule

There will be no interim statistical analysis given the small preliminary nature of the study. We do not expect dramatic efficacy or significant difference in serious adverse effects that would require stopping the trial early. The study medication is a safe and non-toxic agent commonly used for the prophylaxis of oral chronic GVHD and other oral mucosal conditions. All the procedures specifically related to this protocol are standard diagnostic procedures and are associated with few complications. In the event of a serious adverse event related to the study medication or procedure the IRB will be notified immediately.

8.8 Off Study Criteria

8.8.1 Subject choice*:*

Subjects may withdraw from the study at their request any time. The risks of withdrawing will be discussed, as will alternative treatment options. Those subjects who choose to withdraw will be strongly encouraged to participate in post therapy assessments until he/she initiates alternative chronic GVHD therapy or through the 6 month off study medication mark to assess for late occurring adverse events.

8.8.2 Principal investigator decision:

Subjects will be taken off study during the 3 month study drug administration period:

- if they develop oral chronic GVHD in the period between baseline assessments and first dose of study drug (subjects will be reassessed between post-transplant days 90 and 100 to ensure absence of oral chronic GVHD and reinforce protocol procedures)
- if they are unable to comply with the study visits or become severely ill and cannot comply with the intervention
- if they reach the primary endpoint of clinically significant oral chronic GVHD
- if they have relapse of their underlying disease (leukemia subjects, see section 10.3.1 risk of tumor lysis)

These subjects will be asked to complete the required off study drug evaluations and end of study assessments.

8.8.3 Completion of the study

Upon completion of the 6 months off study assessments subjects will have completed study participation and be taken off study.

9. DATA AND SAFETY MONITORING PLAN

9.1 Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by Joseph Clara, M.D., the Principal Investigator.

***NIH Intramural IRB*.** Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to Title 45 CFR 46. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects, or data analysis continues.

NHLBI DSMB*:* The NHLBI Data Safety and Monitoring Board will review the protocol at an interval to be determined by the DSMB. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

9.2 Adverse Events

Because dexamethasone is an FDA approved drug with known toxicity profile, any observed or volunteered adverse events will not be captured in the database unless (1) the adverse event is previously unknown (not on the label) or more severe than on the label and deemed possibly, probably or definitely related to the study medication or (2) meets the criteria for a severe adverse event. Adverse events that are complications of the primary transplant protocol and deemed unlikely or unrelated to dexamethosone will not be captured in the database for this protocol, but will be collected and reported in the transplant protocol. The collection of AE information will begin on the first day of initiation of the study drug.

The NCI Common Terminology Criteria for Adverse Events Version 4 (CTCAE) will be used for toxicity and adverse event reporting. All appropriate treatment areas have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Adverse event is defined according to 21 (CFR 312.32(a)) as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event can also be any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome, or disease temporally associated with the use of a drug, without any judgment about causality. Adverse event either occurs during the study participation, having been absent at baseline or if present at baseline, appears to worsen.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

9.3 Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening, requires or prolongs hospitalization
- causes persistent or significant disability/incapacity
- results in congenital anomalies/birth defects
- or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others.

Only those SAEs that are determined possibly, probably or definitely related to this protocol will be reported on this ancillary study. Those determined possibly, probably or definitely to post transplant complications will be reported on the transplant protocol.

9.4 Serious Adverse Events Reporting

PI: All serious adverse events will be reported to Joseph Clara, M.D., Principal Investigator and Medically Responsible Investigator

Joseph Clara, M.D.
Bldg 10, CRC, Room CRC 4-5140
Phone: 301-312-3731

NHLBI DSMB: Reports of serious adverse events that are unexpected and thought to be possibly, probably or definitely related to this protocol to be reported on this ancillary study will also be forwarded no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). All SAEs that are considered at least possibly related to the study agent will be included for reviewed by the DSMB.

SAEs determined to be related to post transplant complications will be reported on the transplant protocol.

9.5 Unanticipated Problems and Protocol Deviations

An unanticipated problem is any incident, experience, or outcome that is:

1. unexpected in terms of nature, severity, or frequency in relation to:

   a) the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure or other study documents, and
   b) the characteristics of the subject population being studied, and

2.related or possibly related to participation in the research, and
3.places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (An AE with a serious outcome will be considered increased risk.)

A protocol deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol.

Reports to the IRB and CD

Expedited Reporting
Events requiring expedited reporting (serious protocol deviations, unanticipated problems, instances of non-compliance) will be submitted to the IRB per Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review:
The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:
The PI or designee will refer to NHLBI DIR Policy to determine CD reporting requirements and timelines.

10. HUMAN SUBJECT PROTECTIONS

10.1 Rationale for Subject Selection

No subjects will be excluded from participation based on gender, race or ethnicity. The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. From previous transplant protocol recruitment patterns at NIH we expect the population may be distributed as follows:

- by gender: 40% females; 60% males
- by age: ages 11-73, median 38; 7% ages 10-17; 20% ages 18-30; 28% ages 31-40; 22% ages 41-50 and 23% ages 51-73
- by race/ethnicity: 11% Asian, 8% Black, 44% Hispanic, 37% White

Recruitment: Subjects will be recruited from NIH Clinical Center and the surrounding hematopoietic stem cell transplant clinics.

Reimbursement for protocol participation, travel, food, and lodging: Reimbursement will occur on this protocol; subject reimbursement will be according to the NHLBI travel policy reimbursement and will be consistent with NIH and NHLBI guidelines.

10.2 Participation of Children

When a pediatric subject reaches age 18, continued participation will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of

time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects;
   a) Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;
   a) Samples and data will be kept in secure locations in the laboratory of Dr. Young. Retention of samples or data does not affect the welfare of subjects.
(3) The research could not practicably be carried out without the waiver or alteration; and
   a) Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
   a) We only plan to request a waiver of re-consent for those subjects who have been lost to follow-up.

If the subject is an affected minor (level of risk is Category 3), both parents must provide parental permission unless one parent is deceased, unknown, incompetent, or not reasonably available (i.e., incarcerated), or when only one parent or guardian has legal responsibility for the care and custody of the child. In the event that one parent is not present to provide consent, telephone consent from one parent may be obtained.

If the subject is an unaffected minor (level of risk is Category 1) we request that only one parent be required to provide parental permission. However, in cases where parents share joint legal custody for medical decisions for a child (e.g., by custody agreement or court order), both parents must give their permission regardless of the level of risk of the research. Exceptions may be made if one parent has since died, become incompetent, or is not reasonably available (e.g., incarcerated).

This study will be limited to subjects aged 12 or older. Given the preliminary nature of the study and requirement for precise compliance with the instructions to minimize swallowing and systemic absorption of dexamethasone, children < 12 who are less likely to comply will be excluded. In addition, oral rinses such as topical fluorides are not recommended for young children; and children < 12 are less likely to develop GVHD. Oral biopsy for research purposes will be limited to subjects 18 years old and older.

Pregnancy testing will be done in all females of childbearing potential. We will inform any applicable minors during the assent process that for safety, we need to do a pregnancy test. She will be told that if the pregnancy test is positive, we will counsel her and help her tell her parents if she wishes. If the minor does not want to proceed, she will be advised not to sign the assent and her study participation will end.

10.3 Risks and Discomforts

10.3.1 Related to dexamethasone

The side-effects of topical steroids are usually limited to Candida (yeast) infection. This is relatively uncommon in our subject population (<5%), easily treatable and does not require discontinuation of the prophylaxis.

Low levels of topical steroids are absorbed through the oral mucosa, but the exact rate is not known. Possible risks associated with absorbed drug include:

- Adrenal suppression*:* While adrenal suppression is very rare with topically applied corticosteroids, it is physiologically possible. Cases of adrenal suppression following topically-applied steroid creams have been reported with long-term use on extensive body surface primarily in young children. In other reports, it was difficult to determine if there was true adrenal suppression. Symptoms of adrenal suppression are non-specific and include fatigue, nausea and abdominal discomfort. Since these symptoms are extremely common in the general hospital population, routine monitoring in the context of this study is not indicated. In this study, blood pressure will be monitored at every visit, and adrenal function will be assessed at the end of prophylaxis. Given the very short duration of this study, we do not expect significant side effects.

  In the unlikely event of significant adrenal suppression as assessed by repeat ACTH stimulation test, Clinical Center Endocrinology Service will be consulted (see section 8.4.6, Assessment of Adrenocortical Function [Cortisol] levels).

- Growth and bone health*:* Impairment of childhood growth with an approximate cortisone dose of 1.5 mg/kg/day was first described over 40 years ago; osteopenia in children receiving a prednisolone dose of less than 0.16 mg/kg/day has also been reported.[52, 53] Loss of bone and deterioration in short term growth are dependent on the type and dose of glucocorticoid and occur most prominently over the first six months of treatment. [54-56]

  We will monitor pre-pubertal pediatric subjects by use of growth chart (length for age, weight for age percentiles and body mass index for age percentiles) and bone age (hand x-ray) if the results are available on the parent transplant protocol.

- Acute tumor lysis: Two cases of acute tumor lysis syndrome in patients with acute leukemia have been reported in the literature following dexamethasone for prophylaxis of peri-operative nausea and vomiting; one of which was fatal (patient had a new onset of undiagnosed acute leukemia)[57, 58]. Although the dexamethasone on this study is administered as an oral rinse (not swallowed), low levels (exact rate unknown) of topical steroids are absorbed through the oral mucosa. Although it is very unlikely that acute tumor lysis would occur in our study, in the event of disease relapse in leukemia subjects, the study drug will be stopped, and the subject will go off-study (see section 8.8.2)

10.3.2 Related to clotrimazole

Side-effects associated with clotrimazole troches are rare and may include nausea, vomiting, unpleasant mouth sensations, and pruritus.

10.3.3 Related to blood collection

Minor complications including bleeding, pain, and hematoma formation at the site of blood draws or infections may rarely occur.

10.3.4 Related to tissue biopsy

Oral mucosal punch biopsy is a minor surgical procedure that may be associated with temporary bleeding, hematoma at the site, local infection and postoperative discomfort. These risks are small (generally <5%) and transient.

10.3.5 Related to pregnancy and nursing mothers

Although dexamethasone will be applied topically to the oral cavity in this study, some amount of medication may be absorbed into the bloodstream. Strong corticosteroids have caused birth defects in animals. It is not known whether topical steroids are absorbed in sufficient amounts to appear in breast milk. Therefore, pregnant and nursing women will not be eligible to participate in this study. Women of childbearing potential will be required to use an effective method of contraception.

10.3.6 Related to saliva collection

Subjects who have undergone transplant may have decreased salivary function and find it difficult to produce a saliva sample. A mild citric acid solution will be placed on the tongue to elicit salivation, and the saliva will be collected using mild suction. Subjects might experience a sour taste and mild discomfort from the citric acid and oral suctioning.

10.3.7 Related to quality of life assessments

There are little to no risks involved in completing the quality of life assessment measures.

10.4 Risks in Relation to Benefits

10.4.1 For adult subjects

The risks of participating in this trial are limited to side effects of dexamethasone and clotrimazole, the risks of standard diagnostic procedures (oral mucosal biopsy), the collection of saliva, the completion of quality of life assessments and an additional blood sample to be used strictly for research purposes. Samples for clinical monitoring will be collected during sample collection procedures that are part of their routine post transplant care.

The benefits to the subjects could be prevention of oral chronic GVHD resulting in improved quality of life, decreased morbidity associated with oral chronic GVHD (should it develop), and potentially, treatment with other more toxic therapies could also be avoided or postponed. Subjects will also receive direct health benefits due to thorough post transplant oral examinations, early diagnosis of oral chronic GVHD, and therefore earlier access to oral chronic GVHD management.

10.4.2 For pediatric subjects

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: as follows:

(a) "The risk represents a minor increase over minimal risk". The risks of participating in this trial are limited to side effects of dexamethasone and the collection of saliva. Samples for clinical monitoring will be collected during sample collection procedures that are part of their routine post transplant care. Only those laboratory tests approved by the IRB and involving not greater than minimal risk will be conducted. Research will not include genetic testing. Therefore, there is no genetic testing associated risk.

(b) *The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches*. Post transplant subjects who co-enroll in this protocol are familiar with blood collection and hospital procedures due to their frequent need for monitoring for their primary disease and for post transplant care. The monitoring that will be done is with the monitoring for the primary transplant protocol. It only minimally exceeds what would be done post transplant (increased oral surveillance and saliva samples)

(c) *Adequate provisions are made for soliciting the assent of the children* and permission of their parents or guardians, as set forth in 46.408. An assent form is available.

10.5 Informed Consent

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject. The potential subject will be educated regarding the nature of the condition, proposed intervention, and outcome measures. Study subjects will be informed that participation is entirely voluntary and that withdrawal from the study can be made at any time without penalty of benefits to which they may be entitled.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective subject participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

We anticipate the enrollment of non-English speaking research participants into our study. The IRB approved full consent document will be translated into that language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use a short form oral consent process as described in MAS Policy M77-2, 45CFR 46.117(b)(2), 21CFR50.27. The summary that will be used is the English version of the extant IRB approved consent document.
We request prospective IRB approval of the use of the short form for up to 3 participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the short form. Should we reach a threshold of 3, we will notify the IRB of the need for an additional usage of the short form and that we will have that consent document translated into the given inherent language.

10.6 Compensation

Compensation will be provided for those subjects participating in the study based on the below values consistent with NIH and NHLBI guidelines. Subject who are ineligible or choose not to have the procedure will receive only partial payment.

Per Subject Enrolled

Procedure(s)/Test(s)	**IU***	**$**	**Frequency**	**Total $**
Blood Draw	1	10	3	$30
Serial Blood Draw	1	10	1	$10
Research Blood Draw	1	10	3	$30
Patient Questionnaire #1	1	10	3	$30
Patient Questionnaire #2	1	10	3	$30
Patient Questionnaire #3	1	10	3	$30
Patient Questionnaire (for adults) #4	1	10	3	$30
Oral Exam	2.5	25	3	$75
Buccal Mucosal Bx (research)	5	50	3	$150
Research Salvia Sample	1	10	3	$30
Drug Administration, General	2	20	2	$40
Telephone Follow Up	1	10	7	$70
OUTPATIENT- 1st HOUR	NA	**20**	**3**	**$60**
OUTPATIENT TIME- **Not to Exceed More than 4 Hours**	1	10	6	$60

ESCORT FEE- INPATIENT				
OUT PATIENT				
TOTAL $				**$675**

10.7 Travel Reimbursement

Reimbursement for local travel, US travel, food and lodging will be in accordance with NHLBI travel policy reimbursement and will be consistent with NIH and NHLBI guidelines.

10.8 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team have reported a potential conflict of interest.

11. PHARMACEUTICALS

11.1 Dexamethasone Oral Solution

Other: Dexamethasone sodium phosphate, Decadron.
Classification: Corticosteroid. 07-H
Action: Anti-inflammatory and immunosuppressive. Dexamethasone is a corticosteroid with mainly glucocorticoid activity; 750 micrograms of dexamethasone is equivalent in anti-inflammatory activity to about 5 mg of prednisone.
Supply / availability: Commercially available dexamethasone 0.01% (0.5mg/5 mL) solution will be repackaged, labeled, and dispensed by NIH Clinical Center Pharmacy under USP <795> guidelines and FDA 503a regulations.
Product description: Dexamethasone oral solution 0.01% (100mcg/mL or 0.5 mg/5 mL) is a clear, colorless solution available commercially in a 500 mL bottle.
Active ingredient: dexamethasone sodium phosphate.
Inert ingredients: Flavoring, glycerin, propylene glycol, methylparaben, propylparaben, sorbitol water.
Preparation: The commercially available solution will be repackaged in 8 ounce amber bottle and relabeled as study drug for the purposes of this study and to allow blinding of the subject, clinical staff, and investigators.
Storage: Store at room temperature (20o to 25oC , 68o to 77oF, controlled room temperature).
Administration: Rinse oral cavity for 2 minutes and spit out. Use 3 times daily after meals. DO NOT SWALLOW. Do not eat or drink for 30 minutes after use.
Dose: 10 mL (1mg).
Toxicities: see section 10.3.1.

11.2 Study Placebo Oral Solution

Supply: The placebo solution will be repackaged, labeled, and dispensed by NIH Clinical Center Pharmacy under USP <795> guidelines and FDA 503a regulations for the purpose of this study and to allow blinding to subject, clinical staff, and investigators.
Product description: The placebo oral solution contains the following ingredients: glycerin, propylene glycol, methylparaben, propylparaben, sorbitol, cherry flavoring, and water for injection.

Storage: Store at room temperature (20o to 25oC , 68o to 77oF controlled room temperature).
Administration: Rinse oral cavity for 2 minutes and spit out. Use 3 times daily after meals. DO NOT SWALLOW. Do not eat or drink for 30 minutes after use.
Dose: 10 mL.

11.3 Clotrimazole Oral Troches

Other: Clotrimazole oral lozenge; Mycelex®(Bayer).
Classification: Antifungal.
Action: Antifungal.
Supply / availability: Commercially available; various manufacturers.
Product description: Clotrimazole oral troches 10mg.
Active ingredient:
Clotrimazole ([1-(o-chloro-α,α-diphenylbenzyl) imidazole].
Inert ingredients:
Dextrates
Cellulose, Microcrystalline
Modified Cellulose Gum
Povidone
Magnesium Stearate.
Storage: Store at room temperature (20° to 25°C ,68° to 77°F controlled room temperature).
Administration: Dissolve in the oral cavity twice daily. Do not eat or drink for 30 minutes after use.
Dose: 10 mg.
Toxicities: see section 10.3.2.

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APPENDIX A : SCALE FOR ORAL PAIN

Scale for Oral Pain

On a 0 to 10 scale, how SENSITIVE OR PAINFUL was your mouth at its WORST in the past month? Please circle.

0 1 2 3 4 5 6 7 8 9 10

Scale for Xerostomia

On a 0 to 10 scale, how DRY was your mouth at its WORST in the past month? Please circle.

0 1 2 3 4 5 6 7 8 9 10

Patient-assessed Global Scale

Compared to one month ago, the overall condition of your mouth today is (please circle)

Much worse A little worse Same A little better Much better

APPENDIX B : GVHD SEVERITY SCALE

GVHD Severity Scale (adapted from Piboonniyom S et al, 2005)

The severity of the lesions in each site was scored according to the presence of reticular/hyperkeratotic, erosive/erythematous, and/or ulcerative lesion(s) as follows: reticular/hyperkeratotic lesions were scored from 0 to 1 (0 = no white striations, 1 = presence of white striations or keratotic papules); erosive/erythematous areas were scored from 0 to 3 by area of involvement (0 = no lesion, 1 = lesions less than 1 cm^2, 2 = lesions from 1 to 3 cm^2, 3 = lesions greater than 3 cm^2); ulcerative areas were scored from 0 to 3 by area of involvement (0 = no lesion, 1 = lesions less than 1 cm^2, 2 = lesions from 1 to 3 cm^2, 3 = lesions greater than 3 cm^2).

Site	**Reticular area**		**Erythematous area**				**Ulcerative area**			
Upper/lower labial mucosa	0	1	0	1	2	3	0	1	2	3
Right buccal mucosa	0	1	0	1	2	3	0	1	2	3
Left buccal mucosa	0	1	0	1	2	3	0	1	2	3
Dorsal tongue	0	1	0	1	2	3	0	1	2	3
Ventral tongue	0	1	0	1	2	3	0	1	2	3
Floor of mouth	0	1	0	1	2	3	0	1	2	3
Hard palate mucosa	0	1	0	1	2	3	0	1	2	3
Soft palate/tonsillar pillars	0	1	0	1	2	3	0	1	2	3
Maxillary gingiva	0	1	0	1	2	3	0	1	2	3
Mandibular gingiva	0	1	0	1	2	3	0	1	2	3
Total										

For each of the 3 clinical signs, a score was derived by summation of the scores of all 10 areas: reticular score = ΣR, erythema score = ΣE, and ulcerative score = ΣU (REU score) with a total weighted score of ΣR + Σ(E × 1.5) + Σ(U × 2.0).

APPENDIX C : ORAL GVHD SEVERITY SCALE

Lichen Planus (Oral GVHD) Severity Scale (adapted from Thongprasom K et al, 2003)

Score 5: White striae with erosive area > 1 cm^2
Score 4: White striae with erosive area < 1 cm^2
Score 3: White striae with erythematous area > 1cm^2
Score 2: White striae with erythematous area < 1 cm^2
Score 1: Mild white striae only
Score 0: No lesions, normal mucosa

APPENDIX D : OHIP-14

Oral Health Impact Profile (OHIP-14)

	Subject Name_____					
	Medical Record Number_____					
	Date_____					
#	**Because of problems with your teeth, denture or mouth have you…**	**Never (0)**	**Hardly ever (1)**	**Occasionally (2)**	**Often (3)**	**Very Often (4)**
1	Had trouble pronouncing words					
2	Felt sense of taste has worsened					
3	Had painful aching in the mouth					
4	Found it uncomfortable to eat any foods					
5	Have been self-conscious					
6	Felt tense					
7	Had an unsatisfactory diet					
8	Had to interrupt meals					
9	Found it difficult to relax					
10	Have been a bit embarrassed					
11	Have been irritable with other people					
12	Had difficulty doing usual jobs					
13	Felt life in general was less satisfying					
14	Have been totally unable to function					
	Total _____					

APPENDIX E : ABBREVIATIONS

The List of Abbreviations Used in the Protocol

GVHD – Graft versus Host Disease
cGVHD - Chronic Graft versus Host Disease
GVT – Graft versus Tumor
OHIP-14 – Oral Health Impact Profile-14
VAS – Visual Analog Scale
ACTH – Adreno Corticotropic Hormone
PBSC - Peripheral Blood Stem Cell
BMSC - Bone Marrow Stem Cells
SCCA – Squamous Cell Carcinoma
SCT – Stem Cell Transplantation
H&E – Hematoxylin and Eosin
NK – Normal Killer
DC – Dendritic Cell
pDC – Plasmacytoid Dendritic Cell
Th-1/Th-2 – T helper 1-2
IL- Interleukin
PCR – Polymerase Chain Reaction
STR - Short Tandem Repeats
RNA – Ribonucleic Acid
ELISA – Enzyme Linked Immunosorbent Assay
SELDI-TOF - Surface Enhanced Laser Desorption – Time of Flight Mass Spectrometry
2D-DIGE - 2-dimensional difference in gel electrophoresis
rDNA – Ribosomal Desoxyribonucleic Acid
CFR – Code of Federal Regulations
SAE – Serious Adverse Events
AE - Adverse Events

APPENDIX F: Oral Mucosa Biopsy Scoring Matrix

Oral Buccal Mucosa Biopsy Scoring Matrix
Case Number:

Epithelium			
Thickness	☐ **Normal**		
	☐ **Atrophic**		
	☐ **Hyperkeratosis**		
	☐ **Acanthosis**		
Basilar Vaculopathy	**Yes**	**No**	
	☐ **generalized**		
	☐ **localized**		
Apoptosis/Eosinophilic Bodies	**Yes (>1/10x field)**	**Occasional (<1/10x field)**	**None**
Spongiosis	**Yes**	**No**	
Keratinocytic Atypia	**Yes**	**No**	
Exocytosis (5 lymphocytes/10x field)			
Lymphocytes	**Yes**	**No**	
Other Inflammatory Cells	**Yes**	**No**	
Thickening of Basilar Lamina	**Yes**	**No**	
Lamina Propria			
Inflammation	**Generalized**	**Localized**	
Cell Type			
Lymphocytes	**Yes**	**No**	
Plasma Cells	**Yes**	**No**	
Eosinophils	**Yes**	**No**	
Neutrophils	**Yes**	**No**	
Mast Cells	**Yes**	**No**	
Distribution			
Perivascular	**Yes**	**No**	
Periductal (excretory)	**Yes**	**No**	
Interstitial	**Yes**	**No**	
Bandlike (interface, submucosal, obscuring the junction)	**Yes**	**No**	
Width of mucosal surface	_____	**Number of serial sections**	_____

FINAL DIAGNOSIS	
No GVHD	☐
Possible GVHD	☐
Likely GVHD	☐

Comments:
DDX
Reasons for favored Dx
Issues with sample
Other

Original assessment form published as part of the supplemental material from Shulman HM, Kleiner D, et. al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. Biol Blood Marrow Transplant. 2006 Jan;12(1):31-47. Modified from Form H: Evaluation of Mucosal Biopsies to include information regarding oral buccal mucosa only and to fit the revised 2014 Consensus Criteria from the Pathology Working Group Report