# A Phase 1/2 Clinical Trial to Assess the Safety and Preliminary Efficacy of Lipoxin Analog BLXA4-ME Oral Rinse for the Treatment of Gingivitis

**NIDCR Protocol Number: 14-020-E**

**NIDCR Grant Number:**

U01 DE026934

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**IND Number:**
107061

**Funding Agency:**
National Institute of Dental and Craniofacial Research (NIDCR)

**Version Number: 8.0**

**18 May 2017**

Forsyth IRB approved:
June 8, 2017

## STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations (CFR) on the Protection of Human Subjects (45 CFR Part 46), and the National Institute of Dental and Craniofacial Research (NIDCR) Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subjects protection training.

## SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator or Clinical Site Investigator:

Signed: Date: _____

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | adverse event |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| ATLa | 15-epi-16-*para*-fluoro-phenoxy-$LXA_4$, aspirin-triggered lipoxin analog |
| BOP | bleeding on probing |
| CAL | clinical attachment level |
| CCTR | Center for Clinical and Translational Research, Forsyth Institute |
| CFR | Code of Federal Regulations |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DNA | deoxyribonucleic acid |
| DSMB | Data and Safety Monitoring Board |
| ELISA | enzyme-linked immunosorbent assay |
| FDA | Food and Drug Administration |
| GCF | gingival crevicular fluid |
| GM-CEJ | distance from free gingival margin to cementoenamel junction |
| ICH | International Conference on Harmonisation |
| ICMJE | International Committee of Medical Journal Editors |
| IL-1β | interleukin-1β |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |
| $LXA_4$ | lipoxin $A_4$ |
| MGI | modified gingival index |

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|---|---|
| NIDCR | National Institute of Dental and Craniofacial Research |
| NIH | National Institutes of Health |
| NSAID | nonsteroidal anti-inflammatory drug |
| OHRP | Office for Human Research Protections |
| OMAS | Oral Mucositis Assessment Scale |
| PD | probing depth |
| PI | plaque index |
| RBC | red blood cell |
| SAE | serious adverse event |
| SPM | specialized pro-resolution mediator |
| UP | unanticipated problem |
| WBC | white blood cell |

## PROTOCOL SUMMARY

**Title:** A Phase 1/2 Clinical Trial to Assess the Safety and Preliminary Efficacy of Lipoxin Analog BLXA4-ME Oral Rinse for the Treatment of Gingivitis

**Précis:** The study comprises 3 groups in a randomized, placebo-controlled, double-blind clinical trial design. The treatment group (1.0 μM BLXA4-ME oral rinse) and the placebo rinse group will each include 50 subjects. The no-rinse control group will consist of 25 subjects. Subjects in the treatment and placebo rinse groups will receive oral rinse (BLXA4-ME or placebo) to be applied once daily after morning teeth brushing. Safety parameters will be assessed before treatment and 3, 7, 14, 21, and 28 days after initial treatment. Efficacy parameters will be assessed before treatment and 14 and 28 days after initial treatment.

**Objectives:** The primary objective is to evaluate the safety of an investigational compound, BLXA4-ME, topically applied as a daily oral rinse in adults with gingivitis. Safety will be assessed by the incidence of adverse events, including mucosal inflammation and irritancy, and findings from safety laboratory tests. Subjects will be monitored for development or progression of periodontitis and oral flora will be analyzed to detect an increase in opportunistic organisms.

The secondary objective is to assess preliminary efficacy of the oral rinse, by monitoring changes in the plaque index (PI), modified gingival index (MGI), bleeding on probing (BOP), andlevels of interleukin-1β (IL-1β) in gingival crevicular fluid (GCF). Changes in pocket depth (PD) and clinical attachment level (CAL) will be assessed as exploratory efficacy analysis.

**Population:** 125 healthy adults, aged 18 through 65 years, with existing gingivitis (MGI ≥ 2.0)

**Phase:** 1/2

**Number of Sites:** 1

**Description of Intervention:** The active preparation (test rinse) will contain BLXA4-ME at 1.0 μM in a formulated oral rinse containing 10% ethanol and 0.25% sodium lauryl sulfate. The compound BLXA4-ME is an analog of lipoxin $A_4$, a locally acting lipid mediator biosynthesized from the essential fatty acid, arachidonic acid. In BLXA4-ME, a fused o-substituted benzo-ring is incorporated between

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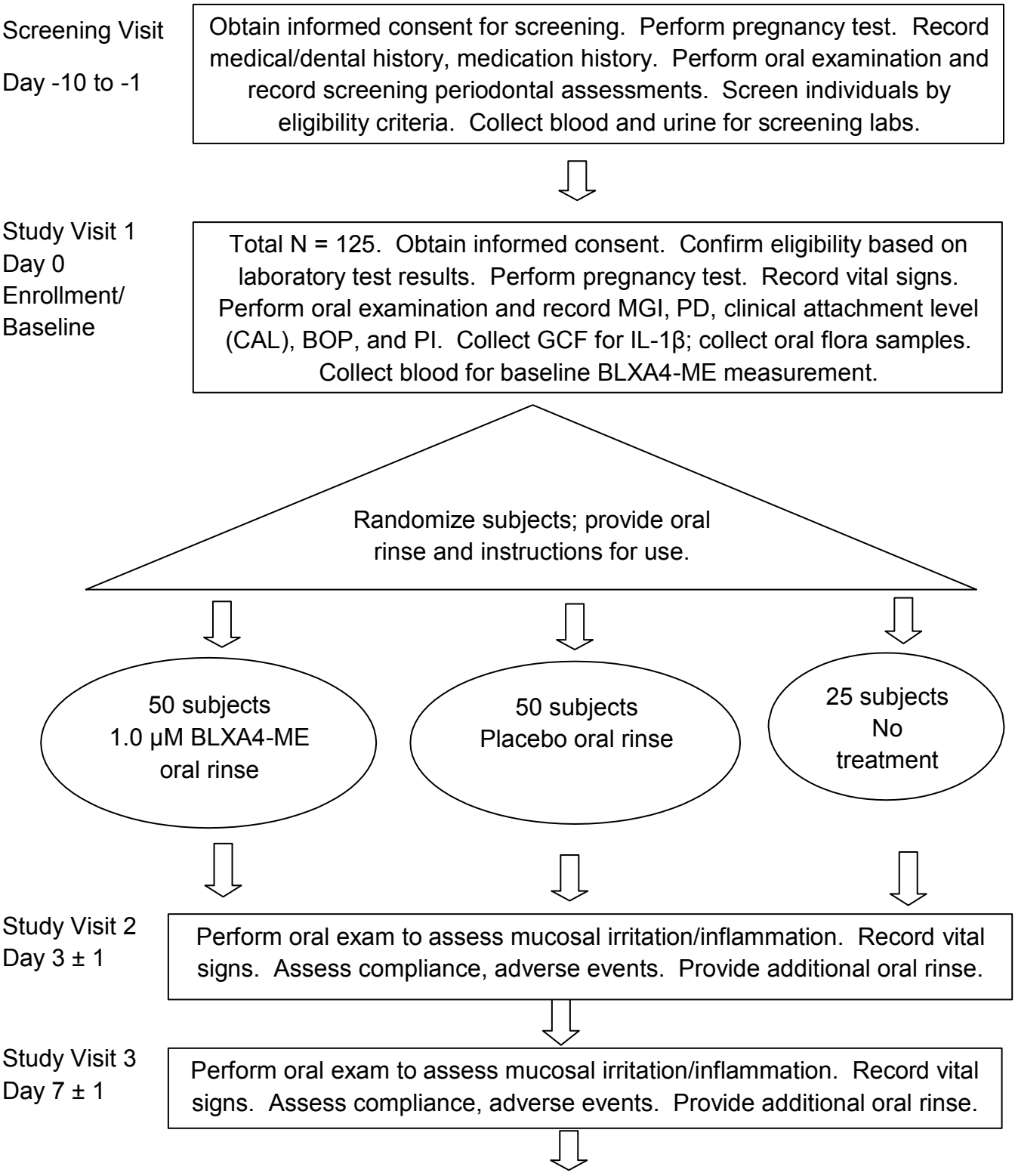
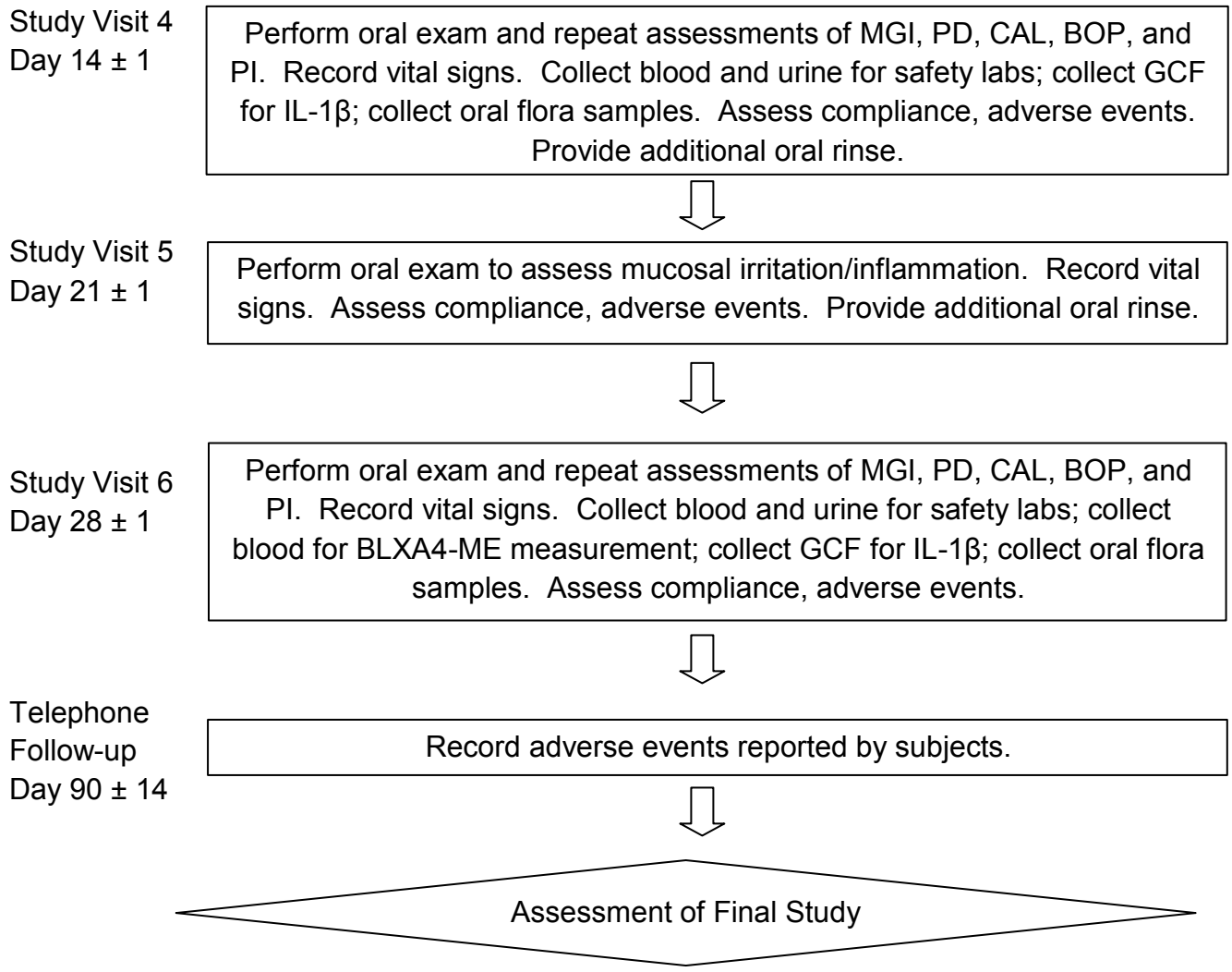
| | 2 positions of the tetraene unit of native lipoxin $A_4$. The placebo preparation will consist of formulated oral rinse without BLXA4-ME. |
| **Study Duration:** | 34 months |
| **Subject Participation Duration:** | Approximately 3 months (visits through 28 days plus safety follow-up phone call 2 months after last product use) |
| **Estimated Time to Complete Enrollment:** | 30 months |

**Schematic of Study Design**




Study Visit 4
Day 14 ± 1
Perform oral exam and repeat assessments of MGI, PD, CAL, BOP, and PI. Record vital signs. Collect blood and urine for safety labs; collect GCF for IL-1β; collect oral flora samples. Assess compliance, adverse events. Provide additional oral rinse.
Study Visit 5
Day 21 ± 1
Perform oral exam to assess mucosal irritation/inflammation. Record vital signs. Assess compliance, adverse events. Provide additional oral rinse.
Study Visit 6
Day 28 ± 1
Perform oral exam and repeat assessments of MGI, PD, CAL, BOP, and PI. Record vital signs. Collect blood and urine for safety labs; collect blood for BLXA4-ME measurement; collect GCF for IL-1β; collect oral flora samples. Assess compliance, adverse events.
Telephone Follow-up
Day 90 ± 14
Record adverse events reported by subjects.
Assessment of Final Study

# 1 KEY ROLES AND CONTACT INFORMATION

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# 2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

## 2.1 Background Information

Inflammation is the normal host tissue response to infection and injury. However, uncontrolled and unresolved inflammation contributes to a range of acute and chronic human conditions such as arthritis and cardiovascular diseases. The chronic inflammation in these conditions is characterized by the production of inflammatory cytokines, arachidonic acid-derived eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other oxidative derivatives), reactive oxygen species, and adhesion molecules (Gallin and Snyderman 1999, Tilley, Coffman et al. 2001, Nathan 2002). Periodontitis is a similar progressive inflammatory disease in which microbial etiologic factors induce an inflammatory cascade that leads to destruction of the organ supporting the teeth, the periodontium, including soft tissues and bone (Van Dyke, Lester et al. 1993, Giannobile, Lynch et al. 1995, Kornman, Page et al. 1997, Kinane and Lappin 2002). As with all inflammatory diseases, the arachidonate-derived eicosanoids play a key role in the initiation and pathogenesis of the inflammatory lesion in periodontitis (Offenbacher, Odle et al. 1986, Birkedal-Hansen 1993, Birkedal-Hansen, Moore et al. 1993). In the case of periodontitis, the associated gram negative bacteria, such as *Porphyromonas gingivalis* (*P. gingivalis*), initiate an influx of neutrophils and neutrophil cyclooxygenase-2 activation leading to increased prostaglandin E2 in situ (Pouliot, Clish et al. 2000). Indeed, many of the early pathophysiological events in periodontal disease and its chronicity can be attributed to lipid mediators (Offenbacher 1996). Leukotriene B4, produced mainly by activated leukocytes, initiates accumulation and superoxide generation by neutrophils within inflamed sites, stimulating the release of granule-associated enzymes and bone resorption (Samuelsson, Dahlen et al. 1987, Varani and Ward 1994). Prostaglandin E2 is a potent activator of osteoclast-mediated bone resorption, the hallmark of periodontal disease (Offenbacher, Odle et al. 1986, Tsai, Hong et al. 1998). Together with other eicosanoids, it mediates inflammation and periodontal tissue destruction (Smith, Braswell et al. 1993, Gronert, Kantarci et al. 2004).

Resolution of inflammation is an actively regulated program rather than the passive termination of inflammation, as was once widely believed (Levy, Clish et al. 2001, Serhan 2004, Bannenberg, Chiang et al. 2005, Serhan and Savill 2005). The crucial identification of the cellular events and molecular signals that determine the end of inflammation and beginning of resolution has led to a new appreciation of pathogenesis in inflammatory diseases (Serhan, Clish et al. 2000, Lawrence, Willoughby et al. 2002,

Heasman, Giles et al. 2003, Serhan, Brain et al. 2007). Neutrophils are present mainly in inflamed or injured tissues, and their effective elimination is a prerequisite for complete resolution of an inflammatory response (Ariel, Fredman et al. 2006). Most current therapeutic approaches attempt to block activation of inflammation using anti-inflammatory drugs that are pathway inhibitors (nonsteroidal anti-inflammatory drugs [NSAIDs], or tumor necrosis factor inhibitors), or to promote healing with agents such as transforming growth factor β1, bridging molecules, and phagocyte receptors (Kantarci, Hasturk et al. 2006). Prostaglandins and leukotrienes play essential roles in orchestrating inflammation and are well-appreciated autacoids or locally acting mediators (Samuelsson, Dahlen et al. 1987). Cyclooxygenase inhibitors are examples of widely used anti-inflammatory drugs that act by blocking prostaglandin biosynthesis (Flower 2003); however, their use may actually attenuate the active resolution pathways mediated by lipoxins, resolvins, and protectins (Levy, Clish et al. 2001, Serhan 2004).

A rapidly emerging body of evidence demonstrates that endogenous pro-resolving lipid mediators actively participate in regulating host responses and orchestrate resolution of inflammation (Serhan, Hong et al. 2002, Hong, Gronert et al. 2003). Lipoxins, the product of lipoxygenase and lipoxygenase interactions, are trihydroxy derivatives of arachidonic acid that actively drive resolution of inflammation; they are typified by lipoxin $A_4$ ($LXA_4$). In addition, the previously unappreciated role of aspirin-triggered transformation circuits in producing the endogenous anti-inflammatory 15-epi- form of $LXA_4$ has led to a better understanding of pro-resolution signaling networks, including a series of complex cellular and chemical reactions and tissue trafficking events (Serhan 2007). For example, lipoxins not only reduce the influx of neutrophils, but also stimulate the nonphlogistic uptake of apoptotic neutrophils by tissue macrophages (Godson, Mitchell et al. 2000, Serhan 2004).

## 2.2 Preclinical Studies on Treatment of Inflammatory Periodontal Disease

Current therapies for gingivitis and periodontitis remain inadequate. To examine the potential of pro-resolving molecules for the treatment of inflammatory oral diseases, a rabbit model of periodontitis has been used (Serhan, Jain et al. 2003, Hasturk, Kantarci et al. 2006, Hasturk, Kantarci et al. 2007). In this model system, *P. gingivalis* induced significant periodontal disease. An initial study examined an $LXA_4$ analog (aspirin-triggered lipoxin analog [15-epi-16-*para*-fluoro-phenoxy-$LXA_4$], 15-epi-$LXA_4$ analog, denoted ATLa) to treat 6 rabbits with experimentally induced periodontitis. A topical dose of 6 µg 3 times per week for 6 weeks around the second premolar was found to reduce microbe-initiated, neutrophil-mediated tissue damage and bone destruction (Serhan, Jain et al. 2003).

Based on these observations, a series of chemically and metabolically stable benzo-$LXA_4$ analogs were produced (Petasis, Keledjian et al. 2008). These analogs featured a modification of the tetraene portion via substitution of a benzo-fused ring system while retaining the biological activity of the lipid chain moieties. Noted advantages of this series of compounds were longer half-life in vitro and ease of synthesis through iterative palladium-mediated coupling methods. In comparison with native $LXA_4$, BLXA4-ME and related analogs were not readily converted to inactive forms in an in vitro stability assay system containing eicosanoid oxido-reductase, and this particular analog was most effective at inhibiting polymorphonuclear neutrophil infiltration in a murine peritonitis model (approximately 32% inhibition [n = 5-10, $p < 0.005$ compared with the vehicle control]). In the same model system, the benchmark compound, ATLa, gave 40% inhibition of polymorphonuclear neutrophil infiltration ($p < 0.05$) (Sun, Tjonahen et al. 2009). Based on these preliminary efficacy assessments, BLXA4-ME was chosen as the lead compound for clinical development in this series.

The observation of reduced tissue damage and bone loss in rabbits treated with a lipoxin analog suggests a paradigm shift in periodontitis treatment approaches—a move away from a purely mechanistic and antimicrobial approach to one that considers the driving force of the disease, namely uncontrolled inflammation. In this emerging paradigm, it is suggested that the infection can be controlled if the inflammation can be controlled. In one study, pushing the inflammatory response of an experimental periodontitis lesion towards a resolution of inflammation resulted not only in tissue repair/regeneration, but also in significant reversion of the subgingival microflora to one consistent with health (Hasturk, Kantarci et al. 2007). This shift in microflora occurred in the absence of any antimicrobial intervention, and it highlights the potential of resolution agonists like BLXA4-ME to play an active role in antimicrobial activities in resolving gingival inflammation (Campbell, Serhan et al. 2011). The expected outcome of treating gingivitis with BLXA4-ME is resolution of gingival inflammation with a return of the gingival microflora profile to one associated with health.

The remarkable potency of lipoxins and their analogs compared with NSAIDs supports a new mechanistic modality for the treatment of oral inflammatory conditions such as gingivitis and periodontitis through the resolution of inflammation rather than through interference with regulatory circuits controlling the initiation and maintenance of the inflammatory response. Although less active than native $LXA_4$, benzo-lipoxin analogs have proven activity in the nano- to microgram range in murine assays, and these doses are logarithmic orders of magnitude more potent than traditional NSAIDs, such as aspirin or indomethacin, and steroids (both classes requiring milligram/kilogram doses in vivo). The use of pro-resolving agonists could result in new therapies that reduce

inflammation without the unwanted side effects of traditional anti-inflammatory drugs (e.g., NSAIDs and steroids).

## 2.3 Investigational Product for the Current Study

The investigational compound BLXA4-ME was designed as a new drug entity with increased chemical and metabolic stability for use as a pro-resolving anti-inflammatory agent. It is an analog of the naturally occurring autacoid $LXA_4$.

The investigational compound, (5S, 6R, E)-methyl 5,6-dihydroxy-8-(2-((R,E)-3-hydroxyoct-1-enyl) phenyl) oct-7-enoate, was originally designated as 9,12-LXA4 in certain preclinical studies. The name of the compound has been changed to BLXA4-ME to more accurately reflect the nomenclature used by the manufacturer and analytical laboratory. The "-ME" designation reflects the methyl ester form of the drug substance. BLXA4-ME is converted by nonspecific esterase activity under physiological conditions in vivo to the more active "free-acid" form designated as BLXA4-FA. Both the methyl ester and free-acid forms of BLXA4 are pharmacologically active.

The active preparation (test rinse) for the study will contain BLXA4-ME at 1.0 µM as a formulated oral rinse containing 10% ethanol and 0.25% sodium lauryl sulfate in water. The placebo preparation will consist of formulated oral rinse without the BLXA4-ME.

## 2.4 Rationale

The prevention of gingival inflammation or gingivitis has been an area of intense research and development for many years. In spite of these efforts, only 2 types of oral rinse products have been marketed with an antigingivitis claim, those containing chlorhexidine and those containing essential oils. The chlorhexidine products have significant side effects that limit their routine usefulness. The essential oils have limited effectiveness. Both oral rinse products are antimicrobials; there are no products that directly address the inflammatory aspects of gingival inflammation or gingivitis. The hypothesis to be tested in the current study is that the lipoxin analog BLXA4-ME, applied topically in an oral rinse formulation, will be safe in humans and will enhance the resolution of gingival inflammation.

For reasons of subject compliance and routine oral hygiene habits, a once-a-day application was chosen. The dose was based on preclinical experiments in animals for topical application on a mg/kg-body weight basis (see Investigator's Brochure for preclinical data).

## 2.5 Potential Risks and Benefits

### *2.5.1 Potential Risks*

BLXA4-ME topical oral rinse is intended for administration as an oral rinse (mouthwash) and is not intended for ingestion. For this study, BLXA4-ME will be applied topically as an oral rinse that will be held in the mouth for 45 seconds each day for 28 days. Potential risks include oral irritation from use of the rinse. In a preclinical 28-day oral irritancy study in rats with abraded oral mucosa, transient erythema and edema of the oral mucosa were noted in animals treated with either BLXA4-ME or a saline solution used as a control. Microscopic examination of oral tissues showed no differences between rats treated with BLXA4-ME and control rats treated with saline. In this Phase 1/2 safety study, oral tissues will be closely monitored for signs of inflammation and irritancy, and product administration will be discontinued if toxicity develops. Because the investigational compound may be absorbed through the oral mucosa, blood and urine for safety laboratory tests will be collected from all subjects at screening and 14 and 28 days after initial product administration. Treatment of gingivitis has the potential to conceal the presence of the more serious condition of periodontitis; therefore, subjects will be monitored for development or progression of periodontitis, using clinical periodontal measurements. In addition, oral flora will be analyzed to detect any increase in pathogenic organisms.

Following the Screening Visit, individuals with gingivitis or periodontitis who are not eligible for participation in the trial will be informed of their disease status. Those with gingivitis will be provided with oral hygiene instructions. Those with periodontitis will be advised to seek treatment and will be provided with information about dental facilities that could provide care. Subjects who enroll in the trial will have inflammatory periodontal disease (gingivitis and/or periodontitis), and it is possible they will experience no improvement in their condition during the study. Subjects will be informed of their disease status at enrollment and will be told that they may need to seek periodontal treatment after their participation in the trial is completed. At the conclusion of their study participation, those with gingivitis will be provided with oral hygiene guidance, and those with periodontitis will be advised to seek treatment and will be provided with information about dental facilities that could provide care.

### *2.5.2 Potential Benefits*

As this is a Phase 1/2 safety study, the benefits to subjects from participation are not known. This study has the potential to provide safety and preliminary efficacy information that could support further studies of the BLXA4-ME compound to assess its efficacy in the treatment of gingivitis or periodontal disease.

# 3 OBJECTIVES

## 3.1 Study Objectives

The primary objective is to evaluate the safety of an investigational compound, BLXA4-ME, topically applied for 28 days as a daily oral rinse in adults with gingival inflammation as determined by a modified gingival index (MGI) score of 2.0 or more. Safety will be assessed based on the incidence of adverse events (AEs), including mucosal inflammation and irritancy, and findings from oral examinations and safety laboratory tests. Subjects will be monitored for the development of periodontitis or progression of existing periodontitis. Oral flora will be analyzed to detect any increase in pathogenic organisms.

A secondary objective is to assess preliminary efficacy of the oral rinse. Efficacy will be measured by changes in the plaque index (PI) (Silness and Loe 1964), MGI (Gordon, Lamster et al. 1985), bleeding on probing (BOP), and levels of interleukin-1β (IL-1β) in gingival crevicular fluid (GCF).

## 3.2 Study Outcome Measures

### *3.2.1 Primary*

The primary outcome measure of this study is safety, which will be assessed by the incidence of AEs, development or progression of periodontitis and changes in the oral flora.

Adverse events will be recorded throughout the study, and may include events reported by subjects or changes observed in oral cavity examinations or vital signs (assessed at baseline, Days 3, 7, 14, 21, and 28). Blood and urine will be collected for safety laboratory tests at Days 14 and 28 after product administration. Subjects will undergo close monitoring for mucosal inflammation and irritancy.

Subjects will be monitored for the development of or the progression of existing periodontitis, using standard clinical periodontal measurements. Development or progression of periodontitis will be determined by an increase of 2 mm or more in pocket depth or clinical attachment level (CAL) from the Day 0 baseline measurement (Goodson et al, 1982).

Each subject's oral flora will be analyzed in a pooled plaque sample collected from the mesiobuccal surfaces of the Ramfjord teeth at the Baseline Visit and at Days 14 and 28 of the experimental period. Using the deoxyribonucleic acid (DNA)-DNA hybridization (checkerboard) method (Socransky, Smith et al. 1994, Socransky, Haffajee et al. 2004),

samples will be analyzed for the presence of the following species: *Fusobacterium nucleatum subspecies vincentii, Campylobacter concisus, Campylobacter rectus, Tannerella forsythensis, Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis, Capnocytophaga sputigena, Streptococcus oralis, Actinomyces naeslundii, Actinomyces israeli, Eubacterium brachy, Eikenella corrodens*, and spirochetes.

### *3.2.2 Secondary*

The secondary outcome measures will consist of the change from baseline in the preliminary efficacy measures. These secondary efficiacy measures include MGI (Gordon, Lamster et al. 1985), BOP, PI (Silness and Loe 1964), and levels of IL-1β in GCF. In addition, plaque and gingivitis severity scores will be calculated using methods previously described (Panagakos, Volpe et al. 2005). Levels of IL-1β, a marker of inflammation, will be determined in the GCF collected at baseline and 14 and 28 days after treatment. Gingival crevicular fluid will be collected and quantified, and IL-1β levels in the GCF will be determined by enzyme-linked immunosorbent assay (ELISA).

### *3.2.3 Exploratory*

Differences among the treatment arms with respect to the change from baseline in pocket depth and clinical attachment level at Day 28 will be assessed as exploratory analyses.

## 4 STUDY DESIGN

This Phase 1/2 study comprises 3 groups in a randomized, placebo-controlled, double-blind clinical trial design. Subjects will be healthy adults, aged 18 through 65 years, with gingivitis as defined by MGI ≥ 2.0. The treatment group (1.0 μM BLXA4-ME oral rinse) and the placebo rinse group will each consist of 50 subjects. The no-rinse control group will consist of 25 subjects. The group sizes allow for a 20% dropout rate.

The treatment group will receive the active agent BLXA4-ME at a concentration of 1.0 μM in an oral rinse applied once daily (after morning teeth brushing) for 28 days. The placebo rinse control subjects will rinse once daily after morning teeth brushing with the same rinse formulation, except that it will not contain BLXA4-ME. The no-rinse control group will use no oral rinse to assess the effect of the rinsing action independent of the active ingredients. At the Enrollment/Baseline Visit, eligible individuals will be randomly assigned to 1 of the 3 treatment groups. The baseline clinical measurements, i.e., MGI, probing depth (PD), CAL, PI, and BOP, will be recorded. Plaque, GCF, and blood will be collected as described in the Manual of Procedures. The subjects randomized to rinse groups will be given the BLXA4-ME oral rinse formulation or

placebo rinse with instructions for use in addition to their regular oral hygiene. Subjects in all groups will be instructed to maintain their regular oral hygiene procedures, except for using the study-provided rinse, if applicable.

Subjects will return to the clinic on Days 3, 7, 14, 21, and 28 to undergo an oral examination to assess mucosal irritancy and inflammation, to participate in an interview to solicit AE information, and to renew their supply of oral rinse (excluding Day 28). Additional procedures on Days 14 and 28 will be recording of periodontal measurements to monitor for development of periodontitis, collection of blood and urine for safety laboratory tests, collection of plaque samples for analysis of oral flora, and collection of GCF for measurement of IL-1β. To assess absorption of BLXA4-ME through the oral mucosa, subjects will provide blood samples at Day 0 and Day 28, for measurement of plasma levels of BLXA4-ME, and for profiling of specialized lipid mediators in plasma and serum. Subjects will be contacted via telephone approximately 2 months after completion of treatment for safety follow-up. The total duration of an individual subject's participation in the study is 3 months.

# 5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 125 healthy adult male and female subjects with gingival inflammation, determined by mean full mouth MGI of at least 2.0, will participate in the study. The inclusion and exclusion criteria are as follows:

## 5.1 Subject Inclusion Criteria

1. Signed consent form;
2. Good general health as evidenced by medical history;
3. Age 18 through 65 years;
4. Stable address;
5. Availability for the duration of the study (if a subject does not meet this criterion, he/she may be rescreened for study participation when he/she does meet this inclusion criterion);
6. Minimum of 20 natural teeth, excluding third molars;
7. Mean full mouth MGI of at least 2.0;
8. Willing to use prescribed oral hygiene procedures and products;

9. Stability of medications for chronic conditions for at least 3 months prior to enrollment (if a subject does not meet this criterion, he/she may be rescreened for study participation when he/she does meet this inclusion criterion);

10. For women of reproductive potential, use of licensed hormonal contraception or double-barrier methods;

11. For men of reproductive potential, agreement to use condoms;

12. Liver function test (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, and total bilirubin) levels equal to or less than 1.5 times the upper limit of normal;

13. Serum creatinine levels equal to or less than the upper limit of normal;

14. Subjects with complete blood count levels within 10% of the normal laboratory range and erythrocyte sedimentation rate equal to or less than 2 times the upper limit of normal.

## 5.2 Subject Exclusion Criteria

1. Presence of orthodontic appliances or removable partial dentures;

2. Presence of a soft tissue tumor of the oral cavity;

3. Presence of gross plaque or calculus as determined by the investigator;

4. Presence of extensive restorations that could affect the marginal gingiva (at the investigator's discretion);

5. Preexisting oral pathology requiring immediate treatment or ulcerations of the mucosa;

6. Preexisting carious lesions requiring immediate treatment (if a subject meets this criterion, he/she may be rescreened for study participation when he/she no longer meets this exclusion criterion);

7. Current participation in another clinical trial or product test;

8. Pregnant or breastfeeding;

9. Residence in the same household as a subject currently enrolled in the study (due to potential blinding and compliance issues);

10. Periodontal therapy other than prophylaxis within the past 6 months or endodontic therapy within 1 month of enrollment (if a subject meets this criterion, he/she may be rescreened for study participation when he/she no longer meets this exclusion criterion);

11. History of early onset periodontitis or acute necrotizing ulcerative gingivitis;

12. Chronic disease with concomitant oral manifestations, such as autoimmune or immunosuppressive diseases (e.g., human immunodeficiency virus, severe combined immunodeficiency, neutropenia, juvenile arthritis, systemic lupus erythematosus, sickle cell anemia, Crohn's disease, rheumatoid arthritis, Sjögren's syndrome) or immunocompromised status due to cancer chemotherapy, hematopoietic stem cell or solid organ transplant, head and neck radiotherapy, splenectomy, chronic corticosteroid usage;

13. Recent history of chronic alcohol consumption of more than five 1.5-ounce servings of 80-proof distilled spirits, five 12-ounce servings of beer, or five 5-ounce servings of wine per day;

14. Tobacco use (former tobacco users may be enrolled, provided they have been tobacco-free for 1 year or more);

15. Diabetes mellitus;

16. Subjects with urinalysis results suggestive of infection (if a subject meets this criterion, he/she may be rescreened for study participation when he/she no longer meets this exclusion criterion);

17. Medical conditions that the investigator considers significant and that may interfere with the examination or the safety of the subject;

18. Chronic (2 weeks or more) use of medication known to affect periodontal status within 1 month of enrollment, such as ≥ 81 mg aspirin per day, phenytoin, calcium antagonists such as nifedipine, NSAIDs, warfarin, cyclosporine, ≥ 10 mg/day atorvastatin or equivalent dose of another statin (Subramanian, Emami et al. 2013) (if a subject meets this criterion, he/she may be rescreened for study participation when he/she no longer meets this exclusion criterion);

19. Treatment with antibiotics within 1 month prior to enrollment (if a subject meets this criterion, he/she may be rescreened for study participation when he/she no longer meets this exclusion criterion);

20. Medical condition for which antibiotic treatment during the study period is likely or a condition for which antibiotic prophylaxis is recommended before dental procedures (American Heart Association 2007 guidelines [Geist, Fitzpatrick et al. 2007] will be followed);

21. Known hypersensitivity to any component of the test or placebo products;

22. Anything that, in the opinion of the investigator, would place the subject at increased risk or prevent the subject from fully complying with or completing the study.

The on-site medical monitor will review all laboratory test results and ensure that subjects meet the inclusion criteria for the study and do not meet any exclusion criteria. Additionally, the on-site medical monitor will decide whether subjects with an abnormal laboratory test result should have the laboratory test repeated. Subjects with a second abnormal laboratory test result will be referred to their primary care physician.

### 5.3 Strategies for Recruitment and Retention

Enrollment of 125 subjects is expected to take place over approximately 30 months. Having an already identified subject pool at the Forsyth Institute Center for Clinical and Translational Research (CCTR) should allow recruitment of subjects required to conduct the study in a timely manner. Subjects will be recruited from the CCTR subject pool, from area health centers, and through advertisements in local newspapers and electronic media outlets. All recruitment information and advertising will be submitted to the Institutional Review Board (IRB) prior to release.

Prospective subjects will receive a free periodontal examination and close dental and periodontal surveillance during the study. In addition, each subject will be compensated for visits attended, and parking fees will be paid for individuals who attend screening or study visits.

Subjects who miss a scheduled visit without notifying the study staff will be contacted by telephone, email, or text message to encourage their continued participation in the study and provide them with another appointment. If the subject declines further participation, he/she will be referred to a qualified practitioner for continued care. If a subject cannot be contacted by one of the methods listed above or he/she does not keep the next scheduled appointment, he/she will be sent a certified letter advising of the need for further follow-up and offering referral to a qualified practitioner for continued care.

Every effort will be made to encourage full participation until the end of the study. If a participant appears to be considering ending participation in the study, specific retention activities will be conducted to encourage him or her to continue. Strategies to promote adherence to study requirements will include after-hours visits for those who have tight work or personal schedules. To encourage compliance with study procedures, participants will be provided written instructions for product use and important details about the study protocol, and will be reminded of these at each study visit and with phone calls. Based on the participant's preferred communication method, reminder postcards, emails, telephone calls, and/or telephone texts will be provided prior to clinic visits. Appointment cards with important information on the reverse side (for example, contact person and phone number in case of an AE) will be given to all subjects after each completed visit.

## 5.4 Treatment Assignment Procedures

### *5.4.1 Randomization Procedures*

Subjects will be assigned to treatment groups using a block randomization schedule.

### *5.4.2 Masking Procedures*

The examiner will be masked to the assigned treatment. Subjects receiving oral rinse will not know whether they are in the active or placebo rinse group. A qualified and trained individual not involved in the examination procedures will be responsible for making assignments to treatment groups, dispensing the products to the subjects with instructions, collecting information on subject compliance, and soliciting AE reports from subjects.

Test materials will be coded and the identity of the products concealed, so the examiners and subjects will remain blinded. Subjects will be reminded to avoid discussing product use with their examiners at any time during their participation.

The product code may be broken for an individual subject if he or she experiences a serious adverse event (SAE) and cannot be adequately treated without knowing the identity of the study product. Every effort will be made to inform the study investigators prior to breaking the blind, or in the event of an emergency, as soon as possible thereafter.

## 5.5 Subject Withdrawal

### *5.5.1 Reasons for Withdrawal*

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

An investigator may terminate a study subject's participation in the study if:

- Any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

### *5.5.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention*

Should a subject elect to withdraw from the study and cease use of product, efforts will be made to complete as much follow-up of safety information as possible. Telephone contact with the subject is acceptable if it is not possible to arrange a follow-up appointment. Subjects who withdraw prior to the Day 14 Visit will be replaced.

## 5.6 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator, the Investigational New Drug (IND) sponsor, the National Institute of Dental and Craniofacial Research (NIDCR), and the Food and Drug Administration (FDA). If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

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

- Determination of unexpected, significant, or unacceptable risk to subjects;
- Insufficient adherence to protocol requirements;
- Data that are not sufficiently complete and/or evaluable;
- Plans to modify, suspend, or discontinue the development of the study drug;
- Determination of futility. No formal futility analysis is planned. However, if serious safety concerns require the unblinding of all study subjects, a lack of

efficacy may be considered along with the safety concerns as a reason for the premature termination of the study.

# 6 STUDY INTERVENTION

## 6.1 Study Product Description

### *6.1.1 BLXA4-ME Oral Rinse*

BLXA4-ME is a member of a new class of chemically and metabolically stable lipoxin analogs featuring a replacement of the tetraene unit of native $LXA_4$ with a substituted benzo-fused ring system. The full chemical name of the BLXA4-ME drug substance is (5S, 6R, E)-methyl 5,6-dihydroxy-8-(2-((R,E)-3-hydroxyoct-1-enyl) phenyl) oct-7-enoate.

The topical oral rinse dosage form of BLXA4-ME (also known as ClinRinse-1) will consist of drug substance prepared at a concentration of 1.0 µM in an aqueous vehicle solution containing the inactive components shown in Table 6-1.

**Table 6-1 Inactive Components of BLXA4-ME**

| COMPONENT | AMOUNT (%w/w) |
|---|---|
| Saccharin sodium | 0.03 |
| Ethanol (95%) | 10.00 |
| Propylene glycol | 7.00 |
| Sodium lauryl sulfate | 0.25 |
| Sorbitol | 10.00 |
| Flavoring oil | 0.145 |
| Water | 72.575 |

The physical and chemical properties of BLXA4-ME, its anti-inflammatory and pro-resolving actions, and the procedures for preparing the oral rinses are described in the Investigator's Brochure.

### *6.1.2 Placebo Oral Rinse*

The placebo preparation will consist of formulated oral rinse without BLXA4-ME and will be identical to the test rinse in color, appearance, and taste.

### *6.1.3 Acquisition*

The BLXA4-ME compound and the oral rinse formulation containing the 1.0 μM dose of BLXA4-ME (ClinRinse-1) will be prepared by Avanti Polar Lipids, Alabaster, Alabama, a facility that adheres to the principles of Good Manufacturing Practice. The topical oral rinse will be packaged into amber high-density polyethylene bottles for the clinical studies and shipped to the Forsyth Institute.

### *6.1.4 Formulation, Packaging, and Labeling*

The formulation of ClinRinse-1 and placebo rinse is described in the Investigator's Brochure. Each bottle of study product will contain a volume sufficient for 8 doses plus an excess volume to accommodate for an out-of-window study visit. The residual volume will be measured to assess compliance with product use. Labels on the rinse bottles will comply with the regulations for labeling of an investigational product (21 CFR Part 312.6), and the active and placebo rinses will be identified by codes, to maintain the blinding of subjects and investigators.

### *6.1.5 Product Storage and Stability*

Prior to its distribution to study participants, the BLXA4-ME oral rinse (ClinRinse-1) and placebo rinse will be stored at 2-8°C in high-density polyethylene bottles in a secure area reserved for study materials at the Forsyth Institute CCTR. Subjects will be instructed to store the bottle at room temperature during the week of use. Storage with the subject's toothbrush is intended to encourage compliance with the protocol-specified product use. Testing of the drug product has demonstrated that it is stable at 2-8°C for up to 6 months, and stable at 25°C/60% relative humidity for up to 4 weeks.

## 6.2 Dosage, Preparation, and Administration of Study Product

ClinRinse-1 containing 1.0 μM BLXA4-ME and placebo rinse will be provided to study participants in the original bottles supplied by Avanti. A 30 mL polypropylene dosing cup with volume markings will be provided with the rinse. Subjects will rinse under supervision on the day they pick up study drug. They will be instructed to use the rinse (30 mL for 45 seconds) daily after brushing their teeth in the morning. Subjects will be provided with a timer so that 45 seconds of use can be standardized. Subjects will be resupplied with study drug on Days 3, 7, 14, and 21.

### 6.3 Modification of Study Product Administration for a Subject

See Sections 5.5 and 5.6 for information on product discontinuation.

### 6.4 Accountability Procedures for the Study Product

All study products will be stored at 2-8°C in a secure area reserved for study materials at the Forsyth Institute CCTR. ClinRinse-1 and placebo rinses will be segregated to maintain effective control of study products and ensure proper distribution to subjects. Documentation to maintain study product accountability will occur at all visits. The product number and amount of medication provided will be recorded, and information will be collected to track subject compliance. To assist with product accountability, subjects will be instructed to bring the bottle containing unused product to each follow-up visit. A trained and qualified individual not involved in the examination procedures will distribute product, provide rinse instructions, receive the returned bottles, and measure and record the residual volume of rinse.

### 6.5 Assessment of Subject Compliance with Study Product Administration

Compliance will be recorded at all follow-up study visits by measurement of the volume of remaining product. This will be done to estimate under- and over-usage of study drug. All subjects will be resupplied with rinse preparations at each study visit through the Day 21 Visit. In addition, the subjects will be provided with memory aid cards, and will be queried as to their compliance with the required schedule of product use.

### 6.6 Concomitant Medications/Treatments

Subjects will receive instructions to avoid, during the 28 days of product use, any medications and dental procedures that could affect periodontal status (see exclusion criteria). At each study visit, subjects will be asked to report all concomitant medications and any dental care received or procedures performed during the study period.

## 7 STUDY SCHEDULE

### 7.1 Prescreening

Prescreening of potential subjects may be completed using a telephone script with questions that assess preliminary eligibility.

## 7.2 Screening

**Screening Visit (Day -10 to -1)**

- Obtain and document consent from potential subject on screening consent form.
- Record demographics information.
- Review and record medical/dental history, including alcohol/tobacco use, to determine eligibility based on inclusion/exclusion criteria.
- Review and record medications history to determine eligibility based on inclusion/exclusion criteria.
- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure, weight).
- Perform intraoral and extraoral examinations needed to determine eligibility, including hard and soft tissue assessments (face, lymph nodes, lips, buccal mucosa, floor of the mouth, tongue, hard and soft palate, gingiva, edentulous ridges, and teeth).
- Perform the MGI to ensure mean full mouth MGI of at least 2.0. Subjects with a mean MGI of less than 2.0 will not be enrolled in the study.
- Assess for the presence of gross plaque and calculus. Gross plaque and calculus are based on investigator discretion. Subjects with the presence of gross plaque or calculus will not be enrolled in the study.
- Obtain urine pregnancy test on women of childbearing potential. Study staff will record the test kit lot, expiration date, and result. Test must be documented as negative to meet eligibility criteria.
- Obtain urine for urinalysis.
- Obtain approximately 15 mL blood for complete blood count, erythrocyte sedimentation rate, creatinine, blood urea nitrogen, alkaline phosphatase, AST, ALT, total bilirubin, and electrolytes (sodium, potassium, chloride, and bicarbonate).
- Schedule study visits for individuals who are potentially eligible (eligibility is confirmed after receipt of laboratory results).
- For individuals with periodontal disease who are found to be ineligible, provide, as appropriate, oral hygiene instructions or information about dental facilities that could provide periodontal treatment.

## 7.3 Enrollment/Baseline

**Study Visit 1 - Enrollment/Baseline Visit (Day 0)**

- Obtain and document consent from subject on study consent form.
- Review and confirm medical and dental history, including alcohol and tobacco use history, for study inclusion.
- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure).
- Record current concomitant medications and dental procedures and update medication history for study inclusion.
- Obtain urine pregnancy test on women of childbearing potential. Study staff will record the test kit lot, expiration date, and result. Test must be documented as negative before continuing to conduct baseline procedures that follow.
- Perform intraoral and extraoral examinations, to include hard and soft tissue assessments (face, lymph nodes, lips, buccal mucosa, floor of the mouth, tongue, hard and soft palate, gingiva, edentulous ridges, and teeth).
- Collect GCF for baseline measurements of IL-1$\beta$.
- Assess and record baseline MGI.
- Assess and record baseline PI.
- Obtain supragingival plaque samples for baseline analyses of oral bacteria.
- Assess and record baseline PD and GM-CEJ distance (to calculate CAL).
- Assess and record baseline BOP.
- Reconfirm eligibility prior to subject randomization based on information collected during Screening Visit and study Visit 1.
- Obtain approximately 15 mL blood for baseline BLXA4 level and specialized pro-resolution mediator (SPM) (lipoxins and resolvins) profiling.
- Randomize subject to 1 of the study arms, using a block randomization schedule.
- Provide study drug with instructions for use and a timer to be used while rinsing. A study team member other than the examiner will supervise the subject's first use of the medication.
- Provide subject with the memory aid and instructions for its use.
- Provide subject with a study contact number to call for AEs or questions.

## 7.4 Intermediate Study Visits

**Study Visit 2 (Day 3 ± 1)**

- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure).
- Update medical and dental histories.
- Record concomitant medications and dental procedures.
- Perform intraoral and extraoral examinations; evaluate mucosal irritancy using edema/erythema score and record other findings.
- Review memory aid and document AEs and product experience.
- Review product use and compliance.
- Record volume of unused product in bottle.
- Provide additional product, if needed.

**Study Visit 3 (Day 7 ± 1)**

- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure).
- Update medical and dental histories.
- Record concomitant medications and dental procedures.
- Perform intraoral and extraoral examinations; evaluate mucosal irritancy using edema/erythema score and record other findings.
- Review memory aid and document AEs and product experience.
- Review product use and compliance.
- Record volume of unused product in bottle.
- Provide additional product.

**Study Visit 4 (Day 14 ± 1)**

- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure).
- Update medical and dental histories.
- Record concomitant medications and dental procedures.
- Obtain approximately 15 mL blood for complete blood count, erythrocyte sedimentation rate, creatinine, blood urea nitrogen, alkaline phosphatase, AST,

ALT, total bilirubin, and electrolytes (sodium, potassium, chloride, and bicarbonate).

- Obtain urine for urinalysis.
- Perform intraoral and extraoral examinations; evaluate mucosal irritancy using edema/erythema score and record other findings.
- Collect GCF for measurements of IL-1β.
- Assess and record MGI.
- Assess and record PI.
- Obtain supragingival plaque samples for analyses of oral bacteria.
- Assess and record PD and GM-CEJ distance (to calculate CAL).
- Assess and record BOP.
- Review memory aid and document AEs and product experience.
- Review product use and compliance.
- Record volume of unused product in bottle.
- Provide additional product.

**Study Visit 5 (Day 21 ± 1)**

- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure).
- Update medical and dental histories.
- Record concomitant medications and dental procedures.
- Perform intraoral and extraoral examinations; evaluate mucosal irritancy using edema/erythema score and record other findings.
- Review memory aid and document AEs and product experience.
- Review product use and compliance.
- Record volume of unused product in bottle.
- Provide additional product.

## 7.5 Final Study Visit

**Study Visit 6 (Day 28 ± 1)**

- Record vital signs (temperature, pulse rate, respiratory rate, blood pressure, weight).
- Update medical and dental histories.
- Record concomitant medications and dental procedures.
- Obtain approximately 15 mL blood for complete blood count, erythrocyte sedimentation rate, creatinine, blood urea nitrogen, alkaline phosphatase, AST, ALT, total bilirubin, and electrolytes (sodium, potassium, chloride, and bicarbonate).
- Obtain approximately 15 mL blood for BLXA4 level and SPM (lipoxins and resolvins) profiling.
- Obtain urine for urinalysis.
- Perform intraoral and extraoral examinations; evaluate mucosal irritancy using edema/erythema score and record other findings.
- Collect GCF for measurements of IL-1β.
- Assess and record MGI.
- Assess and record PI.
- Obtain supragingival plaque samples for analyses of oral bacteria.
- Assess and record PD and GM-CEJ distance (to calculate CAL).
- Assess and record BOP.
- Review memory aid and document AEs and product experience.
- Review product use and compliance.
- Record volume of unused product in bottle.

## 7.6 Final Safety Evaluation

**Telephone Call for Safety Follow-up (3 months ± 2 weeks)**

The subject will be contacted via telephone to collect information on any AEs that may have occurred since the final study visit. At the end of the telephone interview, subjects will be reminded that they may need periodontal treatment, and will be provided with information on dental facilities that could provide care.

### 7.7 Withdrawal Visit

If a subject withdraws early or is withdrawn by the investigator, efforts will be made to complete as much follow-up of safety information as possible. The subject will be invited to attend a withdrawal visit at which safety assessments will be completed. Telephone contact with the subject is acceptable if it is not possible to arrange a follow-up appointment.

### 7.8 Unscheduled Visit

Unscheduled visits will be documented, recording the time and the reason for the visit and the disposition of the subject at the end of the visit.

## 8 STUDY PROCEDURES/EVALUATIONS

### 8.1 Clinical Evaluations

#### *8.1.1 Safety Observations and Other Measurements*

A complete medical history will be obtained at the Screening Visit. The medical history will also include demographic information, alcohol and tobacco history, medications, dental procedures, and dental history information.

Vital signs, including oral temperature, pulse rate, respiratory rate, and blood pressure, will be measured at all study visits beginning with the Screening Visit and through the Day 28 Visit. Weight will be collected only at the Screening and Day 28 Visits.

An intraoral and extraoral examination will be completed at each of the visits from Baseline through the Day 28 Visit. During the intraoral examination, the oral cavity will be evaluated for ulceration and erythema using the Oral Mucositis Assessment Scale (OMAS) (Sonis, Eilers et al. 1999). Subjects will have the OMAS scored by 2 independent examiners at each study visit, until 10 subjects have completed the Day 28 visit. The OMAS will be used to evaluate ulceration and erythema at the following sites:

- Upper and lower lips
- Hard and soft palate/fauces
- Right and left buccal mucosa
- Ventral and lateral surfaces on the tongue
- Floor of mouth

Ulceration and erythema will be scored using the system shown in Table 8-1.

**Table 8-1 Scoring System for Ulceration and Erythema**

| **Oral cavity for Ulceration** | | **Oral cavity for Erythema** | |
|---|---|---|---|
| Grade 0 | No lesion | Grade 0 | None |
| Grade 1 | Lesion < 1 $cm^2$ | Grade 1 | Not severe |
| Grade 2 | Lesion 1 to 3 $cm^2$ | Grade 2 | Severe |
| Grade 3 | Lesion > 3 $cm^2$ | | |

Subject's periodontal measurements (Section 8.1.2) will be monitored for the development of or the progression of periodontitis. Safety laboratory tests will be obtained and reviewed by the on-site medical monitor as described in Section 8.2. At each visit except Screening and Baseline, the subject will provide the completed memory aid, and the clinician will interview the subject and collect information regarding solicited AEs. The clinician will also record concomitant medications and confirm that the subject has not had any dental procedures.

### *8.1.2 Clinical Periodontal Measurements*

The clinical periodontal measurements described below will be obtained from the full mouth at Baseline and on Days 14 and 28. In addition, plaque and gingivitis severity scores will be calculated using the method described in (Panagakos, Volpe et al. 2005). These severity scores represent the ratio of surfaces with high PI (scores 2 and 3 assessed with the Silness and Löe PI) and high gingival index (scores 3 and 4 assessed with the MGI) to total scored surfaces.

- Modified gingival index (Gordon, Lamster et al. 1985): The MGI will be assessed at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual aspects of the tooth), and will be recorded as follows:

  0 = absence of inflammation

  1 = mild inflammation; slight change in color, little change in texture of any portion of, but not the entire, marginal or papillary gingival unit

  2 = mild inflammation; criteria as above but involving the entire marginal and papillary gingival unit

3 = moderate inflammation; glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit

4 = severe inflammation; marked redness, edema, and/or hypertrophy of the marginal or papillary gingival unit; spontaneous bleeding, congestion, or ulceration

- Probing depth: Periodontal pocket depth will be determined at 6 sites per tooth using a UNC-15 periodontal probe, with measurements rounded to the next lower whole millimeter.
- Distance from free gingival margin to cementoenamel junction: The GM-CEJ will be measured to calculate the CAL. The GM-CEJ is measured with a periodontal probe at 6 sites per tooth, and is recorded in millimeters rounded to the next lower whole number. Millimeters of recession will be recorded as a negative number, and when the free gingival margin is coronal to the cementoenamal junction, the GM-CEJ will be recorded as a positive number.
- Clinical attachment level: The CAL will be calculated by subtracting the GM-CEJ from the PD.
- Bleeding on probing: BOP is a dichotomous measurement that will be recorded at the same 6 sites per tooth:

  0 = no bleeding in 15 seconds after probing

  1 = bleeding in 15 seconds after probing

- Plaque index (Silness and Loe 1964): Plaque will be assessed at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual aspects of the tooth). The PI will be recorded as follows:

  0 = no plaque

  1 = film at the gingival margin

  2 = moderate (easily visible)

  3 = abundance of material

Periodontal measurements obtained on Days 14 and 28 will be compared with measurements obtained at Baseline to monitor subjects for development of or progression of periodontitis, defined for this study as an increase of 2 mm or more in pocket depth or CAL from the Day 0 baseline measurement.

## 8.2 Laboratory Procedures/Evaluations

Blood and urine for safety laboratory tests will be collected prior to treatment and on Days 14 and 28. Safety laboratory test results will be reviewed by the on-site medical monitor.

### *8.2.1 Clinical Laboratory Evaluations*

#### 8.2.1.1 Pregnancy Test

A urine pregnancy test will be performed for women of childbearing potential at the Screening and Baseline Visits. The results must be available and negative before initiation of product use and continuation in the study.

#### 8.2.1.2 Blood and Urine Analyses for Safety Assessments

Blood will be sent to a clinical laboratory for the following tests:

- Complete blood count: hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count with differential and absolute counts by WBC type, platelet count, and RBC indices (mean corpuscular volume, mean corpuscular hemoglobin concentration, RBC distribution width)
- Erythrocyte sedimentation rate
- Serum chemistry tests: creatinine, blood urea nitrogen, alkaline phosphatase, AST, ALT, total bilirubin, and electrolytes (sodium, potassium, chloride, and bicarbonate).
- Urinalysis: color, appearance, specific gravity, pH, glucose, bilirubin, ketones, occult blood, protein, nitrite, leukocyte esterase, WBCs, RBCs, squamous and other epithelial cells, bacteria, casts, and crystals.

The on-site medical monitor will review the results of safety laboratory tests to determine whether a laboratory test should be redrawn. Subjects with serum creatinine values greater than 50% above their baseline level will be retested, even if within normal range. Subjects with persistent out-of-normal values at the retest will be referred to their primary care physician and will be followed until the values have returned to the normal range or are confirmed as stable with an adequate explanation. Additionally, the on-site medical monitor will review the laboratory results to assist the principal investigator with assessing and monitoring AEs.

### *8.2.2 Special Assays or Procedures*

#### 8.2.2.1 Plaque Sample Collection, Processing and Analysis for Safety Assessments

A plaque sample will be obtained from every subject at the Baseline Visit, Day 14, and Day 28, the conclusion of the experimental period. These plaque samples of oral flora will be analyzed for any increase in opportunistic or pathogenic bacteria. Supragingival plaque will be obtained from 6 sites in the mouth (the mesiobuccal surfaces of the Ramfjord teeth). Samples will be placed in transport media for DNA checkerboard probe analysis (DNA-DNA hybridization assay) and analyzed according to the method of Socransky (Socransky, Smith et al. 1994, Socransky, Haffajee et al. 2004). Samples will be analyzed for the presence of *Fusobacterium nucleatum subspecies vincentii, Campylobacter concisus, Campylobacter rectus, Tannerella forsythensis, Prevotella intermedia, Prevotella nigrescens, Porphyromonas gingivalis, Capnocytophaga sputigena, Streptococcus oralis, Actinomyces naeslundii, Actinomyces israeli, Eubacterium brachy, Eikenella corrodens,* and spirochetes. The technique has been validated by several laboratories and found to be accurate, sensitive (detects $10^4$ or more bacteria), and specific when carefully selected DNA probes are used.

#### 8.2.2.2 Gingival Crevicular Fluid Collection and Analysis

Crevicular fluid samples from the mesiobuccal sites of the 4 first molar teeth will be collected using paper strips (Periopaper) furnished by the manufacturers of the Periotron 8000, the instrument used for measurement of crevicular fluid volume. The sample strip will be placed in a prelabeled vial, snap frozen in liquid nitrogen, and stored at -80°C. Levels of IL-1β in GCF will be determined by ELISA.

#### 8.2.2.3 Plasma Levels of BLXA4-ME and SPM Profiling in Plasma and Serum

Blood will be collected at Baseline and on Day 28 to measure absorption of BLXA4-ME through the oral mucosa. Labeled plasma specimens will be sent to Avanti Polar Lipids for assay using a liquid chromatography-tandem mass spectrometry technique.

In addition, SPMs (lipoxins and resolvins) will be profiled in serum and plasma samples. Labeled plasma and serum specimens will be sent to laboratory of Dr. Charles Serhan at Brigham and Women's Hospital, Boston for lipidomics assay using liquid chromatography-tandem mass spectrometry technique.

### *8.2.3 Specimen Preparation, Handling, and Storage*

Specimen preparation for the various assays is described in the Manual of Procedures. Assays on plaque and GCF samples will be performed at the Forsyth Institute laboratories; these samples are stored at -80°C until time of assay. Blood and urine for safety labs will be sent to a commercial clinical laboratory on the day the samples are collected. Frozen plasma specimens for BLXA4 measurement will be sent to the laboratory at Avanti Polar Lipids. Additional frozen plasma and serum specimens for SPM profiling will be sent to Dr. Serhan's laboratory at Brigham and Women's Hospital, Boston.

## 9 ASSESSMENT OF SAFETY

### 9.1 Specification of Safety Parameters

Parameters used to assess safety will include AEs spontaneously reported by the subject or observed by the investigator, solicited AEs captured by review of the memory aid while interviewing the subject, clinical laboratory values (reviewed by the on-site medical monitor), oral cavity examination, vital signs, and increases in pathogenic bacterial flora identified in plaque samples. All AEs will be coded by system organ class and preferred term according to the terminology of the Medical Dictionary for Regulatory Activities.

Monitoring of AEs will be conducted throughout the study. New AEs, including SAEs, will be captured through the final study visit. In addition, all AEs will be captured at the final telephone follow-up conducted 2 months after the last use of study product. Adverse events will be followed in accordance with Good Clinical Practice. Serious adverse events will be immediately reported to the IND sponsor, to NIDCR, and to the IRB within 24 hours of becoming aware of the event, and will be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es). Unanticipated problems (UPs) will also be captured and reported to the IRB and FDA as required by regulation.

### *9.1.1 Adverse Events*

International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study

personnel during study visits and interviews of a study subject presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs should be captured. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, DO, physician assistant, nurse practitioner, DDS or DMD, dental hygienist), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or stabilization.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

### *9.1.2 Solicited Adverse Events from the Memory Aid*

When the subject returns for follow-up visits, he or she will be instructed to bring the memory aid. Study staff will review the data recorded on the memory aid. The information obtained from the interview will be recorded for the study.

### *9.1.3 Serious Adverse Events*

An SAE is an AE that meets 1 or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All SAEs will be:

- Reviewed and evaluated by a study clinician;
- Recorded for the study;
- Followed through resolution by a study clinician.

### *9.1.4 Unanticipated Problems*

The Office for Human Research Protections (OHRP) considers UPs involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Because the occurrence of an incident, experience, or outcome that meets the above 3 criteria will generally require changes in the research to protect the safety or welfare of subjects or others, any such occurrence will be promptly reported to the IRB, the medical monitor, and the Data and Safety Monitoring Board (DSMB) Chair.

Unanticipated problems will be captured and reported throughout the study.

## 9.2 Time Period and Frequency for Event Assessment and Follow-Up

Adverse events will be followed until resolved or considered stable. Phone calls or extra visits may be performed to follow up the AE. The subject will be questioned on the AE and whether it continues or is resolved at the time of the study visit, and whether the subject received any treatment specifically for the AE. The AE data will be updated and a follow-up report will be submitted to the IND Sponsor, NIDCR, and the IRB.

All unresolved AEs will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the

subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the IND sponsor, NIDCR, and the IRB of any death or AE occurring at any time after a subject has discontinued or terminated study participation, if the death or AE may reasonably be related to this study.

## 9.3 Characteristics of an Adverse Event

All AEs must be graded for relationship to study product, expectedness, and severity.

### *9.3.1 Relationship to Study Intervention*

The clinician's assessment of an AE's relationship to test article is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the following terms:

- Related (possible, probable, definite) events meet 1 or more of the following criteria:
    - The event is known to occur with the study intervention.
    - There is a temporal relationship between the intervention and event onset.
    - The event abates when the intervention is discontinued.
    - The event reappears upon a rechallenge with the intervention.
- Not related (unlikely, not related) events meet 1 of the following criteria:
    - There is no temporal relationship between the intervention and event onset.
    - An alternate etiology has been established.

### *9.3.2 Expectedness*

This trial involves the first use of the BLXA4-ME oral rinse in humans; therefore, no human data exist to classify expectedness of events. In a preclinical 28-day oral irritancy study in rats, transient erythema and edema of the oral mucosa were noted in animals treated with either BLXA4-ME or a saline solution used as a control. For this study, the IND sponsor, the study investigator, and the NIDCR medical monitor will be responsible for assessing AEs that may be related to product use. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described in the protocol or the Investigator's Brochure or discussed with the subject during the informed consent process.

### *9.3.3 Severity of Event*

Severity of AEs will be graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting activities of daily living
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

## 9.4 Reporting Procedures

### *9.4.1 Unanticipated Problem Reporting to IRB and NIDCR*

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of a UP report form. The OHRP recommends that investigators include the following information when reporting an AE, or any other incident, experience, or outcome as a UP to the IRB:

- Appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- A detailed description of the AE, incident, experience, or outcome;
- An explanation of the basis for determining that the AE, incident, experience, or outcome represents a UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

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

- Unanticipated problems that are SAEs will be reported to the IRB within 24 hours and to NIDCR within 1 week of the investigator becoming aware of the event.

- Any other UP will be reported to the IRB and to NIDCR within 2 weeks of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within 1 month of the IRB's receipt of the report of the problem from the investigator.

All UPs will be reported to NIDCR's centralized reporting system via Rho Product Safety:

- Product Safety Fax Line (US): 1-888-746-3293
- Product Safety Fax Line (International): 919-287-3998
- Product Safety Email: rho_productsafety@rhoworld.com

General questions about SAE reporting can be directed to the Rho Product Safety Help Line (available 8:00 AM – 5:00 PM Eastern Time):

- US: 1-888-746-7231
- International: 919-595-6486

### *9.4.2 Serious Adverse Event Reporting to NIDCR*

Any AE meeting the specified SAE criteria will be submitted on an SAE form to NIDCR's centralized safety system via Rho Product Safety. This report may be sent by fax or email. Once submitted, Rho Product Safety will send a confirmation email to the investigator within 1 business day. The investigator should contact Rho Product Safety if this confirmation is not received. This process applies to both initial and follow-up SAE reports.

SAE reporting contact information:

- Product Safety Fax Line (US): 1-888-746-3293
- Product Safety Fax Line (International): 919-287-3998
- Product Safety Email: rho_productsafety@rhoworld.com

General questions about SAE reporting can be directed to the Rho Product Safety Help Line (available 8:00 AM – 5:00 PM Eastern Time):

- US: 1-888-746-7231
- International: 919-595-6486

The study clinician will complete an SAE form and submit via fax or email within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE form and submitted to Product Safety within 24 hours of site awareness.
- Serious adverse events other than death and immediately life-threatening events, regardless of relationship, will be reported by fax within 72 hours of site awareness.

All SAEs will be followed until resolution or stabilization.

### *9.4.3 Reporting of SAEs and AEs to FDA*

Following notification from the investigator, the IND sponsor will report events that are both serious and unexpected and that are related to study product to the FDA within the required timelines as specified in 21 CFR Part 312.32: fatal and life-threatening events within 7 calendar days (by phone or fax) and all other SAEs in writing within 15 calendar days. All serious events designated as “not related” to study product will be reported to the FDA at least annually in a summary format.

### *9.4.4 Reporting of Pregnancy*

Subjects who are pregnant, nursing, or not using an acceptable birth control method at the time of the screening will not be enrolled in the study. A urine pregnancy test will be performed for women of childbearing potential at the Screening and Baseline Visits to rule out pregnancy. The results must be available and negative before study drug dispensation. The investigator will ensure that nonpregnant subjects are advised to avoid pregnancy and nursing during their participation in the research.

Subjects will be advised to notify the principal investigator immediately should they become pregnant. If a woman becomes pregnant or suspects she is pregnant while participating in this study, she must inform her treating dental provider immediately and permanently discontinue study drug. A subject who becomes pregnant during study participation will be discontinued from study participation because of her additional health concerns during pregnancy and because of the need to avoid unnecessary risk to the fetus. If a female subject reports a pregnancy or a male subject reports a partner’s pregnancy, the investigator will report the pregnancy condition to the IND sponsor, safety monitoring team, and the IRB as soon as he/she becomes aware of the event. The investigator will attempt to obtain and report information about the pregnancy and the birth outcome.

### 9.5 Rules for Study Suspension or Discontinuation

The NIDCR medical monitor will review all SAEs and UPs in an expedited fashion. In the event of unacceptable toxicity, the trial will be stopped prematurely to prevent harm to subjects. In addition, any injuries, SAEs, or other unexpected AEs involving risk to subjects or others, and occurring at a frequency above that considered acceptable by the investigators, IND sponsor, NIDCR, or IRB, may result in a temporary or permanent halt to the study. The study may be suspended for a safety review and consideration of study discontinuation if:

- One or more subjects experiences a clinically significant event or laboratory abnormality determined to be Grade 3 or higher and related (possibly, probably, definitely) to use of the investigational product;
- Two of more subjects experience a similar event determined to be Grade 2 or higher and related (possibly, probably, definitely) to use of the investigational product;
- One or more subjects experiences a Grade 2 or higher event that does not resolve within 14 days.

The NIDCR medical monitor or DSMB may request to be unblinded to the entire study when evaluating whether to suspend or discontinue the study. In the event of study unblinding, a non-study statistician will be assigned as the “unblinded statistician” to assist in unblinding and any safety review.

Any proposed changes in the consent form or research procedures resulting from any safety findings are to be submitted by the principal investigator to the IRB for approval.

## 10 STUDY OVERSIGHT

In addition to the principal investigator’s responsibility for oversight, study oversight will be under the direction of an independent DSMB constituted by NIDCR and composed of members with expertise in safety, statistics of early safety studies, and gingival and periodontal disease. The DSMB will operate under the rules of a charter approved at the organizational meeting of the DSMB. Most data elements needed for the DSMB reviews will be collected in the clinical database, and the DSMB will meet at least annually. The DSMB will assess safety and preliminary efficacy data for the study. The DSMB will be convened if a safety signal emerges prior to the completion of study enrollment. The DSMB will provide recommendations to the NIDCR.

# 11 CLINICAL SITE MONITORING

Site monitoring is conducted to ensure that the human subjects protections, study procedures, laboratory procedures, study product administration, and data collection processes are of high quality and meet ICH E6 and regulatory guidelines. The NIDCR is funding the study, and NIDCR or its designee will be responsible for conducting the on-site monitoring visits. A study initiation visit will be performed by NIDCR prior to subject enrollment. The purpose of this visit is to ensure site staff and facilities are prepared to conduct the study as described in this protocol. Interim site monitoring visits will be performed as necessary to evaluate the conduct of the study, quality and integrity of data collection and regulatory documentation, and compliance with Good Clinical Practice. Scheduling of interim site monitoring visits will be dependent upon subject enrollment. Following each site visit, the clinical site will be provided with documentation of actions for follow-up or resolution. A separate monitoring plan document will be developed to further describe the methods for monitoring, the frequency of monitoring, and the level of detail for monitoring.

# 12 STATISTICAL CONSIDERATIONS

## 12.1 Study Hypotheses

No formal statistical hypothesis testing will be performed in the primary safety analysis. The safety data will be summarized as described in Section 12.5.1.

The efficacy hypothesis to be tested is that the efficacy outcomes differ among the study arms at the later post-baseline study visits.

## 12.2 Analysis Populations

### *12.2.1 Safety Population*

The Safety Population will consist of all subjects who are randomized into a study arm and complete their Baseline Visit.

### *12.2.2 Efficacy Population*

The Efficacy Population will consist of all subjects of the Safety Population who have a baseline and at least 1 post-baseline assessment of 1 or more of the secondary efficacy outcome measures.

## 12.3 Sample Size Considerations

Sample size determination is based on the power to detect AEs (the primary safety objective) with additional consideration given to detecting post-baseline study arm differences with respect to the MGI (a secondary efficacy outcome).

The probability of observing a specific AE for the study's sample size can be estimated. This probability depends on the sample size within a treatment group and on the true underlying probability of an individual experiencing the specific AE in that treatment group. Table 12-1 summarizes the probabilities of observing an AE given a sample size and true underlying event rate. For example, if the true probability of a subject experiencing a specific AE is 5%, the probability of observing 1 or more of that specific AE within a treatment group of 40 subjects is 87.1%.

The study's test oral rinse, placebo oral rinse, and no-rinse control arms are assigned treatment group sample sizes of 40, 40, and 20 subjects, respectively. For the test oral rinse and the placebo oral rinse arms, a sample size of 40 subjects per treatment group should have probabilities of 80.5%, 87.1%, 91.6%, and 96.4% of observing an AE having underlying incidence probabilities of 4%, 5%, 6%, and 8%, respectively. The no-rinse control arm is considered to be of lesser importance and, thus, to conserve the overall sample size, is assigned a sample size of 20 subjects, which should have probabilities of 55.8%, 64.2%, 71.0%, and 81.1% of observing an AE having underlying incidence probabilities of 4%, 5%, 6%, and 8%, respectively. Thus, the above sample sizes of 40, 40, and 20 subjects should give adequate power (> 80%) to detect AEs that occur at true underlying frequencies of 4% or greater in the test and placebo oral rinse study arms, and adequate power (> 80%) to detect AEs that occur at true underlying frequencies of 8% or greater in the no-rinse control arm.

**Table 12-1 Probability of Observing One or More Adverse Events within a Study Arm**

| "True" Underlying Probability of an Individual Experiencing an Adverse Event | Probability of Observing One or More Adverse Events within a Study Arm | |
|---|---|---|
| | No-Rinse Control Study Arm (n = 20) | Test and Placebo Oral Rinse Study Arms (n = 40) |
| 0.01% | 0.20% | 0.40% |
| 0.10% | 2.0% | 3.9% |
| 0.25% | 4.9% | 9.5% |
| 0.50% | 9.5% | 18.2% |
| 1.00% | 18.2% | 33.1% |
| 2.00% | 33.2% | 55.4% |
| 3.00% | 45.6% | 70.4% |
| 4.00% | 55.8% | 80.5% |
| 5.00% | 64.2% | 87.1% |
| 6.00% | 71.0% | 91.6% |
| 8.00% | 81.1% | 96.4% |
| 10.0% | 87.8% | 98.5% |

Sample size (n) refers to the final sample size per treatment group of enrolled subjects who complete the study.

Power estimates for the secondary efficacy endpoint (MGI) are found in Table 12-2. In this power analysis, MGI refers to the MGI scores averaged over all tooth sites within each subject and visit. The power estimates in the table are based upon 50,000 simulations of mixed models analyses. The mixed models simulations include the MGI as the dependent variable, and fixed effects for treatment group (test oral rinse, placebo oral rinse, and no-rinse control), visit (Baseline, 14 Days, and 28 Days), and their treatment group-by-visit interaction. The model includes random effects for subject using a compound symmetry variance structure (i.e., exchangeable covariance). These power analyses assume an overall within-study group standard deviation of 0.3 MGI units at each visit and a difference in the mean change from baseline MGI at 14 or 28 days between the test oral rinse and the placebo rinse or between the test oral rinse and the no-rinse control of 0.2 MGI units. Correlation of MGI within subject among visits

was assumed to be 0.3. The treatment group sample sizes of 40, 40, and 20 subjects for the test oral rinse, placebo oral rinse, and no-rinse control arms, respectively, are estimated to have powers of 71% and 54% to detect significant differences of 0.2 in the mean MGI scores between the test oral rinse and placebo oral rinse study arms, and between the test oral rinse and the no-rinse control study arms, respectively. Even though these sample sizes would be considered under-powered for testing efficacy in a Phase 3 clinical trial, for this Phase 1/2 protocol, with efficacy as a secondary objective, these study sample sizes can provide evidence (significant or not) of efficacy and provide efficacy estimates with their associated standard deviations needed for planning future Phase 2 or Phase 3 trials.

It is estimated that 50, 50, and 25 subjects will need to be enrolled to have final sample sizes of 40, 40, and 20 subjects for the test oral rinse, placebo oral rinse, and no-rinse control study arms, respectively, assuming a 20% subject attrition rate before completing the study.

**Table 12-2 Mixed Models Power Analysis for MGI (Secondary Efficacy Outcome) Showing the Power for Several Sample Size Scenarios**

| Enrolled and Eligible Subjects | | | Subjects after 20% Drop-out | | | Power (%) | |
|---|---|---|---|---|---|---|---|
| Test (n) | Placebo (n) | Control (n) | Test (n) | Placebo (n) | Control (n) | Trt – Placebo[a] | Trt – Control[b] |
| 50 | 50 | 25 | 40 | 40 | 20 | 70.9 | 53.8 |

Control = no-rinse control study arm; MGI = modified gingival index; placebo = placebo oral rinse study arm; test = test oral rinse study arm; trt = treatment (placebo or test oral rinse) study arm.

a Trt-Placebo: Power (probability) for finding a statistically significant difference (alpha = 0.05) in the change from baseline MGI at 14 or 28 days between the test oral rinse and the placebo oral rinse study arms.

b Trt-Control: Power (probability) for finding a statistically significant difference (alpha = 0.05) in the change from baseline MGI at 14 or 28 days between the test oral rinse and the no-rinse control study arms.

## 12.4 Planned Interim Analyses

No interim analysis of efficacy endpoints is planned. Safety monitoring will occur throughout the study period.

## 12.5 Final Analysis Plan

### *12.5.1 Primary Safety Analysis*

Safety data will be summarized for all subjects in the Safety Population. Adverse event and SAE reporting requirements are found in Section 9.

Safety for study treatment groups will be evaluated by the incidence, severity, and type of AEs, and by changes from baseline in subjects' oral examination findings, vital signs, and clinical laboratory test results. Concomitant medications taken during the trial will be summarized and listed.

The number and percentage of subjects who experience an AE, SAE, treatment-related AE, treatment-related SAE, and AE leading to discontinuation from the study will be summarized. All AEs will be coded by system organ class and preferred term according to the terminology of the Medical Dictionary for Regulatory Activities, and severity will be graded according to the National Cancer Institute's CTCAE, Version 4.0.

All AEs will be summarized by system organ class and preferred term and by maximum CTCAE severity grade. Summaries will also be presented by relationship to study treatment. For AE summaries, emphasis will be placed on the summary of treatment-emergent AEs, defined as new AEs reported after initiation of treatment or pre-existing conditions that worsen during the treatment period.

Subjects who withdraw from study treatment because of an AE will be listed.

The incidence of development or progression of periodontal disease at Day 28 will be reported for each study arm as part of the primary safety analysis. Development of disease or progression of existing periodontitis is defined as an increase from baseline of 2 mm or more in either the PD or CAL measures.

### *12.5.2 Secondary Efficacy Analysis*

The study is not fully powered to find statistical differences among the study arms with respect to the secondary efficacy outcomes. As such, the main objectives of the secondary efficacy analyses are to: 1) obtain some evidence of efficacy (either a statistically significant effect or a nonsignificant trend); 2) obtain estimates of the size and variance of possible treatment effects that can then be used to plan later Phase 3 clinical trials; and 3) explore which of several outcomes may best be used to measure efficacy. As secondary analyses, no corrections will be made for multiple comparisons.

The secondary efficacy measures of PI, MGI, IL-1β, and percentage of tooth sites with BOP will be analyzed using mixed models with a random effect for subject, and fixed

effects for baseline value, visit, study arm, and a study arm-by-visit interaction. Contrast statements within the mixed models analyses will compare study arms with respect to the efficacy measures. These statistical models allow comparisons to be made between study arms, between visits, and between treatment groups at each visit. These mixed models analyses assume the efficacy outcomes are approximately normal in distribution. If residual analysis shows the normality assumption to be unreasonable for any efficacy outcomes, then generalized linear mixed models with the appropriate links and distributional assumptions will be used to analyze these efficacy outcomes.

## 13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The investigative team at the Forsyth Institute will be responsible for completion of all source documents, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. The investigator will permit authorized representatives of NIDCR, FDA, and other regulatory authorities to inspect facilities and records relevant to this study if needed.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Data collected for this trial will include demographic information; vital signs; medical/dental history; oral examination results; medication history; records of collection for blood, urine, GCF, and plaque samples; measurements of MGI, PD, GM-CEJ, BOP, and PI; study medication administration and accountability records; clinical laboratory results; and AEs.

Source documents will include the original documents, data, and records (e.g., hospital records, clinical and office charts, safety laboratory test results and other laboratory test results, memoranda, subjects' diaries or evaluation checklists, subject files, and records kept at the Forsyth Institute and at the laboratories involved in the clinical trial). Some data will be recorded directly into the study data system. The data captured in the study data system must be consistent with source documents; any discrepancies will be explained.

## 14 QUALITY CONTROL AND QUALITY ASSURANCE

Quality management is the overall process of establishing and ensuring the quality of processes, data, and documentation associated with clinical research activities. It encompasses both quality control and quality assurance activities. All sites conducting research that are funded by NIDCR are required to have a plan in place for assuring the quality of the research being conducted.

This clinical trial will have a separate quality management plan to describe:

- How data will be evaluated for compliance with the protocol and for accuracy in relation to source documents;
- Which documents will be reviewed (e.g., electronic case report forms, product accountability records, specimen tracking logs), who is responsible for reviewing, and the frequency for reviews;
- Who will be responsible for addressing quality assurance issues (correcting procedures that are not in compliance with protocol) and quality control issues (correcting errors in data entry);
- Staff training methods and how such training will be tracked;
- Calibration exercises conducted prior to and during the study to train examiners and maintain acceptable intra- and inter-examiner agreement.

# 15 ETHICS/PROTECTION OF HUMAN SUBJECTS

## 15.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the United States National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

## 15.2 Institutional Review Board

The protocol, consent forms, recruitment materials, and all subject materials will be submitted to the Forsyth Institute IRB for review and approval. Approval of both the protocol and the consent forms must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

## 15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects. A consent form describing in detail the study interventions/products, procedures, and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject

and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

### 15.4 Exclusion of Women, Minorities, and Children (Special Populations)

Adults of any gender or racial/ethnic group may participate.

### 15.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the investigators, study staff, and the IND sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the IND sponsor.

Information from this study and from a subject's medical record may be reviewed and photocopied by the FDA and/or state and federal regulatory agencies such as the OHRP as applicable, and the IRB of the Forsyth Institute. Information from this study and from a subject's medical record may be used for research purposes and may be published; however, a subject's name will not be used in any publications. A subject's data and samples will be protected by using coded designations only. The list that matches a subject's name to the code number will be kept in a password-protected computer file and destroyed permanently after study completion. A subject will not be personally identified by any of the research materials in this study.

The study monitor or other authorized representatives of NIDCR may inspect all study documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

### 15.6 Future Use of Stored Specimens and Other Identifiable Data

No specimens will be retained for future use.

# 16 DATA HANDLING AND RECORD KEEPING

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study subjects, including accurate case report forms and source documentation.

Details of the study data system will be described in a separate Data Management Plan.

## 16.1 Data Management Responsibilities

The investigative team at the Forsyth Institute will be responsible for data management including data entry, data security, and specimen tracking. Unanticipated problems and AEs must be reviewed by the investigator or designee.

## 16.2 Data Capture Methods

Data will be captured and stored electronically in a study-specific, password-protected database, with data quality checks built into the system.

Data including medical and dental history, demographics, AEs, concomitant medications, oral examination results, and vital signs will be entered into a data system designed for this study. Clinical measurements including MGI, PD, GM-CEJ, BOP, and PI will be entered chairside to an electronic data form, using special software installed on a study-designated laptop computer. For chairside data collection, a data recorder will work with the study examiner to enter data during the oral examination. Data from laboratory test results and special assays will be recorded by the laboratory and entered into the data system by study staff.

## 16.3 Types of Data

Data for this study will include demographic and medical/dental history data, vital signs, information on concomitant medications, safety observations (records of AEs), oral examination findings, clinical outcome measures (i.e., PD, CAL, PI, MGI, BOP, plaque and gingivitis severity scores), safety laboratory and other laboratory values (i.e., IL-1β in GCF, BLXA4-ME levels in plasma, SPM profiles in plasma and serum), and microbial identification data.

## 16.4 Schedule and Content of Reports

The primary outcome measures are the safety parameters, which will be assessed by recording all AEs and SAEs, as reported by subjects or observed by oral examination,

measurement of vital signs, and review of safety laboratory results. The clinical investigator and the IND sponsor will review these data and work with the data coordinating center to provide regular reports to NIDCR (the study funder), the DSMB, and the IRB. The NIDCR medical monitor will track safety data in real time. If the trial is terminated prematurely for any reason, an abbreviated report will be prepared. To ensure confidentiality, coding will be used to identify subjects.

## 16.5 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the IND sponsor. It is the responsibility of the IND sponsor to inform the investigator when these documents no longer need to be retained.

Study records will be maintained for at least 3 years from the date that the grant final financial report is submitted to the National Institutes of Health (NIH).

## 16.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2

All deviations from the protocol must be addressed in study subject source documents and promptly reported to NIDCR and the local IRB, according to their requirements. A completed copy of the Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject's source document file. The principal investigator and study staff are responsible for knowing and adhering to the IRB requirements.

## 17 PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. Any publications based on the results of the trial will conform to the NIH Grants Policy and the publication policy of the Forsyth Institute. This study will comply with the *NIH Public Access Policy*, which ensures that the public has access to the published results of NIH-funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and AEs. The ICMJE policy requires that all clinical trials be registered in a public trials registry such as *ClinicalTrials.gov*, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIDCR grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

*United States Public Law 110-85* (Food and Drug Administration Amendments Act of 2007), Title VIII, Section 801 mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials," described as:

- Trials of drugs and biologics: controlled clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;
- Trials of devices: controlled trials with health outcomes of devices subject to FDA regulation, other than small feasibility studies, and pediatric postmarket surveillance required by FDA.

National Institutes of Health grantees must take specific steps to ensure compliance with NIH implementation of the Food and Drug Administration Amendments Act of 2007.

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## APPENDICES

Appendix A: Schedule of Events

**APPENDIX A:**

## SCHEDULE OF EVENTS

| Procedures | Screen<br>Day<br>-10 to -1 | Enrollment/<br>Baseline<br>Visit 1<br>Day 0 | Visit 2<br>Day 3<br>± 1 day | Visit 3<br>Day 7<br>± 1 day | Visit 4<br>Day 14<br>± 1 day | Visit 5<br>Day 21<br>± 1 day | Visit 6<br>Day 28<br>± 1 day | F/U<br>Day 90<br>± 14 days |
|---|---|---|---|---|---|---|---|---|
| Obtain informed consent | X | X | | | | | | |
| Medical/dental history | X[a,b] | X[a] | X | X | X | X | X | |
| Record concomitant medications and dental procedures | X | X | X | X | X | X | X | |
| Urine pregnancy test | X | X | | | | | | |
| Vital signs[c] | X | X | X | X | X | X | X | |
| Intraoral and extraoral examinations | X | X | X | X | X | X | X | |
| Assessment of plaque and calculus | X | | | | | | | |
| Solicit adverse events | | | X | X | X | X | X | X |
| Modified gingival index | X | X | | | X | | X | |
| Gingival crevicular fluid sample for IL-1β | | X | | | X | | X | |
| Probing depth; measure GM-CEJ to determine clinical attachment level | | X | | | X | | X | |
| Bleeding on probing | | X | | | X | | X | |
| Plaque index | | X | | | X | | X | |
| Plaque samples to analyze oral flora | | X | | | X | | X | |
| Blood for plasma BLXA4-ME level and SPM profiling in serum and plasma | | X | | | | | X | |
| Blood for eligibility or safety laboratory tests[d] | X | | | | X | | X | |
| Urine for eligibility or safety laboratory tests[d] | X | | | | X | | X | |

| Procedures | Screen<br>Day<br>-10 to -1 | Enrollment/<br>Baseline<br>Visit 1<br>Day 0 | Visit 2<br>Day 3<br>± 1 day | Visit 3<br>Day 7<br>± 1 day | Visit 4<br>Day 14<br>± 1 day | Visit 5<br>Day 21<br>± 1 day | Visit 6<br>Day 28<br>± 1 day | F/U<br>Day 90<br>± 14 days |
|---|---|---|---|---|---|---|---|---|
| Assess compliance | | | X | X | X | X | X | |
| Provide oral rinse | | X | X | X | X | X | | |
| Hygiene instructions or referral for periodontal treatment | | | | | | | | X |

F/U = safety follow-up telephone call; GM-CEJ = distance from free gingival margin to cementoenamel junction; IL-1β = interleukin-1β; SPM = specialized pro-resolution mediator

a Includes history of alcohol and tobacco use.

b Includes demographic information.

c Pulse rate, respiratory rate, blood pressure, and oral body temperature are collected at all visits marked with "X". Weight is collected at the Screening Visit and Visit 6 (Day 28).

d The on-site medical monitor will review the results of safety laboratory tests to determine whether a laboratory test should be redrawn.