A THREE-MONTH CLINICAL STUDY TO ASSESS THE GINGIVITIS EFFECT OF VARIOUS DENTIFRICES

2024026

20 February 2025

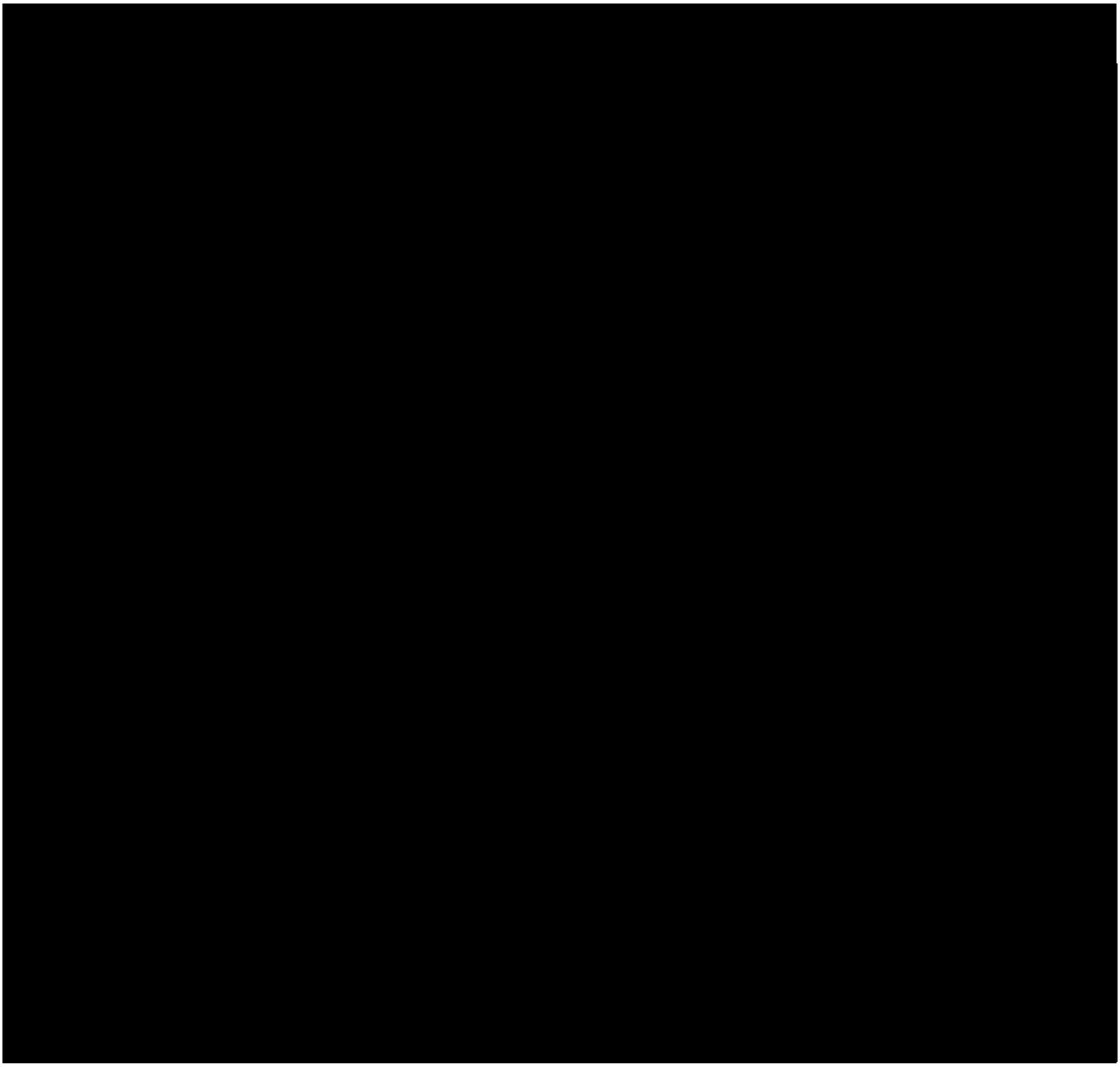
NCT06892834

Investigator's Agreement Statement and Signature

I have read, I understand, and I will conduct the study according to Good Clinical Practices.

[redacted]

Sponsor Signatures



Compliance Statement

This trial will be conducted in compliance with the protocol and in accordance with the ethical principles of Good Clinical Practice (GCP), according to the International Council for Harmonization (ICH) Harmonized Tripartite Guideline and all applicable regulatory requirements.

Table of Contents for the Clinical Study Protocol

List of Abbreviations

AE(s)	Adverse Event(s)
AR	Adverse Reaction
B&A	Balance and Assignment
(e)CRF(s)	(Electronic) Case Report Form(s)
GCP	Good Clinical Practice
ICH	International Council for Harmonization
IRB/IEC	Institutional Review Board/Independent Ethics Committee
ITT	Intent-to-treat
LSGI	Löe-Silness Gingivitis Index
MedDRA	Medical Dictionary for Regulatory Activities
NHPR	Natural Health Products Regulations
NNHPD	Natural and Non-prescription Health Product Directorate
PP	Per Protocol
QC	Quality Control
SAE(s)	Serious Adverse Event(s)
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Standard Deviation
SOP(s)	Standard Operating Procedure(s)
SUAR	Serious Unexpected Adverse Reaction

Background Information

Investigational Drug Product Name

Experimental Toothpaste #1
Experimental Toothpaste #2

Description of Drug Product

Experimental Toothpaste #1 0.243% NaF with 0.1% Hops, *Humulus lupulus*, extract
Experimental Toothpaste #2 0.243% NaF with 0.5% Hops, *Humulus lupulus*, extract

Summary of Findings

Periodontal disease initially presents as gingivitis, a plaque-induced inflammation of the marginal and attached gingiva[1]. If left untreated, gingivitis will progress into periodontitis with periodontal tissue breakdown which ultimately can lead to tooth loss. Gingivitis is highly prevalent globally[2]. The treatment and prevention of gingivitis is directed towards reducing the virulence burden of microbial dental plaque to the gingival tissues[3]. Stannous fluoride (SnF_2) dentifrices have been shown to be effective in reducing the bacteria virulence in the dental biofilm and in controlling gingivitis in clinical studies[4]. Recent studies have shown hop components and hops extracts as potent antimicrobial agents[5].

Risks and Benefits

This study is for research purposes only. There may be no direct benefit to the subjects from participation in the study. Others may benefit from the knowledge gained. There may be other risks of study participation that are unknown. There is no guarantee that the subjects will receive any medical or dental benefits from participating in this research study.

Description of and Justification for the Route of Administration, Dosage, Dosage Regimen and Treatment Period(s)

Subjects will be instructed to brush their teeth thoroughly for 1 minute, twice daily. Subjects should apply enough toothpaste to cover the whole length of the brush head onto their toothbrush, approximately 1.25g. Subjects will use their assigned toothpaste for 12 weeks.

Population Studied

The target population of the study is generally healthy male and female subjects over the age of 18 years with mild-to-moderate gingivitis.

Literature References

1. Abiko Y, Paudel D, Uehara O (2022). Hops components and oral health. J Funct Foods 92:105035 [7pp]. DOI:10.1016/j.jff.2022.105035.
2. Albandar JM, Rams TE (2002). Global epidemiology of periodontal diseases: an overview. Periodontol 2000 29:7-10. DOI:10.1034/j.1600-0757.2002.290101.x.
3. Biesbrock A, He T, DiGennaro J, Zou Y, Ramsey D, Garcia-Godoy F (2019). The effects of bioavailable gluconate chelated stannous fluoride dentifrice on gingival bleeding: Meta-analysis of eighteen randomized controlled trials. J Clin Periodontol 46(12):1205-1216. DOI:10.1111/jcpe.13203.

4. Page RC (1986). Gingivitis. J Clin Periodontol 13(5):345-359. DOI:10.1111/j.1600-051x.1986.tb01471.x.
5. Sanz M, Serrano J, Iniesta M, Santa Cruz I, Herrera D (2013). Antiplaque and antigingivitis toothpastes. Monogr Oral Sci 23:27-44. DOI:10.1159/000350465.

Objective and Purpose

The primary objective of this study is to evaluate the effect of two experimental hops extract-containing dentifrices on gingivitis relative to a positive control 0.454% SnF2 dentifrice and a negative control dentifrice after 12 weeks of product use.

The secondary objective is to assess the effect of two experimental hops extract -containing dentifrices have on gingivitis relative to a positive control 0.454% SnF2 dentifrice and a negative control dentifrice after 4 weeks of product use.

Trial Design

Primary Endpoint

The primary endpoint will be the measurement of the total number of bleeding sites.

Secondary Endpoints

The secondary endpoints will be:

- Percent number of bleeding sites after 4 weeks
- Löe-Silness Gingivitis Index (LSGI) score at 4 and 12 weeks

Trial Design/Type

This is a randomized, four-treatment, parallel-group, double-blind clinical trial. The target population is approximately 120 adult volunteers with mild to moderate gingivitis. At the baseline visit, subjects will undergo a gingivitis assessment using the Löe-Silness Gingivitis Index by a licensed dentist. One hundred twenty (approximately 30 per group) will be randomly assigned to 1 of 4 treatments at baseline. Oral safety and gingival health evaluations will be repeated at 4 weeks and 12 weeks.

Table 1. Study Schedule by Procedure Type and Visit

PROCEDURE	VISIT 1 BASELINE	VISIT 2 WEEK 4	VISIT 3 WEEK 12
Informed Consent	X		
Medical History Review	X	X	X
Inclusion/Exclusion Criteria	X		
Demographics	X		
Concomitant Medications	X	X	X
Urine Pregnancy Test for Females of Child-bearing Potential	X		

Continuance Criteria		X	X
Oral Examination	X	X	X
Löe-Silness Gingivitis Index Evaluation	X	X	X
Adverse Events (AEs)		X	X
Take-home Kit Distribution	X	X	
Take-home Kit Return		X	X
General Comments	X	X	X
Subject Accountability			X

Randomization

Subjects will be stratified based on the baseline number of bleeding sites, age, and gender (bleeding and age cut-offs based on data from 2020054 study). Subjects will be randomly assigned in approximately equal numbers to 1 of the 4 treatment groups using an encoded program supplied by the Sponsor. Subjects residing in the same household will be assigned to the same treatment group. The examiner will be in an area separate from randomization and product distribution.

Table 2. Basic Study Design Information

STUDY DESIGN	N	N/GROUP	B&A PROGRAM	BLOCK SIZE	STRATA	CUT-OFFS	SAME HOUSEHOLD
Parallel	120	30	Yes	4	Bleeding Sites Age Gender	≤30%, >30% and ≤58%, or >58% ≤ 50 or >50 Male or Female	Yes

B&A = Balance and Assignment.

Balance and Assignment (B&A) Program

At the baseline visit, subjects will be randomly assigned to 1 of the 4 treatment groups (up to 30 per group) using an encoded B&A program supplied by the Sponsor. The subjects will be stratified based on total number of bleeding sites, gender, and age. Subjects residing in the same household will be assigned to the same treatment group.

Blinding

Identities of the study investigational products will be disguised with a permanent study label. Products that are purchased from the marketplace (e.g., negative and positive controls) will be over-labeled with a permanent generic white label that is similar to the experimental toothpastes to maintain the study blind. Additionally, toothpastes will be over-labeled by personnel not associated with the study assigned and will be distributed to participants in a concealed to ensure study blinding. When possible, similar sized kit boxes/tubes/bottles will be used to ensure product blinding. All treatment products will be dispensed in a secluded area, away from the study examiner(s), to maintain the study blind.
Subject treatment(s) will only be distributed to select site personnel responsible for usage instruction and supervised use.

White opaque cardboard take-home kit boxes will contain 3 tubes of toothpaste in plain white tubes, a manual toothbrush, timer, and instruction sheet. All treatment products will be used at home twice daily as instructed on the instruction sheet for 12 weeks.

The site will be provided with a code breaker report in a sealed envelope. The sealed code breaker report contains documents that list the kit box number or treatment code, while the identity of the treatment products is hidden by an opaque scratch-off seal. If the study blind needs to be broken, an individual subject's investigational product may be ascertained by opening the sealed code breaker report, locating the subject's kit box number or treatment code and scratching off the opaque seal to reveal the treatment identity. The sealed code breaker report will be opened if a clinically Serious Adverse Event (SAE) occurs or management of the subject requires knowledge of the identity of the investigational product. The Investigator should immediately inform the Sponsor that the code will be broken and record the date, time, and reason for breaking the code in writing. After the study is complete and the study database has been finalized and locked, the site staff will return the code breaker report to the Sponsor using the self-addressed, stamped envelope provided by the Sponsor.

Trial Treatments

- Negative Control—Colgate Cavity Protection toothpaste (0.76% Sodium Monofluophosphate) and Oral-B® Indicator soft toothbrush

- Positive Control—Crest Pro-Health Clean Mint (0.454% stannous fluoride, 0.533% zinc citrate) and Oral-B® Indicator soft toothbrush

- Experimental Toothpaste #1—Procter & Gamble Experimental Toothpaste (0.243% NaF with 0.1% Hops, *Humulus lupulus* extract [0.045% hops β-acids]) and Oral-B® Indicator soft toothbrush

- Experimental Toothpaste #2—Procter & Gamble Experimental Toothpaste (0.243% NaF with 0.5% Hops, *Humulus lupulus* extract [0.225% hops β-acids]) and Oral-B® Indicator soft toothbrush

Dosage of Investigational Product

Subjects will apply enough toothpaste (approximately 1.25 grams) to cover the length of the toothbrush for all treatment groups.

Dosage Regimen of Investigational Product

Subjects will brush for 1 minute with study toothpaste twice daily in the morning and evening.

Dosage Form of Investigational Product

The subjects will self-dose their toothbrush. Subjects will have their first brushing supervised on-site and then continue to use their toothpaste at home for the continuation of the study.

Packaging of Investigational Product

The identity of the toothpaste will be disguised. Subjects will receive 1 kit box containing 3 tubes of toothpaste, a timer, a toothbrush and product usage instructions to be used throughout the duration of this study.

Labeling of Investigational Product

The kit boxes will be labeled with a unique kit number. Kit box labels will also contain the study number, emergency phone number, distributor name/address, appropriate caution statements, content statement**,** and other information as required by internal regulations and clinical Standard Operating Procedures **(**SOPs**)**. The shipping containers will be labeled with the clinical site address and a content statement listing study number and kit box numbers contained within. Supplemental products will be provided to site.

The investigational product will be labeled with the following information in both French and English:

- A statement indicating that the natural health product is an investigational natural health product to be used only by a qualified investigator;
- The expiry date of the natural health product;
- The recommended storage conditions for the natural health product, if any;
- The lot number of the natural health product;
- The name and address of the sponsor; and
- The protocol code or identification.

Because the clinical study uses commercialized toothpastes that are produced by two different manufacturers (i.e., Colgate-Palmolive and Procter & Gamble) and have different brand names, the following information will not be able to be included on the product labels to conserve blinding:

- The brand name or code of the natural health product;
- The name and address of the manufacturer;

However, the toothpaste tubes will be labeled with the study site's information, and be assigned a generic brand name (i.e., experimental toothpaste 1-4), in order to maintain blinding.

Duration of Subject Participation

Screening

Prior to the baseline exam, a sufficient number of subjects may be pre-screened outside of this protocol for mild -to -moderate gingivitis to determine their potential enrollment into this study. Subjects who will be invited for the baseline visit will be instructed to refrain from performing any oral hygiene procedures the morning prior to their scheduled visit.

Visit 1 (Baseline)

Prior to this visit, site staff will instruct subjects to refrain from brushing their teeth, before the morning visit.

Subjects will be asked to read and sign duplicate copies of the informed consent form which will be witnessed by site staff. Subjects will be given a signed copy of the informed consent form, and the other copy will be maintained as site source documentation. Medical history, demographic information, concomitant medications, and study entrance criteria will be obtained and documented on the appropriate Case Report Form (CRF). A urine pregnancy test (Clearblue) will be performed at baseline in females of child-bearing potential to confirm absence of pregnancy.

A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, will be performed, followed by a gingivitis evaluation.

Up to 120 qualified subjects with a range of 10 to 70% bleeding sites will be randomly assigned to a treatment group by a B&A program and will be given a study kit box containing their assigned test products. Product will be dispensed in a protected area that will ensure blinding of the examiner to the identity of the test products. Subjects will be provided with both verbal and written instructions on product usage and will perform their first treatment under the supervision of study personnel to ensure compliance (see Appendix A of Information and Consent Form). They will then take their products home and will be instructed to use them that evening and twice daily for the remainder of the study.

Subjects will be reminded to bring their treatment kit box to their next visit, to refrain from performing any oral hygiene procedures, including flossing if that is part of their regular oral hygiene routine, the morning prior to the next visit and will then be scheduled for their 4-week visit.

General Comments, if any, will be recorded on the appropriate CRF.

Visit 2 (Week 4)

Prior to this visit, site staff will instruct subjects to refrain from performing any oral hygiene before their visit.

Subjects will return to the site with the kit box provided at the baseline visit. The site will visually assess the product to look for product usage compliance. Continuance criteria will be assessed, and concomitant medications will be reviewed or updated in the study database. A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, will be performed, followed by a gingivitis evaluation.

Subjects will be reminded to bring their treatment kit box to their next visit, to refrain from performing any oral hygiene procedures, including flossing if that is part of their regular oral hygiene routine, the morning prior to the next visit and will then be scheduled for their 12-week visit.

General Comments and AEs, if any, will be recorded on the appropriate CRF.

Visit 3 (Week 12)

Prior to this visit, site staff will instruct subjects to refrain from performing any oral hygiene before their visit.

Subjects will return to the site with the kit box provided at the baseline visit. The site will visually assess the product to look for product usage compliance. Continuance criteria will be assessed, and concomitant medications will be reviewed or updated in the study database. A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, will be performed, followed by a gingivitis evaluation.

Any General Comments or AEs will be recorded on the appropriate CRFs. Subject Accountability will be completed, and subjects will be dismissed from the study.

Subject Accountability

A Subject Accountability form will be completed for each subject. If, for any reason, a subject does not complete the study, an explanation will be entered on the Subject Accountability CRF. All data gathered on the subject prior to discontinuation will be made available to the Sponsor.

General Comments and Adverse Event Recording

General Comments and AEs may be recorded at any time during the study. Subjects will be able to contact the study site and Investigator throughout the study to report general comments and AEs. Phone numbers for the Investigator and site will be listed on the informed consent form, the product labels, and kit boxes. All AEs will be recorded by site staff. Any recorded AE that remains unresolved by study completion should be followed up until resolution by the Investigator, and the resolution should be documented as site source documentation by the Investigator. If a subject is unreachable to determine whether the AE has been resolved, the attempts to contact the subject should be documented as site source documentation by the Investigator.

Sequence and Duration of Trial Periods

This is a 12-week product usage study.

Discontinuation Criteria

Discontinued subjects are those who do not complete final evaluations and/or procedures outlined in the protocol because of 1 of the following:

a. Any AE;

b. Significant protocol deviation that that are enumerated in the continuance criteria

or, that in the opinion of the Investigator, may compromise the study results;

c. Voluntary withdrawal; or

d. Withdrawal at the Investigator's discretion.

The Investigator/Sponsor may recommend dropping a subject from the study at any time. Recommendations to drop a subject may include but are not limited to: misuse of study product, not following study procedures, or a protocol violation. Any subject who is discontinued from the study that has a continuing AE will have follow-up communications about the AE until the AE is resolved to the satisfaction of the Investigator/Medical Monitor.

Accountability Procedures for the Investigational Product

Study products will be stored in a secure area, under environmental condition as required by label instructions or as described in this protocol and dispensed only under the authorization of the Investigator. The storage condition shall be properly documented. The study site staff will record and log both the receipt and dispensation of all test products (used and unused) by using forms provided by the Sponsor or suitable forms provided by the site. Study products will be returned to the Sponsor following the trial, or alternatively, they will be destroyed at the clinical site provided the site has an existing SOP for the destruction of clinical materials and prior written approval from the Sponsor.

Maintenance of Trial Treatment Randomization Codes and Procedures for Breaking Codes

All treatment products will be dispensed in a secluded area. The treatment code that each subject will receive will not be disclosed to the Investigator, examiners, study site personnel, or subjects. Only site personnel responsible for usage instruction and supervision will know the treatment codes. The treatment tubes will not be disguised from the study staff member that is dispensing the product.

If the study blind needs to be broken, an investigational product may be ascertained by locating the subject's treatment code and asking the site staff member assigned to product distribution to reveal the treatment identity. The code should only be broken if a clinically SAE occurs or management of the subject requires knowledge of the identity of the investigational product. The Investigator should immediately inform the Sponsor in writing that the code will be broken and record the date, time, and reason for breaking the code as source documentation. After the study is complete and the study database has been finalized and locked, treatment codes will be unblinded.

Identification of Any Data to be Recorded Directly on the Case Report Forms

Data to be recorded directly on the CRFs (*i.e.,* no prior written or electronic record of data) is considered to be source data. Data that will be recorded directly in electronic CRFs (eCRFs) include Medical History, Demographics, Concomitant Medications, Inclusion/Exclusion/Continuance Criteria, Oral Examinations, Gingivitis Examinations, General Comments, Adverse Events (AEs), Adverse Reaction, and Subject Accountability. Paper CRFs will be provided for backup purposes, per the Data Management contingency plan, in case of

system, connectivity, or power outages. Data would then be transcribed from the paper source documents to the eCRFs upon system renewal and source verified by the clinical site.

Selection and Withdrawal of Subjects

Subject Inclusion Criteria

In order to be included in the study, each subject must:

- Be at least 18 years of age;
- Provide written informed consent prior to participation and be given a signed copy of the informed consent form;
- Be in general good health as determined by the Investigator based on a review of the health history/update for participation in the trial;

If female of child-bearing potential, agree to use a medically approved method of birth control for the duration of the study. Acceptable methods of medically approved birth control include: at least 3 months of use of a hormonal birth control (including oral contraceptives, hormone birth control patch, vaginal contraceptive ring, injectable contraceptives, or hormone implant), double-barrier method, intrauterine devices, non-heterosexual lifestyle or agrees to use contraception if planning on changing to heterosexual partner(s), vasectomy of partner at least 6 months prior to screening. *Not applicable to females not of child-bearing potential (females who have undergone a sterilization procedure including hysterectomy, bilateral oophorectomy, bilateral tubal ligation, complete endometrial ablation) or have been post-menopausal for at least 1 year prior*;

- If female and of child-bearing potential, agree to immediately inform the study investigator if they become pregnant;
- Have at least 20 gradable teeth;
- Have mild -to -moderate gingivitis with a range of 10 to 70% bleeding sites;
- Agree to return for scheduled visits and follow the study procedures;
- Agree to refrain from use of any non-study oral hygiene products for the duration of the study; regular floss users can continue to floss in their customary manner.
- Agree to delay any elective dentistry, including dental prophylaxis, until the completion of the study; and
- Agree to refrain from any oral hygiene the morning of each visit.

Subject Exclusion Criteria

Subjects are excluded from study participation due to:

- Having taken antibiotic, anti-inflammatory, or anticoagulant medications within 4 weeks of the baseline visit;
- Having any oral conditions that could interfere with study compliance and/or examination procedures, such as widespread caries, soft or hard tissue tumor of the oral cavity, severe gingivitis, or advanced periodontal disease;
- Females of child-bearing potential who are not using a medically approved method of birth control (*i.e.,* hormonal contraceptives including oral contraceptives, hormone birth control patch, vaginal contraceptive ring, injectable contraceptives, or hormone implant, double-barrier method, intrauterine devices, non-heterosexual lifestyle or agrees to use contraception if planning on changing to heterosexual partner(s), vasectomy of partner at least 6 months prior to screening) or who have not been using hormonal birth control for a minimum of 3 months prior to study enrollment;
- Taking medication that alters gingival appearance or gingival bleeding (*e.g.,* calcium channel blockers, anticonvulsants, or immunosuppressants) within one month prior to study initiation;
- Regularly (>5x/week) using anti-gingivitis treatments (*e.g.,* stannous fluoride toothpaste, or anti-gingivitis mouthwashes, such as those containing chlorohexidine) in the month prior to screening;
- Removable oral appliances;
- Fixed facial or lingual orthodontic appliances;
- Have a positive pregnancy test (Clearblue) at baseline or self-reported pregnancy during the study; ;
- Any diseases or condition that might interfere with the safe participation in the study according to the study investigator;
- Inability to undergo study procedures; and
- Allergic reactions to any of the study toothpastes and/or ingredients in the study toothpastes.

Subject Continuance Criteria

Subjects may be excluded from study or the analysis if they:

- Participated in any other oral care study since their last study visit;

- Received any non-study dentistry, including dental prophylaxis, since their last study visit;
- Performed any oral hygiene prior to the study visit;
- Used any oral care products other than their assigned study products since their last study visit;
- Have taken antibiotics since their last study visit;
- Have taken medication that alters gingival appearance or gingival bleeding (*e.g.,* calcium channel blockers, anticonvulsants, or immunosuppressants);
- Have regularly (>5x/week) taken other anti-gingivitis treatments (*e.g.,* stannous fluoride toothpaste, or anti-gingivitis mouthwashes, such as those containing chlorohexidine);
- Have used removable oral appliances since their last study visit;
- Have used fixed facial or lingual orthodontic appliances;
- Had a positive pregnancy test or self-reported pregnancy since their last study visit;
- Are females who are of child-bearing potential and do not agree to continue to use a medically approved method of birth control during the study; or
- Have been unable or unwilling to comply with product usage instructions for any reason.

Subject Withdrawal Criteria

Subject participation is strictly voluntary, and a subject may withdraw from the research study at any time. Subjects may withdraw or take away permission to use and disclose health information at any time by sending written notice to the Principal Investigator. If a subject withdraws permission, the subject will not be able to continue being in the research study. When permission has been withdrawn, no new health information will be gathered after that date. Information that has already been gathered may still be used and given to Sponsor.

Subjects who withdraw or drop from the research study will not be replaced.

If, for any reason, a subject does not complete the study, an explanation will be entered on the CRF as Subject Accountability. All data gathered on the subject prior to discontinuation will be made available to the Sponsor.

Treatment of Subjects

Treatment to be Administered

Subjects will be randomly assigned to 1 of the 4 treatment groups and will be asked to brush at home twice daily for 1 minute each brushing for the duration of the 12-week study.

Subjects will be randomly assigned to 1 of the following 4 treatment groups:

- Negative Control—Colgate Cavity Protection toothpaste (0.76% sodium monofluorophosphate) and Oral-B® Indicator soft toothbrush
- Positive Control—Crest Pro-Health Clean Mint (0.454% stannous fluoride, 0.533% zinc citrate) and Oral-B® Indicator soft toothbrush
- Experimental Toothpaste #1 —Procter and Gamble Experimental Toothpaste (1,100 ppm NaF with 0.1% hops beta acids) and Oral-B® Indicator soft toothbrush
- Experimental Toothpaste #2 —Procter and Gamble Experimental Toothpaste (1,100 ppm NaF with 0.5% hops beta acids) and Oral-B® Indicator soft toothbrush

Medication(s) Treatment(s) Permitted and Not Permitted

Subjects are to refrain from all non study–related oral hygiene products, other than flossing if that is part of their regular oral hygiene routine.

Subjects who are enrolled in the study should not have used antibiotics 4 weeks prior to the baseline visit. Antibiotic use during the 12-week study period should be reported and assessed by the Investigator for continuing eligibility in the study.

Procedures for Monitoring Subject Compliance

A dispensing log, including treatment code, will be recorded on site source documentation. Study site staff with visually assess toothpaste usage at Visit 2 and 3.

Assessment of Efficacy

Methods and Timing for Assessing, Recording, and Analyzing Efficacy Parameters

Löe-Silness Gingivitis Index

The Löe-Silness Gingivitis Index (LSGI) will be used to evaluate the gingival health based on colour, consistency, and bleeding upon probing. The entire dentition, with the exception of the third molars, will be evaluated. For each tooth, 6 gingival areas (distobuccal, buccal, mesiobuccal, mesiolingual, lingual, and distolingual) will be scored using adequate light, a mouth mirror, and a periodontal probe. Prior to scoring, the teeth and gingiva may be air dried as required to provide adequate visibility. The probe will be inserted about 1 mm into the gingival sulcus and passed from interproximal to interproximal. One aspect (either facial or lingual) of each tooth in a quadrant will first be skimmed with the probe and then graded before passing to the next quadrant. Each of the 6 gingival areas will be given a score of 0 to 3, 8 or 9, as described below. A full mouth LSGI score will be calculated by summing the scores and dividing by the number of sites examined (excludes sites scored 8 or 9).

Table 3 Löe-Silness Gingivitis Index

Score	Criteria
0	Normal gingiva
1	Mild inflammation — slight change in colour, slight edema. No bleeding on probing
2	Moderate inflammation — redness, edema, and glazing. Bleeding on probing
3	Severe inflammation — marked redness, edema. Ulceration. Tendency to spontaneous bleeding
8	Tooth not scored
9	Missing tooth

Due to the nature of the measurement, the LSGI assesses both inflammation and bleeding. Bleeding Scores (BS) will be derived from the LSGI scores at each site. BS will be assigned a value of 0 if the LSGI score is 0 or 1, and a value of 1 if the LSGI score is either a 2 or 3. A full mouth bleeding score is determined by summing the BS and dividing this value by the number of all scored sites (excludes sites scored 8 or 9).

$$Mouth\ Bleeding\ score = \frac{\sum Bleeding\ score\ individual\ teeth}{n_{evaluated\ sites}}$$

Assessment of Safety

Specification of Safety Parameters

Safety will be assessed by the absence of irreversible side effects.

Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Oral Examination

Assessment of the oral soft tissue is conducted *via* a visual examination of the oral cavity and perioral area utilizing a standard dental light, dental mirror, and gauze. The structures examined include the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips, and perioral area. Assessment of the oral hard tissues are conducted *via* a visual examination of the dentition and restorations utilizing a standard dental light, dental mirror, and air syringe. All abnormal findings are recorded and categorized by their location with hard tissue findings categorized as "other-oral." An AE is recorded if a new abnormal finding is noted after treatment application, or any abnormal finding noted prior to treatment application increases in severity after treatment is applied.

Procedures for Eliciting Reports of and for Recording and Reporting Adverse Events and Intercurrent Illnesses

Adverse Event Management

An AE is any any adverse occurrence in the health of a clinical trial subject who is administered a natural health product, that may or may not be caused by the administration of the natural health product, and includes an adverse reaction, a serious adverse reaction and a serious unexpected adverse reaction. Adverse reaction (AR), serious adverse reaction (SAR) and serious unexpected adverse (SUAR) reaction are defined below in Table 4. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally

associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. An SAE is defined as an event which suggests a definite hazard or handicap to the subjects. SAEs are any events resulting in death, life-threatening situation, significant or persistent disability, incapacity or permanent damage, hospitalization or prolongation of existing hospitalization, or congenital anomaly/birth defects; events requiring intervention to prevent permanent impairment/damage; or other serious (important) medical events.

Table 4 – Adverse Reaction Definitions

Term	Regulatory Definition
Adverse Reaction	A noxious and unintended response to a natural health product that occurs at any dose used or tested for the diagnosis, treatment or prevention of a disease or for modifying an organic function.
Serious Adverse Reaction	A noxious and unintended response to a natural health product that occurs at any dose and that requires in-patient hospitalization or a prolongation of existing hospitalization, that causes congenital malformation, that results in persistent or significant disability or incapacity, that is life threatening or that results in death.
Serious unexpected adverse reaction	A serious adverse reaction that is not identified in nature, severity or frequency in the risk information set out on the label of the natural health product.

When an Investigator is notified of a SAR, the Investigator must promptly (within 24 hours) notify the Sponsor (Clinical Trial Manager or the Medical Monitor) of the serious event, regardless of causality. Within 5 working days, a written and/or electronic report describing the circumstances of the event must be submitted to the Sponsor. The Investigator will be responsible for SAE reporting to the Institutional Review Board (IRB). The Sponsor will appropriately inform the Natural and Non-prescription Health Product Directorate (NNHPD) of SARs and SUARs within the timelines indicated in Section 78 of the *Natural Health Products Regulations* (NHPR).

All other AEs that do not fit within the definitions of SAR or SUAR will be considered "non-serious." Unexpected adverse event/reaction is any occurrence the nature or severity of which is not consistent with the AE provided in the current label of the NHP. All other AEs are considered "expected."

When an AE is suspected, all relevant evaluations will be carried out and appropriate treatment will be recommended. Additional follow-up will be performed as necessary and recorded as site source documentation, with the results provided to the Sponsor. Subjects that experience any clinically SAE will remain under medical supervision until the Principal Investigator/Medical Monitor recommends appropriate follow-up treatment or deems the AE to be resolved, stabilized, or no longer serious enough to warrant follow-up.

Adverse Event Recording

When an AE occurs after written informed consent has been obtained but before the first dose of study drug, the AE will be considered a nontreatment-emergent AE. An AE that occurs from the time the subject receives the first dose of study drug until dismissed from the study will be considered a treatment-emergent AE. All AEs will be collected. The severity, action taken, causality, outcome, and category of all recorded AEs will be recorded.

Severity

Severity refers to the extent to which an AE affects daily activities. Severity will be categorized according to the following criteria:

- Mild: Normal activities unaltered;
- Moderate: Normal activities altered;
- Severe: Unable to undertake normal activities.

The term "severity" is not the same as "serious." Seriousness, not severity, serves as the guide for defining regulatory reporting obligations.

Action Taken

If any action was taken due to the AE, it should be recorded.

- None: No action was taken by the subject.
- Discontinued: Investigator recommended or the subject stopped using the study products.
- Reduced/Interrupted: Investigator recommended, or the subject reduced or interrupted the instructed product usage.

Causality

Causality refers to the relationship of the AE to study drug. Assessment of causality is the responsibility of the Principal Investigator at each site. If this responsibility is delegated to a sub-Investigator, this should be appropriately documented in the delegation sheet. Causality will be categorized according to the following criteria:

- Doubtful: There is no medical evidence to suggest that the AE may be related to study drug usage, or there is another more probable medical explanation.
- Possible: There is medical evidence to suggest that the AE may be related to study drug usage. However, other medical explanations cannot be excluded as a possible cause.
- Probable: There is strong medical evidence to suggest that the AE is related to study drug usage.
- Not Related: There is no evidence to suggest the adverse event is related to study drug usage.

Category

Category refers to the region or area where the AE occurred. Categories are listed 0 to 9.

- 0 = Non-oral related
- 1 = Perioral area/lips
- 2 = Labial mucosa/buccal mucosa
- 3 = Mucolabial fold/mucobuccal fold
- 4 = Gingiva/free and attached
- 5 = Palate/hard and soft
- 6 = Oropharynx/uvula
- 7 = Tongue
- 8 = Sublingual
- 9 = Other oral

Type and Duration of the Follow-up of Subjects After Adverse Events

General Comments can be recorded at any time during the study. AEs will be documented in eCRF. Any subject reported AE that remains unresolved by the end of the study should be followed up until resolution by the Investigator/designee. Examiner-observed AEs that are unresolved at the end of the study are followed up to resolution at the discretion of the Medical Monitor. All resolutions should be documented only as source documentation. If a subject is unreachable to determine whether the AE has been resolved, the attempts to contact the subject should be documented as source documentation.

Statistics

Statistical Methods

Summary statistics (*e.g.,* means, standard deviations, frequencies.) of the demographic characteristics will be calculated for each treatment group and overall. Gingivitis scores will be calculated for each treatment group and visit.

Primary Efficacy Analysis

Comparisons to baseline will be investigated using paired-difference t-tests. The treatment groups will be compared using the analysis of covariance method with baseline as a covariate. If normality assumptions are not satisfied, transformation of the data and/or non-parametric analyses may be carried out. Statistical tests for treatment comparisons will be two-sided using a 5% significance level. No adjustment will be made for multiple comparisons. Additional analyses may be performed to fully understand the data.

The following hypotheses will be tested using a serial gatekeeping approach. Using this approach, differences between groups are tested in accordance with a pre-defined hierarchical approach such that the subsequent comparison is statistically tested only if the null hypothesis for the prior test is rejected. The hierarchical approach is detailed below.

For model sensitivity Hypothesis 1 will be tested at Week 12 for the total number of bleeding sites.

Hypothesis 1:
Null: The mean is equal between the Positive Control Group and Negative Control Group.
Alternative: The mean is not equal between the Positive Control Group and Negative Control Group.

If the null hypothesis for Hypothesis 1 is rejected, Hypothesis 2A will be tested at Week 12 for the total number of bleeding sites.

Hypothesis 2A:
Null: The mean is equal between the Experimental Toothpaste #2 Group and Negative Control Group.
Alternative: The mean is not equal between the Experimental Toothpaste #2 and Negative Control Group.

If the null hypothesis for Hypothesis 2A is rejected, Hypothesis 2B will be tested at Week 12 for the total number of bleeding sites.

Hypothesis 2B:
Null: The mean is equal between the Experimental Toothpaste #1 Group and Negative Control Group.
Alternative: The mean is not equal between the Experimental Toothpaste #1 Group and Negative Control Group.

If the null hypothesis for Hypothesis 2B is rejected, subsequent analyses will compare the experimental groups with the positive control group at Week 12. These analyses will focus on the total number of bleeding sites, consistent with the study's primary objective. Percent number of bleeding sites, LSGI score, Week 4 and Week 12 visits, and all other treatment comparisons will also be analyzed. No adjustment will be made for multiple comparisons.

Safety Analysis

Summary tables will be provided for all reported AEs by MedDRA code and treatment group with safety analysis set. Tables will also be provided for all reported AEs by MedDRA code and subject.

Number of Subjects Planned to Be Enrolled

Power analyses were conducted with α=0.05, using a 2-sided test. Up to 120 subjects will be enrolled in the study assuming ~5% dropout rate. Data were utilized from a similarly designed study with the same examiner and endpoints (from 2020054).

Twenty-eight subjects per group completing this trial should provide at least 80% power to detect a difference in number of bleeding sites of at least 8 units between treatments assuming the variability (SD) number of bleeding sites is 10. Assuming the variability (SD) of whole mouth LSGI is 0.1, a sample size of 28 subjects per group should provide at least 80% power to detect a difference in LSGI of at least 0.08 units between treatments.

Level of Significance

The level 0.05 of significance will be used for treatment comparisons.

Termination Criteria

Early termination is not anticipated for this study.

Procedure for Missing, Unused and Spurious Data

Unless noted otherwise, missing data will not be imputed. All analyses will be based on observed data only.

Procedure for Reporting Deviations from the Original Statistical Plan

When differences exist in descriptions or explanations provided in this protocol and the Statistical Analysis Plan (SAP), the SAP takes precedence; the differences will be explained in the clinical study final report.

Subjects to be Included in the Analyses

The following populations are defined for the purpose of the analysis:

Intent-to-treat (ITT) Population

The ITT population will include all randomized subjects who are confirmed to have received study treatment and have at least one post-treatment efficacy measurement. This will be the primary population for the analysis of efficacy endpoints.

Per Protocol (PP): All subjects who received study treatments and completed the study per protocol (also known as "evaluable population").

Safety Analysis Set: All subjects who received study treatments and had oral examinations conducted will be included.

Direct Access to Source Data/Documents

The Investigator has the responsibility for ensuring that all source documents (*i.e.,* study and/or medical records) and CRFs are completed and maintained according to the study protocol and local regulations and are available at the site. Any CRF used as a source document must be identified as such in the Investigator Notebook.

The Data Manager will supply the paper and/or eCRFs to be used in this study. It is the responsibility of the Investigator to maintain and submit accurate and timely CRFs to the Sponsor. All hard copy CRFs will be filled out legibly in ink.

All questions should be answered. For paper CRFs, if an entry requires correction, a single line will be placed through the entry so as not to obscure the original record, the corrected entry will be initialled and dated by the individual making the change, and a reason will be given for the change. There will be no whiteouts or erasures. For eCRFs, if an entry requires correction, the change is made directly to the CRF in the database, the user is prompted to provide a reason for the change, and the correction is logged in by an electronic audit trail.

As necessary, the Data Manager may make specified allowable changes to the database without issuing a query to the site, as agreed upon by study site staff per this protocol. Examples of allowable changes include incorrect date formats, incorrect current year recorded (as in the start of a new year), and unambiguous spelling errors. Changes to common abbreviations and symbols to equivalent text to meet system or coding constraints (*e.g.,* @ = at, ~ = approximately), may also be allowable. Values that are ambiguous or open to interpretation will be queried to the sites' staff. It is the responsibility of the Data Manager to ensure all changes are supported by information contained elsewhere and/or are unambiguous.

Quality Assurance and Quality Control

The following steps will be taken to ensure the accuracy, consistency, completeness and reliability of the data:

- Creating Clinical Trial Risk Assessment and Risk Management Plan;
- Site initiation meeting;
- Routine site and/or data monitoring;
- eCRF review against source documents;
- Data management quality control checks;
- Statistical quality control (QC) checks;
- Continuous data acquisition and cleaning; and
- Internal review of data.

In addition, a Sponsor representative may conduct periodic quality assurance audits of the study processes, including, but not limited to, clinical site visits, laboratories, vendors and contract research organizations, the study database, and Procter & Gamble's final study report. Data monitoring will ensure quality of collected information by detection of inconsistent and missing information. The investigational site staff will follow up on any queries from Procter & Gamble's Data Manager. If a query arises from a subject that was lost to follow-up, the site staff will take action to contact the subject and document any attempts made. The study monitor will ensure that the study was conducted in accordance with the protocol and any amendments, good epidemiological practice, and applicable laws and regulations. The quality assurance and quality control systems implemented to assure the quality of the data should be described.

Ethics Description and Ethical Considerations

Subject participation is voluntary. Subjects have a right to refuse participation in the research study at any stage. The study will be conducted in accordance with all international laws and regulations, and also Canadian national laws and regulations, and in accordance with any applicable guidelines. Informed consent will be obtained from potential subjects of the study. Beforehand, the study site staff should fully inform each subject regarding all the aspects of the clinical study, including objectives, collected data, expected risks and benefits of participation, and voluntary participation in the study.

Conduction of the study will comply with the principles of ICH-GCP. Before the study starts an approval of independent ethical committee for conduction of the present study will be obtained. A list of Investigators and investigational sites will be approved. All documents and data related to

the study are strictly confidential. Protocol contents cannot be disclosed to third parties without written permission of the Sponsor.

Sponsor of the study is obliged to adhere to confidentiality of the study subjects' personal data. Any information identifying the subject's identity should not be disclosed. The necessary personal data of the subjects as part of the study (for example, gender, age) will be collected only for achieving study purposes and in minimum quantity. The study site staff will maintain an identification list of all screened and enrolled subjects. This information will only be accessible to the project team members that perform pertinent study execution tasks and/or GCP monitoring. Names and surnames of subjects will not be disclosed or reported to other project team members. If subject name and surname are mentioned in any document, then before sending a copy of such document to the Sponsor these data will be erased. Results of the study stored in electronic format will be kept in accordance with current information protection laws. Sponsor employees or regulatory authorities' representatives should not contact the subject directly. Before enrollment into the study, subjects will be informed regarding confidentiality provisions and use of their personal data, including necessity of access to them of the monitor and other authorized people (in case of auditing, inspection, *etc.*).

Prior to study initiation, the Investigator must obtain institutional review and approval of the Protocol, the consent form, and other necessary study-related documents in compliance with the Part 4 of the NHPR or the ICH-GCPs Consolidated Guidelines, Chapter 3 and in compliance with Procter & Gamble's Standard Operating Procedure (SOP) CTN-CL-504 ("Institutional Review Board/Independent Ethics Committee Review and Approval"). The Investigator will maintain any original authorization letter(s) which will be available for review by the Sponsor. IRB approval letters should include the study title, Sponsor study number, the address of the IRB, date of request, and the signature of the IRB chairperson or designee. Additionally, the letter must acknowledge that both the Protocol and consent form have been approved by the IRB. The study will not begin until the Sponsor has received confirmation of IRB approval. The IRB shall also review the investigation at least once a year during study execution. The Investigator will notify the IRB when the study is terminated and provide confirmation that the study has been closed with the IRB to the Sponsor.

The Investigator will obtain written informed consent for each subject prior to participation in the study, per Section 74(h) of the NHPR and ICH-GCPs, Chapter 4, Subpart 4.8 and in compliance with Procter & Gamble's SOP CTN-CL-503 ("Informed Consent Form, Ethics Approval and Investigator Use"). Subjects, or their legal guardian, are required to read, sign, and date an IRB approved consent form with the Investigator also maintaining a signed and dated copy. The subject or legal guardian will be given a copy of the consent form. All study procedures must be explained in non-technical terms.

Changes to the Protocol following IRB approval affecting the safety of subjects, scope or objectives of the investigation, or the scientific quality of the study will be documented as amendments. Such changes will require the Sponsor, Investigator, IRB, and NNHPD approval prior to implementation, unless immediate action is required to safeguard subject safety. Administrative or minor changes (*e.g.,* typographical errors, changes in Sponsor personnel, *etc*.) will be documented as revisions but may not need to be submitted as amendments unless required

by the IRB. Any change in the Sponsor's monitoring staff, Clinical Trial Manager or Medical Monitor during the conduct of the study, will be reported to the Investigator.

During the course of the trial, the clinical site will allow monitoring by the Sponsor (Clinical Trial Manager or designee) to check compliance with the Protocol, regulations and guidelines, adequacy of the equipment and facilities, and satisfactory data collection.

Data Handling and Recordkeeping

The Investigator must retain the subject identification codes, informed consent documentation, clinical materials inventory, CRFs (paper or electronic media), medical records and other source data for a minimum of 15 years after the last regulatory approval has been received or the discontinuation of the study. The Investigator must receive written authorization from the Sponsor before destroying any study document. The Investigator will make the records available for inspection and copying upon the request of an authorized employee of a government authority or the Sponsor, at reasonable times. In the event the Investigator retires, relocates, or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to another person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor.

The subject will be identified with a unique identification code assigned by the Investigator to each trial subject to protect privacy. The identification codes are used in lieu of the subject's name when the Investigator reports all AEs and other trial -related data. These codes will be used on all study documents for the subject's confidentiality, as stated in Section 2.11 of the ICH-GCP.

Any advertisements used in recruitment of subjects must receive prior approval from Procter & Gamble and/or the Investigator's IRB. A copy of the IRB-approved advertising and the documentation thereof must be provided to Procter & Gamble.

Following completion of the study, the Investigator shall submit a final report to the Sponsor describing the conduct of the study, deviations from planned conduct, early withdrawals and Subject Accountability, AEs, and other information on study conduct. The Investigator's IRB may require more frequent status reports.

Financial Statement

Financing and insurance can be found in signed and executed clinical study agreement.

Publication Policy

The results of this study may be published in oral presentations, written abstracts, written manuscripts, and/or with regulatory agency.

Supplements

This section is not applicable to this protocol.