Clinical Trial Study Protocol

A Randomized, Double-Blind, Placebo-Controlled Study to Determine the Efficacy SALI-10 Oral Probiotics in Experimental Gingivitis

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Ostia Sciences

1. INTRODUCTION

Gingivitis is an oral disease condition affecting 50% to 90% of adults globally (Albandar and Rams, 2002). Not only is it common in the adult population, but it is a precursor to periodontal disease, which is a more severe condition of gum disease that permanently damages the soft tissues and the bone supporting the teeth (Löe et al., 2009). It is an issue that the investigators do not want to overlook since it has also been proven that periodontitis exacerbates different systemic diseases, such as diabetes and atherosclerotic vascular disease (Lockhart et al., 2012; Mealy et al. 2006). Previous studies and clinical research have shown that combining antimicrobial treatment with non-surgical periodontal therapy, such as scaling and root planning, is an efficient way to tackle periodontal disease (Kapoor et al. 2012). However, modern practitioners are looking for an alternative therapy, as antibiotics are innocuous drugs by nature and can lead to several side effects, damage to our commensal bacterial flora and favour the development of antibiotic-resistant bacteria (De Nies et al. 2023; Soares et al. 2012). Knowing the complexities associated with the treatment and management of periodontal disease, the primary strategy for success lies in its prevention. Therefore, it is also imperative to identify effective methods for treating and preventing gingivitis.

The gingivitis pathology can be reversed by reduction or removal of microbial plaque that accumulates on hard and soft tissues and is considered standard of care in the industry (Berezow Alex and Darveau Richard, 2010, van der Weijden and Slot, 2011). Regular oral hygiene in combination with therapeutics that delivers an anti-microbial benefit is thought to mitigate the onset of gingivitis (van der Weijden and Slot, 2011, Gunsolley, 2006). However, testing therapeutics for prophylaxis benefit to mitigate development of gingivitis has not been fully examined.

The classical model of experimental gingivitis (EG) was developed in 1965 by Loe and Silness who convincingly demonstrated the causative relationship between the accumulation of dental plaque and the development of clinically evident gingivitis in healthy young adults abstaining from all oral hygiene practices for a 21-day period. Furthermore, on resuming customary oral hygiene practices, all study participants demonstrated a return to gingival health within two weeks (Loe et al., 1965). To understand how the participants of this study returned so quickly to gingival health we can reflect on our current understanding that our clinically healthy state is an active and dynamic process.

Neutrophils, a type of white blood cell (leukocyte), represent a key component of the innate defence system that protects periodontal tissue from both gingivitis and periodontitis. Not only are they the first line of cellular defence, but they are among the most abundant leukocytes within the periodontal tissues (Hirschfeld, 2020). For example, gingivitis is associated with a significant increase in the number of neutrophils that migrate to periodontal tissue. In contrast, individuals with too few neutrophils brought about by either congenital deficiencies in neutrophil numbers, or transit (LAD 1 and 2) or have an induced neutropenia by chemical induction with antimitotic agents such as cyclophosamide invariably develop periodontitis. Likewise, studies in KO mice that are defective in neutrophil transit also develop periodontitis. Consistent with the key contribution of neutrophils to both gingivitis and periodontitis, neutrophil transit to gingival tissue is highly regulated. The periodontium contains a highly orchestrated expression of select innate host defense

mediators that facilitate the transit of neutrophils from the highly vascularized gingival tissue to the gingival crevice, where they form a "wall" between the host tissue and the dental plaque biofilm.

However, the prolonged presence of neutrophils in gingival tissue is not tolerated in the healthy state. For example, the failure to down regulate orchestrated neutrophil transit 0results in an increase in neutrophil numbers in gingival tissue and a significant increase in per iodontal bone loss. Therefore, neutrophil homing to the gingival crevice is highly regulated such that under conditions of periodontal health the appropriate amount of neutrophils are present to maintain control of dental plaque bacterial growth and yet not elicit tissue damage. Evaluation of oral and blood neutrophils during experimental gingivitis showed that people with uniquely high inflammatory response had an exaggerated polymorphonuclear neutrophil response both in the oral cavity as well as in the blood (Wellappuli 2017).

Gingivitis is a reversible inflammatory condition caused by the accumulation of dental plaque and the associated disruption of the host–microbial homeostasis. During gingivitis, the microbial community transitions from being dominated by gram-positive health-associated bacteria, such as Streptococcus species, to gram-negative periopathogens, including species of the genera Porphyromonas, Tannerella, Treponema and Prevotella. This dysbiotic shift triggers inflammatory responses, leading to tissue damage and, in some cases, progression to periodontitis. Those pathogenic bacteria also contribute to the production of volatile sulfur compounds; as a result, people with gingivitis have an increased probability of having halitosis by 3.6 times (Lee et al. 2023).

A recent study (Kerns, 2023) on human experimental gingivitis identified three distinct host response phenotypes—high, low, and slow responders—based on clinical, inflammatory, and microbial parameters:
1. High Responders: Rapid plaque accumulation accompanied by a significant increase in gram-negative periopathogens and elevated inflammatory markers, such as interleukin-1β (IL-1β).
2. Low Responders: Similar plaque accumulation to high responders but lower inflammation, suggesting a more muted host response to bacterial dysbiosis.
3. Slow Responders: Delayed plaque accumulation and microbial succession, with prolonged dominance of health-associated Streptococcus species. This group exhibited delayed or reduced inflammation, demonstrating a more resilient microbial community and host response.

The microbial analysis revealed that the persistence of beneficial Streptococcus species, such as S. sanguinis and S. oralis, in slow and low responders correlates with a protective effect against the emergence of periopathogens and the associated inflammatory cascade. Conversely, the loss of these beneficial bacteria in high responders was linked to more severe inflammation, highlighting the critical role of the oral microbiome in modulating gingivitis severity.

Lantibiotic salivaricins are polycyclic peptides containing lanthionine and/or β-methyllanthionine residues that are produced by certain strains of Streptococcus salivarius, which almost exclusively reside in the human oral cavity. These molecules importance stems from their antimicrobial activity towards relevant oral pathogens which has been applied through the development of salivaricin-producing probiotic strains. However, salivaricins may also prove

to be of great value in the development of new and novel antibacterial therapies in this era of emerging antibiotic resistance (Barbour, Wescombe, & Smith, 2020). In a study by Barbour & Philip 2014, they found that the bacteriocin, levan-sucrase production and basic safety features of S. salivarius strains isolated from healthy Malaysian study participants demonstrating their potential for use as probiotics. A new bacteriocin production medium was developed with potential scale up application for pharmaceuticals and probiotics from S. salivarius generating different lantibiotics. This is relevant for the clinical management of oral cavity and upper respiratory tract in the human population. Appendix 1 includes a summary table that lists the relevant clinical trials that utilized S. salivarius.

Proposed Solution: S. salivarius SALI-10 to be used as a probiotic to help prevent gingivitis

We propose using a novel strain, Streptococcus salivarius SALI-10, as a targeted microbial intervention to modulate the oral microbiome and prevent gingivitis. S. salivarius SALI-10 is hypothezised to:

• Maintain a stable population of beneficial streptococci during plaque accumulation.
• Inhibit the growth of periopathogens such as Porphyromonas, Tannerella and Prevotella through competition and production of Salivaricin 10.
• Delay or suppress the dysbiotic shift to gram-negative dominance, thereby reducing the inflammatory response.
By preserving microbial homeostasis, SALI-10 may emulate the microbial resilience observed in slow responders, offering a novel strategy for gingivitis prevention.

2. STUDY OJECTIVES

1) The aim of this study is to evaluate the efficacy of S. salivarius SALI-10 in modulating the oral microbiome and preventing gingivitis, using a placebo-controlled experimental gingivitis model. Specifically, the investigators aim to:
   Microbiome Modulation:
   - Assess the ability of SALI-10 to maintain higher levels of beneficial streptococci (S. sanguinis, S. oralis, S. parasanguinis and SALI-10) during plaque accumulation.
   - Determine whether SALI-10 prevents or delays the increase of gram-negative periopathogens (Tannerella, Prevotella, and Porphyromonas) and dysbiosis.
2) To evalute the effects of probiotic Sali-10 intake regarding clinical parameters of periodontal health during an experimental gingivitis.
3) To assess differences in the onset of gingivitis, the extent of the condition, and its resolution among experimental and control groups.
4) To evaluate the capability to establish or increase oral colonization by *Streptococcus salivarius SALI-10*. The expected outcomes will be attributed to the effect of antimicrobial and immunomodulation metabolites (mainly, the natural product Salivaricin 10) produced by this strain present in the probiotic.
5) To analyze the level of sulfured compounds emitted from the oral cavity with a halimeter (Halimeter PLUS) from each study participant to assess the level of halitosis objectively, then evaluate the difference between the control and test groups as they go through the experimental gingivitis clinical trial.

3. STUDY OVERVIEW

This research study is designed to evaluate a new strain of *Streptococcus salivarius* containing probiotic properties for its effect on gingivitis including the oral neutrophil phenotype and oral microbiome modulation during an induced gingivitis state. A 7-week (8 appointments, 7 weeks) clinical study will be conducted to evaluate a probiotic for therapeutic effects that aim to delay the onset of experimental gingivitis and hasten the resolution of the disease.

4. RECRUITMENT

4.1 Test Site and Investigator

Site: University of Toronto, Canada
Study Originator: Dr. Michael Glogauer (Osteia Sciences)
Principal Investigator: Dr. Michael Goldberg
Co-Investigator: Dr. Howard Tenenbaum

4.2 Study Population

Advertisements (Appendix 2) will be placed at University of Toronto downtown campus to invite volunteers who are interested to participate. All study participants will undergo informed consent process, prior to enrollment in the study. Categorization of participants and sites will be based on the clinical assessments. Other than the study-related oral health history recorded, there is no intention to obtain information from the participants‟ other dental or medical records. Sixty (60) qualifying volunteers that agree to participate and sign all corresponding forms will be enrolled.

The first participant will be assigned a number, and all the following enrolled participants will be assigned consecutive numbers by accession. The following inclusion and exclusion criteria will be utilized in this study.

4.3 Inclusion Criteria:

1) Male or female volunteers aged 18-70 years
2) In good general health, ASA I
3) No clinical signs of gingival inflammation at >90% of sites observed
4) Absence of Periodontal Pockets, Probing Depth (PD) < 3.0 mm on all teeth/site
5) Absence of Clinical Attachment Loss (CAL) = 0 mm
6) No periodontal disease history
7) Have at least 20 gradeable teeth
8) Non-smokers
9) Fluent in English
10) For study participants of childbearing potential, both men and women, at least one of the following birth control measures must be used: abstinence, hormonal birth control (oral, injectable, transdermal, intra-vaginal), intrauterine devices, confirmed successful vasectomy, or condoms.

4.4 Exclusion Criteria:

1) Presence of orthodontic bands.

2) Presence of partial or full dentures.
3) Tumour(s) of the soft or the hard tissues of the oral cavity.
4) Cavitated carious lesions requiring immediate restorative treatment.
5) History of allergy to a consumer or personal care products or dentifrice ingredients as determined by the dental profession monitoring the study.
6) Participation in any other clinical study or test panel within one month before entering the study.
7) Medical condition which requires pre-medication before dental visits/procedures
8) Current use of anti-inflammatory, antibiotics, or antimicrobial drugs or within the last 30 days of enrolment.
9) History of periodontal disease.
10) History of systemic inflammatory, immune conditions and immunocompromised conditions
11) Pregnant or nursing women or those planning to get pregnant
12) Use of tobacco products.
13) Long-term antibiotic or anti-inflammatory therapy.
14) Medication or Natural Health Products (NHPs) that could affect the gingiva like calcium channel blockers, anti-epileptic therapy etc.
15) Medical condition or any current usage of medication that the investigator considers may compromise the study participant's safety as well as the quality of the study results
16) Allergy to any of the following ingredients: Streptococcus Salivarius, Sorbitol, Isomalt, Calcium phosphate dibasic, Potato starch, Mint, Glyceryl dibehenate, Stevia, Maltodextrin
17) Use of other probiotics
18) Taking anticoagulant medications and those suffering from blood and bleeding disorders
19) Recently experienced or will be experiencing dental, oral or any type of surgery.
20) Use of anti-plaque/ anti-gingivitis products
21) Presence of active infections
22) Participants who are experiencing nausea, fever, vomiting, bloody diarrhoe or severe abdominal pain.
23) Use of any antibiotics.

4.5 Continuing Criteria

Each study participant must meet continuance criteria at each visit in order to continue in the study. Study participants will be released from further participation for the following reasons:

- use of non-study dentifrice during treatment periods (acceptable dentifrice will be provided during the study)
- use of oral care products (other than those permitted) during the treatment periods including mouth rinse products and chewing gum.
- non-compliance with study procedures
- actively participating in another clinical study unless permission has been granted by the Principal Investigator
- development of a medical condition or taking a medication which would affect the study outcome (such as an antibiotic) as detailed in the exclusion criteria.
- Subject fails to substantially comply with the protocol requirements.
- Subject fails to report for a scheduled examination.

- Subject is treated with medications during the course of the study, which may interfere with the parameters of the study.
- Subject develops an adverse reaction. The study Investigator will immediately notify the study monitor and information will be recorded on Adverse Reaction Form.
- Subject receives emergency dental or medical treatment, which may interfere with the parameters of the study.
- Sponsor elects to terminate the study.
- Subject elects to terminate participation in the study. No pregnant women will intentionally be enrolled in this study. All female subjects being considered for this study will be asked about their pregnancy status. the clinical/research investigator if they become pregnant during the course of the 9-week study. In the event a woman enrolled in this clinical research study becomes pregnant at any time during this study, participation in this study will be terminated upon the clinical staff's notification of the event.
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4.6 Concomitant Therapy

If a study participant takes concomitant medications as a matter of necessity for the treatment of a medical condition, then such medication may be permitted for the duration of the study at the discretion of the investigator. However, it is the responsibility of the investigator to disqualify from entering the study any study participant who, upon screening, is using medication or consumer product that might obscure the interpretation of study results. All medications currently used by the study participant at enrollment, or any time through the end of the study, will be recorded on the visit report form (Appendix VI). Study participants may receive medication to treat adverse events as deemed necessary by the investigator or the study participant's physician.

4.6 Missed Doses

If a study participant misses their dose, they will be asked to take it if no more than 3 hours has passed since they brushed their teeth. If more than 3 hours have passed, they will be asked to skip the dose completely. Do not double up on the next dose. Do not exceed 2 lozenges per day.

5. TEST PRODUCTS/INTERVENTIONS

Each participant randomized to the intervention group will receive mint-flavoured SALI-10 lozenges. They will be instructed to take a lozenge twice daily after brushing by letting it dissolve in the mouth. The participants will be using the SALI-10 lozenges throughout the study.

5.1 SALI-10 Lozenges Manufacturer Information

The placebo and probiotics lozenges will be manufactured by
Lallemand Health Solutions,
17 975 rue des Gouverneurs
Mirabel, Québec, Canada
J7J 2K7
Tel : 450-433-9139

Content of the placebo lozenge:

Excipients	Content per 1 gram- lozenge (in mg)
Sorbitol	310
Isomalt	310
Maltodextrin	175
Potato starch	90
Dibasic calcium phosphate	83
Mint flavour	20
Glyceryl dibehenate	10
Steviol glycosides	2

Content of the probiotic lozenge:

Active ingredient	Content per 1 gram- lozenge (in mg)
Streptococcus salivarius	200* Min. Bi CFU/lozenge at manufacturing
Excipients	
Sorbitol	310
Isomalt	310
Dibasic calcium phosphate	83
Potato starch	QSP (65*)
Mint flavour	20
Glyceryl dibehenate	10
Steviol glycosides	2

5.2 Dispensing, Storage, and Accountability

All product(s) are distributed by the study coordinator at the clinical test site. The study participant will follow provided instructions on when and how to brush their teeth. These instructions are explained in the procedure section.

One of the study personnel, Dr. Martin Lambert, will be responsible for dispensing and collecting the containers of the SALI-10 lozenges at each visit.

6. STUDY PROCEDURES

6.1 Randomized Assignment and Product Blinding

Qualified study participants will be randomly assigned to one of two study groups, either Group A or Group B, using randomization software. To maintain blinding, the manufacturer will produce both probiotics and placebo lozenges, dispense them in identical, opaque bottles, and label them as either Group A or Group B. The assignment of probiotics to one of these groups will be determined by the manufacturer and disclosed only at the study's conclusion. Each bottle will be labeled with a code (either letter A or B, followed by a number) and emergency contact information. The products will be distributed in sealed boxes that look identical to prevent any differences in appearance or packaging between study groups. Each product inside the box will be overwrapped and include instructions for at-home use and safety information, including emergency contact details.

To monitor study participant compliance, the investigators will use a pharmaceutical scale to ensure the number of lozenges remaining in the bottle matches the expected count at each study visit. A scale will be used to avoid the operator from seeing the lozenges, in order to preserve blindness in this study (placebo and probiotic lozenges are not perfectly identical).

Study participants will be assigned a unique identification number in chronological order (e.g., from 1 to 60) as they enroll in the study. These identification numbers have been pre-assigned to a study group based on a computer-generated randomization list provided by an independent statistician. Cohabitant study participants will be assigned to the same study group and receive the same products. Study participants will be instructed to avoid using any other oral hygiene products. There will be no dietary restrictions during the study.

6.2 Product Dispensing and Use at Home

Study participants will be instructed to brush their teeth twice daily (morning and evening) for two minutes each time with a toothpaste provided by the study and toothbrush and floss. The participants will be informed as to when home oral hygiene should be performed and the times they will be instructed to refrain from using oral hygiene products during certain times during the study. Study participants There will be no restrictions regarding diet habits during the course of the study.

The SALI-10 lozenges and placebo lozenges will be pre-packed when given to each participant.

6.3 Telephone Pre-Screening

Potential study participants who contact the clinical trial coordinator for more information about the study via email will then be contacted via telephone. At this stage the Recruitment Questionnaire (Appendix 3) will be completed to determine whether they are eligible for the screening phase of the study. If the study participant is eligible for the study the screening visit will be scheduled.

6.4 In-Person Screening

Before any participant screening takes place, they will be given the Informed Consent Form to read and ask any questions they may have and sign. If signed, the remainder of the visit entails completion of the Personal Information Form (Appendix 4), and the Health Questionnaire form

(Appendix 5) and completion of an oral exam of both the soft and hard tissues (Screening Oral Assessment Form – Appendix 6). Female participants will be asked to take a urine pregnancy test. This is done to determine if the study participant is eligible for the study. The study personnel will then complete the screening form (hard copy).. The screening will be carried out until sixty (60) study participants are enrolled.

6.5 Pre-Induction Phase (Health Baseline – Study participant maintain dental hygiene at home for 14 days)

6.5.1 Visit 1 (t_{-2}) Day -14

Study participants will undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimens collection will include gingival crevicular fluid (GCF), sub-gingival plaque, saliva and a saline oral rinse. All study participants will receive a prophylaxis (cleaning) which involves scaling and polishing to remove all supra- and subgingival plaque and calculus. Study participants will receive their assigned study product for use at home with directions. The participants will take two lozenges daily, one after their morning brushing and one after evening brushing. They will allow the lozenges to dissolve without biting or swallowing and avoid eating and drinking for one hour. The participants will be instructed on proper oral hygiene and will be instructed to continue their home oral hygiene in addition to use of their assigned product for 14 days (2 weeks). The participants will be instructed to return the lozenges' containers at each visit for protocol compliance check. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.5.2 Visit 2 (t_{-1}) (Day -7)

The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.6 Induction Phase (No Oral Hygiene – for 21 days)

6.6.1 Visit 3 (t_0) (Day 0)

The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. Additionally, study participants will be asked to refrain from any oral hygiene for 21 days and to continue using the lozenges they were assigned, one during the morning, and one during the evening. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.6.2 Visit 4 (t_1) (Day 7)
The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. The participants will again be reminded to refrain from all forms of oral hygiene. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.6.3 Visit 5 (t_2) (Day 14)
The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. The participants will again be reminded to refrain from all forms of oral hygiene. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.7 Resolution (Study participants resume to regular dental hygiene home care for the rest of the study)

6.7.1 Visit 6 (t_3) (Day 21)
The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. All study participants will receive a prophylaxis (cleaning) which involves scaling and polishing to remove all supra- and subgingival plaque and calculus. The study participants will be instructed to continue using the lozenges they were assigned and resume home oral hygiene for the next 14 days (2 weeks). Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

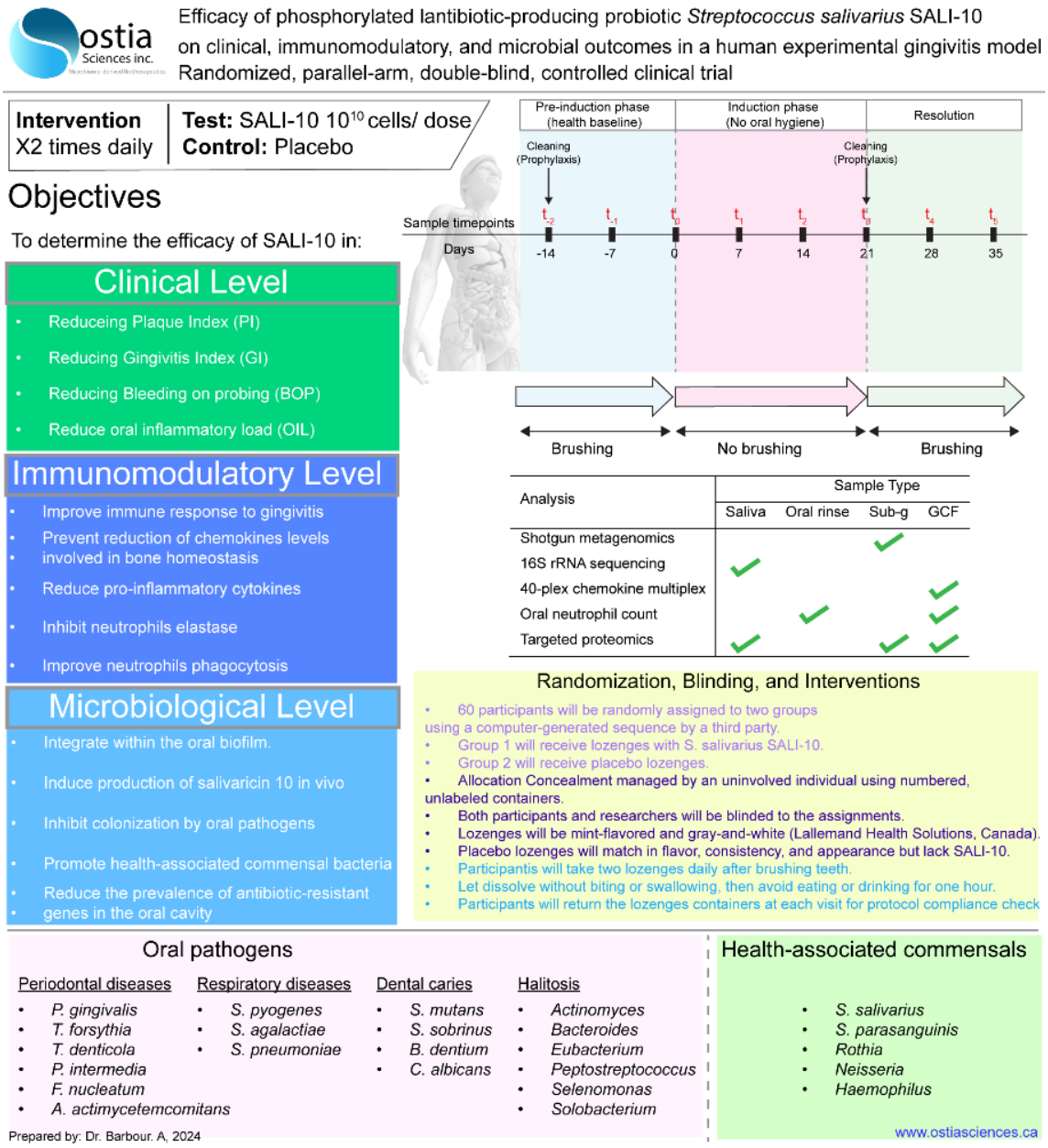
6.7.2 Visit 7 (t_4) (Day 28)
The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. The study participants will be reminded to continue at home oral hygiene. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

6.7.3 Visit 8 End of Study (t_5) (Day 35)

The study participants will return the containers containing the lozenges to a member of the study team. Study participants will then undergo an oral hard and soft tissue exam as well as measurements of probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP) and recorded on the Oral Assessment Form (Appendix 7). The biospecimen collection will include gingival crevicular fluid (GCF), plaque, saliva and a saline oral rinse. The study participants will be informed that this is the end of the study. They will be given their compensation and told that they may resume their regular oral hygiene. Once the study has been completed, the manufacturer will reveal to which group was the probiotic assigned to (either A or B). Afterwards, the investigators will inform participants to which group they were assigned and will ask for their feedback in regards to their experience. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Please refer to figure 1. Illustrating the breakdown and timeline of the study as well as the relevant study procedures at each time point and the objectives.

Figure 1. Study Flow Infographic



7. STUDY PROCEDURES AND CLINICAL REGISTRATIONS

7.1 Abbreviated periodontal health assessment.

Clinical data will be documented based on probing depth (PD), attachment level (AL), visible plaque index (VPI), gingival index (GI) and bleeding on probing (BOP). All clinical measurements will be conducted using a manual periodontal probe.

7.2 Gingival Bleeding Index

Bleeding on Probing (BOP): Absence or presence of bleeding after gentle probing will be recorded within 20 seconds of probing. All six sites of the all the teeth will be probed to determine BOP.

7.3 Visible Plaque Index
The surfaces of the study teeth will be scored and given either a "0" no plaque or "1" plaque assessment: 0 = No plaque; 1 = Visible Plaque

7.4 Loe and Silness Gingival Index (GI)
The surfaces of the study teeth will be evaluated using light air and a periodontal probe. A score is given based on the following scale: 0 = Normal gingiva; 1 = Mild inflammation, a slight change in colour, slight edema, no bleeding on probing; 2 = Moderate inflammation, moderate glazing, redness, bleeding on probing; 3 = Severe inflammation, marked redness and hypertrophy, ulceration, a tendency to spontaneous bleeding.

7.5 Gingival Crevicular Fluid (GCF) Samples
Before GCF collection, the plaque index will be recorded. The sites to be sampled will be isolated with cotton rolls and gently air-dried. GCF samples will be collected with sterile paper strips (Periopaper strips, Oraflow Inc.) that will be inserted into the gingival crevice until mild resistance is felt and left in place for 30 seconds. GCF will be collected from the mesiobuccal and mesiopalatal surfaces of teeth (#'s 3, 4, 5, 12, 13, 14). Paper strips visibly contaminated with saliva and blood will be excluded from the study. All samples will be collected in microcentrifuge tubes. They will be transported on ice and stored within 24 hours at -80ºC until processing. At our lab at Dental Sciences building, at University of Toronto, Faculty of Dentistry, these samples will undergo 40-plex chemokine analysis, oral neutrophil count and targeted proteomics

7.6 Sub-gingival Plaque Samples
Sub-gingival plaque will be collected using sterile paper points inserted in the gingival sulcus for 30 seconds from the mesiobuccal and mesiopalatal surfaces of teeth numbers 14, 15, 16, 24, 25, and 26). Plaque samples will be placed in microcentrifuge tubes then transported on ice to -80ºC freezer in our lab at Dental Sciences building, at University of Toronto, Faculty of Dentistry. There they will be processed and analyzed by shotgun metagenomics and targeted proteomics.

7.7 Saliva and Oral Rinse Samples
Saliva samples (5 ml) will be collected at all visits and placed on ice to -80ºC freezer. Samples will be sent our lab at Dental Sciences building, at University of Toronto, Faculty of Dentistry. There the saliva samples will be processed and undergo 16S rRNA sequencing and targeted proteomics analyses. The oral rinse samples will undergo oral neutrophil count.

7.8 Halitosis Measurement
The Halimeter PLUS is a specialized instrument designed and manufactured by Interscan Corporation to measure the concentration of volatile sulfur compounds (VSC), particularly hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, in a person's breath. These compounds are often associated with oral malodor, commonly known as bad breath. The Halimeter contains electrochemical sensors that detects the concentration of VSCs in breath. When a person exhales into the device, the sensor measures the levels of these sulfur compounds, providing a quantifiable reading. Elevated levels of VSCs indicate the presence of oral bacteria and potential oral health issues.

Since all research projects will be done on de-identified samples, it will not be possible to return individual results to participants. Unless participants ask to withdraw from the study, samples may be retained indefinitely. Because all research will be done on de-identified, aggregated data, it will not be possible to withdraw the data from a specific participant when samples are withdrawn from the study.

8.0 STATISICAL ANALYSES

Correlations between tissue and blood neutrophil subsets and standard periodontal disease parameters from oral exam will be assessed. Data will be complied in SPSS version 20. Changes in the clinical parameters of experimental gingivitis such as bleeding on probing, gingival index, plaque index and probing depths during day 0, 7, 14, 21, 28 and 35 will be compared using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison.

Oral neutrophil counts as well as neutrophil subset formation during day 0, 7, 14, 21, 28 and 35 in oral rinse samples as well as blood samples will be compared using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison.

Multiple sites within each study participant will be evaluated using a Gingival Bleeding Index (BOP) at baseline and 3-week post product use exams. The gingivitis bleeding scores will be averaged to provide a mean score per study participant for each exam period. Product means and standard deviations will be calculated for each time period. Analysis of Variance techniques (possibly including Analysis of Covariance using the baseline scores as the covariant) will be used to compare the difference between the two test treatments using the 95% confidence level ($p \leq 0.05$). The correlation of baseline to post-treatment results will be assessed graphically. If there is evidence of correlation, the baseline result will be included in the ANOVA model to adjust for baseline differences.

9.0 REPORT OF ADVERSE EVENTS

Study participants will be told of any possible adverse reactions or side effects from exposure to this method or product. Oral irritation is possible. If side effects occur, it is expected to be mild and temporary. Any event will stop when the study participant stops being exposed to the instrument or returns to their normal oral hygiene. At all examinations, each participant will be asked if they have experienced any discomfort or oral irritation. The investigators will record any and all adverse reactions and report this documentation to the Product Safety Assurance Department. The nature of the reaction and any correlation with product usage will be assessed. If the evidence indicates that the adverse reaction may be due to product usage, the study participant will be instructed to discontinue product use and appropriate treatment will be provided. In the event of an adverse experience, emergency or other problems or questions regarding your participation in this study participants can contact the following investigators:

Dr. Michael Golderg at (416) 979-4928 ext. 4408 or the Research Oversight and Compliance Office - Human Research Ethics Program at ethics.review@utoronto.ca or Tel: 416 946-3273
In the event of a medical emergency, please contact your physician

9.1 Definitions

Adverse Events (AEs) and Serious Adverse Events (SAEs) are defined by the ICH Guideline for Good Clinical Practice (ICH GCP) as follows:

Adverse Event: Any untoward medical occurrence in a study participant or clinical investigations study participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

AEs include any clinically significant deterioration of a study participant's medical status, after being enrolled and signing an Informed Consent Form. The AE may involve any organs or systems and can be represented by the new onset or the deterioration of a disease, a syndrome, a symptom, a physical sign, as well as by findings and results of instrumental examinations and laboratory tests. Any medically relevant and untoward change from study enrollment, including frequency or pattern changes for a fluctuating condition (e.g., migraine), occurring after signing an informed consent is an adverse event. All such occurrences must be recorded and reported accordingly, whether they appear causally related to the study medication, or not.

Serious Adverse Event: Any adverse event occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening adverse event
- Instudy participant hospitalization, or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Temporary loss of daily function

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the study participant or study participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of such important medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in instudy participant hospitalization, or the development of drug dependency or drug abuse.

IMPORTANT NOTES: The concepts of Adverse Event / Experience[1] (AE) and Serious Adverse Event / Experience (SAE) represent **regulatory** instruments used to evaluate and monitor the safety of clinical trial study participants. Therefore, these terms only apply in light of their regulatory definition. The term "serious", in a regulatory sense, does not necessarily mean "severe". All adverse events (serious and non-serious) reported during a study will be taken into account when analyzing the study data and establishing the safety profile of the investigational drug, and will be included in the final study report. The SAE concept is primarily used to identify,

during the conduct of the trial, those adverse events that may require an expedited reporting procedure to regulatory authorities.

Death: The outcome of death requires that the AE that resulted in death be reported as an SAE. Death, in and of itself, is not an AE; it is only an outcome. The cause of death is the AE; therefore, the investigator should make every effort to obtain and document the cause of death for all study participants who die during the study. If, despite all efforts, the cause of death remains unknown, the AE it should be documented as "unspecified fatal event".

Life-threatening Adverse Event: Any adverse event that places the study participant or study participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred (*i.e.,* it does not include reaction that had it occurred in a more severe form, might have caused death).

Hospitalization: It should be noted that hospitalization, in and of itself, does not represent an SAE. It is the adverse event leading to the study participant's hospitalization that becomes "serious" when it requires instudy participant care. Consequently, SAE should not be reported in case of pre-planned hospitalizations for pre-existing conditions that did not worsen during the study.

Disability: A substantial disruption of a person's ability to conduct normal life functions.

9.2 Documenting and Reporting Adverse Events

9.2.1 General Procedures for All Adverse Events

All clinical complaints, symptoms, or signs that meet the adverse event definition will be recorded on the Adverse Reaction Form (Appendix VIII) using a recognized medical term or diagnosis that accurately reflects the event. Source documentation should be maintained that allows for clear identification of each adverse event and the following parameters required for the form:

- AE description
- Date of onset
- Date of resolution
- Outcome
- Severity
- Seriousness
- Relationship to study drug (causality)
- Actions taken

Adverse events will be assessed by the investigator or designee for severity, relationship to the study product, possible etiologies, and whether the event meets the criteria as a serious adverse event and therefore requires immediate notification of the sponsor.

For data collection purposes, the outcome of all adverse events recorded on the Adverse Reaction Form will be designated as of the completion of the final evaluation or examination. However, the investigator is responsible for following all adverse events until resolution or until no longer of clinical concern, and providing these data to the sponsor.

9.2.2 Reporting Procedures for Serious Adverse Events

Any adverse event that is serious or potentially serious requires additional detailed reports and follow-up. A serious adverse event must be reported via telephone to the sponsor's representative immediately (within 24 hours) so as to facilitate discussion and implementation of necessary follow-up measures, and to enable the sponsor to submit necessary reports to regulatory authorities and other investigators. Following the initial telephone notification, the investigator must complete and submit a Serious Adverse Event Report Form to the sponsor within one calendar day. Serious Adverse Event
Report Forms will be provided to the investigator upon initiation of the study. Once the sponsor reviews the Serious Adverse Event Report Form, additional information may be requested from the investigator to allow appropriate medical evaluation and determine the regulatory reporting requirements.
The investigator is responsible for following all adverse events, especially those deemed "serious", until resolution or until the event is no longer of clinical concern, and for providing these data to the sponsor in an agreed-upon format. The investigator is also responsible for reporting all serious adverse events to the Institutional Review Board (IRB) overseeing the conduct of the study at the respective study center, according to the rules and procedures established by the IRB.

Safety will be evaluated by oral examinations, clinical laboratory evaluations, descriptive analysis of AEs (including incidence, severity, seriousness, andrelatedness), and immune response, over the entire period of the study.

9.2.3 Reporting Adverse Events to Health Canada

All Adverse Events will be reported to Health Canada as per the Natural Health Product Regulations. This report must be submitted to Health Canada by the researchers no later that 7 days after they have been notified. The online form is available at:

https://www.canada.ca/en/health-canada/services/drugs-health-products/medeffect-canada/adverse-reaction-reporting/natural-health-products.html

10. COMPENSATION

Participants of the study will receive monetary compensation of 1000 CAN$ after completion of all trial visits. In addition, they will receive 2 professional dental cleaning treatments prior and subsequent to stopping all oral hygiene procedures. They will also receive proper oral hygiene instructions and 3 kits for at-home daily hygiene during the trial.

11. QUALITY ASSURANCE/CONDUCT OF THE STUDY

This clinical research study will be conducted in compliance with this protocol and U.S. Federal Regulations governing informed consent (21 CFR 50), Institutional Review Board (21 CFR 56), applicable regulations governing Investigator conduct (21 CFR 312) and/or any local regulatory agency (where applicable).

It is the responsibility of the Investigator to ensure that all study participant data are collected and reported according to the study protocol. Study participant records will indicate study

participant and examination information such as visit dates, examiners, etc. that is unique to this study and the study participants. Proper documentation of all adverse events and final resolutions will be maintained. Case report forms will be used for recording all clinical data. All CRFs used in this study will be provided by the study sponsor. The Investigator will be responsible for maintaining original consent forms, case report forms (CRFs), and other source documentation.

These are examples of case report forms that can be used to collect information/data while conducting this study:

- Study participant Assignment Log (Randomization chart)
- Demographic Log
- Inclusion/Exclusion Criteria Form
- Continuance Criteria
- Adverse Event Form
- Oral Hard & Soft Tissue Exam Form

Forms may be added or deleted, if deemed necessary, by agreement between the PI (principal investigator) and study sponsor prior to the study start date.

12. DATA MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical research site staff under the direction of the Principle Investigator. The Investigator will keep a copy of every document (clinical and laboratory) related to the research study.

Data generated at the clinical site will be transferred to the sponsor by the Principal Investigator via mail or electronic email for statistical analyses. The principal Investigator and study manager are responsible for the review and interpretation of the analyzed data.

12.2 Study participant Termination/Procedures

A genuine effort will be made to determine the reason(s) why a study participant fails to return for the necessary visit(s) or is dropped from the study. Study participants could be dropped from the study if any of the following occur:

1. Study participant fails to substantially comply with the protocol requirements.
2. Study participant fails to report for a scheduled examination.
3. Study participant is treated with medications during the course of the study, which may interfere with the parameters of the study.
4. Study participant develops an adverse reaction. The study Investigator will immediately notify the study monitor and information will be recorded on Adverse Reaction Form.
5. Study participant receives emergency dental or medical treatment, which may interfere with the parameters of the study.
6. Sponsor elects to terminate the study.
7. Study participant elects to terminate participation in the study.

12.3 Removal of Study participants From Study

Study participants will be dropped from the study if they receive emergency dental treatment, which in the opinion of the monitor could influence the parameters of the study. Any study participant treated with an antibiotic during the study will be dropped from the study. Either the investigator or the sponsor may terminate the study at any time for well

documented reasons, provided written notice is submitted at a reasonable time in advance of the intended termination.

12.4 Pregnancy

No pregnant women will intentionally be enrolled in this study. All female study participants being considered for this study will be screened with a urine pregnancy test before the start of the trial. All enrolled female study participants will be required to report to the clinical/research investigator if they become pregnant during the course of the 9-week study. In the event a woman enrolled in this clinical research study becomes pregnant at any time during this study, participation in this study will be terminated upon the clinical staff's notification of the event. The study participant's medical records used in this study will be updated to reflect the pregnancy and there will be follow-up contact until the end of the pregnancy to record the outcome in the clinical file.

12.5 Ethical and Regulatory Requirements/Human Study participant Protection

Participants may opt out of the study at any time and request the destruction of all stored samples. Sample destruction and disposal will be completed and properly documented by Coriell. All samples and data will be relayed to researchers in the absence of identifying information; therefore, no personal information or results will be relayed back to participants. The testing done in this study is for research purposes only and not intended to make any medical diagnosis.

12.6 Protocol Approval, Study Monitoring and Compliance

Prior to initiation of the study, the Investigator will obtain approval from the UofT Health Sciences Research Ethics Board for the study protocol, the informed consent document, study instructions, and any forms of advertising in compliance with regulations. The reviewers will also review any change(s) in the protocol before the change is initiated.

12.7 Adherence to Protocol/Amendment(s)

The Investigator will be required to adhere to the final protocol. Any changes to the protocol, except those necessary to eliminate apparent hazards, will require prior approval by the local reviewers through the submission of a protocol amendment. These changes to the protocol must be implemented only through formal written protocol amendments and only upon joint approval by the sponsor and investigator. If a protocol amendment requires changes to the informed consent form, the revised consent form must also be approved by any local board.

Departures from eligibility requirements may be allowed on a case-by-case basis by the medical monitor or other authorized sponsor representative. Such departures must be medically and scientifically justified, must be pre-authorized, and must be documented in the case report form and tracked as official eligibility waivers.

12.8 Institutional Review Board

Prior to initiation of the study, the Investigator will obtain Institutional Review Board approval for the study protocol, the informed consent document, study instructions, and any forms of advertising in compliance with regulations. The IRB will review the investigation at least once a year and will review any significant change in the protocol before the change is initiated. The Investigator will maintain all original correspondence. IRB approval for this study will be obtained

from the U of T Health Sciences Research Ethics Board Tel: +1 416 946-3273 ethics.review@utoronto.ca

12.9 Advertising

No newspaper, radio, or television advertising will be used for recruitment purposes. A flyer was created to recruit participants and will be approved by an Institutional Review Board/Ethics Board prior to posting or dissemination (Appendix 1).

12.10 Informed Consent Process

Written informed consent will be obtained from all study participants prior to their enrollment into the study. The purpose and description of the study in lay language, possible adverse reactions, risks and benefits of participation and the study participant's right to withdraw without prejudice at any time must be explained to each study participant in the presence of a witness. The study participant must read, understand and sign the informed consent form provided (Appendix 10)/The informed consent form and any other written information for study participants should meet local requirements of language and interpretation (i.e., non-English speaking study participants must be presented with informed consent forms in a language that they can understand). The consent form will comply with all applicable regulations governing protection of the participating study participants in the study, and include basic elements specified in the U. S. Code of Federal Regulations, 21 CFR 50.25(a) and 50.27 and ICH-GCPs, Chapter 4, subpart 4.8. The Informed Consent form will be reviewed and approved by the clinical site's IRB.

Each study participant will be given unlimited time to read the consent form and ask questions. Study participants who agree to participate will be asked to sign and date an IRB-approved informed consent form. A copy of the signed and dated consent form will be given to each study participant prior to their participation in the study. The original signed and dated informed consent document will be retained by the Investigator. All informed consent forms will be documented in a log by date and study participant ID; the log will be kept as source documentation. All study procedures must be explained in non-technical terms. Study personnel will assure that participants are clearly informed regarding their roles and obligations to protect vulnerable study participants and ensure they are not under coercion or undue influence. Study participants have the right to withdraw consent at any time.

12.11 Confidentiality

All records of study participant participation in this study are confidential and these records are available only to the investigator, specifically trained site personnel, supervising dentist/examiner, potentially the sponsoring company and Ethics/Institutional Review Board (IRB). In addition, the identity of participating study participants must be protected.

Only investigators (and specifically trained clinical site staff) will collect and have access to a study participant's private information (e.g., name, medical records, etc.). Investigators will assign a study number to study participants which will be used to conceal their identity on all case report forms and other documents prior to their sharing with the broader study team, including the sponsor. Documents that identify the study participant by name (e.g., the signed informed consent form or health questionnaire) will not be transferred or submitted to the sponsor and will be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing

by the sponsor personnel to verify study participant, product safety and study compliance. The results of the study may be published in a scientific journal or a government public clinical database. If any publication occurs, only the study participant's study number/ID, gender and/or age may be used.

12.12 New Findings

Study participants will be informed of any significant new findings related to study products or procedures when they become known during the course of this clinical research study. Such information may affect the study participant's decision to continue participation in the study.

ADMINISTRATIVE ASPECTS

12.13 Curriculum Vitae

The investigator will complete the FDA form 1572 and provide the sponsor with copies of his/her curriculum vitae and those of all sub-investigators listed on the form, at sponsor's request.

12.14 Data Collection in the Case Report Form

All study data will be recorded in the case report form supplied by the sponsor. All entries will be written clearly in black ink. Only the principal investigator, sub-investigators, or study coordinators may make entries in the case report forms. If erroneous data are entered on the case report forms, corrections to the data must be made by crossing out the incorrect entry with a single line (such that the initial entry remains legible) and entering the correction. All corrections on a case report form will be initialed and dated by the investigator, sub-investigator, or study coordinator making the correction.

12.15 Documentation of Consent and Storage of Study Documents

All informed consent forms will be documented in a log by date and study participant ID; the log will be kept as source documentation. Informed consent forms will be stored in a secure locked room designated for research charts storage in the clinical site. The Investigator will provide each study participant with a clear and understandable consent letter regarding the processing in connection with the study of personal data (i.e., any information relating to an identified or identifiable individual) by the investigator, sponsor and other persons involved in the study (Appendix II). Each study participant will be given a copy of the consent letter/form which will be referenced as part of the informed consent process.

Any deviation to the consent letter as regards processing by the sponsor (including its vendors, monitors and other representatives) must be agreed with the sponsor. In addition, the Investigator is responsible for ensuring that all study study participants understand and complete the consent letter/form, and that it includes any local requirements regarding data security and privacy laws and regulations applicable to the study and the study participants. Should the consent letter/form fail to meet any applicable local requirements, the Investigator is responsible for amending the consent letter/form to bring it into compliance with local applicable regulations and the Investigator (including Principal and sub-Investigators) agrees to indemnify and be liable to the Sponsor for any damages resulting from such non-compliance. Study participants' study charts will be stored in a secure locked room designated for research charts storage in the clinical site. The chart room is a limited- access area and only delegated study personnel will have access to the study charts and study participants' data.

12.16 Study Management
Under the direct supervision of the Principal Investigator, certain duties may be delegated during the course of the study. These responsibilities will be documented on the Transfer of Responsibilities form maintained in the Investigator's clinical file for the study.

12.17 Study Monitoring
Appropriate phone calls or visits will be made by the monitor to the investigative site in order to review the protocol and to ascertain that the facility is adequate for satisfactory conduct of the study, as well as to discuss the obligations of both the sponsor and the investigator.

The investigator will permit a representative of the sponsor or the sponsor's designate and the local regulatory authorities, if requested, to inspect all case report forms and corresponding portions of the study study participants' original office, hospital and/or clinic medical records at regular intervals throughout the study. These inspections are for the purpose of assessing the progress of the study, verifying adherence to the protocol, determining the completeness and exactness of the data being entered on the case report forms and assessing the status of study drug storage and accountability. During on-site visits, case report forms will be examined by the study monitor(s) and the data verified by comparison with corresponding source data (such as clinic, hospital and/or office records). Due to the geographic distance of the clinical site from the sponsor, phone conferences or teleconferences may be used to complete the monitoring requirement.

12.18 Final Report
Following the completion of the study, the Investigator shall prepare a final study report. The final report will include a general description of the conduct of the study including protocol deviations, study participant withdrawals, discussion of any adverse events, safety and efficacy data, laboratory data, and statistical analysis of the data if available. This report will be approved and signed by the Principal Investigator.

12.19 Record Retention and Access to Source Data/Documents
Source documents must be kept for at least fifteen (15) years after terminating the study. The Investigator will maintain all study documentation for all study participants entered into the study in a secure area ensuring the confidentiality of the information collected. Securing records includes placing written forms in locked file cabinets and/or sealed and labeled storage boxes in a locked room. Access will be denied to all persons with the exception of the Principal Investigator and his designees. The Sponsor will be notified before any destruction of study documents occurs.

12.20 Publication
All manuscripts or presentations based upon this study, including press statements and internal public notices and memoranda must be submitted to the sponsor for review and approval prior to release for publication or presentation. This review period will be up to 60 days in duration. Review of an abstract may be expedited in some circumstances.

13. References

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APPENDICES

Appendix 1. Table Clinical Trials Utilizing S. Salivarius

Study Design	Population	Dose and Duration	Safety-Related Outcomes	Reference
Prospective, randomized, single-blind, placebo-controlled pilot study	Children (M & F; 6 to 11 y) who had at least 3 episodes of microbiologically documented infections with clinical symptoms suggesting Group A beta-hemolytic Streptococcus (GABHS) pharyngitis. Initial n = 84 Final n = 82	**Test:** *S. salivarius* 24SMB and *S. oralis*. The mix suspension consisted of a minimum of 125 × 10^9 (CFU/mL) in 10 mL of saline. **Control:** matched placebo 2 puffs once a day, with an oral spray that provided 2 × 10^9 CFU per puff. For 3 months	All study participants were compliant to the assigned intervention to them (> 80% compliance). Only four study participants reported an adverse event—three in the probiotic group and one in the control group. A mild cough was reported by two study participants in the probiotic group versus one study participant in the control group. Only one study participant in the probiotic group reported nausea.	(Andaloro et al., 2019)
Double-blind, randomized, two-arm parallel-group	Healthy adults (M & F; 20 to 24 y) Initial n = 31 Final n = 30	**Test:** *S. salivarius* K12 (>1 x 10^9 CFU in 1 tablet) **Control:** matched placebo lozenge 1 lozenge per day For 4 weeks	One participant from the probiotic group was lost in follow-up due to sickness not related to the intervention (COVID-19). No adverse effects were registered.	(Babina et al., 2022)
Randomized, open-label, controlled, parallel	Children (M & F; 4 to 10 y) with black teeth stains initial n = 58 (29/group) final n = 54	**Test:** no less than 1×10^9 CFU/day **Control:** no intervention was administered to the	Four participants (one in the test group and three in the	(Bardellini et al., 2020)

		control group For 3 months	control group) were excluded from the study because they started antibiotic therapy. However, the study authors did not report any adverse events observed by the participants.	
Randomized, triple-blind, placebo-controlled, parallel	Participants (M & F; 10 to 30 y) wearing orthodontic braces initial n = final n = 64	**Test:** 7.2×10^9 CFU/day **Control:** matched placebo lozenge For 1 month	According to the study authors, no adverse events were observed or recorded during the trial.	(Benic et al., 2019)
Randomized, double-blind, placebo controlled	Healthy adults performing regular exercise training (M & F; 20 to 25 y) Initial n = 24 Final n = 20	**Test:** $5x10^9$ CFU/day **Control:** matched placebo tablet For 30 days	The study authors did not report any adverse events observed by the participants. Four study participants abandoned the study for personal reasons. The authors showed an adherence rate of 94%.	(Bertucciol i et al., 2023)
Randomized, parallel. Participants were blinded. Blinding of investigators was not reported.	Healthy adults (18 y and older; average age 19 y; gender not reported) initial n = 75 final n = not reported	**Test 1:** 1×10^6 CFU/day **Test 2:** $1x10^7$ CFU/day **Test 3:** $1x10^8$ CFU/day **Test 4:** $1x10^9$ CFU/day For 28 days	The study authors did not provide information on any adverse events that may have been experienced by the study participants.	(Burton, Wescombe et al., 2013)
Randomized, double-blind, placebo controlled, parallel	Children (M & F; 5 to 10 y) with a history of dental caries initial n = 100 final n = 83	**Test:** 7.2×10^9 CFU/day **Control:** matched placebo lozenge For 3 months	11 participants withdrew for various reasons, including not liking the taste of the lozenges	(Burton et al., 2013)

			(n=6), protocol deviations (n=1), and being lost to follow-up (n=4). In addition, data from six participants were excluded from the analysis because they did not comply with the study requirements, consuming less than 75% of the prescribed lozenges per month.	
Open-label, single-arm	Healthy adults (M & F) n = 14	4×10^9 CFU/day, 3 days	No adverse effects	(Burton et al., 2006)
Open-label, observational	Healthy adults (M & F) n = 23	**Test:** $>1\times10^9$ CFU/lozenge **Control:** placebo lozenge 3-day regimen of CHX rinsing, followed by intake of lozenges (test, control) at 2h intervals over 8h for 3 days ($>4\times10^9$ CFU/day). Subsequently, study participants in the test group (n=13) took the lozenge twice daily (morning & night) for 2 weeks. Two of these study participants continued to take 2 lozenges/day for 28 days ($>2\times10^9$ CFU/day). For 3 days, 2 weeks (test group only), 28 days (2 study participants in test group only)	The study authors did not report any adverse events observed by the participants.	(Burton, Chilcott et al., 2006)

Randomized, parallel Blinding not reported	Healthy adults (M & F; mean age 19 y) initial n = 100 final n was not reported	**Test 1:** 1.5×10^{9} CFU/day **Test 2:** 1.1×10^{8} CFU/day **Test 3:** 2×10^{7} CFU/day **Test 4:** 1×10^{6} CFU/day **Test 5:** 7.5×10^{4} CFU/day for 14 days	The participants did not report any adverse reactions.	(Burton et al., 2010)
Randomized, double-blind, placebo controlled, parallel	Healthy adults (M & F; age 20 to 60 y) initial n = 56 final n = 53	**Test:** 1.1×10^{10} CFU/day **Control:** matched placebo For 28 days	There was no significant difference found between the test and control groups in terms of oral health and gastrointestinal symptoms assessed using a 10-point VAS. No serious adverse events occurred in either group and the proportion of participants reporting any adverse events was similar in both groups. Hematology and clinical chemistry parameters showed no significant difference between the groups. However, the specific gravity of the urine was slightly higher in the placebo group compared to the test group, but still within normal limits.	(Burton et al., 2011)

Double-blind, randomized placebo-control, prospective trial	Healthy adults (M & F; age 21 to 45 y) who practice good oral hygine Initial n = 25 Final n = 24	**Test:** powder sachet (1.5 g) contained approximately 7.77 x 10^9 CFU of *L. acidophilus* DDS-1®, 8.25 x 10^9 CFU of *B. lactis* UABla-12, and 2 x10^9 CFU of *S. salivarius* BLIS K12™ **Control:** matched placebo one daily dose (one 1.5 g sachet) of probiotic powder mixed with any liquid. For 2 weeks	The authors found that the treatment was well tolerated by the study population except for one participant who experienced hives during supplementation which disappeared when supplementation ceased. All other participants were compliant. The overall attrition rate for this study was 4%.	(Cernioglo et al., 2021)
Randomized, double-blind, placebo controlled, parallel	Infants (M & F, 7 to 13 months) with high risk of acute otitis media initial n = 224 final n = 202 by 2-month visit, 166 by 12-month visit	**Test:** follow-up formula containing proB (*S. thermophilus* NCC 2496, *S. salivarius* DSM 13084 [K12], *L. rhamnosus* LPR CGMCC 1.3724) and preB [Raftilose/Raftiline]) **Control:** follow-up formula only Formula contained 2.5×10^7 CFU/g *S. salivarius* (1×10^9 to 2×10^9 CFU/day according to GRN 807). For 12 months	The study authors reported that both the test and control formulas were well-tolerated. The main reason for discontinuation was non-compliance with the study protocol, specifically, missing three consecutive days per month with less than 300 mL of milk consumed per day. The majority of the reported adverse events (93.1%) were not considered related to the study. There were five adverse events considered	(Cohen et al., 2013)

			likely related, with four in the test group and one in the control group. These included lack of appetite for milk, regurgitation, dry skin, chronic diarrhea, and abdominal pain. One adverse event, constipation, was considered related, but no further details were provided.	
Open-label Study was not randomized and not blinded.	Healthy study participants (M & F; 19 to 64 y) Initial n = Final n = 23	**Test:** *Streptococcus salivarius* 24SMBc and *Streptococcus oralis* 89a nasal spray (10^9 CFU/dose) two bilateral spray injections into each anterior nostril/day For 7 days.	No severe side effects were recorded in any enrolled study participants after the treatment. Only 10% of the study participant suffered from allergic cold, and 80% did not show any problems breathing. However, 90% of people reported nasal dripping immediately after the treatment administration.	(De Grandi et al., 2019)
Open-label Study was randomized but not blinded.	Children (M & F; 6 to 17 y) at high risk for dental caries initial n = final n = 76	**Test:** no less than 1×10^9 CFU/day **Control:** no intervention was administered to the control group For 90 days	No participants withdrew. *S. salivarius* M18 was found to be safe with no treatment-related side effects, and no participants dropped out. The majority	(Di Pierro, Zanvit et al., 2015)

			of participants (35 out of 38) rated the tolerability of the treatment as "good" or "very good," and the remaining three participants rated it as "acceptable."	
Open-label Study was not randomized and not blinded.	Children (M & F; 3 to 12 y) with and without recurrent streptococcal pharyngitis and/or tonsillitis initial n = 82 final n = 78	**Test:** 5×10^9 CFU/day **Control 1:** no intervention was administered to controls with recurrent illness **Control 2:** no intervention was administered to controls without recurrent illness For 90 days	The test tablet was reported to be well-tolerated, and there were no observed side effects. However, four participants in the test group were excluded from the analysis due to non-adherence to the study protocol, specifically, missing over 20 days of treatment.	(Di Pierro et al., 2012)
Open-label Study was not randomized and not blinded.	Adults (M & F; 18 to 65 y) with recurrent oral streptococcal pharyngitis initial n = final n = 40	**Test:** 5×10^9 CFU/day **Control:** no intervention was administered to the control group For 90 days	All 20 study participants who received the test tablets successfully completed the study, and no participants dropped out. The study authors reported that the test tablet was well tolerated, and no side effects related to the treatment were reported.	(Di Pierro et al., 2013)
Open-label Study was randomized	Children (M & F; 3 to 13 y) with recurrent oral streptococcal disorders	**Test:** no less than 1×10^9 CFU/day **Control:** no intervention was	The study authors reported that the test tablet	(Di Pierro et al., 2014)

but not blinded.	initial n = 61 final n = 60	administered to the control group For 90 days	was well tolerated and did not cause any notable side effects. However, one study participant dropped out of the study immediately after enrolment due to the poor taste of the test product.	
Open-label, single-arm	Children (M & F; 3 to 9 y) with recurrent secretory otitis media initial n = final n = 22	**Test:** no less than 1×10^9 CFU/day For 90 days	The safety profile of *S. salivarius* K12 was reported as very good, with no treatment-related side effects and no study participant drop out. Tolerability was rated as good or very good in 20 of the 22 study participants, with the remaining 2 study participants rating it as acceptable.	(Di Pierro, Di Pasquale et al., 2015)
Open-label Study was randomized but not blinded.	Children (M & F; 3 to 10 y) with recurrent streptococcal pharyngotonsillitis initial n = final n = 124	**Test:** no less than 1×10^9 CFU/day **Control:** no intervention was administered to the control group For 90 days	According to the study authors, the use of *S. salivarius* K12 was well-tolerated and highly compliant among the participants, and there were no reported side effects observed during the trial.	(Di Pierro, Colombo et al., 2016b)

Open-label Study was randomized but not blinded.	Healthy children (M & F; 33 to 45 months) initial n = final n = 222	**Test:** no less than 1×10^9 CFU/day **Control:** no intervention was administered to the control group For 180 days	All children who were enrolled in the study completed it. The study authors noted that there were no observable side effects in the treatment group, both during the treatment period and the follow-up period.	(Di Pierro, Colombo et al., 2016a)
Retrospective observational	Children (M & F; 3 to 14 y) with recurrent non-streptococcal infection n = 133	**Test:** no less than 1×10^9 CFU/day For 90 days	The study authors reported that compliance and tolerability were excellent. They also noted only one side effect, which occurred in a 6-year-old boy who experienced a mild episode of bronchospasm after a few days of treatment with S. salivarius K12. However, the study participant was able to continue with the study without any further incidents.	(Di Pierro et al., 2018)
Randomized, placebo controlled, parallel Blinding not reported	Children at high risk of acute rheumatic fever (M & F; 5 to 14 y) initial n = 1314 final n = 1137	**Test:** 2.5×10^9 CFU/day **Control:** matched placebo lozenge (*b) 209 days	The study authors did not provide any information about adverse events experienced by the participants. However, they reported that	(Doyle et al., 2018)

			most children found the lozenges to be well accepted, with only two children refusing to take them regularly.	
Retrospective observational	Children (M & F; 3 to 7 y) with recurrent group A beta-hemolytic streptococci pharyngo-tonsillar infections n = 130	**Test:** 1×10^9 CFU/day **Control:** no intervention was administered to the control group For 90 days	All children were able to complete the study without stopping the test tablet before the intervention period ended.	(Gregori et al., 2016)
Randomized, double-blind, placebo controlled, parallel	Adults (M & F; 18 y and older) with severe acute pharyngotonsillitis initial n = 60 final n = 53	**Test:** 4×10^9 CFU/day **Control:** matched placebo tablet 10 days	Seven participants were excluded from the study due to noncompliance with the treatment, but the authors did not mention if any adverse events were observed by the participants.	(Gilbey et al., 2015)
Randomized, double-blind, placebo controlled, parallel	Adults (M & F; 23 to 44 y) with tongue-coating associated halitosis initial n = 33 final n = 28	**Test:** 2×10^9 CFU/day **Control:** matched placebo tablet For 30 days	All participants in the study did not experience any adverse events. However, five participants were excluded from the study, with two participants in the control group and three in the test group, one of which was using antibiotics, and four were lost to follow-up.	(He et al., 2020)
Not applicable (single study participant)	Single healthy adult (M), 40 y old	4×10^{10} CFU/day For 3 days	No adverse events were reported	(Horz et al., 2007)

Randomized, double-blind, placebo controlled, parallel	Adults (M & F; >18 y) with oral candidiasis initial n = 56 final n = 49 (safety-analyses)	**Test:** ≥2×10^9 CFU/day **Control:** matched placebo lozenge For 4 weeks	The study did not report any severe adverse events. However, AEs were reported by 6 study participants in the test group and 8 study participants in the control group. One study participant in the K12 group reported borborygmus and pharyngeal discomfort, which was considered a possible drug-related adverse event by the study authors.	(Hu et al., 2019)
Randomized, double-blind, placebo controlled, parallel	Adults (M & F: 18 y and older) with spondyloarthritis initial n = final n = 63	**Test:** powder containing 1×10^8 CFU/g of *S. salivarius* K12, 4×10^8 CFU/g of *B. lactis* LAFTI B94, and 4×10^8 CFU/g of *L. acidophilus* LAFTI L10 **Control:** matched placebo powder Participants were told to take 1 spoonful of powder (ca. 0.8 g) by mouth twice daily, corresponding to ca. 1.6×10^8 CFU/day of *S. salivarius* K12 For 12 weeks	All participants in the study completed it successfully. Minor and self-limiting adverse events were reported by 43.8% (14/32) of the test group and 38.7% (12/31) of the placebo group, with no significant difference between them. The most common AE in both groups was a change in bowel habit, reported by 7 participants in the test group and 6 in the placebo group. No serious AEs occurred	(Jenks et al., 2010)

			during the study. At the end of the study, there was no significant difference between the test and control groups in terms of fecal calprotectin or change in bowel symptom questionnaire scores.	
Double-blinded, placebo controlled human clinical trial	Healthy adults (M & F; 18 to 75 y) Initial n = 60 Final n = 53	**Test:** 1 x 10^{10} CFU/g BLIS K12 **Control:** matched placebo single 1 g dose mixed with 30 mL water and swallowed directly. For 1 day	The dose chosen for use in this study was relatively high, but safe, dose to administer. No adverse reactions were reported by the authors.	(Laws et al., 2021)
Open-label, single-arm	Study participants with rheumatoid arthritis taking stable doses of sulfasalazine (M & F; mean age = 56 y) initial n = final n = 12	A powder blend (BioRestore™) containing *S. salivarius* K12 at 1×10^8 CFU, *L. acidophilus* L10 at 4×10^8 CFU, *B. lactis* B94 at 4×10^9 CFU. The powder was taken twice a day for total *S. salivarius* K12 of 2×10^8 CFU/day. For 7 days	During the intervention period, four study participants reported adverse events (AEs), with three reporting gastrointestinal disturbances and one experiencing a flare-up of rheumatoid arthritis. The reported AEs were of mild to moderate severity.	(Lee et al., 2010)
Randomized, non-blinded, controlled, parallel	Adults with oral lichen planus (M & F; 22 to 79 y) initial n = final n = 40	**Test:** no less than 2×10^9 CFU/day **Comparator:** topical 0.1% triamcinolone acetonide dental paste	No adverse reactions were observed.	(Li et al., 2020)

		4 weeks		
Randomized, double-blind, placebo-controlled study	Healthy adults (M & F; 18 to 65 y) Initial n = 64 Final n = 60	Test: *S. salivarius* DB-B5 at 10^9 CFU Control: matched placebo 1 sachet in approximately 4 ounces of bottled water daily For 4 weeks.	The safety was confirmed. 4 individuals did not complete the study: One participant in the test group discontinued from the study because they changed their mind about participation. The remaining 3 participants were from the placebo group, and they were discontinued from the study for the following reasons: physician decision due to elevated eosinophils in the baseline blood sample (n = 1), occurrence of adverse event (n = 1), and lost to follow-up (n = 1). There was a total of 15 adverse events reported in 6 participants in the study. In the test group, 2 participants reported a total of 5 adverse events throughout the study that were considered "possibly related" to the interventions. All the events were mild in	(Li et al., 2021)

			nature and resolved on their own. Moreover, a similar frequency of adverse events was reported in the placebo group, in which 4 participants reported a total of 10 adverse events, including one individual discontinued from the study due to the occurrence of mild urticaria.	
A prospective, single-open, multi-centre study	Caucasian children with recurrent respiratory infections (RRIs) (M & F; 1 to 12 y) Inicial n = 100 Final n = 91	**Test:** *Streptococcus salivarius* 24SMBc and *Streptococcus oralis* 89a nasal spray (10^9 CFU/dose) 2 puffs for nostril twice/day for 7 days/months. For 3 months.	Although a good tolerability profile was reported, 9 children experienced burning nose, leading to interruption of therapy. However, none of the children were withdrawn from the study because of adverse events. The authors suggest that this treatment is safe and seems to be effective on short-term in the treatment of RRIs	(Manti et al., 2020)
Open-label Study was randomized. Blinding not reported.	Adults (M & F; 67 to 83 y) who are denture wearers. initial n = final n = 50	**Test:** BactoBlis™ containing *S. salivarius* K12 (10^9 CFU/day) **Control:** no intervention was administered to the	The study authors did not provide information on whether any adverse events were reported	(Passariello et al., 2020)

		control group For 30 days	by the study participants.	
Open-label, single-arm	Infants (age and sex not reported) prone to otitis media scheduled to undergo ventilation tube placement n = 19	**Test:** powdered formulation with *S. salivarius* K12 (reported as 1×10^{10} to 3.4×10^{10} CFU/day in GRN 581) 1 teaspoon was placed on the child's tongue twice daily. For 10 days	The study authors did not provide information on whether any adverse events were reported by the study participants.	(Power et al., 2008)
Open-label Study was randomized. Microbiological analyses were blinded.	Children (M & F; 1 to 6 y) attending daycare centers initial n = 121 final n reported as number of biological samples collected at 1-month and 2-month time period	**Test (children ≤3y old):** powdered formulation with *S. salivarius* K12 (5×10^9 CFU/sachet) **Test (older children):** Chewable tablet containing *S. salivarius* K12 (1×10^9 CFU/tablet) **Control:** no intervention was administered to the control group Daily dose provided was 5×10^9 CFU/day (powder) and 1×10^9 CFU/day (tablet). For 30 days	The administration of *S. salivarius* K12 did not cause any changes in the diversity of the nasopharyngeal or saliva microbiome. However, there was a temporary increase in the proportion of S. salivarius in the saliva of children who received the product containing S. salivarius K12.	(Sarlin et al., 2021)
Randomized, double-blind, placebo controlled	Adults (M & F; mean age 53.5 y in placebo, 53.3 y in test) who had received radiotherapy in the previous 6 months initial n = 17 final n = 13	**Test:** 3.5×10^9 CFU/day **Control:** matched placebo lozenge For 4 weeks	Four participants dropped out of the study: three from the placebo group and one from the test group. Two participants were lost to follow-up, one received	(Vesty et al., 2020)

			antibiotics, and one did not take the lozenges as instructed. The study authors did not mention whether any adverse events were reported by the participants.	
randomised double-blind				

Appendix 2. Recruitment Flyer/Advertisement

NOVO PROBIOTICS SALI-10 FOR THE **PREVENTION OF GINGIVITIS**: A CLINICAL TRIAL

Why are the investigators doing this?

Gum disease is a common condition in the mouth. This study is looking to see if using probiotics can help prevent gum disease in adults.

Who can participate?

Healthy adults (male/female) who don't have gum disease (gingivitis/periodontitis).

What participants must do?

Take the lozenges (tablets) provided daily. Participants will either have the probiotic or placebo lozenges. Study volunteers will come once a week (for a total of 7 weeks) to the Faculty of Dentistry for a clinical exam and biospecimen collection (saliva, dental plaque). Two weeks into this clinical trial, participants will refrain from oral hygiene (brushing + flossing) for three weeks. They will also get a **free professional dental cleaning (scaling + prophylaxis) twice**: at the beginning of the study and after the three weeks of oral hygiene abstinence.

Where will this study take place?

Faculty of Dentistry, University of Toronto

Will I be compensated?

Yes! Financial compensation of 1000$ will be provided for those who complete the clinical trial!

How do I sign up?

Please email us at martin.lambert@mail.utoronto.ca expressing your interest in taking part in this study*

Sostia Sciences inc.
Microbiome-derived Biotherapeutics

*No personal information should be shared via email

Appendix 3. Recruitment Questionnaire

Recruitment Questionnaire

1. Name _____

2. Age (in years) _____

3. Profession _____

4. **Oral Hygiene Habits:**
   Do participant brush your teeth? Yes No
   If so, how many times a day? _____
   Do participant use dental floss? Yes No
   If so how often? _____

5. **Dental Visit History:**
   Have participant visited a dentist before? Yes No
   When was your last visit to the dentist (if applicable)? _____
   At your last dental visit what was done (if applicable)? _____

6. **Smoking History:**
   Do participant smoke cigarettes? Yes No
   Do participant use any other tobacco product(s)? Yes No
   Do participant smoke anything other than cigarettes? Yes No

7. **General Health:**
   Do participant suffer from any major illness? Yes No
   If so what are they? _____
   Do participant take any medications? Yes No
   If so what are they? _____
   Do participant have any allergies?
   If so what are they? _____

8. Are all your teeth missing? Yes No

9. Do participant wear a complete denture? Yes No

10. Do participant wear a partial denture? Yes No

11. Are participant pregnant (if applicable)? Yes No

12. Are participant currently nursing (if applicable)? Yes No

13. Are participant currently using a method of birth control? Yes No

14. English Language Proficiency:
Do participant require English translation? Yes No
Are participant able to read in English? Yes No

15. Participants in this trial will be asked to be available for 8 visits over a 7-week span. Do participant think participant will be able to follow through with such a request? Yes No

16. Participants in this trial will be asked not to practice any kind of oral hygiene for a 3-weeks. This will include no tooth brushing, no mouth rinse, no dental floss or any other oral hygiene procedures. Do participant think participant will be able to follow through with such a request? Yes No

17. To those who are found eligible for the initial screening visit please be aware that in the case participant are not eligible to participate in the trial, participant will not be compensated for the screening visit. However, a full intra-oral examination* will be performed during that visit. Do participant agree to this? Yes No

    * Intra-oral examination will include:
        - assessment of soft tissues (including lips, gums, gi, hard palate, soft palate, back of the throat, tongue, and floor of the mouth),
        - evaluation of gum health via measure gum pockets
        - and dental assessment to evaluate the condition of the teeth, filling, crowns, bridges and/or implants

Eligible for the initial screening visit? Check One	
YES ☐	**NO** ☐ **Reason(s) (violation of inclusion/exclusion criteria):**

If **YES**, scheduled date of initial screen (Month/Day/Year): _____

Name of Assessor: _____

Signature of Assessor: _____

Date: _____

Appendix 4. Personal Information Form

Participants Trial Number
Office use only

Personal Information

Surname	First Name	
Middle Name	Date of birth	Male ☐ Female ☐
Occupation		

Mailing Address

Address (#, street, unit)			
City	Province	Postal Code	Country

Phone Numbers/E-mail Address

Home Phone	Cell Phone
E-mail Address	

** This form will be filled out once participants have consented to participate in this study.*

Appendix 5. Health Questionnaire

Confidential Medical History

Health Questionnaire Form

Study Participant ID No…….....…

Before beginning any study, the investigators need participant to complete and return this form.

Please tick the appropriate response

A yes answer does not necessarily mean participant will not be able to do the study.

1. Are participant attending or receiving treatment from a doctor? ..... YES___NO___

2. Are participant taking or using any medicines, pills, tablets, ointments, injections or any other drug, either from your doctor or on your own accord?.....

   YES___NO___

3. Are participant allergic to or have participant ever had any unfavorable reaction to any medicine, food or any other substance?.....

   YES___NO___

4. Have participant had any serious illnesses as a child or

adult? .....YES___NO___

5. Have participant ever been a hospital in-patient or ill at home for a long period? ...

   YES___NO___

6. Do participant have or have participant ever had any heart or blood pressure problems? .....

YES___NO___

7. Have participant ever had rheumatic fever or chorea (St. Vitus` dance)? .....

   YES___NO___

8. Do participant have a heart murmur? .....

   YES___NO___

9. Do participant have a heart pacemaker? .....

   YES___NO___

10. Have participant ever had any heart surgery? .....

    YES___NO___

11. Do participant have any chest or breathing problems? .....

    YES___NO___

12. Do participant suffer from eczema, asthma or any form of allergy? .....

YES___NO___

13. Do participant suffer from fainting attacks, fits or seizures? .....

YES___NO___

14. Have participant ever suffered from hepatitis, jaundice, liver or kidney

disease? ….....YES___NO___

15. Are participant diabetic? .....

..... YES___NO___

16. Have participant had any problems arising from a blood sample, a blood donation or transfusion……..YES___

NO___

17. Do participant carry a warning card from your doctor or specialist? .....

YES___NO___

18. Is there anything else concerning your health, such as a joint replacement, participant think the investigators should know

about?..... YES___NO___

19. Following extraction, surgery or injury have participant or any other member of your family bled for such a time as to cause participant to be worried?.....

YES___NO___

20. Do participant suffer from a dry mouth when eating food i.e. do participant have to drink liquids to swallow easily?.....

YES___NO___

21. Do participant currently suffer from an active infections, or are experiencing nausea, fever, vomiting, bloody diarrhoea or severe abdominal pain?.....
YES___NO___

Please inform us immediately if there is any change in this information

To the best of my knowledge this information is correct. I understand this information may be inspected by authorized personnel and will be treated in strict confidence

Please sign Here …….. **Date …..……**

Checked by.....…… **Date ……**

Confidential Medical History Continued

Please write information on questions overleaf in the box below:

Question Number	Medication	Description	Study participant Initials	Date

Medical History Review Record

Visit	Date	Any change in MH- details	Staff to initial and date	Study participant to initial and date	Study Dentist to sign and date *

Appendix 6. Screening Oral Assessment Form

Screening Oral Assessment Form

Study participant ID No: _____

Male ☐ **Female** ☐

***Only if there has been a change in medical history**

Visit Number: _____

Tick the appropriate box

	AREA	NORMAL	ABNORMAL
1.	Perioral area/lips	☐	☐
2.	Buccal mucosa	☐	☐
3.	Labial mucosa	☐	☐
4.	Sublingual mucosa	☐	☐
5.	Gingiva free/attached	☐	☐
6.	Tongue	☐	☐
7.	Palate hard/soft	☐	☐
8.	Uvula	☐	☐
9.	Oropharynx	☐	☐
10.	All other soft/hard tissues	☐	☐

DESCRIBE ANY IRREGULARITIES:

_____

_____

_____

Name of Assessor: _____

Signature of Assessor: _____

Date: _____

Appendix 7. Oral Assessment Form

Oral Assessment Form

Study Participant ID No: _____

Visit Number: _____

Male ☐ **Female** ☐

**Changes in the medical history or changes in the oral examination will be recorded.*

Tick the appropriate box

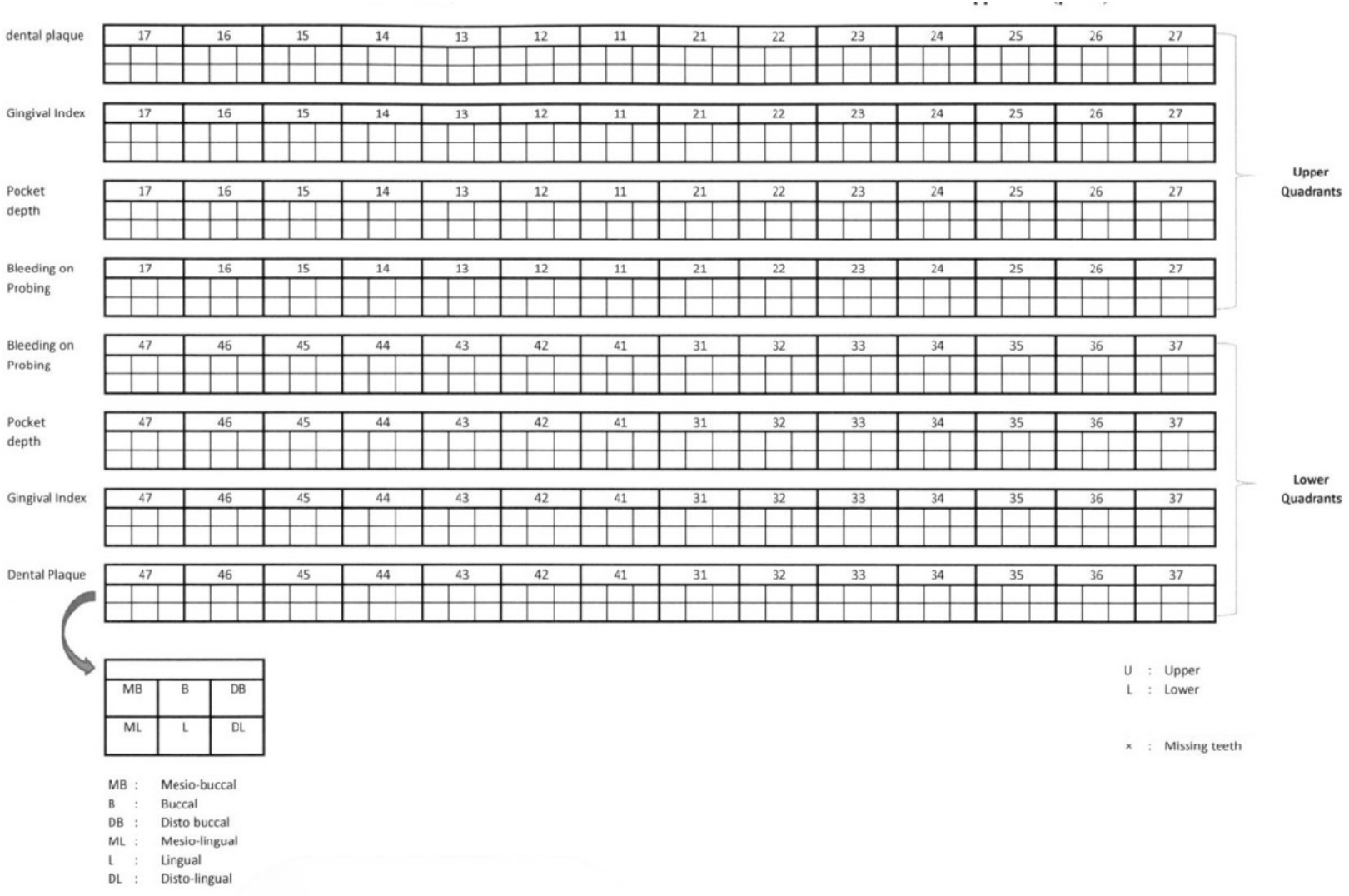
	AREA	NORMAL	ABNORMAL
1.	Perioral area/lips	☐	☐
2.	Buccal mucosa	☐	☐
3.	Labial mucosa	☐	☐
4.	Sublingual mucosa	☐	☐
5.	Gingiva free/attached	☐	☐
6.	Tongue	☐	☐
7.	Palate hard/soft	☐	☐
8.	Uvula	☐	☐
9.	Oropharynx	☐	☐
10.	All other soft/hard tissues	☐	☐

DESCRIBE ANY IRREGULARITIES:

_____

_____

_____


dental plaque
17 16 15 14 13 12 11 21 22 23 24 25 26 27
Gingival Index
17 16 15 14 13 12 11 21 22 23 24 25 26 27
Pocket depth
17 16 15 14 13 12 11 21 22 23 24 25 26 27
Bleeding on Probing
17 16 15 14 13 12 11 21 22 23 24 25 26 27
Upper Quadrants
Bleeding on Probing
47 46 45 44 43 42 41 31 32 33 34 35 36 37
Pocket depth
47 46 45 44 43 42 41 31 32 33 34 35 36 37
Gingival Index
47 46 45 44 43 42 41 31 32 33 34 35 36 37
Lower Quadrants
Dental Plaque
47 46 45 44 43 42 41 31 32 33 34 35 36 37
MB B DB
ML L DL
MB : Mesio-buccal
B : Buccal
DB : Disto buccal
ML : Mesio-lingual
L : Lingual
DL : Disto-lingual
U : Upper
L : Lower
× : Missing teeth

Appendix 8. Adverse Events Form

Ostia Sciences Non-Serious Adverse Event/ Adverse Reaction Form

Instructions: Do not leave any field blank. Please indicate if information is unknown, not provided or not available (refused). Please note that this form is expandable.

Date of Awareness (dd/mmm/yyyy):

Protocol #:

Protocol Title:

Indication of use protocol (if applicable):

Investigator:

Study Originator/Director:

Type of study:

☐Clinical	☐Consumer	☐Panel

Product Category:

☐Fabric care	☐Household Surface care	☐Oral Care
☐Personal care	☐Other:	

Phase of study the earliest event (s) occurred during:

☐After consent	☐ Pre-randomization	☐ Randomization: no product exposure
☐ Randomization: product exposure	☐Other:	

Study participant/Study participant information:

ID	Initials	Sex	Age	Weight	Ethnic group

Product information:

Product name (PIM#):	
Start date (dd/mmm/yyyy):	Stop Date or Duration (dd/mmm/yyyy):
Dosage:	Frequency:
Randomization group:	

Reaction/Event information:

Onset date (dd/mmm/yyyy)	Stop date or duration (dd/mmm/yyyy):	Severity (mild, moderate, severe)	Relationship to product (Possibly related, Related, Unrelated, Unknown)

Describe reaction(s)/event(s) in detail:

Outcome:

☐Resolved, Date (dd/mmm/yyyy):	☐Resolving	☐Unknown
☐Not resolved	☐Resolved with sequelae	☐Other:

Action taken with the product:

☐ Continued	☐ Reduced
☐ Discontinued	☐ Unknown
☐ Temporarily discontinued	☐ Other:

Did the event(s) abate after product was stopped or dose reduced (yes/no)?

Did the event(s) reappear after product was reintroduced (yes/no)?

☐ Protocol Continued ☐ Protocol Discontinued

Treatment rendered for the event(s):

Relevant Medical History Data: ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown
(Medical history with onset dates)

Relevant Concomitant Medications: ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown
(Medication name, dose, frequency, start/stop dates [dd/mmm/yyyy] or duration of therapy)

Relevant Lab data: ☐ Yes (List below) ☐ None ☐ Not provided ☐ Unknown
(Lab test, results, dates [dd/mmm/yyyy])

Serious Adverse Events Report Form for Studies on Humans

Instructions: Please complete all fields of this form. Please indicate if 'Not provided,' 'Not Available' or 'Unknown.' (Date eg: dd/mmm/yyyy 01/Dec/2009)

Case-ID-No:

A) Study site details	
Study No.:	Centre Name:
Study type : clinical / consumer test / panel test	Investigator:
If clinical, please define:	Address:
Study product:	
Dosage form and strength:	Country of occurrence:
Indication if appropriate:	

B) Reporter Information	
Sender / Reporter	
Name:	
Address:	Health professional: YES ☐ NO ☐
	Profession (Speciality):
Tel.:	Date of Awareness (dd/mmm/yyyy):
Fax:	Date of report:(dd/mmm/yyyy)
e-mail:	

C) General information			
Which product is concerned?			
☐ Test Product	☐ Comparator (Control product)	☐ Placebo	☐ Unknown
Specify when the event occurred during the study?			
☐ After consent	☐ Pre-randomization		
☐ Randomized with no product initiated		☐ Randomized with product initiated	
Study participant concerned:			
Study participant's ID-number:			
Sex: ☐ Male ☐ Female	Year of birth (dd/mmm/yyyy):		
In case of presumption or intoxication: Weight (kg):			
If Female, Pregnancy: ☐ NO ☐ YES, For how many months?			

D) Information on SAE

Reason for serious adverse event report

☐ Death ☐ Hospitalisation/Prolonged Hospitalization

☐ Life Threatening ☐ Congenital Anomaly

☐ Disability/Incapacity ☐ Other, specify:

☐ Suspected transmission via a medicinal product of an infectious agent

If hospitalisation, give dates (dd/mmm/yyyy) From: To:

☐ Hospitalization ongoing

Diagnosis / Symptoms

Please, indicate diagnosis or main symptom (s) and list serious most significant adverse event first:

1.

2.

3.

4.

Date of primary symptom (dd/mmm/yyyy) :

Duration of taking the product:

Outcome of serious adverse event :

☐ Not resolved ☐ Resolving ☐ Resolved with sequelae

☐ Resolved, date: (dd/mmm/yyyy) ☐ Unknown/ lost to follow up ☐ *Death, date: (dd/mmm/yyyy)

Cause of death if known:

Autopsy: ☐ YES ☐ NO ☐ Unknown ☐ Outcome:

Action taken:

☐ Protocol continuation ☐ Clinical study product dose reduced, new dose:

☐ Clinical study product discontinued ☐ Medical intervention

☐ Exclusion of the study participant ☐ Other:

Event abated after drug stopped or dose reduced?

☐ YES ☐ NO ☐ N.A.

Event reappeared after drug reintroduction?

☐ YES ☐ NO ☐ N.A.

Causality

☐ Related ☐ Possibly related ☐ Not related* ☐ Unknown

*If not related, please provide an alternative causality:

Code broken (unblinded)

☐ YES ☐ NO ☐ N.A.

E) Case narrative

Please provide full details of the serious adverse event, dechallenge/rechallenge information and vital signs.
Attach any relevant reports from the source document or hospitalisation file. In case of death, report cause and attach a copy of the autopsy report, if performed.

Information enclosed: ☐ NO ☐ YES, specify:

F) Relevant medical history

Description	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

G) Concomitant medication

Please report the medication taken in the last 4 weeks prior to the SAE(s)

Drug	Dose [unit]	Route	Frequency	Indication	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

H) Clinical study products Please indicate if Test product, Comparator (control product) or Placebo						
Product	Lot. No.	Dose [unit]	Route of administration	Frequency	Start Date (dd/mmm/yyyy)	Stop Date (dd/mmm/yyyy)

I) Relevant laboratory data & other test procedure for SAE		
Test	Date (dd/mmm/yyyy)	Result (normal, abnormal, clinically significant)

This report has to be sent within **1 Calendar day (No later than 1 business day)**:

- By the Investigator to the Study Manager:
  - Address
  - Phone
  - E-mail
- By the Study Manager to **contact@osia-sciences.com**
  - Email:

Appendix 9. Study participant Withdrawal Form

Study Participant Withdrawal Form

This form should be completed for all study participants who do not complete full participation of the study.

Study participant ID Number	
Date of Withdrawal	
Person completing withdrawal form	
Date informed of withdrawal	
Name of person reporting the withdrawal	

Withdrawal was made at the request of (tick one :)

Study participant _____ Investigator _____ Study Dentist _____ Assessor _____

REASON FOR WITHDRAWAL (Tick all that Apply)

	PROTOCOL VIOLATION (Give details)
	NON-COMPLIANCE (Give details)
	ADVERSE EVENT (Please attach copy of completed AE form)
	MEDICAL REASONS AND/OR EXCLUSION CRITERIA (Give details)
	STUDY PARTICIPANT'S PERSONAL REASONS (Give details)
	OTHER (Give details)

Person reporting withdrawal _____ Date_____

Investigators signature _____ Date_____

Product returned Yes No NA (circle as appropriate)

COMMENTS: _____

Appendix 10. Study Termination Form

Study Termination Form

STUDY PARTICIPANT ID No_____

MEDICATION HISTORY

Does the study participant currently take any medications (including OTC products)? Yes No (Circle One)

If **YES**, please list all medications below:

Medication	Total Daily Dose	Date Started	Date Stopped (circle C if continuing)	Indication
			C	
			C	
			C	
			C	

ORAL EXAMINATION

Tissue Examined	**Normal**	**Abnormal**	**If abnormal, comment below:**
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. Floor of mouth	_____	_____	_____
9. Other	_____	_____	_____
10. Hard Tissues	_____	_____	_____

_____	_____
Date	Clinical Examiner's Signature

Appendix 11. Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY (CLINICAL TRIAL)

Title	Clinical study to assess the efficacy of SALI-10 Probiotics Experimental Gingivitis
Principal Investigator	Dr. Michael Goldberg University of Toronto, Faculty of Dentistry Tel: (416) 979-4928 ext. 4408
Sponsor	Ostia Sciences Tel: (647) 643-7547

INTRODUCTION

Participant are being invited to take part in a type of research study called a clinical trial. The information below will tell participant about the study, what will be asked of participant and if there are any risks and benefits to you. It is up to participant to decide whether participant would like to participate in the study. Please read the consent form very carefully and make sure that all of your questions are answered before participant consent to take part. Before participant make your decision, feel free to talk about this study with anyone participant wish. Participation in this study is voluntary.

BACKGROUND AND PURPOSE

Clinical trials are necessary to gain or expand knowledge about the efficacy and tolerability of medicinal products. The clinical trial, which the investigators are presenting to participant here, has been reviewed and approved by the Health Sciences Research Ethics Board, University of Toronto and Health Canada, to be conducted in Canada. The study was initiated and is funded by Ostia Sciences the sponsor of this study.

We are conducting this clinical trial with a new oral probiotic in the form of dissolvable oral lozenges (like a tablet) that is being evaluated for improving oral health and promoting healthy gum tissue. Gingivitis (gum disease), or the inflammation of the gums, is commonly due to infection of the gum tissues that is caused by bacteria that attach to the teeth. When these bacteria are not properly removed (by brushing and flossing), the bacterial accumulation may cause the inflammation of the gums. Clinical signs of gum disease include (but are not limited to) redness or swelling of the gums, and bleeding from the gums, usually after irritation such as brushing or flossing. Gum disease is a completely reversible condition with no long-term complications if adequate daily hygiene is performed. However, it is currently estimated that 80% of adolescents and adults have gum disease.

The purpose of the study is to evaluate an oral probiotic named SALI-10 for its effect on preventing gum disease. This new probiotic has been harvested, isolated, and then sanitized from the oral cavity of a healthy individual

The results from this study will provide additional information for the use of SALI-10 oral lozenge probiotic in study participants to help prevent gum disease.

The focus of this study is to analyze and prevent induced gum disease caused by bacteria in dental plaque of healthy individuals participant have been given this information sheet to read because participant are in good general and oral health; with no braces or dentures; and a non-smoker. Under these circumstances, participant may be a good candidate to participate in the study, which will be confirmed after a clinical exam.

STUDY DESIGN

This study is described as a Randomized, Double Blind, Single-center and controlled study to investigate the effect SALI-10 oral lozenge probiotic on the resolution of gum disease compared to a placebo (a lozenge with no SALI-10 probiotic or any form of active ingredient). Gingivitis (gum disease) is a reversible condition causing inflammation of the gums. In this study, gum disease will be induced by having study participants refrain from using any form of oral hygiene, including any treatments from a dental professional, i.e., dentist, hygienist. A description of these terms is given below:

Randomized Trial: Sometimes because the investigators do not know which way of treating study participants is best, the investigators need to make comparisons. Participants in this study will be put into 2 groups and then compared. The groups are selected by a computer, which has no information about the individual. Therefore, participant have an equal chance to be in either group - like the toss of a coin. Participants will receive either the probiotic lozenges or the placebo lozenges, which they will have to take daily. The placebo lozenge will look identical to the probiotic one, but it will not contain the probiotic (active ingredient) that the investigators are testing.

Single center: The study is planned at only one center, the Faculty of Dentistry, University of Toronto, and it will involve a total of around 60 people in it.

Double-blind trial: In a blind trial participant will not know which treatment group participant are in. If the trial is a double-blind trial, neither participant nor the researcher and sponsor will know in which treatment group participant are (although, if the researcher needs to find out i.e. in an emergency, he/she can do so).

STUDY VISITS AND PROCEDURES

If participant decide to participate, you'll be asked to sign the consent form. At the start of the study, you'll be asked to fill out a health questionnaire, and your mouth, teeth and gums will be examined to determine if participant are eligible to participate in this study.

For the clinical trial, the health questionnaire will inquire about any known illnesses, current medications, and any allergies that participant may have.

The intra-oral examination using a mirror, a dental probe and a dental explorer will include:

- assessment of soft tissues (including lips, mucosa, gingiva, hard palate, soft palate, oro-pharynx, tongue, and floor of the mouth),
    - This will involve a visual examination
- evaluation of gum health (via measuring the gum pockets),
- dental assessment (to evaluate the condition of the teeth and any restorations i.e., fillings, crowns, bridges, Implants).
- and plaque levels where the dental explorer will pick-up (if visible) plaque from different tooth surfaces and a plaque index score will then be given.

The professional dental cleanings will be done by Dr. Martin Lambert, a member of the study team, who is a licensed dentist and periodontics resident at the Faculty of Dentistry, University of Toronto. The professional dental cleanings will be done to ensure all study participants start at the same gum health prior to when the gum disease is induced and also done at the end of the study to restore study participants' gum health to normal and healthy.

To measure bad breath, similar to a breathalyzer, participant will breathe through a tube connected to a machine (Halimeter PLUS) that will quantify the degree of bad breath. By measuring the level of sulfur compounds produced (responsible for the unpleasant odour) from the mouth, the investigators can evaluate how the probiotic SALI-10 battles against the bacteria responsible for foul breath.

All examinations and treatments are performed with sterile instruments (sterilized by an autoclave at the Faculty of Dentistry of the University of Toronto).

If participant are accepted into the study, participant will be required to follow a specific set of instructions. participant will agree to use only the oral lozenges given to participant for the duration of the study, as well as only the toothpaste provided. participant will not perform or receive any oral hygiene (other than that described below) or dental care throughout the study, except emergency care, until this study is completed. If participant must receive emergency dental treatment, participant will notify the principal investigator Dr. Micheal Goldberg at (416) 979-4928 ext. 4408. There will be no restrictions regarding your dietary habits.

Study participants who qualify for the study will be randomized in one of the two possible groups in a 1:1 ratio. As a result, your chance of receiving the SALI-10 probiotic lozenge is about 50%. The study researcher and participant will not know to which group participant are assigned.

All examinations and procedures associated with the study will take place in a dental chair, similar to when participant visit your dentist, within a dental operatory at the Faculty of Dentistry, University of Toronto.

Your participation in the clinical study will last up to 7 weeks as described below:

Visit 1 In-Person Screening

Here participant will read over and complete the consent form. Female participants will also be asked to take a urine pregnancy test to confirm they are not pregnant. Then participant will complete a health questionnaire form and an oral exam will be done to see if participant are eligible to take part in the study. If participant are deemed eligible to take part in the study participant will be invited to the next visit of the study.

Visit 2 (Day -14)

Participant cannot eat/drink for 2 hours before the visit. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form. A clinical assessment will be carried out to assess the level of gum disease and the level of plaque in your teeth. Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. participant will receive a professional dental cleaning to remove all soft (dental plaque) and hard (tartar) deposits from your teeth. participant will be assigned the lozenges and instructed on how to use them as well as provided with at-home oral hygiene instructions. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Visits 3 (Day -7)

Participant cannot eat/drink for 2 hours before the visit. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form. A clinical assessment will be carried out to assess the level of gum disease and the level of plaque in your teeth. Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. participant will resume at-home oral hygiene. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Visits 4, 5, 6 (Day 0, 7, 14)

Participant cannot eat/drink for 2 hours before the appointment. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form. A clinical assessment will be carried out to assess the level of gum disease and the level of plaque in your teeth. Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. Bad breath level with be measured using a non-invasive intra-oral device called the Halimeter PLUS. At this point participant will be instructed to continue the use of the lozenges assigned to participant but will be told to refrain from all forms of home oral hygiene for the next 21 days. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Visit 7 (Day 21)

Participant cannot eat/drink for 2 hours before the appointment. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form A clinical assessment will be carried out to assess the level of gum disease and the level of plaque in your teeth Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. Bad breath level with be measured using a non-invasive intra-oral device called the Halimeter PLUS. participant will receive a professional dental cleaning to remove all soft (dental plaque) and hard (tartar) deposits from your teeth. participant will continue taking the lozenges assigned to you. After this visit participant can return to doing at-home oral hygiene.

Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Visit 8 (Day 28)

Participant cannot eat/drink for 2 hours before the appointment. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form A clinical assessment will be carried out to assess the level of gum disease and the level of plaque in your teeth Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. Bad breath level with be measured using a non-invasive intra-oral device called the Halimeter PLUS. participant will continue taking the lozenges assigned to participant as well as continue at-home oral hygiene. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Visit 9 (Day 35)

Participant cannot eat/drink for 2 hours before the appointment. participant will undergo an oral soft and hard tissue assessment and the results will be recorded on the appropriate form. A clinical assessment will be carried out to assess the level of gum disease and the level of plaque. Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected. Bad breath level with be measured using a non-invasive intra-oral device called the Halimeter PLUS. After completion of the study, participant will receive compensation, and a study personnel will reveal to participant what lozenges participant had been assigned and ask for your feedback with regards to your experience in the study. Researchers will query participants on their use of NHPs, as well as any adverse effects they experienced.

Any questions or concerns participant may have will be addressed in this visit and participant will then be formally exited from the study

While participating as a study participant in this clinical research, participant cannot participate as a study participant in any other clinical studies. In addition, participant cannot use drugs, other than over the counter analgesics, during the course of the study. participant agree to inform the investigator about any new medications participant are planning to take, including but not limited to antibiotics, antiseptics, decongestants and antihistamines.

This product falls into the regulatory category of natural health products (NHP). There may be unknown risks with taking the investigational NHP. Study participants agree to inform the study researchers of any new medications and natural health products that they begin taking during the study. Study participants do not give up any legal rights by participating in this trial and they do not release the Investigators from liability for negligence. Records identifying the study participants will be kept confidential for 15 years.

Unscheduled visits

If the researcher believes that participant should have additional visits for your safety, for example in the event of a new symptom or side effect, participant may be asked to come for an additional visit. If necessary, additional tests related to such a safety concern may be performed. If the safety concern goes beyond the scope of dentistry, study participants will be referred to the appropriate healthcare provider.

Missed Doses

If a study participant misses their dose, they will be asked to take it if no more than 3 hours has passed since they brushed their teeth. If more than 3 hours have passed, they will be asked to skip the dose completely. Do not double up on the next dose. Do not exceed 2 lozenges per day.

Description of sample collection
Samples of dental plaque, saliva, oral rinse, and fluid from your gums will be collected on visits 2-9 and are described as follows:

- **Dental Plaque:** sterile paper points (these made of thin paper) will be placed in the space between the gum and the tooth for 30s and then removed. Six teeth will be sampled.
- **Saliva:** you'll be asked to chew on a gum and then spit into a tube (up to 15ml).
- **Oral Rinse:** you'll be asked to rinse with 10ml of a salted solution for 30 seconds and then spit into a tube (30ml).
- **Fluid from Gums:** sterile paper strips (germ-free tiny piece of paper) will be placed in the space between the gum and the tooth for 30s and then removed. Six teeth will be sampled.

Calendar of Visits

Visit	Day	Health Questionnaire (Pregnancy test)	Clinical Exam	Oral soft/ hard tissue assessment	Clinical Data Collection	Sample collection	Professional dental cleaning	Time (mins)
Visit 1	Screening	X	X					60
Visit 2	-14		X	X	X	X	X	90
Visit 3	-7		X	X	X	X		60
Visits 4,5.6	0, 7, 14		X	X	X	X		60
Visit 7	21		X	X	X	X	X	90
Visits 8, 9	28, 35		X	X	X	X		60

RISKS RELATED TO PREGNANCY

Pregnant women must not take part in this study, neither should women who plan to become pregnant during the study. Pregnancy status will be checked through a urine pregnancy test before the start of the trial. Study participants of child bearing potential, both men and women, must practice medically acceptable methods of birth control, which are: abstinence, hormonal birth control (oral, injectable, transdermal, intra-vaginal), intrauterine devices, confirmed successful vasectomy, or condoms. Any woman who finds that she has become pregnant while taking part in the study should immediately stop taking the investigators product immediately and inform the study researcher. In the event a woman enrolled in this clinical research study

becomes pregnant during the course of the study, participation in this study will be terminated upon the clinical staff's notification of the event. Your medical records used in this study will be updated to reflect the pregnancy and participant will have follow-up contact until the end of the pregnancy to record the outcome in your file. Pregnant and nursing women have an elevated level of hormones (oestrogen and progesterone), which favour gum disease and are therefore excluded from this study.

VOLUNTARY PARTICIPATION

Participant are under no obligation whatsoever to participate in this study and that your participation in this study is strictly voluntary. participant may withdraw or discontinue participation at any time and may decline to answer any question or participate in any parts of the procedure. There will be no consequences if participant choose to withdraw from the study other than the following compensation effects:

- No financial compensation will be awarded if participant have not completed the trial period
- In case participant choose to withdraw before visit 7, participant will not receive the second dental cleaning treatment

Participant may also withdraw consent for the use of your data, but participant understand that participant must do this in writing. participant understand that the investigator has the right to withdraw participant from the study at any time because it would not be in your best interest to stay in it. The study researcher can stop treatment even if participant are willing to stay in the study. Any data that has already been sent to the sponsor of the study cannot be withdrawn because there may not be any identifiers with the data.

If participant decide to pull out of the study for any reason, participant may be asked to return for at least one additional visit for safety reasons.

COMPENSATION

Participation in research is voluntary. Compensation of $1000 will be given to study participants; $500 will be given after the 21 days of experimental gingivitis (gum disease) and the remaining $500 will be given at the end of the study. This amount will be given to also cover costs that study participants' may have while taking part in the study i.e., travel costs. It is the responsibility of the participant to comply with the Income Tax rules with respect to this monetary compensation.

Participant will receive 2 professional dental cleaning treatments for free prior and subsequent to stopping all oral hygiene procedures (approximate value of $650). participant will also receive proper oral hygiene instructions.

BENEFITS

Participation in this study may not benefit participant personally. The results of the study may allow improved methods to detect and treat gum disease and disease-causing plaque in the early stages and the investigators would like to thank participant for considering participating in this study.

ALTERNATIVES TO PARTICIPATING IN THIS STUDY

There are no alternatives to participating in this study as participation in this study is on a voluntary basis and participant may choose to not participate in this study.

DISCOMFORTS AND RISKS

Summary of Risks:

- Bleeding gums
- Discomfort during dental examination and probing of the gums
- Gum pain or sensitivity due to the inflammation
- Bad breath
- Temporary tooth staining
- Dental plaque build-up on the gums
- Allergic reaction to the SALI-10 probiotic oral lozenges

These events are expected to be mild and temporary and will stop when participant receive the professional dental cleaning the end of the study.

In the event that study participants experience any fever, vomiting, bloody diarrhea or severe abdominal pain, or symptoms of digestive upset such as diarrhea lasting more than 3 days, participants should consult the study doctors and the decision to discontinue participation in the study will be taken on a case by case basis.

Additionally, at each examinations participant will be asked if participant have experienced any discomfort, pain, irritation in the mouth and/or any other concern(s) that participant may have. The investigators will record any adverse reactions and report this documentation to the sponsor. The nature of the reaction and any correlation with product usage will be assessed. If the evidence indicates that the adverse reaction may be due to product usage, participant will be instructed to discontinue product use and appropriate treatment will be provided. In the event of an adverse experience, emergency or other problems or questions regarding your participation in this study participant can contact the principal investigator Dr. Micheal Goldberg at: (416) 979-4928 ext. 4408 or the Research Oversight and Compliance Office - Human Research Ethics Program at ethics.review@utoronto.ca or 416-946-3273. In the event of a medical emergency, please contact your physician.

As mentioned above, gum disease is a completely reversible condition, as was demonstrated in many studies. Despite the inflammation of the gums, study participants experience no pain and no irreversible pathology. Experimental gum disease is a common and safe clinical tool which is used in many fields of oral research.

Bad breath may occur during the 21 day-period of no oral hygiene. Yet, mouthwash and gum chewing are not allowed until the end of the study.

Participant will be under close monitoring, and if any unexpected disease arises, it will be treated as soon as it is diagnosed. participant can also call the 24-hour number at 581-991-0184 to address any concerns as they arise.

No discomfort or side effects are expected from the collection of dental plaque, saliva, oral rinse oral fluid and measurement of bad breath using the Halimeter PLUS.

NEW FINDINGS

Sometimes during the course of a research study, new information becomes available about the treatment/drug that is being studied. participant will be informed of any significant new findings related to study products or procedures as soon as they are known. If this happens, the study researcher will tell participant about it and discuss with participant whether participant want to continue in the study. If participant decide to continue in the study, participant will be asked to sign an updated consent form.

Also, on receiving new information the study researcher might consider it to be in your best interests to withdraw participant from the study, and explanations will be given accordingly.

CONFIDENTIALITY

If participant agree to join this study, the study researcher and his study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify participant and may include your name, address, date of birth, and new or existing medical records, that includes types, dates and results of medical tests or procedures.

The results of this study may be published and/or submitted to Health Canada and/or local regulatory agencies in other countries, including US FDA. Your identity will be kept confidential, only your study participant number/ID, gender and/or age may be used participant in connection with any such publication of the study results. No documents that identify participant by name (e.g., the signed informed consent form and health questionnaire) will be transferred or submitted to the study sponsor (Ostia Sciences) but will be maintained in strict confidence by the investigator in a locked filing cabinet in a locked room at the Faculty of Dentistry. However, the investigator must allow auditing/monitoring by the sponsor personnel to verify study participant enrollment, product safety and study compliance. If any sponsor monitoring occurs, study documents can be looked at, but no private information will be copied or removed from the clinical site.

The following people may come to the Faculty to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines:

- The study sponsor or its representatives/partner companies;
- Representatives of the University of Toronto Research Ethics Board;

Some study information will be sent outside of the Faculty to the Sponsor. Prior to disclosing such personal data to the larger study team, including the sponsor of the study, investigators and their team will code your personal data. Therefore, any information about participant that is sent out of the Faculty will have a code and will not show your name or address, or any information that directly identifies you.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. participant will not be named in any reports, publications, or presentations that may come from this study.

There will be no future use of data that is collected from participant for the purposes of this study,

If participant decide to leave the study, the information about participant that was collected before participant left the study will still be used. No new information will be collected without your permission.

What will happen to the results of the research study?

The results of this study may be published online, in a medical journal and shown at medical meetings. participant will not be identified (by name or any other personal means) in any of these publications.

If participant would like to know the study results, the study researcher will inform participant when the results are available.

CONFLICT OF INTEREST

Ostia Sciences is sponsoring this clinical trial.

The Institution and study researcher will be paid to run this study.

All of these people have an interest in completing this study. Their interests should not influence your decision to participate in this study. participant should not feel pressured to join this study.

QUESTIONS ABOUT THE STUDY

If participant have any questions, concerns or would like to speak to the study team for any reason, please contact Dr. Martin Lambert through email: martin.lambert@mail.utoronto.ca. Please note that the security of e-mail messages is not guaranteed. Messages may be forged, forwarded, kept indefinitely, or seen by others using the Internet. Do not share personal information via email.

If participant have any questions about your rights as a research participant or have concerns about this study, please contact the Research Oversight and Compliance Office - Human Research Ethics Program at ethics.review@utoronto.ca or call 416 946 3273. The Research Ethics Board is a group of people who oversee the ethical conduct of research studies. These people are not part of the study team. Everything that participant discuss will be kept confidential.

INFORMED CONSENT

Participant will be asked to sign the consent form at the end of this study participant information sheet. If participant take part in this study, participant should follow the study procedures and attend all study visits.

I, _____ have read and understand the
(PRINT NAME)
information provided above and have been given an opportunity to ask questions and they were satisfactorily answered. I have been provided with a copy of this informed consent. By my signature below, I consent to participate in the study and agree to keep confidential any and all information disclosed to me, either directly or indirectly, about this study, including but not

limited to the purpose of the study, any procedures and any toothpaste. I agree that I will follow the instructions.

(Participant)
By signing this form, I confirm that:
• This research study has been fully explained to me and all of my questions have been answered to my satisfaction
• I understand the requirements of participating in this research study
• I have been informed of the risks and benefits, if any, of participating in this research study
• I have been informed of any alternatives to participating in this research study
• I have been informed of the rights of research participants
• I have read each page of this form
• I authorize access to my personal health information and research study data as explained in this form
• I have agreed to participate in this study.

_____	_____	_____
Participant's Name (Print)	Signature	Date

(Person obtaining consent)
By signing this form, I confirm that:
• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

_____	_____	_____
Person Obtaining Consent (Print)	Signature	Date

Appendix 12. Contact Information for Study Withdrawal

Study Withdrawal Contact Form

Title	Clinical study to assess the efficacy of SALI-10 Probiotics Experimental Gingivitis
Principal Investigator	Dr. Michael Goldberg University of Toronto, Faculty of Dentistry Tel: (416) 979-4928 ext. 4408
Sponsor	Ostia Sciences Tel: (647) 643-7547

If participant choose to withdraw from the study, please contact one of the study research personnel, Dr. Martin Lambert, via email:

martin.lambert@mail.utoronto.ca

Please indicated in your email if participant want to be contacted by phone or email.

Please do not share personal information via email.

After receiving your email, a member of the study will contact participant based on the preference participant gave in the email a formal withdrawal interview with you.