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Changes in oral health and dental aesthetic in smokers switching to combustion-free nicotine alternatives: protocol for a multi-center prospective, randomized, controlled trial

Gianluca Conte, Sebastiano Antonio Pacino, Salvatore Urso, Doris Greiling, Pasquale Caponnetto, Eugenio Pedullà, Luigi Generali, Ugo Consolo, Vittorio Checchi, Stefan Gospodaru, Gheorghe Bordeniuc, Valeriu Fala, Jan Kowalski, Maciej Nowak, Renata Górska, Amaliya Amaliya, Iain Chapple, Michael Milward, Robert Maclure, Gianna Maria Nardi, Riccardo Polosa

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Abstract

Objective: This multi-center, prospective, randomized, controlled, three parallel-arms trial will investigate whether switching from CC to C-F NA will lead to measurable improvements in oral health parameters and dental aesthetics over a period of 18 months in adult smokers with limited gum disease.
Methods and Analysis:
Regular smokers not intending to quit and without clinical signs of periodontitis will be randomly assigned (1:4 ratio) to either standard of care with brief cessation advice (Control Group; Arm A) or C-F NA use (Intervention Group; Arm B). The study will also include a reference group of never-smokers (Reference Group; Arm C). The primary endpoint is the change in Modified Gingival Index (MGI) score from baseline between control arm (Arm A) and intervention arm (Arm B) at the 18-month follow-up. Additionally, the study will analyze the within- and between-group (Arm A, B and C) changes in MGI assessment, plaque imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires measured at each study time point. This multicenter study will be conducted in four dental clinics in four countries. Subject recruitment has started in October 2021 and enrolment is expected to be completed by August 2023. Results will be reported in 2025. This will be the first study providing key insights into oral health benefits or risks associated with using C-F NA in smokers who are seeking alternatives to cigarette smoking.

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Changes in oral health and dental aesthetic in smokers switching to combustion-free nicotine alternatives: protocol for a multi-center prospective, randomized, controlled trial

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Keywords: Electronic cigarettes, heated tobacco products, Tobacco Harm Reduction, smoking, oral health, gingivitis, periodontitis, MGI, dental plaque imaging, dental shade

Clinical Trial Registration: NCT04649645

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ABSTRACT (word count: 410)

Background:

Although the detrimental effects of conventional combustible cigarettes (CC) on oral health and dental aesthetics are well-known, there is limited information about the long-term impact of combustion-free nicotine alternatives (C-F NA) like electronic cigarettes or heated tobacco products.

Objective:

This multi-center, prospective, randomized, controlled, three parallel-arms trial will investigate whether switching from CC to C-F NA will lead to measurable improvements in oral health parameters and dental aesthetics over a period of 18 months in adult smokers with limited gum disease.

Methods:

Regular smokers not intending to quit and without clinical signs of periodontitis will be randomly assigned (1:4 ratio) to either standard of care with brief cessation advice (Control Group; Arm A) or C-F NA use (Intervention Group; Arm B). The study will also include a reference group of never-smokers (Reference Group; Arm C). The primary endpoint is the change in Modified Gingival Index (MGI) score from baseline between control arm (Arm A) and intervention arm (Arm B) at the 18-month follow-up. Additionally, the study will analyze the within- and between-group (Arm A, B and C) changes in MGI assessment, plaque imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires measured at each study time point. All participants will attend a total of seven clinic visits: screening, enrollment and randomization (Visit 0), baseline visit – day 14 (Visit 1), day 90 (Visit 2), day 180 (Visit 3), day 360 (Visit 4) and day 540 (Visit 5). This multicenter study will be conducted in four dental clinics in four countries. The statistical analysis will involve descriptive statistics for continuous and categorical data. Primary endpoints will undergo tests for normality and, based on distribution, either a two-sided t-test or Mann-Whitney U test. Linear Mixed Model (LMM) with the random factors center and study arms-by-center will be also applied. Secondary endpoints, including MGI assessment and quality of life, will be subjected to similar tests and comparisons. Only if one value of the parameter MGI is missing after Day 1, the last available observation will be carried forward (LOCF). The analysis will be performed on the substituted data. Secondary parameters will not have missing value replacement.

Results:

Subject recruitment has started in October 2021 and enrolment is expected to be completed by June 2023. Results will be reported in 2025.

Conclusions:

This will be the first study providing key insights into oral health benefits or risks associated with using C-F NA in smokers who are seeking alternatives to cigarette smoking.

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Ethics and Dissemination:

The study protocol, informed consent forms, and relevant documents were approved by four Research Ethics Committees. Study results will be disseminated through articles published in high-quality, peer-reviewed journals, and presentations at conferences.

INTRODUCTION

Periodontal diseases, commonly including gingivitis and periodontitis, arise in response to accumulations of oral bacteria in the gingival sulcus and are characterized by tissue changes in the periodontium occurring as part of the inflammatory process [1]. Gingivitis is characterized by inflammation such as redness, swelling or bleeding on gentle provocation of the gingival sulcus, whereas periodontitis exhibits increased probing depth, clinical attachment loss and radiographic alveolar bone loss, reflecting the destructive aspects of the disease process [2,3].

Periodontal disease is a well-known independent risk factor for cardiovascular disease [4,5] and reducing gingivitis and periodontitis is likely to have an overall positive impact on human health in general.

There are multiple risk factors for periodontal disease, and cigarette smoking is considered among the key independent risk factors for the development and progression of periodontal disease [6-9]. Depending on disease definition and extent of exposure to cigarette smoke, the risk to develop destructive periodontal disease is 5- to 20-fold higher for smokers compared to non-smokers [10]. Cigarette smokers appear to be prone to more severe periodontal manifestations - even after adjusting for age, education level, history of diabetes, BMI, alcohol consumption, perceived mental stress and oral hygiene levels [11]. Besides periodontal diseases, cigarette smoking can cause visible dental manifestations including dental discoloration and tobacco stains, the intensity of which mainly depends on smoking duration and frequency [12]. In a large cross-sectional study in the UK [13], smokers were more likely to report dental discoloration and being dissatisfied with their own tooth color compared to non-smokers. Dissatisfaction with teeth appearance (due to enamel discoloration and tobacco stains) is often perceived as a significant social problem for smokers [14,15].

The importance of dental care and oral health for healthy longevity is stated in the 2015 Tokyo declaration of the WHO: “Greater advocacy is needed to increase the prominence of oral health on the global health agenda. Reducing oral health issues calls for stronger policies addressing the determinants of oral diseases and no communicable diseases and to tackle inequalities through inclusive universal health care access”. More recently WHO has provided policy recommendations for integrating brief tobacco interventions into oral health programs in primary care in accordance with the WHO Oral Health Program and as part of the WHO Global action plan on the prevention and control of no communicable diseases, particularly as pertains to the needs of low- and middle-income countries [16].

Although it is clear that abstaining from smoking will have beneficial effects on oral health and overall dental aesthetic, most smokers are reluctant to seek formal treatment for stopping smoking with the vast majority making attempts to quit without assistance [17,18]. Consequently, the need for novel and more efficient approaches is required.

Although not authorized as medications for smoking cessation, combustion-free technologies for nicotine delivery, such as e-cigarettes (ECs) and heated tobacco products (HTPs), have become *de facto* harm reduction tools from cigarette smoke (19,20) and aids for quitting smoking [21,22].

Combustion-free technologies for nicotine delivery like e-cigarettes (ECs) and heated tobacco products (HTPs) offer substantial reduction in exposure/harm to harmful constituents compared to tobacco cigarettes [23-26], but there is limited information about the long-term impact on oral health and dental aesthetics in

people who use combustion-free nicotine alternatives (C-F NA).

This multi-center, prospective, randomized, controlled, three parallel-arms trial will be the first to determine whether adult smokers who switch to combustion-free nicotine alternatives (C-F NA) will experience measurable improvements in oral health parameters and dental aesthetics. A group of never smokers will be also recruited for comparative measures of study parameters.

Data from this study will provide valuable insights into the overall potential of C-F NA to reduce the risk of periodontal diseases and consequential cardiovascular risk. In addition, findings may have important implications for reducing smoking burden globally, especially for those smokers for which bad breath and/or poor dental aesthetic is a significant concern. For these individuals, an oral-centric narrative (such as achieving a healthier and brighter smile) may serve as a more compelling reason to quit smoking than the fear of future lung cancer or cardiopulmonary diseases.

METHODS

This is a multi-center, three parallel-arms, randomized, controlled trial of 18 months' duration designed to assess whether cigarette smokers switching to combustion free-nicotine alternatives (C-F NA) will undergo measurable improvements in oral health parameters and dental aesthetics as a consequence of avoiding exposure to cigarette smoke. It is intended to assess differences in study endpoints of oral health parameters and dental aesthetics at multiple study time points between smokers switching to C-F NA and smokers who continue to smoke.

Study Population

Eligible participants will be healthy adult regular cigarette smokers (self-reported daily smoking of >10 cigarettes/day for at least 5 consecutive years) and never smokers without clinical signs of periodontitis.

Smokers will be offered access to free smoking cessation programs. Only those who refuse to participate in the smoking cessation program and express willingness to switch to C-F NA will be eligible for randomization.

Never-smokers (who have smoked <100 tobacco cigarettes in their lifetime) will be also included as reference group.

Participants will be required to satisfy all of the following criteria:

<u>Inclusion criteria – Smokers (Study Arms A and B)</u>

- Adults (18 - 50 years old)
- Cigarette smokers of ≥ 10 cigarettes/day
- Smoked for at least five consecutive years prior to screening.
- Verified smoking status by exhaled breath CO ≥ 7 ppm at screening
- Willingness to switch to a C-F NA, if required by the randomization
- Refusal to participate in smoking cessation programs
- Presence of at least 10 natural anterior teeth in total (cuspid to cuspid, lower and upper jaw)
- Healthy subjects, not taking regular medications for chronic medical conditions
- Accepting to comply with the requirements of the study including installing an APP on their smartphone

<u>Inclusion criteria – Never-smokers (Study Arm C)</u>

- Adults (18 - 50 years old)
- Have not smoked > 100 tobacco cigarettes in their lifetime
- Verified non-smoking status by exhaled breath CO < 7 ppm at screening
- Presence of at least 10 natural anterior teeth in total (cuspid to cuspid, lower and upper jaw)
- Healthy subjects, not taking regular medications for chronic medical conditions
- Accepting to comply with the requirements of the study including installing an APP on their smartphone

Exclusion criteria – Smokers (Study Arms A and B) and Never-smokers (Study Arm C)

- Significant oral soft tissue pathology or any type of gingival overgrowth, other than plaque-induced gingivitis
- Periodontitis based on 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [27], which require:
    - Detectable Interdental Clinical Attachment Loss (CAL) ≥ 3 mm at ≥ 2 non-adjacent teeth.
    - Buccal or Oral CAL ≥3 mm with pocketing ≥3 mm detectable at ≥2 teeth
    - Only subjects with mild-moderate gingivitis will be recruited
- Fixed and removable orthodontic appliance or removable dentures
- Any other medical condition that, in the opinion of the Principal Investigator would jeopardize the subject's safety or diminish the validity of the study results
- A course of treatment with any medications which:
    - interfere with the cyclooxygenase pathway (e.g. anti-inflammatory drugs including aspirin and ibuprofen) within 3 days prior to each visit
    - are known to have antibacterial activity (e.g. antibiotics) within 7 days prior to each visit
- Significant history of alcohol or drug abuse within 24 months prior to screening, as determined by the Investigator
- For smokers, planning to quit smoking within the next 6 months
- For smokers, regular use of any nicotine (e.g., e-cigarettes, NRT, nicotine pouches) or tobacco product (e.g., heated tobacco products - HTPs, oral smokeless) other than their own cigarettes within 14 days of screening
- Pregnant or breast feeding or intention to become pregnant during the study
- Active participation in another clinical trial

Study Design

This is an 18-month prospective, multi-center, open-label study with three parallel arms, employing randomization and control to assess a range of oral health and dental aesthetic metrics (**Figure 1**). The primary objective is to compare these metrics between cigarette smokers transitioning to combustion-free nicotine alternatives (C-F NA) and those who persist in smoking.

Eligible smokers will be allocated randomly into either the control group (Arm A), which will receive standard care inclusive of cessation counseling (i.e., very brief advice - VBA), or the intervention group (Arm B), which will be given an C-F NA of their choice and will also receive VBA. Individuals who have never smoked will form a reference group (Arm C). Throughout the study, participants from all three groups will undergo regular assessments of oral health and dental aesthetic. The study will be conducted in four dental clinics across four different countries (Italy, Poland, Moldova, and Indonesia).

The study's protocol adheres to the guidelines stipulated by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines

The study flow is illustrated in **Figure 2**.

All participants will attend a total of seven clinic visits:

- Day -28 to Day -1 - Screening
- Day 0 - Enrollment and Randomization (Visit 0)
- Day 14 - Baseline Visit (Visit 1)
- Day 90 – Week 12 (Visit 2)
- Day 180 – Week 24 (Visit 3)
- Day 360 – Week 52 (Visit 4)
- Day 540 – Week 76 (Visit 5)

Screening visit will be performed within 28 days prior to Enrollment to verify eligibility criteria (Visit 0) (**Figure 2**). During the screening visit, socio-demographic data, medical and oral health history, detailed information about nicotine/tobacco consumption, and interest in trying C-F NA will be noted. A screening for periodontal disease will be carried out to exclude periodontitis, as per the definition of the guidelines of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [27]. Smokers will be offered smoking cessation program as per local guidelines. Any smokers who expresses the intention of booking for the cessation program or to quit smoking in the next 6 months will be urged to do so and not be recruited in the study. Subjects taking part in the study will be informed that they are free to quit smoking and withdraw from the study at any time. Moreover, smokers will be encouraged to quit smoking at every contact throughout the whole study.

Within 28 days of the screening visit, eligible participants will be invited to attend an Enrollment visit (Visit 0). Inclusion and exclusion criteria will be verified again. Smokers will be reminded of the risks associated with smoking prior to enrolment onto the study and that they are free to voluntarily quit smoking/nicotine and/or withdraw from the study at any time. All participants will be undergoing a series of measurements (for details see **Table 1a, Table 1b, Table 1c**) followed by dental scaling and polishing procedures to remove calculus, plaque and stain.

Smokers not intending to quit will be then randomly assigned to either the control (Arm A) or the intervention (Arm B) group. The randomization sequence will be generated by computer, with an allocation ratio of 1:4 (Arm A: Arm B) to accommodate for the estimated 25% proportion of participants achieving a sustained reduction in cigarette consumption of at least 90% in Arm B (as detailed in the "Statistical Considerations" section). Smokers in the control group (Arm A) will receive standard care, inclusive of cessation counseling (i.e., very brief advice - VBA) at each study visit. Smokers in the intervention group (Arm B) will have the option to try and choose a preferred C-F NA (e-cigarettes, HTPs) from a given pool of popular options present in the respective markets and will also receive VBA.

Individuals who have never smoked will be allocated to the reference group (Arm C). Never smokers in the reference group will serve as comparators for assessing any background changes in the primary study endpoint (i.e., MGI) that may occur over time. Although never smokers will not undergo randomization, efforts will be made to achieve a comparable distribution of sex and age across all study arms.
The trial is designed as an unblinded trial due to the nature of the intervention, where participants and trial staff cannot be blinded to the specific intervention being provided. However, data analyses will be conducted blind to Study Arms allocation. All other trial staff who have access to outcome data will remain blinded until pre-specified data analyses will be completed.

Baseline measurements will be performed at Visit 1 (Baseline Visit), 14 days after scaling and polishing procedures at Enrolment (Visit 0). Study measurements will include: MGI assessment, plaque Imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires (for details see **Table 1a, Table 1b, Table 1c**).

Smokers in Arm B will be supplied with a C-F NA of their choice. They will receive training and counseling on how to use the chosen C-F NA and will be provided with a full 2-week supply of the required consumables (cartridges/pods or e-liquids refill bottles for e-cigarettes, tobacco sticks for HTPs). Participants wishing to use a heated tobacco product (HTP) will receive one kit and supply of tobacco sticks of their choice; they will receive the number of tobacco sticks per day corresponding to the number of cigarettes smoked per day at baseline. Participants wishing to use a vaping product will receive one vaping kit and supply of e-liquids of their choice. Products will be supplied at each subsequent visit throughout the whole duration of the study according to the study product supply schedule in **Table 2**.
After the baseline visit, participants will be invited to attend four more clinical visits (Visits 2-5) to repeat study measurements (for details see **Table 1a, Table 1b, Table 1c**).

Throughout the study, monitoring of cigarette consumption, daily C-F NA usage, and oral hygiene routine will be carried out regularly with the tracker APP and personal diaries. Monitoring of cigarette consumption and daily C-F NA usage will be also achieved by asking participants in Arm B to return all empty, part-used, and unused consumables (tobacco sticks, e-cigarette cartridges, e-liquid refill bottles) at each study visit. The tracker application will also identify any protocol violations, collect adverse events and send reminders (e.g. next scheduled appointment, study restrictions, instructions) throughout the study duration. The use of a dedicated tracker application adds an innovative element to continuously collect data and enhances adherence to the study protocol.

Before each study visit (Visits 1-5), all participants will be required to: refrain from scaling and polishing procedures; avoid modifying their own habitual oral hygiene (e.g. mouthwash, mouth-rinse, interdental floss); refrain from flossing for at least 72 hours prior to each study visit; refrain from mouth-rinsing for at least 24 hours prior to each study visit; refrain from tooth brushing for at least 2 hours prior to each study visit; refrain from eating and drinking (except water) for at least 2 hours before each visit; abstain from smoking for 2 hours before each visit. This is to minimize any potential interference with study outcomes and to maintain consistency in data collection. Throughout the study, any modifications in participants' oral hygiene routine will be recorded via the Tracker App.

A detailed description of the measurements that will be specifically carried out at each trial visits is described in **the Annex**.

Study Endpoints

Primary Endpoints: The primary objective of the study is to compare changes in Modified Gingival Index (MGI) by Lobene [28] between baseline and end of study (i.e. 18 months' time point) among participants who continue to smoke tobacco cigarette (Arm A) and participants who switch to C-F NA (Arm B).

Additionally, the study will analyze the number of secondary objectives including within- and between-group variations at each study time point from baseline for MGI assessment, plaque Imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires.

Study Measurements and Calibration

A detailed description of the study measurements that will be specifically carried out at dental clinic is described in **the Annex**.
All Assessors will be a trained on MGI scoring, tooth stain assessment, and on the correct use of technologies/software used to quantify dental plaque, teeth discoloration, eCO monitoring and eCRF use. Assessors will be also periodically calibrated on MGI scoring and tooth stain assessment. To ensure

consistency in the measurements, the same Assessor will collect all study endpoints in the same participant over the whole study period.

Data Monitoring and Study Safety

A trial monitoring plan will be developed based on the trial risk assessment and includes on-site monitoring. The clinical research organization (CRO) will arrange an independent monitor, separate from investigators and the sponsor, to ensure compliance with trial protocols and policies, participant protection, and accurate data collection.

Adverse Events (AEs) and Serious Adverse Events (SAEs) (e.g. related to oral conditions, tobacco cigarette smoking, C-F NA use, or nicotine withdrawal symptoms or nicotine overdosing), will be recorded on the adverse event page of the eCRF throughout the study. Participants will be interviewed at each visit to investigate signs or symptoms. Signs or symptoms will be elicited at each visit by open questioning, such as “How have you been feeling since your last visit?” “How have you been feeling regarding your oral cavity?”. Participants will also be encouraged to spontaneously report AEs occurring at any other time during the study via the dedicated mobile APP or the communication channels provided for the study. The investigator must gather sufficient information to determine the outcome and causality of AEs/SAEs and promptly notify the competent authority if necessary.

Study Withdrawal

Participants may be withdrawn from the study prematurely for the following reasons: 1) experience a severe adverse event (SAE); 2) develop a concurrent disease which at the discretion of the Investigator no longer justifies the participant’s participation in this study; 3) sustain any uncorrectable protocol deviations during the study, 4) decides to stop their participation; 5) become pregnant; 6) exhibit uncooperative behavior or non-attendance. After consultation with the CRO, the Investigator will notify subjects’ discontinuation from the study.

Data Collection and Protection

For data collection, each individual subject will be allocated an electronic case report form (eCRF). Anonymized data from each study visit will be entered directly onto the eCRF, which will then serve as a source document for the trial. This data entry process ensures that data is collected accurately and consistently at all study sites.
Each participant will be assigned a unique study identification number (subject ID). Participants' personal data will not be linked to the research results, and only a limited number of members of the research team will have access to the decoding list that links participant IDs to their personal information. All information obtained during the study procedures, including participant data and personal details, will be treated as private and confidential, in accordance with ethical and privacy regulations.

The trial will reach its formal conclusion on the date of the final visit of the last subject in the last country involved in the study.

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Ethical considerations and results dissemination

The study will be conducted according to the Principles of Good Clinical Practice (GCP) and Declaration of Helsinki. All four local Ethic Review Boards reviewed and approved the study and - where appropriate - translated relevant documentation (informed consent form, subject's information sheet, etc). The trial is registered with ClinicalTrails.gov Identifier: NCT04649645.

The intention is to disseminate the study results through articles in peer-reviewed journals and conference presentations. A summary of results will be available on the study website for public access. The anonymized data will be available to researchers upon reasonable request.

Statistical Considerations

Sample Size Calculation

For a valid sample size calculation, a literature research was performed for the primary endpoint of the study (i.e. MGI). Unfortunately, no data was available for such a study. However, data from studies with mouth-rinse were available, and based on these studies [29,30], a mean MGI change of 0.08 with a standard deviation of 0.18 of the MGI seems relevant.

However, in these studies higher mean MGI changes were observed because active treatment was given, which is not the case in this study. Thus, several scenarios with different mean MGI change and standard deviations will be applied.

The following hypotheses will be tested with a one-sided independent t-Test:
H0: $\mu A \leq \mu B$ versus H1: $\mu A > \mu B$

Where μA denotes the mean change MGI from baseline to after 18 months of regular smoking and the μB denotes the mean MGI from baseline to after 18 months of C-F NA use.

For the sample size calculation SAS software version 9.4 was used and several scenarios were applied, assuming that the data is normally distributed. The results are summarized in Table A, which presents the calculated sample sizes for the comparison of μA versus μB by a one-sided independent t-Test and a significance level of 5% and a power of 90% dependent on the expected MGI change and standard deviation.

***Table A**: Summary table including the sample size for each arm of the study depending on the mean MGI change and standard deviation.*

Nominal Power	Mean MGI Change	Standard Deviation					
		0.20	0.21	0.22	0.23	0.24	
0.9	0.10	70	77	84	92	100	Sample Size per group
0.9	0.15	32	35	38	41	45	
0.9	0.20	18	20	22	24	26	

Since no active treatment is given in the present study, a lower effect compared to the observed values needs to be assumed. Therefore, a mean MGI change of 0.1 with a standard deviation of 0.21 was chosen. Hence, a

*sample size of 77 per treatment group is required. Considering a dropout rate of 20% over two years' observation phase [31,32], a sample size of at least 92 per treatment group is required. In addition, it is estimated that about 25% of those switching to C-F NA use will abstain or substantially (≥ 90%) reduce smoking [33] and only these participants deliver valid results. Therefore, four times more participants should be recruited in the C-F NA group (4*92 = 368). These results in a total sample size of 92 (Arm A) + 368 (Arm B) = 460.*

In Arm C, we have determined a sample size of 40, empirically with no formal statistical analysis, as this cohort's primary study endpoint (i.e. MGI) is expected to be stable throughout the duration of the study.

Statistical Analyses

*Statistical methods to be used in the analysis of this study will include descriptive statistic with d**escriptive presentation of N, mean, standard deviation, median, minimum, maximum and 95 % confidence limits for (pseudo-) continuous data and for categorical data. Further evaluations will be summarized in tables by counts and percentage of scores.*

Statistical methods for primary study endpoints will include:

- Test of normality with Shapiro-Wilk on calculated values
    - In case of normal distribution: two-sided independent t-test
    - In case of no normal distribution: two-sided Mann-Whitney U test

- Linear Mixed Model (LMM) with the random factors center and study arms-by-center

Designated as Study Arm C, the never-smoker group will establish a baseline reference for oral health parameters. This group is anticipated to provide insights into underlying changes in the primary study endpoint (MGI) that may emerge over time in individuals who have never smoked.

Statistical methods for secondary study endpoints will include:

- For comparisons of each study arms for parameters like MGI assessment, plaque Imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires:
    - Test of normality with Shapiro-Wilk on calculated values
        - In case of normal distribution: two-sided independent t-test
        - In case of no normal distribution: two-sided Mann-Whitney U test
- For comparisons of each assessment time for parameters like MGI assessment, plaque Imaging, dental shade quantitation, tooth stains scores, and oral health-related quality of life questionnaires:
    - Test of normality with Shapiro-Wilk on calculated values
        - In case of normal distribution: two-sided independent t-test
        - In case of no normal distribution: two-sided Mann-Whitney U test
- Accounting of Missing, Unused and Spurious Data

Only if one value of the parameter MGI is missing after Day 1, the last available observation will be carried forward (LOCF). The analysis will be performed on the substituted data. For secondary parameters, no replacement of missing values will be applied.

A generalized linear regression model will be used to adjust for all identified confounders. To identify possible predictors of primary and secondary study endpoints, a regression model will be estimated in which each study endpoint will be entered as the dependent variable. Possible predictors will be entered in the model as

independent variables (including study groups, age, gender, cigarette consumption, C-F NDS usage, and frequency of personal oral hygiene – tooth brushing, mouth washing, and dental flossing frequency) to assess the interactions. Following the results obtained from each regression model, comparisons between and within study groups will be performed using ANCOVA adjusted for age, gender and frequency of personal oral hygiene followed by Tukey's post hoc comparison test.

The goal of our study is to help smokers to abstain from cigarette smoking to assess changes in oral health regardless of the product used. The study is not designed to compare efficacy between HTPs and ECs in term of smoking cessation. Nonetheless, we will consider a secondary analysis that takes into account potential different impact of HTPs vs ECs in term of oral health outcomes.

RESULTS

Subject recruitment has started in October 2021 and enrolment is expected to be completed by August 2023. Results will be reported in 2025.

DISCUSSION

Although the negative effects of cigarette smoking on oral health and teeth discoloration are well-known [34,35], there is only limited data about the impact of combustion-free nicotine alternatives (C-F NA) like e-cigarettes (ECs) and heated tobacco products (HTPs). In particular, there are no long-term studies assessing the impact on oral health and teeth appearance when substituting conventional cigarettes for these combustion-free alternatives. The study was specifically designed to address these research questions. In particular, this study tests the hypothesis that avoiding exposure to cigarette smoke toxicants may translate into measurable amelioration in gingival response, dental plaque build-up, enamel discoloration, and tooth staining in subjects with mild-moderate gingivitis by comparing subjects who smoke tobacco cigarette with those who switch to using C-F NA or subjects who never smoked.

Besides the obvious relevance to health and aesthetic concerns, the study seeks also to expand on regulatory science. Regulatory authorities (e.g. FDA in the US) recommend investigating oral health effects of novel tobacco/nicotine products to better understand their impact at individual and population level as well as exploring additional study endpoints for the assessment of their short- and long-term effects in oral health studies [36]. Our study is designed to provide improved design knowledge for oral health switching studies and, most importantly, streamline new innovative cutting-edge technologies that can be used for future studies on existing and emerging tobacco/nicotine products (including oral smokeless products).

The study's design incorporates several significant and groundbreaking characteristics. First, we aim to enhance adherence to C-F NA and optimize overall compliance with the study's instructions through the provision of a diverse range of products, encompassing the most popular options available in the market. This approach allows participants to tailor their own gratifying "nicotine experience" by selecting the C-F NA that aligns with their preferences. By doing so, we anticipate not only fostering a transition to the latest technology, but also facilitating the reduction of cigarette smoking and bolstering long-term prevention of relapse [37-41]. It is worth highlighting that this aspect of personal choice is notably absent in studies of harm reduction. Furthermore, it is important to emphasize that the outcomes of this research will not be product-specific, thereby minimizing constraints on generalizability.

Second, the study is being run in many different locations across the globe and so the challenge was to find a primary endpoint that could be measured by different operators in different sites, using a standardized measurement system that has been used previously and so has provenance. The index to be used had to be simple, reproducible and relatively easy to compare between different sites and different operators. Ideally

the assessors should all be able to calibrate in the index to be used and the calibration results compared. The Modified Gingival Index (MGI) is a very widely used, industry standard index used to determine changes in the gingival health of volunteers in clinical studies [28,42,43]. This index is simple non-invasive and reproducible and it is hoped to be possible to train different examiners in different locations to align in their assessments thus making the results comparable. Since the MGI is noninvasive there is no gingival probing, and it is thought to be easier to calibrate examiners and compare kappa scores. Although still subjective in nature the lack of probing removes one of the variables present in the using pressure to probe the gingivae. It is also though to afford greater sensitivity in determining therapeutic efficacy [44]. The study will be the first one to investigate the long-term impact of smoking or CF-NA intervention on MGI.

Third, the selection of secondary study endpoints is strategic, because it takes into account what drives smokers to turn to cleaner nicotine/tobacco products. This is particularly persuasive for young adults for whom a cardiovascular-cancer-respiratory risk-based narrative is either ineffective or even counterproductive and for whom concern about bad breath and poor dental aesthetic (due to enamel discoloration and “tar” stains) may be a much more significant reason to refrain from smoking. Although experimental work comparing the effect of C-F NA on tooth staining and discoloration has been published [45-47], this study is the first to consider this powerful narrative of oral health in a clinical trial; we have therefore included innovative 21st century technologies for objective and consistent quantitation of dental shade discoloration (by calibrated spectrophotometry) and dental plaque changes (by digital imaging technology; QLF - Quantitative Light-induced Fluorescence) among the study endpoints to investigate if switching completely from cigarettes to C-F NA can improve gum health, reduce bad breath and restore teeth appearance. Small-scale clinical trials have been recently conducted at the Center of Excellence for the Acceleration of Harm Reduction (CoEHAR), of the Catania University to confirm the validity and good reproducibility of dental shade assessment by digital spectrophotometry and dental plaque quantitation by light-induced fluorescence technology, in current, former, and never smokers [48,49].

Fourth, maximizing the magnitude of observable changes of study endpoints is critically important. Given that several factors (including duration of smoking exposure, variations in oral hygiene practices, type of diet, level of alcohol consumption) can significantly affect assessments of gum health and dental esthetics, we reduced baseline variability by using data obtained after the visit during which scaling and polishing was carried out. By removing dental plaque, calculus and stains, we give all study participants the best possible oral health status (gum health and teeth appearance) at the beginning of the study. Considering that a 14-day interval is generally required to allow gingival restoration from tissue trauma caused by scaling and polishing [50], measurements that will be considered for this normalized baseline will be obtained 14 days after scaling and polishing. Any progressive change in gum health or teeth appearance will be compared to the reference data of this baseline. In addition to the inclusion of a full scale and polish at the beginning of the study in an attempt to maximize the magnitude of observable changes of study endpoints, it is also important to consider and that the length of the study is adequate and that the study population will have to be one which allows the possibility of measuring such a change. Although the long-term impact of smoking and smoking cessation on gingival health and teeth appearance has never been investigated in a prospective trial, the planned 18 months’ duration of the study is deemed to be adequate for the detection of significant changes in study endpoints. Chronic periodontal disease is common in smokers and is an irreversible condition which may stabilize with active treatment [51], but is unlikely to actually improve with the simple measure of smoking cessation alone. Therefore, subjects with periodontitis will be excluded and only subjects with mild-moderate gingivitis will be recruited, as they are more likely to show measurable changes in study endpoints with continuing to smoke, smoking cessation and/ or the use of C-F NA.

Fifth, personal oral hygiene and dietary patterns can significantly influence both primary and secondary study endpoints. To mitigate this impact, a standardized approach to oral hygiene will be implemented within the study parameters. Participants will be explicitly advised against altering their established oral hygiene practices for the duration of the study. Furthermore, adherence to specific restriction criteria before each

scheduled study visit will be emphasized, aiming to prevent any confounding of the collected data. Recognizing the variable effects stemming from individual oral hygiene and dietary habits, we have incorporated a cohort of never-smokers as a reference group. This inclusion serves to establish a benchmark for comparison and analysis. To tackle the inherent challenge of ensuring consistent measurement of study endpoints, especially in cases involving diverse operators across multiple sites, we will provide comprehensive training and implement meticulous calibration procedures.

Last but not least, compliance with the research protocol is important because it would decrease or nullify the anticipated improvements in study endpoints if cigarettes were not fully or largely replaced with C-F NA. Participants will be reminded on the importance of adhering to their randomized product allocation and of abstaining from or substantially reducing the daily consumption of cigarettes (by at least 90% of their regular cigarette smoking at baseline) at every study visit. A significant feature of switching studies is close reporting of cigarette consumption and/or C-F NA use; participants will record the consumption of cigarettes and use of C-F NA on each visit in their study diary. In addition, subjects will be asked to return all empty, part-used, and unused consumables. Throughout the study, smoking and C-F NA usage will also be monitored via APP technology. The SMILE tracker App is an integral component of the Smile Study, designed to monitor participants' behaviors and lifestyle choices. Through daily prompts, the app assists in tracking cigarette consumption, usage of combustion-free nicotine delivery systems (C-F NDS), and regular oral hygiene practices such as brushing, flossing, and mouthwash use. Additionally, it functions as a personal diary for participants to log any alterations in their oral care routine. Moreover, the SMILE Tracker App features a system to monitor protocol adherence, identify deviations, and gather data on any potential Adverse Events (A/E) and Serious Adverse Events (SA/E). It sends automated reminders to participants for their upcoming appointments, study restrictions, and provides instructions to ensure adherence and accurate data collection during the study. Furthermore, it includes a step-counter to encourage physical activity among users. Of note, non-compliance with study items is an interesting outcome in itself (particularly in consideration of the wide selection of different products offered in the study). Although compliance with this study is not expected to be significantly different compared to other comparable studies, our power calculations are overestimated to take account of a 75% non-compliance rate. The C-F NA population would therefore be overrepresented by recruiting four times the number of subjects in the C-F NA category (i.e., for every subject randomized in the control population, four will be randomized in the C-F NA population).

The acknowledgment of the impact of COVID-19 restrictions on recruitment is duly noted. However, it is deemed unlikely that these restrictions will exert a significant impact. This assertion is grounded in the fact that recruitment proceedings were initiated in October 2021, a period during which well-defined guidelines had already been established at the dental clinic across all four participating sites.

CONCLUSION

This study represents a pioneering effort in assessing the long-term effects of smoking and smoking abstinence on oral health. Through a prospective approach, it aims to provide novel insights into the relationship between smoking habits and oral well-being. The data derived from this study will significantly enhance our existing understanding of the impact of smoking on oral health. Moreover, the study's outcomes hold the potential to shed light on the role of C-F NA as a potentially cleaner nicotine alternative to tobacco smoking. This is particularly relevant for individuals who prioritize concerns such as bad breath and dental aesthetics. The study's emphasis on oral health aligns with a broader objective of alleviating the burden of smoking. Several parameters measured in this study (including MGI and dental plaque build-up) are linked to the development of periodontal disease. It is noteworthy that the progression from chronic gingivitis to periodontitis raises the risk of cardiovascular disease [4,5], thereby underscoring the broader health implications of the study's results.

DECLARATIONS

Ethics approval and consent to participate
The study was approved by the ethics review board of the coordinating center ("Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione "Garibaldi"). Informed consent was obtained from each subject.

Consent for publication
Not applicable.

Availability of data and materials
Data sharing not applicable to this article as no datasets were generated or analyzed yet during the current study. The datasets used and/or analysed during the current study will be available from the corresponding author on reasonable request.

Competing interests
RP is full tenured professor of Internal Medicine at the University of Catania (Italy) and Medical Director of the Institute for Internal Medicine and Clinical Immunology at the same University. He has received grants from U-BIOPRED and AIR-PROM, Integral Rheumatology & Immunology Specialists Network (IRIS), Foundation for a Smoke Free World, Pfizer, GlaxoSmithKline, CV Therapeutics, NeuroSearch A/S, Sandoz, Merk Sharp & Dohme, Boehringer Ingelheim, Novartis, Arbi Group Srl., Duska Therapeutics, Forest Laboratories, Ministero dell Universita' e della Ricerca (MUR) Bando PNRR 3277/2021 (CUP E63C22000900006) and 341/2022 (CUP E63C22002080006), funded by NextGenerationEU of the European Union (EU), and the ministerial grant PON REACT-EU 2021 GREEN- Bando 3411/2021 by Ministero dell Universita' e (MUR) – PNRR EU Community. He is founder of the Center for Tobacco Prevention and Treatment (CPCT) at the University of Catania and of the Center of Excellence for the Acceleration of Harm Reduction at the same university. He receives consultancy fees from Pfizer, Boehringer Ingelheim, Duska Therapeutics, Forest Laboratories, CV Therapeutics, Sermo Inc., GRG Health, Clarivate Analytics, Guidepoint Expert Network, and GLG Group. He receives textbooks royalties from Elsevier. He is also involved in a patent application for ECLAT Srl. He is a pro bono scientific advisor for Lega Italiana Anti Fumo (LIAF) and the International Network of Nicotine Consumers Organizations (INNCO); and he is Chair of the European Technical Committee for Standardization on "Requirements and test methods for emissions of electronic cigarettes" (CEN/TC 437; WG4).
SAP is a professional dentist and the Chairman of the Board of Directors of ECLAT srl, the spin-off of the University of Catania that sponsors this study through a grant from the Foundation for a Smoke-Free World Inc, a US nonprofit 501(c)(3) private foundation.
PC has been affiliated to the CoEHAR since December 2019 in a pro bono role. He is co-author of a protocol paper supported by an Investigator-Initiated Study award program established by Philip Morris International in 2017.
All other authors have no conflict of interest to declare.

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Authors' contributions

GC, DG, PC, RM, and RP conceived and designed the study. GC, DG, and RM formalized the statistical analysis plan. GC, DG, SU, and RP wrote the first draft. SU designed Figures and Tables. All authors provided critical revisions of the working draft. All authors read and approved the submitted manuscript.

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Legend to Figures

Fig. 1 Study design. Smokers not intending to quit and without clinical signs of periodontitis were randomized to either standard care (Arm A) or C-F NA (Arm B). A reference group of never-smokers will be also included, but not randomized (Arm C). Subjects were prospectively reviewed at four dental clinics for cigarette consumption and C-F NA use, oral health and dental aesthetic measurements, vital signs, and adverse events for up to 18-months.

Fig 2. Study flow diagram of study participants.

Figure 3.: Macpherson modification of Lobene

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Table 1a: Study Schedule of Procedures (Control Group - Arm A)

Procedure	**Screening**	**Enrolment (V0)** Day 0	**Baseline (V1)** Day 14±3	**Week 12 (V2)** Day 90 ±5	**Week 24 (V3)** Day 180 ±7	**Week 52 (V4)** Day 360 ± 7	**Week 76 (V5)** Day 540 ±7
Eligibility Criteria Check	**X**	**X**					
Medical/Oral Health Hx	**X**						
Smoking Hx	**X**						
Intention to Quit Smoking	**X**						
Smoking Cessation Advice	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Cigarette Consumption	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Exhaled CO	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Interest to switch to C-F NA	**X**						
Informed Consent		**X**					
FTND		**X**					
Oral hygiene check	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Periodontal Examination	**X**						
Scaling & Polishing		**X**					**X**
Randomization		**X**					
Tracker APP installation		**X**					
BP, HR, Weight, Height, BMI		**X**	**X**	**X**	**X**	**X**	**X**
MGI Assessment		**X**	**X**	**X**	**X**	**X**	**X**
Plaque Score Imaging		**X**	**X**	**X**	**X**	**X**	**X**
Dental Shade quantitation		**X**	**X**	**X**	**X**	**X**	**X**
Tooth Stain Assessment		**X**	**X**	**X**	**X**	**X**	**X**
QoL questionnaires (OHQOL, EQ VAS)		**X**	**X**	**X**	**X**	**X**	**X**
Safety reporting		**X**	**X**	**X**	**X**	**X**	**X**

Abbreviations

V=visit; Hx=history; CO=carbon monoxide; BMI=body mass index; FTND= Fagerstrom Test for Nicotine Dependence; MGI=Modified Gingival Index; OHQoL=Oral Health Quality of Life; EQ VAS= EuroQoL Visual Analog Scale;

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Table 1b: Study Schedule of Procedures (Intervention Group - Arm B)

Procedure	**Screening**	**Enrolment (V0)** Day 0	**Baseline (V1)** Day 14±3	**Week 12 (V2)** Day 90 ±5	**Week 24 (V3)** Day 180 ±7	**Week 52 (V4)** Day 360 ± 7	**Week 76 (V5)** Day 540 ±7
Eligibility Criteria Check	**X**	**X**					
Medical/Oral Health Hx	**X**						
Smoking Hx	**X**						
Intention to Quit Smoking	**X**						
Smoking Cessation Advice	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Cigarette Consumption	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Exhaled CO	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Interest to switch to C-F NA	**X**						
Informed Consent		**X**					
FTND		**X**					
Oral hygiene check	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Periodontal Examination	**X**						
Scaling & Polishing		**X**					**X**
Randomization		**X**					
Familiarization with C-F NA			**X**				
C-F NA use				**X**	**X**	**X**	**X**
Tracker APP installation		**X**					
BP, HR, Weight, Height, BMI		**X**	**X**	**X**	**X**	**X**	**X**
MGI Assessment		**X**	**X**	**X**	**X**	**X**	**X**
Plaque Score Imaging		**X**	**X**	**X**	**X**	**X**	**X**
Dental Shade quantitation		**X**	**X**	**X**	**X**	**X**	**X**
Tooth Stain Assessment		**X**	**X**	**X**	**X**	**X**	**X**
QoL questionnaires (OHQOL, EQ VAS)		**X**	**X**	**X**	**X**	**X**	**X**
Safety reporting		**X**	**X**	**X**	**X**	**X**	**X**

Abbreviations

V=visit; Hx=history; CO=carbon monoxide; BMI=body mass index; FTND= Fagerstrom Test for Nicotine Dependence; MGI=Modified Gingival Index; OHQoL=Oral Health Quality of Life; EQ VAS= EuroQoL Visual Analog Scale

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Table 1c: Study Schedule of Procedures (Reference Group - Arm C)

Procedure	**Screening**	**Enrolment (V0)** Day 0	**Baseline (V1)** Day 14±3	**Week 12 (V2)** Day 90 ±5	**Week 24 (V3)** Day 180 ±7	**Week 52 (V4)** Day 360 ± 7	**Week 76 (V5)** Day 540 ±7
Eligibility Criteria Check	**X**	**X**					
Medical/Oral Health Hx	**X**						
Smoking Hx	**X**						
Cigarette Consumption	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Exhaled CO	**X**						
Informed Consent		**X**					
Oral hygiene check	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Periodontal Examination	**X**						
Scaling & Polishing		**X**					**X**
Assignment to Arm C		**X**					
Tracker APP installation		**X**					
BP, HR, Weight, Height, BMI		**X**	**X**	**X**	**X**	**X**	**X**
MGI Assessment		**X**	**X**	**X**	**X**	**X**	**X**
Plaque Score Imaging		**X**	**X**	**X**	**X**	**X**	**X**
Dental Shade quantitation		**X**	**X**	**X**	**X**	**X**	**X**
Tooth Stain Assessment		**X**	**X**	**X**	**X**	**X**	**X**
QoL questionnaires (OHQOL, EQ VAS)		**X**	**X**	**X**	**X**	**X**	**X**
Safety reporting		**X**	**X**	**X**	**X**	**X**	**X**

Abbreviations

V=visit; Hx=history; CO=carbon monoxide; BMI=body mass index; FTND= Fagerstrom Test for Nicotine Dependence; MGI=Modified Gingival Index; OHQoL=Oral Health Quality of Life; EQ VAS= EuroQoL Visual Analog Scale

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Table 2: Study Schedule of C-F NA Supply and Use Checks (only for Arm B)

Procedure	Screen	Enrol (V0)	BL (V1)	Wk4	Wk8	Wk12 (V2)	Wk20	Wk24 (V3)	Wk32	Wk40	Wk48	Wk52 (V4)	Wk60	Wk68	Wk76 (V5)
C-F NA use			X	X	X	X	X	X	X	X	X	X	X	X	X
Provide C-F NA Device			X												
Hand out 2 wks supply of consumables*			X												
Hand out 4 wks supply of consumables*				X	X		X				X				
Hand out 2 x 4 wks supply of consumables*						X		X	X	X		X	X	X	X
Product use checks (collect used and unused consumables*)				X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations

Screen= screening; Enrol=enrollment; BL=baseline; V=visit; Wk=week

* Participants wishing to use a heated tobacco product (HTP) will receive one kit and supply of tobacco sticks of their choice; they will receive the number of tobacco sticks per day corresponding to the number of cigarettes smoked per day at baseline. Participants wishing to use a vaping product will receive one vaping kit and supply of e-liquids of their choice.

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ANNEX 1 - Study Activities

General Aspects

All participants will have to agree to:

- refrain from scaling and polishing procedures for the whole duration of the study (except for those provided in the protocol)
- avoid modifying their own habitual oral hygiene (e.g., mouthwash, mouth-rinse, interdental floss) for the whole duration of the study.
- refrain from flossing for at least 72 hours prior to each study visit.
- refrain from mouth-rinsing for at least 24 hours prior to each study visit.
- refrain from tooth brushing for at least 2 hours prior to each study visit.
- refrain from eating and drinking (except water) for at least 2 hours before each visit.
- abstain from smoking for 2 hours before each visit.
- complete questionnaire via the automated Tracker App during the entire period of the study.

Participants in Arm A will have to agree to:

- refrain from use of any nicotine or tobacco product other than their own conventional cigarettes brand.

Participants in Arm B will have to agree to:

- refrain from smoking or using any nicotine/tobacco product other than their assigned C-F NA.
- report any change in using their C-F NA or conventional cigarettes (as per automated Tracker App).
- return study devices, chargers and other accessories supplied to them for this study at the last visit.

All visits will need to be conducted in the morning, if possible, around the same time.

Screening Visit

The participant will be invited to attend a Screening Visit.
The objective of the research will be explained to the participant by a clinical site staff member, and the subject will be asked to take the time to read and review the study-specific Subject Information Sheet.
The subject will be asked to sign the study-specific Informed Consent Form (ICF) in the presence of a clinical site operations staff member prior to any screening procedures being performed. A participant, determined to be eligible during the Screening Visit, is being considered as enrolled into the study after signature of the ICF. Procedures conducted at the Screening Visit are presented in the Schedule of Assessments (see Table 2) and listed here:

- Documentation of socio-demographic data
- Review of smoking history
- Confirmation of participant not intending to quit smoking within the next 30 days -

for regular smokers only

- Smoking cessation advice - for regular smokers only
- Number of cigarettes smoked /day
- Carbon Monoxide breath test
- Question of participant about interest in switching to C-F NA (participants will be shown C-F NA devices at screening) - for regular smokers only
- Administration of Fagerström Test for Cigarette Dependence – for regular smoker only
- Documentation of medical history
- Review of Inclusion and Exclusion criteria check list
- Review of pregnancy status by self-reporting
- Review of oral hygiene history by questionnaire (see Appendix 2)
- Periodontal examination
- Instruction of participant to bring the toothpaste they are using to the next visit

Enrollment and Randomization (Visit 0)

Within 28 days of the screening visit, eligible participants will be invited to attend a study visit. A trained site staff member will perform following tasks (Schedule of Assessments; Table 2):

- Confirmation of participant not intending to quit smoking within the next 30 days - for regular smokers only
- Smoking cessation advice - for regular smokers only
- Number of cigarettes smoked /day
- Carbon Monoxide breath test - for regular smokers only
- Review of Inclusion and Exclusion criteria check list
- Review of pregnancy status by self-reporting
- Review of oral hygiene history
- Participant’s name and brand of toothpaste will be noted
- Randomization of regular smokers to Arm A or B, and assignment of never-smokers to Arm C
- Smokers assigned to Arm B will be asked to familiarize with the C-F NA of their own choice
- Upload of the Tracker APP on participant’s smartphone and complete the questionnaire
- Administration of OHQOL (Oral Health Quality of life) questionnaire

- Administration of EQ VAS – QoL questionnaire
- Measure of vital signs (blood pressure and heart rate)
- Determine height, weight
- Determination of MGI
- Plaque Score Imaging
- Tooth Stains Assessment
- Assessment Dental Discolorations
- Dental Scaling & Polishing to remove plaque, calculus and stains
- Review of AEs and SAEs
- Instruction of participants to bring the toothpaste he/she is using to the next visit

Participants will be reminded not modify their own habitual oral hygiene (e.g., mouthwash, mouth-rinse, interdental floss) pattern for the whole duration of the study. They will be also asked to not change the brand of toothpaste during the study. However, if this has occurred, participants will have to inform the study site investigator.

Smokers randomized into Arm B (switching to C-F NA use) will be asked to try and familiarize themselves with the selection of C-F NA provided by the study investigator in order to choose the product of their preference. They will be trained and counselled on the chosen C-F NA.

After installing the APP for behavioral/lifestyle tracking on participants' smartphones, they will be given instructions and asked to familiarize themselves with the Tracker APP.

Baseline Visit (Visit 1)

At day 14, all participants will attend a baseline visit, during which they will undergo the following assessments (Schedule of Assessments; Table 2):

- Smoking cessation advice - for Arm A and B only
- Number of cigarettes smoked /day
- Carbon Monoxide breath test - for Arm A and B only
- Review of oral hygiene history
- Participant's name and brand of toothpaste will be noted
- Administration of OHQOL (Oral Health Quality of life) questionnaire
- Administration of EQ VAS – QoL questionnaire
- Measure of vital signs (blood pressure and heart rate)

- Determination of MGI
- Plaque Score Imaging
- Tooth Stains Assessment
- Assessment Dental Discolorations
- Switch to C-F NA – for Arm B only
- Check of AEs and SAEs (to be reported in eCRF)
- Instruct participants to bring the toothpaste he/she is using to the next visit

Visit 2 to Visit 5

Participants will return to the clinic for their scheduled visits and at each visit they will undergo the following assessments (Schedule of Assessments; Table 2):

- Smoking cessation advice - for Arm A and B only
- Number of cigarettes smoked /day
- Review of C-F NA use – for Arm B only
- Carbon Monoxide breath test - for Arm A and B only
- Review of pregnancy status by self-reporting
- Review of oral hygiene history
- Participant's name and brand of toothpaste will be noted
- Administration of OHQOL (Oral Health Quality of life) questionnaire
- Administration of EQ VAS – QoL questionnaire
- Measure of vital signs (blood pressure and heart rate)
- Determine height, weight
- Determination of MGI
- Plaque Score Imaging
- Tooth Stains Assessment
- Assessment Dental Discolorations
- Check of AEs and SAEs (to be reported in eCRF)
- Instruct participant to bring the toothpaste he/she is using to the next visit (except last visit)

At the end of or after Visit 5, all participants will be offered a final dental scaling & polishing

to remove plaque, calculus and stains.

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Annex 2 - STUDY PROCEDURES

Specific Restrictions/Requirements

Oral Hygiene

In relation to their oral hygiene habits (e.g. tooth brushing, mouth rinse, interdental), participants will have to adhere to the following restrictions:

- avoid scaling and polishing procedures for the whole duration of the study (except for those provided in the protocol)
- avoid modifying their own habitual oral hygiene (e.g., mouthwash, mouth rinse, interdental floss) for the whole duration of the study.
- refrain from flossing for at least 72 hours prior to each study visit.
- refrain from mouth rinsing for at least 24 hours prior to each study visit.
- refrain from tooth brushing for at least 2 hours prior to each study visit.

Oral hygiene habits will be documented at the baseline and any change in products has to be reported to the Investigator and documented in the Tracker App.

Smoking Status

Self-reported smoking status (i.e. number of cigarettes smoked on a daily basis) will be objectively verified by performing an exhaled breath CO test. Regular smokers in Arms A to B will only be enrolled into the study if their exhaled breath CO level is greater than 7 ppm. Participants will be instructed to refrain from smoking for 2 hours prior to each clinical visit.

Diet and alcohol Restrictions

Participants will be instructed not to eat and drink (except water) for at least 2 hours prior to each day of assessments

Clinical Assessments

All measurements will be taken at the days indicated in the Schedule of Procedures (Table 1a-c)

Demographic Data

Socio-demographic data (sex, age, race) will be recorded at the Screening Visit.

Smoking History and Interest to Switch

Participants will be asked about their smoking history at Screening. This will include questions to:

- evaluate whether the participant has smoked for at least the last 5 consecutive years,
- determine the number of conventional cigarettes smoked by the participant per day on average,
- check that the participant has smoked their chosen brand of conventional cigarette consistently for at least 6 months, and
- determine if the participant regularly uses any nicotine or tobacco product

other than their habitual used commercially manufactured filter cigarettes.
- assess whether the participant is planning to quit smoking within the next 30 days
- assess if he/she is interested to switch to C-F NA.

Participant's name and brand cigarette pack will be noted in the eCRF.

Medical History and Concomitant Diseases

Co-morbidities and significant, uncontrolled medical condition, as determined by the Investigator, will be documented at screening. Gingivitis, however, will not be added as a co-morbidity in the medical history.

Vital Signs

Supine blood pressure and supine pulse rate will be measured at the times indicated in the in the schedule of assessments (Table 2) and can also be performed at other times if judged to be clinically necessary.
Blood pressure and pulse rate will be measured using automated monitors. Participants must be at rest and supine for at least 5 minutes before blood pressure and pulse rate measurements.

Height, Body Weight, and BMI

Height in meters (to the nearest cm), and body weight (BW) in kilograms (to the nearest full kilogram) in underclothing and without shoes will be measured (if possible) or asked to the participant at Screening. The BMI will be calculated by the eCRF by using the formula: BW (kg) / [height (m)]^2

Oral Health Parameters and Evaluations

Modified Gingival Index (MGI)

The MGI, as descripted by Lobene et al. (1986) adopt the following criteria:

- 0 = absence of inflammation;
- 1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary;
- 2 = mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary;
- 3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary;
- 4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.

The MGI of the individual will be obtained by adding the values of each tooth and dividing

by the number of teeth examined. Therefore, the MGI will be assessed in all scorable teeth, except the third molars.

Dental Plaque Imaging

Fluorescence photographs of the vestibular aspect of the anterior teeth (cuspid to cuspid, upper and lower jaw) in end-to-end position will be taken using Q-ray cam Pro (Co., AIOBIO, Seoul, Republic of Korea) and cheek retractors (Henry Schein, Gillingham, UK, Double end large, 106-7079). The Q-ray cam Pro will be vertically placed to the anterior tooth surface with the cone of the device closely attached to the mouth to take images with a standardized depth of focal length (0.32). Focus will be adjusted to the maxillary lateral incisor and canine.
All the analysis of the QLF-D images will be performed in Catania by a single, blinded operator, using a specific centralized software setting. Dental plaques will be assessed with subscores of ΔR30 and ΔR120, according to the fluorescence intensity. QLF images will not be uploaded in the e-CRF.

Tooth Stain Assessment

The tooth stain assessment according to Macpherson modification of Lobene [24] consists of dividing each analyzed tooth into 4 separate sites (Fig. 3):

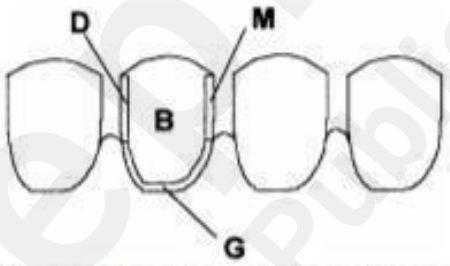


Figure 3.: Macpherson modification of Lobene

1. gingival (G): 2 mm wide strip running parallel to the gingival margin. The limit towards the incisal edge given by the end of the interdental papilla;
2. body of tooth (B): central area of buccal/lingual aspect, between gingival and distal/ mesial sites, extending to incisal edge;
3. mesial (M): visible area between line angle and adjacent tooth, ending at the interdental papilla (i.e. start of gingival site);
4. distal (D): as for mesial (M)site

The index consists of a scoring point for each site analyzed representing the intensity and the extent of the stain.
The criteria for the intensity are:

. no stain present, natural tooth color
1. faint stain
2. clearly visible stain, orange to brown
3. dark stain, deep brown to black

The area criteria and codes extent are:

1. up to 1/3 of area affected
2. between 1/3 and 2/3 of area affected
3. more than 2/3 area affected

Before scoring, participants will be asked to rinse their mouth with water to remove food debris. The index teeth will be dried using a chair-side air syringe and kept dry throughout the examination.
The index will be assessed on the facial surface of the anterior teeth (cuspid to cuspid, upper and lower jaw) recorded and calculating the mean directly on the e-CRF.

Dental Discolorations

Color change of social six (cuspid to cuspid, up and lower jaw) will be measured using a calibrated spectrophotometer. CIELAB [lightness ranging from 0 (black) to 100 (white)], a* (red/green), b* (yellow/blue)] color parameters will be calculated for each anterior tooth. A standardized protocol for tooth preparation and in vivo spectrophotometric evaluation will be adopted for all participants. Measurements will be performed in the same examination room with standardized lighting conditions by the same operator.
The spectrophotometer will be calibrated according to the manufacturer's instructions and a sterile mouthpiece will be placed on the optic handpiece for each subject. Three consecutive measurements will be taken for each parameter and the mean after measurements will be considered the baseline value. The CIELab color difference (ΔE*ab) will be calculated as follows: ΔE*ab = [(ΔL*)2 + (Δa*)2 + (Δb*)2]1/2; where ΔL*, Δa*, and Δb* correspond to the difference between each visit and the baseline L*, a*, and b* values, respectively. All the data will be collected as tables of numerical values and directly imported in the e-CRF.

Periodontal Evaluation

In order to exclude participants with early periodontal disease, participants who meet one of the following clinical periodontal parameters [23] will be excluded from study participation:

- Detectable Interdental Clinical Attachment Loss (CAL) ≥ 3 mm at ≥ 2 non-adjacent teeth.
- Buccal or Oral CAL ≥3 mm with pocketing ≥3 mm detectable at ≥2 teeth.

The observed CAL will be ascribed to non-periodontal causes if it will be aroused by:

1. gingival recession of traumatic origin;
2. dental caries extending in the cervical area of the tooth;
3. the presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar,
4. an endodontic lesion draining through the marginal periodontium;
5. the occurrence of a vertical root fracture.

The periodontal evaluation will be performed with a standardized periodontal probe in all scorable teeth, except third molars.
Finally, as part of oral hygiene history, the numbers of missing, decayed and filled teeth will be also recorded.

https://preprints.jmir.org/preprint/53222 [unpublished, peer-reviewed preprint]

Other Assessment

Exhaled Carbon Monoxide

The participants' smoking status will be objectively verified using an exhaled CO measurement (exhaled CO ≥7 ppm). Exhaled CO (eCO) levels will be measured using a portable CO monitor (MicroCO, Vyaire). Participants will not be allowed to use any study products/smoke any conventional cigarettes within 2 hours prior to CO level measurements.

Oral Health Quality of Life (OHQoL)

The subjective evaluation of participants' oral health will be assessed by using the Oral health related quality of life (OHQoL) questionnaire. The questionnaire was developed based on a general population's perceptions of how oral health affects life quality [(25)]. OHQoL consists of a pool of 3-items: daily work, social activities and communication problems. OHQoL questionnaire will be administered at study visits indicated in the Schedule of Procedures (Table 1 a-c)

Fagerström Test for Cigarette Dependence (FTCD)

Potential nicotine dependence will be assessed via a questionnaire at Screening and the study visits indicated in the Schedule of Procedures (Table 1 a-c) using a FTCD questionnaire in its revised version (Appendix 4). The questionnaire consists of 6 questions which will be answered by the participant himself/herself. The scores obtained on the test permit the classification of nicotine dependence into 3 levels: Mild (0-3 points), moderate (4-6 points), and severe (7-10 points).

EuroQol Visual Analog Scale (EQ VAS)

Quality of life and self-reported health condition will be assessed using EQ VAS questionnaire (Appendix 5). EQ VAS questionnaire will be administered at study visits indicated in the Schedule of Procedures (Table 1 a-c)

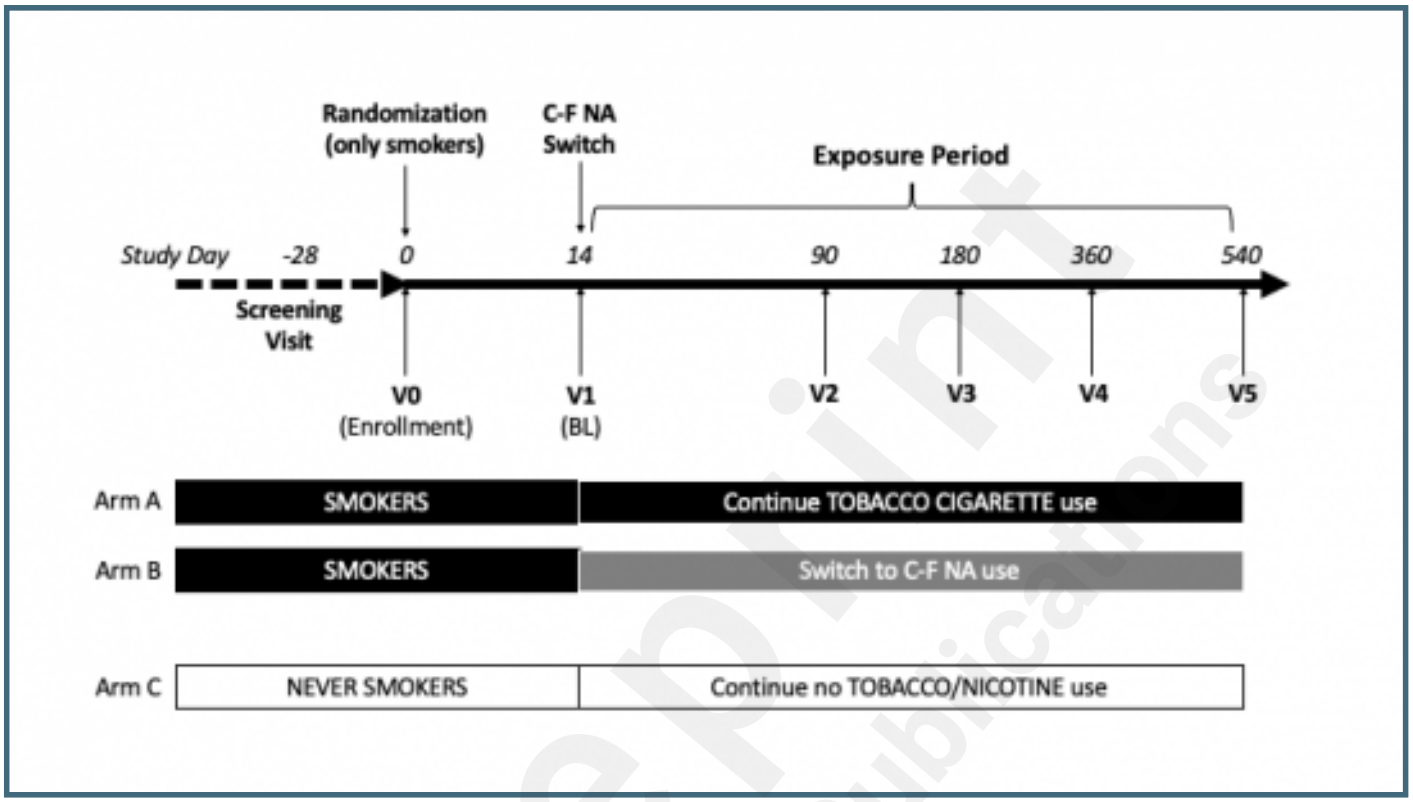
Supplementary Files

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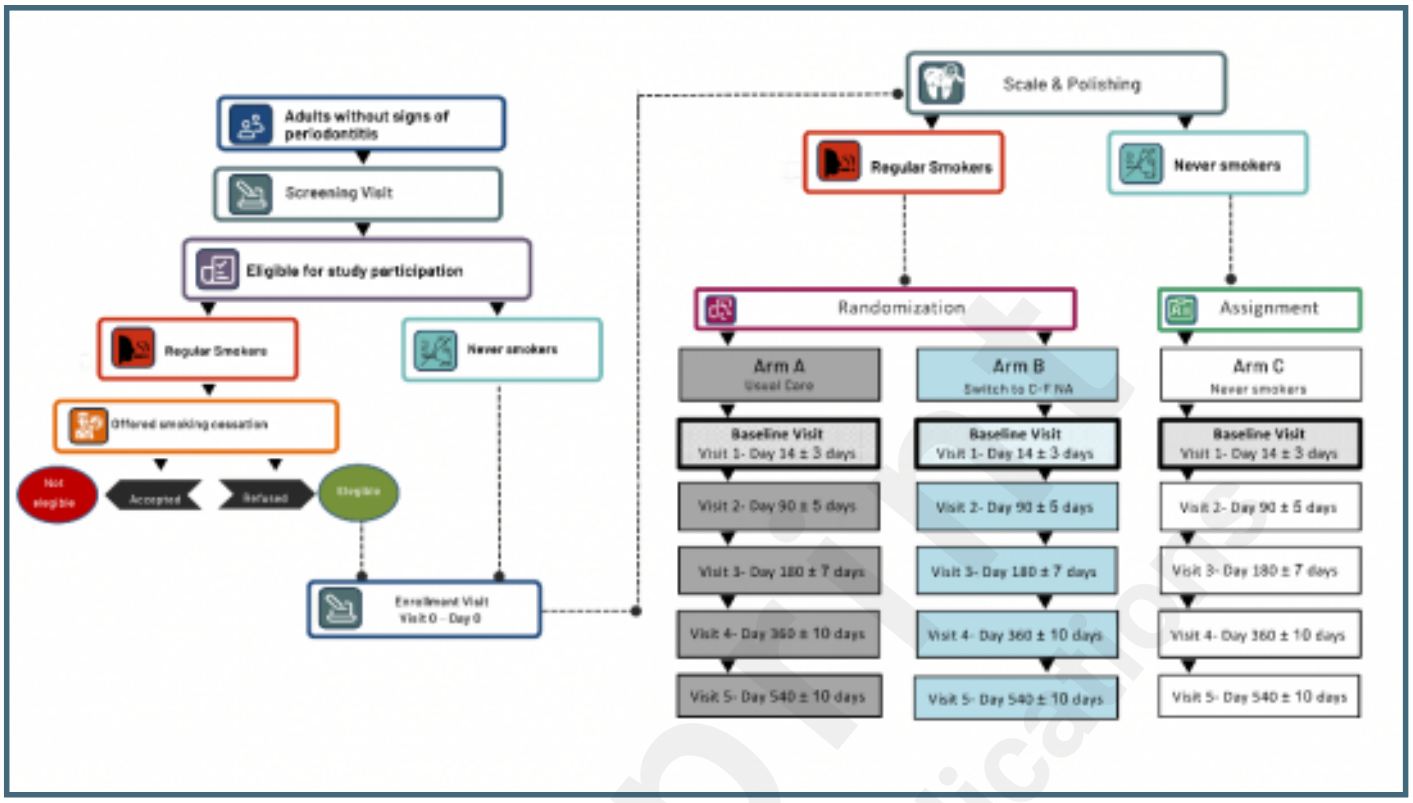
Figures

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Study design. Smokers not intending to quit and without clinical signs of periodontitis were randomized to either standard care (Arm A) or C-F NA (Arm B). A reference group of never-smokers will be also included, but not randomized (Arm C). Subjects were prospectively reviewed at four dental clinics for cigarette consumption and C-F NA use, oral health and dental aesthetic measurements, vital signs, and adverse events for up to 18-months.

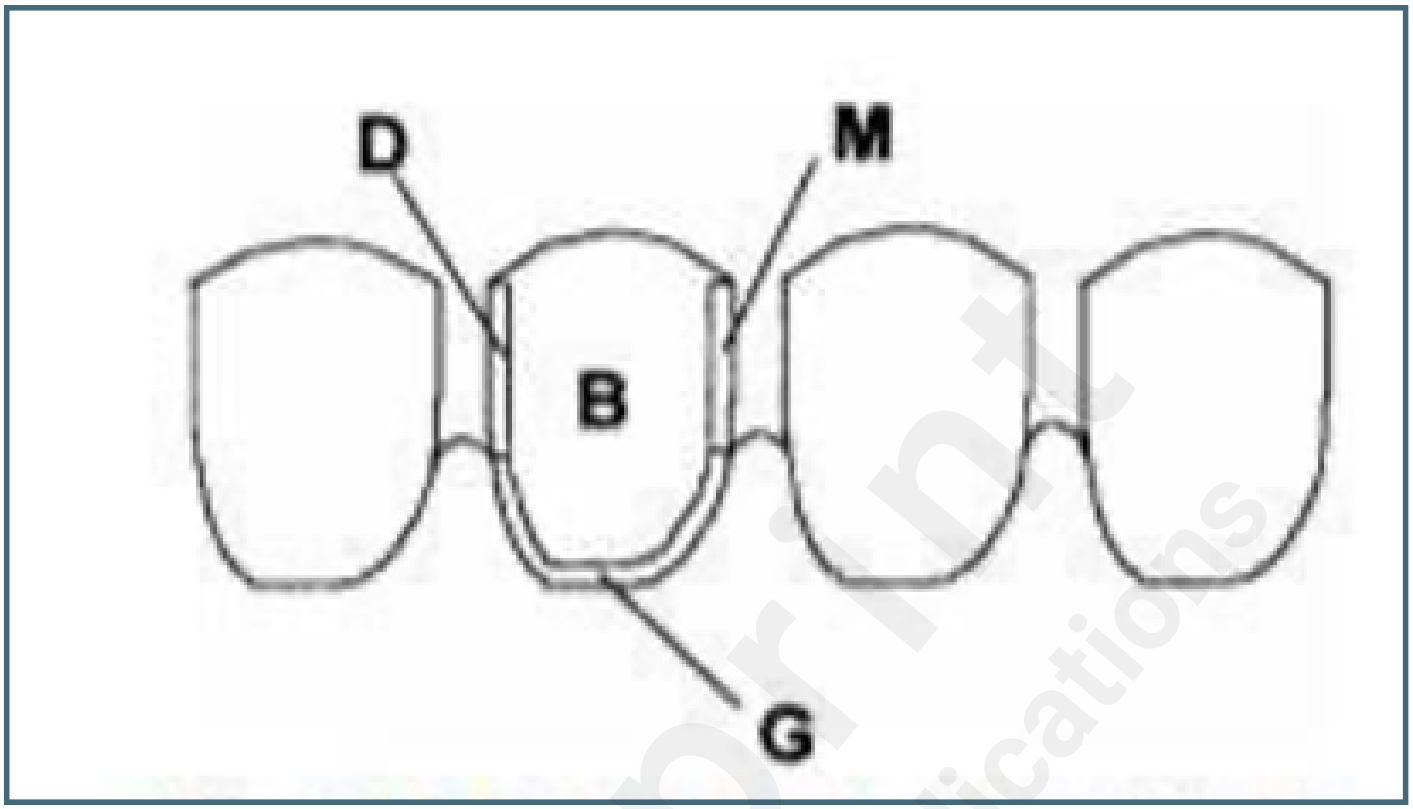


Study flow diagram of study participants.



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Macpherson modification of Lobene.



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