Clinical Study Protocol:
Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

This supplement contains the following items:

1. First protocol approved by Ethics Commission (version 2.0), last protocol approved (version 9.0), summary of changes (revision history).

2. Original statistical analysis plan (version 1.0), final statistical analysis plan (version 2), summary of changes (version 2 with tracked changes)

Clinical Study Protocol

Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

Study Type:	Other clinical trial
Study Categorisation:	Risk Category B
Study Registration:	Clinicaltrials.gov, Swiss National Clinical Trials Portal (SNCTP)
Study Identifier:	Swiss National Science Foundation # 173552
Sponsor-Investigator:	Prof. Dr. med. Reto Auer Berner Institut für Hausarztmedizin (BIHAM) Universität Bern Gesellschaftsstrasse 49 3012 Bern Schweiz Tel: +41 31 631 58 79 Fax: +41 31 631 58 71 reto.auer@biham.unibe.ch
Investigational Product:	Electronic nicotine delivery systems as smoking cessation aid
Protocol Version and Date:	2.0; 12th March 2018

Signature Page(s)

Study number	Swiss National Science Foundation # 173552
Study Title	Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

The Sponsor-Investigator and trial statistician have approved the protocol version 2.0, 12.03.2018, and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor-Investigator:

Prof. Dr. med. Reto Auer

Place/Date	Signature

Trial Statistician and Methodologist:

PD Dr. med. Sven Trelle

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site:

Principal investigator:

Place/Date	Signature

Table of Contents

STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	Prof. Dr. med Reto Auer (Sponsor-Investigator)
Study Title:	Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial
Short Title / Study ID:	ESTxENDS
Protocol Version and Date:	2.0, 12.03.2018
Trial registration:	Clinicaltrials.gov, Swiss national clinical trials portal (SNCTP)
Study category and Rationale	Other clinical trial, risk category B. ENDS and e-liquids are considered alimentary good in Switzerland and thereby regulated by the law on alimentary good. Sale and use of ENDS and e-liquids exempt from nicotine is permitted in Switzerland. Swiss residents can order nicotine-containing e-liquids from online stores abroad for personal use. Direct sale of nicotine-containing e-liquids is forbidden in Switzerland. Because Swiss law considers ENDS and e-liquids as alimentary good, and not as medicinal products or medical devices, Swissmedic has waived approval and surveillance. We will follow the rules set by the Federal Office of Public Health (FOPH) and the Federal Food Safety and Veterinary Office (FSVA) for the import of nicotine-containing e-liquids and have participants order the liquids from abroad and have the e-liquids sent directly to their home.
Clinical Phase:	n.a.
Background and Rationale:	Cigarette smoking is the leading cause of preventable death in Switzerland. Recently, electronic nicotine delivery systems (ENDS or vaporizers, also called e-cigarettes) have become popular with smokers who want to switch from tobacco cigarettes to ENDS to reduce their exposure to toxic compounds or to stop smoking. Only two rigorous RCTs have been published so far. They have promising, yet inconclusive results, as they were based on small samples. The safety and potential adverse effects of ENDS are also largely unknown. While the aerosol the users inhale appears safe in laboratory conditions, the difference in exposure to toxins (such as measures of exposure to organic compounds) and effect of toxins on the body (measures of oxidative stress) between smokers who quit (with or without ENDS) and those who use ENDS for a long time have not yet been assessed in an RCT.
Objective(s):	Test ENDS on: 1) the efficacy for cigarette smoking cessation and reducing the number of cigarettes smoked over 6 months of follow-up; 2) the safety of ENDS on adverse events; 3) the effect of ENDS on reducing exposure to inhaled toxic compounds; 4) the effect of ENDS on health-related outcomes (clinical outcomes: respiratory symptoms; surrogate outcomes: oxidative stress, risk factors for heart disease).

Outcome(s):	- The primary outcome will be the continuous smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report (self-report of no smoking from target quit date) and confirmed by exhaled CO level (CO<10 ppm) and urinary level of cotinine, anabasine (<3 ng/ml) and NNAL (10 pg/mg creat). - Secondary smoking cessation outcome will alternate methods to define the smoking cessation outcome: as the primary outcome, but allowing up to 5 cigarettes in total and a 2-week`grace period' after the target quit date, self-reported 7-day point prevalence abstinence, all confirmed by the exhaled CO level, urinary level of cotinine and anabasine, and change in the self-reported number of cigarettes smoked per day (CPD) with 50% reduction in CPD from baseline to follow-up considered as successful reduction. - Concentrations of urinary tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), aldehydes and polycyclic aromatic hydrocarbons (PAHs) for all participants. Participants will complete detailed questionnaire to account for other sources of contaminants. - Respiratory symptoms (self-report) - Oxidative stress assessed by 8-OHdG and 8-isoprostane concentrations in both exhaled breath condensate (EBC) and urine; - Cardiovascular risk factors (CVRF) assessed by HDL- and LDL-cholesterol, HbA1c, creatinine and glucose concentrations, blood pressure levels, waist circumference, and body mass index. - Adverse events (AEs), serious adverse events (SAEs) following international standards. - Mood, anxiety and sleep quality (Self report)
Study design:	Open-label randomized controlled trial
Inclusion / Exclusion criteria:	Inclusion criteria: - Informed Consent as documented by signature - Persons aged 18 or older - Currently smoking 5 or more cigarettes a day for at least 12 months - Willing to try to quit smoking within the next 3 months, - Personsn providing a valid phone number, a valid email address and/or a valid postal address. Exclusion criteria: - Known hypersensitivity or allergy to contents of the e-liquid - Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected - Women who are pregnant or breast feeding - Intention to become pregnant during the course of the study - Persons having used ENDS regularly in the 3 months preceding the baseline visit - Persons having used nicotine replacement therapy (NRT) or other drug therapy helping smokers quit (varenicline, bupropion) within the 3 months preceding the baseline visit - Plans to move out of the country within the next 6 months, or cannot attend the 6- month follow-up visit for any reason - Cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures

Measurements and procedures:	An overview on the study measurements and procedures is provided in the study schedules. The main measurements and procedures are the following: We plan to include 1172 smokers in a pragmatic RCT that will add ENDS to smoking cessation counselling (SCC), and compare ENDS to SCC alone. Participants in the intervention arm will receive free ENDS and nicotine-containing e-liquids ad libitum. Participants in both arms will receive SCC in four sessions over two months over the phone, and be allowed to use NRTs and other smoking cessation help in parallel. At baseline and 6-month follow-up, we will distribute questionnaires and perform a battery of clinical tests, including tests for cardiovascular risk factors (blood pressure, lipids, HbA1c, creatinine and glucose; body mass index), determine urinary biomarkers for tobacco-specific nitrosamines (TSNAs), nicotine metabolites (nicotine, cotinine, anabasine), metabolites of polycyclic aromatic hydrocarbons (PAHs) (1-hydroxypyrene, 1-and 2-naphthol)), volatile organic compounds (VOCs) and oxidative stress markers in urine and exhaled breath condensates (EBC) (8-hydroxy-2'-deoxyguanosine and 8- iso-prostaglandin F22α).
Study Product / Intervention:	Participants in the intervention arm will receive free ENDS and nicotine-containing e-liquids *ad libitum*; plus smoking cessation counseling (SCC). Participants will be allowed to choose the flavor and nicotine concentration of the e-liquids. Use of ENDS during the 6 months will be ad libitum and will be monitored by use of e-liquids in ml. Study nurses will provide the technical support for the use of ENDS and schedule a phone call on the participant's target quit date. On the target quit date as well as at week 1, 2, 4 and 8 after target quit date, study nurses will call participants for SCC and further technical support (see below).
Control Intervention:	Participants in the control group will receive SCC (standard of care). Nurses will be trained to deliver standardized SCC. SCC will be provided in person at the first clinical visit (baseline visit) and then over the phone at the target quit date) and again at week 1, 2, 4 and 8 after target quit date.
Number of Participants with Rationale:	Number of participats projected for the entire study: 1172; Intervention group: 586, control group: 586. Rationale see in section "Statistical considerations".
Study Duration:	3 years
Study Schedule:	First participant in: 04/18 Last participant out: 03/20

Investigator(s):	**Prof. Dr. med Reto Auer** Berner Institut für Hausarztmedizin (BIHAM) Universität Bern Gesellschaftsstrasse 49 3012 Bern +41 31 631 58 79 reto.auer@biham.unibe.ch **PD Dr. Nancy B. Hopf** Institut Universitaire de Santé au Travail University of Lausanne and Geneva Rue de la Corniche 2 Biopôle 1 1066 Epalinges +41 21 314 74 21 Nancy.Hopf@hospvd.ch **Prof. Dr. med. Jaques Cornuz** Policlinique Médicale Universitaire Université de Lausanne Rue du Bugnon 44 1011 Lausanne +41 21 314 47 32 Jacques.Cornuz@chuv.ch **Prof. Dr. med. Nicolas Rodondi** Klinik und Poliklinik für Allgemeine Innere Medizin Inselspital Freiburgstrasse 20 3010 Bern +41 31 632 41 63 Nicolas.Rodondi@insel.ch **PD Dr. med. Jean-Paul Humair** Département de médecine interne HUG Clinique de Médicine II Rue Gabrielle-Perret-Gentil 4 1211 Genève +41 22 372 95 10 Jean-Paul.Humair@hcuge.ch
Study Centre(s):	Three sites in Switzerland including University Hospitals Bern, Geneva, and Lausanne.

Statistical Considerations:	*Sample size:* Based on the results of a systematic review and meta-analysis of previous trials, we expect a RR of smoking cessation of 1.6; based on our experience with RCTs in the setting of smokers willing to quit and contacting health professionals to seek help receiving SCC alone, we expect a 12% quit rate in the control group and thus 19% in the intervention group (ENDS + SCC) (7% absolute difference in abstinence rate). With a two-sided alpha of 0.05 and a power of 90%, we will include a minimum 557 smokers per group, for a total of 1114 smokers, to find a significant difference in quit rates. Previous experience suggests we should assume a 5% loss to follow-up, so we will increase our sample size by 5% (59 smokers). We will thus recruit 1172 smokers. We will consider participants lost to follow-up to be continuing smokers, and our primary analysis will be intention-to-treat. Though participants in the control group will not be actively provided with ENDS and asked not to use ENDS during the first 6 months of the study, we expect 5% will still purchase ENDS on their own, and thus cross over from the control to the intervention group. The sample size of the entire trial is based on the primary outcome; the power to find a statistically significant difference for secondary outcomes is limited to the total number of participants. *Efficacy analysis:* To answer the primary research question, we will compare the rates of quitting among smokers randomized to ENDS vs. those receiving usual care, on an intention-to-treat basis. Continuous smoking cessation from target quit date will be the main outcome, confirmed by biomarkers of exposure (exhaled carbon monoxide (CO), urinary cotinine, anabasine and NNAL).[1] We will calculated quit rates, relative risks (RR), and absolute risks for ENDS +SCC (intervention group) compared to SCC alone (control group). We will further compare groups using multivariate regression models adjusting for appropriate co-variates. Participants lost during follow-up will be categorized as ongoing smokers in the intention-to-treat analysis. We intend to obtain full follow-up data on all randomized participants (see Follow-up and Retention). Participants who stopped smoking, and who are ongoing ENDS users, will be considered quitters. We will present per-protocol analyses in sensitivity analyses given that participants in the control group might start using ENDS in the first six months. We will repeat these calculations for the secondary smoking cessation outcomes such as alternate definitions of the outcome of having quit smoking: a.) <5 cig from target quit date and b.) 7-days point abstinence (no cigarettes within the 7 days before outcome assessment)). To test the effect of ENDS on reducing the number of cigarettes smoked per day, we will define a binary predictor of $\geq$50% smoking reduction from baseline to follow-up smoking and repeat the analyses for the main outcome of quitting. We will also use joint multivariate random-effects (JMRE) models allowing to model CPD and smoking cessation. In addition, we will use mixed linear effect model to analyze the change in CPD modelled as a continuous outcome over time. We will compare the proportion of adverse events and serious adverse events between groups using recommended methods. For the measures of changes in measures of urinary biomarkers, exhaled breath measures, CVRFs and respiratory symptoms from baseline to the 6-months visit, we will first compare the changes in these measures between the intervention and control group using two-sample t-tests or Wilcoxon rank-sum test, whenever appropriate. We will then fit univariate and multivariate mixed linear effect models. We will use multivariate linear and multinomial logistic regression models to compare groups according to their exposure to tobacco smoking and ENDS.

GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

STUDY SUMMARY IN LOCAL LANGUAGE

Hintergrund

Rauchen ist die häufigste vermeidbare Todesursache in der Schweiz. Immer mehr Raucher, die sich den giftigen Substanzen des Tabakrauchs weniger aussetzen möchten oder einen Tabakrauchstopp anstreben, steigen ganz oder teilweise auf tabakfreie Vaporizer (e-Zigaretten, engl. auch electronic nicotine delivery systems, ENDS) um, mit denen nikotinhaltige Flüssigkeit (e-Liquids) verdampft werden. Momentan ist jedoch noch umstritten, ob ENDS als Unterstützung zur Rauchentwöhnung wirklich generell von Nutzen sind und welche Risiken der Konsum von ENDS birgt.

Ziele des Projekts

Im Rahmen einer kontrollierten, randomisierten klinischen Studie werden wir 1172 RaucherInnen, die entweder eine Rauchstoppberatung alleine oder in Kombination mit ENDS erhalten werden über jeweils 6 Monate begleiten. Wir werden die Effektivität von ENDS zur Tabakentwöhnung prüfen, indem wir bestimmen, ob die Anzahl gerauchter Zigaretten mit Hilfe der ENDS gesenkt werden kann bzw. ein totaler Rauchstopp erreicht wird. Gleichzeitig werden allfällige Nebenwirkungen erfasst, um die Sicherheit der ENDS zu beurteilen. Weiter wird erhoben, inwiefern sich die Schadstoffexposition beim Konsum von ENDS im Vergleich zum Tabakrauchen ändert, und ob sich gesundheitliche Aspekte wie Atemwegsbeschwerden sowie Blut- und Urinwerte, beispielsweise bezüglich oxidativem Stress oder kardiovaskulären Risikofaktoren, verbessern.

Bedeutung

Bisher wurden zwei klinische Studien zu Rauchentwöhnung mit ENDS publiziert. Bezüglich Nutzen der ENDS zur Rauchentwöhnung sind die Resultate zwar vielversprechend, aber noch nicht genug aussagekräftig, da die Anzahl untersuchter Personen gering war. Daten zu Sicherheit und Schadstoffen der ENDS liegen noch kaum vor. Die Positionen der Gesundheitsfachleute und Entscheidungsträger der Politik sind kontrovers: Einige vertreten die Ansicht, dass ENDS als sichere Alternative zum konventionellen Tabakrauchen aktiv angepriesen werden sollten; andere sind der Meinung, dass man von deren Gebrauch abraten sollte, da sie gesundheitsschädlich sein könnten und nicht zum Rauchstopp beitragen. Die Resultate unserer Studie sollen demnach Konsumenten, Gesundheitsfachpersonen und Politikern unabhängige und fundierte Information zu Sicherheit und Schadstoffen von ENDS liefern und zu der Entscheidung beitragen, ob ENDS aktiv als Alternative zum Tabakrauchen angepriesen werden sollen. Die Studie soll zeigen, ob sich ENDS zur Rauchentwöhnung eignen und damit Raucher-assoziierte Erkrankungen und Folgekosten für das Gesundheitswesen reduziert werden und langfristig etliche Leben gerettet werden könnten, die durch Tabakrauchen gefährdet sind.

ABBREVIATIONS

AE	Adverse Event
ASR	Annual Safety Report
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CRF	Case Report Form
CO	Carbon monoxide
CPD	Cigarettes per day
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
CVRF	Cardiovascular risk factors
DSUR	Development safety update report
EBC	Exhaled breath condensates
ENDS	Electronic nicotine delivery devices
GCP	Good Clinical Practice
IB	Investigator's Brochure
HbA1c	Gylcated hemoglobin
HDL-cholesterol	High-density lipoprotein cholesterol
Ho	Null hypothesis
H1	Alternative hypothesis
HRA	Federal Act on Research involving Human Beings
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention to treat
LDL- Cholesterol	Low-density lipoprotein cholesterol
MD	Medical device
NRT	Nicotine replacement therapy
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	Nicotine-derived nitrosamine ketone (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone)
NNN	N'–nitrosonornicotine
PAH	Polycyclic Aromatic Hydrcarbons
PI	Principal Investigator
RCT	Randomized Clinical Trial
SCC	Smoking Cessation Counselling
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction

TMF	Trial Master File
TNSAs	Tobacco-specific nitrosamines
VOCs	Volatile organic compounds

STUDY SCHEDULE

Procedure	**Screening** ☽	**Baseline visit**	**Day 0** ☽	**Week 1** ☽	**Week 2** ☽	**Week 4** ☽	**Week 8** ☽	**Outcome Visit Month 6**
Visit timing Day 0 is target quit date (TQD)	**-x to -8 days**	**-7 days**	**Day 0** (TQD)	**+ 7 days**	**+14 days**	**+ 28 days**	**+ 56 days**	**+ 180 days**
Visit window				±2 days	±3 days	±4 days	±1 week	-4/+12 weeks
ENROLLMENT								
Eligibility screen	x	x						
Explanation of project and protocol	x	x						
Send informed consent	x							
Sign informed consent		x						
Randomization to ENDS (E) or control		x						
Set target quit date (TQD)	x							
ASSESSMENT								
Demographics	x	x						
Medical History		x						
Physical activity		x						x
Smoking history	x	x						
Nicotine dependence		x						x
Withdrawal symptoms		x						x
Environmental pollution (e.g. second hand smoke)		x						x
Alcohol and illegal drug use		x						x
Anxiety		x						x
Depression symptoms		x						x
Sleep quality		x						x
Quality of life		x						x
Women: pregnancy test		x						
INTERVENTION								
ENDS distribution		E						
E-liquid order by participants		E			E	E	E	
E-liquid sent to their homes		E			E	E	E	

ENDS instruction		E						
ENDS technical support			E	E	E	E	E	
Structured smoking cessation counselling		x	x	x	x	x	x	
OUTCOME MARKERS								
Tobacco use (questionnaires)	x	x	x	x	x	x	x	x
Estimated ENDS use (ml e-liquids used)			E	E	E	E	E	E
Exhaled CO		x						x
Urinary nicotine metabolites		x						x
Urinary metabolite of Nitrosamines (TSNAs)		x						x
Adverse events, serious adverse events		x	x	x	x	x	x	x
VOC, aldehyde and PAH metabolites in urine		x						x
Oxidative stress markers in urine		x						x
Oxidative stress in exhaled breath (Lausanne site only)		x						x
Blood pressure, heart rate, height, weight (BMI), waist circumference		x						x
Blood lipids, HbA1c, creatinine & glucose		x						x
Respiratory symptoms		x						x

E: ENDS group only; C: Control group only

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor-Investigator

Prof. Dr. med. Reto Auer
Berner Institut für Hausarztmedizin (BIHAM)
Universität Bern
Gesellschaftsstrasse 49
3012 Bern
Tel: +41 31 631 58 79
reto.auer@biham.unibe.ch

1.2 Principal Investigator

Prof. Dr. med. Nicolas Rodondi
Klinik und Poliklinik für Allgemeine Innere Medizin
Inselspital
Freiburgstrasse 20
3010 Bern
+41 31 632 41 63
Nicolas.Rodondi@insel.ch

1.3 Statistician ("Biostatistician")

PD Dr. med. Sven Trelle
CTU Bern, Faculty of Medicine, University of Bern
Finkelhubelweg 11, 3012 Bern, Switzerland
Tel: +41 31 631 35 04
sven.trelle@ctu.unibe.ch

1.4 Laboratory

Inselspital
Universitätspital Bern
Zentrum für Labormedizin
3010 Bern

For urine and EBC samples:
PD Dr. Nancy B. Hopf
Institut universitaire romand de Santé au Travail (IST)
Route de la Corniche 2
1066 Epalinges
Laboratoire IST
Rue du Bugnon 19
1005 Lausanne

1.5 Monitoring institution

CTU Bern, Faculty of Medicine, University of Bern

Finkelhubelweg 11, 3012 Bern, Switzerland

Tel: +41 31 631 33 72

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Any other relevant Committee, Person, Organisation, Institution

Data management

CTU Bern, Faculty of Medicine, University of Bern

Finkelhubelweg 11, 3012 Bern, Switzerland

Tel: +41 31 631 33 72

Study Steering Commitee

The study steering committee will be responsible for the overall supervision of the trial and will regularly meet to discuss scientific and logistic aspects of the trial. The study steering committee consists of 5 persons, one person from each center involved in the study.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents will be submitted to a properly constituted Competent Ethics Committee (CEC) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.

The decision of the CEC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registered in the Clinical Trials Registry Platform of the National Institute of Health (NIH), clinicaltrial.gov. In addition, it will be registered in the Swiss National Clinical Trials Portal.

2.2 Categorisation of study

Other clinical trial, Risk category B.

ENDS and e-liquids are considered alimentary good in Switzerland and thereby regulated by the law on alimentary good. Sale and use of ENDS and e-liquids exempt from nicotine is permitted in Switzerland. Swiss residents can order nicotine-containing e-liquids from online stores abroad for personal use. Direct sale of nicotine-containing e-liquids is forbidden in Switzerland. Because Swiss law considers ENDS and e-liquids as alimentary good, and not as medicinal products or medical devices, Swissmedic has waived approval and surveillance. We will follow the rules set by the Federal Office of Public Health (FOPH) and the Federal Food Safety and Veterinary Office (FSVA) for the import of nicotine-containing e-liquids and have participants order the liquids from abroad and have the e-liquids sent directly to their home.

2.3 Competent Ethics Committee (CEC)

The sponsor-investigator will ensure that approval from the appropriate constituted CECs is sought for the clinical study. Yearly intermediary reports (annual safety reports) will be forwarded to the CEC. All unanticipated problems involving risks to humans will be reported to the CEC within 7 days. No changes will be made to the protocol without prior Sponsor approval and, in case of substantial amendments, CEC approval, except where necessary to eliminate apparent immediate hazards to study participants. Amendments will be reported according to section 2.10.

Premature study end or interruption of the study will be reported to the CEC within 15 days. The regular end of the study will be reported to the CEC within 90 days, the final study report shall be submitted within one year after study end.

2.4 Competent Authorities (CA)

Given the intervention of this randomized controlled trial (RCT), no approval from any competent authority will be sought. No reporting duties apply.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

There are no conflicts of interest. This study is entirely funded by the Investigator-Initiated Clinical Trial (IICT) funding scheme from the SNSF which support studies on topics that are not in the industry focus and have no direct commercial interests. The application has underwent scrutiny by the SNSF that the applicants comply with good practice aimed at preventing conflicts of interests and the applicants will continue to follow these good practices for this clinical trial.

2.7 Patient Information and Informed Consent

The investigators or a representative of the study team will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

Each participant will be informed that his/her medical records may be examined by authorised individuals other than their treating physician.

All potential study participants will be provided with a participant information sheet and a consent form describing the study and providing sufficient information for the subject to make an informed decision about their participation in the study. Interested subjects will either call themselves or, if they contacted us via email, receive a phone call where the study will be explained. If eligible, a date for their first visit will be set, and the patient information sheet will be sent to them by mail. During the first visit, the study information will be repeated and questions will be discussed. After this, the consent form will be signed. Thus, at least 24 hours will lie between receiving the information and signing the consent form, and therefore participants will be given enough time to decide whether or not to participate.

The patient information sheet and the consent form will be submitted to the CEC to be reviewed and approved. The formal consent of a participant, using the approved consent form, will be obtained before the participant is submitted to any study procedure.

The participant will read and consider the statement before signing and dating the informed consent form, and will be given a copy of the signed document. The consent form will also be signed and dated by the investigator (or his designees) and it will be retained as part of the study records.

Study participants in the intervention group can keep the device and the remaining e-liquids or return them to the investigators at the end of the study whereas the study participants in the control group will receive a voucher (“BERNcity Geschenkcard”) of similar monetary value of the ENDS and e-liquids after the completion of the study.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority, or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants’ medical history.

2.9 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.:

- ethical concerns,
- financial issues,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,

- early evidence of benefit or harm of the experimental intervention
- or any other reason that would prevent the project execution according to the research plan.

Premature study end or interruption of the study will be reported to the CEC within 15 days.

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the CEC.

Under emergency circumstances, deviations from the protocol to protect the rights, safety, and well-being of human subjects may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.

All Non-substantial amendments are communicated to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Research questions

1) How effective are ENDS in helping smokers quit, and reducing the number of cigarettes per day smoked over 6 months of follow-up?
2) Do ENDS result in adverse events when smokers use them as quitting aids?
3) Does using ENDS effectively reduce exposure to inhaled toxic compounds?
4) Does using ENDS improve health-related clinical outcomes (respiratory symptoms) and surrogate outcomes (oxidative stress, cardiovascular risk factors)?

Background

Smoking is the leading cause of avoidable death worldwide, is one of the top five causes of morbidity and lowered disability-adjusted life-years.[2] Cigarette smoking eventually kills one in two smokers, mostly through cancer, heart disease and respiratory failure.[3] More than a quarter of the Swiss population still smokes cigarettes.[4]

ENDS are electrically-driven devices that reproduce many features of tobacco cigarettes such as release of smoke (aerosol for ENDS), mimic the gesture of smoking and provoke similar mouth experiences such as the “throat hit”, typical of cigarette smoking.[5] They consist of a battery part, a liquid storage tank, and an atomizer that aerosolizes the liquid by generating heat to a resistance.[6] The liquid consists of propylene glycol, glycerol, distilled water, flavorings, ethanol and nicotine. Consumers (commonly called ‘vapers’) may choose from several types of ENDS, nicotine strengths, and a long and growing list of flavors. Compared to traditionally used nicotine replacement therapy like nicotine patches, gums and inhalers, ENDS may have different effects, since they better replicate the experience of traditional cigarette smoking and more quickly deliver nicotine to the blood.

ENDS were invented in China over fifteen years ago, but were not marketed in Europe or the USA until a decade ago.[7] Sales dramatically increased around 2010 and for the past few years, the prevalence of ENDS use has been stable in Switzerland, France, and the UK. ENDS changed rapidly, and are now in their fourth generation. First-generation ENDS resemble cigarettes. The devices have low-capacity batteries and a heating element surrounded by a “cartomizer.” First generation ENDS produce a lower quality and amount of vapor than second generation ENDS. Second generation ENDS typically have a high-capacity battery, an “atomizer”, and a refillable “clearomizer”.[8] Parts, including resistance, wick and batteries, can be replaced, lowering costs for users. Third generation modified ENDS, also called ‘mods’, have large-capacity lithium batteries and users deliver more or less power to the atomizer. Vapour production and nicotine delivery are more efficient.[6] Users can control the temperature of the coils in fourth generation ENDS, which might allow them to minimize the amount of toxins they inhale. These latest devices have large coils that again improved vapour production and nicotine delivery.

Because ENDS use appears to be safer than tobacco smoking, healthcare professionals have been interested in ENDS as a smoking cessation or harm reduction device. Most smoking cessation drugs and devices were developed, tested, and promoted by the pharmaceutical industry. Large prospective RCTs have evaluated the efficacy of nicotine replacement therapies (NRTs) and other smoking cessation drugs. The pharmaceutical industry has not yet invested in ENDS, probably because ownership of ENDS patents is uncertain. ENDS are produced, sold, and developed in China’s essentially unregulated, highly competitive market, usually by independent companies.[7] They are cheap to make. The competitive market keeps the price of ENDS low and companies that produce ENDS may not be interested in, and are likely not to have the money to invest in large studies that evaluate the effectiveness and safety of ENDS. The fast growing ENDS market is in competition with the market for tobacco products, so the tobacco industry may be hostile to ENDS.

Healthcare professionals are increasingly asked to counsel smokers who want to use ENDS to help them quit smoking tobacco. But since no large, well conducted studies exist to back up that recommendation yet, they are in a difficult position.

The tobacco industry has funded studies on ENDS in the past, but healthcare professionals are reasonably cautious about using data generated by the tobacco industry, since this industry still promotes tobacco smoking through advertisements and lobbies to block anti-tobacco policies designed to improve public health. There are currently sharp tensions among professionals who want to reduce the health burden of tobacco smoking. Some argue that ENDS are a safe device for helping smokers quit, and others advise to wait until we have more data on the health consequences of prolonged use.[7]

If healthcare workers are going to base their recommendations for ENDS use on evidence rather than conjecture, we need to provide data on the effectiveness of ENDS in helping smokers quit, and on ENDS safety (for example, in terms of adverse events). We must provide health-related outcomes in both the short- and long-term.

Efficacy of ENDS to help smokers quit and reduction of number of cigarettes smoked

Studies on the efficacy of ENDS to help smokers quit are scarce; only two rigorous randomized controlled trials have been published.[5, 9-11] The ECLAT trial found that self-reported abstinence from tobacco smoking at 12 months was higher in participants using ENDS varying nicotine contents than in the ENDS group without nicotine.[9] The second study, published by Bullen et al, found no significant difference in six-month CO-validated continuous abstinence in any of its three arms: 1) ENDS (7.3%); 2) nicotine patches (5.8%); and, 3) placebo e-cigarette (4.1%).[10] A Cochrane systematic review that pooled results from 662 participants in the 2 RCTs found that participants who used ENDS were more likely to have smoked no cigarettes for at least six months (9%) than participants who used placebo ENDS (4%; RR 2.29, 95% CI 1.05 to 4.96).[5] Since this review included only two studies, evidence scored 'low' on GRADE, which means that more research is necessary to increase confidence in effect estimates, and that including data from future studies will probably change the estimate.[5] Only first generation ENDS have been tested in this two RCTs. More recent and effective generation ENDS have so far not been rigorously evaluated in RCTs.

The recently published Cochrane systematic review on the efficacy of ENDS for helping smokers quit identified 15 ongoing studies that test the effect of ENDS.[5] While most studies are underpowered to show significant differences between groups, two large ongoing studies are likely to provide important information the efficacy of ENDS for smoking cessation. One study in New Zealand plans to randomize 1600 participants into three groups.[12] One receiving standard quit advice and NRTs (N=360); quit or substitute advice and NRTs (N=630); or quit or substitute advice and NRT or ENDS (N=630).[12] Another large trial is planned in the UK which will compare 2nd generation ENDS to NRT in addition to in person smoking cessation counselling in 886 smokers. Their sample size is based on a RR to detect a difference between ENDS of 1.7 with expected smoking cessation rates of 14% in the NRT group and 24% in the ENDS group at 12 months follow-up.[5] No RCT plans to test changes in exposure to toxic compounds secondary to ENDS exposure. The only study that plans to measure exposure to biomarkers of tobacco and carcinogens is a prospective cohort study of participants who smoke exclusively tobacco cigarettes (n=175), and dual users of ENDS and tobacco cigarettes (n=275). Without randomization, we cannot know the cause of changes in exposure to biomarkers and carcinogens. The study we plan will be adequately powered to test for the efficacy of ENDS, and provide comprehensive data on the exposure of ENDS users to the toxic compounds they may release.

Monitoring exposure to toxic compounds from ENDS use

Most studies that identified pollutants generated by ENDS were based on aerosol laboratory analyses.[13, 14] Very few human studies have used biomonitoring to assess exposure to pollutants and to estimate the internal dose of toxins delivered by ENDS. None have done so in an RCT setting. Several VOCs (e.g.,propylene glycol, glycerine, ethylene glycol, diethylene glycol, benzene, 1,3-butadiene, ethylene oxide, acrylonitrile, acrolein, propylene oxide, acrylamide) and aldehydes (e.g., formaldehyde, acetaldehyde, acetone, propanal, crotonaldehyde) generated by ENDS are usually quantified in aerosol. We will additionally measure metabolites of VOCs and metabolites of aldehydes in urine. This will allow us to determine differences in urinary concentrations of these compounds in ENDS users, smokers and quitters.

Analysis of air pollutants generated by ENDS use

Studies found ENDS delivers 9- to 450-fold lower levels of toxic substances than conventional cigarettes according to one study.[15] In conventional cigarettes toxins are produced by combustion, but in ENDS they are produced by turning liquid to vapor.[6, 16] Previous studies measured carcinogens and heavy metals. Carinogens were: carbonyl compounds (formaldehyde, acetaldehyde and acrolein), volatile organic compounds (VOCs) and tobacco-specific nitrosamines (TNSAs) (N'–nitrosonornicotine (NNN) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)). Heavy metals were cadmium, lead and mercury.[13, 15-27] Aerosolized flavorings in e-liquid solutions also contain potentially toxic contaminants with unknown health effects,[6, 15-17] though only traces of polycyclic aromatic hydrocarbons (PAHs) have been detected in ENDS aerosols, and sometimes not even that.[6, 16]

TSNAs and 1-and 2-naphthol (PAHs) are of special interest because they are believed to contribute to lung cancer.[28, 29] The TSNAs NNK and NNN are indicators of relative combustible tobacco and ENDS use, since their levels are higher in conventional cigarette use than in ENDS.[17] In urine, NNK and its

metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) can be quantified, both being specific biomarkers of tobacco smoke exposure.[30] The PAHs 1- and 2-Naphtol, metabolites of naphthalene, are higher in smokers than in non-smokers.[31] The level of 1-OHP glucuronide, the main urinary metabolite of pyrene, is twice as high in the urine of smokersthan in non-smokers or ENDS users, thought levels are also influenced by environmental pollution and diet.[28, 31]

Distinguishing exposure to toxins and nicotine from tobacco smoke and ENDS vapor

Biomonitoring may allow us to distinguish exposure to nicotine from exposure to tobacco smoke and ENDS. Anabasine, an alkaloid found in tobacco, can be measured in the urine of tobacco smokers. Since anabasine is present in e-liquids at extremely low levels only, [20, 32] anabasine may be helpful in differentiating nicotine exposure from tobacco and ENDS.[33, 34] We will measure carbon monoxide (CO) released by combustion processes typical of tobacco exposure and not ENDS. We will also test TSNAs, especially NNN, NNK and its metabolite NNAL, nicotine and nicotine degradation products (cotinine and anabasine), VOCs, and three PAHs, 1-hydroxypyrene (1-OHP) and 1- and 2-naphthol, will help us distinguish smokers from ENDS users, continuing smokers, and quitters.

Effect of ENDS use on oxidative stress, CVRFs and respiratory symptoms

Data on the safety-risk profile of ENDS are limited. Studies suggest that ENDS use improves health outcomes, such as reducing respiratory symptoms, and presents only minimal risks, like mild throat irritation and dry cough.[6] Prospective studies that systematically assessed the effect of ENDS on safety outcomes did not show a statistically significant increase in serious adverse events (SAE) like unscheduled visits to the family practitioner, or hospitalization.[9, 10] The self-reports from the ECLAT RCT study did show that, by Week 2, shortness of breath was reduced from 20% to 4% in all participants, but there were no differences between intervention groups.[9]

Effect of ENDS on oxidative stress

Smoking induces acute[35] and chronic oxidative stress and inflammation both in vitro and in vivo.[36] We believe we will be the first to compare oxidative stress from ENDS use to cigarette use in the context of a RCT. Data on oxidative stress induced by inhalation of toxic compounds usually comes from studies on environmental air pollution and tobacco cigarette smoking. Tobacco-smoke contains strong pro-oxidants (stable radicals and toxic substances) that generate reactive oxygen species,[37] and may promote recruitment of phagocytes, which also generate free radicals. All these compounds can induce oxidative stress, which likely plays a key role in causing airways and related pathologies linked to tobacco-smoke exposure.[38]

Oxidative stress can be assessed by measuring 8-iso-prostaglandin F2α (8-isoprostane),[39] a marker of lipoperoxidation, and 8-OHdG, a biomarker of DNA oxidation.[40, 41] 8-isoprostane results mainly from the nonenzymatic action of free radical attack on arachidonic fatty acids; thus can be considered a lipoperoxidation biomarker. The measurement of 8-isoprostane may be useful for evaluating both acute and chronic oxidative stress.[42, 43] [44] Urinary 8-isoprostane has been shown to be greater in smokers than in non-smokers.[42] 8-OHdG is a urinary biomarker of oxidative DNA damage, a predictor of lung cancer.[45-47] Disadvantages with measuring 8-OHdG concentration are its low specificity due to confounders (e.g. age, gender, diet, physical activity, vitamin status).[48, 49] Information regarding known confounders will be collected and lead to better interpretation of 8-OHdG as a marker of cigarette smoking exposure.[39-42, 48-51]

Effect of ENDS on cardiovascular risk factors (CVRFs).

Cardiovascular diseases (CVD) are a leading cause of death in cigarette smokers; quitting smoking is associated with reduced CVD.[3] Cigarette smoking increases CVD through complex mechanisms, mostly on an increase in atherosclerosis and the effect appears unrelated to nicotine. There is currently no evidence that ENDS use affects CVD outcomes. The ECLAT trial showed no difference in body weight, resting heart rate, or systolic/diastolic blood pressure between those who used ENDS or not from baseline to the end of the study.[9] Two other studies evaluated the short-term effects of ECs on the cardiovascular system. One study suggested impairment in diastolic ventricular function with tobacco cigarettes and not with ENDS.[52] Both ENDS and tobacco cigarettes increased diastolic blood pressure, potentially mediated through nicotine exposure, but an increased systolic blood pressure was found only in cigarette smokers. Interventions helping smokers quit have shown that quitting is associated with increased HDL-cholesterol, [53] increased blood pressure, [54, 55] weight gain[56], higher blood glucose, and higher diabetes risk.[57] No randomized trials have tested the effect of ENDS on blood glucose, blood cholesterol and other markers of cardiovascular risk.

Effect of ENDS on respiratory symptoms

In the ECLAT trial, respiratory symptoms similarly improved in all studied groups.[9] Null findings on respiratory symptoms in the ECLAT may be explained by the low number of participants who quit smoking. One study compared the short term effects of cigarette smoking to ENDS use and found that cigarette smoking led to an acute reduction in lung function which was not observed with ENDS.[58] Findings on short-term airway resistance is conflicting.[59, 60] Short term increase in resistance in ENDS users might be caused by aerosolizing the liquid, and not by the same substances that harm lung function in cigarette smokers.[60] Smokers who shifted from tobacco cigarettes to ENDS have offered anecdotes of dramatically improved lung function, but animal models suggest that ENDS liquids can increase markers of asthma.[61] The larger study we propose will provide more useful data on changes in respiratory symptoms.

Measuring markers of oxidative stress in exhaled breath condensates (EBCs)

During exhaled breath condensate (EBC) collection, exhaled breath is directed through a cooling device, which traps the exhaled breath constituents in a liquid or solid phase depending on the condenser temperature.[62] Biomarkers of oxidative stress have been measured in EBCs (pH, H2O2, malondialdehyde, leukotrienes, 8-isoprostanes, cytokines).[62] EBC requires special equipment and trained personnel compared to urinary oxidative stress biomarkers which can be more easily obtained. However, measures in EBCs can more reliably assess oxidative stress in the lungs than measures in the urine, which measures oxidative stress on a systemic level. [63] Thus markers of oxidative stress might be more reliable to measure potential adverse lung health outcomes such as asthma and COPD.[64] ECB is useful for monitoring airway inflammation, although the collection process needs to be standardized.[62] Two studies have reported on 8-OHdG concentrations in EBC[65, 66] and showed increased 8-OHdG concentrations in asbestos-exposed workers[65] and in smokers[66] compared to non-smokers. Our project proposal will add to the body of knowledge regarding the applicability of this biomarker in EBC for evaluating DNA oxidation in lung.

Scientific impact

Despite the ready availability of ENDS in Europe and the USA, at local stores and on the Internet, there are significant gaps in our knowledge. These devices have been commercially available for less than a decade, and only gained popularity in the last few years. They are also evolving rapidly. No researchers have independently evaluate the effectiveness, safety, toxicological profile, and effect on health-related outcomes of ENDS in the setting of a randomized controlled trial. Doing so would help Swiss and international policymakers make evidence-based decisions about the safety profile of ENDS.

Data on exposition to potential toxins from ENDS in daily life.

We will inform smokers interested in quitting with ENDS, and policymakers, about the toxins measured in the urine and blood of participants who use ENDS for smoking cessation under real life conditions. While data on the safety and risk-benefit profile of ENDS appear promising under laboratory conditions,[5, 6] ENDS does release some toxins. To opponents of ENDS, the small amount of released toxins in unacceptable; supporters see it as negligible. Under real life conditions, the exposure of ENDS users to toxins maybe much less than daily exposure to toxins from other environmental pollutants. Users might also misuse ENDS under real life conditions, and thus receive higher exposure to toxins than under laboratory conditions. Our study will help validate or disprove the claims supporters and opponents make about ENDS.

Data on the consequences of potential toxins from ENDS on the body

Even if we find no increase in exposure to toxins among ENDS users, or when we compare those who quit using ENDS to those who quit smoking without ENDS, it is still possible ENDS have adverse physical effects. Some toxins are quite hard to detect, but we can measure oxidative stress in urine and exhaled breath. Measures of oxidative stress are biomarkers of the effect of exposure to toxins on the body. Oxidative stress increases risk of cancer and heart diseases, the leading causes of disease and death worldwide. Because it takes years for cancer and heart disease to be detectable, and because ENDS are a recent development, we can measure oxidative stress in ENDS users, so that users, clinicians, and policymakers can understand the possible long-term consequences of increasing ENDS use. We will also measure the effect of ENDS on CVRFs and respiratory symptoms, to explore the short term effects of ENDS on health.

ENDS use is controversial among health professionals and policy makers. ENDS advocates are comfortable with widespread use and claim it is a safe alternative to cigarettes, others fear ENDS is a gateway to smoking and may harm health users' health in the long term.[7] For smokers, the choice of

ENDS could be a safe alternative to tobacco cigarette smoking and an alternative to smoking cessation. Given the stagnating trend in smoking prevalence, ENDS could make a significant dent into smoking prevalence and save many lives from smoking. Swiss tobacco experts have recently recommended making ENDS available in Switzerland and regulating them.[67] But current recommendations are not well-supported by evidence; our study will help users, policy makers, and health professionals decide whether ENDS should be actively promoted as an alternative to tobacco cigarettes.

3.2 Investigational Product (treatment, device) and Indication

Device:

We will use a third generation nicotine vaporizer, the Innokin Endura T20-S starter kit. The device is authorized for sale in Switzerland and follows the CE declaration of Comformity, essential to ensure safety of the participants. The Endura T20-S kit will come in a user packet with a 1,500mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro Micro USB DC 5V/1A cable, a USB mural adapter and an instruction manual in French or German, respectively. (See also section 8.1.1. for pictures of the tested device)

E-liquids:

We will use the e-liquids produced by the company Gaïatrend in France (https://www.gaiatrend.fr/en/). The e-liquids “Alphaliquids” are produced following rigorous standards. Nicotine concentrations available will be 6 mg/ml, 11 mg/ml and 19.6 mg/ml. The following aromas will be available to be chosen by the study participants: FR-4 (tobacco flavor) and FR-M (tobacco flavor), FR-MINT (menthol flavor), and RED FRUITS (fruity flavor) (https://www.alfaliquid.com/en). The proportion of propylene glycol and vegetal glycerin will be 76/24 for all e-liquids. The components of the e-liquids will be propylene glycol, vegetal glycerin, medical-quality nicotine, alcohol and aromas. The full list of ingredients and their concentration is provided in the section 17 appendices.

Gaïatrend obtained ISO8317 certification for its e-liquid flasks (See also section 8.1.1. for pictures of the flasks containing the e-liquids). This standard certifies that flasks are equipped with child-resistant closures (See the Certificate of Test n°”IBE-BVI” – CR-14.021 from June 26th, 2014 on tests conducted by the Belgian Institute of packaging on children in the section 17 appendices). The vegetable glycerine is guaranteed free of genetically modified organisms (GMOs). It has also obtained EP certification (European Pharmacopoeia). The propylene glycol in the e-liquids is 99.5% pure, organic and guaranteed free of GMOs. This product has also obtained EP and USP certification. Organic ethyl alcohol made from cereals is another ingredient used in the e-liquids and has obtained Ecocert N° FR-BIO-01 certification. It is also guaranteed free of GMOs. Concerning the nicotine that is contained in the e-liquids, it comes from the only nicotine supplier to have obtained certification from the European Pharmacopoeia (see section 17 appendices). The aromas used in the e-liquids comply with standards of quality from CE N°1334/2008 (see section 17 appendices).

In order to comply with the Swiss law, participants will order their e-liquid at the baseline visit with a prefilled and the e-liquid will be sent directly to their home by batches of maximum 150ml e-liquids.

In order to comply with the Swiss law, participants will need to order their e-liquid by themselves. At the baseline visit, study participants will fill in a pre-filled ordering form indicating the chosen e-liquid and their postal mail address, which will then be scanned and transmitted to Gaïatrend. Gaïatrend will receive a list of new participants included on a daily basis and asked to mail batches of 150 ml of e-liquids to their home directly.

Indication for using the device and e-liquids:

The indication for using the device and e-liquids is for replacing tobacco cigarettes smoking by use of the device and e-liquids in the intention to help participants stop smoking tobacco cigarettes at 6 month follow-up as the main outcome or to reduce the number of tobacco cigarettes smoked as a secondary outcome. Other expected secondary outcomes are a reduction in exposure to inhaled toxins measured in the urine, reduction in oxidative stress measured in urine and improvement in clinical outcomes such as respiratory symptoms and improvement in cardiovascular risk factors. We hypothesize that the use of device and e-liquids instead of tobacco cigarettes will not lead to an increase in adverse events. (see detailed description of main and secondary outcomes in Sections 5).

Training and support of participants for using the device and e-liquids:

Study participants in the intervention group will be advised by trained study nurses about how to get started with the device, how to fill the device with e-liquid, how to charge the device and how to change the coil every two weeks. They will also be instructed to wait 5 minutes after changing coils and filling the tanks with e-liquids in order to wait for the cotton of the coils to be soaked in e-liquid before use. A user manual will be provided for every study participant. Study participants will also be provided with a direct phone number of the study nurses for technical support between scheduled phone visits and will have the possibility to go on the study website to obtain the information on the use of the device.

3.3 Preclinical Evidence

Not applicable.

3.4 Clinical Evidence to Date

Evidence of ENDS use to help smokers quit has been reviewed in detail in section 3.1.

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Rationale for the device chosen:

We will use an easy to use and safe ENDS produced by one of the leaders of ENDS producers worldwide. The Innokin T20-S is a starter kit that allows mouth-to-lung experience similar to smoking regular cigarettes. Its simplicity, which comes along with restrictions on variations of the airflow and wattage, will ensure minimizing the risk of misuse and technical issues by users. The top-filling capacity will ensure ease and safety of filling e-liquids on a daily basis. The Endura T20-S kit comes with a 1,500mAh internal Li-Po battery which allows users to vape during a full day without need for charging. It has integrated safety protection which is important in order to avoid the production of “dry-hits”, when the coil gets too hot and pyrolyzes the e-liquid, which is at the origin of the formation of harmful VOCs such as acrolein and formaldehyde. The device will be mounted with a 0.8 Ohm coil in order to optimize the concentration of nicotine in the vapour and limit the exposure of users to vapour.[68] The 0.8 Ohm coil enables lowering the temperature of the vapour to improve the experience of inhaling the vapour by participants. The lower power needed to heat the e-liquids also ensures lower the temperature of the entire heating system and spare the battery to ensure that one battery charge lasts for one full day of use. The tri-color LED power indicator clearly shows the remaining power levels and charging status. We will provide particpiants with a mural USB charger in order to avoid improper charging with defective chargers. Participants will have the choice of three colors of the device to furher improve uptake. Colors available will be Black, Purple, and Blue. Finally, the low price of the device, not obtained at the expense of quality, will ensure generalizability of the results to the overall population, as new users often select a cheap and safe device when trying vaping devices before buying more refined and adjustable vaporizers if they continue vaping over a prolonged time.

Rationale for the chosen e-liquids, their nicotine concentrations and flavors:

The choice of Gaïatrend is based on the outstanding quality of the e-liquids they produce, following the most rigorous standards to ensure safety of the inhaled products. We follow the choice of academic scientists in France who have carefully reviewed the production processes and visited in person the factory for a similar RCT conducted in France in 2018.

The use of ENDS, as for other smoking cessation studies testing the effect of nicotine replacement therapy, will be ad libitum. Participants will be able to choose the nicotine concentration of the e-liquids (6 mg/ml, 11 mg/ml or 19.6 mg/ml), though we will encourage them to use the highest nicotine concentration in order to ensure optimal nicotine substitution while reducing the volume of e-liquids used and vapor inhaled [68, 69]. The choice of nicotine concentration is essential to ensure uptake of the device by participants. We expect those who continue vaping will reduce the nicontine concentration over time. The 6 mg/ml will ensure that participants continue to use the e-liquids provided within the study and avoid using another e-liquid bought online from potential unsafe sellers.

Participants will also be allowed to choose between different aromas in order to improve uptake. While most smokers start with a tobacco-flavored e-liquids, over time, as their sense of taste and smell comes

back through smoking cessation, most shift to other flavors, the most popular being menthol and fruity flavors. Thus providing participants with the choice of aromas over time may increase the chance they will continue using the vaporizers and thus avoid relapse into smoking, which is the main outcome of the study.

3.6 Explanation for choice of comparator (or placebo)

We will compare the combination of nicotine-containing ENDS and smoking cessation counseling (SCC) (intervention group) to SCC alone (control group). SCC is the standard of care. We intend to emulate the real-life conditions for people who want to use ENDS to quit cigarette smoking, and who contact a health care professional for support. Participants in both groups (intervention and control group) are free to use other NRTs or smoking cessation aids, but we will not pay for aids or medication, as is currently the case in clinical practice in Switzerland. At the end of the 6 months, participants in the control group will receive a voucher ("BERNcity Geschenkcard") worth CHF30. The amount of CHF30 is of similar monetary value to what the intervention group will receive in form of the ENDS device and the e-liquids.

3.7 Risks / Benefits

Data on the safety-risk profile of ENDS use are limited and mostly come from observational and laboratory data from the last decade. Previous RCTs suggest that ENDS use improves health outcomes, such as reducing respiratory symptoms, and presents only minimal risks, including mild throat irritation and dry cough.[6] Prospective studies that systematically assessed the effect of ENDS on safety outcomes did not show any statistically significant increase in serious adverse events (SAE) such as unscheduled visits to the family practitioner, or hospitalization.[9, 10] The self-reports from the ECLAT RCT study showed that, by Week 2, shortness of breath was reduced from 20% to 4% in all participants, but there were no differences between intervention and control groups.[9]

ENDS devices chosen for this study have been on the market for more than 6 months. The devices comply with EU regulations (CE-labelled). The ENDS may be the cause for accidents as they are composed of lithium batteries. Reports of accidental explosions of batteries have occurred, although these are mostly caused by misuse of the device by users, prolonged charging, improper chargers or by design defects. We will instruct participants to only use the charger and cable provided in the kit and to avoid trying to open up the battery part. The risk of accidental explosion is however similar to the risk with other common battery-driven devices such as smartphones. In addition, the risk of fire through misuse of regular tobacco cigarettes and use of lighters is estimated to be much higher. Evidence from the Fire Department in London suggests use of vaporizer was associated with a reduction in home fires related to cigarette smoking.

One of the aims of this study is to identify and evaluate the toxicological profile of ENDS as a smoking cessation aid within a RCT to provide further evidence on the risk-benefit profil of using ENDS instead of tobacco cigarettes for smoking cessation.

Data from observational studies indicate that there is very low risk in using ENDS, probably without any clinical significance.[6] The use and sale of ENDS and e-liquids has been allowed by Swiss authorities after safety analyses. The main risks concern the use of nicotine with the ENDS, as well as the use of nicotine replacement therapy (e.g., patches, gums) if participants wish to do so. Generally, nicotine replacement therapy is tolerated well, however, if smoking is continued during the use of NRT, side effects could occur due to the high nicotine levels absorbed. Side effects of nicotine absorption from NRT are similar to those from smoking (e.g., nausea, palpitations, dizziness, sleep problems).

Nicotine contained in the e-liquid is irritable for the mouth, throat and the eyes, therefore it is possible that the use of ENDS leads to irritations of the eyes or the respiratory system (e.g., dry cough). Moreover, inadequate use of ENDS could lead to thermogenic degradation (pyrolysis) of the e-liquids and consequently lead to an inhalation of irritable compounds such as acrolein and formaldehyde. This happens more often when cotton coils are not changed as recommended, also called "dry puff". Qualitative data indicate that ENDS users immediately recognize when dry puffs occur and stop inhalation to exchange the coil. To ensure that participants recognize when coils need to be replaced, we will instruct them to recognize dry puffs when handing out the ENDS during their first visit. We will also instruct them how to change the coils every two weeks and provide them with spare coils at the baseline visit and be sent additional coils by mail over follow-up if needed.

All e-liquids bottle will follow standards by the EU and be limited to 10 ml. They will include a safety notice and logos to indicate that the product is toxic, that bottles should not be used by minors under 18 and not to be used by pregnant women. The bottle will contain information on the composition, the

nicotine concentration, the flavour and have a warning reading: “The Nicotine contained in this product creates a strong dependence. Its use by non-smokers is not recommended”. The bottles are and have been tested to be completely child-proof (ISO 8317 certified cap child resistant packaging). The safety of the e-liquids bottles with children has been evaluated by the producer (see section 17 appendices).

Participants will be instructed to avoid contact with the skin and other mucosa when filling the device and to wash hands immediately in case of spilling. Recent evidence suggest penetration of nicotine through the skin.[70] However, the risk is estimated to be minimal and similar to the application of a nicotine patch regularly used by millions for smoking cessation.[6]

We will also instruct participants not to ingest the e-liquids and to keep them out of reach of children, as ingestion can be a source of overdosing of nicotine.[6] Participants will be provided with the contact of the emergency services and to instruct the investigators immediately in case of accidental ingestion of e-liquids. The lethal dosis of nicotine is estimated at 500-1000 mg.[6] Thus the swallowing of the content of one bottle of 19.6 mg/ml will not suffice for a lethal dose should a participant accidentally ingest one.

Although the e-cigarettes and e-liquids are considered an alimentary product, and thus the evaluation of the safety of e-cigarettes is outside of the jurisdiction of Swissmedic, we will apply the same rigor to the assessment of side effects and risks as for a therapeutic product. In particular, the systematic collection of information on side effects will be based on documents used for pharmacological safety studies.

3.8 Justification of choice of study population

We will include participants who are ≥ 18 years old, smoke > 5 cigarettes a day since > 1 year, and are willing to quit smoking within the next three months. We will restrict to smokers smoking 5 or more cigarettes per day in order to randomize smokers with sufficient tobacco cigarete exposure to adequately detect harmful components of tobacco cigarettes in the urine and to smokers who are expected to need nicotine substitution by ENDS in order to stop smoking cigarettes. We successfully applied similar restrictions in previous RCTs for smoking cessation trials conducted by our group.[71, 72]

Exclusion criteria are listed under section 7.1. Eligibility criteria. We thrive at limiting the number of exclusion criteria to the minimum to ensure the external validity of the study findings to the general population interested in using ENDS for smoking cessation. We will exclude pregnant or breast feeding women; not because ENDS should be particularly harmful in such populations, in particular in comparison to the known harms from smoking tobacco cigarettes for pregnant and breastfeeding women and their foetuses and babies, but because we expect dedicated trials in this population will be conducted by other groups in the future.

4. STUDY OBJECTIVES

4.1 Overall Objective

The study aims to test the efficacy, safety and toxicology of ENDS for helping smokers quit smoking or reduce the number of cigarette smoked per day over a 6-month follow-up period.

4.2 Primary Objective

The study seeks primarily to determine the efficacy of ENDS for helping smokers quit smoking or reduce the number of cigarette smoked per day over a 6-months follow-up period.

4.3 Secondary Objectives

Secondary objectives are to test the effect of using ENDS on exposure to inhaled toxic compounds and test the effect of ENDS use on health-related outcomes, including clinical health-related outcomes (respiratory symptoms) and surrogate health-related outcomes (oxidative stress, risk factors for heart disease).

4.4 Safety Objectives

The study aims to assess the safety of ENDS in terms of adverse events.

5. STUDY OUTCOMES

5.1 Primary Outcome

The primary outcome will be the continuous smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report (self-report of no smoking from target quit date) and confirmed by exhaled CO level (CO<10 ppm) and urinary level of cotinine and anabasine (<3 ng/ml) and NNAL (10 pg/mg creat).[1, 33, 34]

5.2 Secondary Outcomes

The secondary outcomes are as follows:

- Secondary smoking cessation outcome:
    - Same as the primary outcome (continuous smoking abstinence from target quit date to the 6-month follow-up visit) but allowing up to 5 cigarettes in total and a 2-week 'grace period' after the target quit date;
    - Self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), again all confirmed by the exhaled CO level and the urinary level of cotinine and anabasine;
    - Change in the self-reported number of cigarettes smoked per day (CPD) with 50% reduction in CPD from baseline to follow-up considered as successful reduction.
- Concentrations of urinary TSNAs (NNN, NNK and its metabolite NNAL), VOCs and PAHs (1- and 2-Naphtol and 1-OHP) for all participants
- Respiratory symptoms assessed by questionnaire (CAT, mMRC, ACT, ECRHS) [73-76]
- Oxidative stress assessed by 8-OHdG and 8-isoprostane concentrations in both EBC and urine
- CVRFs assessed by HDL- and LDL-cholesterol, triglycerides, HbA1c, creatinine and glucose concentrations, blood pressure levels, heart rate, waist circumference and body mass index.

5.3 Other Outcomes of Interest

Other variables of interest will be assessed by questionnaires:

- Physical activity (International Physical Activity Questionnaire, IPAQ) [77]
- Withdrawal symptoms (Minnesota Nicotine Withdrawal Scale (MNWS) [78, 79]
- Legal and illegal drug use: Alcohol consumption [80], illegal drug use (cannabis, cocaine, other) [81]
- Sleep quality (Pittsburgh sleep quality inventory PSQI) [82]
- Quality of life (EQ-5D questionnaire) [83]
- Depression symptoms (PHQ-9)[84]
- Anxiety (GAD-7) [85]
- Environmental pollution exposure (e.g. second hand smoke) [86, 87]

5.4 Safety Outcomes

Safety outcome variables are adverse events (AEs) and serious adverse events (SAEs) following international standards.[88]

Safety outcomes are defined as follows:

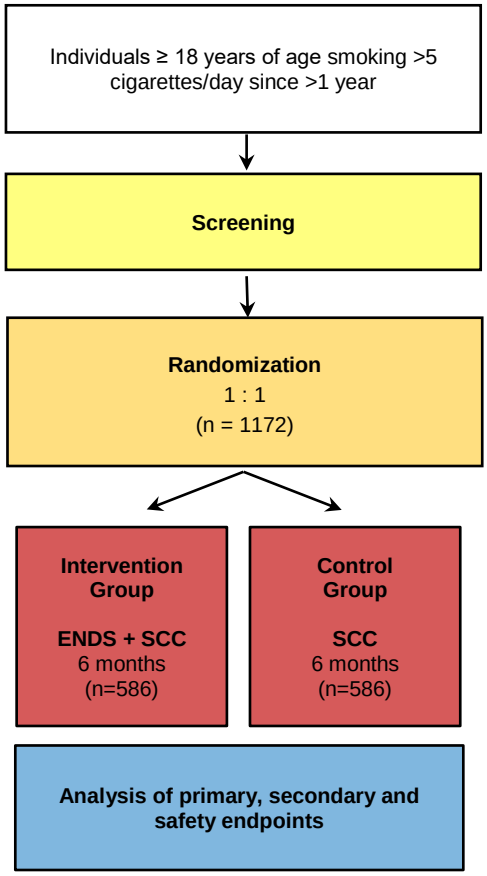
- Pre-specified AEs of special interest:
  dry mouth
  cough/dry cough
  shortness of breath (dyspnea)
  throat / mouth irritation
  headache
  dizziness
  increased heart rate / palpitations
- SAEs
- Unscheduled visits to the family practitioner
- ENDS malfunctioning

6. STUDY DESIGN

6.1 General study design and justification of design

The ESTxENDS trial is a multicentre, pragmatic, open-label randomized controlled trial with two parallel groups allocated in a 1:1 ratio. The trial will assess superiority of ENDS + SCC for smoking cessation to SCC alone in adult (≥18 years) smokers. The intervention group will receive ENDS and nicotine-containing e-liquids to use ad libitum, plus SCC. The control group will receive SCC only. At the end of the 6 months, participants in the control group will receive a voucher ("BERNcity Geschenkcard") worth CHF30 in order to maximize retention of participants in the control group. A total of 1172 smokers will be included in the study, 586 in each group. Recruitment of the participants is planned to occur over 13-18 months at all 3 study sites. Our main outcome will be self-reported continuous smoking cessation after target quit date over a 6-month follow-up period, with blinded biochemical verification by exhaled carbon monoxide (CO), urinary cotinine, anabasine, VOCs, TSNAs (NNN, NNK and its metabolite NNAL) and PAHs (1- and 2-Naphtol and 1-OHP) levels. Statisticians and laboratory personnel will be blinded to group allocation. Participants and clinicians will not be blinded. We will use the methodology of clinical trial investigating a medicinal product to test the efficacy and safety of ENDS. We will mimic the methods of a pharmacovigilance and safety study.

Figure 6.1-1: ESTxENDS trial design schematic



6.2 Methods of minimising bias

6.2.1 Randomisation

We will use a centralized computed randomization process to randomize participants into the intervention and control arm at a 1:1 ratio. The central server will be located in a protected environment

at CTU Bern.

6.2.2 Blinding procedures

Participants, nurses and investigators will not be blinded. We will blind both the statisticians who perform the main analyses and the laboratory personnel who analyse urinary cotinine, anabasine, VOCs, TSNAs (NNN, NNK and its metabolite NNAL) and PAHs (1- and 2-Naphtol and 1-OHP) levels.

6.2.3 Other methods of minimising bias

To minimize bias of safety outcomes, all clinical events (hospitalizations) and other serious adverse events will be verified by a blinded external committee whenever possible (see 1.7.3).

6.3 Unblinding Procedures (Code break)

Not applicable.

7. STUDY POPULATION

7.1 Eligibility criteria

Subjects fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Age 18 or older
- Currently smokes 5 or more cigarettes per day, for a least 12 months
- Willing to try to quit smoking within the next 3 months
- Persons providing a valid phone number, a valid email address and/or a valid postal address.

The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

- Known hypersensitivity or allergy to contents of the e-liquid
- Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected
- Women who are pregnant or breast feeding
- Intention to become pregnant during the course of the study
- Persons having used ENDS regularly in the 3 months preceding the baseline visit
- Persons having used nicotine replacement therapy (NRT) or other drug therapy helping smokers quit (varenicline, bupropion) within the 3 months preceding the baseline visit
- Plans to move out of the country within the next 6 months, or cannot attend the 6- month follow-up visit for any reason
- Cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures

7.2 Recruitment and screening

Target population of the study are individuals who smoke 5 or more cigarettes/day since more than one year and are willing to try and quit within the next three months. Individuals will be recruited through media advertisements or directly at the study sites when seeking help for smoking cessation. Interested smokers will be screened over the phone by a trained nurse to review eligibility criteria. Smokers will also announce their target quit date and, if eligible and if they have no contra-indications for participating in the study, will be sent a participant information sheet and the informed consent form. The baseline visit will be scheduled a week before the quit date they named over the phone. At the baseline visit, trained study nurses will again verify the eligibility criteria, explain the trial and have participants sign the informed consent form.

7.3 Assignment to study groups

We will randomize participants to either intervention or control group at the end of the baseline visit, after participants have signed the consent form and we have taken all baseline measures. Nurses will collect all demographic parameters from participants online before they can request centralized randomization. Once all parameters are entered, the nurse will request a randomization allocation in presence of the participant. The central server located in a protected environment at CTU Bern will then allocate participants in the intervention and control group in a 1:1 ratio. Nurses and participants will learn about the allocation group directly on the screen, hence the nurse and participants will not be blinded. The randomization lists stored on the server will be only accessible by system administrators and not to study personnel involved in the recruitment of participants. The centralization of process and necessity to enter all demographic data before being able to randomize participants in the intervention and control group will ensure the reliability and concealment of the randomization process.

7.4 Criteria for withdrawal / discontinuation of participants

Discontinuation of treatment

Treatment will be discontinued if:

- A participant reports she is pregnant.

Treatment may be discontinued if:

- Participants do not comply with the instructions by the research team;

- We suspect a treatment-related SAE;
- The protocol has been irretrievably violated, as determined by the sponsor-investigator.

Subjects who permanently discontinue study treatment are encouraged to continue in the follow-up period and to attend the protocol-specified follow-up visit at 6 months in accordance with the intention-to-treat principle.

Discontinuation of participation in the study

Participation in the trial will be stopped if:

- participants make a request to discontinue their participation

Participation in the trial may be stopped if:

- the responsible study investigator decides that continued participation in the study could be harmful to the subject's wellbeing

A participant who decides to discontinue the study participation prematurely will be defined as a dropout if they have already been randomized. A study participant who terminates the study before being randomized will be defined as a screening failure.

Participants who cannot be reached over the phone at follow-up visits will not be excluded. Participants who do not present at the 6-month visits will be contacted by phone, by letter, and, finally, we will contact two contact persons and the primary care provider listed by participants at the baseline visit. If we cannot contact participants for more than three months, we will consider them lost to follow-up.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

8.1.1 Experimental Intervention (treatment / device)

Device:

We will use the Innokin Endura T20-S starter kit. The Endura T20-S kit will come in a user packet with a 1,500 mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro USB DC 5V/1A cable and an instruction manual in French or German, respectively. Colors availble will be Black, Purple, and Blue.

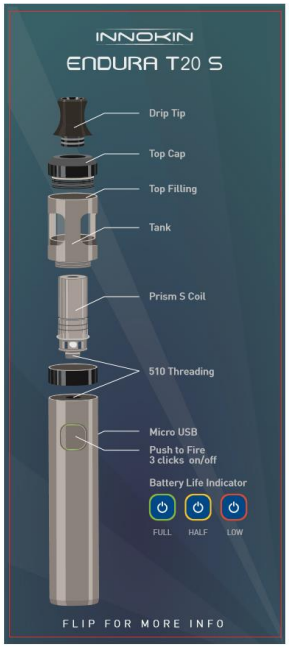
https://www.innokin.com/vaporizers/endura-t20-s/

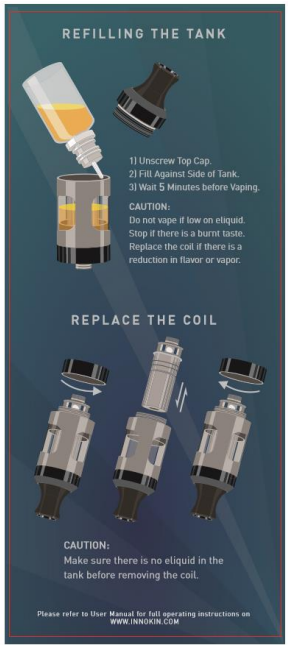
E-liquids:

We use the e-liquids produced by the company Gaïatrend in France (https://www.gaiatrend.fr/en/). Nicotine concentrations available will be 6 mg/ml, 11 mg/ml and 19.6 mg/ml. Following aromas will be available to be chosen by the study participants: FR-4 (tobacco flavor) and FR-M (tobacco flavor), FR-MINT (menthol flavor) or RED FRUITS (fruity flavor).The proportion of propylene glycol and vegetal glycerin will be 76/24 for all e-liquids. The components of the e-liquids will be propylene glycol, vegetal glycerin, medical-quality nicotine, alcohol and aromas.

https://www.gaiatrend.fr/en/

Study participants in the intervention group will be advised by trained study nurses how to get started with the device, how to fill the device with e-liquid, how to charge the device and how to change the coil every two weeks. A user manual will be provided for every study participant.





http://www.innokin.com/manuals/Endura-T20S.pdf

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Participants in the control intervention will receive SCC only. SCC is as follows: Study nurses will provide SCC based on motivational interviewing and cognitive counseling (see e.g., [89]). Trained nurses will follow a standardized course. SCC will be provided at the first clinical visit and then over the phone at the target quit date, at week 1, 2, 4, and 8. Average duration of counseling is expected to be 15 minutes over the phone and 30 minutes during the in-person visit at baseline.

8.1.3 Packaging, Labelling and Supply (re-supply)

Device:

The Endura T20-S kit will come in a user packet with a 1,500mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro Micro USB DC 5V/1A cable, a USB charger and an instruction manual. Colors availble will be Black, Purple, and Blue (see image of the kit below and also images of the device in section 8.1.1.).

The kits will be ordered at Innokin and stored in each center. They will be handled directly in person by the study nurse at the first visit for the intervention group at no charge. In case of defective devices which need to be replaced, or if the device is lost or otherwise unusable, participants will either directly visit the centers to pick up replacement devices, or participants will get the possibility to re-order a device through the study nurse at any time during follow-up by mail. In all cases the devices will be free of charge for participants.

E-liquids:

All e-liquid bottles will follow standards by the EU and be limited to 10 ml. They will include a safety notice and logos to indicate that the product is toxic, that bottles should not be used by minors under 18 and not to be used by pregnant women. The bottle will contain information on the composition, the nicotine concentration, the flavour and have a warning reading: “This product contains nicotine, which is a highly addictive substance”. Gaïatrend obtained ISO8317 certification for its e-liquid flasks (See also section 8.1.1. for pictures of the flasks containing the e-liquids). This standard certifies that flasks are equipped with child-resistant closures (See Section 3.7 Risk/benefit).

In order to comply with the Swiss law, participants will need to order their e-liquid by themselves. At the baseline visit, study participants will be allowed to taste and choose between four e-liquid flavors and between three different nicotine concentrations. Once they have tested and chosen their e-liquid, study participants will fill in a pre-filled ordering form indicating the chosen e-liquid and their postal mail address, which will then be scanned and transmitted to Gaïatrend. Gaïatrend will receive a list of new participants included on a daily basis and asked to mail batches of 150 ml of e-liquids to their home directly.

At regular phone intervals from study nurses to study participants participants will be allowed to switch between flavor and nicotine concentration during the course of the study. Participants will be allowed to make maximum two switches over 6 months. In addition, if participants use all the e-liquid during follow-up, and given that the use of e-liquids will be ad-libitum, participants will be allowed to reorder e-liquids through calling the study nurses. Participants will then fill in an ordering form online or send it to the nurse by mail which will be transferred to Gaïatrend to have them send the e-liquids directly at home. In order to comply with Swiss Law, the size of the batches of e-liquids will be limited to 150 ml for each order.

Batches of e-liquids will be shipped and mailed by Gaïatrend to participants through the company Colissimo International (https://www.laposte.fr/particulier/courriers-colis/produits-et-services/colissimo-international). Experience from Gaïatrend with orders sent to Switzerland shows that batches arrive to the users within 5 office days from the date of order.

8.1.4 Storage Conditions

The devices will be stored in each center at room temperature in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees.

E-liquids will be produced and stored at Gaïatrend following rigorous quality standards. Batches of e-liquids will be shipped and mailed by Gaïatrend directly to participants through the company Colissimo International (https://www.laposte.fr/particulier/courriers-colis/produits-et-services/colissimo-international) through regular mail. Samples of e-liquids of the four flavours (two different tobacco flavors, menthol flavor and a fruity flavor) and different nicotine concentrations (6 mg/ml, 11 mg/ml or 19.6 mg/ml) will be stored in each center in order that study participants can try vaping the e-liquids in pre-filled vaporizers at the baseline intervention before ordering their e-liquids. E-liquids will be stored in each center in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees between inclusion visits.

Participants will be instructed to store the e-liquids in a dry place, at room temperature and out of reach of children. After study termination, participants will be allowed to keep their devices and spare e-liquid or return them in each center if not completely used.

In case of malfunction of the devices or identification of damage to the e-liquids bottles by participants during shipping, participants will be encouraged to call the study nurse, to return the devices or e-liquids and be sent new devices or e-liquids by mail.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

After the ramdomization process, participants in the intervention group will receive a structured intervention for smoking cessation and in addition, a device and e-liquids at no charge and advice on how to use them by trained study nurses. Participants will be explained the rationale behind delivering nicotine through the e-liquids instead of the cigarettes and be trained in using the device, for example how to turn in on and off, charge the device, refill the 2 ml tank with e-liquids, replace the coil every two weeks of use. Participants will be allowed to taste and choose between the various nicotine

concentrations and flavors. We will encourage study participants to use the highest nicotine concentration in order to ensure optimal nicotine substitution while reducing the volume of e-liquids used and vapor inhaled. Study nurses will teach particpiants how to recognize withdrawal symptoms (craving, changes in mood etc.) and instruct them to use more liquid or a higher concentration if they experience withdrawal symptoms. Once they have decided of one nicotine concentration (either 6 mg/ml, 11 mg/ml or 19.6 mg/ml) and one aroma (among two different tobacco flavors, menthol flavor or a fruity flavor), they will order a batch of 150ml e-liquids (expected to suffice at least for the first month of use) to the company Gaïatrend. They will fill in a pre-filled form for ordering the liquids indicating their postal mail address which will then be scanned and transmitted to the company Gaïatrend along with their mail address. Participants will be informed that they will receive the e-liquids directly to their home within 5 office days, thus at about the time of their target quit date, which is scheduled one week after the baseline visit. Participants will also be provided with an explanatory booklet and explanatory videos on the study website. The video will explain the basic functionalities of the device, how to charge the device and the method to refill the tank. The second video will explain participants how to change the coil after each 2 weeks of use. Participants will be free to use their ENDS as much as they wish. They will be informed that they can switch nicotine concentrations and flavors at any time during follow-up for a maximum of two times. They will also be informed that they can re-order e-liquids as much as they want over follow-up. We will document ENDS use by monitoring the use of e-liquids in ml.

After this first visit, study nurses will contact participants in both study groups over the phone on the day of the target quit date, then again after 1 week, 2 weeks, 4 weeks and 8 weeks. During these follow-up calls, study nurses will provide SSC and ask participants about their use of tobacco cigarettes and ENDS and answer technical questions, ask if participants need additional e-liquids or if they want to shift to another nicotine concentration or flavor. Study nurses will record the time spent on the phone with each participant, and will ensure that the same time is spent on the phone with intervention and control participants. Participants will then be able to fill a form online or on-paper to be sent by mail so that Gaïatrend can send them additional e-liquids directly at home. Nurses will also encourage participants the change of coil every 2 weeks if they continue using ENDS regularly. Between these scheduled visits, participants will have the possibility to contact study nurses anytime during office hours, send an email to study nurses for any technical issues with the device or smoking cessation support. Nurses will answer emails within 24 hours of receipt on office days.

Average duration of counseling is expected to be 15 minutes over the phone and 30 minutes during the in-person visit at baseline. Participants will be allowed to use other NRT, smoking cessation medication or smoking cessation help at their own expenses. They will also be encouraged to go on the stop-tabac.ch (www.stop-tabac.ch) website for smoking cessation support if they which online support, as is currently performed in smoking cessation counseling in Switzerland.

8.2.2 Control Intervention

Participants in the control intervention will receive SCC only in person at the baseline visit and then over the phone.

After this first visit, study nurses will contact participants in both study groups over the phone on the day of the target quit date, then again after 1 week, 2 weeks, 4 weeks and 8 weeks. During these follow-up calls, study nurses will provide SSC and ask participants about their use of tobacco cigarettes. Study nurses will record the time spent on the phone with each participant, and will ensure that the same time is spent on the phone with intervention and control participants.

Average duration of counseling is expected to be 15 minutes over the phone and 30 minutes during the in-person visit at baseline. Participants will be allowed to use other NRT, smoking cessation medication or smoking cessation help at their own expenses. They will also be encouraged to go on the stop-tabac.ch (www.stop-tabac.ch) website for smoking cessation support if they wish online support, as is currently performed in smoking cessation counseling in Switzerland.

8.3 Dose / Device modifications

Participants will be free to choose between three nicotine concentrations (6 mg/ml, 11 mg/ml or 19.6 mg/ml), 3 flavors (two different tobacco flavors, menthol flavor or a fruity flavor) and administer through ENDS ad libitum. They will have the possibility to switch to other predefined nicotine concentrations and flavouring in order to maximise the adherence to ENDS. We will teach them how to recognize withdrawal symptoms (changes in mood etc) and instruct them to use more liquid or a higher nicotine concentration if they experience withdrawal symptoms.

Criteria for discontinuing the study treatment or study discontinuation see section 7.4.

8.4 Compliance with study intervention

Study nurses will be trained to deliver a structured intervention that includes explanations on how to use the device and e-liquids. Study participants will also receive an instructional leaflet and we will develop two explanatory videos available online on the study website. The video will explain the basic functionalities of the device, how to charge the device and the method to refill the tank. The second video will explain participants how to change the coil after each 6 weeks of use. We will optimize adherence of study participants to ENDS by offering participants a choice of nicotine content and e-liquid flavors. This should maximize the effect of ENDS and more closely match real life, since smokers select products based on their own needs and preferences. We also allow participants the opportunity to switch e-liquid flavors, as real life consumers might. Second, in order to avoid participants stop using ENDS for technical reasons, study nurses will be reachable during regular working hours. In case of defective ENDS-devices which need to be replaced, participants can either directly visit the centers to pick up replacement devices, or we will ship replacement devices directly to the participants' home as fast as possible.

We will monitor participant's use of e-liquids in ml during follow-up calls after 1 week, 2 weeks, 4 weeks and 8 weeks after the target quit date and at the 6-months visit. During follow-up calls study participants will be asked about any problem with regard to using the ENDS. At the 6-months visit, we will verify participant exposure to tobacco cigarettes vs. other sources of nicotine (ENDS, patches, etc.) by measuring cotinine, anabasine, CO and NNAL.

To encourage participants in the control group to attend their 6-months visit, we will offer a voucher ("BERNcity Geschenkcard") worth CHF 30 after the study ends. Following participants for 6 months instead of 12 months should also limit the number of participants who cross over to ENDS in the control group or who are lost to follow-up. However, we will ask participants in the control group if they use an ENDS at each follow-up call and follow-up visit.

8.5 Data Collection and Follow-up for withdrawn participants

A participant who decides to discontinue the study participation prematurely will be defined as a dropout if they have already been randomized. A study participant who terminates the study before being randomized will be defined as a screening failure.

Participants who cannot be reached over the phone at follow-up visits will not be excluded. Participants who do not present at the 6-month visits will be contacted by phone, by letter/e-mail, and, finally, we will contact their relatives or general practitioner. If we cannot contact participants for more than three months, we will consider them lost to follow-up.

In case of withdrawal, the biological material and health-related personal data of the person concerned will be anonymised after data evaluation has been completed.

8.6 Trial specific preventive measures

We will perform pregnancy tests in pre-menopausal women during the baseline visit. We will also specifically ask participants if they have known allergies to the tested products.

8.7 Concomitant Interventions (treatments)

Participants both in the control and intervention group will be allowed to use any NRT (patches, gums) or other smoking cessation medication (bupropion, varenicline) they wish at their own costs. Their use will be recorded in the eCRF and considered as a covariate in data analysis.

8.8 Study Device Accountability

The investigator or designee will maintain an inventory record of ENDS at the site level (received from manufacturer, returned to manufacturer) and at the patient level (dispensed to the patient, returned by the patient). The pre-filled form for ordering the liquids indicating the study participants postal mail

address which will then be scanned and transmitted to the company Gaïatrend along with their mail address will be maintained in the inventory record.

8.9 Return or Destruction of Study Device

Upon completion or termination of the trial, ENDS and spare e-Liquids will remain in the participants' possession, or if desired, can be returned to the sponsor-investigator.

9. STUDY ASSESSMENTS

9.1 Table of study procedures and assessments

Procedure	**Screening** ☽	**Baseline visit**	**Day 0** ☽	**Week 1** ☽	**Week 2** ☽	**Week 4** ☽	**Week 8** ☽	**Outcome Visit Month 6**
Visit timing Day 0 is target quit date (TQD)	**-x to -8 days**	**-7 days**	**Day 0** (TQD)	**+ 7 days**	**+14 days**	**+ 28 days**	**+ 56 days**	**+ 180 days**
Visit window				±2 days	±3 days	±4 days	±1 week	-4/+12 weeks
ENROLLMENT								
Eligibility screen	x	x						
Explanation of project and protocol	x	x						
Send informed consent	x							
Sign informed consent		x						
Randomization to ENDS (E) or control		x						
Set target quit date (TQD)	x							
ASSESSMENT								
Demographics	x	x						
Medical History		x						
Physical activity		x						x
Smoking history	x	x						
Nicotine dependence		x						x
Withdrawal symptoms		x						x
Environmental pollution (e.g. second hand smoke)		x						x
Alcohol and illegal drug use		x						x
Anxiety		x						x
Depression symptoms		x						x
Sleep quality		x						x
Quality of life		x						x
Women: pregnancy test		x						
INTERVENTION								
ENDS distribution		E						
E-liquid order by participants		E			E	E	E	
E-liquid sent to their homes		E			E	E	E	
ENDS instruction		E						
ENDS technical support			E	E	E	E	E	
Structured smoking		x	x	x	x	x	x	

cessation counselling								
OUTCOME MARKERS								
Tobacco use (questionnaires)	x	x	x	x	x	x	x	x
Estimated ENDS use (ml e-liquids used)			E	E	E	E	E	E
Exhaled CO		x						x
Urinary nicotine metabolites		x						x
Urinary metabolite of Nitrosamines (TSNAs)		x						x
Adverse events, serious adverse events		x	x	x	x	x	x	x
VOC, aldehyde and PAH metabolites in urine		x						x
Oxidative stress markers in urine		x						x
Oxidative stress in exhaled breath (Lausanne site only)		x						x
Blood pressure, heart rate, height, weight (BMI), waist circumference		x						x
Blood lipids, HbA1c, creatinine & glucose		x						x
Respiratory symptoms		x						x

E: ENDS group only; C: Control group only

9.2 Assessments of outcomes

Participants will be seen in a healthcare facility in each center twice, at baseline and after 6 months. Data will be collected by study nurses, supervised by the responsible investigator at each site, based on protocols standardized across sites. All data will be directly collected via centralized computerized data entry forms. Laboratory analyses of urine samples will be centralized at the Institute for Work and Health (Institut universitaire romand de Santé au Travail, IST) laboratory in Lausanne. IST laboratory is accredited ISO 17025 for nicotine quantification in urine, VOCs quantification, and it will use validated methods for the other compounds in urine and oxidative stress analyses. Additional to the urine sample, a full blood sample will be sent to IST to be stored and used for further research. The samples are only stored and continued to be used with the participants consent independent from the study (see section 12.6). The other blood sample will remain in the main laboratory of each health center where routine laboratory analysis of blood samples (blood lipids, HbA1c, creatinine & glucose) will be performed. Analyses of exhaled breath condensateswill only be performed in Lausanne and thus centralized at IST laboratory. CTU Bern personnel will monitor quality. We will inform participants that some random phone calls will be recorded for quality monitoring. Quality monitoring will include reviewing calibration procedures for carbon monoxide (CO) and blood pressure measuring devices, weight and height scales.

9.2.1 Assessment of primary outcome

The primary outcome will be the continuous smoking abstinence from target quit date to the 6-month

follow-up visit, ascertained by self-report (self-report of no smoking from target quit date) and confirmed by exhaled CO level (CO<10 ppm) and urinary level of cotinine and anabasine (<3 ng/ml) and NNAL (10 pg/mg creat).

- CO will be measured with a Micro Smokerlyser®; Bedfont Scientific Ltd
- Complete Urine samples will be collected in a flask. The Institute for Work and Health (IST) laboratory in Lausanne has a routine analytical method for measuring nicotine metabolites in urine and participates regularly in external quality control testing.

9.2.2 Assessment of secondary outcomes

The secondary outcomes will be measured as described for the primary endpoint.

Secondary smoking cessation outcome will be measured with a self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), again all confirmed by the exhaled CO level, urinary level of cotinine and anabasine, and change in the self-reported number of cigarettes smoked per day (CPD) with 50% reduction in CPD from baseline to follow-up considered as successful reduction, measured as above.

Respiratory symptoms will be assessed by questionnaires:

- COPD Assessment Test (CAT), Modified Medical Research Council (MMRC) Scale, Asthma Test (ACT/ ECRHS)

Each Urine sample will be split into six aliquots to quantify cotinine and anabasine (see above), PAH metabolites, VOCs, propylene glycol, TSNAs, 8-OHdG and 8-isoprostanes, since no analytical method can measure all the compounds together. The different compounds will be estimated as follows:

- Concentrations of urinary cotinine and anabasine, VOCs, TSNAs and PAHs will be measured for all participants
- Oxidative stress will be assessed by quantifying 8-OHdG and 8-isoprostane concentrations in both EBC and urine
- We will adapt the method for analyzing urinary TSNAs from Xia et al.[29] After enzymatic hydrolysis with a β-glucuronidase solution, the urine sample will be extracted using solid phase extraction (SPE) columns, and analyzed by LC/MS/MS.
- We will use a high performance liquid chromatography (HPLC) method developed by the IST laboratory to analyze PAHs (1- and 2-Naphtol and urinary 1-OHP).

Exhaled breath condensates (EBC) other than CO will be performed in all participants at the Lausanne site during the first as well as the outcome visit (baseline and 6 months), based on guidelines from the American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force for EBC collection[62]. EBC will be sampled with an EcoScreen II system (total exhaled volume of about 120 L). After collection, each sample will be aliquoted. To avoid auto-oxidation of fatty acids (potential impact on 8-isoprostane levels), we will add tert-butyl hydroxytoluene and rapidly freeze the sample. The samples will be stored at -70°C for a maximum of 1 month.[65]

OHdG and 8-isoprostane will be simultaneously determined in EBC, using a method adapted to Syslova et al.[65] After adding internal standards (isotopically labeled 8-OHdG and 8 isoprostane), 1 ml of EBC will be lyophilized and the residue dissolved in a water:methanol solution and immediately analyzed by a liquid chromatography–atmospheric pressure ionization tandem mass spectrometry (LC-ESI-MS/MS) method available at IST.

Cardiovascular risk factors (CVRF) will be assessed as follows:

- Blood collection: We will measure cardiovascular risk factors in blood by routine methods in clinical practice: : Total-, LDL- and HDL-cholesterol, blood glucose, triglycerides, HbA1c and creatinine.
- Blood pressure and heart rate will be measured by standard sphygmomanometer. After a 10-minute rest, the right arm blood pressure of a seated participant will be assessed at three one-minute intervals. The two final blood pressures will be averaged to reduce variability.
- Height, weight (calculation of body mass index, BMI), and abdominal circumference

Several questionnaires will be used to assess further parameters:

- Demographics: Age, gender; Socio-economic: education level, marital status; medical history, including medication use and healthcare utilization.

- Smoking history,[90, 91] including Fagerström questionnaire to assess nicotine dependence[92] and the Minnesota nicotine withdrawal scale.[93]
- Physical activity (International Physical Activity Questionnaire, IPAQ) [77]
- legal and illegal drug use: Alcohol consumption (AUDIT-C) [80, 94], illegal drug use (cannabis, cocaine, other) [81]
- Quality of life (EQ-5D questionnaire)[83]
- Depression symptoms (PHQ-9)[95]
- Anxiety (GAD-7) [85]
- Sleep Quality (PSQI) [82]
- Environmental pollution exposure (e.g. second hand smoke) [86, 87]

9.2.3 Assessment of other outcomes of interest

See above.

9.2.4 Assessment of safety outcomes

Information on safety outcomes (AEs of special interest, SAEs, visits to the family practitioner, in-patient hospitalizations / prolongation of existing hospitalizations), and ENDS deficiencies will be collected proactively during the phone calls and the 6-months in person visit using standardized questionnaires and international guidelines.[88]

9.2.4.1 Adverse events

For this trial, AEs of special interest as well as all SAEs will be collected, fully investigated, and documented in the source documents and the eCRF for all participants form the date of ICF signature until the last protocol-specific procedure has been completed. AEs of special interest include dry mouth, cough/dry cough, shortness of breath (dyspnea), throat / mouth irritation, headache, dizziness and increased heart rate / palpitations.

9.2.4.2 Laboratory parameters

See above (assessment of secondary outcomes)

9.2.4.3 Vital signs

See above (assessment of secondary outcomes)

9.2.5 Assessments in participants who prematurely stop the study

Subjects who permanently discontinue study treatment are encouraged to continue in the follow-up period and to attend the protocol-specified follow-up visit at 6 months in accordance with the intention-to-treat principle.

9.3 Procedures at each visit

9.3.1 Screening phone call at -x to -8 days to target quit date:

- Study information and explanation
- Eligibility check (inclusion/exclusion criteria)
- Scheduling of target quit date and baseline visit
- Smoking history

9.3.2 Visit 1, Baseline visit at -7 days to target quit date

- Patient information and delivery of written study information to subject
- Verification of eligibility, including urine pregnancy test for premenopausal women
- Informed consent
- Demographics
- Relevant medical history including current medication
- Smoking history and symptoms linked to smoking, nicotine dependence (Fagerström questionnaire), withdrawal symptoms
- Body weight, height, calculation of BMI, waist circumference

- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illegal drug use
    - Environmental pollution exposure
- Exhaled breath composition (CO, oxidative stress markers in a subset of participants)
- Blood collection (lipids, glucose, HbA1c & creatinine)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
- Randomization to study group
- Distribution and instruction of ENDS and e-liquids (intervention group only)
- Smoking cessation counselling
- Scheduling of phone calls

9.3.3 Phone calls at target quit date, week 1, week 2, week 4, and week 8

Phone calls at target quit date (corresponding to day 0) and after 1 week (±2 days), 2 weeks (±3 days), 4 weeks (±4 days), and 8 weeks (+/-1 week):

- Smoking status: cigarettes / ENDS
- Assessment of other NRT use
- Assessment of SAE and AEs of special interest
- Smoking cessation counselling
- ENDS technical trouble shooting
- Scheduling of phone calls and outcome visit

9.3.4 Outcome visit (month 6)

Visit at 6 months (corresponding to 24 weeks (-4 weeks/+12 weeks) after the target quit date):

- Current medication
- Smoking status, nicotine dependence (Fagerström questionnaire), withdrawal symptoms
- Assessment of AEs of special interest
- Body weight, height, calculation of BMI, waist circumference
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illicit drug use
    - Environmental pollution exposure
- Exhaled breath composition (CO, oxidative stress markers in a subset of participants)
- Blood collection (lipids, glucose, HbA1c & creatinine)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
-

10. SAFETY

10.1 Drug studies

Not applicable

10.2 Medical Device Category C studies

Not applicable

10.3 Medical Device Category A studies

Not applicable

10.4 Other clinical trial Category B

During the entire duration of the study, all adverse events (AE) of special interest (see section 9.2.4.1) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and case report forms (eCRF). Study duration encompassed the time from when the participant signs the informed consent form until the last protocol-specific procedure has been completed.

10.4.1 Definition and assessment of (serious) adverse events

An **AE** is any untoward medical occurrence in a clinical investigation participant administered an intervention and which does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the intervention, whether or not related to the intervention. [ICH E6 1.2, adapted]

A **SAE** is classified as any event which:

- requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
- result in permanent or significant incapacity or disability;
- is life-threatening or results in death; or
- causes a congenital anomaly or birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious.

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination will be further followed up until recovery or until stabilisation of the disease after termination, or until the participant is lost to follow-up.

Assessment of Causality

The assessment of causality of SAEs will be done by both, investigator and Sponsor-Investigator based on the criteria listed in the ICH E2A guidelines:

Relationship	Description

Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Assessment of Severity

Classification and severity grading scale in this study will be performed in accordance with "Common Terminology Criteria for Adverse Events CTCAE Version 4.03" terminology.

10.4.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

All SAEs will be reported immediately and within a maximum of 24 hours of learning of its occurrence to the Sponsor-Investigator of the study. Reporting is done via the eCRF which generates an automatic email notification to the Sponsor-Investigator once an SAE is entered (and saved) in the system.
Any SAE for which it cannot be excluded that the event is attributable to the study intervention is reported by the Sponsor-Investigator to the CEC and local ECs (as applicable) within 15 days.

Reporting of safety and protective measures

If immediate safety and protective measures have to be taken during the conduct of the trial, the investigator notifies the CEC of these measures, and of the circumstances necessitating them, within 7 days. The Sponsor-Investigator must immediately inform all participating Investigators about all safety and protective measures. The other in the trial involved Ethics Committees will be informed about safety and protective measures via the Sponsor-Investigator.

Periodic reporting of safety

An annual safety report is submitted by the Sponsor-Investigator once a year to the responsible Ethics committees and is also provided to all participating Investigators.

11. STATISTICAL METHODS

11.1 Hypothesis

The primary hypothesis of this trial is that providing smokers with ENDS and SCC leads to a higher rate of smokers who quit than usual care (i.e., smoking cessation councelling).

11.2 Determination of Sample Size

Primary outcome: The primary hypothesis of this trial is that providing smokers with ENDS and SCC leads to a higher rate of smokers who quit than usual care. Based on the results of a systematic review and meta-analysis of previous trials,[5] we expect a RR of smoking cessation of 1.6; based on our experience with RCTs in the setting of smokers willing to quit and contacting health professionals to seek help receiving SCC alone, we expect a 12% quit rate in the control group and thus 19% in the intervention group (ENDS + SCC) (7% absolute difference in abstinence rate). Targeting a 2-sided alpha of 0.05 and a power of 90% would require we include a minimum 557 smokers per group, for a total of 1114 smokers, to find a significant difference in quit rates. Previous experience suggests we should assume a 5% loss to follow-up, [96, 97] so we will increase our sample size by 5% (59 smokers). We will thus recruit 1172 smokers. We will consider participants lost to follow-up to be continuing smokers,[1] and our primary analysis will be intention-to-treat.

Secondary outcomes: The sample size of the entire trial is based on the primary outcome; the power to find a statistically significant difference for secondary outcome is limited to the total number of participants. For safety outcomes, we do not expect to find statistically different results between groups on SAEs. For toxicological outcomes of ENDS, such as measures of urinary biomarkers, we will compare participants based on ENDS exposure. Table 11.2-1 presents the number of participants we expect to fall into each category of ENDS and cigarette smoking over follow-up. In the intervention group, we expect a 19% of smokers who quit and 30% use of ENDS at the 6-month follow-up.[10] In the control group, we expect a 12% of smokers who quit at 6-month follow-up. Though participants in the control group will not be actively provided with ENDS during the first 6 months of the study, we expect 5% will purchase ENDS on their own, and thus cross over from the control to the intervention group.

Table 11.2-1: Expected distribution of participants over follow-up

	Intervention group				Control group			
Group	Ai	Bi	Ci	Di	Ac	Bc	Cc	Dc
ENDS	+	+	-	-	+	+	-	-
Tob smk	-	+	-	+	-	+	-	+
Baseline		586						586
6 months: N	59	117	53	357	6	12	64	504
% of group	10%	20%	9%	61%	1%	2%	11%	86%

Legend:
Ai= ENDS use, stop smoking, intervention
Bi= ENDS use, smoking, intervention
Ci= No ENDS, stop smoking, intervention
Di=No ENDS, smoking, intervention
Ac= ENDS use, stop smoking, control
Bc= ENDS use, smoking, control
Cc= No ENDS, smoking, control
Dc=No ENDS, stop smoking, control

Expected numbers of smokers who quit at 6 months between groups: 112 vs 70 (19% vs. 12%)

Expected total number of ENDS users at 6 months: 176 vs 18 (30% vs. 3%)

For measures of carcinogenicity, such as measures of incomplete combustion agents (VOCs and PAH: 1-OHP, 1- and 2-NAPH in urine) [28, 29] and exposure to TNSAs (NNK and NNN in urine),[17] and measures of oxidative stress in urine (8-OHdG and 8-isoprostane),[40, 66, 98] we will compare groups that stopped smoking with or without ENDS in intention to treat analyses (Ai+Ci vs. Ac+Cc; 112 vs. 70) and in per protocol analyses (Ai+Ac vs. Ci+Cc; 65 vs. 117). Based on previous cross sectional studies contrasting past smokers to smokers,[51, 98, 99] we estimate a 48% power to detect significant

differences between quitters and continuing smokers for 8-OHdG and 100% for 8-isoprostane between baseline and follow-up, at alpha 0.05 and an assumed correlation between baseline and follow-up of 0.7 (Table 11.2-2).

For the 6-months changes in cardiovascular risk factors, we do not have direct data from RCT of ENDS use. Using data from previous RCT on nicotine replacement therapy, we expect a mean increase in HDL-cholesterol of 0.107 mmol/l (95% confidence interval 0.057 to 0.158 mmol/l) among quitters,[53] an increase in systolic blood pressure of 1.65 mm Hg and in diastolic blood pressure of 0.90 mm Hg after 6 months in quitters.[54, 55] Considering data from the CoLaus study, a study on cardiovascular risk factors in a random sample of the Lausanne population, we expect to only have a 10 to 40% chance to detect a difference between ENDS users and continuing smokers.[100] We expect to be able to have 100% power to detect changes in respiratory symptoms.[9, 101]

Table 11.2-2: Power calculation and sample size required to detect statistically significant differences between past- and current smokers for secondary outcome measures

Measure	Matrix	Non smoker Mean (SD)	Past smoker Mean (SD)	Current smoker Mean (SD)	Reference	Power *	N **
Inhaled compounds							
1-OHP	Urine [µg/g creat]	0.08 (0.11)	0.11 (0.11)	0.2 (0.25)	Bartolomé et al. 2015[102]	98%	37
	Urine [µg/g creat]		0.04 (0.002)	0.12 (0.005)	Naufal et al. 2011[103]	100%	1
1-naphtol	Urine [ng/g creat]		1.6 (0.1)	7.2 (0.4)	Naufal et al. 2011[103]	100%	1
2-naphtol	Urine [ng/g creat]		1.8 (0.05)	8.9 (0.5)	Naufal et al. 2011[103]	100%	1
Oxydative stress							
8OHdG	Urine [ng/mg creat]	2.75 (1.6)	3.62 (2.41)	4.24 (3.3)	Sauvain et al., 2011[98]	48%	174
	EBC [ng/ml]	0.36 (0.09)	0.31 (0.10)	0.52 (0.15)	Doruk et al., 2011[66]	100%	3
8-isoprostane F2a	Urine [µg/g creat]	0.51 (0.04)	0.74 (0.07)	1.1 (0.1)	Harman et al., 2003[51]	100%	1
	Urine [µg/g creat]	0.7 (2.7)		4.48 (2.7)	Seet et al., 2011[42]	100%[t]	5
	EBC [pg/ml]	10.8 (0.8)		24.3 (2.6)	Montuschi et al., 2000[64]	100%[t]	1

*Power to detect statistically significant difference between quitters (past smokers) and continuing smokers (current smokers) between baseline and follow-up at alpha 0.05, an assumed correlation between baseline and follow-up of 0.7 and 182 participants (112 vs. 70) for urine analyses.

[t] Calculations above in * based on data from never smokers compared to current smokers.

** Number of participants per group needed to detect statistically significant differences at alpha of 0.05 and beta (power) of 0.80.

11.3 Statistical criteria of termination of trial

Not applicable as no formal interim analysis is planned.

11.4 Planned Analyses

The statistical analysis of the main results of the trial will be done at CTU Bern by a statistician blinded to the allocation. After the start of the trial but before recruitment ends, a statistical analysis plan will be written. The plan will determine all necessary data preparation steps (e.g. additional validations, generation of new variables), definitions (e.g. analysis sets), and statistical analyses (e.g. models, outputs such as tables and graphs).

All statistical analyses will be presented as effect measure plus 95% confidence interval. A significance level of 5% will be used.

11.4.1 Datasets to be analysed, analysis populations

All analyses will be done on the intention-to-treat principle set whereby all randomized patients will be analyzed in the allocated group regardless of any protocol violations such as cross-overs or early treatment discontinuations.

11.4.2 Primary Analysis

To answer the primary research question, we will compare the rates of quitting among smokers randomized to ENDS vs. those receiving usual care, on an intention-to-treat basis. Continuous smoking cessation from target quit date will be the main outcome, confirmed by biomarkers of exposure (exhaled carbon monoxide (CO) and urinary cotinine, anabasine and NNAL). We will calculated quit rates, relative risks (RR), and absolute risks for ENDS +SCC (intervention group) compared to SCC alone (control group). We will further compare groups using multivariate regression models adjusting for appropriate co-variates. Participants who stopped smoking, and who are continuous ENDS users, will be considered quitters. We will present per-protocol analyses in sensitivity analyses given that participants in the control group might start using ENDS in the first six months.

11.4.3 Secondary Analyses

We will repeat the calculations described in 11.4.2 for the secondary smoking cessation outcomes. To test the effect of ENDS on reducing the number of cigarettes smoked per day, we will define a binary predictor of >50% smoking reduction from baseline to follow-up smoking and repeat the analyses for the main outcome of quitting. We will also use joint multivariate random-effects (JMRE) models allowing to model CPD and smoking cessation. In addition, we will use mixed linear effect model to analyze the change in CPD modelled as a continuous outcomes over time.

For the measures of changes in measures of urinary biomarkers, exhaled breath measures, CVRFs and respiratory symptoms from baseline to the 6-months visit, we will first compare the changes in these measures between the intervention and control group using two-sample t-tests or Wilcoxon rank-sum test, whenever appropriate. We will then fit univariate and multivariate mixed linear effect models. We will use multivariate linear and multinomial logistic regression models to compare groups according to their exposure to tobacco smoking and ENDS.

11.4.4 Interim analyses

No formal interim analysis is planned. We do not anticipate undertaking formal interim analyses in this trial. In particular, even if a significantly large number of participants stop smoking in the intervention group, we will not stop the trial.

11.4.5 Safety analysis

We will compare the proportion of adverse events and serious adverse events between groups using recommended methods.

11.4.6 Deviation(s) from the original statistical plan

Deviations from the statistical analysis plan will be stated and justified in the final analysis report.

11.5 Handling of missing data and drop-outs

Participants lost during follow-up will be categorized as persistent smokers in the intention-to-treat analysis. We intend to obtain full follow-up data on all randomized participants. For missing data on the main and secondary outcomes, we will perform sensitivity analyses computing inverse probability of censoring weights (IPCWs) in the multivariate regression models, modeling the probability of missing data to weight the available measurement. Missing data on covariates will be handled using multiple imputation techniques.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

The Investigators will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The Principal Investigator, Sub-investigator, and Clinical Research Nurses or Coordinators will have access to the records. The Principal Investigators will permit authorized representatives of the Sponsor and regulatory agencies as applicable to examine clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

12.1.1 Case Report Forms

The CRF will be electronic. All data requested on the eCRF will be recorded and the investigators will ensure the recorded data will be consistent with the source documents or the discrepancies will be explained. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported in the eCRF and all other required reports. Generally, the eCRF will be completed within two weeks of completion of a participant's visit/ follow-up phone call.

12.1.2 Specification of source documents

Source documents will be available at the site to document the existence of the study participants and will include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

For all data captured in the eCRF, the location of the source will be documented on a list of source documents (source data location list), which will be stored in the investigator site file at each study site.

If certain data are directly entered into the eCRF (and are thus considered as source data) this will be specified on the source data location list accordingly.

Any change or correction to source data will be dated, initialed, and explained (if necessary) and will not obscure the original entry.

12.1.3 Record keeping / archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. The Investigators will take measures to prevent accidental or premature destruction of these documents.

12.2 Data management

12.2.1 Data Management System

The CRFs in this trial will be implemented electronically using a dedicated electronic data capturing (EDC) system (secuTrial). The EDC system will be activated for the trial only after successfully passing a formal test procedure conducted by the CTU Bern. All data entered in the CRFs will be stored on a Linux server in a dedicated Oracle database at the CTU Bern.

Responsibility for hosting the EDC system and the database will lie with Inselspital Bern.

12.2.2 Data security, access and back-up

The server hosting the EDC system and the database will be kept in a locked server-room. Only the system administrators will have direct access to the server. A role concept with personal passwords (site investigator, statistician, monitor, administrator etc.) will regulate permission for each user to use the system and database as he/she requires.

All data entered into the CRFs will be transferred to the database using Secure Sockets Layer (SSL) encryption. Each data point will have attributes attached to it identifying the user who entered it with the exact time and date. Retrospective alterations of data in the database will be recorded in an audit table. Time, table, data field and altered value, and the person will be recorded (audit trail). A multi-level back-

up system will be implemented.

12.2.3 Analysis and archiving

Final analyses and data files will be extracted from the database into statistical packages to be analyzed. The status of the database at this time will be recorded in special archive tables.

The study database with all archive tables will be securely stored by CTU and Inselspital Bern. The sponsor also will keep the Trial Master File and interim/final reports for at least 10 years.

12.2.4 Electronic and central data validation

Data will be checked by the EDC system for completeness and plausibility. Furthermore, selected data points will be cross-checked for plausibility with previously entered data for that participant. In addition, central data reviews will be performed on a regular basis to ensure completeness of the data collected and accuracy of the primary outcome data.

Before database lock the PI will validate the collected data with his signature.

12.3 Monitoring

For quality control of the study conduct and data retrieval, all study sites will be visited on-site by appropriately trained and qualified Monitors from the CTU Bern. Any findings and comments will be documented in site visit reports and communicated to the local Investigator and to the Sponsor as applicable. Investigators at the participating study sites will support the Monitor in his/ her activities. Prior to study start (first participant enrolled) a plan detailing all monitoring-related procedures will be developed.

All source data and relevant documents will be accessible to Monitors and questions of Monitors are answered during site visits.

12.4 Audits and Inspections

Source data/ documents will be made available to audits by the Sponsor or designee or to inspections by the CEC or regulatory authorities. If an inspection is requested, the Investigator will inform the Sponsor-Investigator immediately that this request has been made. The Investigators at the participating sites will support the inspectors in their activities and will answer questions from inspectors as needed. All involved parties will keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

The Investigator ensures anonymity of the patients; patients will not be identified by names in any study documents leaving the study site. Subject confidentiality will be ensured by utilizing subject identification codes consisting of three random letters and three random numbers, such as toj838. Signed informed consent forms and patient enrollment log will be kept strictly confidential to enable patient identification at the site.

12.6 Storage of biological material and related health data

The IST will be responsible for the storage of the full blood and urine samples. This will be stated in the contract between the IST and Sponsor-Investigator. The time frame for sample storage will remain undefined. The samples are only stored and continued to be used with the participants consent independent from the study.

The blood samples that are analyzed in the study centers (University Hospitals Bern, Geneva, and Lausanne) will remain in their responsibility. Also these samples are only stored and continued to be used with the participants consent independent from the study.

13. PUBLICATION AND DISSEMINATION POLICY

The trial protocol will be published in an open access journal. We will present study results at national and international conferences, and submit scientific articles to high impact, peer-reviewed journals; we will favor submission to Open Access journals.

When we complete the analysis, we will disseminate trial results to all participants.

We will report publicly through press releases and specific meetings of the ENDS user and tobacco cessation communities. No more than 6 months after publication of the trial data, anonymized datasets corresponding to each publication will be made freely available in an online data repository and in line with the Open Access (OA) rules of the SNSF.

14. FUNDING AND SUPPORT

14.1 Funding

This trial is financed by the Swiss National Science Foundation via the “Investigator-initiated clinical trials – IICT” grant # 173552.

14.2 Other Support

n.a.

15. INSURANCE

Insurance will be provided by the Sponsor.

16. REFERENCES

1. West, R., et al., *Outcome criteria in smoking cessation trials: proposal for a common standard.* Addiction, 2005. **100**(3): p. 299-303.
2. Murray, C.J. and A.D. Lopez, *Measuring the global burden of disease.* N Engl J Med, 2013. **369**(5): p. 448-57.
3. Jha, P., et al., *21st-century hazards of smoking and benefits of cessation in the United States.* N Engl J Med, 2013. **368**(4): p. 341-50.
4. Gmel, G., et al., *Monitorage suisse des addictions: Consommation d'alcool, de tabac et de drogues illégales en Suisse en 2012* 2013, Addiction Suisse: Lausanne.
5. Hartmann-Boyce, J., et al., *Electronic cigarettes for smoking cessation.* Cochrane Database Syst Rev, 2016. **9**: p. CD010216.
6. Farsalinos, K.E. and R. Polosa, *Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review.* Therapeutic advances in drug safety, 2014. **5**(2): p. 67-86.
7. Fagerstrom, K., J.F. Etter, and J.B. Unger, *E-cigarettes: a disruptive technology that revolutionizes our field?* Nicotine Tob Res, 2015. **17**(2): p. 125-6.
8. Farsalinos, K.E., et al., *Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices.* Sci Rep, 2014. **4**: p. 4133.
9. Caponnetto, P., et al., *EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: a prospective 12-month randomized control design study.* PLoS One, 2013. **8**(6): p. e66317.
10. Bullen, C., et al., *Electronic cigarettes for smoking cessation: a randomised controlled trial.* Lancet, 2013. **382**(9905): p. 1629-37.
11. Malas, M., et al., *Electronic Cigarettes for Smoking Cessation: A Systematic Review.* Nicotine & Tobacco Research, 2016.
12. Fraser, D., R. Borland, and C. Gartner, *Protocol for a randomised pragmatic policy trial of nicotine products for quitting or long-term substitution in smokers.* BMC Public Health, 2015. **15**: p. 1026.
13. McAuley, T.R., et al., *Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality.* Inhal Toxicol, 2012. **24**(12): p. 850-7.
14. Schober, W., et al., *Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers.* Int J Hyg Environ Health, 2014. **217**(6): p. 628-37.
15. Goniewicz, M.L., et al., *Levels of selected carcinogens and toxicants in vapour from electronic cigarettes.* Tob Control, 2014. **23**(2): p. 133-9.
16. Orr, M.S., *Electronic cigarettes in the USA: a summary of available toxicology data and suggestions for the future.* Tob Control, 2014. **23 Suppl 2**: p. ii18-22.
17. Walton, K.M., et al., *NIH electronic cigarette workshop: developing a research agenda.* Nicotine Tob Res, 2015. **17**(2): p. 259-69.
18. Bertholon, J.F., et al., *[Comparison of the aerosol produced by electronic cigarettes with conventional cigarettes and the shisha].* Rev Mal Respir, 2013. **30**(9): p. 752-7.
19. Etter, J.F. and C. Bullen, *Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy.* Addiction, 2011. **106**(11): p. 2017-28.
20. Etter, J.F., E. Zather, and S. Svensson, *Analysis of refill liquids for electronic cigarettes.* Addiction, 2013. **108**(9): p. 1671-9.
21. Flouris, A.D., et al., *Acute effects of electronic and tobacco cigarette smoking on complete blood count.* Food Chem Toxicol, 2012. **50**(10): p. 3600-3.
22. Ingebrethsen, B.J., S.K. Cole, and S.L. Alderman, *Electronic cigarette aerosol particle size distribution measurements.* Inhal Toxicol, 2012. **24**(14): p. 976-84.
23. Kirschner, R.I., R. Gerona, and K.R. Jacobitz, *Nicotine content of liquid for electronic cigarettes*, in *2013 Annual Meeting of the North American Congress of Clinical Toxicology (NACCT)*. 2013. p. 575-724.

24. Pellegrino, R.M., et al., *Electronic cigarettes: an evaluation of exposure to chemicals and fine particulate matter (PM).* Ann Ig, 2012. **24**(4): p. 279-88.
25. Romagna, G., et al., *Characterization of chemicals released to the environment by electronic cigarettes use (ClearStream-AIR project): is passive vaping a reality?*, in *XIV Annual Meeting of the SRNT Europe 2012*. 2012: Helsinki, Finland.
26. Trehy, M.L., et al., *Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities.* Journal of Liquid Chromatography & Related Technologies, 2011. **34**(14): p. 1442-1458.
27. Uchiyama, S., et al., *Determination of carbonyl compounds generated from the E-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography.* Anal Sci, 2013. **29**(12): p. 1219-22.
28. Feng, S., et al., *Evaluation of urinary 1-hydroxypyrene, S-phenylmercapturic acid, trans,trans-muconic acid, 3-methyladenine, 3-ethyladenine, 8-hydroxy-2'-deoxyguanosine and thioethers as biomarkers of exposure to cigarette smoke.* Biomarkers, 2006. **11**(1): p. 28-52.
29. Xia, B., et al., *Quantitative analysis of five tobacco-specific N-nitrosamines in urine by liquid chromatography-atmospheric pressure ionization tandem mass spectrometry.* Biomed Chromatogr, 2014. **28**(3): p. 375-84.
30. Hu, C.W., et al., *Direct analysis of tobacco-specific nitrosamine NNK and its metabolite NNAL in human urine by LC-MS/MS: evidence of linkage to methylated DNA lesions.* Arch Toxicol, 2014. **88**(2): p. 291-9.
31. Hecht, S.S., *Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer.* Carcinogenesis, 2002. **23**(6): p. 907-22.
32. Lisko, J.G., et al., *Chemical Composition and Evaluation of Nicotine, Tobacco Alkaloids, pH, and Selected Flavors in E-Cigarette Cartridges and Refill Solutions.* Nicotine Tob Res, 2015. **17**(10): p. 1270-8.
33. Suh-Lailam, B.B., et al., *Reference interval determination for anabasine: a biomarker of active tobacco use.* J Anal Toxicol, 2014. **38**(7): p. 416-20.
34. Jacob, P., 3rd, et al., *Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(12): p. 1668-73.
35. van der Vaart, H., et al., *Acute effects of cigarette smoke on inflammation and oxidative stress: a review.* Thorax, 2004. **59**(8): p. 713-21.
36. Rahman, I., *Redox effects of cigarette smoke in lung inflammation*, in *Cigarette Smoke and Oxidative Stress*, P.H. Halliwell B, Editor. 2006, Springer: New York. p. 113-164.
37. Pryor, W.A. and K. Stone, *Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite.* Ann N Y Acad Sci, 1993. **686**: p. 12-27; discussion 27-8.
38. Yamaguchi, Y., et al., *Oxidants in the gas phase of cigarette smoke pass through the lung alveolar wall and raise systemic oxidative stress.* J Pharmacol Sci, 2007. **103**(3): p. 275-82.
39. Campos, C., et al., *Urinary biomarkers of oxidative/nitrosative stress in healthy smokers.* Inhal Toxicol, 2011. **23**(3): p. 148-56.
40. Lowe, F.J., E.O. Gregg, and M. McEwan, *Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never-smokers.* Clin Chem Lab Med, 2009. **47**(3): p. 311-20.
41. Haswell, L.E., et al., *A cross-sectional analysis of candidate biomarkers of biological effect in smokers, never-smokers and ex-smokers.* Biomarkers, 2014. **19**(5): p. 356-67.
42. Seet, R.C., et al., *Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress?* Free Radic Biol Med, 2011. **50**(12): p. 1787-93.
43. Morrow, J.D. and L.J. Roberts, 2nd, *The isoprostanes. Current knowledge and directions for future research.* Biochem Pharmacol, 1996. **51**(1): p. 1-9.
44. Basu, S., *F-2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications.* Antioxidants & Redox Signaling, 2008. **10**(8): p. 1405-1434.
45. Lloyd-Jones, D., et al., *Heart Disease and Stroke Statistics--2010 Update. A Report From the American Heart Association.* Circulation, 2009: p. CIRCULATIONAHA.109.192667.

46. Lowe, F.J., K. Luettich, and E.O. Gregg, *Lung cancer biomarkers for the assessment of modified risk tobacco products: an oxidative stress perspective.* Biomarkers, 2013. **18**(3): p. 183-195.
47. Wu, L.L., et al., *Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics.* Clin Chim Acta, 2004. **339**(1-2): p. 1-9.
48. Loft, S., et al., *Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index.* Carcinogenesis, 1992. **13**(12): p. 2241-7.
49. Hakim, I.A., et al., *Gender difference in systemic oxidative stress and antioxidant capacity in current and former heavy smokers.* Cancer Epidemiol Biomarkers Prev, 2012. **21**(12): p. 2193-200.
50. Prieme, H., *Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion.* Carcinogenesis, 1998. **19**(2): p. 347-351.
51. Harman, S.M., et al., *Urinary excretion of three nucleic acid oxidation adducts and isoprostane F(2)alpha measured by liquid chromatography-mass spectrometry in smokers, ex-smokers, and nonsmokers.* Free Radic Biol Med, 2003. **35**(10): p. 1301-9.
52. Farsalinos, K.E., et al., *Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes.* BMC Cardiovasc Disord, 2014. **14**: p. 78.
53. Forey, B.A., et al., *The effect of quitting smoking on HDL-cholesterol - a review based on within-subject changes.* Biomark Res, 2013. **1**(1): p. 26.
54. Bartholomew, H.C. and M.W. Knuiman, *Longitudinal analysis of the effect of smoking cessation on cardiovascular risk factors in a community sample: the Busselton Study.* J Cardiovasc Risk, 1998. **5**(4): p. 263-71.
55. Gerace, T.A., et al., *Smoking cessation and change in diastolic blood pressure, body weight, and plasma lipids. MRFIT Research Group.* Prev Med, 1991. **20**(5): p. 602-20.
56. Aubin, H.J., et al., *Weight gain in smokers after quitting cigarettes: meta-analysis.* BMJ, 2012. **345**: p. e4439.
57. Stein, J.H., et al., *Smoking cessation and the risk of diabetes mellitus and impaired fasting glucose: three-year outcomes after a quit attempt.* PLoS One, 2014. **9**(6): p. e98278.
58. Flouris, A.D., et al., *Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function.* Inhal Toxicol, 2013. **25**(2): p. 91-101.
59. Vardavas, C.I., et al., *Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide.* Chest, 2012. **141**(6): p. 1400-6.
60. Palamidas, A., et al., *Acute effect of an e-cigarette with and without nicotine on lung function. Poster P1054*, in *European Respiratory Society's Annual Congress*. 2013.
61. Lim, H.B. and S.H. Kim, *Inhallation of e-Cigarette Cartridge Solution Aggravates Allergen-induced Airway Inflammation and Hyper-responsiveness in Mice.* Toxicol Res, 2014. **30**(1): p. 13-8.
62. Horvath, I., et al., *Exhaled breath condensate: methodological recommendations and unresolved questions.* Eur Respir J, 2005. **26**(3): p. 523-48.
63. Yanbaeva, D.G., et al., *Systemic effects of smoking.* Chest, 2007. **131**(5): p. 1557-66.
64. Montuschi, P., *Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications.* Ther Adv Respir Dis, 2007. **1**(1): p. 5-23.
65. Syslova, K., et al., *LC-ESI-MS/MS method for oxidative stress multimarker screening in the exhaled breath condensate of asbestosis/silicosis patients.* J Breath Res, 2010. **4**(1): p. 017104.
66. Doruk, S., et al., *Oxidative status in the lungs associated with tobacco smoke exposure.* Clin Chem Lab Med, 2011. **49**(12): p. 2007-12.
67. Blaser, J. and J. Cornuz, *Experts' consensus on use of electronic cigarettes: a Delphi survey from Switzerland.* BMJ Open, 2015. **5**(4): p. e007197.

68. Wagener, T.L., et al., *Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users.* Tob Control, 2016.
69. Etter, J.F., *A longitudinal study of cotinine in long-term daily users of e-cigarettes.* Drug Alcohol Depend, 2016. **160**: p. 218-21.
70. Frasch, H.F. and A.M. Barbero, *In vitro human epidermal permeation of nicotine from electronic cigarette refill liquids and implications for dermal exposure assessment.* Journal Of Exposure Science And Environmental Epidemiology, 2016. **27**: p. 618.
71. Bize, R., et al., *Participation in a population-based physical activity programme as an aid for smoking cessation: a randomised trial.* Tob Control, 2010. **19**(6): p. 488-94.
72. Rodondi, N., et al., *Impact of carotid plaque screening on smoking cessation and other cardiovascular risk factors: a randomized controlled trial.* Arch Intern Med, 2012. **172**(4): p. 344-52.
73. COPD Assessment Test. 2016; Available from: http://www.catestonline.org/.
74. Mahler, D.A. and C.K. Wells, *Evaluation of clinical methods for rating dyspnea.* Chest, 1988. **93**(3): p. 580-6.
75. Asthma Control Test. Available from: http://www.asthmacontroltest.com/Europe/Switzerland/German/adult/start#.
76. ECRHS European Community Respiratory Health Survey. Available from: http://www.ecrhs.org/.
77. Craig, C.L., et al., *International physical activity questionnaire: 12-country reliability and validity.* Med Sci Sports Exerc, 2003. **35**(8): p. 1381-95.
78. Hughes, J.R., *Tobacco withdrawal in self-quitters.* J Consult Clin Psychol, 1992. **60**(5): p. 689-97.
79. Hughes, J.R. and D. Hatsukami, *Signs and symptoms of tobacco withdrawal.* Arch Gen Psychiatry, 1986. **43**(3): p. 289-94.
80. Pletcher, M.J., et al., *Alcohol consumption, binge drinking, and early coronary calcification: findings from the Coronary Artery Risk Development in Young Adults (CARDIA) Study.* Am J Epidemiol, 2005. **161**(5): p. 423-33.
81. Auer, R., et al., *Association between Lifetime Marijuana use and Cognitive Function in Middle Age: the Coronary Artery Risk Development in Young Adults (CARDIA) Study.* JAMA-Internal Medicine, 2016. **In Press**.
82. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.
83. Perneger, T.V., C. Combescure, and D.S. Courvoisier, *General population reference values for the French version of the EuroQol EQ-5D health utility instrument.* Value Health, 2010. **13**(5): p. 631-5.
84. Kroenke, K., R.L. Spitzer, and J.B. Williams, *The PHQ-9: validity of a brief depression severity measure.* J Gen Intern Med, 2001. **16**(9): p. 606-13.
85. Spitzer, R.L., et al., *A brief measure for assessing generalized anxiety disorder: the GAD-7.* Arch Intern Med, 2006. **166**(10): p. 1092-7.
86. Leuenberger, P., et al., *Passive smoking exposure in adults and chronic respiratory symptoms (SAPALDIA Study). Swiss Study on Air Pollution and Lung Diseases in Adults, SAPALDIA Team.* Am J Respir Crit Care Med, 1994. **150**(5 Pt 1): p. 1222-8.
87. Kunzli, N., et al., *Association of environmental tobacco smoke at work and forced expiratory lung function among never smoking asthmatics and non-asthmatics. The SAPALDIA-Team. Swiss Study on Air Pollution and Lung Disease in Adults.* Soz Praventivmed, 2000. **45**(5): p. 208-17.
88. *International Conference on Harmonisation Working Group. ICH harmonised tripartite guideline: guideline for good clinical practice E6 (R1)*. in *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*. 1996. Washington, DC.
89. Auer, R., et al., *Uptake and efficacy of a systematic intensive smoking cessation intervention using motivational interviewing for smokers hospitalised for an acute coronary syndrome: a*

multicentre before-after study with parallel group comparisons. BMJ Open, 2016. **6**(9): p. e011520.
90. Cornuz, J., et al., *Efficacy of resident training in smoking cessation: a randomized, controlled trial of a program based on application of behavioral theory and practice with standardized patients.* Ann Intern Med, 2002. **136**(6): p. 429-37.
91. Cornuz, J., et al., *Smoking cessation counseling by residents in an outpatient clinic.* Prev Med, 1997. **26**(3): p. 292-6.
92. Heatherton, T.F., et al., *The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire.* Br J Addict, 1991. **86**(9): p. 1119-27.
93. West, R. and P. Hajek, *Evaluation of the mood and physical symptoms scale (MPSS) to assess cigarette withdrawal.* Psychopharmacology (Berl), 2004. **177**(1-2): p. 195-9.
94. Bush, K., et al., *The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test.* Arch Intern Med, 1998. **158**(16): p. 1789-95.
95. Radloff, L.S., *The CES-D Scale: A Self-Report Depression Scale for Research in the General Population.* Applied Psychological Measurement, 1977. **1**(3): p. 385-401.
96. Rodondi, N., et al., *The Impact of CAROtid plaque Screening on Smoking (CAROSS) cessation and control of other cardiovascular risk factors: Rationale and design of a randomized controlled trial.* Contemp Clin Trials, 2008. **29**(5): p. 767-73.
97. Auer, R., et al., *Acceptability and Efficacy of a Systematic Intensive Smoking Cessation Intervention Using Motivational Interviewing for Smokers Hospitalized for Acute Coronary Syndromes.*, in *SwissFamilyDocs*. 2014: Zürich.
98. Sauvain, J.J., et al., *Biomarkers of oxidative stress and its association with the urinary reducing capacity in bus maintenance workers.* J Occup Med Toxicol, 2011. **6**(1): p. 18.
99. Liang, Y., et al., *Quantification of 8-iso-prostaglandin-F2α and 2,3-dinor-8-iso-prostaglandin-F2α in human urine using liquid chromatography-tandem mass spectrometry.* Free Radical Biology and Medicine, 2003. **34**(4): p. 409-418.
100. Le Boudec, J., et al., *Smoking cessation and the incidence of pre-diabetes and type 2 diabetes: a cohort study.* J Diabetes Complications, 2016. **30**(1): p. 43-8.
101. Rajkumar, S., et al., *Effect of a smoking ban on respiratory health in nonsmoking hospitality workers: a prospective cohort study.* J Occup Environ Med, 2014. **56**(10): p. e86-91.
102. Bartolome, M., et al., *Urinary polycyclic aromatic hydrocarbon metabolites levels in a representative sample of the Spanish adult population: The BIOAMBIENT.ES project.* Chemosphere, 2015. **135**: p. 436-46.
103. Naufal, Z.S., et al., *Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999-2008.* Biomarkers, 2011. **16**(3): p. 222-35.

17. APPENDICES

17.1 Certifications and specifications of e-liquids

Ingredients and concentrations

Vegetable Glycerin
Also called glycerol, vegetable glycerin is a non-toxic, viscous, colorless and odorless liquid which tastes sweet. Used in the pharmaceutical, cosmetic and food industries, also generates the dense, full steam produced by e-vaporisers.

Propylene-Glycol
Propylene-glycol is a slightly viscous, little volatile, colorless and practically odorless liquid. Commonly used as a preservative in the pharmaceutical, cosmetic and food industries and for body hygiene products, propylene-glycol fills two major functions in e-liquids: it generates a light, fine vapor at low

temperature and serves as a flavour enhancer by very faithfully reproducing the authentic aroma of the flavour.

Nicotine
The purpose behind using nicotine is to create the throat hit sensation which corresponds to the contraction of the larynx when the vapour is inhaled. It is added in products made for smokers and ex-smokers.

Flavoring
The flavouring is what gives the e-liquid its individual flavour. It can be made up of natural or synthetic flavourings or a mixture of both. It reproduces the required flavour as faithfully as possible: fruit, drink, sweet or complex cocktail. Flavouring represents approximately 2 to 10% of an e-liquid.

See attachement “GAIATREND_eliquids_ingredients”

Children safety of e-liquids

See attachement “GAIATREND_eliquids_childrenSafety”

Certification of nicotine supplier

See attachement “GAIATREND_eliquids_nicotine”

Technical reports of flavour FM-M

See attachement “GAIATREND_eliquids_technicalReport_FR-M”

See attachement “GAIATREND_eliquids_technicalReport_FR-4”

See attachement “GAIATREND_eliquids_technicalReport_FR-MINT”

See attachement “GAIATREND_eliquids_technicalReport_ FRUITS ROUGES”

Clinical Study Protocol

Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

Study Type:	Other clinical trial
Study Categorisation:	Risk Category B
Study Registration:	Clinicaltrials.gov, Swiss National Clinical Trials Portal (SNCTP)
Study Identifier:	Swiss National Science Foundation # 173552
Sponsor- Investigator :	Universität Bern Berner Institut für Hausarztmedizin (BIHAM) Represented by: Prof. Dr. med. Reto Auer Mittelstrasse 43 3012 Bern Schweiz Tel: +41 31 684 58 79 Fax: +41 31 684 58 71 reto.auer@biham.unibe.ch Unisanté Centre universitaire de médecine générale et santé publique, Lausanne Rou du Bugnon 44 1011 Lausanne
Investigational Product:	Electronic nicotine delivery systems as smoking cessation aid
Protocol Version and Date:	9.0; 23rd July 2021

Signature Page(s)

Study number	Swiss National Science Foundation # 173552
Study Title	Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

The Sponsor- Investigator, the trial statistician, the group leader of the laboratory at the Centre universitaire de médecine générale et santé publique, Lausanne, Département Santé Travail - Environnement and the senior doctor at the department of Pneumology and the senior doctor at the department of Radiology have approved the protocol version 9.0, 23.07.2021, and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor- Investigator:

Prof. Dr. med. Reto Auer

Place/Date	Signature

Trial Statistician and Methodologist:

PD Dr. med. Sven Trelle

Place/Date	Signature

Group leader of laboratory at Centre universitaire de médecine générale et santé publique, Lausanne, Département Santé Travail - Environnement, responsible for urine, EBC and micronuclei samples :

PD Dr. Nancy B. Hopf

Place/Date	Signature

Senior Doctor at the Department of Pneumology, Inselspital, responsible for pulmonary function tests and MBW measurements:

PD Dr. med. Manuela Funke

Place/Date	Signature

Senior Doctor at the Department of Radiology, Inselspital, responsible for MRI measurements:
Prof. Dr. med. Lukas Ebner

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site: Klinik und Poliklinik für Allgemeine Innere Medizin
Inselspital, Bern

Principal investigator: Prof. Dr. med. Nicolas Rodondi

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site: Service de Médecine de premier recours
Hôpitaux Universitaires de Genève, Genève

Principal investigator: Prof. Dr. med. Jean-Paul Humair

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site: Unisanté

Centre universitaire de médecine générale et santé publique, Lausanne

Département formation, recherche et innovation

Principal investigator: Prof. Dr. med. Reto Auer

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site: Lungenzentrum, Klinik für Pneumologie und Schlafmedizin, Kantonsspital St. Gallen (KSSG)

Principal investigator: Prof. Dr. med. Martin Brutsche

Place/Date	Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site: Epidemiology, Biostatics and Prevention Institute (EBPI), University of Zurich

Principal investigator: PD Dr. Phil. Anja Frei

Place/Date	Signature

Table of Contents

STUDY SYNOPSIS

Sponsor :	Universität Bern, Berner Institut für Hausarztmedizin (BIHAM), represented by Prof. Dr. med Reto Auer
Study Title:	Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial
Short Title / Study ID:	ESTxENDS
Protocol Version and Date:	9.0, 23.07.2021
Trial registration:	Clinicaltrials.gov, Swiss national clinical trials portal (SNCTP)
Study category and Rationale	Other clinical trial, risk category B. ENDS and e-liquids are considered alimentary good in Switzerland and thereby regulated by the law on alimentary good. Sale and use of ENDS and e-liquids containing nicotine are permitted in Switzerland Because Swiss law considers ENDS and e-liquids as alimentary good, and not as medicinal products or medical devices, Swissmedic has waived approval and surveillance. We will follow the rules of the Federal Office of Public Health (FOPH) and the Federal Food Safety and Veterinary Office (FSVA) for the import of nicotine-containing e-liquids.
Clinical Phase:	n.a.
Background and Rationale:	Cigarette smoking is the leading cause of preventable death in Switzerland. Recently, electronic nicotine delivery systems (ENDS or vaporizers, also called e-cigarettes) have become popular with smokers who want to switch from tobacco cigarettes to ENDS to reduce their exposure to toxic compounds or to stop smoking. Only two rigorous RCTs have been published so far. They have promising, yet inconclusive results, as they were based on small samples. The safety and potential adverse effects of ENDS are also largely unknown. While the aerosol the users inhale appears safe in laboratory conditions, the difference in exposure to toxins (such as measures of exposure to organic compounds) and effect of toxins on the body (measures of oxidative stress) between smokers who quit (with or without ENDS) and those who use ENDS for a long time have not yet been assessed in an RCT.
Objective(s):	Test ENDS on: 1) the efficacy for cigarette smoking cessation and in reducing the number of cigarettes smoked over 6 months of follow-up; 2) the safety of ENDS; 3) the effect of ENDS on exposure to inhaled toxic compounds; 4) the effect of ENDS on health-related outcomes (clinical outcomes: respiratory symptoms; surrogate outcomes: oxidative stress, risk factors for heart disease).

Outcome(s):	Primary outcome - The primary outcome at 6 month will be the continuous smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report (self-report of no smoking from target quit date) and confirmed by urinary levels of anabasine (<3 ng/ml). - The primary outcome at 12- and 24- months will be the self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), confirmed by urinary levels of anabasine (<3 ng/ml). Secondary outcomes - Secondary smoking cessation outcome will alternate definitions of the smoking cessation outcome: as the primary outcome, but allowing up to 5 cigarettes in total and a 2-week`grace period' after the target quit date, self-reported 7-day point prevalence abstinence, all confirmed by urinary level of anabasine. We will also apply alternate definitions of the validation of exposure to tobacco smoking with: exhaled CO levels (CO<10 ppm) and NNAL (<10 pg/ml creat) and exposure to nicotine (from tobacco cigarettes, ENDS or NRTs) with urinary levels of cotinine. We will further report on change in the self-reported number of cigarettes smoked per day (CPD), with 50% reduction in CPD from baseline to follow-up considered as successful reduction. - Concentrations of urinary tobacco-specific nitrosamines (TSNAs), volatile organic compounds (VOCs), aldehydes and polycyclic aromatic hydrocarbons (PAHs) for all participants. Participants will complete a detailed questionnaire to account for other sources of exposure to these chemicals. - Respiratory symptoms (self-report) - Oxidative stress assessed by 8-OHdG and 8-isoprostane concentrations in both exhaled breath condensate (EBC) and urine; - Cardiovascular risk factors (CVRF) assessed by HDL- and LDL-cholesterol, HbA1c, creatinine, blood pressure levels, waist circumference, and body mass index. - Adverse events (AEs), serious adverse events (SAEs) following international standards. - Mood, anxiety and sleep quality (Self report) - Pulmonary function, measured by conventional pulmonary function tests and by multiple breath washout (MBW). - Structural and functional outcomes as ventilation and perfusion of the lung, measured by MRI. - Micronuclei (MN) in buccal epithelium - Olfactory function assessed by the Burghart Sniffin' Sticks (odor identification score (OIS)). - Dry-hit recognition - Detection of inflammatory biomarkers
Study design:	Open-label randomized controlled trial

Inclusion / Exclusion criteria:	Inclusion criteria: - Informed Consent as documented by signature - Persons aged 18 or older - Currently smoking 5 or more cigarettes a day for at least 12 months - Willing to try to quit smoking within the next 3 months - Persons providing a valid phone number, a valid email address and/or a valid postal address. Exclusion criteria: - Known hypersensitivity or allergy to contents of the e-liquid - Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected - Women who are pregnant or breast feeding - Intention to become pregnant during the course of the scheduled study intervention, i.e. within the first 6-months of the study - Persons having used ENDS or tobacco heating systems regularly in the 3 months preceding the baseline visit - Persons having used nicotine replacement therapy (NRT) or other medications with demonstrated efficacy as an aid for smoking cessation such as varenicline or bupropion within the 3 months preceding the baseline visit - Persons who cannot attend the 6-month follow-up visit for any reason - Persons who cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures
Measurements and procedures:	An overview on the study measurements and procedures is provided in the study schedules. The main measurements and procedures are the following: We plan to include 1172 smokers in a pragmatic RCT that will add ENDS to standard of care (SOC) for smoking cessation, and compare ENDS to SOC alone. Participants in the intervention arm will receive free ENDS and nicotine-containing e-liquids *ad libitum* until 6 months of follow-up. Participants in both arms will receive smoking cessation counselling (SCC) in four sessions over two months over the phone, and be allowed to use NRTs and other smoking cessation help (medication and other (any) non medication therapies) in parallel. At baseline and at 6-, 12- and 24- month follow-up, we will distribute questionnaires and perform a battery of clinical tests, including tests for cardiovascular risk factors (blood pressure, lipids, HbA1c, creatinine; body mass index), determine urinary biomarkers for tobacco-specific nitrosamines (TSNAs), urinary levels of nicotine, cotinine and anabasine, metabolites of polycyclic aromatic hydrocarbons (PAHs) (1-hydroxypyrene, 1- and 2-naphthol), volatile organic compounds (VOCs) and oxidative stress markers in urine, exhaled breath condensates (EBC) (8-hydroxy-2'-deoxyguanosine and 8- iso-prostaglandin F22α), micronuclei (MN) in buccal epithelium, measure pulmonary function (FEV1, FVC, FEV1/FVC, PEF, FEF25-75%, TLC, FRC, RV/TLC, DLCO) and multiple breath washout (MBW) (LCI, S_{cond} and S_{acin}). Lung MRI will be done in a subsample of participants at the 6-month follow-up and in healthy non-smoker volunteers never smokers/non-vapers who are not part of the RCT to assess functional and structural information of the lung at two timepoints- before and after smoking/vaping, if smoking/vaping. We will further assess olfactory function (odor indentification score (OIS)) and “dry-hit” recognition and measure inflammatory biomarkers in the blood.

Study Product / Intervention:	Participants in the intervention arm will receive free ENDS and nicotine-containing e-liquids *ad libitum* until 6-month follow-up; plus standard of care (SOC) for smoking cessation. Participants will be allowed to choose the flavor and nicotine concentration of the e-liquids. Use of ENDS will be *ad libitum* and will be monitored by use of e-liquids in ml. Study nurses will provide the technical support for the use of ENDS and schedule a phone call on the participant's target quit date. On the target quit date as well as at week 1, 2, 4 and 8 after target quit date, study nurses will call participants for SCC and further technical support and provide SCC and technical support in person at the first clinical visit (baseline visit) and at 6, 12 and 24 month follow-up visits (see below).
Control Intervention:	Participants in the control group will receive standard of care (SOC) for smoking cessation. Nurses will be trained to deliver standardized SCC. SCC will be provided in person at the first clinical visit (baseline visit), at 6, 12 and 24 month follow-up visits and over the phone at the target quit date) and again at week 1, 2, 4 and 8 after target quit date.
Number of Participants with Rationale:	Number of participats projected for the entire study: 1172; Intervention group: 586, control group: 586. Rationale see in section "Statistical considerations".
Study Duration:	5 years
Study Schedule:	First participant in: 06/18 Last participant out: 05/22

Investigator(s):	**Prof. Dr. med Reto Auer** Unisanté Centre universitaire de médecine générale et santé publique, Lausanne Département formation, recherche et innovation Rue du Bugnon 44 1011 Lausanne +41 21 314 60 60 reto.auer@unisante.ch **Prof. Dr. med. Nicolas Rodondi** Klinik und Poliklinik für Allgemeine Innere Medizin Inselspital Freiburgstrasse 20 3010 Bern +41 31 632 41 63 Nicolas.Rodondi@insel.ch **Prof. Dr. med. Jean-Paul Humair** Service de Médecine de premier recours Hôpitaux Universitaires de Genève Rue Gabrielle-Perret-Gentil 4 1211 Genève 14 +41 22 372 95 10 Jean-Paul.Humair@hcuge.ch **PD Dr. Phil Anja Frei** Universität Zürich Institut für Epidemiologie, Biostatistik und Prävention Hirschengraben 84 8001 Zürich +41 44 634 43 60 Anja.Frei@uzh.ch **Prof. Dr. med. Martin Brutsche** Kantonsspital St. Gallen (KSSG) Lungenzentrum, Klinik für Pneumologie und Schlafmedizin Rorschacher Strasse 95 9007 St. Gallen +41 71 494 99 44 Martin.Brutsche@kssg.ch
Study Centre(s):	Five sites in Switzerland including University Hospitals Bern, Geneva, Lausanne, Zürich and St. Gallen.

Statistical Considerations:	*Sample size:* Based on the results of a systematic review and meta-analysis of previous trials, we expect a RR of smoking cessation of 1.6; based on our experience with RCTs in the setting of smokers willing to quit and contacting health care professionals to seek help receiving SOC alone, we expect a 12% quit rate in the control group and thus 19% in the intervention group (ENDS + SOC) (7% absolute difference in abstinence rate). With a two-sided alpha of 0.05 and a power of 90%, we will include a minimum 557 smokers per group, for a total of 1114 smokers, to find a significant difference in quit rates. Previous experience suggests we should assume a 5% loss to follow-up, so we will increase our sample size by 5% (59 smokers). We will thus recruit 1172 smokers. We will consider participants lost to follow-up to be continuing smokers, and our primary analysis will be intention-to-treat. Though participants in the control group will not be actively provided with ENDS and asked not to use ENDS during the first 6 months of the study, we expect that 5% will still purchase ENDS on their own. The sample size of the entire trial is based on the primary outcome; the power to find a statistically significant difference for secondary outcomes is limited to the total number of participants. *Efficacy analysis:* To answer the primary research question, we will compare the rates of quitting among smokers randomized to ENDS vs. those receiving SOC, on an intention-to-treat basis. Continuous smoking cessation from target quit date will be the main outcome, confirmed by urinary anabasin levels.[1] We will calculate quit rates, relative risks (RR), and absolute risks for ENDS + SOC (intervention group) compared to SOC alone (control group). We will further compare groups using multivariate regression models adjusting for appropriate co-variates. Participants lost during follow-up will be categorized as ongoing smokers in the intention-to-treat analysis. We intend to obtain full follow-up data on all randomized participants (see Follow-up and Retention). Participants who stopped smoking, and who are ongoing ENDS users, will be considered quitters. We will also present per-protocol analyses given that some participants in the control group might start using ENDS in the first six months. We will apply the same data analysis methods for the secondary smoking cessation outcomes such as: a.) <5 cig from target quit date and b.) 7-days point prevalence abstinence (no cigarettes within the 7 days before outcome assessment)). To test the effect of ENDS on reducing the number of cigarettes smoked per day, we will define a binary predictor of $\geq$50% cigarettes per day of reduction (cpd) from baseline to follow-up. We will also use joint multivariate random-effects (JMRE) models allowing to model CPD and smoking cessation. In addition, we will use mixed linear effect model to analyze the change in CPD modelled as a continuous outcome over time. We will compare the proportion of adverse events and serious adverse events between groups using recommended methods. As to changes in measures of urinary biomarkers, exhaled breath measures, CVRFs and respiratory symptoms from baseline to the 6-, 12- and 24-months visit, we will first compare the changes in these measures between the intervention and control group using two-sample t-tests or Wilcoxon rank-sum test, whenever appropriate. We will then fit univariate and multivariate mixed linear effect models. We will use multivariate linear and multinomial logistic regression models to compare groups according to their exposure to tobacco smoking and ENDS.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

STUDY SUMMARY IN LOCAL LANGUAGE

Hintergrund

Rauchen ist die häufigste vermeidbare Todesursache in der Schweiz. Immer mehr Raucher, die sich den giftigen Substanzen des Tabakrauchs weniger aussetzen möchten oder einen Tabakrauchstopp anstreben, steigen ganz oder teilweise auf tabakfreie Vaporizer (e-Zigaretten, engl. auch electronic nicotine delivery systems, ENDS) um, mit denen nikotinhaltige Flüssigkeit (e-Liquids) verdampft werden. Momentan ist jedoch noch umstritten, ob ENDS als Unterstützung zur Rauchentwöhnung wirklich generell von Nutzen sind und welche Risiken der Konsum von ENDS birgt.

Ziele des Projekts

Im Rahmen einer kontrollierten, randomisierten klinischen Studie werden wir 1172 RaucherInnen, die entweder eine Rauchstoppberatung alleine oder in Kombination mit ENDS erhalten werden über jeweils 24 Monate begleiten. Wir werden die Effektivität von ENDS zur Tabakentwöhnung prüfen, indem wir bestimmen, ob die Anzahl gerauchter Zigaretten mit Hilfe der ENDS gesenkt werden kann bzw. ein totaler Rauchstopp erreicht wird. Gleichzeitig werden allfällige Nebenwirkungen erfasst, um die Sicherheit der ENDS zu beurteilen. Weiter wird erhoben, inwiefern sich die Schadstoffexposition beim Konsum von ENDS im Vergleich zum Tabakrauchen ändert, und ob sich gesundheitliche Aspekte wie Atemwegsbeschwerden sowie Blut- und Urinwerte, beispielsweise bezüglich oxidativem Stress oder kardiovaskulären Risikofaktoren, verbessern.

Bedeutung

Bisher wurden zwei klinische Studien zu Rauchentwöhnung mit ENDS publiziert. Bezüglich Nutzen der ENDS zur Rauchentwöhnung sind die Resultate zwar vielversprechend, aber noch nicht genug aussagekräftig, da die Anzahl untersuchter Personen gering war. Daten zu Sicherheit und Schadstoffen der ENDS liegen noch kaum vor. Die Positionen der Gesundheitsfachleute und Entscheidungsträger der Politik sind kontrovers: Einige vertreten die Ansicht, dass ENDS als sichere Alternative zum konventionellen Tabakrauchen aktiv angepriesen werden sollten; andere sind der Meinung, dass man von deren Gebrauch abraten sollte, da sie gesundheitsschädlich sein könnten und nicht zum Rauchstopp beitragen. Die Resultate unserer Studie sollen demnach Konsumenten, Gesundheitsfachpersonen und Politikern unabhängige und fundierte Information zu Sicherheit und Schadstoffen von ENDS liefern und zu der Entscheidung beitragen, ob ENDS aktiv als Alternative zum Tabakrauchen angepriesen werden sollen. Die Studie soll zeigen, ob sich ENDS zur Rauchentwöhnung eignen und damit Raucher-assoziierte Erkrankungen und Folgekosten für das Gesundheitswesen reduziert werden und langfristig etliche Leben gerettet werden könnten, die durch Tabakrauchen gefährdet sind.

ABBREVIATIONS

AE	Adverse Event
ASR	Annual Safety Report
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CRF	Case Report Form
CO	Carbon monoxide
CPD	Cigarettes per day
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
CVRF	Cardiovascular risk factors
DLCO	Diffusion Capacity of the Lung for Carbon Monoxide
DSUR	Development safety update report
EBC	Exhaled breath condensates
ENDS	Electronic nicotine delivery devices
FEF25-75%	Forced expiratory flow at 25-75% of the pulmonary volume
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
FRC	Functional Residual Capacity
GCP	Good Clinical Practice
IB	Investigator's Brochure
HbA1c	Gylcated hemoglobin
HDL-cholesterol	High-density lipoprotein cholesterol
Ho	Null hypothesis
H1	Alternative hypothesis
HRA	Federal Act on Research involving Human Beings
ICDAS	International Caries Detection and Assessment System
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention to treat
LCI	Lung clearance index
LDL- Cholesterol	Low-density lipoprotein cholesterol
MBW	Multiple breath washout
MD	Medical device
MN	Micronuclei
MRI	Magnetic resonance imaging
NRT	Nicotine replacement therapy
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	Nicotine-derived nitrosamine ketone (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone)
NNN	N'–nitrosonornicotine
OIS	Olfactory Identification Score
PAH	Polycyclic Aromatic Hydrcarbons

PEF	Peak expiratory flow
PI	Principal Investigator
PSR	Periodontal Screening and Recording
RCT	Randomized Clinical Trial
RV/TLC	Residual Volume/Total Lung Capacity
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
S_{acin}	Specific markers of ventilation inhomogeneity in intermediate zone acinar airways
S_{cond}	Specific markers of ventilation inhomogeneity in proximal conducting airways
SCC	Smoking Cessation Counselling
SOC	Standards of care (for smoking cessation)
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
TLC	Total Lung Capacity
TMF	Trial Master File
TNSAs	Tobacco-specific nitrosamines
VOCs	Volatile organic compounds

STUDY SCHEDULE

Procedure	**Screening** ◑	**Baseline visit**	**Day 0** ◑	**Week 1** ◑	**Week 2** ◑	**Week 4** ◑	**Week 8** ◑	**Outcome Visit Month 6**	**Outcome visit Month 12**	**Outcome visit Month 24**
Visit timing Day 0 is target quit date (TQD)	**-x to -8 days**	**-7 days**	**Day 0** (TQD)	**+ 7 days**	**+14 days**	**+ 28 days**	**+ 56 days**	**+ 180 days**	**+ 360 days**	**+ 720 days**
Visit window				±2 days	±3 days	±4 days	±1 week	-4/+12 weeks	-4/+12 weeks	-4/+12 weeks
ENROLLMENT										
Eligibility screen	x	x								
Explanation of project and protocol	x	x								
Send informed consent	x									
Sign informed consent		x								
Randomization to ENDS (E) or control		x								
Set target quit date (TQD)	x									
ASSESSMENT										
Demographics	x	x								
Medical History		x						X*	X*	X*
Physical activity		x						X*	X*	X*
Smoking history	x	x								
Nicotine dependence		x						X*	X*	X*

Procedure	**Screening** ◑	**Baseline visit**	**Day 0** ◑	**Week 1** ◑	**Week 2** ◑	**Week 4** ◑	**Week 8** ◑	**Outcome Visit Month 6**	**Outcome visit Month 12**	**Outcome visit Month 24**
Withdrawal symptoms		x						X*	X*	X*
Environmental pollution (e.g. second hand smoke)		x						X*	X*	X*
Alcohol and illegal drug use		x						X*	X*	X*
Anxiety		x						X*	X*	X*
Depression symptoms		x						X*	X*	X*
Sleep quality		x						X*	X*	X*
Quality of life		x						X*	X*	X*
Women: pregnancy test		x								
Pulmonary function tests and MBW		x						x	x	x
MRI of the lung+								x		
Micronuclei assessment in buccal epithelium		x						x	x	x
Olfactory function		x						x	x	x
"Dry-hit" recognition								E		
Inflammatory biomarkers		x						x		
INTERVENTION										
ENDS & e-liquid distribution		E								
E-liquid order by participants				E	E	E	E	E		
E-liquid sent to their homes					E	E	E	E		
ENDS instruction		E								
ENDS technical support			E	E	E	E	E	E		
Structured smoking cessation counselling		x	x	x	x	x	x	x	x	x
OUTCOME MARKERS										
Tobacco use (questionnaires)	x	x	x	x	x	x	x	X*	X*	X*
Estimated ENDS use (ml e-liquids used)			E	E	E	E	E	E*	E*	E*
Exhaled CO		x						x	x	x
Urinary nicotine, cotinine, anabasine		x						x	x	x
Urinary meta-bolites of nitros-amines (TSNAs)		x						x	x	x

Procedure	**Screening** ◑	**Baseline visit**	**Day 0** ◑	**Week 1** ◑	**Week 2** ◑	**Week 4** ◑	**Week 8** ◑	**Outcome Visit Month 6**	**Outcome visit Month 12**	**Outcome visit Month 24**
Adverse events, serious AE			x	x	x	x	x	X*	X*	X*
VOC and PAH metabolites in urine		x						x	x	x
Oxidative stress markers in urine		x						x	x	x
Oxidative stress in exhaled breath (Lausanne site only)		x						x		
Blood pressure, heart rate, height, weight (BMI), waist circumference		x						x	x	x
Blood lipids, HbA1c (only for participants with diabetes), creat.		x						x	x	x
Respiratory symptoms		x						X*	X*	X*
Pulmonary function test parameters (FEV1, FVC, FEV1/FVC, PEF, FEF25-75%, TLC, FRC, RV/TLC, DLCO) (Bern site only)		x						x	x	x
Multiple breath washout (MBW) parameters (LCI, Scond, Sacin) (Bern site only)		x						x	x	x
Parameters of ventilation, perfusion and structural changes of the lung (MRI; Bern site only)+								x		
Micronuclei frequency (micronuclei assessment)		x						x	x	x
Olfactory function parameters (odor identification score (OIS))		x						x	x	x
“Dry-hit” recognition								E		
Inflammatory biomarkers		x						x		

E: ENDS group only; C: Control group only

* possibility of having participants fill the questionnaire online

+ A aged matched group of healthy never smoking/non-vaping volunteers (N=10) will perform once two consecutive MRI measurements. These volunteers are not participants of the RCT.

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor - Investigator

Universität Bern
Berner Institut für Hausarztmedizin (BIHAM)
Represented by: Prof. Dr. med. Reto Auer
Mittelstrasse 43
3012 Bern
Tel: +41 31 631 58 79
reto.auer@biham.unibe.ch

1.2 Principal Investigator

Bern site:
Prof. Dr. med. Nicolas Rodondi
Klinik und Poliklinik für Allgemeine Innere Medizin
Inselspital
Freiburgstrasse 20
3010 Bern
+41 31 632 41 63
Nicolas.Rodondi@insel.ch

Lausanne site:
Prof. Dr. med. Reto Auer
Unisanté
Centre universitaire de médecine générale et santé publique, Lausanne
Département formation, recherche et innovation
Rue du Bugnon 44
1011 Lausanne
+41 21 314 60 60
reto.auer@unisante.ch

Geneva site:
Prof. Dr. med. Jean-Paul Humair
Service de Médecine de premier recours
Hôpitaux Universitaires de Genève
Rue Gabrielle-Perret-Gentil 4
1211 Genève 14
+41 22 372 95 10
Jean-Paul.Humair@hcuge.ch

Zürich site:
PD Dr. Phil Anja Frei
Universität Zürich
Institut für Epidemiologie, Biostatistik und Prävention
Hirschengraben 84
8001 Zürich
+41 44 634 43 60
Anja.Frei@uzh.ch

St. Gallen site:
Prof. Dr. med. Martin Brutsche
Kantonsspital St. Gallen (KSSG)
Lungenzentrum, Klinik für Pneumologie und Schlafmedizin
Rorschacher Strasse 95
9007 St. Gallen
+41 71 494 99 44
Martin.Brutsche@kssg.ch

1.3 Statistician ("Biostatistician")

PD Dr. med. Sven Trelle
CTU Bern, Faculty of Medicine, University of Bern
Mittelstrasse 43, 3012 Bern, Switzerland
Tel: +41 31 631 35 04
sven.trelle@ctu.unibe.ch

1.4 Laboratory

Inselspital
Universitätspital Bern
Zentrum für Labormedizin
3010 Bern

Hôpitaux Universitaires de Genève
Médecine de laboratoire
Rue Gabrielle-Perret-Gentil 4
1205 Genève

CHUV
Département des laboratoires
Rue du Bugnon 21
1011 Lausanne

KSSG
Zentrum für Labormedizin St. Gallen
Frohbergstrasse 3
9001 St. Gallen

Universitätsspital Zürich
Universitäres Zentrum für Labormedizin und Pathologie
Rämistrasse 100
8091 Zürich

For urine, EBC and micronuclei samples:
PD Dr. Nancy B. Hopf
Unisanté
Centre universitaire de médecine générale et santé publique, Lausanne
Département Santé Travail - Environnement
Route de la Corniche 2
1066 Epalinges
Laboratoire Unisanté
Rue du Bugnon 19
1005 Lausanne

For pulmonary function tests and MBW measurements:
PD Dr. med. Manuela Funke
Universitätsklinik für Pneumologie
Inselspital, 3010 Bern

For MRI measurements:
Prof. Dr. med. Lukas Ebner
Department of Radiology
Inselspital, 3010 Bern

For additional blood samples:
Biobank Bern
Inselspital Bern
Direktion Lehre und Forschung, 3010 Bern

Biobank Genève
Hôpitaux Universitaires de Genève
Sérothèque/ Biothèque SML
Rue Gabrielle Perret Gentil 4
1211 Genève 14

1.5 Monitoring institution

CTU Bern, Faculty of Medicine, University of Bern
Mittelstrasse 43, 3012 Bern, Switzerland
Tel: +41 31 631 33 72

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Any other relevant Committee, Person, Organisation, Institution

Data management

CTU Bern, Faculty of Medicine, University of Bern,
Mittelstrasse 43, 3012 Bern, Switzerland, Tel: +41 31 631 33 72

Study Steering Commitee

The study steering committee will be responsible for the overall supervision of the trial and will regularly meet to discuss scientific and logistic aspects of the trial. The study steering committee consists of 7 persons, at least one person from each center involved in the study.

Data and Safety monitoring board

A committee of clinical research experts and patient advocates who monitor the progress of the clinical trial and review safety and effectiveness data while the trial is ongoing will be formed. This committee is independent of the people, organizations, and institutions conducting the clinical trial. The committee can recommend that the trial is stopped early because of concerns about participant safety.

Adjudication Committee

The adjudication committee is an independent group of experts that reviews serious adverse events and adverse events of interest in order to give expert opinions about clinical safety.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents will be submitted to a properly constituted Competent Ethics Committee (CEC) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.

The decision of the CEC concerning the conduct of the study will be made in writing to the Sponsor before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registered in the Clinical Trials Registry Platform of the National Institute of Health (NIH), clinicaltrial.gov. In addition, it will be registered in the Swiss National Clinical Trials Portal.

2.2 Categorisation of study

Other clinical trial, Risk category B.

ENDS and e-liquids are considered alimentary good in Switzerland and thereby regulated by the law on alimentary good. Sale and use of ENDS and e-liquids containing nicotine is permitted in Switzerland. Because Swiss law considers ENDS and e-liquids as alimentary good, and not as medicinal products or medical devices, Swissmedic has waived approval and surveillance. We will follow the rules of the Federal Office of Public Health (FOPH) and the Federal Food Safety and Veterinary Office (FSVA) for the import of nicotine-containing e-liquids.

2.3 Competent Ethics Committee (CEC)

The sponsor will ensure that approval from the appropriate constituted CECs is sought for the clinical study. Yearly intermediary reports (annual safety reports) will be forwarded to the CEC. All unanticipated problems involving risks to humans will be reported to the CEC within 7 days. No changes will be made to the protocol without prior Sponsor approval and, in case of substantial amendments, CEC approval, except where necessary to eliminate apparent immediate hazards to study participants. Amendments will be reported according to section 2.10.

Premature study end or interruption of the study will be reported to the CEC within 15 days. The regular end of the study will be reported to the CEC within 90 days, the final study report shall be submitted within one year after study end.

2.4 Competent Authorities (CA)

Given the intervention of this randomized controlled trial (RCT), no approval from any competent authority will be sought. No reporting duties apply.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

There are no conflicts of interest. This study is entirely funded by the Investigator-Initiated Clinical Trial (IICT) funding scheme from the SNSF which support studies on topics that are not in the industry focus and have no direct commercial interests. The application has underwent scrutiny by the SNSF that the applicants comply with good practice aimed at preventing conflicts of interests and the applicants will continue to follow these good practices for this clinical trial.

2.7 Patient Information and Informed Consent

The investigators or a representative of the study team will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

Each participant will be informed that his/her medical records may be examined by authorised individuals other than their treating physician.

All potential study participants will be provided with a participant information sheet and a consent form describing the study and providing sufficient information for the subject to make an informed decision about their participation in the study. Interested subjects will either call themselves or, if they contacted us via email, receive a phone call where the study will be explained. If eligible, a date for their first visit will be set, and the patient information sheet will be sent to them by mail. During the first visit, the study information will be repeated and questions will be discussed. After this, the consent form will be signed. Thus, at least 24 hours will lie between receiving the information and signing the consent form, and therefore participants will be given enough time to decide whether or not to participate.

The patient information sheet and the consent form will be submitted to the CEC to be reviewed and approved. The formal consent of a participant, using the approved consent form, will be obtained before the participant is submitted to any study procedure.

The participant will read and consider the statement before signing and dating the informed consent form, and will be given a copy of the signed document. The consent form will also be signed and dated by the investigator (or his designees) and it will be retained as part of the study records.

Study participants in the intervention group can keep the devices and the remaining e-liquids or return them to the investigators at the end of the study whereas the study participants in the control group will receive a voucher (“BERNcity Geschenkcard” or similar voucher for Lausanne,Geneva, Zürich and St. Gallen) of similar monetary value of the ENDS and e-liquids at the Baseline visit.

At the clinical visit at 12 and 24 months follow-up, all participants coming for a personal visit will receive a voucher worth 30 CHF in oder to ensure adequate follow-up rates.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor, a competent authority, or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants’ medical history.

2.9 Early termination of the study

The Sponsor may terminate the study prematurely according to certain circumstances, e.g.:

- ethical concerns,
- financial issues,
- insufficient participant recruitment,

- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention
- or any other reason that would prevent the project execution according to the research plan.

Premature study end or interruption of the study will be reported to the CEC within 15 days.

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the CEC.

Under emergency circumstances, deviations from the protocol to protect the rights, safety, and well-being of human subjects may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.

All Non-substantial amendments are communicated to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Research questions

1) How effective are ENDS in helping smokers quit, and reducing the number of cigarettes per day smoked over 6, 12 and 24 months of follow-up?
2) Do ENDS result in adverse events when smokers use them as quitting aids?
3) Does using ENDS effectively reduce exposure to inhaled toxic compounds?
4) Does using ENDS improve health-related clinical outcomes (respiratory symptoms) and surrogate outcomes (oxidative stress, cardiovascular risk factors)?

Background

Smoking is the leading cause of avoidable death worldwide, is one of the top five causes of morbidity and lowered disability-adjusted life-years.[2] Cigarette smoking eventually kills one in two smokers, mostly through cancer, heart disease and respiratory failure.[3] More than a quarter of the Swiss population still smokes cigarettes.[4]

ENDS are electrically-driven devices that reproduce many features of tobacco cigarettes such as release of smoke (aerosol for ENDS), mimic the gesture of smoking and provoke similar mouth experiences such as the "throat hit", typical of cigarette smoking.[5] They consist of a battery part, a liquid storage tank, and an atomizer that aerosolizes the liquid by generating heat to a resistance.[6] The liquid consists of propylene glycol, glycerol, distilled water, flavorings, ethanol and nicotine. Consumers (commonly called 'vapers') may choose from several types of ENDS, nicotine strengths, and a long and growing list of flavors. Compared to traditionally used nicotine replacement therapies such as nicotine patches, gums, sprays, lozenges and inhalers, ENDS may have different effects, since they better replicate the experience of traditional cigarette smoking and more quickly deliver nicotine to the blood.

ENDS were invented in China over fifteen years ago, but were not marketed in Europe or the USA until a decade ago.[7] Sales dramatically increased around 2010 and for the past few years, the prevalence of ENDS use has been stable in Switzerland, France, and the UK. ENDS changed rapidly, and are now in their fourth generation. First-generation ENDS resemble cigarettes. The devices have low-capacity batteries and a heating element surrounded by a "cartomizer." First generation ENDS produce a lower quality and amount of vapor than second generation ENDS. Second generation ENDS typically have a high-capacity battery, an "atomizer", and a refillable "clearomizer".[6] Parts, including resistance, wick and batteries, can be replaced, lowering costs for users. Third generation modified ENDS, also called 'mods', have large-capacity lithium batteries and users deliver more or less power to the atomizer. Vapour production and nicotine delivery are more efficient.[6] Users can control the temperature of the coils in fourth generation ENDS, which might allow them to minimize the amount of toxins they inhale. These latest devices have large coils that again improved vapour production and nicotine delivery.

Because ENDS use appears to be safer than tobacco smoking, healthcare professionals have been interested in ENDS as a smoking cessation or harm reduction device. Most smoking cessation drugs and devices were developed, tested, and promoted by the pharmaceutical industry. Large prospective RCTs have evaluated the efficacy of nicotine replacement therapies (NRTs) and other smoking cessation drugs. The pharmaceutical industry has not yet invested in ENDS. ENDS are produced, sold, and developed in China's essentially unregulated, highly competitive market, usually by independent companies.[7] They are cheap to make. The competitive market keeps the price of ENDS low and companies that produce ENDS may not be interested in conducting large studies that evaluate the effectiveness and safety of ENDS and have no obligations to run such studies. The fast growing ENDS market is in competition with the market for tobacco products, so the tobacco industry may be hostile to ENDS.

Healthcare professionals are increasingly asked to counsel smokers who want to use ENDS to help them quit smoking tobacco. But since no large, well conducted studies exist to back up that recommendation yet, they are in a difficult position.

The tobacco industry has funded studies on ENDS in the past, but healthcare professionals are reasonably cautious about using data generated by the tobacco industry, since this industry still promotes tobacco smoking through advertisements and lobbies to block anti-tobacco policies designed to improve public health. There are currently sharp tensions among professionals who want to reduce the health burden of tobacco smoking. Some argue that ENDS are a safe device for helping smokers quit, and others advise to wait until we have more data on their effect on smoking cessation and on the

health consequences of prolonged use.[7] If healthcare workers are going to base their recommendations for ENDS use on evidence rather than conjecture, we need to provide data on the effectiveness of ENDS in helping smokers quit, and on ENDS safety (for example, in terms of adverse events). We must provide health-related outcomes in both the short- and long-term.

Efficacy of ENDS to help smokers quit and reduction of number of cigarettes smoked

Studies on the efficacy of ENDS to help smokers quit are scarce; only two rigorous randomized controlled trials have been published.[5, 8-10] The ECLAT trial found that self-reported abstinence from tobacco smoking at 12 months was higher in participants using ENDS varying nicotine contents than in the ENDS group without nicotine.[8] The second study, published by Bullen et al, found no significant difference in six-month CO-validated continuous abstinence between any of its three arms: 1) ENDS (7.3%); 2) nicotine patches (5.8%); and, 3) placebo e-cigarette (4.1%).[9] A Cochrane systematic review that pooled results from 662 participants in these 2 RCTs found that participants who used ENDS were more likely to have smoked no cigarettes for at least six months (9%) than participants who used placebo ENDS (4%; RR 2.29, 95% CI 1.05 to 4.96).[5 5, 11]But the review included only two studies, so its evidence had a 'low' GRADE score. In addition, another meta-analysis by another group but including the same 2 studies did not confirm these results, leaving the efficacy issue open.[11] Since then, a large RCT conducted in the UK compared 2nd generation ENDS to NRTs in 886 smokers[12] and found the 1-year abstinence rate was 18% in the e-cigarette group and only 10% in the nicotine-replacement group (RR: 1.83; 95% confidence interval [CI], 1.30 to 2.58). While these results are promising, we urgently need more research to increase confidence in effect estimates, in particular about long-term effects of ENDS over two years.[5] Only the most recent RCTs tested 2nd generation ENDS; the others were conducted with first-generation ENDS[11] so newer and possibly more effective ENDS need to be rigorously evaluated.

The recently published Cochrane systematic review on the efficacy of ENDS for helping smokers quit identified 15 ongoing studies that test the effect of ENDS.[5] While most studies are underpowered to show significant differences between groups, one large ongoing study is likely to provide important information the efficacy of ENDS for smoking cessation. One study in New Zealand plans to randomize 1600 participants into three groups.[13] One receiving standard quit advice and NRTs (N=360); quit or substitute advice and NRTs (N=630); or quit or substitute advice and NRT or ENDS (N=630).[13 5]No RCT plans to test changes in exposure to toxic compounds secondary to ENDS exposure. The only study that plans to measure exposure to biomarkers of tobacco and carcinogens is a prospective cohort study of participants who smoke exclusively tobacco cigarettes (n=175), and dual users of ENDS and tobacco cigarettes (n=275). Without randomization, we cannot know the cause of changes in exposure to biomarkers and carcinogens. The study we plan will be adequately powered to test for the efficacy of ENDS, and provide comprehensive data on the exposure of ENDS users to the toxic compounds they may release.

Monitoring exposure to toxic compounds from ENDS use

Most studies that identified pollutants generated by ENDS were based on aerosol laboratory analyses.[14, 15] Very few human studies have used biomonitoring to assess exposure to pollutants and to estimate the internal dose of toxins delivered by ENDS. None have done so in an RCT setting. Several VOCs (e.g.,propylene glycol, glycerine, ethylene glycol, diethylene glycol, benzene, 1,3-butadiene, ethylene oxide, acrylonitrile, acrolein, propylene oxide, acrylamide) and aldehydes (e.g., formaldehyde, acetaldehyde, acetone, propanal, crotonaldehyde) generated by ENDS are usually quantified in aerosol. We will additionally measure metabolites of VOCs and metabolites of aldehydes in urine. This will allow us to determine differences in urinary concentrations of these compounds in ENDS users, smokers and quitters.

Analysis of air pollutants generated by ENDS use

Studies found ENDS delivers 9- to 450-fold lower levels of toxic substances than conventional cigarettes according to one study.[16] In conventional cigarettes toxins are produced by combustion, but in ENDS they are produced by turning liquid to vapor.[6, 17] Previous studies measured carcinogens and heavy metals. Carinogens were: carbonyl compounds (formaldehyde, acetaldehyde and acrolein), volatile organic compounds (VOCs) and tobacco-specific nitrosamines (TNSAs) (N'–nitrosonornicotine (NNN) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)). Heavy metals were cadmium, lead and mercury.[14, 16-28] Aerosolized flavorings in e-liquid solutions also contain potentially toxic contaminants with unknown health effects,[6, 16-18] though only traces of polycyclic aromatic hydrocarbons (PAHs) have been detected in ENDS aerosols, and sometimes not even that.[6, 17]

TSNAs and 1-and 2-naphthol (PAHs) are of special interest because they are believed to contribute to

lung cancer.[29, 30] The TSNAs NNK and NNN are indicators of relative combustible tobacco and ENDS use, since their levels are higher in conventional cigarette use than in ENDS.[18] In urine, NNK and its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) can be quantified, both being specific biomarkers of tobacco smoke exposure.[31] The PAHs 1- and 2-Naphtol, metabolites of naphthalene, are higher in smokers than in non-smokers.[32] The level of 1-OHP glucuronide, the main urinary metabolite of pyrene, is twice as high in the urine of smokersthan in non-smokers or ENDS users, thought levels are also influenced by environmental pollution and diet.[29, 32]

Distinguishing exposure to toxins and nicotine from tobacco smoke and ENDS vapor

Biomonitoring may allow us to distinguish exposure to nicotine from exposure to tobacco smoke and ENDS. Anabasine, an alkaloid found in tobacco, can be measured in the urine of tobacco smokers. Since anabasine is present in e-liquids at extremely low levels only, [21, 33] anabasine may be helpful in differentiating nicotine exposure from tobacco and ENDS.[34, 35] We will measure carbon monoxide (CO) released by combustion processes typical of tobacco exposure and not ENDS. We will also test TSNAs, especially NNN, NNK and its metabolite NNAL, nicotine and nicotine degradation products (cotinine and anabasine), VOCs, and three PAHs, 1-hydroxypyrene (1-OHP) and 1- and 2-naphthol, will help us distinguish smokers from ENDS users, continuing smokers, and quitters.

Effect of ENDS use on oxidative stress, CVRFs and respiratory symptoms

Data on the safety-risk profile of ENDS are limited. Studies suggest that ENDS use improves health outcomes, such as reducing respiratory symptoms, and presents only minimal risks, like mild throat irritation and dry cough.[6] Prospective studies that systematically assessed the effect of ENDS on safety outcomes did not show a statistically significant increase in serious adverse events (SAE).[8, 9] The self-reports from the ECLAT RCT study did show that, by Week 2, shortness of breath was reduced from 20% to 4% in all participants, but there were no differences between intervention groups.[8]

Effect of ENDS on oxidative stress

Smoking induces acute[36] and chronic oxidative stress and inflammation both in vitro and in vivo.[37] We believe we will be the first to compare oxidative stress from ENDS use to cigarette use in the context of a RCT. Data on oxidative stress induced by inhalation of toxic compounds usually comes from studies on environmental air pollution and tobacco cigarette smoking. Tobacco-smoke contains strong pro-oxidants (stable radicals and toxic substances) that generate reactive oxygen species,[38] and may promote recruitment of phagocytes, which also generate free radicals. All these compounds can induce oxidative stress, which likely plays a key role in causing airways and related pathologies linked to tobacco-smoke exposure.[39]

Oxidative stress can be assessed by measuring 8-iso-prostaglandin F2α (8-isoprostane),[40] a marker of lipoperoxidation, and 8-OHdG, a biomarker of DNA oxidation.[41, 42] 8-isoprostane results mainly from the nonenzymatic action of free radical attack on arachidonic fatty acids; thus can be considered a lipoperoxidation biomarker. The measurement of 8-isoprostane may be useful for evaluating both acute and chronic oxidative stress.[43, 44 45] Urinary 8-isoprostane has been shown to be greater in smokers than in non-smokers.[43] 8-OHdG is a urinary biomarker of oxidative DNA damage, a predictor of lung cancer.[46-48] Disadvantages with measuring 8-OHdG concentration are its low specificity due to confounders (e.g. age, gender, diet, physical activity, vitamin status).[49, 50] Information regarding known confounders will be collected and lead to better interpretation of 8-OHdG as a marker of cigarette smoking exposure.[40-43, 49-52]

Effect of ENDS on cardiovascular risk factors (CVRFs).

Cardiovascular diseases (CVD) are a leading cause of death in cigarette smokers; quitting smoking is associated with reduced CVD.[3] Cigarette smoking increases CVD through complex mechanisms, mostly by an increase in atherosclerotic plaque formation and the effect appears unrelated to nicotine. There is currently no evidence that ENDS use affects CVD outcomes. The ECLAT trial showed no difference in body weight, resting heart rate, or systolic/diastolic blood pressure between those who used ENDS or not from baseline to the end of the study.[8] Two other studies evaluated the short-term effects of ECs on the cardiovascular system. One study suggested impairment in diastolic ventricular function with tobacco cigarettes and not with ENDS.[53] Both ENDS and tobacco cigarettes increased diastolic blood pressure, potentially mediated through nicotine exposure, but an increased systolic blood pressure was found only in cigarette smokers. Interventions helping smokers quit have shown that quitting is associated with increased HDL-cholesterol, [54] increased blood pressure, [55, 56] weight gain[57], higher blood glucose, and higher diabetes risk.[58] No randomized trials have tested the effect of ENDS on blood glucose, blood cholesterol and other markers of cardiovascular risk.

Effect of ENDS on respiratory symptoms

Effects of ENDS on respiratory symptoms: The recent trial in the UK prespecified respiratory symptoms as an outcome of interest. The incidence of cough and phlegm production declined from baseline in both trial groups at 52 weeks. But among participants who reported cough or phlegm at baseline, significantly more in the e-cigarette group were symptom-free at the 52-week follow-up than in the nicotine-replacement group. We need to know more about the effect of ENDS on respiratory symptoms, in particular cough and phlegm, since these are critical symptoms of chronic obstructive pulmonary disease (COPD), a well-known serious health condition induced by tobacco smoking.[59]

A recent prospective study of 2736 current or former smokers and controls who had never smoked measured their respiratory symptoms with the COPD Assessment Test (CAT; scores range from 0 to 40, with higher scores indicating greater severity of symptoms).[60] Cough and phlegm are the two first questions on the CAT. Authors explored whether current or former smokers who had preserved pulmonary function assessed by spirometry and had symptoms (CAT score, ≥10) were at higher risk of respiratory exacerbations than current or former asymptomatic (CAT score, <10) smokers with preserved pulmonary function. Respiratory symptoms were present in 50% of current or former smokers with preserved pulmonary function. The mean rate of respiratory exacerbations among symptomatic current or former smokers was significantly higher than the rates among asymptomatic current or former smokers and among controls who never smoked. Thus, COPD symptoms assessed by the CAT are needed to assess the effects of ENDS on COPD for those who quit. The only other data from an RCT assessing the effects of ENDS for smoking cessation on respiratory symptoms comes from the ECLAT trial, where respiratory symptoms similarly improved in all studied groups.[8] Null findings on respiratory symptoms in the ECLAT may be explained by the low number of participants who quit smoking. One study compared the short term effects of cigarette smoking to ENDS use and found that cigarette smoking acutely reduced lung function; ENDS did not.[61] Findings on short-term airway resistance is conflicting.[62, 63] Short term increase in resistance in ENDS users might be caused by aerosolizing the liquid, and not by the substances that harm lung function in cigarette smokers.[63] Smokers who shifted from tobacco cigarettes to ENDS have offered anecdotes of dramatically improved lung function, but animal models suggest that ENDS liquids can increase markers of asthma.[64]

We will survey all participants of the ESTxENDS RCT with the CAT and structured asthma symptoms questionnaires, along with other systematic data collection on COPD exacerbations within standardized SAE assessments and other respiratory symptoms within standardized AE assessments. We will further query all participants about their drug treatments for respiratory disease. We will perform follow-up assessments at 6, 12, and 24 months in order to assess potential longer-term effects of ENDS on COPD and asthma in smokers who quit.

Effect of ENDS on SARS-CoV2 infection and COVID-19-associated SAEs

By April 2020, the COVID-19 pandemic raged worldwide. Europe is now an active site with a steeply increasing rate of deaths from the disease as it spreads through the population. SARS-CoV-2 is a positive-sense RNA coronavirus that can cause severe acute respiratory syndrome (COVID-19),[65] and is especially dangerous to older patients with comorbid conditions. Mortality is higher in men than women.[66, 67] Since men are more likely to be smokers than women, tobacco smoking might contribute to the higher risk of mortality in men. This is the case for influenza: hospitalized smokers likelier to have poor outcomes.[68] It is unclear if tobacco smoking poses an independent risk or if poorer outcomes result from underlying health conditions caused by long-term tobacco smoking. Tobacco smoking has an immunosuppressive effect,[69] and there may also be a biological explanation for increased SARS-CoV-2 infection among smokers.[70, 71] But we can only verify these suppositions by studying the effects of SARS-CoV-2 among smokers of tobacco cigarettes and comparing outcomes with those who use electronic nicotine delivery systems (ENDS) that do not produce tobacco smoke (vaping). Whilst recent randomised controlled trials (RCT) suggest ENDS effectively help smokers quit, the largest RCT to date found a worrisome, albeit not statistically significant increase in rate of hospitalisation for infection-related diseases in participants allocated to ENDS use.[12] Experiments conducted in the lab suggest ENDS vapour, like traditional cigarette smoke, might lead to immunosuppression.[72, 73] We have no data on the association between ENDS use and SARS-CoV-2 infection rates. In addition to scheduled outcomes collected in the trial, we will query all participants about symptoms of infection, SAEs, and any antibiotic prescriptions.

Effect of ENDS on lung function and MBW

One study compared the short term effects of cigarette smoking compared to ENDS smoking and found that cigarette smoking led to a higher acute reduction in FEV1/FVC compared to ENDS.[61] Short term airway resistance has been studied in various small, short term studies, with conflicting results.[62, 63] This

short term increase in resistance might be due to aerosolization of liquids and not to harmful substances on lung function such as cigarette smoking.[63] However, numerous anecdotal evidence from patients who shifted from tobacco cigarettes to ENDS suggests a dramatic improvement in lung function, possibly by reduced inhalation of the toxic tobacco smoke. An improvement of FEF25-75% in a healthy population and of lung function and quality of life in asthmatics, as well as a reduction of the most common respiratory symptoms associated with cigarette smoking was reported after switching to ENDS.[8, 74, 75] Animal models suggest on the contrary that ENDS liquids could impair markers of asthma.[64] There is currently no study comparing the lung function outcomes between participants who continue smoking, shift to ENDS or stop smoking and ENDS completely.

Multiple breath washout (MBW) is an emerging technique for assessment of peripheral airway function with a better accuracy than conventional spirometry. In this method an inert intrinsic gas (nitrogen) is washed out by breathing 100% oxygen. Impaired lung ventilation results in more tidal breaths and expired volumes to eliminate the inert gas, reflected by an increasing lung clearance index (LCI) as a marker of peripheral airway disease.[76] Specific markers of ventilation inhomogeneity in proximal conducting airways (S_{cond}) and in intermediate zone acinar airways (S_{acin}) are derived from the alveolar phase of the inert gas expirogram.[77, 78] S_{cond} and S_{acin} are higher in smokers with normal lung function compared to never-smokers [79, 80] and a persisting S_{cond} normalization was observed after 1 week of smoking abstinence.[81] MBW measures have never been performed to test the effects on ENDS. Since the technique is more accurate than conventional spirometry, MBW might provide invaluable information on the potential effect of ENDS for smoking cessation on lung function compared to continuing smoking or quitting without ENDS.

Effect of ENDS on ventilation and perfusion in the lung

Recent imaging studies showed that magnetic resonance imaging (MRI) scans can both detect structural and functional changes in the lung.[82-85] The foremost advantage of functional lung imaging is the possibility to depict regional lung function. By comparison, “traditional” lung function testing provides a global metric of function. With regard on subclinical, early disease stages, MR imaging studies have the potential to generate new biomarkers for the depiction of lung function.[82, 83, 86]
Performing functional MRI among participants in ESTxENDS will provide essential information for the scientific community at large on the changes in lung function after shifting from tobacco smoking to vaporizer use.
Functional MRI data using the matrix pencil (MP) decomposition method will be used. The technique provides images of fractional ventilation reflecting lung parenchyma changes during respiration, as well as images of regional parenchymal perfusion and pulmonary blood arrival time. In addition a quantitative data evaluation which allows to estimate the proportion of lung volume with impaired ventilation and perfusion will be performed. Additionally we will examine the lung parenchyma for structural changes in the lung. To assess the repeatability and compare MRI results of participants from the RCT we will include an age matched group of healthy volunteers who are never smokers/non-vapers. These volunteers are not part of the RCT. This population is needed to compare the short-term (within 2 hours) natural variability and repeatability in MRI-derived measures of lung structure, ventilation and perfusion between participants of the RCT and healthy volunteers.

Measuring markers of oxidative stress in exhaled breath condensates (EBCs)

During exhaled breath condensate (EBC) collection, exhaled breath is directed through a cooling device, which traps the exhaled breath constituents in a liquid or solid phase depending on the condenser temperature.[87] Biomarkers of oxidative stress have been measured in EBCs (pH, H2O2, malondialdehyde, leukotrienes, 8-isoprostanes, cytokines).[87] EBC requires special equipment and trained personnel compared to urinary oxidative stress biomarkers which can be more easily obtained. However, measures in EBCs can more reliably assess oxidative stress in the lungs than measures in the urine, which measures oxidative stress on a systemic level. [88] Thus markers of oxidative stress might be more reliable to measure potential adverse lung health outcomes such as asthma and COPD.[89] ECB is useful for monitoring airway inflammation, although the collection process needs to be standardized.[87] Two studies have reported on 8-OHdG concentrations in EBC[90, 91] and showed increased 8-OHdG concentrations in asbestos-exposed workers[90] and in smokers[91] compared to non-smokers. Our project proposal will add to the body of knowledge regarding the applicability of this biomarker in EBC for evaluating DNA oxidation in lung.

Measuring micronuclei in buccal epithelium, a surrogate measure of future cancer risk

The micronucleus assay, a surrogate measure of future cancer risk: The micronucleus cytologic assay test was developed to screen for drug toxicity in bone marrow samples from mammals. The technique identifies micronuclei on smears obtained by oral cavity exfoliation and indicate genomic instability.

Buccal MNs may predict cancer risk for the upper aerodigestive tract.[92] The HUman MicroNucleus project on eXfoLiated buccal cells (HUMNXL) found MN counts gradually increased during the progression from normal mucosal to precancerous lesions to carcinoma.[93] MN assessment discriminates between exposure to tobacco smoke in smokers and in non-smokers, and has been tested on a small number of ENDS users.[94-97] We can measure MNs with standardized protocols for buccal cells.[98-100]

Measuring olfactory function and its effect on the efficiency of ENDS

Olfactory function impairment is strongly associated with smoking[105-108], but its prevalence has yet to be established. There are observations that olfactory function may improve after smoking cessation[105, 106, 109], but only a few prospective studies among smokers have compared change in olfactory function in smokers and quitters. Futhermore it is unknown, if a preserved olfactory function will support smoking cessation with ENDS, as they offer different aromas. The olfactory function will be measured by using the Burghart Sniffin'Sticks 16-item Identification-test.[110-112]

Measuring "dry-hit" recognition in ENDS users

High quantities of carbonyls and aldehydes can be generated by ENDS if the liquid-level is too low and needs to be replaced or if the temperature on the coil is too hot. This is the source of a well-known phenomenon among ENDS users called "dry-hit"[113, 114]. In this case the ENDS is said to leave a burnt taste or smell which is then recognized by users.[114] However, it is unclear if ENDS users with an olfactory dysfunction can adequately recognize such a "dry-hit". ENDS users not recognizing a "dry-hit" are exposed to higher amounts of inhaled toxic substances and might therefore be exposued to higher health risks than ENDS users with an intact "dry hit" recognition. On a voluntary basis we will test whether ENDS users can recognize a "dry hit" generated by the test ENDS generating such aerosol. This will later enable us to test whether olfactory dysfunction is linked to an impaired dry-hit recognition and test whether "dry hit" recognition is associated with higher levels of biomarkers of exposure to carbonyl and aldehyde metabolites in urine.

Effects of ENDS on inflammation

Tobacco smoke upregulates inflammatory pathways and downregulates anti-inflammatory pathways.[36, 115, 116] Tobacco smoke also generates reactive oxygen species and may recruit phagocytes that generate free radicals, possibly contributing to airway and other pathologies by disturbing the balance between free radicals and antioxidants and inducing oxidative stress.[36-38] Most of the damage tobacco smoke causes is derived mainly by inhaling pyrolyzed tobacco and other organic components.[117] Replacing tobacco cigarettes with ENDSs thus decrease user exposure to toxicants and carcinogens; the components of the aerosol and of traditional cigarette smoke differ significantly, and also have different effects on inflammation and oxidative stress. Assessing the effects of ENDS on the full range of inflammatory biomarkers will help understand the effect of ENDS on on health, including unforseen outcomes. Blood samples will be analyzed with Olink Proteomics' unique protein detection system,[118] the Proximity Extension Assay (PEA), which allows us to perform highly specific and sensitive multiplex immunoassays to detect 92 inflammation-related protein biomarkers.

Scientific impact

Despite the ready availability of ENDS in Europe and the USA, at local stores and on the Internet, there are significant gaps in our knowledge. These devices have been commercially available for less than a decade, and only gained popularity in the last few years. They are also evolving rapidly. No researchers have independently evaluate the effectiveness, safety, toxicological profile, and effect on health-related outcomes of ENDS in the setting of a randomized controlled trial. Doing so would help Swiss and international policymakers make evidence-based decisions about the safety profile of ENDS.

Data on exposition to potential toxins from ENDS in daily life.

We will inform smokers interested in quitting with ENDS, and policymakers, about the toxins measured in the urine and blood of participants who use ENDS for smoking cessation under real life conditions. While data on the safety and risk-benefit profile of ENDS appear promising under laboratory conditions,[5, 6] ENDS does release some toxins. To opponents of ENDS, the small amount of released toxins in

unacceptable; supporters see it as negligible. Under real life conditions, the exposure of ENDS users to toxins maybe much less than daily exposure to toxins from other environmental pollutants. Users might also misuse ENDS under real life conditions, and thus receive higher exposure to toxins than under laboratory conditions. Our study will help validate or disprove the claims supporters and opponents make about ENDS.

Data on the consequences of potential toxins from ENDS on the body

Even if we find no increase in exposure to toxins among ENDS users, or when we compare those who quit using ENDS to those who quit smoking without ENDS, it is still possible ENDS have adverse physical effects. Some toxins are quite hard to detect, but we can measure oxidative stress in urine and exhaled breath. Measures of oxidative stress are biomarkers of the effect of exposure to toxins on the body. Oxidative stress increases risk of cancer and heart diseases, the leading causes of disease and death worldwide. Because it takes years for cancer and heart disease to be detectable, and because ENDS are a recent development, we can measure oxidative stress in ENDS users, so that users, clinicians, and policymakers can understand the possible long-term consequences of increasing ENDS use. We will also measure the effect of ENDS on CVRFs and respiratory symptoms, to explore the short term effects of ENDS on health.

ENDS use is controversial among health professionals and policy makers. ENDS advocates are comfortable with widespread use and claim it is a safe alternative to cigarettes, others fear ENDS is a gateway to smoking and may harm health users' health in the long term.[7] For smokers, the choice of ENDS could be a safe alternative to tobacco cigarette smoking and an alternative to smoking cessation. Given the stagnating trend in smoking prevalence, ENDS could make a significant dent into smoking prevalence and save many lives from smoking. Swiss tobacco experts have recently recommended making ENDS available in Switzerland and regulating them.[126] But current recommendations are not well-supported by evidence; our study will help users, policy makers, and health professionals decide whether ENDS should be actively promoted as an alternative to tobacco cigarettes.

3.2 Investigational Product (treatment, device) and Indication

Device:

We will use a third generation nicotine vaporizer, the Innokin Endura T20-S starter kit. The device is authorized for sale in Switzerland and follows the CE declaration of Comformity, essential to ensure safety of the participants. The Endura T20-S kit will come in a user packet with a 1,500mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro Micro USB DC 5V/1A cable, a USB mural adapter and an instruction manual in French or German, respectively. (See also section 8.1.1. for pictures of the tested device)

E-liquids:

We will use the e-liquids produced by the company Gaïatrend in France (https://www.gaiatrend.fr/en/). The e-liquids "Alphaliquids" are produced following rigorous standards. Nicotine concentrations available will be 0 mg/ ml, 6 mg/ml, 11 mg/ml and 19.6 mg/ml. The following aromas will be available to be chosen by the study participants: FR-4 (tobacco flavor) and FR-M (tobacco flavor), FRESH MINT (menthol flavor), RASPBERRY#2 (fruity flavor), RED FRUITS (fruity flavor) and GREEN APPLE (fruity flavor) (https://www.alfaliquid.com/en). The proportion of propylene glycol and vegetal glycerin will be 76/24 for all e-liquids. The components of the e-liquids will be propylene glycol, vegetal glycerin, medical-quality nicotine, alcohol and aromas. The full list of ingredients and their concentration is provided in the section 17 appendices.

Gaïatrend obtained ISO8317 certification for its e-liquid flasks (See also section 8.1.1. for pictures of the flasks containing the e-liquids). This standard certifies that flasks are equipped with child-resistant closures (See the Certificate of Test n°"IBE-BVI" – CR-14.021 from June 26th, 2014 on tests conducted by the Belgian Institute of packaging on children in the section 17 appendices). The vegetable glycerine is guaranteed free of genetically modified organisms (GMOs). It has also obtained EP certification (European Pharmacopoeia). The propylene glycol in the e-liquids is 99.5% pure, organic and guaranteed free of GMOs. This product has also obtained EP and USP certification. Organic ethyl alcohol made from cereals is another ingredient used in the e-liquids and has obtained Ecocert N° FR-BIO-01 certification. It is also guaranteed free of GMOs. Concerning the nicotine that is contained in the e-liquids, it comes from the only nicotine supplier to have obtained certification from the European Pharmacopoeia (see section 17 appendices). The aromas used in the e-liquids comply with standards of quality from CE N°1334/2008 (see section 17 appendices).

At the baseline visit, study participants will have the opportunity to test various flavors and nicotine concentrations and will receive a first batch of nicotine containing e-liquids of their choice in preparation to the smoking cessation attempt. Participants will be advised to only use the e-liquids provided by the study personnel.

Indication for using the device and e-liquids:

The indication for using the device and e-liquids is for replacing tobacco cigarettes smoking by use of the device and e-liquids in the intention to help participants stop smoking tobacco cigarettes as the main outcome or to reduce the number of tobacco cigarettes smoked as a secondary outcome. Other expected secondary outcomes are a reduction in exposure to inhaled toxins measured in the urine, reduction in oxidative stress measured in urine and improvement in clinical outcomes such as respiratory symptoms and improvement in cardiovascular risk factors. We hypothesize that the use of device and e-liquids instead of tobacco cigarettes will not lead to an increase in adverse events. (see detailed description of main and secondary outcomes in Sections 5).

Training and support of participants for using the device and e-liquids:

Study participants in the intervention group will be advised by trained study nurses about how to get started with the device, how to fill the device with e-liquid, how to charge the device and how to change the coil every two weeks. They will also be instructed to wait 5 minutes after changing coils and filling the tanks with e-liquids in order to wait for the cotton of the coils to be soaked in e-liquid before use. A user manual will be provided for every study participant. Study participants will also be provided with a direct phone number of the study nurses for technical support between scheduled phone visits and will have the possibility to go on the study website to obtain the information on the use of the device.

3.3 Preclinical Evidence

Not applicable.

3.4 Clinical Evidence to Date

Evidence of ENDS use to help smokers quit has been reviewed in detail in section 3.1.

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Rationale for the device chosen:

We will use an easy to use and safe ENDS produced by one of the leaders of ENDS producers worldwide. The Innokin T20-S is a starter kit that allows mouth-to-lung experience similar to smoking regular cigarettes. Its simplicity, which comes along with restrictions on variations of the airflow and wattage, will ensure minimizing the risk of misuse and technical issues by users. The top-filling capacity will ensure ease and safety of filling e-liquids on a daily basis. The Endura T20-S kit comes with a 1,500mAh internal Li-Po battery which allows most users to vape during a full day without need for charging. It has integrated safety protection which is important in order to avoid the production of “dry-hits”, when the coil gets too hot and pyrolyzes the e-liquid, which is at the origin of the formation of harmful VOCs such as acrolein and formaldehyde. The device will be mounted with a 0.8 Ohm coil in order to optimize the concentration of nicotine in the vapour and limit the exposure of users to vapour.[127] The 0.8 Ohm coil enables lowering the temperature of the vapour to improve the experience of inhaling the vapour by participants. The lower power needed to heat the e-liquids also ensures lower the temperature of the entire heating system and spare the battery to ensure that one battery charge lasts for one full day of use. The tri-color LED power indicator clearly shows the remaining power levels and charging status. We will provide particpiants with a mural USB charger in order to avoid improper charging with defective chargers. Participants will have the choice of five colors of the device to furher improve uptake. Colors available will be Black, Grey, Red, Purple and Blue. Participants in the Intervention group will receive two devices in oder to get a spare device and avoid nicotine withdrawal symptoms which might lead to a relapse in tobacco smoking if encountering technical issues with one of the devices. Finally, the low price of the device, not obtained at the expense of quality, will ensure generalizability of the results to the overall population, as new users often select a cheap and safe device when trying vaping devices before buying more refined and adjustable vaporizers if they continue vaping over a prolonged time.

Rationale for the chosen e-liquids, their nicotine concentrations and flavors:

The choice of Gaïatrend is based on the outstanding quality of the e-liquids they produce, following the most rigorous standards to ensure safety of the inhaled products. We follow the choice of academic scientists in France who have carefully reviewed the production processes and visited in person the factory for a similar RCT conducted in France in 2018.

The use of ENDS, as for other smoking cessation studies testing the effect of nicotine replacement therapy, will be *ad libitum*. Participants will be able to choose the nicotine concentration of the e-liquids (0 mg/ml, 6 mg/ml, 11 mg/ml or 19.6 mg/ml), though we will encourage them to use the highest nicotine concentration in order to ensure optimal nicotine substitution while reducing the volume of e-liquids used and vapor inhaled [127, 128]. The choice of nicotine concentration is essential to ensure uptake of the device by participants. We expect those who continue vaping will reduce the nicontine concentration over time. The 0 and 6 mg/ml will ensure that participants continue to use the e-liquids provided within the study and avoid using another e-liquid bought online from potential unsafe sellers.

Participants will also be allowed to choose between different aromas in order to improve uptake. While most smokers start with a tobacco-flavored e-liquids, over time, as their sense of taste and smell comes back through smoking cessation, most shift to other flavors, the most popular being menthol and fruity flavors. Thus providing participants with the choice of aromas over time may increase the chance they will continue using the vaporizers and thus avoid relapse into smoking, which is the main outcome of the study.

3.6 Explanation for choice of comparator (or placebo)

We will compare the combination of nicotine-containing ENDS and SOC for smoking cessation (intervention group) to SOC alone (control group). We intend to emulate the real-life conditions for people who want to use ENDS to quit cigarette smoking, and who contact a health care professional for support. SOC for smokers who contact a healthcare professional in Switzerland includes smoking cessation counseling (SCC) based on cognitive behavior therapy and motivational interviewing with counseling of NRTs and other effective smoking cessation medication aids.[129, 130] Participants in both groups (intervention and control group) will be free to use other NRTs or any other smoking cessation help (medication and other (any) non medication therapies), but we will not pay for NRTs or other medication, as is currently the case in clinical practice in Switzerland. At the Baseline visit , participants in the control group will receive a voucher (“BERNcity Geschenkcard” or similar voucher for Lausanne, Geneva, Zürich and St. Gallen) worth CHF50. The amount of CHF50 is of similar monetary value to what the intervention group will receive at baseline in form of the two ENDS devices and the e-liquids.

3.7 Risks / Benefits

Data on the safety-risk profile of ENDS use are limited and mostly come from observational and laboratory data from the last decade. Previous RCTs suggest that ENDS use improves health outcomes, such as reducing respiratory symptoms, and presents only minimal risks, including mild throat irritation and dry cough.[6] Prospective studies that systematically assessed the effect of ENDS on safety outcomes did not show any statistically significant increase in serious adverse events (SAE) [8, 9] The self-reports from the ECLAT RCT study showed that, by Week 2, shortness of breath was reduced from 20% to 4% in all participants, but there were no differences between intervention and control groups.[8]

The ENDS device chosen for this study has been on the market for more than 6 months. The devices comply with EU regulations (CE-labelled). The ENDS may be the cause for accidents as they are composed of lithium batteries. Reports of accidental explosions of batteries have occurred, although these are mostly caused by misuse of the device by users, prolonged charging, improper chargers or by design defects. We will instruct participants to only use the charger and cable provided in the kit and to avoid trying to open up the battery part. The risk of accidental explosion is however similar to the risk with other common battery-driven devices such as smartphones. In addition, the risk of fire through misuse of regular tobacco cigarettes and use of lighters is estimated to be much higher. Evidence from the Fire Department in London suggests use of vaporizer was associated with a reduction in home fires related to cigarette smoking.

One of the aims of this study is to identify and evaluate the toxicological profile of ENDS as a smoking cessation aid within a RCT to provide further evidence on the risk-benefit profil of using ENDS instead

of tobacco cigarettes for smoking cessation.

Data from observational studies indicate that there is very low risk in using ENDS, probably without any clinical significance.[6] The use and sale of ENDS and e-liquids has been allowed by Swiss authorities after safety analyses. The main risks concern the use of nicotine with the ENDS, as well as the use of nicotine replacement therapy (e.g., patches, gums) if participants wish to do so. Generally, nicotine replacement therapy is tolerated well, however, if smoking is continued during the use of NRT, adverse effects could occur due to the high nicotine levels absorbed. Adverse effects of nicotine absorption from NRT are similar to those from smoking (e.g., nausea, palpitations, dizziness, sleep problems).

Nicotine contained in the e-liquid is irritable for the mouth, throat and the eyes, therefore it is possible that the use of ENDS leads to irritations of the eyes or the respiratory system (e.g., dry cough). Moreover, inadequate use of ENDS could lead to thermogenic degradation (pyrolysis) of the e-liquids and consequently lead to an inhalation of irritable compounds such as acrolein and formaldehyde. This happens more often when cotton coils are not changed as recommended, also called “dry puff”. Qualitative data indicate that ENDS users immediately recognize when dry puffs occur and stop inhalation to exchange the coil. To ensure that participants recognize when coils need to be replaced, we will instruct them to recognize dry puffs when handing out the ENDS during their first visit. We will also instruct them how to change the coils every two weeks and provide them with spare coils at the baseline visit and be sent additional coils by mail over follow-up if needed.

All e-liquids bottle will follow standards by the EU and be limited to 10 ml. They will include a safety notice and logos to indicate that the product is toxic, that bottles should not be used by minors under 18 and not to be used by pregnant women. The bottle will contain information on the composition, the nicotine concentration, the flavour and have a warning reading: “The Nicotine contained in this product creates a strong dependence. Its use by non-smokers is not recommended”. The bottles are and have been tested to be completely child-proof (ISO 8317 certified cap child resistant packaging). The safety of the e-liquids bottles with children has been evaluated by the manufacturer (see section 17 appendices).

Participants will be instructed to avoid contact with the skin and mucosas when filling the device and to wash hands immediately in case of spilling. Recent evidence suggest penetration of nicotine through the skin.[131] However, the risk is estimated to be minimal and is expected to be similar to the application of a nicotine patch regularly used by millions for smoking cessation.[6]

We will also instruct participants not to ingest the e-liquids and to keep them out of reach of children, as ingestion can be a source of overdosing of nicotine.[6] Participants will be provided with the contact of the emergency services and to instruct the investigators immediately in case of accidental ingestion of e-liquids. The lethal dosis of nicotine is estimated at 500-1000 mg.[6] Thus the swallowing of the content of one bottle of 19.6 mg/ml will not suffice for a lethal dose should a participant accidentally ingest one. As of today, to our knowledge theres are no reports of completed suicide with e-liquids containing nicotine.

Although the e-cigarettes and e-liquids are considered an alimentary product, and thus the evaluation of the safety of e-cigarettes is outside of the jurisdiction of Swissmedic, we will apply the same rigor to the assessment of adverse effects and risks as for a therapeutic product. In particular, the systematic collection of information on adverse effects will be based on documents used for pharmacological safety studies.

Magnetic Resonance Imaging (MRI) is a non–invasive and radiation free diagnostic test used in clinical setting in children and adults. The measurement is noisy. However, there are no known risks or side-effects for this test. General contraindications for MR imaging will be considered.

The Burghart Sniffin’ Stick 16-Item Identification Test is a validated, easy to administer and non-invasive screening tool to recognize olfactory dysfunction. There are no known side-effects for this test.

3.8 Justification of choice of study population

We will include participants who are ≥ 18 years old, smoke > 5 cigarettes a day since > 1 year, and are willing to quit smoking within the next three months. We will restrict to smokers smoking 5 or more cigarettes per day in order to randomize smokers with sufficient tobacco cigarete exposure to be able to adequately detect harmful components of tobacco cigarettes in the urine and to smokers who are expected to need nicotine substitution by ENDS in order to stop smoking cigarettes. We successfully applied similar restrictions in previous RCTs for smoking cessation trials conducted by our group.[132, 133]

Healthy age-matched never-smoking/non-vaping volunteers (N=10) will be included for the MRI sub-

study. Healthy volunteers will be never-smoker (<100 cigarettes per lifetime), non-vapers (no use of ENDS on more than 50 occasions and not in the last 12 months), no history of lung disease, no use of inhalation medication and no lung infection in the last four weeks. Most studies define a never-smoker as one who either has never smoked at all or has smoked <100 cigarettes (or the equivalent amount of tobacco) in his or her lifetime, which is in line with that proposed by the World Health Organization. Volunteers will be aged matched to our sub-group of participants who undergo MRI assessments within the RCT. Exclusion criteria are listed under section 7.1. Eligibility criteria. We thrive at limiting the number of exclusion criteria to the minimum to ensure the external validity of the study findings to the general population interested in using ENDS for smoking cessation. We will exclude pregnant or breast feeding women at baseline or intending to become pregnant during the first 6-months of the clinical study; not because ENDS should be particularly harmful in such populations, in particular in comparison to the known harms from smoking tobacco cigarettes for pregnant and breastfeeding women and their foetuses and babies, but because we expect dedicated trials in this population will be conducted by other research teams in the future.

4. STUDY OBJECTIVES

4.1 Overall Objective

The study aims to assess the efficacy, safety and toxicology of ENDS for helping smokers quit smoking or reduce the number of cigarette smoked per day over a 24-month follow-up period with follow-up visits done at 6-, 12- and 24- months.

4.2 Primary Objective

The study seeks primarily to determine the efficacy of ENDS for helping smokers quit smoking or reduce the number of cigarette smoked per day over a 24-months follow-up period with follow-up visits done at 6-, 12- and 24- months.

4.3 Secondary Objectives

Secondary objectives are to assess the effect of using ENDS on exposure to inhaled toxic compounds and assess the effect of ENDS use on health-related outcomes, including clinical health-related outcomes (respiratory symptoms, pulmonary function,, olfactory function, inflammatory biomarkers) and surrogate health-related outcomes (oxidative stress, cardiovascular risk factors, micronuclei in mouth epithelium).

4.4 Safety Objectives

The study aims to assess the safety of ENDS in terms of adverse events.

5. STUDY OUTCOMES

5.1 Primary Outcome

The primary outcome for the main analysis at 6-months follow-up will be the continuous smoking abstinence from target quit date (TQD) to the 6-month follow-up visit, ascertained by self-report (self-report of no smoking from target quit date) and confirmed by urinary level of anabasine (<3 ng/ml).[1, 34, 35] If anabasine is missing, validation will be done by exhaled carbon monoxide (CO) (<10 ppm).

The primary outcome for the extended follow-ups at 12- and 24-months will be the self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), again confirmed by urinary level of anabasine (<3 ng/ml). If anabasine is missing, validation by exhaled carbon monoxide (CO) (<10 ppm).

5.2 Secondary Outcomes

The secondary outcomes are as follows:

- Secondary smoking cessation outcomes:

  6 month follow-up:

  - Continuous smoking abstinence. Self-report of having smoked no cigarettes from TQD, validated by urinary levels of NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol). If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

  - Self-reported smoking abstinence allowing a 2-week`grace period' after the TQD

  - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.

  - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

  - Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD.

  - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.

  - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).

  - Self-reported 7-day point prevalence abstinence at 6 months, Self-report of having smoked no cigarettes in the past seven days.

  - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled carbon monoxide (CO).

  - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).

  - Number of cigarettes smoked per day (CPD), self-reported, at baseline, TQD, phone visits and 6 months visit

  - Change in number of cigarettes smoked per day (CPD), self-reported. Successful reduction defined as 50% reduction in CPD.

  - Concentrations of urinary TSNAs (NNN, NNK and its metabolite NNAL), VOCs and PAHs (1- and 2-Naphtol and 1-OHP)

  - Respiratory symptoms assessed by questionnaire (CAT, mMRC, ACT, ECRHS) [134-137]

  - Oxidative stress assessed by 8-OHdG and 8-isoprostane concentrations in both EBC and urine

  - CVRFs assessed by HDL- and LDL-cholesterol, triglycerides, HbA1c (only for participants with diabetes), creatinine, blood pressure levels, heart rate, waist circumference and body mass index.

  - Pulmonary function, measured by conventional pulmonary function tests and by the more

sensitive techniques of multiple breath washout and MRI

- Micronuclei assessment in buccal epithelium

- Olfactory function assessement (olfactory identification score (OIS)). Cut-off for hyposmia 11 points, cut-off for anosmia 8 points.

- “Dry-hit” recognition at 6 month-follow up

- Inflammatory biomarkers

12- and 24- months follow-up:

- Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

- Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

- Self-reported 7-day point prevalence abstinence. Self-report of having smoked no cigarettes in the past seven days.

- Continuous smoking abstinence. Self-report of having smoked no cigarettes from quit date, validated by urinary levels of anabasine. If anabasine is missing, validation by exhaled CO.

- Continuous smoking abstinence. Self-report of having smoked no cigarettes from TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

- Self-reported smoking abstinence allowing a 2-week`grace period'. Smoking abstinence allowing a 2-week`grace period' after the TQD.

- Validated smoking abstinence allowing a 2-week`grace period'. Smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.

- Validated smoking abstinence allowing a 2-week`grace period'. Smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

- Self-reported smoking abstinence allowing up to 5 cigarettes. Smoking abstinence allowing up to 5 cigarettes in total after the TQD.

- Validated smoking abstinence allowing up to 5 cigarettes. Smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled carbon monoxide (CO).

-Validated smoking abstinence allowing up to 5 cigarettes. Smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.

- Number of cigarettes smoked per day (CPD), self-reported.

- - Change in number of cigarettes smoked per day (CPD), self-reported. Successful reduction defined as 50% reduction in CPD.Concentrations of urinary TSNAs (NNN, NNK and its metabolite NNAL), VOCs and PAHs (1- and 2-Naphtol and 1-OHP)
- Respiratory symptoms assessed by questionnaire (CAT, mMRC, ACT, ECRHS) [134-137]
- SARS-CoV-2 infection through serology and related SAEs
- Oxidative stress assessed by 8-OHdG and 8-isoprostane concentrations in both EBC and urine
- CVRFs assessed by HDL- and LDL-cholesterol, triglycerides, HbA1c (only for participants with diabetes), creatinine, blood pressure levels, heart rate, waist circumference and body mass index.
- Pulmonary function, measured by conventional pulmonary function tests and by the more sensitive techniques of multiple breath washout and MRI
- Micronuclei assessment in buccal epithelium
-
- Olfactory function assessement (olfactory identification score (OIS)). Cut-off for hyposmia 11 points, cut-off for anosmia 8 points.

5.3 Other Outcomes of Interest

Other variables of interest will be assessed by questionnaires:

- Physical activity (International Physical Activity Questionnaire, IPAQ) [138]
- Withdrawal symptoms (Minnesota Nicotine Withdrawal Scale (MNWS) [139, 140]
- Legal and illegal drug use: Alcohol consumption [141], illegal drug use (cannabis, cocaine, other)[142]
- Sleep quality (Pittsburgh sleep quality inventory PSQI) [143]
- Quality of life (EQ-5D questionnaire) [144]
- Depression symptoms (PHQ-9)[145]
- Anxiety (GAD-7) [146]
- Environmental pollution exposure (e.g. second hand smoke) [147, 148]

5.4 Safety Outcomes

Safety outcome variables are adverse events (AEs) and serious adverse events (SAEs) following international standards.[149]

Safety outcomes are defined as follows:

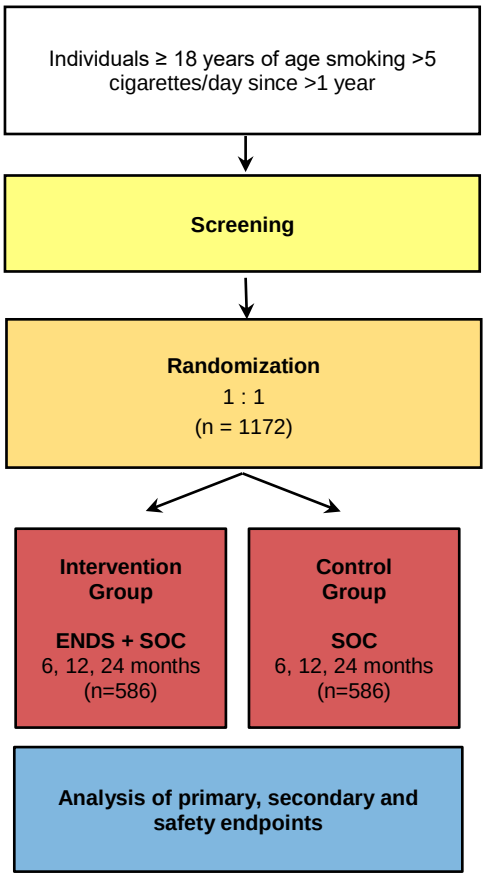
- Adverse events
- ENDS deficiencies

6. STUDY DESIGN

6.1 General study design and justification of design

The ESTxENDS trial is a multicentre, pragmatic, open-label randomized controlled trial with two parallel groups allocated in a 1:1 ratio. The trial will be based on a superiority hypothesis of ENDS + SOC for smoking cessation to SOC alone in adult (≥18 years) smokers. The intervention group will receive ENDS and nicotine-containing e-liquids to use *ad libitum* until 6 months visit, plus SOC. The control group will receive SOC only. At the baseline visit, participants in the control group will receive a voucher ("BERNcity Geschenkcard" or similar voucher for Lausanne,Geneva, Zürich and St. Gallen) worth CHF50. The amount of CHF50 is of similar monetary value to what the intervention group will receive in form of the two ENDS devices and the e-liquids. A total of 1172 smokers will be randomized in the study, 586 in each group. Recruitment of the participants is planned to occur over 13-18 months at all 5 study sites. Our main outcome at the 6-month visit will be self-reported continuous smoking cessation after target quit date, with blinded biochemical verification by exhaled carbon monoxide (CO), urinary cotinine, anabasine, VOCs, TSNAs (NNN, NNK and its metabolite NNAL) and PAHs (1- and 2-Naphtol and 1-OHP) levels. At the 12-month and 24-month visit, the main outcome will be the self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), again all confirmed by the exhaled CO level and the urinary level of cotinine and anabasine. Statisticians and laboratory personnel will be blinded to group allocation. Participants and clinicians will not be blinded. We will use the methodology of a clinical trial investigating a medicinal product to test the efficacy and safety of ENDS.

Figure 6.1-1: ESTxENDS trial design schematic



6.2 Methods of minimising bias

6.2.1 Randomisation

We will use a centralized computed randomization process to randomize participants into the intervention and control arm at a 1:1 ratio. The central server will be located in a protected environment at CTU Bern.

6.2.2 Blinding procedures

Participants, nurses and investigators will not be blinded. We will blind both the statisticians who perform the main analyses and the laboratory personnel who determine urinary cotinine, anabasine, VOCs, TSNAs (NNN, NNK and its metabolite NNAL) and PAHs (1- and 2-Naphtol and 1-OHP) levels.

6.2.3 Other methods of minimising bias

To minimize bias of safety outcomes, all clinical events (hospitalizations) and other serious adverse events will be verified by a blinded external committee whenever possible (see 1.7.3).

6.3 Unblinding Procedures (Code break)

Not applicable.

7. STUDY POPULATION

7.1 Eligibility criteria

Subjects fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Persons aged 18 or older
- Currently smoking 5 or more cigarettes a day, for a least 12 months
- Willing to try to quit smoking within the next 3 months
- Persons providing a valid phone number, a valid email address and/or a valid postal address.

The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

- Known hypersensitivity or allergy to contents of the e-liquid
- Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected
- Women who are pregnant or breast feeding
- Intention to become pregnant during the course of the scheduled study intervention, i.e. during the first 6 months of the intervention
- Persons having used ENDS or tobacco heating systems regularly in the 3 months preceding the baseline visit
- Persons having used nicotine replacement therapy (NRT) or other medications with demonstrated efficacy as an aid for smoking cessation such as varenicline or bupropion within the 3 months preceding the baseline visit within the 3 months preceding the baseline visit
- Persons who cannot attend the 6-month follow-up visit for any reason
- Persons who cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures

7.2 Recruitment and screening

Target population of the study are individuals who smoke 5 or more cigarettes/day since more than one year and are willing to try and quit within the next three months. Individuals will be recruited through media advertisements and communications. Interested smokers will be screened over the phone by a trained nurse to review eligibility criteria. Smokers will also announce their target quit date and, if eligible and if they have no contra-indications for participating in the study, will be sent a participant information sheet and the informed consent form. The baseline visit will be scheduled a week before the quit date they named over the phone. At the baseline visit, trained study nurses will again verify the eligibility criteria, explain the trial and have participants sign the informed consent form.

7.3 Assignment to study groups

We will randomize participants to either intervention or control group at the end of the baseline visit, after participants have signed the consent form and we have taken all baseline measures. Nurses will collect all demographic parameters from participants online before they can request centralized randomization. Once all parameters are entered, the nurse will request a randomization allocation in presence of the participant. The central server located in a protected environment at CTU Bern will then allocate participants in the intervention and control group in a 1:1 ratio. Nurses and participants will learn about the allocation group directly on the screen, hence the nurse and participants will not be blinded. The randomization lists stored on the server will be only accessible by system administrators and not to study personnel involved in the recruitment of participants. The centralization of process and necessity to enter all demographic data before being able to randomize participants in the intervention and control group will ensure the reliability and concealment of the randomization process.

7.4 Criteria for withdrawal / discontinuation of participants

Discontinuation of treatment

Treatment will be discontinued if:

- A participant reports she is pregnant.

Treatment may be discontinued if:

- Participants do not comply with the instructions by the research team;
- A treatment-related SAE is suspected;
- The protocol has been irretrievably violated, as determined by the sponsor.

Subjects who permanently discontinue study treatment are encouraged to continue in the follow-up period and to attend the protocol-specified follow-up visit at 6-, 12- and 24- month follow-up visits in accordance with the intention-to-treat principle.

Discontinuation of participation in the study

Participation in the trial will be stopped if:

- participants make a request to discontinue their participation

Participation in the trial may be stopped if:

- the responsible study investigator decides that continued participation in the study could be harmful to the subject's wellbeing

A participant who decides to discontinue the study participation prematurely will be defined as a dropout if he/she has already been randomized. A study participant who terminates the study before being randomized will be defined as a screening failure.

Participants who cannot be reached over the phone at follow-up visits will not be excluded. Participants who do not present at the 6/12/24-month visits will be contacted by phone, by letter, and, finally, we will contact two contact persons and the primary care provider listed by participants at the baseline visit. If we cannot contact participants for more than three months, we will consider them lost to follow-up.

Participants who have finished their study at 6 months according to their signed informed consent will be contacted by phone or by e-mail to ask if they would like to continue their study participation for the 12- and 24-months visit. If they don't wish to continue or if they are unreachable no futher contact attemps will be performed. These participants will be considered as having finished the study according to the protocol.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

8.1.1 Experimental Intervention (treatment / device)

Device:

We will use the Innokin Endura T20-S starter kit. The Endura T20-S kit will come in a user packet with a 1,500 mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro USB DC 5V/1A cable and an instruction manual in French or German, respectively. Colors availble will be Black, Purple, Grey, Red and Blue.

https://www.innokin.com/vaporizers/endura-t20-s/

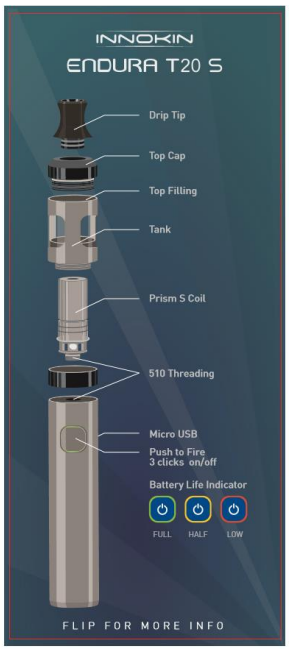
E-liquids:

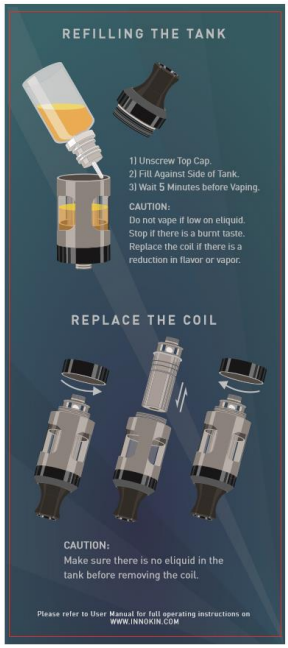
We use the e-liquids produced by the company Gaïatrend in France (https://www.gaiatrend.fr/en/). Nicotine concentrations available will be 0 mg/ml, 6 mg/ml, 11 mg/ml and 19.6 mg/ml. Following aromas will be available to be chosen by the study participants: FR-4 (tobacco flavor) and FR-M (tobacco flavor), FRESH MINT (menthol flavor), RASPBERRY#2 (fruity flavor), RED FRUITS (fruity flavor) or GREEN APPLE (fruity flavor). The proportion of propylene glycol and vegetal glycerin will be 76/24 for all e-liquids. The components of the e-liquids will be propylene glycol, vegetal glycerin, medical-quality nicotine, alcohol and aromas.

https://www.gaiatrend.fr/en/

Study participants in the intervention group will be advised by trained study nurses how to get started with the device, how to fill the device with e-liquid, how to charge the device and how to change the coil every two weeks. A user manual will be provided for every study participant.

Participants will be advised to only use liquids we provide. To avoid odor disturbances, e-liquids should be stored in a food storage plastic or glass box. The box should to be sealed and not stored with food. After use, the box cannot be used for storing food. The box should be stored out of reach of children as normally done with cleaning products and detergents.





http://www.innokin.com/manuals/Endura-T20S.pdf

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Participants in the control intervention will receive SOC only. SOC is as follows: Study nurses will provide SCC based on cognitive behavior therapy and motivational interviewing(see e.g., [150]). Trained nurses will follow a standardized course. SCC will be provided by person at the first clinical visit (baseline visit) and then over the phone at the target quit date, at week 1, 2, 4, and 8. Then again at the 6-, 12- and 24-months follow-up visits. Average duration of counseling is expected to be 15 minutes over the phone and at the follow-up visits and 30 minutes during the baseline in-person visit. Participants will be allowed to use other NRT, smoking cessation medication or smoking cessation help at their own expenses. Participants interested in other evidence-based smoking cessation drug therapy (varenicline or bupropion) will be allowed to consult their primary care physician (PCP) or another health professional to get a prescription for these drugs.They will also be encouraged to go on the stop-tabac.ch (www.stop-tabac.ch) website for smoking cessation support if they wish online support, as is currently performed in smoking cessation counseling in Switzerland.

8.1.3 Packaging, Labelling and Supply (re-supply)

Device:

The Endura T20-S kits will come in a user packet with a 1,500mAh internal Li-Po battery, a Prism S Coil (0.8 ohm) atomizer, a spare drip tip, a micro Micro USB DC 5V/1A cable, a USB charger and an instruction manual. Colors availble will be Black, Purple, and Blue (see image of the kit below and also images of the device in section 8.1.1.).

The kits will be ordered at Innokin and stored in each center. They will be handled directly in person by the study nurse at the first visit for the intervention group at no charge. Participants will receive two devices at the baseline visit. In case of defective devices which need to be replaced, or if the device is lost or otherwise unusable, participants will either directly visit the centers to pick up replacement devices, or participants will get the possibility to re-order a device through the study nurse at any time during the first 6-months follow-up by mail. In all cases the devices will be free of charge for participants during the 6-months follow-up. If participants are interested in getting a new device provided by the study after the 6-months follow-up visit, they will have to purchase them at their own cost wherever available. Free E-cigarettes and coils will not be provided anymore by the study personnel after the 6-months visit.

E-liquids:

All e-liquid bottles will follow standards by the EU and be limited to 10 ml. They will include a safety notice and logos to indicate that the product is toxic, that bottles should not be used by minors under 18 and not to be used by pregnant women. The bottle will contain information on the composition, the nicotine concentration, the flavour and have a warning reading: “This product contains nicotine, which is a highly addictive substance”. Gaïatrend obtained ISO8317 certification for its e-liquid flasks (See also section 8.1.1. for pictures of the flasks containing the e-liquids). This standard certifies that flasks are equipped with child-resistant closures (See Section 3.7 Risk/benefit).

At the baseline visit, study participants will be allowed to taste and choose between six e-liquid flavors and between four different nicotine concentrations. Once they have tested and chosen their e-liquid, they will directly receive a first batch of e-liquids.

At regular phone intervals from study nurses to study participants participants will be allowed to switch between flavor and nicotine concentration during the course of the study. Study nurses will support participants in the progressive reduction in nicotine concentrations over time to avoid withdrawal symptoms, in particular the management of the liquid concentrations used. In addition, if participants use all the e-liquid during follow-up, and given that the use of e-liquids will be *ad libitum*, participants will be allowed to reorder e-liquids through contacting the study nurses during the first 6 months. For those still interested to use e-liquids after the first 6 months, they will have to purchase them wherever available at their own cost. Free e-liquids will not be provided anymore by the study personnel after the 6-months visit.

Batches of e-liquids will be shipped and mailed by Gaïatrend to the study centers.

8.1.4 Storage Conditions

Batches of e-liquids will be shipped and mailed by Gaïatrend to the study centers. The devices and e-liquids will be stored in each center at room temperature in a securely locked cabinet or enclosure. Access will be limited to investigators and their designees.

Participants will be instructed to store the e-liquids in a dry place, at room temperature and out of reach of children and pets. After study termination, participants will be allowed to keep their devices and spare e-liquid or return them in each center if not completely used. To avoid odor disturbances, e-liquids should be stored in a food storage plastic or glass box. The box should to be sealed and not stored with food. After use, the box cannot be used for storing food. The box should be stored out of reach of children as normally done with cleaning products or detergents.

In case of malfunction of the devices or identification of damage to the e-liquids bottles by participants during shipping, participants will be encouraged to call the study nurse, to return the devices or e-liquids and be sent new devices or e-liquids by mail.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

After the ramdomization process, participants in the intervention group will receive a structured intervention for smoking cessation and in addition, two devices and e-liquids at no charge and advice on how to use them by trained study nurses. Participants will be explained the rationale behind delivering nicotine through the e-liquids instead of the cigarettes and be trained in using the device, for example how to turn in on and off, charge the device, refill the 2 ml tank with e-liquids and replace the coil at

regular intervals. Participants will be allowed to taste and choose between the various nicotine concentrations and flavors. We will encourage study participants to use the highest nicotine concentration in order to ensure optimal nicotine substitution while reducing the volume of e-liquids used and vapor inhaled. Study nurses will teach particpiants how to recognize withdrawal symptoms (craving, changes in mood etc.) and instruct them to use more liquid or a higher concentration if they experience withdrawal symptoms. Once they have decided of the nicotine concentration (either 0 mg/ml, 6 mg/ml, 11 mg/ml or 19.6 mg/ml) and the aroma (among two different tobacco flavors, menthol flavor or three different fruity flavors), they will receive a first batch of e-liquids directly at the baseline visit in preparation of their smoking cessation attempt scheduled one week later. Participants will be free to use their ENDS as much as they wish. They will be informed that they can switch nicotine concentrations and flavors at any time during follow-up. They will also be informed that they can re-order e-liquids as much as they want over the first 6 months of follow-up. We will document ENDS use by monitoring the use of e-liquids in ml.

After this first visit, study nurses will contact participants in both study groups over the phone on the day of the target quit date, then again after 1 week, 2 weeks, 4 weeks and 8 weeks. During these follow-up calls, study nurses will provide SSC and ask participants about their use of tobacco cigarettes and ENDS and answer technical questions, ask if participants need additional e-liquids or if they want to shift to another nicotine concentration or flavor. Study nurses will support participants in the progressive reduction in nicotine concentrations over time to avoid withdrawal symptoms, in particular the management of the liquid concentrations used. Study nurses will record the time spent on the phone with each participant. Nurses will also encourage participants the change of coil at regular intervals if they continue using ENDS. Between these scheduled visits, participants will have the possibility to contact study nurses anytime during office hours, send an email to study nurses for any technical issues with the device or smoking cessation support. Nurses will answer emails within 24 hours of receipt on office days.

Average duration of counseling is expected to be 15 minutes over the phone and during the in-person visits at 6-, 12- and 24- month follow-up visits and 30 minutes during the in-person visit at baseline. Participants will be allowed to use other NRT, smoking cessation medication or smoking cessation help at their own expenses. Participants interested in other evidence-based smoking cessation drug therapy (varenicline or bupropion) will be allowed to consult their primary care physician (PCP) or another health professional to get a prescription for these drugs.They will also be encouraged to go on the stop-tabac.ch (www.stop-tabac.ch) website for smoking cessation support if they wish online support, as is currently performed in smoking cessation counseling in Switzerland.

8.2.2 Control Intervention

Participants in the control intervention will receive SOC only in person at the baseline visit and then over the phone.

After this first visit, study nurses will contact participants in both study groups over the phone on the day of the target quit date, then again after 1 week, 2 weeks, 4 weeks and 8 weeks. During these follow-up calls, study nurses will provide SSC and ask participants about their use of tobacco cigarettes. Study nurses will record the time spent on the phone with each participant.

Average duration of counseling is expected to be 15 minutes over the phone and during the in-person visits at 6-, 12- and 24- month follow-up visits and 30 minutes during the in-person visit at baseline. Participants will be allowed to use other NRT, smoking cessation medication or smoking cessation help at their own expenses. Participants interested in other evidence-based smoking cessation drug therapy (varenicline or bupropion) will be allowed to consult their primary care physician (PCP) or another health professional to get a prescription for these drugs.They will also be encouraged to go on the stop-tabac.ch (www.stop-tabac.ch) website for smoking cessation support if they wish online support, as is currently performed in smoking cessation counseling in Switzerland.

8.3 Dose / Device modifications

Participants will be free to choose between four nicotine concentrations (0 mg/ml, 6 mg/ml, 11 mg/ml or 19.6 mg/ml), 6 flavors (two different tobacco flavors, menthol flavor or three different fruity flavors) and administered through ENDS ad libitum during the first 6 months of follow-up. They will have the possibility to switch to other predefined nicotine concentrations and flavouring in order to maximise the adherence to ENDS. We will teach them how to recognize withdrawal symptoms (changes in mood etc) and instruct them to use more liquid or a higher nicotine concentration if they experience withdrawal symptoms.

Criteria for discontinuing the study treatment or study discontinuation see section 7.4.

8.4 Compliance with study intervention

Study nurses will be trained to deliver a structured intervention that includes explanations on how to use the device and e-liquids. We will optimize adherence of study participants to ENDS by offering participants a choice of nicotine content and e-liquid flavors. This should maximize the effect of ENDS and more closely match real life, since smokers select products based on their own needs and preferences. We also allow participants the opportunity to switch e-liquid flavors, as real life consumers might. Second, in order to avoid participants stop using ENDS for technical reasons, study nurses will provide 2 devices at the baseline visit and be reachable during regular working hours. In case of defective ENDS-devices which need to be replaced, participants can either directly visit the centers to pick up replacement devices, or we will ship replacement devices directly to the participants' home as fast as possible.

We will monitor how many e-liquids containing bottles are distributed by the study nurses and participant's use of e-liquids in ml during follow-up calls after 1 week, 2 weeks, 4 weeks and 8 weeks after the target quit date and at the 6months visit. We will continue to monitor use of ENDS and e-liquids at the 12- and 24- months visit by monitoring the type of device they use and the type of e-liquids they use. During follow-up calls study participants will be asked about any problem with regard to using the ENDS. At the 6-, 12 and 24- months visit, we will verify participant exposure to tobacco cigarettes vs. other sources of nicotine (ENDS, NRTs, etc.) by measuring cotinine, anabasine, CO and NNAL.

We will offer a voucher ("BERNcity Geschenkcard" or similar voucher for Lausanne,Geneva, Zürich and St. Gallen) worth CHF 50 at the baseline visit to participants in the control group. Requesting participants in the control group to avoid using ENDS for 6 months instead of 24 months should also limit the number of participants who will purchase an ENDS on their own in the control group or who are lost to follow-up. However, we will ask participants in the control group if they use an ENDS at each follow-up call and follow-up visit.

Participants in the additional MRT data collection at the 6 month visit will receive 90 CHF given that these are additional measures and that participants will need to come back on another day. The 90 CHF corresponds to 2X1.5 hours visits a 25 CHF per hour (75 CHF) and 15 CHF for transportation costs. Healthy volunteers will receive a voucher of 90CHF and a voucher for a lunch at the Inselspital, Bern.

At 12- and 24- months follow-up, we will offer a voucher to all participants coming for a personal visit worth 30 CHF in oder to encourage them to come at the clinical visit in person to assess and validate outcomes.

Adherence to the allocated product is defined as not having used the non-allocated product on a minimum of 5 days during the last 7 days, on at least 1 visit.

8.5 Data Collection and Follow-up for withdrawn participants

A participant who decides to discontinue the study participation prematurely will be defined as a dropout if they have already been randomized. A study participant who terminates the study before being randomized will be defined as a screening failure.

Participants who cannot be reached over the phone at follow-up visits will not be excluded. Participants who do not present at the 6-, 12- and 24-month visits will be contacted by phone, by letter/e-mail, and, finally, we will contact their relatives or general practitioner. If we cannot contact participants for more than three months, we will consider them lost to follow-up.

In case of withdrawal, the biological material and health-related personal data of the person concerned will be anonymised after data evaluation has been completed.

8.6 Trial specific preventive measures

We will perform pregnancy tests in pre-menopausal women during the baseline visit. We will also specifically ask participants if they have known allergies to the tested products.

8.7 Concomitant Interventions (treatments)

Participants both in the control and intervention group will be allowed to use any NRT (patches, gums) or other smoking cessation medication (bupropion, varenicline) they wish at their own costs. Their use will be recorded in the eCRF and considered as a covariate in data analysis.

8.8 Study Device Accountability

The investigator or designee will maintain an inventory record of ENDS and e-liquids at the site level (received from manufacturer, returned to manufacturer) and at the patient level (dispensed to the patient, returned by the patient).

8.9 Return or Destruction of Study Device

Upon completion or termination of the trial, ENDS and spare e-Liquids will remain in the participants' possession, or if desired, can be returned to the sponsor.

9. STUDY ASSESSMENTS

9.1 Table of study procedures and assessments

Procedure	**Screening** ☽	**Baseline visit**	**Day 0** ☽	**Week 1** ☽	**Week 2** ☽	**Week 4** ☽	**Week 8** ☽	**Outcome Visit Month 6**	**Outcome visit Month 12**	**Outcome visit Month 24**
Visit timing Day 0 is target quit date (TQD)	**-x to -8 days**	**-7 days**	**Day 0** (TQD)	**+ 7 days**	**+14 days**	**+ 28 days**	**+ 56 days**	**+ 180 days**	**+ 360 days**	**+ 720 days**
Visit window				±2 days	±3 days	±4 days	±1 week	-4/+12 weeks	-4/+12 weeks	-4/+12 weeks
ENROLLMENT										
Eligibility screen	x	x								
Explanation of project and protocol	x	x								
Send informed consent	x									
Sign informed consent		x								
Randomization to ENDS (E) or control		x								
Set target quit date (TQD)	x									
ASSESSMENT										
Demographics	x	x								
Medical History		x						X*	X*	X*
Physical activity		x						X*	X*	X*
Smoking history	x	x								
Nicotine dependence		x						X*	X*	X*
Withdrawal symptoms		x						X*	X*	X*
Environmental pollution (e.g. second hand smoke)		x						X*	X*	X*
Alcohol and illegal drug use		x						X*	X*	X*
Anxiety		x						X*	X*	X*
Depression symptoms		x						X*	X*	X*
Sleep quality		x						X*	X*	X*
Quality of life		x						X*	X*	X*
Women: pregnancy test		x								
Pulmonary function tests and MBW		x						x	x	x
MRI of the lung+								x		
Micronuclei assessment in buccal epithelium		x						x	x	x
Olfactory function		x						x	x	x
“Dry-hit” recognition								E		
Inflammatory biomarkers		x						x		

Procedure	**Screening** ◑	**Baseline visit**	**Day 0** ◑	**Week 1** ◑	**Week 2** ◑	**Week 4** ◑	**Week 8** ◑	**Outcome Visit Month 6**	**Outcome visit Month 12**	**Outcome visit Month 24**
INTERVENTION										
ENDS & e-liquid distribution		E								
E-liquid order by participants				E	E	E	E	E		
E-liquid sent to their homes					E	E	E	E		
ENDS instruction		E								
ENDS technical support			E	E	E	E	E	E		
Structured smoking cessation counselling		x	x	x	x	x	x	x	x	x
OUTCOME MARKERS										
Tobacco use (questionnaires)	x	x	x	x	x	x	x	X*	X*	X*
Estimated ENDS use (ml e-liquids used)			E	E	E	E	E	E*	E*	E*
Exhaled CO		x						x	x	x
Urinary nicotine, cotinine, anabasine		x						x	x	x
Urinary metabolites of nitrosamines (TSNAs)		x						x	x	x
Adverse events, serious adverse events			x	x	x	x	x	X*	X*	X*
VOC and PAH metabolites in urine		x						x	x	x
Oxidative stress markers in urine		x						x	x	x
Oxidative stress in exhaled breath (Lausanne site only)		x						x		
Blood pressure, heart rate, height, weight (BMI), waist circumference		x						x	x	x
Blood lipids, HbA1c (only for participants with diabetes), creatinine		x						x	x	x
Respiratory symptoms		x						X*	X*	X*
Pulmonary function test parameters (FEV1, FVC, FEV1/FVC, PEF, FEF25-75%, TLC, FRC, RV/TLC, DLCO) (Bern site only)		x						x	x	x

Procedure	Screening ◑	Baseline visit	Day 0 ◑	Week 1 ◑	Week 2 ◑	Week 4 ◑	Week 8 ◑	Outcome Visit Month 6	Outcome visit Month 12	Outcome visit Month 24
Multiple breath washout (MBW) parameters (LCI, Scond, Sacin) (Bern site only)		x						x	x	x
Parameters of ventilation, perfusion and structural changes of the lung (MRI; Bern site only) +								x		
Micronuclei frequency (micronuclei assessment)		x						x	x	x
Olfactory function parameters ((olfactory discrimination score (ODS))		x						x	x	x
"Dry-hit" recogntion								E		
Inflammatory biomarkers		x						x		

E: ENDS group only; C: Control group only

* possibility of filling the survey online

+ A aged matched group of healthy never smoking/non-vaping volunteers (N=10) will perform once two consecutive MRI measurements. These volunteers are not participants of the RCT.

9.2 Assessments of outcomes

Participants will be seen in a healthcare facility in each center four times, at baseline, after 6-, 12- and 24- months. Data will be collected by study nurses, supervised by the responsible investigator at each site, based on protocols standardized across sites. All data will be directly collected via centralized computerized data entry forms. Laboratory analyses of urine samples will be centralized at the laboratory in Lausanne (Unisanté, Centre universitaire de médecine générale et santé publique, Lausanne). The laboratory is accredited ISO 17025 for nicotine quantification in urine, VOCs quantification, and it will use validated methods for the other compounds in urine and oxidative stress analyses. The samples are only stored and continued to be used with the participants consent independent from the study (see section 12.6). The blood samples from which analysis of blood lipids, HbA1c (only for participants with diabetes), creatinine will be performed, will remain in the main laboratory of each health center. The other blood sample for the analyses of inflammatory biomarkers from the centers in St. Gallen and Bern will be transported and stored in the biobank in Bern. Blood samples from participants in Geneva are stored at the local biobank in Geneva. Analyses of exhaled breath condensates will only be performed in Lausanne and thus centralized at the laboratory in Lausanne (Unisanté, Centre universitaire de médecine générale et santé publique, Lausanne). CTU Bern personnel will monitor quality. We will inform participants that some random phone calls will be recorded for quality monitoring. Quality monitoring will include reviewing calibration procedures for carbon monoxide (CO) and blood pressure measuring devices, weight and height scales.

The table below shows which outcome markers listed in 9.1 are collected in which center:

Tobacco use (questionnaires)	Bern, Geneva, Lausanne, St. Gallen, Zürich
Estimated ENDS use (ml e-liquids used)	Bern, Geneva, Lausanne, St. Gallen, Zürich
Exhaled CO	Bern, Geneva, Lausanne, St. Gallen, Zürich
Urinary nicotine, cotinine, anabasine	Bern, Geneva, Lausanne, St. Gallen, Zürich

Urinary metabolites of nitrosamines (TSNAs)	Bern, Geneva, Lausanne, St. Gallen, Zürich
Adverse events, serious adverse events	Bern, Geneva, Lausanne, St. Gallen, Zürich
VOC and PAH metabolites in urine	Bern, Geneva, Lausanne, St. Gallen, Zürich
Oxidative stress markers in urine	Bern, Geneva, Lausanne, St. Gallen, Zürich
Oxidative stress in exhaled breath (Lausanne site only)	Lausanne
Blood pressure, heart rate, height, weight (BMI), waist circumference	Bern, Geneva, Lausanne, St. Gallen, Zürich
Blood lipids, HbA1c (only for participants with diabetes), creatinine	Bern, Geneva, Lausanne, St. Gallen
Respiratory symptoms	Bern, Geneva, Lausanne, St. Gallen, Zürich
Pulmonary function test parameters (FEV1, FVC, FEV1/FVC, PEF, FEF25-75%, TLC, FRC, RV/TLC, DLCO) (Bern site only)	Bern (150 participants)
Multiple breath washout (MBW) parameters (LCI, Scond, Sacin) (Bern site only)	Bern (150 participants)
Parameters of ventilation, perfusion and structural changes of the lung (MRI; Bern site only)	Bern (40 participants & 10 healthy volunteers)
Micronuclei frequency (micronuclei assessment)	Bern, Lausanne
Olfactory function parameters ((olfactory discrimination score (ODS))	Bern, Geneva, Lausanne, St. Gallen, Zürich
“Dry-hit” recogntion	Bern, Geneva, Lausanne, St. Gallen, Zürich
Inflammatory biomarkers	Bern, Geneva, St. Gallen

9.2.1 Assessment of primary outcome

- The primary outcome at 6-month will be the continuous smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report at the 6-months visit (self-report of no smoking from target quit date) and confirmed by urinary level of anabasine (<3 ng/ml). Complete Urine samples will be collected in a flask. The laboratory in Lausanne (Unisanté, Centre universitaire de médecine générale et santé publique, Lausanne) has a routine analytical method for measuring nicotine metabolites in urine and participates regularly in external quality control testing.

- The primary outcome at 12- and 24- months follow-up will be the self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), confirmed by urinary level of anabasine (<3 ng/ml).

9.2.2 Assessment of secondary outcomes

The secondary outcomes will be measured as described for the primary endpoint.

Secondary smoking cessation outcome will be measured with a self-reported 7-day point prevalence abstinence (not having smoked cigarettes within the preceding week), again confirmed by urinary level of anabasine, and change in the self-reported number of cigarettes smoked per day (CPD) with 50% reduction in CPD from baseline to follow-up considered as successful reduction, measured as above.

For alternate validation methods of the primary and secondary outcomes, we will use CO-levels, which will be measured with a Micro Smokerlyser®; Bedfont Scientific Ltd.

Respiratory symptoms will be assessed by questionnaires:

- COPD Assessment Test (CAT), Modified Medical Research Council (MMRC) Scale, Asthma Test (ACT/ ECRHS)

Each Urine sample will be split into six aliquots to quantify cotinine and anabasine (see above), PAH metabolites, VOCs, propylene glycol, TSNAs, 8-OHdG and 8-isoprostanes, since no analytical method can measure all the compounds together. The different compounds will be estimated as follows:

- Concentrations of urinary cotinine and anabasine, VOCs, TSNAs and PAHs will be measured
- Oxidative stress will be assessed by quantifying 8-OHdG and 8-isoprostane concentrations in both EBC and urine
- We will adapt the method for analyzing urinary TSNAs from Xia et al.[30] After enzymatic hydrolysis with a β-glucuronidase solution, the urine sample will be extracted using solid phase extraction (SPE) columns, and analyzed by LC/MS/MS.
- We will use a high performance liquid chromatography (HPLC) method developed by the IST laboratory to analyze PAHs (1- and 2-Naphtol and urinary 1-OHP).

Exhaled breath condensates (EBC) other than CO will be performed in all participants at the Lausanne site during the first as well as the outcome visit (baseline and 6 months), based on guidelines from the American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force for EBC collection[87]. EBC will be sampled with an EcoScreen II system (total exhaled volume of about 120 L). After collection, each sample will be aliquoted. To avoid auto-oxidation of fatty acids (potential impact on 8-isoprostane levels), we will add tert-butyl hydroxytoluene and rapidly freeze the sample. The samples will be stored at -70°C for a maximum of 1 month.[90]

OHdG and 8-isoprostane will be simultaneously determined in EBC, using a method adapted to Syslova et al.[90] After adding internal standards (isotopically labeled 8-OHdG and 8 isoprostane), 1 ml of EBC will be lyophilized and the residue dissolved in a water:methanol solution and immediately analyzed by a liquid chromatography–atmospheric pressure ionization tandem mass spectrometry (LC-ESI-MS/MS) method available at IST.

Cardiovascular risk factors (CVRF) will be assessed as follows:

- Blood collection: We will measure cardiovascular risk factors in blood by routine methods in clinical practice: : Total-, LDL- and HDL-cholesterol, triglycerides, HbA1c (only for participants with diabetes) and creatinine.
- Blood pressure and heart rate will be measured by standard sphygmomanometer. After a 10-minute rest, the right arm blood pressure of a seated participant will be assessed three times at one-minute intervals. The two last blood pressures measures will be averaged to reduce variability.
- Height, weight (calculation of body mass index, BMI), and abdominal circumference

.We will query all participants if they had SARS-CoV-2 testing during the outbreak.

Pulmonary function will be assessed in a subgroup of 150 participants at the Bern site using conventional pulmonary function tests and by multiple breath washout. Pulmonary function test results will be interpreted according to the current ERS/ATS guidelines [151] and in the case of evidence for COPD according to the current GOLD report[152]. MBW results will be analyzed according to the current ERS/ATS consensus statement [77].

MRI and additional pulmonary function testing will be done in a subsample of at least 40 participants at the 6-month follow-up at the Bern site to assess functional and structural changes in the lung. Participants of both groups, (randomized participants who switched to vaping, who continued smoking and quitters of both smoking and vaping) will be invited to perform a MRI measurement and lung function measurement before and after vaping/smoking. Participants will be asked not to vape/smoke 3 hours prior to the first pulmonary function and MRI measurements. The participants will then be asked to vape/smoke (unless they've quit smoking/vaping) and the second pulmonary function and the MRI measurement will be performed less than 30 minutes after vaping/smoking. MRI is a non–invasive and radiation free diagnostic test used in clinical setting in children and adults. MRI provides images of different layers from the body. Measurements with a new developed implication for the MRI called the matrix pencile method (MP-MRI) will be performed. The method of MP-MRI allows simultaneous assessment of regional lung perfusion and ventilation in humans. For this technique, neither administration of intravenous contrast agents nor inhalative gaseous media is needed. Beside functional changes structural changes of the lung parenchyma will be examined. The MRI exams will be performed on a standard clinical whole body MRI with 1.5 Tesla field strength (MAGNETOM Avanto, Siemens Healthcare at the university hospital Basel; MAGNETOM Aera, Siemens Healthcare at the university hospital Bern). Participants coming on both days for the pulmonary function testing and MRI will be reimbursed with 90 CHF. To compare stability over a brief period of time (2 hours) of MRI outcome

measures, we will include a group of healthy volunteers (N=10) who will undergo two consecutive MRI measurements. Healthy volunteers will be never smokers/non-vapers (<100 cigarettes per lifetime), no history of lung disease, no use of inhalation medication and no lung infection in the last four weeks. Volunteers will be aged matched to our sub-group of participants of former smokers, tobacco smokers and participants using ENDS products. Most studies define a never-smoker as one who either has never smoked at all or has smoked <100 cigarettes (or the equivalent amount of tobacco) in his or her lifetime, which is in line with that proposed by the World Health Organization.[153]

Micronuclei assessment in buccal epithelium, a surrogate measure of future cancer risk, will be done at baseline, and at the 6-, 12- and 24-months follow-up visits. Buccal cells will be harvested with a cytobrush. Participants will harvest their own cells by rotating the cytobrush on the inner cheeks of the mouth. The cytobrushes will be transferred or mailed to the laboratory in a buffer. The buccal cells will then be centrifuged, separated mechanically with tissue homogenizer, filtered, transferred onto microscope slides and left to air dry. The cells will be fixed in ethanol/glacial acetic acid and stained with DAPI (4′,6-diamidino-2-phenylindole). An automatic MN counting system will be applied. MN frequency will be calculated as number of cells that contain MNs divided by the total number counted. A minimum of 1000 cells will be counted per participant.

The olfactory function will be assessed at baseline and and at the 6-, 12- and 24-months follow-up visits by using the Burghart Sniffin' Sticks 16-item Identification-test.[110-112, 154] The participants will be asked to not have eaten, drunk anything but water, smoked, used ENDS or chewed gum for at least 15 minutes before the olfactory function test. 16 commonly known odours will be presented to the participants for around 3-4s each, after which the participants will be asked to select the correct answer from 4 possible answers (forced choice). The highest possible score olfactory identification score (OIS) is 16; the cut-off for hyposmia is 11 points and cut-off for anosmia is 8 points.This will be performed at baseline and and at the 6-, 12- and 24-months follow-up visits.

For the "dry-hit" recognition, voluntary participants will be given two ENDS at the 6 months follow-up visit – one with correct filling of the e-liquid and one with a too low e-liquid level and therefore producing a "dry hit" which would be recognized as a burnt smell when vaping. They are asked to asses which one is the ENDS producing a "dry-hit". Both ENDS will be filled with a tobacco flavored e-liquid without nicotine (0mg/ml). The participants will be instructed to take at least 1 puff of 2 seconds (maximum of 4 puffs of 2 seconds each with a 30 second break in-between) from each ENDS. Participants will not be informed about the sequence of the ENDS. Participants are allowed to go back and forth between the two ENDS before they decide. After their decision, they will be informed, if they were able to recognize the ENDS which generates a "dry hit".The inflammatory biomarkers will be measured in blood serum using Olink Proteomics' unique protein detection system,[118] the Proximity Extension Assay (PEA), which allows us to perform highly specific and sensitive multiplex immunoassays to detect 92 inflammation-related protein biomarkers.

Several questionnaires will be used to assess further parameters:

- Demographics: Age, gender; Socio-economic: education level, marital status; medical history, including medication use and healthcare utilization.
- Smoking history,[155, 156] including Fagerström questionnaire to assess nicotine dependence[157] and the Minnesota nicotine withdrawal scale.[158]
- Physical activity (International Physical Activity Questionnaire, IPAQ) [138]
- legal and illegal drug use: Alcohol consumption (AUDIT-C) [141, 159], illegal drug use (cannabis, cocaine, other) [142]
- Quality of life (EQ-5D questionnaire)[144]
- Depression symptoms (PHQ-9)[160]
- Anxiety (GAD-7) [146]
- Sleep Quality (PSQI) [143]
- Environmental pollution exposure (e.g. second hand smoke) [147, 148]

9.2.3 Assessment of other outcomes of interest

See above.

9.2.4 Assessment of safety outcomes

Information on safety outcomes like Adverse Events and ENDS deficiencies will be collected proactively

during the phone calls and the 6-, 12- and 24- month follow-up visits in person visit using standardized questionnaires and international guidelines.[149]

9.2.4.1 Adverse events

For this trial, all AEs as well as all SAEs will be collected, fully investigated, and documented in the source documents and the eCRF for all participants form the date of ICF signature until the last protocol-specific procedure has been completed.

9.2.4.2 Laboratory parameters

See above (assessment of secondary outcomes)

9.2.4.3 Vital signs

See above (assessment of secondary outcomes)

9.2.5 Assessments in participants who prematurely stop the study

Subjects who permanently discontinue study treatment are encouraged to continue in the follow-up period and to attend the protocol-specified follow-up visit at 6 months in accordance with the intention-to-treat principle.

9.3 Procedures at each visit

9.3.1 Screening phone call at -x to -8 days to target quit date:

- Study information and explanation
- Eligibility check (inclusion/exclusion criteria)
- Scheduling of target quit date and baseline visit
- Smoking history

9.3.2 Visit 1, Baseline visit at -7 days to target quit date

- Patient information and delivery of written study information to subject
- Verification of eligibility, including urine pregnancy test for premenopausal women
- Informed consent
- Demographics
- Relevant medical history including current medication
- Smoking history and symptoms linked to smoking, cigarette dependence (Fagerström Test for Cigarette Dependence), withdrawal symptoms
- Body weight, height, calculation of BMI, waist circumference
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest sitting)
- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illegal drug use
    - Environmental pollution exposure
- Exhaled breath composition (CO, oxidative stress markers in a subset of participants)
- Blood collection (lipids, HbA1c (only for participants with diabetes) & creatinine, inflammatory biomarkers)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
- Randomization to study group
- Distribution and instruction of ENDS and e-liquids (intervention group only)
- Smoking cessation counselling

- Scheduling of phone calls
- Pulmonary function tests (conventional and MBW in a subset of participants)
- Micronuclei assessment in buccal epithelium
- Olfactory function: Burghart Sniffin'Sticks 16-Item Identifiaction Test
- Inflammatory biomarkers
- .

9.3.3 Phone calls at target quit date, week 1, week 2, week 4, and week 8

Phone calls at target quit date (corresponding to day 0) and after 1 week (±2 days), 2 weeks (±3 days), 4 weeks (±4 days), and 8 weeks (+/-1 week):

- Smoking status: cigarettes / ENDS
- Assessment of smoking cessation medication use: NRT, proven effective drug therapies (varenicline, bupropion) and other interventions without demonstrated efficacy (e.g. acupuncture, hypnose, sophrology etc)
- Assessment of AEs, ENDS deficiencies
- Smoking cessation counselling
- ENDS technical trouble shooting
- Scheduling of phone calls and outcome visit

9.3.4 Outcome visit (month 6)

Visit at 6 months (corresponding to 24 weeks (-4 weeks/+12 weeks) after the target quit date):

- Current medication
- Antibiotics within the last 6 months
- Smoking status, nicotine dependence (Fagerström questionnaire), withdrawal symptoms
- Assessment of AEs, ENDS deficiencies Body weight, height, calculation of BMI, waist circumference
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illicit drug use
    - Environmental pollution exposure
    -
- Exhaled breath composition (CO, oxidative stress markers in a subset of participants)
- Blood collection (lipids, HbA1c (only for diabetic participants) & creatinine, inflammatory biomarkers)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
- Pulmonary function tests (conventional and MBW in a subset of participants)
- MRI (in a subset of participants)
- Micronuclei assessment in buccal epithelium
- Olfactory function: olfactory indentification score (OIS)
- "Dry-hit" recognition
-

9.3.5 Outcome visit (month 12)

Visit at 12 months (corresponding to 48 weeks after the target quit date):

- Current medication
- Antibiotics within the last 6 months
- Smoking status, nicotine dependence (Fagerström questionnaire), withdrawal symptoms
- Assessment of AEs, ENDS deficiencies
- Body weight, height, calculation of BMI, waist circumference
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)

- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illicit drug use
    - Environmental pollution exposure
- Blood collection (lipids, HbA1c (only for participants with diabetes) & creatinine)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
- Pulmonary function tests (conventional and MBW in a subset of participants)
- Micronuclei assessment in buccal epithelium
- “Dry-hit” recognition
- Olfactory function: Burghart Sniffin’Sticks 16-Item Identifiaction Test

9.3.6 Outcome visit (month 24)

Visit at 24 months (corresponding to 96 weeks after the target quit date):

- Current medication
- Antibiotics within the last 6 months
- Smoking status, nicotine dependence (Fagerström questionnaire), withdrawal symptoms
- Assessment of AEs, ENDS deficiencies
- Body weight, height, calculation of BMI, waist circumference
- Vital signs (heart rate, systolic and diastolic blood pressure at the right arm after at least 5 minutes at rest)
- Questionnaires
    - Respiratory symptoms
    - Quality of life
    - Physical activity
    - Anxiety
    - Depression
    - Sleep quality
    - Alcohol and illicit drug use
    - Environmental pollution exposure
- Blood collection (lipids, HbA1c (only for participants with diabetes) & creatinine)
- Urine collection (nicotine metabolites, VOCs, PAHs, nitrosamines, oxidative stress markers)
- Pulmonary function tests (conventional and MBW in a subset of participants)
- Micronuclei assessment in buccal epithelium
- “Dry-hit” recognition
- Olfactory function: Burghart Sniffin’Sticks 16-Item Identifiaction Test

10. SAFETY

10.1 Drug studies

Not applicable

10.2 Medical Device Category C studies

Not applicable

10.3 Medical Device Category A studies

Not applicable

10.4 Other clinical trial Category B

During the entire duration of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and case report forms (eCRF). Study duration encompassed the time from when the participant signs the informed consent form until the last protocol-specific procedure has been completed.

10.4.1 Definition and assessment of (serious) adverse events

An **AE** is any untoward medical occurrence in participant administered an intervention a clinical investigation and which does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the intervention, whether or not related to the intervention. [ICH E6 1.2, adapted]

A **SAE** is classified as any event which:

- requires inpatient treatment of at least 24 hours or extends a current hospital stay;
- results in permanent or significant incapacity or disability;
- is life-threatening or results in death; or
- causes a congenital anomaly or birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation (regardless of the duration of the hospitalization), but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious.

A hospitalisation or a prolongation of a current hospitalisation for a diagnostic or elective (surgical) procedure that was already planned prior to the participant's enrolment into the study is not classified as an SAE.

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination will be further followed up until recovery or until stabilisation of the disease after termination, or until the participant is lost to follow-up.

<u>Assessment of Causality</u>

The assessment of causality of SAEs will be done by both, investigator and Sponsorbased on the criteria listed in the ICH E2A guidelines below. For events of special interest (all SAEs, respiratory events, cardiovascular events, AEs with prescription of systemic antibiotics and AEs for which a causal relationship between the AE and vaping or smoking is not excluded) a smoking assessement questionnaire will be filled out in relationship to the event to facilitate the assessment of causality.

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Assessment of Severity

Classification and severity grading scale in this study will be performed in accordance with "Common Terminology Criteria for Adverse Events CTCAE Version 5 terminology.

Document collection for (S)AE adjudication

(S)AE Guidelines have been established to ensure complete document collection by the sites to allow proper adjudication by the adjudication committee for all SAEs and specific AEs (important cardiovascular events, important respiratory events, new cancer diagnosis and AE with at least possible relationship to vaping).

10.4.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

All SAEs for which it cannot be excluded that the events are attributable to the study intervention will be reported immediately and within a maximum of 24 hours of learning of its occurrence to the Sponsor of the study. Reporting is done via the eCRF which generates an automatic email notification to the Sponsor once an SAE is entered (and saved) in the system. In addition these events are also reported by the Sponsor to the CEC and local ECs (as applicable) within 15 days.

Reporting of safety and protective measures

If immediate safety and protective measures have to be taken during the conduct of the trial, the investigator notifies the CEC of these measures, and of the circumstances necessitating them, within 7 days. The Sponsor must immediately inform all participating Investigators about all safety and protective measures. The other in the trial involved Ethics Committees will be informed about safety and protective measures via the Sponsor.

Periodic reporting of safety

An annual safety report is submitted by the Sponsor once a year to the responsible Ethics committees and is also provided to all participating Investigators.

11. STATISTICAL METHODS

11.1 Hypothesis

The primary hypothesis of this trial is that providing smokers with ENDS and SOC leads to a higher rate of smokers with continuous smoking cessation from target quit date than SOC alone.

11.2 Determination of Sample Size

Primary outcome:

Sample size calculation of the ESTxENDS trial at 6 months follow-up: Based on the results of a systematic review and meta-analysis of previous trials, we expect an RR of smoking cessation of 1.6. Based on our previous RCTs, we expect a 12% quit rate in the control group and a 19% quit rate in the intervention group (ENDS + SOC) (7% absolute difference in continuous abstinence rate from target quit date [TQD]) for the main outcome in ESTxENDS at 6 months. With a two-sided alpha of 0.05 and a power of 90%, we therefore planned to include a minimum 557 smokers per group, for a total of 1,114 smokers, to find a significant difference in quit rates. Previous experience suggests we should assume a 5% loss to follow-up, so we increased our sample size by 5% (59 smokers). We will thus recruit 1,172 smokers. Though participants in the control group will not be actively provided with ENDS and asked not to use ENDS during the first 6 months of the study, we expect that 5% will still purchase ENDS on their own. The sample size of the entire trial is based on the primary outcome; the power to find a statistically significant difference for secondary outcomes is limited to the total number of participants.

Estimated power to detect significant differences in groups on smoking cessation at 12- and 24-months follow-up: Assuming 10% will have quit smoking in the control group at 12 months (continuous smoking cessation rate from TQD), and applying the RR of 1.6, we estimate 16% will have quit in the intervention group. Assuming 7% loss to follow-up, we will have 86% power to detect such a difference. Based on the literature and previous experience in our own trials,[133, 163] we expect a 10% relative increase in absolute smoking cessation rate by choosing 7-days point prevalence as the main outcome with no change in RR estimate.[163] We will have 90% power to detect a significant difference by choosing the 7-days point prevalence as a main outcome for the 12-months follow-up. We thus expect an 11% quit rate in the control group and 17% in the intervention group at the 12-month follow-up (6% absolute difference). At the 24-month follow-up, we can expect 8% in the control group will have stopped smoking and 12% in the intervention group. Assuming a 10% loss to follow-up rate, we will have 62% power to detect a significant difference between groups.

Secondary outcomes: The sample size of the entire trial is based on the primary outcome; the power to find a statistically significant difference for secondary outcome is limited to the total number of participants. For safety outcomes, we do not expect to find statistically different results between groups on SAEs. For toxicological outcomes of ENDS, such as measures of urinary biomarkers, we will compare participants based on ENDS exposure. Table 11.2-1 presents the number of participants we expect to fall into each category of ENDS and cigarette smoking over follow-up. In the intervention group, we expect that 19% of smokers would quit and 30% would use of ENDS at the 6-month follow-up.[9] In the control group, we expect that 12% of smokers who quit at 6-month follow-up. Though participants in the control group will not be actively provided with ENDS during the first 6 months of the study, we expect 5% will purchase ENDS on their own.

Table 11.2-1: Expected distribution of participants over follow-up

	Intervention group					Control group				
Group	**Ai**	**Bi**	**Ci**	**Di**	**Ei**	**Ac**	**Bc**	**Cc**	**Dc**	**Ec**
ENDS	+	+	-	-		+	+	-	-	
Tob smk	-	+	-	+		-	+	-	+	
Baseline		**586**							**586**	
6 months: N	**56**	**111**	**50**	**340**	**29**	**11**	**17**	**56**	**473**	**29**
% of group	10%	20%	9%	61%	5%	2%	3%	10%	85%	5%
12 months: N	**49**	**93**	**38**	**365**	**41**	**11**	**16**	**44**	**474**	**41**
% of group	9%	17%	7%	67%	7%	2%	3%	8%	87%	7%
24 months: N	**37**	**79**	**32**	**380**	**59**	**11**	**16**	**26**	**475**	**59**
% of group	7%	15%	6%	72%	10%	2%	3%	5%	90%	10%

Legend:
Ai= ENDS use, stop smoking, intervention
Bi= ENDS use, smoking, intervention
Ci= No ENDS, stop smoking, intervention
Di= No ENDS, smoking, intervention
Ei= Lost to follow-up (LTFU), intervention
Ac= ENDS use, stop smoking, control
Bc= ENDS use, smoking, control
Cc= No ENDS, stop smoking, control
Dc= No ENDS, smoking, control
Ec= LTFU, control

For measures of carcinogenicity, such as measures of incomplete combustion agents (VOCs and PAH: 1-OHP, 1- and 2-NAPH in urine) [29, 30] and exposure to TNSAs (NNK and NNN in urine),[18] and measures of oxidative stress in urine (8-OHdG and 8-isoprostane),[41, 91, 164] and MN in buccal epithelium, we will compare groups that stopped smoking with or without ENDS in intention to treat analyses (Ai+Ci vs. Ac+Cc; 112 vs. 70) and in per protocol analyses (Ai+Ac vs. Ci+Cc; 65 vs. 117). Based on previous cross sectional studies contrasting past smokers to smokers,[52, 164, 165] we estimate a 48% power to detect significant differences between quitters and continuing smokers for 8-OHdG and 100% for 8-isoprostane between baseline and follow-up, at alpha 0.05 and an assumed correlation between baseline and follow-up of 0.7 (Table 11.2-2). We will perform 3 determinations of buccal MN on 2000 cells for each participant. Based on published results, [99, 100, 166-168] we expect a baseline frequency among non-smokers of 0.74 MNs per thousand cells and a between-subject GSD of 1.3. We will have 80% power to detect a significant difference between smokers and non-smokers for a frequency ratio (FR) of 1.5.

For the 6-months changes in cardiovascular risk factors, we do not have direct data from RCT of ENDS use. Using data from previous RCT on nicotine replacement therapy, we expect a mean increase in HDL-cholesterol of 0.107 mmol/l (95% confidence interval 0.057 to 0.158 mmol/l) among quitters,[54] an increase in systolic blood pressure of 1.65 mm Hg and in diastolic blood pressure of 0.90 mm Hg after 6 months in quitters.[55, 56] Considering data from the CoLaus study, a study on cardiovascular risk factors in a random sample of the Lausanne population, we expect to only have a 10 to 40% chance to detect a difference between ENDS users and continuing smokers.[169] We expect to be able to have 100% power to detect changes in respiratory symptoms.[8, 170]

We do not expect to have adequate power to detect changes in incidence of cancer. However, we will apply strict open access rules and post the data on rigorous and systematic assessment of self-reported cancer in an online repository to enable the international community to pool results with other datasets and answer more definite answers on these important outcomes

Table 11.2-2: Power calculation and sample size required to detect statistically significant differences between past- and current smokers for secondary outcome measures

Measure	Matrix	Non smoker Mean (SD)	Past smoker Mean (SD)	Current smoker Mean (SD)	Reference	Power *	N **
Inhaled compounds							
1-OHP	Urine [µg/g creat]	0.08 (0.11)	0.11 (0.11)	0.2 (0.25)	Bartolomé et al. 2015[171]	98%	37
	Urine [µg/g creat]		0.04 (0.002)	0.12 (0.005)	Naufal et al. 2011[172]	100%	2
1-naphtol	Urine [ng/g creat]		1.6 (0.1)	7.2 (0.4)	Naufal et al. 2011[172]	100%	2
2-naphtol	Urine [ng/g creat]		1.8 (0.05)	8.9 (0.5)	Naufal et al. 2011[172]	100%	2
Oxydative stress							
8OHdG	Urine [ng/mg creat]	2.75 (1.6)	3.62 (2.41)	4.24 (3.3)	Sauvain et al., 2011[164]	48%	174
	EBC [ng/ml]	0.36 (0.09)	0.31 (0.10)	0.52 (0.15)	Doruk et al., 2011[91]	100%	3
8-isoprostane F2a	Urine [µg/g creat]	0.51 (0.04)	0.74 (0.07)	1.1 (0.1)	Harman et al., 2003[52]	100%	2
	Urine [µg/g creat]	0.7 (2.7)		4.48 (2.7)	Seet et al., 2011[43]	100%[t]	5
	EBC [pg/ml]	10.8 (0.8)		24.3 (2.6)	Montuschi et al., 2000[89]	100%[t]	2

*Power to detect statistically significant difference between quitters (past smokers) and continuing smokers (current smokers) between baseline and follow-up at alpha 0.05, an assumed correlation between baseline and follow-up of 0.7 and 182 participants (112 vs. 70) for urine analyses.

[t] Calculations above in * based on data from never smokers compared to current smokers.

** Number of participants per group needed to detect statistically significant differences at alpha of 0.05 and beta (power) of 0.80.

11.3 Statistical criteria of termination of trial

Not applicable as no formal interim analysis is planned.

11.4 Planned Analyses

The statistical analysis of the main results of the trial will be done at CTU Bern by a statistician blinded to the allocation. After the start of the trial but before recruitment ends, a statistical analysis plan will be written. The plan will determine all necessary data preparation steps (e.g. additional validations, generation of new variables), definitions (e.g. analysis sets), and statistical analyses (e.g. models, outputs such as tables and graphs).

All statistical analyses will be presented as effect measure plus 95% confidence interval. A significance level of 5% will be used.

11.4.1 Datasets to be analysed, analysis populations

All analyses will be done on the intention-to-treat principle set whereby all randomized patients will be analyzed in the allocated group. This means regardless of any protocol violations such as participants in the control group purchasing an ENDS on their own or early treatment discontinuations in the intervention group.

11.4.2 Primary Analysis

To answer the primary research question, we will compare the rates of quitting among smokers randomized to ENDS vs. those receiving usual care, on an intention-to-treat basis. Continuous smoking cessation from target quit date will be the main outcome, confirmed by biomarkers of exposure (anabasine). We will calculate quit rates, relative risks (RR), and absolute risks for ENDS +SOC (intervention group) compared to SOC alone (control group). We will further compare groups using multivariate regression models adjusting for appropriate co-variates. Participants who stopped smoking, and who are continuous ENDS users, will be considered quitters.

11.4.3 Secondary Analyses

We will repeat the calculations described in 11.4.2 for the secondary smoking cessation outcomes. To test the effect of ENDS on reducing the number of cigarettes smoked per day, we will define a binary predictor of >50% smoking reduction from baseline to follow-up smoking and repeat the analyses for the main outcome of quitting. We will also use joint multivariate random-effects (JMRE) models allowing to model CPD and smoking cessation. In addition, we will use mixed linear effect model to analyze the change in CPD modelled as a continuous outcomes over time.

We will present modified per-protocol sensitivity analyses, in particular for participants in the control group who might have started using ENDS in the first six months.

For the measures of changes in measures of urinary biomarkers, exhaled breath measures, CVRFs and respiratory symptoms and pulmonary function from baseline to the 6-months visit, we will first compare the changes in these measures between the intervention and control group using two-sample t-tests or Wilcoxon rank-sum test, whenever appropriate. We will then fit univariate and multivariate mixed linear effect models. We will use multivariate linear and multinomial logistic regression models to compare groups according to their exposure to tobacco smoking and ENDS.

11.4.4 Interim analyses

No formal interim analysis is planned. We do not anticipate undertaking formal interim analyses in this trial. In particular, even if a significantly large number of participants stop smoking in the intervention group, we will not stop the trial.

11.4.5 Safety analysis

We will compare the proportion of adverse events and serious adverse events between groups using recommended methods.

11.4.6 Deviation(s) from the original statistical plan

Deviations from the statistical analysis plan will be stated and justified in the final analysis report.

11.5 Handling of missing data and drop-outs

Participants lost during follow-up will be categorized as persistent smokers in the intention-to-treat analysis. We intend to obtain full follow-up data on all randomized participants. For missing data on the main and secondary outcomes, we will perform sensitivity analyses computing inverse probability of censoring weights (IPCWs) in the multivariate regression models, modeling the probability of missing data to weight the available measurement. Missing data on covariates will be handled using multiple imputation techniques.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

The Investigators will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. The Principal Investigator, Sub-investigator, and Clinical Research Nurses or Coordinators will have access to the records. The Principal Investigators will permit authorized representatives of the Sponsor and regulatory agencies as applicable to examine clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

12.1.1 Case Report Forms

The CRF will be electronic. All data requested on the eCRF will be recorded and the investigators will ensure the recorded data will be consistent with the source documents or the discrepancies will be explained. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported in the eCRF and all other required reports. Generally, the eCRF will be completed within two weeks of completion of a participant's visit/ follow-up phone call.

12.1.2 Specification of source documents

Source documents will be available at the site to document the existence of the study participants and will include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

For all data captured in the eCRF, the location of the source will be documented on a list of source documents (source data location list), which will be stored in the investigator site file at each study site.

If certain data are directly entered into the eCRF (and are thus considered as source data) this will be specified on the source data location list accordingly.

Any change or correction to source data will be dated, initialed, and explained (if necessary) and will not obscure the original entry.

12.1.3 Record keeping / archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. The Investigators will take measures to prevent accidental or premature destruction of these documents.

12.2 Data management

12.2.1 Data Management System

The CRFs in this trial will be implemented electronically using a dedicated electronic data capturing

(EDC) system (secuTrial). The EDC system will be activated for the trial only after successfully passing a formal test procedure conducted by the CTU Bern. All data entered in the CRFs will be stored on a Linux server in a dedicated Oracle database at the CTU Bern.

Responsibility for hosting the EDC system and the database will lie with Inselspital Bern.

12.2.2 Data security, access and back-up

The server hosting the EDC system and the database will be kept in a locked server-room. Only the system administrators will have direct access to the server. A role concept with personal passwords (site investigator, statistician, monitor, administrator etc.) will regulate permission for each user to use the system and database as he/she requires.

All data entered into the CRFs will be transferred to the database using Secure Sockets Layer (SSL) encryption. Each data point will have attributes attached to it identifying the user who entered it with the exact time and date. Retrospective alterations of data in the database will be recorded in an audit table. Time, table, data field and altered value, and the person will be recorded (audit trail). A multi-level back-up system will be implemented.

We will provide participants with the possibility of fillng some questionnaires online themselves. The online questionnaires will be filled by participants through SurveyMonkey. As a global organization, SurveyMonkey is subject to the General Data Protection Regulation (GDPR) in order to protect personal data. The data entered in the surveymonkey will be entered into the dedicated electronic data capturing System by the study nurses of the corresponding study site. No personal data such as name and address will be entered into SurveyMonkey instead the study participants ID will be used for identification.

12.2.3 Analysis and archiving

Final analyses and data files will be extracted from the database into statistical packages to be analyzed. The status of the database at this time will be recorded in special archive tables.

The study database with all archive tables will be securely stored by CTU and Inselspital Bern. The sponsor also will keep the Trial Master File and interim/final reports for at least 10 years.

12.2.4 Electronic and central data validation

Data will be checked by the EDC system for completeness and plausibility. Furthermore, selected data points will be cross-checked for plausibility with previously entered data for that participant. In addition, central data reviews will be performed on a regular basis to ensure completeness of the data collected and accuracy of the primary outcome data.

Before database lock the PI will validate the collected data with his signature.

12.3 Monitoring

For quality control of the study conduct and data retrieval, all study sites will be visited on-site by appropriately trained and qualified Monitors from the CTU Bern. Any findings and comments will be documented in site visit reports and communicated to the local Investigator and to the Sponsor as applicable. Investigators at the participating study sites will support the Monitor in his/ her activities. Prior to study start (first participant enrolled) a plan detailing all monitoring-related procedures will be developed.

All source data and relevant documents will be accessible to Monitors and questions of Monitors are answered during site visits.

12.4 Audits and Inspections

Source data/ documents will be made available to audits by the Sponsor or designee or to inspections by the CEC or regulatory authorities. If an inspection is requested, the Investigator will inform the Sponsor immediately that this request has been made. The Investigators at the participating sites will support the inspectors in their activities and will answer questions from inspectors as needed. All

involved parties will keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

The Investigator ensures anonymity of the patients; patients will not be identified by names in any study documents leaving the study site. Subject confidentiality will be ensured by utilizing subject identification codes consisting of three random letters and three random numbers, such as toj838. Signed informed consent forms and patient enrollment log will be kept strictly confidential to enable patient identification at the site.

12.6 Storage of biological material and related health data

The IST will be responsible for the storage of the full blood and urine samples. This will be stated in the contract between the IST and Sponsor. The time frame for sample storage will remain undefined. The samples are only stored and continued to be used with the participants consent independent from the study.

The blood samples that are analyzed for lipids, creatinine and Hb1Ac (for participants with diabetes only) in the study centers (University Hospitals Bern, Geneva,Lausanne, and St. Gallen) will remain in their responsibility. The other blood sample for the analyses of inflammatory biomarkers from the centers in St. Gallen and Bern will be transported and stored in the biobank in Bern. Blood samples from participants in Geneva are stored at the local biobank in Geneva. These samples are continued to be used for yet unknown purposes only with the participants consent independent from the study.

13. PUBLICATION AND DISSEMINATION POLICY

The trial protocol will be published in an open access journal. We will present study results at national and international conferences, and submit scientific articles to high impact, peer-reviewed journals; we will favor submission to Open Access journals.

When we complete the analysis, we will disseminate trial results to all participants.

We will report publicly through press releases and specific meetings of the ENDS user and tobacco cessation communities. No more than 6 months after publication of the trial data, anonymized datasets corresponding to each publication will be made freely available in an online data repository and in line with the Open Access (OA) rules of the SNSF.

14. FUNDING AND SUPPORT

14.1 Funding

This trial is financed by the Swiss National Science Foundation via the “Investigator-initiated clinical trials – IICT” grant # 173552.

The pulmonary function tests (conventional and MBW) in a subgroup of 150 participants in Bern are financed by Lungenliga Bern.

MRI measurements in a subgroup of 40 participants and in ten healthy volunteers are financed by the Clinical Trial Unit research Grant and Lungenliga Bern.

The extension of follow-up clinical visits after 12- and 24 months are financed by the Swiss Cancer Research # KFS4744-02-2019 and the Tobacco Prevention Fund TPF #19.017477

The analysis of inflammation biomarkers is financed by the Swiss Heart Foundation (FF20122)

14.2 Other Support

n.a.

15. INSURANCE

Insurance will be provided by the Sponsor.

16. REFERENCES

1. West R, Hajek P, Stead L and Stapleton J. Outcome criteria in smoking cessation trials: proposal for a common standard. *Addiction*. 2005;100:299-303.
2. Murray CJ and Lopez AD. Measuring the global burden of disease. *N Engl J Med*. 2013;369:448-57.
3. Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN, McAfee T and Peto R. 21st-century hazards of smoking and benefits of cessation in the United States. *N Engl J Med*. 2013;368:341-50.
4. Gmel G, Kuendig H, Notari L, Gmel C and Flury R. Monitorage suisse des addictions: Consommation d'alcool, de tabac et de drogues illégales en Suisse en 2012 2013.
5. Hartmann-Boyce J, McRobbie H, Bullen C, Begh R, Stead LF and Hajek P. Electronic cigarettes for smoking cessation. *Cochrane Database Syst Rev*. 2016;9:CD010216.
6. Farsalinos KE, Spyrou A, Tsimopoulou K, Stefopoulos C, Romagna G and Voudris V. Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Scientific reports*. 2014;4:4133.
7. Fagerstrom K, Etter JF and Unger JB. E-cigarettes: a disruptive technology that revolutionizes our field? *Nicotine Tob Res*. 2015;17:125-6.
8. Caponnetto P, Campagna D, Cibella F, Morjaria JB, Caruso M, Russo C and Polosa R. EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: a prospective 12-month randomized control design study. *PLoS One*. 2013;8:e66317.
9. Bullen C, Howe C, Laugesen M, McRobbie H, Parag V, Williman J and Walker N. Electronic cigarettes for smoking cessation: a randomised controlled trial. *Lancet*. 2013;382:1629-37.
10. Malas M, van der Tempel J, Schwartz R, Minichiello A, Lightfoot C, Noormohamed A, Andrews J, Zawertailo L and Ferrence R. Electronic Cigarettes for Smoking Cessation: A Systematic Review. *Nicotine & Tobacco Research*. 2016.
11. El Dib R, Suzumura EA, Akl EA, Gomaa H, Agarwal A, Chang Y, Prasad M, Ashoorion V, Heels-Ansdell D, Maziak W and Guyatt G. Electronic nicotine delivery systems and/or electronic non-nicotine delivery systems for tobacco smoking cessation or reduction: a systematic review and meta-analysis. *BMJ Open*. 2017;7:e012680.
12. Hajek P, Phillips-Waller A, Przulj D, Pesola F, Myers Smith K, Bisal N, Li J, Parrott S, Sasieni P, Dawkins L, Ross L, Goniewicz M, Wu Q and McRobbie HJ. A Randomized Trial of E-Cigarettes versus Nicotine-Replacement Therapy. *N Engl J Med*. 2019;380:629-637.
13. Fraser D, Borland R and Gartner C. Protocol for a randomised pragmatic policy trial of nicotine products for quitting or long-term substitution in smokers. *BMC Public Health*. 2015;15:1026.
14. McAuley TR, Hopke PK, Zhao J and Babaian S. Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. *Inhal Toxicol*. 2012;24:850-7.
15. Schober W, Szendrei K, Matzen W, Osiander-Fuchs H, Heitmann D, Schettgen T, Jorres RA and Fromme H. Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. *Int J Hyg Environ Health*. 2014;217:628-37.
16. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, Prokopowicz A, Jablonska-Czapla M, Rosik-Dulewska C, Havel C, Jacob P, 3rd and Benowitz N. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23:133-9.
17. Orr MS. Electronic cigarettes in the USA: a summary of available toxicology data and suggestions for the future. *Tob Control*. 2014;23 Suppl 2:ii18-22.
18. Walton KM, Abrams DB, Bailey WC, Clark D, Connolly GN, Djordjevic MV, Eissenberg TE, Fiore MC, Goniewicz ML, Haverkos L, Hecht SS, Henningfield JE, Hughes JR, Oncken CA, Postow L, Rose JE, Wanke KL, Yang L and Hatsukami DK. NIH electronic cigarette workshop: developing a research agenda. *Nicotine Tob Res*. 2015;17:259-69.
19. Bertholon JF, Becquemin MH, Roy M, Roy F, Ledur D, Annesi Maesano I and Dautzenberg B. [Comparison of the aerosol produced by electronic cigarettes with conventional cigarettes and the shisha]. *Rev Mal Respir*. 2013;30:752-7.
20. Etter JF and Bullen C. Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. *Addiction*. 2011;106:2017-28.
21. Etter JF, Zather E and Svensson S. Analysis of refill liquids for electronic cigarettes. *Addiction*. 2013;108:1671-9.
22. Flouris AD, Poulianiti KP, Chorti MS, Jamurtas AZ, Kouretas D, Owolabi EO, Tzatzarakis MN, Tsatsakis AM and Koutedakis Y. Acute effects of electronic and tobacco cigarette smoking on complete blood count. *Food Chem Toxicol*. 2012;50:3600-3.
23. Ingebrethsen BJ, Cole SK and Alderman SL. Electronic cigarette aerosol particle size distribution measurements. *Inhal Toxicol*. 2012;24:976-84.

24. Kirschner RI, Gerona R and Jacobitz KR. Nicotine content of liquid for electronic cigarettes. Paper presented at: 2013 Annual Meeting of the North American Congress of Clinical Toxicology (NACCT); 2013.
25. Pellegrino RM, Tinghino B, Mangiaracina G, Marani A, Vitali M, Protano C, Osborn JF and Cattaruzza MS. Electronic cigarettes: an evaluation of exposure to chemicals and fine particulate matter (PM). *Ann Ig*. 2012;24:279-88.
26. Romagna G, Zabarini L, Barbiero L, Boccietto E, Todeschi S, Caravati E, Voster D and Farsalinos K. Characterization of chemicals released to the environment by electronic cigarettes use (ClearStream-AIR project): is passive vaping a reality? *XIV Annual Meeting of the SRNT Europe 2012*. 2012;XIV Annual Meeting of the SRNT Europe 2012.
27. Trehy ML, Ye W, Hadwiger ME, Moore TW, Allgire JF, Woodruff JT, Ahadi SS, Black JC and Westenberger BJ. Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities. *Journal of Liquid Chromatography & Related Technologies*. 2011;34:1442-1458.
28. Uchiyama S, Ohta K, Inaba Y and Kunugita N. Determination of carbonyl compounds generated from the E-cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. *Anal Sci*. 2013;29:1219-22.
29. Feng S, Roethig HJ, Liang Q, Kinser R, Jin Y, Scherer G, Urban M, Engl J and Riedel K. Evaluation of urinary 1-hydroxypyrene, S-phenylmercapturic acid, trans,trans-muconic acid, 3-methyladenine, 3-ethyladenine, 8-hydroxy-2'-deoxyguanosine and thioethers as biomarkers of exposure to cigarette smoke. *Biomarkers*. 2006;11:28-52.
30. Xia B, Xia Y, Wong J, Nicodemus KJ, Xu M, Lee J, Guillot T and Li J. Quantitative analysis of five tobacco-specific N-nitrosamines in urine by liquid chromatography-atmospheric pressure ionization tandem mass spectrometry. *Biomed Chromatogr*. 2014;28:375-84.
31. Hu CW, Hsu YW, Chen JL, Tam LM and Chao MR. Direct analysis of tobacco-specific nitrosamine NNK and its metabolite NNAL in human urine by LC-MS/MS: evidence of linkage to methylated DNA lesions. *Arch Toxicol*. 2014;88:291-9.
32. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis*. 2002;23:907-22.
33. Lisko JG, Tran H, Stanfill SB, Blount BC and Watson CH. Chemical Composition and Evaluation of Nicotine, Tobacco Alkaloids, pH, and Selected Flavors in E-Cigarette Cartridges and Refill Solutions. *Nicotine Tob Res*. 2015;17:1270-8.
34. Suh-Lailam BB, Haglock-Adler CJ, Carlisle HJ, Ohman T and McMillin GA. Reference interval determination for anabasine: a biomarker of active tobacco use. *J Anal Toxicol*. 2014;38:416-20.
35. Jacob P, 3rd, Hatsukami D, Severson H, Hall S, Yu L and Benowitz NL. Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy. *Cancer Epidemiol Biomarkers Prev*. 2002;11:1668-73.
36. van der Vaart H, Postma DS, Timens W and ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax*. 2004;59:713-21.
37. Rahman I. Redox effects of cigarette smoke in lung inflammation. In: P. H. Halliwell B, ed. *Cigarette Smoke and Oxidative Stress* New York: Springer; 2006: 113-164.
38. Pryor WA and Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci*. 1993;686:12-27; discussion 27-8.
39. Yamaguchi Y, Nasu F, Harada A and Kunitomo M. Oxidants in the gas phase of cigarette smoke pass through the lung alveolar wall and raise systemic oxidative stress. *J Pharmacol Sci*. 2007;103:275-82.
40. Campos C, Guzman R, Lopez-Fernandez E and Casado A. Urinary biomarkers of oxidative/nitrosative stress in healthy smokers. *Inhal Toxicol*. 2011;23:148-56.
41. Lowe FJ, Gregg EO and McEwan M. Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never-smokers. *Clin Chem Lab Med*. 2009;47:311-20.
42. Haswell LE, Papadopoulou E, Newland N, Shepperd CJ and Lowe FJ. A cross-sectional analysis of candidate biomarkers of biological effect in smokers, never-smokers and ex-smokers. *Biomarkers*. 2014;19:356-67.
43. Seet RC, Lee CY, Loke WM, Huang SH, Huang H, Looi WF, Chew ES, Quek AM, Lim EC and Halliwell B. Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress? *Free Radic Biol Med*. 2011;50:1787-93.
44. Morrow JD and Roberts LJ, 2nd. The isoprostanes. Current knowledge and directions for future research. *Biochem Pharmacol*. 1996;51:1-9.
45. Basu S. F-2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications. *Antioxidants & Redox Signaling*. 2008;10:1405-1434.

46. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger V, Rosamond W, Sacco R, Sorlie P, Stafford R, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J, Committee obotAHAS and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics--2010 Update. A Report From the American Heart Association. *Circulation*. 2009:CIRCULATIONAHA.109.192667.
47. Lowe FJ, Luettich K and Gregg EO. Lung cancer biomarkers for the assessment of modified risk tobacco products: an oxidative stress perspective. *Biomarkers*. 2013;18:183-195.
48. Wu LL, Chiou CC, Chang PY and Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta*. 2004;339:1-9.
49. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K and Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*. 1992;13:2241-7.
50. Hakim IA, Harris R, Garland L, Cordova CA, Mikhael DM and Sherry Chow HH. Gender difference in systemic oxidative stress and antioxidant capacity in current and former heavy smokers. *Cancer Epidemiol Biomarkers Prev*. 2012;21:2193-200.
51. Prieme H. Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion. *Carcinogenesis*. 1998;19:347-351.
52. Harman SM, Liang L, Tsitouras PD, Gucciardo F, Heward CB, Reaven PD, Ping W, Ahmed A and Cutler RG. Urinary excretion of three nucleic acid oxidation adducts and isoprostane F(2)alpha measured by liquid chromatography-mass spectrometry in smokers, ex-smokers, and nonsmokers. *Free Radic Biol Med*. 2003;35:1301-9.
53. Farsalinos KE, Tsiapras D, Kyrzopoulos S, Savvopoulou M and Voudris V. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes. *BMC Cardiovasc Disord*. 2014;14:78.
54. Forey BA, Fry JS, Lee PN, Thornton AJ and Coombs KJ. The effect of quitting smoking on HDL-cholesterol - a review based on within-subject changes. *Biomark Res*. 2013;1:26.
55. Bartholomew HC and Knuiman MW. Longitudinal analysis of the effect of smoking cessation on cardiovascular risk factors in a community sample: the Busselton Study. *J Cardiovasc Risk*. 1998;5:263-71.
56. Gerace TA, Hollis J, Ockene JK and Svendsen K. Smoking cessation and change in diastolic blood pressure, body weight, and plasma lipids. MRFIT Research Group. *Prev Med*. 1991;20:602-20.
57. Aubin HJ, Farley A, Lycett D, Lahmek P and Aveyard P. Weight gain in smokers after quitting cigarettes: meta-analysis. *BMJ*. 2012;345:e4439.
58. Stein JH, Asthana A, Smith SS, Piper ME, Loh WY, Fiore MC and Baker TB. Smoking cessation and the risk of diabetes mellitus and impaired fasting glucose: three-year outcomes after a quit attempt. *PLoS One*. 2014;9:e98278.
59. Thun MJ, Carter BD, Feskanich D, Freedman ND, Prentice R, Lopez AD, Hartge P and Gapstur SM. 50-year trends in smoking-related mortality in the United States. *N Engl J Med*. 2013;368:351-64.
60. Woodruff PG, Barr RG, Bleecker E, Christenson SA, Couper D, Curtis JL, Gouskova NA, Hansel NN, Hoffman EA, Kanner RE, Kleerup E, Lazarus SC, Martinez FJ, Paine R, 3rd, Rennard S, Tashkin DP, Han MK and Group SR. Clinical Significance of Symptoms in Smokers with Preserved Pulmonary Function. *N Engl J Med*. 2016;374:1811-21.
61. Flouris AD, Chorti MS, Poulianiti KP, Jamurtas AZ, Kostikas K, Tzatzarakis MN, Wallace Hayes A, Tsatsakis AM and Koutedakis Y. Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. *Inhal Toxicol*. 2013;25:91-101.
62. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN and Behrakis PK. Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest*. 2012;141:1400-6.
63. Palamidas A, Gennimata S, Kaltsakas G, Tsikrika S, Vakali S and Gratziou C. Acute effect of an e-cigarette with and without nicotine on lung function. Poster P1054. Paper presented at: European Respiratory Society's Annual Congress; 2013.
64. Lim HB and Kim SH. Inhallation of e-Cigarette Cartridge Solution Aggravates Allergen-induced Airway Inflammation and Hyper-responsiveness in Mice. *Toxicol Res*. 2014;30:13-8.
65. Patel AB and Verma A. COVID-19 and Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers: What Is the Evidence? *JAMA*. 2020.
66. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, Liu L, Shan H, Lei C-l, Hui DSC, Du B, Li L-j, Zeng G, Yuen K-Y, Chen R-c, Tang C-l, Wang T, Chen P-y, Xiang J, Li S-y, Wang J-l, Liang Z-j, Peng Y-x, Wei L, Liu Y, Hu Y-h, Peng P, Wang J-m, Liu J-y, Chen Z, Li G, Zheng Z-j, Qiu S-q, Luo J,

Ye C-j, Zhu S-y and Zhong N-s. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*. 2020.
67. Liu W, Tao Z-W, Lei W, Ming-Li Y, Kui L, Ling Z, Shuang W, Yan D, Jing L, Liu H-G, Ming Y and Yi H. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chinese Medical Journal*. 2020;Publish Ahead of Print.
68. Godoy P, Castilla J, Soldevila N, Mayoral JM, Toledo D, Martin V, Astray J, Egurrola M, Morales-Suarez-Varela M, Dominguez A, Cases C and Controls in Pandemic Influenza Working Group S. Smoking may increase the risk of influenza hospitalization and reduce influenza vaccine effectiveness in the elderly. *Eur J Public Health*. 2018;28:150-155.
69. Kaur G, Pinkston R, McLemore B, Dorsey WC and Batra S. Immunological and toxicological risk assessment of e-cigarettes. *Eur Respir Rev*. 2018;27.
70. Oakes JM, Fuchs RM, Gardner JD, Lazartigues E and Yue X. Nicotine and the renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol*. 2018;315:R895-R906.
71. Cai G. Tobacco-Use Disparity in Gene Expression of ACE2, the Receptor of 2019-nCov. *Preprints*. 2020:2020020051.
72. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, Fry RC and Jaspers I. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. *Am J Physiol Lung Cell Mol Physiol*. 2016;311:L135-44.
73. Clapp PW, Pawlak EA, Lackey JT, Keating JE, Reeber SL, Glish GL and Jaspers I. Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. *Am J Physiol Lung Cell Mol Physiol*. 2017;313:L278-L292.
74. Cravo AS, Bush J, Sharma G, Savioz R, Martin C, Craige S and Walele T. A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks. *Regul Toxicol Pharmacol*. 2016;81 Suppl 1:S1-S14.
75. Polosa R, Morjaria JB, Caponnetto P, Caruso M, Campagna D, Amaradio MD, Ciampi G, Russo C and Fisichella A. Persisting long term benefits of smoking abstinence and reduction in asthmatic smokers who have switched to electronic cigarettes. *Discov Med*. 2016;21:99-108.
76. Subbarao P, Milla C, Aurora P, Davies JC, Davis SD, Hall GL, Heltshe S, Latzin P, Lindblad A, Pittman JE, Robinson PD, Rosenfeld M, Singer F, Starner TD, Ratjen F and Morgan W. Multiple-Breath Washout as a Lung Function Test in Cystic Fibrosis. A Cystic Fibrosis Foundation Workshop Report. *Ann Am Thorac Soc*. 2015;12:932-9.
77. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR and Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J*. 2013;41:507-22.
78. Usemann J, Yammine S, Singer F and Latzin P. Inert gas washout: background and application in various lung diseases. *Swiss Med Wkly*. 2017;147:w14483.
79. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, Salome CM and King GG. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. *Respirology*. 2018;23:512-518.
80. Verbanck S, Schuermans D, Meysman M, Paiva M and Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. *Am J Respir Crit Care Med*. 2004;170:414-9.
81. Verbanck S, Schuermans D, Paiva M, Meysman M and Vincken W. Small airway function improvement after smoking cessation in smokers without airway obstruction. *Am J Respir Crit Care Med*. 2006;174:853-7.
82. Bauman G, Lutzen U, Ullrich M, Gaass T, Dinkel J, Elke G, Meybohm P, Frerichs I, Hoffmann B, Borggrefe J, Knuth HC, Schupp J, Prum H, Eichinger M, Puderbach M, Biederer J and Hintze C. Pulmonary functional imaging: qualitative comparison of Fourier decomposition MR imaging with SPECT/CT in porcine lung. *Radiology*. 2011;260:551-9.
83. Bauman G, Puderbach M, Heimann T, Kopp-Schneider A, Fritzsching E, Mall MA and Eichinger M. Validation of Fourier decomposition MRI with dynamic contrast-enhanced MRI using visual and automated scoring of pulmonary perfusion in young cystic fibrosis patients. *Eur J Radiol*. 2013;82:2371-7.
84. Sommer G, Bauman G, Koenigkam-Santos M, Draenkow C, Heussel CP, Kauczor HU, Schlemmer HP and Puderbach M. Non-contrast-enhanced preoperative assessment of lung perfusion in patients with non-small-cell lung cancer using Fourier decomposition magnetic resonance imaging. *Eur J Radiol*. 2013;82:e879-87.

85. Bauman G and Bieri O. Matrix pencil decomposition of time-resolved proton MRI for robust and improved assessment of pulmonary ventilation and perfusion. *Magn Reson Med*. 2017;77:336-342.
86. Nyilas S, Bauman G, Sommer G, Stranzinger E, Pusterla O, Frey U, Korten I, Singer F, Casaulta C, Bieri O and Latzin P. Novel magnetic resonance technique for functional imaging of cystic fibrosis lung disease. *Eur Respir J*. 2017;50.
87. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jobsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, Wirtz H and Condensate AETFoEB. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*. 2005;26:523-48.
88. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G and Wouters EF. Systemic effects of smoking. *Chest*. 2007;131:1557-66.
89. Montuschi P. Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. *Ther Adv Respir Dis*. 2007;1:5-23.
90. Syslova K, Kacer P, Kuzma M, Pankracova A, Fenclova Z, Vlckova S, Lebedova J and Pelclova D. LC-ESI-MS/MS method for oxidative stress multimarker screening in the exhaled breath condensate of asbestosis/silicosis patients. *J Breath Res*. 2010;4:017104.
91. Doruk S, Ozyurt H, Inonu H, Erkorkmaz U, Saylan O and Seyfikli Z. Oxidative status in the lungs associated with tobacco smoke exposure. *Clin Chem Lab Med*. 2011;49:2007-12.
92. Bloching M, Hofmann A, Lautenschlager C, Berghaus A and Grummt T. Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral Oncol*. 2000;36:550-5.
93. Casartelli G, Bonatti S, De Ferrari M, Scala M, Mereu P, Margarino G and Abbondandolo A. Micronucleus frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. *Analytical and quantitative cytology and histology*. 2000;22:486-92.
94. Franco T, Trapasso S, Puzzo L and Allegra E. Electronic Cigarette: Role in the Primary Prevention of Oral Cavity Cancer. *Clin Med Insights Ear Nose Throat*. 2016;9:7-12.
95. Nersesyan A, Muradyan R, Kundi M and Knasmueller S. Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. *Mutagenesis*. 2011;26:295-301.
96. El-Setouhy M, Loffredo CA, Radwan G, Abdel Rahman R, Mahfouz E, Israel E, Mohamed MK and Ayyad SB. Genotoxic effects of waterpipe smoking on the buccal mucosa cells. *Mutat Res*. 2008;655:36-40.
97. Tolbert PE, Shy CM and Allen JW. Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users. *Am J Epidemiol*. 1991;134:840-50.
98. Bolognesi C, Knasmueller S, Nersesyan A, Thomas P and Fenech M. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay - an update and expanded photogallery. *Mutat Res*. 2013;753:100-13.
99. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, Holland N, Kirsh-Volders M, Knasmueller S, Zeiger E, Carnesoltas D, Cavallo D, da Silva J, de Andrade VM, Demircigil GC, Dominguez Odio A, Donmez-Altuntas H, Gattas G, Giri A, Giri S, Gomez-Meda B, Gomez-Arroyo S, Hadjidekova V, Haveric A, Kamboj M, Kurteshi K, Martino-Roth MG, Montero Montoya R, Nersesyan A, Pastor-Benito S, Favero Salvadori DM, Shaposhnikova A, Stopper H, Thomas P, Torres-Bugarin O, Yadav AS, Zuniga Gonzalez G and Fenech M. The HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutat Res*. 2011;728:88-97.
100. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S and Fenech M. Buccal micronucleus cytome assay. *Nature protocols*. 2009;4:825-37.
101. Cheng KW, Shang C, Lee HM, Chaloupka FJ, Fong GT, Borland R, Heckman BW, Hitchman SC, O'Connor RJ, Levy DT and Cummings KM. Costs of vaping: evidence from ITC Four Country Smoking and Vaping Survey. *Tob Control*. 2020.
102. Thompson ME, Fong GT, Boudreau C, Driezen P, Li G, Gravely S, Cummings KM, Heckman BW, O'Connor R, Thrasher JF, Nahhas G, Borland R, Yong HH, McNeill A, Hitchman SC and Quah ACK. Methods of the ITC Four Country Smoking and Vaping Survey, wave 1 (2016). *Addiction*. 2019;114 Suppl 1:6-14.
103. Cheng K-W, Chaloupka FJ, Shang C, Ngo A, Fong GT, Borland R, Heckman BW, Levy DT and Cummings KM. Prices, use restrictions and electronic cigarette use—evidence from wave 1 (2016) US data of the ITC Four Country Smoking and Vaping Survey. *Addiction*. 2019;114:115-122.
104. Jackson SE, Shahab L, Kock L, West R and Brown J. Expenditure on smoking and alternative nicotine delivery products: a population survey in England. *Addiction*. 2019;114:2026-2036.

105. Ajmani GS, Suh HH, Wroblewski KE and Pinto JM. Smoking and olfactory dysfunction: A systematic literature review and meta-analysis. *Laryngoscope*. 2017;127:1753-1761.
106. Frye RE, Schwartz BS and Doty RL. Dose-related effects of cigarette smoking on olfactory function. *JAMA*. 1990;263:1233-6.
107. Siegel JK, Wroblewski KE, McClintock MK and Pinto JM. Olfactory dysfunction persists after smoking cessation and signals increased cardiovascular risk. *Int Forum Allergy Rhinol*. 2019.
108. Vennemann MM, Hummel T and Berger K. The association between smoking and smell and taste impairment in the general population. *J Neurol*. 2008;255:1121-6.
109. Dinc AS, Sengezer T, Cayonu M and Sahin MM. Smoking cessation improves olfactory functions. *Laryngoscope*. 2019.
110. Rumeau C, Nguyen DT and Jankowski R. How to assess olfactory performance with the Sniffin' Sticks test((R)). *Eur Ann Otorhinolaryngol Head Neck Dis*. 2016;133:203-6.
111. Hummel T, Sekinger B, Wolf SR, Pauli E and Kobal G. 'Sniffin' Sticks': Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem Senses*. 1997;22:39-52.
112. Hummel T, Kobal G, Gudziol H and Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur Arch Oto-Rhino-L*. 2007;264:237-243.
113. Farsalinos KE and Gillman G. Carbonyl Emissions in E-cigarette Aerosol: A Systematic Review and Methodological Considerations. *Front Physiol*. 2017;8:1119.
114. Farsalinos KE, Voudris V and Poulas K. E-cigarettes generate high levels of aldehydes only in 'dry puff' conditions. *Addiction*. 2015;110:1352-6.
115. Crotty Alexander LE, Shin S and Hwang JH. Inflammatory Diseases of the Lung Induced by Conventional Cigarette Smoke: A Review. *Chest*. 2015;148:1307-1322.
116. Shields PG, Berman M, Brasky TM, Freudenheim JL, Mathe E, McElroy JP, Song MA and Wewers MD. A Review of Pulmonary Toxicity of Electronic Cigarettes in the Context of Smoking: A Focus on Inflammation. *Cancer Epidemiol Biomarkers Prev*. 2017;26:1175-1191.
117. Zeller M, Hatsukami D and Strategic Dialogue on Tobacco Harm Reduction G. The Strategic Dialogue on Tobacco Harm Reduction: a vision and blueprint for action in the US. *Tob Control*. 2009;18:324-32.
118. www. olink.com.
119. Baelum V, van Palenstein Helderman W, Hugoson A, Yee R and Fejerskov O. A global perspective on changes in the burden of caries and periodontitis: implications for dentistry. *J Oral Rehabil*. 2007;34:872-906; discussion 940.
120. Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ and Marcenes W. Global burden of untreated caries: a systematic review and metaregression. *J Dent Res*. 2015;94:650-8.
121. Chapple IL, Bouchard P, Cagetti MG, Campus G, Carra MC, Cocco F, Nibali L, Hujoel P, Laine ML, Lingstrom P, Manton DJ, Montero E, Pitts N, Range H, Schlueter N, Teughels W, Twetman S, Van Loveren C, Van der Weijden F, Vieira AR and Schulte AG. Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J Clin Periodontol*. 2017;44 Suppl 18:S39-S51.
122. Zhang J, Leung KCM, Sardana D, Wong MCM and Lo ECM. Risk predictors of dental root caries: A systematic review. *J Dent*. 2019;89:103166.
123. Salzer S, Alkilzy M, Slot DE, Dorfer CE, Schmoeckel J, Splieth CH, Chairs of Working G and Orca. Socio-behavioural aspects in the prevention and control of dental caries and periodontal diseases at an individual and population level. *J Clin Periodontol*. 2017;44 Suppl 18:S106-S115.
124. Leite FRM, Nascimento GG, Scheutz F and Lopez R. Effect of Smoking on Periodontitis: A Systematic Review and Meta-regression. *Am J Prev Med*. 2018;54:831-841.
125. Sadri G and Mahjub H. Tobacco smoking and oral cancer: a meta-analysis. *J Res Health Sci*. 2007;7:18-23.
126. Blaser J and Cornuz J. Experts' consensus on use of electronic cigarettes: a Delphi survey from Switzerland. *BMJ Open*. 2015;5:e007197.
127. Wagener TL, Floyd EL, Stepanov I, Driskill LM, Frank SG, Meier E, Leavens EL, Tackett AP, Molina N and Queimado L. Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users. *Tob Control*. 2016.
128. Etter JF. A longitudinal study of cotinine in long-term daily users of e-cigarettes. *Drug Alcohol Depend*. 2016;160:218-21.
129. Cornuz J, Jacot-Sadowski I, Humair J, Clair C, Schuurmans M and Zellweger J. Désaccoutumance au tabac: mise à jour 2011 2ème partie. *Swiss Med Forum*. 2011;11:172-6.

130. Cornuz J, Jacot-Sadowski I, Humair J, Clair C, Schuurmans M and Zellweger J. Désaccoutumance au tabac: mise à jour 2011 1ère partie. *Swiss Med Forum*. 2011;11:156-9.
131. Frasch HF and Barbero AM. In vitro human epidermal permeation of nicotine from electronic cigarette refill liquids and implications for dermal exposure assessment. *Journal Of Exposure Science And Environmental Epidemiology*. 2016;27:618.
132. Bize R, Willi C, Chiolero A, Stoianov R, Payot S, Locatelli I and Cornuz J. Participation in a population-based physical activity programme as an aid for smoking cessation: a randomised trial. *Tob Control*. 2010;19:488-94.
133. Rodondi N, Collet TH, Nanchen D, Locatelli I, Depairon M, Aujesky D, Bovet P and Cornuz J. Impact of carotid plaque screening on smoking cessation and other cardiovascular risk factors: a randomized controlled trial. *Arch Intern Med*. 2012;172:344-52.
134. Assessment Test; 2016; http://www.catestonline.org/.
135. Mahler DA and Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest*. 1988;93:580-6.
136. Control Test; http://www.asthmacontroltest.com/Europe/Switzerland/German/adult/start#.
137. European Community Respiratory Health Survey; http://www.ecrhs.org/.
138. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF and Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35:1381-95.
139. Hughes JR. Tobacco withdrawal in self-quitters. *J Consult Clin Psychol*. 1992;60:689-97.
140. Hughes JR and Hatsukami D. Signs and symptoms of tobacco withdrawal. *Arch Gen Psychiatry*. 1986;43:289-94.
141. Pletcher MJ, Varosy P, Kiefe CI, Lewis CE, Sidney S and Hulley SB. Alcohol consumption, binge drinking, and early coronary calcification: findings from the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Am J Epidemiol*. 2005;161:423-33.
142. Auer R, Vittinghoff E, Yaffe K, Künzi A, Kertesz S, Levin D, Albanese E, Whitmer R, Jacobs D, Sidney S and Pletcher M. Association between Lifetime Marijuana use and Cognitive Function in Middle Age: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *JAMA-Internal Medicine*. 2016;In Press.
143. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR and Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989;28:193-213.
144. Perneger TV, Combescure C and Courvoisier DS. General population reference values for the French version of the EuroQol EQ-5D health utility instrument. *Value Health*. 2010;13:631-5.
145. Kroenke K, Spitzer RL and Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16:606-13.
146. Spitzer RL, Kroenke K, Williams JB and Lowe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006;166:1092-7.
147. Leuenberger P, Schwartz J, Ackermann-Liebrich U, Blaser K, Bolognini G, Bongard JP, Brandli O, Braun P, Bron C, Brutsche M and et al. Passive smoking exposure in adults and chronic respiratory symptoms (SAPALDIA Study). Swiss Study on Air Pollution and Lung Diseases in Adults, SAPALDIA Team. *Am J Respir Crit Care Med*. 1994;150:1222-8.
148. Kunzli N, Schwartz J, Stutz EZ, Ackermann-Liebrich U and Leuenberger P. Association of environmental tobacco smoke at work and forced expiratory lung function among never smoking asthmatics and non-asthmatics. The SAPALDIA-Team. Swiss Study on Air Pollution and Lung Disease in Adults. *Soz Praventivmed*. 2000;45:208-17.
149. International Conference on Harmonisation Working Group. ICH harmonised tripartite guideline: guideline for good clinical practice E6 (R1). *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*. 1996;10.
150. Auer R, Gencer B, Tango R, Nanchen D, Matter CM, Luscher TF, Windecker S, Mach F, Cornuz J, Humair JP and Rodondi N. Uptake and efficacy of a systematic intensive smoking cessation intervention using motivational interviewing for smokers hospitalised for an acute coronary syndrome: a multicentre before-after study with parallel group comparisons. *BMJ Open*. 2016;6:e011520.
151. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF and Wanger J. Interpretative strategies for lung function tests. *Eur Respir J*. 2005;26:948-68.
152. Aho K, Gordin A, Palosuo T, Punsar S, Valkeila E, Karvonen M, Inkovaara J and Pasternack A. Thyroid autoimmunity and cardiovascular diseases. *Eur Heart J*. 1984;5:43-6.
153. WHO. Guidelines for controlling and monitoring the tobacco epidemic. Geneva, Switzerland: World Health Organization. 1998.

154. Hummel T, Konnerth CG, Rosenheim K and Kobal G. Screening of olfactory function with a four-minute odor identification test: reliability, normative data, and investigations in patients with olfactory loss. *Ann Otol Rhinol Laryngol*. 2001;110:976-81.
155. Cornuz J, Humair JP, Seematter L, Stoianov R, van Melle G, Stalder H and Pecoud A. Efficacy of resident training in smoking cessation: a randomized, controlled trial of a program based on application of behavioral theory and practice with standardized patients. *Ann Intern Med*. 2002;136:429-37.
156. Cornuz J, Zellweger JP, Mounoud C, Decrey H, Pecoud A and Burnand B. Smoking cessation counseling by residents in an outpatient clinic. *Prev Med*. 1997;26:292-6.
157. Heatherton TF, Kozlowski LT, Frecker RC and Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict*. 1991;86:1119-27.
158. West R and Hajek P. Evaluation of the mood and physical symptoms scale (MPSS) to assess cigarette withdrawal. *Psychopharmacology (Berl)*. 2004;177:195-9.
159. Bush K, Kivlahan DR, McDonell MB, Fihn SD and Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. *Arch Intern Med*. 1998;158:1789-95.
160. Radloff LS. The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measurement*. 1977;1:385-401.
161. International Caries Detection and Assessment System; https://www.iccms-web.com/content/icdas.
162. The Periodontal Screening and Recording Index (PSR); https://psr.uaperio.org/en/manual.
163. Hughes JR, Carpenter MJ and Naud S. Do point prevalence and prolonged abstinence measures produce similar results in smoking cessation studies? A systematic review. *Nicotine Tob Res*. 2010;12:756-62.
164. Sauvain JJ, Setyan A, Wild P, Tacchini P, Lagger G, Storti F, Deslarzes S, Guillemin M, Rossi MJ and Riediker M. Biomarkers of oxidative stress and its association with the urinary reducing capacity in bus maintenance workers. *J Occup Med Toxicol*. 2011;6:18.
165. Liang Y, Wei P, Duke RW, Reaven PD, Harman SM, Cutler RG and Heward CB. Quantification of 8-iso-prostaglandin-F2α and 2,3-dinor-8-iso-prostaglandin-F2α in human urine using liquid chromatography-tandem mass spectrometry. *Free Radical Biology and Medicine*. 2003;34:409-418.
166. Nersesyan A, Parsadanyan G, Zalinyan G and Chobanyan N. Cytogenetic Biomonitoring in Buccal Mucosa Cells of Young Smokers. *Acta cytologica*. 2017;61:87-88.
167. Bolognesi C and Fenech M. Micronucleus assay in human cells: lymphocytes and buccal cells. *Methods Mol Biol*. 2013;1044:191-207.
168. Fenech M, Holland N, Zeiger E, Chang WP, Burgaz S, Thomas P, Bolognesi C, Knasmueller S, Kirsch-Volders M and Bonassi S. The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells--past, present and future. *Mutagenesis*. 2011;26:239-45.
169. Le Boudec J, Marques-Vidal P, Cornuz J and Clair C. Smoking cessation and the incidence of pre-diabetes and type 2 diabetes: a cohort study. *J Diabetes Complications*. 2016;30:43-8.
170. Rajkumar S, Stolz D, Hammer J, Moeller A, Bauer GF, Huynh CK and Roosli M. Effect of a smoking ban on respiratory health in nonsmoking hospitality workers: a prospective cohort study. *J Occup Environ Med*. 2014;56:e86-91.
171. Bartolome M, Ramos JJ, Cutanda F, Huetos O, Esteban M, Ruiz-Moraga M, Calvo E, Perez-Gomez B, Gonzalez O, Bioambient.Es and Castano A. Urinary polycyclic aromatic hydrocarbon metabolites levels in a representative sample of the Spanish adult population: The BIOAMBIENT.ES project. *Chemosphere*. 2015;135:436-46.
172. Naufal ZS, Marano KM, Kathman SJ and Wilson CL. Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999-2008. *Biomarkers*. 2011;16:222-35.

17. APPENDICES

17.1 Certifications and specifications of e-liquids

Ingredients and concentrations

Vegetable Glycerin
Also called glycerol, vegetable glycerin is a non-toxic, viscous, colorless and odorless liquid which tastes sweet. Used in the pharmaceutical, cosmetic and food industries, also generates the dense, full steam produced by e-vaporisers.

Propylene-Glycol
Propylene-glycol is a slightly viscous, little volatile, colorless and practically odorless liquid. Commonly used as a preservative in the pharmaceutical, cosmetic and food industries and for body hygiene products, propylene-glycol fills two major functions in e-liquids: it generates a light, fine vapor at low temperature and serves as a flavour enhancer by very faithfully reproducing the authentic aroma of the flavour.

Nicotine
The purpose behind using nicotine is to create the throat hit sensation which corresponds to the contraction of the larynx when the vapour is inhaled. It is added in products made for smokers and ex-smokers.

Flavoring
The flavouring is what gives the e-liquid its individual flavour. It can be made up of natural or synthetic flavourings or a mixture of both. It reproduces the required flavour as faithfully as possible: fruit, drink, sweet or complex cocktail. Flavouring represents approximately 2 to 10% of an e-liquid.

See attachement “GAIATREND_eliquids_ingredients”

Children safety of e-liquids

See attachement “GAIATREND_eliquids_childrenSafety”

Certification of nicotine supplier

See attachement “GAIATREND_eliquids_nicotine”

Technical reports of flavours

See attachement “GAIATREND_eliquids_technicalReport_FR-M”

See attachement “GAIATREND_eliquids_technicalReport_FR-4”

See attachement “GAIATREND_eliquids_technicalReport_MENTHE FRAICHE”

See attachement “GAIATREND_eliquids_technicalReport_FRAMBOISE_2”

See attachement “GAIATREND_eliquids_technicalReport_ FRUITS ROUGES”

See attachement “GAIATREND_eliquids_technicalReport_POMME VERTE”

History of changes to protocol approved by central ethics commission (CEC)

Date	Event	Comment
April 27th, 2018	First approved protocol, approval by CEC	Protocol version 2.0, Dated March 12th, 2018
June 6th, 2018	Approval of changes by CEC: 1. Due to a recent change of the Swiss law, the handling of the e-liquids was changed. 2. Addition of "Raspberry" aroma 3. Start of recruitment set to June 2018	Protocol version 3.0, Dated May 9th, 2018
June 21st, 2018	Approval of changes: 1. Change from principal-investigator (PI) to sponsor and coordinating person	Protocol version 4.0, Dated June 12th, 2018. Administrative change of role of Prof. Dr. med Reto Auer from Sponsor-investigator to Sponsor and coordinating person.
August 21st, 2018	Approval of study site Geneva	Protocol version 4.0, Dated June 12th, 2018. Study site Geneva allowed to recruit in addition to study site Bern.
December 18th, 2018	Approval of study site Lausanne Approval of changes: 1. Primary outcome specified 2. Change of PI at study site in Lausanne 3. Addition of e-liquid nicotine concentration: 0mg/ml	Protocol version 5.0, Dated October 30th, 2018. Study site Lausanne allowed to recruit in addition to study site Bern and Geneva. Primary outcome validation was changed from CO level and urinary level of cotinine, anabasine and NNAL to urinary level of anabasine and CO for consistency.
June 27th, 2019	Approval of changes: 1. Addition of "Apple" aroma 2. Systematic questions on antibiotics prescriptions and reasons for prescriptions 3. Adding preference scale	Protocol version 6.0, Dated May 9th, 2019. 1. During consultations, some participants asked for "Apple" aroma in addition to the other aromas provided. We added this aroma to ensure adherence to the e-liquids provided by the study nurses. 2. The publication of a large trial testing efficacy of ENDS for smoking cessation suggested a trend towards more respiratory infections. We added a question at follow-up visits to further detect AE related to respiratory infections.
October 10th, 2019 / November 26th 2019	Approval of changes: 1. Study site Zürich (Nov 26th) and St-Gallen (Oct 10th). 2. Extension of follow-up to 12- and 24-monts. 3. Vouchers to control group given at baseline instead of giving them the voucher after completion of the 6-months visit. 4. Adaptations of documentation of Adverse Events	Protocol version 7.0, Dated September 10th, 2019. 1. Extension of recruitment to study site Zürich and St-Gallen to meet recruitment targets. 2. Additional funding obtained by Tobacco Prevention Fund and Swiss Cancer Research enabling extension of follow-up to 12- and 24-months follow-up 3. We noticed selective attrition through withdrawal in the control group. We decided the control group should receive a voucher at baseline instead of 6-months follow-up to mitigate deception in control group. 4. Further adaptations in documentation of AE by study nurses with systematic queries to hospitals and attending physicians in case of AE and not only SAE
October 11th, 2019	Information to CEC related to EVALI epidemic in USA: 1. Letter sent to all participants asking them to report any respiratory symptoms	1. In light of the reports in the USA about EVALI cases, the research team reviewed already collected SAE and AE, reviewed the literature and reports coming out of the USA and decided to pursue the study as planned. We sent a letter to participants informing them about the situation in the USA, which was most likely due to use of illicit e-liquids or e-liquids used for vaping cannabis and that they should continue to report adverse events to study nurses. 2.
March 2020, until July 2020	Incremental lockdown measures related to COVID pandemic decided by Federal Council of Switzerland. 1. Pause in recruitment of new participants from: March to July 2020 2. Shift as much as possible clinical follow-up visit from in-person visits to phone follow-ups.	1. COVID pandemic required us to pause recruitment of new participants. 2. Study nurses continued to follow-up participants at scheduled times over the phone, following up participants in person if allowed locally.
July 14th, 2020	Approval of changes: 1. Set up of Data and Safety monitoring Board (DSMB) to review SAE and AE collection processes	Protocol version 8.0, Dated May 7th, 2020. We initially did not plan a DSMB and had no stopping rules for the study set. We still set up a DSMB to have international independent experts review the method of data collection of SAE and AE in place and review the already collected AE and SAE.

	2. Set up of an adjudication committee	We set up an adjudication committee of independent experts to review SAEs and AEs of interest.
September 9th, 2021	Approval of changes: 1. Change of the role of Prof. Dr. med. Reto Auer from Sponsor and coordinating person to Sponsor-investigator.	Protocol version 9.0, Dated July 23rd, 2021. Administrative change of role of Prof. Dr. med Reto Auer from Sponsor and coordinating person to Sponsor-Investigator. Prof. Auer took over the role of PI at the study site in Lausanne.

UNIVERSITÄT BERN
Faculty of Medicine
CTU Bern

Statistical Analysis Plan (SAP)

Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

ESTxENDS

Administrative Information

Project number:	746
Trial registration number:	Swiss National Science Foundation # 173552 Clinicaltrials.gov: NCT03589989 (primary outcome). Further outcomes are registered separately, but not part of this SAP.
SAP version:	1.0 of 2020-06-05
Protocol version:	8.0; May 7th, 2020

Contributors

Name	Affiliation	Role in SAP writing
Lenz Armando	CTU Bern	Author
Limacher Andreas	CTU Bern	Reviewer
Odile Stalder	CTU Bern	Co-Author
Reto Auer	BIHAM	Reviewer
Anna Schöni	BIHAM	Reviewer
Cindy Groen	BIHAM	Reviewer

Revision history

Revision	Justification	Timing

Approved by

Name	Affiliation	Study Role	Date and Signature (wet ink)
Odile Stalder	CTU Bern	Trial Statistician	
Limacher Andreas	CTU Bern	Senior Statistician	
Auer Reto	BIHAM	Sponsor / Coordinating person	

Contents

1. Introduction

1.1 Background and rationale

Cigarette smoking is the leading cause of preventable death in Switzerland. Recently, electronic nicotine delivery systems (ENDS or vaporizers, also called e-cigarettes) have become popular with smokers who want to switch from tobacco cigarettes to ENDS to reduce their exposure to toxic compounds or to stop smoking. Only three rigorous RCTs have been published so far. They have promising, yet inconclusive results, as they were based on small samples. The safety and potential adverse effects of ENDS are also largely unknown. While the aerosol the users inhale appears safe in laboratory conditions, the difference in exposure to toxins (such as measures of exposure to organic compounds) and effect of toxins on the body (measures of oxidative stress) between smokers who quit (with or without ENDS) and those who use ENDS for a long time have not yet been assessed in an RCT.

1.2 Objectives

Test ENDS on:
1) the efficacy for cigarette smoking cessation and in reducing the number of cigarettes smoked over 6 months of follow-up;
2) the safety of ENDS
3) the effect of ENDS on exposure to inhaled toxic compounds;
4) the effect of ENDS on health-related outcomes (such as blood pressure, weight and respiratory symptoms).

2. Study methods

2.1 Trial design

The ESTxENDS trial is a multicentre, pragmatic, open-label randomized controlled trial with two parallel groups allocated in a 1:1 ratio. The trial will assess superiority of Electronic Nicotine Delivery Systems (ENDS) + standard of care (SOC) for smoking cessation to SOC alone in adult (≥18 years) smokers. The intervention group will receive free ENDS and nicotine-containing e-liquids to use ad libitum, plus SOC. The control group will receive SOC only.

2.2 Randomization

Simple randomization in a 1:1 ratio to the two arms.

2.3 Sample size

Details on the sample size calculation can be found in the protocol in section 11.2. In short, the sample size is based on the primary outcome, that providing smokers with ENDS and SOC leads to a higher rate of smokers who quit than usual care. Sample size was calculated for a 2-sided alpha of 0.05 and a power of 90%. Accounting for 5% drop-out, the sample size of the trial was defined to be 1172 smokers, 586 in both groups.

2.4 Framework

The trial will assess superiority of ENDS + SOC for smoking cessation to SOC alone in adult (≥18 years) smokers.

2.5 Statistical interim analyses and stopping guidance

Not applicable as no formal interim analysis is planned.

2.6 Timing of final analysis

All outcomes will be analysed collectively after 6 months of follow-up, once all 6-month follow-up activities are completed, all data is entered in the database, and all data is statistically validated and cleaned in the database. Before starting the analysis a final database export will be done.

2.7 Timing of outcome assessments

Study visits are scheduled at baseline and at 6 months (180 days – 4 weeks / + 12 weeks). Telephone visits are scheduled at target quit date (Day 0), Week 1 (7 ± 2 days), Week 2 (14 ± 3 days), Week 4 (28 ± 4 days), and Week 8 (56 ± 7 days).

The primary outcome is assessed at month 6 and needs to be derived from all telephone visits as well as the month 6 follow-up visit.

Secondary outcomes are assessed at months 6.

Further, outcomes are assessed at 12, and 24 months. These outcomes are not part of the current SAP.

2.8 Blinding

Participants, nurses and investigators will not be blinded. The laboratory personnel will be blinded for the determination of all urinary metabolites of nicotine (anabasine, cotinine, nornicotine, 3-OH cotinine). Any adverse event occurred in this study that generated care by a physician will be verified by a blinded external committee whenever possible. The statistician who authors this SAP and performs the blinded

primary ITT and secondary per-protocol analysis of the primary and secondary smoking cessation outcomes will be blinded. All other statisticians and data managers will not be blinded. The laboratory personnel will not be blinded for further urinary measures of exposures such as metabolites of polycyclic aromatic hydrocarbons (PAHs), of VOCs, creatinine and metabolites of TSNAs (NNAL, NNN, NNK).

There will be an automatic import of these urinary lab values into the database, programmed by a CTU data manager. Specific procedure and time points will be defined.

Breaking the blind:

The blinded statistician will be unblinded when all outcomes with blinding of the primary analysis are completely analysed. An unblinded statistician will have to download and label the data and prepare the analysis sets for the blinded analysis.

3. Data management

3.1 Data export

Data are entered in the SecuTrial EDC system. Data will be exported using the SecuTrial data export tool, as semicolon delimited file, and are labelled using the codebook provided by the same export by an unblinded statistician. Further, all analysis sets need to be defined by the unblinded statistician, since adherence to the study (needed to define analysis sets) is recorded in the "smoking and e-cigarettes" (cig_ecig) form, from which the treatment group becomes instantly obvious.

The unblinded statistician needs to provide datasets to the blinded statistician without any direct or indirect information about the group allocation but an encoded group allocation variable will be provided.

3.2 Data validation

Data validation will follow the principles defined in the SOP Statistical data validation.

All variables used in the analysis, including the derived variables, will be checked for:

- Missing values (frequency per parameter, consistency between related parameters within patient, report on missing values for parameters where no missing values were allowed) including responses such as "unknown", "no answer", "unspecific", etc.

- Identification of duplicates

- Outliers (range of values (univariate or multivariate), shape of frequency distribution of data, extremes (inspection of data below the 2.5th and above the 97.5th percentile, i.e. data points on both ends of their cumulative distribution))

- Inconsistencies (chronological dates, suspiciously high frequencies of first day of month, first day of the year, in order to investigate whether incomplete time or date variables were cut)

- Consistency in the measurement units and consistency between variables

For multi-centre trials any of the above items should be analyzed separately by centre and then compared.

All deviations will be queried if no appropriate comment is given in the SecuTrial EDC database system.

A report will be produced which documents:

- The types of quality checks implemented
- The findings (including the distinction between clearly wrong and suspicious data)
- The date and/or database version the checks were applied to.

4. Statistical principles

4.1 Confidence intervals and *P* values

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. Estimates and effect measures will be presented with a two-sided 95% confidence interval.

4.2 Analysis populations

This document describes the patient populations and estimands that are used for the primary and secondary analysis or primary and secondary outcomes. Further patient sets and estimands may be addressed in further exploratory analyses, which will not be done by CTU Bern but by the sponsor-investigator.

4.2.1 Full analysis set (FAS)

The full analysis set (FAS) will include all randomized subjects. Following the intention-to-treat principle, subjects will be analysed according to the treatment they are assigned to at randomization, regardless of any protocol violations or discontinuations of the intervention.

4.2.2 Per-protocol (PP)

The per-protocol population consists of all subjects in the FAS who do not have any protocol deviations that could confound the interpretation of analyses conducted on the FAS. The following are common major protocol deviations:

TABLE 1: **DERIVATION OF PROTOCOL DEVIATIONS.**

Protocol deviation	eCRF sheet	Variable	Variable type	Derivation
Violation of inclusion or exclusion criteria				
Age is less than 18 years	eligibility	ic_age18	Continuous: years	ic_age18 == No
Smoking of less than 5 cigarettes a day	eligibility	ic_cigmore5, ic_cigday,	binary, continuous, continuous	ic_cigmore5 == No OR ic_cigday < 5
Non adherence to intervention				
Regular use of an ENDs or tobacco heating systems device in the control group (on a minimum of 5 days during the last 7 days, on at least 1 visit)	cig_ecig [day 0, week 1, 2, 4, 8, month 6]	quit_ecig_use, quit_ecig_7days, quit_ecig_7d_nxwk	binary, binary continuous	sum(quit_ecig_use == Yes AND quit_ecig_7days == Yes AND quit_ecig_7d_nxwk >= 5) >= 3 visits
No regular use of ENDs or tobacco heating systems device in the intervention group (a minimum of 5 days during	cig_ecig [week 1, 2, 4, 8, month 6]	quit_ecig_use, quit_ecig_7days, quit_ecig_7d_nxwk,	binary binary continuous	sum(quit_ecig_use == No OR (quit_ecig_7days == Yes AND quit_ecig_7d_nxwk < 5)) == 0 visits

Protocol deviation	eCRF sheet	Variable	Variable type	Derivation
the last 7 days, on at least 1 visit is required)				
Primary outcome is outside of time window of 152 to 264 days from target quit date (180 days -4/+12 weeks) or missing	Smoking, smoking + E-cigarette	cig_quitdate, mnpvisfdt	binary	cig_quitdate- mnpvisfdt >=152 AND cig_quitdate- mnpvisfdt <=264

4.2.3 Safety population

Safety data will be analysed in the ITT and PP patient sets. Moreover, separate listings of all the serious adverse events (SAEs) and adverse events (AEs) according to the consumption preceding the event (cigarettes, ENDS, both, none) will be provided.

4.3 Estimands

4.3.1 Treatment policy estimand

Outcome of interest: The primary analysis of the primary outcome (binary) will focus on the treatment policy estimand,

Patient set of interest: using the full analysis set following the intention-to-treat principle, i.e. subjects will be analysed according to the randomized group, irrespective of the treatment actually received.

Intercurrent events: Intercurrent events will be disregarded. Missing values in the primary outcome will be categorized as persistent smoker (i.e. treatment failures).

Summary measure: The difference between the two groups will be presented as a relative risk.

The primary analysis of secondary outcomes will focus on the treatment policy estimand as well. Missing values in secondary smoking cessation outcomes will be treated as for the primary outcome. Missing values in other outcomes will be imputed. Effect sizes of binary outcomes will be presented as relative risk. Effect sizes of continuous outcomes will be presented as mean difference.

4.3.2 Principal strata estimand

Outcome of interest: The secondary analysis of the primary outcome (binary) will focus on the principal strata estimand

Patient set of interest: using the per protocol analysis set. Subjects with protocol violations will be excluded from the analysis.

Intercurrent events: Intercurrent events will be disregarded. Missing values in the primary outcome will be categorized as persistent smoker (i.e. treatment failures).

Summary measure: The difference between the two groups will be presented as a relative risk.

5. Trial Population

5.1 Screening data

Screening data is not captured in the EDC.

5.2 Eligibility

Subjects fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Age 18 or older
- Currently smokes 5 or more cigarettes per day, for a least 12 months
- Willing to try to quit smoking within the next 3 months
- Persons providing a valid phone number, a valid email address and/or a valid postal address.

The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

- Known hypersensitivity or allergy to contents of the e-liquid
- Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected
- Women who are pregnant or breast feeding
- Intention to become pregnant during the course of the study
- Persons having used ENDS or tobacco heating systems regularly in the 3 months preceding the baseline visit
- Persons having used nicotine replacement therapy (NRT) or other medications with demonstrated efficacy as an aid for smoking cessation such as varenicline or bupropion within the 3 months preceding the baseline visit
- Persons who cannot attend the 6- month follow-up visit for any reason
- Persons who cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures

5.3 Recruitment

A CONSORT patient flow diagram will be drawn following the CONSORT 2010 standards (http://www.consort-statement.org/consort-2010).

The flow chart will consider specifically:

- N assessed for eligibility
- N not eligible (with reasons)
- N randomized

- Ns allocated to intervention and control
- Ns not receiving allocated intervention
- Ns lost to follow-up (with reasons)
- Ns analysed
- Ns excluded from primary analysis (with reasons)

5.4 Baseline patient characteristics

Please note: P-values, standard errors, and confidence intervals are not shown in baseline tables since any significant difference can be explained by the play of chance if the randomization was performed properly.

Table 2: Baseline table.

Description	form	Variable	Type
Demography			
Alter	eligibility	ic_age	Continuous: Jahre
Geschlecht	demographics	gender	Categorical: Männlich, Weiblich, Trans
Zivilstand	demographics	martial_status	Categorical: Ledig, Verheiratet, Verwitwet, Geschieden, gerichtlich getrennt, registrierte Partnerschaft, aufgelöste registrierte Partnerschaft
Arbeitssituation	demographics	profession	Categorical: Vollzeitbeschäftigt, Teilzeitbeschäftigt, Selbstständig, Hausfrau/Hausmann, In Ausbildung (Lehre, Studium), Auf Arebitssuche, Andere
Höchster Bildungsabschluss	demographics	education	Categorical: Obligatorische Schule, Lehre, Gymnasiale Maturität/Berufsmaturität, Höhere Fachschule/PädagogischeHochschule, Universitärer Abschluss, Keine, Andere
Medical history			
Medication	medication	med_yn, med_name	Binary: yes – no If yes free text to be categorized according to categories of use (most prevalent ones).
Arztbesuch in letzten 6 monaten	hospitalization	med_visit_6m	Binary: yes – no If yes 4 additional questions
Hospitalisation in letzten 6 monaten	hospitalization	hosp_stay_6m	Binary: Anzahl
Notfallstation in letzten 6 monaten	hospitalization	hosp_emergency_6m	Binary: yes – no If yes 3 additional questions

Description	form	Variable	Type
Tagesklinik in letzten 6 monaten	hospitalization	hosp_day_6m	Binary: yes – no If yes 1 additional question
Leiden Sie momentan an einer Infektion, Fieber oder einer akuten Erkrankung?	healthstatus	hs_current	Binary: yes – no
Allergien	healthstatus	allergy	Binary: yes – no
Bluthochdruck	healthstatus	hypertension	Binary: yes – no
Hypercholesterinämie	healthstatus	hypercholesterol	Binary: yes – no
Diabetes	healthstatus	diabetes	Binary: yes – no
früherer Herzinfarkt	healthstatus	cardiovascular1	Binary: yes – no
Perkutane koronare Intervention	healthstatus	pci	Binary: yes – no If yes categorical
Koronararterien-Bypass	healthstatus	cabg	Binary: yes - no
Herzinsuffizienz (in Behandlung, frühere Hospitalisation)	healthstatus	cardiovascular3	Binary: yes - no
Herzklappenoperation	healthstatus	valve_op	Binary: yes - no
Transitorische ischämische Attacke in der Vorgeschichte	healtstatus	tia	Binary: yes - no
Angina pectoris	healthstatus	cardiovascular2	Binary: yes – no
Periphere arterielle Verschlusskrankheit der unteren Extremitäten	healthstatus	arterial_lower	Binary: yes – no
Andere arterielle Verschlusskrankheiten	healthstatus	arterial_other	Binary: yes – no
Schlaganfall	healthstatus	stroke	Binary: yes – no If yes categorical
Andere Herzgefässerkrankungen	healthstatus	cardiovas_other	Binary: yes – no
Lungenembolie	healthstatus	pe	Binary: yes – no
Tiefe Venenthrombose	healthstatus	dvt	Binary: yes – no
COPD	healthstatus	copd	Binary: yes – no
Chronische Bronchitis	healthstatus	chronic_bronch	Binary: yes – no
Asthma	healthstatus	asthma	Binary: yes – no
Obstruktives Schlafapnoesyndrom	healthstatus	osa	Binary: yes – no If yes categorical
Andere Lungenerkrankungen	healthstatus	pulmonary_other	Binary: yes – no
Krebs	healthstatus	cancer	Binary: yes – no
Andere Krankheiten	healthstatus	disease_other	Binary: yes – no
Andere wichtige gesundheitliche Ereignisse in der Vorgeschichte (z.B. Operationen)	healthstatus	med_hist_other	Binary: yes – no
Wie schätzen Sie Ihren Gesundheitszustand im Allgemeinen ein	healthstatus	health_perception	Categorical
Herzinfarkt oder Angina pectoris bei Verwandten 1. Grades?	healthstatus	hrtattack_angina	Binary: yes – no

Description	form	Variable	Type
Schlaganfall bei Verwandten 1. Grades?	healthstatus	genetic_stroke	Binary: yes – no
Respiratory symptoms*			
CAT	Respiratory symp-toms	sum of all items (q1 to q8)	continuous (score)
MMRC	Respiratory symp-toms	q10 if q9 == yes	ordinal (score)
COPD	Respiratory symp-toms	q1_copd	Binary: yes – no
Asthma test			
ECRHS	asthma	q1_ecrhs to q7_ecrhs	continuous (score, number of yes)
ACT	asthma	q1 to q5	continuous (score)
MINNESOTA NICOTINE WITH-DRAWAL SCALE	withdrawal symptoms	q1 to q15	continuous (score)
Depressed	withdrawal symptoms	q3	ordinal
Irritable	withdrawal symptoms	q1	ordinal
Restless	withdrawal symptoms	q2	ordinal
Hungry	withdrawal symptoms	q5	ordinal
Poor concentration	withdrawal symptoms	q4	ordinal
Lab values			
Metabolites of nicotine:			
Anabasine	To be defined	To be defined	Continuous
Metabolites of TSNAs:			
NNAL	To be defined	To be defined	Continuous

*References for the different scores:
- CAT: The CAT has a scoring range of 0-40. See CAT User Guide & FAQs for cut-offs- MMRC: Scores from 0-4. 0 = Ich bekomme nur Atemnot bei sehr starker Belastung; […] 4 = Ich kann wegen meiner Atemnot das Haus nicht verlassen …
- COPD: y/n: If y: Number of COPD exacerbations/hospitalisation due to COPD and number of COPD exacerbations/hospitalisation due to COPD in last 6 months.
- ECRHS: y/n
- ACT: scoring range of 5-25. See ACT-Erwachsene 2017-08 for cut-offs.
- MINNESOTA NICOTINE WITHDRAWAL SCALE: 0 = überhaupt nicht, 1 = ganz leicht, 2 = etwas, 3 = mittel, 4 = sehr

5.5 Procedural characteristics (if applicable)

Not applicable.

5.6 Adherence and protocol deviations

Adherence

Adherence in the control group is defined as not having used an ENDs device on 5 or more days during the last 7 days of any visit.

Adherence in the intervention group is defined as having used an ENDs device on 5 or more days during the last 7 days of at least one visit.

Adherence will be presented in a table as number (and %) of patients not smoking cigarette, smoking cigarettes only, smoking ENDS only, and smoking cigarettes and ENDS for both groups at each time point (Weeks 1, 2, 4, 8, and month 6). In addition, the number (and %) of patients using any NRT (patches, gums) or other smoking cessation medication (bupropion, varenicline) during the 24 hours before the visit will be presented in a similar table.

Protocol deviations

Protocol deviations are defined in Table 1. All of these protocol deviations will be presented in a table with the number of patients (%) having a protocol deviation for the control and intervention group.

5.7 Withdrawal/follow-up

Subjects will be withdrawn from intervention, if she reports to be pregnant. A subject may be withdrawn from the intervention, when:

- he/she does not comply with instructions by the research team
- a treatment related SAE is expected
- the protocol has been irretrievably violated, as determined by the sponsor

Subjects will be withdrawn from follow-up if they withdraw consent. A subject may be withdrawn from follow-up if the responsible study investigator decides that continued participation in the study could be harmful to the subject's wellbeing.

Withdrawal from intervention or follow-up is recorded in the end of study form. The number of patients not finishing the study according to the protocol due to:

- withdrawal of consent
- lost to follow up
- exclusion by PI
- SAE
- death, and

- other reason

will be presented in a table for both groups and each time point (derived from lastcontact_date in end form). Other reasons will be listed as free text.

A summary of the follow-ups will present the numbers of calls performed as well as the visits performed at each time point.

6. Analysis

6.1 Outcome definitions

Primary outcome: The primary outcome will be the continuous cigarette smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report at the 6-month follow-up exam (self-report of no smoking from target quit date) and confirmed by urinary level of anabasine (<3 ng/ml). If anabasine is missing, validation by exhaled carbon monoxide (CO). The outcome is a binary outcome.

Core secondary outcomes:

- Secondary smoking cessation outcomes to the 6-month follow-up visit:
    - Continuous smoking abstinence. Self-report of having smoked no cigarettes from TQD, validated by urinary levels of NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol). If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.
    - Self-reported smoking abstinence allowing a 2-week`grace period' after the TQD
    - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.
    - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.
    - Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD.
    - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.
    - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).
    - Self-reported 7-day point prevalence abstinence at 6 months, Self-report of having smoked no cigarettes in the past seven days.
    - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled carbon monoxide (CO).
    - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).

  - Number of cigarettes smoked per day (CPD), self-reported, at baseline, TQD, phone visits and 6 months visit
  - Change in number of cigarettes smoked per day (CPD), self-reported. Successful reduction defined as 50% reduction in CPD.
- Respiratory symptoms assessed by questionnaires:
  - Change in COPD Assessment Test (CAT) from baseline to month 6, score from 0-40 (regarded as continuous)
- Change in cardiovascular risk factors from baseline to month 6, assessed by
  - Total cholesterol (continuous)
  - HDL-cholesterol (continuous),
  - LDL-cholesterol (calculated, continuous),
  - triglycerides (continuous),
  - blood pressure levels (continuous),
  - heart rate (continuous),
  - waist circumference (continuous) and
  - body mass index (continuous).

- AEs (in categories from adjudication committee)
- SAEs (in categories from adjudication committee)

6.2 Derivation of Outcomes

Comments to secondary smoking cessation outcomes:

- Smoking is allowed during the grace period.
- The number of cigarettes smoked since the last visit is recorded as a categorical variable (1-5 cigarettes, >5 cigarettes). If the participant smoked more than once a day cigarettes in the last 7 days, the value is recorded as numeric variable. For secondary smoking cessation outcomes, we will assume the median value for the categorical variable. I.e. if a subject records 2 times to smoke 1-5 cigarettes, we assume him to be over the limit of 5 cigarettes.

Table 3: Derivation of primary and secondary outcomes.

Outcome	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Primary: continuous smoking abstinence from target quit date to the 6-month follow-up visit AND	cig_ecig	quit_smoke_again	Binary: No, Yes	{[Week 1/2/4/8, and month 6] quit_smoke_again == No AND quit_tobacco_inhale ==	Binary
		quit_tobacco_inhale	Binary: No, Yes		
	cig_ecig_6m	quit_smoke_again	Binary: No, Yes		
		quit_tobacco_inhale	Binary: No, Yes		

urinary level of anabasine (<3 ng/ml) OR	to be defined	to be defined	to be defined	No} AND {x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	vital_6m	vs_co2_level	Continuous		
Secondary outcomes					
continuous smoking abstinence from target quit date to the 6-month follow-up visit AND	Cig_ecig, cig_ecig_6m	quit_smoke_again, quit_tobacco_inhale	Binary: No, Yes, Binary:No, Yes	{[Week 1/2/4/8, and month 6] quit_smoke_again == No AND quit_tobacco_inhale == No} AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if y==missing & x==missing & vs_co2_level!=missing }	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Continuous smoke abstinence allowing a 2-week grace period after TQD	cig_ecig / cig_ecig_6m	quit_smoke_again quit_tobacco_inhale	Binary: No, Yes	[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2	
Continuous smoke abstinence allowing a 2-week grace period after TQD AND	cig_ecig / cig_ecig_6m to be defined	quit_smoke_again quit_tobacco_inhale quit_smoke_n	Binary: No, Yes Binary: No, Yes categorical: 1-5 cigarettes, > 5 cigarettes	{[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2 AND sum(quit_smoke_n == 1-5) <= 1} AND	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined	{ x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Continuous smoke abstinence allowing a 2-week grace period after TQD AND	cig_ecig / cig_ecig_6m to be defined	quit_smoke_again quit_tobacco_inhale quit_smoke_n	Binary: No, Yes Binary: No, Yes categorical: 1-5 cigarettes, > 5 cigarettes	{[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2 AND sum(quit_smoke_n == 1-5) <= 1} AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{ y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		

CO level	Vital, Vital_6m	vs_co2_level	continuous	y==missing & x==missing & vs_co2_level!=missing }	
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD	cig_ecig / cig_ecig_6m	quit_smoke_n	categorical	sum(quit_smoke_n == 1-5) <= 1	Binary
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD AND	cig_ecig / cig_ecig_6m	quit_smoke_n	Categorical	sum(quit_smoke_n == 1-5) <= 1 AND	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined	{ x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD AND	cig_ecig / cig_ecig_6m	quit_smoke_n	Categorical	sum(quit_smoke_n == 1-5) <= 1 AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{ y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if y==missing & x==missing & vs_co2_level!=missing }	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported 7-day point prevalence abstinence at 6 months	cig_ecig_6m	quit_smoke_again, quit_smoke_7days	Binary: No, Yes	quit_smoke_again == No AND quit_smoke_7days == No	Binary
Self-reported 7-day point prevalence abstinence at 6 months AND	cig_ecig_6m	quit_smoke_again quit_smoke_7days	Binary: No, Yes Binary: No, Yes	{ quit_smoke_again == No AND quit_smoke_7days == No} AND { x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported 7-day point prevalence abstinence AND	cig_ecig_6m	quit_smoke_again, quit_smoke_7days	Binary: No, Yes	{quit_smoke_again == No AND quit_smoke_7days == No} AND {y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined		
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		

CO level	Vital, Vital_6m	vs_co2_level	continuous	y==missing & x==missing & vs_co2_level!=missing}	
Number of cigarettes smoked per day (CPD)	cig_ecig / cig_ecig_6m	quit_more1perday	continuous (number of cigarettes per day in the last 7 days)	[BL, TQD, phone visits and 6-month visit] quit_more1perday	Continuous
Change in the self-reported number of cigarettes smoked per day in the last 7 days	cig_ecig / cig_ecig_6m	quit_more1perday	continuous (number of cigarettes per day in the last 7 days)	(quit_more1perday[month 6] / quit_more1perday [day 0]) >= 0.5 IF [month 6]{contact_estab_yn == Yes AND quit_smoke_again == Yes AND quit_smoke_7days == Yes AND quit_smoke_nperday == ">=1x/Tag"} ELSE TRUE	Binary
CAT	cat / cat_6m	q1 to q8	radio (0 to 5)	sum(q1 to q8)[cat_6m] – sum(q1 to q8)[cat]	continuous (score scale 0-40, difference can be 0-40 as well)
Cholesterin (total)	vital vital_6m	lab_choles_total	continuous (mmol/L)	lab_choles_total[vital_6m] – lab_choles_total[vital]	continuous (mmol/L)
LDL cholesterol	calculated	calculated	Continuous (mmol/L)	lab_choles_total[vital_6m]-lab_choles_hdl[vital_6m]-(lab_triglyceride[vital_6m] /5)-lab_choles_total[vital]-lab_choles_hdl[vital]-(lab_triglyceride[vital] /5) [Friedewald formula]	continuous (mmol/L)
HDL cholesterol	vital vital_6m	lab_choles_hdl	continuous (mmol/L)	lab_choles_hdl[vital_6m] – lab_choles_hdl[vital]	continuous (mmol/L)
triglycerides	vital vital_6m	lab_triglyceride	continuous (mmol/L)	lab_triglyceride[vital_6m] - lab_triglyceride[vital]	continuous (mmol/L)
blood pressure	vital vital_6m	vs_bpressure_sys1-3, vs_bpressure_dias1-3	continuous (mmHg)	mean(vs_bpressure_sys1-3), mean(vs_bpressure_dias1-3)	continuous (mmHg)
heart rate (mean of the second and third measure)	vital vital_6m	vs_heart_rate_2 vs_heart_rate_3	continuous (bpm)	mean(vs_heart_rate_2, vs_heart_rate_3)[vital_6m] – mean(vs_heart_rate_2, vs_heart_rate_3)[vital]	continuous (bpm)
waist circumference	vital vital_6m	vs_waist_cm	continuous (cm)	vs_waist_cm[vital_6m] - vs_waist_cm[vital]	continuous (cm)
BMI	vital vital_6m	bmi	continuous (kg/m^2)	bmi[vital_6m] – bmi[vital]	continuous (kg/m^2)
Lab values					
Metabolites of nicotine:					

Anabasine	To be defined	To be defined	dichotomized at <3 ng/ml	anabasine < 3	Binary: <3ng/ml, ≥3ng/ml
Metabolites of TSNAs:					
NNAL	To be defined	To be defined	dichotomized at <10pg/ml	NNAL < 10	Binary: <10pg/ml, ≥10pg/ml

6.3 Analysis methods

6.3.1 Primary analysis

The primary analysis is based on the treatment policy estimand, using the full analysis set according to the intention to treat principle.

The primary outcome will be presented as proportion with 95% Wilson confidence interval in each intervention group and compared between groups using the chi-square test. The effect size will be presented as relative risk with 95% Koopman confidence interval, as well as risk difference with 95% Newcombe-hybrid-score confidence interval.

Secondary smoking outcomes will be analysed as the primary outcome.

Continuous secondary outcomes will be analysed using linear models, adjusted for the outcome measure at baseline, if it is available. Missing values in continuous outcomes will be imputed. Thus, the estimates of the complete data models are averaged using Rubin's rule (Rubin 1987). Effect sizes will be presented as mean difference with 95% confidence interval.

6.3.2 Secondary analyses

Secondary analyses are based on the principal strata estimand, using the per-protocol analysis set. Binary outcomes will be presented as proportion with 95% Wilson confidence interval in each intervention group and compared between groups using the chi-square test. Effect sizes will be presented as relative risk with 95% Koopman confidence interval, as well as risk difference with 95% Newcombe-hybrid-score confidence interval. Continuous outcomes will be analysed using linear models, adjusted for outcome measures at baseline, if available, with mean differences (95% confidence interval) as effect sizes. If secondary outcomes are missing, we will use the imputed datasets generated for the primary analysis.

6.3.3 Sensitivity analyses

As sensitivity analysis, we will do an available case analysis of the primary outcome, using the full analysis patient set. No further sensitivity analyses will be performed by CTU Bern.

6.3.4 Subgroup analyses

No subgroup analyses are planned to be performed by CTU Bern.

6.3.5 Additional analyses

No further analyses are planned to be performed by CTU Bern.

6.3.6 Assessment of statistical assumptions

To test model assumptions for continuous secondary outcomes, we will visually check residuals in QQ-plots and residual versus fitted plots.

If model assumptions are violated, transformation of the outcome (e.g. log-transformation), more robust methods (e.g. robust standard errors or robust regression) or non-parametric methods (i.e. Wilcoxon rank-sum test) will be considered.

6.4 Interim analyses

No interim analysis is planned

6.5 Missing data

Missing values in the primary outcome will be categorized as persistent smokers. We intend to obtain full follow-up data on all randomized participants.

Missing data in secondary smoking cessation outcomes will be treated as the primary outcome. If more than 5% of data is missing, all other secondary outcomes will be imputed, assuming values to be missing at random. Each outcome will be imputed separately to ensure robust imputation models. We will use all baseline variables (see Table 2) and outcome measures at all time points as predictors in the imputation models. The two intervention groups will be imputed separately. Variables with more than 50% missing values will not be used for the imputation model. Binary variables with a frequency of less than 5% in one category will be omitted from the predictors, levels of categorical variables with a frequency of less than 5% in one category will be combined with another level in a sensible way. Continuous variables will be log-transformed if it improves normality (checked by Shapiro-Wilks test and QQ plots). If predictors are too highly correlated among each other, we will only consider the predictor which is more strongly correlated with the outcome. We will use multiple imputation by chained equation. We will impute values using predictive mean matching for continuous and ordinal variables, logistic regression for binary variables, and a multinomial regression model for categorical variables with more than two levels. In total, fifty imputed data sets will be generated, which will be analysed as described using Rubin's rules (Rubin 1987).

6.6 Safety evaluation

Safety outcomes will be analysed using the ITT and PP set.

The safety analysis will list the number of patients dropping out for both treatment arms, including the reasons, and a list of events for patients dropping out, with the treatment group, relation, grade, type of AE and potential free text.

AEs and SAEs will be summarized by presenting for both treatment arms the number of any AE, any SAE, and the number and percentage of patients with any AE or any SAE.

Further, we will present the

- grade and relation of AEs and SAEs (number of patients with events and the number of events),
- category and grade of AEs and SAEs
- category and relation of AEs and SAEs
- AEs and SAEs stratified by consumption (cigarettes, ENDS, both, none)

6.7 Subproject

Subprojects and further outcomes of interest are registered in clinicaltrials.gov under separate registration numbers. These subprojects and outcomes are not part of the current SAP, nor will they be analysed by CTU Bern.

6.8 Statistical software

All analyses will be done using the current version of Stata (StataCorp. 2019 Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.). The version of Stata and all used packages will be listed in the statistical report.

6.9 Quality control

The primary analysis of the primary and secondary outcomes will be double programmed by a second, unblinded statistician.

7. Changes from the protocol

The SAP is consistent with principle features of the statistical methods described in the protocol. The SAP only describes the primary and secondary analysis of primary and secondary outcomes. Any deviation from the protocol is detailed hereunder with reason. Further analysis of these outcomes, as well

as for additional outcomes are planned by the PI and described in the protocol, but will not be further specified in this SAP.

Table 4: Changes from protocol

Header	Change	Reason
6.1 Outcome definitions	This SAP only focuses on the primary outcome for the main analysis at 6-month follow-up. The primary outcome for the extended follow-ups at 12- and 24-months is not part of this SAP.	Initial outcome defined in the initial protocol was at 6-month follow-up.

8. References

Estimands and SOPs

European Medicines Agency, Committee for Human Medicinal Products; Draft ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials, step 2b - Revision 1. 30 August 2017.

SOP Data preparation and programming, CS_STA_SOP_05, version 04, 11.09.2018

SOP Statistical data validation, CS_STA_SOP_02, version 03, 11.09.2018

References

StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC.

UNIVERSITÄT BERN

Faculty of Medicine

CTU Bern

Statistical Analysis Plan (SAP)

Efficacy, Safety and Toxicology of Electronic Nicotine Delivery Systems as an aid for smoking cessation: The ESTxENDS multicentre randomized controlled trial

ESTxENDS

Administrative Information

Project number:	746
Trial registration number:	Swiss National Science Foundation # 173552 Clinicaltrials.gov: NCT03589989 (primary outcome). Further outcomes are registered separately, but not part of this SAP.
SAP version:	1.0 of 2020-06-05, 2.0 of 2023-06-05
Protocol version:	8.0; May 7th, 2020

Contributors

Name	Affiliation	Role in SAP writing
Lenz Armando	CTU Bern	Author of initial SAP
Limacher Andreas	CTU Bern	Reviewer of initial SAP
Odile Stalder	CTU Bern	Co-Author of initial SAP
Reto Auer	BIHAM	Reviewer of both initial and final SAP
Anna Schöni	BIHAM	Reviewer of initial and final SAP
Cindy Groen	BIHAM	Reviewer of initial SAP
Stéphanie Barrio	BIHAM	Reviewer of final SAP

Revision history

Revision	Justification	Timing
Final version, 2.0	SAP taken over by study team	5.6.2023

Approved by

Name	Affiliation	Study Role	Date and Signature (wet ink)
~~Odile Stalder~~	~~CTU Bern~~	~~Trial Statistician~~	
~~Limacher Andreas~~	~~CTU Bern~~	~~Senior Statistician~~	
Auer Reto	BIHAM	Sponsor / Coordinating person	
Stéphanie Baggio	BIHAM	Senior statistician	
Anna Schöni	BIHAM	Lead Statistician	

Contents

1. Introduction

1.1 Background and rationale

Cigarette smoking is the leading cause of preventable death in Switzerland. Recently, electronic nicotine delivery systems (ENDS or vaporizers, also called e-cigarettes) have become popular with smokers who want to switch from tobacco cigarettes to ENDS to reduce their exposure to toxic compounds or to stop smoking. Only three rigorous RCTs have been published so far. They have promising, yet inconclusive results, as they were based on small samples. The safety and potential adverse effects of ENDS are also largely unknown. While the aerosol the users inhale appears safe in laboratory conditions, the difference in exposure to toxins (such as measures of exposure to organic compounds) and effect of toxins on the body (measures of oxidative stress) between smokers who quit (with or without ENDS) and those who use ENDS for a long time have not yet been assessed in an RCT.

1.2 Objectives

Test ENDS on:
1) the efficacy for cigarette smoking cessation and in reducing the number of cigarettes smoked over 6 months of follow-up;
2) the safety of ENDS
3) the effect of ENDS on exposure to inhaled toxic compounds;
4) the effect of ENDS on health-related outcomes (such as blood pressure, weight and respiratory symptoms).

2. Study methods

2.1 Trial design

The ESTxENDS trial is a multicentre, pragmatic, open-label randomized controlled trial with two parallel groups allocated in a 1:1 ratio. The trial will assess superiority of Electronic Nicotine Delivery Systems (ENDS) + standard of care (SOC) for smoking cessation to SOC alone in adult (≥18 years) smokers. The intervention group will receive free ENDS and nicotine-containing e-liquids to use ad libitum, plus SOC. The control group will receive SOC only.

2.2 Randomization

Simple randomization in a 1:1 ratio to the two arms.

2.3 Sample size

Details on the sample size calculation can be found in the protocol in section 11.2. In short, the sample size is based on the primary outcome, that providing smokers with ENDS and SOC leads to a higher rate of smokers who quit than usual care. Sample size was calculated for a 2-sided alpha of 0.05 and a power of 90%. Accounting for 5% drop-out, the sample size of the trial was defined to be 1172 smokers, 586 in both groups.

2.4 Framework

The trial will assess superiority of ENDS + SOC for smoking cessation to SOC alone in adult (≥18 years) smokers.

2.5 Statistical interim analyses and stopping guidance

Not applicable as no formal interim analysis is planned.

2.6 Timing of final analysis

All outcomes will be analysed collectively after 6 months of follow-up, once all 6-month follow-up activities are completed, all data is entered in the database, and all data is statistically validated and cleaned in the database. Before starting the analysis a final database export will be done.

2.7 Timing of outcome assessments

Study visits are scheduled at baseline and at 6 months (180 days – 4 weeks / + 12 weeks). Telephone visits are scheduled at target quit date (Day 0), Week 1 (7 ± 2 days), Week 2 (14 ± 3 days), Week 4 (28 ± 4 days), and Week 8 (56 ± 7 days).

The primary outcome is assessed at month 6 and needs to be derived from all telephone visits as well as the month 6 follow-up visit.

Secondary outcomes are assessed at months 6.

Further, outcomes are assessed at 12, and 24 months. These outcomes are not part of the current SAP.

2.8 Blinding

Participants, nurses and investigators will not be blinded. The laboratory personnel will be blinded for the determination of all urinary metabolites of nicotine (anabasine, cotinine, nornicotine, 3-OH cotinine). Any adverse event occurred in this study that generated care by a physician will be verified by a blinded external committee whenever possible. ~~The statistician who authors this SAP and performs the blinded~~

~~primary ITT and secondary per-protocol analysis of the primary and secondary smoking cessation outcomes will be blinded.~~ All ~~other~~ statisticians and data managers will not be blinded. The laboratory personnel will not be blinded for further urinary measures of exposures such as metabolites of polycyclic aromatic hydrocarbons (PAHs), of VOCs, creatinine and metabolites of TSNAs (NNAL, NNN, NNK).

There will be an automatic import of these urinary lab values into the database, programmed by a CTU data manager. Specific procedure and time points will be defined.

~~*Breaking the blind:*~~

~~The blinded statistician will be unblinded when all outcomes with blinding of the primary analysis are completely analysed. An unblinded statistician will have to download and label the data and prepare the analysis sets for the blinded analysis.~~

3. Data management

3.1 Data export

Data are entered in the SecuTrial EDC system. Data will be exported using the SecuTrial data export tool, as semicolon delimited file, and are labelled using the codebook provided by the same export by an unblinded statistician. Further, all analysis sets need to be defined by the unblinded statistician, since adherence to the study (needed to define analysis sets) is recorded in the "smoking and e-cigarettes" (cig_ecig) form, from which the treatment group becomes instantly obvious.

The unblinded statistician needs to provide datasets to the blinded statistician without any direct or indirect information about the group allocation but an encoded group allocation variable will be provided.

3.2 Data validation

Data validation will follow the principles defined in the SOP Statistical data validation.

All variables used in the analysis, including the derived variables, will be checked for:

- Missing values (frequency per parameter, consistency between related parameters within patient, report on missing values for parameters where no missing values were allowed) including responses such as "unknown", "no answer", "unspecific", etc.

- Identification of duplicates

- Outliers (range of values (univariate or multivariate), shape of frequency distribution of data, extremes (inspection of data below the 2.5th and above the 97.5th percentile, i.e. data points on both ends of their cumulative distribution))

- Inconsistencies (chronological dates, suspiciously high frequencies of first day of month, first day of the year, in order to investigate whether incomplete time or date variables were cut)

- Consistency in the measurement units and consistency between variables

For multi-centre trials any of the above items should be analyzed separately by centre and then compared.

All deviations will be queried if no appropriate comment is given in the SecuTrial EDC database system.

A report will be produced which documents:

- The types of quality checks implemented
- The findings (including the distinction between clearly wrong and suspicious data)
- The date and/or database version the checks were applied to.

4. Statistical principles

4.1 Confidence intervals and *P* values

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. Estimates and effect measures will be presented with a two-sided 95% confidence interval.

4.2 Analysis populations

This document describes the patient populations and estimands that are used for the primary and secondary analysis or primary and secondary outcomes. Further patient sets and estimands may be addressed in further exploratory analyses, which will not be done by CTU Bern but by the sponsor-investigator.

4.2.1 Full analysis set (FAS)

The full analysis set (FAS) will include all randomized subjects. Following the intention-to-treat principle, subjects will be analysed according to the treatment they are assigned to at randomization, regardless of any protocol violations or discontinuations of the intervention.

4.2.2 Per-protocol (PP)

The per-protocol population consists of all subjects in the FAS who do not have any protocol deviations that could confound the interpretation of analyses conducted on the FAS. The following are common major protocol deviations:

TABLE 1: **DERIVATION OF PROTOCOL DEVIATIONS.**

Protocol deviation	eCRF sheet	Variable	Variable type	Derivation
Violation of inclusion or exclusion criteria				
Age is less than 18 years	eligibility	ic_age18	Continuous: years	ic_age18 == No
Smoking of less than 5 cigarettes a day	eligibility	ic_cigmore5, ic_cigday,	binary, continuous, continuous	ic_cigmore5 == No OR ic_cigday < 5
Non adherence to intervention				
Regular use of an ENDs or tobacco heating systems device in the control group (on a minimum of 5 days during the last 7 days, on at least 1 visit)	cig_ecig [day 0, week 1, 2, 4, 8, month 6]	quit_ecig_use, quit_ecig_7days, quit_ecig_7d_nxwk	binary, binary continuous	sum(quit_ecig_use == Yes AND quit_ecig_7days == Yes AND quit_ecig_7d_nxwk >= 5) >= 3 visits
No regular use of ENDs or tobacco heating systems device in the intervention group (a minimum of 5 days during	cig_ecig [week 1, 2, 4, 8, month 6]	quit_ecig_use, quit_ecig_7days, quit_ecig_7d_nxwk,	binary binary continuous	sum(quit_ecig_use == No OR (quit_ecig_7days == Yes AND quit_ecig_7d_nxwk < 5)) == 0 visits

Protocol deviation	eCRF sheet	Variable	Variable type	Derivation
the last 7 days, on at least 1 visit is required)				
Primary outcome is outside of time window of 152 to 264 days from target quit date (180 days -4/+12 weeks) or missing	Smoking, smoking + E-cigarette	cig_quitdate, mnpvisfdt	binary	cig_quitdate- mnpvisfdt >=152 AND cig_quitdate- mnpvisfdt <=264

4.2.3 Safety population

Safety data will be analysed in the ITT and PP patient sets. Moreover, separate listings of all the serious adverse events (SAEs) and adverse events (AEs) according to the consumption preceding the event (cigarettes, ENDS, both, none) will be provided.

4.3 Estimands

4.3.1 Treatment policy estimand

Outcome of interest: The primary analysis of the primary outcome (binary) will focus on the treatment policy estimand,

Patient set of interest: using the full analysis set following the intention-to-treat principle, i.e. subjects will be analysed according to the randomized group, irrespective of the treatment actually received.

Intercurrent events: Intercurrent events will be disregarded. Missing values in the primary outcome will be categorized as persistent smoker (i.e. treatment failures).

Summary measure: The difference between the two groups will be presented as a relative risk.

The primary analysis of secondary outcomes will focus on the treatment policy estimand as well. Missing values in secondary smoking cessation outcomes will be treated as for the primary outcome. Missing values in other outcomes will be imputed. Effect sizes of binary outcomes will be presented as relative risk. Effect sizes of continuous outcomes will be presented as mean difference.

4.3.2 Principal strata estimand

Outcome of interest: The secondary analysis of the primary outcome (binary) will focus on the principal strata estimand

Patient set of interest: using the per protocol analysis set. Subjects with protocol violations will be excluded from the analysis.

Intercurrent events: Intercurrent events will be disregarded. Missing values in the primary outcome will be categorized as persistent smoker (i.e. treatment failures).

Summary measure: The difference between the two groups will be presented as a relative risk.

5. Trial Population

5.1 Screening data

Screening data is not captured in the EDC.

5.2 Eligibility

Subjects fulfilling all of the following inclusion criteria are eligible for the study:

- Informed Consent as documented by signature
- Age 18 or older
- Currently smokes 5 or more cigarettes per day, for a least 12 months
- Willing to try to quit smoking within the next 3 months
- Persons providing a valid phone number, a valid email address and/or a valid postal address.

The presence of any one of the following exclusion criteria will lead to exclusion of the subject:

- Known hypersensitivity or allergy to contents of the e-liquid
- Participation in another study with investigational drug within the 30 days preceding the baseline visit and during the present study where interactions are to be expected
- Women who are pregnant or breast feeding
- Intention to become pregnant during the course of the study
- Persons having used ENDS or tobacco heating systems regularly in the 3 months preceding the baseline visit
- Persons having used nicotine replacement therapy (NRT) or other medications with demonstrated efficacy as an aid for smoking cessation such as varenicline or bupropion within the 3 months preceding the baseline visit
- Persons who cannot attend the 6- month follow-up visit for any reason
- Persons who cannot understand instructions delivered in person or by phone, or otherwise unable to participate in study procedures

5.3 Recruitment

A CONSORT patient flow diagram will be drawn following the CONSORT 2010 standards (http://www.consort-statement.org/consort-2010).

The flow chart will consider specifically:

- N assessed for eligibility
- N not eligible (with reasons)
- N randomized

- Ns allocated to intervention and control
- Ns not receiving allocated intervention
- Ns lost to follow-up (with reasons)
- Ns analysed
- Ns excluded from primary analysis (with reasons)

5.4 Baseline patient characteristics

Please note: P-values, standard errors, and confidence intervals are not shown in baseline tables since any significant difference can be explained by the play of chance if the randomization was performed properly.

Table 2: Baseline table.

Description	form	Variable	Type
Demography			
Alter	eligibility	ic_age	Continuous: Jahre
Geschlecht	demographics	gender	Categorical: Männlich, Weiblich, Trans
Zivilstand	demographics	martial_status	Categorical: Ledig, Verheiratet, Verwitwet, Geschieden, gerichtlich getrennt, registrierte Partnerschaft, aufgelöste registrierte Partnerschaft
Arbeitssituation	demographics	profession	Categorical: Vollzeitbeschäftigt, Teilzeitbeschäftigt, Selbstständig, Hausfrau/Hausmann, In Ausbildung (Lehre, Studium), Auf Arebitssuche, Andere
Höchster Bildungsabschluss	demographics	education	Categorical: Obligatorische Schule, Lehre, Gymnasiale Maturität/Berufsmaturität, Höhere Fachschule/PädagogischeHochschule, Universitärer Abschluss, Keine, Andere
Medical history			
Medication	medication	med_yn, med_name	Binary: yes – no If yes free text to be categorized according to categories of use (most prevalent ones).
Arztbesuch in letzten 6 monaten	hospitalization	med_visit_6m	Binary: yes – no If yes 4 additional questions
Hospitalisation in letzten 6 monaten	hospitalization	hosp_stay_6m	Binary: Anzahl
Notfallstation in letzten 6 monaten	hospitalization	hosp_emergency_6m	Binary: yes – no If yes 3 additional questions

Description	form	Variable	Type
Tagesklinik in letzten 6 monaten	hospitalization	hosp_day_6m	Binary: yes – no If yes 1 additional question
Leiden Sie momentan an einer Infektion, Fieber oder einer akuten Erkrankung?	healthstatus	hs_current	Binary: yes – no
Allergien	healthstatus	allergy	Binary: yes – no
Bluthochdruck	healthstatus	hypertension	Binary: yes – no
Hypercholesterinämie	healthstatus	hypercholesterol	Binary: yes – no
Diabetes	healthstatus	diabetes	Binary: yes – no
früherer Herzinfarkt	healthstatus	cardiovascular1	Binary: yes – no
Perkutane koronare Intervention	healthstatus	pci	Binary: yes – no If yes categorical
Koronararterien-Bypass	healthstatus	cabg	Binary: yes - no
Herzinsuffizienz (in Behandlung, frühere Hospitalisation)	healthstatus	cardiovascular3	Binary: yes - no
Herzklappenoperation	healthstatus	valve_op	Binary: yes - no
Transitorische ischämische Attacke in der Vorgeschichte	healtstatus	tia	Binary: yes - no
Angina pectoris	healthstatus	cardiovascular2	Binary: yes – no
Periphere arterielle Verschlusskrankheit der unteren Extremitäten	healthstatus	arterial_lower	Binary: yes – no
Andere arterielle Verschlusskrankheiten	healthstatus	arterial_other	Binary: yes – no
Schlaganfall	healthstatus	stroke	Binary: yes – no If yes categorical
Andere Herzgefässerkrankungen	healthstatus	cardiovas_other	Binary: yes – no
Lungenembolie	healthstatus	pe	Binary: yes – no
Tiefe Venenthrombose	healthstatus	dvt	Binary: yes – no
COPD	healthstatus	copd	Binary: yes – no
Chronische Bronchitis	healthstatus	chronic_bronch	Binary: yes – no
Asthma	healthstatus	asthma	Binary: yes – no
Obstruktives Schlafapnoesyndrom	healthstatus	osa	Binary: yes – no If yes categorical
Andere Lungenerkrankungen	healthstatus	pulmonary_other	Binary: yes – no
Krebs	healthstatus	cancer	Binary: yes – no
Andere Krankheiten	healthstatus	disease_other	Binary: yes – no
Andere wichtige gesundheitliche Ereignisse in der Vorgeschichte (z.B. Operationen)	healthstatus	med_hist_other	Binary: yes – no
Wie schätzen Sie Ihren Gesundheitszustand im Allgemeinen ein	healthstatus	health_perception	Categorical
Herzinfarkt oder Angina pectoris bei Verwandten 1. Grades?	healthstatus	hrtattack_angina	Binary: yes – no

Description	form	Variable	Type
Schlaganfall bei Verwandten 1. Grades?	healthstatus	genetic_stroke	Binary: yes – no
Respiratory symptoms*			
CAT	Respiratory symptoms	sum of all items (q1 to q8)	continuous (score)
MMRC	Respiratory symptoms	q10 if q9 == yes	ordinal (score)
COPD	Respiratory symptoms	q1_copd	Binary: yes – no
Asthma test			
ECRHS	asthma	q1_ecrhs to q7_ecrhs	continuous (score, number of yes)
ACT	asthma	q1 to q5	continuous (score)
MINNESOTA NICOTINE WITHDRAWAL SCALE	withdrawal symptoms	q1 to q15	continuous (score)
Depressed	withdrawal symptoms	q3	ordinal
Irritable	withdrawal symptoms	q1	ordinal
Restless	withdrawal symptoms	q2	ordinal
Hungry	withdrawal symptoms	q5	ordinal
Poor concentration	withdrawal symptoms	q4	ordinal
Lab values			
Metabolites of nicotine:			
Anabasine	To be defined	To be defined	Continuous
Metabolites of TSNAs:			
NNAL	To be defined	To be defined	Continuous

*References for the different scores:
- CAT: The CAT has a scoring range of 0-40. See CAT User Guide & FAQs for cut-offs- MMRC: Scores from 0-4. 0 = Ich bekomme nur Atemnot bei sehr starker Belastung; […] 4 = Ich kann wegen meiner Atemnot das Haus nicht verlassen …
- COPD: y/n: If y: Number of COPD exacerbations/hospitalisation due to COPD and number of COPD exacerbations/hospitalisation due to COPD in last 6 months.
- ECRHS: y/n
- ACT: scoring range of 5-25. See ACT-Erwachsene 2017-08 for cut-offs.
- MINNESOTA NICOTINE WITHDRAWAL SCALE: 0 = überhaupt nicht, 1 = ganz leicht, 2 = etwas, 3 = mittel, 4 = sehr

5.5 Procedural characteristics (if applicable)

Not applicable.

5.6 Adherence and protocol deviations

Adherence

Adherence in the control group is defined as not having used an ENDs device on 5 or more days during the last 7 days of any visit.

Adherence in the intervention group is defined as having used an ENDs device on 5 or more days during the last 7 days of at least one visit.

Adherence will be presented in a table as number (and %) of patients not smoking cigarette, smoking cigarettes only, smoking ENDS only, and smoking cigarettes and ENDS for both groups at each time point (Weeks 1, 2, 4, 8, and month 6). In addition, the number (and %) of patients using any NRT (patches, gums) or other smoking cessation medication (bupropion, varenicline) during the 24 hours before the visit will be presented in a similar table.

Protocol deviations

Protocol deviations are defined in Table 1. All of these protocol deviations will be presented in a table with the number of patients (%) having a protocol deviation for the control and intervention group.

5.7 Withdrawal/follow-up

Subjects will be withdrawn from intervention, if she reports to be pregnant. A subject may be withdrawn from the intervention, when:

- he/she does not comply with instructions by the research team
- a treatment related SAE is expected
- the protocol has been irretrievably violated, as determined by the sponsor

Subjects will be withdrawn from follow-up if they withdraw consent. A subject may be withdrawn from follow-up if the responsible study investigator decides that continued participation in the study could be harmful to the subject's wellbeing.

Withdrawal from intervention or follow-up is recorded in the end of study form. The number of patients not finishing the study according to the protocol due to:

- withdrawal of consent
- lost to follow up
- exclusion by PI
- SAE
- death, and

- other reason

will be presented in a table for both groups and each time point (derived from lastcontact_date in end form). Other reasons will be listed as free text.

A summary of the follow-ups will present the numbers of calls performed as well as the visits performed at each time point.

6. Analysis

6.1 Outcome definitions

Primary outcome: The primary outcome will be the continuous cigarette smoking abstinence from target quit date to the 6-month follow-up visit, ascertained by self-report at the 6-month follow-up exam (self-report of no smoking from target quit date) and confirmed by urinary level of anabasine (<3 ng/ml). If anabasine is missing, validation by exhaled carbon monoxide (CO). The outcome is a binary outcome.

Core secondary outcomes:

- Secondary smoking cessation outcomes to the 6-month follow-up visit:
  - Continuous smoking abstinence. Self-report of having smoked no cigarettes from TQD, validated by urinary levels of NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol). If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.
  - Self-reported smoking abstinence allowing a 2-week`grace period' after the TQD
  - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.
  - Validated smoking abstinence allowing a 2-week`grace period' after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled CO.
  - Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD.
  - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled CO.
  - Validated smoking abstinence allowing up to 5 cigarettes in total after the TQD, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).
  - Self-reported 7-day point prevalence abstinence at 6 months, Self-report of having smoked no cigarettes in the past seven days.
  - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of anabasine. If anabasine is missing validation by exhaled carbon monoxide (CO).
  - Validated 7-day point prevalence abstinence. Confirmation of having smoked no cigarettes in the past seven days, validated by urinary levels of NNAL. If NNAL is missing, validation by urinary levels of anabasine or exhaled carbon monoxide (CO).

  - Number of cigarettes smoked per day (CPD), self-reported, at baseline, TQD, phone visits and 6 months visit
  - Change in number of cigarettes smoked per day (CPD), self-reported. Successful reduction defined as 50% reduction in CPD.
- Respiratory symptoms assessed by questionnaires:
  - Change in COPD Assessment Test (CAT) from baseline to month 6, score from 0-40 (regarded as continuous)
- Change in cardiovascular risk factors from baseline to month 6, assessed by
  - Total cholesterol (continuous)
  - HDL-cholesterol (continuous),
  - LDL-cholesterol (calculated, continuous),
  - triglycerides (continuous),
  - blood pressure levels (continuous),
  - heart rate (continuous),
  - waist circumference (continuous) and
  - body mass index (continuous).

- AEs (in categories from adjudication committee)
- SAEs (in categories from adjudication committee)

6.2 Derivation of Outcomes

Comments to secondary smoking cessation outcomes:

- Smoking is allowed during the grace period.
- The number of cigarettes smoked since the last visit is recorded as a categorical variable (1-5 cigarettes, >5 cigarettes). If the participant smoked more than once a day cigarettes in the last 7 days, the value is recorded as numeric variable. For secondary smoking cessation outcomes, we will assume the median value for the categorical variable. I.e. if a subject records 2 times to smoke 1-5 cigarettes, we assume him to be over the limit of 5 cigarettes.

Table 3: Derivation of primary and secondary outcomes.

Outcome	eCRF sheet	Variable	Variable type	Derivation	Outcome type
Primary: continuous smoking abstinence from target quit date to the 6-month follow-up visit AND	cig_ecig	quit_smoke_again	Binary: No, Yes	{[Week 1/2/4/8, and month 6] quit_smoke_again == No AND quit_tobacco_inhale ==	Binary
		quit_tobacco_inhale	Binary: No, Yes		
	cig_ecig_6m	quit_smoke_again	Binary: No, Yes		
		quit_tobacco_inhale	Binary: No, Yes		

urinary level of anabasine (<3 ng/ml) OR	to be defined	to be defined	to be defined	No} AND {x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	vital_6m	vs_co2_level	Continuous		
Secondary outcomes					
continuous smoking abstinence from target quit date to the 6-month follow-up visit AND	Cig_ecig, cig_ecig_6m	quit_smoke_again, quit_tobacco_inhale	Binary: No, Yes, Binary:No, Yes	{[Week 1/2/4/8, and month 6] quit_smoke_again == No AND quit_tobacco_inhale == No} AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if y==missing & x==missing & vs_co2_level!=missing }	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Continuous smoke abstinence allowing a 2-week grace period after TQD	cig_ecig / cig_ecig_6m	quit_smoke_again quit_tobacco_inhale	Binary: No, Yes	[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2	
Continuous smoke abstinence allowing a 2-week grace period after TQD AND	cig_ecig / cig_ecig_6m to be defined	quit_smoke_again quit_tobacco_inhale quit_smoke_n	Binary: No, Yes Binary: No, Yes categorical: 1-5 cigarettes, > 5 cigarettes	{[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2 AND sum(quit_smoke_n == 1-5) <= 1} AND	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined	{ x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Continuous smoke abstinence allowing a 2-week grace period after TQD AND	cig_ecig / cig_ecig_6m to be defined	quit_smoke_again quit_tobacco_inhale quit_smoke_n	Binary: No, Yes Binary: No, Yes categorical: 1-5 cigarettes, > 5 cigarettes	{[Week 4, Week 8, and 6 months] sum(quit_smoke_again == No) >= 2 AND sum(quit_tobacco_inhale == No) >= 2 AND sum(quit_smoke_n == 1-5) <= 1} AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{ y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		

CO level	Vital, Vital_6m	vs_co2_level	continuous	y==missing & x==missing & vs_co2_level!=missing }	
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD	cig_ecig / cig_ecig_6m	quit_smoke_n	categorical	sum(quit_smoke_n == 1-5) <= 1	Binary
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD AND	cig_ecig / cig_ecig_6m	quit_smoke_n	Categorical	sum(quit_smoke_n == 1-5) <= 1 AND	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined	{ x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported smoking abstinence allowing up to 5 cigarettes in total after the TQD AND	cig_ecig / cig_ecig_6m	quit_smoke_n	Categorical	sum(quit_smoke_n == 1-5) <= 1 AND	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined	{ y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if y==missing & x==missing & vs_co2_level!=missing }	
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported 7-day point prevalence abstinence at 6 months	cig_ecig_6m	quit_smoke_again, quit_smoke_7days	Binary: No, Yes	quit_smoke_again == No AND quit_smoke_7days == No	Binary
Self-reported 7-day point prevalence abstinence at 6 months AND	cig_ecig_6m	quit_smoke_again quit_smoke_7days	Binary: No, Yes Binary: No, Yes	{ quit_smoke_again == No AND quit_smoke_7days == No} AND { x < 3 if x!=missing, vs_co2_level<10 if x==missing & vs_co2_level ==missing}	Binary
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		
CO level	Vital, Vital_6m	vs_co2_level	continuous		
Self-reported 7-day point prevalence abstinence AND	cig_ecig_6m	quit_smoke_again, quit_smoke_7days	Binary: No, Yes	{quit_smoke_again == No AND quit_smoke_7days == No} AND {y < 10 if y!=missing, x<3 if y==missing & x!=missing, vs_co2_level<10 if	Binary
urinary level of NNAL (<10 pg/ml creat) OR	To be defined	To be defined	To be defined		
urinary level of anabasine (<3 ng/ml) OR	To be defined	To be defined	To be defined		

CO level	Vital, Vital_6m	vs_co2_level	continuous	y==missing & x==missing & vs_co2_level!=missing}	
Number of cigarettes smoked per day (CPD)	cig_ecig / cig_ecig_6m	quit_more1perday	continuous (number of cigarettes per day in the last 7 days)	[BL, TQD, phone visits and 6-month visit] quit_more1perday	Continuous
Change in the self-reported number of cigarettes smoked per day in the last 7 days	cig_ecig / cig_ecig_6m	quit_more1perday	continuous (number of cigarettes per day in the last 7 days)	(quit_more1perday[month 6] / quit_more1perday [day 0]) >= 0.5 IF [month 6]{contact_estab_yn == Yes AND quit_smoke_again == Yes AND quit_smoke_7days == Yes AND quit_smoke_nperday == ">=1x/Tag"} ELSE TRUE	Binary
CAT	cat / cat_6m	q1 to q8	radio (0 to 5)	sum(q1 to q8)[cat_6m] – sum(q1 to q8)[cat]	continuous (score scale 0-40, difference can be 0-40 as well)
Cholesterin (total)	vital vital_6m	lab_choles_total	continuous (mmol/L)	lab_choles_total[vital_6m] – lab_choles_total[vital]	continuous (mmol/L)
LDL cholesterol	calculated	calculated	Continuous (mmol/L)	lab_choles_total[vital_6m]-lab_choles_hdl[vital_6m]-(lab_triglyceride[vital_6m] /5)-lab_choles_total[vital]-lab_choles_hdl[vital]-(lab_triglyceride[vital] /5) [Friedewald formula]	continuous (mmol/L)
HDL cholesterol	vital vital_6m	lab_choles_hdl	continuous (mmol/L)	lab_choles_hdl[vital_6m] – lab_choles_hdl[vital]	continuous (mmol/L)
triglycerides	vital vital_6m	lab_triglyceride	continuous (mmol/L)	lab_triglyceride[vital_6m] - lab_triglyceride[vital]	continuous (mmol/L)
blood pressure	vital vital_6m	vs_bpressure_sys1-3, vs_bpressure_dias1-3	continuous (mmHg)	mean(vs_bpressure_sys1-3), mean(vs_bpressure_dias1-3)	continuous (mmHg)
heart rate (mean of the second and third measure)	vital vital_6m	vs_heart_rate_2 vs_heart_rate_3	continuous (bpm)	mean(vs_heart_rate_2, vs_heart_rate_3)[vital_6m] – mean(vs_heart_rate_2, vs_heart_rate_3)[vital]	continuous (bpm)
waist circumference	vital vital_6m	vs_waist_cm	continuous (cm)	vs_waist_cm[vital_6m] - vs_waist_cm[vital]	continuous (cm)
BMI	vital vital_6m	bmi	continuous (kg/m^2)	bmi[vital_6m] – bmi[vital]	continuous (kg/m^2)
Lab values					
Metabolites of nicotine:					

Anabasine	To be defined	To be defined	dichotomized at <3 ng/ml	anabasine < 3	Binary: <3ng/ml, ≥3ng/ml
Metabolites of TSNAs:					
NNAL	To be defined	To be defined	dichotomized at <10pg/ml	NNAL < 10	Binary: <10pg/ml, ≥10pg/ml

6.3 Analysis methods

6.3.1 Primary analysis

The primary analysis is based on the treatment policy estimand, using the full analysis set according to the intention to treat principle.

The primary outcome will be presented as proportion with 95% Wilson confidence interval in each intervention group and compared between groups using the chi-square test. The effect size will be presented as relative risk with 95% Koopman confidence interval, as well as risk difference with 95% Newcombe-hybrid-score confidence interval.

Secondary smoking outcomes will be analysed as the primary outcome.

Continuous secondary outcomes will be analysed using linear models, adjusted for the outcome measure at baseline, if it is available. Missing values in continuous outcomes will be imputed. Thus, the estimates of the complete data models are averaged using Rubin's rule (Rubin 1987). Effect sizes will be presented as mean difference with 95% confidence interval.

6.3.2 Secondary analyses

Secondary analyses are based on the principal strata estimand, using the per-protocol analysis set. Binary outcomes will be presented as proportion with 95% Wilson confidence interval in each intervention group and compared between groups using the chi-square test. Effect sizes will be presented as relative risk with 95% Koopman confidence interval, as well as risk difference with 95% Newcombe-hybrid-score confidence interval. Continuous outcomes will be analysed using linear models, adjusted for outcome measures at baseline, if available, with mean differences (95% confidence interval) as effect sizes. If secondary outcomes are missing, we will use the imputed datasets generated for the primary analysis.

6.3.3 Sensitivity analyses

As sensitivity analysis, we will do an available case analysis of the primary outcome, using the full analysis patient set. No further sensitivity analyses will be performed by CTU Bern.

6.3.4 Subgroup analyses

No subgroup analyses are planned to be performed by CTU Bern.

6.3.5 Additional analyses

No further analyses are planned to be performed by CTU Bern.

6.3.6 Assessment of statistical assumptions

To test model assumptions for continuous secondary outcomes, we will visually check residuals in QQ-plots and residual versus fitted plots.

If model assumptions are violated, transformation of the outcome (e.g. log-transformation), more robust methods (e.g. robust standard errors or robust regression) or non-parametric methods (i.e. Wilcoxon rank-sum test) will be considered.

6.4 Interim analyses

No interim analysis is planned

6.5 Missing data

Missing values in the primary outcome will be categorized as persistent smokers. We intend to obtain full follow-up data on all randomized participants.

Missing data in secondary smoking cessation outcomes will be treated as the primary outcome. If more than 5% of data is missing, all other secondary outcomes will be imputed, assuming values to be missing at random. Each outcome will be imputed separately to ensure robust imputation models. We will use all baseline variables (see Table 2) and outcome measures at all time points as predictors in the imputation models. The two intervention groups will be imputed separately. Variables with more than 50% missing values will not be used for the imputation model. Binary variables with a frequency of less than 5% in one category will be omitted from the predictors, levels of categorical variables with a frequency of less than 5% in one category will be combined with another level in a sensible way. Continuous variables will be log-transformed if it improves normality (checked by Shapiro-Wilks test and QQ plots). If predictors are too highly correlated among each other, we will only consider the predictor which is more strongly correlated with the outcome. In case of missing baseline covariates, ~~W~~we will use multiple imputation by chained equation. We will impute values using predictive mean matching for continuous and ordinal variables, logistic regression for binary variables, and a multinomial regression model for categorical variables with more than two levels. In total, fifty imputed data sets will be generated, which will be analysed as described using Rubin's rules (Rubin 1987). We will compute stabilized inverse probability of censoring weights to take into account potentially informative censoring or outcome data.

6.6 Safety evaluation

Safety outcomes will be analysed using the ITT and PP set.

The safety analysis will list the number of patients dropping out for both treatment arms, including the reasons, and a list of events for patients dropping out, with the treatment group, relation, grade, type of AE and potential free text.

AEs and SAEs will be summarized by presenting for both treatment arms the number of any AE, any SAE, and the number and percentage of patients with any AE or any SAE.

Further, we will present the

- grade and relation of AEs and SAEs (number of patients with events and the number of events),
- category and grade of AEs and SAEs
- category and relation of AEs and SAEs
- AEs and SAEs stratified by consumption (cigarettes, ENDS, both, none)

6.7 Subproject

Subprojects and further outcomes of interest are registered in clinicaltrials.gov under separate registration numbers. These subprojects and outcomes are not part of the current SAP, nor will they be analysed by CTU Bern.

6.8 Statistical software

All analyses will be done using the current version of Stata (StataCorp. 2019 Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.). The version of Stata and all used packages will be listed in the statistical report.

6.9 Quality control

The primary analysis of the primary and secondary outcomes will be double programmed by a second, unblinded statistician.

7. Changes from the protocol

The SAP is consistent with principle features of the statistical methods described in the protocol. The SAP only describes the primary and secondary analysis of primary and secondary outcomes. Any deviation from the protocol is detailed hereunder with reason. Further analysis of these outcomes, as well

as for additional outcomes are planned by the PI and described in the protocol, but will not be further specified in this SAP.

Table 4: Changes from protocol

Header	Change	Reason
6.1 Outcome definitions	This SAP only focuses on the primary outcome for the main analysis at 6-month follow-up. The primary outcome for the extended follow-ups at 12- and 24-months is not part of this SAP.	Initial outcome defined in the initial protocol was at 6-month follow-up.
2.8. Blinding	All analyses will be unblinded	In unplanned interim analyses, we noticed the low yield of blinded analyses. The study team, aware of allocation took over all analyses.

8. References

Estimands and SOPs

European Medicines Agency, Committee for Human Medicinal Products; Draft ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials, step 2b - Revision 1. 30 August 2017.

SOP Data preparation and programming, CS_STA_SOP_05, version 04, 11.09.2018

SOP Statistical data validation, CS_STA_SOP_02, version 03, 11.09.2018

References

StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC.

Changes from originally formulated Statistical analysis plan (SAP)

The originally formulated SAP defined the methods for the main analyses, registered on Clinicaltrials.gov: NCT03589989, to be performed by statisticians at CTU (Clinical trials unit, University of Bern) blinded to randomized groups. During the trial, in the context of the e-cigarette or vaping use-associated lung injury (EVALI) epidemic, there were concerns from scientists and experts worldwide about a potential causal link of ENDS on respiratory outcomes. The study team performed unscheduled interim analyses to verify if participants randomized so far might be harmed by the study products delivered in the intervention group. In the ESTxENDS RCT protocol, we had not formulated interim analyses and had no formal pre-defined stopping rules of the RCT because of futility or potential harm to participants. During these analyses, we exchanged with members of the CTU analysis team and noticed blinding of study allocation would not add substantial benefit to the analyses, making the need for the blinded statistical analyses void. The ESTxENDS research team performed all analyses.

Compared to the last version of the SAP, we made following adjustments:

- Lead statistician: Stephanie Baggio, PhD, BIHAM, University of Bern
- Statistician performing all analyses: Anna Schöni, PhD, BIHAM, University of Bern
- 2.8. Blinding: All analyses were unblinded to allocated groups.
- 6.5. Missing data: Instead of multiple imputation by chained equation (MICE), we computed stabilized inverse probability of censoring weights since there were no missing data at baseline.