STATISTICAL ANALYSIS PLAN

Final

Cess@Tion – Combining cytisine and nicotine vapes: a randomised trial in smoking cessation

Trial Registration Number: NCT05311085

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NIHI
The National Institute for Health Innovation

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1. STUDY OBJECTIVES

The primary objective of this trial is to evaluate the effectiveness, safety, and acceptability of combining cytisine with nicotine e-cigarettes plus text-based behavioural support (TBS), compared to cytisine plus TBS or nicotine e-cigarettes plus TBS, on six-month smoking abstinence.

We hypothesise that cytisine plus nicotine e-cigarettes and TBS is more effective at helping smokers quit than cytisine plus TBS. Furthermore, cytisine plus nicotine e-cigarettes and TBS is more effective at helping smokers quit than nicotine e-cigarettes plus TBS.

2. STUDY DESIGN

Please refer to the study protocol for a full description of the trial design. This is a single-blind, pragmatic three-arm, parallel group, randomised trial. Participants will be recruited nationwide, using multi-media advertising with targeted promotion to reach Māori, Pacific and low socio-economic groups given their disproportionately higher smoking prevalence.

Potential participants interested in the trial will register on a trial website. Participants will be asked for on-line consent to complete an online screening questionnaire to determine their eligibility for the trial and verify their phone number. Eligible and interested participants will then provide online consent to enter the trial.

A copy of the consent form and participant information sheet will be automatically emailed by the online system to the participant for their records. Participants will also be asked to provide online consent to inform their general practitioner that they are participating in the trial. Via the online platform the baseline data will be collected, and then participants will immediately be randomised to either 12 weeks of: 1) cytisine plus nicotine e-cigarette, 2) cytisine; or 3) nicotine e-cigarette.

All 12-weeks of trial products will be couriered to participants immediately after randomisation. The courier company will notify the study centre immediately after the courier pack has been delivered, which will trigger the scheduling of the ‘quit date’ follow-up call and the start of the text-based behavioural support programme. Participants will be asked to begin their treatment the day after they receive their courier pack and to reduce their smoking *ad libitum* over the first four days of treatment so that they are not smoking at all by the fifth day (‘quit date’).

Participants will be called on their quit date to verify they are quitting on that day (and to collect outcome data). If a participant states that they are not quitting on the scheduled ‘quit date’, they will be given one chance to reset their quit date within the next seven days, with this date becoming their new ‘quit date’ (which will trigger the scheduling of all subsequent follow-up calls). For participants who are unable to be contacted at quit date, their ‘quit date’ will be set in the system as six days after they received their courier pack (which will trigger the scheduling of all subsequent follow-up calls). Participants will be advised to continue with their allocated treatment irrespective of any lapses back to smoking.

Outcome assessments will be undertaken at one, three, and six months after the Quit date, and at 12 months in a subsample. At the six-month follow-up, in-person carbon monoxide tests will be performed for participants who live in the Auckland region to verify self-reported continuous smoking abstinence.

2.1. Eligibility criteria

Participants will be eligible for inclusion if they:

- Are daily smokers who live in New Zealand
- Are at least 18 years of age
- Want to quit smoking in the next 2 weeks
- Are able to provide online consent
- Have access to a mobile telephone that can text and have access to the internet via a computer or smart phone
- Are willing to use cytisine or an e-cigarette or both products to help quit smoking

Exclusion criteria:

- Are pregnant or breastfeeding or if they are women trying to become pregnant in the next three months
- Currently using smoking cessation medication (including daily use of an e-cigarette for the last month)
- Are enrolled in another smoking cessation program or trial
- Have known hypersensitivity to cytisine, or nicotine e-cigarettes
- Have a strong preference to use or not to use cytisine and/or e-cigarettes in their quit attempt
- Self-report that they have moderate or severe renal impairment, are undergoing treatment for active or latent tuberculosis, have experienced a myocardial infarction, stroke, or severe angina within the previous two weeks, have uncontrolled high blood pressure (>150 mmHg systolic, >100 mmHg diastolic) or have a history of seizures
- Have another person in their household involved in the trial

2.2. Study intervention

Participants allocated cytisine will be instructed to follow the manufacturer's 25-day dosing regimen:

- days 1-3: one tablet (1.5mg) every two hours through the waking day (six tablets/day)
- days 4-12: one tablet every 2.5 hours (five tablets/day)
- days 13-16: one tablet every three hours (four tablets/day)
- days 17-20: one tablet every 4-5 hours (three tablets/day)
- days 21-25: one tablet every six hours (two tablets/day)

However, a maintenance dose of cytisine will be added for day 26 to week 12 (one tablet every six hours: two tablets/day).

Participants allocated a nicotine e-cigarette will be instructed to follow the manufacturer's instructions for use, with ad libitum use over 12 weeks. The e-cigarette device used in the trial will be the 'UpOX', a closed pod system with a 3% nicotine salt (30mg/mL). A tobacco flavour e-liquid will be provided as this is the usual flavour chosen by smokers when they are transitioning away from tobacco. Participants will be advised that they should try and use only the product provided,

but if they are finding the nicotine strength is not sufficiently addressing their carvings (or the flavour is distasteful) they are free to try alternative nicotine strengths or flavours, but that this will be at their own cost). Participants will be asked at follow-up about any switching of products. The content of the nicotine e-liquid supplied will be independently assessed (by LabTech Scientific and Technical Services, Auckland) to verify nicotine content is as labelled. Batch-to-batch variability in nicotine content in the e-liquids will also be assessed.

All participants will receive the text-based behavioural support STOMP programme (developed and tested by the National Institute of Health Innovation, University of Auckland). This text message programme provides personalised smoking cessation advice, support, and motivation to support individuals to quit smoking and maintain cessation. The programme includes messages covering information relevant to quitting, tips to cope with cravings, advice on avoiding smoking triggers, and motivational support. The programme includes 2-way functionality to support individuals during cravings and is personally tailored. Regular, personalised text messages providing smoking cessation advice, support, and distraction will be delivered over a six-month period (five messages a day for six weeks, then three per week until the end of the 26th week – i.e., six-month follow-up).

2.3. Randomisation

All participants who fulfil eligibility criteria will be randomly allocated in a 3:3:2 ratio to one of the 3 treatment groups using block randomisation, and stratified by ethnicity (Māori, non-Māori). The randomisation sequence will be prepared by the study statistician and loaded into a secure database.

2.4. Sample size

A sample size of 800 (N=300 in the cytisine group, N=300 in the cytisine plus nicotine e-cigarette group, and N=200 in the nicotine e-cigarette group) will provide 90% power at two-sided p=0.05 to detect an absolute difference of 13% in six-month smoking abstinence rates between the combination treatment group and the cytisine group, and 16% difference between the combination treatment group and the nicotine e-cigarette group (taking account of multiple testing).

The predicted difference is based on trial evidence for six month verified continuous abstinence quit rates of 9% for nicotine e-cigarettes, 12% for cytisine, and 25% for combination cessation treatment (averaged). The sample size accounts for a 28% loss-to-follow-up at six months.

3. BASELINE DATA

The following baseline data will be collected online via the trial website:

- **Demographic information**: Date of birth, gender, ethnicity, and level of education. Also self-reported height and weight, and body mass index (BMI) will be calculated.
- **Smoking history**: Age when started, number of cigarettes smoked per day, number of years as regular smoker, number of previous attempts to give up in past 12 months (and the longest time they stayed quit and the method used), type of cigarettes smoked per day (e.g. roll-your-own or factory-made) and pack size and how long pack lasts (for roll-your-own users), and whether they had cut down the number of cigarettes they smoked in the past 12 months.

- **Motivation to quit** in the next two weeks, measured using a five-point Likert Scale, where 1=very low motivation and 5=very high.
- **Level of cigarette dependence**: will be determined using the Heaviness of Smoking Index (HSI), which is a two-item measure based on the number of cigarettes smoked per day (categorised as: 0=10 or fewer, 1=11-20, 2=21-30, 3=31 or more) and the time to first cigarette of the day. The HSI total score is calculated by summing these two items and has range 0 to 6 where higher scores indicate greater dependence severity. The HSI total score will be categorised as low addiction (0-2), moderate addiction (3-4) and high addiction (5-6).
- **Other smoking related information**: household smoking, self-rated chances of quitting (measured using a five-point Likert Scale, where 1=extremely low and 5=extremely high), and whether they are around others that use e-cigarettes.
- **General Health**: Self-reported shortness of breath, cough, asthma, chronic pain, and Chronic Obstructive Pulmonary Disease (COPD), and current or history of mental health (including depression, schizophrenia, and anxiety).
- **Alcohol use**: Measured using the Alcohol Use Disorders Identification Test (AUDIT-C). The AUDIT-C score is calculated by summing all three items and has range 0 to 12 where higher scores indicate greater risk of alcohol dependence. Note participants that tick 'Never' for the first AUDIT-C item (How often do you drink alcohol in the past year) then skip the next two AUDIT-C items, so for these AUDIT-C score to be set to value 0. For men, an AUDIT-C score ≥ 4 indicates an increased risk of hazardous drinking or alcohol dependence, while in women it is a score of ≥ 3. And an AUDIT-C score ≥ 8 indicates a very high risk for both men and women.
- **Health related quality of life:** measured using the NZ EQ-5D
- **Signs and symptoms of nicotine withdrawal and urge to smoke:** measured using the Mood and Physical Symptoms Scale (MPSS) which consists of the following:
    - 5-point ratings of depressed mood, irritability, restlessness, hunger and difficulty concentrating. MPSS score is calculated by summing all 5 items and has a range of 5 to 25 where higher scores indicate greater nicotine withdrawal.
    - 6-point ratings of time spent with urges to smoke and strength of urges to smoke. Note the strength of urges question has values 1 to 5. Participants who tick 'Not at all' for time spent with urges to smoke have to then skip to the next section of questions and don't answer the question on strength of urges. So for these participants need to set strength of urges to equal value 0. [Therefore, this question will then have values 0 to 5]. The urge score is calculated by summing the two urges questions and has a range 0 to 10 where higher scores indicate greater urge to smoke.
- **Cannabis use**: Participants will be asked if they have used cannabis for recreational or non–medical purposes in the last 12 months.

4. STUDY OUTCOMES

4.1. Primary outcome

Verified six-month continuous abstinence (using Russell Standard) defined as self-report of smoking not more than five cigarettes from the Quit date and confirmed by an expired air carbon-monoxide (CO) measurement with a Bedfont Smokerlyzer (≤9 ppm signifying abstinence). Biochemical validation of self-reported cessation for Auckland participants will involve a researcher visiting participants within 72 hours if they claim to be abstinent, to obtain a CO reading. For participants outside of Auckland who self-report that they are smokefree, verbal consent will be sought from them to provide their contact details to their region's community-based smoking cessation service, so that they can undertake the CO monitoring within 72 hours. Sensitivity analysis will be undertaken looking at different cut-offs for the CO measurement, given lack of consensus about the best reading to use

4.2. Secondary outcomes

The following secondary outcome measures will be collected over the phone on the quit date and at one, three, six and 12 months post-quit date:

- **Continuous abstinence** rates (using Russell standard) defined as self-report of smoking not more than five cigarettes in total from quit date (not collected at quit date)

  Note if previous follow-up forms state they smoke more than 5 cigarettes since Quit date then subsequent follow-ups will be set to Not quit. Also, if a follow-up form is missing but the subsequent follow-up form(s) says they have smoked less than 5 cigarettes since Quit date (and no other previous follow-up forms have said they smoke more than 5 cigarettes since Quit date), then the missing follow-up form will have continuous abstinence set to 'Quit'.

- **Seven-day self-reported point prevalence abstinence** rates defined as no cigarettes, not even a single puff in the last seven days (not collected at quit date)

- **Change in average number of cigarettes smoked per day** (CPD) for participants still smoking

- **Proportion of participants who have significantly reduced smoking level** defined as reducing consumption by at least 25% (in terms of numbers of cigarettes per day)

- **Time to first lapse** (six and 12 months) defined as time to first cigarette smoked (even a single puff). From first follow-up form with question "Have you smoked any cigarettes at all in the last seven days, even a single puff?" equal to "Yes". Duration is calculated as the days since actual quit day to date of first lapse in the follow-up form.

  Participants that withdraw will be censored on the last available visit, and participants that have not lapsed by the six (or 12) month follow-up will be censored on the six (or 12) months visit date. Note the first follow-up form with a lapse date will be taken since this will be more accurately remembered by participants than later visits.

- **Time to first relapse** defined as time to smoking more than five cigarettes a day for three or more days in a row. From first follow-up form with question "Have you smoked more than 5 cigarettes per day for 3 or more days in a row?" equal to "Yes". Duration is calculated as the days since actual quit day to date of first relapse in the follow-up form

  Participants that withdraw will be censored on the last available visit, and participants that have not relapsed by the six (or 12) month follow-up will be censored on the six (or 12) months visit date. Note the first follow-up form with a relapse date will be taken since this will be more accurately remembered by participants than later visits.

- **Use of other medication and other methods of cessation** (participants will be asked about their use of other methods of cessation, such as NRT, Zyban, clonidine, nortriptyline, e-cigarettes, acupuncture etc)

- **Health-related quality of life** (three, six and 12 months) measured using the New Zealand EQ-5D

- **Treatment use and compliance** (quit date, one and three months): use of the allocated product. This will include daily use (and reasons for not using daily), and number of pills or pods remaining.

  For participants allocated to cytisine, compliance is defined as having taken ≥80% of the required number of tablets over the three-month intervention period.

- **Signs and symptoms of nicotine withdrawal, and urge to smoke** (three months): measured using the MPSS

- **Crossover** where participants in the cytisine-only group will be asked whether they accessed and used an e-cigarette (with or without nicotine) during the six (or 12) month study period, and participants in the e-cigarette group will be asked whether they accessed and used cytisine during the trial.

- **Change in e-cigarette use**: Participants allocated to the e-cigarette groups will be asked whether they changed the type of e-cigarette device and/or the nicotine strength and/or flavor they used in the e-cigarettes provided. If they did, they will be asked when they did this, and what the device type, nicotine strength and/or flavor was.

- **Dual use** (not collected at quit date): defined as daily use of both their allocated treatment and continued smoking of cigarettes every day.

- **Continuation of product use** (at six and 12 months in a subsample) defined as continued use of their allocated treatment after the end of the designed three-month treatment period.

- **Acceptability of the product** (three months): Participants will be asked for their views on the use of their allocated product as a cessation aid

- **Recommendations** (three months): Participants will be asked whether they would recommend their allocated treatment to another smoker who wanted to quit.

- **Adverse events and serious adverse events**: self-reported adverse events are collected at each follow-up call, with adverse events coded using MedDRA. In addition, participants can at any time report an adverse event via a web-based adverse event diary.

- **Change from baseline in weight and body mass index** (three and six months)
- **Change from baseline in general health** (three, six and 12 months): shortness of breath, cough, asthma, chronic pain, COPD, and mental health. Measured for participants who answered Yes to the general health question at baseline, and is measured using a five-point scale (much worse, a bit worse, about the same, a bit better, much worse).
- **Concomitant medication**: other medications taken during the course of the study
- **Cost outcomes**: cost-per-quitter, cost-per-person reducing their daily cigarette consumption, and the incremental cost-effectiveness ratio
- **Text-based behavioral support STOMP program received:** total number received up until end of the six month follow-up or they opt out of receiving the messages.

5. BIOSTATISTICS QUALITY ASSURANCE

Well in advance of study data-lock, programming across all analyses will commence with dummy data. This will allow sufficient time to turn around the 'real' analyses as quickly as possible. Early programming will also allow for a preliminary run at merging of the various datasets into the correct data structure required for analysis. Specifically, the datasets will be taken before data-lock and passed through the necessary biostatistical processes (which will include checking that all the datasets have been imported correctly into SAS and are all able to be correctly merged together).

6. ANALYSIS POPULATIONS

6.1. Intention to Treat

All treatment evaluations will be performed on the principle of 'Intention To Treat' (ITT) unless otherwise specified. The ITT population will consist of all randomised participants regardless of whether they actually satisfied the entry criteria, the treatment actually received, and subsequent withdrawal or deviation from the protocol.

6.2. Per Protocol

A per protocol analysis will also be performed on the primary outcome in order to check the robustness of the results. A per protocol analysis involves looking at the major protocol violations such as cross-over treatments, withdrawals and lost to follow-up. Criteria for defining the per protocol population will include consideration of the following: those who have complied with the treatment allocated, were exposed to the intervention as planned (i.e., used only assigned intervention), were not lost to follow-up, and who do not have major protocol deviations pertaining to eligibility or the assessment of the treatment difference.

7. STATISTICAL ANALYSIS

All statistical analyses will be performed using SAS version 9.4 and R version 3.4.2. Data collected in the NIHI Redcap database will be extracted into SAS for the analyses. All statistical tests will be two-tailed and a 5% significance level throughout the analyses. No adjustments for multiplicity are planned for any of the outcomes.

Summaries of continuous variables which are normally distributed will be presented as means and standard deviations or medians and inter-quartiles for skewed data, while categorical variables will be presented as frequencies and percentages. Continuous variables will be compared with t-tests or Mann-Whitney tests and categorical data with chi-squared tests as appropriate.

A separate EXCEL file containing the SAP tables specific to this study will be provided based on all the analyses stated below (see Appendix I).

All regression analyses will be conducted for the following two comparisons:

- Cytisine plus nicotine e-cigarette group vs Cytisine only group
- Cytisine plus nicotine e-cigarette group vs Nicotine e-cigarette only group

Exploratory analyses will also be conducted comparing the Cytisine only and Nicotine e-cigarette only groups for the primary outcome.

7.1. CONSORT flowchart

All participants who registered online for the trial will be accounted for and a CONSORT flow chart prepared as Figure 1 for the main paper. The reasons for non-participation will be discussed in relation to the external validity of the study and the pattern of protocol violations considered as potential sources of bias. Reasons for early withdrawal will be listed for all participants that prematurely discontinued their allocated intervention or the study. The number of participants that were registered but not randomised will also be presented.

7.2. Participant accountability

Tables describing patient accountability will be produced. The number of participants who were registered, fulfilled eligibility criterion, together with reasons for exclusion will be summarised. The status of participants at each follow-up visit, and the number of protocol violations will also be summarised.

7.3. Baseline characteristics

Baseline variables will be summarised for each intervention group. Since any differences between the groups at baseline could only have occurred by chance, no formal significance testing will be conducted.

7.4. Concomitant medications

Concomitant medications (coded to the Anatomical Therapeutic Chemical, ATC, classification system) by number of events and people will be summarised for each intervention group. Separate tables will be given for general ATC categories, and for the detailed ATC codes.

7.5. Primary outcome analysis

The proportion of participants that have been biochemically verified as continuously abstinent at six months by group will be analysed using log-binomial regression, and the risk in each group, relative risks, absolute risk differences, and corresponding 95% confidence intervals will be calculated. All analyses for the

primary outcome will be ITT (unless otherwise stated) and include all randomised participants. Missing outcomes will be imputed using multiple imputation assuming the data is missing at random. Multiple imputed datasets will be created (n=50) and the imputation model will include baseline age, sex, and group, and the fully conditional specification logistic regression method will be used to impute the missing outcomes. The imputed datasets will be analysed using log-binomial regression and combined to output one inference. Adjusted analyses for potential covariates will be conducted if needed.

To check the robustness of the primary outcome the following sensitivity analyses will be conducted:

- self-reported continuous abstinence at 6 months (not biochemically verified)
- using the following varying cut-offs used for CO measurements: ≤ 3 ppm, ≤ 5 ppm, ≤ 8 ppm

The following sensitivity analyses will also be conducted for the primary outcome where groups will be compared using chi-squared tests:

- complete case analysis
- per protocol analyses where participants that have any major protocol violations will be excluded (see 6.2)
- assuming all missing outcomes are still smoking instead of using multiple imputation

Tests for heterogeneity will be used to examine if there is any difference in the groups on the unadjusted primary outcome for the following subgroups: baseline age (will be dichotomised based on the median), sex, ethnicity (Māori, non-Māori), education (< 12 years of attending school, 12 or more years of attending school), type of cigarettes smoked (RYO, factory made, both), AUDIT-C (low, high), cannabis use, motivation to quit, level of cigarette dependence (using HSI), and level of behavioural support received (will be categorised based on the data). Subgroup analyses will also be conducted by batch number of product received (Batch 1: early 2022, Batch 2: early 2023) to assess if there were any differences by batch.

7.6. Secondary outcomes analysis

Descriptive summary statistics for each follow-up visit and group will be presented for all secondary outcomes. For all secondary outcomes (unless otherwise stated) participants with missing outcome data will be excluded from the analyses.

Incidence rates, risk difference, relative risks and 95% confidence intervals will be calculated for self-reported continuous abstinence (not biochemically verified), seven-day point prevalence abstinence and reduced CPD consumption by at least 50% outcomes at each follow-up visit. Both complete case analysis and ITT analysis (where missing outcomes will be imputed using the same multiple imputation method described above for the primary outcome) will be conducted.

MPSS at 3 months (for 6-month continuous abstainers only) will be analysed using linear regression and include baseline MPSS in the model. The change from baseline in each of the repeated continuous outcomes (average number of cigarettes smoked each day for smokers, weight and BMI) will be analysed using mixed models. Each model will include group and the baseline measure. The appropriate covariance structure to be used will be assessed by the likelihood ratio test. The method of maximum likelihood will be employed to ensure patients with missing data are included in the model assuming the data is missing at random. However, if all follow-up data is missing then the participant will be excluded from the analyses. Intervention by visit interaction effects will be tested. Note the

distribution of these continuous outcomes will be first assessed for normality and skewed data will be subjected to an appropriate transformation before analysis. Non-parametric analysis (Mann-Whitney tests) will be used for the continuous outcomes if data is skewed and cannot be transformed to be normally distributed.

The time to first lapse and relapse outcomes will be analysed using Kaplan-Meier curves, the log rank test, and Cox proportional hazards regression analysis.

If the primary outcome is significant then cost analyses will be conducted. The cost per quitter, cost per person reducing their daily cigarette consumption and incremental cost-effectiveness ratio will be compared with New Zealand data from Quitline and other NRT service providers, and international studies, and will take a health sector perspective. However, the tobacco expenditure savings to individual smokers will also be calculated (for those who quit and cut down) to give a more societal perspective on the benefits. This calculation will use data on the daily amount smoked prior to quitting and the price of the particular products smoked. For those who cut down their consumption by a significant margin (i.e., 25% or more), the cost per person reducing their daily cigarette consumption will be calculated.

7.7. Adverse events

All adverse event data (serious and non-serious) for the duration of the study will be reported and coded to MedDRA classification system.

Adverse event counts by number of events and people will be summarised by group. Also, adverse event counts by categories collected in the adverse event form (type, relationship to study treatment and seriousness) will be summarised and separate table will also be given for the incidence rate ratios comparing the intervention arms. Line listings will also be produced in order to examine all serious adverse events and multiple adverse events for a participant on the same date.

Additional adverse event analysis related to the CONSORT harms extension will be conducted where possible.

8. PEER REVIEW

The statistical analysis and report for the primary outcome will be peer reviewed by an independent member of the NIHI Biostatistics team (not involved in the study).