Study Statistical Analysis Plan

Vascular Effects of Smoking Usual cigarettes Versus electronIc cigaretteS

(The VESUVIUS Trial)

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By signing this document I am confirming that I have read, understood and approve the protocol for the above study.

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Chief Investigator _____ Signature _____ Date

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Trial Statistician _____ Signature _____ Date

Contents

1 ABBREVIATIONS

EC	Electronic Cigarettes
FMD	Flow Mediated Dilation
TC	Traditional Cigarettes
LDL	Low Density Lipoprotein
hs-CRP	High sensitivity C-Reactive Protein
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
NHS	National Health Service
PWA	Pulse Wave Analysis
PT	Preferred Term
PWV	Pulse Wave Velocity
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System organ class
SOP	Standard Operating Procedure

2 SUMMARY

Electronic cigarettes (EC) are gaining popularity as an alternative to Traditional Cigarettes (TC). Despite not containing all the harmful substances seen in TC, EC are known to contain impurities that may have a detrimental impact on human health. The effects of ECs compared with TCs on vascular function, inflammation and oxidative stress are unknown. It is increasingly recognised that nicotine itself has significant atherothrombotic effects. Therefore, clinicians are unable to confidently recommend ECs as a method of smoking cessation. We will study 108 smokers with no significant past medical history in a parallel group design randomized to continue their TC, switch to EC plus nicotine or EC minus nicotine for one month. We will measure baseline and post study endothelial function using flow mediated dilatation which will be performed by a single blinded researcher.

3 STUDY POPULATION

Smokers with no clinical diagnosis of established cardiovascular disease

4 STUDY DESIGN

The VESUVIUS trial is an explanatory randomised, single blinded, placebo controlled single-centre study conducted in NHS Tayside to compare the effect of electronic cigarettes with nicotine, electronic cigarettes without nicotine and traditional cigarettes. Participants will be enrolled in this trial for a period of 4 weeks. To ensure balanced assignment across critical variables, a minimisation algorithm will be employed, using baseline age (≤40 years; >40 years), sex (male; female) and smoking pack years (≤20 pack years; >20 pack years).

4.1 PRIMARY OUTCOME

- Change in Flow mediated Dilation (FMD) between the TC group and the EC-nicotine and EC-nicotine free groups

4.2 SECONDARY OUTCOMES

- Change in FMD between EC-nicotine and EC-nicotine free groups
- Change in Pulse Wave Velocity between the TC group, EC-nicotine and the EC-nicotine free groups
- Change in Augmentation Index@75bpm between the TC group, EC-nicotine and the EC-nicotine free groups
- Change in oxidised LDL, hs-CRP, tPA and PAI-1 between the TC, EC-nicotine free and EC-nicotine groups

5 STATISTICS AND DATA ANALYSIS

5.1 SAMPLE SIZE CALCULATION

The primary endpoint measurement is change in brachial artery FMD, which is expressed as the maximum FMD percentage change from baseline. Based on our existing data for this measure (mean response 7.8%, SD=3.5%), and assuming mean FMD reductions of 2%, 1% and 0% in the EC-Nicotine-free, EC-Nicotine, and TC groups respectively with a SD of the change in FMD =3.0% (based on our previous study[1]), and a using a sample size calculation using Linear Contrast Tests[2] a sample size of 36 subjects in each group will have 80% power to detect an improvement in FMD of 2.0% and 1.0% in the EC-nicotine-free and EC-nicotine groups respectively compared to the TC group at 5% significance. Assuming 10%, 15%, and 20% drop-out rates in the TC, EC-nicotine & EC-nicotine-free groups respectively, we will require 135 completed subjects. All drop-outs will be replaced to achieve 36 completed subjects in each group to fulfil the explanatory nature of the trial.

5.2 STATISTICAL ANALYSES

Descriptive statistics in the form of means and standard deviations for continuous variables and percentages and denominators for categorical variables will be tabulated for baseline and at the 4 week visit. The dependent variable will be assessed for approximation to a normal distribution and transformed if necessary. Pre-specified subgroup analyses will be completed by fitting the appropriate interaction term in the regression model and if significant outcomes will be presented separately by level of subgroup. All comparisons will be between treatment groups (EC-nicotine vs EC-nicotine free vs TC) at the final visit (4 weeks), adjusted for the baseline measure of the outcome.

The statistical analysis will be carried out in accordance to TASC SOP 57 "The implementation of statistical analysis in clinical research" by the study team statistician.

5.3 MISSING DATA

The primary analysis will be on a per protocol basis as the trial is essentially explanatory. We will perform an intention to treat analysis as a secondary analysis. The extent of missing data will be examined and the reason for drop-out ascertained and tabulated. Multiple imputation may be used to impute missing values if the assumptions for missing at random (MAR) data are met.

6 GENERAL CONSIDERATIONS

6.1 TIMING & BLINDING OF ANALYSES

The final analysis will be performed after all data have been entered and the database has been locked. Determination of the outcome was blinded. The trial statistician will follow the SAP and so blinding of the statistician is not necessary.

6.2 ANALYSIS POPULATIONS

The primary analysis will be based on the evaluable dataset in an explanatory trial and so is a per-protocol population. A secondary intention-to-treat analysis will also be implemented.

6.3 BASELINE DEMOGRAPHIC, MEDICAL CONDITIONS AND OTHER VARIABLES

Descriptive statistics of the above characteristics will be presented in a table(s).

7 TECHNICAL DETAILS

All analysis will be performed using IBM SPSS 22. Data, analysis programmes and output will be securely backed up. Final analysis programmes will be run without error.

8 PRIMARY ANALYSIS

The FMD response relationship will be assessed by a linear contrast test, and a multiple linear regression on FMD at 4 weeks including the baseline FMD level and experimental group as covariates. The model will also include the minimisation variables; baseline age (≤40 years; >40 years), sex (male; female) and smoking pack years (≤20 pack years; >20 pack years).

8.1 SECONDARY ANALYSES

1) Changes in Oxidised LDL between baseline and Visit 4
2) Changes in High-sensitivity CRP between baseline and Visit 4
3) Changes in Tissue Plasminogen Activator between baseline and Visit 4
4) Changes in Platelet Activation Inhibitor-1 (PAI-1) between baseline and Visit 4

Subgroup analyses: interaction terms of treatment in men vs women, upper tertile baseline smoking versus lower tertile comparisons for all primary and secondary endpoints
All secondary outcomes will be analysed in the same manner as the primary outcome. Results will be presented as between group differences in measured parameters between the baseline and final visit.

8.2 TREATMENT ADHERENCE

We will measure Carbon Monoxide (CO) levels as a recent paper (Yan et al. Regul Toxicol Pharmacol. 2015 Feb;71(1):24-34) has shown that ECs do not affect CO levels.

CO breath test will be measured as an indicator of treatment allocation adherence and will be added to the primary model to assess the effect of adherence.

8.3 ADVERSE EVENTS

All serious adverse events will be presented in a table and in which study arm they occurred . AE's will be coded by SOC and presented in a table with number and study arm.

9 REPORTING CONVENTIONS

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

References:

1. Khan, F., et al., *Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: A randomized controlled trial of antioxidant- and polyphenol-rich blackcurrant juice.* Free Radic Biol Med, 2014. **72**: p. 232-7.
2. Dmitrienko A, C.-S.C., *Pharmaceutical Statistics Using SAS: A Practical Guide (SAS Press) SAS Institute*2007.