

A Longitudinal Ambulatory Study to Assess Changes in Cigarette Consumption Behavior and Biomarkers of Exposure during a 6-Week Switch to Very Low Nicotine Cigarettes

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GCP Statement

This study is to be performed in full compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements. All required study documentation will be archived as required by regulatory authorities.

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PROTOCOL HISTORY

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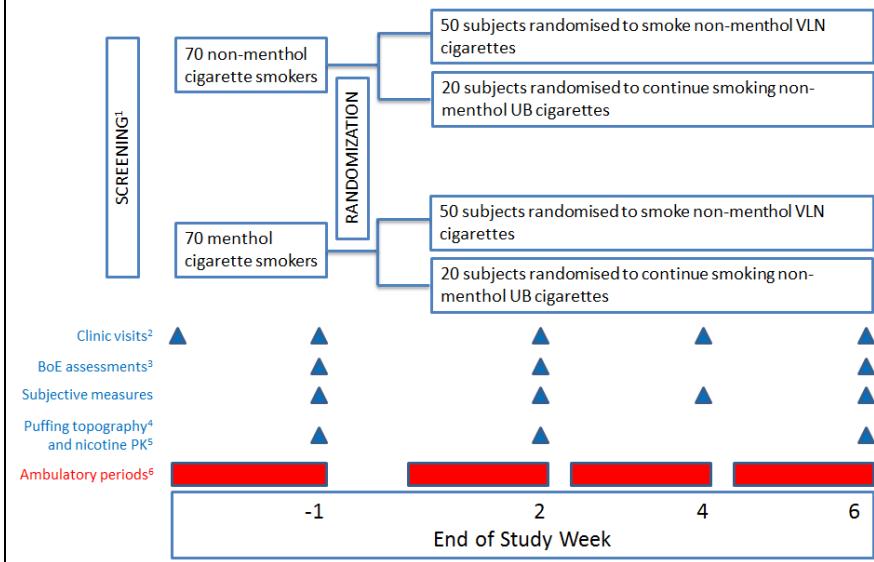
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SYNOPSIS

Study Objectives	<p>Primary objectives:</p> <ol style="list-style-type: none">1. To characterize cigarette consumption behavior (cigarettes per day and smoking topography) before, during and after a switch from usual brand (UB) to very low nicotine (VLN) cigarettes for 6 weeks. <p>Secondary objectives:</p> <ol style="list-style-type: none">1. To evaluate changes in tobacco-related biomarkers of exposure (BoE) before, during and after a 6-week switch from UB to VLN cigarettes.2. To evaluate nicotine pharmacokinetics (PK) before, during and after a 6-week switch from UB to VLN cigarettes.3. To evaluate changes in subjective effects before, during and after a 6-week switch from UB cigarettes to VLN cigarettes
Study Design	<p>This is an open-label, non-randomized, forced-switching study to be conducted at multiple study sites. Seventy (70) self-affirmed exclusive filtered king size non-mentholated cigarette smokers and 70 self-affirmed exclusive filtered king size mentholated cigarette smokers will be enrolled and begin the study at Week -1.</p> <p>All potential subjects will provide informed consent and successfully complete the Screening procedures prior to participation in the study. Subjects will also engage in a brief product trial with the VLN cigarettes. Subjects who react negatively (<i>i.e.</i>, unwilling to use and/or cannot tolerate the product [experience adverse events (AEs) that will prevent them from continuing to use the product as judged by the Investigator]) to the VLN cigarettes during the product trial period will not continue in the study.</p> <p>At the start of Week -1, all subjects will be asked to smoke their UB cigarettes as per their usual daily consumption for the following week. Subjects will receive an electronic diary (e-diary) to record daily cigarette use (cigarettes per day [CPD]). Training in completion of the e-diary will be provided at the visit at the start of Week -1.</p> <p>Subjects will return at the end of Week -1, at the time indicated by the clinical research unit (CRU), for collection of blood and 24-hour urine samples for baseline BoE assessments. Subjective questionnaires for dependence, withdrawal symptoms, urges to smoke, and perceived health risk will also be completed at scheduled times. A subset of 18 non-menthol and 18 menthol smoker subjects will complete an assessment of puffing topography with their UB cigarettes during this visit. A further subset of 12 of the non-menthol and 12 of the menthol smoker subjects who complete the topography assessment will also complete a nicotine PK assessment at the end of this visit. Subjects who undergo topography and PK assessments will be assigned to switch to smoking VLN cigarettes.</p> <p>On Day 1 of Week 1, subjects will be randomly selected to either remain smoking their non-menthol (20 subjects) or menthol (20 subjects) UB cigarettes, or to switch to smoking non-menthol (50 subjects) or menthol (50 subjects) VLN cigarettes as per their UB cigarette flavor. Subjects will return at the end of Weeks 2 and 6, at the time indicated by the CRU, for collection of blood and 24-hour</p>

	<p>urine samples for BoE assessments. Subjective effects questionnaires will also be completed at scheduled times. Subjects will continue recording their CPD in their e-diaries. A subset of 18 non-menthol and 18 menthol smoker subjects will complete an assessment of puffing topography with the VLN cigarettes at these visits, and a further subset of 12 non-menthol and 12 menthol smoker subjects will also complete an assessment of nicotine PK at the end of these visits. Subjects undergoing topography and PK assessments will have been assigned to switch to smoking VLN cigarettes.</p> <p>Additionally, all subjects will visit the clinic at the end of Week 4 to receive further supplies of cigarettes (if assigned to the VLN groups) and to complete subjective effects questionnaires.</p> <p>Subjects randomized to the VLN groups will be provided with a supply of VLN cigarettes at each visit, which will be 150 % of their usual daily consumption as reported during Week -1. If a subject runs out of cigarettes between clinic visits, they may visit the clinic to receive further cigarettes. All subjects will be asked to smoke their cigarettes <i>ad libitum</i>, recording their actual daily consumption in their e-diaries. Non-compliant nicotine product consumption should also be recorded. Used cigarette butts will also be collected during ambulatory periods to verify product use and/or assess compliance. During Week -1 (all subjects) and all subsequent weeks (subjects randomized to continue smoking UB cigarettes) subjects will be asked not to change their UB cigarette brand or flavor.</p> <p>The CRU will attempt to contact all subjects who participated in the study (including subjects who terminate the study early) using their standard procedures approximately 7 days after the last contact to determine if any AE has occurred since the last study visit.</p>  <pre> graph TD A[70 non-menthol cigarette smokers] --> B[70 menthol cigarette smokers] B --> C[50 subjects randomised to smoke non-menthol VLN cigarettes] B --> D[20 subjects randomised to continue smoking non-menthol UB cigarettes] C --> E[50 subjects randomised to smoke non-menthol VLN cigarettes] C --> F[20 subjects randomised to continue smoking non-menthol UB cigarettes] C --> G[Clinic visits²] C --> H[BoE assessments³] C --> I[Subjective measures] C --> J[Puffing topography⁴ and nicotine PKs⁵] C --> K[Armulatory periods⁶] F --> G F --> H F --> I F --> J F --> K E --> G E --> H E --> I E --> J E --> K J --> L[End of Study Week] L --> M[-1] L --> N[2] L --> O[4] L --> P[6] </pre> <p>¹Including trial of VLN cigarettes. ²E-diary training at start of Week -1 ³24h urine BoE, blood COHb and plasma cotinine ⁴Subset of 18 subjects assigned to smoke non-menthol VLN cigarettes and 18 subjects assigned to smoke menthol VLN cigarettes ⁵Subset of 12 subjects assigned to smoke non-menthol VLN cigarettes and 12 subjects assigned to smoke menthol VLN cigarettes ⁶E-diary completion for cigarette consumption, and used cigarette butt collection</p>
Study Population	Seventy (70) self-affirmed, exclusive smokers each of non-mentholated and mentholated King-size cigarettes will be enrolled into the study.

Duration of Study Conduct	Screening events will occur within 28 days prior to Day 1 of Week -1. Subjects will participate in the study for a period of 7 weeks after the start of Week -1, with a follow-up call approximately 7 days after the last study visit.
Study Products and Summarization	<p>The study products are:</p> <ul style="list-style-type: none">• Subject's UB non-mentholated filtered king size cigarettes• Subject's UB mentholated filtered king size cigarettes• Non-mentholated VLN cigarettes• Mentholated VLN cigarettes <p>VLN cigarettes contain 0.4mg nicotine per gram of tobacco.</p> <p>Subjects will smoke their assigned products <i>ad libitum</i> throughout the study. Product use will be listed by subject and summarized by time point (study week).</p>
Consumption and Biomarker Data Parameters and Summarization	<p>During ambulatory periods between clinic visits, subjects will record daily cigarette use (CPD) in their e-diary, including both compliant and non-compliant (nicotine containing products other than their assigned product) use. Subjects will also collect used cigarette butts during these periods.</p> <p>Twenty-four (24)-hour urine collections will be performed during clinic visits at the end of Weeks -1, 2 and 6, for BoE (total NNAL, total NNN, 3-HPMA, S-PMA, 1-OHP and T_{neq}) analysis. Creatinine will also be measured in each 24-hour collection and may be used to adjust the concentration values of the BoE if necessary. Blood samples for carboxyhemoglobin (COHb) and cotinine measurement will also be taken during these visits.</p> <p>All data will be listed by subject and summarized by time point using descriptive statistics.</p>
Puffing topography Data Parameters and Summarization	<p>Puffing topography will be measured in a randomly-selected subset of 18 non-menthol and 18 menthol cigarette smokers during an <i>ad libitum</i> usage session at the clinic visits at the end of Week -1 (UB cigarette) and Weeks 2 and 6 (VLN cigarettes). These subjects will be/have been assigned to switch to smoking VLN cigarettes. Puffing topography will be assessed using the mobile smoking puff analyzer (SPA-M; Sodim) during the confinement periods. Topography assessment will only be performed at one of the two clinical sites (Lincoln).</p> <p>The following topography parameters will be assessed:</p> <ul style="list-style-type: none">• Puff duration• Puff volume• Peak puff flow rate• Average flow rate• Inter-puff interval <p>All data will be listed by subject and summarized using descriptive statistics.</p>

Nicotine PK Data Parameters and Summarization	<p>Plasma nicotine levels will be assessed in a subset of 12 non-menthol and 12 menthol cigarette smokers before, during and after a 5-minute <i>ad libitum</i> smoking session with UB (Week -1) and VLN (Weeks 2 and 6) cigarettes. These subjects will be/have been assigned to switch to smoking VLN cigarettes and will be randomly selected from the subjects selected to undergo topography assessments. Blood samples will be drawn at -5, 2, 5, 7, 10, 12, 15, 20, 30, 45, 60, 90, 120, 150 and 180 minutes relative to the start of cigarette smoking a single cigarette. Nicotine PK assessment will be performed on the day following 24-hour confined urine collection and after an overnight abstinence from cigarette smoking.</p> <p>Plasma nicotine PK parameters, Cmax, Tmax and AUC will be computed from the individual plasma concentrations.</p> <p>Nicotine concentrations and PK parameters will be listed by subject and summarized by study product and sex using descriptive statistics.</p>
Subjective Effects Data Collection and Summarization	<p>Measurement of subjective effects will be made during the clinic visits at Weeks -1, 2, 4 and 6, with the single administration of the Fagerström Test for Cigarette Dependence (FTCD), the Brief Questionnaire of Smoking Urges (QSU-Brief), the Minnesota Nicotine Withdrawal Scale - Revised (MNWS-R) and a perceived health risk scale.</p> <p>All data will be listed by subject and summarized by time point using descriptive statistics.</p>
Safety Assessments and Summarization	<p><u>Baseline visit:</u> At the start of Week -1, evaluations will include vital signs, exhaled breath CO measurement, urine drug and alcohol screen, urine cotinine test and a serum pregnancy test (females only), and body weight.</p> <p><u>On study:</u> At the end of Weeks -1, 2, 4 and 6, evaluations will include vital signs, a urine drug and alcohol screen, and a urine pregnancy test (females only). End of study (or early discontinuation) evaluations will include a 12-lead ECG and vital signs.</p> <p><u>Throughout the study:</u> AEs spontaneously reported by the subjects or observed by the Investigator or other study personnel will be monitored from after the product trial at Screening until the End of Study (or upon early discontinuation), and will be queried by study staff at the follow-up call. Any concomitant medications taken from screening through the end of study (or early discontinuation) will also be recorded. A symptom-driven physical examination may be performed at any time, at the discretion of the Investigator or designee.</p> <p>AEs will be tabulated and summary statistics for vital signs and clinical laboratory tests may be computed and provided, as deemed clinically appropriate.</p>

SUMMARY OF STUDY EVENTS

STUDY EVENTS/ASSESSMENTS	Screening ¹	Clinic Visits					Out-of-CRU Between Clinic Visits	End of study ²	Follow-up Call ³
		Start of Week -1	End of Week -1 (baseline)	End of Week 2	End of Week 4	End of Week 6			
Informed Consent	X								
Demographics	X								
Smoking/Tobacco/Nicotine Product Use History	X								
Usual Brand Cigarette and Flavor Documentation ⁴	X	X	X	X ⁵	X ⁶	X ⁷			
Medical History	X								
Review of Inclusion / Exclusion Criteria	X								
Review of Concomitant Medications						X			
Physical Examination ⁸	X							X ⁹	X ¹⁰
Height, Body Weight, BMI	X	X ¹¹						X ¹²	
Vital Signs	X	X	X	X	X	X		X	
12-Lead ECG	X							X	

¹ Up to 28 days prior to first study visit (start of Week -2).

² At end of Week 6 clinic visit or upon early discontinuation.

³ Approximately 7 days after their last clinic visit, including subjects who discontinue from the study early.

⁴ Including taking a color photocopy of the front and back of the subject's UB cigarette pack along with a ruler.

⁵ For subjects randomized to continue smoking UB cigarettes only.

⁶ For subjects randomized to continue smoking UB cigarettes only.

⁷ For subjects randomized to continue smoking UB cigarettes only.

⁸ Standard physical examination including oral cavity and oropharynx. A symptom driven examination can be performed at other time, at Investigator or designee's discretion.

⁹ Symptom driven and if deemed necessary.

¹⁰ Symptom driven and if deemed necessary based on the follow-up call. Subjects will visit the clinic for this to take place.

¹¹ Body weight only at Start of Week -1

¹² Body weight only at end of study

STUDY EVENTS/ASSESSMENTS	Screening ¹	Clinic Visits					Out-of-CRU Between Clinic Visits		
		Start of Week -1	End of Week -1 (baseline)	End of Week 2	End of Week 4	End of Week 6			
Clinical Chemistry ¹³ , Hematology, Urinalysis	X							X	
Urine Drug and Alcohol Screen	X	X	X	X	X	X		X	
HIV, HBsAg, and HCV Serology	X								
Serum Pregnancy Test (females)	X								
Serum FSH Test ¹⁴	X								
Urine Cotinine Screen	X	X							
Exhaled CO Measurement	X	X							
Test Product (VLN cigarette) Trial	X								
Provide Smoking Cessation Information ¹⁵	X							X	X
Review of AEs	X ¹⁶	X	X	X	X	X		X	X
Urine Pregnancy Test (females)		X	X						
Review Restrictions on Smoking and Nicotine Product Use	X	X	X	X	X	X			
Reminder Contacts to return to the CRU ¹⁷		X ¹⁸					X ¹⁹		
Test Product Dispensing			X	X	X				

¹³ Clinical chemistry tests will be performed after at least an 8 hour fast; however, in case of dropouts or rechecks, subjects may not have fasted for 8 hours prior to the clinical chemistry sample being taken.

¹⁴ Postmenopausal females only

¹⁵ Subjects will be referred to the Quit Assist® website

¹⁶ AE recording to begin immediately after the product trial at Screening.

¹⁷ Phone calls or texts or instant messages (as per site preference) reminding subjects of clinic visits.

¹⁸ The day prior to clinic visit at start of Week -1 subjects will be contacted.

¹⁹The day prior to clinic visits at end of Weeks -1, 2, 4 and 6 subjects will be contacted.

STUDY EVENTS/ASSESSMENTS	Screening ¹	Clinic Visits					Out-of-CRU Between Clinic Visits		
		Start of Week -1	End of Week -1 (baseline)	End of Week 2	End of Week 4	End of Week 6			
Dispense Canisters for Used Cigarette Butt Collection		X	X	X	X				
Used Cigarette Butt Collection							X		
Used Cigarette Butt Canister Return			X	X	X	X			
24-hour Urine Collection for BoE analysis			X	X		X			
Plasma Sample for Cotinine Analysis			X	X		X			
Blood Sample for COHb Analysis			X	X		X			
Puffing Topography ²⁰			X	X		X			
Blood Samples for Nicotine PK Analysis ²¹			X	X		X			
Training on e-diary use		X							
Daily e-diary use to record cigarette consumption		X	X	X	X	X	X		
Fagerström Test for Cigarette Dependence (FTCD)			X	X	X	X			
Brief Questionnaire of Smoking Urges (QSU-brief)			X	X	X	X			
Minnesota Nicotine Withdrawal Scale - Revised (MNWS-R)			X	X	X	X			

²⁰ In a randomly-selected subset of 18 non-menthol and 18 menthol cigarette smokers at a single site (Lincoln). These subjects will be/have been assigned to switch to smoking VLN cigarettes. To be performed during the clinic confinement period.

²¹ In a randomly-selected subset of 12 non-menthol and 12 menthol cigarette smokers at a single site (Lincoln). These subjects will be/have been assigned to switch to smoking VLN cigarettes and will be randomly selected from the subjects selected to undergo topography assessments. To be performed on the day following 24-hour confined urine collection and after an overnight abstinence from cigarette smoking/nicotine product use.

		Clinic Visits					Out-of-CRU Between Clinic Visits		
STUDY EVENTS/ASSESSMENTS	Screening ¹	Start of Week -1	End of Week -1 (baseline)	End of Week 2	End of Week 4	End of Week 6	Weeks -1 to 6	End of study ²	Follow-up Call ³
Perceived health risk scale			X	X	X	X			
Confinement to CRU for 24 hours			X	X		X			
Ambulatory Visits to CRU	X	X			X		X		

ABBREVIATIONS

1-OHP	1-hydroxypyrene
3-HPMA	3-hydroxypropylmercapturic acid
AE	Adverse event
ANPRM	Advanced Notice of Proposed Rule Making
AUC	Area under the concentration curve
BMI	Body mass index
BoE	Biomarker of exposure
bpm	Beats per minute
°C	Degree Celsius
CDMS	Clinical Data Management System
CFR	Code of Federal Regulations
CPD	Cigarettes per day
Cmax	Maximum plasma concentration
CO	Carbon monoxide
COHb	Carboxyhemoglobin
CRF	Case report form
CRU	Clinical research unit
CV	Coefficient of variability
CYP	Cytochrome P450
e-diary	Electronic diary
ECG	Electrocardiogram
eCO	Exhaled carbon monoxide
eCRF	Electronic case report form
EDC	Electronic data capture
°F	Degree Fahrenheit
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
FTCD	Fagerström Test for Cigarette Dependence
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus

HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intent to treat
K ₂ EDTA	Dipotassium ethylenediaminetetraacetic acid
MedDRA	Medical Dictionary for Regulatory Activities
mmHg	Millimeters of mercury
MNWS-R	Minnesota Nicotine Withdrawal Scale - Revised
n	Sample size
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNN	total N-Nitrosonornicotine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
PK	Pharmacokinetic
PP	Per protocol
ppm	Parts per million
QSU-brief	Brief Questionnaire of Smoking Urges
RCF	Relative centrifugal force
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SOP	Standard operating procedure
S-PMA	S-phenylmercapturic acid
T _{max}	Time to maximum plasma concentration
T _{neq}	Total nicotine equivalents
UB	Usual brand
US	United States of America
VLN	Very low nicotine

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1. INTRODUCTION AND BACKGROUND

1.1 Background

A gradual reduction of nicotine levels in cigarettes was first proposed by Benowitz and Henningfield in 1994 (1). In this paper, the authors proposed a long-term (over many years) reduction in cigarette nicotine levels as a measure to reduce smoking addiction and to aid cessation for smokers. The underlying assumption of this proposal was that there is a nicotine exposure threshold, below which reinforcing effects of nicotine are insufficient to cause or sustain addiction. The absolute level of such a threshold has not yet been determined. Hatsukami et al (2) published results of a study where subjects were switched to VLN cigarettes for six weeks. Compensatory smoking behavior, biomarkers of exposure (BoE), and tobacco dependence withdrawal were assessed. VLN cigarettes did not result in compensatory smoking behavior. Cigarettes per day and biomarkers of exposure were reduced after 6 weeks. VLN cigarettes were associated with a greater relief of withdrawal than nicotine lozenges.

In March 2018, the Food and Drug Administration (FDA) announced through an Advanced Notice of Proposed Rule Making (ANPRM) that a tobacco standard for lowering nicotine in cigarettes is being considered (3). As new cigarettes with reduced amounts of nicotine in the tobacco blend are developed, clinical studies are required to assess whether they lead to reductions in cigarette consumption and whether this is associated with reductions in biomarkers of exposure to cigarette smoke toxicants and changes in cigarette dependence. Furthermore, subjective assessments of urges to smoke, withdrawal symptoms and perceived health risks are also useful indicators of whether low nicotine cigarettes will give rise to reduced addiction to cigarette smoking.

1.2 Study Purpose

The purpose of this study is to examine whether measures of cigarette consumption behavior (cigarettes per day and smoking topography) are changed when smokers switch from smoking conventional cigarettes to smoking cigarettes with a very low nicotine (VLN) content (0.4 mg nicotine per gram of tobacco). A secondary purpose is to examine changes in BoE, nicotine pharmacokinetics and subjective measures of dependence, smoking urges, withdrawal symptoms and perceived health risks, when subjects switch to smoking VLN cigarettes.

1.3 Hypothesis

It is anticipated that when adult smokers switch to smoking VLN cigarettes, after habitually consuming their usual quantity and brand of cigarettes, their daily cigarette consumption will be reduced. It is further anticipated that puffing topography parameters will be unchanged following smoking VLN cigarettes for 6 weeks *i.e.* no smoking compensation will occur.

2. STUDY OBJECTIVES

The primary objectives of this study are:

1. To characterize cigarette consumption behavior (cigarettes per day and puffing topography) before, during and after a switch from UB to VLN cigarettes for 6 weeks.

The secondary objectives of this study are:

1. To evaluate changes in tobacco-related BoE before, during and after a 6-week switch from UB to VLN cigarettes.
2. To evaluate nicotine pharmacokinetics before, during and after a 6-week switch from UB to VLN cigarettes.
3. To evaluate changes in subjective effects before, during and after a 6-week switch from UB cigarettes to VLN cigarettes.

2.1 Design and Procedures

This is an open-label, non-randomized, forced-switching study to be conducted at multiple study sites. Seventy (70) self-affirmed exclusive filtered king size non-mentholated cigarette smokers and 70 self-affirmed exclusive filtered king size mentholated cigarette smokers will be enrolled and begin the study at Week -1.

All potential subjects will provide informed consent and successfully complete the Screening procedures prior to participation in the study. Subjects will also engage in a brief product trial with the VLN cigarettes. Subjects who react negatively (i.e., unwilling to use and/or cannot tolerate the product [experience adverse events (AEs) that will prevent them from continuing to use the product as judged by the Investigator]) to the VLN cigarettes during the product trial period will not continue in the study.

At the start of Week -1, all subjects will be asked to smoke their UB cigarettes as per their usual daily consumption for the following week. Subjects will receive an electronic diary (e-diary) to record daily cigarette use (cigarettes per day [CPD]). Training in completion of the e-diary will be provided at the visit at the start of Week -1.

Subjects will return at the end of Week -1, at the time indicated by the clinical research unit (CRU), for collection of blood and 24-hour urine samples for baseline BoE assessments. Subjective questionnaires for dependence, withdrawal symptoms, urges to smoke, and perceived health risk will also be completed at scheduled times. A subset of 18 non-menthol and 18 menthol smoker subjects will complete an assessment of puffing topography with their UB cigarettes during this visit. A further subset of 12 of the non-menthol and 12 of the menthol smoker subjects who complete the topography assessment will also complete a nicotine PK assessment at the end of this visit. Subjects who undergo topography and PK assessments will be assigned to switch to smoking VLN cigarettes.

On Day 1 of Week 1, subjects will be randomly selected to either remain smoking their non-menthol (20 subjects) or menthol (20 subjects) UB cigarettes, or to switch to smoking non-menthol (50 subjects) or menthol (50 subjects) VLN cigarettes as per their UB cigarette flavor. Subjects will return at the end of Weeks 2 and 6, at the time indicated by the CRU, for collection of blood and 24-hour urine samples for BoE assessments. Subjective effects questionnaires will also be completed at scheduled times. Subjects will continue recording their CPD in their e-diaries. A subset of 18 non-menthol and 18 menthol smoker subjects will complete an assessment of puffing topography with the VLN

cigarettes at these visits, and a further subset of 12 non-menthol and 12 menthol smoker subjects will also complete an assessment of nicotine PK at the end of these visits. Subjects undergoing topography and PK assessments will have been assigned to switch to smoking VLN cigarettes.

Additionally, all subjects will visit the clinic at the end of Week 4 to receive further supplies of cigarettes (if assigned to the VLN groups) and to complete subjective effects questionnaires.

Subjects randomized to the VLN groups will be provided with a supply of VLN cigarettes at each visit, which will be 150 % of their usual daily consumption as reported during Week -1. If a subject runs out of cigarettes between clinic visits, they may visit the clinic to receive additional test cigarettes. All subjects will be asked to smoke their cigarettes *ad libitum*, recording their actual daily consumption in their e-diaries. Non-compliant nicotine product consumption should also be recorded. Used cigarette butts will also be collected during ambulatory periods to verify product use and/or assess compliance. During Week -1 (all subjects) and all subsequent weeks (subjects randomized to continue smoking UB cigarettes) subjects will be asked not to change their UB cigarette brand or flavor.

The CRU will attempt to contact all subjects who participated in the study (including subjects who terminate the study early) using their standard procedures approximately 7 days after the last contact to determine if any AE has occurred since the last study visit.

2.2 Study Endpoints

Product use endpoints are:

- Cigarettes smoked per day (CPD). Both compliant and non-compliant smoking should be captured in the e-diaries. For a definition of non-compliant smoking, see [Section 5.12.7.1](#).
- Number of collected used cigarette butts (apparent compliant, apparent non-compliant, and total).
- Puffing topography parameters (puff duration, puff volume, peak puff flow rate, average flow rate and inter-puff interval).

Nicotine PK endpoints are:

- C_{max}
- T_{max}
- AUC

BoE endpoints are:

- In urine
 - Total NNAL (NNK biomarker)
 - Total NNN
 - 3-HPMA (acrolein biomarker)
 - S-PMA (benzene biomarker)

- 1-OHP (hydroxypyrene biomarker)
- Total nicotine equivalents (T_{neq})
- Creatinine concentration (for normalization of urinary BoE endpoints)
- Carboxyhemoglobin in blood
- Cotinine in plasma

Subjective effects endpoints are:

- Fagerström Test for Cigarette Dependence (FTCD)
- Brief Questionnaire of Smoking Urges (QSU-Brief)
- Minnesota Nicotine Withdrawal Scale - Revised (MNWS-R)
- Perceived health risk scale

Safety endpoints include:

- Adverse events (AEs)

3. SUBJECT SELECTION

3.1 Inclusion Criteria

Potential subjects must fulfill all of the following inclusion criteria to be eligible for participation in the study.

1. Is a healthy adult male or female adult smoker, 26 to 65 years of age, inclusive, at Screening.
2. Has been a smoker for at least 5 years prior to Screening. Brief periods of non-smoking (e.g., up to 7 consecutive days due to illness, trying to quit, participation in a study where smoking was prohibited) during that time will be permitted at the discretion of the Investigator.
3. Reports smoking an average of 10 or more manufactured combustible cigarettes per day at Screening.
4. Usual brand (UB) of cigarette is a filtered king size cigarette (with an approximate length of 84 mm (\pm 3 mm)).
5. Has a positive urine cotinine (\geq 500 ng/ml) at Screening.
6. Has an exhaled CO $>$ 10 ppm at Screening.
7. If female, has a negative serum pregnancy test at Screening.
8. A female subject of childbearing potential must have been using one of the following forms of contraception and agree to continue using it through completion of the study:
 - hormonal (e.g., oral, vaginal ring, transdermal patch, implant, or injection) consistently for at least 3 months prior to Screening;
 - double barrier method (e.g., condom with spermicide, diaphragm with spermicide) consistently for at least 14 days prior to Screening;
 - intrauterine device for at least 3 months prior to Screening;
 - Essure[®] or similar nonsurgical sterilization procedure at least 6 months prior to Screening

- a partner who has been vasectomized for at least 6 months prior to Screening;
 - abstinence beginning at least 14 days prior to Screening and through the End of Study.
9. A female subject of non-childbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to Screening:
- hysteroscopic sterilization;
 - bilateral tubal ligation or bilateral salpingectomy;
 - hysterectomy;
 - bilateral oophorectomy;
- Or be postmenopausal with amenorrhea for at least 1 year prior to Day 1 and follicle-stimulating hormone (FSH) levels consistent with postmenopausal status.
10. Willing to comply with the requirements of the study, including a willingness to use the test products.
11. Voluntary consent to participate in this study documented on the signed informed consent form (ICF).
12. Subject is not planning to leave the area during the course of the study.

3.2 Exclusion Criteria

Subjects may be excluded from the study if there is evidence of any of the following criteria at Screening, start of Week -1 (first clinic visit), or during the study as noted, in the opinion of the Investigator.

1. History or presence of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, urologic, pulmonary (especially bronchospastic diseases and asthma), immunologic, psychiatric, or cardiovascular disease, or any other condition that, in the opinion of the Investigator, would jeopardize the safety of the subject or impact the validity of the study results.
2. Clinically significant abnormal findings on the physical examination, medical history, ECG, or clinical laboratory results, in the opinion of the Investigator.
3. Positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV).
4. An acute illness (e.g., upper respiratory infection, viral infection) requiring treatment with prescription medication(s) within 14 days prior to Screening or first clinic visit.
5. Fever (>100.5°F) at Screening or first clinic visit.
6. Body mass index (BMI) greater than 40.0 kg/m² or less than 18.0 kg/m² at Screening.
7. History of drug or alcohol abuse or has used medical/recreational marijuana within 12 months of Screening.
8. Diabetes mellitus that is not controlled by diet/exercise alone, in the opinion of the Investigator.
9. Seated heart rate is lower than 40 bpm or higher than 99 bpm at Screening, unless deemed not clinically significant by the PI.

10. Seated systolic blood pressure <90 mmHg or >150 mmHg, diastolic blood pressure <40 mmHg or >95 mmHg at Screening, unless deemed not clinically significant by the PI.
11. Positive urine screen for drugs of abuse or alcohol at Screening or at the first clinic visit.
12. Female subjects who are pregnant, lactating, or intend to become pregnant from Screening through the End of Study.
13. Use of medications known to interact with cytochrome p450 2A6 (including, but not limited to, amiodarone, desipramine, isoniazid, ketoconazole, miconazole, phenobarbital, rifampin, tranylcypromine, methoxsalen) within 3 months prior to Screening and throughout the study.
14. Use of inhalers to treat any medical condition within 3 months prior to Screening and throughout the study.
15. Use of nicotine-containing products other than factory manufactured cigarettes [e.g., roll-your-own cigarettes, e-vapor products, bidis, snuff, nicotine inhaler, pipe, cigar, chewing tobacco, nicotine patch, nicotine spray, nicotine lozenge, or nicotine gum] within 28 days prior to Screening.
16. Use of any prescription smoking cessation treatments, including, but not limited to, varenicline (Chantix[®]) or bupropion (Zyban[®]) within 3 months prior to Screening.
17. Self-reported puffer/non-inhaler (*i.e.*, a smoker who draws smoke from the cigarette into the mouth and throat but does not inhale).
18. Planning to quit smoking during the study period or postponing a quit attempt in order to participate in the study.
19. Plasma donation within 7 days prior to Screening or at any time during the study.
20. Donation of blood or blood products (with the exception of plasma as noted above), had significant blood loss, or received whole blood or a blood product transfusion within 56 days prior to Screening.
21. Participation in a previous clinical study for an investigational drug, device, biologic, or tobacco product within 30 days prior to Screening.
22. Subject or a first-degree relative (*i.e.*, parent, sibling, child) is a current or former employee of the tobacco industry or a named party or class representative in litigation with the tobacco industry.
23. Subject or a first-degree relative (*i.e.*, parent, sibling, child) is a current employee of the clinic sites.

3.3 Study Restrictions

3.3.1 Concomitant Medications

Except for those medications noted in the exclusion criteria (See [Section 3.2](#)), stable doses (*i.e.*, no dosage adjustments within approximately 30 days prior to Check-in) of prescription or over-the-counter medications required to treat an Investigator-approved disease or condition (e.g., hypertension) are permitted at the discretion of the Investigator. Hormonal contraceptives (e.g., oral, vaginal ring, transdermal patch, implant, injection) and hormonal replacement therapy are permitted. Occasional use of over-the-counter analgesics (e.g., acetaminophen, ibuprofen), antihistamines, nasal decongestants, and

dietary supplements are permitted. Exceptions may be permitted at the discretion of the Investigator in consultation with the Sponsor, providing the medication in question would have no impact on the study. Any exceptions will be documented.

All concomitant medications (and reasons for their use) taken by subjects during the study will be recorded and listed.

3.3.2 Foods and Beverages

Consumption of foods and beverages containing the following substances should not be consumed as indicated below.

- Xanthines/caffeine: throughout each clinic visit, apart from one approximately 250ml caffeinated beverage with each meal.
- Alcohol: 48 hours prior to and throughout each clinic visit.

Exceptions may be permitted at the discretion of the Investigator.

3.3.3 Activity

Subjects will be instructed to refrain from strenuous physical activity outside of their usual routine for the whole study period between Screening and the end of Week 6.

3.4 Subject Numbering

Subjects will be assigned a unique screening number. Upon enrollment into the study, a definitive subject number will be assigned. Replacement subjects may be used at the discretion of the Sponsor.

3.5 Subject Early Discontinuation or Withdrawal

Subjects will be advised that they are free to withdraw from the study at any time. In addition, subject participation in this trial may be discontinued for any of the following reasons.

- Adverse event
- Lost to follow-up
- Non-compliance with study procedures
- Protocol violation
- Pregnancy for female subjects
- Study terminated by Sponsor, US Food and Drug Administration (FDA), or other regulatory authorities
- Withdrawal of consent
- Investigator's discretion, including a severe laboratory abnormality or other medical condition that indicates to the Investigator that continued participation is not in the best interest of the subject

Subject's e-diary completion rates will be checked at each study visit. If the percentage of days on which the e-diary was completed is below 70%, subjects may be withdrawn from the study.

Other protocol deviations/violations should not lead to subject withdrawal unless they indicate a significant risk to the subject's safety or jeopardize the scientific integrity of the study.

If premature withdrawal from the study occurs for any reason, the Investigator must determine the primary reason and record this information in the case report form (CRF). Additionally, subjects withdrawing after study product use will undergo all end-of-study safety procedures as feasible and as deemed necessary by the Investigator.

A subject withdrawn from the study due to any AE or clinically significant abnormal laboratory test values will be evaluated by the Investigator or other monitoring physician and will be treated and/or followed up until the symptoms or values return to normal or acceptable levels or until lost to follow-up, as appropriate in the opinion of the Investigator.

Subjects withdrawing or removed from this study cannot re-enter.

4. STUDY PRODUCTS/MATERIALS

4.1 Description of Study Products

The following products will be used during this study.

- Subjects UB non-mentholated filtered king size cigarettes sourced by the subjects
- Subjects UB mentholated filtered king size cigarettes sourced by the subjects
- Non-mentholated very low nicotine (VLN) cigarettes sourced by 22nd Century Group, Inc
- Mentholated VLN cigarettes sourced by 22nd Century Group, Inc
-

VLN cigarettes contain 0.4 mg nicotine per gram of tobacco. Subjects will be provided with a supply of VLN cigarettes, up to 150 % of their usual daily consumption as reported during Week -1.

4.2 Study Product Accountability

Usual brand (UB) cigarettes will be provided by the study subjects. All VLN study products will be provided by the Sponsor. The pharmacy staff at the site will coordinate receipt of products from the subjects and the Sponsor. The pharmacy will document the date on which products were received and record this in the inventory records. The site pharmacy will document and reconcile the total number of products received at the site, the total number of products dispensed during the study, and the total number of products remaining at the end of clinical conduct.

Subjects will be instructed to not bring any tobacco- or nicotine-containing products into the clinic, other than UB cigarettes at the end of Week -1 visit or VLN cigarettes at the end of Weeks 2 and 6 visits. After their Week -1 visit, subjects assigned to the VLN groups will be requested only to smoke VLN cigarettes during all the clinic visits and the ambulatory periods.

All investigational products will be stored in locked, limited-access areas in the clinic site's pharmacies at controlled room temperature (defined as 20°-25°C [68°-77°F], with excursions permitted to 15°-30°C [59°-86°F]).

Individual study product dispensing records will be maintained by the site pharmacy for each subject. During clinic visits, used cigarette butts will be returned to the staff following each product use for accountability purposes and will be disposed of following accountability by the pharmacy staff. During ambulatory periods, used cigarette butts will be collected by subjects for the purposes of assessing cigarette consumption, checking compliance and study product accountability, and will be disposed of following accountability checking.

Unused VLN cigarettes will be disposed of as directed by the Sponsor. Unused UB cigarettes may be returned to the study subjects at the end of the study, according to site policy. All returns of products will be documented. Subjects will not return unused UB.

4.3 Labelling

The containers housing the VLN cigarettes will be labelled to include the following information:

- Study number
- Product identifier
- Administration requirements/handling directions
- Storage conditions
- Expiry date
- Sponsor's name and address
- Name of PI
- "For Clinical Trials use only"
- "Keep out of reach of children"

4.4 Blinding

This is an open-label study.

4.5 Randomization

A randomization scheme will be provided by Celerion for the clinical sites. A suitably qualified person will monitor the randomization to ensure the study groups are balanced in terms of age and gender, as far as practicable. Each study group will include no fewer than 10 subjects of either gender and no fewer than 10 subjects from each age group (\leq 40 years old and $>$ 40 years old).

4.6 Product Handling

While at the sites, products used during the study will be kept in their packages until use and stored under temperature controlled conditions (See Section 4.2). All VLN products will be securely stored at the clinical sites, in areas which are locked and with restricted access. No special procedures are required for the safe handling of the UB products.

During ambulatory periods, subjects will be asked to keep the study products at room temperature.

The VLN cigarettes in this study do not have a specified shelf life. They were manufactured in March 2018.

5. STUDY PROCEDURES

5.1 Screening

Potential subjects will undergo Screening procedures to ensure that they meet the requirements for inclusion in the study within 28 days prior to their first study visit at the start of Week -1.

Screening procedures will include the following:

- Sign ICF
- Demographics
- Smoking/tobacco/nicotine product use history
- UB cigarette and flavor documentation, including taking a color photocopy of the front and back of the subject's UB cigarette pack along with a ruler
- Medical history
- Review of Concomitant Medications
- Standard physical examination including oral cavity and oropharynx
- Review of Inclusion/Exclusion criteria
- Clinical laboratory tests (clinical chemistry, hematology, urinalysis)
- HIV, HBsAg, and HCV serology
- Urine drug and alcohol screens
- Exhaled breath CO measurement
- Urine cotinine screen
- Vital signs
- Height, body weight, and BMI
- 12-lead ECG
- Serum pregnancy test for females
- Serum FSH for postmenopausal females
- Review of smoking and nicotine product restrictions
- VLN cigarette trial
- Begin AE recording after product trial
- Provide smoking cessation information

5.2 Baseline Visit (Start of Week -1)

Subjects will visit the clinic at the start of Week -1 at a time specified by the clinic. The following procedures will take place during this visit:

- Verification that inclusion and exclusion criteria have not been violated since Screening
- UB cigarette documentation
- Review of AEs and concomitant medication use
- Urine drug and alcohol screens
- Urine cotinine screen and eCO measurement
- Vital signs
- Symptom-driven physical examination, if applicable
- Body weight
- Serum pregnancy test for females
- Training on e-diary use
- Dispense canisters for used cigarette butt collection
- Review smoking and nicotine product use restrictions

5.3 Ambulatory Periods Between Clinic Visits

Subjects will be required to record their daily cigarette consumption in electronic diaries and to collect all used (both compliant and non-compliant) cigarette butts in the provided canisters. Subjects will receive reminder contact to return to the CRU. At each visit, concomitant medications and AEs will be reviewed.

5.4 Clinic Visits (End of Weeks -1, 2 and 6)

Subjects will check in to the clinic at the end of Weeks, -1, 2 and 6 at times specified by the clinic. Subjects will be housed in the clinic until after the 24-hour urine collection and other study procedures. The following procedures will take place during this visit:

- Verification that inclusion and exclusion criteria have not been violated since last visit
- UB cigarette documentation for subjects assigned to continue smoking UB cigarettes
- Urine drug and alcohol screens
- Vital signs
- Urine pregnancy test for females
- Review of AEs and concomitant medication use.
- Usual brand cigarette documentation (only at Week -1).
- Return canister with used cigarette butts for counting
- 24-hour urine collection for BoE analysis
- Plasma sample for cotinine analysis
- Blood sample for carboxyhemoglobin (COHb) analysis
- Daily recording of CPD in the e-diary.
- Completion of subjective effects questionnaires
- Puffing topography assessment (subset of subjects)
- Nicotine PK assessment (subset of subjects)

- Dispense new canister for collecting used cigarette butts during next ambulatory period, except at Week 6
- Review smoking and nicotine product use restrictions
- Test product dispensing for the following ambulatory period; except at Week 6

All subjects will have their personal belongings thoroughly checked at the start of these clinic visits.

5.5 Clinic Visit (End of Week 4)

Subjects will visit the clinic at the end of Week 4 at a time specified by the clinic. The following procedures will take place during this visit:

- Verification that inclusion and exclusion criteria have not been violated since last visit
- UB cigarette documentation for subjects assigned to continue smoking UB cigarettes
- Urine drug and alcohol screens
- Vital signs
- Urine pregnancy test for females
- Review of AEs and concomitant medication use.
- Return canister with used cigarette butts for counting
- Completion of subjective effects questionnaires
- Dispense new canister for collecting used cigarette butts during next ambulatory period
- Review smoking and nicotine product use restrictions
- Test product dispensing for the following ambulatory period

5.6 End-of-Study (End of Week 6 Visit or on Early Discontinuation)

At the end of the study, the following procedures will be performed:

- Review of AEs and concomitant medication use.
- Vital signs
- Body weight
- 12-lead ECG
- Clinical chemistry, hematology and urinalysis
- Symptom-driven physical examination, if applicable
- Provide smoking cessation information

5.7 Follow-Up Call

The CRU will attempt to contact all subjects who participated in the study (including subjects who terminate the study early) using their standard procedures approximately 7 days after the last contact. This will be to:

- Review AEs and concomitant medication use

- Provide smoking cessation information

A symptom-driven physical examination may also be required, in which case subjects will return to the clinic for this to take place.

5.8 Confinement

Subjects will check in to the clinic at the end of Weeks, -1, 2 and 6, at times specified by the clinic. Subjects will be housed until after the 24-hour urine collection, nicotine PK assessment, puffing topography and other study procedures.

5.9 Tobacco Product Use

Except as required by the protocol, consumption of tobacco- or nicotine-containing products will not be permitted during the entire duration of the study from the start of Week -1 through discharge from the study. During the baseline period, subjects will be asked to confine their smoking to one brand which will be deemed their UB. While in the CRU during 24-hour confinement periods, cigarette consumption will be tracked by the number of products dispensed.

5.10 Product Trial

At screening, subjects will have an opportunity to smoke 2-3 VLN cigarettes over a 1-hour period to ensure that they can tolerate smoking those cigarettes for a period of 6 weeks. The number of cigarettes smoked will be documented, as will any abnormal events (e.g., excessive coughing) during the product trial. Subjects who react negatively (*i.e.*, unwilling to use and/or cannot tolerate the product [experience adverse events (AEs) that will prevent them from continuing to use the product as judged by the Investigator]) to the VLN cigarettes during the product trial period will not continue in the study.

5.11 Meals

Standard meals and snacks will be served at appropriate times as determined by the clinic during confinement. Meals each day should be provided at similar times. One approximately 250 ml, caffeinated beverage will be allowed to be taken with each meal. During confinement, water will be allowed *ad libitum* and subjects will be encouraged to maintain their usual hydration habits.

5.12 Study Assessments

All study assessments will take place at the times noted in the [Summary of Study Events](#) tables above unless otherwise noted below.

5.12.1 Medical History/Demographic Data/Record of Concomitant Medication

Self-reported medical history and socio-demographic data, including name, sex, age (each subject must show proof of age with government-issued identification [e.g., driver's license or Verified Clinical Trials]), race, ethnicity, address, and phone number will be recorded at Screening for each subject.

Medical history will include any clinically significant neurological, gastrointestinal, renal, hepatic, cardiovascular, psychiatric, respiratory, metabolic, endocrine, hematological or other major disorders. Medical history is defined as any condition that started prior to Screening and ended prior to the product trial. A concomitant disease is any disease that started prior to, and which was still ongoing at Screening.

Any concomitant medications taken from 30 days prior to Screening through discharge will be recorded in the CRF and source documents.

5.12.2 Tobacco/Nicotine Product Use History

Subjects will be required to report previous tobacco- and nicotine-product use histories to satisfy the study inclusion and exclusion criteria. In addition, the subject will be asked if he/she is planning to quit smoking within the next 3 months. Those planning to quit will be excluded from participating in the study.

The subject's tobacco use and smoking history (including a color photocopy of their UB cigarette package along with a ruler) will be recorded at Screening. Verification of the subject's UB cigarette brand will be made at the end of Week -1 clinic visit.

5.12.3 Urine collections for BoE Assessments

Urine will be collected over 24-hour periods for urine BoE measurements, during the clinic visits at the end of Weeks, -1, 2 and 6. Creatinine in urine will also be measured and used to report BoE as amount per unit of creatinine.

Prior to the first urine sample collection, each subject will be instructed as to the urine collection methods. All urine during an interval is to be collected and subjects will be asked to empty their bladder before the start of the urine collection period.

The urine samples collected during a single 24-hour period will be pooled together and weighed. The pooled samples will be thoroughly mixed before providing aliquots for analyses. A separate document will be provided for handling and processing of the samples.

The total weight of all urine collected over each 24-hour period will be documented. Urine aliquots will be prepared within 60 minutes from removal of the pooled collection from the refrigerator and then stored at approximately -20°C pending analysis, according to the table below:

Analyte	Volume per aliquot	Number of aliquots
Total NNAL	10ml	2
Total NNN	10ml	2
T _{neq}	10ml	2

3-HPMA	10ml	2
S-PMA	10ml	2
1-OHP	10ml	2
Creatinine	10ml	2

5.12.4 Blood sampling for BoE assessments

Blood samples for COHb in whole blood and cotinine in plasma will be collected via direct venipuncture at the scheduled visits as delineated in the [Summary of Study Events Tables](#). Samples will be collected at approximately 13:00, following the subject's lunchtime meal, and will be preceded by at least a 30-minute abstention from study product use.

Samples for COHb will be collected into two 4 ml sodium heparin vacutainers. Immediately following collection, the blood samples will be inverted gently to mix between 8 and 10 times. The whole blood samples will be stored at 5°C (± 3°C) until analyzed by the Celerion Bioanalytical Laboratory (Lincoln, Nebraska).

Samples for plasma cotinine will be collected into a 4 ml plastic K₂EDTA (lavender top) vacutainer tube. Immediately following the collection, blood samples will be gently mixed by inverting the tubes 8 times. The samples may be kept at ambient temperature prior to centrifuge, and will be centrifuged at 1000 - 1300 RCF for 10 minutes at 5°C within 60 minutes of collection. After centrifugation, approximately 1 ml of plasma will be transferred using into 2 appropriately labeled 3.5 ml polypropylene screw top tubes.

Samples will then be stored at approximately -20°C within 120 minutes of collection and until analyzed by Celerion Bioanalytical Services (Lincoln, Nebraska). Samples will be analyzed using a validated analytical method with the appropriate quality controls in accordance with FDA Good Laboratory Practice (GLP) regulations (Title 21 Code of Federal Regulations [CFR] Part 58).

Approximately 36 ml of blood will be required for blood BoE assessments during the entire study.

Additional instructions for collection, processing, and shipping of blood BoE samples will be provided separately.

5.12.5 Puffing topography measurements

At clinic visits at the end of Weeks -1, 2 and 6, a randomly-selected subset of 18 non-menthol and 18 menthol smoker subjects will complete a puffing topography evaluation session with either their UB (Week -1) or the VLN (Weeks 2 and 6) cigarettes. These subjects will be/have been assigned to switch to smoking VLN cigarettes. These assessments will only be performed at a single clinical site (Lincoln) and will take place during the 24-hour confinement period.

During the puffing topography assessment, subjects will engage in a 1-hour *ad libitum* smoking session with their UB (Week -1) or the VLN (Weeks 2 and 6) cigarettes. In these sessions, subjects will smoke cigarettes with the mobile smoking puff analyzer (SPA-M; Sodim). The topography device will be monitored to ensure the device is actively recording during each session.

Topography assessment will be started at least one hour following either the subject's breakfast or lunch. The puffing topography session for each subject at Weeks 2 and 6 should be within \pm 2 hours of the time of their Week -1 session.

Additional details and instructions for puffing topography procedures will be provided separately.

The following topography parameters will be assessed:

- Puff duration
- Puff volume
- Peak puff flow rate
- Average flow rate
- Inter-puff interval

5.12.6 Blood sampling for nicotine PK assessments

At clinic visits at the end of Weeks -1, 2 and 6, a randomly-selected subset of 12 non-menthol and 12 menthol smoker subjects will complete a nicotine pharmacokinetic assessment session with either their UB (Week -1) or the VLN (Weeks 2 and 6) cigarettes. These subjects will be/have been assigned to switch to smoking VLN cigarettes and will be selected from the subjects who undergo topography assessment. These assessments will only be performed at a single clinical site (Lincoln) and will take place on the morning following the 24-h confinement period.

Following the completion of the 24-hour urine collection period, selected subjects will remain in the clinic for a further night, in which they will abstain from smoking any cigarettes/using any nicotine-containing products for at least 12 hours prior to the PK assessment session. During this session, subjects will smoke a single cigarette *ad libitum* during a 5-minute period. A 4 ml blood sample for plasma nicotine analysis will be drawn into a plastic K2-EDTA (lavender top) vacutainer tube at -5, 2, 5, 7, 10, 12, 15, 30, 45, 60, 90, 120, 150 and 180 minutes relative to the start of cigarette smoking, as delineated in the [Summary of Study Events Tables](#). In total, approximately 144 ml of blood will be drawn from each of the selected subjects during the entire study for nicotine pharmacokinetic analysis.

The blood samples collected for plasma nicotine analysis may be kept at room temperature prior to centrifugation, and will be centrifuged at approximately 1000-1300 RCF at \sim 5°C for approximately 10 minutes, within 60 minutes from collection. After centrifugation, the plasma will be transferred to two methanol prewashed 3.5 ml polypropylene screw cap tubes. Samples will then be stored at approximately -20°C within 120 minutes of collection and until analyzed by Celerion Bioanalytical Services (Lincoln, Nebraska).

Samples will be analyzed using a validated liquid chromatography coupled to tandem mass spectrometry detection analytical method with the appropriate quality controls in

accordance with FDA Good Laboratory Practice (GLP) regulations (Title 21 Code of Federal Regulations [CFR] Part 58).

Additional instructions for collection, processing, and shipping of samples for nicotine PK assessments will be provided separately.

5.12.7 Product use recording

5.12.7.1 Electronic diary for cigarette consumption

Subjects will be provided with e-diaries to record their daily cigarette consumption, both when in clinical confinement during study visits and during ambulatory periods. Subjects will be instructed to record both compliant and non-compliant (if any) daily numbers of cigarettes smoked. Non-compliant consumption in the subjects randomized to smoke VLN cigarettes is defined as nicotine product consumption other than their UB cigarettes during the baseline period (Week -1) or VLN cigarettes following switching (Weeks 1 to 6). Non-compliant consumption in the subjects randomized to continue to smoke their UB cigarettes is defined as nicotine product consumption other than their UB cigarettes between Week -1 and Week 6.

For the purposes of data analysis, the e-diary record of cigarette consumption will be used as the primary variable.

5.12.7.2 Collection of used cigarette butts

Subjects will be provided with metal canisters in which to store their used cigarette butts during the ambulatory study periods. This is for the purposes of assessing both compliance with the study requirements and the accuracy of their self-reported (electronic diary) cigarette consumption. Subjects will be requested to collect butts of all cigarettes smoked, both compliant and non-compliant.

5.12.8 Subjective measures

Questionnaires examining subjective effects will be completed using an electronic data collection system (Clinical Ink).

5.12.8.1 Fagerström Test for Cigarette Dependence Questionnaire (FTCD)

The FTCD ([Appendix 1](#)) will be completed at the end of each clinic visit, at the end of Weeks, -1, 2, 4 and 6, as delineated in the [Summary of Study Events Tables](#).

5.12.8.2 Brief Questionnaire of Smoking Urges (QSU-Brief)

The QSU-Brief ([Appendix 2](#)) will be completed at the end of each clinic visit, at the end of Weeks, -1, 2, 4 and 6, as delineated in the [Summary of Study Events Tables](#).

5.12.8.3 Minnesota Nicotine Withdrawal Scale - Revised (MNWS-R)

The MNWS-R ([Appendix 3](#)) will be completed at the end of each clinic visit, at the end of Weeks, -1, 2, 4 and 6, as delineated in the [Summary of Study Events Tables](#).

5.12.8.4 Perceived health risk scale

The perceived health risk scale ([Appendix 4](#)) will be completed at the end of each clinic visit, at the end of Weeks, -1, 2, 4 and 6, as delineated in the [Summary of Study Events Tables](#).

5.12.9 Exhaled Carbon Monoxide

Exhaled CO levels will be measured using a Bedfont Micro+ Smokerlyzer, or similar device, as delineated in the [Summary of Study Events Tables](#).

5.12.10 Safety Assessments

Safety assessments in addition to those below may be obtained as necessary at the discretion of the Investigator. In the case of an early subject withdrawal, discharge safety assessments should be collected to the extent possible.

5.12.10.1 Physical Examination

A standard physical examination (including oral cavity and oropharynx) assessing the general physical well-being will be completed at Screening by a qualified member of the clinic staff. A symptom-driven physical examination may be conducted at discharge (or upon early discontinuation), if required, as delineated in the [Summary of Study Events Tables](#).

5.12.10.2 Height, Body Weight, and BMI

Body height will be measured in cm and body weight will be measured in kilograms. BMI will be recorded as kg/m².

5.12.10.3 Clinical Laboratory

Collection and processing of samples for these purposes will be performed using standard clinical laboratory procedures at an approved laboratory or using a CLIA approved kit on site. Approximately 25 ml of blood will be required for clinical laboratory purposes. Values for the clinical laboratory parameters are to be within the laboratory normal ranges or deemed not clinically significant in the opinion of the Investigator.

Clinical Chemistry ¹	Urinalysis ²	Additional Tests
<ul style="list-style-type: none">• Albumin• Alkaline phosphatase• Alanine aminotransferase• Aspartate aminotransferase• Bicarbonate• Blood urea nitrogen• Creatinine• Glucose (fasting)• Potassium• Sodium• Total bilirubin• Total protein• Uric acid	<ul style="list-style-type: none">• Bilirubin• Blood• Glucose• Ketones• Leukocyte esterase• Nitrite• pH• Protein• Specific gravity• Urobilinogen	<ul style="list-style-type: none">• Serology<ul style="list-style-type: none">○ HIV○ HBsAg○ HCV• Serum pregnancy test³• Serum FSH⁴• Urine cotinine• Urine drug screen<ul style="list-style-type: none">○ Amphetamines○ Cannabinoids○ Cocaine○ Opiates○ Alcohol• Urine pregnancy test⁵

Hematology

- Hematocrit
- Hemoglobin
- Platelet count
- Red blood cell count
- White blood cell count with differential

¹Clinical chemistry tests will be performed after at least an 8-hour fast; however, in case of dropouts or rechecks, subjects may not have fasted for 8 hours prior to the clinical chemistry sample being taken.

²A microscopic examination for red blood cells, white blood cells, bacteria, and casts will be performed if an abnormality is noted in leukocyte esterase, protein, blood, or nitrite.

³At Screening and baseline clinic visit (start of Week -1); females only.

⁴For postmenopausal females; at Screening only.

⁵At start of each clinic visit (end of Weeks -1, 2, 4 and 6); females only.

5.12.10.4 Vital Signs

Vital signs (respiratory rate, heart rate, blood pressure, and oral temperature) will be measured following a rest period of at least 10 minutes in a seated position, and at least 15 minutes after the subject has smoked a cigarette.

5.12.10.5 Electrocardiogram

12-lead ECGs will be taken following resting in the supine position for at least 10 minutes. ECGs will be interpreted, signed, and dated by the Investigator or a qualified designee.

5.12.11 Adverse Events

An AE is any untoward medical occurrence associated with the use of the study product, whether or not considered study product-related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study product, whether or not related to the study product.

5.12.11.1 Monitoring

The subjects will be instructed to inform the study physician or staff of any AEs and intercurrent illnesses experienced during the study. Additionally, a specific inquiry regarding AEs will be conducted during clinic visits, at End-of-Study (or upon early discontinuation), or during the follow-up telephone call. The inquiry will be posed in a non-specific manner using open-ended questions so as not to bias the response (e.g., How are you feeling today?).

A subject who has any clinically significant AE or clinically significant abnormal laboratory test value will be evaluated by the Investigator or other monitoring physician and will be treated and/or followed up until the symptoms or values return to normal or acceptable levels (as appropriate in the opinion of the Investigator), or until the subject is lost to follow-up. Where appropriate, medical tests and examinations will be performed to document resolution of the event(s).

5.12.11.2 Reporting

All AEs occurring during this clinical trial after the subject has used study products (including the product trial at Screening) must be recorded on the CRF, including the date and time of onset, action taken, outcome (resolved, improved, unchanged, worse, fatal, or unknown/lost to follow-up), duration, relationship to product administration, and severity for each event. AEs will be coded using most current version of the Medical Dictionary for Regulatory Activities (MedDRA[®]) available at Celerion.

Events captured between Screening until the first product use (Product Trial) will be documented as baseline signs and symptoms.

The Investigator will review each event and assess its relationship to product administration as unrelated, unlikely, possibly, probably, or likely.

In addition, each sign or symptom reported will be graded on a 3-point severity scale using mild, moderate, or severe.

5.12.11.3 Serious Adverse Events

A serious adverse event (SAE) is any AE that in the view of either the Investigator (or designee) or Sponsor, results in any of the following outcomes: death, a life threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,

or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the above definition.

Life threatening is defined as an AE that in the view of the Investigator (or designee) or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

Unexpected is defined as an AE that is not consistent with the known risk information associated with the study product.

All SAEs, whether or not considered study-related, must be reported by telephone and by fax or e-mail to the Sponsor within 24 hours of the site's learning of the SAE or, at the latest, on the following workday. The Sponsor's representative to contact about this study is provided in the list of study contacts. The Investigator must also inform the IRB, in compliance with GCP reporting guidelines, and the site monitor of any SAE.

5.12.11.4 Pregnancy

A pregnancy occurring in a female study subject during the study will be documented as an unanticipated problem to the Institutional Review Board (IRB). Pregnancy itself is not a SAE. The Investigator or designee will discontinue the pregnant subject from the study and will advise her to seek prenatal care and counseling from her primary care provider. Advice given will be documented in the subject's source document.

The site clinical staff will request the pregnant subject to notify the site of the outcome of the pregnancy (*i.e.*, birth, loss, or termination). To help ensure this, the site clinical staff will follow up with the subject until the end of pregnancy, if in compliance with the site's standard operating procedures (SOPs) and with the subject's consent. This request and the subject's response will be documented in the subject's source documents.

5.13 Smoking Cessation Information

At Screening and prior to discharge (or upon early withdrawal) all subjects will be advised that to reduce the health effects of smoking, the best thing to do is to quit. Subjects will be directed towards further information on the QuitAssist website (www.quitassist.com).

6. DATA ANALYSIS

Data will be handled and processed according to Celerion SOPs, which are written based on the principles of GCP. A brief description of the statistical analysis is included below; detailed methodology for all summary and statistical analyses of the data collected in this trial will be documented in a Statistical Analysis Plan (SAP) prepared by Celerion and agreed upon by the Sponsor. The SAP may modify the plans outlined in the protocol; however, any major modifications of the primary endpoints and/or their analysis will also be reflected in a protocol amendment. If deemed appropriate, additional statistical analyses other than those described in this section may be performed and included in the plan.

6.1 Sample Size Estimation

6.1.1 Cigarette consumption

An intended sample size of 50 subjects in each of the two groups who switch from smoking usual brand to VLN cigarettes has been set for this study. This is based on powering the primary objective of a within-group comparison of cigarette consumption at baseline and end-of-study. The calculation was based on the number of pairs required to perform a two-tailed paired t-test with 80% power and an alpha level of 0.05 for a decrease in cigarette consumption of 3 CPD compared to available historical consumption data (2, 4). The sample size was determined to be adequate based on potential non-compliance with the use of VLN cigarettes (2). A lower number (20) of smokers will be randomized to the continue-to-smoke usual brand groups, given the lower variability in consumption data and lower non-compliance anticipated in these groups. This will provide sufficient power for the secondary objective of between-group comparisons in cigarette consumption.

6.1.2 Puffing topography

An intended sample size of 12 subjects in each of the two groups who switch from smoking usual brand to VLN cigarettes has been set for this study. This is based on powering the primary objective of a within-group comparison of puffing topography parameters at baseline and end-of-study. The calculation was based on the number of pairs required to perform a two-tailed paired t-test with 80% power and an alpha level of 0.05 to detect significant differences between UB and VLN cigarettes and is based on available puffing topography data (5). This sample size will also provide sufficient power to detect differences in nicotine PK between UB and VLN cigarettes.

6.2 Analysis Populations

The statistical analysis will be based on separate, hierarchically organised, analysis populations defined as the following:

Safety population. All subjects who smoked at least one VLN cigarette

Intent-to-treat (ITT) population. All subjects who had at least one valid recording of cigarette consumption (a period of one week of >70% completion of their e-diary).

Per-protocol (PP) population. All subjects who had valid recording of cigarette consumption and completed the study according to the protocol. Data will be excluded from this population:

- If a subject self-reports smoking a significant number of cigarettes other than those which they have been assigned according to the protocol, or if this is apparent following checking of their collected used cigarette butts
- If there is a significant discrepancy between the self-reported number of cigarettes smoked per day and the number of used cigarette butts collected
- Based on a published method of assessing compliance (6). After switching, subjects will be deemed non-compliant if their ratio of [plasma cotinine/CPD VLN]/[plasma cotinine/CPD baseline] exceeds 0.2.

If it is determined that a subject is pregnant during the study, the pregnant subject's data will be listed but excluded from all PK parameter summaries.

6.3 Data Analysis and Summarization

SAS software (version 9.3 or higher, Cary, North Carolina) will be used for all data presentation and summarization including statistical analyses, summary tables, graphs, and data listings. In general, all data will be listed by subject and time point and summarized by group and time point using descriptive statistics appropriate for the endpoint. Absolute and percent change from baseline values will also be listed and summarized where appropriate. Figures will be used to display the data graphically.

Missing data will not be imputed. Where individual data points are missing because of dropouts or other reasons, the data will be considered missing at random and summarized based on reduced denominators.

Within-group comparisons will be made for all groups for endpoints that have baseline (end of Week -1) and post-baseline measurements.

6.3.1 Cigarette Consumption

Study product use will be listed and summarized by subject using descriptive statistics (n, arithmetic mean, standard deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), minimum, median, and maximum).

6.3.2 Urine Biomarkers of Exposure

The urine biomarker data will be presented as follows for the assessments at the end of Weeks -1, 2 and 6:

- Measured concentration
- Total mass excreted per 24 hours
- Total mass excreted per 24 hours absolute change from baseline
- Total mass excreted per 24 hours percent change from baseline
- Creatinine-normalized excretion level
- Creatinine-normalized change from baseline
- Creatinine-normalized percent change from baseline

Note: the appropriate total mass excreted variables will be used in the statistical analyses.

The amount excreted data will be reported for subjects who have an incomplete urine collection during a sampling interval but will not be included in the summary statistics or analysis for any of the urine biomarkers in that interval. Such subjects will be included in the summarization of the creatinine-normalized data.

The measured urine biomarker concentrations will be converted to total mass excreted per 24 hours by multiplying the concentration by the total urine volume of the interval. All values reported as below the limit of quantitation (BLQ) will be set to one-half of the limit of quantitation (LOQ) for summarization and analysis.

Nicotine equivalents will be calculated as the molar sum of nicotine, cotinine, trans-3'hydroxycotinine and their glucuronides excreted in urine over 24 hours:

$$\text{Nicotine} = (\text{free nicotine [mg]} + \text{nicotine gluc [mg]}) + (\text{free}$$

$$\text{Equivalents (mg)} = \text{cotinine [mg]} + \text{cotinine gluc [mg]} + (\text{free trans-3'-hydroxycotinine [mg]} + \text{trans-3'-hydroxycotinine gluc [mg]})$$

The concentration of each metabolite will first be adjusted by the 24-hour urine volume to obtain the total amount excreted in 24 hours, then divided by the molecular weight of the metabolite to obtain the total amount of each in moles. The sum in moles will then be converted to mass of nicotine equivalents by multiplying by the molecular weight of nicotine:

$$\text{Nicotine equivalents (mg/24h)} = [\text{nicotine (mg/162.23 [mg/mmol]} + \text{nicotine-gluc (mg/338.36 [mg/mmol]} + \text{cotinine (mg/176.22 [mg/mmol]} + \text{cotinine-gluc (mg/352.34 [mg/mmol]} + \text{trans-3'-hydroxycotinine (mg/192.22 [mg/mmol]} + \text{trans-3'-hydroxycotinine-gluc (mg/368.34 [mg/mmol]}) \times 162.23 (\text{mg/mmol})$$

Urine creatinine will also be measured in each 24-hour collection and used to adjust the biomarker concentration values as appropriate according to the following formula:

$$\text{Biomarker (mass/ml)} = \frac{\text{Biomarker (mass/ml)} \times 100}{\text{creatinine (mg/dl)}}$$

All data will be listed by subject and summarized by time point using descriptive statistics. Change from baseline will be calculated for each post-baseline time point.

6.3.3 Blood Biomarkers of Exposure

All data will be listed by subject and summarized by product and time point. Change from baseline will be calculated for each post-baseline time point.

6.3.4 Nicotine pharmacokinetics

Nicotine PK parameters will be determined from the individual plasma concentrations applying a non-compartmental approach using appropriate validated PK software (e.g., Phoenix® WinNonlin® version 6.3 or higher).

For each study product, the following PK parameters will be calculated from the nicotine concentration-time data:

- C_{max} Maximum measured plasma concentration over the duration of the measurement interval.
- T_{max} Time to reach the maximum measured plasma concentration over the duration of the measurement interval. If the maximum value occurs at more than one time point, T_{max} is defined as the first time point with this value.
- AUC₀₋₁₂₀ Area under the nicotine concentration-time curve from time zero (defined as the start of product use) to 120 minutes (or the last quantifiable concentration during that interval) as calculated by the linear trapezoidal method.

Baseline adjustments will be performed. Concentration values below the lower limit of quantitation (LOQ) will be set to one-half of the LOQ for the calculation of the descriptive statistics of unadjusted plasma nicotine concentrations. Data will be listed by subject and summarized by product and time point using descriptive statistics.

6.3.5 Puffing topography

Data will be listed by subject and summarized by product and time point using descriptive statistics.

6.3.6 Subjective Effects Questionnaires

Data will be listed by subject and summarized by product and time point. Change from baseline will be calculated for each post-baseline time point.

6.3.7 Statistical Methods for the Primary Endpoints

Paired t-tests will be used to compare cigarette consumption (CPD) and puffing topography parameters at the Week 2 and 6 time points with the baseline (Week -1) values. This analysis will be conducted on the evaluable subjects in the PP population. If data are unexpectedly found not to be normally distributed, an appropriate non-parametric test will be performed. Differences will be considered statistically significant at an alpha level of 0.05.

In addition, a linear mixed model analysis of variance will be used to compare the between-cohort differences in the absolute change from baseline values of the consumption and puffing topography parameters.

6.3.8 Statistical Methods for the Secondary Endpoints

Paired t-tests will be used to compare urinary NNAL, NNN, 3-HPMA, S-PMA, 1-OHP, T_{neq} mass excreted, blood COHb, nicotine PK parameters, and subjective effects measures at the Week 2 and 6 time points with the baseline (Week -1) values. This analysis will be conducted on the evaluable subjects in the PP population. If data are unexpectedly found not to be normally distributed, an appropriate non-parametric test will be performed. Differences will be considered statistically significant at an alpha level of 0.05.

In addition, a linear mixed model analysis of variance will be used to compare the between-cohort differences in the absolute change from baseline values of each of the urinary and blood BoEs.

6.4 Safety

All clinical safety data will be listed by subject and time point as appropriate. The following items will be summarized using n, arithmetic mean, SD, median, minimum, and maximum for continuous data and frequency counts for categorical data.

- Subject disposition
- Subject demographics
- Tobacco product use history

- Vital signs

All events captured in the database will be listed in by-subject data listings. However, only study product use-emergent AEs will be summarized. A study product use-emergent AE is defined as an AE that is starting or worsening at the time of or after the first study product use (i.e., Product Trial).

Frequencies of subjects with study product use-emergent AEs, regardless of relationship to study product will be summarized and sorted by system organ class. Frequencies of subjects with study product use-emergent serious AEs will be likewise summarized. Frequencies of study product use-emergent AEs will be summarized by severity and relationship to study product.

Changes in physical examinations (if any) will be described in the text of the final report. All concomitant medications recorded during the study will be listed by subject.

7. STUDY ADMINISTRATION

7.1 Ethics

7.1.1 Ethical Conduct of the Study

This protocol will be reviewed by an IRB and the study will not start until the Board has approved the protocol or a modification thereof. The Board is constituted and operates in accordance with the principles and requirements described in the US Code of Federal Regulations (21 CFR Part 56). The Board is compliant with the International Conference on Harmonization (ICH).

This research will be carried out in accordance with the protocol, the US CFR (21 CFR Parts 50, 56, and 312), the ICH harmonized tripartite guideline regarding GCP (E6 Consolidated Guidance, April 1996), and the ethical principles set forth in the Declaration of Helsinki.

7.1.2 Subject Information and Consent

All prospective subjects will have the study explained by the PI or his/her designee and will be required to read, sign, and date an IRB-approved ICF prior to undergoing any screening or other study procedures. This consent form will provide the subjects in non-technical terms with the purpose of the study, procedures to be carried out, and potential hazards. The subjects will be assured that they may withdraw from the study at any time without jeopardizing medical care related to or required as a result of study participation. Subjects will be given a signed and dated copy of their ICF. Subjects will be given a copy of their signed ICF.

7.2 Termination of the Study

The Sponsor reserves the right to discontinue this study at any time. The Investigator, in collaboration with the Sponsor, reserves the right to discontinue the study for safety reasons at any time.

7.3 Monitoring

The responsible study monitor(s) will contact and visit the study sites as necessary, and he/she will be allowed, upon request, to inspect and verify all records of the study (e.g.,

source document, ICFs, CRFs, regulatory documents) in a manner consistent with GCP and all other applicable state and federal law.

It will be the study monitor's responsibility to inspect the source documents to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered in the CRF. The monitor will verify that each subject has consented in writing prior to any study procedures being performed. Where the terms of the Informed Consent, GCP, and all other applicable state and federal law permit, the monitor should have access to laboratory test reports and other subject records needed to verify the entries on the CRF. The Investigator (or his/her designee) agrees to cooperate with the monitor to ensure that any issues detected in the course of these monitoring visits are resolved.

In addition, the Sponsor's internal auditors (or designee) and government inspectors may evaluate the study and must be allowed access to CRFs, source documents, and other study files.

The Investigator must notify the Sponsor (or designee) promptly of any inspections of the study or activities related to the study scheduled by regulatory authorities, allow the Sponsor (or designee) to be present, and promptly forward copies of inspection reports to the Sponsor (or designee).

7.4 Data Quality Assurance

SOPs are available for all activities performed at Celerion relevant to the quality of this study. Designated personnel of Celerion will be responsible for maintaining quality assurance (QA) and quality control to ensure that the trial is conducted, and that data are generated, documented and reported in compliance with the study protocol, GCP, and GLP requirements as well as applicable regulatory requirements. All activities performed will be reported in the QA certificate and will be included in the study report.

All clinical data will undergo a 100% quality control check prior to clinical database lock. Edit checks are then performed for appropriate databases as a validation routine using SAS to check for missing data, data inconsistencies, data ranges etc. Corrections are made prior to database lock.

7.5 Direct Access to Source Data/Documents

The Investigator will prepare and maintain adequate and accurate source documents and agree that the Sponsor, its representatives, the IRB, and representatives from worldwide regulatory agencies will have the right to review and inspect pertinent records related to the clinical trial both during and after the clinical trial. Examples of source documents may include the study product inventory, study product label records, CRFs, recorded data from automated instruments, and other primary records that are relevant to this clinical trial. These documents are designed to record all observations and other pertinent data for each subject enrolled in this clinical trial.

7.6 Data Handling and Record Keeping

Each CRF will be reviewed and signed off by the Investigator within the electronic data capture system.

All raw data generated in connection with this study, together with the original copy of the final report, will be retained by the sites until at least 5 years after the last approval of a

marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 5 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

7.7 Publication Policy

All unpublished information given to Celerion by the Sponsor shall not be published or disclosed to a third party without the prior written consent of the Sponsor.

The data generated by this study are considered confidential information and the property of the Sponsor. This confidential information may be published only in collaboration with participating personnel from the Sponsor or upon the Sponsor's written consent to publish the information.

8. DATA MANAGEMENT

Data management activities will be detailed in the Data Management Plan (DMP). Each vendor involved with this study will adhere to Good Documentation Practices and their standard operating procedures covering their respective activities relevant to participation in this study. The Investigator will ensure that all data related to the conduct of this study at his/her site is attributable, legible, contemporaneous, original, accurate, enduring, and readily accessible.

8.1 Database Design and Creation

An appropriate database will be designed and created within a validated Clinical Data Management System (CDMS). Electronic data capture will be used for this study and electronic case report form (eCRFs) will be developed according to the study protocol specifications. Clinical and analytical laboratory data will be collected external to CDMS as external data files.

8.2 Data Coding

AEs and medical history coding will be undertaken using most current version of MedDRA®. Concomitant medications coding will be undertaken using the WHO Drug Dictionary. Each dictionary version will remain the same throughout the trial. Coding will be completed by qualified members of the Celerion staff.

8.3 Data Entry and Verification

Data will be transcribed from original sources by the Investigator or Investigator's staff into the eCRF. External data received from clinical and analytical laboratories (clinical laboratory results) will be integrated into the Clinical Data Interchange Standards Consortium study data tabulation model datasets.

8.4 Study Results Data Transfer

Study data transfers will be sent to the Sponsor or their designee, electronically on a schedule and in a format mutually agreed upon by the Sponsor or their designee, and Celerion for the analysis of these study data. No personally identifiable information will be transferred to the Sponsor at any point in the study.

8.5 Data Validation

After the data have been entered and source data verified by the monitor, various edit checks (including manual review of listings) will be performed to ensure the accuracy, integrity and validation of the database against the eCRF as described in the DMP.

Inconsistencies that arise from these edit checks will be resolved with the Investigator or designee.

8.6 Database Lock

On study completion, after data entry is complete, the data has been pronounced clean, and the Investigators have reviewed and provided approval via signature, the database will be locked and final write access will be removed.

The Sponsor will be required to provide database lock approval.

Any changes to the data following database lock will be documented and approved by the Sponsor prior to unlocking the database to make changes to the data.

The final transfer of all study data (without subject personally identifying information) to the Sponsor will be in SAS format with supporting SAS documentation according to the specifications of the Sponsor. Subject initials, serology results, date of birth (except year), and other personal identifiers will be removed from this data transfer file; any such information removed will be documented at the time of transfer.

9. REPORTING

9.1 Case Report Forms

CRFs will be used for each screened subject whether or not he/she has completed the study. The Investigator will assure complete and accurate entries on the forms. All CRFs will be reviewed and signed by the Investigator.

9.2 Study Report

According to the ICH Harmonized Tripartite Guideline (Organization of the Common Technical Document for the Registration of Pharmaceuticals for Human Use M4 and the ICH M2 Expert Working Group), the final report will be written according to the ICH E3 Guideline (Structure and Content of Clinical Study Reports).

The report will be provided by Celerion to the Sponsor. The report will include a description of the clinical conduct of the study, safety evaluation, analytical methods and results, and the statistical analysis described in the statistical methodology section of the protocol and the SAP.

At the time the draft study report is completed, Celerion's QA unit will audit the report against the SAS data and the raw data. At the completion of the audit, a QA report will be issued internally allowing any findings to be addressed before report finalization.

10. GENERAL

10.1 Confidentiality

All members of the Investigator's staff and vendors will have signed confidentiality agreements with Celerion. Celerion will regard all information provided to the Investigators dealing with the study and information obtained during the course of the study as confidential.

The CRUs will not supply to the Sponsor any subject names, initials, date of birth (except year), or other personal identifiers.⁷ All such information appearing on any study document must be redacted before a copy of the document is supplied to the Sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed during the consenting process that representatives of the Sponsor, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence and in accordance with local data protection laws.

10.2 Responsibility of the Investigator

The Investigator is responsible for ensuring that the clinical study is performed in accordance with GCP based on the current ICH Guideline for GCP, the corresponding sections of the US CFR governing Protection of Human Subjects (Title 21 CFR Part 50), Institutional Review Boards (Title 21 CFR Part 56), the Basic Principles of the Declaration of Helsinki, and other applicable legal and regulatory requirements.

The Investigator should ensure that all persons assisting with the study are qualified for the duties assigned, adequately informed and trained on the protocol and amendments to the protocol, the study products, and their study-related duties and functions.

The Investigator will maintain a list, including signatures, of sub-investigators and other appropriately qualified persons to whom significant study-related duties are delegated. Any personnel change in this list during the course of the study will be documented. All study related training will be documented.

10.3 Procedure for Amendments to Protocol

No deviations from this protocol will be permitted, except in a medical emergency. The Investigator and the Sponsor will discuss any amendment to this study. If agreement is reached concerning the need for modification, this agreement will be made in a formal amendment to the protocol.

All revisions and/or amendments to the protocol must be approved, if applicable, in writing by Chesapeake IRB.

All persons who are affected by the amendment to the protocol will be retrained if deemed necessary.

10.4 Institutional Review Board

This protocol and ICFs will be reviewed and approved in writing by the IRB prior to commencement of the study. The study will not be initiated without the approval from the IRB. Any amendments after protocol approval, if applicable, will be reviewed and approved by the IRB prior to implementation. The IRB operations are in compliance with Title 21 CFR Part 56. Notice that the IRB approved protocol, ICF and any amendments to the protocol and ICF will be in the final study report.

10.5 Study Record Retention

Investigator-specific essential documents and all primary data and copies thereof (e.g., CRFs, laboratory records, data sheets, correspondence, photographs, computer records,), which are a result of the original observations and activities of the study and are necessary for the reconstruction and evaluation of any study report will be retained in the investigative site's archives for a minimum of 20 years after the completion or termination of the study. It is the responsibility of the Sponsor to inform the Investigator as to when these documents no longer need to be retained. The study report and final database will be retained in Celerion's archives for a minimum of 20 years after the completion or termination of the study and will be available for inspection at any time by the Sponsor. At completion of the study (i.e., at issuance of final study report), the final database will be transferred to the Sponsor. Subject initials, serology results, date of birth (except year), and other personal identifiers) will be redacted from this data transfer file; any such information removed will be documented at the time of transfer.

11. REFERENCES

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APPENDIX 1. Fagerström Test For Cigarette Dependence (FTCD)

Please answer each question below by checking (x) your answer in the box (□).

1. How soon after you wake up do you smoke your first cigarette?
 Within 5 minutes
 6 - 30 minutes
 31 - 60 minutes
 After 60 minutes
2. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in church, at the library, in the cinema, etc.)?
 Yes
 No
3. Which cigarette would you hate most to give up?
 The first one in the morning
 Any other
4. How many cigarettes per day do you smoke?
 31 or more
 21 - 30
 11 - 20
 10 or less
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?
 Yes
 No
6. Do you smoke if you are so ill that you are in bed most of the day?
 Yes
 No

APPENDIX 2: Brief Questionnaire of Smoking Urges (QSU-Brief)

For each item, please indicate how you feel RIGHT NOW.

1. I have a desire to smoke right now.

Strongly
Strongly
Disagree
Agree

2. Nothing would be better than smoking right now.

Strongly
Strongly
Disagree
Agree

3. If it were possible, I probably would smoke right now.

Strongly
Strongly
Disagree
Agree

4. I could control things better right now if I could smoke.

Strongly
Strongly
Disagree
Agree

5. All I want right now is a cigarette.

Strongly
Strongly
Disagree
Agree

6. I have an urge for a cigarette.

Strongly
Strongly
Disagree
Agree

7. A cigarette would taste good now.

Strongly
Strongly
Disagree
Agree

8. I would do almost anything for a cigarette now.

Strongly
Strongly
Disagree
Agree

9. Smoking would make me less depressed.

Strongly
Strongly
Disagree
Agree

10. I am going to smoke as soon as possible.

Strongly
Strongly
Disagree
Agree

APPENDIX 3: Minnesota Nicotine Withdrawal Scale - Revised (MNWS-R)

Please rate yourself for the last day

Scale: 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe

1. Angry, irritable, frustrated	0	1	2	3	4
2. Anxious, nervous	0	1	2	3	4
3. Depressed mood, sad	0	1	2	3	4
4. Desire or craving to smoke	0	1	2	3	4
5. Difficulty concentrating	0	1	2	3	4
6. Increased appetite, hungry, weight gain	0	1	2	3	4
7. Insomnia, sleep problems, awakening at night	0	1	2	3	4
8. Restless	0	1	2	3	4
9. Impatient	0	1	2	3	4
10. Constipation	0	1	2	3	4
11. Dizziness	0	1	2	3	4
12. Coughing	0	1	2	3	4
13. Dreaming or nightmares	0	1	2	3	4
14. Nausea	0	1	2	3	4
15. Sore throat	0	1	2	3	4

APPENDIX 4: Perceived Health Risk Scale

On the scale below, indicate your perception of the risk of becoming addicted to the cigarette you are currently using.

Very
low
risk

Very
high
risk