

## **Clinical Trial Protocol**

# **Evaluation of the Abuse Liability of Very Low Nicotine (VLN) Mentholated Cigarettes with Characterization of Nicotine Exposure Profiles in Adult Smokers**

VERY LOW NICOTINE (VLN) MENTHOL CIGARETTES

Original Protocol: 1.0, 04APR2018

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**22ND CENTURY GROUP, INC.:**

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Williamsville, NY 14221

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## 1. SPONSOR AND KEY PERSONNEL CONTACT INFORMATION

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## 2. PROTOCOL SYNOPSIS

<b>Name of Sponsor/Company:</b> 22nd Century Group Inc.
<b>Name of Finished Product:</b> Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)
<b>Study Title:</b> Evaluation of the Abuse Liability of Very Low Nicotine (VLN) Mentholated Cigarettes with Characterization of Nicotine Exposure Profiles in Adult Smokers
<b>Principal Investigator:</b> Debra Kelsh, MD
<b>Study Center:</b> Vince & Associates Clinical Research, Inc. 10103 Metcalf Avenue Overland Park, Kansas, 66212, USA
<b>Phase of Development: I</b> <b>Objectives:</b> The primary objective of the study is: <ul style="list-style-type: none"><li>To evaluate the abuse liability of VLN mentholated cigarettes (0.4 mg nicotine/gram of tobacco) relative to own-brand mentholated cigarettes and 4 mg nicotine polacrilex gum under controlled use and uncontrolled (ad libitum) use conditions.</li></ul> The secondary objectives of the study are: <ul style="list-style-type: none"><li>To compare the nicotine pharmacokinetic (PK) profiles of VLN mentholated cigarettes relative to own-brand mentholated cigarettes and nicotine polacrilex gum under controlled use and uncontrolled use conditions.</li><li>To characterize product use behavior of VLN mentholated cigarettes, own-brand mentholated cigarettes, and nicotine polacrilex gum.</li></ul>
<b>Methodology:</b> This study will be a randomized, two-part, 3-way crossover designed to evaluate the abuse liability, PK, and product use behavior associated with study products, including VLN mentholated cigarettes, subjects' own-brand mentholated cigarettes, and nicotine polacrilex gum in healthy adult male and female exclusive smokers. Subjects will participate in a standard Screening visit and one 7-day Confined Assessment Phase, which will include a product trial session (Day -1), and two study parts (Part A and Part B). Following the Screening visit, eligible subjects will check-in to the study site on Day -1. Following the polacrilex gum training session, subjects will be required to abstain from nicotine- and tobacco-containing products for approximately 20 hours until the first product use session on Days 1 to 3; use of other nicotine-containing products will be prohibited throughout the study. No additional tobacco or nicotine products will be provided after the second product use on Days 4 to 6.

<b>Name of Sponsor/Company:</b> 22nd Century Group Inc.
<b>Name of Finished Product:</b> Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)
<p>On Day 1, subjects will be randomized to one of three product sequence groups in Part A, which will consist of an ad libitum product use session for each of the following study products for 4 hours in a randomized crossover manner (Days 1 to 3; one product per day):</p> <ul style="list-style-type: none"><li>▪ Product A: VLN Menthol cigarette</li><li>▪ Product B: Own-brand menthol filtered standard king size cigarette</li><li>▪ Product C: 4 mg Nicotine polacrilex gum (Nicorette® White Ice Mint™)</li></ul> <p>A pharmacodynamic measure (“use product again” visual analog scale [VAS]) will be administered at the end of each ad libitum product use period. Product use behaviors (i.e., number of units consumed, duration of gum in mouth) will be collected throughout each ad libitum product use period.</p> <p>Upon completion of Part A, subjects will be randomized to one of three product sequence groups in Part B, which will consist of 3 study days (Days 4 to 6), with one product per day. Each study day will consist of: 1) Controlled Product Use Session (10 puffs from their own-brand menthol cigarette or VLN menthol cigarette [maximum <math>3 \pm 2</math> seconds per puff] at approximately <math>30 \pm 5</math>-second interpuff intervals, or chew the nicotine polacrilex gum using the “chew and park” method for 10 minutes); and 2) Uncontrolled Product Use Session (ad libitum use for 10 minutes). The Controlled Product Use Session and Uncontrolled Product Use Session will be separated by approximately 6 hours. During Part B, pharmacodynamic measures, PK, and product use behavior (Uncontrolled only) will be collected at various time points each day (See <a href="#">Table 1</a>).</p> <p>Safety assessments including adverse events (AEs), physical examinations, vital signs (respiratory rate, pulse rate, blood pressure, and oral temperature), electrocardiogram (ECG), clinical laboratory tests (clinical chemistry, hematology, urinalysis, and serology), and urine drug and alcohol screens will be collected at designated time points throughout the study. Subjects will be discharged from the clinic on Day 6, once all procedures are completed (or at Early Termination).</p>
<b>Number of Subjects (Planned):</b> <p>An appropriate number of subjects will be randomized on Day 1 (Part A) to ensure that a minimum of 54 subjects complete the study. Replacement subjects may be enrolled to ensure that the minimum number of subjects complete the study.</p>
<b>Criteria for Inclusion/Exclusion:</b> <p>Subjects will be required to meet each one of the following inclusion criteria in order to be eligible for participation in the study:</p> <ol style="list-style-type: none"><li>1. Must provide written informed consent prior to the initiation of any protocol-specific procedures.</li><li>2. Male and female adults, between 22 to 65 years of age, inclusive.</li><li>3. Body mass index (BMI) within 18.0 to 35.0 kg/m<sup>2</sup>, inclusive (minimum weight of at least 50.0 kg at Screening).</li><li>4. Healthy, as determined by no clinically significant medical history, physical examination, 12-lead ECG, vital signs or laboratory (including hematology, clinical chemistry, urinalysis, and serology) findings at Screening, as judged by an investigator.</li></ol>

<b>Name of Sponsor/Company:</b> 22nd Century Group Inc.
<b>Name of Finished Product:</b> Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)
<ol style="list-style-type: none"><li>5. Smoking history of an average of at least 10 manufactured menthol flavored filtered standard (i.e., not slim) king size combustible cigarettes daily for at least 1 year prior to Screening. Brief periods (i.e., up to 7 consecutive days) of non-smoking during the 3 months prior to Screening (e.g., due to illness or participation in a study where smoking was prohibited) will be permitted.</li><li>6. Self-reporting of desire to smoke within approximately 30 minutes of waking.</li><li>7. Positive urine cotinine (<math>\geq 500</math> ng/mL) at Screening.</li><li>8. Negative pregnancy test at Screening and Day -1 (check-in) for all female subjects.</li><li>9. Female subjects of non-childbearing potential must be surgically sterile or 1 year postmenopausal (as confirmed by serum Follicle Stimulating Hormone [FSH] <math>&gt; 35</math> U/L). A subject is considered to be surgically sterile if she has had a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy or bilateral oophorectomy, or hysterectomy with bilateral salpingo-oophorectomy. If the subject is of childbearing potential, she must be using a medically accepted method of contraception and agree to continued use of this method for the duration of the study and for 30 days after completion of the study. Acceptable methods of contraception include abstinence, birth control pill, or an intrauterine device (known to have a failure rate of less than 1% per year) or double barrier method of contraception (e.g., male condom in addition to a diaphragm, contraceptive sponge or spermicide).</li><li>10. Able to speak, read, and understand English sufficiently to allow completion of all study assessments.</li><li>11. Must be willing to comply with the requirements and restrictions of the study.</li></ol> <p>Subjects will not be eligible to participate in this study if any one of the following exclusion criteria is met:</p> <ol style="list-style-type: none"><li>1. Inability to tolerate 4 mg nicotine polacrilex gum during product use trial on Day -1 (check-in) or dentition prevents subjects from chewing gum.</li><li>2. History or presence of any clinically significant cardiac, psychiatric, endocrine, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, renal, or other major disease at Screening, which in the opinion of an investigator would jeopardize the safety of the subject or the validity of the study results.</li><li>3. History or presence of any type of malignant tumors.</li><li>4. Clinically significant abnormal findings on the vital signs, physical examination (including oral exam), medical history, or clinical laboratory results, in the opinion of an investigator.</li><li>5. Positive serology test results for human immunodeficiency virus (HIV-1/HIV-2) Antibodies, hepatitis B surface antigen (HbsAg), or hepatitis C Antibody (HCVAb).</li><li>6. An acute illness (e.g., upper respiratory infection, viral infection) requiring treatment within 2 weeks prior to Day -1 (check-in).</li><li>7. Drug or alcohol abuse or dependence within the 24 months prior to Screening (except nicotine), as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> edition text</li></ol>

<b>Name of Sponsor/Company:</b> 22nd Century Group Inc.
<b>Name of Finished Product:</b> Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)
revision (DSM-IV-TR), or any self-reported dependence or “addiction” within the subject’s lifetime (except nicotine or caffeine). 8. Subjects who have ever been in treatment for substance use disorder(s) or who are currently seeking treatment for substance use disorder(s). 9. Positive urine drug screen (UDS) or urine alcohol test at Screening or Day -1 (check-in). 10. History or any current conditions that may interfere with drug absorption, distribution, metabolism, or excretion. 11. History of severe allergic reaction (including anaphylaxis) to any substance, or previous status asthmaticus, or food allergies/intolerances/restrictions, or special dietary needs which, in the judgment of an investigator, contraindicates the subject’s participation in the study. 12. Requires concomitant treatment with prescription or non-prescription products that contain pseudoephedrine (e.g., nasal/sinus decongestants). 13. Self-reported use of nicotine polacrilex gum, or other nicotine replacement therapy products in the 30 days prior to Day -1 (check-in). Isolated incidents within 30 days prior to Day -1 (check-in) may be permitted at the discretion of the investigator. 14. Subject has unsuitable or difficult venous access or is unwilling or unable to undergo direct venipuncture or catheter insertion. 15. Subject has donated or lost 100 to 499 mL whole blood within 30 days or more than 499 mL whole blood within 56 days preceding entry into the Confined Assessment Phase. 16. Subject is an employee of the sponsor or research site personnel directly affiliated with this study or their immediate family member defined as a spouse, parent, child or sibling, whether biological or legally adopted. 17. Subject is lactating and or breast feeding. 18. A subject who, in the opinion of an investigator, is considered unsuitable or unlikely to comply with the study protocol for any reason.
<b>Test Product:</b> VLN menthol cigarettes (0.4 mg nicotine/gram of tobacco)
<b>Reference products:</b> Own-brand menthol-flavored combustible cigarettes Nicorette® White Ice Mint™ nicotine polacrilex gum (4 mg)
<b>Duration of Study:</b> Each subject will participate in the study for up to approximately five weeks, from Screening to Discharge.
<b>Criteria for Evaluation:</b> <i>Pharmacodynamic Endpoints:</i>

<b>Name of Sponsor/Company:</b> 22nd Century Group Inc.
<b>Name of Finished Product:</b> Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)
The primary pharmacodynamic endpoints are: <ul style="list-style-type: none"><li>▪ <b>Controlled Product Use (Part B):</b><ul style="list-style-type: none"><li>○ <math>E_{\max\_urge(\text{controlled})}</math>: The maximum reduction in VAS score for the question “Urges to smoke” (Tobacco/Nicotine Withdrawal Questionnaire) between pre-use and post-use (i.e., <math>VAS_{\text{pre-use1}} - VAS_{\text{post-use1}}</math>) during the first product use in Part B.</li><li>○ <math>E_{\max\_plst(\text{controlled})}</math>: The largest VAS score recorded for the response to the question “Is the product pleasant right now?” (Direct Effects of Product Questionnaire) during the first product use in Part B.</li></ul></li></ul>
The secondary pharmacodynamic endpoints are: <ul style="list-style-type: none"><li>▪ <b>Part A (Ad Libitum Product Use)</b><ul style="list-style-type: none"><li>▪ Use the Product Again VAS score</li><li>▪ Product use behavior (number of units consumed, duration of gum in mouth)</li></ul></li><li>▪ <b>Part B (Controlled and Uncontrolled Product Use)</b><ul style="list-style-type: none"><li>▪ Tobacco/Nicotine Withdrawal Questionnaire:<ul style="list-style-type: none"><li>○ <math>E_{\max\_item(\text{controlled})}</math>: The maximum reduction in VAS score for each item<sup>a</sup> between pre-use and post-use (i.e., <math>VAS_{\text{pre-use1}} - VAS_{\text{post-use1}}</math>) during the first product use in Part B.</li><li>○ <math>E_{\max\_item(\text{uncontrolled})}</math>: The maximum reduction in VAS score for each item between pre-use and post-use (i.e., <math>VAS_{\text{pre-use2}} - VAS_{\text{post-use2}}</math>) during the second product use in Part B.</li></ul></li><li>▪ Direct Effects of Product Questionnaire:<ul style="list-style-type: none"><li>○ <math>E_{\max\_item(\text{controlled})}</math>: The largest VAS score recorded for the response to each item<sup>b</sup> during the first product use in Part B.</li><li>○ <math>E_{\max\_item(\text{uncontrolled})}</math>: The largest VAS score recorded for the response to each item during the second product use in Part B.</li></ul></li><li>▪ Product use behavior<ul style="list-style-type: none"><li>○ Uncontrolled Product Use Sessions: number of inhalations, duration of inhalations [per puff], duration of polacrilex gum in mouth</li></ul></li></ul></li></ul>
<b>Pharmacokinetic Endpoints:</b> <ul style="list-style-type: none"><li>▪ <math>C_{\max(\text{controlled})}</math>: Maximum measured plasma nicotine concentration during the Controlled Use Session.</li><li>▪ <math>C_{\max(\text{uncontrolled})}</math>: Maximum measured plasma nicotine concentration during the Uncontrolled Use Session.</li></ul>

<sup>a</sup> Tobacco/Nicotine Withdrawal Questionnaire items (secondary):  $E_{\max\_urge}$  (uncontrolled only),  $E_{\max\_anx}$ ,  $E_{\max\_diffct}$ ,  $E_{\max\_impat}$ , and  $E_{\max\_crav}$ .

<sup>b</sup> Direct Effects of Product Questionnaire items (secondary):  $E_{\max\_plst}$  (uncontrolled only),  $E_{\max\_stf}$ ,  $E_{\max\_calm}$ ,  $E_{\max\_conc}$ ,  $E_{\max\_awake}$ ,  $E_{\max\_sick}$ ,  $E_{\max\_hunger}$ , and  $E_{\max\_more}$ .

**Name of Sponsor/Company:** 22nd Century Group Inc.

**Name of Finished Product:** Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)

- $AUC_{(controlled)}$ : Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time zero (defined as the start of controlled use) to 180 minutes (or the last quantifiable concentration during that interval).
- $AUC_{(uncontrolled)}$ : Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time 360 (defined as the start of uncontrolled use) to 540 minutes (or the last quantifiable concentration during that interval).
- $T_{max (controlled)}$ : Time of the maximum measured plasma nicotine concentration during the Controlled Use Session.
- $T_{max(uncontrolled)}$ : Time of the maximum measured plasma nicotine concentration during the Uncontrolled Use Session.
- $Kel_{(controlled)}$ : Apparent first-order terminal nicotine elimination rate constant calculated from a semi-log plot of the plasma concentration-time curve of the Controlled Use Session.
- $Kel_{(uncontrolled)}$ : Apparent first-order terminal nicotine elimination rate constant calculated from a semi-log plot of the Uncontrolled Use Session.
- $T_{1/2(controlled)}$ : Apparent first-order terminal nicotine elimination half-life calculated as  $0.693/Kel$  of the plasma concentration-time curve from time zero (defined as the start of controlled use) to 180 minutes.
- $T_{1/2(uncontrolled)}$ : Apparent first-order terminal nicotine elimination half-life calculated as  $0.693/Kel$  of the plasma concentration-time curve from time zero (defined as the start of uncontrolled use) to 540 minutes.

**Safety:**

The following safety endpoints will be evaluated:

- Incidence, frequency, severity, and relationship to product of AEs
- Clinical laboratory assessments (hematology, biochemistry, urinalysis)
- Vital signs (respiratory rate, pulse rate, blood pressure, and oral temperature)
- 12-lead ECG
- Physical examination results
- Concomitant medications

**Statistical Methods (Data Analysis):**

**Determination of Sample Size:**

Using a 2-sided Type I error rate at  $\alpha=0.05$  and assuming a mean difference in  $E_{max\_urge(controlled)}$  between own-brand cigarette and nicotine polacrilex gum of 15.85 points and standard deviation (SD) of 28.44, 54 completed subjects will be required to detect a significant difference between own-brand cigarette and nicotine polacrilex gum with greater than 80% power.

Using a 2-sided Type I error rate at  $\alpha=0.05$  and assuming a mean difference in  $E_{max\_plst(controlled)}$  between own-brand cigarette and nicotine polacrilex gum of 35.21 points and SD of 31.82, estimate based on

**Name of Sponsor/Company:** 22nd Century Group Inc.

**Name of Finished Product:** Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)

data obtained from the study site, 16 completed subjects will be adequate to detect a significant difference between own-brand cigarette and nicotine polacrilex gum with greater than 80% power.

The study analysis populations will be defined as follows:

*Randomized Population (Part A):* All subjects who are randomized into Part A.

*Randomized Population (Part B):* All subjects who are randomized into Part B.

*Safety Population:* All randomized subjects who use at least one of the study products. This will include Day -1 (check-in and product trial) and Study Days after the randomization. The Safety Population will be used for the summary of subject demographics, baseline characteristics, safety information, and AEs.

*Pharmacodynamic Population:* All subjects who use any study product and have pre-use (Tobacco/Nicotine Withdrawal questionnaire) and at least one post-use (for Tobacco/Nicotine Withdrawal and Direct Effects of Product) VAS score for the Controlled Use Session of Part B. This population will be used for statistical analyses of the pharmacodynamic measures.

*PK Population:* All subjects who use any study product and have pre- and at least one post-use plasma nicotine concentration value for the Controlled Use Session of Part B. The PK Population will be used for statistical analyses of the PK parameters.

*Completer Population:* All randomized subjects who complete all product use periods and have PK and pharmacodynamic data in the Controlled Use Session of Part B. This dataset will be used for analyses of the two primary pharmacodynamic measures endpoints as a sensitivity analysis to the primary analysis.

***Pharmacodynamic Measures:***

*Part A:*

Responses to Use the Product Again VAS will be summarized by study product using descriptive statistics. A summary table of frequencies will also be provided.

Product use behavior (number of units, duration of gum in mouth) will be summarized by study product using descriptive statistics.

*Part B:*

Responses to each Tobacco/Nicotine Withdrawal Questionnaire and Direct Effects of Product Questionnaire item will be summarized by time point for each study product, and product use condition. For Tobacco/Nicotine Withdrawal VAS scores, descriptive statistics for the difference from pre-use at each time point will also be reported. Derived parameters ( $E_{max}$ ) for each questionnaire item will be summarized using descriptive statistics.

**Name of Sponsor/Company:** 22nd Century Group Inc.

**Name of Finished Product:** Very Low Nicotine (VLN) menthol cigarettes (0.4 mg nicotine/gram of tobacco)

A linear mixed effects model for analysis of variance/covariance (Proc Mixed) will be performed on  $E_{\max\_urge(\text{controlled})}$  and  $E_{\max\_plst(\text{controlled})}$ . The model will include  $E_{\max}$  as the response variable, sequence, study product, and period as fixed model effects, baseline score as a covariate (for  $E_{\max\_urge}$  only) and subject nested-within sequence as a random effect. Sequence will be tested using subject nested-within-sequence as the error term. Least square mean (LSM) and 95% confidence interval (CI) for each Study Product group will be provided. Comparisons will be made for Product A vs. B, A vs. C and B vs. C. The comparison between Product A and Product C will be used as a comparison for internal validity. The LSM difference, p value and 95% CI of the difference will be provided.

The same model will be used for  $E_{\max\_urge(\text{uncontrolled})}$  and  $E_{\max\_plst(\text{uncontrolled})}$ , and all  $E_{\max}$  of other questions in the Tobacco/Nicotine Withdrawal Questionnaire and Direct Effects of Product Questionnaire for the controlled and uncontrolled product uses in Part B.

Product use behavior will be summarized by product using descriptive statistics.

***Pharmacokinetics:***

Descriptive statistics of plasma nicotine concentrations will be tabulated by time point for each study product and product use condition. Derived PK parameters (both unadjusted for baseline and baseline-adjusted) will be summarized using descriptive statistics. Summaries and individual data will be provided for plasma nicotine-adjusted concentrations.

A linear mixed model for analysis of variance (Proc Mixed) will be performed on the baseline-adjusted log transformed nicotine PK parameters in Part B. The model will include sequence, study product, and period as fixed effects and subject nested within sequence as a random effect. Sequence will be tested using subject nested-within sequence as the error term. From each model, the geometric LSM and 95% CI will be calculated for each study product. Separate analyses will be performed on the parameters calculated for the controlled and uncontrolled product use sessions. Geometric mean ratios for each pair comparison, 95% CI and p value will be provided.

The same linear mixed effect model as used for the analysis of  $C_{\max(\text{controlled})}$  will be used for  $C_{\max(\text{uncontrolled})}$  of second product use in Part B, and for AUC following all product uses. The Hodges-Lehmann's method will be used to estimate the median differences and the 95% CI of  $T_{\max}$  and  $T_{1/2}$  between the products.

***Safety:***

A by-subject AE data listing, including verbatim term, preferred term, study product, severity, and relationship to study product, will be provided. Study product use-emergent AEs will be summarized with tables. Frequencies of subjects with AEs, serious adverse events (SAEs) and incidence of those events, regardless of relationship to study product, will be summarized by study product and sorted by system organ class. Frequencies of AEs will be summarized by severity and relationship to study product. Clinical laboratory evaluations (serum chemistry, hematology, and urinalysis), vital signs measurements (blood pressure, pulse, respiration, and oral temperature) and ECG measurements (VR, PR, QRS, QT, and QTcF intervals) will be summarized descriptively. All concomitant medications recorded during the study will be listed by subject. Physical examinations will be listed by subject and time point of collection.

### **3. TABLE OF CONTENTS**

<b>1. Sponsor and Key Personnel Contact Information.....</b>	<b>2</b>
<b>2. Protocol Synopsis .....</b>	<b>3</b>
<b>3. Table of Contents .....</b>	<b>11</b>
<b>4. List of Abbreviations and Definition of Terms.....</b>	<b>14</b>
<b>5. Introduction.....</b>	<b>16</b>
5.1. Background .....	16
5.2. Study Rationale .....	16
<b>6. Study Objectives .....</b>	<b>18</b>
6.1. Primary Objective .....	18
6.2. Secondary Objectives.....	18
<b>7. Investigational Plan .....</b>	<b>19</b>
7.1. Overall Study Design and Plan .....	19
7.1.1. Screening Phase (Visit 1) .....	19
7.1.2. Confined Assessment Phase (Visit 2).....	19
7.1.3. End of Study or Early Termination .....	20
7.2. Discussion of Study Design .....	25
<b>8. Selection of Study Population .....</b>	<b>27</b>
8.1. Inclusion Criteria.....	27
8.2. Exclusion Criteria.....	28
8.3. Removal of Subjects from or Assessment.....	29
8.4. Study Restrictions .....	30
<b>9. Materials.....</b>	<b>31</b>
9.1. Study Products .....	31
9.2. Identity of Investigational Product(s).....	31
9.2.1. Handling, Storage, and Accountability .....	31
9.2.2. Dispensing and Administration .....	31
9.3. Method of Assigning Subjects to Study Groups .....	31
9.4. Selection and Timing of Product Use.....	32
9.4.1. Part A (Days 1 to 3).....	32
9.4.2. Part B (Days 4 to 6).....	33
9.5. Meal Schedule .....	33
9.6. Blinding.....	34
9.7. Prior and Concomitant Therapy .....	34
9.8. Product Compliance .....	34

<b>10. Study Procedures and Assessments</b> .....	<b>35</b>
10.1. Demographics and Other Baseline Characteristics .....	35
10.1.1. Informed Consent.....	35
10.1.2. Demographics.....	35
10.1.3. Medical History.....	35
10.1.4. Medication and Smoking History .....	35
10.2. Pharmacodynamic Measures.....	35
10.2.1. Tobacco/Nicotine Withdrawal Scale .....	35
10.2.2. Direct Effects of Product Questionnaire .....	36
10.2.3. Use the Product Again Questionnaire .....	36
10.2.4. Product Use .....	36
10.3. Safety Assessments .....	37
10.3.1. Adverse Events and Serious Adverse Events.....	37
10.3.2. Pregnancy .....	40
10.3.3. Clinical Laboratory Assessments .....	40
10.3.4. Urine Drug Screen and Alcohol Testing.....	41
10.3.5. Vital Signs .....	42
10.3.6. 12-lead Electrocardiograms.....	42
10.3.7. Physical Examination.....	42
10.4. Pharmacokinetic Assessments.....	42
10.5. Appropriateness of Measures.....	43
10.6. Pharmacodynamic Variables .....	43
10.6.1. Primary Pharmacodynamic Endpoints .....	43
10.6.2. Secondary Endpoints.....	43
10.7. Pharmacokinetic Variables .....	44
10.8. Safety Variables.....	45
<b>11. Data Quality Assurance</b> .....	<b>46</b>
11.1. Data Collection.....	46
11.2. Study Auditing and Monitoring .....	46
<b>12. Statistical Methods and Determination of Sample Size</b> .....	<b>48</b>
12.1. Statistical and Analytical Plans .....	48
12.2. Determination of Sample Size .....	48
12.3. Analysis Populations .....	48
12.4. Planned Analyses .....	49
12.4.1. Subject Disposition .....	49
12.4.2. Demographics and Other Baseline Characteristics .....	49
12.4.3. Analysis of Pharmacodynamic Measures .....	49
12.4.4. Analysis of Pharmacokinetics .....	50

12.4.5. Analysis of Safety Assessments .....	51
<b>13. Study Administration and Investigator Responsibilities.....</b>	<b>52</b>
13.1. Regulatory and Ethical Considerations .....	52
13.1.1. Ethical Conduct of the Study .....	52
13.1.2. Regulatory Authority and Ethics Approval .....	52
13.1.3. Subject Informed Consent.....	52
13.2. Privacy and Confidentiality.....	53
13.3. Study and Site Closure .....	53
13.4. Regulatory Documents and Records Retention .....	54
13.5. Delegation of Responsibilities and Adequate Resources .....	54
13.6. Protocol Amendments .....	55
13.7. Financial Disclosure.....	55
<b>14. Sponsor Approval Page .....</b>	<b>56</b>
<b>15. Investigator Protocol Agreement Page.....</b>	<b>57</b>
<b>16. References.....</b>	<b>58</b>
<b>17. Appendices .....</b>	<b>60</b>
17.1. Summary of Blood Volume Requirements.....	60
17.2. Pharmacodynamic Measures/Scales .....	61
17.2.1. Tobacco/Nicotine Withdrawal Questionnaire .....	61
17.2.2. Direct Effects of Product Questionnaire .....	62
17.2.3. Use the Product Again Questionnaire .....	62

### **Table of Tables**

Table 1: Schedule of Assessments .....	22
Table 2: Clinical Laboratory Assessments .....	41

### **Table of Figures**

Figure 1: Overview of Study Design .....	21
--	----

#### 4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
AUC <sub>(controlled)</sub>	Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time zero (defined as the start of controlled use to 180 minutes, or the last quantifiable concentration during that interval).
AUC <sub>(uncontrolled)</sub>	Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time 360 (defined as the start of uncontrolled use to 540 minutes, or the last quantifiable concentration during that interval).
BMI	Body mass index
CI	Confidence interval
C <sub>max</sub> (controlled/uncontrolled)	Maximum measured plasma concentration during the controlled/uncontrolled use session
CRF	Case Report Form (may include electronic data capture systems or paper forms)
CSA	Clinical study agreement
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4 <sup>th</sup> edition text revision
ECG	Electrocardiogram
EDC	Electronic data capture
E <sub>max_item</sub>	Maximum reduction in VAS score for each item between pre-use and post-use (i.e., VAS <sub>pre-use1</sub> – VAS <sub>post-use1</sub> )
E <sub>max_plst</sub>	Maximum score for Pleasant VAS
E <sub>max_urge</sub>	Maximum reduction “Urges to smoke” (Tobacco/Nicotine Withdrawal Questionnaire) between pre-use and post-use
ENDS	Electronic nicotine delivery system
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
HbsAg	Hepatitis B surface antigen
HCVAb	Hepatitis C Antibody

HIV	Human Immunodeficiency Virus
ICF	Informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
$K_{el(\text{controlled/uncontrolled})}$	Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration-time curve of the controlled/uncontrolled use session.
LSM	Least square mean
PK	Pharmacokinetic
QA	Quality assurance
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
$T_{1/2(\text{controlled})}$	Apparent first-order terminal elimination half-life calculated as $0.693/K_{el}$ of the plasma concentration-time curve from time zero (defined as the start of controlled use) to 180 minutes.
$T_{1/2(\text{uncontrolled})}$	Apparent first-order terminal elimination half-life calculated as $0.693/K_{el}$ of the plasma concentration-time curve from time zero (defined as the start of controlled use) to 540 minutes.
THC	tetrahydrocannabinol
$T_{\max(\text{controlled/uncontrolled})}$	Time of the maximum measured plasma concentration during the controlled /uncontrolled use session.
VAS	Visual analog scale
VLN	Very low nicotine
UDS	Urine drug screen

## 5. INTRODUCTION

### 5.1. Background

With the introduction of the 2009 Family Smoking Prevention and Tobacco Control Act, the Food and Drug Administration (FDA) was granted the authority to regulate tobacco products including the nicotine content of cigarettes. In July 2017, FDA Commissioner Scott Gottlieb, M.D., announced the FDA's intention to limit nicotine in cigarettes to minimally or non-addictive levels. During his announcement, Dr. Gottlieb stated that it has long been known that nicotine is the primary addictive component of cigarettes and that if smokers lose their addiction to cigarettes, they will be free to choose when and how often to smoke. A key component in the FDA's announced plan is to provide current smokers who are unable or unwilling to quit using nicotine with other, less harmful, means to obtain it. In other words, to provide a migration route away from traditional cigarettes to other types of tobacco or nicotine products such as cigarettes based on heat not burn technology, traditional smokeless, Snus, or electronic nicotine delivery system (ENDS) products.

Historically, tobacco harm reduction initiatives have focused on efforts to prevent or reduce tobacco initiation rates, increase quit rates, and decrease recidivism. The proposed reduction of nicotine cigarettes is believed to represent a viable policy tool to impact cigarette initiation rates and cigarette quit rates. Studies conducted to date indicate that reducing the nicotine content of cigarettes to non-addictive levels would potentially limit the reinforcing effects of cigarettes. A number of recent studies suggests that cigarettes with very low nicotine (VLN) content are associated with a positive set of outcomes, including reductions in the number of cigarettes smoked, reductions in nicotine exposure and nicotine dependence, increased abstinence from smoking, reduced exposure to toxicants, and fewer adverse events (AEs) ([Rupprecht et al., 2017](#); [Donny et al., 2007](#); [Donny et al., 2015](#); [Benowitz et al., 2007](#); [Benowitz et al., 2009](#); [Benowitz et al., 2012](#); [Hatsukami et al., 2013](#)). These studies evaluated cigarettes with nicotine content ranging from 0.4 to 15.8 mg nicotine per gram of tobacco with the typical combustible cigarette tobacco content ranging from approximately 8 mg to 20 mg.

The cigarettes included in the reported studies were manufactured by 22nd Century Group, Inc. under contract to RTI/National Institute on drug Abuse (NIDA). 22nd Century Group, Inc. is currently evaluating the commercial potential of a menthol and non-menthol VLN cigarette (0.4 mg nicotine/gram of tobacco). It is anticipated that these cigarettes will provide similar benefits to a consumer that switches from their usual brand to the VLN cigarette as those reported in the literature ([Rupprecht et al., 2017](#); [Donny et al., 2007](#); [Donny et al., 2015](#); [Benowitz et al., 2007](#); [2009](#); [2012](#); [Hatsukami et al., 2013](#)).

### 5.2. Study Rationale

The purpose of the current study is to provide an abuse liability assessment of VLN mentholated cigarettes compared with own-brand mentholated cigarettes and nicotine polacrilex gum in current manufactured filtered king size cigarette smokers, as recommended in "FDA Draft Guidance for Industry Modified Risk Tobacco Products ([March 2012](#))". Results of this study

may provide supporting evidence for lower abuse liability of VLN mentholated cigarettes relative to own-brand mentholated cigarettes.

## **6. STUDY OBJECTIVES**

### **6.1. Primary Objective**

The primary objective of the study is:

- To evaluate the abuse liability of VLN mentholated cigarettes (0.4 mg nicotine/gram of tobacco) relative to own-brand mentholated cigarettes and nicotine polacrilex gum under controlled use and uncontrolled use conditions.

### **6.2. Secondary Objectives**

The secondary objectives of the study are:

- To compare the nicotine pharmacokinetic (PK) profiles of VLN mentholated cigarettes relative to own-brand mentholated cigarettes and nicotine polacrilex gum under controlled use and uncontrolled use conditions.
- To characterize product use behavior of VLN mentholated cigarettes, own-brand mentholated cigarettes, and nicotine polacrilex gum.

## **7. INVESTIGATIONAL PLAN**

### **7.1. Overall Study Design and Plan**

This study will be a randomized, two-part, 3-way crossover designed to evaluate the abuse liability, PK, and product use behavior associated with study products, including VLN mentholated cigarettes, subjects' own-brand mentholated cigarettes, and nicotine polacrilex gum in healthy adult male and female exclusive smokers. The study will consist of 3 phases: Screening, a Confined Assessment Phase consisting of product training session, Part A, and Part B, and an End of Study Phase.

#### **7.1.1. Screening Phase (Visit 1)**

The Screening Phase will be completed during a clinic visit within 28 days of the Confined Assessment Phase and will consist of a standard medical screen.

#### **7.1.2. Confined Assessment Phase (Visit 2)**

##### **7.1.2.1. Check-in and Product Trial (Day -1)**

Subjects who successfully complete the Screening Phase will return to the clinical unit on Day -1 for check-in and to complete a product trial session. Subjects will engage in a 10-minute product training session with the nicotine polacrilex gum in order to familiarize themselves with the “chew and park” method, which requires subjects to chew the gum until they experience a tingling sensation, park the gum between the cheek and gum until the tingling subsides, and then begin chewing again. On Day -1, subjects will also complete a training session on the pharmacodynamic questionnaires. Subjects will be required to abstain from using nicotine- and tobacco-containing products for approximately 20 hours prior to each product use session in Part A.

##### **7.1.2.2. Part A (Day 1 to 3)**

On Day 1, subjects will be randomized to one of three product sequence groups in Part A, which will consist of an ad libitum product use session of each of the following study products for 4 hours in a randomized crossover manner (one product per day):

- Product A: VLN menthol cigarette
- Product B: Own-brand menthol cigarette
- Product C: Nicorette® White Ice Mint™

A pharmacodynamic measure (“use product again” visual analog scale [VAS]) will be administered at the end of each ad libitum product use period and product use behaviors (i.e., number of units consumed, duration of gum in mouth) will be collected throughout each ad libitum product use period (See [Table 1](#)).

### **7.1.2.3. Part B (Day 4 to 6)**

Upon completion of Part A, subjects will be randomized to one of three product sequence groups in Part B, which will consist of 3 study days (Days 4 to 6), with one product per day. Each study day will consist of: 1) Controlled Product Use Session (10 puffs from their own-brand menthol cigarette or VLN menthol cigarette [maximum  $3 \pm 2$  seconds per puff] at approximately  $30 \pm 5$ -second interpuff intervals<sup>a</sup>, or chew the nicotine polacrilex gum using the “chew and park” method for 10 minutes); and 2) Uncontrolled Product Use Session (use of one unit of a product ad libitum for 10 minutes). The Controlled Product Use Session and Uncontrolled Product Use Session will be separated by approximately 6 hours. During Part B, pharmacodynamic measures, PK samples, and product use behavior (Uncontrolled Product Use Session only) will be collected at various time points each day (See [Table 1](#)).

Safety assessments, including AEs, physical examinations, vital signs (respiratory rate, pulse rate, blood pressure, and oral temperature), electrocardiogram (ECG), clinical laboratory tests (clinical chemistry, hematology, urinalysis, and serology), urine drug screen (UDS), and alcohol test will be collected at designated time points throughout the study.

### **7.1.3. End of Study or Early Termination**

Subjects will be discharged from the clinic on Day 6 once all procedures are completed (or at Early Termination).

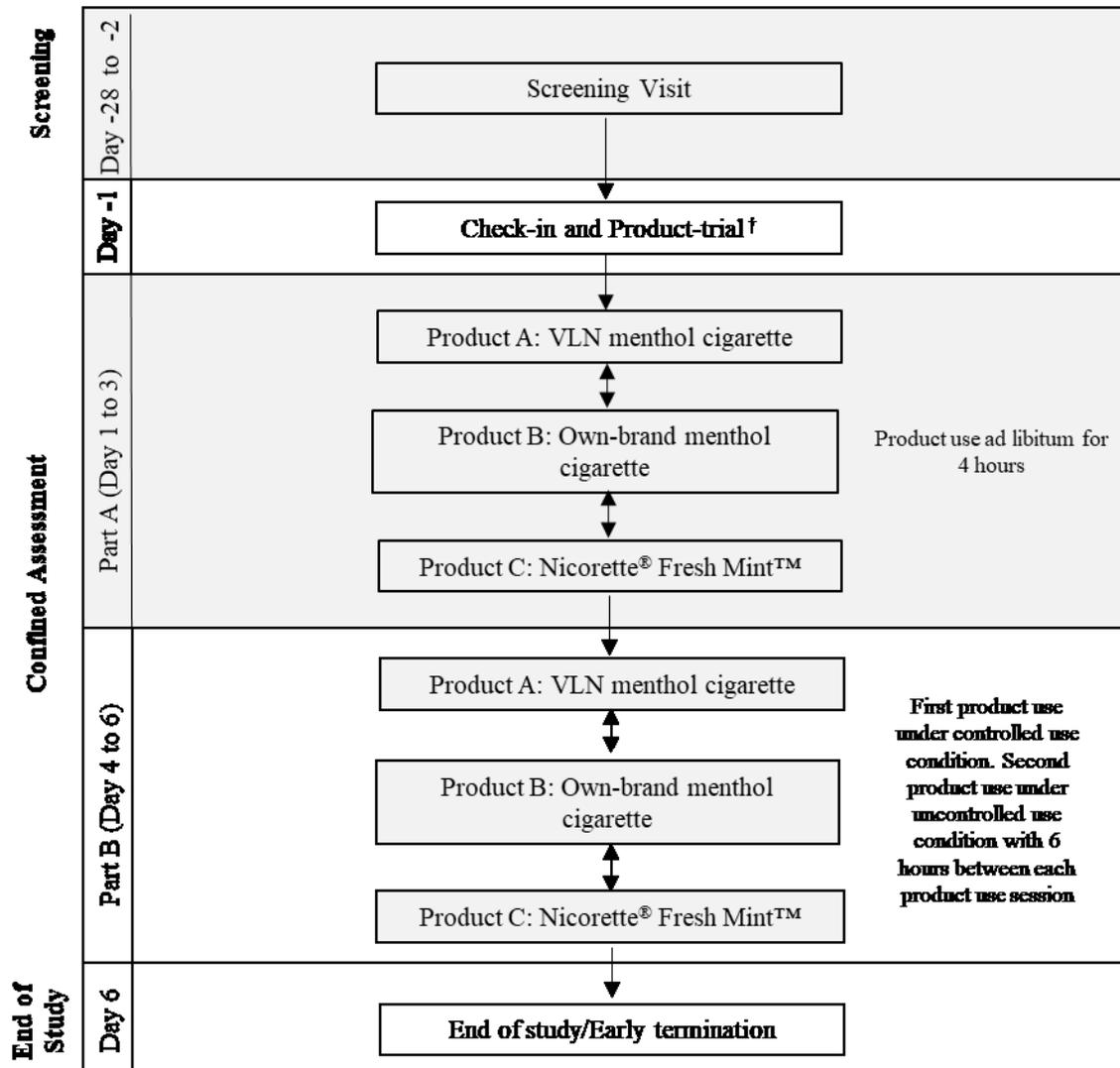
The maximum duration of subject participation, including Screening will be approximately 5 weeks.

An overview of the study design is provided in [Figure 1](#).

Study assessments and procedures will be performed at the visits and time points outlined in the Schedule of Assessments ([Table 1](#)).

<sup>a</sup> In the event that the subject completes the cigarette in less than 10 puffs, no additional cigarette will be provided.

**Figure 1: Overview of Study Design**



<sup>†</sup> Ad libitum use of the nicotine gum for 10 minutes. Subjects will be instructed on how to correctly use the nicotine gum using the “chew and park” method.

**Table 1: Schedule of Assessments**

	Screening	Check-in	Part A	Part B														End of Study/ET		
Day:	-28 to -2	-1	1 to 3	4 to 6 (Daily controlled use and uncontrolled use sessions)														6		
Assessment	Assessment timepoints (minutes <sup>a</sup> )																			
Informed consent	X																			
Demographics	X																			
Medical history	X																			
Review of eligibility	X	X																		
Physical examination	X	X <sup>b</sup>																		X <sup>b</sup>
Height, weight, BMI	X																			
HIV, Hepatitis B/C	X																			
Pregnancy test	X <sup>c</sup>	X <sup>d</sup>																		
FSH (post-menopausal women)	X																			
Vital signs <sup>e</sup>	X	X																		X
Oral temperature	X	X																		X
12-lead ECG	X																			X
Urine cotinine screen	X																			
Urine drug and alcohol test	X	X																		

<sup>a</sup> All listed timepoints are minutes after the start of product use

<sup>b</sup> Abbreviated (symptom-directed) physical examination performed at the investigator’s discretion

<sup>c</sup> Serum pregnancy test

<sup>d</sup> Urine pregnancy test

<sup>e</sup> Vital signs include respiratory rate, pulse rate and blood pressure



	Screening	Check-in	Part A	Part B														End of Study/ET	
Day:	-28 to -2	-1	1 to 3	4 to 6 (Daily controlled use and uncontrolled use sessions)														6	
Assessment				Assessment timepoints (minutes <sup>a</sup> )															
Direct Effects of Product Questionnaire <sup>o</sup>				pre			5			15		30		60	90				
Use the product again VAS			X <sup>p</sup>												90 <sup>o</sup>				
Amount of product used			X <sup>q</sup>	< <sup>r</sup>	-	-	-	>											
Tobacco cessation information																			X
Admission		X																	
Discharge																			X

NOTE: Pre-use assessments (not including PK) may be conducted up to 60 minutes prior to product use. When assessments coincide at any given timepoint during a product use session, the order of assessments should be pharmacodynamics assessments followed by PK. The pharmacodynamic assessments should be conducted at the nominal timepoint (±2 minutes up to 30 minutes postdose and ±5 minutes postdose thereafter). AE=adverse event; BMI=body mass index; ECG=electrocardiogram; ET=early termination; FSH=follicle stimulating hormone; HIV=human immunodeficiency virus; pre=pre-use

<sup>p</sup> Questionnaire administered at end of each product use session, within 10 minutes of completing the product use session (i.e., 4 hours ± 10 minutes)

<sup>q</sup> Number of units consumed and duration of gum in mouth

<sup>r</sup> Uncontrolled Product Use Sessions only; number of inhalations per cigarette, duration of inhalations [per puff], duration of gum in mouth

## 7.2. Discussion of Study Design

The overall study design is consistent with similar publicly available clinical studies conducted with cigarettes, non-combustible nicotine products (i.e., e-cigarettes) or nicotine replacement therapy in that it will be conducted using a randomized, cross-over design. This approach controls for inter-individual variability with each subject acting as his/her own control (Vansickel et al., 2010; McPherson et al., 2016; Stiles et al., 2017). Randomization will be used to avoid bias in the assignment of subjects to order of product use, to increase the likelihood that known and unknown subject attributes (e.g., demographics and baseline characteristics) are evenly balanced across groups, and to enhance the validity of statistical comparisons across groups.

Subjects will be healthy current combustible manufactured filtered king size menthol cigarette smokers, using their own-brand of menthol cigarettes as the positive control. This should serve as the most highly reinforcing product for cigarette smokers and is more representative of a “real-world” scenario. Consistent with the “continuum of risk” of tobacco and nicotine products, which suggests that nicotine replacement therapies provide a lower risk of abuse (Fagerstrom and Eissenberg, 2012), addiction and harm relative to combustible cigarettes, this study will also include nicotine polacrilex gum as the negative control comparator. Nicotine polacrilex gum has been shown to have low to moderate reinforcing effects compared with combustible cigarettes (Stiles et al., 2017). Although traditional abuse liability studies of pharmaceutical products include a placebo comparator, the nature of the products in the current study will be apparent to the subjects, therefore, it is not possible to implement a true placebo control. Rather, the study includes both a positive and a negative control to test the sensitivity of the model, and to achieve a direct relative comparison of the abuse liability of VLN mentholated cigarettes to own-brand mentholated cigarettes and nicotine polacrilex gum.

The current study will include a pre-specified period of abstinence prior to each study product administration day (approximately 20 hours in Part A and prior to controlled-use session in Part B, and 6 hours between controlled and uncontrolled-use sessions in Part B) to minimize the impact that residual nicotine concentrations may have on baseline pharmacodynamic, PK and physiological measurements. Consistent with previously published studies, the current study will include an ad libitum product use session, which will allow the subject to use the product as they would in a “real-world” scenario with no limitation on amount of product used, for up to 4 hours. Controlled and uncontrolled product use sessions are also included in order to compare the pharmacodynamic effects and patterns of use under conditions where subjects are instructed on how to use the product (i.e., [maximum  $3 \pm 2$  seconds per puff] at approximately  $30 \pm 5$ -second interpuff intervals) versus how they would use the product in a typical situation.

The current study will use measures of urge to smoke, positive effects, intent to use the product again, and negative effects as proxy measures for the relative reinforcing efficacy of VLN mentholated cigarettes, own-brand mentholated cigarettes and 4 mg nicotine polacrilex gum. The ability of the VLN mentholated cigarettes to satisfy the urge to smoke over the short term will be assessed using the “Urge to Smoke” question in the Tobacco/Nicotine Withdrawal Questionnaire measure and positive effects will be assessed using the “Is the product ‘Pleasant’ right now?” question in the Direct Effects of Product Questionnaire; both will be administered as 100-point

VAS. The “Use Product Again” bipolar VAS has been included at the end of the ad libitum use session in Part A and 90 minutes after product use in Part B to evaluate overall reinforcement effects of the test products. Additional subjective effects will be assessed with “at-the-moment” VAS (Direct Effects of Product Questionnaire, Tobacco/Nicotine Withdrawal Questionnaire) during the Controlled and Uncontrolled Use Sessions to evaluate the magnitude, onset, and offset of product effects and withdrawal symptoms during and after controlled use of the study products.

## **8. SELECTION OF STUDY POPULATION**

An appropriate number of subjects will be randomized on Day 1 (Part A) to ensure that a minimum of 54 subjects complete the study.

### **8.1. Inclusion Criteria**

Subjects must meet each one of the following inclusion criteria in order to be eligible for participation in the study:

1. Must provide written informed consent prior to the initiation of any protocol-specific procedures.
2. Male and female adults, between 22 to 65 years of age, inclusive.
3. Body mass index (BMI) within 18.0 to 35.0 kg/m<sup>2</sup>, inclusive (minimum weight of at least 50.0 kg at Screening).
4. Healthy, as determined by no clinically significant medical history, physical examination, 12-lead ECG, vital signs or laboratory (including hematology, clinical chemistry, urinalysis, and serology) findings at Screening, as judged by an investigator.
5. Smoking history of an average of at least 10 manufactured menthol flavored filtered standard (i.e., not slim) king size combustible cigarettes daily for at least 1 year prior to Screening. Brief periods (i.e., up to 7 consecutive days) of non-smoking during the 3 months prior to Screening (e.g., due to illness or participation in a study where smoking was prohibited) will be permitted.
6. Self-reporting of desire to smoke within approximately 30 minutes of waking.
7. Positive urine cotinine ( $\geq 500$  ng/mL) at Screening.
8. Negative pregnancy test at Screening and Day -1 (check-in) for all female subjects.
9. Female subjects of non-childbearing potential must be surgically sterile or 1 year postmenopausal (as confirmed by serum Follicle Stimulating Hormone [FSH]  $> 35$  U/L). A subject is considered to be surgically sterile if she has had a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy or bilateral oophorectomy, or hysterectomy with bilateral salpingo-oophorectomy. If the subject is of childbearing potential, she must be using a medically accepted method of contraception and agree to continued use of this method for the duration of the study and for 30 days after completion of the study. Acceptable methods of contraception include abstinence, birth control pill, or an intrauterine device (known to have a failure rate of less than 1% per year) or double barrier method of contraception (e.g., male condom in addition to a diaphragm, contraceptive sponge or spermicide).
10. Able to speak, read, and understand English sufficiently to allow completion of all study assessments.
11. Must be willing to comply with the requirements and restrictions of the study.

## 8.2. Exclusion Criteria

Subjects will not be eligible to participate in this study if any one of the following exclusion criteria is met:

1. Inability to tolerate 4 mg nicotine polacrilex gum during product use trial on Day -1 (check-in) or dentition prevents subjects from chewing gum.
2. History or presence of any clinically significant cardiac, psychiatric, endocrine, hematologic, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, renal, or other major disease at Screening, which in the opinion of an investigator would jeopardize the safety of the subject or the validity of the study results.
3. History or presence of any type of malignant tumors.
4. Clinically significant abnormal findings on the vital signs, physical examination (including oral exam), medical history, or clinical laboratory results, in the opinion of an investigator.
5. Positive serology test results for human immunodeficiency virus (HIV)-1/HIV-2 Antibodies, hepatitis B surface antigen (HbsAg), or hepatitis C Antibody (HCVAb).
6. An acute illness (e.g., upper respiratory infection, viral infection) requiring treatment within 2 weeks prior to Day -1 (check-in).
7. Drug or alcohol abuse or dependence within the 24 months prior to Screening (except nicotine), as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> edition text revision (DSM-IV-TR), or any self-reported dependence or “addiction” within the subject’s lifetime (except nicotine or caffeine).
8. Subjects who have ever been in treatment for substance use disorder(s) or who are currently seeking treatment for substance use disorder(s).
9. Positive urine drug screen (UDS) or urine alcohol test at Screening or Day -1 (check-in).
10. History or any current conditions that may interfere with drug absorption, distribution, metabolism, or excretion.
11. History of severe allergic reaction (including anaphylaxis) to any substance, or previous status asthmaticus, or food allergies/intolerances/restrictions, or special dietary needs which, in the judgment of an investigator, contraindicates the subject’s participation in the study.
12. Requires concomitant treatment with prescription or non-prescription products that contain pseudoephedrine (e.g., nasal/sinus decongestants).
13. Self-reported use of nicotine polacrilex gum, or other nicotine replacement therapy products in the 30 days prior to Day -1 (check-in). Isolated incidents within 30 days prior to Day -1 (check-in) may be permitted at the discretion of the investigator.

14. Subject has unsuitable or difficult venous access or is unwilling or unable to undergo direct venipuncture or catheter insertion.
15. Subject has donated or lost 100 to 499 mL whole blood within 30 days or more than 499 mL whole blood within 56 days preceding entry into the Confined Assessment Phase.
16. Subject is an employee of the sponsor or research site personnel directly affiliated with this study or their immediate family member defined as a spouse, parent, child or sibling, whether biological or legally adopted.
17. Subject is lactating and or breast feeding.
18. A subject who, in the opinion of an investigator, is considered unsuitable or unlikely to comply with the study protocol for any reason.

### **8.3. Removal of Subjects from or Assessment**

A subject is free to withdraw his/her consent and discontinue participation in the study at any time for any reason.

A subject may also be discontinued from the study, at the discretion of an investigator and/or sponsor, for any of the following reasons:

- Entered the study in violation of the protocol;
- Safety reasons, including AEs;
- Use of unacceptable concomitant medication(s);
- Non-compliance or major protocol violation;
- It is not considered in the best interest of the subject to continue;
- Pregnancy;
- Positive UDS or alcohol test;
- Administrative reasons (e.g., termination of enrollment or study);
- Difficulties with blood collection.

An investigator must maintain a record of all subjects who discontinue from the study prior to completion; the reason(s) for study discontinuation will be documented. In the event that a subject chooses to withdraw from the study, an investigator should make a reasonable attempt to obtain and record the reason(s) for withdrawal, if possible, although the subject is not obligated to provide such a reason.

In the event that a subject is discontinued while at the clinical site, the early termination procedures shown in the Schedule of Assessments ([Table 1](#)) should be performed prior to discharge from the study site. For any case of early discontinuation (whether or not the subject is at the clinical site), an investigator should ask the subject to return to complete the end of study procedures, provided that the subject has not withdrawn consent for those procedures. If a subject refuses to complete early termination procedures and/or the End of Study Phase, this information will be recorded.

## 8.4. Study Restrictions

In addition to the inclusion/exclusion criteria described in Section 8, the subject must agree to abide by the following study restrictions:

- Subjects will be asked to abstain from alcohol for 48 hours prior to Screening and prior to admission to the Confined Assessment Phase until discharge from the Confined Assessment Phase. Abstinence will be confirmed via alcohol test.
- Subjects will be asked to abstain from recreational drug use from Screening until discharge from the Confined Assessment Phase. Abstinence will be confirmed via UDS.
- Subjects will be asked to abstain from strenuous physical activity for 48 hours prior to admission to the Confined Assessment Phase until discharge from the Confined Assessment Phase.
- Subjects will be asked to abstain from consuming poppy seeds and grapefruit products from 7 days prior to first product use in Part A until discharge from the Confined Assessment Phase.
- Subjects will be requested to avoid food or beverages containing xanthines (i.e. tea, coffee, cola drinks, energy drinks or chocolate) during the Confined Assessment Phase.
- Subjects will not be permitted to consume beverages of any kind during each controlled and uncontrolled product use session in Part B. Water will be permitted in 125 mL increments at the discretion of the investigator.
- Subjects will be required to abstain from tobacco or nicotine use for approximately 20 hours prior to the ad libitum product use on Days 1 to 3 (Part A).
- No tobacco- or nicotine-containing products will be permitted from Day -1 (check-in) until the End of Study except during the designated product use sessions.
- Subjects will be asked to refrain from blood donation from Screening until 30 days after discharge from the Confined Assessment Phase.

## **9. MATERIALS**

### **9.1. Study Products**

The following products will be used during the Confined Assessment Phase:

- Product A: VLN menthol cigarette (0.4 mg nicotine/gram of tobacco)
- Product B: Subjects' Own-brand menthol filtered standard king size cigarettes (reference product)
- Product C: Nicorette® White Ice Mint™ nicotine polacrilex gum (4 mg; reference product)

### **9.2. Identity of Investigational Product(s)**

The VLN menthol cigarettes will be provided by the Sponsor. Subjects will supply their own-brand menthol cigarettes with 2 unopened packs (20 cigarettes/pack x 2). The nicotine polacrilex gum will be acquired by the study site pharmacy. The study site will coordinate shipping of the study products from the Sponsor.

#### **9.2.1. Handling, Storage, and Accountability**

Study products (not including own-brand menthol cigarettes) will be transported, received, stored, and handled strictly in accordance with the container or product label, the instructions provided to the research site, the site's standard operating procedures, and applicable regulations. Appropriate storage temperature and transportation conditions will be maintained for the study product from the point of manufacture up to delivery of the product.

#### **9.2.2. Dispensing and Administration**

Individual study product dispensing records will be maintained by the site pharmacy for each subject. Study products for dispensing to subjects will be prepared each day. Fresh packs of cigarettes will also be used each study day from which the cigarettes will be provided (Product A and Product B). For product use on Days 1 through 3, the pharmacy will maintain records of the number of VLN and own-brand menthol cigarettes or nicotine polacrilex gum pieces dispensed for each subject.

Opened and unopened packages of VLN menthol cigarettes and nicotine polacrilex gum products will be returned to the Sponsor or destroyed at the direction of the Sponsor. All returns or destruction of study products will be documented. Unused subjects' own-brand menthol cigarettes may be returned to subjects at End of Study or Early Termination.

### **9.3. Method of Assigning Subjects to Study Groups**

Each potential subject will be assigned a unique number in the screening process (subject number). This number will be used to identify the subject throughout the study. Algorithm

Pharma Inc. will prepare the randomization schedule with a computer program according to the study design, the number of subjects and the sequence of product use.

On Day 1, subjects will be randomized to one of three sequence groups (ABC, BCA, CAB, where A = VLN menthol cigarette, B=subject's own-brand menthol cigarette; and C= nicotine polacrilex gum), with approximately the same number of subjects per sequence group. On Day 4, subjects will be re-randomized into one of three sequence groups (ABC, BCA, CAB), with approximately the same number of subjects per sequence group.

#### **9.4. Selection and Timing of Product Use**

First product administration will occur in the morning on each study day (Days 1 through 6), after approximately 20 hours of nicotine abstinence. Subjects will be segregated in different areas of the facility based on the product that they are receiving so that odors and sight of products will not interfere with the subjects' assessments.

##### **9.4.1. Part A (Days 1 to 3)**

On each of the 3 days, subjects will use only one of the 3 study products according to their randomization sequence. No other use of tobacco- or nicotine-containing products will be allowed from check-in on Day -1 through to End of Study discharge except as required by this protocol, and subjects will be instructed to not bring to the study site any form of tobacco- or nicotine-containing products except 2 unopened packs of their own cigarettes.

##### **9.4.1.1. Product A: VLN Menthol Cigarettes**

Subjects will be assigned an unopened pack of VLN menthol cigarettes. They will be presented to the subjects unbranded. Subjects will have open access to the cigarettes and may smoke the cigarettes as desired during the 4-hour session. Study staff will record the number of cigarettes dispensed, time dispensed, and number of butts returned.

##### **9.4.1.2. Product B: Own-brand Menthol Cigarettes**

Subjects will be assigned an unopened pack of their own-brand menthol cigarettes. Subjects will have open access to the cigarettes and may smoke the cigarettes as desired during the 4-hour session. Clinic staff will record the number of cigarettes dispensed, time dispensed, and number of butts returned.

##### **9.4.1.3. Nicotine Polacrilex Gum**

Subjects will be assigned unopened pack of 4 mg nicotine polacrilex gum. Subjects will have open access to nicotine polacrilex gum and may chew the gum as desired during the 4-hour session. Clinic staff will record the number of gum pieces dispensed to subjects and will record the amount of time subjects used each piece of gum by documenting the time dispensed and time discarded.

#### **9.4.2. Part B (Days 4 to 6)**

On each of the 3 days, subjects will use only one of the 3 study products according to their randomization sequences. No other use of tobacco- or nicotine-containing products will be allowed.

##### **9.4.2.1. Product A: VLN Menthol Cigarettes**

During the first product use session (i.e., Controlled Use), subjects will take 10 puffs of one of the VLN menthol cigarettes at approximately 30-second intervals ( $\pm 5$  seconds) based off the start of inhalation, inhalation duration will not exceed  $3 \pm 2$  seconds per puff. The clinic staff will indicate to the subjects when to start each inhalation at each 30-second interval, will document the total number of inhalations (if less than 10) and any reasons for missed inhalations. During the second product use session (i.e., uncontrolled use), subjects will smoke one VLN menthol cigarette ad libitum for 10 minutes and the clinic staff will document the total number of inhalations and the duration of inhalations. The used butts may be discarded after adequate accountability has been completed.

##### **9.4.2.2. Product B: Own-brand Menthol Cigarettes**

During the first product use session (i.e., Controlled Use), subjects will take 10 puffs of one of their own-brand menthol cigarettes at approximately 30-second intervals ( $\pm 5$  seconds) based off the start of inhalation, inhalation duration will not exceed  $3 \pm 2$  seconds per puff. The clinic staff will indicate to the subjects when to start each inhalation at each 30-second interval, will document the total number of inhalations (if less than 10) and any reasons for missed inhalations. During the second product use session (i.e., Uncontrolled Use), subjects will smoke one own-brand menthol cigarette ad libitum for 10 minutes and the clinic staff will document the total number of inhalations and the duration of inhalations. The used butts may be discarded after adequate accountability has been completed.

##### **9.4.2.3. Product C: Nicotine Polacrilex Gum**

During the first product use session (i.e., Controlled Use), subjects will chew the 4 mg nicotine polacrilex gum product according to the package instructions: The gum is to be chewed slowly until a tingling sensation is felt in the mouth, "parked" between the cheek and gum until the tingling sensation subsides, and then resume chewing. Subjects will follow this chewing regimen for the 10-minute product use session. During the second product use session (i.e., Uncontrolled Use), subjects will chew the nicotine polacrilex ad libitum for 10 minutes. The chewed gum may be discarded upon completion of each product use session, after adequate accountability has been completed.

#### **9.5. Meal Schedule**

On Day -1, meals and snacks will be served at appropriate times as determined by the clinic based on the time of Check-in. Standard meals will be served on Days 1 to 3.

Subjects will be served a light breakfast each morning and must complete breakfasts on the mornings of Days 4 to 6 at least 1 hour prior to the first study product use session. The breakfast

menu should be consistent (same menu items) on Days 4 to 6. Lunch, dinner, and snacks will be served at appropriate times as determined by the study site, with lunch scheduled to be served at some point after completion of the final blood draw of the first product use session or end of the ad libitum session on each day.

Subjects may not consume beverages of any kind during each Controlled and Uncontrolled Product Use Session on Days 4 to 6. Water will be provided in 125 mL increments at the discretion of the investigator.

Subjects will also be permitted to consume hard candy during the confinement period at the discretion of the investigator. Hard candy will not be permitted during any product use sessions in Part A or Part B.

Fasting requirements and dietary restrictions are described in Section 8.4.

## **9.6. Blinding**

The study design is not blinded since subjects will know if the product is a VLN menthol cigarette, their own menthol cigarette or a nicotine polacrilex gum. The study product codes will be open to all study staff and to the Sponsor. The randomization code will not be available to the personnel of the bioanalytical facility until the bioanalytical tables have been finalized and audited by the Quality Assurance (QA) department.

## **9.7. Prior and Concomitant Therapy**

All medications, including prescription, over-the-counter, or herbal therapies used by the subjects will be documented for the 30 days prior to Screening and throughout the study. An investigator will determine if the prior/concomitant medication(s) affect the subject's eligibility to participate or continue to participate in the study.

On a case-by-case basis, an investigator is permitted to allow the use of some concomitant medications, for example, to treat an AE, as long as an investigator determines that the medication will not affect the subject's safety or study integrity (e.g., topical medications). Wherever possible, an investigator should obtain approval from the Sponsor's medical monitor prior to administering the medication. Stable doses (i.e., no dosage adjustments within 30 days prior to Day -1) of prescription or over-the-counter medications required to treat an investigator-approved disease or condition will be permitted at the discretion of an investigator.

## **9.8. Product Compliance**

Study products will be administered under the supervision of study personnel, and compliance with protocol-specified procedures during the Controlled Use Sessions will be monitored by study personnel.

## **10. STUDY PROCEDURES AND ASSESSMENTS**

All study assessments will be performed at the visits and timepoints outlined in the Schedule of Assessments ([Table 1](#)); the following sections outline the details and procedures associated with the assessments. Other logistical considerations (e.g., sequence of events, assessment windows) will be outlined in study-specific procedures.

### **10.1. Demographics and Other Baseline Characteristics**

#### **10.1.1. Informed Consent**

The nature of the study and its risks and benefits will be explained to the subject by an investigator or designated study personnel. The subject must provide written informed consent on an ethics-approved informed consent form (ICF), prior to performing any study-related procedures. A copy of the ICF will be provided to the subject.

#### **10.1.2. Demographics**

The following demographics will be recorded: age (birthdate), sex, race, and ethnicity.

#### **10.1.3. Medical History**

The complete medical history will include histories of acute, chronic, or infectious disease; surgical or oncologic histories; and any reported conditions affecting major body systems. All findings on medical history will be evaluated by an investigator for clinical significance. Medical histories will be obtained according to the site's standard operating procedures.

#### **10.1.4. Medication and Smoking History**

All medications (prescription and non-prescription, herbal medications/natural health products, or investigational drugs) taken by the subjects during the 30 days prior to Screening will be recorded in the source documentation as medication history.

A history of tobacco use/history and smoking history will be recorded for each subject.

DSM-IV-TR modules will be included as a part of the recreational drug/alcohol use history and used to screen for alcohol and substance dependence.

### **10.2. Pharmacodynamic Measures**

On Day -1, subjects will complete a training session during which they will complete the subjective questionnaires in order to familiarize themselves with the questions, appropriate use of the VAS, and use of the computerized tablet system.

#### **10.2.1. Tobacco/Nicotine Withdrawal Scale**

The Tobacco/Nicotine Withdrawal items are administered as 100 point VAS, and are intended to measure withdrawal symptoms and craving (adapted from [Hughes & Hatsukami, 1986](#);

Appendix 17.2.1). The VAS is anchored with “Not at All” on the left and “Extremely” on the right. The questionnaire items are as follows:

1. Urges to Smoke
2. Anxious
3. Difficulty Concentrating
4. Impatient
5. Craving a Cigarette

### **10.2.2. Direct Effects of Product Questionnaire**

The Direct Effects of Product items are administered as 100 point VAS, and are intended to measure the effects of the product being sampled at the moment (Appendix 17.2.2). The VAS is anchored with “Not at All” on the left and “Extremely” on the right. The questionnaire items are as follows:

1. Is the product “Pleasant” right now?
2. Is the product “Satisfying” right now?
3. Is the product making you feel “Calm” right now?
4. Is the product helping you “Concentrate” right now?
5. Is the product making you feel more “Awake” right now?
6. Is the product making you feel “Sick” right now?
7. Is the product reducing your “Hunger” for food right now?
8. Would you like “More” of the product right now?

Items are selected based on measures of product effects used in previous trials with tobacco products and conventional cigarettes ([Hanson, Connor & Hatsukami 2009](#)).

### **10.2.3. Use the Product Again Questionnaire**

The Use the Product Again questionnaire is a bipolar 100 point VAS used to assess how much a subject would be willing to use the sampled product again (Appendix 17.2.3). The VAS is anchored by “Definitely Would Not” on the left and “Definitely Would” on the right; the neutral point is also labeled with an anchor (“Don’t Care”). The VAS is adapted from the Take Drug Again VAS administered in human abuse potential studies of pharmaceutical products ([Griffiths, Bigelow & Ator, 2003](#)).

### **10.2.4. Product Use**

Number of own-brand menthol cigarettes and VLN menthol cigarettes smoked and number of nicotine polacrilex gum pieces chewed and duration of gum in mouth will be recorded during the ad libitum product use sessions in Part A. During the Uncontrolled Use Sessions in Part B, number of inhalations and duration of each inhalation will be recorded for own-brand menthol cigarettes and VLN menthol cigarettes; duration of gum in mouth will be recorded for nicotine polacrilex gum.

### **10.3. Safety Assessments**

#### **10.3.1. Adverse Events and Serious Adverse Events**

An investigator and research site staff are responsible for the detection, documentation, classification, reporting, and follow-up of events meeting the definition of an AE or serious adverse event (SAE). All AEs will be recorded following informed consent (at Screening) until the End of Study (or the required follow up period for a specific AE or SAE).

##### **10.3.1.1. Adverse Events**

An AE is any untoward medical occurrence in a clinical investigation subject administered a product and may not necessarily have a causal relationship with the administered treatment. An AE can, therefore, be any unfavorable and unintended sign (including a clinically significant laboratory abnormality), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. During the study, an AE can also occur outside the time that the investigational product(s) was given (e.g., during a washout period). Pre-existing conditions, diseases, or disorders are not considered AEs unless there is a change in intensity, frequency, or quality.

##### **10.3.1.2. Serious Adverse Events and Serious Unexpected Adverse Events**

An SAE is any untoward medical occurrence that at any product use:

- Results in death;
- Is life-threatening (at the time of the event);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity; or
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse product experience when, based upon appropriate medical judgment, they may jeopardize the study subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A serious and unexpected AE is an SAE that is not identified in nature, intensity, or frequency in the risk information generally known for nicotine gum and cigarettes.

Any SAE—expected or unexpected, irrespective of relationship to study treatments, including death due to any cause—experienced by a study subject will be reported to 22<sup>nd</sup> Century Inc. or designee by an investigator within 24 hours of learning of the event. Information regarding the SAE will be transmitted to 22nd Century Inc. or designee, as follows:

James Swauger

22nd Century Group, Inc.

8560 Main Street

Williamsville, NY 14221

Email:Jswauger@xxiicentury.com

22nd Century Inc. or designee assumes responsibility for appropriate reporting of AEs to the regulatory authorities. 22nd Century Inc. or designee will also report to an investigator all SAEs that are unlisted and associated with product use. The investigator (or 22nd Century Inc., where required) must report these events to the appropriate Institutional Review Board (IRB) that approved the protocol (unless otherwise required and documented by the IRB). Follow-up evaluations for SAEs will also be reported to 22nd Century Inc. or designee.

#### **10.3.1.3. Clinical Laboratory Abnormalities and Other Abnormal Assessments**

Abnormal clinical laboratory findings (e.g., clinical chemistry, hematology, or urinalysis) or other abnormal assessments (e.g., from vital signs or ECG), judged as clinically significant by an investigator will be recorded as AEs or SAEs, if they meet the definitions provided previously. Abnormal laboratory or other findings present at baseline that significantly worsen following the start of the study will be reported as AEs or SAEs. However, abnormal findings present at the start of the study that do not worsen will not be reported as AEs or SAEs, unless an investigator or designee judges them as more severe than expected for the subject's condition.

#### **10.3.1.4. Classification of Adverse Event Intensity and Causality**

For each recorded AE or SAE, an investigator must make an assessment of intensity based on the following criteria:

- Mild:** An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate:** An event that is alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- Severe:** An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living or significantly affects clinical status. The event poses a significant risk of harm to the subject and hospitalization may be required.

An investigator must make an assessment of causality based on the following criteria to determine the relationship between the AE/SAE and study product:

- Unrelated:** Clearly and incontrovertibly due only to extraneous causes (e.g., disease, environment, etc.) and does not meet the criteria for study product relationship listed under probable, possible, or unlikely.
- Unlikely:**
- Does not follow a reasonable temporal sequence from administration of the study product;
  - May readily have been produced by the subject's clinical state, environmental, or toxic factors, or other modes of therapy administered to the subject;
  - Does not follow a known pattern of response to the study product;
  - Does not reappear or worsen when the product is used again.
- Possible:** The connection to the product appears unlikely but cannot be ruled out with certainty:
- Follows a reasonable temporal sequence from administration of the study product;
  - May have been produced by the subject's clinical state, environmental, or toxic factors, or other modes of therapy administered to the subject;
  - Follows a pattern of response to the suspected study product.
- Probable:** The connection to the product can be made with a high degree of certainty:
- Follows a reasonable temporal sequence from administration of the study product;
  - Cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental, or toxic factors, or other modes of therapy administered to the subject;
  - Disappears or decreases upon cessation or reduction in use of the product (note that there are important exceptions when an AE or SAE does not disappear upon discontinuation of use the study product, yet relatedness to study product clearly exists, e.g., bone marrow depression or tardive dyskinesias);
  - Follows a known pattern of response to the study product;
  - Reappears upon re-challenge.

#### **10.3.1.5. Follow-up of Adverse Events and Serious Adverse Events**

All unresolved AEs will be followed for a minimum of 14 days after the subject's final Study Day, unless an investigator's judgment dictates otherwise, the event has resolved or stabilized before the 14-day period, or the subject is lost to follow-up.

All AEs that result in discontinuation and SAEs will be followed until the event resolves, stabilizes (according to the judgment of an investigator), returns to a baseline value (if a baseline value is available), or can be attributed to agents other than the study product or to factors unrelated to study conduct.

When it becomes unlikely that any additional information can be obtained (e.g., subject or health care practitioner refuses to provide additional information, the subject is lost to follow-up), an investigator or designee will ensure that the follow-up includes any pertinent supplemental investigations (e.g., laboratory tests or investigations, histopathological examinations or consultation with other health care professionals) to elucidate the nature and/or causality of the AE or SAE.

Investigators are not obligated to actively seek AEs or SAEs in former study subjects that occur following the AE or SAE Follow-up period. However, if an investigator learns of any AE or SAE at any time after a subject has been discharged from the study and the event is considered as reasonably related to the study product, an investigator will notify 22nd Century Inc.

For each recorded AE or SAE, an investigator must make an assessment of outcome at the time of last observation, as follows:

Fatal:	The subject died.
Resolved	The AE or SAE has ended.
Resolved with Sequelae	The AE or SAE has ended but changes are noted from baseline.
Unresolved – Ongoing:	The AE or SAE has not ended and is ongoing at the end of the reporting period (i.e., 14 days after the final Study Day) and an investigator deems that further follow up is not medically required
Unresolved – Lost to Follow:	Lost to follow-up after repeated unsuccessful attempts to contact the subject.

### **10.3.2. Pregnancy**

If a subject becomes pregnant during the study or for 14 days after the final Study Day, an investigator will report the pregnancy to 22nd Century Inc. or designee within 24 hours of learning of the event. Any subject who becomes pregnant during the study will be immediately withdrawn from the study.

Follow-up information will be obtained where possible (with the consent of the subject or their partner) regarding the course and outcome of the pregnancy, including any post-natal sequelae in the infant.

### **10.3.3. Clinical Laboratory Assessments**

Blood and urine samples will be collected, processed, and shipped according to the research site's standard operating procedures and instructions from the safety laboratory. Samples will be

segregated in such a way as to minimize the impact of the potential loss of samples during shipping. All clinical laboratory data will be reviewed by an investigator for clinical significance.

Blood volumes required per draw, laboratory test, and study phase are available in Appendix 17.1. Additional laboratory samples may be taken at the discretion of an investigator if the results of any tests fall outside reference ranges or clinical symptoms necessitate testing to ensure safety. Specific hematology, biochemistry, and urinalysis assessments are listed in Table 2.

**Table 2: Clinical Laboratory Assessments**

Hematology	Biochemistry	Urinalysis
Hematocrit	Sodium	Color
Hemoglobin	Potassium	pH
Red blood cell count	Glucose (random)	Specific gravity
Total and differential (absolute) white blood cell count	Creatinine	Ketones
Platelets	Total protein	Protein
	Blood urea nitrogen	Glucose
	Albumin	Bilirubin
	Total bilirubin	Nitrite
	Alanine transferase	Urobilinogen
	Aspartate transferase	Occult blood
	Lactic dehydrogenase	Microscopic examination of sediment, <i>only if urinalysis dipstick results are abnormal</i>
	Gamma-glutamyl transferase	
	Alkaline phosphatase	
	Creatine phosphokinase	
	FSH <sup>a</sup>	

<sup>a</sup> Postmenopausal women at Screening only.

In addition to the clinical laboratory tests, pregnancy testing for the presence of  $\beta$ -human chorionic gonadotropin in serum or urine will be performed for all women. Results of serum or urine pregnancy tests will be reported and determined to be negative prior to study continuation.

A blood sample for a serology panel testing for hepatitis B surface antigen (HbsAg), anti-hepatitis C antibodies (HCVAb), and human immunodeficiency virus (HIV) will be performed for all subjects. Only subjects with negative viral serology tests will be eligible for the study. Positive results will be managed according to local regulatory requirements and the site's standard operating procedures.

#### 10.3.4. Urine Drug Screen and Alcohol Testing

Urine drug screens will test for the following drugs of abuse: tetrahydrocannabinol (THC), opioids, amphetamines, cocaine, and benzodiazepines. The opioid panel will include morphine, codeine, heroin, hydrocodone, hydromorphone, and oxycodone.

Urine alcohol and cotinine testing will be performed according to the site's standard operating procedures. If there is any doubt or concern regarding alcohol use, research site staff may request a test for alcohol measures at any time during the study.

### **10.3.5. Vital Signs**

Vital signs will consist of blood pressure (systolic and diastolic blood pressure, mmHg), pulse rate (beats per minute), and respiratory rate (breaths/min). Oral temperature (°C) will also be taken at some time points, as specified in [Table 1](#). Vitals signs will be collected and managed according to the site's standard operating procedures.

### **10.3.6. 12-lead Electrocardiograms**

ECGs will be performed after the subject has been resting in a recumbent/supine position for at least 3 minutes. The ECG variables will include ventricular heart rate and the PR, QRS, QT, QTcB and QTcF intervals. 12-lead ECGs will be performed and interpreted according to the site's standard operating procedures.

### **10.3.7. Physical Examination**

A complete physical examination, assessing the subject's overall health and physical condition, will be performed at Screening according to the site's standard operating procedures. A brief, symptom-driven physical will be conducted on Day -1 and End of Study Day 6.

Height, weight and BMI will be assessed as described in [Table 1](#).

## **10.4. Pharmacokinetic Assessments**

Venous blood samples will be collected to determine the plasma concentrations of nicotine at the timepoints outlined in the Schedule of Assessments ([Table 1](#)). Samples will be collected, processed, and shipped according to the site's standard operating procedures and instructions from the sponsor or bioanalytical laboratory. The time of blood sample collection will be calculated according to the start of product use of each session (Controlled and Uncontrolled).

Blood volumes required for PK sampling are available in [Appendix 17.1](#).

The plasma samples will be analyzed by Algorithme Pharma Inc. using validated methods.

Plasma samples will be shipped frozen on dry ice from the research site to the following address:

Algorithme Pharma Inc., An Altasciences Company  
575 Armand-Frappier Blvd.  
Laval, Quebec, Canada  
H7V 4B3

Samples will not be shipped without prior arrangement with the bioanalytical laboratory and/or notification to the Sponsor.

## 10.5. Appropriateness of Measures

The selection of abuse liability measures in this study, including the primary endpoints of “Urges to Smoke” and “Pleasant”  $E_{max}$  during the Controlled Use Session are consistent with previous studies of this type. The Use the Product Again VAS is being used as this is thought to indicate the subject’s willingness to use the product again and to reflect the user’s overall impression of the product. Other subscales of the Tobacco/Nicotine Withdrawal Questionnaire and the Direct Effects of Product Questionnaire will measure positive, negative and other subjective effects including craving to assess the subjective effects of the products for comparative purposes. Measures of product use behavior have been included during the ad libitum sessions and the Uncontrolled Use Sessions in order to evaluate the amount and duration of product used under both conditions.

Standard safety assessments have been included to evaluate the safety profile of VLN menthol cigarettes. PK blood sampling has been included to evaluate the PK profile of nicotine following controlled and uncontrolled use of VLN menthol cigarettes, in comparison with own-brand menthol cigarettes and nicotine polacrilex gum.

## 10.6. Pharmacodynamic Variables

### 10.6.1. Primary Pharmacodynamic Endpoints

The primary endpoints of this study are as follows:

- Controlled Product Use (Part B)
  - $E_{max\_urge(controlled)}$ : The maximum reduction in VAS score for the question “Urges to smoke” (Tobacco/Nicotine Withdrawal Questionnaire) between pre-use and post-use (i.e.,  $VAS_{pre-use1} - VAS_{post-use1}$ ) during the first product use in Part B.
  - $E_{max\_plst(controlled)}$ : The largest VAS score recorded for the response to the question “Is the product “Pleasant” right now?” (Direct Effects of Product Questionnaire) during the first product use in Part B.

### 10.6.2. Secondary Endpoints

The secondary endpoints of this study are as follows:

- Ad libitum Product Use (Part A)
  - Use the Product Again VAS score
  - Product use behavior (number of units consumed, time spent per unit, number of inhalations per cigarette, duration of gum in mouth)
- Controlled and Uncontrolled Product Use (Part B)
  - Tobacco/Nicotine Withdrawal Questionnaire

- $E_{\max\_item(\text{controlled})}$ : The maximum reduction in VAS score for each item<sup>a</sup> between pre-use and post-use (i.e.,  $VAS_{\text{pre-use1}} - VAS_{\text{post-use1}}$ ) during the first product use in Part B.
- $E_{\max\_item(\text{uncontrolled})}$ : The maximum reduction in VAS score for each item between pre-use and post-use (i.e.,  $VAS_{\text{pre-use2}} - VAS_{\text{post-use2}}$ ) during the second product use in Part B.
- Direct Effects of Product Questionnaire
  - $E_{\max\_item(\text{controlled})}$ : The largest VAS score recorded for the response to each item<sup>b</sup> during the first product use in Part B.
  - $E_{\max\_item(\text{uncontrolled})}$ : The largest VAS score recorded for the response to each item during the second product use in Part B.
- Product use behavior (Uncontrolled Use Sessions; number of inhalations, duration of inhalations [per puff], duration of gum in mouth).

## 10.7. Pharmacokinetic Variables

The PK parameters of nicotine will include, but are not limited to, the following:

- $C_{\max(\text{controlled})}$ : Maximum measured plasma nicotine concentration during the Controlled Use Session.
- $C_{\max(\text{uncontrolled})}$ : Maximum measured plasma nicotine concentration during the Uncontrolled Use Session.
- $AUC_{(\text{controlled})}$ : Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time zero (defined as the start of controlled use) to 180 minutes (or the last quantifiable concentration during that interval).
- $AUC_{(\text{uncontrolled})}$ : Area under the nicotine concentration-time curve calculated using linear trapezoidal summation from time 360 (defined as the start of uncontrolled use) to 540 minutes (or the last quantifiable concentration during that interval).
- $T_{\max(\text{controlled})}$ : Time of the maximum measured plasma nicotine concentration during the Controlled Use Session.
- $T_{\max(\text{uncontrolled})}$ : Time of the maximum measured plasma nicotine concentration during the Uncontrolled Use Session.
- $K_{el(\text{controlled})}$ : Apparent first-order terminal nicotine elimination rate constant calculated from a semi-log plot of the plasma concentration-time curve of the Controlled Use Session.

<sup>a</sup> Tobacco/Nicotine Withdrawal Questionnaire items (secondary):  $E_{\max\_urge}$  (uncontrolled only),  $E_{\max\_anx}$ ,  $E_{\max\_diffct}$ ,  $E_{\max\_impat}$ , and  $E_{\max\_crav}$ .

<sup>b</sup> Direct Effects of Product Questionnaire items (secondary):  $E_{\max\_plst}$  (uncontrolled only),  $E_{\max\_stf}$ ,  $E_{\max\_calm}$ ,  $E_{\max\_conc}$ ,  $E_{\max\_awake}$ ,  $E_{\max\_sick}$ ,  $E_{\max\_hunger}$ , and  $E_{\max\_more}$ .

- $K_{el(\text{uncontrolled})}$ : Apparent first-order terminal nicotine elimination rate constant calculated from a semi-log plot of the Uncontrolled Use Session.
- $T_{1/2(\text{controlled})}$ : Apparent first-order terminal nicotine elimination half-life calculated as  $0.693/K_{el}$  of the plasma concentration-time curve from time zero (defined as the start of controlled use) to 180 minutes.
- $T_{1/2(\text{uncontrolled})}$ : Apparent first-order terminal nicotine elimination half-life calculated as  $0.693/K_{el}$  of the plasma concentration-time curve from time zero (defined as the start of uncontrolled use) to 540 minutes.

## 10.8. Safety Variables

The safety endpoints include the following:

- AEs (incidence, frequency, severity and relationship to product), SAEs, and AEs leading to discontinuation;
- Vital signs (heart rate, blood pressure, respiratory rate, oral temperature);
- 12-lead ECG;
- Clinical laboratory tests (clinical chemistry, hematology, urinalysis);
- Physical examination findings;
- Concomitant medications.

## **11. DATA QUALITY ASSURANCE**

This study will be conducted under Good Clinical Practice (GCP) and all applicable regulatory requirements. To ensure compliance, the Sponsor or designee may conduct a quality assurance audit, as outlined in Section 11.2.

Actions to ensure the accuracy and reliability of data include the selection of qualified Investigators and appropriate study centers; the review of protocol procedures with an investigator and study personnel prior to study start; the design of suitable source documents with appropriate instructions for use (where applicable); the internal audit of source data according to GCP and internal procedures to ensure their accuracy, completeness, and verifiability; as well as the periodic site monitoring by the Sponsor. Written instructions will be provided for collection, preparation, and shipment of blood, plasma, and urine samples. The Sponsor or designee will review source documents for accuracy and completeness during on-site monitoring visits and after their return to the Sponsor; any discrepancies will be resolved with an investigator, as appropriate.

Significant and/or repeated non-compliance will be investigated, and remedial action instituted when appropriate. Failure to comply with remedial actions may result in investigator site termination and regulatory authority notification.

### **11.1. Data Collection**

Source documents include but are not limited to original documents, data and records such as hospital/medical records (including electronic health records), clinic charts, lab results, subject diaries, data recorded in automated instruments, microfilm or magnetic media, and pharmacy records, etc. This study will use electronic data capture (EDC) or paper case report forms (CRFs). At a minimum, all data required by the protocol should have supporting source documentation for entries in the EDC system, unless that data can be recorded directly in the EDC system or other device.

All CRFs will be completed by the site staff prior to review by the Sponsor's monitor or designated representative. The Sponsor's monitor or designated representative will review all source records on-site and compare them to the data collected on the CRF. All entries, corrections, and alterations will be made by an investigator or other authorized study personnel. All data entries will be verified for accuracy and correctness by independent monitors. The EDC system maintains a full audit trail.

### **11.2. Study Auditing and Monitoring**

Monitoring of the study site (including, but not limited to, reviewing CRFs for accuracy and completeness) will be performed by the Sponsor's monitor. The extent, nature, and frequency of on-site visits will be based on such considerations as the study objectives and/or endpoints, the purpose of the study, study design complexity, and enrollment rate. By signing the protocol, an investigator agrees that, within local regulatory restrictions and institutional and ethical considerations, authorized representatives of the Sponsor, a regulatory authority, and/or an IRB

may visit the site to perform audits or inspections, including the product storage area, study product stocks, product accountability records, subject charts and source documents, and other records related to study conduct. The purpose of the Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether the study-related activities were conducted, and data recorded, analyzed, and accurately reported according to the protocol, the site's standard operating procedures, GCP guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. An investigator should contact the Sponsor immediately if contacted by a regulatory agency regarding an inspection.

## 12. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

### 12.1. Statistical and Analytical Plans

Complete details of the statistical analyses to be performed will be documented in a statistical analysis plan (SAP), which will be finalized prior to completion of the study. This document will include more detail of analysis populations, summary strategies, and any amendments to the proposed analyses listed here, if necessary. Any changes to the SAP will be outlined in the final study report.

### 12.2. Determination of Sample Size

Sample size estimation was performed on both pharmacodynamic endpoints.

Using a 2-sided Type I error rate at  $\alpha=0.05$  and assuming a mean difference in  $E_{\max\_urge(\text{controlled})}$  between own-brand menthol cigarette and nicotine polacrilex gum of 15.85 points and standard deviation (SD) of 28.44, 54 completed subjects will be required to detect a significant difference between own-brand menthol cigarette and nicotine polacrilex gum with greater than 80% power. Using a 2-sided Type I error rate at  $\alpha=0.05$  and assuming a mean difference in  $E_{\max\_plst(\text{controlled})}$  between own-brand menthol cigarette and nicotine polacrilex gum of 35.21 points and SD of 31.82, estimate based on data obtained from the study site, 16 completed subjects will be adequate to detect a significant difference between own-brand menthol cigarette and nicotine polacrilex gum with greater than 80% power.

An appropriate number of subjects will be randomized on Day 1 (Part A) to ensure that a minimum of 54 subjects complete the study. Replacement subjects may be enrolled to ensure that the minimum number of subjects complete the study.

### 12.3. Analysis Populations

The study analysis populations will consist of

- **Randomized Population (Part A):** All subjects who are randomized into Part A.
- **Randomized Population (Part B):** All subjects who are randomized into Part B.
- **Safety Population:** All randomized subjects who use at least one of the study products. This will include Day -1 (Check-In and product trial) and study days after the randomization. The Safety Population will be used for the summary of subject demographics, baseline characteristics, safety information and AEs.
- **Pharmacodynamic Population:** All subjects who use any study product and have pre-use (Tobacco/Nicotine Withdrawal questionnaire) and at least one post-use (for Tobacco/Nicotine Withdrawal and Direct Effects of Product) VAS score for the Controlled Use Session of Part B. This population will be used for statistical analyses of the pharmacodynamic measures.

- **Pharmacokinetic Population:** All subjects who use any study product and have pre- and at least one post-use plasma concentration value for the Controlled Use Session of Part B. The PK Population will be used for statistical analyses of the PK parameters.
- **Completer Population:** All randomized subjects who complete all product use sessions and have pharmacodynamic and PK data in the Controlled Use Session of Part B. This dataset will be used for analyses of the two primary pharmacodynamic endpoints as a sensitivity analyses to the primary analyses.

## 12.4. Planned Analyses

### 12.4.1. Subject Disposition

Disposition of each analysis population will be summarized using descriptive statistics. Data from subjects who discontinue from the study will also be summarized by last product used and reason for discontinuation.

### 12.4.2. Demographics and Other Baseline Characteristics

Descriptive statistics will be reported for continuous demographic variables (age, weight, height, and BMI) and frequency counts will be tabulated for categorical demographics variables (gender, ethnicity, and race). In addition, number of cigarettes smoked per day, as well as the duration of tobacco use (derived as the interval between tobacco use start date and the ICF signature date), will be summarized by descriptive statistics. Responses for smoking history and number of tobacco products used per day will be listed in the substance usage listing.

### 12.4.3. Analysis of Pharmacodynamic Measures

#### 12.4.3.1. Part A

Responses to Use the Product Again VAS will be summarized by study product using descriptive statistics (n, mean, SD, median, Q1, Q3, min, max). A summary table of frequencies may also be provided.

Descriptive statistics (n, mean, SD, median, Q1, Q3, min, max) will be provided for amount of product used by product (number of cigarettes for own-brand and VLN menthol cigarettes, and number of nicotine gum pieces), duration of time used (time spent per cigarette and duration of gum in mouth), and number of inhalations (puffs) per cigarette for own-brand and VLN menthol cigarettes.

#### 12.4.3.2. Part B

Responses to each Tobacco/Nicotine Withdrawal Questionnaire and Direct Effects of Product Questionnaire item will be summarized by time point for each study product, and product use condition. For Tobacco/Nicotine Withdrawal VAS scores, descriptive statistics (n, mean, SD, median, Q1, Q3, min, max) for the difference from pre-use at each time point will also be reported. Derived parameters ( $E_{max}$ ) for each questionnaire item will be summarized using descriptive statistics.

A linear mixed effects model for analysis of variance/covariance (Proc Mixed) will be performed on  $E_{\max\_urges(controlled)}$  and  $E_{\max\_plst(controlled)}$ . The model will include  $E_{\max}$  as the response variable, sequence, study product, and period as fixed model effects, baseline score as a covariate (for  $E_{\max\_urges}$  only), and subject nested-within sequence as a random effect. Sequence will be tested using subject nested-within-sequence as the error term. Least square mean (LSM) and 95% confidence interval (CI) for each Study Product group will be provided. Comparisons will be made for Product A vs. B, A vs. C and B vs. C. The comparison between Product A and Product C will be used as a comparison for internal validity. The LSM difference, p-value and 95% CI of the difference will be provided.

The same model will be used for  $E_{\max\_urges(uncontrolled)}$  and  $E_{\max\_plst(uncontrolled)}$ , and all  $E_{\max}$  of other questions in the Tobacco/Nicotine Withdrawal Questionnaire and Direct Effects of Product Questionnaire for the controlled and uncontrolled product uses in Part B. Responses to Use the Product Again VAS will be summarized by study product using descriptive statistics (n, mean, SD, median, Q1, Q3, min, max). A summary table of frequencies may also be provided.

Descriptive statistics (n, mean, SD, median, Q1, Q3, min, max) will be provided on duration of time a product is used (time spent per cigarette and duration of gum in mouth), number of inhalations (puffs), and duration of inhalation (per puff) per cigarette for own-brand and VLN menthol cigarettes.

#### **12.4.3.3. Adjustment for Covariates**

Baseline (pre-use) score will be included as a covariate for all pharmacodynamics measures for which a pre-use score is collected.

#### **12.4.3.4. Handling of Dropouts or Missing Data**

Missing pharmacodynamic data, including reasons for the missing data, will be listed by subject and examined on a case-by-case basis to determine if these affect subject allocation. No imputation of missing pharmacodynamic data will be performed.

#### **12.4.3.5. Interim Analyses and Data Monitoring**

No interim analyses will be performed.

#### **12.4.3.6. Multiple Comparison/Multiplicity**

Multiple comparison adjustments will not be made.

#### **12.4.3.7. Tabulation of Individual Response Data**

Individual subject pharmacodynamic data will be listed by measure and timepoint. A by-subject listing of derived pharmacodynamic endpoints (e.g.,  $E_{\max}$ , etc.) will also be provided.

#### **12.4.4. Analysis of Pharmacokinetics**

Refer to the SAP for details on analysis of PK.

Descriptive statistics of plasma nicotine concentrations will be tabulated by time point for each study product and product use condition. Derived PK parameters (both unadjusted and baseline

adjusted) will be summarized using descriptive statistics. Summaries will be provided for plasma nicotine-adjusted concentrations.

A linear mixed model for analysis of variance (Proc Mixed) will be performed on the baseline-adjusted log transformed nicotine PK parameters in Part B. The model will include sequence, study product, and period as fixed effects and subject nested-within sequence as a random effect. Sequence will be tested using subject nested-within sequence as the error term. From each model, the geometric LSM and 95% confidence intervals will be calculated for each study product. Separate analyses will be performed on the parameters calculated for the Controlled and Uncontrolled Use sessions. Geometric mean ratios for each pair comparison, 95% confidence interval and p value will be provided.

The same linear mixed effect model as used for the analysis of  $C_{\max(\text{controlled})}$  will be used for  $C_{\max(\text{uncontrolled})}$  of second product use in Part B, and for AUC following all product uses. The Hodges-Lehmann's method will be used to estimate the median differences and the 95% confidence interval of  $T_{\max}$  and  $T_{1/2}$  between the products.

#### **12.4.5. Analysis of Safety Assessments**

A by-subject AE data listing, including verbatim term, preferred term, study product, severity, and relationship to study product, will be provided. Study product use-emergent AEs will be summarized with tables. Frequencies of subjects with AEs, SAEs and incidence of those events, regardless of relationship to study product, will be summarized by study product and sorted by system organ class. Frequencies of AEs will be summarized by severity and relationship to study product. Clinical laboratory evaluations (serum chemistry, hematology, and urinalysis), vital signs measurements (blood pressure, pulse, respiration, and temperature) and ECG measurements (VR, PR, QRS, QT, and QTcF intervals) will be summarized descriptively. All concomitant medications recorded during the study will be listed by subject. Physical examinations will be listed by subject and time point of collection.

Details can be found in the SAP.

### **13. STUDY ADMINISTRATION AND INVESTIGATOR RESPONSIBILITIES**

Additional details may be outlined in the Clinical Study Agreement (CSA) between the Sponsor and the investigational site.

#### **13.1. Regulatory and Ethical Considerations**

##### **13.1.1. Ethical Conduct of the Study**

An investigator will conduct the study in accordance with GCP and all applicable regulations, including, where applicable, the Declaration of Helsinki. The study will also be carried out in keeping with applicable national and local laws and regulations. This may include an inspection by the Sponsor's representatives and/or regulatory authority's representatives at any time.

##### **13.1.2. Regulatory Authority and Ethics Approval**

The investigational site's IRB must meet all relevant regulatory requirements. The study protocol and ICF will be reviewed by the IRB prior to enrolling subjects into the study; written approval from the committee must be received by the Sponsor before study product will be released to an investigator. An investigator is responsible for submitting all protocol or ICF changes and SAE reports to the IRB according to local procedures.

In accordance with applicable local regulatory requirements, an investigator may be obligated to provide periodic safety updates on the conduct of the study at his/her research site and notification of study closure to the IRB. Such periodic safety updates and notifications are the responsibility of an investigator and not of the Sponsor. The Sponsor will be provided with copies of all notifications sent to the IRB.

All relevant correspondence from the IRB will be forwarded by the respective study site to the Sponsor in a timely fashion.

##### **13.1.3. Subject Informed Consent**

An investigator (or authorized designee) will ensure that the subject (or the subject's legal representative) is given full and adequate oral and written information about the nature, purpose, potential and possible risks and benefits of the study. Each prospective subject will receive an IRB-approved ICF that summarizes the pertinent study information and will be given ample time to read the form and ask questions about the study. All information is to be provided in a language understandable to the subject and must not include any language that waives the subject's legal rights. Prospective subjects must also be informed of their right to withdraw consent without prejudice at any time during the study. If the subject chooses to participate, he/she must sign and date the ICF before any study-related procedures are performed. The time of signing will be recorded on the ICF

Significant changes to the protocol or product safety information may require a revision of the ICF, which must be reviewed and signed by all applicable study subjects.

The time that informed consent is obtained must be documented. An investigator must maintain the original, signed ICF in the subject's source documents. A copy of the signed ICF must be given to the study subject.

### **13.2. Privacy and Confidentiality**

An investigator is responsible for complying with applicable privacy regulations, per his or her jurisdiction. Only information identified in this protocol will be collected. The information collected will only be used for the purposes identified in this protocol.

To ensure anonymity and to limit disclosure, subjects will be assigned a unique identifier at their first assessment. This identifier will be cross-referenced in the subject's chart. The identifier will not contain any potentially identifiable information. An identifier log will be maintained, linking each subject's name to the corresponding identifier. This log will be stored at the research site in a secure location.

The knowledge gained through this study is the property of the Sponsor. The Sponsor, representatives and affiliated companies of the Sponsor, the IRB, and regulatory agencies (such as the United States FDA) may inspect medical records related to the study to check the validity and accuracy of the data gathered in this study. Subject medical records (with subject's initials and/or date of birth) may be copied. Confidentiality of subject records will be maintained except where release of information is required by law.

The results of this study will be reported in such a manner that subjects will not be identifiable in any way. Published reports or presentations will refer to grouped data or coded individual data and not to any identifiable individuals. Study reports sent to the Sponsor or drug regulatory agencies will not include subject names.

By signing the ICF, the subject consents to the collection, access, use, and disclosure of his or her information as described in the ICF document. If a subject withdraws consent, some of the subject's information may still be collected, used, and disclosed by those involved in this study, per applicable laws.

By signing this protocol, an investigator affirms that he/she will maintain in confidence information furnished to him or her by the Sponsor and will divulge such information to his or her respective IRB or Independent Ethics Committee under an appropriate understanding of confidentiality with such board. All data will be considered the sole property of the Sponsor. Please refer to the CSA for details.

### **13.3. Study and Site Closure**

Upon completion of the study, all study data will be provided to the Sponsor following review of site study records for completeness, and data clarifications and resolutions. Accounting, reconciliation, and final disposition of used and unused study products will be performed, as applicable.

In addition, the Sponsor reserves the right to temporarily suspend or prematurely discontinue this study at any time and for any reason. If such action is taken, the Sponsor will discuss this with an investigator (including the reasons for taking such action) at that time. The Sponsor will promptly inform any other investigators and/or institutions conducting the study, if the study is suspended or terminated for safety reasons and will inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, an investigator will inform the IRB promptly and provide the study subjects with the reason for the suspension or termination. If the study is prematurely discontinued, all study data will be returned to the Sponsor.

#### **13.4. Regulatory Documents and Records Retention**

An investigator is responsible for creating and/or maintaining all study documentation required by 21 CFR 50, 54, 56 and 312, ICH E6 section 8, as well as any other documentation defined in the protocol or CSA. An investigator must provide key documents to the Sponsor prior to the start of the study. A complete list of required regulatory documents will be supplied by the Sponsor or its representative.

Federal and local regulations require that an investigator retain a copy of all regulatory documents and records that support the data for this study for whichever of the following is the longest period of time:

- A period of 2 years following the final date of approval by the FDA or other regulatory agency of the study product for the purposes that were the subject of the investigation; or
- A period of 5 years following the date on which the results of the investigation were submitted to the FDA or other regulatory agency in support of, or as part of, an application for a research or marketing permit for the study product for the purposes that were the subject of the investigation.

The Sponsor will notify investigators once one of the above two timeframes has been satisfied.

If the investigation does not result in the submission of the data in support of, or as part of, an application for a research or marketing permit, records must be retained for a period of 2 years following notification by the Sponsor that the entire clinical investigation (not merely an investigator's portion) is completed, terminated, or discontinued.

If an investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Study records should not be destroyed without consultation with the Sponsor.

#### **13.5. Delegation of Responsibilities and Adequate Resources**

An investigator should have adequate time to conduct the study properly and should have an adequate number of qualified staff to assist with the conduct of the study.

The term “investigator” used throughout this protocol refers to the principal investigator and/or qualified sub-investigators. However, the investigator may delegate responsibilities to other investigational site personnel. An investigator shall delegate tasks only to individuals qualified by education, training and experience to perform the delegated tasks. An investigator shall have direct oversight of all delegated activities and shall document delegation of responsibilities. An investigator is responsible for ensuring all delegated staff have been properly trained on the protocol and their assigned study responsibilities. A delegation log identifying all delegated duties and the individual to whom they have been delegated will be maintained at the investigational site.

### **13.6. Protocol Amendments**

Approval of a protocol amendment by an investigator’s IRB must be obtained before implementation of the protocol amendment, unless a change is necessary to eliminate an apparent immediate hazard to the subject or when the change involves logistical or administrative aspects of the study. The protocol amendment must be signed and dated by both the Sponsor and an investigator. The Sponsor or designee will submit protocol amendments to the appropriate regulatory authorities, if required.

### **13.7. Financial Disclosure**

Clinical investigators are required to provide financial disclosure information for the submission of certification or disclosure statements required under 21 CFR § 54. As defined in 21 CFR § 54.2, a clinical investigator is a listed or identified investigator or sub-investigator who is directly involved in the treatment or evaluation of research subjects. The term also includes the spouse and each dependent child of an investigator. In addition, investigators must promptly update financial disclosure information if any relevant changes occur during the course of the investigation and for 1 year following completion of the study.

**14. SPONSOR APPROVAL PAGE**

**EVALUATION OF THE ABUSE LIABILITY OF VLN MENTHOLATED  
CIGARETTES IN ADULT SMOKERS**

*Version:* 1.0

*Date:* 04APR2018

**22nd Century Group, Inc.**

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James Swauger  
SVP Science and Regulatory Affairs

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Date

**15. INVESTIGATOR PROTOCOL AGREEMENT PAGE**

**EVALUATION OF THE ABUSE LIABILITY OF VLN MENTHOLATED  
CIGARETTES IN ADULT SMOKERS**

*Version:* 1.0

*Date:* 04APR2018

I agree to conduct the study in accordance with the protocol and with all applicable government regulations and International Conference on Harmonisation/Good Clinical Practice guidances.

Principal Investigator's  
Name  
(please print or type)

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Principal Investigator's Signature

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Date

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## 17. APPENDICES

### 17.1. Summary of Blood Volume Requirements

	Pharmacokinetic Sampling	Safety			Total
		Clinical Chemistry	Hematology	Serology	
<i>~ volume per sample (mL)</i>	<i>4 mL</i>	<i>5 mL</i>	<i>4 mL</i>	<i>8.5 mL</i>	
	<b>Blood Volume (mL) [Number of Samples]</b>				
<i>Screening</i>	--	5 [1]	4 [1]	8.5 [1]	17.5
<i>Part A</i>	--	--	--	--	--
<i>Part B</i>	360 [90]*	--	--	--	360
<i>End of study</i>		5 [1]	4 [1]		9
<b>Total</b>	<b>360</b>	<b>10</b>	<b>8</b>	<b>8.5</b>	<b>386.5</b>

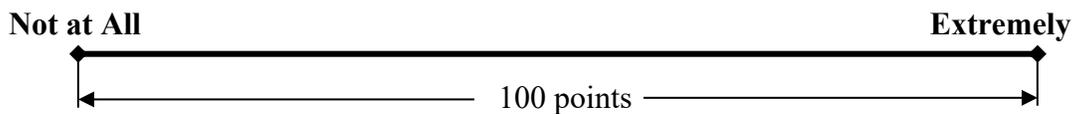
\*Blood volumes shown in the table are approximate and do not include approximately 90 mL catheter waste. Additional blood samples may be taken if needed to follow-up on individual subject safety.

## 17.2. Pharmacodynamic Measures/Scales

### 17.2.1. Tobacco/Nicotine Withdrawal Questionnaire

#### VAS Tobacco/Nicotine Withdrawal Scale <sup>c</sup>

Note: Each question will be paired with a VAS. The VAS anchored with “Not at All” on the left and “Extremely” on the right. Subjects place a vertical line at a place along the VAS based on how he feels in the moment.



These phrases may or may not describe how you feel right now. Please respond to each word or phrase with how you feel RIGHT NOW by drawing a vertical mark anywhere along the horizontal line.

1. Urges to Smoke
2. Anxious
3. Difficulty Concentrating
4. Impatient
5. Craving a Cigarette

<sup>c</sup> Items on this scale have been adapted from [Hughes & Hatsukami 1986](#)

