2. SYNOPSIS

NAME OF SPONSOR/COMPANY
22nd Century Group, Inc.

NAME OF FINISHED PRODUCT(S)
Very low nicotine (VLN) cigarettes

Title of Study:	A Longitudinal Ambulatory Study to Assess Changes in Cigarette Consumption Behavior and Biomarkers of Exposure during a 6-Week Switch to Very Low Nicotine Cigarettes
Investigators:	Allen Hunt, MD (Previously Dr. Philip Mathew, MD) Melanie Fein, MD
Study Centers:	Celerion 621 Rose Street Lincoln, Nebraska 68502, USA High Point Clinical Trials Center 4160 Mendenhall Oaks Parkway, Suite 105 High Point, North Carolina 27265, USA
Publication (Reference):	Not applicable.

Studied Period:

(date of first enrollment)
29 June 2018
(**date of last completed**)
08 March 2019

Objectives:
Primary Objective:

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

Secondary Objectives:

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

Methodology:
This was an open-label, randomized, forced-switching study conducted at two study sites. Seventy-two (72) self-affirmed exclusive filtered non-mentholated cigarette smokers and 70 self-affirmed exclusive filtered mentholated cigarette smokers with no intention to quit during the study period were enrolled in the study. At the start of Week -1, all subjects were asked to smoke their UB cigarettes as per their usual daily consumption for the following week. Subjects

received an e-diary to record daily cigarettes smoked per day (CPD). Subjects continued recording their CPD in their e-diaries through the end of Week 6.

Subjects returned at the end of Week -1 (baseline) for collection of blood and 24-hour urine samples for baseline BoE assessments. Subjective questionnaires for dependence, withdrawal symptoms, urges to smoke, and perceived health risks were also completed at scheduled times. A subset of 18 non-menthol and 18 menthol smoker subjects completed an assessment of puffing topography with their UB cigarettes during this visit. A further subset of 12 of the non-menthol and 12 of the menthol smoker subjects who completed the topography assessment also completed a nicotine PK assessment at the end of this visit.

On Day -1 of Week 1, subjects were randomly selected to either remain smoking their non-menthol (22 subjects) or menthol (20 subjects) UB cigarettes, or to switch to smoking non-menthol (50 subjects) or menthol (50 subjects) VLN cigarettes as per their UB cigarette flavor. Subjects returned at the end of Weeks 2 and 6 for collection of blood and 24-hour urine samples for BoE assessments. Subjective effects questionnaires were also completed at scheduled times. The same subset of subjects who completed the topography and PK assessments at Week -1 also were to complete these events at the Week 2 and Week 6 visits. Subjects who underwent topography and PK assessments were assigned to switch to smoking VLN cigarettes.

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

Number of Subjects (Planned and Analyzed):
A total of 142 smokers were enrolled in the study at Week -1 and 124 subjects completed the study. All 142 randomized subjects were included in the intent-to-treat (ITT) population based on product use. Out of these, 71 subjects were included in the Per-protocol (PP) population and 71 were excluded.

Diagnosis and Main Criteria for Inclusion:
All subjects enrolled in this study were judged by the Principal Investigator (PI) to be normal, healthy volunteers who met all inclusion and none of the exclusion criteria.

Test Product and Batch Number:
The test products were identified as PARE Regular King Box (Part (b) (4)) and the PARE Menthol King Box (Part (b) (4)) both manufactured by 22nd Century Group, Inc. PARE was the previous name for VLN.

Duration of Product Use:
The total duration of product use was from the start of Week -1 through end of Week 6.

Reference Product:
The reference product was Subjects' usual mentholated combustible cigarette brand.

Criteria for Evaluation:

Product Use: Subjects were provided with e-diaries to record their daily cigarette consumption, both when in clinical confinement during study visits and during ambulatory periods.

Subjects were instructed to record both compliant and non-compliant (if any) daily numbers of

cigarettes smoked. For the purposes of data analysis, the e-diary record of cigarette consumption was used as the primary variable.

Puffing Topography: At clinic visits at the end of Weeks -1, 2, and 6, a subset of 18 non-menthol and 18 menthol smoker subjects who had been assigned to switch to smoking VLN cigarettes completed a puffing topography evaluation. During the puffing topography assessment, subjects engaged in a 1-hour ad libitum smoking session with their UB (Week -1) or the VLN (Weeks 2 and 6) cigarettes with the mobile smoking puff analyzer (SPA-M; Sodim). The following topography parameters were assessed: Puff duration, puff volume, peak puff flow rate, average flow rate, and inter-puff interval.

Biomarkers of Exposure: Urine was collected over 24-hour periods for urine BoE measurements, during the clinic visits at the end of Weeks -1, 2, and 6. The urine samples collected during a single 24-hour period were pooled together and weighed. Concentrations of the following biomarkers were measured: total NNAL, total NNN, 3-HPMA, S-PMA, 1-OHP, and total nicotine equivalents (Tneq). Creatinine in urine was also measured and used to report BoE as amount per unit of creatinine. Blood samples for COHb in whole blood and cotinine in plasma were also collected on Weeks -1, 2, and 6. Plasma cotinine was collected to assess compliance.

Pharmacokinetics: PK blood sampling occurred in a subset of 12 non-menthol and 12 menthol smoker subjects following the completion of the 24-hour urine collection period on clinic visits at Weeks -1, 2, and 6. The selected subjects remained in the clinic for an additional night, during which they abstained from smoking any cigarettes or using any nicotine-containing products for at least 12 hours prior to the PK assessment session. During the PK session, subjects smoked a single cigarette (either their UB on Week -1 or the VLN on Weeks 2 and 6) ad libitum during a 5-minute period. Serial venous blood samples were collected for plasma nicotine analysis at 5 minutes prior to and at 2, 5, 7, 10, 12, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes relative to the start of cigarette smoking. The following noncompartmental PK parameters were derived from the baseline-adjusted plasma nicotine concentration-time data: AUC0-180, Cmax, and Tmax.

Subjective Effects: The Fagerström test for cigarette dependence (FTCD), Brief questionnaire of smoking urges (QSU-Brief), Minnesota Nicotine Withdrawal Scale-Revised (MNWS-R), and perceived health risk scale questionnaires were completed at the end of each clinic visit, at the end of Weeks -1, 2, 4, and 6.

Safety: Safety was evaluated by adverse events (AEs) and serious AEs (SAEs), electrocardiograms (ECG), vital signs, clinical chemistry, urinalysis, and hematology.

Statistical Methods:

Product Use, topography, BoE, and subjective effects:

Paired t-tests were used to compare CPD, puffing topography parameters, urinary NNAL, NNN, 3-HPMA, S-PMA, 1 OHP, Tneq mass excreted, blood COHb measures, and subjective effects measures at the Week 2 and Week 6 time points with the baseline (Week -1) values for each VLN product group. Product groups were VLN mentholated, VLN non-mentholated, and VLN combined (the pooled non-mentholated and mentholated groups). The primary analysis was based on the VLN combined product group. This analysis was conducted on the evaluable subjects in the ITT and PP populations. Differences were considered statistically significant at an

alpha level of 0.05. The Means of the differences and their associated 95% confidence interval (CI) and p-values were presented.

In addition, a linear mixed model analysis of variance with the factors product group, week, and product group by week interaction was used to compare the between-product group differences in the absolute change from baseline values of CPD, puffing topography parameters, absolute change from baseline values of each of the urinary and blood BoEs, and in the absolute change from baseline values of each of the subjective effect measures. The least-squares mean (LSM) difference, 95% CI, and p-value were provided for the product group difference.

Pharmacokinetics:
Paired t-tests were also used to compare baseline-adjusted nicotine PK parameters (AUC0-180 and Cmax) at the Weeks 2 and 6 time points with the baseline (Week -1) values. This analysis was conducted on subjects in the PK population (from the ITT and PP populations). Differences were considered statistically significant at an alpha level of 0.05. Geometric mean ratio (GMR), 95% CI for GMR, and p-value were presented. The analysis was based on the log-transformed data.

Safety:
All CRF data were listed by subject and chronologically by assessment time points. This included rechecks, unscheduled assessments, and early termination.

Applicable continuous variables were summarized using sample size (n), arithmetic mean, standard deviation (SD), minimum, median, and maximum.

SUMMARY – CONCLUSIONS

Compliance with Protocol
Out of the 142 randomized subjects, 71 subjects were excluded from the PP Population due to a restrictive compliance criterion (having a ratio of [plasma cotinine/CPD VLN]/[plasma cotinine/CPD baseline] > 0.2). Additionally, 1 subject reported smoking ≥ 50% non-assigned cigarettes on ≥ 50% of post baseline days. The final sample size in the PP population was therefore much lower than planned.

Out of the 36 subjects who participated in the puffing topography evaluation and were included in the ITT population, 9 subjects were included in the PP population. Out of the 140 subjects who participated in the urine BoE evaluation and were included in the ITT population, 69 subjects were included in the PP population. Out of the 24 subjects who participated in the PK evaluation and were included in the ITT population, 6 subjects were included in the PP population. Out of the 142 subjects who participated in the blood BoE and subjective effect evaluations and were included in the ITT population, 71 subjects were included in the PP population. As a result, the power to detect significant differences in the PP population was reduced and statistical comparisons performed in the ITT population were more likely to show statistical significance. Nonetheless, the same directional trends were observed in the ITT and PP populations. Moreover, PK, urine and blood BoE, topography, and subjective effects assessments took place in the CRU where smoking conditions were monitored. As such, these assessments were conducted under compliant smoking conditions.

Time Continuum of Results
Changes observed at Week 2 were not always sustained at Week 6, therefore Week 2 results

should be viewed as an indication of the trend whereas Week 6 results should be viewed as the final determination of effects.

Pharmacokinetic Results:

Product Use Assessment: In the PP population, there were statistically significant increases from baseline in CPD for VLN non-mentholated (7.1 CPD increase) and VLN combined (3.4 CPD increase) at Week 2, and for VLN non-mentholated (5 CPD increase) at Week 6. There were no significant changes noted in VLN mentholated or any of the UB groups. Regarding product comparisons, significantly greater CPD were noted in VLN non-mentholated and VLN combined compared to UB non-mentholated and UB combined, respectively, at Week 2, and for VLN non-mentholated compared to UB non-mentholated at Week 6.

In the ITT population, there were statistically significant decreases from baseline in CPD for VLN mentholated and VLN combined at Week 6, with no significant changes for VLN non-mentholated or any of the UB groups. There were no statistically significant changes for any of the groups at Week 2. There were no statistically significant comparisons between VLN and UB mentholated or non-mentholated at Weeks 2 or 6 in the ITT population.

Topography Assessment: For subjects in the PP population (smokers of either non-mentholated and mentholated filtered cigarettes combined), puff duration and volume (average and total) and average peak puff flow rate, average flow rate, and average number of puffs did not change significantly from baseline following the switch from UB to VLN cigarettes. There was a trend towards lower puff flow rates and lower puff volumes but the decreases were not significant. Significantly shorter inter-puff intervals were observed in Week 2 following the switch to VLN cigarettes and were still observed in Week 6. The sample size for the PP population was small (N = 3 to 8 for each cohort) making it difficult to robustly establish statistical significance.

The trends were more statistically evident in the ITT population, which had a larger sample size than the PP population, with significantly shorter total puff durations, significantly smaller total puff volumes, and significantly shorter inter-puff interval observed at Weeks 2 and 6 following the switch from UB to VLN cigarettes. Average puff duration, average puff volume, and average peak puff flow rate and average flow rate were not significantly changed though were typically lower at Weeks 2 and 6 compared to baseline. A small but significant decrease in the average number of puffs taken was observed on Week 6 but not on Week 3 when compared to baseline.

Taken together, these data indicate that the subjects did not compensate for the much smaller nicotine content on the VLN cigarettes by changing their puffing behavior in a compensatory manner with the exception of a slightly shorter inter-puff interval.

Pharmacokinetic Assessment: For subjects in the PP population (smokers of non-mentholated and mentholated cigarettes combined), nicotine peak and overall exposures (as measured by geometric mean Cmax and geometric mean AUC0-180) were significantly lower following the switch to VLN cigarettes. The decrease was over 99% for AUC0-180 on Weeks 2 and 6. Median Tmax values following use of VLN cigarettes were within the same range as those observed following UB cigarettes.

For subjects in the ITT population, with a larger sample size, a similar significant decrease in nicotine exposure (> 98% decrease for AUC0-180 and > 97% decrease for Cmax) was observed.

Biomarkers of Exposure:

NNAL

At Weeks 2 and 6, in both PP and ITT populations, significant decreases in urinary NNAL (56% to 70% in the PP population and 38% to 49% in the ITT population) were observed for subjects who switched to smoking mentholated or non-mentholated VLN cigarettes and in the combined data from smokers of mentholated and non-mentholated cigarettes. Decreases were greater at Week 6 in the PP population and less at Week 6 in the ITT population. No significant changes were observed for subjects who remained on UB.

NNN

For subjects in the PP population, at Week 2 significant decreases in urinary NNN were observed in subjects who switched to VLN non-mentholated cigarettes (43%). At Week 6, significant decreases in urinary NNN (> 52%) were observed for all subject groups who switched to VLN (smokers of mentholated and non-mentholated cigarettes and combined smokers). Significant decreases of lesser magnitude were also observed for smokers who remained on UB (smokers of mentholated cigarettes and combined smokers).

The same trends were observed for subjects in the ITT population: the decreases in urinary NNN observed at Week 2 (approximately 29%) were significant for some subject groups who switched to VLN (smokers of non-mentholated cigarettes and combined). At Week 6, significant decreases (31% to 53%) were observed in all subject groups who switched to VLN (smokers of mentholated and non-mentholated cigarettes and combined smokers) and for some who remained on UB (smokers of mentholated cigarettes and combined smokers showing 29% and 14% decreases, respectively) with greater changes observed in subjects who switched (VLN non-mentholated and VLN combined).

3-HPMA

For subjects in the PP and ITT populations, at Week 2 significant decreases (9% to 26%) in urinary 3 HPMA were observed in all product use groups except in subjects who switched to non-mentholated VLN cigarettes. Of note, significant decreases in 3-HPMA (9% to 13%) were also observed in the UB groups at Week 2. At Week 6, decreases in urinary 3-HPMA were only significant in subject groups who switched to VLN cigarettes (smokers of mentholated cigarettes and combined smokers in PP population showing 35% and 26% decrease, respectively; smokers of mentholated, non-mentholated cigarettes and combined smokers in ITT population showing 18% to 37% decrease).

S-PMA

For subjects in the PP and ITT populations, no significant changes from baseline in urinary S-PMA were observed except in subjects who switched to non-mentholated VLN cigarettes at Week 2 (PP and ITT populations) and saw a significant increase in urine S-PMA (37% and 17% in the PP and ITT populations, respectively). At Week 6, a significant decrease was observed in subjects who switched to VLN cigarettes (smokers of mentholated cigarettes and combined smokers showing 20% and 10% decrease, respectively) in the ITT population only.

1-OHP

For subjects in the PP and ITT population, at Week 2 significant decreases in urinary 1 OHP were observed in subjects who switched to VLN cigarettes (smokers of mentholated and non-mentholated cigarettes and combined smokers showing approximately 21% to 30% decreases) as well as in subjects who remained on UB (smokers of mentholated cigarettes and

combined smokers showing 12% and 17% decreases, respectively). At Week 6, the only significant decreases in urinary 1-OHP were seen in subjects who switched to VLN cigarettes (smokers of mentholated and non-mentholated cigarettes and combined smokers), with 30% to 39% decreases in the PP population and 19% to 33% decreases in the ITT population.

Tneq

Significant decreases in urinary Tneq were observed in subjects who switched to VLN cigarettes (smokers of mentholated and non-mentholated cigarettes and combined smokers) in the PP population (approximately 93% decrease at Weeks 2 and 6) and in the ITT population (69% to 73% at Week 2 and 48% to 58% and Week 6). No significant changes were noted in subjects who remained on UB.

COHb

For subjects in the PP population, a non-significant increase in blood COHb was observed at Week 2 in smokers of non-mentholated cigarettes who switched to VLN. There were no major changes observed for smokers of mentholated cigarettes who switched to VLN or for those who remained on UB. No major changes were observed when looking at overall data (combined data from smokers of mentholated and non-mentholated cigarettes) at Week 2. At Week 6, a decrease in blood COHb was observed in all subject groups except smokers who remained on non-mentholated UB. The decrease in COHb was significant for smokers who switched to VLN (combined smokers of mentholated and non-mentholated cigarettes) and for those who remained on mentholated UB cigarettes.

Subjects in the ITT population followed the same trend as those in the PP Population. At Week 2, there was a significant increase in blood COHb for smokers who switched to VLN (smokers of non-mentholated cigarettes and combined) while no major changes were observed for other groups. At Week 6, there was a significant decrease in blood COHb for smokers who switched to VLN (smokers of mentholated cigarettes and combined) and for those who remained on mentholated UB cigarettes

Subjective Effects Results

FTCD Questionnaire

In the PP population, there were no statistically significant differences from baseline in mean FTCD score for any study product group at Weeks 2 or 6. Regarding product comparisons, mean FTCD score was significantly greater in VLN non-mentholated compared to UB non-mentholated at Week 2, and was significantly lower in VLN mentholated compared to UB mentholated at Week 6. All other comparisons were not statistically significant. These results suggest that degree of addiction, as measured by FTCD, was not affected by use of VLN over 6 weeks.

In the ITT population, there was a statistically significant increase from baseline in mean FTCD score for VLN non-mentholated at Week 2, and a significant decrease from baseline in mean FTCD score for VLN mentholated at Week 6. There were no significant changes for UB cigarette. Regarding product comparisons, mean FTCD score was significantly higher in VLN non-mentholated compared to UB non-mentholated at Week 2. There were no significant comparisons for mentholated products.

QSU-Brief Questionnaire
In the PP population, decreases from baseline in the QSU-Brief factor score 'anticipation of pleasure from smoking' (Factor 1) were statistically significant for VLN mentholated and VLN combined, and for UB mentholated at Week 6. There were no statistically significant changes from baseline at Week 2. Regarding product comparisons, mean 'anticipation of pleasure from smoking' was significantly greater for VLN non-mentholated compared to UB non-mentholated at Week 2, and was significantly lower in VLN mentholated and VLN combined compared to UB mentholated and UB combined, respectively, at Week 6.

'Relief of nicotine withdrawal' (Factor 2) values were significantly lower than baseline for VLN mentholated and VLN combined at Week 6, and for UB mentholated and UB combined at Weeks 2 and 6, indicating greater relief of withdrawal compared to baseline. There were no statistically significant changes for VLN or UB non-mentholated. Regarding product comparisons, mean 'relief of nicotine withdrawal' was significantly lower for VLN mentholated and VLN combined, compared to UB mentholated and UB combined, respectively, at Week 6. There were no significant comparisons for non-mentholated products.

In the ITT population, increases from baseline in the QSU-Brief factor score 'anticipation of pleasure from smoking' (Factor 1) were statistically significant for VLN non-mentholated and VLN combined at Week 2, with a significant decrease from baseline for UB mentholated at Week 6. Regarding product comparisons, mean 'anticipation of pleasure from smoking' was significantly greater for VLN non-mentholated compared to UB non-mentholated at Week 2. There were no significant comparisons for mentholated products.

The increase from baseline for 'relief of nicotine withdrawal' (Factor 2) was statistically significant for VLN non-mentholated at Week 2. Decreases from baseline were significant for VLN mentholated at Week 6, and for UB mentholated and UB combined at Weeks 2 and 6. Regarding product comparisons, mean 'relief of nicotine withdrawal' was statistically significantly greater for VLN non-mentholated compared to UB non-mentholated at Week 2, and was significantly lower for VLN mentholated compared to UB mentholated at Week 6.

MNWS-R Questionnaire
In the PP population, decreases from baseline in the MNWS-R total scores were statistically significant for VLN non-mentholated and VLN combined, and for UB mentholated at Week 6. There were no statistically significant changes at Week 2. There were no significant comparisons in MNWS-R total score for any products.

In the ITT population, increases from baseline in the MNWS-R total score were statistically significant for VLN non-mentholated and VLN combined at Week 2, and decreases from baseline in the MNWS-R total score were significant for VLN mentholated and UB mentholated at Week 6. Regarding product comparisons, mean MNWS-R total score was statistically significantly greater for VLN non-mentholated and VLN combined compared to UB non-mentholated and UB combined, respectively, at Week 2, and for VLN non-mentholated compared to UB non-mentholated at Week 6.

Perceived Health Risk Questionnaire
In the PP population, decreases from baseline for perceived health risk were statistically significant for VLN mentholated and VLN combined at Week 2, and for VLN mentholated, VLN non-mentholated, and VLN combined at Week 6. There were no statistically significant changes

for UB mentholated or non-mentholated. Regarding product comparisons, with the exception of a non-significant lower value for VLN non-mentholated compared to UB non-mentholated at Week 2, mean perceived health risk was significantly lower for VLN non-mentholated, VLN mentholated, and VLN combined, compared to UB non-mentholated, UB mentholated, and UB combined, respectively, at Weeks 2 and 6.

In the ITT population, decreases from baseline for perceived health risk were statistically significant for VLN non-mentholated, VLN mentholated, and VLN combined at Weeks 2 and 6. There were no statistically significant changes for UB mentholated or non-mentholated. Regarding product comparisons, mean perceived health risk was significantly lower for VLN non-mentholated, VLN mentholated and VLN combined, compared to UB non-mentholated, UB mentholated, and UB combined, respectively, at Weeks 2 and 6.

Safety Results:
There were no deaths reported in this study. One subject experienced an SAE (subarachnoid hemorrhage) after study completion that was not product related. The subject made a complete recovery. One subject was discontinued by the PI due to experiencing several AEs.

During the Baseline period (Week -1), in which subjects smoked their usual brand cigarettes, 19 (13.4%) subjects experienced a total of 30 AEs; with headache being the most frequent event, reported by 12 (8.5%) subjects. The majority (16) of subjects experienced events that were mild in severity and three subjects experienced moderate events (migraine, presyncope, and vomiting). The PI considered two events (dyspepsia and headache) in the same subject to be possibly related to study product and the events in the remaining subjects unlikely/not related.

After study product group randomization, 45 of 142 (31.7%) subjects across study groups experienced a total of 83 AEs. Headache was the most frequently reported AE, experienced 24 times by a total of 22 (15.5%) subjects; all remaining AEs were experienced by seven or fewer (< 5%) subjects each. Regarding maximum severity, one subject experienced a severe AE (subarachnoid hemorrhage), nine subjects experienced moderate AEs (including five subjects with headache), and the remaining 35 subjects experienced only mild AEs. The PI considered an AE in one subject (nausea [VLN non-mentholated]) to be likely related to study product, AEs in 12 subjects to be possibly related to study product, and the remaining 32 subjects only experienced events considered unlikely/not related to study product.

Conclusions:

- Exposure to nicotine and other BoEs was significantly reduced following the switch to VLN cigarettes and subjects did not exhibit behavior that was typical of compensation (increased number of cigarettes, puff duration, puff volume, or flow rate). An observed decrease in inter-puff interval may have suggested compensation but it was not associated with a higher number of puffs taken indicating that compensation did not take place following the switch to VLN cigarettes. Similarly, an increase in CPD was observed in Week 2 but it was not sustained in Week 6.
- Overall, the use of the VLN products under the study conditions appeared to be well tolerated by the healthy adult smokers in this study.

Date of Report: 08 July 2019