

Section II Contents

A. Table of Contents

Section I. Cover Letter

Section II. Contents

- A. Table of Contents
- B. List of Abbreviations
- C. Glossary

Section III. Executive Summary

- A. Background
- B. Product Description
- C. Summary of Analytical Studies and Quantitative Risk Assessments
- D. Summary of Other Nonclinical Studies
- E. Summary of Clinical Studies
- F. Projected Effects on the Population as a Whole
- G. Effect of VLN™ and its Marketing on Tobacco Use Behavior
- H. Addiction and Cessation
- I. Cited Literature

Section IV. Descriptive Information

- A. Product Description
- B. Product Formulation
 - 1. Listing of Design Features
 - 2. Listing of Materials
 - 3. Confidential Listing of Ingredients
 - i. Nicotine in Filler Specification
 - ii. Tobacco
 - iii. (b) (4)
 - iv. (b) (4)
 - v. (b) (4)
 - vi. (b) (4)
 - vii. Ingredients in Cigarette Material Components
 - (a) Side Seam Adhesive

- (b) Tipping Paper
- (c) Tipping Adhesive
- (d) Filter Plug

- 1) Tow
- 2) (b) (4) Plug Wrap
- 3) (b) (4) Plug Wrap
- 4) (b) (4) Filter
- 5) (b) (4)
- 6) (b) (4)
- 7) (b) (4)
- 8) (b) (4)
- 9) (b) (4)

4. List of Packaging Materials

C. Confidential Manufacturing Information

- 1. Production and Facilities
- 2. Managerial Oversight and Employee Training
- 3. Processes and Controls for Product Design and Changes
- 4. Identifying Suppliers
- 5. Validation and Verification to Specifications
 - i. Acceptance Criteria for Incoming Materials
 - ii. Acceptance Criteria for Parts and Finished Product During Assembly Process
- 6. Frequency of Product Specification Validation
- 7. Diagnostic and Measuring Device Validation
- 8. Testing procedures carried out before the product is released to market:
- 9. Handling of complaints, nonconforming products and processes, and corrective and preventative actions:

D. Conditions for Using the Product

- 1. Narrative
- 2. Description of Length of Time to Consume
- 3. Specific Instructions on How to Use and Store the Product
- 4. Specific Instructions on How to Avoid Using the Product in a Way that Reduces the Benefit or Increases the Risk

E. How Consumers Actually Use the Product

F. Overview of Vector 21-41 Tobacco Variety

- 1. Summary of Technology

- 2. History
- 3. Nicotine Biosynthesis
- 4. Modified Nicotine Synthesis: NtQPT antisense construct
- 5. Agrobacterium Transformation
- 6. Characterization of Vector 21-41
- 7. USDA Deregulation of Vector 21-41
- G. Bibliography
- VI. Environmental Assessment
 - A. Description of Proposed Actions
 - B. Description of Facility and Grounds
 - C. Introduction of Substances into the Environment.
 - D. External Impact on Community
 - E. Summary of Environmental Effects as a Result of Manufacturing Due to the Proposed Action
 - F. Environmental Introduction as a Result of Use Due to the Proposed Action
 - G. Environmental Introduction as a Result of Disposal After Use
 - 1. Disposal of Packaging Material
 - 2. Disposal of Used Cigarettes Following Use
 - 3. Air Emissions from Disposal
 - H. Summary of Environmental Effects as a Result of Use of the Products Due to the Proposed Action
 - I. Alternatives to the Proposed Action
 - J. List of Preparer/Agencies Consulted
 - K. Conclusion
 - L. Bibliography
- VII. Summary of All Research Findings
 - A. Health Risks of the Tobacco Product.
 - 1. Summary
 - 2. The Effect of Marketing VLN™ with Modified Exposure Message on Consumer Perception of the Product
 - 3. The Effect of Marketing VLN™ with Modified Exposure Message on Initiation among Never Smokers
 - 4. The Effect of Marketing VLN™ with a Modified Exposure Message on Youth
 - 5. The Effect of Marketing VLN™ with Modified Exposure Message on Initiation among Former Smokers
 - 6. The Effect of Marketing VLN™ with Modified Exposure Message among Current Smokers
 - 7. HPHC's and Quantitative Risk Assessments

- 8. Unintended Consequences
 - i. Weight Gain
 - ii. Platelet Activation
- B. Abuse Liability
 - 1. Background
 - 2. VLN™ King and VLN™ Menthol King
- C. Effect on Tobacco Use Behavior among Current Users
 - 1. Summary
 - 2. Reduced Cigarette Consumption
 - 3. Smoking Urges and Cravings
 - 4. Adolescents
 - 5. Sex Differences
 - 6. Nondaily Smokers
 - 7. Normal and Slow Metabolizers
 - 8. Dual Use
 - i. Conventional Cigarettes
 - ii. NRT
 - 9. Co-Use
 - i. Alcohol
 - ii. Cannabis
 - iii. Opioids
 - 10. Sensitive Populations
 - i. Schizophrenia
 - ii. Affective Disorders
 - iii. Clinically Depressed
 - iv. Chronic Health Conditions
- D. Effect on VLN™ Use Initiation among Non-Users
- E. Effect of Marketing on Consumer Understanding and Perceptions
- F. Effect on the Population as a Whole
- G. Label Development and Claims Support
 - 1. Label development
 - 2. “95% Less Nicotine”
 - 3. “Helps you reduce your nicotine consumption” and “...greatly reduces your nicotine consumption.”
- H. Bibliography

VIII. Scientific Studies and Analyses

A. Background

B. Product Analysis

1. Nicotine in Tobacco

- i. Batch Analysis
- ii. Filler Nicotine Analysis of Top 100 Brands
- iii. Storage Stability

2. Nicotine in Smoke and HPHC Analysis

- i. HPHC Analysis on VLN™ under ISO and Canadian Intense Smoking Conditions
- ii. Smoke Metal Analysis under ISO and Canadian Intense Conditions
- iii. HPHC Analysis on Market Leading Brands

3. Quantitative Risk Assessment

- i. Quantitative Risk Assessment - Cancer
- ii. Quantitative Risk Assessment – Non-Cancer
 - (a) Quantitative Risk Assessment – Respiratory Disease
 - (b) Quantitative Risk Assessment – Reproductive and Developmental Diseases
 - (c) Quantitative Risk Assessment – Cardiovascular Disease
- iii. Overall Risks Compared to All Other Products on the Market

4. Bibliography

C. Non-clinical Studies

1. Background

2. Summary

3. Individual Study Summaries

- i. In Vitro Studies
 - (a) Activation of platelets exposed to shear stress in the presence of smoke extracts of low-nicotine and zero-nicotine cigarettes: the protective effect of nicotine.
 - (b) In vitro model of platelet-endothelial activation due to cigarette smoke under cardiovascular circulation condition
 - (c) Toxicological Analysis of Low-Nicotine and Nicotine free Cigarettes
 - 1) Smoke Chemistry
 - 2) Salmonella/microsome plate incorporation Assay (Ames Test)
 - 3) LDH release assay, WST-8 conversion assay and cell growth assay
 - 4) Gap junction intercellular communication (GJIC)

- 5) Effects of CSC and nicotine on apoptosis
- 6) Cell cycle analysis
- 7) Comet assay
- 8) Western blotting

(d) Comparison of toxicity of smoke from traditional and harm-reduction cigarettes using mouse embryonic stem cells as a novel model for preimplantation development

- 1) Effects on Embryonic cell attachment
- 2) Effects on Embryonic cell proliferation and survival

(e) Comparison of the Toxicity of Smoke from Conventional and Harm Reduction Cigarettes Using Human Embryonic Stem Cells

- 1) Evaluation of Cytotoxicity of MS and SS Smoke from Conventional and Harm Reduction Cigarettes
- 2) Conventional and Harm Reduction SS Cigarette Smoke Inhibited hESC Colony Attachment at a Noncytotoxic Dose (0.1 PE)
- 3) MS and SS Smoke from Conventional and Harm Reduction Cigarettes Inhibited hESC Colony Growth at a Noncytotoxic Dose (0.1 PE)

(f) Effect of full flavor and denicotinized cigarettes exposure on the brain microvascular endothelium: a microarray-based gene expression study using a human immortalized BBB endothelial cell line, with the exception of Nicotine, both full flavor (3R4F) and denicotinized (ULN) cigarettes activated inflammatory and oxidative stress pathways.

ii. Animal Studies

- (a) Tobacco extract but not nicotine impairs the mechanical strength of fracture healing in rats.
- (b) Potentially Reduced Exposure Cigarettes Accelerate Atherosclerosis: Evidence for the Role of Nicotine

4. Bibliography

D. Clinical Studies

- 1. Background
- 2. Summary
- 3. Individual Study Summaries

i. Evaluation of the Abuse Liability of Very Low Nicotine Cigarettes (NCT0359751)

- (a) Study Design
- (b) Results
- (c) Conclusions

- ii. Evaluation of the Abuse Liability of Very Low Nicotine Mentholated Cigarettes (NCT03559725)
 - (a) Study Design
 - (b) Results
- iii. A Longitudinal Ambulatory Study to Assess Changes in Cigarettes Consumption Behavior and Biomarkers of Exposure during a 6-Week switch to Very Low Nicotine (NCT03571724)
 - (a) Study Design
 - (b) Results
- iv. A prospective, double-blind, randomized, active controlled, parallel group, multicenter phase II clinical trial to evaluate the effectiveness of X-22 as a smoking cessation aid (NCT01400815).
- v. A randomized trial of nicotine replacement therapy in combination with reduced-nicotine cigarettes for smoking cessation (IND 69,185).
- vi. Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation (NCT00777569).
- vii. Randomized trial of reduced-nicotine standards for cigarettes (NCT01681875).
- viii. Transient compensatory smoking in response to placebo cigarettes.
- ix. A Randomized Controlled Trial of Progressively Reduced Nicotine Content Cigarettes on Smoking Behaviors, Biomarkers of Exposure, and Subjective Ratings.
- x. New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure.
- xi. The acute effects of nicotine on the subjective and behavioural responses to denicotinized tobacco in dependent smokers
- xii. The effects of nicotine, denicotinized tobacco, and nicotine-containing tobacco on cigarette craving, withdrawal, and self-administration in male and female smokers.
- xiii. Smoking Topography Characteristics of Very Low Nicotine Content Cigarettes, With and Without Nicotine Replacement, in Smokers with Schizophrenia and Controls.
- xiv. The impact of smoking very low nicotine content cigarettes on alcohol use.
- xv. Nondaily smokers' changes in cigarette consumption with very low-nicotine-content cigarettes. A randomized double-blind clinical trial (NCT02228824).
- xvi. Response to varying the nicotine content of cigarettes in vulnerable populations: An initial experimental examination of acute effects.
- xvii. Nicotine and Anatabine Exposure from Very Low Nicotine Content Cigarettes.
- xviii. Alcohol-induced increases in smoking behavior for nicotine and denicotinized cigarettes in men and women.

- xix. Greater reductions in nicotine exposure while smoking very low nicotine content cigarettes predict smoking cessation.
- xx. Reduced nicotine content cigarettes and use of alternative nicotine products: Exploratory trial.
- xxi. Abrupt nicotine reduction as an endgame policy: A randomized trial.
- xxii. Evaluation of a reduced nicotine product standard: Moderating effects of and impact on cannabis use.
- xxiii. Treating smokers before the quit date: Can nicotine patches and denicotinized cigarettes reduce cravings?
- xxiv. Effects of 6-week use of reduced-nicotine content cigarettes in smokers with and without elevated depressive symptoms.
- xxv. A prospective, double-blind, randomized, active controlled, parallel group, multicenter phase II clinical trial to evaluate the effectiveness of Quest alone and in combination with nicotine replacement therapy as a smoking cessation aid (IND 69,185).
- xxvi. Sex differences in acute relief of abstinence-induced withdrawal and negative affect due to nicotine content in cigarettes.
- xxvii. Reduced Nicotine Content Cigarettes and Nicotine Patch.
- xxviii. Reduced nicotine cigarettes: Smoking behavior and biomarkers of exposure in smokers not intending to quit.
- xxix. Prolonged exposure to denicotinized cigarettes with or without transdermal nicotine.
- xxx. The combined effect of very low nicotine content cigarettes, used as an adjunct to usual Quitline care (nicotine replacement therapy and behavioural support), on smoking cessation: a randomized controlled trial (ACTRN126080004103580).
- xxxi. Reduced-nicotine cigarettes in young smokers: Impact of nicotine metabolism on nicotine dose effects.
- xxxii. Pharmacodynamic effects of new de-nicotinized cigarettes.
- xxxiii. Smoking topography in response to denicotinized and high-yield nicotine cigarettes in adolescent smokers.
- xxxiv. Sex differences in response to reduced nicotine content cigarettes.
- xxxv. Complementing the standard multicomponent treatment for smokers with denicotinized cigarettes: A randomized trial.
- xxxvi. Cognitive effects of very low nicotine content cigarettes, with and without nicotine replacement, in smokers with schizophrenia and controls.
- xxxvii. Mouth-level intake of benzo[a]pyrene from reduced nicotine cigarettes.
- xxxviii. Dose-Response Effects of Spectrum Research Cigarettes.

- xxxix. Effects of reduced nicotine content cigarettes on individual withdrawal symptoms over time and during abstinence (NCT01681875).
- xl. Nicotine and non-nicotine smoking factors differentially modulate craving, withdrawal and cerebral blood flow as measured by arterial spin labeling.
- xli. Experimental evidence for a causal relationship between smoking lapse and relapse.
- xlii. Decreasing nicotine content reduces subjective and physiological effects of smoking.
- xlili. Evaluating the acute effects of oral, non-combustible potential reduced exposure products marketed to smokers.
- xliv. The airway sensory impact of nicotine contributes to the conditioned reinforcing effects of individual puffs from cigarettes.
- xlvi. Placebo cigarettes in a spaced smoking paradigm.
- xlvi. Reducing nicotine exposure results in weight gain in smokers randomized to very low nicotine content cigarettes.
- xlvi. Separate and combined effects of very low nicotine cigarettes and nicotine replacement in smokers with schizophrenia and controls.
- xlvi. Adolescent smokers' response to reducing the nicotine content of cigarettes: Acute effects on withdrawal symptoms and subjective evaluations.
- xlix. Smoking in the absence of nicotine: behavioral, subjective and physiological effects over 11 days.
- I. Estimations and predictors of non-compliance in switchers to reduced nicotine content cigarettes.
- li. Preliminary test of cigarette nicotine discrimination threshold in non-dependent versus dependent smokers.
- lii. Effects of low nicotine content cigarettes on smoke intake.
- liii. Age moderates smokers' subjective response to very-low nicotine content cigarettes: Evidence from a randomized controlled trial.
- liv. Sex differences in tobacco withdrawal and responses to smoking reduced-nicotine cigarettes in young smokers.
- lv. Response to reduced nicotine content cigarette among smokers differing in tobacco dependence severity.
- lvi. Cigarette nicotine content as a moderator of the relationship between negative effect and smoking (NCT01681875).
- lvii. Effects of acute abstinence and nicotine administration on taste perception in cigarette smokers.
- lviii. Threshold dose for discrimination of nicotine via cigarette smoking.
- lix. Estimation of compliance with exclusive smoking of very low nicotine content cigarettes using plasma cotinine.

- lx. Addiction potential of cigarettes with reduced nicotine content in populations with psychiatric disorders and other vulnerabilities to tobacco addiction.
- lxi. The influence of nicotine dose and nicotine dose expectancy on the cognitive and subjective effects of cigarette smoking.
- lxii. Effect of Immediate vs Gradual Reduction in Nicotine Content of Cigarettes on Biomarkers of Smoke Exposure: A Randomized Clinical Trial.
- lxiii. Preliminary Validity of the Modified Cigarette Evaluation Questionnaire in predicting the reinforcing effects of cigarettes that vary in nicotine content.
- lxiv. Reduced-nicotine cigarettes increase platelet activation in smokers in vivo: A dilemma in harm reduction.
- lxv. Pharmacokinetic Profile of Spectrum Reduced Nicotine Cigarettes.
- lxvi. Response to reduced Nicotine Content in Vulnerable Populations: Effect of Menthol Status (NCT02250534).
- lxvii. Evaluation of menthol per se on acute perceptions and behavioral choice of cigarettes differing in nicotine content.
- lxviii. The Impact of Nicotine Dose on the Reinforcing Value of Cigarettes in Adolescents.

4. Adverse Events

- i. Evaluation of the Abuse Liability of Very Low Nicotine Cigarettes
 - (a) Adverse Events
 - (b) Serious Adverse Events
 - (c) Discontinuations
- ii. Evaluation of the Abuse Liability of Menthol Very Low Nicotine Cigarettes
 - (a) Adverse Events
 - (b) Serious Adverse Events
 - (c) Discontinuations
- iii. Effect of Immediate vs Gradual Reduction in Nicotine Content of Cigarettes on Biomarkers of Smoke Exposure: A Randomized Clinical Trial
 - (a) Adverse Events
 - (b) Serious Adverse Events
 - (c) Discontinuations
- iv. Reduced Nicotine Standards for Cigarettes: A Randomized Trial
 - (a) Adverse Events
 - (b) Serious Adverse Events
 - (c) Discontinuations
- v. A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of Quest Alone and in

Combination with Nicotine Replacement Therapy as a Smoking Cessation Aid (IND 69185 [pg297])

- (a) Adverse Events
- (b) Serious Adverse Events
- (c) Discontinuation

vi. A Prospective, Double-Blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid (IND 103589 [pg291])

- (a) Adverse Events
- (b) Serious Adverse Events
- (c) Deaths
- (d) Discontinuations Due to Adverse Events

5. Ongoing Studies

- i. Facilitating Smoking Cessation With Reduced Nicotine Cigarettes
- ii. Novel Approaches to Reducing Tobacco Related Harm
- iii. Abuse Liability of Reduced Nicotine Content Cigarettes in the Context of Concurrent E-Cigarette Use
- iv. Effects of Nicotine Reduction on Smoking Behavior in ADHD Smokers
- v. Evaluation of Very Low Nicotine Content Cigarettes in Adolescent Smokers
- vi. Manipulating Tobacco Constituents in Female Menthol Smokers
- vii. Manipulating Tobacco Constituents in Male Menthol Smokers
- viii. Project 1, Study 2: Extended Exposure to Low Nicotine Content Cigarettes in Childbearing Age Women
- ix. Project 1, Study 2: The Combined Impact of Nicotine Replacement and Spectrum Cigarettes
- x. Project 2, Study 2: Extended Exposure to Low Nicotine Content Cigarettes in Opioid Abusers
- xi. Project 3, Study 2: Extended Exposure to Low Nicotine Content Cigarettes in People With Current Affective Disorders
- xii. Reduced Nicotine Content Cigarettes in Smokers of Lower Socioeconomic Status
- xiii. Reduced Nicotine Cigarettes in Smokers With Mood and Anxiety Disorders
- xiv. Strengthening Instrumental Extinction to Prevent Smoking Relapse (VLNCCue)
- xv. Switching to Reduced Oxidant or Nicotine Content Cigarettes in Smokers
- xvi. Very-Low Nicotine Cigarettes and Non-Daily Smokers
- xvii. Very Low Nicotine Cigarettes in Smokers With Schizophrenia
- xviii. Very Low-Nicotine Cigarettes in Smokers With SUD

- xix. Neuroimaging Reward, Behavioral Treatment, and Smoking Cessation
- xx. Abuse Liability of Reduced Nicotine Content Cigarettes Within a Complex Tobacco Marketplace: Experiment 1
- xxi. Concomitant Use of Very Low Nicotine Content Cigarettes and e-Cigarettes
- xxii. Reactions to Reduced Nicotine Cigarettes in Young Adult Low-Frequency Smokers (NicRed)
- xxiii. Reduced Nicotine Cigarettes in Smokers With and Without Alcohol Use Disorder (RedNic)
- xxiv. Switching to Very Low Nicotine Content Cigarettes vs Reducing Cigarettes Per Day

6. Bibliography

E. Effect of Marketing on Consumer Understanding and Perceptions

1. Qualitative Study to Develop PARE / VLN™ Claims within the United States

- i. Phase I
- ii. Phase II
- iii. Phase III
- iv. Phase IV

2. Quantitative Study to Develop VLN™ Hypothetical Product Messages Among U.S. Adult Cigarette Smokers, Adult Former Cigarette Smokers and Adult Never Cigarette Users

- i. Risk of Addiction
- ii. The Effect of Marketing VLN™ with Modified Exposure Message on Consumer Perception of the Product
- iii. The Effect of Marketing VLN™ with Modified Exposure Message on Initiation among Never Smokers
- iv. The Effect of Marketing VLN™ with a Modified Exposure Message on Youth
- v. The Effect of Marketing VLN™ with Modified Exposure Message on Initiation among Former Smokers
- vi. The Effect of Marketing VLN™ with Modified Exposure Message among Current Smokers
- vii. Conclusion

3. Bibliography

F. Effect on the Population as a Whole

- 1. Summary
- 2. Background
- 3. A Simulation Model to Evaluate the Impact of VLN™ Cigarettes on the Population as a Whole
- 4. Bibliography

- IX. All Documents Related to Research Findings
 - A. Summary
 - B. External Document Search
 - 1. Search Strategy
 - 2. Methodology
 - 3. Search Results
 - i. Initial Literature Search – Titles Only Information Research Report
 - ii. Initial Literature Search – Struck Titles
 - iii. Relevant Literature Search after Review with Abstracts
 - C. Internal Document Search
 - 1. Strategy
 - 2. Emails - Strategy
 - 3. Emails – Results
 - 4. Internal Printed Documents - Strategy
 - 5. Internal Documents – Printed – Results
- X. Post-market Surveillance Program
 - A. Background
 - B. Post-market Surveillance Program
 - 1. Serious and Unexpected Adverse Event Reporting
 - 2. A cross-sectional survey of knowledge and use of VLN™ cigarettes
 - 3. A prospective non-interventional study to evaluate reduction in cigarette use
 - 4. Manufacturing Deviations
 - 5. Periodic Reporting
 - C. Bibliography