

22nd Century Limited, LLC

IND 103589

September 25, 2014

Bob Rappaport, M.D., Director
Food and Drug Administration
Center for Drug Evaluation and Research Division of Anesthesia, Analgesia, and Addiction Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: IND 103589 (SN006) X-22 Smoking Cessation Product
Annual Report

Dear Dr. Rappaport,

Reference is made to our Initial IND 103,589 for X-22 Smoking Cessation Product, submitted June 21, 2011 (SN0000), for our investigational drug X-22 Smoking Cessation Product for the indication of smoking cessation.

We are submitting herewith the Annual Report for the period September 2, 2013 to September 1, 2014 for this IND.

If you have additional questions or require further information, please do not hesitate to contact me by e-mail at (b) (6) or by telephone at (b) (6)

Kind regards

(b) (6)

Michael R. Moynihan, Ph.D.
Vice President, Research and Development

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(Title 21, Code of Federal Regulations (CFR) Part 312)

Form Approved: OMB No. 0910-0014
Expiration Date: April 30, 2015
See PRA Statement on page 3.

NOTE: No drug/biologic may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40)

1. Name of Sponsor
22nd Century Limited, LLC

2. Date of Submission *(mm/dd/yyyy)*
09/26/2014

3. Sponsor Address

Address 1 *(Street address, P.O. box, company name c/o)*
9530- Main Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City
Clarence

State/Province/Region
NY

Country
USA

ZIP or Postal Code
14031

4. Telephone Number *(Include country code if applicable and area code)*
(b) (6)

5. Name(s) of Drug *(Include all available names: Trade, Generic, Chemical, or Code)*
X-22 Smoking Cessation Product

Continuation Page for #5

6. IND Number *(If previously assigned)*
103589

7. (Proposed) Indication for Use
Smoking Cessation

Is this indication for a rare disease (prevalence <200,000 in U.S.)? ☐ Yes ☐ No

Does this product have an FDA Orphan Designation for this indication? ☐ Yes ☑ No

If yes, provide the Orphan Designation number for this indication:

Continuation Page for #7

8. Phase(s) of Clinical Investigation to be conducted ☐ Phase 1 ☑ Phase 2 ☐ Phase 3 ☐ Other *(Specify)*: _____

9. List numbers of all Investigational New Drug Applications (21 CFR Part 312), New Drug Applications (21 CFR Part 314) , Drug Master Files (21 CFR Part 314.420) , and Biologics License Applications (21 CFR Part 601) referred to in this application.

10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted..

Serial Number
0006

11. This submission contains the following *(Select all that apply)*

☐ Initial Investigational New Drug Application (IND)
☐ Request For Reactivation Or Reinstatement
☐ Development Safety Update Report (DSUR)
☐ Response to Clinical Hold
☑ Annual Report
☐ Other *(Specify)*: _____
☐ Response To FDA Request For Information
☐ General Correspondence

Protocol Amendment(s)
☐ New Protocol
☐ Change in Protocol
☐ New Investigator
☐ PMR/PMC Protocol

Information Amendment(s)
☐ Chemistry/Microbiology
☐ Pharmacology/Toxicology
☐ Clinical ☐ Statistics
☐ Clinical Pharmacology

Request for
☐ Meeting
☐ Proprietary Name Review
☐ Special Protocol Assessment
☐ Formal Dispute Resolution

IND Safety Report(s)
☐ Initial Written Report
☐ Follow-up to a Written Report

12. Select the following only if applicable. *(Justification statement must be submitted with application for any items selected below. Refer to the cited CFR section for further information.)*

Expanded Access Use, 21 CFR 312.300

☐ Emergency Research Exception From Informed Consent Requirements, 21 CFR 312.23 (f)
☐ Charge Request, 21 CFR 312.8
☐ Individual Patient, Non-Emergency 21 CFR 312.310
☐ Individual Patient, Emergency 21 CFR 312.310(d)
☐ Intermediate Size Patient Population, 21 CFR 312.315
☐ Treatment IND or Protocol, 21 CFR 312.320

For FDA Use Only

CBER/DCC Receipt Stamp	DDR Receipt Stamp	Division Assignment
		IND Number Assigned

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13. Contents of Application – This application contains the following items *(Select all that apply)*

- [x] 1. Form FDA 1571 *(21 CFR 312.23(a)(1))*
- [x] 2. Table of Contents *(21 CFR 312.23(a)(2))*
- [x] 3. Introductory statement *(21 CFR 312.23(a)(3))*
- [x] 4. General Investigational plan *(21 CFR 312.23(a)(3))*
- [ ] 5. Investigator's brochure *(21 CFR 312.23(a)(5))*
- [ ] 6. Protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] a. Study protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] b. Investigator data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
  - [ ] c. Facilities data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
- 6. Protocol(s) *(Continued)*
  - [ ] d. Institutional Review Board data (21 CFR 312.23(a)(6)(iii)(b)) or completed Form(s) FDA 1572
- [ ] 7. Chemistry, manufacturing, and control data *(21 CFR 312.23(a)(7))*
  - [ ] Environmental assessment or claim for exclusion *(21 CFR 312.23(a)(7)(iv)(e))*
- [ ] 8. Pharmacology and toxicology data (21 CFR 312.23(a)(8))
- [x] 9. Previous human experience *(21 CFR 312.23(a)(9))*
- [ ] 10. Additional information *(21 CFR 312.23(a)(10))*
- [ ] 11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
- [ ] 12. Clinical Trials Certification of Compliance *(Form FDA 3674)*

14. Is any part of the clinical study to be conducted by a contract research organization? [ ] Yes [x] No

If Yes, will any sponsor obligations be transferred to the contract research organization? [ ] Yes [ ] No

If Yes, provide a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred *(use continuation page)*. **Continuation Page for #14**

15. Name and Title of the person responsible for monitoring the conduct and progress of the clinical investigations

Michael R. Moynihan, Ph.D., VP Research and Development, 22nd Century Limited, LLC

16. Name(s) and Title(s) of the person(s) responsible for review and evaluation of information relevant to the safety of the drug

(b) (4), (b) (6) Medical , (b) (4) [redacted]

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold or financial hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

17. Name of Sponsor or Sponsor's Authorized Representative

Michael R. Moynihan

18. Telephone Number *(Include country code if applicable and area code)*

(b) (6) [redacted]

19. Facsimile (FAX) Number *(Include country code if applicable and area code)*

716-877-3064

20. Address

Address 1 *(Street address, P.O. box, company name c/o)*: 9530 Main Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*:

City: Clarence | State/Province/Region: NY | Country: USA | ZIP or Postal Code: 14031

21. Email Address

(b) (6) [redacted]

22. Date of Sponsor's Signature *(mm/dd/yyyy)*

09/26/2014

23. Name of Countersigner

24. Address of Countersigner

Address 1 *(Street address, P.O. box, company name c/o)*:

Address 2 *(Apartment, suite, unit, building, floor, etc.)*:

City: | State/Province/Region: | Country: United States of America | ZIP or Postal Code:

WARNING : A willfully false statement is a criminal offense (U.S.C. Title 18, Sec. 1001).

25. Signature of Sponsor or Sponsor's Authorized Representative

(b) (6) [redacted] **Sign**

26. Signature of Countersigner **Sign**

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1 Vogel RI, Hertsgaard LA, Dermody SS, Luo X, Moua L, Allen S, al'Absi M, Hatsukami DK. Sex differences in response to reduced nicotine content cigarettes. *Addict Behav*. 2014; 39(7):1197-204. 24

2 Dermody SS, Donny EC, Hertsgaard LA, Hatsukami DK. Greater reductions in nicotine exposure while smoking very low nicotine content cigarettes predict smoking cessation. *Tob Control.* Published online September 5, 2014, doi:10.1136/tobaccocontrol-2014-051797. 32



9530 Main Street
Clarence, NY 14031
(716) 270-1523

2014 IND ANNUAL REPORT

IND No.: 103,589

Serial No.: 0006

Product Name: X-22 Smoking Cessation Product

Indication: Smoking Cessation

Reporting Period: 02 September 2013 to 01 September 2014

Date of Report: 27 Sep 2014

CONFIDENTIAL

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1 INDIVIDUAL STUDY INFORMATION

There were no clinical studies of X-22 Smoking Cessation Product (X-22) active during the reporting period.

1.1 Studies in Progress

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were being conducted during the past year.

1.2 Completed Studies

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were completed during the past year.

One clinical study was completed during an earlier reporting period, Study X-22-201 (Table 1). The clinical study report is in preparation and will be submitted to the FDA when available.

Table 1 IND Studies

Protocol Number	Title	No. Planned/ Entered	Demographics			No. Completed/ Discontinued	Study Status
			Subject Age Range (years)	Gender	Race		
X-22-201	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	216/234	20-65	128 M 106 F	169 W 59 B 3 A 3 O	175/59	Completed

Abbreviations: A=Asian, B=Black, F=female, M=male, O=other, W=white
Source: X-22-201 draft clinical study report Table 14.1.1.2

1.3 Foreign or other Clinical Trials Conducted by 22nd Century Limited, LLC Not Conducted Under IND 103,589.

There are no clinical trials of X-22 Smoking Cessation Product being conducted outside of the United States.

1.4 Non-22nd Century Limited, LLC Sponsored Studies

There are no clinical trials of the X-22 Smoking Cessation Product being conducted by sponsors other than 22nd Century Limited, LLC.

1.5 Non-22nd Century Limited, LLC Sponsored Studies with Other Very Low Nicotine Cigarettes

A number of independent clinical studies have been carried out recently with other Very Low Nicotine (VLN) cigarettes. These include studies with Quest®, Xodus®, and Spectrum® cigarettes.

1.5.1 Studies of Smoking Cessation

1.5.1.1 An Exploratory Secondary Analysis of Sex Differences in Response to VLN Cigarettes

An exploratory secondary analysis of sex differences in responses to VLN cigarettes and NRT in a previously reported smoking cessation trial carried out by the University of Minnesota was published.[1] A total of 235 subjects, 136 (58%) of whom were female, were randomized to three treatment groups: a) VLN cigarettes; b) 21mg nicotine patch, and 3) VLN cigarettes plus 21mg nicotine patch. The treatment period was 6 weeks. Continuous abstinence, defined as self-report of no smoking of usual brand cigarettes during the treatment period and no tobacco use after the treatment period, verified by exhaled CO <6 ppm and urinary total cotinine <35 ng/mL, was assessed at weeks 12, 24, and 36 (6, 18 and 30 weeks after start of treatment).

Continuous abstinence at each time point differed statistically significantly by sex, as show in the table below. For males, continuous abstinence was highest in the nicotine patch group (20.6%), and lower in the VLN cigarette plus nicotine patch (6.1%) and VLN cigarette only (3.1%) groups. For females, continuous abstinence was highest in the VLN cigarette group (21.3%), followed by the VLN cigarette plus nicotine patch group (14.0%) and nicotine patch (8.7%) groups.

Continuous abstinence rates by sex.

CO and cotinine verified continuous abstinence													
Week	Males						Females						p-value *
	VLNC (n = 32)		Nicotine patch (n = 34)		Combination (n = 33)		VLNC (n = 47)		Nicotine patch (n = 46)		Combination (n = 43)		
	# abstinent	%	# abstinent	%	# abstinent	%	# abstinent	%	# abstinent	%	# abstinent	%	
12	1	3.1	7	20.6	2	6.1	10	21.3	4	8.7	6	14.0	0.029
24	1	3.1	6	17.7	1	3.0	8	17.0	4	8.7	5	11.6	0.059
36	1	3.1	5	14.7	1	3.0	7	14.9	3	6.5	5	11.6	0.078

* P-value for treatment by sex interaction.

Table 2 from Vogel *et al*., *Addict Behav* 39(7):1197-204

1.5.1.2 A Secondary Analysis of the Relationship Between Nicotine Exposure While Smoking VLN Cigarettes and Abstinence

A secondary analysis of the relationship between nicotine exposure while smoking VLN cigarettes and abstinence in two previously reported cessation studies carried out by the University of Minnesota that included VLN cigarette only treatment arms was published.[2]

The analysis included individuals assigned to use VLN cigarettes only who used the products for at least 1 week (n=112, out of 132 initially randomized to VLN cigarettes only). Two different VLN cigarettes were used, Quest 3 (47% of subjects) and Xodus (53%) due to a lack of sufficient Quest 3 cigarettes to complete the later study. The two VLN cigarettes have similar nicotine concentrations (1.0 and 1.2 mg/g), but a larger difference in machine smoking nicotine yields (0.05 an 0.09 mg/cigarette) due to other differences in the cigarette designs (filter type and filler weight).

Abstinence at week 12 (6 weeks after the end of the 6 week treatment period) was defined as no VLN cigarettes or non-study cigarettes smoked during the past 7 days and exhaled carbon monoxide <6 ng/mL (i.e. 7-day point prevalence abstinence. Nicotine exposure was assessed at baseline, week 6 (end of treatment) and week 12 as total urinary cotinine. The retention rate was 74% for week 6 and 63% for week 12.

Lower urinary total cotinine level at week 6 (end of the VLN cigarette treatment) was associated with higher odds of abstinence at week 12 (OR=0.52, 95% CI 0.34 to 0.80, p=0.003). Reported use of even a small number of non-study cigarettes at week 6 was associated with higher cotinine and lower abstinence. Subjects who smoked the study cigarettes with nicotine yields of 0.09 mg/g were underrepresented in the lower cotinine levels at week 6, comprising 29% of those with cotinine scores less than 6.

Table 1 Key sample characteristics (n=83) at each observed week 6 cotinine level

	Natural log of week 6 cotinine									
	0	1	2	3	4	5	6	7	8	9
Sample size	2	0	1	2	16	21	24	6	5	6
Percentage in 2013 study	100		100	0	56.3	47.6	75.0	83.3	100	66.7
Percentage smoking 0.09 mg yield	0		100	0	25.0	33.3	70.8	66.7	60.0	66.7
Study CPD (week 6)	0		2.6	7.9	8.7	16.9	17.8	17.2	11.4	18.0
Non-study CPD (week 6)	0		0	0	0	0	0.2	0.7	4.2	6.7
Percentage non-compliant	0		100	0	12.5	14.3	33.3	50.0	80.0	66.7
Percentage abstinent (week 12)	100		100	100	81.3	57.1	47.1	16.7	20	16.7

Participants who completed the week 6 visit (n=83) were divided into 10 groups based on their natural log of week 6 cotinine levels (binned by rounding to the nearest whole number). Each column in the table provides descriptive statistics (means or proportions) of study outcomes for individuals who had the specified cotinine level (refer to sample size for n). The data provide qualitative support for factors that may explain the linear relation between cotinine level and quit rates. Of note, only two participants stopped smoking the very low nicotine content (VLNC) cigarettes before week 6 because they had quit smoking all cigarettes. Furthermore, the number of VLNC cigarettes appeared to differentiate individuals at cotinine level 4 from those at cotinine level 5, whereas non-compliance and a higher nicotine yield of the study cigarette (0.05 mg vs 0.09 mg) appeared to differentiate individuals at cotinine level 5 from those at cotinine level 6.
CPD, cigarettes per day.

Table 1 from Dermody *et al.*, *Tob Control*, published online September 5, 2014

Use of conventional cigarettes during the treatment period may have undermined the effects of VLN cigarettes on cessation; individuals who smoked conventional cigarettes had a 6.3% abstinence rate compared to 51.2% for those who reported using only VLN cigarettes. However, it may be that subjects less likely to quit for other reasons were less likely to be compliant with treatment.

1.5.1.3 A Study of the Effect of Use of Cigarettes with Progressively Reduced Nicotine Content on Nicotine Dependence in Smokers of Low Socioeconomic Status

A study examining the effect of smoking a series of cigarettes with progressively reduced nicotine content on nicotine dependence in subjects of lower socioeconomic status is being sponsored by the Milton S. Hershey Medical Center.[3] A total of approximately 400 subjects with fewer than 16 years of education will be randomized to one of two conditions: a) 4 weeks each of cigarettes with 8, 4, 1.7, 0.9 mg nicotine/cigarette and followed by 8 weeks of VLN cigarettes with 0.3 mg nicotine/cigarette (corresponding to ca. 0.5 mg/g), or b) research cigarettes with 11.4 mg nicotine/cigarette (ca. 16 mg/g).

The primary outcome measure is level of urinary nicotine metabolites. Other outcome measures include relapse to smoking conventional cigarettes and biomarkers of stress.

1.5.2 Other Clinical Studies Using VLN Cigarettes

A number or other clinical studies using VLN cigarettes have been carried out or are in progress using Spectrum® or Quest 3® cigarettes. The studies summarized below did not directly investigate the use of VLN cigarettes as a tool for smoking cessation.

22nd Century is providing the National Institute on Drug Abuse (NIDA) with SPECTRUM®
research cigarettes that have eight different levels of nicotine content in the tobacco filler (from
0.4 to 25 mg/g) for ongoing research on how different nicotine levels in cigarettes affect smoking behavior and the results of smoking. The two lowest nicotine levels, 0.4 mg/g and 1.3 mg/g, are similar to the nicotine concentrations in VLN cigarettes that have been used in clinical studies of VLN cigarettes and smoking cessation to date.

Although Quest 3® VLN cigarettes have not been sold by the manufacturer since 2008, results of a number of additional clinical studies using Quest 3 have been reported.

1.5.2.1 A Study of Modulation of Craving, Withdrawal and Cerebral Blood Flow by Nicotine and Non-nicotine Smoking Factors

A study led by Duke University Medical Center used VLN cigarettes (Quest 3) and nicotine patches to examined the effects of nicotine and non-nicotine smoking factors on craving, withdrawal and regional cerebral blood flow (CBF) using a within-subjects factorial design.[4] CBF scans were performed on thirty-five smokers on four separate occasions following a) 24 h of wearing either a 21 mg/day nicotine or a placebo patch combined with b) 24 h of smoking denicotinized cigarettes or remaining abstinent from smoking. A control group of nonsmokers were scanned once to provide a comparison group for validating assessment of global CBF.

Both nicotine patches and smoking VLN cigarettes decreased craving, negative affect and withdrawal compared to the placebo patch/abstinent condition, with VLN cigarettes having

a more robust effect. Average scores for craving, negative affect and withdrawal were lower, but not significantly different from either treatment alone, in the VLN cigarette plus nicotine patch condition.

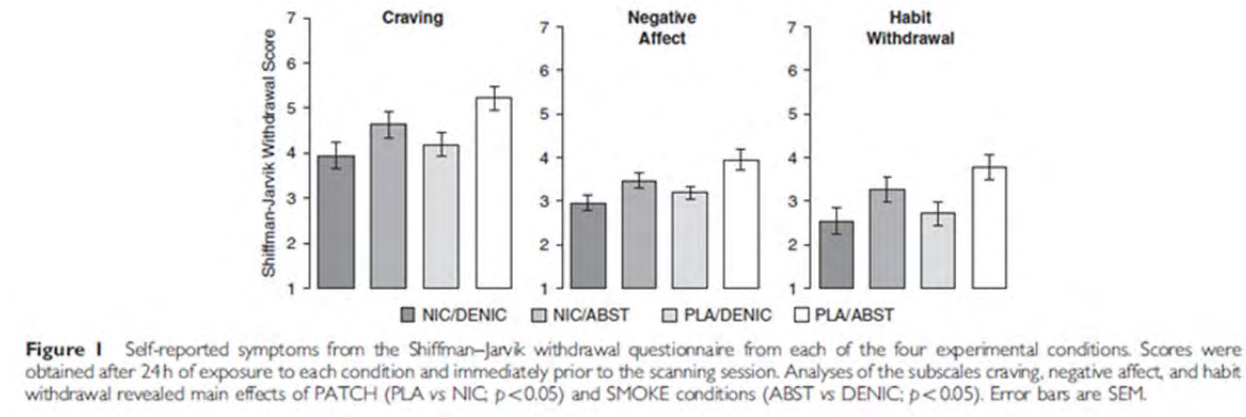


Figure 1 Self-reported symptoms from the Shiffman–Jarvik withdrawal questionnaire from each of the four experimental conditions. Scores were obtained after 24 h of exposure to each condition and immediately prior to the scanning session. Analyses of the subscales craving, negative affect, and habit withdrawal revealed main effects of PATCH (PLA *vs* NIC; $p<0.05$) and SMOKE conditions (ABST *vs* DENIC; $p<0.05$). Error bars are SEM.

Figure 1 from Addicott *et al*., *Neuropsychopharmacology*, published online May 13, 2014

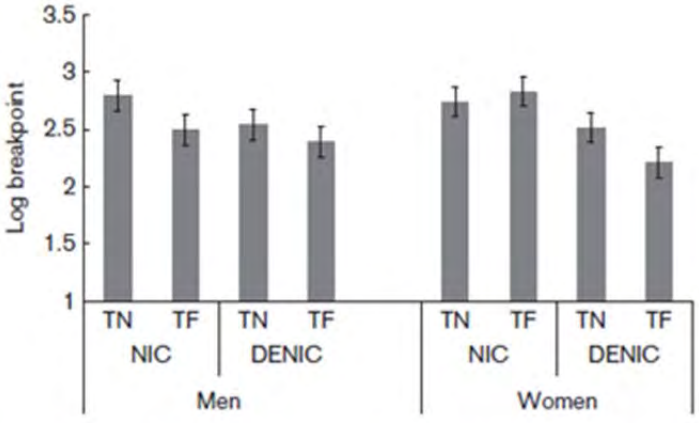
There was little effect of the treatment conditions on regional CBF (rCBF), with a single cluster of 176 voxels in the left temporal lobe showing greater rCBF in the placebo patch conditons than in the nicotine patch conditions. However, there were correlations between differences in withdrawal scores in the abstinent and VLN cigarette conditions and magnitudes of differences in rCBF throughout the frontal, parietal, and temporal cortices, extending into the occipital cortex and cerebellum. Reports of negative affect were positively correlated with rCBF in the right temporal lobe and left cerebellum. Habit withdrawal was negatively correlated with rCBF in the cerebellum/brain stem. The investigators suggest that smoking VLN cigarettes results in reduced activity in a fronto-temporo-cerebellar circuit that underlies the sensation of craving, and that non-nicotine non-nicotine psychoactive constituents of smoke and sensorimotor factors of smoking contribute to craving reduction.

1.5.2.2 A Study of the Effect of Nicotine Content Information on Subjective and Behavioral Responses to VLN and Conventional Cigarettes

Researchers at Dalhousie University examined the effects of nicotine content information on subjective and behavioral responses to Quest 3 VLN cigarettes (1 mg/g nicotine) or Quest 1 cigarettes (15 mg/g nicotine) using a balanced placebo design in which subjects were assigned to one of the two cigarette types, and participated in two laboratory sessions in which they received the same cigarette type, but in one they were told that they received nicotine-containing cigarettes and in the other nicotine-free cigarettes. Subjects included 30 nicotine-dependent (Fagerström Test for Nicotine Dependence (FTND) scores >3) and 30 nondependent smokers (FTND scores 0-2) with no intention of quitting in the next six months.

In each session subjects completed subjective assessments of cigarette craving and cigarette effects, smoked three puffs of the assigned cigarette, and repeated the subjective assessments. They were then allowed to earn puffs of the same cigarette type in a 90-minute computerized progressive ratio task.

Subjects worked harder to earn puffs and administered more puffs when they were told the cigarettes contained nicotine than when they were told the cigarettes were nicotine free (mean puffs administered 15.5, SE=0.8 vs. 14.5, SE=1.0). However, in males the effect of the instructions was only significant in the Quest 1 (NIC) condition, while in women the effect of instructions was only significant in the VLN Quest 3 condition (DENIC).



Performance on the progressive ratio task. Participants worked harder to earn puffs when told the cigarettes contained nicotine (TN) than when told the cigarettes were nicotine-free (TF). A significant Instructions by Pharmacology by Sex interaction on log breakpoint ($P<0.05$) revealed that in men, this increased self-administration when told the cigarettes contained nicotine was only significant in the nicotine condition (NIC; $P<0.05$), whereas in women it was only evident in the denicotinized condition (DENIC; $P<0.05$).

Figure 1 from Darredeau *et al*., *Behav Pharmacol* 24(4):291-7

Nicotine dependent smokers gave higher post-sampling ratings of pleasantness to cigarettes that matched the description, i.e. those told the cigarettes contained nicotine and given Quest 1 rated them higher after sampling, and those told the cigarettes were nicotine-free and given VLN Quest 3 cigarettes rated them higher after sampling.

Fifty of the subjects competed a subjects completed a questionnaire following their second session that included question about which type of cigarette they received in the first session, and the strength of the cigarette in the second section compared to the first. All of the subjects reported receiving different cigarettes in the two sessions, and 78% reported that the cigarettes were stronger in the session in which they were told the cigarettes contained nicotine.

Given the division of the subjects into dependent and non-dependent smokers, two types of cigarettes, and apparent differences by gender, the number of subject is low, and the

exposure to the VLN cigarettes is limited. Nevertheless, it adds to the literature that indicates that gender and expectancies may play roles in responses to VLN cigarettes.

1.5.2.3 A Study of Smoking Behavior and Biomarkers of Exposure During Smoking of Cigarettes of Progressively Lower Nicotine Content by Smokers Not Intending to Quit

Researchers at the University of Waterloo and Roswell Park Cancer Institute monitored smoking behavior, nicotine dependence, and biomarkers of smoke exposure in 101 Canadian smokers with no intention to quit during weeks of smoking cigarettes of progressively lower nicotine content. During week 1 they were provided with usual brand cigarettes free of charge. During weeks 2-4 they were provided with Quest 1 (15 mg nicotine/cigarette, yield 0.6 mg/cigarette), Quest 2 (8 mg nicotine/cigarette, yield 0.3 mg/cigarette), and Quest 3 cigarettes (0.6 mg nicotine/cigarette, yield 0.05). Seventy two subjects (71%) completed the smoking protocol and had complete biomarker data.

There was no significant difference in the number of cigarettes consumed per day as reported by diaries of smoking on the first and last day of each study period. The proportion of subjects reporting non-study cigarette use increased during the weeks of smoking Quest cigarettes; week 2/Quest 1, 27.8%, week 3/Quest 2, 30.6%, week 4/Quest 3, 44.4%. The number of non-study cigarettes smoked by those who used non-study cigarettes also increased from 2.5 $\pm$ 2.8 to 2.9 $\pm$ 4.2) to 4.1 $\pm$ 3.9 (mean + SD).

Puffing behavior during smoking of a single cigarette of the type used the previous week was measured using a CReSSmicro device with a removable mouthpiece connected to the device with tubing during weekly visits. Puff count and total puff volume were lower for the Quest cigarettes compared to usual brand.

Table 2. Smoking behavior (n = 72)

	Own brand (N = 72)	Quest 1 (N = 71)	Quest 2 (N = 71)	Quest 3 (N = 72)
Puff number				
Mean	16.1	14.5	13.8	13.2
SD	(6.3)	(4.6)	(4.3)	(4.5)
Puff volume (mL)				
Mean	63.5	64.3	64.2	69.3
SD	(22.4)	(22.3)	(17.2)	(18.8)
Total puff volume per cigarette (mL)				
Mean	977.9	910.1	834.2	890.7
SD	(420.8)	(520.3)	(190.4)	(353.6)
CPD				
Mean	20.0	19.9	21.8	20.3
(SD)	(8.9)	(8.4)	(9.4)	(10.2)

Table 2 from Hammond and O'Connor, *Cancer Epidemiol Biomarkers Prev*, published online August 22, 2014

No significant difference was observed in exhaled CO, or in urine 1-hydroxypyrene, a marker for polycyclic aromatic hydrocarbons, across cigarette types. Creatinine adjusted urine cotinine levels were 12.1 ± 7.1 µg/mL after smoking usual brand, and 11.9 ± 7.4 , 7.9 ± 6.0) and 5.4 ± 5.6 µg/mL after smoking Quest 1, Quest 2 and Quest 3, with the values after smoking Quest 2 and Quest significantly different.

The investigators conclude that substantial reductions in nicotine content can result in reduction of nicotine exposure without significant increases in cigarette consumption or intake of other smoke components. However, as the authors point out, the Quest cigarettes have American blend type fillers, while most Canadian smokers use cigarettes containing only flue-cured Virginia type tobacco. Differences in the taste of usual brand and Quest cigarettes may have influenced consumption of study cigarettes and compliance and retention.

1.5.2.4 A Comparison of the Effects of Smoking VLN and Conventional Cigarettes on Enhancement of Reward Value

A study carried out by researchers at the University of Pittsburgh compared the reinforcement enhancing effects of smoking Quest 1 and Quest 3 cigarettes for music, video and monetary awards.[7] Twenty smokers each participated in three experimental conditions, continued abstinence, smoking Quest 1, and smoking Quest 3, in separate sessions following overnight abstinence from smoking (confirmed by exhaled CO). In each session the subject completed four 15 minute trials of a computer-based progressive responding task with different reward conditions in each trial (30 sec of preferred music, 30 sec of preferred video, counter on monitor incrementing by $0.10, or no reward). Prior to each of the trials during the smoking condition session, participants self-administered six puffs of the assigned cigarette, one puff every 30 sec guided by computer-presented instructions via a portable Clinical Research Support System which recorded puff volume, timing and duration.

Responding for the music reward increased in the Quest 1 smoking condition, while responding in the Quest 3 smoking condition was not significantly different from the continued abstinence condition. Similar results were obtained for the music reward. Responding for the monetary reward was highest in the Quest 1 smoking condition, but the differences between the two cigarette conditions or was not statistically significant. The investigators conclude that the reinforcement-enhancing effects of smoking are due to nicotine intake, not general responses to smoking behavior, and the strength of the effect may be higher for sensory rewards than some other reward types. The reinforcement-enhancing effects of nicotine do not appear to be due to relief of withdrawal, as the both smoking conditions resulted in similar declines in withdrawal scores.

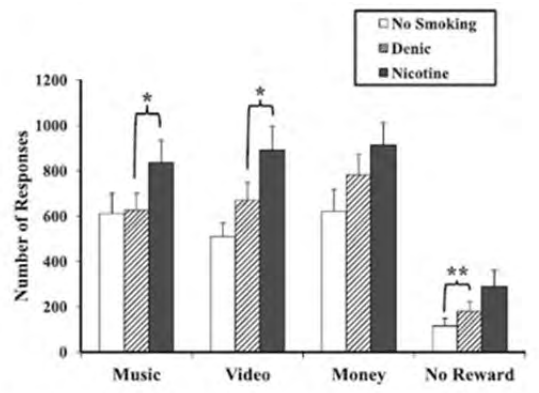


Figure 1. Mean ± *SE* number of responses for reinforcers, by reward type and smoking condition (N = 20). A priori paired comparisons between the nicotine and denic cigarette isolated effects of nicotine per se, whereas comparisons between the denic cigarette and no smoking isolated effects of smoking behavior per se. Music and video rewards, but not money or no reward, were significantly increased by nicotine intake. No reward, but not other rewards, was increased by smoking behavior. * $p <$ .05, ** $p <$ .01.

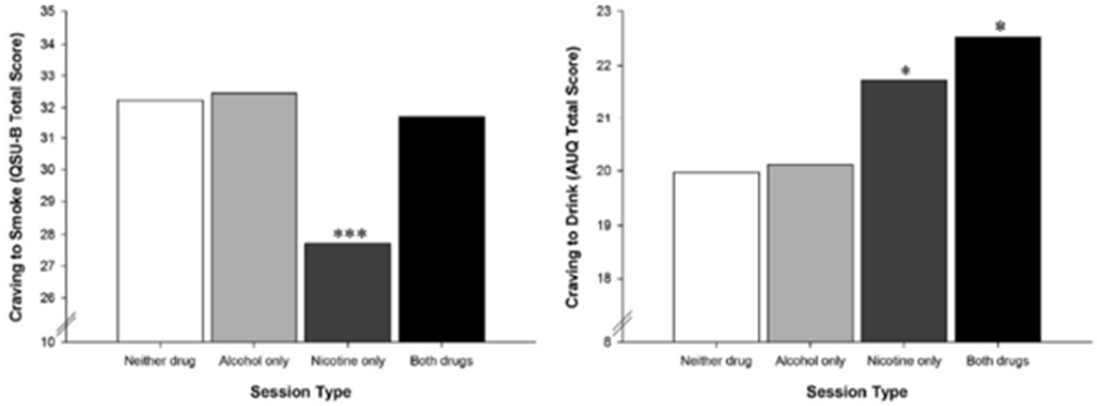
Figure 1 from Perkins and Karelitz, *Exp Clin Psychopharmacol*, published online September 1, 2014

1.5.2.5 A Study of the Interaction of Nicotine and Alcohol on Craving, Liking and Mood

Oliver *et al*. investigated the separate and combined effects of nicotine and a low dose of alcohol on substance use motivation using VLN cigarettes.[8] Eight seven participants with a range of smoking and drinking patterns completed four experimental sessions during which they consumed an low-dose alcohol or placebo beverage and smoked a single Quest 1 cigarette or a Quest 3 VLN cigarette.

Overall, craving to smoke was significantly reduced in the placebo beverage- Quest 1 cigarette condition compared to the placebo beverage-VLN cigarette condition. Craving to smoke in the alcohol beverage- VLN cigarette condition did not differ from the placebo beverage-VLN cigarette condition. However, alcohol blocked the reduction in craving seen with the higher nicotine cigarette when both were administered. Higher nicotine (Quest 1) increased craving to drink with both the placebo beverage and alcohol beverage.

Interactive effects of alcohol and nicotine on cravings may impact smoking cessation attempts.



From Figure 1, Oliver et al., *J Abnorm Psychol* 122(4):1154-65

1.5.2.6 A Study of the Effects of Alcohol on Responses to Cigarettes of Different Nictine Content in Daily and Non-daily smokers

Barrett *et al*. also investigated interactions of alcohol and nicotine in 40 smokers using Quest 1 and Quest 3 cigarettes.[9] Seventeen tobacco-dependent daily smokers and 23 nondependent nondaily smokers participated in four separate sessions in different beverage-cigarette combinations were administered (alcohol or placebo beverage) x (Quest 1 or Quest 2 cigarette). In each session subjects completed baseline assessments (T1), consumed their assigned beverage and completed a second set of assessments (T2), and then smoked a single assigned cigarette and completed a third set of assessments (T3). They were then allowed to earn additional puffs of the same cigarette type in a computerized progressive ratio (PR) task over 60 minutes.

In nondependent smokers, cigarette craving scores were low at baseline, as expected. Craving scores increased after consuming alcohol, but were not significantly affected by smoking. In dependent smokers, cigarette craving was higher at baseline and did differ not significantly after beverage consumption, but declined significantly after smoking in all conditions except the alcohol beverage-Quest 1 condition, indicating that alcohol in combination with nicotine blocked craving relief.

Gender differences in the reponses were also observed. Both men and women showed decreased after smoking in the placebo beverage + Quest 1 cigarette condition ($ps < 0.003$), only women showed significantly reduced craving at T3 in the VLN cigarette conditions ($ps < 0.001$).

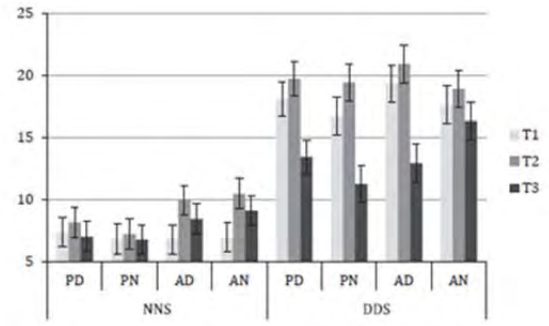


Fig. 2. Factor 2 craving ratings in nondependent nondaily smokers (NNS) and dependent daily smokers (DDS) in the placebo beverage–denicotinized tobacco (PD), placebo beverage–nicotine tobacco (PN), alcohol–denicotinized tobacco (AD), alcohol–nicotine tobacco (AN) conditions at baseline (T1), post-beverage (T2), and post-experimental cigarette (T3). The values reported are the unadjusted estimated marginal means (±SE) from the fitted model. In DDS, there were decreases in craving at T3 in the PD, PN, and AD conditions (p-values < 0.003) but not the AN, while in NNS, post-cigarette ratings did not vary across conditions.

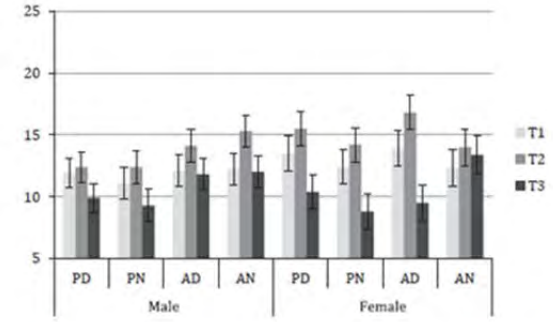


Fig. 3. Factor 2 craving ratings in males and females in the placebo beverage–denicotinized tobacco (PD), placebo beverage–nicotine tobacco (PN), alcohol–denicotinized tobacco(AD), alcohol–nicotine tobacco (AN) conditions at baseline (T1), post-beverage (T2), and post-experimental cigarette (T3). The values reported are the unadjusted estimated marginal means (±SE) from the fitted model. Both men and women experienced relatively decreased craving at T3 in the PN condition, but only women experienced significantly reduced craving at T3 in both of the PD and AD conditions (p-values < 0.003). There was also a tendency for decreased craving at T3 in men (p = 0.004) but not women in the AN condition.

Figures 2 and 3 from Barrett *et al*., Alcohol Clin Exp Res 37(8):1402-9

1.5.2.7 A Study of the Effects of Abstinence on Craving, Withdrawal, and Preference for Cigarettes of Moderate or Very Low Nicotine Content in Smokers With and Without Schizophrenia

A total of 55 smokers with schizophrenia (SS, n=28) or without (CS, n=27) smoked Quest 1 and Quest 3 cigarettes in a nicotine preference test to assess reinforcing effects of nicotine in laboratory session before and after a 72-hour abstinence period in a study reported by Tidey *et al*.[10] In each of the laboratory sessions subjects were allowed to sample four puffs from
"Cigarette A" using a CReSS smoking topography device and completed subjective assessments. After a 20 min rest, the sampling procedure was repeated with "Cigarette B." After another 20 min break, subjects completed 4 choice trials, 20 min apart, in which they invited to take four puffs from either an A or B cigarette.

The higher nicotine Quest 1 cigarettes were chosen more often in all conditions After the 72-hr abstinence period, the percentage of subjects choosing Quest 1 was significantly higher for smokers with schizophrenia (85.0 ± 7.7) than for control smokers (61.3 ± 8.6), but not before (SS:69.1 ± 8.3; CS: 77.7 ± 9.3). While the percentage rose for SS and declined for CS after abstinence, the differences were not statistically significant. Across all subjects Quest 1 puffs were given higher ratings. Average puff volume was somewhat larger for VLN cigarette puffs, 64.0 ± 15.1 vs. 57.7 ± 15.7 ml.

1.5.2.8 A Study of Cognitive Effects of VLN Cigarettes, With and Without Nicotine Patches, in Smokers With and Without Schizophrenia

AhnAllen *et al*. compared performance in cognitive function tasks and cigarette craving, withdrawal symptoms in five conditions: usual brand cigarettes, VLN cigarettes (Quest 3) with nicotine (two 21 mg patches) or placebo patches, and nicotine or placebo patch only, in

smokers with schizophrenia (SS, n=29) or without (CS, n = 28).[11] Each experiment laboratory session included 5 hours during which participants were cued to smoke according to the rate of their usual-brand smoking from the a baseline evaluation, after which they completed measures of cigarette craving, nicotine withdrawal symptoms and cognitive performance, and an assessment of usual-brand smoking behavior.

The acute use of VLN cigarettes, compared to usual-brand cigarettes, negatively affected attention, inhibitory control, processing speed and response time variability in both SS and CS. The impairment was reversed by nicotine provided by nicotine patches.

1.5.2.9 A Study of the Effect of Variation in the α5 Nicotinic Acetylcholine Receptor Subunit Gene on Smoking Topography of Cigarettes of Moderate or Very Low Nicotine Content

Macqueen *et al*. examined the relationship between a single nucleotide polymorphism (SNP) in the α5 nicotinic acetylcholine receptor subunit gene associated with smoking and respiratory health phenotypes and smoking behavior while smoking Quest 1 or Quest 3 cigarettes.[12]

Eight three smokers were recruited to participate in two 2.5 hour experimental sessions in which four cigarettes of one of the two types were smoked through a mouthpiece that was connected to a smoking topography device at 40 min intervals. Subjects were fitted with an electroencephalogram cap as part of the primary study between smoking bouts 1 and 2 and were required to undergo tasks of attention and working-memory between smoking bouts 2 and 3 and bouts 3 and 4. Seventeen participants were excluded from the analysis due to either procedural errors in the smoking topography equipment or missing genotype data.

Participants who did not carry a minor allele at the rs16969968 locus produced significantly reduced puff volumes when smoking nicotine-containing cigarettes relative to placebo cigarettes (12.01%, $p < .001$). Total puff volume did not differ by nicotine content among carriers ($p > .05$).

2 SUMMARY INFORMATION

There were no active clinical trials during this reporting period.

3 NONCLINICAL STUDIES

3.1 Pharmacology

3.1.1 Ongoing Studies

There are currently no nonclinical pharmacology studies ongoing.

3.1.2 Completed Studies

There were no nonclinical pharmacology studies conducted with X-22 Smoking Cessation Product during this reporting period.

3.2 Pharmacokinetics/Absorption, Distribution, Metabolism, and Excretion

3.2.1 Ongoing Studies

There are currently no nonclinical pharmacokinetic/absorption, distribution, metabolism, and excretion (ADME) studies ongoing.

3.2.2 Completed Studies

There were no nonclinical pharmacokinetic/ADME studies conducted with X-22 Smoking Cessation Product during this reporting period.

3.3 Toxicology

3.3.1 Ongoing Studies

There are currently no toxicology studies ongoing.

3.3.2 Completed Studies

There were no toxicology studies conducted with X-22 Smoking Cessation Product during this reporting period.

4 CHEMISTRY, MANUFACTURING, AND CONTROLS

There is no new chemistry, manufacturing and controls information for this report.

5 GENERAL INVESTIGATIONAL PLAN

5.1 Broad Objectives for X-22 Smoking Cessation Product Development

The goal of the ongoing Phase II development program will be to evaluate the safety and efficacy profile of X-22 Smoking Cessation Product, the pharmacokinetics of nicotine in the X-22 cigarette, and to determine the optimum dosing regimen in order to design the pivotal Phase III clinical trials for the desired indication.

The ongoing study of the effects of Spectrum® research cigarettes containing tobacco blends with various nicotine concentrations[3] includes cigarettes with very low nicotine concentrations equivalent to those in cigarettes used in the 22nd Century Phase II trial (0.4 mg/g) and in the recently completed Queen Mary University of London trial[16] and University of Minnesota trial[1] (1.5 mg/g). Plans for further studies to evaluate the effects of nicotine concentration in the filler on nicotine intake, smoking behavior, and compliance will be finalized after the review of these studies.

Effects of nicotine concentration in the filler will be evaluated in Phase II studies. Single and multiple dose pharmacokinetics of nicotine in X-22 cigarette and relative bioavailability of nicotine from X-22 cigarettes compared to other sources of NRT will be finalized and the studies will be carried out prior to the commencement of Phase III studies.

Pase III studies will be conducted to further evaluate the safety and efficacy of X-22 cigarettes as an aid to smoking cessation.

5.2 Clinical Studies Planned for the Coming Year

22nd Century plans to initiate 2 clinical studies in the coming year. One will be a Phase II study of comparative effects of cigarettes with different filler nicotine content. The other will be effects of an open-label, randomized, crossover design study to evaluate the pharmacokinetics of nicotine obtained from X-22 Smoking Cessation Product in healthy smokers. Following completion of these studies, 22nd Century plans to start the first Phase III study of X-22 Smoking Cessation Product.

6 INVESTIGATOR BROCHURE

No changes were made to the Investigator's Brochure during this reporting period. The Investigator's brochure will be updated prior to initiation of the next clinical trial.

7 PHASE 1 PROTOCOL MODIFICATIONS

No amendments to a phase 1 study were made during this reporting period.

8 FOREIGN MARKETING DEVELOPMENTS

No foreign marketing applications for X-22 Smoking Cessation Product have been made or withdrawn nor has X-22 Smoking Cessation Product been suspended from marketing in any foreign country.

9 OUTSTANDING BUSINESS WITH RESPECT TO IND 103,589

There is no outstanding business related to IND 103,589.

10 REFERENCES

1. Vogel RI, Hertsgaard LA, Dermody SS, Luo X, Moua L, Allen S, al'Absi M, Hatsukami DK. Sex differences in response to reduced nicotine content cigarettes. *Addict Behav*. 2014; 39(7):1197-1204.
2. Dermody SS, Donny EC, Hertsgaard LA, Hatsukami DK. Greater reductions in nicotine exposure while smoking very low nicotine content cigarettes predict smoking cessation. *Tob Control*. Published online September 5, 2014, doi:10.1136/tobaccocontrol-2014-051797.
3. NCT01928719. Reduced Nicotine Content Cigarettes in Smokers of Lower Socioeconomic Status. http://www.clinicaltrials.gov/ct2/show/NCT01928719, accessed September 9, 2014.
4. Addicott MA, Froeliger B, Kozink RV, Van Wert DM, Westman EC, Rose JE, McClernon FJ. Nicotine and Non-Nicotine Smoking Factors Differentially Modulate Craving, Withdrawal and Cerebral Blood Flow as Measured with Arterial Spin Labeling. *Neuropsychopharmacology*. Published online May 13, 2014, doi:10.1038/npp.2014.108.
5. Darredeau C, Stewart SH, Barrett SP. The effects of nicotine content information on subjective and behavioural responses to nicotine-containing and denicotinized cigarettes. *Behav Pharmacol*. 2013; 4(4):291-7.
6. Hammond D, O'Connor RJ. Reduced Nicotine Cigarettes: Smoking Behavior and Biomarkers of Exposure among Smokers Not Intending to Quit. *Cancer Epidemiol Biomarkers Prev*. Published Online August 22, 2014, doi: 10.1158/1055-9965.EPI-13-0957.
7. Perkins KA, Karelitz JL. Sensory Reinforcement-Enhancing Effects of Nicotine via Smoking. *Exp Clin Psychopharmacol*. Published online September 1, 2014, doi: 10.1037/a0037823.
8. Oliver JA, Blank MD, Van Rensburg KJ, Macqueen DA, Brandon TH, Drobes DJ. Nicotine interactions with low-dose alcohol: Pharmacological influences on smoking and drinking motivation. *J Abnorm Psychol*. 2013; 122(4):1154-65.
9. Barrett SP, Campbell ML, Roach S, Stewart SH, Darredeau C. The Effects of Alcohol on Responses to Nicotine-Containing and Denicotinized Cigarettes in Dependent and Nondaily Smokers. *Alcohol Clin Exp Res*. 2013; 37(8):1402-9.
10. Tidey JW, Colby SM, Xavier EM. Effects of Smoking Abstinence on Cigarette Craving, Nicotine Withdrawal, and Nicotine Reinforcement in Smokers With and Without Schizophrenia. *Nicotine Tob Res*. 2014; 16(3):326-34.
11. AhnAllen CG, Bidwell LC, Tidey JW. Cognitive Effects of Very Low Nicotine Content Cigarettes, With and Without Nicotine Replacement, in Smokers with Schizophrenia and Controls. *Nicotine Tob Res*. Published online August 20, 2014, doi: 10.1093/ntr/ntu163.
12. Macqueen DA, Heckman BW, Blank MD, Janse Van Rensburg K, Park JY, Drobes DJ, Evans DE. "Variation in the α5 nicotinic acetylcholine receptor subunit gene predicts cigarette smoking intensity as a function of nicotine content. Pharmacogenomics J. 2013; 14(1):70-6.