ANTICANCER RESEARCH
International Journal of Cancer Research and Treatment
ISSN: 0250-7005

# Elimination of Cigarette Smoke-derived Acetaldehyde in Saliva by Slow-release L-Cysteine Lozenge Is a Potential New Method to Assist Smoking Cessation. A Randomised, Double-blind, Placebo-controlled Intervention

KARI SYRJÄNEN[1,2], JOHANNA SALMINEN[1], ULLA ARESVUO[1,3], PANU HENDOLIN[4], LEA PALOHEIMO[4], CARITA EKLUND[4], MIKKO SALASPURO[5] and OSMO SUOVANIEMI[1,4]

*Departments of [1]Clinical Research, and [4]Research and Development, Biohit Oyj, Helsinki, Finland;*
*[2]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, SP, Brazil;*
*[3]Helsinki Metropolitan University of Applied Sciences, Helsinki, Finland;*
*[5]Research Unit on Acetaldehyde and Cancer, University of Helsinki, Helsinki, Finland*

*Reprinted from*
ANTICANCER RESEARCH *36*: 2297-2306 (2016)

# ANTICANCER RESEARCH

International Journal of Cancer Research and Treatment

HighWire Press® STANFORD UNIVERSITY

**ISSN (print): 0250-7005**
**ISSN (online): 1791-7530**


## Editorial Board




**Editorial Office:** International Institute of Anticancer Research, 1st km Kapandritiou-Kalamou Rd., Kapandriti, P.O. Box 22, Attiki 19014, Greece. Tel / Fax: +30-22950-53389.

**U.S. Branch:** Anticancer Research USA, Inc., 111 Bay Avenue, Highlands, NJ 07732, USA.

E-mails: Editorial Office: journals@iiar-anticancer.org
Managing Editor: editor@iiar-anticancer.org

ANTICANCER RESEARCH supports: (a) the establishment and the activities of the INTERNATIONAL INSTITUTE OF ANTICANCER RESEARCH (IIAR; Kapandriti, Attiki, Greece); and (b) the organization of the International Conferences of Anticancer Research. The IIAR is a member of UICC. For more information about ANTICANCER RESEARCH, IIAR and the Conferences, please visit the IIAR website: www.iiar-anticancer.org

**Publication Data:** ANTICANCER RESEARCH (AR) is published monthly from January 2009. Each annual volume comprises 12 issues. Annual Author and Subject Indices are included in the last issue of each volume. ANTICANCER RESEARCH Vol. 24 (2004) and onwards appears online with Stanford University HighWire Press from April 2009.





**Annual Subscription Rates 2016 per volume:** Institutional subscription US$ 1,898.00 (online) or US$ 2,277.00 (print & online). Personal subscription US$ 897.00 (online) or US$ 1,277.00 (print & online). Prices include rapid delivery and insurance. The complete previous volumes of Anticancer Research (Vol. 1-35, 1981-2015) are available at 50% discount on the above rates.

**Subscription Orders:** Orders can be placed at agencies, bookstores, or directly with the Publisher. (e-mail: subscriptions@iiar-anticancer.org)

**Advertising:** All correspondence and rate requests should be addressed to the Editorial Office.

**Book Reviews:** Recently published books and journals should be sent to the Editorial Office. Reviews will be published within 2-4 months.

Articles in ANTICANCER RESEARCH are regularly indexed in all bibliographic services, including Current Contents (Life Sciences), Science Citation Index, Index Medicus, Biological Abstracts, PubMed, Chemical Abstracts, Excerpta Medica, University of Sheffield Biomedical Information Service, Current Clinical Cancer, AIDS Abstracts, Elsevier Bibliographic Database, EMBASE, Compendex, GEOBASE, EMBiology, Elsevier BIOBASE, FLUIDEX, World Textiles, Scopus, Progress in Palliative Care, Cambridge Scientific Abstracts, Cancergram (International Cancer Research Data Bank), MEDLINE, Reference Update - RIS Inc., PASCAL-CNRS, Inpharma-Reactions (Datastar, BRS), CABS, Immunology Abstracts, Telegen Abstracts, Genetics Abstracts, Nutrition Research Newsletter, Dairy Science Abstracts, Current Titles in Dentistry, Inpharma Weekly, BioBase, MedBase, CAB Abstracts/Global Health Databases, Investigational Drugs Database, VINITI Abstracts Journal, Leeds Medical Information, PubsHub, Sociedad Iberoamericana de Información Científíca (SIIC) Data Bases.





D.T.P. BY IIAR

PRINTED BY ENTYPO, ATHENS, GREECE. PRINTED ON ACID-FREE PAPER

# Elimination of Cigarette Smoke-derived Acetaldehyde in Saliva by Slow-release L-Cysteine Lozenge Is a Potential New Method to Assist Smoking Cessation. A Randomised, Double-blind, Placebo-controlled Intervention

KARI SYRJÄNEN[1,2], JOHANNA SALMINEN[1], ULLA ARESVUO[1,3], PANU HENDOLIN[4], LEA PALOHEIMO[4], CARITA EKLUND[4], MIKKO SALASPURO[5] and OSMO SUOVANIEMI[1,4]

*Departments of [1]Clinical Research, and [4]Research and Development, Biohit Oyj, Helsinki, Finland; [2]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, SP, Brazil; [3]Helsinki Metropolitan University of Applied Sciences, Helsinki, Finland; [5]Research Unit on Acetaldehyde and Cancer, University of Helsinki, Helsinki, Finland*

**Abstract.** *Background/Aim: Harmans are condensation products of acetaldehyde and biogenic amines in saliva. Like other monoamine oxidase inhibitors, harmans help maintain behavioral sensitization to nicotine and mediate the addictive potential of cigarette smoke-derived acetaldehyde. The aim of this study was to test the hypothesis that effective elimination of acetaldehyde in saliva by slow-release L-cysteine (Acetium™ lozenge; Biohit Oyj, Helsinki, Finland) blocks the formation of harmans and eliminates acetaldehyde-enhanced nicotine addiction in smokers. Study design: A double-blind, randomized, placebo-controlled trial comparing Acetium lozenges and placebo in smoking intervention was undertaken. Materials and Methods: A cohort of 423 cigarette smokers were randomly allocated to intervention (n=212) and placebo arms (n=211). Smoking-related data were recorded by questionnaires, together with nicotine dependence testing by Fagerström scale. The participants used a smoking diary to record the daily number of cigarettes, test lozenges and sensations of smoking. The data were analyzed separately for point prevalence of abstinence and prolonged abstinence endpoints. Results: Altogether, 110 study participants completed the trial per protocol, 234 had minor violations, and the rest (n=79) were lost to follow-up. During the 6-month trial, 65 participants quit smoking; 38 (17.9%) in the intervention arm and 27 (12.8%) in the placebo arm [odds ratio (OR)=1.48; 95% confidence intervals (CI)=0.87-2.54; p=0.143]. Success in the per protocol group was better (42.9% vs. 31.1%, respectively; OR=1.65, 95% CI=0.75-3.62; p=0.205) than in the modified intention-to-treat group: 13.5% vs. 7.4% (p=0.128). Conclusion: If the efficacy of Acetium lozenge can be confirmed in an adequately powered study, this new approach would represent a major breakthrough in smoking quit intervention because slow-release L-cysteine is non-toxic with no side-effects or limitations of use.*



*Correspondence to:* Professor Kari Syrjänen, MD, Ph.D., FIAC, Head, Department of Clinical Research, Biohit Oyj, Laippatie 1, FIN-00880 Helsinki, Finland. Tel: +358 405566810, Fax: +358 97732867, e-mail: kari.syrjanen@biohit.fi

*Key Words:* Smoking intervention, clinical trial, randomized, double-blind, placebo-controlled, L-cysteine, slow-release, lozenge, smoking quit.

The relative risk of lung cancer among cigarette smokers (1, 2) increases in a dose-dependent manner up to 30-40-fold among heavy smokers (2, 3), being amplified by exposure to other carcinogens, *e.g.* asbestos (4, 5) or oncogenic human papillomaviruses (HPV) (6). The risk of lung cancer remains increased for several years after smoking cessation (2-5), but gradually decreases to the level of non-smokers, making cessation meaningful even after long-term smoking (7, 8). Although smoking rates fell in many Western countries during the 1970s-1980s, this trend seems to be leveling off and instead is increasing in countries like China (9-11). It is estimated that 1.1 billion adults are smokers, making effective smoking cessation interventions essential to reduce the major public health impact (cancer, chronic obstructive pulmonary disease) of cigarette smoking (2, 5, 7, 11).

Smoking cessation can be achieved by two principally different approaches: i) with, and ii) without assistance from healthcare professionals (12, 13). Which of the available intervention methods is the most effective remains under debate (14, 15). Nicotine is the main psychoactive component of tobacco, and adolescents in particular seem to

be more sensitive to the rewarding effects of nicotine and develop nicotine addiction (16). This addiction develops when nicotine acts on nicotinic acetylcholine receptors in the central nervous system (CNS) to release neurotransmitters *e.g.* dopamine, glutamate and gamma-aminobutyric acid (16).

Dependence on smoking is much more complex than simply nicotine addiction (16). Recent experimental evidence implicates that acetaldehyde, a major carcinogenic (class I) compound of tobacco smoke (17, 18), enhances behavioral, endocrine and neuronal responses to nicotine in adolescent and adult rats (19-21). In these experiments, however, the acetaldehyde concentrations used far exceeded those reached in the saliva by humans while smoking (22, 23). Given that smoke-derived acetaldehyde is not absorbed into the circulation, its direct CNS interaction with nicotine described in these animal experiments can be excluded (19-21), suggesting that other indirect mechanisms must exist to explain the reinforcing effects of acetaldehyde in nicotine addiction.

In 2007, Talhout *et al.* (24) suggested that harman and salsolinol (two condensation products of acetaldehyde with biogenic amines), might be the mediators of smoking dependence-maintaining effects of acetaldehyde found in animal experiments (19-21). Both harman and salsolinol inhibit monoamine oxidase (MAO), and some other MAO inhibitors are known to increase nicotine self-administration and maintain behavioral sensitization to nicotine (24). Blood harman levels among smokers appear to be 2-10-times higher than in non-smokers, and since readily passing the blood–brain barrier, harmans are the prime culprits for the lower MAO activity observed in the brain of smokers (24). This led to the reasoning that acetaldehyde in cigarette smoke may increase the addictive potential of tobacco products *via* formation of acetaldehyde-biogenic amine adducts (harmans) *in vivo*.

This hypothesis has never been validated in human smokers until now. It is tempting to speculate, however, that elimination of acetaldehyde in the saliva during cigarette smoking using L-cysteine (25) might effectively i) block (or reduce) the formation of harmans, ii) reduce their high blood levels seen in smokers, and iii) by reducing MAO inhibition, minimize the reinforcing effects of acetaldehyde on smoking dependence. Indeed, a patented formulation based on slow-release L-cysteine lozenge is currently available (Acetium lozenge® 3 mg; Biohit Oyj, Helsinki, Finland), shown to be highly effective in eliminating cigarette smoke-derived acetaldehyde in the saliva by converting it to inactive 2-methylthiazolidine-4-carboxylic acid compound (23, 24).

The present study was a randomized, double-blind, placebo-controlled intervention trial designed to validate the concept that systematic use of Acetium lozenges concomitantly with cigarette smoking supports the decision to quit smoking.

## Materials and Methods

*Study design.* This randomized, double-blind, placebo-controlled trial was designed to evaluate the efficacy of Acetium lozenge intervention in cessation of cigarette smoking. Current cigarette smokers (personally motivated to quit) were invited by public invitations (using different media) to participate in the trial, and randomly allocated to two study arms (Acetium and placebo). At baseline, all participants were requested to fill in a structured questionnaire recording their detailed smoking history and other clinical data pertinent to this study. An essential research tool was a smoking diary recorded on a daily basis by each participant and submitted to the study monitor at the end of each month, for recording study compliance and smoking-related covariates. The study was approved by the Helsinki University Hospital Coordinating Ethical Committee (DNo: 288/13/03/00/13; November 5, 2013).

*Study participants.* Between December 2013 and April 2015, a cohort of 423 current cigarette smokers were enrolled. Participants eligible for the study were current smokers (adult women and men) who were motivated to quit smoking, with no limitations in smoking duration and daily cigarettes (pack-years, PY). However, the following individuals were considered non-eligible: i) those who smoked a type of tobacco other than cigarettes, ii) those who refused to sign written consent, iii) those who were not motivated to quit smoking, and iv) those who did not commit themselves to not using other interventions during the 1-year follow-up.

The enrolled participants were randomly allocated into two study arms receiving either Acetium lozenges (n=212) or placebo (n=211), in a double-blind setting, where both the examiners and the participants were blinded to the test substance. For randomization, a random number generator was used, with a block size of 4 and creating unique randomization codes for each subject (https://www.sealedenvelope.com/simple-randomiser/v1/lists). A printed list (CSV Excel) of codes was sealed in an envelope and stored in a safety box until opened at completion of the study in October 2015. Before enrolment, all participants signed a written consent and agreed to use of the lozenges (Acetium or placebo) concomitantly with every single smoked cigarette throughout the whole intervention period, without adopting any other intervention methods.

*Baseline data.* Having consented to participate, each study participant was requested to fill in (with assistance of the study monitor) a structured questionnaire recoding their medical and smoking history, including details of previous intervention measures used to attempt quitting smoking. This questionnaire also included a more objective estimation of the nicotine dependence, evaluated by using the modified Fagerström Test for Nicotine Dependence (FTND) at baseline (26).

*Follow-up records by smoking diary.* For accurate monitoring of the smoking practices during the trial, all study participants were given a smoking diary. The participants were asked to submit the diaries to the study monitor on monthly basis, so as to confirm the compliance of each individual with the study protocol, to record the date of eventual smoking quit events, violations in the protocol or censoring due to other reasons. Apart from the detailed records on the number of cigarettes smoked per day and the number of

lozenges consumed concomitantly, the participants were asked to subjectively assess how they felt about each cigarette and estimate the degree of smoking-related pleasure, using the scale 1-10. This monthly diary provided an overall estimation of each month of smoking, recording all these variables at the conclusion of the month, total number of cigarettes and lozenges used, as well as the summary of pleasure scale. Two additional questions of each month were: i) Did your smoking habits change? ii) Has the sensation of smoking changed or not? iii) What is the smoking-associated pleasure using the scale 1-10?

*Breath carbon monoxide (CO) monitoring*. CO being a significant component of cigarette smoke, breath CO monitoring can be used to detect recent cigarette use (27). In the present study, CO concentration (ppm) and percentage of carboxy-hemoglobin (CO-Hb) in the blood were monitored at each follow-up visit using MicroCO monitor (Care Fusion, San Diego, CA, USA).

*Application of the FTND*. Originally introduced in 1978, the latest modification of this test is based on six simple questions recording the key variables of the smoker's daily practices (26). This test has been validated in several studies and shown to be valuable in monitoring the psychological dependence on nicotine. The FTND was recorded on each occasion when the participants returned their monthly smoking diaries. For statistical treatment, the FTND scores (from 1-10) were used as categorical variables: 0-2: very low; 3-4: low; 5: moderate; 6-7: high; and 8-10: very high (26).

*Study compliance*. Inherent to all longitudinal study designs, loss to follow-up is an inevitable outcome for a proportion of participants. Consisting of participants who terminated the trial due to a wide variety of reasons (extreme cases only completed interview but never initiated the trial), this group with no recordable outcome measure is not compatible with full or even partial data analysis, and was treated as a separate compliance category named LFU (lost to follow-up). As usual for randomized controlled trials (RCTs), the two categories of compliance as the target of the analysis were: i) per protocol (PP), and i) modified intention to treat (mITT). The former include all participants (in both arms) who were compliant with the study protocol, with only minor violations in taking the test substances (lozenges), and in recording their data. The mITT category includes all those who were not fully compliant with the protocol, but who completed the follow-up of at least 6 months and for whom the study endpoints were available. Following the usual practice for RCTs, the results were analyzed separately for the PP and mITT groups.

*Primary study endpoints*. The two most common outcome measures in smoking intervention trials are: i) prolonged abstinence (PA) and ii) point prevalence of abstinence (PPA) (28). PA, a sustained or continuous abstinence, is typically defined as not smoking for a period of several months after a quit attempt. PPA is typically defined as not smoking on the day of concluding the follow-up. In the present study, the results were analyzed separately for these two primary study endpoints, using the 2-month cut-off for a positive record of PA (28).

*Statistical analysis*. All statistical analyses were performed using SPSS 22.0.0.2 for Windows (IBM, NY, USA) and STATA/SE 14.1 software (STATA Corp., TX, USA). The descriptive statistics were procured according to routine procedures. Frequency tables were analyzed using the Chi-square test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables. Differences in the means of continuous variables were analyzed using non-parametric (Mann–Whitney or Kruskal–Wallis) test for two and multiple independent samples, respectively. The risk estimates of PA and PPA in the two study arms were calculated using conventional univariate regression models, expressed as odds ratios (ORs), and their 95% confidence intervals (95% CI). The time to quit (TTQ) and duration of quit (quit time, equivalent to length of PA) in the two study arms (and compliance groups) were estimated using univariate survival (Kaplan–Meier) analysis, comparing the stratum-specific estimates using the log-rank (Mantel–Cox) statistics.

The data were arranged in the panel format, subjected to analyses using generalized linear models, *e.g.* generalized estimation equation or Poisson regression. In this study, the covariates of smoking quit were estimated using population-averaged Poisson regression model, where study subjects were clustered by their participant ID, follow-up visit (=monthly diary) as the time variable, and incident quit events (events/person days at risk) as the dependent count variable (29,30). The exchangeable within-group correlation structure for the Poisson model, with robust variance estimator (of 95% CI) to account for the within-participant correlation was the best-fit covariance pattern (29,30). The results for all covariates were expressed as the incidence rate ratio (IRR) statistics (with 95% CI). All covariates recorded at the baseline questionnaire (fixed variables) and all smoking-related variables from the smoking diaries (random variables) were first tested in univariate Poisson model. The final multivariate model was adjusted for the covariates that were significant in univariate analysis. All statistical tests were two-sided and declared significant at $p$-value of less than 0.05.

## Results

The participants in the two arms were practically identical in most of their smoking-related variables, indicating an effective randomization (Table I). Importantly, this applies to their mean age, compliance with the intervention (PP, mITT, LFU groups), alcohol drinking habits, age of smoking onset, daily smoking habits and regularity, previous attempts to quit, type of interventions used for assisting quit, as well as compliance with previous interventions and their efficacy (*e.g.* the longest time of smoking abstinence). Most importantly, the participants in the intervention and the placebo arm were practically identical as to their PY of smoking (10.9 and 10.1 PYs, respectively ($p$=0.464), as well as to their nicotine dependence measured by the FTND score ($p$=0.895). In addition, the length of the follow-up of the two study arms was similar: 161 and 166 days, respectively, and an equal number of study participants in both arms reported adverse effects experienced during the intervention ($p$=0.396). The two arms were different in two respects only: i) gender distribution (more males in the placebo arm, $p$=0.020), and ii) weekly consumed alcohol equivalents (higher in the intervention arm, $p$=0.046).

The outcomes of intervention are summarized in Table II, separately for the two study arms, compliance groups (PP,

Table I. *Key characteristics of the study participants in the intervention and placebo arms.*

| Variable | Intervention arm (n=212) | Placebo arm (n=211) | *p*-Value |
|---|---|---|---|
| Gender | | | |
| Women | 109 (51.4%) | 132 (62.6%) | 0.020 |
| Men | 103 (48.6%) | 79 (37.4%) | |
| Mean age (SD), years | 43.8 (11.3) | 44.6 (11.9) | 0.504 |
| Compliance | | | |
| PP | 49 (23.1%) | 61 (28.9%) | 0.220 |
| ITT | 126 (59.4%) | 108 (51.2% | |
| LFU | 37 (17.5%) | 42 (19.9%) | |
| Education | | | |
| Basic schooling only | 28 (13.2%) | 43 (20.5%) | 0.153 |
| Professional training | 75 (35.4%) | 64 (30.5%) | |
| Student examination (no further) | 18 (8.5%) | 26 (12.4%) | |
| High school/technical university | 72 (34.0%) | 62 (29.5%) | |
| Academic degree | 19 (9.0%) | 15 (7.1%) | |
| General health | | | |
| Healthy (no systemic disease) | 161 (75.9%) | 160 (75.8%) | 0.978 |
| Any systemic disease (regular med) | 51 (24.1%) | 51 (24.2%) | |
| Mental health | | | |
| No diagnosed ailment | 190 (89.6%) | 192 (91.0%) | 0.633 |
| Yes, on regular medication | 22 (10.4%) | 19 (9.0%) | |
| Alcohol intake | | | |
| Never | 20 (9.4%) | 22 (10.4%) | 0.814 |
| Social drinker | 183 (86.3%) | 178 (84.4%) | |
| Daily intake (moderate) | 5 (2.4%) | 8 (3.8%) | |
| Risk user (regular excessive) | 4 (1.9%) | 3 (1.4%) | |
| Mean weekly alcohol intake (SD)[1] | 5.9 (7.3) | 4.6 (5.3) | 0.046 |
| Mean age initiated smoking (SD), years | 16.9 (4.6) | 16.2 (3.4) | 0.079 |
| Regular smoker since start | | | |
| No | 182 (85.8%) | 168 (79.6%) | 0.122 |
| Yes | 30 (14.2%) | 43 (20.4%) | |
| If not regular | | | |
| Quit once (followed by relapse) | 42 (23.1%) | 38 (22.6%) | 0.766 |
| Quit twice (followed by relapse) | 32 (17.6%) | 35 (20.8%) | |
| Several attempts to quit (all failed) | 108 (59.3%) | 95 (56.5%) | |
| Smoking habits since initiation | | | |
| Daily no. of cigarettes remained stable | 65 (30.7%) | 53 (25.1%) | 0.246 |
| Daily no. of cigarettes increased | 126 (59.4% | 128 (60.7% | |
| Daily no. of cigarettes decreased | 21 (9.9%) | 30 (14.2%) | |
| Daily urgency to smoke | | | |
| No | 23 (10.8%) | 16 (7.6%) | 0.244 |
| Yes | 189 (89.2%) | 195 (92.4%) | |
| Wake up at night to smoke | | | |
| No | 200 (94.3%) | 198 (93.8%) | 0.827 |
| Yes | 12 (5.7%) | 13 (6.2%) | |
| Smoking at home (inside) | | | |
| No | 193 (91.0%) | 193 (91.5%) | 0.875 |
| Yes | 19 (9.0%) | 18 (8.5%) | |
| Smoking by household members | | | |
| Yes | 131 (61.8%) | 132 (62.5) | 0.541 |
| No | 81 (38.2%) | 79 (37.5%) | |
| Previous attempts to quit | | | |
| No | 9 (4.2%) | 17 (8.1%) | 0.110 |
| Yes | 203 (95.8%) | 194 (91.9%) | |
| Mean no. of previous quit attempts (SD) | 7.6 (9.6) | 7.8 (15.9) | 0.849 |
| Any intervention ever used for quit attempt | | | |
| No | 7 (3.3%) | 13 (6.3%) | 0.175 |
| Yes | 203 (96.7% | 193 (93.7%) | |

Table I. *continued*

Table I. *continued*

| Variable | Intervention arm (n=212) | Placebo arm (n=211) | *p*-Value |
|---|---|---|---|
| Intervention type offered | | | |
| Personal techniques | 107 (93.0%) | 114 (95.0%) | 0.526 |
| Group-based techniques | 8 (7.0%) | 6 (5.0%) | |
| Compliance with the intervention methods | | | |
| Poor | 11 (11.6%) | 11 (12.1%) | 0.910 |
| Moderate | 24 (25.3%) | 20 (22.0%) | |
| Good | 60 (63.2%) | 60 (65.9%) | |
| Longest ever period without smoking (months) | 16.8 (27.8) | 14.1 (30.8) | 0.364 |
| Mean PY of smoking (SD) | 10.9 (10.6) | 10.1 (9.7) | 0.464 |
| FTND at baseline | | | |
| 0-2 | 33 (15.6%) | 35 (16.6%) | 0.895 |
| 3-4 | 50 (23.6%) | 48 (22.7%) | |
| 5 | 33 (15.6%) | 40 (19.0%) | |
| 6-7 | 76 (35.8%) | 70 (33.2%) | |
| 8-10 | 20 (9.4%) | 18 (8.5%) | |
| Mean blood CO (ppm) at baseline (SD) | 24.9 (11.8) | 24.8 (11.3) | 0.965 |
| Mean blood COHb (%) at baseline (SD) | 4.0 (1.9) | 3.9 (1.8) | 0.968 |
| Mean follow-up (days) (SD)[2] | 160.9 (138.9) | 165.9 (126.9) | 0.729 |
| Adverse effects during intervention[3] | | | |
| No | 176 (88.4%) | 166 (85.6%) | 0.396 |
| Yes | 23 (11.6) | 28 (14.4%) | |

PP, Per protocol; mITT, modified intention to treat; LFU, lost to follow-up; PY: pack-years; FTND, Fagerström test for nicotine dependence; CO: carbon monoxide; COHb, carboxyhemoglobin. [1]Glass of wine equivalent; [2]calculated for PP and ITT groups only; [3]recorded in detail in the smoking diary.

mITT) and stratified by the study arm and compliance. The distribution of the six possible outcomes was similar in the two arms. Altogether, 5.1% more participants quit smoking in the intervention arm (17.9% *vs*. 12.8%) than in the placebo arm, whereas a higher proportion of participants reduced smoking or quit but relapsed in the placebo arm ($p$=0.120). When stratified by study compliance, significantly more individuals stopped smoking in the PP group than in the mITT group, 36.4% and 10.7%, respectively.

When analyzed separately, in the PP group, 11.8% more participants (42.9% *vs*. 31.1%) quit smoking in the intervention arm than in the placebo arm, but the overall outcome pattern was not significantly different ($p$=0.107). In the mITT group, this difference in the effect size was only 6.1% in favor of the intervention arm, 13.5% and 7.4%, respectively.

The primary study endpoints (PPA and PA) in the two study arms and stratified by study compliance are shown in Table III. PPA and PA were found to be closely correlated (R=0.816; $p$=0.0001; Spearman's rho). PPA in the intervention arm as compared to the placebo arm had an OR of 1.48 (95% CI=0.87-2.54), PA was less frequent in the intervention than placebo group (OR=0.82; 95% CI=0.42-1.41). The probability of quitting smoking was significantly ($p$=0.0001) higher in the PP than in the mITT group (OR=4.77), and the same is true for experiencing PA (OR=6.26). Of the two endpoints, PPA was a more consistent outcome measure than PA in both the PP and mITT groups, with OR=1.65 and OR=1.95, in favor of Acetium intervention over placebo.

To disclose the significant covariates of smoking quit, all smoking history-related variables recorded at baseline, as well as the study-level covariates (recorded by the smoking diaries), were analyzed using univariate and multivariate Poisson regression for panel data (Table IV). In this analysis, quit event was used as a count variable reported in the smoking diaries during the intervention. In the univariate model, six covariates proved to be significant predictors of the smoking quit event: i) age (older less likely to quit), ii) gender (male more likely to quit), iii) education (higher education, less likely to quit), iv) number of previous quit attempts (more attempts, more likely to quit), v) daily number of cigarettes smoked during intervention (higher number, less likely to quit), and vi) subjective sensations of smoking (changed sensations favor quit). When all these significant univariates were entered in the multivariate Poisson model, i) daily number of cigarettes during intervention, and ii) changed sensations of smoking remained the only significant independent predictors of the quit event, with IRR=0.89 (95% CI=0.82-0.97) and IRR=2.44 (95%CI=1.15-5.20), respectively.

Table II. *The study outcomes in the intervention and placebo arms and related to study compliance.*

| | Study outcome, n (%) | | | | | |
|---|---|---|---|---|---|---|
| | Quit smoking | Reduced smoking | Quit but relapsed | No objective effect | Moved to other method | Lost to follow-up |
| *Study arm | | | | | | |
| Intervention | 38 (17.9%) | 30 (14.2%) | 7 (3.3%) | 89 (42.0%) | 11 (5.2%) | 37 (17.5%) |
| Placebo | 27 (12.8%) | 39 (18.5%) | 15 (7.1%) | 73 (34.6%) | 15 (7.1%) | 42 (19.9%) |
| Total | 65 (15.4%) | 69 (16.3%) | 22 (5.2%) | 162 (38.3%) | 26 (6.1%) | 79 (18.7%) |
| | | | $p$=0.129 (Likelihood ratio statistics) | | | |
| Compliance | | | | | | |
| PP | 40 (36.4%) | 22 (20.0%) | 12 (10.9%) | 29 (26.4%) | 7 (6.4%) | NA |
| mITT | 25 (10.7%) | 47 (20.1%) | 10 (4.3%) | 133 (56.8%) | 19 (8.1%) | NA |
| Total | 65 (18.9%) | 69 (20.1%) | 22 (6.4%) | 162 (47.1%) | 26 (7.6%) | 344 |
| | | | $p$=0.0001 (Likelihood ratio statistics) | | | |
| Study arm by compliance | | | | | | |
| PP | | | | | | |
| Intervention | 21 (42.9%) | 6 (12.2%) | 4 (8.2%) | 17 (34.7%) | 1 (2.0%) | NA |
| Placebo | 19 (31.1%) | 16 (26.2%) | 8 (13.1%) | 12 (19.7%) | 6 (9.8%) | NA |
| Total | 40 (36.4%) | 22 (20.0%) | 12 (10.9%) | 29 (26.4%) | 7 (6.4%) | 110 |
| | | | $p$=0.056 (Fisher's exact test) | | | |
| mITT | | | | | | |
| Intervention | 17 (13.5%) | 24 (19.0%) | 3 (2.4%) | 72 (57.1%) | 10 (7.9%) | NA |
| Placebo | 8 (7.4%) | 23 (21.3%) | 7 (6.5%) | 61 (56.5%) | 9 (8.3%) | NA |
| Total | 25 (10.7%) | 47(20.1%) | 10 (4.3%) | 133 (56.8%) | 19 (8.1%) | 234 |
| | | | $p$=0.358 (Fisher's exact test) | | | |

PP, Per protocol; mITT, modified intention to treat; NA, not applicable. Those lost to follow-up were omitted from analysis. *All enrolled participants (n=423) included.

## Discussion

In a recent meta-analysis evaluating unassisted smoking intervention techniques, the quit rate from unaided methods was quite modest, only 7.3% after an average of 10 months of follow-up (31). These figures are substantially inferior to the efficacy of assisted intervention methods, with 25%-33% quit rates for over 6 months (12). In a recent comprehensive review and meta-analysis by Lemmens *et al.*, evidence of effectiveness was found for the following assisted strategies: group behavioral therapy (OR=2.17, 95% CI=1.37-3.45), bupropion (OR=2.06, 95% CI=1.77-2.40), intensive physician advice (OR=2.04, 95% Cl=1.71-2.43), nicotine replacement therapy (OR=1.77, 95% CI=1.66-1.88), individual counselling (OR=1.56, 95% CI=1.32-1.84), telephone counselling (OR=1.56, 95% CI=1.38-1.77), nursing interventions (OR=1.47, 95% CI=1.29-1.67) and tailored self-help interventions (OR=1.42, 95%CI=1.26-1.61) (14). Interestingly, comprehensive clean (smoke-free) indoor laws increased the quit rates by 12-38% (14).

This meta-analysis did not include the studies on varenicline tartrate (Pfizer), which is one of the most widespread intervention medications in the US (Chantix) and outside (Champix) (14). Two meta-analyses found varenicline to be more effective than nicotine replacement therapy or bupropion, varenicline (2 mg/day) giving the highest abstinence rate (33.2%) of any stand-alone therapy (32, 33). Superiority of varenicline over bupropion was also confirmed in a Cochrane review of 15 studies in 2011 (34). However, both these principal stand-alone medications have serious adverse effects or limitations for use. In double-blind studies, varenicline increased the risk of serious adverse cardiovascular events as compared with placebo (35). It may also cause neuropsychiatric side-effects, *e.g.* possible suicidal behavior, which should seriously limit its long-term use (35). Similarly, bupropion (Zyban, GSK), another FDA-approved medication in this indication is contraindicated *e.g.* in epilepsy and other seizures, anorexia/bulimia, in those taking antidepressant drugs (MAO inhibitors), and those with abrupt discontinuation of ethanol or sedatives (including benzodiazepines) (36). Needless to say, any new method with equal efficacy but devoid of these serious adverse effects would represent a major impact on the global public health burden due to smoking (2, 5, 7, 11).

The Acetium lozenge tested in the present RCT is based on a completely novel hypothesis, supported by animal experiments and convincing clinical results (18-25). In brief: i) acetaldehyde (class I carcinogen) (17) is the principal

Table III. *The primary study endpoints of point prevalence of abstinence (PPA) and prolonged abstinence (PA) in the intervention and placebo arms and related to study compliance.*

| | Primary study endpoints | | | | | |
|---|---|---|---|---|---|---|
| | PPA | | | PA* | | |
| | Yes, n (%) | No, n (%) | OR (95% CI), *p*-Value | Yes, n (%) | No, n (%) | OR (95% CI), *p*-Value |
| **Study arm | | | | | | |
| Intervention | 38 (17.9%) | 174 (82.1%) | 1.48 (0.87-2.54), *p*=0.143 | 29 (13.7%) | 183 (86.3%) | 0.82 (0.48-1.41), *p*=0.482 |
| Placebo | 27 (12.8%) | 184 (87.2%) | | 34 (16.1%) | 177 (83.9%) | |
| Compliance | | | | | | |
| PP | 40 (36.4%) | 70 (63.6%) | 4.77 (2.70-8.43), *p*=0.0001 | 42 (38.2%) | 68 (61.8%) | 6.26 (3.47-11.30), *p*=0.0001 |
| mITT | 25 (10.7%) | 209 (89.3%) | | 21 (9.0%) | 213 (91.0%) | |
| Study arm by compliance | | | | | | |
| PP | | | | | | |
| Intervention | 21 (42.9%) | 28 (57.1%) | 1.65 (0.75-3.62), *p*=0.205 | 20 (40.8%) | 29 (59.2%) | 1.22 (0.56-2.64), *p*=0.610 |
| Placebo | 19 (31.1%) | 42 (68.9%) | | 22 (36.1%) | 39 (63.9%) | |
| mITT | | | | | | |
| Intervention | 17 (13.5%) | 109 (86.5%) | 1.95 (0.80-4.71), *p*=0.128 | 9 (7.1%) | 117 (92.9%) | 0.61 (0.24-1.52), *p*=0.290 |
| Placebo | 8 (7.4%) | 100 (92.6%) | | 12 (11.1%) | 96 (88.9%) | |

*2-Month cut-off. **All enrolled participants (n=423) included.

carcinogenic substance in cigarette smoke (18), being present at a concentration half that of nicotine (37, 38); ii) a synergistic interaction seems to exists between nicotine and acetaldehyde in self-administration in rats (20); iii) acetaldehyde enhances behavioral, endocrine, and neuronal responses to nicotine in rats (19); iv) in concentrations reached in the saliva during cigarette smoking, acetaldehyde is not absorbed into circulation (22, 23), excluding the possibility of direct central interactions with nicotine; v) an indirect link mediating the central nicotine-reinforcing effects of salivary acetaldehyde might be provided by harmans - beta-carboline alkaloids exhibiting a wide range of biological, psychopharmacological and toxicological actions; vi) these beta-carbolines are known to be synthesized as condensation products of alcohol- or cigarette smoke-derived acetaldehyde and biogenic amines (*e.g.* tryptamine) (39, 40).

Among smokers, blood harman levels appear to be 2-10-times higher as compared to non-smokers. Both harman and salsolinol inhibit MAO. MAO inhibitors are known to increase nicotine self-administration and maintain behavioral sensitization to nicotine, and given that harmans readily pass the blood–brain barrier, these may be the agents contributing to the lower MAO activity observed in the brain of smokers (24). This led Talhout *et al*. to propose that acetaldehyde might increase the addictive potential of tobacco products *via* formation of these acetaldehyde-biogenic amine adducts (harmans) (24). The logical next step to validate this concept is to assess whether elimination of acetaldehyde in the saliva could be an effective blocker of these central effects of acetaldehyde in cigarette smokers.

Some 40 years ago, it was demonstrated that cysteine (a non-essential amino acid) is highly effective in eliminating acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (25). Subsequently, this simple principle was patented by Biohit Oyj (Helsinki, Finland) in their Acetium™ products (capsule and lozenge) designed for reduction of harm due to alcohol intake and smoking, by eliminating acetaldehyde in the stomach and saliva, respectively (22, 23, 41).

The present RCT was designed to validate the concept that elimination of acetaldehyde in the saliva during cigarette smoking by sucking L-cysteine-containing lozenges might reduce the acetaldehyde-associated nicotine addiction among smokers (24). In this double blind, placebo controlled intervention trial, a cohort of 423 volunteer current smokers were randomly allocated to intervention (Acetium) and placebo arms, n=212 and n=211, respectively. This randomization was highly effective, building up two study arms with individuals who were practically identical in all key smoking-related variables, including the total PY and the level of nicotine dependence assessed by the FTND at baseline (Table I).

Following the adopted practice in many smoking intervention studies, two primary study endpoints were used: PPA and PA (28). Both PPA and PA are typically tied to the follow-up time (that continues a variable length after a recorded quit date), but both can also be tied to the end of

Table IV. *Predictors of smoking quit* in panel Poisson regression[1] run in univariate model and as adjusted for all significant univariates.*

| Covariate | Quit smoking | | | | | |
|---|---|---|---|---|---|---|
| | Crude IRR | 95% CI | *p*-Value | Adjusted IRR** | 95% CI | *p*-Value |
| Age at study entry (cont.) | 0.97 | 0.95-0.99 | 0.008 | 0.99 | 0.95-1.2 | 0.683 |
| Intervention (placebo=ref) | 1.46 | 0.90-2.35 | 0.120 | | | |
| Compliance (PP=ref) | 0.70 | 0.43-1.14 | 0.160 | | | |
| Gender (women=ref) | 1.79 | 1.11-2.86 | 0.016 | 1.03 | 0.43-2.47 | 0.937 |
| Education (basic=ref) | 0.75 | 0.63-0.91 | 0.003 | 0.88 | 0.65-1.17 | 0.373 |
| General health (not=ref) | 1.01 | 0.57-1.81 | 0.947 | | | |
| Mental health (not=ref) | 1.13 | 0.45-2.82 | 0.791 | | | |
| Alcohol intake (social=ref) | 0.85 | 0.50-1.43 | 0.547 | | | |
| Alcohol weekly dose (cont.) | 1.01 | 0.98-1.04 | 0.322 | | | |
| Age initiated smoking (cont.) | 0.97 | 0.91-1.03 | 0.371 | | | |
| Regular smoker since start (no=ref) | 0.56 | 0.23-1.33 | 0.195 | | | |
| Attempts to quit (one=ref) | 0.95 | 0.85-1.11 | 0.543 | | | |
| Smoking habits since initiation (stable=ref) | 0.93 | 0.60-1.44 | 0.761 | | | |
| Daily urgency of smoking (no=ref) | 0.89 | 0.45-1.79 | 0.765 | | | |
| Nightly wake-up for smoking (no=ref) | 0.53 | 0.13-2.14 | 0.378 | | | |
| Smoking at home (inside) (no=ref) | 0.31 | 0.07-1.30 | 0.111 | | | |
| Smoking by household members (spouse=ref) | 0.85 | 0.65-1.12 | 0.267 | | | |
| Previous attempts to quit (no=ref) | 1.36 | 0.43-4.32 | 0.595 | | | |
| Number of previous quit attempts (cont.) | 1.03 | 1.02-1.05 | 0.0001 | 1.00 | 0.96-1.04 | 0.812 |
| Intervention ever used for quit attempt (no=ref) | 1.49 | 0.37-6.00 | 0.573 | | | |
| Type of intervention (personal=ref) | 0.45 | 0.07-2.95 | 0.408 | | | |
| Compliance with the intervention methods (poor=ref) | 1.13 | 0.58-2.19 | 0.702 | | | |
| Longest ever period without smoking (cont.) | 1.00 | 0.99-1.01 | 0.212 | | | |
| Pack years of smoking (cont.) | 0.99 | 0.97-1.01 | 0.552 | | | |
| FTND at FU visits (graded, 0-2 ref) | 0.82 | 0.65-1.07 | 0.143 | | | |
| CO blood level (ppm) at FU visits (cont.) | 0.98 | 0.95-1.02 | 0.440 | | | |
| COHb level (%) in blood at FU visits (cont.) | 0.91 | 0.73-1.14 | 0.441 | | | |
| Adverse effects during intervention (no=ref) | 0.56 | 0.22-1.43 | 0.231 | | | |
| Cigarettes per day during intervention (cont.) | 0.88 | 0.81-0.95 | 0.002 | 0.89 | 0.82-0.97 | 0.009 |
| Smoking habits changed during intervention (no=ref) | 0.48 | 0.22-1.04 | 0.064 | | | |
| Pleasure obtained from smoking (scale 1-10) (cont.) | 0.92 | 0.75-1.12 | 0.427 | | | |
| Sensations of smoking changed during intervention (no change=ref) | 3.22 | 1.45-7.16 | 0.004 | 2.44 | 1.15-5.20 | 0.020 |
| Level of pleasure (scale 1-10) obtained from smoking (cont.) | 0.92 | 0.75-1.12 | 0.427 | | | |

*Count outcome (quit event), as defined by the quit event reported in the smoking diaries during intervention; [1]Population average model, clustered by subject ID number, monthly diaries, follow-up visits (FU) as the time variable, exchangeable within-group correlation structure, 95% CI=calculated by robust estimation. IRR= incidence rate ratio; cont., continuous variable.**Adjusted for age and all other significant covariates of smoking quit in univariate model.

intervention, or time prior to assessment of results. PA is typically defined as not smoking for a period of several months after a quit attempt. Both PPA and PA have their supporters in the literature (28). Albeit closely correlated, these two outcomes give somewhat different estimates for quit rates, and a recent meta-analysis recommends using both endpoints in smoking intervention studies (28). This was confirmed in the present study, where PPA and PA were closely correlated (R=0.816; $p$=0.0001), but despite this, slightly different results were obtained, PPA being more powerful (Table III). This is because PA (2-month cut-off) was calculated both for those who showed permanent quit, and those who quitted but relapsed. Duration of PA (7.1 and 1.9 months), respectively, among these two groups was significantly different ($p$=0.0001), and the likelihood of having a PA of more than 2 months was higher among those who quit rather than those who relapsed: 83.1% and 36.4%, respectively (OR=8.6, 95% CI=2.9-25.4; $p$=0.0001). Otherwise, TTQ was shorter (3.7 months) in the intervention arm than that (4.5 months) in the placebo arm ($p$=0.214), but the duration of quit (PA) did not differ significantly between the two arms: 5.7 and 5.9 months, respectively ($p$=0.860).

As evident from Table II, the six outcomes had a similar distribution in the two study arms ($p$=0.129). Altogether, 38 individuals (17.9%) in the Acetium arm and 27 (12.8%) in the placebo arm reported smoking quit, *i.e.*, 5.1% difference

in favor of intervention. Study compliance was significantly related to quitting smoking ($p$=0.0001), being higher in the PP than the mITT group. Importantly, much of this difference in compliance seems to be attributable to the intervention itself, as shown in analysis of the PP group, where 42.9% reported smoking quit in the intervention arm as compared with 31.1% in the placebo arm (*i.e.* 11.8% difference). The difference in quit prevalence between the two study arms is less marked (6.1%) in the mITT group, but still in favor of the Acetium intervention. When applied to primary endpoints (PPA, PA), the likelihood of PPA for intervention had an OR=1.65 and OR=1.95, in the PP and mITT groups, respectively (not significant).

Finally, the significant covariates of smoking quit were estimated using Poisson regression in univariate and multivariate mode. Altogether, 65 quit events were reported during a total of 55.730 pdr (quit rate: 1.16/1000 pdr), being higher in the intervention arm (1.37/1000 pdr) than in the placebo arm (0.96/1000 pdr) (IRR=1.42; 95% CI=0.84-2.42; $p$=0.079). Six variables proved to be significant covariates of smoking quit in univariate analysis (Table IV). When all significant univariates were entered in the multivariate Poisson model, only two covariates remained significant: daily number of cigarettes smoked during intervention, and the changed sensation of smoking (Table IV).

The present RCT testing the efficacy of Acetium lozenge intervention in smoking cessation provided evidence substantiating the study hypothesis (24). Accordingly, elimination of cigarette smoke-derived acetaldehyde in the saliva with slow-release L-cysteine might block the central acetaldehyde–nicotine interactions mediated by potent MAO inhibitors, harmans, borne as condensation products of acetaldehyde and biogenic amines and maintaining smoking dependency (24, 42). Once smoke-derived acetaldehyde is eliminated in the saliva, harmans are not formed, their blood level is decreased and the central interactions with nicotine are inhibited. The direct evidence to demonstrate this can be obtained by measuring the blood (and urine) levels of harmans (39), with and without concomitant use of slow-release L-cysteine during the smoking session.

The efficacy of Acetium lozenge, with OR ranging from 1.48-1.95 compared to placebo (Table III) favorably competes with most of the commonly used smoking intervention methods, including medications (14, 32-36). One weakness of the present RCT is the insufficient statistical power to confirm the significance of these results. The power calculations were based on more conservative estimates on i) the effect size and ii) the effect size difference between the two arms. The original cohort size of 500 (n=250 per study arm) was calculated to be adequately powered to detect a true difference (in PPA or PA) of 10% between the two arms, within the PPA/PA prevalence range of 10%-20%. Within this range, the power was shown to be sensitive to any decrease in the effect size difference, but would allow less difference (7.5%) if the quit rate in the two arms was between 5% and 15%. Although this was almost the case in the crude comparison between the two arms (17.9% *vs*. 12.8%), and in the mITT group (13.5% *vs*. 7.4%), the success rates (42.9% *vs*. 31.1%) in the PP group far exceeded our assumptions. With the current effect size difference of 5.1% between the two study arms, an adequate statistical power would necessitate a minimum of 782 participants in both arms. In the PP group, however, current PPA rates would ensure adequate power with 262 participants in both study arms.

Another (potential) weakness of the present RCT is the imbalance in the gender distribution in the intervention arm (Table I). Albeit due to pure chance only, it can be argued that this might have an impact on the success rate in the intervention arm because male gender proved to be a significant ($p$=0.003) covariate of quit events (IRR>1.7; Table IV). Although this effect was confounded by the other covariates in the multivariate model, this potential bias is better handled by also randomizing the study arms by gender. Needless to say, the results of the present proof-of-concept RCT are encouraging enough to prompt a design of such an adequately powered study in the near future.

## Conflicts of Interest

KS, JS, UA, PH, LP and CE are employees of Biohit Oyj; MS is a member of the Board, and OS is the Chairman of the Board, Biohit Oyj.

## References

1 Doll R, Peto R, Wheatley K, Gray R and Sutherland I: Mortality in relation to smoking: 40 years' observations on male British doctors. Br Med J *309*: 901-911, 1994.

2 Peto R, Lopez AD, Boreham J, Thun M and Heath C Jr.: Mortality from tobacco in developed countries: indirect estimation from national vital statistics. Lancet *339*: 1268-1278, 1992.

3 Doll R, Peto R, Boreham J and Sutherland I: Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ *328*: 1519, 2004.

4 Thun MJ, Henley SJ and Calle EE: Tobacco use and cancer: an epidemiologic perspective for geneticists. Oncogene *21*: 7307-7325, 2002.

5 Thun MJ, Carter BD, Feskanich D, Freedman ND, Prentice R, Lopez AD, Hartge P and Gapstur SM: 50-year trends in smoking-related mortality in the United States. N Engl J Med *368*: 351-364, 2013.

6 Syrjänen KJ: Detection of human papillomavirus (HPV) in lung cancer: systematic review and meta-analysis. Anticancer Res *32*: 3235-3250, 2012.

7 Pirie K, Peto R, Reeves GK, Green J and Beral V: The 21st century hazards of smoking and benefits of stopping: a prospective study of one million women in the UK. Lancet *381*: 133-141, 2013.

8 Peto R, Darby S., Deo H, Silcocks P, Whitley E and Doll R: Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. BMJ *321*: 323-329, 2000.

9 Mackay JL: The fight against tobacco in developing countries. Tuber Lung Dis *75*: 8-24, 1994.

10 Samet JM: Tobacco smoking: the leading cause of preventable disease worldwide. Thorac Surg Clin *23*: 103-112, 2013.

11 Chen ZM, Peto R, Iona A, Guo Y, Chen YP, Bian Z, Yang L, Zhang WY, Lu F, Chen JS, Collins R and Li LM: Emerging Tobacco-Related Cancer Risks in China: A Nationwide, Prospective Study of 0.5 Million Adults. Cancer *21(Suppl 17)*: 3097-3106, 2015.

12 Guide to quitting smoking. American Cancer Society. http://www.cancer.org/docroot/ped/content/ped_10_13x_guide_for_quitting_smoking. Retrieved 15.9.2015.

13 Chapman S and MacKenzie R: The global research neglect of unassisted smoking cessation: causes and consequences. PLoS Medicine *7(2)*: e1000216, 2010.

14 Lemmens V, Oenema A, Klepp KI and Brug J: Effectiveness of smoking cessation interventions among adults: a systematic review of reviews. Eur J Cancer Prev *17*: 535-544, 2008.

15 Stead LF, Perera R, Bullen C, Mant D and Lancaster T: Nicotine replacement therapy for smoking cessation. Cochrane Database of Systematic Reviews Issue 1. Art. No.: CD000146, 2008.

16 Benowitz NL: Nicotine addiction. N Engl J Med *362*: 2295-2303, 2010.

17 Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L and Cogliano V: WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens-Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol *10(11)*: 1033-1034, 2009.

18 Haussman HJ: Use of hazard indices for a theoretical evaluation of cigarette smoke composition. Chem Res Toxicol *25*: 794-810, 2012.

19 Cao J, Belluzzi JD, Loughlin SE, Keyler DE, Pentel PR and Leslie FM: Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. Neuropsychopharmacol *32*: 2025-2035, 2007.

20 Belluzzi JD, Wang R and Leslie FM: Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacol *30*: 705-712, 2005.

21 Quertemont E, Tambour S and Tirelli E: The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. Prog Neurobiol *75*: 247-274, 2005.

22 Salaspuro V, Hietala J, Kaihovaara P, Pihlajarinne L, Marvola M and Salaspuro M: Removal of acetaldehyde from saliva by a slow-release buccal tablet of L-cysteine. Int J Cancer *97*: 361-364, 2002.

23 Salaspuro VJ, Hietala JM, Marvola ML and Salaspuro MP: Eliminating carcinogenic acetaldehyde by cysteine from saliva during smoking. Cancer Epidemiol Biomarkers Prev *15*: 146-149, 2006.

24 Talhout R, Opperhuizen A and van Amsterdam JG: Role of acetaldehyde in tobacco smoke addiction. Eur Neuropsychopharmacol *17*: 627-636, 2007.

25 Sprince H, Parker C, Smith CJ and Gonzales L: Protective action of ascorbic acid and sulfur compounds against acetaldehyde toxicity: implications in alcoholism and smoking. Agents Actions *5*: 164-173, 1975.

26 Heatherton TF, Kozlowskı LT, Frecker RC and Fagerström KO: The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict *86*: 1119-1127, 1991.

27 Jamrozik K, Vessey M, Fowler G, Nicholas W, Parker G, and van Vunakis H: trials of three different anti-smoking interventions in general practice. Brit Med J *288*: 1499-1503, 1984.

28 Hughes JR, Carpenter MJ and Naud S: Do point prevalence and prolonged abstinence measures produce similar results in smoking cessation studies? A systematic review. Nicotine Tobacco Res *12*: 756-762, 2010.

29 Diggle PJ, Liang K-Y and Zeger SL: Analysis of longitudinal data, 1st ed. Oxford: Oxford University Press, 1994.

30 Hardin J and Hilbe JM: Generalized estimating equations. (Eds). Boca Raton, FL: Chapman & Hall, 2003.

31 Baillie AJ, Mattick RP and Hall W: Quitting smoking: estimation by meta-analysis of the rate of unaided smoking cessation. Aust J Public Health *19*: 129-131, 1995.

32 Wu P, Wilson K, Dimoulas P and Mills EJ: Effectiveness of smoking cessation therapies: a. BMC Public Health *6*: 300, 2006.

33 Mills EJ, Wu P, Spurden D, Ebbert JO and Wilson K: Efficacy of pharmacotherapies for short-term smoking abstinence: a systematic review and meta-analysis. Harm Reduct J *6*: 25, 2009.

34 Cahill K, Stead LF, Lancaster T. *In*: Cahill, Kate (Ed). Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev *2(2)*: CD006103, 2011. Retrieved 25.10.2015.

35 Singh S, Loke Y, Spangler J and Furberg C: Risk of serious adverse cardiovascular events associated with varenicline: a systematic review and meta-analysis. Can Med Assoc J (CMAJ) *183*: 1359-1366, 2011.

36 Hughes JR, Stead LF and Lancaster T: Antidepressants for smoking cessation. Cochrane Database Syst Rev *(1)*: CD000031, 2007.

37 Hoffmann D and Hecht S: Advances in tobacco carcinogenesis. *In*: Cooper CS, Grover P (Eds). Handbook of experimental pharmacology. Heidelberg: Springer Verlag; pp. 63-102, 1990.

38 Hoffmann D and Hoffmann I: The changing cigarette, 1950-1995. J Toxicol Environ Health *50*: 307-364, 1997.

39 Airaksinen MM and Kari I: Beta-carbolines, psychoactive compounds in the mammalian body. Part I: Occurrence, origin and metabolism. Med Biol *59*: 21-34, 1981.

40 Herraiz T, Guillén H and Arán VJ: Oxidative metabolism of the bioactive and naturally occurring beta-carboline alkaloids, norharman and harman, by human cytochrome P450 enzymes. Chem Res Toxicol *21*: 2172-2180, 2008.

41 Linderborg K, Marvola T, Marvola M, Salaspuro M, Färkkilä M and Väkeväinen S: Reducing carcinogenic acetaldehyde exposure in the achlorhydric stomach with cysteine. Alcohol Clin Exp Res *35*: 516-522, 2011.

42 Rommelspacher, H., Meier-Henco, M., Smolka, M and Kloft C: The levels of norharman are high enough after smoking to affect monoamineoxidase B in platelets. Eur J Pharmacol *441*: 115-125, 2002.


*Received February 26, 2016*
*Revised April 7, 2016*
*Accepted April 11, 2016*

# Instructions for Authors 2016

*General Policy*. ANTICANCER RESEARCH (AR) will accept original high quality works and reviews on all aspects of experimental and clinical cancer research. The Editorial Policy suggests that priority will be given to papers advancing the understanding of cancer causation, and to papers applying the results of basic research to cancer diagnosis, prognosis, and therapy. AR will also accept the following for publication: (a) Abstracts and Proceedings of scientific meetings on cancer, following consideration and approval by the Editorial Board; (b) Announcements of meetings related to cancer research; (c) Short reviews (of approximately 120 words) and announcements of newly received books and journals related to cancer, and (d) Announcements of awards and prizes.

The principal aim of AR is to provide prompt publication (print and online) for original works of high quality, generally within 1-2 months from final acceptance. Manuscripts will be accepted on the understanding that they report original unpublished works in the field of cancer research that are not under consideration for publication by another journal, and that they will not be published again in the same form. All authors should sign a submission letter confirming the approval of their article contents. All material submitted to AR will be subject to review, when appropriate, by two members of the Editorial Board and by one suitable outside referee. The Editors reserve the right to improve manuscripts on grammar and style.

The Editors and Publishers of AR accept no responsibility for the contents and opinions expressed by the contributors. Authors should warrantee due diligence in the creation and issuance of their work.

*NIH Open Access Policy*. The journal acknowledges that authors of NIH-funded research retain the right to provide a copy of the published manuscript to the NIH four months after publication in ANTICANCER RESEARCH, for public archiving in PubMed Central.

*Copyright*. Once a manuscript has been published in ANTICANCER RESEARCH, which is a copyrighted publication, the legal ownership of all published parts of the paper has been transferred from the Author(s) to the journal. Material published in the journal may not be reproduced or published elsewhere without the written consent of the Managing Editor or Publisher.

*Format*. Two types of papers may be submitted: (i) Full papers containing completed original work, and (ii) review articles concerning fields of recognisable progress. Papers should contain all essential data in order to make the presentation clear. Reasonable economy should be exercised with respect to the number of tables and illustrations used. Papers should be written in clear, concise English. Spelling should follow that given in the “Shorter Oxford English Dictionary”.

*Manuscripts*. Submitted manuscripts should not exceed fourteen (14) pages (approximately 250 words per double - spaced typed page), including abstract, text, tables, figures, and references (corresponding to 4 printed pages). Papers exceeding 4 printed pages will be subject to excess page charges. All manuscripts should be divided into the following sections:
(a) *First page* including the title of the presented work [not exceeding fifteen (15) words], full names and full postal addresses of all Authors, name of the Author to whom proofs are to be sent, key words, an abbreviated running title, an indication “review”, “clinical”, “epidemiological”, or “experimental” study, and the date of submission. (Note: The order of the Authors is not necessarily indicative of their contribution to the work. Authors may note their individual contribution(s) in the appropriate section(s) of the presented work); (b) *Abstract* not exceeding 150 words, organized according to the following headings: Background/Aim - Materials and Methods/Patients and Methods - Results - Conclusion; (c) *Introduction;* (d) *Materials and Methods/Patients and Methods;* (e) *Results;* (f) *Discussion;* (g) *Acknowledgements;* (h) *References*. All pages must be numbered consecutively. Footnotes should be avoided. Review articles may follow a different style according to the subject matter and the Author's opinion. Review articles should not exceed 35 pages (approximately 250 words per double-spaced typed page) including all tables, figures, and references.

*Figures*. All figures should appear at the end of the submitted document file. Once a manuscript is accepted all figures and graphs should be submitted separately in either jpg, tiff or pdf format and at a minimum resolution of 300 dpi. Graphs must be submitted as pictures made from drawings and must not require any artwork, typesetting, or size modifications. Symbols, numbering and lettering should be clearly legible. The number and top of each figure must be indicated. Pages that include color figures are subject to color charges.

*Tables*. All tables should appear at the end of the submitted document file. Once a manuscript is accepted, each table should be submitted separately, typed double-spaced. Tables should be numbered with Roman numerals and should include a short title.

*References*. Authors must assume responsibility for the accuracy of the references used. Citations for the reference sections of submitted works should follow the standard form of “Index Medicus” and must be numbered consecutively. In the text, references should be cited by number. Examples: 1 Sumner AT: The nature of chromosome bands and their significance for cancer research. Anticancer Res 1: 205-216, 1981. 2 McGuire WL and Chamnes GC: Studies on the oestrogen receptor in breast cancer. In: Receptors for Reproductive Hormones (O' Malley BW, Chamnes GC (eds.). New York, Plenum Publ Corp., pp 113-136, 1973.

*Nomenclature and Abbreviations*. Nomenclature should follow that given in "Chemical Abstracts", "Index Medicus", "Merck Index", "IUPAC –IUB", "Bergey's Manual of Determinative Bacteriology", The CBE Manual for Authors, Editors and Publishers (6th edition, 1994), and MIAME Standard for Microarray Data. Human gene symbols may be obtained from the HUGO Gene Nomenclature Committee (HGNC) (http://www.gene.ucl.ac.uk/). Approved mouse nomenclature may be obtained from http://www.informatics.jax.org/. Standard abbreviations are preferable. If a new abbreviation is used, it must be defined on first usage.

*Clinical Trials*. Authors of manuscripts describing clinical trials should provide the appropriate clinical trial number in the correct format in the text.
For International Standard Randomised Controlled Trials (ISRCTN) Registry (a not-for-profit organization whose registry is administered by Current Controlled Trials Ltd.) the unique number must be provided in this format: ISRCTNXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by "ISRCTN"). Please note that there is no space between the prefix "ISRCTN" and the number. Example: ISRCTN47956475.

For Clinicaltrials.gov registered trials, the unique number must be provided in this format: NCTXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by 'NCT'). Please note that there is no space between the prefix 'NCT' and the number. Example: NCT00001789.

*Ethical Policies and Standards*. ANTICANCER RESEARCH agrees with and follows the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors in 1978 and updated in October 2001 (www.icmje.org). Microarray data analysis should comply with the "Minimum Information About Microarray Experiments (MIAME) standard". Specific guidelines are provided at the "Microarray Gene Expression Data Society" (MGED) website. Presentation of genome sequences should follow the guidelines of the NHGRI Policy on Release of Human Genomic Sequence Data. Research involving human beings must adhere to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, effective December 13, 2001. Research involving animals must adhere to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society. The use of animals in biomedical research should be under the careful supervision of a person adequately trained in this field and the animals must be treated humanely at all times. Research involving the use of human foetuses, foetal tissue, embryos and embryonic cells should adhere to the U.S. Public Law 103-41, effective December 13, 2001.

*Submission of Manuscripts*. Please follow the Instructions for Authors regarding the format of your manuscript and references.
Manuscripts must be submitted only through our online submission system at: http://www.iiar-submissions.com/login.html
In case a submission is incomplete, the corresponding Author will be notified accordingly.
Questions regarding difficulties in using the online submission system should be addressed to: email: journals@iiar-anticancer.org

*Galley Proofs*. Unless otherwise indicated, galley proofs will be sent to the corresponding Author of the submission. Corrections of galley proofs should be limited to typographical errors. Reprints, PDF files, and/or Open Access may be ordered after the acceptance of the paper. Authors of online open access articles are entitled to a complimentary online annual subscription to Anticancer Research for the current year. Requests should be addressed to the Editorial Office. Galley proofs should be returned corrected to the Editorial Office by email within two days.

## *Specific information and additional instructions for Authors*

1. Anticancer Research (AR) closely follows the new developments in all fields of experimental and clinical cancer research by (a) inviting reviews on topics of immediate importance and substantial progress in the last three years, and (b) providing the highest priority for rapid publication to manuscripts presenting original results judged to be of exceptional value. Theoretical papers will only be considered and accepted if they bear a significant impact or formulate existing knowledge for the benefit of research progress.
2. Anticancer Research will consider the publication of conference proceedings and/or abstracts provided that the material submitted fulfils the quality requirements and instructions of the journal, following the regular review process by two suitable referees. (For further information please click here)
3. An acknowledgement of receipt, including the article number, title and date of receipt is sent to the corresponding author of each manuscript upon receipt. If this receipt is not received within 20 days from submission, the author should call or write to the Editorial Office to ensure that the manuscript (or the receipt) was not lost in the mail or during electronic submission.
4. Each manuscript submitted to AR is sent for review in confidence to two suitable referees with the request to return the manuscript with their comments to the Editorial Office within 12 days from receipt. If reviewers need a longer time or wish to send the manuscript to another expert, the manuscript may be returned to the Editorial Office with a delay. All manuscripts submitted to AR, are treated in confidence, without access to any person other than the Managing Editor, the journal's secretary, the reviewers and the printers.

5. All accepted manuscripts are peer-reviewed and carefully corrected in style and language, if necessary, to make presentation clear. (There is no fee for this service). Every effort is made (a) to maintain the personal style of the author's writing and (b) to avoid change of meaning. Authors will be requested to examine carefully manuscripts which have undergone language correction at the pre-proof or proof stage.

6. Authors should pay attention to the following points when writing an article for AR:
   - The Instructions to Authors must be followed in every detail.
   - The presentation of the experimental methods should be clear and complete in every detail facilitating reproducibility by other scientists.
   - The presentation of results should be simple and straightforward in style. Results and discussion should not he combined into one section, unless the paper is short.
   - Results given in figures should not be repeated in tables.
   - Figures (graphs or photographs) should be prepared at a width of 8 or 17 cm with legible numbers and lettering.
   - Photographs should be clear with high contrast, presenting the actual observation described in the legend and in the text. Each legend should provide a complete description, being self-explanatory, including technique of preparation, information about the specimen and magnification.
   - Statistical analysis should be elaborated wherever it is necessary. Simplification of presentation by giving only numerical or % values should be avoided.
   - Fidelity of the techniques and reproducibility of the results, should be points of particular importance in the discussion section. Authors are advised to check the correctness of their methods and results carefully before writing an article. Probable or dubious explanations should be avoided.
   - Authors should not cite results submitted for publication in the reference section. Such results may be described briefly in the text with a note in parenthesis (submitted for publication by... authors, year).
   - The References section should provide as complete a coverage of the literature as possible including all the relevant works published up to the time of submission.
   - By following these instructions, Authors will facilitate a more rapid review and processing of their manuscripts and will provide the readers with concise and useful papers.

7. Following review and acceptance, a manuscript is examined in language and style, and galley proofs are rapidly prepared. Second proofs are not sent unless required.

8. Authors should correct their galley proofs very carefully and preferably twice. An additional correction by a colleague always proves to be useful. Particular attention should be paid to chemical formulas, mathematical equations, symbols, medical nomenclature etc. Any system of correction marks can be used in a clear manner, preferably with a red pen. Additions or clarifications are allowed provided that they improve the presentation but do not bring new results (no fee).

9. Articles submitted to AR may be rejected without review if:
   - they do not fall within the journal's policy.
   - they do not follow the instructions to authors.
   - language is unclear.
   - results are not sufficient to support a final conclusion.
   - results are not objectively based on valid experiments.
   - they repeat results already published by the same or other authors before the submission to AR.
   - plagiarism is detected by plagiarism screening services.

   (Rejection rate (2015): 64%).

10. Authors who wish to prepare a review should contact the Managing Editor of the journal in order to get confirmation of interest in the particular topic of the review. The expression of interest by the Managing Editor does not necessarily imply acceptance of the review by the journal.

11. Authors may inquire information about the status of their manuscript(s) by calling the Editorial Office at +30-22950-53389, Monday to Friday 9.00-16.00 (Athens time), or by sending an e-mail to journals@iiar-anticancer.org

12. Authors who wish to edit a special issue on a particular topic should contact the Managing Editor.

13. Authors, Editors and Publishers of books are welcome to submit their books for immediate review in AR. There is no fee for this service.

(This text is a combination of advice and suggestions contributed by Editors, Authors, Readers and the Managing Editor of AR).