ADDICTION

SSA SOCIETY FOR THE STUDY OF ADDICTION

MONOGRAPH doi:10.1111/add.14721

Cytisine for nicotine addiction treatment: a review of pharmacology, therapeutics and an update of clinical trial evidence for smoking cessation

Piotr Tutka[1,2,3], Denis Vinnikov[4,5], Ryan J. Courtney[3] & Neal L. Benowitz[6,7]

Department of Experimental and Clinical Pharmacology, University of Rzeszów, Rzeszów, Poland,[1] Laboratory for Innovative Research in Pharmacology, University of Rzeszów, Rzeszów, Poland,[2] National Drug and Alcohol Research Centre, University of New South Wales, Sydney, NSW, Australia,[3] School of Public Health, Al-Farabi Kazakh National University, Almaty, Kazakhstan,[4] Biological Institute, National Research Tomsk State University, Tomsk, Russia,[5] Division of Clinical Pharmacology and Experimental Therapeutics, Department of Medicine, University of California San Francisco, San Francisco, CA, USA[6] and Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA, USA[7]

ABSTRACT

Aims To review cytisine's history of use, pre-clinical evidence, clinical pharmacokinetics, efficacy, adverse reactions (ARs) and safety for smoking cessation. **Methods** A synoptic review of the use of cytisine as a smoking cessation medication, mechanism of action, pharmacokinetics and safety. Relevant literature on data included in these sections were identified through a search of 11 databases with additional literature obtained from reports and monographs. Three databases (PubMed, EMBASE and www.elibrary.ru) were systematically searched for studies published from 2012 to August 2018 in any language to provide an updated meta-analysis of cytisine's efficacy and ARs for smoking cessation compared with placebo. We pooled the relative risks (RR) of abstinence in the efficacy analysis and RR of ARs, either reported by the authors or calculated from the reports. **Results** Cytisine has been in use since 1964 and is currently marketed in 18 countries. Systemic bioavailability from oral ingestion is high and clearance is primarily renal, with minimal or no metabolism. Brain uptake in animal models is moderate. The plasma half-life averages 4.8 hours. Eight studies were included for meta-analysis of efficacy. With heterogeneous results, the overall RR versus placebo of successful continuous abstinence at the longest follow-up was 1.74 [95% confidence interval (CI) = 1.38–2.19]. Nausea, vomiting, dyspepsia, upper abdominal pain and dry mouth that were mild or moderate were the most common ARs, with RR versus placebo 1.10 (95% CI = 0.95–1.28). The cost of cytisine in eastern and central Europe is several-fold less than that of other smoking cessation medications. **Conclusions** Cytisine is a low-cost medication found to increase the likelihood of smoking cessation. The most frequently reported ARs of cytisine involve gastrointestinal symptoms that are mostly reported as either mild or moderate in severity.

Keywords Addiction, cessation, cytisine, low-/middle-income country, nicotine, nicotinic receptor partial agonist.

Correspondence to: Piotr Tutka, Department of Experimental and Clinical Pharmacology, Faculty of Medicine, University of Rzeszów, Al. Kopisto 2a, PL 35-959 Rzeszów, Poland. E-mail: tutka@umlub.pl; piotr.tutka1@gmail.com
Submitted 5 October 2018; initial review completed 8 January 2019; final version accepted 18 June 2019

INTRODUCTION

Tobacco smoking is the leading preventable cause of death [1]. Globally, tobacco use causes nearly 6 million deaths per annum [2] and is projected to cause more than 8 million deaths per annum by 2030 [1]. Smoking cessation reduces the risk of premature death associated with continued smoking by approximately 90% [3]. However, smoking cessation is extremely difficult for several reasons, including the complexity of nicotine addiction. While patterns of cigarette consumption vary among countries, including low- to middle-income countries (LMICs), cigarette consumption in some countries and regions is steadily increasing, e.g. China and the World Health Organization (WHO) eastern Mediterranean region, with Africa presenting the greatest risk for potential future growth [4]. An integral component to successful tobacco control includes both high access and wide availability of cost-effective pharmacological treatments. Unfortunately, such treatments are either inaccessible or unavailable in most LMICs [5]. For the select few LMICs where pharmacological treatment(s) are available, generally they are cost-prohibitive and associated with high out-of-pocket expenses, or not affordable due to constrained and financially limited health-care budgets [6]. Two-thirds of smokers live in countries whereby the average household income is less than US $200 per week and smoking-cessation treatments are not paid for by insurance plans or national health-care systems [7]. Given the limitations in the efficacy, availability and affordability of anti-smoking pharmacotherapies, developing alternative pharmacotherapies for smoking cessation continues to be a research priority.

Cytisine, considered to be the oldest medication used for smoking cessation [8,9], has been licensed and used as a smoking cessation aid in some eastern/central European and central Asian countries for more than 50 years [8,10]. In eastern/central European countries it is available as an inexpensive over-the-counter (OTC) medicine for the equivalent of US$8–17 [11]. In western countries, this compound has been subject to extensive pharmacological research, including studies that have examined treatment strategies to establish the impact of cytisine action on nicotinic acetylcholine receptors (nAChRs) [12]. Health professionals and researchers from countries where cytisine has been used for smoking cessation have tried to generate interest in cytisine in western Europe and the United States. To date, however, these attempts have not been successful because cytisine has been considered as a unknown medicinal compound or viewed as toxic, with only slight brain penetration [13,14], and thus most probably devoid of efficacy as a smoking cessation aid. Since 2006 cytisine has drawn much wider interest, after a review first highlighted its anti-smoking potential [15], with a series of commentaries/reports, research agenda [16], reviews [8,17,18], meta-analyses [10,19,20] and clinical trials [7,21–23] demonstrating efficacy and safety for smoking cessation.

Cytisine, despite its long-standing use as a smoking cessation aid in some parts of the world, has had an unconventional history of use in medicine. This is largely attributable to pre-clinical studies that would typically precede clinical studies and marketing not being conducted, or if so, data not released or critiqued in the public domain. Only more recently has significant progress been made that led to increased knowledge and understanding of the therapeutic potential of cytisine for nicotine addiction treatment.

The purpose of this review is to comprehensively describe what is currently known about cytisine as a smoking cessation aid, with four core aims specifically related to:

i summarizing the pre-clinical properties, current and emerging clinical evidence relating to cytisine's use for smoking cessation;
ii offering a new meta-analysis of clinical trials to gather updated evidence on cytisine's efficacy and adverse reactions (ARs) and to extract further clinical evidence that can guide future policy;
iii evaluating the implications of the updated evidence base, and the current and future impact of cytisine for tobacco dependence treatment; and
iv discussing future opportunities to close evidence-practice gaps, and describe where further research is warranted.

METHODS

This is a synoptic review of the use of cytisine as a smoking cessation medication, mechanism of anti-smoking action, pharmacokinetics and overdose and toxicity. We systematically reviewed the literature to conduct meta-analysis of cytisine efficacy and ARs versus placebo. The two final sections of this monograph are presented in a narrative review structure and examine cytisine treatment regimens/adherence and affordability and cost-effectiveness.

Pre-clinical, pharmacokinetic and overdose/toxicity data search

A literature search for the pre-clinical data, pharmacokinetics and overdose and toxicity was conducted using 11 electronic databases: PubMed, PsycINFO, Cochrane Library, EBSCO, Medscape, ProQuest, Science Direct, Scopus, Springer, Web of Science and Wiley Online Library for studies published until August 2018, although we have included a few key studies published after this date. The cytisine-specific search terms were 'cytisine', 'tabex','desmoxan', 'cytisus laburnum' or 'nicotine receptor partial agonist' in the title or abstract or as keywords. The search terms did not include other names for cytisine, such as baptitoxine, laburnine, sophorine and ulexine. We also examined the reference sections of five earlier reviews of cytisine/nicotine receptor partial agonists [6,8,17,19,20]. Moreover, we examined the reference lists of the final set of articles included in our monograph. Database searches for pre-clinical data and overdose/toxicity were undertaken by P.T. and for pharmacokinetics by N.L. B. and P.T., who independently examined all article titles and subsequently reviewed abstracts to assess their potential relevance to the monograph. Full text articles and studies were obtained, including journal articles, books chapters and publicly available reports. The articles available in Bulgarian, German and Russian were translated into English by two authors (P.T. and D.V.). We contacted authors of reports for additional information where necessary. A total of 2234 references, excluding duplicates, were identified. One hundred and fifteen articles or reports were screened in full.

Clinical efficacy, adverse events and safety data search

Three meta-analyses have been previously completed [10,19,20], in which each corresponding review covered the following time-frames and included the following number of studies: 1968–1974 (n = 3) [10], 1971–2014 (n = 4) [19] and 1968–2011 (n = 7) [20]. The Etter [10] meta-analysis was released before publishing modern trials and included only studies from the 1960s and 1970s [10]. The most recent review was completed by Cochrane Database of Systematic Reviews [19] in 2016, with records extracted until May 2015, and only four controlled trials were included [7,21,22,24]. However, more studies were included in a preceding review completed by Hajek *et al.* [20] in 2013, in which they identified seven controlled trials [7,21,24–28] yielding two analyses: a pooled relative risk model and cytisine ARs. The Cochrane [19] and Hajek *et al.* [20] reviews differ in the number of searched databases and types of search terms used (e.g. 'cytisine' or 'Tabex' or'nicotine receptor partial agonist' in the Cochrane review [19] and 'cytisine' or 'cytisus laburnum' or 'Tabex' in the review by Hajek *et al.* [20]). The Cochrane review included randomized controlled trials which compared cytisine with placebo or nicotine replacement therapy (NRT), and excluded trials which did not report a minimum follow-up period of 6 months from start of treatment. In contrast, Hajek *et al.* [20] included controlled trials; however, the trials in which the longest follow-up period was shorter than 6 months were not excluded from the meta-analysis.

In the current analysis we chose to update an earlier review [20] rather than the most recent Cochrane review [19] because of the greater number of searched databases and greater number of controlled studies included. Therefore, in order to update Hajek *et al.*'s review [20], PubMed and EMBASE were searched using the terms 'cytisine' or 'cytisus laburnum' or 'tabex' or 'desmoxan' for records from July 2012 to August 2018. We also searched the Russian electronic library database (www.elibrary.ru) for all records on cytisine since commencement. We searched on-line clinical trials registers to update information on new cytisine clinical trials.

One reviewer (D.V.) screened the titles and abstracts of publications identified by the search. The full texts of potentially eligible articles were then screened, and those meeting our pre-specified inclusion/exclusion criteria were included. Two reviewers (D.V. and P.T.) independently performed the full-text review. No discrepancies in identifying relevant articles occurred between the reviewers.

Articles eligible for inclusion in meta-analysis were those that contained original data from randomized controlled clinical trials examining the use of cytisine for smoking cessation in adult smokers. The search was not restricted by language of publication. Translations of the articles written in Polish and Russian were completed by two authors (P.T. and D.V.). Reviews, meta-analyses, conference reports, commentaries, case reports and letters to the editor, as well as translations of already included controlled trials, were excluded. Other exclusion criteria included: no smoking cessation assessment, cessation not an outcome, the outcome data was not reported in a useable form, cessation not reported as an outcome or use of cytisine for conditions other than smoking cessation. For each study, data were extracted using the strictest outcome measure at the longest follow-up period. The full sample was included as the denominator, with participants who dropped out of the trials or were lost to follow-up classified as continuing smokers. Data on ARs were also extracted and analyzed.

We ran the analysis using *metan* command in Stata using a random-effects model, given very high between-study heterogeneity. For the relative risk (RR) calculation, the longest follow-up reported by authors either with or without biochemical verification was used. To estimate the bias originating from self-reported abstinence, we performed stratification analysis into two groups of studies (biochemically verified versus self-report). Similarly, we divided studies into those reporting effect within 3 months versus more than 3 months. Heterogeneity was assessed with the I^2 statistic, and potential role of publication bias was estimated via Egger's and Begg's tests.

The use of cytisine as a smoking cessation medication

Cytisine [(−)-cytisine, (1R,5S)-1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido (1,2a)(1,5)diazocin-8-one] (Fig. 1) is an alkaloid originating from some plants belonging to the *Leguminosae* (*Fabaceae*) family [29,30] In 1862, Husemann & Marme isolated pure cytisine from seeds of common garden decorative plants *Laburnum anagyroides* (*Cytisus laburnum*; Golden Rain) [31]. In more recent times, cytisine is most commonly extracted from the *Laburnum* and *Sophora* species [8,12,32].

Plants containing cytisine have been used as a natural remedy and a medicinal agent for various purposes for centuries [8]. Archaeological research has found that plant species *Sophora secundiflora*, which contain cytisine, were also used as one of the first psychotropic agents in North America (approximately 8000 years BC†) [33]. During the Second World War, the leaves of *Laburnum anagyroides*, which contain cytisine, were used as a tobacco substitute [34]. For smoking cessation, cytisine was first authorized

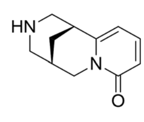

Figure 1 The structure of (−)-cytisine

for use in 1964 in Bulgaria, where the first clinical studies were conducted [35].

With the entry of several former Socialist economy countries to the European Union (EU) cytisine was withdrawn from the market in Czech Republic, Slovakia, Hungary and Romania. Only in Bulgaria and Poland has cytisine been used continuously as a prescription-only or OTC medicine. Consequently, the most comprehensive clinical experience and documented history for cytisine use for smoking cessation is found in Poland, where it has been used since the 1970s [36,37]. Table 1 shows the history of anti-smoking use and research on cytisine.

Cytisine is manufactured according to EU Good Manufacturing Practice as two products available in the OTC category: Tabex® (Sopharma, Bulgaria) and Desmoxan® (Aflofarm, Poland). Cytisine in tablet form as Tabex® has been manufactured from 1964 and is now marketed in 18 countries: four EU (Bulgaria, Latvia, Lithuania and Poland) and eight non-EU countries (Azerbaijan, Armenia, Ukraine, Belarus, Georgia, Moldova, Russia and Serbia), and in five countries in Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan). Total sales data of Tabex® in Poland from January 2005 to March 2014 shows that 1 657 800 packs were sold with a value of €14 093 064 [45]. One pack contains 100 tablets to cover a full 25-day treatment period.

In Poland cytisine is undergoing a resurgence in use, largely attributable to a new cytisine product, Desmoxan®. At the turn of 2012/13 Desmoxan® was introduced onto the Polish market as the first micronized cytisine-based capsule. This product is currently covered by a European patent application. During 2017, 447 863 packs of Desmoxan® were sold, accounting for 60.7% of the Polish cytisine market [11,45].

More than 14.2% of tobacco smokers in Poland have been documented as using cytisine for smoking cessation in a 1.5-year period and nearly 30% of smokers (equating to more than 2.6 million people) have been treated with cytisine in Poland since 2005 [45]. In 2015, the number of Desmoxan® and Tabex® packs sold accounted for 37.5% of the total number of packs of all sold anti-smoking medicines in Poland [45].

As shown in Fig. 2, the sale of cytisine in Poland shows a continuous upward trend. Since 2000 cytisine use has grown by a factor of 10. In 2013, sales increased dramatically following Desmoxan's availability OTC, and extensive product marketing.

Cytisine's increased use has been posited as an important factor responsible for reduced smoking rates in

Table 1 Milestones in cytisine research and use for smoking cessation.

1818: Discovery (Chevreul) [38]
1862: Isolation (Husemann & Marme) [31]
1912: Description of cytisine as a component of *Laburnum anagyroides* (Dale & Laidlaw) [30]
1931: Description of chemical structure (Ing) [39]
1953: Characteristics of pharmacological properties and use in the treatment of nicotine addiction (Paskov & Dobrev) [40]
1964: Tabex® introduced to Bulgarian market
1965: First clinical trial for smoking cessation (Stoyanov & Yanachkova) [35]
1968–1974: Placebo-controlled clinical trials demonstrating efficacy in nicotine addiction (in Bulgaria and Germany) [24–26,41]
2004: First scientific conference on cytisine for smoking cessation (Cracow, Poland) [15]
2006: First review on the potential of cytisine for smoking cessation (Tutka *et al.*) [15]
2006: First complex review published in English-language peer-reviewed literature calling for consolidated efforts towards global access to cytisine (Tutka & Zatoński) [8] and a report of anti-smoking potential (McRobbie *et al.*) [17]
2006: First meta-analysis of placebo-controlled trials (Etter) [10]
2006: First evidence-based medicine (EBM) uncontrolled clinical trial (in Poland) (Zatoński *et al.*) [23]
2008: Research agenda (Etter *et al.*) [16] and review comparing cytisine with varenicline (Tutka) [6]
2008: First EBM placebo-controlled trial (n = 171) (in Kyrgyzstan) (Vinnikov *et al.*) [21]
2011: Largest EBM placebo-controlled trial (n = 740) (British–Polish conducted in Poland) (West *et al.*) [7]
2013: World's first micronized form of cytisine (Desmoxan®) introduced to Polish market
2013: Meta-analysis and review of cytisine-related clinical data (Hajek *et al.*) [20]
2014: Largest EBM non-inferiority trial of cytisine versus nicotine replacement therapy (NRT) (n = 1310) (in New Zealand) (Walker *et al.*) [22]
2014: An international workshop on cytisine for smoking cessation (SRNT Meeting, Seattle)
2015: The creation of a world-wide network of scientists working on cytisine (Cytisine for Smoking Cessation Group)
2015: First description of cytisine pharmacokinetics in humans (Jeong *et al.*) [42]
2016: Editorial published in *Addiction* calling for cytisine licensing to ensure its life-saving potential is reached globally (Walker *et al.*) [43]
2017: Cytisine Clinical Development Program announced for potential global development and commercialization of cytisine for smoking cessation (Achieve Life Sciences)
2017: Cytisine launched as an over-the-counter (OTC) herbal product (Cravv®) in Canada [44]

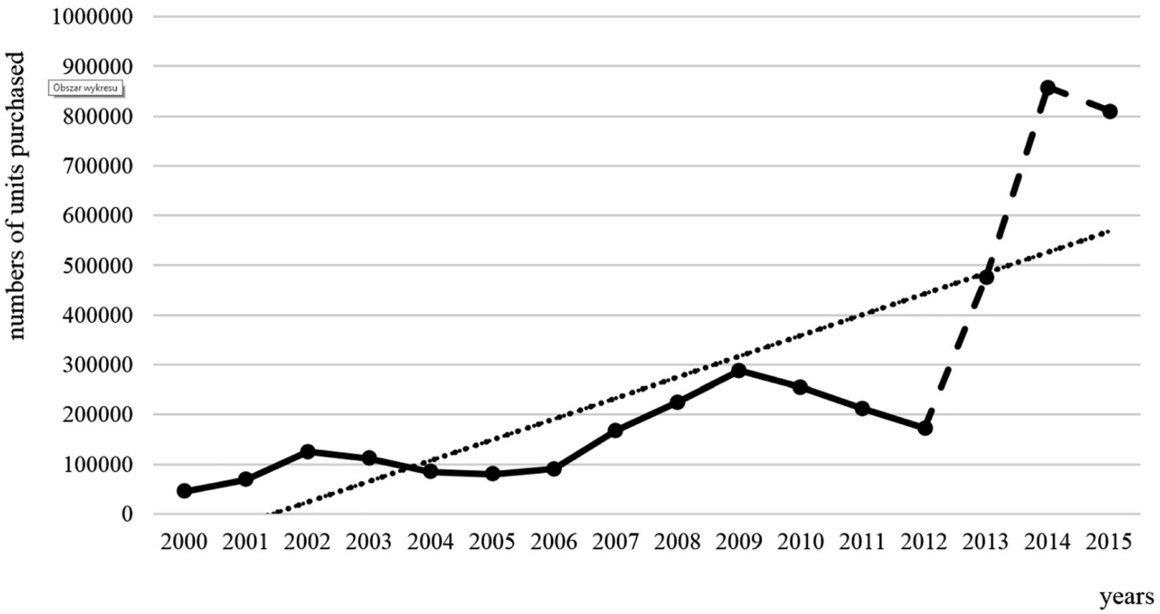

Figure 2 The number of cytisine packs purchased for smoking cessation in Poland from 2000 to 2015. The solid line shows the number of Tabex® packs sold and when at 2012/13 Desmoxan® entered the market (illustrated by corresponding broken line marking). The second dotted line shows the overall trend packaged data of cytisine sales for both Desmoxan® and Tabex® combined over time (Poland National Sales Data 2016) [45]

Poland [46]. However, current evidence is limited to expert opinion provided in a recent commentary [46]. Further studies are needed at a population level that examine smoking prevalence and implementation of all tobacco control measured over time to elucidate a more robust estimation of cytisine's impact on smoking rates [11,46].

In Canada, cytisine has been recently launched (August 2017) as an OTC herbal product in capsule form under the brand name Cravv®. The recommended treatment period is the same as for Desmoxan® or Tabex® [44]. To date no published research exists on this product and its current marketed price is C$50 for a full course of treatment.

Mechanism of anti-smoking action

In-vitro studies

Nicotine is the main psychoactive component of tobacco responsible for addiction [47].

Nicotine addiction is associated with repeated nicotine ingestion from cigarettes leading to stimulation of brain nAChRs and release of dopamine and other neurotransmitters from the nucleus accumbens, ventral tegmental area (VTA) and frontal cortex area [48]. The nAChRs are a critical target for medication development. Cytisine binds predominantly to α4β2* (*denotes the presence of other possible subunits in the receptor complex) subtype of nAChRs which mediates, at least partially and probably mainly, the rewarding and reinforcing effects of nicotine. Receptor binding data show that the affinity of cytisine to α4β2* is approximately sevenfold higher than that of nicotine [49] but fivefold lower than that of varenicline [50], another nAChRs partial agonist commonly used for smoking cessation.

Cytisine has been classified as a selective partial agonist at α4β2* nAChRs. The alkaloid binds with higher activity to α4β2* nAChRs (K = 1.65 nm) than nicotine [13], but has only partial efficacy at these receptors in comparison with a full agonist, i.e. nicotine. The agonist activity of cytisine is determined by both receptor activation and receptor desensitization. In a study on the functional effects of cytisine, at a 10-μM concentration and measuring the current evoked at α4β2*, Coe *et al*. estimated agonistic effects of the alkaloid at 56% of the response to 10 μM nicotine [49]. Another study carried out on human α4β2* nAChRs expressed in *Xenopus* oocytes demonstrated that a brief application of cytisine resulted in activation of these receptors, whereas sustained exposure desensitized the receptors. Desensitization of α4β2* nAChRs was observed at lower concentrations than those needed for activation [50]. Cytisine is less potent in desensitizing α4β2* nAChRs than varenicline [13]. Cytisine acts as an antagonist at α4β2* nAChRs; antagonist activity refers to competition with inhaled nicotine for receptor occupancy. When cytisine and nicotine were co-administered, cytisine partially blocked (30% inhibition) the activity of nicotine [49]. For comparison, varenicline blocked the activity of nicotine by 34% [49].

The interaction of cytisine with α4β2* nAChRs results in the modulation of the release of mesolimbic dopamine associated with pleasure and smoking satisfaction. An efficacy study demonstrated that cytisine elicits dopamine release from striatal slices, with the maximum release reaching approximately 50% of that seen with nicotine [51].

Recent studies have demonstrated that α6β2* nAChRs are subtypes that mediate nicotine-evoked dopamine release in nucleus accumbens and VTA and nicotine self-administration in rodents contributing to dependence [52]. Cytisine binds with high affinity at α6β2* nAChRs [13]. The limited data on the functional effects of cytisine at the α6β2* nAChRs suggest that cytisine is a partial agonist at these receptors with relative agonist efficacy to the full agonist acetylcholine (E_{max}) value 40% [13]. Cytisine can activate α6β2* subtypes at 10–100 times lower concentrations than needed for α4β2* nAChRs activation [53]. The drug causes extensive α6β2* nAChRs desensitization at similar concentrations to varenicline (approximately 40% of the fraction of α6β2* nAChRs desensitized by nicotine from smoking) [13]. Cytisine has been shown to antagonize α6β2* nAChRs. However, the percentage decrease for nicotine receptors occupancy is significantly lower compared to varenicline [53]. Thus, it remains unknown whether the antagonist activity of cytisine at α6β2* nAChRs contributes to its anti-smoking effects.

In addition to receptors containing the β2 subunits, the α7 nAChRs have been implicated in mediating some pharmacological effects of nicotine [54]. According to studies on recombinant human receptors, cytisine is a full agonist of α7 nAChRs [55]. The potent high-efficacy activation of α7 nAChRs may be important in the clinical effects of cytisine as a smoking cessation drug. However, *in-vivo* pharmacological experiments are needed to confirm this speculation.

Of note, cytisine exerted negligible affinity for non-nicotinic neurotransmitters, transporters, modulatory binding sites and hormone receptors [8].

In-vivo studies

The partial agonism of cytisine at the α4β2* nAChRs has also been shown in animal studies. Coe *et al*. assessed the efficacy of cytisine and varenicline by measurement of dopamine turnover (a measure of the synthesis and utilization of dopamine) in the nucleus accumbens of rats treated with cytisine or varenicline [49]. When cytisine was administered alone subcutaneously at a dose of 5.6 mg/kg, the dopamine turnover increased with an efficacy amounting to 40% of the maximum dopamine turnover response for nicotine (1 mg/kg). In animals treated concurrently with nicotine, cytisine inhibited the nicotine effect by 36%. By comparison, varenicline given alone was able to enhance the dopamine turnover to 32% of the maximal nicotine effect. Varenicline administered concurrently with nicotine inhibited the nicotine response by 66%. In another study, cytisine was administered orally in a dose-dependent manner which increased dopamine turnover in rat nucleus accumbens, but its effect compared to varenicline was weaker [50].

The partial agonist effect of cytisine at α4β2* nAChRs has been confirmed in pre-clinical addiction-related behavioral models. Brain α4β2* nAChRs play a key role in discriminative stimulus effects of nicotine in contrast to other central nicotinic receptors (e.g. α3β4* and α7). The drug discrimination assay provides a tool to directly assess both agonist and antagonist functions of cytisine. Animal studies showed that cytisine in a nicotine discrimination assay produced agonist-like effects [14,53,56–60] and relatively weak antagonist effects [14,53,58,59]. A nicotine-discrimination assay in rhesus monkeys did not detect a difference in agonist efficacy between cytisine, nicotine and varenicline but showed evidence of involvement of dopamine in the action of all agonists [61]. In another study in rats, cytisine exhibited lower antagonism of nicotine's discriminative stimulus effects than varenicline [53]. Several lines of evidence suggest that cytisine, like nicotine, could serve as a robust discriminative stimulus, although it was found to be much less potent than nicotine in the behavioral studies [14,58].

Intracranial self-stimulation (ICSS) is a behavioral procedure used to study the abuse potential of drugs in which the operant response is maintained by pulses of electrical brain stimulation. The acute administration of a drug of abuse diminishes the ICSS threshold, which is indicative of a potentiation of brain reward function [62]. In contrast, drug of abuse withdrawal is associated with elevations in ICSS thresholds and anhedonia, which is a hallmark symptom of depression. ICSS studies of the VTA in rats have found that nicotine reduces the stimulation threshold determined using rate-independent methods. Unlike nicotine and varenicline, cytisine was unable to change the ICSS threshold [60,62]. These findings suggest that cytisine does not potentiate brain reward function in rats [62]. The effect of cytisine on the brain reward function in nicotine-withdrawing rats has been determined in two studies. Cytisine, similarly to varenicline, reversed threshold elevations associated with nicotine withdrawal [60,62]. As suggested by a study by Igari *et al*., cytisine may prevent smoking relapse by diminishing the negative mood state caused by smoking cessation [62].

The systemic application of nicotine stimulates the spontaneous locomotor activity [63]. Cytisine (up to 3 mg/kg) has no effect on locomotor activity in rats [60,64], but is able to suppress fully the nicotine-facilitated locomotion [60]. However, in another behavioral model based upon locomotor activity, cytisine is more potent than nicotine when it is administered by direct injection into the central tegmental area of the rat brain [65,66]. This effect seems to be mediated by the mesolimbic dopamine system [66].

Acute presentation of nicotine is known to reinstate nicotine seeking behavior (self-administration) in animals [60]. This self-administration behavior is believed to model drug use in humans. Earlier studies demonstrated that drug-naive mice self-administered cytisine intravenously, which suggested that cytisine had reinforcing effects [67]. In a more recent study by Radchenko *et al*. cytisine failed to reinstate nicotine-seeking behavior in rats [60]. This finding suggests that cytisine is unlikely to induce relapse to nicotine-seeking during abstinence. However, the number of animal studies on the reinforcing and rewarding effects of cytisine is limited, and further studies are necessary to resolve this issue.

Addiction to tobacco is multi-factorial, and can be considered as a combination of a desire for the direct pharmacological actions of nicotine and learned associations and relief of withdrawal symptoms when nicotine is not available [47]. Theoretically, cytisine acting as a partial agonist of α4β2* nAChRs and substituting for nicotine would moderately increase the dopamine level and turnover in the mesolimbic system, and consequently attenuate the withdrawal symptoms [6,49]. So far, however, experimental data has been limited and inconclusive. A statement of behavior—whether cytisine attenuates the withdrawal symptoms—is important from a clinical point of view because it will allow modification of a treatment regimen (e.g. extending the period of cytisine use post quitting to reduce these symptoms). It is also not known whether cytisine *per se* can precipitate some withdrawal symptoms.

Until recently, it was believed that the behavioral profile of cytisine was very similar to that of varenicline and other partial agonists of nAChRs. Interestingly, new studies have found cytisine to produce a relatively unique pattern of *in-vivo* effects compared to varenicline in rats [60,62].

Of note, the lower efficacy of cytisine in causing dopamine release compared with nicotine suggests that cytisine should be less addictive than nicotine. Although previous clinical observations do not indicate that cytisine has addictive properties, it is necessary to confirm this based on behavioral studies and trials.

In conclusion, *in-vivo* cytisine data are in relative concordance with binding and functional data reported for cytisine via *in-vitro* studies.

Pharmacokinetics

Understanding the pharmacokinetics and pharmacodynamics of a drug is useful in determining the optimal therapeutic dose, dosing interval and physiological or disease factors that might influence drug response. Pharmacokinetic parameters of interest with respect to cytisine dosing and pharmacodynamics include oral bioavailability, rate and routes of clearance, tissue distribution characteristics and half-life.

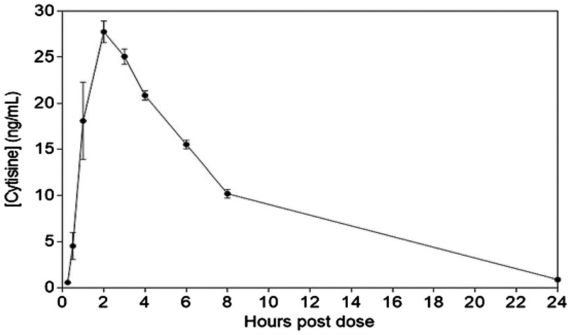

Figure 3 Mean plasma concentrations (ng/ml) of cytisine over 24 hours following a single 3-mg oral dose. Values are shown as mean ± standard error of the mean (SEM) (*n* = 7) (Jeong *et al.* 2015) [42]. We thank the copyright holders, Wiley Publishing Group, for permission to use this figure

The standard 25-day cytisine dosing recommendation for smoking cessation is 1.5 mg every 2 hours throughout the waking day (up to six tablets) on days 1–3; 1.5 mg every 2.5 hours (up to five tablets) on days 4–12; 1.5 mg every 3 hours (up to four tablets) on days 13–16; 1.5 mg every 4–5 hours (three tablets) on days 17–20; and 1.5 mg every 6 hours (two per day) on days 21–25. The dosing regimen includes direction to not smoke by the fifth day. With a 25-day treatment period, cytisine has the shortest treatment duration of any of the currently approved smoking cessation medication [16].

The pharmacokinetics of cytisine in people have been described in four studies [42,68–70]. In the first study (Fig. 3), oral tablets of cytisine (Tabex®) were administered in a dose of 3–7 mg among healthy male smokers [42]. Bioavailability (F) was found to be high based on recovery of 90–95% of the dose in a 24-hour urine collection. Clearance was found to be primarily renal, with minimal or no metabolism. The oral clearance averaged 16.7 l/hour (278 ml/min). As this is considerably greater than glomerular filtration rate (100 ml/min), there is net renal secretion. The volume of distribution (Vd/F) averaged 115l (approximately 2 l/kg), indicating moderate tissue distribution. The plasma half-life averaged 4.8 hours. Of note, cytisine was still measurable in blood (1 ng/ml) at 24 hours, suggesting slow tissue release [42].

A second study determined cytisine plasma concentrations in 10 smokers while taking a recommended 25-day cytisine (Tabex®) dosing regimen for smoking cessation [68]. Blood was collected at 0, 2, 4, 8 and 10 hours on day 1 then prior to the first morning dose on subsequent visits (day of and day following each change in dosing schedule) to measure plasma cytisine concentrations. Accumulation of cytisine was observed with repeated dosing of cytisine on day 1. The mean ± standard error of the mean (SEM) plasma cytisine concentration measured at 10 hours was 50.8 ± 4.7 ng/ml. After modest accumulation over the first 3 days, due to subsequent dose tapering, there was an overall decrease in plasma cytisine concentration over the remainder of the treatment period [68].

A third study examined the dose-proportionality and safety of higher doses of cytisine, comparing 1.5, 3.0 and 4.5 mg (one, two or three Desmoxan® capsules) [70] in groups of six smokers at each dose. Dose-proportionality for maximal concentration and area under the plasma concentration–time curve was confirmed. Adverse effects were mild, with dizziness noted by two of six participants receiving 4.5 mg cytisine. These data support the idea that doses higher than 1.5 mg, dosed less frequently than every 1.5–2 hours, are feasible for future smoking cessation trials.

A recent study evaluated the bioavailability of 3 mg cytisine (Tabex®) under fed and fasted conditions in 24 healthy volunteer subjects [69]. As reported: 'Cytisine was extensively absorbed after oral administration with the maximum cytisine concentration being observed in the blood within less than an hour. Study results demonstrated bioequivalence when cytisine was administered with or without food'. However, at the time of this review, the detailed results of this study were not yet published.

Prior studies in animals also demonstrated high oral bioavailability and minimal metabolism. In rats, cytisine circulates unbound in plasma [50]. The drug has a pKa of 7.8, meaning that 72% circulates in the freebase form at physiological pH. For comparison, nicotine has a pKa of 8.0 and varenicline a pKa of 9.3, so 80 and 99% of nicotine and varenicline are present in the freebase form at physiological pH. Cytisine is less lipophilic than either nicotine or varenicline. Both the drug concentration in freebase form and lipophilicity can influence brain uptake. In rats, the brain concentration of cytisine are approximately 30% that of plasma [14], while for nicotine and varenicline brain concentrations are higher than blood concentrations. Thus, for any given plasma level, brain concentrations of nicotine and varenicline are much higher than those of cytisine. Studies in rats indicate that cytisine is not transported by P-glycoprotein 1 (Pgp) or breast cancer resistance protein (BCRP) efflux transporters [50].

As noted previously, cytisine is taken orally over 25 days, starting from one tablet every 2 hours tapering to two tablets per day. It is conceivable, as demonstrated by Jeong *et al.*, that with a half-life of approximately 5 hours and with regular dosing, accumulation of cytisine in the plasma and tissues over 24 hours would occur, then stabilization [68]. As the dosing interval becomes longer during the period of treatment, for example one to two doses per day, it is expected that the plasma levels and pharmacological effects for much of the day would be quite small. However, as noted below, even very low levels of cytisine might be able to sustain nAChRs desensitization.

Based on minimal metabolism and no effect of transporters, one would anticipate no significant pharmacokinetic drug interactions. Given the renal route of clearance, dose adjustment should be considered in people with severe renal insufficiency.

Of note, a new open-label, randomized, multi-dose study is currently in progress in Wales to evaluate the pharmacokinetic profile and pharmacodynamic effect of cytisine when administered at doses of 1.5 mg and 3.0 mg following the standard 25-day schedule [71].

EFFICACY FOR SMOKING CESSATION

Clinical trials evaluating cytisine as a smoking cessation aid

The first clinical studies of cytisine for smoking cessation were conducted more than 40 years ago prior to the advent of Good Clinical Practice (GCP) guidelines [24–26,35,36,41,72,73]. Only three trials were placebo-controlled [24–26]. A description of earlier placebo-controlled studies is provided in the Etter [10], Tutka [6], Hajek *et al.* [20] and Cochrane [19] reviews.

Evidence of the efficacy of cytisine (used as Tabex®) have come from several clinical trials. An open-label, uncontrolled trial (25-day treatment regimen with a single session of brief behavioral support) in 436 Polish participants demonstrated that the 12-week self-reported (not biochemically verified) abstinence was 27.5%, whereas 12-month verified exhaled carbon monoxide (CO) abstinence was 13.8% [23]. A Kyrgyzstan-based randomized, placebo-controlled trial conducted among mining company workers (n = 171, mostly male participants, 25-day treatment course with individual counseling with brochure) showed that at 26 weeks 10.6% of participants were continuously abstinent (self-report) in the cytisine group compared to 1.2% in the placebo group (P = 0.01) [relative ratio (RR) = 8.73, 95% confidence interval (CI) = 1.13–67.61] [21]. In a large (n = 740, 25-day treatment course, with minimal amount of behavioral counselling) Polish single-center, randomized, placebo-controlled trial, the sustained 12-month CO-verified abstinence rate was 8.4% with cytisine and 2.4% with placebo (risk difference 6.0, 95% CI = 1.66–7.13, P = 0.001) [7]. None of the clinical trials evaluating cytisine's efficacy for smoking cessation published to date have been sponsored by pharmaceutical companies manufacturing cytisine.

It is difficult to compare cytisine as a smoking cessation aid to other cessation medications, because only data from one pragmatic randomized clinical trial are available. This large (n = 1310) open-label, non-inferiority trial tested

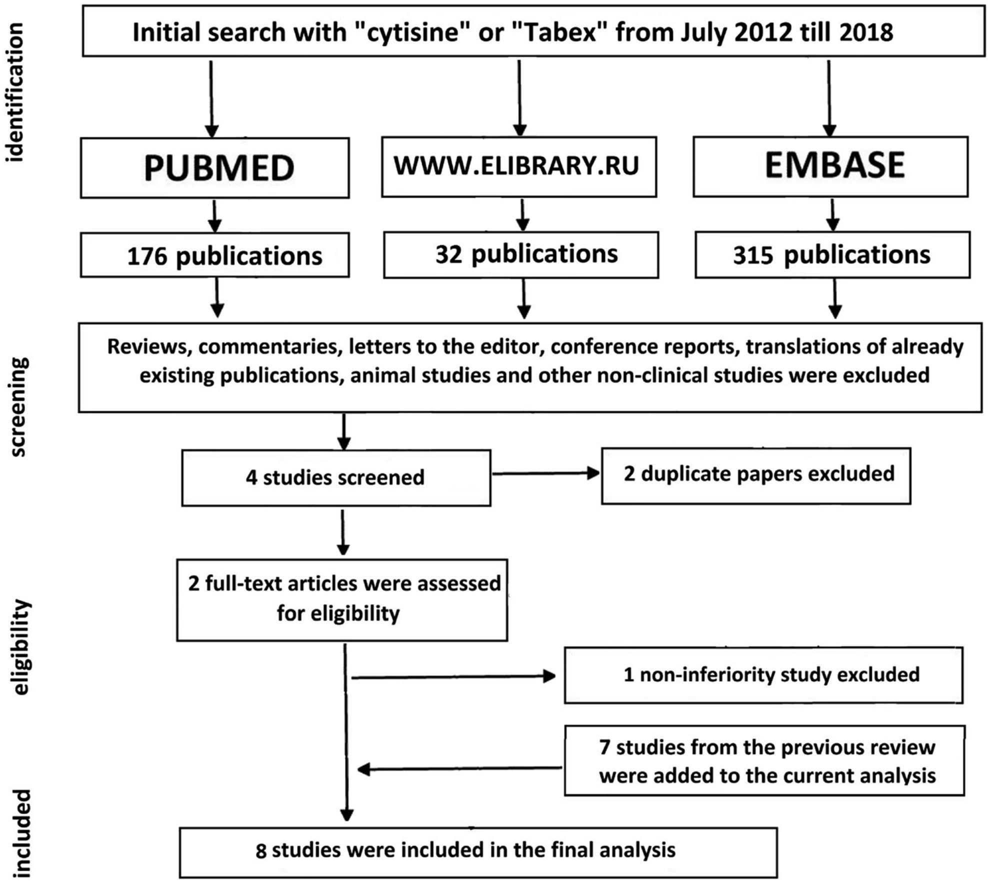

Figure 4 Flow-chart demonstrating the filtering process used to select eligible studies investigating efficacy of cytisine for smoking cessation in the review

cytisine's (Tabex®) effectiveness compared to NRT in New Zealand [22]. Cytisine was superior to NRT in helping smokers to quit at every follow-up: 1 week (60 versus 46%, $P < 0.001$), 2 months (31 versus 22%, $P < 0.001$) and 6 months (22 versus 15%, $P = 0.002$). At 1 month post-quit date the self-reported continuous abstinence rate was 40% in the cytisine group and 31% in the NRT group (risk difference = 9.3, 95% CI = 4.16–4.48). At 6 months the risk difference was 6.6 (95% CI = 2.4–10.8) [22]. Evidence that cytisine is superior to NRT is also supported by results from a recent cross-sectional study using data from a nationally representative household survey of smokers from Russia. Self-reported smoking abstinence rates for cytisine and NRT were 50 and 36% at 30 days and 25 and 11% at 90 days, respectively. After adjusting for age and gender, cytisine was found to be more effective than NRT for 90-day abstinence with odds ratio (OR) = 2.91 (95% CI = 1.28–6.59, $P = 0.011$) [74].

Meta-analysis of cytisine efficacy versus placebo

Our literature search identified 315 publications in EMBASE and 176 publications in PubMed since 2012, along with 32 publications in www.elibrary.ru (Fig. 4). Following article screening two additional publications [22,75] were identified among the three databases (EMBASE, PubMed and www.elibrary.ru) searched. However, one of these two newly identified studies employed a non-inferiority design comparing cytisine to NRT [22]; therefore, it could not be included in the final meta-analysis with the remaining eight superiority studies comparing cytisine to placebo. The second newly identified study was conducted by Levshin *et al.* in Russia [75], and had not been considered in any of the preceding reviews. Levshin *et al.* [75] randomized participants to either cytisine (Tabex®) ($n = 92$) or placebo ($n = 104$), although 14 more participants were initially randomized but were lost after the first visit. The participants were offered medication or placebo. Subjects received behavioral support (described as 'standard psychological support') and were invited to follow-up visits at the end of weeks 1 and 4, and contacted by telephone after 3 and 6 months of treatment. By the end of 12 weeks, 23 (25%) patients in the cytisine group and 12 (11.5%) patients in the placebo group reported being continuously abstinent (RR = 2.16, 95% CI = 1.13–4.12). The authors stated that the follow-up visits included measurement of exhaled CO, but it is unclear if it was used after 3 and 6 months of

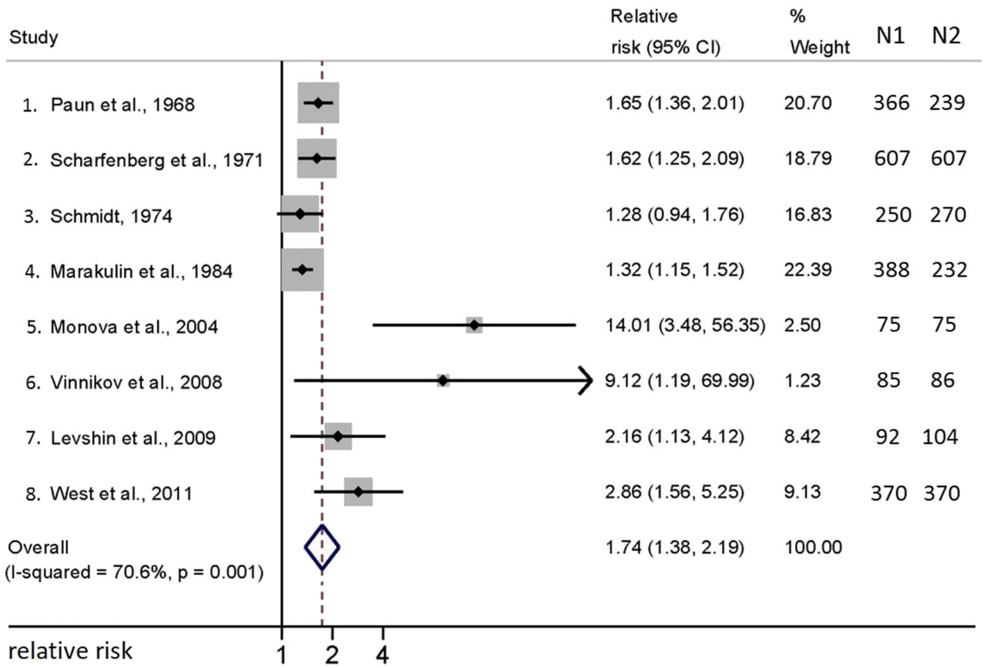

Figure 5 Forest plot of efficacy in eight included studies (cytisine versus control group). N1 = sample size of the cytisine group; N2 = sample size of the placebo group; CI = confidence interval [Colour figure can be viewed at wileyonlinelibrary.com]

treatment, so it is most likely that a verified continuous abstinence outcome was not adopted.

Our updated meta-analysis adds the Levshin *et al.* study [75] to an earlier seven controlled trials [7,21,24–27,76] in the review of Hajek *et al.* [20] and a total of eight superiority studies are now analyzed in the current update. We extracted data from the Levshin *et al.* study to calculate the RR as an effect measure with its corresponding 95% CI. The Preferred Reporting Items for Systemic Review and Meta-Analyses (PRISMA) [77] approach was used to report our findings, and the PRISMA checklist is included as Supporting information.

Figure 5 shows the results of our updated meta-analysis. The overall analysis of eight included studies confirmed previously published data that cytisine was an effective medication, increasing the likelihood of successful treatment by 75% compared to placebo. With heterogeneous results, the overall RR of successful continuous abstinence in a random-effects model was 1.74 (95% CI = 1.38–2.19). Studies with biochemically verified abstinence yielded much higher cytisine efficacy, with RR = 3.33 versus 1.53 (Table 2). Of note, self-reported abstinence could explain some heterogeneity, because studies with biochemical verification were somewhat homogenous. When limited to studies estimating abstinence at longer follow-up, between-study heterogeneity was reduced. Despite these differences, stratification in groups of longer versus shorter abstinence did not notably change the overall RR of quitting. With regard to publication bias, Begg's (P = 0.09) and Egger's tests yielded contradictory results, indicative of some publication bias present, i.e. some likelihood of smaller studies not to be published.

Table 2 Stratification analysis of studies for self-report versus biochemically verified and 3 months as a cut-off for abstinence.

	Random effects				*Heterogeneity*	
Group	*n*	*RR*	CI_L	CI_U	*P*	I^2
Self-report	6	1.53	1.29	1.83	0.01	63.0%
Biochemically verified	2	3.33	1.53	7.23	0.29	12.2%
Less 3 months	5	1.80	1.25	2.58	0.002	79.9%
More 3 months	3	1.60	1.24	2.06	0.07	53.9%

Self-report group includes studies 1–5 and 7 from Fig. 5. Biochemically verified group includes studies 6 and 8 from Fig. 5. Less than 3 months' duration studies were studies 1,3–5 and 7, whereas more than 3 months' duration were studies 2, 6 and 8 from Fig. 5. RR = relative risk; CI_l = lower confidence interval; CI_u = upper confidence interval.

Active clinical trials of cytisine for smoking cessation

There is no information as to whether or not cytisine is as effective as varenicline. Non-inferiority trials (n = 2) comparing cytisine to varenicline for smoking cessation are under way in Australia (n = 1266 smokers; standard 25-day course of Desmoxan®) [78] and New Zealand (n = 2140; Māori smokers and their family members; 12-week course of Tabex®) [79]. Another pilot randomized study comparing cytisine to varenicline in the primary care settings in Croatia and Slovenia has been announced (n = 380; standard 25-day treatment regimen; trade name not announced) [80]. A randomized, placebo-controlled trial funded by the National Institutes of Health (NIH) studying the effects of cytisine (Tabex®; standard 25-day course) in comparison with varenicline and NRT on alcohol and tobacco use in 400 HIV-infected patients is under way in Russia [81]. Further, a multi-centre randomized controlled trial is being conducted to assess cytisine (Desmoxan®; standard 25-day course) for smoking cessation in patients with tuberculosis (n = 2388) in Bangladesh and Pakistan [82]. Another study on cytisine versus nortriptyline has recently commenced recruitment in Thailand [83]. An oral cytisine form is used in this study, manufactured by the Thailand Government Pharmaceutical Organization; however, the treatment regimen has not been announced. Very recently, a multi-centre trial assessing the effectiveness of cytisine versus placebo for smoking cessation among secondary care cancer patients has been announced in Poland, but further details are not available [80]. The findings from these trials will be vital for informing policy makers around the world and for wider global dissemination of cytisine.

Of note, almost all cytisine trials have been conducted in participants smoking 10 or more cigarettes per day. For smokers who smoke < 10 cigarettes per day, it is important to assess the efficacy of cytisine in this population in future studies. In five of eight trials included in our meta-analysis, smokers received only minimal or no behavioral support. Given that the success rate of smoking cessation is associated with intensity of support [84], it is very likely that more intensive support may significantly increase the long-term abstinence rates for cytisine [20].

SAFETY AND ADVERSE REACTIONS

Overdose and toxicity

In 1877 it was known that the consumption of certain parts of plants containing cytisine or drinking of tea made from the flowers or leaves of the plants could cause toxic effects resembling nicotine poisoning in children or the elderly [29].

We found 10 studies reporting toxicity of plant material containing cytisine in humans [85–94]. Among these studies, two reports described fatal cases of *Laburnum* poisoning [89,92]. The first report of *Laburnum* seed poisoning described a fatal case of a 50-year-old schizophrenic man who had swallowed 23 pods (the most likely absorbed dose of cytisine at approximately 50 mg) [92]. The second fatal cytisine intoxication case was described in a 20-year-old man who had drunk tea prepared from *Laburnum*, and cytisine was quantified in post-mortem specimens [89]. Two recent reports of toxicity were not discussed in the previous cytisine reviews [93,94]. Three boys, aged 5–6 years, were accidentally poisoned from ingesting a variable number of *Spartum junceum* (*Genista juncea*, Spanish broom) seeds containing several quinolizidine alkaloids, including cytisine. The intake of one to eight seeds was accompanied by moderate symptoms, including digestive problems, and in one case neurological symptoms and minor metabolic acidosis. The children responded well to a gastric lavage and activated charcoal and were discharged 24 hours after ingestion [94]. Two patients were poisoned after simultaneously ingesting *Baptisia* plant material which contained cytisine. In addition to symptoms resembling nicotine toxicity, profound ataxia was present in both [93].

Analysis of all case reports indicates that poisoning symptoms with cytisine are analogous to symptoms of nicotine intoxication. In the early phase of cholinergic stimulation they include nausea, vomiting, abdominal pain, general weakness, tachycardia, hypertension, respiratory stimulation, pupil dilatation and tremor. The second inhibitory phase consists of bradycardia, hypotension and dyspnea, finally followed by coma and respiratory failure. Exposure to plants containing cytisine can result in severe poisoning, but management with primary emphasis on cardiovascular and respiratory supportive care should result in a full recovery [90].

Despite widespread use in many countries, there have been no recorded deaths in humans caused by the consumption of excessive doses of Tabex® or Desmoxan®. The lethal dose of cytisine in humans is still unknown. According to one quoted report in the literature of overdose and attempted suicide, intake of approximately 40–50 (60–75 mg) and 90 (135 mg) Tabex® tablets did not result in fatal or serious health consequences. In another case, ingestion of 30 mg of cytisine with excessive doses of the antidepressants moclobemide, venlafaxine and mianserin also did not cause any severe or persistent health consequences [95]. In a New Zealand-based trial two people reported that they took all 100 tablets of Tabex® in 1 week and reported no ARs [22].

Clinical trials evaluating cytisine safety and adverse reactions

Serious adverse events

Six trials provided data on serious adverse events (SAEs) [7,21–23,26,28]. An open-label, uncontrolled trial [7] and placebo-controlled study by Vinnikov *et al.* [21] reported no SAEs. Schmidt reported three SAEs in the cytisine group and one in the placebo group [26]. Monova *et al.* reported one serious adverse event (rhabdomyolysis) in the cytisine group and none in the placebo group [28]. In the trial by West *et al.* there were seven SAEs (four among participants receiving cytisine and three among those receiving placebo), five deaths (two in the cytisine group, due to lung cancer and cardiac arrest and three in the placebo group, due to lung cancer, hemorrhagic stroke, and chronic obstructive pulmonary disease) and two hospitalizations (both in the cytisine group, for stroke and tracheal cancer). Most events took place after the treatment phase of the trial and involved participants with longstanding illnesses. None of the SAEs was deemed to be related to cytisine.

There were no significant differences in the frequency of SAEs between cytisine and placebo in controlled trials [7,21,26,28].

In the trial comparing cytisine to NRT, SAEs were self-reported in 6.9% of participants in the cytisine group and 6.0% in those in the NRT group (RR = 1.25, 95% CI = 0.85–1.85) [22]. There were two deaths: one person in the cytisine group died of alcohol-related asphyxiation during the treatment period and one in the NRT group died of a heart attack during follow-up. Neither death was deemed to be treatment-related.

Cytisine sales data from IMS Poland [45] indicate that exposure to cytisine is greater than 30 million patient-days. A recent periodic safety update did not identify any serious and life-threatening signals for cytisine (based on a sample of more than 7 million exposed smokers).

Adverse reactions

There was no difference in overall ARs between cytisine used at its therapeutic dose (1.5–9 mg per day for 25 days) and placebo in clinical trials. The RRs of ARs for each placebo-controlled study included in our meta-analysis are presented in Fig. 6.

In studies conducted in the 1960s and 1970s, the percentage of drug discontinuation was approximately 6% [96] and the most common reason for discontinuation was nausea [8,26]; however, monitoring of frequency of drug discontinuation and ARs led to fragmented results and some doubt surrounding tolerance. In the Polish uncontrolled open-label cytisine trial discontinuation due to ARs was 15.5% [23]. In two new placebo-controlled trials, the percentage of withdrawal or reduction of the dose were 4.7% for cytisine versus 4.7% for placebo [21] and 6.2% for cytisine versus 4.6% for placebo (RR = 1.3, 95% CI = 0.7–2.5) [7]. Levshin *et al.* did not report a discontinuation rate [75]. The discontinuation rate due to ARs observed with cytisine seems to be comparable to discontinuation rates observed with varenicline and bupropion [7].

Among five modern trials [7,21–23,75], in four of these trials [7,21–23] the most frequently reported ARs were gastrointestinal (GI) symptoms (dry mouth, nausea, dyspepsia and upper abdominal pain). In observational uncontrolled trials the most frequent AR was mouth dryness, which occurred in 35% of patients taking cytisine [23]. In two placebo-controlled studies the occurrence of GI symptoms was 3.5% for cytisine versus 3.5% for placebo [21] and 13.8% for cytisine versus 8.1% for placebo [7]. GI symptoms were found to be mild and transient [7,23] In the Levshin *et al.* study most frequent ARs were headache and dizziness, which occurred in 11.3% of participants taking cytisine and 2.5% of participants taking placebo [75].

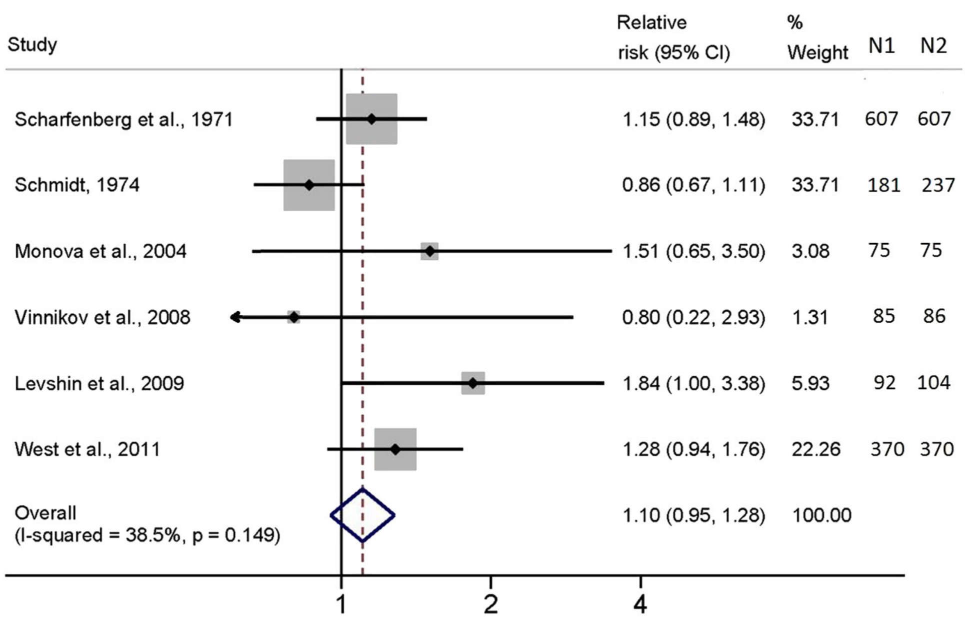

Figure 6 Forest plot of all adverse reactions in six included studies (cytisine versus placebo). N1 = sample size of the cytisine group; N2 = sample size of the placebo group; CI = confidence interval [Colour figure can be viewed at wileyonlinelibrary.com]

In the trial comparing cytisine to NRT, the frequency of all ARs was higher among participants in the cytisine arm (31%) than in those in the NRT arm (20%) [22]. Nausea, vomiting and sleep disorders occurred more frequently in the cytisine group than in the group receiving NRT (4.6 versus 0.03%, respectively) [22]. Among smokers in the cytisine group who reported an adverse event, more than 82% stated they would still recommend cytisine to a smoker who wanted to quit [22].

The frequency and severity of ARs with cytisine compared to varenicline will be determined when current active comparator trials are complete [78,79].

Figure 6 shows the results of our meta-analysis of ARs. Of eight studies in the efficacy analysis, the studies of Paun & Franze [25] and Marakulin *et al*. [27] did not report ARs; therefore, our pooled AR analysis is built on the data from six studies. As shown in Fig. 6, the overall random-effects pooled RR of all ARs in the treatment group did not statistically differ from placebo (RR = 1.10, 95% CI = 0.95–1.28). Because one study [26] used a different approach in adverse event risk calculation, we also performed a sensitivity analysis excluding this study from the analysis, which resulted in the overall RR = 1.25 (95% CI = 1.05–1.49).

So far, clinical studies have not shown that cytisine under its current dosing regimen causes significant cardiovascular ARs [7,22,23]. Also, a recent pharmacokinetic study with cytisine administered at a dose twice the maximum single dose showed no significant effect on cardiovascular parameters [42]. However, caution here is warranted, as data capture is incomplete and no studies have evaluated ARs of cytisine in patients with cardiovascular diseases.

Of note, the modern placebo-controlled trials of cytisine for smoking cessation excluded participants with schizophrenia and schizoaffective disorder and severe forms of major depression and bipolar disorder. However, two observations on the action of cytisine in patients hospitalized in psychiatric clinics have been reported [97]. A patient who had taken 90 tablets of Tabex® for 3–4 days every time he felt a desire to smoke developed psychosis. The psychosis had a polymorphic symptomatology and a fluctuating course with prevailing oneiric, angry and catatonic elements. During remission, another patient, a woman with a schizoaffective disorder, developed psychotic symptoms with maniac-like characteristics within a few days after she had begun taking Tabex® (number of tablets unknown).

In one very small Bulgarian study, Tabex® was used for smoking cessation in five women with depression [97]. A swift increase of working activity followed by an improvement of mood was observed in these patients. This observation is particularly interesting in the context of pre-clinical reports, finding that cytisine works as an antidepressant in animal models [98,99].

However, safety of cytisine in patients with psychoses and affective disorders is unknown and should be considered for future research.

An influence of cytisine on the action of other drugs has been investigated only in one clinical study [72]. No unfavorable interactions with insulin, neuroleptics and antidepressants were observed. Recent studies showed that cytisine antagonized the anticonvulsant activity of lacosamide, lamotrigine, levetiracetam, phenytoin and pregabalin in experimental models of human seizures in mice, and the observed effects of cytisine most probably resulted from the pharmacodynamics mechanism(s) [100,101]. Future research attention is needed on the safety of cytisine use in combination with other medications.

As a drug with a benign safety profile, cytisine seems to be suitable for purchase with minimal or no medical supervision, as is currently the case in Poland, Russia and Canada [102].

Treatment regimen and adherence

First, adherence to the current treatment regimen for cytisine is poor and undermines treatment success, despite its use being associated with increased rates of quitting [20,22]. Very few studies have evaluated treatment adherence, but in two studies that have evaluated medication use, smokers are using fewer cytisine tablets than recommended [20,22]. The most recent randomized clinical trial evaluation of cytisine use (n = 655 smokers) found that at 1 week after quit date, participants reported taking a mean of 49 tablets of the 63 tablets recommended; at 4 weeks, participants reported taking a mean of 72 tablets of the 100 tablets recommended. Only half of participants (53%) were adherent to treatment guidelines (i.e. taking 80 or more tablets). The median time to relapse was significantly longer for those participants who were adherent with treatment (127 days) compared to those who were not adherent (20 days) [22]. Therefore, enhanced treatment adherence strategies are needed to improve quit rates for the traditional 25-day course of cytisine.

Secondly, the dosing regimen for cytisine was first established in the 1960s on an unknown basis, and an optimal dosing schedule has not been scientifically validated. Only in 2015 were the first pharmacokinetic data in humans published [42]. It seems that the current dosage regimen is unlikely to be optimal and requires modification to potentially improve patient compliance. Findings from a New Zealand dose escalation study suggest that dose modifications to the current standard dosing regimen are worthy of further investigation. It may be possible to simplify the current dosing regimen by increasing a single dose (e.g. to 4.5 mg), reducing the dosing frequency and increasing the dosing interval [70]. Studies to explore a simplified cytisine dosing regimen are warranted to improve treatment adherence, which may lead to better treatment efficacy in smokers.

Thirdly, due to a lack of empirical research, there is a need to explore variations in the drug regimen. Changes in dosing regimen might increase efficacy of cytisine. A short duration of cytisine treatment seems to be an advantage as most people do not use smoking cessation medications in accordance with guideline recommendations [103]. Conversely, the administration of cytisine for longer than 25 days (as with varenicline) may provide improved outcomes. Application of maintenance doses or initiation of therapy during smoking may also optimize treatment outcomes [6]. A maintenance dose is being tested in the current cytisine versus varenicline trial in New Zealand [79]. An interesting observation is that some smokers at the end of a 25-day treatment take cytisine *ad hoc* in situations when they have craving (desire to smoke a cigarette) (Tutka, unpublished data, 2019). It is difficult to gauge if such use is problematic, as no study has been undertaken to address this question.

In all clinical trials, cytisine was used at the maximum daily dose not exceeding 9 mg. In a study by Vlaev [97], cytisine (Tabex®) was used in smokers with depression in ascending doses that were increased every day to a maximum daily dose of 22.5 mg. There was no significant increase in ARs compared to traditional treatment regimens identified at the exception of a slight decrease in arterial blood pressure. Both a small sample size (n = 5) and limited study details make it difficult to reach a conclusion on the maximum daily dosage. Consequently, the safety and efficacy of doses, apart from those recommended by the manufacturers, remains unknown.

In Europe, cytisine is currently commercially available in a tablet and capsule form. In Australia, cytisine can be purchased on-line as oral strips [104], but their pharmacokinetics and efficacy has not been studied. It is possible that other forms of the drug, such as buccal films, transdermal patches, inhalers or nasal sprays, could be more effective and/or safer than tablets/capsules. To date, all studies on cytisine were conducted on the oral tablet formulation. Only research in Russia has been carried out on the buccal films containing cytisine alone (1.5 mg), anabasine (a tree tobacco (*Nicotiana glauca*) alkaloid with agonist activity to nAChRs) alone (1.5 mg) or cytisine (0.75 mg) in combination with anabasine (0.75 mg) in a 2-week regimen in 62 smokers. Forty-seven per cent of patients receiving films were reported abstinent at 15 days [105]. Results by group were not indicated, but it was stated that the films containing cytisine alone or cytisine in combination with anabasine were more effective than the films containing anabasine alone. Data from another study using cytisine, anabasine or cytisine/anabasine buccal films in a 2-week regimen, collected after 6 months in 201 smokers (including some psychiatric patients), demonstrated an abstinence or partial effect (defined as a twofold reduction in the number of smoked cigarettes) in 50–75% of participants. However, the results by groups were not given [106]. New formulations might also show an increase of treatment efficacy through improved adherence to the treatment regimen.

Affordability and cost effectiveness

The case for making cytisine available makes sound economic sense. Cytisine is much cheaper than other medicines for smoking cessation that are cost-prohibitive in many developing countries [107,108]. Varenicline, considered as the most effective drug for smoking cessation, has been available since 2006, but its high cost has been a barrier for its wider use, even in countries where there is insurance coverage [9,109]. In Poland, one course of Tabex® is

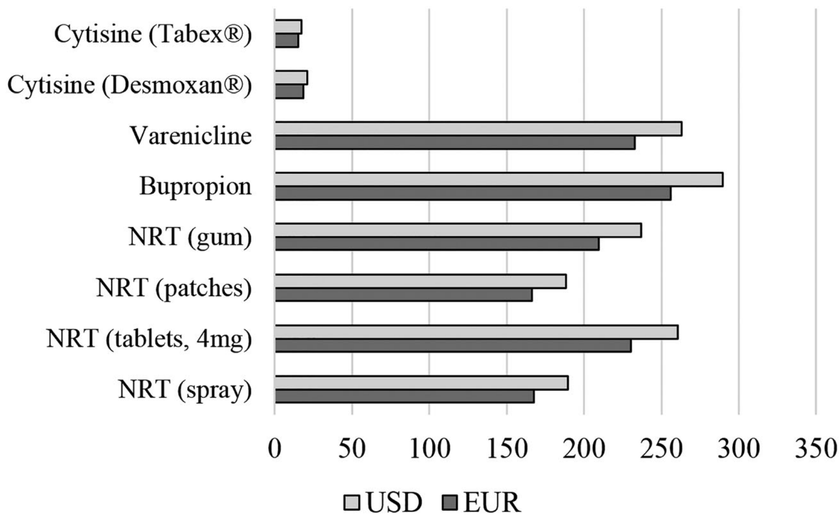

Figure 7 Estimated mean costs of full course of anti-smoking therapies in Poland (01/2017)

Table 3 The cost of treatment with anti-smoking agents in relation to the average monthly wage in Poland.

Treatment	*% of the average monthly salary*
Cytisine (Tabex®)	1.6
Cytisine (Desmoxan®)	1.9
NRT (patches)	17.7
NRT (spray)	17.9
NRT (gum)	22.3
NRT (tablets, 4 mg)	24.6
Varenicline	24.8
Bupropion	27.3

NRT = nicotine replacement therapy.

US$11–17 and Desmoxan® is US$17–21. A comparison of the cost of anti-smoking therapies in Poland is presented in Fig. 7. The cost of one cytisine course is 1.7–2.1% of the average monthly salary, approximately 15 times lower than the 38.5% for a course of varenicline. Table 3 lists the cost of treatment of various anti-smoking agents in relation to the average monthly wage in Poland. The cost of a cytisine treatment course in Bulgaria, Kazakhstan, Kyrgyzstan and Russia is US$8, 13, 9 and 8, respectively.

Due to its efficacy and low price, cytisine has the potential to have an immense benefit on the health-care system budget [110]. The cost savings will be of particular benefit to health-care systems and to consumers in LMICs. Cytisine has been shown to have a lower cost per quality-adjusted life year (QALY) saved than NRT [111]. Recent economic modeling suggests that cytisine may even be more clinically effective and cost-effective than varenicline [107]. This modeling relied upon the results from one placebo-controlled trial [107], and the authors (along with a Health Technology Assessment commissioned by the NHS) conclude that confirmatory trials are necessary to reduce uncertainty about cost-effectiveness [112]. Recent analysis by Anraad *et al*. confirms that smoking cessation services can benefit economically from the inclusion of cytisine as a cessation medication [113]. Interestingly, it has been shown in New Zealand that cytisine (as originating from plants) may be attractive for smokers who prefer natural medicines, e.g. Indigenous people [114] or for those people who do not want to use nicotine-based products.

CONCLUSIONS

Cytisine, a plant-based alkaloid, has been used for several decades in central and eastern Europe as a smoking cessation aid. Activation and desensitization of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (agonist activity) and the reduction of nicotine occupancy at $\alpha 4\beta 2^*$ nAChRs (antagonist activity) seem to be key factors underlying the anti-smoking effects of the drug [13]. Cytisine moderately increases dopamine levels in the mesolimbic system and aids smoking cessation by reducing the severity of nicotine withdrawal symptoms that often precipitate relapse [8]. In addition, cytisine can act as a competitive antagonist by competing with nicotine for receptor occupancy and, as a result, can prevent nicotine from producing its maximal rewarding effect and satisfaction associated with smoking [49,50].

Cytisine has demonstrated positive results from several recent placebo-controlled trials in moderate to heavy smokers. Our updated meta-analysis confirms significant anti-smoking efficacy of cytisine compared to placebo. The latest cytisine trial has found the drug to be superior to NRT for smoking cessation. Cytisine has been well tolerated in clinical trials with no serious safety signals, but the treatment is likely to be associated with more ARs than NRT treatment. Nausea, vomiting, dyspepsia, upper abdominal pain and dry mouth that were mild or moderate were the most common ARs of cytisine. Ultimately, although randomized clinical trials comparing efficacy and safety of cytisine versus varenicline should be a priority, to elucidate how cytisine's efficacy and safety compares to varenicline.

The broad range of topics covered, from basic science to implementation trials, distinguishes our review from previous reviews. This review consolidates and provides new data related to the nicotinic receptor interaction profile of cytisine, its pharmacological behavioral properties, pharmacokinetics and toxicity in humans, and updates clinical evidence of cytisine efficacy for smoking cessation. Future reviews should collect and discuss more comprehensive safety data, especially related to smokers with neuropsychiatric and cardiovascular disorders and use of other medications. A comparison of cytisine efficacy with existing anti-smoking treatments should also be a review priority. Future trials of cytisine may also test more intensive behavioral support.

Cytisine is currently considered a first-line pharmacotherapy for smoking cessation in countries, where access to combined NRT or varenicline is limited due to availability or cost.

The introduction of cytisine as a cheap and cost-effective smoking cessation aid could potentially make a major contribution to reduce smoking rates globally, particularly in developing countries where a cost-effective pharmacotherapy is urgently needed [6,20,43,109,111,115]. World-wide, cytisine's wider availability could lead to a cheaper, more affordable and effective cessation treatment for all smokers.

Declaration of interests

P.T. has undertaken consultancy for Aflofarm, a manufacturer of cytisine. N.L.B. has been a consultant to pharmaceutical companies that market smoking cessation medications, including Achieve Life Sciences, which is developing cytisine for smoking cessation, and a paid expert witness in litigation against tobacco companies. R.J.C. and P.T. are involved in a trial of Desmoxan®, for which Desmoxan® is provided by the manufacturer. D.V. declares no conflicts of interest.

Acknowledgements

R.J.C. is supported by a National Health and Medical Research Council Career Development Fellowship (GNT148497). The National Drug and Alcohol Research Centre at the University of New South Wales (UNSW), Australia is supported by funding from the Australian Government under the Substance Misuse Prevention and Service Improvements Grants Fund and by infrastructure support from the UNSW, Australia. Special thanks go to Drs Dorota Bartusik and David Aebisher for linguistic advice and Patrycjusz Kołodziejczyk for the assistance in drafting the paper. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Health Consequences on Smoking and Tobacco Use; Fact Sheet; Fast Facts. Smoking and Tobacco Use. Available at: http://www.cdc.gov/tobacco/data_statistics/fact_sheets/fast_facts/ (accessed 29 September 2018).
2. World Health Organization (WHO) Tobacco. WHO. Available at: http://www.who.int/mediacentre/factsheets/fs339/en/ (accessed 29 September 2018).
3. Jha P., Ramasundarahettige C., Landsman V., Rostron B., Thun M., Anderson R. N. *et al*. 21st-century hazards of smoking and benefits of cessation in the United States. *N Engl J Med* 2013; **368**: 341–50.
4. Cigarette Use Globally. The Tobacco Atlas. Available at: http://www.tobaccoatlas.org/topic/cigarette-use-globally/ (accessed 29 September 2018).
5. Courtney R. J., Naicker S., Shakeshaft A., Clare P., Martire K. A., Mattick R. P. Smoking cessation among low-socioeconomic status and disadvantaged population groups: a systematic review of research output. *Int J Environ Res Public Health* 2015; **12**: 6403–22.
6. Tutka P. Nicotinic receptor partial agonists as novel compounds for the treatment of smoking cessation. *Expert Opin Investig Drugs* 2008; **17**: 1473–85.
7. West R., Zatonski W., Cedzynska M., Lewandowska D., Pazik J., Aveyard P. *et al*. Placebo-controlled trial of cytisine for smoking cessation. *N Engl J Med* 2011; **365**: 1193–200.
8. Tutka P., Zatoñski W. Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy. *Pharmacol Rep* 2006; **58**: 777–98.
9. Prochaska J. J., Das S., Benowitz N. L. Cytisine, the world's oldest smoking cessation aid. *BMJ* 2013; **347**: f5198.
10. Etter J.-F. Cytisine for smoking cessation: a literature review and a meta-analysis. *Arch Intern Med* 2006; **166**: 1553–9.
11. Tutka P., Wahl H., Dabrowa M. *Cytisine as an Effective Treatment for Nicotine Addiction in Poland: 50 Years of Experience*. Chicago, IL, USA: SRNT Conference; 2016, p. 125.
12. Bartusik D., Aebisher D., Tutka P. A review of the organic synthesis and medicinal applications of the natural product cytisine. *Mod Organ Chem Res* 2016; **1**: 10–23.
13. Rollema H., Hurst R. S. The contribution of agonist and antagonist activities of α4β2* nAChR ligands to smoking cessation efficacy: a quantitative analysis of literature data. *Psychopharmacology (Berl)* 2018; **235**: 2479–505.
14. Reavill C., Walther B., Stolerman I. P., Testa B. Behavioural and pharmacokinetic studies on nicotine, cytisine and lobeline. *Neuropharmacology* 1990; **29**: 619–24.
15. Tutka P., Mroz K., Zatonski W. Cytisine—renaissance of well known alkaloid. Pharmacological aspects of efficacy in the treatment of tobacco dependence. *Farmakoter Psychiat Neurol* 2006; **1**: 33–9.
16. Etter J.-F., Lukas R. J., Benowitz N. L., West R., Dresler C. M. Cytisine for smoking cessation: a research agenda. *Drug Alcohol Depend* 2008; **92**: 3–8.
17. McRobbie H., Hajek P., Bullen C., Feigen V. Rapid review of non-NHS treatments for smoking cessation. 2007. Available at: https://www.nice.org.uk/guidance/ng92/evidence/non-nhs-treatments-for-smoking-cessation-pdf-4788923439 (accessed 15 July 2019).
18. Tzankova V., Danchev N. Cytisine—from ethomedical use to the development as a natural alternative for smoking cessation. *Biotechnol Biotechnol Equip* 2007; **21**: 151–60.
19. Cahill K., Lindson-Hawley N., Thomas K. H., Fanshawe T. R., Lancaster T. Nicotine receptor partial agonists for smoking cessation. In: *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd; 2016.
20. Hajek P., McRobbie H., Myers K. Efficacy of cytisine in helping smokers quit: systematic review and meta-analysis. *Thorax* 2013; **68**: 1037–42.
21. Vinnikov D., Brimkulov N., Burjubaeva A. A double-blind, randomised, placebo-controlled trial of cytisine for smoking cessation in medium-dependent workers. *J Smok Cessat* 2008; **3**: 57–62.
22. Walker N., Howe C., Glover M., McRobbie H., Barnes J., Nosa V. *et al*. Cytisine versus nicotine for smoking cessation. *N Engl J Med* 2014; **371**: 2353–62.
23. Zatonski W., Cedzynska M., Tutka P., West R. An uncontrolled trial of cytisine (Tabex) for smoking cessation. *Tob Control* 2006; **15**: 481–4.
24. Scharfenberg G., Benndorf S., Kempe G. Cytisine (Tabex) as a pharmaceutical aid in stopping smoking. *Dtsch Gesundheit* 1971; **26**: 463–5.
25. Paun D., Franze J. Breaking the smoking habit using cytisine containing 'Tabex' tablets. *Dtsch Gesundheit* 1968; **23**: 2088–91.
26. Schmidt F. Medical support of nicotine withdrawal. Report on a double blind trial in over 5000 smokers (author's translation). *Munch Med Wochenschr* 1974; **116**: 557–64.
27. Marakulin V. S., Komarov V. M., Chuprin V. V. Treatment of nicotinism. *Voen Med Zh* 1984; **1**: 55–8.
28. Monova, D., Nikolov, R. Safety and tolerability of cytisine as a treatment to assist smoking cessation. Kusadasi, Turkey: ESRNT Conference; 2006.
29. Wood H. Preliminary notes on a new medicinal plant and its alkaloid. *Phil Med Times* 1877; **7**: 510.
30. Dale H. H., Laidlaw P. P. The physiological action of cytisine, the active alkaloid of laburnum (*Cytisus laburnum*). *J Pharmacol Exp Ther* 1912; **3**: 205–21.

31. Husemann A., Marmé W. Cytisine. *NED J* 1862; **7**: 908–1025.
32. McDougal O. M., Heenan P. B., Jaksons P., Sansom C. E., Smallfield B. M., Perry N. B. *et al*. Alkaloid variation in New Zealand kōwhai, *Sophora* species. *Phytochemistry* 2015; **118**: 9–16.
33. Adovasio J. M., Fry G. F. Prehistoric psychotropic drug use in northeastern Mexico and trans-Pecos Texas. *Econ Bot* 1976; **30**: 94–6.
34. Seeger R. Cytisine as an aid for smoking cessation. *Med Monatsschr Pharm* 1992; **15**: 20–1.
35. Stoyanov S., Yanachkova M. Treatment of nicotinism with the Bulgarian drug Tabex. *Chimpharm* 1965; **2**: 13.
36. Maliszewski L., Straczynski A. Therapeutic use of Tabex. *Wiadomosci Lek Wars Pol 1960* 1972; **25**: 2207–10.
37. Granatowicz J. Smoking cessation through the use of cytisine and other therapy. *World Smok Health* 1976; **1**: 8–11.
38. Cytisine. Available at: http://eb11.co.uk/index.php?p=CYTISINE38709-h.htm (accessed 29 September 2018).
39. Ing H. Cytisine. Part I. *J Chem Soc* 1931; **0**: 2195–203.
40. Paskov D., Dobrev K. Pharmacological studies on citysine isolation in Bulgaria from *Cytisus laburnum*. *Izv Na Meditsinskite Instituti Bull Inst Med Bulg Akad Na Nauk Sofia Otd Za Biol Meditsinski Nauki* 1953; **8**: 17–28.
41. Paun D., Franze J. Tabex, registering and treatment of smokers with chronic bronchitis in the consultation against tobacco smoking. *Medicobiol Inf* 1970; **3**: 14–9.
42. Jeong S. H., Newcombe D., Sheridan J., Tingle M. Pharmacokinetics of cytisine, an α4β2 nicotinic receptor partial agonist, in healthy smokers following a single dose. *Drug Test Anal* 2015; **7**: 475–82.
43. Walker N., Bullen C., Barnes J., McRobbie H., Tutka P., Raw M. *et al*. Getting cytisine licensed for use world-wide: a call to action. *Addiction* 2016; **111**: 1895–8.
44. Inc Zpharm. Now available: new over-the-counter natural product promises to make quitting smoking easier for millions of Canadians. GlobeNewswire News Room. Available at: http://globenewswire.com/news-release/2017/08/07/1081288/0/en/Now-available-new-over-the-counter-natural-product-promises-to-make-quitting-smoking-easier-for-millions-of-Canadians.html. Published 8 July 2017 (accessed 29 September 2018).
45. Poland National Sales Data 2016/07. IQVIA Pharmascope 2017/12. 2016.
46. Zatoński W., Zatoński M. Cytisine versus nicotine for smoking cessation. *N Engl J Med* 2015; **372**: 1072.
47. Benowitz N. L. Nicotine addiction. *N Engl J Med* 2010; **362**: 2295–303.
48. Corrigall W. A., Franklin K. B., Coen K. M., Clarke P. B. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacology (Berl)* 1992; **107**: 285–9.
49. Coe J. W., Brooks P. R., Vetelino M. G., Wirtz M. C., Arnold E. P., Huang J. *et al*. Varenicline: an α4β2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem* 2005; **48**: 3474–7.
50. Rollema H., Shrikhande A., Ward K. M., Tingley F. D. III, Coe J. W., O'Neill B. T. *et al*. Pre-clinical properties of the α4β2 nicotinic acetylcholine receptor partial agonists varenicline, cytisine and dianicline translate to clinical efficacy for nicotine dependence. *Br J Pharmacol* 2010; **160**: 334–45.
51. Grady S. R., Murphy K. L., Cao J., Marks M. J., McIntosh J. M., Collins A. C. Characterization of nicotinic agonist-induced [3H]dopamine release from Synaptosomes prepared from four mouse brain regions. *J Pharmacol Exp Ther* 2002; **301**: 651–60.
52. Marks M. J., Grady S. R., Salminen O., Paley M. A., Wageman C. R., McIntosh J. M. *et al*. α6β2*-subtype nicotinic acetylcholine receptors are more sensitive than α4β2*-subtype receptors to regulation by chronic nicotine administration. *J Neurochem* 2014; **130**: 185–98.
53. LeSage M. G., Shelley D., Ross J. T., Carroll F. I., Corrigall W. A. Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacol Biochem Behav* 2009; **91**: 461–7.
54. Mansvelder H. D., McGehee D. S. Cellular and synaptic mechanisms of nicotine addiction. *J Neurobiol* 2002; **53**: 606–17.
55. Slater Y. E., Houlihan L. M., Maskell P. D., Exley R., Bermúdez I., Lukas R. J. *et al*. Halogenated cytisine derivatives as agonists at human neuronal nicotinic acetylcholine receptor subtypes. *Neuropharmacology* 2003; **44**: 503–15.
56. Stolerman I. P., Garcha H. S., Pratt J. A., Kumar R. Role of training dose in discrimination of nicotine and related compounds by rats. *Psychopharmacology (Berl)* 1984; **84**: 413–9 .
57. Craft R. M., Howard J. L. Cue properties of oral and transdermal nicotine in the rat. *Psychopharmacology (Berl)* 1988; **96**: 281–4.
58. Chandler C. J., Stolerman I. P. Discriminative stimulus properties of the nicotinic agonist cytisine. *Psychopharmacology (Berl)* 1997; **129**: 257–64.
59. Smith J. W., Mogg A., Tafi E., Peacey E., Pullar I. A., Szekeres P. *et al*. Ligands selective for α4β2 but not α3β4 or α7 nicotinic receptors generalise to the nicotine discriminative stimulus in the rat. *Psychopharmacology (Berl)* 2007; **190**: 157–70.
60. Radchenko E. V., Dravolina O. A., Bespalov A. Y. Agonist and antagonist effects of cytisine in vivo. *Neuropharmacology* 2015; **95**: 206–14.
61. Cunningham C. S., Javors M. A., McMahon L. R. Pharmacologic characterization of a nicotine-discriminative stimulus in rhesus monkeys. *J Pharmacol Exp Ther* 2012; **341**: 840–9.
62. Igari M., Alexander J. C., Ji Y., Qi X., Papke R. L., Bruijnzeel A. W. Varenicline and cytisine diminish the dysphoric-like state associated with spontaneous nicotine withdrawal in rats. *Neuropsychopharmacology* 2014; **39**: 455–65.
63. Clarke P. B. S., Kumar R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br J Pharmacol* 1983; **78**: 329–37.
64. Stolerman I. P., Garcha H. S., Mirza N. R. Dissociations between the locomotor stimulant and depressant effects of nicotinic agonists in rats. *Psychopharmacology (Berl)* 1995; **117**: 430–7.
65. Reavill C., Stolerman I. P. Locomotor activity in rats after administration of nicotinic agonists intracerebrally. *Br J Pharmacol* 1990; **99**: 273–8.
66. Museo E., Wise R. A. Cytisine-induced behavioral activation: delineation of neuroanatomical locus of action. *Brain Res* 1995; **670**: 257–63.
67. Rasmussen T., Swedberg M. D. Reinforcing effects of nicotinic compounds: intravenous self-administration in drug-naive mice. *Pharmacol Biochem Behav* 1998; **60**: 567–73.
68. Jeong S. H., Sheridan J., Newcombe D., Tingle M. Plasma concentrations of cytisine, a commercially available plant-

based alkaloid, in healthy adult smokers taking recommended doses for smoking cessation. *Xenobiotica* 2017; **0**: 1–4.
69. ivps4. Achieve Announces Results of Clinical Study Demonstrating Similar Bioavailability of Cytisine in Fed and Fasted Subjects. Achieve Life Sci. Available at: http://achievelifesciences.com/2017/11/14/achieve-announces-results-of-fed-fasted-clinical-study/ (accessed 29 September 2018).
70. Jeong S. H., Sheridan J., Bullen C., Newcombe D., Walker N., Tingle M. Ascending single dose pharmacokinetics of cytisine in healthy adult smokers. *Xenobiotica* 2019; https://doi.org/10.1080/00498254.2018.1557760
71. A Pharmacokinetic and Pharmacodynamic Evaluation of Cytisine in Healthy Smokers—Full Text View—Clinical Trials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03303911 (accessed 29 September 2018).
72. Stoyanov S., Yanachkova M. Tabex—therapeutic efficacy and tolerance. *Savr Med* 1972; **23**: 6.
73. Benndorf S., Kempe G., Scharfenberg G., Wendekamm R., Winkelvoss E. Results of pharmacological smoking cessation using cytisine (Tabex). *Dtsch Gesundheitsw* 1968; **23**: 2092–6.
74. Castaldelli-Maia J. M., Martins S. S., Walker N. The effectiveness of cytisine versus nicotine replacement treatment for smoking cessation in the Russian Federation. *Int J Drug Policy* 2018; **58**: 121–5.
75. Levshin V., Slepchenko I., Radkevich N. Randomized controlled trial of cytisine for treatment of tobacco dependence. *Vopr Narkol* 2009; **5**: 13–22.
76. Dobreva D., Danchev N., Nikolova I. Tabex–Sopharma—natural alternative for smoking cessation and treatment of nicotine dependence. *Pharmacia* 2005; **52**: 30–5.
77. Moher D., Liberati A., Tetzlaff J., Altman D. G., Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLOS Med* 2009; **6**: e1000097.
78. Thomas D., Farrell M., McRobbie H., Tutka P., Petrie D., West R. *et al*. The effectiveness, safety and cost-effectiveness of cytisine versus varenicline for smoking cessation in an Australian population: a study protocol for a randomised controlled non-inferiority trial. *Addiction* 2018; **114**: 923–33.
79. Walker N., Smith B., Barnes J., Verbiest M., Kurdziel T., Parag V. *et al*. Cytisine versus varenicline for smoking cessation for Māori (the indigenous people of New Zealand) and their extended family: protocol for a randomized non-inferiority trial. *Addiction* 2019; **114**: 344–52.
80. Global Research Awards for Nicotine Dependence (GRAND). Available at: http://www.grandawardsprogram.org/previous-awards/previous-grand-awards.html (accessed 29 September 2018).
81. Studying Partial-agonists for Ethanol and Tobacco Elimination in Russians With HIV (St PETER HIV—Full Text View—ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02797587 (accessed 29 September 2018).
82. Dogar R., Barua D., Boeckmann M., Elsey H., Fatima R., Gabe R. *et al*. The safety, effectiveness and cost-effectiveness of cytisine in achieving six-month continuous smoking abstinence in tuberculosis patients—protocol for a double-blind, placebo-controlled randomized trial. *Addiction* 2018; **113**: 1716–26.
83. TCTR, Thai Clinical Trials Registry. Available at: http://www.clinicaltrials.in.th/index.php?tp=regtrials&menu=trialsearch&smenu=fulltext&task=search&task2=view1&id=1899 (accessed 29 September 2018).
84. Treating Tobacco Use and Dependence: 2008 Update. US Department of Health and Human Services; 2008.
85. Mitchell R. G. Laburnum poisoning in children report on ten cases. *Lancet* 1951; **258**: 57–8.
86. Forrester R. M. Have you eaten laburnum? *Lancet* 1979; **1**: 1073.
87. Morkovský O., Kucera J. Mass poisoning of children in a nursery school by the seeds of *Laburnum anagyroides*. *Cesk Pediatr* 1980; **35**: 284–5.
88. Furet Y., Ernouf D., Brechot J. F., Autret E., Breteau M. Collective poisoning by flowers of laburnum. *Presse Medicale Paris Fr 1983* 1986; **15**: 1103–4.
89. Musshoff F., Madea B. Fatal cytisine intoxication and analysis of biological samples with LC–MS/MS. *Forensic Sci Int* 2009; **186**: e1–e4.
90. Schep L. J., Slaughter R. J., Beasley D. M. G. Nicotinic plant poisoning. *Clin Toxicol* 2009; **47**: 771–81.
91. Aston B. C. The poisonous, suspected, and medicinal plants of New Zealand. *NZJ Agric* 1923; **26**: 149–56.
92. Richards H. G. H., Stephens A. A fatal case of laburnum seed poisoning. *Med Sci Law* 1970; **10**: 260–6.
93. Anderson M. J., Kurtycz D. F., Cline J. R. Baptisia poisoning: a new and toxic look-alike in the neighborhood. *J Emerg Med* 2015; **48**: 39–42.
94. Giménez N., Magro N., Cortés N., Guitart R. Poisoning after ingestion of Spartium junceum seeds: dose-dependent effects in three boys. *J Emerg Med* 2017; **53**: e41–e44.
95. Majewska M., Szponar J., Pyra E., Kostek H., Kujawa A. Serotonin syndrome in the course of drug-poisoning—case presentation. *Przegl Lek* 2011; **68**: 523–6.
96. Tabex. Product Monograph. Sofia, Bulgaria: Sopharma; 2006.
97. Vlaev S. *Possibilities and limits of application of Tabex to depressive patients*. Sofia: Institute of Neurology, Psychiatry and Neurosurgery; 1993.
98. Mineur Y. S., Einstein E. B., Seymour P. A., Coe J. W., ONeill B. T., Rollema H. *et al*. α4β2 nicotinic acetylcholine receptor partial agonists with low intrinsic efficacy have antidepressant-like properties. *Behav Pharmacol* 2011; **22**: 291–9.
99. Han J., Wang D., Liu S., Zhao M. Cytisine, a partial agonist of α4β2 nicotinic acetylcholine receptors, reduced unpredictable chronic mild stress-induced depression-like behaviors. *Biomol Ther* 2016; **24**: 291–7.
100. Tutka P., Kondrat-Wróbel M. W., Zaluska K., Żółkowska D., Florek-Łuszczki M., Łuszczki J. J. Cytisine inhibits the protective activity of various classical and novel antiepileptic drugs against 6 Hz-induced psychomotor seizures in mice. *Psychopharmacology (Berl)* 2017; **234**: 281–91.
101. Tutka P., Mróz T., Bednarski J., Styk A., Ognik J., Mosiewicz J. *et al*. Cytisine inhibits the anticonvulsant activity of phenytoin and lamotrigine in mice. *Pharmacol Rep* 2013; **65**: 195–200.
102. West R., Raw M., McNeill A., Stead L., Aveyard P., Bitton J. *et al*. Health-care interventions to promote and assist tobacco cessation: a review of efficacy, effectiveness and affordability for use in national guideline development. *Addiction* 2015; **110**: 1388–403.
103. Wiggers L. C., Smets E. M., Oort F. J., de Haes H. C., Storm-Versloot M. N., Vermeulen H. *et al*. Adherence to nicotine replacement patch therapy in cardiovascular patients. *Int J Behav Med* 2006; **13**: 79–88.

104. TBX-Free Stop Smoking Aid Oral Strips—how to quit smoking. Men's Health Cures. Available at: https://www.menshealthcures.com/tbx-free/. Published 5 April 2017 (accessed 29 September 2018).
105. Ostrovskaya T. P. Clinical trial of antinicotine drug-containing films. *Biomed Eng* 1994; **28**: 168–71.
106. Metelitsa V. I. Pharmacological agents in controlling smoking. *BiulletenVsesoiuznogo Kardiologicheskogo Nauchnogo Tsentra AMN SSSR* 1986; **10**: 109–12.
107. Leaviss J., Sullivan W., Ren S., Everson-Hock E., Stevenson M., Stevens J. W. *et al*. What is the clinical effectiveness and cost-effectiveness of cytisine compared with varenicline for smoking cessation? A systematic review and economic evaluation. *Health Technol Assess* 2014; **18**: 1–120.
108. Aveyard P., Raw M. Improving smoking cessation approaches at the individual level. *Tob Control* 2012; **21**: 252–7.
109. Aveyard P., West R. *Cytisine and the Failure to Market and Regulate for Human Health*. London: BMJ Publishing Group Ltd and British Thoracic Society; 2013.
110. Cornuz J., Gilbert A., Pinget C., McDonald P., Slama K., Salto E. *et al*. Cost-effectiveness of pharmacotherapies for nicotine dependence in primary care settings: a multinational comparison. *Tob Control* 2006; **15**: 152–9.
111. Stapleton J. A. The case for licensing cytosine now for smoking cessation is overwhelming. *BMJ* 2013; **347**: f5736.
112. Stapleton J. A., West R. A direct method and ICER tables for the estimation of the cost-effectiveness of smoking cessation interventions in general populations: application to a new cytisine trial and other examples. *Nicotine Tob Res* 2012; **14**: 463–71.
113. Anraad C., Cheung K.-L., Hiligsmann M., Coyle K., Coyle D., Owen L. *et al*. Assessment of cost-effective changes to the current and potential provision of smoking cessation services: an analysis based on the EQUIPTMOD. *Addiction*; **113**: 96–105.
114. Thompson-Evans T. P., Glover M. P., Walker N. Cytisine's potential to be used as a traditional healing method to help indigenous people stop smoking: a qualitative study with Māori. *Nicotine Tob Res* 2011; **13**: 353–60.
115. Rigotti N. A. Cytisine—a tobacco treatment hiding in plain sight. *N Engl J Med* 2014; **371**: 2429–0.