Review Article | Basic and Mechanism Sciences

A systematic review of preclinical studies evaluating the association between nicotine and the initiation and progression of cancer

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Background: The association between cigarette smoking and the increased risk of many cancers is well established. Conversely, epidemiological studies of smokeless tobacco demonstrate decreased risk, or no elevated risk, of certain cancers versus smoking. However, it is unclear what role, if any, nicotine plays in these associations. The objective of this systematic review was to synthesize the available evidence from preclinical studies that examined the potential association between nicotine and the initiation and/or progression of cancer.

Methods: MEDLINE, Embase, PsychInfo, and Cochrane Database of Systematic Reviews were searched for articles published from inception until February 13, 2022. Studies were eligible for inclusion if they evaluated animal cancer or tumor models, compared nicotine and non-nicotine groups, and evaluated measures of cancer initiation or progression.

Results: Among 1,137 identified articles, 61 were included in qualitative synthesis. Twelve studies reported data on tumor initiation, and 54 studies reported data on tumor progression. The majority of the tumor initiation studies did not identify an association between nicotine exposure and an increased risk of spontaneous tumor initiation. Results of tumor progression studies were inconsistent and varied across the reported measures, cancer type being evaluated, and animal cancer model used. Overall, the quality of reporting was poor, with many studies not demonstrating a high level of internal and/or external validity.

Conclusions: In conclusion, although animal models have provided invaluable data for human health risk assessments of chemical exposures, the heterogeneity across the studies included in this systematic review make the interpretation and generalizability of the results difficult.

Keywords: Cancer; nicotine; preclinical; tumor initiation; tumor progression

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Introduction

Background

Cancer is among the leading causes of death and disability globally (1,2). According to GLOBOCAN estimates, there were 19.3 million new cases of cancer in 2020 (3). Risk factors for cancer vary by cancer type and include modifiable and non-modifiable risk factors (4,5). Specifically, non-modifiable risk factors include age, sex, family history, and genetics, while modifiable risk factors include environmental, dietary, and lifestyle factors (4,5). Among modifiable risk factors, cigarette smoking accounted for the highest proportion (19%) of preventable cases of cancer (5).

Rationale and knowledge gap

The association between cigarette smoking and an increased risk of cancer is supported by a large body of evidence. Systematic reviews and meta-analyses that evaluated the evidence on the association between cigarette smoking and cancer have concluded that current cigarette smokers, compared to never-smokers, have a significantly higher risk of developing some cancer types, including lung (6-8), breast (8), pancreatic (8,9), colorectal (8,10), gastric (8,11), and head and neck (8), and a significantly higher mortality from breast (8,12), colorectal (8,10), lung (8), head and neck (8), gastric (8), and pancreatic (8) cancer. Evidence also shows that the increased risk of some cancers is related to the number of cigarettes smoked, with those who smoke the highest number of cigarettes per day having a greater risk of lung (7,13), colorectal (10), and gastric (11) cancer compared with those who smoke fewer cigarettes per day.

Carcinogenesis is a multi-stage process that includes tumor initiation, promotion, malignant conversion, and progression (14). Tumor initiation involves irreversible genetic damage or epigenetic changes (14,15). While tumor initiation is an irreversible process, exposure to a tumor initiator is not in itself sufficient to result in tumor formation (16). Tumor promotion involves a multi-stage, reversible process that results in proliferation of initiated cells (14-16). Tumor promoters are not mutagenic, and are not carcinogenic alone, but can accelerate tumor formation after exposure to a tumor initiator, increase the number of tumors formed in that tissue, or induce tumor formation in conjunction with a dose of an initiator that is too low to be carcinogenic alone (14). A compound that is capable of acting as both an initiator and a promoter in the same tissue is defined as a complete carcinogen (16). Malignant conversion involves the transformation of a pre-neoplastic cell into a malignant phenotype and requires further genetic changes (14-16). Repeated administration of a tumor promoter is more consequential than the total dose, and if administration is discontinued before the conversion into a malignant phenotype, premalignant lesions may regress. Tumor progression requires further genetic changes and involves the expression of the malignant phenotype and the acquisition of more aggressive characteristics by malignant cells over time, including metastasis (14-16).

Carcinogenicity testing involves a comprehensive and integrated approach that aims to evaluate a role of a substance in tumor initiation and promotion, or progression (17). Although data from human studies are considered as the highest level of evidence for carcinogenic potential of a substance, experimental animal studies can provide sufficient evidence on carcinogenic risks to humans in cases when human data are not available (18).

Cigarette smoke contains more than 60 carcinogens that have been evaluated by the International Agency for Research on Cancer (IARC) as having sufficient evidence of carcinogenicity in either laboratory animals or humans (19). All of these compounds are carcinogenic in laboratory animals, and 15 are rated as carcinogenic in

Highlight box

Key findings

- The majority of the tumor initiation studies did not identify an association between nicotine exposure and increased risk of spontaneous tumor initiation. Results of tumor progression studies were inconsistent and varied across the reported measures, cancer type being evaluated, and animal cancer model used.

What is known and what is new?

- The objective of this systematic review was to synthesize the evidence from preclinical studies that examined the potential association between nicotine and the initiation/progression of cancer. The association between cigarette smoking and increased risk of many cancers is well established.
- Conversely, epidemiological studies of smokeless tobacco demonstrate decreased risk/no elevated risk, of certain cancers versus smoking. However, it is unclear what role, if any, nicotine plays in these associations.

What is the implication, and what should change now?

- Although animal models have provided invaluable data for human health risk assessments of chemical exposures, the heterogeneity across the studies included in this systematic review limit the interpretation and generalizability of the results in this evidence base.

humans by the IARC. Notably, nicotine—the addictive component of tobacco smoke—is not included in the IARC list of carcinogens. Indeed, it is unclear what role nicotine plays, if any, in the carcinogenicity of tobacco smoke. Although some *in vitro* studies have shown that nicotine may lead to cellular changes associated with cancer initiation and progression (20-25), it is unclear whether these cellular changes are associated with cancer development *in vivo* in animals, or in humans.

Objective

The primary objective of this systematic review was to synthesize the available evidence from preclinical studies examining the potential association between nicotine and the initiation and/or progression of cancer. We conducted this review in accordance with the A MeaSurement Tool to Assess systematic Reviews-2 (AMSTAR-2) guidelines. We present this article in accordance with the PRISMA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1710/rc) (26).

Methods

The protocol for this review was registered with the PROSPERO international prospective register of systematic reviews on February 4, 2022 (PROSPERO 2022 CRD42022308897; available at: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=308897). The key question for this review was predefined as: *Does evidence from preclinical studies support an association between nicotine and the initiation and progression of cancer?*

Literature search

The literature search was conducted by an information specialist who has credentials as a health sciences librarian and is qualified in conducting systematic literature searches. Search terms were developed using medical subject headings (MeSH) and text words related to the associations between nicotine and the initiation and progression of cancer. The search strategy included the use of synonyms of search terms, truncation, wild card symbols, Boolean logic, proximity operators, and limits, in order to focus the search towards the most relevant preclinical literature.

The following online databases were searched for relevant articles published from inception to February 13, 2022: MEDLINE, Embase, PsychINFO, and Cochrane Database of Systematic Reviews (included as part of the Embase search). The literature search strategy can be found at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section A).

Other methods used for identifying relevant research included: a grey literature search; searching of bibliographies of included studies and of relevant published systematic reviews and meta-analyses; searching of trial registries; and contacting experts in the field.

Inclusion criteria

The PICOS (Population or participants and conditions of interest, Interventions or exposures, Comparisons or control groups, Outcomes of interest, and Study designs) review method was used, as it is an objective, non-biased, systematic review method (27,28). The following inclusion criteria were applied:

- Population or participants and conditions of interest:
  - *In vivo* animal cancer or tumor models.
- Interventions or exposures:
  - Nicotine exposure.
- Comparisons or control groups:
  - No nicotine exposure.
- Outcomes of interest:
  - Measures of cancer initiation and progression, including but not limited to:
    - Tumor initiation;
    - Tumor promotion;
    - Tumor growth;
    - Tumor invasion;
    - Tumor angiogenesis;
    - Metastasis;
    - Extravasation;
    - Intravasation.
- Study designs:
  - Controlled or uncontrolled preclinical animal studies.

The intervention criterion allowed for nicotine administered by various means, such as intraperitoneally or in drinking water. However, the intervention did not allow for nicotine to be administered using a tobacco product or byproduct, such as cigarette smoke, in order to isolate the effects of nicotine.

Control groups were required to have a regimen that did not administer nicotine in any form. As the criteria for the control was strictly "no nicotine exposure", this allowed for any vehicle that was administered in the same manner as the intervention group.

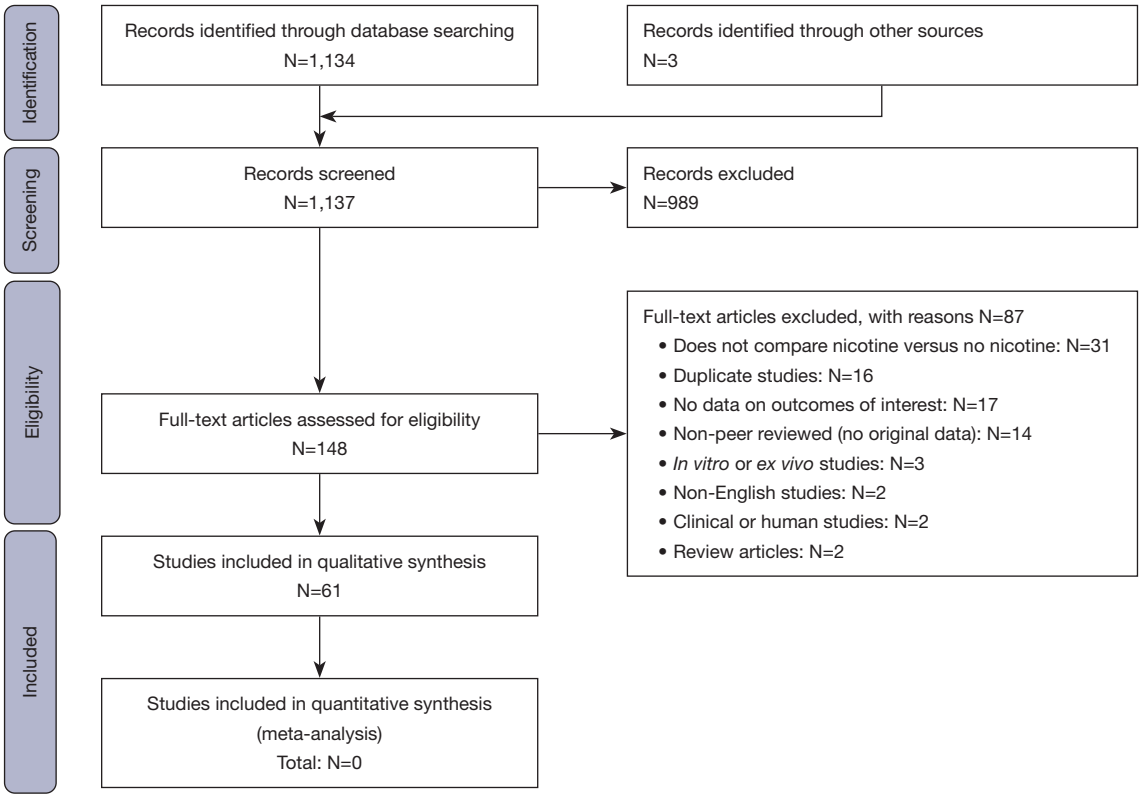

Figure 1 PRISMA flow diagram.

Exclusion criteria

The following studies were excluded from the systematic review:

(I) Clinical or human studies, including post-mortem studies.
(II) Review articles, systematic reviews, and meta-analyses (unless these articles contained original data not previously identified).
(III) Letters to the editor, opinions, editorials, press releases, manufacturers' advertisements, and other non-peer reviewed publications, unless the publication contains original data from preclinical studies.
(IV) *In vitro* or *ex vivo* studies.
(V) Articles in which the abstract and full text is non-English.
(VI) Duplicate articles or articles with the exact same study outcome data as another published article.

Data management

This review was conducted in the systematic review software platform DistillerSR® (Evidence Partners, Ottawa, Canada). The PRISMA flowchart (*Figure 1*) specifies the numbers of studies identified and examined, per PRISMA reporting guidelines (29), and the flowchart was generated in DistillerSR®.

Review methods

Study selection process

Title/abstract review was completed for level 1 review. Subsequently, full text articles were obtained, including those for any articles that could not be excluded based on the title/abstract alone. Each article was independently screened by two reviewers, according to the inclusion criteria and any inclusion/exclusion disagreements between reviewers were resolved in a meeting between reviewers based on mutual agreement. Any disagreements that could not be resolved between the reviewers were decided by a third reviewer at Thera-Business Inc. with reasons for exclusion documented.

Data extraction

Data were independently extracted by one reviewer and checked by a second reviewer with any disagreements resolved through discussion between the two reviewers. Any unresolved disagreements were resolved by a third team member, when necessary. Data extraction forms were hosted on DistillerSR® and information regarding the study characteristics and outcomes—as defined in the PICOS—were extracted for each study. The extracted information included: year of publication; location; funding; study design; acclimation period; wash-out/pre-treatment period; sample size; animal model (species, sex, weight, age, comorbidities, cancer/tumor model, and cancer cell line injected); study methodology; intervention(s) and control(s); study duration; and study outcomes. Any supplementary materials provided by publications were reviewed for relevant data and extracted accordingly, when applicable. Study authors were contacted for any needed clarification on reported data when necessary.

Risk of bias assessment

This review used the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) Risk of Bias tool to assess risks of bias from the following sources: sequence generation (selection bias); baseline characteristics (selection bias); allocation concealment (selection bias); random housing (performance bias); blinding (performance bias); random outcome assessment (detection bias); blinding (detection bias); incomplete outcome data (attrition bias); selective outcome reporting (reporting bias); and other sources of bias (30). These potential sources of bias were each graded as either "low", "high", or "unclear" risk; subsequently, an overall risk of bias grade was given for each study.

Two reviewers independently performed the risk of bias assessments by two researchers. In instances of disagreement, the scoring was discussed and resolved in a meeting between the two reviewers, and a joint decision was made. Any further unresolved disagreements between the reviewers were decided by a third reviewer. The overall risk of bias was determined for each study as: "low"—the study was judged to be at "low" risk across all evaluation domains; "high"—the study was rated as "high" risk in at least one domain; and "unclear"—the study was assessed as "unclear" risk in at least one bias domain, and no bias domains were assessed as "high" risk.

Strength of evidence (SOE)

In the context of preclinical studies, assessment of the certainty in the evidence may also facilitate the translation of findings to clinical studies and, ultimately, clinical practice. To grade the confidence in the overall conclusions for each outcome, a systematic, objective, and transparent assessment of the overall SOE was planned. The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach—the most widely used approach to evaluate certainty of evidence—provides a systematic, transparent and explicit framework for assessing strength of clinical evidence; a modified version of the GRADE tool to rate the certainty and SOE of preclinical (animal) studies was considered for use in this systematic review (31).

Adequacy evaluation

Adequacy of the included studies, evaluated using the criteria based on the current international guidelines for carcinogenicity testing (32) and as described by Haussmann and Fariss (33) was done as a secondary analysis. The evaluation was based on the following criteria: route of administration, group size, dose-response, average daily dose, duration of exposure (studies of tumor initiation only), and study quality. If the minimum criteria were met, the study was assigned a plus score. If the criteria were not met, or information was lacking, the study was assigned a minus score. An overall adequacy score was determined by totaling the number of plus values for individual studies. Studies with an overall score ≥ 3 were considered to be high adequacy, and those with an overall score of ≤ 2 were considered to be low adequacy.

Meta-analysis

This review was designed to pool data from included studies in meta-analyses, where appropriate. Studies were considered for meta-analyses when head-to-head data for outcomes measured were similar, and when studies used the same species, tumor model, and type of intervention and comparator.

Protocol deviations

An update to the protocol was registered with PROSPERO on August 4th, 2022, reflecting the addition of the adequacy

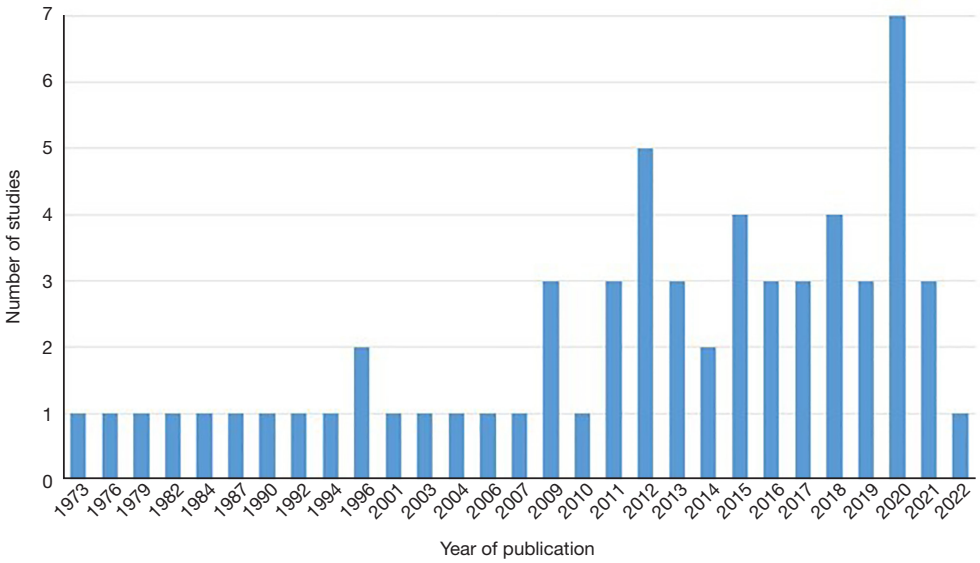

Figure 2 Included studies by publication year.

evaluation of the included studies based on current international guidelines for carcinogenicity testing (32) as a secondary analysis.

Results

Search results

A total of 1,134 articles were retrieved from the databases, and three additional articles were identified through other sources (34-36), bringing the total number of potentially relevant articles to 1,137. Of these potentially relevant articles, 989 were excluded at the title/abstract screening level, resulting in 148 articles being screened at the full-text level [available at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section B) for titles and abstracts of articles screened at the full-text level]. Of these 148 articles, 87 were excluded at the full text level [available at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section C) for table of studies excluded at the full text level and reasons for exclusion], resulting in 61 relevant studies eligible for inclusion in the review [available at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section D) for listing of included studies]. The weighted overall kappa for inter-rater reliability at level 2 screening was 0.82.

All selection steps are presented as a PRISMA flow diagram (*Figure 1*). All 61 studies were included in the qualitative synthesis of evidence: 12 studies for tumor initiation, 54 studies for tumor progression; five studies reported data for both tumor initiation and tumor progression. Quantitative synthesis was not performed due to a lack of studies that used the same animal models, tumor models, and method and dose of nicotine administration.

Characteristics of the included studies

The supplementary material (section E) is available at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx contains summary tables with the complete study and sample characteristics for each of the included studies. The supplementary material (sections F and K) available at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx contains further details on the characteristics of the included studies.

Publication dates ranged from 1973 to 2022, with 18 studies published between 2018 and 2022, 15 studies published between 2013 and 2017, 13 studies published between 2008 and 2012, four published between 2003 and 2007, and 11 studies published in 2001 or earlier (*Figure 2*). The highest proportion of studies were conducted in North America, with the rest of the studies coming from Asia, Europe, South America, or multiple regions (*Figure 3*).

The majority of included studies used mice, eight studies used rats (35,37-43), and three studies used hamsters (44-46). Across the 50 of the 61 included studies that reported sample size for the nicotine and control groups,

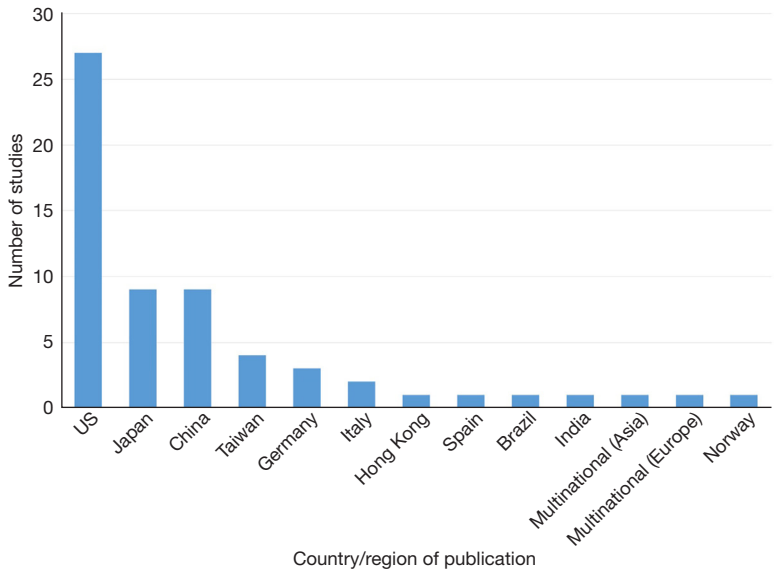

Figure 3 Included studies by country/region of publication.

total sample size ranged from six to 858 animals, for a total of 3,987 animals included across all studies that reported sample size. Of these, a total of 1,414 animals were allocated to a nicotine group, and 1,043 animals were allocated to a control group. Study duration ranged from 7 days to 24 months.

Among the 61 included studies, 45 studies were randomized controlled trials (RCTs), and the remaining 16 studies were controlled, parallel group studies. The most common method of administration was oral administration of nicotine in drinking water (36,42,45,47-66), followed by intraperitoneal injections (34,40,41,55,67-83), subcutaneous injections or subcutaneous continuous infusions (37-39,43,84-90), and buccal administration (46,91,92). Other methods of nicotine administration, used in one study each, included application of a dermal patch (70), intravenous injections (36), gavage (93), and inhalation (35). Across the 53 studies that reported treatment duration, treatment duration ranged from 7 days (88,93) to 24 months (35,46,85). Thirteen studies evaluated biomarkers of nicotine exposure (34,35,42,50,51,54,55,57,60,66,70,72,86).

Across the 61 included studies, 19 studies evaluated digestive cancers (44,45,48,51,56,61,64-68, 71,72,76,79,80,84,87,93), 19 studies evaluated lung cancers (36,47,49,50,52,54,55,57,58,62,70,73,77,81,83,86,88-90), six studies evaluated breast cancers (34,38,53,60,74,75), six studies evaluated head and neck cancers (46,69,78,91,92,94), and two studies evaluated urinary tract cancers (42,82). Nine studies evaluated other cancer types, or evaluated cancers at multiple sites (35,37,39-41,43,59,63,85). Most studies used a xenograft or an allograft tumor model, 14 studies used a carcinogen-induced tumor model (37,38,42, 45,46,55,57,58,65,70,73,84,87,91), and four studies used a genetic tumor model (51,55,59,62). Four studies used more than one tumor model within the same study: three studies used a xenograft and a carcinogen-induced tumor model (58,70,91), and one study used a carcinogen-induced tumor model, a genetic tumor model, and an allograft tumor model (55). Thirteen studies evaluated the spontaneous occurrence of tumors after the administration of nicotine (35,37-39,41,43,44,46,55,57,63,85,91).

Although all studies included relevant outcome measures of cancer initiation and/or progression, these outcomes were the primary evaluations in only approximately a third of the studies (35,37,38,41,42,44,46,50,52, 55-57,63,70,74,84,85,87,89,93). In most studies, the effects of nicotine on tumor signaling pathways were the primary evaluation of interest. In the remaining studies, the primary evaluations of interest were: the effects of nicotine on cancer therapy (78,79,88,90); the effects of perinatal nicotine administration on age at death and disease onset (39); the effects of nicotine on anti-inflammatory pathways, food intake, and body composition in a model of anorexia-cachexia (40); and, the chronic effects of nicotine administration on body weight, organ weights, and pathology (43).

Overall risk of bias grades for the 61 included studies

Table 1 Summary of results of tumor initiation studies by direction of outcome

Outcomes (n studies)	No tumors in either group	Nicotine higher	Control higher	No difference	Result unclear[†]
Tumor incidence (n=12)	4	1[‡]	0	5[§]	2[¶]
Tumor multiplicity (n=2)	–	0	0	1	1
Tumor volume (n=1)	–	0	0	1	0
Tumor induction period (n=1)	–	0	0	0	1

[†], includes studies that did not perform statistical analysis. [‡], statistical analysis was not done, however, tumors were observed in 11 of 14 animals in the nicotine group and 0 of 5 animals in the control group. [§], in two studies (55,57), statistical analysis was not done; however, the number of animals with tumors was similar between groups and therefore it is assumed that there were no differences. [¶], statistical analysis was not performed, however, tumor incidence was numerically higher in the nicotine group than in the control group in one study (43), and numerically lower in the nicotine compared with the control group in one study (63).

were as follows: no studies were graded as having a "low" risk of bias, 18 studies (30%) were graded as having a "high" risk of bias and 43 studies (70%) were graded as having an "unclear" risk of bias. The risk of bias assessment with full scoring for individual studies is provided at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section G).

Of the 12 studies of tumor initiation, five studies had high adequacy scores, while seven studies had low adequacy scores. Of the 54 tumor progression studies, 16 studies had high adequacy scores, while the remaining 38 studies had low adequacy scores. The study adequacy scores for individual studies are provided at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section H).

Tumor initiation and progression

Qualitative synthesis of the included studies that evaluated cancer initiation and/or progression is presented below. The complete data extractions, with study characteristics and outcome data are provided in the summary tables in https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx (section F). The supplementary materials (section I) at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx contains full details on the characteristics of the included tumor initiation studies, and (section J) at https://cdn.amegroups.cn/static/public/atm-23-1710-1.docx contains full details on the characteristics of the included tumor progression studies.

Tumor initiation

Overall, tumor initiation studies failed to show a difference in tumor initiation between the nicotine and control groups, although there were some inconsistencies in the results across studies (*Table 1*). Of the 12 studies that evaluated tumor initiation, nine showed that the incidence of tumors was not different between the nicotine and control groups (35,37-39,41,44,55,57,91), with no tumors being observed in either group in four of those studies (38,39,44,91). None of the remaining three studies conducted statistical analysis, and therefore it is unclear whether tumor incidence was significantly different between the nicotine group and the control group (43,63,85). However, in one of these three studies, skeletal muscle and uterine muscle sarcomas were observed in 11 of 14 animals in the nicotine group and 0 of 5 animals in the control group following administration of an LD_{50} dose of nicotine (3 mg/kg) to mice subcutaneously 5 days per week for 24 months (85). One of the two remaining studies showed numerically lower tumor incidence (63), while the other showed numerically higher tumor incidence (43) in the nicotine group compared with the control group.

Findings of tumor multiplicity were inconsistent: one of the two studies that evaluated it showed no differences between the nicotine and control groups (55), while the other study did not perform statistical analysis (57). One of these studies also evaluated tumor volume and observed no differences between the nicotine and control group (55). In the only study that evaluated tumor induction period, the time to tumor induction was numerically shorter in the nicotine group compared with the control group, although statistical comparison was not provided (41).

Tumor progression

Overall, results of tumor progression studies varied across the outcome measures reported, and at least some degree of inconsistency in results was observed among the majority of outcomes assessed (*Table 2*). Additionally, given that some

Table 2 Summary of results of tumor progression studies by direction of outcome

Outcomes (n studies)	Nicotine higher	Effect dose-related[†]	Control higher	No difference	Result unclear[‡]
Tumor volume (n=32)	24	0/2	0	4	4
Tumor weight (n=14)	10	0/1	0	3	1
Metastasis (n=13)	8	0/1	0	3	2
Proliferation (n=10)	8	0/3	0	1	1
Tumor incidence (n=11)[§]	4	1/1	0	5	1
Angiogenesis (n=6)	3	1/1	0	1	1
Tumor multiplicity (n=8)	1	1/1	1	4	1
Tumor growth (n=5)	3	0/1	0	2	0
Tumor area (n=3)	2	0/1	0	1	0
Metastasis-free survival (n=2)	2	NA	0	0	0
Micrometastasis (n=1)	0	NA	0	1	0
Time to appearance (n=1)	0	NA	0	1	0

All studies (n=54). [†], the denominator represents the number of studies that used more than one dose of nicotine. NA indicates that no studies evaluated the outcome with more than one dose of nicotine. [‡], includes studies that did not statistically compare groups. [§], incidence of nervous system tumors was lower in the nicotine than in the control group in one study, however, there were no differences between groups in overall incidence of tumors. NA, not applicable.

studies did not perform statistical comparison, it was unclear whether there were any significant differences between groups. However, generalizations about some outcomes can be made, given that the majority of studies evaluating those outcomes showed the same direction of effect. Tumor volume, evaluated by the largest number of studies, was significantly higher in the nicotine group compared with the control group in the majority (24 of 32) of studies (47-50,54,56,58,64-71,73,74,78,79,82,83,86,93,94), although four studies reported no differences between groups (52,55,88,90), and results were unclear in the remaining four studies (36,53,54,75). Similarly, the majority of studies showed that nicotine-treated animals had significantly higher tumor weight (10 of 13 studies) (34,36,53,64,65,67-69,74,80), metastasis (8 of 13 studies) (34,60,65,70,72,78,79,81), and tumor proliferation (8 of 10 studies) (42,52,54,56,61,67,68,75) compared with control animals. Metastasis-free survival was significantly shorter in the nicotine group than in the control group, however, it was only evaluated by two studies (34,81).

Findings for other outcomes were inconsistent. Tumor incidence, evaluated by 11 tumor progression studies that used carcinogen-induced or genetic tumor models, was not different between study groups in five studies (37,45,55,57,58), significantly higher in the nicotine group than in the control group in four studies (46,65,84,91), and was unclear in one study (87). In the remaining study, there were no differences in tumor incidence between the lower nicotine dose group and the control group, however, tumor incidence was significantly higher in the higher nicotine dose group compared with the control group (42). Notably, one study showed no differences between the overall tumor incidence between the nicotine and the control group, however, incidence of nervous system tumors was significantly lower in the nicotine group (37).

Tumor multiplicity was not different between the nicotine and the control group in four (45,55,57,62) of eight studies that evaluated it; for the remaining four studies, one study showed a dose-related effect (42), one study showed significantly lower tumor multiplicity in the nicotine group compared with the control group (87), one study showed significantly higher tumor multiplicity in the nicotine group compared with the control group (73), and the result was unclear in one study (58). Inconsistent findings were also reported for tumor angiogenesis, tumor growth, and tumor area.

Other outcomes were evaluated by only one [i.e., micrometastasis (51), time to tumor appearance (38)] or two [i.e., metastasis-free survival (34,81)] studies, and therefore results could not be interpreted with any degree of confidence.

Progression results by cancer model

The studies included in this systematic review included xenograft/allograft models that included allografts, human tumor cell line-derived xenografts, and patient-derived xenografts; carcinogen-induced models; and genetic models of cancer.

Inoculation of xenografts or allografts either subcutaneously or orthotopically into the tissue of interest are the most commonly used animal models of cancer, however, these methods are associated with some notable limitations and therefore, results of these studies should be interpreted with caution (95). A key drawback of xenograft/allograft models is that they do not recapitulate the histology of tumors, which exist as mixtures of tumor cells, neighboring healthy tissue, stromal cells, supporting vasculature, and infiltrating immune cells (95-98). Carcinogen-induced tumor models are generally thought to better mimic progression of human disease compared with xenograft or allograft models (99). Rodent models of chemically induced cancer have been shown to reliably mimic the mechanisms of carcinogenesis, and to resemble the clinical course of human cancers in terms of morphology, histopathology, and molecular changes. However, studies have shown that inbred strains of mice vary substantially in their susceptibility to chemically-induced neoplasia in various tissues, including lung, liver, skin, and colon (100). Additionally, given that the development of cancer often results from interactions between genetic and environmental factors, recent reviews have indicated that the combined use of chemical carcinogens and genetic models of cancer is the optimal approach to unravelling human disease (100). Genetic models of cancer mimic the characteristics observed in human tumors including progression from benign hyperplastic lesions into aggressive tumors and are generally preferable over xenograft or allograft models of cancer (100-102). These models provide a means of investigating the genetic basis of cancer in immunocompetent hosts, and the interaction between genetic and environmental factors in the development and progression of cancer. However, these models show variability in tumor latency and penetrance. Furthermore, availability of genetically engineered mice is low and their use may be costly, and thus their use is not always feasible (102).

(I) Xenograft/allograft cancer models

Results of studies that used xenograft or allograft cancer models generally showed similar trends to the summary results discussed above for all tumor progression studies (*Table 3*). Thirty-one studies that evaluated tumor volume used xenograft models, with 23 of these studies showing significantly higher tumor volume in the nicotine group compared with the control group (47-50,54,56,58,64-71,74,78,79,82,83,86,93,94), four studies showing no differences (52,55,88,90), and four studies reporting unclear results (36,53,54,75). The majority of studies evaluating each outcome also reported that, compared with the control group, the nicotine group had significantly higher tumor weight (10 of 13 studies) (34,36,53,64,65,67-69,74,80), tumor proliferation (7 of 9 studies) (52,54,56,61,67,68,75), and metastasis (8 of 12 studies) (34,60,70,72,78,79,81,91). Metastasis-free survival (34,81) and tumor area (61,70), evaluated by two studies each, were also significantly higher in the nicotine group than in the control group.

Results for angiogenesis, evaluated by six studies (49,50,52,61,66,93), and tumor growth, evaluated by five studies (34,55,77,79,89), were inconsistent, with some studies showing significant differences between the nicotine and control groups and other studies showing no differences. Lastly, micrometastasis was evaluated by only one study, which showed no differences between the nicotine and control groups (51).

(II) Carcinogen-induced tumor models

Results of studies that used carcinogen-induced models were inconsistent across all outcome measures that were assessed by more than one study (*Table 3*). Tumor incidence, the most frequently evaluated outcome (n=11 studies), did not differ between study groups in five studies (37,45,55,57,58), was significantly higher in the nicotine group compared with the control group in four studies (46,65,84,91), showed a dose-dependent result in one study (42), and the results were unclear in one study (87). Notably, in one study, the overall incidence of tumors was not significantly different between treatment groups, however, the incidence of nervous system tumors was significantly lower in the nicotine group compared with the control group (37). Results of the seven studies that evaluated tumor multiplicity were also inconsistent, with three studies showing no differences between the nicotine and control groups (45,55,57), one study showing a significantly lower tumor multiplicity in the nicotine group compared with the control group (87), one study showed significantly higher tumor multiplicity in the nicotine group compared with the control group (73), and one study showing unclear results (58). In the remaining study, tumor multiplicity was significantly higher in the nicotine group compared with the control group only when a high dose of nicotine was administered (i.e.,

Table 3 Summary of results of tumor progression studies by direction of outcome according to cancer model

Outcomes (n studies)	Nicotine higher	Effect dose-related[†]	Control higher	No difference	Result unclear[‡]
Xenograft/allograft cancer models (n=42)					
Tumor volume (n=31)	23	0/2	0	4	4
Tumor weight (n=13)	10	NA	0	2	1
Proliferation (n=9)	7	0/2	0	1	1
Angiogenesis (n=6)	3	2/2	0	1	1
Metastasis (n=12)	8	NA	0	2	2
Micrometastasis (n=1)	0	NA	0	1	0
Metastasis-free survival (n=2)	2	NA	0	0	0
Tumor area (n=2)	2	0/1	0	0	0
Tumor growth (n=5)	3	0/1	0	2	0
Carcinogen-induced tumor studies (n=13)					
Tumor incidence (n=11)[§]	4	1/1	0	5	1
Tumor volume (n=4)	3	NA	0	1	0
Tumor weight (n=1)	1	NA	0	0	0
Proliferation (n=2)	1	NA	0	1	0
Metastasis (n=2)	1	NA	0	1	0
Tumor multiplicity (n=7)	1	1/1	1	3	1
Tumor area (n=1)	1	NA	0	0	0
Tumor growth (n=1)	0	NA	0	1	0
Genetic model studies (n=4)					
Tumor incidence (n=1)	0	NA	0	1	0
Tumor volume (n=1)	0	NA	0	1	0
Tumor weight (n=1)	0	0/1	0	1	0
Proliferation (n=1)	0	NA	0	1	0
Metastasis (n=2)	0	0/1	0	2	0
Micrometastasis (n=1)	0	NA	0	1	0
Tumor multiplicity (n=2)	0	NA	0	2	0
Tumor area (n=1)	0	NA	0	1	0
Tumor growth (n=1)	0	NA	0	1	0

[†], the denominator represents the number of studies that used more than one dose of nicotine. NA indicates that no studies evaluated the outcome with more than one dose of nicotine. [‡], includes studies that did not statistically compare groups. [§], incidence of nervous system tumors was lower in the nicotine than in the control group in one study, however, there were no differences between groups in overall incidence of tumors. NA, not applicable.

Table 4 Lung tumor progression studies by direction of outcome

Outcomes (n studies)	Nicotine higher	Effect dose-related[†]	Control higher	No difference	Result unclear[‡]
Tumor volume (n=13)	8	NA	0	4	1
Metastasis (n=4)	2	NA	0	2	0
Tumor multiplicity (n=5)	1	NA	0	3	1
Tumor growth (n=3)	2	0/1	0	1	0
Proliferation (n=3)	1	NA	0	1	1
Angiogenesis (n=3)	1	NA	0	1	1
Tumor incidence (n=3)	0	NA	0	3	0
Tumor area (n=2)	1	NA	0	1	0
Tumor weight (n=2)	2	NA	0	0	0
Metastasis-free survival (n=1)	1	NA	0	0	0

[†], the denominator represents the number of studies that used more than one dose of nicotine. NA indicates that no studies evaluated the outcome with more than one dose of nicotine. [‡], includes studies that did not statistically compare groups. NA, not applicable.

40 ppm), but not when lower doses (i.e., 10 or 20 ppm) were used (42). Tumor volume was significantly higher in the nicotine group compared with the control group in three studies (58,70,73), and not different between the two groups in one study (55). Across the three outcomes that were each assessed by a single study that used carcinogen-induced tumor models, tumor area (70) and tumor weight (65) were significantly higher in the nicotine group than in the control group, while tumor growth was not significantly different between groups (55).

(III) Genetic tumor models

Only four studies used genetic models of cancer. Across these studies, two outcomes [i.e., tumor multiplicity (55,62) and metastasis (55,59)] were assessed by two studies each, and the remaining outcomes [i.e., tumor incidence (55), volume (55), weight (55), proliferation (55), area (62), growth (55), and micrometastasis (51)] were assessed by only one study. Given the small number of studies assessing each outcome, conclusions from the results cannot be drawn with any degree of certainty. However, it is notable that there were no differences between the nicotine and control groups in any of the outcomes assessed.

Progression results by cancer class

(I) Lung cancer

Summary of results from progression studies of lung cancer is provided in *Table 4*. Generally, findings from progression studies of lung cancer were not consistent across studies for the majority of assessed outcomes. Tumor volume, the outcome evaluated by the largest number of studies, was significantly higher in the nicotine group than in the control group in eight of the 13 studies that evaluated it (36,47,50,54,58,70,73,83), four studies reported no statistically significant differences between groups (52,55,88,90), and the result was unclear in the remaining study (36). Studies of metastasis (n=4 studies) (55,70,77,81), tumor growth (n=3 studies) (55,77,89), tumor proliferation (n=3 studies) (49,52,55), angiogenesis (n=3 studies) (49,50,52), and tumor area (n=2 studies) (62,70) similarly reported inconsistent results, with some studies reporting significantly higher outcomes in the nicotine group compared with the control group, some studies reporting no differences, and some studies having unclear results.

Of the five studies that evaluated lung tumor multiplicity, three studies reported no differences between the nicotine and control groups (55,57,62), one study reported significantly higher multiplicity in the nicotine group compared with the control group (73), and in one study the results were unclear (58). Three studies that evaluated tumor incidence (55,57,58) reported no differences between the nicotine and control groups.

(II) Digestive cancer

Overall, findings from progression studies that evaluated digestive cancers showed that nicotine administration was associated with tumor progression for the majority of outcomes assessed (*Table 5*). Compared with control animals, nicotine-treated animals had significantly higher tumor volume (10 of 11 studies) (48,56,64-68,71,79,93), tumor weight (5 of 5 studies) (64,65,67,68,80), tumor proliferation

Table 5 Digestive tumor progression studies by direction of outcome

Outcomes (n studies)	Nicotine higher	Effect dose-related[†]	Control Higher	No difference	Result unclear[‡]
Tumor volume (n=11)	10	0/2	0	0	1
Tumor weight (n=5)	5	NA	0	0	0
Proliferation (n=5)	4	0/2	0	0	0
Tumor incidence (n=4)	2	NA	0	1	1
Angiogenesis (n=3)	2	2/2	0	0	0
Metastasis (n=3)	2	NA	0	0	1
Tumor multiplicity (n=2)	0	NA	1	1	0
Micrometastasis (n=1)	0	NA	0	1	0
Tumor area (n=1)	1	0/1	0	0	0
Tumor growth (n=1)	1	NA	0	0	0

[†], the denominator represents the number of studies that used more than one dose of nicotine. NA indicates that no studies evaluated the outcome with more than one dose of nicotine. [‡], includes studies that did not statistically compare groups. NA, not applicable.

(5 of 5 studies) (56,61,67,68,76), and angiogenesis (3 of 3 studies) (61,66,93). Notably, one study showed a dose-related effect of nicotine on tumor angiogenesis (66), and one study showed a dose-related effect on tumor proliferation (61). Inconsistent results were observed for tumor incidence (45,65,84,87), metastasis (71,72,79), and tumor multiplicity (45,87). Micrometastasis (51), tumor area (61), and tumor growth (79) were evaluated by one study each.

(III) Head and neck cancer

Overall, the results of the six studies that evaluated head and neck cancer showed that nicotine treatment was associated with increased cancer progression, although results were not consistent for all outcomes (46,69,78,91,92,94). Specifically, nicotine-treated mice had significantly higher tumor volume (69,78,94) and weight (69) compared to control mice. Two studies also showed that the incidence of carcinogen-induced tumors was significantly higher in the nicotine-treated group compared with the control group (46,91). Two studies that assessed metastasis outcomes reported conflicting results, with one study showing no statistically significant differences between the nicotine group and the control group in the incidence of lymph node metastasis (78), whereas, another study showed that the rate of metastasis was significantly higher in the nicotine group compared with the control group (92).

(IV) Breast cancer

Overall, the six studies that evaluated breast cancer reported inconsistent results (34,38,53,60,74,75). The only consistent result was tumor metastasis, which was significantly higher in the nicotine group compared with the control group in the two studies that evaluated it (34,60). Tumor weight, evaluated by five studies was significantly higher in the nicotine group compared with the control group in three studies (34,53,74), was not different between the study groups in one study (60), and was not statistically compared between the study groups in the remaining study (75). Tumor volume was not statistically compared between study groups in two studies (53,75), and was significantly higher in the nicotine group compared with the control group in one study (74). The remaining outcomes were each evaluated by a single study: tumor proliferation (75) and tumor growth (34) were significantly higher, and metastasis-free survival (34) was significantly shorter in the nicotine group compared with the control group, while there were no differences between study groups in the time to tumor appearance (38).

(V) Urinary tract cancer

Urinary tract cancers were evaluated by only two studies and therefore the results cannot be interpreted with any degree of confidence (42,82). One study reported dose-related effects for tumor incidence and multiplicity, with no differences being observed between the lower dose of nicotine and the control groups, and significantly higher tumor incidence and multiplicity between the higher dose of nicotine and the control groups (42). Conversely, tumor proliferation was higher in the nicotine group compared with the control group regardless of the nicotine dose. The

other study reported significantly higher tumor volume in the nicotine group compared with the control group (82).

(VI) Metastatic melanoma

Metastatic melanoma was evaluated by only one study, which reported that tumor volume was significantly higher in the nicotine group compared with the control group (86). The study also evaluated metastasis; however, study groups were not compared statistically.

(VII) Undefined or multiple cancer sites

Overall, the three studies that did not define the cancer type evaluated, or that evaluated cancers at multiple sites reported no differences between study groups in tumor incidence (37), tumor weight (40,59), or metastasis (59). However, in one of these studies, although there was no difference in the overall incidence of tumors between study groups, the incidence of nervous system tumors was significantly lower in the nicotine group compared with the control group (37). The authors noted that there was no obvious explanation for the result.

Strength of evidence

Although the current systematic review intended to evaluate the quality of evidence with the GRADE tool, inherent limitations in the evidence base hindered the application of GRADE. Briefly, limitations in the evidence base discussed above including poor reporting by included studies, and heterogeneity between study methods and assessments, collectively hindered a comprehensive and appropriate assessment of the GRADE domains across the studies. Therefore, GRADE was not feasible for this review.

Discussion

The current systematic review identified 61 preclinical animal studies that evaluated the potential association between nicotine and the initiation (n=12 studies) and/or progression (n=54 studies) of cancer. Consistent with the findings of a previous review (33), the majority of the tumor initiation studies did not identify an association between nicotine exposure and an increased risk of spontaneous tumor initiation, while results of tumor progression studies were inconsistent and inadequate to support or dismiss an association. Specifically, results of tumor progression studies concluded mixed results across the outcome measures reported, the cancer type being evaluated, and the animal cancer model utilized, although some trends were observed. For example, in xenograft/allograft cancer models, outcomes related to cancer progression were generally higher in nicotine-treated animals compared with untreated animals, while studies using carcinogen-induced cancer models showed inconsistent results, and studies using genetic cancer models showed no differences with nicotine treatment. Notably, the latter two cancer models are better at recapitulating the tumor microenvironment, including the surrounding anatomy and immune system, and mimicking progression of the disease compared with xenograft/allograft models (96,99,100,103). However, the bulk of the evidence came from the xenograft/allograft models, which are less representative of human cancers.

Although elucidation of physiological pathways that may mediate the association between nicotine exposure and cancer initiation and progression was outside of the scope of this review, several studies included in this review proposed hypotheses that may explain this association. The proposed mechanisms vary according to the tumor model used, and include a variety of processes that are activated through activation of nicotinic acetylcholine receptors and its downstream signaling pathways, such as promotion of cell proliferation, migration, invasion, and epithelial-to-mesenchymal transition (67,68,79,92), increased endoplasmic reticulum stress (69,70), inducing cell de-differentiation (71), modulation of immune cell functions (34,86), growth factor secretion and receptor activation (36,50,52,61,78), increased cytokine release (40), and suppression of apoptosis (58).

Given the limitations in trying to mimic human cancer in mouse models, several improvements have been made in the past years and new animal models have emerged in an effort to bridge the gap between animals and humans (95). For example, humanized mouse models are an important development because species differences can be circumvented through reconstituting a human immune system in immunodeficient mice; for example, through engraftment of human peripheral blood mononuclear cells or human $CD34^+$ hematopoietic stem cells (104). The humanized mouse models can be combined with patient-derived xenografts to further improve the translation between mice and human cancer models. While some issues with external validity can be minimized with appropriate animal models, the issue of species differences can never be fully overcome; these issues will always impact external validity and the reliability of translating preclinical findings to humans (105).

Regarding different cancer types, there was limited evidence that the effects of nicotine may vary according to the cancer type being evaluated. For lung cancers, findings

were mixed across the two study groups, whereas, for digestive cancers, the majority of outcomes were higher in nicotine-treated animals compared with untreated animals. For the remaining cancer types, there was an insufficient number of studies to observe any possible trends. Future reviews should consider stratifying the data according to cancer type so that trends associated with specific cancer types can be uncovered.

Although animal models have been and continue to be extensively used in preclinical cancer research, general issues associated with their use remain. While mice and humans are at least 95% identical at the genomic level, this similarity obviously does not prevent their respective phenotypes from being very different (106). These distinct disparities limit the ability to clearly predict the performance of a compound in humans. Differences in size and physiology, as well as variations in the homology of targets between mice and humans, may lead to translational limitations (106,107). This poses the 'mouse to man' problem; that is, the problem of extrapolation of risk, particularly related to chemical exposures, from one species to another for many reasons, including but not limited to, size, metabolic rate, life history, diet, microbiomes, and pathogens (106,108,109). For example, the differences in metabolic rate between mice and humans correspond to anatomic, physiologic, and biochemical differences. Therefore, in the case of this review, it is important to recognize the inherent differences that may limit the translation of animal model findings when examining the potential role of nicotine in human carcinogenesis (109).

Adding to this, the dose and the delivery system of nicotine used in the included animal studies may not translate to what is observed in humans. Only 13 of the 61 included studies reported biomarker of nicotine exposure data (34,35,42,50,51,54,55,57,60,66,70,72,86), and six additional studies did not provide data but related the dose of nicotine administered to levels observed in human smokers (47,48,61,68,73,78). The remaining studies did not evaluate biomarker data and therefore it is unclear whether the administered dose was applicable to that used by humans. In addition, only two studies used nicotine products that are similar to human use: the study by Davis *et al.* (70) used nicotine patches, and Waldum *et al.* (35) exposed rats to a stream of air containing nicotine vapor. The study by Davis *et al.* (70) delivered nicotine by cutting 14 mg nicotine patches into 30 equal sized squares representing 0.45 mg of patch to mice with an average weight of 18 grams, resulting in a dose of 25 mg/kg daily. In the study by Waldum *et al.* (35), rats inhaled nicotine through a chamber for 20 hours a day, 5 days per week for 2 years, with nicotine concentrations in the air of 501±151 μg/m^3. The air concentration delivered provided the rats with twice the plasma concentration as that found in heavy smokers. The other included studies delivered nicotine across different modes of administration, frequency, and duration, which consequently is expected to result in variations in outcomes recorded. For example, the study by Lee *et al.* (53) provided nicotine through drinking water for 6 weeks at a dose of 10 mg/mL, far greater than many studies utilizing 100 μg/mL. A primary concern for the different nicotine levels provided is the possibility of toxic levels for some studies. Toxic levels of nicotine could lead to extensive organ damage that not only systemically influences the outcomes reported, but ultimately does not inform on any possible associations between nicotine and cancer (110,111).

Establishing a relationship between preclinical and clinical outcomes requires diligence in preclinical modeling, with an appropriate study design that optimizes internal and external validity. Despite innate differences between rodents and humans for investigations on nicotine, certain considerations can be taken to improve the applicability of future studies. One important process to achieve an improved representation is to apply known techniques in allometric scaling to adequately account for both the weight and metabolic difference in humans and rodents (112,113). Techniques, such as allometric scaling may have also prevented heterogeneity between studies as doses and their corresponding outcomes could have been better administered, recorded, and transformed for human applicability.

Another limitation of the evidence base is the lack of multiple doses being used in individual studies to determine a dose-response relationship. It has been proposed that the individual steps of carcinogenesis are both time- and dose-dependent (114). Indeed, biologically based models of cancer risk assessment require that the dose-response relationship be determined (114). To do so effectively, dose-response relationships should be generated with low doses selected, multiple doses, and long study times (115). Of the 61 included studies, only six studies evaluated more than a single dose of nicotine, with five studies evaluating two nicotine doses (59,61,63,66,89), and one study evaluating three doses of nicotine (64). Establishing dose-response curves of potential tumor promoters allows for findings of particular interest and utility to be examined, such as

non-linear relationships and the existence of a threshold for biological response. However, establishing complete dose-response curves may not always be feasible as a large number of animals may be required, especially to maintain adequate statistical power at lower doses. Thus, the issue of extrapolation of risk to low doses often persists.

As with the clinical heterogeneity discussed above, methodological diversity between the studies must also be accounted for when interpreting the evidence base. First, 28% of included studies (n=17) did not report whether animals were randomized to study groups. Moreover, nearly 70% of the included studies (n=42) had unclear risk of bias suggesting an increased possibility of differences in, or a failure to, account for vital methodological processes such blinding, and concealment of allocation. The exaggeration of intervention effect when a study does not address possible biases has been observed for a long time in humans, and more recently in mouse models (116-118). For example, an umbrella review and meta-analyses of 31 systematic reviews on animal study testing found that randomization significantly reduces effect sizes [standardized mean difference (SMD) =–0.07, 95% confidence interval (CI): –0.12 to –0.02, I^2=89.1%, P=0.008] (119).

In addition to possible differences in randomization, allocation concealment, and blinding, studies assessing the same outcome used different methods of measurement. One of the most prominent differences was observed in the measurement of tumor volumes where some studies measured the tumors with the aid of classic techniques and tools like calipers, while a few used bioluminescent and magnetic resonance imaging (MRI) imaging. The differences between these techniques are stark, given that manual calipers are inaccurate and inconsistent compared with bioluminescence-based measurements (120). Factors such as sensitivity to underlying adipose tissue, epidermis, and irregular borders, are all potential inaccuracies associated with use of calipers in tumor measurement in rodent models (121-123).

Overall, the quality of reporting was poor, with many studies not displaying high levels of internal and/or external validity and were scored as having either a high or unclear risk of bias. Thus, at this time the evidence for an association between nicotine exposure and outcomes related to cancer initiation or progression appears inadequate to draw any firm conclusions.

Despite numerous limitations and the complexity of the evidence base, the current systematic review exhibited four major strengths. First, a detailed protocol was developed a priori and registered. Strict compliance with this protocol protects against bias due to hindsight. The second strength is the comprehensive search methodology that yielded many available studies, allowing for the investigation of multiple cancer types. Additionally, the clearly defined PICOS of the current review assured the identification of the strongest evidence relevant to the research question. Lastly, this study is considered to be robust based on its strict adherence to AMSTAR-2 and PRISMA guidelines. A critical appraisal tool for systematic reviews, AMSTAR-2 provides "*a broad assessment of quality*, *including flaws that may have arisen through poor conduct of the review (with uncertain impact on findings)*", whereas PRISMA is tailored towards proper reporting—as opposed to conduct—in systematic review (29,124). Thus, adhering to these guidelines ensured that the current systematic review was conducted according to the highest standards with regards to methodological rigor, comprehensive reporting, and transparency. Overall, the highest standards were placed on the methodology of the current systematic review, strengthening the validity of the synthesis reported and the conclusions derived.

Only one previous review by Haussmann and Fariss [2016] assessed the potential association between nicotine and cancer initiation and progression (33). The review noted that there is "*suggestive but still limited evidence*" that suggests no association between long-term nicotine exposure and a complete carcinogenic effect—the ability of nicotine to initiate tumors (33). Regarding tumor progression, the review authors concluded that there was inadequate evidence to support an association between nicotine exposure and the presence of or lack of a modulating effect on carcinogenesis. Overall, the results of this systematic review are in agreement with those of Haussmann and Fariss.

However, several differences between the Haussmann and Fariss review and the current systematic review are worth noting. Specifically, the Haussmann and Fariss review also included *in vitro* related outcomes and included studies where xenografts were pretreated with nicotine. However, the animals themselves were not exposed to nicotine; results were not stratified by the specific cancer type; study design details were not reported; detailed outcome data according to specific outcome measures (e.g., tumor volume, tumor multiplicity) were not reported; and benign or malignant tumor status was not specified. In addition, and importantly, although Haussmann and Fariss did evaluate the adequacy and quality of the studies based on international guidelines for carcinogenicity testing (125), a formal risk of bias

assessment was not performed.

Notably, the results of this systematic review are also in agreement with the 2014 Surgeon General's report on the health consequences of smoking, which stated that the evidence is sufficient to infer that nicotine activates multiple biological pathways through which smoking increases risk for disease, however, the evidence is inadequate to infer the presence or absence of a causal relationship between exposure to nicotine and risk for cancer (126).

Conclusions

In conclusion, the heterogeneity across the studies in terms of sample characteristics, cancer models, and evaluated outcomes make the interpretation and generalizability of the results difficult. Further, although animal models have provided invaluable data for human health risk assessments of chemical exposures, the distinct disparities between animals and humans limit our ability to clearly predict the performance of a compound in humans.

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Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-23-1710/rc

Peer Review File: Available at https://atm.amegroups.com/article/view/10.21037/atm-23-1710/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1710/coif). M.M.K. serviced as a full-time employee of Reynolds American until March 31, 2023 and received restricted company shares as part of the company benefits package. In April of 2023, M.M.K. began a new full-time position at Thera-Business. Thera-Business Inc. has provided regulatory science consulting services to RAI Services Company, a wholly-owned subsidiary of British American Tobacco. I.S. is an employee of Thera-Business. Thera-Business Inc. has provided consulting services to RAI Services Company, a wholly-owned subsidiary of British American Tobacco. R.T.D.M. is an employee of Thera-Business. Thera-Business Inc. has provided consulting services to RAI Services Company, a wholly-owned subsidiary of British American Tobacco. A.D.J. is a full-time employee of Reynolds American. R.P. is a full-time employee of Reynolds American and receives restricted shares as a part of the benefits package. Reynolds American is a sponsor that has paid Thera-Business for contracted regulatory research services. C.S.J. is a full-time employee of Reynolds American, and receive restricted shares as a part of the benefits package. Reynolds American is a sponsor that has paid Thera-Business for contracted regulatory research services. T.B. has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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