Supplementary Material

# Appendices

## Literature Search Strategy

Database(s): **Embase**1974 to 2022 February 13; **Ovid MEDLINE® ALL** 1946 to February 13, 2022; **PsycINFO**1806 to February Week 2 2022

| **Set#** | **Searches** | **Results** |
| --- | --- | --- |
| S1 | ((AB,TI(Cancer\* or carcinoma\* or adenocarcinoma\* or neoplasm\* or tumor\* or tumour\* OR carcinogen\*))) | 8411294\* |
| S2 | MESH(nicotine) | 38236\* |
| S3 | EMB(nicotine) | 62937\* |
| S4 | AB,TI(nicotine) | 117292\* |
| S5 | S4 OR S3 OR S2 | 144765\* |
| S6 | S5 AND S1 | 9519\* |
| S7 | MESH("drug evaluation, preclinical") | 55217\* |
| S8 | EMB("drug evaluation, preclinical") | 3° |
| S9 | MESH("animal experimentation") | 4463° |
| S10 | EMB("animal experimentation") | 5° |
| S11 | MESH("models, animal") | 448389\* |
| S12 | EMB(animal model) | 1906003\* |
| S13 | AB,TI(preclinical or pre-clinical or “in vivo” or animals or animal or mice or mouse or rabbit\* or guinea pig\*or rat\* or rodent\*or xenograft\*) | 7627223\* |
| S14 | S13 OR S12 OR S11 OR S10 OR S9 OR S8 OR S7 | 8446875\* |
| S15 | S14 AND S6 | 1134° |

\* Duplicates are removed from the search, but included in the result count.

° Duplicates are removed from the search and from the result count.

## Literature Search Output: Articles Reviewed at the Full-Text Level (n=148)

| **RefID** | **Bibliography** |
| --- | --- |
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## Excluded Full-Text Articles (n=87)

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| **RefID** | **Bibliography** | **Reason for Exclusion** |
| 3 | AACR-IASLC Joint Conference on Molecular Origins of Lung Cancer 2010. Journal of Thoracic Oncology. 2010;5:. | Clinical or human study, including post-mortem studies. |
| 4 | American Society of Andrology - 35th Annual Meeting. Journal of Andrology. 2010;31:. | Clinical or human study, including post-mortem studies. |
| 9 | 12th Biennial Congress of the European Association of Oral Medicine. Oral Diseases. 2014;20:. | Study does not address an outcome of interest (i.e., tumor initiation, promotion, growth, invasion, angiogenesis, metastasis, extravasation, intravasation). |
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## Summary Tables of Characteristics and Outcome Measures of Included Studies

### Characteristics of Included Studies and Outcome Measures for Studies Reporting on Tumor Initiation

| RefID | First author, year; location; funding | Study Details | Intervention/Treatment | Outcomes |
| --- | --- | --- | --- | --- |
| 89 | Berger et al., 1987;  Germany;  NR | Study Design  RCT  Experimental groups:  Prenatal nicotine  Prenatal + postnatal nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NA  Sample size:  Total: n=360 subjects  Male: n=182 subjects  Female: n=178 subjects  Prenatal nicotine:  Total: n=60 subjects  Male: n=30 subjects  Female: n=30 subjects  Prenatal and postnatal nicotine:  Total: n=60 subjects  Male: n=30 subjects  Female: n=30 subjects  Control:  Total: n=60 subjects  Male: n=30 subjects  Female: n=30 subjects  Animal Model  Species: Rats (Sprague‑Dawley)  Sex: Both  Weight: NR  Age: Gestational Day 1  Comorbidities: NR  Cancer/tumor model: Undefined (tumors at any location were included in the analysis)  Cancer cell line injected: NA  [Note: This study is also included in the progression outcomes table] | Study Methodology  Pregnant dams were randomized to intervention groups on gestational Day 1. The offspring were examined for the presence of tumors twice daily.  Intervention  Prenatal nicotine group: Pregnant dams received 0.4 mg/kg nicotine s.c. daily on gestational Days 14-20.  Prenatal + postnatal nicotine group: Pregnant dams received 0.4 mg/kg nicotine s.c. daily on gestational Days 14-20, and postpartum Days 1-20; 0.4 mg/kg nicotine was administered to offspring twice per week on postpartum Weeks 4-26  Control Group: No intervention was administered to pregnant dams or offspring  Study Duration  452 days (longest survival time) | Tumor incidence (benign tumors)\*  Males:  Prenatal nicotine: 0 of 30 (0%)  Prenatal + postnatal nicotine: 0 of 30 (0%)  Control: 0 of 30 (0%)  Females:  Prenatal nicotine: 1 of 30 (3%); nervous system 1 of 30 (3%)  Prenatal + postnatal nicotine: 2 of 30 (7%); nervous system 1 of 30 (3%), mammary gland 1 of 30 (3%), other sites 0 of 30 (0%)  Control: 2 of 30 (7%); nervous system 0 of 30 (0%), mammary gland 2 of 30 (7%), other sites 0 of 30 (0%)  (statistical comparison between groups was performed, no statistical differences were noted)  \*Time point not specified, however, end of study is assumed  Tumor incidence (malignant tumors)\*  Males:  Prenatal nicotine: 0 of 30 (0%)  Prenatal + postnatal nicotine: 0 of 30 (0%)  Control: 1 of 30 (3%); nervous system: 1 of 30 (3%), mammary gland 0 of 30 (0%), kidney 0 of 30 (0%)  Females:  Prenatal nicotine: 2 of 30 (7%), nervous system 0 of 30 (0%), mammary gland 2 of 30 (7%), kidney 0 of (0%)  Prenatal + postnatal nicotine: 1 of 30 (3%); nervous system 0 of 30 (0%), mammary gland 1 of 30 (3%), kidney: 0 of 30 (0%)  Control: 0 of 30 (0%)  (statistical comparison between groups was performed, no statistical differences were noted)  \*Time point not specified, however, end of study is assumed |
| 174 | Chen et al., 1994;  US;  Public Health Service grants | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: NR  Sample size:  Total: n=52 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model  Species: Hamsters (Golden Syrian)  Sex: Male  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: Cheek pouch epithelium and gastric tumors  Cancer cell line injected: NA | Study Methodology  Hamsters were divided into intervention groups. After 13 months of treatment, the hamsters were euthanized and the cheek pouches and forestomach removed and examined clinically.  Intervention  Nicotine: 50 µL of a 6% solution of nicotine in sesame oil was applied on each cheek pouch of hamsters 3 times per week for up to 13 months.  Control: Control animals were treated with sesame oil alone.  Study Duration  13 months | Tumor incidence at the end of the study (13 months)  Nicotine: 0 of 9  Control: 0 of 10  (statistical comparison between groups was not performed)  [Note: One animal in the nicotine group died before the end of the experiment.] |
| 320 | Galitovskiy et al., 2012;  US;  NIH, Lung Cancer Research Foundation | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=20 subjects  Nicotine: n=15 subjects  Control: n=5 subjects.  Animal Model  Species: A/J mice  Sex: Female  Weight: NR  Age: 6 to 8 weeks  Comorbidities: NR  Cancer/tumor model: Muscle sarcoma  Cancer cell line injected: NA | Study Methodology  Mice were randomized in a 3 to 1 ratio to nicotine and control groups, respectively. Mice were euthanized at the first visible sign of tumor development. Tumors were excised and examined histologically.  Intervention  Nicotine: Daily s.c. injections of the LD50 dose of nicotine hydrogen tartrate (3 mg/kg; 50 μl) in PBS, 5 days per week for 24 months.  Control: PBS was administered s.c. into the upper back, 5 days per week for 24 months.  Study Duration  24 months | Tumor incidence during 24 months of the study  Nicotine: 11 of 14\* (78.6%)  Control: 0 of 5 (0%)  Of the 11 nicotine-treated mice with tumor masses, 3 originated from the uterus (leiomyosarcomas), and 8 from skeletal muscle (rhabdomyosarcomas).  (statistical comparison between groups was not performed)  \*One nicotine-treated mouse died from nicotine toxicity prior to completion of the study, and was thus not considered in the outcomes. |
| 366 | Habs and Schmahl, 1984;  Germany;  NR | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=180 subjects  Nicotine Group 1: n=30 subjects  Nicotine Group 2: n=30 subjects  Control: n=30 subjects  Animal Model  Species: Rat (Sprague-Dawley)  Sex: Female  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: Mammary tumors  Cancer cell line injected: NA | Study Methodology  Rats were divided into intervention groups. Starting on Day 52 postpartum, animals were palpated daily for identification of gross masses twice per week. The occurrence of nodules in the mammary line was assessed. Animals were euthanized when the tumor mass led to a significant loss of body weight or necrosis of the adjacent skin. The remaining animals were euthanized on Day 202 and tumors were examined histologically.  [Note: NMU-induced tumor model is summarized in the progression outcomes table]  Intervention  Nicotine Group 1: 0.4 mg/kg nicotine tartrate was administered s.c. Nicotine was administered on postpartum Days 46-52 (n=30).  Nicotine Group 2: 0.4 mg/kg nicotine tartrate was administered s.c. Nicotine was administered twice per week on postpartum Days 55-145 (n=30).  Control: No treatment was administered  Study Duration  202 days | Tumor incidence during the 202 days of the study  Nicotine Group 1: 0 of 30 (0%)  Nicotine Group 2: 0 of 30 (0%)  Control: 0 of 30 (0%)  (statistical comparison between groups was not performed) |
| 632 | Maier et al., 2011;  US;  NR | Study Design  RCT  Experimental groups:  Spontaneous tumor experiment:  Nicotine  Control  Sample size:  Total: n=20 subjects (expanded to 40 subjects)  Nicotine: n=10 subjects (expanded to 30 subjects)  Control: n=10 subjects  Animal model:  Species:  Spontaneous tumor experiment: AB6F1 mice  Sex: NR  Weight: NR  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model:  Lung tumors  Cancer cell line injected:  NA  [Note: This study is also included in the progression outcomes table] | Study Methodology  Spontaneous tumor experiment:  Mice received nicotine or control treatment in drinking water.  Intervention  Nicotine: 100 µg/mL nicotine administered in drinking water for the duration of the study  Control: Drinking water  Biomarkers of nicotine exposure  Average serum cotinine after 12 weeks of treatment:  Oral nicotine group: 137 ng/mL  [Note: it is unclear if cotinine concentration was assessed in all animals, or only those in the NNK-induced tumor model]  Study Duration  16 weeks | Tumor incidence after 12 weeks of treatment  Spontaneous tumor experiment:  Nicotine: 1 of 30 (3%)  Control: 0 of 10 (0%)  [Note: The study noted that there were no differences in tumor incidence between nicotine and control groups, even though 1 of 10 mice in the nicotine-alone group developed one lung tumor (specific tumor type NR). When this group was expanded to 30 mice, no additional mice developed lung tumors. Statistical analysis was not provided]  Number of tumors per lung after 12 weeks of treatment (tumor multiplicity)  Spontaneous tumor experiment:  Nicotine treatment did not alter tumor multiplicity  (data represented graphically, p value not provided).  Tumor volume after 12 weeks of treatment  Spontaneous tumor experiment:  Nicotine treatment did not alter tumor volume  (data represented graphically, p value not provided). |
| 644 | Martin et al., 1979;  US;  NIA/NIH | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n= 80 subjects  Nicotine: n=16 subjects  Control: n=16 subjects  Animal Model  Species: Rats (Sprague-Dawley)  Sex: Male  Weight: NR  Age: ≥28 days  Comorbidities: NR  Cancer/tumor model:  Undefined (tumors at any location were included in the analysis)  Cancer cell line injected: NA | Study Methodology  Pregnant dams received nicotine or control treatment. Male offspring (study subjects) were euthanized throughout the study at various ages and assessed for the presence of tumors at autopsy.  Intervention  Nicotine: 5 mg/mL nicotine (3.0 mg/kg) in 0.9% isotonic saline as administered s.c. to pregnant dams (n=30) twice daily for 21 days. After a 2-day recovery period, injections resumed for an additional 19 days until weaning.  Control: 0.9% isotonic saline as administered s.c. to pregnant dams (n=13) twice daily for 21 days. After a 2-day recovery period, injections resumed for an additional 19 days until weaning.  Study Duration  Nicotine: ≤68 weeks  Control: ≤101 weeks | Tumor incidence at autopsy  Nicotine: 0 of 16 subjects (age at sacrifice: mean: 51 weeks; range: 30-68 weeks)  Control: 0 of 16 subjects (mean age at sacrifice: NR; range: 75-101 weeks)  (statistical comparison between groups was not performed) |
| 683 | Murphy et al., 2011;  US;  Masonic Cancer Center and Animal Care and Research Program at the University of Minnesota and NIH | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: 7 days  Wash-out/pre-treatment period: NR  Sample size:  Total: n=240 subjects  Nicotine: n= 20 subjects (one death at Week 3 for unknown cause)  Control: n=20 subjects (only 19 analyzed)  Animal Model:  Species: Mice (A/J mice)  Sex: Female  Weight: NR  Age: 4 weeks  Comorbidities: NR  Cancer/tumor model:  Lung tumors  Cell line injected: NA  [Note: This study is also included in the progression outcomes table] | Study Methodology  Nicotine or control treatment was administered in drinking water. Mice were euthanized 46 weeks later. Lungs were excised and tumors were counted. Tumors were scored based on location (lung lobe) and the widest tumor diameter.  Intervention  Nicotine: 200 µg/mL (0.44 µmol/mL) nicotine hydrogen tartrate and sodium potassium tartrate were provided in drinking water for 46 weeks. Water was available *ad libitum*.  Control: Drinking water was provided *ad libitum.*  Biomarkers of nicotine exposure  *Urine concentration in the nicotine group at 1-2 weeks:*  Nicotine: 1,360±1,040 ng/mL (range: 65-3,570 ng/mL)  Cotinine: 1,260±1,240 ng/mL(range: 116-4,700 ng/mL)  Trans-3’-hydroxycotinine: 12,200±9,010 ng/mL (range: 1,600‑34,000 ng/mL)  *Urine concentration in the nicotine group at 5 months:*  Nicotine: 1,270±1,170 ng/mL (range: 217-3,620 ng/mL)  Cotinine: 4,400±3,200 ng/mL (range: 450-10,600 ng/mL)  Trans-3’-hydroxycotinine: 27,900±9,820 ng/mL (range: 17,100‑49,600 ng/mL)  *Plasma concentration in the nicotine group on Day 18:*  Nicotine: 0.61±0.5 ng/mL (range: 0.2-2.4 ng/mL)  Cotinine: 40±45 ng/mL(range: 7-198 ng/mL)  Trans-3’-hydroxycotinine: 34±21 ng/mL (range: 13-109 ng/mL)  *Plasma concentration in the nicotine group on Week 46:*  Nicotine: 0.40±0.46 ng/mL (range: 0.1-1.9 ng/mL)  Cotinine: 19±20 ng/mL (range: 3-72 ng/mL)  Trans-3’-hydroxycotinine: 48±36 ng/mL (range: 6-111 ng/mL)  Study Duration  46 weeks | Lung tumor incidence (% mice)\*  Nicotine: 5 of 19 (26%)  Control: 6 of 19 (31%)  (statistical comparison between groups was not performed)  \*Time point not specified, however, end of study is assumed  Lung tumor multiplicity at the end of the study (number of tumors per mouse; mean±SD) categorized by tumor diameter:  Total number of tumors  Nicotine: 0.32±0.1  Control: 0.53±0.1  Number of tumors <0.5 mm  Nicotine: 0.00  Control: 0.05  Number of tumors 0.5-1.0 mm  Nicotine: 0.21  Control: 0.32  Number of tumors 1.0-2.0 mm:  Nicotine: 0.05  Control: 0.11  Number of tumors >2.0 mm:  Nicotine: 0.05  Control: 0.05  (statistical comparison between groups was not performed)  Incidence of lung tumors categorized by tumor type (% mice)\*  Adenoma:  Nicotine: 1 of 13 (8%)  Control: 1 of 14 (7%)  Adenoma with dysplasia:  Nicotine: 0 of 13 (0%)  Control: 1 of 14 (7%)  Carcinoma:  Nicotine: 2 of 13 (15%)  Control: 0 of 14 (0%)  (statistical comparison between groups was not performed)  \*Time point not specified, however, end of study is assumed  Tumor multiplicity at the end of the study (number of tumors per mouse; mean±SD) categorized by tumor type:  Adenoma:  Nicotine: 0.08±0.28 (n=13)  Control: 0.07±0.27 (n=14)  Adenoma with dysplasia:  Nicotine: 0.0 (n=13)  Control: 0.07±0.27 (n=14)  Carcinoma:  Nicotine: 0.15±0.40 (n=13)  Control: 0.0 (n=14)  (statistical comparison between groups was not performed) |
| 850 | Schmahl and Habs, 1976;  Germany;  NR | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n= 858 subjects  Nicotine group: n=67 subjects  Control group: n=69 subjects  Animal Model  Species: Rats (Sprague-Dawley)  Sex: Male and Female  Weight: NR  Age: 12 days  Comorbidities: NR  Cancer/tumor model:  Undefined (tumors at any location were included in the analysis)  Cell lines injected:  NA | Study Methodology  Animals were assigned to intervention groups and observed until natural death. Only animals that lived > 200 days were considered for analysis. Post-mortem analysis of the organs was conducted.  Intervention  Nicotine: Nicotine (2 mg/kg/week) was administered i.p. (duration not specified, however, it is assumed that treatment was administered for the duration of the study)  Control: Vehicle was administered (route of administration and volume NR).  Study Duration  Until animals’ natural death (>200 days). | Tumor (malignant) incidence (% rats)\*  Males:  Nicotine: 2 of 35 (6%±4%)  Control: 1 of 36 (3%±3%)  Females:  Nicotine: 2 of 32 (6%±4%)  Control: 3 of 33 (11%±5%)  (statistical comparison between groups was performed, no statistical differences were noted)  \*Time point not specified, however, end of study is assumed  Tumor (malignant) localization and induction period:  Males:  Nicotine: 1 mammary, 583 days; 1 leucosis, 426 days  Control: 1 hemangioendothelioma of the liver, 637 days  Females:  Nicotine: 2 mammary, 545±117 days  Control: 3 mammary, 717±99 days  (statistical comparison between groups was not performed) |
| 966 | Thompson et al., 1973;  US;  American Medical Association Education and Research Foundation, NIH, Los Angeles County Heart Association | Study Design  RCT  Experimental groups:  *2-month treatment* experiment:  Nicotine  Control  *22-month treatment experiment:*  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=60 subjects  *2-month treatment experiment:*  Nicotine: n=6 subjects  Control: n=6 subjects  *22-month treatment experiment*:  Nicotine: n=38 subjects  Control: n=10 subjects  Animal Model  Species: Rats (Fischer-F344)  Sex: Male  Weight: 142.0 g ± 2.7 g  Age: 8 weeks (approximately)  Comorbidities: NR  Undefined tumor types (tumors at any location were included in the analysis)  Cancer cell line injected: NA | Study Methodology  To study the chronic effects of nicotine in male Fischer-344 rats, nicotine or placebo was injected daily for 2 or 22 months. Rats were euthanized and analyzed after treatment.  Intervention  Nicotine: 1,000 µg base/mL/kg/day in 6% gelatin was injected s.c. daily for 2 or 22 months.  Control: 0.85 g/L physiologic saline in 6% gelatin was injected s.c. daily for 2 or 22 months.  Study Duration  2 months, and 22 months. | Neoplasms  *2-month treatment experiment:*  Nicotine: 2 of 10 (n=1 adenocarcinoma; n=1 chromophobe adenoma)\*  Control: 0 of 6  *22-month treatment experiment:*  Nicotine: 9 (in 8 rats) of 28 (n=3 pheochromocytoma; n=4 epidermoid carcinoma of the skin; n=1 acute lymphocytic leukemia; n=1 fibrosarcoma)\*\*  Control: 0 of 6  \*Note: Data taken from table which does not match the text; text reports these data for the 22-month control group.  \*\*2 metastatic lung tumors were observed, 1 from acute lymphocytic leukemia, and 1 from fibrosarcoma and pheochromocytoma |
| 976 | Toth, 1982;  US;  National Toxicology Program, National Institute of Environmental Health Sciences | Study Design  RCT  Experimental groups:  0.09375% nicotine  0.0625% nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=400  0.09375% nicotine:  Female: n=50  Male: n=50  0.0625% nicotine:  Female: n=50  Male: n=50  Control:  Female: n=100  Male: n=100  Animal Model  Species: Mice (Swiss albino)  Sex: Male and female  Weight: NR  Age: 5-7 weeks  Comorbidities: NR  Cancer/tumor model: Undefined (tumors at any location were included in the analysis)  Cancer cell line injected: NA | Study Methodology  Mice received nicotine hydrochloride in drinking water starting at 5 or 7 weeks of age. Tumor incidences in the lungs, lymphoreticular tissue, and blood vessels of the three groups were compared with an untreated control.  Intervention  0.09375% nicotine: 0.09375% nicotine hydrochloride was administered in drinking water starting at 7 weeks of age.  0.0625% nicotine: 0.0625% nicotine hydrochloride was administered in drinking water starting at 5 weeks of age.  Control: Drinking water alone was provided for life.  Study Duration  Lifetime of mice (up to 120 weeks) | Tumor incidence at autopsy (% mice)  *Lungs*  0.09375% nicotine:  Female: 9 of 50 (18%)  Male: 6 of 50 (12%)  0.0625% nicotine:  Female: 6 of 50 (12%)  Male: 6 of 50 (12%)  Control:  Female: 15 of 100 (15%)  Male: 22 of 100 (22%)  *Lymphoreticular tissue (malignant lymphoma)*  0.09375% nicotine:  Female: 2 of 50 (4%)  Male: 0 of 50 (0%)  0.0625% nicotine:  Female: 5 of 50 (10%)  Male: 2 of 50 (4%)  Control:  Female: 20 of 100 (20%)  Male: 8 of 100 (8%)  *Blood vessels*  0.09375% nicotine:  Female: NR  Male: 1 of 50 (2%)  0.0625% nicotine:  Female: 3 of 50 (6%)  Male: 1 of 50 (2%)  Control:  Female: 8 of 100 (8%)  Male: 5 of 100 (5%)  *Other tissues:*  0.09375% nicotine:  Female: n=1 adenocarcinoma of the breast; n=1 subcutaneous fibrosarcoma  Male: n=1 fibrosarcoma  0.0625% nicotine:  Female: n=1 subcutaneous fibrosarcoma  Male: n=1 subcutaneous fibrosarcoma; n=1 papilloma of the forestomach; n=1 seminoma  Control:\*  Female: n=2 subcutaneous fibrosarcomas; n=2 sex cord mesenchyma tumors; n=1 adenoacanthoma of ovary; n=1 adenoma of ovary; n=1 adenoma of thyroid; n=1 adenocarcinoma of breast; n=1 adenocarcinoma of duodenym; n=1 carcinoma of glandular stomach.  Male: n=2 subcutaneous fibrosarcomas; n=2 adenomas of thyroids; n=1 adenoma of parathyroid  \*Data reported in Toth, 1979; Mycopathologia, 68(2), 121–128.  (statistical comparison between groups was not performed) |
| 1136 | Waldum et al., 1996;  Norway;  NR | Study Design  Controlled, parallel group study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=59 subjects  Control: n= 25 subjects  Animal Model  Species: Rats (Sprague‑Dawley)  Sex: Female  Weight: 240 g  Age: NR  Comorbidities: NR  Cancer/tumor model: Undefined (tumors at any location were included in the analysis)  Cancer cell line injected: NA | Study Methodology  Rats were divided into two intervention groups: nicotine and control groups. After 6, 12, and 18 months, some control and nicotine exposed rats were anesthetized, blood was drawn then these rats were grossly examined for tumors in the brain, lungs, gastrointestinal tract, liver, kidney and ovaries. At the end of the study, the remaining rats were similarly sacrificed during anesthesia and examined for tumors, and effects on the pulmonary neuroendocrine cells.  Intervention  Nicotine group:  Rats were exposed to nicotine vapor by bubbling medical quality air (2.2 L/min) through a reservoir with approximately 60 mL of nicotine (> 99% pure) (Fluka AG, Buchs, Switzerland) at 25°C. The stream of air containing the nicotine vapor was passed through a drop catch bottle before being mixed with the main air stream.  Rats were exposed to nicotine for 20 hours/day for 5 days/week (not on the weekends).  Control group:  NR (likely medical quality air only)  Biomarker of nicotine exposure  After five days of nicotine exposure, controls and nicotine-exposed rats were anaesthetized, and blood was collected by heart puncture for determination of nicotine concentration in plasma.  Plasma nicotine concentrations (mean±SD):  Week 1:  Nicotine group: 108.4±55.1 ng/mL (n=4)  Control group: not detectable (n=2)  Week 103:  Nicotine group: 129.8±43.0 ng/mL (n=17)  Control group: not detectable (n=6)  (detection limit is 2 ng/mL)  Study Duration  24 months | Tumor incidence after up to 24 months nicotine exposure (number of rats with tumors)  Nicotine: 21/59 (36%)  Control: 6/25 (24%)  p=NS  Benign tumor incidence (by site and type)  *Mammary gland*  Fibroadenoma:  Nicotine: 9/59  Control: 6/25  *Pituitary gland*  Adenoma:  Nicotine: 4/59  Control: 0/25  Atypical adenoma:  Nicotine: 1/59  Control: 0/25  *Lungs*  Nicotine: 0/59  Control: 0/25  Malignant tumor incidence (by site and type)  *Mammary gland*  Adenocarcinoma:  Nicotine: 1/59  Control: 0/25  *Ovary*  Granulosa-theta cell tumor:  Nicotine: 1/59  Control: 0/25  Adenocarcinoma:  Nicotine: 2/59  Control: 0/25  *Skin*  Histiocytoma  Nicotine: 1/59  Control: 0/25  *Metastasis (unknown origin)*  Liver:  Nicotine: 1/59  Control: 0/25  Abdominal cavity:  Nicotine: 1/59  Control: 0/25  *Lungs*  Nicotine: 0/59  Control: 0/25 |
| 1028 | Wang et al., 2017;  China;  National Natural Science Foundation of China, Beijing Natural Science Foundation of China | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=140 subjects  Spontaneous tumor experiment:  Nicotine: n=20 subjects  Control: n=10 subjects  Animal Model  Species: Mice (wild type C57BL/6)  Sex: NR  Weight: NR  Age: 6-8 weeks  Comorbidities: NR  Cancer/tumor model: oral precancerous lesions  Cancer cell line injected: NA  [Note: This study is also included in the progression outcomes table] | Study Methodology  Spontaneous tumor experiment:  Mice received nicotine or control treatment for 16 weeks. At the end of treatment, mice were euthanized and tongues were removed for analysis.  Intervention  Spontaneous tumor experiment:  Nicotine: 5% nicotine was smeared on the tongue 3 times per week for 16 weeks.  Control: Distilled water was smeared on the tongue 3 times per week for 16 weeks.  Study Duration  16 weeks | Incidence of oral squamous cell carcinoma at the end of the study  Nicotine: 0 of 20 (0%)  Control: 0 of 10 (0%)  (statistical comparison between groups was performed, no statistical differences were noted) |

Abbreviations: µg = micrograms; µL=microliters; i.p. = intraperitoneal; intraperitoneally; kg = kilograms; LD50 = lethal dose, 50%; mg = milligrams; mL = milliliters; mm = millimeters; NA = not applicable; NIA = National Institute on Aging; NIH = National Institutes of Health; NNK = nitrosamine ketone (4‑(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NR = not reported; PBS = phosphate buffered saline; RCT = randomized controlled trial; s.c. = subcutaneous/subcutaneously; SD = standard deviation; US = United States.

### Characteristics of Included Studies and Outcome Measures for Studies Reporting on Tumor Progression

| **RefID** | **First author, year; location; funding** | **Study Details** | **Intervention/Treatment** | **Outcomes** |
| --- | --- | --- | --- | --- |
| 44 | Al-Wadei et al., 2012;  US;  National Lung Cancer Partnership; LUNGevity Foundation, State of Tennessee Centre of Excellence in Livestock Diseases and Human Health | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=40 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model  Species: Mice (athymic nude mice)  Sex: Male  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: NSCLC xenograft model.  Cancer cell line injected: Human NSCLS cells from cell lines NCI-H322 (histological subtype: adenocarcinoma) with activating point mutations in K-Ras or NCI-H441 without K-Ras mutation  [Note: Study reported each mouse was injected with NCI-H322 or NCI-H441 cell line.] | Study Methodology  Human NSCLC cells (3×106 cells suspended in 0.2 mL PBS, viability >95%) from either the NCI-H322 or NCI-H441 cell line were injected s.c. Animals were randomized to receive either nicotine or control starting 1 day after injection of the tumor cells.  Intervention  Nicotine: 1 µM (200 µg/mL)\* nicotine administered in sterile drinking water and available ad libitum for 30 days.  Control: Sterile drinking water available ad libitum for 30 days.  \*The study reported nicotine concentration of 200 mg/mL, however, 1 µM corresponds to 200 µg/mL. A study by the same group (RefID45) also reported nicotine concentration of 200 µg/mL.  Study Duration  30 days | Tumor incidence on Day 30  Nicotine (NCI-H322 and NCI-441): 10 of 10  Control (NCI-H322 and NCI-H441): 10 of 10  Tumor volume (mean) on Day 30  Tumors from NCI-H322 cell line:  Nicotine: 4.2-fold increase vs. control (volume NR; p<0.001 vs. control)  Control: 154±36mm3  Tumors from NCI-H441 cell line:  Nicotine: 3-fold increase vs. control  Control: 137±48mm3  Tumor volume was significantly larger in the nicotine group compared to the control group at Weeks 1, 2, 3, and 4 (data represented graphically; p<0.0001). |
| 45 | Al-Wadei et al., 2009;  US;  National Cancer Institute, State of Tennessee Center of Excellence for Human and Livestock Diseases | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=40 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model  Species: Mice (athymic nude mice)  Sex: Male  Weight: NR  Age: NR  Comorbidities:  Cancer/tumor model: Pancreatic ductal adenocarcinoma xenograft model  Cancer cell line injected: Panc-1 human pancreatic ductal adenocarcinoma cell line | Study Methodology  Human pancreatic duct adenocarcinoma cell line Panc-1 (3×106 cells suspended in 0.2 mL PBS, viability >95%) were injected s.c. Mice were randomized to receive either nicotine or control treatment starting 1 day after implantation of tumor cells.  Intervention  Nicotine: 200 µg/mL nicotine administered in sterile tap water available ad libitum.  Control: Sterile tap water available ad libitum.  Study Duration  30 days. | Tumor incidence at 2 weeks (after cancer cell injection)  Nicotine: 8 of 10  Control: 8 of 10  Change in tumor volume (fold increase at 30 days compared with baseline)  Control: 0.9±0.2 cm3  Nicotine: 4.2-fold increase vs. control (volume NR; p<0.001 vs. control). |
| 85 | Ben et al., 2020;  China;  None | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: Until tumor volume reached 75‑125 mm3 (time not specified)  Sample size:  Total: n=15 subjects  Nicotine: n=5 subjects  Control: n=5 subjects  [Note: 5 subjects from a YAP1 knockout were not relevant to this analysis, and were therefore not included.]  Animal Model  Species: Mice (BALB/c)  Sex: Male  Weight: NR  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model: Pancreatic tumor xenograft model  Cancer cell line injected: Panc-1 cells | Study Methodology  Log phase Panc-1 cells (5.0×106/100 µL) were inoculated s.c. into the dorsal flank of mice. When tumor volume reached 75-125 mm3, mice were randomized to receive either nicotine or control.  Intervention  Nicotine: Nicotine (0.25 mg/kg) was administered i.p. 3 times per week for 3 weeks (volume not specified).  Control: DMSO was administered i.p. 3 times per week for 3 weeks (volume not specified).  Study Duration  22 days | Tumor volume  Tumor volume was significantly higher in the nicotine group compared to the control group on Day 25 (values NR; data represented graphically; p<0.01).  [Note: Methods specified that experiment was completed on Day 22, however, Figure 2 shows differences on Day 25.]  Tumor weight  Tumor weight was significantly higher in the nicotine group compared to control group at Day 22 (p<0.01; data represented graphically).  Tumor proliferation at the end of the study (relative expression of Ki-67)  Relative expression of Ki-67, a marker of cellular proliferation, was significantly higher in the nicotine-treated group than in control group (values NR; data represented graphically; p<0.01). |
| 86 | Ben et al., 2020;  China;  None | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: Until tumor volume reached 75‑125 mm3 (time not specified)  Sample size:  Total: n=15 subjects  Nicotine: n=5 subjects  Control: n=5 subjects  Animal Model  Species: Mice (BALB/c)  Sex: NR  Weight: 20 g  Age: 6-8 weeks  Comorbidities: NR  Cancer/tumor model: Pancreatic tumor xenograft model  Cancer cell line injected: Panc-1 cells | Study Methodology  Log phase Panc-1 cells (2.0×106/100 µL) were inoculated s.c. into the dorsal flank of nude mice. When tumor volume reached 75-125 mm3, mice were randomized to receive either nicotine or control, and that day was defined as Day 1.  Intervention  Nicotine: Nicotine (0.25 mg/kg) was administered i.p. 3 times per week for 3 weeks (volume not specified)  Control: DMSO was administered locally by direct injection into the xenografts (volume not specified).  Study Duration  22 days | Tumor volume  Tumor volume was significantly higher in nicotine group compared to control group on Day 22 (values NR; data represented graphically; p<0.01).  Tumor weight\*  Tumor weight was significantly higher in nicotine group compared to control group on Day 22 (p<0.01; data represented graphically).  Tumor proliferation at the end of the study (relative expression of Ki-67)  Relative expression of Ki-67, a marker of cell proliferation, was significantly higher in the nicotine-treated group compared to control (values NR; data represented graphically; p<0.05).  \*Given that the tumor volumes in the study of RefID #85 are similar, it appears that the scale for tumor weight is incorrect by a factor of 10 (i.e., 1 g should be 0.1 g). Author was contacted and did not provide clarification. |
| 89 | Berger et al., 1987;  Germany;  NR | Study Design  RCT  Experimental groups:  NMU-induced tumor model:  NMU + prenatal nicotine  NMU + prenatal + postnatal nicotine  NMU  Acclimation period: NR  Wash-out/pre-treatment period: NA  Sample size:  Total: n=360 subjects  Male: n=182 subjects  Female: n=178 subjects  NMU-induced tumor model:  NMU:  Total: n=60 subjects  Male: n=30 subjects  Female: n=30 subjects  NMU + prenatal nicotine:  Total: n=60 subjects  Male: n=31 subjects  Female: n=29 subjects  NMU + prenatal nicotine + postnatal nicotine:  Total: n=60 subjects  Male: n=31 subjects  Female: n=29 subjects  Animal Model  Species: Rats (Sprague‑Dawley)  Sex: Both  Weight: NR  Age: 20±2 weeks  Comorbidities: NR  Cancer/tumor model: NMU-induced tumor model  Cancer cell line injected: NA  [Note: This study is also included in the initiation outcomes table] | Study Methodology  NMU-induced tumor model:  Pregnant dams were randomized to intervention groups on gestational Day 1. The offspring from NMU-treated dams were examined for the presence of tumors twice daily.    Intervention  NMU-induced tumor model:  NMU group: Pregnant dams received 30 mg/kg NMU by gavage on gestational Day 20.  NMU + prenatal nicotine group: Pregnant dams received 30mg/kg NMU by gavage on gestational Day 20, and 0.4 mg/kg nicotine s.c. daily on gestational Days 14-20.  NMU + prenatal + postnatal nicotine group: Pregnant dams received 30mg/kg NMU by gavage on gestational Day 20, and 0.4 mg/kg nicotine s.c. daily on postpartum Days 1-20. The offspring received 0.4 mg/kg nicotine twice per week on postpartum Weeks 4-26.  Study Duration  452 days (longest survival time) | Tumor incidence (benign tumors)  (time point not specified, however, end of study is assumed)  NMU-induced tumor model:  Males:  NMU + prenatal nicotine: 7 of 31 (23%); nervous system 1 of 31 (3%), mammary gland 1 of 31 (3%), other sites 5 of 31 (16%)  NMU + prenatal + postnatal nicotine: 3 of 31 (10%); nervous system 0 of 31 (0%), mammary gland 0 of 31 (0%), other sites 3 of 31 (10%)  NMU: 7 of 30 (23%); nervous system 0 of 30 (0%), mammary gland 0 of 30 (0%), other sites 7 of 30 (23%)  (statistical comparison between groups was performed, no statistical differences were noted)  Females:  NMU + prenatal nicotine: 7 of 29 (24%); nervous system 0 of 29 (0%), mammary gland 3 of 29 (10%), other sites 4 of 29 (14%)  NMU + prenatal + postnatal nicotine: 14 of 29 (48%), nervous system 1 of 29 (3%), mammary gland 6 of 29 (20%), other sites 7 of 29 (24%)  NMU: n=5 (17%); nervous system: 0 of 30 (0%); mammary gland 2 of 30 (7%), other sites 3 of 30 (10%)  (statistical comparison between groups was performed, no statistical differences were noted)  Tumor incidence (malignant tumors)  NMU-induced tumor model:  Males:  NMU + prenatal nicotine: 21 of 31 (68%); nervous system 16 of 31 (52%), mammary gland 0 of 31 (0%), kidney 1 of 31 (3%)  NMU + prenatal + postnatal nicotine: 22 of 31 (71%); nervous system 18 of 31 (58%)\*, mammary gland 0 of 31 (0%), kidney: 1 of 31 (3%)  \*Significantly different from the NMU group, p=0.0015; significantly different from males of the NMU group, p=0.0008. No other statistical differences were noted  NMU: 26 of 30 (87%); nervous system 23 of 30 (77%), mammary gland 0 of 30 (0%), kidney: 1 of 30 (3%)  Females:  NMU + prenatal nicotine: 20 of 29 (69%); nervous system: 14 of 29 (48%), mammary gland 3 of 29 (10%), kidney 0 of 29 (0%)  NMU + prenatal + postnatal nicotine: 16 of 29 (55%); nervous system 10 of 29 (34%)\*, mammary gland: 1 of 29 (3%), kidney 2 of 29 (7%)  \*Significantly different from NMU group, p=0.0015  NMU: 25 of 30 (83%); nervous system 14 of 30 (47%), mammary gland 9 of 30 (30%); kidney: 1 of 30 (3%) |
| 94 | Bersch et al., 2009;  Brazil;  Research and Events Incentive Fund of the Clinics Hospital of Porto Alegre (FIPE); National Counsel of Technological and Scientific Development (CNPq), Brazil | Study Design  RCT  Experimental groups:  Nicotine  Control (historical)  Acclimation period: 7 days  Pre-treatment period: 15 days  Sample size:  Total: n=114 subjects  Nicotine: n=51 subjects  Control: n=NR  Animal Model  Species: Mice (CF1)  Sex: Male  Weight: 20-40g  Age: >60 days  Comorbidities: NR  Cancer/tumor model: DMBA induced pancreatic cancer  Cancer cell line injected: NA | Study Methodology  1 mg of DMBA crystals was implanted in the pancreatic head of mice. The animals were randomized before surgery to the DMBA nicotine group and another study group.  [Note: Control group from a previously published study by the same group was used. Original reference: Osvaldt et al., 2009 Surgery 140(5):803]  Intervention  Nicotine: 2 mg/kg nicotine was administered s.c. twice per day for 45 days  Control: NR  Study Duration  56 days  Nicotine: 45 days | Occurrence of adenocarcinoma  (timing not specified, however, end of study is assumed)  Nicotine: 14 of 27 (52%)  Control: 4 of 24 (17%)  Fisher exact test p<0.001  [Note: Study reported total preoperative death n=3 (6%) and postoperative death n=21 (44%) in the nicotine group; NR for control group. The number of animals available for analysis was n=27 for nicotine and n=24 for control.] |
| 150 | Cedillo et al., 2019;  Spain;  Ministry of Economy, Industry and Competitiveness, Government of Spain; “Mutua Madrileña Investigación Biomédica” Foundation, Spain; Ministry of Education, Culture and Sports, Government of Spain; Autonomous University of Madrid; Ministry of Economy, Industry and Competitiveness, Government of Spain | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: 10 days  Sample size:  Total: n=NR  Nicotine: n=5-6 subjects  Control: n=5-6 subjects  Animal Model  Species: Mice (NU-Foxn1nu athymic)  Sex: Female  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: Lung adenocarcinoma xenograft model  Cancer cell line injected: A549 cell line | Study Methodology  A suspension of wild-type A549 cells (2×106 cells) was injected s.c. into the left flank of each mouse 10 days before mice were randomized to intervention treatment.  Intervention  Nicotine: 1 µM nicotine was administered in drinking water  Control: 2% saccharin was administered in drinking water  Study Duration  37 days | Tumor volume at Day 37  Tumor volume was significantly higher in nicotine treated group compared to the untreated control group (p<0.05; data represented graphically)  Tumor proliferation at the end of the study (relative expression of Ki-67)  Qualitative analysis showed that Ki-67 staining was more robust in tumor tissue from nicotine-treated than control mice (quantitative data NR; data shown as photomicrographs of tumor tissue).  Angiogenesis (VEGF staining) at the end of the study  Qualitative analysis showed that VEGF staining was more robust in tumor tissue from nicotine-treated mice than control mice (quantitative data NR; data shown as photomicrographs of tumor tissue). |
| 175 | Chen et al., 1990;  US;  National Institute of Dental Research, National Institutes of Health, Department of Health and Human Services | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=40  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model  Species: Syrian Golden Hamsters  Sex: Male  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: DMBA-induced tumor model  Cancer cell line injected: NA | Study Methodology  40 male Syrian golden hamsters were randomized into four groups of 10 animals each. Both cheek pouches of each hamster were treated and were examined once a week from the 4th week on for evidence of gross changes. After 12 weeks, the hamsters were killed; the cheek pouches were removed, and the number and size of lesions were recorded. The cheek pouches were then processed for routine histological examination.  Intervention  DMBA+nicotine: 50 µL of 1% DMBA in 6% nicotine in sesame oil three times a week  DMBA alone: 50 µL of DMBA in sesame oil three times a week  Study Duration  12 weeks | Tumor incidence after 12 weeks of treatment  Number of tumors was significantly higher in the DMBA+nicotine group compared to the control group (p<0.001; values NR)    Tumor size  The number of larger tumors (≥ 3-mm diameter) was greater in the DMBA+nicotine group compared to the DMBA group (p<0.05; chi-square distribution). |
| 182 | Chien et al., 2021;  Taiwan;  Ministry of science and Technology; National Defense Medical Center; Tri-Service General Hospital; Chi Mei Medical Center | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: Until tumor size reached 100 mm3 (time not specified)  Sample size:  Total: n=20 subjects  Nicotine: n=5 subjects  Control: n=5 subjects.  Animal Model  Species: Mice (nude)  Sex: Male  Weight: NR  Age: 4 weeks  Comorbidities: NR  Cancer/tumor model: OSCC xenograft model  Cancer cell line injected: Human OSCC cell line (SAS) | Study Methodology  SAS cells (1.5×106) were injected s.c. into the right flank of the mice. When the tumor size was about 100 mm3, the mice were randomized to receive either nicotine or control. The length and width of tumors were measured every 3 days.  Intervention  Nicotine: Nicotine (1 mg/kg) dissolved in PBS was administered i.p. daily for 1 month (volume not specified).  Control: Vehicle (PBS) was administered i.p. daily for 1 month (volume not specified).  Study Duration  29 Days | Tumor volume on Day 29  Tumor volume was significantly higher in the nicotine-treated group compared with the control group (p<0.05; data represented graphically).  Tumor weight on Day 29  Tumor weight was significantly higher in the nicotine-treated group compared with control group (p<0.05; data represented graphically). |
| 236 | Davis, 2009;  US;  National Cancer Institute, Bankhead-Coley Grant, American Heart Association | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Tumor growth experiments:  Nicotine delivered i.p.:  Total n=16\* subjects  Nicotine: n=8 subjects  Control: n=8 subjects  Nicotine delivered via a dermal patch:  Total: n=16\* subjects  Nicotine: n=NR  Control: n=NR  [\*Methods indicate n=8 for nicotine and n=8 for control (patch or i.p. injection), however, it is unclear how many animals received nicotine i.p. and via a transdermal patch].  Tumor recurrence and metastasis experiments:  Total: n=32 subjects  Nicotine: n=16 subjects  Control: n=16 subjects  NNK-induced tumor experiments:  Total: n=16 subjects  Nicotine: n=8 subjects  Control: n=8 subjects  Animal Model:  Tumor growth and tumor recurrence and metastasis experiments:  Species: Mice (syngenic BALB/c mice).  Sex: Female  Weight: NR  Age: 26-30 days.  Comorbidities: NR  Cancer/tumor model: Lung adenocarcinoma allograft model  Cancer cell line injected:  Line 1 mouse adenocarcinoma cells (lung)  NNK-induced tumor experiments:  Species: Mice (A/J mice).  Sex: Female  Weight: NR  Age: 4-6 weeks  Comorbidities: NR  Cancer/tumor model:  Mouse model of lung cancer  Cancer cell line injected:  NA | Study Methodology  Tumor growth experiments:  Line 1 mouse adenocarcinoma cells (lung; 1×106) were injected s.c. Animals were randomized to receive either nicotine or vehicle treatment. Tumor growth was measured 3 times per week.  Tumor recurrence and metastasis experiments:  Tumors were removed 3 weeks after injection of Line 1 mouse adenocarcinoma cells and animals continued to receive nicotine or vehicle for 2 more weeks. At the end of the study, mice were euthanized and lungs were examined for the number of lung tumors.  NNK-induced tumor experiments:  NNK (100 mL/kg) was administered to mice once a week for 5 weeks. Mice were randomized into nicotine and control groups. At the end of the study, mice were euthanized and lungs were examined for the number of lung tumors.  Intervention  Tumor growth experiments:  Nicotine i.p.: 1 mg/kg administered i.p. 3 times per week (vehicle not specified)  Control i.p.: Vehicle administered i.p. 3 times per week.  Nicotine patch: 25 mg/kg nicotine administered daily in a nicotine patch.  Control patch: NR  Tumor recurrence and metastasis experiments and NNK-induced tumor experiments:  Nicotine i.p.: 1 mg/kg administered i.p. 3 times per week (vehicle not specified)  Control i.p.: Vehicle i.p. 3 times per week.  Biomarkers of nicotine exposure  Average urine cotinine concentration in mice receiving nicotine intervention  Nicotine i.p. 1 mg/kg 3 times per week: 3,000 ng/mL cotinine  Nicotine patch, 25 mg/kg daily: 5,000 ng/mL cotinine  Study duration:  Tumor growth experiments: 2 weeks.  Tumor recurrence and metastasis experiments: 5 weeks.  NNK-induced tumor experiments: 33 weeks | Tumor volume (tumor growth experiments)  Nicotine i.p. (tumor volume assessed on Day 18):  Control i.p.: 695±98 mm3  Nicotine i.p.: 2,267±369 mm3  p=0.002  [Note: n=10 indicated in figure legend; n=8 indicated on the graph; unclear if this refers to total n or n per group]  Nicotine dermal patches (tumor volume assessed on Day 11):  Control patch: 530±59 mm3  Nicotine patch: 871±106 mm3  p=0.019  [Note: n=14 indicated in the figure legend and on the graph; n=16 indicated in the methods]  Rate of tumor recurrence 2 weeks after removal of initial tumors\*  Control i.p.: 19%±7%  Nicotine i.p.: 59%±3%  p=0.01  \*Calculated as the percentage of recurring tumors out of the total number of tumors removed.  Number of metastatic foci in the lungs at the end of the study  Nicotine i.p. experiment (n=16; n per group NR):  Control i.p.: 0.9±0.2  Nicotine i.p.: 8.1±1.7  p=0.001  Nicotine dermal patches experiment (n=14; n per group NR):  Control patch: 6.7±2.1  Nicotine patch: 20.6±4.9  p=0.02  [Note: n=16 indicated in figure legend; n=14 indicated on the graph]  Growth of NNK-induced tumors  Number of lung tumors per mouse at the end of the study:  Mice in the nicotine group had significantly more tumors than mice in the control group (p=0.01)  Nicotine: 16±3.0 tumors per mouse (n=8 indicated on the graph)  Control: 10±3.0 tumors per mouse (n=NR)    Tumor area at the end of the study:  Tumors from the nicotine group were larger than those from the control group (values NR; data represented graphically; p value NR, text indicates that there was a significant difference) |
| 247 | Delitto et al., 2016;  US;  Bristol-Meyers Squibb; Cracchiolo Foundation | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre-treatment period: NR  Sample size:  Total: n=20 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model  Species: Mice (NOD-SCID IL2 receptor gamma chain knockout [NSG])  Sex: Female  Weight: NR  Age: 8 weeks  Comorbidities: NR  Cancer/tumor model: Patient-derived pancreatic adenocarcinoma  Cancer cell line injected: NA | Study Methodology  A 2x2 mm section of a surgically resected primary pancreatic adenocarcinoma or 2 mm core biopsy was implanted s.c. into mice. On postoperative Day 5, mice with visible tumors were equally randomized by size to receive either nicotine or control intervention. Tumor dimensions were measured 3 times per week using calipers. Pulmonary metastasis was evaluated using a hematoxylin and eosin staining.  Intervention  Nicotine: Nicotine (1 mg/kg) was administered i.p. 3 times per week (volume not specified).  Control: PBS was administered i.p. 3 times per week (volume not specified).  Study Duration  45 days | Tumor volume  Tumor volume was significantly higher in the nicotine-treated group than in the control group from ~ Days 33-45 (p<0.05; data represented graphically).  Pulmonary metastasis (evaluated using hematoxylin and eosin staining; timing not provided but assumed to be end of study)  Nicotine: 5 of 8 animals (62%)  Control: 0 of 8 animals (0%) |
| 366 | Habs and Schmahl, 1984;  Germany;  NR | Study Design  RCT  Experimental groups:  NMU induced tumor model:  NMU + Nicotine  NMU  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=180 subjects  NMU induced tumor model:  NMU + Nicotine: n=30 subjects  NMU: n=30 subjects  Animal Model  Species: Rat (Sprague-Dawley)  Sex: Female  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: Mammary tumors  Cancer cell line injected: NA | Study Methodology  Rats were divided into intervention groups. For the NMU-induced tumor model, NMU (50 mg/kg) was administered i.v. on Day 52 postpartum. Animals were palpated daily for identification of gross masses twice per week. Animals were euthanized when the tumor mass led to a significant loss of body weight or necrosis of the adjacent skin. The remaining animals were euthanized 150 days after the administration of NMU and tumors were examined histologically.  Intervention  NMU + Nicotine: NMU (50 mg/kg) was administered on postpartum Day 52. Nicotine (0.4 mg/kg) was administered s.c. twice per week on postpartum Days 55-145.  NMU: NMU (50 mg/kg) was administered on postpartum Day 52.  Study Duration  202 days  Nicotine: 90 days | Time to tumor development  The time to tumor development was not different between NMU + Nicotine and NMU groups (values NR; data represented graphically; statistical analysis not provided).  Tumor type:  Histologically, all tumors were of epithelial origin; >80% were classified as adenocarcinomas; the remaining tumors were adenomas or adenofibromas.  Nicotine treatment did not have any influence on the histologic type of NMU-induced neoplasms. |
| 377 | Hanaki et al., 2016;  Japan;  Japan Society for the Promotion of Science | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: 2 weeks  Wash-out/pretreatment period: 2 weeks  Sample size:  Total: n=20 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Animal Model:  Species: Mice (athymic nude)  Sex: Male  Weight: NR  Age: 4 weeks.  Comorbidities: NR  Cancer/tumor model: Pancreatic cancer xenograft model.  Cancer cell line injected: BxPC3 and Panc1 pancreatic cancer cells | Study Methodology  BxPC3 and Panc1cells (1×107 cells) from a human pancreatic cancer cell line were transplanted into the abdominal cavity. 2 weeks after transplantation, mice were randomized to receive either nicotine or control treatment.  Intervention  Nicotine: 1 mg/kg in PBS (150‑200 µL/mouse) administered i.p. 3 times per week.  Control: PBS administered i.p. 3 times per week.  Biomarkers of Nicotine Exposure  Serum cotinine 6 weeks after start of nicotine intervention  Nicotine group: 1,635.00±147.22 pg/mL (n=5)  Control group: 1.47±1.11 pg/mL (n=3)  p<0.01 for control vs. nicotine group  Study Duration:  12 weeks  Nicotine: 10 weeks | Number of peritoneal nodules (peritoneal dissemination)  At 12 weeks, the number of nodules in the peritoneum was significantly higher in the nicotine group than in the control group (values NR; data represented graphically; p<0.05).  Nodule size  At 12 weeks, the diameter of nodules in the peritoneum was significantly larger in the nicotine group than in the control group (values NR; data represented graphically, p<0.05). |
| 380 | Hao et al., 2013;  China;  International Science Cooperative Project of China; National Basic Research Program of China; Program for New Century Excellent Talents in University of China; NFSC; NIH; Key Project of Natural Science Foundation of Tianjin Province; Key Project of Chinese Ministry of Education | Study Design  Controlled study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=15-18 subjects  Control: n=15-18 subjects  Animal Model  Species: Mice (C57BL/6, genotype: RAG2–/–)  Sex: Female  Weight: Upper end of weight was ~30g  Age: 6-8 weeks old  Comorbidities: NR  Cancer/tumor model: Metastatic lung cancer model (melanoma xenograft)  Cancer cell line injected: melanoma B16 cells expressing luciferase (B16-F10-luc2 (B16) melanoma cell line) | Study Methodology  C57BL/6 RAG2-/- mice received nicotine or control treatment for at least 14 days before receiving an i.v. injection of B16-melanoma cells (varying concentrations from 1×105 to 2.5×106 cells). Tumor volume was monitored using bioluminescence and MRI imaging.  Intervention  Nicotine: 100 mg/mL nicotine in PBS was administered s.c. using miniosmotic pumps, at a rate of 3.6 µL per day for 6 weeks (0.39 mg of nicotine free base per day).  Control: PBS was administered s.c. with miniosmotic pumps at a rate of 3.6 µL per day for 6 weeks.  [Note: Study reported that some animals in the control group received PBS via direct injection rather than through the pump]  Biomarkers of Nicotine Exposure  Plasma nicotine level after nicotine infusion  [Note: the study noted that plasma nicotine levels in mice are ~100-200 ng/mL (~0.6-1.2 mM) after infusion of ~2-4 mg/kg/hr of drug, and ~45 ng/mL (280 nM) after infusion at ~0.5 mg/kg/hr, however, it is unclear if plasma nicotine was measured in this study, or if these values were extrapolated from previous studies]  Study Duration  60 Days  Nicotine: 2 weeks prior to cancer cell line injection and 6 weeks post-injection | Number of lung metastases\*  Day 14 (n=6 per group):  Nicotine: 110±15  Control: 102±13  p=NR  Day 21 (n=6 per group):  Nicotine: 263±28  Control: 178±23  p=NR  \*Note: Results presented for the transplantation of 1 X 106 B16 melanoma cells, which (according to the investigators) provided the most efficient comparison of tumor burden.  Tumor volume†  Tumor volume was significantly higher in mice treated with nicotine compared with untreated control (data represented graphically, tumor volume was measured via high field MRI; p<0.05)  †Time point not recorded. |
| 390 | Hayashi et al., 2013;  Japan;  Japan Society for the Promotion of Science (KAKENHI) Grants | Study Design  Controlled study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: NR  Colitis-associated cancer + nicotine: n=10-13 subjects  Colitis-associated cancer + vehicle: n=10-13 subjects  Animal Model  Species: Mice (BALB/c)  Sex: Male  Weight: NR  Age: 8-10 weeks  Comorbidities: NR  Cancer/tumor model: Colitis associated cancer  Cancer cell line injected: NA | Study Methodology  Colitis-associated cancer was induced by a combination of azoxymethane and repeated DSS treatment. Mice received 12 mg/kg azoxymethane i.p. and, 5 days later, 2% DSS in drinking water for 5 days (with nicotine administered daily during this period), followed by 16 days of regular water. This cycle was repeated 3 times. On Day 90 after azoxymethane administration, mice were euthanized and the colon was excised for evaluation. The visible tumors (>1 mm in the major axis) were counted in the mid to distal colon of each mouse.  Intervention  Nicotine: 3 mg/kg per day s.c. for 5 days during DSS treatment  Control: Not treated with nicotine  Study Duration  90 days  Nicotine: three 5 day periods, each followed by 16 days of control treatment. | Tumor number per mouse (tumor multiplicity) at Day 90, mean ±SE\*  Nicotine: 8.8±1.1  Control: 17.3±2.0  p<0.05  \*means of 10–13 mice  Tumor size at Day 90  Tumors were reduced in size in nicotine-treated mice compared with those in vehicle-treated mice (n=6; values NR; data represented in a photomicrograph of hematoxylin & eosin stained tissue; statistical analysis NR).  Adenocarcinomas at Day 90  No difference in the incidence of adenocarcinomas in the distal colon was observed between nicotine- and vehicle-treated mice (n=6; values NR; data represented in a photomicrograph of hematoxylin & eosin stained tissue; statistical analysis NR). |
| 399 | Heeschen et al., 2001;  US;  National Heart, Lung and Blood Institute, Tobacco Related Disease Research Program, German Research Council | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out pretreatment period: NR  Sample Size:  n=NR  Animal Model:  Species: Mice (C57BL6J wild-type)  Sex: NR  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: Lung carcinoma allograft model (Lewis lung carcinoma mouse model)  Cancer cell line injected: Lewis lung carcinoma 3LL cells | Study Methodology  Lewis lung carcinoma cells (1×106 cells/mouse) were injected s.c. into the flank or percutaneously introduced into the lung parenchyma. Animals were randomized to receive either nicotine or control treatment. When tumors grew to a size >1.0 cm3, mice were euthanized and histological evaluation was performed.  Intervention  Nicotine: 100 µg/mL nicotine administered in the drinking water available ad libitum  Control: Drinking water available ad libitum  Biomarkers of Nicotine Exposure  Serum cotinine:  Nicotine group: 216.5 ng/mL (95% CI 189.8-236.2 ng/mL)  Control group: NR  Study Duration  Study was terminated when tumors reached a size of 1.0 cm3. | Tumor volume  s.c. injection of lung carcinoma cells:  On Day 7, there were no differences in tumor volume between nicotine and control groups (data represented graphically; values NR).  On Day 16, tumors were significantly larger in the nicotine group than in the control group (data represented graphically; values NR; p<0.01).  Orthotopic implantation of cancer cells into lung parenchyma:  Tumor volume on Day 12:  Nicotine: 0.51 cm3 (95% CI 0.17-0.73)  Control: 0.22 cm3 (95% CI 0.18-0.45)  p<0.001  Capillary density  s.c. injection of lung carcinoma cells on Day 16:  Nicotine: 1.1 capillaries/kilopixel (95% CI 0.8‑1.7)  Control: 0.2 capillaries/kilopixel (95% CI 0.1‑0.4)  p<0.001  Orthotopic implantation of cancer cells into lung parenchyma on Day 12:  Nicotine: 1.8 capillaries/kilopixel (95% CI 0.9‑2.5)  Control: 0.5 capillaries/kilopixel (95% CI 0.2‑0.9)  p<0.001 |
| 403 | Hermann et al., 2014;  Spain, Germany, and UK;  European Research Council, European Community’s Seventh Framework Programme, Fonde de Investigacion Sanitaria, Programa Nacional de Internacionalizacion, Spanish Ministry of Economy and Competitiveness. | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=NR  Control: n=NR  Animal Model:  Species:  Circulating pancreas-derived cells experiment:  KPC mice  Micrometastasis frequency experiment:  NU-Foxn1nu nude mice  Sex: NR  Weight: NR  Age: 12-14 weeks  Comorbidities: NR  Cancer/tumor model: Pancreatic cancer.  Cancer cell line injected: Circulating pancreas-derived cells experiment:  NA  Micrometastasis frequency experiment:  Pancreatic ductal adenocarcinoma cells | Study Methodology  Circulating pancreas-derived cells experiment:  Studies was conducted in a Pdx-1-Cre mouse model of pancreatic cancer. After 2 weeks of treatment, blood was extracted and the presence of cells positive for red fluorescent protein or epithelial cell adhesion molecule was assessed.  Micrometastasis frequency experiment:  Red fluorescent protein/firefly luciferase-expressing pancreatic ductal adenocarcinoma cells (5× 104 cells) were injected into the spleen of NU-Foxn1nu nude mice. Animals were randomized to receive nicotine or control treatment.  Intervention  Nicotine: 100 µg/mL administered in the drinking water supplemented with 2% sucrose. Water was available ad libitum.  Control: NR  Biomarkers of Nicotine Exposure  Urine cotinine:  Values NR (data represented graphically; p<0.05 vs. control)  [Note: Study reported that cotinine levels were comparable with those in intermediate smokers, ~200 ng/mL)  Study Duration:  Circulating pancreas-derived cells experiment: 2 weeks.  Micrometastasis frequency experiment: 3 weeks. | Number of circulating pancreas-derived cells after 2 weeks  The number of circulating pancreatic cells positive for the red fluorescent protein or for epithelial cell adhesion-molecule was significantly higher in the nicotine group than in the control group (data represented graphically, p value not provided).  Micrometastasis frequency (%) at 3 weeks  There was no significant difference in the dissemination of pancreas-derived carcinoma cells in the liver between the nicotine group and the control group; (data represented graphically, p=0.0513).  [Note: the study stated that there was an increased dissemination of pancreas-derived cells to the liver in nicotine-treated mice, however, p value provided was 0.0513; level of statistical significance was not defined in the study] |
| 444 | Hsu et al., 2020;  Taiwan;  Ministry of science and technology, National Defense Medical Center, Tri-Service General Hospital, Cardinal Tien Hospital, Chi Mei Medical Center | Study Design  Controlled study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: NR  Nicotine: NR  Control: NR  Animal Model  Species: Mice (BALB/cAnN.Cg-Foxn1nu/Cr1Nar1 nude)  Sex: Male  Weight: Mean 20g  Age: 4-5 weeks  Comorbidities: NR  Cancer/tumor model: OSCC xenograft model  Cancer cell line injected: OEC-M1 squamous cell carcinoma cell line | Study Methodology  OEC-M1 cells (1×106 cells) were injected s.c. into the flanks of nude mice. Mice were euthanized 4 weeks after xenograft implantation.  Intervention  Nicotine: 1.5 mg/kg per day (volume not specified)  Control: PBS (volume not specified)  Study Duration  4 weeks | Tumor volume at 4 weeks  Tumor volume was significantly larger in the nicotine group than in the control group (values NR; data represented graphically; p<0.05). |
| 458 | Iskandar et al. 2013;  US;  NIH grant and the U.S. Department of Agriculture | Study Design  Controlled, parallel group study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=16 subjects  Control: n= 16 subjects  Animal Model  Species: A/J mice  Sex: Male  Weight: 240g  Age: 5-6 weeks old  Comorbidities: NR  Cancer/tumor model: carcinogen-induced lung tumors  Cancer cell line injected: NA | Study Methodology  Mice received a single i.p injection of NNK (100 mg/kg), and after 2 weeks, mice were divided into two interventions groups: control and nicotine treatment. After the experimental periods, the mice were terminally exsanguinated under anesthesia. Lung tumor lesions were quantified by determining the multiplicity of the pulmonary surface tumors. The diameter of each tumor was calculated using a caliper. The volume of each lung surface tumor was calculated on the basis of the assumption that the tumor was spherical.  Intervention  NNK+Nicotine group: i.p. injection of NNK (100 mg/kg), and after 2 weeks, i.p. nicotine injections (1 mg/kg) 3 times weekly for 10 weeks (daily dose of ~ 0.43 mg/kg)  NNK Group: i.p. injection of NNK (100 mg/kg).  Study Duration  12 weeks | Tumor volume (mean±SEM)  NNK+ Nicotine: 2.32 ± 0.48 mm3 (p<0.01 vs. NNK group)  NNK: 1.29 ± 0.07 mm3  (data represented graphically; p<0.05 on the graph, p<0.01 in the text)  Tumor multiplicity (mean±SEM)  NNK+ Nicotine: 9.7 ± 1.7 (p<0.01 vs. NNK group)  NNK: 4.3 ± 0.5  (data represented graphically; p<0.05 on the graph, p<0.01 in the text) |
| 475 | Jarzynka et al., 2006;  US;  National Cancer Institute Specialized Programs of Excellence in Lung Cancer | Study Design  Controlled, parallel group trial (randomization not specified)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=32 subjects  Nicotine: n=8 subjects  Control: n=8 subjects  Animal Model  Species: Mice (ovariectomized nude mice)  Sex: Female  Weight: NR  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model: Lung tumor xenograft model  Cancer cell line injected: Human A549 bronchioloalveolar carcinoma cells | Study Methodology  A549 cells were injected s.c. (1×107 cells suspended in PBS) into the flank of mice and mice were divided into intervention groups. Tumor volume was estimated every 5 days. At the end of the study, tumors were excised and evaluated.  Intervention  Nicotine: 200 µg/mL nicotine administered in drinking water.  Control group: Drinking water was administered  Study Duration  36 days | Tumor volume at Day 36  Nicotine: 507±79.6 mm3  Control: 418±50.4 mm3  Graphical representation of data shows 1.2× increase in tumor volume in nicotine group compared to control (difference not significant)  Angiogenesis (microvascular density) at Day 36  Nicotine enhanced microvascular density compared to control (not significant)  Graphical representation of data shows 1.8× increase in tumor microvascular density in nicotine treated group compared to untreated control (2 experiments with 8 mice per group in each experiment, difference not significant).  [Note: Qualitative analysis was performed by CD31 Immunohistochemistry staining]  Tumor proliferation (relative expression of Ki-67) at Day 36  Immunohistochemistry analysis shows >2-fold increase in the expression of the cellular marker for proliferation (Ki67) in nicotine treated group compared to control (2 experiments with 8 mice per group in each experiment, p<0.05; data represented graphically). |
| 483 | Jimenez et al., 2020;  US  NIH; Urban Health Initiative, National Institute on Minority Health and Health Disparities; California Tobacco-Related Disease Research Program | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: 2 weeks  Pre-treatment period: NR  Sample size:  Total: n=40 subjects  HCC70 group:  Nicotine: n=5 subjects  Control: n=5 subjects  HCC1806 group:  Nicotine: n=5 subjects  Control: n=5 subjects  Animal Model  Species: Mice (immunodeficient nude)  Sex: NR  Weight: NR  Age: 6-8 weeks  Comorbidities: NR  Cancer/tumor model: Breast cancer xenograft model  Cancer cell line injected: Human breast cancer cell lines HCC1806 and HCC70 | Study Methodology  Breast cancer cell lines HCC70 and HCC1806 (2×106 cell/100µL) were implanted s.c. in nude mice. Mice received either nicotine or saline. Tumor volume was measured weekly for 10 weeks.  Intervention  HCC70 group; HCC1806 group:  Nicotine: Nicotine (0.75 mg/kg; 300 nM) was administered i.p. twice a day (volume not specified).  Control: Saline was administered i.p. twice a day (volume not specified).  Study Duration  10 weeks | Tumor volume at Week 8  HCC70 group:  Control: 231±46 mm3  Nicotine: 372±32 mm3  p≤0.05  HCC71806 group:  Control: 229±24 mm3  Nicotine: 372±32 mm3  p≤0.05  Tumor weight  HCC70 group:  Control: 305±26 mg  Nicotine: 357±24 mg  p≤0.01  HCC71806 group:  NR |
| 533 | Kumari et al., 2018;  India;  Department of Biotechnology (Government of India); Department of Science and Technology (Government of India); Institute of Life Science (India) | Study Design  Controlled, parallel group trial (not randomized to control)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=24 subjects  Nicotine: n=12 subjects  Control: n=12 subjects  Animal Model  Species: Mice (BALB/c nude mice)  Sex: Female  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: Human breast carcinoma xenograft model  Cancer cell line injected: MDA-MB-231 breast cancer cell line | Study Methodology  MDA-MB-231 cells in matrigel (107 cells) were injected s.c. into the flank of mice. Mice started receiving intervention treatment 3 days later. Tumor volume was measured every 4 days with a digital caliper  Intervention  Nicotine: Nicotine (0.25mg/kg) was administered i.p. twice per week  Control: PBS was administered i.p. twice per week.  Study Duration  80 days | Incidence of well-established\* tumors on Day 60  Nicotine: 10 of 12 mice  Control: 1 of 12 mice  \*100 mm3 was considered a well-established tumor  Tumor volume at Day 80  Tumor volume was numerically higher in the nicotine compared to control group (data represented graphically; unclear if the groups were compared statistically)  Tumor weight at Day 80  Tumor weight was numerically higher in nicotine treated group compared to control group (data represented graphically; unclear if the groups were compared statistically)  Tumor proliferation at Day 80 (relative expression of Ki-67)  The percentage of Ki-67-positive cells was significantly higher (22.5%) in the nicotine than in control (values NR; data represented graphically; p<0.005) |
| 542 | Kyte et al., 2018;  US;  NIH; Massey Cancer Center Pilot Project Grant | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=5-6 subjects  Control: n=5-6 subjects.  Animal Model  Species: Mice (immunocompetent C57BL/6J adult mice)  Sex: Male  Weight: NR  Age: 8 weeks  Comorbidities: NR  Cancer/tumor model: Lewis lung carcinoma tumor allograft model  Cancer cell line injected: Lewis lung carcinoma | Study Methodology  Mice were injected s.c. with 1.5×106 Lewis lung carcinoma cells in both flanks. Once tumor formed (13 days post-tumor cell injection), s.c. osmotic minipumps were implanted on Day 0 to release nicotine or saline daily for a total of 7 days.  Intervention  Nicotine: Nicotine (24 mg/kg per day) was administered s.c. through osmotic minipumps for up to 7 days.  Control: Saline was administered s.c. through osmotic minipumps for up to 7 days.  Study Duration  20 days  Nicotine: 7 days | Tumor volume  No significant difference in fold-change in tumor volume from baseline was observed between control and nicotine treated animals at different time points (n=5-6 per group; data represented graphically from Day 0 to Day 8; no interaction between time and treatment [F(4,39) = 2.560, p=0.054])  [Note: The tumor volumes (left and right flank) were compared with the respective baseline tumor volumes to calculate fold change; the fold change values were averaged for each mouse] |
| 562 | Lee et al., 2010;  Taiwan;  National Science Council; Cathy Medical Center | Study Design  Controlled, parallel group study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=50 subjects  Nicotine: n=5 subjects  Control: n=5 subjects  Animal Model  Species: Mice (NOD.CB17-PRKDC(SCID)/J(NOD-SCID)) Sex: NR  Weight: NR  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model: Breast cancer xenograft model  Cancer cell line injected: MDA-MB-231 breast cancer cell lines | Study Methodology  MDA-MB-231 cell lines (5×106) were implanted s.c. After tumor transplantation, mice were treated with or without nicotine for 6 weeks.  Intervention  Nicotine: After tumor transplantation, nicotine (10 mg/mL) was administered via drinking water for 6 weeks.  Control: Not treated with nicotine  Study Duration  6 weeks | Tumor volume at 6 weeks  Nicotine: 2993.2 mm3  Control: NR  Tumor weight at 6 weeks  Nicotine: 4.38 g  Control: NR  Tumors in the nicotine-treated mice were heavier compared with the control mice (p=0.027; data represented graphically). |
| 573 | Li et al., 2022;  Taiwan;  NR | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: Until tumor volume reached 100 mm3 (time not specified)  Sample size:  Total: n=27 subjects  Nicotine: n=6 subjects  Control: n=6 subjects  Animal Model:  Species: Mice (athymic nude)  Sex: Male  Weight: NR  Age: 4 weeks  Comorbidities: NR  Cancer/tumor model:  Hepatocellular carcinoma xenograft model  Cancer cell line injected:  HepG2 cells | Study Methodology  Mice received an s.c. injection of HepG2 (1×106) cells into the right flank. When the tumor volume was approximately 100 mm3, mice were randomly divided into control and nicotine groups. Tumor volume was measured once per week for 8 weeks.  Intervention  Nicotine: 200 mg/kg nicotine was administered i.p. 5 times per week for 4 weeks  Control: 100μL normal saline and 100μL 1% DMSO was administered i.p. 5 times per week for 4 weeks  Study Duration  8 weeks  Nicotine: 4 weeks | Tumor volume at Week 8  Nicotine-treated mice showed numerically higher tumor volume compared with saline control (data represented graphically; statistical analysis not performed)  Tumor proliferation (relative expression of Ki-67) at Week 8  Ki-67 expression was significantly higher in the nicotine than in the control group (data represented graphically; p<0.05)  [Note: Expression of Ki-67 in tumor tissue was assessed using polymerase chain reaction.] |
| 576 | Li et al., 2015;  Japan;  Research Institute for Diseases of the Chest, Kyushu University (Fukuoka, Japan) | Study Design  RCT  Experimental groups:  i.v. nicotine  Oral nicotine  Control  Acclimation period: NR  Pre‑treatment period: 3 days  Sample size:  Total: n=NR  i.v. nicotine: n=5-6\* subjects  Oral nicotine: n=6 subjects  Control: n=6 subjects  \*As reported in the reference  Animal Model:  Species: Mice (BALB/cAJc1-nude)  Sex: Female  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: NSCLC xenograft model  Cancer cell line injected: PC9 cells | Study Methodology  Mice received an s.c. injection of PC9 cells (5×106 cells) 3 days before the start of the intervention. Tumor size was measured twice weekly; tumor volume was calculated using the formula V=ab2/2, where a and b are tumor length and width, respectively.  Intervention  i.v. nicotine: 0.6 mg/kg nicotine was administered i.v. 5 times per week for 18 days  Oral nicotine: 100 µg/mL nicotine administered in drinking water for 18 days  Control: Mice received vehicle administered i.v. 5 times per week for 18 days (i.v. control; amount not specified), or drinking water for 18 days (oral control).  Biomarkers of Nicotine Exposure:  Serum cotinine level at the end of the study  i.v. nicotine: 372.37±42.3 ng/mL; p<0.05 vs. control  Oral nicotine: 36.75±5.50 ng/mL; p vs control NR  Control: 13.85±0.69 ng/mL  Study Duration  20 days  Nicotine: 18 days | Tumor volume after 18 days  i.v. nicotine: 746±24 mm3  Oral nicotine: 811±53 mm3  Control: 600-630 mm3 (range; p<0.05 vs. both nicotine groups) |
| 600 | Liu et al., 2015  China;  National Natural Science Foundation of China | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=NR  Control: n=NR  Animal Model  Species: nude BALB/c mice  Sex: Male  Weight: NR  Age: 5-8 weeks  Comorbidities: NR  Cancer/tumor model: xenograft-induced lung tumor models  Cancer cell line injected: A549 cells | Study Methodology  A549 cells (5×106 cells/mouse in 0.2 mL PBS) were injected s.c. into each flank, and then mice were divided into intervention groups. All treatments started 1 day after subcutaneous inoculation of the tumor cells and the animals were observed for 20 days. Body weights and tumor growth were recorded every 5 days, and at the end of the 20-day observation period  Intervention  Nicotine: 1 µmol/L of nicotine in drinking water  Control: drinking water  Study Duration  21 days | Tumor weight at 20 days  Tumor weight significantly increased in the nicotine-treated mice compared to control mice (p<0.01; data represented graphically, values NR).  Tumor volume at 20 days  Tumor volume was numerically higher in the nicotine-treated mice compared to control mice (data represented graphically, values NR; p-values NR) |
| 632 | Maier et al., 2011;  US;  NR | Study Design  RCT  Experimental groups:  Nicotine  Control  Sample size:  Carcinogen-induced model:  Total: n=40 subjects  Nicotine: n=10 subjects  Control: n=10 subjects  Transgenic model:  Total: n=10-12 subjects  Nicotine: n=5-7 subjects  Control: n=5 subjects  Allograft model:  Total: n=10 subjects  Nicotine: n=5 subjects  Control: n=5 subjects  Animal Model:  Species:  Carcinogen-induced tumor model: AB6F1 mice  Transgenic model:  KrasLA2/+ mice on a C57Bl/6 background.  Xenograft model:  AB6F1 mice injected with CL13, IO33 or CL25 cells.  Sex: NR  Weight: NR  Age:  Carcinogen-induced model: 6 weeks  Transgenic model: 3 weeks  Xenograft model: 6-10 weeks.  Comorbidities: NR  Cancer/tumor model:  Lung tumors.  Cancer cell line injected:  CL13, IO33, or CL25 lung adenocarcinoma cells (xenograft model) | Study Methodology  Carcinogen-induced model:  Animals received 3 weekly i.p. injections of 100 mg/kg NNK followed by nicotine administration in drinking water.  Transgenic model:  KrasLA2/+ mice on a C57Bl/6 background were treated for 2 or 6 weeks (longer chronic studies), starting at 3 or 6 weeks of age. At the end of the study, peripheral lung tumors were counted and measured.  Allograft model:  CL13, IO33, or CL25 lung adenocarcinoma cells (1× 105 cells) were injected s.c. into AB6F1 mice. One day after implantation, mice were randomized to receive oral nicotine or control water for the remainder of the study (i.p. details not provided). Tumor size was measured every other day and tumor volume was calculated.  Intervention  NNK-induced model and transgenic model:  Nicotine: 100 µg/mL nicotine administered in drinking water for 2-6 weeks.  Control: Drinking water  Allograft model:  i.p. nicotine administration:  Nicotine: 0.8 mg/kg nicotine administered i.p. (frequency of administration NR)  Control group: NR  Oral nicotine administration:  Nicotine: 100 µg/mL nicotine administered in drinking water.  Control: Drinking water  Biomarkers of Nicotine Exposure  Average serum cotinine concentration after 12 weeks of treatment:  Oral nicotine group: 137 ng/mL  [Note: it is unclear if cotinine concentration was assessed in all animals, or only those in the NNK-induced tumor model]  Study Duration  Carcinogen-induced model: 16 weeks  Transgenic model: 2 or 6 weeks  Xenograft model: not specified. | Tumor incidence (% mice) at 12 weeks  NNK-induced tumor model:  Nicotine group had numerically higher tumor incidence than control group (data represented graphically; differences reported as not significant; p=NR)  Number of tumors per lung (tumor multiplicity) at the end of the study  NNK-induced tumor model:  In animals treated with nicotine for 12 weeks, nicotine treatment did not alter tumor multiplicity (data represented graphically, p value not provided).  Transgenic model:  In animals treated with nicotine for 6 weeks, there were no differences between nicotine and control groups in peripheral tumor multiplicity (data represented graphically, p value NR)  In animals treated with nicotine for 2 weeks, nicotine did not change tumor multiplicity compared to control treatment (data represented graphically, p value NR)  Tumor volume  NNK-induced tumor model:  Nicotine treatment did not alter tumor size at 12 weeks (data represented graphically, p value not provided).  Transgenic model:  In animals treated with nicotine for 2 weeks or 6 weeks, there were no differences between nicotine and control groups in tumor size (data represented graphically, p value NR)  Allograft model:  There were no significant differences in tumor volume at ~ 25-32 days after implantation between oral nicotine and control groups (data represented graphically, p value not provided).  There were no differences in tumor growth between oral nicotine administration (100 µg/mL) or i.p. administration (0.8 mg/kg nicotine), and control group (~18 days post implantation; data represented graphically, p value not provided).  Tumor proliferation (relative expression of Ki-67)  NNK-induced tumors:  Nicotine did not increase Ki-67 staining at 12 weeks (data represented graphically, p value not provided).  Transgenic model:  Nicotine did not increase Ki-67 staining (time point not provided; data represented graphically, p value not provided).  Incidence of metastasis  Xenograft model:  There were no differences in the incidence of metastasis between nicotine and control groups in animals that received injections of CL25 or CL13 cell lines (time point not provided; data represented graphically, p value NR). |
| 645 | Martinez et al., 2017;  US;  The Central Texas Veterans Health Care System, US Department of Veterans Affairs Biomedical Laboratory Research and Development Service | Study Design  Controlled, parallel group study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre‑treatment period: 7 days  Sample size:  Total: n=18 subjects  Nicotine 50 µmol/L : n=6 subjects  Nicotine 200 µmol/L: n=6 subjects  Control: n=6 subjects  Animal Model:  Species: Mice (BALB/c nude)  Sex: Male  Mean weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: Cholangiocarcinoma xenograft model  Cancer cell line injected:  Mz-ChA-1 cells | Study Methodology  Mice received an s.c. injection of Mz-ChA-1 cells (5×106 cells) into the right and left rear flanks. 7 days later, volume of each tumor was measured and mice were divided into intervention groups.  Intervention  Nicotine 50 µmol/L: 50 µmol/L nicotine administered in drinking water available ad libitum.  Nicotine 200 µmol/L: 200 µmol/L nicotine administered in drinking water available ad libitum.  Control: Drinking water available ad libitum  Study Duration  38 days | Tumor volume  Tumor volume was significantly larger in the 200 µmol/L nicotine group than in the control group from Day 29 to Day 38 (data represented graphically; p<0.05)  Tumor volume was significantly larger in the 50 µmol/L nicotine group than in the control group from Day 34 to Day 38 (data represented graphically; p<0.05).  Tumor proliferation (relative expression of Ki-67) at the end of the study  Tumors in the 50 µmol/L and 200 µmol/L nicotine groups had significantly more Ki-67‑positive cells at Day 38 compared with the control group (n=6, two tumors per mouse; data represented graphically; p<0.05 for 50 µmol/L nicotine vs. control and for 200 µmol/L nicotine vs. control). |
| 672 | Molfino et al., 2011;  Italy;  Ministero dell’Istruzione, dell’ Universita della Ricerca, Italy | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: 10 days  Wash-out/pre-treatment period: 8 days  Sample size:  Total: n=16 subjects  Nicotine: n=8 subjects  Control: n=8 subjects  Animal Model:  Species: Fischer rats  Sex: Male  Weight: 260-280 g  Age: NR  Comorbidities: NR  Cancer/tumor model: Sarcoma allograft model  Cancer cell line injected:  MCA sarcoma cells | Study Methodology  Viable MCA sarcoma cells (1×106) were inoculated into the right flank. Animals were then randomly assigned to intervention groups.  Intervention  Nicotine: Nicotine hydrogen tartrate salt (200 mg/kg/day) in 500 µL of saline was administered i.p. on Days 8, 9,10,15,16, and 17 after tumor inoculation.  Control: 500 µL saline was administered i.p. on Days 8, 9,10,15,16, and 17 after tumor inoculation.  Study Duration  19 days. | Tumor burden (weight) on Day 19  Nicotine group: 59.5±16.0 g  Control group: 45.6±6.6 g  p=NS |
| 683 | Murphy et al., 2011;  US;  Masonic Cancer Center and Animal Care and Research Program at the University of Minnesota and NIH | Study Design  RCT  Experimental groups:  NNK-induced tumor model:  NNK + Nicotine  NNK Control  Acclimation period: 7 days  Wash-out/pre-treatment period: NR  Sample size:  Total: n=240 subjects  NNK-induced tumor model:  NNK + Nicotine: n=40 subjects  NNK Control: n=40 subjects  Animal Model:  Species: Mice (A/J mice)  Sex: Female  Weight: NR  Age: 4 weeks  Comorbidities: NR  Cancer/tumor model:  Lung tumors  Cell line injected: NA | Study Methodology  NNK-induced tumor model:  NNK (10 µmol in 0.1 mL PBS) was administered i.p for 2 weeks. Mice were euthanized 44 weeks later. Lungs were excised and tumors were counted. Tumors were scored based on location (lung lobe) and the widest tumor diameter  Intervention  NNK-induced tumor model  NNK + Nicotine: 2 weeks after NNK injection, 200 µg/mL (0.44 µmol/mL) nicotine hydrogen tartrate and sodium potassium tartrate were provided in drinking water for 44 weeks.  NNK Control: Drinking water was provided ad libitum.  Biomarkers of Nicotine Exposure  Urine concentration in the nicotine group at 5 months:  Nicotine: 840±714 ng/mL (range: 62-2,560 ng/mL)  Cotinine: 5,910±4,140 ng/mL(range: 820‑14,700 ng/mL)  Trans-3’-hydroxycotinine: 37,800±19,400 ng/mL (range: 9,750‑83,400 ng/mL)  Plasma concentration in the nicotine group on Day 18:  Nicotine: 0.65±0.76 ng/mL (range: 0.1-2.5 ng/mL)  Cotinine: 32±33 ng/mL(range: 1-123 ng/mL)  Trans-3’-hydroxycotinine: 15±14 ng/mL (range: 0-45 ng/mL)  Plasma concentration in the nicotine group on Week 46:  Nicotine: 0.26±0.28 ng/mL (range: 0.1-1.1 ng/mL)  Cotinine: 29±19 ng/mL(range: 6-53 ng/mL)  Trans-3’-hydroxycotinine: 62±28 ng/mL (range: 28-105 ng/mL)  Study Duration  46 weeks.  Nicotine: 44 weeks. | Tumor incidence (% mice) at 46 weeks  NNK-induced tumor model (n=20 per group)  NNK + Nicotine: 20 of 20 (100%)  NNK Control: 18 of 18 (100%)  Tumor multiplicity (number of tumors per mouse; mean±SD) categorized by tumor diameter at 46 weeks:  NNK-induced tumor model (NNK + Nicotine, n=20; NNK Control, n=16 [2 died prematurely])  Total number of tumors:  NNK + Nicotine: 20.4±5.4  NNK Control: 18.4±4.5  Number of tumors <0.5 mm  NNK + Nicotine: 0.28  NNK Control: 0.50  Number of tumors 0.5-1.0 mm  NNK + Nicotine: 7.89  NNK Control: 6.50  Number of tumors 1.0-2.0 mm:  NNK + Nicotine: 11.3  NNK Control: 10.1  Tumors >2.0 mm:  NNK + Nicotine: 0.94  NNK Control: 1.39  Incidence of tumors categorized by tumor type (% mice) at 46 weeks:  NNK-induced tumor model (NNK + Nicotine, n=20; NNK Control, n=18):  Adenoma:  NNK + Nicotine: 89%  NNK Control: 83%  Adenoma with dysplasia:  Nicotine: 61%  Control: 56%  Carcinoma:  Nicotine: 44%  Control: 28%  Tumor multiplicity (number of tumors per mouse; mean±SD) at 46 weeks:  NNK-induced tumorigenesis experiments (NNK + Nicotine, n=20; NNK Control, n=18):  Adenoma:  NNK + Nicotine: 4.4±2.7  NNK Control: 3.4±2.4  Adenoma with dysplasia:  Nicotine: 1.0±1.1  Control: 0.83±0.92  Carcinoma:  Nicotine: 0.67±0.91  Control: 0.44±0.78 |
| 691 | Nakada et al. 2012;  Japan;  Ministry of Education | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Allograft-induced model  Nicotine: n=NR  Control: n=NR  NNK-induced model  Nicotine: n=12 subjects  Control: n=12 subjects  Animal Model  Species: C57BL/6 and A/J mice  Sex: Female  Weight: NR  Age:  C57BL/6 mice: 10 weeks  and  A/J mice: 7 weeks  Comorbidities: NR  Cancer/tumor model: xenograft- and carcinogen-induced lung tumor models  Cancer cell line injected: Lewis lung carcinoma cells | Study Methodology  Allograft-induced model  Lewis Lung carcinoma cells (1×106 cells/mouse) were injected s.c. into each flank of C57BL/6 mice, and then mice were divided into intervention groups. At day 14, tumors were dissected and the tumor volume was measured and calculated.  NNK-induced model  Female A/J mice were treated with NNK (i.p. injection of 2 mg/0.1 mL saline/mouse). The next day, the mice were divided into intervention groups. The experiment was terminated after 16 weeks after the first NNK treatment and the surviving mice were killed under anesthesia. At autopsy, lungs were excised, weighed, macroscopically inspected, and lung nodules were counted. Lung lobes were examined histopathologically.  Intervention  Allograft-induced model  Nicotine: 100 µg/mL of nicotine in 2% saccharine given in drinking water  Control: 2% saccharine given in drinking water  NNK-induced model:  NNK+Nicotine: i.p. injection of 2 mg/0.1 mL saline/mouse followed the next day with 100 µg/mL of nicotine in 2% saccharine given in drinking water  NNK alone: i.p. injection of 2 mg/0.1 mL saline/mouse followed the next day, by 2% saccharine given in drinking water  Study Duration  Xenograft-induced model: 14 days (2 weeks)  NNK-induced model: 111 days (16 weeks minus one day) | Allograft-induced model  Tumor volume at 2 weeks  Nicotine-treated mice had significantly higher tumor volume compared with control mice (p=0.013)  NNK-induced model  Tumor incidence (%) at 16 weeks  NNK+Nicotine: 12/12 (100%)  NNK alone: 12/12 (100%)  Numbers of tumors per mouse (by type) at 16 weeks  Adenoma:  NNK+Nicotine: 2.7±1.6  NNK alone: 2.3±0.9  Hyperplasia+Adenoma:  NNK+Nicotine: 4.0±3.0  NNK alone: 2.8±1.3  (values reported in table; statistical analysis performed but p-values for above comparisons NR) |
| 699 | Natori et al., 2003;  Japan;  Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labor and Welfare; Motor Vehicle Trust Fund for Research on Heart Diseases. | Study Design  Controlled, parallel study (randomization NR)  Experimental groups:  Allograft model:  Nicotine  Control  Allograft and bone marrow transplantation:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=20 subjects  Allograft model:  Nicotine: n=6 subjects  Control: n=6 subjects  Allograft and bone marrow transplantation:  Nicotine: n=4 subjects  Control: n=4 subjects  Animal Model:  Species: Mice (C57BL/6 mice)  Sex: Male  Weight: NR  Age: 8 weeks  Comorbidities: NR  Cancer/tumor model: Colon cancer allograft model  Cancer cell line injected: CMT93 cells, mouse allograft colon cancer cells | Study Methodology  Allograft model  Mice were treated with either nicotine or control prior to tumor inoculation. 5 days later, mouse allograft colon cancer cells CMT93 (6×107 cells) suspended in 0.1 mL of extracellular matrix gel were injected s.c. into the left flank. Tumor size was measured daily for 11 days after implantation, tumors were excised and capillary density was visualized using CD31 staining.  Allograft and bone marrow transplantation  CMT93 (1×107 cells) were inoculated into wild-type mice whose bone marrow had been replaced with that of ROSA26 mice (expressing LacZ reporter), and the mice were treated with vehicle or nicotine (concentration and route of administration NR) for 7 days.  Intervention  Allograft model  Nicotine: 20 mg/kg nicotine was administered by gavage daily starting 5 days before tumor inoculation.  Control: Vehicle (0.5% carboxymethylcellulose) was administered by gavage daily starting 5 days before tumor inoculation.  Allograft and bone marrow transplantation  Nicotine: Wild-type mice whose bone marrow had been replaced with that of ROSA26 mice were treated with nicotine for 7 days.  Control: Wild-type mice whose bone marrow had been replaced with that of ROSA26 mice were treated with vehicle for 7 days.  Study Duration  16 days.  Nicotine: 5 days prior to cancer cell inoculation (allograft model); 7 days after inoculation (allograft and bone marrow transplantation) | Tumor volume at 11 days  Allograft model:  Tumor volume in the nicotine-treated group significantly exceeded that in the vehicle group 11 days after implantation (values NR; data reported graphically; p<0.01).  Capillary density at 11 days  Allograft and bone marrow transplantation:  Nicotine: 496±29 capillaries/mm2  Control: 269±25 capillaries/mm2  p<0.01 |
| 720 | Nishikawa et al., 1992;  Japan;  Ministry of Health and Welfare | Study Design  Controlled, parallel group study  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=270 subjects  Nicotine: n=30 subjects  Control (BOP alone): n=30 subjects  Animal Model:  Species: Hamsters (Syrian golden)  Sex: Female  Weight: <100 g  Age: 6 weeks  Comorbidities: NR  Cancer/tumor models:  BOP-induced pancreatic tumors  Cell line injected:  NA | Study Methodology  BOP (10 mg/kg) was injected s.c. once a week for 3 weeks before being assigned to intervention groups. At Week 40, animals were euthanized, pancreatic tissue was removed and examined for the presence of adenocarcinomas.  Intervention  Nicotine: 25 ppm nicotine administered in drinking water ad libitum for 37 weeks.  Control: drinking water available ad libitum  Study Duration  40 weeks  Nicotine: 37 weeks | Tumor incidence (% animals) at Week 40  Adenocarcinoma:  Nicotine: 12 of 28 (43%)  Control: 11 of 28 (39%)  p=NS  Tumor multiplicity (number of tumors per animal; means±SD) at Week 40  Adenocarcinoma:  Nicotine: 0.6±0.9  Control: 0.4±0.6  p= NS  Distribution of adenocarcinomas in pancreatic lobes (n, %) at Week 40:  Splenic lobe lesions:  Nicotine: 9 of 18 (50%)  Control: 4 of 12 (33%)  p= NS  Gastric lobe lesions:  Nicotine: 3 of 18 (17%)  Control: 5 of 12 (42%)  p=NS  Duodenal lobe lesions:  Nicotine: 0 of 18 (0%)  Control: 0 of 12 (0%)  p= NA  Pancreatic head lesions:  Nicotine: 6 of 18 (33%)  Control: 3 of 12 (25%)  p= NS |
| 761 | Pillai et al., 2015;  US;  NCI | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=12 subjects  Nicotine: n=6 subjects  Control: n=6 subjects  Animal Model:  Species: Mice (SCID mice)  Sex: NR  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model:  NSCLC xenograft model  Cell line injected:  A549 cancer cell line | Study Methodology  Luciferase-expressing A549 cancer cells (2×105 cells/100 µL, viability >90%) were implanted into the left lung of SCID- mice. Mice were then randomized into intervention groups. Tumor growth was monitored weekly by in-vivo imaging system.  Intervention  Nicotine: Nicotine was administered i.p. every other day for 7 weeks (dose NR).  Control: Vehicle (type and volume NR) was administered i.p. every other day for 7 weeks  Study Duration  7 weeks | Tumor growth (assessed with mean luminescence in photon flux) at 7 weeks  Tumors in the nicotine group were significantly larger than those in the control group (p<0.05; data presented graphically).  Tumor metastasis (assessed as mean luminescence in photon flux) at 7 weeks  There were no significant differences in metastasis to the lung, brain, adrenal glands, or liver between the nicotine and control groups (values NR; data presented graphically)  [Note: the study noted that metastases in each tissue were numerically higher in the nicotine-than in the control group]. |
| 769 | Pratesi et al., 1996;  Italy;  NR | Study Design  Controlled parallel group (randomization NR)  Experimental groups:  Nicotine 20 µg  Nicotine 200 µg  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size: NR  Animal Model:  Species: Mice (athymic Swiss mice)  Sex: male and female  Weight: NR  Age: 6-10 weeks  Comorbidities: NR  Cancer/tumor model:  SCLC xenograft model  Cell line injected:  NCI-N592 SCLC cell line | Study Methodology  Mice received s.c. injections of NCI-N592 cells (106 cells) into both flanks. Growing tumor fragments were serially transplanted into new recipient mice. Nicotine or control intervention was administered on the same day (Day 0; early tumor progression experiment), or on Day 12 (established tumor progression experiment) after cancer cell line implantation. Tumor growth was monitored biweekly.  Intervention  20 µg/day nicotine experiment:  Nicotine: 20µg/day nicotine in water was administered s.c. via a continuous infusion for 14 days using osmotic pump.  Control: Vehicle was administered s.c. via continuous infusion for 14 days using an osmotic pump.  200 µg/day nicotine experiment:  Nicotine: 200 µg/day nicotine in water was administered s.c. via a continuous infusion for 14 days using osmotic pump.  Control: Vehicle was administered s.c. via continuous infusion for 14 days using an osmotic pump.    Study Duration  14 days  [Note: Two separate identical experiments were performed for the 200 µg/day nicotine and early tumor progression experiment.] | Early tumor progression\*  20 µg/day nicotine experiment:  Nicotine: 22.7±4.8 days  Control: 19.1±9.1 days  p=NS  200 µg/day nicotine experiment:  Nicotine 1: 12.3±3.9 days; Nicotine 2: 13.6±6.6 days  Control 1: 12.5±2.9 days; Control 2: 12.4±3 days  p=NS  [Note: Two separate identical experiments were performed for the 200 µg/day nicotine experiment; data from both experiments are presented.]  \*Early tumor progression was defined as the time between intervention (Day 0) and the tumor reaching 50 mm3 in size. Intervention administered for 2 weeks starting Day 0.  Established tumor progression†  20 µg/day nicotine experiment:  20 µg/day nicotine: 27.8±6.7 days  20 µg/day control: 25.0±8.9days  p=NS  200 µg/day nicotine experiment:  200 µg/day nicotine: 25.8±7.9 days  200 µg/day control: 25.0±8.9 days  p=NS  [Note: The same control group is used for the 20 µg/day and 200 µg/day nicotine experiments.]  †Established tumor progression was defined as the time for an established tumor >100 mm3 in size to reach 1000 mm3. Intervention administered for 2 weeks starting Day 12. |
| 775 | Prueitt et al., 2016;  US;  NIH, NCI, Center for Cancer Research,  Department of Defense, National Science Foundation, and Cancer Prevention and Research Institute of Texas Metabolomics Core | Study Design  Controlled parallel group (randomization NR)  Experimental groups:  Nicotine 100 µg/mL  Nicotine 250 µg/mL  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=65  Nicotine 100 µg/mL: n=22 subjects  Nicotine 250 µg/mL: n= 23 subjects  Control: n=20 subjects  Animal Model:  Species: Mice (TRAMP mice)  Sex: Male  Weight: NR  Age: 8-9 weeks  Comorbidities: NR  Cancer/tumor model:  Transgenic adenocarcinoma of the mouse (TRAMP) prostate cancer model.  Cell line injected:  NA | Study Methodology  Male TRAMP mice received either nicotine or control treatment. Mice were euthanized after 80 days or when they became moribund. Prostate glands and lungs were collected for analysis of primary tumor growth and metastasis.  Intervention  100 µg/mL nicotine: 100 µg/mL nicotine was administered in tap water available ad libitum  250 µg/mL nicotine: 250 µg/mL nicotine administered in tap water available ad libitum  Control: Tap water was administered ad libitum.  Study Duration:  80 days (or when animals became moribund) | Urogenital tract weight without seminal vesicles (indicator of tumor size) at 80 days  100 µg/mL nicotine: 513±195 mg  250 µg/mL nicotine: 532±462 mg  Control: 592±231 mg  p=NS  Lung metastasis (n, %) at 80 days  100 µg/mL nicotine: 6 of 22 (27%)  250 µg/mL nicotine\*: 7 of 23 (30%)  Control: 0 of 20 (0%)  \*p=0.046 (Fisher exact test for trend)  Adenocarcinoma with metastasis to the lung (n, %) at 80 days  100 µg/mL nicotine: 1 of 22 (5%)  250 µg/mL nicotine: 2 of 23 (9%)  Control: 0 of 20 (0%)  p=NR  Neuroendocrine carcinoma with metastasis to the lung (n, %) at 80 days  100 µg/mL nicotine: 5 of 22 (23%)  250 µg/mL nicotine: 5 of 22 (22%)  Control: 0 of 20 (0%)  p=NR  Lymph node metastasis (lymph nodes examined, %)  100 µg/mL nicotine: 2 of 7 (29%)  250 µg/mL nicotine: 1 of 3 (33%)  Control: 0 of 3 (0%)  p=NR |
| 824 | Ross et al., 2020;  US;  NIH, National Cancer Institute, Center for Cancer Research | Study Design  Controlled parallel study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: 7 days  Sample size:  Total: n=20  Nicotine group: n=10 subjects  Control group: n=10 subjects  Animal Model:  Species: Mice (FVB/NJ mice)  Sex: Female  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model:  Metastatic breast cancer allograft model  Cell lines injected:  6DT1 cells (mouse mammary carcinoma cell line) | Study Design  6DT1 (1×105) cells in 100 μL PBS were implantation into the fourth mammary fat pad 7 days before the start of the intervention. Primary tumors were resected 2 weeks after the implantation. Mice were euthanized 42–45 days post-implantation.  Intervention  Nicotine: 100 μg/mL nicotine was administered in drinking water starting on Day 7 after tumor injection for ~21 days.  Control: Drinking water was administered.  Biomarkers of Nicotine Exposure  Mean serum nicotine levels during 4 weeks (range):\*  Nicotine: 13.48 ng/mL (1.17-76.66 ng/mL)  Control: 2.278 ng/mL (1.34-3.07 ng/mL)  Mean serum cotinine levels during 4 weeks (range):\*  Nicotine: 54.98 ng/mL (0-254.13 ng/mL)  Control: 0 ng/mL (range NA)  \*Serum levels were determined once per week for 4 weeks and the average of the 4 values was reported.  Study Duration  42-45 days.  Nicotine: ~21 days | Primary tumor weight at 2 weeks  There were no significant differences in primary tumor weight between control and nicotine groups (data represented graphically; p=NS).  Pulmonary metastasis at 42-45 post implantation  The number of pulmonary metastases was significantly higher in the nicotine than in the control group (values NR; data represented graphically; p=0.0105)  Metastases normalized to primary tumor weight  There number of metastases per gram of tumor weight was significantly higher in the nicotine than in the control group (values NR; data represented graphically; p=0.009) |
| 888 | Shimizu et al., 2019;  Japan;  Ministry of Education, Culture, Sports, Science, and Technology of Japan; Wesco Scientific Promotion Foundation | Study Design  Controlled, parallel group study (randomization NR)  Experimental groups:  Nicotine  Control  Acclimation period: NR  Pre‑treatment period: NR  Sample size:  Total: n=50 subjects  i.p. nicotine: n=10 subjects  Control: n=10 subjects  Animal Model:  Species: Mice (athymic nu/nu)  Sex: Male  Mean weight: 19.5 g  Age: 5 weeks  Comorbidities: NR  Cancer/tumor model: HNSCC lymph node metastasis xenograft model  Cancer cell line injected:  OSC-19 cells, a tongue squamous cell carcinoma line | Study Methodology  Athymic mice received an i.p. injection of 8×105 OSC-19 cells into the hind footpad. Tumor sizes and body weights were measured weekly, and tumor volume was calculated. The footpad tumor tissues and popliteal lymph nodes were isolated after 42 days.  Intervention  Nicotine: Mice received i.p. injection of nicotine (30 µg/mouse) daily for 42 days (volume not specified).  Control: Mice received i.p. injection of PBS daily for 42 days (volume not specified).  Study Duration  42 days. | Tumor volume on Day 42  Nicotine: 950.0±188.9 mm3  Control: 615.2±65.3 mm3  p<0.05 (nicotine vs. control).  Incidence of lymph node metastasis:  Nicotine-treated group: 6 of 10 (60%)  Control group: 1 of 10 (10%)  (q=0.057, q values were calculated with the false discovery rate method controlled by the Benjamini-Hochberg procedure, q<0.05 considered to be significant) |
| 894 | Shin et al., 2004;  Hong Kong, Taiwan;  University of Hong Kong, Hong Kong Research Grants Council | Study Design  RCT  Experimental groups:  Nicotine 50 µg/mL  Nicotine 200 µg/mL  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total n= 50 subjects  Nicotine 50 µg/mL: 10 subjects  Nicotine 200 µg/mL: n=10 subjects  Control: n= 10 subjects  Animal Model:  Species: Mice (athymic nude)  Sex: NR  Weight: NR  Age: 4-6 weeks  Comorbidities: NR  Cancer/tumor model: Gastric cancer xenograft model.  Cancer cell line injected: AGS cells | Study Methodology  AGS cells (2×107 cells/mL) from a poorly differentiated human gastric adenocarcinoma cell line, were inoculated into the gastric wall. The no tumor control group did not receive an inoculation of AGS cells. Animals were randomized to intervention groups. At the end of the study, mice were euthanized, tumors growing on the stomach were removed, and tumor area was measured.  Intervention  Nicotine: 50 µg/mL or 200 µg/mL nicotine administered in tap water available ad libitum.  Tumor control: Tap water available ad libitum.  No tumor control: Mice were not inoculated with AGS cells.  Study Duration  3 months. | Tumor area after 3 months (mean±SD)  Tumor control: 18.05±3.27 mm2  50 µg/mL nicotine: 25.34±1.02 mm2 (p<0.05 vs. tumor control)  200 µg/mL nicotine: 29.91±2.23 mm2 (p<0.01 vs. tumor control)  No tumor control: NA  Number of proliferative (PCNA-positive) cells in gastric tissue (mean±SD) after 3 months:  Tumor control: 16.02±0.60 cells (p<0.05 vs. no tumor control)  50 µg/mL nicotine: 22.61±1.73 cells (p<0.05 vs. no tumor control)  200 µg/mL nicotine: 28.56±2.20 cells (p<0.05 vs. no tumor control; p<0.01 vs. tumor control).  No tumor control: 11.20±2.19 cells  Angiogenesis after 3 months  Density of microvessels was significantly higher in the 200 µg/mL nicotine group than in the tumor control group (data represented graphically, p<0.05) or the no tumor control group (p<0.05).  There were no significant differences in the density of microvessels between the 50 µg/mL nicotine group and either control groups (data represented graphically, p value NR). |
| 940 | Suzuki, 2018;  Japan;  Nagoya City University | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: 1 week  Wash-out/pre-treatment period: NR  Sample size:  Total n = 61 subjects  Nicotine (10 ppm): n=15 subjects  Nicotine (20 ppm): n=15 subjects  Nicotine (40 ppm): n=16 subjects  Control: n=15 subjects  Animal Model  Species: Rats (F344)  Sex: male  Weight: NR  Age: 5 weeks  Comorbidities: NR  Cancer/tumor model: Carcinogen (BBN)-induced model of bladder cancer.  Cancer cell line injected: NR | Study Methodology  Animals received drinking water containing 0.05% BBN for the first 4 weeks of the study before being randomized to receive either nicotine or control treatment.  Intervention  Nicotine: Nicotine hydrogen tartrate was administered at a concentration of 28.5, 57.2, or 114 ppm in drinking water, corresponding to 10, 20, and 40 ppm nicotine, respectively. Drinking water was available ad libitum.  Control: Drinking water available ad libitum.  Biomarkers of Nicotine Exposure  Serum cotinine level (mean ±SE):  10 ppm nicotine: 83.8±5.3 ng/mL  20 ppm nicotine: 199.5±10.5 ng/mL  40 ppm nicotine: 347.4±15.9 ng/mL  Control: NA  Study Duration:  36 weeks.  Nicotine: 32 weeks. | Incidence of urothelial carcinoma (n, %) at 36 weeks  10 ppm nicotine group: 4 of 15 (27%)  20 ppm nicotine group: 6 of 15 (40%)  40 ppm nicotine group: 9 of 16 (56%); p<0.05 vs. control group  Control group: 1 of 15 (7%)  Number of urothelial carcinomas (mean±SE) at 36 weeks  10 ppm nicotine group: 0.3±0.2  20 ppm nicotine group: 0.5±0.2  40 ppm nicotine group: 0.6±0.2; p<0.05 vs. control group  Control group: 0.1±0.1  Tumor proliferation (relative expression of Ki-67) at 36 weeks  The proportion of Ki-67-positive cells was significantly higher in all nicotine groups (ie, 10, 20, or 40 ppm nicotine) than in the control group (data represented graphically, p<0.001 for each nicotine group). |
| 975 | Torres-Gonzalez et al., 2014;  US;  NIH | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=NR  Control: n=NR  Animal Model  Species: Mice (Kras)  Sex: Male  Weight: NR  Age: NR  Comorbidities: NR  Cancer/tumor model: Kras mice lung cancer model  Cancer cell line injected: NA | Study Methodology  Kras mice were given water with nicotine for 90 days. After treatment, number and size of lung tumors developed were compared to matched controls.  Intervention  Nicotine: 100 µg/mL nicotine administered in drinking water for 90 days.  Control: Water alone for 90 days.  Study Duration  90 days | Tumor number (n)  (timing not specified)  Nicotine: 10.37±3.6  Control: 10.57±4.2  p=0.95  Tumor area(µm2)  (timing not specified)  Nicotine: 320,626 µm2±252,865 µm2  Control: 244,774 µm2±455,926 µm2  p=0.37 |
| 982 | Trevino et al., 2012;  US;  National Cancer Institute | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total n=NR.  Animal Model:  Species: Mice (SCID)  Sex: Female  Weight: NR  Age: 8 weeks  Comorbidities: NR  Cancer/tumor model:  Pancreatic adenocarcinoma xenograft model.  Cell line injected: L3.6pl metastatic variant pancreatic cancer cell line | Study Methodology  Cells from the L3.6pl metastatic variant pancreatic cancer cell line were orthotopically implanted into the pancreas of SCID mice. One week after implantation, mice were randomized to receive either nicotine or control treatment. Tumor growth and metastasis to the liver were assessed using bioluminescence. At the end of the study (4 weeks after implantation), primary tumors, regional pancreatic lymph nodes, and livers were examined for tumor cells by ex vivo imaging.  Intervention  Nicotine: 1 mg/kg administered i.p. 3 days per week.  Control: PBS administered i.p. 3 times per week.  Study Duration:  4 weeks.  Nicotine: 3 weeks | Tumor volume at 4 weeks  Tumor volume was significantly larger in the nicotine group than in the control group (data represented graphically, p<0.01).  Tumor growth over 3 weeks  Nicotine significantly augmented tumor growth compared with control treatment (data represented graphically, p<0.001).  Metastasis to the liver (assessed using bioluminescence) at 4 weeks  Metastasis was significantly higher in the nicotine than in the control group (data represented graphically, p<0.001). |
| 1135 | Tyagi et al., 2021;  US;  National Institutes of Health. | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample Size:  Spontaneous metastasis model:  Total: n= 12  Nicotine: n=6  Control: n=7  Experimental metastasis model:  Total: n = 14  Nicotine: n = 7  Control: n = 7  Experimental pre-metastasis model:  Total n = 16  Nicotine: n = 8  Control: n = 8  Nicotine abstinence model:  Total n = 12  Nicotine pretreatment 1 day: n = 4  Nicotine pretreatment 15 days: n = 4  Nicotine pretreatment 30 days: n = 4  Animal Model:  Species: Mice (BALB/c or C57BI/6)  Sex: NR  Weight: NR  Age: 5-6 weeks.  Cancer/tumor model: Mouse models of breast cancer (allograft models)  Cancer cell line injected: 4T1 cells or E0771 cells | Study Methodology  Spontaneous metastasis model:  4T1 (204 cells/mouse) or E0771 (0.5×106 cells/mouse) mouse mammary cancer cells were injected into mammary fat pads. Animals were randomized to receive either nicotine or control treatment.  Experimental metastasis model:  4T1 tumor cells (204 cells/mouse) or E0771 tumor cells (0.5×106 cells/mouse) were injected into the tail vein. Animals were randomized to receive either nicotine or control treatment.  Experimental pre-metastasis model:  Mice were exposed to nicotine or control treatment for 10 days before receiving an i.v. injection of 4T1 or E0771 tumor cells.  Nicotine abstinence model:  Mice were pre-treated with nicotine for 10 days before receiving an injection of 4T1 cells. Cells were injected 1 day, 15 days, or 30 days after nicotine treatment.  Intervention  Spontaneous and experimental metastasis models:  Nicotine: 2 mg/kg administered i.p. every other day.  Control: Phosphate buffered saline administered i.p. every other day.  Experimental pre-metastasis model:  Nicotine: 2 mg/kg administered i.p. for 10 days before the injection of tumor cells.  Control: Phosphate buffer saline administered i.p. for 10 days before the injection of tumor cells  Nicotine abstinence model:  2 mg/kg nicotine was administered i.p. for 10 days before the injection of tumor cells. Tumor cells were injected 1 day, 15 days, or 30 days after nicotine treatment.  Biomarkers of nicotine exposure  Serum cotinine  Value NR, data represented graphically.  [Note: The study indicated that serum cotinine levels were comparable to those in adult smokers]  Study duration:  28 days. | Primary tumor growth  Spontaneous metastasis model:  4T1luc cells  Primary tumor growth was significantly larger in the nicotine group than in the control group on Day 24 (data represented graphically, p=0.04) and Day 28 (p=0.0001) (<10-fold increase in nicotine vs. control).  E0771 cells  Primary tumor growth was significantly larger in the nicotine than in the control group on Day 24 (data represented graphically, p=0.393) and Day 28 (p=0.0001).  [Note: Graph indicates statistical difference on Day 24, however, p=0.393 was reported and significance level was set at p<0.05].  Experimental metastasis model:  4T1luc cells:  Primary tumor growth assessed by in vivo (bioluminescence) was significantly larger than in the control group (<10-fold increase; p=0.0003)  Primary tumor weight  Spontaneous metastasis model:  4T1luc cells:  Primary tumor weight was significantly higher in the nicotine group than in the control group (Day 28; data represented graphically, p=0.02).  E0771 cells:  Primary tumor weight was higher in the nicotine group than in the control group (Day 28; data represented graphically, p=NS).  Lung metastatic burden  Spontaneous metastasis model:  4T1luc cells:  The lung metastatic burden evaluated ex vivo was significantly greater in the nicotine group than in the control group (Day 28; >100-fold increase, data represented graphically, p=0.01).  E0771 cells:  The lung metastatic burden evaluated ex vivo (bioluminescence) was greater in the nicotine group than in the control group (Day 28; data represented graphically, p=0.349).  [Note: Graph indicates statistical difference, however, p=0.349 was reported and significance level was set at p<0.05; there appears to be a typo and should be 0.0349].  Experimental metastasis model:  4T1luc cells:  The lung metastatic burden assessed ex vivo was significantly greater in the nicotine group than in the control group (Day 28; >100-fold increase, data represented graphically, p=0.362).  [Note: Graph indicates a statistical difference, however p=0.362 was reported and significance level was set at p<0.05].  The lung metastatic burden assessed in vivo was significantly higher in the nicotine group than in the control group on Day 24 (p=0.038) and Day 28 (p=0.0003)  The number of lung metastatic nodules was significantly greater in the nicotine group than in the control group (Day 28; data represented graphically, p<0.0001).  Experimental pre-metastasis model:  4T1luc cells:  Lung metastasis assessed in vivo was significantly larger in the nicotine group than in the control group on Day 24 (data represented graphically, p=0.04) and Day 28 (p=0.0001).  The lung metastatic burden (assessed ex vivo) was significantly greater in the nicotine group than in the control group (Day 28; data represented graphically, p=0.01).  E0771 cells:  Lung metastasis was significantly greater in the nicotine group than in the control group on Day 24 (data represented graphically, p=0.026) and Day 28 (p<0.0001).  The lung metastatic burden (assessed ex vivo) was significantly greater in the nicotine group than in the control group (Day 28; data represented graphically, p=0.004).  Nicotine abstinence model:  4T1luc cells:  Lung metastasis (assessed in vivo) was significantly higher when nicotine treatment was stopped 1 day before the injection of 4T1luc cells compared to 15 days before the injection (data represented graphically, p=0.033 vs. 1 day) and compared to 30 days before the injection (p<0.0001 vs. 1 day)  Lung metastasis (assessed in vivo) was significantly higher when nicotine treatment was stopped 15 days before the injection compared to 30 days before the injection (data represented graphically, p=0.025).  Lung metastasis (assessed ex vivo) was significantly higher when nicotine was stopped 1 day after the injection of cells compared to 15 days (data represented graphically, p=0.046) or 30 days before the injection of cells (p=0.032),  There were no significant differences between groups in which nicotine treatment was stopped 15 days vs 30 days before the injection of tumor cells (data represented graphically, p=0.15)  Metastasis-free survival  Spontaneous metastasis model:  4T1luc cells:  Lung metastasis-free survival was significantly shorter in the nicotine than in the control group (data represented graphically, p=0.001).  Experimental metastasis model:  4T1luc cells:  Lung metastasis-free survival was significantly shorter in the nicotine than in the control group (data represented graphically, p=0.01). |
| 998 | Underwood et al., 2020;  US;  NCI, NIH | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: 14 days  Sample size:  Total: n=26 subjects  Nicotine: n=9 subjects  Control: n=12 subjects  Animal Model  Species: Mice (non-obese, diabetic SCID-gamma mice)  Sex: Male and female  Weight: NR  Age: 12 weeks  Comorbidities: Diabetes, severe combined immunodeficiency  Cancer/tumor model: Pancreatic cancer patient-derived xenograft model  Cancer cell line injected: Pancreatic ductal adenocarcinoma | Study Methodology  Pancreatic cancer patient-derived xenograft cells were implanted into the pancreas of 12-week old mice. 14 days later, mice received nicotine or control treatment. After 6 weeks of treatment, mice were euthanized, tumors were collected and weighed.  Intervention  Nicotine: 1 mg/kg nicotine in PBS was administered i.p. 3 days per week over 6 weeks (method of injection NR).  Control: PBS was administered i.p. 3 days per week over 6 weeks (method of injection NR).  Study Duration  8 weeks  6 weeks of nicotine treatment | Tumor weight at 8 weeks  Nicotine: 3.08 g ± 1.07 g  Control: 1.75 g ± 1.31 g  p=0.02 |
| 1025 | Wan et al., 2018;  China;  National Natural Science Foundation of China, Science and Technology Planning Project of Jiangsu Province, Science and Technology Project of Nantong | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=6 subjects  Nicotine: n=4 subjects  shPP1y lentivirus present: n=2 subjects  shPP1y lentivirus not present: n=2 subjects  Control: n=2 subjects  Animal Model  Species: Mice (BALB/c)  Sex: Female  Weight: NR  Age: 4 weeks  Comorbidities: shPP1y lentivirus  Cancer/tumor model: Hepatocellular carcinoma xenograft model  Cancer cell line injected: Human hepatocarcinoma SMMC-7721 cells | Study Methodology  SMMC-7721 cells (1×106 cells) infected with control or shPP1γ lentivirus were injected s.c. into the flanks of mice before nicotine or control intervention. Tumor volumes were measured every 5 days using a Vernier caliper. 40 days after injection, the tumors were excised for analyses.  Intervention  Nicotine: 200 μg/mL nicotine in sterile drinking water for 40 days.  Control: Drinking water alone for 40 days.  Study Duration  40 days | Tumor volume on Day 35  Tumor volume was significantly greater in the nicotine than in the control group (values NR, data represented graphically, p<0.05).  Tumor weight on Day 35  Tumor weight was significantly greater in the nicotine than in the control group (values NR, data represented graphically, p<0.05). |
| 1028 | Wang et al., 2017;  China;  National Natural Science Foundation of China, Beijing Natural Science Foundation of China | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=140 subjects  Nicotine: n=20 subjects  Control: n=20 subjects  Animal Model  Species: Mice (wild type C57BL/6)  Sex: NR  Weight: NR  Age: 6-8 weeks  Comorbidities: NR  Cancer/tumor model: 4NQO-induced oral precancerous lesions  Cancer cell line injected: NA | Study Methodology  4NQO with or without nicotine was administered onto mouse tongues 3 times per week for 16 weeks. At the end of treatment, mice were euthanized and tongues were removed for analysis.  Intervention  Nicotine: 5% nicotine and 50 µg/mL 4NQO were smeared on the tongue 3 times per week for 16 weeks.  Control: 50 µg/mL 4NQO smeared on the tongue 3 times per week for 16 weeks.  Study Duration  16 weeks | Incidence of carcinoma in situ/OSCC  Nicotine: 5 of 20 (40%)  Control: 1 of 20 (5%)  p<0.05 |
| 1034 | Wang et al., 2021;  China;  National Natural Science Foundation of China, Natural Science Foundation of Guangdong Province of China, Special Project on the Integration of Industry, Education and Research of Guangdong Province, China Postdoctoral Science Foundation, Flagship Specialty Construction Project-General Surgery | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: 16 weeks of 4NQO  Sample size:  Total: n=16 subjects  Nicotine: n=8 subjects  Control: n=8 subjects  Animal Model  Species: Mice (C57BL/6 and nude mice)  Sex: Female  Weight: NR  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model: 4NQO-induced and human xenograft models of esophageal squamous cell carcinoma  Cancer cell line injected: Human esophageal squamous cell carcinoma TE1 cells. | Study Methodology  4NQO-induced model:  Mice were given 100 µg/mL of 4NQO in drinking water for 16 weeks to induce esophageal squamous cell carcinoma before receiving nicotine or control treatment for 16 weeks. Mice were euthanized 12 weeks after the end of treatment and the number of tumor per mouse was assessed.  Human xenograft model:  Human esophageal squamous cell carcinoma xenograft was injected into nude mice (timing relative to intervention NR). Tumor volume and weight were assessed.  Intervention  Nicotine: 200 µg/mL nicotine administered orally for 16 weeks.  Control: No nicotine administered orally during 16 week treatment period.  Study Duration  4NQO-induced tumor model: 44 weeks (16 weeks of nicotine treatment)  Human xenograft model: NR | Tumor incidence at 44 weeks  4NQO-induced model:  Number of tumors per mouse was significantly higher in the nicotine than in the control group (values NR; data represented graphically; p<0.001).  Tumor volume  Human xenograft model  Tumor volume was significantly higher in the nicotine group than in the control group at Week 2 (p<0.01), Week 3 (p<0.001), Week 4 (p<0.001), and Week 5 (p<0.001) (values NR; data represented graphically).  Tumor weight  Human xenograft model  Tumor weight was significantly higher in the nicotine group than in the control group (p<0.001) (values NR; data represented graphically). |
| 1037 | Wang et al., 2019;  China;  Beijing Natural Science Foundation of Chine, National Natural Science Foundation of China | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n = 100 subjects  Nicotine: n = 20 subjects  Control: n= 20 subjects  Animal Model:  Species: Mice (BALB/c nude)  Sex: Male and female  Weight: NR  Age: 5 weeks.  Comorbidities: NR  Cancer/tumor model: Oral squamous cell carcinoma xenograft model.  Cancer cell line injected: Human oral cancer CAL27 cells | Study Methodology  Human oral cancer CAL27 cells (5×106 cells) were transplanted into the tongue. Metastasis to the lymph nodes was assessed starting on Day 11 after implantation.  Intervention  Nicotine: 10 µL nicotine in 6% corn oil administered orally 3 times per week.  Control: 6% corn oil administered orally 3 times per week.  Study Duration:  13 days. | Tumor metastasis  Metastasis rate was significantly higher in the nicotine group than in the control group 13 days after implantation of CAL27 cells (data represented graphically, p ≤ 0.05 vs. control). |
| 1047 | Warren et al., 2012;  US;  American Cancer Society; American Society of Oncology Foundation (Conquer Cancer Foundation), Young Investigators Award | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Nicotine: n=NR  Control: n=NR  Animal Model  Species: Mice (Foxn 1nu athymic nude)  Sex: Male  Weight: 25-28 grams  Age: 8-10 weeks  Comorbidities: NR  Cancer/tumor model: Human lung cancer xenograft model  Cancer cell line injected: H460 lung cancer cells | Study Methodology  Human H460 lung cancer xenografts (1.5 million cells suspended in 50 µL of media) were implanted into the right flank of mice. When tumors reached 5 mm in maximal dimension, mice were randomized to intervention groups. Tumor volumes were estimated using orthogonal measurements every other day.  Intervention  Short-term nicotine: Nicotine (60 µg) was administered s.c. every other day for 6 days.  Long-term nicotine: Nicotine (60 µg) was administered every other day until tumor was 15 mm in greatest linear dimension or a maximum of 28 days of growth after reaching 5 mm in greatest dimension.  Control: Saline was administered s.c. every other day for 6 days.  Study Duration  28 days. | Tumor Volume at 28 days  There were no significant differences in tumor volume between short-term nicotine, long-term nicotine, and control groups (values not reported, data represented graphically; p-value NR). |
| 1072 | Wong et al., 2007;  Hong Kong;  University of Hong Kong, Hong Kong Research Grants Council. | Study Design  RCT  Experimental groups:  Nicotine 50 µg/mL  Nicotine 200 µg/mL  Control    Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=NR  Animal Model:  Species: Mice (BALB/c nu/nu)  Sex: Female  Weight: NR  Age: 4-6 weeks.  Cancer/tumor model: Colon tumor xenograft model.  Cancer cell line injected: HT-29 cells | Study Methodology  HT-29 cells (3×106 cells) from a human colon cancer cell line were implanted s.c. into the right flank. Animals were randomized to receive nicotine (50 or 200 µg/mL) or control treatment.  Intervention  Nicotine: 50 µg/mL or 200 µg/mL administered in tap water.  Control: Tap water (not specified if available ad libitum).  Biomarkers of Nicotine Exposure  Plasma cotinine on Day 25:  Nicotine 50 µg/mL group: 43.2±7.10 ng/mL (p<0.05; vs control group)  Nicotine 200 µg/ml group: 169.4±12.21 ng/mL(p<0.005; vs control group)  Control group: 8.7 ± 0.66 ng/mL  Study Duration:  25 days. | Tumor volume on Day 25, mean±SE  Control: 0.77±0.13 cm3  Nicotine 50 µg/mL: 1.10±0.11 cm3 (p<0.05 vs. control)  Nicotine 200 µg/mL: 1.78±0.17 cm3 (p<0.005 vs. control).\*  \*Taken from text. Table 2 reported final tumor volume as 1.82±0.22 cm3 for the Nicotine 200 µg/mL group.  Mean number of blood vessels on Day 25  The number of blood vessels per mm2 was significantly higher in the 200 µg/mL nicotine group than in the control group (data represented graphically, p<0.005).  There were no differences in the number of blood vessels between the 50 µg/mL nicotine and control groups (data represented graphically). |
| 1076 | Wu et al., 2020;  US;  NIH | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: 3 days  Sample size:  Total: n=18  Nicotine: n=9  Control: n=9  Animal Model  Species: Mice (BALB/c, C57BL/6, and nude)  Sex: Female  Weight: NR  Age: 5-6 weeks  Comorbidities: NR  Cancer/tumor model: Lung cancer xenograft/allograft model  Cancer cell line injected: H2030BrM human lung cancer cells and LL/2 mouse lung cancer cells | Study Methodology  Intracranial injection of mouse LL/2 lung cancer cells into BALB/c mice:  BALB/C mice received an intracranial injection of luciferase-labelled LL/2 mouse lung cancer cells. Nicotine or control treatment started 3 days after cell injections. Tumor growth was monitored every week by bioluminescence for 40 days. At the end of the study, whole brain was removed and metastatic tumor size was assessed by bioluminescence.  Intracardiac injection of mouse LL/2 lung cancer cells into C57BL/6 mice:  C57BL/6 mice received intracardiac injections of luciferase-labelled LL/2 mouse lung cancer cells, and nude mice received intracardiac injections of luciferase-labelled H2030BrM human lung cancer cells. Nicotine or control treatment started 3 days after cell injections. Tumor growth was monitored every week by bioluminescence for 40 days. At the end of the study, whole brain was removed and metastatic tumor size was assessed by bioluminescence.  Intracardiac injections of H2020BrM human lung cancer cells into nude mice:  Nude mice received intracardiac injections of luciferase-labelled H2030BrM human lung cancer cells. Nicotine or control treatment started 3 days after cell injections. Tumor growth was monitored every week by bioluminescence for 60 days. At the end of the study, whole brain was removed and metastatic tumor size was assessed by bioluminescence.  Intervention  Nicotine: 1 mg/kg of nicotine in PBS was injected i.p. once every 3 days (BALB/c and C57BL/6)  Control: PBS was injected i.p. every 3 days for the duration of the study.  Study Duration  40 days (BALC/c and C57BL/6 mice ) or 60 days (nude mice) | Metastatic tumor growth (flux assessed every week)  Intracranial injection of mouse LL/2 lung cancer cells into BALB/c mice:  Growth of brain metastases was significantly greater in the nicotine group than in the control group (values NR; data represented graphically; p<0.01 on Day 40)  Intracardiac injection of mouse LL/2 lung cancer cells into C57BL/6 mice:  Growth of brain metastases was significantly greater in the nicotine group than in the control group after intracardiac injection of LL/2 cells in C57BL/6 mice (values NR; data represented graphically; p<0.01 on Day 40)  Intracardiac injections of H2020BrM human lung cancer cells into nude mice:  Growth of brain metastases was significantly greater in the nicotine group than in the control group after intracardiac injections of H2030BrM cells into nude mice (values NR; data represented graphically; p<0.001 on Day 60)  Metastatic tumor size at endpoint (photon flux)  Intracranial injection of mouse LL/2 lung cancer cells into BALB/c mice:  Brain metastatic lesions were significantly bigger in the nicotine group than in the control group on Day 40 (data represented graphically; p<0.01)  Intracardiac injection of mouse LL/2 lung cancer cells in C57BL/6 mice:  Brain metastases were significantly larger in the nicotine group than in the control group on Day 40 (values NR; data represented graphically; p<0.001)  Intracardiac injections of H2020BrM human lung cancer cells into nude mice:  Brain metastases were significantly larger in the nicotine group than in the control group on Day 60 (values NR; data represented graphically; p<0.001)  Metastasis-free survival  Intracranial injection of mouse LL/2 lung cancer cells into BALB/c mice:  Brain metastasis-free survival was significantly shorter in the nicotine-treated group than in the control group (values NR; data represented graphically; p<0.05)  Intracardiac injection of mouse LL/2 lung cancer cells into nude mice:  Brain metastasis-free survival was significantly shorter in the nicotine-treated group than in the control group (values NR; data represented graphically; p<0.05)  Intracardiac injection of human H2030BrM lung cancer cells into nude mice:  Brain metastasis-free survival was significantly shorter in the nicotine-treated group than in the control group (values NR; data represented graphically; p<0.05)  Incidence of metastasis (n)  Intracardiac injection of mouse LL/2 lung cancer cells into BALB/c mice:\*  Bone metastasis:  Nicotine: 3 of 9 (33%),  Control: 1 of 9 (11%)  p=0.25  Brain Metastasis:  Number of mice with metastasis to the brain:  Nicotine: 7 of 9 (77%),  Control: 2 of 9 (22%)  p=0.01  \*Note: text indicates that intracranial injections were made, whereas Figure 2 legends states that intracardial injections were done. |
| 1109 | Yuge et al., 2015;  Japan;  Ministry of Education, Culture, Sport, Science, and Technology of Japan | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total: n=40 subjects  Nicotine: n=10 subjects  Control: n=10 subjects.  Animal Model:  Species: Mouse (nude athymic BALB/c)  Sex: Not specified  Weight: 20 g  Age: 6 weeks  Comorbidities: NR  Cancer/tumor model: Bladder cancer xenograft model.  Cancer cell line injected: T24 cells from a human bladder cancer cell line | Study Methodology  T24 cells (2×106 cells) from a human bladder cancer cell line were implanted s.c. in the flank of each mouse. Tumor size was assessed twice per week  Intervention  Nicotine: 1 mg/kg administered i.p. 3 times per week (vehicle not specified)  Control: Vehicle administered i.p. 3 times per week  Study Duration:  21 days. | Tumor volume on Day 21, mean±SE  Control: 470.3±73.4 mm3  Nicotine: 929.1±180.2 mm3  p=0.039 |
| 1114 | Zhang et al., 2017;  China;  Foundation of Shanghai Jiao Tong University School of Medicine, Foundation of Shanghai Pharmaceutical Association | Study Design  RCT  Experimental groups:  Nicotine  Control  Acclimation period: NR  Wash-out/pre-treatment period: NR  Sample size:  Total n=21  Nicotine: n=4  Control: n=4  Animal Model:  Species: Mice (nude)  Sex: Male  Weight: Mean: 18±2 g  Age: NR  Cancer/tumor model: NSCLC xenograft model.  Cancer cell line injected: H1299 cells from a human NSCLC cell line | Study Methodology  H1299 cells (2.5×107 cells/mL) from a human non-small cell lung cancer cell line were injected s.c. in to the right axilla of each mouse. Animals were randomized to receive either nicotine or control treatment. Tumor volume was assessed once per week.  Intervention  Nicotine: 1 mg/kg dissolved in saline administered i.p. 3 times per week for 6 weeks.  Control: Saline administered i.p. 3 times per week for 6 weeks.  Study Duration:  6 weeks | Tumor volume  Tumor volume was significantly larger in the nicotine group than in the control group (data represented graphically; p<0.05) |

Abbreviations: 4NQO = 4-nitroquinoline 1-oxide; µg = micrograms; µL=microliters; µM = micromolar; BBN = N-butyl-N-(4-hydroxybutyl) nitrosamine; BrdU=bromodeoxyuridine; BOP = N‑nitrosobis(2-oxopropyl)amine; CD31 = cluster of differentiation 31; CPRIT = Cancer Prevention and Research Institute of Texas; DMBA = 7,12-dimethylbenzanthracene; DMSO = dimethyl sylfoxide; DSS=dextran sulfate sodium; g = grams; H2030BrM = human lung adenocarcinomas brain metastatic cell; HNSCC = head and neck squamous cell carcinoma; hr(s)=hour(s); i.p. = intraperitoneal; intraperitoneally; i.v. = intravenous/intravenously; kg = kilograms; Ki‑67 = antigen-67; KPC = Kras+/LSLG12D;Trp53+/LSLR172H;Pdx-1-Cre; L = liter; LL/2 = Lewis lung carcinoma cell; mg = milligrams; mL = milliliters; mM = millimolar; mm = millimeters; MRI = magnetic resonance imaging; NA = not applicable; NCI = National Cancer Institute; NIA = National Institute on Aging; NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; NMU = N‑methylnitrosourea; NNK = nitrosamine ketone (4‑(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NR = not reported; NS = not significantly different; NSCLC = non-small cell lung cancer; OSCC=oral squamous cell carcinoma; PBS = phosphate buffered saline; PCNA = proliferating cell nuclear antigen; RCT = randomized controlled trial; s.c. = subcutaneous;subcutaneously; SCID = severe combined immunodeficient mice; SCLC = small cell lung cancer; SD = standard deviation; SE=standard error; shRNA = short hairpin RNA; siRNA = small interfering RNA; US = United States; VEGF=vascular endothelial grown factor.

## Study Characteristics of Included Studies

### Study Designs

Among the 61 included studies, 45 studies were RCTs1-43 and the remaining 16 studies were controlled, parallel group studies that did not specify whether animals were randomized to study groups43-58.

### Study Evaluations

Although all studies included outcome measures of cancer initiation and/or progression relevant to this systematic review, these outcomes were not the primary evaluations of interest of all of the included studies. The primary evaluations of interest in approximately a third of the studies (n=20) were related to tumor initiation and/or progression5, 6, 9, 11, 13, 17, 19, 22, 36, 38, 40, 41, 44, 47, 49, 52, 53, 55, 59, 60. Tumor initiation was the primary evaluation of interest in three of these studies40, 44, 59, while tumor progression was the primary evaluation in the remaining 16 studies5, 6, 9, 11, 13, 17, 19, 22, 36, 38, 47, 49, 52, 53, 55, 60. In the majority of studies (n=34), the effects of nicotine on tumor signaling pathways was the primary evaluation of interest1-4, 7, 8, 10, 12, 15, 16, 20, 21, 23, 25-30, 32-35, 42, 43, 45, 46, 48, 50, 51, 54, 56, 57, 61. In four of the studies, the primary evaluation of interest was the effects of nicotine on cancer therapy14, 24, 31, 58. In the remaining three studies, the primary evaluations of interest included: the effects of perinatal nicotine administration on age at death and disease onset37; the effects of nicotine on anti-inflammatory pathways, food intake, and body composition in a model of anorexia-cachexia18; and, the chronic effects of nicotine administration on body weight, organ weights, and pathology39.

### Outcomes Measures

With regards to outcome measures reported among the 61 included studies, 12 studies reported data on tumor initiation5, 17, 19, 28, 36-40, 44, 59, 60, and 54 studies reported data on tumor progression1-35, 41-43, 45-58, 60, 61 ([**Table 1**.](#Table41)). All 12 studies that evaluated tumor initiation reported tumor incidence5, 17, 19, 28, 36-40, 44, 59, 60. Two studies also reported data on tumor multiplicity (i.e., number of tumors per animal)17, 19, and one study reported data on tumor volume17.

Among the 54 studies that reported on tumor progression, outcome measures included tumor volume (n=32 studies)1-4, 7-11, 13-17, 24, 27, 29, 31, 32, 34, 35, 42, 43, 46, 48-53, 58, 61, metastasis or micrometastasis (n=14 studies)9, 10, 12, 17, 20, 24, 25, 30, 33, 45, 46, 56-58, tumor incidence (n=11 studies)5, 6, 17, 19, 22, 28, 29, 41, 43, 47, 54, tumor weight (n=14 studies)3, 4, 8, 13, 18, 25-27, 29, 42, 50, 51, 56, 57, tumor proliferation (n=10 studies)3, 4, 7, 15, 17, 21, 22, 49, 50, 52, tumor growth (n=5 studies)17, 20, 24, 25, 42, 43, 55, angiogenesis or vascularization (n=6 studies)7, 11, 21, 32, 49, 53, tumor multiplicity (n=8 studies)17, 19, 22, 23, 43, 47, 54, 61, tumor area (n=3 studies)9, 21, 23, and time to tumor appearance (n=1 study)60

Table 1: Number of Studies According to Outcome Measures

| **Measure** | **Study count\*** | **List of studies** |
| --- | --- | --- |
| ***Tumor Initiation (n=12)*** | | |
| Tumor incidence | 12 | *Spontaneous model (n=12):* Berger et al., 1987; Chen et al., 1994; Galitovskiy et al., 2012; Habs and Schmahl, 1984; Maier et al., 2011; Martin et al., 1979; Murphy et al., 2011; Schmahl and Habs, 1976; Thompson et al., 1973; Toth, 1982; Waldum et al., 1996; Wang et al., 2017 |
| Tumor multiplicity | 2 | *Spontaneous model (n=2):* Maier et al., 2011; Murphy et al., 2011 |
| Tumor volume | 1 | *Spontaneous model (n=1):* Maier et al., 2011 |
| Time to tumor induction | 1 | *Spontaneous model (n=1):* Schmahl and Habs, 1976 |
| ***Tumor Progression (n=54)*** | | |
| Tumor volume | 32 | *Human xenograft model (n=25):* Al-Wadei et al., 2012; Al-Wadei et al., 2009; Ben et al., 2020a; Ben et al., 2020b; Bersch et al., 2009; Cedillo et al., 2019; Chien et al., 2021; Delitto et al., 2016; Hsu et al., 2020; Jarzynka et al., 2006; Jimenez et al., 2020; Kumari et al., 2018; Lee et al., 2010; Li et al., 2022; Li et al., 2015; Liu et al., 2015; Martinez et al., 2017; Shimizu et al., 2019; Trevino et al., 2012; Wan et al., 2018; Wang et al., 2021; Warren et al., 2012; Wong et al., 2007; Yuge et al., 2015; Zhang et al., 2017  *Allograft model (n=7):* Davis et al., 2009; Hao et al., 2013; Heeschen et al., 2001; Kyte et al., 2017; Maier et al., 2011; Nakada et al., 2012; Natori et al., 2003  *Carcinogen-induced model (n=2):* Iskandar et al., 2013; Maier et al., 2011  *Genetic model (n=1):* Maier et al., 2011 |
| Metastasis | 13 | *Human xenograft model (n=7):* Delitto et al., 2016; Maier et al., 2011; Hanaki et al., 2016; Pillai et al., 2015; Shimizu et al., 2019; Trevino et al., 2012; Wang et al., 2019  *Allograft model (n=5)*: Davis et al., 2009; Hao et al., 2013; Ross et al., 2020; Tyagi et al., 2021; Wu et al., 2020  *Genetic model (n=2):* Maier et al., 2011; Prueitt et al., 2016 |
| Tumor incidence | 11 | *Carcinogen-induced model (n=11):* Berger et al., 1987; Bersch et al., 2009; Chen et al., 1990; Hayashi et al., 2013; Maier et al., 2011; Murphy et al., 2011; Nakada et al., 2012; Nishikawa et al., 1992; Suzuki et al. 2018; Wang et al., 2017; Wang et al., 2021 |
| Tumor weight | 14 | *Human xenograft model (n=10):* Ben et al., 2020; Ben et al., 2020; Chien et al., 2021; Jimenez et al., 2020; Kumari et al., 2018; Lee et al. 2010; Liu et al., 2015; Underwood et al., 2020; Wan et al., 2018; Wang et al., 2021  *Allograft model (n=3):* Molfino et al., 2011; Ross et al., 2020; Tyagi et al., 2021;  Genetic model (n=1): Prueitt et al., 2016 |
| Tumor proliferation | 10 | *Human xenograft model (n=8)*: Ben et al., 2020; Ben et al., 2020; Cedillo et al., 2019; Jarzynka et al., 2006; Kumari et al., 2018; Li et al., 2022; Martinez et al., 2017; Shin et al., 2005  *Carcinogen-induced model (n=2):* Maier et al., 2011; Suzuki et al., 2018  Genetic model (n=1): Maier et al., 2011 |
| Tumor growth | 5 | *Human xenograft model (n=3):* Pillai et al., 2015; Pratesi et al., 1996; Trevino et al., 2012  *Mouse allograft model (n=2):* Maier et al., 2011; Tyagi et al., 2021; |
| Angiogenesis/vascularization | 6 | *Human xenograft model (n=5):* Cedillo et al., 2019; Jarzynka et al., 2006; Shin et al., 2004; Wong et al., 2007  *Allograft model (n=2)*: Heeschen et al., 2001; Natori et al., 2003 |
| Tumor multiplicity | 7 | *Carcinogen-induced model (n=6):* Hayashi et al. 2013; Iskandar et al., 2013; Maier et al., 2011; Murphy et al., 2011; Nakada et al., 2012 Nishikawa et al., 1992; Suzuki et al., 2018  *Genetic model (n=1):* Maier et al., 2011; Torres-Gonzales et al., 2014 |
| Time to tumor appearance | 1 | Carcinogen-induced model (n=1): Habs and Schmahl, 1984 |
| Tumor area | 3 | Human xenograft model (n=1): Shin et al., 2004  Carcinogen-induced model (n=1): Davis et al., 2009  *Genetic model (n=1):* Torres-Gonzalez et al., 2014 |
| ***Other (n=1)*** | | |
| Micrometastasis | 1 | *Genetic model (n=1):* Hermann et al., 2014; |

a Note: some studies evaluated more than one outcome measure of interest.

### Study Sample Sizes

Across the 50 of the 61 included studies that reported sample size for the nicotine and control groups1-5, 7-10, 13-22, 25-30, 33-41, 44-47, 49-54, 56-61, total sample size ranged from six27 to 858 animals38, 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. The number of animals per group ranged from four27 to 200 animals59 for the nicotine group, and from two27 to 200 animals40 for the control group. Sample size was not reported in the remaining 11 studies6, 11, 12, 23, 24, 31, 32, 42, 43, 47, 48, 55.

### Study Population

Characteristics of the study population for the included studies are listed in **Table 2** and **Table 3**. Among the 61 included studies, 50 studies used mice1-21, 23-35, 40-43, 45-53, 55-62, eight studies used rats5, 18, 22, 37-39, 44, 60, and three studies used hamsters36, 41, 54.

Across the 50 studies that used mice, more than a half of the studies (n=28 studies) used spontaneous mutant immunodeficient mice strains (i.e., nude, athymic nude, athymic Swiss mice, NU-Foxn1nu athymic, BALB/c nude athymic, BALB/cAJcl-nu/nu athymic, BALB/c nude, BALB/cAJc1 nude, SCID, NOD-SCID)1-4, 7, 8, 13, 15, 16, 20, 21, 24, 29-35, 42, 45, 48-52, 55, 58, 63, 17 studies used inbred mice strains (i.e., AB6F1, A/J, BALB/c, C57BL6, and FVB/NJ)9, 11, 14, 17, 19, 25, 27, 28, 30, 33, 43, 47, 48, 53, 57, 59, 61, two studies used outbred mice stocks (i.e., Swiss albino, and CF1)6, 40, and seven studies used genetically engineered mice (i.e., Kras, KPC, TRAMP, NOD-SCID-gamma, and C57BL6 RAG2-/- mice)10, 12, 17, 23, 26, 46, 56. Description of the mouse models used in the included studies is provided in [**Appendix**](#_Characteristics_of_National) **K.**

Across the eight studies that used rats, all used wild-type strains: five studies used Sprague-Dawley rats 5, 18, 37, 38, 44, 60, and three studies used Fischer (F344) rats18, 22, 39. Both studies that used hamsters used Syrian golden hamsters36, 54.

Of the 61 included studies, 51 studies reported the age of the animals at the start of the study. Forty nine of these studies used animals that were between 4 and 12 weeks old[[1]](#footnote-1) 3, 4, 6-10, 12-17, 19, 21, 22, 24-34, 36, 37, 39-51, 53-56, 58, 59, 61. The remaining two studies included one study where nicotine administration started at 12 days of age38, and one study where nicotine administration started on gestational Day 1 and continued postpartum5. Ten studies did not report the age of the animals 1, 2, 11, 18, 20, 23, 35, 52, 57, 60, with one of these studies reporting that mice weighed 18±2 grams at the start of the study35.

Twenty-five of the 61 included studies used male animals1-4, 6, 8, 14, 15, 18, 22, 23, 35-37, 39, 41, 42, 45, 47, 48, 52, 53, 56, 58, 61, 20 used female animals7, 9, 10, 16, 19, 24, 27, 29, 32, 33, 43, 44, 46, 49-51, 54, 57, 59, 60, and six studies used animals of both sexes5, 26, 30, 38, 40, 55. Ten studies did not report the sex of the animals11-13, 17, 20, 21, 25, 28, 31, 34.

#### Tumor Models

Across the 61 included studies, most studies (n=42 studies) used a xenograft or an allograft tumor model1-4, 7-18, 20, 21, 24-27, 29-35, 42, 43, 45, 46, 48-53, 55, 57, 58, 14 studies used a carcinogen-induced tumor model5, 6, 9, 17, 19, 22, 28, 29, 41, 43, 47, 54, 60, 61, and four studies used a genetic tumor model12, 17, 23, 56. Four studies used more than one tumor model within the same study: three studies used a xenograft and a carcinogen-induced tumor model9, 29, 43, and one study used a carcinogen-induced tumor model, a genetic tumor model, and an allograft tumor model17. Thirteen studies evaluated the spontaneous occurrence of tumors after the administration of nicotine5, 17, 19, 28, 36-41, 44, 59, 60.

Across the 42 studies that used xenograft or allograft tumor models, 42 different cell lines were used to induce tumors1-4, 7-18, 20, 21, 24-27, 29-35, 42, 43, 45, 46, 48-53, 55, 57, 58. Of these 42 studies, 30 used human xenografts1-4, 7, 8, 10, 13, 15, 16, 20, 21, 24, 26, 27, 29-32, 34, 35, 42, 45, 48-52, 55, 58, 11 used allografts9, 11, 12, 14, 17, 18, 25, 43, 46, 53, 57, and one study used both xenografts and allografts33. Descriptions of cell lines used in these studies are provided in [**Appendix**](#_Mouse_Models,_Cell) **K**. The most commonly used cell line was the human A549 cell line used in four studies7, 20, 42, 49, followed by the mouse Lewis lung carcinoma cell line used in three studies11, 14, 43, and the human MDA-MB-231 breast cancer cell line used in two other studies50, 51. Four studies used multiple cell lines to induce tumors: one study used NCI-H322 and NCI-H441 human non-small cell lung cancer (NSCLC) cell lines1, one study used CL13, IO33, and CL25 mouse lung adenocarcinoma cell lines17, one study used 4T1 or E0771 mouse breast cancer cell lines25, and one study used HCC1806 and HCC70 human breast cancer cell lines13.

Across the 14 studies that used carcinogen-induced tumor models, five studies used nitrosamine ketone (4 (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)9, 17, 19, 43, 61, two used N methylnitrosourea (NMU)5, 60, two studies used 4-nitroquinoline 1-oxide (4NQO)28, 29, and two studies used 7,12 dimethylbenzanthracene (DMBA)6, 41. Other carcinogens, used in one study each, included, azoxymethane and dextran sulfate sodium (DSS)47, N nitrosobis(2-oxopropyl)amine (BOP)54, and N butyl-N-(4-hydroxybutyl) nitrosamine (BBN)22. Descriptions of carcinogens used in these studies are provided in [**Appendix**](#_Characteristics_of_National) **K.**

Table 2: Animal and Cancer Model Characteristics among the Studies Evaluating Tumor Initiation

| **RefID** | **Author, Year** | **Species and Strain** | **Age** | **Sex** | **Cancer/Tumor Class** |
| --- | --- | --- | --- | --- | --- |
| ***Mouse models (n=5)*** | | | | | |
| 320 | Galitovskiy et al., 2012 | A/Ja | 6-8 weeks | Female | Other/undefined/multiple tissues |
| 683 | Murphy et al., 2011 | A/Ja | 4 weeks | Female | Lung |
| 632 | Maier et al., 2011 | AB6F1b | 6 weeks | NR | Lung |
| 976 | Toth, 1982 | Swiss albino | 5-7 weeks | Male and female | Other/undefined/multiple tissues |
| 1028 | Wang et al., 2017 | C57BL/6d | 6-8 weeks | NR | Head and neck |
| ***Rat models (n=6)*** | | | | | |
| 89 | Berger et al., 1987 | Sprague-Dawley | 20±2 weeks | Male and female | Other/undefined/multiple tissues |
| 366 | Habs and Schmahl, 1984 | Sprague-Dawley | NR | Female | Breast |
| 644 | Martin et al., 1979 | Sprague-Dawley | ≥28 days | Male | Other/undefined/multiple tissues |
| 850 | Schmahl et al., 1976 | Sprague-Dawley | 12 days | Male and female | Other/undefined/multiple tissues |
| 966 | Thompson et al., 1973 | Fischer (F344) | 8 weeks | Male | Other/undefined/multiple tissues |
| 1136 | Waldum et al., 1996 | Sprague-Dawley | ~2 months | Female | Other/undefined/multiple tissues |
| ***Hamster models (n=1)*** | | | | | |
| 174 | Chen et al., 1994 | Golden Syrian | 5-6 weeks | Male | Digestive |

Abbreviations: NR=not reported.

a A/J inbred mice are widely used to model cancer and for carcinogen testing given their high susceptibility to carcinogen-induced tumors 64.

b AB6F1 mice are the F1 progeny of mating A/J and C57BL/6 mice 17

c The strain was created from 2 outbred albino males and 7 outbred albino females. These mice are widely used in biomedical research 65.

d This is the most widely used wild-type inbred strain. These mice are refractory to many tumors but provide a permissive background for maximal expression of most mutations66.

Table 3: Animal and Cancer Model Characteristics among the Studies Evaluating Tumor Progression

| **RefID** | **Author, Year** | **Species and Strain** | **Age** | **Sex** | **Cancer/Tumor Class** | **Model Type** |
| --- | --- | --- | --- | --- | --- | --- |
| ***Mouse models (n=48)*** | | | | | | |
| 45 | Al-Wadei et al., 2009 | Athymic nudea | NR | Male | Digestive | Xenograft |
| 44 | Al-Wadei et al., 2012 | Athymic nudea | NR | Male | Lung | Xenograft |
| 85 | Ben et al., 2020a | BALB/c nudeb | 6 weeks | Male | Digestive | Xenograft |
| 86 | Ben et al., 2020b | BALB/c nudeb | 6-8 weeks | Male | Digestive | Xenograft |
| 94 | Bersch et al., 2009 | CF1c | >60 days | Male | Digestive | Carcinogen-induced |
| 150 | Cedillo et al., 2019 | NU-Foxn1nu athymicd | 5-6 weeks | Female | Lung | Xenograft |
| 182 | Chien et al., 2021 | Nudee | 4 weeks | Male | Head and neck | Xenograft |
| 236 | Davis et al., 2009 | BALB/cf | 26-30 days | Female | Lung | Allograft and carcinogen-induced |
| 247 | Delitto et al., 2016 | NOD-SCIDg | 8 weeks | Female | Digestive | Xenograft |
| 377 | Hanaki et al., 2016 | BALB/cAJcl-nu/nu athymich | 4 weeks | Male | Digestive | Xenograft |
| 380 | Hao et al., 2013 | C57BL/6 RAG2-/-I, u | 6-8 weeks | Female | Lung | Allograft |
| 390 | Hayashi et al., 2013 | BALB/cf | 8-10 weeks | Male | Digestive | Carcinogen-induced |
| 399 | Heeschen et al., 2001 | C57BL/6Ji | NR | NR | Lung | Allograft |
| 403 | Hermann et al., 2014 | KPCj and nudee | 10-12 weeks | NR | Digestive | Genetic model and allograft |
| 444 | Hsu et al., 2020 | BALB/cf | 4-5 weeks | Male | Head and neck | Xenograft |
| 458 | Iskandar et al., 2013 | A/Jk | 5-6 weeks | Male | Lung | Carcinogen-induced |
| 475 | Jarzynka et al., 2006 | Nudee | 6 weeks | Female | Lung | Xenograft |
| 483 | Jimenez et al., 2020 | Nudee | 6-8 weeks | NR | Breast | Xenograft |
| 542 | Kyte et al., 2018 | C57BL/6Ji | 8 weeks | Male | Lung | Allograft |
| 533 | Kumari et al., 2018 | BALB/c nudeb | 5-6 weeks | Female | Breast | Xenograft |
| 562 | Lee et al., 2010 | NOD-SCIDg | 6 weeks | NR | Breast | Xenograft |
| 573 | Li et al., 2022 | Athymic nudea | 4 weeks | Male | Digestive | Xenograft |
| 576 | Li et al., 2015 | BALB/cAJc1 nudeh | 5-6 weeks | Female | Lung | Xenograft |
| 600 | Liu et al. 2015 | BALB/c nudeb | 5-8 weeks | Male | Lung | Xenograft |
| 632 | Maier et al., 2011 | AB6F1l, KrasLA2/+ on a C57Bl/6 backgroundm | 6 weeks | NR | Lung | Carcinogen-induced, allograft, and genetic |
| 645 | Martinez et al., 2017 | BALB/c nudeb | NR | Male | Digestive | Xenograft |
| 683 | Murphy et al., 2011 | A/Jm | 4 weeks | Female | Lung | Carcinogen-induced |
| 691 | Nakada et al., 2012 | C57BL/6i and A/J micem | 10 weeks | Female | Lung | Allograft, and carcinogen-induced |
| 699 | Natori et al., 2003 | C57BL/6i | 8 weeks | Male | Digestive | Allograft |
| 761 | Pillai et al., 2015 | SCIDn | NR | NR | Lung | Xenograft |
| 769 | Pratesi et al., 1996 | Athymic Swisso | 6-7 weeks | Male and female | Lung | Xenograft |
| 775 | Prueitt et al., 2016 | TRAMPp | 8-9 weeks | Male | Other/undefined/multiple tissues | Genetic model |
| 824 | Ross et al., 2020 | FVB/NJq | NR | Female | Breast | Allograft |
| 888 | Shimizu et al., 2019 | Athymic nu/nur | 5 weeks | Male | Head and neck | Xenograft |
| 894 | Shin et al., 2004 | Athymic nudea | 4-6 weeks | NR | Digestive | Xenograft |
| 975 | Torres-Gonzalez et al., 2014 | Krass | NR | Male | Lung | Genetic model |
| 982 | Trevino et al., 2012 | SCIDn | 8 weeks | Female | Digestive | Xenograft |
| 1135 | Tyagi et al., 2021 | BALB/cf and C57BL/6i | 5-6 weeks | NR | Breast | Allograft |
| 998 | Underwood et al., 2020 | Non-obese, diabetic SCID-gammat | 12 weeks | Male and female | Digestive | Xenograft |
| 1025 | Wan et al., 2018 | BALB/cf | 4 weeks | Female | Digestive | Xenograft |
| 1028 | Wang et al., 2017 | C57BL/6i | 6-8 weeks | NR | Head and neck | Carcinogen-induced |
| 1034 | Wang et al., 2021 | BALB/c nudeb | 6 weeks | Female | Digestive | Carcinogen-induced, xenograft |
| 1037 | Wang et al., 2019 | C57BL/6i and nudee | 5 weeks | Male and female | Head and neck | Xenograft |
| 1047 | Warren et al., 2012 | Athymic nudea | 8-10 weeks | Male | Lung | Xenograft |
| 1072 | Wong et al., 2007 | BALB/c nudeb | 4-6 weeks | Female | Digestive | Xenograft |
| 1076 | Wu et al., 2020 | BALB/cf, C57BL/6i, and nudee | 5-6 weeks | Female | Lung | Allograft and xenograft |
| 1109 | Yuge et al., 2015 | BALB/c nude athymicb | 6 weeks | NR | Urinary tract | Xenograft |
| 1114 | Zhang et al., 2017 | Nudee | NR | Male | Lung | Xenograft |
| ***Rat models (n=4)*** | | | | | | |
| 89 | Berger et al., 1987 | Sprague-Dawley | 20±2 weeks | Male and female | Other/undefined/multiple tissues | Carcinogen-induced |
| 366 | Habs and Schmahl, 1984 | Sprague-Dawley | NR | Female | Breast | Carcinogen-induced |
| 672 | Molfino et al., 2011 | Fischer (F344) | NR | Male | Other/undefined/multiple tissues | Allograft |
| 940 | Suzuki et al., 2018 | Fischer (F344) | 5 weeks | Male | Urinary tract | Carcinogen-induced |
| ***Hamster models (n=2)*** | | | | | | |
| 175 | Chen et al., 1990 | Golden Syrian | 5-6 weeks | Male | Head and neck | Carcinogen-induced |
| 720 | Nishikawa et al., 1992 | Golden Syrian | 6 weeks | Female | Digestive | Carcinogen-induced |

Abbreviations: KPC = Kras+/LSLG12D;Trp53+/LSLR172H;Pdx-1-Cre; NOD-SCID = Non-obese, diabetic-severe combined immunodeficient; NR = not reported; nu/nu = homozygous nude mice; SCID = severe combined immunodeficient mice; TRAMP = Transgenic Adenocarcinoma of Mouse Prostate.

a Immunodeficient nude mouse model that is maintained as an outbred mouse and is not associated with any stock/strain. The animal lacks a thymus and is unable to produce T-cells67.

b Inbred, BALB/c nude mouse. The animal lacks a thymus, is unable to produce T-cells, and is therefore immunodeficient 68.

c This strain is white (albino) and carries brown behind its albino gene. It is a general multipurpose mouse model 69

d Immunodeficient nude mouse model maintained as an outbred mouse and is not associated with any stock or strain. The animal lacks a thymus and is unable to produce T-cells67.

e The first immunocompromised mouse strain to be used in cancer research. These mice lack a normal immune system and the thymus, and have a repressed immune system due to reduced number of T cells. These mice have no rejection responses and are hairless, making it easier to identify tumors70.

f A commonly used wild-type inbred mice71.

g The SCID mutation has been transferred onto a NOD background. Animals homozygous for the SCID mutation have impaired T and B cell lymphocyte development. The NOD background additionally results in deficient natural killer cell function. These mice are commonly used for studying tumor biology and xenograft research.

h BALB/cAJcl (also known as BALB/cAJc1 nude mice) is a sub-strain lineage from BALB/c. These mice are athymic, hairless, and lack T-cell function72.

i C57BL/6 is the most widely used wild-type inbred strain. These mice are refractory to many tumors, but provide a permissive background for maximal expression of most mutations66.

j KPC mouse model of PDAC reproduces many of the key features of the immune microenvironment observed in human PDAC. This model is the most extensively studied genetic model of PDAC for evaluation of immunotherapy, and has reproduced clinical observations seen in PDAC patients treated with several immune oncology drugs73.

k A/J inbred mice are widely used to model cancer and for carcinogen testing given their high susceptibility to carcinogen-induced tumors.

l AB6F1 mice are the F1 progeny of mating A/J and C57BL/6 mice

m Mice heterozygous for the Kras LA2 allele have a reduced lifespan compared with wildtype controls. All KrasLA2/+ mice develop extensive tumors, most frequently in the lungs, with 100% of animals developing multifocal tumors at one week of age. Tumor multiplicity and size increase with age, ultimately resulting in respiratory distress and death. KrasLA2/+ mice are also prone to thymic lymphoma (30%) and skin papilloma (40%)74, 75.

n SCID mice have a genetic immune deficiency that affects their B and T cells. Due to the lack of mature B and T lymphocytes, these mouse models are commonly used for xeno-engraftment of human cells and tissue 76.

o These mice resulted after a mutation had occurred in a colony of albino outbred mice. These nude mice originate from the Swiss strain and lack the thymus. They are hairless, and used to study tumor biology and xenograft research77.

p These mice express the TRAMP transgene and develop progressive forms of prostate cancer with distant site metastasis. The TRAMP model closely mirrors the pathogenesis of human prostate cancer. Mice with F1 background show earlier onset of the phenotype compared to mice carrying the transgene on the C57BL/6 background. Male TRAMP mice uniformly and spontaneously develop orthotopic prostate tumors following the onset of puberty 78-80.

q A widely used multipurpose inbred strain that is homozygous for the retinal degeneration 1 allele of Pde6brd1, resulting in blindness by wean age81.

r This immunodeficient nude mouse originated from NIH and has a BALB/c genetic strain background. The animal lacks a thymus, is unable to produce T-cells and is therefore immunodeficient.

s Genetically engineered mouse models of *Kras* mutant model critical aspects of cancer and are widely used for preclinical research. The Kras oncogene is mutated at a high frequency in human cancers including PDAC (95%), colon cancers (50%), and NSCLC (30%) 82, 83.

t Immunodeficient NOD-SCID mice bearing a targeted mutation in the gene encoding the interleukin -2 receptor gamma chain gene (IL2rγnull) engraft readily with human peripheral blood mononuclear cells 84.

u uRAG2 knock-out mice produce no mature T cells or B cells. Their phenotype can be described as a "non-leaky" immune deficiency

### Treatment Regimens

#### Treatments Evaluated

All of the 61 included studies compared nicotine treatment with control treatment. The majority of the studies (n=51) compared one nicotine group to one control group1-4, 6-15, 17-20, 23-31, 33-38, 41-54, 57-61, and 10 of the studies evaluated multiple nicotine groups versus one control group5, 16, 21, 22, 32, 39, 40, 52, 55, 56.

Across the ten studies that compared multiple nicotine groups with a control group, six studies evaluated two nicotine doses21, 32, 40, 52, 55, 56, one study evaluated three nicotine doses22, two studies evaluated two durations of nicotine treatment5, 39, and one study evaluated two modes of nicotine administration16. Treatment regimens, including method of nicotine administration, dose, frequency, and treatment duration are described in **Table 1‑4** and **Table 1‑5**.

#### Mode of Nicotine Administration

Mode of nicotine administration in the included studies is described in **Table 1‑4** and **Table 1‑5**. Across the 61 included studies, the most common method of administration was oral administration of nicotine in drinking water (n=23 studies)1, 2, 7, 11, 12, 16, 17, 19, 21-23, 27, 29, 32, 40, 42, 43, 49, 51, 52, 54, 56, 57, 61, followed by intraperitoneal injections (n=21 studies)3, 4, 8-10, 13, 15, 17, 18, 20, 24-26, 33-35, 38, 45, 50, 58, 61, and subcutaneous injections or subcutaneous continuous infusions (n=11 studies)5, 6, 14, 31, 37, 39, 46, 47, 55, 59, 60. Four studies administered nicotine buccally by applying it on the tongue or the cheek pouch 30, 36, 41, 85. Other methods of nicotine administration, used in one study each, included application of a dermal patch9, intravenous injections16, gavage 53, and inhalation44. One study did not specify the mode of nicotine administration48.

#### Duration of Treatment Periods

Treatment durations in the included studies are listed in **Table 1‑4** and **Table 1‑5**. Treatment duration varied considerably across studies and across different methods of nicotine administration. Eight of the 61 included studies did not report treatment duration11, 30, 32, 34, 38, 40, 52, 56. Across the 53 studies that reported treatment duration, treatment duration ranged from 7 days14 to 24 months41, 44, 59. The most common treatment duration was approximately 1 month, used in 10 studies1, 2, 5, 7-9, 15, 18, 25, 48, followed by 3 weeks used in six studies3, 4, 9, 24, 37, 57.

Of the 23 studies that administered nicotine orally, treatment duration was reported by 18 studies1, 2, 7, 12, 16, 17, 19, 21-23, 27, 29, 42, 43, 49, 51, 54, 57. In these studies, treatment duration ranged from 18 days16 to 44 weeks19. Of the 22 studies that administered nicotine intraperitoneally, treatment duration was reported by 19 studies3, 4, 8-10, 13, 15, 17, 18, 20, 24-26, 33, 35, 45, 50, 58, 61. Treatment duration in these studies ranged from 3 weeks3, 4, 24 to 10 weeks45, 61. Across the 11 studies that administered nicotine subcutaneously, treatment duration ranged from 7 days14 to 24 months59. In the four studies that administered nicotine buccally, treatment duration was 12 weeks in one study41, 16 weeks in one study28, 13 months in one study 36, and was not reported by one study30. In the studies that used other modes of nicotine administration, treatment duration was 3 weeks in the study that administered nicotine via a patch9, 18 days in the study that administered nicotine intravenously16, 12 days in the study that administered nicotine by gavage53, and up to 24 months in the study that administered nicotine by inhalation44. In the study that did not specify the mode of administration, treatment duration was 4 weeks48.

#### Study Interventions

The dose of nicotine used varied considerably across the 61 included studies (**Table 1‑4** and **Table 1‑5**), and across the different methods of administration. Additionally, there was variability across the studies in the reporting of nicotine concentrations, with studies expressing nicotine concentrations using different units.

Across the 23 studies in which nicotine was administered orally in drinking water 1, 2, 7, 11, 12, 16, 17, 19, 21-23, 27, 29, 32, 40, 42, 43, 49, 51, 52, 54, 56, 57, 61, 17 studies reported the concentration of nicotine as weight per volume1, 2, 7, 11, 12, 16, 17, 19, 21, 23, 27, 29, 32, 43, 49, 51, 56, 57: The concentration of nicotine in these studies ranged from 50 µg/mL21, 32 to 10 mg/mL51. The most common concentrations of nicotine (by weight) in drinking water were 100 µg/mL (n=8 studies)11, 12, 16, 17, 23, 43, 56, 57 and 200 µg/mL (n=7 studies)1, 2, 19, 21, 27, 29, 32, 49. In three studies that reported the concentration of nicotine in drinking water as percent or parts per million (ppm) solution in water[58](#_ENREF_58), [75](#_ENREF_75), [85](#_ENREF_85), one study administered nicotine at concentrations of 10 ppm, 20 ppm, and 40 ppm22, one study administered 25 ppm nicotine[85](#_ENREF_85), and one study administered 0.0625% and 0.09375% solutions of nicotine[75](#_ENREF_75). In one study, where the concentration of nicotine was reported as micromoles per liter, nicotine was administered at concentrations of 50 µmol/L and 200 µmol/L52. Two studies administered nicotine at a concentration of 1 µM7, 42.

Six studies administered more than one concentration of nicotine in the drinking water21, 22, 32, 40, 52, 56: two studies administered 50 µg/mL and 200 µg/mL nicotine21[67](#_ENREF_67), one study administered 50 µmol/L and 200 µmol/L 52, one study administered 0.09375% and 0.06250% solutions of nicotine40, one study administered 100 µg/mL and 250 µg/mL nicotine56, and one study administered 10 ppm, 20 ppm, and 40 ppm nicotine22.

Across the 21 studies in which nicotine was administered intraperitoneally3, 4, 8-10, 13, 15, 17, 18, 20, 24-26, 33-35, 38, 45, 50, 58, 61, one study did not report the dose of nicotine administered20. Another study reported that 30 µg nicotine was administered per mouse, with the specific dose per body weight not reported but the mean body weight of the mice reported as 19.5 grams58. Across the remaining 18 studies, the doses of nicotine administered ranged from 0.25 mg/kg administered three times per week for 3 weeks in two studies3, 4, and two times per week for 57 days in one study50, to 200 mg/kg administered five times per week for 4 weeks in one study15, and three times per week for 2 weeks in another study18. The most commonly used dose of nicotine administered intraperitoneally was 1 mg/kg (n=10 studies), which was administered daily for 3 weeks in two studies3, 4, 8, 9, three times per week for 3 weeks in two studies3, 4, 24, 61, three times per week for 6 weeks in two studies26, 35, three times per week for 40 days in one study10, and three times per week for 10 weeks in two studies45, 61. The remaining study did not report the duration of nicotine treatment34. All 21 studies administered only a single dose of nicotine.

Eleven studies administered nicotine subcutaneously via injections or continuous infusion with osmotic pumps5, 6, 14, 31, 37, 39, 46, 47, 55, 59, 60. Across the eight studies that reported the dose of nicotine in weight (milligrams) per kilogram body weight5, 6, 14, 37, 39, 47, 59, 60, the dose of nicotine ranged from 0.4 mg/kg administered daily5 or twice per week for 7 or 90 days60, to 24 mg/kg per day administered continuously for 7 days14. For the remaining three studies, 100 mg/mL nicotine per day was administered continuously for 8 weeks in one study46, 20 µg or 200 µg was administered per day for 14 days in one study55, and one study administered the maximal tolerated dose (60 µg) every other day for 6 or 28 days31.

In the four studies in which nicotine was administered buccally30, 36, 41, 85, one study applied a 5% nicotine solution on the tongue85, one study applied 10 µL of a nicotine solution on the tongue, although the concentration was not specified30, and two studies applied 50 µL of a 6% nicotine solution on the cheek pouch36, 41.

In the four studies that used other modes of nicotine administration, one study applied a dermal patch containing 25 mg/kg nicotine three times per week for 3 weeks9, one study administered 0.6 mg/kg nicotine intravenously five times per week for 18 days16, one study administered 20 mg/kg nicotine by gavage daily for 5 or 7 days53, and one study administered nicotine in the air at a concentration of approximately 500 µg/m3 for up to 24 months44. One study, which did not specify the mode of nicotine administration, administered 1.5 mg/kg nicotine daily for 4 weeks48.

#### Biomarkers of nicotine exposure

Across the 61 included studies, 13 studies evaluated biomarkers of nicotine exposure9, 11, 12, 16, 17, 19, 22, 25, 32, 44-46, 57. Eight of these studies evaluated biomarker levels in serum (n=5 studies)11, 16, 17, 22, 57, urine (n=1 study)12, plasma (n=1 study)32, or both urine and plasma (n=1 study)19 following oral administration of nicotine in drinking water. In four of the five studies assessing serum, nicotine was administered at a concentration of 100 µg/mL and serum levels of cotinine were assessed11, 16, 17, 57. The mean serum cotinine concentration ranged from 36.75±5.50 ng/mL16 to 216.5 ng/mL (95% CI: 189.9-236.2 ng/mL)11. One of these studies reported that the mean serum cotinine levels (137 ng/mL) were comparable to those reported for 22 mg nicotine patch users. In the remaining study assessing serum, nicotine was administered orally in drinking water at concentrations of 10 ppm, 20 ppm, and 40 ppm 22. The mean serum cotinine concentration was 83.8±5.3 ng/mL after administration of 10 ppm nicotine, 199.5±10.5 ng/mL after administration of 20 ppm, and 347.3±15.9 ng/mL after administration of 40 ppm nicotine. The authors noted that the cotinine level in active smokers is generally in the range of 250 to 300 ng/mL. One study that assessed serum also reported serum nicotine concentration of 54.98 ng/mL after oral administration of nicotine in drinking water (100 µg/mL for 21 days)57.

Two studies evaluated urine concentrations of biomarkers of exposure following oral administration of nicotine in drinking water12, 19. One study, in which 100 µL nicotine was administered for 2 weeks, represented cotinine concentrations graphically without providing specific values 12. However, the study noted that cotinine levels were comparable to those in intermediate smokers, and were approximately 200 ng/mL. In the other study, administration of 200 µg/mL nicotine resulted in a urine cotinine concentration of 4,400±3,200 ng/mL, nicotine concentration of 1,270±1,170 ng/mL, and trans-3’-hydroxycotinine concentration of 27,900±9,820 ng/mL at 5 months19. The same study also reported plasma levels of biomarkers of nicotine exposure at 44 weeks: cotinine was 19±20 ng/mL; average nicotine was 0.40±0.46 ng/mL; and, trans-3’-hydroxycotinine was 48±36 ng/mL19. One other study in which nicotine was administered orally in drinking water reported plasma levels of cotinine. The average cotinine concentration in plasma was 43.2±7.10 ng/mL after administration of 50 µg/mL nicotine for 25 days, and 169.4±12.21 ng/mL after administration of 200 µg/mL nicotine for 25 days32. Study authors noted that the doses of nicotine administered mimicked the daily intake of nicotine in cigarette smokers.

Three studies evaluated cotinine levels following intraperitoneal administration of nicotine9, 25, 45. One of these studies represented serum cotinine concentrations graphically without providing specific values, although the study noted that serum cotinine levels were comparable to those in adult smokers25. In another study, 1 mg/kg of nicotine was administered three times per week for 10 weeks and the mean serum cotinine concentration was 1,635±142.22 pg/mL45. In the third study, intraperitoneal administration of 1 mg/kg nicotine three times per week for 5 weeks resulted in a urine cotinine concentration of 3,000 ng/mL9. The authors noted that previous studies reported cotinine levels in human smokers that ranged from 1,500 to 8,000 ng/mL. In the same study, a separate group of animals also received nicotine (25 mg/kg daily) via a dermal patch, resulting in a urine cotinine concentration of 5,000 ng/mL9.

One study administered nicotine (100 mg/mL; 3.6 µL/day) subcutaneously using an osmotic pump for 10 weeks46. The study reported that plasma nicotine levels were about 100 to 200 ng/mL (~0.6-1.2 mM) after infusion of approximately 2 to 4 mg/kg/hr of drug, and about 45 ng/mL (280 nM) after infusion of approximately 0.5 mg/kg/hr; however, it was unclear if plasma nicotine was measured in this study, or if these values were extrapolated from previous studies. The authors noted that these levels were comparable to peak plasma nicotine levels in human smokers, which were reported to be 10 to 50 ng/mL, corresponding to 60 to 310 nM nicotine.

One study administered nicotine (0.6 mg/kg) intravenously 5 times per week for 18 days, and reported that the mean serum cotinine concentration was 372.37±42.3 ng/mL16.

One study administered nicotine via inhalation (500 µg/m3) for up to 24 months, and reported a mean plasma nicotine concentration of slightly over 100 ng/mL in the plasma of rats (108.4±55.1 ng/mL at Week 1 and 129.8±43.0 ng/mL at Week 103)44.

Six additional studies did not evaluate biomarkers of nicotine exposure, however, they related the dose of nicotine administered to levels observed in human smokers1, 2, 4, 18, 21, 31, 58, 61. Two of these studies reported that the dose of nicotine administered corresponded to levels observed in heavy smokers1, 2, two studies used doses that corresponded to nicotine levels in smokers without specifying the amount of smoking21, 58, one study reported that the dose of nicotine corresponded to that in regular smokers4, and in one study, the amount of nicotine administered corresponded to smoking 25 cigarettes per day61. One study noted that the dose of nicotine administered was intended to achieve as high an intratumoral concentration as possible31, and one study reported that the dose of nicotine administered did not cause toxicity18.

Table 4: Nicotine Intervention among the Studies Evaluating Tumor Initiation

| **RefID** | **Author, Year** | **Mode of Administration** | **Nicotine dosage** | **Frequency of administration** | **Treatment Duration** |
| --- | --- | --- | --- | --- | --- |
| ***Oral administration (n=3)*** | | | | | |
| 632 | Maier et al., 2011 | Oral | 100 µg/mL | Ad libitum | 2-6 weeks |
| 683 | Murphy et al., 2011 | Oral (in drinking water) | 200 mg/mL (0.44 µmol/mL) | Ad libitum | 44 weeks |
| 976 | Toth, 1982 | Oral (in drinking water) | 0.09375%; 0.06250% | Ad libitum | NR |
| ***Subcutaneous administration (n=5)*** | | | | | |
| 89 | Berger et al., 1987 | Subcutaneous | 0.4 mg/kg | Daily | 14-20 days |
| 320 | Galitovskiy et al., 2012 | Subcutaneous | 3 mg/kg | 5 days per week | 24 months |
| 366 | Habs and Schmahl, 1984 | Subcutaneous | 0.4 mg/kg | 2 times per week | 46-52 days (group 1); 55-145 days (group 2) |
| 644 | Martin et al., 1979 | Subcutaneous | 5 mg/ml (3.0 mg/kg) | 2 times per day | 21 days |
| 966 | Thompson et al., 1973 | Subcutaneous | 1000 µg/mL/kg | Daily | 2 or 22 months |
| ***Intraperitoneal administration (n=1)*** | | | | | |
| 850 | Schmahl et al., 1976 | Intraperitoneal | 2 mg/kg/week | NR | NR |
| ***Buccal administration (n=2)*** | | | | | |
| 174 | Chen et al., 1994 | Buccal (applied on cheek pouch | 50 µL of a 6% solution of nicotine in sesame oil | 3 times per week | 13 months |
| 1028 | Wang et al., 2017 | Buccal (applied on tongue) | 5% | 3 times per week | 16 weeks |
| ***Inhalation (n=1)*** | | | | | |
| 1136 | Waldum et al., 1996 | Inhalation | 500 µg/m3 | 20 hours per day, 5 days per week | Up to 24 months |

Abbreviations: µg=micrograms; µl=microliters; µM=micromolar; µmol=micromole; DSS=dextran sulfate sodium; kg=kilograms; m3 = cubic meters; mg=milligrams; ml=milliliters; mM=millimolar; NA=not applicable; nM=nanomolar; NR=not reported; ppm=parts per million.

Table 5: Nicotine Intervention among the Studies Evaluating Tumor Progression

| **RefID** | **Author, Year** | **Mode of Administration** | **Nicotine Dosage** | **Frequency of administration** | **Treatment Duration** |
| --- | --- | --- | --- | --- | --- |
| ***Oral administration (n=22)*** | | | | | |
| 44 | Al-Wadei et al., 2012 | Oral (in drinking water) | 200 µg/mL | Ad libitum | 30 days |
| 45 | Al-Wadei et al., 2009 | Oral (in drinking water) | 200 µg/mL | Ad libitum | 30 days |
| 150 | Cedillo et al., 2019 | Oral (in drinking water) | 1 µM | NR (but likely *Ad libitum*) | 27 days |
| 399 | Heeschen et al., 2001 | Oral (in drinking water) | 100 µg/mL | Ad libitum | NR |
| 403 | Hermann et al., 2014 | Oral (in drinking water) | 100 µg/mL | Ad libitum | 2 weeks |
| 475 | Jarzynka et al., 2006 | Oral (in drinking water) | 200 µg/mL | NR (but likely *Ad libitum*) | 36 days |
| 562 | Lee et al., 2010 | Oral (in drinking water) | 10 mg/mL | NR (but likely *Ad libitum*) | 6 weeks |
| 576 | Li et al., 2015 | Oral | 100 µg/mL | Ad libitum | 18 days |
| 600 | Liu et al. 2015 | Oral (in drinking water) | 1 µmol/L (1 µM) | NR (but likely *Ad libitum*) | 20 days |
| 632 | Maier et al., 2011 | Oral | 100 µg/mL | Ad libitum | 2-6 weeks |
| 645 | Martinez et al., 2017 | Oral (in drinking water) | 50 µmol/L; 200 µmol/L | Ad libitum | NR |
| 683 | Murphy et al., 2011 | Oral (in drinking water) | 200 mg/mL (0.44 µmol/mL) | Ad libitum | 44 weeks |
| 691 | Nakada et al., 2012 | Oral (in drinking water) | 100 µg/mL | NR (but likely *Ad libitum*) | 14 days |
| 720 | Nishikawa et al., 1992 | Oral (in drinking water) | 25 ppm | Ad libitum | 37 weeks |
| 775 | Prueitt et al., 2016 | Oral (in drinking water) | 100 µg/mL; 250 µg/mL | Ad libitum | NR |
| 824 | Ross et al., 2020 | Oral (in drinking water) | 100 μg/mL | NR (but likely *Ad libitum*) | 21 days |
| 894 | Shin et al., 2004 | Oral (in drinking water) | 50 µg/mL; 200 µg/mL | Ad libitum | NR |
| 940 | Suzuki, 2018 | Oral (in drinking water) | 10 ppm; 20 ppm; 40 ppm | Ad libitum | 32 weeks |
| 975 | Torres-Gonzalez et al., 2014 | Oral (in drinking water) | 100 µg/mL | NR (but likely *Ad libitum*) | 90 days |
| 1025 | Wan et al., 2018 | Oral (in drinking water) | 200 μg/mL | Ad libitum | 40 days |
| 1034 | Wang et al., 2021 | Oral (in drinking water) | 200 µg/mL | NR (but likely *Ad libitum*) | 16 weeks |
| 1072 | Wong et al., 2007 | Oral (in drinking water) | 50 µg/mL; 200 µg/mL | NR (but likely *Ad libitum*) | NR |
| ***Intraperitoneal administration (n=20)*** | | | | | |
| 85 | Ben et al., 2020 | Intraperitoneal | 0.25 mg/kg | 3 times per week | 3 weeks |
| 86 | Ben et al., 2020 | Intraperitoneal | 0.25 mg/kg | 3 times per week | 3 weeks |
| 182 | Chien et al., 2021 | Intraperitoneal | 1 mg/kg | Daily | 1 month |
| 236 | Davis, 2009 | Intraperitoneal | 1 mg/kg | Daily | 1 month |
| 247 | Delitto et al., 2016 | Intraperitoneal | 1 mg/kg | 3 times per week | 45 days |
| 377 | Hanaki et al., 2016 | Intraperitoneal | 1 mg/kg | 3 times per week | 10 weeks |
| 458 | Iskandar et al., 2013 | Intraperitoneal | 1 mg/kg | 3 times per week | 10 weeks |
| 483 | Jimenez et al., 2020 | Intraperitoneal | 0.75 mg/kg | 2 times per day | 10 weeks |
| 533 | Kumari et al., 2018 | Intraperitoneal | 0.25 mg/kg | 2 times per week | 57 days |
| 573 | Li et al., 2022 | Intraperitoneal | 200 mg/kg/day | 5 times per week | 4 weeks |
| 632 | Maier et al., 2011 | Intraperitoneal | 0.8 mg/kg | 3 times per week | NR |
| 672 | Molfino et al., 2011 | Intraperitoneal | 200 mg/kg/day | Days 8, 9,10,15,16, and 17 | 29 days |
| 761 | Pillai et al., 2015 | Intraperitoneal | NR | Every 2 days | 7 weeks |
| 888 | Shimizu et al., 2019 | Intraperitoneal | 30 µg/mouse | Daily | 42 days |
| 982 | Trevino et al., 2012 | Intraperitoneal | 1 mg/kg | 3 times per week | 3 weeks |
| 1135 | Tyagi et al., 2021 | Intraperitoneal | 2 mg/kg | Every 2 days | 28 days |
| 998 | Underwood et al., 2020 | Intraperitoneal | 1 mg/kg | 3 times per week | 6 weeks |
| 1076 | Wu et al., 2020 | Intraperitoneal | 1mg/kg | Every 3 days | 40 days (BALC/c and C57BL/6 mice ); 60 days (nude mice) |
| 1109 | Yuge et al., 2015 | Intraperitoneal | 1 mg/kg | 3 times per week | NR |
| 1114 | Zhang et al., 2017 | Intraperitoneal | 1 mg/kg | 3 times per week | 6 weeks |
| ***Subcutaneous administration (n=8)*** | | | | | |
| 89 | Berger et al., 1987 | Subcutaneous | 0.4 mg/kg | Daily | Gestational days 14-20 days; Postpartum days 1-20 |
| 94 | Bersch et al., 2009 | Subcutaneous | 2 mg/kg | 2 times per day | 45 days |
| 366 | Habs and Schmahl, 1984 | Subcutaneous | 0.4 mg/kg | 2 times per week | 90 days (postpartum days 55-145) |
| 380 | Hao et al., 2013 | Subcutaneous (osmotic pump) | 100 mg/mL (3.6 µl/day) | Continuous infusion using an osmotic pump | 2 weeks prior to cancer cell line injection and 6 weeks post-injection |
| 390 | Hayashi et al., 2013 | Subcutaneous | 3 mg/kg | Daily | Three 5-day periods (during DSS treatment), each followed by 16 days of control treatment. |
| 542 | Kyte et al., 2017 | Subcutaneous (osmotic pump) | 24 mg/kg/day | Daily | 7 days |
| 769 | Pratesi et al., 1996 | Subcutaneous (osmotic pump) | 20 µg/day; 200 µg/day | Continuous infusion using an osmotic pump | 14 days |
| 1047 | Warren et al., 2012 | Subcutaneous | 60 µg | Daily; every 2 days | 28 days |
| ***Buccal administration (n=3)*** | | | | | |
| 175 | Chen et al., 1990 | Applied on cheek pouch | 6% | 3 times per week | 12 weeks |
| 1028 | Wang et al., 2017 | Applied on tongue | 5% | 3 times per week | 16 weeks |
| 1037 | Wang et al., 2019 | Applied on tongue | 10 µL | 3 times per week | NR |
| ***Other modes of administration (n=3)*** | | | | | |
| 236 | Davis, 2009 | Patch | 25 mg/kg | 3 times per week | 3 weeks |
| 576 | Li et al., 2015 | Intravenous | 0.6 mg/kg | 5 times per week | 18 days |
| 699 | Natori et al., 2003 | Gavage | 20 mg/kg | Daily | 5 days prior to cancer cell inoculation (tumor model); 7 days (bone marrow transplantation) |
| ***Unspecified mode of administration (n=1)*** | | | | | |
| 444 | Hsu et al., 2020 | NR | 1.5 mg/kg | Daily | 4 weeks |

Abbreviations: µg=micrograms; µl=microliters; µM=micromolar; µmol=micromole; DSS=dextran sulfate sodium; kg=kilograms; mg=milligrams; ml=milliliters; mM=millimolar; NR=not reported; ppm=parts per million.

### Cancer Classifications and Type

Cancers evaluated in the included studies were classified by cancer site according to the ICD-10 classification86. Across the 61 included studies, 19 studies evaluated digestive cancers2-4, 6, 10, 12, 15, 21, 24, 26, 27, 29, 32, 36, 45, 47, 52-54, 19 studies evaluated lung cancers1, 7, 9, 11, 14, 16, 17, 19, 20, 23, 31, 33, 35, 42, 43, 46, 49, 55, 61, six studies evaluated breast cancers13, 25, 50, 51, 57, 60, six studies evaluated head and neck cancers8, 28, 30, 41, 48, 58, and two studies evaluated urinary tract cancers22, 34. Nine studies evaluated other cancer types, or evaluated cancers at multiple sites5, 18, 37-40, 44, 56, 59.

Regarding cancer types, 42 studies used xenografts or allografts derived from malignant cell lines, and therefore evaluated malignant tumors1-4, 7-18, 20, 21, 24-27, 29-35, 42, 43, 45, 46, 48-53, 55, 57, 58. Of these 42 studies, five studies also evaluated non-xenograft/allograft tumor models9, 12, 17, 29, 43: two of these studies evaluated malignant tumors12, 29, one study evaluated benign tumors43 and two studies did not specify whether lung tumors were benign or malignant9, 17.

In 17 studies that did not use xenografts or allografts, nine studies evaluated malignant tumors6, 22, 28, 38, 41, 47, 54, 56, 59, four studies evaluated both benign and malignant tumors5, 19, 39, 44, no tumors were observed in two studies36, 37, and two studies did not specify whether tumors were benign or malignant23, 61. For the last two studies that did not use xenografts or allografts, one study evaluated carcinogen-induced benign and malignant tumors, and evaluated the occurrence of spontaneous tumors and reported no tumors60, and one study evaluated tumors at multiple sites and reported both malignant and benign tumors at some sites and unspecified tumor type at other sites40.

### Risk of Bias Assessment

Overall risk of bias grades for the 61 included studies 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 bias4, 6, 10, 11, 13, 15, 25, 26, 29, 35, 37, 39, 44, 46, 49, 50, 54, 59, and 43 studies (70%) were graded as having an “unclear” risk of bias1-3, 5, 7-9, 12, 14, 16-24, 27, 28, 30-34, 36, 38, 40-43, 45, 47, 48, 51-53, 55-58, 60, 61. The complete risk of bias assessments for each study are provided in **Appendix G**.

### Study Adequacy Evaluation

Of the 12 studies of tumor initiation, five studies had high adequacy scores17, 19, 39, 40, 44, while seven studies had low adequacy scores5, 36-38, 59, 60, 85. Of the 54 tumor progression studies, 16 studies had high adequacy scores11, 12, 14, 16, 17, 19, 21, 22, 32, 46, 49, 52, 54-57, while the remaining 38 studies had low adequacy scores1-10, 13, 15, 18, 20, 23-31, 33-35, 41, 43, 45, 47, 48, 50, 51, 53, 58, 60-62. The complete study adequacy evaluation for each study is provided in [**Appendix**](#_Study_Adequacy_Evaluation) **H.**

## Risk of Bias Assessment

|  | **Sequence ganeration (selection bias)** | **Baseline chaacteristics (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)** | **Other sources of bias (other)** | **Overall Assessment†** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Al-Wadei et al., 2012a |  |  |  |  |  |  |  |  |  |  |  |
| Al-Wadei et al., 2009b |  |  |  |  |  |  |  |  |  |  |  |
| Ben et al., 2020c |  |  |  |  |  |  |  |  |  |  |  |
| Ben et al., 2020d |  |  |  |  |  |  |  |  |  |  |  |
| Berger et al., 1987e |  |  |  |  |  |  |  |  |  |  |  |
| Bersch et al., 2009f |  |  |  |  |  |  |  |  |  |  |  |
| Cedillo et al., 2019g |  |  |  |  |  |  |  |  |  |  |  |
| Chen et al., 1990h |  |  |  |  |  |  |  |  |  |  |  |
| Chen et al., 1994i |  |  |  |  |  |  |  |  |  |  |  |
| Chien et al., 2021j |  |  |  |  |  |  |  |  |  |  |  |
| Davis et al., 2009k |  |  |  |  |  |  |  |  |  |  |  |
| Delitto et al., 2016l |  |  |  |  |  |  |  |  |  |  |  |
| Galitovskiy et al., 2012m |  |  |  |  |  |  |  |  |  |  |  |
| Habs and Schmahl, 1984n |  |  |  |  |  |  |  |  |  |  |  |
| Hanaki et al., 2016o |  |  |  |  |  |  |  |  |  |  |  |
| Hao et al., 2013p |  |  |  |  |  |  |  |  |  |  |  |
| Hayashi et al., 2014q |  |  |  |  |  |  |  |  |  |  |  |
| Heeschen et al., 2001r |  |  |  |  |  |  |  |  |  |  |  |
| Hermann et al., 2014s |  |  |  |  |  |  |  |  |  |  |  |
| Hsu et al., 2020t |  |  |  |  |  |  |  |  |  |  |  |
| Iskandar et al.,2013u |  |  |  |  |  |  |  |  |  |  |  |
| Jarzynka et al., 2006v |  |  |  |  |  |  |  |  |  |  |  |
| Jimenez et al., 2020w |  |  |  |  |  |  |  |  |  |  |  |
| Kumari et al., 2018x |  |  |  |  |  |  |  |  |  |  |  |
| Kyte et al., 2018y |  |  |  |  |  |  |  |  |  |  |  |
| Lee et al., 2010z |  |  |  |  |  |  |  |  |  |  |  |
| Li et al., 2022aa |  |  |  |  |  |  |  |  |  |  |  |
| Li et al., 2015bb |  |  |  |  |  |  |  |  |  |  |  |
| Liu et al., 2015cc |  |  |  |  |  |  |  |  |  |  |  |
| Maier et al., 2011dd |  |  |  |  |  |  |  |  |  |  |  |
| Martin et al., 1979ee |  |  |  |  |  |  |  |  |  |  |  |
| Martinez et al., 2017ff |  |  |  |  |  |  |  |  |  |  |  |
| Molfino et al., 2011gg |  |  |  |  |  |  |  |  |  |  |  |
| Murphy et al., 2011hh |  |  |  |  |  |  |  |  |  |  |  |
| Nakada et al., 2012ii |  |  |  |  |  |  |  |  |  |  |  |
| Natori et al., 2003jj |  |  |  |  |  |  |  |  |  |  |  |
| Nishikawa et al., 1992kk |  |  |  |  |  |  |  |  |  |  |  |
| Pillai et al., 2015ll |  |  |  |  |  |  |  |  |  |  |  |
| Pratesi et al., 1996mm |  |  |  |  |  |  |  |  |  |  |  |
| Prueitt et al., 2016nn |  |  |  |  |  |  |  |  |  |  |  |
| Ross et al., 2020oo |  |  |  |  |  |  |  |  |  |  |  |
| Schmähl and Habs, 1976pp |  |  |  |  |  |  |  |  |  |  |  |
| Shimizu et al., 2019qq |  |  |  |  |  |  |  |  |  |  |  |
| Shin et al., 2004rr |  |  |  |  |  |  |  |  |  |  |  |
| Suzuki et al., 2018ss |  |  |  |  |  |  |  |  |  |  |  |
| Thompson et al., 1973tt |  |  |  |  |  |  |  |  |  |  |  |
| Torres-Gonzalez et al., 2014uu |  |  |  |  |  |  |  |  |  |  |  |
| Toth, 1982vv |  |  |  |  |  |  |  |  |  |  |  |
| Trevino et al., 2012ww |  |  |  |  |  |  |  |  |  |  |  |
| Tyagi et al., 2021xx |  |  |  |  |  |  |  |  |  |  |  |
| Underwood et al., 2020yy |  |  |  |  |  |  |  |  |  |  |  |
| Waldum et al., 1996zz |  |  |  |  |  |  |  |  |  |  |  |
| Wan et al., 2018aaa |  |  |  |  |  |  |  |  |  |  |  |
| Wang et al., 2017bbb |  |  |  |  |  |  |  |  |  |  |  |
| Wang et al., 2021ccc |  |  |  |  |  |  |  |  |  |  |  |
| Wang et al., 2019ddd |  |  |  |  |  |  |  |  |  |  |  |
| Warren et al., 2012eee |  |  |  |  |  |  |  |  |  |  |  |
| Wong et al., 2007fff |  |  |  |  |  |  |  |  |  |  |  |
| Wu et al., 2020ggg |  |  |  |  |  |  |  |  |  |  |  |
| Yuge et al., 2015hhh |  |  |  |  |  |  |  |  |  |  |  |
| Zhang et al., 2017iii |  |  |  |  |  |  |  |  |  |  |  |

Key: Low risk of bias; High risk of bias; Unclear risk of bias

**†**The overall assessment of bias was: “low” if all bias domains were assessed as “low” risk; “high” if at least one bias domain was assessed as “high” risk; and “unclear” if at least one bias domain was assessed as “unclear” risk, and no domains were assessed as “high” risk.

a Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

b Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

c Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

d Some control mice treatment differently (i.e. directly injected with PBS rather than via minipump).

e Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

f Control group selected from the previous study (i.e. not true randomization, unbalanced baseline characteristics). DBMSA-S and DBMSA-N groups were treated differently. Circumstances during intervention are different between groups. Outcome assessment was not blinded Animals added during study to replace losses; losses by group not reported. Not all primary outcomes have been reported for the control group. Multi-arm study in which the same comparisons are not reported for all outcomes.

g Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

h Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Blinding of outcome assessors not reported.

i Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported.

j Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

k Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Study reported 16 BALB/c were randomized into two groups, but data was shown for only 14 mice; study did not justify whether missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups.

lTwo animals per group missing from analysis without explanation.

m Control and experimental groups were not identical in size. Study reported "three experimental mice did not develop any tumors and one experimental mouse died from nicotine toxicity prior to completion of the study".

n Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

o Randomization not reported. Baseline characteristics not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

p Some control mice treatment differently (i.e. directly injected with PBS rather than via minipump).

q Randomization not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported.

r Loss of one animal in the Nicotine + rofecoxib group was not explained. Potential conflict of interest (authors have patent on the use of nicotine for therapeutic angiogenesis).

s Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Random selection of animals for assessment not reported.

t Randomization not reported. Baseline characteristics not reported. Allocation concealing not reported.Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

u Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported.

v Randomization not reported. Random selection of animals for assessment not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

w Expected difference in visible effects between groups (i.e. mice fed high-fat diets).

x Study reported the outcome of only 1 of 12 animals in the vehicle or control group.

y Randomization process not described. Allocation concealing not reported. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

z Randomization not reported. Baseline characteristics not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

aa Only 15 of 27 mice mentioned in the results; no explanation provided.

bb Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods (i.e. cannot confirm if outcome data are complete).

cc Randomization process not described. Allocation conealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

dd Randomization process not described. Age of animals reported, but not sex. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified for all experiments in methods (i.e. cannot confirm if outcome data are complete).

ee Animal housing was not randomized. Pathology rating was deemed "unclassifiable" for some organs and these were excluded from the analysis; no attempt was made to balance numbers across intervention groups and data were not imputed.

ff Randomization not reported. Age and weight of animals not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

ggRandomization process not described. Allocation concealing not reported. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in results (i.e. cannot confirm if outcome data is complete).

hh Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in results (i.e. cannot confirm if outcome data is complete).

ii Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in results (i.e. cannot confirm if outcome data is complete).

jj Randomization not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods (i.e. cannot confirm if outcome data is complete).

kk Not all animals included in the analysis.

ll Randomization process not described. Age and sex of animals not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

mm Randomization not reported. Age of animals reported, but not sex. Allocation concealing not reported. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

nn Randomization not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

oo Randomization not reported. Sex of animals reported, but not age. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

pp Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

qq Randomization not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

rr Randomization not reported. Age of animals reported, but not sex. Allocation concealing not reported. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods (i.e. cannot confirm if outcome data is complete).

ss Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

tt Some animals died spontaneously during the trial; cause of death was not determined (although was noted that they did not die from metastatic neoplastic disease).

uu Randomization process not described. Baseline characteristics not reported. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

vv Randomization process not described. Allocation concealing not reported. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

ww Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

xx Three mice in the treatment group and one mouse in the control group not included in the results (without explanation).

yy Three mice in the treatment group died (without explanation) and were not included in the analysis.

zz Allocation of animals to groups was not concealed.

aaa Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

bbb Randomization process not described. Age of animals reported, but not sex. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods (i.e. cannot confirm if outcome data is complete).

ccc Blinding to treatment was not performed. Blinding of outcome assessor was not performed.

ddd Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in results (i.e. cannot confirm if outcome data is complete).

eee Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in results (i.e. cannot confirm if outcome data is complete).

fff Randomization process not described. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods or results (i.e. cannot confirm if outcome data is complete).

ggg Randomization process not described. Allocation concealing not reported. Not specified if animals from different groups housed together. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported. Number of animals not specified in methods (i.e. cannot confirm if outcome data is complete).

hhh Randomization process not described. Age of animals reported, but not sex. Allocation concealing not reported. Details of animal housing not provided. Blinding to treatment not reported. Random selection of animals for assessment not reported. Blinding of outcome assessors not reported.

iii Twenty-one mice were purchased but only 17 were analyzed (with no explanation for discrepancy).

## Study Adequacy Evaluation

| **RefID** | **Author, Year** | **Route of administrationa** | **Group sizeb** | | **Dose responsec** | | **Daily dosed** | | **Duration of exposuree** | | **Study Qualityf** | | **Overall  adequacy scoreg** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Tumor initiation studies*** | | | | | | | | | | | | | | |
| 89 | Berger et al., 1987 | + | + | | - | | - | | - | | - | | 2 | |
| 174 | Chen et al., 1994 | + | - | | - | | + | | - | | - | | 2 | |
| 320 | Galitovskiy et al., 2012 | - | - | | - | | + | | + | | - | | 2 | |
| 366 | Habs and Schmahl, 1984 | - | - | | - | | - | | - | | - | | 0 | |
| 632 | Maier et al., 2011 | + | - | | - | | + | | - | | + | | 3 | |
| 644 | Martin et al., 1979 | - | - | | - | | + | | - | | - | | 1 | |
| 683 | Murphy et al., 2011 | + | - | | - | | + | | - | | + | | 3 | |
| 850 | Schmahl and Habs, 1976 | - | + | | - | | - | | - | | + | | 2 | |
| 966 | Thompson et al., 1973 | - | - | | - | | + | | + | | + | | 3 | |
| 976 | Toth, 1982 | + | + | | + | | + | | + | | + | | 6 | |
| 1028 | Wang et al., 2017 | + | - | | - | | + | | - | | - | | 2 | |
| 1136 | Waldum et al., 1996 | + | - | | - | | + | | + | | + | | 4 | |
| ***Tumor Progression studies*** | | | | | | | | | | | | | | |
| 44 | Al-Wadei et al., 2012 | + | | - | | - | | - | | NA | | + | | 2 |
| 45 | Al-Wadei et al., 2009 | + | | - | | - | | - | | NA | | + | | 2 |
| 85 | Ben et al., 2020a | - | | - | | - | | - | | NA | | - | | 0 |
| ., 86 | Ben et al., 2020b | - | | - | | - | | - | | NA | | - | | 0 |
| 89 | Berger et al., 1987 | - | | + | | - | | - | | NA | | - | | 1 |
| 94 | Bersch et al.,2009 | - | | - | | - | | + | | NA | | - | | 1 |
| 150 | Cedillo et al., 2019 | - | | - | | - | | - | | NA | | - | | 0 |
| 175 | Chen et al., 1990 | + | | - | | - | | + | | NA | | - | | 2 |
| 182 | Chien et al., 2021 | - | | - | | - | | + | | NA | | + | | 2 |
| 236 | Davis et al., 2009 | + | | - | | - | | - | | NA | | + | | 2 |
| 247 | Delitto et al., 2016 | - | | - | | - | | - | | NA | | - | | 0 |
| 366 | Habs and Schmahl, 1984 | - | | - | | - | | - | | NA | | - | | 0 |
| 377 | Hanaki et al., 2016 | - | | - | | - | | - | | NA | | + | | 1 |
| 380 | Hao et al., 2013 | + | | - | | - | | + | | NA | | + | | 3 |
| 390 | Hayashi et al., 2013 | - | | - | | - | | + | | NA | | + | | 2 |
| 399 | Heeschen et al., 200 | + | | - | | - | | + | | NA | | + | | 3 |
| 403 | Hermann et al., 2014 | + | | - | | - | | + | | NA | | + | | 3 |
| 444 | Hsu et al., 2020 | - | | - | | - | | + | | NA | | - | | 1 |
| 458 | Iskandar et al., 2013 | - | | - | | - | | - | | NA | | + | | 1 |
| 475 | Jarzynka et al., 2006 | + | | - | | + | | + | | NA | | - | | 3 |
| 483 | Jimenez et al., 2020 | - | | - | | - | | + | | NA | | - | | 1 |
| 533 | Kumari et al., 2018 | - | | - | | - | | - | | NA | | - | | 0 |
| 542 | Kyte et al., 2018 | + | | - | | + | | + | | NA | | - | | 3 |
| 562 | Lee et al., 2010 | + | | - | | - | | + | | NA | | - | | 2 |
| 573 | Li et al., 2022 | - | | - | | - | | + | | NA | | - | | 1 |
| 576 | Li et al., 2015 | + | | - | | - | | + | | NA | | + | | 3 |
| 600 | Liu et al., 2015 | + | | - | | - | | - | | NA | | - | | 1 |
| 632 | Maier et al., 2011 | + | | - | | - | | + | | NA | | + | | 3 |
| 645 | Martinez et al., 2017 | + | | - | | + | | + | | NA | | - | | 3 |
| 672 | Molfino et al., 2011 | - | | - | | - | | + | | NA | | + | | 2 |
| 691 | Nakada et al., 2012 | + | | - | | - | | + | | NA | | - | | 2 |
| 699 | Natori et al., 2003 | + | | - | | - | | + | | NA | | - | | 2 |
| 720 | Nishikawa et al., 1992 | + | | - | | - | | + | | NA | | + | | 3 |
| 761 | Pillai et al., 2015 | - | | - | | - | | - | | NA | | - | | 0 |
| 769 | Pratesi et al., 1996 | + | | - | | + | | + | | NA | | - | | 3 |
| 775 | Prueitt et al., 2016 | + | | - | | + | | + | | NA | | + | | 4 |
| 824 | Ross et al., 2020 | + | | - | | - | | + | | NA | | + | | 3 |
| 683 | Murphy et al., 2011 | + | | - | | - | | + | | NA | | + | | 3 |
| 888 | Shimizu et al., 2019 | - | | - | | - | | + | | NA | | + | | 2 |
| 984 | Shin et al., 2004 | + | | - | | + | | + | | NA | | + | | 4 |
| 940 | Suzuki et al., 2018 | + | | - | | + | | + | | NA | | + | | 4 |
| 975 | Torres-Gonzales et al., 2014 | + | | - | | - | | + | | NA | | - | | 2 |
| 982 | Trevino et al., 2012 | - | | - | | - | | - | | NA | | - | | 0 |
| 998 | Underwood et al., 2020 | - | | - | | - | | - | | NA | | + | | 1 |
| 1025 | Wan et al., 2018 | + | | - | | - | | + | | NA | | - | | 2 |
| 1028 | Wang et al., 2017 | + | | - | | - | | + | | NA | | - | | 2 |
| 1034 | Wang et al., 2021 | + | | - | | - | | + | | NA | | - | | 2 |
| 1037 | Wang et al., 2019 | + | | - | | - | | - | | NA | | + | | 2 |
| 1047 | Warren et al., 2012 | - | | - | | - | | + | | NA | | + | | 2 |
| 1072 | Wong et al., 2007 | + | | - | | + | | + | | NA | | + | | 4 |
| 1076 | Wu et al., 2020 | - | | - | | - | | - | | NA | | + | | 1 |
| 1109 | Yuge et al., 2015 | - | | - | | - | | - | | NA | | - | | 0 |
| 1114 | Zhang et al., 2017 | - | | - | | - | | - | | NA | | - | | 0 |
| 1135 | Tyagi et al., 2021 | - | | - | | - | | + | | NA | | + | | 2 |

a Adequacy was scored with plus (+) if nicotine was administered via inhalation, oral, or subcutaneous injections.

b Adequacy was scored with plus (+) if at least 50 animals per sex, or 100 animals of a single sex were included in the study.

c Adequacy was scored with plus (+) if at least two doses of nicotine were used in the study.

d Adequacy was scored with plus (+) if the average daily dose of nicotine was ≥1 mg/kg per day, or if animals were eliciting signs of toxicity.

e For tumor initiation studies, adequacy was scored with plus (+) if the duration of exposure was ≥18 months.

f Study quality was based on a subjective judgement. A plus (+) score was assigned if nicotine biomonitoring was performed, or if body weight was monitored during the study.

g The overall adequacy score was determined by summing all the plus values. Scores ≥3 indicate high adequacy, while scores ≤2 indicate low adequacy.

## Study Characteristics of Tumor Initiation Studies

Of the 61 included studies, 12 studies evaluated tumor initiation5, 17, 19, 28, 36-40, 44, 59, 60. All 12 studies performed spontaneous tumor experiments, in which nicotine or control interventions were the only treatments provided. Eleven studies were RCTs, and one study44 was a controlled, non-randomized study. Source of funding was reported by seven studies and included national agencies28, 36, 37, 39, 40, 59, research foundations39, and research/educational institutions19; the remaining five studies did not report the source of funding5, 17, 38, 44, 60.

All 12 studies evaluated nicotine versus control treatment. Nine of these 12 studies included two intervention groups – a nicotine group and a control group17, 19, 28, 36-39, 44, 59. The remaining three studies included three groups: two studies evaluated two nicotine treatment durations versus control treatment5, 60, and one study evaluated two concentrations of nicotine versus control treatment40.

Across these 12 studies, five studies used mice17, 19, 40, 59, 85, six studies used rats5, 37-39, 44, 60, and one used hamsters36. The total sample sizes across the 12 studies ranged from 20 animals59 to 858 animals38. The number of animals in the nicotine-treated group ranged from six39 to 10040, while the number of animals in the control group ranged from five59 to 20040. A total of 641 animals were allocated to a nicotine group, and 471 were allocated to a control group.

Across the 12 tumor initiation studies, the most common method of nicotine administration was subcutaneous injections, performed in five of the 12 studies5, 37, 39, 59, 60. Across these five studies, dose of nicotine administered ranged from 0.4 mg/kg daily5 to 3 mg/kg twice daily37, and treatment duration ranged from 14 to 20 days5 to 24 months59. Three studies provided oral nicotine via drinking water that was available to animals *ad libitum*: one study administered 100 µg/mL of nicotine for 12 weeks17, one study administered 200 µg/mL nicotine for 46 weeks19, and one study administered a solution of either 0.09375% or 0.0625% nicotine for life, up to 120 weeks40. In one study, nicotine (2 mg/kg) was administered intraperitoneally once per week until the animals’ natural death38. In another study, nicotine (500 µg/m3) was administered via inhalation for 24 months, 20 hours per day, 5 days per week44. The two remaining studies administered nicotine buccally: in one study, 50 µL nicotine in a 6% solution was smeared onto the cheek pouch three times per week for up to 13 months36, and in the other study, a 5% solution of nicotine was smeared on the tongue three times per week for 16 weeks28.

Two of the 12 studies reported data on biomarkers of nicotine exposure19, 44. In one study, after 5 months of 200 mg/mL nicotine in drinking water, the average urine concentration of nicotine was 1,270±1,170 ng/mL, cotinine was 4,400 ± 3,200 ng/mL, and trans-3’hydroxycotinine was 27,900±9,820 ng/mL. Plasma concentration of biomarkers at the end of the study (i.e., 46 weeks) was 0.40±0.46 ng/mL for nicotine, 19±20 ng/mL for cotinine, and 48±36 ng/mL for trans-3’-hydroxycotinine19. In the second study, the average concentration of nicotine in the plasma was slightly over 100 ng/mL (108.4±55.1 ng/mL at Week 1 and 129.8±43.0 ng/mL at Week 103) following nicotine inhalation (500 µg/m3 for up to 24 months, 20 hours per day and 5 days per week)44.

Of the 12 studies that evaluated tumor initiation, no tumors were observed in four studies 28, 36, 37, 60, four studies evaluated both benign and malignant tumors5, 19, 39, 44, two studies evaluated malignant tumors38, 59, and one study did not specify whether tumors were benign or malignant 17. One study reported both benign and malignant tumors for some tumor sites, but did not report the tumor type for other tumor sites40.

Regarding outcome measures in studies that evaluated tumor initiation, all 12 studies reported data on tumor incidence. Two studies also reported data on tumor multiplicity (i.e., number of tumors per animal)17, 19, and one study reported data on the tumor induction period38. Statistical comparison between nicotine and control groups was performed in four studies5, 17, 28, 44; the remaining eight did not perform statistical comparison between nicotine and control groups19, 36-40, 59, 60.

The risk of bias was graded as “unclear” in eight of 12 studies5, 17, 19, 28, 36, 38, 40, 60, and high in the remaining four studies37, 39, 44, 59.

Quality of reporting was generally poor, with many studies not reporting on key study characteristics, including sample sizes, weight of the animals, or duration of treatment. Additionally, several inconsistencies in reporting were identified, including inconsistencies in reporting of sample sizes, p values, and units of measure.

### Lung Cancer

Across the 12 studies that evaluated tumor initiation, two studies evaluated lung cancers17, 19. Both studies were RCTs and both used mice. In the study by Maier et al.17, mice in the nicotine group received nicotine in the drinking water (100 µg/mL) and the control group received drinking water alone. In the nicotine group, the mean serum cotinine level was 137 ng/mL – the study authors noted that according to previous studies this serum cotinine level was comparable to a 22 mg nicotine patch dose in humans. After 12 weeks of treatment, one of the 30 mice in the nicotine group and none of the 10 mice in the control group developed tumors; statistical analysis was not performed. The study noted that there were no differences between groups in tumor multiplicity (i.e., number of tumors per lung) or tumor volume, although p values were not provided. The study by Murphy et al.19 administered nicotine in drinking water at a dose of 200 µg/mL for 46 weeks. At 5 months, urine concentration of biomarkers of nicotine exposure were as follows: nicotine, 1,270±1,170 ng/mL; cotinine, 4,400±3,200 ng/mL; and trans-3’hydroxycotinine, 27,900±9,820 ng/mL. Plasma concentrations of biomarkers of exposure, evaluated on Week 46 were as follows: nicotine: 0.40±0.46 ng/mL; cotinine 19±20 ng/mL; and trans-3’-hydroxycotinine 48±36 ng/mL. Twenty-six percent of mice in the nicotine group and 31% of mice in the control group developed tumors, however, statistical comparison between the two groups was not performed. The study noted that there were no differences in tumor multiplicity between groups, although statistical analysis was not performed.

### Breast Cancer

One study evaluated initiation of breast cancer in female rats60. The study was an RCT that administered nicotine (0.4 mg/kg) or control treatment subcutaneously on postpartum Days 46 to 52, or twice per week between postpartum Days 55 to 145. None of the rats in the nicotine or the control groups developed mammary tumors during the study.

### Digestive Cancer

One study evaluated initiation of gastric cancer in male hamsters36. The study was an RCT that applied nicotine or control treatment on each cheek pouch three times per week for up to 13 months. None of the hamsters in either group developed tumors by the end of the study.

### Head and Neck Cancer

An RCT in mice evaluated initiation of oral squamous cell carcinoma28. Nicotine (5% solution) or distilled water was smeared on the tongue three times per week for 16 weeks. None of the mice in either group developed tumors.

### Undefined Cancer Sites

The remaining seven studies, six of which were RCTs, did not pre-define the tumor site, but evaluated incidence of tumors at multiple sites5, 37-40, 44, 59. Five of the seven studies used rats5, 37-39, 44. The study by Berger et al.5 included two nicotine groups: in the prenatal nicotine group, nicotine (0.4 mg/kg) was administered to pregnant dams on gestational Day 14; in the prenatal and postnatal nicotine group, pregnant dams received nicotine on gestational Days 14 to 20 and postpartum Days 1 to 20, and the offspring received nicotine twice per week on postpartum Weeks 4 to 26. The control groups consisted of untreated dams and offspring. There were no statistically significant differences in the incidence of benign or malignant tumors in rat offspring between the prenatal nicotine group, the prenatal and postnatal nicotine group, and the control groups.

In the study by Martin et al.37, nicotine (3 mg/kg) or control treatment was administered to pregnant dams twice daily during gestation for 3 weeks and continued from 2 days postpartum (a 2-day recovery period of no treatment was provided at delivery) until weaning, and the incidence of tumors was evaluated in the male rat offspring. None of the rats in either group developed tumors during the study (up to 68 weeks duration in the nicotine group, and 101 weeks in the control group).

Schmahl et al.38 administered nicotine (2 mg/kg) or control treatment intraperitoneally once per week until the rats’ natural death. No statistically significant differences in the incidence of malignant tumors were noted between the two treatment groups. In the nicotine-treated group, two of 35 male rats developed tumors (one mammary tumor [tumor induction period 583 days], and one leucosis [tumor induction period 426 days]), and two of 32 female rats developed mammary tumors (tumor induction period 545±117 days). In the control group, one of 36 male rats developed hemangioendothelioma of the liver (tumor induction period 637 days), and three of 33 females developed mammary tumors (tumor induction period 717±99 days). Tumor incidence and tumor induction periods were not statistically compared between the nicotine and control groups.

Thompson et al.39 administered nicotine (1,000 µg/mL/kg) or control treatment subcutaneously daily to rats for 2 or 22 months. At the end of the 2-month treatment, one lung adenocarcinoma and one pituitary chromophobe adenoma were observed among 10 rats in the nicotine group, while no tumors were observed among six rats in the control group. At the end of the 22-month treatment, three adrenal pheochromocytomas and one fibrosarcoma were observed among 28 rats in the nicotine group, while no tumors were observed in the control group. Statistical comparison between groups was not performed. It should be noted that this study had poor reporting, and the data for the 2-month treatment group reported above was taken from the table in the published article but in the text these data are reported for the 22-month control group and no tumors are reported for the 2-month treatment group.

Waldum et al.44 administered nicotine via inhalation (500 µg/m3) for up to 24 months starting at 2 months of age; the control group inhaled regular air. Rats were sacrificed at 6, 12, 18, and 24 months and examined for tumors. The overall incidence of tumors was not significantly different between the nicotine group and the control group (36% vs 24%, p=NS). No tumors were found in the lungs of either treatment group. Five malignant tumors (mammary gland adenocarcinoma [n=1], granulosa-theta cell tumor [n=1], ovary adenocarcinoma [n=2], histiocytoma [n=1]) were reported in the nicotine group (n=59), and no malignant tumors were reported in the control group (n=25; statistical comparison not provided). In addition, there were two reports of metastasis of unknown origin (one in the liver and one in the abdominal cavity) of the nicotine group. It should be noted that seven animals in the control group (n=25, 28.0%), and five animals in the nicotine group (n=59, 8.4%) were not examined, which introduces bias into the results.

Two of the six studies that evaluated the incidence of tumors at multiple sites used mice40, 59. In the study by Galitovskiy et al.59, nicotine (3 mg/kg) or control treatment was administered subcutaneously 5 days per week for 24 months. At the end of the study, 11 of 14 mice in the nicotine group, and none of the five mice in the control group developed tumors. Of the 11 mice that developed tumors, three had tumors in the uterus, and eight in the skeletal muscle. Statistical comparison between the groups was not performed.

In the study by Toth et al.40, nicotine was administered in drinking water at concentrations of 0.09375% or 0.0625% to mice starting at 5 or 7 weeks of age and continued until the animals’ natural death. The control group received untreated drinking water. At the end of the study, the percentage of mice with tumors was similar between the three study groups, although statistical comparison was not performed. Lung tumors were observed in a total of 15 of 100 mice in the 0.09375% nicotine group, in 12 of 100 mice in the 0.0625% nicotine group, and in 37 of 200 mice in the control group. Malignant lymphoma was observed in two of 100 mice in the 0.09375% nicotine group, seven of 100 mice in the 0.0625% nicotine group, and 28 of 200 mice in the control group. Tumors were observed in blood vessels of one of 50 male mice in the 0.09375% nicotine group (data for female mice not reported), four of 100 mice in the 0.0625% nicotine group, and 13 of 200 mice in the control group. In other tissues, tumors were observed in three of 100 mice in the 0.09375% nicotine group, four of 100 animals in the 0.0625% nicotine group, and 15 of 200 animals in the control group.

## Study Characteristics of Tumor Progression Studies

Of the 61 included studies, 54 studies evaluated tumor progression1-35, 41, 43, 45-58, 60-62. Thirty-eight of these studies were RCTs1-35, 41, 42, 60, and the remaining 16 studies used a controlled, parallel group study design, but did not specify whether randomization was performed42, 43, 45-58, 61. Source of funding was reported by 47 studies and included national agencies1, 2, 6-9, 11-14, 18-21, 23-30, 32-34, 41-43, 45-51, 53, 54, 56-58, research foundations1, 10, 31, 35, 49, 53, 58, and research/educational institutions7, 8, 14, 17, 19, 21, 22, 32, 46, 48, 51, 52, 56. The remaining seven studies did not report the source of funding3-5, 15, 17, 55, 60.

All of the 54 included progression studies compared nicotine treatment with control treatment. The majority of the studies (n=45) compared one nicotine group to one control group1-4, 6-15, 17-20, 23-30, 33-35, 41, 43, 45-51, 53, 54, 57, 58, 60-62, and nine of the studies evaluated multiple nicotine groups versus one control group5, 16, 21, 22, 31, 32, 42, 52, 55, 56, 61. Across the nine studies that compared multiple nicotine groups with a control group, five studies evaluated two nicotine doses21, 32, 52, 55, 56, one study evaluated three doses22, two studies evaluated two durations of nicotine treatment5, 31, and one study evaluated two modes of nicotine administration16. One study used two modes of nicotine administration, however, the two groups were not compared to each other17. Treatment regimens, including method of nicotine administration, dose, frequency, and treatment duration are described in **Table 1‑5**.

Across the 54 studies that evaluated tumor progression, 48 studies used mice1-4, 6-17, 19-21, 23-35, 42, 43, 45-53, 55-58, 61, four studies used rats5, 18, 22, 60, and two studies used hamsters41, 54. Description of the mouse models used in the included studies is provided in **Appendix K**.

The total sample sizes across the 54 studies ranged from six27 to 3605 animals. The number of animals per group ranged from four27 to 605 for the nicotine group, and from two27 to 605 for the control group. Across the studies that reported sample size, a total of 773 animals were allocated to a nicotine group, and 572 were allocated to a control group.

The most common method of nicotine administration was oral administration in drinking water available *ad libitum,* whichwas used in 22 studies1, 2, 7, 11, 12, 16, 17, 19, 21-23, 27, 29, 32, 42, 43, 49, 51, 52, 54, 56, 57. Across these 22 studies, the dose of nicotine administered ranged from 50 µg/mL21, 32, 52 to 10 mg/mL51, and the treatment duration ranged from 2 weeks17 to 44 weeks19. Twenty studies administered nicotine intraperitoneally3, 4, 8-10, 13, 15, 17, 18, 20, 24-26, 33-35, 45, 50, 58, 61. One of these studies did not report the dose of nicotine administered20, while another study reported administering 30 µg nicotine per mouse but the dose per body weight was not reported58. Across the remaining 18 studies, the dose of nicotine administered ranged from 0.25 mg/kg50 to 200 mg/kg15. Treatment duration was reported in 18 studies3, 4, 8-10, 13, 15, 18, 20, 24-26, 33, 35, 45, 50, 58, 61 and ranged from 2 weeks9 to 10 weeks 13, 45, 61. Eight studies administered nicotine subcutaneously5, 6, 14, 31, 46, 47, 55, 60. Five of these studies reported the nicotine dose as weight per kilogram body weight5, 6, 14, 47, 60, and the dose of nicotine administered ranged from 0.4 mg/kg5, 60, to 24 mg/kg14. One study administered 100 mg/mL nicotine via a continuous infusion for a total of 3.6 µL per day46, one study administered 20 µg or 200 µg nicotine per day via a continuous subcutaneous infusion55, and one study administered 60 µg nicotine daily via subcutaneous injections, noting that this was the maximal tolerated dose 31. Treatment duration for the subcutaneous studies ranged from 7 days14 to 90 days60. Two studies of tumor progression administered nicotine buccally: one study applied a 5% solution of nicotine on the tongue of animals for 16 weeks28, and one study applied 10 µL of nicotine, but did not specify the concentration or the duration of treatment30.

Of the four studies that used other modes of nicotine administration, one study administered nicotine via a dermal patch containing 25 mg/kg nicotine for 3 weeks9, one study administered 0.6 mg/kg nicotine intravenously five times per week for 18 days16, one study administered 20 mg/kg nicotine by gavage daily for 12 days53, and one study administered 6% nicotine solution onto the cheek pouch41. One study did not specify the method of administration, but did specify that nicotine was administered at a dose of 1.5 mg/kg daily for 4 weeks48.

Across the 54 studies of tumor progression, 12 studies evaluated biomarkers of nicotine exposure9, 11, 12, 16, 17, 19, 22, 25, 32, 45, 46, 57. Eight of these studies administered nicotine orally via drinking water11, 12, 16, 17, 19, 22, 32, 57. One of these studies also administered nicotine via intravenous injections16, and one study also administered it via intraperitoneal injections17. Three additional studies administered nicotine via intraperitoneal injections9, 25, 45, with one of these studies also administering nicotine via a dermal patch9. One study administered nicotine via subcutaneous infusions with an osmotic pump46. Of the eight studies that evaluated biomarker levels following oral administration of nicotine in drinking water, five studies evaluated levels in serum11, 16, 17, 22, 57, one evaluated levels in urine12, one in plasma32, and one in both urine and plasma19. In four of the five studies assessing serum, nicotine was administered at a dose of 100 µg/mL and serum levels of cotinine were assessed11, 16, 17, 57. The mean serum cotinine concentration ranged from 36.75±5.50 ng/mL16 to 216.5 ng/mL (95% CI: 189.9-236.2 ng/mL)11. One of the studies observed a mean serum cotinine concentration of 137 ng/mL, and indicated that the serum levels were comparable to those in 22 mg nicotine patch users17, and one study reported that the mean serum cotinine concentration was 54.98 ng/mL (range: 0-254.13 ng/mL), and the mean serum nicotine concentration was 13.48 ng/mL (range: 1.17-76.66 ng/mL) during the 4 weeks of treatment57. In the same study, the control group had a mean serum cotinine concentration of 0 ng/mL (range NA), and a mean nicotine concentration of 2.278 ng/mL (range: 1.34-3.07 ng/mL)57. In the remaining study assessing serum, nicotine was administered orally in drinking water at a dose of 10 ppm, 20 ppm, and 40 ppm22. The mean serum cotinine concentration was 83.8±5.3 ng/mL after administration of 10 ppm nicotine, 199.5±10.5 ng/mL after administration of 20 ppm, and 347.3±15.9 ng/mL after administration of 40 ppm nicotine. According to the investigators, at the higher doses these levels were comparable to those of active smokers, which they stated range from 250 to 300 ng/mL22. One of the above studies that assessed serum cotinine levels also reported a mean serum concentration of nicotine of 54.98 ng/mL after oral administration of nicotine in drinking water (100 µg/mL for 21 days)57.

Two studies evaluated urine concentrations of biomarkers of exposure following oral administration of nicotine in drinking water12, 19. One study, in which 100 µL nicotine was administered for 2 weeks, represented cotinine concentrations graphically without providing specific values 12. However, the study noted that cotinine levels were comparable to those in intermediate smokers, and were approximately 200 ng/mL. In the other study, administration of 200 µg/mL nicotine resulted in a mean urine concentration of cotinine of 5,910±4,140 ng/mL, a mean nicotine concentration of 840±714 ng/mL, and a mean trans-3’-hydroxycotinine concentration of 37,800±19,400 ng/mL at 5 months19. The same study also reported plasma levels of biomarkers of nicotine exposure at 44 weeks: mean cotinine concentration of 29±0.19 ng/mL; mean nicotine concentration of 0.26±0.28 ng/mL; and, mean trans-3’-hydroxycotinine concentration of 62±28 ng/mL19. One other study in which nicotine was administered orally in drinking water reported plasma levels of cotinine32. The average cotinine concentration in plasma was 43.2±7.10 ng/mL after administration of 50 µg/mL nicotine for 25 days, and 169.4±12.21 ng/mL after administration of 200 µg/mL nicotine for 25 days32. The doses of nicotine used mimicked the daily intakes of cigarettes in smokers reported in previous studies.

Three studies evaluated cotinine levels following intraperitoneal administration of nicotine9, 25, 45. One of these studies represented serum cotinine concentrations graphically without providing specific values, noting that serum cotinine levels were comparable to those in adult smokers25. In another study, intraperitoneal administration of 1 mg/kg nicotine three times per week for 5 weeks resulted in a urine cotinine concentration of 3,000 ng/mL9. The study authors noted that previous studies showed that cotinine concentrations in smokers range from 1,500 ng/mL to 8,000 ng/mL9. In the third study, 1 mg/kg of nicotine was administered three times per week for 10 weeks and the mean serum cotinine concentration was 1,635±142.22 pg/mL45.

In one of the studies in which nicotine was administered intraperitoneally, a separate group of animals also received nicotine (25 mg/kg daily) via a dermal patch, resulting in a urine cotinine concentration of 5,000 ng/mL9 (time of urine cotinine assessment was not specified).

One study administered nicotine (100 mg/mL; 3.6 µL/day) subcutaneously using an osmotic pump for 6 weeks46. The study reported that mean plasma nicotine levels were about 100 to 200 ng/mL (0.6-1.2 mM) after infusion of approximately 2 to 4 mg/kg/hr of drug, and about 45 ng/mL (280 nM) after infusion of approximately 0.5 mg/kg/hr, noting that these plasma nicotine levels were comparable to those of human smokers, which range from 10 to 50 ng/mL (60‑310 nM). However, it was unclear if plasma nicotine levels were measured in this study, or if these values were extrapolated from previous studies.

One study administered nicotine (0.6 mg/kg) intravenously five times per week for 18 days, and reported that the mean serum cotinine concentration was 372.37±42.3 ng/mL16.

Six additional studies did not evaluate biomarkers of nicotine exposure, however, they related the dose of nicotine administered to levels observed in human smokers1, 2, 4, 18, 21, 31, 58, 61. Two of these studies reported that the dose of nicotine administered corresponded to levels observed in heavy smokers1, 2, two studies used doses that corresponded to nicotine levels in smokers without specifying the amount of smoking21, 58, one study reported that the dose of nicotine corresponded to that in regular smokers4, and in one study, the amount of nicotine administered corresponded to smoking 25 cigarettes per day61. One study noted that the dose of nicotine administered was intended to achieve as high an intratumoral concentration as possible31, and one study reported that the dose of nicotine administered did not cause toxicity18.

Across the 54 studies of tumor progression, 18 studies evaluated digestive cancers2-4, 6, 10, 12, 15, 21, 24, 26, 27, 29, 32, 45, 47, 52-54, 18 studies evaluated lung cancers1, 7, 9, 11, 14, 16, 17, 19, 20, 23, 31, 33, 35, 42, 43, 49, 55, 61, six studies evaluated breast cancers13, 25, 50, 51, 57, 60, six studies evaluated head and neck cancers8, 28, 30, 41, 48, 58, two studies evaluated urinary tract cancers22, 34, and one study evaluated metastatic melanoma46. Three studies of tumor progression evaluated other cancer types, or evaluated cancers at multiple sites5, 18, 56.

Regarding cancer types, 42 studies used xenografts or allografts derived from malignant cell lines, and therefore evaluated malignant tumors1-4, 7-18, 20, 21, 24-29, 31-35, 42, 43, 45, 46, 48-53, 55, 57, 58. Five of these 42 studies also evaluated non-xenograft/allograft tumor models9, 12, 17, 29, 43. Of these four studies, two studies evaluated malignant tumors12, 29, one study evaluated benign tumors43, and two studies did not specify whether lung tumors were benign or malignant9, 17.

In the remaining 12 studies, seven studies evaluated malignant tumors6, 22, 28, 41, 47, 54, 56, three studies evaluated both benign and malignant tumors5, 19, 60, and two studies did not specify whether tumors were benign or malignant23, 61.

Among the 54 studies that evaluated tumor progression, outcome measures included tumor volume (n=32 studies)1-4, 7-11, 13-17, 24, 27, 29, 31, 32, 34, 35, 42, 43, 46, 48-53, 58, 61, metastasis or micrometastasis (n=14 studies)9, 10, 12, 17, 20, 24, 25, 30, 33, 45, 46, 56-58, tumor incidence (n=12 studies)5, 6, 17, 19, 22, 28, 29, 41, 43, 47, 50, 54, tumor weight (n=14 studies)3, 4, 8, 13, 18, 25-27, 29, 42, 50, 51, 56, 57, tumor proliferation (n=10 studies)3, 4, 7, 15, 17, 21, 22, 49, 50, 52, tumor growth (n=5 studies)17, 20, 24, 25, 42, 43, 55, angiogenesis or vascularization (n=6 studies)7, 11, 21, 32, 49, 53, tumor multiplicity (n=8 studies)17, 19, 22, 23, 43, 47, 54, 61, tumor area (n=3 studies)9, 21, 23, and time to tumor appearance (n=1 study)60.

The overall risk of bias grades for the 54 tumor progression studies were as follows: no studies were graded as having a “low” risk of bias, 14 studies (26%) were graded as having a “high” risk of bias4, 6, 10, 11, 13, 15, 25, 26, 29, 35, 46, 49, 50, 54, and 40 studies (74%) were graded as having an “unclear” risk of bias1-3, 5, 7-9, 12, 14, 16-24, 27, 28, 30-34, 41-43, 45, 47, 48, 51-53, 55-58, 60, 61.

### Lung Cancer

Of the 54 tumor progression studies, 19 studies evaluated lung cancer1, 7, 9, 11, 14, 16, 17, 19, 20, 23, 31, 33, 35, 42, 43, 46, 49, 55, 61. Of these 19 studies, 14 were RCTs2, 7, 9, 11, 14, 16, 17, 19, 20, 23, 31, 33, 35, 42, and five studies were controlled, parallel-design studies that did not specify whether animals were randomized to study groups43, 46, 49, 55, 61. All studies used mice.

Generally, findings of progression studies of lung cancer were not consistent across studies. Tumor volume was the most frequently evaluated outcome (n=13 studies)1, 9, 11, 14, 16, 17, 19, 31, 35, 42, 43, 49, 61. Eight of these 13 studies reported that tumor volume was significantly higher in the nicotine group compared with the control group1, 9, 11, 16, 19, 35, 43, 61, four studies14, 17, 31, 49 reported no statistically significant differences between groups, and in one study, tumor volume was numerically higher in the nicotine compared with the control group, however, statistical analysis was not performed42. Other outcomes measures included lung tumor proliferation – assessed by relative expression of Ki-67 – evaluated by three studies7, 17, 19, 49, 61; angiogenesis, evaluated by three studies7, 11, 49; tumor size, evaluated by three studies;17, 20, 23; tumor number or tumor multiplicity, evaluated by five studies17, 19, 23, 43, 61; metastasis, evaluated by four studies9, 17, 20, 33. Findings for each of these outcomes were inconsistent, with some studies reporting statistically significant differences, and others reporting no significant differences between the nicotine and the control group. Other outcome measures, included tumor growth over time (n=3 studies)17, 43,55, tumor weight (n=1 study)42, and tumor incidence in a carcinogen-induced tumor model (n=2 studies)17, 43.

Specific findings of lung cancer progression studies are detailed below and grouped by the cancer model utilized.

#### Xenograft/Allograft Cancer Models

##### Oral Administration of Nicotine

In seven studies that used xenograft/allograft models, nicotine was administered orally via drinking water and the control group received drinking water alone2, 7, 11, 16, 17, 43, 49. The dose of nicotine administered was 100 µg/mL in three studies11, 16, 17, and 200 µg/mL in two studies1, 49; the remaining study administered 1 µM of nicotine7. Treatment duration ranged from 14 days16, 43 to 12 weeks17. Three studies assessed biomarkers of nicotine exposure11, 16, 17.

In a xenograft study by Al-Wadei et al.1, human NSCLC cells from either the NCI-H322 or the NCI-H441 cell lines were injected into mice before mice were randomized to either the nicotine (200 µg/mL) or the control group. At Day 30, tumor volume was significantly higher in the nicotine group compared with the control group in animals with xenografts of either the NCI-H322 cells (p<0.001) or the NCI-H441 cells (p<0.0001).

In a xenograft study by Cedillo et al.7, mice were injected with human bronchioalveolar carcinoma A549 cells before being randomized to receive either nicotine 1 µM or control treatment for 37 days. At Day 37, tumor volume was significantly higher in the nicotine group compared with the control group (p<0.05). Tumor proliferation was assessed qualitatively by relative expression of Ki-67, a marker of tumor proliferation. Staining was qualitatively more robust in tumor tissue from mice treated with nicotine than from those treated with control. Similarly, qualitative assessment of angiogenesis – evaluated using VEGF staining – showed more robust staining in tissue from nicotine-treated mice than from control mice.

In a xenograft study by Jarzynska et al.49, mice were also injected with the A549 cell line before being randomized to receive either 200 µg/mL nicotine in drinking water or drinking water alone for 36 days. At Day 36, there were no statistically significant differences in tumor volume between the nicotine and control group. Regarding angiogenesis, the study authors stated that nicotine enhanced microvascular density compared with control treatment, although the difference was not statistically significant. There was also a two-fold increase in the relative expression of Ki-67, a marker of tumor proliferation, in the nicotine group compared with the control group (p<0.05).

In an allograft study, Heeschen et al.11 injected mouse Lewis lung carcinoma cells, and then randomized mice to receive either nicotine (100 µg/mL) in drinking water or drinking water alone. Serum cotinine levels in the nicotine group were 216.5 ng/mL (95% CI 189.8-236.2 ng/mL); serum cotinine levels in the control group were not reported. On Day 16, nicotine-treated mice had significantly larger tumor volumes (p<0.01) and capillary densities (p<0.001) compared with control mice. In a separate group of mice, orthotropic implantation of cancer cells into the lung parenchyma was performed. Similar to the findings after subcutaneous injections of lung carcinoma cells, tumor volume (p<0.001) and capillary density (p<0.001) were significantly higher on Day 12 in nicotine-treated mice compared with control mice.

In the xenograft study by Li et al.16, human PC9 cells were injected into mice to create NSCLC xenografts, and mice were randomized to receive either oral nicotine in drinking water (100 µg/mL) or control treatment (normal drinking water). Sample size of the study was not reported. Serum cotinine was 36.75±5.50 ng/mL in the oral nicotine group compared with 13.85±0.69 ng/mL in the control group. On Day 18, tumor volume was significantly larger in the oral nicotine group (811±53 mm3) compared with control group (range: 600-630 mm3; p<0.05).

In an allograft study by Nakada et al. 43, Lewis lung carcinoma cells were injected into mice and mice were randomized to receive either nicotine (100 µg/mL) in drinking water with 2% saccharine or drinking water with 2% saccharine alone for 2 weeks. After 2 weeks, tumor volume was significantly higher in the nicotine group compared with the control group (data represented graphically).

In an allograft study by Maier et al.17, CL13, IO33, or CL25 mouse lung adenocarcinoma cells were injected into AB6F1 mice, and mice were randomized to receive either oral nicotine (100 µg/mL in drinking water) or control (drinking water alone). Average serum cotinine levels were reported as 137 ng/mL in the nicotine-treated mice. There was no statistically significant difference in tumor volume between the oral nicotine and control groups approximately 25 to 32 days after xenograft implantation. Additionally, there was no difference in the incidence of metastasis between the oral nicotine and control groups in mice with xenograft injections of the CL25 or CL13 cell lines, however, the time point of assessment was not provided and the p value was not reported.

Lastly, in a xenograft study by Liu et al.42, human bronchioalveolar carcinoma A549 cells were injected into mice, and mice were then randomized to receive either 1 µM nicotine or control treatment for 20 days in the drinking water. At Day 20, tumor volume was numerically higher in the nicotine-treated mice compared with control mice (p value not reported), whereas tumor weight significantly increased in the nicotine-treated mice compared to control mice (p<0.01).

##### Intraperitoneal Administration of Nicotine

Five progression studies of lung cancer used xenograft/allograft models and administered nicotine via intraperitoneal injections9, 17, 20, 33, 35. The intraperitoneal dose administered was 1 mg/kg in three studies9, 33, 35, 0.8 mg/kg in one study17, and not reported in one study20. Treatment duration ranged from 2 weeks9 to 12 weeks17. Two studies reported biomarker data9, 17.

In the study by Pillai et al.20, NSCLC human A549 cells were injected into mice, and nicotine or control treatment was administered by intraperitoneal injections every other day for 7 weeks, although the dose was not specified. At 7 weeks, tumors in the nicotine group were significantly larger than those in the control group (p<0.05). There were no statistically significant differences in metastasis to the lung, brain, adrenal glands, or liver at 7 weeks between the nicotine and control groups, although the authors noted that metastases in each tissue were numerically higher in the nicotine group compared with the control group.

In a xenograft study by Zhang et al.35, H1299 cells from a human NSCLC cell line were injected into nude mice prior to administration of intraperitoneal injections of either nicotine (1 mg/kg) or control treatment for 6 weeks. At 6 weeks, tumor volume was significantly larger in the nicotine group compared with the control group (p<0.05).

In the allograft study by Wu et al.33, mouse LL/2 lung cancer cells were injected intracranially into BALB/c mice or intracardially into C57BL/6 mice, and human H2030BrM lung cancer cells were injected intracardially into nude mice. Three days later, mice started receiving daily intraperitoneal injections of either nicotine (1 mg/kg) or control treatment (phosphate buffered saline [PBS]). In all three experimental conditions, growth of brain metastases on Day 40 (assessed by bioluminescence) was significantly higher (p<0.01 for LL/2 intracranial or intracardiac cell injections; p<0.001 for H2030BrM intracardial cell injections), and brain metastatic lesions were significantly bigger (p<0.01 for intracranial injections of LL/2 cells; p<0.001 for intracardiac injections of LL/2 cells; p<0.001 for intracardial injections of H2030BrM cells), whereas metastasis-free survival was significantly shorter (p<0.05 for all three cell lines) in the nicotine group compared with the control group. Incidence of bone and brain metastasis was also assessed following intracardiac injections of LL/2 lung cancer cells: the incidence of brain metastasis on Day 40 was significantly higher in the nicotine (77%) than in the control group (22%; p=0.01), while there were no statistically significant differences in the incidence of bone metastasis on Day 40 between the nicotine (33%) and control groups (11%; p=0.25).

In the allograft study by Davis et al.9, mouse Line-1 lung adenocarcinoma cells were injected into BALB/c mice. Nicotine (1 mg/kg) or control treatment was administered via intraperitoneal injections three times per week, resulting in a urine cotinine concentration of 3,000 ng/mL. At Day 18, tumor volume was significantly higher in the nicotine group compared with the control group (p=0.002). In a separate experiment, tumors were removed 3 weeks after allograft injections and mice continue to receive nicotine or control treatment for two more weeks. Rate of tumor recurrence, calculated as the percentage of recurring tumors out of the total number of tumors removed, was significantly higher in the nicotine group (59% ± 3%) compared with the control group (19% ± 7%; p = 0.01). The number of metastatic foci in the lungs at the end of the study was also significantly higher in the nicotine group (8.1±1.7) compared with the control group (0.9±0.2; p = 0.001).

Lastly, in the fifth study that used a xenograft model,17 human IO33 or CL25 lung adenocarcinoma cells were injected into AB6F1 mice, and mice were randomized to receive either nicotine (0.8 mg/kg) via an intraperitoneal injection (frequency of administration not reported) or control treatment (drinking water alone). Approximately 18 days after xenograft implantation, there were no statistically significant differences in tumor growth between the nicotine group and the control group.

##### Subcutaneous Administration of Nicotine

Three lung tumor progression studies used xenograft/allograft models and administered nicotine subcutaneously14, 31, 55. The subcutaneous dose administered was 24/mg/kg/day in one study14, 20 µg or 200 µg in a second study55, and 60 µg in the third study31. Treatment duration ranged from 7 days14 to 28 days31. None of the studies assessed biomarkers of nicotine exposure.

In one study, mice received injections of mouse Lewis lung carcinoma cells and 13 days later received either nicotine (24 mg/kg/day) or control treatment (saline) subcutaneously via osmotic pumps for up to 7 days14. There were no statistically significant differences in the change in tumor volume (at Day 8) from baseline between the nicotine and control groups (p=0.054).

In a xenograft study by Pratesi et al.55, the human small cell lung cancer (SCLC) cell line NCI-N592 was injected into both flanks of mice . Growing tumor fragments were serially transplanted into new recipient mice, who received either 20 µg/day nicotine, 200 µg/day nicotine, or control treatment via a continuous subcutaneous infusion for 14 days. There were no statistically significant differences between the 20 µg/day nicotine or the 200 µg/day nicotine group and the control group in early tumor progression – defined as the time between the implantation of tumors into mice and the tumor reaching 50 mm3 in size – or established tumor progression – defined as the time for a tumor >100 mm3 in size to reach 1,000 mm3.

Warren et al.31 injected a human lung cancer cell line H460 xenograft into the right flank of mice. When tumors reached 5 mm in maximal dimension, mice were randomized to receive nicotine (60 µg) for either 6 days (short-term group) or 28 days (long-term group), or control treatment (saline) via subcutaneous injections. According to the investigators, this dose of nicotine was chosen based on previous dose escalation experiments, which showed that 60 µg per mouse was the maximum tolerated dose of nicotine (data not shown). At 28 Days, there were no statistically significant differences in tumor volume between the short-term nicotine, long-term nicotine and control groups (p values were not reported).

##### Dermal Patch Administration of Nicotine

In the allograft study by Davis et al9, where mouse Line-1 lung adenocarcinoma cells were injected into BALB/c mice9, mice were also randomized to receive nicotine via a dermal patch (25 mg/kg daily) or a control patch. Urine cotinine concentration following transdermal nicotine treatment was 5,000 ng/mL. Tumor volume at Day 11 was significantly larger in the nicotine patch group (871±106 mm3) compared with the control patch group (530±56 mm3; p = 0.019). Additionally, the number of metastatic foci in the lungs was significantly greater in the nicotine patch group (20.6±4.9) compared with the control patch group (6.7±2.1; p=0.02).

##### Intravenous Administration of Nicotine

In the NSCLC xenograft study by Li et al.16, where PC9 cells were injected into mice, mice were randomized to receive either intravenous nicotine (0.6 mg/kg five times per week) or control treatment (intravenous vehicle five times per week). Sample size was not reported. Serum cotinine levels at the end of the study were significantly higher in the intravenous nicotine group (372.37±42.3 ng/mL) compared with the control group (13.85±0.69 ng/mL; p<0.05). On Day 18, tumor volume was significantly larger in the intravenous nicotine group (746±24 mm3) compared with the control group (range: 600-630 mm3; p<0.05).

#### Carcinogen-Induced Cancer Models

##### Oral Administration of Nicotine

In three studies that used carcinogen-induced cancer models, nicotine was administered orally via drinking water and the control group received drinking water alone17, 19, 43. The dose of nicotine administered was 100 µg/mL in two studies17, 43, and 200 µg/mL in the other study19. Treatment duration was 14 days in one study43, 12 weeks in one study17, and 44 weeks in the other study19. Two studies assessed biomarkers of nicotine exposure17, 19.

The study by Maier et al.17 evaluated lung tumor progression in a carcinogen-induced model of lung cancer, in addition to using an allograft model. In the carcinogen-induced cancer experiments, mice received three weekly intraperitoneal injections of 100 mg/kg NNK, followed by either nicotine in drinking water (100 µg/mL) or drinking water alone. Average serum cotinine levels were reported as 137 ng/mL in the nicotine-treated mice. At 12 weeks, there were no significant differences between the nicotine and the control group in tumor incidence, tumor multiplicity, tumor size, or Ki-67 staining (tumor proliferation).

In the study by Murphy et al.19, mice received intraperitoneal injections of NNK (10 µmol in 0.1 mL PBS) for 2 weeks. Two weeks after NNK injections, mice were randomized to receive either 200 µg/mL nicotine in drinking water or drinking water alone for 44 weeks. At the end of the study, 20 of 20 mice (100%) in the nicotine group and 18 of 18 mice (100%) in the control group developed tumors. Tumor multiplicity – the number of tumors per mouse – was 20.4±5.4 in the nicotine group and 18.4±4.5 in the control group (statistical comparison between groups was not performed). Tumor multiplicity categorized by tumor diameter was also similar between the two groups, although statistical comparison was not performed. When tumor were categorized by type, the incidence and multiplicity of adenomas, adenomas with dysplasia, and carcinomas was similar between the nicotine and the control group, although statistical comparison was not performed.

The study by Nakada et al.43, evaluated lung tumor progression in a carcinogen-induced model of lung cancer, in addition to using an allograft model. Mice received a single intraperitoneal injection of 2 mg of NNK, followed by either nicotine (100 µg/mL) in drinking water with 2% saccharine (nicotine group) or drinking water with 2% saccharine alone (control group). After 16 weeks, the incidence of tumors in mice was 100% in both study groups. The mean number of adenomas per mouse was 2.7±1.6 in the nicotine group and 2.3±0.9 in the control group. The mean number of adenomas with hyperplasia per mouse was 4.0±3.0 in the nicotine group and 2.8±1.3 in the control group. With regards to this outcome, no differences between the study groups were indicated but it is unclear if these two groups were statistically compared.

##### Intraperitoneal Administration of Nicotine

In the study by Iskandar et al.61, mice received a single intraperitoneal injection of NNK (100 mg/kg) and after 2 weeks received no treatment (control group) or intraperitoneal injections (1 mg/kg) three times weekly for 10 weeks (nicotine group). After 10 weeks, both tumor volume and tumor multiplicity were significantly higher in the nicotine group compared with the control group (p<0.05 for both).

#### Genetic Cancer Models

##### Oral Administration of Nicotine

In two studies that used genetic models of cancer, nicotine was administered orally via drinking water and the control group received drinking water alone17, 23. The dose of nicotine administered was 100 µg/mL in both studies17, 23. Treatment duration was 2 weeks or 6 weeks in one study17, and 90 days in the other study23. One study assessed biomarkers of nicotine exposure17.

The study by Maier et al.17 evaluated lung tumor progression in a genetic model of lung cancer in KrasLA2/+ mice on a C57BL/6 background, in addition to using both allograft and carcinogen-induced cancer models. These mice develop extensive tumors, most frequently in the lungs. Mice were treated with either nicotine in drinking water or drinking water alone for 2 or 6 weeks starting at 3 or 6 weeks of age. There were no statistically significant differences between nicotine and control groups in peripheral tumor multiplicity, or Ki-67 staining. Notably, these findings were observed after both 2 and 6 weeks of treatment.

Another study used the Kras mouse genetic model of lung cancer and administered nicotine orally23. Mice were given either 100 µg/mL nicotine in drinking water or drinking water alone for 90 days. There were no statistically significant differences between the nicotine and control groups in tumor number (p=0.95) or tumor size (p=0.37) (time of assessment not specified but assumed to be 90 days).

### Digestive Cancer

Of the 54 tumor progression studies, 18 studies evaluated digestive cancer 2-4, 6, 10, 12, 15, 21, 24, 26, 27, 29, 32, 45, 47, 52-54. Thirteen of these studies were RCTs2-4, 6, 10, 12, 15, 21, 24, 26, 27, 29, 32, and the remaining five studies were controlled, parallel group studies that did not specify whether animals were randomized to study groups 45, 47, 52-54. Seventeen studies used mice2-4, 6, 10, 12, 15, 21, 24, 26, 27, 29, 32, 45, 47, 52, 53, and the remaining study used hamsters54.

Overall, findings of progression studies of digestive cancers showed that nicotine administration was associated with tumor progression. Compared with control animals, animals treated with nicotine had significantly higher tumor volumes in 10 of 11 studies 2-4, 10, 24, 27, 29, 32, 52, 53, with the remaining study showing a numerically higher tumor volume in the nicotine group, although statistical analysis was not performed15. In addition, significantly higher tumor proliferation with nicotine treatment was observed in five studies3, 4, 15, 21, 52, although in one study a difference was only observed after administration of 200 µg/mL nicotine, but not 50 µg/mL nicotine21. Furthermore, significantly higher angiogenesis or vascularization was observed with nicotine treatment in three studies21, 32, 53, and significantly higher tumor weight was observed in all five studies that evaluated it26, 27, 29 3, 4. Moreover, two of three studies evaluating metastasis reported significantly higher metastasis in nicotine-treated animals compared with control animals20, 45, and one study reported numerically higher outcomes, but did not perform statistical analysis10. Inconsistent results – that is, some studies showing statistically significant differences and other studies showing no differences between the nicotine and control groups – were reported for other outcomes, including incidence of tumors in carcinogen-induced models6, 47, 54, and tumor multiplicity47, 54. Other outcomes measures, evaluated by one study each, included rate of tumor growth20, tumor area21, and micrometastasis12.

Specific findings of digestive cancer progression studies are detailed below and grouped by the cancer model.

#### Xenograft/Allograft Cancer Models

##### Oral Administration of Nicotine

Seven of the 18 progression studies that evaluated digestive cancers used xenograft/allograft models and administered nicotine orally in drinking water available *ad libitum*2, 12, 21, 27, 29, 32, 52. Three studies administered two doses of nicotine (50 µg/mL and 200 µg/mL or 50 µmol/L and 200 µmol/L)21, 32, 52, three studies administered 200 µg/mL nicotine2, 27, 29, and one study administered 100 µg/mL12. The duration of treatment ranged from 2 weeks12 to 3 months21. Two studies assessed biomarkers of nicotine exposure12, 32.

In the xenograft study by Al-Wadei et al.2, the human pancreatic ductal adenocarcinoma cell line (Panc-1) was injected into athymic nude mice, and mice were randomized to receive either nicotine (200 µg/mL) in drinking water or drinking water alone. At Day 30, there was a 4.2-fold increase in the volume of tumors from mice treated with nicotine (p<0.001 vs. baseline), while there was no change in the volume of tumors from control mice.

In the xenograft study by Wan et al.27, human SMMC-7221 hepatocarcinoma cells were injected, and mice were randomized to receive either nicotine (200 µg/mL) in drinking water or drinking water alone. At Day 35, tumor volume and tumor weight were significantly higher in nicotine-treated mice compared with control mice (p<0.05 for tumor volume and tumor weight).

In another xenograft study, the human Mz-ChA-1 biliary epithelial cell line was injected into BALB/c nude mice52. Mice were then assigned to receive either 50 µmol/L nicotine in drinking water, 200 µmol/L nicotine in drinking water, or drinking water alone. Tumor volume was significantly larger in the 50 µmol/L nicotine group than in the control group from Day 34 to Day 38 (p<0.05), and in the 200 µmol/L nicotine group than in the control group from Day 29 to Day 38 (p<0.05). The number of Ki-67-positive cells, a marker of tumor proliferation, at the end of the study was significantly higher in both nicotine groups than in the control group (p<0.05 for both 50 µmol/L nicotine and 200 µmol/L nicotine vs. control).

In the xenograft study by Shin et al.21, human gastric adenocarcinoma AGS cells were inoculated into the gastric wall of athymic nude mice, and mice were randomized to receive either 50 µg/mL or 200 µL/mL nicotine in drinking water, or drinking water alone for 3 months. At the end of the study, tumor area was significantly larger in mice treated with 50 µg/mL nicotine (p<0.05) and mice treated with 200 µg/mL nicotine (p<0.01), compared to control mice. The number of proliferative cells in tumor tissue was assessed using proliferating cell nuclear antigen (PCNA) staining; PCNA staining was significantly higher in the 50 µg/mL compared with the no tumor control tissue (p<0.05), but was not statistically significantly different from staining in tumor control tissue (p value not reported). In the 200 µg/mL nicotine group, the number of PCNA-positive cells was significantly higher compared to both no tumor control tissue (p<0.01) and tumor control tissue (p<0.05). Density of microvessels after 3 months of treatment was significantly higher in the 200 µg/mL nicotine group compared with the tumor control group (p<0.05) and the no tumor control group (p<0.05), however, there were no statistically significant differences between the 50 µg/mL nicotine group and either the tumor or the no tumor control groups.

In the xenograft study by Wong et al.32, human HT-29 colorectal adenocarcinoma cells were implanted subcutaneously, and mice were randomized to receive either 50 µg/mL or 200 µg/mL nicotine in drinking water, or drinking water alone for 25 days. At Day 25, plasma cotinine concentration was significantly higher in the 50 µg/mL nicotine group (43.2±7.10 ng/mL; p<0.05), and 200 µg/mL nicotine group (169.4±12.21 ng/mL; p<0.005) compared with the control group (8.7 ± 0.66 ng/mL). At Day 25, tumor volume was significantly larger in the 50 µg/mL nicotine group (p<0.05) and the 200 µg/mL nicotine group (p<0.005), compared with the control group. The mean number of blood vessels on Day 25 was significantly higher in the 200 µg/mL nicotine group compared with the control group, however, there were no statistically significant difference between the 50 µg/mL nicotine group and the control group.

In a study that used an allograft model, fluorescent-labelled mouse pancreatic ductal adenocarcinoma cells were injected into the spleen of mice, and mice were randomized to receive either 100 µg/mL nicotine in drinking water supplemented with sucrose, or control treatment (not specified)12. Urine cotinine levels were reported graphically and were approximately 200 ng/mL. After 3 weeks of treatment, there were no statistically significant differences in the dissemination of pancreas-derived carcinoma cells in the liver between the nicotine and control groups (p = 0.0513).

In the seventh xenograft/allograft study that evaluated digestive cancer, a human esophageal squamous cell carcinoma xenograft was injected into nude mice, and mice received 200 µg/mL nicotine in drinking water or drinking water alone for 16 weeks29. Tumor volume was significantly higher in the nicotine group compared with the control group starting at Week 2 (p<0.01) through Week 5 (p<0.001). Correspondingly, tumor weight was significantly higher in the nicotine group compared with the control group at the end of the study (specific time point not specified; p<0.001).

##### Intraperitoneal Administration of Nicotine

Seven of the 18 progression studies that evaluated digestive cancer used xenograft models and administered nicotine via intraperitoneal injections3, 4, 10, 15, 24, 26, 45. The dose of nicotine administered ranged from 0.25 mg/kg3, 4 to 200 mg/kg15, and the duration of treatment ranged from 3 weeks3, 4, 24 to 10 weeks45. Only one study reported the levels of biomarkers of exposure45.

In a study by Ben et al.4, human pancreatic ductal adenocarcinoma (PDAC) Panc-1 xenograft cells were injected into the dorsal flank of nude mice. When tumor volume reached 75 to 125 mm3, mice were randomized to receive either nicotine (0.25 mg/kg) or vehicle via intraperitoneal injections three times per week for 3 weeks. On Day 22, tumors from nicotine-treated mice had significantly higher volume (p<0.01), weight (p<0.01), and relative expression of tumor proliferation marker Ki-67 (p<0.05) compared with tumors from control-treated mice. In another study by the same group3, the same study design was used: Panc-1 cells were inoculated into nude mice, and mice were randomized to receive either nicotine (0.25 mg/kg) or control treatment when tumors reached the volume of 75 to 125 mm3. Consistent with the findings from the previously study by the same group, at Day 22, tumors from nicotine-treated mice had significantly higher volume (p<0.01), weight (p<0.01), and relative expression of Ki-67 (p<0.05) compared with tumors from control-treated mice. Despite the consistent findings between these two studies, an inconsistency in reporting was noted between the two publications: although tumor volume appeared similar at similar time points in both studies, the scale for the graph in one study had the tumor weight higher by a factor of 10. Authors were contacted for clarification, however, no reply was provided.

In the xenograft study by Hanaki et al.45, human Panc-1 pancreatic ductal adenocarcinoma cells and human BxPC3 pancreatic adenocarcinoma cells were inoculated into the abdominal cavity of mice. Two weeks later, mice were randomized to receive nicotine (1 mg/kg) or control treatment via intraperitoneal injections three times per week for 12 weeks. At the end of the treatment period, the number and the diameter of peritoneal nodules was significantly higher in the nicotine group compared with the control group (p<0.05 for both outcomes).

In the xenograft study by Li et al.15, human HepG2 hepatocellular carcinoma cells were injected into the flank of athymic nude mice. When the tumor volume reached approximately 100 mm3, mice were randomized to receive intraperitoneal injections of nicotine (200 mg/kg) or control treatment five times per week for 4 weeks. At Week 8, nicotine-treated mice had a numerically higher tumor volume compared with control mice, however, statistical comparison of the two groups was not performed. Expression of tumor proliferation marker Ki-67, assessed using polymerase chain reaction, was significantly higher in the nicotine group compared with the control group (p<0.05).

In the study by Trevino et al.24, a L3.6pl cell xenograft, a human cell line metastatic variant of a squamous cell carcinoma of the pancreas, was implanted into the pancreas of SCID mice. One week after xenograft implantation, mice were randomized to receive intraperitoneal injections of either nicotine (1 mg/kg) or control treatment 3 days per week. Nicotine-treated mice had significantly greater tumor growth over 3 weeks (p<0.001), significantly larger tumor volume at 4 weeks (p<0.01), and significantly higher metastasis evaluated using bioluminescence (p<0.001), than control-treated mice.

In the xenograft study conducted by Underwood et al.26, patient-derived PDAC xenografts were injected into the pancreas of non-obese, diabetic SCID-gamma mice 14 days before randomizing the mice to receive either nicotine (1 mg/kg) or control treatment via intraperitoneal injections 3 days per week for 6 weeks. At 8 weeks, the weight of tumors from nicotine-treated mice (3.08 ± 1.07 g) was significantly higher than the weight of tumors from control mice (1.75 ± 1.31 g; p = 0.02)

In the final xenograft study by Delitto et al.10, a 2 x 2 mm section of a surgically resected primary pancreatic adenocarcinoma or a 2 mm core biopsy was implanted subcutaneously into mice (NOD-SCID IL2 receptor gamma chain knockout mice). On postoperative Day 5, mice with visible tumors were randomized by size to receive either nicotine (1 mg/kg) or control treatment via intraperitoneal injections three times per week. On Days 33 to 45, tumor volume was significantly higher in the nicotine-treated group compared with the control group (p<0.05). Pulmonary metastasis, evaluated using hematoxylin and eosin staining, was observed in five of eight (62%) nicotine-treated mice and in zero of eight (0%) control mice.

##### Administration of Nicotine by Gavage

In the allograft study by Natori et al.53, mice received either nicotine (20 mg/kg) or vehicle by gavage and 5 days later, mice CMT93 colon cancer cells were injected subcutaneously into the left flank. At Day 11, tumor volume in the nicotine-treated mice was significantly higher than in the control mice (p<0.01). In a separate experiment, mice CMT93 colon cancer cells were injected into wild-type mice whose bone marrow had been replaced with that of ROSA26 mice, and the mice were treated with vehicle or nicotine (concentration not reported) for 7 days. Capillary density, assessed using CD31 immunostaining and reported collectively for the allograft and bone marrow transplantation experiments, was also significantly higher in the nicotine group (496 ± 29 capillaries/mm3) compared with the control group (269 ± 25 capillaries/mm3; p<0.01).

#### Carcinogen-Induced Cancer Models

##### Oral Administration of Nicotine

Two of the 18 progression studies that evaluated digestive cancers used carcinogen-induced cancer models and administered nicotine orally in drinking water available *ad libitum*29, 54. One study administered 200 µg/mL nicotine29, and one study administered 25 ppm nicotine54. The duration of treatment was 37 weeks in one study54 and 44 weeks in the other study29. Neither of the two studies reported biomarker data.

One study that used a carcinogen-induced model of cancer, also used a xenograft model which is discussed above29. In the carcinogen-induced tumor experiments, 100 µg/mL of 4NQO was administered to mice in drinking water for 16 weeks before mice received 200 µg/mL nicotine in drinking water or drinking water alone. At 44 weeks, the number of tumors per mouse was significantly higher in the nicotine group compared with the control group (p<0.001).

In another study that used a carcinogen-induced model of pancreatic cancer, 10 mg/kg of BOP was administered subcutaneously once per week for 3 weeks before hamsters were assigned to either the 25 ppm nicotine or control group54. At Week 40, there were no statistically significant differences in the incidence (43% in the nicotine group, 39% in the control group) or tumor multiplicity (nicotine 0.6±0.9; control 0.4±0.6) of pancreatic adenocarcinoma between the nicotine and control groups (p values not reported). Regarding the distribution of adenocarcinomas in pancreatic lobes at Week 40, there were no statistically significant differences between the nicotine group and the control group in the number of lesions in the splenic lobe, gastric lobe, duodenal lobe, or pancreatic head (p values not reported).

##### Subcutaneous Administration of Nicotine

Two carcinogen-induced cancer model studies administered nicotine via subcutaneous injections6, 47. Neither study reported data on biomarkers of nicotine exposure6, 47. In the study by Bersch et al.6, 7,12-dimethylbenzanthracene (DMBA) crystals were implanted into the pancreatic head of CF1 mice. Mice were randomized to receive either subcutaneous injections of nicotine (2 mg/kg) twice per day for 45 days or control treatment (details of control treatment were not provided). Occurrence of adenocarcinoma was significantly higher in the nicotine group (14 of 27 mice; 52%) compared with the control group (4 of 24 mice, 17%; p<0.001). Although timing of the assessment was not specified, it was assumed that it was performed at the end of the study.

The second study used a colitis-associated tumor model and also administered nicotine via subcutaneous injections47. Colitis-associated cancer was induced by administering 12 mg/kg azoxymethane via an intraperitoneal injection, and 5 days later, administering 2% dextran sulfate sodium (DSS) in drinking water for 5 days. Mice received either nicotine (3 mg/kg) during DSS treatment or control treatment. At Day 90, the number of tumors per mouse was significantly lower in the nicotine group (8.8 ± 1.1) compared with the control group (17.3±2.0; p<0.05). Additionally, tumor size at Day 90 was reduced in nicotine-treated mice compared with control mice, although statistical analysis was not reported. There were no apparent differences between treatment groups in the incidence of adenocarcinomas in the distal colon, although statistical analysis was not conducted. The authors proposed that the suppressive action of nicotine on colitis-associated colon cancer were mediated by the reduced expression of inflammatory mediators by nicotine47.

#### Genetic Cancer Models

##### Oral Administration of Nicotine

One tumor progression study of digestive cancer used a genetic model of cancers, in addition to using an allograft cancer model which is described above12. The study used the KPC mouse model of pancreatic cancer, which reproduces many of the key features of the immune microenvironment observed in human pancreatic duct adenocarcinomas. Mice were randomized to receive either nicotine (100 µg/mL) in drinking water or drinking water alone for 2 weeks. Urine cotinine levels were reported graphically and were approximately 200 ng/mL. At the end of the treatment period, the number of circulating pancreatic cells was reported as being significantly higher in the nicotine group compared with the control group, however, the p value was not provided.

### Head and Neck Cancer

Of the 54 tumor progression studies, six studies evaluated head and neck cancers8, 28, 30, 41, 48, 58. Of these five studies, four were RCTs8, 28, 30, 41, and two were controlled, parallel group studies that did not specify whether animals were randomized to study groups48, 58. Four studies used mice8, 28, 30, 41, 48, 58, and one study used hamsters41. None of the studies evaluated biomarkers of nicotine exposure. Overall, these studies showed that nicotine-treated mice had significantly higher tumor volumes8, 48, 58 and weights8 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 group28, 41. Two studies that assessed metastasis outcomes reported conflicting results: a study that used a xenograft model of head and neck squamous cell carcinoma showed no statistically significant differences between the nicotine group and the control group in the incidence of lymph node metastasis58, whereas, a study that used a xenograft model of OSCC showed that the rate of metastasis was significantly higher in the nicotine group compared with the control group30.

Specific findings of head and neck cancer progression studies are detailed below and grouped by the cancer model.

#### Xenograft/Allograft Cancer Models

##### Intraperitoneal Administration of Nicotine

In two xenograft studies, nicotine was administered via intraperitoneal injections8, 58. In one of the xenograft studies, mice received an injection of human tongue squamous cell carcinoma SAS cells before being randomized to receive either nicotine (1 mg/kg) or control treatment8. At Day 29, tumor volume and tumor weight were significantly higher in the nicotine group compared with the control group (p<0.05 for both outcomes).

In the second xenograft study58, mice received injections of a metastatic variant of a human head and neck squamous cell carcinoma OSC-19 cell line and were randomized to receive either nicotine (30 µg per mouse; dose per body weight not reported) or PBS via intraperitoneal injections for 42 days58. At Day 42, tumor volume was significantly higher in the nicotine group (950.0±188.9 mm3) compared with the control group (615.2±65.3 mm3; p<0.05). Incidence of lymph node metastasis was not statistically significantly different between the nicotine (6 of 10 mice) and the control (1 of 10 mice; q=0.057[[2]](#footnote-2)) groups.

##### Buccal Administration of Nicotine

One xenograft study used a buccal mode of nicotine administration. In the study by Wang et al.30, human oral squamous cell carcinoma (OSCC) cell line CAL27 xenografts were injected into the tongue of mice. Mice were then randomized to receive either nicotine (10 µL applied onto the tongue) or control treatment. Thirteen days after xenograft implantation, the rate of metastasis was significantly higher in the nicotine group compared with the control group (p≤0.05).

##### Unspecified Route of Administration

The mode of nicotine administration was not specified in one xenograft study48. Human OSCC OEC-M1 cells were injected into mice before mice were randomized to receive either nicotine (1.5 mg/kg) or control treatment. At 4 weeks (end of the study), tumor volume was significantly larger in the nicotine group compared with the control group (p<0.05).

#### Carcinogen-Induced Cancer Models

##### Buccal Administration of Nicotine

Two studies used a carcinogen-induced cancer model and administered nicotine buccally28, 41. In one of the studies, 4NQO (50 µg/mL) was applied onto the tongue three times per week for 16 weeks. Mice in the nicotine group also received nicotine (5% solution) with 4NQO. The incidence of OSCC *in situ* was significantly higher in the nicotine group (5 of 20 mice; 40%) compared with the control group (1 of 20 mice, 5%; p<0.05).

In the study by Chen et al.41, cheek pouches were treated three times per week with sesame oil containing 1% DMBA and 6% nicotine (nicotine group) or 1% DMBA alone (control group). After 12 weeks of treatment, the number of tumors, as well as the number of larger tumors (≥3 mm), were significantly higher in the nicotine treated group compared with the control group (p<0.001 for tumor incidence and p<0.05 for tumor size).

### Breast Cancer

Of the 54 tumor progression studies, six studies evaluated breast cancers13, 25, 50, 51, 57, 60. Of these six studies, three were RCTs13, 25, 60, and three studies were controlled, parallel-design studies that did not specify whether animals were randomized to study groups50, 51, 57. Five studies13, 25, 50, 51, 57 used mice and one study used rats60.

Specific findings of breast cancer progression studies are detailed below and grouped by the cancer model.

#### Xenograft/Allograft Cancer Models

##### Oral Administration of Nicotine

In two xenograft/allograft studies, nicotine was administered orally via drinking water51, 57. One study administered 10 mg/mL51, and the other study administered 100 µg/mL57. Treatment duration was 6 weeks in one study 51, and 3 weeks in the other study57. One study reported biomarker data57.

In the xenograft study by Lee et al.51, mice received injections of human MDA-MB-231 breast cancer cell lines before being randomized to receive either nicotine (10 mg/mL) in drinking water or drinking water alone. At Week 6, tumor volume was 2993.2 mm3 in the nicotine group and was not reported for the control group. Tumor weight was significantly higher in the nicotine group (4.38 g) compared with the control group (tumor weight not reported; p=0.027).

In the allograft study by Ross et al.57, mice received injections of mouse 6DT1 breast cancer cells into the fourth mammary fat pad. Seven days later, mice received either nicotine (100 µg/mL) or control treatment. The mean serum nicotine levels during the 4 weeks of nicotine treatment was higher in the nicotine group compared with the control group (nicotine: 13.48 ng/mL; control: 2.28 ng/mL). Likewise, serum cotinine levels during the 4 week course of nicotine treatment was 54.98 ng/mL for the nicotine group and 0 ng/mL for the control group. At Week 2, there was no significant difference in tumor weight between the nicotine and control groups (p value not reported). The number of metastases at 42 to 45 days after allograft implantation was significantly higher in the nicotine group compared with the control group (p=0.0105). Additionally, the number of metastases per gram of tumor weight was significantly higher in the nicotine group compared with the control group (p=0.009).

##### Intraperitoneal Administration of Nicotine

In three xenograft/allograft studies, nicotine was administered via intraperitoneal injections13, 25, 50. One study administered 2 mg/kg25, one study administered 0.75 mg/kg13, and one study administered 0.25 mg/kg50. Treatment duration was 10 days or 28 days in one study25, 10 weeks in the second study13, and 57 days in the third study50. One of the three studies reported biomarker data[35](#_ENREF_35).

In the xenograft study by Jimenez et al.13, mice were randomized to receive either nicotine (0.75 mg/kg) or control treatment via intraperitoneal injections twice daily for 10 weeks. After two weeks, mice received subcutaneous injections of either the human HCC1806 or the HCC70 breast cancer cell line. At Week 8 after cell line inoculation, tumor volume was significantly larger in the nicotine group compared with the control group for both HCC70 (nicotine group: 372±32 mm3; control group: 231±46 mm3; p<0.05) and HCC1806 xenografts (nicotine group: 372±324 mm3; control group: 229±24 mm3;p≤0.05). In addition, tumor weight was significantly larger in the HCC70 xenograft nicotine group (357±24 mg) compared with the control group (305±26 mg; p≤0.01).

In another xenograft study, human MDA-MB-231 breast carcinoma cells were injected into the flank of mice50. Mice were then randomized to receive either nicotine (0.25 mg/kg) or control treatment via intraperitoneal injections (twice weekly) for 57 days. At Day 60, well-established tumors, defined as tumors 100 mm3 in volume, were observed in 10 of 12 nicotine-treated mice and in one of 12 control mice. However, statistical comparison between groups was not conducted. At Day 80, tumor volume and tumor weight were numerically higher in the nicotine group than in the control group, however, the two groups were not compared statistically. Tumor proliferation, assessed as the percentage of Ki-67-positive cells, was significantly higher (22.5%) in the nicotine group compared with the control group (p<0.05).

A study by Tyagi et al.25 used various allograft models to investigate the effects of nicotine exposure. In a spontaneous metastasis model, mouse mammary cancer cell lines 4T1 or E0771 were injected into the mammary fat pads of mice. Mice were then randomized to receive either nicotine (2 mg/kg) or control treatment via intraperitoneal injections every other day for 28 days. Biomarker data were reported graphically (serum cotinine levels were ~90 ng/mL after administration and decreased to ~5-10 ng/mL after ~47 hours). In this model, primary tumor growth was significantly larger in the nicotine group compared with the control group at Day 24 (4T1 cell allograft: p=0.04; E0771 cell allograft: p=0.0393[[3]](#footnote-3)), and at Day 28 (4T1 cell allograft: < 10-fold increase, p=0.0001; E0771 cell allograft: p=0.0001). Accordingly, primary tumor weight was significantly higher in the nicotine group than in the control group in the 4T1 allograft group (p=0.02), but not in the E0771 allograft group (p value NR). Lung metastatic burden evaluated *ex vivo* at Day 28 was significantly greater in the nicotine group than in the control group for both 4T1 (p=0.01) and E0771 (p=0.0349[[4]](#footnote-4)) allograft. Additionally, lung metastasis‑free survival was significantly shorter in the nicotine 4T1 allograft group compared with the control group (p=0.001); metastasis‑free survival was not evaluated in the E0771 allograft group.

In an experimental metastasis model, a separate group of mice received injections of either 4T1 or E0771 cells into the tail vein before being randomized to either nicotine (2 mg/kg) or control treatment via intraperitoneal injections every other day for 28 days25. In animals that received 4T1 cells, primary tumor growth assessed *in vivo* was significantly larger in the nicotine group compared with the control group (<10-fold increase; p=0.0003). The lung metastasis burden assessed *ex vivo* at Day 28 was significantly greater in the nicotine group compared with the control group (>100 fold increase, p=0.0362[[5]](#footnote-5)). Similarly, the lung metastasis burden assessed *in vivo* was significantly higher in the nicotine group compared with the control group at Day 24 (p=0.038) and at Day 28 (p=0.0003). Lastly, the number of lung metastatic nodules at Day 28 was significantly greater in the nicotine group compared with the control group (p<0.0001). Data were not reported for E0771 allografts.

In an experimental premetastatic model, mice received either nicotine (2 mg/kg) or control treatment via intraperitoneal injections every other day for 10 days before receiving injections of 4T1 or E0771 allograft cells into the tail vein25. In both the 4T1 and E0771 allograft groups, lung metastasis assessed *in vivo* was significantly larger in the nicotine groups compared with the control groups at Day 24 (4T1 allograft: p=0.04; E0771 allografts: p=0.026) and Day 28 (4T1 allograft: p=0.01; E0771 allograft: p<0.0001). Additionally, lung metastasis assessed *ex vivo* at Day 28 was significantly greater in nicotine group compared with the control group (4T1 allograft: p=0.01; E0771 allograft: p=0.04).

In a nicotine pre-treatment model (the nicotine abstinence model), mice received either nicotine (2 mg/kg) or control treatment via intraperitoneal injections every other day for 10 days before receiving allograft injections of 4T1 cancer cells25. Cancer cells were injected at either Day 1, Day 15, or Day 30 after nicotine treatment. Lung metastasis assessed *in vivo* was significantly higher when nicotine treatment was stopped one day before the allograft injection compared with 15 days (p=0.033), or 30 days before the allograft injection (p<0.0001). Additionally, lung metastasis assessed by *in vivo* was significantly higher when nicotine treatment was stopped 15 days before the allograft injection of cells compared with 30 days before the allograft injection (p=0.025). When assessed *ex vivo,* lung metastasis was significantly higher when nicotine was stopped one day after the allograft injection compared with 15 days (p=0.046) or 30 days before the allograft injection (p=0.032). There were no statistically significant differences between groups when nicotine treatment was stopped 15 days before the allograft injection of cancer cells compared with 30 days before the allograft injection (p=0.15).

The study noted that serum cotinine levels were comparable to those in adult smokers, however, serum cotinine levels were not specified25.

#### Carcinogen-Induced Cancer Models

One study used a carcinogen-induced model for breast cancer and administered nicotine via subcutaneous injections60. NMU was administered via intravenous injections on postpartum Day 52. Rats were randomized to the nicotine group received nicotine (0.4 mg/kg) via subcutaneous injections twice per week on postpartum Days 55-145; control rats received NMU alone. Biomarker data were not reported. Rats were palpated daily for identification of gross masses twice per week, and were euthanized when the tumor mass led to a significant loss of body weight or necrosis of the adjacent skin. The investigators reported that the time to tumor development was not different between the nicotine and the control groups, however, statistical analysis was not provided. Histological analysis showed that all tumors were of epithelial origin; > 80% were classified as adenocarcinoma; the remaining tumors were adenoma or adenofibromas. Nicotine treatment did not have any influence on the histologic type of NMU-induced tumors.

### Urinary Tract Cancer

Two studies evaluated progression of urinary tract cancers22, 34. Both studies were RCTs. One study was a xenograft study in mice34, and the other study was a carcinogen-induced cancer model study in rats22. One of the two studies reported biomarker data22.

In a xenograft study, Yuge et al.34 injected the human T24 bladder cancer cell line into the flank of mice. Mice were then randomized to receive either nicotine (1 mg/kg) or control treatment via intraperitoneal injections three times per week. At Day 21, tumor volume was significantly higher in the nicotine group (929.1±180.2 mm3) compared with the control group (470.3±73.4 mm3; p=0.039).

In the carcinogen-induced cancer model study by Suzuki et al.22, urothelial carcinoma was induced by administration of 0.05% BBN in drinking water for 4 weeks. Following BBN treatment, rats were randomized to receive either nicotine (10 ppm, 20 ppm, or 40 ppm nicotine) in drinking water or drinking water alone. At Week 36, the incidence of urothelial carcinoma was significantly higher in the 40 ppm nicotine group (9 of 16 rats, 56%) compared with the control group (1 of 15 rats, 7%; p<0.05 vs). The concentration of cotinine in the serum of the 10, 20 and 40 ppm nicotine treated rats in this study was 83.8±5.3 ng/mL, 199.5±10.5 ng/mL, and 347.4±15.9 ng/mL, respectively. There were no statistically significant differences in the incidence of urothelial carcinoma between the 10 ppm nicotine (4 of 15 rats, 27%) or the 20 ppm nicotine (6 of 15 rats, 40%), and the control group (p value not provided). The number of urothelial carcinomas at Week 36 was significantly higher in the 40 ppm nicotine group (0.6 ± 0.2) compared with the control group (0.1 ± 0.1; p<0.05), with no statistically significant differences between the 10 ppm nicotine (0.3 ± 0.2) or 20 ppm nicotine (0.5 ± 0.2) groups, and the control group (p values not provided). Tumor proliferation, assessed as relative expression of Ki-67, was significantly higher in all nicotine groups (i.e., 10 ppm, 20 ppm , or 40 ppm nicotine groups) than in the control group (p<0.001 for each nicotine group).

### Metastatic Melanoma

One controlled, parallel-design study evaluated metastatic melanoma46. In this study, luciferase-expressing B16 melanoma cells were injected into mice, and mice received either nicotine (100 mg/mL) or control treatment (PBS) via an osmotic pump. The study reported that mice in the nicotine group had significantly greater tumor burden on Day 21 compared with control mice, however, the p value was not reported. Additionally, tumor volume was significantly higher in mice treated with nicotine than in control mice (p<0.05), although the time point of assessment was not provided.

### Undefined Cancer Sites

Of the 54 tumor progression studies, three studies evaluated progression of other cancers, or cancers at undefined or multiple sites5, 18, 56. None of these studies evaluated biomarkers of nicotine exposure. One of these studies was an allograft cancer model study in mice18, one study was a carcinogen-induced cancer model study in rats5, and one study was a genetic cancer model study in mice56.

#### Xenograft/Allograft Cancer Models

##### Intraperitoneal Administration of Nicotine

One allograft study that evaluated tumor progression in undefined tumors administered nicotine via intraperitoneal injections18. Mouse MCA sarcoma cells were injected into the right flank of Fischer rats. Rats were randomized to receive either nicotine (200 mg/kg) in 500 µL saline or saline alone via intraperitoneal injections on Days 8, 9, 10, 15, 16, and 17 after allograft cancer cell inoculations. Biomarker data were not reported. On Day 19, there were no statistically significant differences in tumor burden between the nicotine group (59.5±16.0 g) and the control group (45.6±6.6 g) (p value reported as non-significant).

#### Carcinogen-Induced Cancer Models

##### Subcutaneous Administration of Nicotine

One carcinogen-induced cancer model study evaluated tumor progression in undefined tumor types and administered nicotine subcutaneously5. Tumors were induced by the administration of 30 mg/kg NMU by gavage to pregnant rats on gestational Day 20. One group of dams also received 0.4 mg/kg nicotine via subcutaneous injections on gestational Days 14 to 20 (prenatal nicotine group). Another study group also received 0.4 mg/kg nicotine subcutaneously on postpartum Days 1 to 20, and the offspring received 0.4 mg/kg nicotine via subcutaneous injections on postpartum Weeks 4 to 26 (prenatal + postnatal nicotine group). Biomarker data were not reported. Incidence of benign and malignant tumors was assessed in the offspring. The control groups consisted of offspring from untreated dams that only received 30 mg/kg NMU on gestational Day 20. There were no statistically significant differences in the incidence of benign tumors between the prenatal nicotine or the prenatal + postnatal nicotine groups and the control group. The incidence of malignant nervous system tumors was significantly lower in the prenatal + postnatal nicotine group than in the control group in male offspring (p=0.0015 vs. full control group, p=0.0008 vs. males in the control group) and female offspring (p=0.0015). However, there were no differences in the incidence of other malignant tumor types (i.e., mammary gland and kidney) between the two nicotine groups and the control group. The authors noted that there was no obvious explanation for the lower incidence of nervous system tumors in the nicotine group compared with the control group.

#### Genetic Cancer Models

##### Oral Administration of Nicotine

The study by Prueitt et al. 56, used a genetic cancer model in TRAMP mice, who develop progressive forms of prostate cancer with distant site metastases. Mice were assigned to receive either 100 µg/mL nicotine, 250 µg/mL nicotine in drinking water or drinking water alone. At 80 days, there were no statistically significant differences in urogenital tract weight without seminal vesicles – an indicator of tumor size – between the 100 µg/mL nicotine (513±195 mg), 250 µg/mL nicotine (532±462 mg), and control (592±231 mg) groups (p value not reported). Additionally, there were no differences between the tree groups in the incidence of adenocarcinoma with metastasis to the lung (100 µg/mL nicotine: 1 of 22 [5%]; 250 µg/mL nicotine: 2 of 23 [9%]; control: 0 of 20 [0%;] p value not reported), neuroendocrine carcinoma with metastasis to the lung (100 µg/mL nicotine: 5 of 22 [23%]; 250 µg/mL nicotine: 5 of 22 [22%]; control: 0 of 20 [0%] p value not reported), or lymph node metastasis (100 µg/mL nicotine: 2 of 7 [29%]; 250 µg/mL nicotine: 1 of 3 [33%]; control: 0 of 3 [0%]; p value not reported). However, there was a trend of significantly higher incidence of lung metastasis in the 250 µg/mL nicotine group (7 of 23 [30%]) compared with control group (0 of 20, 0%; p=0.046, Fisher exact test for trend).

## Mouse Models, Cell Lines, and Carcinogens Used in Included Studies

### Description of Mouse Models Used in Included Studies

|  |  |
| --- | --- |
| **Mouse model** | **Description** |
| A/J mice | A/J inbred mice are widely used to model cancer and for carcinogen testing given their high susceptibility to carcinogen-induced tumors 64. |
| AB6F1 | AB6F1 mice are the F1 progeny of mating A/J and C57BL/6 mice 17 |
| Athymic BALB/c nu/nu mice | This immunodeficient nude mouse originated from NIH and has a BALB/c genetic strain background. The animal lacks a thymus, is unable to produce T-cells and is therefore immunodeficient 87. |
| Athymic nude mice  (includes NU Foxn1nu athymic mice) | This immunodeficient nude mouse model is maintained as an outbred mouse and is not associated with any stock or strain. The animal lacks a thymus and is unable to produce T-cells67. |
| Swiss albino mice | The strain was created from 2 outbred albino males and 7 outbred albino females. These mice are widely used in biomedical research 65. |
| Athymic Swiss mice | These mice resulted after a mutation had occurred in a colony of albino outbred mice. These nude mice originate from the Swiss strain and lack the thymus. They are hairless, and used to study tumor biology and xenograft research 77. |
| Nude mice | Nude mice were the first immunocompromised mouse strain to be used in cancer research. These mice lack a normal immune system and the thymus, and have a repressed immune system due to reduced number of T cells. Nude mice are ideal for tumor and tissue studies because they have no rejection responses and are hairless, making it easier to identify tumors70. |
| BALB/c | A commonly used wild-type inbred mice71. |
| C57BL/6 | This is the most widely used wild-type inbred strain. These mice are refractory to many tumors, but provide a permissive background for maximal expression of most mutations66. |
| BALB/c nude mice | This mouse is inbred, and genetic monitoring results confirm it to be a BALB/c nude mouse. The animal lacks a thymus, is unable to produce T-cells, and is therefore immunodeficient 68. |
| BALB/cAJcl-nu/nu athymic mice | BALB/cAJcl (also known as BALB/cAJc1 nude mice) is a sub-strain lineage from BALB/c. BALB/cAJcl-nu/nu athymic mice. These mice are athymic, hairless, and lack T-cell function72. |
| FVB/NJ mice | A widely used multipurpose inbred strain. FVB/NJ mice are homozygous for the retinal degeneration 1 allele of Pde6brd1, resulting in blindness by wean age 81. |
| CF1 mice | This strain is white (albino) and carries brown behind its albino gene. It is a general multipurpose mouse model 69. |
| SCID mice | SCID mice have a genetic immune deficiency that affects their B and T cells. Due to the lack of mature B and T lymphocytes, these mouse models are commonly used for xeno-engraftment of human cells and tissue 76. |
| NOD-SCID mice | The SCID mutation has been transferred onto a NOD background. Animals homozygous for the SCID mutation have impaired T and B cell lymphocyte development. The NOD background additionally results in deficient natural killer cell function. These mice are commonly used for studying tumor biology and xenograft research 88. |
| NOD-SCID-gamma mice | Immunodeficient NOD-SCID mice bearing a targeted mutation in the gene encoding the interleukin -2 receptor gamma chain gene (*IL2rγnull*) engraft readily with human peripheral blood mononuclear cells 84. |
| TRAMP mice | These mice express the TRAMP transgene and develop progressive forms of prostate cancer with distant site metastasis. The TRAMP model closely mirrors the pathogenesis of human prostate cancer. Mice with F1 background show earlier onset of the phenotype compared to mice carrying the transgene on the C57BL/6 background. Male TRAMP mice uniformly and spontaneously develop orthotopic prostate tumors following the onset of puberty78-80. |
| Kras | Genetically engineered mouse models of *Kras* mutant model critical aspects of cancer and are widely used for preclinical research. The Kras oncogene is mutated at a high frequency in human cancers including PDAC (95%), colon cancers (50%), and NSCLC (30%)82, 83. |
| KrasLA2/+ | Mice heterozygous for the Kras LA2 allele have a reduced lifespan compared with wildtype controls. All KrasLA2/+ mice develop extensive tumors, most frequently in the lungs, with 100% of animals developing multifocal tumors at one week of age. Tumor multiplicity and size increase with age, ultimately resulting in respiratory distress and death. KrasLA2/+ mice are also prone to thymic lymphoma (30%) and skin papillomas (40%)74, 75. |
| Kras+/LSLG12D;Trp53+/LSLR172H;Pdx-1-Cre (KPC) | KPC mouse model of PDAC reproduces many of the key features of the immune microenvironment observed in human PDAC. This model is the most extensively studied genetic model of PDAC for evaluation of immunotherapy, and has reproduced clinical observations seen in PDAC patients treated with several immune oncology drugs73. |

Abbreviations: NIH = National Institutes of Health; NOD = non-obese diabetic mice; NSCLC= non-small cell lung cancer; PDAC= Pancreatic ductal adenocarcinoma, Pde6brd1= cGMP-specific 3', 5'-cyclic phosphodiesterase subunit beta; SCID = severe combined immune-deficient mice; TRAMP = transgenic adenocarcinoma of mouse prostate.

### Description of Xenograft Cell Lines Used in Included Studies

| **Cell line** | **Description** |
| --- | --- |
| NCI-H322 cell line | Human cell line that was isolated from a primary bronchioalveolar carcinoma of the lung from a 52-year old male prior to treatment. These cells produce tumours in athymic mice89. |
| NCI-H441 cell line | Human cell line that was isolated from the pericardial fluid of a male patient with papillary adenocarcinoma of the lung. This cell line has slightly more than the normal diploid number of chromosomes (hyperdiploid)90. |
| Panc-1 cell line | Human pancreatic ductal adenocarcinoma cell line that exhibits epithelial morphology. It was isolated from the pancreatic duct of a 56-year-old, White, male with epithelioid carcinoma91. |
| A549 cell line | This human bronchiolalveolar carcinoma cell line exhibits epithelial-like morphology and was isolated from the lung tissue of a 58-year-old Caucasian male with NSCLC92. |
| SAS cells | This human cell line was isolated from a 69-year-old female with tongue squamous cell carcinoma93. |
| Line 1 mouse adenocarcinoma cells | Cells that were isolated from subcutaneous tumors in BALB/c mice and can metastasize to the lungs. The ability of primary tumors to suppress the growth of metastases develops slowly in this system, such that the metastases that are shed within the first week of tumor growth survive and ultimately prove lethal to the host9. |
| BxPC3 cells | This human cell line exhibits epithelial morphology, and was isolated from the pancreas of a 61-year-old, female patient with adenocarcinoma. These cells are tumorigenic in athymic nude mice94. |
| B16-F10-luc2 cell line | Luciferase expressing cell line derived from mouse skin cells. This cell line is used for in vivo bioluminescence imaging of xenograft animal models to study human cancer and to monitor the activity of anti-cancer drugs95. |
| Lewis lung carcinoma cells (also known as LL/2 or LLC1) | This cell line is established from the lung of a C57BL mouse bearing a tumor resulting from an implantation of primary Lewis lung carcinoma. This cell line is widely used as a model for metastasis96. |
| OEC-M1 cells | This human cell line was derived from surgical resection of a primary tumor of a Taiwanese male patient with squamous cell carcinoma. It harbors a missense mutation in the p53 tumor suppressor gene, displays low epidermal growth factor receptor expression, and is tumorigenic in nude mice97. |
| HCC1806 cells | This epithelial human cell line isolated from the mammary gland of a 60-year-old, Black, female patient with acantholytic squamous cell carcinoma, TNM Stage IIB, grade 2 with no lymph node metastasis. These cells are poorly differentiated98. |
| HCC70 cell line | This epithelial human cell line was isolated from a primary ductal carcinoma of a 49-year-old, Black female. The tumor was classified as TNM Stage IIIA, grade 3, invasive ductal carcinoma with metastases in 4 of 17 lymph nodes. These cells are poorly differentiated99. |
| MDA-MB-231 cell line | This epithelial human breast cancer cell line that was established from a pleural effusion of a 51-year-old Caucasian female with a metastatic mammary adenocarcinoma and is one of the most commonly used breast cancer cell lines in cancer and immuno-oncology research100. |
| HepG2 cells | This human cell line exhibits epithelial-like morphology, and was isolated from a hepatocellular carcinoma of a 15-year-old, White, male youth with liver cancer. HepG2 cells are non-tumorigenic cells with high proliferation rates and an epithelial-like morphology101 |
| PC9 cells  (Formerly known as PC-14) | This human cell line is derived from lung adenocarcinoma cells (differentiated type)102. |
| Human umbilical cord mesenchymal stem cells | Mesenchymal stem cells are exist in many tissues of the body, including the umbilical cord. Collection of these cells from the human umbilical cord is convenient and not associated with legal or ethical issues. They can renew themselves continuously and, under certain conditions, differentiate into one or more cell types constituting human tissues and organs103. |
| CL13 cells | This mouse lung adenocarcinoma cell line is derived from lung tumors induced in A/J mice by treatment with NNK104. |
| IO33 cells | This mouse lung adenocarcinoma cell line is derived from lung tumors induced in A/J mice by treatment with NNK104. |
| CL25 cells | This mouse lung adenocarcinoma cell line is derived from lung tumors induced in A/J mice by treatment with NNK104. |
| Mz-ChA-1 cells | This human cholangiocarcinoma cell line was isolated from a 55-year-old female with gallbladder and extrahepatic biliary tract carcinoma. It was derived from the abdominal wall metastatic site105. |
| MCA cells  (Also named MCA205 cell line) | This mouse sarcoma cell line is derived from 3-methylcholanthrene-induced sarcoma in C57BL/6 mice106. |
| CMT93 cells | This mouse colon cancer cell line has been isolated from a 19-month old male mouse (C57BL/1CRF), which had received an i.p. injection of methylazoxymethanol acetate weekly for 18 months. The explant culture is derived from the 4th in vivo passage. 6 million cells as inoculum produce large tumours in nude mice after 1 month107. |
| NCI-N592 cell line | This human cell line was isolated from a 55-year-old male with a SCLC, and is derived from a pleural effusion metastatic site89.  [Note: This cell has been shown to be contaminated and reported to be a NCI-H69 derivative.] |
| 6DT1 cells | The mammary carcinoma cell line is derived from a mouse mammary tumor virus -c-Myc transgenic mouse model108 108. |
| OSC-19 cells | This human cell line was isolated from a 61-year-old male with a tongue squamous cell carcinoma, and is derived from a cervical lymph node metastatic site109. |
| AGS cells | This human gastric adenocarcinoma cell line is moderately differentiated and has slightly more than the normal diploid number of chromosomes (hyperdiploid). It was derived from fragments of a tumor resected from a 54-year-old female patient who had received no prior therapy. AGS is a common model used in gastric cancer-related studies110. |
| L3.6pl cells | This human cell line was isolated from a 77-year-old female with a squamous cell carcinoma of the pancreas, and is derived from a celiac lymph node metastatic site111. |
| 4T1 cells | This mouse tumor-derived cell line serves as an animal model that mimics very closely stage IV human breast cancer based on the tumor growth and metastatic spread of 4T1 cells in BALB/c mice. 4T1-induced tumors can be used as a post-operative model as well as a nonsurgical model because the 4T1-induced tumor metastasizes spontaneously in both models with similar kinetics112. |
| E0771 cells  (also named EO771) | The E0771 mouse mammary cancer cell line was originally isolated from a spontaneous tumour in C57BL/6 mouse. This cell line can be used to analyze genes that regulate metastasis of breast cancer and/or drugs that might reduce spontaneous metastasis in immune competent C57BL/6 mice113. |
| PDAC PDX | PDXs are generated by direct implantation of human tumor tissue into immunocompromised mice. Compared to the patient tumors, PDAC PDX at either the subcutaneous or orthotopic site show a tendency toward enrichment of tumor cells compared to stromal components. Metastases are not observed at either the subcutaneous or orthotopic tumor growth sites, although at orthotopic sites, larger tumors may invade into adjacent organs, such as intestines, spleen and liver114. |
| SMMC-7721 cells | The human hepatoma cell line that was derived from an Asian male patient with hepatocellular carcinom115-117.  [Note: This cell has been shown to be contaminated and reported to be a HeLa derivative. |
| TE-1 cells | The human cell line was derived from a 58-year old male patient with squamous cell carcinoma of the esophagus118. |
| CAL27 cells | This human cell line was isolated from a 56-year-old, White male with a lesion in the middle of the tongue prior to treatment. These cells are epithelial, polygonal and tumorigenic with solid tumors developing within 6 weeks in nude mice inoculated with 2 million cells subcutaneously119. |
| H460 cells | The human H460 cell line was isolated from lung tissue120. |
| HT-29 cells | This human cell line with epithelial morphology was isolated from a primary tumor obtained from a 44-year-old, White, female patient with colorectal adenocarcinoma. This cell line is tumorigenic in nude mice and forms well differentiated adenocarcinoma consistent with colonic primary (grade I) tumors121. |
| Luciferase-labelled LL/2 cells (LL/2-Luc2) | This mouse lung cancerreporter-labeled cell line has stable luciferase expression, and is used for *in vivo* bioluminescence imaging of xenograft animal models122. |
| Luciferase-labelled H2030BrM cells | This human cell line exhibits epithelial morphology, and was isolated from the lungs of a non-smoking male with NSCLC. As areporter-labeled cell line, it is used for *in vivo* bioluminescence imaging of xenograft animal model to study human lung cancer brain metastatic activity123. |
| T24 cells | This human cell line isolated from an 81-year old female is established from a urinary bladder cancer patient, exhibiting high-grade and invasive transitional cell carcinoma124. |
| H1299 cells | This human NSCLC [cell line](https://en.wikipedia.org/wiki/Non-small_cell_lung_carcinoma) is derived from a metastatic lymph node in from a 43-year old male patient who had received prior radiation therapy125. |

Abbreviations: i.p. = intraperitoneally; NNK= nitrosamine ketone (4‑ (methylnitrosamino)-1-(3-pyridyl)-1-butanone; NSCLC= non-small cell lung cancer; PDAC= pancreatic ductal adenocarcinoma; PDX = patient-derived xenograft; SCLC= small cell lung cancer; TNM=tumor, lymph nodes, metastasis (cancer staging).

### Description of Carcinogens Used to Induce Tumors in Included Studies

|  |  |
| --- | --- |
| **Carcinogen** | **Description** |
| 4NQO | 4NQO is a water-soluble carcinogen that produces squamous cell carcinoma in rodents. The 4NQO mouse model specifically mimics the stepwise progression observed in OSCC patients126, 127. |
| Azoxymethane/DSS | AOM/DSS is the most common model used to induce and study inflammation-associated colorectal carcinogenesis128. |
| BBN | BBN is the most-used urothelial chemical carcinogen. BBN belongs to the nitrosamine family, a wide group of alkylating agents that are able to induce bladder tumours in laboratory animals129. |
| BOP | BOP is a reagent commonly used in the synthesis of peptides. BOP is a known pancreatic carcinogen that was shown to induce only a few neoplasms of the lung, liver, and kidney and none in the nasal cavity, larynx, and trachea. Hence, BOP constitutes a specific model for pancreatic carcinogenesis studies130, 131. |
| DMBA | DMBA, a polycyclic aromatic hydrocarbon is a widely studied model carcinogen for the induction of tumors in rodents132. |
| NMU | NMU is a DNA [alkylating agent](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/alkylating-agent) that causes severe damage to hematopoietic, lymphoid, and other tissues that have rapid rates of cell turnover. It produces tumors at various sites, including the nervous tissue, stomach, esophagus, pancreas, respiratory tract, intestine, lymphoreticular tissues, skin, and kidney. It induces benign and malignant tumors following its administration by different routes, including [ingestion](https://www.sciencedirect.com/topics/medicine-and-dentistry/ingestion). It is classified as a group 2A carcinogen by the IARC (i.e., probably carcinogenic to humans)133. |
| NNK | NNK is a tumor-promoting, nicotine-derived, tobacco-specific nitrosamine ketone resulting from tobacco pyrolysis (burning). It is classified as a group 1 carcinogen by the IARC (i.e., carcinogenic to humans)134. |

Abbreviations: 4NQO = 4-nitroquinoline 1-oxide; BBN = N‑butyl-N-(4-hydroxybutyl) nitrosamine; BOP = N‑nitrosobis(2-oxopropyl)amine; DMBA = 7,12 dimethylbenzanthracene, DNA = deoxyribonucleic acid; DSS = azoxymethane/dextran sulfate sodium; IARC = International Agency for Research on Cancer; NMU = N-methylnitrosourea; NNK = 4‑(methylnitrosamino)-1-(3‑pyridyl)-1-butanone (nitrosamine ketone); OSCC = oral squamous cell carcinoma.

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1. Laboratory rats are weaned at postpartum Day 21, reach adolescence on postpartum Day 35, and young adulthood around Day 63 (McCutcheon et al. Eur J Neurosci. 2009; 29:997). Laboratory mice are weaned at postpartum Day 28, reach puberty on Day 42, and adulthood at Day 70 (Dutta and Sengupta 2016; Life Sci 152:244). Golden Syrian hamsters are weaned at postpartum Day 21, attain puberty at postpartum Day 42, and adulthood at 6 months (Dutta and Senbupta 2019; Biomed Pharmacol J 12). [↑](#footnote-ref-1)
2. q values were calculated with the false discovery rate method controlled by the Benjamini-Hochberg procedure, q<0.05 considered to be significant [↑](#footnote-ref-2)
3. Graph indicated a significant difference on Day 24, p value of 0.393 was reported, but there appears to be a typo and should be 0.0393 [↑](#footnote-ref-3)
4. Graph indicated a significant difference on Day 28, p value of 0.349 was reported, but there appears to be a typo and should be 0.0349 [↑](#footnote-ref-4)
5. Graph indicated a significant difference on Day 28, p value of 0.362 was reported, but there appears to be a typo and should be 0.0362. [↑](#footnote-ref-5)