

## Minireviews

# The Influence of Nicotine on Lung Tumor Growth, Cancer Chemotherapy, and Chemotherapy-Induced Peripheral Neuropathy

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### ABSTRACT

Studies in animal models have suggested that nicotine, an agonist of nicotinic acetylcholine receptors, may have the potential to prevent and/or reverse the peripheral neuropathy induced by cancer chemotherapeutic drugs, such as paclitaxel and oxaliplatin. However, a large body of evidence suggests that nicotine may also stimulate lung tumor growth and/or interfere with the effectiveness of cancer chemotherapy. Whereas the reported proliferative effects of nicotine are highly variable, the antagonism of antitumor drug efficacy is more consistent, although this latter effect has been demonstrated primarily in cell culture studies. In contrast, *in vitro* and *in vivo* studies from our own laboratory

indicate that nicotine fails to enhance the growth of nonsmall cell lung cancer cells or attenuate the effects of chemotherapy (paclitaxel). Given the inconsistencies in the literature, coupled with our own findings, the weight of evidence suggests that caution may be warranted in proposing to use nicotine to mitigate chemotherapy-induced peripheral neuropathy in cancer patients receiving chemotherapy. Conversely, clinical trials could be performed in patients who have completed therapy and are considered to be disease-free to determine whether nicotine, in the form of commercially available patches or gum, is effective in alleviating peripheral neuropathy symptoms.

### Introduction

**Nicotine Action in the Nervous System and in Tumor Cells.** Nicotine is an agonist of the nicotinic acetylcholine receptors (nAChRs), which are pentameric ligand-gated ion channels located on the membranes of various cells in the nervous and immune systems, as well as in lung tumor cells. These receptors can be homomeric, with five subunits of the same type ( $\alpha 7$ ,  $\alpha 9$ ), or heteromeric, with a combination of both  $\alpha$  and  $\beta$  subunits (including  $\alpha 1-7$ ,  $\alpha 9-10$ , and  $\beta 1-4$ ). Binding of an agonist such as nicotine to a nAChR induces a conformational change that allows for the influx of sodium and calcium ions. In neurons, this ion flux results in depolarization of the cell and initiation of an action potential. In tumor cells, both calcium-dependent and calcium-independent downstream signaling pathways of nAChRs appear to be activated; stimulation of these signaling pathways has been reported to contribute to proliferative and antiapoptotic actions of nicotine [see reviews

by Egleton et al. (2008), Improgo et al. (2011), Schaal and Chellappan (2014), and Czyżykowski et al. (2016)].

**Antinociceptive and Analgesic Actions of Nicotine.** Both human and animal studies have demonstrated that nicotine possesses analgesic and antinociceptive properties, respectively. For example, randomized placebo-controlled clinical trials have revealed that nicotine can reduce postoperative pain scores in nonsmokers, as well as decrease morphine consumption (Flood and Daniel, 2004; Habib et al., 2008). In rats, Di Cesare Mannelli et al. (2013) demonstrated that acute administration of nicotine can reverse trauma-induced neuropathic pain as well as oxaliplatin-induced cold and mechanical allodynia, both of which are characteristic of chemotherapy-induced peripheral neuropathy (CIPN). Our laboratory, in collaboration with the Damaj group, has also shown that nicotine can both prevent and reverse paclitaxel-induced mechanical allodynia in mice following chronic and acute administration, respectively (Kyte et al., 2018). These two reports are, to our knowledge, currently the only publications investigating the use of nicotine in CIPN animal models, indicating that there is a need to explore the antiallodynic property of nicotine with other classes of cancer chemotherapy drugs that cause CIPN, such as the vinca alkaloids and bortezomib.

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**ABBREVIATIONS:** CIPN, chemotherapy-induced peripheral neuropathy; nAChR, nicotinic acetylcholine receptor; NRT, nicotine replacement therapy; NSCLC, nonsmall cell lung cancer.

**The Potential Utility of Nicotine for Mitigation of Chemotherapy-Induced Peripheral Neuropathy.** Further investigation of the promising actions of nicotine in suppressing the development of and/or reversing the symptoms of CIPN could be compromised by the extensive body of literature, largely focused on lung cancer, which suggests nicotine can either promote tumor growth and/or reduce the antitumor effects of cancer chemotherapy. If these properties of nicotine translate to the clinic, then its use may be limited to patients who have previously undergone cancer therapy and are currently considered to be disease-free, because CIPN symptoms can persist for over 6 months after cancer chemotherapy administration has been completed (Seretny et al., 2014). Therefore, even patients with cancer in complete remission may still be experiencing neuropathic pain and could benefit from nicotine treatment. If, however, nicotine could also be administered in combination with chemotherapy to *prevent* the development of CIPN in cancer patients, this would potentially provide an even greater patient benefit.

In our recent publication establishing the antinociceptive actions of nicotine in a mouse model of paclitaxel-induced peripheral neuropathy (Kyte et al., 2018), we also reported that nicotine does not stimulate proliferation of nonsmall cell lung cancer (NSCLC) or ovarian cancer cells *in vitro*, nor enhance NSCLC tumor growth *in vivo*. This work also demonstrated that nicotine fails to interfere with the antiproliferative and cytotoxic actions of paclitaxel in NSCLC cells in culture, whereas our recent unpublished studies have reproduced these findings in tumor-bearing mice (manuscript in preparation). These observations are in conflict with a large body of evidence that argues against the use of nicotine within the framework of tumor growth or the utilization of cancer chemotherapy [see reviews by Catassi et al. (2008) and Grando (2014)]. More specifically, nicotine has been shown to be capable of promoting tumor cell proliferation, invasion and metastasis, angiogenesis, and resistance to apoptotic cell death via various signaling pathways. To evaluate the potential utilization of nicotine for the alleviation of CIPN symptoms in cancer patients and/or cancer survivors, this review will summarize the previous literature that investigates the effects of nicotine on lung cancer progression both alone and in combination with antitumor drugs. It should be emphasized that this review is not addressing the potential roles of nicotine and nAChRs in carcinogenesis [see reviews by Dang et al. (2016) and Haussmann and Fariss (2016)], but rather focuses on

the interaction of nicotine with established tumors and its impact on the antitumor properties of cancer chemotherapy.

## Studies in Cell Culture

**Nicotine Alone.** Approximately half of the publications relating to nicotine and lung cancer *in vitro* have reported significant increases in various assays assessing lung cancer cell progression (Tables 2 and 3); the lung cancer type for each cell line used in these studies is indicated in Table 1. However, the experimental systems used are not uniform. Almost half of the *in vitro* experiments were conducted under conditions of serum deprivation or serum starvation with the purpose of eliminating exogenous growth factors and/or inducing quiescence to synchronize the cell cycle. This approach creates an environment where enhanced proliferation induced by nicotine is likely to be more pronounced (Rosner et al., 2013); however, the physiologic relevance may be limited. The majority of serum starvation/deprivation studies show an increase in lung tumor cell viability (viable cell number), proliferation, growth, invasion, and/or migration following nicotine exposure over a wide range of nicotine concentrations (10 nM to 500  $\mu$ M; Table 3). In contrast, a number of studies reported no effects of nicotine (1 pM to 100  $\mu$ M for 48–72 hours) on lung cancer cell viability, growth, or proliferation even under the relatively nonphysiologic condition of serum deprivation (Heeschen et al., 2001; Jarzynka et al., 2006; Mucchietto et al., 2017). In our own studies, nicotine exposure (1  $\mu$ M for 24 hours) under either serum deprivation or serum starvation conditions had essentially no influence on NSCLC cell viability (Kyte et al., 2018).

If the administration of nicotine via nicotine patches or gum could prove to have utility for the prevention or treatment of CIPN, then it is necessary to evaluate the previous literature within the framework of plasma nicotine concentrations in patients using nicotine replacement therapy (NRT). Nicotine patches (21 mg) deliver peak plasma concentrations of 18–23 ng/ml or 111–142 nM nicotine within 8 hours of use, after which the levels gradually decline until the patch is removed at 24 hours postapplication (Fant et al., 2000); 2–4 mg nicotine gum provides maximum nicotine concentrations of 6–17 ng/ml or 37–105 nM after 30 minutes of chewing (Benowitz et al., 1987). Although e-cigarettes are unlikely to be considered for therapeutic use, these devices can generate circulating nicotine concentrations of 7–25 ng/ml or 43–154 nM (Wagener et al., 2017). These values suggest that concentrations of nicotine in cell culture studies between 35 and 200 nM would encompass the range of plasma nicotine levels that

TABLE 1

Lung cancer cell lines grouped by species and lung cancer type

The cell lines indicated as primary were derived from human lung cancer tissue samples and not purchased commercially.

Species	Lung Cancer Type	Lung Cancer Cell Lines
Human	Nonsmall cell lung cancer	A549, H23, H157, H358, H460, H1299, H1703, H1975, H5800, PC9, 11–18
	Small cell lung cancer	DMS-53, H446, N417, N592
	Adenocarcinoma	HCC827, T1 (primary), 201T (primary)
	Bronchoalveolar carcinoma	H1650
	Papillary adenocarcinoma	H441
	Squamous cell carcinoma	SW900
Mouse	Lewis lung carcinoma	LLC
	Adenocarcinoma	LKR, Line1

would be achieved in patients using NRT. However, the majority of studies have tested nicotine concentrations from 100 nM to 1  $\mu$ M, a range that is comparable to or slightly higher than the plasma nicotine levels of 20–60 ng/ml or 100–400 nM observed after tobacco cigarette smoking (Benowitz et al., 2009). Overall, the studies shown in Table 2 demonstrate the capacity of nicotine to increase lung cancer

cell viability, growth, proliferation, invasion, migration, and/or angiogenesis following 30-minute to 2-week exposure to 0.1–1  $\mu$ M nicotine. However, only half of these publications demonstrate significant increases in characteristics of tumor growth, ranging from a 20% to a 750% increase, whereas half of the studies do not demonstrate significant enhancement. When considering nicotine levels achieved during NRT use

TABLE 2  
In vitro effects of nicotine on lung cancer

Lung Cancer Cell Line	Nicotine ( $\mu$ M)	Duration of Treatment	Serum Concentration	Cellular Response (Assay)	Result (Relative to Control)	Reference
14 SCLC and NSCLC lines	0.1–1	5 days	10%	Viability (MTT)	No effect	Maneckjee and Minna (1990)
H460, H157	0.1–1	7 days	10%	Viability (MTT)	No effect	Chen et al. (2002)
201T	1	48 h	10%	Viability (MTS)	No effect	Carlisle et al. (2007)
H460	0.1, 1	5 days	10%	Viability (Cell Titer-Glo)	20%, 25% increase*	Zheng et al. (2007)
A549	1	24 h	10%	Viability (MTT)	20% increase*	Zhang et al. (2009)
A549, H1299	0.1, 1	72 h	Not indicated	Growth ( $^3$ H]-thymidine)	H1299: 20%, 5% increase <sup>†</sup> A549: 10%, 15% increase <sup>†</sup>	Puliyappadamba et al. (2010)
		72 h		Growth ( $^3$ H]-thymidine)	H1299: 15%, 5% increase <sup>†</sup> A549: 20%, 10% increase <sup>†</sup>	
		Previously treated for 72 h, then seeded		Proliferation (colony formation)	A549: 175% increase (1 $\mu$ M) <sup>†</sup>	
H441, H1299	1	30 min or 7 day <sup>a</sup>	10%	Viability (MTT)	100%, 75% increase (30 min),* 375%, 250% increase (7 days)*	Al-Wadei et al. (2012)
H446	0.1–1	12–72 h	10%	Viability (MTT)	8, 5% increase at 12 h (0.1, 0.25 $\mu$ M), <sup>†</sup> no effect at 24–48 h, 8% decrease at 72 h (0.5, 1 $\mu$ M) <sup>†</sup>	Zeng et al. (2012)
A549	1	3–5 days	10%	Viability (MTT)	40%–80% increase*	Wu et al. (2013)
		24 h		Invasion (Boyden)	60% increase*	
A549	0.1, 1	24 h	10%	Viability (MTS)	40%, 55% decrease*	Gao et al. (2016)
LKR, H5800	1	2 wk <sup>b</sup>	10%	Proliferation (colony formation)	13%, 24% increase <sup>†</sup>	Nishioka et al. (2010)
SW900	1	24 h	Not indicated	Proliferation (cell counting)	275% increase*	Chernyavsky et al. (2015)
A549	1	24 h	10%	Invasion (Transwell)	7% increase	Sun and Ma (2015)
		8 or 24 h		Migration (wound healing)	10% increase (8 h), 28% increase (24 h)*	
A549, H460, LLC, T1	0.1–1	24 h	10%	Viability (MTS, MTT)	No effect	Kyte et al. (2018)
A549, H460	1	48–96 h		Viability (MTS, MTT)	No effect	
	1	48 h		Proliferation (cell counting)	No effect	
	1	24 h		Proliferation (colony formation)	No effect	
A549	0.5, 1	16 h	10%	Angiogenesis (HIF-1 $\alpha$ )	350%, 750% increase*	Zhang et al. (2007)
				Angiogenesis (VEGF)	14% increase (0.5 $\mu$ M), 43% increase (1 $\mu$ M)*	
A549, H1299, H1975	0.1, 1	24 h	10%	Viability (MTT)	A549: 39, 52% increase* H1299: 13% increase (0.1 $\mu$ M), 20% increase (1 $\mu$ M)* H1975: 30% increase (0.1 $\mu$ M), 52% increase (1 $\mu$ M)*	Ma et al. (2014)
A549	0.1–1	16 h		Angiogenesis (HIF-1 $\alpha$ )	20%–40% increase (0.1, 0.5 $\mu$ M), 100% increase (1 $\mu$ M)*	
A549	0.1–1	16 h		Angiogenesis (VEGF)	75%, 125% increase (0.1, 0.5 $\mu$ M), 175% increase (1 $\mu$ M)*	

HIF-1 $\alpha$ , hypoxia-inducible factor 1- $\alpha$ ; LLC, Lewis lung carcinoma; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SCLC, small cell lung cancer; T1, primary human lung carcinoma; VEGF, vascular endothelial growth factor.

<sup>a</sup>Nicotine was replaced every 24 h.

<sup>b</sup>Nicotine was replenished every 4 days.

\*Statistically significant.

<sup>†</sup>Statistical significance not indicated.

(35–200 nM), only a third of the studies report significant increases in lung cancer cell viability, proliferation, migration, and/or invasion, with approximately half of these experiments having been performed under conditions of serum deprivation or serum starvation (Tables 2 and 3). When excluding studies performed under serum deprivation/starvation conditions and limiting our analysis to the lower, therapeutically relevant concentrations of nicotine, it may be surmised that the effects of nicotine on lung tumor progression with nicotine patch or gum use are likely to be negligible.

In contrast, approximately 40% of publications testing 0.1–1  $\mu\text{M}$  nicotine under full serum conditions report no effects or modest, nonsignificant effects of nicotine on tumor cell viability, growth, and/or proliferation following 12 hours to 2 weeks of nicotine exposure (Table 2). In addition, studies using nicotine concentrations between 100 nM and 1  $\mu\text{M}$  for 24–72 hours under full serum conditions (Zeng et al., 2012; Gao et al., 2016) have reported that nicotine decreases lung tumor cell viability and growth; these reports also showed decreases in lung cancer cell viability with 2.5–15  $\mu\text{M}$  nicotine. However, the impact of nicotine at higher nonphysiologic and nonpharmacological concentrations is likely the result of off-target effects and general toxicity; ultrastructural analysis of A549 NSCLC cells treated with 10  $\mu\text{M}$  nicotine revealed shrunken nuclei, an increase in both nucleoli and lysosomes, swollen mitochondria, and changes in endoplasmic reticulum morphology after 24 hours (Gao et al., 2016).

**Nicotine in Combination with Cancer Chemotherapy.** Nearly three quarters of cell culture studies assessing the influence of nicotine on sensitivity to chemotherapy in lung cancer cells show significant interference with chemotherapy (Tables 4 and 5). A nicotine-induced resistance to chemotherapy (average of 50% decrease in apoptosis with 1  $\mu\text{M}$  nicotine) has been observed with annexin V–propidium iodide staining, caspase activity, and DNA fragmentation assays (enzyme-linked immunosorbent assay and cell cycle analysis for sub-G1 population), as well as standard viability assays (Table 4). Lung cancer cells exposed to both cancer chemotherapy and nicotine over the range of 0.1–1  $\mu\text{M}$  have been shown to exhibit increased viability and decreased apoptosis, although statistical significance was only reported for about two-thirds of these studies. In contrast, our findings that nicotine (1  $\mu\text{M}$  for 24–48 hours with 10% serum) does not attenuate paclitaxel-induced growth arrest or apoptosis (Kyte et al., 2018) are consistent with studies by other laboratories that have shown a lack of significant effects of nicotine (0.1–1  $\mu\text{M}$  for 1 hour to 1 week with 10% serum) on cisplatin-induced DNA fragmentation (apoptosis) and decreased viability, or on gefitinib-induced decreases in lung cancer cell viability (Carlisle et al., 2007; Nishioka et al., 2010; Zeng et al., 2012; Togashi et al., 2015). Nevertheless, it is apparent that antiapoptotic and prosurvival effects can occur as the concentration of nicotine increases (Table 5). Surprisingly, only one study has been conducted with nicotine in the NRT range, in this case 100 nM nicotine, in combination with chemotherapy (Zeng et al., 2012). This report demonstrated that 100 nM nicotine induces only a modest increase in viability in the presence of 10  $\mu\text{M}$  cisplatin and has no effect on cisplatin-induced apoptosis.

## Studies in Tumor-Bearing Animals

As with the cell culture work, studies regarding the effects of nicotine on lung tumor growth and sensitivity to cancer chemotherapy drugs in tumor-bearing animals vary greatly in their design, given the use of both human and murine lung tumor xenografts, carcinogen-induced tumor development, and oncogene-induced spontaneous tumor formation. Excluding studies of nicotine-exposed lung cancer cell xenografts, where the cells were treated with nicotine *ex vivo* before implantation, approximately two-thirds of the publications show that chronic nicotine administration can significantly increase lung tumor incidence/recurrence, size, weight, and/or metastasis, as well as Ki-67 and angiogenic factor expression *in vivo* (Table 6). One study included the use of 14 mg NicoDerm CQ patches that were cut to represent 0.45 mg or 25 mg/kg nicotine (Davis et al., 2009). These transdermal patches were applied to the lower dorsal region of female immunocompetent tumor-bearing mice daily for 2 weeks during tumor growth. Cotinine, a predominant metabolite of nicotine, was quantified in the urine of these mice (5000 ng/ml) and was shown to be comparable with urine cotinine concentrations in human smokers (1500–8000 ng/ml). Although this animal model well represents cancer patients receiving NRT, the dose of nicotine appears to be higher than what would be expected clinically since nonpregnant women receiving nicotine via a 22 mg patch have been reported to produce 2240 ng cotinine in their urine (Ogburn et al., 1999). In addition, the remaining third of the literature has shown that chronic nicotine administration does not enhance lung tumor incidence, multiplicity, volume, and/or growth (Ki-67<sup>+</sup> population) in mice (Pratesi et al., 1996; Maier et al., 2011; Murphy et al., 2011), as also reported in our own studies (Kyte et al., 2018).

Surprisingly, to our knowledge, only one study has been published involving systemic coadministration of nicotine and cancer chemotherapy *in vivo*. Li et al. (2015) observed significant increases in PC9 human lung adenocarcinoma tumor volume in BALB/c nude mice following administration of erlotinib (100 mg/kg, by mouth) for 10 days in combination with 100  $\mu\text{g}/\text{ml}$  nicotine in the drinking water or given *i.v.* (0.6 mg/kg, 5 $\times$ /week) when compared with erlotinib alone.

Collectively, a possible explanation for these incongruent outcomes with nicotine alone or in combination with chemotherapy relates to differences in the route and duration of nicotine administration. The literature presents studies where nicotine was administered via *s.c.*, intraperitoneal, and *i.v.* injections, as well as *s.c.* minipump infusions, intake via drinking water, and transdermal absorption via nicotine patches, with all lasting anywhere from 6 days to 46 weeks. Although osmotic minipumps allow for steady-state plasma levels of nicotine similar to those achieved in humans either between cigarettes or during NRT (Matta et al., 2007), only a few publications used this technology; another group used a transdermal patch, which releases nicotine in a similar manner as the *s.c.* pump (Davis et al., 2009). Approximately half of the studies were performed with nicotine being ingested via the drinking water, which achieves a similar effect as the minipump, with relatively stable plasma concentrations of nicotine when compared with intermittent injections (Rowell et al., 1983).

TABLE 3  
 In vitro effects of nicotine on lung cancer under nonphysiologic conditions and/or with nonpharmacological concentrations of nicotine

Lung Cancer Cell Line	Nicotine	Duration of Treatment	Serum Concentration	Cellular Response (Assay)	Result (Relative to Control)	Reference
H460, H157	0.01–  1 mM	7 days	10%	Viability (MTT)	H460: 5% increase (10, 100 $\mu$ M), 5% decrease (1 mM) H157: 5% decrease (10 $\mu$ M), 5% increase (0.1–1 mM)	Chen et al. (2002)
201T H460	10 $\mu$ M 10 nM, 0.01–1 mM	48 h 5 days	10% 10%	Viability (MTS) Viability (Cell Titer-Glo)	No effect 12.5%–50% increase (10 nM, 10–100 $\mu$ M),* no effect (1 mM)	Carlisle et al. (2007) Zheng et al. (2007)
A549, H1299	1 nM to 10 mM	72 h	Not indicated	Viability (MTT)	A549: 5%–18% increase (1 nM to 10 $\mu$ M), no effect (100 $\mu$ M), 5%–40% decrease (1–10 mM) <sup>†</sup> H1299: 10%–30% increase (1–100 nM), no effect (1–100 $\mu$ M), 40%–80% decrease (1–10 mM) <sup>†</sup>	Puliyappadamba et al. (2010)
H446 A549	2.5–15 $\mu$ M 0.01, 10 $\mu$ M	12–72 h 24 h	10% 10%	Viability (MTT) Viability (MTS)	0%–85% decrease <sup>†</sup> No effect (0.01 $\mu$ M), 75% decrease (10 $\mu$ M)*	Zeng et al. (2012) Gao et al. (2016)
A549, H1975	10 nM to 100 $\mu$ M	48 h	0% for 72 h, then treated	Viability (MTS)	A549: 12.5% increase (50 nM to 100 $\mu$ M),* H1975: no effect	Mucchietto et al. (2017)
A549	0.5–10 $\mu$ M	72 h	0%	Proliferation (cell counting)	A549: 33%–66% increase,* H1975: no effect	
A549	0.5–10 $\mu$ M	72 h	0%	Growth (BrdU)	0%–9% increase	Jarzynka et al. (2006)
Line1	1 $\mu$ M	18 h	0% for 72 h, then treated	Growth (BrdU)	180% increase <sup>†</sup>	Davis et al. (2009)
LKR	1 $\mu$ M	24 h	0.2% for 24 h, then treated	Growth ( <sup>3</sup> H-thymidine)	200% increase <sup>†</sup>	Nishioka et al. (2010)
A549, H1299	1 nM to 100 $\mu$ M	24 h	Not indicated	Growth ( <sup>3</sup> H-thymidine)	5%–20% increase (1 nM to 1 $\mu$ M), <sup>†</sup> 5%–20% decrease (10–100 $\mu$ M) <sup>†</sup>	Puliyappadamba et al. (2010)
A549	1 $\mu$ M	18 h	0% for 36 h, then treated	Growth (BrdU)	150% increase*	Dasgupta et al. (2011)
A549, H1650	1 $\mu$ M	18 h 24 h	0% for 24 h, then treated	Growth (BrdU) Invasion (Boyden)	175%–180% increase <sup>†</sup> 90%–100% increase <sup>†</sup>	Pillai et al. (2011)
A549, H1650	1 $\mu$ M	18 h 24 h	0% for 24 h, then treated	Growth (BrdU) Invasion (Boyden)	75%, 100% increase <sup>†</sup> 75%, 150% increase <sup>†</sup>	Nair et al. (2014)
LLC	1 pM to 100 $\mu$ M	Not indicated	0.1%	Proliferation (cell counting)	No effect	Heeschen et al. (2001)
H157, H1703	100 nM	3 days <sup>a</sup>	0.1%	Proliferation (cell counting)	50%–95% increase*	Tsurutani et al. (2005)
H1299	10 nM	Previously treated for 72 h, then seeded	Not indicated	Proliferation (colony formation)	150% increase <sup>†</sup>	Puliyappadamba et al. (2010)
A549	0.01–10 $\mu$ M	18 h  24 h	0% (before and during treatment)  0% (during treatment)	Invasion (Boyden)  Migration (wound healing)	10% decrease (10 nM), 50%–160% increase (0.1–1 $\mu$ M), 90% increase (10 $\mu$ M) <sup>†</sup> 10%–100% increase (0.01–1 $\mu$ M), 25% increase (10 $\mu$ M) <sup>†</sup>	Dasgupta et al. (2009)
N417	500 $\mu$ M	Previously treated for 7 days, then seeded	10%  0.5%	Proliferation (colony formation) Migration (Transwell)	130% increase* 55% increase*	Martínez-García et al. (2010)
A549, H1299	0.1–1 $\mu$ M	36 h	0% for 24 h, treated, then seeded	Proliferation (cell counting) Migration (wound healing)	50%–200% increase* 30% increase*	Liu et al. (2015)

(continued)

TABLE 3—Continued

Lung Cancer Cell Line	Nicotine	Duration of Treatment	Serum Concentration	Cellular Response (Assay)	Result (Relative to Control)	Reference
A549, H1650, H1975, H23, H358	1 $\mu$ M	24 h	0% for 36 h, then treated	Invasion (Transwell) Invasion (Boyden)	20% increase* 120%–430% increase*	Pillai et al. (2015)
A549, H1299	1 $\mu$ M, 10 nM	48 h 48 h	0% for 12 h, then treated	Viability (CCK-8) Invasion (Transwell)	25%, 40% increase* 75% increase*	Gong et al. (2014)
A549, H1299	1 $\mu$ M	48 h 72 h		Migration (wound healing)	25%, 30% increase*	
A549, H460, LLC, T1	5, 10 $\mu$ M	24 h	10%	Viability (MTS, MTT)	No effect	Kyte et al. (2018)
A549, H460	1 $\mu$ M	48–96 h	0%–5%	Viability (MTS, MTT)	A549: no effect, H460: 25% increase with 0% serum at 96 h*	
A549	5 $\mu$ M	48 h	10%	Invasion (QCM)	950% increase*	Zhang et al. (2007)
	5, 10 $\mu$ M	16 h		Angiogenesis (HIF-1 $\alpha$ )	1000%, 1100% increase*	
	5, 10 $\mu$ M	16 h		Angiogenesis (VEGF)	130%, 170% increase*	
A549, H1299, H1975	10, 50 $\mu$ M	24 h	10%	Viability (MTT)	A549: 40% increase (10 $\mu$ M),* no effect (50 $\mu$ M) H1299: 13%, 14% increase, H1975: 65% increase (10 $\mu$ M),* 40% increase (50 $\mu$ M)	Ma et al. (2014)
A549	5 $\mu$ M	16 h		Angiogenesis (HIF-1 $\alpha$ )	25% increase	
A549	5 $\mu$ M	16 h		Angiogenesis (VEGF)	150% increase	
A549	5 $\mu$ M	36 h	10%	Invasion (Transwell)	230% increase*	Shi et al. (2015)
		16 h		Angiogenesis (VEGF protein, mRNA)	25% increase,* 700% increase*	
		16 h		Angiogenesis (HIF-1 $\alpha$ mRNA)	100% increase*	

BrdU, bromodeoxyuridine; CCK-8, cell counting kit-8; HIF-1 $\alpha$ , hypoxia-inducible factor 1- $\alpha$ ; LLC, Lewis lung carcinoma; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; QCM, QCM (TM) collagen-based cell invasion assay; T1, primary human lung carcinoma; VEGF, vascular endothelial growth factor.

\*Nicotine was replaced every 24 h.

\*Statistically significant.

†Statistical significance not indicated.

The route of administration could play a role in how the nAChRs are responding to nicotine over time. For example, chronic exposure of nAChRs to nicotine via a s.c. minipump or via drinking water could cause prolonged desensitization of nAChRs, which has been shown to occur in neuroblastoma cells chronically treated with nicotine (Sokolova et al., 2005). In contrast, Sokolova et al. (2005) also showed that acute exposure to nicotine could produce nAChR activation, followed by rapid desensitization and/or reduced responsiveness. After washout and repeat exposure to nicotine, the nAChRs recover sensitivity to nicotine; this response could be occurring during intermittent injections of nicotine. Therefore, it is possible that the duration of tumor exposure to nicotine, which can be influenced by the route of administration, could be contributing to the induction or inhibition of nAChR-mediated signaling.

However, unless the plasma concentration of nicotine is monitored over time, it is difficult to determine how much nicotine the mice are receiving systemically. AlSharari et al. (2013) determined the plasma concentration of nicotine following various dosing regimens in C57BL/6J mice: 0.5–2 mg/kg s.c. twice daily for 10 days (51–163 ng/ml or 314–1005 nM),

2.5–25 mg/kg per day s.c. via 14-day minipump (13–97 ng/ml or 80–598 nM), and 25–100  $\mu$ g/ml by mouth for 10 days (18–27.5 ng/ml or 111–170 nM). Although direct comparisons cannot be made between animals and humans, this study demonstrates that the nicotine concentrations being achieved via s.c. or oral administration in mice, the predominant animal model for cancer and CIPN studies, are similar to that of circulating nicotine levels in humans using NRT and are expected to be predictive of patient response.

### The Complexity of the Problem

It is challenging to determine which specific experimental factors and/or properties of nicotine are responsible for the contradictory observations in the literature. One possibility worthy of consideration involves the initial transient response to nicotine, including the phosphorylation of Akt, a key player in proliferative and antiapoptotic pathways. Jin et al. (2004) demonstrated a peak of Akt phosphorylation at 30–60 minutes postnicotine (1  $\mu$ M) treatment in A549 NSCLC cells that returns to baseline levels at 120 minutes.

TABLE 4  
In vitro effects of nicotine in combination with chemotherapy on lung cancer

Lung Cancer Cell Line	Nicotine	Chemotherapy	Duration of Treatment	Serum Concentration	Cellular Response (Assay)	Result (Relative to Chemotherapy Alone)	Reference
A549	1 $\mu$ M	Cisplatin 40 $\mu$ M	24 h	10%	Apoptosis (annexin V)	30% decrease <sup>†</sup>	Jin et al. (2004)
A549, H157	1 $\mu$ M	Cisplatin 40 $\mu$ M	6%–48 h 24 h	10%	Apoptosis (annexin V)	0%–40% decrease <sup>†</sup>	Xin and Deng (2005)
LKR	1 $\mu$ M	Cisplatin 5 $\mu$ M	Nicotine for 1 h, then cisplatin for 24 h Nicotine for 1 wk, then cisplatin for 24 h	10%	Apoptosis (sub-G1)	20% decrease <sup>†</sup> 5% decrease <sup>†</sup>	Nishioka et al. (2010)
H446	0.1–1 $\mu$ M	Cisplatin 10 $\mu$ M	12–72 h 36 h	10%	Viability (MTT) Apoptosis (AV/PI)	13%–20% increase <sup>†</sup> No effect (0.1–0.5 $\mu$ M), 15% decrease (1 $\mu$ M)*	Zeng et al. (2012)
H5800, LKR	0.5 $\mu$ M	Cisplatin 0.6 $\mu$ M	Nicotine for 24 h, then cotreatment of 48 h	10%	Apoptosis (annexin V)	60% decrease*	Nishioka et al. (2014)
A549	1 $\mu$ M	Cisplatin 20 $\mu$ M	Nicotine for 24 h, then cisplatin for 24 h	10%	Apoptosis (AV/PI)	40% decrease*	Liu et al. (2015)
A549	1 $\mu$ M	Cisplatin 35 $\mu$ M Etoposide 20 $\mu$ M Cisplatin 35 $\mu$ M Etoposide 20 $\mu$ M	Nicotine for 24 h, then cotreatment of 24 h	10%	Viability (MTT) Apoptosis (DNA fragmentation ELISA)	25% increase* 35% increase* 35% decrease* 20% decrease*	Zhang et al. (2009)
H1299	1 $\mu$ M	Cisplatin 40 $\mu$ M Etoposide 40 $\mu$ M	96 h	10%	Apoptosis (annexin V)	40% decrease* 30% decrease*	Zhao et al. (2009)
A549	1 $\mu$ M	Doxorubicin 10 $\mu$ M	Nicotine for 1 h, then cotreatment of 48 h	10%	Viability (XTT) Apoptosis (caspase-Glo 3/7)	25% increase* 300% decrease*	Nakada et al. (2012)
PC9, HCC827	1 $\mu$ M	Erlotinib 1 nM to 5 $\mu$ M	72 h	10%	Viability (MTS)	IC <sub>50</sub> 31 nM → 43 nM (PC9),* IC <sub>50</sub> 46 nM → 140 nM	Li et al. (2015)
201T	1 $\mu$ M	Gefitinib 35 $\mu$ M	48 h	10%	Viability (MTS)	30% increase	Carlisle et al. (2007)
PC9, 11–18	1 $\mu$ M	Gefitinib 5 nM to 50 $\mu$ M	72 h	10%	Viability (MTT)	IC <sub>50</sub> 24 nM → 22 nM, 0.35 $\mu$ M → 0.33 $\mu$ M IC <sub>50</sub> 24 nM → 76 nM,* 0.35 $\mu$ M → 1.09 $\mu$ M*	Togashi et al. (2015)
A549, H460	1 $\mu$ M	Paclitaxel 50 nM Paclitaxel 50 nM Paclitaxel 100 nM Paclitaxel 100 nM	Nicotine for 3 mo, then cotreatment of 72 h Paclitaxel for 24 h, 24-h drug-free, nicotine for 24 h Nicotine for 24 h, then 24-h cotreatment 48 h 48 h	10%	Proliferation (colony formation) Proliferation (cell counting) Apoptosis (AV/PI) Apoptosis (sub-G1)	No effect No effect No effect No effect	Kyte et al. (2018)

AV/PI, annexin V/propidium iodide; ELISA, enzyme-linked immunosorbent assay; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; XTT, 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt.

<sup>†</sup>Statistical significance not indicated.

\*Statistically significant.

Depending on the time of observation postnicotine treatment, it is possible that activation of the phosphatidylinositol 3-kinase/Akt pathway is contributing to a temporary enhancement of proliferation, which dissipates even in the presence of nicotine. In addition, chronic nicotine treatment may be inducing prolonged alterations in nAChR expression. For example, exposure to 100 nM to 10  $\mu$ M nicotine for 96 hours leads to a significant upregulation of  $\alpha 7$  nAChR expression in H520 small cell lung cancer cells (Brown et al.,

2013). Yet it appears that this increased receptor expression does not persist in the absence of nicotine. Studies in human bronchial epithelial cells revealed that 100 nM nicotine significantly increases the expression of genes that encode nAChR subunits, including *CHRNA1*, *CHRNA5*, and *CHRNA7* within 72 hours, but, following removal of nicotine, the expression levels return to baseline at 144 hours (Lam et al., 2007). This observation raises the question of how quickly we might expect to observe similar changes in

TABLE 5

In vitro effects of nicotine in combination with chemotherapy on lung cancer under nonphysiologic conditions and/or with nonpharmacological concentrations of nicotine

Lung Cancer Cell Line	Nicotine	Chemotherapy	Duration of Treatment	Serum Concentration	Cellular Response (Assay)	Result (Relative to Chemotherapy Alone)	Reference
A549	1 $\mu\text{M}$	Cisplatin 20 $\mu\text{M}$	24 h	0% for 36 h, then treated	Apoptosis (TUNEL)	40% decrease*	Dasgupta et al. (2011)
H446	2.5–15 $\mu\text{M}$	Cisplatin 10 $\mu\text{M}$	12–72 h	10%	Viability (MTT)	10%–20% increase (2.5 $\mu\text{M}$ ), 0%–50% decrease (5–15 $\mu\text{M}$ ) <sup>†</sup>	Zeng et al. (2012)
			36 h		Apoptosis (AV/PI)	25%–50% decrease*	
A549, H1299, H23	1 $\mu\text{M}$	Cisplatin 20 $\mu\text{M}$ Gemcitabine 20 $\mu\text{M}$ Paclitaxel 20 $\mu\text{M}$	36 h	0%	Apoptosis (TUNEL)	20%–40% decrease <sup>†</sup> 20%–25% decrease <sup>†</sup> 25%–50% decrease <sup>†</sup>	Dasgupta et al. (2006)
N417	Previous nicotine exposure (500 $\mu\text{M}$ for 7 days)	Cisplatin (5–100 $\mu\text{M}$ ) Etoposide (5–100 $\mu\text{M}$ ) Mitomycin (5–50 $\mu\text{M}$ ) Paclitaxel (5–100 $\mu\text{M}$ )	48 h	10%	Viability (MTT)	50% increase* 50% increase* IC <sub>50</sub> 10 $\mu\text{M}$ → 20 $\mu\text{M}$ * IC <sub>50</sub> 35 $\mu\text{M}$ → 70 $\mu\text{M}$ *	Martínez-García et al. (2010)
201T	10 $\mu\text{M}$	Gefitinib 35 $\mu\text{M}$	48 h	10%	Viability (MTS)	47% increase (10 $\mu\text{M}$ )*	Carlisle et al. (2007)
A549	1 $\mu\text{M}$	Gemcitabine 10 $\mu\text{M}$	36 h	0% for 24 h, then treated	Apoptosis (TUNEL)	20% decrease*	Guo et al. (2013)
H157, H1703	10 $\mu\text{M}$	Paclitaxel 100 nM Etoposide 100 $\mu\text{M}$	48 h	0.1%	Apoptosis (sub-G1)	8% decrease* 15% decrease*	Tsurutani et al. (2005)

AV/PI, annexin V/propidium iodide; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

\*Statistically significant.

<sup>†</sup>Statistical significance not indicated.

nAChR expression in the lung tumors of cancer patients, as well as how the initial nAChR expression profile differs from patient to patient and possibly determines nicotine's predominant effect.

There is also evidence that nicotine can induce both p53 and p21 tumor suppressor proteins, which could be responsible for the lack of enhanced proliferation reported by some research groups. It has previously been shown that nicotine can induce p53 and p21 at concentrations ranging from 1 nM to 1  $\mu\text{M}$  in A549 NSCLC cells (Puliyappadamba et al., 2010). Both of these proteins are induced when the cell is undergoing stress, including the presence of reactive oxygen species, which has been observed in HT-29 colon cancer cells following treatment with 100 nM nicotine (Pelissier-Rota et al., 2015). The cellular response to stress involves upregulation of p21, which inhibits the cyclins that normally allow for retinoblastoma protein (Rb) phosphorylation and subsequent E2F transcription factor-mediated initiation of DNA synthesis and progression through the cell cycle (Giacinti and Giordano, 2006). Conversely, it has been observed that nicotine can activate E2F via the nAChR- $\beta$ -arrestin-Src-Raf-Rb pathway [see review by Schaal and Chellappan (2014)]. If the p21-mediated antiproliferative pathway is being stimulated by nicotine, then any proliferative signaling induced downstream of the nAChRs could be offset, resulting in little or no stimulation of tumor cell growth.

Another possibility is that the nicotine-mediated activation of the prosurvival and antiapoptotic nAChR downstream

signaling is counterbalanced by inhibition of this same signaling downstream of the  $\alpha 9$  nAChR. It has been known for decades that nicotine can act as an antagonist at the  $\alpha 9$  nAChR, as shown by Elgoyhen et al. (1994), where  $\alpha 9$  nAChR-expressing *Xenopus* oocytes were exposed to increasing concentrations of nicotine in the presence of acetylcholine, which led to a dose-dependent decrease in acetylcholine-evoked currents. It has also been shown in MDA-MB-231 metastatic breast cancer cells that CRISPR-Cas9 knockout of  $\alpha 9$  nAChR expression leads to a significant decrease in both migration and invasion of these cells (Huang et al., 2017). Therefore, the nAChR subtype expression profile in different lung cancer cell lines may play a role in the varying outcomes following nicotine exposure.

## Conclusions

Although the findings pertaining to the effects of nicotine alone on lung tumor cells in culture are somewhat inconclusive, the evidence supporting nicotine-induced chemoresistance in vitro is relatively strong. However, additional studies with nicotine in the low nanomolar range in combination with cancer chemotherapy would provide much-needed clarity. Furthermore, there is a deficiency of data relating to the interaction of nicotine with cancer chemotherapeutic agents in vivo. Therefore, erring on the side of caution, our analysis of the literature suggests that nicotine could be tested safely in patients exhibiting CIPN who have



TABLE 6  
In vivo effects of nicotine on lung cancer

Lung Cancer Model	Mouse Strain	Nicotine Dose, Route of Administration	Duration of Treatment	Tumor Measurement	Result (Relative to Control)	Reference
N592	Nude	20 or 200 $\mu\text{g}/\text{d}$ , s.c. (osmotic minipump)	14 days	Volume	No effect	Pratesi et al. (1996)
N417 (nicotine-treated, 500 $\mu\text{M}$ for 7 days)	Nude	—	—	Volume Growth (Ki-67 <sup>+</sup> )	100% increase* 30% increase	Martínez-García et al. (2010)
DMS-53	Nude	24 mg/kg per day, s.c. (osmotic minipump)	1 mo	Volume	250% increase*	Improgo et al. (2013)
A549	Nude, ovariectomized	200 $\mu\text{g}/\text{ml}$ in drinking water	38 days	Volume Growth (Ki-67 <sup>+</sup> ) Microvascular density	20% increase 300% increase* 80% increase	Jarzynka et al. (2006)
H460	Foxn1 <sup>nu</sup>	60 $\mu\text{g}$ , s.c., every other day	6 or 28 days	Volume Angiogenesis (HIF-1 $\alpha$ )	No effect 75% increase (acute), 1300% increase (chronic)*	Warren et al. (2012)
A549	SCID-Beige	i.p., every other day (dose not indicated)	7 wk	Size (luminescence)	120% increase*	Pillai et al. (2015)
A549	Nude BALB/c	1 $\mu\text{M}$ in drinking water	20 days	Lung metastasis Volume	75% increase <sup>†</sup> 88% increase <sup>†</sup>	Liu et al. (2015)
A549 (nicotine-treated, 5 $\mu\text{M}$ )	Nude BALB/c	—	—	Weight Angiogenesis (hemoglobin)	185% increase* 170% increase*	Shi et al. (2015)
PC9	BALB/cA/Jc1-nu/nu	0.6 mg/kg, i.v., 5 $\times$ /wk or 100 $\mu\text{g}/\text{ml}$ in drinking water, then combination with erlotinib (100 mg/kg, p.o.)	Nicotine for 18 days  Nicotine + Erlotinib for 10 days	Volume	24% and 39% increase for i.v. and p.o., respectively* 200% and 300% increase for i.v. Nic + ER and p.o. Nic + ER, respectively, compared with ER alone*	Li et al. (2015)
Line1	BALB/c	1 mg/kg, i.p., 3 $\times$ /wk	2 wk	Volume Tumor recurrence	225% increase* 200% increase*	Davis et al. (2009)
		25 mg/kg per day via transdermal patch		Lung metastasis Volume	700% increase* 65% increase*	
LLC	C57BL/6J	100 $\mu\text{g}/\text{ml}$ in drinking water	16 days	Lung metastasis Volume	230% increase* 100% increase*	Heeschen et al. (2001)
LLC	C57BL/6	100 $\mu\text{g}/\text{ml}$ in drinking water	14 days	Volume	75% increase*	Nakada et al. (2012)
LLC	C57BL/6J	24 mg/kg per day, s.c. (osmotic minipump)	7 days	Volume	No effect	Kyte et al. (2018)
NNK, i.p.	A/J	1 mg/kg, i.p., 3 $\times$ /wk	4 wk	Area Lung metastasis	135% increase <sup>†</sup> 60% increase*	Davis et al. (2009)
NNK, i.p.	Ab6F1 (A/J $\times$ C57BL/6J)	100 $\mu\text{g}/\text{ml}$ in drinking water	12 wk	Multiplicity Volume Incidence Growth (Ki-67 <sup>+</sup> )	No effect No effect 35% increase No effect	Maier et al. (2011)
NNK, i.p.	A/J	200 $\mu\text{g}/\text{ml}$ in drinking water	2, 44, or 46 wk	Volume Multiplicity Incidence	No effect No effect No effect	Murphy et al. (2011)
NNK, i.p.	A/J	1 mg/kg, i.p., 3 $\times$ per week	10 wk	Incidence Volume	125% increase* 80% increase*	Iskandar et al. (2013)
Spontaneous tumor	Kras <sup>LA2/+</sup> C57BL/6J	100 $\mu\text{g}/\text{ml}$ in drinking water	6 wk	Multiplicity Growth (Ki-67 <sup>+</sup> )	No effect No effect	Maier et al. (2011)

HIF-1 $\alpha$ , hypoxia-inducible factor 1- $\alpha$ ; i.p., intraperitoneal; LLC, Lewis lung carcinoma; NNK, nicotine-derived nitrosamine ketone; p.o., oral.

\*Statistically significant.

<sup>†</sup>Statistical significance not indicated.

completed chemotherapy and are cancer-free by using Food and Drug Administration–approved, commercially available nicotine patches or gum, thereby eliminating the concern for tumor growth promotion or interference with the effectiveness of chemotherapy. Finally, it should be noted that human studies have reported nicotine

replacement therapy as not being a significant predictor of cancer (Murray et al., 2009).

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## Authorship Contributions

Performed data analysis: Kyte.

Wrote or contributed to the writing of the manuscript: Kyte, Gewirtz.

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