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The Noscapine Chronicle: A Pharmaco-Historic Biography of the Opiate Alkaloid Family and its Clinical Applications

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Abstract

Given its manifold potential therapeutic applications and amenability to modification, noscapine is a veritable “Renaissance drug” worthy of commemoration. Perhaps the only facet of noscapine’s profile more astounding than its versatility is its virtual lack of side effects and addictive properties, which distinguishes it from other denizens of *Papaver somniferum*. This review intimately chronicles the rich intellectual and pharmacological history behind the noscapine family of compounds, the length of whose arms was revealed over decades of patient scholarship and experimentation. We discuss the intriguing story of this family of nontoxic alkaloids, from noscapine’s purification from opium at the turn of the 19th century in Paris to the recent torrent of rationally designed analogs with tremendous anticancer potential. In between, noscapine’s unique pharmacology; impact on cellular signaling pathways, the mitotic spindle, and centrosome clustering; use as an antimalarial drug and cough suppressant; and exceptional potential as a treatment for polycystic ovarian syndrome, strokes, and diverse malignancies are catalogued. Seminal experiments involving some of its more promising analogs, such as amino-noscapine, 9-nitronoscapine, 9-bromonoscapine, and reduced bromonoscapine, are also detailed. Finally, the bright future of these oftentimes even more exceptional derivatives is described, rounding out a portrait of a truly remarkable family of compounds.

Keywords

noscapine; noscapinoids; microtubule targeting drugs; antineoplastic drugs; centrosome declustering drugs

1. DISCOVERY OF NOSCAPINE: EXTRACTION OF A PRECIOUS GEM FROM A PLAIN POPPY POD

Many ancient civilizations consumed and smoked opium for euphoric and medicinal purposes. As early as 4000 BC, the poppy plant was refined and utilized by various societies.¹ The poppy grew in popularity, infiltrating India and China, and its use spread

throughout Europe by the 16th century. The Renaissance-era physician Paracelsus extracted morphine from the poppy, which along with opium made its way into pharmacies.² The clinical utility of opium-derived medicines persists today because of many investigators who over the years attempted to extract biologically active constituents from the poppy. One such derivative is the particularly salubrious compound noscapine.³ In 1803, the Parisian pharmacist Jean-François Derosne separated a crystalline substance from opium containing noscapine, although it was likely admixed with morphine meconate. Pierre-Jean Robiquet, a professor at the Paris École de Pharmacie, is nonetheless generally credited with the discovery of noscapine because in 1817 he successfully isolated it from Derosne's salt.⁴ In the late 19th century, opium was used as an antimalarial medicine in India, both for prophylaxis and treatment, until a comprehensive study was published in 1930 debunking its supposed antimalarial properties.⁵ Thus, after enjoying a transient period of fame in India, noscapine was decried as a mostly useless compound. However, the consensus that noscapine was of no medicinal use was equally mistaken, and the broad clinical applications of noscapine were slowly uncovered over the next few decades, as detailed in the timeline in Figure 1. Indeed, noscapine and its manifold derivatives are still revealing their wealth of curative powers today, which we will consider at length later in this review. Herein, we will describe noscapine chemically, then follow it on its journey from the initial discovery of its antitussive properties, to compelling recent evidence of its potential to treat strokes, a variety of cancers, and polycystic ovarian syndrome (PCOS) and its pharmacology, and end with novel noscapine analogs that have been synthesized and their possible medicinal utility.

2. NOSCAPINE CHEMISTRY: A PORTRAIT OF THE PROTAGONIST

Noscapine is a phthalideisoquinoline alkaloid comprising 4–12% of the latex of *Papaver somniferum*, the opium poppy. Its IUPAC name is (3S)-6,7-dimethoxy-3-[(5R)-4-methoxy-6-methyl-7,8-dihydro-5H-[1,3]dioxolo[4,5-g]isoquinolin-5-yl]-3H-2-benzofuran-1-one. In addition to the name “noscapine,” it has also been called Capval, Coscopin, Terbenol, Tusscapine, Narcompren, and Narcosine.⁶ It is a member of the benzyloisoquinoline alkaloids. Its molecular formula is C₂₂H₂₃NO₇ and molecular weight is 413.42052 g/mol. Noscapine is a fine white powder that is odorless, bitter, melts at 176°C, and has a density of 1.395 g/mL. It is insoluble in water; practically insoluble in vegetable oils; slightly soluble in alcohol, ether, NH₄OH, and hot solutions of KOH and NaOH; and soluble in benzene and acetone.⁷ Noscapine HCl is freely water soluble,⁸ which is important for clinical applications.

3. BIOSYNTHESIS OF NOSCAPINE: THE NATURAL ALCHEMY THAT MANUFACTURES A MEDICINAL HERO

The proposed synthesis pathway of noscapine starts from (S)-reticuline and includes (S)-scoulerine, (S)-canadine, and (S)-N-methylcanadine as intermediates.⁹ In their pursuit to fully understand noscapine biosynthesis in the opium poppy, Dang et al. have characterized four sequential enzymes that transform 1-hydroxy-N-methylcanadine to narcotoline hemiacetal. Cytochrome P450 CYP82Y1 was isolated and characterized to catalyze the 1-hydroxylation of (S)-N-methylcanadine to 1-hydroxy-N-methylcanadine as the first committed step in the formation of noscapine in the opium poppy.¹⁰ Another cytochrome

P450, CYP719A21, converts (S)-tetrahydrocolumbamine to (S)-canadine.¹¹ Two cytochromes P450 catalyze hydroxylations at C13 and C8 on the protoberberine scaffold, with the C8 hydroxylation opening a ring and forming an aldehyde moiety. In noscapine biosynthesis in the opium poppy, acetylation at C13 before C8 hydroxylation creates a protective group in noscapine biosynthesis in the opium poppy that is subsequently hydrolyzed by a carboxylesterase, triggering rearrangement to a cyclic hemiacetal.¹² The final enzyme in noscapine biosynthesis was discovered to be a short-chain dehydrogenase/reductase,¹³ designated noscapine synthase, which catalyzes dehydrogenation of narcontinehemiacetal to noscapine and is located in the laticifers in the opium poppy.¹⁴

4. NOSCAPINE COUGHS OUT ITS FIRST MEDICINAL USE: ANTITUSSIVE

Noscapine's antitussive activity was discovered in 1930. Outside the United States, various regulatory bodies have approved the drug to treat cough, although noscapine is not FDA approved for any indication.¹⁵ In addition to being a highly efficacious antitussive, it also lacks significant analgesic, sedative, hypnotic, and euphoric effects, rendering it nonaddictive. This distinguishes it from codeine, which is often a drug of abuse due to its analgesic and addictive properties, which derive from its metabolite codeine-6-glucuronide.¹⁶ The μ -opioid receptor produces the clinical effects associated with opioids.¹⁷ One study found that noscapine lost its antitussive properties dose-dependently when the σ -opioid receptor was antagonized.¹⁸ This finding suggests noscapine may not bind to the μ -opioid receptor like the stronger, more addictive opioids such as morphine and codeine. Noscapine has been especially effective in suppressing angiotensin-converting enzyme inhibitor (ACEI)-induced coughs. Mahmoudian et al. conducted a study that uncovered that noscapine antagonizes bradykinin-mediated constriction of guinea pig ileum. Dry cough has been attributed in part to the accumulation of bradykinin, the degradation of which is inhibited by ACEIs. Bradykinin can stimulate bronchoconstriction and mucous production. Furthermore, activation of bradykinin's B2 receptor initiates histamine release, resulting in inflammation and irritation and thereby stimulating cough. Noscapine has been shown to reduce the histamine-induced contractility of human umbilical cord artery.¹⁹ According to Mooraki et al., patients' severe ACEI-induced cough was essentially eliminated with noscapine. Ninety percent of patients experienced a complete response within 10 days.²⁰ Altogether, noscapine has potent antitussive activity due in part to its actions on bradykinin responses, which, as described below, may additionally render it a useful therapy for strokes.

5. NOSCAPINE AS A NOVEL TREATMENT FOR STROKE: NEW SWING FOR AN OLD SWORD

In a majority of developed countries, stroke is one of the most common causes of death.^{21, 22} Recombinant tissue plasminogen activator (rt-PA), the only FDA-approved drug for ischemic strokes, must be administered within 3 hr of stroke onset. In addition, there are many contraindications (e.g., bleeding diathesis, intracranial or subarachnoid hemorrhage, major surgery or trauma within 3 months). The narrow treatment window and strict exclusion criteria severely limit the patient population that can receive rt-PA, which has created demand for novel stroke treatments. Bradykinin is released from the brain following focal ischemia in mice, and mice lacking bradykinin B2 receptors experience less morbidity

(e.g., smaller infarct volume, decreased cerebral edema, less motor deficit) and improved survival.²³ Noscaphine is a noncompetitive antagonist of bradykinin receptors, and it has been reported to protect some organs against ischemia–reperfusion injury.²⁴ Consequently, a bradykinin antagonist such as noscaphine might mitigate stroke sequelae and decrease mortality. To investigate noscaphine’s potential in this regard, a rat model of brain edema was employed.²¹ Noscaphine was effective in reducing cerebral injury in neonatal rats experiencing hypoxic ischemia. Furthermore, a small clinical study found that oral noscaphine reduced mortality in patients who had signs and symptoms of ischemic stroke for less than 12 hr from 80 to 20%.²¹ Hemorrhaging did not occur, and surviving noscaphine-treated patients experienced improved recovery relative to controls.

6. NOSCAPINE: A NEW ADDITION TO THE ANTICANCER ARSENAL

Noscaphine has been shown to have negligible toxicity and does not suppress humoral or cell-mediated immunity.²⁵ This evidence sparked interest in determining other functions of noscaphine aside from its antitussive and antistroke properties. Many studies have been conducted highlighting its effectiveness as an antineoplastic remedy for a wide range of cancers, and noscaphine is currently in Phase I/II clinical trials. In higher concentrations than those used for suppressing cough, noscaphine induces apoptosis and metaphase arrest in dividing cells.²⁶ Rather than affecting microtubule polymerization, noscaphine was found to alter steady-state dynamics of microtubule assembly, lengthening the time microtubules spent in an attenuated state.²⁷ Zhou et al. found that noscaphine, even at high concentrations, did not alter the tubulin polymer/monomer ratio in HeLa cells. The same study discovered noscaphine’s ability to cause a 30% reduction in the tension across kinetochores, which could activate the spindle checkpoint and block mitotic progression. The research team examined cellular localization patterns of spindle checkpoint proteins Mad2, Bub1, and BubR1 in noscaphine-treated cells, and found that the checkpoint protein levels decreased by 138.0-, 3.7-, and 3.9-fold, respectively, at the kinetochore region when chromosomes aligned.²⁷ When noscaphine was added to HeLa cells, mitotic spindles were abnormal and chromosomal congression at the metaphase plate failed. Some cells also exhibited multiple spindle poles. Cells arrested in mitosis and appeared apoptotic, showing large fragmented nuclei after 48 hr of noscaphine treatment. According to Ye et al., noscaphine elicited this effect in various cell types. Noscaphine’s stoichiometric binding induces a conformational change in tubulin and alters microtubule assembly without affecting the microtubule mass. By altering this assembly, microtubule dynamicity becomes attenuated, activating the spindle assembly checkpoint, which is a cellular surveillance mechanism that monitors the integrity of the mitotic spindle.²⁸ Spindle assembly checkpoint activation triggers mitotic arrest and apoptosis, underlying noscaphine’s anticancer activity.²⁶ In the following sections, research of noscaphine’s potential effectiveness against various types of cancer will be detailed.

A. Noscaphine’s Therapeutic Magic: A Potential Treatment for Thymoma

Tumors of the thymus are exceedingly rare such that they are considered an orphan disease. Nonetheless, they are not uncommon in individuals with myasthenia gravis, about 10–15% of whom are stricken with this malignancy.²⁹ Noscaphine may offer a treatment for thymoma sufferers. In one study, noscaphine injected intraperitoneally or intragastrically decreased

tumor size in mice implanted subcutaneously with E.G7-OVA thymoma cells.³⁰ DNA fragmentation was observed within 8 h of noscapine application, and apoptotic bodies increased 50% in 24 hr. Ye et al. also used TUNEL assay to detect DNA fragmentation in situ. Apoptotic nuclei were identified in both HeLa cells and E.G7-OVA cells. Further research could pave the road to clinical trials of this drug for use in thymoma patients.

B. Noscapine: A Promising Seed of Hope for Ovarian Cancer

Human ovarian carcinoma cells can be resistant to standard chemotherapeutics such as paclitaxel and cisplatin (CIS), and these drugs generally have concerning toxicities and low aqueous solubility. Thus, there is a need for new forms of chemotherapy for ovarian cancer. Beta-tubulin mutations are present in some ovarian carcinoma cells, impairing the interaction between paclitaxel and tubulin and causing cellular resistance to paclitaxel. Zhou et al. showed that noscapine binds to tubulin at a different site than paclitaxel and causes mitotic arrest in paclitaxel-resistant ovarian carcinoma cells.³¹ Noscapine was also shown to reverse tumoral resistance toward vincristine and doxorubicin in OVCAR3 cell lines and to potentiate the effectiveness of vincristine and doxorubicin.³² Along with the activation of c-Jun NH₂-terminal kinases (JNKs), which are implicated in regulating cell proliferation, noscapine can induce apoptosis and diminish the proliferation of ovarian carcinoma cells.³¹ In another study, noscapine was added to human ovarian carcinoma cells for 18 hr or vehicle was added as the control. The mitotic indices in drug-treated 1A9, 1A9PTX10, and 1A9PTX22 cells were 37.6, 35.2, and 35.8%, respectively. The control cells showed typical radial microtubule arrays with most in interphase and only 5.4% (1A9), 5.0% (1A9PTX10), and 5.1% (1A9PTX22) of the cells were in mitosis.³¹ Shen et al. conducted a study investigating the cytotoxic effects of the combination of noscapine and CIS in CIS-resistant human ovarian cancer SKOV3 cells in vitro and in an vivo null mouse xenograft model. Noscapine inhibited proliferation of the SKOV3 and SKOV3/CIS-resistant cells dose-dependently.³³ The experiments by Shen et al. also showed that noscapine (i) increases sensitivity of the drug-resistant cells to CIS, (ii) changes protein and mRNA levels of apoptotic genes (XIAP, survivin, NF- κ B, and proapoptotic caspsase-3), (iii) increases the proportion of drug-resistant cells in the G2/M phase, indicating arrested cells, (iv) inhibits tumor growth in nude mice, (v) increases apoptosis of tumor cells in nude mice, and (vi) regulates protein expression of apoptotic factors in xenografts.³⁴ Another study found that noscapine sensitizes CIS-resistant ovarian cancer cells by inhibiting hypoxia inducible factor-1 alpha (HIF-1 α).³⁵ Thus, noscapine could be a useful treatment to increase sensitivity of paclitaxel- and CIS-resistant ovarian cancer.

C. Noscapine Has the Nerve to Stand Up to Glioblastoma

One cancer that is notoriously difficult to treat is glioblastoma since it is challenging to find therapeutic agents that can cross the blood–brain barrier without being cytotoxic to the rest of the brain tissue. The current chemotherapeutics poorly penetrate this barrier. Patients with glioblastoma live on average only another 9–12 months because astrocytic tumor growth is so infiltrative that complete surgical resection is difficult. Often, 90% of the tumors recur within 2 cm of the original tumor.³⁶ Importantly, noscapine can cross the blood–brain barrier and inhibit glioblastoma cell proliferation.³⁷ The downside would be the short plasma half-life and rapid elimination, requiring frequent injections for a successive

chemotherapeutic approach. Madan et al. investigated noscapine-bearing solid lipid nanoparticles (Nos-SLN) and poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscapine (Nos-PEG-SLN). Nos-PEG-SLN increased plasma half-life ~11-fold, while Nos-SLN saw a ~5-fold increase and lower IC₅₀ values when compared with noscapine, suggesting a new approach for noscapine administration, requiring further study.³⁷ Noscapine significantly inhibits proliferation of C6 glioma cells. Interestingly, noscapine causes C6 glioma cells to re-enter S phase and accumulate abnormal amounts of DNA, which leads to mitotic arrest and apoptosis.³⁶ When noscapine was administered orally to mice that were implanted with rat C6 glioblastoma tumor cells, tumor volume was reduced significantly.³⁸ Microtubule-targeting agents can cause peripheral neuropathy such as tubulovascular accumulations and axonal degeneration. Looking at the peripheral motor and sensory nerves, Landen et al. found no evidence of any peripheral neuropathy with noscapine. An important finding was that noscapine caused little toxicity to dorsal root ganglia cultures, which was measured by inhibition of neurite outgrowth. The glioblastoma-ridden mice experienced no toxicity in duodenum, spleen, liver, or hematopoietic cells.³⁶ Landen et al. did observe vasodilation of noscapine-treated brain tissue, which could be concerning for glioblastoma patients with already elevated intracranial pressure, although further research is needed to determine whether intracranial pressure indeed rises. Noscapine synergized with diltiazem, a calcium channel-blocking antiarrhythmic drug, in decreasing the proportion of paclitaxel-treated C6 glioma cells in S phase.³⁹ Altogether, noscapine may be a useful and non-neurotoxic treatment for glioblastoma patients.

Cells derived from the extremely lethal cancer glioblastoma multiforme (GBM) have been shown to be vulnerable to treatment with noscapine. GBM carries an alarming 3.3% overall survival rate, with survival lasting only 14.6 months after diagnosis with the best care currently. Conventional chemotherapeutics can include temozolamide (TMZ), *bis*-chloroethylnitrosourea (BCNU), or CIS.⁴⁰ TMZ is the current standard of care but its efficacy has been challenged.⁴¹ GBMs typically recur and then are resistant to the TMZ treatment, further dwindling the survival rate.⁴¹ The need for a new GBM treatment protocol is dire. Noscapine showed its strength as an anticancer drug by inhibiting the growth of TMZ-resistant glioma cells by causing G2/M arrest. When given in combination with the current chemotherapeutics, noscapine increases the antiproliferation effects of TMZ, BCNU, and CIS in U87MG human glioblastoma cells *in vitro*.⁴⁰ Qi et al. also found the combined treatments to greatly enhance apoptosis, and levels of activated caspase-3 and PARP. Jhaveri et al. discovered noscapine's ability to decrease endothelial cell migration in the brain by blocking the migration of IL-8-activated endothelial cells. Noscapine can also decrease the migration and invasion of glioma cells while decreasing endothelial cell migration; noscapine thus strikingly increases the survival of the animals afflicted with TMZ-resistant GBM. However, even at the highest experimental dose, noscapine had little effect on the survival and proliferation of normal or glioma-associated brain endothelial cells.⁴¹ One study found an antagonistic combination involving noscapine with the T98G human glioblastoma cell line. When administered alongside imatinib mesylate (IM), a tyrosine kinase inhibitor, noscapine could not achieve as strong a therapeutic effect as when administered alone, and levels of midkine, an antiapoptotic protein, rose.⁴² Thus, noscapine's effects may depend to an extent on tyrosine kinases in glioblastoma cells.

Importantly, noscapine improves radiation sensitivity of gliomas, causing significant growth delay and a decrease in tumor vessel density and tubule formation; so this drug may prove especially useful in glioblastoma patients who are candidates for radiation therapy.⁴³

D. Noscapine Launches a Big Attack on Non-small Cell Lung Cancer

Because of the poor clinical results with current treatments, noscapine has also been studied for its antitumor activity in non-small cell lung cancer. After Jackson et al. saw positive results with noscapine in vitro, they designed an in vivo model. With doses ranging from 300 to 550 mg/kg/day, 49–86% reductions were seen in the xenografted H460 tumor volumes. After a 28-day study, 49, 65, 86, and 93% reductions were seen in the tumor volumes. The noscapine-dependent tumor growth suppression was accompanied by upregulation of PARP, Bax, and caspase-3 and repression of Bcl2 expression. These increased protein expressions are known to be proapoptotic, indicating noscapine's role in inducing apoptosis in these cancerous cells. The involvement of mitochondria-mediated apoptosis is suggested by the increased Bax/Bcl2 ratio, increased chromatin condensation and fragmentation, upregulation of cytochrome c, downregulation of survivin, and the activation of caspase-3 and caspase-9.⁴⁴ Research has indicated, in human neuroblastoma cells having wild-type or null p53, noscapine's role in suppressing survivin as a critical mediator of noscapine-induced apoptotic cell death with activation of caspase-3 and cleavage of PARP.⁴⁵ Evidence also suggests that noscapine enhances CIS's anticancer activity synergistically. It was shown that together noscapine and CIS increased p53, caspase-3, cleaved caspase-3, p21 Waf1/Cip1, cleaved PARP, and Bax in non-small cell lung cancer cell lines and xenografted tumor lysates. The two drugs also decreased PARP, Bcl-2, and survivin greater than either one used alone.⁴⁶ Chougule et al., in another study, showed noscapine potentiating the anticancer activity of gemcitabine in an additive to synergistic way when administered together against non-small cell lung cancer via antiangiogenic and apoptotic pathways.⁴⁷ Madan et al. synthesized and evaluated sterically stabilized gelatin microassemblies (SSGMS) of noscapine for targeting human non-small cell lung cancer A549 cells. In vitro, the SSGMS correlated with a lower IC₅₀ value than GMS and free noscapine. This study provides evidence that SSGMS could be better for noscapine delivery in lung cancer.⁴⁸ Therefore, noscapine may represent a useful chemotherapeutic for non-small cell lung cancer patients.

E. Noscapine May Help Combat Gastric Cancer

One of the most common cancers worldwide is gastric cancer. It occurs more in Eastern Asia, Eastern Europe, and the Andean regions of South America, with an average of 9 million cases reported worldwide annually. The issue with gastric cancer is that it has a high rate of recurrence post-treatment and, therefore, new treatment options that carry higher efficacy through different mechanisms of action are greatly needed.⁴⁹ Since noscapine has been shown to have potent anticancer properties by multiple mechanisms, it was studied for its effectiveness in gastric cancer. Liu et al. showed that gastric cancer cells underwent apoptosis upon administration of noscapine. Four cell lines (BGC823, SGC7901, MGC803, and HGC27) were tested. Via the colorimetric MTT assay, cell viability was shown to greatly decrease after treatment with noscapine. Treatment with noscapine greatly diminished the number of viable BGC823 cells. Mice were chosen with tumor xenografts

approximately 100 mm³ and were divided in three groups. Each group was administered noscapine intravenously every 3 days, which resulted in formation of smaller tumors than in controls. Noscapine upregulated Bax and cytochrome c and downregulated Bcl2 protein, thereby increasing the Bax/Bcl2 ratio. Noscapine also activated capases-3 and 9, suggesting that apoptosis was aided by mitochondrial pathways.⁴⁹ Gastric cancer patients may benefit from noscapine therapy based on these promising preclinical findings.

F. Noscapine Shows Potential in Eliminating Colon Cancer

Colorectal cancer is considered relatively resistant to current chemotherapeutic treatments.⁵⁰ Aneja et al. found that the sensitivity of human colon cancer cells (HCT116) to noscapine and the extent of noscapine-induced G2/M arrest and apoptosis depend on the p53/p21 status. Multiple types of HCT116 cells (p53-wt, p53-null, p21-null, and BAX-null) were examined with p53-wt cells appearing most sensitive to noscapine and its antiproliferative effects. P53-null were the most resistant cells. Treatment with noscapine altered cell cycle and apoptosis regulatory protein levels (p53, BAX, Bcl-2, p21, and cyclin B1). Interestingly, if p53 is reintroduced into the p53-null cells, noscapine-induced apoptosis is restored, supporting the hypothesis of p53-dependence. Even with high levels of p53, p21-null cells remained resistant to apoptosis which (i) suggests a proapoptotic role for p21 and (ii) indicates that p53 is necessary but not sufficient for noscapine-mediated apoptosis.⁵⁰ Additionally, noscapine can induce mitochondria-mediated apoptosis in human LoVo colon cancer cells. Noscapine inhibited proliferation of LoVo cells and inhibited tumor growth via apoptosis through mitochondrial pathways in a xenograft tumor model in mice.⁵¹ These results warrant further investigation of noscapine as a possible therapeutic agent to treat colon cancer.

G. Noscapine Could Provide Firepower in the War against Prostate Cancer

A major cancer affecting unsuspecting men is prostate cancer, and metastatic prostate cancer remains largely untreatable even though early detection, diagnosis, and understanding of this cancer have increased over the years.⁵² Currently, the prevalent antimicrotubule therapeutic approach includes docetaxel being administered at its maximum-tolerated dose, which can still bring debilitating toxicities such as myelosuppression, immunosuppression, gastrointestinal toxicity, and peripheral neuropathy.⁵³ Therefore, the search for a novel chemotherapeutic agent to effectively battle metastatic prostate cancer is underway. Noscapine was shown to reduce tumor volume in prostate cancers in mice. Over the course of 2 months, Barken et al. found significantly diminished tumor weights, including the primary tumors and metastatic tumors, compared to the control mice. The incidence of metastasis was also lower in a group of noscapine-treated animals with prostate cancer. The animals were given 300 mg/kg noscapine orally every day. The control group saw 90% incidence of metastasis, while the treated group's metastasis was only 30%. The effectiveness seen in the treated group suggests that noscapine may reduce or stall the metastasis of prostate cancer to the lymph nodes. Contrastingly, no significant difference in metastasis to the lung was seen between the control and treated groups,⁵² so further study is needed to determine whether this drug can improve metastasis-free survival in humans.

H. Noscaphine Can Take Up the Battle against Breast Cancer

Noscaphine has shown great promise against breast cancer cells in vitro and in vivo. It was shown to inhibit the growth of murine and human breast tumors implanted into mice by inducing apoptosis.²⁶ In vitro proliferation assays were used to test noscaphine's ability to inhibit growth of human MCF-7 breast cancer cells, which are hormone receptor positive. In vivo, noscaphine caused 80% regression of human breast tumors implanted in athymic mice. Noscaphine also displays potency against hormone-insensitive, triple-negative breast cancer cells. It was effective in shrinking tumors from MDA-MB-231 xenografts in nude mice. Excitingly, it evinced synergism when combined with doxorubicin.⁵⁴ These promising preclinical data merit consideration of noscaphine combined with doxorubicin for clinical trials involving triple-negative breast cancer patients, for whom treatment options are presently limited, although hormone receptor-positive cases may also derive benefit from noscaphine. Due to short biological half-life, poor absorption, low aqueous solubility, and extensive first-pass metabolism, noscaphine-loaded estrone-conjugated gelatin nanoparticles (Nos-ES-GN) were designed for targeting estrogen receptor-positive breast cancer MCF-7 cells. Nos-ES-GN had an IC₅₀ value about ~50% less than the free drug. The same study showed a greater accumulation of estrone-conjugated noscaphine-loaded gelatin nanoparticles in estrogen receptor-positive (MCF-7) cells than in the estrogen receptor-negative MDA-MB-231 cells, indicating that estrone-conjugated nanoparticles have the potential to target estrogen receptor-positive-breast cancer cells.⁵⁵

7. THE WIZARDRY CONTINUES: MORE BENEFICIAL ACTIVITIES ATTRIBUTED TO NOSCAPINE

Not only does noscaphine have success as an antitussive and potential as an anticancer agent, it has more potential clinical benefits. Noscaphinoids can inhibit dopamine biosynthesis, which could be useful for treating diseases such as pheochromocytoma (a neuroendocrine tumor of the adrenal medulla), in which extremely high levels of dopamine are secreted. In one study of noscaphine's effect on dopamine biosynthesis, noscaphine treatment of PC12 rat adrenal pheochromocytoma cells produced a significant reduction in dopamine content without being cytotoxic.⁵⁶ Also, noscaphine studies have expanded into other clinical opportunities, such as a possible treatment for PCOS because noscaphine possesses antiangiogenic properties. A team of researchers radiolabeled noscaphine with Technetium-99m (Tc-99m) in an effort to observe the organ biodistribution and uptake kinetics in rat and rabbit models.⁵⁷ The liver, spleen, kidney, and then ovary showed the most accumulation of noscaphine as the blood cleared 80% by the first hour. The researchers discovered a doubled uptake of noscaphine in the ovaries of PCOS rats compared to normal, cyclic rats, which the team backed up with scintigraphy. Noscaphine's antiangiogenic properties combined with ovarian uptake may make it a useful therapeutic approach for treating PCOS. It has also been hypothesized that noscaphine could be used as an antiviral due to its disruption of cytoskeletal components.⁵⁸ However, noscaphine was unable to disrupt viral replication in three diverse families of viruses. Matthews et al. suggest that this finding reveals that viruses do not need a functional cytoskeletal or use alternate methods to replicate when damaged. Noscaphine's inability to prevent viral replication brings to light the need for stronger antivirals for cancer-causing viruses, such as the human papilloma virus.

8. THE SECRET TO ITS SUCCESS: PEERING INTO NOSCAPINE'S PHARMACOLOGY

Noscapine is often administered orally due to its fast and effective absorption by this route. It has been shown to yield a maximum plasma concentration after 1 hr. Independent of gender, bi-exponential kinetics were present in healthy human volunteers in a study done by Dahlstrom et al., in which noscapine was infused intravenously with a dosage of 66 mg or orally as 150 mg rapidly dissolving tablets. After intravenous administration, the average plasma distribution half-life was found to be 13 min with a range from 7 to 22 min, and the average plasma elimination half-life was 156 min with a range from 96 to 236 min. The total plasma clearance was 22 mL/min/kg and the volume of distribution was 4.7 L/kg. The scientists found the absolute oral bioavailability to be 30% with 3.6-fold interindividual variation.⁵⁹ A later study used tablets of noscapine to determine bioavailability, and data indicated dependence on the dose, which could be attributed to saturable first-pass metabolism of the drug. The authors found a disproportionate increase in the area under the curve (AUC) when the tablets were administered; when dosage increased threefold, a ninefold AUC increase was observed. Karlsson's team also administered noscapine in solution, which yielded a significantly higher maximal concentration and earlier and higher AUC compared to the corresponding tablet dose. The terminal half-life found in this study was independent of formulation or dose size and was measured to be 4.5 hr.⁶⁰

Amore recent study conducted by Aneja et al. found mean plasma noscapine concentration in mice to be 7.88 µg/mL 5 min after a 10 mg/kg intravenous noscapine bolus. After 4 hr, the levels were undetectable, indicating the mean total body clearance was 4.78 L/h with a mean volume of distribution of 5.05 L. The mean oral bioavailability found in this study was 31.5%.⁶¹ In an effort to augment the bioavailability of noscapine, an inclusion complex utilizing β-cyclodextrin (β-CD) was prepared. Compared with free noscapine, the noscapine in the β-CD inclusion complex showed a 1.87-fold increase in bioavailability.⁶² Therefore, the addition of the inclusion complex can increase the efficacy per dose of noscapine. One team studied epithelial transport of noscapine across colon cancer Caco-2 and Madin-Darby canine kidney cell monolayers and the influence of absorption enhancers on in vitro permeation and bioavailability. After administration of captisol, significant increases in oral bioavailability were seen in Sprague-Dawley rats implicating intestinal absorption, suggesting a viable approach for reducing the required dose of noscapine with an enhanced oral bioavailability.⁶³

Noscapine's metabolism has been mapped and its bioactivation studied, notably by Fang et al. Mice were administered noscapine orally. Several metabolites were detected including *N*-demethylated, two hydroxylated, and bis-demethylated metabolites, and one metabolite that underwent both demethylation and cleavage of the methylenedioxy group. Glucuronides were also present along with several cytochromes P450, flavin-containing mono-oxygenase 1, and the UDP-glucuronosyltransferases UGT1A1, UGT1A3, UGT1A9, and UGT2B7, which were confirmed with recombinant enzyme screening.⁶⁴ Glutathione trapping uncovered the existence of an ortho-quinone reactive intermediate that formed due to catechol metabolite oxidation. It was found that this bioactivation does not occur in vivo.

Altered glutathione levels in the liver and serum biochemistry showed no damage to the liver, demonstrating that in mice noscapine induces no hepatotoxicity through bioactivation.⁶⁴

One of the necessary and vital pathways in progressing through the cell cycle is the activation of p34^{cdc2}, a cyclin-dependent kinase activated by noscapine. A study showed that inhibition of p34^{cdc2} activity with olomoucine prevented noscapine-induced apoptosis in FM3A murine mammary carcinoma cells. Ye et al. concluded that the p34^{cdc2} kinase needs to be inactivated in order for cells to exit mitosis. In the experiment, a prolonged activation of the p34^{cdc2} kinase was seen in the mitotically-arrested cells, suggesting its involvement in inducing apoptosis. It was shown that this kinase and its activation were essential for noscapine-induced apoptosis.⁶⁵

In paclitaxel-resistant cancers, noscapine treatment has been found to activate the JNK pathway to induce apoptosis. JNK is activated when cells are exposed to proinflammatory cytokines or environmental stress or undergo growth factor withdrawal; they are also called stress-activated protein kinases.³¹ It has been shown that JNK activation occurs when antimicrotubule agents are used, and the JNK pathway has been shown to be required for antimicrotubule agent-induced apoptosis. Endogenous phosphorylated c-Jun, a marker of JNK activation, was present when noscapine treatment was performed in a study by Joshi's team. They showed that inhibiting JNK activity with antisense oligonucleotide or transfection with dominant-negative JNK blocks noscapine-induced apoptosis.³¹

Often, cancer cells are hypoxic and express stable HIF-1 α , which is the main transcription factor activated by hypoxia.¹⁵ When HIF-1 α is stabilized and binds to HIF-1 β , the transcriptionally active HIF dimer is formed and angiogenesis is stimulated. HIF-1 α drives the transcription of vascular endothelial growth factor (VEGF), which acts as a potent angiogenesis promoter. Noscapine has been shown to inhibit HIF-1 α and, consequently, VEGF expression, thereby reducing angiogenesis.¹⁵

Another pathway that has been connected to noscapine is the NF- κ B signaling pathway, where NF- κ B acts as a transcription factor made of five different proteins.⁶⁶ This pathway has been linked to angiogenesis and chemoresistance. Therefore, a study was conducted using leukemia cells to examine the pathway in relation to the administration of noscapine. Results from Sung et al. showed noscapine suppresses tumor necrosis factor (TNF) induced NF- κ B-dependent antiapoptotic proteins (cIAP-1, XIAP, TRAF1, Bcl-2, and survivin), which when overexpressed would help the cancerous cells survive longer by evading apoptosis. Noscapine also represses TNF-induced NF- κ B-dependent cell proliferation proteins cyclin D1 and COX-2, which would otherwise be overexpressed by TNF. It also suppresses TNF-induced proteins involved in invasion and angiogenesis including intercellular adhesion molecule 1 (ICAM-1), matrix metalloproteinase 9 (MMP-9), and VEGF. Lastly, it inhibits TNF-induced NF- κ B activation in dose- and time-dependent manners. Noscapine was also shown to inhibit IKK activation by inhibiting TNF induction.⁶⁶

Recently, noscapine has been discovered to have antifibrotic effects.⁶⁷ In cultured human lung fibroblasts (HLFs), noscapine inhibited TGF- β -induced differentiation. With therapeutic doses of noscapine, a significant attenuation of pulmonary fibrosis was seen in the bleomycin model. Kach et al. found noscapine to inhibit TGF- β -induced stress fiber formation without affecting the gross microtubule content of the HLFs. This team showed that noscapine stimulates protein kinase A (PKA) activation, mediating the antifibrotic effect in HLFs; noscapine, however, did not activate PKA in the human bronchial or alveolar epithelia cells. EP₂ prostaglandin E₂ receptor antagonist, PF-04418948, blocked the PKA activation and noscapine's antifibrotic capabilities; these data suggest that EP₂ prostaglandin E₂ receptor mediated activation of PKA is responsible for noscapine's antifibrotic activity in pulmonary fibroblasts.⁶⁷ The mechanism by which noscapine antagonizes fibrosis and its various other molecular mechanisms discussed in this section are shown schematically in Figure 3.

9. PROFILING NOSCAPINE'S SAFETY: STUDYING THE SIDE EFFECTS

There have been multiple tests demonstrating noscapine's low toxicity to organs and tissues. With very high doses, the side effects have only consisted of nausea and abdominal discomfort in a small percentage of patients. A study done by Dr. Lasagna at Johns Hopkins University in 1961 treated terminally ill cancer patients; noscapine was administered at doses up to 3000 mg daily and only 20% of patients experienced side effects, which were limited to mild sedation and abdominal discomfort.⁶⁸ Noscapine has been administered alongside morphine, with which it acts synergistically to increase morphine's sedative and pain-relieving effects by as much as threefold. Some physicians outside the United States are now pairing the two, so a safer, lower dose of morphine can be used but with a stronger effect than morphine alone.¹⁵

10. CAUTION! NOSCAPINE TEAMS UP WITH WARFARIN

Noscapine can also interact with warfarin, warranting a warfarin dosage adjustment when co-administered with noscapine. It has been theorized to possibly increase the anticoagulative effects of warfarin.⁶⁹ It has been shown that noscapine is a competitive inhibitor of the (S)-warfarin 7-hydroxylation reaction by CYP2C9 and it does so in an NADPH- and time-dependent fashion. Noscapine is two- to threefold more efficient at inactivating CYP2C9.2 and 3 variants than of the wild type. The time-dependent inhibition of CYP3A4 and CYP2C9 by noscapine could explain the clinical noscapine-warfarin interaction.⁷⁰ Because of noscapine's inhibitory effects, (S)-warfarin exposure is predicted to increase up to sevenfold when administered alongside noscapine.⁷¹ These pharmacokinetic interactions between warfarin and noscapine indicate that careful monitoring of the international normalized ratio for blood coagulation will be required when noscapine is combined with warfarin.

11. NOSCAPINOIDS CAN FUNCTION AS CLUSTERBOMBS AND INDUCE CENTROSOME DECLUSTERING

Since noscapine has shown success as an anticancer agent, various analogs have been derived via modification of five sites on the parent compound (Figure 2). This newly emerging family has a promising career fighting cancer since its members are often potent centrosome declustering agents that modulate microtubule dynamicity.⁷² Cancer cells often contain amplified centrosomes and cluster these organelles into two groups to survive mitosis, as otherwise a highly multipolar spindle results and causes mitotic catastrophe or inviable daughter cells. These declustering drugs prevent amplified centrosome from clustering, ultimately leading to high-grade spindle multipolarity, G2/M phase arrest, and cell death. Noscapine analogs bind to microtubules inducing minor alterations in the innate dynamic instability and interfere with their attachment to kinetochores during mitosis, thus activating the spindle assembly checkpoint, which can lead to mitotic arrest and cell death.⁷³ Noscapine binds to and causes a conformational change in the tubulin heterodimer. It has been found to function as a tubulin-stabilizing agent that interacts strongest with the lateral and longitudinal segments of the tubulin dimer, affecting the monomeric interactions in nearby protofilaments.⁷⁴ Additionally, bromonoscapine (Br-Nos) and reduced bromonoscapine (Red-Br-Nos) have been shown to induce centrosome amplification by increasing the expression of Plk4, which mediates centrosome amplification.⁷² Ogden et al. found that noscapine induced and further propagated centrosome amplification along with supernumerary centrosome declustering. Noscapine and its analogs also generated acentrosomal spindle poles. Both situations—declustered centrosomes and acentrosomal spindle poles—resulted in spindle multipolarity and cancer cell death. Thus, noscapine and its analogs could be very useful as cancer-selective chemotherapeutics since supernumerary centrosomes are a cancer-specific anomaly.

12. ALL IN THE FAMILY: NOSCAPINE ANALOGS

Noscapine and its analogs not only act on centrosome clustering mechanisms and spindle poles to induce cell death but also on autophagic mechanisms. For instance, Red-Br-Nos was found to induce autophagy in human prostate cancer PC-3 cells within 12 hrs. This noscapine analog also triggered the release of reactive oxygen species (ROS), upon which induction of autophagy depended.⁷⁵ Karna et al. first reported the protective role of ROS-mediated autophagy following Red-Br-Nos treatment in PC-3 cells. They also discovered that Red-Br-Nos induces apoptosis in a manner dependent on ROS but independent of caspases. Researchers from the same lab studied Red-Br-Nos and found that it induced DNA damage in a ROS-dependent fashion, resulting in high-grade centrosome amplification and spindle multipolarity in PC-3 cells.⁷⁶ This study was the first to document that Red-Br-Nos generated centrosome amplification via de novo centrosome formation, which occurred during a brief S/G2 stall. The result was the formation of abnormal mitotic spindles with high-grade multipolarity, which triggered cell death. This mechanism distinguishes Red-Br-Nos from other ROS-inducers, DNA-damaging drugs, and antimicrotubule agents.⁷⁶ Madan et al. encapsulated Red-Br-Nos with β -CD and methyl- β -cyclodextrin (methyl- β -CD) in bioresponsive guar gum microspheres (GGM) to improve solubility and colonic delivery. In

a phosphate buffer saline, the Red-Br-Nos solubility increased ~10.7- and 21.2-fold when combined with β -CD and methyl- β -CD, respectively. In human colon HT-29 cells, the increased solubility correlated with a decline in the IC₅₀ by approximately two- and threefold for Red-Br-Nos- β -CD-GGM and Red-Br-Nos-methyl- β -CD-GGM, respectively, compared to the analogs free of GGM.⁷⁷

Although noscapinoids can cause ROS release, they nonetheless possess intriguing anti-inflammatory activity. Noscapine resembles colchicine, another microtubule-binding drug that has anti-inflammatory properties. Molecular models of competition binding of Br-Nos and colchicine have provided insight into the noscapinoid tubulin binding site, suggesting, most simply, that Br-Nos binds tubulin at a site overlapping with, or in close proximity to, the colchicine-binding site of tubulin.⁷⁸ Noscapine along with brominated analogs were studied by Zughaier et al. for anti-inflammatory activity in vitro. Brominated noscapine analogs were shown to inhibit the release of the cytokine TNF α and chemokine IP-10/CXCL10 from macrophages, thereby reducing inflammation without decreasing macrophage viability.⁷⁹ This research team also found that anti-inflammatory properties of brominated noscapine analogs persisted regardless of whether the cells were challenged with TLR- or non-TLR binding ligands, which resulted in the induction of autophagy.

Multiple other halonoscapine derivatives have also been studied. For instance, 9-chloronoscapine is easy to synthesize, and it was the most effective derivative against the glioma U87 cell line as compared with other halonoscapine analogs (specifically, 9-bromo- and 9-iodonoscapine). It has also been shown to increase G2/M populations in MCF-7 breast cancer and PC3 prostate cancer cell lines.⁸⁰ Another halonoscapinoid is 5-Br-Nos, which interrupts mitosis and inhibits the proliferation of HeLa cells.⁸¹ EM015, a 9-chloronoscapine derivative, binds to tubulin and is a more active analog of noscapine.³⁸ The analogs 9-chloronoscapine, 9-Br-Nos, and 9-iodonoscapine induced a greater extent of apoptosis in U-87 human glioblastoma cells than noscapine.³⁸

While the derivative 9-Br-Nos has been shown to increase G2/M population in MCF-7 breast cancer cells,⁸⁰ it may also be useful against hormone-insensitive human breast cancers since it can induce apoptosis after aG2-M arrest in estrogen- and progesterone receptor negative breast cancer cells. This derivative was also effective against human tumors implanted in nude mice as xenografts of MDA-MB-231 cells, which are negative for estrogen and progesterone receptors. Treatment group mice were administered 9-Br-Nos orally by gavage while the control was administered water. The experimental group experienced significant reductions in tumor volume. The mice treated with this analog also showed a fourfold increase in survival.⁸¹ The potency of this noscapinoid has been determined to be 40-fold stronger than the parent compound for inhibiting the proliferation of MCF-7 cells.⁸² The 9-Br-Nos analog was also effective against drug-resistant tumor cells, although it produced no cytotoxic effects in healthy tissues, including those that are highly proliferative like the spleen.²⁸ This same analog was effective against hormone-refractory human prostate cancer cells implanted in the tibias of nude mice.⁸³ Aneja et al. found no detectable toxicity in normal tissues that proliferate rapidly such as the gut and bone marrow. Hormone-independent PC-3 cells were used alongside three other cell types that had differing metastatic potential: the parental androgen-responsive nonmetastatic LNCaP

cells, androgen-independent C4-2 cells, and bone-metastatic C4-2B cells.⁸³ The same study revealed that this analog arrests the prostate cancer cell lines at the G2/M boundary and binds to tubulin with a greater affinity than noscapine. Interestingly, 9-Br-Nos decreases the mitochondrial transmembrane potential, resulting in induction of the intrinsic apoptotic cascade in PC-3 cells. In addition, it changes the expression on Bcl2 proteins that can modulate the release of downstream factors and activate caspase-dependent apoptosis. The same team saw inhibition of tumor growth in the intratibial prostate cancer xenografts after oral administration of 9-Br-Nos.⁸³ Another study discovered the synergistic relationship between 9-Br-Nos and docetaxel for treating prostate cancer. In the presence of noscapine, lower doses of docetaxel produced greater proapoptotic activity than when docetaxel was used alone. This finding suggests that a combinatorial approach may reduce toxicity and improve quality of life for docetaxel-treated patients.⁵³ Madan et al. encapsulated 9-Br-Nos with β -CD and methyl- β -CD. Cycloencapsulating this analog leads to ~11-fold (β -CD) and ~21-fold (methyl- β -CD) solubility increase in phosphate buffer solution. These modified bromonoscapinoids exhibited a more potent therapeutic index by lowering the IC₅₀ value.⁸⁴ An inhalable rapid-release nanostructured lipid particle species of 9-bromonoscapine (9-Br-Nos-RR-NLP) was synthesized since this noscapinoid alters tubulin polymerization better than the parent compound in non-small cell lung cancer cells.⁸⁵ These 9-Br-Nos-RR-NLPs showed enhanced cytotoxicity, apoptosis, and cellular uptake in A549 non-small cell lung cancer cells according to Jyoti et al. They found the IC₅₀ value to be less than that of the nonrapid release form, 9-Br-Nos-NLP, and 9-Br-Nos suspension.⁸⁵ Novel 9-alkyl and 9-arylnoscapine derivatives have been synthesized and showed similar cytotoxic potency in comparison to 9-Br-Nos.⁸⁶

An analog that has been found to be extremely potent with potential to treat some forms of drug-resistant cancer is 9-nitronoscapine. Other microtubule-binding drugs often fail when pitted against drug-resistant tumors. 9-Nitronoscapine effectively inhibits the proliferation of lymphoblastoid cell lines that are resistant to vinblastine, teniposide, and paclitaxel while not altering normal human fibroblast cell cycles. This analog was shown to induce G2/M arrest, altering the cell cycle profile of the cancerous cells. 9-Nitronoscapine was also found to activate caspase-3, the executioner caspase, demonstrating the potent proapoptotic properties of this analog. Immunohistochemistry showed extensive apoptosis after this noscapine derivative had been administered to the lymphoma cells.⁸⁷

A major mechanism of acquired drug resistance is the pumping of drugs from cancer cells via efflux pumps such as the P-glycoprotein (Pgp) multidrug resistance pump (MRP). CEM/VLB100 cells are vinblastine-resistant variants with Pgp-overexpression that show 270-fold resistance to vinblastine.⁸⁸ CEM/VM-1-5 cells overexpressing MRP are 400-fold resistant to teniposide.⁸⁹ Aneja et al. discovered 9-nitronoscapine to be active against not only the parental CEM cells but also the aforementioned drug-resistant variants. MTS assay was used to determine the IC₅₀ values for CEM, CEM/VLB100, and CEM/VM-1-5 cells, which were lower, demonstrating the greater potency of the 9-nitronoscapine analog compared with the parent noscapine compound.⁸⁷

Amino-noscapine is one of the most potent tubulin-binding noscapinoids to date with one of the highest binding scores of the noscapinoids only rivaled by Br-Nos.⁹⁰ One study found

amino-noscapine to be an extremely potent substoichiometric tubulin assembly inhibitor.⁹¹ As an anticancer agent, it is effective against a panel of 60 human cancer cell lines available from the National Cancer Institute through its Developmental Therapeutics Program. The IC₅₀ values for amino-noscapine are much lower than for noscapine. For leukemia, non-small lung cancer, colon cancer, CNS cancer, ovarian cancer, renal cancer, breast cancer, and prostate cancer cell lines, these values do not exceed the IC₅₀ value for amino-noscapine. Amino-noscapine thus proved to have superior potency over the parent compound, noscapine.⁹²

Studies have been conducted to test the efficacy of noscapine derivatives. Debono et al. investigated the potency and efficacy of *N*-alkyl, *N*-acyl, *N*-carbamoyl, *N*-thiocarbamoyl, and *N*-carbamate noscapine analogs against prostate cancer (PC3), breast cancer (MCF-7), and colon cancer (Caco-2). The noscapinoid with an *N*-substituted ethyl urea functional group showed potency against all cell lines.⁹³ In another study, the moiety of interest was the benzofuranone ring. Multiple analogs were derived by substituting functional groups in the ring's position-7. It was discovered that with increased size of the substituted group, the number and magnitude of steric clashes at position-7 increased, which could decrease tubulin binding to the functional group at that site and diminish drug efficacy.⁹⁴ It was demonstrated in vitro that benzofuranone ring substituted noscapinoids inhibited tubulin polymerization. Mishra et al. demonstrated that all five analogs inhibited the light scattering signal at 350 nm in a concentration-dependent manner, indicating that these noscapinoids can bind to tubulin and inhibit microtubule assembly. The five derivative substituents were acetyl (compound 3), benzoyl (compound 4), ethylcarbamato (compound 5), phenylcarbamato (compound 6), and benzylcarbamato (compound 7). Generally, the most effective derivative against most cell lines (with the exception of breast cancer cells) was 7-acetyl-noscapine. Pancreatic cancer MIA PaCa-2 cells were sensitive to acetyl-, benzoyl-, and phenylcarbamato- derivatives. Compounds 3–5 proved strong toward lung cancer A549 cells, while compound 5 was most effective against CEM lymphoma cells. MCF-7 was found to be resistant to the analogs. None of the analogs tested inhibited cell proliferation in normal human dermal fibroblasts. 7-Acetyl-noscapine was found to be the strongest overall except in MCF-7, the breast cancer cell line, suggesting a high degree of interline variability.⁹⁴ Higher IC₅₀ values for 7-phenylcarbamato-noscapine indicated the significance of bulky substituents for the efficacy of the drug. When compared with noscapine, these second-generation benzofuranone ring-substituted noscapine analogs are generally more effective and deserve further research.

Natural α -noscapine has been proven to possess weak anticancer efficacy with a relatively safe toxicity profile.⁹⁵ Third-generation α -noscapine analogs were synthesized and *N*-(3-bromobenzyl)-noscapine binds with affinity ~4.0 times higher than noscapine, and is slightly more potent than the first-generation clinical candidate EM011, 9-Br-Nos.⁹⁶ Multiple studies have been conducted synthesizing and evaluating novel biaryl type α -noscapine congeners where an aryl unit is added to the tetrahydroisoquinoline of the natural α -noscapine core, the scaffold structure of noscapine. Through palladium catalyzed Suzuki cross-couplings of 9-bromo α -noscapine with aryl boronic acids, multiple noscapinoids were recovered and three were found to have potent cytotoxic and tubulin-binding activity against

human breast epithelial (MCF-7), human cervical cancer (HeLa), and human lung adenocarcinoma epithelial (A549) cell lines.^{95, 97}

13. THE FUTURE OF THE FAMILY: A RICH TAPESTRY OF POSSIBILITIES

It is now well established that, beyond treating cough, noscapine has a great variety of uses that can be beneficial to a wide range of patients, especially those with malignancies. Noscapine has synergistic properties with other anticancer treatments, and the newly derived analogs have produced tremendous results. Although side effects are possible with extremely high doses of noscapine (nausea and abdominal discomfort have been reported), at usual doses it produces no noticeable untoward effects.¹⁵ Multiple teams have conducted experiments testing the toxicity of noscapine in vitro, in animal models, and in patients, and they have confirmed that the drug has minimal toxicity.^{98–101} The future of noscapine and its derivatives as anticancer agents is promising because they tend to subtly modulate microtubule dynamicity rather than impacting the monomer–polymer ratio, rendering them gentler than other microtubule-targeting drugs currently on the market. The ability to manipulate noscapine and synthesize new derivatives with success against multiple cancer cell lines reveals the drug's versatility and foreshadows the possible armory of anticancer noscapinoids. Noscapine has a short biological half-life, poor absorption, extensive first pass metabolism, and limited water solubility, which hinders its development into a promising oral anticancer drug.^{102, 103} Novel third-generation water-soluble noscapinoids have been synthesized with negatively charged sulfonate and positively charged quaternary ammonium groups using noscapine, 9-Br-Nos, and 9-aminonoscapine as scaffolds. The water-soluble analogs have been found to be superior microtubule-interfering agents with enhanced antiproliferative activity, inhibiting proliferation of cancer cells more potently than noscapine and Br-Nos.¹⁰³ The efficacy of noscapine and its analogs has been enhanced through nanoscale-based drug delivery systems, such as the enveloped gelatin nanoparticles, poly(ethylene glycol)-grafted gelatin nanoparticles, the inclusion complex of noscapine in β -CD, human serum albumin nanoparticles, and noscapine-loaded magnetic polymeric nanoparticles.^{104–107} These compounds should be further researched to find new modifications increasing efficacy and to identify more optimal combinations of noscapinoids with other cytotoxins and targeted agents to design preclinical studies and clinical trials. By the amalgamation of sophisticated new methods in computational biology, bioinformatics, pharmacogenomics, engineering, and/or nanotechnology, the sky is truly the limit for this extraordinary family of molecules.

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ABBREVIATIONS

ACEI	angiotensin-converting enzyme inhibitor
rt-PA	recombinant tissue plasminogen activator

JNKs	c-Jun NH ₂ -terminal kinases
HIF-1α	hypoxia inducible factor-1 alpha
Nos-SLN	noscapine-bearing solid lipid nanoparticles
Nos-PEG-SLN	noscapine poly (ethylene)-glycol conjugated solid lipid nanoparticles
GBM	glioblastoma multiforme
TMZ	temozolamide
BCNU	<i>bis</i> -chloroethylnitrosourea
CIS	cisplatin
IM	imatinib mesylate
SSGM	sterically stabilized gelatin microassemblies
Nos-ES-GN	noscapine-loaded estrone-conjugated gelatin nanoparticles
Tc-99m	Technetium-99m
PCOS	polycystic ovarian syndrome
AUC	area under the curve
VEGF	vascular endothelial growth factor
PKA	protein kinase A
HLF	human lung fibroblast
TNF	tumor necrosis factor
ICAM-1	intercellular adhesion molecule 1
MMP-9	matrix metalloproteinase 9
ROS	reactive oxygen species
GGM	guar gum microspheres
Br-Nos	bromonoscapine
Red-Br-Nos	reduced bromonoscapine
β-CD	β -cyclodextrin
methyl-β-CD	methyl- β -cyclodextrin
9-Br-Nos-RR-NLP	9-bromonoscapine nanostructured lipid particle
Pgp	P-glycoprotein
MRP	multidrug resistance pump

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Biographies

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Dillon LiVecche earned his bachelor's degree in Biology in 2014 at Georgia State University, where he worked as an undergraduate research assistant in the lab of Dr. Ritu Aneja. He graduated summa cum laude with advanced honors and a minor in Chemistry. In addition, he received the Biology Department Student Award for Outstanding Achievement. Dillon is currently pursuing admission into medical school, with ambitions to study general surgery or emergency medicine.

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Jun Zhou received his bachelor's degree in Genetics from Fudan University in 1997, after which he was a master's student studying Cell Biology at Peking University. He received his Ph.D. in Biochemistry and Cell and Developmental Biology from Emory University in 2002, where he studied the impact of noscapinoids and taxanes on microtubule dynamics, cellular checkpoints, and signaling pathways in cancer cells. Thereafter, he worked at Genentech, Inc. as a postdoctoral fellow in Molecular Oncology and at Emory University as an Instructor in Cancer Biology and Pharmacology. He returned to China in 2005 to take a professor position in the Department of Genetics and Cell Biology at Nankai University College of Life Sciences. His lab currently investigates microtubule-regulated cellular processes and the role of microtubules in disease pathogenesis, diagnosis, and treatment. He received the National Science Fund for Distinguished Young Scholars in 2008 and the Fok Ying Tung Young Faculty Award in 2010. He served as Visiting Professor in the Department of Biomedical Engineering at the Georgia Institute of Technology in 2014.

Ritu Aneja is a Professor in the Department of Biology at Georgia State University in Atlanta where she also serves as the Graduate Director and Interim Molecular Basis of Disease Program Area of Focus Director. She obtained her Ph.D. in Chemistry in 1997 from the University of Delhi in India. Later, she did her postdoctoral studies at Yale and Emory before joining as a tenure-track faculty at Georgia State in 2008. Her laboratory is actively engaged in the identification and preclinical development of novel centrosome-based "druggable" targets with an overall goal to contribute "kinder and gentler" chemotherapeutic modalities that have superior pharmacological profiles to the clinic. Equally important is her focus on the development and clinical validation of biomarkers for early cancer detection, prognosis, and prediction of metastasis risk. Her research projects are indeed a promising step forward in the application of cell biology to human disease since they have strong clinical and translational relevance.

Figure 1.

Timeline of noscapine-related discoveries. (A) 1803: Jean-François Derosne separates a noscapine-containing salt that he calls "narceine" from poppies. (B) 1817: Pierre-Jean Robiquet purifies noscapine from Derosne's salt. (C) 1895–1930: Noscapine is widely used as an antimalarial based on the recommendations of Sir Robert Williams of the 1893–1895 Royal Commission on Opium. (D) 1930: The antitussive action of noscapine is discovered. (E) 1954: Hans Lettr é describes "narcotine" as a weak mitotic poison whose antimetabolic activity synergizes with that of *N*-methyl-colchicine. (F) 1997: Noscapine's ability to antagonize dopamine biosynthesis in PC12 cells is discovered. (G) 1998: Mouse models reveal the in vivo anticancer activity of noscapine against various malignancies. (H) 2003: Noscapine's antistroke activity is discovered. (I) 2003: Brominated noscapine analogs are synthesized that potentially inhibit mitosis and proliferation in cancer cells. (J) 2006: A variety of noscapine haloderivatives are synthesized and characterized. (K) 2006: A nitro-derivative of noscapine is synthesized that selectively kills chemotherapy-resistant cancer cells. (L) 2010: Brominated noscapine analogs are found to induce autophagy in macrophages and

prostate cancer cells. (M) 2010: Experiments reveal that noscapine may have therapeutic value in polycystic ovarian syndrome. (N) 2011: Amino-noscapine is synthesized and characterized. (O) 2011: Poly(ethylene glycol)-grafted gelatin nanoparticles customized to deliver noscapine intracellularly with long circulating half-lives are synthesized and characterized. (P) 2011: Second-generation benzofuranone ring-substituted derivatives of noscapine analogs are synthesized and characterized. (Q) 2014: Third-generation water-soluble derivatives of noscapine are synthesized and characterized. (R) 2015: Inhalable nanostructured lipid particles containing a brominated noscapine derivative are synthesized and characterized.

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Figure 2.

Analogues of noscapine. (1a–e) Biaryl derivatives resulting from addition of an aryl unit with a variable R substituent to position-9 in the tetrahydroisoquinoline portion of noscapine. (2a–j) Third-generation analogs resulting from derivatization of the nitrogen (position-6) in noscapine's isoquinoline ring system. (3a–e) Second-generation analogs resulting from derivatization of position-7 in the benzofuranone ring system. Only a few of the many O-alkylated derivatives are pictured, including two water-soluble analogs (d, e). (4a–c) Substitution of position-9 in a modified noscapine core in which the benzofuranone lactone

ring has been reduced to a cyclic ether yields various derivatives, including haloderivatives (b) and a nitro-derivative (c). (5a–l) First-generation analogs resulting from modification of position-9 in noscapine's isoquinoline ring system.

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Figure 3. Molecular mechanisms of noscapine. The mechanisms by which noscapine antagonizes potentially and certainly pathological processes, including cough, ischemic cerebral injury, angiogenesis, fibrosis, and tumor formation (indicated in blue boxes), are depicted.