Invited Reviews

Camptothecin and Taxol: Historic Achievements in Natural Products Research[⊥]

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The research team of Dr. Monroe E. Wall and Dr. Mansukh C. Wani of Research Triangle Institute discovered two first-in-class life-saving chemotherapeutic agents. Camptothecin, first isolated and identified from *Camptotheca acuminata*, was found to kill cancer cells uniquely via topoisomerase I poisoning. Presently, two first-generation analogues of camptothecin are used to treat ovarian, colorectal, and small-cell lung cancers, and several second-generation analogues are in clinical trials. Taxol, first isolated and identified by Wall and Wani from *Taxus brevifolia*, was found to inhibit cancer cell growth via the stabilization of microtubules. In 1992, taxol was approved for refractory ovarian cancer and today is used against breast and non-small cell lung cancers and in Kaposi's sarcoma. While there have been numerous reviews of these molecules individually, this review offers an integrated account of the research team of "Wall and Wani" and the significance of their discoveries to chemistry, biology, and clinical medicine.

Introduction

The research team headed by Dr. Monroe E. Wall and Dr. Mansukh C. Wani of Research Triangle Institute is responsible for the discovery of two life-saving compounds for the treatment of cancer. In 1966, Drs. Wall and Wani and colleagues reported the first of these, which they termed camptothecin, from the Chinese tree *Camptotheca acuminata*.[1] Nearly 20 years later, the unique mode of action for this potently cytotoxic compound was found to be the inhibition of DNA topoisomerase I.[2] Presently, the first-generation analogues of camptothecin, Hycamtin (topotecan) and Camptosar (irinotecan or CPT-11), marketed by GlaxoSmithKline and Pharmacia (now Pfizer), respectively, are used for the treatment of ovarian and colon cancers.[3,4] Perhaps more well known, the structure of taxol, isolated from the Pacific yew tree, *Taxus brevifolia*, was reported in 1971 by Drs. Wall and Wani and colleagues.[5] The unique mode of action for this compound was found to be the stabilization of microtubule assembly.[6,7] In 1992, Bristol-Myers Squibb received approval to market taxol (now known by the generic name paclitaxel and the trade name Taxol) for the treatment of refractory ovarian cancer, and subsequently, it was approved for the treatment of metastatic breast and lung cancers and Kaposi's sarcoma.[8] The unique action of taxol spurred the development of a second-generation semisynthetic taxane, docetaxel (Taxotere), approved in 1996 for anthracycline-refractory advanced breast cancer and now also used in lung cancer regimens. The events surrounding the discovery and development of these drugs provide numerous examples of the power of natural products to uncover new therapeutic agents and define novel drug targets. Moreover, central to all of these lessons are the drive and persistence of two complementary researchers whose dedication to science transcended the obstacles encountered during the 30-plus-year journey of these molecules from bench to bedside. Individually, both of these discoveries are seminal accomplishments, and taken together, this work represents a truly historic achievement in natural products research.

The Research Team of Wall and Wani

Dr. Monroe E. Wall received his B.S., M.S., and Ph.D. degrees from Rutgers University in the 1930s, and in 1941 he joined the Eastern Regional Research Laboratory of the U.S. Department of Agriculture (USDA). He had a successful career there, and during the 1950s most of his research focused on the search for phytosteroids that could serve as precursors for cortisone. In doing so, he amassed a collection of thousands of plant extracts. During the mid to late 1950s, the National Cancer Institute (NCI) started a program to test compounds for anticancer activity, and this included the testing of plant extracts. Dr. Jonathan Hartwell of the NCI learned of Dr. Wall's plant extract collection and requested that aliquots of these be screened for anticancer activity. From the initial testing of a thousand plant extracts, *Camptotheca acuminata* displayed strong in vivo activity against a murine adenocarcinoma model.[8] Since the USDA was not supportive of his anticancer research, Dr. Wall moved to the then new Research Triangle Institute (RTI) in 1960 to found the Natural Products Laboratory. Through a contract with the NCI, his research group began the search for anticancer compounds from plants, and *C. acuminata* was the initial plant they studied. In 1966, they described camptothecin from this plant as a "novel alkaloidal leukemia and tumor inhibitor".[1] Through these initial investigations, Dr. Wall noted a strong correlation between in vitro cytotoxicity against the 9KB (human oral epidermoid carcinoma)[9] cell line and in vivo anticancer activity.[10] Thus, he requested that the NCI

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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send plants to him that displayed cytotoxicity. In the early 1960s, extracts of *Taxus brevifolia* were studied for their in vivo anticancer activity. Although these extracts were not particularly active against NCI's in vivo models, they showed good activity against 9KB in vitro. Thus, in 1965, a large sample of *T. brevifolia* bark was assigned to Dr. Wall's research group, and by 1971 Wall and Wani described taxol from this plant as a "novel antileukemic and antitumor agent".[5]

Dr. Mansukh Wani received his B.S. and M.S. degrees from the University of Bombay in 1947 and 1950, respectively. He then spent eight years as an instructor of chemistry at Bhavan's College before coming to the United States to pursue a Ph.D. at Indiana University. After completing his degree in 1961 and working as a postdoctoral student at the University of Wisconsin, Dr. Wani joined Dr. Wall's research group at RTI in 1962. Dr. Wani's contributions to the discoveries of both camptothecin and taxol were dependent on his laboratory acumen. Camptothecin has a unique pentacyclic structure. Since sophisticated analytical techniques for structural determination were not available in the 1960s, its structure was determined via single-crystal X-ray analysis of an analogue, camptothecin iodoacetate. For this, Dr. Wani's expertise in recrystallization was crucial for obtaining a sample suitable for the X-ray analysis, which was conducted by Drs. George Sim and Andrew McPhail at the University of Illinois. Beginning work on *T. brevifolia* in 1965, he isolated taxol by 1966, and its purification and bioactivity were discussed at an American Chemical Society meeting in Miami Beach, Florida, the following year.[11] However, the complex structure of taxol required more work than that of camptothecin to be solved. Once more, the RTI team resorted to single-crystal X-ray crystallography to determine the structure of taxol, again working with Dr. Andrew McPhail, who was then at Duke University. This work proved considerably more challenging, and it was only through the persistence of Dr. Wani that the structure was finally determined in early 1971. By cleaving the amino acid side chain from the core of the molecule, Dr. Wani was able to make derivatives of both the amino acid side chain and the core molecule that were suitable for X-ray analysis. From these data and using other derivatizations, he determined the structure in early 1971 in room 212 of the Hermann Building on the present site of RTI.[5]

Drs. Wall and Wani have been the recipients of numerous awards for their discoveries. Individually, Dr. Wall has won the American Chemical Society (ACS) Alfred Burger Award in Medicinal Chemistry, the Research Achievement Award from the American Society of Pharmacognosy (ASP), and several honorary doctorate degrees, to name just a few. Likewise, Dr. Wani has been the recipient of the Pride of India Award, the Indo-American Pharmaceutical Award, the Ranbaxy Award, and the Indiana University Distinguished Alumni Award. Together, Drs. Wall and Wani have both received the Distinguished Speaker Award from the North Carolina Section of the ACS, Honorary Membership in the ASP, the Bruce F. Cain Memorial Award from the American Association for Cancer Research, the City of Medicine Award from Durham, NC, the NCI Award of Recognition, and the Charles F. Kettering Prize from the General Motors Cancer Research Foundation.

Perhaps more impressively, both of these gentlemen worked well past retirement age, and even today, Dr. Wani's enthusiasm for research continues unabated. Since the discovery of camptothecin and taxol, three and a half decades ago, they have searched for other novel, bioactive compounds from plants, synthesized hundreds of second-generation analogues of camptothecin, synthesized steroids as possible antifertility agents, and developed standards for herbal drugs, to name only a few of their numerous research projects. Moreover, throughout their long and fruitful careers, they have inspired, taught, and mentored several generations of scientists, including, most recently, the authors of this review.

Historical Significance of the Research

When it comes to anticancer drug discovery, the most obvious metric for achievement comes from the prolongation and improvement of the quality of human life. With both camptothecin and taxol, this aim has been and continues to be achieved. In the case of camptothecin, the parent molecule isolated originally from the plant was not ideal for pharmaceutical development, mostly due to its extremely poor solubility. Early attempts at clinical trials with a sodium salt of camptothecin were unsuccessful. Later, it was determined that the lactone ring moiety must be intact for activity and that this ring was being opened in the preparation of the sodium salt.[12] Moreover, restoration of the lactone linkage in the acidic environment of the bladder led to severe cases of cystitis that stalled clinical development in the early 1970s. As discussed below, the unique mode of action of camptothecin rekindled interest in this molecule in the mid-1980s. First-generation analogues of camptothecin, Camptosar (irinotecan or CPT-11) and Hycamtin (topotecan), which are water-soluble derivatives of camptothecin with an intact lactone ring, were approved for use by the U.S. Food and Drug Administration (FDA) in 1996. These are marketed in the United States by Pharmacia (Pfizer) and GlaxoSmithKline, respectively, for the treatment of metastatic colorectal, primary colon, and metastatic ovarian cancers, and in 2002, their combined annual sales were nearly $750 million[13] and many sources expect them to approach $1 billion by 2003. For cancer patients, these compounds have improved therapies associated with several types of cancers, and currently, there are nearly a dozen second-generation analogues of the camptothecins in different phases of clinical trials. A timeline for the development of the camptothecins is included (Figure 1), and several books are referenced for more specific details about camptothecin research.[14−16]

The development of taxol from basic science discovery to viable pharmaceutical application took a circuitous route. Entire books have been written on this subject,[17,18] and the American Chemical Society (ACS) held its first symposium on taxol in 1992, while three divisions of the ACS held symposia on taxane research in 1994.[19] As with the camptothecins, taxol's unique mode of action was instrumental in driving much of the drug development efforts (see below). The original extract of *Taxus brevifolia* was not very active against the in vivo anticancer models favored by the NCI in the 1960s and 1970s. However, because Dr. Wall had noted a strong correlation between in vitro 9KB cytotoxicity and in vivo anticancer activity during the camptothecin studies, his research group pursued its bioactivity-directed fractionation, and taxol was isolated by 1966 and characterized by 1971.[5,11] Taxol showed strong activity in vivo against P1534 leukemia, but the NCI did not consider this predictive of clinical activity, especially since taxol was not very active against either L1210 or P388 leukemia models.[20] Other problems, such as poor solubility and limited supply, hampered development as well. However, as the NCI tumor panel was expanded to include human tumor xenografts in nude mice,

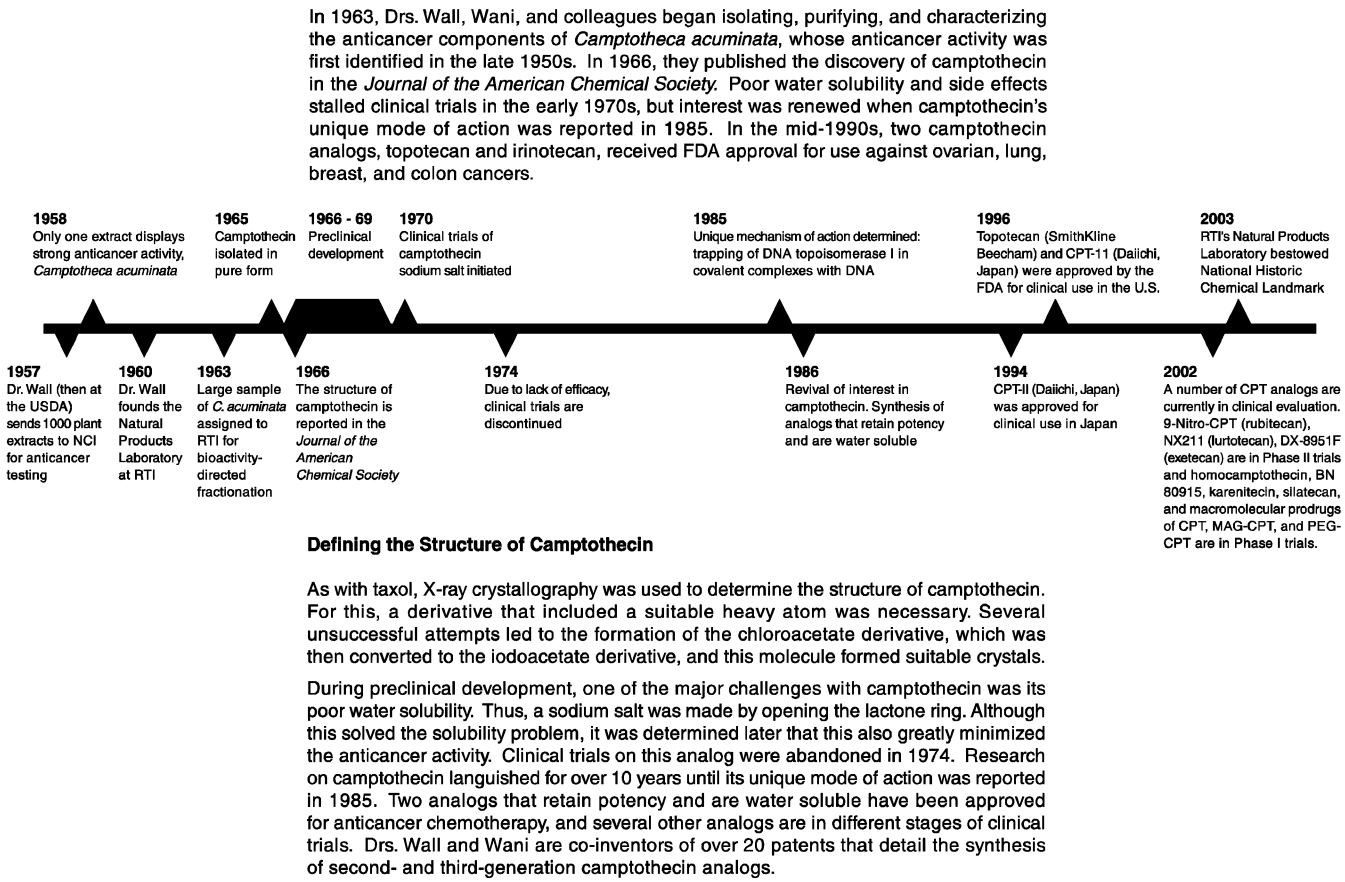

Figure 1. Timeline for camptothecin discovery and development.

taxol displayed impressive activity.[20] This moved taxol into preclinical development (see timeline in Figure 2). From this solid tumor activity and the unique mode of action (discussed below), taxol advanced into clinical trials in the early 1980s. Throughout the 1980s, taxol progressed through Phases I, II, and III of clinical trials, and in 1992, the FDA approved its use for refractory ovarian cancer. Today, taxol is approved also for the treatment of breast and colon cancers and Kaposi's sarcoma. It has been a multibillion dollar drug, with annual sales reaching nearly $2 billion in 2000.[21,22] By adding the present market value of the camptothecins, taxol, and docetaxel (Taxotere) the discoveries of Drs. Wall and Wani can be attributed to nearly one-third of the global antineoplastic agent market, which has been estimated at approximately $9 billion/year.[23]

Cancer chemotherapeutic agents are rarely used as single agents, and clinical trials compare new drugs to standard treatments rather than to placebo controls, for obvious ethical reasons. However, there are several examples in the literature that describe the benefits of the camptothecins and taxol, particularly in second-line therapy where cancer patients experience recurrent disease. Their novel mechanisms of action have led to numerous clinical trials demonstrating improved patient survival where durable responses can no longer be realistically achieved. For example, metastatic pancreatic cancer, which has single-agent response rates of less than 10% and median survival of 4−5 months, is one of the most challenging settings for oncologists. In a recent trial where irinotecan (CPT-11) was added to regimens in patients with progressive disease, 24% of patients experienced a partial response and 21% had stable disease with a median survival of 10.3 months.[24] Similarly, taxol has found utility in recurrent ovarian cancer, where median survival is usually less than 2 years.[25] In patients who failed platinum-based first-line chemotherapy, a combination of carboplatin and taxol has been shown to increase response rates to 70% with a 3-year survival rate of 72%.[26] The utility of these agents in the face of such formidable opponents as metastatic pancreatic cancer and recurrent ovarian cancer is strongly indicative of their life-prolonging activity. These two examples demonstrate that the camptothecins and taxol have provided substantial hope for patients to whom a death sentence has been declared more than once.

Novel Mechanisms of Action

From a basic science perspective, the discoveries of camptothecin and taxol are impressive examples of the power of natural products to lead to the elucidation and exploitation of novel mechanisms of antitumor action. Although both of these agents were isolated originally via standard cytotoxicity screening assays, they were found later to inhibit cancer cell growth via unique mechanisms of action, arresting the cancer proliferation in ways that scientists had not previously imagined or determined. In the present post-genomic period of biology, where scientists are faced with thousands of potential drug targets seeking interaction with small-molecule ligands, the contribution of camptothecin and taxol in directing the biological investigation of novel anticancer targets cannot be understated. In the last century, there may have been only two other pairs of discoveries of the same magnitude: Sir James Black, 1988 Nobel Laureate, for the discovery of histamine H_2 antagonists and β_1-adrenoceptor antagonists and Otto Loewi, 1936 Nobel Laureate, for adrenergic and cholinergic agonists.

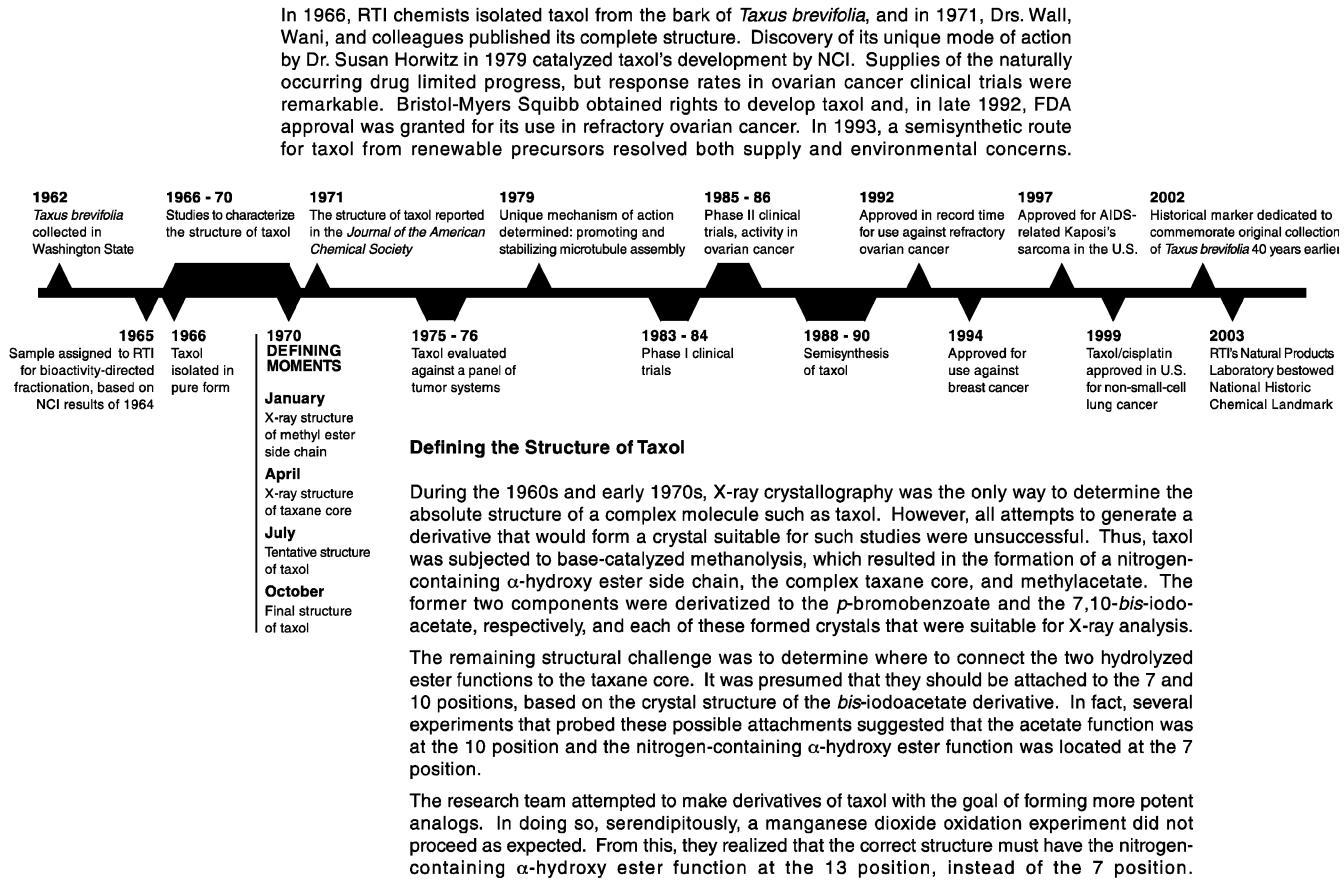

Figure 2. Timeline for taxol discovery and development.

The lesson provided by camptothecin is perhaps more striking, since the compound was discovered half a decade before taxol, although its mechanism of action took much longer to elucidate. In the early 1970s, camptothecin was known to inhibit RNA and DNA synthesis, but a specific enzyme could not be identified as its site of activity. Interestingly, Dr. Susan Band Horwitz of the Albert Einstein College of Medicine, most well known for her ground-breaking studies on taxol described elsewhere in this special issue of the *Journal of Natural Products*, contributed to some of the early biological evaluations of camptothecin.[27−29] In 1979, it was recognized that antitumor drugs such as doxorubicin (Adriamycin) promote covalent linkage of protein to DNA in tumor cells, and it was postulated that identifying these proteins would reveal the proximal targets of these drugs.[30] Contemporaneously, Dr. Leroy F. Liu, then of Johns Hopkins University, had been studying the action of enzymes called DNA topoisomerases, which modulate DNA superhelicity during transcription and replication by relieving the torsional strain introduced by separation of DNA strands as the transcriptional or replication apparatus proceeds.[31] Topoisomerases act by cleaving one or both DNA strands, allowing the passage of an unbroken strand or DNA duplex through the breakage site prior to resealing the break. It was determined in earlier studies with the bacterial type II topoisomerase, DNA gyrase, that the enzyme exists in a covalent complex with the 5′-phosphate of the broken DNA strand via a conserved tyrosine residue in the enzyme during the course of its catalytic cycle. Cozzarelli's group demonstrated that the antibacterial agent nalidixic acid stabilized DNA gyrase in this covalent enzyme-DNA complex, and this prevented the resealing of the DNA break.[32] A type II topoisomerase activity had been identified in mammalian cells, and Liu's group demonstrated subsequently that doxorubicin trapped topoisomerase II on DNA at therapeutically relevant concentrations.[33] Camptothecin, a compound that also produced protein-linked DNA strand breaks, was tested via this same mechanism but was inactive.[34] However, while working with the related type I topoisomerase, a group led by Liu demonstrated that this enzyme was the target for camptothecin.[2] Moreover, camptothecin was discovered to be more than a simple inhibitor of topoisomerase I activity. By stabilizing the enzyme in its catalytic intermediate covalently bound to DNA, camptothecin transformed this normally useful enzyme into an intracellular, cytotoxic poison, and hence, these agents are termed topoisomerase "poisons" to distinguish them from conventional enzyme inhibitors.[31] Liu and Wang demonstrated later that these covalent cleavage complexes stabilized by camptothecin act as physical barriers to DNA synthesis and kill cells as a result of replication fork collision.[35] While many structurally diverse agents have been shown to act via topoisomerase II poisoning (doxorubicin, etoposide, mitoxantrone, amsacrine), camptothecin's discovery as a topoisomerase I poison was unprecedented. Continued development of topoisomerase I poisoning drugs is essential, as topoisomerase II poisons fall out of favor owing to their risk of secondary, therapy-related leukemias, a long-term side effect not shared by the camptothecins.[36]

It is notable that while topotecan and CPT-11 have achieved nearly $750 million in annual sales,[13] the general opinion in clinical oncology circles is that the potential of the camptothecins as anticancer agents has yet to be realized fully.[37] Newer analogues with improved and more predictable bioavailability relative to these two existing

agents are certain to expand the use of this natural product drug class. Moreover, recent advances in the basic and clinical pharmacology of the camptothecins have renewed enthusiasm on a number of fronts. First, NCI researchers recently screened 2000 diverse structural entities for functional inhibition of the hypoxia-inducible factor 1 (HIF-1), a master regulator of the cancer cell's ability to survive under oxygen deprivation. Only four compounds exhibited HIF-1 inhibitory activity, and three of these were camptothecins.[38] Hence, camptothecins may have other desirable activities against solid tumors that are independent of topoisomerase I poisoning. In the clinical setting, a trial based at Mayo Clinic−Jacksonville (NCCTG 963255) showed that CPT-11 was unusually effective in breast cancer patients whose disease had become unresponsive to anthracyclines and taxanes.[39] Camptothecins had not been thought previously to be useful in breast cancer, but this study is striking in that activity was observed against the more challenging drug-resistant form of the disease. Moreover, camptothecins possess dose-limiting toxicities distinct from those of anthracyclines and taxanes and normally do not require the use of hematopoietic growth factors to rescue blood cell progenitors in the bone marrow. Therefore, camptothecins are ripe for evaluation as primary agents in breast cancer, a disease that afflicts nearly 200 000 women *and* men in the United States annually and 1.2 million worldwide. The discovery of camptothecin and progress with first-generation derivatives topotecan and CPT-11, as well as future second- and third-generation analogues, will continue to maximize the clinical utility of a peculiar but extremely useful mechanism of antitumor drug action.

By studying taxol, biologists also revealed a unique mechanism of antitumor activity. In a manner that was distinct and opposite from the *Vinca* alkaloids, taxol was found to act on microtubules. Perhaps due to the highly visual effect of taxol on microtubule dynamics, its mechanism of action was discovered relatively shortly after its structure was determined. In contrast to topoisomerase assays, microtubule assembly assays had been available for years before the discovery of taxol, and these assays required far less sophisticated equipment. However, the action of taxol is far from pedestrian. In 1979, the laboratory of Dr. Susan Band Horwitz at the Albert Einstein College of Medicine demonstrated that taxol could substitute for GTP in promoting the assembly of tubulin into its polymeric form.[7] In fact, taxol was the first known agent to interact specifically with the polymerized form of tubulin. Shortly thereafter, Schiff and Horwitz demonstrated that promotion of microtubule assembly occurs in intact cells treated with taxol and prevents proliferating cells from completing mitosis.[6] Following the recent X-ray crystallographic solution of the structure of β-tubulin, taxol was shown to interact with the H7 helix of the molecule, a structure also implicated in GTP binding and hydrolysis.[40] Remarkably, taxol, which bears no remote resemblance to GTP, targets microtubules but in a manner that does not duplicate the mode of action of the *Vinca* alkaloids, which were already in clinical use at that time. In fact, the action of taxol is even more striking than simply inhibiting mitosis. Taxol misleads the tumor cell into passing the G1/S checkpoint and into another cycle of DNA replication.[41] DNA replication in the absence of cytokinesis is called endoreduplication and results in the generation of giant cells with 4N, 8N, 16N, and even 32N DNA content, and this leads subsequently to delayed apoptosis. As more natural products are screened for activity, there have been a number of novel compounds found to act in a manner similar to that of taxol.[42] For example, epothilones A and B, which were isolated from the myxobacterium *Sorangium cellulosum*,[43] have been shown to promote microtubule assembly but without the risk of resistance from cellular export by the MDR efflux pump. As with camptothecin, the discovery of taxol and elucidation of its mechanism has been a watershed in our understanding of means by which tumor cells can be eradicated.

Akin to the camptothecins, taxol continues to be refined for improved activity, and its discovery has led to the development of second-generation analogues. Most notably, in 1996, the FDA approved docetaxel (Taxotere), a structural analogue first synthesized in the late 1980s by Dr. Pierre Potier and colleagues.[44,45] The unique action of taxol had stimulated French academic and industrial researchers to investigate other *Taxus* species for renewable precursors for producing taxol and related compounds. Beginning with 10-deacetylbaccatin III isolated from the needles of the English yew, *Taxus baccata*, docetaxel was one of a number of analogues synthesized that was selected ultimately for development by Rhone-Poulenc Rorer (now Aventis). While docetaxel possesses a pharmacologic and toxicologic profile that is somewhat different from taxol, the parent molecule continues to be the subject of therapeutic development. For example, a solvent-free, nanoparticle-based delivery system developed for taxol (ABI-007; Abraxane) bypasses the need for premedication to prevent hypersensitivity reactions to the Cremophor EL vehicle used in first-generation taxol formulations.[46] In another arena, nanoparticle taxol in the form of a drug-eluting stent is likely to gain FDA approval for preventing restenosis following balloon angioplasty treatment of coronary arterial blockage.[47] This novel indication stems from observations that several proto-oncogenes (i.e., c-*myc*, c-*myb*) activated in cancer are also expressed in neointimal cells following angioplasty, and proliferation of these cells then causes reocclusion of the coronary vessels over a period of months. Already approved for use in several European countries, local delivery of taxol via this drug-eluting stent (Taxus; Boston Scientific) prevents this neointimal hyperplasia and minimizes the need for subsequent surgery.

Hence, the novel mechanisms of action through which camptothecin and taxol act represent far more than intellectual curiosities to chemists and biologists. Most importantly, these discoveries have given oncologists and their patients additional choices, both in first-line chemotherapy as well as when standard chemotherapy has failed. All chemotherapeutic combination regimens are designed so that each agent acts via a distinct mechanism of action to provide synergistic activity against tumor cells that have accumulated numerous growth-regulatory anomalies and to prevent the emergence of drug resistance via any one pathway. When standard treatments fail, the drug choices for oncologists in "salvage therapy" are limited because the resistant cancer will often exhibit cross-resistance to agents acting via the same mechanism. The major clinical contribution of camptothecin and taxol lies in that they are founding members of drug classes with novel and previously unpredicted mechanisms of action. While these agents and their analogues first found utility in second-line therapy (such as irinotecan in cisplatin-refractory ovarian cancer), their true therapeutic potential is now being realized in first-line therapy as well. Novel agents that act via topoisomerase I poisoning or promotion of microtubule assembly will continue to be refined in efforts to improve activity and reduce side effects. Yet, without

the seminal discoveries of these two natural products, these novel targeting mechanisms surely would have gone unrecognized for decades.

Bioactivity-Directed Fractionation as a Viable Natural Products Drug Discovery Tool

Seminal discoveries should also lead to the development of new ways to think about a problem at hand and open doors to new ideas, and, in this regard, camptothecin and taxol are notable examples. Their isolation from crude plant extracts was driven by bioactivity.[10] During the time when camptothecin and taxol were first discovered, many natural products chemists used a more phytochemical approach, wherein the bioactivity of the compounds was evaluated only after purification. Drs. Wall and Wani pioneered the idea of using the bioactivity of the crude extract to direct the fractionation toward the most potent compounds. While other contemporaries were experimenting also with these same techniques, Drs. Wall and Wani led the charge with the examples of camptothecin and taxol. Today, such bioactivity-directed fractionation procedures are used routinely by natural products chemists around the world to find novel, bioactive compounds, but in the 1960s these techniques were in their infancy. Thus, Drs. Wall and Wani used novel techniques to discover novel compounds with novel modes of action. As a result, not only have new treatments for cancer emerged, but also, a new paradigm has been established that is being utilized to find many other bioactive compounds from natural sources.

Summary and Conclusions

The above-described achievements represent monumental discoveries for the treatment of cancer, and thus the site of these discoveries, the Natural Products Laboratory of Research Triangle Institute, has been recognized recently by the American Chemical Society as a National Historic Chemical Landmark.[48] Most significantly, taxol and compounds derived from the camptothecin structure have been responsible for saving the lives of hundreds of thousands of people afflicted with cancer. Many statistics could be cited in this regard, but one of the more telling relates to the treatment of ovarian cancer. Since the inclusion of taxol in the treatment regimen for this disease, the survival rate has more than doubled.[49] Furthermore, both taxol and camptothecin were found to inhibit cancer cell growth via novel mechanisms of action. Prior to their discovery, neither the stabilization of microtubule assembly nor the trapping of topoisomerase I-DNA intermediates was known to be an effective way to circumvent the uncontrolled growth of cancer cells. Thus, these compounds have led to the identification of new cancer drug targets. Finally, from the chemical laboratory perspective, both of these compounds were discovered using the principles of bioactivity-directed fractionation, especially in vitro cytotoxicity as a predictor of in vivo efficacy.[10] As regular readers of the *Journal of Natural Products* realize, these techniques are used routinely today in natural product laboratories around the world to discover bioactive compounds from plant, marine, and microbial origin. In summary, Dr. Wall and Dr. Wani, through the isolation and structure elucidation of the novel, bioactive natural products taxol and camptothecin, improved the lives of people afflicted with cancer, unearthed new mechanisms of action for inhibiting cancer cell growth, and established new principles for the discovery of other, bioactive compounds from natural sources.

While working for Research Triangle Institute, a private, nonprofit research environment, Drs. Wall and Wani are responsible for the discovery of two compounds that are being produced today by major pharmaceutical companies to treat human diseases. They showed great scientific persistence in the face of difficulty in isolating and determining the structures of these unique compounds. Moreover, they saw the promise of these molecules, and they pushed for their development toward the pharmaceuticals they are today. Their chemical discoveries, which were reported initially in the *Journal of the American Chemical Society*,[1,5] have led to new drugs, new modes of action to kill cancer cells, and new ideas for the discovery of bioactive compounds from natural sources. Their chemistry has led to new science and new medicine, and thus, it seems very fitting that it has been recognized as a National Historic Chemical Landmark.[50]

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