Camptothecin and Taxol

The Story Behind the Science

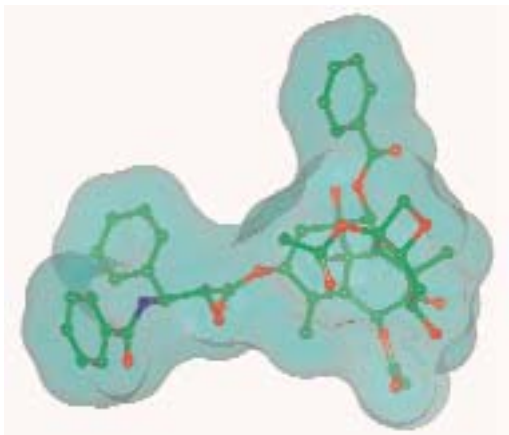
by Nicholas H. Oberlies, Sharla Flora, and Anna L. Weaver

On 23 April 2003, many researchers and friends gathered on the campus of Research Triangle Institute (RTI) International in Research Triangle Park, NC, for a ceremony designating the site as an American Chemical Society (ACS) National Historic Chemical Landmark. This honor recognizes the work of RTI's Natural Products Laboratory and its scientists Dr. Mansukh Wani, the late Dr. Monroe Wall, and their colleagues, whose dedication and innovation led to the discovery of the unique cancer-fighting compounds Taxol® and camptothecin™.[1] Present to laud these accomplishments were representatives of ACS and eminent researchers in the fields of natural products chemistry, cancer pharmacology, clinical oncology, and others.

As with many groundbreaking scientific developments, the stories behind the discoveries of Taxol and camptothecin are compelling. They provide more than good scientific narrative; these compounds have far-reaching impact both on individuals afflicted with cancer and on the field of natural products chemistry.

Intuition and Interest: Beginning with Camptothecin

Working for the U.S. Department of Agriculture (USDA), Dr. Wall spent much of the 1950s researching a large collection of plants, searching for phytosteroids that could serve as cortisone precursors. Fortunately, Dr. Wall had the foresight to save all the extracts from his studies. Later that decade, the National Cancer Institute (NCI) launched a program to screen plants for anticancer activity. Of the 1000 samples Dr. Wall initially sent to NCI for testing, one demonstrated particularly promising anticancer activity: *Camptotheca acuminata*, a plant native only to China and Tibet, where it is known as xi shu ("happy tree").



Source: S. Wayne Mascarella, Ph.D., of RTI

A modeller view of Taxol.

Given its focus on agriculture, the USDA did not share Dr. Wall's interest in studying cancer-fighting plants. Therefore, with the promise of funding from NCI, Dr. Wall took his passion to the newly formed RTI. In 1962, Dr. Wall was joined by Dr. Wani—and a scientific partnership that would last over four decades was born.

RTI's Natural Products Laboratory acquired a large sample of *C. acuminata* and began work to isolate and determine the structure of the active agent. One of the more formidable challenges was to make a crystal suitable for X-ray analysis. In this area, among others, Dr. Wani demonstrated unusual skill. The research team persisted, and success came in 1966, when Dr. Wall, Dr. Wani, and colleagues published the isolation and structure elucidation of camptothecin.

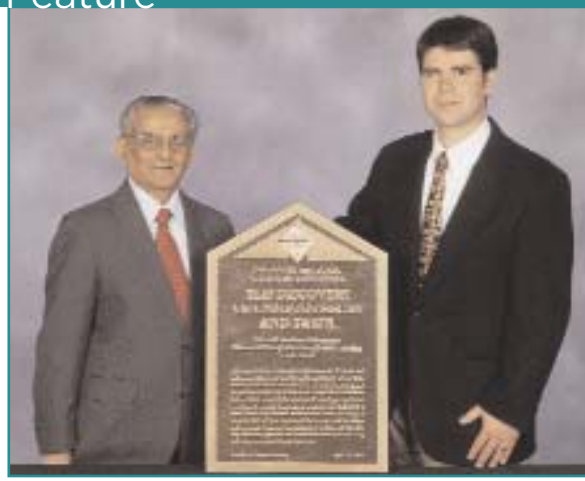
This paper was their first publication on an anticancer compound from a plant source. Interestingly, Dr. Wall presented results at the annual meeting of IUPAC in Stockholm, Sweden, that same year. The seminal nature of this initial publication is rather remarkable, and it would lead eventually to camptothecin analogs used today in the fight against cancer.

Another obstacle encountered by the research team involved the natural form of camptothecin. Specifically, its poor water solubility makes drug delivery extremely difficult. Clinical trials with camptothecin were initiated in the late 1960s using an analog (the sodium salt). Although this compound is quite water soluble, its anticancer activity is substantially diminished. Clinical trials were abandoned due to high toxicity and low efficacy, halting progress of camptothecin for more than a decade until its unique mode of action was discovered in 1985.

Lessons Learned: Early Years of Taxol Research

One of the early observations noted by Drs. Wall and Wani during their work with *C. acuminata* was a strong correlation between *in vitro* cytotoxicity and *in vivo* anticancer activity. Based on this insight, Dr. Wall

[1] Taxol, a word coined by Monroe Wall, is a registered trademark of Bristol-Myers Squibb. Camptothecin is a trademark of the Research Triangle Institute.



Dr. Wani (left) and Dr. Oberlies (article author) stand with a plaque designating the Research Triangle Institute a National Historic Chemical Landmark.

requested that RTI be assigned strongly cytotoxic plant extracts from the NCI. In contrast, other research groups shunned the cytotoxic extracts, wrongly presuming that general cytotoxicity would not lead to promising drug leads.

NCI's rationale for selecting plants to study is also a story worth mentioning: with limited funding for gathering samples, NCI chose to focus on sources available in the United States and on unique plants that had yet to be studied in depth; *Taxus brevifolia* met these qualifications. Thus, *T. brevifolia*, a species of yew tree that grows in the Pacific United States, was assigned to RTI for investigation.

By 1971, Drs. Wall and Wani had succeeded in isolating and determining the structure of the active compound from *T. brevifolia*, which they named taxol. Publication of their results would mark their sixth paper on a natural product with promising anticancer activity. Twenty years later, this compound would become a valuable weapon in the fight against cancer.

Failures, Perseverance, and a Late Correction

The structural characterization of camptothecin was certainly challenging, especially given the limited spectroscopic tools of the day. However, once a crystal suitable for X-ray analysis was prepared, the structure elucidation moved fairly rapidly. In contrast, the structure elucidation of Taxol was extremely difficult. The molecule had to be broken into parts—a large, complex core and a side chain. Even then, determining how the pieces were assembled based on X-ray structures of the parts was not straightforward.

At the time, camptothecin was advancing into early clinical trials. In addition, there were many other promising plants to study, while Taxol's structure remained elusive. At one point, Dr. Wall urged Dr. Wani to move on to other projects and to work on Taxol only as a low priority. Dr. Wani persisted, often on his own time, and his perseverance led to success. RTI researchers determined a tentative structure of Taxol in late 1970.

During one final experiment, as Wani and colleagues attempted to modify Taxol to increase its potency, they realized that the tentative structure was incorrect. They had misplaced the side chain. In the end, skill, determination, and luck came together for the greater good, and the research team corrected their error a scant few months before publication.

Solving the Problem of Supply

For naturally occurring substances such as Taxol and camptothecin, the question of supply can become central to their evolution into commercially available drugs. This is particularly true for Taxol, whose complex structure renders its total synthesis economically infeasible.

Taxol's supply problem remained until the late 1980s, when researchers found a way to extract a similar compound from the needles (a renewable resource) of another species of yew. That compound—10-deacetylbaccatin III—consisted of the complex core molecule of Taxol to which researchers successfully combined a synthetic version of the relatively simple side chain.

Yesterday's Novel Methods, Today's Best Practice

To isolate and characterize both camptothecin and Taxol, RTI researchers used a then-novel method known as bioactivity-directed fractionation. In this process, a promising plant extract is fractionated, and the fractions are tested for activity. This process is performed iteratively—further purifying the active fractions and retesting—until the active compound(s) is isolated.[2] Drs. Wall and Wani also pioneered the use of *in vitro* cytotoxicity as a predictor of *in vivo* efficacy. By using these bioassays to predict whether plant extracts or natural product compounds would have anticancer activity, they were

[2] In contrast, a phytochemical approach involves isolating numerous compounds from a plant without regard to bioactivity.

able to focus and accelerate their research toward new treatments. Today, these techniques are used routinely in natural products laboratories around the world to discover bioactive compounds from numerous sources, such as plants, marine life, fungi, etc.

Unique Mechanisms of Action

One of natural products research's greatest contributions is its ability to identify new ways to attack disease. For example, Taxol and camptothecin each act on cancer cells in ways that were unimagined prior to the compounds' discoveries. Both compounds inhibit tumor growth differently than all other known anticancer agents. Camptothecin impedes DNA replication by trapping a key enzyme, topoisomerase I. In contrast, Taxol stimulates the development of microtubules causing cells to be unable to coordinate cell division. Through very different mechanisms of action, both compounds lead to cancer cell death.

Taxol and Camptothecin Today

In the decades that followed the initial work of Drs. Wall and Wani, many researchers would prove instrumental in the development of successful cancer treatments from Taxol and camptothecin. Efforts to improve the methods for synthesizing the compounds and to reduce the side effects associated with the drugs are ongoing.

Today, Taxol is approved by the U.S. Food and Drug Administration for treatment of refractory ovarian cancer, metastatic breast and lung cancers, and Kaposi's sarcoma, while first-generation analogs of camptothecin (topotecan and irinotecan) are approved for treatment of ovarian and colorectal cancer. Today, one-third of all cancer treatments are derived from work in RTI's Natural Products Laboratory. Meanwhile, many other Taxol and camptothecin analogs are currently undergoing clinical trials and may yield new cancer-fighting drugs.

Camptothecin and Taxol: Timeline

1960-1966	Isolation of active compound from amptotheca *acuminata*; determination of structure of *camptothecin*
1962-1971	Isolation of active compound from *Taxus brevifolia*; determintion of structure of Taxol
1979	Determination of mechanism of action of Taxol
1985	Determination of mechanism of action of camptothecin
1988	Supply problem of Taxol abated via semisynthesis of Taxol
1992	FDA approval of Taxol for use in ovarian cancer, then subsequently breast and lung cancers and Kaposi's sarcoma
1996	FDA approval of two analogs of camptothecin for treatment of ovarian, lung, breast, and colon cancer

Recognizing the Impact

In recognition of their keen scientific intuition, their perseverance, and their incontrovertible achievements, Drs. Wall and Wani have received a number of prestigious accolades, including the 2000 Charles F. Kettering Prize. The most recent honor—designation of RTI's Natural Products Laboratory as one of fewer than 50 National Historic Chemical Landmarks—is particularly fitting for a scientific achievement of such singularity.

Taxol and camptothecin have prolonged and saved the lives of hundreds of thousands of cancer patients. Moreover, in the course of discovering these potent anticancer agents, researchers identified novel mechanisms of action for killing cancer cells, and the scientific community benefited from their new ideas and methods for finding and refining bioactive compounds from natural sources.

A Living Legacy

The resolve and innovation evident in the breakthrough research of Drs. Wall and Wani is a legacy that persists. Research into natural products has contributed 65% of today's anti-infective and anti-cancer drugs. In fact, fully 25% of the drugs in use today originated from sources in nature. Dr. Wani remains an active mentor in RTI's Natural Products Laboratory, where investigations in medicinal chemistry continue.

In addition to its active research efforts, RTI is working to ensure the Wall/Wani legacy by endowing fellowships in natural products research in their name, with the support of the American Society of Pharmacognosy and other private donors. Building on the accomplishments of the past 40 years, RTI will keep its Natural Products Laboratory at the forefront of the field, through research, mentoring, and fellowships, for the next four decades and beyond.

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