Chapter 12. The Mitotic Inhibitor Story – vinca - taxol 221009dj3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 12

The Mitotic Inhibitor Story: taxol and vinca.

This chapter is about anticancer drugs that were discovered as toxins in certain plants or sea creatures and that were found to block the microtubules that pull the chromosomes apart during mitosis. Microtubules also convey essential molecules down the axons of nerve cells, which is why these same drugs can damage nerve cells.

Anti-cancer drugs from natural products

The natural world of animals, plants, and microorganisms is full of biological warfare agents in conflicts between various species. Natural poisons serve to ward off predators and competitors. Some were used by people over the ages, both to poison and to cure. A few became useful as medicines for treatment of cancer (Cragg and Newman, 2004; Vindya et al., 2015). Since those medicines are also poisons, the amounts given to patients, as with most drugs used in cancer chemotherapy, must be carefully adjusted to give significant effect against the cancer without producing too much toxicity.

So, how do those microtubule poisons work? During mitosis, the newly formed chromosome pairs are pulled apart by fibers, called microtubules. Each daughter cell then gets one of the newly formed chromosome pairs, although cancer cells often have abnormal mitosis that yields cells with abnormal sets of chromosomes. A major action of anti-microtubule drugs is to impair cell division at mitosis. As with most cancer chemotherapy, however, those microtubule-binding drugs are effective only against those cancers that are more sensitive to them than critical normal tissues. I will tell the stories of two classes of anti-microtubule drugs that became

important in anticancer therapy and of a class of more recently discovered mitotic inhibitors.

Drugs derived from plants and animals in nature often have more complicated chemical structures than what chemists can easily synthesize in the laboratory. Although making those complicated compounds artificially in the laboratory can be challenging, living creatures have enzymes that put together surprisingly complicated structures from simple building blocks using enzymes that occur naturally in their cells.

Moreover, evolution provided enormous opportunity for selection of compounds that would help a species to survive and beat off the competition. That is why useful medicines can be derived from nature that our chemists would be unlikely to discover on their own. These drugs however evolved as poisons, and so it is not surprising that they have toxic side-effects in patients. But why they have anticancer activity is not entirely clear. This chapter is about the discovery, mechanism of action, therapeutic opportunities and toxic limitations of some of those complicated drug structures

Many anticancer drugs act by damaging DNA or blocking its synthesis, but the mitotic inhibitors do not act on DNA; rather, they interfere with the process of cell division itself. They impair the function of the mitotic spindle, which is made up of microtubules that normally assure that each daughter cell gets precisely one pair of the newly made chromosomes. When the mitotic spindle is perturbed by these drugs, the cell cannot divide normally and often dies. The first two classes of mitotic inhibitors to be discovered and developed for cancer treatment were the vinca alkaloids and the taxanes. I will now begin their stories.

The story of Vinca and the periwinkle.

The renowned Madagascar periwinkle (*Vinca rosea*, also known as *Catharanthus roseus*) is a colorful flowering plant (Figure 12.1) that has a venerable provenance beginning with the writings of Albartus Magnus (circa 1200-1280), a German Catholic Dominican friar and philosopher.

Here is a 16th century English translation of what Albartus wrote about the periwinkle in *The Boke of Secretes of Albartus Magnus of the Vertues of Herbs, Stones and certaine Beastes: "Perwynke when it is beate unto pouder with worms of ye earth wrapped about it and with an herbe called houslyke, it induceth love between man and wyfe if it bee used in their meales . . . if the sayde confection be put in the fyre it shall be turned anone unto blue coloure"*. (From Botanical.com, A Modern Herbal).

In Chaucer, we read:

And fresshe pervinke, riche of hewe,
And floures yelowe, whyte, and rede;

The periwinkle also has world-wide medicinal traditions: "In India, they treated wasp sting with the juice from the leaves. In Hawaii they prescribed an extract of the boiled plant to arrest bleeding. In Central America and parts of South America, they made a gargle to ease sore throat and chest ailments and laryngitis. In Cuba, Puerto Rico, Jamaica and other islands, an extract of the flower was commonly administered as an eyewash for the eyes of infants. In Africa, leaves are used for menorrhagia and rheumatism. Surinamese boil ten leaves and ten flowers together for diabetes. Bahamians take flower decoction for asthma and flatulence, and the entire plant for tuberculosis. In Mauritius, the leaves infusion is given for dyspepsia and indigestion. In Vietnam, it is taken for diabetes and malaria. Curacao and Bermuda natives take the plant for high blood pressure. Indochinese use the stalks and leaves for dysmenorrhea." (From J. A. Duke, Handbook of Medicinal Herbs, 1985; Magic and Medicine of Plants, 1993; cited by the National Tropical Botanical Garden.)

Obviously, the periwinkle had worldwide reputations for medicinal use for treatment of many ailments. Therefore, it most likely was doing something useful in the sick body – but what, exactly? Among all of those cited uses there is no mention of cancer!

Vinca rosea

Figure 12.1. The Madagascar periwinkle (*Vinca rosea,* also known as *Catharanthus roseus*), the source of vinblastine and vincristine.

Road to Discovery: from the periwinkle to an anticancer drug.

We transition now from ancient lore and tradition to scientific knowledge and medical application. The fascinating history of the discovery of anti-cancer ingredients in the Madagascar periwinkle was summarized in 1958 by Noble (Noble

et al., 1958) and in 1968 by Johnson (Johnson, 1968). As early as 1910, Theodore Pickolt, a naturalist and pharmacist, had already described the medicinal use of the periwinkle in Brazil (Johnson et al., 1960). However, the story leading to anticancer drugs from the periwinkle began in a surprising way in 1949, when J. H. Cutts at the University of Western Ontario in Canada learned that in the West Indies a tea made from the leaves was used as a remedy for diabetes (Noble et al., 1958). When he investigated this in diabetic rats, however, there was no trace of any effect of the tea on diabetes. Undeterred, Cutts tried administering a stronger dose by injecting it instead of just giving it to the rats to sip. To his surprise, the tea-injected rats died within a week!

The rats were found to be dying of infection, which was in turn caused by marked loss of infection-fighting white blood cells (leucocytes). Depletion of leucocytes was evident not only in the blood stream, but also in the bone marrow where these blood cells are made (Figure 12.2). Cutts may have known that depletion of white blood cells occurred in mustard gas-exposed sailors and a few years earlier was associated with the discovery of the anti-cancer activity of nitrogen mustard (see Chapter 1). Hence, it made sense to purify the white-count-suppressing ingredients from the periwinkle extracts and to test them against cancer.

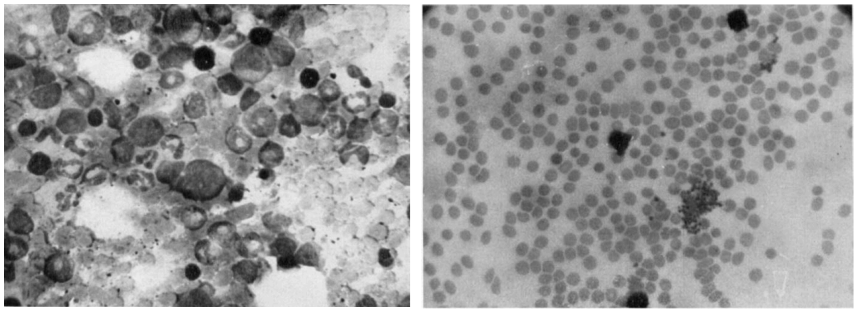


Figure 12.2. Bone marrow from a normal rat (left) and a rat treated with the active material purified from *Vinca rosea* (right). The bone marrow from treated rats (right) shows many red blood cells but very few of the large developing white blood cells such as those seen in normal bone marrow on the left (Noble et al., 1958).

To give an idea of how R. L. Noble, C. T. Beer, and J. H. Cutts at the University of Western Ontario, Canada, purified the active compound from extracts of the plant material in 1953, here is a brief summary of their procedure: The plant material was first soaked in a solvent, such as alcohol or ether. The material in the resulting solutions was then separated into different "fractions". The researchers then injected samples of each fraction from various stages of purification into rats and monitored white blood cell counts in blood drawn daily from the rat's tail. Injecting the original extract caused the rat's leukocyte count to drop after 2-3 days and then to recover about a week

later. In the first step of purification, an acid extract (ethanol with 10% acetic acid) of the dried leaves was made alkaline, whereupon something precipitated that was more active than the original extract; thus, the active ingredient had been partially purified. Further steps of purification eventually yielded a pure highly potent needle-like crystalline compound, which they named vincaleukoblastin, later shortened to vinblastine (Figure 12.3). The chemical structure of vinblastine, like that of many natural products, took a lot of work to unravel, because it is very complicated with many interlocking rings and asymmetric centers (Figure 12.4).

Even at this early stage on the road to vinca alkaloids as important anticancer drugs, the investigators already had clues to how the drug work and the drug's limiting toxicities. In addition to the blood count suppression, there was damage to the cells that line the inner surface of the intestines. We now know that these drugs tend to kill dividing cells and that active cell division is required to maintain blood counts and to replenish cells that are continually being sloughed off from the intestinal lining (mucosa). Blood count suppression and damage to the intestinal mucosa are two major toxicities of many anticancer drugs. Thus, the vinca story highlights the fact that attack on dividing cancer cells also impacts normal dividing cells and particularly normal tissues that critically rely on continual production of new cells. The damage to dividing cells in normal tissues is the main cause of dose-limiting toxicity by most anticancer agents.

(Gastrointestinal toxicity in the case of vinca alkaloids turned out not to be a major clinical problem, most likely because other toxicities supervened, and patients rarely received dosage high enough to cause troublesome gastrointestinal toxicity.)

In addition to vinblastine, several other alkaloids of related chemical structure were isolated, of which the most important, vincristine, differed from vinblastine only by addition of an oxygen atom at an important location in the molecule (Figure 12.4). Even though the change in chemical structure was tiny, the two drugs differed in the cancers they were most effective against. Most striking was the greater effectiveness of vincristine against acute leukemia (Johnson et al., 1963). Also, there was surprisingly little cross-resistance between the two vinca drugs; thus, when patients stopped responding to one of the vinca drugs, they sometimes subsequently responded to the other.

In the 1980's Pierre Potier and his colleagues at the CNRS in France prepared another anti-cancer vinca drug, vinorelbine. The periwinkle makes several chemically related compounds ("alkaloids"). They started with one of those (not the one from which vinblastine was derived) and chemically modified it to obtain the active drug. Compared to vinblastine, vinorelbine lacks an oxygen and a carbon atom (CH_2 group), indicated by the blue arrows in Figure 12.4. So, we see that the three vinca drugs are chemically very similar, and they were found to inhibit microtubules in essentially the same way. Nevertheless, they differed in the cancers for which they were most useful.

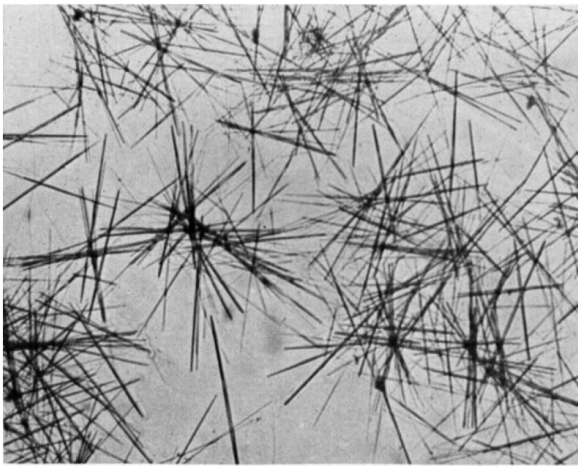
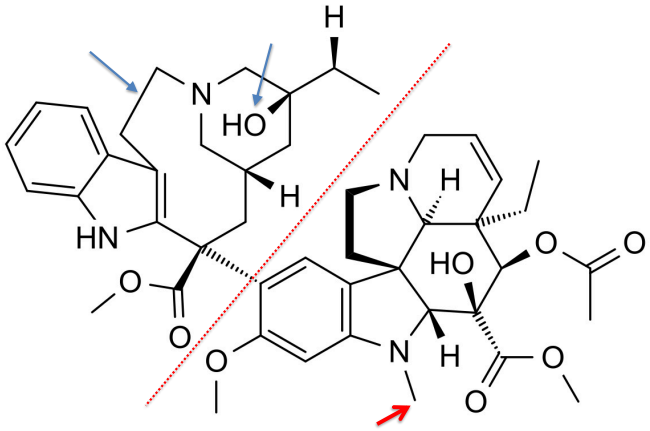


Figure 12.3. Needle-like crystals of vinblastine purified from the periwinkle *Vinca rosea* (Noble et al., 1958).



Vinblastine and the changes in Vincristine and Vinorelbine

Figure 12.4. The vinblastine molecule consisted of two parts: a "catharanthine nucleus" to the upper left of the red line and a "vindoline nucleus" to the lower right of the line. Vincristine differed only in having an oxygen atom added at the red arrow (thus vincristine had a formyl group instead of a methyl group attached to the nitrogen at that position). The dashed bond that connects the 2 parts of the molecule indicated that the upper left half of the molecule is above the plane of the page, while lower right half is below the plane; only the correct 3D geometry worked. Vinorelbine was like vinblastine, except that it lacked the OH to which a blue arrow points and has only one instead of two carbons connecting two rings indicated by another blue arrow.

How the vinca drugs block mitosis.

A major clue to how the vinca's work soon emerged, when it was found that vinblastine blocked the cell division process. Moreover, the block was not at the DNA duplication stage, where most of the previously known anti-cancer drugs, such as methotrexate and 5-fluorouracil, block cells. Instead, the block was at mitosis, during which the chromosomes segregate into the two daughter cells. More precisely, the block was at the metaphase stage of mitosis, where the chromosomes are fully condensed and line up, ready to separate into their respective daughter cells (Figures 12.5 and 12.6) (Palmer et al., 1960) (Johnson et al., 1960) (Cutts, 1961). The block was due to the fact that the vinca drugs impaired the microtubules that make up the mitotic spindle, the structure that lines up the chromosomes and separates them equally into the two daughter cells. The vinca drugs were found to bind to the microtubules and thereby to block mitosis.

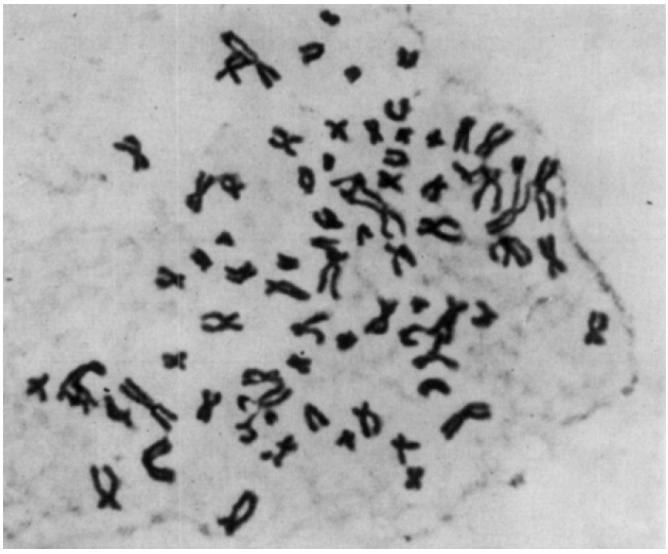


Figure 12.5. A vinblastine-treated cell arrested in a spindle-deficient metaphase. The chromosomes are condensed as in normal mitosis, but the microtubules of the mitotic spindle is absent; therefore, the chromosomes are scattered instead of being lines up as they would be in a normal metaphase (Palmer et al., 1960).

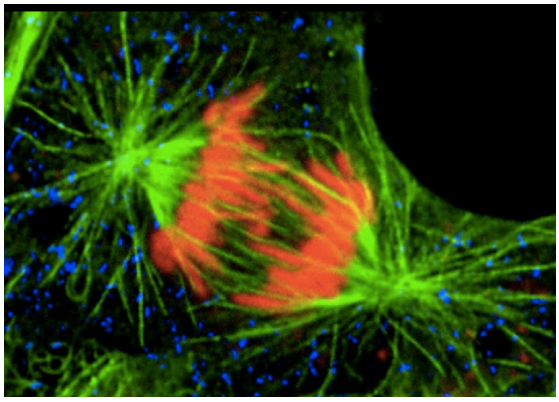


Figure 12.6. This beautiful picture shows a critical phase of a normal mitosis ("anaphase" – immediately after metaphase), when the microtubules of the mitotic spindle pull the chromosomes apart so as to give to each daughter cell one copy of each duplicated chromosome. The chromosomes are stained red; the microtubules, green. A vinca-treated cell lacks microtubules and therefore could not proceed from metaphase to anaphase and would not show in this picture.

Although the idea that mitotic block was plausible as the cause of cell killing by vinblastine, it was mere conjecture, because the drug had other actions as well. But the conjecture was soon supported by direct evidence: the extent of cell killing by various doses correlated quantitatively with the extent of microtubule inhibition; therefore the two effects were correlated and their causes were probably related (Said and Tsimberidou, 2014; Tucker et al., 1977).

Further investigation revealed the molecular details of how the vinca alkaloids block mitosis. During mitosis, the chromosomes normally divide equally between the two daughter cells. Each chromosome set is pulled into its corresponding daughter cell through the action of the mitotic spindle (Figure 12.6). The mitotic spindle is made up of microtubules, which in turn are made up of tubulin molecules (Figure 12.7). That is where the vinca's attack: they bind to the tubulins and prevent them from assembling into microtubules. Instead of adding to the microtubules, the tubulins become bound by vincristine or vinblastine and aggregate into "paracrystalline" structures that were of no use to the cell (Na and Timasheff, 1982) (Figures 15.8 and 15.9).

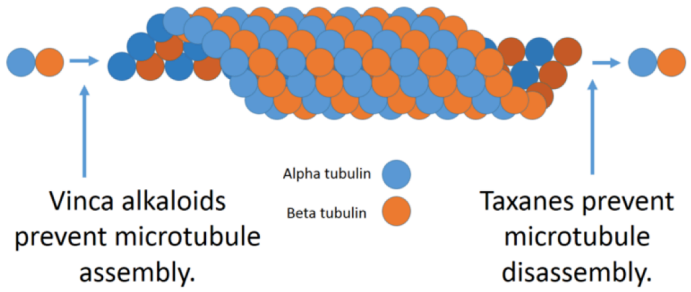


Figure 12.7. Microtubule are made up of alpha and beta tubulin units (blue and red). The Vinca alkaloids, vinblastine and vincristine, were found to prevent tubulins from assembling into microtubules, whereas taxanes were found to lock the tubulins in place, so that the microtubules could not function. Either way, the progress of mitosis was blocked. Microtubule function required that tubulin units be able to add at one end and be removed at the other end. The vincas made microtubules shrink and disappear, whereas the taxanes caused microtubules to accumulate in functionless bundles (Lobert et al., 1996).

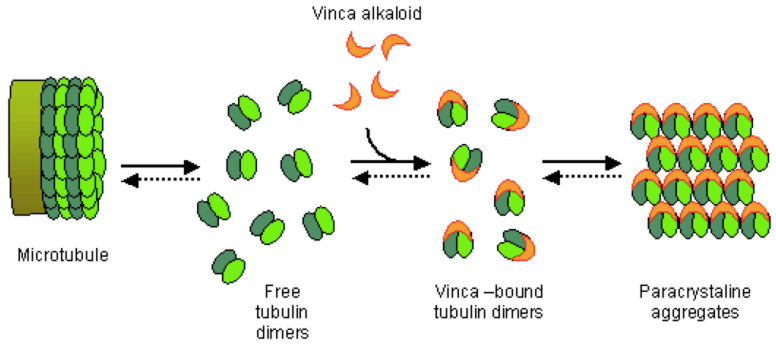


Figure 12.8. How vincristine or vinblastine sequestered tubulin into useless paracrystalline aggregates. The end of a microtubule is depicted on the left. It consists of alpha- and beta-tubulin units (blue and green), which pair up. Vinca molecules (orange crescents) bind tubulin pairs (heterodimers) and assemble them into useless paracrystalline aggregates (right). (Source: Wikipedia.)

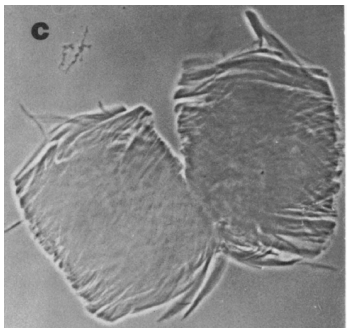


Figure 12.9. Paracrystalline bodies formed by vinblastine-bound tubulins (Na and Timasheff, 1982)

Vinblastine is found to be effective against lymphomas.

In the early 1950's, Cutts had already noted that vinca extracts and the purified vinblastine -- the first vinca alkaloid to be isolated and tested -- inhibited the production of blood cells in the bone marrow of rats (Figure 12.2). He thought it might therefore work against leukemias, which are malignancies arising in the bone marrow. When given to leukemic mice, the drug indeed extended their lifespan (Cutts et al., 1960; Johnson et al., 1960).

In view of the impressive activity of vinblastine in mice, clinical investigators at the Ontario Cancer Institute in Toronto, Canada administered the drug to patients with advanced stages of leukemia, lymphoma, or other malignant tumors (Warwick et al., 1960). At about the same time, a preliminary clinical trial of vinblastine was also carried out at the Indiana University Medical Center (Hodes et al., 1960). In both studies, a few patients had partial remissions, but the results, although encouraging, were insufficient for firm conclusions. The main toxicity, as expected, was suppression of white blood cells (leukopenia). Since that time vinblastine has not been very useful in cancer treatment. But its sister drug, vincristine, became very useful indeed.

Vincristine is the star.

Vinblastine and vincristine differed in how effective they were against different malignant tumor, possibly due to differences in how much of the drug enters particular types of tissues (Zhou et al., 1990). The most striking difference however was the extraordinary effectiveness of vincristine against acute leukemia.

Even though vincristine differed from vinblastine only in the addition of an oxygen atom to an important part of the molecule (Figure 12.4), it became much more useful in cancer treatment, especially for acute leukemia. The first clinical study of vincristine for the treatment of acute leukemia in children was carried out in 1962

at the National Cancer Institute by Myron R. Karon, Emil J Freireich, and Emil ("Tom") Frei (Karon et al., 1962). Karon unfortunately died of a cerebral hemorrhage in 1974 at the age of 42 at the height of his career as a leading researcher and pediatric oncologist (Hersh et al., 1975).

Frei and Freireich went on to lead the development of cancer chemotherapy and the cure of childhood leukemia. In the initial study, with Myron Karon, they escalated the vincristine dose slowly while closely monitoring the blood counts and bone marrow of their patients. When dangerous toxicity threatened, they lowered the dose of vincristine, and transfused whole blood, platelet-rich plasma, or leukocytes as needed. Of 12 children with acute lymphocytic leukemia who were treated with vincristine in that first study, 8 had a complete remission. This experience was the first indication that vincristine would become a major part of the cure of acute leukemia (Said and Tsimberidou, 2014).

Vincristine had a leading role in the cure of acute lymphoblastic leukemia in children as part of the VAMP combination: Vincristine +Amethopterin (methotrexate) + 6-Mercaptopurine (6MP) + Prednisone. The VAMP story is told by John Laszlo in his book about the cure of childhood leukemia (Laszlo, 1995).

But vincristine damages the nervous system.

Researchers were pleased that vincristine rarely produced serious toxicity to the blood-cell-forming bone marrow. However, they were not at all pleased that the amount of drug given to patients had to be limited to avoid damage to the nervous system (neurotoxicity) (Legha, 1986; Rosenthal and Kaufman, 1974). The first sign of neurotoxicity was numbness and tingling in the fingers and toes (Legha, 1986). The reason was simple. In addition to depleting the microtubules of the mitotic spindle, vincristine attacked the microtubules that fill the nerve cell's axon; the nerve cell needs those axonal microtubules to function and survive. The axons long enough to reach the tips of fingers and toes contain the longest microtubules and therefore were the most vulnerable to the drug. Vincristine caused the axons to degenerate and the nerve cells to die.

How might the neurotoxicity of vincristine be avoided?

Much effort was devoted to trying to reduce vincristine's neurotoxicity. At early stages of treatment, the ill-effects on peripheral nerves were reversible if the drug was discontinued; therefore, the dosage had to be kept within those limits. Several substances were investigated for possibly reducing this toxicity. Of those, glutamic acid received the greatest attention, and early studies were encouraging (Jackson et al., 1988). However, recent studies unfortunately failed to confirm that hope (Bradfield et al., 2015).

In recent attempts to reduce toxicity in general and neurotoxicity in particular, vincristine has been incorporated into sub-microscopic fatty globules called liposomes (Raj et al., 2013). Toxicities may be less for the liposomal-vincristine, but the extent to which it reduced toxicity in relation to therapeutic efficacy remained uncertain. Within acceptable dosage, however, vincristine maintained an important role in cancer therapy, particularly in the cure of acute leukemia of children.

Danger of inadvertent injection of vincristine into the spinal fluid.

Even though air travel has become very safe, a serious accident sometimes happens. Similarly, much care is required to eliminate serious medical mistakes due to human error. Since vincristine attacks the microtubules in nerve axons, one of the worst medical mistakes would be if the drug were accidentally injected into the spinal fluid. How could that happen? Here is what happened not so long ago in Thailand to a 12-year old girl with acute lymphoblastic leukemia who was receiving treatment that would probably have cured her and saved her life (Chotsampancharoen et al., 2015). On the day of the error, she was to receive an intravenous injection of vincristine and a spinal injection of methotrexate as an essential part of the leukemia cure: methotrexate kills any leukemic cells in the central nervous system and does not damage normal brain cells. Vincristine fortunately is kept out of the central nervous system by the blood-brain barrier. Thus, vincristine acts safely against the leukemic cells outside of the central nervous system. Methotrexate is injected directly into the spinal fluid in order to kill any leukemic cells that may lurk within the central nervous system. But it is disastrous if the two drugs were accidentally mixed up as to which was injected where – which is what happened in this tragic case. The injection kit that was provided for treatment of the leukemic child was provided with 2 syringes, each properly labeled for what it contained: vincristine or methotrexate. Somehow, the administering team mixed up the 2 syringes and used the vincristine syringe for the spinal injection. The team realized their mistake almost immediately and tried to flush the drug out of the spinal canal. Nevertheless, despite all efforts for supportive care, the child suffered badly and died 5 days later. This case, as well as previous cases, were published with suggestions for additional safeguards to avoid such errors (Chotsampancharoen et al., 2015; Gilbar, 2012; Gilbar, 2014).

Taxol and the Pacific yew tree.

To find new anti-cancer drugs from nature, the National Cancer Institute began in 1960 under the direction of Jonathan L. Hartwell an ambitious program to collect natural products and screen them for their ability to kill cancer cells. If something kills cancer cells, however, it doesn't mean that it necessarily has anti-cancer activity, because the substance might kill normal cells just as well. Some of the toxic

extracts of plants or animals were selected to test whether they prolonged the life of tumor-bearing mice, which would suggest that the material was killing the cancer without killing the animal. The road from there to a useful anti-cancer drug however could be long and tortuous, and is well illustrated by the story of taxol, or "paclitaxel" as it is now called.

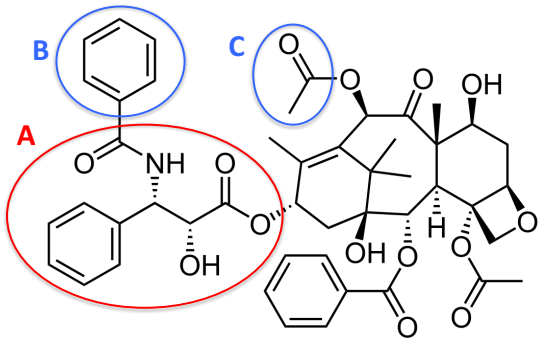
It is remarkable that three scientists who discovered taxol and initiated an understanding of how it worked had also contributed similarly to the discovery of another natural product, the major anticancer drug, camptothecin, which was the subject or the previous chapter. The three were organic chemists Monroe E. Wall and Mansukh C. Wani, and biochemist Susan Band Horwitz (Figures 11.1 and 11.3). Wani and Horwitz described how they purified the drug and determined its chemical structure and mechanism of action (Wani and Horwitz, 2014). Other details of the story, including its political aspects, were told by Jordan Goodman and Vivien Walsch in *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug* (Goodman and Walsh, 2001).

Figure 12.10. Left: The Pacific yew tree (*Taxus brevifolia*), the source of paclitaxel (Taxol). Right: Peeling the bark (image from the National Cancer Institute. Public domain.)

The Taxol story began in 1962, when Arthur S. Barklay, a botanist working for the U.S. Department of Agriculture (USDA) collected bark from a Pacific yew tree (*Taxus brevifolia*) in Washington State (Figure 12.10). The USDA had been commissioned by the National Cancer Institute to collect samples of plants from which extracts were to be prepared and tested for activity against a cancer cell line. Extracts of the yew bark indeed killed cancer cells in culture.

There was not enough material to test it adequately in tumor-bearing mice, so they resorted to testing their materials for action against cells in culture. A natural products chemist however might be inclined to go after a biologically active compound even without knowing whether it may turn out be useful. Monroe E. Wall and his colleagues at Research Triangle Park in North Carolina however hoped that an anti-cancer agent was lurking within the bark extracts, and they had the skills, patience and determination to go after it. In 1964 they started a huge effort to carry this out. It required testing on cancer cells at all stages of a seemingly endless separation sequence. After 2-3 years of painstaking work, they had a purified material that prolonged the lives of cancer-bearing mice. They named the new drug "taxol," in honor of the genus name of the tree it came from, and by 1971 they had determined its chemical structure (Figure 12.11) (Wani et al., 1971). The drug was later renamed "paclitaxel" when Taxol became the brand name.

A glance at the complicated chemical structure of the taxanes (Figure 12.11) gives an appreciation of the difficulty of solving the structure. Not only does the molecule have a great many atoms, but the atoms are arranged in a complicated way with interlocking rings and many asymmetric centers (indicated by the thick and hatched bonds).



Paclitaxel (Taxol)

A, microtubule binding site
B, replaced by $(CH_3)_3CO-$ in docetaxel
C, removed in docetaxel

Figure 12.11. Structure of paclitaxel (Taxol) and the changes in docetaxel (Taxotere). The part of the molecule in the red circle (A), which is the same in paclitaxel and docetaxel, is where microtubules bind (de Weger et al., 2014). The blue circles show the parts of the paclitaxel molecule that were changed in going from paclitaxel to docetaxel. Removing the atoms in C left a hydrogen attached to the oxygen; that H can dissociate, leaving a negative charge on the oxygen, which can be shared with the oxygen to its right. The resulting negative charge makes docetaxel more water-soluble than paclitaxel.

More studies of paclitaxel and a new taxane: docetaxel.

Taxol (paclitaxel), like the vinca drugs, bound and blocked microtubules (Figure 12.7). Although the mechanism of the blockage was different, the net effect was to prevent microtubule function in the mitotic spindle and in the long axons of neurons. Taxol, like the vincas, was toxic to the central nervous system. Moreover, very little of the drug would dissolve to allow it to be injected. These problems spurred the search for new paclitaxel-like drugs that would have less toxicity and better solubility.

A promising candidate was developed in 1981 from a compound extracted from another species of yew tree, the European yew *Taxus piccata* (de Weger et al., 2014). The extracted compound was itself inactive, but chemists had the insight to modify its structure in a way that conferred paclitaxel-like actions. The new drug, docetaxel (brand name Taxotere) had a chemical structure like paclitaxel with two simple modifications (Figure 12.11) that gave the drug better solubility and better clinical results: thus, it became mainstream in cancer treatment and research.

Clinicians did not give up on paclitaxel, however, because when one of the taxanes didn't work, sometimes the other did. Also, the paclitaxel solubility problem was attacked in another way that turned out had other advantages. The drug was combined with albumin in aggregates of sub-microscopic clusters called nanoparticles (Henderson and Bhatia, 2007). Albumin is a blood protein having high solubility that also has the ability to bind many kinds of low-solubility molecules and distribute them through the blood stream. The new advantage was that these nanoparticles tended to leak out of blood vessels in tumors more easily than from blood vessels in normal tissues; therefore, the drug was somewhat selective for delivery into cancer tissues. Nab-paclitaxel, as the albumin conjugated form was called, was found to be clinically more effective than conventional paclitaxel (Henderson and Bhatia, 2007).

Nab-paclitaxel became first-line treatment, for example, of metastatic pancreatic cancer. But it extended life expectancy by a mere 2 to 8 months and was more toxic (peripheral neurotoxicity and fatigue in about 17% of patients) relative to previously available treatment (Hoy, 2014). This disease was extremely deadly and difficult to treat, so any positive effect was seen as a clue to better therapy.

How the taxanes produced their anticancer effects.

Interest in taxol languished for several years because it was difficult to acquire enough bark material from which to isolate the drug, and because the drug's activity in leukemia test systems in mice was deemed mediocre (Wani and Horwitz, 2014). The chemical structure of taxol (Figure 12.11) was too complicated for chemists to

synthesize routinely in the laboratory. However, interest in the drug mounted when taxol was found to work unusually well against a mouse melanoma called B16.

Susan Horwitz then made an important discovery. Working at the Albert Einstein College of Medicine in New York, she found that taxol blocked cells in mitosis by perturbing the function of the microtubules that make up the mitotic spindle; moreover, the way in which the drug affected microtubules was novel (Figure 12.7) (Schiff et al., 1979; Schiff and Horwitz, 1980).

(For many years, we had a small Taxol tree, acquired I think by our lab chief David Rall, growing in the hall of our laboratory building – 5th floor of building 37 on the NIH campus in Bethesda. The tree was located in the Northwest corner of the hall that went all around the exterior next to the windows before the interior was rebuilt. That exterior hall had added cheer to our windowless labs and allowed a pleasant walk around as brief relief from long laboratory hours; some of us used to - gather to view the sunset and share our latest ideas. The redesign was in part fired by the misguided notion that the exterior hall was wasted space.)

Microtubules are composed of two types of subunits: the protein molecules alpha and beta-tubulin, which associate in pairs in a manner that produces alternating alpha-beta pairs in intact microtubules (Figures 15.7). Taxol binds to a specific site on beta-tubulin in the intact microtubule (Figure 12.12). The microtubules of the mitotic spindle are a framework on which various motor proteins apply forces to move the chromosomes appropriately during mitosis. It is a complicated process in which tubulin molecules are added to one end of a microtubule and removed from the other end. That is how a microtubule grows and shrinks and moves to its proper place in the spindle. Essentially, the way taxol blocks microtubule function and the difference from the vincas is shown in Figure 12.7. But, anyone brave enough to read all the details -- including the role of GTP in microtubule function, which is not shown in the Figure -- could find them in a comprehensive review article by Walczak and Heald (Walczak and Heald, 2008). More details can also be found in the early articles by Susan Horwitz and coworkers (Schiff et al., 1979; Schiff and Horwitz, 1980) and in more recent review articles (Orr et al., 2003) and (Wani and Horwitz, 2014).

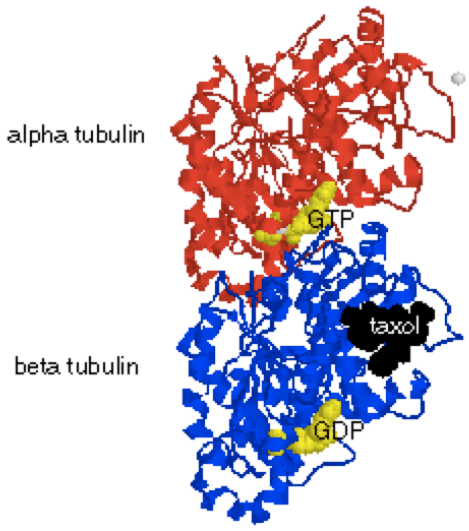


Figure 12.12. Structure of an alpha and beta tubulin pair (dimer) showing the backbones of those protein molecules. The place where Taxol binds, which is on the beta subunit, is shown in black. Red, alpha-tubulin; blue, beta-tubulin. In yellow, are GDP and GTP, which are small energy-bearing molecules that are essential for the structure and function of microtubules.

Therapy with vinca and taxane drugs.

Paclitaxel (as Taxol had by then been renamed) was becoming promising for treatment of several common cancers that were unresponsive or had developed resistance to other drugs. But two problems emerged that limited the dose levels that could be given to patients (Rowinsky and Donehower, 1995). First, was neurotoxicity: nerve damage, especially to the long nerves leading to the tips of fingers and toes. As already noted for vincristine, those long nerves are especially sensitive to anti-microtubule drugs, because long nerve axons contain long microtubules (Kudlowitz and Muggia, 2013) (Kudlowitz and Muggia, 2014). Those microtubules carry essential molecules from the neuron's cell body where they are made all the way to synapses at the end of the axon.

The second problem was more specific to paclitaxel. Many patients developed hypersensitivity akin to the severe reactions that some people have to shellfish or beestings. The reactions to paclitaxel were sometimes even life-threatening. Here is how that problem arose: Paclitaxel was so insoluble that an injectable preparation could not be made directly. Therefore, the drug was mixed with an oily substance called cremaphore, which is a chemical modification of castor oil. That solved the solubility problem, but created another problem: the hypersensitivity reactions that

patients were experiencing turned out not to be due to paclitaxel itself, but to the cremaphore additive (Rowinsky and Donehower, 1995).

Taxanes in combination chemotherapy.

Cancer of the ovary had shown, albeit meager, responses to alkylating agents such as chlorambucil, and the addition of cisplatin was found improve the responses. In the mid-1990's, the standard of treatment for advanced ovarian cancer was the combination of cisplatin and the alkylating agent, cyclophosphamide. However, the outlook for the patients improved when paclitaxel replaced cyclophosphamide in the combination (McGuire et al., 1996) (Figure 12.13).

There was however a caveat to this kind of clinical trial. The possibility was not excluded that increasing the dosage of cyclophosphamide a little or administering it on a different time schedule might have given results as good as paclitaxel in the cisplatin-combination. That was one reason that multiple trials under slightly different conditions would be needed for firm conclusions.

Although early phase I trials that combined paclitaxel with topotecan suggested that this combination merited further investigation (Lilenbaum et al., 1995; O'Reilly et al., 1997), adding topotecan to a paclitaxel-carboplatin combination in treatment of phase III ovarian cancer gave no benefit (Bookman et al., 2009). (Carboplatin equaled cisplatin in effect against the cancer, but it was less toxic.)

The standard of care of advanced ovarian cancer became surgery followed by treatment with a combination of paclitaxel and carboplatin. After that drug treatment had been established, clinical researchers searched for and determined the best time schedule by which to administer the drugs (Lee and Tan, 2018). That was one of the better results of a 2-drug combination with a taxane. Evidently, there was a long way to go in the treatment of advanced cancers.

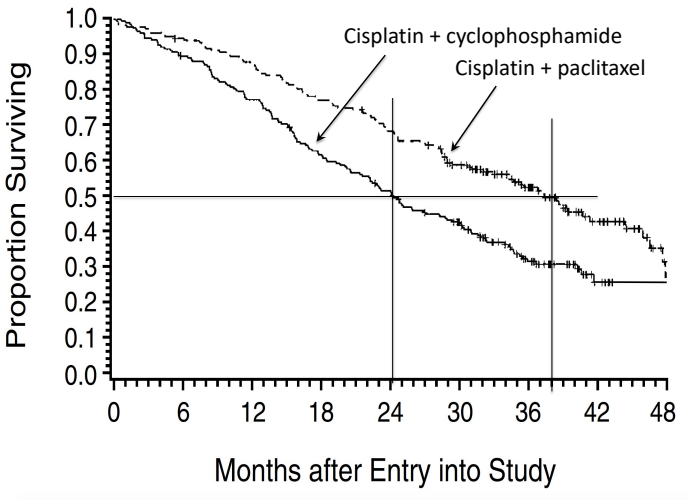


Figure 12.13. Paclitaxel + cisplatin was better than cyclophosphamide + cisplatin for treatment of ovarian cancer that had spread locally or metastasized to distant sites (stage III or IV) (from (McGuire et al., 1996), annotated). When paclitaxel replaced cyclophosphamide in the combination, the median survival time (the time when 50% of the patients were still living) went from 24 months to 38 months. Their life-expectancy went from 2 years to more than 3 years.

In retrospect, much was accomplished in discovering and developing the taxanes. As with many other conventional anticancer drugs, the taxanes also attacked dividing normal cells. Thus, paclitaxel and docetaxel, either as single agent or in combination with other drugs, sometimes slowed the progression of some of the most common cancers. But the benefit was to prolong life usually only for a few months and only in a fraction of the patients. The tumors soon stopped responding to the drug by acquiring mutations that lowered the ability of the drugs to bind microtubules or to inhibit their function (Orr et al., 2003). Moreover, the quality of life during those few added months was often degraded by the toxicity and side effects of the treatment. Much was learned about the taxanes and how they affect cancer as well as normal cells. But the impact on the most common cancers was painfully limited. That might not be surprising, considering that these materials evolved as biological warfare poisons in nature.

From a marine sponge, a new microtubule inhibitor, halichondrin B, and from it a synthetic derivative, eribulin.

In the quest for new and better anti-cancer drugs, the National Cancer Institute began collecting invertebrate marine animals and tested extracts for ability to kill cancer cells (Vindya et al., 2015). They hoped that some of the toxins made by those creatures in their natural biological warfare might be useful against cancers. One of

the most promising came from a rare Japanese sponge called *Halichondria okadai* (Figure 12.14) (Hirata and lJemura, 1986; Swami et al., 2015). Extracts from this organism were extraordinarily potent in killing cancer cells in culture, and the active component became a new anticancer drug, halichondrin B. The isolation of this rare molecule and the determination of its complicated chemical structure were themselves a *tour de force* (Figure 12.15). But, coupled with the novel way its mechanism of action was unraveled makes this a truly remarkable achievement. The NCI team credited with this work is depicted in Figure 12.16.

Chemists isolated the most active toxin, which they named halichondrin B, and determined its complicated chemical structure (Figure 12.15). The drug held promise, because it suppressed several human tumors transplanted into immune-deficient mice ("xenograft tumors") (Fodstad et al., 1996). Further progress was hampered however, because it was difficult to obtain enough material from that rare sponge, and the chemical structure was too complex to prepare routinely in the laboratory. Therefore, chemists prepared simpler structures by leaving out parts of the full Halichondrin B molecule, hoping to hit upon a compound that had the desired activity and that was feasible to synthesize in sufficient quantity. That effort yielded a promising new anti-cancer drug: eribulin (Figure 12.15) (Dybdal-Hargreaves et al., 2015) (Thara and Gitlitz, 2014). The chemists could be congratulated for having the insight that allowed them to select a small part of the halichondrin molecule that was active and that they could synthesize in the laboratory. Moreover, the new synthetic drug, eribulin, had better solubility than the parent halichondrin.

Halichondria

Figure 12.14. Halichondria, the type of marine sponge from which halichondrin B was extracted. (From Wikipedia.)

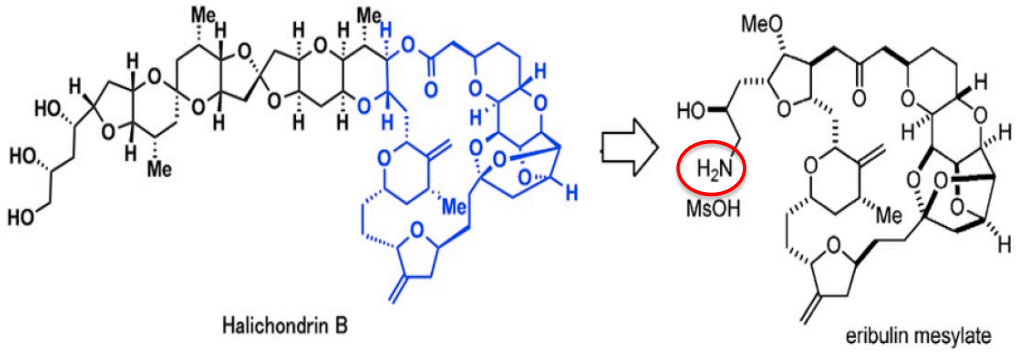


Figure 12.15. Chemical structures of the natural product Halichondrin B (left) and its synthetic derivative Eribulin (right). The latter has a positive charge on the NH2 group (red circle), which is paired with the negatively charged mesylate (MsOH) ion . The additional charged group makes eribulin more soluble than halichondrin. The part of the halichondrin B molecule that is preserved in eribulin is shown in blue (Swami et al., 2015). (*Permission needed.*)

Figure 12.16. The National Cancer Institute's halichondrin team in 1992. From left to right: Robert Shoemaker, Ernest Hamel, George Pettit, Kenneth Paull, Michael Boyd (Shoemaker, 2006). (All were NCI staff, except for George Pettit who was Professor of Chemistry at Arizona State University and worked under NCI contract.)

How Halichondrin B was found to be a microtubule inhibitor.

That halichondrin was a mitotic inhibitor was first indicated by cell toxicity assays in the National Cancer Institute's 60 human cell lines (NCI-60) (see Chapter 20) (Figure 12.17). The pattern of toxicity (inhibition of cell growth and/or increase in cell killing) among the cell lines showed that halichondrin B had similar effects to

those of maytansine, a known microtubule inhibitor. This mechanism of action was confirmed by Earnest Hamel at the NCI, who showed that halichondrin B binds tubulin and inhibits its assembly into microtubules in a manner similar to vinca alkaloids (Bai et al., 1991).

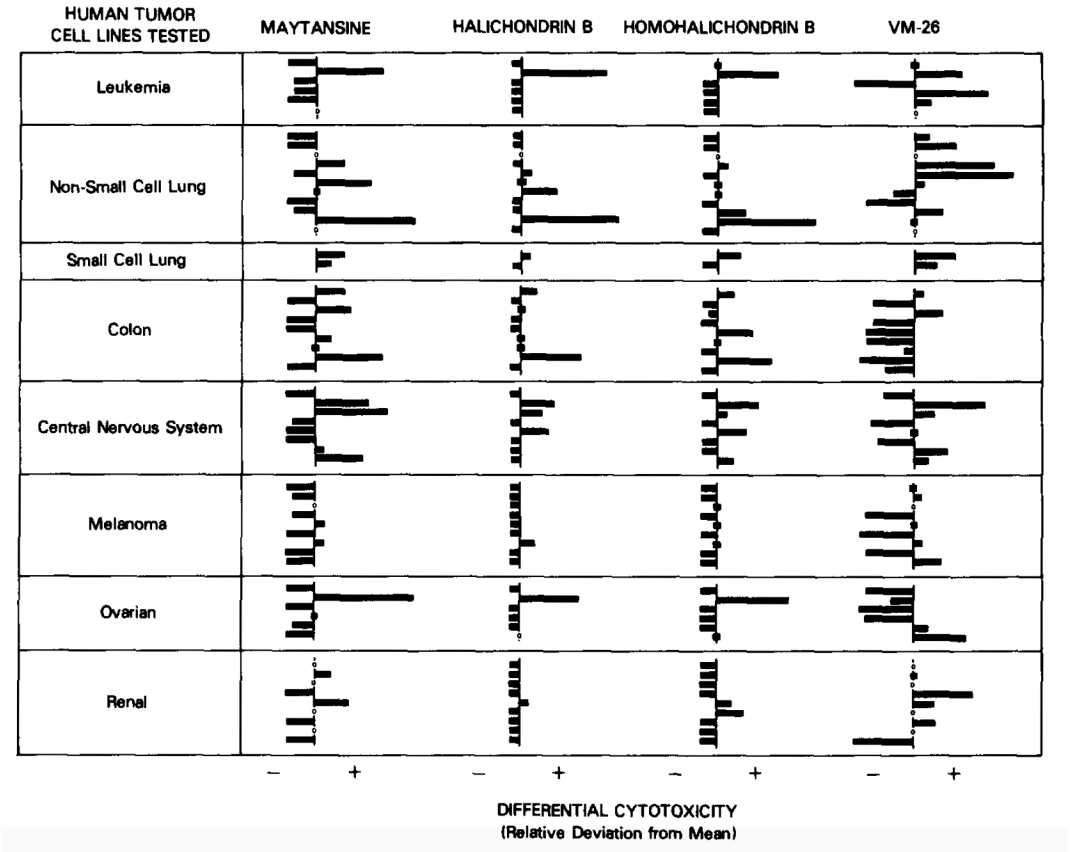


Figure 12.17. Toxicity patterns (inhibitions of cell growth and/or increase in cell killing) in the National Cancer Institute's 60 cell line panel (NCI-60) (Chapter 20). The pattern for halichondrin (2nd from the left) resembled that of maytansine (a known microtubule-targeted drug) (left-most) and differed from the pattern of VM26 (teniposide, a topoisomerase II targeted drug) (Bai et al., 1991). This was the first clue that halichondrin targets mitotic microtubules. This method of comparing anticancer cell activities was developed by NCI's Kenneth Paull.

Other mitotic inhibitors: prospects and surprises

Many other microtubule-targeted inhibitors were isolated from plants and animals or made in the laboratory by chemists who continued to modify their structures in hope of finding new useful drugs (Jiang et al., 2006; Jordan et al., 1998). Several have been tested in early clinical trials (phase 1 or 2), but none of them have, as of this writing, become part of our anti-cancer armamentarium.

Aside from microtubules, there are other essential mitotic spindle components that are being investigated as potential anti-cancer targets (Jiang et al., 2006). Although none have yet been demonstrated to have useful anti-cancer action, we can briefly state what those targets are inhibitors that target (1) kinesin motor proteins that move chromosomes during mitosis by attaching to and pulling on spindle microtubules, (2) aurora kinases that are required to initiate the formation of the mitotic spindle, and (3) polo-like kinases that are required to turn on the machinery that initiates mitosis. This gives an idea of the extensive terrain remaining for researchers to explore for new mitotic inhibitors.

A small change converts a microtubule blocker into a topoisomerase blocker.

Podophyllin has a long history of medicinal use by Native Americans -- it was used as a suicide agent by the Iroquois (Kelly and Hartwell, 1954). In recent times, it became the source of two very different types of drugs, although, remarkably, both types derived from the same chemical structure. First, podophyllin (podophyllotxin) was found to be a mitotic inhibitor like colchicine and vinblastine. A small change in chemical structure however yielded the important anti-cancer epipodophyllotoxins etoposide and teniposide, which work in an entirely different way: they target topoisomerase II (Chapter 10).

Podophyllin resin is made from the mayapple, also known as the American mandrake. The active podophyllotoxin is in the plant's roots, leaves, and creeping underground rhizomes, which were used by Native Americans as an emetic and cathartic and to expel parasitic worms from the intestinal tract (Small and Catling, *Canadian Medicinal Crops*, NRC Research Press 1999, cited by Wikipedia). The first U.S. Pharmacopeia (1820) listed podophyllin as a cathartic but was dropped in the 12th revision (1942). Interest revived when it as found an effective dermatologic treatment of condyloma acuminata (genital warts now known to be caused by certain human papilloma viruses (HPV)) (Kelly and Hartwell, 1954; King and Sullivan, 1946).

Investigating why podophyllin was effective against genital warts, King and Sullivan (King and Sullivan, 1946) applied it to the skin of rabbits and noted unusual changes in the nuclei of the skin cells. They thought that many of the cells were in a distorted state of mitosis, similar to that produced by colchicine. These simple observations, reported in a brief note in *Science* in 1946, led the researchers to a correct idea about what the drug does to cells.

In a subsequent note in *Science*, Sullivan and Wechsler (Sullivan and Wechsler, 1947) looked at the effects of podophyllin in onion root tips, a convenient tissue for study of mitosis in the rapidly proliferating cells of the growing root. They

confirmed the colchicine-like block of mitosis and thought podophyllin useful for cell division studies; they noted that "podophyllin is readily available at pharmaceutical supply houses and may be obtained at approximately 90 cents for four ounces."

Podophyllin was tested in a variety of experimental systems, including tumors in mice, and clinically, especially in treatment of various skin conditions, but the main lasting clinical application has been for genital warts. The extensive history of podophyllin studies and trials was compiled by Margaret Kelly and Jonathan Hartwell in the NCI's former Laboratory of Chemical Pharmacology (Kelly and Hartwell, 1954).

Colchicine revisited.

Colchicine, a product of *Colchicum automnale*, a plant of the Lily family, has long been used in the treatment of gout, tracing back to the Byzantine physician Alexander of Tralles in 550 AD (Kumar et al., 2017). It has many actions in therapy of inflammatory diseases, largely based on its ability to inhibit the polymerization of tubulin by binding to a site at the interface between the alpha and beta subunits (Cheng et al., 2020). The colchicine binding site is distinct from the binding sites of taxol and the vincas. The drug has been reported to bind at a terminus of the microtubule, thereby preventing elongation at that end and leading to its depolymerization. The higher dose required for antitumor activity, however, is close to the toxicity level: an unfavorable therapeutic ratio. The drug therefore has not been useful in cancer therapy. In view of its unique microtubule binding site, the colchicine molecule has been synthetically modified in attempts to create new anticancer drugs, but so far without clinical success.

Mitotic inhibitors: overview

The unusual sources and chemistry of the major mitotic inhibitor drugs may at first be puzzling. Unlike DNA damaging agents, they do not have highly reactive (covalent bond-forming) chemistries. Unlike the DNA synthesis inhibitors, they are not analogs of vitamins or molecules of the cell's normal biochemistry. They are not antibiotics such as are produced by microorganisms. Instead, they are complicated molecules that do not at all resemble any of the cell's normal molecules. Also, they derive almost exclusively from plants or animals, including marine invertebrates. They almost all come from creatures whose cells have a nucleus ("eukaryotes") that undergoes mitosis; in other words, mitotic inhibitors are made almost exclusively by organisms that engage in mitosis. They seem to be poisons used in the competition (biological warfare if you will) among eukaryotes in nature.

This chapter was about 3 classes of mitotic inhibitors: vinca alkaloids, taxanes, and halichondrins that have established roles in cancer chemotherapy. However, there are other classes of mitotic inhibitors, most of them from eukaryotic animals or plants (Jordan et al., 1998). Several are or have been in clinical development; some have been discarded as ineffective or too toxic, but several remain promising and are still being studied.

Mitotic inhibitors bind and disable the microtubules whose function is required for cell division. But microtubules also have other important functions in the cell, functions that do not involve cell division. Inhibition of mitosis however seems to be the major anti-cancer action of these drugs. We have seen that some of these drugs disrupt microtubules in the axons of nerve cells, resulting in sometimes severe neurotoxicity.

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