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Microtubule Targeting Anti-mitotic Agents as Anti-Cancer Drugs: A Review

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ABSTRACT:

Microtubules are highly dynamic cytoskeletal fibers that are composed of tubulin subunits. Dynamic microtubules continue to be one of the most successful cancer chemotherapeutic targets. Cancer cells, in bare contrast to the usual controlled cell proliferation of normal cells, continue to grow and divide to new abnormal cells or tumors. They also possess an even more dangerous property of invading nearby tissues and metastasizing at distant sites in the body. Interestingly, a large number of chemically diverse substances bind to soluble tubulin and/or directly to tubulin in the microtubules. Most of these compounds are antimitotic agents and inhibit cell proliferation by acting on the polymerization dynamics of spindle microtubules, the rapid dynamics of which are essential to proper spindle function. Among the most successful microtubule-targeted chemotherapeutic drugs are paclitaxel and the Vinca alkaloids, which act through the suppression of microtubule dynamics, rather than increasing or decreasing microtubule-polymer mass. Microtubule-active drugs generally bind to one of three main classes of sites on tubulin, the paclitaxel site, the Vinca domain and the colchicine domain. Many new drugs that target microtubules are in clinical trials and large numbers of microtubule-active compounds are being developed.

***Keywords**: Microtubules, tubulin, cancer, mitosis, paclitaxel, colchicine, vinblastine*

1. INTRODUCTION:

1.1 What are microtubules (MTs)?

Microtubules, key components of the cytoskeleton, are long, filamentous, tube-shaped protein polymers that are essential in all eukaryotic cells (1). They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signalling, and in cell division and mitosis. Microtubules are composed of α-tubulin and β-tubulin heterodimers (of dimensions 4 nm ×5 nm × 8 nm and 100,000 daltons in mass) arranged in the form of slender filamentous tubes that can be many micrometres long (FIGS 1).

They are highly dynamic polymers and their polymerization dynamics are tightly regulated both spatially and temporally (2). The functional diversity of microtubules is achieved in several ways: through the binding of various regulatory proteins, including microtubule associated proteins (MAPs), to soluble tubulin and to the microtubule surfaces and ends; by expression of different tubulin isotypes, which have different functions; and through several post-translational modifications of tubulin.

The extensive involvement of microtubules in mitosis and cell division makes them an important target for anticancer drugs (3). Microtubules and their dynamics are targets of

chemically diverse groups of anti-mitotic drugs that have been used with great success in the treatment of cancer (4). In fact, it has been argued that microtubules represent the best cancer target to be identified so far, and it seems likely that drugs of this class will continue to be important chemotherapeutic agents.

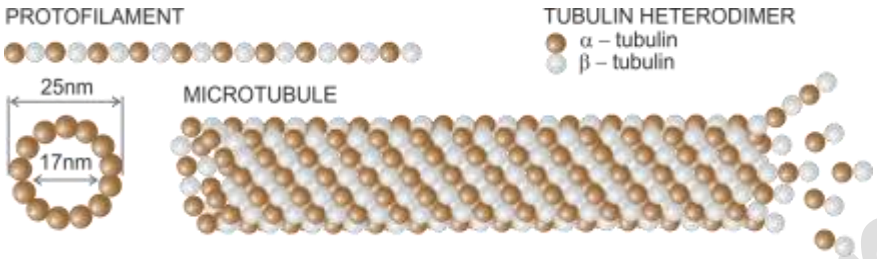

Figure 1: **Polymerization of microtubules.** Heterodimers of α- and β-tubulin assemble to form a short microtubules nucleus

1.2 What is Cancer?

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death (6).

Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

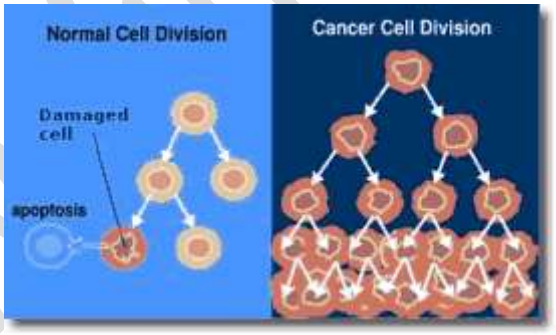

Figure 2: Normal Cell division vs. Cancer Cell division

Cancer starts when cells in a part of the body start to grow out of control. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells (Figure 2). Cancer cells can also invade other tissues, something that normal cells cannot do. Growing out of control and invading other tissues are what makes a cell a cancer cell (6).

Cells become cancer cells because of DNA (deoxyribonucleic acid) damage. DNA is in every cell and it directs all the cell's actions. In a normal cell, when DNA gets damaged the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired,

and the cell doesn't die like it should. Instead, the cell goes on making new cells that the body doesn't need. These new cells all have the same abnormal DNA as the first cell does.

People can inherit abnormal DNA, but most DNA damage is caused by mistakes that happen while the normal cell is reproducing or by something in the environment. Sometimes the cause of the DNA damage may be something exposure to carcinogens. But it's rare to know exactly what caused any one person's cancer. In most cases, the cancer cells form a tumor. Some cancers, like leukemia, rarely form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow.

Cancer cells often travel to other parts of the body where they begin to grow and form new tumors. This happens when the cancer cells get into the body's bloodstream or lymph vessels. Over time, the tumors replace normal tissue. The process of cancer spreading is called metastasis.

1.3 What is Mitosis?

Mitosis is the process by which a cell duplicates the chromosomes in its cell nucleus in order to generate two, identical, daughter nuclei. It is followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two daughter cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the mitotic (M) phase of the cell cycle (Figure 3). Mitosis is a normal cellular process necessary to sustain life, but its deregulation in one form or another is found in all cancer cells. Mitosis can often become abnormal by the change in, or absence of, the normal mitotic checkpoints (5).

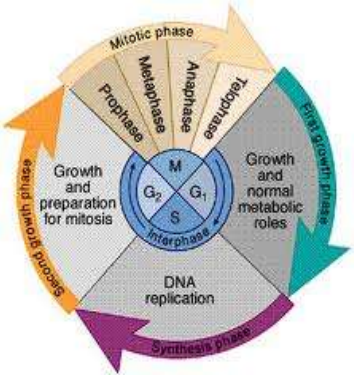

Figure 3: Cell cycle in Mitosis

1.4 Mitotic Checkpoints:

Mitotic checkpoints are points in the cell cycle which act to ensure correct transmission of genetic information during cell division. These checkpoints look for abnormalities within the cycle, specifically chromosomal aberrancy. Checkpoints take place towards the end of each phase of mitosis and must be passed before the cell can get clearance to enter into the next stage of mitosis (7).

If errors are found during checkpoints, the cell acts quickly to correct them, arresting cell growth and not proceeding with mitosis until the error has been fixed. If these errors cannot be fixed, the cell normally undergoes apoptosis, or programmed cell death (8).

When carcinogens or other factors lead to mutations, those accumulate over time and extend past the cell cycle to the cellular machinery itself. The cell is allowed to move through the cell cycle and grow unchecked.

These mutations, in combination with the genetic mutations accrued through abnormal mitotic progression, eventually cause the cell to be completely deregulated in its growth and proliferation. It becomes unstoppable and even immortal.

2. ANTIMITOTIC AGENTS: ONE POSSIBLE TREATMENT

Anti-tumor agents inhibit the function of microtubules through the binding of their subunits or through direct cessation of their growth. Microtubules are created during normal cell functions by assembling (through polymerization) tubulin components, and are disassembled when they are no longer needed. One of the important functions of microtubules is to move and separate chromosomes and other components of the cell for cell division (mitosis). Mitotic inhibitors interfere with the assembly and disassembly of tubulin into microtubule polymers. This interrupts cell division, usually during the mitosis (M) phase of the cell cycle when two sets of fully formed chromosomes are supposed to separate into daughter cells. The crucial involvement of MTs in mitosis makes them a prime target for anti-cancer agents.

The specific effects of individual microtubule-targeted drugs on the microtubule polymer mass and on the stability and dynamics of the microtubules is complex. Microtubule-targeted antimitotic drugs are usually classified into two main groups. One group, known as the microtubule-destabilizing agents, inhibits microtubule polymerization at high concentrations and includes several compounds – such as the Vinca alkaloids (vinblastine, vincristine, vinorelbine, vindesine and vinflunine), cryptophycins, halichondrins, estramustine, colchicine and combretastatins— that are used clinically or are under clinical investigation for treatment of cancer (Table 1). The second main group is known as the microtubule-stabilizing agents. These agents stimulate microtubule polymerization and include paclitaxel (the first agent to be identified in this class), docetaxel (Taxotere), the epothilones, discodermolide, the eleutherobins, sarcodictyins, laulimalide, rhazinalam, and certain steroids and polyisoprenyl benzophenones.

3. SPECIFIC ACTION OF FEW DRUGS:

3.1 Taxanes: Isolated from the bark of the Western yew tree in 1971, the compound paclitaxel (Figure 3) became useful in the treatment of cancer when it was discovered that it possessed the unique ability to promote the formation of microtubules by binding to their β-tubulin subunit and antagonizing their disassembly. However, the amount of paclitaxel in yew bark was small, and extracting it was a complicated and expensive process. Its development for clinical use was impeded by limited supplies of the natural compound until procedures for its semi-synthesis form of paclitaxel (docetaxel) was found, derived from the needles and twigs of the Himalayan yew tree *Taxus bacatta*, which is a renewable resource. The FDA approved docetaxel in 1995 and it is now widely used to treat breast and ovarian cancer, non-small-cell lung cancer and Kaposi's sarcoma.

The binding site for paclitaxel is in the β-subunit, and its location, which is on the inside surface of the microtubule, is known with precision because determination of the electron crystal structure of tubulin was carried out with tubulin complexed with paclitaxel (9). Paclitaxel prevent the growth of cancer cells by affecting microtubules. They enhance microtubule formation, and then they stop the microtubules from being broken down so that the cells become so clogged with microtubules that they cannot continue to grow and divide (10). This result in the cell's arrest in mitosis which eventually leads to cell death or apoptosis.

Figure 3: Paclitaxel, Docetaxel and Western yew

The clinical success of the taxanes has led to a search for other drags that enhance microtubule polymerization, yielding several promising compounds, including the epothilones, discodermolide, the sarcodictyins, eleutherobin and laulimalide. Some of these compounds compete with paclitaxel for binding to microtubules and are said to bind at or near the taxane site.

Resistance to taxanes is a complicating factor to successful treatment and is often associated with increased expression of the *mdr-1* gene and its product, the P-glycoprotein. In addition, some resistant cells also display increased aurora kinase, an enzyme that promotes completion of mitosis. These drugs are very expensive and must be administered in large amounts at once cannot be tolerated in many patients.

3.2 Vinca Alkaloids: The Vinca alkaloids are all derived from the Madagascan periwinkle plant, *Vinca rosea*. The plant was reputed to be useful in the treatment of diabetes. Attempts to verify the antidiabetic properties of the plant's extracts, in the 1950's, led instead to the discovery and isolation of vinblastine. Scientists first observed its anticancer properties in a lab in 1962 with the observation of regression of lymphocytic leukemia in rats. Several years later, the successful purification of the plant's alkaloids yielded three other active dimers: vincristine, vinorelbine, vinrosidine (Figure 4).

Tubulin and microtubules are the main targets of the Vinca alkaloids, which depolymerize microtubules and destroy mitotic spindles at high concentrations leaving the dividing cancer cells blocked in mitosis with condensed chromosomes (11). The Vincas work through their ability to bind to the β-tubulin subunit of microtubules, blocking their ability to polymerize

with the α-tubulin subunit to form complete microtubules at low but clinically relevant concentrations, vinblastine does not depolymerize spindle microtubules, yet it powerfully blocks mitosis and cells die by apoptosis.

Vinblastine is used in the treatment of: bladder and testicular cancers, Kaposi's sarcoma, neuroblastoma and Hodgkin's disease, vincristine for pediatric leukemias and lymphomas, non-Hodgkin's lymphoma, neuroblastoma and rhabdomyosarcoma and Vinorelbine used in the treatment of lung carcinoma, breast cancer (12).

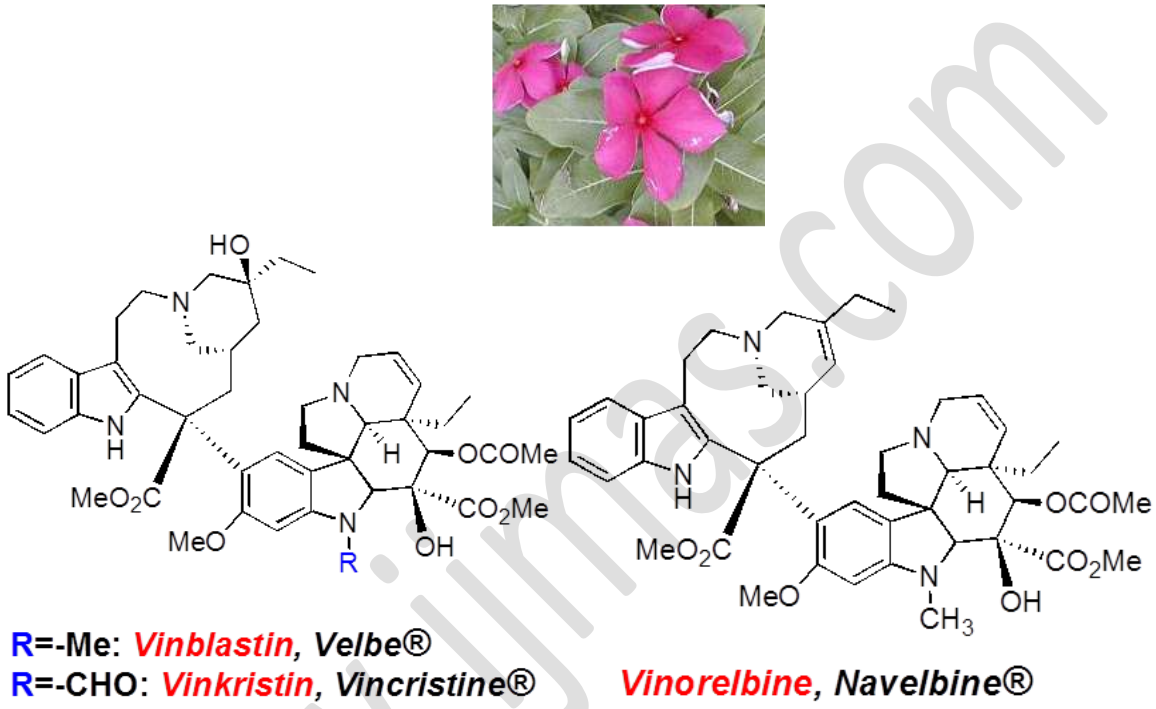

Figure 4: Vinblastin, Vinkristine, Vinorelbine and Vinca rosea

Resistance to the Vinca alkaloids comes in the form of cross-resistance due to the structural similarity of the four compounds, and their antitumor effects are blocked by multidrug resistance in which tumor cells become cross-resistant to a wide variety of agents after exposure to a single drug. Also, because of the heavy concentration of microtubules in the brain and the drug's disruption of this, patients treated with Vinca alkaloids can experience severe neurotoxicity.

3.3 Colchicine: The colchicine alkaloid was initially isolated in 1820 from Meadow-saffron, *Colchicum autumnale* (Tidlos) seeds and was found to bind tubulin, the protein subunit of microtubules (Figure 5). While it has been shown to kill cancer cells, the drug's usefulness in the treatment of cancer is hindered by its cytotoxicity (13). Colchicine has proven to have a fairly narrow range of effectiveness as a chemotherapy agent, so it is only FDA-approved to treat gout. The interaction of colchicine with tubulin and microtubules presents yet another variation in the mechanisms by which microtubule-active drugs inhibit microtubule function (14). Colchicine is believed to bind to soluble tubulin, induces slow conformational changes in the tubulin and ultimately forms a poorly reversible final-state tubulin–colchicine complex,

which then copolymerizes into the microtubule ends in small numbers along with large numbers of free tubulin molecules. The ends remain competent to grow but their dynamics are suppressed (15).

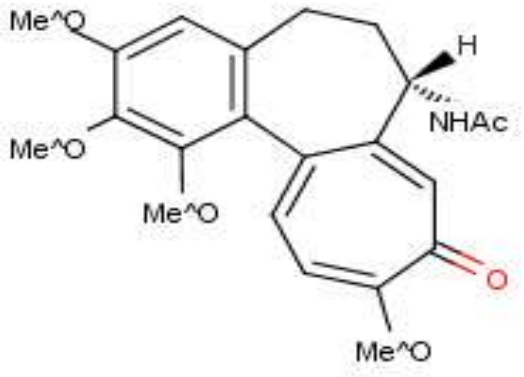

Figure 5: Colchicine and Colchicum autumnale

4. CONCLUSION:

So, despite the differences between the effects at high concentrations of the Vinca/colchicine-like drugs and the taxane-like drugs, nearly all of the microtubule-targeted antimitotic drugs stabilize microtubule dynamics at their lowest effective concentrations. Stabilization of microtubule dynamics correlates with blocking of the cell cycle at mitosis and in sensitive tumour cells, ultimately resulting in cell death by apoptosis. Therefore, the most potent mechanism of nearly all of the microtubule-targeted drugs seems to be stabilization of dynamics of mitotic-spindle microtubules.

Side effects with antimitotic agents, as with much chemotherapy, can be debilitating and even fatal. Chemotherapy targets rapidly-dividing cells, which includes cancer cells but also hair and gut cells. This results in hair loss and nausea in patients. Much research remains to be done in this area of cancer treatment to minimize toxicity.

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