**Review Article**

# Cancer: An Overview

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**ABSTRACT:**Cancer is the second leading causes of deaths in western countries after heart disease. In US alone more than one million people are diagnosed with cancer annually and more than half result in death. Cancer is a medical condition where abnormal cells in body divided without any control. Cancers cells do not respond normally to the body's control mechanism, so they divide excessively and invade the neighboring tissues. Cancer starts when a normal cell is instantly detected by body immune system. However, if the abnormal cells remain at the original site, it will result in the less severe condition termed as benign tumor. Benign tumor can be completely removed by surgery and pose little threat. Malignant tumors have capability to enter the blood stream invading other tissues and disrupting the normal function of various systems in the body. They are difficult to treat may become life threatening. Cancer cells can spread to distinct locations from the original site by a process called metastasis.

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## CANCER

Cancer is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth causing a lump called a tumor. This is true of all cancers except leukemia (blood cancer). If left untreated, tumors can grow and spread into the surrounding normal tissue, or to other parts of body via the bloodstream and lymphatic systems and can affect the digestive, nervous and circulatory systems [1].

**TYPES OF TUMORS:** Tumors (lumps) can be benign or malignant

- Benign tumors are not cancerous and rarely threaten life [2].They tend to grow quite slowly, do not spread to other parts of the body and are usually made up of cells quite like normal/healthy cells [3]. They will only cause a problem if they grow very large, becoming uncomfortable or press on other organs, for example a brain tumor inside the skull.

- Malignant tumors are faster growing than benign tumors and can spread and destroy neighboring tissue. Cells of malignant tumors can break off from the main (primary) tumor and spread to other parts of the body through a process known as metastasis. Upon invading healthy tissue at the new site, they continue to divide and grow. These secondary sites are known as metastases and the condition is referred to as metastatic cancer [4].

## CLASSIFICATION OF CANCER:

Cancer can be classified according to the following categories: [4]

**Carcinoma -** A cancer that arises from the epithelial cells (the lining of cells that helps protect or enclose organs). Carcinomas may invade the surrounding tissues and organs and metastasis to the lymph nodes and other areas of the body. The most common forms of cancer in this group are breast, prostate, lung and colon cancer.

- **Sarcoma –** A type of malignant tumor of the bone or soft tissue (fat, muscle, blood vessels, nerves and other connective tissues that support and surround organs). The most common forms of sarcoma are leiomyosarcoma, liposarcoma and osteosarcoma.

- **Lymphoma –** Lymphoma is a cancer of the lymphatic system, which runs all through the body and can therefore occur anywhere. The two main forms are non-Hodgkin's which begins with uncontrolled growth of the white blood cells- lymphocytes of the immune system and Hodgkin's lymphoma in which cells of the lymph nodes become cancerous.

- **Leukemia –** Leukemia is a cancer of the white blood cells and bone marrow, the tissue that forms blood cells. There are several subtypes; common are lymphocytic leukemia and chronic lymphocytic leukemia.

**CAUSES OF CANCER:**

- **Cancer Causing substances (Carcinogens) –** Genes are coded messages inside a cell that tell it how to behave (i.e. which proteins to make). Mutation or changes to the gene, such as damage or loss can alter how that cell behaves. For example, a mutation may mean that too much protein is made or that protein is not made at all. Significantly, there needs to be a number of genetic mutations [4]. Within a cell before it becomes cancerous. Something that damages a cell, changing its behavior and makes it more likely to be cancerous is called a 'carcinogen'.

- **Age –** Many types of cancer become more prevalent with age. The longer people live, the more exposure there is to carcinogens and the more time there is for genetic changes or mutations to occur within their cells[3].

- **Genetics –** Some people are unfortunately born with a genetically inherited high risk for a specific cancer (genetic predisposition). This does not mean developing cancer is guaranteed but a genetic predisposition makes the disease more likely. For example, women that carry the BRCA1 and BRCA2 (breast cancer genes 1 and 2) breast cancer genes have a higher predisposition to developing this form of cancer than women with a normal breast cancer risk [2]. However, less than 5% of all breast cancer is known to be due to genes. So, although women with one of these genes are individually more likely to develop breast cancer, most cases are not caused by a high risk inherited gene fault. This is true of other common cancers where some people have a genetic predisposition. For example, colon (large bowl) cancer.

- **The immune system –** People who have weakened immune systems are more at risk of developing some types of cancer. This includes people who have had organ transplants and take drugs to suppress their immune systems to stop organ rejection, plus people who have HIV or AIDS or other medical conditions which reduce their immunity to disease.

Certain lifestyles and environmental factors are also known to cause mutations that can cause cancer.Lifestyle and environmental causes are to a large extent controllable or avoidable.

- **Body weight, diet and physical activity –** Cancer experts estimate that maintaining a healthy bodyweight, making changes to our diet and taking regular physical activity could prevent about one in three deaths from cancer. Many people eat too much red and processed meat and not enough fresh fruit and vegetables. This type of diet is known to increase the risk of cancer.

- **Overweight or obesity –** 'Obese' means being more than about 25% overweight. Overweight or obese people have an increased risk of bowel and pancreatic cancer, probably due to tendency towards higher insulin levels. Obesity can also increase the risk of cancer of the food pipe (esophageal cancer), kidney and gall bladder cancer as well as breast or womb (uterine) cancer in women.

- **Alcohol –** The evidence that all types of alcoholic drinks are a cause of several cancers is now stronger than ever before [5]. Alcohol can increase the risk of several cancers including mouth, throat (which includes pharyngeal cancer), laryngeal and cancer of the food pipe, plus liver, breast and bowel cancer (in men). Even moderate alcohol intake increases the risk of cancer.

- **Tobacco –** Tobacco smoke contains at least 80 different cancer-causing substances (carcinogenic agents). When smoke is inhaled the chemicals enter the lungs, pass into the blood stream and are transports throughout the body [3]. Therefore, smoking or chewing tobacco not only causes lung cancer and mouth cancers but is also related to many other cancers. The more a person smokes, the younger they start and the longer they keep smoking, all further increase the risk of cancer.

- **Ionizing radiation –** Manmade sources of radiation can cause cancer and are a risk for workers. The main risk is however, prolong and unprotected exposure to ultraviolet radiations from the sun which can lead to melanoma and skin malignancies [6]. Fair skinned people, those with a lot of moles or who have a relative who has had melanoma or non-melanoma skin cancer are at highest risk.

- **Work place hazards –** Some people risk being exposed to a cancer-causing substance because of the work that they do. For example, workers in the chemical dye industry have been found to have a higher incidence than normal of bladder cancer. Asbestos is a well-known work place cause of cancer,

particularly a cancer called mesothelioma [3]. which most commonly affects the covering of the lungs (pleura).

- **Infection –** A proportion of cancers can be caused by infection with a virus. However, this does not mean that these cancers can be caught like an infection rather the virus can cause changes in cells that make them more likely to become cancerous.

Examples include cervical cancer linked to the Human Papilloma Virus, primary liver cancer which can be caused by the Hepatitis B and Hepatitis C virus and lymphomas linked to the Epstein-Barr virus [4].

Bacterial infections have not been thought of as cancer causing agents in the past. But studies have shown that people who have *Helicobacter pylori* infection of their stomach develop inflammation of the stomach lining, which increases the risk of stomach cancer.

## SIGN AND SYMPTOMS OF CANCER:

As there are so many different types of cancer so the symptoms are varied and depend on where the disease is located. However, there are some key signs and symptoms[7] including:

- **Lumps –** Some cancers can be felt through the skin. Cancerous lumps are often painless and may increase in size as the cancer progresses.

- **Coughing, breathlessness –** Persistent coughing episodes and breathlessness can be associated with lung cancer.

- **Changes in bowel habit –** Symptoms of bowel cancer may include blood in the stools and a change in bowel habits such as constipation and diarrhea.

- **Bleeding –** Any unexpected bleeding can be a sign of cancer.

  o Bleeding from the anal passage may be a sign of bowel cancer.

  o Bleeding from the cervix may be a sign of cervical cancer.

  o Blood present in the urine may be a sign of kidney or bladder cancer.

- **Unexplained weight loss –** A large amount of unexplained weight loss over a short period of time (a couple of months) can be a sign of cancer.

- **Fatigue –** Fatigue is extreme tiredness and a severe lack of energy. If fatigue is due to cancer, sufferers normally also have other symptoms.

## METASTASIS OF CANCER:

Metastasis, the spread of cancer from its primary site to a distant organ is responsible for the major cause of morbidity

and mortality of cancer suffering patients rather than the primary tumors. Five-year survival rates for breast cancer drop from nearly 100% when the cancer is localized to less than 25% when the cancer has colonized distant sites.

When cancer is detected at an early stage, prior to its wide spreading, surgery excision or irradiation with the primary tumor followed by chemotherapy and immunotherapy could have successful treatment effects to patients. However, when cancer is detected to be in metastatic stages such treatments are much less effective. Furthermore, those patients in whom there is no evidence of metastasis at the time of their initial diagnosis, metastases could still be detected in a later time along with the cancer recurrence. The breast cancer, for example, is likely to occur metastatic growth even years after the patient has been declared cancer free[8] and nearly one-third of breast cancer patients would have positive for metastatic disease at the time of initial diagnosis[9].This suggests that a sub-population of tumor cells had undergone therapy resistance and disseminated survived at secondary distant sites to recommence uncontrolled growth. The transformation from a non-malignant to a malignant metastatic phenotype is an evolutionary process in which tumor cells progressively acquire aggressive characteristics that result from or are a consequence of selection of a sub-population of cells that are eventually capable of completing all the metastatic cascade steps[10].

Typically, the process of metastasis has been viewed as a series of sequential and interrelated biological steps. These steps include dissociation of malignant cells in the primary tumor, local invasion, angiogenesis, intravasation of invading cells into the vasculature or lymphatic systems survival in these channels, extravasation and proliferation at a distant site. The formation of primary tumor lesion is a dominant requirement for metastasis and it is estimated that approximately $1 \times 10^6$ cells escape into circulation per gram of primary tumor daily [11]. Only a fraction of cells leaving the primary tumor survive in circulation and even fewer cells colonize at the secondary sites [12].

As a primary tumor grows, it needs to develop a blood supply that can support its high proliferation with sufficient amount of oxygen and nutrient and metabolic needs – a process termed angiogenesis. Meanwhile, these new blood vessels also provide an escape channel by which cells can leave the tumor and enter into the circulation system [13]**.** Besides, tumor cells could also enter the circulation system indirectly via the lymphatic system. The action that tumor cells escape from the primary site to circulation system via either blood vessels or lymphatic vessels is usually called intravasation. While circulating in the blood system, tumor cells can arrest in a new organ by extravasting from the circulation into the surrounding tissue. The arrival of the tumor cells at the secondary site implies that the tumor cells have completed all antecedent steps in the metastatic cascade.

Upon disseminated, tumor cells are believed to be regulated by their immediate micro-environment whereas there is emerging evidence that pre-conditioning of arresting sites may occur before tumor cells reach the secondary site. It has been evidenced that melanoma cells released soluble factors that stimulated lung fibroblasts to secrete fibronectin creating an attachment site for the arrival of α4β1/α4β7 integrins, vascular

endothelial growth factor receptor (VEGFR) positive progenitor cells. These progenitor cells then secreted metalloproteinases which released from the matrix, including stromal derived factor-1 (SDF-1) that attracted chemokine receptor 4 (CXCR4) positive tumor cells [14].

Formation of a tumor mass at the secondary site is believed to follow some of the same steps as in primary tumor growth. The term 'colonization' is used herein to implicit the combined influences of tumor cell proliferation, apoptosis. Dormancy and angiogenesis in the formation of a progressively growing lesion in distant organs. However, the major distinction is that these disseminated cells grow in an ectopic environment. Hence, only those cancer cells 'seed' that are able to adopt to the new environment – 'soil'; or those that already possess the ability to respond to microenvironmental signals at the 'foreign' site; or those cells that can modify the new microenvironment can be expected to thrive. In 1889, Stephen Paget reported the 'seed-soil' model to illustrate the tumor metastasis processes [15]. Paget hypothesized that their interaction determines metastatic outcome: "When a plant goes to seed, its seeds are carried in all directions but they can only live and grow if they fall on congenial soil".

This observation predicted that the tissue environment, composed of a myriad of specialized cell types, extracellular matrices and cells recruited to the site, may facilitate tumor metastasis and contribute to the organ selectively sometimes seen in metastatic colonization. However, this idea was challenged in the 1920s by James Ewing, who suggested that circulatory patterns between a primary tumor and specific secondary organs were sufficient to account for organ specific metastasis [16]. In a later experiment, Hart and Fidler confirmed Paget's hypothesis of non-random tumor cell growth by implanting kidney, lung and ovary tissue fragments in the thighs of mice and showed that B16 melanoma cells injected intravenously grew only in implanted lung or ovarian tissue [17]. These preliminary but pioneering experiments demonstrated that disseminated cells arrested in the implants at essentially similar numbers but still showed organ selectively for the growth. Therefore, the role of vasculature in organ selective tumor seeding and growth, although important may be only secondary site and the tumor cell itself.

In fact, these two theories are not mutually exclusive and current evidence supports a role for both factors. In a series of autopsy studies, Leonard Weiss documented that larger numbers of bone metastases than would be expected based solely on blood flow patterns were identified for both breast and prostate cancer, for example. In contrast, fewer numbers of skin metastasis than expected based on blood flow patterns were found for osteosarcomas, stomach and testicular cancers [18].

Out of the 16 primary tumor types and 8 target organs that were analyzed, metastasis in 66% of the tumor-type-organ pairs seemed to be adequately explained based on blood flow alone whereas the remainders were not. For these remaining tumors, negative interactions that is, fewer metastasis than expected based on blood flow between the primary and secondary sites between cancer cells and the environment of the metastatic site were found in 14% of cases and positive interactions were found in 20% of cases[19].

To associate a molecular event with metastasis, its occurrence in primary tumors or disseminated cells is correlated with survival or other indicators such as disease-free survival or the presence of regional lymph node metastases. Most mechanistic insights into metastasis are derived from xenograft studies in rodents [20].

Typically, a tumor cell line known to metastasize in vivo is manipulated to change the expression or mutation status of a single gene. In spontaneous assays, the tumor cells are injected into a site, a primary tumor forms and metastases develop. It is preferable to inject cells into an orthotropic location, the tissue of origin. This assay measures the complete metastatic process but suffers from poor quantification and slow completion. In experimental metastasis assays, tumor cells are injected into the blood stream from the tail vein or other sites. Metastases form more quickly than in spontaneous assays and in greater numbers, facilitating statistical analysis.

However, a drawback of experimental metastasis assays is that only part of the metastatic process, the post intravasation stage is modeled. To overcome the inherent shortage, several transgenic mouse strains are developed to have both primary tumors and spontaneous metastases and are crossed to other genetically engineered mice to determine effects on metastasis. In preclinical studies, a compound is administered to animals either just after injection of the tumor cells or after metastases has formed, constituting prevention and treatment studies, respectively. Veterinary animals including dogs are increasingly used to test therapeutics in the metastatic setting and for a subset of cancers, their Pathophysiology may more closely resemble to that of humans[21].

## SCREENING METHODS OF ANTICANCER ACTIVITY:

### *IN VITRO* METHODS

**Trypan blue dye exclusion assay** [22]

The trypan blue dye exclusion assay is the most commonly utilized test for cell viability. In this assay, the cells are washed with HBSS (Hank's Buffered Salt Solution) and centrifuged for 10-15 min at 10,000 rpm. The procedure is repeated thrice. The cells are suspended in known quantity of HBSS and the cell count is adjusted to 2 □ 106 cells/ml. The cell suspension is distributed into Eppendorf tubes (0.1 ml containing 2 lakhs cells). The cells are exposed to drug dilutions and incubated at 37 oC for 3 hours. After 3 hours, dye exclusion test i.e. equal quality of the drug treated cells are mixed with tryphan blue (0.4%) and left for 1 min. It is then loaded in a hemocytometer and viable and non-viable count is recorded within 2 min. Viable cells do not take up color, whereas dead cells take up color. However, if kept longer, live cells also generate and take up color. The percentage of growth inhibition is calculated using the following formula:

$$\text{Growth inhibition (\%)} = 100 - \frac{(\text{Total cells - Dead cells})}{\text{Total cells}} \times 100$$

**LDH (Lactic Dehydrogenase) Assay** [23]

Lactic Dehydrogenase activity is spectrophotometrically measured in the culture medium and in the cellular lysates at 340 nm by analyzing NADH reduction during the pyruvatelactate transformation. Cells are lysed with 50 mM Tris-HCl buffer, pH 7.4 + 20 mM EDTA +.05% Sodium Dodecyl Sulfate (SDS), further disrupted by sonication and centrifuged at 13,000 X g for 15 min.

The assay mixture (1 ml final volume) for the enzymatic analysis consists of 33 µl of sample in 48 mM PBS, pH 7.5 + 1 mM pyruvate and 0.2 mM NADH. The percentage of LDH released is calculated as percentage of total amount, considered as the sum of the enzymatic activity present in the cellular lysate and that in the culture medium.

## MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] Assay [24]

The MTT assay, based on the conversion of the yellow tetrazolium salt- MMT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of $2 \square 105 \ mL^{-1}$ per well in 100 µL of RPMI 1640 and allowed to grow in $CO_2$ incubator for 24 hours (37$^o$C, 5 % $CO_2$). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 hours. The cells are incubated for 24-48 hours (37$^o$C, 5% $CO_2$). Then, 20 µL MTT {[3-(4,5-dimethylyhiazol-yl)-2,5-diphenyltetrazolium bromide]} stock solution (5 mg/ml in PBS) is added to each well and incubated for 5 hours. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560 nm. Untreated cells (basal) are used as a control of viability (100%) and the results are expressed as % viability (log) relative to the control.

## XTT assay [25]

To measure the proliferation response of the cell, the [2,3-bis(2-Methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide] inner salt (XTT) assay is used. The tetrazolium salt, XTT is especially useful in quantifying viable cells. This assay is designed for the spectrophotometric quantification of cell growth and viability without the use of radioactive isotopes and is based on the cleavage of yellow tetrazolium salt, XTT, to form an orange formation dye by metabolically active cells. XTT cleavages into an orange formazan dye by the mitochondrial enzyme, dehydrogenase occurs exclusively in living cells. Cells are grown in growth medium with 10% FBS in 96-well plates until 70-80% confluence. They are then treated with the appropriate drug sample for 24 hours. XTT assay is performed at the end of incubation. Briefly, 50 ml of XTT labeling mixture solution is add to each well and the cells are incubated at 37 oC for 4 hours. The formazan dye formed is soluble in aqueous solutions and the optical density at 450 nm is compared with that of control wells with a screening multiwall apectrophotometer enzyme linked immunosorbent assay (ELISA) reader. The reference wavelength is 650 nm.

## Sulforhodamine B assay [26]

Sulforhodamine B assay is a bright pink amino xanthene dye that binds to basic amino acids in mild acidic conditions and dissociates under basic conditions. Cells are plated in 96 well flat bottom plates at 5000-10000 cell/well. The difference in cell numbers plated adjust for differences in the growth rates of various cell lines. Cells are allowed to adhere to the wells overnight, then the samples are added to triplicate wells in serial 3-fold dilutions.

Water is added to the control wells at a 1:10 dilution in medium. These plates are incubated at 37 oC, 5% CO2 for 3 days and then assayed for growth inhibition using a sulforhodamine B (SRB) assay. The cells are fixed by the addition of cold 50% trichloroacetic acid to a final concentration of 10%. After 1-hour incubation at 4$^o$C, the cells are washed five times with deionized water. The cells are then stained with 0.4% SRB (Sigma) dissolved in 1% acetic acid for 15-30 min and subsequently washed five times with 1% acetic acid to remove unbound stain. After the plates are air dries at room temperature, the bound dye is solubilized with 10 mm Tris base and the plates are analyzed on a microplate reader (Molecular Devices) at 595 nm.

## *IN VIVO* MODEL [27]

### Induction of Ehrlich Ascites Carcinoma

Antitumor activity of the test compounds may determine using Ehrlich ascites carcinoma (EAC) tumor model in mice. The ascetic carcinoma bearing mice (donor) are used for the study, 15 days after tumor transplantation. The animals are divided into groups of 12 animals each:

(a) Normal mice
(b) Tumor bearing mice
(c) Tumor bearing mice treated with standard drug
(d) Tumor bearing mice groups treated with test drug.

The ascetic fluid is drawn using an 18-gauge needle with sterile syringe. A small amount is testing for microbial contamination. Tumor viability is determined by Trypan blue exclusion test and cells are counted using hemocytometer. The ascetic fluid is suitably diluted in normal saline to get a concentration of 106 cells/ml of tumor cell suspension. This is injected intraperitoneally to obtain ascetic tumor. The mice are weighed on the day of tumor inoculation and then once in three days thereafter. Treatment is started on the tenth day of tumor inoculation. Standard (one dose) is injected on tenth day intraperitoneally. The drug is administered from tenth day for 5 days intraperitoneally. After the administration of last dose followed by 18 hours fasting, six mice from each group are sacrifice for the study of antitumor activity and hematological parameters. The remaining animals in each of the groups are kept to check the mean survival time (MST) of the tumor bearing hosts. Antitumor effects of drugs are assessed by observation of following parameters –

i. Percentage increase in weight as compared to day-0 weight.

ii. Median survival time and increase in lifespan (% ILS).

iii. Hematological parameters.

**Ectopic tumor xenograft model**

Human cancer cells are subsequently injected into the hind leg or back of mice. In an ectopic tumor xenograft model (ectopic model), the transplanted site is different from the origin of cultured cells. Tumor volume (V) is calculated from the largest length and the shortest length of the tumor. From several parameters based on this data, anticancer activity can be evaluated.

The ration of treated group (T) to control group (C) (optical % T/C), tumor growth delay and tumor regression were utilized. Drug-related deaths (DRD) and body weight change as parameters of toxicity were determined. DRD was presumed animal deaths within 15 days and over 20% loss of treated mouse body weight compared to control was considered an adverse effect.

$$V\ (mm^3) = \frac{(\text{The largest length})\ X\ (\text{The shortest length})^2}{1}$$

These parameters help to draw the lead compound from drug screening. Occasionally, drug response depending on cancer types could be compared without individual differences because two types of cancer cells could be transplanted spontaneously into the same mouse and the two tumors can show differences in growth. Furthermore, the ectopic model is very reproducible, homogenous and amenable to use.

**Orthotopic tumor xenograft model**

In the orthotopic model, the human cancer cells are transplanted into the same origin site of the tumor. For instance, lung cancer cells were directly injected into the mouse lung for the orthotopic model. Moreover, orthotopic models are limited to measurement of tumor growth without sacrifice unlike subcutaneous ectopic models. Currently, orthotopic models with cancer cell lines expressing fluorescence or luciferase are observed by optical imaging, computerized tomography (CT) or magnetic resonance imaging (MRI). To assess carcinogenesis and determine the tumor growth without sacrifice, expensive equipment is required, so the availability of this model is limited.

**ANTICANCER AGENTS**

- **Alkylating agents:** These highly reactive compounds produce their effects by covalently linking an alkyl group ($R-CH_2$) to a chemical species in nucleic acids or proteins. The site at which the cross-links are formed and the number of cross-links formed is drug specific. Most alkylating agents are bipolar, i.e. they contain two groups capable of reacting with DNA. They can thus form bridges between a single strand or two separate strands of DNA, interfering with the action of the enzymes involved in DNA replication. The cell then either dies or is physically unable to divide or triggers apoptosis. The damage is most serious during the S-phase, as the cell has less time to remove the damaged fragments. Example include –

  o Nitrogen mustards (e.g. Melphalan and Chlorambucil)

  o Oxazaphosphorenes (e.g. Cyclophosphamide, Ifosfamide)

  o Alkyl Alkane Sulphonates (e.g. Busulphan)

  o Nitrosureas (e.g. Carmustine (Bcnu), LomustineCcnu)

  o Tetrazines (e.g. Dacarbazine, Mitozolomide and Temozolomide)

  o Aziridines (e.g. Thiopeta, Mitomucin C)

  o Procabazine

- **Heavy metal**

  o **Platinum agents** – These include carboplatin, cisplatin and oxaliplatin. Cisplatin is an organic heavy metal complex. Chloride ions are lost form the molecule after it diffuses into a cell allowing the compound to cross-link with the DNA strands, mostly to guanine groups. This causes intra- and inter-strand DNA cross-links, resulting in inhibition of DNA, RNA and protein synthesis. Carboplatin has the same platinum moiety as cisplatin but is bonded to an organic carboxylate group. This leads to increased water solubility and slower hydrolysis that has an influence on its toxicity profile. It is less nephrotoxic and neurotoxic but causes more marked myelosuppressio. Oxaliplatin belongs to a new class of platinum atom complexed with oxalate and a bulky diaminocyclohexane (DACH) group. It forms reactive platinum complexes that are believed to inhibit DNA synthesis by forming interstrand and intrastrand cross-linking of DNA molecules. Oxaliplatin is not generally cross-resistant to cisplatin or carboplatin, possibly due to the DACH group.

- **Antimetabolites:** Antimetabolites are compounds that bear a structural similarity to naturally occurring substances such as vitamins, nucleosides or amino acids. They complete with the natural substrate for the active site on an essential enzyme or receptor. Some are incorporated directly into DNA or RNA. Most are phase-specific, acting during the S-phase of the cell cycle. Their efficacy is usually greater over a prolonged period of time, so they are usually given continuously. There are three main classes-

  o **Folic acid antagonists** - Methotrexate competitively inhibits dihydrofolate reductase, which is responsible for the formation of tetrahydrofolate from dihydrofolate. This is essential for the generation of a variety of coenzymes that are involved in the synthesis of purines, thymidylate, methionine and glycine. A critical influence on cell division also appears to be inhibition of the production of thymidine monophosphate which is essential for

DNA and RNA synthesis. The block is activity of dihydrofolate reductase can be bypassed by supplying an intermediary metabolite, most commonly folinic acid. This is converted to tetrahydrofolate that is required for thymidylate synthetase function.

o **Pyrimidine analogues -** These drugs resemble pyrimidine molecules and work by either inhibiting the synthesis of nucleic acids (e.g. fluorouracil) inhibiting enzyme involved in DNA synthesis (e.g. cytarabine, which inhibits DNA polymerase) or by becoming incorporated into DNA (e.g. gemcitabine), interfering with DNA synthesis and resulting in cell death.

o **Purine analogues –** These are analogues of the natural purine bases and nucleotides, 6-mercaptopurine (6MP) and thioguanine are derivatives of adenine and guanine respectively. A sulfur group replaces the keto grouo on carbon-6 in these compounds. In many cases, the drugs require initial activation. They are then able to inhibit nucleotide biosynthesis by direct incorporation into DNA.

- **Cytotoxic antibiotics:** Most antitumor antibiotics have been produced from bacterial and fungal cultures (often Streptomyces species). They affect the function and synthesis of nucleic acids in different ways-

o **Anthracyclines –** (e.g. doxorubicin, daunorubicin, epirubicin) intercalate with DNA and affect the topoiosmerase II enzyme. This DNA gyrase splits the DNA helix and reconnects it to overcome the torsional forces that would interfare with replication. The anthracyclines stabilize the DNA tomoisomerase II complex and thus prevent reconnection of the strands.

o **Actinomycin D –** intercalates between guanine and cytosine base pairs. This interferes with the transcription of DNA at high doses. At low doses DNA-directed RNA synthesis is blocked.

o **Bleomycin –** consists of a mixture of glycopeptides that cause DNA fragmentation.

o **Mitomycin C –** inhibits DNA synthesis by cross-linking DNA, acting like an alkylating agent.

- **Spindle poison:** Vinca alkaloids, the two prominent agents in this group are vincristine and vinblastine that are extracted from the periwinkle plant. They are mitotic spindle poisons that act by binding block of the microtubules. This inhibits further assembly of the spindle during metaphase, thus inhibiting mitosis. Although microtubules are important in other cell functions (hormone secretion, axonal transport and cell motility), it is likely that the influence of this group of drugs on DNA repair contributes most significantly to

their toxicity. Other newer examples include vindesine and vinorelbine.

- **Taxoids:** Paclitaxel (Taxol) is a drug derived from the bark of the pacific yew, *Texusbrevifolia*. It promotes assembly of microtubules and inhibits their disassembly. Direct activation of apoptotic pathways has also been suggested to be critical to the cytotoxicity of this drug. 2-Docetaxel (Taxotere) is a semisynthetic derivative.

- **Topoisomerase inhibitors:** Topoisomerases are responsible for altering the 3D structure of DNA by a cleaving/unwinding/rejoining reaction. They are involved in DNA replication, chromatid segregation and transcription. It has previously been considered that the efficacy of topoisomerase inhibitors in the treatment of cancer was based solely on their ability to inhibit DNA replication.

It has now been suggested that drug efficacy may also depend on the simultaneous manipulation of other cellular pathways within tumor cells. The drugs are phase specific and prevent cells from entering mitosis from G2. There are two broad classes-

o **Topoisomerase I inhibitors –** Camptothecin, derived from Camptotheca acuminate (a Chinese tree), binds to the enzyme- DNA complex, stabilizing it and preventing DNA replication. Irinotecan and topetecan have been derived from this prototype.

o **Topoisomerase II inhibitors –** Epipodophyllotoxin derivatives (e.g. etoposide, vespid) are semisynthetic derivatives of *Podophyllum peltatum*, the American mandrake. They stabilize the complex between topoisomerase II and DNA that causes strand breaks and ultimately inhibits DNA replication.

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