INTRODUCTION

1.1 Introduction to cancer

The term cancer refers to several different diseases that share the common biological characteristic of atypical cell growth. These malignant cells, if untreated metastasize to other parts of the body and lead to the death of the patient. Tumors have been identified in dinosaur bones as early as Jurassic period, more than 150 million years ago. Cancer remains a major health problem throughout the globe as the most feared diagnosis [1]. It stands as the second leading cause of death in many developed countries and its occurrence is increasing annually. In spite of the remarkable advances made by medical sciences, treatment of cancer still remains an enigma. The development of more effective drugs for treating patients suffering from cancer has been a major human attempt over the past several years.

The total cancer cases in India are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020. The tobacco-related cancers for males are estimated to go up from 190,244 in the year 2010 to 225,241 in the year 2020. Similarly, the female cancer cases will rise from 75,289 in year 2010 to 93,563 in the year 2020. For the year 2010, the number of cancer cases related to digestive system, for both males and females, were estimated to be 107,030 and 86,606 respectively. For, head and neck cancers, the estimates were 122,643 and 53,148 cases, respectively. The assessments for the lymphoid and hematopoietic system (LHS), for the year 2010, were 62,648 for males and 41,591 for females. Gynaecological-related cancers are estimated to increase from 153,850 in 2010 to
182,602 in 2020. Among females, cancer of breast alone is expected to cross the figure of 100,000 by the year 2020 [2].

1.2 Characteristics of malignant neoplasms [3-7]

The distinction between benign and malignant tumors is based on morphology and ultimately on behavior, using four criteria:

i) Differentiation and anaplasia

ii) Rate of growth

iii) Local invasion

iv) Metastasis

i) Differentiation and Anaplasia: Differentiation is the extent to which tumor cells bear similarity to the normal cells. Cells within most benign tumors closely mimic corresponding normal cells. Lack of differentiation, called anaplasia is a hallmark of malignant cells. The following cytological features characterize anaplastic tumors:

Nuclear and Cellular Pleomorphism: Extensive variation in the shape and size of cells and nuclei.

Hyperchromatism: Darkly stained nuclei that frequently contain prominent nucleoli.

Nuclear-Cytoplasmic Ratio: Approaches 1:1 instead of 1:4 or 1:6, reflecting enlargement of nuclei.

Abundant Mitoses: Reveal proliferative activity. Mitotic figures may be abnormal.

Tumor Giant Cells: Contain a single large polyploid nucleus or multiple nuclei.

Dysplasia refers to disorderly but non-neoplastic growth. It is characterized by pleomorphism, hyperchromatism and loss of normal orientation. When dysplastic changes are marked and include the entire thickness of the epithelium, the lesion is considered a case, of
preinvasive neoplasm and is referred to as carcinoma *in situ*. This is an indication, in many invasive carcinoma.

ii) *Rate of Growth:* Most malignant tumors grow more rapidly than benign tumors. Some cancers grow slowly for years and then enter a rapid growth phase; others expand rapidly from the onset. Quickly growing malignant tumors often contain central areas of ischemic necrosis because the tumor blood supply fails to keep pace with the oxygen needs of the expanding mass of cells.

iii) *Local Invasion:* Malignant neoplasms are invasive and infiltrating. They destroy the normal tissues surrounding them. There is a lack of well-defined area and plane of cleavage. Surgical treatment of such tumors needs removal of a considerable margin of healthy and apparently uninvolved tissue.

iv) *Metastasis:* The process of metastasis involves invasion of the lymphatics, blood vessels and body cavities by the tumor, followed by transport and growth of secondary tumor cell masses that are discontinuous with the primary tumor. This is the single most important feature that distinguishes benign from malignant tumors. Spread of tumors to distant areas occurs by three routes:

a) *Spread into Body Cavities:* This occurs by seeding of surfaces in peritoneal, pleural, pericardial, and subarachnoid spaces. For example, carcinoma of the ovary spreads transperitoneally to the surface of the liver or other abdominal viscera.

b) *Invasion of Lymphatics:* This is followed by transport of tumor cells to regional nodes and eventually, other parts of the body and is common in the initial spread of carcinomas. Thus carcinomas of the breast spread to either axillary or internal mammary lymph nodes depending on the location of the tumor.

c) *Hematogenous Spread:* This is typical of all sarcomas but also is the favored route for certain carcinomas, such as those originating in the kidney. Veins are more frequently
invaded than arteries due to their thinner walls. Lung and liver are common sites of hematogenous metastasis because they receive the systemic and venous outflow. Other major sites of hematogenous spread include brain and bones.

### 1.3 Epidemiology

A variety of factors affect an individual or a population to the development of cancer [3-7]. They are:

i) **Geographic and environmental factors:** Environmental factors considerably influence the manifestation of specific forms of cancer in different parts of the world. In Japan for example, the death rate from stomach cancer is about seven times than in the United States. Conversely, carcinoma of the colon is less common cause of death in Japan. There is increased risk of certain cancers with exposure to asbestos, vinyl chloride, 2-naphthylamine and association of carcinomas of the oropharynx, larynx and lung with cigarette smoking.

ii) **Age:** Cancer is common in those older than 55 years of age. Certain cancers are predominantly seen in children younger than 15 years of age like leukemias, lymphomas, neuroblastomas, wilms tumors etc.

iii) **Heredity:** Heredity plays a role in the development of cancer. Hereditary forms of cancers can be divided into three categories:

a) Inherited cancer syndromes are characterized by inheritance of single mutant genes that escalates the risk of developing a certain type of tumor.

b) Familial cancers are characterized by familial clustering of specific forms of cancer, but the transmission pattern is not clear in an individual case. Familial forms of common cancers (e.g., breast, colon, brain, and ovary) were recorded.
c) Autosomal recessive syndromes of defective DNA repair are characterized by chromosome or DNA instability that increases the predisposition to environmental carcinogens.

iv) Acquired Pre-neoplastic Disorders: Certain clinical conditions are associated with a bigger risk of developing cancers: e.g. Cirrhosis of the liver - hepatocellular carcinoma. Certain benign tumors are also associated with the subsequent development of cancer.

1.4 Molecular basis of cancer

Cancer is a genetic disease [3-7]. The genetic injury may be acquired in somatic cells by environmental agents or inherited in the germ line. Tumors develop as clonal progeny of a single genetically damaged progenitor cell. Carcinogenesis is a multistep process. The attributes of malignancy like invasiveness, excessive growth, and escape from the immune system are acquired in a stepwise fashion called tumor progression. At the genetic level, progression results from accumulation of successive mutations (Fig. 1.1). Four classes of genes are the targets of genetic damage:

i) Oncogenes and cancer: Oncogenes are genes whose products are associated with neoplastic transformation. Proto-oncogenes are normal cellular genes that affect growth and differentiation, which may be converted to oncogenes by one of the three mechanisms: point mutations, chromosomal rearrangements or gene amplification.

ii) Deactivation of cancer-suppressor genes: Cancer may also arise by inactivation of genes that normally suppress cell proliferation (cancer-suppressor genes, or anti-oncogenes). The RB gene located on chromosome 13ql4 is the prototypic cancer-suppressor gene. The RB gene product controls the advancement of cells from the ‘G1’ to ‘S’ phase of the cell cycle. It is pertinent to the pathogenesis of the childhood tumor, retinoblastoma. The p53 tumor-suppressor gene called the “guardian of the genome” is mutated in greater than 50 % of all
human cancers. People who inherit a mutated copy of p53 gene (Li-Fraumeni syndrome) are at a great risk of developing a malignant tumor by inactivation of the second normal allele in somatic cells. Normal p53 gene prevents the proliferation of genetically damaged cells. When the DNA is impaired by chemicals, UV light or irradiation, the normal p53 gene is up-regulated, and it starts transcription of several genes that cause cell cycle arrest and DNA repair. If during the gap in cell cycle the DNA can be repaired, the cell is allowed to enter ‘S’ phase; however, if DNA damage cannot be repaired, p53 induces apoptosis by increasing transcription of the pro-apoptotic gene. With homozygous loss of p53, DNA damage goes unrepaired, and cells carrying mutant genes continue to divide and ultimately lead to cancer.

Fig. 1.1: Flow chart depicting scheme of molecular basis of cancer [3]
iii) *Genes that regulate apoptosis:* The prototypic gene, BCL-2, prevents apoptosis. Over expression of BCL-2 apparently extends cell survival, and if the cells are genetically damaged, they suffer added mutations in oncogenes and cancer-suppressor genes.

iv) *Genes that regulate DNA repair:* The DNA repair genes act indirectly by correcting damages in DNA that occur naturally during cell division or those that follow exposure to mutagenic chemicals or irradiation. When errors involving DNA mismatch accumulate in proto-oncogenes and tumor suppressor genes, the carcinomas develop.

A single genetic alteration is insufficient to induce cancers. Multiple controls exerted by oncogenes, tumor-suppressor genes, and apoptosis-regulating genes must be lost for the appearance of cancer cells.

1.5 Biology of tumor growth [3-7]

In every population of cell, there are 3 sub-populations. The first group that continuously proliferates by mitosis is cycling cells. The second is terminally differentiated cells that leave the growth cycle and die without dividing again. The third is subpopulation of non-dividing cells that can re-enter the cell cycle, if an appropriate stimulus is applied. The cell cycle is an ordered series of events comprising of several successive phases: the DNA synthetic phase (S), postsynthetic gap (G2), mitosis (M) and presynthetic gap (G1) (Fig. 1.2).

*DNA synthesis phase:* During ‘S’ phase, the nucleus replicates its DNA content. The first check point in the late ‘S’ phase will rectify any DNA damage.

*Post synthetic gap (G2) phase:* Significant biosynthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis.

*Mitosis:* Mitosis is a continuous process that consists of four stages namely prophase, metaphase, anaphase and telophase. Somatic cells whose DNA has been replicated in ‘S’
phase are equally distributed between daughter cells formed in mitosis. The mitotic check point controls spindle formation.

Fig. 1.2: Normal cell cycle [3]

*Presynthetic gap (G1) phase:* Cells synthesize nucleotides and enzymes. This phase has restriction points, which works between middle and late ‘G1’ phase to determine the fate of cell division. The cells may leave cell cycle during ‘G1’ and then either cease proliferation, differentiate and die or enter quiescent phase (G0) from which they may be recruited back into cell cycle at a later time.

*Quiescent (G0) phase:* This is the resting period for the cells. The cells which are likely to divide enter into this phase. Quiescent cells may activate into ‘G1’ by chemical stimuli associated with damage. The impetus for a cell to start off on the cell cycle (i.e., move from G0 to G1) can be provided by several stimuli, the most important being growth factor action.

Most human cells complete the cell cycle in approximately 24 h. Cell division requires controlled timing of two critical events of cell cycle, ‘S’ phase and ‘M’ phase. Entry...
into each of these phases is carefully regulated and this gives rise to two checkpoints in the cell cycle: one at the start of ‘S’ phase and one at the start of ‘M’ phase.

Cells can proliferate by any one of three mechanisms: reducing the length of the cell cycle causing more cell production per unit time; decreasing the rate of cell death and moving ‘G0’ cells into cell cycle again. In cancer, there is sometimes a shortening of cell cycle compared with normal cells or there is an increase in the growth fraction or a decrease in the rate of cell loss.

1.6 Kinetics of tumor cell growth

Three variables influence tumor cell growth [3-7]:

i) Doubling time of tumor cells: The cell cycle of transmuted cells has the same five phases (G0, G1, S, G2, and M) noted in normal cells. The total cell cycle time for many tumors is equal to or longer than that of corresponding normal cells. Hence, rapid tumor growth cannot always be attributed to a shortening of tumor cell cycle time.

ii) Growth fraction (GF): This is the proportion of cells within the tumor population that are in the replicative pool. Most cells within clinically detectable tumors are not in the proliferative pool. Even in some rapidly growing tumors, the GF is approximately 20%. Cells leave the replicative pool by being shed, by differentiating and by reverting to ‘G0’. Thus progressive tumor growth cannot be ascribed to an extremely high GF.

iii) Cell production and loss: Tumor cell accumulation resulting in growth of tumors can be explained by an imbalance between cell production and cell loss.

The rate of tumor growth depends on the GF and the degree of imbalance between tumor cell production and loss. As most antineoplastic agents act on proliferating cells, tumors with higher GFs are the most susceptible to anticancer agents. They are also the most rapidly growing, if left untreated. If all descendants of an originally transformed cell
remained in the replicative pool, most tumors would become clinically detectable within a few months after the initiation of tumor cell growth. The accumulation of tumor cells is a relatively slow process as most tumor cells leave the replicative pool which results in a latent period of several months to years before a tumor becomes clinically detectable.

1.7 Tumor angiogenesis [2-8]

Since tumor cells also need oxygen to survive, vascularization of tumors has a profound influence on tumor growth. In rapidly growing tumors, the rate of growth sometimes exceeds the pace of vascularization, forming areas of ischemic necrosis. Vascularization of tumors is provoked by the release of tumor-associated angiogenic factors derived from tumor cells or inflammatory cells (e.g., macrophages) that enter the tumors. The two most important tumor angiogenic factors are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (BFGF). In addition to angiogenic factors, tumor cells or host cells also produce anti-angiogenic factors which include angiostatin, endostatin, and vasculostatin. Tumor growth is controlled by the balance between angiogenic and antiangiogenic factors. The latter is under study on how to retard tumor growth therapeutically.

The sequential steps involved in invasion and metastasis are depicted in Fig. 1.3. Only certain sub clones can complete all the steps outlined in figure and are able to form secondary tumors at distant sites.

a) Invasion of extracellular matrix: Tumor cells must attach to, degrade and penetrate the extra cellular matrix at several steps of the metastatic cascade. Invasion of extra cellular matrix can be resolved into the following four steps: detachment of tumor cells from each other, attachment to matrix components, degradation of extra cellular matrix and migration of tumor cells.
Fig. 1.3: The metastatic cascade [3]
b) *Vascular dissemination and homing of tumor cells:* In the circulation, tumor cells form emboli by aggregation and adhere to circulating leukocytes, particularly platelets, thereby getting protection from the anti-tumor host effector cells. The site where tumor cell emboli lodge and produce secondary growths is influenced by several factors: vascular and lymphatic drainage from the site of the primary tumor, interaction of tumor cells with organ-specific receptors and the microenvironment of the organ or site.

### 1.8 Carcinogenic agents

Radiation, viruses and many chemicals are identified to be carcinogenic in animals and humans [3-8].

i) *Chemical carcinogenesis:* Neoplastic transformation brought about by chemicals. Carcinogen-induced changes in DNA need not necessarily lead to the initiation of carcinogenesis because the damage to DNA can be repaired by cellular enzymes. If the ability to repair DNA is impaired, the risk of cancer development increases. The different carcinogenic chemicals include:

a) Alkylating agents: These comprise of direct-acting agents, such as Cyclophosphamide and Busulfan, used in the treatment of cancer as well as immunosuppressant. Patients receiving such therapy are at increased risk of developing another cancer.

b) Aromatic hydrocarbons: These are present in cigarette smoke and may lead to lung cancer.

c) Azo dyes: 3-naphthylamine, an aniline dye used in the rubber industries.

d) Naturally occurring carcinogens: Aflatoxin B, produced by the fungus *Aspergillus flams*, is a potent hepato-carcinogen.

e) Nitrosamines and Amides: These can be synthesized in the gastrointestinal tract from ingested nitrites or derived from digested proteins and contribute to the induction of gastric cancer.
f) Miscellaneous agents: Asbestos, vinyl chloride, and metals such as nickel are carcinogenic. They predispose exposed individuals to the development of cancer. Hormones such as estrogens may play a role in causing endometrial cancer.

ii) Radiation carcinogenesis: UV rays and ionizing radiations can cause cancer.
   a) Ultraviolet rays: UV radiation, especially UV-B, derived from the sun can cause skin cancer.
   b) Ionization radiation: Electromagnetic and particulate radiations are carcinogenic as they are able to induce mutations. Particulate radiations (such as α particles and neutrons) are more carcinogenic than electromagnetic radiations (X-rays, γ rays).

iii) Viral and microbial carcinogenesis: A variety of DNA and RNA viruses are known to cause cancer in human beings. e.g. Human Papilloma Virus (HPV), Epstein-Barr Virus (EBV) and Hepatitis B Virus (HBV)

1.9 Treatment of cancer

Cancer treatment employs six established principal modalities [9-12]:


ii) Radiotherapy: Conventional type of treatment with X-rays and γ rays.

iii) Chemotherapy: Most anticancer drugs are anti-proliferative and damages DNA, leading to apoptosis.

iv) Endocrine therapy: Prevents cell division by manipulating specific hormone that is responsible for cell proliferation.

v) Immunotherapy: Recent technique to stimulate our body’s natural immune system to fight disease or to protect the body from some of the side effects of cancer treatment.

vi) Photo-dynamic therapy (PDT): A light sensitive drug (haematoporphyrin derivative) is injected into the body. An endoscope is then used to shine laser light on the cancer cells to
produce a chemical reaction which kills only the malignant cells, leaving the surrounding tissues unaffected.

vii) Gene therapy: involves in the manipulation of genes. This is ideal in treating pre-cancerous conditions especially people having a genetic disposition to certain cancers.

1.10 Systemic cancer chemotherapy

The past two decades have witnessed a remarkable revolution in the field of tumor chemotherapy [9-11]. A spectacular wealth of basic knowledge with regard to molecular and cellular biology, better understanding of mechanisms of cellular division, tumor immunology, fundamental factors that involved in both viral and chemical carcinogenesis and above all the improved investigative techniques have ultimately led to the introduction of a substantial number of newer antineoplastic agents.

Cancers originating from different organs of the body differ in their behavior and in their response to treatments. Primary surgery and / or radiotherapy to a localized cancer offer the best chance of cure for patients. Drug treatments in the past were mainly restricted to patients with disseminated, metastatic disease, where a systemic effect is required. Cytotoxic chemotherapy for advanced disease offers cure for only certain types of cancer, e.g. testicular cancers, Wilms tumor etc. Most often, chemotherapy may prolong life, although patients ultimately die of their disease. Palliation may be achieved by treatment in terms of both increased survival and improved quality of life as a consequence of symptom control at least in the short term. There remain a number of types of cancer which are unresponsive to currently available drugs. Chemotherapy depends on developing drugs that kill malignant cells or modify their growth and leave those of the host unharmed or more usually, harmed but capable of recovery. When there is convincing expectation of cure or extensive life prolongation of good quality life, it is suitable to risk severe drug toxicity, e.g. treatment of
testicular cancer patients with possibly life threatening platinum-based combination chemotherapy regimens offers a greater than 85 % chance of cure, even for those with extensive, metastatic disease.

1.10.1 Rationale for cytotoxic chemotherapy [9-11]

Cytotoxic chemotherapy began with sulphur mustards (oily vesicant liquids) which had been developed and used as chemical weapons in World War I (1914-18). Amongst their actions, depression of hematopoiesis and lymphoid tissues were observed and gave rise to the idea of possible efficacy in lymphoid cancers.

Other classes of cytotoxic agents, e.g. anti-metabolites, were subsequently identified and used to treat cancer patients. Their efficacy evidently was limited by their relative nonselectivity for proliferating cells; the narrow therapeutic index of cytotoxic agents means that escalation of drug doses is constrained by damage to normal cells and maximum doses which can be safely administered to patients are often suboptimal to achieve total cancer cell killing. Even so, cytotoxic chemotherapeutic agents remain the mainstay of systemic anticancer treatment; since an understanding of their pharmacology has enabled clinicians to exploit the benefits of these drugs by various means.

1.10.2 Classes of cytotoxic chemotherapy drugs [9-11]

Cytotoxic chemotherapy drugs exert their effect by inhibiting cell proliferation. All proliferating cells, whether normal or malignant, cycle through a series of phases: ‘S’ phase, ‘M’ phase and ‘G1’ phase. Non-cycling cells are quiescent in ‘G0’ phase. Cytotoxic drugs interfere with cell division at various points of the cell cycle, e.g. synthesis of nucleotides from purines and pyrimidines of DNA and RNA, mitosis etc. They are potentially mutagenic. Such drugs ultimately induce cell death by the process of apoptosis. This is a process by which single cells are removed from the midst of living tissue without disturbing its architecture or function, or provoking an inflammatory response by fragmentation into
membrane-bound particles and phagocytized by other cells. The instructions for the response are built into the cell’s genetic material, i.e. ‘programmed cell death’.

In general, cytotoxics are most effective against actively cycling cells and least effective against resting or quiescent cells. The latter are particularly problematic in that, although inactive, they retain the capacity to proliferate and may start cycling again after a completed course of chemotherapy, often leading to rapid re-growth of the cancer at a later date.

Cytotoxic drugs can be classified as either:

i) *Cell cycle nonspecific*: These kill cells whether resting or actively cycling (as in a low growth fraction cancer such as solid tumors, e.g. alkylating agents, Doxorubicin and allied Anthracyclines).

ii) *Cell cycle (Phase) specific*: These kill only cells that are actively cycling (often because their site of action is confined to one phase of the cell cycle, e.g. antimetabolite drugs).

The different classes of chemotherapeutic drugs (Fig. 1.4) are

a) *Alkylating agents*: Alkylating agents (nitrogen mustards) act by transferring alkyl groups to DNA in the N-7 position of guanine during cell division. There follows either DNA strand breakage or cross linking of the two strands so that normal synthesis is prevented. e.g. Busulfan, Carmustine, Chlorambucil (Fig. 1.5). Systemic adverse effects of alkylating agents include nausea, vomiting, and bone marrow depression (delayed with Carmustine and Lomustine), cystitis and pulmonary fibrosis (especially Busulfan). These agents are used widely in the treatment of hematological and non-hematological cancers, with varying degrees of success.

b) *Platinum drugs*: This family of drugs including Cisplatin, Carboplatin, Oxaliplatin (Fig. 1.6) crosslink to DNA similar to alkylating agents. The parent drug, Cisplatin is associated with a variety of adverse effects including severe emetogenicity, nephrotoxicity and
Fig. 1.4: Mechanisms and sites of action of some chemotherapeutic agents [8]

Fig. 1.5: Alkylating agents
ototoxicity. Although second- (e.g. Carboplatin) and third- (e.g. Oxaliplatin) generation platinum agents are now available with improved toxicity profiles, Cisplatin remains a highly effective treatment for germ cell tumors, when many patients may be cured.

Fig. 1.6: Platinum drugs

c) Antimetabolites: Antimetabolites are synthetic analogues of normal metabolites and act by competition. Methotrexate (Fig. 1.7), for example, a folic acid antagonist, competitively inhibits dihydrofolate reductase, preventing the synthesis of tetrahydrofolic acid (the coenzyme that is important in synthesis of amino and nucleic acids). Antimetabolites cause gastrointestinal toxicity including stomatitis and diarrhea as well as bone marrow depression. Renal impairment is the main toxicity associated with Methotrexate.

Fig. 1.7: Methotrexate

d) Spindle poisons: The plant alkaloids like Vincristine, Vinblastine (Fig. 1.8) and toxoids like Paclitaxel (Fig. 1.8) inhibit microtubule assembly and cause cell cycle arrest in mitosis.
They particularly cause bone marrow depression, peripheral neuropathy (Vincristine) and alopecia.

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\text{Vincristine} \quad \text{Vinblastine} \quad \text{Paclitaxel}
\]

\textbf{Fig. 1.8: Spindle poisons}

e) Cytotoxic antibiotics: These antibiotics interfere with DNA and or RNA synthesis. e.g. Bleomycin, Dactinomycin (Fig. 1.9). Cytotoxic antibiotics depress the bone marrow, cause gastrointestinal upsets, stomatitis, alopecia and cardiomyopathy.

f) Topoisomerase inhibitors: Doxorubicin (Fig. 1.10) is a nonspecific inhibitor of topoisomerase I and II. These agents have clinical efficacy in relapsed ovarian and colorectal cancer, respectively. Their dose limiting toxicity is bone marrow depression.
Fig. 1.9: Cytotoxic antibiotics
1.11 Antioxidants in chemotherapy

*In vitro* and *in vivo* data suggested that certain antioxidants selectively hinder the growth of tumor cells, may induce cellular differentiation, and may modify the intracellular redox state, thereby augmenting the effects of cytotoxic therapy [13]. Some investigators argued that average concentrations of antioxidants may be insufficient to counter the higher production of reactive oxygen metabolites, and hence may induce cell proliferation and malignant progression [14,15]. Moreover, the association between beta carotene intake and increased risk of lung cancer in two lung cancer chemoprevention trials had generated concern about the cancer promoting effects of antioxidants [16].

Effects of chemotherapy on antioxidant levels supported the hypothesis that chemotherapy lowers total antioxidant status [17,18]. An explanation for the lack of consistent change in antioxidant status after chemotherapy treatment may be that patients were depleted of antioxidants before initiation of treatment perhaps because cancer cells used antioxidants more efficiently than healthy cells, thus depleting circulating plasma levels of antioxidants. This hypothesis was supported by the studies that found that cancer patients had lower levels of antioxidants than controls, even before the initiation of treatment [19, 20]. These observations proposed that low antioxidant status may be associated with neoplastic
activity and subsequent poor health and supported the idea that antioxidant supplementation could benefit cancer patients.

The initiation of anticancer therapy may also lower the level of antioxidants by affecting dietary intake, but as treatment progresses and the cancer cells burden declines, antioxidant levels may improve [19]. However, the finding that TRAP (a measure of total antioxidant capacity) levels consistently decreased, while the individual antioxidants showed no consistent changes, suggested that factors other than known antioxidants may contribute to changes in the total antioxidant status of the body during chemotherapy. The clinical trials revealed that individual antioxidants administered in combination with conventional therapy may affect serum values for some nutrients but not others, selenium levels in patients undergoing chemotherapy [20, 21].

In theory, antioxidants may decrease the efficacy of chemotherapeutic agents by quenching free radicals. Supplementation with vitamin E altered the metabolism of Doxorubicin [22]. However such interactions may not necessarily reduce treatment efficacy; certain adjunctive agents exert their effects through the quenching of free radicals and do not appear to decrease the efficacy of chemotherapy. Similarly, Amifostine, an agent with antioxidant properties, was utilized to reduce the side effects associated with Cisplatin therapy. Individuals treated with anticancer agents that deplete antioxidant status may require replenishment of antioxidants after treatment, similar to patients who receive high dose Methotrexate require Leucovorin rescue. Antioxidant supplements may reduce the frequency and severity of toxicity associated with anticancer therapy. Antioxidant use might make it possible to administer higher and more effective doses of chemotherapy. Either antioxidant does not reduce toxicity or more potent antioxidants or higher dose of individual antioxidants may be needed to minimize the side effects of anticancer therapy. Timing may also be
important; supplementation may have to be introduced very early in therapy before the cumulative doses reach their peak [23-25].

1.12 Emerging anticancer treatments

Our understanding of the biological processes which govern carcinogenesis is growing rapidly and provides the basis for identifying novel cellular targets for anticancer drug development. New approaches that are designed to exploit biological instabilities unique to the cancer cell are being tested in clinical trials [9-11, 26, 27]. Examples include:

i) Matrix metalloproteinase inhibitors: designed to inhibit invasion of cancer cells and prevent metastasis.

ii) Inhibitors of angiogenesis: Tumors require nutrition and produce angiogenic signals that lead to new vessel formation; the strategy is to prevent new blood vessel formation essential for tumor growth.

iii) Signal transduction inhibitors: Farnesyl transferase, an enzyme crucial for the activation of ras oncogene, is frequently over expressed in cancers. Inhibitors of this enzyme appear effective in inhibiting cancer cell growth.

iv) Designer molecular therapy: A tyrosine kinase inhibitor, Imatinib, is specifically designed to block the deregulated tyrosine kinase hyperactivity produced by the Philadelphia chromosome that is specific for chronic granulocytic leukemia; clinical trials support its efficacy in this disease.

v) Agents that promote apoptosis: are being developed for clinical use.

1.13 Triazole derivatives as pharmaceutical agents

1,2,4-Triazoles, which are by far the best-known class of triazoles, five membered heterocyclic with three nitrogen atoms in the ring comprise wide variety of medicinal
activities like antifungal, antimicrobial, anti-inflammatory, hypoglycemic, antidepressant, antitubercular and anticonvulsant [28,29]. Some of the marketed formulations which contain triazole ring are Terconozole, Itraconazole, Fluconazole, Bittertanol (fungicides), Trazodone (antidepressant) and Triazolam (sedative and hypnotic). 1,2,4-triazole is an important emerging moiety in pharmaceutical study and a lot of work can be carried out on this molecule for obtaining better therapeutic activity.

In the past few decades there has been a hiatus in the momentum of research and discovery of novel antineoplastic drugs [30]. Synthetic organic chemistry has always played a vital role in anticancer drug development; the nature of its contribution has varied over time. The therapeutic effects of 1,2,4-triazole derivatives have been studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis and hypertension [31-34].

The third-generation aromatase inhibitors (AI), developed in the 1990s, include Anastrozole and Letrozole [9-11, 35]. AIs are drugs that inhibit aromatase, the enzyme that carries out the final step in the conversion of androgens to estrogen. Currently, third-generation AIs are most commonly used for the treatment of early stage and advanced breast cancer.

**Anastrozole:** (Fig. 1.11) is a potent and selective triazole AI. It binds competitively and specifically to the heme of the CYP19. The main metabolite of Anastrozole is a Triazole. Anastrozole, 1 or 10 mg administered once daily for 28 days reduces total body aromatization by 96.7 and 98.1 %, respectively. Anastrozole has shown efficacy in the treatment of postmenopausal women with early-stage or advanced, hormone-receptor positive breast cancer.
Fig. 1.11: Anastrazole

_Letrozole:_ In postmenopausal women with primary breast cancer, Letrozole (Fig. 1.12) inhibits whole body aromatization and reduces local aromatization within the tumors. The drug has no significant effect on the synthesis of adrenal steroids or thyroid hormone and does not alter levels of a range of other hormones. It also reduces cellular markers of proliferation significantly. It has a bioavailability of 99.9%. Letrozole is eliminated as an inactive carbinol metabolite mainly _via_ the kidneys.

Fig. 1.12: Letrozole

_Sanazole:_ AK-2123 (Sanazole, N-(2-methoxyethyl)-3-nitro-1H-1,2,4-triazole-1-acetamide) (Fig. 1.13), a nitrotriazole hypoxic cell sensitizer has reportedly improved results in head and neck cancers, uterine cervical cancers and other solid tumors when added to radical radiotherapy [36]. The addition of AK-2123 to radical radiotherapy significantly increased response rates and local tumor control in advanced squamous cell cancer of the uterine cervix without any increase in major toxicity.

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3-(4-chlorobenzylsulfonfylmethyl)-5-(2-chlorophenyl)-4H-1,2,4-triazol-4-amine (Scheme-1.1) was found to exhibit cytotoxic activity against A549 (lung adenocarcinoma) cells [37].

Substituted triazolo[4,3-a]pyrimidin-6-sulfonamide (Scheme-1.2) with an incorporated thiazolidinone moiety totally inhibited the growth of twenty cell lines at 0.318-9.73 × 10^{-5} concentrations, showing a good selectivity against leukemia panel [38].
Scheme-1.2: Substituted triazolo[4,3-α]pyrimidin-6-sulfonamide
5-[2-(N-Dimethylsulfamoyl)-4,5-dimethoxybenzyl]-4-phenyl-s-triazole-3-thione (Scheme 1.3) was found to be an excellent antifungal agent against *Aspergillus flavus* and *Trichoderma viride* [39].

**Scheme-1.3**: 5-[2-(N-Dimethylsulfamoyl)-4,5-dimethoxybenzyl]-4-phenyl-s-triazole-3-thione

1-(4-Chlorophenyl)-1H-1,2,3-triazole-4-carbaldehyde exhibited excellent anti-tuberculosis profile (Scheme-1.4) [40].
3-[(4-chloro-2-methylphenoxy)methyl]-7-phenyl-6,7-dihydro-5H-imidazo[2,1-c][1,2,4]triazole synthesized by the reaction between (2E)-2-hydrazinylidene-1-phenylimidazolidine and (4-chloro-2-methylphenoxy)acetic acid (Scheme-1.5) was found to be active against human Caucasian colon adenocarcinoma cell line (LS180) [41].

2-(4-amino-5-oxo-3-phenyl-4,5-dihydro-1H-1,2,4-triazol-1-yl)-N'-[(E)-(2,4-dichlorophenyl)methylidene]acetohydrazide (Fig. 1.14) showed potent therapeutic activity in the treatment of breast cancer [42].
Triazoyl derivative of pregnenolone (Fig. 1.15) exhibited excellent anticancer activity against seven human cell lines [43].

1.14 Pyrazole derivatives as pharmaceutical agents

Pyrazole refers to heterocyclic moiety characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions. Drugs based on a pyrazole ring have often been occupying a position in the list of best-selling pharmaceutical products since the beginning of this decade. Many pyrazole derivatives have been reported to display an array of diverse pharmacological activities such as anti-inflammatory, antimicrobial, antihypertensive, tuberculostatic, analgesic, antidiabetic, antileishmanial and antitumor activity.
4-\{(E)-2-phenylethenyl\}-1\Hpyrazol-3-yl\}piperidine (Scheme-1.6) was found to exhibit potent antibacterial activity against \textit{S. aureus} and \textit{E. coli} [44].

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme1.6}
\end{center}

\textbf{Scheme-1.6}: 4-\{(E)-2-phenylethenyl\}-1\Hpyrazol-3-yl\}piperidine

\textit{N-cyclopropyl-3-(4-methoxyphenyl)-5-(1\Hpyrrol-2-yl)-4,5-dihydro-1\Hpyrazole-1-carbothioamide} (Scheme-1.7) was found to be a potent MAO-B inhibitor and anti-inflammatory agent [45].

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme1.7}
\end{center}

\textbf{Scheme-1.7}: \textit{N-cyclopropyl-3-(4-methoxyphenyl)-5-(1\Hpyrrol-2-yl)-4,5-dihydro-1\Hpyrazole-1-carbothioamide}

\textit{Li-Chen et al} had synthesized \textit{Seleno-pyrazole} (Scheme-1.8) which was found to exhibit excellent cytotoxicity against A-498 cells [46].
Scheme-1.8: Selenolo[3,2-\textit{c}]pyrazole derivative

2-\([(3,5\text{-dimethyl-}4,5\text{-dihydro-1H-pyrazol-1-yl)methoxy}]\text{ethanol (Fig. 1.16)}\) displayed good antibacterial activity [47].

Fig. 1.16: 2-\([(3,5\text{-dimethyl-}4,5\text{-dihydro-1H-pyrazol-1-yl)methoxy}]\text{ethanol}
3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-1H-pyrazole-1-carbothioamide \,(\text{Scheme-1.9})\, was shown to be an excellent anticancer agent [48].

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{OHC} \quad \text{OCH}_3 \\
\text{H}_3\text{C} & \quad \text{OCH}_3 \\
\text{C} & \quad \text{OHC} \\
\text{OCH}_3 & \quad \text{40\% NaOH, ethanol} \\
\end{align*}
\]

\[\text{thiosemicarbazide} \quad \text{ethanol, reflux} \]

\textbf{Scheme-1.9:} 3-(3,4-dimethylphenyl)-5-(4-methoxyphenyl)-1H-pyrazole-1-carbothioamide

Sahu \textit{et al} synthesized a pyrazole derivative (\textit{Fig. 1.17}) which displayed excellent antimicrobial activity [49].

\[
\begin{align*}
\text{OH} & \quad \text{NH} \quad \text{N} \\
\text{N} & \quad \text{H}_3\text{CO} \quad \text{H}_3\text{CO} \\
\text{NH} & \quad \text{N} \quad \text{Cl} \\
\end{align*}
\]

\textbf{Fig. 1.17:} 4-\{(5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino\}phenol

\subsubsection*{1.15 Scope and objectives of present work}

Though third generation aromatase inhibitors are emerging as potential endocrine agents for the treatment of breast cancer, there is plenty of scope and need for improvement. Attempts are being made to overcome the toxicity problems and minimize side effects – first by developing new approaches based on the advances in knowledge of the biology of cancer cells, the second by using selective targeting of anticancer compounds, the third by
developing agents which reverse multidrug resistance and also by boosting the host immune response to tumor. However most of these drugs, either used as a chemotherapeutic agent or as a hypoxic cell sensitizer, are toxic at optimum dose. Moreover most of the chemotherapeutic drugs are expensive. Therefore, research in this direction is still going on, to find an alternative drug which can target the malignant tissues, less toxic and affordable by common man.

A systematic investigation of heterocyclic lead revealed that pyrazole and triazole containing pharmaco-active agents play important role in medicinal chemistry. Hence this research work encompasses the synthesis, characterization and study of the anticancer and antioxidant potential of new triazole derivatives with pyrazole moiety incorporated into them.

The main objectives of the present research work are as follows:

- Synthesis of new triazole derivatives with an incorporated pyrazole moiety.
- Structural elucidation - Spectral techniques:
  - IR, NMR, Mass Spectrometry.
- Thin Layer Chromatography, C, H, N analyses and melting point determination.
- Evaluation of \textit{in vitro} and \textit{in vivo} anticancer properties.
- Study of \textit{in vitro} antioxidant properties.
- Mechanism of anticancer and antioxidant activity.
- Study of structure-activity relationship with reference to anticancer activity.

The work presented in the thesis is broadly divided into eight chapters.

Chapter-1: an introductory chapter on cancer, different chemotherapeutic agents and the importance of triazoles and pyrazoles as potent pharmaceutical agents.

Chapter-2: includes the materials and methods employed for the study.

Chapter-3: encompasses the synthesis of twelve Schiff bases and ten Mannich bases, their characterization and \textit{in vitro} anticancer and antioxidant studies.
Chapter-4: describes the synthesis, characterization, *in vitro* antineoplastic and antioxidant activities of triazolo-thiadiazoles.

Chapter-5: synthesis, characterization, *in vitro* anticancer and antioxidant studies of triazolo-thiazolidin-4-ones form the principal study in this chapter.

Chapter-6: incorporates the synthesis, characterization and *in vitro* anticancer and antioxidant studies of triazolo-thiadiazepines.

Chapter-7: deals with the *in vivo* anticancer studies of the potent Schiff bases and thiadiazoles in EAC cells in mice.

Chapter-8: focuses on the conclusions of the research work.
References


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