CHAPTER I
INTRODUCTION

1.1 General

Cancer is a disease in which the control of growth is lost in one or more cells, leading either to a solid mass of cells known as a tumour or to a liquid cancer (i.e. blood or bone marrow-related cancer). It is one of the leading causes of death throughout the world, in which the main treatments involve surgery, chemotherapy, and/or radiotherapy. Cancer originates in the cells of the body, which are constantly dividing and multiplying to replace old or damaged cells. In few incidences, cells begin to divide unnecessarily, forming excess tissue known as ‘tumor’. In most cases tumors are benign, i.e. they are not cancerous. Benign tumors are not life threatening but they may cause some health problems depending on their size and location. On the other hand if an abnormal cell begins to divide it may form a malignant or cancerous tumor. These malignant tumors grow quite rapidly and invade the nearby organs and tissues. Cancerous cells can spread from its original site and travel through the bloodstream to other regions of the body and the process is known as metastasis.

An important characteristic of cancer cells is their uncontrolled proliferation, which does not respond to normal growth inhibition signals which in normal case limit the cell division. Another important characteristic of cancer cells is their tendency to metastasize which is the detachment of cells from the site of tumor growth and their development in distant body parts. Cancer cells possess the ability to migrate from the site of origin and invade surrounding and distant tissues thereby forming tumor masses at the distant sites in the body. Cancer cells do not grow faster than normal cells but they continue to divide when normal cells would not. Hence, as cancer proliferates in the body its demand for nutrients can literally starve the host. Additionally cancer tends to weaken the immune system, making the host susceptible to other infections and can also interfere with the normal functioning of various organs.
1.2 Types of Cancers

There are various types of cancers which are classified on the basis of their tissue of origin, appearance of cancer cells under the microscope and their tendency to invade surrounding organs and tissues.

All types of cancers are thus classified into following four broad categories:

a) Carcinomas: These tumors arise in the tissues that line the body's organs. Nearly 80% of all cancers belong to this type.

b) Sarcomas: The tumors that originate in bone, muscle, cartilage, fibrous tissue or fats are classified as sarcomas.

c) Leukaemia: These belong to cancers of the blood or blood-forming organs.

d) Lymphomas: Cancers that affect the lymphatic system, which forms network of vessels and nodes that act as the body's filters are classified as lymphomas. The lymphatic system distributes nutrients to blood and tissue, and prevents bacteria and other foreign invaders from entering the bloodstream.

1.3 Process of Carcinogenesis

Carcinogenesis is a multi-stage process where a series of changes take place in normal cells after the initiation step producing factor commonly referred as carcinogen. Cancer seems to arise from the combined effects of two different kinds of carcinogens, one comprising of agents that damage genes involved in controlling cell proliferation and migration while the other involving compounds which selectively enhance the growth of cancer cells. The process of carcinogenesis is initiated when a single cell accumulates several mutations, usually over many years and finally escapes from most of the cellular restraints on the processes of proliferation. These mutations allow the cell and its descendants to develop some additional alterations allowing them to accumulate in increasingly large numbers forming a “new growth” normally referred as a tumor that consists of these abnormal cells. Some of the important stages in the process of carcinogenesis are summarized in Figure 1.
Figure 1: Conversion of normal cell into tumor cell (Adapted from National Cancer Institute, USA)

1.4 Cell Proliferation

Two important aspects of cellular metabolism include DNA synthesis and mitosis to produce new cells as well as cell differentiation, which produces specialized cells. A normal cell has mechanisms to control these two processes and hence it is referred as the non-transformed cell. Chemical signals from growth factors or growth inhibitors produced by the cell regulate cell growth and differentiation as well as its division processes. Consequently, many cells have a negative feedback loop to counter-balance the effects of growth factors. The growth factors and inhibitors exert their effects by binding to various cell surface receptors. In cancer cells, these regulatory processes are aberrant. The activation of growth factors or decreased expression of growth inhibitors leads to the loss of normal growth control, resulting in abnormal and increased proliferation. The root causes of these aberrations, at the cellular levels have not been completely understood. However, the general belief is that the proto-oncogenes, which control normal proliferation and differentiation, are transformed into oncogenes. In turn, oncogenes alter the cellular control mechanisms thereby stimulating processes that support cellular proliferation.
1.5 Cell Cycle and Growth Regulation

In recent years some impressive evidences have been uncovered related to the destination of stimulatory and inhibitory pathways in the cell. These converge on a molecular apparatus in the cell nucleus that is referred to as “cell cycle clock”. The cell cycle is the progression of events that takes place during generation of two daughter cells from one parental cell (Figure 2). It is described in four major phases consisting of the First gap phase (G1), DNA synthesis stage (S), the Second gap phase (G2) and the Mitosis phase (M) respectively. The time period that cell remains in the G1 phase depends on the tissue type and whether it is normal or tumor cell. If the cell is a proliferating cell it will quickly move into the synthesis phase (S). During this phase the DNA is replicated and at the end of the S phase, two copies of DNA are present in the cell. The next phase is the G2 phase where largely preparations are made for the final cell cycle phase, the M phase or the mitosis phase. There are two major control points in the cell cycle. One of these is at the G1/S stage when cells commit to replicate while the second is at G0/M stage when cells commit to divide. Of these two major points in the cells cycle, the G1/S stage is of major importance in understanding cancer and chemotherapy. During the G1 phase cell can take one of the three possible routes. For example, the cell may enter the S phase or a cell in the G1 phase may enter into the G0 phase in which the cells remain in the quiescent state or the cell may terminally differentiate and die respectively.

Figure 2: The Cell Cycle shows four phases: G1, S, G2 and M phases where S is the DNA synthesis step and M is the Mitotic phase. G1 and G2 are gap phases. (Adapted from Cooper G.M.)
1.6 Cell cycle network

There is a complex cell cycle network that has a number of signalling systems that control whether or not a cell enters the cell cycle. These signalling systems determine the choices that are available when cells exit from the cell cycle. They can follow four possible pathways, resulting in markedly different cell fates:

- Senescence
- Apoptosis
- Differentiation
- Cell proliferation

It is the last option of cell proliferation that is controlled by the proliferative signalling pathways that induce resting (G0) or stem cells to enter the cell cycle. These proliferation signalling pathways are highly dynamic systems, with both positive and negative elements. Many of the positive elements are proto-oncogenes, which often are mutated to become constitutively active to form the oncogenes found in many tumour cells. Conversely, there are negative elements, and many of these are tumour suppressors that are inactivated in many tumour cells. There also are anti-proliferative signalling pathways that inhibit cells from entering the cell cycle. An important example is the Smad signalling pathway controlled by the transforming growth factor-β (TGF-β) superfamily, which not only prevents cells from entering the cell cycle, but also drives cells to differentiate.

Presiding over the operation of the cell cycle is the p53 surveillance system, which is constantly checking on the performance of all cell cycle processes, and particularly those concerned with DNA synthesis. The p53 system is also responsive to cell stress from external sources such as irradiation, which often results in causing damage to DNA. All such cell defects are relayed through p53, which then takes appropriate action, depending on their severity. If the damage is not too severe, p53-induced cell cycle arrest occurs while the DNA damage is repaired. However, if damage is more severe, the cell is driven towards senescence or p53-induced apoptosis. With regard to cell proliferation, the main question to focus on is how the information flowing into the cell from the proliferative signalling pathways activates the endogenous cell cycle signalling system responsible for guiding the cell through rest of the cell cycle. This growth factor signalling/cell cycle interface is of central importance because it deals with how the growth factor signalling...
systems instructs the cell cycle signalling system to proceed with another cycle of cell division.

Such a holistic view of the cell cycle network is critical for understanding the causes of cancer, because tumorigenesis develops from multiple alterations in components distributed throughout the signalling network that regulates the cell cycle.

1.7 Cellular Signalling Pathways

Cells use a large number of clearly defined signalling pathways to regulate their activity. These signalling pathways fall into two main groups depending on how they are activated. Most of them are activated by external stimuli and function to transfer information from the cell surface to internal effector systems. However, some of the signalling systems respond to information generated from within the cell, usually in the form of metabolic messengers. For all of these signalling pathways, information is conveyed either through protein-protein interactions or it is transmitted by diffusible elements usually referred to as second messengers. Cells often employ a number of these signalling pathways, and cross-talk between them is an important feature.

1.8 Intracellular Signalling Pathways

There are a large number of intracellular signalling pathways responsible for transmitting information within the cell. They fall into two main categories, majority of which respond to external stimuli arriving at the cell surface, usually in the form of a chemical signal (neuro-transmitter, hormone or growth factor), which is received by receptors at the cell periphery that function as molecular antennae embedded in the plasma membrane. All of these signalling pathways generate an internal messenger that is responsible for relaying information to the sensors which then engage the effectors that activate cellular responses. During cancer growth it is commonly observed that these signaling molecules are up-regulated or over-expressed in cancer cells. In the following discussion we have provided a precise description of the signalling pathways and how they play an important role in cancer proliferation.
1.9 Apoptosis Pathway in Cancer

Apoptosis is a physiological process of programmed cell death that is essential for normal tissue development and homeostasis. It is a process through which damaged, unattached, mutant and aged cells are eliminated. Aberrations in the pathway can lead to a variety of diseases including cancer as well as degenerative and autoimmune disorders. Apoptosis is a highly regulated process with specific and well-described morphological changes. The process was first described in 1842 by Carl Vogt but it was not until 1965 that Lock-shin and Williams introduced the concept of ‘programmed cell death’ to describe the coordinated death of larval muscles during their transformation to adult moths. Nearly 10 years later, the term apoptosis was coined by Kerr et al. who described a series of morphological changes, similar to those described by Lockshin and Williams that were associated with the death of a range of tissues. These changes start with consolidation of nuclear chromatin, followed by condensation of the cytoplasm, DNA degradation, membrane blebbing and fragmentation of the cell into apoptotic bodies. The apoptotic bodies are taken up by surrounding cells and degraded in their lysosomes in the absence of inflammation. The biochemical changes include double stranded cleavage at the linker regions between nucleosomes, leading to the formation of multiple DNA fragments, phosphatidly serine externalization and a range of genes and protein expression changes. Apoptosis is critical for many physiological processes including cell development, proliferation, differentiation, regulation of the immune system and removal of defective and harmful cells. Diminished apoptosis is seen in autoimmune diseases, viral infections and cancer. Targeting components of the apoptotic pathway as a therapeutic approach in cancer is supported by the fact that aberrant apoptosis is central to the growth of tumors and development of resistance to anti-cancer therapies. Current anti-cancer treatments including cytotoxic agents and radiotherapy lead to destruction of cells by inducing apoptosis although mutations of key proteins in the pathway may result in the development of resistance to these therapies. Therefore, evolving novel targeting agents and approaches may therefore lead to cell death, reversal of resistance or induction of sensitization of current agents towards tumor cells.

There are two known signalling pathways mediating apoptosis: the extrinsic and intrinsic pathways (Figure 3). The extrinsic pathway is mediated by cell surface death
receptors, whilst the intrinsic pathway is initiated in the mitochondria. The central regulatory proteins in both pathways are the caspases (cysteine aspartic acid specific proteases). These proteins are synthesized as inactive zymogens which are cleaved into active enzymes in a cascading manner culminating in the activation of what are termed ‘executioner’ caspases that are common to both signalling pathways. These executioner caspases go on to cleave a variety of proteins essential for cell survival such as cytoskeletal proteins and DNA repair proteins resulting in cell death.

**Figure 3:** Schematic diagram of the extrinsic and intrinsic pathways of apoptosis (Adapted from Cryns et al., 1998)

**a) The extrinsic pathway**

The extrinsic pathway of apoptosis is mediated by ligands activating death receptors (DR). DRs are members of the tumor necrosis factor (TNF) receptor superfamily and include functional receptors and decoy receptors (DcR). The receptor superfamily includes TNF-R1, Fas/APO1, DR3, TNF-related apoptosis-inducing ligand receptors-1 (TRAIL-R1, DR4), -2(TRAIL-R2, DR5), and DR6. Others including TNF receptor, FasL/APO1/CD95 receptor, and TRAIL/APO2L receptor regulate other biological functions including cell metabolism, proliferation and cytokine production.
b) The intrinsic pathway

The intrinsic or mitochondrial pathway is so called as it is initiated within the cell, and in particular, within the mitochondria. It is activated in response to cellular stress signals arising from, e.g., DNA damage, hypoxia, a defective cell cycle and loss of cell survival factors. The pathway is tightly regulated by a balance of pro-apoptotic and anti-apoptotic members of the Bcl-2 super-family of proteins (from B cell lymphoma 2, as it is the second member of a range of proteins initially described in chromosomal translocations involving chromosomes 14 and 18 in follicular lymphomas). The anti-apoptotic Bcl-2 proteins include Bcl-2 related geneA1 (A1), Bcl-2, Bcl-2-related gene, long isoform (Bcl-XL),Bcl-w, and myeloid cell leukemia 1 (MCL-1). The primary function of these proteins is to maintain the integrity of the outer mitochondrial membrane (OMM). The intrinsic pathway, when activated by cellular stress signals, leads to up-regulation of pro-apoptotic BH-3 only proteins such as BAD (Bcl-2 antagonist of the cell death), BID (BH3 interacting domain death agonist), BIM (Bcl-2 interacting mediator of the cell death) BMF (Bcl-2 modifying factor), PUMA (p53 up-regulated modulator of apoptosis), and Noxa. These proteins in turn bind to the anti-apoptotic members of the family and inhibit their actions.

c) Targeting apoptosis: Selected anticancer strategies

The selective modulation of both apoptotic pathways has proven to be a challenge in cancer drug development. Unlike most oncogenes that work by promoting proliferation, Bcl-2 functions by preventing programmed cell death. These proteins therefore provide therapeutic targets where their inhibition can lead to the induction of apoptosis. However, the significant challenge is that many of these targets are protein–protein interactions and difficult to modulate. Despite this, there are several molecules targeting components of the apoptotic pathway that have now entered the clinic, and are under investigation as single agents or in combination with other anti-cancer therapies.

1.10 Epidermal Growth Factor Receptor (EGFR) pathway

Epidermal growth factor receptors (EGFRs) are a large family of receptor tyrosine kinases (TK) expressed in several types of cancer, including breast, lung, esophageal, and
head and neck. EGFR and its family members are the major contributors of a complex signaling cascade that modulates growth, signaling, differentiation, adhesion, migration and survival of cancer cells. Due to their multidimensional role in the progression of cancer, EGFR and its family members have emerged as attractive candidates for anti-cancer therapy. Specifically the aberrant activity of EGFR has shown to play a key role in the development and growth of tumor cells, where it is involved in numerous cellular responses including proliferation and apoptosis. Stanley Cohen, Nobel Prize Laureate in Physiology/Medicine, discovered epidermal growth factor (EGF) 25 years ago and elucidated its role in cell growth.

A) General mechanism of EGFR signaling activation and initiation of a diverse array of cellular pathways

Activation of EGFR signaling (Figure 4) is triggered by ligand-induced receptor dimerization following which the tyrosine residues present in the intrinsic kinase domain of one receptor cross phosphorylates specific residues in the C-terminal tail of the partnering receptor, thus providing a scaffold for the recruitment of effector proteins. This occurs via the Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains on the effector proteins and the phosphotyrosine motif present on the intracellular tyrosine kinase domain of the receptor. On subsequent dissociation, the activated adaptor and effector proteins will further stimulate their corresponding signaling cascades, which include the KRAS-BRAF-MEK-ERK pathway, phosphoinositide 3-kinase (PI3K), phospholipase C gamma protein pathway, the anti-apoptotic AKT kinase pathway and the STAT signaling pathway, which leads to cell proliferation, angiogenesis, migration, survival, and adhesion. These cellular processes are often deregulated in malignant cells due to the several mutations harbored in various genes involved in these pathways.
B) **EGFR targeted therapies**

Given the functional involvement of EGFR in diverse cellular processes, several approaches have been developed that target and interfere with EGFR mediated effects. Two distinct therapeutic approaches currently employed for targeting EGFR in various human malignancies are the use of monoclonal antibodies and small molecule tyrosine kinase inhibitors. Each of these approaches have distinct mechanism of action; while anti-EGFR antibodies bind to extracellular domains and TK inhibitor target the intra cellular TK domain. Recent studies have indicated the use of various chemopreventive agents in down-regulating EGFR at gene level. Furthermore, several studies have substantiated and conferred significant benefits of anti-EGFR agents in several types of solid tumors.
including colorectal, head and neck cancer and pancreatic cancer in terms of overall survival, progression free survival and overall response rate\textsuperscript{22,23}.

1.11 Vascular Endothelial Growth Factor (VEGF) Pathway

Vascular endothelial growth factor (VEGF) was identified and isolated as an endothelial cell-specific mitogen that has the capacity to induce physiological and pathological angiogenesis\textsuperscript{24,25} (Figure 5). It is a factor that promotes vascular hyperpermeability, vascular permeability factor, was isolated and later shown to be identical to VEGF\textsuperscript{26,27}. This VEGF is now known as VEGFA and is a member of a larger family of growth factors that also includes VEGFB, VEGFC, VEGFD and placental growth factor (PLGF). These family members differ in their expression pattern, receptor specificity and biological functions\textsuperscript{28}. VEGFA, which is often referred to as VEGF, has been studied more than the other members of this family and it has several distinct variants (VEGF121, VEGF145, VEGF148, VEGF165, VEGF183, VEGF189 and VEGF206). These variants occur because of alternative splicing, and they also differ in receptor specificity and function\textsuperscript{28}. Un-surprisingly, the role of VEGFs in angiogenesis and lymph-angiogenesis has dominated the VEGF research field since the initial discovery of VEGFs, and these studies have provided considerable insights into the mechanisms that underlie the complex process of angiogenesis\textsuperscript{29}. Importantly, these studies provided the foundation for the development of anti-angiogenic therapies that target VEGF and VEGF receptors\textsuperscript{30,31}. It has become apparent that the function of VEGF is not limited to angiogenesis and vascular permeability\textsuperscript{32}. VEGF, for example, can affect the function of immune cells that are present in the tumour microenvironment and, consequently, it can affect the host response to tumours. One of the most interesting developments is the discovery that autocrine and paracrine VEGF signalling occur in tumour cells and that this signalling contributes to key aspects of tumorigenesis, especially the function of cancer stem cells, independently of angiogenesis.

A) VEGF-Mediated Functions in Tumour Cells

VEGF signalling in tumour cells markedly affects tumour function and that this is independent of VEGF-mediated angiogenesis and vascular permeability. The initial
reports that described the effects of VEGF on tumour cells showed that autocrine VEGF signalling—particularly signalling that is mediated by VEGF receptor tyrosine kinases (RTKs) and neuropilins (NRPs)—can promote the growth, survival, migration and invasion of cancer cells. Most of these studies implicated dominant signalling pathways (for example, the PI3K–AKT and MAPK pathways) as the mechanism by which VEGF influences these processes; for example, VEGFR1 promotes the migration and the invasion of colorectal carcinoma cells by stimulating the activation of ERK1 or ERK2 as well as the activation of JNK and the consequent translocation of the p65 (also known as RELA) subunit of nuclear factor-κB (NF-κB) into the nucleus. VEGFR1 can also sustain the survival of colorectal carcinoma cells that have undergone an EMT. Several studies have described the ability of NRP-mediated VEGF signalling to affect the survival of tumour cells by activating the PI3K–AKT pathway; for example, NRP1-mediated VEGF signalling is able to sustain the survival of breast carcinoma cells. VEGF may also regulate autophagy because it has been shown that NRP2-mediated VEGFC signalling mediated by mTOR complex activates an autophagic mechanism that combats chemotherapy-induced stress, which has implications for the role of VEGF signalling in therapy resistance.

Figure 5: Schematic representation of VEGF signalling pathway. (Adapted from 24)
B) Targeting VEGF

Targeting of VEGF family members has the potential to be a very effective approach for inhibiting tumour cell function. This is supported by the report that bevacizumab treatment of patients with locally advanced breast cancer significantly increased tumour cell apoptosis. In addition, antibody-mediated inhibition of PLGF in medulloblastomas had a direct antitumour effect in vivo and caused tumour regression, and it had minimal side effects. Similar results were achieved by blocking NRP1, but VEGFR1 inhibition had no effect. These findings substantiate the feasibility of using antibodies that are specific for other VEGF family members that block binding to NRPs. The potential of targeting VEGF is validated by the suppression of pancreatic carcinoma cell tumorigenesis by using a ‘VEGF-trap’ that sequesters VEGF. The above findings are tempered by the report that anti-angiogenic therapy involving inhibition of either VEGF or VEGF RTKs increased tumour invasion and metastasis. Although more work is needed to understand these fundamental issues, it is becoming evident that combined modes of therapy will be necessary to target VEGF signalling in tumour cells. The development of more effective strategies will probably involve approaches that target tumour cells more specifically as well as the use of a combination of therapeutic reagents that overcome the resistance caused by targeting single molecules.

1.12 NF-κβ Signalling pathway

The transcription factor NF-κβ was discovered in 1986 as a nuclear factor that binds to the enhancer element of the immunoglobulin kappa light-chain of activated B cells (thereby coining the abbreviation NF-κβ). The role for NF-κβ in cancer cells appears to involve regulation of cell proliferation, control of apoptosis, promotion of angiogenesis, and stimulation of invasion/metastasis. Consistent with this role are the observations that inhibition of NF-κβ alone or in combination with other cancer therapies leads to tumor cell death or growth inhibition. However, other experimental data indicate that NF-κβ can play a tumor suppressor role in certain settings and that it can be important in promoting an apoptotic signal downstream of certain cancer therapy regimens.
A) The NF-κβ signaling pathway in inflammation and cancer

Inflammation is the process of innate immunity in response to physical, physiological and/or oxidative stress and is associated with activation of the canonical NF-κβ signaling pathway, which is conserved in all multi-cellular animals. Inflammation in general and NF-κβ in particular have a double-edged role in cancer. On one hand, activation of NF-κβ is part of the immune defence, which targets and eliminates transformed cells. This seems to be particularly true for acute inflammatory processes, where full activation of NF-κβ is accompanied by a high activity of cytotoxic immune cells against cancer cells. On the other hand, NF-κβ is constitutively activated in many types of cancer and can exert a variety of pro-tumorigenic functions. NF-κβ activation usually results in the up-regulation of anti-apoptotic genes thereby providing cell survival mechanism to withstand the physiological stress that triggered the inflammatory response. Furthermore, NF-κβ induces cytokines that regulate the immune response (such as TNF-α, IL-1, IL-6 and IL-8), as well as adhesion molecules, which leads to the recruitment of leukocytes to sites of inflammation. In addition to its role in innate immunity NF-κβ signalling was shown to control variety of other cellular processes, including cell proliferation and apoptosis. The contribution of inflammation in general and NF-κβ in particular to cancer initiation and progression is manifold and complex. Moreover, NF-κβ signaling was shown to contribute to cancer progression by controlling epithelial to mesenchymal transition and metastasis. Finally, NF-κB can also contribute to tumor progression by controlling vascularization of tumors via up-regulation of VEGF (vascular endothelial growth factor) and its receptors.

![Diagram of NF-κβ signaling pathway](image)

**Figure 6:** Representation of NF-κβ dependent genes involved in different aspects of oncogenesis. (Adapted from 43)
B) **NF-κB as target in drug combination therapies of cancer**

Given its role in the initiation and progression of cancer, the NF-κB signaling pathway is a potent mode of pharmacological interference in the clinics. Figure 7 shows the cross-talk of NF-κβ pathway with other signalling pathways leading to cancer cell proliferation. Since NF-κB is also an essential player in the immune response against cancer, there had always been a reluctance to use NF-κB inhibitors in the treatment of malignancies. Nevertheless, combining classical chemotherapeutics with inhibitors of NF-κB activation seems to result in promising synergies. Most cancer drugs are cytotoxic agents that drive proliferating cells into apoptosis, e.g. by interfering with DNA synthesis. Elevated NF-κB activity in cancer cells provides a survival mechanism by up-regulating the anti-apoptotic genes, thereby representing a major causative factor for drug resistance\(^50\). Inhibition of NF-κB is also thought to be at least one mechanism of action of proteasome inhibitors in cancer treatment as activation of NF-κB requires the proteasomal degradation of IκB molecules\(^51\).

![Figure 7: Crosstalk of the canonical NF-κB pathway with other signaling processes.](image)

(Adapted from \(^50\))

### 1.13 Hedgehog Signalling Pathway

Since its first description in Drosophila by Nusslein-Volhard and Wieschaus in 1980, hedgehog (Hh) signaling has been implicated in regulation of cell differentiation, proliferation, tissue polarity, stem cell maintenance, and carcinogenesis. The first link of Hh signaling to cancer was established through studies of Gorlin syndrome in 1996 by two
independent teams. Later, it was shown that Hh signaling may be involved in many types of cancer, including skin, leukemia, lung, brain, and gastrointestinal cancers. In early 2012, the US Food and Drug Administration approved the clinical use of Hh inhibitor Erivedge/vismodegib for treatment of locally advanced and metastatic basal cell carcinomas. With further investigation, it is possible to see more clinical applications of Hh signaling inhibitors. As an essential pathway during development, the Hh pathway is critical for maintaining tissue polarity and stem cell population. The first link between Hh signaling and cancer was shown in tumor-prone Gorlin syndrome in 199652-54. The general signaling mechanisms of the Hh pathway are conserved from flies to humans55. Mammalian Hh signaling molecules include ligands (sonic Hh, Indian Hh, and desert Hh), patched receptors (PTCH1, PTCH2), signal transducer smoothened (SMO), and transcription factors (Gli1, Gli2, Gli3).

A) Hedgehog (Hh) signaling in tumor initiation, promotion, and metastases

Hh signaling (Figure 8) plays different roles in different types of cancer. The functions of Hh signaling during cancer development are basically of three types: the tumor driver, the tumor promoter, or the regulator for residual cancer cells after therapy. For example, In pancreatic cancer, inhibition of Hh signaling does not affect tumor formation but can promote tumor metastasis56. For other cancer types, Hh signaling may regulate the number of cancer stem cells or the tumor microenvironment, such as leukemia and liver cancer57. Increasing evidence indicates that Hh signaling plays an important role during tumor metastasis in several types of cancer, such as pancreatic and breast cancers58.

![Figure 8: Hedgehog signalling in mammalian cells (Adapted from 52)](image-url)
B) Inhibition of Hedgehog pathway

More than 200 compounds have been disclosed to have inhibitory effects on Hh signaling. Of these, eight have been used for clinical trials. Rapid advancement in the discovery of novel Hh signaling inhibitors has provided many opportunities for developing novel cancer therapeutic strategies. It is not surprising to learn that several major challenges still exist to prevent the use of Hh signaling inhibitors in clinics. These challenges include a lack of basic understanding of the molecular mechanisms by which Hh signaling mediates carcinogenesis; no clear criteria to identify the right tumors for therapeutic application; only a few reliable, physiologically relevant, and reproducible mouse models for cancer metastases to test and optimize drug dosages in order to minimize side effects; and a lack of clear strategies to mitigate drug resistance.

1.14 Wnt/β-catenin signalling pathway

Wnt/β-catenin signaling (Figure 9) is a branch of an extensive functional network that developed around a class of proteins - called armadillo proteins - that dates back to the first anaerobic metazoans. Wnt/β-catenin signaling is involved in a broad range of biological systems, including stem cells biology, developmental biology, and adult organ systems. The first detail of the Wnt/β-catenin network was reported in 1982 with the identification of the proto-oncogene int-1 in mice\textsuperscript{59}. Later its homolog in Drosophila, Wingless, was shown to be required for proper wing formation\textsuperscript{60}. In 1989 injection of Wnt1 mRNA in Xenopus was shown to cause body axis duplication, and demonstrated the functional conservation of the pathway\textsuperscript{61}. Since then, the functional importance of Wnt/β-catenin signaling has been shown in a plethora of developmental and organ systems including the cerebral cortex, the hippocampus, the eye, the lens, the spinal cord, limbs, bone, cartilage, somites, the neural crest, skin, teeth, the gut, the lungs, the heart, the pancreas, the liver, the kidneys, the mammary glands, the hematopoietic system and the reproductive system\textsuperscript{62}. De-regulation of Wnt/β-catenin signaling is implicated in a wide spectrum of diseases including degenerative diseases, metabolic diseases and cancer\textsuperscript{63}. The key mediator of Wnt signaling, the armadillo protein β-catenin, is found in a dynamic mode at multiple sub-cellular localizations, including junctions where it contributes to stabilize cell-cell contacts, the cytoplasm where β-catenin levels are tightly controlled by
protein stability regulating processes and the nucleus, where β-catenin is involved in transcriptional regulation and chromatin interactions. Central extracellular regulators of β-catenin levels are the Wnt morphogens. However, multiple other processes, including hepatocyte growth factor, prostaglandines, PKA (Protein Kinase A), E-cadherin, and hypoxia, can also influence β-catenin levels. β-catenin itself is a specialized member of the larger armadillo protein family that consists of three subfamilies: the p120 subfamily, the beta subfamily (β-catenin and plakoglobin) and the more distant alpha subfamily. The functional interplay between members of this protein family is not well understood, but an involvement of p120 and plakoglobin in Wnt/β-catenin signaling has been shown. The regulation of the presence and stability of β-catenin and functionally convergent armadillo proteins – in particular p120 – at the various cellular localizations as well as their shuffling within the cell provides alternative intervention points for therapeutic reagents. The broad implications of Wnt/β-catenin signaling in development, the adult body and in disease renders it a prime target for pharmacological research and development. The armadillo protein β-catenin is the central denominator of Wnt/β-catenin (canonical Wnt) signaling. The levels of β-catenin at different subcellular localizations are regulated by a variety of processes including site-specific phosphorylation of β-catenin. In particular, the control of the turnover of cytoplasmic β-catenin by the destruction complex and the control of the destruction complex by the Wnt signalosome have been studied extensively. Other important mechanisms regulating sub-cellular β-catenin thresholds are those controlling its mobilization from adherens junctions and its translocation to the nucleus. One of the central end points of the Wnt/β-catenin signaling pathway is the regulation of transcription through the binding of β-catenin to members of the Tcf-1/lymphoid enhancer factors family of transcription factors in the nucleus.64

A) Wnt/β-catenin signaling in diseases and potential therapeutics

In view of the critical roles of Wnt/β-catenin signaling in development and homeostasis it is no surprise that mutations of the Wnt pathway components are associated with many hereditary disorders, cancer and other diseases. Association of deregulated Wnt/β-catenin signaling with cancer has been well documented, particularly with colorectal cancer.63 Constitutively activated β-catenin signaling, due to APC deficiency or
β-catenin mutations that prevent its degradation, leads to excessive stem cell renewal/proliferation that predisposes cells to tumorigenesis. Blocking β-catenin signaling for cancer treatment has thus generated significant interests. Indeed the beneficial effect of non-steroidal anti-inflammatory drugs (NSAIDS) in colorectal cancer prevention and therapy has been attributed partially to the perturbation of TCF/β-catenin signaling through the ability of NSAIDS to inhibit Prostaglandin E2 production, which enhances TCF/β-catenin-dependent transcription \(^{65,66}\). Small molecules that disrupt TCF/β-catenin or β-catenin/coactivator (CBP) interaction\(^{67}\) and thereby block TCF/β-catenin signalling have been described.

**Figure 9:** Wnt-beta catenin signalling pathway. (Adapted from \(^{59}\))
Studies of different animal models and human diseases have established a complex Wnt signaling network far beyond a linear pathway, with many components having multiple distinct roles and acting in different cellular compartments, and many modulators feeding into and cross-regulating within this network. The patterns of dynamic and kinetic protein phosphorylation/modification and complex assembly/disassembly are beginning to emerge. Challenges and excitement include Specific small molecular inhibitors or activators with defined targets and mechanisms would provide not only leads for therapeutics but also research tools to manipulate the Wnt pathway in precise temporal and spatial manners. A better understanding of Wnt/β-catenin signaling will have broad impact on biology and medicine.

1.15 Progress and Recent Developments in Anticancer Drug Design

The field of anticancer drug development has undergone profound changes in the last two decades. Current efforts in the pharmaceutical industries are directed at lowering the hit to lead drug time process and increasing the number of lead candidates with the aid of new techniques in drug discovery and clinical development. This includes investments in genomics, High Throughput Screening (HTS) and combinatorial chemistry for improved identification and validation of therapeutic targets, predictive ADME methods and physical methods such as x-ray crystallography and NMR used as tools in structural biology. Virtual Screening is another technique that is being increasingly used and is considered as a complementary to HTS that enhances the success of lead identification. Structure-based virtual screening can be broadly classified as:

a) Ligand-based: In this approach the strategy is to use the information provided by a compound or set of compounds, which are known to bind the target. This is further used to identify other compounds in the database with similar properties. This can be achieved by several methods like substructure searching, pharmacophore matching and shape matching.

b) Receptor-based: This approach is followed when structure of the target protein is known. This involves docking of ligands into the binding site of the target that can produce predictive binding mode for every compound in the database. These advances have led to the decline of empirical approaches to the new drug discoveries in the past.
with more focus on well-defined and disease related targets. There is also an upward trend in delegation of more financial resources for drug development from both governmental agencies and pharmaceutical industries which has been prompted by the emergence of cancer as the major cause of morbidity and mortality among the ageing population in the industrialized countries. Consequently, a single laboratory or a small team of researchers can no longer successfully accomplish anticancer drug development. The process has become rather a complex interplay between the drug discovery staff and preclinical formulation group, toxicology and metabolism group, combinatorial synthesis and purification group, the analytical group, the clinical group and the people dealing with regulatory affairs. The chances of uncertainty prevail at each step where the lead candidate may fail despite enormous effects. As a consequence, target validation, focused drug development and well-designed clinical trials have become critical for the rapid development of new active anticancer compounds. The process has thus become an exercise in partnership between academia, government and industry. Finally, in the field of anticancer drug development, approximately 500 compounds are now in development with potential of thousands of entities. To select appropriate compounds requires well-integrated processes from the initial synthesis through clinical trials. In the following discussion we have provided a brief account of predictive designs for anticancer drug development both chemically and biologically. For the purpose of convenience we have categorized the existing anticancer drugs and recent exploratory agents into a few broad categories depending upon their site of action or mechanism so that proper perspective for the work described in the present dissertation can be provided.

1.16 Traditional Chemical Approaches to Cancer Therapy

A) DNA-RNA interactive anticancer agents

(i) Synthetic alkylating agents: Nitrogen Mustards\(^\text{68}\) (1) were used in World war I as the chemical warfare agents due to their toxic and vesicant properties on the skin, lungs and eyes respectively. They were noted to produce atrophy of lymphoid and myeloid tissues, which lead to their exploratory use for treating lymphomas and leukemias. Later analogs such as melphalan (2), clorambucil (3), cyclophosphamide endoxan (4) were found to be
useful in the treatment of number of cancers including those of breast, ovary, uterine, leukemia, multiple myeloma, Hodgkin and Non-Hodgkin cancers\textsuperscript{69}. To overcome the severe toxicity of nitrogen mustards, the N-nitrosourea drugs were developed during the 1950s. The presence of nitroxyl species in N-nitrosoureas was found to greatly increase their cytotoxicites while reducing their general toxicities and hence enhancing their therapeutic index\textsuperscript{70;71}. A comprehensive review on nitrosourea anticancer drug is presently available. According to these review four drugs, viz. carmustine (5), lomustine (6), semustine (7), and streptozotocin (8) respectively have been used in the clinical oncology.

(ii) Platinum compounds

The anticancer properties of platinum complexes were discovered serendipitously during studies on the effects of electrical current on the bacterial growth using platinum electrodes\textsuperscript{72}. The parent compound “Cisplatin” (9) has been found to be active against many malignancies such as non-small-cell lung, small-cell lung, breast, penile, head and neck, gastric, uterine, ovarian, testicular and osteosarcomas respectively. It has been well established that the major target of cisplatin drugs is the DNA molecule and the interaction results in the formation of intrastrand cross-links causing a bent formation and a local distortion of the DNA helix\textsuperscript{73;74}. Although introduction of cisplatin in the clinical oncology had a major impact on cancer chemotherapy, the drug has been found to exhibit various toxicities towards normal cells even at low doses. As a result several new cisplatin analogs have been synthesized. However, only two among these, viz. carboplatin (10) and oxaloplatin (11) have been cleared for clinical use.

\[
\begin{align*}
\text{R} & = \text{CH}_2H \quad \text{Nitrogen mustard} (1) \\
\text{R} & = \text{NH}_2 \quad \text{Melphalan} (2) \\
\text{R} & = \text{O(CH}_2)_3C_6H_5 \quad \text{Chlorambucil} (3) \\
\text{R} & = \text{C}_6H_4\text{NH} \quad \text{Cyclophosphamide endoxan} (4) \\
\text{R} & = \text{Cl} \quad \text{Carmustine} (5) \\
\text{R} & = \text{H}_2 \quad \text{Lomustine} (6) \\
\text{R} & = \text{H}_2 \quad \text{Semustine} (7) \\
\text{R} & = \text{OH} \quad \text{Streptozotocin} (8)
\end{align*}
\]

\textbf{Figure 10:} Synthetic Alkylating agents
(iii) **Minor groove binding agents**

The anticancer antibiotic duocarmycin\(^\text{75}(12)\) and congeners represent a group of DNA minor groove binding agents. These compounds contain a cyclopropane pyrrole-indole (CPI) moiety, which is connected by the amide linkages to either one or several repeat pyrrole-indole subunits. The DNA-reactive cyclopropane ring interacts with the N3 position of the adenine in the minor groove of the DNA. However, these antibiotics produce severe liver and kidney toxicities and hence new therapeutic agents such as adozelesin \((13)\), bizelesin and carzelesin have been developed for small-cell lung, melanoma and gastric cancers, which are in phase II clinical trials\(^\text{76,77}\). The DNA minor groove binding antibiotic, Bleomycin has also been used extensively in clinical oncology against squamous cell carcinoma, reticulum cell sarcoma, lymphosarcoma and testicular carcinoma respectively\(^\text{78}\).

![Figure 11: Platinum compounds and minor groove binding agents](image)

(iv) **Topoisomerase inhibitors**

DNA topoisomerases I and II and their enzyme \(\alpha\) and \(\beta\) forms are nuclear enzymes which control, maintain, and modify the structure and topology of DNA during the replication and translation processes of genetic materials\(^\text{79}\). The topoisomerases induce transient cuts in DNA, which enable the strands to pass through the nicks and then rejoin...
the nicked strands to DNA. During this process covalent links are formed between topoisomerases and DNA, which are called as “cleavable complexes”. Drugs, which stabilize such complexes, tend to inhibit topoisomerases and result in strand scission and inhibition of DNA synthesis. Different drugs induce site-specific DNA damages because they bind DNA at different sites. The well-known topo I inhibitor is a plant alkaloid called Camptothecin\(^8\) (14) and its synthetic analogs topotecan (15) and irinotecan (16)\(^8\). All these compounds contain a lactone ring, which is critical for their activity. Topotecan has been found to possess activity against small-cell lung cancers and ovarian cancers while irinotecan was shown to have promising anticancer activity against metastatic colon and cervical cancers.

The other class of potent topoisomerase II inhibitors includes the anthracyclin antibiotics such as adriamycin (17), daunomycin (18), idarubicin (19) and epirubicin (20)\(^8\). However, their anticancer activity has been attributed to several other factors including their free radical generating abilities through redox cycling. Most agents belonging to this class of compounds were discovered accidentally and suffer from a lack of selectivity and specificity in general. They are also less effective against solid cancers.

![Topoisomerase inhibitors](image)

**Figure 12**: Topoisomerase inhibitors
1.17 Novel Approaches To Anticancer Drug Discovery

A) **Signal transduction inhibitors**

It is well known fact that cell signalling pathways play important role in tumor cell proliferation. Thus a novel approach to targeted drug discovery involves targeting the various check points of cell signalling proteins. Signalling pathways include both extracellular and intracellular events where receptors for various growth factors such as epidermal growth factor (EGDF), platelet –derived growth factor (PDGF), macrophage colony stimulatory factor (M-CSF) for antigens and cytokines and the G protein-coupled receptors, located on the cell surface play a major role in the relaying of stimulus to the interior of the cell. The growth factor receptors are composed of transmembrane proteins with cytoplasmic protein-tyrosine kinase (PTK) domains which become excellent cellular targets for design of anticancer compounds.
B) Tyrosine kinase inhibitors

The tyrosine kinases are dominant signalling molecules in cells since they are present on the membrane as a part of growth factor receptors, i.e. receptor tyrosine kinases (RTKs) and could be a target in cancer therapy. Monoclonal antibodies for tyrosine kinase proteins have been developed which down-regulate the expression of this protein and cause inhibition of cancer cell proliferation both in vitro and in vivo. The octapeptide somatostatin (21) and its analogs were found to decrease the growth of cancer cells by activating the tyrosine phosphatases, which dephosphorylates and halts the growth signal. Natural products such as quercetin (22), genistein (23), lavendustin A (24), erbstatin (25), herbimycin A (26), Lovastatin (27), Limonene (29) and many others also.

Figure 13: Signal transduction inhibitors
exhibit a broad but non-specific inhibition of tyrosine kinases by binding site in the protein domain\textsuperscript{85}. Attempts have been made to design inhibitors, which would bind to the substrate binding site and not the ATP-binding site. Newer selective inhibitors of RTKs include di-anilinophalamide (DHAP-1), 4-anilinoquinazoline, 4-ar(alk)ylamino] pyridopyrimidine derivatives\textsuperscript{86}, which act as selective inhibitors of EGFR tyrosine kinase.

C) **Cell cycle regulators**

Cell cycle regulation is important in the maintainence of normal cells, whereas abnormal regulation of the cycle can result in neoplastic transformation. A series of protein kinase complexes and cyclin dependent kinases (cdks) are positive regulators of the cell cycle\textsuperscript{87}. In normal non-cancerous cells there is a mechanism to ensure certain stoppages in the cell cycle to allow the repair of DNA damaged by radiation, carcinogens and cytotoxic drugs. In the cancerous cells these inhibitory controls are lost and hence the mutated DNA can proceed through the cell cycle. As a result the cancerous cells can multiply without corrections\textsuperscript{88}. One of the cell arrest mechanism involves the transforming growth factor (TGF-β) which blocks the synthesis of cdk-4 in the G1 phase thereby, preventing the formation of the Cyclin d/CDK complex and the phosphoryaltion of Rb (retinoblastoma). This effective growth arrest leads to the accumulation of the cells in the G1 phase. Based on this mechanism, inhibitor compounds of the cyclins or cdk enzymes have been prepared, most of which bind to the ATP pocket of the kinases. Among these Flavopyridol (30), which is a polyhydroxylated, flavone, is an efficient inhibitor of protein kinases\textsuperscript{89}. It is competitive with ATP, non-competeive with the peptide substrate, cytostatic to a variety of cancer cell lines and cytotoxic to certain cell lines\textsuperscript{90}. The compound is in clinical evaluations. However, there is a need for more improved synthetic compounds with growth inhibitory activities but without the toxicities observed with such compounds.
Other inhibitory compound includes the alkaloid, staurosporine (31) and purine derivatives such as olomucine (32) and roscovitine (33) respectively. The cdk inhibitors can act as cytostatic and cytotoxic agents or can enhance the anticancer activity of the cytotoxic agents by inducing cancer cells to either arrest or delay progress through the cell cycle phases in which they are more sensitive than the cytotoxic drugs.

D) **Inhibitors acting through the apoptosis pathway**

Apoptosis or programmed cell death is another process for the elimination of cancer cells. In normal cells process of mitosis and apoptosis balance each other under steady state conditions. A large number of diverse natural and synthetic inhibitors of apoptosis have been discovered such as phorbol esters, growth factors, granulocyte stimulating factors (GM-CSF), serum factors, cytokines, certain viruses, RNA protein synthesis inhibitors, PKC tyrosine phosphorylation inhibitors, endonucleases inhibitors of transglutaminases and proteases. Additional important inducers and inhibitors of apoptosis
include certain proto-oncogenes and the p53 cancer suppressor gene\textsuperscript{93}. The c-myc and c-fos proto-oncogenes have also been implicated in the induction of apoptosis\textsuperscript{94}.

It has been pointed out that the relationship between some of the apoptosis inducing proteins like p53 and Bcl gene family is important for understanding apoptosis in cancer cells\textsuperscript{95}. It is becoming increasingly clear that the anti-apoptotic effects of bcl-2 protein can be easily reversed by p53 protein contributing to the success of the treatment in patients with some types of cancers. It has been further observed that activation of another gene, viz. bax-2, can result in restoring apoptosis and thereby limiting extent of cancer cell malignancy\textsuperscript{96}. Thus, work in this area is making it obvious that drug target interactions alone are not important and stimulation of cell signals that lead to apoptosis are equally crucial.

E) Angiogenesis inhibitors

Angiogenesis is a process whereby endothelial cells divide and migrate to form new capillaries. This process can be observed under normal conditions such as wound healing, and in disease states such as arthritis, psoriasis, duodenal ulcers, certain disorders of female reproductive system and cancers. In the cancerous state, the new capillaries are thin-walled and leaky and provide access to the circulation for group of cancer cells, which can metastasize and also acquire the angiogenic phenotype. Among various angiogenic agents two proteins appear to be most important for sustaining cancer growth, viz. the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). When released from the cancer cells, these can trigger the growth of new capillary vessels. Additionally, migrating endothelial cells also produce matrix metalloproteinases (MMPs), which are thought to be responsible for the breakdown of basement membranes and extracellular matrices. Since cancer cells require an additional blood supply for growth, inhibition of angiogenesis is a logical approach to control of the cancers. Angiogenesis inhibitors are grouped according to their mechanism of action. One class of compounds such as tecogalan, pentosan sulfate (PPS) and fumagillin, block the production of angiogenic growth factors. Another class of angiogenic compounds includes the signal transduction inhibitors, which inhibit angiogenesis and mitosis by interfering with the transduction of growth stimulus signals across cellular membranes. This novel
approach of targeted anticancer drug discovery is crucial in providing lead molecules which can be further taken to clinical trials. Moreover multi-targeted compounds provide an edge in the field of anticancer drug discovery. Further studies are required to make this approach successful and develop new molecules which can enter the markets for treatment of cancer and related diseases.

1.18 Toxicities Related to Anticancer Compounds

One of the characteristics that distinguish anticancer agents from other drugs is the frequency and severity of side effects at therapeutic doses. Most cytotoxic drugs target rapidly multiplying cells and the putative targets are the nucleic acids and their precursors, which are rapidly synthesised during cell division. Many solid tumours have a lower growth fraction than the normal bone marrow, gastrointestinal lining, reticulo-endothelial system and gonads. Anticancer drugs affect these tissues in a dose dependent manner and there is also an individual susceptibility. So toxicities are more frequently associated with these tissues. The side effects may be acute or chronic, self-limited, permanent, mild or potentially life-threatening. Management of these side effects is of utmost importance because they affect the treatment, tolerability and overall quality of life. Various chemotherapeutic agents are used widely for the treatment of variety of neoplastic diseases either as single or in combination or as adjuvant with surgery, radiotherapy and immunotherapy. Chemotherapy presently plays different roles in different clinical settings like induction treatment for advance diseases, as an adjunct to local methods of treatment, or as primary treatment for the localised disease. Clinically useful anti-neoplastic agents exhibit selective toxicity to malignant cells. Many regenerating tissues possess high proliferative capacity rivalling malignant tissues and on exposure to chemotherapy, such tissues like bone marrow elements, gastrointestinal tract mucosa, hair follicles have to endure these toxic effects. The anti-neoplastic agents have the lowest therapeutic indices of any drug and as such they cause frequent and predictable multi-system toxicity. The acute toxicity in immediate post therapy periods is usually reversible while the long-term toxicity is generally delayed and irreversible. Consequently, these increase the morbidity and mortality of treatment. Chemotherapy drugs are generally administered in combination with a view of inducing rapid cytoreduction and the overlapping of toxicities.
is considered when drugs are used in optimal dose and schedules. Approaches to the reduction of chemotherapy-induced toxicity include dose reduction, use of alternate drugs or their analogs, growth factors, and cytoprotective agents. Common toxicities encountered include haematological, gastrointestinal, epidermal and hair follicle toxicity, nervous system toxicity, local toxicity, metabolic abnormalities, hepatic toxicity, urinary tract toxicity (nephrotoxicity), cardiac toxicity, pulmonary toxicity, gonadal toxicity and many others. Some of these are discussed in brief below:

A) **Haematological Toxicity**

Peripheral cytopenia from bone marrow suppression is a frequent dose limiting side effect of chemotherapy and can manifest as acute and chronic marrow damage. Chemotherapy may result in the destruction of activity of proliferating haematopoietic precursor cells, leading to deprivation of formed elements, and incidence of life threatening haemorrhage and infection. Some of the anticancer drugs causing such hematological toxicities are listed below (Figure 15)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug</th>
<th>Drug</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboplatin</td>
<td>Dactinomycin</td>
<td>Vinblastin</td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Mitomycin</td>
<td>Melphelan</td>
<td>Gemcitabine</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Fludarabine</td>
<td>Oxaliplatin</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Mustine</td>
<td>Idarubicin</td>
<td>Daunorubicin</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>Topotecan</td>
<td>Irinotecan</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>Vinorelbine</td>
<td>Fluoro-uracil</td>
<td>Rituximab</td>
<td>Hydroxyurea</td>
</tr>
<tr>
<td>Ifosamide</td>
<td>Mitoxantrone</td>
<td>Transtuzumab</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 15:** Common drugs causing haematological toxicities

B) **Gastrointestinal Toxicity**

Anorexia, nausea and vomiting are frequently observed after chemotherapy. It is not a pathological process but rather a physiological process in which the body attempts to rid itself of toxic substances. This reaction is controlled by a reflex that are with multiple afferent limbs, a coordinating area (vomiting centre) and multiple efferent pathways that
activate and coordinate the muscle group necessary for a successful emetic response. Patients who are about to begin chemotherapy are apprehensive about nausea and vomiting. Nausea and vomiting can be distressing enough to the patient to cause extreme physiologic and psychological discomfort culminating in withdrawal from therapy. Commonly nausea begins 4 to 6h after the treatment and lasts for 1 to 2 days. Chemotherapeutic agents are classified into highly emetic, moderately emetic, mild emetic according to their emetic potential. The drugs implicated in gastrointestinal toxicities are listed below. (Figure 16)

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin</td>
<td>Docetaxel</td>
<td>Cyclophosphamide</td>
<td>Carboplatin</td>
</tr>
<tr>
<td>Busulfan</td>
<td>Etoposide</td>
<td>Doxorubicin</td>
<td>Carmustine</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>Gemcitabine</td>
<td>Epirubicin</td>
<td>Cisplatin</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Methotrexate</td>
<td>Idarubicin</td>
<td>Dacarbazine</td>
</tr>
<tr>
<td>Vinblastine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Figure 16: Drugs Implicated In Gastrointestinal Toxicities)

C) Nervous System Toxicity

The incidence of neurotoxicity associated with chemotherapy is increasing because of greater use of high dosage chemotherapy and higher neurotoxicity caused by new drugs used in combination weakening the barrier found with the brain. Vincristine is one of the most important anti neoplastic agent that has dose limiting neurotoxicity which can result in Paresthesia of hands and feet and loss of deep tendon reflexes in almost all patients. All these are reversible and are observed mostly in older patients. Peripheral sensations and motor nerve axons are at risk for damage by those chemotherapeutic agents that disrupt microtubules. Subjects with diabetics, alcohol abuse and those with inflammation and toxic neuropathy are at risk for neurotoxicity.
Hepatic Toxicity

Hepatic toxicity is a common problem in cancer chemotherapy. Virtually most of the patients undergoing chemotherapy have exposure to hepatotoxins including medication and alcohol. Some may have coexisting liver disease. Some because of immunocompromised state may be prone to infection. Hepatotoxicity reactions may occur in a varying pattern including parenchymal cell injury with necrosis, fibrosis, ductal injury with cholestasis, & blocking of hepatic veins. Chemotherapeutic agents that cause hepatotoxicity produce a predictable pattern of injury where the mechanism is direct or idiosyncratic.

<table>
<thead>
<tr>
<th>Acute myelopathy</th>
<th>Cerebellar syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathecal cytarabine</td>
<td>Cytarabine</td>
</tr>
<tr>
<td>Intrathecal methotrexate</td>
<td>Procarbazine</td>
</tr>
<tr>
<td>Intrathecal thiotepa</td>
<td>5-Flourouracil</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Autonomic neuropathy</th>
<th>Cranial nerve toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>Vindesine</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Vinblastine</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>Vincristine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Encephalopathy</th>
<th>Peripheral neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmustine</td>
<td>Vindesine</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>Vinblastine</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Vincristine</td>
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<table>
<thead>
<tr>
<th>Figure 17: Drugs causing nervous system toxicity</th>
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<table>
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<tr>
<th>Figure 18: Drugs causing hepatic toxicity</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Cyclophosphamide</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steptozotocin</td>
<td>6-Mercaptopurine</td>
</tr>
<tr>
<td>5-Flourouracil</td>
<td>Doxorubicine</td>
</tr>
</tbody>
</table>
E) Pulmonary Toxicity

Chemotherapeutic drugs can damage directly or indirectly, lung tissue both of endothelial and epithelial cells\textsuperscript{104}. Clinical presentations of pulmonary toxicity are acute pneumonitis, pulmonary fibrosis, hypersensitivity pneumonitis, non cardiogenic pulmonary edema. Many patients exhibit symptoms and histological findings of more than one type of toxicity.

\begin{figure}[h!]
\centering
\begin{tabular}{|l|l|}
\hline
**Acute pneumonitis** & **Pulmonary fibrosis** \\
Bleomycin & Bleomycin \\
Vinca alkaloids & \\
Methotrexate & \\
Procarbazine & \\
Carmustine & \\
Mitomycin & \\
\hline
**Hypersensitivity pneumonitis** & **Non cardiogenic pulmonary edema** \\
Procarbazine & Cyclophosphamide \\
Azathioprine & Methotrexate \\
Bleomycin & Cytarabin \\
Methotrexate & Mitomycin \\
\hline
\end{tabular}
\caption{Drugs causing pulmonary toxicity\textsuperscript{100}}
\end{figure}

F) Cardiac Toxicity

Cardiomyopathy is the most common chemotherapy associated cardiac toxicity and is thought to be due to free radical mediated injury. Myocardial ischemia, pericarditis, arrhythmia, ECG changes and angina also occur but much less frequently\textsuperscript{105}. The anthracyclines used as anticancer drugs have the highest risk for cardiomyopathy which is related to cumulative dose response. Acute effects occur within hours of bolus administrations include arrhythmia, sinus tachycardia, ECG changes which are transient and no dose related. Sub-acute cardiomyopathy may present during or weeks to months
after therapy or within one year of treatment. Later onset cardiac toxicity occurs one to five years after treatment.

G) Renal Toxicity

Major risk factors for renal toxicity in patients with cancer include nephrotoxic chemotherapeutic drugs, age, nutritional status, use of nephrotoxic drugs, and pre-existing renal dysfunction\textsuperscript{106}. Of these impaired renal function is the most significant risk factor. Acute renal failure and hemolytic uremic syndrome are serious and fatal complications. The reason lies in the functional properties of the kidney which makes it susceptible to injury. The kidney is an organ characterized by a large volume of blood supply (20-25\% of the cardiac output) which ensures a high level of toxicant delivery over a period of time. The extensive re-absorptive capacity of the tubule with specialized transporters promotes cellular uptake of the toxicant. Concentrating capability of the tubule produces high concentrations in the medullary lumen and interstitium. High metabolic rates and workload increases the sensitivity to toxicants. Metabolic alteration may also produce highly toxic metabolites or reactive intermediates. The nephrotoxic chemicals damage specific portions of the tubule with the brunt being borne by the proximal tubule.

Segment specific targets exist for nephrotoxicants. The reasons for segmental specific nephrotoxicity of agents may be attributable to the differences in toxicant delivery, differences in transport and uptake among segments and differences in biotransformation among segments. The end result of the nephrotoxic effect on the epithelial cells is cell injury and death. Many recent excellent reviews on the potential nephrotoxicity and the renal handling of cancer chemotherapeutic drugs are available in the literature.

1.19 Nature and Scope of Present Thesis

The present thesis consists of three parts dealing with the central theme of cancer drug design but encompassing different themes:

1. The first part of the thesis deals with designing novel colon cancer drugs for targeting HA/CD44v6 signalling pathway which plays a crucial role in regulating COX-2/PGE2 mediated cell survival, motility, and drug resistance in colon cancer. The reversal of HA/CD44v6 signalling modulates the cancer phenotype and adenoma growth in
ApcMin/+ mice by inhibiting CD44v6/ErbB2/COX-2-PGE2 pathway. These compounds are found to be dual COX-LOX inhibitors active against panel of colon cancer cells at micromolar concentrations. Molecular docking studies revealed confirmation of their strong binding in the cavities of COX and LOX enzymes.

2. The second part of the thesis deals with the drug design strategies for inhibition of anti-apoptotic bcl-2 family of proteins which operate through the apoptotic pathway and increase resistance towards anticancer drugs. The compounds consisted of sulfonamide-gallic acid hybrids which were tested against variety of cell line including triple negative breast cancer (MDA-MB231) cells, Prostate cancer (PC-3) cells and pancreatic cancer (Bx-PC-3) cells. Molecular docking studies revealed that the new compounds moderately bind in the bcl-xl cavity with comparable binding energies.

3. The final part of the thesis presents an account of the protective capability of synthetic Mn-SOD mimics against Xanthine-oxidase induced kidney toxicities which are commonly found in nephropathy in diabetic patients. The novel synthetic complexes were effective in increasing the viability of kidney cells during high ROS stressed conditions even at low concentration as low as 10nM. These compounds were shown to operate mechanistically through quenching of ROS and enhancing Mn-SOD expression in mitochondria.
1.20 References


