1. Cancer

A cluster of diseases featured by uncontrolled growth of cells invading and destructing adjacent tissues and thereby, leading to formation of tumor (solid mass), which can either be malignant or benign, or metastatic via connective tissues can be defined as cancer (Nussbaumer *et al*., 2011; Perfézou *et al.,* 2012). Abnormalities in the genetic makeup of the transformed cells, intake of tobacco, smoking, exposure to harmful radiation, infective agents, chemicals, etc. can aggravate the disease conditions. These cancer causing agents are also termed as carcinogens. The old or damaged cells are replaced by the cancerous cells that divide and multiply consistently. Although benign tumors are not lethal but may cause serious health conditions owing to their size and location. On the other hand, an abnormal cell may divide to form a malignant or cancerous tumor which is invasive in nature, and thereby, growing and invading the healthy organs and tissues in the periphery through the process of metastasis. Metastasis is the process by which the cancerous cells spread from its original site to other bodily areas through the connective tissue (Farsinejad *et al.,* 2015; Behrens *et al.,* 1992). It has been established that nearly 13% of all human deaths are caused due to various types of cancer. Global annual death due to the cancer is approximately around 3 lakh, and an average of 7 lakh new cases reported. By 2030, a fatality of 13.1 million is estimated to be caused by cancer (Bleyer *et al.,* 2008). Although breast cancer is not epidemic, yet the reports in recent years are spine chilling with a total of 232, 340 females and 2, 240 males suffering from breast cancer in the USA and second highest rate of cancer incidence is reposed in India, after the USA (Mazumdar *et al.,* 2018). As per the National Cancer Control Program, at any given point of time nearly 2-2.5 million people in India are suffering from this deadly disease. Skin cancers are also one of the most common types of cancers, reported to have a higher prevalence.

1.2. Types of Cancers

The cancer is classified on the basis of tissue origin, morphological appearance of cancer cells and propensity to attack neighbour organs and tissues.

a) Carcinomas: This carcinoma occurred in the skin or in the tissue which line up the body's organs and covers the surface of internal organs and glands. These cancers are usually solid tumors and are the most common type of cancers. For example: breast, prostate, lung and colorectal cancer.

The mortality (50%) of death due to cancer in India is due to prostrate, lung and colorectal cancer in men, whereas breast, lung and colorectal cancer are more prominent in women (Saranath, *et al.,* 1991). Current progress in cancer treatment has led to better prognosis of the cancer patients and reduced incidence and mortality rates. One of the primary reasons for cancer-induced mortality is the ability to metastasize as well as colonize to distant organs and disrupting their normal function (Jemal, *et al*., 2009; Resche, *et al.,* 1997).

Further research in cancer prevention, early detection and treatment need to be encouraged to control cancer induced mortality across all segments of the world population.

b) Sarcomas: Sarcomas instigate in the tissue or organ that connect the body and developed in the nerves, bone, joints, muscle, lymph, vessels, cartilage or bone, fibrous tissue or fats.

c) Leukaemia: Leukaemia is a cancer of the blood.

d) Lymphomas: Lymphomas are the cancers that begin in the lymphatic system. The lymphatic system is a network of vessels, glands which supply nutrients to blood and tissue that help fight infection against bacteria as well as other foreign attacker entering into the bloodstream.

1.3. Metastasis and Invasive Properties of Cancer Cells

Metastasis is an important characteristic of cancer cells, which involves the detachment of cells from the site of tumor growth and their development in distant body parts. Cancer cells have the capability to migrate from the site of origin and invade surrounding and distant tissues thereby forming tumor masses at the distant sites in the body (Rehm *et al.*, 2009). Cancer cells do not grow faster than normal cells but divide continuously, and hence demand more nutrients, which can make the host starve. Additionally, cancer attacks the body's defensive system making the host vulnerable to other infections and can also interfere with the normal functioning of various organs. Both benign and malignant tumor is the result of uncontrolled proliferation of cells. The treatment strategy of malignant cancer lies in the inhibition of metastasis, invasion and angiogenesis. The process of metastasis involves the following steps-migration, intravasation, transport, extravasations, metastatic colonization, and angiogenesis. For the cancer cells to segregate from a solid tumor, the catenin-cadherin junction (junction between tumor cell and neighbor cell) and integrin junction (junction between extracellular matrix and tumor cell) have to be branched (**Figure 1.1**). A change in the junction can result in the free movement of cancer cell and adhere it to other extracellular matrices.

Following its separation from the solid tumor and extracellular matrix, the cancer cell may reach the peripheral organs by penetrating blood and lymphatic vessels through the process of intravasation. The signals between cancer cells and endothelial cells regulate extravasations, by the process known as "seed and soil''.

Extravasations involve adherence of cancer cells to the endothelium, penetration to the vessels, and infiltration of the intestine. Penetration of the vessels from the interior side by the cancer cells can initiate its colonization.

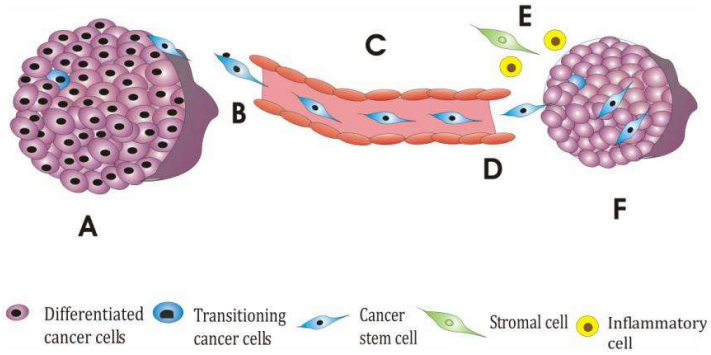


Figure 1.1: Metastasis pathway of cancer cells

1.4. Process of Carcinogenesis

Carcinogenesis is a complex process involving multiple changes in normal cells after the initiation step producing factor commonly referred to as a carcinogen. Two different kinds of carcinogens have pivotal role in developing cancer- i) agents damaging genes involved in controlling cell proliferation and migration and ii) compounds selectively enhancing the growth of cancer cells.

The process of carcinogenesis is initiated usually over many years of a single cell accumulating several mutations, and finally escaping from most of the cellular restraints on the processes of proliferation (Basu *et al.*, 2018; Haschek *et al.,* 2013). Surplus alterations are developed by the cell and its descendants, and are accumulated in huge numbers giving rise to a new growth consisting of abnormal cells usually termed as tumor (Yates *et al.*, 2012). Some of the important stages in the process of carcinogenesis are summarized in **Figure 1.2**.

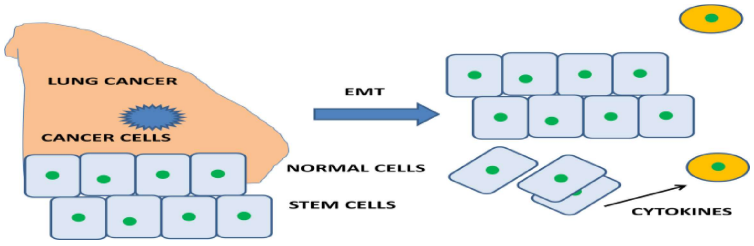


Figure 1.2: Conversion of a normal cell into a tumor cell

1.5. Cell Proliferation

Cellular metabolism involves two vital processes like a) DNA synthesis and b) mitosis to produce new cells as well as cell differentiation. Generally, normal cells have regulatory mechanisms to control these two processes and hence they are referred to as the non-transformed cells. 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, as well as inhibitors, exert their effects by binding to various cell surface receptors (Johnson *et al.,* 2007). In cancer cells, these regulatory processes are anomalous. The increased activation of growth factors or decreased expression of growth inhibitors leads to an 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, which in turn alter the cellular control mechanisms thereby leading to uncontrolled cellular proliferation (Cairns *et al.,* 2011; Tanaka *et al.,* 1994).

1.6. Cell Cycle and Growth Regulation

In recent years some significant evidence have come out related to the converging of stimulatory and inhibitory pathways in the cell on a “cell cycle clock”, a molecular apparatus in the cell nucleus. The cell cycle is nothing but the progression of events which takes place during the generation of two daughter cells from one parental cell. The cell cycle comprises of four major phases like a) First gap phase (G1), b) DNA synthesis stage (S), c) the Second gap phase (G2) and d) the Mitosis phase (M) respectively (Cooper *et al.,* 2000). The time duration which the cell spent in the G1 phase depends on the tissue type and whether it is normal or tumor cell. If the cell is a

proliferating, then it will quickly enter into the synthesis phase (S). In the S phase, replication of DNA takes place and two copies of DNA are produced. The next phase is the G2 phase where the cells are prepared 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. Out of these two major points in the cells cycle, the G1/S stage is of utmost benefit in underscoring cancer and chemotherapy. During the G1 phase, cell can take one of the three possible routes, like a) the cell may enter the S phase or b) cell may enter into the G0 phase in which the cells remain in the quiescent state or c) cell may terminally differentiate and die respectively (Stein *et al.*, 2004).

1.7. Cell Cycle Network

Signalling systems are the key to cell cycle network which decide whether a cell will enter a cell cycle or exit the same. There are four possible pathways to move into different gates

(Barr *et al.,* 2004).

- Senescence
- Apoptosis
- Differentiation
- Cell proliferation

Resting (G0) or stem cells proceed into cell cycle by the proliferative signalling pathways, which are much active. These signalling pathways are highly active, and divided into positive and negative elements. Positive elements are represented by proto-oncogenes, which get mutated to oncogenes and found in tumor cells. On the other side, negative elements constituted by tumor suppressors which suppress tumor. Specifically other signalling pathways are anti-proliferative signalling pathway, which

prevents cell from entering the cell cycle. For example- TGF-β is associated with differentiation. p53 surveillance system constantly monitors the performance of all cell cycle processes. External sources such as radiation cause cancer. It also relates through p53. Thus, understanding of the cell cycle network is significant for understanding the causes of cancer (Sever *et al.,* 2015; Duronio *et al.*, 2013).

1.8. Cellular Signalling Pathways

The signalling pathways are of two types depending on their mode of activation. External stimuli activate the cell surface and pass the information from the cell surface to internal effectors systems (Jordan *et al.,* 2000). Metabolic messengers activate the signalling systems. Conveyance of information occurs through the protein protein interactions or the application of diffusible elements referred as second messengers (Bar-Sagi *et al.,* 2000).

1.9. Intracellular Signalling Pathways

The intracellular signalling pathways, which transmit information within the cell, are stimulated by external stimuli (neurotransmitter, hormone or growth factor) that arrives at the cell surface in the form of chemical signal (Kraus *et al.,* 2001). The internal messenger system generated by the signalling pathways, relay information to the sensors activating cellular responses. Generally, in cancer these signalling molecules are up-regulated or over-expressed in cancer cells (Lahiani *et al.*, 2017).

1.10. Apoptosis Pathway in Cancer

Apoptosis, a physiological process of regulated cell death whereby aged, unattached, damaged, mutant and aged cells are eliminated for homeostasis and normal tissue development (Renehan *et al.,* 2001; Basmaciyan *et* al., 2019). In 1842, Carl Vogt (Peter *et al*., 1997) first described the process of apoptosis but later Lock-shin and Williams who described and briefed out the concept of 'programmed cell death in 1965

(Lockshin *et al.*, 1965). After 10 years, Kerr *et al.*, coined the term "apoptosis" and described the same as Lockshin and Williams (Kerr *et al.,* 1972). The irregularities in this important process give rise to cancer as well as degenerative and autoimmune disorders (Wong *et al.,* 2011). The morphological changes comprised of (i) consolidation of nuclear chromatin (ii) condensation of the cytoplasm (iii) DNA degradation (iv) membrane blebbing (v) fragmentation of the cell into apoptotic bodies. In the absence of inflammation, the surrounding cells take up the apoptotic bodies and are degraded in their lysosomes (Zhang *et al.,* 2018). Cleavage occurs at the linker regions between nucleosomes giving rise to multiple fragments of DNA, and change in the expression of proteins and genes.

Physiological processes like development, proliferation and differentiation of cell, removal of defective and harmful cells, and regulation of the immune system is critically monitored by apoptosis (Wallach *et al.,* 2016). Apoptosis is lessened in viral infections, autoimmune diseases and cancer (Ren *et al.,* 2017). A newer approach to treat cancer is to target the components of aberrant apoptotic pathway, which plays a central role in tumor growth and development of resistance to anti-cancer therapy (Hanahan *et al.*, 2011). The cytotoxic agents and radiation therapy induce apoptosis in the cancerous cells thereby destructing the cells. However, resistance to these therapies might develop due to the mutation of key proteins in the apoptotic pathway. Therefore, role of novel targeting agents or approaches to reverse the drug resistance is important for better management of disease. Apoptosis is mediated by two pathways (a) extrinsic pathway and (b) intrinsic pathway (Pistritto *et al.,* 2016) (**Figure 1.3**). The death receptors on cell surface mediate extrinsic pathway while the mitochondria does so to the intrinsic pathway. The caspases (cysteine aspartic acid specific

proteases), also termed as "executioner" is common to both the pathways (Guicciardi *et al.,* 2009).

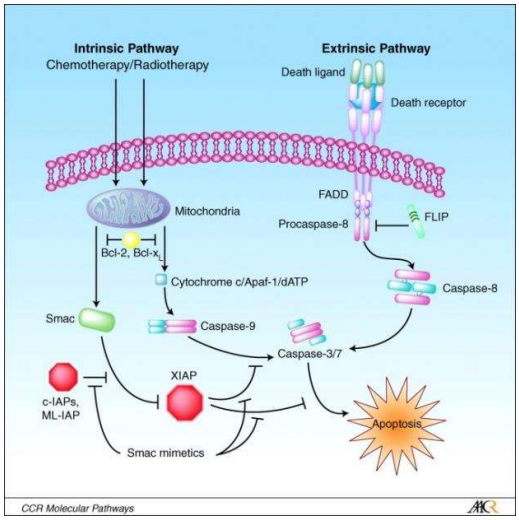


Figure 1.3: Schematic diagram of the extrinsic and intrinsic pathways of apoptosis

1.10.1. The extrinsic pathway

Ligands activating death receptors (DR) mediate the extrinsic pathway of apoptosis. DRs are members of the tumor necrosis factor (TNF) receptor super-family, and include decoy receptors (DcR) and functional receptors. The receptor super-family includes TNF-related apoptosis-inducing ligand receptors-1 (TRAIL-R1, DR4), -2(TRAIL-R2, DR5), TNF-R1, Fas/APO1, DR3, and DR6. Other receptors like TRAIL/APO2L, TNF and FasL/APO1/CD95 regulate cell metabolism, proliferation and cytokine production.

1.10.2. The intrinsic pathway

The intrinsic pathway also called as "mitochondrial pathway" as is initiated in the mitochondria, is activated in response to cellular stress signals from loss of cell survival factors, hypoxia, DNA damage, and defective cell cycle. A balance between the pro-apoptotic and anti-apoptotic members of the Bcl-2 (B-cell lymphoma 2) super family of proteins is needed to regulate the pathway (Kuribayashi *et al.*, 2006). The anti-

apoptotic Bcl-2 proteins maintain the integrity of outer mitochondrial membrane (OMM). Examples of such proteins are Bcl-2, Bcl-2 related gene, Bcl-2 related gene A1, long isoform Bcl-XL, Bcl-w and myeloid cell leukaemia (MCL-1). Upon activation, the intrinsic pathway up regulates the pro-apoptotic BH-3 proteins such as BID (BH3 interacting domain death agonist), PUMA (p53 up regulated modulator of apoptosis), BIM (Bcl-2 interacting mediator of the cell death), BAD (Bcl-2 antagonist of the cell death), BMF (Bcl-2 modifying factor) and Noxa. It may further bind to anti-apoptotic proteins and inhibit their action (Green *et al.*, 2004; Kuribayashi *et al*., 2006).

1.11. Receptor Tyrosine Kinases

RTKs are the transmembrane glycoprotein which comprise of three portions i.e. i) extracellular portion ii) transmembrane region iii) cytoplasmic portion. The cytoplasmic portion is the segment bearing the tyrosine kinase catalytic activity (**Figure 1.4**). RTKs exist in monomeric form in the absence of binding of extracellular ligand (Hubbard *et al.,* 2007; Lemmon *et al.,* 2010). The Met and its family members, and the insulin receptor and its family members are the exceptions, which include a short α chain disulfide-linked to a membrane-spanning β chain and two extracellular α chain disulfide-linked to two membrane-spanning β chains respectively. The chains are also connected to each other by disulfide-linkage forming a α2β2 hetero tetramer. Polypeptide ligands for RTKs are mainly soluble. Some exceptions are found like, the ephrins, the ligands for the Eph receptor family, which either distance the cell membrane or are tethered to the membrane via a GPI (glycosyl-phosphatidylinositol) linkage (Longati *et al.*, 2001; Hubbard *et al.*, 2000). The extracellular portion of RTKs typically contains a varied array of disconnected globular domains such as immunoglobulin (Ig)-like domains, fibronectin type III– like domains, cysteine-rich domains, and EGF-like domains. In disparity, the cytoplasmic portion of RTKs is

simpler, it consist of a juxtamembrane region (just after the transmembrane helix), afterward the tyrosine kinase catalytic domain and a carboxy-terminal region. Key factors are the juxtamembrane and carboxy-terminal regions which vary in length among RTKs. Along with the tyrosine kinase insert, these regions contain tyrosine residues that are autophosphorylated upon ligand binding (Schlessinger *et al*., 2003; Fantl *et al.*, 1993). NRTKs are short of extracellular ligand-binding domain and a transmembrane-spanning region, because they are restricted in the cytoplasm. Some NRTKs are anchored to the cell membrane through amino terminal modification, such as myristoylation or palmitoylation. In addition to a tyrosine kinase domain, NRTKs possess domains that mediate protein-protein, protein-lipid, and protein-DNA interactions **(Figure 1.4)**. The most commonly found protein-protein interaction domains in NRTKs are the Src homology 2 (SH2) and 3 (SH3) domains (Neet *et al.,* 1996; Park *et al.,* 2016).

Mode of activation is by binding to cognate ligands and they transduce the extracellular signal to the cytoplasm by phosphorylating tyrosine residues on the receptors themselves (autophosphorylation) and on downstream signalling proteins.

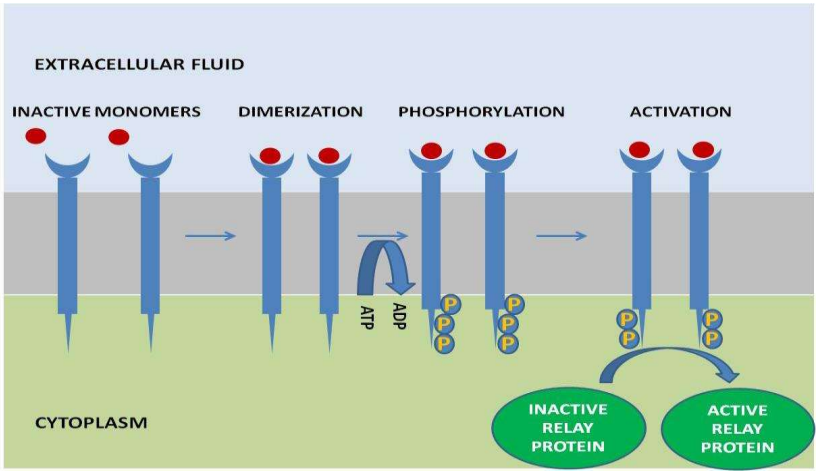


Figure 1.4: Receptor tyrosine kinase

Numerous signalling pathways within cells, which lead to cell proliferation, differentiation, migration, or metabolic changes, are activated by RTKs (Hubbard *et al.,* 2007). The RTK family includes the receptors for insulin and for many growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and nerve growth factor (NGF). The NRTKs are integral components of the signalling cascades triggered by RTKs and by other cell surface receptors such as G protein-coupled receptors and receptors of the immune system.

1.11.1. Regulation of Receptor Tyrosine Kinases

1.11.2. Tyrosine Autophosphorylation

RTKs are activated by mainly two processes like augmentation of intrinsic catalytic activity and creation of binding sites to recruit downstream signalling proteins. In majority of RTKs, activation is accomplished by autophosphorylation on tyrosine residues; which is a consequence of ligand-mediated oligomerization (Tonks *et al.,* 1996). In general, autophosphorylation of tyrosines in the activation loop within the kinase domain results in stimulation of kinase activity, whereas autophosphorylation of tyrosines in the juxtamembrane, kinase insert, and carboxy-terminal regions generates docking sites for modular domains that recognize phosphotyrosine in specific sequence contexts. There are two well-established phosphotyrosine-binding modules SH2 domain and the phosphotyrosine-binding (PTB) domain present within signalling proteins.

1.12. Non Receptor Tyrosine Kinases (NRTK)

Src family is the largest subfamily of NRTKs, with nine members. Its members participate in mitogenesis, T- and B-cell activation, and cytoskeleton restructuring. Multiple *in-vivo* substrates have been described for Src and include, among others, the

PDGF and EGF receptors; the NRTK focal adhesion kinase (Fak); p130Cas, an adapter protein involved in integrin- and growth factor-mediated signalling; (Ziemska *et al.*, 2016). Src is implicated in several human carcinomas, including breast, lung, and colon cancer. NRTKs are critical components in the regulation of the immune system. The Jak families of NRTKs are non-covalently associated with the cytoplasmic domain of cytokine receptors, such as the interferon-γ receptor, and they are activated by ligand-induced receptor oligomerization. Activated Jaks then phosphorylate the cytokine receptors with which they are associated, providing binding sites for the Stat family of transcription factors. Phosphorylation of Stats by Jaks leads to Stat dimerization, translocation to the nucleus, and transcription of specific genes (Tonks *et al.,* 1996).

1.12.1. Regulation of Nonreceptor Tyrosine Kinases

The most common theme in NRTK regulation, as in RTK regulation, is tyrosine phosphorylation. With few exceptions, phosphorylation of tyrosines in the activation loop of NRTKs leads to an increase in enzymatic activity. Activation loop phosphorylation occurs via trans-autophosphorylation or phosphorylation by a different NRTK. Phosphorylation of tyrosines outside of the activation loop can negatively regulate kinase activity. PTPs restore NRTKs to their basal state of activity or, in some cases, positively regulate NRTK activity.

1.13. Epidermal Growth Factor Receptor (EGFR) Pathway

Epidermal growth factor receptors (EGFRs) belong to ErbB family of receptor tyrosine kinases (TK) articulated in a number of types of cancer (Wee *et al.,* 2017; Kuribayashi *et al*., 2006), including breast (Osada *et al.*, 2018), lung, colon, head, neck, blood, and esophageal (Steuer *et al.*, 2015; Li *et al.,* 2006). They are the major contributors of a complex signalling cascade that regulate growth, signalling, differentiation, adhesion, migration and endurance of cancer cells. EGFR and its family members have emerged

as attractive candidates for anti-cancer therapy due to their multi dimensional role in the progression of cancer (Hirsch *et al.*, 2009). Stanley Cohen (Nobel Prize Laureate in Physiology/Medicine) discovered epidermal growth factor (EGF) 25 years ago and elucidated its role in cell growth. Specifically, the deviant activity of EGFR is seen in growth of tumor cells.

1.13.1. Mechanism of EGFR Signalling Activation

Ligand-induced receptor dimerization triggers the activation of EGFR signalling, followed by the cross phosphorylation of specific residues in the C-terminal tail of the partnering receptor by the tyrosine residues present in the intrinsic kinase domain of one receptor **(Figure 1.5)**, thus providing a scaffold for the recruitment of effector proteins (Tsai *et al.,* 2019; Siwak *et al.,* 2010). This occurs via the Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains on the effector proteins as well as the phosphotyrosine motif present on the intracellular tyrosine kinase domain of the receptor.

On subsequent dissociation, the activated adaptor and effector proteins will stimulate their corresponding signalling cascade. This further leads to cell proliferation, angiogenesis, migration, survival, and adhesion (Yarden *et al.*, 2001; Seshacharyulu *et al.*, 2012). The genes involved in these pathways harbor mutations leading to deregulation of above cellular processes.

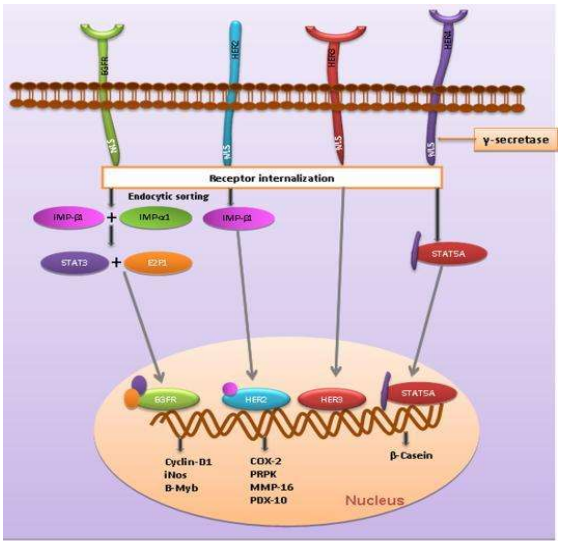


Figure 1.5: Schematic representation of EGFR signalling pathway

1.13.2. EGFR Targeted Therapies

Owing to the functional involvement of EGFR in various cellular processes, two distinct therapeutic approaches are utilized for targeting EGFR in human malignancies- (a) use of small molecules as tyrosine kinase inhibitors (b) use of monoclonal antibodies. Both act through different mechanism of action- TK inhibitor target the intra cellular TK domain, while the anti-EGFR antibodies bind to extracellular domains. Anti-EGFR agents have proved to be useful in the treatment of different types of cancers like pancreatic, colorectal, head and neck with respect to the survival, progression and overall response rate (Petrelli *et al.,* 2011).

1.14. Vascular Endothelial Growth Factor (VEGF) Pathway

Vascular endothelial growth factor (VEGF), also termed as vascular permeability factor (VPF) is an endothelial cell-specific mitogen (Bates *et al.,* 2010), that is produced by keratinocytes (Frank *et al*., 1995), tumor cells (Boocock *et al.,* 1995), platelets (Verheul *et al.,* 1994), macrophages (Itakura *et al.,* 2010), and renal mesangial cells (Iijima *et al.,* 1993). The variants like VEGF121, VEGF145, VEGF148, VEGF165, VEGF183,

VEGF189 and VEGF206 occur as a result of alternative splicing, and are distinct in their receptor specificity and function (Chung *et al.,* 2011; Takahashi *et al.,* 2011).

Ever since its discovery, considerable studies have been made to understand the role of VEGF and VEGF receptors in various physiological and pathological functions like wound healing (Chintalgattu *et al.,* 2003), haematopoiesis (Ferrara *et al.,*2009), bone formation, vascular permeability (Koch *et al.,* 2012), lymph angiogenesis and angiogenesis.

Based on these studies VEGF and VEGF receptor targeted anti-angiogenic (Leung *et al.,* 1989; Ferrara *et al.,* 2009) therapies have been developed (Gerber *et al.,* 1999; Reichardt *et al.,* 1991; Ellis *et al.,* 2008).

1.14.1. VEGF-Mediated Functions in Tumour Cells

The autocrine and paracrine VEGF signaling contributes to tumorigenesis independent of angiogenesis and vascular permeability. Neuropilins (NRPs) and VEGF receptor tyrosine kinases (RTKs) mediate the autocrine signaling. VEGF accelerate the growth, survival, migration and invasion of cancer cells with the mechanistic aid of dominant signaling pathways (Moghaddam *et al.,* 2012; Yang *et al.,* 2018; Bachelder *et al.,* 2001). For example, the survivality of breast carcinoma cells is sustained by the NRP1-mediated VEGF signaling. It also affects the survival of tumor cells by activating the PI3K-AKT pathway. ERK1 or ERK2 as well as JNK are activated by the VEGFR1 (Fanm *et al*., 2005, thereby promoting the migration and invasion of colorectal carcinoma cells (Barr *et al.,* 2008; Perrot-Applanat *et al.*, 2012; Stanton *et al.,* 2013).

- **Targeting VEGF**

VEGF family members can be additional target to inhibit the functioning of tumor cell. 'VEGF trap' sequestering VEGF can suppress the genesis of pancreatic carcinoma cell tumor and thereby, highlights the significance of VEGF targeting (Pàez-Ribes *et al.,*

2009). Contradictory reports have also been reported as in the case of anti-angiogenic therapy, where the VEGF or VEGF RTKs' inhibitions lead to an increase in tumour invasion and metastasis (Fukasawa *et al.*, 2004). Therefore, deep learning of the same is necessary to understand the fundamentals behind it (Wedam *et al.,* 2013; Snuderl *et al.,* 2013).

1.15. Inhibition of the Hedgehog pathway

In 1980, Christiane Nusslein-Volhard and Eric F Weischaus discovered hedgehog (Hh) gene while screening for mutations disrupting the larval body plan of Drosophila (Afiattalab *et al.,* 1980; Varjosalo *et al.,* 2008). The short and spiked phenotype of the cuticle of the Hh mutant Drosophila larvae resembling the spikes of a hedgehog is the source of its name (Bryden *et al.,* 1971). The Hh family of proteins may either act as morphogens or mitogens inducting specified cell fates within a target field and regulating cell proliferation controlling the form of developing organs respectively. These proteins are the key mediators of vertebrate embryonic developmental processes. Defects in the signaling pathway may give birth to foetuses with brain, facial and midline defects such as cleft palate, cyclopia, holoprosencephaly or microencephaly (Ingham *et al.,* 2001). A teratogenic plant alkaloid, cyclopamine was discovered in lambs whose mothers had ingested corn lilies, a phenotype similar to Sonic Hedgehog (Shh) knockout mice. This was the first Hh pathway inhibitor to be identified. It caused holoprosencephaly and cyclopia in the lamps (Rubin *et al.,* 2006; Roessler *et al.,* 1996).

1.16. Approaches For The Treatment of Cancer

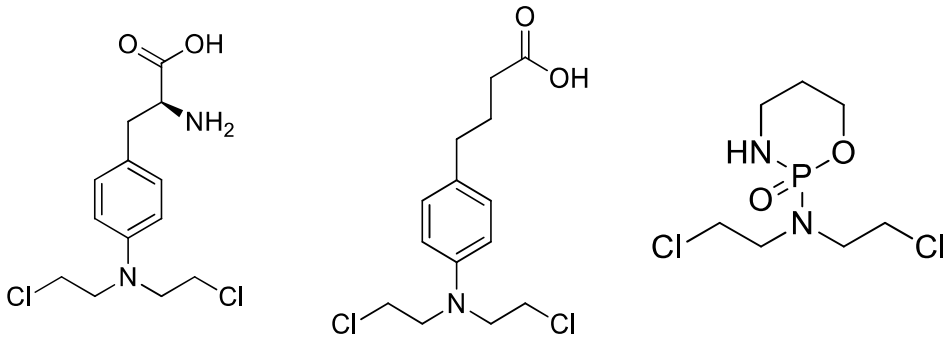
1.16.1. Alkylating Agents

These are frequently used in the treatment of cancer. These agents are non-cell cycle specific and target all the phases of cell cycle. They damage the nuclear as well as mitochondrial DNA by addition of alkyl groups leading to breakage of DNA strands or

point mutations. Such alterations in the DNA result in programmed cell death (apoptosis). Alkylating agents are used for a wide range of cancer treatments especially Leukemia and slow-growing solid tumors (Kintzel *et al*. 1995).

In the World war I these were used as the chemical warfare agents due to their toxic and vesicant properties on the eyes, skin and lungs as these agents produce atrophy of lymphoid and myeloid tissues, which lead to their exploratory use for treating lymphomas, leukaemias of breast, ovary and uterine, multiple myeloma, Hodgkin and Non-Hodgkin cancers (**Figure 1.6).**

Examples: Melphalan, Clorambucil, Cyclophosphamide, Carmustine and Lomustine, Semustine



Melphalan **Clorambucil** **Cyclophosphamide**

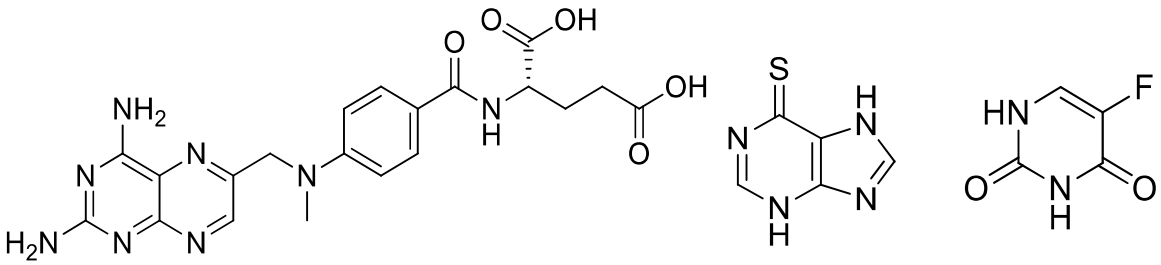
Figure 1.6: Alkylating agents used for the treatment of cancer

1.16.2. Antimetabolites

These molecules are the metabolites of nucleic acids. They hold a structural resemblance with the synthetic related with cell assimilation drew in with cell division. They similarly damage the DNA by mimicking a purine, augmenting into DNA or RNA and as such interfere with the DNA replication frames. Antimetabolites are cell cycle unequivocal authorities and as such target express time of the cell cycle. They are regularly used in the treatment of hemolytic malignancies, for instance, chronic and

acute leukemia. Beside this, they are used in treatment of breast disease, gastrointestinal tumors, head and neck malignancies (**Figure 1.7)**.

Examples: Methotrexate, Mercaptopurine, 5-Fluorouracil



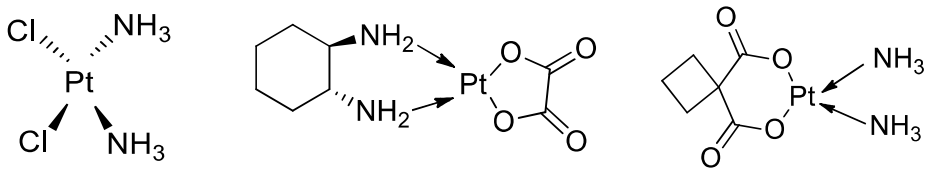
Methotrexate **Mercaptopurine** **5-Fluorouracil**

Figure 1.7: Antimetabolite drugs used for the treatment of cancer

1.16.3. Platinum Compounds

The parent compound "Cisplatin" has been seen to be viable against various malignancies, for instance, osteosarcomas, small cell lung, gastric, non-small cell lung, head and neck, breast, uterine, penile, ovarian and testicular individually. It has been deductively settled that the genuine focal point of cisplatin drugs is the DNA molecule and the participation results in the improvement of intrastrand cross-intersections causing a bowed course of action and a close-by winding of the DNA helix. Disregarding the way that action of cisplatin in the clinical oncology significantly influenced threat chemotherapy, the drug has been found to show various toxicities towards common cells even at low fixations (Iglesias *et al.,* 2018; Tseng *et al.,* 2009). Consequently a couple of new cisplatin analogs have been fused **(Figure 1.8)**.

Examples: Cisplatin, Oxaliplatin and Carboplatin

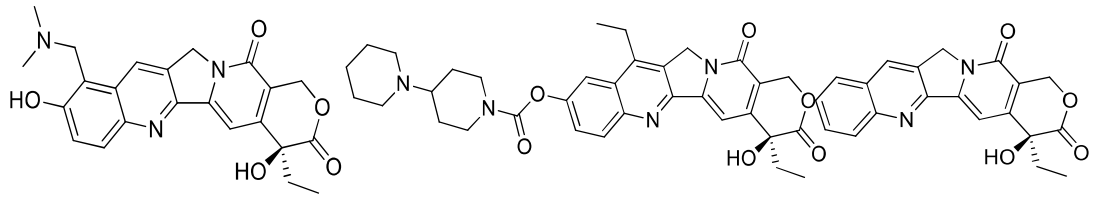


Cisplatin **Oxaliplatin** **Carboplatin**

Figure 1.8: Platinum drugs used for the treatment of cancer

1.16.4. Topoisomerase Inhibitors

At the time of replication and translation, the DNA topoisomerase I and II and their isoforms modulate the structure and topology of DNA. The transient cuts in DNA induced by topoisomerases enable the strands to pass through the nicks, and get rejoined. At this time of nicking and rejoining of DNA, 'cleavable complexes' are formed. Drugs, which stabilize such cleavable complexes, tend to inhibit topoisomerases and are called as topoisomerase inhibitors, and finally DNA synthesis get inhibited (Kawano *et al.,* 2014; Hande *et al.*, 1998; Du *et al*., 2011). The well-known topo I inhibitor, is a plant alkaloid called camptothecin and its synthetic analogues topotecan and irinotecan. The parent moiety contains a lactone ring, which is needed for its activity. Topotecan is active against ovarian and small-cell lung cancers while irinotecan exhibits potent activity against metastatic colon and cervical cancers (**Figure 1.9)**.

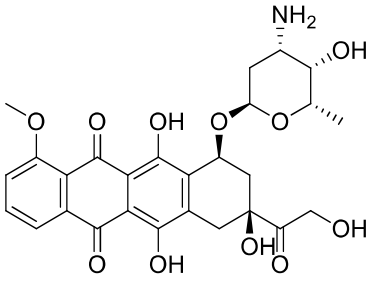


Topotecan **Irinotecan** **Camptothecin**

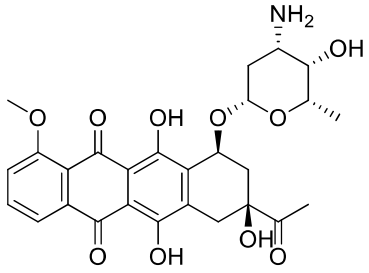
Figure 1.9: Topoisomerase I inhibitors used for the treatment of cancer.

The different class of intense topoisomerase II inhibitors incorporates anthracyclin anti-infection agents, for example, Doxorubicin, daunomycin, idarubicin and epirubicin .Be that as it may, their anticancer action has been ascribed to a few different components including their free radical creating capacities through redox cycling. Most specialists having a place with this class of mixes were found incidentally and experience the ill effects of an absence of selectivity and explicitness when all is said in done. They are additionally less powerful against (**Figure 1.10)**.

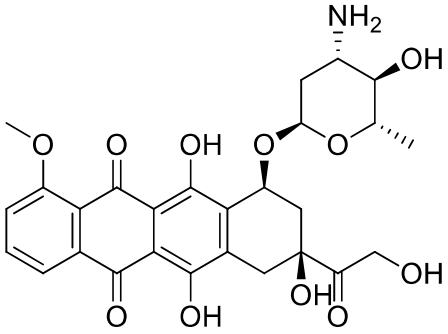
Examples: Doxorubicin, Daunomycin, Epirubicin and Idarubicin



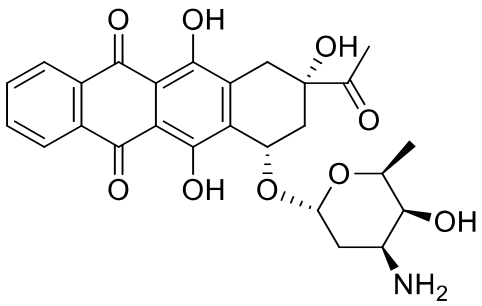
Doxorubicin



Daunomycin



Epirubicin



Idarubicin

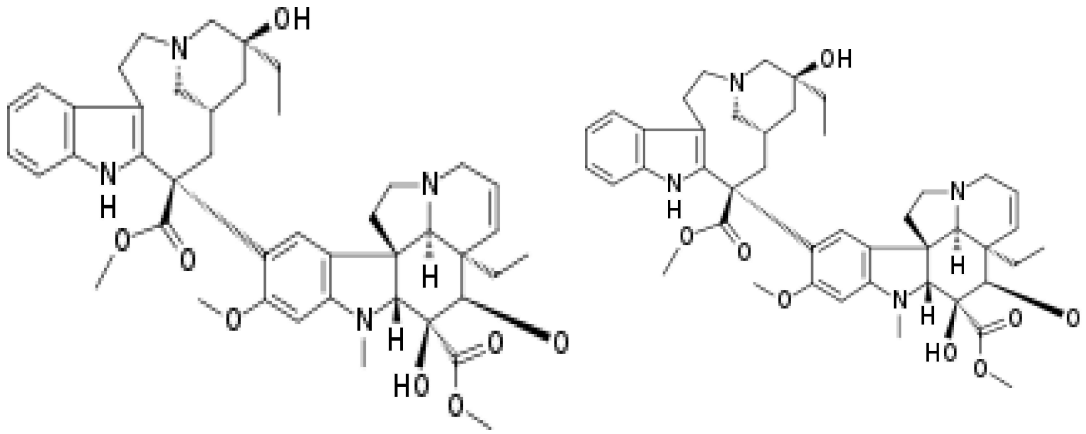
Figure 1.10: Topoisomerase II inhibitors used for the treatment of cancer

1.16.5. Plant Alkaloids

These are the chemotherapeutic agents derived from plants. They are cell cycle specific agents however; the target phase of cell cycle varies with the compound being used. Plant alkaloids act by blocking cell division thus slowing the tumor growth and

progression. The vinca alkaloids specifically target the M phase of cell cycle and act by blocking tubulin assembly into microtubules in the mitotic spindle during cell division (**Figure 1.11)**. They are used for the treatment of testicular carcinomas, Ewing's sarcoma and acute lymphoblastic leukemia (Wilson *et al.,* 2004; Bhatia *et al*., 2007).

Examples: Vinblastine and Vincristine



Vinblastine **Vincristine**

Figure 1.11: Plant Alkaloids used as chemotherapeutic agents

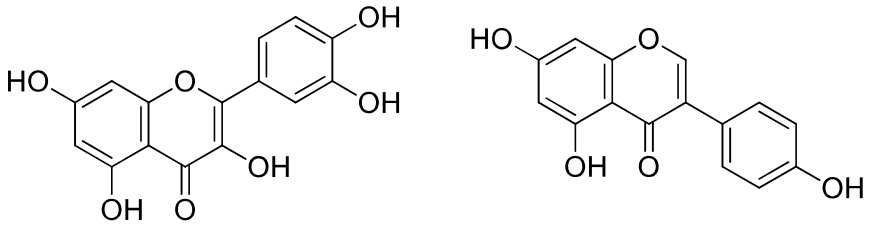
1.17. Novel Approaches to Anticancer Drug Discovery

1.17.1. Signal transduction Inhibitors

It is well known that cell signalling pathways play remarkable role in tumour 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 platelet-derived growth factor (PDGF), macrophage colony stimulatory factor (M-CSF) for antigens and cytokines, epidermal growth factor (EDGF) and G protein-coupled receptors located on the cell surface play an important role in the relaying of stimulus to the interior of the cell (Scapin *et al.,* 2002; Demoulin *et al.,* 2012).

Cytoplasmic protein-tyrosine kinase (PTK) domains located on the growth factor receptors are an excellent target for the design of anti-cancer drugs (**Figure 1.12)**

Examples: Genistein and Quercetin



Quercetin **Genistein**

Figure 1.12: Signal transduction inhibitors

1.17.2. Tyrosine Kinase Inhibitors

Being present on the cell membrane as a part of growth factor receptor, the tyrosine kinases are called receptor tyrosine kinases and are predominant as signalling molecule in cell. Monoclonal antibodies inhibit cancer cell proliferation both *in vitro* and *in vivo* by down-regulating the expression of tyrosine kinase protein (Buchdunger *et al.,*1996). Tyrosine phosphatase upon activation by somatostatin (octapeptide) gets dephosphorylated and ceases the cancer growth signal. Natural products such as quercetin, genistein, lavendustin, erbstatin, herbimycin, Lovastatin, Limonene and many others also exhibit a broad but non-specific inhibition of tyrosine kinases by binding site in the protein domain (Levitzki *et al.,* 1995).

Designing inhibitors has been specific about the substrate binding site but not the ATP-binding site. Some selective inhibitors of RTK include Dasatinib **(**Araujo *et al.,* 1999**)**, Imatinib (Kano *et al.,* 2009), Gefitinib (Kaur *et al*., 2013) and Erlotinib (Mandal *et al.,* 2016) etc., which act as selective inhibitors of EGFR tyrosine kinase **(Figure 1.13)**.

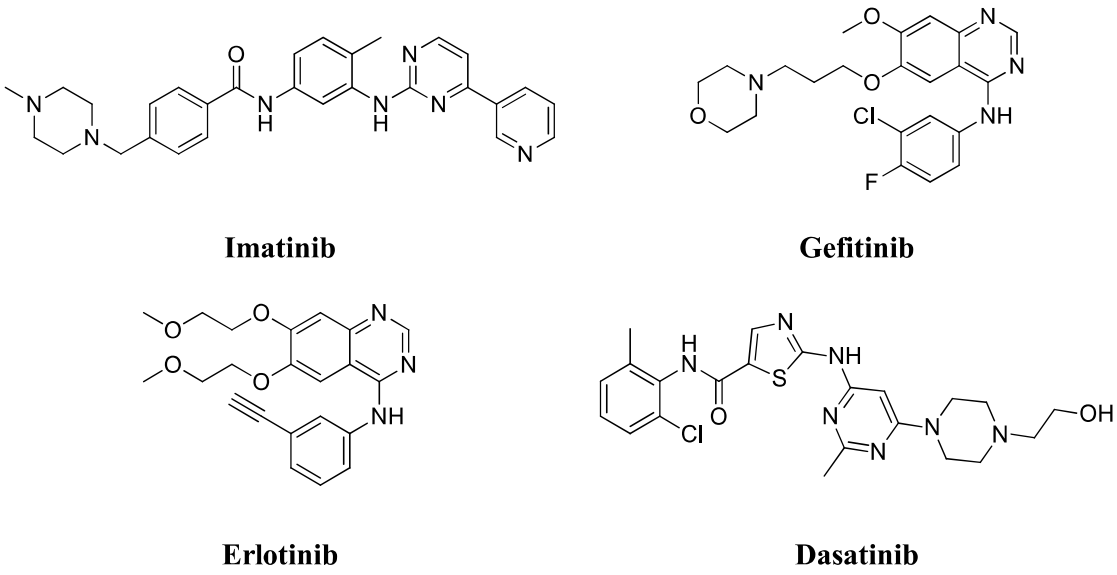
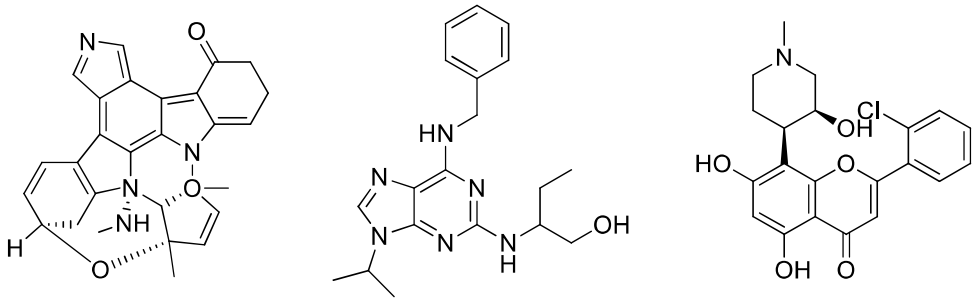


Figure 1.13: Tyrosine kinase inhibitors

1.17.3. Cell Cycle Regulators

Neoplastic transformation is a result of abnormal regulation of the cell cycle. So, to maintain normal cell regulation of cell cycle is of utmost importance. A series of protein kinase complexes and cyclin dependent kinases (cdks) are positive regulators of the cell cycle (Bruyère *et al.,* 2013). Radiation, carcinogens and cytotoxic drugs damage DNA which is repaired in normal non-cancerous cells at certain stoppages in the cell cycle whereas in cancerous cell the inhibitory control is lost. As a result, the mutated DNA gets replicated without correction. Transforming growth factor (TGF-β) prevents the formation of the Cyclin d/CDK complex and the phosphoryaltion of Rb (retinoblastoma) by blocking the synthesis of cdk-4 in the G1 phase and thereby, leading 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 (Shapiro *et al.,* 1999; Senderowicz *et al.,* 2002). Among these Flavopyridol, which is a polyhydroxylated flavone is an efficient inhibitor of protein kinases **(Figure 1.14)**.



Staurosporine **Seliciclib** **Flavopiridol**

Figure 1.14: Cell cycle regulators

Other inhibitory compound includes the alkaloid, staurosporine (Gadbois *et al.,* 1992; Ōmura*et al.,* 2008) and purine derivative such Seliciclib **(**MacCallum *et al.,* 2005**)**. cdk inhibitors are more sensitive than the cytotoxic drugs in the cell cycle phase, thereby, arresting or delaying progress by acting as cytotoxic and cytostatic agents or enhancing the anticancer activity of the cytotoxic agents.

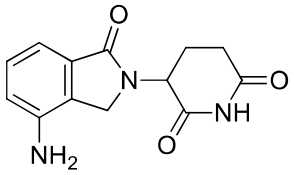
1.17.4. Inhibitors Acting Through the Apoptosis Pathway

Apoptosis or modified cell death is another procedure for the disposal of disease cells. Equilibrium between the mitosis and apoptosis process endures state condition in normal cells. An enormous number of assorted characteristic and engineered inhibitors of apoptosis have been found, for example, development factors, serum factors, phorbol esters, cytokines, certain infections, RNA protein union inhibitors, granulocyte animating components (GM-CSF), PKC tyrosine phosphorylation inhibitors, endonucleases inhibitors of transglutaminases and proteases. Additional important inducers and inhibitors of apoptosis include the p53 cancer suppressor gene and certain proto-oncogenes (Brown *et al.,* 1999; Dang *et al.,* 1999).

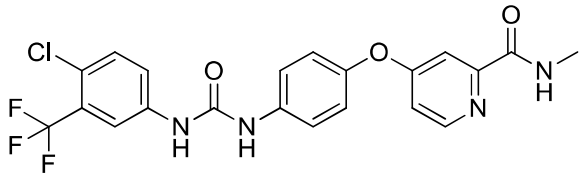
1.17.5. Angiogenesis Inhibitors

Angiogenesis is a procedure where new blood vessels are formed and endothelial cells gap and relocate to shape new vessels. This procedure can be seen under typical

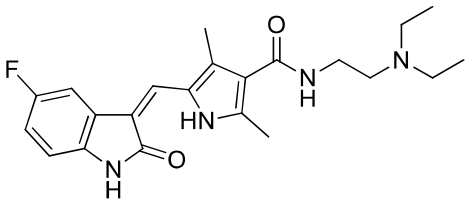
conditions, for example, wound recuperating and in ailment states, for example, joint inflammation, psoriasis, duodenal ulcers, certain scatters of female regenerative framework and diseases. In the harmful express, the new vessels are delicate walled and broken and give access to the flow to a gathering of malignant growth cells, which can metastasize and furthermore obtain the angiogenic phenotype (Wang *et al.,* 2017). The vascular endothelial development factor (VEGF) and essential fibroblast development factor (bFGF) is two most significant proteins that support malignancy development (Ke *et al.,* 2000). At the point when they get detached from the malignancy cells, these can trigger the development of new narrow vessels. Moreover, relocating endothelial cells additionally produce lattice metalloproteinases (MMPs), which are believed to be in charge of the breakdown of storm cellar layers and extracellular grids **(Figure 1.15)**. Since malignant growth cells require an extra blood supply for development, hindrance of angiogenesis is a legitimate way to deal with control of the diseases. **Example:** Lenalidomide, Sorafenib, Sunitinib and Axitinib



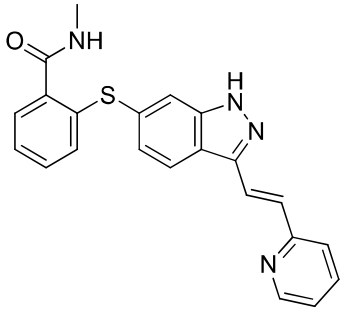
Lenalidomide



Sorafenib



Sunitinib



Axitinib

Figure 1.15: Angiogenesis inhibitors

1.18. Recent Developments in Anticancer Drug Design

1.18.1. Drug Design

Rational drug design or simply drug design is a biological target based invention of new medications. The process of drug design is started once the hypothesis is correlated with the biological activity and chemistry of a molecule or series of molecules. Based on structural similarities and differences of the test molecules (both active and inactive) and without much learning the biochemical processes involved, the hypothesis is designed. Compounds having maximum functional groups or features relevant for the particular biological activity are selected for synthesis. Design of new compounds is totally hypothetical based discovery of a new drug and it consist of the following strategies-

- Ligand based drug design
- Structure based drug design

Structure based drug design is dependent on the three dimensional structure of the biological target, while ligand based design to like molecules binding to biological target of interest. Both of these strategies are important for better and efficient drug design (Fons *et al.,* 2017; Mandal *et al.*, 2009). The SBDD process has been discussed in the following section.

1.18.2. Structure-Based Drug Design (SBDD)

Structure-based drug design (SBDD), an important tool of the rational drug design toolbox utilizes the knowledge of known 3D dimensions of proteins in developing new drug compounds. It selects molecules involved in a particular cell signalling or metabolic pathway related to the disease conditions under study. Proteins and enzymes involved in these pathways are the common drug targets. The designed drugs tend to restore, inhibit or alter the structure and behaviour of disease-related proteins and

enzymes (Vulpetti *et al.,* 2009). X-ray crystallography or Nuclear magnetic resonance (NMR) techniques resolve the 3D structure of targeted proteins to a few Angstroms, which help in precise examination of the interaction between atoms of potential protein targets and drug compounds that bind to that proteins. This potential of SBDD to study the proteins and drug compounds under high magnification make it one of the most robust methods in drug design (Schneider *et al.,* 2005; Mandal *et al.*, 2009).

1.18.3. Docking

A SBDD methodology that takes into consideration the predicted orientation of one molecule to a second when bound to each other to form a stable complex is known as docking (Jorgensen *et al.,* 2004). Molecular docking can be related to "lock-and-key", wherein the protein acts as "lock" and the ligand as "key". The lock will open up only with correct relative orientation of the key, once it is inserted in the key hole located on the surface of the lock **(Figure 1.16)**.

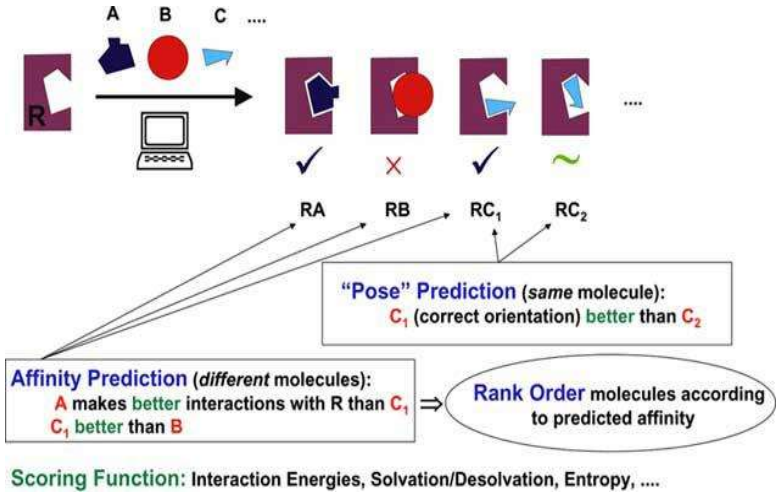


Figure 1.16: Illustration of docking and scoring. R symbolises a receptor structure, A, B and C represent small molecules to be docked into the receptor

Molecular docking programs can be of great aid in predicting the orientation with which a small molecule drug candidate binds to their protein targets. The bioactivity and affinity of the test molecule can also be determined without performing any

laboratory experiment (Lengauer *et al.,* 1996; Halperin *et al.,* 2002). Thus, docking is of prime importance in rational designing of drugs. The core compartments of docking software are-

Optimization algorithm: Also known as search algorithm. It helps to detect the best conformation of the protein and ligand system. Conformation represents the orientation and position of the ligand with respect to protein, while in flexible docking, the internal flexible structure of ligand and sometimes the protein is also reported. Sophisticated search techniques like Genetic Algorithms and Monte Carlo simulations are applied to screen out the possible conformations.

Score function: Also known as evaluation score. This provides a predictive measure of the interactive strength of a given ligand to that of a particular protein. Energy force field is the frequently opted evaluation function. Components from multiple scoring functions can also be combined in hybrid scoring functions. Experimental binding mode may be screened out from the searching algorithm with the application of scoring function. AMBER, OPLS or CHARMM utilizes molecular mechanics force fields. Knowledge based functions or empirical free energy scoring functions are also available (Jain *et al.,* 2006).

1.18.4. Approaches for the Docking

Identification of protein and ligand: The protein and ligand molecule is identified based on their description about the matching surface. This description makes it easier to find the docking position of the target and ligand molecule.

Replicating the original process of docking by which the ligand and protein of calculated energies interact: Simulation of the docking process is a complex approach whereby the ligand moves in its conformational space unless it fits itself in to one of the active sites of protein. Translational, rotational, energy level as well as internal changes

are incorporated by this motion of ligand. Hence, the total energy of the system should be calculated after every move (Shoichet *et al.,* 1992; Robertson *et al.,* 2007).

1.19. Quinones

After chlorethyl alkylating agents, quinones are the second largest class of cytotoxic agents used as an anticancer drug (O'brien *et al*., 1991), from soil fungi of streptomyces strains. It was first isolated in the 1950s. In 1963 daunorubicin antibiotic was first isolated as an anthraquinone glycosidic inhibitor for the treatment of antileukemia. 1969 was the year of discovery of doxorubicin antibiotic, it was isolated and found to a better antitumor activity against various cancer such as prostate, bladder, lung, neck, head, breast cancer and ovary cancer (Wakharde *et al.*, 2018).

The mitomycin C antibiotic was isolated in 1958 used for the antitumor efficacy but the anticancer activity was found for a short period of time. Both the doxorubicin and mitomycin C antibiotic were found to be higher effective against hypoxic cancerous cells. Mitomycin C was mostly accredited to 'bioreductive alkylation' via substitution or addition on the quinone ring. Quinone moiety based various drug are used for the treatment of solid cancer for example mitomycin, daunorubicin, saintopin, doxorubicin, anthracyclines and mitoxantrones.

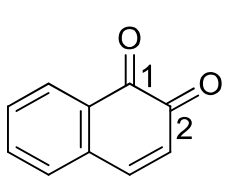
1,4-Naphthoquinones moiety with electron accepting or electron donating substituent produce anion radical that form redox couple which is responsible for production of hydrogen peroxide and superoxide to damage cells. It is most accepted scaffolds usually present in various natural products having different biological effectiveness. The structure activity relationship (SAR) of heterocyclic quinones containing nitrogen atoms is very important for the biological properties. Quinones are one of the most accepted scaffolds, extensively distributed in nature, and obtained from microbes, animals and plants. It is commonly used as anti-inflammatory (McNamara *et al.,* 2005),

laxative agents (Srinivas *et al.,* 2007), antifeedant (Akhtar *et al.,* 2012), antiplatelet (Fuentes *et al.,* 2018), anticancer (Epifano *et al.,* 2014), etc.

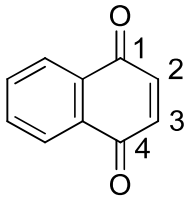
The Quinone scaffolds have not only fascinated research interests but also led to the approval of number of commercial drugs by the FDA for the treatment of various diseases. Several FDA approved drugs along with various biological activities are mentioned in **Figure 1.17** based on quinone moieties.

1.20. Naphthoquinone

Naphthoquinone, derived from naphthalene is subclasses of quinine having planar geometry. They have aromatic ring fused to a quinone. Mainly two types of naphthoquinone i.e., 1,2-Naphthoquinone and 1,4-Naphthoquinone are found in nature.

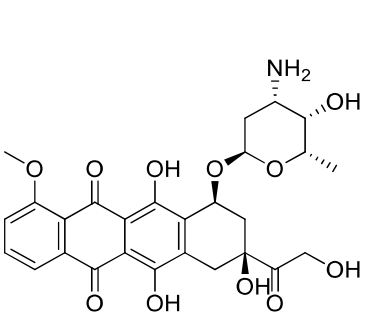


1,2-Naphthoquinone

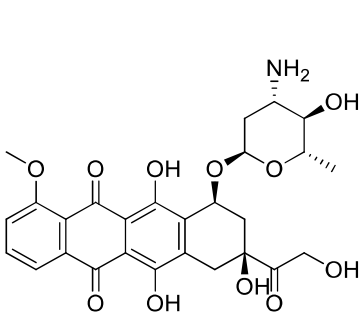


1,4-Napthoquinone

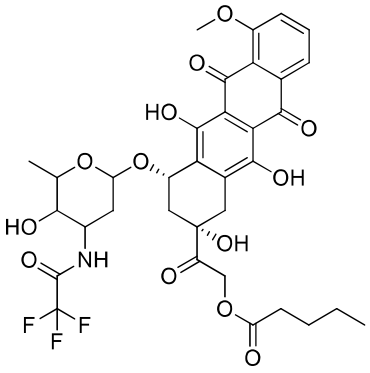
The molecules containing naphthoquinone scaffolds used as a protein kinase, coenzyme, and vitamin K-dependent carboxylase inhibitors. It can also been used as a growth stimulator for bifidobacteria.



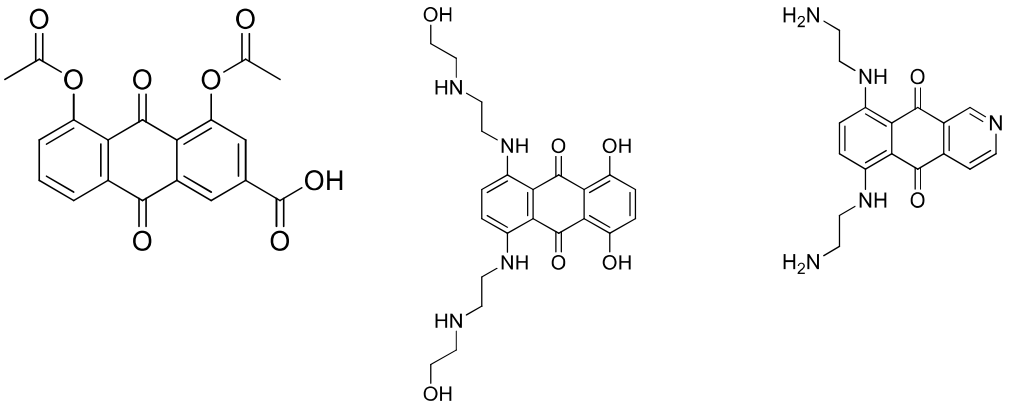
Doxorubicin



Epirubicin



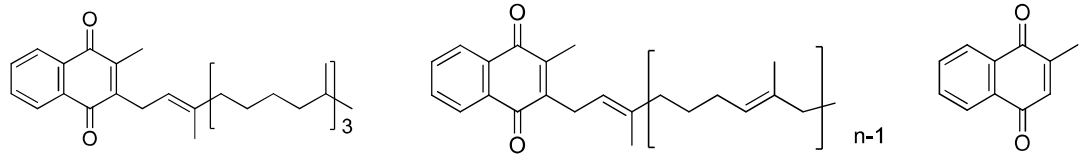
Valrubicin



Diacerein **Mitoxantrone** **Pixantrone**

Figure 1.17: Commercial drugs containing quinone scaffold

Vitamin K1 (Phylloquinone) is a naturally occurring class of 1,4-naphthoquinone molecule (**Figure 1.18).** It includes vitamin K1, vitamin K2 (menaquinone) and vitamin K3 (menadione). It is essential for the biological system as it plays a key role in the coagulation of blood. Menaquinone is essential for bone health. Ascorbic acid in combination with Menadione is under clinical trial for the treatment of cancer. Therefore, naphthoquinone scaffold is very interesting for research in medicinal chemistry because a variation of substitution could be significantly changing its biological responses.



Phylloquinone **Menaquinone** **Menadione**

Figure 1.18: Vitamin K series molecules

1.21. Factors Affecting Cancer and Naphthoquinone-Based Anticancer Drugs

1.21.1. Oxidative Stress

An imbalance between reactive oxygen species (ROS) and antioxidant protection causes oxidative stress (Ott *et al.,* 2017). The excess amount of ROS defeats the

protection by antioxidant, and pile up in the cells harming and changing diverse cell segments, such as proteins, lipids, RNA and DNA. A high concentration of ROS is undesired, while a low fixation is needed to maintain the subcellular processes, for example, disulfide bond development, catalyst actuation, enzyme activation and gene transduction (Schieber *et al.,* 2014; Rahman *et al.,* 2013).

1.21.2. Reactive Oxygen Species (ROS)

A mass of reactive free oxygen radical and its derivatives such as hydroxyl radical, single oxygen, peroxides and superoxide radical anion together constitute the reactive oxygen species (ROS). A reduction of one-electron from the molecular oxygen results in the formation of superoxide radical, which changes over to hydrogen peroxide. Of all the ROS, hydrogen peroxide is the least reactive and therefore, its life span in the cells is longer than any other ROS (Sharma *et al.,* 2012).

1.21.3. Level of ROS in Cancer Cell *vs.* Normal Cell

Cancer cells are quickly separating and metabolically extremely active than the ordinary cells, which have less amount of endogenous ROS. The increased metabolic rate in tumor cells lead to the greater demand of ATP than the normal cell lines. This unwanted metabolic burden on ETC triggers the raise of ROS level, which can harm the mitochondrial DNA and result in the mutation. Therefore, the mutation causes extra electrons to run away from ETC forming extra ROS (Kumari *et al.,* 2018; Diehn *et al.,* 2009).

1.21.4. Mechanism of Cell Death Involving Quinone

The toxicity of quinones depends upon its ROS producing capability, and the tendency to work as a Michael acceptor. The carbonyl group on quinone may accept one or two electrons and protons, and form semiquinone, which in turn reduces the molecular oxygen forming ROS.

1.22. Modification of Signal Transduction by Quinones

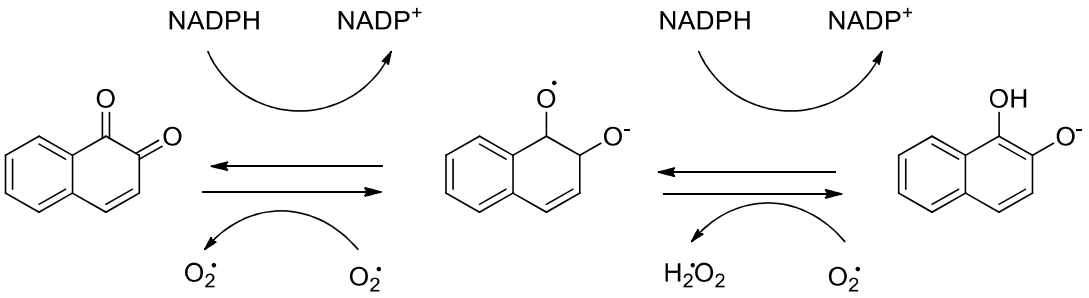
Quinones have been shown to modify cell signalling pathways by reacting with key regulatory proteins, either directly as electrophiles or indirectly through the generation of ROS (Kar *et al.,* 2003). For examples such regulatory proteins, protein tyrosine phosphatase (PTP) and Kelch-like ECH-associated protein 1 (Keap1).

1.23. Quinone as Michael Acceptor

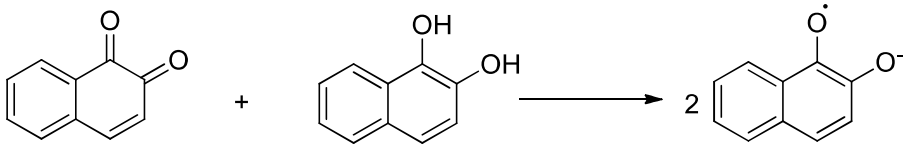
Quinones react with regulatory proteins, either directly as electrophile or indirectly through the generation of ROS to modify the signalling pathways of the cell. Kelch-like ECH-associated protein 1 (Keap1) and protein tyrosine phosphatase (PTP) is such regulatory proteins. Quinone derivatives having open α,β-unsaturated ketones bind to nucleophiles through Michael addition. For example, menadione thioester is produced through Michael addition reaction when menadione binds covalently to β-93 thiol groups of human haemoglobin inducing haemolysis of erythrocytes (Lin *et al.,* 2015). Upon bio-activation compounds like doxorubicin and daunorubicin lacking α,β-unsaturated site alkylate DNA and other nucleophilic macromolecules *via* Michael addition. The sugar moiety undergoes reductive cleavage to form α, β-unsaturated site at ring D.

1.24. Quinones as Electron Transfer Agents

Quinones can initiate the free radical chain reactions. Free radicals contain atleast one unpaired electron, which can be transferred to other species. Such reactions involving oxygen lead to the formation of reactive oxygen species (ROS) like superoxide hydroxyl radical and hydrogen peroxide. In the biological system, quinones get reduced to semiquinones and free radicals, and further to hydroquinone with the aid of cytochrome P450 reductase and other flavoprotein enzymes (Kumagai *et al.,* 2012) **(Figure 1.19)**.



Reaction 1



Reaction 2

Figure 1.19: Mechanism of electron transfer of quinones