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REVIEW ARTICLE

Holistic Molecular Approaches for Anticancer Therapy

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ABSTRACT

Cancer is a threat concomitant with human history. Although we have stepped into the very advanced twenty first century with considerable progress in cancer treatment, it is still a very difficult disease to treat and is the second most common disease that causes mortality. In recent years, the discovery of new anticancer drugs has evolved from a dramatic shift from cell based screening for anti proliferative effects to a more mechanistically based approach that targets the specific molecular lesions thought to be responsible for the development and maintenance of the malignant phenotype in various forms of cancer. The development of molecularly targeted drugs has improved the efficacy and selectivity of cancer treatment by exploiting the differences between cancer cells and normal cells. Targeted therapies are now a component of treatment for many types of cancer, including breast, colorectal, lung and pancreatic as well as lymphoma, leukemia and multiple myeloma. In order to enhance the specificity and efficacy of current cancer therapies, the aim of this review is to shed light on some of the important molecular targets.

KEYWORDS: Cancer, Apoptosis, Cell signalling, Therapeutic targets.

INTRODUCTION

Cancer is the second largest health problem of the world after cardiovascular diseases in both developed and developing countries. Some of the common cancer causing agents are given in the figure below (Fig 1). According to the World Health Organization 7.6 million people died of cancer worldwide in the year 2007, which accounts for 13 percent of all the deaths, and the incidences, are expected to increase by 12 million deaths in 2030. As per the reports of Parkin et al., 2005 (1) the most commonly diagnosed cancers worldwide are lung (1.35 million), breast (1.15 million) and colorectal (1million), and the most common causes of deaths due to cancers are lung cancer (1.18 million deaths), stomach cancer (700,000 deaths) and liver cancer (598,000 deaths). Breast cancer (4.4 million survivors up to 5 years following diagnosis) has been reported to be the most prevalent cancer in the world (1). According to World Health Organization 460,000 females died from breast cancer in 2008, while close to 610,000 males and females died from colorectal cancer. Compared to the western countries, cancer rates lower in India, but the incidences are increasing with increasing rural population to the cities, increase in life expectancy and changes in life style. Data from population based cancer registries in India have shown that the most commonly reported cancer sites in males are lung, stomach, oesophagus and larynx, however in case of females these include, cancer of cervix, breast, ovary and oesophagus (2) . Cancer has been the main focus of research from last three decades and still continues to be a challenge. Depending on the type and stage of cancer, the most common treatments involve surgical removal of the tumor, radiation therapy, chemotherapy, immunotherapy and combinations thereof (Fig 2). Surgical resection is the primary procedure to remove cancers large enough to detect and manipulate. However, surgical resection alone in most cases cannot remove every cancer cell present; it leaves behind some microscopic tumor deposits that over time result in relapse and recurrent disease (3) . Radiation therapy on the other hand is the dosing of radiations to kill cancer cells and to stop or slow down their growth. It has been observed that radiation not only kills or slows down the growth of cancer cells; however it also affects the nearby healthy cells. Radiation therapy can be used along with surgery. Radiation shrinks the size of the cancer before surgery, or it may be used after surgery to kill cancer cells that remain in the body. Chemotherapy as distinct from the other forms of treatment uses certain antineoplastic agents to treat cancer cells locally and systemically. Broadly, most chemotherapeutic drugs work by impairing mitosis (cell division), thus effectively targeting fast dividing cells. Chemotherapy is delivered through a central line, giving more reliable access to the circulatory system while preventing phlebitis in peripheral veins. Years of testing and research have proved chemotherapy to be an effective cancer treatment. The majority of chemotherapeutic drugs can be divided into alkylating agents, anti-metabolites and plant alkaloids. Alkylating agents interfere with DNA integrity and function in rapidly proliferating tissues. The five major alkylating agents used in the chemotherapy are nitrogen mustard, ethylene amines, alkyl sulfonates, nitrosourea and triazenes. Mechlorethamine, Cyclophosphamide, Ifosfamide, Melphalan and Chlorambucil are the most reactive drugs of nitrogen mustard family whereas

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Triethlenenemelamine, Thiotepa, Altretamine are ethyleneamines. Anti-metabolites like folic acid analogues (methotrextrate), pyrimidine analogues (5FU, cytarabine) and Purine analogues (mercaptopurine, azathioprine, thioguanine, fludarabine phosphate, pentostatin and cladribine) are the most active anti- neoplastic agents. Although over the past 50 years, great strides have been made for treating cancer, it still continues to be a major health concern and therefore extensive efforts have been devoted for searching new therapeutic approaches. The major difficulty in the treatment is that cancerous and normal cells are remarkably similar. Even though cancer cells harbour mutated genes and resultant mutated proteins that affect cell division and/or contribute to oncogenesis, tumor cells and their normal counterparts share the same DNA and major metabolic pathways. Thus traditional chemotherapeutic compounds that attack DNA replication or cell division in a cancer cell can also attack a normal dividing cell, resulting in serious side effects such as bone marrow and gastrointestinal toxicity. Therefore efforts are made to select only those candidate drug molecules which affect cancer cells without harming the normal ones. Within the sphere of cancer, a number of important new commercialised drugs have been obtained from natural sources, by either modifying them structurally or by synthesizing new compounds taking them as models. The etiology of major cancers is still largely unknown and there is a need for more effective and less toxic chemotherapeutic agents. The search for improved cytotoxic agents therefore continues to be an important line in the discovery of modern anticancer drugs.

A vast number of multi-pronged strategies are designed to eradicate cancer, these include target based chemotherapy utilizing modern bioinformatic tools for drug designing, immunotherapy using designer cancer vaccines based on tumor associated cell surface antigen, anti-angiogenesis therapy and development of tumor specific vehicles for drugs. A significant progress had been made in the development of targeted therapy drugs that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. Mostly it is influenced by the type of cancer, stage or extent of the disease. In addition the presence of specific molecular markers can also be useful in the treatments. Recent advances in deciphering the human genome have launched a tremendous potential for cancer treatment. New drugs are now being designed to target cancer cell's specific molecular features, including genetic mutations, gene expressions, changes in protein structure and signalling pathways (4) **.** Molecularly targeted interventions can be classified into five general strategies that will guide future research to discover and develop new anticancer agents that specifically target the cancer cells without affecting the normal cells. Targeting these events should have potent and specific therapeutic consequences. One of these events in cell deregulation is obligate compensatory suppression of apoptosis (programmed cell death, PCD), which provides support for neoplastic progression.

2. APOPTOSIS AS AN IMPORTANT MOLECULAR EVENT FOR ANTICANCER DRUG TARGETTING:

Apoptosis (programmed cell death) is an important regulator of cell growth and proliferation. It is an essential cellular homeostasis mechanism that ensures correct development and function of a multicellular organism particularly during embryogenesis and metamorphosis (5, 6) . The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidential nature but follows a sequence of controlled steps leading to defined self-destruction (7) . Apoptosis is beneficial as a natural anticancer mechanism. It has been found that, once the DNA of a cell gets damaged it undergoes apoptosis to preserve the healthy state of an organism, further, cells becoming irrepairably damaged due to a disease also undergo apoptosis. Studies have revealed that most of the known cancers carry mutations in one of the key regulators of the apoptotic pathways and the resistance towards apoptosis is a key factor for their survival. It has also been observed that defects in the apoptosis signalling further contribute to the drug resistance of tumor cells. Targeting apoptosis has therefore become a major goal for oncologic treatments and it has turned out to be an exciting challenge to translate the growing knowledge of apoptotic pathways into clinical applications (8) . Since apoptosis is a normal physiological process, it therefore affects the life span of both normal and cancer cells. It is believed that a successful anticancer drug kills or incapacitates cancer cells without causing excessive damage to normal cells. Most of the cancer chemotherapeutic drugs have been found to induce cancer cell to death by apoptotic pathways. Defects in apoptosis plays an important role in tumor pathogenesis, by allowing neoplastic cells to survive beyond their normally intended lifespan, subverting the need for exogenous survival factors, providing protection from hypoxia and oxidative stress as tumor mass expands, and allowing time for accumulative genetic alterations that deregulate cell proliferation, interfere with differentiation, promote angiogenesis, and increase cell motility and invasiveness during tumor progression (9) . A drug that activates apoptosis achieves a suitable therapeutic index in several ways. First, it activates a death cascade *via* a drug target

that is uniquely expressed in a cancer cell, alternatively it is delivered to the target tissue in a manner that is selective for the cancer cell and finally it exploits a pathway that is activated by oncogenes, in order to provoke apoptosis selectively in cancer cells.

2.1 MOLECULAR MECHANISM OF APOPTOSIS:

Apoptosis is a normal physiological process involving many genes and requires the active consumption of energy in the form of ATP to safely dispose of the cells once they have fulfilled their intended biological role. Apoptotic cell death can be induced by a variety of stimuli, including ligation of cell surface receptors, serum deprivation, growth factor deprivation, heat shock, hypoxia, exposure to ultraviolet radiation, DNA damage, viral infection and exposure to cytotoxic or chemotherapeutic agents. The process of apoptosis is characterized by a number of stereotypical morphological features which include cell shrinkage, nuclear and cytoplasmic condensation, release of cytochrome c from mitochondria, caspase activation, plasma membrane blebbing, phosphatidylserine externalization on the plasma membrane, DNA fragmentation and the formation of apoptotic bodies (10) . Nuclear condensation during apoptosis is usually accompanied by the activation of nucleases that first degrade chromosomal DNA into large 50 to 300 kb subunits and then into smaller units of ~180 base pairs (11) . Since the plasma membrane integrity is maintained throughout the process of apoptosis, therefore, this form of cell death is normally not associated with an inflammatory response. The signalling for apoptosis occurs *via* both intracellular (Intrinsic) and extracellular (Extrinsic) mediators that are in turn initiated from triggering events from either within or outside the cell (12) (Fig 3). Although, the intrinsic and the extrinsic pathways are known to occur independently, however, a cross-talk between the two pathways has also been observed. The death ligands/receptors and their respective intracellular signalling pathways have been found to act *via* specific death signals and all these signalling pathways converge on a common machinery of cell destruction that is activated by a family of cystein proteases known as caspases (13) , which play a key role in the progression towards the final morphological changes taking place in this process. Although cancer can arise due to the dysfunctioning of any of the two pathways, however, due to its sensitivity cancer arises more oftently *via* intrinsic than the extrinsic pathway. The extrinsic pathway (death receptor pathway) gets activated by clustering of various death receptors on the cell surface. The binding of the ligands to the respective receptors e.g. Fas ligand (FasL) to Fas receptor and Tumor Necrosis Factor (TNF) to TNF receptor triggers the formation of a death inducing signalling complex (DISC) that recruits and activates pro-caspase 8 to active caspase-8 (14,15) , which further activates caspase-3 and thus initiating the final step of apoptosis (Fig. 3). An intrinsic pathway on the other hand is a mitochondria dependant pathway as here most of the responses of this pathway get derived from this organelle (15, 16) . In this pathway the oligomerization of the bax results in the permeabilization of the outer membrane of mitochondria (17) leading to the release of pro-apoptotic materials like cytochrome c and apoptosis inducing factors (AIF) from the mitochondria into the cytosol. These pro-apoptotic factors are considered to be the central players of this pathway (14, 15,16) . After its release from mitochondrion, cytochrome c gets combined with the apoptotic protease activating factor (Apaf-1) and caspase-9 resulting in the formation of apoptosome which further triggers the activation of caspase-3 (15) and thus resulting in apoptosis (Fig.3). The intrinsic pathway of apoptosis is mainly controlled by the proteins of Bcl-2 family by regulating the exit of cytochrome c from mitochondria (15) , but once the cytochome c gets released the process of apoptosis goes on with no return (18) .

2.2 DIFFERENT APOPTOTGENIC FACTORS AS TARGETS FOR ANTICANCER DRUG THERAPY:

2.2.1 CASPASES:

Caspases are known to be the initiators of apoptosis. Caspases are a set of cysteine proteases possessing an active cysteine site and cleave their substrates at aspartic acid residues. Caspases have been found to mediate over 100 substrates in a cell which are very important and have a well defined function. During the process of apoptosis caspase-3 removes the inhibitory subunit of ICAD allowing CAD (Caspase activated DNase) to cut the genomic DNA into 180 base pair fragments which forms the basis of the DNA laddering test for apoptosis. Further nuclear shrinking, budding and active blebbing are due to cleavage of nuclear lamins (19,20) and PAK2 (21) by caspases respectively. Although caspases are the main initiators of apoptosis, their activation is in turn tightly regulated by other apoptotic regulators such as proteins from IAP and Bcl-2 family (15) . Based on the pro apoptotic function of caspases, these are further divided into two groups i.e. initiator and effector caspases. The initiator (apical) caspases including caspase -2, -8, -9, -10 and -11 activate the effector caspases including caspase -3,-6 & -7. (Fig3). The effector (downstream) caspases further degrade multiple substrates including the structural and regulatory

proteins in cell nucleus, cytoplasm and cytoskeleton which leads to the deregulation of vital cell processes and ultimately to cell death. It has been observed that caspase-1 promotes the activation of effector caspases-3 -7 and -6. Caspase-9 on the other hand undergoes activation of caspases -2, -3, -6, -7, -8 and -10 in a cytochrome c dependant manner. Caspase-3 has been found to activate caspases-2,-6,-8,-10 and also cytochrome c dependent activation of caspase-9 (22) . Further, caspase-8 activates caspases-1, -2, -3, -6, -7, -9 and -11. In the final stage of caspase cascade, caspase-6 catalyzes the activation of caspase-8 &-10 and caspase-2, -7, -8 and -10 cleave the target protein substrates directly (22) . Caspases and their regulators are therefore potentially attractive targets for the development of new cancer therapies.

2.2.2 CYTOCHROMEC:

Cytochrome c is a component of the electron transport chain and is involved in the production of ATP. Both *in vitro* and *in vivo* studies have demonstrated the release of cytochrome c from mitochondria during apoptosis (23, 24) . After getting released, cytochrome c interacts with Apaf-1 and procaspase-9 in the cytosol thus forming an apoptosome complex (25) , which further undergoes cleavage and activation of procaspase-9 and other procaspases responsible for the executive stages of apoptotic cell death (Fig 3).

2.2.3 POLY (ADP-RIBOSE) POLYMERASE:

Poly (ADP-ribose) polymerase (PARP) is an essential DNA repair enzyme playing its role in the repair of single-stranded breaks in DNA *via* the base excision repair pathway. PARP has emerged as an important target in cancer therapy. It has been observed that the cleavage of PARP during apoptosis facilitates cellular disassembly and in particular of the nucleus, ensuring the completion and irreversibility of this process. The cleavage of PARP has been observed in almost all forms of apoptosis (26) . During apoptosis the fragmentation of nuclear DNA produces numerous single-strand nicks in the linker regions of chromatin since PARP interacts preferentially with single-stranded DNA breaks, it recruit the base excision repair (BER) proteins to repair the damaged DNA. Studies by (27) have shown that the cleavage of the PARP by caspase-3 results in the isolation of its DNA-binding domain and therefore it binds irreversibly to the internucleosomal DNA in apoptotic cells (Fig 4). Further it has been proposed that the cleavage of PARP by caspases can promote apoptosis *via* two ways, either, the absence of PARP disables the key aspects of the cellular genomic surveillance mechanism and prevents the unnecessary DNA repair that would delay chromatin degradation (28) or the cleavage of the PARP improves the access of the endonuclease to the chromatin. The localization of PARP to the nuclear envelope has also suggested that its cleavage during apoptosis participates in nuclear disassembly and facilitates downstream events which are otherwise delayed (29) .

2.2.3 BCL-2 FAMILY:

Bcl-2 family is a group of evolutionary conserved proteins that regulate apoptotic processes converging on the mitochondria as well as endoplasmic reticulcum (ER) (30) **.** Bcl-2 the first recognised member of the family was found in B-cell lymphoma hence the name Bcl. The proteins are divided into three subgroups determined by their structure and function. The antiapoptotic fractions including Bcl-2, Bcl-$x_{L,}$ Bcl-w, A1, Mcl-1 and Boo. The proapoptotic Bax/ Bak- like group comprising Bad, Bik, Bid, Hrk, Bim, Bmf, Noxa and Puma. Most anti-apoptotic proteins like Bcl-2 and Bcl-x_L show strong sequence conservation in all four domains, whereas pro-apoptotic proteins, including Bad, Bik and Bid frequently lack the BH4 domain. The later are divided in the Bax subfamily, members of which contain BH1, BH2 and BH3 and the 'BH3-domain-only' proteins that show sequence homology only within the BH3 domain (Bid, Bad). Inviable cells and anti-apoptotic proteins are localized in membranes such as the outer mitochondrial membrane, the endoplasmic reticulum or the nuclear membrane while pro-apoptotic proteins are found in the cytosol (31) . The formation of Bcl-2/Bax heterodimers and the interaction of Bad with Bcl-2-Bcl-x_L within the outer mitochondrial membrane, respectively suggests a neutralising competition of the proteins (32) . Furthermore Bad, Bcl-2 and Bcl-x_L can be inactivated by phosphorylation (33) . In response to apoptotic signal, Bax, for example dimerises and translocates to mitochondria where it becomes an integral pore forming membrane protein (34) . Aditionally, following CD95 treatment, Bid is cleaved at its amino terminus by caspase-8. Truncated Bid (tBid) also translocates to the mitochondria, inserts into the membrane and interacts with bax to cause mitochondrial permeability transition (35) . In contrast, Bcl-2 antagonises the pore forming activity of the Bax, thus preventing the efflux of apoptosis-activating factors (36) .

3. IDENTIFICATION OF NEW THERAPEUTIC TARGETS FOR RATIONAL TARGET BASED DRUG DISCOVERY:

The selection of targets for drug development cannot be empirically random but must be based on information giving some credibility to a probable role the target might

have in the life of a cancer cell i.e. a key role in cancer cell growth and survival. In general, the areas in which molecular mechanisms are evaluated as possible targets for drug development include gene transcription (the function and specificity of transcription factors and of the transcription machineries with emphasis on the protein-protein interactions involved in the formation of transcription complexes), cell immortality factors and genomic instability (mechanism of DNA replication and repair as well as the role of telomerases), post-transcriptional mechanisms of mRNA processing (stability and function as well as cellular antisense mechanisms of mRNA control), signaling cascades (receptor functions, cytoplasmic and nuclear signalling as well as cross-talks among signaling pathways), cell cycle mechanisms of control (factors conditioning progression), the proper function of check points (the mechanisms by which cells make decisions about their fate, for example to undergo differentiations, to die by apoptosis, or to proliferate), factors affecting resistance to single or multiple drugs (ranging from the expression of responsible genes, to mechanisms of drug uptake or retention, to changes in the target of drug action, to changes in DNA repair or apoptotic processes), mechanisms of tumor angiogenesis (production, metabolism and function of the factors and pathways involved as well as the role of the related receptors), mechanisms unique to the metastatic process (those concerned with invasion and/or attachment at sites of dissemination) and the complexities of tumor immunity (tumor-induced immunosuppression and tumor escape mechanisms, the role of cytokines as effectors and regulators, the mechanisms of antigen presentation, the cellular and humoral processes involved in antitumor action). In the current review, stress has been given on some of the important molecular targets, which will lead to the development of potentially new therapeutic approaches for targeting cancer.

Targeted cancer therapies interfere with cancer division and spread in different ways. Many of these therapies focus on proteins that are involved in cell signalling pathways, which form a complex communication system that governs basic cellular functions and activities such as cell division, cell movement, cell responses to specific external stimuli, and even cell death. Cell signals are translated from the cell surface to the nucleus through second messengers and interacting enzyme systems. The best studied second messengers are cAMP, Ca^{2+} and the inositol phosphates and one of the interacting types of enzymes are protein kinases (PK), which are specific either for serine/ threonine or for tyrosine phosphoacceptors, regulating the phosphorylation status, and consequently the activity, of specific target proteins. The importance of phosphorylation cascades has been reflected by the findings that many kinases, phosphatases, and the signal transduction pathways in which they participate have been highly conserved during the course of evolution (37) . From the past few years, much of the research has focused on the role of protein phosphorylation in the control of the cell cycle (38) ; a number of cellular protooncogenes have been found to encode members of the serine (threonine) kinase family (39,40,41) and it has become increasingly clear that certain serine(threonine) kinases function as key components of the cell cycle regulatory network (42) . Therefore, the complete delineation of these pathways is an important aim for the understanding of oncogenesis and tumor progression. In recent years, tyrosine kinases, that catalyze the transfer of the γ phosphate group from adenosine triphosphate to target proteins have gained the interest of cancer researchers. These kinases play an important role in diverse normal cellular regulatory processes. Tyrosine kinases can be classified as receptor protein kinases and non receptor protein kinases. The receptor tyrosine kinases are membrane-spanning cell surface proteins that play critical roles in the transduction of extracellular signals to the cytoplasm (43) . There are approximately 60 receptor tyrosine kinases that have been identified, and they are divided into some 20 subfamilies as defined by receptor and/or ligand (44) . The binding of the ligand induces dimerization of these receptor tyrosine kinases, resulting in autophosphorylation of their cytoplasmic domains and activation of tyrosine kinase activity. Multiple cytoplasmic signaling pathways, including the Ras/ Raf mitogen-activated protein kinase pathway, the phosphoinositol 3′-kinase/Akt pathway, the signal transducer and activator of transcription 3 pathway, the protein kinase C pathway, and scaffolding proteins have been found to get activated (45, 46) . Intracellular mediators in these pathways transduce signals from membrane receptors through the cytosol and into the nucleus, culminating in altered DNA synthesis and cell division as well as effects on a variety of biological processes, including cell growth, migration, differentiation, and death (47,48) .

3.1 TARGETING PHOSPHATIDYLINOSITOL-3-KINASE SIGNALLING PATHWAY IN CANCER:

Phosphoinositides (PtdIns) are a class of rare lipid molecules whose phosphorylation is carried out by a large family of lipid kinases. The Phosphatidylinositol-3-kinase (PI3K) family of lipid kinases phosphorylate the 3'OH group of phosphatidylinositols. On the basis of their structure and preferred substrates, these lipid kinases are classified into

three classes. Class IA of PI3Ks is the most widely implicated class in cancer. Class I PI3Ks catalyse the conversion of phosphatidylinositol-3,4-bisphosphate (PtdIns-4,5-P_2) to phosphatidylinositol-3,4,5-triphosphate (PtdIns-3,4,5-P_3). PtdIns-3, 4, 5-P_3 is absent or undetectable in resting cells but is acutely increased in response to multiple stimuli that activate type I PI3K. Phosphorylated lipids are produced at cellular membranes during signalling events and contribute to the recruitment and activation of various signalling components (Fig. 5). Phosphoinositide 3-kinase (PI3K) catalyzes the production of phosphatidylinositol-3, 4, 5-trisphosphate, in cell survival pathways, regulation of gene expression, cell metabolism and cytoskeletal rearrangements. The acute phosphorylation of phosphatidylinositol lipids at the D-3 position of the inositol ring in response to cell stimulation by growth factors and hormones sets in motion a coordinated set of events leading to cell growth, cell cycle entry, cell migration, and cell survival. It has been observed that various signaling proteins, including protein serine threonine kinases, protein tyrosine kinases and exchange factors that regulate heterotrimeric guanosine triphosphate (GTP)–binding proteins (G proteins), have domains that specifically bind to D-3 phosphorylated phosphoinositides. These proteins are located in the cytosol of unstimulated cells but, in response to lipid phosphorylation, accumulate at the plasma membrane because of their ability to associate with the newly formed phosphoinositides. At the membrane, these proteins become activated and initiate various local responses, including polymerization of actin, assembly of signaling complexes, and priming of protein kinase cascades. PI3K is tightly regulated in normal tissue but it is estimated to be constitutively active in upto 50% of human cancers. Akt, a serine-threonine kinase that is directly activated in response to PI3K, is a major effector of PI3K in cancers (Fig 5). There are three different Akt isoforms in mammalian cancers, and emerging data suggest that they have overlapping and distinct roles in cancers. Akt signalling leads to increased cellular growth and survival. Although Akt is the PI3k effector that is most widely implicated in cancer, there are Akt- independent pathways activated by PI3K, which include the Bruton tyrosine kinase (BTK) (49) . Receptor tyrosine kinases (RTKs) and somatic mutations are the two main events responsible for PI3K–Akt activation in human cancers. One of the consequence of PI3K/AKT activation is engagement of an anti-apoptotic pathway. There are a variety of substrates lying downstream of AKT, that are inhibited or activated to prevent apoptosis. For example, AKT prevents release of cytochrome c from mitochondria, inactivates forehead transcription factors, preventing their nuclear transcolation and subsequent activation of downstream pro-apoptotic proteins including Bim and FAS ligands. Phosphorylation by AKT inactivates a prodeath protease caspase-9 and the anti-apoptotic factor BAD. Furthermore AKT induces nuclear translocation of the survival protein NF-KB (*via* IKK) and MDM2, thus targeting the tumor suppression gene P53 for degradation by proteosome (50) . One of the major effector downstream of Akt is mToR (Mammalian Target of Rapamycin) complex 1 (mTORC1) (Fig 5). mTORC1 is often not only under the control of PI3K-Akt signalling, however, mTORC1 integrates many inputs, including growth factor signalling, the energy state of the cell and nutrient and oxygen availability. PI3K/mTOR signalling has been found to play a key role in angiogenesis pathways, as the mTOR inhibitor Rapamycin has been found to reduce induction of VEGF and tube formation in response to cytokines, leading to significant inhibition of angiogenesis, tumor growth and metastasis *in vivo* (51) . From the therapeutic point of view the complex regulation of mTORC1 is important, as some PI3K inhibitors in development directly block both PI3K and mTOR, where as others inhibit only PI3K (XL147, PX866) (52) . Dual PI3K-mTOR inhibitors like BEZ235, BGT226 might offer a therapeutic advantage in cancers in which PI3K is not the main regulator of mTORC1. Although, PI3K pathway inhibitors are just entering the clinic, there are emerging preclinical studies that suggest how they should be most appropriately used (53, 54) . PTEN (Phosphatase and Tensin Homologue Deleted from Chromosome-10) which is one of the most frequently mutated tumor suppressors in human cancer, functions primarily as a cytoplasmic phosphatase to regulate crucial signal transduction pathways involving growth, adhesion, migration, invasion and apoptosis (55) . The major function of tumor suppression by PTEN is achieved by the down regulation of the oncogenic Akt pathway (56) by encoding a phosphatidylinositol- 3,4,5-trisphosphate (PIP3) 3'-phosphatase that turns off the PI3K pathway (57) . A major role for PI3K pathway activation in human tumors has been more recently established following both the positional cloning of the PTEN tumor suppressor gene, and the discovery that the PTEN protein product downstream signalling through Akt. Another important target of Akt is glycogen synthase kinase 3 (GSK3) (Fig 5). This protein kinase is constitutively active in unstimulated cells and phosphorylates many proteins (including glycogen synthase, c-Myc, and cyclin D) to keep them in inactive states or promote their degradation. Phosphorylation of GSK3 (both alpha and beta isoforms) by Akt turns off the catalytic activity of this enzyme, resulting in the activation of pathways that are normally repressed by GSK3 (58) .

PI3K pathway is a major survival pathway activated in cancer. The development of inhibitors for this particular pathway has become a major concern in cancer chemotherapy. PI3K inhibitors are among the rapid molecules entering into clinical trials, suggesting PI3K signalling pathway a potential therapeutic target in cancer cells.

3.2 TARGETING EPIDERMAL GROWTH FACTOR RECEPTOR IN CANCER:

Epidermal growth factor receptor (EGFR; human epidermal growth factor receptor, HER 1) is a member of the ErbB family of receptors that also includes HER2, HER3 and HER4. EGFR and its ligands function in diverse cellular functions including cell proliferation, differentiation, motility, and survival. EGFR signaling is important for the development of many tissues, including skin, lungs, intestines, and the craniofacial skeleton. Basically, EGFR is a transmembrane glycoprotein and acts as a receptor for the members of the epidermal growth factor (EGF) family possessing tyrosine kinase activity. EGFR binds several ligands including epidermal growth factor (EGF), transforming growth factor-α, betacellulin, epiregulin, and amphiregulin. Binding of EGF to EGFR induces the formation of homodimers and heterodimers and tyrosine autophosphorylation (59,60) which triggers the activation of downstream signaling pathways, such as the phosphoinositide-3 kinase (PI3K)/Akt pathway (among others) (61) . EGFR is commonly over expressed in several epithelial cancers, and its over expression has largely been correlated with poor prognosis in patients. EGFR is a rational target in solid tumors. Activation of the EGFR promotes processes responsible for tumor growth and progression, including proliferation and maturation, angiogenesis, invasion, metastasis, and inhibition of apoptosis. Agents targeting members of the human epidermal growth factor receptor family have shown encouraging therapeutic efficacy. Infact, tyrosine kinase inhibitors directed against the epidermal growth factor receptor (EGFR) are the first molecular targeted agents to be approved in the US and other countries for the treatment of advanced non-small-cell lung cancer after failure of chemotherapy. The first to be approved by the US Food and Drug Administration (FDA) in 1998 was trastuzumab (Herceptin) for the treatment of HER-2 (ErbB-2)-positive breast cancer (62) . Over the past few years, three EGFR (EGFR1/ErbB-1)- specific agents have also received regulatory approval: cetuximab (Erbitux) for metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck (SCCHN), erlotinib (Tarceva) for advanced or metastatic pancreatic cancer and non-small-cell lung cancer (NSCLC), and gefitinib (Iressa) for advanced or metastatic NSCLC. However, FDA approval for gefitinib was recently withdrawn after it failed to demonstrate a survival benefit either alone or with chemotherapy in three phase III trials (63, 64, 65) . Erlotinib and gefitinib both selectively and reversibly inhibit phosphorylation of the EGFR tyrosine kinase without inducing EGFR internalization or degradation (Fig 6). Inhibition of EGFR downstream signaling by erlotinib and gefitinib exerts antitumor activity through inhibition of proliferation and tumor angiogenesis and through induction of apoptosis (66) . EGFR and its downstream signalling pathways seem to contribute directly to growth and behaviour of most kinds of malignancies. Taken together, EGFR represents a promising molecular target for exploitation in the cancer treatment.

3.3 TARGETTING CELL CYCLE & CHECK POINT CONTROL:

At the centre of cellular proliferation is the cell division cycle, the process by which a cell grows, replicates its DNA and then divides to give two daughter cells. This process is divided into four sequential phases including G_1, S, G_2 & M phase. During eukaryotic cell division, in order for each daughter cell to inherit one and only one copy of each chromosome, the mother cell must replicate its chromosomes exactly once in the synthetic phase, and then must separate the replicated chromosomes evenly at the end of the mitotic phase to the two daughter cells. Defects in the coordination of chromosome replication and chromosome segregation can have severe consequences leading to genetic instability and aneuploidy, and eventually fostering tumor malignancy (67, 68, 69) . To ensure faithful transmission of chromosomes during cell division, eukaryotic cells have evolved cellular regulatory mechanisms termed cell cycle checkpoints (70) . The checkpoints prevent or delay cell cycle progression if certain cellular processes or proteins are disrupted, to gain time to repair the damage before cell division occurs. When the damage is irreparable, the cell undergoes apoptosis through the triggering of specific biochemical pathways (71) . However, cancer cells often harbour defective cell cycle checkpoints allowing for uncontrolled cell proliferation, even when cell division does not occur properly. The collective results from studies in various eukaryotes have demonstrated that progression through the cell-division cycle is driven by activation and inactivation of cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs) which trigger the transition to subsequent phases of the cycle (Fig 7). The periodic accumulation and degradation of cyclins activate

the CDKs, resulting in the phosphorylation of retinoblastoma (RB) protein and releasing an important nuclei transcription factor E2F therefore initiating the DNA replication in S-phase. It is known that cyclin family consists of 11 known cyclins, among which cyclin D & E regulate the G1/S transition, cyclin A controls the S phase progression and cyclin B is related to M phase regulation. Opposite to cyclin effect, CKIs including p21, p27, p16 & P15 can inhibit the kinase activity of their specific CDKs and thus negatively regulate the cell cycle (Fig 7). They, together with other regulating proteins, create a complex signal pathway that drives the cell passing through its four phases. The presence of two major checkpoints at G1/S and G2/M boundaries ensures the precise copy of DNA. Whenever cells are impaired, the checkpoints limit the cell cycle progression so that the self repair mechanism can have time to do their job. If the cells are not able to repair the damage, they start the apoptotic process. The cell cycle regulation, is therefore very important in cell proliferation and apoptosis regulation, thus determining its role in cancer cell cycle and tumorigenesis. Cyclin D is the first cyclin that has been found to relate to the tumorigenesis, including breast cancer, esophagus cancer and stomach cancer (72) . Besides cyclin D, studies have shown that cyclin A, C, E and B1 are also related to tumorigenesis (73,74,75,76) . Further, amplification of CDK4 and low levels of CKI p27 protein has been reported as a poor prognosis in both colon and breast cancer (77, 78) . In addition to CDK, cyclin and CKIs, there are numerous proteins such as p53, pRB, c-myc, Bcl-2, c-jun etc that are also involved in cell cycle regulations and DNA replication. The signalling pathways which regulate cyclin D-dependent kinase activity links some of these proteins with cancer. As has been observed, the Ras genes themselves, the PIK3CA gene encoding the p110α subunit of PI3 kinase and the tumour suppressor gene PTEN which acts as a lipid phosphatase and reverses the PI3 kinase reaction, have been found to be mutated in cancer (79,80) . These genetic mutations have the ability to cause activation of the cyclin D dependent kinases leading to inappropriate phosphorylation of pRb and misregulation of the restriction point. CDKs lying downstream of cyclin D-dependent kinase signalling, their regulators, as well as the gene encoding pRb itself (RB) are all cancer targets, as most tumors contain a genetic alteration in one of these genes. The cell cycle progression is a complex result coordinated by various control factors. Studying the role of these cell cycle regulators in drug induced cell cycle arrest and apoptosis will provide a better understanding of the drug anticancerous mechanism. All this culminates into a statement that, effective cancer treatment can be achieved by drugs that target these check points or proteins impinging on the cell cycle machinery (81) .

3.4 TARGETTING PROTEOSOMES:

Proteasomes are multisubunit, cylindrical proteases found in eukaryotes, eubacteria, and archaebacteria. The proteasome's active sites face a central chamber buried within the cylindrical particle. Thus, the proteasome is an ideal intracellular protease because cellular proteins can only be degraded if they are actively transferred to the enzyme's central chamber. Proteasome substrates include misfolded or misassembled proteins as well as short-lived components of signaling cascades that regulate cell proliferation and survival pathways. Inhibition of the proteasome results in the accumulation of these substrate proteins and leads to cell death. Eukaryotic proteasomes come in two sizes, the 20S proteasome and the considerably larger ATP-dependent 26S proteasome. The latter is formed when the 20S proteasome binds one or two multisubunit ATPase-containing particles known as 19S regulatory complexes (Fig.8A). The catalytic core of the proteasome includes three proteolytic activities that are commonly described by their substrate selectivities (82) chymotrypsin like, trypsin like and caspase-like. Each proteasome active site uses the side chain hydroxyl group of an NH_2-terminal threonine as the catalytic nucleophile, a mechanism that distinguishes the proteasome from other cellular proteases (83) . 26S proteasome is responsible for degrading ubiquitylated proteins and is therefore essential for a vast array of cellular processes including cell-cycle traverse, control of transcription, regulation of enzyme levels, and apoptosis. Ubiquitin (Ub) is a small, evolutionarily conserved eukaryotic protein that can be attached to a wide variety of intracellular proteins, including itself. Although Ub serves nonproteolytic roles, such as histone modification or viral budding, its major function is targeting proteins for destruction. For ubiquitination of proteins the carboxyl terminus of ubiquitin is activated by an ATP-consuming enzyme (E1) and is transferred to one of several small carrier proteins (E2s) in the form of a reactive thiolester. The carboxyl terminus of an activated Ub then forms an isopeptide bond with lysine amino groups on proteolytic substrates (S) that have been selected by members of several large families of Ub ligases or E3s. Chains of Ub are formed, and the Ub-conjugated substrate is recognized by the 26S proteasome and degraded. Being the key protease of the ubiquitin system, the 26S proteasome also impacts a number of human diseases, especially cancer. Interestingly, transformed cells display greater susceptibility to proteasome inhibition than non-malignant cells (84) .

Therefore, proteasome inhibition holds promise as a novel approach to the treatment of cancer. Several important proteins that are regulated by the proteasome include the inhibitor of nuclear factor κB (NFκB; IκB), the tumor suppressor p53, the cyclin-dependent kinase inhibitors p21 and p27, and the proapoptotic protein Bax. Accumulation of these substrates on proteasome inhibition leads to decreased NFκB-dependent transcription of genes crucial to the promotion of tumorigenesis, increased p53-mediated transcription of genes important to apoptosis and negative regulation of the cell cycle, p21 and p27-mediated induction of cell cycle arrest, and promotion of apoptosis *via* the inhibition of Bcl-2 by Bax (Fig 8B). Proteasome inhibitors also down-regulate signaling through the p44/42 mitogen-activated protein kinase (MAPK), a pathway crucial to the promotion of tumorigenesis in a number of model systems (84) . It has been observed that fibroblasts transformed with *ras* and *c-myc*, and lymphoblasts transformed with *c-myc*, were up to 40-fold more susceptible to proteasome inhibitor-induced apoptosis than primary fibroblasts or immortalized, nontransformed human lymphoblasts (85) . Further, studies have shown that proteasome inhibitors synergize with DNA damaging agents by inhibiting the transcription of genes involved in DNA damage repair (86) . Also, since, ubiquitin modification of substrate proteins is achieved by the activity of E1 activating, E2 conjugating and E3 ligase enzymes these enzymes have been implicated in cancer and are thus attractive targets for anticancer drug discovery. It has been observed that overexpression of MDM2 (mouse double minute 2) a self ubiquitinylating molecule, possessing E3 ubiquitin ligase activity, facilitates cell transformation by preventing p53 increase in response to oncogenic stimuli. MDM2 amplification has been observed in 20% of soft tissue tumors and 16% of osteosarcomas (87) . MDM2 therfore appears as an attaractive target for treating such types of cancers. Overall, ubiquitin proteasome system (UPS) has emerged as a one of the promising drug target in cancer therapy. Indeed the proteasome inhibitor bortezomib (also known as PS-341 or Velcade) is currently used as a successful drug in the treatment of multiple myeloma and mantle-cell lymphoma (88) . It is a covalent, slowly reversible inhibitor that primarily targets the chymotrypsin-like activity of the proteasome (89) . The cellular mechanism(s) responsible for the clinical efficacy of bortezomib remain unclear, but may include disruption of cell adhesion and cytokine-dependent survival pathways, in part through suppression of NF-ƙB activity, inhibition of angiogenesis, and/or activation of a misfolded protein stress response (Fig 8B). The clinical efficacy of the proteasome inhibitor bortezomib toward multiple myeloma and other hematologic malignancies provides the "proof of concept" that targeting the proteasome is a promising strategy for cancer treatment. Several other proteasome inhibitors have also been identified from natural resources, such as marine microbial metabolites, green tea polyphenols, flavonoids, and medicinal compounds. Additionally, the use of metal complexes as proteasome inhibitors has also been investigated as a potential anticancer strategy (90) .

3.5 TARGETTING TOPOISOMERASES:

Topoisomerases are the enzymes that modulate superhelicoidal density of DNA and act by introducing single (type I) or double (type II) strand DNA breaks. These enzymes are involved in DNA repair, replication, transcription and chromosome segregation during mitosis. In prokaryotes these topoisomerases maintain DNA in a supercoiled state by altering the linking number without changing its primary structure. In higher organisms the wrapping of DNA around histones requires the action of DNA topoisomerases to resolve the topological constraints imposed during wrapping and thus maintaining the supercoiled structure. During cell division DNA topoisomerase ensures that DNA does not come under too much of torsional stress, thus the agents targeting this mechanism will act against rapidly dividing cells. Although the biological functions of topoisomerases are important for ensuring genomic integrity, the ability to interfere with topoisomerases and generate enzyme-mediated DNA damage is an effective strategy for cancer chemotherapy. Topoisomerases are the targets of an increasing number of anticancer drugs that block the reaction that reseals the breaks in the DNA mediated by these enzymes. Drugs targetting topoisomerase can either be classified as topo posions or topo catalytic inhibitors. The former acts by stabilizing the enzyme DNA cleavable complexes leading to DNA break and the latter acts by stabilizing the enzyme where both DNA strands remain intact and no DNA breaks occur. The binding of drug is often found to be reversible, however, once the replication fork runs into the blocked topoisomerase, a piece of gapped DNA strand which is not bound by the topoisomerase gets released, creating a permanent breakage in the DNA and thus leading to the cell death. It has been found that topoisomerase I inhibitors induce single-strand breaks into DNA and show their inhibitory activity *via* different mechanisms. Certain drugs like camptothecin (CPT) inhibit the dissociation of topoisomerase and DNA (91) resulting in a replication-mediated DNA damage which is repaired more efficiently in normal cells than in cancer cells (deficient for DNA repair). Topoisomerase I inhibitors have also been

observed to cause gene inactivation through chromatid aberrations. Topoisomerase II has held the interest of cancer researchers owing to the discovery that it is targeted by active anticancer drugs, notably etoposide and doxorubicin. Being the potent inducers of double strand breaks in DNA (92) , these drugs arrest the cell cycle at the G_2 stage by disrupting the interaction between topoisomerase II and regulators of the cell cycle such as Cdc2. These inhibitors result in a wide range of chromosomal aberrations and show their activity by either stabilising topoisomerase II-DNA complexes that can easily be cleaved or by interfering with the catalytic activity of the enzyme. The inhibitory action of CPT and ETO has widely been studied on various cell lines (93) . Dual inhibitors like Intoplicin, XR11576, & PKBA target both topoisomerase I & II (94, 95) . These inhibitors work either by recognising structural motifs present on both enzymes, by linking separate topoisomerase inhibitors together into a hybrid drug, or by using inhibitors that bind to and intercalate DNA. This mechanism of action has been reported to be advantageous, because selective inhibition of topoisomerase I has been reported to increase topoisomerse II enzyme activity and vice-versa, which may be important for the development of drug resistance. Topoisomerases are the targets of several clinically important anticancer drugs and much of the research effort has been devoted to discovering new drugs targeting these enzymes, making topoisomerase an incredible target for cancer chemotherapy.

3.6 TARGETING TELOMERASES:

Telomerase, a reverse transcriptase, appears to be required in essentially all tumors for immortilization of a subset of cells and is therefore an attractive cancer target. Telomerase synthesizes telomeric DNA, the terminal DNA (telomeres), at chromosome ends which, together with telomere-binding proteins, confers stability to chromosomes. Telomeres consist of long TTAGGG nucleotide repeats and an associated protein complex, termed shelterin (96) . The shelterin complex has been found to protect chromosome ends from end-to-end fusion and degradation forming special t-loop like structures and thus masking the linear ends of chromosome from being recognised as single and/or double-strand DNA breaks. Due to oxidative damage and other end processing events the TTAGGG repeats have been found to get shorten with each cell division (97,98) and the critical shortening of few telomers result in growth arrest state thus triggering the signal of DNA damage and cellular senescence (99) . In the absence of other changes, cells can remain in a quiescent/senescent state for years and this can be thought of as a potent anticancer protection mechanism for long-lived species such as humans. Telomerase activation is therefore, one of the important tumour escape mechanisms to circumvent the telomere-dependent pathways of cell mortality, replicative senescence and crisis (Fig.9) (100) . Telomerase is composed of an RNA component (hTR or hTERC) and a catalytic protein (hTERT). Telomerase has been detected in approximately 90% of all malignant tumors (101, 102) , making it a highly attractive target for the development of mechanism-based cancer therapeutics (103) . Human hTERT-specific epitopes are expressed on cancer cells but not on normal cells (104) . Telomerase (hTERT) is thus regarded as a universal tumor antigen owing to its expression in almost all cancers. Phase I clinical trials have demonstrated that most patients with advanced breast or prostate carcinoma have induced hTERT-specific cytotoxic T lymphocytes (CTLs) following vaccination to mobilize dendritic cells that were pulsed with hTERT peptide or telomerase RNA. In this study, no significant toxic side effects were observed (104) . Furthermore, several studies have shown that inactivation of hTR/hTERT by dominant-negative mutants or antisense strategies resulted in an inhibition of tumor cell proliferation, thus providing genetic validation of telomerase as an anticancer target (105) . It has also been observed that antisense oligonucleotide mediated inhibition of hTERT resulted in rapid reduction in cell growth and induction of apoptosis without telomere shortening in human prostate cancer cells (106) . In a study, a reverse transcriptase inhibitor 3ȧzido- 3ˊ-deozythymidine (AZT) has been found to inhibit telomerase activity (107) and also decreased telomerase activity and increased apoptosis oral squamous and mammary carcinoma cells (108) . The elevated telomerase activity in most of the tumors, resulting into larger telomers and hence larger life span of cancer cells sets telomerase a potent target in cancer therapy. Targetting telomerase has introduced an advanced strategy for handling this disease.

3.7 TARGETTING TUMOR MICROENVIRONMENT:

The tumour microenvironment consists of normal cells and molecules that surround cancer cells. In broader sense, tumor microenvironment is a complex system of many cells, including endothelial cells and their precursors, pericytes, smooth-muscle cells, fibroblasts of various phenotypes, myofibroblasts, neutrophils and other granulocytes (eosinophils and basophils), mast cells, T, B and natural killer lymphocytes, and antigen-presenting cells such as macrophages and dendritic cells which all participate in tumor progression. The process by which normal cells become benign tumor cells, benign tumor cells

are transformed to malignant cells, and malignant cells turn metastatic depends on the molecular signals between the cells and the surrounding area. It has been reported that a cancer cell is dependent on the microenvironment for its proliferation, progression and metastasis (109, 110) . The cells, vessels, and molecules that surround a tumor influence the tumor cells, and the microenvironment can be changed by the tumor. Carcinogenesis and tumour angiogenesis result not only from the interaction of cancer cells with endothelial cells, however, microenvironment is the primary factor determining whether epithelial cells grow continuously and invade or, at the opposite extreme, are eliminated. Tumors can circumvent inhibitory signals during their progression and even exploit the surrounding cells to grow, invade, and metastasize (Fig.10). In the past few years, the role of the cellular microenvironment in tumorigenesis has become an intense area of research. This is in part due to studies demonstrating that genetic abnormalities, such as loss of heterozygosity (LOH), occur not only in cancer cells, but in stromal cells as well (111,112,113) . Transforming growth factor-beta (TGFbeta) signalling regulates cancer through mechanisms that function either within the tumour cell itself or through host-tumour cell interactions. Studies of tumour-cell-autonomous TGFbeta effects show clearly that TGFbeta signalling has a mechanistic role in tumour suppression and tumour promotion. In addition, factors in the tumour microenvironment, such as fibroblasts, immune cells and the extracellular matrix, influence the ability of TGFbeta to promote or suppress carcinoma progression and metastasis. The complex nature of TGFbeta signalling and crosstalk in the tumour microenvironment presents a unique challenge, and an opportunity to develop therapeutic intervention strategies for targeting cancer. There are many transcription factors which are important molecular targets in the microenvironment, and many drugs are known to interact with these targets, these include signal transducers and activators of transcription (STATs), nuclear factor κB (NFκB) and hypoxia-inducible factor 1α (HIF1α). These transcription factors are known to be intimately involved in regulating inflammation, wound healing and angiogenesis. STATs are constitutively overexpressed in many cancers; their phosphorylation, which is required for transcriptional activity, is regulated by a set of kinases (JAKs), phosphatases and binding proteins, all of which are targets for drug development (114) , thus indicating that the use of therapeutic agents to targetting these factors in the microenvironment will be an important approach to the overall control of cancer.

The normal cellular microenvironment is known to inhibit malignant cell growth, however, the modifications that occur in the tumor microenvironment (Massive Cell Death, Hypoxia, Low pH, Low Glucose Levels) synergistically supports cell proliferation. Tumors can shape their microenvironment and support the development of both tumor cells and non-malignant cells. As the microenvironment has such a crucial role in carcinogenesis and metastasis, it represents a crucial target not only for cancer therapy but also for preventive strategies. The rationale for chemoprevention is simple and straightforward: it is preferable to fix something in its early stages of dysfunction, before it is beyond repair. In a holistic view, microenvironment of a developing carcinoma is an obvious target for chemoprevention, although in the past, most attention in cancer research has been given to controlling the dysfunctional epithelium. There is already a wealth of information about specific cells and molecules in the tumour microenvironment that are targets for cancer therapy at present (115,116) . These targets should now be investigated for their use in chemoprevention. Molecular targeting has thus emerged as a new approach with tremendous potential to make an impact on the control of this disease. Most of the cellular targets for anticancer drug therapy have already been discussed above, some of the other molecular targets and their respective chemotherapeutic agents are categorised in the table below (Table 1).

To summarize, targeted therapy provides a new approach for cancer therapy that has the potential for avoiding some of the drawbacks associated with cytotoxic chemotherapy. The validation of a particular cancer target encompasses information on the prevalence and role of the target or pathway in human cancer i.e. the modulation target should have a direct impact on a single or multiple anticancer phenotypes such as growth inhibition, induction of apoptosis or prevention of angiogenesis, migration or invasion. In recent years, this strategy has resulted in some notable success stories with newly approved molecularly targeted drugs that have made a significant effect in lengthening the survival of cancer sufferers, for example, imatinib in chronic myelogenous leukemia, trastuzumab in breast cancer, and bevacizumab in colorectal cancer. Unfortunately, several of the inhibitors used in targeted therapy have their drawbacks and limitations and have more similarities than differences to the current cytotoxic drugs. However, knowledge of their effects will facilitate the development of improved targeted agents that can circumvent these limitations. Also, it is important for future studies to focus on the discovery of new molecular targets for the development of better anticancer therapeutics

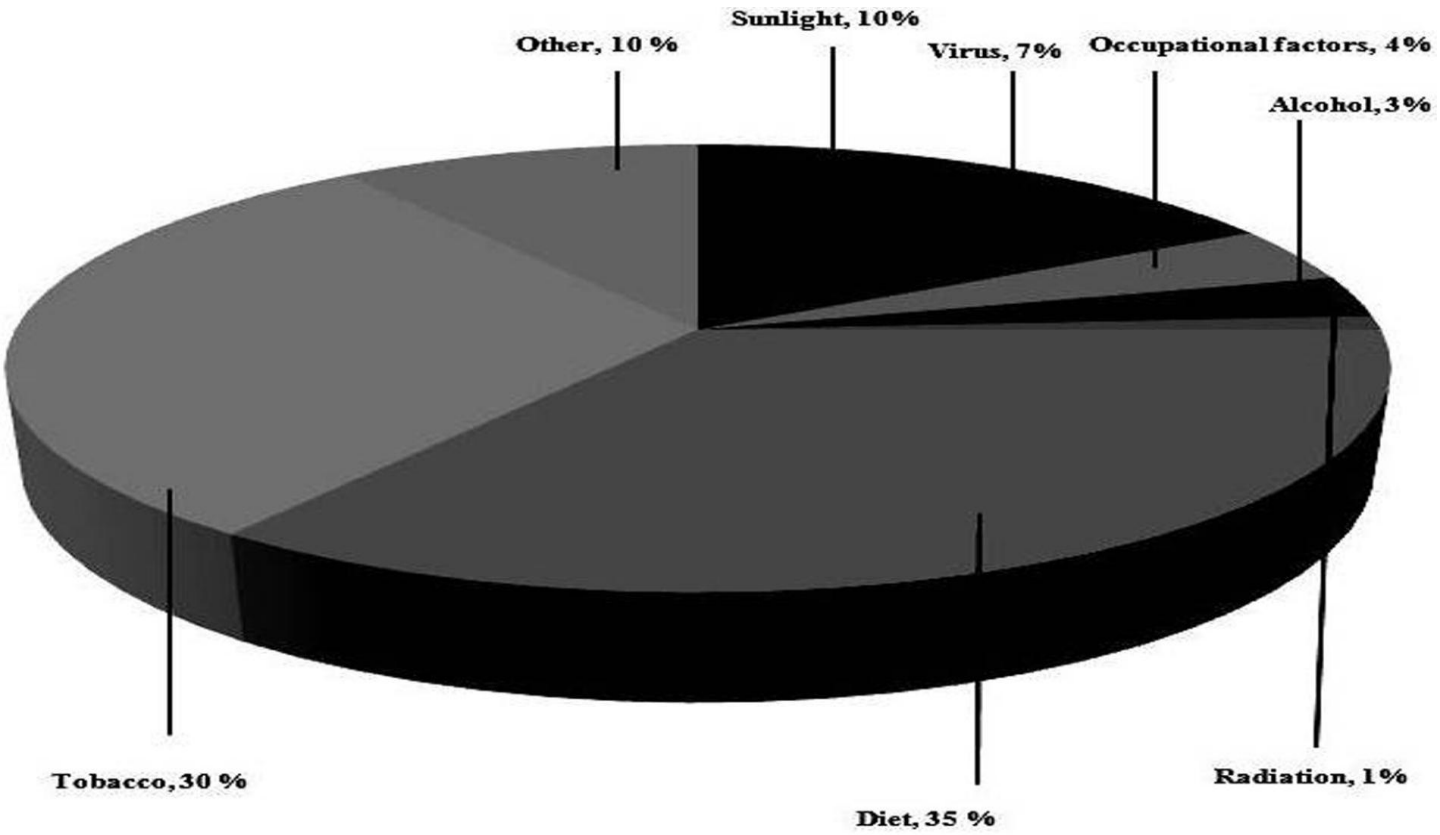

Figure No. 01: Some of the known cancer causing agents

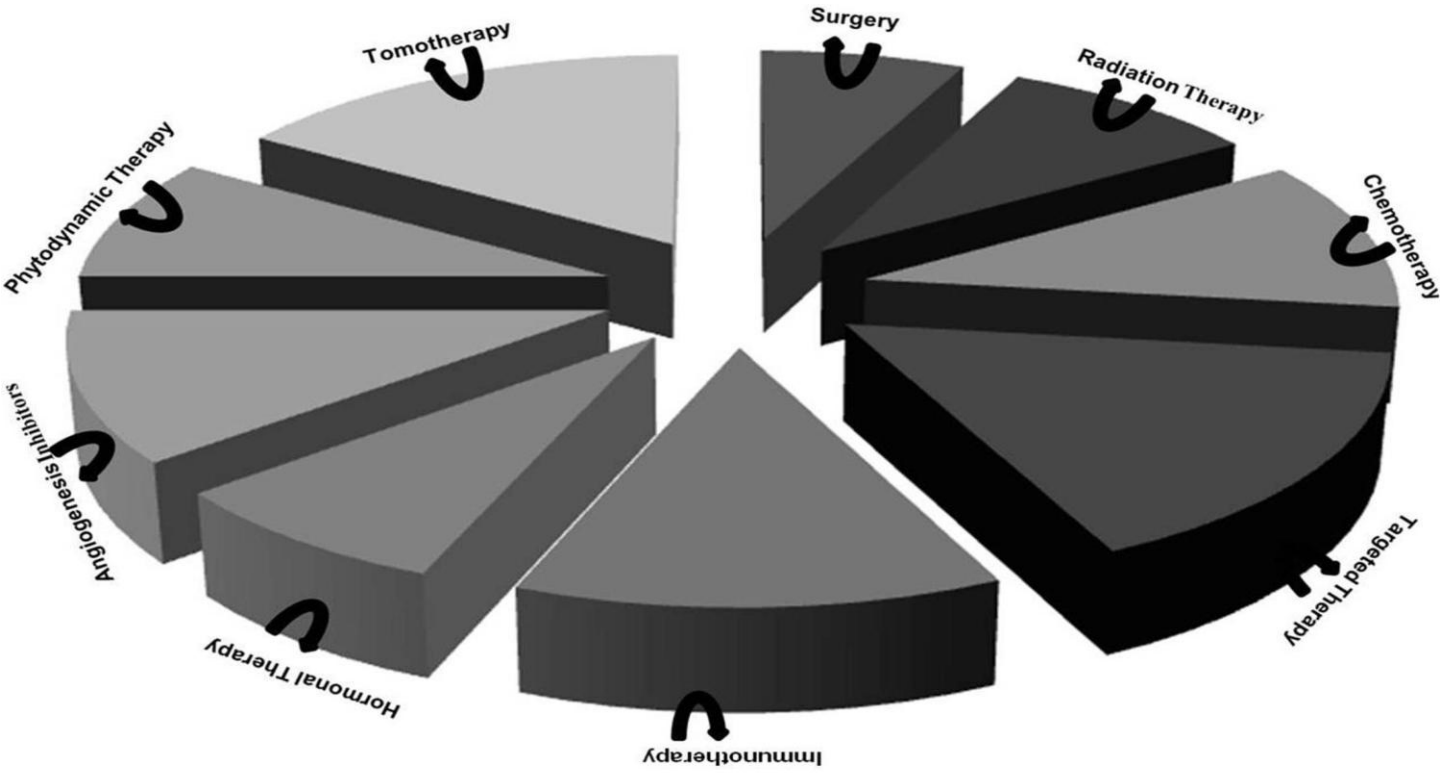

Figure No. 02: Effective Cancer Therapies

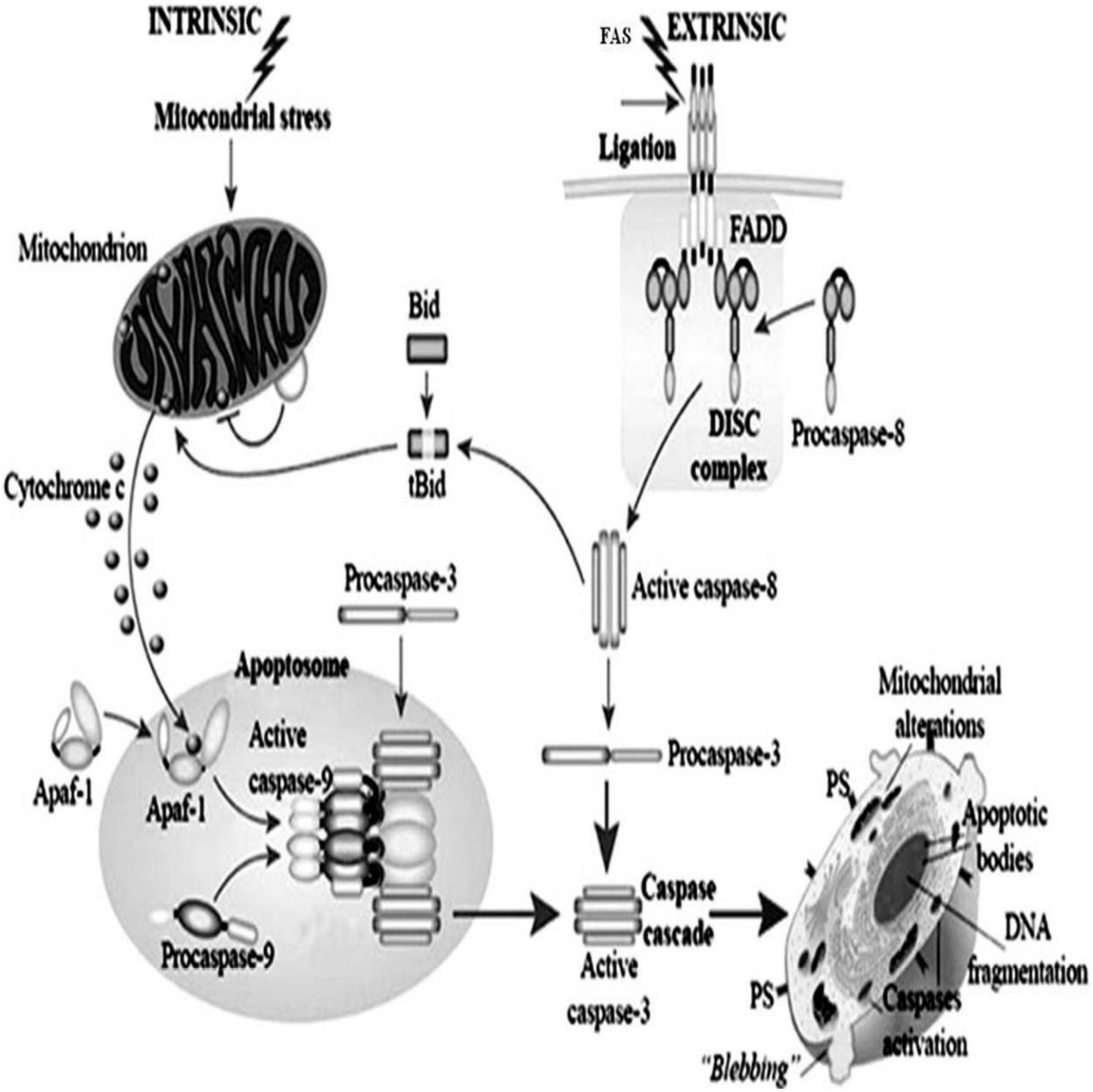

Figure No. 03: Molecular Mechanism of Apoptosis. The extrinsic and intrinsic pathways require specific triggering signals to begin an energy-dependent cascade of molecular events. The extrinsic signaling pathways involves death receptors e.g. FAS. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD. FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8. The activated caspase-8 further activates the downstream caspasess-3,-6 &-7, cluminating into apoptosis. The intrinsic signaling pathways involves a diverse array of non-receptor-mediated stimuli which cause changes in the inner mitochondrial membrane, resulting in an opening of the mitochondrial permeability transition (MPT) pore. Loss of mitochondrial transmembrane potential releases the normally sequestered pro-apoptotic proteins like cytochrome c from the intermembrane space into the cytosol. Cytochrome c binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome" The clustering of procaspase-9 in this manner leads to caspase-9 activation which later activates the down stream caspases. Bad acts as a crosstalk between the two pathways.

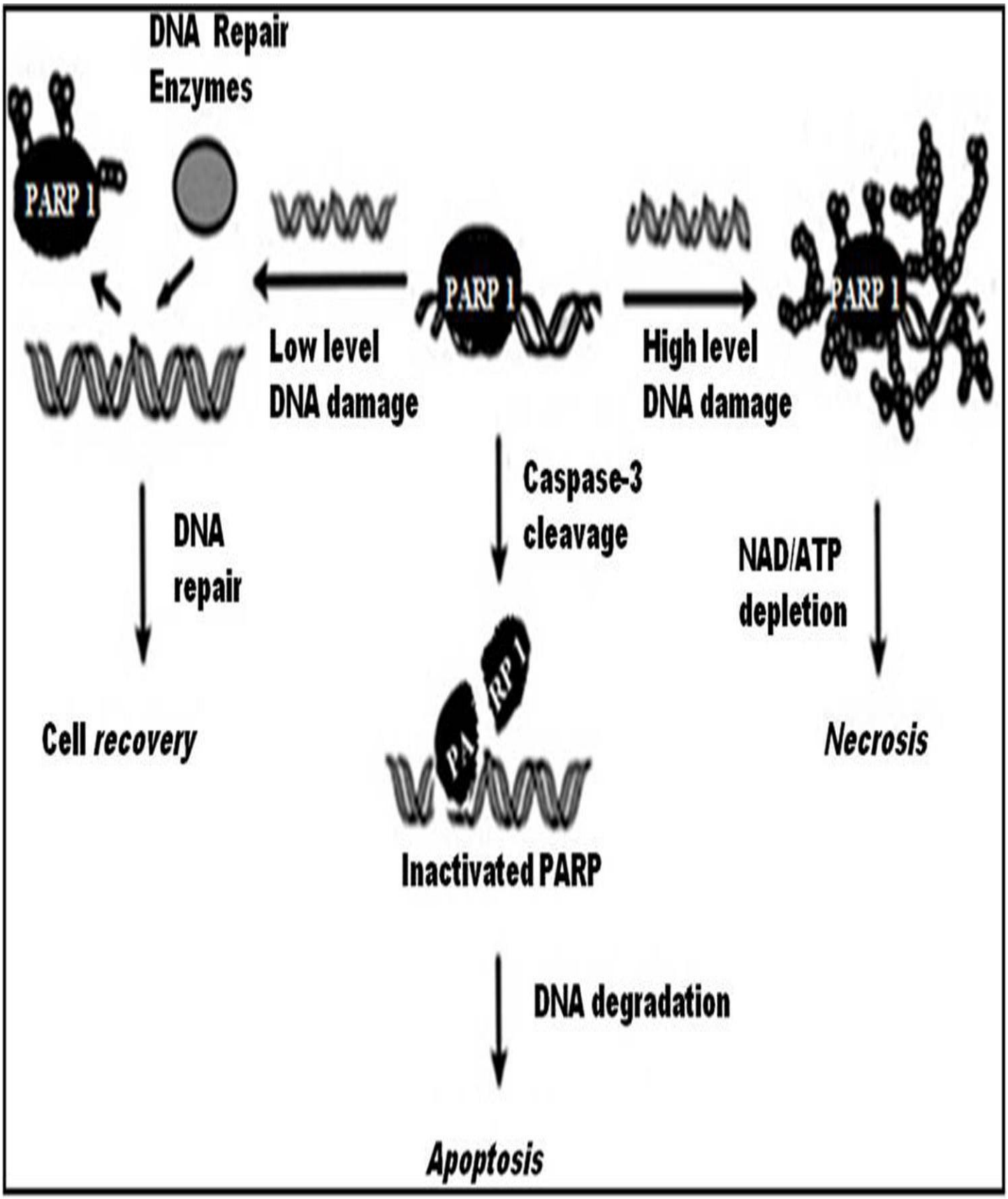

Figure No. 04: PARP is an essential DNA repair enzyme. In molecular targetting of cancer, the cleavage of PARP by caspase-3 prevents unwanted DNA repair, leading to the fragmentation of cancer cell DNA.

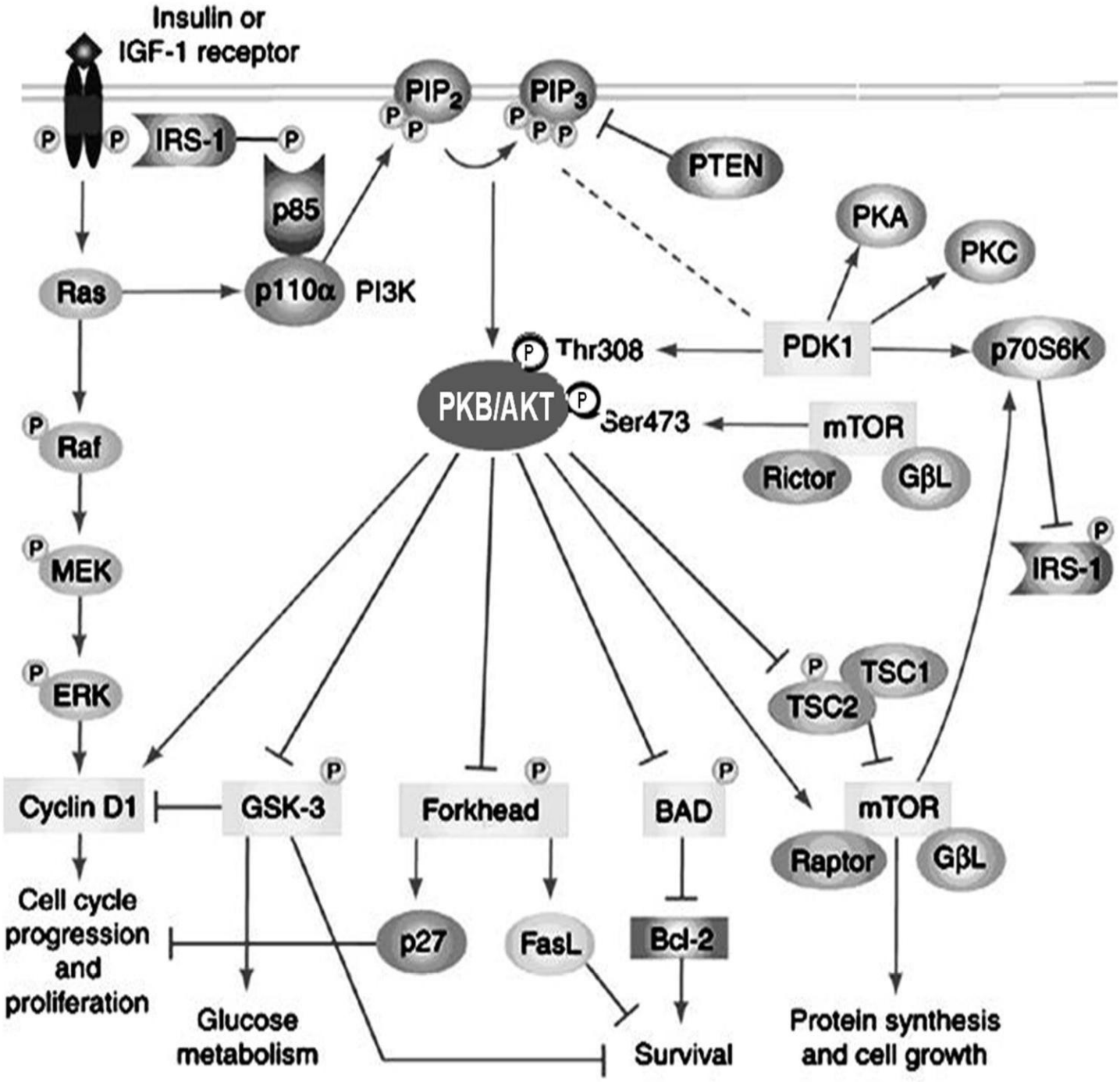

Figure No. 05: PI3K signalling and Cancer**.** Phosphoinositide 3-kinases (PI3Ks) play a key role in cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which, in turn, are involved in cancer. Class I PI3Ks are heterodimers composed of various combinations of catalytic and regulator subunit isoforms (85 kDa adaptor subunit that facilitates interaction with receptor tyrosine kinases (RTK) and either p110α, p110β, or p110γ catalytic subunit. Upon stimulation by receptor tyrosine kinases ligands such as insulin the preffered substrate of class 1PI3ks, phosphoinositide (4,5) bisphosphate (PIP2) gets phosphorylated to phosphoinositide (3,4,5)triphosphate (PIP3). PIP3 and PIP2, are important second messengers that coordinate to promote cell survival, growth, protein synthesis, mitosis, and motility. Cell survival, mitosis, and protein synthesis are promoted by PI3K dependent activation of the PDK/AKT(PKB) pathway *via* inactiavtion of different forehead transcription factors including p27, FAS L, Bcl-2, cyclins and an important glucose synthase kinase (GSK3). mTOR (mammalian target of Rapamycin) is an important downsteam effector of AKT activation. mTOR complex 1 (mTORC1) is composed of mTOR, Raptor, GβL (mLST8), and Deptor. Active mTORC1 shows number of downstream biological effects including translation of mRNA via the phosphorylation of downstream targets (4E-BP1 and p70 S6 Kinase). The mTOR complex 2 (mTORC2) is composed of mTOR, Rictor, GβL, Sin1, PRR5/Protor-1, and Deptor and promotes cellular survival by activating Akt. mTORC2 also regulates cytoskeletal dynamics by activating PKCα and regulates ion transport and growth via SGK1 phosphorylation. PTEN, a cytoplasmic phosphatase negatively regulates the PI3K pathway.

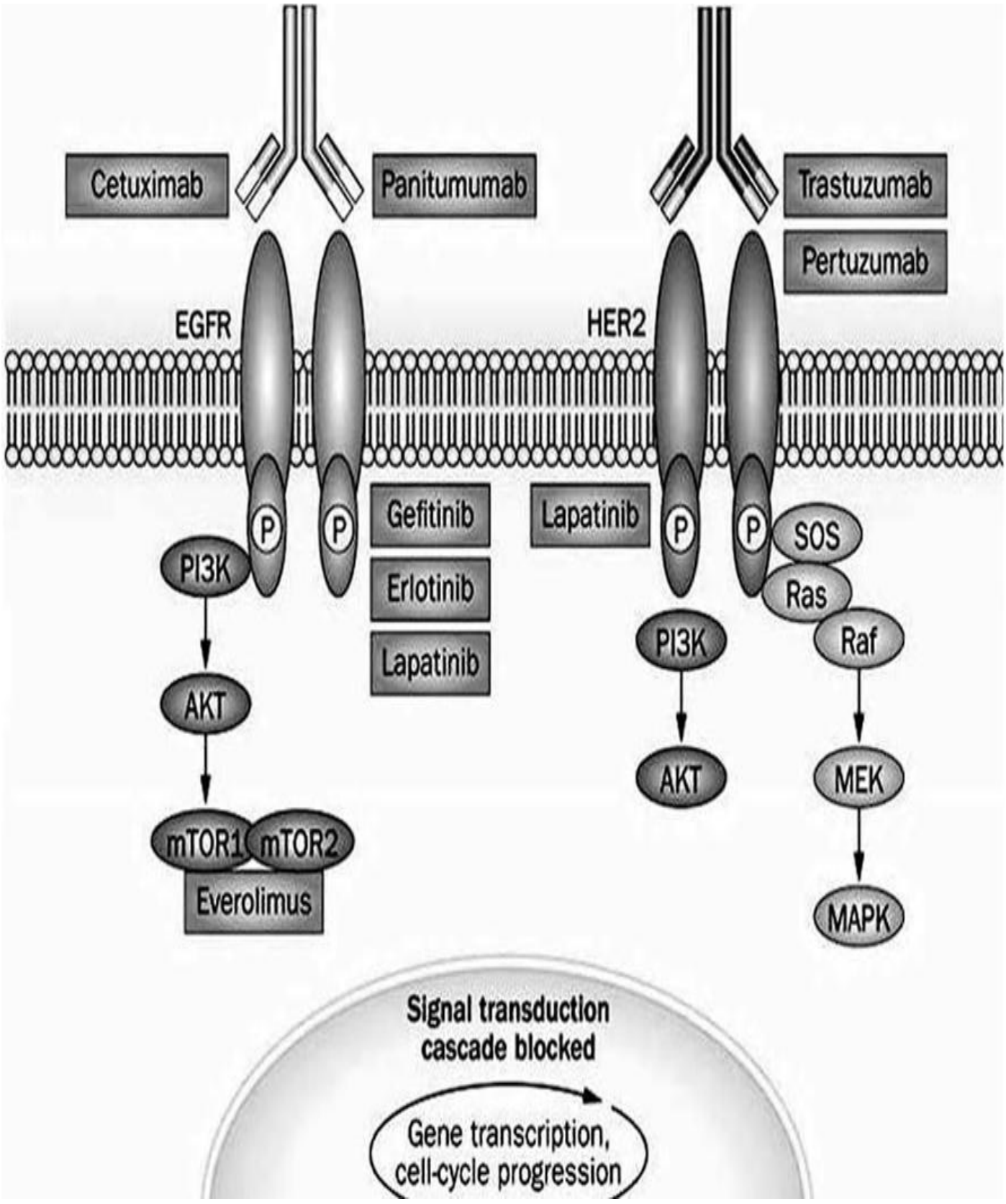

Figure No. 06: Drugs Targetting EGFR in cancer. The binding of EGF to EGFR induces formation of homo and heterodimers and ultimately to tyrosine autophosphorylation, triggering the activation of downstream siganlling pathways, more importantly the PI3K/AKT siganlling pathway. EGFR pathway can be inhibited at several steps, which can switch off this pathway. Gefitinib, Erlotinib and Lapitinib reversibly inhibits the phosphorylation of EGFR tyrosine kinase. Trastuzumab, Pertuzumab, Cetuxumab and Panitumumab binds specifically and selectively to the EGFR, preventing binding of activating ligand, EGF.

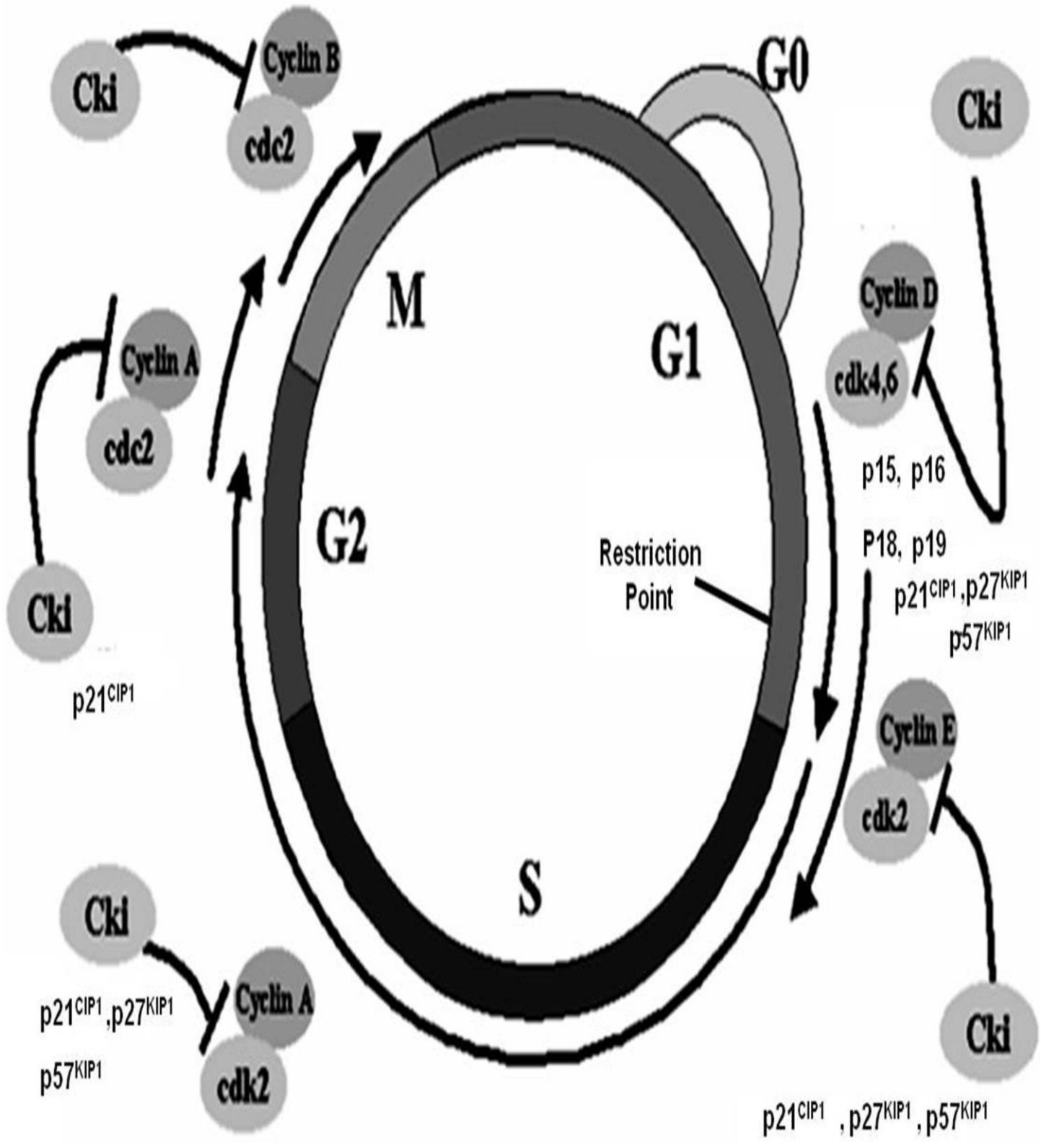

Figure No .07: Cell cycle and checkpoint control is an essential target in cancer cells. Cell cycle is a sequence of events transferring cell through four phases: G_1, S, G_2 & M to complete the cycle. The sub G_1 or G_0 is a quiescent/ resting phase of the cycle. Cyclins, Cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs) control the transition of cell from one phase of cell cycle to another. Cyclin D & E regulate the G1/S transition, Cyclin A controls the S phase progression and cyclin B regulates the M phase transition. CKIs: p21, p27, p16 & P15 negatively regulate the cell cycle by inhibiting the kinase activity of their specific CDKs. The regulation of cell cycle plays an important role in cell proliferation and apoptosis and therefore in cancer.

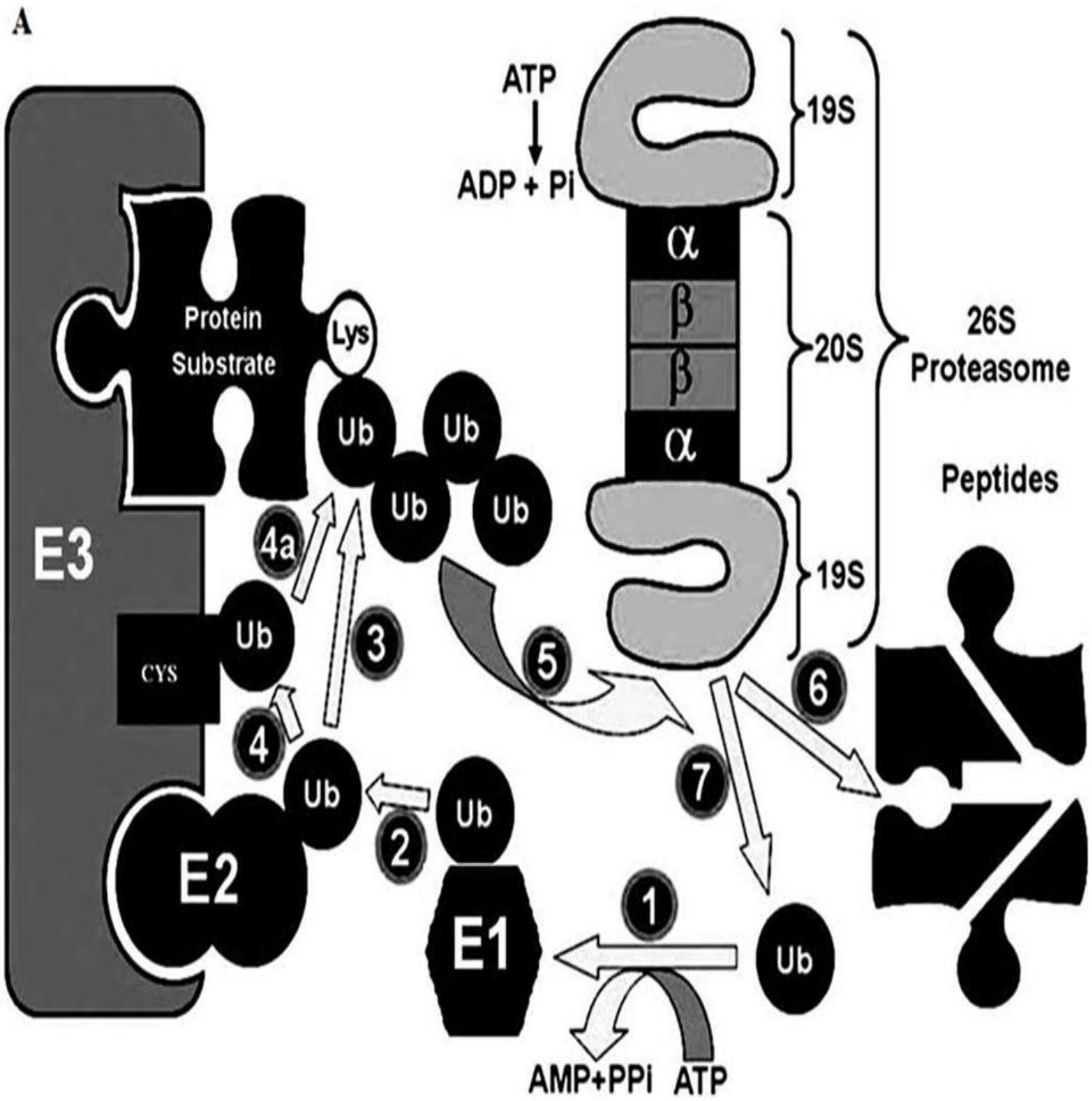

Figure No. 08 A: Proteasome Pathway. The proteasome is a massive protein complex (multicatalytic protease complex) that removes unnecessary proteins by breaking them down into short peptides. It consists of a tunnel like catalytic core (20S) with a regulatory cap at each end (19S), forming a larger ATP-dependent 26S proteasome. The 20S core consists of 2α and 2β subunits. The caps recognize and bind to the targeted proteins and inject them into the central core that acts as a degradation chamber. The pathway involves 1.Activation of ubiquitin by the ubiquitin-activating enzyme E1 and ATP. 2. A ubiquitin-carrier protein, E2 (ubiquitin-conjugating enzyme, UBC) forms a conjugate with E1and ATP. The product of this reaction is a high-energy E2~ubiquitin thiol ester intermediate. 3. Binding of the protein substrate, via a defined recognition motif, to a specific ubiquitin-protein ligase, E3. 4. Multiple (*n*) cycles of conjugation of ubiquitin to the target substrate and synthesis of a polyubiquitin chain. E2 transfers the first activated ubiquitin moiety directly to the E3-bound substrate, and in following cycles, to previously conjugated ubiquitin moiety.5. Transfer of polyubiquitylated proteins into the catalytic core. 6: Degradation of the ubiquitin-tagged substrate by the 26S proteasome complex with release of short peptides. 7: Ubiquitin is recycled *via* the activity of deubiquitinating enzymes (DUBs).

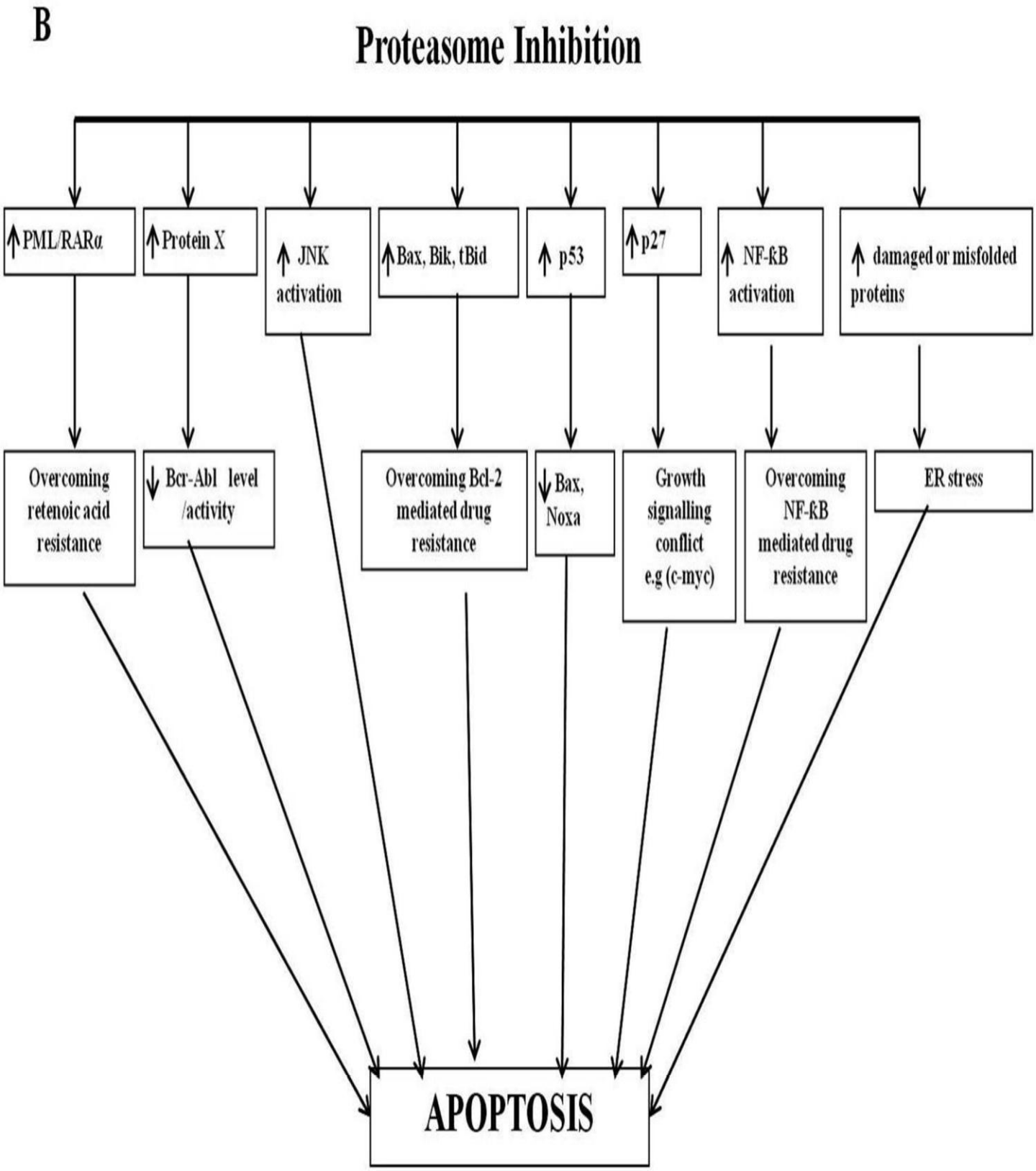

Figure No. 08 B: The proteasome mediated steps in apoptosis is located upstream of mitochondria and can involve in different systems Bcl-2, Jun N-terminal kinase, heat shock proteins, Myc, p53, and other factors. Proteasome inhibitors increase p53 activity. Loss of function of MDM-2 (responsible for its degradation) results in accumulation of P53. The synchronized proteolysis of cyclins and cyclin-dependent kinase inhibitors is critical for cell cycle progression. Disruption of this process in proliferating cells leads to cell cycle arrest at G1/S, G2/M, or both depending on the cell type. The failure to degrade IκB blocks the signaling action of the transcription factor NF-κB.

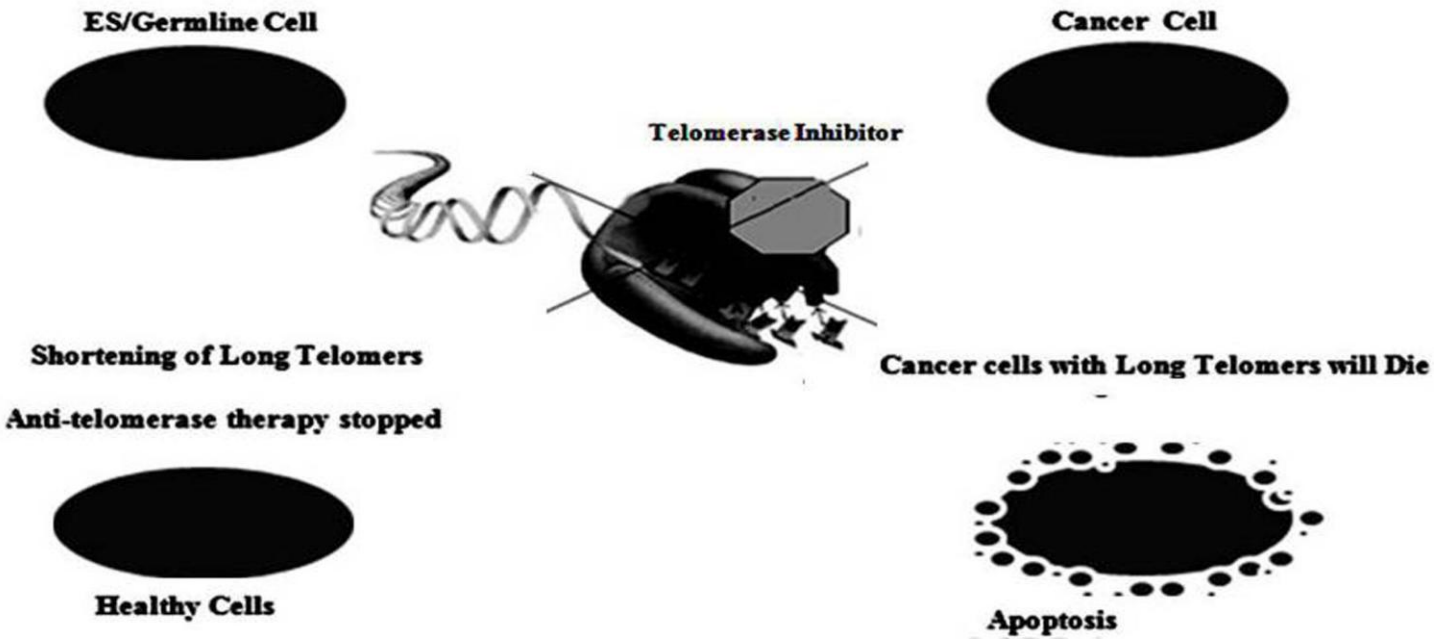

Figure No. 09: Inhibiting Telomerase in cancer. Telomerase inhibition shortens the length of telomere in germline cells and retains them healthy after the therapy is stopped. In case of cancer cells, the inhibition of telomerase induces apoptosis in cancer cells thus terminating their growth and proliferation.

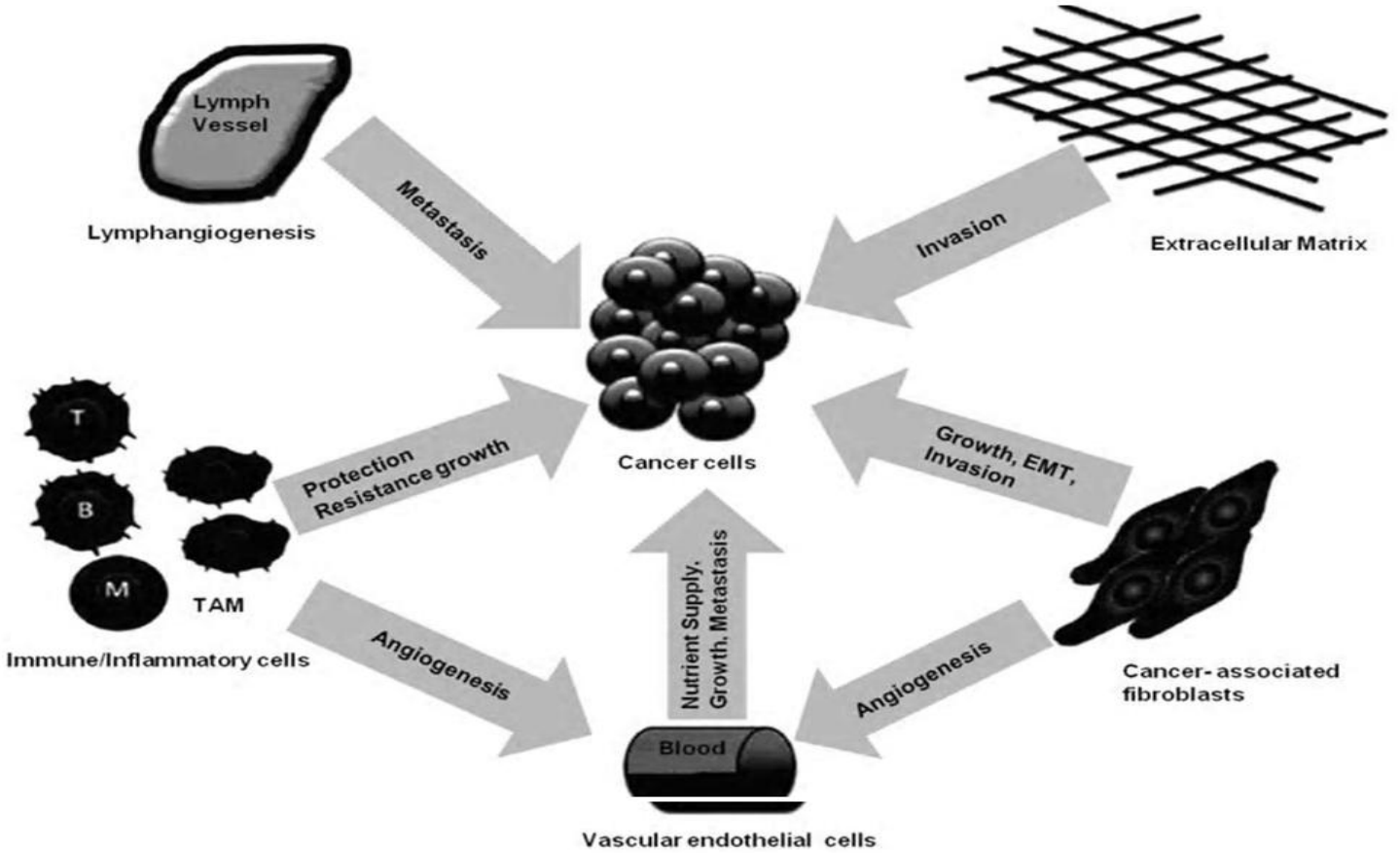

Figure No. 10 Tumor Microenvironemnt.The micrienviroment of a tumor cell is an important factor in determining the proliferation, progression and metastasis of tumor. The miroenvironment provides the signals for sustained proliferative signaling *via* cancer associated fibroblasts, evading growth suppressors by supressing immune and inflammatory cells , resisting cell death, enabling replicative immortality, inducing angiogenesis by providing nutrient supply *via* new blood vessels (angiogenesis) using vascular endothelial cells, activating invasion by extracellular matrix and metastasis *via* lymph vessels.

Sr. No.	Molecular Targets	Chemopreventive agents
1.	Her-2	Trastuzumab , Lapatinib , Pertuzumab
2.	Bcr/abl,	Imatinib Mesylate (Gleevec), Dasatinib
3.	PDGFR	Imatinib Mesylate (Gleevec), Sunitinib
4.	EGFR	Gefitinib (Iressa), Erlotinib (Tarveca) , Cetuximab , Lapatinib
5.	Oestrogen receptors	Tamoxifen; raloxifene; arzoxifene
6.	Akt and NFKB	Curcumin; *N*-acetyl cysteine; silibinin; xanthohumol; deguelin; EGCG; resveratrol
7.	NRF2–KEAP1	Sulphoraphane; oltipraz
8.	COX2	Rofecoxib; celecoxib; EGCG
9.	COX1/2	Aspirin and other NSAIDs
10.	Histone deacetylases	Sulphoraphane
11.	TGF pathway	CDDO-Imidazolide
12.	HIF1	EGCG; resveratrol; apigenin; sulphoraphane
13.	STATs	CDDO-Imidazolide
14.	VEGF	Sulphoraphane; EGCG; fenretinide, Bevacizumab, Sunitinib
15.	Antiapoptotic gene, Bcl_2	G3139 (Genta, Berkley)
16.	mTOR	Rapamycin RAD001
17.	RAF Kinase	BAY43-9006

Table No. 1: Some of the Known Molecular targets and the effective anticancer drugs against these targets are are listed below

4. CONCLUSION:

Cancer is one of the most common diseases in both developed and developing countries. The induction of apoptosis has long been a central goal of chemotherapy and radiation treatment. The discovery of molecular targets have allowed for potentially greater flexibility when approaching cancers. The future of cancer therapy requires an understanding of new molecular targets and genetic defects that lower the efficacy of current therapeutics to effective molecular targeting.

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