Part-1

Introduction
1. Cancer

Cancer is the term given to a large group of diseases that vary in type and location. It refers to a continuous, abnormal and uncontrolled cell proliferation, which is due to permanent alteration of some cells that gets transmitted to the cell family. It is a large and complex family of malignancies that can affect virtually every organ in the body. Principally, it is a genetic disease caused by multiple steps of processes involving activation of oncogenes, loss of function of tumor suppressor genes, alteration of modifier genes, genes involved in DNA repair and genomic stability (Croce, 2001). Over millions of new cases which are diagnosed every year, half of them occur in the lung, prostate, breast, colon and rectum. Cancer can strike at any age, although it is most common in people above 50 years of age. Worldwide, breast cancer is the fifth most common (after lung, stomach, liver and colon cancer) and second most common among women, excluding non-melanoma skin cancers, cause of cancer related deaths. According to the World Health Organization, approximately more than 1.7 million people will be diagnosed with breast cancer this year worldwide. Though less common, breast cancer also occurs in men. In 2007, about 2,030 new cases of breast cancer were expected in men in USA only (Cancer Facts and Figures 2007). There are around 200 different types of cancer. Two in five people, at some point in their lives, are susceptible to it. It is a disease wherein a cell loses its control over the reproduction capacity and rather than dividing in a controlled and programmed manner, cell continues to divide, leading to multiplication abnormally, which finally leads to tumor development. This new growth can be either \textit{benign} or \textit{malignant}. Benign tumors do not spread, or \textit{metastasize}, to other parts of the body and so are not cancerous. They can often be removed and are rarely a threat to life. A malignant tumor however can spread and is
cancerous. Malignant cells break off and travel through the blood lymph system to other parts of the body, resulting in a secondary tumor and finally metastasis. The name given to the cancer is reflective of origin of the cancer. For example, if breast cancer spreads to the lung, the disease is called metastatic breast cancer only, not lung cancer.

1.1 Classification: All cancers fall in four broad categories listed below:

1.1.1 Carcinoma
Carcinoma is a malignant neoplasm of epithelial origin. It is a tumor that arises in the tissues which line the body's organs like nose, colon, penis, breasts, prostrate, urinary bladder, and the ureter. About 80% of all cancer cases are carcinomas.

1.1.2 Sarcoma
Sarcomas are tumors that originate in bone, muscle, cartilage, fibrous tissue or fat. Ewing sarcoma (family of tumors) and Kaposi's sarcoma are the common types of sarcomas.

1.1.3 Leukemias
Leukemias are cancers of the blood or blood-forming organs. When leukemia develops, the body produces a large number of abnormal blood cells. In most types of leukemia, the abnormal cells are white blood cells.

1.1.4 Lymphomas
Lymphomas affect the lymphatic system, a network of vessels and nodes that act as the body's filter. The lymphatic system distributes nutrients to blood and tissues, and prevents bacteria and other foreign "invaders" from entering the bloodstream. There are over 20 types of lymphomas. Hodgkin's disease is a type of lymphoma.
1.2 Breast Cancer

Breast cancer is a cancer that starts in the cells of the breast. Because breast is composed of identical tissues in males and females, breast cancer also occurs in males. Incidences of breast cancer in men are approximately 100 times less common than in women, but men with breast cancer are considered to have the same statistical survival rates as women (Male Breast Cancer Treatment - National Cancer Institute, 2006). It is estimated that in 2008 itself, 182,460 (female) and 1,990 (male) new cases of breast cancer occurred which led to estimated 40,480 (female) and 450 (male) deaths. The number of breast cancer patients has increased significantly worldwide.

Both external and internal factors can cause cancer. Factors such as chemicals, radiation, viruses, hormones and inherited mutations may act together to initiate cancer and are thus referred to as carcinogens. Ten or more years may pass between exposure to carcinogenic agents and detectable cancer. Present study mainly focuses on breast cancer which can be classified into four major groups according to different purposes of its study:

1.2.1 Pathology

This classification depends mainly upon invasiveness or malignant stage of a specific tumor. It may include invasive ductal carcinoma, which is malignant cancer in the breast's ducts, and invasive lobular carcinoma, which represents malignant cancer in the breast's lobules.

1.2.2 Tumor Grade

Can be classified as low grade (well-differentiated) and high grade (poorly differentiated), means in place of normal tissue, tumor is composed of disorganized cells.
**TNM classification**: Is used to define the stage (grade) of a tumor.

1.2.2.1 Tumor
Can be classified as five tumor classification values (Tis, T1, T2, T3 or T4) which depend on the presence or absence of invasive cancer, invasion outside the breast and further by the dimensions of the invasive cancer.

1.2.2.2 Lymph Node
Based on the number, size and location of breast cancer cell deposits in lymph nodes, there are four lymph node classification values (N0, N1, N2 or N3).

1.2.2.3 Metastases
There are two metastatic classification values (M0 or M1) which represent presence or absence of cancerous cells in locations other than the breast and lymph nodes (so-called distant metastases, e.g. to bone, brain, lung).

1.2.3 Protein & gene expression status
A particular cancer can also be classified as presence or expression of different gene or protein like HER2/neu proteins, estrogen receptor (ER) and progesterone receptor (PR). All breast cancers should be tested for these genes.

1.3 Risk factors for breast cancer

1.3.1 Age
The incidences of breast cancer increase with age. Compared with other cancers, the incidence of breast cancer is higher at younger age. It seems that age is one of the most important deciding factors for breast cancer incidence.
1.3.2 Geographical variation

Age adjusted incidence and mortality for breast cancer varies by up to a factor of five between countries. The difference between Far Eastern and Western countries is diminishing but is still about fivefold. Studies of migrants from Japan to Hawaii show that the rates of breast cancer occurrence in migrants assume the rate in the host country within one or two generations, indicating that environmental factors are of greater importance than genetic factors.

1.3.3 Age at first pregnancy

It has been proven that mother's age at first birth influences the lifetime incidences of breast cancer. The risk of breast cancer in women who have their first child after the age of 30 is about twice that of women who have their first child before the age of 20. The highest risk groups are those who have a first child after the age of 35. An early age at birth of a second child further reduces the risk of breast cancer.

1.3.4 Family history

It is not yet known how many breast cancer genes are present. Two breast cancer genes, BRCA1 and BRCA2, located on the long arms of chromosomes 17 and 13 respectively, have been identified and account for a substantial proportion of very high risk families i.e. those with four or more breast cancers among close relatives. Both genes are very large and mutations can occur at almost any position, so that molecular screening to detect mutation for the first time in an affected individual or family is technically demanding. A woman's risk of breast cancer is two or more times greater if she has a first degree relative (mother, sister, or daughter) who developed the disease before the age of 50, and the younger the relative when she developed breast cancer the greater the risk.
1.3.5 Lifestyle

1.3.5.1 Diet

Although there is a close correlation between the incidences of breast cancer and dietary fat intake in populations, the true relation between fat intake and breast cancer does not appear to be particularly strong or consistent.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Risk</th>
<th>High risk group</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>&gt; 10</td>
<td>Elderly</td>
</tr>
<tr>
<td>Geographical location</td>
<td>5</td>
<td>Developed country</td>
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<tr>
<td>Age at menarche</td>
<td>3</td>
<td>Menarche before age 11</td>
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<tr>
<td>Age at menopause</td>
<td>2</td>
<td>Menopause after age 54</td>
</tr>
<tr>
<td>Age at first full pregnancy</td>
<td>3</td>
<td>First child in early 40s</td>
</tr>
<tr>
<td>Family history</td>
<td>&gt;2</td>
<td>Breast cancer in first degree relative.</td>
</tr>
<tr>
<td>Previous benign disease</td>
<td>45</td>
<td>Atypical hyperplasia</td>
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<tr>
<td>Cancer in other breast</td>
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<tr>
<td>Diet</td>
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<td>High intake of saturated fat</td>
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<tr>
<td>Premenopausal</td>
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<td>Body mass index &gt; 35</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>2</td>
<td>Body mass index &gt; 35</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>1.3</td>
<td>Excessive intake</td>
</tr>
<tr>
<td>Exposure to ionizing radiation</td>
<td>3</td>
<td>young females after age 10</td>
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<tr>
<td>Oral contraceptives</td>
<td>1.24</td>
<td>Current use</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td>1.35</td>
<td>Use for &gt;10 years</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>2</td>
<td>Use during pregnancy</td>
</tr>
</tbody>
</table>

Table 1: Established and probable risk factors for breast cancer (McPherson et. al., 2000).

1.3.5.2 Weight

Obesity is associated with a two fold increase in the risk of breast cancer in postmenopausal women whereas among premenopausal women it is associated with a reduced incidence.
1.3.5.3 Alcohol intake
A link between alcohol consumption and incidence of breast cancer has been suggested by some studies but the relation is inconsistent and the association may be with other dietary factors rather than alcohol.

1.3.5.4 Smoking
Smoking is of no importance in the etiology of breast cancer however there is some evidence that exposure to tobacco smoke is most problematic between puberty and first childbirth. The reason that breast tissue appears most sensitive to chemical carcinogens in this phase is that breast cells are not fully differentiated until lactation.

All risk factors including these as well as others can be summarized as given below (Table. 1):

1.4 Genetics of Breast cancer
Heredity or family background of a woman has an important role to play in the occurrence of breast cancer. Among all the women who develop breast cancer in their lifetime, more than 5% breast cancers occur from a hereditary predisposition of disease. The risk of breast cancer in women with affected relatives is higher when the diagnosis is made at an early age and when the disease is bilateral. About 30% of daughters of women with early onset, bilateral breast cancer inherit the susceptibility. Out of total number of breast cancer patients, 10 to 20% of patients have a first or second-degree relative with one of these diseases (Madigan et. al., 1995; Wooster and Weber, 2003).

The most extensive efforts to identify a breast cancer susceptibility gene have focused on chromosomes 17q21. Two major genes associated with susceptibility to breast cancer namely, breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) have been identified to date (Miki et. al., 1994). Mutations in
either of these genes confer a lifetime risk of breast cancer in people between 60 and 85 age groups. However, mutations in these genes account for only 2 to 3 percent of all breast cancers (Friedman et. al., 1995; Newman et. al., 1997), and susceptibility alleles in other genes, such as TP53, PTEN, and STK11/LKB1, are even less common causes of breast cancer (Fig. 1). The prediction that there are common DNA sequence variants that confer a small but appreciable enhanced risk of cancer has been validated with the recent discovery of the 1100delC mutation in the cell cycle checkpoint kinase gene (CHEK2) (Meijers-Heijboer et. al., 2002). There is convincing evidence that additional high-penetrance genes that increase susceptibility to breast cancer exist. Many additional genetic variants in low-penetrance susceptibility alleles may moderately increase the risk of breast cancer. These genetic variants are much more common in the population than are high-penetrance gene mutations and, thus, in aggregate may make a substantially greater contribution to breast cancer in the population than mutations in high-risk genes (Dunning et. al., 1999).
1.5 Development of spontaneous breast cancer

Normally breast development is regulated by a sense of balance between cell growth, proliferation and apoptosis, and it is also known that tumor growth is not just a result of uncontrolled growth but also of reduced apoptosis (Morrow and Gradishar, 2002). Breast cancers, as with other human tumors are believed to result from genetic alterations that lead to specific growth advantage to preneoplastic mammary glandular epithelial cells. During carcinogenesis in epithelial tissue, genetic mutations accumulate and it finally results in loss of cellular functions. The phenotype of the cells change from normal through a series of malignant lesions to superficial cancers and finally invasive disease (Fig. 2). In general, these processes are protracted and are believed to occur up to 30 years before the clinical appearance of breast cancer (Kelloff et. al., 2000).

2.0 Cell signaling pathways: importance in cancer therapy

During the course of tumor progression, cancer cells gain a number of characteristic alterations. These include proliferation independent of exogenous growth promoting or growth inhibitory signals to invade surrounding tissue and metastasize to distant site, to elicit an angiogenic response and to evade mechanisms that limit cell proliferation, such as apoptosis and senescence. One of the reasons for these is alterations in cellular signaling pathways that occur in normal cells, e.g., controlled cell proliferation, mortality and survival. Intracellular signaling pathways that perform these jobs are diverse and complex.
There is significant cross talk between these signaling pathways and up or down regulation of one of them may trigger coordinate response in another one. Thus, inhibition of one component of signal transduction pathway may be compensated by regulation of another pathway. These observations increase the long list of signal transduction components that are known to be altered in cancers. There are clear evidences that disruption of signal transduction pathways is a commonly observed event in human cancers and

Fig. 2: Formation of invasive ductal carcinoma. From normal breast, atypical ductal hyperplasia (intraepithelial neoplasia) through to intraductal carcinoma in situ and invasive ductal or lobular carcinoma. Adopted from Parton et. al., 2001)
provides a target for therapeutic intervention. Typically, a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen. Advancements in signal transduction and genomics research promise to identify the molecular mechanisms primarily involved in many human diseases. Coupled with the development of modern drug development strategies such as high throughput screening and sophisticated synthetic or computation chemistry efforts, these advances are facilitating the development of potent and selective modulators of many signal transduction pathways. Many of the molecules currently under investigation as possible target for cancer therapy are signaling proteins that are component of these pathways.

3.0 Apoptosis in treatment of cancer: first hope of cure

A common feature of cancer cells is their ability to evade apoptosis as a result of alternations that block cell death signaling pathways. Apoptosis is a kind of cellular suicide that is commonly seen in nature. This phenomenon can be induced by variety of stimuli, including hormones, virus, cytokines, and toxic insults. Abnormal inhibition of apoptosis is a hallmark of cancer and autoimmune diseases whereas excessive cell death has been implicated in number of neurodegenerative disorders (Thompson, 1995). Chemotherapeutic drug are known to work by inducing apoptosis in the cells. Different mechanisms of apoptosis are depicted in Fig. 3. Over past two years, extensive research efforts elucidated apoptosis inducing signaling pathways have set the stage for the development of therapeutic agents that either kill cancer cells selectively or reset their apoptotic threshold.
3.1 Morphological and molecular changes during apoptosis

Many molecular changes occur during apoptosis which include chromatin condensation, cytoplasmic shrinkage and active membrane blebbing (Kerr et. al., 1972; Wyllie et. al., 1980a). Some other important changes include release of cytochrome C (Kluck et. al., 1997; Yang et. al., 1997), an apoptosis inducing factor, an oxidoreductase related flavoprotein, from the mitochondrial intermembrane space. Molecular changes induced during apoptosis include internucleosomal DNA cleavage, by cysteine dependent aspartate directed protease termed caspase (Wyllie, 1980b) and randomization of the distribution of phosphatidyl serine (PS) between the inner and outer leaflet of plasma membrane. These changes can be detected by staining of cell surface exposed PS with annexin V and technique based on detecting these changes such as terminal deoxynucleotidyl transferase mediated incorporation of labeled
nucleotide at DNA break. These changes are initiated by many types of death stimuli and this common death effector machinery seems to be evolutionarily conserved (Herr and Debatin, 2001).

3.2 Molecular regulators of apoptosis

3.2.1 The cell biology of caveolae

Caveolae (little cave) are cholesterol enriched 50-100 nm invaginations of plasma membrane and caveolins are the structural proteins used by cells to form caveolae. The caveolae have generated a great deal of attention during last decade, as it appears to mediate multiple cellular functions, including clatherin-independent endocytosis, cholesterol efflux and regulation of intracellular signaling pathways. Caveolae are related to membrane microdomains called lipid rafts. Because of their unique lipid composition that differs from bulk cellular membranes in being enriched with cholesterol and sphingolipids. The liquid-ordered phase of rafts and caveolar membranes is quite useful as it confers upon the membrane insolubility in certain non-ionic detergents at 40°C. This property enables the fractionation of cellular proteins in discontinuous sucrose density flotation gradient, in which cold detergent insoluble proteins that are localized in lipid rafts and caveolar membranes float into low density fractions and are well resolved from most other cellular proteins (Anderson, 1998).

3.2.1.1 Caveolin-1

Plasma membrane is one of the most promising targets of chemotherapeutic drugs. It plays important role in drug influx or efflux and in regulation of different cellular signaling, important in drug trafficking. Cholesterol enriched invaginations of plasma membrane regulate cellular signaling by its structural gene Caveolin-1 (Cav-1). Cav-1 is a 21-24 kDa protein which works as an inhibitory clamp and entraps many important signaling molecules through its scaffolding
binding domain thereby negatively regulating signaling (Fig. 4). Its role as a “signaling hub” generates many possibilities of its functional involvement in regulation of disease like cancer, which mainly is initiated by deregulated cellular signaling. In past few years Cav-1 has generated a great deal of attention in cancer biology as it appears to be associated with multiple aspects of human cancer progression. Available experimental evidences (from cultured cells, animal models, and human tumor samples) indicate that it is a multitasking molecule having controversial roles. Some preliminary reports in mouse fibroblast NIH 3T3 cells claim that transformation of these cells by various oncogenes leads to reduction in cellular levels of Cav-1 gene (Koleske et. al., 1995). Up regulation of Cav-1 negatively regulates the activation state of one of the important MAP Kinase, p42/44 (Galbiati et. al., 1998). Interestingly, the human Cav-1 gene is localized to a
suspected tumor suppressor locus (7q31.1) suggesting that it might be a candidate tumor suppressor gene in many tumors. In contrast many reports in bladder and prostate tumors indicate that Cav-1 may function as a tumor promoter since studies relate Cav-1 gene expression to the pathological grade. Moreover these studies also suggest that over-expression of Cav-1 is associated with established features of prostate cancer and its aggressiveness. Thus, Cav-1 expression might help identify patients at high risk of developing aggressive prostate cancer recurrence and metastasis (Galbiati et. al., 1998; Koleske et. al., 1995). Recently, Cav-1 is being investigated for its involvement in multiple aspects of chemotherapy, especially with multi-drug resistance. It is reported that overexpression of Cav-1 changes the state of the cells from drug-resistant to drug-sensitive by inhibiting P-glycoprotein transport activity. In parallel to its relation with MDR, some reports demonstrate that Cav-1 sensitizes cells to apoptosis by regulating cell cycle progression and activation of the apoptotic signaling molecules such as Bcl-2, p53 and p21 (Lavie et. al., 1998; Shajahan et. al., 2006). It is also reported that NIH/3T3 cells harboring antisense Cav-1 are resistant to staurosporine-induced apoptosis (Linge et. al., 2007; Wu et. al., 2007). Moreover, in drug-sensitive lung cancer cells (A549, Calu-6 or NCI-H69), exposed to cytotoxic drugs (taxol, doxorubicin or etoposide) up-regulation of Cav-1 and 2 proteins have been demonstrated (Belanger et. al., 2003). Cav-1 expression is found to be related to chemosensitivity in oral squamous cell carcinoma (OSCC) and its overexpression may provide novel diagnostic markers associated with cisplatin sensitivity (Nakatani et. al., 2005). In addition to these, one recent report in breast cancer showed that Cav-1 tyrosine phosphorylation enhances paclitaxel-mediated cytotoxicity. Taken together, all these reports are indicative of need for further exhaustive studies (Shajahan et. al., 2006).
3.2.2 Cyclin-dependent kinase 5 (Cdk5)

Cell division and apoptosis are key aspects of cancer biology. The combination of increased cell proliferation and reduced cell death lies very close to the reason why cancer is a deadly disease. The importance of apoptosis and cell division in tumor biology has made these cellular phenomenon potential targets of new anti-cancer therapies. Although the cell division cycle and apoptosis might appear to be quite different from a physiological perspective and thus provide independent targets for therapy, recent evidence suggests that they are intertwined, and that an enzyme in one system might also have an important role in the other system. Protein kinase complexes known as cyclin dependent kinases (Cdks) are the first example of such enzymes (Cicero and Herrup, 2005). Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase that was discovered by its homology to Cdc2 (Cdk1) (Hellmich et. al., 1992). Despite its structural homology with the traditional cyclin-dependent kinases, Cdk5 is not believed to function in a normal cell cycle. Cyclin-dependent kinase-5 (Cdk5) regulates cell differentiation and morphology rather than cell division. Alzheimer's disease (AD) involves neuronal cell death, the formation of beta-amyloid plaques and neurofibrillary tangles. Parkinson's disease (PD) is characterized by loss of dopaminergic neurons. Traditionally, cyclin proteins can bind Cdk5, but they do not activate it (Miyajima et. al., 1995; Xiong et. al., 1992; Zhang et. al., 1993). Activity requires the association of p35 or p39, both of these possess structural but not sequence homologies to typical cyclins. The substrates of Cdk5/p35 include cytoskeletal and cell-signaling proteins (Dhavan and Tsai, 2001; Homayouni and Curran, 2000; Kwon and Tsai, 2000). The known functions of Cdk5 are diverse including neuronal migration (Gilmore et. al., 1998), axonal growth (Paglini et. al., 1998), neurite extension (Nikolic et. al., 1996), stress response (Bibb et. al., 2001), and cellular senescence.
(Alexander et. al., 2004). Cdk5 was originally identified and cloned from cervical cancer HeLa cells (Maccioni et. al., 2001). Till now interestingly, very little is known about this neuron-specific Cdk5 and its functional role in cell cycle regulation or in cancer, though recently expression and activity of Cdk5 has been reported in prostate (Lin et. al., 2004; Strock et. al., 2006), breast (Goodyear and Sharma, 2007) and other cancer cells (Lin et. al., 2007). Some of emerging reports not only document the presence of Cdk5 activity in cancer cells but also suggest its role in different important cellular processes like proliferation, apoptosis or metastatic potential of cells (Strock et. al., 2007). All these studies collectively suggest that in addition to the role of Cdk5/p35 in central nervous system, it also seems to have extra-neural function(s) particularly in cancer cells.

3.2.3 The mitogen-activated protein (MAPK) kinase cascades

The mitogen activated protein kinases (MAPK) are evolutionarily conserved kinases that coordinate between extracellular signals to the machinery that controls fundamental cellular processes such as growth, proliferation, differentiation, migration and apoptosis (Dhillon et. al., 2007; Raman et. al., 2007). These are the large family of serine/threonine specific protein kinases that were originally described as proteins that become phosphorylated following mitogen stimulation of eukaryotic cells. Activation occurs when proteins are phosphorylated on the threonine and tyrosine in a region called T-loop, which is located in the kinase domain. MAP kinases are conserved from yeast to mammals and are the last components of three-tiered, protein kinase cascade (Dhillon et. al., 2007; Raman et. al., 2007). In mammals, to date, six families of MAPKs have been identified; these are extracellular signal regulated protein kinases (ERK1 and ERK2), the c-Jun N-terminal Kinases (JNK 1, 2, 3), the p38 MAPKs, ERK3/4,
ERK5 and ERK7/8 (Dhillon et. al., 2007; Raman et. al., 2007). Fundamentally ERK signaling is associated with cell growth and differentiation. JNK and p38 families, by contrast are associated with cellular stress. However these distinctions are not fully complete as ERK also is found to be associated with death. Like the MAPKs, a number of MAPKKs have also been identified. These are called MEK1, MEK2, MKK3, MKK4, MEK5, MKK6 and MKK7 (Davis, 2000). The specificity within these modules occurs because the different MAPKKs are restricted in the MAPKs that they can phosphorylate and activate. Thus, ERK1/2 are likely to be only physiological substrates for MEK1 and MEK2 and the JNKs are likely the only physiological substrates for MKK4 and MKK7, whereas the p38 MAPKs are preferred substrates for MKK3 and MKK6 (Davis, 2000; Lee et. al., 1999). Furthermore, despite being most similar to MEK1/2, MEK5 only activate ERK5 and MEK1/2 can not activate ERK5; the MAPKK for ERK3 has yet to be characterized (Fig. 5). The MAPKKs responsible for activating the JNKs and p38 MAPKs are less well defined. The MLKs, MEKK1-4, TAK1 can all activate JNK and p38 MAPKs. But the mechanism of activation of these MAPKKs is less well characterized than Raf-1.

3.2.3.1 ERK signaling and Cancer

ERK (Extra cellular signal-regulated kinase), is the best studied of mammalian MAPK pathways, and is found deregulated in approximately one-third of all human cancers (Fig. 6). Earlier, ERK signaling was synonymous with cell proliferation as it was shown that ERK activity was elevated in cell lines transformed by expression of oncogenes such as v-ras, v-raf, and v-src (Dhillon et. al., 2007). Interfering mutants of ERK (in which T-loop sites were mutated to prevent their phosphorylation) or suppression of ERK expression using anti-sense constructs or specific SiRNA were used to inhibit growth factor mediated DNA synthesis and cell proliferation.
In these pathways, growth factors stimulate receptor tyrosine kinases (RTKs), which are embedded in the plasma membrane and lead to activation of Ras proteins. These are small guanine nucleotide binding proteins that are bound to inner surface of the plasma membrane and are activated by exchange of GDP for GTP, which induce the conformational change in Ras protein and allow them to bind to downstream effector proteins. One family of Ras effector proteins are the Raf proteins, in presence of inactive Ras, Raf proteins are cytosolic, but they are recruited to the plasma membrane in presence of active Ras, GTP. The Raf proteins are the MAPKKKs of the ERK signaling cascade and Raf-1 is the most highly studied isoform. Raf-1 is activated at the plasma membrane in a Ras dependent fashion, by its binding to Ras. GTP is not sufficient to activate it, and other proteins, including Raf-1 phosphorylation, oligomerization, association
with other proteins, interaction with membrane lipids, and, possibly, a Ras-induced conformational change is required. Active Raf phosphorylates and activates MEK1 and MEK2 which in turn activate ERK1 and ERK2. The Raf proteins are therefore responsible for coupling growth factor mediated RTK stimulation at the plasma membrane to cytosolic activation of ERKs (Dhillon et. al., 2007; Roberts and Der, 2007; Raman et. al., 2007).

<table>
<thead>
<tr>
<th>EGFR overexpression</th>
<th>ERBB2 overexpression</th>
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<tbody>
<tr>
<td>• Most carcinomas (&gt;50%)</td>
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<tr>
<td>Ras mutation</td>
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<tr>
<td>• Pancreas (90%)</td>
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<td>• Lung adenocarcinoma (35%)</td>
<td></td>
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<tr>
<td>• Throid; follicular (55%) (non-small cell)</td>
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<td>• Thyroid; undifferentiated papillary (60%)</td>
<td></td>
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<td>• Seminoma (45%)</td>
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<tr>
<td>• Melanoma (15%)</td>
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<tr>
<td>• Bladder (10%)</td>
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<tr>
<td>• Liver (30%)</td>
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<tr>
<td>• Kidney (10%)</td>
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<tr>
<td>• Myelodysplastic syndrome (40%)</td>
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<tr>
<td>• Acute myelogenous leukemia (30%)</td>
<td></td>
</tr>
<tr>
<td>BRAF mutation</td>
<td></td>
</tr>
<tr>
<td>• Melanoma (66%)</td>
<td></td>
</tr>
<tr>
<td>• Colorectal (12%)</td>
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Fig. 6: Cancer-associated lesions in ERK signaling pathway in cancer. Adopted from Downward, 2003

Historically, ERK signaling was synonymous with cell proliferation but now it is clear that it centered on multiple signal transduction pathways to regulate different types of functions. Like other members of MAP kinase family, ERK is also reported to be activated in response to stresses for the induction of apoptosis (Cagnol et. al., 2006; Singh et. al., 2007; Tang et. al., 2002; Wang et. al., 2000). Interestingly activation of ERK through different pathways promotes either cell
survival or cell death (Bergmann et. al., 1998). Because of its signaling complexity, upstream as well as downstream regulators of ERK cascade in response to DNA-damage stresses are still unclear. Because of its importance in cancer, the ERK pathway has been focus for almost 15 years with Ras, Raf, and MEK as the main target (Downward, 2003; Kohno and Pouyssegur, 2006). The most promising drug targeting Raf kinase is Sorafenib (BAY 43-9006), CI-1040, the first MEK inhibitor to enter clinical trials. Second hope is on PD0325901, a second generation MEK1/2 inhibitor, with improved pharmaceutical and pharmacological properties.

3.2.3.2 p38 Map Kinase

p38 MAPKs belong to mammalian stress-activated MAPK family. There are four p38 kinases $\alpha$, $\beta$, $\gamma$, and $\delta$. The p38 enzyme is the best characterized and is expressed in most cell types. The p38 kinases were first defined in a screen for drugs inhibiting tumor necrosis factor mediated inflammatory responses (Lee et. al., 1994). MAPK families play an important role in cytokine production and stress response. Recent reports have also demonstrated additional functions for p38 MAPKs. The mammalian p38 MAPK families are activated by cellular stress including UV irradiation, heat shock, high osmotic stress, lipopolysaccharide, protein synthesis inhibitors, proinflammatory cytokines such as IL-1 (Interleukin-1) and TNF-Alpha (Tumor Necrosis Factor-Alpha)) and certain mitogens (Dhillon et. al., 2007; Raman et. al., 2007). Following its activation, p38 translocates to the nucleus and directly or indirectly activates multiple downstream effector pathways to generate biologic responses. p38 MAPK downstream targets include several kinases, transcription factors and cytosolic proteins. Among the kinases that are activated by p38 MAPK are MAPKAPK2 (MAPK-Activated Protein Kinase-2), MAPKAPK3 (MAPK-Activated Protein Kinase-3), PRAK (p38-related/activated protein kinase), MSK1 (Mitogen- and Stress-Activated Protein Kinase-1) and MNK1/2 (MAP
kinase-interacting kinase 1/2). The kinase MAPKAPK2 is perhaps the best characterized p38 MAPK substrate. Together with MAPKAPK3, it belongs to the RSK family of serine-threonine kinases and was the first kinase substrate found to be activated by the p38MAPK. Several studies have highlighted the role of p38 activation in the induction of cell-cycle checkpoints following DNA damage (Wang et al., 2000; Bulavin et al., 2001; Manke et al., 2005). One study also documented the role of MEK6 and p38 in the activation of G2/M cell cycle checkpoints following ionizing radiation (Wang et al., 2000). Like other MAP kinase family members, p38 pathways are all molecular targets for drug development, and inhibitors of these important pathways will undoubtedly be one of the next group of drugs developed for the treatment of human diseases.

3.2.4 Akt kinase

Since the discovery that the Akt/protein kinase B (PKB) serine/threonine protein kinase is a target of phosphoinositide 3-kinase (Franke et al., 1995; Toker and Yoeli-Lerner, 2006) and Akt increases cell survival in a PI3K-dependent manner (Dudek et al., 1997), recent experimental evidence suggests, Akt plays an important role in the pathogenesis of degenerative diseases and cancer (Franke et al., 1995). It became rapidly evident that Akt signaling pathway could be an effective target for anti-cancer therapies. Akt controls a variety of cellular responses, and that the three Akt isoforms, Akt1 (PKBa), Akt2 (PKBh), and Akt3 (PKBg), are ubiquitously expressed in all cell types and tissues, Akt regulates both growth and survival mechanisms in normal as well as cancer cells by phosphorylating a large number of substrates. The phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B, PKB) signaling pathway plays a critical role in cell growth and survival (Marte and Downward, 1997). Many cell surface receptors induce the production of second messengers that activate
phosphoinositide 3-kinase (PI3K). Akt is located downstream of PI3K
and therefore, functions as part of a wortmannin-sensitive signaling
pathway. Numerous Akt substrates have been reported in recent
years. First identified was the Bcl-2 family member, Bad, a pro-
apoptotic protein that binds and inhibits anti-apoptotic Bcl-2 molecules
when Bad is not phosphorylated (Datta et. al., 1997; Downward, 1999).
Another notable substrate of Akt is the death protease caspase-9
(Brunet et. al., 1999). Phosphorylation of caspase-9 decreases
apoptosis by directly inhibiting the protease activity. Further the
downstream targets of Akt also include eNOS (Nitric Oxide Synthase),
mTOR (Mammalian Target of Rapamycin), IKK (I-KappaB Kinase), NF-
KappaB (Nuclear Factor-KappaB), MDM2 (Mouse Double Minute-2),
p21(CIP1) (Cyclin Dependent Kinase Inhibitor-p21), p27(KIP1) (Cyclin
Dependent Kinase Inhibitor-p27), Chk1 (Cell Cycle Checkpoint Kinase-
1), Raf1 (v-Raf1 Murine Leukemia Viral Oncogene Homolog-1) (Toker
and Yoeli-Lerner, 2006; Zhou et. al., 2001).
Recently, constitutively active PI3K/Akt signaling has been firmly
established as a major determinant for cell growth and survival in an
array of cancers. Blocking the constitutively active PI3K/AKT signaling
pathway provides a new strategy for targeted cancer therapy. Thus,
inhibitors of this signaling pathway would be potential anti-cancer
agents, particularly for cancer cells whose survival and growth are
dependent on constitutively active PI3K/Akt signaling (Brunet et. al.,
1999).

3.2.5 NF-κB; Mechanism of action

The eukaryotic transcription factor NF-κB was identified as a protein
that is bound to a specific decameric DNA sequence (GGG ACT TTC
C), within the intronic enhancer of the immunoglobulin kappa light
chain in mature B- and plasma cells but not pre B-cells (Sen and
Baltimore, 1986). NF-κB's transcription factors are induced in response
to many signals that leads to cell growth, differentiation, inflammatory response, regulation of apoptosis and neoplastic transformation (Pahl, 1999). Today, the study of NF-κB signaling is essentially an industry, complete with website (www.NF-κB.org), many patents and more than 25,000 publications. NF-κB has been detected in most cell types, and

<table>
<thead>
<tr>
<th>NF-κB subunit</th>
<th>Size</th>
<th>Special Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>p50</td>
<td>50 kDa</td>
<td>Synthesized as the precursor IκB protein p105; p50 does not possess TD.</td>
</tr>
<tr>
<td>p52</td>
<td>52 kDa</td>
<td>Synthesized as the precursor IκB protein p100; p52 does not possess TD.</td>
</tr>
<tr>
<td>p65</td>
<td>65 kDa</td>
<td></td>
</tr>
<tr>
<td>(RelA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RelB</td>
<td>68 kDa</td>
<td>Does not possess PKA phosphorylation site, but an additional N-terminal leucin-zipper-like region which affects its transcriptional activity.</td>
</tr>
<tr>
<td>c-Rel</td>
<td>69 kDa</td>
<td></td>
</tr>
</tbody>
</table>

Table. 2: Indicating different important subunits of NF-κB transcription factor

specific NF-κB binding sites have been identified in promoters and enhancers of a big number of inducible genes.

3.2.5.1 Rel/NF-κB proteins

The transcription factor NF-κB consists out of homo- or heterodimers of different subunits. The subunits are members of a family of structurally related proteins (Rel/NF-κB proteins). Five different Rel proteins (also called Rel/NF-κB proteins) namely p50, p52, p65, RelB, and c-Rel have been identified so far (Table. 2). Only RelA (p65), RelB
and c-Rel contain potent transactivation domains (TDs) within sequences C-terminal to the RHD. The TDs consist of abundant serine, acidic and hydrophobic amino acids which are essential for transactivation activity. In contrast, p50 and p52 do not possess TDs, and therefore can not act as transcriptional activators by themselves. Homo- or heterodimers of p50 and p52 were even reported to repress NF-κB site-dependent transcription in vivo (Lernbecher et. al., 1993), possibly by competing with other transcriptionally active dimers (e.g. p50/RelA) for DNA binding. Interestingly, κB-site-dependent transcriptional activation by p50/p50 has been demonstrated in vitro (Lin et. al., 1995).

3.2.5.3 Functional regulation of NF-κB action

In most cell type Rel/NF-κB complex exist in inactive form in cytoplasm. Here it is found to be bound with several related IκB inhibitor proteins including IκBα, IκBβ, IκBε, IκBγ, p105, p100, and Bcl-3. Among variety of pathways followed by NF-kB for its activation, most common occur via induced phosphorylation of IκB at two N-terminal Ser residue by a large IκB kinase complex (IKK), and phosphorylation of IκB leads to its degradation by proteasome. The free Rel/ NF-κB complex can then enter the nucleus and bind to DNA. (Margaret, 1999). Moreover there are many reports indicating that many tumor cells have constitutive NF-κB site binding and transactivation activity (Rayet and Gelinhas 1999).

3.2.5.4 Rel/NF-κB inducers and NF-κB-dependent genes

Rel/NF-κB transcriptional factors are induced in response to many signals that lead to cell growth, differentiation, inflammatory response and regulation of apoptosis (Pahl, 1999). Some examples are the response to and induction of IL-2, the induction of TAP1 and MHC molecules by NF-κB, and many aspects of the inflammatory response, e.g. induction of IL-1 (alpha and beta), TNF-alpha and leukocyte
adhesion molecules (VCAM-1 and ICAM-1). Moreover, NF-κB is involved in many aspects of cell growth, differentiation and proliferation via the induction of certain growth and transcription factors (e.g. c-myc, Ras and p53). Some other important NF-κB target genes involved in regulation of apoptosis are; Fas ligand, Fas receptor, Bcl-2 family proteins, etc. (Barkett and Gilmore 1999). NF-κB itself is induced by stimuli such as pro-inflammatory cytokines and bacterial toxins (e.g. LPS, exotoxin B) and a number of viruses/viral products (e.g. HIV-1, HTLV-I, HBV, EBV, Herpes simplex) as well as pro-apoptotic and necrotic stimuli (oxygen free radicals, UV light, gamma-irradiation).

3.2.5.5 Inhibitors of NF-κB

NF-κB dimers are formed by non-covalent interactions and are normally present in the cytosol of cells with a class of inhibitor proteins, called IκBs. Till today, seven IκBs have been identified: IκB-alpha, IκB-beta, IκB-gamma, IκB-epsilon, Bcl-3, p100 and p105. All signals that induce NF-κB activity resulted as phosphorylation of IκBs, their dissociation and subsequent degradation, allowing NF-κB proteins to enter the nucleus and induce target gene expression. This ubiquitination and subsequent degradation is mediated by the multicatalytic ATP-dependent 26S proteasome complex. Two kinases have been identified, that are responsible for this modification of the IκBs: IKK-alpha and IKK-beta. Both kinases were identified to be members of a high molecular complex which also contains IKK-gamma (also called NEMO, IKKAP) and IKAP. IKK-alpha and IKK-beta share significant sequence homology and contain identical structural domains. By their leucine-zipper domains they form heterodimers, in vivo (May and Gosh 1999). In summary NF-κB is a collective name for the complexes formed by multi gene NF-κB Rel family. In mammalian cells there are five NF-κB subunits Rel A (p65), Rel B, c-Rel, p105-p50 (NF-κB1) and p100-p52. In unstimulated normal cells,
NF-κB subunits are held in an inactive cytoplasmic form. NF-κB dimers are sequestered in the cytosol of unstimulated cells via non-covalent interactions with a class of inhibitor proteins, called IκBs. Signals that induce NF-κB activity cause the phosphorylation of IκBs, their dissociation and subsequent degradation, allowing NF-κB proteins to enter the nucleus and induce gene expression (Karin et. al., 2002; Wang et. al., 2000). NF-κB signaling is involved in regulating many aspects of cellular activity, in stress, injury and especially in pathways of the immune response. Additionally, constitutive NF-κB activity, detected commonly in many solid tumors is connected with multiple aspects of tumorigenesis and also for resistance to chemotherapy or radiation (Wang et. al., 1999). Thus, inhibitors of active NF-κB, may function as better cancer preventive compound and generate need to be explored (Fig. 7).
3.2.6 p53

Since the discovery in 1979, p53 is considered as one of the most important molecule in cancer cell biology. Following more than 25
years of extensive research, we now know that p53 is a member of family of proteins that has three members: p53, p63, and p73. Mutations in the p53 gene are the most commonly detected alterations in human tumors. The frequency of p53 mutation varies among types of tumor. For most types, mutant p53 has been detected in 20-50% of the cases, but with some types as many as 80% of tumors harbor mutant p53 gene. All class of mutations (insertions, deletions, substitutions) has been found in p53 in human cancers, but point mutations leading to amino acid substitutions are the most prevalent. The stage at which p53 mutation occurs during tumorigenesis depends on the tumor type. p53 mutation seems to be an early event in development of cancer of breast, cervix, esophagus, lung and stomach (Horn and Vousden, 2007). p53 plays a central role in regulating cell fate. Level of p53 increases in response to various stresses, either genotoxic (DNA alterations induced by irradiation, UV, carcinogens, cytotoxic drugs) or not (hypoxia, nucleotide depletion, oncogene activation, microtubule disruption, loss of normal cell contacts. This increase in p53 is attributed mainly to an increase in p53 stability which is achieved through post transcriptional modifications (e.g., phosphorylation, acetylation etc.) and reduced interaction with the MDM2 protein that normally targets p53 for degradation via ubiquitin-mediated proteasome pathway. The protein may be viewed as a node for the stress signals, which are then transduced, mainly through the ability of p53 to act as a transcription factor. p53 exerts its anti-proliferative action by inducing reversible or irreversible (senescence) cell cycle arrest, or apoptosis. It may also enhance DNA repair and inhibit angiogenesis (Braithwaite, 2005; Lacroix et. al., 2006; Levesque and Eastman, 2007). As p53 is a transcriptional factor, it is able to both initiate and suppress gene expression following cellular stress. p53 is of course, able to elevate the expression of genes whose promoters contain p53-binding site. Pro-apoptotic genes in which a p53
responsive element has been reported include Bax, DR5/ER, Fas/Apo-1, Noxa, Apaf1, and PUMA etc. the product of these genes initiate apoptosis by number of mechanism; for example Bax, PUMA, Noxa localize to mitochondria and promote the loss of mitochondrial membrane potential which results in cytochrome-C release and apoptosis (Fig. 8). p53 can also induce apoptosis via transcriptionally independent pathways. Recently, there have been number of reports demonstrating a direct localization of p53 to the mitochondria following DNA damage or hypoxia, where p53 can interact directly with anti-apoptotic proteins such as Bcl2 and Bcl-XL.

3.2.6.1 Stabilization and activation of p53

p53 phosphorylation has been widely investigated. In most cases, it is associated with stabilization of p53 protein. p53 is phosphorylated at many sites including three N-terminal sites, Ser15, Thr18, and Ser20. When p53 get phosphorylated at these sites the interaction between p53 and its major negative regulator, MDM2, is diminished. In addition, it promotes the binding of the acetyltransferase p300, which finally leads to increase in the level and stability of p53. Ser15 may be phosphorylated by UV (via ataxia-telangiectasia and Rad3-related; ATR) or IR (via ataxia-telangiectasia mutated; ATM). These stresses also lead to Ser20 phosphorylation, through the action of cell cycle checkpoint kinase 2 (CHK2) and CHK1 respectively. In fact, besides IR and UV, almost all stresses have been shown to induce Ser15 phosphorylation, which is thought to nucleate a series of subsequent p53 posttranslational modifications (Lacroix et. al., 2006; Meek, 2004).

3.2.6.2 Acetylation

Acetylation has been shown to augment p53 DNA binding and to stimulate p53-mediated transactivation of target genes through the recruitment of coactivators. Acetylation also contributes to p53
stabilization by impairing ubiquitination of the acetylated residues. Intriguingly, while all evidences so far indicate that acetylation positively regulates p53 function (Brooks and Gu 2003), this modification seems also to regulate p53 subcellular localization, at least in part by activating its nuclear export.

3.2.6.3 Ubiquitination

In normal cells, degradation is the only mechanism that abrogates all functions of p53, and this appears to be accomplished, in part, by the ubiquitin-26S proteasome system (the other way is ubiquitin-independent). The highly conserved protein, ubiquitin, targets substrate proteins for degradation by the 26S proteasome to peptides.
Ubiquitin ligases realize the last step of ubiquitination. These enzymes exhibit a high level of target specificity.

### 3.2.6.4 Sumoylation

The p53 residue Lys386 may be sumoylated. SUMO (small ubiquitin-related modifier) is a ubiquitin related protein that covalently binds to other proteins using a mechanism analogous to, but distinct from, ubiquitin. In contrast to ubiquitination, sumoylation is not involved in protein degradation. Sumoylation affects target protein function by altering sub-cellular localization of the protein or by antagonizing other modifications (for example ubiquitination at the same acceptor site). Sumoylation most frequently correlates with decreased transcriptional activity and thus repression of target genes.

### 3.2.7 Bcl-2 Family

Bcl-2 was the first identified cellular protein that functions as an oncogene by blocking apoptotic cell death. In C. elegans, ced-3 and ced-4 were identified as genes essential for programmed cell death, while ced-9 was found to be a regulator of cell death by preventing apoptosis. The first mammalian homolog for ced-3 was described in 1988 as Bcl-2 which is involved in B-cell lymphomas (Vaux and Adams, 1988). The Bcl-2 family is the best characterized protein family involved in the regulation of apoptotic cell death. Interestingly this family consists of both anti-apoptotic and pro-apoptotic members. Thus, the Bcl-2 family of proteins acts as a critical life-death decision point within the common pathway of apoptosis (Tsujimoto, 1998).

#### 3.2.7.1 The members of the family

Main Bcl2 family members of can be divided into three subfamilies:

1. Bcl-2 subfamily (pro-survival): Bcl-2, Bcl-XL, Bcl-w and Mcl-1
2. Bax subfamily (pro-apoptotic): Bax, Bak and Bok
3. BH3 subfamily (pro-apoptotic): Bad, Bid, Bik, Blk, Hrk, BNIP3 and BimL.
The \textit{bcl-2} gene codes for a 25 kDa protein. The C terminal 21 amino acids encode a group of hydrophobic amino acids. This stretch is mainly responsible for membrane docking: Bcl-2 present on the cytoplasmic face of the mitochondrial outer membrane, the nuclear envelop, and the endoplasmic reticulum. Deletion of the C terminus does not abrogate Bcl-2 survival function. Most Bcl-2 homologs have this hydrophobic C terminal domain, though they not necessarily are located on membranes but are cytosolic (e.g. Bax). Many homologs of Bcl-2 have been identified and it became apparent that the Bcl-2 family can be identified by the presence of conserved motifs known as Bcl-2 homology domains (BH1 to BH4). While Bcl-2 and its most similar pro survival homologs Bcl-XL and Bcl-w contain all four BH domains, the other pro-survival members contain at least BH1 and BH2. While the members of the Bax subfamily contain BH1, BH2 and BH3, and resemble Bcl-2 fairly closely, the seven mammalian members of the BH3 subfamily possess only the central short (9 - 16 residue) BH3 domain and are unrelated to any known protein (Fig. 9). The BH3

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{Schematic representation of the structural features of anti-apoptotic and pro-apoptotic Bcl-2 proteins. Adopted from Burlacu et. al., 2003.}
\end{figure}

subfamily members may well represent the physiological antagonists of the pro-survival proteins, since programmed cell death in \textit{C. elegans} requires EGL-1 (the one non-mammalian BH3 family member) and Bid
was reported to link caspase-8 activity to cytochrome c release (Adams and Cory 1998).

3.2.7.2 Mechanisms of action

Bcl-2 family member play their important role by following ways (Fig. 11).

3.2.7.2.1 Bcl-2 and Bcl-XL bind to Apaf-1

Caspase-9 is a central part of apoptosis by mitochondria. The BH4 domain of Bcl-2 and Bcl-XL can bind to the C terminal part of Apaf-1

![Fig. 10: Speculative model for differential regulation of cell death by Bcl-2 proteins.](image)

(to the CED-4 like part and the WD-40 domain), thus inhibiting the association of Caspase-9 with Apaf-1 (Hu et. al., 1998; Huang et. al., 1998; Pan et. al., 1998). This process seems to be conserved from nematodes to humans since in *C. elegans* CED-9 binds to CED-4, preventing it from binding and activating CED-3 (Fig. 10).
3.2.7.2.2 Bcl-2 family members regulate Cytochrome c release

The pro-survival proteins also seem to maintain organelle integrity since Bcl-2 directly or indirectly prevents the release of cytochrome c from mitochondria (Yang et. al, 1997). On the other hand, the pro-apoptotic BH3 subfamily member BID was reported to mediate the

Fig. 11: Schematic model of apoptosis signaling pathways involving proapoptotic Bcl-2 members. Activation of death receptors by their trimerization leads to cleavage of Bid, generating a p15 tBid that translocates to the mitochondria. Growth factor deprivation induces Bax dimerization and translocation to the mitochondria membrane. The same kind of stimulus can inhibit the kinases that phosphorilate Bad and lead to Bad dephosphorylation. Bad translocate in the mitochondria gets associated with Bcl-XL Bcl-2. Each of these proapoptotic proteins, once translocated in the mitochondria can induce the release of cytochrome c, which will activates the caspase cascade. Adopted from Burlacu, 2003.
release of cytochrome c (without evoking mitochondrial swelling and permeability transition). Interestingly, BID is able to bind to pro-apoptotic members of the Bcl-2 family (e.g. Bax) as well as to pro-survival members Bcl-2 and Bcl-XL (Luo et al., 1998).

3.2.7.2.3 Bax may be involved in Caspase-Independent Death

Bax exists in an inactive conformation, and a death stimulus induces a modification resulting in (i) the exposure of its amino terminus, (ii) the orientation of the BH3 domain hydrophobic surface to increase the accessibility, (iii) the dimerization and (iv) the translocation to the mitochondria membrane.

3.3 Cancer treatment

3.3.1 Surgery

This is the oldest form of treatment for cancer. Surgery is performed in order to remove the cancerous tumor as well as some of the surrounding tissue and lymph nodes near it.

3.3.2 Radiation therapy

Radiation therapy (also called radiotherapy) uses high-energy particles or waves, such as x-rays or gamma rays, to destroy cancer cells.

3.3.3 Chemotherapy

This is the use of medicines to treat cancer. Systematic chemotherapy uses anticancer drugs that enter the bloodstream and reach all areas of the body, making this treatment potentially useful for cancer that has spread.

3.3.3.1 Cancer chemotherapy

Normally, cells grow and die in a controlled way. Cancer cells keep growing without control. Chemotherapy is drug therapy that can stop these cells from multiplying. However, it can also harm healthy cells, which causes side effects, which mainly depend on the type and dose.
of chemotherapy. Healthy cells usually recover after chemotherapy, so most side effects gradually go away. Chemotherapy can be categorized as follow:

**Combined modality chemotherapy** is the use of drugs with other cancer treatments, such as radiation therapy or surgery. Most cancers are now treated in this way. Combination chemotherapy is a similar practice which involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimizing the chances of resistance developing to any one agent.

**Neoadjuvant chemotherapy** (preoperative treatment): in this class of combination therapy, first line of chemotherapy is aimed for shrinking the primary tumor, thereby rendering local therapy (surgery or radiotherapy) less destructive or more effective.

**Adjuvant chemotherapy** (postoperative treatment) can be used when there is very less chance of cancer presence, but there is risk of recurrence. This can help reduce chances of resistance developing if the tumor does develop. It is also useful in killing any cancerous cells which have spread to other parts of the body. This is often effective as the newly growing tumors are fast-dividing, and therefore, very susceptible. All chemotherapy regimens require that the patient be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is required.
4.0 Chemotherapeutic Drugs

4.1 5-Fluorouracil

5-Fluorouracil (5-FU) is useful in the treatment of carcinomas of breast, ovary, esophagus, colon and skin. 5-FU initiates apoptosis by targeting thymidylate synthase (TS) and by direct incorporation of 5-FU metabolites into DNA. 5-Fluorouridine monophosphate which is incorporated into RNA inhibiting its function. 5-Fluorouracil, 15 mg/kg, by intravenous injection daily for 5 days is a typical regimen, but there are wide varieties of dosage schedules. It is also given by hepatic artery infusion in patients with hepatic metastases to produce high hepatic level without correspondingly high systemic levels. Dose modification is required with liver dysfunction. 5-Fluorouracil activation occurs in target cells and thus defines the specificity of the drug. Inactivation occurs mainly in liver. 5-Fluorouracil is initially reduced and then cleared non-enzymatically to inactive forms that are excreted in the urine. It is cleared from plasma with a t1/2 of 10-20 min. About 20% is excreted unchanged in the urine and the remainder is metabolized. (Longley et. al., 2003)

4.2 Carboplatin

An anticancer drug that belongs to the family of drugs called platinum compounds.
Carboplatin (cis-diammine-1,1- cyclobutane- dicarboxylatoplatinum (II)) (Carb)) is a platinum compound, which acts as an alkylating agent. It forms inter and intrastrand cross-links and interstrand adducts with DNA by reacting with N⁷ guanine residue. Alkylation at N⁷ position of guanine causes depurination and leads to strand breakage. Platinum analogues have become the mainstay of treatment for many tumors including ovarian cancer, lung cancer (both non-small-cell and small-cell), germ cell tumors, head and neck cancer, bladder cancer, breast cancer and gastric cancer. Carb is the only cisplatin (cis-diammine-dichloroplatinum(II)) derivative currently available for the treatment of cancer worldwide. The higher stability of the carboxylate ligand compared to the coordinated chloride in cisplatin results in a reduced reactivity of the molecule (Boulikas and Vougiouka, 2003).

4.3 Vinblastine

Vinblastine (Vin) is the salt of a naturally-occurring vinca alkaloid obtained from the flowering herb periwinkle. Vinca alkaloids act by preventing the polymerization of tubulin to form microtubules, as well as inducing depolymerization of formed tubules and works by stopping the cancer cells from separating into two new cells. It is a chemical analogue of vincristine. Vin may also interfere with amino acid, cyclic AMP, and glutathione metabolism; calmodulin-dependent Ca⁺⁺ transport ATPase activity; cellular respiration; and nucleic acid and lipid biosynthesis. Vinca alkaloids are cell cycle phase-specific for M phase and S phase. Vin exerts some immunosuppressive activity.
Cross-resistance with vincristine has been reported. Vin is commonly used to treat some types of lymphomas, Hodgkin’s disease, testicular cancer, breast cancer, choriocarcinoma, mycosis fungoides, Kaposi’s sarcoma, and Letterer-Siwe disease (David 1964).

4.4 Side Effects of Chemotherapy

Chemotherapy drugs are good at killing cancer cells, but they can damage some healthy cells as well. The damage to normal cells induces side effects in cancer patients. Most commonly seen side effects of chemotherapy are fatigue, weak immunity, infection, insomnia, nausea and vomiting and / or pain.

4.4.1 Fatigue

Fatigue is a common side effect of chemotherapy and is characterized by a general tiredness or irresistible lack of energy. It may be associated with an increased need for rest, an inability to regain energy with rest, difficulty concentrating, or a disinterest in events. Chemotherapy-related fatigue is caused by anemia, or a low white blood cell count. Such fatigue may be acute or chronic.
4.4.2 Weak Immunity

Despite the fact that chemotherapy treatment destroys cancer cells it also destroys cancer patients’ healthy cells resulting in the side effects such as weakened immune system, low white blood cell counts and fatigue. Some drugs are given to boost the immune system and increase the body’s white blood cells.

4.4.3 Infection

A powerful chemotherapy agent kills cancer cells, but it is also harmful to rapidly dividing healthy cells. Most of the common side effects of chemotherapy are caused by this type of damage. Human body normally keeps producing a certain number of white blood cells, which are involved to the body in fighting infections. Unfortunately, white blood cells (WBC) are killed along with the cancer cells. The WBC count decreases too rapidly to be replaced during the chemotherapy treatment, so patient’s body is at risk for simple infections that could become very serious.

4.4.4 Insomnia

This problem is commonly seen in most of the cancer patients. The term “Insomnia” is generally used for inability to obtain sufficient normal sleep and it keep patients to feel tired and sleepy throughout the day. Therefore patients on chemotherapy can end up with difficulty in sleeping at night. Sometimes sleep problems exist because of other medications prescribed to combat side effects of the chemotherapy.

4.4.5 Nausea, vomiting and pain

Nausea and vomiting are frequent side effects of chemotherapy and radiation therapy. Nausea is feeling queasy or sick to your stomach. Vomiting is emptying your stomach by throwing up. Pain may also be a side effect of cancer or cancer treatment. If not adequately managed, pain may have a tremendous effect on quality of life. Modern medicine can help control moderate to severe pain. Patients also have other
methods of controlling it, including exercise, acupuncture, massage, and taking natural herbal medicine. There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.
Objective of the study

Side effects associated with high-dose regimen and the lack of specificity towards their respective molecular targets are two major problems of cancer chemotherapeutic drugs. The objective of the present study is to offer a better and more targeted approach towards cancer treatment with chemotherapeutic drugs. Comprehensive understanding of the proteins and signaling pathways, involved in cytotoxic action of a particular drug, may provide the necessary information to improve existing therapies for individual tumor types. Present study aims to identify some compounds/drugs, which show additive or synergistic cytotoxic effects or enhance the drug uptake in combination with other conventional cytotoxic drugs to facilitate reduction of drug dosage. In addition to investigate the functions of some important regulatory proteins, present study also aims to explore novel targets of drugs and their involvement in the induction of cell death initiated by chemotherapeutic drugs having different mode of action.