Introduction

Cancer Overview: Cancer is a rapid uncontrolled proliferation of transformed cells which are able to invade or spread to other parts of the body [1]. The difference between the cancer cells and their normal counterpart is the loss of growth control mechanisms. It is predicted that a substantive increase to 19.3 million new cancer cases per year due to growth and ageing of the global population. Cancer can be caused by exogenous (e.g., UV radiation and viruses) and endogenous (e.g., genetic predisposition, immunodeficiency and chronic inflammation) factors [2]. These factors induce changes in the genome of the cells, which are the main reason for cancer. Cancer is caused by modifications leading to activation of oncogenes, thereby contributing new properties to the cells, such as proliferation, enhanced cell division and inhibition of programmed cell death.

Additionally, tumour suppressor genes may be inactivated, which disturb the normal cell functions such as control of cell division, cell adhesion [3]. All these properties of cancer cells are due to abnormalities in multiple cell regulatory systems and signaling cascades [4].

People have in their minds that cancer is very scariest diseases. Common people thought that it is very uncurable and untreatable diseases. Though it is undoubtedly a serious and potent life-threatening disease, there exists misconception regarding its cure in people's mind. Treatment varies depending upon the type cancer and how far it spread to other part of the body. Today, cancer is eliminated partially without impacting on patient's life.

The three most common treatments are:

Radiotherapy: It uses high doses of radiation to inhibit the cancer cells from multiplying and shrink tumors.

Chemotherapy: It involves the use of anti-cancer medicines to stop cells from multiplying. There are different types of drugs used for chemotherapy.

Surgery: It is possibly to remove a malignant tumor.

Recent treatments for cancer include:

Stem cell transplant: Only established therapy using stem cells is hematopoietic stem cell transplantation in blood cancer.

Hormone therapy: The therapy uses medicines to block the effects of hormones promoting cancer and is used in case of cancers of breast, prostate etc.

Immunotherapy: This therapy boost affected the immune system so that immune system kills the cancer cells efficiently. This immunotherapy involves the antibodies aiming to destroy the cancer cells. Vaccine is used for the stimulation one's own immune system to make antibodies against cancer cells.

Gene therapy: This therapy involves silencing, repairing or replacing the abnormal genes responsible for promoting the disease.

Types of Cancer:

Cancer may be classified by cells type and origin of Tumor. These include carcinoma, sarcoma, lymphoma, myeloma and leukemia.

Carcinoma: It refers to the malignant neoplasm of epithelial origin. Carcinomas are divided into two major subtypes: adenocarcinoma which develops in an organ or gland, and squamous cell carcinoma, which originates in the squamous epithelium. Carcinoma includes mainly prostate, Breast, lung, Pancreas and colon.

Sarcoma: This cancer arising from connective tissue (Example: Bone, cartilage, fat, nerve). It develops from the cells originating in mesenchymal cells. Some examples include osteosarcoma (bone), fibrosarcoma (fibrous tissue), angiosarcoma (blood vessels) and glioma (neurogenic connective tissue found in the brain).

Lymphoma: Lymphomas develop in the glands or nodes of the lymphatic system, a network of vessels, nodes, and organs (specifically the spleen, tonsils, and thymus) that purify bodily fluids and produce infection-fighting white blood cells, or lymphocytes. The lymphomas are Hodgkin lymphoma and non-Hodgkin lymphoma.

Myeloma: Itis a cancer of plasma cells, a type of white blood cell that normally produces antibodies**.**

Leukemia: In leukemia**,** overproductionof immature WBC is produced in bone marrow [5] This immature WBC do not function properly. So, patients are often prone to infection. Leukemia occurs most often in adults older than 55, but it is also the most common cancer in children younger than 15.

Types of Leukemia:

Leukemia is grouped according to the rapidness of disease progression as well as by the cell lineage involved.

Acute Leukemia and Chronic Leukemia:

In acute Leukemia, a rapid onset and progression of malignancy is occurred. It is characterized by the proliferation of immature leukemic cells which are known as blast cells.

In Chronic leukemia, this disease develops and progress over long period of time. It is characterized by the increase in the matured leukemic cells which involve white blood cells.

Lymphoid and Myeloid Lineage in Leukemia:

In Lymphoid Leukemia Cells involved are lymphocytes. Acute leukemia involves immature lymphoblasts while chronic leukemia involves mature lymphocytes.

In Myeloid Leukemia Cells involved are myelogenous or non-lymphoid leukocytes. Acute leukemia involves immature myeloblasts while chronic leukemia involves mature myeloid cells.

The four most common types of leukemia are:

I) Acute lymphocytic leukemia.

II) Acute myeloid leukemia.

III) Chronic lymphocytic leukemia.

IV) Chronic myeloid leukemia.

Acute lymphocytic leukemia: It is a cancer of lymphoid lineage of blood cells. It is a characterized by the development of large number of immature lymphoblast. Normal lymphoblasts develop mature B cells and T cells also called lymphocyte. In ALL, both the normal development of some lymphocytes and the control over the number of lymphoid cells become defective. In ALL, single lymphoblast gains many mutations to the genes that could affect blood cell development and proliferation. In childhood ALL, some of these genes are inherited. These genes, in turn, increase the risk that more mutations to occur in developing lymphoid cells. KMT2A (formerly *MLL*) gene rearrangements are most commonly occurred in embryo. These rearrangements result in increased expression of blood cell development genes by promoting gene transcription. In childhood ALL, one more mutation is found beside KMT2A rearrangement [6].

Chronic lymphocytic leukemia: In this cancer, bone marrow makes too many mature lymphocytes. The cause of CLL is exposure to certain chemicals like Agent Orange and long-

term exposure to some pesticides. Males are more likely than females to get CLL. CLL is more common in people of North American or European decent than people of Asian descent. B cells Chronic lymphocytic leukemia is recognized morphologically homogenous disease of mature and resting B lymphocytes. CLL cells are recognized by expression of cell surface antigens CD5, CD23 and CD27 and low-level surface Immunoglobulin that are not pattern of normal B cells. CLL cases are heterogeneous. First, the rearrange of Ig variable region (IgV) of CLL can be mutated either somatically or unmutated, implying that the precursor cells may be originated from either T cells dependant or T cells independent response. IgV somatic mutation is correlated with clinical course. So, somatically mutated CLL shows better prognosis. Second, CLL shows differential expression of CD38 cell surface antigen. Third, CLL exhibits genetic lesion, genetic alternation, and mostly chromosomal deletions. In CLL, B cells do not function properly and crowd out the healthy blood cells. More infection is seen in CLL patients [7].

Acute myeloid leukemia:

Acute Myeloid Leukemia (AML), represents biologically and clinically heterogeneous group of disease. It is caused by a malignant genetic alteration in the hematopoietic stem cell. These genetic alterations can disrupt the normal function of differentiation of the cell or may cause excessive production of blast cells [8]. These blasts suppress usual haematopoiesis and invade different organs and tissues. Furthermore, proliferative benefits of leukemic stem cells are inhibition of apoptosis and genetic alterations [9]. It leads to the accumulation of immature cells in bone marrow. Genomic instability, indefinite self-renewal, inhibition of differentiation and multiorgan dissemination are some of the properties of this leukemic cells. Many myeloid cells accumulate in the blood, that causes the blockage of blood vessels. In AML, patients primarily treated with chemotherapy result in inducing remission. Acute Myelogenous Leukemia (AML) accounts for approximately 30% of all leukemias in the Western world. The occurrence of AML is low as compared to other cancers in India and it increases with age.

Chronic Myeloid Leukemia: An Overview

CML is clonal myeloproliferative diseases. CML arises from neoplastic transformation of a pluripotent bone marrow stem cell that's why excessive growth of myeloid cells and their progenitors are occurred [10]. CML is most adult leukemia. 1.2 to 1.5 million

people are currently living with CML worldwide. The common symptoms are night sweat, Heat intolerance, urticaria, Gouty arthritis, left upper quadrant and left shoulder pain and hyper leukocyte syndrome etc.

The abnormal chromosomal rearrangement being linking with cancer was first demonstrated by Peter Nowell and David Hungerford in1960 [11]. Nowell and Hungerford in 1960 named the "minute chromosome" as the Philadelphia (Ph) chromosome which is associated with CML. In 1970, Rowley discovered the cytogenetic anatomy of Philadelphia chromosome by chromosome banding method. In 1973, Rowley proved that cytogenetic changes were drivers of leukemogenesis [12]. The molecular event behind this cytogenesis is that the part of BCR gene from chromosome 22 is fused with Abl portion of chromosome 9. The 'abnormal fusion' gene encodes the abnormal protein molecular weight p210 or p185. Because abl is a tyrosine kinase. So, abl add phosphate group to the tyrosine residue. BCR-ABL fusion gene encodes constitively active tyrosine kinase protein [13] [Fig1].

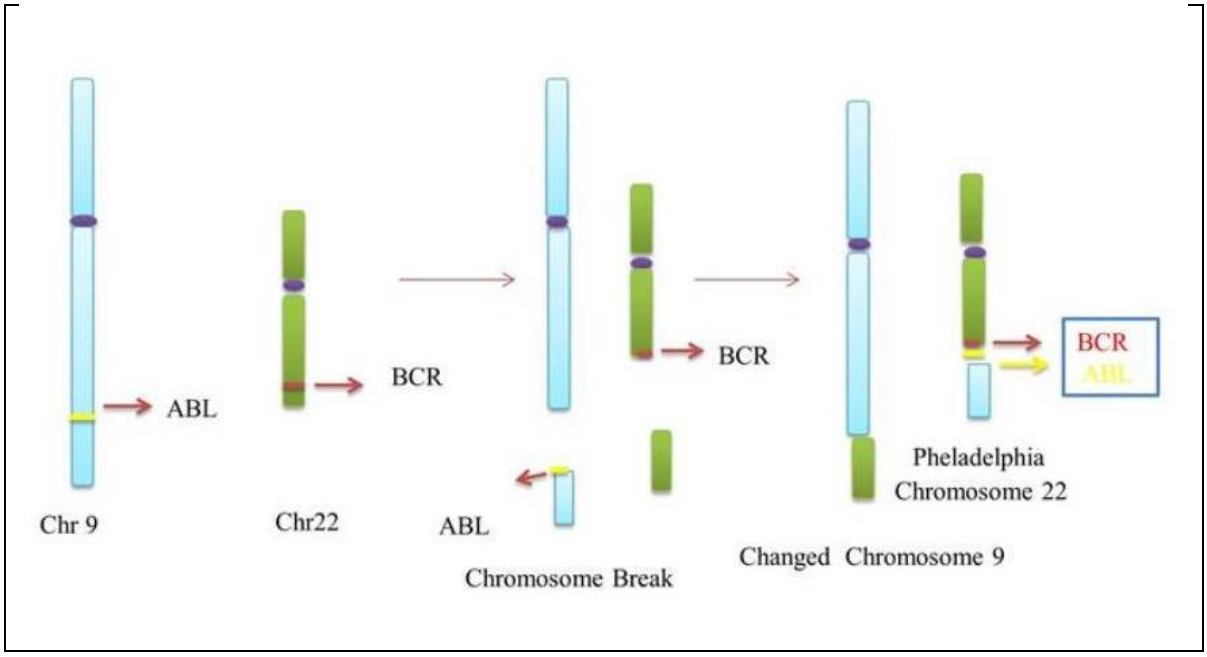

Fig 1. Diagram explains that Philadelphia chromosome is created by translocation between chromosome 9 and chromosome 22

Phases of CML: Three types of CML.

i) Chronic Phase

ii) Accelerated Phase

iii) Blast phase

I) **Chronic Phase:** In the chronic phase, fewer than 10 percent of the cells in the blood and bone marrow are blasts. In this phase, most patients are asymptomatic. Incidental leucocytosis/splenomegaly is seen. Bleeding and infectious complication are uncommon in this phase.

II) **Accelerated Phase:** The number of blast cells in the Bone marrow and /or peripheral blood is higher than normal. People with accelerated phase CML have an increased white blood cell count due to the accumulation of blast cells, a decrease in the number of platelets, Experience fatigue, new chromosome changes (mutations), worsened anemia, enlarged spleen, other complications.

III) **Blast phase:** The number of blast cells increases in both blood and bone marrow People with blast crisis phase CML have low red blood cell, platelet and neutrophil counts, the spread of blast cells outside the blood and/or the bone marrow and into other tissues [14].

Tyrosine Kinase Inhibitor Therapy for CML:

Tyrosine kinase inhibitors (TKIs) are drugs that target the abnormal BCR-ABL protein. BCR-ABL protein is encoded by BCR-ABL fusion gene. BCR-ABL fusion protein is Tyrosine Kinase protein. TKIs inhibit the BCR-ABL protein from sending the signals that cause the growth of abnormal cells. Three TKI drugs are approved as initial therapy for chronic phase CML. These are

1. Imatinib mesylate (Gleevec®)
2. Dasatinib (Sprycel®)
3. Nilotinib (Tasigna®).

Imatinib: First ever targeted therapy

Treatment mode of CML is changed dramatically over the decades. In the pre Imatinib era, the CML patient's symptoms are controlled for improving the quality of patient's live. In 1996, STI571 (Gleevec, Glivec, imatinib) was invented. Imatinib is 2-phenylaminopyrimidine derivative. Imatinib inhibits BCR-ABL tyrosine kinase. The constitutively active BCR-ABL tyrosine kinase transfers phosphate from ATP to tyrosine residues on the substrate that cause excessive growth signal to myeloid cells that cause chronic myeloid leukemia. Imatinib blocks

the binding of ATP to BCR-ABL tyrosine kinase. Thus, tyrosine kinase activity of BCR-ABL is inhibited. The tyrosine residue is not phosphorylated and excessive growth signal is prohibited [15]. Imatinib, now termed as 1st generation tyrosine kinase inhibitor (TKI), substantially and reduces the number of CML cells in the chronic phase.

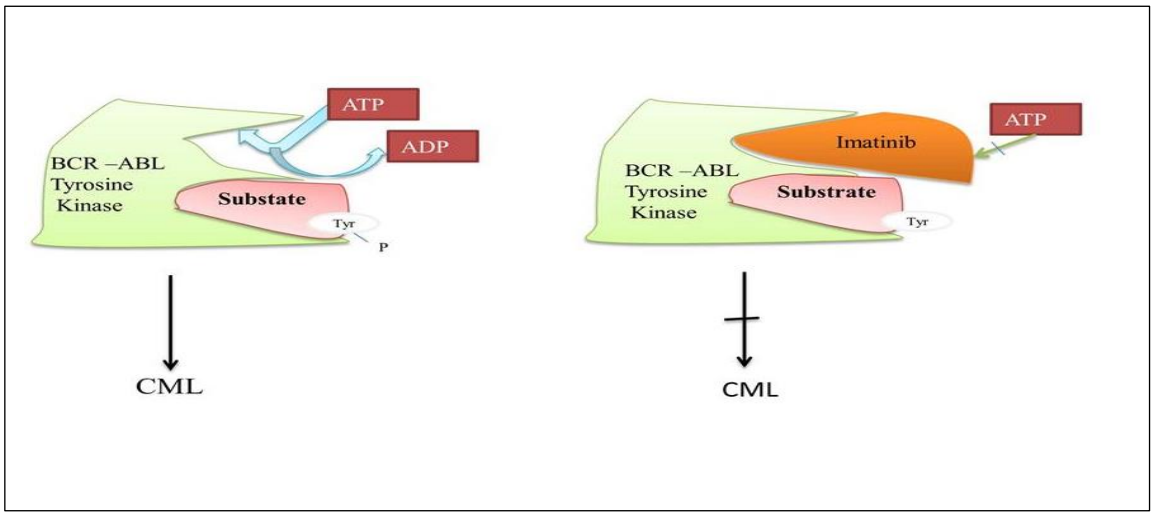

Fig 2: Mechanism of action of Imatinib. (Source: Druker, 2008)

Some CML patientsshow imatinib resistance or relapse after an initial response. The common mechanisms responsible for Imatinib resistance are that amplification of BCR-ABL1, overexpression of the multidrug-resistant P-glycoprotein (MDR-1) and the development of BCR-ABL1-independent pathways of signal transduction and the mutations in the ABL-kinase domain [16]. In diagnosed patients with chronic phase CML, the rate of resistance to imatinib at 4 years was up to 20%, increasing to 70% to 90% for patients in the accelerated/blastic phase. Resistance to imatinib led to the development of novel TK inhibitors such as Dasatinib (Sprycel), nilotinib (Tasigna) etc.

Dasatinib (Sprycel), nilotinib (Tasigna):

The second-generation tyrosine kinase inhibitors are Dasatinib (Sprycel), nilotinib (Tasigna). All are potent BCR- ABL tyrosine kinase inhibitor than Imatinib. All have been approved by FDA (US Food and Drug Administration) for the treatment for the Philadelphia chromosome positive (Ph1+). Dasatinib is a potent, small-molecule, second-generation, multitarget kinase inhibitor of BCR-ABL. It has greater activity against unmutated ABL kinase than imatinib. It binds to the ATP-binding site, but it extends in the opposite direction from imatinib and binds

the inactive and active conformation of the ABL kinase domain and requires fewer contact points with ABL, and has a greater affinity to the ABL kinase domain compared to imatinib [17]. Dasatinib is used for CML in chronic, blastic or accelerated phase that is resistantto imatinib. Nilotinib is second generation drug and it binds to and stabilizes the inactive conformation of the kinase domain of the Abl protein of the Bcr-Abl fusion protein. The Bcr-Abl-mediated proliferation of Philadelphia chromosome-positive (Ph+) of CML cells is inhibited [18].

Development of resistance to these tyrosine kinase inhibitors drugs is major problem for the treatment of patients with CML. So, alternative therapeutical approaches might prove effective treatment choice. TRAIL is Tumor Nacrosis Factor Related Apoptosis Inducing Ligands. TRAIL acts as promising anticancer drugs as TRAIL shows high cytotoxicity toward cancer cells, but low cytotoxicity toward normal cells. TRAIL is in phase 3 clinical trial. TRAIL induces extrinsic apoptotic pathway. If caspase 3, caspase 8 activation is blocked by IAPs (Inhibitor of apoptotic proteins), TRAIL mediated extrinsic apoptotic pathway is inhibited. So, we targeted IAPs proteins to enhance TRAIL mediated Extrinsic apoptotic pathway. Now, it is discussed about IAPs proteins and its role.

Inhibitor of apoptotic protein and its role:

IAPs belong to the large and heterogeneous group of antiapoptotic proteins. IAPs are overexpressed in lymphomia, leukemia. IAP proteins control the cell's decision to live or die. IAPs proteins may be considered as promising targets for therapeutical intervention. The first discovered cellular, non-viral, IAP is the mammalian gene NAIP, IAP family has rapidly expanded to seven other members: cIAP1, cIAP2 and XIAP, ILP2, BRUCE, survivin and livin [19]. As the name indicate that IAP suppress apoptosis induced by a variety of stimuli e.g., growth factor withdrawal, death receptor activation, ionizing radiation, viral infection, genotoxic damage and endoplasmic reticulum stress. Inhibitors of apoptosis (IAPs) has function in a wide range of cellular roles, from the promotion of cell-cycle progression to the inhibition of caspases. These diverse functions may be linked to the many different domains that are contained within different proteins in the family [20]. IAP proteins are composed of three structural domains are made of the Really Interesting New Gene (RING) domain, baculoviral IAP repeat (BIR) domain and Caspase Activating and Recruitment Domain (CARD). IAP family contains a BIR domain. BIR domain is a protein-protein interaction motif and is responsible for the binding of IAP proteins to caspases [21]. The RING domain harbors

E3 ubiquitin ligase activity. The RING domain is responsible for ubiquitination and proteasomal degradation of various substrates e.g., caspases, Smac and IAP proteins are degraded via auto- or heteroubiquitination [22]. The Caspase Activating and Recruitment Domain is protein-protein interaction domain. The CARD mediates auto- or heterodimerization with other Caspase Activating and Recruitment Domain-containing proteins [23]. Other unique protein domains are found within the IAP family, including leucine-rich repeat (LRR) and a nucleotide-binding and oligomerization domain (NOD) in NAIP (neuronal apoptosis inhibitory protein), a ubiquitin-conjugating (UBC) domain in Apollon. Apollon is also known as BIRC6[24].

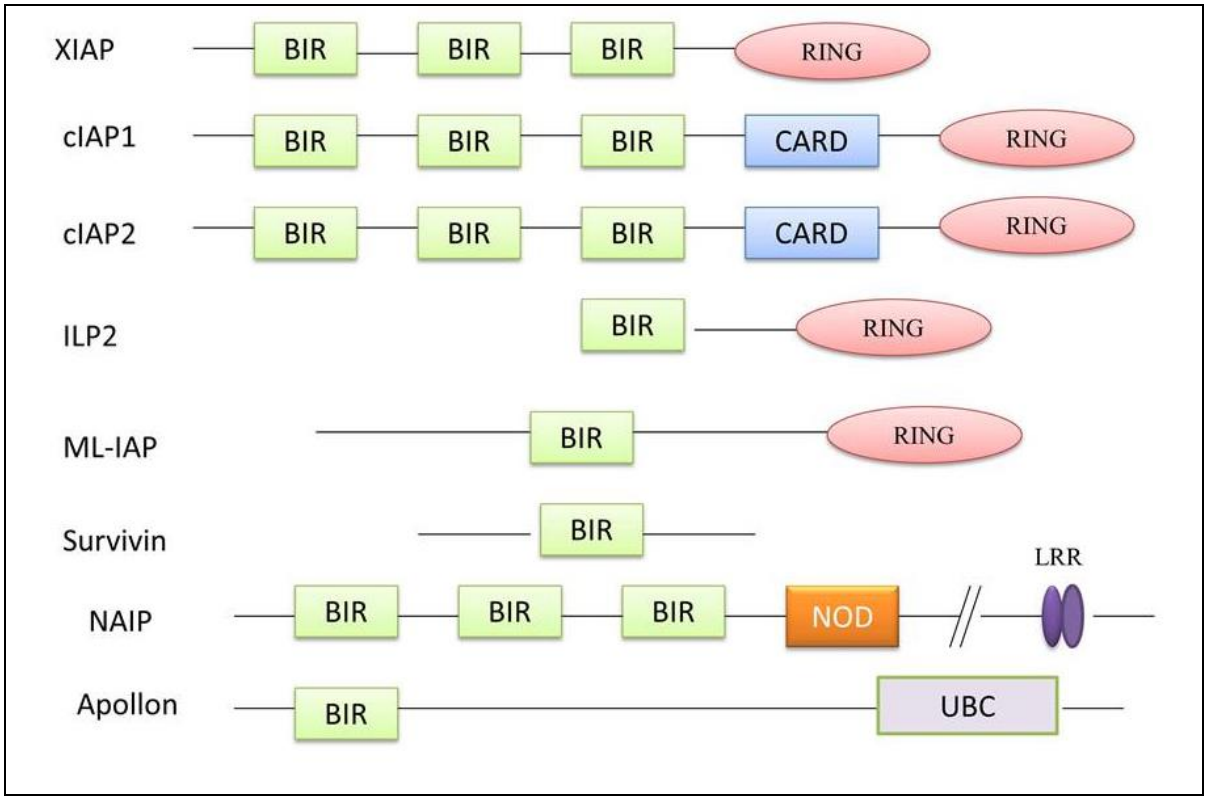

Fig 3: Structure of Different type of IAPs

Among the IAP family proteins, XIAPisone with the strongest antiapoptotic functions. XIAP inhibits the activity of caspase-3, -7 and -9 through its BIR2 and BIR3 domains. The E3 ligase activity of XIAP stimulates the proteasomal degradation of different types of proapoptotic proteins, including caspases and Smac [25] (**Figure 4**). XIAP stimulate nuclear factor-kappa B (NF-kB) activation that contributes to its antiapoptotic functions. cIAP1 and cIAP2 act as E3

ligases and promote canonical NF-kB activation. cIAP1 and cIAP2 promote canonical NF-kB activation [26]. How do cIAP1 and cIAP2 promote canonical NF-kB activation. Tumor necrosis factor (TNF) superfamily receptors that utilize TNF receptor associated factor 2 (TRAF2) as an adaptor. It can recruit cIAP1 or cIAP2, which can then positively modulate the classical pathway [27]. NF-kB dimers **(**eg. p50/RelA) are present in the cytoplasm and interacts with IkB. In response to the binding of a ligand to a TNF receptor (TNFR), TRAF are recruited to TNFR. The ubiquitination of receptor-interacting protein (RIP) kinase (RIP1/RIPK1) is mediated by the RING domain E3 ligase of cIAP1 and cIAP2. The IKK complex contain IKKa, IKKb and NEMO (IKKgamma). Then IKK is recruited and activated. The activated IKK complex phosphorylates the inhibitor of kB (IkB), leading to its degradation. NF-kB (p50/RelA) is released and translocate into the nucleus for transcriptional activation of targeted genes. In this way classical NF-kB pathway is activated [28]. By contrast, cIAP 1 and cIAP 2 inhibits the Alternative NF-kB pathway. cIAP1 and cIAP2, TRAF2 and TRAF3 promote the ubiquitination and degradation of NFkB- inducing kinase (NIK) [29]. Other side NIK is required for phosphorylation of p100 in the p100/RelB complex. Phosphorylated p100 is processed into p52, and NF-kB (that is, the p52/RelB dimer) translocates into the nucleus for transcriptional activation of targeted genes. cIAP1 and cIAP2 are the true E3 ligases that constitutively target NIKfor K48-linked polyubiquitination and proteasomal degradation, plays a central role in the regulation of alternative NF-kB pathways [30]. ML-IAPcontains a single N-terminal BIR domain and a RING domain at its C-terminus. ML-IAP block apoptosis by inhibiting caspases and by sequestering Smac. Two ML-IAP splice variants are present. e, g. ML-IAPa and ML-IAPb. ML-IAPa has additional 18 amino acids at the linker region between the BIR and RING domains. Caspases proteolytically cleave ML-IAP [31]. Survivin contains one single BIR domain and inhibits apoptosis by binding to Smac and also stabilizes XIAP protein [32]. It has main role in cell homeostasis: cell-cycle regulation and inhibition of apoptosis. Survivin is upregulated in cancer cells. Although it is possible that this upregulation is simply indicative of cancer cells cycling at a greater rate than the rest of the cell population, because survivin is regulated in a cell-cycle-dependent manner.The loss of the survivin protein resulting in arrest or mitotic catastrophe in cycling cells.Survivin binds to the inner centromere protein (INCENP) and AuroraB kinase during cytokinesis and promotes cell division.Survivin can bind to smac to its single BIR to inhibit cell death, removing the ability of Smac to inhibit XIAP [33].

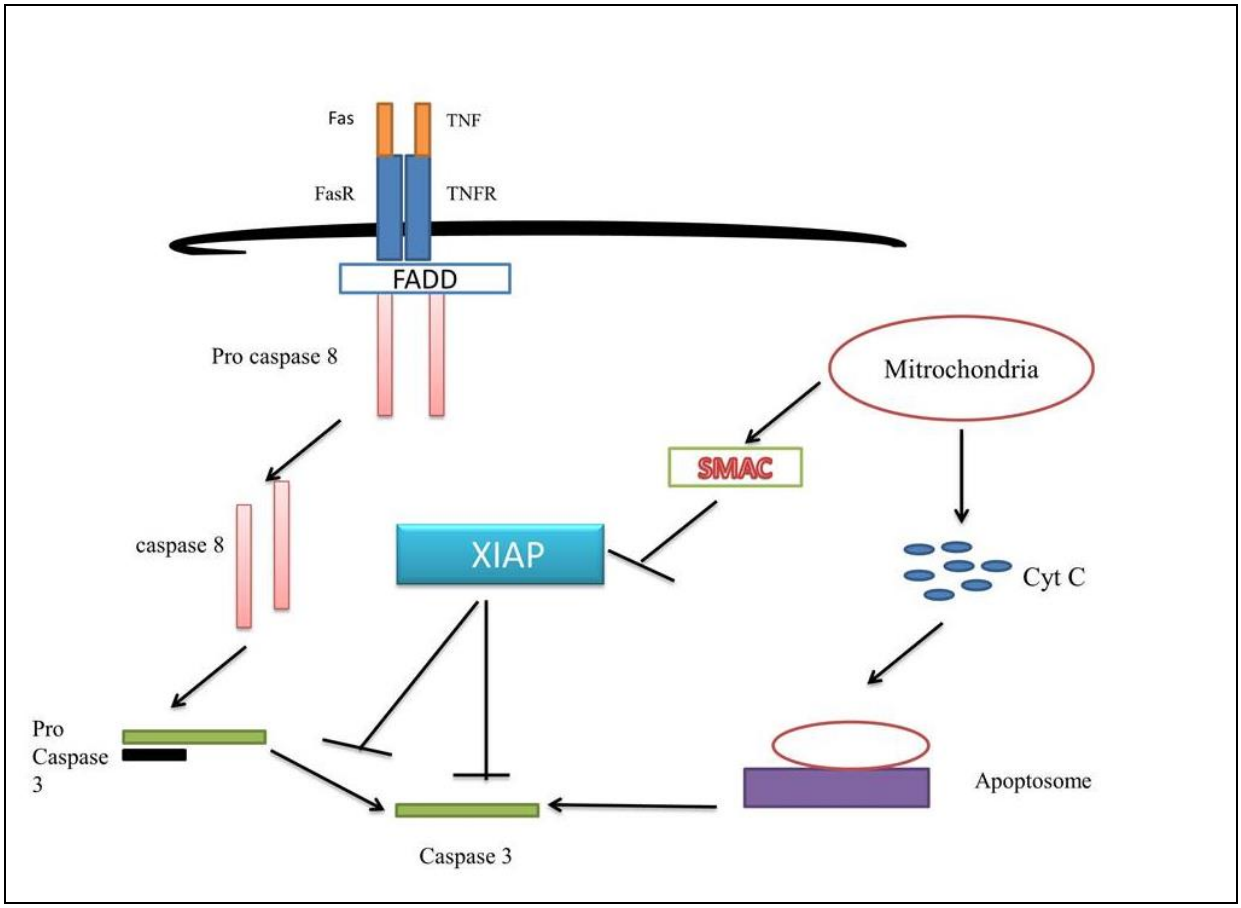

Fig 4: IAPs regulate apoptotic signalling pathway. The binding of death receptor ligandstotheir respective death receptor, caspase 8 is activated. Thereby caspase3 is activated in Extrinsic apoptotic Pathway. In intrinsic pathway, cytochrome c is and smac are released by mitochondrial outer membrane permeabilization. Smac neutralize IAPs and caspase activation is activated.

Targeting the IAPs for cancer therapy:

The acquired resistance of cancer cells to apoptosis is one of the hallmarks of cancer [34]. Cell death is suppressed by oncogene activation. The primary or acquired resistance to chemotherapeutic-based treatments is a main problem to effective cancer therapy [35]. Although there are many biochemical and genetic alternation occur in cancer cells, in vitro experiments explain that upregulation of IAP expression increases resistance to radiation and chemotherapeutic resistance [36]. So, IAPs has role in apoptosis regulation. It suggests that the inhibition of IAP expression and function may be promising therapeutic target for cancer and proliferative treatment [37]. Different types of approaches are used for inhibition of IAP expression in cancer treatment. These approaches are

1. AS ODNs (Antisense oligonucleotides) to XIAP or survivin as therapeutics.
2. SMCs antagonist to IAP

3. cIAP1 and cIAP2 modulators in cancer treatment
4. Survivin antagonist in cancer treatment
5. Survivin immunotherapy

1. **AS ODNs (Antisense oligonucleotides) to XIAP or survivin as therapeutics**

Applications of single-stranded antisense oligodeoxynucleotides (AS ODNs) are used for targeting IAPs in tumours. It is an important challenge to researchers. The AS ODNs are short stretches of synthetic DNA, approximately 12–30 nucleotides long and are complementary to a specific mRNA strand. Hybridization of the AS ODNs to the mRNA by Watson–Crick base pairing prevents the target gene from being translated into protein, thereby blocking the action of the gene, and resulting in the degradation of the target mRNA [38]. The specificity in the AS ODN approach is based on the fact that any sequence of approximately 17 bases in DNA and 13 bases in RNA is estimated to be represented only once in the human genome. Additional proof-of-principle studies were performed with newer double-stranded RNAi-based technologies. It shows that siRNA mediated downregulation of XIAP gene expression enhances apoptosis in cultured MCF-7 breast cancer cells and also enhances the killing effects of doxorubicin and etoposide [39]. In another study, short-hairpin RNAs as an RNAi is used against XIAP. It showed that XIAP mRNA is reduced by 85% in some breast carcinoma cell lines. This reduction in XIAP sensitizes these cell lines to taxane and to TRAIL induced killing [40]. Expression of Survivin is restricted during development and is absent in most healthy, differentiated adult tissues. Survivin is expressed in fetal tissues, stem cells, thymus, testes, regenerating hepatocytes and endothelial cells. Survivin is re-expressed during malignant transformation. The expression of survivin in cancer is a predictor of decreased survival time and is implicated in conferring radio and chemo-resistance phenotypes to tumor cells. Survivin and EPR-1 (effector cell protease receptor- 1) are encoded by mRNAs transcribed from opposite strands of the same chromosome locus. When epr-1 cDNA is overexpressed in HeLa cells, survivin is downregulated, the rate of spontaneous apoptosis increases and cell proliferation is inhibited [41]. ASODNs targeting survivin protein decrease survivin protein levels in human lung adenocarcinoma cell lines in a dose-dependent manner stimulate higher levels of caspase-3 activation, induce apoptosis, increase the sensitivity of cells to chemotherapeutics [42]. Using different AS ODNs spanning the entire survivin gene induces caspase-dependent or –independent apoptosis pathway depending on the neuronal tumor type [43]. Current phase II clinical trials of a surviving AS ODN are initiated by Eli Lilly

and Company. The importance of XIAP in cancer prognosis is not clearer in contrast to survivin. XIAP is the most potent of the IAPs. It has potential to inhibit caspase activation and to suppress apoptosis potentially. On the other hand, Survivin's death-suppressing effects may be mediated by XIAP stabilization and so it is very weak apoptotic inhibitor. Furthermore, XIAP has been correlated with poor prognosis. So, XIAP may be an attractive target for cancer treatment. AS ODNs targeting XIAP sensitize the tumor cells to the several chemotherapeutics' cytotoxic effects. The one study shows that XIAP AS ODNs enhance tumor regression in conjunction with radiotherapy in a mouse model of lung cancer [44]. A second generation, mixed backbone AS ODN (AEG35156/GEM640) downregulate XIAP expression in the Panc-1, the pancreatic carcinoma cell line. This approach induces sensitive to TRAIL-induced killing [45]. Overall, these promising data indicate that when antisense or RNAsi approach reduces XIAP protein expression, it decreases apoptotic threshold to an array of chemotherapeutics [46]. This approach is used inmultiple phase I or II clinical trials in the United Kingdom, Canada and the United States for solid tumors, lymphoma and leukemia.

2**. SMCs antagonist to IAP**: Binding of XIAP to caspases is antagonized by several mitochondrial proteins such as smac (second mitochondrial activator of caspases). Smac is released after the loss of mitochondrial integrity during Apoptosis [47]. This XIAP-Smac interaction to sensitize cells to apoptosis. This concept leads to discover molecules that would mimic Smac and inhibits XIAP [48]. Several small molecules have been developed that can induce death in tumor cells, either alone or in combination with established chemotherapeutics. TRAIL (Tumor-necrosis factor (TNF)-related apoptosis-inducing ligand) resistant tumor cells can be sensitized to TRAIL-induced death when used in combination with a Smac mimetic drug [49]. From the previous study, we know that under normal conditions, c-IAP1 is an E3 ubiquitin ligase (probably through its RING domain) and degrades nuclear factor-kB (NF-kB)-inducing kinase (NIK) and low basal levels of NIK is maintained and preventalternative NF-kB pathway. The loss of c-IAP1 (by Smac or Smac mimetics) results in phosphorylation and activation of downstream signaling molecules results in phosphorylation and activation of downstream signaling molecules e.g., NIK. leading to the translocation of NF-kB dimers into the nucleus. NF-kB activation induces the expression of TNF [50]. In the absence of c-IAP1, TNF- TNFR receptor ligation results in the inhibition of ubiquitylation of the receptor-associated kinase RIP1 by the tumor suppressor protein CYLD (cylindromatosis) and in the induction of a death-inducing complex which includes RIP1, FADD (Fas-associated death-

domain protein) and activated caspase-8 causing in cell death through caspase-3 activation [51].

3. **cIAP1 and cIAP2 modulators in cancer treatment**: cIAP1 protein destabilizers present in the form of analogs of bestatin (Ubenimex), an aminopeptidase inhibitor used in the treatment of leukemia. The bestatin analogs bind to BIR3 of cIAP1 and degrade cIAP1 in the micromolar range [52]. Another, Ro106-9920, small molecule inhibitor of NF-kB activation is an inhibitor of IkBa ubiquitination requires cIAP2 or a cIAP2-associated protein for activity. But it is not demonstrated that cIAP2 E3 ligase is directly inhibited by Ro106-9920 [53].

4. **Survivin antagonist in cancer treatment**: Several small molecule antagonists of survivin are under clinical trial. YM155 is identified as a small molecule to suppress the activity of the survivin promoter [54]. It is currently in phase II clinical trial in patient with cancer. A semisynthetic derivative of plant lignin that is Terameprocol (EM- 1421, M4N) suppresses survivin gene expression. It affects Sp1-dependent gene expression. It not only supresses survivin expression but also effect on cell cycle regulator Cdc2 [55]. The molecular chaperone protein, hsp90 interacts with and stabilizes survivin. Antibody-mediated disruption of the survivin-hsp90 complex or Global suppression of hsp90 chaperone function results in proteasomal degradation of survivin that cause cell cycle arrest [56]. Shepherdin, a small molecule peptidomimetic was designed to disrupt the hsp90–survivin interaction. It affects survivin expression and destabilizes other hsp90 client proteins [57]. Cell cycle disruption effects and polyploidy are observed after treatment with survivin antagonists. Survivin is well known a chromosomal passenger protein involved in chromosome alignment and segregation during mitosis and cytokinesis. Survivin antagonism may increase the aggressiveness of the surviving tumor cells by altering or increasing their ploidy, leading to increased dosage of oncogenes or reductions in tumor suppressor gene number [58].

5. Survivin immunotherapy: Cancer vaccines, a novel approach to stimulate host's immune system targeting cancer-restricted epitopes. Survivin is a tumor-associated antigen expressed in a variety of malignancies. Survivin epitopes are main target for the development cancer vaccine development. This approach is currently undergoing preclinical and clinical

evaluation [59]. Several phase I trials, involving either administration of survivin peptides or survivin-directed autologous CTLs, have concluded and progressed on to larger phase II trials.

Immunotherapy: Immunotherapy is type of cancer treatment. It helps our immune system to fight cancer [60]. Immunosystem detects and destroys abnormal cells and prevents growth of many cancers. Sometimesimmune cells are found in and around tumour. These cells called Tumour infiltrating lymphocytes or TILs. This is a sign that immune systems are responding to tumours. Although immune system can prevent cancer grow, cancer cells also have ways to prevent destruction by Immunosystem. Because cancer cells have protein on surface that turn off immune cells. Cancer cells also have genetic changes that make cells less visible to immune system [61].

There are different types of immunotherapies. These include

T cell transfer therapy:

This treatment boostsnatural ability of T cells to fight cancer. In this treatment, Immune cells are taken from patient's body. Those that are more active against cancer are selected and changed in lab to better attack to those patient's cancer cells and grown in large batches. Then put back toward into those patient's body through in vain. T cell therapy is also called adaptive cell therapy [62].

Monoclonal antibodies therapy:

Monoclonal antibodies are created in lab that are designed to binds to specific targets on cancer cells. Some monoclonal antibodies marks cancer cells so that this cancer cells will be better seen and destroy by immune system. These Monoclonal types of antibodies are types of immunotherapies. These monoclonal antibodies are also called therapeutic monoclonal antibodies [63].

Immune system modulators

It enhances body's immune system against cancer. Some of modulators affect specific parts of immune system whereas other affects immune system in more general way [64].

Treatment of vaccine: It works against cancer by boosting immune system's response to cancer cells.

TRAIL therapy: TRAIL (Tumour Nacrosis Factor Related Apoptosis Inducing Ligands) and its death receptors TRAIL R1 and TRAIL R2 selectively triggers apoptotic cell death in tumour. For that reason, TRAIL is a promising anticancer drug. TRAIL is in Phase II clinical trial. But there are some limitations in TRAIL based therapy. To deliver TRAIL based therapies with higher antitumour potential, several novel TRAIL derivates and modifications are designed [65]. Soluble recombinant versions of TRAIL molecules exhibited specific tumoricidal activity against varieties of tumour alone or in combination with other cancer treatment. In recent literature, natural role of TRAIL was explored in tumour and also allogeneic bone marrow transplantation in mouse. TRAIL effector pathway appears vital component of immunosurveillance of spontaneous or resident tumour cells by T cells and NK cells that stimulates more hope that manipulating TRAIL activity a natural path to improve cancer immunotherapy.

Reactive oxygen species: It defined as oxygen-containing, reactive chemical species. There are two types of ROS. Free radicals, which contain one or more unpaired electron(s) in their outer molecular orbitals. Non-radical ROS do not have unpaired electron(s), are chemically reactive. It can be converted to radical ROS. Example: Radical ROS are nitric oxide and hydroxyl radicals, superoxide and commonly seen in biological systems and nonradical ROS include hydrogen peroxide, ozone, peroxynitrate and hydroxide. ROS are also generated during mitochondrial oxidative metabolism. Oxidative stress also refers to imbalance due to excess ROS over the capacity of cell to mount an effective antioxidant response. There are several ROS molecules such as superoxide anion (O_2-), hydroxy radicals ($HO^{\cdot}$) and Hydrogen per oxide (H_2O_2) [66].

Redox status in haematological malignancies:

Elevated ROS has been detected in a variety of diverse pathologic states, including atherosclerosis, rheumatoid arthritis, and amyotrophic lateral sclerosis and in Fanconi anemia (FA), several cancers, including chronic and acute myeloid malignancies. In the case of chronic myeloid leukemia (CML), overexpression of BCR-ABL alone induce ROS production in hematopoietic cells [67]. Oxidative stress markers such as malondialdehyde and protein-carbonyls are elevated in the plasma of CML patients compared with normal. During the accelerated phase of CML, oxidative stress markers are increased whereas nonenzymatic

antioxidants in the plasma are decreased [68]. Furthermore, individuals with a polymorphism leading to reduced activity of glutathione S-transferase π (GSTP1, an enzyme important in detoxifying by-products of DNA oxidation) showed a significantly increased risk of developing CML and were more likely to have a poorer prognosis [69]. Elevated ROS production and oxidative DNA damage were observed in a murine model of AML driven by expression of mutant N-Ras and B-cell CLL/lymphoma-2 (Bcl-2) [70]. In haematological cancer cells, Ras mutation activate NOX2 oxidases. So, ROS is increased. Ras mutation is common molecular marker in AML patients. Increased ROS is seen in AML patients [71]. An increase in several oxidative stress markers was detected in relapsed AML patients compared with samples obtained at first diagnosis [72]. The gene encoding thioredoxin (TRX)–interacting protein (TXNIP) was a common retrovirus insertion site in mice that developed AML [73]. Thioredoxin (TRX)–interacting protein (TXNIP) is overexpressed in AML patients. So high oxidative stress is also related.

Targeting redox alterations in cancer:

Most of cancer cells exhibit increased aerobic glycolysis (the Warburg effect) and oxidative stress, these features could be important in the development of new anticancer strategies. Many evidence suggests that, many types of cancer cell have increased levels of reactive oxygen species (ROS) compare with normal cells [74]. A moderate increase in ROS can promote cell proliferation and differentiation whereas excessive amounts of ROS can cause oxidative damage to lipids, proteins and DNA [75]. Therefore, ROS homeostasis is very crucial for normal cell growth and survival. An increase in ROS is associated with abnormal cancer cell growth and reflects a disruption of redox homeostasis due either to an elevation of ROS production or to a decline of ROS-scavenging capacity, a condition known as oxidative stress [76]. Excessive levels of ROS stress can also be toxic to the cells. Cancer cells with increased oxidative stress are likely to be more susceptible to damage by further ROS insults induced by exogenous agents [77]. So, it is concluded that redox modulation is a way to selectively kill cancer cells without causing significant toxicity to normal cells [78-80].

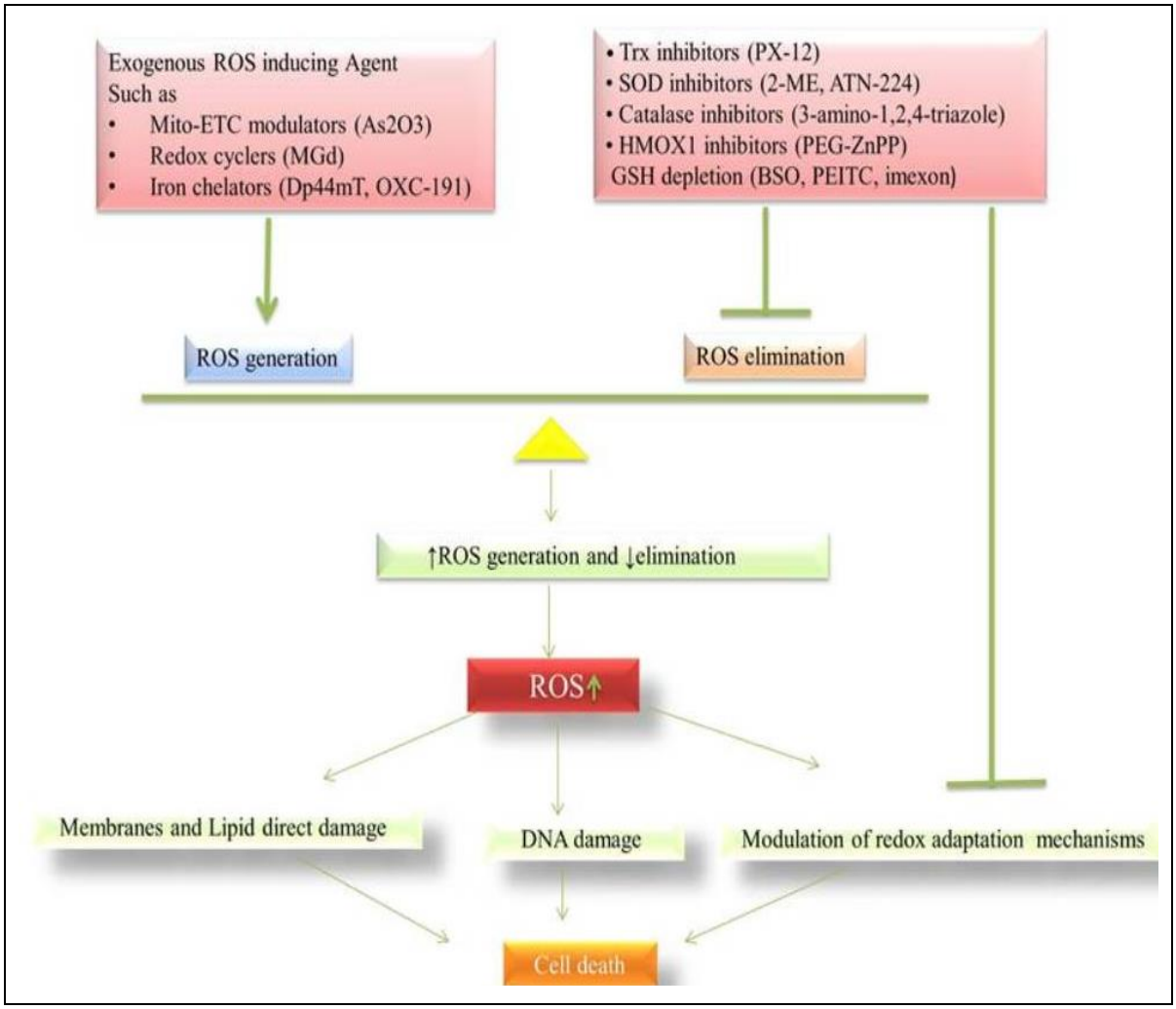

Fig 5: Therapeutic targeting cancer cells through ROS-mediated mechanisms: Exogenous agents increase ROS generation or inhibit ROS elimination. These exogenous agents increase the accumulation of ROS in cancer cells that lead to oxidative damage of membrane, lipid and DNA and cell death.

Mechanism of ROS production in cells:

Exogenousagents such as arsenic trioxide, which impair the function of the respiratory complex of mitochondrial membrane. Then electrons leaked from the impaired mitochondrial membrane are major source of ROS production [81]. Other side, redox intermediates are formed from the 'redox cyclers. Example of redox cyclers are that daunorubicin, doxorubicin and motexafin gadolinium etc. The radical intermediates that are created by the redox cyclers may interact with cytochrome P450 reductase and NAD(P)H: quinine oxidoreductase (NQO1) in the presence of reduced NADPH. In this reaction, superoxide is generated in the presence of molecular oxygen. In cancer cells, increased activity of NOX complex (NADPH oxidase) is

seen [82]. The synthetic retinoid N-(4-hydroxyphenyl) retinamide (4HPR) shows its cytotoxic effect against cancer cells by elevating the p67phox subunit of NOX [83]. Elesclomol (STA-4783), a novel compound that showed therapeutic activity in malignant melanoma and prolonged the progression-free survival time in a Phase II clinical trial. It interacts with the electron transport chain (ETC) to generate high levels of ROS within the cells and cause cell death [84].

Redox status: a biological basis for therapeutic selectivity:

Under physiological conditions, normal cells maintain redox homeostasis with a low level of basal ROS by controlling the balance between ROS generation (pro-oxidants) and elimination (antioxidant capacity) [85]. Normal cells can tolerate a certain level of exogenous ROS stress owing to their 'reserve' antioxidant capacity, which can be mobilized to prevent the ROS level from reaching the cell-death threshold. In cancer cells, the increase in ROS generation from metabolic abnormalities and oncogenic signalling may trigger a redox adaptation response, leading to an upregulation of antioxidant capacity and a shift of redox dynamics with high ROS generation and elimination to maintain the ROS levels below the toxic threshold. ROS-generating agents increased endogenous ROS, raising oxidative stress over the threshold of toxicity, showed selective toxicity in tumour cells [86]. Combinations of ROS-generating agents and drugs that inhibit ROS elimination could be a potent strategy to promote ROS accumulation in cells and enhance cancer cell cytotoxicity [87]. Redox adaptation is an important concept that, to large degree, explains the mechanisms by which cancer cells survive under persistent endogenous ROS stress and become resistant to certain anticancer agents [88]. Due to the increased intracellular antioxidant capacity, which can be provided by GSH and thioredoxin as a result of redox adaptation, So, this cause tumour cells associated with resistance to many anticancer agents [89]. Redox biology of cancer stem cells suggests the use of redox-modulating strategies to eliminate cancer stem cells. The potential of using a redox-modulating strategy to eliminate this subpopulation of malignant cells could have major implications in cancer treatment [90].

ROS mediated Signalling Pathway:

Oxidants, such as H_2O_2, acts as second messenger. It plays central roles in the regulation of different type of cellular functions such as proliferation, differentiation and migrationand also apoptosis [91]**.** At normal redox status, cells will respond properly to endogenous and exogenous stimuli. When redox homeostasis is disturbed it may lead to disease development

such as cancer and degenerative disorders. Different type of antioxidants such as SOD, catalase and GSH will eliminate excessive ROS and help maintaining the redox homeostasis. H_2O_2 acts mainly through reversible cysteine oxidation on formation of cysteine oxidative adduct. Main targets of oxidant are that different type of signalling adaptor, protein serine/threonine kinases, protein tyrosine kinase, phosphatase etc.

I) **Stress induced MAPK pathway:**

There are three well-defined mitogen-activated protein kinases (MAPKs): the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 MAPKs. The tight regulations of MAPK pathway are involved in both cell growth and cell death. MAPK pathways have been activated in the cells not only by receptor ligand interactions but also the presence of different stressors. MAPK phosphatases (MKPs) dephosphorylate and deactivate MAPKs [92]. Previous studies indicate that ROS can activate the MAPK pathway [93]. The different type of stimuli that induces ROS production can activate the MAPK pathway in multiple cells type [94]. The application of antioxidant prevents the MAPK pathway after cell stimulation with cellular stimuli. So, it indicates that the involvement of ROS in activation of MAPK pathways. Direct exposure of cells to exogenous H_2O_2, to mimic oxidative stress, leads to activation of MAPK pathways [95]. The mechanism, by which ROS can activate the MAPK pathways, is not well known. ROS can alter protein structure and function by modifying critical amino acid residues of proteins, the oxidative modification of signaling proteins by ROS may be one of the possible mechanisms for the activation of MAPK pathways [96]. Another potential mechanism is that ROS may inactivate the MAPK phosphatase (MKP) that inactivates the whole MAPK pathway. The intracellular H_2O_2 cause oxidation of the catalytic cysteine residue and inactivates the MKPs which lead to sustained activation of JNK pathway Choi et al. [97] showed that glutamate-induced oxidative stress induces sustained activation of ERK pathway through a mechanism that involves degradation of MKP-1.There are relatively fewer reports of endogenous ROS regulating the ERK MAPK pathway[98]. Wilmer et al. showed that IL-1-induced activation of ERK2 and JNK in human mesangial cells was inhibited by antioxidants, suggesting that ligand-stimulated ROS may be involved in mediating this effect. In cell death induced by the Ras ⁄ Raf ⁄ERK pathway, ERK activation is unusually prolonged. The delayed treatments with U0126, a MEK inhibitor, have revealed that ERK activity is continuously required to induce cell death. Chemical oxidants, such as H_2O_2, peroxynitrite ONOO) induce ERK, whereas many stimuli implicating ERK in cell death promote the production of ROS [99]. ERK activation requires ROS production to induce cell

death. It is proved by using the different ROS inhibitor [100]. Thus, ROS-mediated prolonged ERK activation might be the important mechanism in the the Ras/Raf/ERK pathway mediated cell death.

The activation of MAPK pathways is extremely complex and involve multiple MAPKK, more than 10 different MAPKKK, and a variety of other interacting regulatory proteins [101]. Changes the redox status in cells are main cause for the activation of the MAPK pathway under the oxidative stress [102]. Apoptosis signal-regulating kinase 1 (ASK1), a MAPKKK involved in both JNK and p38. In normal condition, thioredoxin (Trx), a redox regulatory protein binds to the ASK1 so that activity of ASK1 is inhibited. So, MAPK pathway is not activated [103]. CDC25A, a phosphatase that binds and inhibits ASK1 activity .Then JNK pathway is inhibited. So cell death by oxidative stress is reduced. Other studies indicated that under normal non stressed condition, glutathione S-transferase (GST) bind and inhibit JNK activity. Under oxidative stress, binding between GST and JNK is less, So, JNK pathway is activated. Gene disruption procedure has been useful to define the role of JNK in oxidative stress-induced apoptosis. For the example, UVC-induced apoptosis was completely ablated in Mouse Embryonic Fibroblast (MEF) derived from JNK1-/- and JNK2-/- double knockouts mice [104]. ASK1 deletion inhibits hydrogen peroxide induced JNK activation, renders ASK1-/-MEF resistant to apoptosis by the oxidant. In this way ROS plays at multiple levels in JNK signalling pathway to regulate its activity. The role of P38 in oxidant induced apoptosis may also be agent specific. Pre incubation of cells with SB203580, a specific inhibitor of p38, inhibited DNA fragmentation induced by singlet oxygen but not by H_2O_2. P38 activation required for apoptosis is induced by singlet oxygen not by hydrogen per oxide [105].

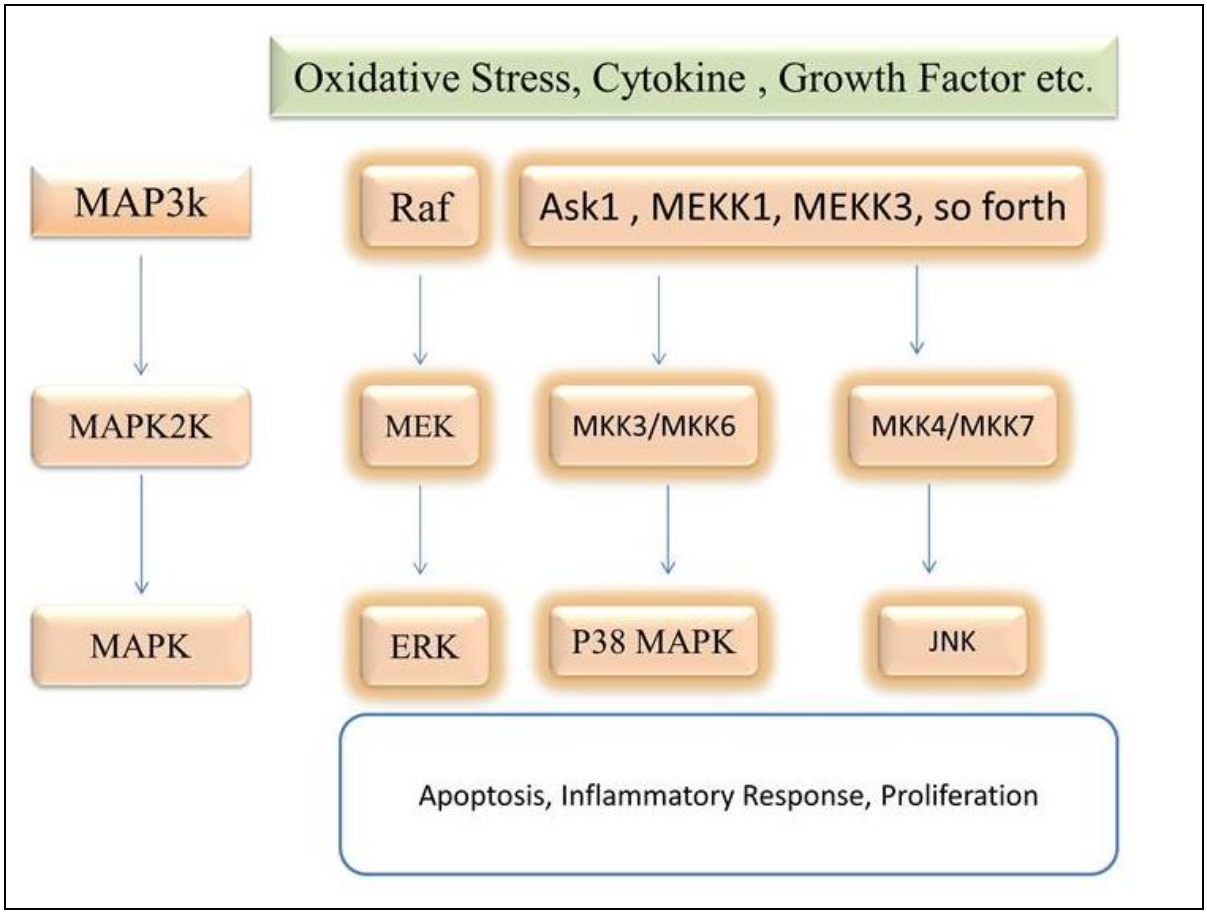

Fig 6: MAPK signalling pathways mediates intracellular signalling initiated by extra or intra cellular stimuli.

ROS and PI3K-Akt Signaling Pathway:

The phosphoinositide-3-kinase- (PI3K-) Akt pathway has been involved in many functions in cells such as cell cycle progression, proliferation, protein synthesis, apoptosis, autophagy, and drug resistance [106]. The binding of growth factor to its receptors directly stimulates class 1A PI3Ks via their regulatory subunit or adapter molecules such as the insulin receptor substrate (IRS) proteins. IRS proteins trigger activation of PI3K. The activated PI3K catalyzes the synthesis of phosphatidylinositol 3,4,5-triphosphate (PIP3), from phosphatidylinositol 4,5-bisphosphate (PIP2) [107]. The membranal PIP3, a signaling molecule, recruits and activates proteins such as the phosphoinositide-dependent protein kinase (PDK) and protein kinase B (Akt) serine/threonine kinases. This activated PDK and AKT perform their functions [108]. The unphosphorylated form of Akt is inactive, and phosphorylation of AKT at Thr-308 and Ser-473 shows its activity [109]. Akt binds with Heat shock proteins (HSP90) and complex is formed. HSP90 is a molecular chaperone. In this complex HSP90 protects Akt from protein

phosphatase 2A (PP2A)-mediated de phosphorylation [110]. Some studies suggested that ROS induces the intra molecular disulphide bond of Akt. So, Akt changes it's conformation. So that interaction between AKT and HSP90 is inhibited and increases Akt dephosphorylation catalysed by PP2A. So, enzymatic activity of Akt is decreased [111].

ROS and protein Tyrosine kinase (PTK) and protein Tyrosine Phosphatase (PTP):

The function of Protein tyrosine kinase (PTK) is opposite from the function of protein Tyrosine Phosphatase (PTP). All PTP contains cysteine residue in active site. The oxidative modifications of cysteine residue in active site of PTP leads to inactivation of activity of PTPs. PTPs are divided into two groups; tyrosine specific phosphatases and dual specificity phosphatases [112]. Tyrosine specific phosphatases are PTP1B, low molecular weight and Src homology 2 domain containing PTPs (SHP2) and dual specificity phosphatases include mitogen activated protein kinase (MAPK) phosphatases. H_2O_2 regulates the activity of PTP1B in insulin pathway. insulin- stimulated H_2O_2 production reversibly inhibits activity of phosphatase (PTP1B) and thus enhances the early insulin cascade [113].

PTK (Protein Tyrosine kinase) are divided into two. That is Receptor Family and non-receptor family. PTK are important into cell growth, differentiation, migration, and metabolism. The receptor tyrosine family includes epidermal growth factor (EGF) receptor, platelet-derived growth factor (PDGF) receptor and the non-receptor family includes Src, Focal adhesion kinase (FAK) etc [114]. ROS can activate PTKs in different mechanisms. ROS cause oxidative modification on Cys on active site of Protein phosphatase. So, PTP is inactivated. So, This can lead to tyrosine phosphorylation of the kinases and thus affect the kinase activities. In other way, ROS induces proteolysis of regulatory proteins that may inhibit tyrosine kinase activity [115].

The molecular mechanism of chemoresistance that is different from chemosensitive

Chemoresistance causes disease relapse and metastasis. It remains the main obstacle to cancer therapy. Therefore, it is very important to understand its molecular mechanisms behind the chemoresistance, and find out novel therapeutic approaches for cancer therapy. Overcoming intrinsic, acquired drug resistance is major challenge for the cancer patients' treatment. The molecular aspects of multi-resistance may be oncogenes (EGFR, PI3K/Akt, Erk and NF-κB), mitochondrial alteration, DNA repair, tumor suppressor gene (p53), transporter pumps, exosome etc [116]. The chemoresistance-related proteins are localized to extracellular ligand,

membrane receptor, cytosolic signal messenger, and nuclear transcription factors for proliferation, apoptosis and exosome [117]. The regulatory effects of EGFR-Akt-NF-κB signal pathway on the transcription of Bcl-2, Bcl-xL and survivin or EMT-related stemness is observed [118]. The different mechanisms are involved in chemoresistance. They are summerized in below:

I) Transporter: ABC proteins are ATPase transporter or channel protein and responsible for the translocation of various substrates (Such as amino acids, peptides, lipids, sugars, xenobiotic and ions) across the cell membrane. The structural analysis of ABC transporters minimally contains 2 nucleotide-binding domains and 2 transmembrane domains. The transmembrane domains recognize and transports a various kind of substrate via conformational changes and nucleotide-binding domains has ATP binding site [119]. The proteins include P-glycoprotein (P-gp). MVP, ABCG2.

P-glycoprotein acts as ATP-dependent efflux pump in intestinal epithelium, renal proximal tubule, capillary endothelial cells and liver cells. Pgp detoxificate cytotoxic drugs in cancer cells through efflux and GSH [120]. It transports a broad range of substrates such a lipids, steroids, bilirubin, digoxin, dexamethasone, colchicine, tacrolimus, quinidine, etoposide, doxorubicin, vinblastine. P-gp overexpression is observed in different kinds of hematological and solid tumors eg leukemia, ovarian, breast cancers and neuroblastomas. P-gp contributes chemoresistance.

BCRP/ABCG2 or ABCP or MXR1 (Breast cancer resistance protein) is a member of ABC superfamily. Its overexpression has been observed in hematopoietic progenitor, other stem cells and chemoresistant cancer cells for the efflux of cytotoxic drugs (daunorubicin, doxorubicin and mitoxantrone etc.). ABCG2 was overexpressed in the mitoxantrone (MX)-resistant MCF-7/MX in comparison to the normal parental cells. Estrogen up-regulated the tolerance of MCF-7 cancer cells to MX by inducing ABCG2 expression, but not after the inhibition of estrogen receptor α (ERα). These findings indicated that estrogen induced ABCG2 expression through ERα, and ABCG2 overexpression made MCF-7 more tolerant to MX [121]. MVP (major vault protein) is a 110 Kda drug transporter protein, observered in doxorubicin-resistant lung cancer cells. It localizes to nuclear pore complexes and has interaction with PTEN and PARP and ERα [122]. MVP may contribute chemoresistance by modulating the nucleocytoplasmic transport of chemoresistant drugs. It's overexpression is observed in

glioma, lymphoma and non-small cell lung carcinoma. MVP transports cytotoxic DNA-targeting drugs outside nucleus and contributes chemoresistance.

II) Oncogenes

Growth factor receptor in chemoresistance: Epidermal growth factor receptor (EGFR) can activate PI3K/Akt/mTOR, JAK/stat3, Src/FAK /ROS and SOS/Grb2/Ras pathways. It involved in differentiation, proliferation, survival and transformation. EGFR overexpression can activate AKT, NF-κB and STAT3, which leads to chemoresistance [123]. EGF-triggered and HER3-mediated Akt activation in chemoresistant cells. cisplatin resistance was associated with heme oxygenase (HO)-1 in lung cancer cells through EGFR-mediated PI3K/Akt and NF-κB pathways [124]. NSCLC cells with wild-type EGFR were treated with EGFR tyrosine kinase inhibitor continuously, induce chemoresistance to paclitaxel, gemcitabine, and cisplatin via STAT 3 pathway activation [125]. CD133 overexpression conferred the chemoresistance by stabilizing EGFR-Akt signalling [126]. miR-20b reduced 5-FU resistance by inhibiting ADAM9/EGFR pathway in colon cancer cells [127].

PI3K/Akt in chemoresistance: Akt is a serine/threonine-specific protein kinase involved in proliferation, transcription and migration. AKT activates NF-κB and up-regulate the transcription of pro-survival genes [128]. Akt1 overexpression results in chemodrug resistance in NSCLC cells. Akt is also phosphorylated at T308 and S473 and ubiquitinated partly by E3 ligase NEDD4 for proteasomal degradation. Akt-overexpressing cells displayed inactivation of p53 signaling pathway and hyperexpression of the antiapoptotic Bcl-xL in cisplatin resistance cells [129]. Akt 1 and Akt 2 overexpression cause ovarian cancer cells more highly resistant to paclitaxel. Doxorubicin and etoposide, and wortmannin inactivates PI3K/Akt pathway and cause sensitivityof gastric cancer cells to chemotherapy [130]. Activation of endoplasmic reticulum stress suppress PI3K/Akt/mTOR signaling pathway and increase chemosensitivity of small cell lung cancer cells [131]. FKBP51 could promote PHLPP-Akt interaction and following Akt dephosphorylation at ser473, so that USP49 enhanced cellular response to gemcitabine through FKBP51-Akt signalling [132]. miRNA-130b target PTEN and enhances chemoresistance to Adriamycin in breast cancer cells via activation of PI3K/Akt pathway [133].

NF-κB in chemoresistance: NF-κB have a transactivation domain in their C-terminus and Rel homology domain in their N-terminus. In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm by IκBs as an inactive form [134]. In stimulated cells, polyubiquitination and

proteasomal degradation of IκB protein is occurred. NF-κB is activated by phosphorylation. ROS, TNFα, isoproterenol, cocaine, ionizing radiation, viruses, chemo reagents, IL-1β, LPS acts as inducers of NF-κB activity [135]. Phosphorylated NFkB enters the nucleus and up-regulates the transcription of Bcl-xL, XIAP, survivin and Akt and Bcl-2[136]. In this colonic cancer cells, chemoresistance to 5-FU was strongly dependent on NF-κB activation [137]. DNA-PKCs downregulation suppressed P-gp expression and inhibit Akt/NF-κB pathway and enhances cisplatin chemosensitivity in osteosarcoma cells [138]. Aspirin inhibits NF-κB-IL6 or NF-κB-ABC transporter axis suppress acquired chemoresistance [139].

II)Tumor suppressor gene

p53 in chemoresistance: p53 involvement cisplatin-induced apoptosis is confirmed in testicular cancer cells by up-regulating the expression of pro-apoptotic genes and by down-regulating the expression of anti-apoptotic genes. Cisplatin treatment increases p53 expression. p53 significantly down-regulates survivin in lung cancer cells and binds to the promoter of survivin and suppress the expression of survivin at transcription level [140] that causes Caspase-3 activation and inhibited cell proliferation with response to Adriamycin. Mutant p53 induced drug resistance via miR-223 down-regulation in breast and colon cancer cells by the transcription control of Zeb-1 on miR-223 promoter because miR-223 targets microtubule-modulated stathmin-1 for chemoresistance [141]. MageA2 protein suppress p53 transactivation in HDAC3 and MageA2/p53 complex and that cause etoposide resistance of melanoma cells. p53 mutant Arg282Trp bind to the promoter of ERP29 and enhances its expression and cause cisplatin resistance [142]. WP1130 attenuated cisplatin resistance through USP9X-p53 ubiquitination-mediated degradation pathways [143].

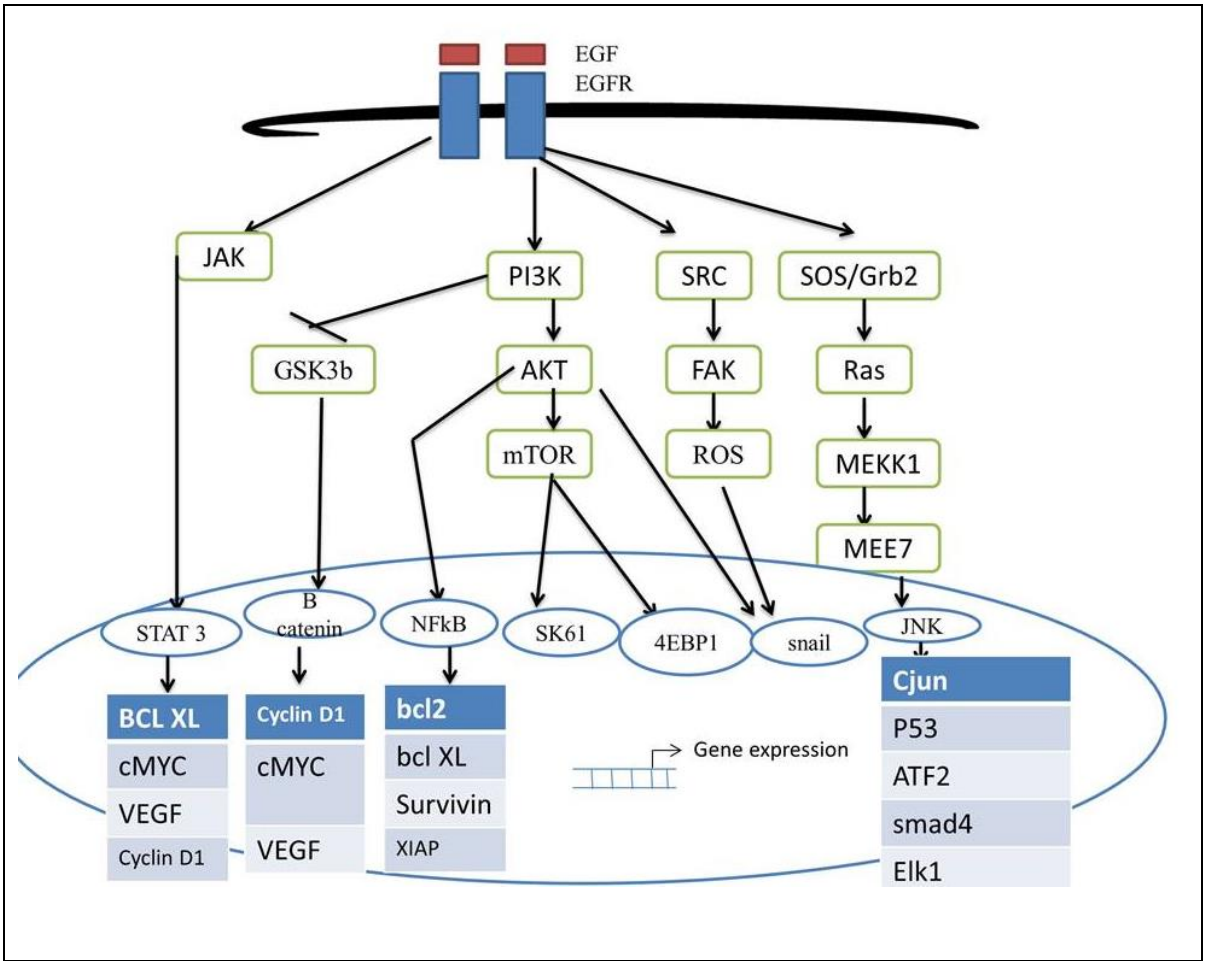

Fig 7: The interaction of epidermal growth factor receptor (EGFR) and its ligand induces the dimerization, autophosphorylation of EGFR and EGFR is activated. Then JAK/stat3, PI3K/Akt/mTOR and src/FAK/ROS, SOS/Grb2/Ras pathways areactivated. Stat3, β-catenin, NF-κB, SK61, 4EBP1 and JNK are translocated into cytoplasm thereby these all proteins regulate downstream genes's expression.

DNA repair in chemoresistance: DNA repair is a biological event that cell corrects the damage to the DNA molecules. DNA damage is induced by ultraviolet radiation, x- and gamma rays, endogenous ROS, mutagenic chemicals, and chemotherapeutic agents. Two types of DNA repair base excision repair and nucleotide excision repair can contribute the drug resistance to DNA-targeting chemo drugs. The insufficiencies in DNA damage repair were involved in cisplatin chemoresistance via Wip1. Wip1 is suppressor of the ATM-dependent signaling pathway. Wip1 silencing attenuated DNA damage repair and strengthened the cisplatin chemosensitivity of oral squamous cell carcinoma cells [144]. The strand separation and nuclease activities of YB-1 is related chemoresistance signatures of breast carcinoma cells [145]. Protein reversion less 3-like (REV3L) acts as catalytic subunit of DNA polymerase (pol)

ζ and has role in error-prone translesion synthesis. Low expression of REV3L enhanced the chemosensitivity of esophageal squamous carcinoma cells to 5-FU by G_1 phase arrest and apoptotic induction [146]. ERCC1-XPF enzyme complex that participates in DNA repair and recombination by nucleotide excision repair pathway. ERCC1 overexpression is linked with platinum-based chemotherapy resistance. cisplatin regulated the MAPK kinase pathway to induce ERCC1 overexpression and increase melanoma chemoresistance [147]. miR-770-5p target ERCC2, cisplatin chemoresistance in ovarian cancer is overcome. ERCC1- XPF could repair cisplatin-induced DNA lesions in melanoma cells via interstrand crosslink repair pathways and nucleotide excision repair [148]. O (6)-methylguanine DNA methyltransferase (MGMT) is DNA repair enzyme. Overexpression of MGMT cause chemoresistance via Wnt signalling pathway. Inhibition of WNT signalling pathway cause MGMT DNAenzyme downregulation and restore the chemosensitivity to DNA-alkylating drugs [149].

If we understand the chemoresistance signal network, we able to establishment of valid therapeutic targets and potential chemosensitivity biomarkers in cancer therapeutics.