CHAPTER – 2

Review of literature
Chapter 2

Review of Literature

Cancer is an uncontrolled growth of uncharacteristic cells in the body which may conquer or extend to other parts in the body. Number of cancers have been reported in literature viz. breast cancer, lymphatic cancer, skin cancer, lung carcinoma, colon cancer, hepatic cancer and prostate cancer. Signs and symptoms vary according to the type of cancer. Cancer cure may comprise chemotherapy, radiation therapy, and/or surgery.

2.1 Different types of cancer

2.1.1 Lung cancer

Lung Cancer is a major type of cancer attributed to cancer-related deaths. It comprises of a wicked tumor distinguished by over expression of cell growth in lung tissues [53]. Nearly >80% patients are affected by non-small cell lung cancer. Main stream of cases in lung cancer are linked with tobacco inhalation, while about 15-25% patients get affected with other reasons which do not include tobacco use. The symptoms of lung cancer include wheezing, coughing, shortness of breath and blood in mucus [54].

2.1.2 Liver cancer

Liver cancer is another cancer type in which mainly the liver gets affected. The main causative agents or the risk factors associated with liver cancer are excessive alcohol intake, hepatitis, and diabetes. It’s the second most leading cause of mortality especially in the developing countries [55]. The symptoms are abdominal pain, vomiting, white colored stool, loss of appetite, weight loss etc [56].

2.1.3 Bladder cancer

Bladder cancer is the cancer type which starts with urothelial cells lined in the bladder. Bladder cancer occurs more frequently in men than in women mainly affecting older people. The symptoms include blood in urine, frequent urination and pain [57]. The risk factors associated with bladder cancer are environmental factors, dietary and life style, smoking-derived carcinogenic agents, rubber compounds, certain dyes, and tobacco smoke. Other risk factors include radiation therapy to the pelvic region, environmental pollution, urinary schistosomiasis, and certain chemotherapeutic agents such as cyclophosphamide [58].
2.1.4 Renal cancer
Renal Cancer is another cancer type which affects the kidney cells severely. The main indications of occurrence of kidney cancer includes mass in the abdomen area, incidences of blood in the urine, loss of appetite, tiredness, loss of body weight, high temperature, pain in the abdomen. Factors that are associated with kidney cancer include smoking, regular use of NSAIDs, obesity, family history, dialysis patients and hepatitis C [59-60].

2.1.5 Ovarian cancer
Ovarian Cancer is another type that initially takes place in the ovaries but can spread to cells of the fallopian tubes. The symptoms of ovarian cells include pain in the pelvis region, pain in the lower abdomen, back pain, frequent urination, and pain during sexual intercourse, weight loss, fatigue, loss of appetite, heartburn, nausea [61]. Women with endometriosis are more prone to the ovarian cancer, in comparison to that of other women. The other risk factors are obesity and hormone replacement therapy which further increases the women's risk of developing ovarian cancer [62].

2.1.6 Thyroid cancer
Thyroid Cancer is an uncommon endocrine type of cancer which targets mainly the thyroid gland. The utmost common symptoms are a lump in the neck, pain in the neck, inflammation of the thyroid gland, hoarseness, and sore throat along with difficulty in swallowing [63]. The factors that can cause thyroid cancer are radiation exposure, having a previous non-cancerous breast condition or patients having any history for the same type [64].

2.1.7 Prostate cancer
Prostate Cancer is another cancer type which has an impact on the prostate (gland of male reproductive organ). The most common symptoms are frequent urination, difficulty in urination, blood in the urine, pain during urination, pain in the pelvic region, weakness of the legs, urinary incontinence etc. [65].

There are many signaling pathway reportedly attributed to breast cancer such as EGFR/PI3K/Akt signaling pathway, AKT-ERK Pathway, NF-κB pathway, Ras/Raf/ERKs signaling pathway, GPR30/PI3K/MAPK/STAT signaling pathway, HIF-1α pathway, CUX1/FGF1/HGF signaling pathway and PAF/PTAFR signaling pathway.
2.2 mTOR pathway, structure and its function:
The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which is an imperative component as a principal supervisory body of cell proliferation, growth and metabolic rate by signaling through protein complexes such as mTORC1 and mTORC2. mTOR complex 1/2 is evolutionary well-kept from yeast to mammals. The introduction of mTOR inhibitors marked a pivotal development for the treatment of cancers especially breast cancer [66]. Oncogenic triggering of PI3K/AKT/mTOR pathway is mainly known to occur through variety of mechanisms often comprising mutation or deletion of genes encoding RTKs [e.g., HER2 (ERBB2) and EGFR (ERBB1)], subunits of PI3K (such as p110β, p110α, p85β and p85α encoded by PIK3CB, PIK3CA, PIK3R2 and PIK3R1 respectively), AKT1, or activating isoforms of RAS. Loss of expression or function of PTEN through deletions, mutations or epigenetic silencing is also quite common [67]. Rapamycin, a macrolide is reported to be produced by the microorganism, *Streptomyces hygroscopius* which is known to have anticancer, antifungal and immunosuppressant properties. Rapamycin derivatives such as rapalogs were further developed as first generation mTOR inhibitors having similar therapeutic effects in a range of preclinical models as rapamycin but with better hydrophilicity and therefore could be used for intravenous and oral administration [68]. Due to partial inhibition of mTOR, rapalogs were not adequate for attaining wide and vigorous anticancer effects [69]. The partial inhibition of mTORC1 by rapalogs results in a negative feedback loop inhibition which further results in phosphorylation and activation of AKT [70]. Due to these restrictions, the second generation of ATP-competitive mTORC1/mTORC2 dual inhibitors were developed [71]. These inhibitors inhibit the kinase-reliant utilities of mTORC1 and mTORC2 and consequently, suppress the response galvanization of PI3K/AKT signaling while rapalogs only inhibit the activity of mTORC1. In addition, some naturally occurring compounds have been found to down regulate mTOR signaling [72]. Keeping in view of the above facts, the function of PI3K/AKT/mTOR pathway in pathogenesis of breast cancer, preclinical as well as in vitro findings with respect to PI3K/AKT/mTOR inhibitors have been discussed so as to develop such inhibitors as potential biomarkers against breast cancer.
2.2.1 Structural Preview into mTOR and Pathway

mTORC1 and mTORC2 are the two structurally and functionally different complexes of mTOR which play very important role in the pathway at various levels.

2.2.1.1 mTORC1

mTORC1 comprises of mainly five units:

(i) Mammalian lethal with Sec13 protein 8 (mLST8)
(ii) Raptor (mTOR; regulatory-associated protein)
(iii) Proline rich AKT substrate 40 kDa (PRAS40)
(iv) Deptor (DEP-domain-containing mTOR-interacting protein) and mTOR.

Raptor enrolls substrates for mTOR regulating the complex establishment. mTOR is the catalytic part of mTORC1 [73].

mTORC1 is negatively regulated by both PRAS40 and Deptor. Enrollment of both PRAS40 and DEPTOR to mTORC1 upholds the reticence of the complex when there is reduction in mTORC1 activity. PRAS40 directly inhibits mTORC1 kinase activity by binding to the substrate [74]. Activation leads to phosphorylation of PRAS40 and Deptor by mTORC1, which demonstrated an incomplete physical relations with mTORC1 and further encourages mTORC1 signaling [75].

mTORC1 acts as energy and nutrient sensor, and also reported to be included in regulation of biogenesis, lipid formation, autophagy, mitochondrial metabolism etc. Also various amino acids, serum, insulin and other growth factors are known to forbid the activity of mTORC1 [Fig. 1A].

2.2.1.2 mTORC2

mTORC2 complex entails six units:

(i) mTOR,
(ii) Rapamycin-insensitive companion of mTOR (Rictor)
(iii) mSIN1
(iv) mLST8
(v) Protor-1
(vi) Deptor

Some of the main components: mTOR, mLST8 and Deptor are commonly present concerning both mTORC1/C2 complexes. Rictor and mSIN1 are known to alleviate each other. Deptor regulates mTORC2 activity negatively as in mTORC1 [76]. mLST8 is a significant element of mTORC2 which decreases the activity and stability of this complex [77]. mTORC2 stimulates the paxillin, Rac1,
Cdc42, F-actin stress fibers, and RhoA, therefore is an essential regulator of the cytoskeleton [78]. [Fig. 1B]

**2.2.1.3 mTOR domain structure:**

The mTOR domain structure is having six functional domains:

(i) HEAT domain acts as a mediator in protein-protein interactions

(ii) FAT domain

(iii) FRB domain ((FKBP12-rapamycin binding domain)

(iv) PIKK domain

(v) Repressor domain

(vi) FATC domain [79]. [Fig. 1C]

---

**Figure 1A**

**Figure 1B**

**Figure 1C**

**Figure 2.1: Domain Organization of mTOR**

mTOR N terminal comprises of twenty tandem HEAT repeats and C-terminal half consists of the kinase domain which is a catalytic domain. In mTOR, starting 1350 residues are made up of HEAT repeats and next 650 residues are made up of FAT domain which contain many tetratricopeptide repeats (TPRs) [80-81]. FRB domain mainly consists of one hundred residues and the same lies amongst the FAT and kinase domain, and binds to RAPTOR and RICTOR [82-83]. The kinase domain is bilobed (N-terminal lobe and C-terminal) and consists of 550 residues having a clefsteduck
between which impasses to ATP. The FATC domain is located at C-terminus and comprises of 35 residues which creates an obligatory site for mLST8 [84]. The repressor domain is flanked by the kinase and FATC domain and the deletion of this domain is obligatory for the instigation of mTOR [85].

2.2.1.4 Essence of Pathway (PI3K/AKT/mTOR Pathway)

PI3K/AKT/mTOR; a cell signaling pathway which is a fundamental controller of cell growth, metabolism and propagation. The pathway stimulation begins with cellular developments like angiogenesis, tumor establishment etc. PI3K/AKT complex stimulates mTORC1 and is repressed by multipart TSC1/TSC2 while mTORC2 is stimulated by growth factors. mTORC1 concludes the phosphorylation, and inactivation of 4EBP1 while on other hand the phosphorylation and activation of S6K [86-88].

Figure 2.2: PI3K/AKT/mTOR Pathway.
mTOR is a cell growth, cell proliferation and cell metabolism regulator, and the pathway regulation is done or completed by various growth factors in which main regulators are as: ATP, amino acids and Oxygen levels [89]. Signaling in mTOR gets triggered by AKT upon its phosphorylation [90]. PtdIns (3,4,5) P3 production is due to Class I PI3K, alternatively known as the second messenger. After phosphorylation processes it to confine to the pleckstrin-homology (PH) domain of AKT. This upon enwrapping takes the kinase to cell membrane [91].

PTEN negatively reins activation of AKT, that transforms PtdIns (3,4,5) P3 to PtdIns(4,5) P2, subsequent in reduction in employment of AKT to cell membrane [92-93]. FOXO transcription factors, TSC2 and GSK3 as well as a number of downstream substrates are known for AKT activation. Phosphorylation of TSC2 disrupts the complex establishment of TSC1/TSC2 that further converts GTPase Rheb into the GTP-confined active state, and result in mTORC1 instigation at Ser2448 position. As PRAS40 gets phosphorylated also inhibited by AKT the mTORC1 gets negatively regulated by its galvanization by Rheb. Both S6K1 and 4EBP1 phosphorylation resulted in mTORC1 induction which was also activated by the raptor and TOR signaling motif in both S6K and 4EBP. The phosphorylation of S6K1 activates the same through mTORC1 and importantly sites of phosphorylation are altogether hindered by inhibitors of mTOR [94-95].

S6K1 is phosphorylated by the activated form of mTORC1, that supplementary phosphorylates 40S ribosomal protein. S6K1 includes ribosomal proteins, elongation factors along with insulin growth factor 2 [96-97]. Binding of 4EBP1 to eIF4E (eukaryotic translation initiation factor 4E) results in its deactivation, so the protein translation stops [98].

2.3 Diverse Classes of PI3K/AKT/mTOR Inhibitors and Their Therapeutic Potential

NVP-BKM120 is a new generation of Class 1 PI3K-specific inhibitors displaying activity to PI3Kα and mTOR. The blockage of NF-κB expression and PI3K/AKT signaling was reportedly induced by BKM120, while stimulated caspase-9 and caspase-3/7, further modifying the apoptosis-related gene expression. In chemo-resistant breast cancer, BKM120 trounced the MDR phenotype through cell apoptosis induction [99-100]. NVP-BEZ235 (PI3K/mTOR inhibitor) was further reported to have suppressive effects on the pathway, AKT and p-70S6K. This inhibitor also subdued cell propagation and prompted apoptosis and autophagy in breast cancer cells [101-102].

Jolkinolide B (root extraction of *Euphorbia fischeriana Steud*), a medicinal herb robustly impedes the PI3K/AKT/mTOR pathway. On the other hand apoptosis in MDA-MB-231 cells were stimulated. In order to study in vivo antitumor property of Jolkinolide B (JB), MCF-7 cells were subcutaneously
immunized into nude mice [103]. MCF-7 cells growth was restricted in the S phase by JB. The tumor volume was significantly dwindled in nude mice vaccinated with MCF-7 cells [104]. It was observed that handling with JB induced down regulation of cyclinD1, cyclinE, p-PI3K, mTOR and p-Akt, regulation of PTEN expression and p-eIF4E [105-106].

N-Hydroxyphthalimide (NHPI) exhibits effectual and anti-proliferative effects by suppressing human breast carcinoma BT-20 cells.

The proliferation of BT-20, HT-29 cells and LoVo cells was reported to be inhibited when incubated with NHPI (0-40 μM) for 48 hours in a concentration-dependent manner while MCF-10A cells proliferation was not affected when treated with NHPI [107]. There were morphological changes observed in BT-20, LoVo and HT-29 cell lines after treatment with NHPI and cells were shrunk and became round shaped but there was no morphological change observed in MCF-10A cells (human normal breast epithelial). This data indicated the NHPI suppresses the LoVo, HT-29 along with BT-20 cells propagation [108]. NHPI promoted G2/M phase cell cycle arrest by cyclin cdc2 and B1 expression. At higher absorptions of NHPI for 24 hours, cells (BT-20 and LoVo) were arrested in G2/M phase. The cells arrested in G2/M phase coincided with cell count decrease in S phase of the cell cycle [109]. Cyclin B1 protein expression at cellular level along with cdc2 level were reported to be repressed when BT-20 and LoVo cells were cured for 24 hours. The percentage of apoptotic cells was increased when BT-20 cells were treated with NHPI for 48 hours. Reduction in anti-apoptotic activity in Bcl-xL and significant establishment of caspase 9 and caspase 3 was observed with treatment of NHPI to BT-20 cells for 24 hours along with the increased levels of poly ADP-ribose polymerase (PARP) cleavage. Phosphorylation of substrates of mTORC1 (S6K1 and 4E-BP1) and S6K1 (S6) were also inhibited with NHPI [110]. NHPI restricted PI3K-mediated Akt phosphorylation at Thr308. The nuclear translocation of eIF4E was also induced when BT-20 cells were treated with NHPI for 6 hours [111]. As per the results indicated, NHPI reportedly suppressed mTORC1/mTORC2. Tumor volume/weight was significantly reduced when treated with 40 mg/kg NHPI. The tumor weight was decreased to 45% in BT-20 xenografts in comparison to the control [112].

ZSTK474 is a new class I phosphatidylinositol 3-kinase supressor which is known to induce G1 arrest in human MCF-7 cells. Effective inhibition of cancer xenografts (humans) treated with ZSTK474,has been shown in vivo as well [113]. The expression of phospho-Akt was also suppressed with ZSTK474 indicating PI3K inhibition and the reduced expression of nuclear cyclin D1 and Ki67. But TUNEL-
positive apoptotic cells were not increased when treated with ZSTK474 [114]. Marked G(0)/G(1) arrest was also induced as a result of the treatment with ZSTK474. PI3K was found to be inhibited with ZSTK474 at low concentrations [115]. Molecular modeling further signified that ZSTK474 binds to the ATP-binding pocket of PI3Kγ and acted as an ATP-competitive inhibitor. At higher concentration (100 µM), ZSTK474 inhibited mTOR activity as well [116]. The propagation of human breast cancer cells, MCF-7 was also inhibited by ZSTK474. At G1 phase (Cell cycle arrest) was also induced in consequent to treatment with ZSTK474 as revealed by flow cytometric analysis. ZSTK474 could induce autophagy process in MCF-7 cells as demonstrated using different assays including transmission electron microscopy (TEM), monodansylcadaverine (MDC) staining etc. Autophagy-inducing effect may further cause reticence of class I PI3K of mTOR [117].

AZD8835 is another potential inhibitor of PI3Kα and PI3Kδ. With chronic oral administration at 25mg/kg b.i.d, AZD8835 exhibited in vivo tumor growth inhibition and pharmacodynamic modulation of AKT phosphorylation in a murine H1047R PI3Kα mutated SKOV-3 xenograft tumour model [118-119]. Moreover, alternating high-dose arrangement of AZD8835 achieved greater inhibition of pathway along with induction in apoptosis. Thus AZD8835 exhibited dual action comprising proapoptotic and antiproliferative effects [120-121].

SZC015 is a derivative of oleanolic acid that in MCF-7 breast cancer cells encourages both apoptosis and autophagy. Cytotoxic mechanism of SZC015 was investigated in MCF-7 cells. Decrease in MCF-7 cell viability by SZC015 was observed resulting in intrinsic apoptosis activation, which was induced by caspase9, caspase 3, cleavage of PARP, release of cytochrome C and increased ratio of Bax/Bcl-2 [122]. Melittin (MEL), a bee venom constituent and a peptide was reported to be useful for the inhibition of EGF-induced invasion. MEL was also reported to block NF-κB and PI3K/Akt/mTOR. Moreover, MEL inhibited mTOR/p70S6K/4E-BP1 pathway and thereby considerably censored the EGF-induced FAK phosphorylation [123-124].

Trisubstituted-Imidazoles [2-chloro-3-(4, 5-diphenyl-1H-imidazol-2-yl) pyridine (CIP)] targeted oncogenic PI3K/Akt/mTOR Pathway and persuade apoptosis in human breast cancer cells. CIP censored phosphorylation of PDK to induce apoptosis [125].

INK128 is another novel molecule active-site mTORC1/2 dual kinase inhibitor which was found to inhibit cell propagation in breast cancer cells [126]. INK128 also inhibited primary tumor growth significantly in both VEGF-driven MCF-7 and non-VEGF (ML20; p = 0.05) xenograft models. Altogether, in preclinical models of breast cancer, INK128 decreased TORC1/2-dependent signaling
INK128 was found to be a superior inhibitor in blocking mTORC1/2 signaling in comparison to Rapamycin. The cell viability was found to be decreased in MCF-7 cells when exposed to INK128 [128]. Tanshinone IIA (T2A), is an antiangiogenic agent and the bioactive compound that has been derived from the traditional Chinese medicine (TCM). T2A inhibited the mTOR phosphorylation of RPS6 (Ser235/236 and Ser240/244), p70S6K (Thr421/Ser424) and 4E-BP1 (Thr37/46) in the environment when there were hypoxic conditions. Further T2A was found to inhibit the HIF-1α (transcriptional activity) and repression of HIF-1α expression at the translational level along with VEGF expression suppression [129]. Tan I exhibited anti proliferative activities resulting in S phase arrest, displaying association with that of cyclin-dependent kinase inhibitors p21Cip1 and p27Kip1 along with regulation. Tan I showed upregulation of apoptotic components and down regulation of anti-apoptotic mechanism of PI3K/Akt/mTOR signaling pathway [130]. Metformin displayed antitumor effects mainly through mTOR inhibition and AMPK establishment. Along with these factors, this compound also decreased the production of insulin, inflammatory cytokines bringing forth its anti-mitotic, anti-angiogenetic and anti-inflammatory effects as reported in earlier studies [131]. Simvastatin treatment suppressed proliferation and induction of apoptosis by deregulating caspase cascades, which strongly suppressed PI3K/Akt/mTOR by dephosphorylation of mTOR, Akt, S6RP, p70S6K and 4E-BP1 by enhancing PTEN expression. Moreover, simvastatin dephosphorylated e-Raf, MEK1/2 and ERK1/2 and significantly inhibited MAPK/ERK pathway [132].

Ibrutinib is an irreversible, specific and prohibitor of Burton's tyrosine kinase (BTK). The viability of ErbB2+ cell lines was significantly reduced by treatment with Ibrutinib with IC50 values at nanomolar concentrations. The cell viability of ErbB2+ was synergistically reduced with Ibrutinib when combined with dual PI3K/mTOR inhibitor BEZ235 [133].

AZD5363 (an AKT inhibitor) is a novel pyrrolopyrimidine-derived compound which was found to inhibit all AKT isoforms. The phosphorylation of the substrates was inhibited by AZD5363 with IC50 values of 0.06 to 0.76 μmol/L in three cell lines tested. The S6 phosphorylation and 4E-BP1 in cell lines was reported to be effectively inhibited by AZD5363, however AKT phosphorylation at both ser473 and thr308 was increased by the said AKT inhibitor. The ability of AZD5363 to induce nuclear translocation of FOXO3a in BT474c cells was also measured [134-135]. The phosphorylation of FOXO3a was prevented with inhibition of AKT and this led to translocation of FOXO3a to the nucleus where it switches on the countenance of genes such as FasL, p27 and BIM, which collectively
induced apoptosis and cell-cycle arrest. Moreover, FOXO3a nuclear translocation was induced by AZD5363 in BT474c cells with a half-maximal effective concentration (EC50) value of 0.69 μmol/L [136-138].

Benzo[b]furan and its derivatives (26 and 36) targeted the mTOR signaling pathway in humans which also induced apoptosis linked to breast cancer cell lines (MDA MB-231 and MCF-7). Cell cycle analysis demonstrated that in MCF-7 cells, these compounds tempted arrest at G2/M phase. Western blot analysis demonstrated that these compounds inhibited the mTOR signaling pathway in human breast cancer cells (MCF-7) [139]. P7170 is another inhibitor of mTOR/PI3K/ALK1 in TNBC model and its antitumor activity was further evaluated. P7170 hindered the proliferation in TNBC cell lines by the use of propidium iodide (PI) assay [140]. In MDA-MB-231 cells, PI3K-mTOR pathway proteins pAkt were modestly inhibited by P7170 while pS6 and p4EBP1 were potently inhibited as assessed by Western blot. In a trans well migration assay, migration of MDA-MB-231 cells was inhibited by P7170. P7170 at 1/3rd dose of MTD (5 mg/kg) resulted in 63% tumor growth inhibition. P7170 significantly reduced tumor CDC25A gene by 2-folds and reduced tumor p4EBP1 [141-142].

M2698 a dual-inhibitor of p70S6K and Akt, which in mouse models affects tumor growth. M2698 blocked p70S6K and inhibited PAM. M2698 demonstrated dose-dependent tumor growth inhibition. M2698 inhibited phosphorylation of p70S6K1 and Akt substrates in the human breast cancer-derived cell line. The phosphorylation of S6 was inhibited and significant reduction in p70S6k activity in the tumors of mice treated with M2698. The levels of pAkt were increased in tumor tissue after treatment with M2698 activating Akt following p70S6k inhibition; also portentous that Akt activity was blocked by M2698 [143].

2.4 Other Targeted Therapies

Loss of expression of ARID1A presents confrontation to some drugs which either suppresses or inhibits HER2/PI3K/mTOR signaling cascade. ARID1A loss starts with the activation of annexin A1 (ANXA1) expression. It was found that in patients where cancer is due to HER2+ are resistant to adjuvant trastuzumab–based therapy due to this compound. Therefore activated ANXA1 serves for trastuzumab-based treatment, may also acts as a predictive biomarker [144].

p70S6K1 (serine-threonine kinase) regulates protein translation and a marker for mTOR activity. MCF-7 and HER2 treatment caused a radical reduction with varying concentrations of Lapatinib. Lapatinib-resistant MCF-7/HER2-Lap10 cells exhibited a lapatinib-insensitive p70S6K1 hyperphosphorylation and co-treatment with rapamycin, suppressed p70S6K1 hyperactivation.
*FBXW7* gene is a p53-dependent tumor suppressor in which the genetic status of *FBXW7* and *PTEN* genes in a panel of (53) breast cancer cell lines was examined in one study. It was found that 23 out of 53 cancer cell lines loss of *FBXW7* but not of *PTEN*. *FBXW7* or *PTEN* exhibited significant sensitivity to killing by Rapamycin [145].

The effect of anastrozole (aromatase inhibitor), and AZD0530 (Src inhibitor), either alone or in combination was investigated in the study. Both these two drugs together lead to upregulated p27 levels and proliferative arrest in both in vivo and cell culture in a breast cancer xenograft model than either drug alone. The analysis of xenocraft tumors at proteomic levels demonstrated potential predictive markers for drug response. Also they displayed activation of the pathway in tumors resistant to AZD0530 monotherapy. Anastrozole at varying concentrations were used to treat MCF-7Arom5 and MCF-Ca cell lines with 1 μmol/L AZD0530, or both. The cell cycle progression was interrupted by 100 μmol/L anastrozole, which also showed gradual reduction in % S-phase cells and increasing the % G0-G1. AZD0530 (Src inhibitor) [146]. Also there was a moderate fall of % S-phase cells i.e from 52% to 45%. G1 arrest within 48 hours was initiated by both the drugs together than using the drug alone. Anastrozole used alone had little effect on dMAPK phosphorylation, while on the other hand AZD0530 showed an unanticipated increase in pMAPK which was reduced by the co treatment with anastrozole [147]. AZD0530 was shown to decrease pSrc but anastrozole paradoxically increased pSrc. However anastrozole and AZD0530 together initiated Src inhibition. The combinatorial treatment of anastrozole and AZD0530 inhibited Src, Akt and MAPK activities without affecting total kinase levels. Breast cancer growth in-vivo was also reportedly inhibited by both the compounds [148].

PCI-24781 (histone deacetylase) inhibitor effect in combination to tamoxifen on AKT was evaluated in cells (breast cancer) and this combination resulted in down regulation of AKT protein. ER positive MCF7 cells were delighted with tamoxifen and PCI-24781 that increased the cell death along with escalating PCI-24781 concentrations [149-151]. The increased concentrations of PCI-24781 abridged Akt expression and activity (PS473) of downstream targets (FoxO1-PS256 and mTOR-PS2448). Treatment of breast cancer cells with tamoxifen or PCI-24781 alone can lead to reticent decrease in ER, PRb, and Cyclin D1 expression whereas combination of these two drugs can lead to reduction in ER, PRb, and Cyclin D1 levels. Therefore they could act as novel biomarker for predicting response [152].

HER3 and p95HER2 (p95) are predictive and potential biomarkers on trastuzumab therapy. In trastuzumab-treated metastatic HER2-positive breast cancer (MBC) patients, those patients which
articulated prominent levels of p95 (p95-high) practiced less complimentary consequences measured up to the subgroup with tumors that also articulated low p95 levels (p95-low) [153].

AR expression can be an autonomous prognostic biomarker for response to NVP-BEZ235, the same compound at higher dosage levels, NVP-BEZ235 inhibited proliferation of tumor cell. *PIK3CA* mutations or *HER2* amplification in breast cancer cells, NVP-BEZ235 induces cell death, while all the *PTEN* obliterated, mutated, or silenced cell lines. Dihydrotestosterone (DHT) and NVP-BEZ235 not only subdued tumor growth, along with the reduction in tumor size, along with inhibition of the activity of PI3K/AKT/mTOR pathway at variant dosage on other hand the same compound at lower dosages, decreases androgen receptor (AR) activity and suppress androgen receptor (AR) expression in breast cancer (AR+/ER+). Suppression of NVP-BEZ235–induced androgen receptor (AR) showed expression level decrease in PTEN and KLLN in AR+/ER+ (breast cancer cells). NVP-BEZ235 at varying and high concentrations suppresses AKT phosphorylation also this at low dosage upsurges the levels of AKT phosphorylation (Ser473) [154].

Hypoxia inducible factor 1 (HIF-1) can be explored as a potential biomarker of response to therapeutic targets. The investigations were performed for the functional role and its regulation by human epidermal growth factor receptor-2 (HER2). Under nonhypoxic conditions Long-term letrozole-treated (LTLTCa) cells had higher HIF-1α protein expression than that of MCF-7Ca cells. HER2-activated PI3K/Akt/mTOR pathway standardizes HIF-1α expression in LTLTCa cells. Lapatinib an inhibitor of HER2 kinase which doesn’t effect HER2 expression but shows reduction in HER2 activation of downstream kinase pathway (for example PI3K/Akt/mTOR pathway) [155-156].

Usnic acid +/- is a lichen-derived metabolite which is having a property of distinguishing dibenzofuran scaffold, has been shown to have the most favorable binding activity at the active pocket of mTOR kinase assisted by various contacts with amino acids [157].

Paris saponin XA-2 is a prospective and a novel therapeutic agent which in breast cancer showed functional anticancer activity. Paris saponin XA-2 induced autophagy encourages apoptosis of breast cancer cells by the activation of Poly (ADP-ribose) polymerase in Akt/mTOR signaling pathway. BEZ235 and Trichostatin a (TSA) (histone deacetylase inhibitor) are dual inhibitors for PI3K/mTOR, which resulted in anti-tumor activity. Different breast cancer cell lines upon exposure to TSA or BEZ235 resulted in the reduction in S6 (S240/244) phosphorylation, mTOR (S2448), Akt (S473) and 4EBP1 (S65) in main cultured cells of the above mentioned cell lines. On the other hand, the only TSA treatment could not affect the protein phosphorylation in PI3K/AKT/mTOR pathway, however in
combination it again inhibited the mTOR (S2448), Akt (S473) and 4EBP1 (S65) in breast cancer cells [158].
In Breast Cancer, Receptor Tyrosine Kinases (RTKs) incorporation establishes sensitivity to PI3Kα-selective Inhibitors. RTKs over-expression (also including c-MET, FGFR, HER3 and EGFR) re-established ERK phosphorylation and the feasibility of the cell concealed by BYL719. This further suggested the proper and authenticated function of RTKs in cell proliferation and signaling. More concisely, the over activation of c-MET may result in EGFR to show resistance, on the other hand over-activation of IGF-1R and/or HER2 showed some sensitivity to BYL719 in breast cancer cells. The efficacy/safety profile of RTKs could be potentially developed as a predictive biomarker for the effectiveness of PI3Kα inhibitors [159].
Another inhibitor, DYRK2 has been reported previously whose uprooted expression encouraged Thr631 phosphorylation for the degradation of mTOR. Also for the Everolimus sensitivity, Tyrosine Phosphorylation Regulated Kinase 2 could potentially be used as a prognostic marker [160]. General action mechanism of the PI3K/AKT/mTOR inhibitors has been proposed in Figure 3.

Due to various factors worldwide breast cancer has become a foremost threat to the lives of women. Several molecular pathways and inhibitors in breast carcinogenesis are constantly being acknowledged. mTOR is a kinase protein involved in cell growth, proliferation and metabolism.
Upregulation of mTOR increases propagation of cells and tumor growth in breast cancer, and FRB Domain in mTOR suppresses cell proliferation and tumor growth via the PI3K/AKT/mTOR pathway. The present work recommends that mTOR plays a vital role in tumor proliferation and the cell cycle.