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PI3K/AKT/mTOR pathway inhibitors in triple-negative breast cancer: a review on drug discovery and future challenges

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Triple-negative breast cancer (TNBC) is a highly malignant subtype of breast cancer associated with poor prognosis. Although conventional chemotherapy regimens have shown some effectiveness in early TNBC cases, the outcome in advanced stages is poor. The PI3K/AKT/mTOR pathway is one of the important and active pathways involved in chemoresistance and survival of TNBC. This pathway is speculated to play an important part in malignant transformation and has been considered as a potential molecular target for the design of therapeutic agents to treat TNBC. This review discusses the potentials and drug discovery perspectives of PI3K/AKT/mTOR as a therapeutic target for effective management of TNBC with anticipated challenges.

Introduction

Cancer is the second-leading cause of death globally despite extensive research being carried out worldwide to combat this dreadful and fatal disease. Breast cancer is the second-leading cause of death among cancers in women. In 2015, it accounted for the death of 570 000 women worldwide. More than 232 000 new breast cancer cases were expected to report in the USA with ~40 000 deaths in the year 2018. Despite extensive research, early diagnosis is the best approach to preventing breast cancer. The common problem with breast cancer is that it can metastasize to distant vital organs of the body including the brain, bone, lung and liver, among others [1–3]. Breast cancer could be divided into different subtypes on the basis of expression of receptors that also have therapeutic and prognostic associations. Normally, breast cancer patients show expression of estrogen receptor (ER), progesterone receptor (PR) and amplified human epidermal growth factor receptor (HER2) – this subtype is referred as triple positive. Some cases of breast cancer lack expression of these receptors and this subcategory of breast cancer is referred to as triple-negative breast cancer (TNBC). Out of these two subcategories, the former has better prognosis. The third subtype of breast cancer is one that shows HER2 amplification [1,2,4].

TNBC patients commonly suffer from the problem of disease recurrence and resistance to chemotherapy and this resistance is speculated to be caused by cancer stem-like cells (CSCs) [1,2,4,5]. TNBC is very heterogeneous in nature with its own pattern of disease dynamics and metastasis leading to high relapse and an overall shorter survival period than other subtypes of breast cancer. Despite some effectiveness, shown by conventional chemotherapy in early-stage cases of TNBC, the response in advanced stages remains poor. The FDA-approved regimens for TNBC chemotherapy in adjuvant and neoadjuvant settings include anti-metabolites, taxanes and anthracyclines. Although there is agreement among researchers that TNBC has comparatively higher chemosensitivity for cytotoxic regimens than ER-positive breast cancer, no optimal cytotoxic regimen has been recognized [3,5].

Major challenges in TNBC therapy: need to discover new drugs

TNBC represents the most aggressive phenotype of breast cancer characterized by poor prognosis as a result of the high risk of

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metastasis and recurrence [6–9]. A lot of challenges are associated with the treatment of TNBC including: (i) unavailability of effective chemotherapeutic agents; (ii) poor prognosis; and (iii) lack of therapeutic targets.

Inefficacy of chemotherapeutic drugs used in treatment of breast cancer

Poor prognosis is one of the major concerns in TNBC cases because it is refractory to endocrine therapy. Current chemotherapy protocols for TNBC include platinum compounds, anthracyclines and taxanes. A combination of anthracycline and taxane is the common first-line treatment approach for TNBC followed by capecitabine at the time of progression. Currently, the standard agents can prove effective for a limited number of early TNBC patients who show chemosensitivity. However, patients with advanced disease typically respond poorly to current chemotherapeutic agents. Even in cases that respond to standard chemotherapy regimens, rapid disease progression is observed later [1,2,6]. Development of resistance to chemotherapy plays an important part in metastasis and recurrence of cancer in TNBC patients. It has been reported that heterogenecity and chemoresistant CSCs could be responsible for resistance to chemotherapy in TNBC patients resulting in metastasis and recurrence of tumors leading to high mortality [1,2,7]. The treatment gap created by nonavailability of a clinically efficient and molecularly targeted therapy has been a major concern. The identification of new molecular targets and targeted agents is an urgent need for patients with TNBC.

Poor prognosis

TNBC is considered as the most aggressive phenotype of breast cancer owing to high risk of metastasis and recurrence. It is a fatal disease with the frequent cause of fatality being lung metastasis. It has been observed using whole-genome sequencing with functional screening that lung metastasis could be related to upregulated expression of the ENY2 gene in TNBC. A high cancer stem cell population could be correlated with malignancy and invasiveness of TNBC [7–9]. Scientists are continuously exploring the molecules, mechanisms or pathways associated with poor prognosis of the TNBC phenotype as well as also investigating these biomarkers as a druggable target for discovering new medicine for treatment of TNBC. According to reports the aggressiveness of TNBC could be associated with deregulation of immunological responses involving tumor necrosis factor (TNF)α and interleukin (IL)-1β [7]. Maiti *et al*. [10] reported that vasculogenic mimicry could be a reason for poor prognosis in TNBC — formation of a vessel-like structure that is angiogenesis-independent is known as vasculogenic mimicry. Li *et al*. [11] observed a correlation between poor prognosis of invasive ductal carcinoma subtype of TNBC with p53 expression in a cohort study. Molecules or receptors including LAPTM4B/p27kip1 expression, receptor tyrosine kinase AXL and G-protein-coupled estrogen receptor 1 (GPER) signaling/Ezrin phosphorylation molecules are being investigated by scientists, and could be associated with poor prognosis of TNBC [12–14]. Ramchandani *et al*. [15] investigated microRNA (miR-708), a metastasis suppressor, carrying gold nanoparticles to reduce lung metastasis in TNBC with successful results. In the conclusion of this study, the authors reported that this antimetastasis-based therapeutic approach could be beneficial for TNBC patients. He *et al*. [16] observed a ribosomal protein: RPLP1, to be associated with high risk of metastasis and recurrence in TNBC, probably owing to its effect on epithelial–mesenchymal transition (EMT) of cancer cells.

Metastasis, poor prognosis and recurrence are the main challenges to be tackled with the treatment of TNBC. In other subtypes treatment is comparatively effective owing to availability of targeting molecules and receptors for which the FDA has approved certain therapies that target these molecules and receptors. The next challenge in the design of promising therapeutic molecules for treatment of TNBC is lack of therapeutic targets, discussed below.

Lack of targeted therapies and therapeutic targets

One more problem with treatment of TNBC is absence of effective therapeutic targets, which is also a reason for unavailability of targeted therapies approved by the FDA for TNBC [8,15]. Absence of PR, ER and amplified HER2 makes treatment of TNBC a difficult task because they are the main targets for treating the other subtypes of breast cancer. This problem enforced scientists to explore new targets specific to TNBC, such as PI3K/AKT/mTOR (PAM), RAS/MAPK, poly(ADP-ribose) polymerase (PARP), cell cycle and growth factor receptor, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), androgen receptor, protein tyrosine kinases, phosphatases, proteases, microRNAs (miRs) and alteration in the metabolic pathway, among others, to develop new therapeutic agents and preventive approaches [4,17,18]. An enhanced Warburg effect and dependence on glutamine are examples of altered metabolism in TNBC. One of the glutaminase inhibitors: CB839, which is currently in Phase I/II clinical trials, has shown a promising therapeutic response in some TNBC tumors but not all TNBCs. Further tumor resistance is also a problem with this drug as well as the mechanism of resistance not being clear [18]. Further, it has been reported that TNBC cells unresponsive to CB839 shows amplified carnitine palmitoyl transferase 2 (CPT2) protein and carnitine palmitoyl transferase CPT1 activity and showed inhibition of cell proliferation and migration on inhibition of glutaminase and CPT1 [18]. Overall, the treatment of TNBC is still a challenge for healthcare professionals and one of the promising strategies to overcome this challenge is the search for promising targets and design of effective and safe drug molecules acting on these targets. In the next section of this review, we discuss two more promising strategies to design a successful therapy for TNBC.

Strategies to overcome the challenges associated with TNBC therapy

Scientists are working on various approaches to solve the problems associated with treatment of TNBC like the development of nanotechnology-based formulations, targeted drug delivery systems and discovery or identification of potential and druggable targets particularly pertinent to TNBC [8,17].

Nanotechnology-based formulations

The main therapy for hormone-responsive or receptor-positive breast cancer is endocrine therapy, which is based on selective ER modulators (SERMs), selective ER downregulators (SERDs) and aromatase inhibitors (AIs) [4]. Tamoxifen and trastuzumab, the two most widely used chemotherapeutic agents in treatment of

breast cancer, act by targeting ER and HER2, respectively. TNBC, the most severe category of breast cancer with poor prognosis due to metastasis and recurrence, does not express these hormone receptors and, hence, these hormone-responsive therapies are ineffective. Poor solubility and bioavailability, lack of intracellular transport within cancer cells, and development of resistance with adverse effects on normal cells are major problems associated with conventional therapies of TNBC. Hence, scientists are exploring various nanodrug delivery systems with multiple functionalities such as nanoparticles, nanocrystal carbon nanotubes, quantum dots, dendrimers, among others, to fight this lethal disease with the objective of solving various problems associated with conventional therapies [1,4,19,20]. These nanodrug delivery systems have been shown to increase bioavailability with favorable pharmacokinetic and pharmacodynamic responses as well as intracellular uptake by cancer cells. These nanomaterials are also being investigated for personalized drug delivery, 3D printing and theranostic applications with the ability to target cancer cells with imaging [21–23].

Juneja *et al.* [24] investigated multifunctional and stimuli-responsive polysilsesquioxane (PSilQ) nanoparticles to deliver RNA interference, curcumin and protoporphyrin, simultaneously, within the cancer cells for combined chemotherapy and phototherapy along with gene delivery for treatment of TNBC. Pal *et al.* [25] explored biodegradable and folic-acid-conjugated poly(lactic-co-glycolic acid) (PLGA) microspheres for the delivery of curcumin to increase its bioavailability for the therapy of TNBC with expected advantages of enhanced circulation time and greater accumulation into tumor cells. In the results, these microspheres induced apoptosis in TNBC cells via perturbation of mitochondrial membrane potential, downregulation of p-AKT and upregulation of cleaved caspase-3, as well as greater tumor regression in a xenograft tumor model in BALB/c mice. Yang *et al.* [26] synthesized polyamidoamine (PAMAM)-dendrimer-based unimolecular micelles for simultaneous therapeutic and diagnostic (i.e., theranostic) applications as a vehicle for the delivery of the anticancer drug doxorubicin via a hydrazone bond and imaging agent (positron-emitting isotope ^{64}Cu) to be detected by positron emission tomography. The selectivity of this carrier system toward MDA-MB-231 breast cancer cells was further enhanced by conjugation with F3 peptide. The presence of a hydrazine bond in this carrier between dendrimer and drug, doxorubicin, rendered it sensitive to altered pH conditions in the tumor. In the results for this research, scientists reported this carrier system to be efficient for the delivery of doxorubicin preferentially in cancer cells with promising results for imaging applications. Baghi *et al.* [6] investigated the possibility of a synergistic effect between p53 delivered exogenously and dendrosomal nanocurcumin for an anticancer effect in TNBC cell line MDA-MB-231 via MTT assay and also evaluated the effect of p53 overexpression on cytotoxicity induced by dendrosomal curcumin. Results of a different experiment showed superiority of and synergistic effect of a combination (i.e., dendrosomal nanocurcumin with exogenous p53) in terms of increase in apoptosis induction, decrease in cancer cell migration and higher gene expression with significant anticancer effect, as studied on MDA-MB-231. All these studies showed that some improvement in the therapy of TNBC can be observed with the use of nanotechnology.

Potential targetable pathways for TNBC

As we discussed previously, many molecules are involved in survival and metastatic transformation of TNBC and, hence, are overexpressed selectively compared with normal healthy cells. For instance, SPAG5 expression has been observed to be upregulated in TNBC cells as compared with noncancerous cells [8]. Further STAT3 has been reported to be overexpressed in TNBC cells and to play a crucial part from instigation and growth to metastasis and immune evasion of TNBC. It plays an important part in the survival, proliferation, antiapoptosis, resistance and recurrence of TNBC along with immunosuppression. Inhibitors of STAT3 have been developed by scientists that also showed inhibition of a TNBC tumor model in in vitro and in vivo preclinical studies [17].

Protein arginine methyltransferase 5 (PRMT5) is an example of a target explored to design a drug molecule for treatment of TNBC. It is an enzyme that catalyzes conversion of arginine to histone and non-histone proteins via the process of methylation. Inhibition of PRMT5 has been correlated with impaired cellular proliferation of TNBC cells by acting on various stages of the cell cycle leading to reduced formation of the mammosphere. It has also shown a potentiating effect with EGFR as the target [27]. Another emerging class is the Chk1 inhibitors. A few Chk1 inhibitors have reached the clinical stage. Chk1 inhibitors target the DNA damage repair pathways or replicative stress in TNBC cells [28]. The nonselective cation channels known as transient receptor potential (TRP) channels, which play an important part in cellular physiology, have been found to be crucial in breast cancer as well. There has been a positive correlation between increased proliferation of a number of breast cancer subtypes and dysregulated (or rather upregulated) TRP channels [29]. Hematological and neurological expressed 1 like (HN1L) expression has also been demonstrated to bear a positive correlation with poor prognosis of TNBC, whereas knockdown has been found to be associated with better survival [30]. A significant amount of work is going on in this direction and a summary of recently explored potential targets and ongoing trials of new drugs against various TNBC targets is given in Tables 1 and 2, respectively. Further, some important target molecules identified for TNBC are presented in Fig. 1.

PAM pathway and its inhibitors: therapeutic intervention in TNBC

Ongoing clinical and translational research in the area of signal transduction pathways has identified pathways involved in survival of TNBC through chemoresistance. The PAM pathway is an important signaling pathway involved in motility regulation, metabolism, cell proliferation, migration and survival of TNBC cells. It is also found to be activated in breast cancer. The PAM pathway is involved in regulation of various physiological and cellular processes in breast cancer, including malignant transformation. It has been widely studied in several cancer types and a number of PAM pathway inhibitors are being investigated to be developed as therapeutic agents against cancer. The structures of some of these inhibitors are given in Fig. 2. Overall, the upregulation of PAM signaling in TNBC is reported to induce resistance to chemotherapy, hormone treatment and HER2-targeted treatment [31]. Several PAM inhibitors have been screened and assessed against breast cancer cell lines. The breast cancer cell lines with

TABLE 1

Potential targets being explored for their role in TNBC resistance, invasion and metastasis

Target	Role in TNBC	Inhibitor	Refs
Protein arginine methyltransferase 5 (PRMT5)	There is higher localization of PRMT5 mRNA and proteins in TNBC compared with other breast tumors or healthy tissue. The higher expression has also been associated with poor prognosis. Inhibition of PRMT5 can prevent progression of the cell cycle, induce apoptosis The PRMT5 inhibitor has been found to show a potentiating effect in combination with EGFR-targeting agents	EPZ015666	[27]
Checkpoint kinase 1 (CHK1)	CHK1 is an important mediator of DNA damage associated checkpoint signaling response. It has shown positive correlation with several DNA damage response (DDR) biomarkers e.g., BRCA1, DNA-PKCα, RAD51 and ATM	Prexasertib (LY2606368)	[28]
Transient receptor potential isoform 3 (TRPC3)	Plasma membranes of several TNBC cell lines overexpress TRPC3. Inhibitor of TRPC3 induced apoptosis, prevented growth and reduced drug resistance by improving sensitivity It has been found that TRPC3 decreases Ras GTPase-activating protein 4 (RASA4) which is a suppressor of the Ras/MAPK pathway. Thus, a novel TRPC3-RASA4-MAPK signaling cascade could be a novel target	Pyr3	[29]
Hematological and neurological expressed 1 like (HN1L)	HN1L is a breast cancer stem cell transcription regulator gene found to play a significant part in reducing relapse-free survival of TNBC patients. It regulates the LEPR/STAT3 signaling axis through upstream binding to STAT3, leptin receptor and microRNA-150 sequences	–	[30]
Programmed cell death 1 (PD-1)/ programmed death ligand 1 (PD-L1)	PD-L1 is an immunoregulatory receptor. TNBCs overexpress PD-L1 which also correlates positively with the tumor infiltrating lymphocyte level. Activation of the Ras/MAPK pathway in TNBC has been associated with immune evasion and sustainable results in advanced cases have been observed with immunotherapy targeting PD-1 and PD-L1. Reports also suggest that oncogenic transmembrane mucin MUC1 which is mediates immune escape are upregulated in TNBC. MUC1 can elevate PD-L1 transcription through NF-κB, p65 and MYC recruitment and has emerged as an important target for PD-L1 supression in TNBC	Atezolizumab	[53,54]
E3 ubiquitin ligase (UBR5)	UBR5 has been found to be overexpressed in TNBC. Knockout of the UBR5 gene in murine models has been found to annul tumor growth and metastasis. UBR5 loss also resulted in impaired angiogenesis and upregulated tumor apoptosis. Reduced E-cadherin expression in absence of UBR5 has also been found to drastically reduce tumor metastasis	–	[55]
Nectin-4	The adhesion molecule, nectin-4 expression has inverse correlation with prognosis of TNBC. Nectin-4 expression has been found in >60% of TNBCs. The antibody-conjugated nectin-4 inhibitor has been found to show sustained cytotoxic effect on nectin-4-positive xenograft TNBC of primary, metastatic as well relapse types. Further, the efficacy of the inhibitor was dependent upon nectin-4 expression	Anti-nectin-4 monoclonal antibody conjugated to monomethyl auristatin-E (N41mab-vcMMAE)	[56]
PIM1	It belongs to PIM kinase family associated with cellular proliferation, migration and apoptosis. It has no effect on normal physiology. PIM1 expression has been found to show positive correlation with several transcription factors e.g., oncogene MYC. PIM1 expression is elevated in primary TNBC. Evidence has suggested a regulatory role of PIM1 on the JAK/STAT pathway and a number of cell cycle and apoptotic genes	AZD1208	[57]
Guanylate-binding protein 1 (GBP1),	GBP1 is under regulatory control of EGFR. It is regarded as a cytoskeletal path for resistant cancer. It has potential to be a target in cases of EGFR overexpression	–	[58]
Cancerous inhibitor of protein phosphatase 2A (Cip2a)	Expression of Cip2a inversely correlates with the survival of TNBC patients. Cip2a inhibition can inhibit G2/M phase causing cell cycle arrest. It can regulate cyclin-dependent kinase inhibitor 1B (p27Kip1) by recruiting c-myc and inhibiting Akt-associated PP2A activity in TNBC	–	[59]
Deubiquitinase (DUB3)	DUB3 has been identified as a target of cyclin-dependent kinase (CDK4/6) which has been implicated in metastasis of TNBC. It regulated SNAIL 1 stabilization essential for mesenchymal transition	–	[60]
Syndecan-1	Syndecan-1 is a proteoglycan mediated tumor progression and cancer stemness by acting as a chemokine and growth factor co-receptor. Owing to its role in inflammation, it has emerged as an important target for inflammatory TNBC. Syndecan-1 upregulation correlates with enhanced EGFR, Notch-1 and CD44 expression and knockout of syndecan-1 also suppressed STAT3 and NF-κB which are constitutively activated in inflammatory types of TNBC	–	[61]
Kisspeptin (KISS1 or GPR54)	KISS-1 receptor is a G-protein-coupled receptor that regulates the reproductive axis. Kisspeptin propeptides are produced by the KISS-1 gene and converted to Kisspeptin-10 (KP-10) through MMP cleavage. KP-10 promotes invasive behavior through MAPK/Erk activation. It also produced positive feedback on TGFβ, further promoting invasion	–	[62]

TABLE 2

Summary of ongoing trials of new drugs against various TNBC targets and PI3K/AKT/mTOR inhibitors as combination therapy in TNBC

Trials of new drugs against various TNBC targets

Target	Drug	Monotherapy/combination	Phase	Identifier
Checkpoint Inhibitor	LAG525	Combination with spartalizumab and carboplatin	Phase II	NCT03499899
AKT/ERK	ONC201	Monotherapy with or without methionine restricted diet	Phase II	NCT03733119
VEGFR 2	TTAC0001	Combination with pembrolizumab	Phase Ib	NCT03720431
Carbohydrate antigen Globo H	Adagloxad simolenin (OBI 822) OBI821	Combination therapy	Phase III	NCT03562637
PD-L1	Atezolizumab	Combination with anthracycline/taxanes	Phase III	NCT03498716
PD-L1/TGFβ RII	M7824	Monotherapy	Phase I	NCT03579472
PD-1	SHR1210	Combination therapy with apatinib	Phase II	NCT03394287
Protein tyrosine kinase PI3K/mTOR	PTK7ADC Gedatolisib	Combination therapy	Phase I	NCT03243331
Receptor tyrosine kinase	TT00420	Monotherapy	Phase I	NCT03654547
Tumor-cell apoptosis factor	Nerofe	Monotherapy	Phase I/II	NCT03634150
Chemokine receptor 5 (CCR5)	Leronlimab	Combination	Phase Ib/II	NCT03838367
EGFR	SCT200	Monotherapy	Phase II	NCT03692689
Cyclin-dependent kinase (CDK)	PF06873600	Mono/combination therapy with endocrine drugs	Phase II	NCT03519178
Histone deacetylase (HDAC)	Entinostat	Monotherapy	Phase I	NCT03361800
TTK protein kinase	S81694	Monotherapy Combination therapy with paclitaxel	Phase I/II	NCT03411161
TNF-related apoptosis inducing ligand (TRAIL)	ONC201	Monotherapy	Phase II	NCT03394027
Glutaminase	Telaglenastat	Combination therapy with talazoparib	Phase I/II	NCT03875313

Trials on PI3K/AKT/mTOR inhibitors as combination therapy in TNBC

Identifier	Combination treatment	Trial phase
NCT01623349	BKM120 (PI3K inhibitor) + olaparib BYL719 (PI3K inhibitor) + olaparib	Phase I
NCT02616848	Everolimus (mTOR inhibitor) + eribulin	Phase I
NCT02723877	PQR309 (dual PI3K/mTOR inhibitor) + eribulin	Phase I & II
NCT02457910	Enzalutamide + taselisib (PI3K inhibitor)	Phase I & II
NCT01111825	Neratinib + temsirolimus (mTOR inhibitor)	Phase I & II
NCT02646748	Pembrolizumab + INCB050465 (PI3K inhibitor)	Phase I
NCT02423603	Paclitaxel + AZD5363 (AKT inhibitor)	Phase II
NCT01964924	GSK2141795 trametinib	Phase II
NCT02301988	Ipatasertib (AKT inhibitor) + paclitaxel	Phase II
NCT02162719	Ipatasertib (AKT inhibitor) + paclitaxel	Phase II
NCT02208375	Olaparib + AZD2014 (mTORC1/2 inhibitor) Olaparib + AZD5363 (AKT inhibitor)	Phase I Phase II
NCT01562275	Ipatasertib (AKT inhibitor) + cobimetinib	Phase I
NCT02476955	ARQ092 (pan-AKT inhibitor) + carboplatin + paclitaxel ARQ092 + paclitaxel ARQ092 + anastrozole	Phase I
NCT02583542	AZD2014 (mTORC1/2 inhibitor) + selumetinib	Phase I Phase II

alteration in the PAM pathway as a result of PIK3CA mutations or HER2 amplification are sensitive to these inhibitors. Preclinical findings also suggest that AKT can activate the ER pathway independent of estrogen availability. Some PAM inhibitors are in clinical trials, emphasizing its significance to improve the prognosis of therapy in TNBC. Furthermore, activation of certain pathways can facilitate resistance through escape mechanisms. Thus, a combination of therapeutics with multiple targeting of intracellular machinery can be a promising approach against TNBC [31–34].

One of the major components of the PAM pathway: phosphoinositide 3-kinase (PI3K), is a heterodimeric molecule from a larger family of lipid kinases that phosphorylate the 3-hydroxyl group of phosphoinositides. PI3K consists of a catalytic (p110) and a regulatory (p85) subunit [32]. Relationships between AKT-mediated PI3K signaling and mTOR signaling mediators are presented diagrammatically in Fig. 3. The PI3K signaling pathway is activated as a result of binding of a growth factor or ligand to a number of membrane-associated receptor tyrosine kinases (RTKs), including

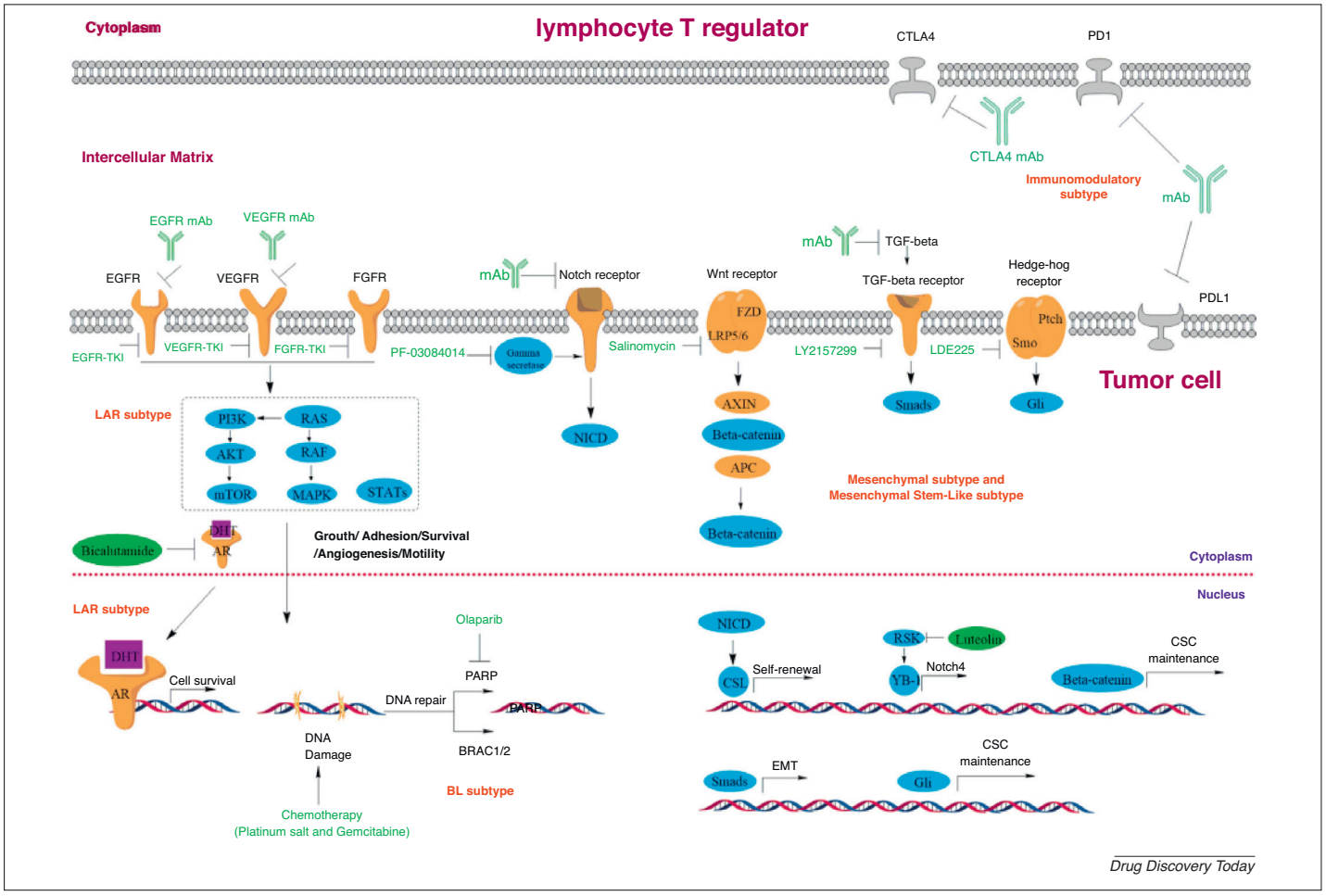

FIGURE 1

Diagrammatic representation of different target molecules or receptors for triple-negative breast cancer (TNBC). Reproduced, with permission, from [63].

IGF-1 receptors and HER proteins [33]. Activation of RTK leads to the recruitment of the p85 subunit and subsequent conformational change allowing the p110 subunit to catalyze 4,5-phosphoinositide (PIP2) phosphorylation to 3,4,5-phosphoinositide (PIP3). PIP3 presents a docking site for AKT and activates AKT. Some RTKs require adaptor molecules (e.g., IRS1) to interact with p85. PTEN phosphatase is the main protein that negatively controls PI3K by converting PIP3 to PIP2. Another tumor suppressor, inositol polyphosphate 4-phosphatase type II (INPP4B), also dephosphorylates PIP3 to PIP2 and regulates PIP3 levels [34].

PIP3 activates the downstream PI3K pathway by colocalization of the serine/threonine kinases PDK1 and AKT to the cell membrane via their plekstrin homology (PH) domains. AKT activation leads to protein synthesis and cell growth by activating the downstream effector mTOR through TSC1/2. Activated mTOR forms two functionally different complexes: mTOR complex (mTORC)1 and mTORC2. mTORC1 mediates growth-stimulatory effects of mTOR by promoting mRNA translocation and activating protein translation. It is also involved in lipid synthesis and metabolism. By contrast, mTORC2 regulates AKT phosphorylation and stimulates further AKT activation and is involved in the organization of the actin cytoskeleton [35].

Analysis of The Cancer Genome Atlas (TCGA) samples has shown PTEN loss and activation of PI3K pathway signaling in TNBC. Many research works have reported that the incidence of PTEN and PI3K mutations is much higher in TNBC compared with other classes of breast cancer [36]. mTOR is another target of fundamental importance in the clinical setting because many drugs selectively target mTORC1, which regulates the expression of factors involved in TNBC tumorigenesis. The activation of the mTOR pathway in TNBC is correlated with poor prognosis [37]. Role of PI3K inhibitor dactolisib has also been identified in controlling the whole mTOR pathway [38]. PIK3CA mutations having AKT-independent and/or AKT-dependent mechanisms, inactivating mutations in PTEN and PI3K/AKT activation, have been identified as common events in TNBC [3,39,40].

The major advantage of the PAM pathway as a druggable target is its potential benefit against the emergence of resistance after therapy. Disease progression in a significant number of patients is associated with resistance to endocrine therapy. However, the data suggest that there is coordinated reciprocal regulation between PI3K and ER pathways. Further, increased PAM pathway activity is observed in patients who underwent endocrine and HER2-targeted therapies. In such cases of resistance, dual PI3K–mTOR inhibitors offer the potential advantage of increased clinical efficacy as a result of complete inhibition of the PAM pathway [41]. PAM pathways are reported to regulate several hallmarks of cancer including genomic instability, motility, metabolism, survival

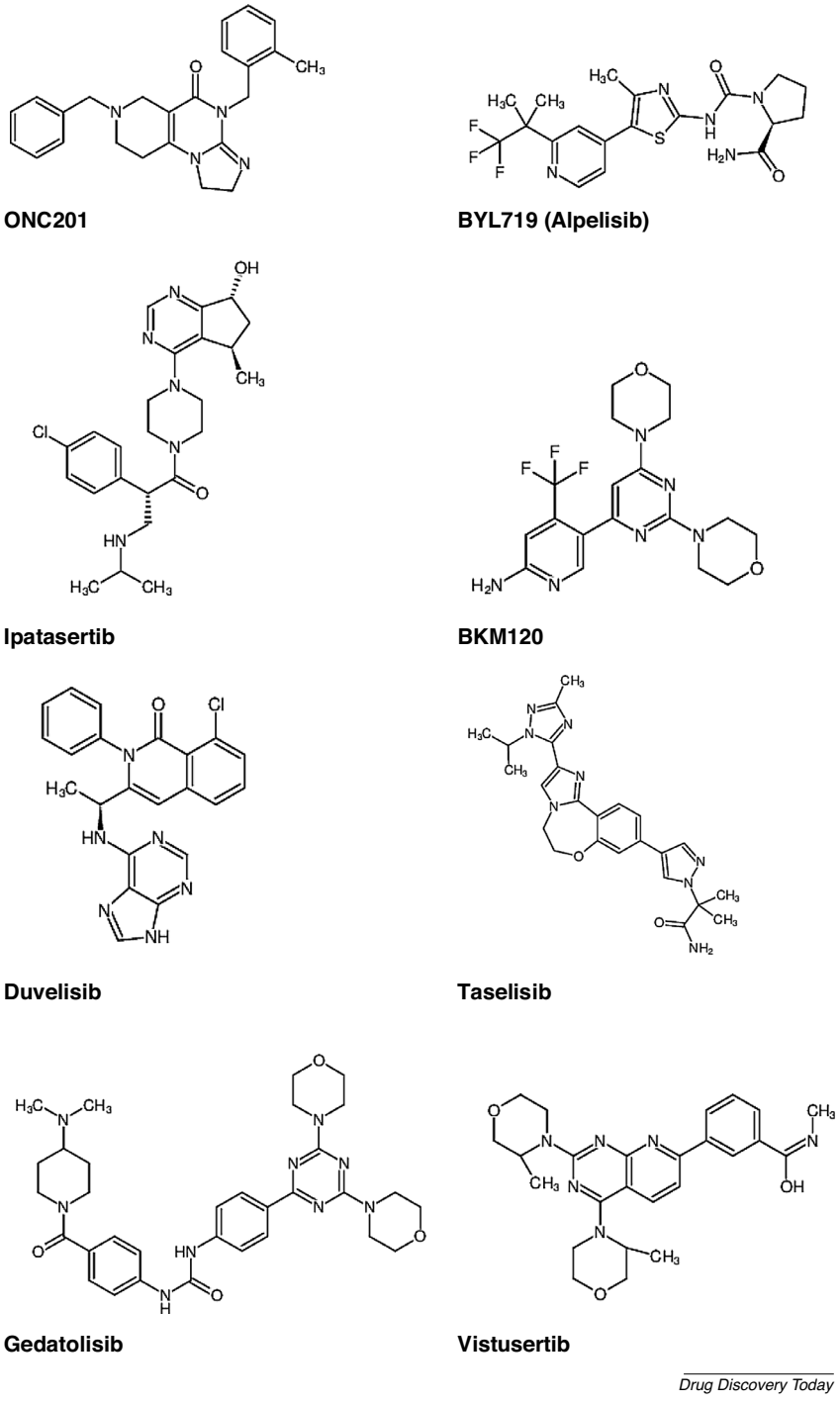

FIGURE 2

Structure of some of the PI3K/AKT/mTOR (PAM) inhibitors being investigated for treatment of triple-negative breast cancer (TNBC).

and the cell cycle [12]. Further, its role in regulating the immunosuppressive microenvironment has also been indicated. Although there has been success of immunotherapeutic targets (checkpoint, PD-1/PDL-1), the relapse with these therapies has led to identification of a new hallmark of cancer (i.e., immunoevasion). PAM has not only been identified to affect cancer proliferation but also suppress immunosuppressive pathways and consequently enhance tumor immunosurveillance [42]. The PAM pathway has been reported to be deregulated in TNBC. This not only makes the PAM pathway a potential target for pharmacologic treatment but also underscores the need for mutation profiling of individualized therapies. This will also provide a scientific rationale for selecting TNBC patients for individualized targeted therapy.

Clinical translational scope of PAM inhibitors

The PAM pathway can be targeted by upstream inhibition of PI3K and AKT. Further, inhibitors of AKT can widen the spectrum of PI3K inhibitors. Although there are agents that can inactivate PI3K

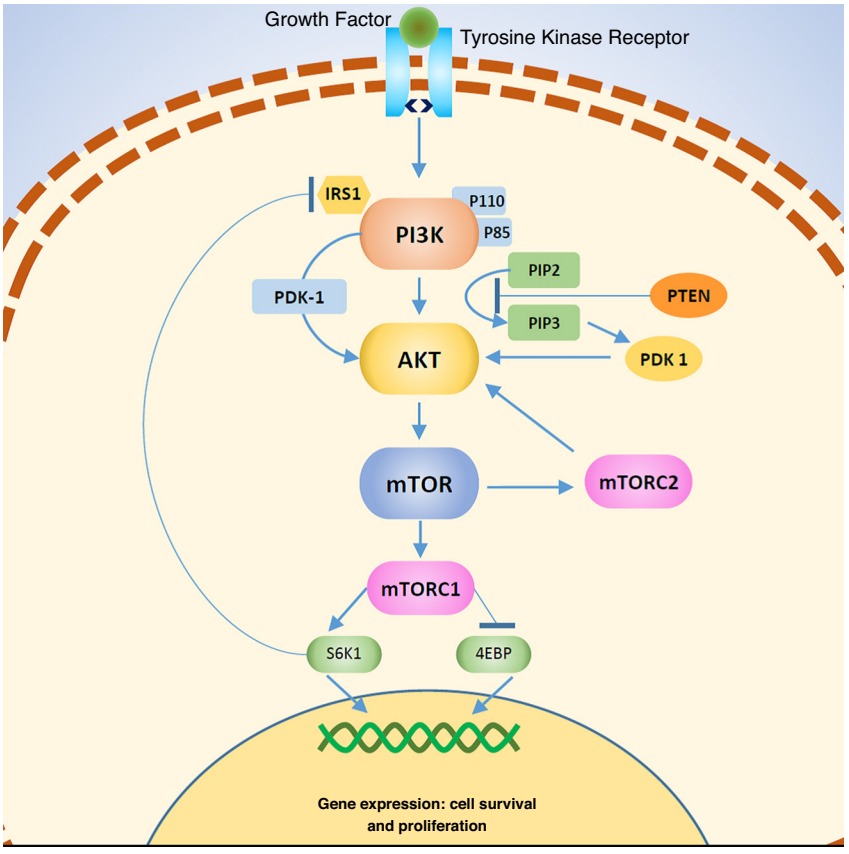

FIGURE 3

Diagrammatic presentation of the PI3K/AKT/mTOR pathway.

and mTOR, there are concerns related to increased toxicity, which can hinder further development [43]. A Phase I/III of AKT inhibitor MK2206, which included stage I-III TNBC cases, was to be suspended owing to development of hyperglycaemia, pruritus, fever, rash and mucositis despite reduction of dose twice [44].

A further setting in which the PI3K–AKT inhibitors can prove their usefulness could be in association with PARP inhibitors (PARPis) in TNBC patients who did not exhibit BRCA1/2 function loss [45]. This is because PARP has been found to be active in tumors showing alterations in the BRCA1/2 genes leading to deficient homologous recombination (HR) mechanisms [46]. However, apart from upstream inhibition, multiple mediators within the PI3K signaling cascade provide the advantage of multiple drug-specific targets with relatively safer therapy. Several PI3K pathway inhibitors have been developed in the past decade.

There are not many reports on clinical studies with mTOR-specific inhibitors in TNBC, although, in preclinical studies, inhibitors against mTOR could also arrest growth in patient-derived xenograft TNBC models. Further, preclinical reports of breast cancer cells with basal-like expression have been found sensitive to everolimus [47]. The mTOR inhibitor rapamycin was the first inhibitor of the PAM pathway to reach the clinical trials stage. Activity of mTOR inhibitor temsirolimus was only modest in unselected metastatic breast cancer cases [48].

Cases of PIK3CA mutation or PTEN loss where the conformation of AKT is unchanged can be the potential target for allosteric inhibitors of AKT that bind to the PH domain. MK2206 is one such allosteric inhibitor. However, in cases involving disruption of the PH domain or AKT3 fusion, AKT-kinase inhibitors related to GSK690693 could be the potential agents [49]. In a recent study, three antipsychotic drugs belonging to the phenothiazine category, including fluphenazine, trifluoperazine and thioridazine, showed considerable inhibition of growth, proliferation, viability, cell invasion and metastasis, as well as enhanced apoptosis in TNBC cells, by decreasing PAM and ERK signaling [12].

Apart from TNBC, the PAM inhibitors have been evaluated in other types of breast cancers as well. Preclinical studies using copanlisib monotherapy reported that 100% complete tumor regression rate was achieved in HER2-amplified, PI3KCA-mutant KPL4 breast tumors. The allosteric inhibitor MK2206 has also been under study as mono and combination therapy against HER2-positive types. Further, AZD5363 is also being studied for its potential against ER-positive breast cancers. Similarly, another study reported a positive effect of mTOR inhibitor ridaforolimus in HER2-positive breast cancer, wherein 41% patients achieved stable disease when it was combined with trastuzumab. The studies on the PAM pathway inhibitors against various types of breast cancer have been reviewed by Kenna *et al.* [50]. From the above discussion, it could be concluded that systematically designed preclinical and clinical studies investigating PAM inhibitors in treatment

of TNBC could lead to some positive and promising treatment strategies.

PAM-inhibitor-based combination therapy in TNBC: opportunities and challenges

Despite promising potential of PAM pathway inhibitors in TNBC, effectiveness of these agents is limited by multiple factors like mutations or copy number changes in genes such as TP53, MYC and RB. Further, single-agent therapy with inhibitors of the PAM pathway has not been totally successful. Yuan *et al.* [51] reported that, although the PAM pathway is commonly altered in TNBC patients, substantial therapeutic efficacy was not observed with the use of a PAM inhibitor alone. BYL719, a PAM inhibitor, was found effective in restraining growth of T47D breast cancer cells which are positive for PR, ER and HER2 amplification. By contrast, BYL719 showed restricted efficiency in inhibiting proliferation of TNBC cells attributed to limited inhibition of p-S6 and permanent phosphorylation of p-RB. A combination of LEE011, CDK4/6 inhibitor, with BYL719 showed significant obstruction of TNBC proliferation.

Zhang *et al.* [52] reported that the WNT/β-catenin signaling pathway mediated regulation of the PAM pathway and could induce drug resistance of TNBC cells to PI3K inhibitors. PI3K and AKT signaling is the most common oncogenic abnormality observed with TNBC. Some of the randomized trials have shown promising results for combination of first-line chemotherapy with AKT inhibitors in TNBC patients [3]. Moses *et al.* [40] reported that PI3K/mTOR inhibitors showed reduction in migration and growth of mutant melanoma BRAF V600E cells.

CSCs are reported to be involved in the initiation, progression and chemoresistance of cancer cells. Further CSC survival in TNBC is regulated by Notch signaling. It has been reported that resistance to PI3K or mTORC1 inhibitors is the result of Notch-dependent drug-resistant CSCs [5]. Hossain *et al.* [5] concluded from their experimental study that targeting the intersection of the Notch, AKT and NF-κB pathways could be a promising strategy to treat TNBC patients. The ongoing clinical studies involving PAM pathway inhibitors as combination treatment in TNBC are reported in Table 2.

Concluding remarks and future perspectives

TNBC is devoid of three relevant therapeutic targets: ER, PR and HER2. Intra- and inter-tumor heterogeneity of TNBC remain the most significant challenges to the development of targeted therapies. Heterogeneity at the molecular level also translates into pathologic and clinical manifestations. In the advanced settings, the clinical response to such cases remains variable. This could partly be the reason for the unsatisfactory outcome from early trials in unselected TNBC cases treated with newer targeted agents. Recently proposed classification of TNBC into molecule subtypes can be utilized for development of better-targeted therapies. Identification of a newer molecular subtype and drivers that can serve as a therapeutic target needs to be carried out using extensive genomic and molecular analysis in clinical samples. Several advanced gene expression and sequencing technologies are now available; however, widespread utilization of these technologies needs to be promoted. Pathway-specific genomic stratification of patients at the multi-institutional level can also help in developing a connection between the type of TNBC and therapy. Further, the complexity of the disease underscores the fact that no single treatment approach can enhance benefit beyond the conventional therapies. The PAM pathway regulates various physiological and cellular processes in TNBC. Combination of targeted PAM pathway inhibitors and other pathway inhibitors can provide optimum benefit. A recent trial has also emphasized the importance of PAM inhibitors in improving the prognosis of therapy in TNBC. Future research should explore and identify the pathways that can be concomitantly targeted with PAM pathway inhibitors for optimizing outcome in TNBC. Carefully designed preclinical studies using xenografts from newly identified molecular subtypes of TNBC can help achieve this objective.

Conflicts of interest

Authors report no conflicts of interest related to this manuscript.

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GLOSSARY

ER Estrogen receptor
PR Progesterone receptor
HER2 Human epidermal growth factor receptor
TNBC Triple-negative breast cancer
CSCs Cancer stem-like cells
TNF-α Tumor necrosis factor-α
IL-1β Interleukin-1β
GPER G protein-coupled estrogen receptor 1
miR microRNA
EMT Epithelial-mesenchymal transition
PAM PI3K/AKT/mTOR
MAPK Mitogen-activated protein kinase
PARP poly(ADP-ribose) polymerase
EGFR Epidermal growth factor receptor
VEGF Vascular endothelial growth factor
CPT2 Carnitine palmitoyl transferase 2
CPT1 Carnitine palmitoyl transferase 2
SERMs Selective estrogen receptor modulators
SERDs Selective estrogen receptor down regulators
AIs Aromatase inhibitors
PSilQ Polysilsesquioxane
PAMAM Polyamidoamine
PRMT5 Protein arginine methyltransferase 5
TRP Transient receptor potential
HN1L Hematological and neurological expressed 1-like
PI3K Phosphoinositide 3-kinase
RTK Receptor tyrosine kinase

PIP2 4,5-phosphoinositide
IGF-1 Insulin-like growth factor 1
PIP3 3,4,5-phosphoinositide
IRS1 Insulin receptor substrate 1
PTEN Phosphatase and tensin homolog
INPP4B Inositol polyphosphate 4-phosphatase type II
PH Plekstrin homology
mTORC1 mTOR complex 1
mTORC2 mTOR complex 2
TCGA The Cancer Genome Atlas
PD-1 Programmed cell death protein 1
PDL-1 Programmed death-ligand 1
PARP Poly (ADP-ribose) polymerase
HR Homologous recombination
ER Estrogen receptors
PR Progesterone receptor
SPAG5 Sperm-associated antigen 5
STAT Signal transducer and activator of transcription
Chk Checkpoint kinase
TSC $^{1}/_{2}$ Tuberous sclerosis $^{1}/_{2}$
CDK Cyclin-dependent kinases