Abstract

The triple-negative breast cancer (TNBC) subtype, defined clinically by the lack of estrogen, progesterone, and Her2 receptor expression, accounts for 10% to 15% of annual breast cancer diagnoses. Currently, limited therapeutic options have shown clinical benefit beyond cytotoxic chemotherapy. Defining this clinical cohort and identifying subtype-specific molecular targets remain critical for new therapeutic development. The current era of high-throughput molecular analysis has revealed new insights into these targets and confirmed the phosphoinositide 3-kinase (PI3K) as a key player in pathogenesis. The improved knowledge of the molecular basis of TNBC in parallel with efforts to develop new PI3K pathway–specific inhibitors may finally produce the therapeutic breakthrough that is desperately needed.

CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the role of the PI3K signaling pathway in breast cancer, particularly triple-negative breast cancer, and the potential new inhibitors under development against targets in the PI3K pathway.

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Background

The complex network of PI3K signaling in breast cancer

Phosphoinositide 3-kinase (PI3K) is part of a larger family of lipid kinases that phosphorylate the 3-hydroxyl group of phosphoinositides involved in regulation of diverse cellular processes, including cell proliferation, survival, and migration (1). Three classes are known to exist although Class I PI3K are the most frequently mutated in cancer (2). The PI3K are heterodimeric proteins consisting of a catalytic (p110) subunit and regulatory (p85) subunit (1). Figure 1 summarizes some of the key relationships involved in PI3K signaling via AKT and mTOR signaling mediators. Activation of a membrane-associated receptor tyrosine kinase (RTK) by ligand results in the recruitment of the p85 subunit, causing a conformation change allowing the p110 subunit to catalyze 4,5-phosphoinositide (PIP2) phosphorylation to 3,4,5-phosphoinositide (PIP3; ref. 3). Some RTKs require the presence of adaptor molecules (e.g., IRS1) to interact with p85. The tumor suppressors PTEN and INPP4B in turn hydrolyze PIP3 and PIP2, respectively, directly opposing PI3K activity (4, 5). Oncogenic mutations in PIK3CA have been identified in many cancers, including all subtypes of breast cancer (6). The majority of these mutations are within the helical (E545K and E542K) and kinase (H1047R) domains and result in constitutive activation of the kinase (7). Mutations in PIK3CA are also mutually exclusive of PTEN loss in breast tumors (8). The second messenger molecule PIP3 facilitates the colocalization of the serine/threonine kinases PDK1 and AKT to the cell membrane via their plekstrin homology (PH-) domains, leading to downstream PI3K pathway activation (9). PIK3CA mutant cells may contribute to tumor formation by both AKT-dependent and AKT-independent mechanisms (10).

Three AKT isoforms are known to exist (AKT-1, -2, -3), and each has very distinctive roles depending on the specific cell lineage. For a more complete review of AKT isoforms, please refer to the article by Toker (11). AKT1 mutations have only been identified in estrogen receptor (ER)-positive breast tumors, whereas AKT3 is the
dominant isoform expressed in ER-negative tumors (12, 13). AKT activation, either by upstream signals or mutation, alters multiple downstream proteins, including the relief of mTOR repression by the tuberous sclerosis complex (TSC1/2) proteins (14). Activated mTOR forms two unique complexes with other proteins (TORC1 and TORC2; 15). TORC2 containing rictor stimulates further AKT activation (16), whereas TORC1 containing raptor mediates growth-stimulatory effects of mTOR. Multiple feedback mechanisms exist within the PI3K/AKT/mTOR signaling cascade that can complicate therapy. Among these are the activation of PI3K and mitogen-activated protein kinase (MAPK) signaling pathways in the setting of mTOR-specific inhibitors caused by the relief of S6K-mediated repression of IRS1 (17, 18), and reactivation of RTK expression with AKT inhibition (19).

**Defining the triple-negative breast cancer cohort**

Triple-negative breast cancer (TNBC) is defined clinically by the lack of expression of ERs and progesterone receptors and the absence of human EGF2 (Her2) expression/amplification (20, 21). This cohort accounts for 10% to 15% of annual breast cancer diagnoses in a general population and typically presents in younger patients, with more poorly differentiated tumors compared with receptor-positive cases (20). Patients with TNBC are also more likely to experience a recurrence within the first 3 years of diagnosis, usually with distant visceral metastasis, leading to an increased likelihood of breast cancer–specific mortality (21, 22).

Defining TNBC remains a challenge due to significant disease heterogeneity. The classic "triple receptor-negative" phenotype shows significant overlap with, but is not synonymous with, newer breast cancer subtypes defined by gene expression–based classification methods. Perou and colleagues have described four reproducible intrinsic gene expression subtypes: luminal A, luminal B, Her2-enriched, and basal like (23). The luminal subtypes mainly represent ER-expressing epithelial cells, whereas the Her2-enriched subtype reflects HER2 gene activation (23). The basal-like subgroup shows the greatest overlap with TNBC tumors and is associated with the worst prognosis of all the intrinsic
subtypes. A subgroup of TNBC without basal markers has recently been identified. This ‘claudin-low’ group seems to be enriched for mesenchymal and stem cell markers (24). Lehmann and colleagues confirmed the complexity of these tumors at the expression level by showing that TNBC tumors can be further subclassified into six different expression-based cohorts (25). The majority of these cohorts share characteristics of the intrinsic basal-like subtype except a luminal androgen receptor (LAR) cohort of TNBC whose expression profile shows similarity to intrinsic luminal subtypes (25).

The overlap between the clinical immunohistochemistry (IHC) and molecular gene expression cohorts often leads to the labels TNBC and basal like used interchangeably, which is inappropriate given the heterogeneity between these molecular defined cohorts (26). An integrated genomic analysis of 466 breast tumors revealed that 80% of TNBCs were classified as basal like, whereas other common subtypes, mainly the Her2-enriched, represented the remainder (27). The gene expression–based basal-like subtype seems to be more reproducible compared with the IHC-defined TNBC cohort and has been the focus of further genomic exploration as detailed below.

Current treatment of triple-negative breast cancers

Despite lower survival, triple-negative tumors paradoxically show an increased sensitivity to neoadjuvant cytotoxic chemotherapy compared with non-TNBC cases (pathologic complete response rates 22% vs. 11%, respectively; \( P = 0.034; \) ref. 22). These higher responses are seen with both anthracycline and taxane-based regimens used to treat breast cancer and may reflect the higher cell proliferation rates of these tumors (22, 28). In patients who harbor germline mutations in the \textit{BRCA1} gene, greater responses have also been observed with the DNA cross-linking agent cisplatin (29, 30). Patients also seem to benefit from cytotoxic therapy in the adjuvant and metastatic settings, although in the latter group, responses are short lived. The lack of common molecular targets means that less toxic hormonal and targeted therapies that are used for non-TNBC tumors are not available for the treatment of this breast cancer subtype.

The observation of frequent \textit{TP53}, \textit{BRCA1}, and \textit{BRCA2} mutations in TNBC led to the hypothesis that DNA repair, specifically the homologous recombination mechanism used to repair double-stranded DNA breaks, was defective in these tumors. Cells defective in homologous recombination can become dependent on the base excision repair pathway mediated by PARP for DNA repair (31). PARP inhibition produces a synthetic lethal effect in the presence of \textit{BRCA1}/2 mutations resulting in further chromosomal instability, cell-cycle arrest, and apoptosis (31). Early phase I and II clinical trials with PARP inhibitors were encouraging (32, 33), but subsequent phase III studies were less convincing, and the future development of these agents remains unclear. Newer targets for therapy for the TNBC cohort are therefore desperately needed in the clinical setting.

Genomic analysis of breast cancer reveals PI3K pathway aberrations

Defining the genetic heterogeneity of breast cancer has been proposed as key to understanding the pathogenesis of the disease and to identify new targets to guide therapy into the future. Over the past year, several international collaborative groups have reported on high-throughput, genome-scale and cross-platform analyses covering the spectrum of breast cancer expression subtypes (13, 27, 34–36). These studies confirmed that the clinical and molecular heterogeneity of breast cancer is reflected in the cancer genome. However, distinct patterns of genomic alterations have been teased from the data. The most common genes with somatic mutations in breast cancer include \textit{TP53}, \textit{PIK3CA}, \textit{GATA3}, \textit{MAP3K1}, \textit{MLL3}, \textit{CDH1}, \textit{RB1}, \textit{MAP2K4}, \textit{RUNX1}, \textit{PTEN}, \textit{AKT1}, and \textit{CBFB} (13, 27, 34), the majority of which have been previously described in less expansive genomic studies. Only \textit{TP53} and \textit{PIK3CA} mutations are seen at mutation frequencies across expression subtypes exceeding 10% of samples. The remaining mutations, despite occurring at much lower frequencies, had some interesting characteristics. For example, mutations in \textit{MAP3K1}, \textit{MAP2K4}, \textit{GATA3}, \textit{CDH1}, \textit{AKT1}, \textit{RUNX1}, and \textit{CBFB} occurred almost exclusively in luminal and Her2-enriched subtypes associated with hormone receptor–positive disease (13, 27). Also, most mutations in genes with known connections within a cellular pathway (e.g., \textit{PIK3CA}/\textit{AKT1}, \textit{MAP3K1}/\textit{MAP2K4}, or \textit{RUNX1}/\textit{CBFB}) tended to be mutually exclusive, suggesting that pathway disruption rather than the individual gene mutation may be more important (13, 27).

Relative to other expression subtypes, genomic alterations in the basal-like breast cancers appeared to be much more homogeneous. In the study by The Cancer Genome Atlas, 80% of the 98 basal-like tumors harbored mutations in \textit{TP53} (27). Most of these mutations were nonsense or frame shift mutations believed to be more deleterious to \textit{TP53} function. An integrated analysis of datasets suggests that \textit{TP53} function is lost in all basal-like cancers (27). Furthermore, contributing to abnormalities in cell-cycle regulation and DNA repair were loss of genes \textit{RB1}, \textit{BRCA1}, \textit{BRCA2}, and \textit{ATM} (27, 36). These findings are consistent with the observation that basal-like tumors show the greatest genomic instability of all subgroups (13, 27, 36) and provide a partial explanation for why these tumors are more sensitive to DNA-damaging agents. Interestingly, the basal-like expression subtype also had the highest activation of downstream members of the PI3K signaling pathway as determined by both gene expression levels and proteomic arrays (27). Genomic aberrations observed in the PI3K pathway in basal-like tumors include loss/mutation of \textit{PTEN}, loss of \textit{INPP4B}, amplification/mutation of \textit{PIK3CA} (the gene encoding the p110 catalytic subunit of the PI3K), and activating translocations involving the genes \textit{MAGI3} (a scaffolding protein required for \textit{PTEN} activity) and \textit{AKT3} (13, 27). The \textit{PTEN} and \textit{PIK3CA} mutations occur early in breast tumor initiation and seem to be present in dominant tumor clones (35, 37). The \textit{PIK3CA} and \textit{PTEN} mutations in TNBC tumors seem to be more
common in the Pietenpol mesenchymal-like and LAR expression subtypes (25).

Clinical–Translational Advances

Emergence of several PI3K pathway inhibitors

The presence of multiple mediators within the PI3K signaling pathway means that there are several opportunities to drug-specific targets to achieve cell arrest and limit toxicity of therapy. Multiple inhibitors have emerged over the past decade and have been the focus of clinical trials. Table 1 lists some ongoing clinical trials testing the use of PI3K pathway inhibitors in breast cancer cohorts that would include patients with TNBC.

The first drugs to enter clinical trials were the mTOR inhibitors rapamycin and its analogues. Temsirolimus has shown only modest activity in unselected patients with metastatic breast cancer (38). Everolimus has shown clinical benefit in 34% of trastuzumab-resistant patients treated with a combination of everolimus and trastuzumab and has also shown significant improvement in progression-free survival in combination with aromatase inhibition in previously resistant hormone receptor–positive patients (39, 40). Common adverse effects of these therapies include mucositis, rash, nausea, diarrhea, and hyperglycemia, the latter likely a result of PI3K signaling also being important for insulin sensitization. Clinical trials with mTOR-specific inhibitors in TNBC have not yet been reported although in the preclinical setting, tumors with basal-like expression are more sensitive to everolimus inhibition (41). Several trials with mTOR inhibitors alone or in combination with cytotoxic or targeted therapies are currently accruing (Table 1).

PI3K inhibitors related to wortmannin are relatively new to the clinical scene. First-generation inhibitors like BKM120 inhibit all class I PI3K but have no activity against mTOR. A phase I clinical trial in patients with a variety of cancers showed one confirmed partial response to this agent in a patient with TNBC. The most common toxicities reported with this agent have been rash, hyperglycemia, mood alteration, epigastralgia, nausea, fatigue, mucositis, and pruritis (42). Other related compounds, such as BAY 80-6946, GDC-0941, PX-866, and XL147, have been evaluated in phase I trials and have a similar toxicity profile to that of BKM120 (43–46).

Newer dual inhibitors targeting both PI3K and mTOR have arrived in the clinic. Agents like BEZ235, GDC-0980, GSK458, SF1126, and XL765 have all shown evidence of tolerability and target inhibition although specific responses in breast tumors have yet to be seen (47–51). The side effects of the dual inhibitors overlap those seen with PI3K and mTOR-specific agents. TNBC cell lines having mesenchymal-like or LAR expression profiles with frequent PIK3CA mutations and PTEN loss appear more sensitive to dual inhibitors like BEZ235 compared with other TNBC expression subtypes (25).

Table 1. Ongoing clinical trials with PI3K pathway inhibitors that include a TNBC cohort (clinicaltrials.gov)

<table>
<thead>
<tr>
<th>Target</th>
<th>Study drugs</th>
<th>Design</th>
<th>N*</th>
<th>Breast subtype</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTOR Inhibitors</td>
<td>Temsirolimus + cisplatin/erlotinib</td>
<td>Phase I</td>
<td>18</td>
<td>MBC TNBC</td>
<td>NCT00998036</td>
</tr>
<tr>
<td></td>
<td>Temsirolimus + peg-liposomal doxorubicin</td>
<td>Phase I</td>
<td>30</td>
<td>MBC</td>
<td>NCT00982631</td>
</tr>
<tr>
<td></td>
<td>Temsirolimus + neratinib</td>
<td>Phase II</td>
<td>65</td>
<td>MBC TNBC or Her2-</td>
<td>NCT01111825</td>
</tr>
<tr>
<td></td>
<td>Temsirolimus + IMC-A12</td>
<td>Phase II</td>
<td>68</td>
<td>MBC</td>
<td>NCT00699491</td>
</tr>
<tr>
<td></td>
<td>Everolimus + abraxane</td>
<td>Phase II</td>
<td>72</td>
<td>MBC Her2-</td>
<td>NCT00934895</td>
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<tr>
<td></td>
<td>Everolimus + lapatinib</td>
<td>Phase II</td>
<td>43</td>
<td>MBC TNBC</td>
<td>NCT01272141</td>
</tr>
<tr>
<td></td>
<td>Everolimus + FEC/paclitaxel</td>
<td>Phase II</td>
<td>62</td>
<td>Neoadjuvant stage II–III TNBC</td>
<td>NCT00499603</td>
</tr>
<tr>
<td></td>
<td>Everolimus</td>
<td>Phase II</td>
<td>50</td>
<td>Neoadjuvant stage I–III after chemotherapy</td>
<td>NCT01088893</td>
</tr>
<tr>
<td>Par PI3K inhibitors</td>
<td>Everolimus + cisplatin/paclitaxel/bevacizumab</td>
<td>Phase II</td>
<td>120</td>
<td>Neoadjuvant stage II–III TNBC</td>
<td>NCT00930930</td>
</tr>
<tr>
<td></td>
<td>GDC-0941 + paclitaxel/bevacizumab</td>
<td>Phase I</td>
<td>24</td>
<td>Locally recurrent or MBC</td>
<td>NCT00960960</td>
</tr>
<tr>
<td></td>
<td>BKM120 + olaparib</td>
<td>Phase I</td>
<td>56</td>
<td>TNBC or high-grade serous ovarian cancer</td>
<td>NCT01623349</td>
</tr>
<tr>
<td>Dual PI3K + mTOR inhibitors</td>
<td>BKM120</td>
<td>Phase II</td>
<td>50</td>
<td>MBC TNBC</td>
<td>NCT01629615</td>
</tr>
<tr>
<td></td>
<td>BEZ235 + paclitaxel</td>
<td>Phase II</td>
<td>230</td>
<td>MBC Her2-</td>
<td>NCT01495247</td>
</tr>
<tr>
<td>AKT Inhibitors</td>
<td>MK2206</td>
<td>Phase II</td>
<td>30</td>
<td>Neoadjuvant stage I–III</td>
<td>NCT01319539</td>
</tr>
<tr>
<td></td>
<td>MK2206</td>
<td>Phase II</td>
<td>40</td>
<td>MBC with PIK3CA mutation or PTEN loss</td>
<td>NCT01277757</td>
</tr>
</tbody>
</table>

NOTE: This list is restricted to currently recruiting trials. Abbreviations: FEC, 5-fluorouracil, etoposide, and cyclophosphamide; MBC, metastatic breast cancer. N represents the goal for recruitment.
Inhibitors of AKT add to the spectrum of drugs targeting the PI3K signaling pathway. Allosteric inhibitors that bind to the PH-domain of AKT like MK2206 may have utility in cases of PTEN loss or PIK3CA mutation where the protein conformation of AKT remains intact (52). However, AKT-kinase domain inhibitors related to GSK690693 may have a greater role when the PH-domain itself is disrupted such as with the common E17K AKT1 mutations and the reported AKT3 fusion (13).

Combination therapy holds the key for TNBC

Use of PI3K pathway inhibitors as single-agent therapies has proved minimally effective in some diseases. As shown in Fig. 1, the complexity of the PI3K pathway with multiple feedback mechanisms explains some of the drug resistance. Combination drug therapies that simultaneously target these escape mechanisms will likely lead to the greatest clinical success. mTOR inhibition combined with either antiestrogen or anti-Her2 therapies has already shown success in clinical trials (38–40). Numerous other examples of preclinical models of success have been shown in TNBCs. BRCA1-deficient cell lines treated with a PARP inhibitor followed sequentially by dual PI3K/mTOR inhibitor LY294002 showed the greatest inhibition of growth compared with either drug alone (53). Similarly, primary breast tumor xenografts treated with BKM120 are sensitized to PARP inhibition (54). However, even resistance to this combination was observed in some TNBC xenografts, which could only be overcome with the addition of the MAP–ERK kinase inhibitor AZD6244 (54). Similar preclinical TNBC models have also shown the synergistic effect of combining EGF receptor (EGFR) and mTOR inhibition (55). These observations still need to be tested in the clinical setting. Targeted monotherapy against RTKs EGFR, fibroblast growth factor receptor (FGFR), and insulin-like growth factor I receptor (IGF-1R) have shown only limited success in TNBC to date. It is conceivable that with either a more biomarker-driven therapeutic approach or combined with PI3K inhibitors, further therapeutic potential can be achieved (56–58). Although all these combinatorial treatments have theoretical merit, these approaches are often limited by the side effect profiles of each drug and whether the patient can tolerate the combined toxicity profile.

Answers to these questions lie only in carefully planned and executed clinical trials.

Challenges going forward

Intra- and intertumor heterogeneity continues to be a significant challenge in the era of targeted therapy development. Target discovery and routine identification in clinical samples are thus critical elements to modern cancer care. The complexity of TNBC at the clinical and genetic levels illustrates that no single treatment approach will produce universal benefit in this disease beyond what has already been accomplished with broad-spectrum cytotoxic agents. Therapies tied to specific biomarkers, along with the more rational use of targeted therapies, remains the key to improving clinical outcomes for patients with TNBC. Inhibitors specific to components of the PI3K pathway combined with inhibition of other proliferation pathway targets will likely yield the best results. Critical to the use of these drugs are tools to accurately and rapidly identify patients with the specific molecular biomarker that predicts response to the chosen therapy. Many of these tools, including gene expression arrays and next-generation sequencing instruments, are starting to be available through clinical laboratories, but more widespread deployment of these methods will be needed in the future. To parallel these efforts, individuals associated with the diagnosis and treatment of TNBC, and cancer in general, need to be more dynamic so that appropriate therapies can be initiated at the optimal time for individual patients.

Authors’ Contributions

Conception and design: S. Banerji
Development of methodology: S. Banerji
Writing, review, and/or revision of the manuscript: V. Gordon, S. Banerji

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References

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