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REVIEW ARTICLE

Advances in Targeted Therapies for Triple-Negative Breast Cancer

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Abstract
While the outcomes for patients diagnosed with hormone receptor positive (HR+) and/or human epidermal growth factor receptor 2-positive (HER2+) breast cancers have continued to improve with the development of targeted therapies, the same cannot be said yet for those affected with triple-negative breast cancer (TNBC). Currently, the mainstay of treatment for the 10–15% of patients diagnosed with TNBC remains cytotoxic chemotherapy, but it is hoped that through an enhanced characterization of TNBC biology, this disease will be molecularly delineated into subgroups with targetable oncogenic drivers. This review will focus on recent therapeutic innovations for TNBC, including poly-ADP-ribosyl polymerase (PARP) inhibitors, phosphoinositide 3-kinase (PI3K) pathway inhibitors, immune checkpoint inhibitors, and cyclin-dependent kinase (CDK) inhibitors.

Key Points

Cytotoxic chemotherapy remains the mainstay of systemic therapy for most patients with triple-negative breast cancer (TNBC).

Predictive biomarkers have identified subsets of TNBC patients that may respond best to certain targeted therapies and immunotherapies.

Identification of new drug targets and more precise predictive biomarkers are intense areas of clinical and translational research in TNBC.

1 Introduction

Triple-negative breast cancers (TNBCs) are simply defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), and so are a diverse set of malignancies united only by the absence of readily available targeted therapies such as hormone blockade and HER2-specific monoclonal antibodies. Though only about 10–15% of primary breast cancers are triple negative, TNBCs account for a disproportionate number of patient deaths, with a breast-cancer-specific 5-year survival after diagnosis (all stages) of about 83% for TNBC compared with 96% for hormone receptor positive (HR+), HER2-normal (HER2-) breast cancers, 94% for HR+ HER2-positive (HER2+) disease, and 89% for hormone receptor negative (HR−), HER2+ breast cancer based on 2010–2015 statistics available in the Surveillance, Epidemiology, and End Results (SEER) database for female patients with operable invasive breast cancer [1]. Overall, prognoses for women and men with breast cancer are improving with early detection and the development of targeted therapies. This is strong motivation to define subtypes of TNBC, to find their oncogenic drivers, and to develop targeted therapeutic strategies.

Based on gene expression analysis of over 500 TNBCs, six subtypes of TNBC have been proposed, including basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor subtypes [2]. The two basal-like subtypes were characterized by increased proliferation rates, loss of cell-cycle checkpoints, genomic instability, and sensitivity to platinum agents. *BRCA1* and *BRCA2*-deficient breast cancers tend to fall into the basal-like category. The immunomodulatory group was defined by increased expression of immune cell

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signaling processes, such as cytokine signaling and antigen presentation. The mesenchymal subtypes included metaplastic TNBCs and were enriched for activating *PIK3CA* mutations; many drugs targeting the PI3K/AKT/mTOR (phosphoinositide-3 kinase, Akt = protein kinase B, mammalian target of rapamycin) pathway are in clinical development. Cell lines of the luminal androgen receptor (LAR) subtype tended to be sensitive to AR-targeting agents such as bicalutamide. Another research group defined four TNBC subtypes based on DNA and RNA analysis of 198 TNBCs: basal-like immunosuppressed, basal-like immune-activated, mesenchymal, and luminal androgen receptor subtypes [3]. In this review, we will discuss recent research relating to targeted agents for TNBC, including poly-ADP-ribosyl polymerase (PARP) inhibitors, PI3K pathway inhibitors, immune checkpoint inhibitors, and cyclin-dependent kinase (CDK) inhibitors. Summaries of these studies are highlighted in Table 1 with common adverse events summarized in Table 2.

2 Poly-ADP-Ribosyl Polymerase (PARP) Inhibitors

2.1 PARP Biology

PARP-1 and PARP-2 recognize and bind to sites of DNA damage, predominantly during S-phase when DNA is exposed for replication, and catalyze the conversion of nicotinamide adenine dinucleotide (NAD+) into chains of adenosine diphosphate (ADP) on target proteins for the purposes of DNA damage repair [4, 5]. In addition to inhibition of RNA polymerases and activation of the G2/M checkpoint, poly-ADP-ribosylation (PARylation) of histones relaxes the chromatin, and DNA repair proteins are recruited to sites of damage by poly-ADP-ribosyl (PAR)-binding motifs [5–7]. PARP inhibitors are small molecule mimetics of nicotinamide that reversibly bind to the NAD+ site of PARP-1 and PARP-2, preventing PARylation and thus DNA repair processes [8–10]. PARP inhibitors have also been demonstrated to trap PARP-1 on DNA by preventing the auto-PARylation event required for PARP-1 to change configuration and unbind DNA [4, 11, 12], resulting in stalled replication forks with collapse into lethal DNA double-strand breaks (DSBs) during S-phase [13]. This may be one of the reasons why PARP inhibitors appear to be most effective in tumors with defects in homologous recombination repair, including breast and ovarian cancers with deleterious mutations in *BRCA1* and *BRCA2*, as homologous recombination repair predominates over non-homologous end-joining during S-phase as a relatively error-proof mechanism of repairing DNA DSBs. It is important to note that patients with deleterious *BRCA1* mutations more commonly develop TNBCs than HR+ HER2− breast cancers, while patients with deleterious *BRCA2* mutations more commonly develop HR+ HER2− breast cancers than TNBC.

2.2 PARP Inhibitor Clinical Trials

Thus far, two PARP inhibitors (PARPi)—olaparib and talazoparib—have been approved by the United States Food and Drug Administration (FDA) for use in women and men with deleterious germline *BRCA1* or *BRCA2* (g*BRCA1/2*+) mutations and metastatic HER2− breast cancer based on the phase III OlympiAD [14, 15] and EMBRACA [16] trials.

For the OlympiAD trial (NCT02000622), patients with g*BRCA1/2*+, metastatic breast cancer were randomized two to one (2:1) to olaparib 300 mg by mouth twice daily versus physician's choice of chemotherapy (the choices being capecitabine, eribulin, or vinorelbine). Patients had to have received prior therapy with an anthracycline and a taxane (adjuvant or metastatic setting), but no more than two prior lines of cytotoxic chemotherapy in the metastatic setting. Prior exposure to platinum agents was allowed providing the patients had not progressed on platinum therapy. The primary outcome of median progression-free survival (mPFS) by blinded independent central review (BICR) was 7.4 months with olaparib ($n = 205$) versus 4.2 months with cytotoxic chemotherapy ($n = 97$) with a hazard ratio (HR) of 0.58 and a 95% confidence interval (CI) of 0.43–0.80 ($p < 0.001$). In the triple-negative subgroup ($n = 150$), the mPFS HR was 0.43 (95% CI 0.29–0.63) for patients treated with olaparib ($n = 102$) versus chemotherapy ($n = 48$). The overall response rate (ORR) was 59.9% (100/167) for the patients taking olaparib versus 28.8% for chemotherapy (19/66). The overall survival (OS) was not significantly different for the two arms (19.3 months for olaparib versus 19.6 months for chemotherapy, HR 0.90 with 95% CI 0.63–1.29; $p = 0.57$) in the initial analysis published in 2017 [14] or the final analysis published in 2019 (19.3 months for olaparib versus 17.1 months for chemotherapy, HR 0.90 with 95% CI 0.66–1.23; $p = 0.513$) [15], but there was an OS advantage for patients who had not previously been treated with chemotherapy in the final analysis published in 2019. Patients who had received no prior chemotherapy for metastatic breast cancer had a median OS of 22.6 months with olaparib ($n = 30$) versus 14.7 months with chemotherapy ($n = 21$) (HR 0.51; $p = 0.02$). There was no significant difference in OS for patients treated with olaparib versus chemotherapy in the HR+, triple-negative, chemotherapy-exposed, platinum-exposed, or platinum-naïve subgroups, but OlympiAD was not powered to detect OS differences. It is important to note that grade 3 and 4 toxicities were less common with olaparib than chemotherapy (36.6% compared with 50.5%), suggesting an improvement in quality of life that is important to

Table 1 Clinical trials under discussion in manuscript

Drug class	Agent under investigation	Trial	Patient population	Intervention	Outcome measures
PARP inhibitor	Olaparib	OlympiAD [14, 15] NCT02000622 Phase III Randomized 2:1 ($n = 302$)	g*BRCA1/2*+ with metastatic HER2− breast cancer Prior anthracycline and taxane (any setting) required ≤ 2 cytotoxics in metastatic setting	Olaparib 300 mg po bid ($n = 205$) vs Cytotoxic chemotherapy with capecitabine, eribulin, or vinorelbine ($n = 97$)	mPFS (BICR) 7.4 mo ($n = 205$) vs 4.2 mo ($n = 97$) (HR 0.58, $p < 0.001$) ORR 59.9% (100/164) vs 28.8% (19/66) OS (2017 initial analysis) 19.3 mo vs 19.6 mo (HR 0.90, $p = 0.57$) OS (2019 final analysis) 19.3 mo vs 17.1 mo (HR 0.90, $p = 0.513$)
		Pre-specified sub-group:	TNBC ($n = 150$)	Olaparib ($n = 102$) vs chemo ($n = 48$)	mPFS HR 0.43 (95% CI 0.29–0.63) OS 17.4 mo ($n = 72$) vs 14.9 mo ($n = 33$) HR 0.93 (95% CI 0.62–1.43)
PARP inhibitor	Talazoparib	EMBRCA [16] NCT0195775 Phase III Randomized 2:1 ($n = 431$)	g*BRCA1/2*+ with advanced unresectable or metastatic HER2− breast cancer ≤3 cytotoxics in metastatic setting	Talazoparib 1 mg po daily ($n = 287$) vs Cytotoxic chemotherapy with capecitabine, gemcitabine, eribulin, or vinorelbine ($n = 144$)	mPFS (BICR) 8.6 mo vs 5.6 mo (HR 0.54, $p < 0.0001$) ORR 62.6% (137/219) vs 27.2% ($n = 39/144$)
		Pre-specified sub-group:	TNBC ($n = 190$)	Talazoparib ($n = 130$) vs chemo ($n = 60$)	mPFS HR 0.60 (95% CI 0.41–0.87)
PARP inhibitor	Veliparib	BrighTNess [22] NCT02032277 Phase III neoadjuvant Randomized 2:1:1 ($n = 634$)	Stage II or III TNBC	Paclitaxel + carboplatin + veliparib 50 mg* po bid continuously ($n = 316$) vs Paclitaxel + carboplatin + placebo ($n = 160$) vs Paclitaxel + placebo ($n = 158$) (all arms followed by doxorubicin + cyclophosphamide)	pCR 53% (168/316) vs 58% (92/160) vs 31% (49/158) (PCV vs P $p < 0.0001$; PCV vs PC $p = 0.36$)
PI3K inhibitor	Ipatasertib	LOTUS [35] NCT02162719 Phase II Randomized 1:1 ($n = 124$)	Advanced TNBC	Paclitaxel + ipatasertib 400 mg po daily on D1–21 of a 28-day cycle ($n = 62$) vs Paclitaxel + placebo ($n = 62$)	mPFS 6.2 mo vs 4.9 mo (HR 0.60, $p = 0.037$) ORR 40% (25/62) vs 32% (20/62)
		Pre-specified sub-groups:	PTEN-low with IHC 0 in ≥50% of tumor cells ($n = 48$)		mPFS 6.2 mo vs 3.7 mo (HR 0.59, $p = 0.18$) ORR 48% (12/25) vs 26% (6/23)

Table 1 (continued)

Drug class	Agent under investigation	Trial	Patient population	Intervention	Outcome measures
			PIK3CA / AKT / PTEN genetic alterations ($n = 42$)		mPFS 9.0 mo vs 4.9 mo (HR 0.44, $p = 0.041$) ORR 50% (13/26) vs 44% (7/16)
PI3K inhibitor	Buparlisib	BELLE-4 [36] NCT01572727 Phase II Randomized 1:1 ($n = 416$ with 99 TNBC)	Metastatic HER2− breast cancer	Paclitaxel + buparlisib 100 mg po daily ($n = 207$) vs Paclitaxel + placebo ($n = 209$)	mPFS 8.0 mo vs 9.2 mo (HR 1.18, 95% CI 0.82–1.68) ORR 22.6% (38/168) vs 27.1% (46/170)
		Pre-specified sub-groups:	PI3K / AKT pathway activation by PTEN loss (IHC 1+ in ≤10% of cells positive by IHC) or activating mutations in *PIK3CA* in exons 1, 7, 9, or 20		mPFS 9.1 mo ($n = 73$) vs 9.2 mo ($n = 74$) (HR 1.17, 95% CI 0.63–2.17) ORR 22.6% (14/62) vs 27.0% (17/63)
			TNBC ($n = 90$)		mPFS 5.5 mo ($n = 50$ with 17 PI3K-activated) vs 9.3 mo ($n = 40$ with 19 PI3K-activated) (HR 1.86, 95% CI 0.91–3.79)
Immune checkpoint inhibitor	Atezolizumab (PD-L1 inhibitor)	IMpassion130 [43] NCT02425891 Phase III Randomized 1:1 ($n = 902$)	Metastatic TNBC Untreated in metastatic setting (first line)	Nab-paclitaxel + atezolizumab 840 mg IV D1 and D15 of a 28-day cycle ($n = 451$) vs Nab-paclitaxel ($n = 451$)	mPFS 7.2 mo vs 5.5 mo (HR 0.80, $p = 0.002$) ORR 56.0% (252/450) vs 45.9% (206/449) OS 21.3 mo vs 17.6 mo (HR 0.84, $p = 0.08$)
		Pre-specified sub-group:	PD-L1-expressing tumors (≥1% of tumor cells positive by IHC)		mPFS 7.5 mo vs 5.0 mo (HR 0.62, $p < 0.001$) ORR 58.9% (109/185) vs 42.6% (78/183) OS 25.0 mo vs 15.5 mo (HR 0.62, 95% CI 0.45–0.86)
Immune checkpoint inhibitor	Durvalumab (PD-L1 inhibitor)	GeparNuevo [45] NCT02685059 Phase II neoadjuvant Randomized 1:1 ($n = 174$)	Resectable TNBC	Anthracycline-based chemotherapy + durvalumab 1.5 g IV q4 weeks ($n = 88$) vs Anthracycline-based chemotherapy + placebo ($n = 86$)	pCR 53.4% (47/88) vs 44.2% (38/86) ($p = 0.287$)
		Pre-specified sub-groups:	'Window sub-group' with lead-in of durvalumab 2 weeks prior to chemo ($n = 117$)	Durvalumab lead-in to chemo vs placebo lead-in to chemo	pCR 61% vs 41.4% ($p = 0.048$)

Table 1 (continued)

Drug class	Agent under investigation	Trial	Patient population	Intervention	Outcome measures
			PD-L1-expressing tumors (≥1% of tumor cells or TILs positive by IHC) ($n = 138$)	Durvalumab vs placebo	pCR 58.0% for PD-L1 positive (44.4% for PD-L1 negative, $p = 0.445$) vs 50.7% for PD-L1 positive (18.2% for PD-L1 negative, $p = 0.061$)
CDK 4/6 inhibitor	Palbociclib	UPCC 03909 [58] NCT01037790 Phase II Open label ($n = 37$)	*RB1* wild-type metastatic breast cancer Unlimited prior therapies allowed	Palbociclib 125 mg po daily, D1–21 of a 28-day cycle	mPFS 3.7 mo (95% CI 1.9–5.1)
		Pre-specified sub-group:	TNBC ($n = 4$); enrollment of TNBC halted after all 4 progressed rapidly		mPFS 1.5 mo (95% CI 0.62 to infinity)
Trop-2 antibody drug conjugate	Sacituzumab govitecan-hziy (topoisomerase-1 inhibitor SN-38)	NCT01631552 [65] Phase I/II Open label ($n = 108$)	Advanced TNBC ≥2 prior cytotoxic agents in metastatic setting Although not a requirement for enrollment, 88% of patients had moderate to strong expression of Trop-2 by IHC	Sacituzumab govitecan 10 mg/kg D1 and D8 of 21-day cycle	mPFS 5.5 mo (95% CI 4.1–6.3) ORR 33.3% (36/108) with 3 pCR OS 13.0 mo (95% CI 11.2–13.7)

BICR blinded independent central review, *bid* twice daily, *CI* confidence interval, *D* day, *gBRCA1/2+* deleterious germline mutations in *BRCA1* or *BRCA2*, *HER2−* human epidermal growth factor receptor 2 normal, *HR* hazard ratio, *IHC* immunohistochemistry, *ITT* intention to treat, *IV* intravenous, *mo* month, *mPFS* median progression-free survival, *n* number, *ORR* overall response rate, *OS* overall survival, *PARP* poly-ADP-ribosyl polymerase, *pCR* pathologic complete response, *po* per os (by mouth), *PTEN* phosphatase and tensin homolog, *q* every, *RB1* retinoblastoma gene, *RCT* randomized, placebo-controlled trial, *TILs* tumor-infiltrating lymphocytes, *TNBC* triple-negative breast cancer, *vs* versus

*Monotherapy dose of veliparib is 400 mg po bid

Table 2 Common adverse reactions of select therapies

Drug (trial)	Class	Common AEs: all grades (≥ 20%)	Common grade 3/4 AEs (≥ 5%)
Olaparib (OlympiAD) [14]	PARP inhibitor	Nausea (58%) Anemia (40%) Fatigue (29%) Neutropenia (27%) Diarrhea (21%) Headache (20%)	Anemia (16%) Neutropenia (9%)
Talazoparib (EMBRACA) [16]	PARP inhibitor	Anemia (53%) Neutropenia (35%) Thrombocytopenia (27%) Fatigue (50%) Nausea (49%) Headache (33%) Alopecia (25%) Vomiting (25%) Diarrhea (22%) Constipation (22%) Decreased appetite (21%) Back pain (21%)	Anemia (39%) Neutropenia (21%) Thrombocytopenia (15%) Leukopenia (7%)
Ipatasertib + paclitaxel (LOTUS) [35]	AKT inhibitor	Diarrhea (93%) Alopecia (54%) Nausea (49%) Vomiting (28%) Neuropathy (26%) Fatigue (26%) Rash (26%) Asthenia (25%) Myalgia (25%) Neutropenia (21%) Decreased appetite (21%)	Diarrhea (23%) Neutropenia (10%) Neuropathy (5%) Pneumonia (5%)
Buparlisib (BELLE-4) [36]	Pan-PI3K inhibitor	Diarrhea (55%) Alopecia (51%) Rash (43%) Nausea (41%) Hyperglycemia (41%) Fatigue (33%) Decreased appetite (32%) Neutropenia (31%) Stomatitis (28%) Depression (25%) Peripheral neuropathy (25%) Asthenia (24%) Constipation (23%) Anemia (23%) Anxiety (20%)	Neutropenia (15%) Hyperglycemia (9%) Rash (8%) Increased ALT (7%) Fatigue (6%) Diarrhea (5%) Alopecia (5%)
Atezolizumab + nab-paclitaxel (IMpassion130) [43]	PD-L1 inhibitor	Alopecia (56%) Nausea (46%) Cough (25%) Neuropathy (22%)	Neutropenia (8%) Neuropathy (6%)

Table 2 (continued)

Drug (trial)	Class	Common AEs: all grades (≥ 20%)	Common grade 3/4 AEs (≥ 5%)
Sacituzumab govitecan [65]	Trop-2 ADC	Nausea (67%) Neutropenia (64%) Diarrhea (62%) Fatigue (55%) Anemia (50%) Vomiting (49%) Alopecia (36%) Constipation (34%) Decreased appetite (30%) Rash (28%) Abdominal pain (25%) Hyperglycemia (24%) Leukopenia (21%) Headache (21%) Respiratory infection (21%) Back pain (21%) Urinary tract infection (20%) Dizziness (20%)	Neutropenia (42%) Anemia (11%) Leukopenia (11%) Hypophosphatemia (9%) Febrile neutropenia (8%) Fatigue (8%) Diarrhea (8%) Nausea (6%) Vomiting (6%)

ADC antibody-drug conjugate, *AE* adverse event, *ALT* alanine aminotransferase

consider for metastatic patients. The most common toxicities for olaparib and other PARP inhibitors are myelosuppression and gastrointestinal toxicities (Table 2).

The EMBRCA trial (NCT01945775) randomized g*BRCA1/2*+ patients with advanced unresectable or metastatic breast cancer 2:1 to talazoparib 1 mg by mouth daily versus physician's choice of chemotherapy (capecitabine, gemcitabine, eribulin, or vinorelbine) after no more than three prior cytotoxic regimens in the metastatic setting, but with no limitations on prior targeted therapies (e.g., hormone blockade, CDK 4/6 inhibitors, tyrosine kinase inhibitors, monoclonal antibodies). By BICR, mPFS was 8.6 months for patients given talazoparib versus 5.6 months for those treated with chemotherapy (HR 0.54, $p < 0.0001$). In the TNBC subgroup ($n = 190$), the mPFS HR was 0.60 (95% CI 0.41–0.87). The ORR in the talazoparib arm was 62.6% ($n = 219$), including 12 complete remissions (CRs), versus 27.2% ($n = 144$, no CRs) with cytotoxic agents. Patients noted a slower decline in their overall health as assessed by the EORTC QLQ-C30 questionnaire [17, 18] despite greater grade 3 and 4 myelosuppressive toxicities with talazoparib than chemotherapy (55% compared with 39%) [19]. Although much has been made of talazoparib being the most potent of the PARP inhibitors in terms of half maximal inhibitory concentration (IC50) in catalytic inhibition studies and PARP trapping activity in vitro, it remains to be seen if this is of clinical consequence, as increased potency seems to translate into decreased tolerability in humans [20].

Clinical trials combining PARP inhibitors with other therapies are ongoing. It should be noted that due to the dose-limiting myelosuppressive toxicities of PARP inhibitors, data from combination strategies with myelosuppressive cytotoxic agents must be interpreted with caution. Early-stage clinical trials have almost all started with full-dose chemotherapies and titrated up the dose of PARP inhibitors, typically to dose levels far below effective monotherapy doses due to compounded myelosuppressive toxicities [21]. This is typified by the phase III neoadjuvant BrighTNess trial (NCT02032277) evaluating the combination of carboplatin at an area under the curve of 6 (AUC6) every 3 weeks + paclitaxel 80 mg/m^2 weekly ± PARP inhibitor veliparib 50 mg twice daily continuously followed by doxorubicin + cyclophosphamide in patients with stage II or III TNBC [22, 23]. The addition of veliparib did not improve the pathologic complete response rate, but it should be noted that the veliparib dose shown to be effective as a monotherapy is 400 mg twice daily (eight times the dose used in BrighTNess) [24]. Studies to evaluate full doses of PARP inhibitors combined with low-dose chemotherapy are warranted. For advanced or metastatic TNBC, it may also be of interest to treat patients with germline *BRCA1/2* mutations with induction chemotherapy followed by maintenance PARP inhibition, as is currently FDA-approved for ovarian cancer.

3 PI3K/AKT Inhibitors

3.1 PI3K/AKT Pathway

The phosphoinositide 3-kinase (PI3K)/AKT pathway is an important regulator of cell growth and glucose metabolism. Under normal biological circumstances (such as embryological development and maintenance of glucose homeostasis), stimulation of receptor tyrosine kinases (RTK) by growth factors, most importantly insulin, leads to PI3K activation [25–28]. Activated PI3K results in lipid phosphorylation of

phosphatidylinositol-4,5-trisphosphate (PIP2) and conversion to phosphatidylinositol-3,4,5-trisphosphate (PIP3) [26]. PIP3 is membrane-bound and acts as an anchor to which the protein serine-threonine kinase AKT binds via its pleckstrin homology (PH) domain [25]. Anchoring to PIP3 brings AKT into proximity of phosphoinositide-dependent kinase 1 (PDK1), which also expresses a PH domain and is likewise PIP3-anchored. PDK1 then activates AKT, which in turn influences a variety of downstream events and pathways (including mTOR), influencing cell growth, cell-cycle entry, and increased glucose metabolism [26, 28]. This pathway is negatively regulated by the phosphatase and tensin homolog (PTEN) and inositol polyphosphate 4-phosphatase type II (INPP4B) proteins [29]. PTEN/INPP4B reverse the action of PI3K by removing the 3-position phosphate group from PIP3, converting it back to PIP2 [28].

3.2 PI3K/AKT Alterations in Breast Cancer

Because of the complex associations surrounding the PI3K/AKT pathway, a variety of aberrations can lead to inappropriate activation [29, 30]. Overall PI3K pathway activation, regardless of the cause of the hyperactivity, is highest in TNBC [31]. While mutations in *PIK3CA* are common in HR+ and HER2+ breast cancers, they are less common (< 10%) in TNBC. Pathologic activation of the PI3K/AKT pathway in TNBC is more commonly the result of loss of PTEN activity (35%), loss of INPP4B (30%), or amplification of *PIK3CA* [31, 32]. Furthermore, cell lines with PI3K activation due to PTEN loss exhibit more growth inhibition from PI3K inhibitors than cells with *PIK3CA* mutations [30]. With the PI3K/AKT pathway being commonly activated in TNBC and in a fashion that may be more susceptible to PI3K inhibition, TNBC represents an ideal setting in which PI3K inhibitors may be studied [33].

3.3 Clinical Trials of PI3K Pathway Inhibitors in Triple-Negative Breast Cancer (TNBC)

Ipatasertib is an oral, highly selective, competitive AKT inhibitor that has previously shown activity across a variety of malignancies [34]. The results of LOTUS (NCT02162719), a phase II, randomized, placebo-controlled trial, were recently reported by Kim and colleagues [35]. A total of 124 patients with advanced TNBC were enrolled and randomized 1:1 to receive first-line paclitaxel (80 mg/m^2, days 1, 15, and 21 of a 28-day cycle) plus either placebo or ipatasertib 400 mg daily (days 1–21). The co-primary endpoints were progression-free survival (PFS) in the intention-to-treat (ITT) population as well as PFS in the PTEN-low population (defined as an immunohistochemistry [IHC] score of zero in at least 50% of tumor cells), with secondary endpoints of ORR, duration of response (DOR), and OS.

Subjects receiving ipatasertib in the ITT group had a modestly improved PFS compared with those receiving placebo (mPFS 6.2 vs 4.9 months, HR 0.60, 95% CI 0.37–0.98, $p = 0.037$). In the PTEN-low subgroup ($n = 48$), those receiving ipatasertib had a numerically higher mPFS compared with the placebo arm (6.2 vs 3.7 months), but this difference did not reach statistical significance (HR 0.59, 95% CI 0.26–1.32; $p = 0.18$). In the PTEN-low group, the ORR was nearly doubled in the ipatasertib arm compared with the placebo arm (48% vs 26%, respectively). A predefined sub-group analysis of 42 subjects with *PIK3CA/AKT1/PTEN*-altered tumors (based on Foundation One Next-Gen sequencing) revealed a more pronounced difference in PFS between the ipatasertib group (9.0 months) and the placebo group (4.9 months), which was statistically significant (HR 0.44, 95% CI 0.20–0.99; $p = 0.041$). The intervention and placebo groups differed primarily with regard to grade ≥ 3 diarrhea (23% vs 0%), neutropenia (10% vs 2%), pneumonia (5% vs 0%), and febrile neutropenia (2% vs 0%). In LOTUS, the PTEN-low group included many patients with no genetic alteration underlying their loss of PTEN by IHC. While these patients did not have improved survival when exposed to ipatasertib compared with placebo, those with *PIK3CA/AKT1/PTEN* alterations showed a significant 4.1-month increase in survival. This discordance highlights the importance that the specific mechanism of PI3K/AKT pathway activation plays with respect to drug efficacy, as patients with loss of PTEN by IHC did not receive benefit unless there was also an identifiable genetic alteration in the *PIK3CA/AKT1/PTEN* pathway.

The findings of LOTUS contrast with the results of the BELLE-4 study (NCT01572727), which was recently reported by Martín and colleagues [36]. BELLE-4 was a phase II/III study that investigated the addition of buparlisib, an oral pan-PI3K inhibitor, to first-line paclitaxel. BELLE-4 was not limited to TNBC as patients with HR+ HER2− tumors were also eligible. BELLE-4 also had co-primary endpoints of PFS stratified by PI3K/AKT pathway activation status; however, this was defined slightly differently as either *PIK3CA* mutations in exons 1, 7, 9, or 20, and/or PTEN loss (≤ 10%) on IHC. A total of 416 patients were randomized between both arms, and there was no difference in PFS between buparlisib and placebo in either the full population (8.0 vs 9.2 months, respectively; HR 1.18, 95% CI 0.82–1.68) or in the PI3K/AKT-activated population (9.1 vs 9.2 months; HR 1.17, 95% CI 0.63–2.17). Furthermore, in the 99 TNBC patients (23.7% of total study), the buparlisib arm compared with placebo was associated with a slightly worse (but non-significant) PFS of 5.5 versus 9.3 months, respectively (HR 1.86, 95% CI 0.91–3.79).

The study was terminated for futility and did not proceed to phase III.

It is not immediately clear why LOTUS showed clinical benefit of inhibiting the PI3K/AKT pathway, while BELLE-4 did not. The LOTUS findings suggest that the specific lesion in the PI3K/AKT pathway seems to have an impact on drug effectiveness. This has also been shown preclinically, with *PTEN* mutated cell lines, but not *PIK3CA* mutant cell lines, being responsive to PI3K inhibition [30]. Therefore, matching the right mutation with the right drug will be an important part of future drug development in this pathway. Without reliable predictive biomarkers, unselected patient populations may have variable responses to these agents. Downstream AKT inhibition might be a more effective means of inhibiting the PI3K/AKT pathway than pan-PI3K inhibitors [27], which work upstream of AKT, thus allowing for potential alternative activation of AKT. Attention should be paid to identifying the most predictive biomarkers of drug response in these patients so therapies and therapeutic combinations can be further refined.

4 Immune Checkpoint Inhibitors in TNBC

4.1 Overview of PD-1 and PD-L1 Expression

Activation of cytotoxic T cells to promote an anti-tumoral immune response is the primary goal of immune checkpoint inhibition [37, 38]. Inhibitors of the programmed death receptor 1 (PD-1) pathway are some of the most extensively studied and developed drugs within cancer immunotherapy. PD-1 is a cell surface protein expressed on tumor infiltrating lymphocytes (TILs) that induces inhibition of the T cell upon binding by one of its two ligands, PD-L1 and PD-L2 [39, 40]. PD-L1 expression in breast cancer is significantly associated with high grade and hormone receptor negativity [41]. Several early phase trials in advanced TNBC studying combination single-agent chemotherapy plus PD-1 or PD-L1 inhibitors displayed promising response rates in heavily pretreated patients, though the predictive role of PD-L1 expression has been inconsistent among these earlier trials [37, 42].

4.2 Immunotherapy Trials in TNBC

The largest immunotherapy study in TNBC to date that has reported results is the IMpassion130 trial (NCT02425891) [43]. Patients with previously untreated metastatic TNBC were randomized 1:1 to receive nab-paclitaxel ± atezolizumab (a PD-L1 inhibitor) with PFS and OS as co-primary endpoints and patients with PD-L1-expressing tumors (> 1%) as a predefined subgroup. While improvement in PFS modestly favored the atezolizumab group (significant absolute benefit of 1.7 months and 2.5 months in the ITT and PD-L1-positive groups, respectively), atezolizumab was associated with a 9.5-month absolute improvement in OS in the PD-L1-positive group. That said, improvement in OS in the ITT group (a primary endpoint) was not observed.

Several phase II trials deploying immune checkpoint inhibitors in early TNBC have been reported, largely in the neoadjuvant setting. The I-SPY 2 trial (NCT01042379) randomized 69 patients with HER2− early breast cancer to receive neoadjuvant weekly paclitaxel ± pembrolizumab followed by dose-dense doxorubicin and cyclophosphamide [44]. In the 29 patients with TNBC, raw and estimated pathologic complete response (pCR) rates were drastically higher in the pembrolizumab arm (71% and 62%, respectively) compared with the control arm (19% and 22%, respectively). The rate of pCR with standard therapy was lower than expected in I-SPY 2, and results from the GeparNuevo (NCT02685059) further suggest that this difference may be more modest. GeparNuevo similarly added an immune checkpoint inhibitor (durvalumab, a PD-L1 inhibitor) to anthracycline-based neoadjuvant chemotherapy in subjects with early TNBC. In the overall group, durvalumab was associated with a non-significant 9% improvement in pCR compared with standard therapy [45]. However, patients in the 'window subgroup' received durvalumab monotherapy for two weeks prior to chemotherapy in an effort to 'prime' the immune system and had a nearly 20% improvement in pCR. This observation has support in preclinical studies, as drug-induced neoantigens may enhance efficacy of immune checkpoint inhibitors [46]. Nonetheless, the stark difference favoring pembrolizumab in the I-SPY 2 trial provided the basis for a larger phase III trial, KEYNOTE-552 (NCT03036488), which is currently ongoing [47]. Patients in KEYNOTE-552 will receive pembrolizumab or placebo in either the neoadjuvant or the adjuvant setting, and hence pCR and event-free survival (EFS) are co-primary endpoints. Additionally, NSABP B-59/GBG96-GeparDouze (NCT03281954) is a phase III double-blind trial evaluating a neoadjuvant regimen consisting of paclitaxel and carboplatin concurrently with atezolizumab or placebo, followed by an anthracycline plus cyclophosphamide. Patients then resume atezolizumab or placebo after surgery for 6 months. While a number of other early-phase studies are ongoing in early- and late-stage TNBC, ongoing challenges include optimal predictive and prognostic biomarkers that help identify patients within TNBC who will derive the most benefit.

5 Cyclin-Dependent Kinase (CDK) 4/6 Inhibitors

5.1 Overview of Cell Cycle Regulation in Breast Cancer

Progression through the cell cycle is tightly regulated at the G1-S phase transition [48]. To pass through this restriction point (the 'R Point'), retinoblastoma protein (Rb) must be inactivated via hyperphosphorylation by CDK 4/6 [48]. CDK 4/6 inhibitors block hyperphosphorylation of Rb and subsequently inhibit progression from G1 to S phase [49]. Cell line studies suggested that luminal type, HR+ breast cancer cells are particularly sensitive to growth restriction by CDK 4/6 inhibition by palbociclib [50]. This observation provided the basis on which CDK 4/6 inhibitors were studied in advanced HR+ HER2− breast cancer, showing improved PFS when combined with endocrine therapy versus endocrine therapy alone [51–56]. TNBC has many common molecular alternations that suggest resistance to CDK 4/6 inhibition, including frequent loss or mutation of *RB1*, cyclin E1 amplification, and high expression of *CDKN2A* [31]. While, on average, basal-type breast cancer cell lines were more resistant to palbociclib's growth inhibitory effect, 30% of basal cell lines exhibited at least moderate sensitivity to palbociclib in vitro [50].

5.2 CDK 4/6 Inhibitor Trials in TNBC

To date, a few small studies have reported on the use of CDK 4/6 in the metastatic TNBC setting, and others are still ongoing. DeMichele and colleagues investigated single agent palbociclib in 37 patients with *RB1* wild-type metastatic breast cancer (UPCC 03909, NCT01037790) in a single-arm phase II study, which included four patients (11%) with TNBC [57]. Due to rapid progression, enrollment in the TNBC group was halted at four patients. A phase I study of palbociclib in combination with paclitaxel in 27 patients with metastatic breast cancer was recently reported, which included nine patients (33%) with TNBC [58]. Positive Rb expression was a requirement for all patients with TNBC. While one third of the patients with TNBC experienced clinical benefit (partial response or stable disease ≥ 6 months), this response may have been due to paclitaxel alone.

Several studies utilizing CDK 4/6 inhibitors are ongoing in TNBC [59]. Preclinical studies suggest androgen receptor-positive (AR+) TNBC is sensitive to CDK 4/6 inhibition [60, 61], and two ongoing phase I/II single-arm studies are investigating the combination of a CDK 4/6 inhibitor (palbociclib in NCT02605486, ribociclib in NCT03090165) with bicalutamide in AR+ TNBC. Several other phase I/II studies of novel CDK inhibitors, either alone or in combination with chemotherapy or immunotherapy, are currently recruiting patients [59].

6 Sacituzumab Govitecan (IMMU-132)

The tumor-associated calcium signal transducer 2 cell-surface glycoprotein (Trop-2) is both frequently expressed and is a poor prognostic factor in TNBC [62]. Sacituzumab govitecan (IMMU-132) is an antibody-drug conjugate that targets Trop-2 and delivers a topoisomerase-1 inhibiting payload, leading to double-stranded DNA breaks [63]. Preclinical studies suggested activity in a variety of advanced solid malignancies [63], and a first-in-human study of patients with advanced triple-negative breast, colorectal, pancreatic, small-cell lung, and other difficult-to-treat cancers showed promising activity with an acceptable toxicity profile [64].

To evaluate the effectiveness and safety of sacituzumab govitecan in patients with advanced TNBC, Bardia et al. conducted a single-arm, phase I/II study in patients with advanced, pre-treated TNBC [65]. One hundred and eight patients with advanced TNBC who had progressed on at least two prior lines of therapy in the metastatic setting (median of three lines of prior therapy) were dosed with sacituzumab govitecan at 10 mg/kg on days 1 and 8 of a 21-day cycle. A confirmed objective response (complete response + partial response) was seen in 33.3% of patients (36/108, including three CRs), with a clinical benefit rate (confirmed objective response + stable disease ≥ 6 months) of 45.4% (49/108). A subset analysis of patients with archival tumors ($n = 46$) showed that 88% of the patients in the trial had moderate to strong expression of Trop-2 by IHC [66]. All of the responders had moderate to strong staining of Trop-2, while patients with weak to no expression of Trop-2 only had stable disease as the best response.

With regards to safety, the most common grade ≥ 3 event was neutropenia (42%), with a grade ≥ 3 febrile neutropenia rate of 10%. Grade ≥ 3 anemia was seen in 11% of patients. Other common AEs of any grade were nausea (67%), diarrhea (62%), vomiting (49%), and fatigue (55%). Sacituzumab govitecan is currently being developed in an open-label phase III clinical trial in advanced, pre-treated TNBC, with patients being randomized 1:1 to either sacituzumab govitecan or treatment of physician's choice (the ASCENT trial, NCT02574455).

7 Conclusion

In summary, although TNBC is currently a disease with a poor prognosis relative to hormonally driven and HER2-amplifed breast cancers, it is hoped that subsets of TNBC will be revolutionized by targeted approaches as HER2+

breast cancer treatment was revolutionized by the development of the HER2-specific antibody trastuzumab in the 1990s. The only currently FDA-approved targeted agents for TNBC are the PARP inhibitors olaparib and talazoparib for metastatic g*BRCA1/2*-associated cancers, which largely fall into the basal-like molecular biology category. Recent results of the IMpassion130 trial with atezolizumab in combination with nab-paclitaxel suggest a role for immune checkpoint inhibitors for TNBCs over-expressing PD-L1, and neoadjuvant studies defining the potential role of these drugs in early-stage disease are underway. Additional biomarkers for sensitivity to immune checkpoint inhibitors are needed in multiple types of cancer, breast cancer included. With the very recent approval of the PI3K inhibitor alpelisib for HR+ HER2− breast cancer based on the SOLAR-1 trial [67], there is hope that PI3K inhibitors may also be useful in the TNBC setting as well. Some academic and community pathology departments have begun testing for androgen receptor (AR) in triple-negative breast tumors by default, though thus far it isn't clear that AR is a biologically or clinically important target in TNBC. However, if AR overexpression is a driver for some subsets of TNBCs, AR would be a convenient and welcome target given the wealth of androgen deprivation therapies available. If AR is a viable target, perhaps CDK 4/6 inhibitors would be most likely to work in this subpopulation of TNBC, as these are the triple-negative tumors in which the G1/S checkpoint is intact and can be activated. Beyond broad molecular sub-categorizations of triple-negative breast tumors, sometimes treatment revolution occurs through identification of a novel target that might encompass several tumor types as exemplified by the transmembrane glycoprotein Trop-2. Sacituzumab govitecan is a promising Trop-2 targeted antibody drug conjugate with impressive activity in pre-treated, advanced TNBC, and results of a randomized phase III study are pending. The study of TNBC biology and the development of targeted agents is a particularly rich area of research, and we look forward to being able to offer our patients more than cytotoxic chemotherapies.

Compliance with Ethical Standards

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