Molecularly targeted therapy and immunotherapy for hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer (Review)

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Abstract. The advent of targeted therapy for hormone receptor-positive/human epidermal growth factor receptor 2-negative advanced breast cancer ($HR^+/HER2^-$ aBC) provides a novel therapeutic approach other than endocrine therapy. One targeted signaling pathway and three immune-checkpoints have been demonstrated to be in association with tumor proliferation and growth in $HR^+/HER2^-$ aBC. A number of phosphoinositide 3-kinase/AKT/mammalian target of rapamycin signaling pathway inhibitors demonstrate clinical activity against this tumor subtype. The CDK4/6 inhibitors as a single agent or in combination with endocrine therapy have produced promising tumor response with acceptable toxicity in patients with $HR^+/HER2^-$ aBC. Programmed death 1/programmed death ligand 1 (PD1/PD-L1) and cytotoxic T lymphocyte antigen-4 inhibitors can also produce an antitumor immune response, which provides a proof-of-principle for the initial utilization of immunotherapy in breast cancer. The aim of the present review was to discuss the mechanisms of action, clinical efficacy and safety profiles of all the targeted biological therapies and immunotherapies that have been approved or are currently under evaluation for $HR^+/HER2^-$ aBC.

Contents

1. Introduction

Of the 1.5 million new-onset breast cancer cases diagnosed annually worldwide, a large subset are hormone receptor (HR)-positive breast cancers [estrogen receptor (ER)-positive, progesterone receptor-positive, or both, with normal human epidermal growth factor receptor 2 (HER2) expression], accounting for ~60-65% (1). Patients with this disease (>70%) are significantly more commonly diagnosed at an advanced stage compared with those with other subtypes of breast cancer (2). Over several decades, the mainstay in the treatment of women with advanced HR-positive (HR^+) breast cancer has been hormone therapy, which may benefit patients initially, but eventually leads to drug resistance and disease progression. Endocrine therapy-refractory patients are not considered as suitable candidates for a combination of endocrine therapy and chemotherapy, as this combination may accentuate the expression of naïve regulatory T cells (Tregs) that are associated with disease progression or death (3). Clinical studies have demonstrated that everolimus [a mammalian target of rapamycin (mTOR) inhibitor] or tucidinostat (a histone deacetylase inhibitor) in combination with exemestane (an aromatase inhibitor) achieved significant improvement of the

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Abbreviations: HR, hormone receptor; Tregs, regulatory T cells; PFS, progression-free survival; $HR^+/HER2^-$ aBC, HR-positive/human epidermal growth factor receptor 2-negative advanced breast cancer; TNBC, triple-negative breast cancer; TILs, tumor-infiltrating lymphocytes; PD1/PD-L1, programmed death 1/programmed death ligand 1; CTLA-4, cytotoxic T lymphocyte antigen-4; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; *PIK3CA*, PI3K, catalytic, a polypeptide; AEs, adverse events; MTD, maximum tolerated dose; ORR, overall response rate; CBR, clinical benefit rate; ER, estrogen receptor; CDK4/6, cyclin-dependent kinase 4 and 6; Rb, retinoblastoma; TCR, T-cell receptor

Key words: targeted therapy, immunotherapy, hormone receptor, human epidermal growth factor receptor 2, breast cancer

progression-free survival (PFS) of patients with HR+/HER2- advanced breast cancer (aBC), who had become resistant to endocrine therapy (4,5). These findings heightened the interest in the application of targeted therapy for such patients.

Immunotherapy has also shown promising clinical efficacy in breast cancer, specifically in triple-negative breast cancer (TNBC), due to its immune-rich characteristics (6), and is currently the fifth treatment strategy for breast carcinoma after surgery, chemotherapy, radiotherapy and molecularly targeted therapy. By contrast, HR+ breast tumor is an immunologically cold cancer. Metastatic disease due to the escape from immune surveillance at the primary tumor site contributes to the lower immunogenicity of the metastases compared with the primary tumor. Moreover, previous chemotherapy depletes immune-active tumors, giving rise to the development of immunologically cold tumors in aBC. All these underpinnings are associated with a weakened response of HR+/HER2- aBC to immunotherapy. However, in the context of high numbers of tumor-infiltrating lymphocytes (TILs) and immune-related gene expression signatures, these patients may benefit from systematic adjuvant therapy (7). Several clinical trials confirmed the antitumor activities of inhibiting immune-checkpoints, such as programmed death 1/programmed death ligand 1 (PD1/PD-L1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) in breast cancer (8-11). The aim of the present study was to evaluate the mechanisms of action and the therapeutic efficacy and tolerability of targeted therapy and immunotherapy for HR+/HER2- aBC.

2. PI3K/AKT/mTOR signaling pathway inhibitors

The phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway is crucial for cell survival and proliferation via multiple downstream effectors, including mammalian target of rapamycin (mTOR) (12). In HR+ breast cancer, mutations of the activated PI3K, catalytic, a polypeptide (*PIK3CA*) occur frequently, increase the AKT level and may give rise to the insensitivity to antitumor therapy (12,13). Several cytotoxic agents and mTOR inhibitors are available that can reduce the size and even eliminate tumors, although activation of ATK signaling reduces their therapeutic effectiveness (14-17). Estrogen-induced cell proliferation in breast tumors depends on mTOR signaling (18), and mTOR inhibition can down-regulate the expression of ER. Therefore, inhibition of mTOR signaling may effectively reduce cell proliferation in HR+ breast tumors, even when they have been proven to be resistant to hormone therapy (19). In breast cancer xenograft models, a PI3K inhibitor can abolish the activation of ATK signaling induced by mTOR inhibition. Under hypoxic conditions, inhibition of the PI3K/ATK/mTOR signaling pathway can reduce the expression of hypoxia-inducible factor 1a and the synthesis of vascular endothelial growth factor, both of which promote angiogenesis in tumors (Fig. 1) (16). These mechanisms constitute the conceptual framework for investigating the antitumor activity of PI3K inhibitors, ATK inhibitors and mTOR inhibitors for the treatment of patients with HR+ breast cancer.

PI3K inhibitors. Buparlisib (BKM120), an oral reversible PI3K inhibitor, manifests antitumor efficacy as monotherapy and in combination with endocrine therapy for the treatment of patients with HR+ breast cancer independently of the presence of *PIK3CA* mutations (20,21). Two phase III randomized controlled trials (RCTs), BELLE-2 (22) and BELLE-3 (23), demonstrated that buparlisib-fulvestrant significantly prolonged the median PFS of HR+/HER2- endocrine treatment-refractory aBC postmenopausal patients compared with fulvestrant (Table I). However, no additional studies were launched to explore the clinical benefit and safety in this setting, due to the serious toxicity of the combined treatment; the most common grade 3-4 adverse events (AEs) were increased alanine aminotransferase and aspartate aminotransferase levels, and hyperglycemia (Table I). As a result, the clinical application of buparlisib has been limited and several other trials have been initiated to test a-specific PI3K inhibitors in patients.

Alpelisib (BYL719) is an orally selective, bioavailable, α-specific PI3K small-molecule inhibitor, which acts synergistically with hormone therapy against HR+ *PIK3CA*-mutant breast cancer (24). The maximum tolerated dose (MTD) of alpelisib combined with letrozole or fulvestrant is different, 300 and 400 mg daily, respectively (24,25). Both drug combinations have manageable safety profiles, with reversible toxicity. Additionally, these combinations are associated with greater clinical benefits in *PIK3CA*-mutated HR+/HER2- aBC compared with *PIK3CA*-wild-type tumors. However, *FGFR1* amplification is an adverse factor in the antitumor activity of alpelisib in HR+/*PIK3CA*-mutant breast cancer (24), suggesting that alpelisib should not be administered to patients with coexisting genomic alterations. The NEO-ORB phase II clinical study (26) indicated that alpelisib-letrozole did not improve the response of postmenopausal women with early HR+/HER2- breast cancer compared with placebo-letrozole in the neoadjuvant setting, regardless of the patients' *PIK3CA* mutation status (Table I). By contrast, alpelisib-fulvestrant compared with placebo-fulvestrant significantly prolonged the median PFS, increased the overall response rate (ORR; complete or partial response) to ≥24 weeks and increased the clinical benefit rate (ORR + stable disease) to ≥24 weeks (CBR) in postmenopausal patients with endocrine-refractory HR+/HER2- *PIK3CA*-mutant aBC; however, no difference was observed in patients with *PIK3CA*-wild-type tumors (SOLAR-1 trial) (27). The most frequent grade 3-4 treatment-associated AEs in the alpelisib group were hyperglycemia, rash and maculopapular rash (Table I). These findings collectively suggest that alpelisib in combination with endocrine therapy should only be used for the treatment of patients with HR+/HER2-, *PIK3CA*-mutant aBC.

AKT inhibitors. MK-2206, an orally selective and potent allosteric AKT inhibitor (28), induces apoptosis in parental ER+ breast cancer cell lines, but not in those subjected to long-term estrogen deprivation. MK-2206 in combination with endocrine agents for the treatment of HR+/HER2- aBC has achieved a tumor response that is not dependent on the *PIK3CA*-mutant status (29,30). Unexpectedly, this combination therapy does not enhance the antitumor efficacy in early-stage HR+/HER2- breast cancer compared with endocrine therapy alone (31). Overall, further phase II/III clinical trials are required to investigate the clinical activity and safety profile of MK-2206 in HR+/HER2- aBC.

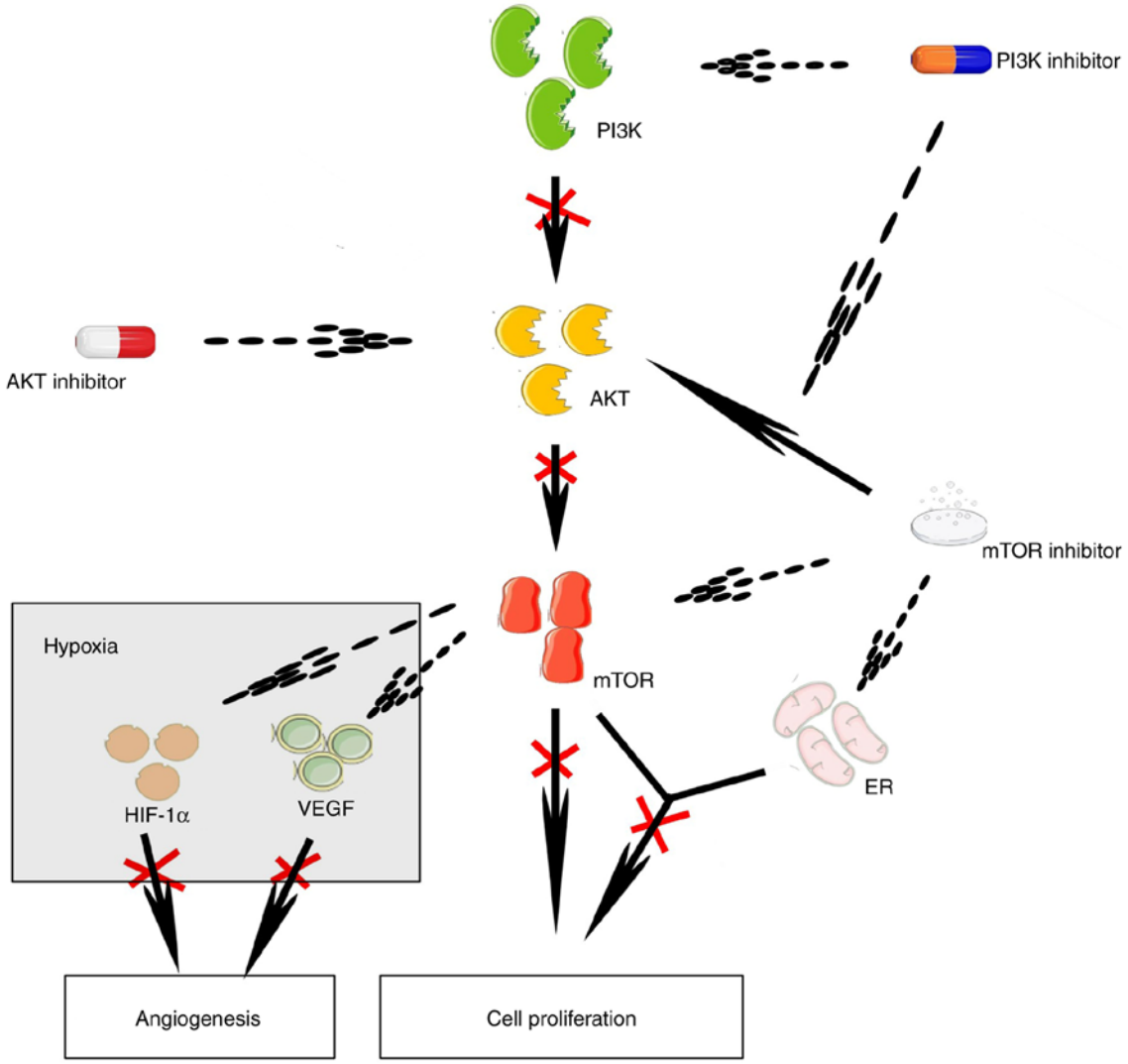

Figure 1. Mechanisms underlying the antitumor effect of CDK4/6 inhibitors. Solid arrow, promoting effect; dashed arrow, inhibiting effect; red cross solid arrow, weakening effect caused by previous step. Gray box represents hypoxic environment. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; ER, estrogen receptor; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor-1α.

Capivasertib (AZD5363) is another orally selective and potent AKT inhibitor, the sensitivity of cancer to which is increased by the presence of the *PIK3CA* mutation (32). Estrogen blockade may be crucial for its antitumor activity, as capivasertib does not improve the median PFS of patients with $HR^+/HER2^-$ aBC receiving paclitaxel without endocrine agents, even in the *PIK3CA*-mutant subpopulation (BEECH trial; Table I) (33). Capivasertib exerts a synergistic effect with fulvestrant in delaying tumor progression in xenograft models of ER^+ breast cancer (34); further investigation of the clinical activity and safety of capivasertib-fulvestrant in patients with $HR^+/HER2^-$ aBC is ongoing (NCT01992952).

mTOR inhibitors. Everolimus (Afinitor), an orally selective and potent allosteric mTOR inhibitor (35), acts synergistically with letrozole to suppress cell proliferation and trigger apoptosis in ER^+ breast cancer cells (18). In the neoadjuvant setting, everolimus can significantly increase the ORR of patients with early $HR^+/HER2^-$ breast cancer who receive letrozole monotherapy (36). Numerous phase II and III clinical trials, including the 4EVER trial (37), GINECO study (38), BOLERO-2 trial (4), BOLERO-4 trial (39) and BOLERO-6 trial (40), have explored the antitumor efficacy and safety profile of everolimus combined with aromatase inhibitors in $HR^+/HER2^-$ aBC (Table I). Taken together, the combination significantly prolongs the median PFS of postmenopausal patients with $HR^+/HER2^-$ aBC compared with aromatase inhibitors or everolimus alone, with acceptable toxicity. This combined strategy appears to be a good first- and second-line treatment option for patients with $HR^+/HER2^-$ aBC, as it is more effective and less toxic compared with most chemotherapy protocols (41), particularly as first-line therapy, as its median PFS is longer compared with that of second-line therapy (39).

3. Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors

The interaction of CDK4/6 with D-type cyclins phosphorylates the retinoblastoma (Rb) tumor suppressor protein to promote the cell cycle progression from the G1 to the S phase (42). However, the cell cycle can be inhibited by CDK4/6 inhibitors, thereby suppressing tumor proliferation (43). The regulation of CDK4/6 activity is associated with the ubiqui-

Table I. Clinical studies on PI3K/AKT/mTOR signaling pathway targeting therapy for $HR^+/HER2^-$ advanced breast cancer.

Trial	St. cohort	Co. cohort	Study type	Clinical efficacy	Safety[a]	(Refs.)
BELLE-2	Buparlisib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS: St. vs. Co. (6.9 vs. 5.0 ms; P<0.05)	Elevated ALT (25%), elevated AST (18%), hyperglycemia (15%)	(22)
BELLE-3	Buparlisib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS: St. vs. Co. (3.9 vs. 1.8 ms; P<0.05)	Elevated ALT (22%), elevated AST (18%), hyperglycemia (12%)	(23)
NEO-ORB	Alpelisib + letrozole	Placebo + letrozole	RCT	ORR in $PIK3CA^+$ cohort: St. vs. Co. (43 vs. 45%); ORR in $PIK3CA^-$ cohort: St. vs. Co. (63 vs. 61%);	Hyperglycemia (27%), rash (12%), MP rash (8%)	(26)
SOLAR-1	Alpelisib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS in $PIK3CA^+$ cohort: St. vs. Co. (11.0 vs. 5.7 ms; P<0.05); Median PFS in $PIK3CA^-$ cohort: St. vs. Co. (7.4 vs. 5.6 ms; P>0.05); ORR in $PIK3CA^+$ cohort: St. vs. Co. (26.6 vs. 12.8%); CBR in $PIK3CA^+$ cohort: St. vs. Co. (61.5 vs. 45.3%)	Hyperglycemia (36.6%), rash (9.9%), diarrhea (6.7%)	(27)
BEECH[b]	Capivasertib + paclitaxel	Placebo + paclitaxel	RCT	Median PFS: St. vs. Co. (10.9 vs. 8.4 ms; P=0.31); Median PFS in PIK3CA + cohort: St. vs. Co. (10.9 vs. 10.8 ms; P=0.76)	Diarrhea (22%), hyperglycemia (13%), neutropenia (11%)	(33)
4EVER	Everolimus + exemestane	NA	Single-arm	Median PFS: 5.6 ms; ORR 8.9%	Stomatitis (8.4%), GPH deterioration (6.7%), dyspnea (4.7%)	(37)
GINECO	Everolimus + tamoxifen	Tamoxifen	Randomized	TTP: St. vs. Co. (8.6 vs. 4.5 ms; P<0.05); CBR: St. vs. Co. (61 vs. 42%).	Pain (18%), fatigue (11%), infection (5%)	(38)
BOLERO-2	Everolimus + exemestane	Placebo + exemestane	RCT	Median PFS: St. vs. Co. (6.9 vs. 2.8 ms; P<0.05)	Stomatitis (8%), anemia (6%)	(4)
BOLERO-4	Everolimus + letrozole	NA	Single-arm	Median PFS in the first-line setting: 22.0 months; Median PFS in the second-line setting: 3.7 months	Anemia (10.4%); hypertension (10%)	(39)
BOLERO-6[c]	Everolimus + exemestane	Everolimus or capecitabine	Randomized	Median PFS: St. vs. Eve. (8.4 vs. 6.8 ms; P<0.05); St. vs. Cap. (8.4 vs. 9.6 ms; P>0.05); Median OS: St. vs. Eve. Vs. Cap. (23.1 vs. 29.3 vs. 25.6 ms; P>0.05); ORR: St. vs. Eve. Vs. Cap. (20 vs. 12 vs. 23%); CBR: St. vs. Eve. Vs. Cap. (57 vs. 42 vs. 52%)	Anemia (13%), elevated γ-GT (9%) stomatitis (9%)	(40)

[a]Grade 3-4 treatment-associated adverse events in the study group. [b]Pre- or perimenopausal women receive a gonadotropin-releasing hormone agonist. [c]Patients are randomly assigned in a 1:1:1 ratio to the treatment groups everolimus + exemestane or everolimus alone or capecitabine alone. St., study; Co., control; ms, months; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; HR, hormone receptor; HER2, epidermal growth factor receptor 2; PIK3CA, PI3K, catalytic, a polypeptide; NA, not applicable; RCT, randomized controlled trial; PFS, progression-free survival; OS, overall survival; ORR, overall response rate (≥24 weeks); CBR, clinical benefit rate (≥24 weeks); ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transferase; MP rash, maculopapular rash; GPH, general physical health.

Table II. Clinical studies on CDK4/6 inhibitor therapy for $HR^+/HER2^-$ advanced breast cancer.

Trial	St. cohort	Co. cohort	Study type	Clinical efficacy	Safety[a]	(Refs.)
PALOMA-1	Palbociclib + letrozole	Letrozole	Randomized	Median PFS: St. vs. Co. (20.2 vs. 10.2 ms; P<0.05); Median OS: St. vs. Co. (37.5 vs. 33.3 ms; P=0.42)	Neutropenia (54%), leukopenia (16%), fatigue (4%)	(51)
PALOMA-2	Palbociclib + letrozole	Placebo + letrozole	RCT	Median PFS: St. vs. Co. (24.8 vs. 14.5 ms; P<0.05); CBR: St. vs. Co. (84.9 vs. 70.3%; P<0.05); ORR: St. vs. Co. (42.1% vs. 34.7%; P=0.06)	Neutropenia (66.4%), leukopenia (24.8%), anemia (5.4%)	(52)
PALOMA-3	Palbociclib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS: St. vs. Co. (9.5 vs. 4.6 ms; P<0.05); CBR: St. vs. Co. (67.0 vs. 40.0%; P<0.05); ORR: St. vs. Co. (19.0 vs. 9.0%; P<0.05)	Neutropenia (65%), leukopenia (28%) anemia (3%)	(53)
MONALEESA-2	Ribociclib + letrozole	Placebo + letrozole	RCT	Median PFS: St. vs. Co. (25.3 vs. 16.0 ms; P<0.05); 18-ms PFS rate: St. vs. Co. (63.0 vs. 42.2%; P<0.05); CBR: St. vs. Co. (79.6 vs. 72.8%; P=0.02); ORR: St. vs. Co. (40.7 vs. 27.5%; P<0.05)	Neutropenia (59.3%), leukopenia (21.0%), elevated ALT (9.3%)	(58)
MONALEESA-3	Ribociclib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS: St. vs. Co. (20.5 vs. 12.8 ms; P<0.05) CBR: St. vs. Co. (70.2 vs. 62.8%; P=0.02); ORR: St. vs. Co. (32.4 vs. 21.5%; P<0.05)	Neutropenia (53.4%), leukopenia (14.1%), anemia (3.1%)	(59)
MONARCH [b]	Abemaciclib	NA	Single-arm	Median PFS: 6.0 ms; Median OS: 17.7 ms; CBR: 42.4%; ORR: 19.7%	leukopenia (27.7%), neutropenia (22.3%), diarrhea (19.7%)	(63)
MONARCH 2[b]	Abemaciclib + fulvestrant	Placebo + fulvestrant	RCT	Median PFS: St. vs. Co. (16.4 vs. 9.3 ms; P<0.05); CBR: St. vs. Co. (72.2 vs. 56.1%; P<0.05); ORR: St. vs. Co. (35.2 vs. 16.1%; P<0.05)	Neutropenia (26.5%), diarrhea (13.4%), leukopenia (8.8%)	(64)

[a]Grade 3-4 treatment-associated adverse events in the study group. [b]Pre- or perimenopausal women received a gonadotropin-releasing hormone agonist. St., study; Co., control; ms, months; CDK, cyclin-dependent kinase; HR, hormone receptor; HER2, epidermal growth factor receptor 2; NA, not applicable; RCT, randomized controlled trial; PFS, progression-free survival; OS, overall survival; ORR, overall response rate (≥24 weeks); CBR, clinical benefit rate (≥24 weeks); ALT, alanine aminotransferase.

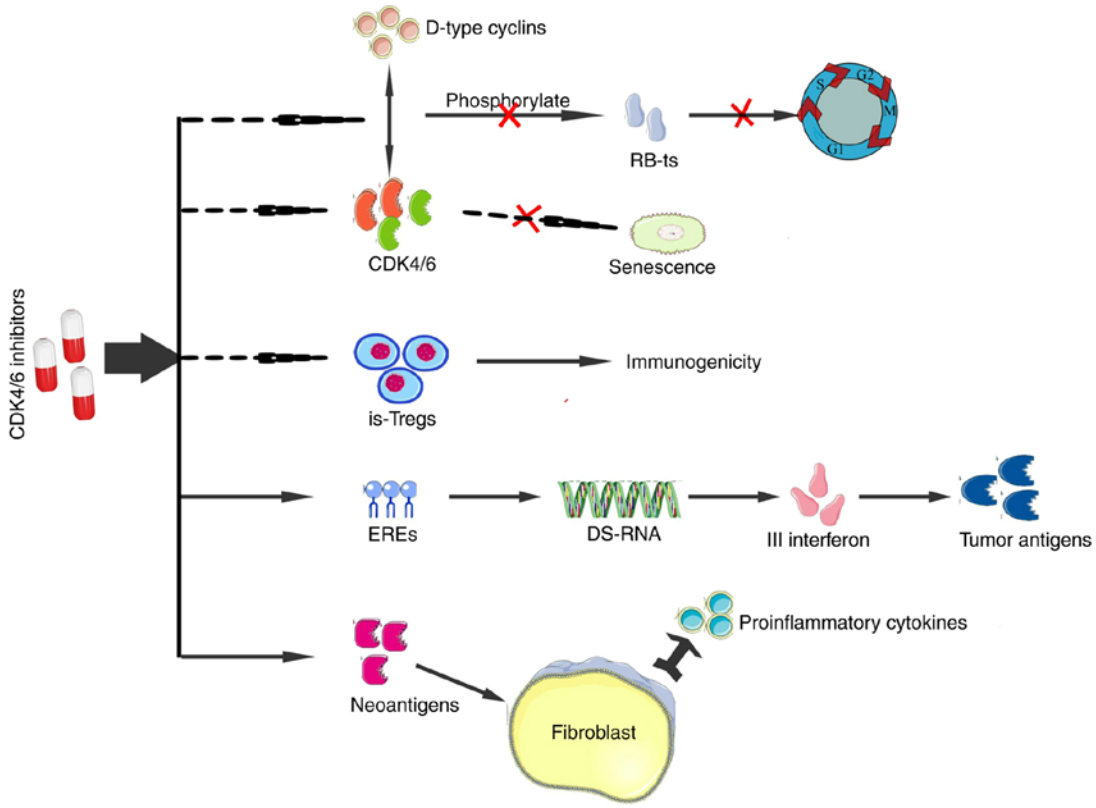

Figure 2. Mechanisms underlying the antitumor effect of PI3K/AKT/mTOR signaling pathway inhibitors. Solid arrow, promoting effect; dashed arrow, inhibiting effect; red cross solid arrow, weakening effect caused by previous step; red cross dashed arrow, enhancing effect caused by previous step. CDK4/6, cyclin-dependent kinases 4 and 6; RB-ts, retinoblastoma tumor suppressor protein; is-Tregs, immunosuppressive regulatory T cells; EREs, endogenous retrovirus elements; DS-RNA, double-stranded RNA.

tination and phosphorylation of endogenous cell inhibitors of the INK4 family (44). However, the regulation is commonly disrupted in tumors by a number of mechanisms, leading to increased CDK4/6 activity that delays senescence (45-47). CDK4/6 inhibitors can promote senescence of cancer cells by decreasing the activity of these kinases. These inhibitors exert a stronger inhibitory effect on the proliferation of immunosuppressive Tregs compared with any other type of T cells, thereby shifting the local immune balance over time to increase tumor immunogenicity (48). They also promote the expression of endogenous retrovirus elements, which is associated with the high level of intracellular double-stranded RNA that stimulates the production of type III interferon, ultimately bolstering the presentation of tumor antigens (48). The expression of MHC I-presented neoantigens may be activated by the CDK4/6 inhibitors, resulting in the release of proinflammatory cytokines by fibroblasts (Fig. 2) (48). Currently, three CDK4/6 inhibitors have been approved by the Food and Drug Administration in the United States for the treatment of HR+/HER2- aBC.

Palbociclib. Palbociclib (Ibrance) is an oral, reversible, small-molecule CDK4/6 inhibitor (49). Palbociclib has been demonstrated to suppress the growth of ER+ breast cancer cell lines more than that of TNBC cell lines *in vitro*; it also arrests the cell cycle at the G0/G1 phase and blocks the phosphorylation of Rb only in sensitive cell lines. In addition, it acts synergistically with tamoxifen in ER+ breast cancer cell lines and even increases the sensitivity to tamoxifen in cell lines resistant to endocrine therapy (50). Based on these findings, two clinical studies, PALOMA-1 (51) and PALOMA-2 (52), were designed, and demonstrated a significantly longer median PFS and higher tumor response of HR+/HER2- aBC postmenopausal patients receiving palbociclib plus letrozole compared with those receiving letrozole alone. The safety profile of the palbociclib-letrozole group was manageable; the most frequent grade 3-4 treatment-related AEs were neutropenia, leukopenia and fatigue (Table II). Similarly, the PALOMA-3 study (53) confirmed the significantly and consistently improved PFS in postmenopausal women with HR+/HER2- endocrine-refractory aBC receiving palbociclib plus fulvestrant compared with fulvestrant plus placebo (Table II). Other clinical trials are currently evaluating the efficacy and safety of palbociclib in combination with fulvestrant in HR+/HER- aBC patients who had previously progressed on palbociclib plus aromatase inhibitor (NCT02738866) and those on palbociclib combined with aromatase inhibitor compared with chemotherapy-based treatment for HR+/HER2- aBC in a Real World Setting (NCT03355157).

Ribociclib. The second small-molecule CDK4/6 inhibitor is ribociclib (LEE011), which is characterized by its oral bioavailability and high selectivity (54). A preclinical study demonstrated the antitumor efficacy of ribociclib combined with letrozole and PI3K inhibitors in HR+ breast cancer *in vivo* (55). Based on these findings, a phase Ib clinical trial of ribociclib plus an endocrine agent also demonstrated clinical

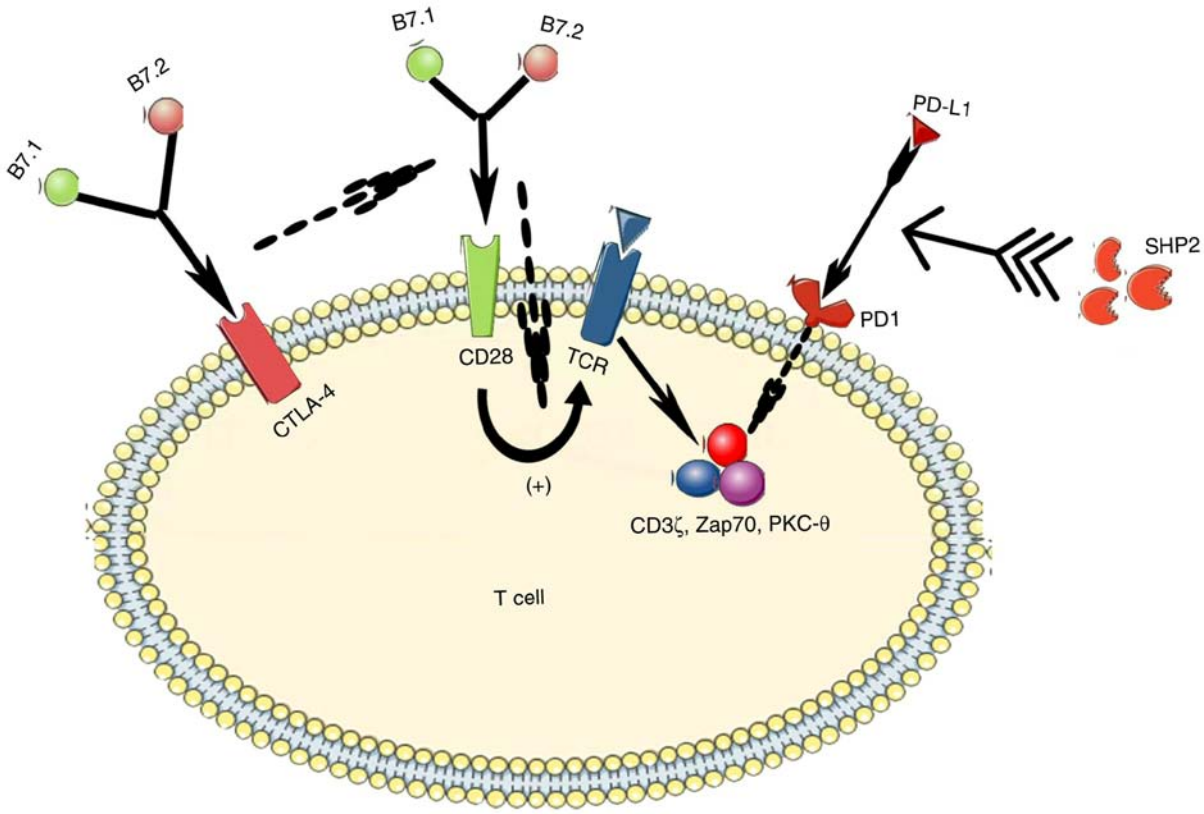

Figure 3. Mechanisms of action of PD1/PD-L1 and CTLA-4 in reducing T-cell proliferation. Solid arrow, promoting effect; dashed arrow, inhibiting effect. CTLA-4, anti-cytotoxic T lymphocyte antigen-4; TCR, T-cell receptor; PD1, programmed death 1; PD-L1, programmed death ligand-1; SHP2, Src homology 2-domain-containing tyrosine phosphatase 2.

efficacy with acceptable toxicity in postmenopausal patients with advanced HR^+ breast tumor (56). This combination also significantly improved the PFS of premenopausal women with $HR^+/HER2^-$ aBC compared with those on placebo plus endocrine therapy (57). Two well-designed phase III RCTs, MONALEESA-2 (58) and MONALEESA-3 (59), demonstrated that ribociclib combined with hormone therapy (letrozole or fulvestrant) outperformed hormone therapy alone in postmenopausal patients with $HR^+/HER2^-$ aBC with regard to the median PFS, ORR and CBR. The drug toxicity in the combination cohort was tolerable, with similar grade 3-4 AEs as those of palbociclib-letrozole (Table II). Of note, the therapeutic efficacy of ribociclib was independent of PIK3CA or TP53 mutations, total Rb, Ki67 or p16 protein expression, and the CDKN2A, CCND1, or ESR1 mRNA levels (60).

Abemaciclib. Abemaciclib (LY2835219) is the third oral, small-molecule CDK4/6 inhibitor, which is structurally distinct from palbociclib and ribociclib and appears to be more selective for CDK4 compared with CDK6 (61). As a single agent, abemaciclib produced an outstanding tumor response with an acceptable safety profile in advanced HR^+ breast cancer (62), which warrants its further development, as monotherapy or in combination with other therapies. The MONARCH 1 phase II single-arm study (63) and the MONARCH 2 phase III RCT (64) investigated the monotherapy with abemaciclib and its combination with fulvestrant, respectively, for patients with $HR^+/HER2^-$, endocrine-refractory aBC. Analysis of the results demonstrated the clinical efficacy and good tolerability of abemaciclib, both as a single agent and combined with fulvestrant. Of note, significant improvement of the median PFS, ORR and CBR was observed in the abemaciclib-fulvestrant cohort compared with fulvestrant alone. Leukopenia and neutropenia were the most common grade 3-4 AEs of abemaciclib (Table II).

4. Anti-PD1/PD-L1

PD1 is expressed on the surface of most T cells (65). Upon binding to its ligand PD-L1, PD1 clusters with the T-cell receptor (TCR) to form a negative costimulatory microcluster that can dephosphorylate and inactivate TCR downstream signaling molecules (CD3ζ, Zap70 and PKC-θ) by recruiting Src homology 2-domain-containing tyrosine phosphatase 2, thereby suppressing T-cell activation and proliferation, and ultimately reducing the antitumor immune response (Fig. 3) (66-68). PD-L1 expression can markedly promote tumorigenesis and tumor invasiveness, which may be associated with the downregulation of the immune response (69).

PD1 is most frequently expressed in TNBC compared with other subsets of breast tumors (70). Previous clinical studies have mainly focused on applying anti-PD1/PD-L1 treatment to advanced TNBC, which demonstrated clinical efficacy (71-73). Recently, two phase I trials, KEYNOTE-028 (8) and JAVELIN Solid Tumor (9), documented that anti-PD1/PD-L1 monotherapy with pembrolizumab (MK-3475) or avelumab (MSB0010718C) also achieved a modest but lasting antitumor response in $HR^+/HER2^-$ aBC (Table III). These findings may provide a new

Table III. Clinical studies on immunotherapy for HR+/HER2- advanced breast cancer.

Trial	Drug	Clinical efficacy	Safety[a]	(Refs.)
KEYNOTE-02[b]	Pembrolizumab	ORR:12.0%; CBR: 20%	Autoimmune hepatitis (4%), elevated γ-GT (4%), muscular weakness (4%), nausea (4%), septic shock (4%)	(8)
JAVELI[b,c]	Avelumab	ORR 2.8%	Anemia (1.8%), autoimmune hepatitis (1.8%), elevated γ-GT (1.8%), fatigue (1.8%)	(9)

[a]Grade 3-4 treatment-associated adverse events in the study group. [b]Pre- or perimenopausal women received a gonadotropin-releasing hormone agonist. [c]The safety profile also includes triple-negative breast cancer patients. HR, hormone receptor; HER2, epidermal growth factor receptor 2; ORR, overall response rate (≥24 weeks); CBR, clinical benefit rate (≥24 weeks); γ-GT, γ-glutamyl transferase.

perspective for the treatment of patients with this disease; a rational recommendation is that future studies aim to evaluate the clinical efficacy and safety profile of anti-PD1/PD-L1 therapy in combination with other therapies, particularly hormone therapy, for patients with HR+/HER2- aBC.

5. Anti-CTLA-4

The CD28-B7 immunoglobulin superfamily is a critical signature in T-cell activation and tolerance, in which CD28 and CTLA-4 are two immunoregulatory molecules that competitively share their two ligands (B7.1 and B7.2) (74). CD28 is expressed in 90% of human CD4+ T cells and 50% of human CD8+ T cells, sending a costimulating signal upon TCR binding that plays a key role in the transmission of a productive immune response in many cases (75). The alteration of the number of bound TCRs is consistent with that of the CD28 presentation. However, CTLA-4 is an important negative regulator of the CD28-dependent T-cell response, with a 500- to 2,500-fold higher affinity for both B7 ligands compared with CD28 (75). As such, an increase in the CTLA-4 expression ultimately suppresses the immune response (Fig. 3). CTLA-4 blockade, in turn, increases the level of CD28 expression, which can enhance the immune response of chronic tumor-reactive T cells in tumors to achieve an antitumor effect (76).

Preclinical *in vivo* studies have indicated that inhibition of the CTLA-4 expression enhanced the endogenous immune response of immunogenic tumors, and acted synergistically with other therapies to produce an antitumor effect in less immunogenic tumors (75). Of note, anti-CTLA-4 alone is ineffective in the more poorly immunogenic tumors, as the antitumor activity of immunotherapy depends on the inherent immunogenicity of the tumor. The similarity of the ORR between anti-CTLA-4 monotherapy and its combination with chemotherapy or radiotherapy (77-79) suggests that the results of the antitumor response may only be associated with anti-CTLA-4 alone.

Tremelimumab (CP-675,206) is a fully humanized IgG2 anti-CTLA-4 monoclonal antibody that accentuates the immune activity of human T-cells by blocking the binding of CTLA-4 to B7.1 and B7.2 (80). The antitumor activity of tremelimumab as a single agent in advanced melanoma has been confirmed in numerous clinical trials (80-84), but it is inferior to that of chemotherapy (85). It appears that tremelimumab in combination with other treatments is likely to achieve maximum clinical efficacy. A single-arm pilot study of tremelimumab plus durvalumab for the treatment of 18 patients with aBC (11 with HR+ breast cancer and 7 with TNBC) demonstrated that ORR was only observed in TNBC patients (43%), but not in HR+ breast cancer patients (10). These findings mirror the results of a previous phase I clinical trial of tremelimumab combined with exemestane in 26 patients with HR+ aBC to investigate its MTD, clinical efficacy and safety (11). The best therapeutic benefit was observed in 11 patients with stable disease for ≥12 weeks; however, no partial or complete response was documented. The MTD of exemestane was 6 mg/kg every 90 days. Diarrhea was the most common grade 3-4 treatment-related AE, but it was not observed in patients treated at the MTD. The treatment benefit was positively associated with the increased expression of inducible costimulator by peripheral CD4+ and CD8+ T cells that may be secondary to the immune activation following CTLA-4 blockade. These disappointing outcomes have limited the utility of tremelimumab for HR+/HER2- aBC patients.

6. Conclusions

HR+/HER2- aBC accounts for the largest proportion of advanced breast tumors. The preferred treatment to date has been endocrine therapy, which is initially effective, but eventually results in disease progression. Over the past decade, a large body of clinical studies has demonstrated the clinical activity and controllable toxicity of several molecularly targeted therapeutic agents, both as monotherapy and in combination with hormone therapy for patients with HR+/HER2- aBC. These agents have been approved and recommended by a number of breast cancer treatment guidelines. Due to the advent and popularity of immunotherapy, particularly with the great strides towards achieving antitumor immune response in TNBC, several clinical studies have employed immunotherapy for the treatment of patients with HR+/HER2- aBC and observed some clinical benefits. Therefore, immunotherapy may become a valuable treatment modality for such patients in the future.

Search strategy and inclusion criteria

Articles published in English were searched in the PubMed and Embase databases using the search terms 'immu-

notherapy' or 'targeted therapy' and 'breast cancer' and 'clinical trial' and 'estrogen receptor-positive'. The publications were retrieved on July 3, 2019. Clinical studies that evaluated the clinical efficacy and safety profile of targeted therapy or immunotherapy for $HR^+/HER2^-$ aBC met the inclusion criteria. We also retrieved relevant clinical studies currently underway in the ClinicalTrial.gov database on July 26, 2019.

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Authors' contributions

YS: Conception and design, writing and final approval of the manuscript. LH: Writing the manuscript and figure charting. YW: Writing the manuscript and drawing the tables. QW: Writing the manuscript. WH: Writing the manuscript and drawing the tables. All authors have reviewed and approved the final version of the manuscript prior to submission.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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