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Review

The Future of ER+/HER2– Metastatic Breast Cancer Therapy: Beyond PI3K Inhibitors

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Abstract. *Most breast cancers express the estrogen receptor (ER) receptor and are negative for the human epidermal growth factor receptor 2 (HER2) receptor. ER+/HER2– cancers are treated with hormone-based therapies in the adjuvant setting and derive significant survival benefit from these therapies in the metastatic setting. However, hormone resistance develops in most metastatic patients. An increased understanding of the biology of ER+/HER2– breast cancers has led to the development of new therapies for this disease including CDK4/6 inhibitors and PI3K inhibitors. Several other neoplastic processes are targeted by novel drugs in clinical development, addressing cancer vulnerabilities. These include newer ways to block the ER and targeting the HER2 receptors in ER+/HER2– cancers expressing HER2 in low levels not qualifying for clinical positivity. In addition, promising therapeutic options include targeting other surface receptors or their downstream pathways, as well as targeting the apoptotic machinery and boosting the immune response which is initially insufficient in these cancers. A selection of new drugs in advanced development for ER+/HER2– breast cancer will be discussed in this review.*

Estrogen receptor positive (ER)+/human epidermal growth factor receptor 2 negative HER2– breast cancers constitute the most common subset of breast cancer representing about three fourths of these cancers. The backbone of their systemic therapy consists of hormonal therapies that block the function of ER through various mechanisms (1). These include blockade of the receptor itself in breast cancer cells, blockade of the production of its major ligand, estradiol, or degradation of the receptor. However, a subset of ER+/HER2– breast cancers, mostly corresponding to the genomic luminal B genotype, are resistant to hormonal manipulations from the outset of therapies. More commonly, resistance eventually develops with protracted use of hormone receptor targeting therapies. Resistance to endocrine therapies, either primary or secondary, represents a major block in the success of ER+/HER2– breast cancer therapeutics and it is thus intensely investigated. Various mechanisms imparting resistance have been identified and elucidated in preclinical models and several have been confirmed in the clinic. Successful development of drugs that prevent or circumvent hormone resistance has been accomplished with the introduction of mTOR inhibitors, CDK4/6 inhibitors and more recently, PI3K inhibitors, for PIK3CA mutated cancers (2, 3). However, these targeted drugs address only specific pathways of resistance and are effective either only in groups of patients with specific molecular defects or for only a defined time period before tumors develop secondary resistance. Median progression free survival (PFS), for example, in the combination arm of the phase III trial of letrozole with the CDK4/6 inhibitor palbociclib in the first line setting was 24.8 months (4). Similarly, median PFS was 25.3 months with the combination of letrozole with ribociclib and 23.8 months with the same combination plus goserelin in pre-menopausal women (5, 6). A third CDK4/6 inhibitor, abemaciclib with letrozole or anastrozole showed a median PFS of 28.1 months in the first line setting (7). In the second line, after progression on hormonal therapy alone, the combination of abemaciclib and fulvestrant produced a median PFS of 16.4 months and the combination of palbociclib and fulvestrant

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showed a median PFS of 9.2 months (8, 9). The combination of exemestane with everolimus showed a median PFS of 6.9 months in the hormone refractory setting (2).

Thus, development of further therapies or combinations in this most common type of breast cancer is an unmet need. The molecular understanding of the mechanisms and pathways of endocrine and other resistance has improved in recent years and is starting to have therapeutic bearings. This paper will discuss leading avenues in future therapeutics of ER+/HER2– breast cancers.

Mutations in *ESR1*: SERDs, SERCAs and PROTACs

Nearly 75% of breast cancers express estrogen receptor alpha (ERα; encoded by gene *ESR1*), a ligand-dependent transcription factor which is an essential driver for breast cancer initiation and progression. ER-directed therapies including selective ER modulators (SERM; tamoxifen), selective ER down regulators (SERD; fulvestrant) and aromatase inhibitors (AI; steroidal; exemestane and non-steroidal; anastrozole and letrozole) represent the cornerstone of systematic therapy in both the adjuvant and metastatic setting for patients with ER+ breast cancer. Despite their demonstrated antitumor efficacy in the clinic, *de novo* or acquired resistance can occur during the treatment, which remains a challenging and substantial barrier to prolonged disease remission. Whereas diverse and complex mechanisms of resistance to endocrine therapies have been identified, it is now established that the gain of function due to point mutations within the ligand-binding domain (LBD) of ERα resulting in constitutive transcriptional activity can confer resistance to aromatase inhibitors (10, 11). Mutations in the *ESR1* LBD, which are rare in primary tumors, are found in up to 40% of metastatic lesions, usually acquired following long-term treatment with AIs or tamoxifen (12). They are often associated with more aggressive disease biology and carry dismal prognosis with shorter overall survival in patients relative to wild-type *ESR1* (13). Two of the most common LBD point mutations, Y537S and D538G, are found in 70% of all *ESR1* mutations identified in patients with recurrent ER+ breast cancers (11, 14). Besides their constitutively activating transcription, these mutations probably contribute to disease progression by exhibiting distinct neomorphic activities (15).

Even if mutant ER is resistant to estrogen deprivation, it is unclear if other endocrine therapies, especially the selective estrogen receptor degraders (SERDs) such as fulvestrant, are effective in *ESR1* mutated tumors, as suggested by preclinical data (11, 16). The randomized phase III SoFEA trial, evaluating the combination of fulvestrant with or without anastrozole *versus* exemestane, showed improved PFS of 5.7 months in the combination arm compared with 2.6 months with exemestane in *ESR1* mutated patients (HR=0.52). In contrast, patients with wild-type *ESR1* had similar PFS in both arms (17). However, in the PALOMA-3 trial, palbociclib added to fulvestrant demonstrated a prolonged PFS irrespectively of the *ESR1* mutational status (18). To further improve the treatment of *ESR1* mutant breast tumors, next-generation oral SERMs, or SERDs with improved pharmacokinetics and oral bioavailability that target both wild-type and mutant ER are currently being studied.

Several novel SERDs have been developed (elacestrant, AZD9496, GDSC-0810, OP-1074, Seragon 14-n, GDC-0927, LSZ102, GDC-0810, and OHBS-N) and elacestrant (also known as RAD1901) is currently in advanced clinical trials. Elacestrant is an orally bioavailable SERD that binds to both mutant and wild-type forms of the ER leading to ER degradation and inhibition of cellular proliferation. Partial responses in previously treated patients with CDK4/6 inhibitors have been observed in a phase I clinical trial (19). Among 39 postmenopausal women who had undergone a median of three previous lines of therapy for metastatic breast cancer, 38% had received fulvestrant, 40% had received CDK4/6 inhibitors, and half of the patients were positive for at least one *ESR1* mutation at their baseline plasma circulating tumor DNA (ctDNA) samples. An overall response rate (ORR) of 27.3% was observed. The median duration of response was 17.4 weeks, with a median PFS of 4.5 months, achieved irrespectively of the ESR1 status. Currently, a phase III trial is ongoing with elacestrant (EMERALD/NCT03778931) in patients previously treated with CDK4/6 inhibitors (20).

AZD9496, an orally bioavailable, potent SERD, consistently outperformed fulvestrant *in vitro* in tumor cells expressing Y537S *ESR1* (11), with its efficacy being further improved when combined with phosphatidylinositol 3-kinase and CDK inhibitors. Potential clinical activity of AZD9496 has been shown in the early phase of its development in heavily pretreated patients with ER+/HER2– breast cancer (NCT02248090). Among the forty-five pre- and postmenopausal women who were treated with escalated doses in this first-in-human phase I trial, stable disease was obtained in 22% of patients at six months and one patient, who had never been treated either with fulvestrant or with CDK4/6 inhibitors, had a partial response (21). A phase I clinical trial is ongoing comparing AZD9496 with fulvestrant in postmenopausal women with ER+/HER2– breast cancer (NCT03236974). Several other SERDs are in early phase clinical development alone or in combination with CDK4/6 inhibitors.

A new category of ER antagonists named selective estrogen receptor covalent antagonists (SERCAs) is represented by H3B-6465, a SERCA that inactivates both wildtype and mutant ER by targeting a unique cysteine residue (C530) in the ligand binding pocket of the receptor,

enforcing an irreversible antagonist conformation (22). *In vivo*, the drug has demonstrated significant single-agent antitumor activity in ER wild-type and ER Y537S mutant breast cancer xenografts, that was superior to fulvestrant. Its clinical activity is currently evaluated in two ongoing clinical trials, either as monotherapy or in combination with palbociclib (NCT03250676/NCT04288089).

Proteolysis targeting chimeric (PROTACs) molecules are a novel family of hetero-bifunctional small molecules that connect a target protein to an E3-ubiquitin ligase leading to the formation of a ternary complex that initiates degradation by ubiquitylation. Effective protein degradation was shown in both wild-type and mutant ERα by binding to a conserved region shared between wild-type and mutant receptors (23). In a preclinical study, PROTAC ARV-471 degraded the clinically relevant *ESR1* variants with Y537S and D538G mutations *in vitro* (24). Moreover, ARV-471 inhibited *in vivo* tumor growth in a human xenograft model harboring ESR1 Y537S (24). A phase I trial in locally advanced and metastatic endocrine receptor-positive breast cancer patients is ongoing (NCT04072952).

HER2 and HER3

The human epidermal growth factor receptor (Erb) family consists of four distinct receptors: EGFR (ErbB1/HER1), ErbB2 (HER2/Neu), ErbB3 (HER3), and ERbB4 (HER4) (25, 26). HER2 proteins have no endogenous ligand but are capable of dimerizing with other family members, such as HER1, HER3, or HER4 and transduce down-stream signals (27). Heterodimers containing HER2 are more stable than other non-HER2 receptor combinations. HER2 serves as an essential biomarker for prognosis (28, 29). *ERBB2* amplification is an established molecular event that leads to reduced sensitivity to antiestrogen treatment, through activation of alternative signal transduction pathways promoting survival *i.e.*, PI3K-AKT and mitogen-activated protein kinase (MAPK) pathways (30). Therefore, ER+/HER2+ tumors are treated with HER2 inhibitors in combination with antiestrogens. More recent data have implicated HER2-activating mutations in both primary and acquired resistance to endocrine therapies (31).

Some ER+/HER2– breast cancers express HER2 at low levels being HER2 1+ or 2+ by immunohistochemistry and non-amplified by *in situ* hybridization. These ER+/HER2– breast cancers do not derive benefit from unconjugated anti-HER2 monoclonal antibodies such as trastuzumab (32). However, novel antibody-drug-conjugates (ADCs) show potential activity in the treatment of HER2 low advanced ER+/HER2– breast cancers. ADCs are a class of targeted agents encopassing a recombinant monoclonal antibody covalently bound to a cytotoxic drug through a chemical linker (33). The primary mechanism of action is mediated by internalization of the ADC freeing the cytotoxic payload into the cytoplasm after cleavage of the linker, allowing specific delivery of the cytotoxic agent to the tumor site and minimizing exposure of normal tissues to the cytotoxic drug. Several ADCs are in active clinical development for breast cancer treatment. Most ADCs target HER2, but also other cell surface receptors such as HER3.

Trastuzumab deruxtecan is a HER2-targeting monoclonal antibody conjugated with a topoisomerase I inhibitor that recently showed impressive results in a single-arm phase II trial with 184 highly pretreated HER2-positive breast cancer patients, with a median of 6 prior lines that prompted accelerated approval by the FDA in early 2020 (34). The drug also demonstrated antitumor activity in patients expressing HER2 at low levels (HER2 IHC 1+ or 2+ with negative ISH assay), a subgroup in which available anti-HER2 treatment has not proven effective and is therefore not recommended. The drug conjugate was tested in a phase I trial enrolling 54 extensively pretreated HER2-low advanced breast cancer patients, with a median of 7.5 prior therapies, resulting in an ORR of 37%, a median PFS of 11.1 months, and median overall survival (OS) of 29.4 months (35). Guided by the substantial antitumor activity seen in early phase trials and its generally manageable safety profile, three phase III trials have been initiated, one of which is in HER2-low patients (36). Moreover, two phase Ib trials are evaluating its activity in combination with anti-PD1 antibodies.

A similar to trastuzumab deruxtecan ADC, trastuzumab duocarmazine (SYD985), another HER2-targeting monoclonal antibody conjugated to a potent duocarmycin analog, seco-DUocarmycin-hydroxyBenzamide-Azaindole (vc-seco-DUBA), showed clinical activity in both highly pretreated HER2 positive breast cancer resulting in promising ORR of 33% and a median PFS of 7.6 months (37), but also in HER2-low breast cancer, with an ORR ranging between 28% and 40% depending on hormone receptor status (38). Several other ADCs warrantee clinical investigation in both HER2-positive and HER2 low breast cancer.

A novel class of monoclonal antibody drugs is bispecific antibodies that target two antigens that may be expressed in different cells and mediate cell-cell interaction. Ertumaxomab is a bispecific antibody that targets HER2 on cancer cells and CD3 in T cells and brings these cells in contact with other immune cells expressing the Fcγ receptor in the tumor microenvironment. Whether this drug will prove to trigger antitumor immunity is still unknown. A phase I trial in fourteen patients with solid tumors including breast cancers and expressing varying degrees of HER2 showed that ertumaxomab had a manageable toxicity profile and resulted in one partial response (PR) and two patients with stable disease (39).

Increased expression of HER3 has been reported in 50–70% of breast cancers and defined as a poor prognostic

factor as it has been associated with endocrine resistance in luminal breast cancers and with reduced sensitivity to HER2 directed therapies in HER2-amplified breast cancers (40, 41). New anti-HER3 monoclonal and bispecific agents that can overcome this resistance are also of interest. The anti-HER3 antibody seribantumab (MM-121) has been investigated in combination with either paclitaxel alone in HER2-negative advanced breast cancers or with taxane/epirubicin in luminal advanced breast cancers (42). Data on the combination in HER2–/ER+ advanced breast cancer showed an improved PFS signal that was reflected in improved OS (43). Lumretuzumab (RG7116, RO-5479599), another anti-HER3 antibody, has been studied in combination with paclitaxel and pertuzumab for the treatment of HER2-low/HER3+ advanced breast cancer (44). The observed ORR was high (55% and 38.5% in different dose-cohorts). However, the therapeutic window of the combination was narrow leading to suspension of further clinical development.

Based on initial encouraging results, there is a strong rationale for exploring U3-1402, an anti-HER3 ADC conjugated with a topoisomerase I inhibitor cytotoxic agent. U3-1402 was investigated in a phase I/II study including 42 heavily pretreated HER3- positive (IHC score 2+/3+) advanced breast cancer patients. This study reported activity of the drug regardless of HER2-positivity (45). Most patients enrolled in the trial were hormone receptor-positive and triple-negative whereas only 16% of patients enrolled were HER2-positive. The dose-expansion part of the trial that enrolled 42 patients obtained an ORR of 42.9% and a median PFS of 8.3 months. Responses were observed in all molecular subtypes, and treatment was well tolerated, with the most common grade 3 and above adverse events being hematological toxicity.

Fibroblast Growth Factor Receptors (FGFR)

The FGFR family of tyrosine kinase receptors may facilitate many cancer-promoting processes such as proliferation, inhibition of apoptosis and migration (46). They share their intracellular signal transduction machinery with other tyrosine kinase receptors such as the EGFR family receptors, including the Ras-Raf-MEK pathway, the PI3K-Akt pathway and PKC. Among the four FGFR with kinase activity, FGFR1 is most commonly amplified in breast cancer in 10% to 15% of cases with a higher prevalence in the ER+/HER2– sub-type (47). Amplifications of the three other receptors, FGFR2, FGFR3 and FGFR4 and mutations in the four receptors are less common. FGFR lesions are associated with hormone therapy resistance and worse outcomes (48). Thus, targeting FGFR in cancers with lesions in the receptors arises as a viable therapeutic option. Selective and less selective, multi-targeted small molecule tyrosine kinase (TKI) inhibitors of FGFR are in development in the space of ER+/HER2– breast cancer.

The two most advanced multi-targeted inhibitors in development in breast cancer is dovitinib, an FGFR, VEGFR, PDGFR, RET and c-kit inhibitor and lucitanib, an FGFR and VEGFR inhibitor both of which have completed phase II trial evaluation (49, 50). Among 97 post-menopausal ER+/HER2– breast cancer patients who had progressed on previous hormonal therapies and were randomized to receive fulvestrant with or without dovitinib, median PFS was equivalent in the two arms. In patients with FGFR amplification PFS was longer in the dovitinib *versus* the placebo group and overall response rates were higher in the dovitinib group (27%) *versus* 10% with placebo (49). The phase II trial of lucitanib monotherapy enrolled both FGFR amplified and non-amplified ER+/HER2– metastatic breast cancer patients (50). The response rate was low even in amplified patients but somewhat enriched in patients with higher levels of FGFR1 amplification (copy number >4). A third non-selective inhibitor, nintedanib, targeting FGFR, VEGFR, PDGFR, and FLT3, has been evaluated in combination with letrozole in a phase 0/I study (51). The study confirmed feasibility but raised concerns for the long-term tolerability of the combination (51).

One of the specific kinase inhibitors, the orally bioavailable erdafitinib (formerly JNJ-42756493), which is already in the market with a breakthrough designation in urothelial cancer, has phase I data from two trials that included breast cancer patients (52). In the most extensive of them, the response rate in the 36 breast cancer patients included was 8.3% (3 of 36 patients) (53). Twenty nine of the 36 breast cancer patients had lesions in FGFR genes and those were mostly amplifications (n=21). The higher response rates were observed in cholangiocarcinomas and urothelial carcinomas where FGFR lesions are mostly mutations and fusions, as opposed to amplifications in breast cancer (53). This concurs with the results of the other smaller phase I study of erdafitinib in which partial responses were observed only in patients with translocations (54). An additional phase Ib trial (NCT03238196) is ongoing and will investigate the combination of erdafitinib with fulvestrant and palbociclib in ER+/HER2–/FGFR amplified breast cancer patients. The development of another selective FGFR inhibitor that was studied with fulvestrant (study NCT01202591) was terminated, given negative signals for the efficacy of the drug in other cancers. Interestingly, in these studies, selective FGFR inhibitors show the on-target adverse effect of hyperphosphatemia, due to FGFR inhibition in renal tubules, as opposed to multi-targeted inhibitors that were not associated with this adverse effect despite pharmacodynamically inhibiting FGFR signaling (46, 55). This suggests that inhibition of other pathways by multi-targeted inhibitors may counteract FGFR inhibition in renal tubules. Whether these multiple inhibitions have similar effects in cancer tissues and thus may be a pharmacodynamic

liability for multitargeted FGFR inhibitors remains to be investigated. Other common adverse effects observed in FGFR inhibitor studies include hepatic enzyme elevation, asthenia, nail toxicity and dysgeusia (46, 49, 50).

Overall, FGFR appears to be a promising therapeutic target in ER+ /HER2– breast cancers. Preclinical *in vitro* and *in vivo* studies have suggested that FGFR inhibitors are effective independently of the genetic lesion in FGFR receptors, be it mutation, amplification or translocation (56). However, the clinical experience in different cancers suggests that FGFR amplified tumors are less responsive to FGFR inhibition. Variable amplification levels are present in FGFR amplified breast cancers and it is conceivable that only tumors with the highest copy numbers become dependent on FGFR signaling and thus sensitive to FGFR inhibitors. Thus, parallel development and validation of biomarkers of response will be instrumental in the clinical success of these drugs. Moreover, even cancers with FGFR mutations or high-level amplifications may be less sensitive to FGFR inhibitors if they possess additional lesions in downstream pathways such as *PIK3CA* mutations. Targeted combinations with drugs addressing these downstream lesions may be able to circumvent resistance. Besides small molecule inhibitors, monoclonal antibodies inhibiting FGFR family members had been developed and brought to early phase clinical trials (57). Further development has not progressed, though, possibly related to efficacy reasons but also the parallel development of small molecule inhibitors that had successfully competed for limited number of patients and resources.

Downstream Signaling: Akt Inhibitors, src Inhibitors

Aberrant activation of the phosphatidylinositol 3-kinase (PI3K) pathway is common in ER+ breast cancers, with mutations in *PIK3CA*, the gene for the PI3K alpha catalytic subunit p110α having a prevalence of approximately 40% of cases (12). In an estimated 7% of ER+ breast cancers, instead of *PIK3CA* mutations, the PI3K pathway is activated by mutations of kinase AKT1. AKT1 E17K figures as the most common (about 80%) alteration and it constitutively activates PI3K signaling by promoting localizing AKT1 to the plasma membrane (58). The ATP-competitive pan-AKT kinase inhibitor capivasertib has shown activity in recent clinical trials in AKT1 E17K-mutant metastatic breast cancer and other cancers (59). Breast cancer xenograft preclinical models suggested that sequence of therapies is important. An improved efficacy was obtained if docetaxel was administered before capivasertib, whereas an antagonist effect was observed if docetaxel was administered after capivasertib (60). Accumulating evidence led to the phase I/II randomized BEECH study evaluating capivasertib in combination with weekly paclitaxel in the first-line setting (61). Weekly paclitaxel was chosen because of superior tolerability compared to docetaxel (62). Despite substantial preclinical data and a phase I study showing a response to capivasertib monotherapy in PIK3CA+ tumors (63, 64), no statistically significant differences in term of PFS (10.8 months) was observed in the overall population or in the PIK3CA+ sub-population (61). The BEECH trial included ER+ breast cancer patients, but no concomitant endocrine therapy was allowed, which may explain the lack of effect of capivasertib in combination with chemotherapy.

In contrast, when combined with hormonal therapy, capivasertib showed positive results. The FAKTION trial reported a doubling of PFS from 4.8 months (95% CI=3.1-7.7) to 10.3 months (95% CI=5.0-13.2, p=0.004) with the addition of capivasertib to fulvestrant in ER+/HER2– negative, advanced breast cancer that had progressed on an aromatase inhibitor (65). The benefit of the combination therapy was observed in both wild-type and mutated PIK3CA and PTEN patients, further highlighting the essential role of AKT1. An analysis of the AKT1 kinase mutation status was not reported in the original publication but is underway (65).

Ipatasertib, a highly selective oral ATP-competitive AKT inhibitor, was tested in combination with paclitaxel in unselected triple negative breast cancers and showed an improved PFS and overall survival (66). This compound is currently evaluated in combination with endocrine therapy and palbociclib in the TACTIK trial in ER+/HER2– metastatic breast cancer patients (NCT03959891). MK-2206, a selective allosteric inhibitor of AKT, showed limited clinical activity as monotherapy in a phase II trial of patients with advanced breast cancer bearing PI3K/AKT pathway mutations (67). Less than optimal efficacy could be the result of cross talk from multiple transduction pathways, including HER2 and insulin growth factor receptor, that overwhelm the drug inhibitory effects (68).

Overexpression and activation of tyrosine kinase Src has been linked to breast carcinogenesis, as well as to the development of resistance to therapy (69, 70). *In vitro* studies in breast cancer cells have shown that a complex of Src with membrane-associated ERα and kinase PI3K promotes growth and endocrine resistance (71). Combining Src inhibitors with endocrine agents can reduce the emergence of acquired resistance in preclinical models. Src has also been implicated in the survival and outgrowth of breast cancer cells in the bone marrow (72). The Src inhibitor bosutinib was evaluated as monotherapy in a phase II clinical study of 73 previously treated advanced or metastatic breast cancer. Four responses occurred in patients with hormone receptor positive disease (73).

Dasatinib is a kinase inhibitor with specificity for many related kinases including Src kinase. It is in the market for

the therapy of Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase and acute lymphoblastic leukemia (74). In a phase II clinical trial, dasatinib monotherapy produced two confirmed partial responses (4.4%) and six disease stabilizations for more than 16 weeks (13.3%) in 45 evaluable patients with advanced, pretreated HR+ breast cancer (75). The drug was also evaluated in a phase II non-comparative study in which 120 patients were randomized to letrozole alone or with dasatinib. Crossover was allowed upon progression on monotherapy. A clinical benefit rate (CBR; defined as complete response rate, partial response rate and stable disease for more than six months) of 71% (95% CI=58-83%) was observed with letrozole and dasatinib *versus* a projected CBR of letrozole alone of 56%. Median PFS with the combination was 20.1 months and 9.9 months with letrozole alone. These results are intriguing and suggest that dasatinib may impede the development of acquired resistance to aromatase inhibitor therapy (76).

Apoptosis as a Target

Apoptosis inhibition is one of the defining characteristics of cancer (77). Two main apoptotic pathways have been characterized that culminate in the activation of caspases, the main enzymes that execute apoptosis. The extrinsic pathway is triggered by ligation of death receptors in the cell surface and the intrinsic pathway is triggered by collapse of mitochondrial membrane potential regulated by the B cell lymphoma 2 (BCL2) family of proteins that include both anti-apoptotic and pro-apoptotic members (78). Anti-apoptotic family members such as BCL2, BCL-xL, and MCL-1 prevent apoptosis initiation by safeguarding the maintenance of mitochondrial membrane potential. Pro-apoptotic BCL2 family members such as BAD, BIM and BIK possess a BCL2 Homology 3 (BH3) domain through which interact and inhibit anti-apoptotic members and trigger apoptosis. Preclinical studies and expression data have addressed the relevance of apoptosis and BCL2, the prototypic anti-apoptotic family member in breast cancer. BCL2 is a target gene of ER mediated transcription and is expressed in the great majority of ER positive breast cancers (79-81). In addition, BCL2 expression is associated with improved survival in stage I to III breast cancer, independently of ER and HER2 positivity (80). BIM knockdown in breast cancer cells did not affect xenograft formation in mice as compared to parental cells but it increased metastatic potential (82). BIM is a direct target of transcription factor Snail, a master regulator of epithelial to mesenchymal transition (EMT) and thus, it acts as a safeguard for apoptosis induction in cells that have inadvertently activated the EMT program (83). In a human ER positive breast cancer xenograft study in mice, the BH3 mimetic drug ABT-737 and the BCL2 selective inhibitor ABT-199 (now known as venetoclax) were efficacious in inhibiting progression of xenografted breast cancers and improving survival of mice, each in doublet combination with tamoxifen, compared with mice that were treated with placebo or with the three drugs as monotherapy (84). Moreover, the addition of the mTOR inhibitor PKI-587 to the combination of tamoxifen and venetoclax further improved survival results.

Venetoclax has been approved for treatment of hematologic cancers including chronic lymphocytic leukemia and acute myeloblastic leukemia (85, 86). In view of the expression of BCL2 and preclinical studies confirming *in vivo* activity, development of venetoclax has been initiated in ER positive breast cancer (87). Results from a phase Ib dose escalation and expansion study of venetoclax and tamoxifen in ER+ and BCL2 positive metastatic breast cancer patients who have received up to three previous lines of therapy are available (87). BCL2 positivity was defined as moderate or strong cytoplasmic staining in at least 10% of cells. The combination of venetoclax and tamoxifen was feasible, and no unexpected toxicities were observed. The expansion cohort was set at 800 mg daily of venetoclax after three patients were treated at 200 mg, 400 mg and 600 mg without dose limiting toxicities. The ORR of the whole cohort of 33 patients was 45%. Among the 24 patients that were treated at the 800mg daily level, responses were observed in 13 patients (ORR=54%) and median duration of response was 42 weeks. Five additional patients had stable disease lasting more than 24 weeks, for an overall clinical benefit rate of 75% (87). A correlative circulating tumor DNA study showed no correlation of the response to therapy with the presence of common mutations of breast cancer such as *PIK3CA*, *GATA3*, *MAP3K1* and *CDH1* or mutations in *ESR1* that commonly develop after hormonal therapy exposure. These efficacy and tolerability results of the venetoclax and tamoxifen combination are promising, especially taking into consideration that patients were pretreated and resistant to hormonal therapies. Confirmation in further studies is eagerly awaited.

Building on the success of venetoclax in hematologic malignancies and early positive results in ER positive breast cancer, further development of apoptosis pathway drugs will require continuous elucidation of the role and regulations of each BCL2 family member. An example is the pro-apoptotic family member BCL2-interacting killer (BIK) which has been paradoxically linked with adverse outcomes in breast cancer across subtypes (88). Moreover, BIK expression is associated with proliferative tumors with high Ki-67 expression (89). In *in vitro* studies in breast cancer cells, suppression of BIK with siRNA interference up-regulated autophagy markers (90). The SERD fulvestrant up-regulates both BIK and another BCL2 pro-apoptotic family member, p53-upregulated modulator of apoptosis (PUMA) in ER+ breast cancer cells MCF7 (91). A similar role of these pro-apoptotic proteins in apoptosis induction has been described

following irradiation and doxorubicin treatment (92). These data, in conjunction with the above described role of BIM as a target of Snail transcription, suggest that the apoptosis network is engaged in multiple interactions and receives significant input from regulators that are embedded in diverse cellular programs. Targeted therapeutic exploitation of apoptosis with venetoclax and, possibly additional drugs that will be brought to clinical development has the potential to reverse hormonal resistance in ER+ breast cancers.

Immunotherapy

ER+/HER2– breast cancer is the sub-type that is the least immunogenic based on the presence of tumor infiltrating lymphocytes (TILs) (93). PD-L1 expression is lower in ER+/HER2– breast cancer, especially luminal A cancers, than other sub-types (94, 95). In addition, the percentage of cases with a high tumor mutation burden (TMB) is less than 5% when using a cut-off of 192 mutations, suggesting restricted ability for neoantigen production (96). Consistent with this low immunogenicity, results of the initial evaluation of the anti-PD-1 monoclonal antibody pembrolizumab in a phase I study in ER+/HER2– advanced breast cancers were disappointing, having a response rate of 12% (97). In addition, a phase II trial that randomized 88 ER+/HER2– breast cancer patients who had received two or more previous lines of therapy to eribulin with or without pembrolizumab showed a higher response rate in the monotherapy arm (34% *versus* 25% with the combination of eribulin and pembrolizumab) (98). PFS was identical in the two arms. In a sub-set analysis of patients with PD-L1 positive tumors, patients whose tumors showed high TILs or high TMB obtained no PFS benefit of pembrolizumab, although the number of patients was low (98). Ongoing trials of pembrolizumab in ER+/HER2– breast cancer focus on combinations with endocrine therapy and CDK inhibitors in the metastatic setting (NCT02778685) or with chemotherapy in the neo-adjuvant setting, for high risk patients (KEYNOTE-756, NCT03725059). Other investigators will explore the combination of pembrolizumab, tamoxifen and the HDAC inhibitor vorinostat (NCT04190056) or the combination of pembrolizumab and paclitaxel, specifically for luminal B patients (NCT03841747). Interestingly, most of the trials do not restrict inclusion to PD-L1 positive patients but include secondary or exploratory analyses for patients with a combined positive score (CPS) of 1% or above.

The PD-1 monoclonal antibody nivolumab is investigated in a phase I study in combination with nab-paclitaxel, with or without the anti-CTLA-4 antibody ipilimumab in metastatic ER+/HER2– breast cancer (NCT04132817). A phase III study in the neoadjuvant setting comparing chemotherapy with or without nivolumab is also planned, similarly to the pembrolizumab study (CheckMate 7FL, NCT04109066).

The only immune checkpoint inhibitor that is currently clinically available for breast cancer in the metastatic triple negative setting is the anti-PD-L1 antibody atezolizumab, which is also being explored in ER+/HER2- breast cancer (99). In the neo-adjuvant setting, a phase II trial of multiple arms compares atezolizumab monotherapy with combinations with the MEK inhibitor cobimetinib or the AKT kinase inhibitor ipatasertib or a combination of ipatasertib and bevacizumab (NCT03395899, ECLIPSE). A study in the metastatic setting is investigating the combinations of atezolizumab with cobimetinib for patients with TP53 mutations or with idasanutlin (an MDM2 inhibitor) in patients without TP53 mutations (NCT03566485).

Immunotherapy for ER+/HER2– breast cancer is evidently in the early phases of investigation and results of these studies will give the directions that the field will take. However, it is clear that the immunologically cold environment of these cancers will require combination therapies to boost the effect of immune checkpoint inhibitors. These could include other immune checkpoint inhibitors or alternative therapeutic manipulations. A relevant target from the tumor microenvironment is the complement system (100). In studies in mice, the complement inhibitor cluster of differentiation 55 (CD55) [also called decay accelerating factor (DAF)] expressed in cancer cells after chemotherapy downregulates ICOS-L expression in B cells which leads to T effector cell down-regulation and T regulatory cell up-regulation (101). These modulations tip the balance of the tumor microenvironment towards immunosuppression. Knockdown of CD55 led to upregulation of ICOS-L in B cells (101). CD55 gene is located at the chromosome arm 1q which is one of the most commonly gained loci in breast cancer. The gene *per se* is amplified in 9% of breast cancer cases, according to breast cancer data from the Cancer Genome Atlas (TCGA) (58, 102, 103). Thus, inhibition with a monoclonal antibody or other drugs could be a plausible means of turning on cold breast cancers to tumors with inflamed microenvironments more responsive to immune checkpoint inhibitors.

Conclusion

While survival and quality of life of women with breast cancer have improved with the discovery of novel targeted therapies for ER+ cancers, overcoming endocrine resistance remains a primary therapeutic need. The ER signaling pathway represents a complex cascade with several regulators and comprehensive crosstalk with other pathways, which favor endocrine therapy resistance development. It is hoped that characterization of genetic lesions commonly associated with progression will guide the discovery of targeted therapeutic agents. Combination therapies that efficaciously inhibit tumor growth by interfering with the function of cell

cycle kinases or with the function of other key kinases participating in cancer-associated signaling pathways have recently been approved. New agents with inhibitory effects on the intracellular pathways mediating endocrine resistance are being tested. Novel endocrine agents, addressing resistance mechanisms impeding efficacy of existing drugs in use, also continue to be investigated as monotherapy and in new combinations. For advanced luminal breast cancers, combinations of endocrine therapies with other biological agents, particularly with CDK4/6 inhibitors, PI3K inhibitors (for mutant cancers) and mTOR inhibitors, are the mainstay therapy and many drugs of these classes have been approved. The confirmed theme in developmental therapeutics of ER+/HER2– breast cancers with hormone therapy resistance is that continuation of endocrine treatment, possibly with an hormonal agent with a different mode of inhibition, and addition of an inhibitor of the resistance pathways is advantageous, compared with therapeutic strategies that do not include endocrine therapies. It is anticipated that several of the newly explored inhibitors of the pathways presented in this paper will soon enter the clinical armamentarium against metastatic ER+/HER2– breast cancers.

The clinical benefit provided by novel agents needs to be constantly weighted against the possible increase in adverse effects. A continuous focus on supportive and palliative therapies, including symptoms derived from adverse effects of these novel therapies, will help preserve the quality of life of patients. As an enlarging pipeline of promising agents provides incremental benefits to ER+/HER2– breast cancer patients, with corresponding prolongation of survival, preservation of quality of life becomes ever more important.

Conflicts of Interest

The Authors declare they have no conflicts of interest.

Authors' Contributions

AS reviewed the literature, wrote and revised the paper. IAV conceived the study, reviewed the literature, wrote and revised the paper.

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