REVIEW

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Clinician's guide to targeted estrogen receptor degradation using PROTAC in patients with estrogen receptor-positive metastatic breast cancer

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Purpose of review
Metastatic breast cancer (MBC) remains a major clinical challenge, necessitating the development of innovative therapeutic strategies. Estrogen receptor (ER) degradation using proteolysis-targeting chimeras (PROTAC) has emerged as a promising approach for overcoming acquired resistance to endocrine therapy. This review will summarize recent findings, highlighting the role of ER degradation by PROTAC in patients with MBC.

Recent findings
The application of PROTAC technology for ER degradation has demonstrated initial success in preclinical and early clinical studies. PROTACs, consisting of an ER-targeting moiety, an E3 ubiquitin ligase-recruiting moiety, and a linker, facilitate ER ubiquitination and subsequent proteasomal degradation. Yet, significant challenges persist in the clinical translation of ER degradation by PROTAC. These include the optimization of PROTAC design, elucidation of mechanisms underlying resistance to PROTAC-induced ER degradation, and identification of predictive biomarkers for patient stratification. Additionally, addressing potential off-target effects and toxicity profiles remains a critical aspect of developing PROTAC-based therapies.

Summary
Recent data demonstrate the potential of ER degradation by PROTAC as a therapeutic strategy for patients with MBC. Continued research efforts and development of synergistic combinations are crucial for further advancing PROTAC-based therapies and improving outcomes in patients with MBC.

Keywords
breast cancer, degradation, estrogen receptor, PROTAC, ubiquitin

INTRODUCTION

Approximately 70% of breast cancers are hormone receptor-positive (HR+); these tumors have estrogen receptors (ER), progesterone receptors, or both. HR+ breast cancers are predominantly luminal and driven by the ER transcriptional network. ERα, encoded by the *ESR1* gene, is a nuclear receptor transcription factor. Following ligand binding to ER, the receptor undergoes dimerization and translocates into the nucleus, where it either binds directly to chromatin at sites with the estrogen responsive elements motif or indirectly through interaction with additional transcription factors, such as AP-1 [1]. ER also interacts with co-regulators and together these complexes mediate the expression of multiple genes that drive tumorigenesis and tumor progression [1,2].

Endocrine therapies targeting ER are the mainstay treatment for HR+ metastatic breast cancer (MBC). Modern endocrine treatments include two main classes of agents: [1] treatments that decrease estrogen production, and [2] treatments that directly decrease ER signaling. The first class is composed of aromatase inhibitors (AI) that suppress the production of estradiol in peripheral tissues by the

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KEY POINTS

- PROTACs consist of an ER-targeting moiety, an E3 ubiquitin ligase-recruiting moiety, and a linker.
- ER-targeting PROTAC facilitates ER ubiquitination and subsequent proteasomal degradation.
- Initial reports from clinical trials utilizing PROTAC in patients with endocrine resistant HR+ MBC demonstrated successful ER degradation with meaningful clinical outcomes.

inhibition of aromatase, the enzyme that converts androgens to estrogen. In premenopausal woman, estradiol levels can be decreased by blocking the production of estradiol in the ovaries by gonadotropin-releasing hormone agonists or surgical removal of the ovaries. Typically, the second class consists of selective estrogen modulators (SERM) and selective estrogen receptor degraders (SERD). SERMs, such as tamoxifen or lasofoxifene, inhibit ER in breast cells but might have different effects on ER in other tissues. For example, tamoxifen has an agonistic effect on endometrial ER, increasing the risk for endometrial cancer. SERDs, such as fulvestrant, are considered pure antagonists and have been shown to promote the degradation of ER [3].

Nowadays, the standard of care (SOC) first-line therapy in patients with HR+, human epidermal growth factor receptor 2 (HER2)-negative MBC is an endocrine therapy (AI/fulvestrant) backbone with a concurrent CDK4/6 inhibitor (CDK4/6i). Recently, the combination of the CDK4/6i ribociclib with an AI administered in the first-line setting was shown to extend median overall survival compared to AI alone. The median overall survival was 63.9 months (95% confidence interval [CI], 52.4–71.0) with ribociclib plus letrozole and 51.4 months (95% CI, 47.2–59.7) with placebo plus letrozole (hazard ratio for death, 0.76; 95% CI, 0.63–0.93; two-sided $P = 0.008$). Yet, despite the potency of these agents in patients with newly diagnosed MBC, ultimately all patients face endocrine resistance and disease progression. The median overall survival following endocrine resistance is limited and in the range of 2–3 years only, highlighting the need for improved endocrine therapies. There are multiple mechanisms of resistance involving ER directly (e.g., *ESR1* activating mutations, *ESR1* fusions), or indirectly through activation of bypassing pathways and mediators (e.g., MAPK, PI3K/AKT/mTOR) [4].

The current review will provide a focused and updated examination on the application of proteolysis-targeting chimera (PROTAC) molecules to degrade ER in patients with HR+ MBC, as a promising approach to tackle endocrine resistance.

THE CURRENT SERD LANDSCAPE IN METASTATIC BREAST CANCER

The first SERD, fulvestrant, was approved by the U.S. Food and Drug Administration (FDA) in 2002. The main drawback of fulvestrant is its intramuscular route of administration, negatively affecting the quality of life of numerous patients [3]. This feature led to a global effort to develop a SERD that can be delivered orally with improved pharmacokinetics, culminating in the approval of the first oral SERD elacestrant in January 2023. In the randomized phase III EMERALD trial, the use of elacestrant as a single agent was evaluated versus SOC endocrine therapy in patients with HR+ MBC who progressed on prior treatment with a CDK4/6i and endocrine therapy [5]. Interestingly, most patients in the control arm received fulvestrant (69%). The median progression-free survival (PFS) among patients treated with elacestrant was improved compared to SOC (2.79 vs. 1.91 months) in the intent-to-treat population ($P = 0.0018$). Among patients with an activating *ESR1* mutation, the PFS was 3.78 months in the elacestrant arm versus 1.87 months in the SOC arm ($P = 0.005$). Following these results, the agent was approved by the U.S. FDA only for patients with *ESR1* mutations. Remarkably, two randomized phase II studies evaluating additional oral SERDs (giredestrant [aceIERA] and amcenestrant [AMEERA-3]) as single agents versus fulvestrant showed only a nonsignificant trend towards improved performance of these oral SERDs in patients harboring *ESR1* mutations, possibly failing to reach statistical significance due to relatively modest sample sizes. Corti *et al.* provide a detailed review of the contemporary development of SERDs in HR+ MBC [6].

THE ERA OF PROTAC IN METASTATIC BREAST CANCER

The PROTAC molecule is a novel approach for the purpose of targeted degradation of a protein of interest. PROTACs are heterodimeric agents that are being developed in various medical scenarios to promote ubiquitination and proteosomal degradation of their targets. The PROTAC is composed of a linker that connects the targeting part (i.e., receptor's ligand) and the E3 ligase recruiting part (Fig. 1). The E3 ligase is a member of a large family of proteins that regulate the final step of an enzymatic cascade leading to proteosomal degradation by the ubiquitin proteosome system [7].

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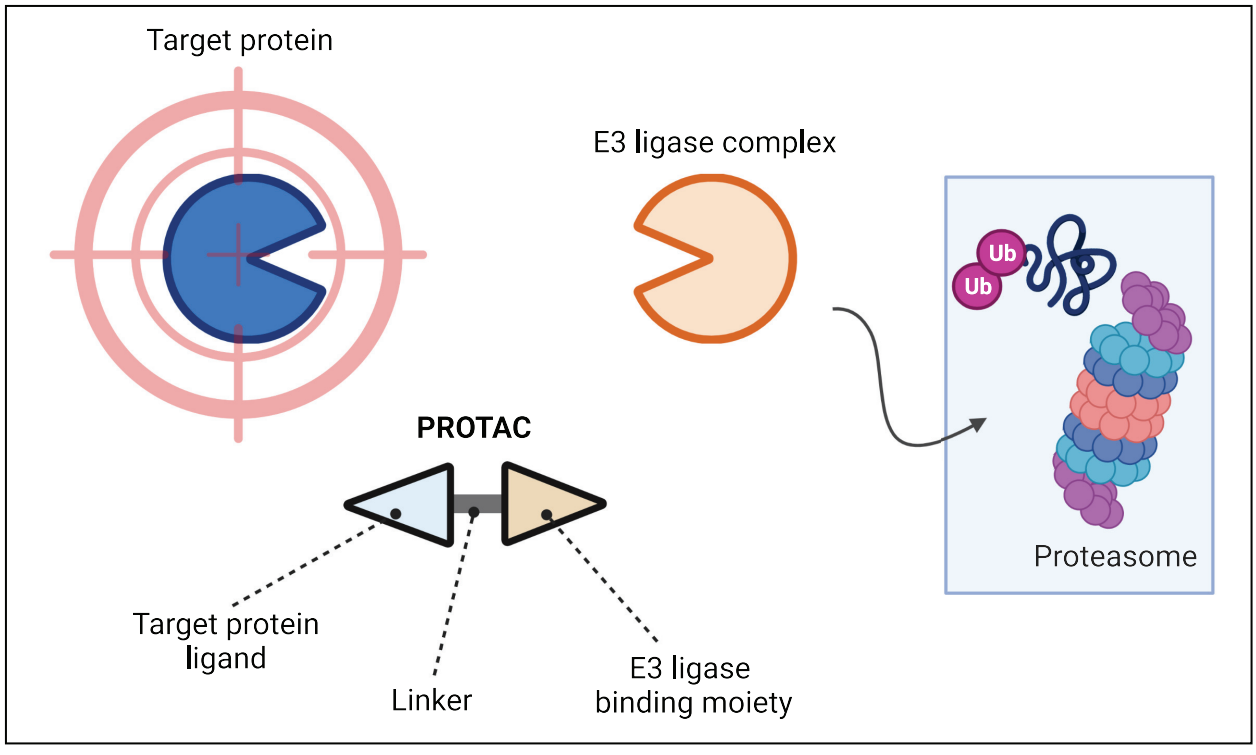

FIGURE 1. PROTAC anatomy. Created by biorender.com. PROTACs, proteolysis-targeting chimeras.

Based on PROTAC's mechanism of action, there are several prerequisites for a successful and clinically meaningful degradation: [1] the utilized E3 ligase should be expressed in the tumor cells [2], the target protein should not be essential to non-cancer cells' function, and [3] degradation of the target protein would lead to a clinically meaningful result. Bekes, Langley and Crews have suggested to classify PROTAC into three categories: classic, bio-PROTAC, and hybrid PROTAC. Classic molecules represent mostly orally available agents that contain small molecules to bind their target; bioPROTAC has a peptide ligand and a hybrid has both classic small molecules and a peptide ligand. Examples for bioPROTAC are fusion proteins that contain ligand-binding domain and E3 activating domain, or molecules that target transcription factors using DNA ligands [8].

ER degradation using PROTAC in patients with MBC is one of the first proof-of-concept studies to show the broad utility of this approach in a clinical setting. ARV-471 (Arvinas) was the first-in-class agent to be tested in human patients starting in 2019. ARV-471 is an orally administered molecule composed of an ER-binding ligand and a second ligand to recruit cereblon (CRBN), an ubiquitously expressed E3 ligase [8]. The results of the dose escalation part of the phase I/II study of ARV-471 monotherapy were reported in 2021 (NCT04072952) [9]. The enrolled patients ($n = 51$) had HR+, HER2-negative MBC and were required to have received at least one prior CDK4/6i treatment, at least two prior endocrine therapies, and up to three prior lines of chemotherapy. During dose escalation, a maximal tolerated dose was not reached, and no dose-limiting toxicities were observed. The most common ($\geq$ 10%) treatment-related adverse events were nausea (24%), fatigue (12%), and vomiting (10%). Only about 10% of evaluable patients developed grade 3 toxicities, and no grade 4–5 adverse events were reported. Promising clinical activity was demonstrated with a clinical benefit rate of 40% (95% CI, 26–56) in 47 evaluable patients.

In 2022, the results of the phase II dose expansion part were presented. Out of 71 patients, 16 (23%) patients developed grade $\geq$3 adverse events, including one (1%) patient suffering from grade 5 toxicity (acute respiratory failure in the setting of progressive disease, probably not related to therapy). The median PFS for the entire cohort was 3.7 months (95% CI, 1.9–8.3), and 5.7 months (95% CI, 3.6–9.4) for patients with *ESR1* mutations. Nine patients with matched pretreatment and on-treatment biopsies underwent quantitative ER assessment that showed a median reduction in ER of 69% (range 28–95%) [10▪]. The results of ARV-471 in combination with palbociclib were not reported. Currently, ARV-471 is being tested in a global

randomized phase III study evaluating ARV-471 monotherapy versus fulvestrant in patients with HR+ MBC who progressed on CDK4/6i with endocrine therapy (VERITAC-2, NCT05654623). Another phase Ib trial is evaluating the tolerability and safety of ARV-471 in combination with everolimus in a similar patient population (NCT05501769).

The second molecule, which is being evaluated in clinical trials starting in 2021, is AC682 (Accutar Biotechnology, NCT05080842 and NCT05489679). Patients with HR+ MBC are enrolled across China and the U.S. AC682 is an ER-targeting oral PROTAC, also activating the CRBN E3 ligase to induce ER degradation. In published data from in-vitro and animal models, AC682 induced potent proteosomal ER degradation and exhibited synergy with CDK4/6 inhibition. Furthermore, activity in tamoxifen-resistant and *ESR1* mutant mouse models was noted [11].

The third molecule to reach clinical development is DT2216 (Dialectic Therapeutics). This agent is being tested in patients with solid tumors (including breast) who are no longer responsive to approved or accepted standard-of-care interventions (NCT04886622). This is a PROTAC targeting the antiapoptotic protein BCL-XL, using the Von Hippel-Lindau (VHL) E3 ligase for degradation [12]. The rationale for this design was to avoid systemic administration of BCL-XL inhibitor, which is known to induce significant thrombocytopenia that limits its clinical use. Platelets express VHL at very low levels, leading to minimal VHL-based degradation of platelets' BCL-XL protein. DT2216 was shown to sensitize VHL-expressing cell lines to chemotherapy, including triple negative cell line (MDA-MB-231) in-vitro and in-vivo [12].

Clinical results are eagerly awaited in the upcoming years for these promising agents.

RESISTANCE MECHANISMS TO ESTROGEN RECEPTOR-TARGETING PROTAC THERAPY

Globally, research efforts to elucidate potential mechanisms of resistance to PROTAC therapy are actively ongoing. Theoretically, the two most plausible mechanisms for acquired resistance are elimination or alterations in the direct PROTAC targets on both sides of the molecule, such as ER loss and CRBN loss. In line with this logic, previous studies in nonbreast cancer models showed that loss of the proteosomal machinery was indeed responsible for acquired resistance to PROTAC [13,14]. However, breast cancer models tell a different story.

The current preliminary data in breast cancer are derived from in-vitro models of ARV-471 resistant cells, from two groups presenting their in-depth analyses at the 2023 annual meeting of the American Association of Cancer Research. Teh *et al.* applied a multiomic profiling approach to describe ARV-471-resistant MCF7 cell line [15▪▪]. The resistant cells exhibited decreased ER expression and increased signaling in bypass pathways, such as MAPK pathway (e.g., *EGFR* overexpression, *NRAS* copy number gain). Interestingly, no changes in *CRBN* expression or appearance of *ESR1* mutations were observed. Moreover, acute decrease in the expression of CRBN did not lead to resistant phenotype, demonstrating the single warhead (ER-binding) activity of the agent. Friel *et al.* performed an elegant genome-wide loss-of-function screen using CRISPR in ER+ breast cancer cell lines treated with ARV-471 [16▪▪]. The genes *NF2*, *PTEN*, and *CRBN* were found as potential ARV-471 resistance mechanisms. Furthermore, the researchers have created resistant cell-line models following a prolonged exposure to ARV-471. Phenotypic characterization revealed that these cells have minimal ER function and an increased MAPK and epithelial-to-mesenchyme pathways signaling. As the cells no longer rely on ER signaling for survival, they are able to evade ARV-471.

Altogether, these findings suggest that in breast cancer models, the main mechanism of acquired resistance is ER loss and activation of bypass pathway signaling. Moreover, as stronger ER degraders are being used, we might observe an increase in the prevalence of HR+ tumors with clinical ER loss. Also, the data imply that carefully designed combination therapies, targeting two or more pathways, should be evaluated in clinical trials to increase the potency and long-term results of PROTAC in patients with HR+ MBC. Considering the published data regarding the moderate toxicity profile of PROTAC, combination therapies with additional agents targeting resistance pathways are warranted.

CHALLENGES IN CLINICAL APPLICATION OF PROTACS

Among the different aspects considered when designing clinical trials utilizing novel therapies, two issues deserve special attention in regard to treatment with PROTACs. First is the ''hook effect'' [17]. The PROTAC is a heterobifunctional molecule that binds to different proteins and creates effective complexes with three parts (target-PROTAC-E3 ligase) to facilitate effective target degradation. When PROTAC is administered beyond the optimal dose, the excess amount of available PROTAC creates ineffective complexes with only two parts (target-PROTAC or E3 ligase-PROTAC) that interfere

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and preclude optimal degradation. Therefore, classic phase I trial designs based on identifying the maximal tolerated dose should be planned and performed with caution. Second, the magnitude of ligase expression is an important factor in PROTAC therapy, affecting both treatment efficacy and toxicity. The most commonly utilized ligases (VHL and CRBN) have variable expression patterns between different tissues with a considerable variability within each tumor histology (Fig. 2). Rational ligase choice can maximize desired protein degradation in parallel to minimizing potential side effects. Interestingly, a novel approach to increase PROTAC specificity is to create an antibody-drug conjugate by attaching the PROTAC to a tumor-targeting antibody [18]. The antibody may limit the exposure of normal tissues to the PROTAC, without the need to identify the tumor-specific E3 ligase. This concept was shown when BRD4-targeting PROTAC was attached to trastuzumab and successfully led to BRD4 degradation only in HER2-expressing tumor cells [18]. In addition, many of the E3 ligases are a part of larger complex; for example, CRBN binds DDB1, CUL4, and RBX1 to form an active machinery [19]. Presumably, expression of all of these partners is a prerequisite for an optimal degradation in the desired tumor cells.

FUTURE PERSPECTIVE

HR+ MBC represents a significant clinical challenge, necessitating the development of novel therapeutic strategies. Among these strategies, the application of novel methods such as ER-targeting PROTAC for efficient ER degradation has emerged as a promising approach (Table 1). However, several major

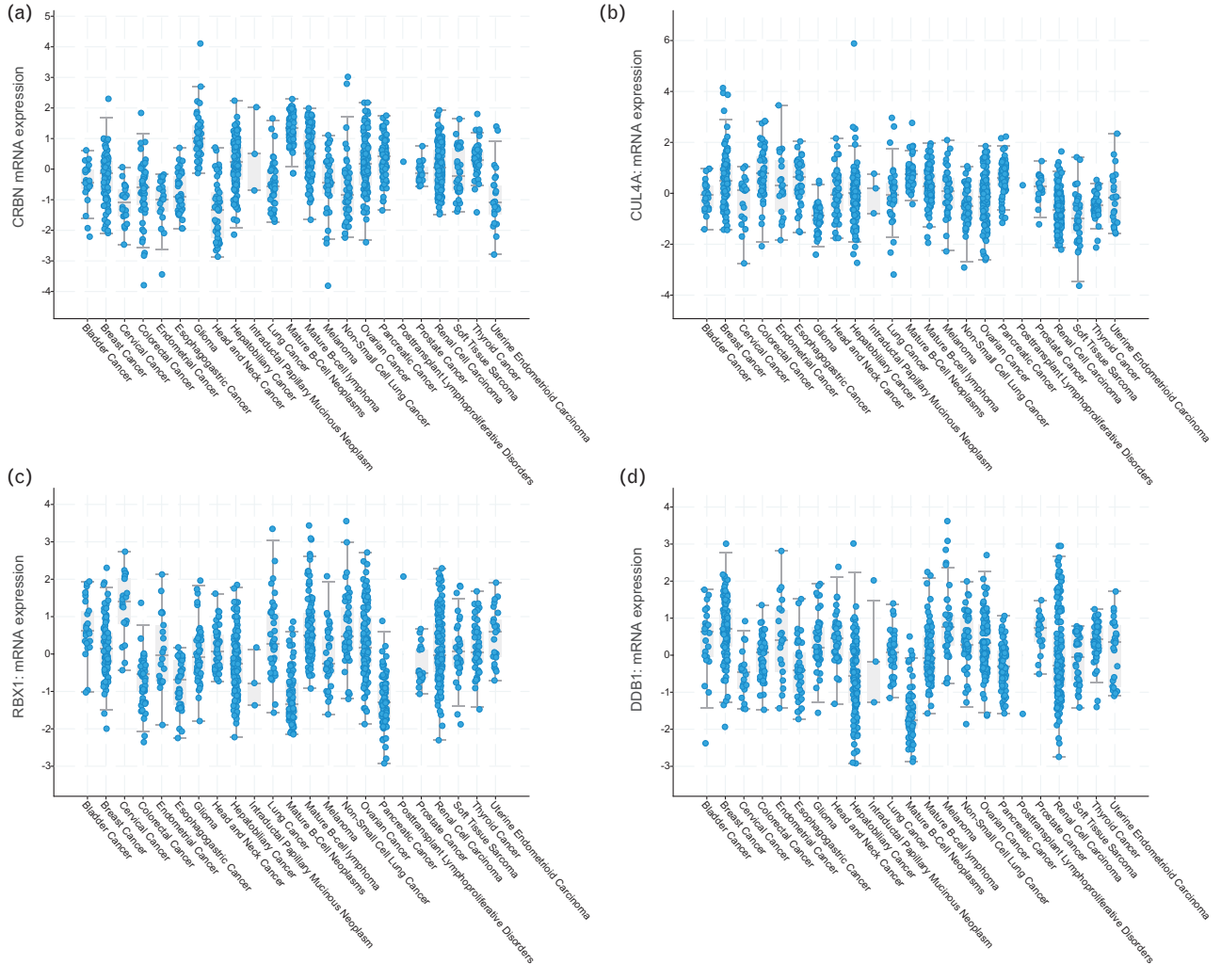

FIGURE 2. The highly heterogeneous gene expression landscape of CRBN (A) and associated proteins across multiple tumor types: CUL4A (B), RBX1 (C), and DDB1 (D). All these proteins are required for an efficient CRBN-based target degradation. The data represent $n = 1210$ cases. Each gene's expression is presented as z-scores relative to all samples (log FPKM). Data was extracted from cBioPortal.org. [21].

Table 1. Pivotal clinical trials evaluating ER degradation using PROTACs

Agent	Phase	Start date	Clinical trials. gov identifier
ARV-471 alone, ARV-471 plus palbociclib	I/II	2019	NCT04072952
ARV-471 plus everolimus	Ib	2022	NCT05501769
ARV-471	III	2023	NCT05654623
AC682	I	2021	NCT05080842
AC682	I	2022	NCT05489679
DT2216	I	2021	NCT04886622

ER, estrogen receptor; PROTACs, proteolysis-targeting chimeras.

challenges must be addressed to effectively translate this approach into the clinic including [1] identifying predictive biomarkers to improve patient selection [2], designing synergistic combinatorial therapies to target diverse pathways for improved efficacy to postpone the development of an acquired resistance, and [3] understanding the long-term safety and potential side effects of robust ER degradation therapy. Rigorous preclinical and clinical studies are necessary to comprehensively evaluate the potential toxicities (e.g., central nervous system ER degradation) and assess the overall risk-to-benefit ratio of this novel therapeutic approach. Additionally, a significant effort should be invested to appraise the extent of ER loss phenomenon in the context of ER-targeting PROTAC. The question whether highly-potent ER degradation might create new luminal tumors with minimal ER expression (perhaps driven by ER fusions lacking the ligand-binding domain [20▪]) or alternatively, aggressive basal-like cancers, remains to be answered. Both possibilities have major consequences on patient outcomes and therapeutic options (e.g., no current therapy for ER fusion), and should be evaluated in cohort studies.

Compared to SERDs, PROTACs offer several advantages in terms of ER degradation. PROTACs provide a more potent and sustained mechanism of ER degradation and offer potential versatility in targeting different regions of the ER protein. While SERDs primarily interact with the ER ligand-binding domain, PROTACs can be potentially designed to target different domains of the ER protein, providing opportunities to overcome resistance mutations or alterations in the receptor structure.

CONCLUSION

ER-targeting PROTACs are a highly promising therapeutic approach to tackle endocrine resistant in patients with HR+ MBC. In the coming months and years, we will gain further insights into the safety, efficacy and overall impact of PROTACs on the lives of patients living with MBC.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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