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Next-Generation Estrogen Receptor–Targeted Therapeutics

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Abstract

Estrogen receptor (ER) α is expressed in the vast majority of breast cancers and is one of the most successfully prosecuted drug targets in oncology, with multiple classes of endocrine therapies approved for the treatment of ER^+ breast cancer. These existing agents are highly active, both as single agents and as combination partners for other targeted therapies, and have significantly benefited patients. However, each of these standard-of-care (SOC) therapies has liabilities that allow for the reengagement of ER signaling as a mechanism of resistance. Data supporting the continued dependence of tumors on ER signaling following exposure to SOC agents have underpinned an extraordinary reenergizing of academic, biotechnology, and pharmaceutical groups pursuing next-generation ER-targeted therapies. The hypothesis that there remains an opportunity to bring further meaningful benefit to patients through fully optimized ER-targeted therapies is currently being investigated in the clinic.

1. INTRODUCTION: THE BIOLOGICAL BASIS FOR THERAPEUTIC TARGETING OF ER IN BREAST CANCER

The estrogen receptor (ER) is a ligand-inducible transcription factor expressed in a subset of luminal cells of the mammary gland ductal epithelium. Estrogen, upon engaging with ER, provides a key mitogenic cue driving the proliferation of ductal cells during normal mammary gland development, evidenced in part through elegant genetic studies conducted in model organisms (Feng et al. 2007). The majority of tumors that arise from the mammary gland, initiated through oncogenic mutations and copy number alterations, maintain expression of ER and, as in mammary development, depend on ER signaling for progression through the cell cycle. This dependency of many breast cancers on the estrogen-ER signaling axis was first probed and therapeutically exploited in the late 1890s, prior to the identification and isolation of estrogen itself, when Dr. George Beatson performed a bilateral oophorectomy to test the hypothesis that the ovaries were a key source of a tumor-stimulatory signal (Beatson 1896). Beaton's hypothesis was borne out by his own experiments, by additional studies of the impact of ovarian suppression through surgery and radiation, and eventually by the evaluation of synthetic analogs of luteinizing hormone releasing hormone (LHRH agonists) in premenopausal women with ER^+ breast cancer. The isolation of estrogen in the 1920s as the "primary ovarian hormone" (Allen & Doisy 1923) followed by the identification and subsequent cloning of ER (Walter et al. 1985), and an enormous amount of preclinical and clinical research contributed by numerous collaborative groups spanning the subsequent decades have together provided evidence, and molecular detail, for the estrogen-ER axis as a major contributor to the pathogenesis of ER^+ breast cancer. Here, we discuss the landscape of ER-targeted therapeutics for the treatment of ER^+ breast cancer, which is currently evolving as clinical investigation of multiple next-generation therapeutics is playing out.

2. FIRST OF THE SMALL-MOLECULE THERAPEUTICS TO BE APPROVED: THE ER MODULATOR TAMOXIFEN

Tamoxifen was the first ER-targeted small molecule to be approved for the treatment of women with ER^+ breast cancer. This molecule had initially been explored for use as an antiestrogenic contraceptive agent based on its activity in rodents but was found to stimulate rather than suppress ovulation in women, halting its progress within Imperial Chemical Industries' (ICI's) contraceptive program. Fortunately, a collaborative group of ICI and academic researchers recognized the potential of tamoxifen for the treatment of breast cancer, leading to a redirection of both preclinical and clinical research, and ultimately to tamoxifen's approval, first as a therapeutic for ER^+ metastatic breast cancer (MBC) and then as the first chemopreventive/adjuvant agent approved for any cancer. The remarkable story of tamoxifen's journey from a failed contraceptive to a highly successful oncology drug has been described in detail by one of the key participants in tamoxifen's story, V. Craig Jordan (Jordan 2021), and also by the respected medical science historian Viviane Quirke (Quirke 2017). At the heart of tamoxifen's unusual development journey is its complex pharmacology, which is also relevant for mechanisms of resistance. Tamoxifen is described as having both ER antagonist and ER agonist potential, leading to its designation as a modulator of ER signaling, i.e., a SERM (selective ER modulator), rather than as an obligate ER antagonist. This curious pharmacology is underpinned by the molecular composition of ER itself.

ER, like other nuclear hormone receptors, contains a central DNA-binding domain, as well as two terminal transactivation domains (Mangelsdorf et al. 1995). At the C terminus of ER is the ligand-binding domain (LBD), a well-structured region encompassing the ligand-binding pocket, to which the natural agonist 17β-estradiol (E2; estrogen), as well as therapeutic ER ligands, bind. Binding of E2 to the ER LBD triggers the release of ER from HSP90, which normally maintains

ER in its inactive state. Release from HSP90 allows for the engagement of ER with chromatin. As well as releasing ER from HSP90, E2 binding promotes a particular conformation of the LBD. Specifically, the key functional helix 12 (H12) is repositioned to create a binding surface for specific coactivator proteins, which in turn recruit additional chromatin modifiers and the general transcriptional machinery to promote expression of ER target genes. In contrast to the structurally defined C-terminal domain, the N terminus, containing the activation function 1 (AF1) domain, is intrinsically disordered. Such intrinsically disordered regions are key functional features of other transcription factors (including those that do not contain a ligand-binding pocket) and are capable of recruiting coactivators, but the detailed mechanisms through which these domains function have only recently begun to be elucidated. In particular, imaging studies, including in live cells, have implicated these domains in assembling highly dynamic signaling hubs defined by weak, transient, multivalent protein-protein interactions, within which proteins exchange at rapid timescales of the order of seconds to minutes (Chong et al. 2018).

Tamoxifen, and in particular the active, metabolized form of tamoxifen 4-hydroxytamoxifen (4-OHT), can outcompete E2 for binding to the LBD and alters the positioning of H12 such that coactivators are not recruited to this domain (Shiau et al. 1998). The suppression of LBD-coactivator interactions, relative to E2-engaged ER, means that tamoxifen reduces ER signaling, i.e., is antagonistic relative to the E2-induced state. However, tamoxifen-bound ER engages with chromatin, and the N-terminal AF1 domain can promote some degree of ER signaling (Liu et al. 2001, McInerney & Katzenellenbogen 1996). Critically, the degree to which the AF1 domain signals in the presence of tamoxifen is context dependent, likely related to the relative abundance of AF1-binding coactivators. Tamoxifen is also capable of binding ER in its HSP90-bound off state, promoting ER-chromatin engagement and the potential of signaling via the AF1 domain, and in this way, it can weakly agonize ER signaling relative to the off state. Thus, tamoxifen can fail to fully antagonize ER signaling relative to the E2-bound on state and can agonize ER signaling relative to the HSP90-bound off state. The partial agonist/modulator feature of tamoxifen is relevant for its safety profile, specifically related to ER agonism in the uterus, and is also relevant for its efficacy and resistance profile. The majority of ER^+ breast cancer cell lines allow for weak agonist activity of tamoxifen, which impacts its antiproliferative potential even in the context of relatively short-duration treatment (Guan et al. 2019). Perhaps even more compelling with respect to the impact of partial agonism on long-term efficacy, in vivo experiments conducted in the late 1980s and 1990s showed that continuous exposure to tamoxifen through multiple rounds of passaging supports the evolution of tumors that are growth-stimulated by tamoxifen, suggesting that its partial agonism can be elevated over time, leading to frank resistance (Gottardis et al. 1989, Osborne et al. 1991, Wolf & Jordan 1994).

3. OVERCOMING THE AGONIST CHALLENGE TO ENABLE FULL ER SUPPRESSION: AROMATASE INHIBITORS AND PURE ANTIESTROGENS AS SOLUTIONS

It has been recognized that while tamoxifen is unquestionably an active and important agent in the treatment of ER^+ breast cancer, further benefit might be brought to patients, from the perspectives of both safety and efficacy, through the avoidance of its partial agonist activity. Two potential solutions have been proposed: (*a*) to maintain ER in its natural off state by suppressing synthesis of peripheral E2, which is relevant for the postmenopausal/ovary-suppressed setting, and (*b*) to identify ER ligands that successfully compete with E2 for binding to ER, thereby antagonizing ER function without the ligands themselves showing any ER agonist potential.

The first potential solution was articulated and pursued initially by the team of Angela and Harry Brodie, who identified 4-hydroxy-androstenedione (4-OH-A) as an inhibitor of the

aromatase enzyme, encoded by the *CYP19A1* gene (Brodie et al. 1977). Aromatase converts androgens to E2 via aromatization and is responsible for the synthesis of peripheral (non-ovarian-derived) E2. The clinical evaluation of 4-OH-A (Coombes et al. 1992), later known as formestane, paved the way for the more potent and selective aromatase inhibitors (AIs) that are in clinical use today: exemestane, letrozole, and anastrozole. A series of clinical trials, including very large adjuvant studies, biomarker-focused neoadjuvant studies, and studies conducted in the metastatic setting, demonstrated superior efficacy of the AIs versus tamoxifen (Breast Int. Group 1-98 Collab. Group et al. 2005, Howell et al. 2005). These clinical studies additionally showed tamoxifen and the AIs to have distinct safety profiles, with the AIs being associated with fewer gynecological and vascular events, but with an increase in arthralgia, bone pain, and bone fractures, consistent with distinct effects on ER signaling in the relevant tissues (i.e., partial ER agonism versus ER suppression). These clinical studies thus provided evidence that overcoming the partial agonist effects of tamoxifen could improve upon the already meaningful benefit imparted by this agent. It should be noted that since the AIs inhibit the production of peripheral but not ovarian E2, their use requires that patients are either naturally ovarian suppressed (i.e., postmenopausal) or co-administered LHRH agonists to chemically induce ovarian suppression. We refer readers to an excellent review by Santen et al. (2009) for a full historical account of the development of the AIs.

The alternative potential solution to the partial agonism of tamoxifen, identifying a fully suppressive ER ligand, was pursued by the team at ICI. In their approach, the rodent uterus, which is highly sensitive to estrogenic action, was creatively and cleverly leveraged as an in vivo screening tool to seek ligands that could block the trophic actions of estrogen and that were devoid of any estrogenic action themselves, unlike tamoxifen and other ER modulators. Screening a collection of estrogen derivatives led to the identification of what was defined as the first pure antiestrogen, ICI 164384, which was further optimized for potency to generate ICI 182780, now known as fulvestrant, named to reflect full estrogen receptor antagonism (Wakeling 1990, Wakeling & Bowler 1992). After its identification as a pure antiestrogen/full ER antagonist, fulvestrant was observed to deplete ER protein, initially in the rodent uterus, subsequently in ER^+ breast cancer cell lines, and finally also in human tumor biopsies (Borras et al. 1996, Gibson et al. 1991, Nicholson et al. 1995). ER depletion mediated by fulvestrant was shown to be via proteasome-mediated degradation of ubiquitinated ER (Preisler-Mashek et al. 2002). Fulvestrant's ability to trigger such ER degradation was proposed as the mechanism by which it avoids ER agonism (further discussed below). As a reflection of this feature, fulvestrant was subsequently described as a selective ER downregulator (SERD), also referred to in the literature as a selective ER degrader. The SERD terminology, highlighting fulvestrant's degradation potential, has, over time, displaced the original language that was at the heart of fulvestrant's identification and development (i.e., its pure antiestrogen functional effect).

Although fulvestrant met ICI's goal in terms of its pure antiestrogenic mechanism of action, it was found to lack oral bioavailability, necessitating administration to patients by intramuscular injection. This route of administration created challenges for the clinical development of fulvestrant, in particular with respect to dose selection/regime. Multiple dosing strategies were evaluated over the course of many years before settling on its currently recommended regime of 500 mg once per month with a loading dose at day 14 (Robertson et al. 2014). Critically, an evaluation of this optimized dosing regime in the FALCON trial (for endocrine therapy–naïve locally advanced or MBC) showed fulvestrant to be superior to the AI anastrozole (Robertson et al. 2016). Altogether, these clinical trials evaluating tamoxifen, the AIs, and fulvestrant suggest not only that avoidance of partial ER agonism allows for improvements over tamoxifen but also that direct targeting of ER with a pure antiestrogen may drive improvements over targeting ER signaling at the level of its ligand, via the AIs, at least in some patient subsets.

4. KEY DRIVERS BEHIND THE DEVELOPMENT OF NEXT-GENERATION ER THERAPEUTICS

Although fulvestrant and the AIs each made gains over tamoxifen that brought further benefit to patients, additional studies revealed that these therapies have their own liabilities that could lead to incomplete or nonsustained ER suppression in patients. Most notably, genomic sequencing of tumors from patients who had been diagnosed with ER^+ breast cancer and exposed to endocrine therapies revealed the emergence of hotspot mutations in the LBD region of *ESR1*, the gene that encodes ERα (Li et al. 2013, Merenbakh-Lamin et al. 2013, Robinson et al. 2013, Toy et al. 2013). The high prevalence of these mutations, in up to 40% of patients in some metastatic cohorts, was particularly striking given the very low prevalence of such mutations in endocrine therapy–naïve patient cohorts. Thoughtful evaluation of these mutations leveraging structural, molecular, and cell biology studies, together with additional sequencing of tumor biopsies and circulating tumor DNA, has led researchers to converge on the conclusion that these mutations enable estrogen-independent activity of ER and arise primarily under the selective pressure created through administration of the AIs. In addition to estrogen independence, the *ESR1* mutations have also been shown to reduce the binding potency of fulvestrant and other antagonist ligands, and it has been hypothesized that this is due to the folding of the ER LBD into its active conformation, which increases the energy barrier for transition to the inactive conformation (Bihani et al. 2017, Joseph et al. 2016, Katzenellenbogen et al. 2018, Toy et al. 2017). Indeed, although clinical data suggest that the selective advantage of *ESR1* mutations appears much weaker for fulvestrant than for the AIs (Schiavon et al. 2015, Spoerke et al. 2016), the emergence of the ER.Y537S mutation in particular, which exhibits the strongest activating phenotype of the *ESR1* mutations, has been shown to be enriched following exposure to fulvestrant (O'Leary et al. 2018). These data suggest that tumor cells expressing ER.Y537S may have a selective advantage in the presence of this therapy. Perhaps less well understood, but also proposed by multiple groups, is that the *ESR1* mutations may impart gain-of-function properties to ER, potentially impacting metastatic phenotypes and functional interactions with other hormone receptors (Bahreini et al. 2017, Jeselsohn et al. 2018). Thus, the acquisition of *ESR1* mutations presents a hurdle to the long-term durability of responses to the AIs, most notably in the metastatic setting, and may also impact the efficacy of fulvestrant. The discovery of the *ESR1* mutations in late lines of MBC further provided key evidence that the ER signaling axis remains engaged even after the failure of standard-of-care (SOC) therapies, and this has been a major driving force behind the development of next-generation ER-targeted therapies over the past decade.

Fulvestrant has undergone considerable dose optimization over the course of its clinical development, but a remaining question concerns whether the currently recommended dose leads to exposures that saturate ER, leading to maximal ER inhibition and efficacy. To address this question, van Kruchten et al. (2015) deployed positron emission tomography/computed tomography with a labeled estradiol, [(18)F]fluoroestradiol (FES), in order to measure residual availability of ER in patients treated with fulvestrant. In a small cohort of 16 cases, it was observed that in 38% of patients, fulvestrant failed to achieve complete suppression of FES uptake in metastatic tumors, suggesting that fulvestrant does not fully saturate ER in every tumor. Importantly, residual ER availability in the presence of fulvestrant was associated with early progression of patients in this cohort. These data suggested that there may be room for improvement over fulvestrant, with drugs that have a similar mechanism of action, but that might be dosed to achieve higher exposures (i.e., through oral bioavailability), allowing for saturation of ER and leading to maximal inhibition of ER activity.

Independent of the hypothesis that there remains an opportunity to improve on the efficacy of fulvestrant, its route of administration has been seen as a barrier to its development in the early

breast cancer setting. Specifically, the community has long been interested in evaluating the SERD mechanism in the adjuvant setting, but the necessity of monthly injections—which are associated with injection site pain, hematomas, and ulcers—over a five-to-ten-year period following surgery has been deemed impractical and not aligned with patient quality of life. Thus, there is some incentive behind the identification of an oral equivalent to facilitate this development path.

The liabilities of fulvestrant, primarily related to its administration/exposure profile, and the discovery of the *ESR1* mutations as a major driver of resistance to SOC therapies have together underpinned considerable reinvestment in the development of ER therapeutics with the expectation that further benefit might be brought to patients through continued targeting of ER as the major mitogenic axis of ER^+ breast cancer.

5. REDEPLOYMENT OF EXISTING LIGANDS AND THE CREATION OF NEW MOLECULAR ENTITIES

With the considerable re-energizing of the community around ER as a therapeutic target, multiple strategies have been explored in an attempt to identify the most promising candidates for development, including major efforts to create new chemical matter with a variety of features and mechanisms, as well as efforts to revisit existing ER ligands that had been deployed earlier for other purposes or indications. Perhaps the most notable ER ligands exemplifying the latter approach are bazedoxifene, lasofoxifene, and elacestrant (RAD-1901; see also **Table 1**). Bazedoxifene and lasofoxifene are both approved for the treatment of postmenopausal osteoporosis, while elacestrant was originally developed for the treatment of vasomotor symptoms associated with menopause; that is, each of these ligands was originally pursued for their SERM features in order to counter the effects of reduced ER signaling that occurs in menopause. However, each of these ligands was additionally shown to promote ER degradation in at least some ER^+ breast cancer cell lines, leading to their designation as SERM/SERD hybrids, and exhibited robust antiproliferative potential in both ER wild-type and mutant settings, (Garner et al. 2015; Lainé et al. 2021; Wardell et al. 2013, 2015). Clinical evaluation of these molecules, now for ER^+ breast cancer, has been progressing and is further described below.

While bazedoxifene, lasofoxifene, and elacestrant had been initially recognized and developed for their SERM properties, with their SERD activity discovered later, the reverse discovery timeline from SERD to SERM has also occurred. In particular, GDC-0810 and AZD9496 were each prospectively optimized for their ER degradation potential in ER^+ breast cancer cells but were found to maintain partial ER agonist features despite that optimization; thus, they were also ultimately designated as SERM/SERD hybrids (Lai et al. 2015, Weir et al. 2016). A preclinical investigation comparing GDC-0810 and AZD9496 to fulvestrant showed that the SERD/SERM hybrids neither exhibited the same consistency of ER degradation across ER^+ breast cancer cell lines nor achieved the same antiproliferative potential as fulvestrant (Guan et al. 2019). Perhaps most importantly, while AZD9496 successfully achieved oral bioavailability, it failed to demonstrate superiority over fulvestrant in a presurgical study of early breast cancer assessing the impact on ER and progesterone receptor protein levels and Ki67 positivity (Robertson et al. 2020). The clinical development of both GDC-0810 and AZD9496 has been halted.

Next in the wave of ER ligands prospectively optimized for ER degradation was GDC-0927 (Kahraman et al. 2019). This molecule was shown to be mechanistically more similar to fulvestrant than GDC-0810 and AZD9496, lacking the partial agonism of the SERM/SERD hybrids, and was also significantly more potent as an ER antagonist than GDC-0810 (Guan et al. 2019). However, GDC-0927 suffered from a suboptimal drug metabolism and pharmacokinetics (DMPK) profile, leading to a high pill burden, and its clinical development was likewise halted. While the stalled clinical development of these molecules was unquestionably disappointing, their identification did

Table 1 ER-targeted therapies recently or currently explored in clinical trials

ET class/MOA	ER-targeted molecule	Molecule features	Approved indications and status of clinical development in ER$^+$ BC	Clinical studies in ER$^+$ BC with efficacy endpoints
SERM/SERD hybrid	Bazedoxifene	ER agonist in bone; retains efficacy for mutant ER	Approved for treatment of postmenopausal osteoporosis; active development in MBC	NCT02448771: ph1/2 palbociclib combination in 1L+ MBC
	Lasofoxifene	Retains efficacy for mutant ER	Approved for treatment of postmenopausal osteoporosis; active development in MBC	NCT04432454: single-arm ph2 abemaciclib combination in 2L+ MBC (ELAINEII) NCT03781063: randomized ph2 versus fulvestrant in m*ESR1* 2L+ MBC (ELAINEI)
	Elacestrant (RAD-1901)	Dose-dependent ER agonist/antagonist activity; high receptor occupancy; retains efficacy for mutant ER	Active development in MBC and EBC; anticipated approval in 2L+ MBC in 2023	NCT03778931: randomized ph3 versus physician's choice ET in 2L+ MBC (EMERALD) NCT05512364: randomized ph3 versus SOC in ctDNA-relapsed EBC (TREAT ctDNA) NCT04797728: early ph1 WoO in EBC (ELIPSE)
	GDC-0810	Weak ER agonist in uterus; inconsistent ER degradation across ER$^+$ breast models	Discontinued development	
	AZD9496	Weak ER agonist in uterus; inconsistent ER degradation across ER$^+$ breast models	Discontinued development	
SERD	GDC-0927	Retains efficacy for mutant ER; high receptor occupancy; low oral exposure	Discontinued development	
	Giredestrant (GDC-9545)	Retains efficacy for mutant ER; high receptor occupancy; more potent than fulvestrant and other SERDs	Active development in MBC and early BC	NCT04576455: randomized ph2 versus physician's choice ET in 2L+ MBC (acelERA) NCT05306340: randomized ph2 everolimus combination versus exemestane+everolimus in 2L+ MBC (evERA) NCT04546009: randomized ph3 palbociclib combination versus letrozole+palbociclib in 1L MBC (persevERA) NCT05296798: randomized ph3 Phesgo combination versus Phesgo in 1L ER$^+$ HER2$^+$ MBC, (heredERA) NCT04436744: randomized ph2 WoO in EBC (coopERA) NCT04961996: randomized ph3 versus physician's choice ET in high-risk EBC (lidERA)

(*Continued*)

Table 1 (*Continued*)

ET class/MOA	ER-targeted molecule	Molecule features	Approved indications and status of clinical development in ER+ BC	Clinical studies in ER+ BC with efficacy endpoints
	Amcenestrant (SAR439859)	Retains efficacy for mutant ER (but with lower potency); high receptor occupancy; inducer of CYP3A4 and drug-drug interactions observed with CDK4/6 inhibitors	Discontinued development; did not meet primary endpoint in AMEERA-3 trial and lack of signal in m*ESR1* subgroup; no evidence of greater Ki67 reduction versus letrozole in AMEERA-4; lack of efficacy in 1L MBC in AMEERA-5	NCT04059484: randomized ph2 versus physician's choice ET in 2L+ MBC (AMEERA-3) NCT04478266: randomized ph3 palbociclib combination versus letrozole+palbociclib in 1L MBC (AMEERA-5) NCT05128773: randomized ph3 in EBC after AI discontinuation (AMEERA-6) NCT04191382: randomized ph2 WoO in EBC (AMEERA-4)
	Camizestrant (AZD9833)	Retains efficacy for mutant ER; predicted bioavailability is 40%	Active development in MBC and early BC	NCT04214288: randomized ph2 versus fulvestrant in 2L+ MBC (SERENA-2) NCT04711252: randomized ph3 palbociclib combination versus anastrozole+palbociclib in 1L MBC (SERENA-4) NCT04964934: randomized ph3 CDK4/6i combination versus AI+palbociclib in 1L MBC with detectable m*ESR1* ctDNA (SERENA-6) NCT04588298: randomized ph2 WoO with different doses in EBC (SERENA-3)
	Imlunestrant (LY3484356)	Retains efficacy for mutant ER (but with lower potency)	Active development in MBC and early BC	NCT04975308: randomized ph3 single agent and abemaciclib combination versus physician's choice ET in 2L+ MBC (EMBER-3) NCT05514054: randomized ph3 versus physician's choice ET in high-risk EBC patients who have received prior adjuvant ET (EMBER-4)
	OP-1250	Retains efficacy for mutant ER	Active development in MBC	NCT05266105: ph1 dose escalation and expansion in combination with palbociclib in MBC NCT05508906: ph1b dose escalation and expansion in combination with ribiciclib and alpelisib in MBC NCT04505826: ph1/2 dose escalation and expansion in MBC
PROTAC	ARV-471	Heterobifunctional molecule that recruits the E3 ligase CRBN; retains efficacy for mutant ER	Active development in MBC	NCT04072952: ph1/2 dose escalation and expansion alone and in combination with palbociclib in 3L+ MBC NCT05501769: ph1b in combination with everolimus in 3L+ MBC

(*Continued*)

Table 1 (*Continued*)

ET class/MOA	ER-targeted molecule	Molecule features	Approved indications and status of clinical development in ER$^+$ BC	Clinical studies in ER$^+$ BC with efficacy endpoints
SERCA	H3B-6545	Covalent ER antagonist; inhibits E2 binding to both WT and mutant ER; partial agonism in bone and uterus	Active development in MBC	NCT03250676: ph1/2 dose escalation and expansion in 2L+ MBC

This table includes clinical trials that have been publicly disclosed on ClinicalTrials.gov (**https://clinicaltrials.gov/ct2/home**), the database of privately and publicly funded clinical studies conducted around the world. Phase I studies are not included for investigational agents that have progressed to phase II and III studies. We have aimed to capture all relevant new molecular entities targeting ER that are being clinically evaluated for the treatment of ER$^+$ breast cancer, and have also included ER ligands previously investigated for other indications but currently being reevaluated for the treatment of ER$^+$ breast cancer.

Abbreviations: 1/2/3L(+), first-/second-/third-line (or later); AI, aromatase inhibitor; BC, breast cancer; ctDNA, circulating tumor DNA; EBC, early breast cancer; ER, estrogen receptor; ET, endocrine therapy; -i, inhibitor; MOA, method of action; MBC; metastatic breast cancer; m*ESR1*, mutant *ESR1*; NCT, National Clinical Trials (clinicaltrials.gov) identifier; ph, phase; PROTAC, proteolysis-targeting chimera; SERCA, selective ER covalent antagonist; SERD, selected ER degrader; SERM, selective ER modulator; SOC, standard of care; WoO, window of opportunity; WT, wild-type.

offer a valuable opportunity to bring additional insights to the research community. The discovery of GDC-0927, in particular, as a true pure antiestrogen in the same mechanistic class as fulvestrant presented an opportunity to further explore the mechanism by which these molecules achieve full ER antagonism. In characterizing the molecular and cellular impact of fulvestrant and GDC-0927 relative to SERMs and SERM/SERD hybrids, we made the discovery that the impacts of these different classes of agents (in particular on chromatin accessibility) diverge prior to ER degradation; i.e., there are elements of mechanistic differentiation of the pure antiestrogen/SERDs that are not obviously explained by loss of ER protein (Guan et al. 2019). This observation was in line with the earlier observation from Donald McDonnell and colleagues that ER degradation by fulvestrant is a saturable process not required for its antagonistic properties; i.e., ER antagonism might be uncoupled from ER degradation (Wardell et al. 2011). These observations, together with highly intriguing data from the Mancini group in which they had leveraged live cell imaging and observed fulvestrant to acutely impact the intranuclear mobility of ER (Stenoien et al. 2000), prompted us to further investigate the molecular underpinnings of ER antagonism by the SERD class. Based on the totality of data, we propose that immobilization of ER is an early event triggered by the pure antiestrogens that disables ER function and precedes ER degradation. Pure antiestrogens that were identified after GDC-0927, including giredestrant, camizenstrant, and amcenestrant, all display the ER immobilization phenotype (C. Metcalfe et al., manuscript in preparation).

The hurdles encountered in the development of the early wave of novel ligands (encompassing GDC-0810, GDC-0927, and AZD9496) highlighted the challenges in creating new molecular entities that encompass the full set of desirable features spanning mechanism, potency, DMPK, and safety features. Importantly, considerable progress has been made with several high-potential orally bioavailable ER ligands emerging from impressive medicinal chemistry campaigns, which are or were under active clinical investigation, including giredestrant [GDC-9545 (Liang et al. 2021)], amcenestrant [SAR439859 (Shomali et al. 2021)], camizestrant [AZD9833 (Scott et al. 2020)], imlunestrant [LY3484356 (Jhaveri et al. 2021)] and OP-1250 (Hodges-Gallagher et al. 2020) [molecular features reviewed by Chen et al. (2022)].

In addition to pursuing monovalent molecules that function in a manner similar to fulvestrant, researchers have also evaluated ARV-471, a heterobifunctional molecule designed to recruit the E3 ligase cereblon directly to ER to induce its degradation, for its utility in ER$^+$ breast cancer (Snyder

et al. 2021). It has been proposed that the distinct pharmacology of this molecule, and in particular the iterative degradation activity imparted by the PROTAC (proteolysis-targeting chimera) mechanism, might allow for differentiation from other ER antagonists. One might imagine that distinct mechanisms of resistance to the -esterant class and ARV-471 might allow for ARV-471 to maintain activity in cases of resistance to those agents, but until such mechanisms of resistance are identified, this remains speculative. Beyond the strategies focusing on ER degradation, H3B-6545, a selective ER covalent antagonist (SERCA) that leverages a reactive cysteine in the ER LBD, is being explored as an alternative mechanism to achieve potent ER inhibition (Furman et al. 2022). Notably, H3B-6545 was designed to maintain potency without relying on covalency, such that mutations in the reactive cysteine are not anticipated to present a major mechanism of resistance to this molecule. While H3B-6545 can potently inhibit binding of E2 to both wild-type and mutant ER, it displays tamoxifen-like partial agonism in bone and the uterus, as might be expected from an LBD antagonist that does not disable the AF1 domain. The balance of efficacy features and potential benefits of bone agonism, on the one hand, against the potential risks associated with ER agonism in the uterus, on the other, are presently being evaluated in the clinic.

6. EMERGING CLINICAL DATA: LATEST DEVELOPMENTS

The hypothesis that there is an opportunity to bring further, meaningful benefit to patients through optimized ER targeting has been a major catalyst driving the rapid progression of multiple candidate ER therapeutics into the clinic (**Figure 1**). Data are now emerging that are

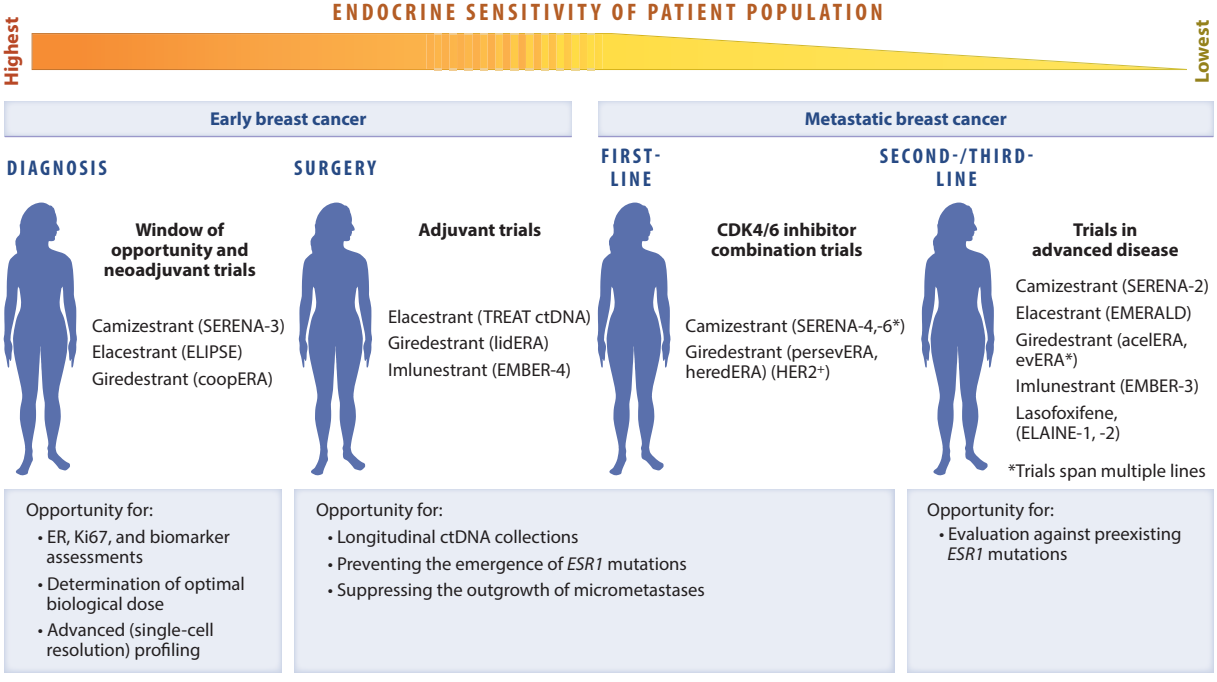

Figure 1

The clinical development of next-generation ER-targeted therapeutics spans the entire patient journey. Multiple agents are being evaluated in many trials across different clinical settings, from highly endocrine-sensitive early breast cancer to second- and third-line metastatic breast cancer, which is enriched for ER-independent disease. Each of these clinical settings provides an opportunity to address a particular patient need and scientific question, in addition to generating key efficacy and safety data. Abbreviation: ctDNA, circulating tumor DNA.

beginning to address some key questions, and perhaps also challenge the community to consider more precisely the contexts in which patients stand to benefit the most from these next-generation therapies. In this section, we describe some of the most thought-provoking recent data from phase II and III studies.

As described above, in addition to new molecular entities, ER ligands that have been previously explored or approved in other settings are being revisited for their potential in ER^+ breast cancer, with lasofoxifene and elacestrant generating particularly notable data. Lasofoxifene has shown promising clinical benefit in a single-arm phase II study (ELAINE-2) that evaluated it in combination with the CDK4/6 inhibitor abemaciclib, specifically in *ESR1*-mutant ER^+ MBC patients who progressed on at least one line of endocrine therapy and received prior treatment with a CDK4/6 inhibitor (Damodaran et al. 2022). Although it was a small study with a limited sample size, ELAINE-2 demonstrated a median progression-free survival (PFS) of 13.9 months with an overall response rate (ORR) of 33% and clinical benefit rate (CBR) of 62% in 29 ER^+ MBC patients with *ESR1* mutations. The reported median PFS of 13.9 months is substantially longer than what has been previously described for ER^+ MBC patients treated with abemaciclib after a prior course of CDK4/6 inhibitor therapy (albeit primarily nonsequential), at 5.3 months (Wander et al. 2021). Lasofoxifene versus fulvestrant has also been investigated in ELAINE-1, a phase II study in patients with ER^+ MBC with *ESR1* mutations that had disease progression after treatment with an AI in combination with a CDK4/6 inhibitor. Although lasofoxifene did not statistically improve PFS compared with fulvestrant [hazard ratio (HR) = 0.699; $p = 0.138$)], lasofoxifene was numerically superior for all primary and secondary clinical outcomes and decreased the *ESR1*-mutant allele fraction, including Y537S (median relative change for all variants: 87.1% for lasofoxifene versus 14.7% for fulvestrant) (Goetz et al. 2022). Lasofoxifene has recently received fast-track designation from the FDA (US Food and Drug Administration), and a phase III combination study with abemaciclib is currently planned in patients with *ESR1*-mutant tumors based on encouraging efficacy and safety from ELAINE-1 and -2.

Elacestrant was evaluated in the phase III EMERALD trial and demonstrated a modest but statistically significant benefit in PFS versus SOC endocrine therapy (including fulvestrant) in ER^+ MBC patients following progression on prior endocrine and CDK4/6 inhibitor therapy (Bidard et al. 2022). The coprimary endpoints were PFS benefit in both the intent-to-treat (ITT) population and the *ESR1*-mutant population. Elacestrant treatment was well tolerated and resulted in a PFS benefit in the ITT population (2.8 versus 1.9 months in SOC; HR 0.70). However, the strongest PFS benefit was observed in patients with *ESR1*-mutant tumors (3.8 versus 1.9 months in SOC; HR 0.55), suggesting that the presence of *ESR1* mutations was driving much of the benefit observed in the ITT. Kaplan-Meier curves show that 40–60% of patients progressed at the 2-month time point (likely when the patients received their first tumor assessment scan), suggesting a high frequency of endocrine resistance in this patient population [also observed in other studies such as AMEERA-3 and acelERA, discussed below; also see Lindeman et al. (2022)]. However, in landmark analyses of PFS rates at 6 and 12 months, elacestrant treatment resulted in higher PFS rates compared to the SOC arm at both time points (34.3% elacestrant versus 20.4% SOC at 6 months; 22.3% versus 9.4% at 12 months), reflecting a differentiation of elacestrant versus SOC in the putative endocrine-sensitive (i.e., ER-relevant) subpopulation. Additional landmark analyses have demonstrated elacestrant benefit in the ITT and *ESR1*-mutant populations irrespective of endocrine comparator (fulvestrant or AI) (Aftimos et al. 2022). In June 2022, Radius Health and the Menarini Group announced that they have submitted an NDA (New Drug Application) to the FDA seeking approval for elacestrant based on EMERALD results, and if accepted, this would make elacestrant the first of the oral SERD/SERM hybrids to make it to market (anticipated in 2023).

Two subsequent studies, also in the context of ER^+ MBC following progression on prior endocrine or CDK4/6 inhibitor therapy, that did not meet their primary endpoints in the ITT population were AMEERA-3 [amcenestrant (Tolaney et al. 2022)] and acelERA [giredestrant (Martin Jimenez et al. 2022)]. In the phase II AMEERA-3 study, amcenestrant demonstrated no statistically significant benefit over physician's choice endocrine therapy (PCET) in second-/third-line (2L/3L) $ER^+/HER2^-$ MBC patients in the ITT population (3.6 versus 3.7 months; HR 1.051) and demonstrated only a marginal improvement in patients with *ESR1*-mutant tumors (3.7 versus 2.0 months; HR 0.9). In the phase II acelERA trial, even though the study did not reach statistical significance, giredestrant showed a numerical improvement over PCET (5.6 versus 5.4 months; HR 0.81) with a consistent treatment effect across most key subgroups and a more pronounced effect in patients with *ESR1*-mutated tumors (5.3 versus 3.5 months; HR 0.60). Secondary efficacy endpoints numerically favored giredestrant in terms of CBR (31.8%, versus 21.1% with PCET) and ORR (12.6%, versus 7.2% with PCET). The encouraging efficacy and safety data support the continued investigation of giredestant in other lines of therapy, such as the phase III persevERA trial in 1L MBC and the phase III lidERA trial in early breast cancer. Results from the phase II SERENA-2 study were presented at the San Antonio Breast Cancer Symposium in December 2022, and they showed camizestrant monotherapy to improve PFS versus fulvestrant in patients previously treated with an endocrine therapy [median PFS was 7.2 months for 75 mg camizestrant (HR 0.58), 7.7 months for 150 mg camizestrant (HR 0.67), and 3.7 months for fulvestrant]. Results from an evaluation of imlunestrant from EMBER-3 are expected in 2024.

In addition to trials evaluating the collection of ER-targeted candidate therapeutics in heavily pretreated populations, there are several studies evaluating these molecules in earlier lines of therapy, as 1L treatment for metastatic disease and in the adjuvant setting—settings where *ESR1* mutations are rarer. Given the tremendous time and resource investments required to execute these very large clinical trials that aim to demonstrate meaningful benefit over already highly active treatment regimes, window-of-opportunity (WoO) and neoadjuvant studies are increasingly being utilized to assess the biological activity of new agents through pharmacodynamic changes and predictive biomarkers in untreated patients. Such biomarker-focused studies have previously demonstrated that changes in the proliferation biomarker Ki67 after short-term treatment with perioperative endocrine therapy can predict long-term outcomes of disease-free survival (Guerrero-Zotano & Arteaga 2017). For example, the POETIC study demonstrated that patients with lower Ki67 scores in their tumors after two weeks of endocrine therapy had lower rates of disease recurrence compared to those patients with tumors that still had high Ki67 levels after the treatment (Smith et al. 2020). To this end, many of these novel ER-targeted therapeutics are being evaluated in WoO and neoadjuvant studies in order for researchers to evaluate their activity and predict their possible success in the adjuvant setting (**Figure 1**).

A WoO study evaluating three dose levels of giredestrant demonstrated a robust and dose-independent suppression of Ki67, a strong decrease in ER and progesterone protein levels, and a strong decrease in ER pathway activity transcriptional scores; this study also supported the conclusion that the 30-mg dose of giredestrant being investigated in other studies is likely saturating for ER functional suppression (Moore et al. 2021). A subsequent study of giredestrant in a randomized phase II study (coopERA) showed that two weeks of single-agent giredestrant treatment resulted in superior Ki67 reduction (a decrease in the geometric mean of 75%) compared to what was achieved with the AI anastrozole [−67%, $p = 0.04$ (Bardia et al. 2022, Fasching et al. 2022)]. These data are conceptually important in supporting the potential of the new agents to outperform SOC in patient populations that are largely *ESR1* wild type, and the data have provided additional supporting rationales for evaluating girdestrant in high-risk early breast cancer in the lidERA adjuvant study. In contrast to the coopERA study evaluating giredestrant, in the AMEERA-4 study,

amcenestrant, evaluated at two dose levels, did not demonstrate greater reduction in Ki67 compared to letrozole (Campone et al. 2022). Results from this early breast cancer trial and the AMEERA-3 trial (described above), together with the lack of improved efficacy in the prespecified interim analysis of the phase III AMEERA-5 trial in 1L $ER^+/HER2^-$ MBC (evaluating amcenestrant plus palbociclib compared with letrozole plus palbociclib), have led to the discontinuation of amcenestrant development. Given the distinct preclinical profiles of the -esterants class (e.g., potency), their unique clinical profiles (e.g., pharmacokinetics and drug-drug interactions), the different outcomes of biomarker-focused trials (e.g., coopERA versus AMEERA-4), and the different clinical trial designs and patient populations, the discontinuation of amcenestrant development does not necessarily affect the likelihood of the other agents' potential success. WoO studies evaluating elacestrant [ELIPSE (Vidal et al. 2022)] and camizestrant [SERENA-3 (Im et al. 2021)] are ongoing.

7. COMBINATION CONSIDERATIONS

While many of the SERM/SERD hybrids and SERDs are initially being assessed as single agents, the investigation of combinations with other targeted agents is an area of high priority. CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) are now considered SOC in ER^+ MBC and as such are being evaluated as combination partners for the next generation of SERDs and related molecules. Aberrant activation of growth factor signaling pathways, including at the level of PI3K, AKT, and mTOR, as well as upstream RTKs (receptor tyrosine kinases) and the CDK7 axis, have been implicated in endocrine therapy resistance. Several SERD combinations with alpha-specific PI3K inhibitors (alpelisib and inavolisib), AKT inhibitors (capivasertib and ipatasertib), and mTOR (everolimus) are thus also being clinically evaluated, in addition to the CDK7 inhibitor samuraciclib. In patients with ER^+ $HER2^+$ breast cancer, oral SERDs are also being paired with HER2-directed therapies.

8. OUTLOOK

The encouraging data available thus far, from patients with *ESR1*-mutant-expressing tumors in the metastatic setting, and from biomarker-focused (Ki67/proliferation) studies in early breast cancer, support the conclusion that the ER-targeted therapeutics currently progressing in the clinic are active, well tolerated, and may bring advances relative to SOC agents. However, the data also emphasize the importance of carefully considering the patient populations in which the candidate therapeutics are being investigated. Data from the EMERALD and acelERA trials converge on the notion that a large proportion of patients following exposure to CDK4/6 inhibitors, and many of those that have not acquired *ESR1* mutations, have likely transitioned to bona fide ER independence, meaning that this is not the most informative population in which to explore the potential benefits of optimized ER-targeted therapies. A hugely impactful advance would be made by strategies that allow for the identification of patients whose tumors retain ER signaling in the metastatic setting, in a more highly resolved fashion that is reflected by the *ESR1* mutations alone, enabling the identification of individuals who might derive the most benefit from the new agents.

At the other extreme end of the patient journey, two weeks of therapy exposure in the highly endocrine-sensitive, treatment-naïve early breast cancer setting may likewise underrepresent the full potential of optimized endocrine therapy, which is designed to overcome liabilities that manifest over longer treatment duration. The data already available in early breast cancer trials also emphasize that the next-generation molecules that have been progressed through clinical trials are not equivalent. A key challenge that needs to be addressed is to appropriately match the next generation of ER-targeted therapies, each of which exhibits a unique combination of features, with particular treatment paradigms and patient populations in order to maximize therapeutic benefit.

DISCLOSURE STATEMENT

All authors are employees of Roche/Genentech and hold shares in Roche/Genentech.

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