The Oncologist, 2023, **28**, 172–179
https://doi.org/10.1093/oncolo/oyac248
Advance access publication 9 December 2022
Precision Medicine Clinic: Molecular Tumor Board

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Clinical Implications and Treatment Strategies for *ESR1* Fusions in Hormone Receptor-Positive Metastatic Breast Cancer: A Case Series

Jamie O. Brett[1], Lauren L. Ritterhouse[2,3], Erik T. Newman[3,4], Kelly E. Irwin[3,5], Megan Dawson[5,6], Lianne Y. Ryan[3], Laura M. Spring[1,3], Miguel N. Rivera[2,3], Jochen K. Lennerz[2,3], Dora Dias-Santagata[2], Leif W. Ellisen[1,3], Aditya Bardia[1,3], Seth A. Wander[*,1,3]

[1]Massachusetts General Hospital Department of Medicine, Harvard Medical School, Boston, MA, USA
[2]Massachusetts General Hospital Department of Pathology, Center for Integrated Diagnostics, Harvard Medical School, Boston, MA, USA
[3]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA
[4]Massachusetts General Hospital Department of Orthopedic Surgery, Harvard Medical School, Boston, MA, USA
[5]Massachusetts General Hospital Department of Psychiatry, Harvard Medical School, Boston, MA, USA
[6]University of Michigan Department of Psychiatry, University of Michigan Medical School, Ann Arbor, MI, USA

*Corresponding author: Seth A. Wander, MD, PhD, Massachusetts General Hospital Cancer Center, Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA. Tel: +1 617 726 6500; E-mail: swander@partners.org

Abstract

In hormone receptor-positive metastatic breast cancer (HR+ MBC), endocrine resistance is commonly due to genetic alterations of *ESR1*, the gene encoding estrogen receptor alpha (ERα). While *ESR1* point mutations (ESR1-MUT) cause acquired resistance to aromatase inhibition (AI) through constitutive activation, far less is known about the molecular functions and clinical consequences of *ESR1* fusions (ESR1-FUS). This case series discusses 4 patients with HR+ MBC with ESR1-FUS in the context of the existing ESR1-FUS literature. We consider therapeutic strategies and raise the hypothesis that CDK4/6 inhibition (CDK4/6i) may be effective against ESR1-FUS with functional ligand-binding domain swaps. These cases highlight the importance of screening for ESR1-FUS in patients with HR+ MBC while continuing investigation of precision treatments for these genomic rearrangements.

Key words: breast cancer; estrogen receptor alpha; gene fusion; cyclin-dependent kinase 4; cyclin-dependent kinase 6.

Key Points

- *ESR1* fusions occur in 1%-10% of HR+ breast cancers, including untreated, early-stage breast cancers.
- These fusions can swap the ERα ligand-binding domain for a different protein fragment to cause constitutive and neomorphic activity, truncate a fusion partner (CCDC170) to activate growth signaling pathways, or be non-functional.
- While resistant to AI and selective estrogen receptor modulators and degraders, functional swaps may be sensitive to CDK4/6i.
- Much remains unknown about the molecular consequences and clinical susceptibilities of *ESR1* fusions.

Patient Stories

Case 1

A 58-year-old postmenopausal woman was diagnosed with ER+ (100%), PR+ (5%-30%), HER2-, grade 2 multifocal invasive carcinoma of the left breast (pT2N1aM0) (Fig. 1A). There were 3 tumor foci: 3.5-cm invasive ductal carcinoma (IDC), 2-cm invasive lobular carcinoma (ILC), and 2.5-cm ILC. Initial imaging for distant metastasis was negative. Additional medical history was significant for bipolar I disorder.

The patient underwent a modified radical mastectomy. One of 8 axillary lymph nodes was involved with 2 cm of IDC with extranodal extension. Margins were negative, and there was no lymphovascular invasion. The patient received adjuvant docetaxel and cyclophosphamide for 4 cycles followed by radiation of the chest wall and regional lymph nodes. Adjuvant endocrine treatment was with anastrozole and later letrozole for 15 months, although with limited adherence due to psychiatric side effects.

Twenty-six months after mastectomy, the patient developed left hip pain. Imaging demonstrated widespread osseous recurrence. This included an 8.8-cm lytic lesion in the left ilium causing a pathologic fracture. Biopsy of the iliac

Received: 11 June 2022; Accepted: 25 October 2022.

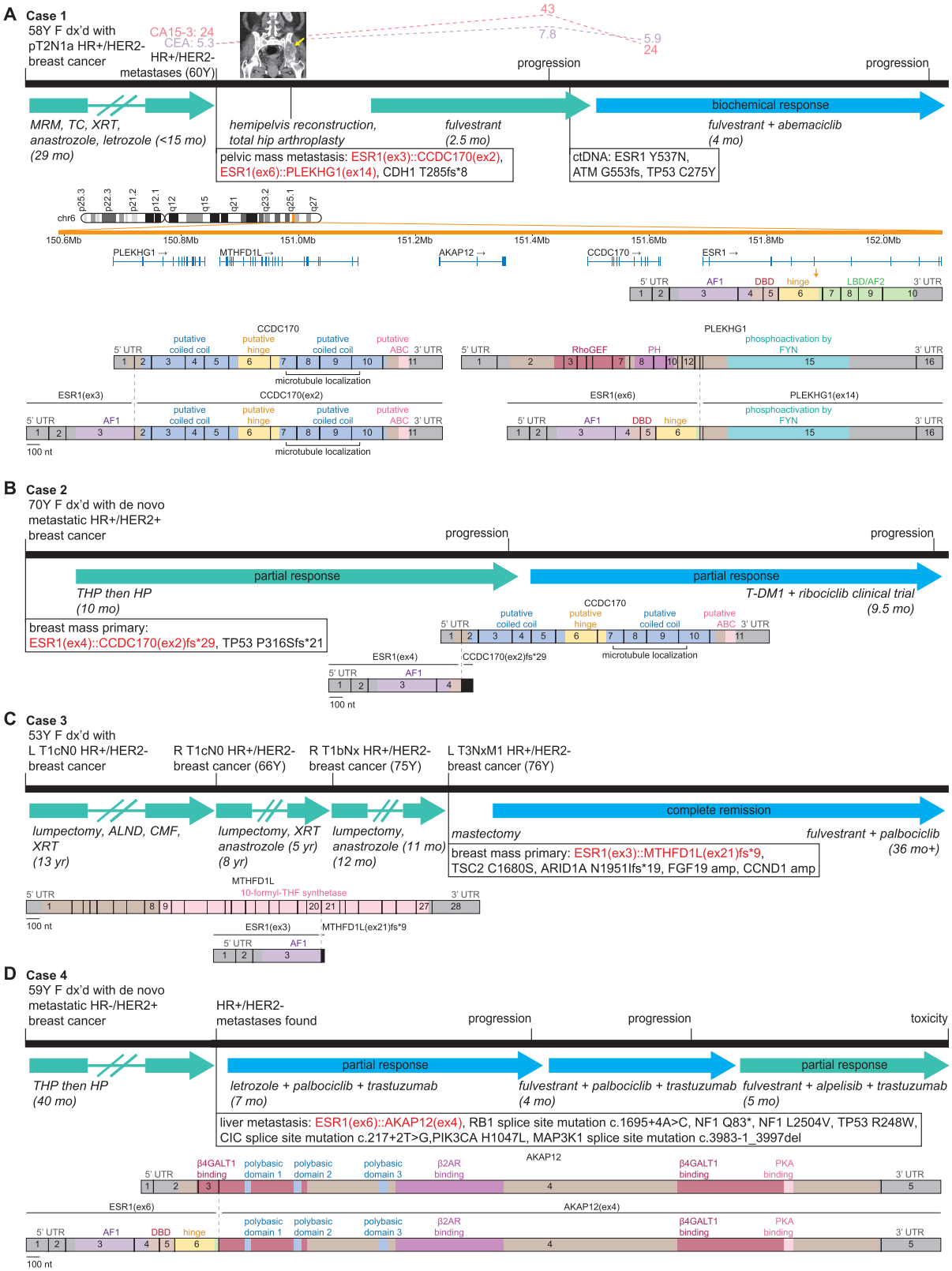

Figure 1. ESR1-FUS MBC clinical histories and fusion products. (**A**) Case 1 had *ESR1*(ex6)::*PLEKHG1*(ex14) and *ESR1*(ex3)::*CCDC170*(ex2) detected at metastatic diagnosis after adjuvant treatment with AI. Coronal CT shows the 8.8-cm left iliac mass (arrow) causing pathologic fracture. The disease initially progressed on fulvestrant. After adding abemaciclib, there was biochemical response with 4 months of stability. Tumor markers are CA15-3 (cancer antigen 15-3, U/mL) and CEA (carcinoembryonic antigen, ng/mL), with line graph height linearly proportional to marker level. Below the vignette is chr6q25.1 showing the genes involved in fusions in the Cases, and the ESR1-WT exon structure. The ESR1::CCDC170 and ESR1::PLEKHG1 fusions for Case 1 are shown. Case 2 (**B**), Case 3 (**C**), and Case 4 (**D**) vignettes and fusions. Abbreviations: ABC, ATP-binding cassette; AF1, Activation Function 1 domain; ALND, axillary lymph node dissection; β2AR, beta-2 adrenergic receptor; β4GALT1, beta-1,4-galactosyltransferase 1; CMF, cyclophosphamide, methotrexate, and fluorouracil; DBD, DNA-binding domain; HP, trastuzumab and pertuzumab; LBD/AF2, ligand-binding/Activation Function 2 domain; MRM, modified radical mastectomy; PH, plekstrin homology domain; PKA, protein kinase A; RhoGEF, Rho guanine nucleotide exchange factor domain; T-DM1, ado-trastuzumab emtansine; TC, docetaxel and cyclophosphamide; THF, tetrahydrofolate; THP, docetaxel, trastuzumab, and pertuzumab; XRT, radiation.

mass showed ER+ (95%), PR-, HER2- lobular breast carcinoma. The patient underwent curettage of the mass, hemipelvis reconstruction, and complex total hip arthroplasty, which was complicated by periprosthetic infection, hypomania, and traumatic prosthesis dislocation.

Molecular profiling of the recurrent tumor revealed an *ESR1*(ex3)::*CCDC170*(ex2) fusion and an *ESR1*(ex6)::*PLEKHG1*(ex14) fusion. Fulvestrant was started 4 weeks after hip reconstruction; integration of CDK4/6i was planned pending clinical course and after radiation of lumbar spine metastases. Two months later, however, restaging scans and tumor markers showed progression of osseous metastases. Circulating tumor DNA profiling by a 74-gene assay (Guardant) revealed additional mutations in *ESR1* (Y537N, 0.09%), *ATM* (G553fs, 1.1%), and *TP53* (C275Y, 0.2%). Abemaciclib was added to fulvestrant, and 2 weeks later tumor markers were decreased.

On fulvestrant and abemaciclib, the patient had clinical stability for 4 months—after the preceding 4 months of orthopedic, infectious, and psychiatric complications and progression on fulvestrant monotherapy. However, the patient then presented with extensive bilateral lower extremity deep venous thromboses, and imaging revealed progressive osseous disease and liver metastases. In the setting of progressive disease and functional decline, the patient chose to focus on comfort and transitioned to hospice care. The patient passed away 3 years after initial diagnosis.

Cases 2-4

After reviewing Case 1, we searched for additional cases of MBC with ESR1-FUS and identified 3 in our clinicopathologic database of tumors with molecular profiling performed at our institution, which included over 800 breast carcinoma specimens. We were interested in the efficacy of various treatment strategies.

Case 2 was a 70-year-old woman diagnosed with de novo metastatic HR+/HER2+ breast IDC, with bone, liver, and lung metastases (Fig. 1B). Molecular profiling of the treatment-naïve tumor showed an *ESR1*(ex4)::*CCDC170*(ex2) frameshift fusion. After 10 months of combination HER2-targeted and taxane therapy with partial response then progression, the patient received ado-trastuzumab emtansine (T-DM1) and ribociclib on a Phase Ib clinical trial[1] with 9.5 months of partial response prior to progression.

Case 3 was a woman with a history of bilateral breast cancer (T1cN0 HR+/HER2- left IDC and T1cN0 HR+/HER2- right ILC) who was diagnosed with T1bNx HR+/HER2- right breast IDC at age 75 years (Fig. 1C). One year later, while on adjuvant anastrozole, the patient developed HR+/HER2- left breast IDC with small metastases to liver and bone. The breast primary was removed with simple mastectomy and found to have an *ESR1*(ex3)::*MTHFD1L*(ex21) frameshift fusion. The patient received treatment with fulvestrant plus palbociclib with complete remission and has remained on treatment for at least 36 months.

Case 4 was a 59-year-old woman diagnosed with de novo metastatic breast cancer involving both breasts, bones, liver, pleura, lungs, and brain (Fig. 1D). As pleural fluid cytology revealed HR-/HER2+ carcinoma, treatment initially was combination HER2-targeted and taxane therapy, with partial response. Forty months later, there was isolated progression of liver lesions, whose biopsy revealed HR+/HER2- disease with an *ESR1*(ex6)::*AKAP12*(ex4) fusion. Therapy was changed to combination letrozole, palbociclib, and trastuzumab with partial response lasting 7 months prior to progression, after which replacing letrozole with fulvestrant did not produce further response. Due to a *PIK3CA* mutation, treatment was changed to combination fulvestrant, alpelisib, and trastuzumab for 5 months, with partial response until hospitalization for colitis, after which the patient passed away.

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Epidemiology of ESR1-FUS in HR+ Breast Cancer

Endocrine resistance in HR+ breast cancer occurs through diverse mechanisms. These include genetic alterations in *ESR1* itself and changes in pathways that bypass the need for ERα.[2] ESR1-MUT occurs in 20-40% of AI-treated HR+ MBC. These mutations restrict therapeutic options, as ESR1-MUT MBC is resistant to AI but likely remains susceptible to selective estrogen receptor modulators (SERMs), selective estrogen receptor degraders (SERDs), and CDK4/6i.[3] On the other hand, how ESR1-FUS impacts molecular signaling and therapy resistance is less clear.[4,5]

The prevalence of ESR1-FUS in different populations with breast cancer is not established. In The Cancer Genome Atlas (TCGA), ESR1-FUS is present in 1%-3% of primary HR+ breast cancers.[6-10] In other early-stage HR+ breast cancer cohorts, ESR1-FUS prevalence is 2%-10%.[8,11] Most fusions occur in luminal B and HR+/HER2+ tumors.[6,7] The most prevalent fusions are between *ESR1* and the adjacent gene *CCDC170*, comprising over half of ESR1-FUS cases; the remaining fusions involve assorted gene partners.[6,8,10,12,13] Analysis of HR+ MBC samples has identified ESR1-FUS at 3-5% prevalence.[14,15] These sample sizes are small, and it is unknown how ESR1-FUS prevalence differs in treated versus treatment-naïve conditions. Other studies suggest higher prevalence limits (up to 22-28%), although likely with higher false-positive rates.[12,13] Of note, some immortalized breast cancer lines also carry *ESR1*::*CCDC170* fusions, including MCF7, HCC1428, and ZR75-1.[6,8,12] In summary, ESR1-FUS may be present in at least 1-10% of primary HR+ breast cancer, typically in luminal B tumors, and the most common are *ESR1*::*CCDC170* fusions.

Little is known about how ESR1-FUS affects clinical outcomes, since the large prevalence analyses thus far lack clinical outcomes data. Individual cases with endocrine resistance have been reported.[4,8,14] In addition, how ESR1-FUS interacts with targeted therapies, such as CDK4/6i, PI3K inhibition, and mTORC1 inhibition, is unknown.

ESR1-FUS Mechanistic Consequences, Clinical Implications, and Treatment Strategies

ESR1-FUS products are heterogeneous and poorly characterized. Wild-type *ESR1* (ESR1-WT) has 10 exons (Fig. 1A, Fig. 2A), encoding the 5' UTR, the Activation Function 1 domain (AF1) that has a modulatory role, the DNA-binding domain (DBD), the hinge region containing the nuclear localization sequence (NLS), the ligand-binding/Activation Function 2 domain (LBD/AF2), and the 3' UTR. The LBD/AF2 binds estrogen, SERMs/SERDs, and transcriptional coactivators, and contains the dimerization interface. ESR1-FUS mechanistic consequences can be organized into 4 categories: LBD/AF2-swapping, partner-truncating, non-functional, and uncharacterized.

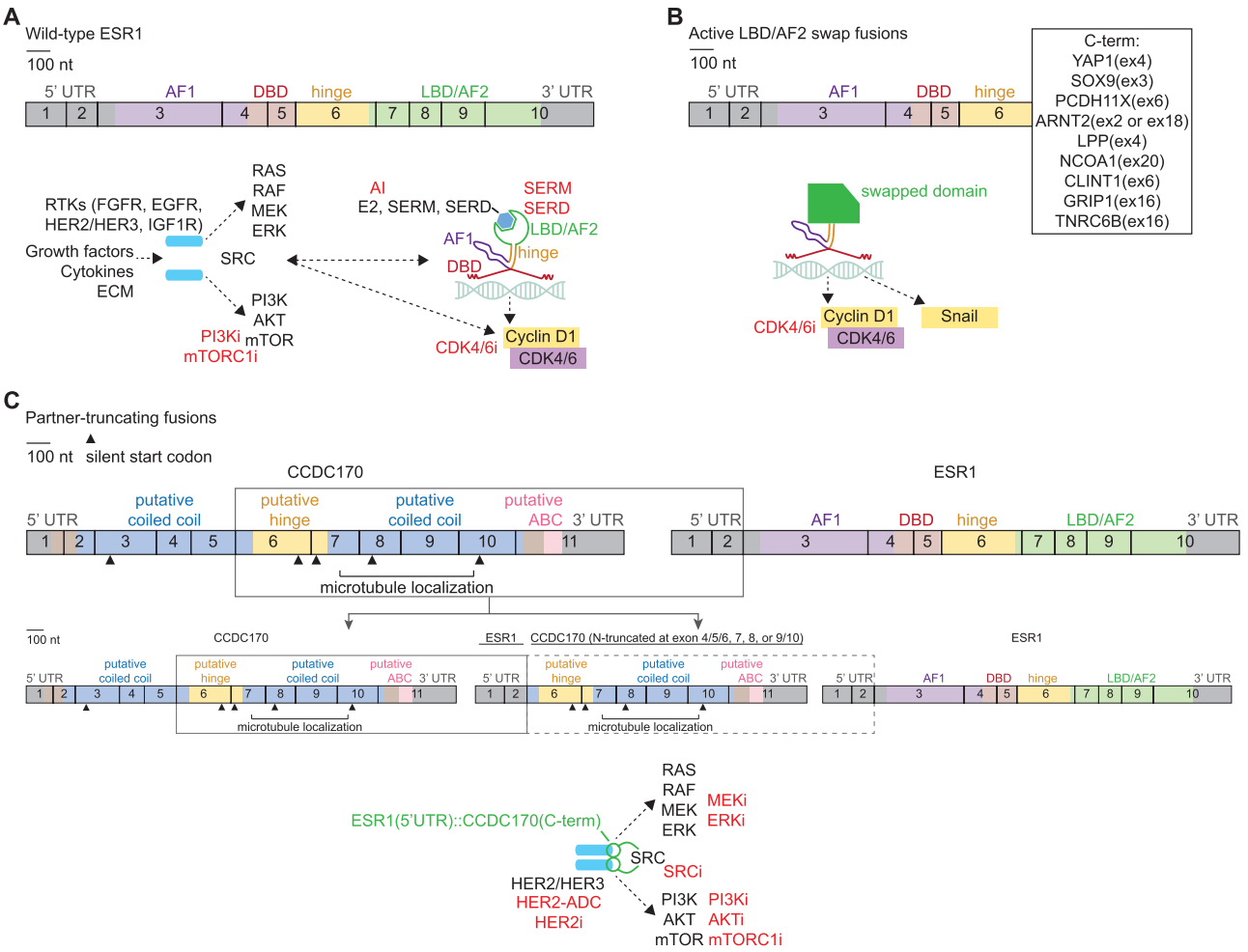

Figure 2. ESR1-FUS types and therapeutic strategies. (**A**) Wild-type *ESR1* exon structure, signaling, and approved treatments. A key downstream mediator of ERα and growth signaling is cyclin D1, which activates CDK4/6. Current non-chemotherapy strategies include AI depletion of estrogen, SERM/SERD targeting of ERα, CDK4/6i, and PI3K or mTORC1 inhibition to block growth signaling. (**B**) ESR1-FUS types that replace the LBD/AF2 after exon 6 with the C-term portion of another protein can hyperactivate ERα targets in an estrogen-independent manner and activate neomorphic target genes. As the LBD is absent, these fusions cannot be bound or inhibited by SERMs/SERDs. A promising treatment strategy is targeting downstream CDK4/6. (**C**) *ESR1* fusion to *CCDC170* through tandem duplication, resulting in the *ESR1* promoter driving transcription of the 5′ UTR of *ESR1* and a truncated C-term portion of *CCDC170*. There are 5 silent start codons (arrowheads) that render certain fusion points (exons 4/5/6 and exons 9/10) equivalent. These fusions cause *CCDC170* mislocalization and abnormal SRC/HER2/HER3 binding and activation. ESR1::CCDC170 fusions thus have complete endocrine resistance but may be susceptible to growth signaling inhibitors and HER2 ADCs. CDK4/6i sensitivity is not known. (**D**) Inactive LBD/AF2 swap fusions that are known to be non-functional. (**E**) *ESR1* fusions that have been detected in patients with unknown molecular consequences. Abbreviations: ABC, ATP-binding cassette; ADC; antibody-drug conjugate; AF1, Activation Function 1 domain; AI, aromatase inhibition; DBD, DNA-binding domain; E2, estradiol; ECM, extracellular matrix; LBD/AF2, ligand-binding/activation function 2 domain; RTKs, receptor tyrosine kinases; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator.

LBD/AF2-Swapping Fusions: AI/SERM/SERD-Resistant ERα Activity

Many *ESR1* fusions involve swapping the LBD/AF2 for the fusion partner (Fig. 2B). The first ESR1-FUS product characterized was *ESR1*(ex6)::*YAP1*(ex3) in a patient-derived xenograft (PDX) with primary fulvestrant resistance.[4] This fusion removed the LBD/AF2 from ERα, making the fusion unresponsive to estrogen and invisible to SERMs/SERDs. However, pure removal of the LBD/AF2 without replacement simply nullifies transcriptional activity.[14,16] Thus, it was LBD/AF2 replacement by the YAP1 transactivation domain that made *ESR1*(ex6)::*YAP1*(ex3) a constitutively active transcription factor.[4,16] Additional fusion partners have since been characterized with similar consequences[14,16] (Fig. 2B). ESR1 LBD/AF2 swap fusions with SOX9, YAP1, PCDH11X, or ARNT2 not only hyperactivate conventional ERα targets but also upregulate epithelial-mesenchymal transition (EMT) genes.[7,16] Thus, LBD/AF2 swapping can cause ligand-independent, SERM/SERD-resistant ERα and EMT gene expression.

Regarding treatments, LBD/AF2 swap fusions are resistant to all endocrine therapies (Fig. 2B). However, downstream of ERα, Cyclin D-CDK4/6 remains central to breast cancer cell viability. Thus, CDK4/6i has been tested experimentally against ESR1-FUS, with encouraging results in cell line and PDX models.[4] Cell lines expressing ESR1::YAP1 and ESR1::PCDH11X that are resistant to estrogen deprivation and fulvestrant remain susceptible to palbociclib, and a PDX model with ESR1::YAP1 was susceptible to palbociclib.[4] Case 1 and Case 4, harboring ESR1::PLEKHG1 and ESR1::AKAP12 swapping fusions, respectively, both had tumor responses to CDK4/6i-containing regimens after prior progression on regimens lacking CDK4/6i, although these ESR1-FUS products have not been characterized experimentally. Thus, CDK4/6i may be effective against at least some LBD/AF2 swap fusions.

Partner-Truncating Fusions: ERα-Independent Partner Activity

The most frequently detected ESR1-FUS in patients involves the *ESR1* promoter and 5' UTR exons (without any *ESR1* coding sequence) truncating a fusion partner[6,8,12,13] (Fig. 2C). The prototype for this is a tandem duplication involving the upstream gene *CCDC170*, a structural maintenance of chromosome (SMC) member that regulates Golgi-associated microtubule organization.[17] ESR1::CCDC170 fusion results in *ESR1* promoter-driven CCDC170 truncation, causing loss of Golgi localization, and AKT and ERK activation possibly via direct binding of the truncated CCDC170 to SRC/HER2/HER3. This causes more aggressive cancer cell growth and resistance to estrogen deprivation, tamoxifen, and fulvestrant.[6,17,18]

In contrast to LBD/AF2 swap fusions, for ESR1::CCDC170 fusions, AKT and ERK activation raise the possibility of CDK4/6i resistance.[6,18] Instead, because ESR1::CCDC170 activates SRC/HER2/HER3, HER2 inhibition (lapatinib), and SRC inhibition (dasatinib) were tested against this fusion and resensitized ESR1::CCDC170 breast cancer cells to endocrine therapy.[18] PI3K and mTORC1 inhibitors, already in use for HR+ breast cancer, or AKT, MEK, and ERK inhibition, are additional treatment strategies (Fig. 2C). Furthermore, an untested idea is that ESR1::CCDC170 fusions sensitize cancer cells to cytotoxic chemotherapy, either through HER2 signaling activation and increased proliferation or the remaining microtubule-binding domains of CCDC170 in the fusion product that might be susceptible to microtubule inhibition (Fig. 2C). Antibody-drug conjugate (ADC) therapies against HER2 are thus another strategy, such as T-DM1 administered in Case 2 (although this Case likely had a non-functional fusion due to a frameshift).

Non-functional Fusions

In contrast to active ESR1-FUS products, several *ESR1*(ex6) fusions have been identified that are more like pure LBD/AF2 removal, resisting SERD degradation but lacking ERα activity and growth promotion[14,16] (Fig. 2D). Such fusions may instead be selected for in tumor evolution due to loss of function or dominant negative inhibition of the fusion partner, which is often a tumor suppressor such as ARID1B or TCF12.[16]

Uncharacterized Fusions

Numerous *ESR1* fusions have been detected in patient samples but have not been characterized[7-11,14-16,19] (Fig. 2E). As described above, these fusions have diverse possible effects, including constitutive ERα activity, neomorphic transcription factor activity, neomorphic signal transduction activity, partner loss of function, and bystander non-functionality. This is problematic for clinical decision-making. A recent study developed a 24-gene expression assay that discriminated between the presence of active versus non-functional LBD/AF2-swapping ESR1-FUS.[16] While promising, this assay has not been tested on ESR1::CCDC170 fusions, and it cannot distinguish between active LBD/AF2-swapping ESR1-FUS versus ESR1-MUT. Thus, this assay may prove useful for Case 4's uncharacterized *ESR1*(ex6)::*AKAP12*(ex4) fusion but not for Case 1, which had an uncharacterized *ESR1*(ex6)::*PLEKHG1*(ex14) fusion but also an *ESR1 Y537N* mutation.

In summary, there are 3 main ESR1-FUS types, occurring in at least 1-10% of HR+ breast cancer. One is exchange of the ERα LBD/AF2 for another protein, conferring ERα hyperactivation and SERD/SERM resistance (Fig. 2B). Second is the truncation of *CCDC170* through fusion with the *ESR1* 5' UTR, conferring AKT/ERK activation and SERD/SERM resistance (Fig. 2C). Third is the exchange of the ERα LBD/AF2 for a protein without apparent effect (Fig. 2D). In addition, other fusions have been detected but not characterized (Fig. 2E).

Molecular Results

In the Cases, gene fusions in tissue samples were detected with the institution's Solid Fusion assay based on anchored multiplex PCR.[20] All ESR1-FUS alterations in the Cases contained fusion partners in the same chromosomal neighborhood as *ESR1* (chr6q25.1) (Fig. 1A), which is consistent with other studies.[8]

In Case 1, 2 fusions were detected (Fig. 1A). One was *ESR1* exons 1-3, including part of the AF1, joined to *CCDC170* exons 2-11. While *ESR1*(ex2)::*CCDC170*(ex4) through *ESR1*(ex2)::*CCDC170*(ex10) create endocrine resistance by AKT/ERK activation via a CCDC170 fragment, it is not clear how a larger CCDC170 fragment fused to the ERα AF1 would behave (Fig. 2E). Wild-type CCDC170 overexpression does not create resistance and in fact leads to breast cancer cell apoptosis.[21] The other fusion was *ESR1* exons 1-6 joined

to *PLEKHG1* exons 14-16. *PLEKHG1* encodes a Rho guanine nucleotide exchange factor (RhoGEF) that activates RAC1 and CDC42 to regulate cell fate and and motility.[22] The RhoGEF and plekstrin homology domains are excluded from the fusion product.

The patient had prompt progression on fulvestrant alone followed by disease stabilization when abemaciclib was added. One hypothesis is that *ESR1*(ex6)::*PLEKHG1*(ex14) confers endocrine resistance via a constitutively active ESR1-FUS LBD/AF2 swap that relies on CDK4/6 (Fig. 2B). As the tumor was HER2-low (IHC 2+, ISH negative), another idea is that targeting HER2, SRC, AKT, or MEK/ERK may have also addressed *ESR1*(ex3)::*CCDC170*(ex2) if this fusion were like known ESR1::CCDC170 fusions (Fig. 2C).

Case 2 (Fig. 1B) contrasts with Case 1 in harboring *ESR1*(ex4)::*CCDC170*(ex2)fs*29. This results in the *ESR1* 5′ UTR and AF1 joined to *CCDC170* in a frameshift with termination after 29 codons. As this is unlikely to produce functional protein, it may be a bystander alteration.

Case 3 (Fig. 1C) had a fusion of the ERα AF1 to MTHFD1L in a frameshift with termination after 9 codons, a likely also nonfunctional product. This tumor recurred on AI but was sensitive to fulvestrant plus palbociclib, still in complete remission after 3 years. MTHFD1L is a rate-limiting enzyme of the 1-carbon cycle, linking mitochondrial and cytoplasmic activities, and is an important mediator of cancer cell growth and tumor progression.[23] *ESR1*(ex3)::*MTHFD1L*(ex21)fs*9 may have come with additional mutations (Fig. 1C) and alterations that caused recurrence on AI; subsequent SERD and CDK4/6i therapy may have unveiled the metabolic disadvantage of MTHFD1L loss. An alternative possibility is that *ESR1*(ex3)::*MTHFD1L*(ex21)fs*9 is simply a bystander mutation. Together with Case 2, this highlights the importance of addressing knowledge gaps in ESR1-FUS characterization and ESR1-FUS effect prediction.

Case 4 (Fig. 1D) had *ESR1* exons 1-6 fused to *AKAP12* exons 4-5. This fusion has been detected frequently in breast cancer samples and associated with AI resistance but has not been molecularly characterized.[7,8,12-14] AKAP12 is a tumor suppressor and protein scaffold that modulates the activity of many other proteins, including PKA, β2 adrenergic receptors, and β-1,4-galactosyltransferase 1.[24] It localizes to cell membranes in a manner dependent on polybasic domains but not N-term myristylation.[25] It is thus possible that *ESR1*(ex6)::*AKAP12*(ex4) creates an active LBD/AF2-swapping fusion (Fig. 2B), an AKAP12 truncation with altered activity (Fig. 2C), neither (Fig. 2D), or both. As the patient received combination therapy, it is not clear which was the case. It is possible that CDK4/6i bypassed an active LBD/AF2-swapping fusion, but it is also possible that endocrine therapy addressed a now-HR+ tumor with a non-functional ESR1-FUS. Finally, the tumor also had a partial response to the addition of alpelisib later, which may be due to the tumor's PI3K-activating mutation or PI3K activation as a known consequence of AKAP12 loss-of-function.[24]

Summary

ESR1-FUS occurs in at least 1-10% of treatment-naïve and treated HR+ breast cancers. These fusions have diverse mechanistic implications, and many have unknown molecular consequences. CDK4/6i may be effective for ESR1-FUS with active LBD/AF2 swaps (Case 1, Case 4), and other fusions may be non-functional or represent loss-of-function effects (Case 2, Case 3). It will be important to detect ESR1-FUS in patients and to develop further strategies for precision treatments of these resistance alterations.

Limitations

This work is limited by the small number and heterogeneity of cases in the series. Although we report new *ESR1* fusion products, no 2 patients had the same exact fusions, and we did not analyze these fusion products in experiments. Thus, our work is only hypothesis-generating as to the functionality and therapy-responsiveness (especially of CDK4/6i) of these *ESR1* fusions. In addition, the 4 cases did not have serial tissue biopsies or circulating tumor DNA samples collected to analyze molecular changes over time. We therefore do not have data on how *ESR1* fusion clonal prevalence may change in response to treatment and which genetic resistance mechanisms may create resistance to initially effective treatments. We hope our case series motivates larger, multi-institutional collections of ESR1-FUS cases with clinical histories and serial molecular samples, facilitated by improved technical and analytic methods for gene rearrangements in liquid in addition to solid biopsies. It will be important to continue work with patient-derived and engineered ESR1-FUS breast cancer models for experiments to elucidate the structures, functions, and sensitivities of these fusions.

Glossary of Genomic Terms and Nomenclature

Gene fusion notation is per the 2021 HUGO Gene Nomenclature Committee recommendations of [5′ gene](last exon)::[3′ gene last exon](first exon). For example, *ESR1*(ex2)::*CCDC170*(ex8) is exons 1-2 of *ESR1* fused 5′ to exons 8-11 of *CCDC170*.

For all genes except *ESR1*, the Ensembl Canonical transcript variant was selected for exon enumeration; Ensembl v105.38 (GRCh38.p13) was used. For *ESR1*, ENST00000440973.5 was used as the most discussed variant in the literature; unlike the Ensembl Canonical variant, this transcript includes the 2 non-coding 5′ UTR exons frequently involved in fusions.

Frameshift notation is per the Human Genome Variation Society with "fs" denoting a frameshift change and "*[codon]" representing a termination after the indicated number of new codons numbered from the first amino acid change. For example, *ESR1*(ex3)::*MTHFD1L*(ex21)fs*9 indicates exons 1-3 of *ESR1* fused 5′ to exon 21 of *MTHFD1L* with a frameshift at the site of fusion followed 9 codons later by a termination codon.

ADC: antibody-drug conjugate
AF1: Activation Function 1 domain
AI: aromatase inhibition
CDK4/6i: CDK4 and CDK6 inhibition
DBD: DNA-binding domain
DCIS: ductal carcinoma in situ
EMT: epithelial-mesenchymal transition
ERα: estrogen receptor alpha
ESR1-FUS: ESR1 fusion
ESR1-MUT: ESR1 point mutation
ESR1-WT: wild-type ESR1
HR: hormone receptor

IDC: invasive ductal carcinoma
ILC: invasive lobular carcinoma
LBD/AF2: ligand-binding/Activation Function 2 domain
MBC: metastatic breast cancer
NLS: nuclear localization signal
PDX: patient-derived xenograft
RhoGEF: Rho guanine nucleotide exchange factor
SERD: selective estrogen receptor degrader
SERM: selective estrogen receptor modulator
T-DM1: ado-trastuzumab emtansine
TCGA: The Cancer Genome Atlas

Funding

National Institutes of Health, National Cancer Institute: K12CA087723 (L.M. Spring); National Comprehensive Cancer Network (L.M. Spring).

Conflict of Interest

Lauren L. Ritterhouse reported consulting for Loxo Oncology, Amgen, Merck, AstraZeneca, Sanofi Genzyme, and EMD Serono. Laura M. Spring reported consulting for Novartis and Puma Biotechnology and institutional research support from Phillips, Merck, Genentech, and Daiichi Pharma/AstraZeneca. Miguel N. Rivera reported research support from Advanced Cell Diagnostics and Merck Serono. Aditya Bardia reported consulting for Pfizer, Novartis, Genentech, Merck, Radius Health, Immunomedics, Taiho, Sanofi, Daiichi Pharma/AstraZeneca, Puma Biotechnology, Biothernostics Inc., Phillips, Eli Lilly, and Foundation Medicine; contracted research (via institution) with Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health, Immunomedics, and Daiichi Pharma/AstraZeneca. Seth A. Wander reported consulting for Foundation Medicine, Veracyte, Eli Lilly, Hologic, Pfizer, and Biovica and institutional research support from Genentech, Eli Lilly, Nuvation, and Regor. The other authors indicated no financial relationships.

Author Contributions

Conception/design: J.O.B., A.B., S.A.W. Provision of study material or patients: L.L.R., E.T.N., K.E.I., M.D., L.Y.R., L.M.S., A.B., S.A.W. Collection and/or assembly of data: L.L.R., L.Y.R., M.N.R., J.K.L., L.D.D.-S., L.W.E., A.B. Data analysis and interpretation: J.O.B., L.L.R., S.A.W. Manuscript writing: J.O.B., L.L.R., S.A.W. Final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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