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## *ESR1* and endocrine therapy resistance: more than just mutations

Estrogen receptor (ER)-positive breast cancer accounts for 70%–80% of all diagnosed breast cancers [1]. The adoption of endocrine therapies, including ER modulators/degraders (SERMs/SERDs), which antagonize ER, and aromatase inhibitors (AIs), which suppress estrogen synthesis, as the mainstay of treatment of ER-positive breast cancer patients has resulted in substantial survival benefit for patients with early stage disease [2]. Treating ER-positive metastatic breast cancer (MBC), however, remains a significant clinical challenge, due to the development of secondary resistance to all modalities of endocrine therapy [3]. Recently, studies have identified recurrent somatic mutations within the ligand-binding domain (LBD) of *ESR1* (encoding ER) in >30% of ER-positive MBC [4–8]. These mutations alter the conformation of ER and produce a constitutively active form of the protein. Mutations at residues 536–538, in particular, promote ER activity in the absence of ligand, resulting in resistance to AIs and reduced sensitivity to SERMs/SERDs [4, 5]. *ESR1* fusion genes have also been reported in ER-positive MBCs; however, a detailed description of their manifestations and clinical prevalence is lacking [9]. In this issue of *Annals of Oncology*, Hartmaier et al. reported the identification of recurrent hyperactive *ESR1* fusion genes in breast cancers resistant to endocrine therapy [10], adding to the diversity of reported *ESR1* alterations.

Hartmaier et al. carried out a retrospective study to discover genomic rearrangements involved in the acquired resistance to ER-targeted therapies [10]. Using mate-pair DNA sequencing and/or RNA sequencing of matched primary-metastasis-normal samples from 6 patients, the authors identified an *ESR1-DAB2* in-frame fusion transcript that fused exons 1–6 of *ESR1* to exons 3–15 of *DAB2*. This fusion was found only in the lymph node metastasis but not in the primary. RNA sequencing analysis of an additional 51 breast cancer metastases revealed an *ESR1-GYG1* fusion gene in a bone metastasis, comprising the *ESR1* exons 1–6, the same involved in the *ESR1-DAB2* fusion gene, and the 3′ end of *GYG1*. Importantly, both fusions were also detectable at the protein level [10].

Prompted by the discovery of recurrent *ESR1* fusion breakpoints, the authors analyzed 9542 breast cancers (including 5216 from metastases) and 254 circulating tumor DNA (ctDNA) samples from advanced breast cancer patients, and identified 7 additional *ESR1* fusion genes. Including the initial cohorts subjected to mate-pair and/or RNA sequencing, 5 fusions were identified in metastatic disease (5/5, 272, 0.09%), 1 in local recurrence after endocrine therapy (1/4, 329, 0.02% of primary tumors) and 3 in ctDNA (3/254, 1.2%). For the 4/9 patients with available clinical histories, all had been treated extensively with AIs. Of note, the *ESR1* breakpoints were all in or between exons 6 and 7, disrupting the LBD of *ESR1*. *In vitro* analysis of 3 of the fusions identified (*ESR1-DAB2*, *ESR1-GYG1*, and *ESR1-SOX9*) demonstrated that all had ligand-independent activity and two were hyperactive [10]. These observations suggest a potential role for the distinct 3′ gene partners in determining resultant ER activity.

With genomic breakpoints frequently located in intronic regions, capture-based targeted sequencing of exons does not always detect fusion genes. Structural rearrangements, however, are frequently associated with copy number alterations. Based on this notion, the authors devised a novel algorithm *copyshift* to detect intra-genic fusion junctions associated with copy number changes in targeted sequencing. When tested in a cohort of lung cancer with known *ALK* rearrangements, the authors showed that *copyshift* was specific (>89% positive predictive value), albeit with limited sensitivity (~85% false negative rate). Applying *copyshift* to the cohort of 9542 breast cancers to interrogate the recurrent *ESR1* breakpoint region, the authors found 83 *copyshift*-positive tumors. These tumors were enriched for ER-positive and

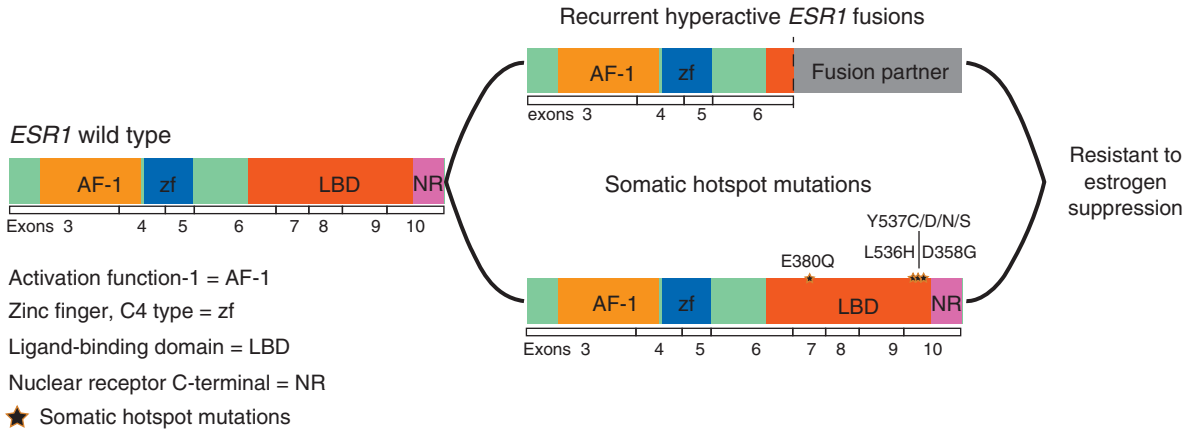


**Figure 1.** Development of resistance to estrogen suppression *via* distinct somatic genetic alterations in the *ESR1* gene. Structural/functional representation of the somatic genetic alterations in *ESR1* leading to resistance to estrogen suppression in breast cancer. The structural domains of the *ESR1* gene are shown in different colors, including the transcription activation function 1 (AF-1) domain, the DNA-binding zinc finger, C4 type domain (zf), ligand-binding domain (LBD), and the nuclear receptor C-terminal domain (NR). The positions of the coding exons 3–10 are illustrated beneath the protein domains. Whilst wild-type *ESR1* consists of intact coding exons 3–10, *ESR1* fusion genes and/or hotspot activating mutations disrupt the ligand-binding domain.

metastatic disease, and were associated with the presence of activating *ESR1* mutations. Taken together, the authors estimated that at least 1% of MBCs harbored *ESR1* fusion genes. With the 85% false negative rate of *copyshift*, the prevalence of 1% may represent an underestimate, since *copyshift* prioritizes specificity over sensitivity.

The discovery of recurrent *ESR1* fusion genes reinforces the concept that resistance to targeted therapies often represents a convergent phenotype (i.e. that resistance, the phenotype observed, may be caused by distinct genetic alterations) [11]. In the case of resistance to endocrine therapy, the identification of recurrent rearrangements adds to the previously described activating mutations to expand the repertoire of genetic alterations affecting *ESR1* (Figure 1). A similar phenomenon has been described for resistance to PARP inhibitors/platinum salts in *BRCA1/2* germline mutation carriers, with distinct *BRCA1/2* intragenic deletions or reversion mutations [12–14] restoring the reading frame and thus resulting in resistance. In fact, multiple activating *ESR1* mutations have been detected in the ctDNA samples of patients harboring activating *ESR1* fusion gene [10], suggesting the existence of polyclonal resistance mechanism, akin to the recent reports of polyclonal *BRCA1/2* reversion mutations in therapy-resistant patients [15, 16].

The results reported by Hartmaier et al. [10] have important and immediate clinical implications. The emergence of *ESR1* mutations as a resistance mechanism to AIs has led to the development of novel molecules and combination regimens that are more effective in suppressing *ESR1*-mutant tumor growth [17–19]. Even these new agents and regimens, however, rely on targeting the LBD. The loss of the LBD by genomic rearrangements may represent a resistance mechanism not readily detectable by common approaches such as ddPCR/BEAMing and targeted sequencing of exonic regions, arguing for the use of methods such as *copyshift* or RNA sequencing based methods to identify such alterations. It should be noted, however, that the biological and clinical significance of the findings by Hartmaier et al. [10] remains to be fully elucidated, given that limited clinical history was available. Furthermore, the authors demonstrated the detection of the chimeric proteins in only a handful of patients and it is possible that some of the *ESR1* fusion genes are not transcribed and/or translated, or may have limited impact on the resistance to endocrine therapies. It would be important to investigate the mutual exclusivity or co-occurrence between *ESR1* mutations and/or fusions and other mechanisms of resistance [20–23] and the existence of multiple subclonal resistance mechanisms in individual patients, and whether the mechanisms resulting in the acquisition of distinct modalities of resistance to endocrine therapy would differ.

S. Piscuoglio[1], C. K. Y. Ng[1,2], B. Weigelt[3], S. Chandarlapaty[4] & J. S. Reis-Filho[3*]

[1]Institute of Pathology and Medical Genetics, University Hospital Basel, Basel; [2]Department of Biomedicine, University of Basel, Basel, Switzerland; [3]Department of Pathology; [4]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, USA

(*E-mail: reisfilj@mskcc.org)

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## Genetic profiling of cell-free DNA from cerebrospinal fluid: opening the barrier to leptomeningeal metastasis in *EGFR*-mutant NSCLC

Over the past decade, remarkable progress has been made in the management of advanced non-small-cell lung cancer (NSCLC), when tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors have been added to the therapeutic armamentarium. Indeed, dramatic responses to epidermal growth factor receptor (EGFR)-TKIs are observed in patients with NSCLC harbouring activating *EGFR* driver mutations [1, 2]. Unfortunately, all tumours ultimately develop secondary resistance, half of these due to the acquirement of the gatekeeper *EGFR* T790M mutation [3]. Fortunately, T790M-induced resistance can now be successfully addressed by use of third-generation EGFR-TKIs that show impressive activity in these patients [4].

Despite the much better outcome of patients with *EGFR*-mutant NSCLC nowadays, central nervous system metastases, and in particular leptomeningeal metastases (LM), remain a devastating complication associated with a poor prognosis and often dramatic effect on quality-of-life [5]. Particularly in *EGFR*-mutant NSCLC, LM appears to occur more frequently with a reported incidence of 9.4% [6]. Information regarding the genetic profile of LM is largely lacking, as these lesions are hard to access. Consequently, little progress has been made in the management of LM disease. Due to recent technological advances, it is now possible to study genetic alterations in widely accessible but relatively challenging sources of tumour DNA, such as circulating cell-free tumour DNA (ctDNA) in plasma or in cerebrospinal fluid (CSF) [7, 8]. This is an important evolution as the detection of tumour-specific mutations in the CSF may increase the diagnostic sensitivity and specificity of LM, allow monitoring of treatment response and improve our understanding of the evolving tumour biology.

In this issue of *Annals of Oncology*, Li et al. report that CSF-derived ctDNA is a valuable source of tumour DNA, allowing the identification of the unique genetic profile of LM in *EGFR*-mutant NSCLC. In their study, ultra-deep targeted next-generation sequencing (NGS) analysis was carried out on different sources of tumour DNA obtained from 26 NSCLC patients diagnosed with LM and carrying *EGFR* driver mutations in their primary tumour. DNA sources included cell-free DNA obtained from CSF and plasma, as well as cellular DNA obtained from CSF precipitates and primary tumour tissue [9]. Of interest, apart from single nucleotide variants and small insertions and deletions (indels), also copy number alterations (CNAs) were investigated.