Estrogen Receptor Mutations Found in Breast Cancer Metastases Integrated With the Molecular Pharmacology of Selective ER Modulators

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Abstract

The consistent reports of mutations at Asp538 and Tyr537 in helix 12 of the ligand-binding domain (LBD) of estrogen receptors (ERs) from antihormone-resistant breast cancer metastases constitute an important advance. The mutant amino acids interact with an anchor amino acid, Asp351, to close the LBD, thereby creating a ligand-free constitutively activated ER. Amino acids Asp538, Tyr537, and Asp351 are known to play a role in either the turnover of ER, the antiestrogenic activity of the ER complex, or the estrogen-like actions of selective ER modulators. A unifying mechanism of action for these amino acids to enhance ER gene activation and growth response is presented. There is a range of mutations described in metastases vs low to zero in primary disease, so the new knowledge is of clinical relevance, thereby confirming an additional mechanism of acquired resistance to antihormone therapy through cell population selection pressure and enrichment during treatment. Circulating tumor cells containing ER mutations can be cultured ex vivo, and tumor tissues can be grown as patient-derived xenografts to add a new dimension for testing drug susceptibility for future drug discovery.
Table 1. A summary of recent literature reports of specific mutations noted in either primary tumors or metastases from patients following the failure of multiple antihormonal therapies (mainly aromatase inhibitors)∗

<table>
<thead>
<tr>
<th>Authors</th>
<th>Samples</th>
<th>No. point mutations</th>
<th>Type of mutation</th>
<th>Total samples, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. Cell Reports. 2013;4:1116–1130</td>
<td>22 total 7 primary 15 mets. (2 from the same patient)</td>
<td>4 mets.</td>
<td>ESR1 YAP1 fusion Tyr537Ser met. Tyr537Ser (grown in mice only one mutant) (wild-type when grown) Glu380Gln Asp538Gly</td>
<td>26.6</td>
</tr>
<tr>
<td>Merenbakh-Lamin et al. Cancer Res. 2013;73:6856–6864.</td>
<td>13 total 5 liver mets.</td>
<td>5 mets.</td>
<td>Ser463Pro/Asp538Glu Val534Glu Pro535His Leu536Arg Tyr537Ser Tyr537Asn Tyr537Cys Tyr537Ser/ Asp538Gly Asp538Gly</td>
<td>38</td>
</tr>
<tr>
<td>Toy et al. Nature Genetics. 2013;45:1439–1445.</td>
<td>183 primary 80 mets.</td>
<td>6 primary 14 mets.</td>
<td>Ser463Pro/Tyr537Asn Val534Glu Pro535His Leu536Arg Tyr537Ser Tyr537Asn Tyr537Cys Tyr537Ser/ Asp538Gly Asp538Gly</td>
<td>3.28% of total primary 17.5% of total mets.</td>
</tr>
<tr>
<td>Yu et al. Science. 2014;345:216–220.</td>
<td>6 patients with CTCs</td>
<td>3 CTCs</td>
<td>Tyr537Ser Asp538Gly</td>
<td>50</td>
</tr>
</tbody>
</table>

∗ CTC = circulating tumor cells; mets. = metastases.

How Do the ER Mutations in Metastases “Upgrade” Prior Knowledge of ER Mechanics?

The identification of the ER protein in breast cancer as the primary estrogen signal transduction pathway created an appropriate target to block estrogen stimulated tumor growth (10). Endocrine therapy using either SERMs or AIs eventually became the standard of care for both the treatment and the prevention of breast cancer (11).

It was clear from early studies (12–14) before the ER sequence was known that the conformation of the receptor complex could be modulated to switch on or switch off target gene function. The bioassay to modulate estrogen-induced prolactin synthesis utilized primary cultures of immature rat pituitary cells as the first system in vitro to describe the structure-function relationships of ER-binding ligands predictably (15). The model of estrogen/antiestrogen action was referred to as the “crocodile model”; estrogen closed the jaws tightly for activation, but the irregular shape of an antiestrogen was a stick in the jaws of the crocodile (16). The antiestrogenic action of antiestrogens was postulated to be because the “anti-estrogenic side chain” of an antiestrogen interacted at the “antiestrogenic region” on the aberrant conformation of the ER complex (12). However, the cloning and sequencing of the human ER (17,18) created opportunities first to understand the organization of the ER and then to understand the mechanics of estrogen activation of the human ER through the resolution of x-ray crystallographic studies (19). The mechanics of antiestrogen action would follow.

There are six major functional areas of the ER identified as A-F domains (Figure 1A). Domains A/B are referred to as activating function 1 (AF-1), and E (Figure 1A) is the ligand-binding domain (LBD) but contains within it activating function 2 (AF-2). Domain C is the DNA-binding domain, with a D domain as a hinge region to link C at aa 302, to the LBD. The F domain appears to modulate the functions of the ER in a ligand-, promoter-, and cell-specific fashion (20–23). The function of the different domains was established by regional deletions and selective mutational analysis for specific amino acids (24,25). However, the originally cloned human ER was subsequently found to contain a mutation at Gly400Val (Figure 1A). This mutation decreases estrogen binding (26), which dramatically reduces its ability to prevent cell replication when stably transfected into an ER-negative breast cancer cell line (27). The mutation does not alter the antiestrogenic activity of raloxifene (28).

Under normal physiologic circumstances, signal transduction is initiated with the planar steroid estradiol correctly positioned in the LBD of the wild-type ER by Glu353, Arg394, and His524 (Figure 1B). This scaffold allows helix 12 to seal the LBD (Figure 1B). The amino acids Tyr537 and Asp538 now of interest (1–6) are shown on the external view of the E, ER complex closely aligned with Asp351 in the LBD (Figure 1C).
The raison d’être for mutational variation in the principal signal transduction pathway of cell survival in breast cancer is simply to provide a growth advantage. It is a straightforward example of Darwinism. By expanding the cancer cell population, the tumors can survive and thrive. The response to estrogen deprivation and mutation of the ER can only be considered an adaptation of the normal creation of the estradiol:ER complex as the optimal growth stimulus.

To survive estrogen deprivation, the ER-positive breast cancer cell has, up until now, been viewed as having two options: either expand the ER population so clones now grow out possibly with the ability to scavenge meager quantities of estrogen (29) or close down the ER regulatory mechanism to enhance the probability of selecting out an ER-negative cell population. Examples of these options are noted in the laboratory despite the fact that there are very few ER-positive cell lines (30). MCF-7 cells and T47D cells regulate the ER in opposite ways (31).

MCF-7 cells respond to estrogen deprivation with expansion of the ER population by upregulation of the ER gene (32) or perhaps gene amplification, as noted in studies with breast cancer samples (33). By contrast, T47D cells are ER positive but require estrogen to increase ER synthesis to remain estrogen dependent. Long-term estrogen deprivation results in the loss of the ER (34) in T47D cells and the creation of a stable ER-negative breast cancer cell line (35).

Early studies of ligand and antiestrogen resistance proposed a truncated ER that still had DNA-binding properties in the absence of a LBD (36-38). Specifically, a report 20 years ago (39) found few alterations in the ER genome; only 2 out of 20 tamoxifen-resistant breast cancers had detectable changes. The 2 aberrations in ER were a 42 base pair replacement in exon 6 and a single base pair deletion in exon 6 of a metastasis but not the primary tumor. Exon 6 is in the LBD. The authors (39) speculated that translation of the ERs could result in a truncated ER with an intact DNA binding domain but a defective LBD, both of
which might have constitutively activated ER-responsive genes. No evidence was provided, but from what we know now this is possibly correct and consistent with current mechanisms of resistance. The finding of a 344insCys (6) and an ESR1-YAP1 translocation (ESR1 aa 1–365 including the DNA binding domain fused with the C-terminus of YAP1 [aa 230–504]) (1) may be the identification of constitutive growth enhancing DNA-binding domain proteins. Fuqua and colleagues (40) provided evidence of constitutive transcriptional activity in an examination of five ER-negative/progesterone receptor (PgR)-positive breast tumors and dominant negative activity in an ER transcript from an ER-positive/PgR-negative tumor (41). Fuqua and colleagues (42) further pioneered studies on ER mutations in metastases when her group identified a single-point mutation Tyr537Asn that was predicted to cause a conformational change in the ER analogous to hormone binding. Evidence was provided. This mutation and Tyr537Ser, Tyr537Cys were noted in the recent clinical studies (Table 1). However, does Tyr537 have any defined role in ER homeostasis and function?

Specific studies demonstrated that Tyr537 in the ER protein is phosphorylated to enhance efficient estrogen action (42,43–45). Current molecular modeling illustrates that the mutant Tyr537Ser in the ER protein can allow helix 12 in the absence of estrogen to close (3). Under normal conditions Tyr537 is phosphorylated by cSrc (43–45), but long-term inhibition of cSrc in estrogen-deprived breast cancer cells causes an accumulation of the ER (46), thereby implying that Tyr537 is an important amino acid for regulation of ER turnover and activation. A recent finding (47) that protein-tyrosine phosphatase H1 dephosphorylates ER at Tyr537 stimulates nuclear accumulation of ER and sensitivity to antiestrogens illustrates the potential role of aa Tyr537 in antihormone resistance.

Mutation Asp538Gly improves the possibility of closure of helix 12 (3). An earlier experimental study (48) with an Asp538Ala mutation demonstrated a reduction of the estrogen-like properties of the 4OHT ER complex and caused the rapid destruction of the 4OHT ER. The 4OHT ER routinely accumulates when liganded to wild-type Asp538 ER. It is tempting to speculate that the mutation of 538 provides the opportunity for Tyr537 to be phosphorylated by cSrc and the ER destroyed.

The fascinating observation made in two of the six new studies (2,3) is that when molecular modeling is performed, the mutation Ser537 and Gly538 found in metastases are drawn to a single amino acid, Asp535, to accomplish the closure of helix 12. It seems that Asp535 is the pivotal anchor for the majority of mutations (Table 1) that facilitate helix 12 closing in the absence of a ligand with mutant ER. This anchor, amino acid Asp535, also has a history in modulating SERM action.

Do These Mutations in the ER Inform About SERM Action?

Prior receptor research is essential to understanding current mutational mechanisms. In this respect, Asp531 is also an important amino acid for the modulation of SERM action at target genes. But how is SERM action regulated?

Two earlier parallel lines of investigation provided the primary evidence for the important role of Asp531 in SERM pharmacology. One line of evidence came from a laboratory study of acquired breast cancer resistance to tamoxifen in an animal model, and the second was a precise understanding of the binding of raloxifene and 4OHT in the LBD of the ER through x-ray crystallography (19,49). The two tracks of evidence ultimately coalesced (Supplementary Table 2, available online) to decipher the molecular pharmacology of SERM action that parallels what we know of their different clinical pharmacology (50,51) in the Study of Tamoxifen and Raloxifene (STAR).

Twenty-five years ago, an animal model of acquired resistance of tamoxifen to ER-positive breast cancer was described using the ubiquitous MCF-7 cell line (52,53). The transplantable tumors with acquired resistance to tamoxifen grew in athymic animals because of tamoxifen, not despite tamoxifen treatment (53,54). The tumors were tamoxifen dependent. There is clinical evidence for tamoxifen-stimulated tumor growth demonstrating a “withdrawal response” when tamoxifen treatment was stopped (55,56). Furthermore, tumors in the laboratory continued to grow with physiologic estrogen after stopping tamoxifen. Further studies demonstrated that either no estrogen (like using an AI) or the use of a pure antiestrogen that binds to and destroys ER (57) stopped tumor growth.

The biology of the laboratory model in vivo replicated the clinical course of acquired resistance, so it was only reasonable to look for mutations in the ER. Despite the fact that the MCF-7 and T47D breast cancer cell lines retain wild-type ER when they become antiestrogen resistant in vitro (60), the theory was simple: The estrogen-stimulated MCF-7 tumor cell uses wild-type ER but would be enriched with appropriate mutations to catalyze tamoxifen-stimulated growth in vivo. However, only a single one of the three tumor lines retransplanted in athymic mice contained high concentrations of a mutant codon that substituted Tyr (61,62) for Asp531 (Supplementary Figure 1A, available online). The Asp351Tyr mutation for ER could be interpreted as providing a Darwinian growth advantage for SERMs as they acquire resistance. At the time, there was no understanding of the three-dimensional conformation of the ER complex, and Asp351Tyr was not located in the AF2 domain (eventually to be described as helix 12) (Figure 1A), so the mutation appeared to be of no functional significance; a chance observation? Apparently not. However, the path to progress needed to solve numerous experimental challenges to create a model to study the molecular pharmacology of SERMs (Supplementary Table 2, available online).

The estrogen response target gene in the new model cell system was transforming growth factor alpha (TGFA) (63) in ER-negative MDA-MB-231 cells stably transfected with wild-type or Asp351Tyr-mutant ER (27,64). The 4OHT ER (wt) complex was promiscuous and activated the TGFA gene exactly like E2, but the less estrogenic SERM raloxifene blocked E2-stimulated TGFA (28,65). By contrast, the mutant Asp351Tyr ER enhanced TGFA synthesis with 4OHT, and raloxifene became an estrogen at the same target. For the first time a natural mutation of the human ER was shown to convert an antiestrogen to an estrogen (28,65). The results with the wild-type ER at a model gene target (28,65) also illustrated how tamoxifen is more estrogen-like and promiscuous at select target sites compared with raloxifene, just as is noted when tamoxifen and raloxifene were compared in a chemoprevention study (50,51).

The subsequent publication of the x-ray crystallographic structures of the raloxifene and 4OHT LBD (19,49) now told us how the antiestrogenic side chain can modulate the estrogenic/antiestrogenic efficacy of the antiestrogenic complex. The “antiestrogenic region” previously postulated (12) was identified. Both raloxifene and 4OHT fit into the hydrophobic pocket of the LBD, but the antiestrogenic side chain prevents the reorientation of helix 12 that must seal the ligand into the receptor before coactivators can bind at AF2 and produce a transcription complex (Figure 1D). The high-affinity antiestrogens raloxifene...
and 4OHT both interact through their phenolic hydroxyls with Glu353 and Arg394 to locate the ligand correctly in the LBD (Figure 1D). This is the same locator system used by E2 (Figure 1B). However, the bulky side chain not only prevents helix 12 from sealing the LBD, a step necessary for coactivator binding to AF2, but also the piperidine ring of raloxifene shields and neutralizes Asp351 (Figure 1, D and E). Additionally, the new SERM lasofoxifene (66) also has an antiestrogenic side chain that shields and neutralizes Asp351 (67). By contrast, 4OHT has a shorter side chain and does not shield or neutralize Asp351 (Figure 1D) (68, 69). As a result, helix 12 can potentially interact with Asp351 or other amino acids to induce estrogen-like actions.

The molecular pharmacology of ligand binding and the importance of Asp351 illustrate the modulation of SERM action through a spectrum of estrogenic and antiestrogenic complexes (49, 68–70). There appears to be a conversation between the side chain of a SERM, Asp 351, and helix 12 based on amino acid substitution at 351. The Asp351Tyr rotomer #2 exposes the phenolic group to interact with Asp538 outside the influence of the piperidine side chain of raloxifene (Figure 2, A–D). This potentially closes helix 12 to promote estrogenic action (Figure 2, C and D). Conversely, substitution of a cyclohexane for the piperidine ring in raloxifene causes estrogen-like actions for the ligand ER complex (70). Asp 351 must be neutralized by the nitrogen of the piperidine ring of raloxifene to prevent Helix 12 closure.

The molecular modeling of the Asp351 substitution with raloxifene (Figure 2) and 4OHT (Figure 3) in the LBD and the interaction with aa in helix 12 parallel the biology at the TGFα gene. The AF2 deletion mutation (68) is consistent with the necessity to close helix 12, as per the modeling. The Asp351Gly mutant cannot close helix 12, and as such the 4OHT Asp351Gly ER is not estrogenic and only antiestrogenic (69) (Figure 3, B–D).

Knowledge of the molecular modulation of Asp351 using either wt ER or Asp351Gly ER has been taken one step further to classify planar (E2) or angular (triphenylethylene) estrogens based on closed or open helix 12 (71). The assay (Supplementary Figure 3, available online), can predict the time course of different structural estrogens to create an activated ER complex and induce apoptosis in antihormone-resistant cells (72, 73). This basic molecular pharmacology has clinical significance (74–77).

Structurally different estrogens were used by Haddow and coauthors (78) to treat postmenopausal breast cancer patients successfully in the 1940s. A series of studies of structurally different estrogens has described the time course of different estrogenic ER complexes (73) to solve the paradox of estrogen-induced tumor apoptosis. The molecular pharmacology is consistent with clinical experience.

The initial laboratory data, the molecular model described here, and current clinical knowledge of antihormone resistance are all consistent to illustrate the clinically relevant molecular pharmacology of acquired drug resistance pivoting around an intact Asp351 anchor in the majority of specific mutations (537, 538) noted in the antihormone-resistant metastases (Table 1; Supplementary Table 1, available online). Nevertheless, this mechanism does not account for the mechanism of a

Figure 2. A summary of interactions of selective estrogen receptor (ER) modulator (SERM) antiestrogen side chains. Interaction of key amino acids at 351 or 537 and 538 amino acids in helix 12 that may hypothetically secure the closing of the ligand-binding domain (LBD) for coactivator binding and subsequent modulation of estrogen action by SERMs. A) Substitution of different amino acids at 351 to modulate a raloxifene ER complex to be estrogenic (68). This research was originally published in (70) the American Society for Biochemistry and Molecular Biology. B) The role of the antiestrogenic side chain of raloxifene to modulate the estrogenic SERM ER complex by neutralizing and shielding the carboxylic group of the surface amino acid Asp351 to produce no estrogen action. C) Long polar amino acids glutamic acid and D. tyrosine at 351 form estrogen-like complexes with raloxifene.
minority of relevant mutations in the ER at 380, 463, 536 (Table 1; Supplementary Table 2, available online). Other processes remain to be discovered. Mutation of ER at 380, 463, and 536 will be considered in detail in the next section.

What Is the Incidence of Clinically Relevant Mutation in Metastatic ER?

It is clear from early studies described previously in this Commentary that functional mutations were a rare event in primary tumors. A summary of the recent reports in Table 1 and Supplementary Table 2 (available online) now supports this view with an expanded database. The primary tumor initially harbors low or undetectable levels of cells with the functional mutation (or at least the cancer cell with a mutated ER is not in sufficient concentrations for adequate detection) but needs selection pressure with estrogen deprivation to ensure metastatic tumor cell survival at a nascent nested site. There is a major fidelity of mutational drift (1–6) to the amino acids 538, 537 in helix 12 of the ligand binding domain. There is also early documentation (Figure 1A) of the potential clinical relevance of laboratory-generated mutations in the human ER (79–86) that paint a reassuring compliment to current clinical findings. There is an expanded structure/functional data base in the literature of artificial mutations in the ER.

Katzenellenbogen’s laboratory characterized the Tyr537Ser and Glu380Gln ER mutants created by site-directed mutagenesis (79,82–85). The mutant ERs were constitutively active and antiestrogen resistant, with the prediction that if found in breast cancer they would pose clinical risks. This indeed occurred, with the majority of mutations at 537 (Table 1) and recent reports of the Glu380Gln mutation (1,6,87). The Glu380Gln has recently been tracked in a single patient when no treatment was given (87) and noted to have a mutant allele fraction (MAF) of 68% in liver metastases but only a 2% MAF in the primary tumor. This rare mutation does not have a role interacting with Asp351, but Katzenellenbogen (84) established constitutive activity for the mutant ER, a hypersensitivity to E2, and a reduced responsiveness to 4OHT. Together the characteristics of this rare mutation create a Darwinian growth advantage for the metastases in the presence of estrogen in the patient’s body and predict failure of AI therapy and potential resistance to tamoxifen. The Ser463Pro mutation found by Toy et al. (3) was noted in three patients but only in association with mutations in Tyr537 and Asp538. Serine 463Pro decreases transactivation observed with Tyr537 and Asp538. Serine 463Pro decreases transactivation observed with Tyr537 and Asp538 alone. The mutations at Leu536 occur in multiple forms (Leu536Arg, Leu536Gln, Leu536Pro) (Table 1; Supplementary Table 1, available online). This amino acid is part of the triplet “Leu crown” (Leu536, Leu539, and Leu540) on helix 12. Leu536 is the closest to the antiestrogenic side chain of 4OHT and raloxifene interacting with Asp351 (Figure 1D). As a result the “Leu crown” has different conformations for helix 12 when 4OHT or raloxifene bind (19,49). The Leu536 has been extensively evaluated with multiple targeted mutations. In general, there is enhanced constitutive activity but attenuated responsiveness...
to E. However, 4OHT is in general ineffective in preventing transactivation of Leu536 mutations at an estrogen response element (86). Mutations of Leu536 may reflect a prior cellular evolution to modulate SERM action during tamoxifen treatment. Nevertheless, the constitutive transactivation of mutations of Leu536 is reduced dramatically by fulvestrant (86), thereby reinforcing the proposition that novel pure antiestrogens should be developed for clinical utility. In a subsequent review, Skafar and Koide (88) stated, “Leu536 essentially ‘reads’ the side chains of tamoxifen, raloxifene and fulvestrant, and so distinguishes the conformations of these SERM-bound or antiestrogen-bound ligand-binding domains.”

The selection pressure with AIs might also encourage amplification of the ER gene in metastatic breast cancer to aid cell survival, and there is some evidence that this occurs (1,89). Mechanistically, it would not be anticipated to observe mutations of Asp351, as this is essential under the current selection pressure of AIs to create a closed conformation of the ER necessary to initiate constitutive replication. It was more likely for the MCF-7 [a metastatic breast cancer cell line from a pleural effusion (90) from a high-dose estrogen-treated patient] to enrich for a Asp351Tyr mutation that enhances the estrogenicity of SERMs to favor tumor growth (Supplementary Figure 1, available online). This would be accomplished by sporadic closing of helix 12 to initiate coactivator binding, however transiently, to activate estrogen-responsive genes (Figures 2D and 3B). Additionally, it would not be anticipated to observe Lys303Arg (first described by Fugua [91]), as this provides only an initial growth advantage with estrogen in the early stages of breast cancer. The Lys303Arg mutations were not found in the reports of AI-treated metastases (1–6).

Conclusions and Clinical Significance

Primary tumors invariably have low or no cells with mutation (Table 1), so it seems reasonable to suggest that random mutations are enriched because of the plasticity of surviving cell populations. Adjuvant estrogen deprivation therapy (AI) ensures mutant ERs in metastases survive. Nevertheless, earlier laboratory studies (77) demonstrate that long-term tamoxifen (SERM) therapy changes the biology of the tumor cell without mutations. Acquired antihormone resistance has numerous, possibly independent, survival mechanisms.

In an earlier Commentary (92), two potential pathways to acquired tamoxifen resistance (Figure 3, D and E) were proposed: 1) Elevated coactivator levels would push the equilibrium towards helix 12 closure; this would result in the enhanced estrogenicity of the tamoxifen-ER complex based on the law of mass action. Increased coactivator concentrations enhance ligand affinity for the ER and are associated with constitutive activity of 537 mutants (85,93). 2) An enhanced ER signal transduction pathway based on a ER mutation at Asp351 to facilitate Helix 12 closure (Figure 3E) despite SERM binding.

Both proposed pathways have veracity, but there is now a more complex model of acquired antihormone resistance based on: 1) different natural ligands for the ER causing breast cancer cell growth, 2) selection of cell populations over time, and 3) different forms of resistance based on ER and growth factor crosstalk. Each will be summarized in turn, but the actual role of the current mutations of interest (Table 1) in each phenotype is not apparent at present.

1. The discovery that the cholesterol metabolite 27-hydroxycholesterol (27-OH cholesterol) can activate the ER (94) is important. 27-OH cholesterol (95,96) can stimulate both wild-type and SERM-resistant breast cancer cell growth. Body mass index and hypercholesterolemia are poor risk factors for breast cancer patients (97), so rigorous strategies of weight loss and use of lipid-lowering drugs must be considered during breast cancer treatment with AIs in the obese patients.

2. Acquired antihormone resistance evolves over time into different forms based on cell selection pressure (98). Laboratory studies on the evolution of acquired resistance to tamoxifen have described two forms: phase I acquired resistance that emerges within a year or two that responds to either estrogen or tamoxifen to sustain tumor growth and survival and a “tamoxifen withdrawal response” (55,56) that occurs following cessation of tamoxifen therapy in advanced disease, and either an aromatase inhibitor or the pure antiestrogen fulvestrant are useful as second-line therapies (58,59). The Confirm Trial (99) demonstrated that high-dose (500 mg) fulvestrant therapy can produce a survival advantage over low-dose therapy (250 mg). Fulvestrant could be of value at high doses in AI-resistant breast cancer with ER mutations (4,86).

The continuing transplantation of MCF-7 tamoxifen-stimulated tumors to replicate five years of adjuvant tamoxifen therapy cause phase II acquired resistance when physiologic estrogen causes tumor regression (77,98,100–102). The new biology of estrogen-induced apoptosis (46,100–103) has several clinical applications that have been reviewed recently (104). Additionally, there is the impact of long-term adjuvant antihormone therapy on ER-positive micrometastatic tumor cell selection. Estrogen-induced apoptosis triggered by natural endogenous ligands for ER would explain (101) the statistically significant (P value of .01) decrease in mortality in the decade after 10 years of adjuvant tamoxifen treatment is stopped in the ATLAS trial (8). However, none of these effects of acquired resistance are linked to mutations in the ER.

3. There is a considerable literature on the ability of growth factor signal transduction pathways to subvert the blockade of the ER signal transduction pathway. This has been extensively reviewed (106). The best example is HER2/neu subverting the action of antihormones at the ER, and this is well documented in the laboratory (107,108). However, a change in cell populations, given different selection pressures, is also a key to acquired resistance and has not been linked to mutations in the ER.

Now, a mosaic of mutations is to be laid on top of the evolution of cell selection to antihormone therapy. Much is already known about the molecular mechanics of the ER in the presence of ligands (19,49,109), so the role of the mutations is defined (4,85,86). One task ahead is to decipher the new therapeutic options for tumors with mutant ER. Regrettably, based on clinical experience with late-stage disease, an intervention with a third- or fourth-line therapy will achieve only limited results, even if directed to the ER with laser precision. Very few patients may benefit from this personalized approach, but not for long. Long-term treatment laboratory models and clinical experience with bulky disseminated disease teach us about the relentless plasticity of resistant cell populations that can rapidly ebb and flow with each selection pressure. The conversion of cell populations from one form of resistance to another in just two months (110–112) (Supplementary Figure 3, available online), ie, exactly the time used clinically to judge clinical benefit to therapy (46), illustrates that selection pressure is hard to stop at a late stage of cellular survival sophistication.
Our interdependent translational research community has made a decisive stride with the detection of mutations in metastases. It is clear, however, that the traditional laboratory breast cancer cell lines are inadequate to study the reality of ER mutations of breast cancer. Dozens of new cell lines derived from patients are now required with long-term, ie, years of therapy to decipher tumor options in the laboratory.

The recent advance by Yu and colleagues (5) to isolate and expand ex vivo circulating tumor cells (CTCs) in patients is potentially useful for studying metastatic breast cancer and creating cell lines. In the proof-of-concept study (5), CTCs from six separate samples produced five CTC lines, of which three were tumorigenic in mice (50%). Additionally, the patient-derived xenograft (PDx) provided a 13% engraftment rate from 152 patients, producing 22 transplantable PDx from 20 patients following surgery (1). By contrast, a clinical study of ER expression in CTCs from metastatic breast cancer patients noted a frequent lack of ER expression, even with an ER-positive primary tumor, thus illustrating a considerable intrapatient heterogeneity (113).

The challenge for the future is to increase sensitivity for micrometastatic primary breast cancer to designer antiestrogens after surgery. Appropriate new therapies, based on clues with ER downregulation with raloxifene, bazedoxifene (112,114), the novel SERM GW5638 (115), or a new novel oral pure antiestrogen could be advanced to adjuvant therapy. It would clearly be an advantage to have compounds that are cidal immediately, and bazedoxifene may have promise (114). New orally active ER downregulators that kill cancer may be superior to AIs that increase ER.

It is clear we are discovering important new knowledge about the methods used by a metastasis (not the primary tumor) to kill and end life. However, the infinite options for cell survival and rapid population changes that occur in response to treatment may exceed our skill to close each new survival escape route. New populations will emerge in response to selection pressure based on increasing tumor burden. If tamoxifen taught us but one lesson, it was that applications of an antiestrogenic therapy at the end of life is palliative (116) for a few, but adjuvant therapy (earlier) is somehow different with very low tumor burdens. Huge numbers of lives were saved with the same drug used correctly (8,117). Early therapeutic intervention to target micrometastic cancer cells to be killed must be the goal to ensure patient survival.

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Notes

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