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ESR1 Mutations in Cell-Free DNA of Breast Cancer: Predictive “Tip of the Iceberg”

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In this issue of *JAMA Oncology*, Chandarlapaty and colleagues report a correlative analysis of the two most frequent hormone-binding domain estrogen receptor (ESR1) mutations (Y537S and D538G) in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer using archival tumor specimens from the BOLERO-2 trial to identify correlations between these mutations and efficacy of the mammalian target of rapamycin complex 1 (mTOR1) inhibitor everolimus². The BOLERO-2 trial was a double-blind, placebo-controlled, phase III trial of patients who recurred and/or progressed during therapy with nonsteroidal aromatase inhibitors (AIs), who were randomized to everolimus plus the steroidal AI exemestane or exemestane only, and the trial demonstrated that everolimus plus exemestane substantially improved progression-free survival (PFS)¹. Using next generation sequencing approximately 5% of evaluable samples (the majority analyzed were primary tumors) from patients in BOLERO-2, as compared to 2% of primary tumors from The Cancer Genome Atlas (TCGA) study contained ESR1 mutations⁵. The Chandarlapaty *et al.* study herein reports a higher ESR1 alteration frequency (28%) using digital drop PCR (ddPCR) mutation detection of cell free (cf) DNA for specific ESR1 mutations in these advanced breast cancer patients, and is consistent with the overall reported frequency of ESR1 mutations in about 20% of metastatic breast cancers⁴. The use of sensitive detection methods such as mutation-specific ddPCR has transformed our understanding of the genomic basis of breast tumors, and has revealed the extreme genomic complexity and heterogeneity of metastatic breast cancer. In the BOLERO-2 study, overall survival (OS) was 32 months for patients with wild-type ESR1 vs. 20.73 months with Y537S/D538G mutations. The reported shorter OS for patients harboring ESR1 mutations suggests that they may represent potential poor prognostic factors in breast cancer, however, it must be stressed that without a no-treatment control arm, significant clinical conclusions whether mutations were predictive of everolimus response cannot be determined.

An important finding from the Chandarlapaty study is that the prevalence of these two ESR1 mutations varied between AI treatments in the adjuvant versus the metastatic settings. A three-fold increase in mutation prevalence was found in patients who had failed first-line therapy for metastatic disease as compared those who were initiating first-line treatment for metastatic breast cancer (33% vs. 11%). These data confirm an earlier smaller study showing that ESR1 mutations were rarely acquired during adjuvant AI treatment, but were frequently

(42%) selected for by these drugs in the metastatic setting⁶. Furthermore, serial monitoring of cfDNA during treatment of metastatic disease demonstrated that ESR1 mutations were selected to become the dominant clone, and patients with ESR1 mutations had a substantially shorter PFS with subsequent AI therapy⁶. Mutation tracking of cfDNA can also predict relapse in early breast cancer, and tracking in serial samples increased sensitivity for prediction of clinical relapse earlier in the course of treatment³. However, we also know that metastatic tumors can simultaneously harbor multiple ESR1 mutations in the same or distant lesions, so that incomplete ESR1 mutation testing as reported by Chandarlapaty *et al.*, ie. the lack of other relatively frequent mutations such as K303R and E380Q, may be just the “tip of the iceberg” in predicting therapy response. Clinically it will be critical to determine whether the alternate use of tamoxifen and/or fulvestrant in the metastatic setting will more effectively block the selection and evolution of ESR1 mutations during therapy, and thus avoid such dominant selection pressures.

Patients with wild-type ESR1 in cfDNA analyses also demonstrated only modest responses to everolimus and exemestane, so that other tumor-intrinsic or microenvironmentally-induced hormone resistance mechanisms must co-exist in the BOLERO-2 cohort². We do not yet know whether specific ESR1 mutations employ alternative therapeutic escape mechanisms, such as activation of growth factor receptor signaling or survival pathways. Importantly, selective ESR1 mutations might also impart more aggressive biological attributes. In the Chandarlapaty study, patients with the D538G ESR1 mutation derived significant clinical benefit from everolimus treatment, but patients with the Y537S mutation did not show reductions in PFS². These results suggest that knowledge of the resistance escape mechanisms co-existing with ESR1 mutant expression will need to be identified to accurately guide targeted therapy of metastatic breast cancer. The armamentarium of potential targeted agents useful for the treatment of advanced breast cancer in the metastatic setting has never been larger, and includes histone deacetylase, heat shock protein 90 (hsp90), cyclin-dependent kinases 4 and 6 (CDK4/6), and phosphoinositide-3-kinase (PI3K) inhibitors. The efficacy of newer selective estrogen receptor degraders in combination with these targeted agents in preclinical models harboring ESR1 mutations provides encouragement that these therapeutic approaches will provide meaningful benefit to metastatic patients⁷. The use of sensitive, yet simple mutation monitoring of plasma as described in this study could provide indispensable predictive information for correcting the course of therapy in advanced or perhaps even early breast cancer. We eagerly await an illumination of what is not just evident on the surface, the “tip” of circulating cfDNA, but also what resistance networks lie beneath and drive the bulk of the metastatic ESR1 mutant-positive tumors in unique distant microenvironments.

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