editorial

Cyclin E mRNA: Assessing Cyclin-Dependent Kinase (CDK) Activation State to Elucidate Breast Cancer Resistance to CDK4/6 Inhibitors

Sarat Chandarlapaty, MD, PhD[1] and Pedram Razavi, MD, PhD[1]

The treatment of estrogen receptor (ER)–positive metastatic breast cancer has been dramatically transformed through the introduction of ATP-competitive inhibitors of the cyclin-dependent kinases 4 and 6 (CDK4/6i) in combination with antiestrogen therapies. Seven phase III studies demonstrated clear improvements in progression-free survival through the addition of these agents in either the first- or later-line setting, with new studies underway to evaluate the benefit of these agents as adjuvant therapy.[1-7] A core scientific premise backing these agents is that in a large subset of tumors, ER inhibition is only partially effective at blocking the G1 checkpoint kinases CDK4/6, either because of ineffective ER inhibition (eg, *ESR1* mutations)[8-10] or hormone-independent inputs into CDK4/6 activation (eg, *NF1* loss).[11] The addition of highly selective CDK4/6 kinase inhibitors enables more potent and durable blockade of the cell cycle, which may accrue additional benefits such as inducing tumor cell senescence or augmenting antitumor immunity.

Despite the clear benefit of this combination, a subset of cancers (10% to 20%) remain insensitive, whereas a much larger group of cancers (70% to 80%) become resistant after 12 to 36 months of therapy.[1-7] Even with such great clinical heterogeneity, there remain virtually no biomarkers to separate these subgroups of patients. This is particularly poignant given the potential efficacy of alternative forms of therapy in ER-positive metastatic breast cancer, including mammalian target of rapamycin inhibition (everolimus)[12] as well as chemotherapy. In the companion article, Turner et al[13] use pretreatment tumor samples from the PALOMA-3 (Palbociclib [PD-0332991] Combined With Fulvestrant In Hormone Receptor+ HER2-Negative Metastatic Breast Cancer After Endocrine Failure) trial of fulvestrant versus fulvestrant plus palbociclib to investigate biomarkers that may identify tumors that are de novo resistant to the fulvestrant plus palbociclib combination and, specifically, find high levels of cyclin E1 (*CCNE1*) associated with an attenuated benefit.

As a backdrop to this investigation, several groups have used preclinical models to help nominate potential genomic alterations that may promote resistance to CDK4/6 inhibitors in the clinic. The work, depicted in Fig 1, has identified: (1) mechanisms that bypass the requirement for G1 checkpoint kinases to phosphorylate Rb and release E2F, and (2) mechanisms that hyperactivate the G1 checkpoint kinases and thereby render the drugs insufficiently potent. With respect to the former, loss of *RB1* expression has been noted in subsets of models and patient samples and ably causes drug resistance.[14-17] However, this event is rare enough in ER-positive breast cancer[11,18-21] that preemptive exclusion of patients with *RB1* loss was largely abandoned in the clinical trials in ER-positive breast cancer, to no apparent detriment.

Apart from *RB1* loss, a number of different mechanisms (Fig 1) have been identified that result in restored Rb phosphorylation, such as amplification of *CDK6*,[22] hyperactivation of growth factor signaling,[23] and aberrant activation of CCNE1-CDK2 downstream of CDK4/6.[23-25] Among these, it is notable that *CCNE1* is not only a potential mediator of resistance. In addition, *CCNE1* overexpression may prove to be a sensor of other mechanisms of resistance. This results in a constitutively bypassed G1 checkpoint because *CCNE1* transcription is induced by E2F itself. In their study, Turner et al[13] attempted to identify transcripts that may reflect these different states of resistance and highlight the finding of high levels of *CCNE1* being associated with reduced response to palbociclib.

To identify potential biomarkers that predicted intrinsic resistance to the combination of palbociclib and fulvestrant in the PALOMA-3 clinical trial,[1] Turner et al[13] made use of 194 samples (92 of 194 metastatic samples) from the combination arm and 108 samples (50 of 108 metastatic samples) from the fulvestrant-only arm. These samples were subjected to a well-validated and relatively small-panel mRNA profiling platform (EdgeSeq Oncology platform; 2,534 genes; HTG Molecular Diagnostics, Inc., Tucson, AZ). The effect of 10 genes relevant to the G1 checkpoint were primarily examined. In this analysis, *CCNE1* mRNA levels correlated with the degree of benefit. Tumors with low levels of *CCNE1* showed marked improvement with the addition of palbociclib (median progression-free survival, 14.1 months in the palbociclib arm *v* 4.8 months in the placebo arm). Tumors with high levels of *CCNE1* had an attenuated benefit (median progression-free survival, 7.6 months in the palbociclib arm *v* 4.0 months in the placebo arm). Curiously, these

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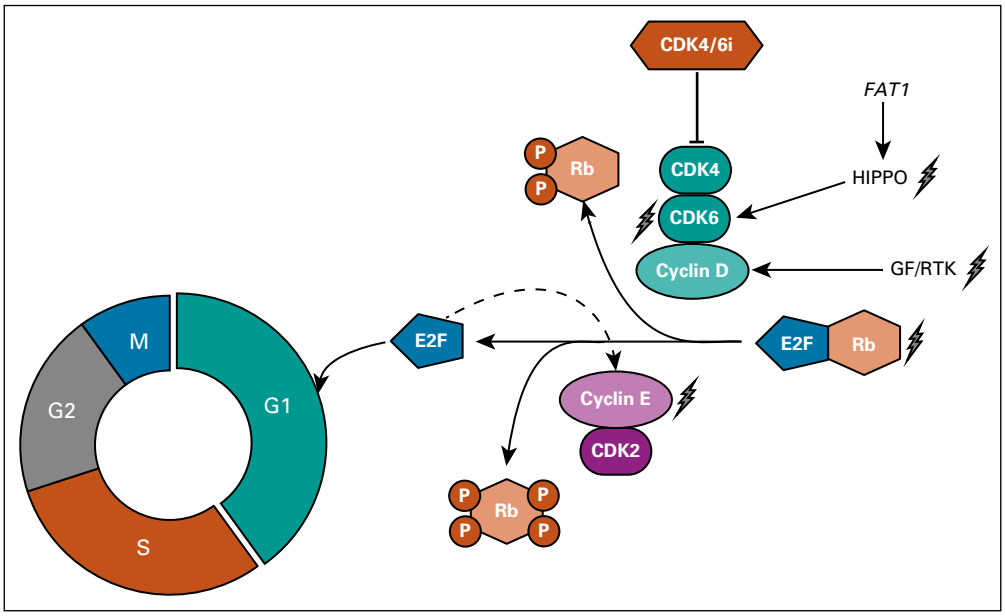

FIG 1. Putative mechanisms of resistance to CDK4/6i. Cartoon shows G1 checkpoint kinases CDK4/6 coupled to D-cyclins promoting cell cycle progression through the G1-S transition by phosphorylating Rb and thereby inducing release of E2F. E2F further supports Cyclin E (CCNE1) expression by increasing Rb phosphorylation and thereby accelerating progression. Potential mechanisms of drug resistance are denoted by lightning symbol with hyperactivation of CDK4/6 activity, loss of Rb, or hyperactivation of CCNE1-CDK2 representing potential mechanisms. CDK, cyclin-dependent kinase; GF, growth factor; P, phosphorylation; RTK, receptor tyrosine kinase.

effects were mainly evident when metastatic tumor samples were used, suggesting that tumor samples collected temporally closer to the start of therapy may more accurately represent the genomic landscape of the disease than archival samples. The other nine target genes examined, including *CDK2* and *RB1*, did not show a significant difference by expression. Importantly, the authors attempted to validate the finding of *CCNE1* mRNA and response in an independent cohort of patients with breast cancer from the Preoperative Palbociclib (POP) adjuvant trial. They found that high *CCNE1* levels were associated with impaired reduction in tumor proliferation (Ki67) by palbociclib treatment. Finally, the authors performed an unbiased screening analysis across the 2,534 genes included in the assay. Interestingly, they found that high levels of p18 (*CDKN2C*) and p19 (*CDKN2D*; both of which are endogenous inhibitors of CDK4/6) were also associated with reduced response to palbociclib.

The work is commendable on multiple levels. First, the trial successfully collected metastatic tissues on a large proportion of patients enrolled in a multicenter phase III trial and then collaborated to validate their key finding in another trial. Second, the search for biomarkers of response used both candidate and unbiased methods, enabling a robust finding of *CCNE1*. Third, the validation of *CCNE1* ties together the biology of CCNE1-CDK2 as an alternative means to Rb phosphorylation and the dynamic activation of *CCNE1* transcription in response to E2F activity. Finally, the work helps bring to the fore a biomarker for a candidate set of tumors for CDK2-selective inhibitors currently in development.

There are a few important cautions and questions for future work, which are naturally raised by the study. First and critically, a top-level statement must be made that even patients with high levels of *CCNE1* derived benefit from the addition of palbociclib to fulvestrant. This may be as the result of many reasons. These include heterogeneity and time required for selection of resistant subclones under the pressure of drug therapy, spatial tumor heterogeneity resulting in incomplete representation of disease status in the biopsied tumor tissue, intrinsic deficiencies of the assay and cut points used, among others. For instance, *CCNE1* mRNA may only partially reflect the true state of CCNE1-CDK2 activity such that other factors (eg, CCNE1 isoforms, localization, and even proteostasis) may be of significance.[26-29] Irrespective of the intriguing results reported by Turner et al,[13] physicians should continue their current practice of adding CDK4/6i to endocrine therapy without reference to any biomarkers.

A second point raised is the lack of accounting for what caused reduced response in patients who derived little benefit but did not have high levels of *CCNE1*. To this point, a single, steady state look at mRNA may prove an inadequate reflection of the underlying biology of the tumor and potential resilience. Perhaps an augmentation to these data will come through the use of provocative biomarkers such as the response of transcripts, circulating tumor DNA, or proteins to a challenge of CDK4/6i exposure. Moreover, integration with less dynamic measures such as DNA mutations (eg, *RB1*) may further help to provide a composite marker of nonresponders.[30] Finally, although studying a panel of 2,534 genes at a single time point is far from trivial, there are a number of other potential inputs into cancer cell growth control. These include unexpected players such as the *FAT1*/Hippo pathway, which was recently identified as a mediator of CDK4/6i resistance.[30] Therefore, ongoing efforts to survey broadly are needed.

We laud Turner et al[13] for conducting this outstanding study of biomarkers of response to CDK4/6i in metastatic breast cancer. The work strongly validates the investigators' prespecified collection of tissues for understanding biology and developing future biomarkers. It nominates *CCNE1* as a specific transcript that, when elevated, seems to predict a reduced response to CDK4/6i.

AFFILIATION

[1]Memorial Sloan Kettering Cancer Center, New York, NY

CORRESPONDING AUTHOR

Sarat Chandarlapaty, MD, PhD, Memorial Sloan Kettering Cancer Center, Medicine, 1275 York Ave, New York, NY 10065; e-mail: chandars@mskcc.org.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Sarat Chandarlapaty
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Pedram Razavi
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