CDK4/6 Inhibitor Biomarker Research: Are We Barking Up the Wrong Tree?

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SUMMARY

CDK4/6 inhibitors have emerged as a significant advance for the treatment of patients with advanced estrogen receptor–positive breast cancer. However, the identification of predictive markers that optimize their use is proving harder than expected. In this commentary we advocate for unbiased discovery and a collaborative approach across trials.

See related article by Finn et al., p. 110

In this issue of *Clinical Cancer Research*, Finn and colleagues describe an analysis of tissue samples from patients enrolled into the PALOMA2 trial, a randomized letrozole/placebo or letrozole/palbociclib first-line treatment study that demonstrated that the addition of palbociclib to letrozole improved median progression-free survival (PFS) by almost 13 months ($P < 0.0001$) in treatment-naïve postmenopausal patients (1). The results from this analysis amply demonstrate the methodologic difficulties of CDK4/6 predictive marker development. Inhibitors of CDK4/6, palbociclib, ribociclib, and abemaciclib, have rapidly established a central role in the management of estrogen receptor (ER)-positive/HER2$^-$ advanced breast cancer (2). The most mature data from trials in the first- and second-line setting, recently presented at the ESMO2019 conference confirmed overall survival (OS) benefits, which will drive guidelines to recommend that these agents are broadly prescribed to patients with advanced ER$^+$ disease (3, 4). Adjuvant trials (PALLAS- NCT02513394, NATALEE - NCT03701334) also underway and it seems likely that these drugs will eventually be used for patients with ER$^+$HER2$^-$ breast cancer at high risk of relapse. CDK4/6 inhibitors are not a panacea, however, with the consequences of chronic neutropenia, QTC prolongation, and diarrhea, as well as other less common side effects in otherwise healthy women are not fully understood. Issues for the clinical application of CDK4/6 inhibitors in the adjuvant setting are the high cost of therapy and the lack of predictive biomarkers that could be used to improve the cost and risk to benefit ratios.

From the PALOMA2 trial, tissues samples from 666 postmenopausal patients with treatment-naïve advanced breast cancer were collected. The tissue samples either belonged to primary or metastatic biopsies and the origin was not recorded, which is a limitation of the study. Using a candidate approach, a variety of RNA and protein-based biomarkers were explored in formalin-fixed tissue samples accrued from study participants with appropriate efforts to control for multiple testing. Biomarkers included genomic loss of the CDK4/6 inhibitor p16, Cyclin D1 amplification, or loss of the target for CDK4/6 action, Rb. None of these biomarkers showed clear promise, except for the rare tumors with complete loss of Rb, which were, as expected, resistant. An RNA level analysis of same targets with inclusion of CDK4/6 also did not produce a clear pattern that associated with palbociclib outcomes, although on the letrozole arm, high levels of CDK4 mRNA (but not CDK6 mRNA) was associated with more rapid progression. The Ki67 proliferation biomarker and gene expression–based surrogates for Luminal A versus B status were also unrevealing. Because these *a priori* hypotheses did not produce a clear result, the rest of the genes identified in the RNA profiling data were explored to produce biomarker candidates for further consideration. This allowed the authors to make some interesting observations regarding the possibility that the PD1 signaling pathway possibly associated with reduced PFS benefit from the addition of palbociclib to letrozole. In contrast, tumors with more active growth factor signaling, perhaps most associated with FGFR2 and ERBB3 mRNA expression, were potentially associated with greater PFS gain. The tests for interaction with treatment were not significant and the lack of an independent validation set limited conclusions.

While the study by Finn and colleagues does not identify new biomarkers for CDK4/6 response, the results highlight some interesting observations worth further consideration. There have been previous studies concerning the role of pRB, RB1 mutations, CCDN1 amplifications, and CCNE1 overexpression as potential biomarkers for CDK4/6 response (5, 6), but these investigations have also not proven significant in the clinical setting. Thus, it would seem the field has been "barking up the wrong tree" and progress would be best served from less biased RNA and DNA analyses so that iterative cycles of discovery and testing could be conducted across CDK4/6 inhibitor trials. This might define entirely new molecular features of tumors where intervention with CDK4/6 inhibition is particularly warranted or futile. Obviously, this would take an unprecedented level of cooperation between pharmaceutical companies but is an entirely necessary step if we are to fully understand the therapeutic window for CDK4/6-based interventions. Indeed, recent evidence in the field derived from DNA and RNA analysis suggests that defects in single-strand break repair in ER$^+$ breast cancer can drive endocrine therapy resistance (**Fig. 1**; refs. 7, 8). The cryptic link between these two processes centers on the DNA damage sensor ATM which, when active, promotes CHK2 activity (8). CDK2 phosphorylates CDC25A at S123, triggering degradation. Without CDC25A, a phosphatase, CDK4/6 is held in an "off state," as inhibitory sites of phosphorylation on CDK4/6 are not removed. Thus CDK4/6 inhibitors are pharmaceutical mimics of the ATM/CHK2/CDC25A pathway. Haricharan and colleagues recently demonstrated that both ATM and CHK2 are required for the efficacy

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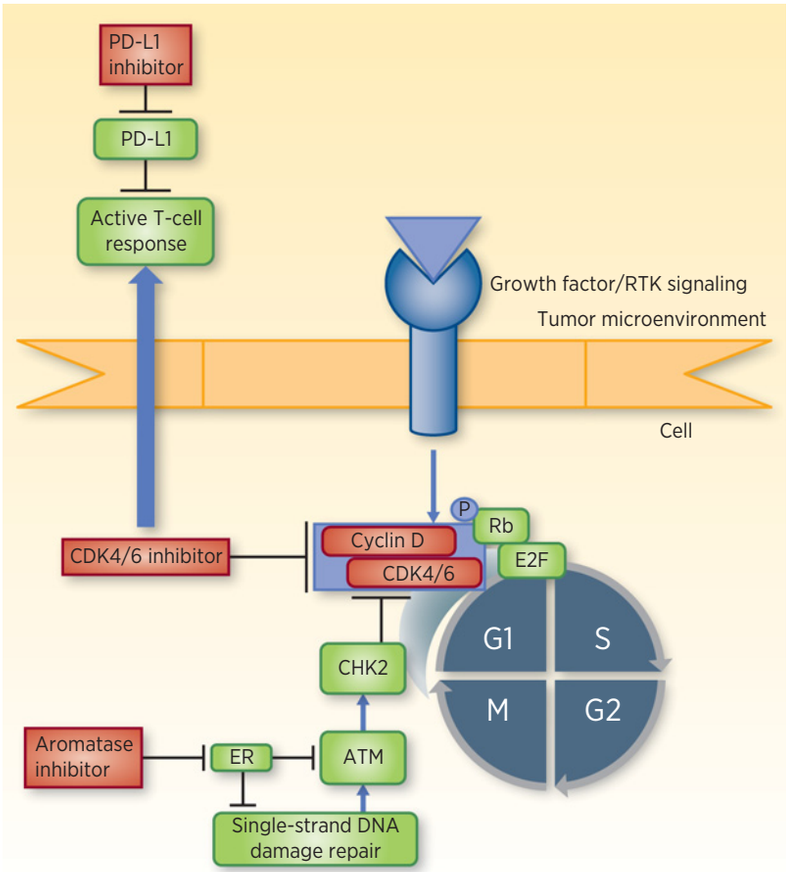

Figure 1.
CDK4/6 inhibitors targeting the CDK4/6 complex of the cell cycle may be most effective in a setting of single-strand break defects because of the interconnectedness of ER, CHK2, and CDK4/6 signaling. Similarly, a role for combinatorial therapeutics involving CDK4/6 inhibitors and immunotherapy is warranted. Further efforts are required to uncover other predictive markers for CDK4/6 inhibitor efficacy.

of endocrine agents as inactivation of either of these negative cell-cycle regulators prevents cell-cycle arrest upon ER inhibition. Interestingly CHK1 knockdown or drug inhibition had no effect in model systems, showing the specificity of this pathway in ER^+ cells. Importantly, in ER^+ breast cancer cells with a single-strand break repair defect, for example mismatch repair, nucleotide excision repair, or base excision repair, ATM and CHK2 are suppressed allowing proliferation to occur despite accumulated DNA damage. This suppression, or lack of robust activation, of ATM/CHK2 effectively leads to "cross-over" endocrine therapy resistance, due to codependency on the ATM/CHK2/CDC25A for negative cell-cycle regulation triggered by endocrine treatment. By addressing a target downstream of ATM and CHK2, CDK4/6 inhibition could therefore be theoretically most effective in ER^+ cells with a defective single-strand break repair (7, 8). Unfortunately, massively increased mutational repertoires in cancer cells with single-stand break repair defects could also mean therapeutic effects are short-lived because of accelerated accumulation of clones encoded with CDK4/6 resistance–driving mutations.

Interestingly Goel and colleagues have shown that CDK4/6 inhibitors may increase tumor immunogenicity (9), potentially by reducing activity of the E2F target, DNA methyltransferase 1. This provides a rationale for combination regimens comprising CDK4/6 inhibitors and immunotherapies and there are several ongoing trials testing combinations of CDK4/6 inhibitors with immunotherapy, including pembrolizumab and avelumab (e.g., NCT02778685; NCT02779751; and NCT03147287). The fact that Finn and colleagues observed an association between PD1 expression and PD1 "signaling," and reduced PFS benefit supports a link between DNA repair defects, immune evasion, and endocrine therapy resistance. Unlike ER^- disease, where PD1 activity is associated with favorable outcome in patients treated with chemotherapy, the opposite seems true in the setting of ER^+ patients treated with CDK4/6 and endocrine therapy. Perhaps CDK4/6 inhibition does not have the proimmune rejection properties of chemotherapy after all, or alternatively the immune microenvironment of ER^+ disease has different modulators in play. It is safe to say that the immunologic response to ER^+ breast cancer remains remarkably understudied. It would be worthwhile for the investigators to follow-up their findings with a tumor-infiltrating lymphocyte analyses, as well as IHC analysis of various immune evasion mechanisms.

In conclusion, while PFS and OS is being thankfully improved with the use of CDK4/6 inhibitors, there is no suggestion, as yet, that this is a curative approach or that we have a good biomarker to direct therapy (other than the presence of ER expression). In the search for therapeutic options in a lethal disease in a randomized clinical trial setting, an awareness of the importance of collection/donation of tumor samples has to be created among patient advocates and care providers. This generates the required thrust for unbiased analysis of multi-omics data deposited in public space for collaborative bioinformatics analysis.

Disclosure of Potential Conflicts of Interest

M.J. Ellis has intellectual property ownership and received royalties for the PAM50-based breast cancer test "Prosigna," is a paid consultant for Bioclassifier LLC, Pfizer, AstraZeneca, Novartis, StemCentrix, and Abbvie, has ownership interest in Bioclassifier LLC, and is an unpaid consultant/advisory board member for GI Therapeutics and Lilly. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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