Peer Review File

Article information: https://dx.doi.org/10.21037/tbcr-21-36

Reviewer Comments

The paper titled "Overview of the clinical development of CDK4/6 inhibitor abemaciclib in breast cancer" is interesting. The review, we focus on the results of preclinical and clinical studies of abemaciclib, describing current indications for treatment, ongoing clinical trials, safety and tolerability, and future perspectives. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: What is the resistant mechanisms of abemaciclib? How to find useful biomarkers to predict the efficiency of the CDK4/6 inhibitors? It is recommended to add relevant content.

Reply 1: Thank you for your comment. Abemaciclib, one CDK4/6 inhibitor, has shown great effects in improving prognosis of HR+/HER2- breast cancer patients. However, not everyone is responsive to the treatment. There are mainly two kinds of mechanisms, one is cell-cycle-specific mechanisms, such as loss of RB, CDK4/6 amplification, CCNE1/2 amplification, CCND1 amplification and/or loss of p16. The other one is cell-cycle-nonspecific mechanisms, such as PIK3CA pathway activation; mouse double minute 2 homolog (MDM2) overexpression, FGFR pathway activation, or ESR1 expression and mutation. Great efforts have been made to identify the mechanism of resistance to CDK4/6 inhibitors, however, no clear mechanism of resistance is sufficiently validated.

Several attempts have been made to find the biomarkers for response prediction through tissue biopsy or liquid biopsy. The circulating biomarkers, including circulating tumor DNA (ctDNA) and RNA (ctRNA), microRNA (miRNA), exosomes, proteins, metabolites and circulating tumor cells (CTCs), are currently being investigated for their potential to identify patients with primary resistance, monitor the effects of treatments. But for now, no clear biomarker is identified.

Comment 2: There have been many studies on breast cancer. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction. It is recommended to add relevant content.

Reply 2: We really appreciate your comment. I assumed the study you mentioned in "the difference between this study and previous studies" above means MONARCH plus. The study was designed to kill two birds with one stone. MONARCH plus trial has two treatment arms, not only as initial treatment for advanced breast cancer patients in combination with AIs, but also in combination with fulvestrant for breast cancer patients who had progressed on prior ET.

Comment 3: What are the determinants of whether to continue using CDK4/6 inhibitors after the disease progression? It is recommended to add relevant content.

Reply 3: Thank you for your comment. This is very inspiring. Given the widespread

use of CDK4/6 inhibitors in the large HR+ MBC population, some patients may benefit from continued CDK4/6-directed therapy. Some studies shown that a part of patients still could benefit from abemaciclib after progression on palbociclib or ribociclib. Apart from this, several studies are ongoing to investigate the treatment mode to change another endocrine therapy other than CDK4/6 inhibitors. However, whether CDK4/6 inhibitor treatment should be continued post progression of disease remains to be defined.

Comment 4: What is the benefit and the risk of CDK4/6 inhibitors plus endocrine therapy for endocrine-sensitive or endocrine-resistant population in metastatic HR+/HER2- breast cancer? Please add relevant content.
Reply 4: Thank you for your comment. This review focus on the clinical development of abemaciclib in breast cancer. In terms of benefit, the combination of CDK4/6 inhibitors and endocrine therapy (ET) significantly improved the prognosis of HR+/HER2- advanced breast cancer patients. CDK4/6 inhibitor, such as abemaciclib, plus ET provided efficacy benefit for both endocrine-sensitive and endocrine-resistant metastatic HR+/HER2- breast cancer patients. We have included the related content in the efficacy part of MONARCH 2 (page 12, line 194-198), MONARCH 3 (page 13, line 217-222) and MONARCH plus (page 16-17, line 285-294) studies.

In terms of risk, the addition of CDK4/6 inhibitor to ET had treatment related adverse events (TEAEs), such as neutropenia, diarrhea and so on.

Comment 5: What is the current use of and potential next directions for CDK4/6 inhibitors in the treatment of patients with HR+/HER2- breast cancer? It is recommended to add relevant content.
Reply 5: The combination of CDK4/6 inhibitors and endocrine therapy has been the standard of care for HR+/HER2- advanced breast cancer patients. And abemaciclib recently got approval for use in HR+/HER2- high-risk early breast cancer patients globally, including China. Abemaciclib is the only CDK4/6 inhibitor approved in EBC for now. Although CDK4/6 inhibitors have rendered greater clinical benefits, patients tend to develop resistance to these drugs. Therefore, how to identify the patients who could benefit from CDK4/6 inhibitors and the mechanism that lead to resistance to CDK4/6 inhibitors is urgently needed. These investigations will shed light on future opportunities for CDK4/6 inhibitors, such as combination with other target therapy, or replace one CDK4/6 inhibitor to another CDK4/6 inhibitor in treatment of HR+/HER2- breast cancer.

Comment 6: How to evaluate the role of neoadjuvant chemotherapy and other prognostic factors? What impact will it have on the prognosis of women who without pathologic complete remission? It is recommended to add relevant content.
Reply 6: Thank you for your comment. This review focus on the preclinical and clinical development of abemaciclib in breast cancer. We involved no information on neoadjuvant chemotherapy or prognostic factors in this manuscript. And we think it will not be suitable for adding this content to this manuscript.

Comment 7: What is the pharmacology and clinical activity in therapy of the CDK4/6 inhibitors? Compared with other inhibitors, what is the biggest advantage of abemaciclib? It is recommended to add relevant content.

Reply 7: As mentioned in comment 4, this review focus on the clinical development of abemaciclib in breast cancer. And we included few detailed information on the other CDK4/6 inhibitors. Abemaciclib is a selective CDK4/6 inhibitor that dephosphorylates RB, resulting in a block of cell-cycle progression from G1 to S phase, preventing proliferation of cancer cells. Abemaciclib is more potent (14 times) to CDK4 than CDK6, suggesting that abemaciclib could strongly inhibit the proliferation of breast cancer cells with lower bone marrow toxicity, which permitting a continuous dosing to anti-tumor continuously.