Chemotherapy modulates CDK4/6 inhibitors resistance in metastatic breast cancer by Rb1 mutations: a case report and literature review

Lu Gao[1#], Xiabo Shen[2,3#], Libin He[1], Jiayi Wu[1], Yiyuan Liu[1], Xiaojia Wang[2,3], Xiying Shao[1,2,3]

[1]The Second Affiliated College of Zhejiang Chinese Medical University, Hangzhou, China; [2]Department of Breast Medical Oncology, Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou, China; [3]Institute of Cancer and Basic Medicine (IBMC), Chinese Academy of Sciences, Hangzhou, China

[#]These authors contributed equally to this work.

Correspondence to: Xiying Shao. Department of Breast Medical Oncology, Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou 310022, China. Email: shaoxy@zjcc.org.cn.

Abstract: Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) plays a major role in breast cancer therapeutics acting through preventing the cell cycle from G1 to the S phase. Recently, Endocrine therapy combined with CDK4/6i represented a major milestone in hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer treatment. However, the resistance of CDK4/6i is clinically common, and the mechanism remains to be clarified. Retinoblastoma (Rb) is a negative regulator of cell cycle. It inhibits cell cycle transition by binding to E2F transcription factors, and prevent cells division in this way. Rb is regulated by phosphorylation. The CDK4/6i have been shown to affect cancer by blocking phosphorylation of Rb. In addition, decreasing estrogen signal has been confirmed to reduce cyclin D-CDK4/6 complexing. Currently, FCN-437c is a new CDK4/6i that is in clinical trials. Here, we present the case of an HR-positive and HER2-negative patient whose disease continued to rapidly progress after receiving FCN-437c. To determine why, we did a series of examinations and found that her Rb1 had mutated after using CDK4/6i. To our surprise, the Rb1 mutations recovered after treatment with eribulin, and CDK4/6i was shown to exert a renewed effect. In this way, a hypothesis was made that eribulin may influence the pathway of cyclin D– CDK4/6– Rb– E2F by effecting in Rb. This case provides new insights into strategies for CDK4/6i therapy resistance options and shows the significance of next-generation sequencing in the clinic.

Keywords: Retinoblastoma (Rb); CDK4/6 inhibitors resistance (CDK4/6i resistance); next-generation sequencing; metastatic breast cancer (MBC); case report

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Introduction

Breast cancer is one of the most common causes of cancer death in women (1). Approximately 75% of breast cancer cases are hormone receptor (HR)-positive breast cancer. Hormone therapy is the most important method for ER+ breast cancer. However, primary or acquired resistance to hormone therapy has inevitably developed. Some 30% of HR+ metastatic breast cancer (MBC) patients show primary resistance, and most of them are initially sensitive to hormonal therapy yet ultimately display resistance (2). In recent years, CDK4/6 inhibitors (CDK4/6i) combined with endocrine therapy have become one of the main treatments for ER-positive, human epidermal growth factor receptor 2 (HER2)-negative MBC (3).

Retinoblastoma (Rb) is a kind of tumor suppressor, which can regulate the cell cycle (4). There is a pathway

known as cyclin D-CDK4/6-Rb-E2F that controls the cell cycle progression (5). Specifically, Rb is regulated by phosphorylation. The activation of the cyclin D-CDK4/6 complex promotes the phosphorylation of Rb, which promotes Rb to bind to the transcription factor E2F and represses transcription (6). And the cells division is prevented in this way.

The main effects of CDK4/6i are exerted through blocking phosphorylation of Rb to prevent the cell cycle from transforming from G1 to the S phase. In this way, though Rb mutation is rare found in HR-positive and HER2-negative patients, Rb is still indispensable in CDK4/6i as a bridge. In addition, CDK4/6i can induce cell senescence, influence cellular metabolism, and alter the tumor microenvironment (TME) (6). There are 3 oral CDK4/6is including ribociclib, palbociclib, and abemaciclib which have been confirmed as able to improve the progression-free survival (PFS) of patients with ER-positive and HER2-negative breast cancer. Nevertheless, CDK4/6i resistance remains a major clinical challenge. Thus, there is an urgent need to clarify the system of CDK4/6i resistance and explore a new CDK4/6i with fewer side effects.

As a second-generation CDK4/6i, FCN-437c is potent and selective to cellular proliferation. In 2017, the Food and Drug Administration (FDA) approved the use of FCN-437c in clinical trials. In past years, the effectiveness and safety of FCN-437c has been preliminarily confirmed. Similar to ribociclib and palbociclib, 60% of the patients showed stable disease (SD) when they received FCN-437c as monotherapy. This suggested that the antitumor activity of FCN-437c might be improved when combined with endocrine therapy (7). Currently, there are several clinical trials underway for further research.

In this report, we present a case of a patient with HR-positive, HER2-negative advanced breast cancer (ABC), whose disease still progressed after receiving FCN-437c due to loss of Rb1. After treatment with eribulin, her Rb1 was recovered and CDK4/6i reactivated. Based on this case, we conjectured that eribulin may overcome CDK4/6i resistance through Rb. This may provide new insights into strategies for CDK4/6i therapy resistance options in the clinic.

We present the following article in accordance with the CARE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-52/rc).

Case presentation

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal. The patient's management is described in *Figure 1*. A 65-year-old woman had found a lump in her left breast 2 weeks prior to presenting at the hospital for treatment. Her positron emission tomography/computed tomography (PET/CT) on 12 February 2019 showed an irregular soft-tissue nodule in the upper inner quadrant of the left breast, multiple nodal metastases (in the left axilla, left supraclavicular, right pelvic wall, right iliac fossa, bilateral carotid-artery sheaths, bilateral hilar regions, and the mediastinum), and bone metastases (in the right proximal femur and right femoral neck). For diagnostic purposes, the patient was biopsied on 14 February 2019, the pathology of which revealed invasive ductal carcinoma (IDC; from the mass on her left breast) and cancer metastasis (from the axillary lymph node). The immunohistochemistry (IHC) report revealed ER (+++, 80%), PR (+, 55%), AR (+, 90%), C-erbB-2 (–), Ki-67 (+, about 30%), CK5/6 (–), E-cadherin (+), GATA-3 (+), and P120 (membrane+). Later, a CT and magnetic resonance imaging (MRI) on 24 February 2019 showed that an irregular nodule in the upper quadrant of the left breast was matched with breast cancer (BI-RADs: 5, tumor size 4.5 cm × 1.9 cm), and the nodal metastasis in the left axilla. The disease stage was cT2N3M1, stage IV.

In this case, from 25 February to 21 May, 2019, the patient underwent first-line chemotherapy with "Nab-paclitaxel 200 mg d1, 8" for 5 cycles. After that, she sequenced maintenance endocrine therapy with fulvestrant 500 mg q4w for 5 circles until 2 September 2019. However, on 29 September 2019, the results of CT showed progressive disease (PD) had occurred in the lymph nodes left axilla and left supraclavicular region, producing a PFS of 7.1 months. The patient refused intravenous chemotherapy for an undisclosed reason. Since 30 September 2019, the patient received second-line chemotherapy consisting of "capecitabine 1.5 g bid d1–14". From 30 September 2019 to 14 February 2020, she continued endocrine therapy with fulvestrant 500 mg q4w for 6–11 cycles. Unfortunately, PD was detected on 12 March 2020, with a PFS of 5.4 months.

Later, she participated in a clinical trial (NCT04488107) of FCN-437c, a new CDK4/6 inhibitor. She started taking FCN-437c 300 mg qd from 27 March 2020. However, CT revealed PD on 28 May 2020 (*Figure 2*). The PFS was

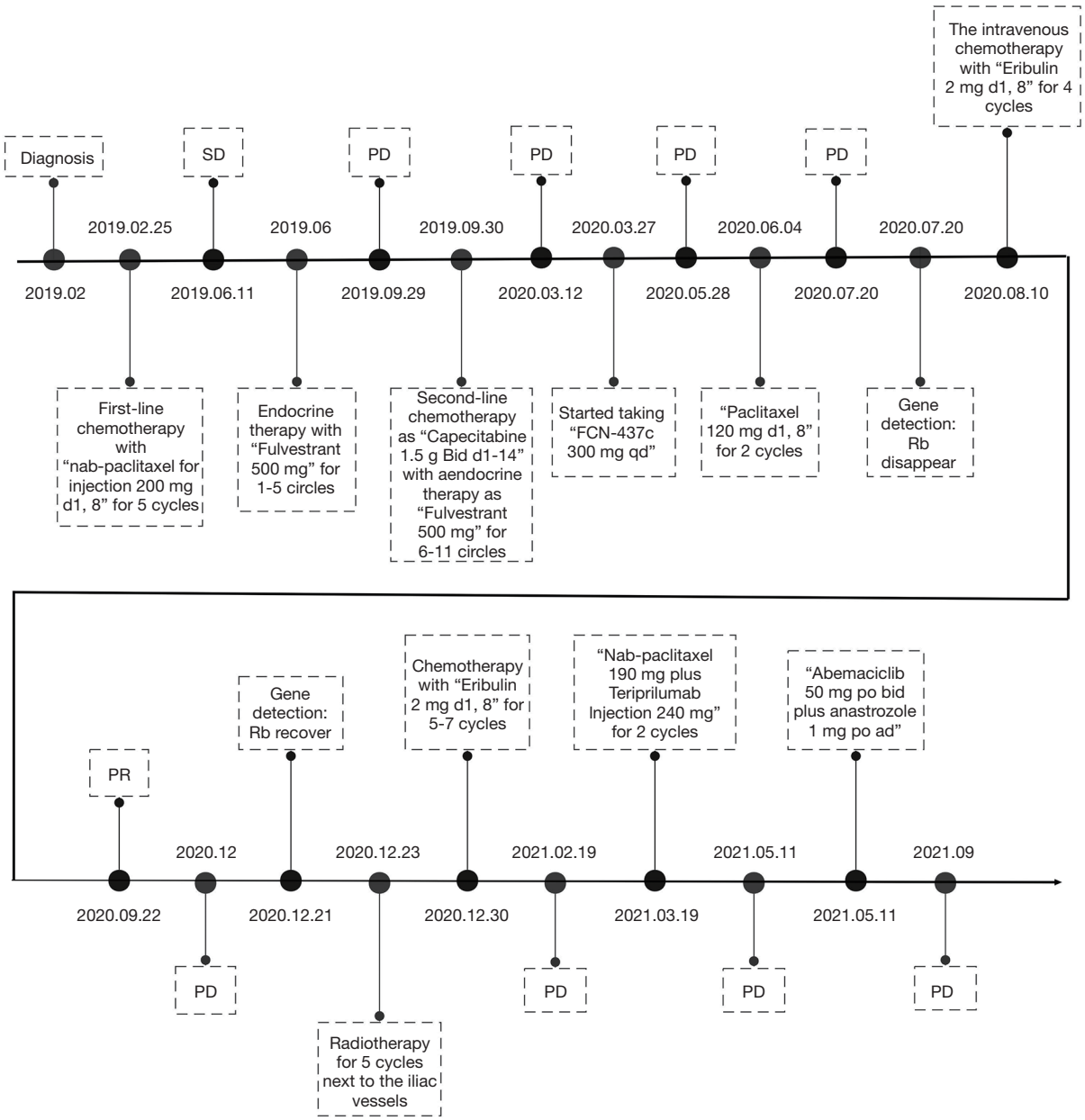

Figure 1 Timeline of the patient management. SD, stable disease; PD, progressive disease; PR, partial response.

only 2 months. Then, she started new-term chemotherapy of paclitaxel 120 mg d1, 8 for 2 cycles. Unfortunately, the CT examination indicated that PD had persisted in lymph nodes on 20 July 2020. In July 2020, the patient underwent an ultrasound-guided biopsy in the lymph nodes of the left supraclavicular region and the left axilla. The IHC confirmed ER (–), PR (–), Her2 (BC, 0), Ki-67 (+, 60%), and GATA-3 (+), hMAM (–). Additional findings included ER (+++, 80%), PR (–), AR (+++, 80%), Her2 (BC, 0), Ki-67 (+, 60%), EGFR (+), and CK5/6 (–).

In order to find the cause of FCN-437c resistance and determine a more suitable treatment for the patient, we conducted Foundation One CDx detection of the lymph nodes. Genomic profiling of the lymph nodes of the left supraclavicular specimen showed a high tumor mutation burden (TMB) level and 9 reportable mutations (*Table 1*). Excessive activation of the PI3K-AKT1 and FGF-CCND1 signaling pathways were also detected. It is worth noting

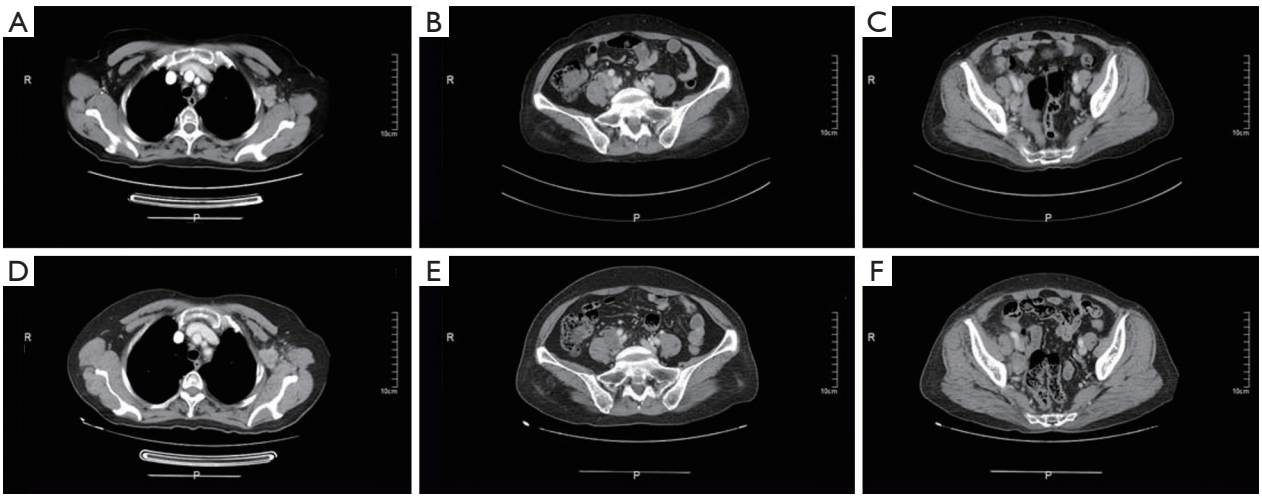

Figure 2 Before FCN-437c treatment (A-C); after FCN-437c treatment disease progressed (D-F).

Table 1 Variant table before eribulin

Gene	Protein	Nucleotide	MAF	Copy number	Exon ratio	CNV type
ATRX	S79*	236C>G	55.87	–	–	–
ARID1A	G933fs*3	2796_2797insA	49.17	–	–	–
RB1	S397*	1190C>A	48.24	–	–	–
AKT1	E17K	49G>A	33.60	–	–	–
PIK3CA	H1047R	3140A>G	34.43	–	–	–
CCND1	–	–	–	8	5 of 5	Amplification
FGF19	–	–	–	8	3 of 3	Amplification
FGF4	–	–	–	8	3 of 3	Amplification
FGF3	–	–	–	8	3 of 3	Amplification
TMB			18 muts/Mb			

*, stop codon. MAF, minor allele frequency; CNV, copy number variations; TMB, tumor mutation burden.

that Rb1 inactivation in the left supraclavicular specimen was found.

After that, the patient consented to chemotherapy with eribulin 2 mg d1, 8 for 4 cycles from 10 August 2020 to 12 October 2020. Follow-up synthetic (S)CT on 22 September 2020 demonstrated the lymph nodes of the left axilla had been reduced. However, the patient withdrew by herself because of her intolerance. Therefore, the disease progressed again according to the results of CT and MRI on 21 December 2020. This period produced a PFS of 4.3 months (*Figure 3*).

On 21 December 2020, the patient underwent biopsy in the lymph nodes of the right iliac vessel, and IHC confirmed the origin from breast cancer for the biopsied tissues and staining of ER (++, 30%), PR (+, 20%), AR (+++, 80%), Her2 (BC, 1+), Ki-67 (+, 30%), SOX10 (–), GATA-3 (+), hMAM (–), Muc-1 (+), Sy (+), CgA (–), and CD5/6 (–). Genomic profiling of the lymph nodes of the right iliac vessel specimen showed a high TMB level and 16 reportable mutations (*Table 2*). More genetic changes appeared after disease progression and multi-line therapy. What piqued our attention was that Rb1 inactivating mutation had not been found in this specimen.

At this time, she underwent radiotherapy for 5 cycles for the iliac vessel lymph nodes from 23 to 28 December 2020. She also underwent chemotherapy with eribulin

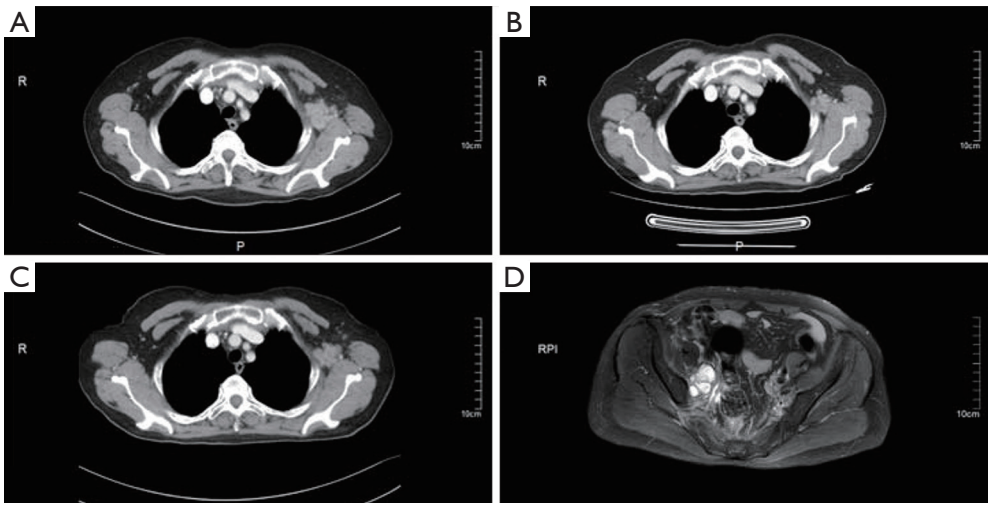

Figure 3 Before eribulin treatment (A); after 2 cycles eribulin treatment (B); after eribulin treatment disease progressed (C,D).

Table 2 Variant table after eribulin

Gene	Protein	Nucleotide	MAF	Copy number	Exon ratio	CNV type
MAP2K1	C121S	361T>A	4.79	–	–	–
TET2	R1516*	4546C>T	1.40	–	–	–
SGK1	G181R	541G>C	4.19	–	–	–
SMAD4	S32fs*1	94_95delAG	3.20	–	–	–
PIK3CA	H1047R	3140A>G	17.01	–	–	–
CDKN2A	P114L	341C>T	3.38	–	–	–
AKT1	E17K	49G>A	31.86	–	–	–
TP53	E326*	976G>T	19.37	–	–	–
CDKN2A	T79I	236C>T	2.86	–	–	–
CDKN2A	R99C	295C>T	2.50	–	–	–
ARID1A	G933fs*3	2796_2797insA	24.01	–	–	–
KRAS	G13D	38G>A	2.91	–	–	–
FGF4	–	–	–	7	3 of 3	Amplification
FGF19	–	–	–	7	3 of 3	Amplification
FGF3	–	–	–	7	3 of 3	Amplification
CCND1	–	–	–	7	5 of 5	Amplification
TMB			19 muts/Mb			

*, stop codon. MAF, minor allele frequency; CNV, copy number variations; TMB, tumor mutation burden.

2 mg d1, 8" for 5–7 cycles. However, eribulin was no longer effective according to the results of CT on 19 February 2021, which showed that the disease had progressed widely in the lymph nodes. Due to her high TMB from next-generation sequencing, we changed the therapy to nab-paclitaxel 190 mg d1, d8 + teriprilumab Injection 240 mg d1 for 2 cycles until 11 May 2021, and the disease progressed again. Considering that her Rb1 had recovered, the patient

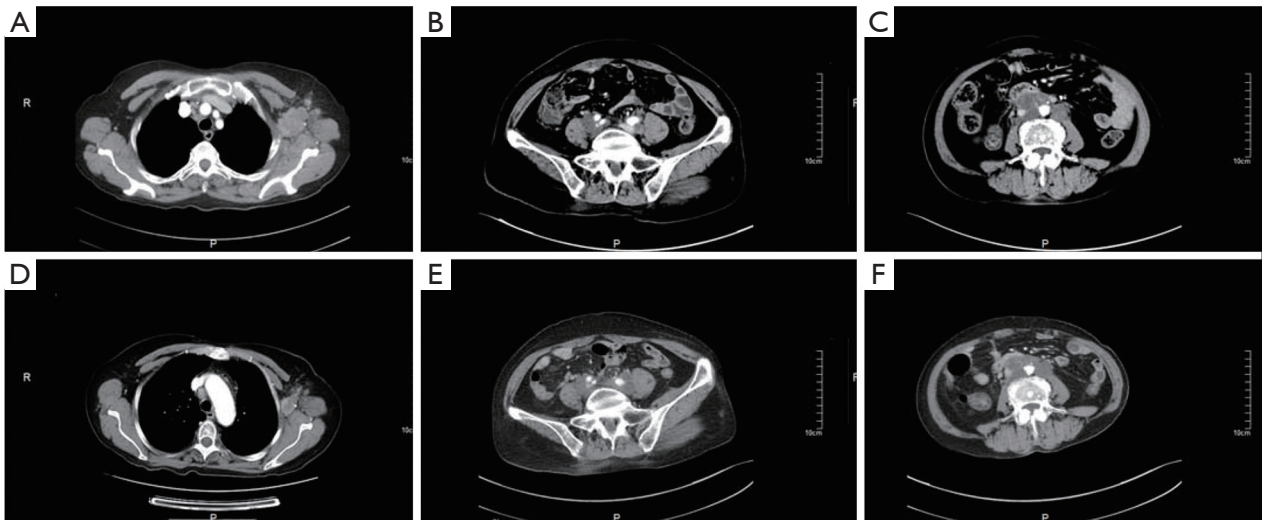

Figure 4 Before abemaciclib + anastrozole treatment (A-C); after abemaciclib + anastrozole treatment disease progressed (D-F).

received abemaciclib 150 mg po bid plus anastrozole 1 mg po qd from 11 May 2021 until PD occurred in September 2021 (*Figure 4*). The latest treatment relieved the pain, restored physical function, and improved health-related quality of life for the patient.

Discussion

Endocrine therapy plus CDK4/6i plays an important role in treating HR-positive and HER2-negative BC. In the past, there have been a series of classical clinical trials such as PALOMA-2, 3, MONALEESA-2, 3, 7, and MONARCH-2, 3, which confirmed that HR-positive and HER2-negative breast cancer patients benefited more from therapy combined endocrine with CDK4/6i, evidenced by improvements in median PFS (5).

The mechanism of CDK4/6i resistance remained to be to clarified, and is likely to be multifactorial. The amplification/overexpression of *FGFR1* was confirmed as one of the mechanisms of CDK4/6i resistance in the study by Formisano (8). It was shown that *FGFR1* prevents fulvestrant/palbociclib-induced growth inhibition and induces cell cycle transcriptional program, which leads to poor outcomes. Besides, *NF1* is a tumor suppressor gene whose loss will activate RAS and the MAPK pathway downstream. The enrichment of inactivating *NF1* mutations in MBC might also contribute to endocrine therapy resistance (9). Studies have shown that overexpression of the PI3K-AKT1 and FGF-CCND1 signaling pathways are associated with endocrine resistance and poor prognosis of breast cancer (10,11).

Inactivation of Rb has been demonstrated to be related to CDK4/6i resistance and a poor prognosis (12). In their study, Wang (13) showed that 30% of patients with Rb mutation had shorter PFS in triple-negative breast cancer (TNBC). O'Leary (14) asserted mutation of Rb occurred following the treatment of CDK4/6i, but it might be subclonal, with low prevalence. In China, there only a 2.7% Rb mutation was found in HR-positive and HER2-negative patients (15). However, the relationship between the mutation of Rb and the resistance of CDK4/6i remains to be further studied.

We have reported about CDK4/6i resistance in HR-positive and HER2-negative patients, who experienced rapid PD after receiving FCN-437c. Our investigations revealed that her Rb1 had disappeared. Therefore, we believed that this patient's insensitivity to FCN-437c may have been caused by Rb1 inactivation. To our surprise, the-second-time gene detection showed that her Rb1 recovered after receiving chemotherapy with eribulin. After that, the patient received a series of therapies as radiotherapy, chemotherapy, and immunotherapy. Though the disease still progressed during these therapies, the process seemed to play an essential role that eventually enabled abemaciclib to have a great therapeutic effect. Most pertinently, the effects of endocrine therapy and CDK4/6i were restored after receiving chemotherapy, radiotherapy, and immunotherapy, especially the chemotherapy with eribulin which recovered Rb1 and all of these treatment processes might have contributed to the change of Rb1.

Owing to the importance of Rb in the cyclin D-CDK4/6-Rb-E2F pathway, we began to suspect that the inactivation of Rb may lead to CDK4/6i resistance, but this resistance could be reversed by chemotherapy. In the past, it has been shown that chemotherapy could modulate endocrine therapy-related resistance mutations in MBC by changing the site and frequency of gene mutation (16). In this way, the case provides new insights into strategies for CDK4/6i therapy resistance options and highlighted the significance of next-generation sequencing in the clinic.

Conclusions

We have presented a case of an HR-positive and HER2-negative breast cancer patient with CDK4/6i resistance. Eribulin recovered her Rb1 mutations and overcame the resistance of CDK4/6i. In the future, further large-scale clinical trials are required to clarify the role of chemotherapy in HR-positive and HER2-negative breast cancer patients with CDK4/6i resistance. And we may work out more methods for MBC by overcoming the resistance of the drugs, which can prolong the effective time of the drugs and the survival time of the patients. Further, next-generation sequencing provides a significant tool and guidance for clinical practice.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-52/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-52/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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