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CASE REPORT

Partial Response to Pyrotinib Plus Capecitabine in an Advanced Breast Cancer Patient with *HER2* Amplification and *R157W* Mutation After Anti-*HER2* Treatment: A Case Report and Literature Review

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Yanchun Qu[1–3,*]
Yufeng Liu[3,*]
Kailin Ding[3]
Yong Li[1,2]
Xiaoyu Hong[4]
Haibo Zhang[1,2]

[1]Department of Oncology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, People's Republic of China; [2]Department of Oncology, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou, People's Republic of China; [3]The Second Clinical Medical College of Guangzhou University of Chinese Medicine, Guangzhou, People's Republic of China; [4]Nanjing Geneseeq Technology Inc, Nanjing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Haibo Zhang
Department of Oncology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, No. 111 Dade Road, Yuexiu District, Guangzhou, 510120, People's Republic of China
Tel +86-020-81887233
Email haibozh@gzucm.edu.cn

Abstract: Human epidermal growth factor receptor2 (*HER2*) overexpression/amplification is associated with high malignancy, rapid disease progression and poor overall survival in breast cancer. The application of anti-*HER2* drugs has greatly improved the survival of patients with *HER2*-positive breast cancer, but drug resistance issues affect the long-term efficacy. The *HER2* mutation is considered to be one of the reasons for resistance to anti-*HER2* therapy, and there is currently no standard treatment. We report for the first time the detection of *HER2* amplification with *R157W* mutation by second-generation sequencing (NGS) in a 57-year-old hormone receptor-negative, *HER2*-positive woman with advanced breast cancer who was resistant to multi-line anti-*HER2* therapies. She subsequently received pyrotinib combined with capecitabine treatment and achieved partial response. The small-molecule pan-*HER* family irreversible inhibitor pyrotinib combined with capecitabine has shown a promising effect in the treatment of *HER2* mutation-induced resistance, but the molecular mechanism and efficacy need to be further verified.

Keywords: breast cancer, HER2 amplification, HER2 R157W mutation, pyrotinib plus capecitabine, anti-HER2 treatment

Introduction

Breast cancer is a malignant tumor with the highest morbidity and second highest mortality rate in women.[1] *HER2*-positive (*HER2* amplification) breast cancer accounts for about 15–20% of all breast cancers.[2] Anti-*HER2* drugs have greatly improved the survival of such patients. These drugs include humanized monoclonal antibodies like trastuzumab and pertuzumab, antibody-drug conjugates (ADC) like T-DM1, and novel oral small-molecule tyrosine kinase inhibitors like pyrotinib, neratinib, lapatinib and so on.

Acquired drug resistance is inevitable in patients with advanced *HER2*-positive breast cancer after first-line and second-line anti-*HER2* treatments, and the mechanism is still under investigation. *HER2* mutations are rare in breast cancer patients. A systematic review showed that the frequency of *HER2* mutation in breast cancer patients was about 3%,[3] while previous gene sequencing results suggested that patients with primary *HER2*-positive breast cancer had a lower frequency of *HER2* mutation.[4,5] Therefore, *HER2* amplification and mutation are considered mutually exclusive in breast cancer patients. In recent years, studies have found that the

HER2 gene mutation rate is higher in breast cancer patients treated with multi-line anti-*HER2* therapies,[6,7] and *HER2* mutation is one of the causes of resistance to anti-*HER2* treatment. There is no standard treatment for *HER2* mutation patients yet. Of these, *HER2* R157W mutation is a rare mutation and there are currently only two reports in the literature. One was somatic mutation found in micropapillary urothelial carcinoma (MPUC),[8] which was not accompanied by *HER2* overexpression and *HER2* amplification. FATHMM prediction determined that it was a pathogenic mutation according to the COSMIC database. The other one was germline mutation found in breast cancer,[9] of which the clinical significance is unknown. This is the first report of a patient with *HER2*-positive advanced breast cancer who developed *HER2*-amplification with *R157W* missense mutation after treatment of lapatinib plus trastuzumab and achieved partial response (PR) after using pyrotinib plus capecitabine.

Case Description

A 57-year-old Chinese female was initially presented to an outside hospital and underwent radical resection of left breast cancer diagnosed with stage IIIC (pTxN3M0) in Jan 2013. Pathology indicated invasive ductal carcinoma of breast estrogen receptor (ER) (-), progesterone receptor (PR) (-), and HER2(3+) by immunohistochemistry. Then, she received one cycle of adjuvant chemotherapy with docetaxel and three cycles of oncolytic virus therapy in other hospital, but the patient could not provide detailed information. In March 2013, PET/CT showed bilateral lung metastases. However, the patient refused chemotherapy and received traditional Chinese medicine (TCM) decoction treatment. In March 2014, follow-up PET/CT revealed multiple metastases in liver, both lungs and bones. A liver biopsy conducted in our department suggested hepatic metastasis of the breast cancer (ER(-), PR(-), HER2(3+)). The patient received eight cycles of first-line-targeted treatment with trastuzumab (8mg/kg IV day 1 followed by 6mg/kg IV day 1 every 21 days) plus paclitaxel (135mg/m^2 IV day 1 every 21 days), followed by maintenance treatment with trastuzumab (6mg/kg IV day 1 every 21 days), achieving partial response and progression-free survival (PFS) for approximately 1 year. Subsequently, she was treated with docetaxel (75mg/m^2 IV day 1 every 21 days), xeloda (1000mg/m^2 PO bid days 1–14 every 21 days), and trastuzumab (6mg/kg IV day 1 every 21 days) as the second-line treatment for six cycles, with the best efficacy of partial response and PFS for 10 months. Upon disease progression, the third-line treatment was gemcitabine (1000mg/m^2 IV day 1 every 21 days) plus S-1 (40mg PO bid days 1–14 every 21 days) based chemotherapy for 4 cycles, with the best efficacy of stable disease (SD) and PFS for nearly 4 months. In the fourth-line treatment, she received epirubicin (80mg/m^2 IV day 1 every 21 days) plus lapatinib (0.25g PO qd). However, after one cycle, due to impaired liver function (with her alanine aminotransferase [ALT] level reaching 1122U/L), use of epirubicin was discontinued. Liver function improved following liver protection therapy, and the patient then continued lapatinib monotherapy (0.5g PO qd), maintaining an SD with PFS of 2 months.

In October 2017, enlarged lesions revealed in the chest and abdomen CT indicated progressive disease (PD). Next-generation sequencing (NGS) of plasma detected *HER2* and *CDK12* amplification, *NSD1* gene fusion, and *TP53 L257R* mutation. The patient began to receive trastuzumab (6mg/kg IV day 1 every 21 days) combined with lapatinib (1g PO qd) as the fifth-line treatment from November 2017. The best efficacy was SD, and PFS was about 9 months. In August 2018, plasma NGS showed *R157W* missense mutation in HER2 exon 4, along with *HER2* amplification and *TP53 L257R* mutation. That September, a chest and abdomen CT confirmed PD. The patient began to receive pyrotinib (400mg PO qd) plus xeloda (1000mg/m^2 PO bid days 1–14 every 21 days) as the sixth-line treatment, with the best efficacy of PR and PFS for 13 months. Plasma NGS monitoring during the period (December 2018) suggested a decrease in *HER2* copy number and abundance of *R157W* mutation. In November 2019, when her disease progressed again, the re-examination of the plasma NGS showed *CDK12* and *HER2* amplification, *DNMT3A* inactivation mutation and *TP53 L257R* mutation. There was no *HER2* mutation.

From November 2019 to January 2020, the patient received the seventh-line treatment with Abraxane (200mg IV day 1 every 14 days), with the optimal efficacy of SD. In February 2020, she began to accept the eighth-line treatment with pertuzumab (840mg IV day 1 followed by 420mg IV day 1 every 21 days) combined with trastuzumab (8mg/kg IV da y1 followed by 6mg/kg IV day 1 every 21 days) and xeloda (1000mg/m^2 PO bid days 1–14 every 21 days). The efficacy has not been evaluated at the time of writing this paper.

Discussion

HER2-amplified breast cancer accounts for about 15–20% of all breast cancers[2] and is often characterized by rapid progression and poor prognosis.[10,11] The precise treatment of

HER2 has completely changed the survival rate for breast cancer with *HER2*-amplification. However, as time goes by, anti-*HER2* treatment will inevitably produce acquired drug resistance, which will affect the survival of patients. Therefore, it is imperative to clarify the mechanism of drug resistance and make targeted treatments. The resistance mechanism of anti-*HER2* drugs is complex, and the resistance caused by *HER2* mutation has attracted more and more attention and may become a new potential therapeutic target.[12] We report a 57-year-old woman with advanced *HER2*-positive breast cancer who received multiple lines of anti-*HER2* therapy and was confirmed to have the *HER2 R157W* missense mutation in exon 4 by plasma NGS, and obtained PR after treatment with pyrotinib and capecitabine. This case provides a new potential treatment option for *HER2* mutations following resistance to *HER2* therapy.

Proto-oncogene *HER2*, also known as *ErbB2*, belongs to the *ERB* family with *ErbB1(EGFR), ErbB3(HER3)*, and *ErbB4(HER4)*. No high-affinity ligand has been found in *HER2* so far, so it must form homologously or as a heterodimer with other members of the family, binding with ATP to activate intracellular tyrosine kinases, thereby initiating the downstream signaling pathway and regulating the proliferation and differentiation of cells.[13–15] *HER2* is the most important driver gene of breast cancer, and the most common positive form is gene amplification. Amplification of *HER2* would lead to increased protein synthesis (ie, overexpression of HER2 protein) or increased protein function, which would lead to overactivation of downstream signaling pathways and overgrowth of cells, playing an important role in the proliferation, invasion, metastasis, and evolution of breast cancer cells.[16–18]

H0648g,[19] M77001,[20] EGF100151,[21] EGF104900[22] et al studies have shown that patients with *HER2*-positive breast cancer can benefit from anti-*HER2* humanized trastuzumab and double *HER2-EGFR* tyrosine kinase inhibitor (TKI) lapatinib. However, approximately 50% of *HER2*-positive patients developed resistance to trastuzumab 1 year after treatment.[23] Lapatinib has achieved certain clinical efficacy in metastatic *HER2*-positive breast cancer treated with trastuzumab, but a significant proportion of patients develop disease progression due to innate or acquired resistance to lapatinib.[24,25] Studies on the molecular mechanisms of trastuzumab and lapatinib resistance[26] found that overexpression of other HER family receptors and their ligands, loss of PTEN leading to activation of the PI3K/Akt/mTOR pathway, PI3KCA mutation, and Akt mutation or amplification were common causes of drug resistance. Drug resistance has become an urgent problem.

The patient developed resistance to lapatinib combined with trastuzumab. The *HER2* gene mutation was not detected before the patient received lapatinib plus trastuzumab treatment, while the *R157W* mutation was found in the disease progression after treatment. Moreover, by comparing the two gene test results (Table 1), only the *HER2* mutation was acquired, so it was believed that the *HER2* mutation was the main mechanism of drug resistance in this case. In recent years, with the gradual deepening of the understanding of *HER2*, it is believed that *HER2* mutation plays an important

Table 1 NGS Results Detected Before and After Treatment with Trastuzumab and Lapatinib

Genes	20171018 Before Trastuzumab with Lapatinib			20180820 After Resistance to Trastuzumab with Lapatinib			Methodology
	Variations	Abundance	CN	Variations	Abundance	CN	
HER2	CNG		2.1	CNG		7.7	NGS
				p.R157W	2.0%		NGS
TP53	p.L257R	6.4%		p.L257R	49.9%		NGS
CDK12	CNG		1.8	Negative			NGS
NSD1	GF	0.2%		Negative			NGS
ESR1	Negative			Negative			NGS
BRCA1	Negative			Negative			NGS
BRCA2	Negative			Negative			NGS

Abbreviations: *HER2*, human epidermal growth factor receptor-2; *TP53*, tumor protein p53; *CDK12*, cyclin-dependent kinase 12; *NSD1*, nuclear receptor binding SET domain protein 1; *ESR1*, estrogen receptor 1; *BRCA1*, breast cancer susceptibility gene 1; *BRCA2*, breast cancer susceptibility gene 2; CNG, copy number gain; CN, copy number; GF, gene fusion; NGS, next-generation sequencing.

role in the incidence, development and resistance of breast cancer.[27,28] The primary *HER2* mutation mostly occurred in *HER2* negative conditions, while in *HER2* positive breast cancer the *HER2* mutation mostly occurred after anti-*HER2* treatment. Fang et al[6] performed *HER2* full-length gene sequencing on the tissues of 198 patients with metastatic breast cancer (MBC) after multiple cycles of treatment and found that the rate of *HER2* mutations in patients treated with trastuzumab was as high as 17.7%. Park et al[7] also carried out NGS tests on the tissues of 36 refractory MBC patients after multi-cycle and multi-drug treatment, and found that 5 out of 6 patients with *HER2* mutation were *HER2* positive and developed drug resistance after receiving anti-*HER2* drugs (trastuzumab, lapatinib).

In the literature, the most common *HER2* mutation sites in breast cancer were *L755S, V777L, D769H, D769Y, G778_P780dup, Y772_A775dup* in the kinase domain, and *S310F* and *S310Y* in the extracellular domain.[3,29] There are no studies to prove the difference between *HER2*-negative combined mutation and *HER2* positive-combined mutation, and most of the mutations can occur in both. The causes of resistance to *HER2* therapy due to *HER2* mutations are not fully understood. A cell-induced drug resistance test suggested that drug resistance of lapatinib might be related to the change of kinase structure caused by *L755S* and *P780L* mutations, which would activate the kinase activity and interfere with the inactive structure required for lapatinib binding.[30,31] It may also be related to the production of larger amino acids after *L726F, L785F*, and other mutations, which interfered with the binding of lapatinib spatially.[30] In addition, *T789* mutation can stabilize the active conformation and directly compete with lapatinib for ATP binding sites, which may also be one of the causes of lapatinib resistance.[30,31] Other studies have found that increased levels of *EGFR-HER3* dimerization and expression of *HER4* can also lead to lapatinib resistance. The extracellular resistance test of trastuzumab suggested that mutations in the *HER2* kinase domain might alter the PI3K/AKT cascade signaling, thereby weakening the inhibition of trastuzumab to the PI3K/AKT pathway.[32] It has also been observed in cell experiments that *L755S* mutation can lead to overactivation of PI3K/AKT/mTOR and MAPK pathways, leading to resistance to lapatinib and neratinib.[33] Hanker B et al[34] reported that a breast cancer patient with *HER2 L869R* mutation got new *T798I* mutation when neratinib resistance happened. Cell models confirmed that the presence of isoleucine at the 798 site leads to steric hindrance, reducing the binding affinity of neratinib. Smyth et al[29] believed that *HER2* mutation accompanied with other changes in *HER* family signaling pathways, such as *HER3* mutation, might lead to drug resistance to neratinib.

This case reported that *R157W* missense mutation happened in the extracellular domain belonging to exon4 of *HER2* which could be found in the cBioPortal database[35,36] within the TCGA data set (Figure 1). Prior to this, only two cases of *HER2 R157W* mutation have been reported in the literature, and that which was found in breast cancer harbored germline mutations. As this residue is presented in the extracellular domain, we think the drug-resistant mechanism may be related to the change of the extracellular domain receptor structure, which interfered with the binding of trastuzumab. It may also strengthen dimerization with other family receptors, such as *HER2/HER3*, and *HER2/HER4* polymerization, thereby weakening the inhibition of lapatinib, which activated downstream pathways and caused a cascade reaction, eventually leading to tumor progression.

There is no standard treatment for *HER2* mutation, but many studies have begun to explore treatment strategies. A number of studies have demonstrated that the pan-*HER2* irreversible inhibitor neratinib has a good inhibitory effect on certain mutations. Zuo et al[28] found that neratinib had a strong

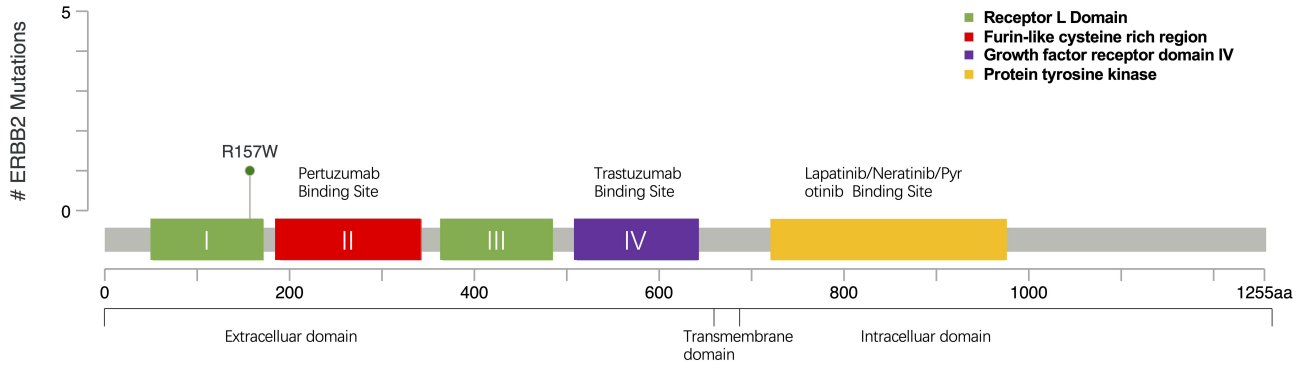

Figure 1 Mutation site map of R157W gene in TCGA dataset from cBioPortal database.[35,36]

inhibitory effect on drug-resistant mutant cell lines bearing *K753E* or *L755S* mutations. Cocco et al[37] demonstrated that neratinib could not only effectively overcome the acquired resistance to *HER2* therapy in breast cancer cells carrying both *HER2* amplification and *L755S* somatic mutation but also significantly inhibit tumor growth in mice which are resistant to trastuzumab and lapatinib as well as carrying both *D769Y* mutation and *HER2* amplification. At the same time, it was observed that neratinib had clinical activity in breast cancer patients with both *HER2* gene amplification and mutation. SUMMIT is a Phase II basket clinical trial involving patients with cancers featuring *HER2* mutations, and the result shows that neratinib has a good clinical effect on patients with breast cancer accompanied by *HER2* mutations but without amplification, with an ORR of 32% at 8 weeks,[4] but resistance to *T798*-related mutations.[33] Overall, neratinib has a certain clinical efficacy against common *HER2* mutations, but has not yet been marketed in China.

Pyrotinib is a new generation of small-molecule irreversible pan-*ErbB* receptor TKI, covalently binding with the ATP binding site in the intracellular kinase regions of *HER1, HER2*, and *HER4*, which prevents the formation of homodimers/heterodimers in the *HER* family, inhibits phosphorylation itself, blocks the activation of downstream signaling pathways, and inhibits tumor cell growth.[38] Its structure and mechanism of action are similar to neratinib, but it has stronger inhibitory activity in vitro. In 2019, Jiang et al, reported a phenix study orally at the ASCO conference, confirming that to *HER2* positive MBC females who previously received paclitaxel and trastuzumab, pyrotinib plus capecitabine, compared to placebo plus capecitabine, can effectively improve the median PFS. In this case, the patient developed disease progression in September 2018 after receiving trastuzumab combined with lapatinib, and received pyrotinib plus capecitabine. Three months

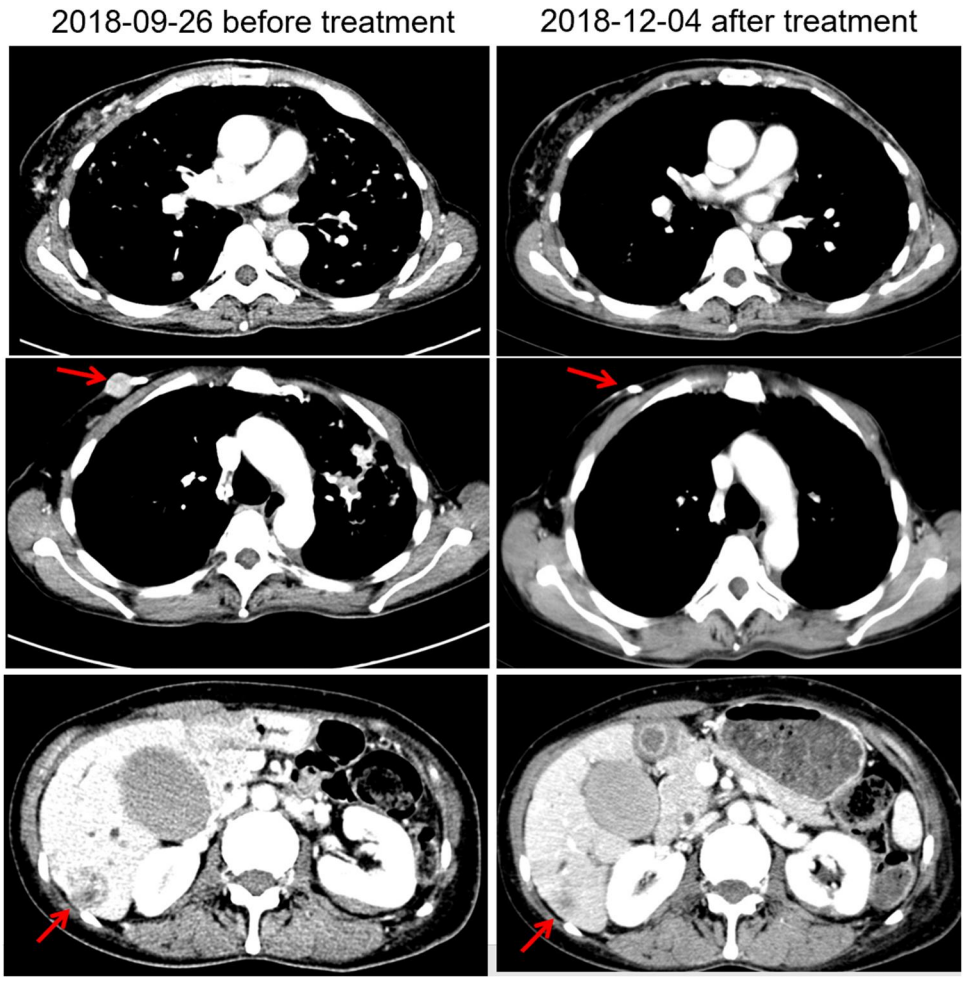

Figure 2 Changes of images before and after treatment. The red arrows indicate the metastatic tumor.

Table 2 NGS Results Detected Before and After Treatment with Pyrotinib and Capecitabine

Genes	20180820 Before Pyrotinib with Capecitabine			20181212 After Pyrotinib with Capecitabine			Methodology
	Variations	Abundance	CN	Variations	Abundance	CN	
HER2	p.R157W	2.0%		p.R157W	1.3%		NGS
	CNG		7.7	CNG		2.1	NGS
TP53	p.L257R	49.9%		p.L257R	5.7%		NGS
ESR1	Negative			Negative			NGS
BRCA1	Negative			Negative			NGS
BRCA2	Negative			Negative			NGS

Abbreviations: *HER2*, human epidermal growth factor receptor-2; *TP53*, tumor protein p53; *ESR1*, estrogen receptor 1; *BRCA1*, breast cancer susceptibility gene 1; *BRCA2*, breast cancer susceptibility gene 2; CNG, copy number gain; CN, copy number; NGS, next-generation sequencing.

later, she got partial response, (Figure 2) and symptoms were relieved. Plasma NGS indicated that both the copy number of *HER2* amplification and the abundance of *R157W* mutations were decreased compared with previous results (Table 2). The relationship between gene dynamics and efficacy is shown in Figure 3.

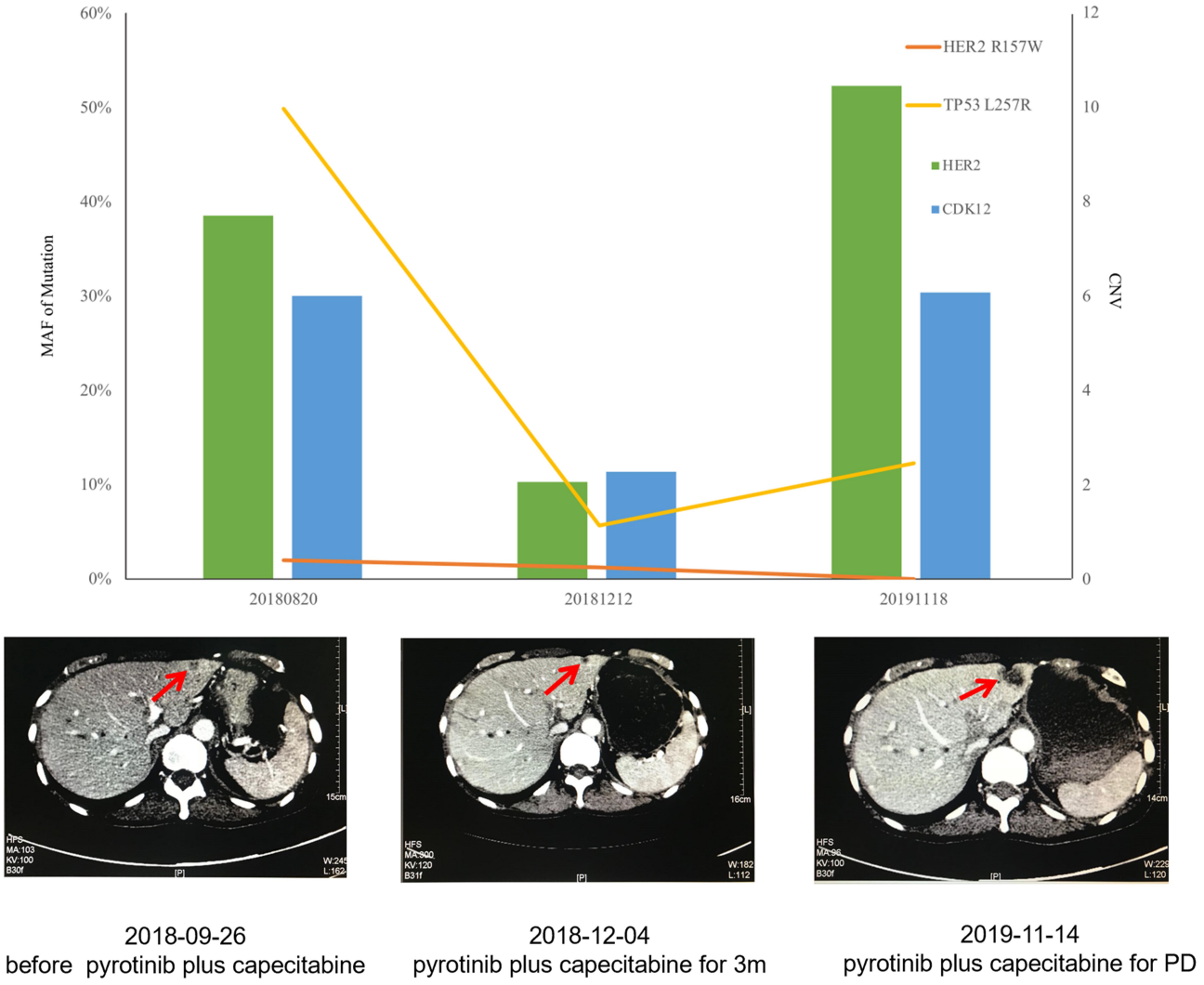

Figure 3 The relationship between gene dynamics and efficacy. The red arrows indicate the metastatic tumor.

Conclusion

Drug resistance has affected the efficacy of anti-*HER2* therapy in breast cancer, and *HER2* mutation is one of the causes. However, there is no standard treatment yet. We report for the first time the occurrence of *HER2* amplification accompanied by R157W mutation after anti-*HER2* treatment. This case is the first clinical report of pyrotinib plus capecitabine effective for *HER2* mutation, which shows a good clinical efficacy against *HER2* resistance accompanied with mutation in MBC patients, and provides a possible new management strategy of anti-*HER2* treatment for patients with *HER2*-positive breast cancer.

Ethical Approval

Institutional approval was not required to publish the case details.

Patient Informed Consent

Written informed consent was obtained from the patient for the publication of her case details and images.

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Disclosure

Xiaoyu Hong is the employee of Nanjing Geneseeq Technology Inc. The other authors have no conflicts of interest that are directly relevant to the content of this article.

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