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Dual-targeting strategy using trastuzumab and lapatinib in a patient with HER2 gene amplification in recurrent metachronous metastatic gallbladder carcinoma

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Abstract

Gallbladder carcinoma (GBC) is a rare and highly aggressive tumor. Early diagnosis is challenging, which results in a poor prognosis using systemic therapy. Recent studies have identified a subset of GBC patients with HER2 gene (ERBB2) amplification that could benefit from HER2-targeted therapy. Here, we report one patient with recurrent metachronous GBC with metastasis, who received the combination of trastuzumab and lapatinib. This approach achieved a partial response for both the brain and the lung metastases. This study demonstrated that HER2 inhibition is a promising therapeutic strategy for GBC with HER2 amplification and, combined with lapatinib, it can effectively target brain metastasis.

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Introduction

Anti-HER2 molecular targeted agents are established for treating breast cancer,[1–3] gastric cancer,[4] and metastatic colorectal cancer[5] in patients with HER2 gene amplification (an increase in the copy number). A combination of two HER2-targeted therapies, such as trastuzumab and lapatinib, has also been demonstrated to have clinical efficacy in advanced HER2-positive metastatic breast cancers[2,3] and colorectal cancers.[5]

Lapatinib (GW572016) is a selective inhibitor of epidermal growth factor receptor (EGFR) and HER-2 tyrosine kinase,[6] and trastuzumab is a humanized monoclonal antibody that targets the HER-2 extracellular domain.[7] The dual targeting of HER2-positive tumors using trastuzumab and lapatinib results from the primary and acquired resistance of these two drugs, their partly non-overlapping mechanisms of action, and the well characterized synergistic interaction between them in HER2 breast-cancer models. Clinically, trastuzumab primarily induces pro-apoptotic effects, while lapatinib inhibits proliferation.[8,9] Clinical evidence shows indirect support for dual HER2 blockade. In patients with refractory breast cancer who are treated with trastuzumab, lapatinib plus trastuzumab prolonged the progression-free survival compared with lapatinib alone. Konecny et al.[10] analyzed the combination of trastuzumab plus lapatinib, and they observed synergistic drug interactions in four HER-2-overexpressing breast cancer cell lines. Additionally, similar results were obtained in the study reported by Baselga et al.,[11] which suggested complementary mechanisms of action and synergistic antitumor activity for the monoclonal antibody in a model of breast cancer that overexpressed HER2. Xu et al.[3] conducted a systematic review and meta-analysis of randomized controlled trials (RCTs), and the results showed that lapatinib plus trastuzumab significantly prolonged the pathological complete response, overall survival, and event-free survival with tolerance in HER2-positive breast cancer patients. Moreover, the combination of trastuzumab and lapatinib was also effective and well tolerated in patients with HER2-positive metastatic colorectal cancer refractory disease.[5]

Gallbladder carcinoma (GBC) is a rare and highly aggressive tumor. Early diagnosis is challenging, which results in a poor prognosis in patients who receive systemic therapy. However, combination therapy with trastuzumab and lapatinib has not been reported in patients with GBC. Here, we present the case of a patient with dual-targeted HER2 therapy including lapatinib that was used to treat advanced GBC with brain metastases.

Case presentation

On 4 January 2016, a 48-year-old Chinese female patient was admitted to the Shaoxing People's Hospital because of nausea, vomiting, dizziness, headache, crooked mouth, and slurred speech. Magnetic resonance imaging (MRI) showed multi-site metastatic brain tumors (Figure 1a) and bilateral adrenal gland metastasis (data not shown). Chest

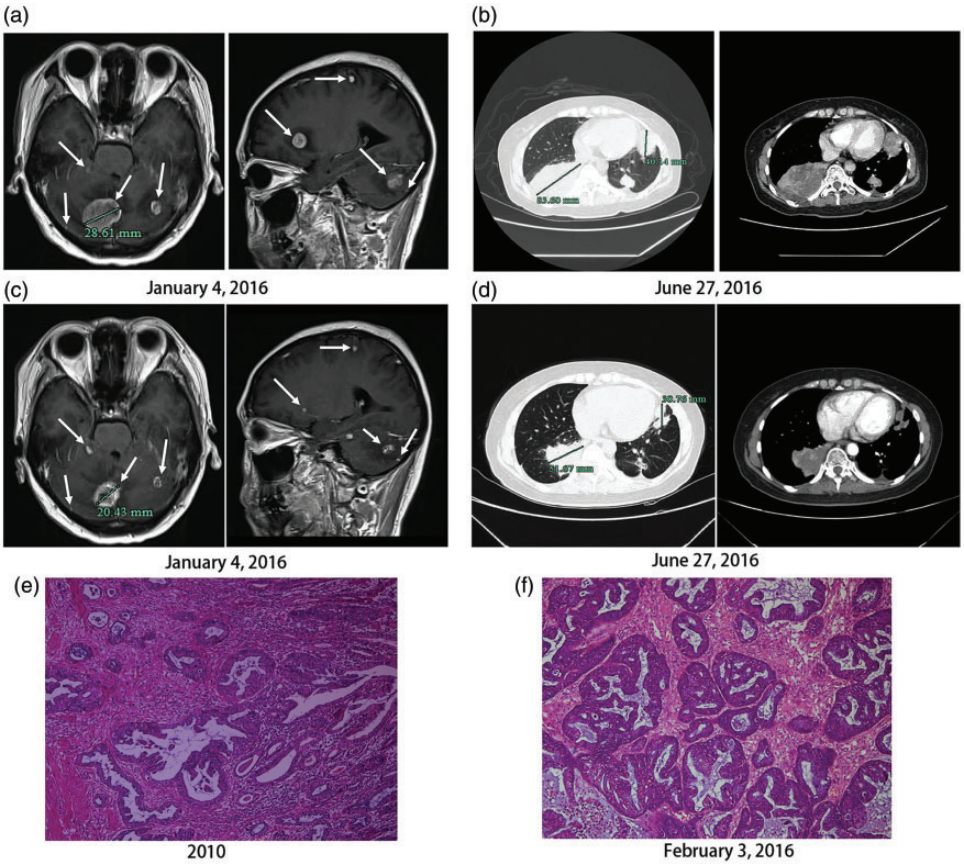

Figure 1. Dual-targeted therapy with trastuzumab and lapatinib were effective in advanced gallbladder carcinoma patients with multi-site brain and lung metastases.
Magnetic resonance imaging showed the status of brain metastases before (a) and after (c) three cycles of dual-HER2 targeted therapy with trastuzumab and lapatinib. Chest computed tomography scans showed the status of lung metastasis before (b) and after (d) three cycles of dual HER2-targeted therapy with trastuzumab and lapatinib. (e) A biopsy identified that the gallbladder tumor was moderate-differentiated adenocarcinoma (hematoxylin and eosin staining; original magnification $\times 100$). (f) A biopsy showed that the metastatic brain tumors were adenocarcinoma (hematoxylin and eosin staining; original magnification $\times 100$).

computed tomography (CT) scans revealed multi-site metastatic lung tumors (Figure 1b). This patient was diagnosed with GBC via cholecystectomy that was performed because of cholecystitis and gallstones in 2010 (pT3N0M0; TNM staging: IIB; Nevin staging: III) (Figure 1e). Lung metastasis was found in 2014, and brain metastasis was detected in 2015. The patient's medical history is shown in Figure 2.

One month after admission, the patient underwent surgery to remove one of the largest metastatic tumors (longest diameter in the transverse plane, 38.96 mm) in the left frontal lobe. The biopsy showed that the metastatic tumor was adenocarcinoma (Figure 1f). The patient was diagnosed with the GBC brain metastasis after a comprehensive review. Genetic profiling of 416 cancer-relevant genes was performed using

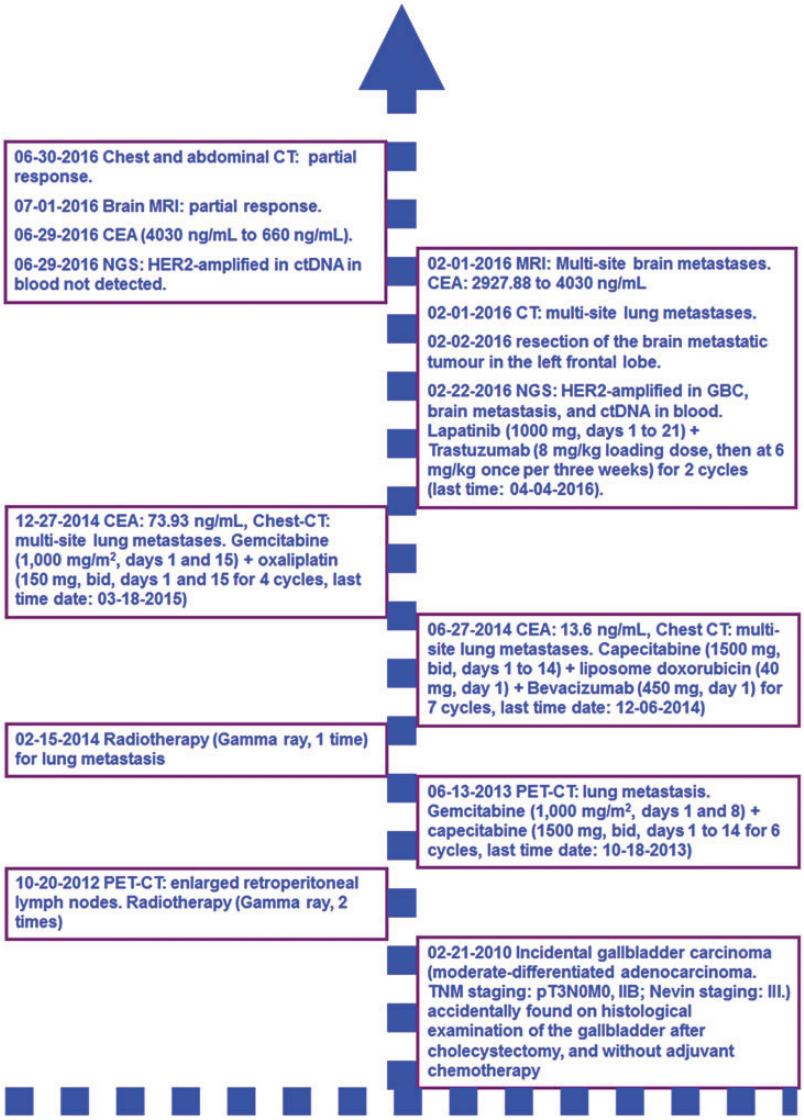

Figure 2. Medical history, clinical interventions, and metastasis development.

targeted next generation sequencing (NGS; see Materials and Methods) to develop potential molecular therapeutic approaches. GBC primary tumor and brain metastatic tissues, as well as the circulating tumor DNA (ctDNA) in the blood were collected from the patient for genetic profiling. The sequencing results showed a significant increase in the HER2 copy number in the GBC primary tumor (8.6×), brain metastasis (14.6×), and ctDNA (2.5×) (Table 1). Thus, dual-targeted therapy was initiated on 6 March, 2016 using trastuzumab (administered intravenously at an initial dose of 8 mg/kg, followed by subsequent doses of 6 mg/kg

Table 1. Genetic alterations identified in samples before/after HER2-targeted therapy.

Sample type	Sample ID	Genetic alterations identified	MAF (%)/ Fold change (×)
Before HER2-targeted therapy			
ctDNA	P16020611144	TP53: p.K291X (c.A871T)	4%
		CDK12: amplification	2.87×
		ERBB2: amplification	2.48×
FFPE sample of gallbladder tumor	F16020611145	TP53: p.K291X (c.A871T)	31%
		CDK12: amplification	9.03×
		ERBB2: amplification	8.40×
FFPE sample of brain tumor	F16020611146	TP53: p.K291X (c.A871T)	72%
		CDK12: amplification	11.28×
		ERBB2: amplification	14.60×
		RB1: Single-copy loss	0.53×
After three cycles of anti-HER2 targeted therapy			
ctDNA	P16051918361	TP53: p.K291X (c.A871T)	0.5%
		CDK12: amplification	Not detected
		ERBB2: amplification	Not detected
		RB1: Single-copy loss	Not detected

MAF, mutant allele frequency; ctDNA, circulating tumor DNA; FFPE, formalin-fixed paraffin-embedded.

once per week for 3 weeks) and lapatinib (administered orally each day at a dose of 1000 mg per day in 21-day treatment cycles), based on previously published work in breast cancer[2,3] (i.e., one dose of trastuzumab each week and one dose of oral lapatinib each day). No concurrent chemotherapy was administrated.

Three weeks after dual HER2 targeted therapy with trastuzumab and lapatinib, the follow-up chest and brain MRI revealed that the patient achieved a partial response in brain metastatic tumors (Figure 1c) and lung metastatic tumors (Figure 1d), after three cycles of treatment based on the Response Criteria Evaluation in Solid Tumors (RECIST) version 1.1. The MRI results showed that the metastatic tumors had shrunk. A concurrent blood sample was collected for genetic testing of ctDNA using targeted NGS. The HER2 copy numbers did not increase at this time (Table 1), suggesting that the tumors were cleared after trastuzumab and lapatinib dual therapy. It was also accompanied by a carcinoembryonic antigen (CEA) response (CEA levels decreased from 4030 ng/mL to 660 ng/mL). The patient developed grade 3 adverse reactions including fatigue, skin rash, and increased bilirubin concentration, but no grade 4 or 5 adverse reactions were observed. In summary, dual-targeted therapy with trastuzumab and lapatinib offered a chemotherapy-free option and achieved tumor suppression with an acceptable safety profile for this patient with HER2 amplification in GBC.

Materials and methods

Patient information

The patient provided written informed consent. The study was approved by the ethics committee at the institute and was performed in strict accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization and Good Clinical Practice guidelines.

Tumor assessment

Tumor measurements were performed by a radiologist (ZXL) before treatment and were assessed every 2 months in accordance with RECIST (version 1.1). The patient was examined using a CT of the chest, abdomen, or pelvis, and MRI brain scans when clinically indicated in accordance with the protocol. All tumor measurements were reviewed by a radiologist (ZXL) who read the MRI or CT scans, and collected, stored, and interpreted the imaging results.

Adverse reaction assessment

The patient's health was continuously monitored and graded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Laboratory test assessments (hematology, serum chemistry, urine analysis, and tumor markers) were performed at baseline (day 1 of every cycle), and at the end of treatment. The left ventricular ejection fraction was examined at baseline (day 1 of every cycle) and at the end of treatment.

DNA extraction

Formalin-fixed paraffin-embedded (FFPE) tissues were sectioned, and hematoxylin and eosin (H&E)-stained slides were reviewed by surgical pathologists (FL and AJS). Tumor tissue areas were dissected from ten serial sections (4 μm) guided by H&E slides as templates. The FFPE tissues were deparaffinized with xylene and genomic DNA was extracted using a QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA, USA). The concentration of DNA was determined using a Qubit dsDNA HS assay kit on the Qubit 3.0 (Life Technologies, Carlsbad, CA, USA), in strict accordance with the manufacturer's protocol. DNA (A260/280 and A260/230) was measured using the Nanodrop-2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Whole blood (8 mL) was collected using EDTA blood collection tubes (BD Biosciences, San Jose, CA, USA) that were centrifuged within 2 hours at 1800 $\times g$ for 10 minutes at $\geq$4°C to collect the plasma. ctDNA was extracted from plasma using a QIAamp circulating nucleic acid kit (Qiagen). The purified ctDNA was quantified using a Qubit dsDNA HS assay kit on the Qubit 3.0 Fluorometer (Life Technologies).

Library construction

The sequencing library was prepared using a KAPA Hyper Prep Kit (Kapa Biosystems, Boston, MA, USA), in accordance with the manufacturing protocol. Briefly, 1 μg genomic DNA sample was fragmented into 350 bp in an Adaptive Focused Acoustics (AFA) fiber snap-cap microTUBE using Covaris M220 (Covaris, Woburn, MA, USA) or 10–50 ng ctDNA underwent end repairing, A-tailing, adapter ligation, size selection, and finally PCR amplification. Library concentration was determined using a Qubit dsDNA HS assay kit on the Qubit 3.0 Fluorometer (Life Technologies).

Hybrid capture and ultra-deep next generation sequencing

The 5′-biotinylated probe solution was used as capture probes, which targeted 416 cancer-related genes (Table 2). The capture reaction was performed using the NimbleGen SeqCap EZ hybridization and Wash Kit (Roche) with 1 μg of pooled libraries, 5 μg of human Cot-1 DNA, 1 unit of adapter-specific blocker DNA, and the capture probes. The solution hybridization was performed for 16–18 hours at 65°C. The captured targets were selected by pulling down the biotinylated probe/target hybrids using

Table 2. Gene targeted in hybridization capture.

ABCB1 (MDR1)	CDK4	ERBB4	HGF	MSH2	PTEN	STK11
ABCC2 (MRP2)	CDK6	ERCC1	HNF1A	MSH3	PTPN11	STMN1
ACTB	CDK8	ERCC2	HNF1B	MSH6	PTPN2	STX11
ADH1B	CDKN1B	ERCC3	HRAS	MTHFR	PTPN6	STXBP2
AIP	CDKN1C	ERCC4	ID3	MTOR	PTPRO	SUFU
AKT1	CDKN2A	ERCC5	IDH1	MUTYH	QKI	SUZ12
AKT2	CDKN2B	ESR1	IDH2	MYC	RAC1	SYN3
AKT3	CDKN2C	ETV1	IGF1R	MYCL1	RAD21	TCN2
ALDH2	CEBPA	ETV4	IGF2	MYCN	RAD50	TEK
ALK	CEP57	EWSR1	IKBKE	MYD88	RAD51	TEKT4
AMER1	CHD4	EXT1	IKZF1	MYNN	RAD51C	TERC
AP3B1	CHEK1	EXT2	IKZF2	NBN	RAD51D	TERT
APC	CHEK2	EZH2	IKZF3	NCSTN	RAF1	TET2
AR	CKS1B	FANCA	IL13	NF1	RARA	TGFBR2
ARAF	CREBBP	FANCB	IL7R	NF2	RASGEF1A	TLE1
ARID1A	CRKL	FANCC	INPP4B	NFKBIA	RB1	TLE4
ARID2	CROT	FANCD2	INPP5D	NKX2-1	RECQL4	TMEM127
ARID5B	CSF1R	FANCE	IRF1	NOTCH1	RELN	TMPRSS2
ASXL1	CSF3R	FANCF	IRF2	NOTCH2	RET	TNFAIP3
ATM	CTCF	FANCG	IRF4	NPM1	RHBDF2	TNFRSF14
ATR	CTLA4	FANCI	IRF8	NQO1	RHOA	TNFRSF17
ATRX	CTNNB1	FANCL	JAK1	NRAS	RICTOR	TNFRSF19
AURKA	CUX1	FANCM	JAK2	NRG1	RNF146	TOP1
AURKB	CXCR4	FAT1	JAK3	NSD1	RNF43	TOP2A
AXIN1	CYLD	FBXO11	JARID2	NT5C2	ROS1	TP53
AXL	CYP19A1	FCGR2B	JUN	NTRK1	RPTOR	TP63
B2M	CYP2B6*6	FGF19	KDM2B	PAG1	RRM1	TPMT
BAP1	CYP2C19*2	FGFR1	KDM5A	PAK3	RTEL1	TRAF2
BARD1	CYP2C9*3	FGFR2	KDR (VEGFR2)	PALB2	RUNX1	TRAF3
BCL2	CYP2D6*3	FGFR3	KIF1B	PARK2	SBDS	TRAF5
BCL2L1	CYP2D6*4	FGFR4	KIT	PAX5	SDHA	TSC1
BCL2L2	CYP2D6*5	FH	KMT2B	PBRM1	SDHAF2	TSC2
BCORL1	CYP2D6*6	FIP1L1	KMT2C	PC	SDHB	TSHR
BCL2L11 (BIM)	CYP3A4*4	FLCN	KRAS	PDCD1	SDHC	TTF1
BLM	CYP3A5*1	FLT1 (VEGFR1)	LEF1	PDCD1LG2 (PD-L2)	SDHD	TUBB3
BMPR1A	CYP3A5*3	FLT3	LMO1	PDGFRA	SERP2	TYMS
BRAF	DAB2	FLT4 (VEGFR4)	LSP1	PDGFRB	SETBP1	TYR
BRCA1	DAXX	GADD45B	LYN	PDK1	SETD2	U2AF1
BRCA2	DDR2	GATA1	LYST	PHF6	SF3B1	UGT1A1
BRD4	DDX1	GATA2	LZTR1	PHOX2B	SGK1	UNC13D
BRIP1	DHFR	GATA3	MAP2K1 (MEK1)	PICK3R1	SH2D1A	VEGFA

(continued)

Table 2. Continued.

BTG2	DICER1	GATA4	MAP2K2 (MEK2)	PIK3C3	SLX4	VHL
BTK	DIS3L2	GATA6	MAP2K4	PIK3CA	SMAD2	WISP3
BTLA	DLG2	GNA11	MAP3K1	PIK3CD	SMAD3	WRN
BUB1B	DMNT3A	GNA13	MCL1	PIK3R1	SMAD4	WT1
c11orf30	DNM2	GNAQ	MDM2	PIK3R2	SMAD7	XIAP
CALR	DOCK1	GNAS	MDM4	PLCE1	SMARCA4	XPA
CBL	DOT1L	GPC3	MECOM	PLK1	SMARCB1	XPC
CCND1	DPYD	GRIN2A	MED12	PMS1	SMC1A	XPO1
CCNE1	DUSP2	GRM3	MEF2B	PMS2	SMC3	XRCC1
CCT6B	EBF1	GSTM1	MEN1	POLD1	SMO	YAP1
CD22	ECT2L	GSTP1	MET	POLD3	SOX2	ZAP70
CD274 (PD-L1)	EED	GSTT1	MGMT	POLE	SPOP	ZBTB20
CD58	EGFR	HBA1	MITF	POT1	SRC	ZNF217
CD70	EGR1	HBA2	MLH1	PPP2R1A	SRSF2	ZNF703
CDA	EP300	HBB	MLL	PRDM1	STAG2	ZRSR2
CDC73	EPCAM	HDAC1	MLLT10	PRF1	STAT3	
CDH1	EPHA3	HDAC2	MLPH	PRKAR1A	STAT5A	
CDK10	**ERBB2 (HER2)**	HDAC4	MPL	PRKCI	STAT5B	
CDK12	ERBB3	HDAC7	MRE11A	PTCH1	STIL	

streptavidin-coated magnetic beads, and the off-target library was removed by washing with wash buffer, followed by PCR amplification of the target-enriched libraries. Sequencing libraries were quantified by qPCR using the KAPA Library Quantification kit (KAPA Biosystems, Boston, MA, USA), sized on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA), and then deep-sequenced on an Illumina HiSeq 4000 using the PE150 kit (Illumina Inc., San Diego, CA, USA).

Sequence alignment and data processing

Quality control was applied using Trimmomatic.[12] High quality reads were mapped to the human genome (hg19, GRCh37 Genome Reference Consortium Human Reference 37) using a modified Burrows-Wheeler Aligner (BWA) version 0.7.12[13] with BWA-MEM algorithm and default parameters. The Genome Analysis Toolkit[14] (GATK, version 3.4-0) was modified and used to locally realign the BAM files at intervals with insertion/deletion (indel) mismatches and to recalibrate the BAM file read base quality scores. Single nucleotide variants (SNVs) and short indels were identified using VarScan2 2.3.9[15] with a minimum variant allele frequency threshold set at 0.01 and a p-value threshold for calling variants set at 0.05 to generate variant call format (VCF) files. All SNVs/indels were annotated using ANNOVAR, and each SNV/indel was manually checked using the integrative genomics viewer (IGV).[16] Copy number variations (CNVs) were identified using ADTEx 1.0.4.[17] The analysis and data are presented as copy number changes in all Tables.

Discussion

Recent studies have identified a subset of patients who are undergoing treatment for

advanced GBC with HER2 overexpression or amplification that could potentially benefit from HER2-targeted therapy.[6,7] Some studies previously reported the use of HER2-targeted treatment to treat HER2-positive gallbladder cancers. One case included a significant reduction in lung metastasis in patients with HER2-amplified metastatic cholangiocarcinoma that was treated with trastuzumab and paclitaxel.[8] Another study retrospectively reviewed GBC patients who received HER2-directed therapy with trastuzumab, lapatinib, or pertuzumab, and showed that HER2 blockage was a promising treatment strategy in HER2-positive GBC patients.[7] Most of those patients received a single HER2-targeted reagent combined with chemotherapy.

Clinical trials have demonstrated that lapatinib can be used as a therapeutic option for advanced HER2 alterations in cancers with brain metastasis.[9] Considering the multi-site brain and lung metastases of the GBC patients, we administrated dual-targeted HER2 therapy with trastuzumab and lapatinib and achieved PR after 4 months of treatment without concurrent chemotherapy. In summary, this is the first study to administer dual-targeted HER2 therapy including lapatinib to treat patients with advanced GBC with brain metastases. Patient follow-up and further multi-center clinical trials are necessary to investigate the long-term efficacy.

Conclusion

HER2 inhibition is a promising therapeutic strategy for GBC with HER2 amplification and used in combination with lapatinib, it can effectively target brain metastasis.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Disclosure

Xue Wu, Yichuan Liu, and Yang W. Shao are the shareholders or employees of Geneseeq Technology Inc.

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