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.
**Introduction**

Anti-HER2 molecular targeted agents are established for treating breast cancer, gastric cancer, and metastatic colorectal cancer 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 and colorectal cancers.

Lapatinib (GW572016) is a selective inhibitor of epidermal growth factor receptor (EGFR) and HER-2 tyrosine kinase, and trastuzumab is a humanized monoclonal antibody that targets the HER-2 extracellular domain. 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. 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. 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., which suggested complementary mechanisms of action and synergistic anti-tumor activity for the monoclonal antibody in a model of breast cancer that overexpressed HER2. Xu et al. 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.

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...
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
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.

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

| Sample type | Sample ID | Genetic alterations identified | MAF (%) | Fold change (×) |
|-------------|-----------|--------------------------------|---------|-----------------|
| Before HER2-targeted therapy | P16020611144 | TP53: p.K291X (c.A871T) | 4% | |
| | | CDK12: amplification | 2.87× | |
| | | ERBB2: amplification | 2.48× | |
| | F16020611145 | TP53: p.K291X (c.A871T) | 31% | |
| | | CDK12: amplification | 9.03× | |
| | | ERBB2: amplification | 8.40× | |
| | 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 | 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.
Tumor assessment

Tumor measurements were performed by a radiologist (ZXLL) 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 (ZXLL) 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 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.

| Gene targeted in hybridization capture |
|----------------------------------------|
| ABCB1 (MDR1)                           |
| ABCB2 (MRP2)                           |
| ACTB                                  |
| ADH1B                                 |
| AIP                                   |
| AKT1                                  |
| AKT2                                  |
| AKT3                                  |
| ALDH2                                 |
| ALK                                   |
| AMER1                                 |
| AP3B1                                 |
| APC                                   |
| AR                                    |
| ARAF                                  |
| ARID1A                                |
| ARID2                                 |
| ARID5B                                |
| ASXL1                                 |
| ATM                                   |
| ATR                                   |
| ATRX                                  |
| AURKA                                 |
| AURKB                                 |
| AXIN1                                 |
| AXIN2                                 |
| AXIN3                                 |
| AXIN4                                 |
| AXIN5                                 |
| B2M                                   |
| BAP1                                  |
| BARD1                                 |
| BCL2                                  |
| BCL2L1                                |
| BCL2L2                                |
| BCORL1                                |
| BCL2L1I (BIM)                         |
| BLM                                   |
| BMPRIA                                |
| BRAF                                  |
| BRCA1                                 |
| BRCA2                                 |
| BRD4                                  |
| BRIP1                                 |

(continued)
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. 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 with BWA-MEM algorithm and default parameters. The Genome Analysis Toolkit (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 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). Copy number variations (CNVs) were identified using ADTEx 1.0.4. 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

| 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           | PLEC1  | SMARCA4| XPA |
| CBL    | DOT1L  | GPC3  | MECOM          | PLK1   | SMARCB1| XPC |
| CCND1  | DPYD   | GRIN2A| MED12          | PMS1   | SMC1A| XPO1 |
| CCNE1  | DUSP2  | GRM3  | MEF2B          | PMS2   | SMC3 | XRCC1 |
| CCT6B  | EBFI   | GSTM1 | MEN1           | POLD1  | SMO  | YAP1 |
| CD22   | ECT2L  | GSTP1 | MET            | POLD3  | SOX2 | ZAP70 |
| CD274  | EED    | GSTD1 | MGMT           | POLE   | SPOP | ZBTB20 |

(PD-L1)  

| 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 |   |
| CDHI1  | EPHA3  | HDAC2 | MLPH           | PRKAR1A| STAT5A|   |
| CDK10  | ERBB2  | HDAC4 | MPL            | PRKCI  | STAT5B|   |

(HER2) 

| CDK12  | ERBB3  | HDAC7 | MRE11A        | PTCH1  | STIL |   |

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advanced GBC with HER2 overexpression or amplification that could potentially benefit from HER2-targeted therapy.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9} Considering the multi-site brain and lung metastases of the GBC patients, we administered 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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