Response of Leptomeningeal Metastasis of Breast Cancer With a HER2/neu Activating Variant to Tucatinib: A Case Report

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ABSTRACT
Metastatic breast cancer demonstrates HER2/neu amplification approximately 15% of the time. However, HER2 mutations, which often stimulate tumor growth, occur in only 3% to 5% of patients, and are seen more frequently in metastatic versus primary tumors. They are more frequent in lobular carcinoma, including triple-negative lobular cancer. Many of these variants are resistant to trastuzumab and lapatinib. However, neratinib can be efficacious, and recent data suggest that antibody–drug conjugates (ADCs) such as ado-trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan may also be helpful. Laboratory and clinical data raise the possibility that simultaneous treatment with ADCs plus neratinib may be even more efficacious. Tucatinib, which has demonstrated significant activity in the central nervous system, has also been shown in vitro to be active against a number of these HER2 variants. This report describes a patient with metastatic estrogen receptor–positive, HER2-nonamplified breast cancer with an activating HER2 mutation whose tumor became resistant to neratinib as well as capecitabine, but whose subsequent leptomeningeal disease had a dramatically successful response to tucatinib plus capecitabine. As the frequency of HER2 mutations increases during the evolution of metastatic breast cancer, it is important to obtain genomic evaluation on these tumors with either repeat tissue or liquid biopsy as they progress over time.

MOLECULAR INSIGHTS IN PATIENT CARE

HER2/neu amplification occurs in approximately 15% of breast cancers. Dramatic advances have occurred in the treatment of these patients with the introduction of a host of target specific therapies, including monoclonal antibodies, tyrosine kinase inhibitors (TKIs), and antibody–drug conjugates (ADCs). HER2/neu somatic mutations, however, occur in only approximately 3% to 5% of untreated breast cancers,1–4 and have an incidence of approximately 10% in lobular carcinoma.3 In patients with lobular breast cancer, the frequency of HER2 mutations has been reported to be increased, particularly when there is a concurrent CDH1 mutation.5,6 Conforti et al,7 combining their own data with those from publicly available datasets, reported that 7 of 35 (20%) triple-negative lobular tumors harbored HER2 mutations. In ductal carcinomas they are more common in estrogen receptor (ER)–positive tumors and in metastatic rather than primary lesions. It is uncommon for them to occur in tumors that are HER2-amplified.3,6 Most, but not all, HER2 variants result in increased HER2 signaling and tumor growth.8 Studies have suggested that HER2 mutations may cause estrogen deprivation resistance independent of any effects on the ER.8,9

Approximately 70% of HER2 mutations occur in the kinase domain between amino acids 755 and 781 (exons 19 and 20), while another 20% are found in the extracellular domain of either amino acid 309 or 310 (exon 8). A small percentage are found to be in the transmembrane or juxtamembrane domains.3,6,8,9 These latter variants, which have been reported in colon, breast, and lung cancers, have been confirmed to be oncogenic and, in contradistinction to the more common variants, sensitive in vitro to standard HER2 blockade.10,11 Trastuzumab and lapatinib have not been very active against the kinase and extracellular domain variants,3,6,8,9 but the TKI neratinib has shown good in vitro and clinical activity.3,6,10,12 The most recently approved TKI, tucatinib, has demonstrated activity in vitro and in patient-derived xenograft (PDX) models against some HER2 mutants,13 but there are no clinical data on its efficacy in HER2-mutant tumors. A phase II basket trial (ClinicalTrials.gov identifier: NCT04579380) is evaluating the efficacy of...
tucatinib + trastuzumab in solid tumors (plus fulvestrant in hormone receptor [HR]–positive HER2-mutant breast cancer) with either HER2 amplification or mutation. This case report presents the successful use of neratinib for systemic disease and subsequent dramatically successful tucatinib use for leptomeningeal disease in a patient harboring the activating p780ins HER2 variant.

Methods
A literature search was performed on December 10, 2021, in Google, Google Scholar, Publons, Science Direct, and PubMed using the keywords HER2 and ERBB2 mutation and variant, breast cancer, and leptomeningeal.

Case Report
A female patient was diagnosed with stage IIA (T2N0) invasive lobular carcinoma of the left breast in 2013 at age 54 years. The tumor was ER-positive, progesterone receptor-negative, and HER2/neu-negative by immunohistochemistry (IHC). Her Oncotype Dx score was 29. She underwent left breast lumpectomy and sentinel lymph node biopsy, followed by adjuvant chemotherapy with 4 cycles of docetaxel and cyclophosphamide and then whole-breast radiation therapy (RT). She began letrozole in April 2014.

In October 2017, liver biopsy confirmed metastatic lobular carcinoma, ER-positive, progesterone receptor-negative, HER2/neu 2+ by IHC. Fluorescence in situ hybridization (FISH) testing showed HER2 1.96/CEP17 1.88 with a FISH ratio of 1.04. She started capecitabine but experienced disease progression within 5 months. She subsequently received fulvestrant and palbociclib with no response. The liver metastasis demonstrated HER2 p780_y781insGSP (Foundation Medicine), an activating HER2/neu variant. She received treatment with docetaxel/trastuzumab/pertuzumab but progressed after 6.5 months. She then experienced a partial clinical response (PR) to vinorelbine/neratinib for 8.5 months, followed by a PR to eribulin/neratinib for 6 additional months without significant toxicity. She started trastuzumab deruxtecan (T-DXd) in May 2020 due to systemic disease progression. After one dose of T-DXd, she developed subacute onset of dysarthria and gait instability. Brain MRI showed extensive abnormal enhancement within the cerebellar folia, with associated sulcal FLAIR hyperintensities, and in the bilateral medial parietal sulci, consistent with leptomeningeal disease (Figure 1). The patient declined a spinal tap and underwent whole-brain RT. Brain MRI 8 weeks after completion of RT had similar distribution of abnormal leptomeningeal enhancement with overall only minimal improvement. The patient continued to deteriorate symptomatically.

The patient started capecitabine and tucatinib in September 2020. Her neurologic symptoms quickly resolved, and results of a brain MRI 3 months later showed resolution of her leptomeningeal disease (Figure 2). Her systemic disease stabilized and remained so throughout the remainder of her life. After 7 months, an equivocal change in brain scan was noted and fulvestrant was added. However, at 10 months there was clear progression by MRI scan. After one unsuccessful dose of T-DM1, she developed rapidly worsening symptomatic central nervous system (CNS) disease and died with her systemic disease still stable.

Discussion
Mutations of HER2 were first described in non–small cell lung cancer (NSCLC) in 200414 and in breast cancer in 2009.15 The highest incidence occurs in bladder cancer (9%–18%), followed by uterine cervix (6%), colorectal (5%–8%), lung (2%–4%), and breast cancer (3%–5%).3,4,14,16–21 For patients with lung cancer, an increased risk of these variants has also been noted in adenocarcinoma,14 non-smokers, women, and persons of Asian descent.22

Patients with NSCLC harboring HER2 mutations have a worse prognosis than those who do not, as well as worse survival than those with other mutations.20,23 Use of TKIs has improved survival,20,23 though response rates have been in the range of 25% to 30% and median duration of responses with TKIs have only been in the range of 4 to 7 months.20,24,25 The utility of TKIs has been shown to vary with the identity of the specific variant,26 as well as with which TKI was used, with poziotinib and pyrotinib showing suggestively higher response rates than neratinib, dacomitinib, and afatinib.25

In the SUMMIT trial, a basket trial utilizing neratinib for HER2-mutant cancers, patients with cervical cancer (n=5) had an objective response rate (ORR) of 20% at 6 months and a median progression-free survival (mPFS) of 20.1 months. The clinical benefit rate (ORR plus stable disease) was 60%.26 Patients with biliary cancer (n=9) demonstrated an ORR of 22.2% at 8 weeks, but the 6-month ORR was 0%; mPFS was 2.8 months. Patients with colorectal, bladder, or endometrial cancer did not demonstrate objective responses.27,28

Several preclinical studies have been performed with HER2 variants in breast cancer. Wang et al29 showed that the activating Y784A mutation was sensitive to trastuzumab and lapatinib in MCF10A cells. Bose et al30 studied 13 additional variants in the same cell line. Three variants (R678Q, I767M, and Y835F) were not functionally active, whereas G309A, L755S, D769H/Y, V777L, V842L, R896Q, and P780insGSP, which our patient’s tumor harbored, were active. All were sensitive in vitro to neratinib. L755S, the most common variant reported in breast cancer, was resistant to lapatinib in this and multiple other studies.3,6,28–30 Kancha et al31 demonstrated a wide range of antitumor activity for lapatinib, depending on the variants being tested, with H878Y and V777L the most
sensitive and T798M and L755S the most resistant. Zuo et al\textsuperscript{32} reported similar findings in both MCF10A cells and xenograft (PDX) models. Cocco et al\textsuperscript{12} studied HER2-mutant strains in SK-Br-3 and BT474 cells and demonstrated good antitumor activity by neratinib but poor activity for lapatinib and trastuzumab, though they noted that preclinical sensitivities did not always correlate well with clinical activity.

Croessmann et al\textsuperscript{8} studied HER2 variants specifically in ER-positive tumors. They hypothesized that HER2 mutations constitute a mechanism of hormone resistance. Studies were performed with MCF7 cells as well as athymic mouse xenografts and the HER2 missense variants G309A, L755S, and V777L. Both estrogen deprivation resistance and fulvestrant resistance in HER2 variants could be reversed by neratinib. HER2 mutations hyperactivated HER3 and the PI3K/AKT/mTOR signaling pathway, resulting in estrogen-independent growth and resistance to endocrine therapy. Treatment with a PI3K\alpha inhibitor, a TORC inhibitor, or HER3 siRNA restored sensitivity to fulvestrant and to estrogen deprivation. These observations are consistent with those of Hanker et al,\textsuperscript{33} who found synergy between alpelisib and neratinib against a HER2 mutant both in cell lines and PDXs. However, this combination has not been evaluated clinically, and due to overlapping toxicities is not likely to be well tolerated. Their work also suggests that HER3 mutations may cause resistance to neratinib in HER2-mutant tumors.\textsuperscript{33,34}

Clinical trials have been performed to study neratinib in nonamplified HER2-mutated breast cancer. In the MutHER trial, 22 patients (21 ER-positive) received neratinib as a single agent. Clinical benefit was seen in 36% of patients, with 1 complete remission (CR), 1 PR, and 3 stable disease (SD) for ≥24 weeks.\textsuperscript{35} In part II of this trial, patients received fulvestrant/neratinib, 42.5% of whom had lobular disease.\textsuperscript{36} The fulvestrant-prior-treatment group (n=21) had 1 CR and 4 PRs with an mPFS of 24 weeks. Fulvestrant-naïve patients (n=10) demonstrated 3 PRs and an mPFS of 20 weeks. Patients in the exploratory triple-negative breast cancer (TNBC) arm (n=4) demonstrated 1 PR and had an mPFS of 8.5 weeks. Trastuzumab was added at disease progression on fulvestrant/neratinib for 4 patients with ER-positive disease and 1 with TNBC.

\textbf{Figure 1.} MRI of brain prior to start of tucatinib + capecitabine.
Clinical benefit was seen in 4 of 5 patients (3 PR, 1 SD >24 weeks) with an mPFS of 28 weeks, suggesting that dual blockade of HER2 is important. The SUMMIT trial is also looking at HR-positive, HER2-nonamplified, HER2-mutated breast cancer. Patients with HR-positive, HER2-negative breast cancer with prior CDK4/6 inhibitor use (n=33) who received fulvestrant/trastuzumab/neratinib had an ORR rate of 42.4% (1 CR, 13 PR). The clinical benefit rate was 51.5%. Median duration of response (DoR) was 14.4 months, and mPFS was 7 months. Tumor shrinkage was seen with mutations in all domains and in both ductal and lobular carcinoma. Responses were seen in patients with co-mutations in PIK3CA and EGFR. Patients in the randomized portion of this trial who received fulvestrant/trastuzumab or fulvestrant showed no objective responses (n=7 in each arm).

For the 18 patients with HER2-mutated TNBC who received neratinib + trastuzumab, the ORR was 33% (1 CR, 5 PR); the best ORR was 38.9%. Median DoR was not evaluable and mPFS was 6.2 months. Median duration of treatment was 4.4 months, and 16.7% of these patients experienced grade 3 diarrhea with no treatment discontinuations.

In the plasmaMATCH trial, circulating tumor DNA (ctDNA) analysis was used to direct therapy for patients with metastatic breast cancer. Cohort B comprised patients with HER2 mutations. All patients in this cohort received neratinib, and those who were ER-positive also received fulvestrant. A total of 5 of 20 patients (18 ER-positive) had confirmed objective responses (1 CR of 29 months’ duration, 4 PR); median DoR was 5.7 months. Three others had unconfirmed responses.

Tucatinib is the most recently approved TKI and is active against amplified wild-type HER2/neu in combination with trastuzumab. In addition, tucatinib has shown significant activity against CNS metastases in breast cancer when combined with trastuzumab. The 3-drug combination of trastuzumab/tucatinib/capecitabine was quite active in the HER2CLIMB trial and confirmed the CNS activity. Tucatinib has now also been studied in preclinical studies against HER2 variants. Peterson et al demonstrated that tucatinib alone was active and the combination of tucatinib and trastuzumab was even more active.
against V777L in colon cancer PDX models and S310Y in gallbladder and gastric cancer PDXs. They also showed tucatinib activity against L755S both as a single agent and combined with trastuzumab. Veeraraghavan et al35 studied TKIs in an ER-positive BT474 lapatinib-resistant cell line with the L755S variant. This study showed that when the cell line was sensitive to neratinib, it was also sensitive to tucatinib. However, when the line became resistant to neratinib, it demonstrated cross-resistance to tucatinib as well.

The neuropharmacokinetics of tucatinib in combination with capecitabine and trastuzumab have been reported for patients with leptomeningeal breast cancer in the TBCR049 trial. Both tucatinib and the parent compound ONT-993 were detectable in cerebrospinal fluid (CSF) within 2 hours after drug administration, with concentrations ranging from 0.57 to 25 ng/mL for tucatinib and 0.28 to 4.7 ng/mL for ONT-993.44 Steady state CSF-to-plasma ratios were 0.83 for tucatinib and were similar for ONT-993.

The patient reported in the current study highlights a number of issues related to HER2 variants. The tumor was an ER-positive lobular carcinoma, a phenotype associated with an increased risk of HER2 mutation. Her tumor was not HER2-amplified, which is most often the case. When the HER2 variant was discovered she was treated with neratinib plus first vinorelbine and then eribulin, with some response to each. Fulvestrant and capecitabine were not used because of prior progression. In the MutHER trial, it is not clear that fulvestrant/neratinib is superior to neratinib.35-37 and in the ongoing SUMMIT trial only the arm with 2 HER2-directed therapies and fulvestrant is showing activity.38 Eribulin45-53 and vinorelbine54-56 have demonstrated activity in HER2-amplified disease both as single agents and in combination with trastuzumab. Awada et al57 reported a 41% ORR for vinorelbine + neratinib in lapatinib-naïve patients and an 8% ORR in prior lapatinib HER2-positive patients. After our patient experienced disease progression on neratinib, tucatinib/capecitabine was used rather than neratinib/capecitabine, as was used in the NALA trial.58

Once fulvestrant had been added to this patient’s regimen, one might have added trastuzumab to tucatinib/capecitabine/fulvestrant, building on the tucatinib/trastuzumab/capecitabine triplet used in HER2CLIMB, but the MutHER part II data regarding patients who experienced progression on neratinib and the updated SUMMIT data were not yet available at the time of this patient’s treatment.37,38 In the SUMMIT trial, only the arm containing both neratinib and trastuzumab is doing well (as opposed to the trastuzumab/fulvestrant and fulvestrant arms).38 Given the data regarding ADCs in lung cancer, the treating physician opted for T-DM1 upon progression on tucatinib/capecitabine/fulvestrant.

Li et al59 reported a 44% response rate to T-DM1 in 18 patients with HER2-mutated lung cancer. Responses were seen in patients with exon 20 mutations and point mutations in the kinase, transmembrane, and extracellular domains; mPFS was 5 months. In cell lines and PDXs from patients with lung cancer, studying the variants L755S and S310F, they showed that HER2 ubiquitination and internalization, rather than overexpression, are key mechanisms promoting endocytosis and efficacy of both ADCs T-DM1 and T-DXd.60 Cotreatment with the irreversible TKIs neratinib and afatinib increased ubiquitination and ADC internalization. This was not seen with lapatinib and tucatinib, both reversible HER2 TKI inhibitors. They also found that T-DXd was active in T-DM1-resistant tumors.

Li et al61 also reported the clinical results of T-DXd in 91 patients with lung cancer with HER2 mutations. Responses were seen with a variety of HER2 mutations, including both kinase domain and extracellular domain variants; 99% of patients had had prior cancer therapy, including 14% with prior TKIs. The ORR was 55% with an median DoR of 3 months. mPFS was 8.2 months and median overall survival was 17.8 months. Mukohara et al62 reported a breast cancer case with both HER2 amplification and the L755S mutation showing excellent responses to T-DM1 and then T-DXd after progression on trastuzumab and lapatinib.

Both T-DM1 and T-DXd have shown activity in patients with HER2-amplified breast cancer with CNS disease.63-66 One basket trial using an ADC for HER2-mutated or HER2-amplified solid tumors is planned (ACE-Pan Tumor-02; ClinicalTrials.gov identifier: NCT05041972). This utilizes ARX788, an experimental drug that uses a noncleavable linker between a humanized HER2-targeting antibody conjugated to a cytotoxic tubulin inhibitor, Amberstatin (AS269).67 This study will enroll patients with stable brain metastases.

Studies are currently exploring tucatinib in combination with both T-DM1 (NCT03975647) and T-DXd (NCT04539938) for patients with breast cancer with HER2 amplification. In a phase Ib study of T-DM1 and tucatinib, Borges et al68 reported a 47% ORR with a median DoR of 6.9 months and a mPFS of 6.5 months at the maximum tolerated dose. Patients with measurable brain metastases had a 36% ORR. Another trial (NCT04752059) is exploring T-DXd specifically in patients with breast cancer with brain metastases. Studies are also examining the efficacy of a number of additional TKIs.69 It will be of interest to examine these combinations in patients harboring HER2 mutants as well.

Finally, this patient demonstrated a dramatic response of leptomeningeal disease to tucatinib, which is known to be active in the CNS. Tucatinib was combined with capecitabine, despite the fact that her disease had previously been treated unsuccessfully with capecitabine. Further, pharmacokinetic studies have suggested that the active
metabolite of capecitabine may not reliably reach therapeu-
tic levels in CSF\textsuperscript{70–72} and levels are expected to be somewhat dependent on dihydropyrimidine dehydro-
ase mutation status.\textsuperscript{73} This suggests tucatinib was the
main driver of response for the current patient and efficacy
not only in the CNS when this variant is present but also
efficacy in a patient whose HER2-mutated tumor had
become resistant to neratinib and capecitabine. One could
question whether the patient’s improvement was a late
effect of RT. However, the lack of any clinical or radio-
graphic response after 2 months and the dramatic and
rapid response after the initiation of tucatinib-based ther-
apy argues against this.

This patient’s particular HER2 mutation has been
investigated in NSCLC both in vitro and in the clinic.
Gow et al\textsuperscript{74} showed sensitivity to afatinib in vitro, and
multiple patients have had significant responses to this
TKI, with some lasting 1 to 2 years.\textsuperscript{20,74,75} Additionally, 4 of
6 patients were reported to have objective responses to
pyrotinib,\textsuperscript{20,76} and 2 patients with this variant have experi-
enced response to dacomitinib.\textsuperscript{77} We are unaware of any
prior reports of treatment of this variant with tucatinib.

Conclusions

HER2-mutated cancers present an unusual and difficult
clinical problem. Neratinib is known to have activity both
in vitro and in vivo in this patient cohort; tucatinib has
shown activity in preclinical studies as well. The case
reported herein demonstrates tucatinib activity in a
patient whose tumor had progressed on neratinib. Tuca-
тинib is likely to be clinically useful against HER2 muta-
tions, especially in the CNS. The sensitivity of specific
variants to tucatinib remains to be determined. In view
of T-DM1 and T-DXd activity in HER2-mutated lung
cancer, it will be of great interest to evaluate both as
single agents and in combination with TKIs in patients
with breast cancer.

As the frequency of HER2 mutations increases during
the evolution of metastatic breast cancer it is important
to obtain genomic evaluation of these tumors with either
repeat tissue or liquid biopsy as they progress over time
given the encouraging response rates with TKIs and
ADCs in patients with HER2 mutations.

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