Introduction

Human epidermal growth factor 2 (HER2 or ErbB2) is a receptor tyrosine kinase of the ERBB family, which also includes EGFR, HER3 and HER4. These receptors each contain an extracellular ligand binding region, a hydrophobic transmembrane domain as well as an intracellular domain with tyrosine kinase catalytic activity. When ligands bind to the extracellular domains of EGFR, HER3 and HER4, these receptors form heterodimers, which in turn activate various signaling cascades including the raf/mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and protein kinase C (PKC) pathways. These pathways play essential roles in cell proliferation, differentiation, angiogenesis and protection from apoptosis. Interestingly, although it is structurally quite similar to its relatives, there are no identified ErbB2-specific ligands. Instead, ErbB2 is constitutively activated, serves as the preferred dimerization partner for the other receptor kinases and heterodimers containing ErbB2 are rather stable and potent (1). As a result, deregulation and overexpression of ErbB2 leads to increased formation of these heterodimers, which in turn activate signaling cascades that potentiate cell proliferation, and ultimately serves as a potent driver of oncogenesis. Therefore, two main strategies have emerged in targeting ErbB2 amplification and mutations: antibodies (and antibody-drug conjugates), which are directed at the extracellular domain, and tyrosine kinase inhibitors (TKIs), which target the intracellular kinase domain. Especially promising among these is poziotinib, a novel, quinazoline-based irreversible TKI, which has shown efficacy in patients with exon 20 mutations.

Case presentation

A 68-year-old gentleman with a remote smoking history and no significant medical comorbidities presented initially with a persistent cough that had worsened over the course of several months. A chest computed tomography (CT) showed a right upper lobe mass with associated adenopathy, and a positron emission tomography (PET)/CT demonstrated PET-avid liver, adrenal and bony metastases. He was referred for a CT-guided biopsy of the right iliac bone, and
results showed a TTF1+ adenocarcinoma compatible with lung origin. Unfortunately, the specimen was not sufficient for further molecular testing. Further workup included a brain magnetic resonance imaging (MRI), which showed multiple small (less than 4 mm) lesions, consistent with brain metastases, and a repeat bone biopsy, which ruled out the presence of EGFR/ALK abnormalities and showed advanced, ErbB2 YVMA mutated lung adenocarcinoma. The patient received stereotactic radiosurgery (SRS) for the brain metastases, and a repeat brain MRI showed resolution of all but one lesion, which showed treatment-related changes. He was started on treatment with carboplatin/pemetrexed/pembrolizumab and initially had an excellent response. However, after approximately 8–9 months of treatment, he was found to have bony progression in the left hemipelvis. Extended molecular testing at that time confirmed the above ErbB2 mutation. He was then given second-line treatment with docetaxel/ramucirumab. The patient developed considerable adverse effects related to this regimen after just 2–3 cycles and was subsequently started on the anti-ErbB2 drug conjugate, ado-trastuzumab emtansine (T-DM1) at an outside institution. However, within several months, he was found to have progression in central nervous system (CNS) metastases and received whole brain radiation therapy. He then resumed care at our institution and was started on poziotinib 16mg daily on a research protocol. After just one cycle of treatment, imaging showed improvement with significant reduction in burden of metastatic disease (Figure 1). Unfortunately, later significant toxicities developed preventing patient from being able to continue study medicine with subsequent decline in performance status.

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Oncogenic activation of ErbB2 occurs primarily by three mechanisms: amplification and overexpression, somatic mutations of the receptor and inhibition of phosphatase activity (2). ErbB2 amplification was first discovered in breast cancer and is found in 15–30% of invasive breast carcinomas. In that setting, it serves both prognostic and predictive value as it is associated with shorter progression-free and overall survival (3). ErbB2 amplification has now been identified in various other cancers as well, including ovarian, uterine, gastric, colon, bladder, lung and is a validated treatment target in gastric adenocarcinoma. More

![Image of imaging results showing significant reduction in burden of disease before and after treatment with poziotinib.](image-url)
recent studies have shown that deregulation of the Erbb2 gene can also result from various somatic mutations. First identified in lung adenocarcinoma, these have now been identified in various other malignancies as well. As with EGFR mutations, which are mutually exclusive with Erbb2 mutations, the latter have more commonly been identified in non-smokers, women, Asians and in adenocarcinoma (4). Activation of Erbb2 can result from missense mutations or insertions in the kinase domain, as well as from missense mutations (such as the S310 single-site substitution) or deletions of the extracellular domain. The most common alterations of Erbb2 involve in-frame insertions in exon 20, such as the YVMA insertion at codon 776. These mutations result in a conformational change in the autoinhibitory αC-β4 loop, which narrows the ATP-binding site and as a result promotes constitutive kinase activity (5). Less common are missense mutations in the extracellular domain. These promote constitutive kinase activity by either inducing disulfide bond formation (such as S335C or G309E) or by increasing C-terminal tail phosphorylation (such as S3105 and S310Y) (6). Lastly, Erbb2 can be activated by inhibition of phosphatase activity, and this is supported by in vitro studies which have shown that inactivation of the PTPN12 phosphatase promotes Erbb2 activation (7).

ErbB2 alterations can be identified using various diagnostic assays including enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), as well as next generation sequencing (NGS) (4,8,9). However, no gold standard exists. Traditionally, the most commonly used methods to detect ErbB2 amplifications have been IHC, to quantify the ErbB2 protein as well as FISH to determine the ErbB2 gene copy number. While the former is widely available and easy to perform, results are variable and depend on a variety of factors including the antibody used as well the cutoffs used to define positivity. FISH, on the other hand, is not as easily available but much more specific. As a result, in breast cancer, IHC is often used a screening test, and a reflex FISH is performed if results are equivocal. In non-small cell lung cancer (NSCLC), however, this approach has not been favored as there is often significant discordance between IHC and FISH results (10). More recently, dual in situ hybridization (DISH) has been shown to be better at detection of ErbB2 amplification compared to FISH (11). ErbB2 mutations, on the other hand, can be detected using Sanger sequencing, NGS as well as mass spectrometry genotyping. Of these, NGS is highly sensitive, and allows for the detection of various mutations, rearrangements as well as amplifications, and is the preferred methodology for molecular testing in lung cancer as it can detect all potential actionable alterations.

Patients with ErbB2 aberrations have a shorter overall survival than those with other oncogenic drivers (12), and there remains a significant unmet need to develop the optimal targeted therapy for these patients. At present, two main strategies have been used in targeting ErbB2 amplification and mutations: antibodies (and antibody-drug conjugates), which are directed at the extracellular domain, and TKIs, which target the intracellular kinase domain (2).

Trastuzumab, a humanized monoclonal antibody that targets the extracellular domain of the ErbB2 receptor, is standard of care in the management of ErbB2-amplified breast cancer. Unfortunately, early studies did not show similar efficacy or survival benefit in NSCLC as a single agent (13) or when combined with gemcitabine-cisplatin (14) or docetaxel (15). However, it is important to note that these early trials selected patients on the basis of ErbB2 positivity on IHC and we now know that ErbB2 mutations, which do not always result in ErbB2 overexpression, likely play a more important role in oncogenesis in NSCLC. More recent retrospective analyses have provided more promising results. A 2013 study identified 65 patients with ErbB2 mutations, 16 of which were treated with trastuzumab-based therapies after conventional chemotherapy, and reported a disease control rate of 96% (16). A similar retrospective analysis of a cohort of 101 patients with ErbB2 exon 20 mutations identified 65 patients who had received ErbB2-directed therapy after conventional chemotherapy. Of these, 57 patients had received trastuzumab in combination with chemotherapy, suggesting a disease control rate of 50%, and supporting the use of combined regimens (17). T-DM1, an ErbB2-targeted antibody-drug conjugate, has shown benefit as well. A study by Li et al. in 2018 showed a partial response rate of 44% and median progression-free survival (PFS) of 5 months in 18 patients with advanced, ErbB2-mutant lung adenocarcinomas who were treated with T-DM1 (18). These patients had various ErbB2 mutations including exon 20 insertions and point mutations in the transmembrane and extracellular domains.

TKIs have been studied in this setting as well. Afatinib, an irreversible inhibitor of EGFR, ErbB2, and HER4, has shown some efficacy in case reports and retrospective reviews. In a series of three patients with various ErbB2 exon 20 mutations, each achieved a partial response, lasting as long as 4 months (19). Another case report showed a more durable, 10-month response in a patient with the common exon 20
Table 1 Summary of efficacy/major AEs with agents/trials

<table>
<thead>
<tr>
<th>Agents</th>
<th>Mechanism of action</th>
<th>Eligibility</th>
<th>Efficacy</th>
<th>AEs</th>
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<tr>
<td>T-DM1</td>
<td>Cytotoxic anti-microtubule agent released within the target cells upon degradation of the human HER2-T-DM1 complex in lysosomes</td>
<td>IHC 3+ Mutation Amplified/mutation</td>
<td>44% (partial response rate)</td>
<td>Fatigue, nausea, bone and joint pain, muscle pain, thrombocytopenia headache, constipation, nerve damage, anemia, hypo-potassium</td>
<td>(18)</td>
</tr>
<tr>
<td>Pertuzumab, trastuzumab</td>
<td>Humanized mAb directed against the extracellular domain of the tyrosine kinase receptor HER2</td>
<td>Amplified Mutation</td>
<td>50–96% (disease control rate)</td>
<td>Cardiac failure, cardiomyopathy, CHF, pulmonary infiltrates, anemia, neutropenia Pulmonary toxicity, acute renal failure, hepatotoxicity, exfoliative dermatitis, hypokalemia</td>
<td>(13-17)</td>
</tr>
<tr>
<td>Afatinib</td>
<td>Irreversibly binds to the intracellular tyrosine kinase domain, subsequently inhibits EGFR, HER2 and HER4 receptors</td>
<td>Mutation</td>
<td>15% (partial response rate)</td>
<td></td>
<td>(19-21)</td>
</tr>
<tr>
<td>Dacomitinib</td>
<td>Irreversible binding at the ATP domain of the EGFR family kinase domains</td>
<td>Mutation</td>
<td>12% (partial response rate)</td>
<td>Interstitial lung disease, diarrhea, rash, hypokalemia</td>
<td>(22)</td>
</tr>
<tr>
<td>TAK788</td>
<td>EGFR antagonists; ERBB 2 receptor Mutation</td>
<td>Exon 20 mutation</td>
<td>20–56% (partial response rate)</td>
<td>Diarrhea, nausea, rash, vomiting, decreased appetite</td>
<td>(28,29)</td>
</tr>
<tr>
<td>Poziotinib</td>
<td>Inhibitor of EGFR and HER2 exon 20 insertion mutants</td>
<td>Exon 20 mutation</td>
<td>50% (partial response rate)</td>
<td>Rash, diarrhea, paronychia</td>
<td>(30,31)</td>
</tr>
</tbody>
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T-DM1, ado-trastuzumab emtansine; IHC, immunohistochemistry; HER2, human epidermal growth factor 2; AEs, adverse event; mAb, monoclonal antibody; CHF, congestive heart failure; EGFR, epidermal growth factor receptor.

YVMA insertion (20), and an international retrospective review of 27 patients with metastatic ErbB2-mutant disease showed a partial response rate of 15% (21). Dacomitinib, a second-generation, irreversible inhibitor of EGFR, ErbB2, and HER4, has been studied as well. A phase two trial enrolled 30 patients with ErbB2 mutations or amplifications and found a partial response rate of 12% that lasted as long as 14 months in patients who had ErbB2-mutated disease. No partial responses occurred in those with ErbB2 metabolic amplification or in those with the most common exon 20 insertion, A775 G776insYVMA (22). Neratinib, another second-generation TKI, produced a response rate of 19% and progression free survival of up to 18-plus months in a cohort of 43 patients with ErbB2 mutations, when it was used in combination with the mammalian target of rapamycin (mTOR) inhibitor temsirolimus (23). Lapatinib, a dual TKI which inhibits ErbB1 and ErbB2, has been shown to be effective in HER2-positive breast cancer but has not demonstrated activity in NSCLC. More recently, preclinical data suggests that osimertinib may be effective in targeting ErbB2 aberrations as well, particularly amplification (24). Tarloxsotinib, a prodrug that releases an irreversible EGFR/ErbB2 TKI under hypoxic conditions, has also shown promising results in a recent in vivo study of murine xenografts of two adenocarcinoma cell lines with exon 20 mutations (25,26). After 4 weeks of treatment with tarloxsotinib, the authors found significant reduction in tumor burden as compared to afatinib which did not alter tumor growth.

Although TKIs have certainly shown some activity, response rates are low and there are no approved agents for ErbB2-mutant lung cancer. Newer data is encouraging. TAK-788 (AP32788), an investigational TKI that targets EGFR and ErbB2 mutations, is being studied in a phase I/II, open-label, multicenter study which administered the drug in dose escalation and expansion cohorts, which were based on tumor genotype. Initial data showed a partial response rate of 21% (n=14) in patients with EGFR exon 20 insertions (27) and more recently published data shows an objective response rate of 54% and disease control rate of 89% in the 26 patients assessed (28). The ongoing clinical trial will help assess efficacy in patients with ErbB2 alterations (29). Equally exciting is data on poziotinib, a novel, quinazoline-based irreversible inhibitor of EGFR, ErbB2 and HER4. This potent, small and flexible inhibitor is hypothesized to be more effective in those with exon 20 mutations as these alterations reduce the size of the drug-binding pocket, rendering most larger TKIs ineffective (30). A phase I/II trial of 50 patients with metastatic, EGFR or ErbB2 exon 20-mutated lung adenocarcinoma showed an objective response rate of 50% and disease control rate of 83% in the latter group (31). The mean PFS for these patients was 5.1 months (Table 1). Further studies are...
needed to determine the efficacy of these various agents.

**Conclusions**

Our patient showed an initial response to poziotinib reflecting the potential actionability of ErbB2 mutations in non-small cell lung cancer, however further data is necessary to assess its effectiveness in patients with the YVMA exon 20 insertion. Our case highlights the importance of developing agents that precisely target the various ErbB2 mutations, in particular the common YVMA exon 20 mutation, as these molecular alterations do not respond similarly to the various ErbB2-targeted agents.

**Acknowledgments**

None.

**Footnote**

**Conflicts of Interest:** 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.

**References**


