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Case report

Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu☆,☆☆

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ABSTRACT

Human epidermal growth factor receptor (HER)2/neu kinase domain mutations are found in approximately 1–4% of lung adenocarcinomas with a similar phenotype to tumors with epidermal growth factor receptor (EGFR) mutations. Afatinib is a potent irreversible ErbB family blocker. We determined the tumor genomic status of the EGFR and HER2 genes in non- or light smokers with lung adenocarcinoma in patients who were entered into an exploratory Phase II study with afatinib. Five patients with a non-smoking history and metastatic lung adenocarcinomas bearing mutations in the kinase domain of HER2 gene were identified, three of which were evaluable for response. Objective response was observed in all three patients, even after failure of other EGFR- and/or HER2-targeted treatments; the case histories of these patients are described in this report. These findings suggest that afatinib is a potential novel treatment option for this subgroup of patients, even when other EGFR and HER2 targeting treatments have failed.

1. Introduction

The presence of activating mutations in the tyrosine kinase domain of the human epidermal growth factor receptor 1 (EGFR/HER1/erbB1) in non-small cell lung cancer (NSCLC) correlates with a clinical phenotype of adenocarcinoma in never or light smokers, and renders the tumor exquisitely sensitive to EGFR tyrosine kinase inhibitors (TKIs) [1–3]. The introduction of targeted drugs for the treatment of NSCLC with EGFR-directed small-molecule TKIs [3] and monoclonal antibodies [4] has led to a significant but relatively small overall improvement in clinical outcome of unselected patients with advanced disease. EGFR mutations and increased EGFR copy number by fluorescence *in situ* hybridization (FISH) are predictive biomarkers that identify patients who are most sensitive to TKIs [5,6].

HER2 kinase domain mutations are rare in NSCLC, and are found in approximately 1–4% of lung adenocarcinomas with a similar phenotype as tumors with EGFR mutations [7–9]. In 229 patients with adenocarcinoma of the lung, with a little or no smoking history, we identified a HER2 mutation in the tumor tissue of five patients (2%), which is 10-fold rarer than the frequency of EGFR mutations in the same cohort of patients [10]. In other cohorts with potentially differing phenotypic selection criteria, the HER2 mutation rate was even lower: in tumors from 830 patients analyzed within the NCI's Lung Cancer Mutation Consortium (LCMC) [11] a HER2 mutation was found in only three cases (1%) compared to 98 cases with an EGFR mutation. In 552 samples analyzed at Massachusetts General Hospital, only one patient with a HER2 mutation was identified [12]. The HER2 mutations found in clinical samples so far are all in exon 20.

Afatinib is a potent, irreversible ErbB family blocker with preclinical activity in Ba/F3 cells expressing an artificial HER2 mutant and in a human lung cancer cell line with an insertional mutation at codon 776 [13].

We determined the tumor genomic status of the EGFR and HER2 genes in non- or light smokers with lung adenocarcinoma by denaturing gradient gel electrophoresis (DGGE)/DNA sequencing of NSCLC tumor tissue or increased copy number of the EGFR gene, as determined by FISH analysis. HER2 FISH was not required

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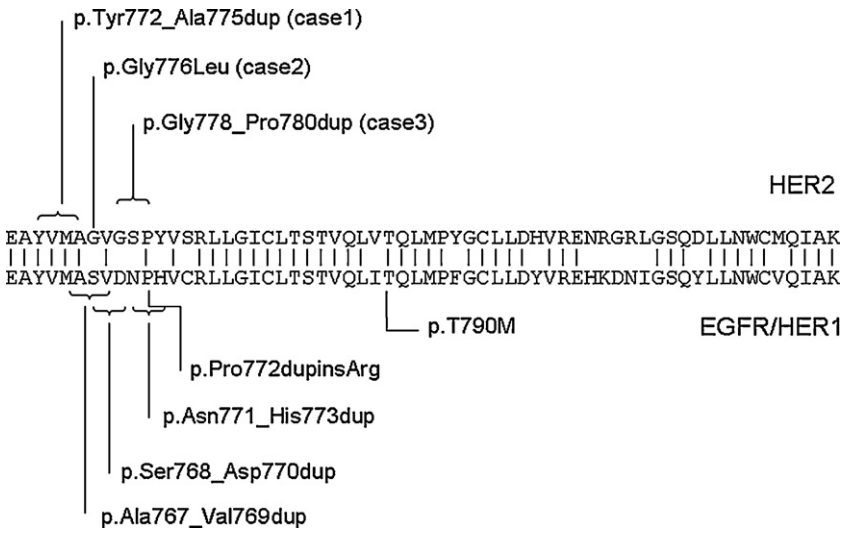

Fig. 1. Examples of HER2 exon 20 mutations.

for entry into the study and therefore not systematically undertaken. In Case 2, HER2 FISH was performed long before inclusion into the current study. Patients were entered into this exploratory Phase II study with afatinib, which, among others, included a cohort of patients with HER2 kinase domain mutations [14]. There were no restrictions in prior therapy for patients with HER2 mutations, although patients had to have at least one measurable tumor lesion that could be accurately measured by computed tomography (CT) scan or magnetic resonance imaging [14]. Here, we report the first therapeutic activity of afatinib in three patients with lung adenocarcinoma and a non-smoking history, whose tumors exhibited activating HER2 mutations in exon 20 (Fig. 1). Treatment with afatinib resulted in an objective remission in all three patients, even after failure of other EGFR- and/or HER2-targeted treatments. Following disease progression, there was an option to combine a lower level of afatinib with weekly paclitaxel at 80 mg/m^2 on a 3/4-week schedule. Five patients were treated in this study; two patients were not evaluable due to early treatment discontinuation. The study was approved by the Ethical Committee of the Universitair Ziekenhuis Brussel and participating centers and patients provided informed consent. Here we report on three evaluable patients.

2. Case 1

A 72-year-old, non-smoking female was diagnosed with a stage III lung adenocarcinoma (right lower lobe) in May 2007. Treatment with four cycles of carboplatin/gemcitabine resulted in a partial remission. Following progressive disease (PD) in January 2008, administration of an additional four cycles of reduced dose carboplatin/gemcitabine resulted in stable disease (SD). In May 2008, the patient was found to have PD in the lung, with symptoms of mildly productive cough. An exon 20 HER2 mutation (p.Tyr772_Ala775dup; Fig. 1) was found in the tumor DNA extracted from the original diagnostic biopsy in May 2007.

Treatment with afatinib (50 mg/day) started in July 2008. After 8 days, positron emission tomography-CT (PET-CT) imaging showed a radiological partial response (PR) and a metabolic complete response that was maintained for 3 months (Fig. 2A). Treatment was interrupted three times due to side effects (diarrhea, dysgeusia and skin adverse events [AEs]; all Common Terminology Criteria for Adverse Events [CTCAE] Grade 2) and prompted successive dose reductions to 30 mg/day. The patient was deemed to have progression after 3 months based on an approximate 20% increase in target lesions above the nadir, although the total tumor burden was below baseline and the patient continued to receive monotherapy with afatinib. Following further progression in May 2009, afatinib was combined with paclitaxel, but the patient showed progression solely due to the occurrence of brain metastases shortly afterwards and died one month after going off study without having received any subsequent therapy. The patient was treated with afatinib for a total of 9 months and survived one year from study entry.

3. Case 2

A 62-year-old, non-smoking female with adenocarcinoma of the right lung was initially diagnosed in 2002. Her tumor cells had increased EGFR/HER1 copy number, as assessed by FISH, as well as mutations in the EGFR kinase domain (exon 21: p.Ala859Thr) and in HER2 (exon 20: p.Gly776Leu). She underwent a lobectomy for a pT2N1 adenocarcinoma and received adjuvant chemotherapy with cisplatin/gemcitabine, followed by radiotherapy. A relapse in the lung and mediastinal lymph nodes in July 2003 was treated with four cycles of the same chemotherapy, resulting in SD. From 2004 through 2008, PD was treated sequentially with docetaxel (six cycles; SD), gefitinib (PD), trastuzumab with paclitaxel (PR), lapatinib, gemcitabine and vinorelbine.

At inclusion in the current study, this patient suffered from dyspnea and retrosternal and right chest wall pain requiring narcotic pain relief, as well as facial and cervical soft-tissue congestion. Her Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 2.

From July 2008, this patient was treated with afatinib (50 mg/day). Within 2 weeks, the cervical soft-tissue swelling decreased with marked improvement in her general condition (ECOG PS: 1). On Day 15, a metabolic response was observed in a PET-CT scan (Fig. 2B). Treatment-related AEs included skin reactions, diarrhea, intermittent nausea and vomiting, pyrosis and epigastric pain, fatigue, mucositis, sialorrhea, hair thinning, nail changes and fissures of the nail bed and fingertip. After 2 months of treatment (August 2008), a PR was observed by CT scan. Treatment was interrupted due to the associated diarrhea, and the dose was reduced successively to 40 mg/day and 30 mg/day (October 2008). At that time, the patient was progressive compared to the nadir of response, but still had a tumor burden reduction (20% decrease in target lesions) by CT scan, compared to baseline. The time to progression on single-agent afatinib was 4 months; in December 2008, she developed further PD in the liver and mediastinal lymph

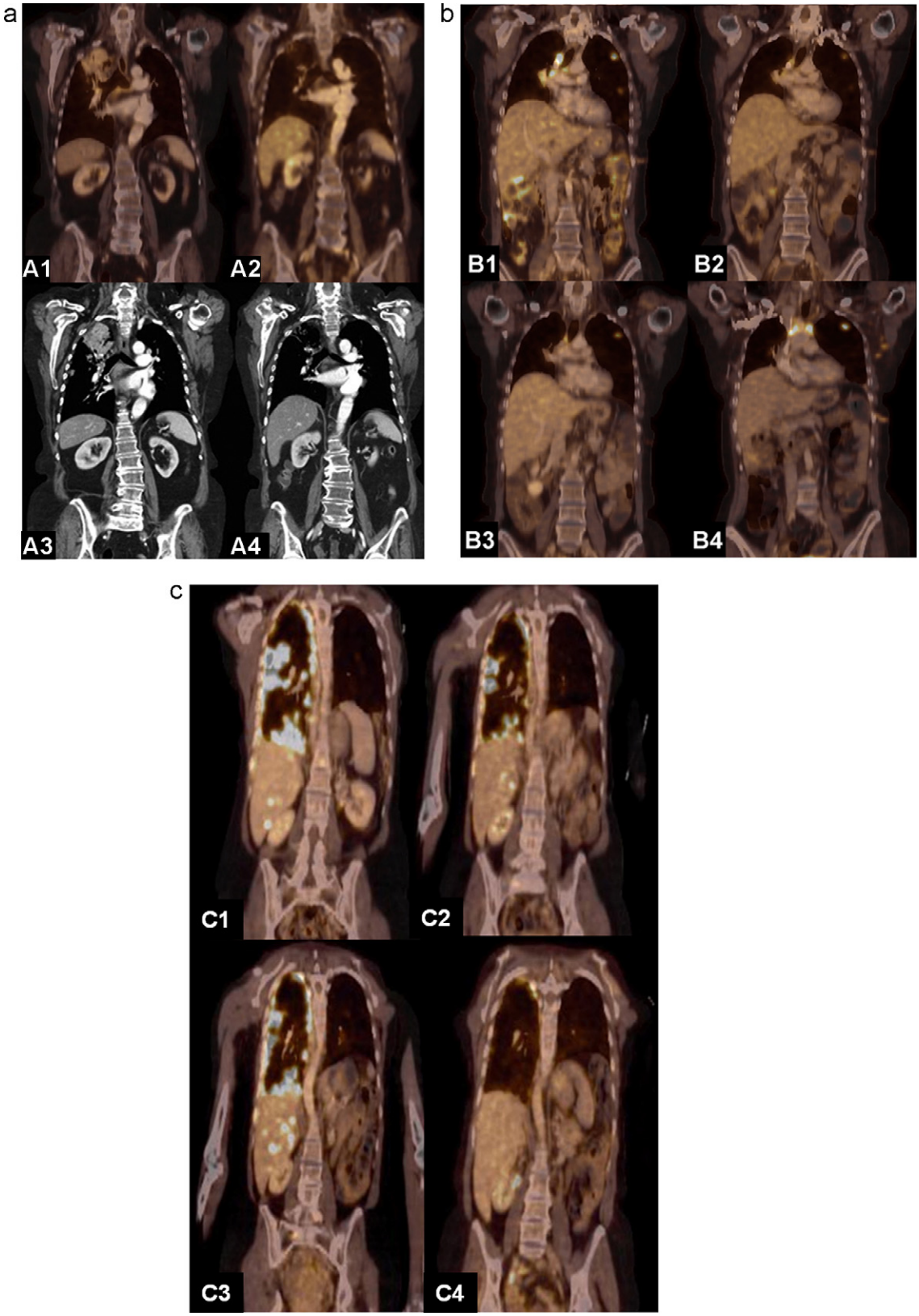

Fig. 2. (A) Case 1 – response to single-agent afatinib. Panels A1 and A3 are the baseline PET-CT and CT scans, respectively. Panels A2 and A4 are the post-treatment PET-CT and CT scans showing the early response to afatinib. (B) Case 2 – response to single-agent afatinib. Panel B1 is a baseline PET-CT image. Panels B2 and B3 are the post-treatment PET-CT scans showing metabolic response on Day 15 of treatment and partial remission after 2 months, with disease progression at 4 months (Panel B4). (C) Case 3 – response to single-agent afatinib and in combination with paclitaxel. Panel C1 is a baseline PET-CT image, panel C2 shows the important response in pleural and liver disease. Panel C3 is a baseline PET-CT image for subsequent combined afatinib–paclitaxel treatment and panel C4 demonstrates the profound response to the combination.

nodes. Weekly paclitaxel was added and the dose of afatinib was reduced to 20 mg. The patient had SD overall, but with a metabolic and radiological response in the liver for 9 months until April 2009, after which she progressed. The time to progression after paclitaxel was added to afatinib was 4 months. The patient died in September 2009, a total of 14 months from study entry.

4. Case 3

In March 2006, a 49-year-old Caucasian, non-smoking woman was diagnosed with stage IV right upper-lobe lung adenocarcinoma with diffuse pleural, liver and soft-tissue metastases. The tumor cells had an increased EGFR gene copy number, as assessed by FISH, with a wild-type sequence. This patient received first-line treatment with erlotinib at 150 mg/day, but clinical and radiological progression occurred within 3 months. From June 2006, she was treated with cisplatin/gemcitabine, with an objective tumor response, but treatment was interrupted due to cumulative toxicity. She then received, sequentially, gemcitabine (PD), carboplatin (transient response, but hematological intolerance), vinorelbine (PD), pemetrexed (transient response) and weekly cisplatin (symptomatic and objective response; treatment stopped because of intolerance). Additional genomic analysis revealed an insertional duplication (p.Gly778_Pro780dup) in exon 20 of the HER2 gene (Fig. 1). At inclusion in the current study in June 2008 [14], the patient was severely symptomatic, with pain in the right chest, right hypochondrium and right shoulder, and anorexia and fatigue. She had also developed asymptomatic bone metastases and had an ECOG PS of 1.

Within 2 weeks of starting afatinib (50 mg/day), the patient had a rapid clinical and symptomatic response, with disappearance of all disease-related symptoms, as well as overall SD with a radiological response in liver and pleura, which was maintained for 3 months (Fig. 2C). Treatment with afatinib (50 mg/day) was associated with skin-related AEs, diarrhea and mucosal inflammation with intermittent epistaxis, aphthous stomatitis and dry eyes.

The time to progression on single-agent afatinib was 4 months; following PD in October 2008, the patient received afatinib (40 mg/day) combined with weekly paclitaxel (80 mg/m^2). After one cycle, disease-related symptoms disappeared and a dramatic partial remission was seen. As of July 2009, this patient had an ECOG PS of 0, a disease volume of less than that at her remission after first-line cisplatin-based chemotherapy 2.5 years earlier (Fig. 2C). Sustained control of carcinoembryonic antigen (CEA) tumor marker levels was also achieved during afatinib treatment. There was an increase in CEA levels during ineffective prior chemotherapy treatment and CEA levels declined rapidly to normal after combination of afatinib and weekly paclitaxel. Afatinib treatment was continued for a total of 15 months, 11 of which were in combination with paclitaxel, after which time the patient developed a brain metastasis without concurrent progression at the other disease sites. Adverse events with afatinib and weekly paclitaxel were mild and included skin reaction, diarrhea, fatigue and hematological AEs.

After going off study in September 2009, the patient received trastuzumab sequentially combined with weekly paclitaxel for 6 months (CEA marker stabilization for 3 months), liposomal doxorubicin for 4 months (marker stabilization for 2 months), weekly cisplatin for three administrations, and oral etoposide for 3 months with no further clinical benefit. In addition, she developed leptomeningeal disease in June 2010, which was treated with four intrathecal administrations of depocyte leading to a durable complete cytological and symptomatic response of her leptomeningeal disease. The patient died in March 2011, with an overall survival of 32 months after inclusion in the study.

5. Additional cases

Two other patients with HER2 mutations were enrolled into the study, but both cases were considered to be non-evaluable. One patient was a 51-year-old woman with a 4 pack-year smoking history (who stopped smoking 29 years before study entry). She was treated with afatinib monotherapy for 7 weeks and discontinued treatment due to the occurrence of Grade 3 rash. Stable disease was observed at this time. The patient received subsequent pemetrexed therapy with disease progression after two cycles, followed by docetaxel with disease stabilization for 5 months, after which the patient was lost to follow-up.

The second patient was a 62-year-old female, never smoker, who received afatinib for only 2 weeks and was discontinued due to Grade 3 diarrhea and deterioration of her general condition. No tumor assessments were undertaken within the study after baseline. The patient was subsequently lost to follow-up.

6. Discussion

We describe the first evidence of clinical benefit from treatment with afatinib in patients with an exon 20 HER2-mutant lung adenocarcinoma who have previously failed various chemotherapy regimens and the EGFR and/or HER2 inhibitors erlotinib, trastuzumab and lapatinib. Five patients were identified with a HER2 mutation, although only three were evaluable for response; mutations in all three patients were in exon 20 (two insertional duplications and one single amino-acid mutation). Analogous mutations in EGFR in exon 20 are relatively insensitive to inhibition by the reversible inhibitor gefitinib [15]. In two patients, a rapid metabolic response was observed within 1–2 weeks. Two patients had genomic activation of both EGFR and HER2.

The most striking response to single-agent afatinib was observed in Case 1, with a p.Tyr772_Ala775dup mutation in HER2. Compared with the other two patients, this patient showed genomic activation of HER2 only. This mutation causes an amino acid change identical to a mutation studied in a recently published preclinical model of mutant HER2-driven lung cancer [16]. In this mouse model, the forced expression of the mutant allele is capable of inducing invasive adenosquamous carcinomas that are restricted to the proximal and distal bronchioles. These cancers were completely dependent on the presence of this mutation and regressed completely when the expression of the mutant gene was reversed. Treatment with afatinib led to significant tumor regression in this preclinical model. In two of our clinical cases, the addition of paclitaxel to afatinib led to additional disease control, with prolonged remission in one patient despite a short response to single-agent afatinib, raising the possibility of synergism. In a xenograft of the HER2 mutant lung cancer cell line H1781, which contains a homozygous single amino-acid insertion in exon 20 [8], administration of afatinib resulted in disease stabilization, in contrast to the tumor regression observed in the preclinical mouse model. Taken together with our clinical experience, this indicates that the human HER2-driven lung cancer may have a more complex molecular pathogenesis than the preclinical HER2-driven mouse model.

The therapeutic effect observed in Case 2 was also of considerable interest, as the tumor showed genomic activation of both EGFR and HER2, and was previously treated with, and had become clinically resistant to, erlotinib, trastuzumab and lapatinib [17].

Although we cannot exclude that the second response, with added paclitaxel, results from the activity of single-agent paclitaxel, the magnitude and duration of the response in patients with disease resistant to multiple other chemotherapies suggests that

the response was to some extent achieved by the combination of afatinib with paclitaxel.

A limited number of studies in NSCLC have attempted to evaluate the activity of HER2-targeting agents, and have been summarized by Kelly et al. [18]. These studies could not reveal a significant benefit from trastuzumab or lapatinib. However, these studies were performed in NSCLC patient populations unselected for HER2 status (HER2 copy number or mutation) and primarily in combination with chemotherapeutic agents, and therefore were not apt to detect clinical benefit in patients with a genomic activation of HER2. There was, however, a report of one patient with a HER2 FISH positive tumor, but no HER2 or EGFR mutation, who achieved a short-lived response (4 weeks) to a pan-HER inhibitor (dacomitinib; PF-00299804) and subsequently progressed following additional treatment with trastuzumab, but who responded after vinorelbine was added. Furthermore, an additional patient with a HER2 mutation responded to trastuzumab plus vinorelbine after failure of platinum-based chemotherapy and gefitinib. However, this case does not allow for the assessment of the independent activity of trastuzumab [19].

This report suggests that the presence of HER2 mutations may characterize a subgroup of NSCLC that is constitutively dependent on the HER2 pathway. Afatinib is a potential novel treatment option for this subgroup of patients, even when other EGFR and HER2 targeting treatments have failed. The rate and duration of response associated with afatinib and the combined activity of afatinib and paclitaxel should be further assessed in earlier lines of treatment in this genomically defined population.

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Conflict of interest statement

Jacques De Grève received honoraria and a research grant from Boehringer Ingelheim. Ijeoma Umelo declared VUB OZR fellowship; and a collaborator in the HER2 trial. No other conflicts of interest to disclose. Caroline Geers, Erik Teugels, Denis Schallier, Henrik Everaert, Daniella Galdermans, Lore Decoster, Johan De Mey and Peter In't Veld have no disclosures to declare.

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