Tyrosine Kinase Inhibitors Could Be Effective Against Non-small Cell Lung Cancer Brain Metastases Harboring Uncommon EGFR Mutations

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Background: The significance of uncommon epidermal growth factor receptor (EGFR) mutations in patients with non-small cell lung cancer (NSCLC) and brain metastasis (BM) remains unclear. Cerebrospinal fluid (CSF) liquid biopsy is a novel tool for assessing EGFR mutations in BM. This study aimed to evaluate the EGFR mutations in patients with NSCLC and newly diagnosed BM and to examine the effect of EGFR tyrosine kinase inhibitors (TKI) on BM harboring CSF-tested uncommon EGFR mutations.

Methods: This was a prospective study of 21 patients with NSCLC and BM diagnosed between 04/2018 and 01/2019. CSF was obtained to detect the BM EGFR mutations by next-generation sequencing. BM characteristics at magnetic resonance imaging (MRI) and EGFR-TKI response were examined.

Results: Of 21 patients with NSCLC, 10 (47.6%) had leptomeningeal metastasis (LM), while 11 (52.4%) had brain parenchymal metastasis (BPM); 13 (61.9%) had confirmed EGFR mutation-positive primary tumors. The uncommon mutation rate in CSF ctDNA was 33.3% (7/21). Among those with EGFR mutation-positive primary tumors, the rate of uncommon EGFR mutations in CSF was 53.8% (7/13). Uncommon EGFR mutations were more common in patients with LM than in patients with BPM (6/11, 54.5% vs. 1/10, 10%), and included G719A, L861Q, L703P, and G575R. TKI was effective for four patients with BMs harboring uncommon EGFR mutations.

Conclusion: In patients with NSCLC and LM, the rate of uncommon EGFR mutation was high. The BMs with uncommon EGFR mutations seem to respond to EGFR-TKI treatment. CSF liquid biopsy could reveal the EGFR genetic profile of the BM and help guide treatment using small-molecule TKI.

Keywords: non-small cell lung cancer, brain metastasis, tyrosine kinase inhibitors, epidermal growth factor receptor, mutation
BACKGROUND

Brain metastases (BM) occurs in 30–50% of patients with non-small cell lung cancer (NSCLC) during the course of their disease (1). About 50% of the BMs are diagnosed at presentation of NSCLC, with 50–60% as the only site of distant metastasis (1). Patients with NSCLC and BMs have a poor prognosis, and the median survival is only 1–2 months (2, 3).

BMs include parenchymal BMs (PBMGs) and leptomeningeal metastases (LMs). LMs are less common than PBMGs, with an occurrence rate of 3.4–3.8% in NSCLC, but their prognosis is worse (4, 5).

The management of BMs from NSCLC mostly includes surgery and radiation therapy; chemotherapy is seldom applied, and targeted drugs could be more effective than chemotherapy (6). In NSCLC, the targeted therapies mainly include tyrosine kinase inhibitors (TKI). TKIs have replaced chemotherapy because of better responses and survival rates (7–9). Recently developed EGFR-TKIs, e.g., osimertinib, specifically address the challenges of acquired drug resistance and low blood-brain barrier (BBB) permeability of first and second-generation TKIs, demonstrating efficacy in the CNS (10). Nevertheless, only NSCLC cells harboring epidermal growth factor receptor (EGFR) sensitizing mutations will respond to EGFR TKIs. Activating mutations in EGFR are found in 20–40% of NSCLC, with exon 19 deletions (45%) and exon 21 L858R mutations (40–45%) as the most common mutations (10). In NSCLC patients with BMs, the prevalence of EGFR mutations has been reported to be 39–63% in Asians (11, 12) and 2–40% in North American and European populations (13, 14).

A retrospective study in China showed that the rate of uncommon mutations [i.e., mutations other than 19Del and L858R (15)] was high, with 12% of 1,837 Chinese patients with NSCLC EGFR mutations having non-classical mutations such as exon 20 insertion (30%), G719X mutation (21%), L858R complex mutation (17%; complex mutation defined as more than one EGFR mutation within a tumor sample) and T790M complex mutation (14%) (16). Importantly, different EGFR mutations respond differently to TKI therapy, and the impact of the uncommon mutations found in Asian patients is unknown (17, 18). Clinical studies so far have focused on the TKI treatment of NSCLC BMs with sensitizing mutations. Gefitinib is indicated in the treatment of EGFR-positive NSCLC BM and erlotinib as the second-line treatment for BM from asymptomatic NSCLC (1). The BRAIN trial (CTONG1201) showed that icotinib significantly improved the progression-free survival (PFS) and intracranial objective response rate (ORR) of patients with EGFR mutation and BMs (19). The ongoing APOLO trial (ClinicalTrials.org #NCT02972333) is examining the efficiency and safety of osimertinib EGFR TKI in the treatment of EGFR mutated patients with BMs. Based on the post hoc analysis of the LUX-Lung 2/3/6 trials (9, 20, 21), the treatment indication for afatinib has been expanded to the first-line treatment of metastatic NSCLC with non-resistant EGFR mutation including L861Q/G719X/S768I. Afatinib is able to cross the BBB in sufficient amounts to induce anti-tumor actions (22, 23).

Several studies showed that EGFR mutation patterns in NSCLC primary lesions and metastases in various body locations are not consistent with that found in the BMs (24–26), possibly because of the specific events required for cancer cell migration to and survival in the brain. Indeed, a primary tumor is composed of various clones (27, 28) and not all of them will have the abilities to spread in circulation, cross the BBB, survive in the brain microenvironment, and invade the brain tissue (1, 29).

These abilities call for specific sets of factors and mutations and therefore the actual tumor mutation status of BMs may differ from the estimation using primary tumor tissue or peripheral blood (12, 30). Indeed, a discordance rate of 16–32% for EGFR mutation status (depending on assay sensitivity for mutational analysis) between the primary site and BMs has been previously reported (12). Recent studies indicated that cerebrospinal fluid (CSF) ctDNA from BMs were present in CSF and that clinically actionable EGFR mutations were also more frequently detected in CSF ctDNA than in plasma in patients with BMs (31). Therefore, there is a possibility that BMs harboring rare mutations (e.g., L861Q, G719X, and S768I) not found in the primary lesion or metastases in other body locations will respond to EGFR-TKIs that are effective against lesions harboring those rare mutations, e.g., afatinib (9, 20, 21).

Therefore, EGFR-TKI can be used for the management of BMs from NSCLC, but the significance of uncommon EGFR mutations on the development and treatment response of BMs is still unclear. There are no studies on the significance of uncommon EGFR mutations in patients with BMs from NSCLC. We hypothesized that EGFR-TKIs could be effective against BMs with uncommon EGFR mutations, as evaluated by CSF ctDNA. The objectives of the present study were: (1) to evaluate the EGFR mutations in patients with NSCLC and newly diagnosed BMs; and (2) to examine the effect of EGFR-TKI on BMs harboring uncommon EGFR mutations.

METHODS

Study Design and Patients

This was a prospective study of 21 consecutive patients with NSCLC and BMs diagnosed between April 2018 and January 2019. The study was approved by the ethics committee of Tianjin Huanhu Hospital. All patients provided written informed consent prior to any study procedure. The inclusion criteria were: (1) NSCLC confirmed by histopathological examination; (2) new diagnosis of BMs by MRI and CSF cytological test with ThinPrep liquid-based cytology test applied in the diagnosis of LM (29); and (3) no prior treatment against BMs.

Data Collection

Demographics, clinical data, pathological data, imaging data, and tumor markers [carcinoembryonic antigen (CEA)] were obtained routinely. The EGFR mutation status of the primary site was obtained from previous medical records.

Abbreviations: BBB, blood-brain barrier; BM, brain metastases; CEA, carcinoembryonic antigen; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; LMs, leptomeningeal metastases; NSCLC, non-small cell lung cancer; ORR, objective response rate; PBMGs, parenchymal BMs; PFS, progression-free survival; TKI, tyrosine kinase inhibitors.
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Samples, DNA Extraction, and Next-Generation Sequencing
CSF samples were obtained from all 21 patients by lumbar puncture and placed in SanMed fixative solution, a patented cell preservation solution (Zhuhau SanMed Diagnostics Inc.), for transport and storage. Total DNA was extracted from CSF using the QiAamp Circumstance Nucleic Acid kit (#55114, Qiagen, Venlo, The Netherlands) according to the manufacturer’s instructions. The reference library was constructed using the Ion AmpliSeq Library Kit 2.0 and the Ion AmpliSeq Cancer HosSpot Panel v2 (#55114 and #4475346, Thermo Fisher Scientific, Waltham, MA, USA) and the Ion Library TaqMan Quantitation kit (#4468802, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer’s instructions. Details on next-generation sequencing are provided in Supplementary File 1.

Statistical Analysis
Due to the relatively small sample size, only descriptive statistics were used. Data are presented as numbers and percentages.

RESULTS
Characteristics of the Patients
Among the 21 patients with NSCLC, there were 10 (47.6%) males and 11 (52.4%) females. The mean age was 59.7 ± 9.9 years. Ten (47.6%) patients had LM, while 11 (52.4%) had PBMs. Thirteen (61.9%) patients had primary tumors confirmed with EGFR mutation.

Uncommon EGFR Mutations
The uncommon mutation-positive rate in CSF ctDNA from all study subjects was 33.3% (7/21) (Figure 1). Among the patients with primary tumors with EGFR mutation, the rate of uncommon mutations was 53.8% (7/13). Six of these seven patients were treated with TKI and showed disease progression in the brain during the course of treatment.

Compared with wild type EGFR, patients with primary tumors with EGFR mutation were more likely to display an uncommon EGFR mutation in CSF ctDNA (7/13, 50% vs. 0/5, 0%). Uncommon mutations were also more common in patients with LM than in patients with PBMs (6/11, 54.5% vs. 1/10, 10%).

Effectiveness of EGFR TKI in Patients With Uncommon Mutations in CSF ctDNA
For the seven patients with uncommon EGFR mutations in CSF ctDNA (regardless of EGFR mutations status in brain/lung tissues), TKI was effective in four cases (57.1%), as shown by MRI and CEA levels.

Case 01 was a male of 34 years of age, with lung adenocarcinoma and with a history of smoking, but quitted 10 years ago (Figure 2). In April 2018, LM was diagnosed, and the EGFR p.G719A mutation was detected in CSF ctDNA (55.6%). The CSF CEA level was 9,470 ng/ml. The patient started afatinib treatment in May 2018, and achieved a partial response by July 2018, with a CSF CEA level of 2,111 ng/ml. The response was maintained in November 2018, with a CSF CEA level of 1,590 ng/ml and CSF EGFR p.G719A mutation at 23.1%.

Case 05 was a male of 71 years of age, with lung adenocarcinoma but without smoking history (Figure 3). The EGFR 19Del mutation was detected in the primary tumor. He received oral icotinib for 8 months before being admitted to the hospital for dizziness and episodes of loss of consciousness and was diagnosed with PBMs. In December 2018, the EGFR p.L703P (2.0%) and EGFR p.T790M (2.1%) mutations, and the EGFR 19Del (86.0%) were detected in CSF ctDNA. The CSF CEA level was 96.1 ng/ml. The patient started osimertinib (80 mg qd) treatment, and the neurological symptoms were alleviated. In January 2019, the CSF CEA level was 8.7 ng/ml.

Case 12 was a female of 57 years of age, with lung adenocarcinoma but without smoking history (Figure 4). She was diagnosed with LM in September 2018. CSF ctDNA analysis revealed the EGFR p.L861Q (46.5%) mutation, and the CSF CEA level was 786.9 ng/ml. She started afatinib treatment. In December 2018, the CSF CEA level was 98.1 ng/ml.

Case 17 was a female of 65 years of age, with lung adenocarcinoma but without smoking history (Figure 5). In November 2018, CSF ctDNA analysis revealed EGFR p.L861Q (62.6%) and TP53 p.C135F (95.5%) mutations, and the CSF CEA level was 168.3 ng/ml. The patient started afatinib treatment. In December 2018, the CSF CEA level was 35.4 ng/ml.

![FIGURE 1](image1.png) Uncommon mutations in the epidermal growth factor receptor (EGFR) gene from cerebrospinal fluid (CSF) circulating tumor DNA (ctDNA) from patients with non-small cell lung cancer (NSCLC). BPM, brain parenchymal metastases; LM, leptomeningeal metastases; TKI, tyrosine kinase inhibitor.
FIGURE 2 | Case 01 was a male of 34 years of age, with lung adenocarcinoma and with a history of smoking, but quit 10 years ago. (A) T2 FLAIR enhanced magnetic resonance imaging (MRI) showed abnormal high signal in the medulla, oblongata, pon, and ventral and dorsal midbrain, suggesting leptomeningeal metastases (LMs). (B) T2 FLAIR enhanced MRI during afatinib treatment showed that the abnormal high signal in the medulla, oblongata, and ventral and dorsal midbrain was lower than before treatment. (C) Carcinoembryonic antigen (CEA) levels before and after afatinib treatment.

FIGURE 3 | Case 05 was a male of 71 years of age, with lung adenocarcinoma but without smoking history. (A) Cerebellar vermis, bilateral cerebral hemispheres, and pia meninges showed abnormal enhancement on magnetic resonance imaging. Leptomeningeal metastasis (LM) was considered. (B) Chest computed tomography revealing the primary lung lesion. (C) Carcinoembryonic antigen (CEA) levels before and after osimertinib treatment.

FIGURE 4 | Case 12 was a female of 57 years of age, with lung adenocarcinoma but without smoking history. (A) In September 2018, the right cerebellopontine angle area, the edge of the tentorium, and the lateral edge of the right arm were abnormally enhanced on magnetic resonance imaging. (B) In December, the enhancement intensity was decreased on the right side, and her condition was improved. (C) Carcinoembryonic antigen (CEA) levels before and after afatinib treatment.

DISCUSSION

The rate of uncommon EGFR mutations in Asian patients with NSCLC is high, comprising 11.9% of all cases in a previous report (31). There were rare previous studies on the significance of EGFR uncommon mutations in patients with NSCLC and BMs. There is a possibility that BMs harboring rare mutations not found in other body locations will respond to EGFR-TKIs
(9, 20, 21). Therefore, the aim of this study was to evaluate the 
EGFR mutations in patients with NSCLC and newly diagnosed 
BMs and examine the effect of EGFR TKI on BMs harboring 
uncommon EGFR mutations. The results showed that the rate of 
uncommon EGFR mutation in patients with NSCLC and BMs 
was high. The BMs with uncommon EGFR mutations seemed 
to respond to EGFR TKI treatment. Taken together, CSF liquid 
biopsy could reveal the EGFR genetic profile of the BM and 
help guide treatment using small-molecule TKI. These results do 
not imply that metastases in other body locations will answer 
or not to the BM-guided therapy, but since survival to BMs is 
short (2, 3), tailoring EGFR-TKI treatment specifically to the 
BMs might have a higher likelihood of prolonging survival in 
those patients.

In this study, the frequency of uncommon EGFR mutations 
was high, with these mutations detected in the CSF ctDNA 
in 33.3% (7/21) patients (considered to be from the BMs). 
These rates are higher than the 12% previously reported in 
patients with NSCLC but not necessarily with BM in China 
(16). This discrepancy might be due to the small sample size 
(selection bias) and the different testing methods. On the 
other hand, EGFR mutations have been reported to be more 
frequent in patients with NSCLC and BM (32). The exact role 
of uncommon EGFR mutations in BM development requires 
further research.

A primary tumor is a mosaic of various clones that evolved 
from the original tumor cell(s) (27, 28). Unlike cytotoxic 
chemotherapies that target all fast-growing cells, targeted 
treatments target specific cells within the tumor, raising the 
possibility of selecting resistant or unaffected clones, which 
can be responsible for relapse and metastasis (33, 34). BMs 
show significant molecular divergence with the primary tumor 
and with extracranial metastases (30, 31, 35–39). The process 
of BM development from the primary tumor necessitates 
specific steps, including crossing the BBB, surviving in the 
brain microenvironment, and invading the brain tissue, all 
of which requiring specific sets of biological aspects (1). The 
development of BMs in lung cancer patients who received 
an anti-EGFR treatment may be due to the TKI effectively 
allocating the cancer cells with the exon 19 deletion or the L858R 
mutation, but the effect of the TKI could be insufficient on 
the cells with uncommon mutation, therefore increasing the 
possibility of these cells contributing to BM development.

Indeed, it has been shown that mutations such as exon 20 
insertions, L861Q, S768I, and G718X have inferior response 
to first- generation EGFR TKIs (40). In the present study, 
six of the seven patients with BMs harboring uncommon 
EGFR mutations had received adjuvant EGFR TKI, supporting 
the hypothesis of clone selection by EGFR TKI. Nevertheless, 
additional studies are necessary to examine this point since 
erlotinib has been shown to reduce the risk of BMs from 
NSCLC (41).

A number of studies indicated the efficacy of EGFR TKI 
treatment against NSCLC BMs (1, 26, 42). The results from 
the LUX-Lung 2/3/6 trials (9, 20, 21) indicate that afatinib 
can be used as first-line treatment of metastatic NSCLC with 
non-resistant EGFR mutation including L861Q/G719X/S768I. 
Of particular interest, afatinib is able to cross the BBB in 
sufficient amounts to induce anti-tumor actions (22, 23). In 
the present study, three patients with uncommon EGFR mutations 
responded well to afatinib, as shown by MRI and CEA levels. A 
good response was also observed with Osimertinib. Additional 
studies are necessary to determine the best treatment approaches 
for BMs harboring uncommon mutations, particularly in the 
context that the frequency of those mutations is high in 
Asia (16).

Obtaining genetic material from BMs is complicated because 
surgical resection and biopsy are often impossible or not 
indicated due to the patient’s condition. The BBB prevents ctDNA 
from brain lesions to pass into the blood circulation and vice 
versa; therefore, the ctDNA found in CSF by liquid biopsy will 
reflect the status of the BMs (38, 43–46). Hence, a liquid biopsy 
of CSF in patients with NSCLC and BMs could provide the actual 
intracranial situation, helping to guide patient management.

FIGURE 5 | Case 17 was a female of 65 years of age, with lung adenocarcinoma but without smoking history. (A) Magnetic resonance imaging (MRI) of the brain. (B) Carcinoembryonic antigen (CEA) levels before and after afatinib treatment.
New technologies such as next-generation sequencing will allow personalized medicine to reach its full potential (38, 44).

It is well-known that LMs are less common than PBMs, but their prognosis is poorer (4, 5). In the present study, the frequencies of LMs and PBMs were similar, hinting toward some possible selection bias. Nevertheless, an important result is that the frequency of uncommon EGFR mutation was higher in LMs than in PBMs. This could explain, at least in part, the poorer prognosis of LMs. The association of uncommon EGFR mutation and LM will have to be examined in future studies.

The present study had limitations. Because uncommon mutations are rarely diagnosed, the sample size was relatively small, and the study was performed in a single center. In addition, follow-up was short. Furthermore, no post-treatment radiological data were available in some cases after patient improvement and discharge, especially non-residents. Moreover, CEA assessment is not widely accepted as a response marker. Finally, patients were administered various TKIs that had different BBB penetration rates.

CONCLUSIONS

EGFR TKI could be effective against uncommon EGFR mutations in NSCLC BMs. Molecular testing of CSF could be helpful in guiding treatment and tracking treatment response. Uncommon mutation might be considered as participating in the process of brain metastases of NSCLC.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of Interest: JZ, CH, DT, XY, and LX were employed by Zuhai SanMed Biotech Ltd. RL and ZL were employed by Zuhai Livzon Gene Diagnostics Ltd.