Lung Cancer 108 (2017) 22–28

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Case report

Longitudinal monitoring of ctDNA *EGFR* mutation burden from urine correlates with patient response to EGFR TKIs: A case series

Nishan Tchekmedyian[a,1,2], Raja Mudad[b,1], Fernando F. Blanco[c], Victoria M. Raymond[c,*], Jordan Garst[d], Mark G. Erlander[c], Eric Haura[a], David Berz[d,*]

[a] H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, FL 33612, United States
[b] Division of Hematology-Oncology, Sylvester Comprehensive Cancer Center, University of Miami School of Medicine, 1475 NW 12 Avenue, Miami, FL 33136, United States
[c] Trovagene, Inc, 11055 Flintkote Avenue, San Diego, CA 92121, United States
[d] Beverly Hills Cancer Center, 8900 Wilshire Blvd, Beverly Hills, CA 90211, United States

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form 14 January 2017
Accepted 16 February 2017

Keywords:
Circulating tumor DNA
Non-small cell lung cancer
Systemic mutation burden
Tyrosine kinase inhibitors
Longitudinal monitoring
EGFR

ABSTRACT

Targetable, somatic *EGFR* mutations are highly prevalent in patients with non-small cell lung cancer (NSCLC), making them eligible for tyrosine kinase inhibitor (TKI) therapy. Circulating tumor DNA (ctDNA), isolated from blood or urine, has been demonstrated to reliably identify somatic tumor associated *EGFR* mutations, specifically in patients with inconclusive biopsy. When conventional imaging modalities are inconclusive, quantitative assessment of systemic ctDNA burden has the potential to assess therapeutic response. We report on the clinical use of non-invasive, urinary ctDNA liquid biopsies for the ultrasensitive detection and longitudinal monitoring of ctDNA *EGFR* systemic mutation burden in five patients with NSCLC treated with *EGFR* TKIs. Urinary ctDNA-based quantitative assessment of systemic *EGFR* mutant allele burden is a non-invasive molecular diagnostic testing modality that has the potential to be utilized as an ancillary tool to assess disease burden and response to therapy.

1. Introduction

Somatic activating mutations in the epidermal growth factor receptor (*EGFR*) gene occur in approximately 10–50% of patients diagnosed with non-small cell lung cancer (NSCLC) and occur more frequently in females with little-to-no smoking history whose tumors exhibit an adenocarcinoma histology [1–3]. Guidelines advocate for patient identification as treatment naïve patients have a greater than 50% initial response rate to first and second generation tyrosine kinase inhibitors (TKIs) [4,5]. Secondary therapeutic resistance due to acquired *EGFR* T790M mutations develop in more than half of treated patients making them eligible for third generation TKI's [6]. A significant challenge in *EGFR* assessment is obtaining sufficient tissue for molecular testing. Inadequate tissue samples and inconclusive biopsies occur in 10–25% of patients [7–9] and it is estimated that up to 25% of patients treated with *EGFR* targeted TKIs receive treatment without mutation documentation [10].

Liquid biopsies, utilizing circulating tumor DNA (ctDNA) isolated from urine and blood samples, can reliably inform on a patient's tumor mutational status, providing an alternative non-invasive molecular testing option with equivalent sensitivity and specificity [11–14]. Additionally, liquid biopsies allow for serial testing to longitudinally monitor ctDNA mutation load, identify residual disease, and inform on disease recurrence, and results correlate with disease burden and response to therapy, often in advance of standard diagnostic imaging modalities [11,15,16].

We report on five patients, who underwent *EGFR* longitudinal monitoring using an ultrasensitive and quantitative analytical platform from urine-derived systemic ctDNA. *EGFR* mutations were quantitatively interrogated by short footprint mutation enriched next-generation sequencing (NGS) assays in a CLIA-certified, CAP-accredited laboratory (Trovagene, San Diego, CA) [12]. Ultra-deep sequencing was performed with a quantitative lower limit of detec-

Abbreviations: ctDNA, circulating tumor DNA; LLoD, lower limit of detection; EGFR, Epidermal growth factor receptor; PCR, polymerase chain reaction; TKI, tyrosine kinase inhibitor; GEq, genome equivalent; CT, computerized tomography; q.d., daily; MRI, magnetic response imaging; RECIST, response evaluation criteria in solid tumors.

* Corresponding authors.
E-mail addresses: vraymond@trovagene.com (V.M. Raymond), Berz2001@yahoo.com (D. Berz).

[1] NT and RM contributed equally to this work.

[2] Present Address: Nishan Tchekmedyian, MD Pacific Shores Medical Group 19582 Beach Blvd., Suite 212 Huntington Beach, CA 92648, United States.

http://dx.doi.org/10.1016/j.lungcan.2017.02.010
0169-5002/

tion (LLoD) of 0.006% for *EGFR* exon 19 deletions and L858R, and 0.01% for *EGFR* T790M. The results were reported in a quantifiable format normalized to 10^5 genomic equivalents (GEq) [12].

Quantitative *EGFR* ctDNA was longitudinally assessed at baseline and at intervals following administration of TKI therapy in three specific scenarios to monitor response to: (a) first-line TKI (Case 1), (b) an approved third-generation TKI upon detection of *EGFR* T790 M at disease progression (Cases 2, 3, and 4), and (c) an experimental third-generation TKI in a newly diagnosed patient who presented with *de novo EGFR* T790M (Case 5).

2. Case presentations

2.1. Case 1

A 51-year-old Hispanic female presented in March 2016 with three weeks of dizziness and headaches. Brain computed tomography (CT) revealed numerous lesions with edema. Chest CT revealed a four centimeter (cm) left upper lung mass, enlarged mediastinal lymph nodes, left adrenal metastases, right renal metastases, and a right iliac bone lesion (Fig. 1A). Biopsy of the right iliac bone was positive for adenocarcinoma consistent with a lung primary. Urine ctDNA analysis in April 2016 identified *EGFR* L858R (788 copies/10^5 GEq) (Fig. 1B,C). She started erlotinib (150 mg daily (q.d)). After two months of therapy, a follow-up chest CT in June 2016 revealed left upper lobe lung mass and mediastinal and hilar lymphadenopathy decrease, but interval progression in the size of lung nodules, and slight increase in the left adrenal gland nodule (Fig. 1A). Repeat urinary *EGFR* ctDNA at that time revealed an increase in *EGFR* L858R (1200 copies/10^5 GEq), without the presence of *EGFR* T790M (Fig. 1B,C). Given that the patient had a mixed CT response, increasing ctDNA mutation levels, but was doing well clinically, she was maintained on erlotinib. A repeat chest CT six weeks later confirmed progression in the main lung mass and mediastinal lymph nodes. Erlotinib was discontinued and systemic chemotherapy was initiated.

2.2. Case 2

A 49-year-old Caucasian female presented with tissue confirmed *EGFR* L858R positive adenocarcinoma, involving the left lung, pleura, bone, and brain. She was treated with whole brain radiation followed by erlotinib (150 mg q.d.) with a partial response lasting 17 months. She subsequently developed severe lower back pain with an unremarkable follow-up chest CT in March 2016. Lumbosacral magnetic resonance imaging (MRI) showed probable disease progression in the sacrum with epidural extension. Under suspicion of progressive disease, the patient underwent urinary ctDNA analysis which was positive for *EGFR* L858R (2412 copies/10^5 GEq) and T790 M (2064/10^5 GEq) (Fig. 2A,B). These results were confirmed by *EGFR* molecular testing conducted on pleural fluid collected via thoracentesis. Based on the urinary ctDNA result, the patient was started on osimertinib (80 mg q.d.) and had near complete resolution of her back pain within one week. Urinary mutational assessment was repeated eight weeks later to assess response (May 2016), revealing 86 copies/10^5 GEq of *EGFR* L858R and 35 copies/10^5 GEq of *EGFR* T790M (Fig. 2A,B). Repeat CT demonstrated a small pleural effusion but no other findings.

2.3. Case 3

A 62-year-old Filipino female with no prior history of smoking presented in September 2014 with dizziness and right-sided weakness. A brain MRI revealed lesions suspicious for metastatic disease. Chest CT revealed a large left upper lobe mass with hilar extension, pronounced confluent hilar and mediastinal adenopathy and multifocal bilateral pulmonary nodularity with small effusion compatible with metastatic carcinoma. She underwent a craniotomy and excision of brain lesions. Tissue molecular analyses was positive for an *EGFR* exon 19 deletion. She underwent whole brain radiation followed by erlotinib (150 mg q.d.) with a response lasting 12 months. In September 2015, she underwent stereotactic brain radiation to treat an enhanced frontal lesion identified by a repeat brain MRI. A contrast-enhanced CT of the chest, abdomen, and pelvis confirmed progression with left upper lobe mass increase and lymph node involvement. Tissue biopsy from a pericardiectomy was positive for *EGFR* T790M. Urinary ctDNA analysis in November 2015 confirmed the presence of the *EGFR* T790M (18.7 copies/10^5 GEq; Fig. 2C,D). She was enrolled on an expanded access osimertinib trial (80 mg q.d.; NCT02451852). Urinary *EGFR* ctDNA was used to longitudinally monitor patient response. After one month on therapy, T790M mutant allele burden dropped below detection, which was predictive of, and consistent with, subsequent imaging studies revealing stable disease. Repeat urinary ctDNA analysis was performed two months (February 2016) and eight months (July 216) after initiation of therapy and T790M mutant allele burden remained undetected (Fig. 2C,D). In July 2016, brain MRI revealed an enhancing nodule in the right posterior hippocampal structure, but overall stable disease.

2.4. Case 4

A 74-year-old Caucasian woman was initially diagnosed with stage IB adenosquamous NSCLC and underwent complete resection with curative intent. She subsequently developed recurrent, widely metastatic, *EGFR* exon 19 deleted bronchogenic adenosquamous carcinoma in 2013 and took erlotinib for two years with multiple treatment interruptions, mainly due to cutaneous toxicity. In November 2015 she presented with bulky intrathoracic and retroperitoneal progression (Fig. 3A). She experienced profound left flank and back pain and severe dyspnea. Given concern for progression, urinary ctDNA analysis was ordered in January 2016 and confirmed the presence of *EGFR* T790 M (2426 copies/10^5 GEq). Based on these results, she was granted access to an extended access osimertinib trial (80 mg q.d.). She reported rapid clinical improvement with relief of her dyspnea, improvement of her musculoskeletal pain, and increased energy level at her first 14 day follow up visit. This was paralleled by an appreciable decline of her urinary mutational ctDNA levels and a striking imaging response at 2.5 months (Fig. 3A). Her urinary T790M ctDNA levels were found to decline to 14, 22 and 13 copies per 10^5 GEq at week 14, 19 and 27, respectively (Fig. 3B,C). She was without clear evidence of disease at last follow-up.

2.5. Case 5

A treatment-naïve 62-year old African American female with previous 5–10 pack year smoking history presented in February 2016 with a right hilar/perihilar mass causing significant narrowing of the right main and segmental bronchi and partial atelectasis of the right lower lobe. Chest CT revealed multiple left lung pulmonary nodules and a cavitary right pulmonary nodule with a trace right pleural effusion, along with indications of metastatic disease evidenced by enlarged subcarinal and precarinal lymph nodes (Fig. 4A). Molecular testing from primary lesion tissue was positive for *EGFR* L858R and T790M, which was confirmed with urinary ctDNA testing (499 copies/10^5 GEq of L858R and 613 copies/10^5 GEq of T790M; Fig. 4B,C) in February 2016. She was randomized to a phase III clinical trial receiving an experimental third-generation T790 M targeted TKI. After six weeks on therapy, her urinary *EGFR* ctDNA revealed a decrease in L858R (64 copies/10^5 GEq) and no detectable T790M, suggesting an early response to therapy