

Clinical Utility of Target Selector™ ctDNA Testing: Detection of EGFR Mutations via Liquid Biopsy Enabled Targeted Therapy Selection for Patients with Advanced NSCLC

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Abstract

Background: Molecular profiling of tumors provides information key for devising personalized therapeutic strategies for managing disease in cancer patients. A liquid biopsy is emerging as a sensitive means to evaluate biomarker status without the complications and costs associated with surgical biopsies, particularly for patients unable or unwilling to undergo invasive procedures.

Materials and Methods: Patient blood specimens were collected in Biocept's proprietary Blood Collection Tubes for liquid biopsy testing in Biocept's CLIA-certified, CAP-accredited laboratory. Dual Circulating Tumor Cell (CTC) and circulating tumor DNA (ctDNA) platforms were utilized to detect ALK or ROS1 gene rearrangements by FISH, or EGFR mutations, respectively.

Results: Described are three metastatic NSCLC patients for which liquid biopsy guided the selection of a targeted therapy when a standard tissue biopsy was inadequate to assess biomarker status. All three patients were prescribed EGFR Tyrosine Kinase Inhibitor (TKI) treatment after an activating EGFR mutation was detected via liquid biopsy. One patient exhibited a complete response for approximately two years. Two patients received osimertinib following emergence of the EGFR T790M resistance mutation, which was also detected via a liquid biopsy.

Conclusion: Liquid biopsy analyses of ctDNA and CTCs can complement tumor testing, identifying potential drivers of a patient's cancer. Clinical utility of liquid biopsy is demonstrated where the first line and subsequent targeted treatments were prescribed based on the identification of genomic alterations in blood. Each patient received a therapeutic benefit that significantly extended survival and enhanced their quality of life.

Keywords Liquid biopsy; EGFR; targeted therapy; NSCLC

Introduction

Lung cancer is the leading cause of cancer death in the USA, with an estimated 154,000 deaths predicted for 2018. To improve patient outcomes, molecular profiling of a patient's tumor can guide the selection of a personalized treatment strategies [1-3]. For example, Non-Small Cell Lung Cancer (NSCLC) represents over 80% of lung carcinomas [1,2] and EGFR tyrosine kinase activating mutations are observed in 15-20% of NSCLC adenocarcinomas in the USA. Treating these patients with an EGFR Tyrosine Kinase Inhibitor (TKI) can extend Progression-Free Survival (PFS) and quality of life compared to platinum-based chemotherapy. Unfortunately, tissue biopsies performed for initial cancer diagnosis often do not yield sufficient amounts of tissue for biomarker analysis. In addition, some patients are either unable or unwilling to tolerate these invasive procedures.

In recent years, technological platforms for performing "liquid biopsies" have emerged, complementing traditional tumor-based diagnostic testing. Recent technological advances in liquid biopsy offer

healthcare providers with sensitive and viable molecular profiling options through the analysis of circulating tumor DNA (ctDNA) or Circulating Tumor Cells (CTCs) from a peripheral blood draw [4,5]. This non-invasive approach permits efficient serial specimen collection for tracking tumor characteristics (e.g., the emergence of resistance mutations at signs of progression), and can detect actionable genetic alterations missed by solid tissue tests. Here we describe three NSCLC cases for which liquid biopsies identified EGFR activating and resistance mutations. Based on liquid biopsy results, targeted therapies were prescribed that dramatically extended patient survival. All patients (or next of kin for the deceased) provided written consent to present their medical information (excluding private information) in this publication.

Materials and Methods

Liquid biopsy blood samples were collected into 10 mL Biocept CEE-Sure(Biocept, Inc.) and maintained at ambient temperature until processed. Specimens were shipped to Biocept's CLIA-certified, CAP-accredited laboratory for processing within 24 hours of receipt. ctDNA

extracted from blood plasma was subjected to Target Selector™ assays specific for the EGFR activating mutations, L858R and exon 19 deletion (Del19), or the T790M mutation, which confers resistance to EGFR TKIs. Proprietary Target Selector™ switch blocker methodology enriches mutant target sequences by specifically blocking PCR amplification of the wild-type allele. Subsequent Sanger sequencing of the Target Selector™ amplicon identifies the specific mutation (Figure 1) [6]. CTC isolation and enumeration were performed as described [7-9]. Captured CTCs were subsequently subjected to FISH analyses within the microchannel to identify ALK or ROS1 gene rearrangements.

Results

Case 1

A 66-year-old male patient presented for medical attention in December 2015 after he had ceased smoking for 30 years. The patient had a 3 × 2.8 cm mass, which was hot by PET scan. Tumor tissue obtained by fine-needle aspiration confirmed NSCLC and was determined to be bronchioalveolar adenocarcinoma. The tissue biopsy was insufficient for biomarker testing and the patient was reluctant to undergo surgery.

The lack of tissue prompted the physician to send a blood sample to Biocept for EGFR ctDNA mutation and CTC analysis. Because liquid biopsy testing detected the EGFR L858R mutation at a Mutant Allele Frequency (MAF) <1.0%, the patient was treated with erlotinib from January until May of 2016. PET imaging performed April 2016 showed persistent glucose uptake (increased SUV from 5.3 to 9.0), although the tumor mass was stable in size. As the patient demonstrated clinical signs of progression, a second Biocept test was ordered. The patient tested positive for EGFR T790M with a MAF <1.0% and L858R at MAF <1.0%, and was subsequently treated with osimertinib from June through the end of August 2016. A PET scan performed August 2016 showed a mass with SUV 10, and a stable tumor size (3.8 × 3.3 cm with cavitation). The patient was convinced to have surgery and presented with another tumor in the same lobe (EGFR negative Stage 2b N1). Histology determined that the new tumor was adenosquamous (unlike the initial bronchioalveolar tumor), with lymphovascular invasion and PD-L1 >90%. The patient then received three cycles of chemotherapy (paclitaxel protein-bound/platinum), and was placed on pemrolizumab in December 2016. Chest X-rays performed February 2017 appeared normal.

Case 2

A female patient with a history of multiple myeloma presented for medical attention in March 2015, and a second primary NSCLC was diagnosed. PET was performed August 2015 for bilateral lung nodules. Tissue was insufficient for biomarker testing. To guide treatment options, the patient's blood was sent to Biocept in August 2015 for EGFR, ALK, and ROS1 biomarker testing. Based on liquid biopsy detection of EGFR L858R with MAF <1.0%, the patient was treated with erlotinib from the end of August 2015 until the time this case study was first being prepared (July 2017). Lung scans normalized completely, and the myeloma was also in remission. Identification of this clinically actionable biomarker and subsequent targeted therapy has, to date, resulted in 23 months of complete response with limited toxicities.

Case 3

A 58-year-old, female non-smoker with pericardial tamponade presented for medical attention in March 2015. The patient had multiple bone metastases at diagnosis. Additionally, PET showed liver metastases. The patient had a pericardium window procedure and fluid drainage. Tissue was confirmed as metastatic NSCLC, but the material was insufficient for biomarker testing. Biocept liquid biopsy was ordered for EGFR mutations plus ALK and ROS1 gene rearrangements, and testing identified EGFR Del 19 in ctDNA with MAF=52.8%. Tumor tissue showed cMET gene amplification. Based on results from Biocept liquid biopsy combined with tumor testing, the patient received bevacizumab+erlotinib, as the tumor was aggressive. In April 2015, therapy was changed to bevacizumab+afatinib since the patient's insurance denied erlotinib. An erlotinib+crizotinib phase I clinical trial was identified for the patient, and treatment was switched to this combination in August 2015. In November 2015, the patient showed signs of progression. Biocept tests detected EGFR Del19 with MAF=32.5%. In early 2016, the patient again showed signs of progression, and a CT-guided biopsy was performed; obtained tissue was insufficient for biomarker testing. In February 2016, the third round of Biocept testing showed significantly higher levels of the EGFR Del19 driver at MAF=72.9%, in conjunction with the EGFR T790M resistance marker (MAF=14.0%). Solely based on liquid biopsy results, the patient received FDA approved osimertinib targeted therapy from March until October of 2016. At progression, the patient developed a cavity in the left upper lung, a bad cough, and sputum full of *Aspergillus*. She was then prescribed voriconazole and chemotherapy with pemetrexed+carboplatin. The patient developed new brain metastases in February 2017, was placed on temozolomide, and received radiation. She died at home two years after her first visit to the oncology clinic. In two instances where the tissue biopsy was insufficient for molecular profiling, the liquid biopsy identified relevant biomarkers, enabling the selection of targeted therapies, which contributes to an overall patient survival of 25 months.

Discussion

Reflecting improved understanding of the genetic causes of NSCLC and the rapid development of therapies targeting these mutations, the National Comprehensive Cancer Network (NCCN) now recommends EGFR mutation and ALK testing (category 1), as well as ROS1, BRAF, and PD-L1 testing (category 2A), for metastatic non-squamous NSCLC patients, and suggests consideration to test for these biomarkers in a subset of squamous cell carcinoma patients [10]. However, significant risk and cost are associated with acquiring sufficient tissue for these molecular analyses. Poor patient health, reluctance to undergo invasive surgical procedures, and inaccessible metastatic lesions are additional barriers to obtaining tissue for molecular profiling. Tumor heterogeneity may also preclude correct assessment of a patient's biomarker status. Thus, relying solely on tissue testing may misclassify patients. Emerging liquid biopsy technologies enable molecular characterization via a simple, peripheral blood draw, affording a more comprehensive analysis of tumor DNA derived from various regions within a tumor and metastatic sites. Furthermore, a recent analysis by the International Association for the Study of Lung Cancer (IASLC) has concluded that technologies for detecting EGFR mutations in peripheral blood are now so reliable that their implementation in the clinic is highly recommended.

Here we have presented three cases of metastatic NSCLC for which traditional biopsy procedures did not provide sufficient tissue for

biomarker testing. Recognizing the potential of liquid biopsy, the physician submitted patient blood samples to Biocept to test clinically actionable biomarkers associated with FDA approved therapies. For all three patients, EGFR TKI therapy was initiated upon detecting an activating EGFR mutation via liquid biopsy across a range of mutant allele frequencies. For one patient, EGFR TKI administration resulted in approximately two years of complete response, a remarkable improvement over typical chemotherapy outcomes. When the other two patients eventually exhibited signs of progression, additional Biocept liquid biopsy testing identified the EGFR T790M resistance mutation; these patients were placed on T790M-specific inhibitors, further extending quality of life and survival.

This case study series highlights the clinical utility of liquid biopsy in both identifying the molecular drivers of a tumor at diagnosis when traditional biopsies fail to recover sufficient tissue for molecular analysis (as was the case for the three patients discussed here), and in monitoring the evolution of tumor mutations to identify drivers of drug resistance and disease progression (resistance to targeted therapies inevitably emerges). Whereas the serial collection of tissue biopsies is frequently infeasible, the longitudinal evaluation of blood samples is well tolerated and can be used to identify changes in mutation frequencies and the emergence of drug-resistant clones. This enables the rapid switching to TKIs that target acquired resistance EGFR mutations (e.g., osimertinib and fourth generation EGFR TKIs that are on the way). It should be noted, however, that a negative liquid biopsy result derived from current technologies should not be considered conclusive, but instead should trigger conventional tissue analyses. Thus, the paired implementation of tissue and liquid biopsies offers the best chance to understand and treat a patient's disease.

Applicable to a wide range of cancers, liquid biopsies represent a state-of-the-art technology for the non-invasive, real-time, and cost-effective identification of genetic drivers of a patient's disease. Importantly, emerging technologies promise to increase the clinical utility of liquid biopsies by enabling, for example, the analysis of other fluids (urine, cerebrospinal fluid, saliva), the capture and analysis of RNA, and the monitoring for residual disease. Analysis of a single liquid biopsy specimen provides a snapshot of the molecular profiling landscape of both primary and metastatic tumors, including intra- and inter-tumor heterogeneities. These data equip physicians with information valuable for devising optimal, personalized therapeutic

strategies that can extend patient survival-as seen for the three NSCLC patients in this case series.

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