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Demonstrated Clinical Utility of Target Selector™ ctDNA Testing: Liquid Biopsy *EGFR* Mutation Detection Enabled Targeted Therapy Selection for Three Advanced NSCLC Patients

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Abstract

Introduction: Tumor molecular profiling is key for personalized medicine strategies to manage disease in cancer patients. Liquid biopsy can sensitively 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 was collected for liquid biopsy testing by Biocept's CLIA-certified, CAP-accredited laboratory. Dual CTC and ctDNA platforms were utilized to detect *EGFR* mutations in ctDNA and *ALK* or *ROS1* gene rearrangements by FISH in CTCs.

Results and Discussion: Described are three metastatic NSCLC patients where liquid biopsy results guided targeted therapy selections when standard tissue biopsy was inadequate to assess biomarker status. For all three patients, EGFR TKI treatment was prescribed upon detecting an activating *EGFR* mutation by liquid biopsy. One patient exhibited complete response for approximately two years. Two patients received osimertinib following emergence of the *EGFR* T790M resistance mutation detected by liquid biopsy.

Conclusion: Liquid biopsy analyses of ctDNA and CTCs in blood can complement tumor testing, providing information on drivers related to a patient's cancer. Clinical utility of liquid biopsy is demonstrated where first line and subsequent targeted treatment was prescribed upon identifying genomic alterations found in blood, and the patients received therapeutic benefit that significantly extended survival and enhanced their quality of life.

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Introduction

Lung cancer is the leading cause of cancer death in the USA, with non-small cell lung cancer (NSCLC) comprising >80% of lung carcinomas [1,2]. Effective targeted cancer management and designing personalized therapies is accomplished through tumor molecular profiling [1-3]. For instance, *EGFR* activating mutations are observed in 15% to 20% of NSCLC adenocarcinoma 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, frequently yield insufficient tissue for biomarker analysis. Additionally, certain patients are either unable or unwilling to tolerate these invasive procedures.

Complementing traditional tumor testing, newer "liquid biopsy" methods offer sensitive and viable molecular profiling options via analyzing circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) from blood [4,5]. This non-invasive approach can detect actionable genetic alterations missed by solid tissue tests, and permits efficient serial specimen analyses to track tumor characteristics (e.g., emerging resistance mutations). Here, we describe three NSCLC cases for which liquid biopsies identified *EGFR* activating and resistance mutations. All patients (or next of kin for the deceased) provided written consent to publish their medical information (excluding private information). Targeted therapies were prescribed based on liquid biopsy results, dramatically

extending patient survival.

Materials and Methods

Blood was collected into 8-mL CEE-Sure™ Blood Collection Tubes (Biocept, Inc.) and maintained at ambient temperature until processed. Specimens were shipped to Biocept's CLIA-certified, CAP-accredited laboratory for processing within 24 hrs of receipt. ctDNA extracted from plasma was used in Target Selector™ assays specific for *EGFR* L858R and exon 19 deletion (Del19) activating mutations, or the T790M mutation conferring EGFR TKI resistance. Proprietary Target Selector™ Switch-Blocker methodology enriches target mutation sequences by specifically amplifying mutant targets while blocking wild-type amplification; Sanger sequencing of the amplicon confirms mutation identity (Figure 1) [6]. CTC isolation and enumeration were performed as previously described [7-9]. Captured CTCs were subjected to FISH analyses within Biocept's patented microchannel to identify *ALK* or *ROS*1 gene rearrangements.

Results

Case 1

A 66-year-old male patient presented for medical attention in December 2015 after he ceased smoking for 30 years. The patient had a 3 cm × 2.8 cm mass, which was hot by PET scan. Tumor tissue from fine-needle aspiration confirmed NSCLC and was determined as bronchioalveolar adenocarcinoma. Tissue biopsy was insufficient for biomarker testing and the patient was reluctant to undergo surgery. Due to lack of tissue, the physician sent blood to Biocept for ctDNA and CTC analysis. Upon detecting *EGFR* L858R in the patient specimen at mutant allele frequency (MAF) <1.0% by liquid biopsy, the patient received erlotinib from January - May 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, liquid biopsy was again ordered. Testing positive for *EGFR* T790M with MAF <1.0% and L585R at MAF <1.0%, the patient received 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 cm × 3.3 cm with cavitations). 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 pembrolizumab 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; 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, patient blood was sent to Biocept in August 2015 for *EGFR*, *ALK*, and *ROS*1 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 through at least July 2017. Lung scans normalized completely, and the myeloma was also in remission. Identifying a clinically actionable biomarker and subsequent targeted therapy resulted in at least 23 months of complete response with limited toxicities.

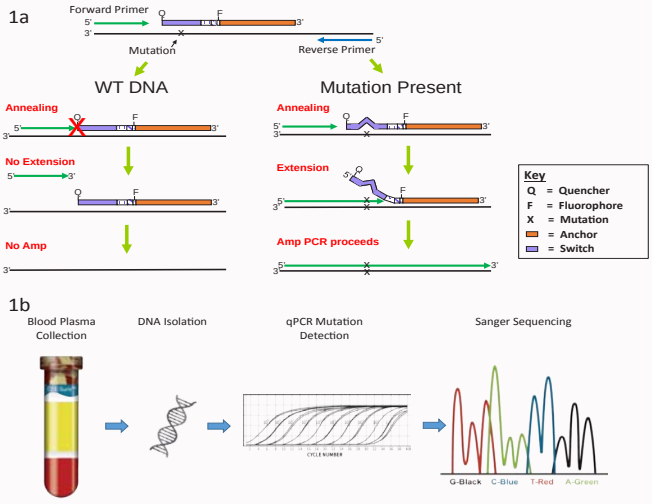

Figure 1: Target Selector™ ctDNA assays
1a : Target Selector™ Switch-Blocker probes block wild-type amplification, permitting specific mutant allele DNA amplification. A mismatch (mutation within the Switch region creates an approximately 20°C temperature discrimination between the mutant and wild-type to enable highly specific mutant amplification.
1b: Target Selector™ ctDNA assay workflow.

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 confirmed metastatic NSCLC, but the material was insufficient for biomarker testing. Liquid biopsy was ordered for *EGFR* mutations plus *ALK* and *ROS*1 gene rearrangements; testing identified *EGFR* Del19 with MAF =52.8%. Tumor tissue showed *cMET* gene amplification. From tissue testing combined with Biocept liquid biopsy results, 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; treatment was switched to this combination in August 2015. In November 2015, the patient showed signs of progression. Liquid biopsy 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, a third liquid biopsy showed significantly higher levels of the *EGFR* Del19 driver at MAF =72.9%, plus the *EGFR* T790M resistance marker with MAF =14.0%. Based solely on liquid biopsy results, the patient received FDA approved osimertinib targeted therapy from March-October 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 oncology clinic visit. Where tissue biopsy was insufficient twice for this patient, liquid biopsy was vital towards identifying relevant biomarkers for the individual to be prescribed targeted therapies, contributing to an overall survival of 25 months.

Discussion

Reflecting better understanding of NSCLC genetic drivers and

the rapid development of associated targeted therapies, the National Comprehensive Cancer Network (NCCN) now recommends *EGFR* mutation and *ALK* testing (category 1), plus *ROS*1, *BRAF* and PD-L1 testing (category 2A), for metastatic non-squamous NSCLC patients [10]. NCCN also suggests testing these biomarkers in a subset of squamous-cell carcinoma patients [10]. However, significant risk and cost are associated with acquiring sufficient tissue for molecular analyses. Poor patient health, reluctance to undergo invasive surgical procedures, and inaccessible metastatic lesions are additional barriers to obtain tissue for molecular profiling. Tumor heterogeneity may also preclude correct biomarker status assessment. Thus, relying solely on tissue testing may misclassify patients. Liquid biopsy technologies enable molecular characterization via a simple blood draw, affording more comprehensive analysis of tumor DNA and CTCs derived from various regions within a tumor and metastatic sites.

Here, we presented three cases of metastatic NSCLC for which traditional biopsies yielded insufficient tissue for biomarker testing. Recognizing the potential of liquid biopsy, the physician had blood samples tested for 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 MAFs. 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 exhibited signs of progression, additional liquid biopsy testing identified *EGFR* T790M; 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 to detect clonal tumor mutations [11] and evaluate longitudinal blood collections for changing mutation frequencies and emergent drug-resistant clones. Applicable to a wide range of cancers, liquid biopsies represent state-of-the-art assessment for non-invasive, real-time, and cost-effective identification of genetic drivers of a patient's disease. Analysis of a single blood 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 valuable information towards 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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