Surrogate or Not: The Role for Cell Free Circulating DNA in Detecting EGFR Mutations Present in Tumor Tissue

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BACKGROUND

- Lung cancer remains the leading cause of cancer mortality globally and in the United States. Non-small cell lung cancer (NSCLC) makes up 85% of all lung cancer cases. Nearly 70% of these patients present with advanced disease unamenable to local resection (1).
- Identification of oncogenic drivers such as EGFR has personalized therapy for patients carrying aberrations in these driver genes, offering targeted treatment possibilities to advanced stage NSCLC patients with previously limited options.
- Almost 90% of mutations within EGFR tend to be deletions in exon 19 and the L858R substitution in exon 21. Both mutations predict sensitivity to first generation tyrosine kinase inhibitors (TKI) such as erlotinib (2).
- Secondary mutations conferring resistance to erlotinib include the T790M mutation in the active site of the EGFR receptor or amplification of the MET bypass pathway (3). Next generation EGFR TKIs have been developed specifically to treat lung cancers with secondary mutations, making determination of the specific mechanism of resistance in an individual patient clinically relevant.
- Post-progression biopsy has been the only definitive means of identifying secondary mechanisms of resistance until now. The process of obtaining a biopsy harbors inherent limitations such as non-trivial post procedural complication rates for patients and the inability to represent complete tumor heterogeneity from a single sample (4).
- Cell free circulating DNA (cfcDNA) represents an alternative non-invasive means of detecting resistant mutations in cancer cells peripherally (5). Initial studies have shown plasma cfcDNA to be very specific for detection of EGFR mutations present in tumor tissue in advanced stage NSCLC patients (6,7).

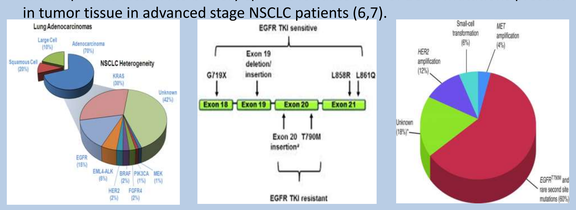

Figure 1: Epidemiology of NSCLC. From left to right, common oncogenic drivers in adenocarcinoma; common EGFR gene mutations; prevalence of secondary EGFR mutations in patients who progress on first generation TKI.

METHODS

OBJECTIVES

- To assess the concordance between Biocept's minimally invasive peripheral blood assay with analytical specificity > 99% and sensitivity > 95% at a mutant allele frequency of 0.05% (7 mutant copies against 14, 000 wild type copies) and tumor tissue in detecting the presence of EGFR T790M mutations from patients who progressed on erlotinib.
- To suggest cfcDNA is an adequate surrogate for tumor tissue in detecting secondary EGFR mutations in patients who progressed on erlotinib.

STUDY DESIGN

- Single institution observational study of 16 patients.

INCLUSION CRITERIA

- Only stage IV lung cancer patients with histologically proven adenocarcinoma were included.
- Only patients with initial sensitizing EGFR mutations (deletions in exon 19 or substitution in exon 21) confirmed by molecular genetics were included.

SCHEME

- Two 8-10 ml tubes of blood were collected from patients who progressed on erlotinib. Patient samples were then tested for T790M using Biocept's minimally invasive Target-Selector ™ assay and MET amplification using a FISH assay.
- The Target-Selector ™ assay combines real-time PCR and sequencing to verify the presence of mutations. Its unique feature is a wild-type PCR blocker which allows the mutant template to be amplified in a high prevalence background of wild-type.
- Results from these "liquid-biopsies" were compared to results obtained on standard tissue biopsy. Sensitivity, concordance and positive predictive value (PPV) were calculated.

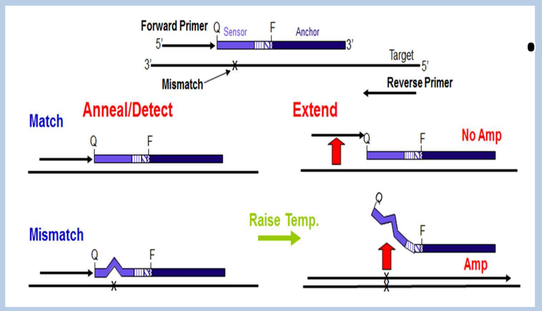

Figure 4: Mechanism of action of the Biocept Target-Selector ™ PCR assay. The forward primer is proximal to a sensor and anchor sequence. The sensor recognizes a sequence in exon 20 of the EGFR gene and anneals tightly to wild-type DNA. When the temperature is increased, due to adherence of the sensor, amplification is blocked. In mutant cfcDNA with T790M in exon 20, the sensor binds weakly. When the temperature is raised, the sensor opens, allowing the forward primer to extend and for amplification to occur.

CONCLUSION

- Biocept's plasma based Target-Selector ™ cfcDNA assay is highly concordant with mutations present in tumor tissue and therefore appears to be a viable non-invasive alternative to identify secondary EGFR mutations such as T790M in patients who progress on first line TKI therapy.
- The Selector assay demonstrates superior and comparable performance to other leading cfcDNA PCR assays cited in the literature with analytical sensitivity >95% and clinical sensitivity ~90% with 88% concordance between mutations detected in plasma and tumor tissue (9).
- Potential advantages of accurately being able to detect new mutations present in tumor tissue via a peripheral assay are many-fold and include:

  1) Avoiding patient complications during repeat tissue biopsies.

  2) Avoiding inadequate biopsy samples that do not represent a tumor's true heterogeneity.

  3)Facilitating serial monitoring to detect the development of resistant mutations prior to clinical or radiographic progression in EGFR mutant lung adenocarcinoma patients on TKI therapy.

  4)Enabling changes in therapy in a more time-efficient manner.

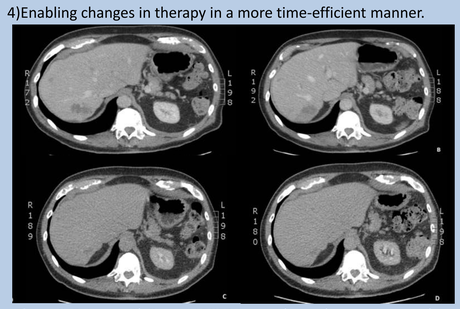

Figure 6: Patient with Stage IV EGFR mutant lung adenocarcinoma who progressed on erlotinib with new hepatic metastases and was found to have T790M initially on cfcDNA before undergoing confirmational tissue biopsy. He was started on AZD-9291, a next generation TKI. (A) Before starting therapy. (B) Two months into therapy. (C) Four months into therapy. (D) Six months into therapy.

CONCEPT

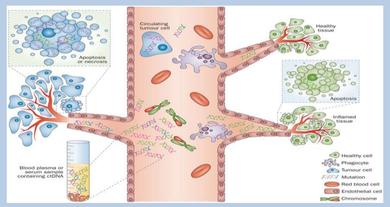

Figure 2: Depiction illustrating the origin of cfcDNA and circulating tumor cells (CTC) from a milieu of apoptotic & necrotic fragments from healthy and diseased tissue (8). The challenge in identifying mutant cfcDNA or isolating CTCs is capturing and amplifying a mutational needle from the background haystack of wild-type.

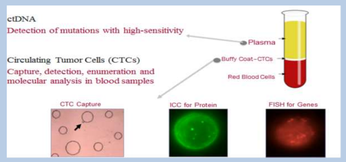

Figure 3: Origins of cfcDNA and CTCs. Following density gradient centrifugation, cfcDNA is detected from the plasma component of the supernatant while CTCs are captured from the buffy coat component.

RESULTS

Study #	Age	Stage	Primary EGFR Mutation	Secondary EGFR Mutation	Months Between Tumor Biopsy and cfcDNA sampling
1	62	IV	EGFR exon 21 L858R	EGFR exon 20 T790M	3.7
2	58	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	0.9
3	59	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	4.8
4	56	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	1.5
5	75	IV	EGFR exon 21 L858R	EGFR exon 20 T790M	1.2
6	68	IV	EGFR exon 21 L858R	Not Obtained	Not obtained
7	56	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	4
8	73	IV	EGFR exon 19 deletion	None Identified	cfcDNA collected on 10/28/2014, biopsy done 2/30/2015.
9	52	IV	EGFR exon 21 L858R	EGFR exon 20 T790M,c-met	49.7
10	62	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	cfcDNA collected 1/2/2015, biopsy done 3/20/2015
11	76	IV	EGFR exon 19 deletion	None Identified	cfcDNA collected 1/20/2015, biopsy not done.
12	74	IV	EGFR exon 19 deletion	None Identified	cfcDNA collected 2/4/2015, biopsy done 3/2/2015
13	56	IV	EGFR exon 19 deletion	EGFR exon 20 T790M	cfcDNA collected 2/5/2015, biopsy done 2/11/2015
14	58	IV	EGFR exon 19 deletion	None Identified	cfcDNA collected 3/4/2015, biopsy done 4/2/2015
15	70	IV	EGFR exon 21 L858R	Not Yet Available (biopsided 7/	cfcDNA collected 3/19/2015, biopsy done 7/17/2015
16	69	IV	EGFR exon 21 L858R	EGFR exon 20 T790M	cfcDNA collected 7/7/2015, biopsy not yet done.

Table 1: Characteristics of all 16 patients enrolled in the trial. All patients underwent cfcDNA sampling however only twelve have been able to undergo successful tissue biopsies with results. Of the four patients without tissue biopsy results, two are awaiting molecular genetic analysis while one is still awaiting scheduling of her biopsy.

Patient	T790M mutant copies detected in approx. 3ml plasma	EGFR copies detected in approx. 3ml plasma	% T790M mutant copies detected in approx. 3ml plasma	Verification
1	2833	169628	1.7	verified by sequencing
2	26	14964	0.2	verified by sequencing
3	90	7785	1.2	verified by sequencing
4	55	4308	1.3	verified by sequencing
5	1697	18725	9.1	verified by sequencing
6	62	4958	1.3	verified by sequencing
7	20507	74296	27.6	verified by sequencing
8	37	5829	0.6	verified by sequencing
9	24	4560	0.5	verified by sequencing
10	0	24968	0	N/A
11	0	7826	0	N/A
12	7	160266	0.004	verified by sequencing
13	68	14677	0.5	verified by sequencing
14	0	10767	0	N/A
15	0	17079	0	N/A
16	261	6525	4.1	verified by sequencing

Table 2: T790M Selector assay data. Quantitative number of T790M copies and relative percentages compared to EGFR wild-type copies detected from cfcDNA in all 16 patients.

		Tissue	
		Positive	Negative
cfcDNA	Positive	8	1
	Negative	1	2

Table 3: 2X2 table used to calculate sensitivity (8/9) and PPV (8/9) of the Selector assay in detecting the T790M mutation peripherally from cfcDNA when compared to tissue biopsy.

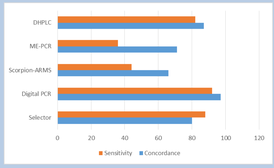

Figure 5: Comparison of sensitivity and concordance between five different assays designed to detect secondary EGFR mutations in plasma (6). DHPLC: denaturing high performance liquid chromatography; ARMS: amplified refractory mutation system.

REFERENCES

1. Molina JR, Yang P, Cassivi SD, Schild SE, and Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clinic Proceedings 2008; 83:584–594.
2. Rosell R, Moran T, Queralt C, Porta R, Cardenal F et al. Screening for Epidermal Growth Factor Receptor Mutations in Lung Cancer. New England Journal of Medicine 2009; 361:958-67.
3. Wang Z, Chen R, Wang S, Zhong J, Wu M et al. Quantification and Dynamic Monitoring of EGFR T790M in Plasma Cell-Free DNA by Digital PCR for Prognosis of EGFR-TKI Treatment in Advanced NSCLC. PLOS ONE 2014; 9(11): e110780.
4. Overman MJ, Modak J, Kopetz S, Murthy R, Yao JC, Hicks ME, et al. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? J Clin Oncol 2013;31:17–22.
5. Diaz L, Bardelli A. Liquid Biopsies: Genotyping Circulating Tumor DNA. Journal of Clinical Oncology 2014; 32(6):579-586.
6. Zhao X, Han R, Zhao J, Wang J, Yang F, Zhong W et al. Comparison of Epidermal Growth Factor Receptor Mutation Statuses in Tissue and Plasma in Stage I-IV Non-Small Cell Lung Cancer Patients. Respiration 2013; 85:119–125.
7. Luo J, Shen L, Zheng D. Diagnostic value of circulating free DNA for the detection of EGFR mutation status in NSCLC: a systematic review and meta-analysis. Scientific Reports 2014; 4(6269):1-7.
8. Crowley E, Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nature Reviews Clinical Oncology 2013; 10:472-484.
9. Alexiadis V, Watanaskul T, Kosco K, Mayer J, Arnold L. The CEE-Selector TM Assay: a tool for the identification of rare allele variants. Paper presented at AACR 2012. Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, Illinois.