Clinical Evaluation of a Rapid Cell-free EGFR Mutation Detection Kit in Plasma from NSCLC Patients

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Introduction

Recent studies have demonstrated the potential utility of circulating cell-free DNA (cfDNA) in plasma and serum for clinical management of patients diagnosed with several different cancers. Detection of mutations in blood, so-called "liquid biopsy" tests, can hold many advantages over traditional methods using tissue sampling. Liquid biopsy may offer a non-invasive method for identifying best responders to targeted therapies, and can be ideal for routine follow-up to monitor disease progression. Additionally, tumor-associated mutations identified in cfDNA may be a better representation of the whole tumor genetic makeup, including metastatic sites that are generally not characterized with the current diagnostic workup. Mutation detection using cfDNA has the capacity to become a sensitive genetic indicator of impending disease flare, preceding radiographic progression. In the setting of EGFR TK-activating NSCLC, mutation detection using cfDNA has the potential to become an actionable clinical test to monitor TKI therapy. Below we describe the results of a 2-arm study using the EntroGen CTEGFR Mutation Detection Kit.

Methods

For Arm 1, cell-free DNA (cfDNA) was isolated using a commercial kit (p/n 55114, Qiagen) from approximately 5ml of plasma collected in Cell-Free DNA BCT tubes (p/n 218962, Streck) from 38 treatment-naïve patients diagnosed with non-small cell lung adenocarcinoma at various disease stages (Table 1). EGFR mutation status of the plasma and tumor were determined using the CTEGFR Mutation Detection Kit (p/n CTEGFR-48, EntroGen) as demonstrated in figure 1 and EGFR Mutation Detection Kit (p/n EGFR-RT52, EntroGen), respectively on a Bio-Rad CFX96 real-time PCR instrument and confirmed with Roche cobas® EGFR v2 (p/n 05985536190). For Arm 2, cell-free DNA was isolated from blood collected from 8 NSCLC patients at the time of progression from EGFR TKI therapy.

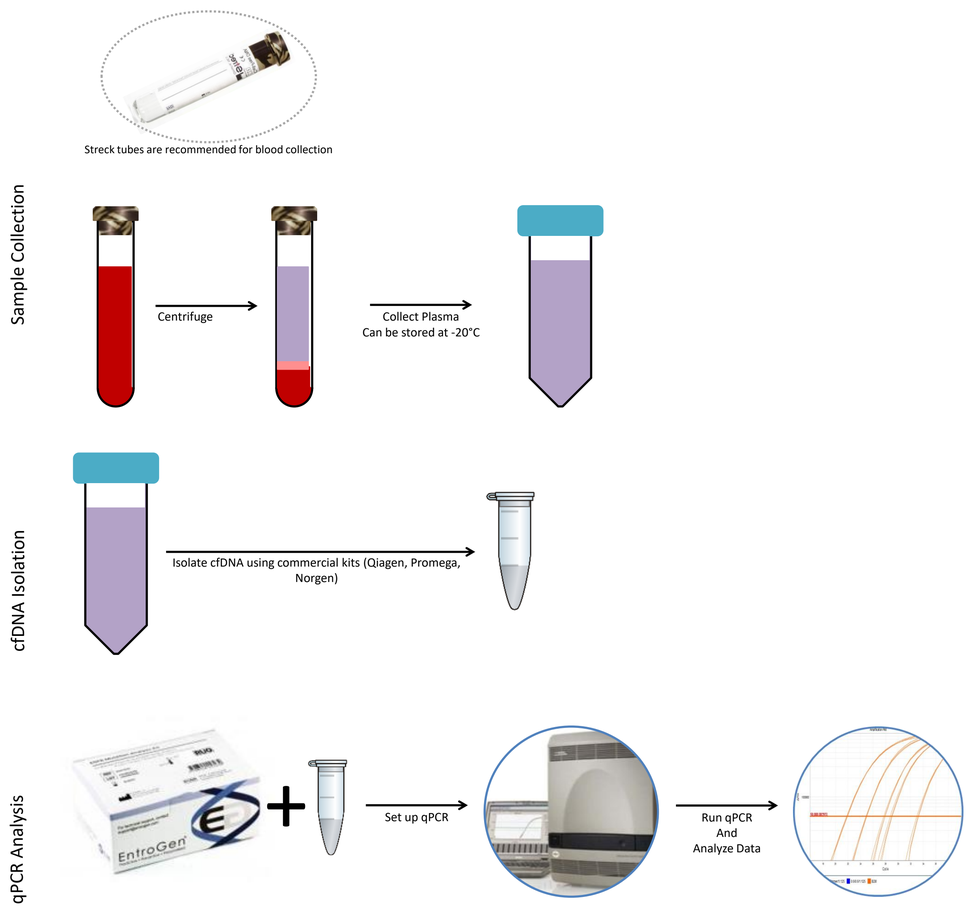

Figure 1. Assay workflow for CTEGFR Mutation Detection Kit.

Results

			FFPE		Plasma	
#	TNM	Stage	cobas EGFR	EntroGen FFPE EGFR	cobas EGFR	EntroGen cfDNA EGFR
1	T2aN0M0	IB	wt	wt	wt	wt
2	T2aN0M0	IB	exon 19 - del	exon 19 - del	wt	wt
3	T3N0M0	IIB	exon 19 - del	exon 19 - del	wt	wt
4	T2aN0M0	IB	exon 21 - L858R	exon 21 - L858R	wt	wt
5	T2aN0M0	IB	wt	wt	wt	wt
6	T1bN0M0	IA	wt	wt	wt	wt
7	T2aN1M0	IIA	wt	wt	wt	wt
8	T3N1M1	IV	wt	wt	wt	wt
9	T2aN0M0	IB	exon 19 - del	exon 19 - del	wt	wt
10	T3N3M1	IV	wt	wt	wt	wt
11	T1bN0M0	IA	exon 19 - del	exon 19 - del	wt	wt
12	T1bN2M0	IIIA	wt	wt	wt	wt
13	T3NxM1b	IV	wt	wt	wt	wt
14	T4N3M0	IIIB	wt	wt	wt	wt
15	T2aN0M0	IB	wt	wt	wt	wt
16	T2aN1M0	IIA	exon 19 - del	exon 19 - del	wt	wt
17	T2aN3M1b	IV	wt	wt	wt	wt
18	T3N1M0	IIIA	wt	wt	wt	wt
19	T2aN0M0	IB	exon 19 - del	exon 19 - del	wt	exon 19 - del
20	T4N2M1	IV	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R
21	T4N3M1	IV	exon 19 - del	exon 19 - del	exon 19 - del	exon 19 - del
22	T2aN1M0	IIA	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R
23	T1aN0M0	IA	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R	exon 21 - L858R
24	T2aN0M0	IB	wt	S768I	wt	wt
25	T2N3M1	IV	wt	wt	wt	wt
26	T2aN0M0	IB	wt	wt	wt	wt
27	T3N2M1	IV	wt	wt	wt	wt
28	T4N3M0	IIIB	wt	wt	wt	wt
29	T1aN0M0	IA	wt	wt	wt	wt
30	T2aN1M0	IIA	exon 19 - del	exon 19 - del	exon 19 - del	exon 19 - del
31	T2aN0M0	IB	wt	wt	wt	wt
32	T2aN0M0	IB	wt	wt	wt	wt
33	T4NxM1	IV	wt	wt	wt	wt
34	T2aN0M0	IB	wt	wt	wt	wt
35	T2N2M1	IV	wt	wt	wt	wt
36	T2aN3M1b	IV	wt	wt	wt	wt
37	T2bN2M0	IIIA	wt	wt	wt	wt
38	T1aN0M0	IB	wt	wt	wt	wt

Table 1. Arm 1 data. Comparison of cobas EGFR (Roche) and EntroGen EGFR tests on FFPE and cfDNA specimens.

Sample ID	Tissue at Dx	Plasma at Progression	Tissue at Progression
1	L858R	L858R	L858R
2	Exon 19 del	Exon 19 del	NA
3	Exon 19 del	Exon 19 del	NA
4	L858R	T790M & L858R	NA
5	L858R	T790M & L858R	T790M & L858R
6	Exon 19 del	Exon 19 del	NA
7	L858R	T790M & L858R	NA
8	L858R	WT	NA

Table 2. Arm 2 data. EGFR mutations detected in plasma and tissue of NSCLC patients at progression with EntroGen's cfEGFR test. NA = tissue not available.

Conclusions

Based on limited Arm 1 data, the cfEGFR assay sensitivity was 66.7% (.41-.87; 95% CI) and specificity was 100% (.54-1.0; 95% CI). Positive predictive value (PPV) was 100%, while negative predictive value (NPV) was 50% (.34-.66; 95% CI). The test had higher sensitivity and NPV in stage III and IV than in stage I and II patient populations while maintaining 100% specificity and PPV. In Arm 2, 7 out of 8 patients harbored the founder activating EGFR mutation at progression. Additionally, 3 cases were found to have acquired T790M resistance mutation. One of these cases was confirmed in tissue re-biopsy.

The clinical performance of the CTEGFR Mutation Detection Kit demonstrates a rapid and reliable alternative to EGFR mutation detection in tissue biopsies, especially for late stage diagnoses where tumor tissue may not be available. Testing of select patients at progression showed promising results for using this test as a monitoring tool during treatment.

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