Original Article

Cell free DNA analysis by SiRe® next generation sequencing panel in non small cell lung cancer patients: focus on basal setting

Pasquale Pisapia[1], Francesco Pepe[1], Riccardo Smeraglio[1], Maria Russo[1], Danilo Rocco[2], Roberta Sgariglia[1], Mariantonia Nacchio[1], Caterina De Luca[1], Elena Vigliar[1], Claudio Bellevicine[1], Giancarlo Troncone[1], Umberto Malapelle[1]

[1]Department of Public Health, University of Naples Federico II, Naples, Italy; [2]AORN Vincenzo Monaldi, Department of Oncology, Naples, Italy

Contributions: (I) Conception and design: All authors; (II) Administrative support: All authors; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Giancarlo Troncone. Department of Public Health University of Naples Federico II, via Sergio Pansini 5, Naples I-80131, Italy. Email: giancarlo.troncone@unina.it.

Background: Non small cell lung cancer (NSCLC) is diagnosed in most cases on small tissue samples, such as cytological preparations and histological biopsies; these limited tissue specimens may be not always sufficient for testing epidermal growth factor receptor (*EGFR*) mutations and other relevant predictive biomarkers. Cell-free DNA (cfDNA) can be used as a surrogate for *EGFR* mutational testing, whenever tissue is unavailable. However, the detection of gene mutations on cfDNA is challenging; in fact, the extremely low concentration of circulating tumor DNA requires the implementation of highly sensitive and validated next generation techniques.

Methods: Thus, we have recently validated a novel next generation sequencing (NGS) assay, employing the SiRe® gene panel to detect on cfDNA mutations of *EGFR* and *KRAS*, *NRAS*, *BRAF*, *cKIT* and *PDGFR* genes. In this current study, we report on a series of NSCLC patients, without available tissue for *EGFR* testing, who prospectively underwent SiRe® NGS analysis.

Results: The results confirm the high clinical performance, in terms of success rate and mutation detection, of NGS based analysis of cfDNA.

Conclusions: SiRe® NGS panel represent an effective diagnostic tool in cfDNA analysis setting.

Keywords: Liquid biopsy; non small cell lung cancer (NSCLC); next generation sequencing (NGS); tyrosine kinase inhibitors (TKIs)

Submitted May 09, 2017. Accepted for publication Jun 16, 2017.
doi: 10.21037/jtd.2017.06.97
View this article at: http://dx.doi.org/10.21037/jtd.2017.06.97

Introduction

Non small cell lung cancer (NSCLC) is diagnosed in most cases at advanced stages of disease. Diagnostic samples are frequently scarcely cellular, being represented by either cytological specimens or small tissue endoscopic biopsies; these limited tissue samples often may be not sufficient for epidermal growth factor receptor (*EGFR*) and other clinical relevant biomarkers, such as *ALK* translocation and PD-L1 expression, whose assessment is required to select patients for first line treatment administration (1,2). In particular for *EGFR* tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib and afatinib the identification of activating *EGFR* mutations in exon 18, 19 and 21 is mandatory before the first line treatment (3-8). To date according to the European Medicines Agency guidelines, in patients without tissue availability, only for *EGFR* TKIs treatment decision making, cell-free DNA (cfDNA) can be used as a fast and non-invasive surrogate for *EGFR* mutational testing (9-13). However, the assessment of

gene mutations in cfDNA is challenging, in particular in basal setting, for the detection of first and second TKIs generation *EGFR* sensitizing mutations, due to the very low concentration of circulating tumor DNA, that represent only a small fraction of the total cfDNA (9,10,12-15). Thus, the clinical implementation of next generation techniques, such as next generation sequencing (NGS) or digital PCR (dPCR) based assay is crucial (9,10,12,13,16,17). In a recent study of ours, we validated the SiRe® NGS panel for mutation detection in *EGFR*, *KRAS*, *NRAS*, *BRAF*, *cKIT* and *PDGFR* starting from cfDNA retrieved from patients with different solid tumors (NSCLC, metastatic colo-rectal cancer, melanoma and gastrointestinal stromal tumor) (13). SiRe®, with a lower limit of detection of 0.01% and a reference range of 568 clinical relevant mutations, showed a higher analytical performance respect to a very sensitive modified TaqMan probe real time PCR based approach (13). In the clinical trials settings and in other published validation studies (18-20), the analysis of cfDNA gene mutations was carried out using as gold standard the mutational status obtained on matched tissue derived DNA, but little is known regarding the application of this approach in clinical setting, in particular in baseline setting of NSCLC patients, prior to *EGFR* TKIs administration, without a referent DNA derived tissue to confirm the mutational data obtained on cfDNA (21).

In this current study, we reviewed the NGS data obtained by using SiRe® NGS panel starting from cfDNA collected in routine NSCLC baseline setting to prospectively select patients, without tissue availability, for first and second generation *EGFR* TKIs treatment administration.

Methods

Our molecular laboratory is an accredited Italian reference center for predictive molecular pathology testing in oncology (22). In particular for NSCLC patients, from January 2017 to March 2017, n=64 liquid biopsy analysis was requested from the oncologists of different South Italy institutions (n=14), following the European Medicines Agency guidelines, for the analysis of *EGFR* mutations on cfDNA inpatients without tissues availability at presentation, to assess the eligibility to first and second generation *EGFR* TKIs (*Table 1*). On the overall n=39 men and n=25 women were analyzed with a mean age of 66 years (range, 36–89 years). For each patient, 10 mL of blood was collected in-house by using EDTA Vacutainer tubes (BD, Plymount, UK) by a dedicated nurse at the Department of Public Health of the University of Naples Federico II. The protocols adopted in this study were previously validated (13). Briefly, before cfDNA extraction, two centrifugation steps (2,300 rpm for 10 min) were carried out to obtain at least 1.2 mL of plasma for each patient. cfDNA was extracted by using the QIAsymphony DSPVirus/Pathogen Midi Kit on the QIAsymphony robot (Qiagen, Venlo Limburg) accordingly with the manufacturer instructions. By using SiRe® panel, following the previously validated protocol, libraries were automated constructed and purified using Ion AmpliSeq DL8 Kit (Thermofisher) on the Ion Chef instrument (Thermofisher) and, after barcoding, purified libraries derived from eight cfDNA plasma samples were diluted and combined with eight additional cfDNA-derived libraries to obtain a 16 Ion Code pooled library, re-loaded into the Ion Chef instrument for template preparation by using the Ion PGM Hi-Q IC Kit (Thermofisher). Finally, templates were loaded into the 316v2 chip and sequenced on Personal Genome Machine (PGM). Signal processing and base calling were carried out using the default base-caller parameters on Torrent Suite (v.5.0.2) and coverage analysis was performed using SiRe® specific bed files with coverage plug-in (v.5.0.2.0). In addition to automatic variant calling analysis, by using SiRe® panel specific optimized variant caller plug-in (v.5.0.2.1) parameters, BAM files were visually inspected with the Golden Helix Genome Browser v.2.0.7 (Bozeman, MT, USA). Only variants with >5× allele coverage and a quality score >20, within an amplicon coverage at least 1,000× alleles, were reported and the relative mutated allele frequency was annotated, considering not only *EGFR*, but also *KRAS*, *BRAF* and *NRAS* gene hot-spots region, relevant for NSCLC and covered by the SiRe® panel.

Written informed consent was obtained from all patients and documented in accordance with the general authorization to process personal data for scientific research purposes from 'The Italian Data Protection Authority' (http://www.garanteprivacy.it/web/guest/home/docweb/docwebdisplay/export/2485392) and all samples were handled in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/).

Results

The clinical performance of the SiRe® panel in basal setting was assessed by prospectively testing the plasma derived cfDNA of n=64 NSCLC patients for whom no tissue was available to test the EGFR mutational status for TKIs

Table 1 Patients characteristics and NGS results

Patient	Sex	Age	Reads	Mean read length (bp)	Number of mapped reads	% reads on target (%)	Average reads per amplicon	Uniformity of amplicon coverage (%)	NRAS (allelic frequency)	BRAF (allelic frequency)	EGFR (allelic frequency)	KRAS (allelic frequency)
1	M	76	325,098	127	324,684	97.31	7,523	100.00	WT	WT	WT	WT
2	F	77	223,871	130	223,209	97.71	5,187	100.00	WT	WT	WT	WT
3	M	68	288,543	127	287,410	97.60	6,679	100.00	WT	WT	WT	WT
4	F	74	26,017	125	25,666	96.70	590.9	97.62	G12S (1.00%)	WT	WT	WT
5	F	36	163,218	128	162,752	98.06	3,800	97.62	WT	WT	WT	G12D (1.50%)
6	F	50	146,656	127	145,906	97.25	3,378	100.00	WT	V600A (0.20%)	WT	WT
7	M	70	173,021	127	172,314	97.55	4,002	97.62	G13D (0.34%)	WT	WT	Q61H (0.20%)
8	M	64	151,445	127	150,370	97.03	3,474	97.62	WT	WT	WT	G12C (1.30%)
9	M	61	75,353	127	74,972	97.68	1,744	97.62	WT	WT	WT	WT
10	M	45	190,620	126	189,708	97.49	4,403	100.00	WT	WT	H773_V774insH (37.0%)	WT
11	M	48	103,023	128	102,573	97.79	2,388	97.62	WT	WT	WT	G12C (0.60%)
12	F	61	163,363	128	162,841	97.75	3,790	97.62	WT	WT	WT	WT
13	F	57	130,596	128	130,250	97.72	3,031	97.62	WT	WT	WT	WT
14	F	51	155,551	128	155,018	97.40	3,595	97.62	WT	WT	WT	WT
15	M	62	60,927	128	60,611	97.77	1,411	97.62	WT	WT	V769_D770insASV (12.30%)	WT
16	F	87	67,571	128	67,404	97.85	1,570	97.62	WT	WT	WT	WT
17	F	77	181,295	129	180,633	98.06	4,217	95.24	WT	WT	WT	WT
18	M	75	104,653	127	104,925	97.79	2,429	97.62	WT	WT	WT	WT
19	M	58	262,771	130	261,202	97.94	6,091	97.62	WT	WT	WT	WT
20	F	38	248,897	128	248,287	97.68	5,775	97.62	WT	WT	WT	WT
21	F	51	169,989	128	169,581	97.44	9,734	97.62	WT	WT	WT	WT
22	M	64	36,774	128	36,693	97.84	854.7	97.62	WT	WT	WT	WT
23	M	74	195,245	128	194,624	97.50	4,518	100.00	WT	WT	L858R (3.20%)	WT
24	F	54	82,480	128	82,303	97.80	1,916	97.62	WT	WT	WT	WT
25	M	84	12,499	127	12,439	97.79	289.6	97.62	WT	WT	WT	WT

Table 1 (*continued*)

Table 1 (*continued*)

Patient	Sex	Age	Reads	Mean read length (bp)	Number of mapped reads	% reads on target (%)	Average reads per amplicon	Uniformity of amplicon coverage (%)	NRAS (allelic frequency)	BRAF (allelic frequency)	EGFR (allelic frequency)	KRAS (allelic frequency)
26	F	59	71,556	129	71,360	97.54	1,657	97.62	WT	WT	WT	G12D (1.30%)
27	M	68	62,909	128	62,723	97.61	1,458	97.62	WT	WT	WT	WT
28	M	56	51,993	128	51,453	97.44	1,194	100.00	WT	WT	WT	WT
29	M	89	21,306	127	21,126	97.12	488.5	97.62	WT	WT	WT	G12D
30	M	53	76	60	66	39.39	0.69	91.45	Failed	Failed	Failed	Failed
31	F	67	49,539	129	49,435	97.50	1,148	97.62	WT	G469A (5.00%)	WT	WT
32	M	62	113,202	130	112,930	98.01	2,635	97.62	WT	WT	WT	G12C (5.63%)
33	M	70	72,936	129	72,752	97.77	1,694	97.62	WT	WT	WT	WT
34	F	71	133,127	129	132,803	97.93	3,096	97.62	WT	WT	WT	WT
35	F	75	78,508	128	78,340	97.55	1,820	97.62	WT	WT	WT	WT
36	M	80	175,862	130	175,177	97.66	4,073	97.62	WT	WT	WT	WT
37	M	66	118,804	129	118,575	97.87	2,763	97.62	WT	WT	WT	WT
38	F	71	139,472	129	139,008	97.23	3,218	100.00	WT	WT	WT	WT
39	M	67	111,500	128	110,909	97.27	2,571	97.62	WT	WT	WT	WT
40	M	64	348,181	135	347,148	97.91	8,093	97.62	WT	WT	WT	G13S (0.20%)
41	M	80	131,590	129	131,257	97.98	3,062	97.62	WT	WT	WT	WT
42	M	80	141,018	129	140,564	98.15	3,285	97.62	Q61P (0.14%)	WT	WT	WT
43	F	76	72,635	128	72,364	97.77	1,685	97.62	WT	WT	WT	G13D (0.30%)
44	M	42	152,707	127	152,031	97.58	3,532	97.62	A59C (0.20%)	WT	WT	WT
45	M	66	74,945	127	74,630	97.64	1,735	97.62	WT	WT	WT	WT
46	F	57	50,945	128	50,607	97.58	1,176	97.62	WT	WT	WT	WT
47	M	67	84,001	130	83,802	97.69	1,949	97.62	WT	WT	WT	WT
48	M	69	133,275	132	132,908	97.57	3,087	97.62	WT	WT	WT	G12S (6.40%)
49	M	65	92,859	126	91,962	96.90	2,122	97.62	WT	WT	WT	G12C (3.30%)
50	M	77	47,845	130	47,653	97.32	1,104	97.62	WT	WT	WT	Q61H (4.50%)

Table 1 (*continued*)

Table 1 (*continued*)

Patient	Sex	Age	Reads	Mean read length (bp)	Number of mapped reads	% reads on target (%)	Average reads per amplicon	Uniformity of amplicon coverage (%)	NRAS (allelic frequency)	BRAF (allelic frequency)	EGFR (allelic frequency)	KRAS (allelic frequency)
51	M	82	23,283	129	23,118	97.44	5,363	97.62	WT	WT	WT	WT
52	F	77	31,713	129	31,599	97.86	9,363	97.62	WT	WT	WT	WT
53	M	84	121,037	129	120,379	97.44	2,793	97.62	WT	WT	WT	G13S (0.20%)
54	F	73	73,937	129	73,230	97.42	1,699	97.62	WT	WT	WT	WT
55	M	66	136,734	131	136,185	97.37	3,157	97.62	WT	WT	WT	WT
56	M	69	102,126	130	101,718	97.30	2,356	97.62	WT	WT	WT	WT
57	M	65	103,211	131	102,957	97.48	2,390	97.62	WT	WT	WT	WT
58	M	65	73,517	129	73,310	97.32	1,699	97.62	WT	WT	WT	WT
59	M	81	163,403	130	162,818	97.33	3,773	97.62	WT	WT	WT	WT
60	M	68	182,161	130	181,087	97.19	4,190	97.62	WT	WT	WT	WT
61	F	54	64,662	127	63,955	97.52	1,485	97.62	WT	WT	WT	WT
62	F	64	119,320	130	118,669	97.21	2,747	97.62	WT	WT	WT	A59V (0.20%)
63	F	67	103,023	128	102,573	97.79	2,388	97.62	WT	WT	ELREA (5.40%)	WT
64	F	61	173,021	127	172,314	97.55	4,002	97.62	WT	WT	ELREA (0.70%)	WT

Patients characteristics, SiRe® next generation sequencing (NGS) panel run metric parameters (reads, mean read length in base pair, number of mapped reads, percentage of read on target, average reads per amplicon, uniformity of amplicon coverage) and genes mutational status with relative mutated allele frequency are reported for each sample.

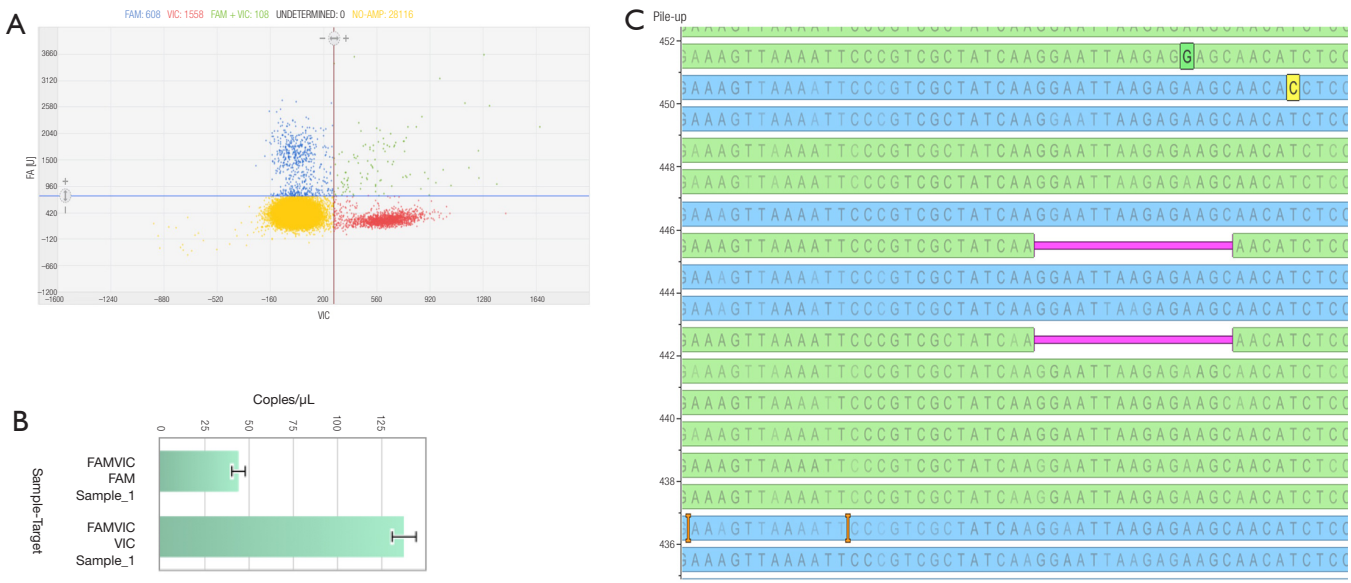

Figure 1 Case n.64 is reported. Digital PCR Quant Studio 3D cloud software (Thermofisher) was used to analyze the scatter plot (A) and the copies of mutated and wild type alleles detected in one µl of the extracted cell-free DNA (cfDNA) (B). In the panel (C), the SiRe® panel next generation sequencing (NGS) result is reported obtained on the same extracted cfDNA and analyzed by using Golden Helix Genome Browser v.2.0.7 (Bozeman, MT, USA) and showing an epidermal growth factor receptor (EGFR) exon 19 deletion (p.E746_A750delELREA).

treatment administration. The SiRe® NGS analysis results were adequate in 98.4% of cases (63/64) accordingly to the quality parameters reported in the methods section and previously validated; only one cases (#30) failed to reach the quality thresholds for data analysis. Regarding the run metrics parameters (*Table 1*), the median number of reads for sample was 120,960, the median number of read length was 127 bp, the median number of mapped reads was 120,498, the mean percentage of reads on target was 97%, the average reads for amplicon was 2,894 and the uniformity of coverage was 98%, in accordance with the data obtained in our previous validation study (13). On the overall, considering *EGFR*, *KRAS*, *NRAS* and *BRAF* genes, 24 patients (38%) showed at least one mutation. Only one patient (#7) showed two concomitant mutations (*NRAS* p.G13D and *KRAS* p.Q61H). In particular, 5 *EGFR* mutations (8%) were detected [n=2, exon 19 deletions (both p.E746_A750delELREA); n=2, exon 20 insertions (p.H773_V774insH and V769_D770insASV); and n=1, p.L858R exon 21 point mutation]; 14 *KRAS* point mutations (22%) [n=11, exon 2 mutations (n=4 p.G12C, n=3 p.G12D, n=1 p.G12S, n=1 p.G13D and n=2 p.G13S); and n=3, exon 3 point mutations (n=1 p.A59V and n=2 p.Q61H)]; n=4 *NRAS* point mutations (6%) [n=2, exon 2 mutations (n=1 p.G12S and n=1 p.G13D); and n=2, exon 3 point mutations (n=1 p.A59C and n=1 Q61P)]; 2 (3%) *BRAF* point mutations [n=1 exon 11 p.G469A mutation and n=1 exon 15 p.V600E mutation]. The mutated allele frequency for each mutation detected is reported in *Table 1*.

Prior to clinical reporting, only the *EGFR* detected mutations by the SiRe® panel were also confirmed by digital PCR based assay. An example of this approach was showed in *Figure 1*.

Discussion

Data, generated by the SiRe® NGS panel on cfDNA, prospectively collected from NSCLC patients, without tissue availability, examined for first and second generation *EGFR* TKIs treatment administration, are here reported; the performance of this NGS panel designed to cover only the current clinical relevant mutations, was more than excellent.

Our data confirm previous validation data. Preliminary, we had prospectively analyzed a total of 79 NSCLC patients on cfDNA. In 46 instances, cfDNA had been derived from NSCLC patients at presentation; in this subset, we detected four *EGFR* mutations (8.7%); more in details, these were

one point mutation in exon 18 (p.G719A), two deletions in exon 19 (both p.E746_A750delELREA) and one insertion in exon 20 (p.H773-V774insH) (13). Here, in this current subsequent study, we detected two exon 19 deletions (both p.E746_A750delELREA), two exon 20 insertions (p.H773_V774insH; V769_D770insASV) and one p.L858R exon 21 point mutation. Thus, we confirm an overall *EGFR* mutation rate of 8.0%. In all instances, the *EGFR* mutations were always confirmed by an independent orthogonal dPCR based assay (*Figure 1*). In addition, in the present study we have also sequenced, in the same sample set, *KRAS*, *NRAS* and *BRAF* NSCLC relevant hot-spot regions, reporting an overall mutation rate of 38%. In particular, we detected 22% *KRAS*, 6% *NRAS* and 3% *BRAF* mutated samples, with only one patient that showed two concurrent mutations (*NRAS* p.G13D and *KRAS* p.Q61H). It is remarkable to note that the mutation distribution in cfDNA of this NSCLC baseline patient series was very similar to that reported on tissues derived DNA by previous studies exploiting a multi-gene assay in NSCLC (18-21).

As a general rule, in the clinical trial settings the analysis of cfDNA had as a reference the mutational status obtained on tissue derived DNA (18-20). Conversely, following the European Medicines Agency guidelines, in baseline setting, the cfDNA analysis is indicated only for those patients in which tissues is not available. For this reason, the ability of SiRe®, to detect also mutation in *KRAS*, *NRAS* and *BRAF* genes, offer an internal control in patients that do not show alterations in *EGFR*, considering that in the most part of the cases these mutations in these genes are mutually exclusive.

In conclusion, our data update and confirm that SiRe® NGS panel represents a robust analytical tool for a centralized laboratory enabling the possibility to test cfDNA mutational status in basal setting of NSCLC patients when no tissue samples are available to assess *EGFR* mutational status for first line treatment decision making.

Acknowledgements

This study was supported by the Department of Public Health, University of Naples Federico II.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by Comitato Etico Università Federico II (No. 257/16) and written informed consent was obtained from all patients.

References

1. Ofiara LM, Navasakulpong A, Ezer N, et al. The importance of a satisfactory biopsy for the diagnosis of lung cancer in the era of personalized treatment. Curr Oncol 2012;19:S16-23.
2. Wang S, Yu B, Ng CC, et al. The suitability of small biopsy and cytology specimens for EGFR and other mutation testing in non-small cell lung cancer. Transl Lung Cancer Res 2015;4:119-25.
3. Malapelle U, Bellevicine C, De Luca C, et al. EGFR mutations detected on cytology samples by a centralized laboratory reliably predict response to gefitinib in non-small cell lung carcinoma patients. Cancer Cytopathol 2013;121:552-60.
4. Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res 2015;4:36-54.
5. Köhler J, Schuler M. Afatinib, erlotinib and gefitinib in the first-line therapy of EGFR mutation-positive lung adenocarcinoma: a review. Onkologie 2013;36:510-8.
6. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
7. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
8. Wu YL, Zhou C, Hu CP, et al. Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:213-22.
9. Crowley E, Di Nicolantonio F, Loupakis F, et al. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 2013;10:472-84.
10. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011;11:426-37.
11. Karachaliou N, Mayo-de las Casas C, Queralt C, et al. Association of EGFR L858R Mutation in Circulating Free DNA With Survival in the EURTAC Trial. JAMA Oncol 2015;1:149-57.

12. Malapelle U, Pisapia P, Rocco D, et al. Next generation sequencing techniques in liquid biopsy: focus on non-small cell lung cancer patients. Transl Lung Cancer Res 2016;5:505-10.
13. Malapelle U, Mayo de-Las-Casas C, Rocco D, et al. Development of a gene panel for next-generation sequencing of clinically relevant mutations in cell-free DNA from cancer patients. Br J Cancer 2017;116:802-10.
14. Qin Z, Ljubimov VA, Zhou C, et al. Cell-free circulating tumor DNA in cancer. Chin J Cancer 2016;35:36.
15. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
16. Bennett CW, Berchem G, Kim YJ, et al. Cell-free DNA and next-generation sequencing in the service of personalized medicine for lung cancer. Oncotarget 2016;7:71013-35.
17. Vendrell JA, Mau-Them FT, Béganton B, et al. Circulating Cell Free Tumor DNA Detection as a Routine Tool forLung Cancer Patient Management. Int J Mol Sci 2017;18. pii: E264.
18. Jänne PA, Yang JC, Kim DW, et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med 2015;372:1689-99.
19. Goss G, Tsai CM, Shepherd FA, et al. Osimertinib for pretreated EGFRThr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. Lancet Oncol 2016;17:1643-52.
20. Mok TS, Wu YL, Ahn MJ, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med 2017;376:629-40.
21. Thress KS, Brant R, Carr TH, et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. Lung Cancer 2015;90:509-15.
22. Malapelle U, de Rosa N, Rocco D, et al. EGFR and KRAS mutations detection on lung cancer liquid-based cytology: a pilot study. J Clin Pathol 2012;65:87-91.

Cite this article as: Pisapia P, Pepe F, Smeraglio R, Russo M, Rocco D, Sgariglia R, Nacchio M, De Luca C, Vigliar E, Bellevicine C, Troncone G, Malapelle U. Cell free DNA analysis by SiRe® next generation sequencing panel in non small cell lung cancer patients: focus on basal setting. J Thorac Dis 2017;9(Suppl 13):S1383-S1390. doi: 10.21037/jtd.2017.06.97

