Review Article

KRAS mutations in the circulating free DNA (cfDNA) of non-small cell lung cancer (NSCLC) patients

Mónica Garzón[1*], Sergi Villatoro[1*], Cristina Teixidó[1], Clara Mayo[1], Alejandro Martínez[2], Maria de los Llanos Gil[2], Santiago Viteri[2], Daniela Morales-Espinosa[2], Rafael Rosell[1,2,3,4,5]

[1]Pangaea Biotech, Laboratory of Oncology, Quirón Dexeus University Hospital, 08028 Barcelona, Spain; [2]Dr Rosell Oncology Institute, Quirón Dexeus University Hospital, 08028 Barcelona, Spain; [3]Cancer Biology & Precision Medicine Program, Catalan Institute of Oncology, Germans Trias i Pujol Health Sciences Institute and Hospital, Crta de Canyet s/n, 08016 Badalona, Spain; [4]Autonomous University of Barcelona (UAB), Campus Can Ruti, Crta de Canyet s/n, 08016 Badalona, Spain; [5]Molecular Oncology Research (MORe) Foundation, 08028 Barcelona, Spain

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: M Garzón, S Villatoro; (V) Data analysis and interpretation: M Garzón, S Villatoro; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Mónica Garzón. Pangaea Biotech, Laboratory of Oncology, Quirón Dexeus University Hospital, C/ Sabino Arana 5-19, 08028 Barcelona, Spain. Email: mogarzon@pangaeabiotech.com.

Abstract: Circulating free DNA (cfDNA) is obtained from serum or plasma by non-invasive methods such as a simple blood draw, a technique known as "liquid biopsy". Genetic analyses of driver alterations in cfDNA have proved very effective to predict survival and treatment response of cancer patients according to tumoral cfDNA burden in blood. Non-small cell lung cancer (NSCLC) patients with higher concentration of tumoral cfDNA in blood have, on average, shorter progression-free survival (PFS) and overall survival (OS). Regarding specific genetic alterations, KRAS proto-oncogene, GTPase (KRAS) is one of the main genes involved in NSCLC and several studies have been performed to determine its value as a predictive and prognostic biomarker in liquid biopsy. Unfortunately, to date no strong conclusions can be drawn since they have yielded contradictory results. Therefore, further investigations are necessary to establish the value of KRAS testing in liquid biopsy as prognostic or predictive factor in NSCLC. Herein, we review the current knowledge on the importance of KRAS as prognostic and predictive biomarker using non-invasive approaches and the scientific data available regarding its application in clinical practice for treatment of NSCLC.

Keywords: Liquid biopsy; circulating free DNA (cfDNA); KRAS; lung cancer; non-small cell lung cancer (NSCLC)

Submitted Aug 10, 2016. Accepted for publication Sep 14, 2016.
doi: 10.21037/tlcr.2016.10.14
View this article at: http://dx.doi.org/10.21037/tlcr.2016.10.14

KRAS in non-small cell lung cancer (NSCLC)

Lung cancer is the most common cancer and the first cause of cancer deaths worldwide (1). NSCLC is the predominant subtype of lung cancer, being adenocarcinoma the most common histology. Unfortunately, almost half of NSCLC patients are diagnosed at advanced stage and have poor prognosis and limited options for treatment, traditionally restricted to chemotherapy (2). However, in recent years, the identification of prognostic and predictive biomarkers has led to improvements in outcome and has set allowed the application of personalized medicine approaches in NSCLC patients. More than 50% of advanced NSCLC patients harbor a driver genetic alteration that, if targetable, changes the therapeutic panorama (3). For this reason, implementing resources for quick, cost-effective, multiplex detection of alterations has recently gained importance for cancer diagnostics.

Numerous mutations have been identified in NSCLC which vary depending on whether histology is adenocarcinoma or squamous-cell carcinoma (SCC), as well as with smoking history and status. The two most important alterations in the carcinogenesis of the lung are somatic mutations in the *epidermal growth factor receptor* (*EGFR*) and *KRAS* proto-oncogene, GTPase (*KRAS*) genes (4). These mutations are more frequent in lung adenocarcinoma than in SCC (5) and have implications for treatment selection. Patients with *EGFR* mutations can be treated with *EGFR* tyrosine kinase inhibitors (TKIs); however, for no drugs have yet been developed to specifically target *KRAS* alterations or show an increased efficacy when a *KRAS* mutation is present.

KRAS mutations were first identified over 30 years ago but it is only in recent years that there have been significant advances in the understanding of the biology of *KRAS* and its downstream effectors (6). The majority of *KRAS*-mutant cases in NSCLC present single point mutations at codon 12, while mutations in others positions are relatively rare (in codons 13 and 61) (7). Within codon 12, the most frequent point mutations are G12C (42%), G12V (21%), G12D (17%) and G12A (7%) (8). Current or former smokers have a significantly higher frequency of *KRAS* mutations than never smokers (9) and it is possible to identify the primary mutagenic signature of DNA damage by tobacco smoke. Smoker patients show substitutions GGT>GTT (G12V) and GGT>TGT (G12C) (purine for a pyrimidine transversions) in comparison with never-smoker patients in whom changes in GGT>GAT (G12D) or GGT>AGT (G12S) (purine for purine transitions) are more common. This suggests that, although some *KRAS* mutations are associated with history of cigarette smoking, others can also occur in never-smokers.

KRAS mutations as a prognostic factor

Prognostic biomarkers can be used as indicators of the natural history of the disease. Traditionally, *KRAS* mutations detected in biopsies of NSCLC patients have been associated with negative prognosis and poor outcomes (10).

However, the value of *KRAS* mutant status as a prognostic marker remains unclear, and seems to depend both on the disease stage at the time of diagnosis and the specific *KRAS* codon mutation. In terms of staging, the prognostic value of *KRAS* for resectable disease does not appear to be significant. However, some prospective data have shown that in resected early-stage NSCLC, *KRAS* mutations were found only in smokers and were associated with worse survival exclusively in stage I disease but not in the whole population (11). By contrast, in stage IV disease, presence of *KRAS* mutations has been associated with shorter survival (6). In terms of mutation, one study has demonstrated no difference in overall survival (OS) when comparing specific amino acid substitutions on codon 12. An interesting finding was that *KRAS* codon 13 mutations seemed to be associated with worse survival compared to codon 12 mutations. Unfortunately, these results were not confirmed by independent validation (12). Finally, according to histology, the presence of *KRAS* mutation in adenocarcinoma subtype appears to be a negative prognostic factor (13).

KRAS mutations as a predictive factor of resistance

In tissue, predictive markers can be used as indicators of response or resistance to a specific targeted treatment. Some data show that adjuvant chemotherapy is unlikely to benefit NSCLC patients harboring *KRAS* mutations. Nevertheless, in a recent study *KRAS* codon 13 mutations appeared to be deleterious and the patients had significantly worse OS with adjuvant chemotherapy (6,8).

In relation to NSCLC, *KRAS* mutations were shown to be significantly associated with inferior outcomes to chemotherapy and EGFR-TKIs (14). However, when *EGFR* mutant patients were excluded, there were no statistical differences between progression-free survival (PFS) to chemotherapy and response rates to *EGFR*-TKIs or chemotherapy. One explanation might be that *KRAS* and *EGFR* mutations are generally mutually exclusive in NSCLC and, consequently, the vast majority of *EGFR* mutations are present in *KRAS* wild-type patients (15,16). Therefore, the absence of *EGFR* alterations, rather than the presence of *KRAS* mutation, can be a negative predictor of response to *EGFR*-TKIs (8). At this respect, in advanced NSCLC some studies have also investigated the influence of *KRAS* mutations on sensitivity to chemotherapy with no significant differences in PFS and OS between *KRAS* wild-type and *KRAS* mutated patients (8). By contrast, other reports suggest that in patients treated with first-line platinum-based chemotherapy, *KRAS* mutations have a negative predictive role. So, all these findings need to be confirmed in a larger population to be of relevance for clinical decision making, highlighting the possibility that

subtype-specific *KRAS* mutation analysis could identify a subgroup of patients who could benefit more from chemotherapy (10).

Mutations in codon 12 seem to confer different responses depending on treatment. While expression of G12C is associated with reduced response to cisplatin and increased sensitivity to taxol and pemetrexed, G12D is only associated with resistance to taxol treatment and sensitivity to sorafenib. Furthermore, G12V mutants show strong sensitivity to cisplatin when compared with wild-type clones and are slightly more resistant to pemetrexed (10). However, expression of different KRAS mutants did not modify the cellular response to the EGFR inhibitor erlotinib or to gemcitabine (17,18).

Taken together, these findings change the clinical point of view since different *KRAS* mutations may lead to different signal transduction cascades in NSCLC and to different carcinogenesis and drug sensitivity. Therefore, it is necessary to define the specific *KRAS* mutation in order to identify those patients with different probabilities of responding to therapy (18). Further research is required to understand *KRAS* mutations and to develop drugs targeted against them (6). Some recent investigations have generated a renewed interest in the development of direct *KRAS* inhibitors (19). For instance, Lito and colleagues (20) achieved blockade of nucleotide exchange factors from activating KRAS. They are working with a compound, ARS-853, which is a selective, covalent inhibitor of $KRAS^{G12C}$ that inhibits mutant KRAS-driven signaling by binding to the GDP-bound oncoprotein and preventing activation. This work could present a significant step toward a direct *KRAS* inhibitor for the patients with $KRAS^{G12C}$ mutation, but nevertheless still further optimization is required to generate compound suitable for *in vivo* studies.

Circulating free DNA (cfDNA) as prognostic and monitoring technique

Unfortunately, surgical lung cancer biopsies are ineffective for showing tumor heterogeneity and are not well tolerated by patients, in addition to having certain related risks. Therefore, performing serial tissue biopsies in order to detect and monitor disease progression is extremely challenging. The answer lies in developing more accessible methodologies that facilitate non- or minimally-invasive detection and monitoring of known NSCLC mutations, as well as characterization of metastatic and/or resistant disease mechanisms, when tissue or re-biopsies are unavailable (10).

Liquid biopsy is an excellent means of identifying and monitoring alterations using a non-invasive diagnostic method. cfDNA presents the same mutations found in the primitive tumor mass since cellular necrosis and apoptosis cause the release of tumoral DNA into the bloodstream (21). In order to assess cancer disease alterations through the capture and analysis of cfDNA, many highly sensitive and specific techniques have been developed. Among these, our laboratory has extensive experience in detection of melanoma, lung and colon cancer biomarkers in cfDNA by Real-Time PNA PCR, particularly in those advanced NSCLC cases in which tumor tissue cannot be obtained by surgical biopsy. Peptide nucleic acid (PNA) is an artificially synthesized polymer analogue to DNA in which deoxyribose-phosphate backbone is replaced with a peptide of amino-ethyl-glycine unit. It forms highly stable complex with complementary DNA, and we designed to inhibit, in a specific manner, the amplification of the wt allele during the PCR amplification. We currently test serum and plasma from cancer patients for mutations in three genes (*EGFR*, *KRAS* and *BRAF*) (22,23) with 75% sensitivity and 100% specificity. Our experience demonstrates that cfDNA offers an alternative, rapid, minimally-invasive option for accurate mutation testing.

The total amount of cfDNA in the bloodstream has been demonstrated to be an effective biomarker of outcome in NSCLC; patients with higher concentrations of total cfDNA have shorter PFS and OS compared with healthy, high-risk individuals (24). By contrast, tumor regression correlates to decreased ctDNA burden in cfDNA. With regard to specific genetic alterations, one clear example is the clinical utility of the detection of *EGFR* mutations in the cfDNA of NSCLC patients treated with gefitinib (25) or erlotinib (26). *EGFR* mutations have also been shown to be of prognostic and predictive value, and patients with an activating mutation in *EGFR* in cfDNA have been reported to respond significantly better to TKIs (27). *KRAS* gene alterations detected in cfDNA have also been used as prognostic biomarkers, mainly in colorectal and pancreatic cancer (28,29). However, their predictive and prognostic value in NSCLC remains undefined, and to an extent controversial, due to the relatively few studies performed. Nevertheless, considering that liquid biopsy techniques are still being developed; new data will be generated that, in all likelihood, will clarify the importance of *KRAS* testing in NSCLC. In addition, all this new information will speed up implementation of potential new treatments. In summary, it can be concluded that the analysis of cfDNA is an essential

Table 1 Survival data according to KRAS status in blood

Author, year	Study population (n)	NSCLC Stage	Therapeutic regimen	Specimen type	PFS (months)	P value (PFS)	OS (months)	P value (OS)
Camps C. *et al.*, (30) 2005	67	IIIB or IV	Chemotherapy	Serum	KRAS +: 7.3 WT: 5.5	0.2300	KRAS +: 11.4 WT: 12.5	0.2800
Gautschi O. *et al.*, (31) 2007	175	I, II, III (A/B) or IV	Surgery + chemotherapy	Plasma	–	–	Worse OS of patients with mutant plasma KRAS	0.0370
Wang S. *et al.*, (32) 2010	120	IIIB or IV	EGFR-TKI	Plasma	KRAS+: 2.5 WT: 8.8	<0.0010	KRAS +: 16.9 WT: 20.3	0.8270
Nygaard AD. *et al.*, (33) 2013	246	III or IV	Chemotherapy	Plasma	KRAS +: 3.0 WT: 5.6	0.0043	KRAS +: 4.8 WT: 9.5	0.0002
Kim ST. *et al.*, (34) 2013	57	IIIB and IV	EGFR-TKI	Serum	–	–	KRAS +: 3.9 WT: 10.4	0.4520
Nygaard AD. *et al.*, (35) 2014	69	III or IV	Chemotherapy	Plasma	KRAS +: 2.1 WT: 5.5	0.0100	KRAS +: 3.6 WT: 8.4	0.0300
Ai B. *et al.*,(16) 2016	meta-analysis (30,31,33,35)	III or IV	Chemotherapy	cfDNA	No significant differences	0.4500	No significant differences	0.8900

NSCLC, non-small cell lung cancer; PFS, progression-free survival; OS, overall survival; EGFR, epidermal growth factor receptor; cfDNA, circulating free DNA.

tool for clinicians to select targeted therapies, and is becoming a powerful means of monitoring somatic changes induced after treatment.

KRAS mutations: prognostic and predictive value of cfDNA in NSCLC

As mentioned, *KRAS* mutations in tissue could be a weak, but valid, predictor of poor prognosis and treatment outcome (14). Therefore, several studies have tried to uncover the same kind of correlation between the presence of *KRAS* mutations in blood and clinical outcome in order to use *KRAS* as a biomarker.

We have reviewed the relevant studies related to *KRAS* mutations in liquid biopsy as predictive or prognostic factors in NSCLC, summarizing all the information in *Table 1*.

Several studies have evaluated *KRAS* mutation status in cfDNA and response to chemotherapy. Three were performed using plasma samples and showed worse PFS and OS in *KRAS* mutated patients (31,33,35) while a study performed in serum did not show any significant differences (30). However, a meta-analysis incorporating data from all the studies concluded that *KRAS* mutations in cfDNA may not be useful to predict response to chemotherapy (16).

The clinical utility of determining *KRAS* mutations in liquid biopsy as a marker of sensitivity to *EGFR*-TKIs in NSCLC has also been studied. So far, two studies, one in plasma and one in serum, failed to show significant differences in terms of OS. However, the plasma study did show that mutant *KRAS* patients had a worse PFS than wild type subjects (32,34). As mentioned, the discrepancies between these studies might be due to the fact that the vast majority of *EGFR* mutations occur in *KRAS* wild-type patients. In consequence, the real value of *KRAS* as prognostic and predictive biomarker might have been overestimated (34). Another reason could be the small number of studies performed which have assessed the prognostic value of *KRAS* mutations in NSCLC in cfDNA. In summary, all the evidence suggests that *KRAS* genotype detected in cfDNA may not be a good prognostic factor of survival in NSCLC patients. However, the predictive or

prognostic role of detection of KRAS mutations in cfDNA remains to be confirmed and warrants further investigation (4). Also, serial testing of *KRAS* mutations in the blood of *KRAS* positive patients can be useful to monitor the course of the disease, as it has already been demonstrated for *EGFR* or *BRAF* mutations. Our laboratory is actively working in this direction, and preliminary results are encouraging (36).

Conclusions

Tissue biopsy is still the gold standard for diagnosis. However, new technologies are improving the isolation and identification of lung cancer-related mutations in blood and therefore leading to new therapeutic options for the management of cancer patients. Currently, the ability to analyze tumoral cfDNA is one the most important breakthroughs in thoracic oncology.

Furthermore, liquid biopsy has the important advantage of being a noninvasive procedure, meaning it can be reproduced, facilitating repeated evaluations of tumor genetic alterations and monitoring of their status throughout the course of the disease. Liquid biopsy has also been shown to be a huge boon to oncologists in terms of early identification of the molecular mechanisms responsible for development of acquired resistance to targeted therapies.

Regarding the detection of *EGFR* or *KRAS* mutations in cfDNA as predictive and prognostic biomarkers, *EGFR* T790M mutations are clearly related to acquire resistance to *EGFR*-TKIs. However, the prognostic and predictive value of *KRA*S mutations in cfDNA as a biomarker is still a matter of debate. Therefore, prospective studies with larger patient research cohorts are still required to draw definitive conclusions.

Acknowledgements

None.

Footnote

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

References

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395-409.
3. 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.
4. Rosell R, Karachaliou N. Large-scale screening for somatic mutations in lung cancer. Lancet 2016;387:1354-6.
5. Roberts PJ, Stinchcombe TE, Der CJ, et al. Personalized medicine in non-small-cell lung cancer: is KRAS a useful marker in selecting patients for epidermal growth factor receptor-targeted therapy? J Clin Oncol 2010;28:4769-77.
6. Wood K, Hensing T, Malik R, et al. Prognostic and Predictive Value in KRAS in Non-Small-Cell Lung Cancer: A Review. JAMA Oncol 2016;2:805-12.
7. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 2002;62:6997-7000.
8. Karachaliou N, Mayo C, Costa C, et al. KRAS mutations in lung cancer. Clin Lung Cancer 2013;14:205-14.
9. Mao C, Qiu LX, Liao RY, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. Lung Cancer 2010;69:272-8.
10. Passiglia F, Bronte G, Castiglia M, et al. Prognostic and predictive biomarkers for targeted therapy in NSCLC: for whom the bell tolls? Expert Opin Biol Ther 2015;15:1553-66.
11. Nelson HH, Christiani DC, Mark EJ, et al. Implications and prognostic value of K-ras mutation for early-stage lung cancer in women. J Natl Cancer Inst 1999;91:2032-8.
12. Yu HA, Sima CS, Shen R, et al. Prognostic impact of KRAS mutation subtypes in 677 patients with metastatic lung adenocarcinomas. J Thorac Oncol 2015;10:431-7.
13. Mascaux C, Iannino N, Martin B, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2005;92:131-9.
14. Pan W, Yang Y, Zhu H, et al. KRAS mutation is a weak, but valid predictor for poor prognosis and treatment outcomes in NSCLC: A meta-analysis of 41 studies. Oncotarget 2016;7:8373-88.
15. Ulivi P, Chiadini E, Dazzi C, et al. Nonsquamous, Non-Small-Cell Lung Cancer Patients Who Carry a Double Mutation of EGFR, EML4-ALK or KRAS: Frequency, Clinical-Pathological Characteristics, and Response to

Therapy. Clin Lung Cancer 2016;17:384-390.
16. Ai B, Liu H, Huang Y, et al. Circulating cell-free DNA as a prognostic and predictive biomarker in non-small cell lung cancer. Oncotarget 2016. [Epub ahead of print].
17. Zhu CQ, da Cunha Santos G, Ding K, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2008;26:4268-75.
18. Garassino MC, Marabese M, Rusconi P, et al. Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. Ann Oncol 2011;22:235-7.
19. Patricelli MP, Janes MR, Li LS, et al. Selective Inhibition of Oncogenic KRAS Output with Small Molecules Targeting the Inactive State. Cancer Discov 2016;6:316-29.
20. Lito P, Solomon M, Li LS, et al. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. Science 2016;351:604-8.
21. 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.
22. 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.
23. Gonzalez-Cao M, Mayo-de-Las-Casas C, Molina-Vila MA, et al. BRAF mutation analysis in circulating free tumor DNA of melanoma patients treated with BRAF inhibitors. Melanoma Res 2015;25:486-95.
24. Wei Z, Shah N, Deng C, et al. Circulating DNA addresses cancer monitoring in non small cell lung cancer patients for detection and capturing the dynamic changes of the disease. Springerplus 2016;5:531.
25. Douillard JY, Ostoros G, Cobo M, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014;9:1345-53.
26. Weber B, Meldgaard P, Hager H, et al. Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. BMC Cancer 2014;14:294.
27. Qiu M, Wang J, Xu Y, et al. Circulating tumor DNA is effective for the detection of EGFR mutation in non-small cell lung cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2015;24:206-12.
28. Perrone F, Lampis A, Bertan C, et al. Circulating free DNA in a screening program for early colorectal cancer detection. Tumori 2014;100:115-21.
29. Tjensvoll K, Lapin M, Buhl T, et al. Clinical relevance of circulating KRAS mutated DNA in plasma from patients with advanced pancreatic cancer. Mol Oncol 2016;10:635-43.
30. Camps C, Sirera R, Bremnes R, et al. Is there a prognostic role of K-ras point mutations in the serum of patients with advanced non-small cell lung cancer? Lung Cancer 2005;50:339-46.
31. Gautschi O, Huegli B, Ziegler A, et al. Origin and prognostic value of circulating KRAS mutations in lung cancer patients. Cancer Lett 2007;254:265-73.
32. Wang S, An T, Wang J, et al. Potential clinical significance of a plasma-based KRAS mutation analysis in patients with advanced non-small cell lung cancer. Clin Cancer Res 2010;16:1324-30.
33. Nygaard AD, Garm Spindler KL, Pallisgaard N, et al. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. Lung Cancer 2013;79:312-7.
34. Kim ST, Sung JS, Jo UH, et al. Can mutations of EGFR and KRAS in serum be predictive and prognostic markers in patients with advanced non-small cell lung cancer (NSCLC)? Med Oncol 2013;30:328.
35. Dowler Nygaard A, Spindler KL, Pallisgaard N, et al. Levels of cell-free DNA and plasma KRAS during treatment of advanced NSCLC. Oncol Rep 2014;31:969-74.
36. de las Casas CM, Cao MG, Ramirez SV, et al. Usefulness of circulating free DNA for monitoring epidermal growth factor receptor mutations in advanced non-small cell lung cancer patients: a case report. Transl Lung Cancer Res 2016;5:532-7.

Cite this article as: Garzón M, Villatoro S, Teixidó C, Mayo C, Martínez A, de Los Llanos Gil M, Viteri S, Morales-Espinosa D, Rosell R. *KRAS* mutations in the circulating free DNA (cfDNA) of non-small cell lung cancer (NSCLC) patients. Transl Lung Cancer Res 2016;5(5):511-516. doi: 10.21037/tlcr.2016.10.14

