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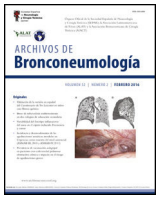

Original Article

Plasmatic *KRAS* Kinetics for the Prediction of Treatment Response and Progression in Patients With KRAS-mutant Lung Adenocarcinoma

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ABSTRACT

Introduction: *KRAS* is the most common driver mutation in lung cancer. ctDNA-based assessment offers advantages over tumor as a minimally invasive method able to capture tumor heterogeneity. Monitoring *KRAS* mutational load in ctDNA may be useful in the management of the patients.
Methods: Consecutive patients diagnosed with *KRAS* mutant lung adenocarcinoma in the tumor biopsy were included in this study. Plasma samples were obtained at different time points during the course of the disease. *KRAS* mutations in plasma were quantified using digital PCR and correlated with mutations in tumor and with radiological response and progression.
Results: Two hundred and forty-five plasma samples from 56 patients were analyzed. The rate of detection of *KRAS* mutations in plasma in our previously characterized *KRAS*-mutant cases was 82% overall, reaching 96% in cases with more than 1 metastatic location. The dynamics of *KRAS* mutational load predicted response in 93% and progression in 63% of cases, 33 and 50 days respectively in advance of radiological evaluation. Progression-free survival for patients in whom ctDNA was not detectable in plasma after treatment initiation was significantly longer than for those in whom ctDNA remained detectable (7.7 versus 3.2 months; HR: 0.44, $p = 0.004$).
Conclusions: The detection of *KRAS* mutations in ctDNA showed a good correlation with that in tumor biopsy and, in most cases, predicted tumor response and progression to chemotherapy in advance of radiographic evaluation. The liquid biopsies for ctDNA-based molecular analyses are a reliable tool for *KRAS* testing in clinical practice.

Estudio de la cinética del *KRAS* plasmático para predecir la respuesta al tratamiento y la progresión en pacientes con adenocarcinoma de pulmón con mutación de *KRAS*

Palabras clave:
KRAS
Adenocarcinoma de pulmón
Biopsia líquida
ADN tumoral circulante
Dinámica clonal

RESUMEN

Introducción: La mutación en *KRAS* es la mutación iniciadora más común en el cáncer de pulmón. La valoración basada en el ctDNA ofrece ventajas frente a la tumoral, al ser un método mínimamente invasivo capaz de capturar la heterogeneidad del tumor. La monitorización de la carga de *KRAS* mutado en el ctDNA puede ser útil en el manejo de los pacientes.

Abbreviations: NSCLC, non-small-cell lung cancer; LA, lung adenocarcinoma; ctDNA, circulating tumor DNA; CT, computed tomography; RECIST, response evaluation criteria in solid tumors; dPCR, digital PCR; cfDNA, circulating-free DNA; GE, genomic equivalents; PFS, progression free survival.

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Métodos: En este estudio se incluyó, mediante selección consecutiva, a pacientes diagnosticados con adenocarcinoma de pulmón con mutación en *KRAS* en la biopsia tumoral. Se obtuvieron muestras de plasma en diferentes momentos durante el curso de la enfermedad. Las mutaciones de *KRAS* en plasma se cuantificaron mediante PCR digital y se correlacionaron con las mutaciones en el tumor y con la respuesta radiológica y la progresión.
Resultados: Se analizaron 245 muestras de plasma de 56 pacientes. La tasa de detección de mutaciones *KRAS* en plasma en aquellos casos previamente definidos con dicha mutación fue del 82% globalmente, porcentaje que alcanzó el 96% en aquellos casos con más de una ubicación metastásica. La dinámica de la carga de *KRAS* mutado predijo la respuesta en el 93% de los casos y la progresión en el 63%, a los 33 y 50 días, respectivamente, anteriores a la evaluación radiológica. La supervivencia libre de progresión para pacientes en los que el ctDNA no era detectable en plasma después del inicio del tratamiento fue significativamente más larga que para aquellos en los que el ctDNA permaneció detectable (7,7 frente a 3,2 meses; HR: 0,44; $p = 0{,}004$).
Conclusiones: La detección de mutaciones *KRAS* en el ctDNA mostró una buena correlación con la de la biopsia tumoral y, en la mayoría de los casos, predijo la respuesta tumoral a la quimioterapia y la progresión antes de la evaluación radiológica. Las biopsias líquidas para análisis moleculares basados en ctDNA son una herramienta fiable para la valoración de *KRAS* en la práctica clínica.

Introduction

Lung cancer is the leading cause of cancer mortality.[1] *KRAS* mutation, the most common driver mutation in NSCLC, is present in approximately 30% of NSCLC patients, mainly in lung adenocarcinoma (LA).[2] *KRAS* mutation has been historically believed to be a bad prognostic factor, but studies shows conflicting results.[3–5] These may be explained by the heterogeneity within *KRAS*-driven NSCLC, with different response to chemotherapy depending on the involved codon,[6] and by the impact of the presence of other mutations such as *TP53* or *STK11* concomitant with *KRAS*.[7–9] Despite many efforts, no *KRAS*-directed targeted therapy has been approved to date.[10,11] Recently has been reported results of a phase 1 trial showing great antitumor activity of the AMG 510 inhibitor in cases with the $KRAS^{G12C}$ mutation.[12] These encouraging results must be confirmed in ongoing clinical trials.

Liquid biopsy to obtain circulating tumor DNA (ctDNA) has been proposed as an alternative to tumor biopsy for molecular assessment. The advantages of ctDNA are several. As a minimally invasive method, it allows a greater frequency of sampling during follow-up and can be used when tumor samples are insufficient for molecular testing. Moreover, the use of ctDNA may better reflect the molecular heterogeneity of the tumor, since it will include ctDNA fragments from all tumor sites.[13] We have previously reported the usefulness of ctDNA monitoring for the prediction of treatment outcome in patients with *EGFR*-mutant NSCLC.[14]

KRAS mutational load in plasma may be used as a surrogate marker of the global tumor burden in *KRAS*-mutated NSCLC, and thus monitoring *KRAS* mutational load in ctDNA may be useful in the management of these patients. The aim of this study was to determine the feasibility of using ctDNA for the detection of *KRAS* mutations both at the time of diagnosis and, specifically to evaluate the use of serial plasma genotyping for monitoring treatment response.

Materials and methods

Study population

This retrospective study included patients diagnosed with tissue characterized *KRAS*-mutant LA and with available plasma samples between November 2012 and February 2017 at our center. Staging of cases was done according to the American Joint Committee on Cancer Staging Manual, 8th edition.[15] Plasma samples were collected before treatment and at different time points during the follow-up (in most cases, every 6 weeks during treatment and every 3 months during follow-up). Treatment response was evaluated with computed tomography (CT) according to RECISTv1.1[16] following local practice (every 8–9 weeks). The study was approved by the local ethics committee (CEIC 2012/4823/I). All participants gave written informed consent.

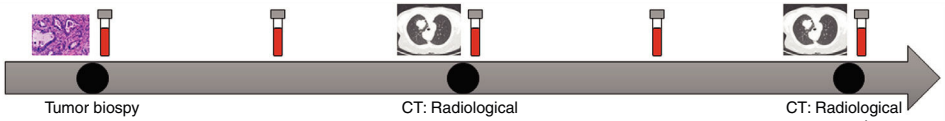
Study design

The primary objective of the study was the correlation between ctDNA- and tumor-based detection of mutations. Findings from plasma samples at the time of tumor biopsy were compared with those of the paired tumor biopsy. Agreement was considered positive when *KRAS*-mutant alleles were detected in plasma in at least 0.1% of frequency. Sensitivity, specificity, positive and negative predictive values were calculated considering tissue biopsy as a gold standard, and using as a negative control plasma samples from 11 cases characterized in tissue as *KRAS* wild type.

The association between the dynamics of the *KRAS* mutational load in plasma and radiological response and progression was analyzed for any treatment line received for each patient. The term "treatment line" refers to any administered treatment, including best supportive care. To analyze correlation with response and progression we used plasma samples obtained at the same time as a CT, whereas plasma samples obtained after the start of treatment and prior to the time of a CT were used for the analysis of prediction of response and progression. A positive correlation with/prediction of response was defined as the lack of detection or any level of decrease of *KRAS* mutational load in the plasma sample at the time (for correlation analysis) or prior to the time (for the prediction study) of the CT showed tumor response. A positive correlation with/prediction of progression was defined as the reappearance or any level of increase of *KRAS* mutational load in the plasma sample at the time (for correlation analysis) or prior to the time of (for the prediction study) the CT showed progression (Fig. 1).

Sample collection and KRAS mutation assessment

KRAS analysis in tumor tissue was performed in paraffin-embedded biopsies. DNA was extracted from macro-dissected sections of each tumor sample with the QIAamp DNA Mini kit (Qiagen). *KRAS* mutations (exon 2 and 3) were analyzed by Sanger sequencing using BigDyev3.1 (Applied Biosystems) on a 3500DX Genetic Analyzer (Applied Biosystems).



	Plasma/tumor biopsy correlation	Radiological response prediction	Radiological response correlation	Radiological progression prediction	Radiological progression correlation
Analyzed cases	51	15	17	19	42
ctDNA positive agreement	42/51 (82%)	14/15 (93%)	15/17 (88%)	12/19 (63%)	30/42 (71%)
Reason for exclusion					
No response	-	30	30	-	-
No progression	-	-	-	4	4
No pre-treatment plasma	-	1	1	-	-
No plasma at the moment of the CT	-	-	4	-	6
No plasma between treatment start and CT	-	6	-	29	-

CT: computed tomography

Fig. 1. Study design. The table shows the number of analyzed cases in each study time point and the reason of exclusion in not analyzed cases.

For ctDNA-based assessment of *KRAS* mutations, 10–15 ml of blood were collected in K2EDTA tubes and processed within 4 h of collection. The tubes were centrifuged twice at 1600 × *g* for 15 min; plasma was collected, aliquoted in 1 ml fractions and frozen at −80 °C. *KRAS* mutations were assessed by digital PCR (dPCR). DNA was extracted from 1 ml of plasma using the MagMax (cfDNA) isolation kit (Applied Biosystems) with manual processing. Isolated DNA was quantified using the QubitdsDNA HS assay kit (Life Technologies). The *KRAS*-mutated allele frequency was quantified by dPCR with a QuantStudio3D dPCR system (Life Technologies) using wet-lab validated dPCR TaqMan Assays (Life Technologies). Used assays are listed in Supplementary Table 1. Mutation analysis was performed with the QuantStudio 3D AnalysisSuite™ Cloud Software (Life Technologies).

Circulating cell-free DNA (cfDNA) refers to the total circulating DNA and ctDNA only to the circulating tumoral DNA. *KRAS* mutational load was quantified with dPCR using the QuantStudio3D Analysis Suite™ software that expresses the allele frequency as the percentage of mutated copies referred to the total number of gene copies.

Statistical analyses

Concordance between tissue and plasma *KRAS* mutation status was calculated as the number of positive plasma samples out of the total number of tissue samples. Bivariate analyses were performed using the chi-square test. The Kaplan–Meier method was used to estimate progression free survival (PFS). All statistical analyses were performed with IBMR SPSSv.22. Significance was set at $p \leq 0.05$.

Results

A total of 245 plasma samples from 56 patients were analyzed. The majority of patients were male (73%), current smokers (64%), had stage IVc disease (70%), and a *KRAS* mutation involving codon 12(96%) (Table 1).

Table 1
Patients' characteristics.

	n = 56
Age, median (range)	60 (45–82)
Sex, *n* (%)	
Male	41 (73%)
Female	15 (27%)
Smoking status, *n* (%)	
Never	4 (7%)
Former	16 (29%)
Active	36 (64%)
Disease stage, *n* (%)	
Stage III	6 (11%)
Stage IVa	10 (18%)
Stage IVb	1 (1%)
Stage IVc	39 (70%)
Number of metastatic locations, *n* (%)	
0	6 (11%)
1	19 (34%)
2	12 (21%)
3	9 (16%)
4	5 (9%)
5	4 (7%)
6	1 (2%)
Liver metastases, *n* (%)	
No	46 (82%)
Yes	10 (18%)
KRAS mutation in tissue at diagnosis, *n* (%)	
Codon 12	53 (95%)
Gly12Cys	24 (43%)
Gly12Val	10 (18%)
Gly12Ala	7 (12%)
Gly12Asp	6 (10%)
Gly12Phe	3 (5%)
Gly12Arg	2 (4%)
Gly12Glu	1 (2%)
Codon 13	3 (5%)
Gly13Cys	2 (4%)
Gly13Asp	1 (2%)

Correlation between ctDNA- and tumor-based detection of KRAS mutations

For this correlation study 51 paired tissue and plasma samples were available from 49 patients (Fig. 1). 49 patients were biopsied once and 2 patients twice (in these two cases, the second biopsy was performed at the time of progression). The median time from tumor biopsy to blood withdrawal was 19 days (range: −56 to 94) (Supplementary Table 2).

A positive correlation between plasma and tumor *KRAS* mutational status was observed in 42 cases (82.4%) (Fig. 1). The rate of detection of *KRAS* mutations in plasma was significantly higher in patients with more than one metastatic location (96.4% vs. 65.2%; $p = 0.004$). The case with more than 1 metastatic location in which plasmatic *KRAS* was not detected, had only 2 metastatic locations (bone and liver). The median cfDNA obtained from the plasma samples was 3595.12 genomic equivalents (GE)/ml (range: 307.4–197,978.54). The rate of *KRAS* mutation detection was significantly lower in samples with a cfDNA burden below the median (76% vs. 100%; $p = 0.009$). The presence of more than one metastatic location was significantly associated with a cfDNA burden above the median ($p = 0.028$). The 6 cases with negative plasma-tissue correlation had the GE/ml below the median and only one metastatic location. Type of *KRAS* mutation (codon12 vs. codon13), presence of liver metastases, sex, smoking history, and time from tumor biopsy to blood withdrawal were not associated with the rate of detection of *KRAS* mutations in plasma (Table 2).

Circulating *KRAS* mutations were not detected in any of the 11 negative control cases. Sensitivity for *KRAS* detection in plasma was 0.82 and specificity 1. The positive and negative predictive values were 1 and 0.55 respectively.

In order to avoid the heterogeneity, data were analyzed excluding on one hand the two biopsies performed at the time of progression, and on the other hand cases with stage III disease. In the 49 cases biopsied at the initial diagnosis, a positive correlation between plasma and tumor *KRAS* mutational status was observed in 40 (81.6%). As shown in the overall population, the rate of detection of *KRAS* mutations in plasma was significantly higher in cases with more than one metastatic location (96.2% vs. 55.6%; $p = 0.002$). No significant differences in the detection rate were found when cases were analyzed according to the different clinical characteristics. When the 6 cases with stage III excluded from the analysis, the rate of KRAS detection in plasma in cases with a stage IV disease stage was 80.4%. This rate was higher in cases with more than one metastatic location (96.4% vs. 55.6%; $p = 0.001$).

Dynamics of KRAS mutational load and disease monitoring

In the analysis of correlation and prediction of response and progression to treatment, plasma samples from 32 patients, who received a total of 52 treatment lines, were included. The median number of treatment lines analyzed per patient was 1 (range: 0–5). Most cases were treated with chemotherapy (72%). In the five patients who did not receive any treatment, plasma samples were collected both at diagnosis and disease progression. The radiological evaluation of 50% of the treatment lines was classified as progressive disease. Clinical characteristics of cases are summarized in Supplementary Table 3, and details on analyzed treatment lines of each case in Supplementary Table 4.

Overall, the mean percentage of circulating *KRAS* mutant alleles was lower at the time of radiological response (0.4%; standard deviation (SD): 0.87) than at pre-treatment (3.32%; SD: 5.46) and higher at radiological progression (4.63%; SD: 7.69) (Supplementary Figure 1).

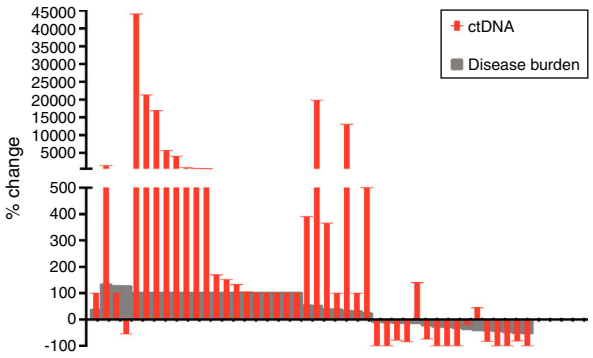


Fig. 2. Waterfall plot showing disease burden and ctDNA dynamics in the 44 treatment lines with available plasma and measurable disease. Cases with progression disease driven by appearance of new lesions are shown as 100% increase in disease burden.

Correlation of KRAS mutational load with radiological response and progression

17 (33%) of the 52 treatment lines were included in the analysis of correlation with radiological response (Fig. 1). In 15 (88%) of the 17 cases, there was a positive correlation between the dynamics of the *KRAS* mutational load and radiological response (Fig. 1). The *KRAS* mutational load decreased in 6 cases and was not detectable in 9 at response evaluation. One of the 2 cases without a positive correlation had stage III disease and the other one had bone as the sole metastatic location.

42 (80%) of the 52 treatment lines were included in the analysis of correlation with radiological progression (Fig. 1). In 30 (71%) of cases, there was a positive correlation between the dynamics of *KRAS* mutational load and radiological progression. The *KRAS* mutational load increased in 21 cases and reappeared in 9. No significant differences in the agreement rate with response or progression were observed when cases were analyzed according to the different clinical characteristics.

The mean reduction of ctDNA at the time of radiological response compared to initial levels in the 16 cases with radiological response and available plasma was 64.7% (range: −100 to 140), whereas the mean increase of ctDNA at the time of radiological progression compared to ctDNA levels at the moment of radiological response (or before treatment initiation in case of non-responders) in the 42 progressing cases with available plasma was 3104% (range: −100 to 44,059) (Fig. 2).

In seven cases within this cohort, no plasma sample was available at the time of tumor biopsy and were thus not evaluable for the ctDNA- and tumor-based correlation study, and only second or subsequent treatment lines could be evaluated. When these 7 cases were excluded from the analysis, the rate of concordance between the dynamics of plasma and radiological response and progression were 93.3% and 68.6% respectively.

KRAS mutational load for prediction of radiological response and progression

15 (29%) of the 52 treatment lines from 32 patients were included in the analysis of prediction of radiological response (Fig. 1). In 14 (93%) of the 15 cases, the dynamics of the *KRAS* mutational load were able to predict radiological response (Fig. 1). The *KRAS* mutational load decreased in 8 cases and was undetectable in 6 at response. The case where radiological response was not predicted had bone as the sole metastatic location. In cases where radiological response was predicted, the median time elapsed between obtaining the plasma for the analysis of prediction of

Table 2
Bivariate analysis between *KRAS* detection ratio and clinical characteristics.

	% *KRAS* detection in plasma	*p*
Sex: male vs. female	28/34 (82.4%) vs. 12/15 (80%)	0.845
Smoking history: yes vs. no	37/45 (82.2%) vs. 3/4 (75%)	0.721
Liver metastases: yes vs. no	8/10 (80%) vs. 34/41 (82.9%)	0.828
Disease limited to thorax: yes vs. no	11/14 (78.6%) vs. 31/37 (83.8%)	0.663
Disease stage: IVa vs. IVb-c[a]	5/8 (62.5%) vs. 32/38 (84.2%)	0.159
KRAS mutation: codon 12 vs. codon 13	41/49 (83.7%) vs. 1/2 (50%)	0.221
Metastatic locations: ≤1 vs. >1	15/23 (65.2%) vs. 27/28 (96.4%)	0.004
Plasma-biopsy interval: below vs. above median	25/27 (92.6%) vs. 20/24 (83.3%)	0.306
GE: below vs. above median	19/25 (76%) vs. 25/25 (100%)	0.009

GE: genomic equivalents.
[a] 5 cases with disease stage III excluded.

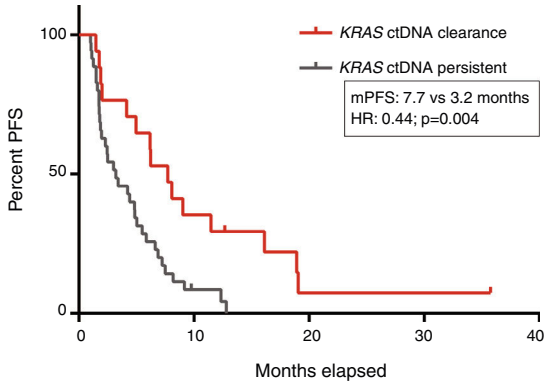


Fig. 3. Kaplan–Meier plot showing progression-free survival according to the ctDNA clearance in plasma during treatment. ctDNA, circulant tumor DNA; mPFS, median progression free survival; HR, hazard ratio.

response and the CT that stated the radiological response was 33 days (range: 14–67).

19 (36%) of the 52 treatment lines were included in the analysis of prediction of radiological progression (Fig. 1). In 12 (63%) of the 19 cases, the dynamics of *KRAS* mutational load were able to predict radiological progression (Fig. 1). The *KRAS* mutational load increased in 8 cases and reappeared in 4. In cases where radiological progression was predicted, time elapsed between obtaining the plasma for the analysis of prediction of progression and the CT that stated the disease progression was 50 days (range: 13–120). No significant differences in the rate of prediction of radiological response or progression were shown when cases were analyzed according to the different clinical characteristics (Supplementary Table 5).

The ratio of prediction of radiological response and progression when the 7 cases without available plasma sample at the time of tumor diagnostic was 92.3% and 61.1% respectively.

PFS was significantly longer for those patients with a ctDNA clearance after treatment initiation, compared to those with a ctDNA persistence during treatment (7.7 versus 3.2 months; HR: 0.44, $p = 0.004$) (Fig. 3). No differences in PFS were shown depending on pre-treatment ctDNA levels. Graphic representation of cases in which plasmatic *KRAS* kinetics were predictive and not predictive of clinical outcome is shown in Fig. 4 and Supplementary Figure 2, respectively.

Discussion

The rate of detection of *KRAS* mutations in plasma was 82% overall, reaching 96% in cases with more than 1 metastatic location. These results are similar to those previously reported by our group in *EGFR*-mutated NSCLC,[14] and better than the 64% accuracy for plasmatic *KRAS* mutation detection reported by Sacher et al.[17] This difference may be explained by the difference in the disease burden between the two populations. The percentage of cases with less than 4 metastatic locations in the study of Sacher et al. was more than 90% compared with 75% in our series. In both studies, the rate of mutation detection increased with the number of metastatic locations.

The correlation between ctDNA- and tumor-based detection on *KRAS* mutations was lower in plasma samples with a low cfDNA burden, which is a surrogate marker of the number of cells represented by the circulating DNA in the plasma sample. Low cfDNA indicates limited DNA shedding from the tumor and limits the sensitivity for detecting mutant tumoral DNA. These findings are in line of previously reported data with *EGFR*-mutated cases,[14,18] and suggests that in cases where *KRAS* mutations are not detected in plasma samples with a low cfDNA burden, either additional sampling may be warranted to increase the amount of evaluable DNA or, alternatively, a tumor biopsy should be performed.

The most novel aspect of this study was the evaluation of the utility of serial plasma genotyping for monitoring treatment response and progression. Our findings confirm the feasibility of ctDNA-based analysis in monitoring treatment outcomes. The decrease of the circulating *KRAS* mutational load correlated with radiological response in 93% and with progression in 71% of cases in our study. Furthermore, the dynamics of *KRAS* mutational load were able to predict radiological response more than one month in advance of radiographic evaluation in almost 90% of cases, and radiological progression in 63% of cases. The lower prediction and correlation with progression may be due to the presence of resistance mechanisms not detectable by our assays. As previously reported in the *EGFR* setting,[14,19–21] the circulating *KRAS* clearance during treatment was associated with longer PFS. The fact that the time between plasma sampling and CT was different among patients suggests that ctDNA clearance may not be detected in all cases and implies that these results must be interpreted with caution.

The utility of liquid biopsy to predict treatment outcomes has been previously reported, mainly in *EGFR*-mutated cases.[21,22] Several studies have evaluated the relationship between plasmatic *KRAS* status and response to chemotherapy with conflicting results.[23–26] Recently, the monitoring of circulating *KRAS* mutations has been proposed as a tool for discriminating pseudo-progression from true progression to immunotherapy in *KRAS*-mutated LA.[27]

As this study was performed in a real-life setting, it has some inherent limitations. Plasma samples were collected when patients attended the outpatient clinic, which depended on the clinical evolution and toxicities, as well as other logistic parameters. Then, plasma sampling times were not scheduled uniformly and were thus not homogeneous for all patients. In addition, the use of a single-gene PCR assay implies that we were not able to evaluate the impact of concurrent mutations other than *KRAS* especially at the time of progression disease. The use of next generation sequencing techniques with panels covering a wide spectrum of hotspot

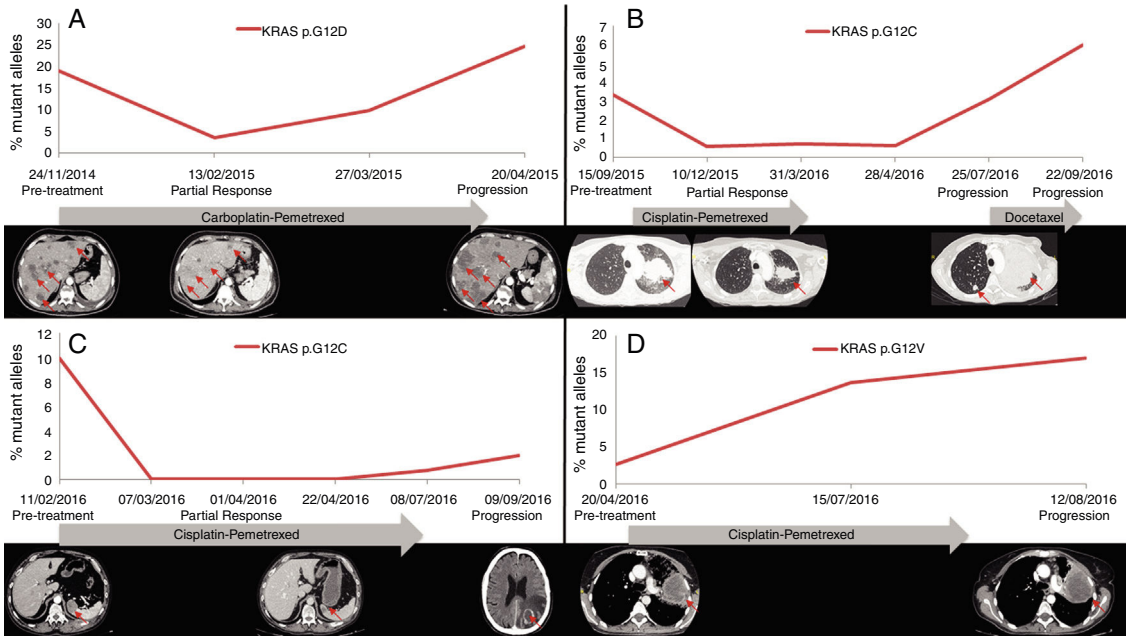


Fig. 4. Dynamics of *KRAS* mutational load in three patients with an initial radiological response followed by disease progression (A–C) and one non-responder patient (D).

genes or even the sequencing of the whole exome or genome, is providing important information about the role of these concomitant mutations, that may have a role as predictive biomarkers for response to immunotherapy.[8,9] The genotyping of plasmatic *KRAS* will be crucial if the long awaited *KRAS* targeted therapy reaches the clinics.

Serum markers like soluble fragment of cytokeratin 19 (CYFRA21-1), carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and cancer antigen (CA)-125, has been studied in lung cancer. Its prognostic significance is controversial, with conflicting results between studies.[28–32] It seems that the elevation of more than one tumor markers at baseline have been related with poor prognosis more consistently that the elevation of one isolated marker.[33–35] Regarding the use of circulating tumor markers for the prediction of response to chemotherapy, the decrease of CYFRA21-1 after treatment initiation has been related with response in some studies.[31,36,37] The use of this circulating tumor markers is not currently recommended for the management of lung cancer.

In conclusion, ctDNA-based assessment of *KRAS* mutations showed a good correlation with tumor-based detection and, in most cases, predicted tumor response and progression well in advance of radiographic evaluation. Our findings indicate that liquid biopsies for ctDNA-based molecular analyses are a reliable tool for *KRAS* testing in clinical practice.

Author contributions

AT identified patients for the study, performed the statistical analyses and wrote the manuscript; PR, AH and EA identified patients for the study; LC, RL, SC, LH and CF processed the samples and performed the mutational analysis; JA, BB and EA supervised the study, wrote and revised the manuscript. All authors read and approved the final manuscript.

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None of the sponsors had any role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Conflict of interest

Authors declare no conflicts of interest related to the current work. AT reports personal fees and non-financial support from Roche and Boehringer-Ingelheim; non-financial support from Lilly and personal fees from BMS, MSD and Pfizer. EA reports personal fees and non-financial support from BMS, personal fees from Astra Zeneca, grants, personal fees and non-financial support from Roche, personal fees and non-financial support from MSD, personal fees from Lilly, grants and personal fees from Pfizer, personal fees from Boehriger-Ingelheim. BB has received honoraria for speaker, consultancy or advisory role from Astra-Zeneca, Biocartis, Merck-Serono, Novartis, Pfizer, Qiagen, La Roche and ThermoFisher.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2020.01.023.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2019;69:7–34.
2. Wood K, Hensing T, Malik R, Salgia R. Prognostic and predictive value in KRAS in non-small-cell lung cancer. JAMA Oncol. 2016;2:805.
3. Slebos RJC, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJLM, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med. 1990;323:561–5.
4. Shepherd FA, Domerg C, Hainaut P, Jänne PA, Pignon J-PP, Graziano S, et al. Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol. 2013;31:2173–81.
5. Mellema WW, Dingemans A-MC, Thunnissen E, Snijders PJFF, Derks J, Heideman DAMM, et al. KRAS mutations in advanced nonsquamous non-small-cell lung cancer patients treated with first-line platinum-based chemotherapy have no predictive value. J Thorac Oncol. 2013;8:1190–5.
6. Mellema WW, Masen-Poos L, Smit EF, Hendriks LEL, Aerts JG, Termeer A, et al. Comparison of clinical outcome after first-line platinum-based chemotherapy

in different types of KRAS mutated advanced non-small-cell lung cancer. Lung Cancer. 2015;90:249–54.
7. Shepherd FA, Lacas B, Le Teuff G, Hainaut P, Jänne PA, Pignon JP, et al. Pooled analysis of the prognostic and predictive effects of TP53 comutation status combined with KRAS or EGFR mutation in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol. 2017;35:2018–27.
8. Arbour KC, Jordan E, Kim HR, Dienstag J, Yu HA, Sanchez-Vega F, et al. Effects of co-occurring genomic alterations on outcomes in patients with KRAS-mutant non-small cell lung cancer. Clin Cancer Res. 2018;24:334–40.
9. Skoulidis F, Goldberg ME, Greenawalt DM, Hellmann MD, Awad MM, Gainor JF, et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. Cancer Discov. 2018. CD-18-0099.
10. Blumenschein GR, Smit EF, Planchard D, Kim DW, Cadranel J, De Pas T, et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC). Ann Oncol. 2015;26:894–901.
11. Jänne PA, van den Heuvel MM, Barlesi F, Cobo M, Mazieres J, Crinò L, et al. Selumetinib plus docetaxel compared with docetaxel alone and progression-free survival in patients with KRAS-mutant advanced non-small cell lung cancer. JAMA. 2017;317:1844.
12. Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature. 2019;575:217–23.
13. Diaz LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32:579–86.
14. Taus Á, Camacho L, Rocha P, Hardy-Werbin M, Pijuan L, Piquer G, et al. Dynamics of EGFR mutation load in plasma for prediction of treatment response and disease progression in patients with EGFR-mutant lung adenocarcinoma. Clin Lung Cancer. 2018.
15. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WEE, et al. The IASLC lung cancer staging project: Proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. J Thorac Oncol. 2016;11:39–51.
16. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–47.
17. Sacher AG, Paweletz C, Dahlberg SE, Alden RS, O'Connell A, Feeney N, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA Oncol. 2016;2:1014–22.
18. Bronkhorst AJ, Aucamp J, Pretorius PJ. Cell-free DNA: preanalytical variables. Clin Chim Acta. 2015;450:243–53.
19. Iwama E, Sakai K, Azuma K, Harada T, Harada D, Nosaki K, et al. Monitoring of somatic mutations in circulating cell-free DNA by digital PCR and next-generation sequencing during afatinib treatment in patients with lung adenocarcinoma positive for EGFR activating mutations. Ann Oncol. 2017;28:136–41.
20. Marchetti A, Palma JF, Felicioni L, De Pas TM, Chiari R, Del Grammastro M, et al. Early prediction of response to tyrosine kinase inhibitors by quantification of EGFR mutations in plasma of NSCLC patients. J Thorac Oncol. 2015;10:1437–43.
21. Oxnard GR, Thress KS, Alden RS, Lawrance R, Paweletz CP, Cantarini M, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol. 2016;34:3375–82.
22. Lee JY, Qing X, Xiumin W, Yali B, Chi S, Bak SH, et al. Longitudinal monitoring of EGFR mutations in plasma predicts outcomes of NSCLC patients treated with EGFR TKIs: Korean Lung Cancer Consortium (KLCC-12-02). Oncotarget. 2016;7:6984–93.
23. Gautschi O, Huegli B, Ziegler A, Gugger M, Heighway J, Ratschiller D, et al. Origin and prognostic value of circulating KRAS mutations in lung cancer patients. Cancer Lett. 2007;254:265–73.
24. Nygaard AD, Garm Spindler K-L, Pallisgaard N, Andersen RF, Jakobsen A. The prognostic value of KRAS mutated plasma DNA in advanced non-small cell lung cancer. Lung Cancer. 2013;79:312–7.
25. Dowler Nygaard A, Spindler K-LG, Pallisgaard N, Andersen RF, Jakobsen A. Levels of cell-free DNA and plasma KRAS during treatment of advanced NSCLC. Oncol Rep. 2014;31:969–74.
26. Ai B, Liu H, Huang Y, Peng P. Circulating cell-free DNA as a prognostic and predictive biomarker in non-small cell lung cancer. Oncotarget. 2016;7:44583–95.
27. Guibert N, Mazieres J, Delaunay M, Casanova A, Farella M, Keller L, et al. Monitoring of KRAS-mutated ctDNA to discriminate pseudo-progression from true progression during anti-PD-1 treatment of lung adenocarcinoma. Oncotarget. 2017;8:38056–60.
28. Moro D, Villemain D, Vuillez JP, Delord CA, Brambilla C. CEA CYFRA21-1 and SCC in non-small cell lung cancer. Lung Cancer. 1995;13:169–76.
29. Arrieta O, Saavedra-Perez D, Kuri R, Aviles-Salas A, Martinez L, Mendoza-Posada D, et al. Brain metastasis development and poor survival associated with carcinoembryonic antigen (CEA) level in advanced non-small cell lung cancer: a prospective analysis. BMC Cancer. 2009;9:119.
30. Kulpa J, Wójcik E, Reinfuss M, Kołodziejski L. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem. 2002;48:1931–7.
31. Ardizzoni A, Cafferata MA, Tiseo M, Filiberti R, Marroni P, Grossi F, et al. Decline in serum carcinoembryonic antigen and cytokeratin 19 fragment during chemotherapy predicts objective response and survival in patients with advanced nonsmall cell lung cancer. Cancer. 2006;107:2842–9.
32. Pujol JL, Molinier O, Ebert W, Dayrès JP, Barlesi F, Buccheri G, et al. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. Br J Cancer. 2004;90:2097–105.
33. Nisman B, Lafair J, Heching N, Lyass O, Baras M, Peretz T, et al. Evaluation of tissue polypeptide specific antigen CYFRA 21-1, and carcinoembryonic antigen in nonsmall cell lung carcinoma: does the combined use of cytokeratin markers give any additional information? Cancer. 1998;82:1850–9.
34. Ando S, Kimura H, Iwai N, Yamamoto N, Iida T. Positive reactions for both Cyfra21-1 and CA125 indicate worst prognosis in non-small cell lung cancer. Anticancer Res n.d.;23:2869–74.
35. Barlési F, Gimenez C, Torre J-P, Doddoli C, Mancini J, Greillier L, et al. Prognostic value of combination of Cyfra 21-1 CEA and NSE in patients with advanced non-small cell lung cancer. Respir Med. 2004;98:357–62.
36. Vollmer RT, Govindan R, Graziano SL, Gamble G, Garst J, Kelley MJ, et al. Serum CYFRA;1; 21-1 in advanced stage non-small cell lung cancer: an early measure of response. Clin Cancer Res. 2003;9:33–1728.
37. Hamzaoui A, Thomas P, Castelnau O, Roux N, Roux F, Kleisbauer JP. Usefulness of longitudinal evaluation of Cyfra 21-1 variations in advanced lung cancer monitoring. Lung Cancer. 1997;16:191–202.