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Short communication

Monitoring *KRAS* mutations in circulating DNA and tumor cells using digital droplet PCR during treatment of *KRAS*-mutated lung adenocarcinoma

Nicolas Guibert [a,b,d], Anne Pradines [b,c], Magali Farella [b,c], Anne Casanova [b,c], Sandrine Gouin [a], Laura Keller [b,c,d], Gilles Favre [b,c,d], Julien Mazieres [a,b,d,*]

[a] Thoracic Oncology Department, Larrey Hospital, University Hospital of Toulouse, France
[b] Inserm, Centre de Recherche en Cancérologie de Toulouse, CRCT UMR-1037, Toulouse, France
[c] Institut Claudius Regaud, IUCT-Oncopole, Laboratoire de Biologie Médicale Oncologique, Toulouse, France
[d] University of Toulouse III (Paul Sabatier), Toulouse, France

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ABSTRACT

Liquid biopsies are a new non-invasive strategy to detect and monitor the biology of non-small-cell lung cancer (NSCLC) in the era of personalized medicine. *KRAS* is an oncogenic driver that is mutated in 30% of NSCLCs and is associated with a poor prognosis. 62 samples from 32 patients, treated for metastatic *KRAS*-mutated lung adenocarcinoma, had DNA extracted from plasma and circulating tumor cells (CTCs) prospectively tested for the presence of *KRAS* mutations using droplet digital PCR. A *KRAS* mutation was detected in 82% of patients. Sensitivity was 78% for circulating free DNA (cfDNA) and 34% for CTCs. The presence of a *KRAS* mutation in cfDNA was correlated with a poor response to chemotherapy or targeted therapy. When a *KRAS*-mutated-DNA was detected and then monitored in cfDNA, its variation during targeted or conventional therapy was correlated with response, according to RECIST criteria, in 87.5% of cases ($n = 14/16$), whereas this correlation was observed in 37.5% of cases for CTCs ($n = 3/8$). We report the usefulness of using digital droplet PCR on liquid biopsies to predict and monitor responses to treatment of *KRAS*-mutated lung adenocarcinoma. ctDNA was much more sensitive than CTCs in this context.
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1. Introduction

Amongst all the theranostic biomarkers for NSCLC, *KRAS* mutations are characterized by their high frequency (nearly 30% of adenocarcinomas), their negative prognostic value, and a lack of an effective targeted therapy. Nevertheless, recent preclinical findings and early phase trials indicate potentially effective strategies using lethal synthetic interactions [1] or by directly targeting KRAS-mutant proteins [2]. These encouraging results suggest the need for new tools that provide non-invasive and iterative information on these tumors. Liquid biopsies, circulating tumor cells (CTCs), and circulating-free DNA (cfDNA) isolated from the blood of patients have been widely studied in this field, but have not yet been adopted into routine clinical practice [3]. Beside their prognostic value and the possibility of cytomorphological analysis, CTCs isolated by ISET (isolation by size of epithelial tumor cells) can detect some molecular alterations in NSCLC, such as *EGFR* mutations (assessed by PCR) [4] or *ALK* rearrangement (assessed by FISH and immunocytochemistry) [5].

cfDNA level is increased in lung-cancer patients and changes during treatment could help monitor tumor burden [6]. Identifying mutated tumor-specific DNA can circumvent the obstacle of its low specificity [7]. Thus, detection of several genomic alterations in cfDNA is possible with good diagnostic accuracy, including *EGFR* [8], *KRAS* [9–13], or *BRAF* mutations [14]. Another potential application for liquid biopsies is the early detection of mechanisms of resistance or their use to dynamically follow-up a mutated clone during targeted therapy [8,14].

KRAS-mutated cfDNA has been detected using many procedures that have variable sensitivities: 76.7% using restriction fragment-length polymorphism [15], 90% using ARMS-qPCR [14], and 97% with a multiplex PCR sequencing system [16]. A study recently compared the diagnostic accuracy of CTCs to cfDNA to detect *KRAS* mutations in blood samples ($n = 26$), and found cfDNA was more

* Corresponding author at: Thoracic Oncology Unit, Respiratory Disease Department, Hôpital Larrey, CHU Toulouse, Chemin de Pouvourville, 31059 Toulouse Cedex, France.
E-mail addresses: nicolas.guibert@hotmail.fr (N. Guibert), mazieres.j@chu-toulouse.fr (J. Mazieres).

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Table 1
Diagnostic accuracy of ddPCR on blood-derived DNA compared to tissue samples.

CfDNA	Results	DCR (RECIST)
Positive	26/32	34.6%
Negative	6/32	100%
Sensitivity	78%	
Specificity	100%	
CTCs		
Positive	11/32	
Negative	21/32	
Sensitivity	34.3%	
Specificity	100%	
Total liquid biopsy		
Positive	27/32	
Negative	5/32	
Sensitivity	81%	
Specificity	100%	

cfDNA = circulating free DNA; CTCs = circulating tumor cells; DCR = disease-control rate; RECIST = Response Evaluation Criteria in Solid Tumors.

sensitive than using CTCs (95% vs. 78% sensitivity, respectively) [17]. Variations in *EGFR*-mutated DNA level seem to be correlated with radiographic response to *EGFR*-TKI [8]. Nevertheless, although the use of plasma digital droplet PCR (ddPCR) for cfDNA has been recently used to detect *KRAS* mutations, and thus determine resistance in patients who have progressed under EGFR-TKI [18], the level of *KRAS* mutation has never been monitored during chemotherapy or targeted therapies using CTCs or cfDNA.

Herein, we compare the sensitivity of *KRAS*-mutated ctDNA and *KRAS*-mutated DNA extracted from CTCs to detect responses to treatment of *KRAS*-mutated lung adenocarcinoma.

2. Material and methods

2.1. Patients and samples

Thirty-two patients treated for a metastatic adenocarcinoma were prospectively included in this study between December 2014 and June 2015. All patients had a *KRAS* mutation previously detected in formalin-fixed paraffin-embedded tissue samples, which had undergone high-resolution melting and the TaqMan assay when a variant was detected. The *KRAS* subtype substitutions were G12C ($n = 15$, 47%), G12D ($n = 8$, 25%), G12V ($n = 6$, 19%), and G12A ($n = 3$, 9%).

Patients were predominantly male (66%). Multiple samples were available from 22 patients, and included a total of 62 samples. All patients gave their informed consent to participate in this study. Initial samples were collected before conventional chemotherapy ($n = 23$, 72%), targeted therapy ($n = 8$, 25%: i.e., *EGFR*-TKI [$n = 3$], cdk4/6 inhibitor [$n = 2$], MEK inhibitor + docetaxel [$n = 3$]), or immunotherapy using anti-PD-1 ($n = 1$). Responses to systemic treatment was evaluated using RECIST 1.1 (Response Evaluation Criteria in Solid Tumors).

2.2. Isolation of DNA from circulating tumor cells and plasma, and analysis of KRAS mutations

For each patient, four 5-mL blood samples were analyzed within 4 h of collection. Two samples underwent ISET to isolate CTCs [5]. Three spots on the ISET filter were used to extract DNA using proteinase K lysing buffer. The two other blood samples were used to isolate cfDNA using the QI-Amp circulating nucleic-acid kit (Qiagen). DNA extracted from both cfDNA and CTCs was tested for the presence of the corresponding *KRAS* mutation using digital droplet PCR (QX200. Bio-Rad). The input DNA was emulsified into

Table 2
KRAS-mutated and wild-type DNA in plasma and circulating tumor cells (CTCs) during treatment for non-small-cell lung cancer.

	Last treatment received (before the time of blood collection)	Mutant copies/mL in CTCs	Negative control (wild-type patients)	Mutant copies/mL in ctDNA	RECIST evaluation
Patient 1 *KRAS* G12D	Carboplatin + Paclitaxel Cdk4/6 inhibitor Cdk4/6 inhibitor Cdk4/6 inhibitor	8 6 0 2.5	0 0 0 0	240 9 4.5 6.5	Partial response Stable disease Progressive disease
Patient 2 *KRAS* G12D	None Cisplatin + Pemetrexed Pemetrexed	9.5 1.5 1.5	0 0 0	16 2.5 2.5	Partial response Stable disease
Patient 3 *KRAS* G12V	Bevacizumab Erlotinib Erlotinib	0 0 0	0 0 0	3 6 6	Progressive disease Stable disease
Patient 4 *KRAS* G12D	Cisplatin + Pemetrexed Docetaxel	2 2 NA	0 0 0	72 776 2960	Progressive disease Progressive disease
Patient 5 *KRAS* G12D	Pemetrexed Carboplatin + Gemcitabine	6.5 0	0 0	256 61	Partial response
Patient 6 *KRAS* G12C	Gemcitabine Erlotinib	0 0	0 0	1232 976	Progressive disease
Patient 7 *KRAS* G12D	Erlotinib Vinorelbine	2 3.5	0 0	0 1.5	Progressive disease
Patient 8 *KRAS* G12D	Pemetrexed Docetaxel + MEK inhibitor	0 1.5	0 0	1464 53	Stable disease
Patient 9 *KRAS* G12D	Pemetrexed Docetaxel + MEK inhibitor	0 4	0 0	6.5 0	Partial response
Patient 10 *KRAS* G12V	Carboplatin + Paclitaxel	0 0	0 0	0 84	Progressive disease
Patient 11 *KRAS* G12C	Carboplatin + Vinorelbine	0 0	0 0	1132 1956	Progressive disease
Patient 12 *KRAS* G12 V	Pemetrexed	0 0	0 0	0 5	Stable disease
Patient 13 *KRAS* G12V	Cdk4/6 inhibitor	0 0	0 0	5760 6860	Progressive disease
Patient 14 *KRAS* G12C	Carboplatin + Pemetrexed	0 0	0 0	3 2	Stable disease
Patient 15 *KRAS* G12D	Gemcitabine	3 0	0 0	6 0	Stable disease
Patient 16 *KRAS* G12C	Carboplatin + Vinorelbine	0 0	0 0	2 0	Partial response
Patient 17 *KRAS* G12C	Carboplatin + Pemetrexed	0 0	0 0	312 160	Partial response

CTCs: circulating tumor cells; cfDNA: circulating free DNA; WT: wild-type; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease; PD: progressive disease; PR: partial response. NA: not available. Negative control: cfDNA extracted from the plasma of patients with *BRAF*-mutated and *KRAS* WT lung adenocarcinomas.

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