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Revolutions in cancer treatment:
how can they be integrated?

Liquid biopsies

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Abstract

Free circulating nucleic acids (fcNAs) constitute one of the most exciting areas in oncology and one of the fastest-developing fields in recent years. Free circulating DNA and RNA (fcDNA/fcRNA) are extracellular DNA and RNA found in the blood. The study of these molecules, particularly fcDNA, may offer tremendous potential in cancer screening and prognosis, and in monitoring the efficacy of cancer treatments. Unlike tissue biopsies, fcDNA is obtained from blood as a liquid biopsy, which offers a number of advantages. First, the preservatives used for tissue biopsies may damage the DNA and hinder genetic analysis. By contrast, the preservatives used for blood samples do not affect current methods of genetic analysis and so plasma samples are suitable for genetic analysis. A second advantage is the simplicity of obtaining a blood sample (less invasive than surgical resection or needle biopsy) and the reduced risk for patients. Blood samples can be obtained at any time during treatment and follow-up. Samples can be taken regularly during routine consultations and do not require trained surgical staff or any specific equipment. This allows dynamic monitoring of molecular changes in the tumour during treatment, rather than simply a snapshot when treatment is started. A third advantage of liquid biopsies is the possibility of detecting heterogeneity: they provide information on all parts of the patient's tumour and on multiple tumour sites in patients with multiple metastases. This wider genetic picture helps to characterise the molecular profile more fully, and can result in principles to guide therapy. Finally, the blood samples contain information on early metastases, while tissue biopsies are only carried out on tumours that have already been detected by imaging or in other ways. Liquid biopsies provide information on minimal residual disease and metastases that may not have been detected yet, allowing doctors to identify those patients who need to be treated. In colorectal cancer, analyses of RAS *and* BRAF *mutations in tumour tissues can be replaced by fcDNA analyses before possibly starting treatment with antibodies that target epidermal growth factor receptor (EGFR), and also for treatment monitoring. A variety of data indicate that fcDNA could be used in future to assess residual disease and support decision-making on adjuvant chemotherapy after colorectal resection. Finally, it is possible to imagine liquid biopsy being used in screening for cancer.*

THE VALUE OF USING CIRCULATING TUMOUR DNA IN STRATEGIES TO MANAGE COLORECTAL CANCER

Liquid biopsy involves identifying tumour biomarkers from a blood test. Two complementary types of approach are used: circulating tumour cells and free circulating nucleic acids (fcNAs). Among these, circulating DNA (fcDNA) is the main subject of this article.

In colorectal cancer, as with the oncological management of other cancers, fcDNA from a liquid biopsy offers a number of benefits and possible applications:
- For theranostics, it can guide the selection of a treatment, in particular prescriptions for anti-EGFR antibodies (mutations of *RAS*);
- For treatment monitoring, it becomes possible to carry out longitudinal blood tests to observe and manage resistance to treatment;
- Recurrences can be monitored and detected at an early stage;
- It allows identification of minimal residual disease and determination of the possible value of adjuvant treatments;
- It could be useful in prognosis;
- It also has a role in screening and very early detection of cancers.

USE IN THERANOSTICS

A blinded, multi-centre study involving 106 patients with metastatic colorectal cancer compared two methods of analysing *KRAS* and *BRAF* mutations in tumour tissue. The standard method used to analyse tumour tissues was employed, and compared with liquid biopsy using a quantitative method based on polymerase chain reaction (PCR) specifically designed to analyse fcDNA.[1] The fcDNA analysis was found to have a specificity and sensitivity of 100% for the *BRAF* V600E mutation, and a 98% specificity and 92% sensitivity with a concordance value of 96% for the seven *KRAS* point mutations tested.[1]

A second prospective, real-time multi-centre clinical study in 11 centres in France used a blinded approach to assess the clinical value of liquid biopsy in traditional therapeutic management of 140 patients with metastatic colorectal cancer.[2] The study examined concordance and time taken to obtain fcDNA results analysed by quantitative PCR in real time versus results from standard analysis of tumour tissues.[2] All *BRAF* and *KRAS* mutations were studied. A greater number of mutations were identified through fcDNA analysis in liquid biopsy than in tissue biopsies; the mean time taken to obtain the results was 16 days with tissue biopsies compared with 2 days for fcDNA analysis.[2] These results suggest that fcDNA analysis could replace tumour tissue analysis in practice, and emphasised the clinical usefulness of this test, which significantly reduces the time required to process and use the data.[2] In terms of concordance, the study design did not include a minimal detection threshold for mutations, unlike the previous study.[1] This increased sensitivity translated into more cases of discordance between the mutations found through fcDNA and those found through tissue biopsies, particularly in the case of *KRAS* exon 2 and *BRAF*.[2] This observation raises the question of the appropriate threshold and identification of the factors "responsible" for resistance to anti-EGFR therapies.

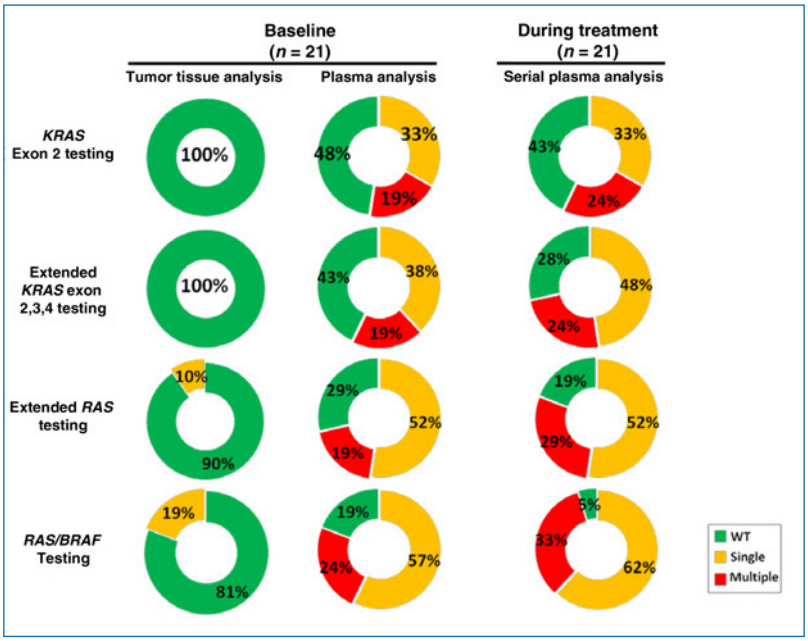
Similarly, the multicentre prospective RASANC study looked at the concordance between liquid biopsies using next-generation sequencing and tissue biopsies in relation to *RAS* mutations.[3] This trial, which included a large sample of patients with colorectal cancer with hepatic metastases (n=425, 77.4% with fcDNA suitable for evaluation) found excellent concordance between the results of the two techniques for determining the *RAS* status of these patients, with a κ coefficient of 0.89 and 94.8% accuracy.[3] This result confirms the value of liquid biopsy in practice, and validates the use of this technology for routine analysis of *RAS* mutations in colorectal cancer.[3] This study also sought to identify clinical factors likely to be associated with efficacy for detecting *RAS* mutations using fcDNA. Results indicated that the proportion of inconclusive tests with plasma was higher in patients with peritoneal carcinosis than those with hepatic metastases. The absence of hepatic metastases was the main clinical factor associated with inconclusive results from fcDNA and conversely, the presence of such metastases was associated with very good test sensitivity.[3]

VALUE OF LONGITUDINAL LIQUID BIOPSIES IN TREATMENT FOLLOW-UP

It was longitudinal blood tests that first led to detection of the clonal evolution of tumours during treatment, which is a potential factor causing resistance. In a retrospective, blinded study in 46 patients with colorectal cancer refractory to cetuximab or dasatinib combined with FOLFOX chemotherapy, blood samples were taken before treatment, at 8 days in the second and fourth cycles of chemotherapy and at the end of treatment.[4] fcDNA analysis from plasma samples (79 different samples in total) showed that in the 21 patients considered as wild-type for *KRAS* exon 2 (*KRAS* not mutated) through tests on tumour tissues, based on plasma testing only 48% had the same result prior to treatment (**Figure 1**).[5] When more mutation tests were carried out (*KRAS* exons 2, 3 and 4, *NRAS* or *BRAF*), differences appeared between the results obtained from tissues and those from fcDNA during treatment, with few patients remaining wild-type for both *KRAS*, *NRAS* and *BRAF* at the end of treatment with cetuximab (**Figure 1**).[5] In patients who had several blood tests during treatment, mutation rates appeared low at the beginning, with only one mutation. After a period of reduction or stabilisation, multiple new mutations developed during treatment, particularly due to selective pressure from the treatment, revealing a convergent evolutionary model.[5]

Figure 1. Distribution of the status of RAS / BRAF mutations based on the analysis of fcDNA before and after treatment with anti-EGFR antibodies.[5]

Reprinted from reference [5], Clin Cancer Res, 2017, 23, 4578-4591, Thierry et al, Circulating DNA demonstrates convergent evolution and common resistance mechanisms during treatment of colorectal cancer, with permission from AACR.



In the case of treatment with an anti-EGFR therapy in metastatic colorectal cancer, the strategic application of longitudinal biopsies could be the following:

- Initially there may be no mutation or a small number of cells with mutations in *RAS*, which may have sensitivity to an anti-EGFR agent.
- During treatment the majority of *RAS* wild-type cells might be destroyed but clone cells with a *RAS* mutation may develop.
- If mutations become predominant, it could be reduced by using an alternative treatment (i.e. not anti-EGFR), creating the possibility of reintroducing an anti-EGFR later on.[6]

This pattern is the principle underlying the prospective Italian CRICKET study involving treatment rechallenge in patients with metastatic colorectal cancer.[7] Patients were wild-type for *RAS* and *BRAF* on FOLFOX chemotherapy, had been given cetuximab + irinotecan as first-line treatment, then bevacizumab as second-line treatment (due to resistance to first-line treatment). The study assessed the activity of cetuximab + irinotecan when reintroduced as a third-line treatment.[7] Using translational techniques fcDNA was analysed; progression-free survival (PFS) and

overall survival were better during re-administration of the treatment in the patients who had remained wild-type than in those who had mutations.[7]
These observations, which need to be confirmed in larger patient numbers, suggest a new treatment strategy based on data from liquid biopsy: treatment with anti-EGFR antibodies can be stopped and then reintroduced depending on the evolution of their *RAS* status during treatment.

VALUE OF EARLY DETECTION OF RECURRENCES AND MINIMAL RESIDUAL DISEASE

The detection of minimal residual disease is one of the most valuable applications for fcDNA. DNA with somatic mutations is strongly specific to tumours and can in theory therefore provide optimum markers. In relation to the number of normal fragments of fcDNA, however, the number of fragments of circulating mutant genes is low, which makes them difficult to detect and quantify with sufficient sensitivity for significant clinical use.[8] In the study by Diehl et al,[8] a highly sensitive approach was used to quantify fcDNA in 18 patients (162 plasma samples) on multimodal therapy for colorectal cancer. There was a significant difference between recurrence-free survival in patients with detectable postoperative levels of fcDNA and those patients whose postoperative fcDNA levels were undetectable (P = 0.006). These results suggest that the measurement of fcDNA can be used to reliably monitor the evolution of tumours in patients with cancer who are undergoing surgery or chemotherapy.[8]

A review by Crowley et al looked at the clinical use of mutations that are detectable by liquid biopsy after diagnosis, and their prognostic value in early detection of residual disease and recurrence.[9] The authors propose that traditional biopsies should be substituted for fcDNA studies and monitoring of tumour-specific molecular abnormalities to facilitate detecting signs of early relapse, predicting the response to treatments and development of acquired resistance.[9]

A large Australian study, carried out in a prospective cohort of 230 patients (1046 plasma samples) with resected stage 2 colon cancer, showed that detection of fcDNA (via a postoperative sequencing test) was able to identify minimal residual disease and predict recurrence.[10] The results from this study show that in patients not treated with adjuvant chemotherapy, when this postoperative test was considered to be negative (fcDNA negative) the recurrence rate was very low (9.8%). However, in the 14 patients who had positive postoperative tests for fcDNA, the survival rate was very low and 79% of these patients had a recurrence during the median 27-month follow-up.[10] A similar result was found after resection of hepatic metastases.[10] The authors concluded that detection of fcDNA after resection of stage 2 colon cancer makes it possible to identify the patients who are most at risk of recurrence and contributes towards decision-making on adjuvant treatment.[10]

Similar results were found in a US study that used a different postoperative technology in a sample of 145 patients with stage 2 (n = 86) and stage 3 (n = 59) colorectal cancer.[11] fcDNA analysis detected minimal residual disease within a short time-frame after complete resection of the tumour and precisely identified which patients were at high risk of recurrence in stage 2 and 3 colorectal cancers. They also suggested that the detection of minimal residual disease using fcDNA sequencing may allow personalisation of adjuvant treatment strategies, and in particular, the avoidance of chemotherapy in patients who do not need it.[11]

PROGNOSTIC VALUE

The analysis of fcDNA can yield qualitative and quantitative data. Qualitative data is used to assess the type of mutations, while quantitative data can assess the total fcDNA level which has a clear correlation with tumour volume. This level can clearly provide a prognostic index, particularly in patients with metastases.

Results from a study by Messaoudi et al in 97 patients with metastatic colorectal cancer provided the first qualitative and quantitative evidence in favour of multi-parameter analysis of fcDNA for prognostic assessment of patients.[12] In this study, the main mutations of *KRAS* exon2 and *BRAF* V600E (qualitative parameters) were determined and at the same time PCR quantitative multi-markers were used to ascertain the total concentrations of fcDNA and mutant fcDNA, the proportion of mutant fcDNA and the fcDNA integrity index (quantitative parameters).[12]

Patients presenting with high levels of fcDNA were found to have a significantly lower overall survival than those with low levels (18.07 months versus 28.5 months, P<0.0087). Furthermore, multivariate analysis showed that a high level of fcDNA is an independent prognostic factor (P<0.034).[12]

The PLACOL study made the same observation. The authors observed large differences in median survival depending on the level of fcDNA.[13] Patients with a high concentration of fcDNA (> 10 ng/mL) before the first cycle of chemotherapy had lower overall survival than those with a low concentration (≤ 0.1 ng/mL): 6.8 vs 33.4 months (P < 0.0001).[13] These results clearly indicate a relationship between tumour volume, tumour extension and the level of fcDNA.

The PRODIGE-14 phase 2 prospective study tested the intensification of chemotherapy in order to resect hepatic metastases that were initially not resectable among 153 patients with metastatic colorectal cancer after resection of hepatic metastases.[14] This trial specifically examined the clinical validity of circulating tumour cells (CTCs) using Cellsearch® and *KRAS* mutations on fcDNA, evaluated by PCR liquid biopsy.[14] In patients with a *KRAS* mutation rate that was undetected before surgery, the R0/R1 resection rate was much higher than for those with a mutant fcDNA level detected before surgery (85% versus 15%); 64% of the latter were unable to undergo resection of their hepatic metastases.[14] On the basis of these results, the evolution of the fcDNA level is an indicator that should be taken into account when making decisions regarding resection surgery of metastases.

POSSIBLE VALUE IN SCREENING FOR CANCER

The screening of fcDNA, and all the techniques developed around this parameter, has potential value in screening for cancer. Moulière et al[15] noted different gradations in the fcDNA level between patients with colorectal cancer at all stages and healthy individuals. This study used a multi-marker (Intplex®) method for fcDNA analysis, which permits sensitive and specific non-invasive analysis of tumour fcDNA.[15] The authors emphasised that this tool could easily be introduced into oncology laboratories,[15] and therefore offers potential in personalised medicine for the screening and management of colorectal cancer.

A number of other studies are also examining the use of these methods for screening, and their results are awaited.

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