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## Is the determination of ctDNA a scientific “spy” that foresees cancer?

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### Abstract

Since 1948, circulating tumour DNA (ctDNA) was first identified in human blood. ctDNA is in fact DNA shed by tumour cells from all metastatic tumour locations throughout the whole body, and is thrown into the bloodstream and can then be isolated by a standard blood draw. Using this technique scientists can obtain a wide view of tumour heterogeneity, identify different mechanisms of drug resistance, what is its predominance and the clinical rational of precision cancer medicine become a part of our daily practice. Secondly, early detection of cancer may also contribute to global decrease in cancer mortality.

**Key words:** Tumour biopsies; Liquid biopsies; Circulating tumour DNA; Precision cancer medicine

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**Core tip:** With the increase development of molecular medicine we may further change our clinical rational to a precise cancer medicine rational way. Consequently, we may improve the quality of life of our patients, with less toxicity, more cost-effectiveness decisions and above all improve response rate and survival. Defining the complete genomic “picture” of all cancerous lesions, in the near future as a standard of care, will require all genetic information concerning each individual cancer.

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### INTRODUCTION

Cancer mortality has decreased globally in the United

States and Europe, as well as the individual risk of dying from cancer, due to recent reliable data<sup>[1]</sup>. Although analysis confirms that decrease in cancer mortality is across all income levels, a difference has also been reported in low and middle income countries in which this decline still needs to be clarified. Preventive strategies in cancer control, specifically addressing risk factors, have been one of the measures that lead to these results. Nevertheless, early detection and intervention in high risk groups, with a non-invasive, accurate, sensitive and specific method such as analysis of circulating tumour DNA (ctDNA), is still the most effective measure to reduce cancer mortality<sup>[2]</sup>.

Since 1948, ctDNA was first identified in human blood<sup>[3]</sup>. At that time scientist were innocent of the real meaning and consequences of this procedure. The discovery of tumour DNA in blood samples, also called "liquid biopsy", may have bypassed the need for traditional invasive measures. The first challenge was how to discriminate ctDNA from normal cell free DNA. The sensitivity of polymerase chain reaction (PCR) based on digital techniques improved along time with the addition of next generation sequencing (NGS). ctDNA have a median half-life round about two hours, and changes in ctDNA levels can be detected for weeks, before changes in imaging or in protein biomarkers. The blood stream is in fact the reservoir of ctDNA of all sites of metastases<sup>[4,5]</sup>.

ctDNA is in fact DNA shed by tumour cells from all metastatic tumour locations throughout the whole body, and is thrown into the bloodstream and can then be isolated by a standard blood draw. Using this technique scientist can obtain a wide view of tumour heterogeneity and identify different mechanisms of drug resistance and what is its predominance in a global view. This open view may be missed by a single lesion tumour biopsy, and only a small perspective of the whole disease may be captured. In other words, additional resistance alterations may not be found<sup>[5]</sup>.

## DISCUSSION

### ***What evidence do we have that justify the benefit of identifying early ctDNA?***

The answer is just because the test exists doesn't mean we all have to do it. There is evidence that cancers that may rapidly lead to resistance such as lung, melanoma and colorectal cancer, should be mandatory to be monitored by genetic analysis of ctDNA<sup>[6]</sup>. Secondly, is there a prognostic and predictive factor in early stage surgical lung cancers patients? As Dr. Karachaliou mentioned at ASCO 2016, in 25% of lung cancer cases, the tissue from small biopsies is insufficient to execute the genotyping sequencing in order to offer a personalizing cancer therapy<sup>[7]</sup>.

The idea that liquid biopsy matches tissue biopsy has been around for several years now. In ASCO 2016, a study funded by Guardian Health Inc., obtained liquid biopsies

from 15000 cancer patients (lung cancer 37%, breast cancer 14%, colorectal cancer 10% and other cancers 38%). From 386 of these patients, tumour biopsies were also available. When compared tissue samples with ctDNA, by sequencing method, the results reported showed, an overall accuracy of 87% (336/386 patients)<sup>[6,7]</sup>.

There has also been established a correlation between quantification of ctDNA with stage and tumour burden in colon, breast and lung cancer. Some investigation has also proven a statistical significance between detection also of ctDNA tumour relapse and resistance to target therapies<sup>[8]</sup>.

Considering surgical stages, the presence of ctDNA in localized disease at the time of sample collection among different types of cancer, levels of ctDNA were detectable in 55%<sup>[8]</sup>. This percentage was lower than in metastatic stages.

In patients submitted to surgical resection of their localized tumour, but before chemotherapy, identification of ctDNA may indicate residual disease. Its absence may identify subgroup of patients at low risk of recurrence, who could be spared of adjuvant treatment and all its consequences: Risk, cost and discomfort. Nevertheless, regular measurements of ctDNA could monitor total systemic tumour burden, as it should decrease after complete surgical resection. As a monitoring tool, it should increase before new radiological lesions become apparent. It has also been reported that micrometastatic lesions, smaller than a few millimetres may also be detected by increase in ctDNA (86% to 100%) and not yet detected by imaging<sup>[8]</sup>.

ctDNA levels can also predict early relapse and early identification of resistance to treatment, namely detect sensitive and resistant EGFR mutations in lung cancer, such as T790M. Concerning EGFR mutation several studies have reported a wide range of concordance rates between ctDNA and tissue samples<sup>[9-12]</sup>. T790M genetic aberrations in EGFR have also been referenced as acquired resistance to target therapies<sup>[13,14]</sup>. For example, as stated by Naygaard *et al.*<sup>[15]</sup> in plasma using the ARMS-qPCR technique KRAS mutations have also been reported in NSCLC. Concerning ALK mutations such as, C1156Y and L1196M have also been identified as acquired resistance to target therapy with crizotinib<sup>[16]</sup>. Understanding the mechanisms of acquired resistance to target agents at molecular levels can allow science to use selective targeted treatments focusing in a modern precision medical care<sup>[17]</sup>.

How should then ctDNA sequences be checked for early detection, evaluate relapse and how often? First it requires detection of a specific mutation or mutations in the tumour tissue to then look for same ones in the ctDNA after surgery and during follow-up. Concerning detection of residual disease after curative surgery, 6 to 8 wk ctDNA should be measured. They were measured in this study during two to five years after surgery. ctDNA can in fact detect upfront specific genetic alterations months before clinical biomarkers or imaging studies<sup>[18]</sup>.



**Table 1 Pros and Cons: Liquid *vs* tumour biopsy<sup>[20]</sup>**

Liquid biopsy	Tumour biopsy
Non-invasive	Invasive
Better compliance	Difficult to tolerate
Several withdrawals	Serial biopsies
Easily performed	Difficult to biopsy
Independent	Dependent
Less expensive	Expensive
Early detection of cancer	
Clonal heterogeneity	Minor sub-clone
Evaluate response to treatment	
Evaluate residual disease	
Evaluate relapse	Non-prognostic
Evaluate therapy resistance	Non-predictive

### **What are the pros and cons in performing liquid biopsies?**

The advantages in performing liquid biopsies are many. First of all, it's a harmless, non-invasive and easily executed technique. Blood samples are easily obtained and patients tolerate well several blood draws and when explained, there is a greater compliance. Secondly, we become less dependent on the original tumour site, since either primary tumour or metastatic lesions, both release DNA into the bloodstream. ctDNA is representative for all sites of metastases. Concerning cost-effectiveness it's a cheap method with a good sensitivity and specificity as demonstrated in several studies<sup>[19]</sup>. As scientists, we may bypass clinical signs and symptoms and various diagnostic invasive and more expensive diagnostic methods by introducing liquid biopsies in our daily clinical practice. Early detection by sequential analysis, in absence of detectable primary tumours or metastases, response or failure to therapy, detection of residual disease after primary surgery and development of resistance during therapy are the fundamental advantages of detection of ctDNA.

The main disadvantages of tumour biopsies, besides being the gold standard for diagnosis, are the facts that it's an invasive procedure, requires an experience technician to target the specific tumour site. On the other hand, being cancer a heterogeneous disease, the biopsy may be limited to a section of the tumour biopsied and analysed. Different sites of the disease have distinct mutational profiles. Some lung locations are difficult to target, even if well localised *via* imaging exams. If the tumour was resected it can no longer be biopsied, only in metastatic locations (stage IV). Serial lung or mediastinal biopsies are not very friendly to be performed. Obviously, we have a problem with surgically treated patients, non-metastatic if further genetically analysis or disease monitoring wants to be performed, unless liquid biopsies can be done as a monitoring tool (Table 1)<sup>[20,21]</sup>.

### **FUTURE PERSPECTIVE OF LIQUID BIOPSIES**

The possibility of identifying neoplastic ctDNA is a silent

inoffensive tool, that acts as a cancer spy and can trigger appropriate reactions even before the disease (the enemy) or the doctors (the fighters), could have the minimal idea of its manifestation, besides all experience and scientific knowledge of time to progression or acquired resistance to target therapies<sup>[22]</sup>.

A good spy (ctDNA) is the one which triggers the alarm discretely but with high sensitivity and specificity, to produce a good defence and an unexpected combat. It should be performed at the appropriate time (fit patient) permitting to use the best armament (target therapies). This is what all clinicians, molecular scientists and patients should wish for: An excellent precision cancer medicine in a silent mode (minimal side effects), in order to preserve and recover benign territory, before further one is damaged or definitely lost.

The turnaround timer is quicker on liquid biopsies than tissue biopsy, which means that the molecular personalized information of this type of cancer is available more rapidly. In other words, liquid biopsies may be useful at early diagnosis, real-time monitoring disease and at estimating risk of relapse (prognostic information) in order to define therapy selection, therapy resistance (predictive information) and secondary therapy selection<sup>[23-25]</sup>.

### **Is the cost-benefit increment of liquid biopsies as a routine practice, an affordable procedure?**

If we think what a normal tissue sample biopsy implies we can enumerate. First of all, the interventional radiologist and all occupational time of the computed tomography (CT) scan or other technique chosen; secondly the cost of the pathologist and all that it implies in time and material to extract DNA, and finally it's analysis. Whilst ctDNA relies on a blood sample withdrawal, happening every day, and the final procedure is the same. Indeed we are removing steps and costs, in favour of a more modern, comfortable and updated technique and also, a more accurate and precise diagnostic method. Based on two articles, both state that tissue biopsies increase cost for patient care and an uncomfortable invasive procedure<sup>[20,21]</sup>.

Unfortunately, it is not yet a standard of care because only, this May 2017, were the guidelines for NGS suggestions for liquid biopsies validated by the College of American Pathologists, by the Association of Molecular Pathology as well as the European Society of Pathology. For these reasons, although they have been added to the testing palette for NSCLC by NCCN, only now has validation occurred and therefore, it is not yet a standard of care. Nevertheless many clinical studies are attempting to empower the utility and benefit of liquid biopsies as a whole, and not only ctDNA<sup>[26]</sup>.

### **CONCLUSION**

With the increase development of molecular medicine and expanding fields in translational cancer research, we may further change our clinical rational to a new era of precision cancer medicine<sup>[20]</sup>. Consequently, we

may improve the quality of life of our patients, with less toxicity, more cost-effectiveness decisions and above all, improve response rate and survival. Defining the whole genetic “picture” or genomic landscape of each patient, in the near future as a standard of care, will require all genetic information concerning each individual cancer, in order to offer personalized medicine by customizing healthcare by molecular analyses.

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