Detecting cancer by monitoring circulating tumor DNA

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DNA that is shed by dead tumor cells into the blood, termed circulating tumor DNA (ctDNA), is a rich resource that could potentially be used for cancer diagnostics and monitoring. A new study describes a sequencing-based method that improves upon the sensitivity and specificity achieved by past techniques for detecting ctDNA and that can be used for monitoring of disease burden in patients with non–small-cell lung cancer (pages 548–554).

Dead cells shed DNA into the bloodstream, and when this DNA is selectively isolated from the plasma, it is termed cell-free DNA (cfDNA). In patients with cancer, a fraction of the cfDNA is from tumor cells and is referred to as ctDNA. Several studies over the last decade have illustrated the potential of ctDNA analysis as a source of information about tumor presence and molecular composition without the need for a direct tumor biopsy[1]. However, the clinical utility of ctDNA in diagnostics and disease monitoring has been limited because such assays have not been sufficiently sensitive. A recent work detected ctDNA in about two-thirds of more than 600 patient samples[2], but ctDNA in many patients with advanced disease, and in nearly half of patients with early disease, could not be detected.

Ash A. Alizadeh, Maximilian Diehn and their colleagues[3] have now developed a clinically relevant approach to ctDNA monitoring that is economically feasible. This approach advances the field by merging ctDNA isolation, optimized preparation of the targeted DNA sample (the 'sequencing library') and massively parallel sequencing. They call their approach cancer personalized profiling by deep sequencing (CAPP-Seq). Although the method's costs are not explicitly disclosed in the article, the group's efforts are, we estimate, economically viable today. Our pricing estimate per plasma specimen would be $500, including reagents and labor. This makes ctDNA analysis very appealing for general tumor screening and monitoring.

The authors first analyzed cancer genome sequencing data from The Cancer Genome Atlas (TCGA) to identify genes that harbored recurrent mutations or gene fusions in non–small-cell lung cancer (NSCLC). They used that information to construct a DNA reagent to capture recurrent mutations or gene fusions by complementary hybridization in solution. This reagent was designed to capture at least one recurrent aberration in ~90% of all patients with NSCLC. This gene-focused approach allows the detection of ctDNA from the highest fraction of patients at the least cost and allows the identification of specific mutations in individual cancers. In total, the capture reagent targeted 125,000 base pairs of sequence from 139 genes, including 521 exons, 13 introns and 8 known gene fusions (**Fig. 1**).

Application of CAPP-Seq to 1 plasma sample from 5 controls and 35 samples from 13 patients with NSCLC correctly identified 85% of patients with stage II–IV disease as having cancer (termed sensitivity) and 96% of cancer-free patients as not having ctDNA (termed specificity). The sensitivity and specificity for patients with phase I disease were 50% and 96%, respectively. The better tumor detection performance for patients with stage II–IV disease is due to the quantitative nature of CAPP-Seq ctDNA analysis. The abundance of ctDNA in the cfDNA is higher in patients with advanced disease, so the sensitivity for tumor detection is better in these patients. In the present study, the abundance of ctDNA in the cfDNA fraction in patients with stage I tumors was measured to be approximately tenfold lower than in patients with more advanced disease. The likely interpretation is that stage I tumors undergo less apoptosis and/or are less vascularized, so they shed less DNA into the blood. Nonetheless, further improvements to the technology might enable much earlier cancer detection.

One particularly exciting result presented by the authors suggested that analysis of ctDNA may complement radiographic imaging in disease burden assessment after treatment. In this part of their study, cancer burden was assessed in patients at several times after treatment using both radiographic imaging and analysis of ctDNA abundance in blood. One patient who appeared to have a large lesion but had no ctDNA detectable survived at least 21

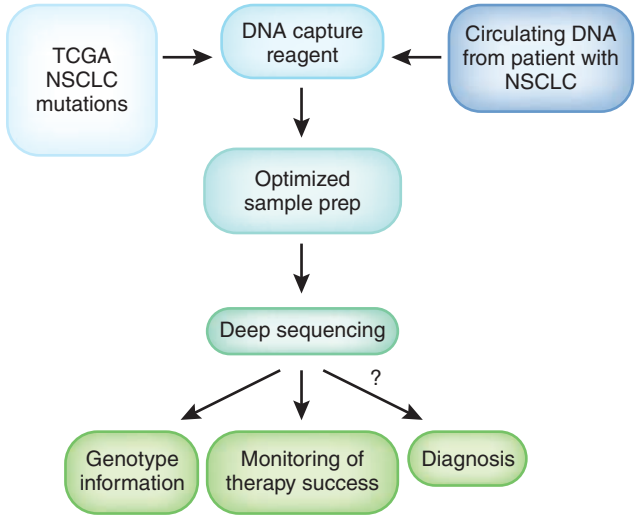

Figure 1 Detecting ctDNA in patients with NSLC. Diehn, Alizadeh and colleagues developed a pipeline they call CAPP-Seq to identify ctDNA in patients with NSLC. Recurring mutations were taken from the TCGA and used in a capture reagent. Capturing the ctDNA from blood of subjects with NLSC and then deep sequencing it illustrated that the tool could be used to monitor tumor progress after treatment—and potentially for diagnosis.

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months without disease progression, whereas another patient with disease detectable by CAPP-Seq but not by imaging progressed rapidly and ultimately succumbed to the disease. This suggests that the presence or absence of ctDNA may predict survival. This type of information argues that it is worth adding ctDNA to the arsenal of care, particularly in monitoring the return of the disease after treatment.

The authors suggest further improvements in their protocol that might be important for widening its applications. First, they highlight the new ultra-accurate sequencing methods that have been developed[4] that might allow DNA sequencing accuracy to one part in 1 billion, that is, if 1 billion bases of input DNA are sequenced, only one error would be present in the data. Accuracy this high would remove concerns about sequencing errors being mistaken for mutations, and, therefore, scoring a sample as having ctDNA when none is present would be unlikely. Second, the authors raise the crucial point that sensitivity is directly tied to input molecules captured, which is linearly related to the amount of blood sampled. Increasing the input to 10 ml or even 100 ml of plasma could improve capabilities 10- to 100-fold. Although 100 ml of blood is probably too much for routine diagnostics, it is far less than the standard US donation volume of 450 ml and could therefore be possible when ctDNA analysis is being used to confirm a suspected diagnosis.

One suggestion that we think would dramatically improve sensitivity is to expand the mutated gene search space. The benefits of the authors' approach are that it is cheap and easy to carry out. However, the sensitivity of CAPP-Seq assays targeted to recurrent mutations is linearly related to the number of mutations screened. This reduces the sensitivity of CAPP-Seq for detection of ctDNA in tumors for which large numbers of recurrently aberrant mutations and fusions do not exist. Indeed, examination of current TCGA data suggests that only a few cancers, such as melanoma, have as many recurrent mutations as NSCLC. The sensitivity of CAPP-Seq could be increased enormously by expanding the capture reagents to encompass the hundreds or thousands of mutations that are present in individual tumors. This would make it useful for analysis of tumors in which the number of recurrent mutations is not high. Such an approach would admittedly add cost and complexity because the capture reagents would have to be customized to each patient. However, procedures for capture reagent preparation are advancing steadily, and we believe that preparation of capture reagents for individual patients will become practical.

This paper and several preceding it make it clear that clinicians are entering the age when ctDNA analysis will be a key part of cancer management. Near-term applications are likely to include assessment of cancer burden and cancer recurrence during treatment and identification of actionable mutations in disseminated metastatic disease that cannot be adequately biopsied. In the longer term, earlier detection of cancer may be possible so that treatments can begin at a time when the tumor will be most amenable to cure.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nm.3564).

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