**Predicting relapse with circulating tumor DNA analysis in lung cancer**

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The authors disclose no potential conflicts of interest.**Summary** (2 sentences): Advances in circulating tumor DNA analysis are revealing new treatment strategies for patients with cancer. Detection of residual disease with ctDNA analysis, predicts relapse with high accuracy after treatment for early stage lung cancer.

Non-invasive liquid biopsies have the potential to transform the management of patients with cancer. Circulating tumour DNA (ctDNA) derived from cancer cells can be identified in over 90% of patients with advanced cancer (1), representing a potential non-invasive source of tumoral DNA to genotype the cancer, select treatment options and in monitor response to treatment. The level of ctDNA is often at a low level in plasma, requiring highly sensitive and accurate assays for ctDNA analysis, some of which have already achieved regulatory approval in the US and Europe.

The most exciting potential applications of ctDNA analysis are in early stage cancer, detecting residual disease to predict who is at risk of relapse, and using ctDNA assays to screen for cancer in unaffected people. In early stages of cancer the level of ctDNA in circulation is very low mainly due to low tumour bulk, and the potential uses of ctDNA in these early cancer settings has been facilitated by rapid development of ctDNA analysis techniques that allow detection of very low levels of ctDNA with high specificity, using PCR based techniques (2,3) and error corrected next generation sequencing techniques (NGS)(4) .

Diehn and colleagues (5) demonstrate the potential of an ultra-sensitive error corrected NGS method (4) in predicting prognosis in patients who have completed potentially curative treatment for early stage lung cancer with surgery or radical radiotherapy. They retrospectively analysed blood samples from a cohort of 40 patients with localized non-small and small cell lung cancers, with plasma samples taken before treatment and every 2-6 months during follow-up(Fig. 1). Circulating tumour DNA was detectable in 93% (37/40) before any treatment, and was detectable in 54% patients after treatment all of whom went on to relapse. Detection of ctDNA post-operatively had a very high risk of future relapse (hazard ratio 43.4, 95% CI 5.7-341), with a median 5.2 month lead time over clinical progression.

The manuscript of Diehn and colleagues joins a number of recent reports illustrating of the potential of ctDNA detection to predict relapse. Similar small proof-of-principle studies have shown that detection of ctDNA in the adjuvant setting predicts relapse with high positive predictive value in patients with breast cancer (6), colon cancer (7) and lung cancer (8). The ctDNA assay used by Diehn and colleagues reports a higher sensitivity than prior studies, and in part this may reflect technical advances. However, Diehn and colleagues did study a high-stage group of patients, with high-tumour bulk, and this does preclude cross-study comparisons. The technique used by Diehn and colleagues offers robust error correction using molecular barcoded duplex sequencing, the current gold-standard for error corrected NGS, combined with a 188Kb panel designed to maximize the number of somatic mutations that can be tracked in plasma. The greater the number of mutations tracked in plasma, the greater the chance through stochastic effects that the residual disease will be detectable. Applying this approach to other cancers will be more challenging. Lung cancers have a particularly high mutational load (9), and much larger panels would be required to identify similar numbers of mutations in other cancers.

There are a number of additional weaknesses. The study is small with very few low stage cancers. Only 7 patients presenting with stage I tumors were included. The ultimate clinical application of such a ctDNA assay will be in stage I-II cancers, to identify who is at risk of relapse and requires further intervention to prevent relapse, and much larger future prospective studies in patients with lower stage cancers are required to fully assess the clinical validity of this approach. Furthermore, in a number of patients the first sample was not taken until four months after completing treatment, during which time the tumour may have proliferated, and it is uncertain whether this affects the sensitivity of the assay compared to samples taken at the end of treatment. The study includes few patients with small cell lung cancer, only 3 patients, precluding any conclusions about the role of the assay in small cell cancer.

Current ctDNA assays are likely limited by the number of mutations tracked. Tracking multiple instead of single mutations can increase the detection rate of ctDNA. It is likely that the current generation of assays could be further improved by further increasing the number of somatic changes tracked, potentially including non-coding variants. As sequencing costs reduce further, it could be conceivable that panels may track 100’s of variants from an individual patient, to develop very robust assays.

The potential of ctDNA detection of residual disease is clear. We may be able to identify who is at risk of relapse with high accuracy, and this information could be used to precisely deliver adjuvant therapy to those patients who need it. However, we are far from routine clinical application. There is no evidence that using ctDNA assays in this setting can improve outcome. We will need trials of ctDNA guided adjuvant therapy that demonstrate improved outcome. In lung cancer this could be trials of EGFR inhibitor or immune checkpoint inhibitors. Whether there can be possible false positive results, ctDNA assay predicting relapse in patients who don’t have residual disease, has not been established robustly any studies as yet. Nevertheless, the work of Diehn et al. demonstrates the potential of non-invasive ctDNA assays to transform how we monitor patients with early stage cancer patients and the potential to tailor adjuvant therapies.

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FIGURE LEGENDS

**Figure 1. Molecular prognosis in lung cancer.** Post-treatment circulating tumor DNA (ctDNA) in circulation could predict disease relapse in localized lung cancer patients. ctDNA analysis applied to plasma sample taken at diagnosis, as well as tumor sequencing, permits the identification of patient-specific somatic alterations that can be used to detect ctDNA in follow-up plasma samples taken after first-line treatments. The presence of ctDNA in the “MRD landmark” (first follow-up time point) and/or follow-up plasma samples predicted very accurately those patients who relapsed. ctDNA analysis is proposed as promising sensitive biomarker to detect molecular residual disease. SMs, somatic mutations; Pre-T, pre-treatment; Post-T, post-treatment.