MILESTONE 2

Tracking cancer in liquid biopsies

Oncologists have long been aware that cancer cells disseminate through the bloodstream. In the early 2000s, substantial efforts were devoted to developing techniques for the reliable and sensitive detection of cancer cells and their components in bodily fluids. As cell-detection systems were optimized, several studies aimed to determine their clinical utility. A study published by Cristofanilli et al. in 2004 was the first to use the CellSearch platform to show that the number of circulating epithelial cells in the blood is markedly higher in women with metastatic breast cancer before starting systemic therapy than in women without breast cancer or with benign breast disease. In analysing survival outcomes, the investigators established the prognostic value of such differences: the durations of progression-free survival (PFS) and overall survival were significantly shorter in patients with cell counts above an established threshold at baseline and, more importantly, at the first follow-up visit during treatment. This study was the first to demonstrate the clinical relevance of circulating tumour cell enumeration for stratifying cancer patients.

Subsequent studies explored more specific approaches to identify the presence of tumour-derived material in blood, such as detecting tumour-related mutations in circulating tumour cells (CTCs). In patients with *EGFR*-mutated non-small-cell lung cancer, Maheswaran et al. have demonstrated the feasibility of using DNA extracted from CTCs for non-invasive monitoring of patients under therapy. Among patients in this study who received EGFR tyrosine-kinase inhibitors, the PFS duration was shorter in those carrying the resistance-related $EGFR^{T790M}$ alteration.

To overcome the technical challenges associated with purifying DNA from CTCs, several groups have focused on refining the detection of somatic mutations in DNA extracted from the cell-free fraction of human blood. Through sensitive detection of tumour mutations in a cohort of patients with colorectal cancer, pioneering work by Diehl et al. has shown an abrupt decrease in circulating tumour DNA (ctDNA) levels in blood samples from patients who had undergone complete surgical resection or chemotherapy. Moreover, the disease recurrence rates were significantly lower in patients with undetectable rather than detectable levels of ctDNA, thus providing the first evidence of the potential value of using ctDNA analysis as a tumour biomarker.

Credit: sorbetto

The diverse clinical applications that derived from analysis of circulating tumour material eventually led to the coining of the term 'liquid biopsy' by Pantel and Alix-Panabières in 2010. In subsequent investigations, researchers assessed the potential of liquid-biopsy tools for non-invasive monitoring of response to therapy in patients with cancer. In a landmark study by Dawson et al. in 2013, serial blood samples from women with metastatic breast cancer undergoing treatment were collected, and CTC quantification and ctDNA analysis were compared side by side for disease monitoring. This work showed that ctDNA has superior sensitivity to that of CTCs as a cancer biomarker and, crucially, changes in ctDNA levels closely paralleled treatment responses: increased ctDNA levels were seen in 89% of the women with progressive disease.

> One of the next challenges in this field will be incorporating liquid biopsies into routine cancer screening protocols to facilitate early cancer diagnosis

The results of another necessary comparison were published a year later by Bettegowda et al. Analysis of patients with cancers across 14 different tissues of origin revealed that ctDNA can be detected in the blood of most patients with solid tumours outside the brain. For some malignancies studied, the percentage of patients with detectable ctDNA was low, thus underscoring the need for developing comprehensive gene panels for liquid-biopsy assays. Expectedly, most patients with metastatic disease had detectable ctDNA; however, ctDNA was also found in a substantial proportion of patients with localized cancers. This observation confirmed that cancer cells and cancer-derived DNA can enter the bloodstream at any stage of disease progression, as had already been proposed, thus drawing new attention to a role for liquid biopsies in enabling early cancer detection.

The encouraging initial results from these and other studies are now prompting clinicians to increasingly use liquid biopsies for a range of clinical applications, including predicting the risk of disease recurrence, as reported by Tie et al., matching patients to treatments while minimizing surgical procedures to obtain tissue biopsies, and tracking the presence of resistance-related mutations. One of the next challenges in this field will be incorporating liquid biopsies into routine cancer screening protocols to facilitate early cancer diagnosis. The feasibility of this approach has already been demonstrated in a pilot intervention by Chan et al. focused on the detection of Epstein–Barr virus–related nasopharyngeal carcinoma, and in a pan-cancer study by Lennon et al. combining positron emission tomography–computed tomography with the detection of biomarkers in blood (including ctDNA).

Diana Romero,
Nature Reviews Clinical Oncology

ORIGINAL ARTICLE Cristofanilli, M. et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N. Engl. J. Med.* **351**, 781–791 (2004).
FURTHER READING Please visit the online article for a full list of further reading.