



An Introduction to Liquid Biopsy

Why Would “Liquid Biopsy” Be Useful?

Standard histopathology requires tumour tissue. The same tissue can be used for molecular testing, which may provide information regarding tumour classification or prognosis, and direct targeted therapy based on specific mutations. However, tissue samples are not always suitable for molecular analysis – for example, when there are few neoplastic cells, or when DNA is damaged by formalin fixation. Moreover, depending on the malignancy site and patient comorbidities, obtaining tissue may not be feasible. Finally, tumours can be heterogeneous between metastatic foci and over time; a sample from a single site and time point may not reflect the whole disease burden.

Testing body fluids such as plasma (‘liquid biopsy’) could overcome many of these problems. It would pose minimal risk to the patient, allowing repeat testing over time. Assuming all tumour deposits release analytes into the circulation, it would also provide a more complete picture of the malignancy. New methods are beginning to enable liquid biopsy in clinical practise, and two approaches are described below.

Circulating Tumour Cells

Circulating tumour cells (CTCs) are promising analytes for metastatic tumours, as they contain all tumour biomolecules (e.g. proteins and lipids, as well as DNA and RNA). CTC xenotransplantation studies have also been carried out in model organisms, potentially enabling an *in vivo* ‘test system’ for individualised therapy.

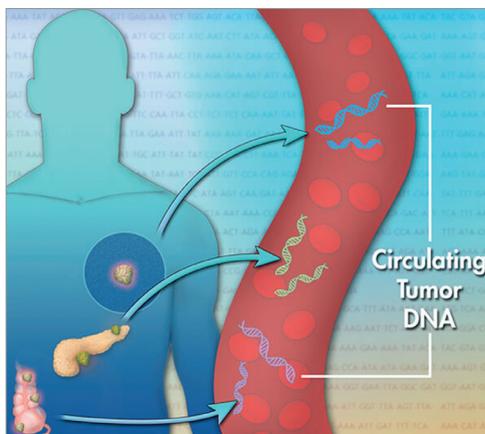
However, CTC testing is currently limited by biological and technical issues. CTCs are present in very small numbers in peripheral blood. Extensive enrichment is required before downstream analysis, and current enrichment methods are imperfect. In addition, the proportion of CTCs released into the circulation appears to vary between tumour types. For these reasons, CTC-based liquid biopsy is not yet in routine clinical use.

Cell-Free DNA

Circulating ‘cell-free’ DNA (cfDNA) consists of small DNA fragments, released mainly from apoptotic cells. Tumour DNA (ctDNA) is mixed

with cfDNA derived from non-malignant cells. Some patients have a high ctDNA burden, but in many cases clinically significant mutations are present in a very low fraction of circulating DNA.

cfDNA can be stabilised in plasma collected using specialised blood tubes. A number of different molecular methods can then be applied, typically allowing highly sensitive detection of a small number of clinically relevant mutation(s), or moderately sensitive detection of a wider range of mutations.



Courtesy: National Human Genome Research Institute

Partly because of its high analytical sensitivity, ctDNA testing has advanced further into clinical practise than CTC analysis - particularly for directing targeted therapies in non-small cell lung cancer (NSCLC). EGFR mutations detected using plasma ctDNA are typically also present in tissue (i.e. the ‘false positive’ rate is low). Importantly, the clinical response to targeted therapy appears to be equivalent, whether an EGFR mutation is detected by testing ctDNA or tumour tissue directly.

However, mutations present in tissue are not always detectable using ctDNA - the negative concordance of ctDNA with tissue testing is 70-80% in many studies. This may be because the mutation is below the limit of detection of current technology, and/or due to biological variability in the release of ctDNA. Testing of tumour tissue is therefore still the ‘gold standard’ for targeted therapies, even for NSCLC.

Future Applications

Several issues need to be addressed before liquid biopsy becomes part of routine cancer care:

- Better understanding of the underlying biology of circulating tumour cells and nucleic acids.
- Improved test methods, especially for CTCs - enhancing sensitivity while maintaining low false positive rates.
- Standardisation of approaches to sample collection, processing and analysis.

Liquid biopsies have been used as a monitoring tool in research protocols, to enable early detection of relapse and to determine the molecular mechanism(s) of treatment resistance. In the more distant future, liquid biopsies might be used as a screening tool, enabling early detection of cancer in at-risk patients (e.g. www.grail.com).

At present, the analytical and clinical validity of liquid biopsy has been demonstrated, particularly for detecting EGFR mutations in NSCLC. However, the clinical utility of this technique has not yet been confirmed by prospective trials, showing improved patient outcomes from tumour testing and/or monitoring by liquid biopsy. If this is shown to be the case, liquid biopsy will become an integral part of cancer care.

Further Reading:
Siravenga et al, *Nat Rev Clin Oncol*, doi:10.1038/nrclinonc.2017.14

Key Points

- Solid tumours release cells and cell-free DNA into the circulation.
- These analytes can be detected and characterised, yielding actionable information.
- There are technical and biological limits to the sensitivity of liquid biopsy.
- Liquid biopsy is currently used in specific clinical circumstances, but its scope is likely to increase in future.

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