Liquid Biopsy

Genomics & Oncology Backgrounder
Tumour biopsy is an essential procedure for patients with cancer, providing crucial information on diagnosis, prognosis, and prediction of response or resistance to treatment. For decades, biopsy has been, and continues to be, the standard of care. Moreover, analysis of biopsy tissue has been the only option to determine the tissue from where the cancer originated, as well as the patient's disease stage. With the development of targeted therapies, it is now also essential to evaluate the presence or absence of specific molecular alterations in conjunction with known histologic features. Companion diagnostics are now routinely used to determine whether treatment with a targeted therapy is appropriate for patients with several tumour types, including non-small cell lung cancer (NSCLC), melanoma, breast cancer, and metastatic colorectal cancer.

As the number of available tests for cancer diagnosis and genetic evaluation has grown, so has the need for additional testing, and hence the need for more tumour tissue. Current biopsy procedures are invasive and, especially in NSCLC, often produce too few cells or tissue sections for extensive analysis; multiple biopsies are not feasible for many patients, such as the elderly and those with comorbidities. Improved biopsy methods that produce adequate sample amounts that are reflective of overall tumour burden and are amenable to frequent testing are clearly needed.
Limitations with Current Tissue Biopsy Techniques

Direct analysis of tumor tissue provides essential information on tumor status and is one of the fundamental best practices for diagnosing cancer. Yet, direct tissue biopsies are limited in utility by several problems, including the difficulty in acquiring adequate amounts of tissue; limited repeatability, and biases that result from sampling a small area of a single tumor, including those due to tissue heterogeneity. Obtaining biopsies can also be hampered by patient comorbidities and lack of patient compliance, thus limiting access to adequate amounts of tissue for analysis.

Although surgical biopsies typically provide the greatest amount of intact tumor tissue for analysis, the procedure is invasive, costly and time-consuming. Surgical biopsy procedures can also lead to clinical complications, with one study finding a 17.1 percent rate of complications for intrathoracic biopsies and 1.6 percent for abdominal/pelvic biopsies, plus an overall major complication rate of 0.8 percent. Fine needle aspiration (FNA) and core needle biopsy (CNB) procedures are less invasive than standard surgical biopsy; however, they provide less tumor sample, query only a portion of the tumor, and can be difficult to analyze because of cellular necrosis or immune cell infiltration. Additionally, most biopsy samples are formalin-fixed and paraffin-embedded for preservation, but this technique can alter the DNA and make it difficult to analyze by routine techniques such as polymerase chain reaction (PCR).

Perhaps the greatest limitation to tissue-based biopsy techniques is that sampling of a single tumor may not capture all of the mutations present. Cancer evolves genetically over time, a property that allows tumors to metastasize and develop resistance to treatments that are initially effective.

For example, treatment with erlotinib is indicated in patients with metastatic NSCLC containing EGFR exon 19 deletions or exon 21 (L858R) substitution; however, resistance to erlotinib typically occurs within several months of initiating treatment, as evidenced by a median progression-free survival (PFS) of 10.4 months. Because cancer cells can readily mutate, genetic heterogeneity can exist not only among different tumor sites within a single patient, but also within a single tumor. This intratumoral heterogeneity has been definitively demonstrated in lung tumors by three different groups, as well as in tumors from other organs. Thus a single tumor biopsy may provide limited information, underscoring the need for less invasive techniques, such as a liquid biopsy, that allows the potential for frequent testing of multiple tumor sites.
Liquid biopsy is a minimally invasive test for assessing cancer genetic status based on the analysis of circulating free DNA (cfDNA) that is present in the plasma component of blood. Because blood samples can be easily obtained, the concept of a blood-based biopsy has long held promise as a less invasive complement to traditional biopsy techniques. One way that tumours release biomarker information is through tumour cell necrosis and the release of dead cells or cell fragments. These cells are engulfed by phagocytes that process the tumour cell DNA, which is then released into the bloodstream. Because cfDNA is derived from the tumour cells, cfDNA can be analyzed for the same genetic aberrations found in tumours, including mutation, amplification, chromosomal rearrangement, and hypermethylation. To maximize sensitivity, liquid biopsy techniques are based on either PCR amplification or next-generation sequencing (NGS) technology. In addition to cfDNA, liquid biopsies may use circulating tumour cells (CTCs) or exosomes as the source for tumour DNA. CTCs are intact tumour cells released into the bloodstream that can provide a source of tumour DNA. Many patients with early-stage cancer may not possess CTCs in their blood, making these cells a less reliable sample source for identifying genetic variations or assessing prognosis. Exosomes are membranous, extracellular vesicles ranging from 50–150nm in size. They are shed by most cell types, including tumour cells, and can also be used in liquid biopsy analysis as a source for tumour DNA, RNA, and protein.

To perform a liquid biopsy, blood is drawn and the cells are removed, yielding plasma. The cfDNA is then isolated from the plasma and prepared for analysis. By requiring only a simple blood draw, liquid biopsy can be repeated numerous times with little impact on the patient. Because liquid biopsy analyzes the tumour DNA directly, it can provide information on the status of cancer-related genes, such as EGFR, KRAS, NRAS, PIK3CA, and BRAF.
In contrast to standard biopsy procedures, liquid biopsies can be performed much more frequently with less risk to the patient. The method has the potential to provide a more complete genetic signature of the patient’s entire tumour burden by providing DNA from more than one tumour. Due to the noninvasive nature of the technique, liquid biopsy could be used for frequent monitoring of patients undergoing treatment, thus providing snapshots of tumour genetics throughout the management of the patient. Currently, oncologists typically rely on imaging, such as computed tomography, or CT scan, and fluorodeoxyglucose-positron emission tomography, or PET scan, both of which are costly and time-consuming. Additionally, disease monitoring typically occurs every 3–6 months. In contrast, blood samples could readily be taken at a more frequent interval (i.e. weekly) to evaluate the patient’s response to treatment and, more frequent biopsies could be used to detect the development of treatment resistance and prompt a change in therapy or the acceleration of diagnostic modalities.

In patients who appear to have achieved a complete response to treatment, liquid biopsy could provide improved monitoring of tumour burden and assessment of minimal residual disease. Tumour burden is usually assessed with imaging and, for some tumour types, by determining levels of circulating protein biomarkers; for example, prostate-specific antigen is a standard biomarker that is monitored for diagnosis, disease recurrence and tumour burden in patients with prostate cancer. Similarly, CA-125 is a protein biomarker for ovarian cancer recurrence.

Many proteins have a relatively long half-life. In contrast, cfDNA has a short half-life of approximately 2 hours, and therefore may have the potential to provide an accurate snapshot of tumour burden at the time of blood withdrawal. As a result, analysis of cfDNA could provide a dynamic assessment of changes to tumour burden and tumour genetics. In some situations, the concomitant use of protein and nucleic-acid based biomarkers may provide complementary insights into the disease state.

Liquid biopsy also presents an attractive means to supplement the limited amount of tissue obtained by FNA or CNB methods, or as an alternative for patients who are not eligible for tissue biopsy. In addition, liquid biopsy has a high positive predictive value; thus a positive result from a validated liquid biopsy test could lead directly to a clinician’s confidence that a targeted therapy is likely to benefit the patient.
Despite the promise of liquid biopsy, several technical challenges need to be overcome for the development of a viable approach based on the analysis of cfDNA. It is thought that many and possibly most tumours shed some DNA into the plasma. However, the amount of tumour-derived cfDNA varies in different patients. If the amount is proportional to the total tumour burden, patients with early stage disease may not have sufficient cfDNA for accurate testing. In some cases, tumour-derived cfDNA has been shown to account for less than 1 percent of circulating DNA. Compounding the problem, normal genomic DNA is also present in cfDNA. For use in patients with early stage disease or for early detection, highly sensitive techniques are needed that can reliably sequence a small amount of mutant, tumour-derived cfDNA in the context of large amounts of normal genomic DNA. The detection of mutations at these ultrasensitive levels also requires extensive validation to be clinically actionable.
Clinical Studies of Liquid Biopsy

To evaluate technical capabilities and limitations, liquid biopsy techniques are now being pursued in clinical studies. Several retrospective analyses suggest that detection of tumour mutations in cfDNA is comparable with results obtained from biopsy. An early retrospective analysis of 34 patient-matched samples demonstrated 100 percent concordance between cfDNA and breast tumour tissue for mutations in the PIK3CA gene. In a phase 2 study that recruited all tumour types, 29 mutations were identified by NGS in biopsy tissue, and 28 (97 percent) of these were also identified by NGS in matched samples of cfDNA.

Liquid biopsy of cfDNA is also being explored to detect the emergence of resistant clones, which limit the remission times achieved in patients treated with targeted therapies, and to correlate findings from cfDNA with patient outcomes. With earlier detection, drug-resistant disease could potentially be addressed by altering therapy, while treatment could be reduced or halted sooner in patients with an early response. In several retrospective studies, treatment-resistant mutations have been detected by liquid biopsy in cfDNA samples taken well before disease progression was evident by imaging. In a study of plasma samples from 57 NSCLC patients and 22 negative controls, a reduction in the level of EGFR mutations present in cfDNA correlated with early response to treatment with a tyrosine kinase inhibitor. In a study of 218 advanced NSCLC patients, multivariate analysis revealed a significant association between elevated baseline cfDNA concentration and overall survival, independently of baseline performance status, disease stage, age, and response rate. Numerous retrospective studies have demonstrated that disease recurrence after resection can be discovered earlier with liquid biopsy than with imaging—often by many months; in addition, a lack of detectable DNA by liquid biopsy after resection often correlates with better prognosis.
Clinical studies such as these highlight the potential power of a liquid biopsy, but a viable assay will have to overcome several challenges to be a reliable diagnostic test. To compete with current options, and to ensure that patients receive appropriate treatment, a successful liquid biopsy assay will have to demonstrate a high positive predictive value. Concomitantly, good sensitivity and excellent specificity will be required to yield acceptable rates of false positives and false negatives. These technical characteristics will have to be evaluated in larger, prospective clinical trials.

Liquid biopsies present a unique opportunity to advance the understanding of metastatic disease development and to further elucidate the signaling pathways involved in cell invasiveness and metastatic competence. Ultimately, these tests could be used in cancer diagnosis and treatment monitoring. Liquid biopsies could revolutionize cancer care, providing clinicians with rapid access to information on a molecular level at diagnosis, and potentially enabling treatment to be more closely tailored to each patient’s disease state. Liquid biopsies are likely to play a major role in the application and monitoring of therapies that are linked to powerful predictive biomarkers of response in metastatic cancer. Thus, the liquid biopsy will allow for a faster, less invasive and more robust test to help oncologists best manage patients with cancer.
References


