

Wet or Dry? Do Liquid Biopsy Techniques Compete with or Complement PET for Disease Monitoring in Oncology?

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The use of blood biomarkers, including prostate-specific antigen in prostate cancer and cancer antigen 125 in ovarian cancer, is well established in clinical practice and generally initiates imaging rather than replaces it. Although often allowing assessment of tumor response, tractable serum biomarkers are not available for every type of cancer, and most provide little or no characterization of tumor biology. The ability to obtain so-called liquid biopsies from a patient has recently attracted major scientific and clinical attention as a means of cancer diagnosis, characterization, and therapeutic monitoring. Currently, liquid biopsy technologies are still being evaluated to determine their clinical utility and whether they can replace other conventional clinical biomarkers. This topic is particularly pertinent to PET, which is increasingly being used as an imaging biomarker (1).

Several forms of liquid biopsy have been proposed, but the dominant technologies currently involve analysis of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs). CTCs are released from a tumor into the blood as single cells or cell clusters and are thought to be precursors of metastases (2). Using specialized instrumentation, CTCs from a blood sample are enriched (e.g., using cell-specific markers such as EpCAM for epithelial cell types), isolated, and then enumerated. Previous studies have shown that CTC numbers are strongly prognostic of overall survival in metastatic breast, colorectal, or prostate cancer (3–5). This finding has led to U.S. Food and Drug Administration approval of the first methodology for enumeration of CTCs from whole blood (CellSearch; Janssen Diagnostics). Isolation of CTCs can also provide an alternative source for molecular characterization instead of tumor biopsy and the opportunity to perform functional studies on CTCs *ex vivo* (6).

ctDNA is also shed from a tumor into the bloodstream, with quantitative levels being associated with disease burden (7,8). Through current genomic technologies, ctDNA can be detected from plasma and act as a surrogate of tumor-derived DNA from conventional biopsy. In contrast to CTC analysis, ctDNA analysis is appealing as there is no prior need to enrich and isolate a rare population of cells. Rather, blood is drawn from a patient and fractionated for plasma. Plasma-derived DNA is then extracted and subjected to a range of genomic technologies to detect genomic alterations specific to the tumor. The application of

ctDNA analysis has gained significant traction in recent years as a potential diagnostic tool, for reasons including the high specificity of ctDNA as a cancer biomarker, the falling costs of genomic technologies, and advances in their ability to detect mutations at low abundance. For example, techniques such as digital polymerase chain reaction can detect single somatic mutations at low mutation allele fractions (<0.1%). Other techniques, such as targeted sequencing and whole-exome or -genome sequencing, can detect a much larger breadth of mutations but at lower analytic sensitivity (~1%–10% mutation allele fraction).

There are several potential advantages of liquid biopsy for cancer treatment monitoring and surveillance in comparison to PET/CT. Certainly from a patient perspective, the convenience of local blood collection, which can be sent to a central testing laboratory for processing and analysis, can facilitate more frequent disease assessment while circumventing or minimizing radiation exposure. This is particularly advantageous in patients who lack easy access to PET. A clear further advantage of liquid biopsy is its ability to capture the genomic landscape of disease, which can influence treatment decisions in the era of precision medicine. In particular, assessing the genomic basis of treatment resistance, which usually arises from a branched and heterogeneous course of evolution, is critical for guiding management. In this context, serial tissue biopsies throughout treatment are usually not feasible, and single biopsies remain limited in their ability to capture intratumoral heterogeneity. In contrast, ctDNA analysis can allow monitoring of genomic changes during therapy and can potentially profile the global pool of genomic changes from many sites of disease.

Nonetheless, compared with PET/CT, liquid biopsies have some limitations. For example, a major challenge of CTC analysis is that it requires an enrichment step, often based on a preselected marker, to allow CTC detection. Accordingly, heterogeneity in cell surface markers across tumor cells can lead to failure of enrichment. Moreover, there is a limitation to the volume of blood that can feasibly be drawn and analyzed. Organ-specific differences in the representation of ctDNA may influence the interpretation of ctDNA results. For example, in metastatic melanoma we have observed that patients with subcutaneous and brain disease do not have high levels of ctDNA (9). Recognition of these limitations will be important if ctDNA monitoring is to be integrated into routine clinical practice. Fundamentally, levels of ctDNA and CTCs are highly dependent on disease burden and their level of release into the circulation. Although preanalytic processing of liquid biopsies and the sensitivity of mutation detection through genomic technologies are constantly improving, future studies need to assess whether current approaches

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are as sensitive and reliable as PET imaging in the detection of ctDNA in patients with localized disease across various cancer types. Furthermore, the ability to localize sites of recurrent or resistant disease through PET/CT imaging may guide locoregional therapies, especially when genomic analysis fails to identify a therapeutic target.

In many ways, the liquid biopsies of today represent a more sophisticated version of traditional circulating tumor markers, and there are lessons to be learned from this observation. First, the notion that liquid biopsy technologies will replace PET/CT is not warranted because, akin to conventional circulating biomarkers, they will most likely play a guiding role in determining when to image and how to interpret imaging findings (e.g., in resolving the question of disease recurrence vs. inflammation). Liquid biopsies may also help identify tumors that have low metabolic activity. Most importantly, because liquid biopsies can contribute clinically important genomic information that PET imaging cannot, there is a sound rationale for performing PET/CT imaging in combination with liquid biopsy analysis. This approach will allow a powerful and complementary strategy for comprehensive disease monitoring in oncology, enabling detection, localization, and characterization of disease through the course of treatment and aiding in selection of the right treatment at the right time.

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