

Non-Invasive Liquid Biopsies Become a Reality in Today's Personalized Cancer Treatment

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Cancer is a complex, heterogeneous disease presenting a worldwide health problem in industrialized and developing nations alike. Treatment has generally relied upon the analysis of primary tumor specimens for the comprehensive characterization to help develop and guide personalized treatment. But due to the heterogeneity of the tumor cell population, sampling error can lead to incomplete and insufficient information regarding the actual complete tumor biology, which can lead to inadequate treatment.

As cancer progresses and becomes refractory to treatment, clinicians can no longer rely solely on the primary tumor to guide next treatment steps, as the biology driving these resistant cells is clearly different from that in its primary stage. Cancer is indeed a constantly evolving and dynamic process and developing a means to detect any such changes to better inform clinical decisions is paramount to achieving the best possible outcomes.

Over the past decade investigations have been made into isolating of cancer biomarkers and evaluating cancer related mutations using physiological biofluids and the answers to achieve better clinical treatments are hidden in the bloodstream where minimally invasive tests, known as "liquid biopsies", [1,2] hold great promise for personalized cancer treatment. In addition to blood, liquid biopsies of other body fluids such as urine, saliva, and Cerebrospinal Fluid (CSF) have been shown to contain tumor-derived genetic materials [3,4].

Much of the early liquid biopsy studies have been in lung, breast and prostate cancers but this technology is anticipated to have an impact on all types of cancer. In the last few years, liquid biopsies have gained significant attention and the technologies applied in liquid biopsy research have been extensively reviewed. A number of circulating biomarkers can be isolated from liquid biopsies. In particular, circulating tumor cells (CTCs) and cell-free DNAs (cfDNAs) also known as circulating tumor DNAs (ctDNAs) are the two main types of potential blood-based biomarkers; alongside microRNAs (miRNAs), exosomes and micro vesicles [5]. To monitor the real-time dynamics of cancer the presence of DNA mutations, epigenetic alterations and other tumor specific abnormalities such as Microsatellite Instability (MSI) and Loss of Heterozygosity (LOH) have diagnostic value. In this context, the ability to detect ctDNA fragments in a patient's plasma that have been shed from any tumor, has enabled clinicians to repeatedly

and non-invasively interrogate the dynamic evolution of human cancers and the plasma ctDNA testing moves into mainstream cancer treatment.

Several novel technologies now facilitate the detection of tumor-associated mutations in CTCs and ctDNAs. Combination of liquid biopsy and next generation sequencing (NGS) enables to provide comprehensive molecular portrait of the tumor and guides therapeutic options. In addition, digital Polymerase Chain Reaction (dPCR) is capable to detect specific genomic footprint at frequencies as low as 0.01% and the technology has been widely adopted for monitoring liquid biopsy samples. Analyzing ctDNAs and profiling of tumor-derived somatic alterations in DNA have become routine. The potential of investigating ctDNA holds promise for early diagnostics and real-time longitudinal monitoring of cancer mutations over time and treatment.

Currently, treatment for advanced Non-Small Cell Lung Cancer (NSCLC) is guided according to the genetic abnormalities and rapid detection of targetable epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and ROS1 alterations [6]. In this context, monitoring of both CTCs and ctDNAs in EGFR mutant NSCLC patients will be beneficial for daily clinical practice. In several clinical studies including the EURTAC trial that was the basis for the approval of erlotinib for the first-line treatment of patients with metastatic NSCLC whose tumors have certain EGFR activating mutations [exon 19 deletions or exon 21 (L858R) substitution], EGFR mutations were detected in the serum in more than 50% tissue positive patients [7]. The analysis of ctDNA has also showed promising early results in the metastatic breast cancer setting where 95% concordance between the PIK3CA mutation status in matched tumor and ctDNA was observed [8]. In patients with metastatic colorectal cancer researchers were able to detect ctDNAs with clinically relevant KRAS gene mutations with 87.2% sensitivity and 99.2% specificity [9]. The combination of liquid biopsy and NGS enables to provide a wealth of sequence data and build understanding of tumor evolution, resulting in significant advances in a wide range of research areas and applications. Whereas the utility of ctDNA is limited to NGS, CTCs can be used not only for NGS, but also used to look for cell-surface expression markers like PD-L1 as well as intracellular expression of Her-2 and many RNA based targets.

Besides CTCs and ctDNAs, a specific class of small RNA

called microRNAs (miRNAs) that are very stable in biofluids and aberrantly expressed in a broad array of human cancers, showing tremendous potential as diagnostic and prognostic biomarkers in cancer management. miRNAs are short non-coding RNAs, approximately 20–25 nucleotides in length, that regulate gene expression at the post-transcriptional level [10]. Although, majority of miRNAs are found intracellularly, a significant number of miRNAs have been observed outside of cells and variety of circulating, cell-free miRNAs as cancer biomarkers have been extensively investigated. Several studies have compared hundreds of circulating miRNAs expression profiles across a range of non-malignant and malignant diseases to identify and distinguish disease-specific expression patterns [11,12]. For example, differences of miR-155 concentrations and increases of miR-21 levels in the bloodstream of patients with various cancers were observed [13]. Several groups reported promising results regarding potential use of circulating miRNAs as cancer biomarkers and their association with various tumor types including mainly miR-21, miR-10b, miR-155, miR-92 and miR-30 family.

Apart from the classical CTCs and ctDNAs we are witnessing novel molecules and vesicle classes that promise to provide cancer related information. Several studies have shown the potential of exosomes, large oncosomes, exoDNAs and Tumor-Educated Platelets (TEPs) in the realm of cancer research. In particular, due to their presence and stability in most bodily fluids, exosomes have a great potential to serve as liquid biopsy biomarkers [14]. Exosomes are tiny vesicles composed of proteins, various RNAs, DNAs and lipids and can be collected at once and provide the dynamic information from the tumors at the time of blood drawing [15]. More recently, Best et al. described potential role of TEPs across six different tumor types by performing mRNA sequencing of TEPs from 283 platelet samples and the TEPs mRNA profiles distinguished mutant KRAS, EGFR, or PIK3CA tumors [16]. Although, the ability of TEPs to find the location of primary tumor is exciting further validation is warranted to establish their clinical utility.

Liquid biopsies provide complementary information to that seen in the primary tumor, as well as offering a means to do real-time monitoring and analysis as a tumor progresses and the surviving cancer clones mutates so as to provide new insight into the existing biology which the primary tumor may not reveal. To date, the field of oncology has evidenced numerous studies that demonstrate the potential of the circulating molecular profile. The emerging liquid biopsy technologies have generated a lot of excitement since they can provide actionable information for decision-making prior to treatment and also ongoing picture of patient's cancer. This will offer valuable information about longitudinal surveillance during and after the treatment regimen to assess for patient response, drug resistance and cancer recurrence that will ultimately allow for even great precision and analysis, which will lead to better healthcare outcomes.

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