Tumor Heterogeneity Drives the Need for Complementary Approaches in LiquidBiopsy® Analyses

Cynvenio Biosystems | April 2015

An experiment in 2012 showed that in one patient, the majority of cancer cells displayed different mutations at every site from which they were recovered, even when cells from different parts of the same biopsy sample were examined\(^1\). This presents oncologists with the challenge of deciding where to biopsy a patient to determine the best treatment.

If every biopsy has the potential to deliver a different list of mutations, treatment decisions are profoundly affected. Therefore, not only is a tissue biopsy a snapshot that cannot be easily repeated to monitor cancer, by its very nature it misses the complexity of cancer in each patient.

LiquidBiopsy is a new approach that uses tumor information derived from a blood sample to supplement a traditional tissue biopsy. Blood pervades every site in the body and therefore reflects the full burden of a cancer, wherever it is located. There are three sources of disease associated information available in blood:

- **Protein biomarkers** have been the standard for a generation, but suffer from low sensitivity and poor specificity. As an example, prostate specific antigen (PSA) can be produced in both men and women for many reasons other than the presence of cancer.

- **Circulating tumor DNA (ctDNA)** is assumed to be derived from the elevated turnover of cancer cells. It has been shown to contain the same mutations found in the biopsy, as well as mutations caused by non-specific damage to the DNA while it is in the blood. While identifying alterations in the DNA is a very specific readout—and the root cause of cancer—ctDNA has noted limitations in this regard. ctDNA is extremely rare, has great variability between patients, and is largely restricted to DNA. In addition, it must be compared to existing tests at limits of detection (i.e. biopsy - a circular comparison) because the provenance of the template is unclear and ctDNA has a significant noise profile.

- **Circulating tumor cells** in blood describe a spectrum of cells that leave the tumor site. Mobilization may be caused by shearing events, mesenchymal transition events that down-regulate “stickiness”, or programmed release of metastatic stem cells - the ultimate cause of morbidity in cancer. All are relevant to understanding the heterogeneity of cancer. Being cells, they contain other useful biomarkers that reflect the tissue of origin, such as proteins, RNA and DNA templates, and are associated with increased metastasis\(^2\).

---

\(^1\) Gerlinger et al. NEJM (2012)\textbf{366}:10,p883

\(^2\) Strauss et al. CancRes (2014)\textbf{74}:8 suppl 1:2846
To date, the analysis of cancer alterations from blood has focused on either ctDNA or counting a sub-population of tumor cells found in blood - but not both:

i) Other than single cell analysis, it has not previously been possible to sequence rare populations of cancer cells from blood - a technical hurdle solved by the LiquidBiopsy platform at Cynvenio and various academic research centers.

ii) Release of cells and ctDNA reflect two different biological processes in a developing tumor. The information obtained from both are, by their nature, complementary.

iii) In a direct comparison study of 60 subjects measuring actionable mutations seen in ctDNA and tumor cells recovered from blood (ctcDNA), the latter produced four-times as many actionable targets.

iv) Additional biomarkers are available in tumor cells ranging from protein biomarkers to RNA based expression readouts leveraging 20 years of research linking the presence of tumor cells in blood with the disease process.

v) The sequencing sensitivity shown to be informative for ctDNA analysis overlaps the sensitivity of the LiquidBiopsy test enabling evaluation of both compartments from a single sample.

New sequencing technologies have changed the way we monitor cancer. Sequencing studies from hundreds of patients emphasize the emerging complexities of the cancer cell population and the need for more targeted therapies. New tools for the changing “genographics” are necessary if we are to improve the treatment of patients with cancer. Furthermore, to change the equation and improve detection of cancer in the earliest stages, new tools to detect the earliest hallmarks of cancer will be needed. There is a biological limit to the number of fragments of DNA or cells that can be detected and no one single answer is correct. Their strength is in being deployed together.

---
