Liquid biopsies

Solid tissue versus liquid biopsies

Traditional tissue biopsies of the primary tumor remains the gold standard for molecular testing of cancer, but may in hindsight not be the best sample type. Tissue biopsies are in fact poor diagnostic samples: they are invasive, cannot be used repeatedly, and are ineffective in understanding metastatic risk, disease progression, and treatment effectiveness.

Solid tumors are also known to be heterogenous, with multiple clones of cancer cells existing side by side.There is a real risk that some clinically important but numerically minor clones go undetected.

Therefore it seems reasonable that blood samples ultimately will be the preferred sample type. Blood samples are minimally invasive, repeated samples can be taken for monitoring any dynamic changes in the disease, they are quick and cheap to take and they represent the spread of the disease.

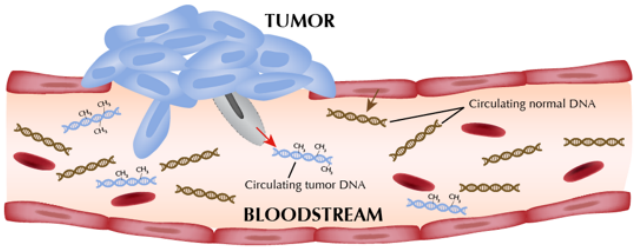
Liquid biopsies also differ in that their essential components, the cells or DNA, are suspended or dissolved in the blood where they are technically easier than solid tissue to separate into their individual components, individual tumor cells or DNA fragments.

It is known how the ctDNA (circulating tumor DNA) and CTC (Circulating Tumor Cells) reach the blood stream. Tumors lose both cells and subcellular components, such as DNA, into the blood stream, where they may be detected and used to guide patient management. They constitute of new sample types that because of recent technical developements have now started to become available. They are in the process of being tested for their utility in complementing or replacing the traditional solid tissue biopsy and/or the traditional circulating bimarkers such as PSA and CA125.

Tumor growth results from cell divisions, which by its nature is logarithmic since one cell divides into two daughter cells which in turn will grow and then divide again into another pair of daugter cells etc. In practice, tumor growth soon slows due to two additional, often overlooked, factors that affect cell growth rate: cell death, which is a normal part of cell life, causing the debris from the dead cells to be washed away from the primary tumor mass by the blood, and cellular invasion of surounding tissue and blood vessels by a sub-group of cancer cells causing also them to be lost from the mass of the primary tumor. This is how ctDNA and CTCs reach the blood stream, ready to be tested.

Circulating tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) is tumor-derived fragmented DNA in the bloodstream that is not associated with cell nuclei. ctDNA should not be confused with circulating cell-free DNA (ccfDNA), a broader term which describes DNA that is freely circulating in the bloodstream, but is not necessarily of tumor origin. ctDNA is a small subset of all ccfDNA. Because ctDNA may reflect the tumor genome, it has gained attention for its potential clinical utility, even though it is not itself part of what spreads the disease.



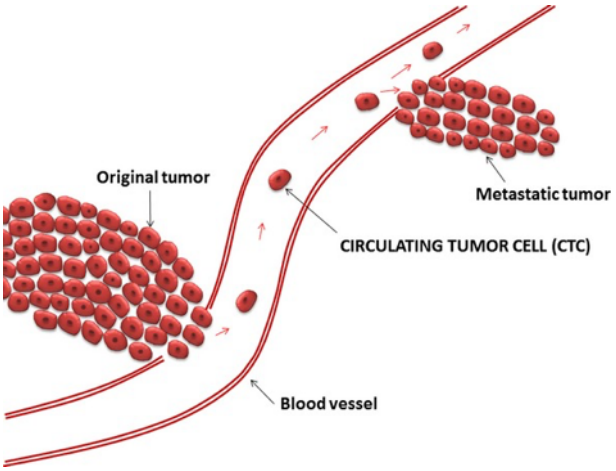
Picture 1. Circulating tumor DNA (ctDNA) in the bloodstream.

The dead cancer cells release their contents, including DNA, that ultimately reaches the blood plasma. By the time it reaches plasma the DNA is degraded into 160 base pair fragments, a small size by molecular standards. Small enough to be cleared through the kidneys. The balance between release from dying cancer cells and clearance in the kidneys is the ctDNA found in blood. It is highly diluted by degraded fragments of normal DNA, that goes through the same process of degradation and excretion, limiting its utility. Since the ctDNA cannot be specifically purified away from the total ccfDNA it can only be tested by massive re-sequencing of the ccfDNA to look for rare sequence variants that correspond to known oncogenic variants, among the otherwise normal sequences. The cost of the massive resequencing and the ultimate sensitivity threshold of having to have at least one oncogenic variant in a blood sample is what utlimately limits the utility of ctDNA as a cancer sample.

Circulating tumor cells (CTCs)

CTCs are cells that have migrated into the vasculature from a primary tumor or metastasis and is carried through much of the body by the blood circulation. CTCs are known to be the seeds for the subsequent growth of any additional distant tumors (metastases), some in vital distant organs, triggering a mechanism that is responsible for the vast majority of cancer-related deaths. I.e. CTCs are part of the disease mechanism of cancer, a property not shared by any

other sampe type, providing a unique advantage for the CTC sample.



Picture 2. Circulating tumor cells (CTCs) in the bloodstream.

Circulating tumor cells are not excreted by the kidneys since they are much to large for that, and therefore remain longer in circulation. Ultimately they either leave the circulation through an active process of invasion into distant organs, to become potential new micrometstases, or dies from similar cell death mechanisms as those that generate ctDNA. The invasive cancer cell clones seen in blood samples are the most representative ones since they are part of the spread of the disease.

The importance of CTCs in modern cancer research began in the mid 1990s with the demonstration that CTC's exist early on in the course of the disease. Those results were made possible by sensitive immuno-affinity enrichment of cells carrying the EpCAM cell surface marker, that typically only occurrs on cells of epithelial origin. Carcinomas, “cancers”, are of epithelial origin. The expectation was that even very small tumors should supply enough invasive cells, including such cells that end up in the blood circulation, to be detectable in blood.

Experimental models of human cancer in experimental animal models have helped clarify the principles of tumor growth and of the nature of the new sample types: CTC and ctDNA.

Analysis of blood samples found a propensity for increased CTC detection as the disease progressed in individual patients. The ability to monitor the disease progression over time could facilitate appropriate modification to a patient's therapy, potentially improving their prognosis and quality of life.

ctDNA versus CTC utility

ctDNA was detectable in >75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers, but in less than 50% of primary brain, renal, prostate or thyroid cancers. In patients with localized tumors, ctDNA was detected in 73, 57, 48, and 50% of patients with colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, respectively. It is hard to see how ctDNA sensitivity could be increased any further, given that the ctDNA, unlike CTCs, cannot be specifically enriched.

While more comparisons of optimized ctDNA and CTC analyses are warranted, perhaps the best results we have today suggest that CTC sensitivity is about 3 times that of ctDNA, and with the cellular context preserved. It now seems likely that the CTC and the ctDNA sample types will complement each other.