Cancer Diagnostics: Tumor Markers, Cancer Genomics, Circulating Tumor Cells, and Circulating Tumor DNA

by Sean Devlin, DO

The statistical peril of developing cancer in one's lifetime is 40.1% in men and 38.7% in women according to the American Cancer Society. The risk of dying from that cancer: 21.34% in men and 18.33% in women.

Cancer is among the leading causes of death worldwide. In 2018, there were 18.1 million new cases and 9.5 million cancer-related deaths worldwide. In 2020, an estimated 1,806,590 new cases of cancer were diagnosed in the United States and some 606,520 people will die from the disease. By 2040, new cancer cases per year are expected to grow to 29.5 million and the number of cancer-related deaths to 16.4 million.

Cancer is a complex illness that can affect any organ system in the body. A variety of factors contribute to the development and growth of cancer – from epigenetic influences like radiation, toxin exposure, obesity, and stress to a patient's genetic background. Combinations of these influences can create and promote cancer in the body. Several mechanisms provide for cancer's survival and growth in the human body. Some of these factors include evading cell death and senescence messaging, a limitless replication potential, the capacity for immune evasion, the ability to spread throughout the body, metabolic stress and genomic instability (see Figure 1).

Minimally invasive cancer surveillance, diagnostics and monitoring are critically important for patient care and have been evolving over the past decade. Many practitioners, from primary care physicians to surgeons and subspecialists, utilize organized approaches to evaluate and diagnose cancer patients.

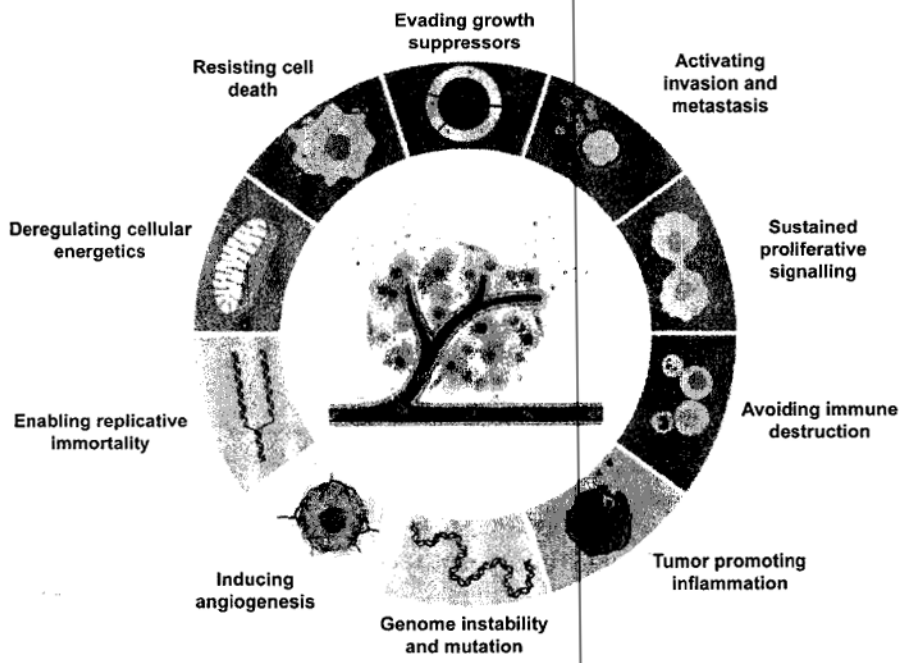
In order to understand the road to a cancer diagnosis, let's start at the beginning. Practice styles may vary, however the pathway most clinicians and surgeons take in diagnosing and staging a patient with the suspicion for cancer includes the following:

Most physicians use the VINDICATE model to develop a differential diagnosis for a given patient's presentation. This template allows physicians to organize the case into distinct categories as they develop their assessment. The acronym VINDICATE stands for the following:

- V – Vascular
- I – Inflammatory
- N – Neoplastic/Cancerous Disease
- D – Degenerative / Deficiency Issue
- I – Idiopathic (Unknown), Intoxication (Drug induced)
- C – Congenital
- A – Autoimmune / Allergic
- T – Traumatic
- E – Endocrine

This differential diagnostic tool starts the clinician on their journey to discover what is the underlying cause of the patient's complaint. In the case of a cancer diagnosis, the clinician initiates a variety of tests and referrals to establish the cancer type, the stage of disease, and the patient's overall clinical situation. Although every case is different, the most logical approaches refine the diagnosis through taking a

Figure 1 'Hallmarks of Cancer'



complete history, performing a physical exam, obtaining a pathology specimen, and gathering appropriate lab work along with medical imaging. A thorough history includes information about physical signs and symptoms, which may be reflective of a mass effect. Common signs like pain and changes in bodily function can be associated with cancer. The history will also identify familial risk factors, like close relatives with cancer diagnoses, which guide clinicians in ordering certain genetic tests.

Some solid tumors, masses, or lesions are found directly upon exam (e.g.: physical exam or colonoscopy). Imaging of an asymptomatic patient may reveal a mass as well (e.g.: through an x-ray, ultrasound or mammography), or in a symptomatic patient (e.g.: abdominal pain, coughing up blood or neurological changes from the presence of a mass) where a CT/PET and/or an MRI is obtained. These scenarios, of course, can vary; but in general this is how most cancer diagnoses come about. Once a solid tumor mass is identified, a biopsy and/or tumor resection (partial or complete) can be performed to further the diagnostic process by examining the macro and microscopic nature of the tissue.

Normal cancer diagnostics include a wide range of pathology tools. Direct histological observation and staining measures can help define the type of cancer that someone is dealing with (see Figure 2). Once pathology is confirmed, the process of staging the cancer can begin. The purpose of staging is to gather prognostic information, which will include further lab work and anatomical imaging, and from there ultimately develop a therapeutic plan.

Advanced imaging technologies such as CT scans, PET scans, and MRI help stage the disease and guide therapeutic planning. Further imaging is used to monitor progression of the cancer and identify disease stability or regression.

Adjunctive lab testing can include measuring tumor markers. Common tumor markers, sometimes called cancer markers, are found in the blood, urine, or bodily tissues. Tumor markers are substances made by cancer cells or normal cells in response to cancer in the body. These markers vary in their value and should be used in concert with imaging and clinical findings to determine a patient's status. Other diagnostic and prognostic parameters include examining cancer cell receptors, gene mutations, and amplifications. Some of the most common cancer markers, receptors, and gene mutations include the following:

Markers and Cancer Type:

- CA15-3, CA27.29 seen in breast cancer
- CEA seen in colorectal cancer
- CA 19-9 seen in pancreatic cancer
- PSA, PAP seen in prostate cancer
- CA 125 seen in ovarian cancer
- Alpha Fetoprotein (AFP) seen in liver, testes, and ovarian cancers

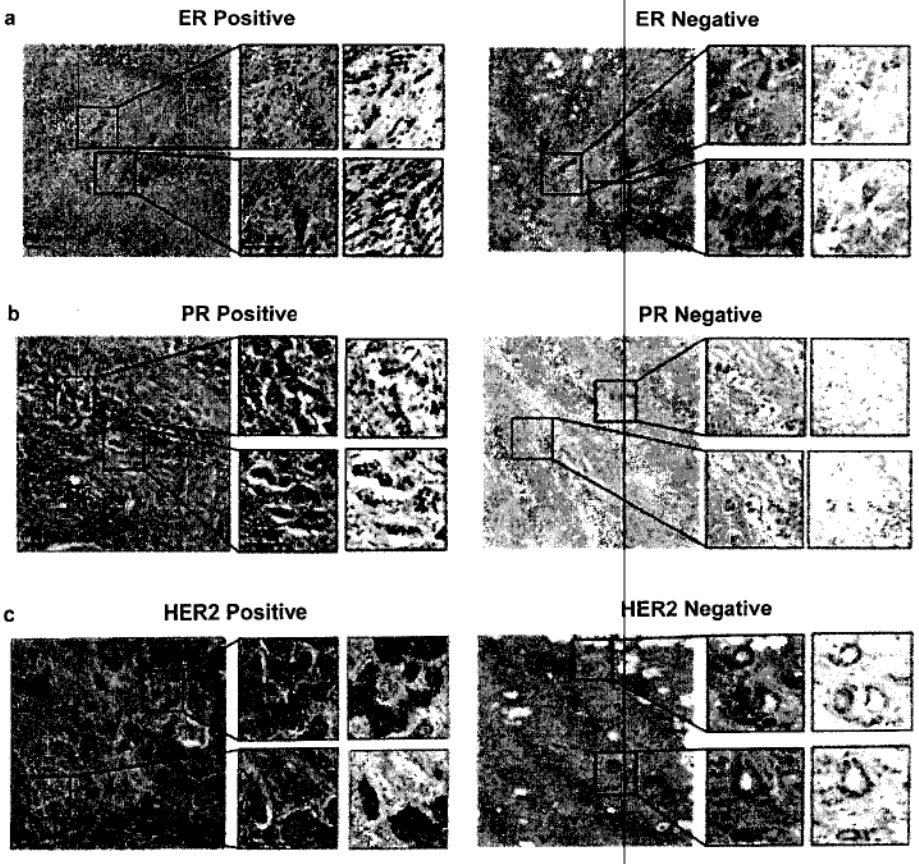
Cancer Cell Receptors and Gene Amplifications (see Figure 3):

- Progesterone receptor positive (PR+) seen in breast cancer
- Estrogen receptor positive (ER+) seen in breast and ovarian cancers
- Androgen receptor positive (AR+) seen in prostate cancer/other hormone sensitive cancers
- HER2/Neu gene amplification/ overexpression seen in breast, ovarian, pancreatic, and gastric cancers
- Programmed Death Ligand (PDL-1) seen in melanoma, GI cancers, lymphomas, and other cancers

Gene Mutations and Cancer Type:

- BRCA1/2 mutations seen in breast and ovarian cancer
- *KRAS* gene mutation seen in some colorectal and non-small cell lung cancer
- MYC gene expression seen in some leukemia and lymphoma
- RAS gene mutation seen in pancreatic, lung, and colorectal cancers
- TP53 gene mutation seen in breast cancer, bone and soft tissue sarcomas, brain tumors, and adrenocortical carcinomas
- EGFR gene mutation seen in non-small cell lung cancer

Figure 2 (Histology Slides) Example of Stained Breast Cancer Cells



➤

- BRAF gene mutations seen in melanoma, non-small cell lung cancer, and colorectal cancer
- BCR-ABL fusion gene seen in chronic myeloid leukemia, acute lymphoblastic leukemia, and acute myelogenous leukemia

Many tumor markers are proteins made by both normal cells and cancer cells, but they are made in higher amounts by cancer cells. Tumor markers include CA-125 (in ovarian cancer), estrogen receptor and progesterone receptor (in breast cancer), CEA (in colon cancer), PCA3 mRNA and PSA (in prostate cancer), EGFR and KRAS gene mutations (in non-small cell lung cancer).

A variety of tumor markers and characteristics, which are seen in and on tumors, may respond to therapies that may not be traditionally used per NCCN guideline treatments. Such markers include PD-L1, HER2/Neu protein, estrogen receptors, androgen receptors, BRCA1/BRCA2 mutations, KRAS mutations, and BRAF mutations.

Advances in cancer diagnostics have led to ever-evolving insights into this insidious disease. Beyond basic pathology and tumor markers, a variety of relatively new testing tools have opened more doors for cancer monitoring and guiding therapeutics.

Oncology is a rapidly evolving field and the direction cancer therapy is moving requires focus on an individual's tumor genetics. Personalized cancer medicine comes from studies of human genetics and the unique gene expression seen in tumors and individual cancer cells. By focusing on an individual's tumor genetics, practitioners can provide more individualized therapies and protocols in treating their patients in an effort to get better results with less potential for side effects.

Tumor genetic studies allow the promise of precision medicine to become a reality. Molecular profiling has become standard of care for many cancer types – and required for certain therapies. We must remember no two tumors are alike and they are created by an interplay between epigenetic factors, the immune system, the tumor microenvironment, and the patient's genetics. Complicating factors are circulating tumor cells and satellite lesions, and the fact that tumor heterogeneity varies within any given mass.

Comprehensive tumor profiling assesses DNA, RNA, and proteins. This provides the highest quality molecular blueprint to guide more precise and individualized treatment decisions, which are proven to extend overall survival.

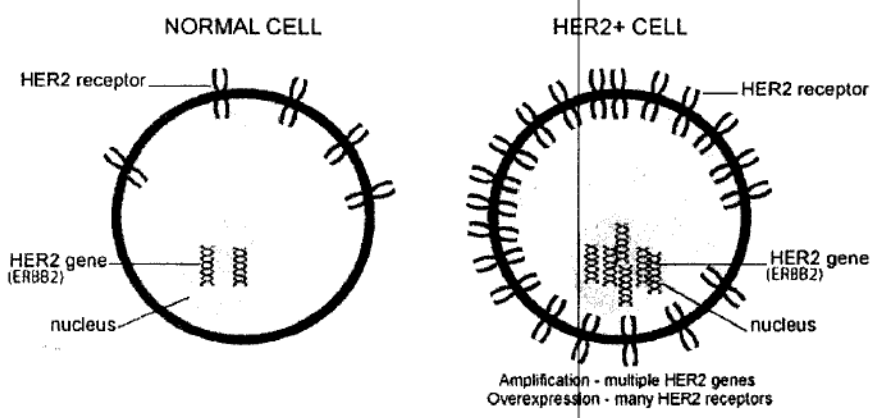
Types of tumor genetic and cancer cell analysis utilized include the following:

- *Immunohistochemistry (IHC)*: detects the presence of specific protein markers which can assist with accurate tumor classification and diagnosis.
- Fluorescence in situ Hybridization (FISH): detects gene deletions, amplifications, translocations and fusions. Evaluating these genetic disturbances can provide improved prognostics and help guide therapies.
- *Next-Generation Sequencing (NGS)*: rapidly examines and detects DNA mutations, copy number variations and gene fusions across the genome. Clinical NGS involves analysis of raw genomic data and rapid clinical interpretations for consideration by the treating clinician. There are three general ways in which NGS can aid a clinician. The first is with diagnosis; tumor subtypes that only a few years ago were defined by morphologic criteria are now defined by genetic mutations. The second is finding an appropriate "targeted therapy," which can personalize the treatment. There are an increasing number of therapies which have indications for use based on DNA sequencing results. The third point at which clinicians stands to benefit is when a patient stops responding to a targeted therapy with known resistance mutations.
- *Sanger Sequencing*: examines strands of DNA to identify mutations by analyzing long contiguous sequencing reads
- *Pyro Sequencing (PyroSeq)*: detects and quantifies mutations, methylation, etc. through sequencing by synthesis
- *Fragment Analysis (FA/Frag. Analysis)*: detects changes in DNA or RNA to indicate the presence or absence of genetic markers

Beyond assessing tumor genetics there is a branch of diagnostic and prognostic testing which slowly grew over the past 20 years. This testing involves looking for and analyzing cancer cells and cancer cellular material that has broken away from the primary tumor (see Figure 4). These cells are referred to as circulating tumor cells and the cellular material in question is referred to as circulating tumor and/or cancer cell DNA.

Circulating tumor cells (CTCs) have long been assumed to be the substrate of cancer metastasis. In recent years, we have begun to leverage the potential of CTCs found in minimally invasive peripheral blood specimens to improve diagnostics, monitoring, and managing care for cancer patients. Over the past several years it has

Figure 3 'HER2 Expression in Normal Cell vs Cancer Cell'



een shown that individual patients who have a sustained and/r elevated circulating tumor cell level are at increased risk or disease progression and recurrence. A high CTC count has een also associated with poor prognosis in several cancers ıcluding breast, lung, and prostate cancer.

In fact, the overall survival and progression-free survival an be reflected by the number of circulating tumor cells resent in a patient.

Liquid biopsies capture whole tumor cells in the blood. The hole cell can provide a lot of information by revealing the enome and transcriptome of the CTC and what downstream roteins could be targeted by anticancer agents.

These cells not only reveal the presence of a tumor; they so indicate that a cancer is progressing or spreading. Most inicians and researchers agree circulating tumor cells are the eds allowing a cancer to spread. This has been challenging prove because circulating tumor cells are rare; they are eterogeneous and may look different from the collection tumor cells they were shed from. However, their distinct llular morphology, genetic characteristics and larger size ake them easy to identify against other circulating cellular aterial. They have also been found in early-stage disease, ading some to believe the primary tumor may not have esented itself alone, but with other surrounding satellite mors. These satellites may pose future risk of recurrence ıd/or new tumor growth.

The CTC count is usually low in non-metastatic cases, and e CTC detection cutoff has regularly been set at ≥ 1 CTC/7.5 L of blood in most studies which validated this technology. lls from solid tumors circulating in blood (CTCs) determine e risk of blood-borne metastases. It is therefore crucial to onitor the response of these cells in order to tailor therapies stematically.

Circulating tumor DNA (ctDNA) is found in the bloodstream ıd refers to DNA that comes from cancerous cells and mors. Most DNA is found inside a cell's ıcleus. As a tumor grows, cells die and e replaced by new ones. It is during these ıases of rapid growth and death in which ncer cells shed DNA.

Researchers and clinician foresee a time ıen ctDNA and circulating tumor cells ll be used in combination with other ore traditional diagnostics to offer the st picture of what's happening with a ncer at any given time inside a patient e Figure 5).

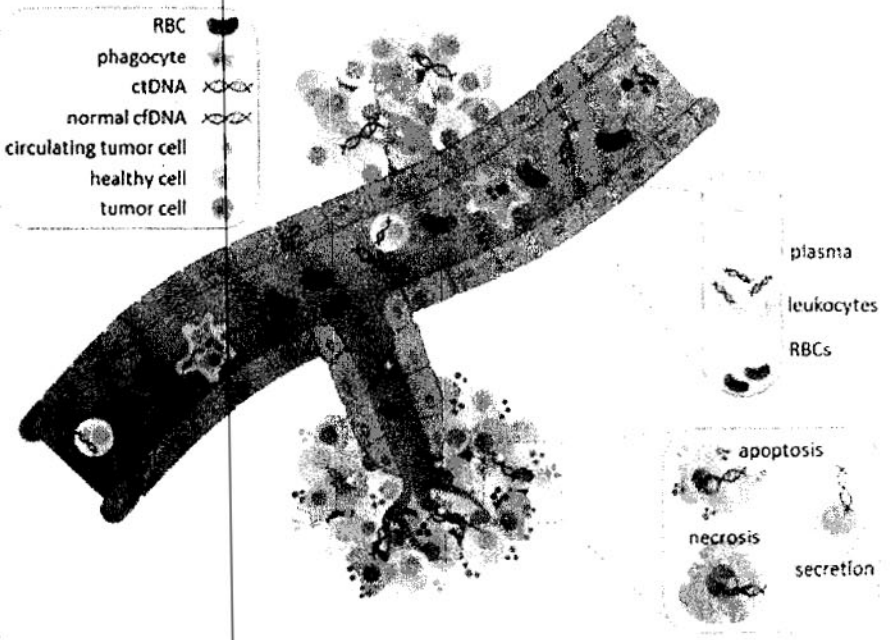
Tumor cell DNA acquires multiple netic changes during tumor development ıding to the tumor's heterogeneous ıte. Therefore, ctDNA may not be an act match to the shedding tumor. The)NA is also not an exact match to the lividual's DNA: finding DNA with genetic ferences aids in tumor detection. ıgnosing the type of tumor using ctDNA ıy reduce the need for getting a sample of the tumor tissue (tumor biopsy) and certainly a better option if the biopsy procedure comes with great risk or simply isn't possible.

Analyzing the genome of tumor cells, ctDNA and DNA fragments can help doctors determine how effective a treatment may be. Some examples include the following:

- Monitoring treatment and seeing a decrease in the quantity of ctDNA may suggest the tumor is shrinking and treatment is successful.
- Monitoring periods with no symptoms (remission of cancer) in which lack of ctDNA in the bloodstream may indicate the cancer has not returned.

Cancer patients usually have a high level of Cell-Free (cfDNA) in their serum or plasma. Cell-free DNA refers to all non-encapsulated DNA in the bloodstream that is a result of cellular necrosis or apoptosis. This material can be from both normal tissue turnover along with that of cancer cells. Since tumor cells divide faster than normal cells, cfDNAs are seen in higher amounts in patients with cancer. Clinicians can tell the difference between the tumor DNA and normal cellular DNA when examining blood samples. One way to distinguish the two is that cfDNA are typically longer strands of DNA while ctDNA are generally shorter length fragments. Specifically, the ratio of total cfDNA to ctDNA is smaller in the cancer patient. A high level of known cancer mutations is found amongst the ctDNA of cancer patients. Gene mutations seen in TP53, EGFR, KRAS, PIK3CA, and BRAF genes were some of the most common. Temporal analysis also suggests that these mutations grew in number as patients received treatment for their cancer – demonstrating chemotherapy and targeted therapy most likely ➤

Figure 4 'Circulating Tumor Cells and Circulating Tumor Cell DNA'



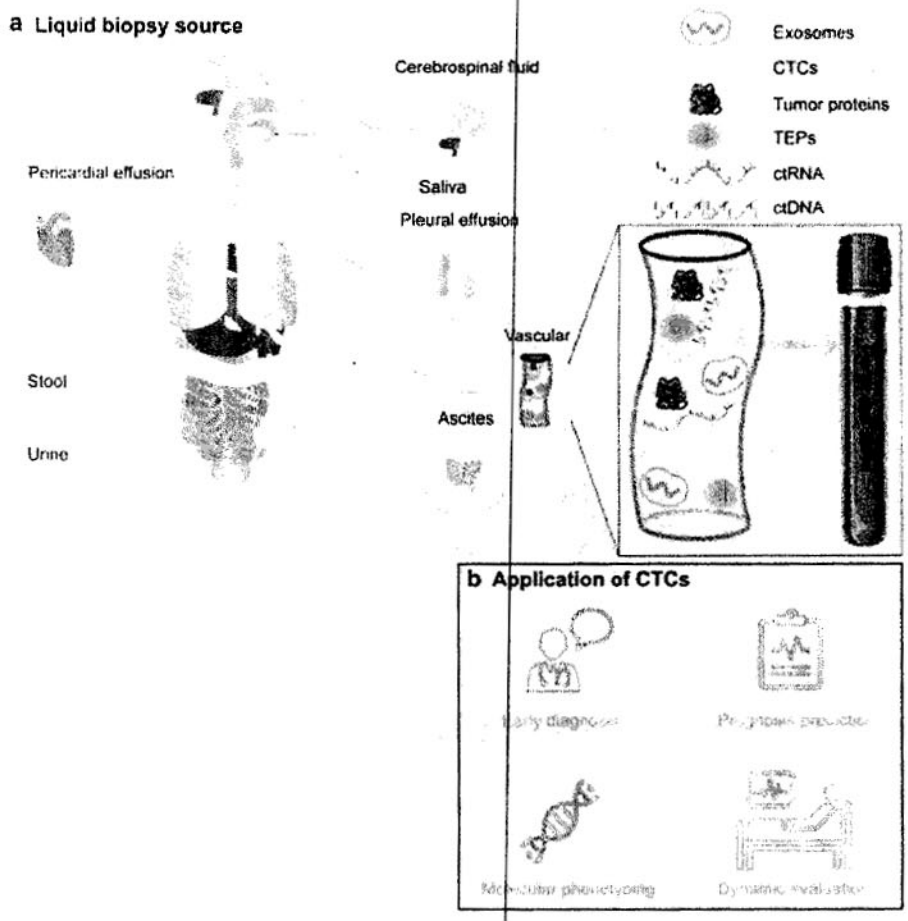
➤

pressured the tumor cells to 'adapt or die'. The importance of patient monitoring is critical and can now rely on blood-based labs that assess disease progression and 'genomic pivots' the cancer is making to evade current treatments.

Future therapies may target circulating tumor cells as well as circulating cancer cell DNA and possibly use them to make personalized cancer vaccines. They will also be used to develop targeted immunotherapies, which have been specifically designed for the cancer patient.

Overall, the journey to a cancer diagnosis, staging, and disease monitoring requires many steps and involves a variety of specialists and technologies working synergistically over time. Our fundamental understanding of cancer has grown dramatically over the past 20 years, we understand it to be a multifaceted and complex illness. Each cancer diagnoses carries with it the opportunity for us to learn more, not only about the disease but also the patient and ultimately the unique interaction between the two. We have seen a vast number of diagnostic tests and precision genomic testing become available in the field of oncology and personalized medicine over the past 20 years. Today there are numerous companies worldwide who are utilizing and refining cutting edge diagnostics by harnessing the information held within the genome of the tumor, the individual circulating cancer cell, and the genetic material from both. By utilizing information about a given patient's cancer, at the microscopic and molecular level, we will be able to fine tune our diagnostic and prognostic capabilities for patients and offer more individualized therapeutics. By unlocking this information, we may find patients qualifying for clinical trials, novel targeted therapies, personalized immunotherapy, chemotherapy and repurposed medications which could prove lifesaving. Through advances in Artificial Intelligence programs, like Deep Learning technology, one will be able to forecast the likelihood of future mutations, make a more accurate overall prognosis for a given patient with cancer, and ultimately know real response rates to a variety of therapeutic agents and interventions.

Figure 5 'Liquid Biopsy to gather CTC's and ctDNA'



References

Castro-Giner F, et al. Tracking Cancer Progression: from circulating tumor cells to metastasis, *Genome Medicine*. 2020;12:31.

Chelakkot C, Yang H, Shin YK. Relevance of Circulating Tumor Cells as Predictive Markers for Cancer Incidence and Relapse. *Pharmaceuticals*. January 3, 2022.

Danfeng Lin, et al. Circulating Tumor Cells: biology and clinical significance. *Signal Transduction and Target Therapy*. 2021;6:404.

Echle A, et al. Deep learning in cancer pathology: a new generation of clinical biomarkers, *British Journal of Cancer*. September 6, 2021.

Hu X, et al. Detection of circulating tumor cells: Advances and critical concerns (Review),. *Oncology Letters*. 2021;21: 422.

Jing Jin, et al. Identification of Genetic Mutations in Cancer: Challenge and Opportunity in the New Era of Targeted Therapy. *Frontiers in Oncology*. April 16 2019; Article 263.

Lianidou E, Pantel K. Liquid biopsies, *Genes Chromosomes and Cancer*. 01 November 2018.

Siravegna G, et al. Integrating liquid biopsies into the management of cancer. *Natural Reviews Clinical Oncology*. 2017; 14: 531–548.

Visal TH, et al. Circulating tumour cells in the –omics era: how far are we from achieving the 'singularity'? *British Journal of Cancer*. March 10, 2022

www.cancer.gov

Yi Jiang, et al. Liquid Biopsy in the OMICS Era of Tumor Medicine. *Journal of Biomedical Applications Basis and Communications*, 2018;1(3).

Sean Devlin, DO, practices integrative oncology and functional medicine and is one the founders of the International Organization of Integrative Cancer Physicians (IOICP), which is a non-profit cancer research and educational foundation. Dr. Devlin is a board-certified and fellowship trained physician. Dr. Devlin holds a master's degree in biochemistry and has pursued doctoral studies in pharmacology with an emphasis on the evaluation of novel anti-neoplastic agents. Dr. Devlin has been honored to lecture nationally and internationally on a variety of medical topics and is regularly sought to provide his expert opinion through direct consultation, speaking engagements, and medical-legal review.

For more information about appropriate testing and specialty laboratories, please contact Dr. Sean Devlin at Gerber Medical Clinic in Reno, Nevada, at 775-826-1900; Highland Springs Wellness Center in Grass Valley, California, at 530-274-2274; or Advanced Dermatology and Cosmetic Surgery in Lake Tahoe at 775-588-5000.