Leveraging Different Liquid Biopsies Markers For Early Cancer Screening And Monitoring

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Abstract

Liquid biopsy is an emerging method for early cancer diagnosis as it analyzes the patient's liquid in an extremely convenient, minimally invasive and reproducible way. Furthermore, it gives scientists reliable data about the patient's specific cancer, which is pivotal to developing many other individualized therapies. Qualification has been recently granted to circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) for its use as diagnostic biomarkers for cancer detection. Alternatively, there are many more components or factors that could enhance the diagnostic potential of liquid biopsy, namely tumor-derived extracellular vesicles (tdEVs), circulating tumor-derived proteins, circulating tumor RNA (ctRNA) and tumor-bearing platelets (TEPs). This review will mainly explore both the benefits and limitations of ctDNA and CTC as biomarkers for cancer monitoring. Moreover, I will discuss the current advancements in the process of separating and analyzing circulating tumor biomarkers alongside the usefulness of non-blood liquid biopsies.

Introduction

It is widely proven and agreed that early detection of cancer is fundamental to improving treatment outcomes. Yet, there lacks sufficient techniques for early detection. The current gold standard for tumor detection is tissue biopsy, which comes with many inconveniences such as difficult-to-obtain tissue samples, low accuracy, and expensive costs [1]. Furthermore, it can not

differentiate heterogeneous tumors from invasiveness, which makes it overall not suitable for long-term clinical monitoring [1]. On the other hand, liquid biopsy is a detection method that could be easily obtained, cheaper, and non-invasive [2]. Thanks to all of these advantages, it has received numerous applications, from screening early cancer tumors to monitoring cancer progression to analyzing drug response to spotting relapse [3].

Since common liquid biopsy markers (LBMs) serve as cancer biomarkers in the early detection of cancer, it is a fundamental basis and requirement for the proper function of liquid biopsy [4]. The best part is that there are many types of LBMs, which allows scientists to do many tests to increase accuracy [4]. Scientists could analyze ctDNA, CTC, TEPs, and tdEVs simultaneously to reaffirm the results obtained from one test [4]. Among many developments that enhance the diagnostic and prognostic potential of liquid biopsies, there are still many clinical applications unmet due to certain limitations [5]. Additionally, there remains an inquiry on the possibility of using non-blood bodily fluids as cancer biomarkers [5].

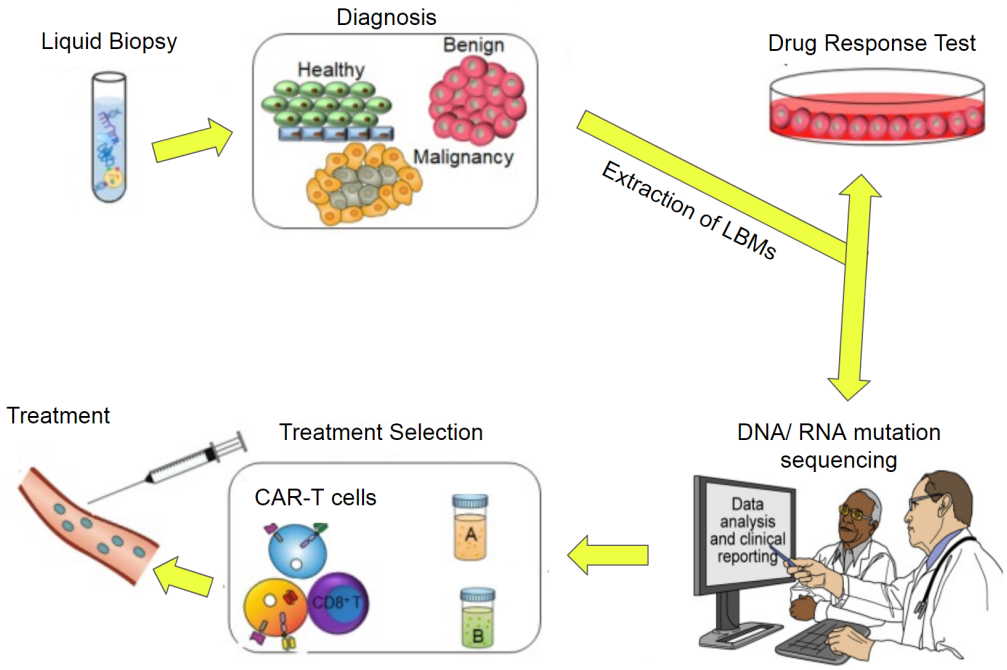

Figure 1: The Clinical Value of LBMs Detection

Circulating Tumor Nucleic Acids

During apoptosis or necrosis, the patient's original tumor produces extracellular nucleic acids which end up circulating in the bloodstream [6]. These tumor-associated ctDNAs/RNAs vastly contrast that of the healthy patient's sera. The mutated KRAS gene, a widely known cancer mutation, actually can be evidenced in the ctDNA captured in the patient's blood [7]. Unsurprisingly, myelodysplastic syndrome and acute myelogenous leukemia (AML) patients' ctDNA also expressed the N-ras mutations [7]. Extracellular-DNA (exDNA) are often indicative of cancer too, especially when they are found in the patient's serum or plasma [8,9]. There are many types of exRNA: microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and messenger RNAs (mRNAs) [9,10]. They can all be very useful playing the role of non-invasive cancer biomarkers [10]. The ctRNA levels detected can help monitor the patient's drug response as it alters regularly following a normal surgery [11]. Detection of gene mutations and

methylation are the two main avenues in which cfDNA/ctDNA technology serves as the major platform. For example, the first clinical ctDNA-detection cancer tests, known as Roche Diagnostics, were able to track the tumor's response to the epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) [12]. Another approved CRC-using diagnostic test functions primarily on the basis of the SEPT9 gene promoter's pattern of methylation [13]. But, because healthy cells can also contain mutations and genetic variations, the use of liquid biopsy to detect gene mutations alone might not be very useful in terms of early detection. Since almost all patients harbor normal tissue's genetic mutations and only a third of all patients harbor cancer-specific mutations, researchers conclude that the use of the cfDNA/ctDNA's methylation is better than gene mutations [14]. Additionally, when detection of genetic mutations are used for other diseases that have mutations at specific genetic loci such as clonal erythropoiesis, each of the detected mutations need to be sorted and analyzed independently from each other, rendering the process inconvenient and rigorous [15].

Since microRNAs modulate gene expression, they play an important role in many biological/ pathological processes such as tumor initiation and development [16]. Many preclinical studies have found that circulating miRNA are very tumor-specific biomarkers [17]. This means that they can serve as a platform for accurate prognosis and the tracing of the metastasis origin [18]. Different microRNA levels found in the serum describe different physiological conditions [19]. For example, it can be used to detect pregnancy and even the specific stage [20]. Analysis of capture ctDNA mainly uses either PCR or next-generation sequencing (NGS). Meanwhile the first attempt at detecting ctDNA utilizes allele-specific PCR, many modified versions have been developed such as the COBAS® EGFR test [21]. To increase the PCR technologies' ctDNA detection accuracy, many cutting edge versions of PCR have also

been developed. Some examples include the ddPCR (droplet digital PCR), BEAMing (beads, emulsion, amplification and magnetics), and dPCR (digital PCR) [22]. These methods are not only highly sensitive, but also convenient and affordable [23]. However, since it is not capable of analyzing bigger gene sequences than a several number of loci simultaneously, these PCR techniques are restrictive in terms of low multiplexing capacity.

The NGS-based ctDNA detection method performs even more poorly since they detect fewer loci compared to other already-restrictive PCR-based technologies [24]. To solve this problem of low sensitivity, researchers use the CAPP-Seq technology, which can spot specific cancer sequences using a larger available library of cancer sequences [25]. To further enhance the efficiency, researchers attach unique molecular identifiers to distinct template sequences and selective nucleases to healthy DNA, which improves differentiation [26]. Moreover, breakthroughs in A.I involving whole-genome sequencing (WGS) are promising avenues for various forms of liquid biopsies, including ctDNA detection [27]. For instance, the DELFI (DNA evaluation of fragments for early interception) platform is an artificial intelligence-based technology that is able to track cancer progression from early diagnosis to relapse [28]. Firstly, tumor-derived ctDNA are matched with particular previously-known mutations.[28] Next, these ctDNA are compared with the rest of the healthy DNA [28]. From here, researchers measure the fragment length distribution to input all the data into the database [28]. Then, more information such as position of chromosome arms, mutant alleles and AGC abundance were inputted and marked on a scale to serve as a baseline for future improvements [29]. All of this information synergizes to create a specific patient's ctDNA profile, which can be used to conveniently separate the healthy patients from the cancer patients [29]. This DELFI platform performs with a 91% detection accuracy in 236 cancer patients and 98% in 245 healthy patients, proving that

artificial intelligence is a promising and powerful tool in the fight against cancer [29]. Despite this, ctDNA detection performs poorly when the patient's cancer is in its earliest stages because the immature tumors produce very few ctDNA. This increase in ctDNA rarity decreases the MAF and detection sensitivity.

Circulating Tumor Cells

During metastasis, the original tumor generates cells to invade nearby tissues or even travel to distant body parts through the bloodstream [30]. These CTCs, often found floating in the patient's blood, create tumor clones and blood vessel walls to support neo-angiogenesis [30]. Since CTCs are indicative of cancer metastasis, it can be used as a reliable biomarker for early detection. From the baseline to the second anti-breast cancer treatment, increased CTCs had been linked to worse cancer prognosis and treatment outcomes [31]. This finding was also confirmed and replicated in other types of metastasis cancer from ovarian to colorectal [32, 33]. Aside from assisting the development of new drugs, the tracking of CTCs population, more importantly, is key to early cancer detection and successful monitoring of the patient's cancer progression and drug responses [34]. Additionally, scientists could use CTCs to separate patients into different risk groups, matching each group with the fittest (neo) adjuvant therapies [35]. Despite these benefits, CTCs are very hard to spot in the cancer patient's peripheral blood because they are only found at a ratio of usually one per million blood cells. To detect CTC, researchers utilized epithelial markers [36]. Specifically, they use the EpCAM-coated magnetic beads to attract and separate the epithelial cells from the patient's blood. Then, these cells get specified through fluorescently-labeled antibodies [36]. Even though not yet approved, emerging systems, detecting stem-cells-associated CTCs, are extremely useful because they alert the doctors of the ongoing epithelial- mesenchymal transition (EMT) and, sometimes, even myschemal

characteristics these CTCs acquired [37]. Traditional CTC detection systems, ones that use epithelial markers, can not detect stem-like CTCs subpopulations, thereby neglecting micrometastasis and metastatic CTCs [38]. So, more research and studies on these CTC subpopulations and how they inform us about their tumor is pivotal to new breakthroughs in therapeutics. CTCs are different in each patient; this allows for more individualized drug screening and treatment administration [39]. Depending on the patient's tumor mutation profiles and drug sensitivity patterns, they will receive the treatments accordingly [39].

Though it gives significant insights for developing clinical treatment, expanding CTCs in vitro is ultimately unuseful due to many reasons. Firstly, there are many factors that could impose unfixable harms to the CTCs' contents, namely enrichment, harsh culture conditions, and pre-sample processing [40]. Secondly, long- term in vitro culture increases the risks of CTCs adopting novel non-tumor-associated traits, rendering the culture a less accurate version of the original tumor [40]. To eliminate this burden, researchers are coming up with ways to upgrade the sorting and enrichment system and the in vitro culture conditions. Furthermore, to understand the mechanisms CTCs use to survive different peripheral blood's components, researchers develop and study the co-culture of CTCs and immune cells or other blood cells [41]. Using these valuable insights, scientists plan to develop new methods to expand the clinical implementation of CTCs beyond liquid biopsy early detection. The most useful aspect of CTCs is their DNA segments that hints back at their original tumor. After CTCs isolation, researchers analyze their genetic components through a variety of techniques including target NGS, dPCR-based mutational spectroscopy technology, genome-wide sequencing technologies, qPCR, and many more [42]. Since CTCs' chromosomal rearrangements are very specific to its tumor, scientists aim to access them by using fluorescence in situ hybridization (FISH), a type of

cytogenetic techniques [43]. To decode how tumor subpopulations interact with its surrounding tissue, researchers use multi omics analyses to dissect the CTCs at the single-cell level [44]. Single CTC genomics and transcriptomics can therefore be considered as an extremely helpful tool in examining tumor heterogeneity. Moreover, it could be used to compare the effectiveness of liquid biopsies versus tissue biopsies.

There are more than one ways of capturing or detecting CTCs: biological and functional. Scientists can apply the strategy that best fits the particular CTC. In the biological method, biomarkers are fundamental. Scientists enhanced EpCAM+ cells and then utilized either cancer biomarkers or CD45+ depletion [45]. In more advanced technology that gives higher CTC yields, the recognition of surface phenotypes are enhanced by microfluidics [45]. To separate CTCs from the patient's normal blood cells, scientists could also look at the difference in size and density [46]. After that, to affirm the initial results, they further examine the CTCs' dielectric properties by a variety of methods, namely di-electrophoresis, microfluidics, filtration, differential centrifugation and many more [47]. In the functional method, assays, which can either employ CAM digestion, capture protein release or analyze the expression of telomerase, are the main platforms [48]. Despite all the advantages that each method has, they still can not sufficiently capture CTCs due to the rare and heterogeneous nature of CTCs. To solve this problem, scientists exploit many methods at once to improve CTC yields.

CTC-Chip

As a technology that receives increasing clinical applications, the CTC-chip takes use of microfluidics in a way that boosts both sensitivity and specificity in separating CTCs from the rest components in the patient's fluid sample [49]. One of its most major strengths is the ability to gently grasp on CTCs, which ensure that isolated CTCs are still in its purest form [50]. As a

controlled nano-scale environment that allows for biological-analysis activity that a macro-scale environment would not, microfluidics performs micro-assays, quickly stimulating the fluid to microscopic distances and leveraging the differences in fluid dynamic properties that occurred as a result [50]. These microfluidics enable researchers to manipulate cells and transport reagents [51]. As a chip, made of silicon, with a relatively small size (that of a standard microscope slide), the CTC-chip serves as the basis for bioengineering. On the chip's surface, there are 78,000 μm-sized posts, many of which form geometric patterns [51]. Some of these posts are covered with the epithelial cell adhesion molecule (EpCAM) to filter the CTCs from the fluid sample [52]. Flow velocity and shear force are two critical factors that influence CTC yields. Faster or slower flow velocity respectively decreases or increases the amount of time that CTC contacts the posts [52]. Similarly, stronger or weaker shear force respectively damages or enhances the maximum cell–micropost attachment [52]. After the patient's blood sample goes towards the surface of the CTC-chip through pneumatic forces, they meet a plethora of microposts [53].

The purposefully-designed geometric arrangement on the chip affects the fluid dynamics so as to ensure that the blood's cellular component travels down a specific pathway, which maximizes the times of contact between CTCs with the EpCAM posts [54]. This way, both the efficiency and effectiveness of the capture process increases. To ensure that the captured cells are CTCs, researchers check the staining which can differentiate leukocytes from epithelial CTCs [55]. After that, CTCs are measured and analyzed at a molecular level. The viability of these CTCs remain unaffected due to the minimal preprocessing required and shear force applied [56,57]. The platform still has many more open avenues for innovations. One promising example is the implementation of different antibodies that could detect different CTCs accordingly, which

will lead to a greater availability of studied CTCs, which will overall enhance our understanding of the original tumor [58].

Conclusion

By serving as an affordable, quick, reproducible and non-invasive method of diagnosing and monitoring cancer, liquid biopsies prove to be a useful tool in the fight against cancer. Compared to tissue biopsies, liquid biopsies can examine the tumor's clonal heterogeneity when the patient's circulating tumor-derived factors are analyzed. Scientists can utilize many liquid biopsy samples so as to reaffirm the accuracy of cancer diagnosis. What's more, the following-administered real-time biopsies can do a huge favor by distinguishing the therapy-resistant tumors from the non-resistant ones. Beyond that, liquid biopsy could be used to detect and track the minimal residual disease, usually occurring after initial therapy. Eliminating the need for long and expensive purification processes, automated chip-based devices or CTC chips are upgraded technology that fundamentally applied liquid biopsies to analyze biomarkers in the patient's fluids sample. But, since there is a deficiency in standardized pre-analytical and analytical variables, liquid biopsies are limited to being used at a wide clinical scale. In summary, liquid biopsies can be a transformative tool, revolutionizing the current reactive cancer treatment paradigm, as it helps oncologists to proactively diagnose, monitor, and develop personalized drugs. Despite all of its potential, there still remains a need for more research and development aiming towards enriching the downstream analysis of circulating biomarkers.

References:

[1] Use of research biopsies in clinical trials: are risks and benefits adequately discussed? Overman MJ, Modak J, Kopetz S, Murthy R, Yao JC, Hicks ME, Abbruzzese JL, Tam AL. J Clin Oncol. 2013 Jan 1; 31(1):17-22.

[2] Liquid Biopsies in Cancer Diagnosis, Monitoring, and Prognosis.De Rubis G, Rajeev Krishnan S, Bebawy M. Trends Pharmacol Sci. 2019 Mar; 40(3):172-186.

[3] Recent Progress on Liquid Biopsy Analysis using Surface-Enhanced Raman Spectroscopy. Zhang Y, Mi X, Tan X, Xiang R. Theranostics. 2019; 9(2):491-525.

[4] Liquid biopsies in gastrointestinal malignancies: when is the big day? Lopez A, Harada K, Mizrak Kaya D, Dong X, Song S, Ajani JA. Expert Rev Anticancer Ther. 2018 Jan; 18(1):19-38.

[5] Integrating liquid biopsies into the management of cancer. Siravegna G, Marsoni S, Siena S, Bardelli A. Nat Rev Clin Oncol. 2017 Sep; 14(9):531-548.

[6] The origin and mechanism of circulating DNA. Stroun M, Maurice P, Vasioukhin V, Lyautey J, Lederrey C, Lefort F, Rossier A, Chen XQ, Anker P. Ann N Y Acad Sci. 2000 Apr; 906():161-8.

[7] Soluble normal and mutated DNA sequences from single-copy genes in human blood. Sorenson GD, Pribish DM, Valone FH, Memoli VA, Bzik DJ, Yao SL. Cancer Epidemiol Biomarkers Prev. 1994 Jan-Feb; 3(1):67-71.

[8] Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Vasioukhin V, Anker P, Maurice P, Lyautey J, Lederrey C, Stroun M. Br J Haematol. 1994 Apr; 86(4):774-9.

[9] Fernández-Domínguez, I. J., Manzo-Merino, J., Taja-Chayeb, L., Dueñas-González, A., Pérez-Cárdenas, E., & Trejo-Becerril, C. (2021). The role of extracellular DNA (exDNA) in cellular processes. Cancer biology & therapy, 22(4), 267–278. https://doi.org/10.1080/15384047.2021.1890319

[10] Monitoring tumour burden and therapeutic response through analysis of circulating tumour DNA and extracellular RNA in multiple myeloma patients. Mithraprabhu S, Morley R, Khong T, Kalff A, Bergin K, Hocking J, Savvidou I, Bowen KM, Ramachandran M, Choi K, Wong BKL, Reynolds J, Spencer A. Leukemia. 2019 Aug; 33(8):2022-2033

[11] The circulating transcriptome as a source of non-invasive cancer biomarkers: concepts and controversies of non-coding and coding RNA in body fluids. Fernandez-Mercado M, Manterola L, Larrea E, Goicoechea I, Arestin M, Armesto M, Otaegui D, Lawrie CH. J Cell Mol Med. 2015 Oct; 19(10):2307-23.

[12] Elevated serum-circulating RNA in patients with conventional renal cell cancer. Feng G, Li G, Gentil-Perret A, Tostain J, Genin C. Anticancer Res. 2008 Jan-Feb; 28(1A):321-6.

[13] The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? Kwapisz D. Ann Transl Med. 2017 Feb; 5(3):46.

[14] Epi proColon® 2.0 CE: A Blood-Based Screening Test for Colorectal Cancer. Lamb YN, Dhillon S. Mol Diagn Ther. 2017 Apr; 21(2):225-232.

[15] RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Yizhak K, Aguet F, Kim J, Hess JM, Kübler K, Grimsby J, Frazer R, Zhang H,

Haradhvala NJ, Rosebrock D, Livitz D, Li X, Arich-Landkof E, Shoresh N, Stewart C, Segrè AV, Branton PA, Polak P, Ardlie KG, Getz G. Science. 2019 Jun 7; 364(6444):.

[16] Park, S., Han, C. R., Park, J. W., Zhao, L., Zhu, X., Willingham, M., Bodine, D. M., & Cheng, S. Y. (2017). Defective erythropoiesis caused by mutations of the thyroid hormone receptor α gene. PLoS genetics, 13(9), e1006991. https://doi.org/10.1371/journal.pgen.1006991

[17] A microRNA expression signature of human solid tumors defines cancer gene targets. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. Proc Natl Acad Sci U S A. 2006 Feb 14; 103(7):2257-61.

[18] MicroRNAs accurately identify cancer tissue origin. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, Benjamin H, Shabes N, Tabak S, Levy A, Lebanony D, Goren Y, Silberschein E, Targan N, Ben-Ari A, Gilad S, Sion-Vardy N, Tobar A, Feinmesser M, Kharenko O, Nativ O, Nass D, Perelman M, Yosepovich A, Shalmon B, Polak-Charcon S, Fridman E, Avniel A, Bentwich I, Bentwich Z, Cohen D, Chajut A, Barshack I.Nat Biotechnol. 2008 Apr; 26(4):462-9.

[19] Serum microRNAs are promising novel biomarkers.Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A. PLoS One. 2008 Sep 5; 3(9):e3148.

[20] Weber, B., Meldgaard, P., Hager, H., Wu, L., Wei, W., Tsai, J., Khalil, A., Nexo, E., & Sorensen, B. S. (2014). Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. BMC cancer, 14, 294. https://doi.org/10.1186/1471-2407-14-294

[21] Incorporating BEAMing technology as a liquid biopsy into clinical practice for the management of colorectal cancer patients: an expert taskforce review. García-Foncillas J, Alba E, Aranda E, Díaz-Rubio E, López-López R, Tabernero J, Vivancos A. Ann Oncol. 2017 Dec 1; 28(12):2943-2949.

[22] The potential of liquid biopsies for the early detection of cancer. Heitzer E, Perakis S, Geigl JB, Speicher MR. NPJ Precis Oncol. 2017; 1(1):36.

[23] Lai, Jinhuo et al. “Next-generation sequencing of circulating tumor DNA for detection of gene mutations in lung cancer: implications for precision treatment.” OncoTargets and therapy vol. 11 9111-9116. 14 Dec. 2018, doi:10.2147/OTT.S174877

[24] An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, Liu CL, Neal JW, Wakelee HA, Merritt RE, Shrager JB, Loo BW Jr, Alizadeh AA, Diehn M. Nat Med. 2014 May; 20(5):548-54.

[25] Development of a highly sensitive liquid biopsy platform to detect clinically-relevant cancer mutations at low allele fractions in cell-free DNA. Gale D, Lawson ARJ, Howarth K, Madi M, Durham B, Smalley S, Calaway J, Blais S, Jones G, Clark J, Dimitrov P, Pugh M, Woodhouse S, Epstein M, Fernandez-Gonzalez A, Whale AS, Huggett JF, Foy CA, Jones GM, Raveh-Amit H, Schmitt K, Devonshire A, Green E, Forshew T, Plagnol V, Rosenfeld N. PLoS One. 2018; 13(3):e0194630.

[26] Imperial, Robin et al. “Matched Whole-Genome Sequencing (WGS) and Whole-Exome Sequencing (WES) of Tumor Tissue with Circulating Tumor DNA (ctDNA) Analysis:

Complementary Modalities in Clinical Practice." Cancers vol. 11,9 1399. 19 Sep. 2019, doi:10.3390/cancers11091399.
[27] Zhang, Zhou, and Wei Zhang. "Fragmentation patterns of circulating cell-free DNA demonstrate biomarker potential for human cancers." Biotarget vol. 3 (2019): 16. doi:10.21037/biotarget.2019.08.02.
[28] Genome-wide cell-free DNA fragmentation in patients with cancer. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, Jensen SØ, Medina JE, Hruban C, White JR, Palsgrove DN, Niknafs N, Anagnostou V, Forde P, Naidoo J, Marrone K, Brahmer J, Woodward BD, Husain H, van Rooijen KL, Ørntoft MW, Madsen AH, van de Velde CJH, Verheij M, Cats A, Punt CJA, Vink GR, van Grieken NCT, Koopman M, Fijneman RJA, Johansen JS, Nielsen HJ, Meijer GA, Andersen CL, Scharpf RB, Velculescu VE. Nature. 2019 Jun; 570(7761):385-389.
[29] A perspective on cancer cell metastasis. Chaffer CL, Weinberg RA. Science. 2011 Mar 25; 331(6024):1559-64.
[30] Circulating tumor cells as early predictors of metastatic spread in breast cancer patients with limited metastatic dissemination. Giuliano M, Giordano A, Jackson S, De Giorgi U, Mego M, Cohen EN, Gao H, Anfossi S, Handy BC, Ueno NT, Alvarez RH, De Placido S, Valero V, Hortobagyi GN, Reuben JM, Cristofanilli M. Breast Cancer Res. 2014 Sep 16; 16(5):440.
[31] Prognostic models based on postoperative circulating tumor cells can predict poor tumor recurrence-free survival in patients with stage II-III colorectal cancer. Wang D, Yang Y, Jin L, Wang J, Zhao X, Wu G, Zhang J, Kou T, Yao H, Zhang Z. J Cancer. 2019; 10(19):4552-4563.
[32] Circulating tumor cells predict progression free survival and overall survival in patients with relapsed/recurrent advanced ovarian cancer.Poveda A, Kaye SB, McCormack R, Wang S, Parekh T, Ricci D, Lebedinsky CA, Tercero JC, Zintl P, Monk BJ. Gynecol Oncol. 2011 Sep; 122(3):567-72.
[33] Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, Shioda T, Ting DT, Ramaswamy S, Getz G, Iafrate AJ, Benes C, Toner M, Maheswaran S, Haber DA. Science. 2014 Jul 11; 345(6193):216-20.
[34] What's new on circulating tumor cells? A meeting report. Lianidou ES, Mavroudis D, Sotiropoulou G, Agelaki S, Pantel K. Breast Cancer Res. 2010; 12(4):307.
[35] EpCAM^{high} and EpCAM^{low} circulating tumor cells in metastatic prostate and breast cancer patients.de Wit S, Manicone M, Rossi E, Lampignano R, Yang L, Zill B, Rengel-Puertas A, Ouhlen M, Crespo M, Berghuis AMS, Andree KC, Vidotto R, Trapp EK, Tzschaschel M, Colomba E, Fowler G, Flohr P, Rescigno P, Fontes MS, Zamarchi R, Fehm T, Neubauer H, Rack B, Alunni-Fabbroni M, Farace F, De Bono J, IJzerman MJ, Terstappen LWMM. Oncotarget. 2018 Nov 2; 9(86):35705-35716.
[36] EMT circulating tumor cells detected by cell-surface vimentin are associated with prostate cancer progression. Satelli A, Batth I, Brownlee Z, Mitra A, Zhou S, Noh H, Rojas CR, Li H, Meng QH, Li S. Oncotarget. 2017 Jul 25; 8(30):49329-49337.

[37] Barriere, Guislaine et al. “Circulating tumor cells and epithelial, mesenchymal and stemness markers: characterization of cell subpopulations.” Annals of translational medicine vol. 2,11 (2014): 109. doi:10.3978/j.issn.2305-5839.2014.10.04
[38] Clinical applications of the CellSearch platform in cancer patients. Riethdorf S, O'Flaherty L, Hille C, Pantel K. Adv Drug Deliv Rev. 2018 Feb 1; 125():102-121.
[39] Progress and challenges of sequencing and analyzing circulating tumor cells. Zhu Z, Qiu S, Shao K, Hou Y. Cell Biol Toxicol. 2018 Oct; 34(5):405-415.
[40] Rivera-Báez, Lianette et al. “Expansion of Circulating Tumor Cells from Patients with Locally Advanced Pancreatic Cancer Enable Patient Derived Xenografts and Functional Studies for Personalized Medicine.” Cancers vol. 12,4 1011. 20 Apr. 2020, doi:10.3390/cancers12041011
[41] Detection of Gene Rearrangements in Circulating Tumor Cells: Examples of ALK-, ROS1-, RET-Rearrangements in Non-Small-Cell Lung Cancer and ERG-Rearrangements in Prostate Cancer. Catelain C, Pailler E, Oulhen M, Faugeroux V, Pommier AL, Farace F. Adv Exp Med Biol. 2017; 994():169-179.
[42] Chrzanowska, Natalia Magdalena et al. “Use of Fluorescence In Situ Hybridization (FISH) in Diagnosis and Tailored Therapies in Solid Tumors.” Molecules (Basel, Switzerland) vol. 25,8 1864. 17 Apr. 2020, doi:10.3390/molecules25081864
[43] Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. Keller L, Pantel K. Nat Rev Cancer. 2019 Oct; 19(10):553-567.
[44] Negative enrichment by immunomagnetic nanobeads for unbiased characterization of circulating tumor cells from peripheral blood of cancer patients. Liu Z, Fusi A, Klopocki E, Schmittel A, Tinhofer I, Nonnenmacher A, Keilholz U. J Transl Med. 2011 May 19; 9():70.
[45] Microfluidic Isolation of Circulating Tumor Cells and Cancer Stem-Like Cells from Patients with Pancreatic Ductal Adenocarcinoma. Varillas JI, Zhang J, Chen K, Barnes II, Liu C, George TJ, Fan ZH. Theranostics. 2019; 9(5):1417-1425.
[46] Detection and cultivation of circulating tumor cells in malignant pleural mesothelioma. Bobek V, Kacprzak G, Rzechonek A, Kolostova K. Anticancer Res. 2014 May; 34(5):2565-9.
[47] RareCyte® CTC Analysis Step 1: AccuCyte® Sample Preparation for the Comprehensive Recovery of Nucleated Cells from Whole Blood. Ramirez AB, U'Ren L, Campton DE, Stewart D, Nordberg JJ, Stilwell JL, Kaldjian EP. Methods Mol Biol. 2017; 1634():163-172.
[48] Sensitive detection of viable circulating tumor cells using a novel conditionally telomerase-selective replicating adenovirus in non-small cell lung cancer patients. Togo S, Katagiri N, Namba Y, Tulafu M, Nagahama K, Kadoya K, Takamochi K, Oh S, Suzuki K,
[49] Sakurai F, Mizuguchi H, Urata Y, Takahashi K. Oncotarget. 2017 May 23; 8(21):34884-34895.
[50] Kozminsky, Molly et al. “The incorporation of microfluidics into circulating tumor cell isolation for clinical applications.” Current opinion in chemical engineering vol. 11 (2016): 59-66. doi:10.1016/j.coche.2016.01.005
[51] Sequist, Lecia V et al. “The CTC-chip: an exciting new tool to detect circulating tumor cells in lung cancer patients.” Journal of thoracic oncology : official publication of the

International Association for the Study of Lung Cancer vol. 4,3 (2009): 281-3. doi:10.1097/JTO.0b013e3181989565

[52] Qian, W., Zhang, Y., & Chen, W. (2015). Capturing Cancer: Emerging Microfluidic Technologies for the Capture and Characterization of Circulating Tumor Cells. Small (Weinheim an der Bergstrasse, Germany), 11(32), 3850–3872. https://doi.org/10.1002/smll.201403658

[53] Chikaishi, Y., Yoneda, K., Ohnaga, T., & Tanaka, F. (2017). EpCAM-independent capture of circulating tumor cells with a 'universal CTC-chip'. Oncology reports, 37(1), 77–82. https://doi.org/10.3892/or.2016.5235

[54] Nagrath, S., Sequist, L. V., Maheswaran, S., Bell, D. W., Irimia, D., Ulkus, L., Smith, M. R., Kwak, E. L., Digumarthy, S., Muzikansky, A., Ryan, P., Balis, U. J., Tompkins, R. G., Haber, D. A., & Toner, M. (2007). Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature, 450(7173), 1235–1239. https://doi.org/10.1038/nature06385

[55] Eslami-S, Zahra et al. “Epithelial Cell Adhesion Molecule: An Anchor to Isolate Clinically Relevant Circulating Tumor Cells.” Cells vol. 9,8 1836. 5 Aug. 2020, doi:10.3390/cells9081836

[56] Batth, Izhar S et al. “CTC analysis: an update on technological progress.” Translational research : the journal of laboratory and clinical medicine vol. 212 (2019): 14-25. doi:10.1016/j.trsl.2019.07.003

[57] Dementeva, N., Kokova, D., & Mayboroda, O. A. (2017). Current Methods of the Circulating Tumor Cells (CTC) Analysis: A Brief Overview. Current pharmaceutical design, 23(32), 4726–4728. https://doi.org/10.2174/1381612823666170616082608

[58] Shahneh F. Z. (2013). Sensitive antibody-based CTCs detection from peripheral blood. Human antibodies, 22(1-2), 51–54. https://doi.org/10.3233/HAB-130270