Clinical Perspective of Liquid Biopsy

Subjects: Oncology

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The term liquid biopsy (LB) refers to the study of circulating tumor cells, circulating tumors nucleic acids free of cells or contained in exosomes, and information about platelets associated with tumors. LB can be performed in different biofluids and allows the limitations of tissue biopsy to be overcome offering possibilities of tumor identification reflecting in real time tumor heterogeneity. In addition, LB allows screening and early detection of cancer, real-time monitoring of therapy, stratification and therapeutic intervention, a therapeutic target and resistance mechanism, and a risk of metastatic relapse.

Keywords: liquid biopsy ; cancer detection ; clinical practice ; molecular profile ; minimal residual disease ; response monitoring ; early detection ; resistance mechanisms ; translational oncology

1. Liquid Biopsy

Cancer disease is an active pathology as a consequence of clonal expansion responsible for the progression of biological processes that trigger tumor development [1]. The ontogeny of cancer is in the accumulation of mutations originating in each of the mechanisms responsible for carcinogenesis. In this way, the evaluation of the genetic mutations related to the tumor could serve as tools in the diagnosis and prognosis. Simultaneous monitoring of pacemakers and treatment linked to these mutations could establish an individualized medicine plan for each patient [2].

Biopsies of tumor tissue are the protocoled way to diagnose cancer. The molecular determination tests and the selection of personalized therapies use the tissue biopsies as a guide. Tissue biopsy consists of the collection of cells from the human body and is a procedure for easy and frequent monitoring of oncogenic mutations [3]. However, it presents limitations: (i) high clinical risk for the patient; (ii) high economic cost; (iii) it is a highly invasive and uncomfortable technique; (iv) there are technical limitations associated with tumor localization; (v) there is difficulty in extracting the cellular subpopulations that make up the tumors; (vi) the dissemination of tumor cells to other organs and tissues inaccessible for tissue biopsy; and (vii) an inability to perform serial testing (e.g., after removal of the primary tumor) [3]. To overcome these limitations an innovative tool is the liquid biopsy (LB) which could be a molecular determination technique to assess the heterogeneity of tumors, their detection and monitoring. LB is a modern biomarker analysis tool that uses the body fluids of patients present in non-solid biological tissue (blood, urine, saliva, urine, cerebrospinal fluid, and pleural effusion). Biomarkers are circulating tumor cells (CTCs), cell-free circulating nucleic acids, exosomes and platelets associated with tumors. All of them are released into the peripheral blood from primary tumor and/or metastatic deposits [3]. These characteristics of the LB establish it as a minimally invasive method, of lower cost than tissue biopsy and without the use of surgery. Some clinical applications for the LB in medical oncology have been suggested, such as determinate molecular profile, diagnosis, response monitoring and early detection of resistance mechanisms, early detection and screening, determination minimal residual disease. Also, this could be used in lung, colorectal, prostate, melanoma, breast and pancreatic cancer [3][4].

However, technological, instrumental and scientific difficulties pose a challenge to LB until it is used in the clinic in a standardized way. For this reason, it is mandatory to validate and establish standardized working protocols for all stages that make up LB (**Figure 1**). In addition, it is necessary to evaluate whether the biomarkers found in the biofluids reproduce the results of tissue biopsies [5][6].

Figure 1. Operative flow of the liquid biopsy: individualized therapeutic strategy for capture dynamics of the tumoral disease.

In this sense, LB is the analysis of the circulating biomarkers, which describe more completely the dynamics of the tumoral disease in real time and which contain tumoral materials from different sites of the disease in the body, requiring different molecular detection technologies: quantitative polymerase chain reaction (q-PCR), BEAMing, safe-sequencing system (Safe-SeqS), cancer personalized profiling by deep sequencing (CAPP-Seq), digital polymerase chain reaction (dPCR), copy number aberrations (CNAs) or point mutations by sequencing the entire genome (whole-genome sequencing, WGS) or sequencing the entire exome (whole-exome sequencing, WES) and tagged-amplicon deep sequencing (TAmSeq) [7] (**Table 1**). These techniques evaluate mutations in the primary tumor and are extremely sensitive, since mutations can be detected at an allele frequency with high specificity showing complete information about the tumor genome and permit to identify new changes that occur during treatment of the tumor and no prior information about the genome of the primary tumor is required [8][9]. These molecular technologies allow positioning the LB as an indispensable clinical test for personalized medicine through the analysis of its biomarkers for prognostic and predictive purposes by non-invasive means, which in the near future will represent a change in the paradigm of molecular biological understanding and the heterogeneity of tumors [10]. Consequently, the LB monitors in real time the dynamics of the cancer disease [11].

Table 1. Properties of the different molecular detection techniques.

Molecular Detection Techniques	Properties
quantitative polymerase chain reaction (qPCR)	qPCR, is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). qPCR is regarded as the 'gold standard' in the quantitative analysis of nucleic acids, be it DNA, RNA or micro-RNA molecules. qPCR has high sensitivity, robustness, good reproducibility, broad dynamic quantification range, and very importantly, affordability.
Safe-Sequencing System (Safe-SeqS)	Safe-SeqS is a Unique Molecular Identifier (UMI) approach to detect rare variants. Safe-SeqS assigns a UMI to each template molecule and amplifies each uniquely tagged template molecule to create UMI families. The abundance of each UMI can be used to distinguish between rare mutations and technical errors, as well as to correct for PCR amplification bias
CAncer Personalized Profiling by deep Sequencing (CAPP-Seq)	CAPP-Seq is a next-generation sequencing based method used to quantify circulating DNA in cancer. CAPP-Seq is an economical and ultrasensitive method and could be routinely applied clinically to detect and monitor diverse malignancies, thus facilitating personalized cancer therapy.
Digital PCR (dPCR)	dPCR is a modification of the qPCR method that can be employed to quantify precisely defined nucleic acid targets. The technique is based on the concept of limiting dilutions, which involves the partitioning of a PCR reaction into multiple sub-reactions such that each sub-reaction either contains none or one or more DNA targets. Following thermal cycling, reactions are classified as either positive (target detected) or negative (no target detected), hence providing the basis for a digital output format. By determining the proportion of empty partitions, Poissonian statistics can be applied and the initial number of target molecules present can be estimated.
Copy number alterations (CNAs)	CNA are somatic changes in chromosome structure that result in gains or losses in copies of DNA sections in somatic tissue, and are prevalent in many cancers. CNA has facilitated the discovery of tumor suppressor genes and oncogenes. Microarray-based CNA assays designed to detect these chromosomes copy number alterations on a high-resolution, genome-wide scale have emerged as a key technology in the genomic era.
Whole-genome sequencing (WGS)	WGS is a comprehensive method for analyzing entire genomes. Whole genome sequencing WGS has revolutionized the biosciences and proven to be essential and invaluable to the identification of gene functions and their involvement in disease. The feasibility of WGS analysis is under the support of next generation sequencing (NGS) technologies, which require substantial computational and biomedical resources to acquire and analyze large and complex sequence data.

Molecular Detection Techniques	Properties
Whole-exome sequencing (WES)	WES is a genomic technique for sequencing all of the protein-coding regions of genes in a genome (known as the exome). WES provides coverage of more than 95% of the exons, which harbor the majority of the large genetic variants and single nucleotide polymorphisms (SNPs) associated with human disease phenotypes. WES strategy starts by narrowing down the details of variants to be studied by filtering against databases such as HapMap, from the approximately 3.5 million SNPs identified in the human genome project. This focus enables a simpler way for discovery and validation of causative genes and common and rare variants.
Tagged-Amplicon deep sequencing (TAm-Seq)	TAM-Seq allows targeted sequencing of entire genes to detect mutations in ctDNA. TAM-Seq is based on a multiplex pre-amplification of tiling short amplicons with target-specific primers and initial eminent of the target regions is performed followed by a selective amplification in individual (singleplex) PCRs in order to exclude non-specific products

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The possibility of performing a molecular perl as simple as a blood extraction, which is non-invasive and can be repeated as many times as necessary, opens the door to a significant number of clinical applications that are developing rapidly and will be used in the coming years [3] (**Figure 2**).

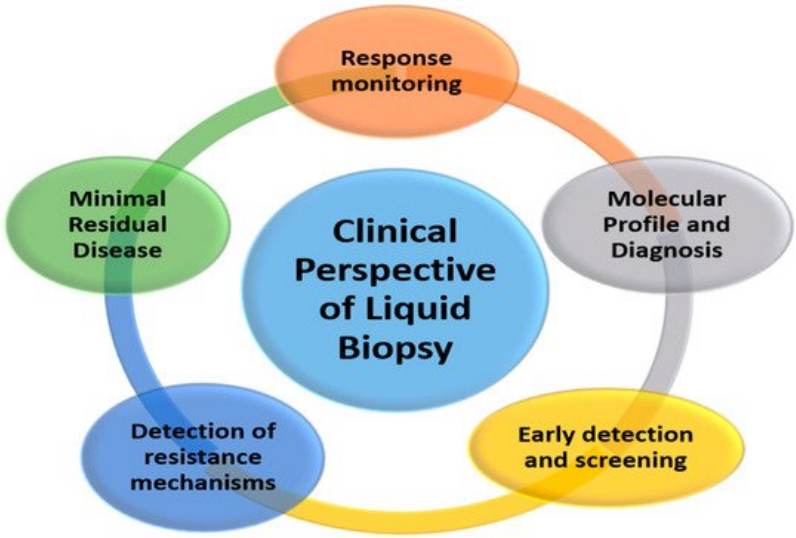

Figure 2. Potential clinical applications of liquid biopsy.

2.1. Molecular Profile and Diagnosis

Cancer treatment is based on histological molecular profile and diagnosis in order to select the most appropriate therapies for each patient's clinical situation. The molecular study of tumor products by LB is mainly based on ctDNA but also CTCs or exosomes that allow the molecular and evolutionary characteristics of the tumor to be established, which will determine its tumor heterogeneity [12]. In addition, LB is increasingly used to generate information on the transcriptome, epigenome, proteome and metabolome. This makes it possible for CTCs and ctDNA to become a clinical and/or research platform to overcome the limitations of current methods of diagnosis and follow-up of cancer patients [6]. For these reasons, LB is a necessity in precision oncology.

Some clinical trials have used cDNA-based molecular profiling as a tool for treatment selection in solid tumors such as lung cancer, prostate cancer and breast cancer [13]. In lung non-small cell lung cancer (NSCLC), the ctDNA determination of mutations of the epidermal growth factor receptor (EGFR) gene including the T790M-resistant allowed to selection the treatment between two drugs (erlotinib or osimertinib) [14]. The ctDNA assay for the determination of the anaplastic lymphoma kinase (ALK) gene mutation (ALK F1174C) in small cell carcinoma of the prostate, establishes a molecular profile that could focus the treatment on the use with ALK inhibitor (alectinib) [15]. In metastatic breast cancer, the use of ctDNA in determining the presence of estrogen receptor 1 (ESR1) mutations was the molecular profile used in the Phase III clinical trials PALOMA-3 and SOLAR-1. These clinical trials evaluate the use of CDK4/6 inhibitors in conjunction with endocrine therapy with Fluvestrant [16]. Thus, the use of ctDNA sequencing seems be adequate in the identification of patients with somatic mutations associated with increased risks of cancer.

In addition, the use of CTCs to establish the molecular profile and diagnosis based on the enumeration and characterization of the CTC. In breast cancer, CTCs were used to select the first line of treatment and established the poor prognosis of the initial number of CTCs [17]. Similarly, in prostate cancer the positive determination androgen receptor splice variant 7 (AR-V7) in CTCs showed a better response to taxanes on display, superior overall survival, and resistance to enzalutamide and abiraterone [18]. We believe that the determination of the molecular profile could improve the ability to diagnose a patient, however, more prospective clinical trials to demonstrate the clinical utility of a LB cancer screening test instead of standard screening programs are essential conditions.

2.2. Response Monitoring and Early Detection of Resistance Mechanisms

LB is an advance in achieving the clinical objectives set because it allows the effectiveness of treatments to be evaluated using new biomarkers in addition to routine clinical indicators (overall survival, or progression-free survival of the disease). These biomarkers serve as a substitute for a clinically significant end-point, which is expected to predict the effect of a therapeutic intervention, by earlier and more manageable criteria in both conventional clinical and clinical trials [6].

In early-stage cancers, the main problem is identifying patients at risk of relapse. These patients may benefit from early surgical or radiotherapy treatments. Patients with pT1-T2 and pN0-N1 stage breast cancer from the National Cancer Database and in the multi-center Phase III clinical trial SUCCESS who were identified as having CTCs were associated with overall survival and disease-free survival by benefiting adjuvant surgery or radiotherapy after breast-conserving surgery [19].

The monitoring of pharmacological therapy using LB makes it possible to determine the tumor phenotype over time, i.e., the evolution of the tumor that is essential when a treatment is administered. Therefore, it allows its effectiveness to be monitored, as well as the possible resistances that could appear in the course of the treatment. The detection of these resistant cell phenotypes allows not only unnecessary toxicities to be avoided, but also the treatment to be individually adjusted before the disease progresses [5]. In addition, the different drugs used against cancer have a very low therapeutic index (therapeutic dose/toxic dose ratio), so the precision must be very high and, additionally, it must have continuity over time. This poses a challenge due to the molecular, cellular, tissue and clinical complexity of cancer disease, which could be resolved by the so-called LB, which conceptually meets all these requirements. This could radically change the diagnostic and therapeutic approach to cancer, as well as the clinical monitoring of this disease [4].

Also, ctDNA analysis has a usefulness in detecting resistance mechanisms [3]. The determination of genomic aberrations in ESR1, mitogen-activated protein kinase (MAPK), Retinoblastoma gene 1 (RB1), Thr790Met (T790M), Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) and proto-oncogene B-Raf (BRAF) genes that appear after chemotherapy treatments in breast cancer with aromatase inhibitors [20] or CDK4/6 inhibitors [21], lung cancer with osimertinib [22], colon cancer with cetuximab [23] and melanoma with BRAF inhibitors [24] may provide a better strategy to delay drug resistance. In general, the LB allows the monitoring of the response and is able to establish early resistance mechanisms which could be achieved up to 10 months prior to radiological progression. This implies the possibility of indicating new treatments or retreatments, allowing the monitoring of clonal evolution and responses to different evolutionary and/or therapeutic pressures, for example, colorectal or breast cancer [10].

2.3. Minimal Residual Disease

The minimal residual disease (MRD), is a clinical state in which the patient has tumor cells disseminated from the primary lesion to distant organs without clinical or radiological signs of metastasis or residual tumor cells [5]. The detection of minimal residual disease (MRD) represents an unsolvable problem in conventional medical oncology. One of the advantages of LB is that it potentially allows MRD. This is especially important in two clinical situations: in adjuvant treatments or after intended surgery or in early detection of relapses [5]. Therefore, the detection of cell-free circulating DNA (cfDNA) could be useful, as demonstrated by Tie et al. [25] in patients with stage II/III colon and rectal cancer where there is no standardized method and/or definition or consensus on the population at risk of micrometastasic disease. In this study it was observed that the percentage of recurrence was 10 times higher when ctDNA was detected after surgery [10].

The possibility offered by the detection and characterization of MRD using CTCs was reflected in the fact that the presence of CTCs was a predictive marker of shorter disease-free survival (DFS) and overall survival (OS) in breast cancer patients after Phase III clinical trial SUCCESS study [26]. Also, CTCs detection after surgery might be a predictive marker of benefit from adjuvant radiotherapy because in patients with CTCs detected before adjuvant therapy radiotherapy was associated with longer OS, loco regional relapse-free survival and disease-free survival (DFS) [19]. Also, colorectal cancer patients, who were found to have CTCs years after surgery had a worse prognosis and increased

recurrence [27]. The lower number of post-treatment CTCs in NSCLC patients was correlated with a higher shorter disease-free survival (DFS) ($p < 0.001$) and overall survival (OS) ($p = 0.009$) [28]. Thus, CTCs counts could early surrogate end point that predicts survival in patients with cancer [5].

2.4. Early Detection and Screening

The last promise, which may come true in a short space of time, about the usefulness of LB is the detection of early disease, long before it appears clinically or radiologically. Until then, work must be done to improve sensitivity in the detection of ctDNA in asymptomatic patients with very early stage tumors [29].

In early stage cancers, LB allows cancer patients to be discriminated from healthy controls, thus screening besides detecting early cancers, and it also permits locating the organ of origin of the tumor. In this sense, the CancerSEEK blood test detect eight biomarkers of circulating proteins and tumour-specific mutations in the ctDNA. In a study of 1000 patients previously diagnosed with cancer and 850 healthy control individuals, CancerSEEK detected cancer with a sensitivity of 69 to 98% (depending on the type of cancer) and 99% specificity [30]. Also the detection cancer-derived (Epstein Barr–virus) EBV DNA in plasma has proven to be a useful screen for early detection of nasopharyngeal carcinoma in asymptomatic subjects, with high sensitivity and specificity analysis of plasma Epstein–Barr virus DNA to screen for nasopharyngeal cancer [31].

References

1. Venesio, T.; Siravegna, G.; Bardelli, A.; Sapino, A. Liquid biopsies for monitoring temporal genomic heterogeneity in bre ast and colon cancers. Pathobiology 2018, 85, 146–154.
2. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. Cell 2011, 144, 646–674.
3. Fernández-Lázaro, D.; García, J.L.; Caballero García, A.; Córdova, A.; Mielgo-Ayuso, J.; Cruz, J.J. Liquid Biopsy as No vel Tool in Precision Medicine: Origins, Properties, Identification and Clinical Perspective of Cancer's Biomarkers. Diag nostics 2020, 10, 215.
4. Siravegna, G.; Marsoni, S.; Siena, S.; Bardelli, A. Integrating liquid biopsies into the management of cancer. Nat. Rev. Clin. Oncol. 2017, 14, 531–548.
5. Buyse, M.; Sargent, D.J.; Grothey, A.; Matheson, A.; De Gramont, A. Biomarkers and surrogate end points—The challe nge of statistical validation. Nat. Rev. Clin. Oncol. 2010, 7, 309–317.
6. Heitzer, E.; Haque, I.S.; Roberts, C.E.; Speicher, M.R. Current and future perspectives of liquid biopsies in genomics-dr iven oncology. Nat. Rev. Genet. 2018, 20, 71–88.
7. Lim, M.; Kim, C.-J.; Sunkara, V.; Kim, M.-H.; Cho, Y.-K. Liquid biopsy in lung cancer: Clinical applications of circulating biomarkers (CTCs and ctDNA). Micromachines 2018, 9, 100.
8. Beaver, J.A.; Jelovac, D.; Balukrishna, S.; Cochran, R.L.; Croessmann, S.; Zabransky, D.J.; Wong, H.Y.; Valda, P.; Cida do, J.; Blair, B.G.; et al. Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin. Cancer Re s. 2014, 20, 2643–2650.
9. Czeiger, D.; Shaked, G.; Eini, H.; Vered, I.; Belochitski, O.; Avriel, A.; Douvdevani, A. Measurement of circulating cell-fr ee DNA levels by a new simple fluorescent test in patients with primary colorectal cancer. Am. J. Clin. Pathol. 2011, 13 5, 264–270.
10. Bardelli, A.; Pantel, K. Liquid biopsies, what we do not know (yet). Cancer Cell 2017, 31, 172–179.
11. Imamura, T.; Komatsu, S.; Ichikawa, D.; Kawaguchi, T.; Miyamae, M.; Okajima, W.; Ohashi, T.; Arita, T.; Konishi, H.; Shi ozaki, A.; et al. Liquid biopsy in patients with pancreatic cancer: Circulating tumor cells and cell-free nucleic acids. Worl d J. Gastroenterol. 2016, 22, 5627–5641.
12. Maltoni, R.; Casadio, V.; Ravaioli, S.; Foca, F.; Tumedei, M.M.; Salvi, S.; Martignano, F.; Calistri, D.; Rocca, A.; Schiron e, A.; et al. Cell-free DNA detected by "liquid biopsy" as a potential prognostic biomarker in early breast cancer. Oncotar get 2017, 8, 16642–16649.
13. Rossi, G.; Ignatiadis, M. Promises and pitfalls of using liquid biopsy for precision medicine. Cancer Res. 2019, 79, 2798 –2804.
14. Rolfo, C.; Mack, P.C.; Scagliotti, G.V.; Baas, P.; Barlesi, F.; Bivona, T.G.; Herbst, R.S.; Mok, T.S.; Peled, N.; Pirker, R.; e t al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): A statement paper from the IASLC. J. Thorac. On col. 2018, 13, 1248–1268.

15. Carneiro, B.A.; Pamarthy, S.; Shah, A.N.; Sagar, V.; Unno, K.; Han, H.; Yang, X.J.; Costa, R.B.; Nagy, R.J.; Lanman, R. B.; et al. Anaplastic lymphoma kinase mutation (ALK F1174C) in small cell carcinoma of the prostate and molecular res ponse to alectinib. Clin. Cancer Res. 2018, 24, 2732–2739.
16. El Sayed, R.; El Jamal, L.; El Iskandarani, S.; Kort, J.; Salam, M.A.; Assi, H. Endocrine and Targeted Therapy for Horm one-Receptor-Positive, HER2-Negative Advanced Breast Cancer: Insights to Sequencing Treatment and Overcoming Resistance Based on Clinical Trials. Front. Oncol. 2019, 21, 510.
17. Schochter, F.; Friedl, T.W.; deGregorio, A.; Krause, S.; Huober, J.; Rack, B.; Janni, W. Are Circulating Tumor Cells (CTC s) Ready for Clinical Use in Breast Cancer? An Overview of Completed and Ongoing Trials Using CTCs for Clinical Tre atment Decisions. Cells 2019, 8, 1412.
18. García, J.L.; Lozano, R.; Misiewicz-Krzeminska, I.; Fernández-Mateos, J.; Krzeminski, P.; Alfonso, S.; Marcos, R.A.; Ga rcía, R.; Gómez-Veiga, F.; Virseda, Á.; et al. A novel capillary nano-immunoassay for assessing androgen receptor splic e variant 7 in plasma. Correlation with CD133 antigen expression in circulating tumor cells. A pilot study in prostate can cer patients. Clin. Transl. Oncol. 2017, 19, 1350–1357.
19. Goodman, C.R.; Seagle, B.-L.L.; Friedl, T.W.; Rack, B.; Lato, K.; Fink, V.; Cristofanilli, M.; Donnelly, E.D.; Janni, W.; Sh ahabi, S.; et al. Association of circulating tumor cell status with benefit of radiotherapy and survival in early-stage breast cancer. JAMA Oncol. 2018, 4, e180163.
20. Fribbens, C.; Garcia Murillas, I.; Beaney, M.; Hrebien, S.; O'Leary, B.; Kilburn, L.; Howarth, K.; Epstein, M.; Green, E.; Rosenfeld, N.; et al. Tracking evolution of aromatase inhibitor resistance with circulating tumour DNA analysis in metast atic breast cancer. Ann. Oncol. 2018, 29, 145–153.
21. Condorelli, R.; Spring, L.; O'shaughnessy, J.; Lacroix, L.; Bailleux, C.; Scott, V.; Dubois, J.; Nagy, R.J.; Lanman, R.B.; I afrate, A.J.; et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic br east cancer. Ann. Oncol. 2018, 29, 640–645.
22. Oxnard, G.R.; Hu, Y.; Mileham, K.F.; Husain, H.; Costa, D.B.; Tracy, P.; Feeney, N.; Sholl, L.M.; Dahlberg, S.E.; Redig, A.J.; et al. Assessment of resistance mechanisms and clinical implications in patients with EGFR T790M–positive lung cancer and acquired resistance to osimertinib. JAMA Oncol. 2018, 4, 1527–1534.
23. Misale, S.; Yaeger, R.; Hobor, S.; Scala, E.; Janakiraman, M.; Liska, D.; Valtorta, E.; Schiavo, R.; Buscarino, M.; Sirave gna, G.; et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Natur e 2012, 486, 532–536.
24. Schreuer, M.; Meersseman, G.; Van Den Herrewegen, S.; Jansen, Y.; Chevolet, I.; Bott, A.; Wilgenhof, S.; Seremet, T.; Jacobs, B.; Buyl, R.; et al. Quantitative assessment of BRAF V600 mutant circulating cell-free tumor DNA as a tool for t herapeutic monitoring in metastatic melanoma patients treated with BRAF/MEK inhibitors. J. Transl. Med. 2016, 14, 95.
25. Tie, J.; Wang, Y.; Tomasetti, C.; Li, L.; Springer, S.; Kinde, I.; Silliman, N.; Tacey, M.; Wong, H.-L.; Christie, M.; et al. Cir culating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon ca ncer. Sci. Transl. Med. 2016, 8, 346ra92.
26. Ignatiadis, M.; Litière, S.; Rothé, F.; Riethdorf, S.; Proudhon, C.; Fehm, T.; Silliman, N.; Tacey, M.; Wong, H.L.; Christie, M.; et al. Trastuzumab versus observation for HER2 nonamplified early breast cancer with circulating tumor cells (EOR TC 90091–10093, BIG 1–12, Treat CTC): A randomized phase II trial. Ann. Oncol. 2018, 29, 1777–1783.
27. van Dalum, G.; Stam, G.-J.; Scholten, L.F.; Mastboom, W.J.; Vermes, I.; Tibbe, A.G.; De Groot, M.R.; Terstappen, L.W. Importance of circulating tumor cells in newly diagnosed colorectal cancer. Int. J. Oncol. 2015, 46, 1361–1368.
28. Krebs, M.G.; Sloane, R.; Priest, L.; Lancashire, L.; Hou, J.-M.; Greystoke, A.; Ward, T.H.; Ferraldeschi, R.; Andrew Hug hes, A.; Clack, G.; et al. Evaluation and prognostic significance of circulating tumor cells in patients with non–small-cell lung cancer. J. Clin. Oncol. 2011, 29, 1556–1563.
29. Rossi, G.; Mu, Z.; Rademaker, A.W.; Austin, L.K.; Strickland, K.S.; Costa, R.L.B.; Nagy, R.J.; Zagonel, V.; Taxter, T.J.; B ehdad, A.; et al. Cell-free DNA and circulating tumor cells: Comprehensive liquid biopsy analysis in advanced breast ca ncer. Clin. Cancer Res. 2018, 24, 560–568.
30. Cohen, J.D.; Li, L.; Wang, Y.; Thoburn, C.; Afsari, B.; Danilova, L.; Douville, C.; Ammar, A.; Javed, A.A.; Wong, F.; et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018, 359, 926–930.
31. Chan, K.A.; Woo, J.K.; King, A.; Zee, B.C.; Lam, W.J.; Chan, S.L.; Chan, S.L.; Chu, S.W.I.; Mak, C.; Tse, I.O.L.; et al. A nalysis of plasma Epstein–Barr virus DNA to screen for nasopharyngeal cancer. N. Engl. J. Med. 2017, 377, 513–522.