Circulating tumor cells and circulating tumor DNA for precision medicine: dream or reality?

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Next-generation sequencing studies have provided further evidence to support the notion that cancer is a disease characterized by Darwinian evolution. Today, we often fail to capture this evolution and treatment decisions, even in the metastatic setting, are often based on analysis of primary tumor diagnosed years ago. Currently, this is considered a major reason for treatment failures in cancer care. Recent technological advances in the detection and characterization of circulating tumor cells and circulating tumor DNA might address this and allow for treatment tailoring based on real-time monitoring of tumor evolution. In this review, we summarize the most important recent findings in the field, focusing on challenges and opportunities in moving these tools forward in clinical practice.

Key words: liquid biopsy, circulating tumor cells, circulating tumor DNA, precision medicine

introduction

Cancer is a disease characterized by Darwinian evolution [1, 2]. Although a significant proportion of patients diagnosed with cancer are cured by surgery alone or surgery and adjuvant systemic treatment, many patients still die due to the development of metastatic disease [3]. There is now evidence from next-generation sequencing (NGS) studies that clonal evolution occurs within the primary tumor and this evolution is at least partly fueled by intratumoral heterogeneity [1, 4]. When metastases develop, clonal evolution continues to occur under the selection pressure of anti-cancer treatments [5]. Patients with certain tumor types, such as luminal breast cancer may develop metastatic disease many years after diagnosis [6]. These late recurrences are due to tumor dormancy, whereby tumor cells that have disseminated to distant organs remain in a dormant state, during which time they accumulate genetic and epigenetic traits that under specific conditions may give rise to overt metastases [7]. Several models have been suggested to describe the metastatic process from models in which tumor cells leave the primary tumor to colonize distant organs when they are fully or partially competent to metastasize, compared with models in which almost ‘normal epithelial cells’ leave preneoplastic lesions very early and evolve in parallel with the primary tumor [3, 8]. These models are not mutually exclusive and in all of them clonal evolution occurs. Our inability to accurately capture spatial and temporal heterogeneity during tumor evolution is considered one of the major reasons for the current failure of cancer systemic treatments whether administered early or later in the disease course. There is hope that a ‘liquid biopsy’ performed at different time-points might be one way to address the above challenge. This is supported by recent technological advances in the field of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA).

The purpose of this review is to summarize recent findings in the liquid biopsy field and highlight challenges and opportunities of applying CTCs and ctDNA as complementary tools to improve the outcome of patients with cancer.

circulating tumor cells
technologies for CTC detection and characterization

There are several challenges associated with CTC detection and characterization: (i) CTCs are rare cells in a background of $10^6$–$10^7$ nucleated blood cells, (ii) an enrichment step is often needed before detection and capturing CTC heterogeneity may not be always feasible if enrichment is based on a preselected marker and (iii) there is a limitation in the blood volume that can be drawn and analyzed.

Although CTCs have been described since 1869 [9], it is only recently that advances in technology have, at least partly, addressed the above challenges [10–13]. The only FDA-cleared technology is the CellSearch® (Veridex, Raritan, NJ). It is based on an initial enrichment of cells expressing the epithelial cell
adhesion molecule (EpCAM) followed by immunofluorescent staining using an epithelial marker (cytokeratin, CK 8, 18, 19), a leucocyte marker (CD45) and DAPI for nuclear staining. The definition of a CTC based on CellSearch® is any EpCAM-positive, intact cell that is at least 4 μm in size, CK+/CD45−, with a nucleus that is at least 50% inside the cytoplasm. However, technologies relying on EpCAM cannot detect CTCs that have undergone epithelial–mesenchymal transition (EMT), a potentially relevant CTC subpopulation with characteristics of chemoresistance [14, 15]. To address this, investigators have used; (i) enrichment based on three antibodies [EpCAM, human epidermal growth factor receptor 2 (HER2) and EGFR] [16], (ii) leucocyte depletion with either immunomagnetic beads [17] or microfluidics (CTC-iChip) [18] or 3) enrichment based on CTC physical properties such as size/deformability (filter technologies, e.g. ISET [19]) and density (ficol/gradient centrifugation [20]).

Another problem limiting the sensitivity of CTC detection is the volume of blood that can be processed ex vivo [21]. To address this, technologies for in vivo isolation of CTCs (e.g. the GILUPI CellCollector™) are currently being evaluated [22]. The technologies for CTC enrichment have been extensively described before [10–12, 23] and are summarized in Figure 1. Following the enrichment step, CTC detection is often carried out using either immunocytochemical or molecular technologies for epithelial- or tumor-specific markers [10–12, 23]. Moreover, functional assays such as in vitro cell culture (e.g. EPISPOT) or xenotransplantation in mouse models are also used [10–12, 23]. These technologies may detect different CTC subpopulations with different sensitivity and purity. The challenge for each particular test is to demonstrate appropriate analytical validity (the accuracy of the test to measure what it is supposed to measure), clinical validity (the value of the test to predict a specific clinical outcome) and ultimately clinical utility (the likelihood that the test will lead to improved clinical outcome when used by physicians) in specific clinical settings [24].

**Figure 1.** Technologies for CTC enrichment.

**Clinical applications of CTCs**

**Clinical utility of CTC enumeration.** The prognostic value of CTC detection using CellSearch® was demonstrated in metastatic breast [25], prostate [26] and colon cancer [27] leading to the FDA clearance of this test as an aid in monitoring patients with the above indications. A recent pooled analysis including almost 2000 women with metastatic breast cancer (MBC), provided level 1 evidence that CTC detection using CellSearch® is associated with worse outcome [28]. The recently reported S0500 trial was the first to test whether randomization based on CTC detection using CellSearch® could improve patient outcome. This trial failed to demonstrate an improvement in overall survival (OS) for MBC patients with persistently elevated CTCs after one cycle of first-line chemotherapy, randomized to switch early to an alternate cytotoxic chemotherapy versus waiting for standard radiological disease progression [29]. The lack of effective treatment options for patients with persistently elevated CTCs might explain these results. Other ongoing randomized trials are evaluating the clinical utility of CTCs (Table 1).

**CTC elimination as an early signal of drug activity.** The role of CTC changes as a surrogate of response to improve OS was evaluated as a secondary end point in a phase III trial of abiraterone, a drug inhibiting androgen biosynthesis, versus placebo in metastatic prostate cancer [30]. The authors demonstrated that a biomarker panel including CTC enumeration by CellSearch® and lactate dehydrogenase measured 12 weeks after starting treatment was significantly associated with reduced OS and was able to assign patients into clinically relevant groups [30]. Independent validation is ongoing to confirm individual patient-level surrogacy for this biomarker panel.

We are exploring the role of CTC elimination as an early signal of drug activity in early breast cancer (EBC) in the ongoing Treat CTC trial (NCT01548677). Historically, new drugs are approved in EBC, years after their approval in the metastatic setting. For approval in the early disease setting,
demonstration of disease-free survival (DFS) or OS improvement in expensive phase 3 trials necessitates long patient follow-up [31]. With the increased availability of new drugs, there is a need to explore novel end points for an early readout of drug activity such as pathological complete response [32] or CTC elimination.

The primary objective of the Treat CTC trial is to explore whether trastuzumab, a monoclonal antibody against HER2, can eliminate chemotherapy-resistant CTCs detected using CellSearch® in women with high-risk HER2-negative EBC. The rational for this trial was based on results from: (i) subset analyses of prospective trials showing that women with HER2-positive EBC by the local laboratory but HER2-negative by the central laboratory derived benefit from trastuzumab [33, 34]; (ii) mouse models suggesting that trastuzumab might be effective in the absence of HER2 amplification by targeting the cancer stem cell population [35]; (iii) studies on CTCs in EBC.

Indeed, several lines of evidence suggest that CTC detection is associated with worse clinical outcome in EBC. This was initially demonstrated using cytokeratin 19 (CK19)-mRNA as a marker for CTC detection [36–39]. The same group showed that a short administration of trastuzumab was able to eliminate chemotherapy-resistant CK19mRNA-positive cells and improve patient outcome in a randomized phase II study including 75 patients with HER2-negative EBC [40, 41]. More recently, several studies including more than 2800 patients have shown that the detection of CTCs by CellSearch® is independently associated with poor prognosis in EBC [42–45]. In this population, the detection rate is around 20% and a median of 1 CTC is detected per sample analyzed [42–45].

Before starting the Treat CTC trial, we carried out an international inter-reader variability study including 14 academic and 2 CellSearch® Veridex laboratories. We demonstrated that although agreement on CellSearch® image interpretation between independent readers and Veridex consensus was high overall (92%), it was lower for patients with EBC and low CTC counts [46]. Therefore, in the Treat CTC, continuous training in image interpretation and independent image review are carried out.

The results from the above studies will indicate whether CTC elimination might be used as an early signal of drug activity to accelerate drug development.

**clinical utility of CTCs characterization using one to four markers.** Beyond enumeration, there is great interest in the genotypic and phenotypic characterization of CTCs. HER2 evaluation at the DNA, mRNA and protein level has been extensively carried out on CTCs [47–51], since there are many drugs available targeting HER2. These studies have shown that although HER2-positive CTCs were more commonly detected in women with HER2-positive disease, there have been still some women with HER2-negative breast cancer and HER2-positive CTCs [47–51]. A phase II study failed to demonstrate any meaningful benefit of single-agent lapatinib in women with HER2-negative MBC and CTCs with HER2-protein overexpression by CellSearch® [52]. The DETECT III trial (NCT01619111) is evaluating the value of adding lapatinib to chemotherapy for a similar indication. A separate trial sponsored by the Institut Curie (NCT01975142) is exploring the value of trastuzumab-emtansine (T-DM1) in women with HER2-negative MBC and
HER2-amplified CTCs by fluorescent in situ hybridization (FISH) [53].

Another group of investigators has developed the CTC-Endocrine therapy index (CTC-ETI), a score based on CTC enumeration and characterization for estrogen receptor (ER), Bcl-2, HER2 and Ki67 using CellSearch® [54]. A high CTC-ETI index is attributed to patients with high CTC counts and CTCs having low expression of ER and Bcl-2 proteins and high expression of HER2 and Ki67 proteins. The COMETI phase II study (NCT01701050) is evaluating the value of the CTC-ETI score to identify women with endocrine refractory MBC (rapid progression within 3 months) after disease progression following at least one line of endocrine treatment.

Beyond breast cancer, CTC characterization has been carried out in other tumor types. Epidermal growth factor (EGFR) mutations conferring drug resistance were detected in CTCs from non-small-cell lung cancer patients who had received tyrosine kinase inhibitors [55]. Androgen receptor mutations were also identified in CTC-enriched peripheral blood samples from castration-resistant prostate cancer (CRPC) patients [56]. Recently, investigators developed an approach to evaluate androgen signaling on CTCs in order to tailor hormonal treatment of patients with prostate cancer [57]. All these are proof of principle studies demonstrating the potential of CTCs characterization for precision medicine. However, further studies are needed to robustly demonstrate clinical validity and utility for these approaches.

Potential for using CTCs for high-throughput molecular analyses. Beyond studies evaluating the clinical utility of CTC enumeration and the characterization of a limited number of markers, we anticipate that in the future CTCs might be evaluated as a tissue source in molecular screening programs.

Several groups have demonstrated the feasibility of analyzing CTC-enriched fractions [58] or pure CTCs [59] for the expression of a number of preselected transcripts. These studies have revealed a profound heterogeneity of CTCs at the transcriptional level. In addition to characterizing gene expression, efforts have also been made to study the mutational profiles of CTCs in various cancer types. A recent study has analyzed CTCs isolated by CellSearch®, primary tumor and metastases from six metastatic colorectal cancer patients using array comparative genomic hybridization and NGS for a panel of 68 cancer genes [60]. They observed that most mutations initially found only on CTCs were also present as subclonal mutations in primary tumors and metastases. We have also demonstrated the feasibility of performing mutational analysis on CTCs detected by CellSearch® and purified using DEPArray™ [61]. Recently, investigators showed the feasibility of CTC whole-exome sequencing and reliable somatic single-nucleotide variant (SNV) calling in patients with metastatic prostate cancer and at least 10 CTCs/7.5 ml of blood. They have employed a modular approach consisting of CTC isolation with the Magsweeper technology [62], single-cell whole-genome amplification, library qualification and a census-based sequencing strategy [63]. This approach allows for confident variant calling when the variant is present in many CTC libraries from the same sample allowing for the identification of truncal mutations but cannot be used to study heterogeneity between single CTCs. The above studies demonstrate the feasibility of performing genomic and transcriptomic analysis on CTCs. Advances in single-cell sequencing [64–66] and the application of these technologies to serial CTC analyses have significant potential to improve our understanding of heterogeneity and disease evolution.

Beyond such analyses, there is now evidence that CTCs can be used as a tissue source for drug sensitivity testing. Indeed, investigators were able to culture CTCs ex vivo and identified activating ESR1 mutations in three of the six CTC-derived cell lines from aromatase inhibitor-pretreated, ER-positive, MBC patients [67]. These mutations are very rarely observed in primary or treatment-naïve ER-breast cancer but were recently suggested to occur at a frequency of 15% in hormone-resistant breast cancer [68, 69]. Using these CTC-derived cell lines, they confirmed previous findings that ESR1–mutant cells were resistant to tamoxifen, raloxifene and fulvestrant and provided novel evidence for antitumor activity using a combination of raloxifene or fulvestrant with an HSP90 inhibitor [67]. The same group carried out drug sensitivity testing in a mouse xenograft model from a CTC cell line harboring a PIK3CA and an FGFR2 mutation. Similarly, other groups have shown that CTCs from patients with breast [70] and small-cell lung cancer (SCLC) [71] were tumorigenic in mouse models. Interestingly, CTC-derived, SCLC mouse models demonstrated similar patterns of response to platinum-based chemotherapy as the original donor-patients.

The clinical utility of the CTC models (CTC-derived cell lines or mouse xenografts) will depend on (i) the percentage of patients in whom this approach is feasible and (ii) whether the
CTC models can always reliably capture response to different drugs. In the future, we could imagine that CTC genomic and transcriptomic analysis together with drug sensitivity testing in CTC-derived cell lines and mouse models might guide personalized treatment approaches (Figure 2).

**circulating tumor DNA technologies for ctDNA analysis**

In parallel to efforts aimed at characterizing CTCs, the benefits of measuring cell-free circulating DNA (cfDNA) for biomarker applications in cancer are actively being explored. Higher levels of cfDNA are identified in cancer patients compared with healthy controls due to the presence of circulating DNA containing tumor-specific sequences that harbor the somatic genomic alterations found in a patient’s tumor (ctDNA) [72, 73]. ctDNA enters the circulation following apoptosis and/or necrosis of tumor cells and is typically fragmented to around 160–180 bp reflecting the degradation of DNA into nucleosomal units which is characteristic of the apoptotic process [74–76]. While ctDNA can theoretically be released into the bloodstream both from tumor tissue and the lysis of CTCs, CTCs and ctDNA represent separate entities, with studies now demonstrating that it is possible to identify ctDNA in the absence of detectable CTCs [77–79]. ctDNA can be detected in a range of different solid malignancies and levels have been shown to increase with disease stage.

The analysis of ctDNA is challenging and requires highly sensitive techniques due to the small fraction of tumor specific DNA present within background levels of normal cfDNA. Importantly, preanalytical factors involved in sample collection can have a significant impact on ctDNA analysis [80]. Whole blood is routinely collected in standard tubes treated with an anticoagulant such as EDTA, and plasma is then separated using centrifugation. Plasma is preferable to serum, and should be processed and stored promptly after blood collection, to prevent increases in cfDNA levels due to cell lysis of normal blood cells that may influence relative levels of ctDNA. ctDNA can then be extracted from plasma using commercially available kits and the analysis of ctDNA can proceed using assays designed to detect somatic genomic aberrations.

Recent advances in genomics technologies are now providing new opportunities for the analysis of ctDNA (Table 2). Types of tumor-specific aberrations that have been followed in ctDNA include somatic SNVs, chromosomal rearrangements and epigenetic alterations [81, 82, 87, 93–95, 97, 98]. The detection of SNVs in plasma DNA has previously been achieved using a variety of PCR approaches [83, 84, 99]; however, digital PCR has now emerged as a sensitive analysis tool for the detection of mutations at low allele fractions [100]. Methods involving the use of digital PCR include droplet-based systems [101], microfluidic platforms [78, 87, 102] and the use of Beads, Emulsions, Amplification and Magnetics (BEAMing) [85, 103]; these approaches are most applicable when a limited number of loci are evaluated.

NGS technologies are now being applied to plasma DNA analysis to allow more comprehensive detection of mutations across wider genomic regions. Targeted deep sequencing using PCR-based (e.g. TAm-Seq [87], Safe-Seq [104], Ion AmpliSeq™ [91, 92] or capture-based (e.g. CAPP-seq [90]) approaches have been

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used to sequence specified genomic regions in plasma DNA. In addition, whole-exome analysis of plasma DNA has opened up new opportunities to comprehensively characterize mutation profiles, without the need to focus on predefined or existing mutations [93]. Chromosomal rearrangements such as translocations or gains/losses of chromosomal regions can also be detected in ctDNA, providing excellent sensitivity and specificity as tumor biomarkers [81, 105]. Personalized analysis of rearrangement ends (PARE) is a method which involves the identification of specific somatic rearrangements in tumor tissue and the subsequent design of PCR-based assays to detect these alterations in plasma DNA [82]. Moreover, in selected cases, whole-genome sequencing has now been directly applied to plasma DNA analysis, to provide an unprecedented view of somatic chromosomal alterations and copy number aberrations in ctDNA genome-wide [94–96]. With continued improvements in the sensitivity of genomic approaches, NGS techniques will undoubtedly play a key role in ctDNA analysis for future clinical applications.

**clinical applications of ctDNA**

**tumor genotyping: tissue versus liquid biopsy.** Studies characterizing the genomic landscape of various malignancies have emphasized the diversity in cancer genomes and highlighted the important role of intratumor heterogeneity [4, 5]. Sampling of a single region at the time of tumor biopsy limits the extent to which the complete spectrum of mutations within a tumor can be assessed. Furthermore, serial sampling of tumor material through repeat biopsies is usually not feasible, hampering efforts to understand patterns of genomic evolution during disease progression and treatment. Our data, together with recent reports, show that somatic mutations identified in ctDNA are widely representative of the underlying tumor genome and can provide an alternative noninvasive method of tumor sampling that can overcome many of these limitations [83, 86, 91, 93, 94, 106, 107].

The most immediate clinical application of this strategy is in the identification of specific genomic alterations to guide the selection of targeted therapies; *EGFR* mutations and *EML4-ALK* rearrangements in non-small-cell lung cancer, *BRAF* mutations in melanoma, *KRAS* mutations in colorectal cancer and *HER2* amplification and *PIK3CA* mutations in breast cancer. The ability to perform blood-based tumor genotyping assays using ctDNA will greatly facilitate the ease with which therapeutic targets can be identified in individual patients to guide treatment decisions and importantly, will also allow these targets to be monitored in real-time during disease progression and therapy (Figure 3). Clinical applications in this arena are likely to expand in coming years with the development of future genotype-based targeted therapies.

**monitoring tumor burden, therapeutic responses and treatment resistance.** A number of recent studies have examined ctDNA dynamics in various solid malignancies to investigate the relationship between ctDNA levels, tumor burden and treatment response [78, 81, 82, 85, 88]. Monitoring treatment response is important in all phases of cancer management to avoid continuing ineffective therapies, to prevent unnecessary side-effects and to determine the benefit of new therapeutics. In MBC, we have recently shown that dynamic changes in ctDNA levels, as
documented by monitoring serial PIK3CA and TP53 mutant ctDNA, closely reflect changes in tumor burden [78]. In these women, ctDNA was a more sensitive biomarker of disease burden than other circulating biomarkers (CA15-3, and CTCs as assessed by CellSearch®), and increases in ctDNA levels often predated the detection of progressive disease using radiological methods by several months. These data on ctDNA need to be validated in a much larger cohort of patients to demonstrate clinical validity for ctDNA to predict clinical outcome in MBC as it was demonstrated for CTCs detection by CellSearch® in the European pooled analysis [28]. The timing of blood and plasma collection in relation to treatment is likely to be an important consideration in the use of ctDNA to assess tumor burden and treatment response [108]. Plasma samples collected immediately before the administration of each treatment cycle may reflect overall tumor burden, whereas the ability to measure increases in ctDNA release immediately after treatment may be prove to be an early indicator of tumor cell death and treatment response. In addition to potential applications of ctDNA as a surrogate marker of treatment response, quantitative assessment of ctDNA levels may also prove to be an important indicator of prognosis. In patients with advanced disease, preliminary data in small patient cohorts data have supported an association between ctDNA levels and prognosis in several malignancies [78, 109], but future long-term studies in larger patient populations will be needed to validate the role of ctDNA as a surrogate biomarker for DFS and OS.

The development of resistance to chemotherapeutic and targeted agents is a major problem currently faced in the care of cancer patients, and represents a key area where ctDNA analysis is likely to play an important role (Figure 3). Treatment resistance, in part, develops due to a continuously evolving spectrum of somatic mutations within the tumor under the selective pressure of treatment. Resistance conferring mutations, if present, are often found in a small fraction of the initial tumor cells and commonly expand to become the dominant clone as the tumor evolves following treatment. Recent studies have demonstrated that ctDNA analysis can allow the emergence of mutations associated with treatment resistance to be assessed noninvasively from plasma DNA, including the identification of T790M-EGFR mutations in non-small-cell lung cancer and the emergence of KRAS mutations and MET amplification in colorectal cancer patients receiving EGFR-based therapies [110–114]. In addition, we have demonstrated the application of serial ctDNA analysis using whole-exome sequencing of plasma DNA to provide a comprehensive and unbiased assessment of genomic changes (through monitoring alterations in mutant allele fractions) during the acquisition of treatment resistance [93]. In patients with advanced cancer, this approach can provide a powerful in vivo strategy to study mechanisms of treatment resistance and gain insights into strategies to overcome or circumvent resistance to current and future therapeutic combinations.

minimal residual disease monitoring and early detection. In haematological malignancies, assessment of recurrent somatic structural alterations such as the BCR-ABL and PML-RARA translocations are used routinely as biomarkers in the diagnostic setting and in the assessment of minimal residual disease to influence treatment decisions [115, 116]. In solid malignancies, tumor-specific genomic alterations identified on an individual basis could be used in an analogous fashion to guide clinical management, although this practice has not been adopted to date. ctDNA analysis has the potential to be used as a biomarker after potentially curative treatment to identify individuals at risk of relapse (Figure 3). Previous studies have shown that by monitoring tumor-specific mutations in plasma following surgical resection, it is possible to identify individuals with residual disease [89] and to detect disease recurrence [84, 85]. The early diagnosis of relapse may allow effective treatment strategies to be introduced at a time when disease burden is still minimal. Alternatively, ctDNA may have a role in stratifying patients at highest risk of relapse to guide the most appropriate selection of adjuvant therapy. Further studies are needed to accurately characterize the relationship between ctDNA levels and disease outcomes following treatment of early-stage malignancies. Finally, the potential use of ctDNA as a biomarker for cancer screening is the most challenging future application of ctDNA assessment as analysis techniques will require the highest levels of sensitivity, specificity and reproducibility to achieve this goal. Ongoing technological advancements may lead to the application of ctDNA analysis as a screening tool to allow the early detection of malignancy at a stage when curative treatments can be offered.

open questions for liquid biopsy

In the future, it is likely that ctDNA and CTCs will have complementary roles as cancer biomarkers although separate approaches may have distinct advantages in specific clinical contexts. ctDNA analysis is appealing due to the ease with which plasma can be collected and analyzed without the prior need to enrich and isolate a rare population of cells. For this reason, ctDNA analysis is likely to be the preferred option for genotyping and monitoring treatment response. The analysis of ctDNA can be applied as a high-throughput strategy for the assessment of clinical samples, but is limited to the analysis of point mutations, structural rearrangements, copy number aberrations and changes in DNA methylation. In contrast, the analysis of CTCs provides the unique opportunity to study the whole cell, allowing DNA, RNA and protein-based molecular profiling, and the opportunity for functional studies to guide personalized treatment selection.

Although the analysis of both ctDNA and CTCs pose several technical challenges, ongoing improvements are expected over coming years, and both approaches hold great promise as biomarkers in various facets of cancer management. Technological advances might allow in the future, the use of a liquid biopsy (CTCs or ctDNA) for earlier cancer diagnosis or detection of disease relapse/progression. However, it is not known if earlier detection will lead to improved patient outcomes. This will become possible only if effective treatments are available to target early recurrences. Another open question is whether the liquid biopsy will complement analysis of primary tumor or metastases for tailoring treatment selection. Ongoing interventional clinical trials using CTC detection and characterization by CellSearch® will provide initial answers on the above question. Future research will be focused on optimizing and standardizing new technologies for both ctDNA and CTC analysis, demonstrating appropriate analytical and clinical validity as well as
establishing the clinical utility of ctDNA and CTC testing through appropriately designed prospective clinical trials.

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