Current Opinion

Liquid biopsy: will it be the ‘magic tool’ for monitoring response of solid tumors to anticancer therapies?

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**Purpose of review**
The aim of this review is to highlight the recent advances (in the past 12 months) concerning circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in oncology.

**Recent findings**
The value of CTCs as a prognostic biomarker is now well validated in breast, colon, and prostate cancer, but no trial has yet demonstrated that modifying treatment according to CTCs is superior to standard of care. Ongoing trials are addressing the clinical utility of CTCs. Moreover, there is emerging evidence about the potential of CTCs as a tumor tissue source to analyze protein and RNA expression, DNA mutations and drug sensitivity. ctDNA is a specific biomarker associated with tumor burden, and small studies have shown an association with worse outcome; prospective clinical studies on the prognostic and predictive value of ctDNA are needed. ctDNA can be used for tumor molecular profiling, with the potential advantage to encompass the spectrum of mutations present in the tumor.

**Summary**
CTCs and ctDNA are promising new biomarkers in oncology, with potential clinical applications for monitoring and for comprehensive molecular profiling of cancer. For each assay, demonstration of analytical and clinical validity, as well as clinical utility in prospective clinical trials is needed before implementation in clinical practice.

**Keywords**
biomarkers, cancer, circulating tumor cells, circulating tumor DNA, liquid biopsy

**INTRODUCTION**
In the past two decades, the medical community has witnessed the rapid development of molecular oncology, with powerful molecular profiling tools enabling a detailed characterization of cancer. However, our knowledge of the molecular evolution of cancer has been limited by the lack of access to tumor tissue throughout disease progression. Liquid biopsies, including analysis of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), are a promising new avenue for real-time monitoring of tumor progression. CTCs have been widely investigated during the past decade, with numerous studies addressing their prognostic value and potential for tumor molecular profiling [1–3]. More recently, ctDNA proved to be associated with tumor burden [4], and to be representative of the underlying tumor genome [5\textsuperscript{*}, 6, 7, 8\textsuperscript{*}]. The aim of this review is to highlight recent breakthroughs (in the past 12 months) concerning the use of CTCs and ctDNA in oncology.

**PROGNOSTIC AND PREDICTIVE VALUE OF CIRCULATING TUMOR CELLS**
The prognostic value of CTCs has been established in many cancers, with the most robust data in breast, prostate, and colon cancer (Table 1) [1]. Recently, in breast cancer, a pooled analysis of CTC levels in 1944 metastatic breast cancer (mBC)
patients provided level-one evidence that CTCs are associated with decreased progression-free survival (PFS) and overall survival (OS) [11]. Analysis of CTCs in 2026 patients with early breast cancer (eBC) also confirmed that CTC detection is associated with worse clinical outcomes in that setting [12]. However, in an inter-reader variability study, agreement for CTC definition between independent readers was lower in eBC compared to mBC setting, suggesting that continuous training and central review should be applied in clinical trials using CTC detection in eBC [43]. A score integrating CTC enumeration with estrogen receptor, HER2, BCL-2, and Ki67 expression was recently developed in estrogen receptor-positive mBC to predict endocrine resistance; its clinical validity will be prospectively evaluated in clinical trials (NCT01701050, NCT02137837) [44].

In metastatic castrate-resistant prostate cancer, the prognostic value of CTCs was recently prospectively validated in two phase III randomized trials [33,34]. In the S0421 trial, higher baseline CTC count (≥ five per 7.5 ml) and CTC count increase at day 21 where both were associated with reduced OS [33]. Likewise, CTC count at 12 weeks combined with lactate dehydrogenase (LDH) level fulfilled all of Prentice’s criteria for OS surrogacy in the COU-AA-301 trial [34].

A recent meta-analysis also confirmed the adverse prognostic value of CTCs in metastatic

colorectal cancer (CRC) [16]. However, the prognostic impact of CTCs in early CRC is unclear. Although small studies demonstrated association between CTC count and relapse in the early setting [17,18], a prospective analysis of CTCs in a cohort of 472 stage III CRC patients did not show association between CTCs, measured after tumor resection and before adjuvant therapy, and outcomes [19]. Differences in timing for CTC count, as well as distinct populations, may explain these conflicting results.

Small studies also explored CTC prognostic value in other tumor types such as lung, melanoma, and esophagogastric cancer, also revealing association with adverse outcomes [20–22,28–30]. Moreover, few meta-analyses provided new evidence supporting the adverse prognostic value of CTCs in pancreas, ovarian, and head and neck cancer [25,26,38,39].

The association between CTCs and poor prognosis triggered attempts to investigate CTC potential to guide treatment decision. The SWOG S0500 trial addressed the benefit of switching therapy preemptively in case of persistent CTCs after 21 days of first-line chemotherapy in mBC [10]. One hundred and twenty-three patients were randomized to continue therapy or to change to an alternate chemotherapy. Although the presence of CTCs was associated with poor prognosis, the early switch strategy failed to improve outcomes. An explanation for these results may be the lack of an efficient alternative therapy. Many ongoing trials are addressing treatment decision based not only CTC enumeration but also on CTC protein expression (Table 2).

**CIRCULATING TUMOR CELLS BEYOND ENUMERATION**

Characterization of CTCs could help provide a better insight on the biology underlying metastasis development. For example, emerging data suggest that CTCs sometimes circulate in clusters with or without stromal cells, and that these clusters are implicated in the process of cancer dissemination [45,46].

There is a growing interest in exploiting CTCs as tumor tissue source to analyze protein expression, RNA expression, and DNA mutations. In prostate cancer, expression of androgen-receptor splice variant 7 RNA (AR-V7) on CTCs proved to be associated with resistance to enzalutamide and abiraterone [47]. Studies in various cancers also demonstrated the feasibility to detect specific mutations such as KRAS, BRAF, EGFR, R0S1, PI3KCA, or TP53 mutations on CTCs [1,48–56]. Moreover, the feasibility to perform comprehensive whole-exome sequencing on CTCs was recently demonstrated and provided evidence that CTC analysis could

<table>
<thead>
<tr>
<th>Trial</th>
<th>Estimated enrollment</th>
<th>Status</th>
<th>Population</th>
<th>Intervention</th>
<th>Primary endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>CirCe01 NCT01349842 phase III</td>
<td>568</td>
<td>Recruiting</td>
<td>Refractory MBC with CTCs positive before starting CT</td>
<td>Therapeutic decision CTCs-driven vs. according to usual clinical and radiologic criteria</td>
<td>OS</td>
</tr>
<tr>
<td>TREAT-CTC NCT01548777 phase II</td>
<td>2175</td>
<td>Recruiting</td>
<td>HER2-negative early BC with CTCs positive after (neo)adjuvant CT</td>
<td>Adjunct trastuzumab vs. placebo</td>
<td>CTC detection (week 18)</td>
</tr>
<tr>
<td>DETECT-III NCT01619111 phase III</td>
<td>120</td>
<td>Recruiting</td>
<td>HER2-negative MBC with HER2-positive CTCs</td>
<td>Standard therapy (CT or ET) vs. physician choice + lapatinib</td>
<td>CTC clearance rate</td>
</tr>
<tr>
<td>VISNU-I NCT01640405 Phase III</td>
<td>350</td>
<td>Recruiting</td>
<td>Metastatic CRC KRAS wild-type, CTCs &gt; 3</td>
<td>FOLFOX6 + Bev vs. FOLFOX6 + Bev</td>
<td>PFS</td>
</tr>
<tr>
<td>STIC-CTC NCT01710605 phase III</td>
<td>1000</td>
<td>Recruiting</td>
<td>MBC, HR+, HER2-negative</td>
<td>Physician choice vs. CTCs-driven choice between ET and CT</td>
<td>PFS economic evaluation</td>
</tr>
<tr>
<td>DETECT-IV NCT02035813 phase II</td>
<td>520</td>
<td>Recruiting</td>
<td>HER2-negative MBC with persistent CTCs</td>
<td>Everolimus + ET or eribulin</td>
<td>PFS</td>
</tr>
<tr>
<td>NCT01975142 phase II</td>
<td>480</td>
<td>Recruiting</td>
<td>HER2-negative MBC with HER2-positive CTCs</td>
<td>T-DM1</td>
<td>Tumor RR</td>
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</tbody>
</table>

**Table 2. Summary of ongoing clinical trials involving therapeutic decision based on circulating tumor cells**

BC, breast cancer; Bev, bevacizumab; CRC, colorectal cancer; CT, chemotherapy; CTCs, circulating tumor cells; ET, endocrine therapy; HR, hormonal receptor; MBC, metastatic breast cancer; OS, overall survival; PFS, progression-free survival; RR, response rate.
reveal a majority of ‘trunk’ mutations present in both metastasis and primary tumor tissue samples [57].

Circulating tumor cells can also be cultured ex vivo for functional analysis [58**,59]. Yu et al. [58**] performed next-generation sequencing (NGS) and drug sensitivity testing on six cultured cell lines derived from CTCs of mBC patients. The results suggested correlation between CTCs’ mutation profile, in-vitro drug resistance, and clinical history [58**]. Additionally, the tumorigenic potential of CTCs after implantation in immunosuppressed mice was demonstrated in breast, colon, and small cell lung cancer (SCLC) [58**,59,60**]. Hodgkinson et al. [60*] showed that in patients with SCLC, the molecular profile of CTCs corresponded to that of CTC-derived explants (CDX), and that CDX reflected patient’s tumor response to platinum-based chemotherapy, providing a model to study drug resistance.

**CHALLENGES IN CIRCULATING TUMOR CELL ANALYSIS**

Many pitfalls need to be addressed before broader use of CTCs in clinical practice. CTC detection is challenging because of the rarity of CTCs among other blood cells; hence, different methods have been developed to enrich and isolate CTCs [3]. The CellSearch system – the only US Food and Drug Administration (FDA)-approved technology for CTC detection, uses the epithelial cell adhesion molecule (EpCAM) to positively enrich CTCs. However, this method is dependent on EpCAM expression on tumor cells. Alternative antigen-independent methods are in development, but clinical validity remains to be demonstrated [61]. Nevertheless, CTCs could be used in prospective clinical trials to select a high-risk population that may benefit from therapy intensification.

Circulating tumor cell characterization may be more promising than CTC detection to guide treatment-related decision. However, molecular analysis on CTCs must be interpreted with caution, considering the clonal heterogeneity of cancer, with evidence that no two cells are genetically identical [62]. Some studies revealed disagreements between mutations identified on CTCs and tumor tissue samples [50,52,63]. There are many unanswered questions; what is the clinical significance of mutations identified on CTCs, but not on tumor tissue? Do CTCs capture the molecular
heterogeneity of metastatic disease? Can CTC models be derived only from patients with highly aggressive tumors? Can these models always capture treatment response?

**CIRCULATING TUMOR DNA: A PROMISING BIOMARKER**

DNA is released into circulation following cell-death processes. ctDNA fragments are typically the size of 160–180 bp, reflecting DNA degradation into nucleosomal units during the process of apoptosis [64]. Detection of ctDNA is challenging since it is found in low levels and has to be discriminated against nontumoral circulating free DNA (cfDNA).

The feasibility of developing personalized ctDNA assay by identifying genomic alteration also present in the corresponding tumor tissue has been demonstrated in a few trials, the larger of which identified genetic alterations in various tumors, allowing ctDNA detection in 82% of the patients [65]. In that study, ctDNA was detected at a high frequency in advanced pancreas, bladder, colon, melanoma, stomach, breast, liver, esophagus, and head and neck cancer, but was detected in less than 50% of patients with medulloblastoma, kidney, prostate, or thyroid cancer, and in less than 10% of patients with glioma. ctDNA was detected in only 55% of patients with localized cancer, but was overall more sensitive than CTCs.

Several small studies have demonstrated the association of ctDNA levels with tumor burden [4,65,66,67–72], and adverse prognosis [67,73–75], in various cancer types. Preliminary data in breast, ovarian and colorectal cancer suggest that ctDNA could be used as a specific biomarker with better performance than classic blood tumor markers, with the potential to improve management of cancer patients (Fig. 1) [4,69,76,77]. To that aim, ctDNA assays need to be integrated in prospective clinical trials to demonstrate clinical validity and utility in various cancer types.

**CIRCULATING TUMOR DNA FOR GENOMIC PROFILING**

The possibility to identify specific mutations on ctDNA has been demonstrated in several tumor types, with the most robust data in lung and CRC (Table 3) [65,75,78,79,81–84,95]. Three meta-analyses addressed the accuracy of *EGFR* mutation assays on cfDNA in lung cancer and demonstrated

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Genomic alteration</th>
<th>Potential indications</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Breast</td>
<td><em>ESR1</em> mutation</td>
<td>Tracking emerging resistance mechanism</td>
<td>[78,79]</td>
</tr>
<tr>
<td></td>
<td><em>PIK3CA</em> mutation</td>
<td>Prediction of response to agents targeting the PI3K/AKT pathway, Prognostic evaluation</td>
<td>[80,81]</td>
</tr>
<tr>
<td>Colorectal</td>
<td><em>KRAS</em> mutation</td>
<td>Prediction of response to anti-EGFR treatment, Tracking emerging resistance mechanism</td>
<td>[65,82–85]</td>
</tr>
<tr>
<td></td>
<td><em>NRAS</em> mutation</td>
<td>Prediction of response to anti-EGFR treatment, Tracking emerging resistance mechanism</td>
<td>[83,85]</td>
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<tr>
<td></td>
<td><em>BRAF</em> muts</td>
<td>Prediction of response to anti-EGFR treatment, Tracking emerging resistance mechanism</td>
<td>[74]</td>
</tr>
<tr>
<td>Esophagogastric</td>
<td><em>APC</em> methylation</td>
<td>Prognostic evaluation</td>
<td>[86]</td>
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<tr>
<td></td>
<td><em>RASSF1A</em> methylation</td>
<td>Association with tumor burden</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td><em>HER2</em> amplification</td>
<td>Prediction of response to anti-HER2 treatment</td>
<td>[87]</td>
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<tr>
<td>Pancreas</td>
<td><em>KRAS</em> mutation</td>
<td>Prognostic evaluation</td>
<td>[88]</td>
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<tr>
<td>Lung</td>
<td><em>EGFR</em> mutation</td>
<td>Prediction of response to anti-EGFR treatment, Tracking emerging resistance mechanism</td>
<td>[89–93]</td>
</tr>
<tr>
<td>Prostate</td>
<td><em>GSTP1</em> methylation</td>
<td>Prognostic evaluation</td>
<td>[94]</td>
</tr>
<tr>
<td>Ovarian</td>
<td><em>ERCC1</em> mutation</td>
<td>Prediction of response to platinum-based chemotherapy, Prognostic evaluation</td>
<td>[55]</td>
</tr>
<tr>
<td>Melanoma</td>
<td><em>BRAF</em> mutation</td>
<td>Prediction of response to anti-BRAF treatment</td>
<td>[95,96]</td>
</tr>
<tr>
<td>Glioma</td>
<td><em>MGMT</em> methylation</td>
<td>Prognostic evaluation</td>
<td>[97]</td>
</tr>
<tr>
<td>Gastro-Intestinal Stromal Tumor</td>
<td><em>KIT</em> mutation</td>
<td>Prediction of response to treatment with tyrosine kinases inhibitors</td>
<td>[98]</td>
</tr>
<tr>
<td><em>PDGFR</em> mutation</td>
<td>Prediction of response to treatment with tyrosine kinases inhibitors</td>
<td>[98]</td>
<td></td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td><em>ALK</em> mutation</td>
<td>Prediction of response to treatment with ALK inhibitors</td>
<td>[99]</td>
</tr>
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</table>
the good specificity (88–93%) but lack of sensitivity (63–67%) of this approach [90,100,101]. However, the diagnostic performance of these assays is improving; for example, a ctDNA EGFR assay demonstrated 78% sensitivity within the EURTAC trial [102]. In the FASTACT2 trial randomizing patients to six cycles of gemcitabine/platinum with intercalated erlotinib versus placebo, the sensitivity of the ctDNA EGFR assay was 75%, and patients allocated to the erlotinib arm harboring EGFR mutation on ctDNA had longer PFS than the wild-type group [89]. The possibility to monitor the acquisition of mutations conferring resistance to targeted therapies was demonstrated in lung, colorectal, and breast cancer [78,79,83,84,91,103], also showing that ‘resistant’ clones are fading following therapy withdrawal, providing rationale for adaptive therapy strategies based on ctDNA molecular profile [83].

The feasibility of performing NGS on cfDNA was recently demonstrated, showing good concordance between plasma and tumor tissue in terms of the identified genomic aberrations [8*,104,105]. Our laboratory demonstrated the feasibility of high-coverage NGS of a 50-gene panel in plasma samples from 17 mBC patients with high sensitivity (detection of mutations at allelic frequencies as low as 0.5%); when we compared tumor and plasma samples collected at the same time-point, the results were concordant in 76% of patients, whereas in 24%, they were discordant and provided complementary information [8*]. An interesting question is whether actionable mutations identified in plasma ctDNA are associated with treatment benefit, especially in cases when the mutation is not identified in matched metastatic biopsy. Another question is whether the detection of subclonal mutations with low allele fraction in plasma ctDNA is relevant for treatment selection. To address the above questions, plasma needs to be tested as an alternative tissue source to metastatic biopsies in molecular screening programs.

**CONCLUSION AND FUTURE PERSPECTIVES**

In summary, clinical applications for liquid biopsies are divided into two main categories. First, quantification of CTCs and ctDNA can provide prognostic information. Second, CTCs and ctDNA may allow accessible molecular profiling of the tumor. ctDNA could be used for the screening of mutations that predict response to therapy, and for real-time monitoring of clonal evolution to allow adaptive treatment strategy. In the presence of high tumor burden, comprehensive characterization of tumor DNA, RNA, and protein expression, along with functional analysis, could be performed on CTCs to optimize therapy selection. However, before application in clinical practice, these assays need to prove analytical and clinical validity, and then clinical utility. Prospective trials integrating these tools are needed to obtain such validation in the metastatic and early setting.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

6. The first study providing evidence that it is feasible to uncover mechanisms of resistance to treatment using exome sequencing on plasma circulating cell-free DNA.

This study demonstrated the feasibility of performing high coverage NGS for a 50-cancer gene panel in plasma from metastatic breast cancer patients.

This is the first published phase III trial evaluating the benefit of an early-switch therapy strategy according to persistence of circulating tumor cells with chemotherapy.


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In this trial, the CAPPM-seq platform was used to detect circulating tumor DNA with high sensitivity and specificity in nonsmall cell lung cancer, with potential application in other malignancies.


