Review

Cancer Research

Promises and Pitfalls of Using Liquid Biopsy for Precision Medicine

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Abstract

New sensitive assays are currently available for the detection of circulating tumor DNA (ctDNA) and circulating tumor cells (CTC). However, there remains a need for standardization of preanalytical issues and cross-platform comparison studies. Liquid biopsies are being evaluated for treatment selection, for monitoring disease response and resistance, for tracking minimal residual disease, and for cancer diagnosis. Multiple studies are underway to assess the clinical utility of CTC and ctDNA in different settings (treatment-naïve vs. resistant, adjuvant vs. metastatic) and for different treatment modalities (systemic therapy, surgery, radiation therapy). This review aims to map the challenges that remain to be addressed before liquid biopsies can be widely used for cancer management.

Introduction

In the era of precision medicine, liquid biopsies are increasingly being studied as a tool that can capture tumor evolution in real time and thus guide systemic treatment. In this article, we will refer to the analysis of circulating tumor DNA (ctDNA) and circulating tumor cells (CTC) only and we will not cover other liquid biopsy biomarkers such as circulating RNAs, proteins, metabolites, and exosomes.

Sampling a patient's blood may give information about the genomic profile of a given cancer (1–3) and provide an assessment of tumor burden (4), without the need of invasive procedures. Over the last few years, several studies have been published supporting the analytical and clinical validity of CTC (5) and ctDNA assays (6) in cancer.

However, according to a recent ASCO review there is still insufficient evidence of clinical utility for the majority of ctDNA assays in advanced cancer and no evidence of clinical utility in early-stage disease or cancer screening (7). Moreover, no CTC assay is currently being used in the clinic.

Recent reviews have addressed the use of liquid biopsies (8–10), focusing especially on the technologies and their analytical and clinical validity (8). This review aims to focus more on the remaining challenges that currently prevent the use of liquid biopsies clinically (demonstration of clinical utility). To that end, we will provide an update (2) on ongoing/completed key clinical studies using CTCs and ctDNA for clinical decision (Table 1). Additionally, the main clinical applications of CTCs and ctDNA that are currently being explored are summarized in Fig. 1A.

Challenges Associated with Preanalytical Issues and the Analytical Validity of Liquid Biopsy Assays

For CTC and ctDNA assays, there is a need to standardize preanalytical variables and for cross-platform comparison studies. To address these challenges, initiatives are underway both in Europe (Cancer-ID; ref. 11) and United States (BloodPAC; ref. 12) aiming to standardize preanalytical issues and compare the performance of different liquid biopsy assays in the same patient samples.

In particular, the low concentration of CTCs and ctDNA limits the use of liquid biopsies in early-stage cancer and drawing a large volume of blood is not always clinically feasible. The addition of extra markers for CTCs, the use of implanted devices containing materials that bind ctDNA (13) or assays that can simultaneously test for multiple mutations in the same reaction (14), appear to be promising in increasing the amount of CTCs/ctDNA detected. Alternative approaches that integrate ctDNA mutations with multiple other blood-based analytes (such as exomes, CTCs, ctDNA epigenetics, metabolites) may also be required.

Clinical Applications of Liquid Biopsy in the Metastatic Disease

Treatment selection

In the metastatic setting, the only ctDNA assay that is currently used in solid tumors is the **cobas** *EGFR* Mutation Test v2. This assay is used for patients with metastatic non–small cell lung cancer (NSCLC) and can detect 42 mutations in exons 18, 19, 20, and 21 of the *EGFR* gene encompassing the *T790M*-resistant mutation. A positive finding—of an actionable mutation in plasma—using this assay can inform treatment selection relating to erlotinib and osimertinib. However, a negative result—such as the absence of the *T790M* mutation in a patient with clinical or

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Table 1. Clinical testing of liquid biopsy

Clinical trials with liquid biopsy-based patient management and translational studies of liquid biopsy in completed clinical trials				
Trial	**Disease and stage**	**Biomarker**	**No. patients enrolled in the study/ translational sub-study**	**Study design**
STIC CTC (NCT01710605) randomized phase III	MBC	CTCs	778	Physician vs. CTCs-driven choice for first-line treatment (HT vs. CT)
SWOG S0500 (NCT00382018) randomized phase III	MBC	CTCs	595	Changing therapy vs. maintaining therapy in patients with persistently increased CTCs
Treat CTC (NCT01548677) randomized phase II	EBC	CTCs	63	Adjuvant trastuzumab for 6 cycles vs. observation
PROPHECY (NCT02269982) nonrandomized, observational	mCRPC	CTCs (and ctDNA)	118	AR-V7+ CTC status as a biomarker of resistance to HT
ctDNA sub-study of SoFEA (NCT00253422) randomized phase III	LABC or MBC	ctDNA	161 (63 *ESR1*+)	Fulvestrant plus anastrozole vs. fulvestrant plus placebo vs. exemestane alone
ctDNA sub-study of PALOMA-3 (NCT01942135) randomized phase III	MBC	ctDNA	360 (91 *ESR1*+)	Fulvestrant plus palbociclib vs. fulvestrant plus placebo
ctDNA sub-study of BOLERO-2 (NCT00863655) randomized phase III	LABC or MBC	ctDNA	550 (238 *PIK3CA*+)	Exemestane plus everolimus vs. exemestane plus placebo
ctDNA sub-study of BELLE-2 (NCT01610284) randomized phase III	LABC or MBC	ctDNA	587 (200 *PIK3CA*+)	Fulvestrant plus buparlisb vs. fulvestrant plus placebo
ctDNA sub-study of SOLAR-1 (NCT02437318) randomized phase III	MBC	ctDNA	549 (186 *PIK3CA*+)	Alpelisib plus fulvestrant vs. fulvestrant plus placebo
Combined ctDNA meta-analysis of SoFEA (NCT00253422) and EFECT (NCT00065325) randomized phase III (22)	LABC or MBC	ctDNA	383 (115 *ESR1*+)	SoFEA: fulvestrant plus anastrozole vs. fulvestrant plus placebo vs. exemestane alone. EFECT: fulvestrant vs. exemestane

Abbreviations: CT, chemotherapy; EBC, early breast cancer; HT, hormone therapy; LABC, locally advanced breast cancer; MBC, metastatic breast cancer; mCRPC, metastatic castration-resistant prostate cancer.

radiological progression—should be considered inconclusive and DNA from a tumor biopsy should also be assessed (15).

The assessment of *PIK3CA* mutations in plasma cell-free DNA may be the first liquid biopsy to be used in the clinic for metastatic breast cancer (MBC). This is based on the analysis of *PIK3CA* mutations in ctDNA among 549 hormone receptor (HR)+/HER2− MBC patients enrolled in the SOLAR-1 trial (16). Progression-free survival (PFS) was significantly prolonged when the PI3KCA selective inhibitor alpelisib was added to fulvestrant in patients with detectable *PIK3CA* mutations in ctDNA. If the FDA approves alpelisib in this setting, *PIK3CA* mutation ctDNA testing will likely become a companion diagnostic test. Moreover, the presence of *PIK3CA* mutations in plasma ctDNA in patients with endocrine-resistant estrogen receptor (HR)+/HER2− advanced breast cancer, identified patients that could benefit from the combination of the pan-PI3K inhibitor buparlisib with fulvestrant (BELLE-2 trial; ref. 17). However, the clinical development of buparlisib has been discontinued for toxicity issues. The value of *PIK3CA* ctDNA genotyping has also been evaluated in other studies. In the BOLERO-2 study, the addition of everolimus to exemestane prolonged median PFS irrespective of *PIK3CA* genotype (18). In the PALOMA-3 study, *PIK3CA* ctDNA levels after 15 days treatment appear to be predictive of PFS on palbociclib and fulvestrant (19).

In patients with MBC, the use of digital PCR assays (19, 20) showed that *estrogen receptor 1* (*ESR1*) mutations in ctDNA are frequently subclonal, and occur later during metastatic aromatase inhibitor (AI) therapy. Results from retrospective ctDNA analysis in the SoFEA trial showed that *ESR1* mutation analysis in plasma might be useful to direct the choice of further endocrine-based therapy; because patients with plasma *ESR1* mutations (63 of 161) have a shorter PFS on subsequent AI-based therapy (18 patients) compared with fulvestrant (45 patients; ref. 21). The results of the above study have been recently extended in 383 patients in a combined meta-analysis of the Sofea and Efect studies (22). However, the benefit of adding CDK4/6 inhibitors to endocrine treatment was largely irrespective of the presence of *ESR1* mutations (21). As the combination of endocrine treatment with CDK4/6 inhibitors has become the new standard of care, the clinical value of *ESR1* mutation detection in patients progressing on AIs is limited to patients that will subsequently receive a second line of endocrine monotherapy without CDK4/6 inhibitors. Moreover, analyses of plasma baseline *ESR1* mutations in the BOLERO-2 trial, demonstrated that the presence of *ESR1* mutations was a marker of poor prognosis (23). In a subgroup analysis, it was suggested that patients with the *Y537S ESR1* mutation did not derive benefit from the addition of everolimus to exemestane, although these data need independent validation in larger series. Currently, there is no clear role for plasma *ESR1* mutations as a tool to guide treatment in patients with MBC.

ctDNA assays have been incorporated in trials for various solid tumors, with lung (24) and prostate cancer (25) being lead indications.

Several studies are also evaluating the role of CTC enumeration and characterization in guiding treatment decision, especially in metastatic breast and prostate cancer. The French STIC CTC study (NCT01710605; refs. 26–28), demonstrated the "noninferiority" of a CTC-based treatment decision vs. a clinician-based treatment decision, in the choice of first-line treatment (single-agent hormone therapy vs. chemotherapy) of HR+ MBC and confirmed the adverse prognostic value of baseline CTC count (27). In the CTC-driven arm, for patients that received chemotherapy because of their high CTC count ($\geq$5 CTCs) PFS was significantly longer than in the clinically-

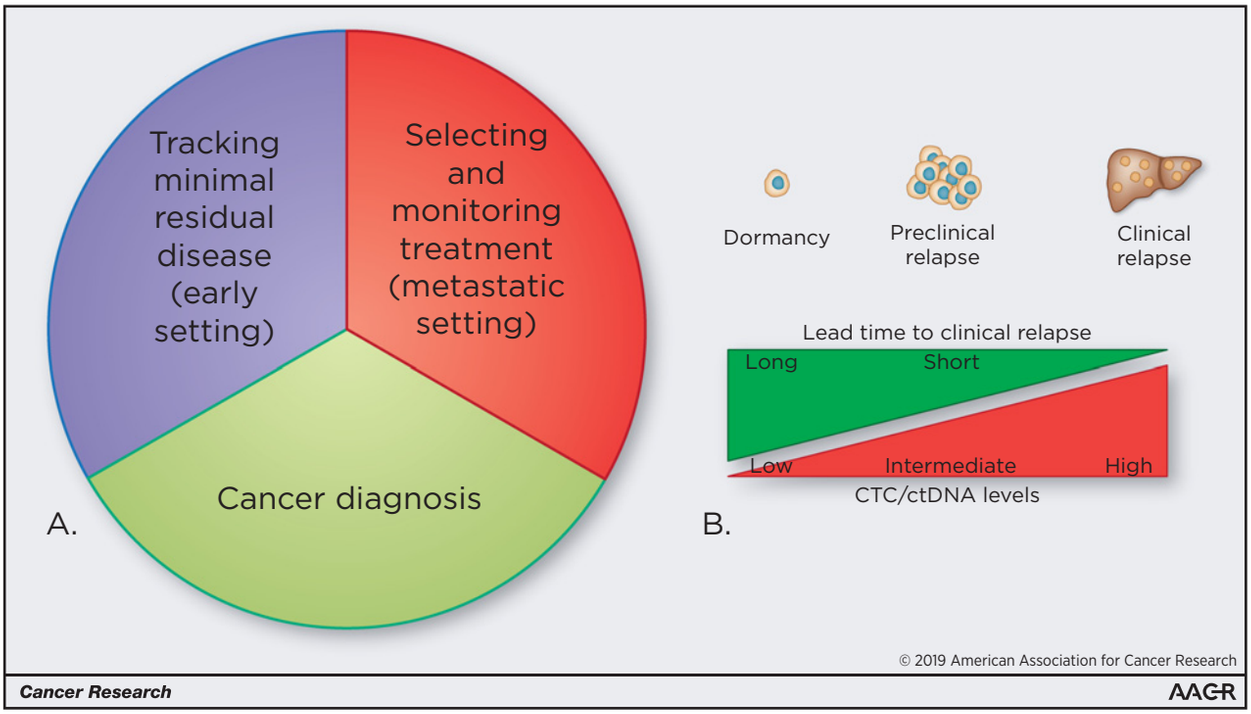

Figure 1.
A, Clinical applications of liquid biopsy. **B,** Liquid biopsy in early and metastatic breast cancer. The figure shows the inverse correlation between ctDNA/CTC levels and the lead time to clinical relapse in three different settings: (i) Dormancy (low ctDNA or CTCs levels), dormant micrometastatic cells that need another oncogenic hit or a more permissive microenvironment to give rise to metastases; long lead time to clinical relapse. (ii) Preclinical relapse (intermediate ctDNA or CTC levels), cancer cells with full malignant potential, short lead time to clinical relapse. (iii) Clinical relapse (high ctDNA or CTC levels), evidence of clinical relapse using standard imaging.

driven arm (HR 0.67; 95% CI, 0.49–0.92; $P = 0.01$; ref. 28). However, interpretation of the results of this study is challenging due to the change in the standard of care for these patients, which is currently the combination of hormone therapy with CDK4/6 inhibitors.

Beyond CTC enumeration, there is preliminary evidence that the molecular analysis of CTCs in patients with MBC with bone (29) or brain (30) metastases might help direct the choice of treatment. Finally, preliminary evidence suggests that the use of highly sensitive assays for analyzing prostate CTC-derived transcripts (31, 32) may help to guide therapies, especially in advanced prostate cancer with bone metastases that cannot be easily biopsied. Patients with prostate cancer with nuclear-localized androgen receptor splice variant 7 (AR-V7)-positive CTCs exhibited resistance to abiraterone and enzalutamide (31) and had a superior overall survival (OS) when treated with taxanes (32). In a recent consensus statement on circulating biomarkers for advanced prostate cancer (33), 31% of the experts preferred the EPIC AR-V7 CTC protein assay—laboratory-developed and validated (32, 34)—as an AR-V7 test to use in clinical practice, whereas 7.67% of the scientists chose the Hopkins/Qiagen AR-V7 RT-PCR mRNA-based assay using the AdnaTest platform (35).

Access to clinical trials through molecular screening programs. Plasma ctDNA analyses using NGS cancer gene panels (either pan-cancer or disease-specific) have been shown to be an attractive alternative to tumor tissue biopsy NGS analysis in molecular screening programs; and have the potential to increase patient access to clinical trials with new compounds (36, 37), as in the case of the basket trial SUMMIT (38).

Treatment monitoring

Alongside treatment selection, another potential application of liquid biopsies is in monitoring treatment response. This might allow for the earlier withdrawal of expensive and potentially toxic drugs when a liquid biopsy test suggests that there will be no benefit. CTC elimination after short-term drug exposure has been demonstrated to be an early response endpoint in metastatic castration-resistant prostate cancer, more reliable than PSA levels, which are affected by modulations in androgen receptor signaling (39). In the SWOG S0500 clinical trial, patients with MBC and persistently increased CTC levels after one cycle of first-line chemotherapy were shown to have a poorer outcome, although an early switch to an alternative chemotherapy regimen did not improve outcome (40). In this setting, it might be interesting to test whether a switch to a therapy targeting specific genomic aberrations, detected using a liquid biopsy test, might be a better approach instead of switching to a different chemotherapy regimen. Beyond CTC enumeration, monitoring of the CTC genomic and epigenomic profile might also better inform treatment selection. Indeed, the presence of *ESR1* methylation in CTCs from serial blood samples was shown to be associated with lack of response to

everolimus/exemestane (41). Finally, serial ctDNA evaluation can be used for treatment monitoring (4, 19).

Immune checkpoint inhibitors, such as inhibitors of programmed-death receptor 1 (PD1) or programmed death receptor ligand (PDL1) have become a standard therapy in several cancer types. Preliminary evidence suggests that longitudinal on-treatment monitoring of CTCs (42), ctDNA (43, 44), or exomal PD-L1 (45) dynamics might be used as a marker of response to identify the patients more likely to benefit from immunotherapy, thus sparing nonresponding patients from such treatment. However, definitive studies to change clinical practice are needed. Blood-based monitoring may also help decide whether to continue immunotherapy or not in cases of pseudoprogression (46).

Understanding the Mechanisms of Drug Resistance

The molecular basis of the acquired resistance to targeted therapies represents one of the main challenges in cancer research, with large implications in the clinical setting. Serial ctDNA analysis has emerged as a promising tool to identify and track the mechanisms of drug resistance (47–49). ctDNA and CTCs have been useful tools to detect multiple genomic alterations (in genes such as *ESR1*, genes of the mitogen-activated protein kinase (MAPK) pathway and the *RB1*, *T790M*, *KRAS*, and *BRAF* genes) that emerge following treatment with various therapies (AI, CDK4/6 inhibitors; osimertinib; cetuximab; BRAF inhibitors) in breast cancer (50–54), lung cancer (55), colon cancer (49), and melanoma (56, 57), respectively. CTC and ctDNA analysis may, therefore, have a complementary utility to detect mechanisms of resistance (58, 59). Results from the above studies suggest that different mechanisms of resistance can often coexist within a given patient (55), with frequent subclonal mutations (50), so targeting only one pathway might result in a transient benefit for the patient. Combination therapy might provide a better strategy to delay drug resistance in this setting.

Clinical Applications of Liquid Biopsy in the Early Disease

Liquid biopsy and systemic therapies

Neoadjuvant setting. A pooled analysis of individual patient data from more than 1,500 patients from 21 studies provided evidence that baseline CTC counts, as determined by Cellsearch, is an independent poor prognostic factor in patients with breast cancer treated with neoadjuvant chemotherapy (60).

Adjuvant setting. The purpose of administering adjuvant treatment after surgery is to eradicate tumor cells undetectable by conventional imaging called minimal residual disease (MRD). Proof-of-principle studies demonstrated that persistent detection of ctDNA after local therapy [surgery or radiotherapy (RT)] is associated with a higher risk of recurrence (14, 61). In this setting, monitoring multiple mutations per patient has been shown to improve the sensitivity for ctDNA detection (14). Moreover, it has been suggested that CTC detection before or after adjuvant chemotherapy is associated with a worse clinical outcome (62–64). Furthermore, the detection of CTCs 5 years or more after diagnosis was recently found to be associated with a higher risk of late recurrence in patients with HR+HER2− localized breast cancer (65). The correlation between ctDNA/CTCs levels and the lead time to clinical relapse is shown in Fig. 1B.

Preliminary data support the design of clinical trials for selection of adjuvant therapy based on MRD detection and characterization, in addition to characterization of the primary tumor (66). The first international trial to test this new model of drug development has been the Treat CTC trial (67). In the Treat CTC trial, we have explored the question of whether six cycles of trastuzumab can eliminate chemotherapy-resistant CTCs following standard (neo)adjuvant chemotherapy and surgery in women with HER2-non-amplified breast cancer. The trial was terminated by an independent data monitoring committee after 63 patients had been randomized because it demonstrated that trastuzumab could not eliminate CTCs in this setting. In line with the Treat CTC trial results, the NSABP B47 phase III trial including more than 3,000 patients demonstrated that 1 year of adjuvant trastuzumab did not improve invasive disease-free survival when added to standard chemotherapy in HER2-negative breast cancer (68). More examples such as the Treat CTC/NSABP B47 example are needed to provide evidence that CTCs or ctDNA elimination after short drug exposure can provide relevant information that can be used in addition to data from the activity of the drug in the metastatic and neoadjuvant setting in order to make more informed decisions before testing this drug in a large phase III adjuvant trial. Moreover, CTCs and ctDNA can be used in the future for designing trials aiming to de-escalate (in CTC/ctDNA-negative patients) or escalate (in CTCs/ctDNA-positive patients) systemic or locoregional treatment.

Liquid biopsy and radiotherapy

In early-stage cancers, the identification of patients at higher risk of recurrence who will benefit from adjuvant RT remains challenging. Promising preliminary results were reported in the recent analysis of patients with stage pT1-T2 and pN0-N1 breast cancer from the National Cancer Database and the multicenter phase III SUCCESS clinical trial (69). Adjuvant RT after breast-conserving surgery was associated with longer OS among patients of both cohorts with detectable CTCs before adjuvant therapy and was also associated with longer locoregional-free survival and disease-free survival in patients with CTCs from the SUCCESS cohort. This benefit was not observed in patients without CTCs and prospective validation of these findings is required.

Liquid biopsy and cancer diagnosis

There are efforts to explore whether ctDNA testing can be used as a tool for cancer diagnosis. For instance, the detection of circulating, cancer-derived (Epstein Barr Virus) EBV DNA in plasma has proven to be a useful screen for nasopharyngeal carcinoma in asymptomatic subjects, with high sensitivity and specificity (70). The use of ctDNA sequencing seems similarly promising in the identification of patients with somatic mutations associated with increased risks of hematologic cancer. However, caution is warranted because clonal hematopoiesis with somatic mutations was detected in 10% of elderly patients, whereas the risk of developing a hematologic malignancy was modest (1% per year; ref. 71). Moreover, new blood tests (72, 73) seem to be useful in discriminating cancer patients from healthy controls and allow for the detection of early cancers and also localize to the organ of origin such as *CancerSEEK* (72) and one further method, based on a genome-wide bisulfite sequencing of plasma DNA, that can

identify the specific methylation profiles of each tissue (73). Another approach to identify the tissue of origin has been mRNA sequencing of tumor-educated platelets (74). There are several ethical issues related to the clinical testing of a cancer diagnostic test. Such a test needs to be sensitive enough to detect surgically resectable tumors but also highly specific, since false-positive results will cause unnecessary imaging workup and severely affect quality of life. Prospective clinical trials to demonstrate the clinical utility of a liquid biopsy cancer screening test in addition to, or instead of, standard screening programs are required (75).

Conclusions and Future Perspective

There are increasing data on the analytical and clinical validity of commercially available liquid biopsy assays; however, demonstration of clinical utility is needed. To this aim, there is a need of clinical trials with inclusion criteria based on ctDNA/CTCs detection and characteristics in order to make decisions of treatment escalation or de-escalation. The detection of *PIK3CA* mutations in plasma ctDNA to guide treatment with the *PIK3CA* inhibitor alpelisib might provide the first example of a ctDNA assay with clinical utility in MBC. In the near future multigene ctDNA assays are expected to be used for guiding treatment selection in the metastatic cancer setting. Moreover, efforts should be made to validate the use of highly sensitive liquid biopsy assays (76) in the early disease setting to identify patients with MRD, who will benefit from adjuvant treatment, or those without MRD, who can be spared the toxicity of adjuvant treatment. Therefore, the development of targeted drugs designed to eliminate dormant tumor cells (77) or maintain them in a quiescent status might be an attractive perspective in order to delay/prevent the progression from MRD to overt metastases. The results from the ongoing trials are expected to improve patient outcomes and change the way we treat cancer patients.

Disclosure of Potential Conflicts of Interest

M. Ignatiadis is a consultant/advisory board member of Novartis, Pfizer, Celgene, Tesaro, and Seattle Genetics. No conflicts of interest was disclosed by the other author.

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References

1. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
2. Ignatiadis M, Lee M, Jeffrey SS. Circulating tumor cells and circulating tumor DNA: challenges and opportunities on the path to clinical utility. Clin Cancer Res 2015;21:4786–800.
3. Chu D, Park BH. Liquid biopsy: unlocking the potentials of cell-free DNA. Virchows Arch 2017;471:147–54.
4. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199–209.
5. Bidard FC, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, Mavroudis D, et al. Clinical validity of circulating tumour cells in patients with metastatic breast cancer : a pooled analysis of individual patients data. Lancet Oncol 2014;15:406–14.
6. Zill OA, Banks KC, Fairclough SR, Mortimer SA, Vowles JV, Mokhtari R, et al. The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clin Cancer Res 2018; 24:3528–38.
7. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol 2018;36:1631–41.
8. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20:71–88.
9. Bardelli A, Pantel K. Liquid biopsies, what we do not know (yet). Cancer Cell 2017;31:172–9.
10. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016;6: 479–91.
11. Cancer –ID Consortium. https://www.cancer-id.eu/.
12. The Blood Profiling Atlas in Cancer (BloodPAC) Consortium. https://www.bloodpac.org/.
13. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer 2017;17:223–38.
14. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 2017;545:446–51. Erratum in: Nature 2017 Dec 20.
15. Rolfo C, Mack PC, Scagliotti GV, Baas P, Barlesi F, Bivona TG, et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. J Thorac Oncol 2018;13:1248–68.
16. Juric D, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib + fulvestrant for advanced breast cancer: Subgroup analyses from the phase III SOLAR-1 trial. Abstract GS3-08. Forty-First Annual CTRC-AACR San Antonio Breast Cancer Symposium; December 4–8, 2018; San Antonio, TX.
17. Campone M, Im SA, Iwata H, Clemons M, Ito Y, Awada A, et al. Buparlisib plus fulvestrant versus placebo plus fulvestrant for postmenopausal, hormone receptor-positive, human epidermal growth factor receptor 2-negative, advanced breast cancer: Overall survival results from BELLE-2. Eur J Cancer 2018;103:147–54.
18. Moynahan ME, Chen D, He W, Sung P, Samoila A, You D, et al. Correlation between PIK3CA mutations in cell-free DNA and everolimus efficacy in HR+, HER2-advanced breast cancer: results from BOLERO-2. Br J Cancer 2017;116:726–30.
19. O'Leary B, Hrebien S, Morden JP, Beaney M, Fribbens C, Huang X, et al. Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nat Commun 2018;9:896. doi: 10.1038/s41467-018-03215-x.
20. Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, Tarazona N, et al. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. Sci Transl Med 2015; 7:313ra182.
21. Fribbens C, O'Leary B, Kilburn L, Hrebien S, Garcia-Murillas I, Beaney M, et al. Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast cancer. J Clin Oncol 2016;34:2961–8.
22. Turner N, Swift C, Kilburn L, Garcia-Murillas I, Johnston S, Budzar A, et al. Baseline circulating ESR1 mutation analysis in the randomised phase III EFECT study of fulvestrant versus exemestane in advanced hormone receptor positive breast cancer. Abstract PD2-04. Forty-First Annual CTRC-AACR San Antonio Breast Cancer Symposium; December 4–8, 2018; San Antonio, TX.
23. Chandarlapaty S, Chen D, He W, Sung P, Samoila A, You D, et al. Prevalence of ESR1 mutations in cell-free DNA and outcomes in metastatic breast cancer: a secondary analysis of the BOLERO-2 clinical trial. JAMA Oncol 2016;2:1310–5.
24. Remon J, Menis J, Hasan B, Peric A, De Maio E, Novello S, et al. The APPLE trial: feasibility and activity of AZD9291 (osimertinib) treatment on

positive plasma T790M in EGFR-mutant NSCLC patients. EORTC 1613. Clin Lung Cancer 2017;18:583–8.
25. Carneiro BA, Pamarthy S, Shah AN, Sagar V, Unno K, Han H, et al. Anaplastic lymphoma kinase mutation (ALK F1174C) in small cell carcinoma of the prostate and molecular response to alectinib. Clin Cancer Res 2018;24:2732–9.
26. Pierga J-Y, Baffert S, Hajage D, Brain E, Armanet S, Simondi C, et al. Abstract 2410: circulating tumor cells to guide the choice between chemotherapy and hormone therapy as first line treatment for metastatic breast cancer patients: the STIC CTC METABREAST trial. Cancer Res 2013;(8 Suppl):2410.
27. Bidard F-C, Brain E, Jacot W, Bachelot T, Ladoire S, Bourgeois H, et al. First line hormone therapy vs. chemotherapy for HR+ HER2− metastatic breast cancer in the phase III STIC CTC trial: clinical choice and validity of CTC count. Ann Oncol, 2016;27(suppl_6):1, 226PD.
28. Bidard F-C, Jacot W, Dureau S, Brain E, Bachelot T, Bourgeois H, et al. Clinical utility of circulating tumor cell count as a tool to chose between first line hormone therapy and chemotherapy for ER+ HER2− metastatic breast cancer: Results of the phase III STIC CTC trial. Abstract GS3-07 and oral presentation. Forty-First Annual CTRC-AACR San Antonio Breast Cancer Symposium; December 4–8, 2018; San Antonio, TX.
29. Aceto N, Bardia A, Wittner BS, Donaldson MC, O'Keefe R, Engstrom A, et al. AR expression in breast cancer CTCs associates with bone metastases. Mol Cancer Res 2018;16:720–7.
30. Boral D, Vishnoi M, Liu HN, Yin W, Sprouse ML, Scamardo A, et al. Molecular characterization of breast cancer CTCs associated with brain metastasis. Nat Commun 2017;8:196. doi: 10.1038/s41467-017-00196-1.
31. Miyamoto DT, Lee RJ, Kalinich M, LiCausi JA, Zheng Y, Chen T, et al. An RNA-based digital circulating tumor cell signature is predictive of drug response and early dissemination in prostate cancer. Cancer Discov 2018;8:288–303.
32. Scher HI, Graf RP, Schreiber NA, Jayaram A, Winquist E, McLaughlin B, et al. Assessment of the validity of nuclear-localized androgen receptor splice variant 7 in circulating tumor cells as a predictive biomarker for castration-resistant prostate cancer. JAMA Oncol 2018;4:1179–86.
33. Sumanasuriya S, Omlin AG, Armstrong AJ, Attard G, Chi KN, Bevan CL, et al. Consensus statement on circulating biomarkers for advanced prostate cancer. JCO 2018;36:6_suppl:299–299.
34. Werner SL, Graf RP, Landers M, Valenta DT, Schroeder M, Greene SB, et al. Analytical validation and capabilities of the epic CTC platform: enrichment-free circulating tumour cell detection and characterization. J Circ Biomark 2015;4:3. doi: 10.5772/60725.
35. Lokhandwala PM, Riel SL, Haley L, Lu C, Chen Y, Silberstein J, et al. Analytical validation of androgen receptor splice variant 7 detection in a clinical laboratory improvement amendaments (CLIA) laboratory setting. J Mol Diagn 2017;19:115–25.
36. Rolfo C, Manca P, Salgado R, Van Dam P, Dendooven A, Machado Coelho A, et al. Multidisciplinary molecular tumour board: a tool to improve clinical practice and selection accrual for clinical trials in patients with cancer. ESMO Open 2018;3:e000398.
37. Rothé F, Laes JF, Lambrechts D, Smeets D, Vincent D, Maetens M, et al. Plasma circulating tumor DNA as an alternative to metastatic biopsies for mutational analysis in breast cancer. Ann Oncol 2014;25:1959–65.
38. Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018;554:189–94.
39. Heller G, McCormack R, Kheoh T, Molina A, Smith MR, Dreicer R, et al. Circulating tumor cells number as a response measure of prolonged survival for metastatic castration-resistant prostate cancer: a comparison with prostate-specific antigen across five randomized phase III clinical trials. J Clin Oncol 2018;36:572–80.
40. Smerage JB, Barlow WE, Hortobagyi GN, Winer EP, Leyland-Jones B, Srkalovic G, et al. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. J Clin Oncol 2014;32:3483–9.
41. Mastoraki S, Strati A, Tzanikou E, Chimonidou M, Politaki E, Voutsina A, et al. ESR1 methylation: a liquid biopsy-based epigenetic assay for the follow-up of patients with metastatic breast cancer receiving endocrine treatment. Clin Cancer Res 2018;24:1500–10.
42. Hong X, Sullivan RJ, Kalinich M, Kwan TT, Giobbie-Hurder A, Pan S, et al. Molecular signatures of circulating melanoma cells for monitoring early response to immunecheckpoint therapy. Proc Natl Acad Sci U S A 2018;115:2467–72.
43. Cabel L, Riva F, Servois V, Livartowski A, Daniel C, Rampanou A, et al. Circulating tumor DNA changes for early monitoring of anti-PD1 immunotherapy: a proof-of-concept study. Ann Oncol 2017;28:1996–2001.
44. Lee JH, Long GV, Boyd S, Lo S, Menzies AM, Tembe V, et al. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. Ann Oncol 2017;28:1130–6.
45. Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exomal PD-L1 contributes to immunosoppression and is associated with anti-PD-1 response. Nature 2018;560:382–6.
46. Lee JH, Long GV, Menzies AM, Lo S, Guminski A, Whitbourne K, et al. Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti-programmed cell death 1 antibodies. JAMA Oncol 2018;4:717–21.
47. Murtaza M, Dawson SJ, Tsui DW, Gale D, Forshew T, Piskorz AM, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013;497:108–12.
48. Weigelt B, Comino-Méndez I, de Bruijn I, Tian L, Meisel JL, García-Murillas I, et al. Diverse BRCA1 and BRCA2 reversion mutations in circulating cell-free DNA of therapy-resistant breast or ovarian cancer. Clin Cancer Res 2017;23:6708–20.
49. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012;486:532–6.
50. Fribbens C, Garcia Murillas I, Beaney M, Hrebien S, O'Leary B, Kilburn L, et al. Tracking evolution of aromatase inhibitor resistance with circulating tumour DNA analysis in metastatic breast cancer. Ann Oncol 2018;29:145–53.
51. Condorelli R, Spring L, O'Shaughnessy J, Lacroix L, Bailleux C, Scott V, et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann Oncol 2018;29:640–5.
52. Paoletti C, Cani AK, Larios JM, Hovelson DH, Aung K, Darga EP, et al. Comprehensive mutation and copy number profiling in archived circulating breast cancer tumor cells documents heterogeneous resistance mechanisms. Cancer Res 2018;78:1110–22.
53. Razavi P, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2018;34:427–38.e6.
54. O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018;8:1390–403.
55. Oxnard GR, Hu Y, Mileham KF, Husain H, Costa DB, Tracy P, 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;4:1527–34.
56. Sullivan RJ, O'Neill VJ, Brinkmann K, Enderle D, Koestler T, Spiel A, et al. Plasma-based monitoring of BRAF mutations during therapy for malignant melanoma (MM) using combined exosomal RNA and cell-free DNA analysis. J Clin Oncol 33;15_suppl:9017–9017.
57. Schreuer M, Meersseman G, Van Den Herrewegen S, Jansen Y, Chevolet I, Bott A, et al. Quantitative assessment of BRAF V600 mutant circulating cell-free tumor DNA as a tool for therapeutic monitoring in metastatic melanoma patients treated with BRAF/MEK inhibitors. J Transl Med 2016;14:95. doi: 10.1186/s12967-016-0852-6.
58. Paoletti C, Schiavon G, Dolce EM, Darga EP, Carr TH, Geradts J, et al. Circulating biomarkers and resistance to endocrine therapy in metastatic breast cancers: correlative results from AZD9496 oral SERD Phase I trial. Clin Cancer Res 2018;24:5860–72.
59. Jordan NV, Bardia A, Wittner BS, Benes C, Ligorio M, Zheng Y, et al. HER2 expression identifies dynamic functional states within circulating breast cancer cells. Nature 2016;537:102–6.
60. Bidard FC, Michielis S, Riethdorf S, Mueller V, Esserman LJ, Lucci A, et al. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis. J Natl Cancer Inst 2018;110:560–7.
61. Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015;7:302ra133.
62. Ignatiadis M, Xenidis N, Perraki M, Apostolaki S, Politaki E, Kafousi M, et al. Different prognostic value of cytokeratin-19 mRNA positive circulating

tumor cells according to estrogen receptor and HER2 status in early-stage breast cancer. J Clin Oncol 2007;25:5194–202.
63. Rack B, Schindlbeck C, Jückstock J, Andergassen U, Hepp P, Zwingers T, et al. Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. J Natl Cancer Inst 2014; 106:pii: dju066.
64. Janni WJ, Rack B, Terstappen LW, Pierga JY, Taran FA, Fehm T, et al. Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. Clin Cancer Res 2016;22:2583–93.
65. Sparano J, O'Neill A, Alpaugh K, Wolff AC, Northfelt DW, Dang CT, et al. Association of circulating tumor cells with late recurrence of estrogen receptor–positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol 2018;4:1700–6.
66. Luskin MR, Murakami MA, Manalis SR, Weinstock DM. Targeting minimal residual disease: a path to cure? Nat Rev Cancer 2018;18: 255–63.
67. Ignatiadis M, Litière S, Rothe F, Riethdorf S, Proudhon C, Fehm T, et al. Trastuzumab versus observation for HER2 nonamplified early breast cancer with circulating tumor cells (EORTC 90091-10093, BIG 1-12, Treat CTC): a randomized phase II trial. Ann Oncol 2018;29:1777–83.
68. Fehrenbacher L, Cecchini RS, Geyer CE, Rastogi P, Costantino JP, Atkins JN, et al. Abstract GS1-02: NSABP B-47 (NRG oncology): Phase III randomized trial comparing adjuvant chemotherapy with adriamycin (A) and cyclophosphamide (C) → weekly paclitaxel (WP), or docetaxel (T) and C with or without a year of trastuzumab (H) in women with node-positive or high-risk node-negative invasive breast cancer (IBC) expressing HER2 staining intensity of IHC 1+ or 2+ with negative FISH (HER2-Low IBC). Cancer Res 2018;(4 Suppl):GS1–02.
69. Goodman CR, Seagle BL, Friedl TWP, Rack B, Lato K, Fink V, et al. Association of circulating tumor cell status with benefit of radiotherapy and survival in early-stage breast cancer. JAMA Oncol 2018;4:e180163.
70. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, Chan SL, et al. Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med 2017;377:513–22. Erratum in: N Engl J Med. 2018 Mar 8;378: 973.
71. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477–87.
72. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359:926–30.
73. Sun K, Jiang P, Chan KC, Wong J, Cheng YK, Liang RH, et al. Plasma DNA tissue mapping by genome-wide methylation sequencing for noninvasive prenatal, cancer, and transplantation assessments. Proc Natl Acad Sci U S A 2015;112:E5503–12.
74. Best MG, Sol N, Kooi I, Tannous J, Westerman BA, Rustenburg F, et al. RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. Cancer Cell 2015;28: 666–76.
75. Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell 2017;168:571–4.
76. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease – latest advances and implications for cure. Nat Rev Clin Oncol 2019. doi: 10.1038/s41571-019-0187-3. Epub ahead of print.
77. Pantel K, Hayes DF. Disseminated breast and tumour cells: biological and clinical meaning. Nat Rev Clin Oncol 2018:15:129–31.