The promise of circulating tumor cells for precision cancer therapy

The rapidly growing array of therapeutic options in cancer requires informative biomarkers to guide the rational selection and precision application of appropriate therapies. Circulating biomarkers such as circulating tumor cells have immense potential as noninvasive, serial 'liquid biopsies' that may be more representative of the complete spectrum of a patient's individual malignancy than spatially and temporally restricted tumor biopsies. In this review, we discuss the current state-of-the-art in the isolation and molecular characterization of circulating tumor cells as well as their utility in a wide range of clinical applications such as prognostics, treatment monitoring and identification of novel therapeutic targets and resistance mechanisms to enable real-time adjustments in the clinical management of cancer.

First draft submitted: 15 July 2016; Accepted for publication: 27 September 2016; Published online: 7 December 2016

Keywords: cancer • circulating tumor cells (CTCs) • precision therapy • predictive marker • prognostic marker

The evolving landscape of cancer therapy creates an urgent need for minimally invasive biomarkers to facilitate early detection, prognosis and prediction of therapeutic response and resistance to enable highly adaptable, real-time precision therapy [1]. The conventional gold standard of using single biopsies of the primary tumor to inform all downstream therapeutic decisions may no longer be adequate given increasing evidence of significant spatial and temporal heterogeneity in tumors [2], especially when placed under the selection pressure of various treatments. Moreover, serial biopsies are often impractical, morbid and technically challenging.

In this review, we focus on the emerging role of circulating tumor cells (CTCs), which can be serially obtained from minimally invasive blood draws or 'liquid biopsies'. CTCs are cancer cells that have been shed into the peripheral circulation from primary or metastatic tumors [3,4]. The first reported obser-

vation of CTCs was in 1869 by Australian pathologist Thomas Ashworth at the autopsy of a patient with metastatic cancer [5]. By comparing the morphology of circulating cells with those from different cancerous lesions, Ashworth came to the prescient conclusion that 'cells identical with those of the cancer itself being seen in the blood may tend to throw some light upon the mode of origin of multiple tumors existing in the same person' [5]. Indeed, molecular analyses of CTCs may be superior to individual tumor biopsies because these circulating cells likely provide a sampling of different regions of the primary tumor as well as metastatic deposits, and therefore are more likely to capture the genetic heterogeneity of a patient's cancer.

While cell-free circulating tumor DNA (ctDNA) is an important, complementary biomarker to CTCs, it has been reviewed extensively elsewhere and will not be discussed in this review [4,6]. Moreover, although ctDNA may in some cases be more reliably

William L Hwang^{1,2}, Katie L Hwang^{2,3} & David T Miyamoto^{*1,2}

¹Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA 02114, USA

²Massachusetts General Hospital Cancer Center, Boston, MA 02114, USA

³Medical Scientist Training Program, Harvard Medical School, Boston, MA 02115, USA

*Author for correspondence:

Tel.: +1 617 726 5866

Fax: +1 617 726 3603

dmiyamoto@mgh.harvard.edu

Future
Medicine

part of
fsg

isolated from patients than CTCs [7], they are largely limited to genomic mutational analysis. In contrast, CTC analysis can provide a wealth of other information including cell morphology, immunocytochemical phenotype, presence of important epitopes, presence of multiple mutations within a single cell to decode tumor heterogeneity and map clonal evolution, and genomic mutational analysis combined with epigenetic/transcriptomic/proteomic/metabolomic profiling. CTC analyses may also enable determination of the functional relevance of novel targets for therapeutic inhibition, and provide an unprecedented opportunity to dissect the biology of metastasis. Additional emerging blood-based biomarkers such as exosomes and platelets are discussed elsewhere and are also beyond the scope of this review [8–10].

Biology of CTCs

CTCs originate from cells within primary or metastatic tumors that acquire the ability to invade through the basement membrane and tissue stroma to enter the bloodstream/lymphatics, a process that is hypothesized to involve epithelial-to-mesenchymal transition (EMT) [11]. Furthermore, only a subset of CTCs have the tumorigenic potential to form distant metastatic deposits [12], which are ultimately responsible for up to 90% of cancer deaths [13]. Understanding the biology of CTCs can provide insights into the evolution of malignancy, development of metastases and identification of exploitable targets for treatment.

Epithelial–mesenchymal transition & metastasis

EMT is a normal process in embryogenesis and wound healing, but in cancer, it is a process whereby epithelial tumor cells are believed to lose their apical-basal polarization and cell–cell adhesions to take on a mesenchymal phenotype with a motile cytoskeleton that enables them to invade through adjacent tissue [14]. Epithelial markers include epithelial cell adhesion molecule (EPCAM) and cytokeratins, whereas mesenchymal markers include vimentin, N-cadherin and O-cadherin, among others. The reason that positive selection approaches for CTC capture generally use epithelial markers is because mesenchymal markers such as vimentin are also expressed on blood cells.

CTCs expressing mesenchymal markers are more frequently found in advanced cancers than early-stage disease, indicating a likely association between EMT and disease progression [15]. Furthermore, a spectrum of breast cancer CTCs spanning a mixed epithelial–mesenchymal phenotype was identified by RNA FISH, with an increase in the representation of the mesenchymal phenotype during disease progression and a decrease during disease regression, further

suggesting mesenchymal CTCs are associated with metastatic progression [16]. Interestingly, metastases often resemble the predominantly epithelial primary tumor, implying that cells may transition from epithelial to mesenchymal phenotypes to migrate, but then revert back to an epithelial state at distant sites (mesenchymal–epithelial transition), which recapitulates the process in organ development [17,18]. Tumor cells that have undergone partial EMT (intermediate phenotype) may have the highest plasticity to adapt to the conditions present at secondary sites [19].

However, it is not as simple as concluding that mesenchymal-type CTCs all have the capability of generating metastases [20]. Indeed, it has been hypothesized that only a small fraction of tumor cells have metastatic capability–adaptations to survive passage through the circulation and generate metastases [21,22]. This subset of cells likely overlaps with cancer stem cells, which have properties of adult stem cells (self-renewal and multipotency), as well as a mesenchymal phenotype as discussed previously, and are capable of initiating tumors when injected into immunocompromised mice [23–25]. These metastatic stem and mesenchymal-like cells should be represented in the CTC population, and identifying and studying their properties in isolation, culture and preclinical animal models would enable a better understanding of these unique cancer-driving cells and increase the odds of eradicating them.

Blood samples depleted of leukocytes from metastatic breast cancer patients have been used to generate CTC-derived xenografts in immunocompromised mice, but the process is highly inefficient. In one study, xenografts with multiorgan metastases were only successful when there were >1000 CTCs per 7.5 ml of blood, measured using the CellSearch® platform (Janssen Diagnostics, Raritan, NJ, USA; discussed in detail below) [26]. This was the first confirmation of a tumorigenic subpopulation of CTCs, which by flow cytometry was characterized as CD45⁺ EPCAM⁺ CD44⁺ CD47⁺ c-MET⁺ [26]. Another similar study on breast cancer uncovered EPCAM⁺ CTCs with tumorigenic properties, causing brain metastases [27]. A group from Manchester, UK recently created a non-small-cell lung cancer (NSCLC) CTC-derived xenograft using blood samples that were devoid of CTCs when using the CellSearch platform; size-based CTC enrichment revealed abundant heterogeneous CTCs, of which 80% expressed the mesenchymal marker vimentin, demonstrating that the absence of epithelial CTCs does not preclude the generation of CTC-derived xenografts [28]. Using an orthotopic mouse model of colorectal cancer that reliably produces metastases and CTCs, Scholch and colleagues cultured the CTCs and

demonstrated their tumorigenic capacity upon re-injection into mice [12]. Reverse transcription PCR expression profiling of these CTCs revealed reduced expression of cell–cell adhesion genes such as *claudin-7* and *CD166*, and increased expression of stem cell markers such as *DLG7* and *BM11* relative to bulk tumor cells derived from hepatic metastases.

Circulating tumor microemboli (CTM) are defined as a cluster of three or more CTCs that circulate together [29]. The presence of heterotypic tumor stroma (fibroblasts, platelets, leukocytes, endothelial cells, pericytes) increases viability of tumor cells within CTM and confers an early growth advantage to metastatic colonies [30], which is supported by the observation that CTM injected into mice have a higher probability of seeding distant metastases than single CTCs [31–33]. Lung cancer CTCs clustered as CTM were found to be nonproliferative (based on absence of Ki-67 expression), but up to 62% of single cells had high Ki-67 expression, suggesting that tumor cells within CTM are in a state of cell-cycle arrest, which may contribute to chemotherapy resistance and enhanced cell survival, resulting in worse prognosis [29].

Recent genomic studies challenge the conventional model that each metastasis arises from a single malignant cell, and instead reveal that metastases can be composed of multiple genetically distinct clones [33–35]. Cheung and colleagues used multicolor lineage tracing to demonstrate that polyclonal seeding by CTM is a frequent mechanism in a breast cancer mouse model, accounting for >90% of metastases [36]. Expression of the epithelial cytoskeletal protein, keratin 14 (K14) plays a critical role in this process since depletion of K14 abrogates distant metastases, suggesting that K14⁺ epithelial tumor cell clusters disseminate collectively to colonize distant organs. In an elegant study from the University of California San Francisco, Headley and colleagues developed an intravital two-photon lung imaging model in mice to directly observe the arrival of CTCs and subsequent host immune interaction [37]. They found that tumor microemboli form in the capillaries within minutes of CTC arrival and then remain attached to the lung vasculature or migrate along the vessels. Interestingly, waves of different myeloid cell derivatives were seen to interact with these CTC microemboli, ingesting them and accumulating in the lung interstitium. While recruited neutrophils and monocytes/macrophages are important to establishing the early metastatic niche, the less abundant lung dendritic cells mediate potent antimetastatic effects.

Evolution & heterogeneity

Reliable detection of mutations plays an important role in personalized therapy selection, since mutations

in genes encoding therapeutic targets or downstream signaling proteins can affect drug efficacy. For example, mutations in EGFR affect anti-EGFR therapy in lung cancer and mutations in KRAS, a protein downstream of EGFR, block efficacy of anti-EGFR therapy in colorectal cancer [38,39]. It can be informative to compare CTC and matched biopsy profiles to determine the degree of overlap or disparity. Furthermore, sequencing analysis of single CTCs circumvents leukocyte contamination and can potentially provide a more global view of cancer heterogeneity than spatially limited biopsies. Single-cell DNA sequencing of CTCs has revealed intra- and inter-patient genetic heterogeneity as well as discordance with primary tumors, metastatic deposits and changes after treatment [38,40–41]. However, most copy number variations and mutations found in CTCs (>80%) can be traced back to either primary or metastatic tumors [42,43]. These initial findings suggested that serial CTC analyses during cancer treatment could potentially guide real-time precision therapy by providing a snapshot of the genetic evolution of a patient's particular cancer and allowing identification of genetic vulnerabilities. For example, the EGFR T790M mutation is acquired by some EGFR-mutant NSCLC as they become resistant to selective tyrosine kinase inhibitors (TKIs), making the newer third-generation TKIs necessary. Sundaresan and colleagues found that combined CTC- and ctDNA-based genotyping detected T790M in 35% of patients in whom concurrent biopsy was negative or indeterminate [44], thereby identifying a population of patients in whom liquid biopsies demonstrably improved real-time precision therapy.

To elucidate the phenotypic diversity of CTCs with regard to differential gene expression profiles, several groups have undertaken single-cell RNA sequencing [45–49]. Powell and colleagues identified two major CTC subgroups in breast cancer patients, one with elevated expression of metastasis-associated genes and the other with increased expression of EMT-associated genes [45]. Using single-cell transcriptomics, Miyamoto and colleagues identified two androgen receptor (AR) independent mechanisms for antiandrogen resistance in prostate cancer: noncanonical Wnt signaling and activation of glucocorticoid receptor signaling [49,50]. Additional examples will be discussed in the 'Clinical application' section.

Capture & characterization of CTCs

The reliable capture of CTCs from whole blood is challenging due to their scarcity, estimated at approximately one CTC per 10⁷ leukocytes per ml of blood [18]. Numerous approaches for their isolation have been devised, which can be loosely grouped into the fol-

lowing categories: immunomagnetic capture (both positive and negative selection); physical separation; high-throughput microscopy; and functional selection [51–53] (Figure 1). The sensitivity and specificity of CTC detection vary for different methods. The most commonly used blood volume in clinical CTC studies is 7.5 ml. Assuming an underlying Poisson distribution, the probability of detecting at least 1 CTC in 7.5 ml of whole blood from a patient with a total of 500 CTCs is approximately 50% [54].

Recently, integrated microfluidic platforms with increased automation incorporating sample preparation and image processing with immunohistochemistry, flow cytometry or DNA/RNA FISH have been reported [55]. While technologies designed to isolate single CTCs can often detect CTMs as well, there have also been efforts toward the specific capture of CTMs [56].

Immunomagnetic capture

Enrichment of CTCs by immunomagnetic capture is the most established strategy. The paradigm of this approach is CellSearch, which uses ferromagnetic beads coated with anti-EpCAM antibodies to enrich for EpCAM-expressing epithelial-type CTCs and then performs a second selection step based on morphology, expression of cytokeratins and absence of leukocyte common antigen (CD45) [57]. To date, CellSearch is the only CTC-capture system that has been extensively validated and deployed in large clinical trials, leading to US FDA clearance [58] as a prognostic tool in metastatic breast, prostate and colorectal cancer [59–61]. Other platforms utilizing EpCAM positive selection of CTCs have been developed including magnetic rods [62], a medical wire that enriches CTCs directly from a peripheral vein [63] and microfluidic chips [64–67]. Non-EpCAM positive selection (e.g., cytokeratins, EGFR, CD19) and negative selection (e.g., CD45) techniques have been demonstrated [66,68–70]. Capturing CTCs using rationally designed aptamer cocktails in a microfluidic platform was recently described [71]. Despite the successful and varied implementation of immunomagnetic capture, the common reliance on expression of epithelial markers such as EpCAM and cytokeratins omits mesenchymal-type CTCs, which are thought to be fundamentally important for metastasis. Hence, there has recently been increased interest in technologies that utilize marker-independent isolation methods.

Physical separation

The primary advantage of label-free approaches is avoiding the selective enrichment of CTC subgroups with particular immunocytochemical features, which

enables isolation of nonepithelial CTCs. Several physical differences between CTCs and leukocytes can be exploited. Size-based filtration harnesses the fact that CTCs tend to be larger than leukocytes [72–74]. CTCs typically have higher membrane capacitance than leukocytes, in large part because of greater total surface area, allowing for their isolation by dielectrophoresis field-flow fractionation [75]. Finally, CTCs are more rigid than leukocytes because of increased nuclear to cytoplasmic ratio (as indicated by the length of time required for them to pass through a microfluidic constriction); hence, using tapered microfluidic constrictions and diagonal oscillatory flow, the resultant ratcheting effect produces distinct flow paths for CTCs, leukocytes and erythrocytes (Figure 1) [76,77].

High-throughput microscopy

Another unbiased, label-free approach has been developed by Epic Sciences (CA, USA) in which CTCs undergo no enrichment other than erythrocyte lysis, are plated on multiple slides, and then identified against a background of excess leukocytes by high-throughput immunofluorescence microscopy [78–80] (Figure 1). This platform was recently used by Scher and colleagues to demonstrate that CTC expression of AR-V7 in patients with metastatic castrate-resistant prostate cancer is associated with longer survival on taxane chemotherapy as opposed to AR-targeted therapies [81].

Functional selection

The Vita-Assay™ (Vitalex, NY, USA) method identifies CTCs based on preferential adhesion of invasive CTCs to a tissue or tumor microenvironment mimic (cell adhesion matrix or CAM), which has been shown to enrich viable CTCs from blood up to 1,000,000-fold [82,83]. Moreover, CAM-captured CTCs can ingest the CAM itself such that the use of fluorescent-labeled CAM allows for direct visualization of cancer cell invasion (Figure 1).

For additional information on the various approaches available for the capture and analysis of CTCs, we refer the reader to comprehensive reviews on this topic [3,52–53,84].

Clinical application of CTCs

Among the many potential clinical applications for CTCs, we will focus on early detection; prognosis and treatment decisions based on CTC enumeration and molecular characterization; monitoring the efficacy of treatment; dynamic identification of therapeutic targets and resistance mechanisms to make real-time changes in clinical management; and direct targeting of CTCs as a therapeutic strategy to eliminate the putative metastatic subpopulation (Figure 2).

Figure 1. Capture of circulating tumor cells. Circulating tumor cells (CTCs) can be enriched from blood samples, or 'liquid biopsies' based on their biological and physical properties. (Left panel) Immunomagnetic capture utilizes antibodies against specific CTC surface markers such as epithelial cell adhesion molecule (EpCAM). (Left-middle panel) Separation of CTCs from other blood cells can also be accomplished through differences in physical properties, such as size, deformability and capacitance. Application of an oscillating flow gradient across a microfluidic chip with tapered constrictions separates cell types based on rigidity. (Right-middle panel) High-throughput microscopy of nucleated blood cells with fluorescent antibody staining allows for CTCs to be detected and isolated. (Right panel) Functional separation of CTCs can be achieved based on their viability in selective cell culture conditions or ability to attach to and invade extracellular matrix. In the Vita-Assay™ method depicted, CTCs preferentially attach to and ingest a fluorescently labeled cell adhesion matrix (CAM), which enables direct visualization by fluorescent microscopy.

Early detection

While early detection of cancer by CTCs is hampered by limited sensitivity and specificity, there have been some successful applications to date, and more are anticipated with improvements in capture and detection technologies discussed above. For example, CTCs were found in 3% of chronic obstructive pulmonary disease patients without clinically detectable lung cancer compared with 0% of healthy and smoking controls [85]. These CTC-positive individuals were

monitored annually by low-dose spiral CT, and lung nodules were detected 1–4 years after CTC detection, leading to prompt surgical resection and histopathologic diagnosis of early-stage lung cancer. The Breast Early Screening Test study obtained 8 ml of blood in 144 women presenting for breast biopsy due to a mammogram BIRADS score (breast imaging reporting and data system) of at least 3, and CTC detection was performed with antimammoglobin immunocytochemistry (positive if at least one CTC

Figure 2. Clinical application of circulating tumor cells. The broad range of applications for circulating tumor cells (CTCs) in clinical decision making are illustrated schematically. Multidimensional molecular characterization of CTCs (outer ring) enables a cycle of utilizing CTCs for early detection, prognosis, prediction of targets/resistance, monitoring of treatment response and detection of tumor evolution/recurrence (inner ring).

detected) [86]. While the CTC screening test detected 87% of invasive cancers, it only detected 50% of *in situ* cancers and none of the intraductal cancers.

Prognosis

Many studies have demonstrated that enumeration of CTCs using the FDA-cleared CellSearch platform before, during and after various systemic therapies has prognostic value for a wide range of malignancies. The majority of studies have focused on the metastatic setting, due to the overall higher yield of CTCs [87]. CTCs above a certain threshold (typically on the order of one to five CTCs per 7.5 ml of blood, except 50 CTCs per 7.5 ml of blood for small-cell lung cancer) at any time point or the lack of a sufficient decrease in CTCs after treatment were associated with worse prognosis in terms of progression-free survival (PFS) and overall survival (OS) for metastatic breast cancer [59,88–91], prostate cancer [60,92–93], colorectal cancer [61], NSCLC [94], small-cell lung cancer (SCLC) [29,95], gastric cancer [96] and neuroendocrine tumors [97] among others. In many cases, CTCs are a superior prognostic indicator to conventional tumor markers. For example, CTC enumeration improved the prognostication of metastatic breast cancer when added to full clinicopathologic predictive models, whereas serum tumor markers (CEA, CA15–3) did not, despite the fact that

these serum markers are frequently used in clinical practice [98]. The presence of CTM may portend an even worse prognosis compared with the presence of CTCs alone [99,100].

More recently, the improvement in detection technologies has shifted more attention to localized cancers. The presence of any CTCs (≥ 1) is associated with unfavorable outcomes in a number of primary malignancies, including breast cancer [90,101–103], bladder cancer [104,105], liver cancer [106], NSCLC [107], head and neck squamous cell carcinoma (HNSCC) [108] and cholangiocarcinoma [109]. In contrast, results to date are inconsistent for a handful of other localized malignancies such as prostate cancer [110,111] and colorectal cancer [112,113]. Another anticipated use of CTC enumeration is in the selection of patients with early-stage cancer who are most likely to benefit from adjuvant therapy.

Molecular analyses of CTCs and CTM can yield distinct prognostic information beyond enumeration alone. Bulfoni and colleagues identified four CTC subpopulations in breast cancer patients: epithelial, epithelial–mesenchymal, mesenchymal and negative [114]. The presence of CTCs coexpressing epithelial and mesenchymal markers was associated with significantly poorer PFS and OS [114]. In metastatic melanoma, the presence of CTM with high expres-

sion of SOX10, CD100 and TRF2 was an independent predictor of shorter survival from time of diagnosis regardless of treatment strategy [115]. Vimentin- and Ki67-positive CTCs are associated with increased mortality in metastatic castration resistant prostate cancer (mCRPC) [116]. Moreover, the combination of stem-like gene expression and CTC enumeration improved prediction of docetaxel treatment efficacy and OS in mCRPC patients [117]. Barbazan and colleagues found that overexpression of nonreceptor guanine nucleotide exchange factors in CTCs isolated from metastatic colorectal cancer was associated with a shorter PFS, implying that hyperactivation of G-protein signaling may be a critical event during metastatic progression [118]. Interestingly, the presence of epithelial-like but not mesenchymal-like CTCs correlated with poorer survival in pancreatic adenocarcinoma; moreover, CTCs with partial EMT phenotype (cytokeratin and vimentin positive) were associated with earlier recurrence [119].

Treatment monitoring

CTC counts have also been used as a surrogate marker of efficacy in clinical trials. For example, a Phase I study of LY2606368, a checkpoint kinase 1 inhibitor, in patients with advanced solid tumors, used CTCs as a measure of pharmacodynamics [120]. In another Phase I study investigating apatersen, an antisense inhibitor targeting heat shock protein 27, in mCRPC and other advanced cancers, CTC enumeration was used as evidence of single-agent activity [121]. Meulendijks and colleagues undertook a multicenter, Phase II study on the feasibility of bevacizumab, docetaxel, oxaliplatin, and capecitabine (B-DOC) chemotherapy for patients with advanced HER2-negative, previously untreated, gastric or gastroesophageal adenocarcinoma and found that CTC enumeration was prognostic in patients treated with B-DOC [122]. In the Phase III trial COU-AA-301, abiraterone was found to prolong OS for patients with mCRPC who had previously received chemotherapy [123]. One of the secondary objectives in this study was to evaluate whether CTC enumeration by CellSearch was a suitable surrogate biomarker of treatment efficacy and OS. Remarkably, Scher and colleagues found on multivariate analysis that a biomarker panel encompassing CTC number and LDH level were prognostic for survival and could be used to risk stratify patients, whereas changes in prostate-specific antigen (PSA) serum levels, currently used in clinical practice to assess therapeutic efficacy, were not relevant [124].

An increase in CTCs from favorable to unfavorable levels after chemotherapy is associated with shorter OS in several cancer types, including mCRPC [60,93].

When such a finding is detected, a switch to another therapy may be warranted, although in a trial with metastatic breast cancer patients, an early switch from one cytotoxic chemotherapeutic agent to another for patients with an increase in CTCs did not improve OS [125]. Notably, this may not reflect a failure of screening by CTCs *per se*, but rather may be an indication that we need more effective treatments for these patients; indeed, one can only optimize decision making up to the asymptote defined by the maximum efficacy of the therapy itself. NCT01710605 is an ongoing trial of hormone receptor (HR) positive, metastatic breast cancer patients randomized to first-line treatment with chemotherapy or endocrine therapy based on CTC count versus clinician decision. In the CTC count arm, patients with high CTC counts (≥ 5 per 7.5 ml blood) receive chemotherapy, whereas those with low CTC counts receive endocrine therapy first. The study is designed to show noninferiority of the CTC arm for PFS and superiority of the CTC arm from a medicoeconomics standpoint.

Beyond systemic therapies, CTCs may also play an important role in monitoring the impact of locoregional cancer interventions. Some studies have shown that surgical manipulation can dislodge CTCs and increase CTC levels in the peripheral blood with the presence of CTM associated with unfavorable outcomes [126] while others have not [127]. Nevertheless, 'no-touch' isolation techniques for certain tumor resections have been developed and proposed as a way to reduce CTC shedding into the circulation [128], although no prospective data on the efficacy of such techniques are currently available.

Similarly, radiotherapy (RT) mediated CTC shedding has also been reported. NSCLC patients treated with RT may release CTCs with elevated γ -H2AX into the circulation, indicating their DNA damage and viability, and provides a rationale for potentially reducing the number of viable CTCs and subsequent treatment failure rate by modulating RT fractionation or by coadministering systemic therapies [129]. As with systemic therapies, CTC enumeration before, during and after RT seems to be reflective of treatment efficacy [130,131]. The mechanism of CTC shedding after local therapy, whether surgery or RT, is not well understood though vascular disruption may play a role.

Therapeutic targets & resistance mechanisms

In addition to serving as prognostic biomarkers, the molecular analysis of CTCs can also open the door to a wealth of additional information that can help identify new therapeutic targets and resistance mechanisms to

guide real-time precision therapy. In the following, we provide representative examples in breast and prostate cancers.

Metastatic breast cancer patients with estrogen receptor (ER) positive primary tumors have been shown to frequently have ER-negative CTCs, which may escape endocrine therapy [132]. To address this issue, Paoletti and colleagues developed a multi-parameter CTC-endocrine therapy index based on CTC enumeration and CTC expression of ER, BCL2, HER2 and Ki67 that may improve prediction of resistance to endocrine therapy in patients with HR-positive metastatic breast cancer and is currently undergoing a prospective trial [133]. In a similar vein, Reijm and colleagues developed an eight-gene mRNA expression profile in CTCs to predict the response to first-line aromatase inhibitors in metastatic breast cancer [134].

HER2-amplification is seen in approximately 20% of primary breast cancers and there is increasing evidence that CTCs and metastatic deposits have disparate HER2 statuses compared with the primary tumor in more than 30% of cases [135]. This has led to multicenter trials investigating whether these discordant patients still benefit from HER2-specific therapies [136,137]. For example, the ongoing DETECT III study is enrolling patients with metastatic breast cancer treated with up to three chemotherapy lines for metastatic disease who are found to have HER2⁺ CTCs (HER2 status of primary tumor and metastatic lesions must be negative) and randomizing them to standard therapy (chemotherapy or endocrine therapy) versus standard therapy plus lapatinib, a dual TKI that interrupts the HER2/neu and EGFR pathways [138]. Resistance to HER2-targeted therapies is often secondary to activation of the PI3K pathway (e.g., mutations in *PIK3CA* gene) and can be detected in CTCs [139–141].

Immune checkpoint inhibitors are highly efficacious in a subpopulation of patients with advanced malignancies, but due to their cost and toxicity, there is a critical need for predictive biomarkers to discriminate responders from nonresponders. PD-L1 is frequently expressed on CTCs in HR-positive, HER2-negative breast cancer and may provide an approach for further clinical trials as a prospective predictive biomarker [142,143].

mCRPC is defined by disease progression despite androgen-deprivation therapy. Next-generation AR-targeted therapies have activity in mCRPC, including enzalutamide and abiraterone, but some patients unfortunately do not benefit. In prostate cancer, PSA and prostate-specific membrane antigen (PSMA) are upregulated following AR activation and suppres-

sion, respectively. PSA/PSMA-based measurements can serve as surrogates for AR signaling in CTCs, and may help predict the response to AR-targeted therapies [144]. Mutations or amplification of the AR can cause resistance to androgen-deprivation therapy and are detectable in CTCs [145]. Moreover, recent studies have shown that mRNA expression of *AR-V7*, a truncated mRNA splice variant of *AR* that lacks the ligand-binding domain but remains constitutively active, in CTCs may predict failure of treatment with enzalutamide and abiraterone [146,147], but retained sensitivity to cytotoxic taxanes such as docetaxel [81,148–150]. In addition, a point mutation (F876L) in the ligand-binding domain of AR confers resistance to enzalutamide [151,152]. Although these results require validation in larger prospective trials, they do suggest that detection of *AR-V7* and *AR* mutations in CTCs can serve as predictive biomarkers that portend poor response to AR-targeted therapies and may prompt clinicians to initiate cytotoxic chemotherapy earlier.

Beyond modifications of the AR, other genomic and transcriptomic alterations have been detected in prostate CTCs including loss of *PTEN* [153], amplification of *MYC* [154] and the *TMPRSS2-ERG* chromosomal translocation [153,155]. *TMPRSS2-ERG* expression in peripheral CTCs does not predict response to abiraterone treatment [156] but interestingly does predict resistance to docetaxel and may be useful in treatment selection to avoid possible toxicities in refractory patients [157]. Moving beyond genetic analysis, Miyamoto and colleagues harnessed single-cell transcriptomics to identify activation of noncanonical Wnt signaling as a potential non-AR mechanism for antiandrogen resistance [49].

CTC-directed therapies

Among the CTCs is a putative metastatic subpopulation, so the CTCs themselves may serve as a promising therapeutic target. Platelets are thought to promote metastasis by protecting CTCs from immune elimination and promoting their arrest at the endothelium, supporting CTC extravasation into secondary sites. TNF-related apoptosis-inducing ligand (TRAIL) is a cytokine known to induce apoptosis specifically in tumor cells. Using synthetic silica particles functionalized with activated platelet membrane with surface conjugation of TRAIL or platelets genetically modified to express surface-bound TRAIL, Li and colleagues demonstrated selective killing of cancer cells *in vitro* and a significant metastatic reduction in mouse models of prostate and breast cancer metastasis [158,159]. This is the first demonstration of a Trojan-horse approach to neutralize CTCs and attenuate

metastasis. In a similar fashion, Wayne and colleagues used nanoscale liposomes conjugated with E-selectin adhesion protein and Apo2L/TRAIL that attach to the surface of leukocytes to rapidly clear viable CTCs and prevent the spontaneous formation and growth of metastatic tumors in an orthotopic xenograft model of prostate cancer [160].

Rather than target endogenous CTCs for destruction, Dondossola and colleagues exploited the self-seeding ability of CTCs to colonize distant organs and form metastases or re-infiltrate primary tumors by engineering cancer cells to release murine TNF [161]. Systemic administration of TNF-expressing tumor cells was associated with decreased growth of both primary tumors and metastatic colonies in immunocompetent mice. This novel proof of concept study demonstrated that genetically modified CTCs can act as targeted vectors to deliver anticancer agents.

Conclusion & future perspective

The majority of biomarkers currently used to guide ongoing treatment decisions in cancer are based on the analysis of primary and metastatic tumor biopsies. However, cancer is constantly evolving at the molecular level, especially under the selection pressure of various treatments, yielding a tremendous amount of spatial and temporal heterogeneity, which is unlikely to be captured by a spatially and temporally limited biopsy. An attractive alternative is the concept of serial 'liquid biopsies' in which peripheral blood is sampled throughout the course of disease to enable molecular analysis of CTCs, which are shed by both primary and metastatic tumors and may be more representative of the total breadth of disease. A wide range of applications using CTCs have already been demonstrated, including illuminating the fundamental processes of epithelial–mesenchymal transition and metastasis, providing prognostic information in numerous malignancies, monitoring treatment efficacy and identifying new therapeutic targets and resistance mechanisms to enable real-time modifications in clinical management.

Over the next decade, we envision the increased clinical adoption of new transformative technologies utilizing CTCs and other circulating tumor biomarkers such as cell-free DNA and exosomes to enable real-time precision medicine. Integrated microfluidic platforms for combined enrichment, detection, analysis of CTCs are on the horizon. Another area of expected growth is in single-CTC multiomics analysis. CTC analysis at the DNA and RNA levels may have a considerable impact on increasing our understanding of the molecular evolution of cancer (Figure 2). Most of the information obtained to date

has been targeted based on known cancer pathways, which has the advantage of lower cost and dropout rate; however, complementary untargeted omics approaches will enable more unbiased discovery. Other salient forms of information can be gleaned from individual CTCs, including epigenetics, proteomics and metabolomics. Single-CTC methylation analysis was recently demonstrated by Huang and colleagues, indicating at a gross level that DNA was hypomethylated and RNA was hypermethylated [162,163]. Along similar lines, methylation patterns in ctDNA has been used to identify tissue-specific cell death [164], and ctDNA detection of aberrant promoter methylation in a six-gene panel has been used for diagnosing breast cancer [165]. Concurrent multiomics analysis of single CTCs in a highly automated and multiplexed fashion using microfluidics and barcoding technology could augment lineage tracing and dissection of the genetic and epigenetic contributions to CTC cell state and dynamics (via the transcriptome readout) [166]. Integrated genome and transcriptome sequencing has recently been achieved in single cells [167,168] as has combined transcriptome and methylome sequencing [169].

CTC cultures [170], organoids [171] and xenografts [172] will facilitate additional testing for therapeutic targets and drug susceptibility to guide real-time therapeutic decisions (Figure 2). The concept of a co-clinical platform would be extremely powerful as each patient could have a personalized CTC-derived xenograft animal avatar at each stage of disease to enable direct testing of potential therapies before administration to the patient [173,174]. One of the challenges that will need to be overcome is the current inefficiency of the process, with hundreds to thousands of CTCs required to establish a cell line or xenograft, which limits the approach to patients with advanced disease.

CTCs represent a powerful, multifaceted tool that can help uncover fundamental principles of cancer biology in the laboratory and provide ongoing therapeutic guidance in the clinic; the journey to unlocking its full potential has only just begun.

Financial & competing interests disclosure

This work was supported by the Department of Defense (DT Miyamoto), Prostate Cancer Foundation (DT Miyamoto) and NIH T32G007753 Training Grant (KL Hwang). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Biology of circulating tumor cells

- Circulating tumor cells (CTCs) originate from cells within primary or metastatic tumors that acquire the ability to invade through the basement membrane and tissue stroma to enter the bloodstream/lymphatics, a process that is hypothesized to involve epithelial-to-mesenchymal transition.
- It is thought that only a small fraction of tumor cells have metastatic capability and CTCs provide an accessible window into this fundamental tumorigenic process, including the epithelial–mesenchymal transition.
- The use of CTCs has led to evidence that metastases are composed of multiple genetically distinct clones likely secondary to polyclonal seeding by CTCs and circulating tumor microemboli and the interplay between host immune cells at the metastatic site and arriving CTCs has been directly observed using intravital two-photon microscopy.
- Genomic and transcriptomic analysis of single CTCs can provide a global view of cancer heterogeneity, disease evolution and tumor biology. Comparing CTC and matched biopsy profiles for the degree of overlap/heterogeneity provides complementary information to guide therapeutic decision-making.

Capture & characterization of CTCs

- The reliable capture of CTCs from whole blood is challenging due to their scarcity, estimated at approximately one CTC per 10^7 leukocytes per ml of blood.
- Immunomagnetic capture uses antibodies directed toward epithelial markers (positive selection) or leukocyte markers (negative selection) and includes the only US FDA-cleared CTC enrichment technology, CellSearch.
- Label-free physical separation of CTCs from hematological cells harnesses the larger size, higher membrane capacitance and increased rigidity of CTCs compared with leukocytes and has the benefit of not enriching for CTC subgroups with specific immunocytochemical features.
- CTCs undergo no enrichment other than erythrocyte lysis, are plated on multiple slides, and then identified against a background of excess leukocytes by high-throughput immunofluorescence microscopy.
- Functional selection of CTCs captures viable CTCs that are capable of growing in culture and invading cell adhesion matrix.

Clinical application of CTCs

- Early detection is generally hampered by low sensitivity, though the prediction of lung cancer development in chronic obstructive pulmonary disease patients has been demonstrated.
- Enumeration of CTCs represents the simplest and most cost-effective level of analysis, and has been shown to provide nonredundant prognostic information for early-stage and metastatic cancers. Moreover, CTC counts can contribute to ongoing therapeutic monitoring, with a persistence or increase in counts motivating a change in treatment.
- Molecular analysis of CTCs provides prognostic information beyond enumeration alone, and importantly, is capable of uncovering new therapeutic targets and resistance mechanisms to optimize precision therapy for individual patients.
- Researchers have validated CTC expression profiles that can predict response to hormone therapy in breast cancer patients. Moreover, the hormone receptor and HER2/neu status of CTCs is often discordant with that of the primary/metastatic biopsy specimen and there is increasing evidence that applying targeted therapies based on the information gleaned from CTCs can improve clinical outcomes.
- In prostate cancer, prostate-specific antigen- and prostate-specific membrane antigen-based measurements can serve as surrogates for androgen receptor (AR) signaling in CTCs, which may help predict the outcome of AR-targeted therapy. Expression of *AR-V7*, a truncated mRNA splice variant of *AR* that lacks the ligand-binding domain but remains constitutively active, in CTCs may predict failure of AR-targeted therapy with enzalutamide and abiraterone but retained sensitivity to taxanes such as docetaxel.
- Surgical manipulation and radiotherapy can dislodge CTCs and increase CTC levels in the peripheral blood, but the clinical significance of this observation is not known.
- Given the putative metastatic CTC subpopulation, there has been recent attention toward targeting CTCs therapeutically. Approaches to date include particles, liposomes or platelets expressing TRAIL to induce CTC apoptosis and engineering CTCs to release TNF such that upon reinjection they home to primary/metastatic tumors and induce massive apoptosis.

References

Papers of special note have been highlighted as: • of interest;
•• of considerable interest

- 1 Haber DA, Gray NS, Baselga J. The evolving war on cancer. *Cell* 145(1), 19–24 (2011).
- 2 Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat. Rev. Cancer* 12(5), 323–334 (2012).
- 3 Miyamoto DT, Sequist LV, Lee RJ. Circulating tumour cells-monitoring treatment response in prostate cancer. *Nat. Rev. Clin. Oncol.* 11(7), 401–412 (2014).

- 4 Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. *Cancer Discov.* 6(5), 479–491 (2016).
- 5 Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. *Aust. Med. J.* 14, 146–147 (1869).
- 6 Ma M, Zhu H, Zhang C, Sun X, Gao X, Chen G. ‘Liquid biopsy’-ctDNA detection with great potential and challenges. *Ann. Transl. Med.* 3(16), 235 (2015).
- 7 Dawson S-J, Tsui DWYYW, Murtaza M *et al.* Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N. Engl. J. Med.* 368(13), 1199–209 (2013).
- 8 Melo SA, Luecke LB, Kahler C *et al.* Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 523(7559), 177–182 (2015).
- 9 Hoshino A, Costa-Silva B, Shen T-L *et al.* Tumour exosome integrins determine organotropic metastasis. *Nature* 527(7578), 329–335 (2015).
- 10 Best MG, Sol N, Koobi I *et al.* RNA-Seq of tumor-educated platelets enables blood-based pan-cancer, multiclass, and molecular pathway cancer diagnostics. *Cancer Cell* 28(5), 666–676 (2015).
- 11 Vincent-Salomon A, Thiery JP. Host microenvironment in breast cancer development: epithelial–mesenchymal transition in breast cancer development. *Breast Cancer Res.* 5(2), 101–106 (2003).
- 12 Schölch S, García SA, Iwata N *et al.* Circulating tumor cells exhibit stem cell characteristics in an orthotopic mouse model of colorectal cancer. *Oncotarget* 7(19), 27232–27242 (2016).
- 13 Weitz J, Koch M, Debus J, Höhler T, Galle PR, Büchler MW. Colorectal cancer. *Lancet* 365(9454), 153–165 (2005).
- 14 Kalluri R, Weinberg RA. The basics of epithelial–mesenchymal transition. *J. Clin. Invest.* 119(6), 1420–1428 (2009).
- 15 Kallergi G, Papadaki MA, Politaki E, Mavroudis D, Georgoulas V, Agelaki S. Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. *Breast Cancer Res.* 13(3), R59 (2011).
- 16 Yu M, Bardia A, Wittner BS *et al.* Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* 339, 580–584 (2013).
- 17 Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 372(6507), 679–683 (1994).
- 18 Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. *Nat. Rev. Clin. Oncol.* 11(3), 129–144 (2014).
- 19 Tam WL, Weinberg RA. The epigenetics of epithelial–mesenchymal plasticity in cancer. *Nat. Med.* 19(11), 1438–1449 (2013).
- 20 Massagué J, Obenauf AC. Metastatic colonization by circulating tumour cells. *Nature* 529(7586), 298–306 (2016).
- 21 Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. *Nat. Rev. Cancer* 3, 453–458 (2003).
- 22 Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* 331(6024), 1559–1564 (2011).
- 23 Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl Acad. Sci. USA* 100(7), 3983–3988 (2003).
- 24 Singh SK, Clarke ID, Terasaki M *et al.* Identification of a cancer stem cell in human brain tumors. *Cancer Res.* 63(18), 5821–5828 (2003).
- 25 Ricci-Vitiani L, Pallini R, Biffoni M *et al.* Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* 468(7325), 824–828 (2010).
- 26 Baccelli I, Schneeweiss A, Riethdorf S *et al.* Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nat. Biotechnol.* 31(6), 539–44 (2013).
- 27 Zhang L, Ridgway LD, Wetzel MDA *et al.* The identification and characterization of breast cancer CTCs competent for brain metastasis. *Sci. Transl. Med.* 5(180), 180ra48 (2013).
- 28 Morrow CJ, Trapani F, Metcalf RL, Bertolini G, Hodgkinson CL. Tumorigenic non-small-cell lung cancer mesenchymal circulating tumour cells: a clinical case study. *Ann. Oncol.* 27(6), 1155–1160 (2016).
- 29 Hou JM, Krebs MG, Lancashire L *et al.* Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. *J. Clin. Oncol.* 30(5), 525–532 (2012).
- 30 Duda DG, Duyverman AM, Kohno M *et al.* Malignant cells facilitate lung metastasis by bringing their own soil. *Proc. Natl Acad. Sci. USA* 107(50), 21677–21682 (2010).
- 31 Fidler IJ. The relationship of embolic homogeneity, number, size and viability to the incidence of experimental metastasis. *Eur. J. Cancer* 9(3), 223–227 (1973).
- 32 Topal B, Roskams T, Fevery J, Penninckx F. Aggregated colon cancer cells have a higher metastatic efficiency in the liver compared with nonaggregated cells: an experimental study. *J. Surg. Res.* 112(1), 31–37 (2003).
- 33 Aceto N, Bardia A, Miyamoto DT *et al.* Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* 158(5), 1110–1122 (2014).
- 34 Maddipati R, Stanger BZ. Pancreatic cancer metastases harbor evidence of polyclonality. *Cancer Discov.* 5(10), 1086–1097 (2015).
- 35 Gudem G, Van Loo P, Kremeyer B *et al.* The evolutionary history of lethal metastatic prostate cancer. *Nature* 520(7547), 353–357 (2015).
- 36 Cheung KJ, Padmanaban V, Silvestri V *et al.* Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *Proc. Natl Acad. Sci.* 113(7), E854–E863 (2016).
- 37 Headley MB, Bins A, Nip A *et al.* Visualization of immediate immune responses to pioneer metastatic cells in the lung. *Nature* 531(7595), 513–517 (2016).
- **Developed a murine intravital two-photon lung imaging model to directly observe the arrival of fluorescently labeled circulating tumor cells (CTCs) and their interaction with the host immune system. Discovered that tumor microemboli form within minutes of CTC**

- arrival and remain attached to the lung vasculature where waves of different myeloid cell derivatives interact with the circulating tumor microemboli in a competitive manner, including prometastatic neutrophils/monocytes/macrophages and antimetastatic dendritic cells.
- 38 Gasch C, Bauernhofer T, Pichler M *et al.* Heterogeneity of epidermal growth factor receptor status and mutations of KRAS/PIK3CA in circulating tumor cells of patients with colorectal cancer. *Clin. Chem.* 59(1), 252–260 (2013).
 - 39 Mostert B, Jiang Y, Sieuwerts AM *et al.* KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue. *Int. J. Cancer* 133(1), 130–141 (2013).
 - 40 Fabbri F, Carloni S, Zoli W *et al.* Detection and recovery of circulating colon cancer cells using a dielectrophoresis-based device: KRAS mutation status in pure CTCs. *Cancer Lett.* 335(1), 225–231 (2013).
 - 41 De Luca F, Rotunno G, Salvianti F *et al.* Mutational analysis of single circulating tumor cells by next generation sequencing in metastatic breast cancer. *Oncotarget* 7(18), 26107–26119 (2016).
 - 42 Heitzer E, Auer M, Gasch C *et al.* Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. *Cancer Res.* 73(10), 2965–2975 (2013).
 - 43 Jiang R, Lu Y, Ho H *et al.* A comparison of isolated circulating tumor cells and tissue biopsies using whole-genome sequencing in prostate cancer. *Oncotarget* 6(42), 44781–44793 (2015).
 - 44 Sundaresan TK, Sequist LV, Heymach JV *et al.* Detection of T790M, the acquired resistance EGFR mutation, by tumor biopsy versus noninvasive blood-based analyses. *Clin. Cancer Res.* 22(5), 1103–1110 (2015).
 - 45 Powell AA, Talasz AAH, Zhang H *et al.* Single cell profiling of circulating tumor cells: transcriptional heterogeneity and diversity from breast cancer cell lines. *PLoS ONE* 7(5), e33788 (2012).
 - 46 Ramsköld D, Luo S, Wang Y *et al.* Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. *Nat. Biotechnol.* 30(8), 777–782 (2012).
 - 47 Cann GM, Gulzar ZG, Cooper S *et al.* mRNA-Seq of single prostate cancer circulating tumor cells reveals recapitulation of gene expression and pathways found in prostate cancer. *PLoS ONE* 7(11), e49144 (2012).
 - 48 Ting DT, Wittner BS, Ligorio M *et al.* Single-cell RNA sequencing identifies extracellular matrix gene expression by pancreatic circulating tumor cells. *Cell Rep.* 8(6), 1905–1918 (2014).
 - 49 Miyamoto DT, Zheng Y, Wittner BS *et al.* RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science* 349(6254), 1351–1356 (2015).
 - Using single-cell RNA sequencing, the authors discovered that activation of noncanonical Wnt signaling (ectopic expression of Wnt5a) was a novel, nonandrogen receptor mediated mechanism underlying resistance to antiandrogen therapy.
 - 50 Arora VK, Schenkein E, Murali R *et al.* Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 155(6), 1309–1322 (2013).
 - 51 Andree KC, van Dalum G, Terstappen LWMM *et al.* Challenges in circulating tumor cell detection by the CellSearch system. *Mol. Oncol.* 10(3), 395–407 (2016).
 - 52 Gabriel MT, Calleja LR, Chalopin A, Ory B, Heymann D. Circulating tumor cells: a review of non-EpCAM-based approaches for cell enrichment and isolation. *Clin. Chem.* 62(4), 571–581 (2016).
 - 53 Green BJ, Saberi Safaei T, Mephram A, Labib M, Mohamadi RM, Kelley SO. Beyond the capture of circulating tumor cells: next-generation devices and materials. *Angew. Chem. Int. Ed. Engl.* 55(4), 1252–1265 (2016).
 - 54 Tibbe AGJ, Craig Miller M, Terstappen LWMM. Statistical considerations for enumeration of circulating tumor cells. *Cytom. Part A* 71(A), 154–162 (2007).
 - 55 Gogoi P, Sepehri S, Zhou Y *et al.* Development of an automated and sensitive microfluidic device for capturing and characterizing circulating tumor cells (CTCs) from clinical blood samples. *PLoS ONE* 11(1), e0147400 (2016).
 - 56 Sarioglu AF, Aceto N, Kojic N *et al.* A microfluidic device for label-free, physical capture of circulating tumor cell clusters. *Nat. Methods* 12(7), 685–691 (2015).
 - 57 Allard WJ, Matera J, Miller MC *et al.* Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin. Cancer Res.* 10, 6897–6904 (2005).
 - 58 Veridex LLC. CellSearch(TM) circulating tumor cell kit. Premarket notification – expanded indications for use – metastatic prostate cancer (2008). www.accessdata.fda.gov/
 - 59 Cristofanilli M, Stopeck A, Matera J *et al.* Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N. Engl. J. Med.* 351(8), 781–791 (2004).
 - Seminal prospective trial of 177 patients with metastatic breast cancer that showed patients with at least five CTCs per 7.5 ml blood before or after treatment had shorter progression-free survival (PFS) and overall survival (OS), paving the way for US FDA approval of CTC enumeration using CellSearch in metastatic breast cancer.
 - 60 de Bono JS, Scher HI, Montgomery RB *et al.* Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* 14(19), 6302–6309 (2008).
 - Prospective trial of 276 patients with castration-resistant prostate cancer (CRPC) that showed patients with at least five CTCs per 7.5 ml blood (unfavorable) before or after treatment had shorter OS and CTC counts were a better predictor of OS than prostate-specific antigen decrement at all time points, and led to FDA approval of CellSearch for castration resistant prostate cancer. Moreover, patients with unfavorable baseline CTC counts who converted to favorable CTC counts after treatment had significantly improved prognosis whereas those who went the other way had poorer prognosis.
 - 61 Cohen SJ, Punt CJA, Iannotti N *et al.* Relationship of

- circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26(19), 3213–3221 (2008).
- Prospective trial of 430 patients with metastatic colorectal cancer, which found that patients with unfavorable baseline or post-therapy CTC counts (≥ 3 CTCs per 7.5 ml blood) had significantly poorer PFS and OS, and conversion of baseline unfavorable CTC counts to favorable with treatment correlated with longer PFS and OS compared with those who did not change groups. These results led to FDA approval of CellSearch for metastatic colorectal cancer.
- 62 Talasz AH, Powell AA, Huber DE *et al.* Isolating highly enriched populations of circulating epithelial cells and other rare cells from blood using a magnetic sweeper device. *Proc. Natl Acad. Sci. USA* 106(10), 3970–3975 (2009).
 - 63 Saucedo-Zeni N, Mewes S, Niestroj R *et al.* A novel method for the *in vivo* isolation of circulating tumor cells from peripheral blood of cancer patients using a functionalized and structured medical wire. *Int. J. Oncol.* 41(4), 1241–1250 (2012).
 - 64 Nagrath S, Sequist LV, Maheswaran S *et al.* Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 450(7173), 1235–1239 (2007).
 - 65 Stott SL, Hsu C-HC-H, Tsukrov DI *et al.* Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. *Proc. Natl Acad. Sci. USA* 107(35), 18392–18397 (2010).
 - 66 Ozkumur E, Shah AM, Ciciliano JC *et al.* Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. *Sci. Transl. Med.* 5(179), 179ra47 (2013).
 - 67 Tang M, Wen C, Wu L *et al.* Lab on a Chip system for the efficient capture and *in situ* identification of circulating tumor cells. *Lab Chip* 16, 1214–1223 (2016).
 - 68 Saliba A-E, Saias L, Psychari E *et al.* Microfluidic sorting and multimodal typing of cancer cells in self-assembled magnetic arrays. *Proc. Natl Acad. Sci. USA* 107(33), 14524–14529 (2010).
 - 69 Zieglschmid V, Hollmann C, Gutierrez B *et al.* Combination of immunomagnetic enrichment with multiplex RT-PCR analysis for the detection of disseminated tumor cells. *Anticancer Res.* 25(3 A), 1803–1810 (2005).
 - 70 Wang Z, Wu W, Wang Z *et al.* *Ex vivo* expansion of circulating lung tumor cells based on one-step microfluidics-based immunomagnetic isolation. *Analyst* 141, 3621–3625 (2016).
 - 71 Zhao L, Tang C, Xu L *et al.* Enhanced and differential capture of circulating tumor cells from lung cancer patients by microfluidic assays using aptamer cocktail. *Small* 12(8), 1072–1081 (2016).
 - 72 Vona G, Estepa L, Bérout C *et al.* Impact of cytomorphological detection of circulating tumor cells in patients with liver cancer. *Hepatology* 39(3), 792–797 (2004).
 - 73 Hou HW, Warkiani ME, Khoo BL *et al.* Isolation and retrieval of circulating tumor cells using centrifugal forces. *Sci. Rep.* 3, 1259 (2013).
 - 74 Warkiani ME, Khoo BL, Wu L *et al.* Ultra-fast, label-free isolation of circulating tumor cells from blood using spiral microfluidics. *Nat. Protoc.* 11(1), 134–148 (2016).
 - 75 Gascoyne PR, Noshari J, Anderson TJ, Becker FF. Isolation of rare cells from cell mixtures by dielectrophoresis. *Electrophoresis* 30(8), 1388–1398 (2009).
 - 76 Shaw Bagnall J, Byun S, Begum S *et al.* Deformability of tumor cells versus blood cells. *Sci. Rep.* 5, 18542 (2015).
 - 77 Park ES, Jin C, Guo Q *et al.* Continuous flow deformability-based separation of circulating tumor cells using microfluidic ratchets. *Small* 12(14), 1909–1919 (2016).
 - 78 Werner SL, Graf RP, Landers M *et al.* Analytical validation and capabilities of the epic CTC platform: enrichment-free circulating tumour cell detection and characterization. *J. Circ. Biomarkers* 4(3), doi:10.5772/60725 (2015).
 - 79 Marrinucci D, Bethel K, Kolatkar A *et al.* Fluid biopsy in patients with metastatic prostate, pancreatic and breast cancers. *Phys. Biol.* 9, 1–9 (2012).
 - 80 Cho EHE, Wendel M, Luttgren M *et al.* Characterization of circulating tumor cell aggregates identified in patients with epithelial tumors. *Phys. Biol.* 9, 1–13 (2012).
 - 81 Scher HI, Lu D, Schreiber NA *et al.* Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. *JAMA Oncol.* doi:10.1001/jamaoncol.2016.1828 (2016) (Epub ahead of print).
 - 82 Lu J, Fan T, Zhao Q *et al.* Isolation of circulating epithelial and tumor progenitor cells with an invasive phenotype from breast cancer patients. *Int. J. Cancer* 126(3), 669–683 (2010).
 - 83 Tulley S, Zhao Q, Dong H, Pearl ML, Chen W. Vita-Assay™ method of enrichment and identification of circulating cancer cells/circulating tumor cells (CTCs). *Methods Mol. Biol.* 1406, 107–119 (2006).
 - 84 Lianidou ES, Markou A. Circulating tumor cells in breast cancer: detection systems, molecular characterization, and future challenges. *Clin. Chem.* 57(9), 1242–1255 (2011).
 - 85 Ilie M, Hofman V, Long-Mira E *et al.* ‘Sentinel’ circulating tumor cells allow early diagnosis of lung cancer in patients with chronic obstructive pulmonary disease. *PLoS ONE* 9(10), 4–10 (2014).
 - 86 Murray NP, Miranda R, Ruiz A, Droguett E. Diagnostic yield of primary circulating tumor cells in women suspected of breast cancer: the BEST (Breast Early Screening Test) study. *Asian Pac J. Cancer Prev.* 16(5), 1929–1934 (2015).
 - 87 Miller MC, Doyle GV, Terstappen LWMM. Significance of circulating tumor cells detected by the CellSearch system in patients with metastatic breast colorectal and prostate cancer. *J. Oncol.* 2010, 617421 (2010).
 - 88 Lv Q, Gong L, Zhang T *et al.* Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis. *Clin. Transl. Oncol.* 18(3), 322–330 (2016).
 - 89 Shiomi-Mouri Y, Kousaka J, Ando T *et al.* Clinical significance of circulating tumor cells (CTCs) with respect to optimal cut-off value and tumor markers in advanced/metastatic breast cancer. *Breast Cancer* 23(1), 120–127 (2016).
 - 90 Lu Y-J, Wang P, Wang X, Peng J, Zhu Y-W, Shen N. The

- significant prognostic value of circulating tumor cells in triple-negative breast cancer: a meta-analysis. *Oncotarget* doi:10.18632/oncotarget.8156 (2016) (Epub ahead of print).
- 91 Zhang L, Riethdorf S, Wu G *et al.* Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin. Cancer Res.* 18(20), 5701–5710 (2012).
 - 92 Scher HI, Jia X, de Bono JS *et al.* Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. *Lancet Oncol.* 10(3), 233–239 (2009).
 - 93 Goldkorn A, Ely B, Quinn DI *et al.* Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: A Phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 32(11), 1136–1142 (2014).
 - 94 Krebs MG, Sloane R, Priest L *et al.* Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell-lung cancer. *J. Clin. Oncol.* 29(12), 1556–1563 (2011).
 - 95 Cheng Y, Liu X, Fan Y *et al.* Circulating tumor cell counts/change for outcome prediction in patients with extensive-stage small-cell-lung cancer. *Future Oncol.* 12(6), 789–799 (2016).
 - 96 Li Y, Gong J, Zhang Q *et al.* Dynamic monitoring of circulating tumour cells to evaluate therapeutic efficacy in advanced gastric cancer. *Br. J. Cancer* 114(2), 138–145 (2016).
 - 97 Khan MS, Kirkwood AA, Tsigani T *et al.* Early changes in circulating tumor cells are associated with response and survival following treatment of metastatic neuroendocrine neoplasms. *Clin. Cancer Res.* 22(1), 79–85 (2016).
 - 98 Bidard F-C, Peeters DJ, Fehm T *et al.* Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol.* 15(4), 406–414 (2014).
 - 99 Mu Z, Wang C, Ye Z *et al.* Prospective assessment of the prognostic value of circulating tumor cells and their clusters in patients with advanced-stage breast cancer. *Breast Cancer Res. Treat.* 154(3), 563–571 (2015).
 - 100 Chang M-C, Chang Y-T, Chen J-Y *et al.* Clinical significance of circulating tumor microemboli as a prognostic marker in patients with pancreatic ductal adenocarcinoma. *Clin. Chem.* 62(3), 505–513 (2016).
 - 101 Rack B, Schindlbeck C, Jückstock J *et al.* Circulating tumor cells predict survival in early average-to-high risk breast cancer patients. *J. Natl Cancer Inst.* 106(5), dju066 (2014).
 - 102 Hall CS, Karhade MG, Bowman Bauldry JB *et al.* Prognostic value of circulating tumor cells identified before surgical resection in nonmetastatic breast cancer patients. *J. Am. Coll. Surg.* doi:10.1016/j.jamcollsurg.2016.02.021 (2016) (Epub ahead of print).
 - 103 Janni W, Rack B, Terstappen LW *et al.* Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. *Clin. Cancer Res.* 22(10), 2583–2593 (2016).
 - 104 Rink M, Chun FK, Dahlem R *et al.* Prognostic role and HER2 expression of circulating tumor cells in peripheral blood of patients prior to radical cystectomy: a prospective study. *Eur. Urol.* 61(4), 810–817 (2012).
 - 105 Gazzaniga P, de Berardinis E, Raimondi C *et al.* Circulating tumor cells detection has independent prognostic impact in high-risk non-muscle invasive bladder cancer. *Int. J. Cancer* 135, 1978–1982 (2014).
 - 106 Schulze K, Gasch C, Staufer K *et al.* Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. *Int. J. Cancer* 133(9), 2165–2171 (2013).
 - 107 Bayarri-Lara C, Ortega FG, Cueto Ladrón de Guevara A *et al.* Circulating tumor cells identify early recurrence in patients with non-small-cell lung cancer undergoing radical resection. *PLoS ONE* 11(2), e0148659 (2016).
 - 108 Wu XL, Tu Q, Faure G, Gallier P, Kohler C, Bittencourt Mde C. Diagnostic and prognostic value of circulating tumor cells in head and neck squamous cell carcinoma: a systematic review and meta-analysis. *Sci. Rep.* 6, 20210 (2016).
 - 109 Yang JD, Campion MB, Liu MC *et al.* Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. *Hepatology* 63(1), 148–158 (2016).
 - 110 Thalgott M, Rack B, Horn T *et al.* Detection of circulating tumor cells in locally advanced high-risk prostate cancer during neoadjuvant chemotherapy and radical prostatectomy. *Anticancer Res.* 35(10), 5679–5685 (2015).
 - 111 Meyer CP, Pantel K, Tennstedt P *et al.* Limited prognostic value of preoperative circulating tumor cells for early biochemical recurrence in patients with localized prostate cancer. *Urol. Oncol.* 34(5), e11–e16 (2016).
 - 112 Denève E, Riethdorf S, Ramos J *et al.* Capture of viable circulating tumor cells in the liver of colorectal cancer patients. *Clin. Chem.* 59(9), 1384–1392 (2013).
 - 113 Hinz S, Röder C, Tepel J *et al.* Cytokeratin 20 positive circulating tumor cells are a marker for response after neoadjuvant chemoradiation but not for prognosis in patients with rectal cancer. *BMC Cancer* 15(1), 953 (2015).
 - 114 Bulfoni M, Gerratana L, Del Ben F *et al.* In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis. *Breast Cancer Res.* 18(1), 30 (2016).
 - 115 Long E, Ilie M, Bence C *et al.* High expression of TRF2, SOX10, and CD10 in circulating tumor microemboli detected in metastatic melanoma patients. A potential impact for the assessment of disease aggressiveness. *Cancer Med.* 5(6), 1022–1030 (2016).
 - 116 Lindsay CR, Le Moulec S, Billiot F *et al.* Vimentin and Ki67 expression in circulating tumour cells derived from castrate-resistant prostate cancer. *BMC Cancer* 16(1), 168 (2016).
 - 117 Chang K, Kong Y, Dai B, Ye D, Qu Y, Wang Y. Combination of circulating tumor cell enumeration and tumor marker detection in predicting prognosis and treatment effect in metastatic castration-resistant prostate cancer. *Oncotarget* 6(39), 41825–41836 (2015).
 - 118 Barbazan J, Dunkel Y, Li H *et al.* Prognostic impact of modulators of g proteins in circulating tumor cells from patients with metastatic colorectal Cancer. *Sci. Rep.* 6, 22112

- (2016).
- 119 Poruk KE, Valero V, Saunders T *et al.* Circulating tumor cell phenotype predicts recurrence and survival in pancreatic Adenocarcinoma. *Ann. Surg.* doi:10.1097/SLA.0000000000001600 (2016) (Epub ahead of print).
 - 120 Hong D, Infante J, Janku F *et al.* Phase I Study of LY2606368, a checkpoint kinase 1 inhibitor, in patients with advanced cancer. *J. Clin. Oncol.* 34(15), 1764–1771 (2016).
 - 121 Chi K, Yu EY, Jacobs C *et al.* A Phase 1 dose-escalation study of apatersen (OGX-427), an antisense inhibitor targeting heat shock protein 27 (Hsp27), in patients with castration resistant prostate cancer and other advanced cancers. *Ann. Oncol.* 27, 1116–1122 (2016).
 - 122 Meulendijks D, De Groot JWB, Los M *et al.* Bevacizumab combined with docetaxel, oxaliplatin, and capecitabine, followed by maintenance with capecitabine and bevacizumab, as first-line treatment of patients with advanced HER2-negative gastric cancer: a multicenter Phase II study. *Cancer* 122(9), 1434–1443 (2016).
 - 123 de Bono JS, Logothetis CJ, Molina A *et al.* Abiraterone and increased survival in metastatic prostate cancer. *N. Engl. J. Med.* 364(21), 1995–2005 (2011).
 - 124 Scher HI, Heller G, Molina A *et al.* Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 33(12), 1348–1355 (2015).
 - **COU-AA-301, a Phase III trial that demonstrated an increase in OS with abiraterone in metastatic castration-resistant prostate cancer, was the first Phase III trial to prospectively evaluate a secondary objective on whether CTC enumeration by CellSearch could serve as a surrogate biomarker for treatment efficacy and OS. Multivariate analysis led to a biomarker panel consisting of CTC count and LDH level, which was used to risk stratify patients. Interestingly, prostate-specific antigen was not a significant prognostic factor.**
 - 125 Smerage JB, Barlow WE, Hortobagyi GN *et al.* Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. *J. Clin. Oncol.* 32(31), 3483–3489 (2014).
 - **Prior trials had noted that patients who remain in the unfavorable CTC count group after treatment may benefit from a change in therapeutic strategy – a hypothesis that was tested in SWOG S0500. Metastatic breast cancer patients with a persistent unfavorable level of CTCs after one cycle of chemotherapy were randomized to remain on the same therapeutic agent or to switch to a different chemotherapy. There was no difference in OS observed though CTC counts were strongly prognostic; this result does not necessarily imply a failure in the predictive power of CTC enumeration but more likely indicates that more effective therapies are needed.**
 - 126 Sawabata N, Funaki S, Hyakutake T, Shintani Y, Fujiwara A, Okumura M. Perioperative circulating tumor cells in surgical patients with non-small-cell lung cancer: does surgical manipulation dislodge cancer cells thus allowing them to pass into the peripheral blood? *Surg. Today* doi:10.1007/s00595–016–1318–4 (2016) (Epub ahead of print).
 - 127 Charitoudis G, Schuster R, Joussem AM, Keilholz U, Bechrakis NE. Detection of tumour cells in the bloodstream of patients with uveal melanoma: influence of surgical manipulation on the dissemination of tumour cells in the bloodstream. *Br. J. Ophthalmol.* 100(4), 468–472 (2016).
 - 128 Kuroki T, Eguchi S. No-touch isolation techniques for pancreatic cancer. *Surg. Today* doi:10.1007/s00595–016–1317–5 (2016) (Epub ahead of print).
 - 129 Martin OA, Anderson RL, Russell PA *et al.* Mobilization of viable tumor cells into the circulation during radiation therapy. *Int. J. Radiat. Oncol.* 88(2), 395–403 (2014).
 - 130 Dorsey JF, Kao GD, MacArthur KM *et al.* Tracking viable circulating tumor cells (CTCs) in the peripheral blood of non-small-cell lung cancer (NSCLC) patients undergoing definitive radiation therapy: pilot study results. *Cancer* 121(1), 139–149 (2015).
 - 131 Lowes LE, Lock M, Rodrigues G *et al.* The significance of circulating tumor cells in prostate cancer patients undergoing adjuvant or salvage radiation therapy. *Prostate Cancer Prostatic Dis.* 18, 358–364 (2015).
 - 132 Babayan A, Hannemann J, Spotter J, Muller V, Pantel K, Joosse SA. Heterogeneity of estrogen receptor expression in circulating tumor cells from metastatic breast cancer patients. *PLoS ONE* 8(9), e75038 (2013).
 - 133 Paoletti C, Muñoz MC, Thomas DG *et al.* Development of circulating tumor cell-endocrine therapy index in patients with hormone receptor-positive Breast Cancer. *Clin. Cancer Res.* 21(11), 2487–2498 (2015).
 - 134 Reijm EA, Sieuwerts AM, Smid M *et al.* An 8-gene mRNA expression profile in circulating tumor cells predicts response to aromatase inhibitors in metastatic breast cancer patients. *BMC Cancer* 16(123), 1–9 (2016).
 - 135 Fehm T, Muller V, Aktas B *et al.* HER2 status of circulating tumor cells in patients with metastatic breast cancer: A prospective, multicenter trial. *Breast Cancer Res. Treat.* 124(2), 403–412 (2010).
 - 136 Riethdorf S, Fritsche H, Müller V *et al.* Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin. Cancer Res.* 13(3), 920–928 (2007).
 - 137 Ignatiadis M, Rothé F, Chaboteaux C *et al.* HER2-positive circulating tumor cells in breast cancer. *PLoS ONE* 6(1), e15624 (2011).
 - 138 Schramm A, Friedl TWP, Schochter F *et al.* Therapeutic intervention based on circulating tumor cell phenotype in metastatic breast cancer: concept of the DETECT study program. *Arch. Gynecol. Obstet.* 293(2), 271–281 (2016).
 - 139 Neves RPL, Raba K, Schmidt O *et al.* Genomic high-resolution profiling of single CKpos/CD45neg flow-sorting purified circulating tumor cells from patients with metastatic breast cancer. *Clin. Chem.* 60(10), 1290–1297 (2014).
 - 140 Pestrin M, Salvianti F, Galardi F *et al.* Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumor cells from metastatic breast cancer patients. *Mol. Oncol.* 9(4), 749–757 (2015).
 - 141 Polzer B, Medoro G, Pasch S *et al.* Molecular profiling of

- single circulating tumor cells with diagnostic intention. *EMBO Mol. Med.* 6(11), 1371–1386 (2014).
- 142 Mazel M, Jacot W, Pantel K *et al.* Frequent expression of PD-L1 on circulating breast cancer cells. *Mol. Oncol.* 9(9), 1773–1782 (2015).
 - 143 David R. PD-L1 expression by circulating breast cancer cells. *Lancet Oncol.* 16(7), e321 (2015).
 - 144 Miyamoto DT, Lee RJ, Stott SL *et al.* Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. *Cancer Discov.* 2(11), 995–1003 (2012).
 - 145 Jiang Y, Palma JF, Agus DB, Wang Y, Gross ME. Detection of androgen receptor mutations in circulating tumor cells in castration-resistant prostate cancer. *Clin. Chem.* 56(9), 1492–1495 (2010).
 - 146 Antonarakis ES, Lu C, Wang H *et al.* AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N. Engl. J. Med.* 371(11), 1028–1038 (2014).
 - **AR-V7 is a splice variant of the androgen receptor that lacks the ligand-binding domain targeted by enzalutamide and abiraterone, but remains constitutively active as a transcription factor. The authors were able to detect AR-V7 in CTCs from metastatic castration-resistant prostate cancer patients using qRT-PCR and showed that the presence of this splice variant in CTCs was a predictive marker of therapeutic resistance to enzalutamide and abiraterone.**
 - 147 Steinestel J, Luedeke M, Arndt A *et al.* Detecting predictive androgen receptor modifications in circulating prostate cancer cells. *Oncotarget* doi:10.18632/oncotarget.3925 (2015) (Epub ahead of print).
 - 148 Thadani-Mulero M, Portella L, Sun S *et al.* Androgen receptor splice variants determine taxane sensitivity in prostate cancer. *Cancer Res.* 74(8), 2270–2282 (2014).
 - 149 Antonarakis ES, Lu C, Lubner B *et al.* Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. *JAMA Oncol.* 1(5), 582–591 (2015).
 - 150 Onstenk W, Sieuwerts AM, Kraan J *et al.* Efficacy of cabazitaxel in castration-resistant prostate cancer is independent of the presence of AR-V7 in circulating tumor cells. *Eur. Urol.* 68(6), 939–945 (2015).
 - 151 Balbas MD, Evans MJ, Hosfield DJ *et al.* Overcoming mutation-based resistance to antiandrogens with rational drug design. *Elife* 2, e00499 (2013).
 - 152 Joseph JD, Lu N, Qian J *et al.* A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. *Cancer Discov.* 3(9), 1020–1029 (2013).
 - 153 Attard G, Swennenhuis JF, Olmos D *et al.* Characterization of ERG, AR and PTEN gene status in circulating tumor cells from patients with castration-resistant prostate cancer. *Cancer Res.* 69(7), 2912–2918 (2009).
 - 154 Leversha MA, Han J, Asgari Z *et al.* Fluorescence *in situ* hybridization analysis of circulating tumor cells in metastatic prostate cancer. *Clin. Cancer Res.* 15(6), 2091–2097 (2009).
 - 155 Stott SL, Lee RJ, Nagrath S *et al.* Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. *Sci. Transl. Med.* 2(25), 25ra23 (2010).
 - 156 Danila DC, Anand A, Sung CC *et al.* TMPRSS2-ERG Status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. *Eur. Urol.* 60(5), 897–904 (2011).
 - 157 Reig O, Marin-Aguilera M, Carrera G *et al.* TMPRSS2-ERG in blood and docetaxel resistance in metastatic castration-resistant prostate cancer. *Eur. Urol.* doi:10.1016/j.eururo.2016.02.034 (2016) (Epub ahead of print).
 - 158 Li J, Sharkey CC, Wun B, Liesveld JL, King MR. Genetic engineering of platelets to neutralize circulating tumor cells. *J. Control. Release* 228, 38–47 (2016).
 - 159 Li J, Ai Y, Wang L *et al.* Targeted drug delivery to circulating tumor cells via platelet membrane-functionalized particles. *Biomaterials* 76, 52–65 (2016).
 - 160 Wayne EC, Chandrasekaran S, Mitchell MJ *et al.* TRAIL-coated leukocytes that prevent the bloodborne metastasis of prostate cancer. *J. Control. Release* 223, 215–223 (2016).
 - 161 Dondossola E, Dobroff AS, Marchiò S *et al.* Self-targeting of TNF-releasing cancer cells in preclinical models of primary and metastatic tumors. *Proc. Natl Acad. Sci. USA* 113(8), 2223–2228 (2016).
 - **Proof of concept study demonstrating the use of genetically modified CTCs to deliver anticancer agents. The authors exploited the self-seeding ability of CTCs by engineering them to release murine TNF such that they home to and reinfiltate primary tumors and metastatic deposits to elicit apoptosis of cancer cells, thereby decreasing the tumor burden in immunocompetent mice.**
 - 162 Huang W, Qi C-B, Lv S-W *et al.* Determination of DNA and RNA epigenetic modifications in circulating tumor cells by mass spectrometry. *Anal. Chem.* 88, 1378–1384 (2015).
 - 163 Lianidou ES. Gene expression profiling and DNA methylation analyses of CTCs. *Mol. Oncol.* 10(3), 431–442 (2016).
 - 164 Lehmann-Werman R, Neiman D, Zemmour H *et al.* Identification of tissue-specific cell death using methylation patterns of circulating DNA. *Proc. Natl Acad. Sci. USA* 113(13), e1826–e1834 (2016).
 - 165 Shan M, Yin H, Li J *et al.* Detection of aberrant methylation of a six-gene panel in serum DNA for diagnosis of breast cancer. *Oncotarget* 7(14), 18485–18494 (2016).
 - 166 Macosko EZ, Basu A, Satija R *et al.* Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. *Cell* 161(5), 1202–1214 (2015).
 - 167 Macaulay IC, Haerty W, Kumar P *et al.* G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. *Nat. Methods* 12(6), 519–522 (2015).
 - 168 Dey SS, Kester L, Spanjaard B, Bienko M, van Oudenaarden A. Integrated genome and transcriptome sequencing of the same cell. *Nat. Biotechnol.* 33(3), 285–289 (2015).
 - 169 Angermueller C, Clark SJ, Lee HJ *et al.* Parallel single-cell sequencing links transcriptional and epigenetic heterogeneity. *Nat. Methods* 13(3), 229–232 (2016).

- 170 Yu M, Bardia A, Aceto N *et al.* *Ex vivo* culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* 345(6193), 216–220 (2014).
- 171 Gao D, Vela I, Sboner A *et al.* Organoid cultures derived from patients with advanced prostate cancer. *Cell* 159(1), 176–187 (2014).
- 172 Williams ES, Rodriguez-Bravo V, Rodriguez-Bravo V *et al.* Generation of prostate cancer patient derived xenograft models from circulating tumor cells. *J. Vis. Exp.* 105, 53182 (2015).
- 173 Malaney P, Nicosia SV, Dave V. One mouse, one patient paradigm: new avatars of personalized cancer therapy. *Cancer Lett.* 344(1), 1–12 (2014).
- 174 Lunardi A, Pandolfi PP. A co-clinical platform to accelerate cancer treatment optimization. *Trends Mol. Med.* 21(1), 1–5 (2015).