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Current Advances in Clinical Application of Liquid Biopsy

Shawn Baldacchino

Abstract

Liquid biopsy solutions are available for niche clinical applications. The patient benefits of such solutions are evident: ease of sampling, acceptable and repeatable. To date a number of solutions have received regulatory approval with more comprehensive, multi-cancer companion diagnostic approaches receiving approval in late 2020. Given these breakthrough advances and the ongoing clinical studies in early detection of cancer, the liquid biopsy field is making strides in technology. While circulating tumour DNA (ctDNA) solutions are quickly penetrating the market, strides in circulating tumour cells (CTC) and extracellular vesicles (EV) technologies is unlocking their potential for liquid biopsy. ctDNA solutions are paving the way towards clinical translation into the distinct applications across the cancer continuum. This chapter presents a detailed review of current approved liquid biopsy tests and provides a summary of advanced-stage prospective technologies within the context of distinctive clinical applications.

Keywords: circulating tumour cells, CTC, extracellular vesicles, EV, cfDNA, ctDNA, methylation, liquid biopsy, cancer screening, precision medicine, companion diagnostics

1. Introduction

Precision medicine is driven by discoveries in cancer biology that enable targeted therapy against specific oncogenic molecular targets. Using small selective inhibitory molecules or monoclonal antibodies, therapies aim to effectively target tumour cells with minor effects on normal cells [1]. Targeted therapies significantly contribute to improved cancer survival, however the results have not been commensurate with expectations [2]. Tumours accumulate mutations, many of which are passenger or dispensable aberrations that can be bypassed to confer resistance. Malignant cells interact and exploit their immediate and distant microenvironment. Tumours exhibit clonal evolution that results in heterogeneity [3, 4]. Cancer is a cell disorder characterised not only by its genetics but transcriptomic, proteomic expression patterns and cellular interactions. This is driving an integrative approach to cancer diagnosis and therapeutic options [5–7].

Until recently, precision medicine was limited to the solid tissue space but is now becoming established in the liquid biopsy field with several approved solutions (**Tables 1** and **2**). The broad term, liquid biopsy, alludes to a test or series of tests that can provide information comparable and potentially beyond the limits of the tissue biopsy harnessing body fluid constituents. Body fluids investigated for liquid biopsy applications are comprehensive including but not limited to blood,

Single Cancer Indication						
Test (Manufacturer)	Technology	Biomarker	Cancer Type	Approval	Application	Sample
CellSearch® (Menarini Silocon Biosystems)	CTC immuno-isolation and detection by immune-fluorescence	CTC with CD45-, EpCAM+ and (CK8, 18 and/or 19)	Metastatic Breast, Colorectal, Prostate	FDA / CE-IVD	Prognostic	Blood
Cobas® EGFR mutation test V2 (Roche)	PCR	EGFR	NSCLC	FDA	CDx	Plasma
Therascreen (Qiagen)	PCR	• PIK3CA	• Breast	FDA / CE-IVD	CDx	Blood
		• KRAS	• CRC			
		• BRAF	• CRC			
		• EGFR	• NSCLC			
		• FGFR	• Urothelial			
Target Selector™ (Biocept) [8]	Switch-Blocker, qPCR, NGS	EGFR	NSCLC	CE-IVD	CDx	Blood / FFPE
OncoBEAM (Sysmex)	Digital PCR	KRAS & NRAS	mCRC	CE-IVD	CDx	Plasma
Idylla (Biocartis)	PCR	• KRAS	• mCRC	CE-IVD	CDx	Plasma
		• NRAS, BRAF	• mCRC			
HCCBlood Test (Epigenomics AG) [9]	Bisulfite converted DNA & PCR	SEPT9 methylation	HCC (patients with liver cirrhosis)	CE-IVD	Diagnostic aid	Plasma
Epi proColon® (Epigenomics AG) [10, 11]	Bisulfite converted DNA & PCR	SEPT9 methylation	CRC	FDA / CE-IVD	Ancillary Screening	Plasma
COLOGUARD (ExactSciences) [12]	QuARTS & Immunoassay	BMP3 & NDRG4 methylation, KRAS, ACTB Haemoglobin	CRC or advanced adenoma	FDA	Ancillary screening	Stool
IntPlex® (DiaDx) [13, 14]	PCR	• BRAF	• mCRC	CE-IVD	CDx	Plasma
		• EFGR	• mCRC			

Single Cancer Indication						
Xpert® Bladder Cancer Detection Xpert® Bladder Cancer Monitor (Cepheid) [15]	RT-PCR	UPK1B, IGF2, CRH, ANXA10, ABL1	Bladder (patients with haematuria) NMIBC	CE-IVD	Diagnostic aid Surveillance for recurrence	Urine
UroVysion (Abbott) [16]	FISH	Aneuploidy 3,7,17 and loss of 9p21 (p16)	Bladder (patients with haematuria)	FDA / CE-IVD	Diagnostic aid	Urine
Uromonitor (Uromonitor)	PCR	FGFR3 and TERT PCR	NMIBC (patients with haematuria)	CE-IVD	Diagnostic aid, Surveillance for recurrence	Urine
Epicheck (Nucleix) [17]	Methylation-sensitive restriction Enzyme digestion, PCR	15 DNA methylation markers	Bladder	CE-IVD	Surveillance for recurrence	Urine
Table provides a general overview and may not be exhaustive [CDx: Companion diagnostic; mCRC: metastatic colorectal carcinoma; CTC: Circulating tumour cells; ddPCR: droplet digital Polymerase chain reaction; FDA: Food & Drug Administration; FISH: Fluorescent in situ hybridisation; HCC: Hepatocellular carcinoma; CE-IVD: In vitro Diagnostic device certification; NMIBC: Non-muscle invasive bladder cancer; NSCLC: Non-small cell lung carcinoma; PCR: Polymerase chain reaction; QuARTS: Quantitative allele-specific real-time target and signal amplification; RT-PCR: reverse transcription PCR] [18, 19].						

Table 1.
Overview of current approved (FDA/IVD) ctDNA liquid biopsy solutions for single cancer indications.

Pan-Cancer / Multi-cancer Indication						
Test (Manufacturer)	Technology	Biomarker	Cancer Type	Approval	Application	Sample
FoundationOne Liquid CDx (FoundationOne) [20]	NGS (324 genes)	• ALK, EGFR	• NSCLC	FDA	CDx	Plasma
		• BRCA1/2	• Ovarian			
		• BRCA1/2 & ATM	• Prostate			
		• PIK3CA	• Breast			
Guardant360 (Guardant Health) [21, 22]	NGS (73 genes)	• Tumour mutation profiling	• Any solid tumour	FDA	CDx	Plasma
		• EGFR	• NSCLC			
Table provides a general overview and may not be exhaustive. [CDx: Companion diagnostic; mCRC: metastatic colorectal carcinoma; FDA: Food & Drug Administration; NGS: Next-generation sequencing; NSCLC: Non-small cell lung carcinoma] [18, 19].						

Table 2.
Overview of current approved (FDA/IVD) ctDNA liquid biopsy solutions indicated for use with 2 or more solid cancers.

Figure 1.
Comparison of workflows of emerging liquid biopsy tools with routine cancer diagnostics by tissue biopsy. Liquid biopsy solutions remain complimentary to the clinical standard of care. [CTC: Circulating tumour cells; EVs: Extracellular vesicles; ctDNA: Circulating tumour DNA; FISH: Fluorescent in situ hybridisation; H&E: Haematoxylin and eosin; IHC: Immunohistochemistry; TAT: Turn-around time; created with BioRender.com].

urine [16], cerebrospinal fluid [23], stool [24], breast milk [25], saliva/sputum, oesophageal brushing, Pap smears/brushing [26], tears [27], pleural effusion [28] and ascitic fluid [29].

Liquid biopsy testing may encompass investigations of circulating tumour DNA (ctDNA) or cell-free DNA (cfDNA) and RNA (ctRNA), circulating tumour cells (CTCs), tumour-derived extracellular vesicles (EVs) and tumour-educated platelets [30]. Rapidly advancing technologies for immunoprofiling of leukocytes and T-cell receptor (TCR) profiling also present a potential liquid biopsy tool with a particular role in metastatic cancer patients for immunotherapy [31, 32].

The potential applications of liquid biopsy are numerous and throughout the cancer journey:

1. Cancer detection for screening or earlier detection [33, 34],
2. Diagnosis / Prognosis/Predictive (Companion Diagnostics, CDx) [30],
3. Therapeutic response monitoring (Detection of resistance mechanisms) [35, 36],

4. Minimal Residual Disease detection [37],

5. Post-remission surveillance to predict/detect relapse, metastases and clonal evolution [37, 38].

The main advantages of a liquid biopsy assay relate to the ease of sampling. Collecting the sample is generally not invasive and repeatable enabling longitudinal monitoring. The risk of complications and pain from sample collection is minimal presenting a very acceptable procedure that beckons better uptake as a screening procedure. Liquid biopsy methods are less laborious than tissue biopsy methods and can be analysed in a much shorter time-frame (**Figure 1**). Moreover, liquid biopsies offer an overall snapshot of the tumour which represents distinct tumour clones, mitigating tumour region selection bias [30]. Monitoring cancer over time also provides insight on the temporal heterogeneity, a potential tool to study mechanisms of response and resistance [32, 39].

Following is a review of the advances of liquid biopsy in the context of the current state of tissue molecular pathology for clinical application. A brief illustration of future prospects is also described based on ongoing clinical studies.

2. Molecular pathology: overcoming challenges for solid and liquid biopsy

Challenges to comprehensively characterise cancer in the clinical setting exist, relating to pre-analytical (sample collection & processing), analytical and post-analytical factors. Molecular pathology of solid cancer on formalin-fixed paraffin embedded (FFPE) tissue presents technical challenges arising from tissue fixation and processing but also sample availability.

A study evaluating factors for next-generation sequencing (NGS) testing failure showed that on average 22.5% of cases do not meet quality requirements. Insufficient tissue or insufficient DNA accounted for 62% and 29% of failures with 6% failing at library preparation [40]. The study highlights increased failure from fine needle aspirates and biopsy specimens with a low failure rate in excisional specimens (1.7%) [41]. Whole genome sequencing approaches show non-uniform coverage in FFPE DNA samples resulting in sub-optimal somatic copy number alteration detection. Nonetheless clinically actionable variants are generally detected [42]. Sensitive NGS applications require good quality DNA to achieve adequate assay performance and coverage. Recent developments in DNA extraction methods and optimisation improve assay performance [42, 43]. In fact NGS solutions have been achieving regulatory approval such like Oncomine Dx (ThermoFisher Scientific) for targeted therapies in non-small cell lung carcinoma (NSCLC) [44]; Praxis (Illumina) characterising 56 KRAS/NRAS mutations for colorectal cancer companion diagnostics (CDx); Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), the first U.S. Food & Drug Administration (FDA) approved tissue profiling test that detecting aberrations across 341 cancer genes for solid cancer tissue diagnostics but not prescriptive for any specific therapeutic product [45]; and FoundationOne CDx which is the first FDA-approved broad CDx test that is clinically and analytically validated for all solid tumours for therapeutic indication and has a success rate of >95% on FFPE [46].

Targeted gene panels or single gene polymerase chain reaction (PCR) assays are more easily translated to clinical application given their very specific intended use. Recent advances can mitigate the effects of DNA fragmentation and PCR

inhibition [47]. Technical challenges are greater for detection of RNA signatures due to high degree of RNA fragmentation and introduced technical bias [48]. A study assessing the performance of PCR on RNA derived from FFPE reports that only 50% (37/74) of samples were informative. RNA profiling on FFPE samples requires alternative technologies that can robustly detect degraded RNA with reduced technical bias [49–51].

Liquid biopsy options involve far less sample processing and better sample quality. Nonetheless tumour signatures are generally rare, similar to finding ‘the needle in a haystack’, and assays require high sensitivity to avoid false negative results. In the search for a sensitive, specific and reliable method, liquid biopsy technologies are becoming more diverse and complex [52–54]. Moreover, pre-analytical considerations are critical to ensure high sensitivity and reproducible performance. These requirements vary depending on the analysed liquid biopsy component. Expert recommendations for minimal requirements for clinical cfDNA testing have been published to emphasise the need for standardisation of the test processes [55].

3. Current state of liquid biopsy application

3.1 Liquid biopsy for companion diagnostics

A particular study of previously untreated metastatic non-small cell lung carcinoma (NSCLC) shows that cfDNA technologies have the potential to detect guideline-recommended biomarkers with a higher sensitivity compared to standard of care tissue genotyping methodology [22]. Currently, ctDNA assays are being recommended for use in lung patients with progression of secondary clinical resistance and in some clinical settings where tissue is limited or insufficient for molecular testing. ctDNA assays are not recommended for the diagnosis of primary lung tumours [56]. ctDNA liquid biopsy solutions are currently approved as additional tools to the standard of care and when results are negative, tissue testing is recommended when available. Lung cancer tissue is not easily available and sampling implies potential serious complications such as pneumothorax, haemorrhage and respiratory failure. Only 50% of cases in the Marker Identification Trial (MERIT) trial had sufficient sample for the planned molecular analyses [40]. This presents a clinical need for liquid biopsy to potentially identify a route for targeted treatment.

Advanced-stage technologies within the liquid biopsy field harness ctDNA. These approaches mainly focus on hallmark mutations or other changes in the DNA (methylation). The first FDA approved ctDNA liquid biopsy was the cobas® EGFR mutation test V2 (Roche) as a companion diagnostic [57]. This was followed by several other targeted panel companion diagnostics (**Table 1**). The main available plasma liquid biopsy solutions detect mutations in clinically actionable biomarkers that predict response to specific targeted therapies. The main biomarkers detected are EGFR, FGFR, KRAS, NRAS, BRAF and PIK3CA.

Selected diagnostic panels are useful as companion diagnostics for clinical trials and patient selection for specific targeted therapeutics. Nonetheless, established targets would then be integrated into larger diagnostic panels that provide a comprehensive and exhaustive approach to cancer diagnostics. Recently the FDA approved the first two NGS-based liquid biopsy solutions: Guardant360 (August, 2020) and FoundationOne CDx (November 2020) (**Table 2**). Unlike PCR-based targeted panels, large NGS panel tests interrogate a large-set of genes generating more clinically useful information but present a challenge to validate and regulate [58]. Similar to tissue-based NGS approaches, generated clinical information assists the definition of a spectrum of potential therapeutic options to identify a sequence of

treatments to achieve optimised response [59]. In contrast, to tissue biopsy molecular analysis, liquid biopsy solutions are expected to generate a collective picture of cancer heterogeneous clones enabling a comprehensive therapeutic approach which may be key to avoid clonal residual disease or recurrence [60].

A recent study evaluated the post-progression ctDNA (Guardant360 assay) with matched multiple lesion biopsies to assess heterogeneity during acquired resistance [61]. This study reveals distinct mutational profiles across metastatic lesions of gastrointestinal origin. The majority of private alterations across lesions could be detected by cfDNA. In another study, combined analysis of solid (192 genes) and liquid biopsies (27 genes) (OncoSTRAT&GO™, OncoDNA, Gosselies, Belgium), only found 40% of variants to be shared between the solid and liquid biopsy, with 51% of variants being exclusive to tissue and 9% to blood [62]. The liquid exclusive variants increased to 14% after a year from tissue sampling reflecting temporal heterogeneity [62]. The disparity in mutation calling may be a result of distinct shedding rates across tumour stage and types or sensitivity of the ctDNA assay. Although further studies are needed, such studies suggest that liquid biopsy can complement tissue molecular pathology to improve the detection of clinically actionable aberrations to overcome spatial and temporal heterogeneity especially in late-stage disease.

3.2 Liquid biopsy for cancer detection

Similar to CDx assays, current solutions for primary cancer diagnosis are either ancillary solutions or to be used when the routine screening/diagnostic test is not an option. Thus, liquid biopsy approaches are currently another tool that assist and improve the overall performance of cancer detection. Approved liquid biopsy solutions for bladder cancer screening and colorectal cancer screening are available (**Table 1**) to support current screening methods. Evidently, when standard of care investigations are not available, liquid biopsy can provide means of detection. For instance, Epi proColon® (Epigenomics) is available only to patients who are unwilling or unable to be screened by recommended methods. This can potentially improve screening uptake with current colorectal cancer screening uptake reported between 53 and 61% [63–65]. The more acceptable, repeatable advantages of liquid biopsy enable multi-line testing or triage testing to select patients for further investigation, similar to the faecal immunochemical testing (FIT) to the colonoscopy procedure. Cologuard (ExactSciences) offers an approved stool molecular test for the detection of colorectal cancer with a reported increased sensitivity for detecting any stage CRC (92%) and 42% sensitivity for advanced precancerous lesions [12]. Specific cases presenting positive Cologuard test and negative follow-up colonoscopy raised concerns for lack of recommendations for patient management in these scenarios [66].

A first-line or triage test should be cost-effective, especially for screening purposes, to achieve a net cost–benefit. A recent health technology assessment evaluates EGFR T790M resistance mutation testing in patients with advanced NSCLC can lead to fewer tissue biopsies although a follow-up confirmatory tissue biopsy is required when liquid biopsy tests negative [67]. EGFR T790M mutation detection from urine has also been shown to be feasible for NSCLC patients to reduce biopsy procedures and mitigate biopsy related complications [68].

In a similar approach, the ExoDx Prostate test (ExosomeDx, a Bio-Techne brand), can be used to assess cancer risk in patients with elevated prostate specific antigen (PSA) to assist the decision to proceed or defer a prostate biopsy. ExoDx is the first exosome-based (RNA biomarkers) liquid biopsy solution to receive a Breakthrough Device Designation by the U.S. FDA [69]. Prostate cancer screening

by PSA has highlighted the risks of over diagnosis and over treatment accompanied by a lack of tangible benefit [70, 71]. This created a need to better inform clinical decisions to follow-up with invasive diagnostic procedures and treatment and accentuates the need for sensitive tests that are also highly specific. Specific clinical applications require performance parameters that balance risk of non-detection with overtreatment depending on the backbone standard of care tests.

3.3 Liquid biopsy for prognosis and therapy intervention

CellSearch® (Menarini Silicon Biosystems), a CTC detection system, was the first liquid biopsy approach to be approved by FDA in 2004. The CellSearch technology immunomagnetically captures CTCs from whole blood, that express the Epithelial cell adhesion molecule (EpCAM) and enumerates CTCs with the profile of CD45 negative and cytokeratin 8, 18, and/or 19 positive [72]. The CellSearch system provides prognostic information for patients with metastatic breast, prostate or colorectal cancer. A major limitation of this method is the reliance on the EpCAM marker. CTCs have been described to be heterogeneous and not all CTCs express EpCAM. Such methods are restrictive to the epithelial phenotype and have intrinsic selection bias [73]. Label-free CTC enrichment solutions, such as Parsortix® (ANGLE) and ClearCell® FX1 system (Biolidics) are European CE marked as *in vitro* diagnostic device (CE-IVD) solutions for CTC enrichment but require downstream analysis to derive clinically relevant information. Moreover, isolated CTC remain viable and can potentially be cultured and studied further although finding optimal conditions for culturing CTC subtypes is challenging [74]. CTC enrichment by size discrimination shows a reduced recovery rate (~60%) for smaller sized cell lines (SKBR3) [75] presenting with a selective enrichment and failing to detect a subset of cells similarly to immunoisolation methods. CTC enrichment by depletion of leukocytes also results in reduced recovery [76]. Current advances in flow cytometry resolution and imaging may enable the suppression of pre-enrichment to enable a quick and efficient detection of CTC [77–79]. These approaches have a definite role in therapeutic monitoring, identifying treatment response and early resistance and are ready for clinical studies [80, 81].

ctDNA abundance, mutation count and a KEAP1, KRAS, MET signature predict overall survival in advanced NSCLC patients (N = 949). Interestingly, patients with at least one ctDNA clearance during the course of treatment had a significantly better progression-free survival and overall survival than patients with consistent ctDNA levels throughout treatment [82]. The prognosis and predictive potential of ctDNA is yet to be translated into practical clinical assays. While the potential role of EV in cancer prognosis has been shown [83], further studies are required to define EV isolation and prognostic correlations in larger patient cohorts.

4. Current outlook for early cancer detection

5-year survival rates for patients diagnosed with stage I and stage IV cancer respectively are 98% and 26% for breast cancer, 92% to 10% for colorectal cancer and 57% to 3% for lung cancer [84]. Earlier diagnosis would greatly improve cancer survivability but is currently a great challenge. Detecting cancer early is a cornerstone of the UK's NHS Long term plan. There have been numerous efforts to achieve early cancer screening, through public awareness (Be Clear on Cancer and Detect Cancer Early campaigns), introducing new screening tests (Bowel screening) and targeted lung health checks (following the NELSON trial) and many more.

Complex approaches, by GRAIL [85], Thrive's CancerSEEK [86], FoundationMedicine, Base Genomics, Freenome aim to expand the potential of early diagnosis from blood. Grail's Galleri, Thrive's CancerSEEK and Natera's Signatera have achieved FDA Breakthrough device status while in the trial stage. Early diagnosis remains a challenge with sensitivity being a critical factor. Achieving early diagnosis in the blood using ctDNA is more complex, mainly because there is a huge amount of "normal" DNA circulating in the blood. The smaller the cancer the smaller and less detectible the cancer signature is in the blood. As any cancer grows, it sheds more DNA, more cellular debris and more cancer cells into the bloodstream which eventually leads to the cancer spreading to distant organs. Although the ctDNA shedding rate can vary among patients, a mathematical model can predict tumour size by assessing haploid genome equivalents per plasma volume (correlation: $R^2 = 0.32$; $P = 2.6 \times 10^{-16}$) [87]. The smaller the tumour, the higher the probability of a false negative result for a particular actionable mutation.

Till date there is no FDA-approved solution for early cancer detection from blood with targeted panel solutions available as ancillary diagnostics from stools for colorectal cancer (ColoGuard & Epi ProColon) and from urine for bladder cancer (Xpert Bladder Cancer detection & Uromonitor). Interestingly, a blood test detecting Septin 9 (SEPT9) methylation to aid the detection of hepatocellular carcinoma (HCC) in patients with cirrhosis, has been CE-IVD marked (HCCBloodTest by Epigenomics) [9].

Following are some illustrative examples of ongoing clinical studies investigating the application of liquid biopsy for multi-cancer detection.

4.1 CancerSEEK, PapSEEK, UroSEEK

A series of liquid biopsy tests for early diagnosis have been developed at the Johns Hopkins University: CancerSEEK, PapSEEK and UroSEEK.

CancerSEEK measures 8 protein biomarkers by immunoassays and mutations on 16 genes by PCR and sequencing in blood samples to detect and localise the cancer. A study of eight cancer types (colorectal, ovary, pancreas, breast, upper gastrointestinal tract, lung and liver) resulted in a median sensitivity of 70%, ranging from 33% in breast and 98% in ovarian cancer. Across stages of the disease the test was 43%, 73% and 78% sensitive respectively [86]. In a following prospective, interventional study (DETECT-A) CancerSEEK was coupled with positron emission tomography-computed tomography (PET-CT) for cancer detection. During this trial, the blood test sensitivity for all cancer types was 27.1% and specificity of 98.9%. Of note, 108 participants out of 10,006 in this study had a positive blood test without cancer, most of who (101) were followed up by PET-CT and 38 also had a subsequent procedure to rule out cancer [34]. This highlights the importance of the high specificity levels required for potential screening tests and clearly defined second-line testing with a good consideration of the risk of overtreatment.

PapSEEK was developed for Pap brush samples or Tao brush samples and detects aneuploidy and somatic mutations on 18 genes by multiplex-PCR (AKT1, APC, BRAF, CDKN2A, CTNNB1, EGFR, FBXW7, FGFR2, KRAS, MAPK1, NRAS, PIK3CA, PIK3R1, POLE, PPP2R1A, PTEN, RNF43, and TP53). 81% endometrial cancer and 29% ovarian cancer were detected by PapSEEK on Pap brush samples which increased to 93% and 45% respectively when intrauterine samples were collected using a Tao brush. False positive rate was 1.4% for Pap brush samples which improved to >99% specificity when using the Tao brush [88].

UroSEEK detects mutations within 11 genes (FGFR3, TP53, CDKN2A, ERBB2, HRAS, KRAS, PIK3CA, MET, VHL, MLL, TERT promoter) as well as aneuploidy. In

an early detection cohort UroSEEK was 83% sensitive and 93% specific while in the surveillance cohort sensitivity was 71% and specificity 80% which was a significant improvement compared to cytology alone [89].

4.2 Galleri

Recently, the UK's National Health Service (NHS) has taken bold steps and will be partnering with GRAIL to confirm Galleri's clinical and economic performance in the NHS system [90]. The study will investigate the effectiveness of the Galleri test on 140,000 asymptomatic, healthy patients and 25,000 participants showing signs and symptoms of cancer. The Galleri test is a genome-wide test interrogating methylation patterns in plasma samples, optimised during the The Circulating Cell-free Genome Atlas Study (CCGA). Methylation patterns, measured by whole genome-bisulfite sequencing, were found to perform better than whole genome and targeted (507genes) sequencing for the detection of cancer [85]. A further study to evaluate the performance of the optimised method, included 6,689 participants with more than 50 cancer types which were approximately equally distributed across stage of the disease (I- IV). The test achieved 99.3% specificity and 55.2% sensitivity across all cancers in the validation sets. Sensitivity improved when detecting more advanced cancer, reporting a detection of 39% of Stage I cancer, 69% of Stage II cancer and 83–92% sensitivity in Stage III & IV cancer. Cancer detection performance varied across different cancer types [85].

Such clinical studies represent landmark studies that paving the way for clinical service to initiate the introduction of liquid biopsy technologies for cancer screening.

5. Potential for EVs and integrative solutions

Tumour derived extracellular vesicles (EV) show great potential for liquid biopsy. EVs carry protein, DNA, RNA and small-RNA cargo shielding it from degradation [33, 91]. The cargoes carried by EVs represents a molecular fingerprint of the cell of origin [30]. A study comparing cfDNA and EV DNA in pleural effusion for EGFR testing by qPCR, shows an improved detection rate when using EV DNA (72.2% vs. 61.1%) [28]. Moreover, research has described that 90% of prostate cancer ctDNA is found in large EVs [92]. EVs are released in abundant quantities presenting an intriguing solution for increase detection sensitivity [30]. TearExo® is a potential solution detecting EV diagnostic and prognostic markers from tears for diagnosis of breast cancer [27].

Despite these advantages, implementation of EVs into clinical cancer diagnostics is hampered by challenges and lack of standardisation in the isolation methods and analytical sensitivity [93]. With improved and standardised technologies and focused efforts, tumour EVs can potentially be used to selectively pick out tumour-derived DNA from a background of normal DNA enhancing ctDNA technology sensitivity but also enable analysis of DNA, RNA and protein from the same sample, potentially for yet earlier detection.

Several challenges remain to be elucidated. EV populations are diverse and the functions and contents of EVs across their size distribution is not well known. The shedding rates across different tumour types or disease states are cannot be assessed without a standardised and accurate method for sizing and specific size isolation. Several concerted efforts are leading the way to technical standardisation to robustly understand the role of EVs [93, 94].

Solutions that integrate multi-modal testing are budding, such as Epic Sciences' Comprehensive cancer profiling that performs CTC, ctDNA and immune-cell analysis from a single blood draw relying characterising protein, morphology and genomics. CancerSEEK integrates protein markers with ctDNA analysis. Such approaches may be key to unlock the full potential of liquid biopsies but present technical, workflow and interpretation challenges [95].

6. Conclusion

Liquid biopsy is currently a clinically useful tool for assisting companion diagnostics, cancer screening programmes and surveillance. There is an evident prevalence of ctDNA solutions which are already available for the companion diagnostic space and are expected to be accessing the earlier diagnostic space soon following clear delineation of the clinical value and applications. CTC solutions, the first approved liquid biopsy tool for clinical use, have a role in defining cancer prognosis and therapeutic monitoring for timely and effective therapeutic decisions. The clinical value and approach remain to be defined by further clinical studies and translation into practical, clinically applicable solutions.

The full potential of EVs is being uncovered with concerted efforts to establish rigour and standardisation driving reproducible research. Apart from the role of EVs for therapeutic applications, EVs show great potential for early diagnosis of cancer, therapeutic monitoring and post-therapeutic surveillance. Versatile and open technologies could facilitate integrated solutions to maximise the potential of liquid biopsy. Nonetheless, translation to the clinical setting will require practical solutions with clearly defined clinical applications.

Promising data is emerging across potential applications for liquid biopsy with multi-cancer early detection solutions expected in the near future.

Author details

Shawn Baldacchino
Applied Biotech Ltd, Cambridge, UK

*Address all correspondence to: shawn.baldacchino@appliedbiotechltd.com;
shawn.baldacchino@gmail.com

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References

- [1] Falzone L, Salomone S, Libra M. Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front Pharmacol*. 2018;9(November 2018):1300.
- [2] Tu SM, Bilen MA, Tannir NM. Personalised cancer care: promises and challenges of targeted therapy. *J R Soc Med*. 2016;109(3):98-105.
- [3] Bremnes RM, Dønnem T, Al-Saad S, Al-Shibli K, Andersen S, Sirera R, et al. The role of tumor stroma in cancer progression and prognosis: Emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. *J Thorac Oncol* [Internet]. 2011;6(1):209-17. Available from: <http://dx.doi.org/10.1097/JTO.0b013e3181f8a1bd>
- [4] Liu Q, Zhang H, Jiang X, Qian C, Liu Z, Luo D. Factors involved in cancer metastasis: a better understanding to “seed and soil” hypothesis. *Mol Cancer* [Internet]. 2017 Dec;16(1):176. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29197379>
- [5] Lopez JS, Banerji U. Combine and conquer: Challenges for targeted therapy combinations in early phase trials. *Nat Rev Clin Oncol*. 2017;14(1):57-66.
- [6] Green S. Cancer beyond genetics: On the practical implications of downward causation [Internet]. 2019. Available from: <http://philsci-archive.pitt.edu/16297/>
- [7] Sandoval GJ, Hahn WC. Going beyond genetics to discover cancer targets. *Genome Biol*. 2017;18(1):17-9.
- [8] Poole JC, Wu SF, Lu TT, Vibat CRT, Pham A, Samuelsz E, et al. Analytical validation of the Target Selector ctDNA platform featuring single copy detection sensitivity for clinically actionable EGFR, BRAF, and KRAS mutations. *PLoS One*. 2019;14(10):1-22.
- [9] Oussalah A, Rischer S, Bensenane M, Conroy G, Filhine-Tresarrieu P, Debard R, et al. Plasma mSEPT9: A Novel Circulating Cell-free DNA-Based Epigenetic Biomarker to Diagnose Hepatocellular Carcinoma. *EBioMedicine* [Internet]. 2018;30:138-47. Available from: <https://doi.org/10.1016/j.ebiom.2018.03.029>
- [10] Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado L V., Roberts WL, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. *BMC Med* [Internet]. 2011;9(1):133. Available from: <https://doi.org/10.1186/1741-7015-9-133>
- [11] Lamb YN, Dhillon S. Epi proColon® 2.0 CE: A Blood-Based Screening Test for Colorectal Cancer. *Mol Diagnosis Ther*. 2017;21(2):225-32.
- [12] Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. *N Engl J Med*. 2014;370(14):1287-97.
- [13] Thierry AR. A targeted Q-PCR-based method for point mutation testing by analyzing circulating DNA for cancer management care. *Methods Mol Biol*. 2016;1392:1-16.
- [14] DiaDx | Liquid Biopsy For Personalized Medecine In Oncology [Internet]. [cited 2021 Jan 12]. Available from: <https://diadx.com/>
- [15] Pichler R, Fritz J, Tulchiner G, Klinglmair G, Soleiman A, Horninger W, et al. Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. *BJU Int* [Internet]. 2018 Jan 1 [cited 2021 Jan 12];121(1):29-37. Available from: <http://doi.wiley.com/10.1111/bju.14019>
- [16] Batista R, Vinagre N, Meireles S, Vinagre J, Prazeres H, Leão R, et al.

Biomarkers for bladder cancer diagnosis and surveillance: A comprehensive review. *Diagnostics*. 2020;10(1):1-19.

[17] Witjes JA, Morote J, Cornel EB, Gakis G, van Valenberg FJP, Lozano F, et al. Performance of the Bladder EpiCheck™ Methylation Test for Patients Under Surveillance for Non-muscle-invasive Bladder Cancer: Results of a Multicenter, Prospective, Blinded Clinical Trial. *Eur Urol Oncol* [Internet]. 2018;1(4):307-13. Available from: <https://doi.org/10.1016/j.euo.2018.06.011>

[18] De Richter P. Conquering Complexity : The Coming Revolution in Oncology Biomarker Testing [Internet]. 2017 [cited 2021 Jan 10]. Available from: https://www.ipsos.com/sites/default/files/2017-06/Ipsos_Healthcare_Conquering_Complexity_June_2017.pdf

[19] List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools) | FDA [Internet]. [cited 2021 Jan 11]. Available from: <https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools>

[20] FDA approves liquid biopsy NGS companion diagnostic test for multiple cancers and biomarkers | FDA [Internet]. [cited 2021 Jan 11]. Available from: <https://www.fda.gov/drugs/fda-approves-liquid-biopsy-ngs-companion-diagnostic-test-multiple-cancers-and-biomarkers>

[21] FDA Approves First Liquid Biopsy Next-Generation Sequencing Companion Diagnostic Test | FDA [Internet]. [cited 2021 Jan 11]. Available from: <https://www.fda.gov/news-events/press-announcements/fda-approves-first-liquid-biopsy-next-generation-sequencing-companion-diagnostic-test>

[22] Leighl NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, et

al. Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-small Cell Lung Cancer. *Clin Cancer Res*. 2019;25(15):4691-700.

[23] De Mattos-Arruda L, Mayor R, Ng CKY, Weigelt B, Martínez-Ricarte F, Torrejon D, et al. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat Commun*. 2015;6:1-6.

[24] Robertson DJ, Imperiale TF. Stool Testing for Colorectal Cancer Screening. *Gastroenterology* [Internet]. 2015;149(5):1286-93. Available from: <http://www.sciencedirect.com/science/article/pii/S0016508515007726>

[25] Halvaei S, Daryani S, Eslami-S Z, Samadi T, Jafarbeik-Iravani N, Bakhshayesh TO, et al. Exosomes in Cancer Liquid Biopsy: A Focus on Breast Cancer. Vol. 10, *Molecular Therapy - Nucleic Acids*. 2018. p. 131-41.

[26] 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(4):223-38.

[27] Takeuchi T, Mori K, Sunayama H, Takano E, Kitayama Y, Shimizu T, et al. Antibody-Conjugated Signaling Nanocavities Fabricated by Dynamic Molding for Detecting Cancers Using Small Extracellular Vesicle Markers from Tears. *J Am Chem Soc*. 2020;142(14):6617-24.

[28] Lee JS, Hur JY, Kim IA, Kim HJ, Choi CM, Lee JC, et al. Liquid biopsy using the supernatant of a pleural effusion for EGFR genotyping in pulmonary adenocarcinoma patients: A comparison between cell-free DNA and extracellular vesicle-derived DNA. *BMC Cancer*. 2018;18(1):1-8.

- [29] Villatoro S, Mayo-de-Las-Casas C, Jordana-Ariza N, Viteri-Ramírez S, Garzón-Ibañez M, Moya-Horno I, et al. Prospective detection of mutations in cerebrospinal fluid, pleural effusion, and ascites of advanced cancer patients to guide treatment decisions. *Mol Oncol* [Internet]. 2019/10/11. 2019 Dec;13(12):2633-45. Available from: <https://pubmed.ncbi.nlm.nih.gov/31529604>
- [30] De Rubis G, Rajeev Krishnan S, Bebawy M. Liquid Biopsies in Cancer Diagnosis, Monitoring, and Prognosis. *Trends Pharmacol Sci* [Internet]. 2019;40(3):172-86. Available from: <https://doi.org/10.1016/j.tips.2019.01.006>
- [31] Hofman P, Heeke S, Alix-Panabières C, Pantel K. Liquid biopsy in the era of immuno-oncology: Is it ready for prime-time use for cancer patients? *Ann Oncol*. 2019;30(9):1448-59.
- [32] Russano M, Napolitano A, Ribelli G, Iuliani M, Simonetti S, Citarella F, et al. Liquid biopsy and tumor heterogeneity in metastatic solid tumors: The potentiality of blood samples. *J Exp Clin Cancer Res*. 2020;39(1):1-13.
- [33] Brock G, Castellanos-Rizaldos E, Hu L, Coticchia C, Skog J. Liquid biopsy for cancer screening, patient stratification and monitoring. *Transl Cancer Res*. 2015;4(3):280-90.
- [34] Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, Cohain AT, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. *Science*. 2020;369(6499):eabb9601.
- [35] Pasini L, Ulivi P. Liquid Biopsy for the Detection of Resistance Mechanisms in NSCLC: Comparison of Different Blood Biomarkers. *J Clin Med*. 2019;8(7):998.
- [36] Ulz P, Heitzer E, Geigl JB, Speicher MR. Patient monitoring through liquid biopsies using circulating tumor DNA. *Int J Cancer*. 2017;141(5):887-96.
- [37] Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease — latest advances and implications for cure. *Nat Rev Clin Oncol* [Internet]. 2019;16(7):409-24. Available from: <http://dx.doi.org/10.1038/s41571-019-0187-3>
- [38] Vacante M, Ciuni R, Basile F, Biondi A. The liquid biopsy in the management of colorectal cancer: An overview. *Biomedicines*. 2020;8(9):308.
- [39] Venesio T, Siravegna G, Bardelli A, Sapino A. Liquid Biopsies for Monitoring Temporal Genomic Heterogeneity in Breast and Colon Cancers. *Pathobiology*. 2018;85(1-2):146-54.
- [40] Reck M, Hermes A, Tan EH, Felip E, Klughammer B, Baselga J. Tissue sampling in lung cancer: A review in light of the MERIT experience. *Lung Cancer* [Internet]. 2011;74(1):1-6. Available from: <http://dx.doi.org/10.1016/j.lungcan.2011.05.002>
- [41] Al-Kateb H, Nguyen TDT, Steger-May K, Pfeifer JD. Identification of major factors associated with failed clinical molecular oncology testing performed by next generation sequencing (NGS). *Mol Oncol*. 2015;9(9):1737-43.
- [42] Robbe P, Popitsch N, Knight SJL, Antoniou P, Becq J, He M, et al. Clinical whole-genome sequencing from routine formalin-fixed, paraffin-embedded specimens: pilot study for the 100,000 Genomes Project. *Genet Med*. 2018;20(10):1196-205.
- [43] McDonough SJ, Bhagwate A, Sun Z, Wang C, Zschunke M, Gorman JA,

et al. Use of FFPE-Derived DNA in Next Generation Sequencing: DNA extraction methods. PLoS One. 2019;14(4):e0211400.

[44] Nucleic Acid Based Tests | FDA [Internet]. [cited 2021 Jan 10]. Available from: <https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests>

[45] Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagnostics [Internet]. 2015;17(3):251-64. Available from: <http://dx.doi.org/10.1016/j.jmoldx.2014.12.006>

[46] Takeda M, Takahama T, Sakai K, Shimizu S, Watanabe S, Kawakami H, et al. Clinical Application of the FoundationOne CDx Assay to Therapeutic Decision-Making for Patients with Advanced Solid Tumors. Oncologist [Internet]. 2020 Dec 16;n/a(n/a):1-9. Available from: <https://doi.org/10.1002/onco.13639>

[47] Dietrich D, Uhl B, Sailer V, Holmes EE, Jung M, Meller S, et al. Improved PCR Performance Using Template DNA from Formalin-Fixed and Paraffin-Embedded Tissues by Overcoming PCR Inhibition. PLoS One. 2013;8(10):1-10.

[48] Minshall N, Git A. Enzyme- and gene-specific biases in reverse transcription of RNA raise concerns for evaluating gene expression. Sci Rep. 2020;10(1):1-7.

[49] Baldacchino S, Saliba C, Scerri J, Scerri C, Grech G. Optimization of a Multiplex RNA-based Expression Assay Using Breast Cancer Archival Material. JoVE [Internet]. 2018;(138):e57148.

Available from: <https://www.jove.com/t/57148>

[50] Knudsen BS, Allen AN, McLerran DF, Vessella RL, Karademos J, Davies JE, et al. Evaluation of the branched-chain DNA assay for measurement of RNA in formalin-fixed tissues. J Mol Diagnostics [Internet]. 2008;10(2):169-76. Available from: <http://dx.doi.org/10.2353/jmoldx.2008.070127>

[51] Veldman-Jones MH, Brant R, Rooney C, Geh C, Emery H, Harbron CG, et al. Evaluating robustness and sensitivity of the nanostring technologies ncounter platform to enable multiplexed gene expression analysis of clinical samples. Cancer Res. 2015;75(13):2587-93.

[52] Campos CDM, Jackson JM, Witek MA, Soper SA. Molecular Profiling of Liquid Biopsy Samples for Precision Medicine. Cancer J (United States). 2018;24(2):93-103.

[53] Jia Y, Ni Z, Sun HAO, Wang C. Microfluidic Approaches Toward the Isolation and Detection of Exosome Nanovesicles. IEEE Access. 2019;7:45080-98.

[54] Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. Mol Oncol [Internet]. 2016;10(3):374-94. Available from: <http://dx.doi.org/10.1016/j.molonc.2016.01.007>

[55] Deans ZC, Butler R, Cheetham M, Dequeker EMC, Fairley JA, Fenizia F, et al. IQN path ASBL report from the first European cfDNA consensus meeting: expert opinion on the minimal requirements for clinical ctDNA testing. Virchows Arch. 2019;474(6):681-9.

[56] Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung

Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer. *J Thorac Oncol* [Internet]. 2018 Mar;13(3):323-58. Available from: <https://doi.org/10.1016/j.jtho.2017.12.001>

[57] Kwapisz D. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? *Ann Transl Med*. 2017;5(3):1-7.

[58] U.S. Food and Drug Administration. Optimizing FDA's Regulatory Oversight of Next Generation Sequencing Diagnostic Tests—Preliminary Discussion Paper [Internet]. Vol. 3. 2015. Available from: <https://www.fda.gov/media/90403/download>

[59] Allegretti M, Fabi A, Buglioni S, Martayan A, Conti L, Pescarmona E, et al. Tearing down the walls: FDA approves next generation sequencing (NGS) assays for actionable cancer genomic aberrations. *J Exp Clin Cancer Res*. 2018;37(1):1-3.

[60] Walens A, Lin J, Damrauer JS, McKinney B, Lupo R, Newcomb R, et al. Adaptation and selection shape clonal evolution of tumors during residual disease and recurrence. *Nat Commun* [Internet]. 2020;11(1):1-15. Available from: <http://dx.doi.org/10.1038/s41467-020-18730-z>

[61] Parikh AR, Leshchiner I, Elagina L, Goyal L, Levovitz C, Siravegna G, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat Med* [Internet]. 2019;25(9):1415-21. Available from: <http://dx.doi.org/10.1038/s41591-019-0561-9>

[62] Finzel A, Sadik H, Ghitti G, Laes J-F. The combined analysis of solid and liquid biopsies provides additional

clinical information to improve patient care. *J Cancer Metastasis Treat*. 2018;4(5):21.

[63] Jones RM, Devers KJ, Kuzel AJ, Woolf SH. Patient-Reported Barriers to Colorectal Cancer Screening. A Mixed-Methods Analysis. *Am J Prev Med*. 2010;38(5):508-16.

[64] Fraser CG, Digby J, McDonald PJ, Strachan JA, Carey FA, Steele RJC. Experience with a two-tier reflex gFOBT/FIT strategy in a national bowel screening programme. *J Med Screen*. 2012;19(1):8-13.

[65] Steele RJ, McDonald PJ, Digby J, Brownlee L, Strachan JA, Libby G, et al. Clinical outcomes using a faecal immunochemical test for haemoglobin as a first-line test in a national programme constrained by colonoscopy capacity. *United Eur Gastroenterol J*. 2013;1(3):198-205.

[66] Mulat B, Boroda K, Hertan H. 1673 Multitarget Stool DNA Test (Cologuard™): A Double-Edged Sword. *Am J Gastroenterol | ACG* [Internet]. 2019;114(p S935-S936). Available from: https://journals.lww.com/ajg/Fulltext/2019/10001/1673_Multitarget_Stool_DNA_Test_Cologuard____A.1674.aspx

[67] (Quality) OH. Cell-Free Circulating Tumour DNA Blood Testing to Detect EGFR T790M Mutation in People With Advanced Non-Small Cell Lung Cancer: A Health Technology Assessment. *Ont Health Technol Assess Ser* [Internet]. 2020 Mar 6;20(5):1-176. Available from: <https://pubmed.ncbi.nlm.nih.gov/32206157>

[68] Sands J, Li Q, Hornberger J. Urine circulating-tumor DNA (ctDNA) detection of acquired EGFR T790M mutation in non-small-cell lung cancer: An outcomes and total cost-of-care analysis. *Lung Cancer* [Internet]. 2017;110(November 2016):19-25.

Available from: <http://dx.doi.org/10.1016/j.lungcan.2017.05.014>

[69] Tutrone R, Donovan MJ, Torkler P, Tadigotla V, McLain T, Noerholm M, et al. Clinical utility of the exosome based ExoDx Prostate(IntelliScore) EPI test in men presenting for initial Biopsy with a PSA 2-10 ng/mL. *Prostate Cancer Prostatic Dis* [Internet]. 2020;23(4):607-14. Available from: <http://dx.doi.org/10.1038/s41391-020-0237-z>

[70] Pinsky PF, Prorok PC, Kramer BS. Prostate Cancer Screening — A Perspective on the Current State of the Evidence. *N Engl J Med*. 2017;376(13):1285-9.

[71] Ilic D, Neuberger MM, Djulbegovic M, Dahm P. Screening for prostate cancer. *Cochrane Database Syst Rev* [Internet]. 2013 Jan 31 [cited 2021 Jan 14];2013(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/23440794/>

[72] Miller MC, Doyle G V., Terstappen LWMM. Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer. *J Oncol*. 2010;2010:1-8.

[73] Wang L, Balasubramanian P, Chen AP, Kummar S, Evrard YA, Kinders RJ. Promise and limits of the CellSearch platform for evaluating pharmacodynamics in circulating tumor cells. *Semin Oncol* [Internet]. 2016;43(4):464-75. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27663478>

[74] Guo T, Wang CS, Wang W, Lu Y. Culture of Circulating Tumor Cells - Holy Grail and Big Challenge. *Int J Cancer Clin Res*. 2016;3(4):065.

[75] Takahashi Y, Shirai K, Ijiri Y, Morita E, Yoshida T, Iwanaga S, et al. Integrated system for detection and molecular characterization of circulating tumor cells. Patel GK, editor.

PLoS One [Internet]. 2020 Aug 13 [cited 2021 Jan 16];15(8):e0237506. Available from: <https://dx.plos.org/10.1371/journal.pone.0237506>

[76] Xu Y, Liu B, Ding F, Zhou X, Tu P, Yu B, et al. Circulating tumor cell detection: A direct comparison between negative and unbiased enrichment in lung cancer. *Oncol Lett*. 2017;13(6):4882-6.

[77] Liu Z, Guo W, Zhang D, Pang Y, Shi J, Wan S, et al. Circulating tumor cell detection in hepatocellular carcinoma based on karyoplasmic ratios using imaging flow cytometry. *Sci Rep*. 2016;6(December):6-15.

[78] Lopresti A, Malergue F, Bertucci F, Liberatoscioli ML, Garnier S, DaCosta Q, et al. Sensitive and easy screening for circulating tumor cells by flow cytometry. *JCI Insight*. 2019;4(14):1-14.

[79] Dent BM, Ogle LF, O'donnell RL, Hayes N, Malik U, Curtin NJ, et al. High-resolution imaging for the detection and characterisation of circulating tumour cells from patients with oesophageal, hepatocellular, thyroid and ovarian cancers. *Int J Cancer*. 2016;138(1):206-16.

[80] Krause S, Friedl T, Romashova T, Fasching P, Schneeweiss A, Müller V, et al. Abstract OT1-10-01: DETECT III/IV study trial – The multicenter study program in patients with HER2-negative metastatic breast cancer and circulating tumor cells. In: *Cancer Research* [Internet]. American Association for Cancer Research (AACR); 2019 [cited 2021 Jan 16]. p. OT1-10-01-OT1-10-01. Available from: https://cancerres.aacrjournals.org/content/79/4_Supplement/OT1-10-01

[81] Stoecklein NH, Fischer JC, Niederacher D, Terstappen LWMM. Challenges for CTC-based liquid biopsies: low CTC frequency and

diagnostic leukapheresis as a potential solution. *Expert Rev Mol Diagn* [Internet]. 2016 Feb 1;16(2):147-64. Available from: <https://doi.org/10.1586/14737159.2016.1123095>

[82] Song Y, Hu C, Xie Z, Wu L, Zhu Z, Rao C, et al. Circulating tumor DNA clearance predicts prognosis across treatment regimen in a large real-world longitudinally monitored advanced non-small cell lung cancer cohort. *Transl Lung Cancer Res*. 2020;9(2):269-79.

[83] Verma M, Lam TK, Hebert E, Divi RL. Extracellular vesicles: Potential applications in cancer diagnosis, prognosis, and epidemiology. *BMC Clin Pathol*. 2015;15(1):1-9.

[84] ONS. Cancer survival in England - Office for National Statistics [Internet]. Office for national statistics. 2019 [cited 2021 Jan 14]. Available from: <https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/cancersurvivalratescancersurvivalinenglandadultsdiagnosed>

[85] Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden M V., Liu MC, et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann Oncol*. 2020;31(6):745-59.

[86] 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* [Internet]. 2018/01/18. 2018 Feb 23;359(6378):926-30. Available from: <https://pubmed.ncbi.nlm.nih.gov/29348365>

[87] Avanzini S, Kurtz DM, Chabon JJ, Moding EJ, Hori SS, Gambhir SS, et al. A mathematical model of ctDNA shedding predicts tumor detection size. *Sci Adv* [Internet]. 2020 Dec

1;6(December):1-10. Available from: <http://advances.sciencemag.org/content/6/50/eabc4308.abstract>

[88] Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, et al. Evaluation of liquid from the papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. *Obstet Gynecol Surv*. 2018;73(8):463-4.

[89] Springer SU, Chen C-H, Rodriguez Pena MDC, Li L, Douville C, Wang Y, et al. Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy. *Elife* [Internet]. 2018 Mar 20;7:e32143. Available from: <https://doi.org/10.7554/eLife.32143>

[90] GRAIL and UK Government to Make Galleri Multi-Cancer Early Detection Blood Test Available to Patients | GRAIL [Internet]. [cited 2021 Jan 15]. Available from: <https://grail.com/press-releases/grail-and-uk-government-to-make-galleri-multi-cancer-early-detection-blood-test-available-to-patients/>

[91] Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—A survey. *Biochim Biophys Acta - Rev Cancer* [Internet]. 2007;1775(1):181-232. Available from: <http://www.sciencedirect.com/science/article/pii/S0304419X0600059X>

[92] Vagner T, Spinelli C, Minciocchi VR, Balaj L, Zandian M, Conley A, et al. Large extracellular vesicles carry most of the tumour DNA circulating in prostate cancer patient plasma. *J Extracell vesicles* [Internet]. 2018 Aug 7;7(1):1505403. Available from: <https://doi.org/10.1080/20013078.2018.1505403>

[93] Théry C, Witwer KW, Aikawa E, Alcaraz MJM, Anderson JDJD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement

of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7(1):1535750.

[94] Geeurickx E, Lippens L, Rappu P, De Geest BG, De Wever O, Hendrix A. Recombinant extracellular vesicles as biological reference material for method development, data normalization and assessment of (pre-)analytical variables. *Nat Protoc [Internet]*. 2021; Available from: <https://doi.org/10.1038/s41596-020-00446-5>

[95] Qiu J, Xu J, Zhang K, Gu W, Nie L, Wang G, et al. Refining cancer management using integrated liquid biopsy. *Theranostics*. 2020;10(5):2374-84.