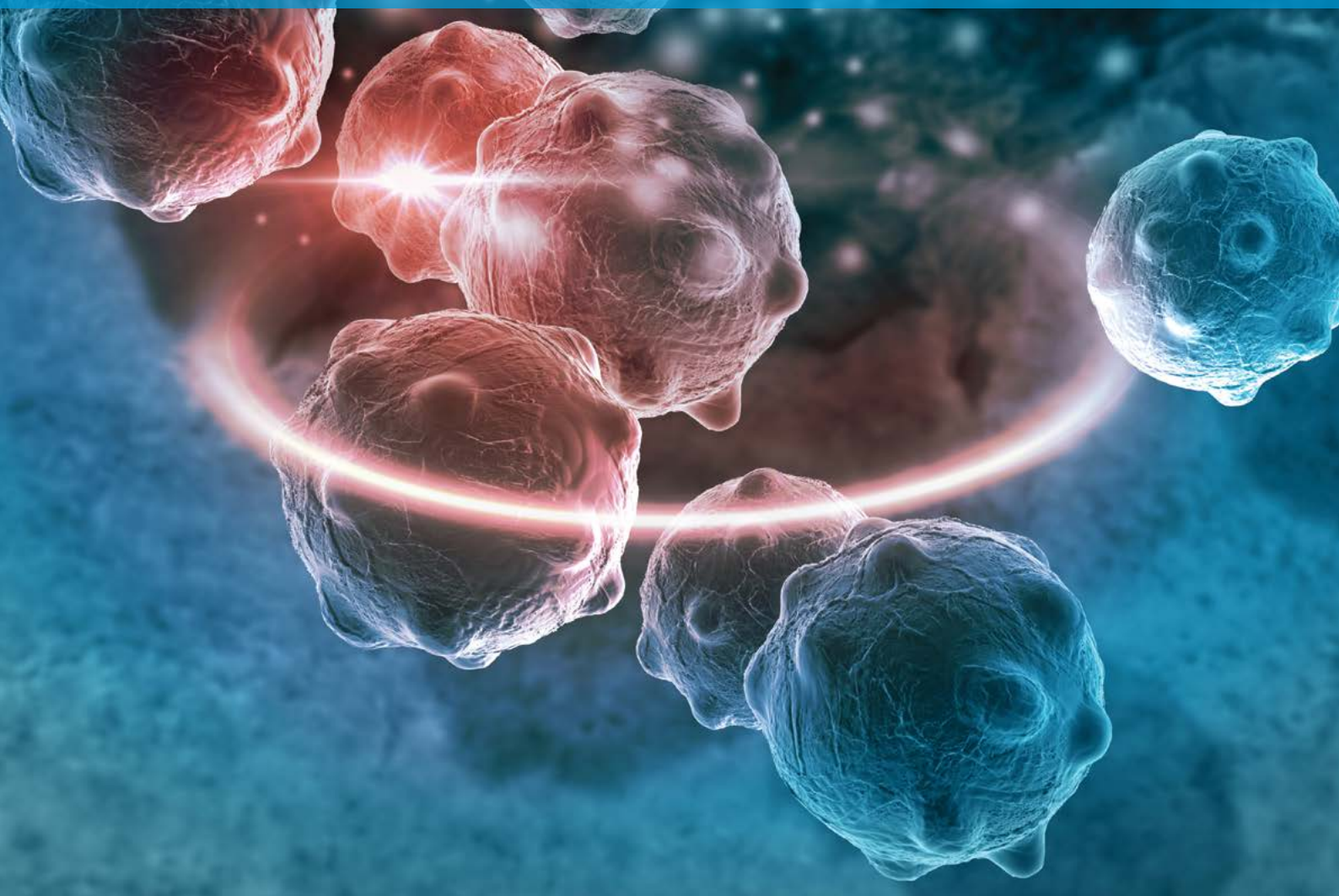
Cambridge Consultants

LIQUID BIOPSY: CURRENT AND FUTURE UTILITY

MEDICAL TECHNOLOGY



INTRODUCTION

The field of medical oncology continues to gather pace, with a record number of targeted therapies approved by the US Food and Drug Administration in 2017[1]. Similarly, molecular diagnostics, driven in large part by oncology, is also maturing with 'liquid biopsy' tests *finally* receiving formal approval as companion diagnostics.

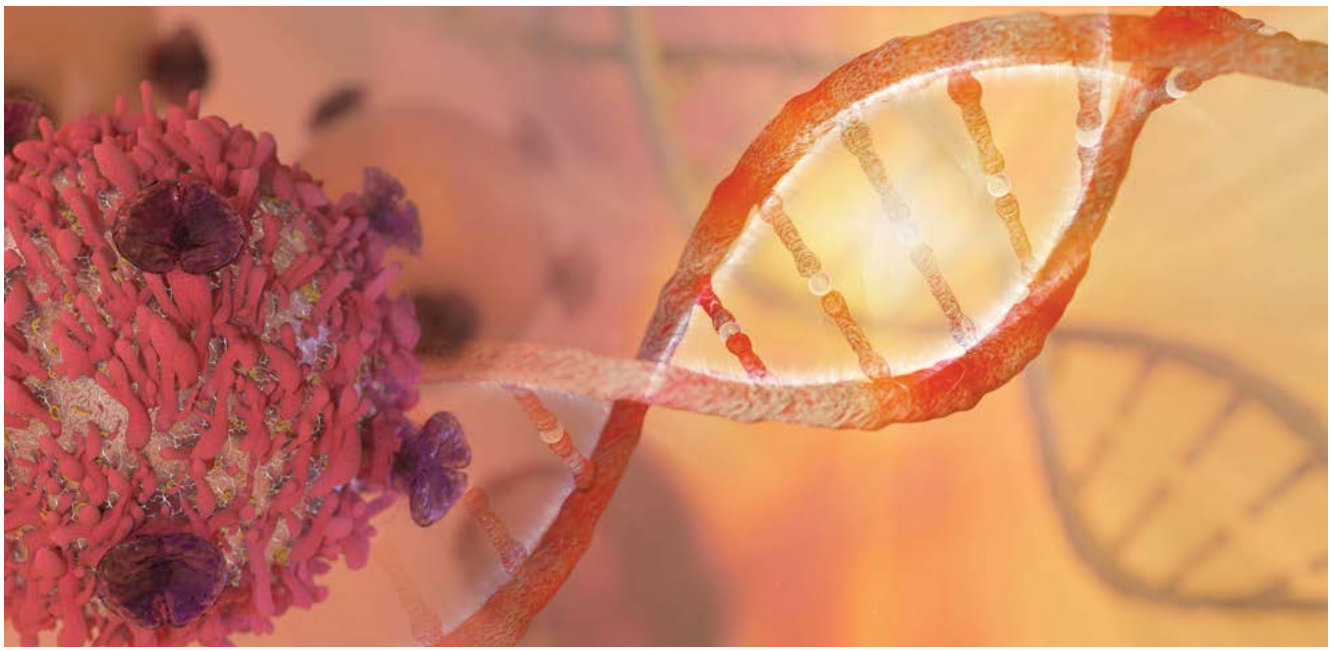
The emergence of 'liquid biopsy' is a key trend that is bringing targeted therapies and molecular diagnostic tests together, in a highly synergistic way. The concept of a liquid biopsy is the ability to capture components from a tumour within mainly blood samples, which can then be analysed to provide diagnostic information about a patient's tumour. Liquid biopsies offer potentially transformative improvements in cancer care, given their ability to rapidly interrogate the molecular basis of disease in a way that classic tissue biopsies cannot.

There is considerable investor and market excitement in technologies relating to liquid biopsy. Biotech firm Grail (a spin-out from Illumina) raised over $900 million in 2017 to push its circulating tumour marker detection platform through clinical studies[2]; investors include Amazon and Merck. The overall global market for liquid biopsies is predicted to reach over $4 billion by 2025 with double-digit annual growth[3]. Announcements of developmental and commercial milestones relating to liquid biopsies are appearing regularly in healthcare industry news flow, as shown in Table 1.

Therefore, Cambridge Consultants sought to understand the current usage and future potential of liquid biopsy, by facilitating a panel discussion that brought in expertise across disciplines. The discussion addressed the following key question:

What is the current and future clinical utility of liquid biopsy-based testing in oncology?

This report presents the main analysis and insights from this panel discussion and goes on to consider how technical bottlenecks to broad clinical adoption of liquid biopsy may be addressed.



1 https://www.cancer.org/latest-news/new-cancer-drug-approvals-from-2017.html
2 http://uk.businessinsider.com/grail-raises-more-than-900-million-2017-3?r=US&IR=T
3 https://globenewswire.com/news-release/2017/12/06/1234086/0/en/4-43-Billion-Liquid-Biopsy-Market-2025.html

PARTNER(S)	DESCRIPTION OF INVESTMENT ACTIVITY
Abbott/Angle	Abbott partnership with Angle on a liquid biopsy study for breast cancer. The trial will assess the technology's utility for harvesting circulating tumour cells in combination with Abbott's HER-2 test. **SOURCE:** http://www.londonstockexchange.com/exchange/news/market-news/market-news-detail/AGL/13522406.html
Biocartis	Biocartis announced an €80 million private placement to expand its product range and sales and manufacturing capacity **SOURCE:** https://biocartis.com/news/article/press-release-biocartis-successfully-raises-eur-80-million-in-an-equity-placement
BMS & NEA/ Personal Genome Diagnostics	Bristol-Myers Squibb and NEA have co-led a $75 million investment in Personal Genome Diagnostics (PGDx). Funding gives PGDx the capital to pursue approvals of both tissue and liquid biopsy cancer tests in global markets. **SOURCE:** http://www.personalgenome.com/wp-content/uploads/2018/01/PGDx-Series-B-Press-Release-for-1.4.18-final-corrected-1.pdf
Microsoft/ Adaptive Biotechnologies	Microsoft has partnered with Adaptive Biotechnologies' to develop blood-based diagnostics. Microsoft is contributing its machine learning and cloud computing capabilities to the effort, and further has made an equity investment in Adaptive Biotechnologies. **SOURCE:** https://www.adaptivebiotech.com/news/adaptive-biotechnologies-announces-partnership-microsoft-decode-human-immune-system-improve
Qiagen/Clinical Genomics	Qiagen has incoporated its circulating cell-free DNA (ccfDNA) blood-collection tubes in Clinical Genomics' liquid biopsy test for the recurrence of colorectal cancer. **SOURCE:** https://clinicalgenomics.com/qiagen-and-clinical-genomics-partner-on-liquid-biopsies-to-monitor-patients-for-recurrence-of-colorectal-cancer/

Table 1 – Recent investment and partnering activity in the field of liquid biopsy

WHAT IS A LIQUID BIOPSY, REALLY?

The term 'liquid biopsy' was originally defined in limited terms by Pantel and Alix-Panabieres in 2010[4]: *"…although promising data from patients with advanced disease demonstrated the value of CTC [circulating tumour cell] analysis as 'liquid biopsy', studies on cancer patients at earlier stages are hampered by low CTC counts…"*.

In this paper the panel defined the term as a two-step process by which circulating tumour cells (CTCs), circulating free tumour DNA (cfDNA) or exosomes are harvested, from a sample of whole blood, plasma or serum. This enriched biological material then becomes the starting point for conventional or next-generation molecular diagnostic analyses (see Figure 1). In this sense, liquid biopsy represents an innovative preanalytical step that enables downstream molecular pathology.

"The term has since become a buzzword for the non-invasive capture and analysis of stray biological components from tumours. It doesn't yet appear to have a fixed definition, at least not one that the FDA or other competent authorities have agreed upon – compared with companion diagnostics or complementary diagnostics"

PANEL MEMBER

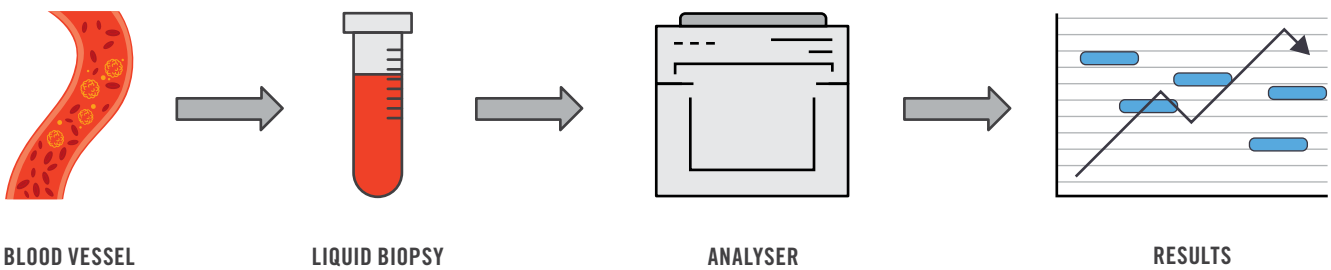


Figure 1 – Circulating tumour markers as basis for liquid biopsy

4 Pantel and Alix-Panabieres (2010). Circulating tumour cells in cancer patients: challenges and perspectives. Trends in Molecular Medicine 16, 398-406

HOW IS LIQUID BIOPSY BEING USED TODAY?

"Liquid biopsies are used today for limited points of clinical investigation along the cancer care pathway, including treatment selection, treatment monitoring and prognostic testing"

PANEL MEMBER

Key fields of investigation, and representative examples, are shown in Figure 2. These include:

- **TREATMENT SELECTION:** mandatory (companion) or discretionary (complementary) diagnostic tests used to steer safe and effective prescribing decisions on the use of targeted therapies.
- **TREATMENT MONITORING:** tests that analyse the activity of a group of genes that can affect how a cancer is likely to behave or respond to medical/surgical treatment
- **PROGNOSTIC TESTING:** tests that enable the monitoring of a patient's molecular basis of disease over time, to evaluate disease 'evolution' (e.g. clonal variation) or minimal residual disease

The use also highlights the different regulatory approaches to liquid biopsy. Kits may be approved for in vitro diagnostic use ('FDA approved' or CE-IVD marked), in which case their application is defined and limited by their intended use or indication described on the product label. Alternatively, they may be approved for research use only (RUO), which provides scope for clinical investigation but cannot be used to directly steer prescribing decisions. Finally, liquid biopsies may be regulated as a laboratory developed test (LDTs) for use in individual pathology laboratories or as a basis for a central 'send-out' service, whereby oncologists will send samples to a dedicated laboratory for analysis.

Recent mainstream news flow has made much of the CancerSEEK diagnostic test, particularly its ability to detect multiple types of cancer from a single blood sample. In reality, further confirmatory tests will be required post-screening before a patient is placed on the current standard of care for their particular cancer. Also, the test's sensitivity falls to 70% for the purposes of screening certain cancers, which is insufficient to drive standalone clinical decisions. Therefore, considerable further development and validation of liquid biopsy-type platforms are required to establish such a 'one stop' diagnostic test.

The detection of circulating tumour markers has greatest utility in the monitoring of certain types of non-localising cancers, e.g. non-small cell lung cancer (NSCLC). NSCLC is the most common form of lung cancer, affecting up to 80% of patients diagnosed with the disease. Other examples include carcinomas, such as

DIAGNOSIS	TREATMENT SELECTION	PROGNOSIS	MONITORING (LONGITUDINAL)
Confirmatory or supporting of cancer based on detection of molecular markers of disease **Example: *CellSearch® Circulating Tumor Cell Kit* (Menarini Silicon Biosystems)** FDA-approved (US-IVD) kit intended for the enumeration of CTCs of epithelial origin in whole blood.	Companion diagnostic tests used to guide prescribing decisions on safe and effective use of targeted therapies **Example: *cobas EGFR® Mutation Test v2* (Roche)** FDA-approved/CE-marked test for the detection of mutations of the EGFR gene in NSCLC patients from plasma; indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with targeted therapies	Tests that analyse the activity of a group of genes that can affect how a cancer is likely to behave or respond to medical/surgical treatment **Example: *FoundationACT®* (Foundation Medicine)** Central 'send-out' service that utilised a laboratory developed test (LDT); liquid biopsy-based ctDNA assay for solid tumours that identifies >60 clinically relevant genomic alterations driving the growth of patient's cancer	Tests that enable the monitoring of a patient's molecular basis of disease over time to evaluate disease 'evolution' (eg clonal variation) or minimal residual diease **Example: *Oncomine™ Lung cfDNA Assay* (ThermoFisher)** Research Use Only (RUO) tumour type-specific, multi-biomarker (NGS) assay that enables detection of somatic mutations in genes found in plasma samples; also offered as LDT by Cancer Genetics, Inc.

Figure 2 – Current settings of interest for liquid biopsy along the cancer care pathway

colorectal cancer. Technically, liquid biopsy doesn't lend itself to the monitoring of all types of cancers. Certain cancers, such as retinoblastoma and glioblastoma, may remain refractive to these tests due to the localising nature of these tumours.

"Liquid biopsies represent a complementary clinical tool in the oncologist's or molecular pathologist's disease management toolbox, rather than a standalone diagnostic solution"

PANEL MEMBER

Precision medicine – the pairing together of companion diagnostics and targeted therapies – is both a key driver and bottleneck to implementation of liquid biopsy in clinical practice. The chief clinical – and therefore commercial – value of liquid biopsy is directly linked to its ability to steer targeted therapy prescribing decisions (an example is provided in Figure 3). Conversely, clinical utility – and therefore commercial value – is largely hamstrung until there emerges a tangible breadth and depth of targeted therapies on offer by pharmaceutical companies (although this is more of a 'when' rather than 'if' argument, at the current rate of approvals). Specifically, liquid biopsy is largely being used to investigate EGFR-based activating mutations; this reflects the relatively narrow range in mechanism of action of the more established targeted therapies. There are a large number of new and emerging therapies, with accompanying diagnostic tests within genes, such as PD/L1, ALK, ROS1 and HER2.

Frequency of use of liquid biopsy approaches is highly limited relative to incumbent methods of investigation, however these platforms are gaining traction. It is used predominantly on a clinical research footing on a RUO-basis to investigate disease pathogenesis (clonality) and drug response. However, cautious optimism in its clinical promise is providing greater acceleration toward this path than many previous concepts in cancer care.

"Currently, liquid biopsy is an emerging platform which hasn't yet been adopted into mainstream clinical practice; it is at a transition point between basic research and clinical practice"

PANEL MEMBER

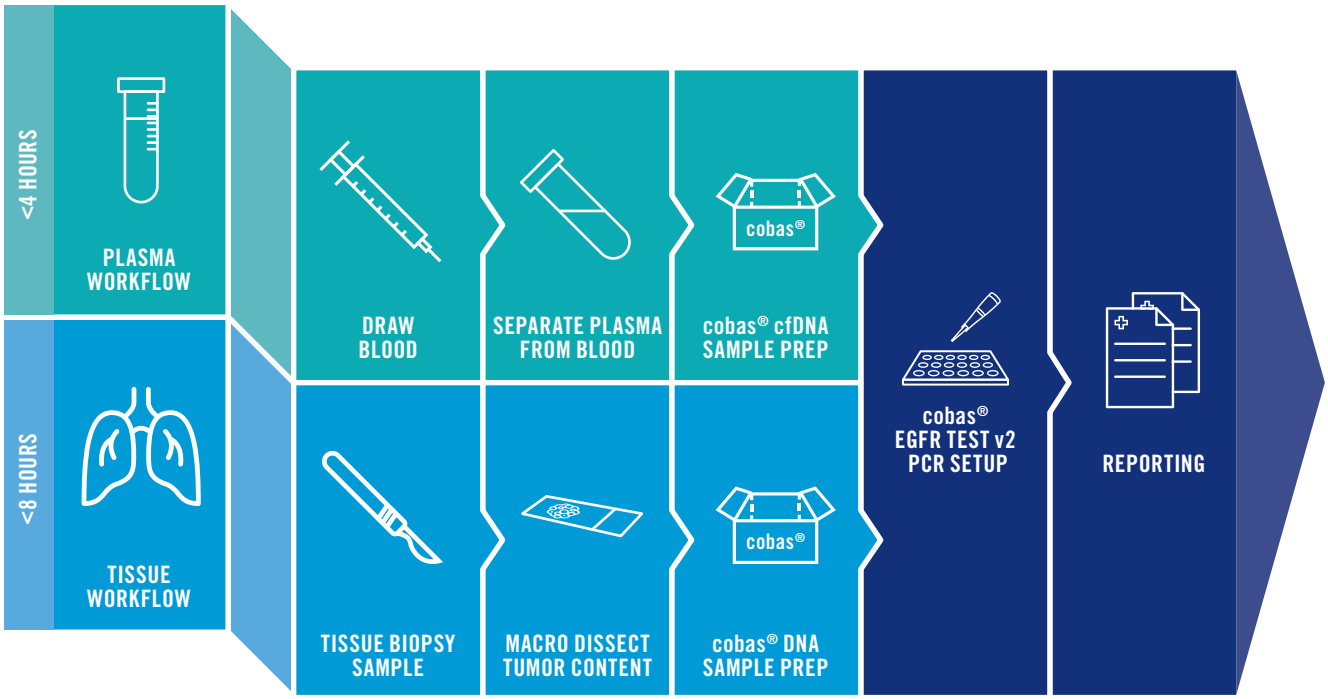


Figure 3 – Roche's cobas® EGFR Mutation Test v2 is the first FDA-approved liquid biopsy companion diagnostic test for Tarceva® and Tagrisso® therapies

HOW DOES WORKFLOW AND PERFORMANCE OF CIRCULATING TUMOUR MARKERS (LIQUID BIOPSIES) COMPARE WITH TISSUE BIOPSIES?

Why compare liquid biopsy approaches with tissue biopsies? The latter represents the gold standard in cancer diagnostics against which new entrants will be judged (on both regulatory and commercial footings).

"Tissue biopsy is the gold standard because it has decades of use to validate its clinical utility. The focus of conventional cancer diagnostics is still heavily based on histological analysis of tissue biopsies"

PANEL MEMBER

Table 2 provides a summary comparison of liquid biopsy versus tissue biopsy, in terms of practical considerations. However, despite inevitable comparisons, any 'liquid biopsy versus tissue biopsy' argument is too simplistic. These two diagnostic approaches shouldn't be seen as market place competitors. Realistically – at least in the short term – the focus is on how the two platforms complement each other. Histopathological and morphological analyses from tissue biopsies provide the oncologist/pathologist with more (qualitative) information about the cancer than liquid biopsies, for instance staging and aggressiveness of the cancer. However, an accurate liquid biopsy process – that is to say a more efficient preanalytical method that is free from contaminating cells or circulating-free DNA – provides a foundation for accurate and sensitive tests, which may ultimately disrupt the testing status quo.

A liquid biopsy test comes into its own in difficult-to-biopsy cases, such as advanced lung cancer, where it may be technically difficult or the patient is not stable enough to extract tissue. In metastatic cancers it would not be practical to capture all the different tumour sites through tissue biopsies. In these cases, relative to tissue biopsy, liquid biopsy performance is seen as 'good enough' by clinical stakeholders, given that the alternative would be no biopsy at all.

Critically, liquid biopsy enables longitudinal analyses: the ability to routinely test patients frequently over prolonged periods of time. According to the panel, this is a key differentiator compared with tissue biopsy as it facilitates routine screening and monitoring of patients throughout the care pathway/journey (such as treatment response or other changes in the molecular basis of their disease).

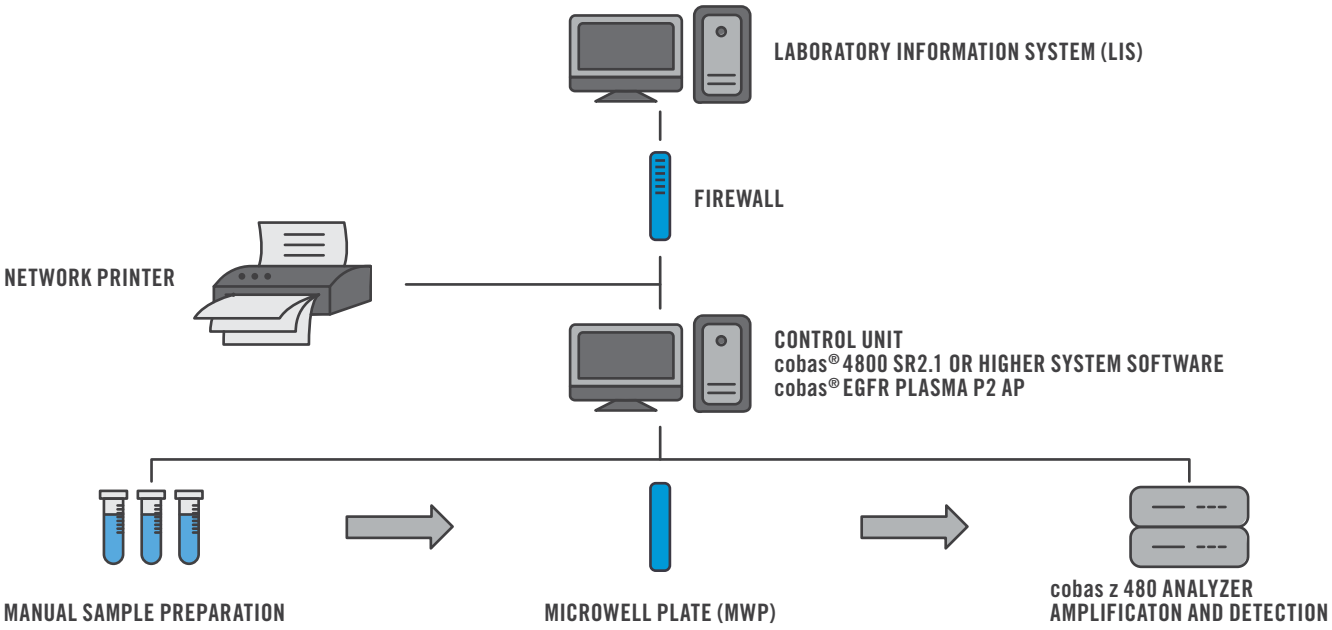
Currently, liquid biopsies and tissue biopsies show poor concordance in terms of their predictive values for specificity and sensitivity as seen in Figure 4. Therefore, based on the variance in these two data sets, liquid biopsies approved for in vitro diagnostic use have received restrictive indications or cannot be a standalone test. Recent research data comparing commercial providers has shown different results from the same patient samples[5]. There is still a need for liquid biopsies to demonstrate and establish trust with the clinical and laboratory communities.

In principle, liquid biopsy approaches have reduced workflow and turnaround time when compared with tissue biopsies. However, this benefit diminishes rapidly depending upon the complexity of downstream analytical processing required. Single gene analysis may be performed with a rapid turnaround, relative to Next Generation Sequencing (NGS). Also, only a relatively small number of labs are equipped to do NGS, which is a bottleneck to its adoption.

USE SCENARIO	LIQUID BIOPSY	TISSUE BIOPSY
Logistics	Easy to draw; requires phlebotomy – inexpensive; permits easy serial testing	Invasive, more difficult to obtain; Requires interventional radiology, pathology, all expensive; Serial testing difficult
Pre-analytical	Easier to control (fixative, anti-coagulant, etc in vacutainer tube)	Processing may cause artefacts (response gene activation, time to fixation, type of fixation, etc)
Sensitivity	CTC rare events (n=~1-10^3/10mL); ctDNA low abundance	10^6-10^8 cells/biopsy
Biology	May represent 'entire organisem', not single site; however, may not represent biology tissue-based cancer	Only represents one site; represents tissue-biology at least at that site

Table 2 – Comparison of liquid biopsy and tissue biopsy: practical considerations

5 https://www.sciencedaily.com/releases/2017/12/171214140825.htm



		cobas® Tissue Test v1		
		EGFR+ (MD)	EGFR- (NMD)	Total
cobas® Plasma Test v2	EGFR+ (MD)	161	4	165
	EGFR- (NMD)	49	217	266
	Total	210	221	431
With only valid result	PPA (95% CI)	**76.7%** (70.5%, 81.9%)		
	NPA (95% CI)	**98.2%** (95.4%, 99.3%)		

Implications for clinical validation

- **[Good specificity, poor sensitivity]** IIn 76.7% of tissue-positive cases, plasma was also positive; in 98.2% of tissue negative cases, plasma was also negative
- As a result, FDA requested a 'reflex' claim in the test's Indications for Use statement:

  (i) Patients with positive plasma test result for L858R mutation eligible for erlotinib treatment;

  (ii) Patients with negative plasma test result for L858R mutation should be reflexed to routine biopsy and testing for EGFR mutations with formalin fixed, paraffin-embedded tissue (FFPET) sample

		cobas® Tissue Test v1		
		T790M+	T790M-	Total
cobas® Plasma Test v2	T790M+	131	22	153
	T790M-	92	89	181
	Total	223	111	334
With only valid result	PPA (95% CI)	**58.7%** (52.2%, 65.0%)		
	NPA (95% CI)	**98.2%** (71.8%, 86.5%)		

Implications for clinical validation

- **[Poor sensitivity]** In 58.7% of tissue-positive cases, plasma was also positive; in 80% of tissue-negative cases, plasmas was also negative
- As a result, FDA requested the following drug labelling: *"Testing for the presence of the [T790M] mutation in plasma specimens is recommended only in patients for whom a tumour biopsy cannot be obtained"*

Abbreviations: MD = mutation detected; NMD = no mutations detected
EGFR = epidermal growth factor receptor [L858R mutation]
PPA = Positive Percent Agreement; NPA = Negative Percent Agreement
T790M = epidermal growth factor receptor (EGFR) activating mutation
PPA = Positive Percent Agreement; NPA = Negative Percent Agreement

Source: FDA Summary Of Safety And Effectiveness Data, cobas® EGFR Mutation Test v2 (28th September, 2016); https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150044B.pdf

Figure 4 – Poor concordance between liquid biopsy and tissue biopsy tests results in labelling restrictions for in vitro diagnostic use

WHAT IS A TYPICAL LIQUID BIOPSY WORKFLOW; WHERE ARE THE KEY POINTS OF VARIATION?

A generalised view of a typical liquid biopsy workflow is shown in Figure 5.

Currently, few device manufacturers have the capability to be leading edge across all key segments of the liquid biopsy workflow. This includes automation of workflow (isolation, preparation, measurement) and integration assay type (CTCs, cfDNA, vesicles). Complete integration of all components from sample prep through to analysis (e.g. NGS) is key to successful, valid clinical decision making. There is space for a commercial player to take ownership of the whole workflow to give greater confidence in the results, in a similar approach to that adopted for histological pathology by major players.

One example is Menarini Silicon Biosystems' (DEP Array) acquisition of CellSearch (Janssen) in 2017[6]. In principle this system gives this required capability for enrichment and analysis of CTCs, cfDNA and exosomes.

In addition to automation and integration, the panel agreed that sensitivity is a key challenge to liquid biopsies in order to optimise the impact on clinical application. It is vital that such systems can detect the presence of molecular targets at the vanishingly small concentrations present in a blood sample – typically dependent upon a 10mL vacutainer. Preanalytical standard-of-care platforms may need to change to accommodate the requirements of liquid biopsy, for example routinely moving to 20mL blood collection systems.

Novel blood collection systems may be required to facilitate this shift in preanalytical workflow, particularly if patient testing is needed on a regular basis. Increased testing rates will place a further burden on the patient and healthcare professional. The process may need to become home-based in order to avoid bottlenecks in the clinic. There are novel blood collection systems in development. For example, TAP, Seventh Sense Biosystems' novel blood draw system which may facilitate longitudinal testing via at-home blood draws without the presence of a health care professional[7].

Finally, liquid biopsy workflows need standardisation. Currently, many of these tests are regulated as Laboratory Developed Tests (LDTs). Most of the commonly-used liquid biopsy based tests are operated by major commercial 'send-out' laboratories such as Guardant360. This represents a flexible but fragmented approach to regulation. As a result, there is considerable variation in protocols and practice associated with liquid biopsy methodology. This makes it difficult for lay users of these systems to gain a foothold into using them.

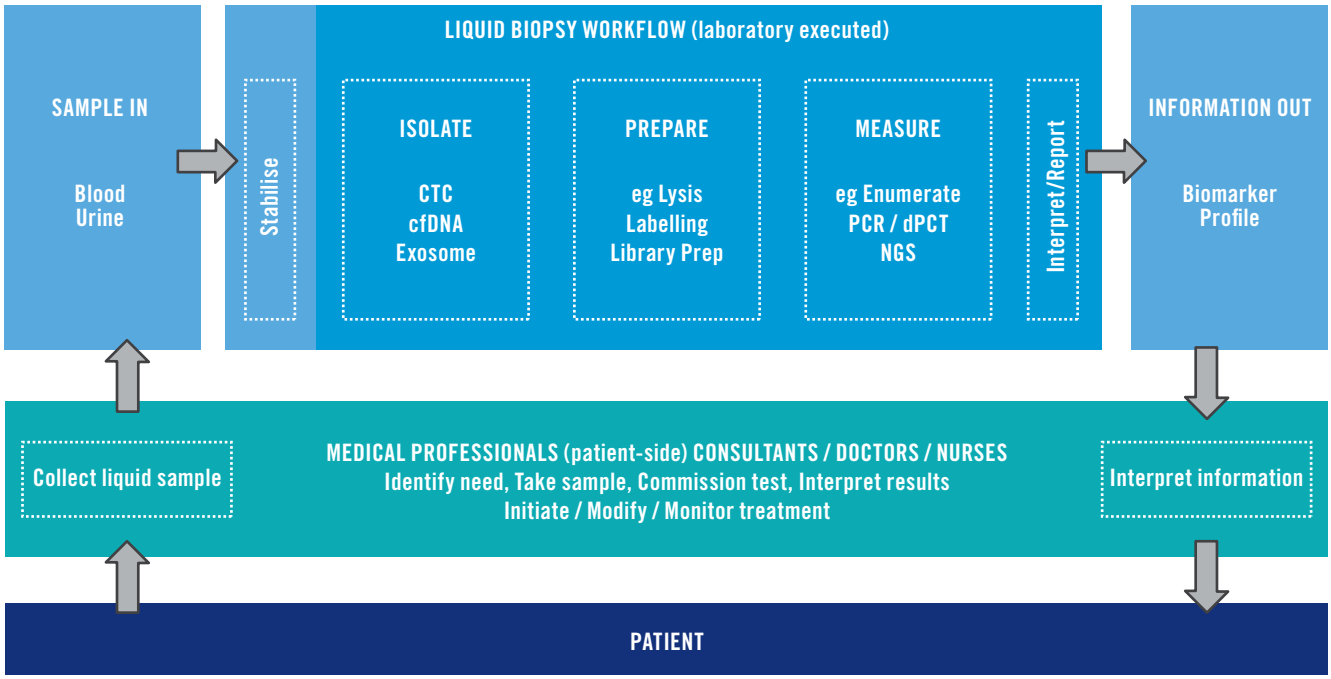


Figure 5 – Typical liquid biopsy workflow

6 https://www.genomeweb.com/business-news/menarini-silicon-biosystems-completes-acquisition-janssen-ctc-system#.WtWh6y7waUk
7 http://www.7sbio.com/

WHERE ARE THE KEY COST POINTS FOR LIQUID BIOPSY?

Liquid biopsy costs are opaque and none of the panel members could provide indicative costs for liquid biopsy kits (e.g. Roche Cobas' liquid biopsy system). Liquid biopsy costs are segmented, and it is the cost of performing NGS that ramps up the absolute cost of performing assays.

"The costs of liquid biopsy and next generation diagnostics are not fully reimbursed, and there is much v ariation in rates of reimbursement according to territory"

PANEL MEMBER

In any case, reference laboratory labour and material costs potentially dwarf liquid biopsy kit costs and may be a bigger constraint on the uptake of liquid biopsy.

WHAT IS THE POTENTIAL CLINICAL UTILITY FOR LIQUID BIOPSY… AND WHAT ARE THE BOTTLENECKS IN ACHIEVING THIS?

Opportunities to expand upon the current utility of liquid biopsy are described in Figure 6. The schematic indicates three major converging trends for expanding upon the clinical utility of liquid biopsy:

1. **Greater impact upon cancer care pathway,** e.g. screening of at-risk/asymptomatic individuals for the presence of susceptibility genes or activating mutations. In addition to consolidation of its clinical potential with patients already in the care pathway, early detection and screening was the panel's consensus view of where liquid biopsy is heading. A key example of this is the Grail initiative – an Illumina spin-out – that utilises liquid biopsy for longitudinal analysis[8].

2. **Greater depth of biomarker analysis,** e.g. qualification/ validation of more biomarkers for diagnosis, monitoring, prognosis, pharmacodynamic/response biomarkers and safety. Liquid biopsy will help in the analysis of a greater number of validated biomarkers, which will give much greater depth of support to clinical decision making.

3. **Expand clinical utility into other therapy areas,** e.g. diagnosis and monitoring of pro-inflammatory cytokines associated with autoimmune disease. To date, liquid biopsy has been most closely associated with oncology. This is not surprising given the largely genetic basis of the disease and its unmet needs. However, as the molecular basis of other complex multifactorial diseases, particularly inflammatory disease and metabolic disorders, are understood, the role for liquid biopsy testing emerges.

Liquid biopsy has enormous potential clinical utility across the cancer care pathway, however its commercial value is hamstrung by the breadth and depth of available targeted therapies. High availability of therapies means need to select the appropriate one and liquid biopsy will become critical in materialising the concept of *"one test, many drugs"*.

The extent to which liquid biopsy emerges as a new gold standard in testing depends largely on the trust that healthcare professionals can place in the results delivered, compared to tissue biopsies. This trust will be gained from multiple technical advances, but also standardisation in all parts of the process – currently there are too many vendors with bespoke protocols and different approaches.

Liquid Biopsy has the potential to revolutionise cancer treatment and management and every day we get a step closer to this vision.

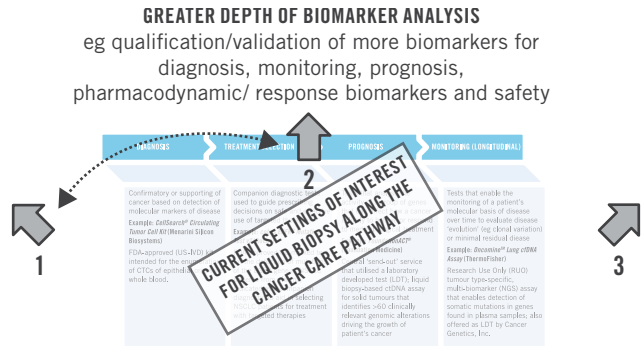


Figure 6 – Opportunities to expand upon current clinical utility of liquid biopsy

8 https://grail.com/

ACKNOWLEDGEMENTS

Cambridge Consultants would like to thank the following individuals for their valuable insights on liquid biopsy that were provided during the roundtable discussion held during October, 2017:

Sandip Patel
Medical Oncologist
UC San Diego Health

Daniel Grosu
Founder, Genopraxis
LLC (ex-Illumina, ex-Sequenom CMO)

Lei Zhang
Board of Director & VP Programs,
San Diego Regulatory Affairs Network

Jan Lotvall
CSO
Codiak BioSciences Inc

Hatim Husain
Asst. Prof. Medical Oncology
UC San Diego Moores Cancer Center

Mark Landers
VP Translational Research
Epic Sciences

Fernando Lopez Diaz
Staff Scientist
Salk Institute

Steve Soper
Professor
University of North Carolina at Chapel Hill

About Cambridge Consultants

Cambridge Consultants is a world-class supplier of innovative product development engineering and technology consulting. We work with companies globally to help them manage the business impact of the changing technology landscape.

With a team of more than 750 staff in the UK, the USA, Singapore and Japan, we have all the in-house skills needed to help you – from creating innovative concepts right the way through to taking your product into manufacturing. Most of our projects deliver prototype hardware or software and trials production batches. Equally, our technology consultants can help you to maximise your product portfolio and technology roadmap.

We're not content just to create 'me-too' products that make incremental change; we specialise in helping companies achieve the seemingly impossible. We work with some of the world's largest blue-chip companies as well as with some of the smallest, innovative start-ups that want to change the status quo fast.

Cambridge Consultants is part of the Altran Group, a global leader in innovation. www.Altran.com

For more information, or to discuss how this approach could fit your business, please contact:

James Blakemore, Senior Consultant, Medical Technology
James.Blakemore@CambridgeConsultants.com

Cambridge Consultants

UK ▪ USA ▪ SINGAPORE ▪ JAPAN

www.CambridgeConsultants.com

Cambridge Consultants is part of the Altran group, a global leader in innovation. www.Altran.com

THE QUEEN'S AWARDS FOR ENTERPRISE: INTERNATIONAL TRADE 2017