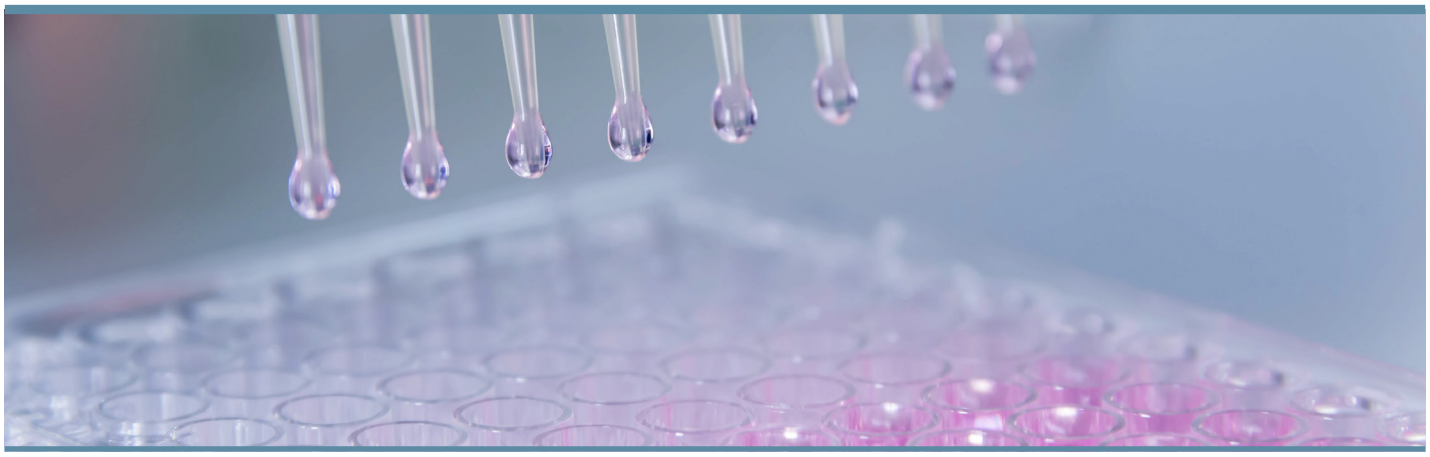

Executive Insights

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Clinical Liquid Biopsy Explained: Applications, Techniques and Players

A minimally invasive diagnostic that can help clinicians detect, diagnose and manage cancer patients has long been the dream of many healthcare practitioners. Over the past few years, highly sensitive molecular technologies — primarily Next Generation Sequencing (NGS) and digital PCR (dPCR) — have enabled researchers and clinicians to detect the presence of cancer genomic material (circulating tumor DNA or ctDNA) in peripheral biofluids, such as blood.

Alas, the field known as liquid biopsy was born on the backs of these novel molecular technologies. But liquid biopsy is not limited to ctDNA, and may include detection of other cancer-derived particles, including exosomes harboring tumor RNA and circulating tumor cells (CTCs).

Despite all the press and hype, the term "liquid biopsy" is often used without a precise understanding of its different applications and technological approaches. In this *Executive Insights*, we lay out the key clinical applications, describe the various liquid biopsy technologies and highlight key companies that are turning this dream into reality.

Liquid biopsy applications span the entire cancer patient journey

Liquid biopsies' potential clinical applications are broad and span the entire cancer patient journey — from screening healthy individuals in hopes of detecting cancer early, all the way through profiling resistance to targeted therapies in late-stage treated cancer patients (see Figure 1).

While therapy guidance is the leading application today, monitoring and early detection are expected to represent the most sizable and impactful applications in the future. Early detection has the potential to downstage cancer detection, enabling definitive intervention (e.g., full tumor resection), and could become a routine part of health checks, similar to mammograms and colonoscopies. Monitoring applications are myriad and have the potential to reduce the current dependence on imaging, as well as potentially detect recurrence or resistance earlier than traditional approaches, again enabling earlier intervention or changes in management.

In aggregate, analysts estimate the market potential for liquid biopsy applications to represent tens of billions of dollars, making liquid biopsy one of the most scalable opportunities diagnostic companies have encountered in decades.

Multiple liquid biopsy techniques exist

Central to liquid biopsy techniques is the capture and analysis of tumor-derived particles, which may include nucleic acids,

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Figure 1
Liquid biopsy applications

Patient journey	Liquid biopsy application		Scale of opportunity	Benefits	Challenges
Health check	Early detection	Routine screen for presence of cancer-derived particles in healthy individuals	$10Bs Large patient base (100M+) Low-cost recurring test	Earlier intervention Definitive localized therapy	Rare-event detection with low false-positive rate Tumor localization Large clinical trials / HECON (start in enriched populations) Low-cost test
Patient workup	Diagnostic aid	Test to inform diagnosis in suspected cases (e.g., pelvic mass, lung nodule)	$100Ms Niche patient base (100K)	Supports diagnosis in biopsy-constrained situations Diagnoses metastatic disease	Tissue-based testing is gold standard
Early-stage disease	Prognostic	Single test to support prognosis	Moderate cost, one-time test	Informs aggressiveness of intervention	Tissue-based testing is gold standard
	Intervention outcome monitoring / MRD	Test to track response to intervention or detect minimal residual disease (surgery, radiation, adjuvant therapy)	$1Bs Large prevalent pool (10Ms) Low-cost, recurring test	Lower cost / risk to imaging	Displace imaging with compelling cost / benefit data
	Surveillance and recurrence monitoring	Longitudinal monitoring of remission patients to detect recurrence earlier		Early detection and intervention (many recurrences are metastatic today) Reduced imaging (cost / toxicity)	Demonstrate ability to downstage recurrence detection and improve patient outcomes Displace imaging with compelling cost / benefit data
Late-stage / metastatic disease	Therapy guidance	Tests, including companion diagnostics and panels to inform therapy decisions	$100Ms Small patient pool (100Ks) High-cost, one-time test	Enables personalized medicine in biopsy-constrained situations Increases predictability if heterogeneous cancer or metastatic disease	Tissue-based testing is gold standard Unclear potential in immuno-oncology where tumor microenvironment is important
	Monitoring	Longitudinal test to monitor response or resistance to therapy	$100Ms Small patient pool (100Ks), moderate cost recurring test	Reduced imaging (cost / toxicity)	Demonstrate ability to achieve improved patient management / outcomes Displace imaging with compelling cost / benefit data

Source: L.E.K. analysis

proteins, exosomes, nucleosomes and cells. Since tumors divide, die, communicate and migrate, it is possible to find traces of their existence in biofluids. However, since these tumor-derived particles are typically rare, analytical approaches require very high sensitivity (NGS or digital PCR) or upfront enrichment (e.g., CTC cell capture).

There are multiple technologies used in liquid biopsy (enrichment and analysis), and it appears diverse approaches (and combinations thereof) may be required to address the breadth of potential applications (see Figure 2). Among all techniques, the combination of deep sequencing circulating tumor DNA or "ctDNA" using next-generation sequencing (NGS) represents the majority of current activity, but targeted (qPCR, digital PCR) approaches to measuring ctDNA are also being leveraged.

Beyond ctDNA, there is also significant activity in capturing and analyzing exosomes (vesicles involved in cell messaging), nucleosomes (chromosomal DNA packaging units) and circulating tumor cells (CTCs), as these contain a broader selection of tumor-derived analytes — including DNA, RNA and proteins — and may bring deeper insight into cancer biology versus ctDNA alone. While not based on detecting tumor-derived particles, capturing and analyzing immune cells is also emerging as a complementary technique in liquid biopsy and may be relevant in early cancer detection, as well as in guiding and managing patients on immunotherapy.

The competitive landscape is broad and dynamic

To date, we have identified over 30 different liquid biopsy competitors (see Figure 3). And while each competitor may have unique differentiators and offerings, we have attempted to categorize them into distinct segments based on a mix of technique/technology and current application focus.

Differentiation in the nascent clinical liquid biopsy space today appears to be driven by a mix of technology (e.g., sequencing

Figure 2
Overview of liquid biopsy techniques

	ctDNA	Particles (exosomes and nucleosomes)	Cells (CTCs and immune cells)
Target analyte	Circulating tumor DNA that spills into bloodstream with tumor cell division / death	Particles containing cancer-derived molecules including exosomes and nucleosomes Exosomes are vesicles involved in cell messaging Nucleosomes are complexes formed in chromosomal DNA packaging	Circulating tumor cells that slough into blood from tumors May also include peripheral immune cells to identify cancer-driven immune response Include broad coverage of cell components within a single cell (DNA, RNA, proteins, structure)
	Increasing analyte class coverage		
Isolation approaches	Nucleic acid sample prep kits optimized for cell-free DNA isolation	Physical separation Immunoaffinity Polymeric precipitation	Physical separation Immunoaffinity Direct visualization
Analytical approaches*	Molecular (NGS, qPCR, etc.)*	Molecular Proteomic	Molecular Proteomic Histology / imaging
Pros	Highly abundant (correlated with tumor size) Validated isolation methods Covers genetic heterogeneity NGS aligned	Highly abundant Covers genetic heterogeneity RNA capture	Broadest analysis possible May not represent heterogeneity
Cons	DNA fragmentation limits some applications (e.g., whole genome, DNA macro structure)	Fragmented nucleic acids limit some applications Challenging to isolate tumor-specific exosomes	Rare / hard to capture cells in quantity for ensemble analysis Lack of consensus on isolation methods

Note: *Molecular includes NGS, digital PCR, qPCR; proteomic includes immuno-diagnostics and mass spec; histology / imaging includes IHC, FISH and other imaging-based approaches.
Source: L.E.K. analysis

chemistry, cell capture technology and informatics) and investment in clinical trials/data to support utility. Commercial differentiation is minimal as few companies have reached the scale where this represents a meaningful differentiator. The intellectual property landscape is also dynamic and expected to support company differentiation in the future.

Looking across the competitive landscape, we define the following clusters:

Early detection using ctDNA. Probably the most exciting segment (given its potential to impact health screening in the general population) includes various players seeking to develop offerings in early cancer detection and screening.

Competitors in this segment are expected to differentiate with clinical data to support test utility. Clinical studies for the general screening population are likely to be massive (~100K patients) in scale and require significant longitudinal analysis.

Grail is the poster child in the early detection space, having raised $1 billion in VC funding and recently embarked on a 120,000-patient study for early breast cancer detection. Many others are eyeing the early detection space, and some may ease their way into the general population applications through trials in at-risk patients, or as a reflex to other screening methods (e.g., low-dose CT, fecal occult blood).

Therapy guidance using ctDNA. This segment is the most crowded and is seeing actual clinical application today in metastatic patients. There is a core group of players deploying NGS-based liquid biopsy panels to support therapy guidance in biopsy-constrained situations. The clear front-runner here is Guardant Health, but many other competitors — several of which also play in the solid-tumor panel NGS space (e.g., Foundation Medicine, Personal Genome Dx) or have some differentiating technology enabling higher sensitivity or smaller sample input amounts — are also present.

Many traditional oncology diagnostics companies (e.g., Roche, Qiagen) have targeted single-gene tests based on PCR in the therapy guidance space, including regulated kits for companion diagnostic biomarkers such as EGFR. Players in this space are also likely to transition into monitoring applications for tested patients (looking at resistance or recurrence in treated metastatic patients).

Figure 3
Overview of liquid biopsy competitors

Analyte	Application	Analysis	Company	Liquid biopsy differentiators	Liquid biopsy offering
ctDNA	Early detection / monitoring	NGS	BiomaRx	Combinatorial biomarker discovery approach	Pancreatic cancer in development
		QUARTS	Exact Sciences	Cancer screening (Cologuard), DNA methylation	Cologuard (CRC), lung cancer in development
		NGS	Freenome	Startup (investors include Andresssen Horowitz)	In development
		NGS	Grail	High-profile Illumina spin-out with massive funding	In development
		mmPCR, NGS	Natera	Leader in NIPT testing, low-cost mmPCR	Involved in TRACERx trial (lung cancer)
		qPCR	Nucleix	Novel content (DNA methylation)	Bladder recurrence, lung cancer in development
	Therapy guidance panels / tests	NGS	Accuragen	High-sensitivity Firefly NGS workflow	In development
		NGS	Biodesix	Rapid turnaround time	Therapy guidance panel for lung
		NGS	Circulogene	Finger stick (low sample input)	Therapy guidance panel
		NGS	Foundation Medicine	Solid tumor NGS lab leader, content, pharma partnerships and commercial channel	Therapy guidance panel (62 genes)
		NGS	Genomic Health	Oncology brand (esp. breast cancer) and channel	Therapy guidance panel (17 genes)
		NGS	Guardant Health	Front-runner in therapy guidance	Therapy guidance panel (73 genes) and monitoring protocol
		NGS	Inivata	NGS chemistry (TAM-Seq), CRUK affiliation	Therapy guidance panel (34 genes)
		NGS	Personal Genome Dx	Technology access (Johns Hopkins), pharma partnerships, lab / kit hybrid model	Therapy guidance panel (64 genes)
		NGS	Resolution Bio	High-sensitivity NGS approach	Therapy guidance panel (lung) and single gene
		NGS	Trovagene	Urine-based detection	Single-gene tests (e.g., EGFR, KRAS)
		qPCR	Biocartis	Sample-to-answer workflow	Single-gene tests (e.g., EGFR)
		qPCR	Qiagen	Scale and molecular diagnostic capabilities	Single-gene tests (e.g., EGFR)
		dPCR	Roche Dx	Scale, technical capabilities and Rx business	Single-gene tests (e.g., EGFR T790M)
		qPCR	Sysmex Inostics	Beaming technology and scale	Single-gene tests (e.g., KRAS, NRAS)
		qPCR	Transgenomic	Novel, highly sensitive ice-cold PCR approach	Single-gene tests
	Agnostic	NGS	Boreal Genomics	Technologies for isolation and analysis of cfDNA	Tools for cfDNA enrichment and analysis
Particles and cells	Various applications	IHC / NGS	Biocept	Cell capture technology deployed for validated content	Therapy guidance and monitoring tests
		NGS, other	Cynvenio	Hybrid approach looking at CTCs, cfDNA, NK cell count	Test for breast cancer monitoring (27 genes)
		Imaging	Epic Biosciences	Hypothesis-free imaging-based approach Investment in prostate cancer	ARV7 test for prostate cancer
		NGS	ExosomeDx	Exosome capture and RNA analysis	Lung therapy guidance test; prostate diagnosis aid; exosomal protein analysis instrument
		Imaging	Menarini	Acquired legacy business	CTC enumeration test (Cellsearch)
		nCounter (GEP)	Oncocyte	RNA / protein focus looking at systematic immune system changes (not tumor-derived)	Lung cancer diagnostic aid (indeterminate CT); also offers breast and bladder cancer tests
		Immuno-assay	Volition Rx	Nucleosome-based diagnostics	Colorectal cancer screening triage test (FOB reflex)
	Isolation focus	Imaging / other	ApoCell	Cell isolation technology	Cell isolation systems and CRO services
		N/A	Caris	Commercial channel; exosome capture technology	Exosome capture technology
		N/A	Clearbridge Biomedics	CTC isolation technology	CTC isolation system
		NGS	Fluxion	CTC isolation technology	Therapy guidance panel (59 genes) CTC capture and analysis platform
		N/A	Vortex Biosciences	CTC isolation technology	CTC isolation system

Source: L.E.K. analysis

Particles and cells (exosomes, nucleosomes, CTCs and immune cells). This is a heterogeneous segment developing varied clinical applications based on analysis of captured particles or cells. In some instances, competitors are enabling existing biomarkers to be measured from blood (e.g., Biocept, Exosome Dx) whereas others are developing novel content (e.g., Epic in prostate cancer and Cynvenio in breast cancer).

Epic is unique in that they do not enrich CTCs; rather, they image cells in a monolayer. Epic has focused efforts on prostate cancer and recently launched an ARV7 test to guide prostate cancer treatment. Cynvenio is interesting in that they are combining multiple techniques for their breast cancer recurrence monitoring test, which includes ctDNA and CTC DNA sequencing, as well as NK cell enumeration. Oncocyte is focused on blood-based immune cell function analysis. Another group of competitors in this space offers tools for CTC capture using various approaches, but is not focused on specific applications.

Significant opportunities lie ahead

The market opportunity associated with liquid biopsy represents one of the largest and most dynamic diagnostics/healthcare opportunities. While we are excited about the market potential and clinical utility, we urge pharma stakeholders, tools companies, diagnostic developers and other market stakeholders to carefully consider the impact liquid biopsy may have on the oncology landscape and what it will take to play and win.

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