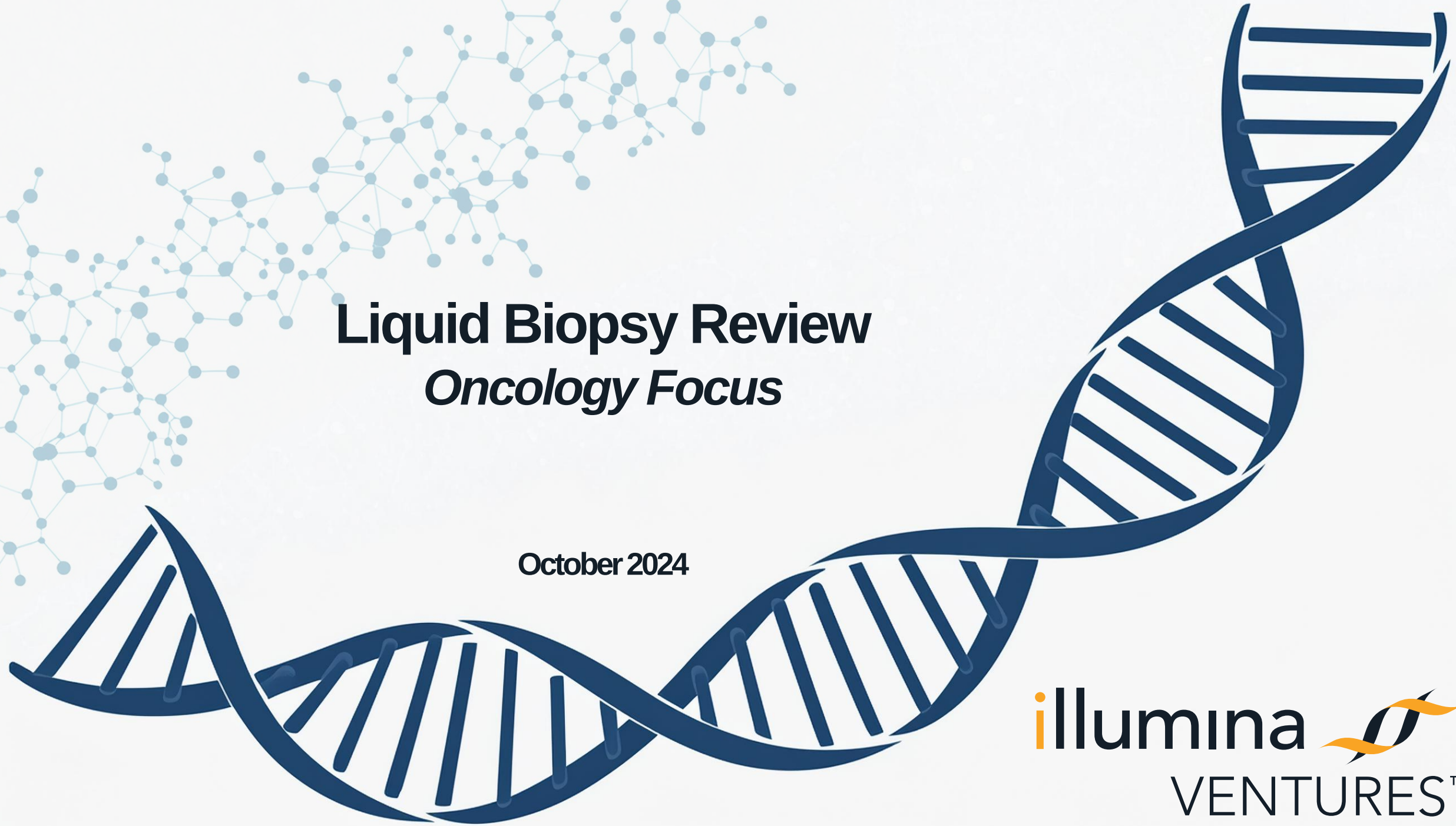
Liquid Biopsy Review

Oncology Focus

October 2024



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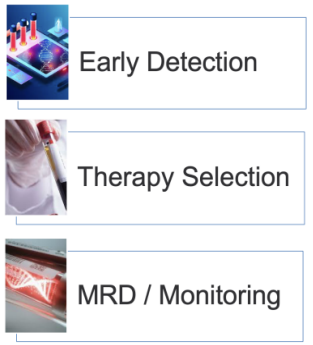
Liquid Biopsy: Agenda

- **Liquid Biopsy Overview**
- Current Products and Research
  - Early Cancer Detection: Single-Cancer and Multi-Cancer
  - Therapy Selection and Response
  - MRD / Monitoring
- Market and Financials
- Looking forward: Opportunities



Liquid Biopsy: Definition

Liquid biopsy is moving to the forefront of precision medicine with a current focus in cancer. Liquid Biopsies utilizing disruptive diagnostic technologies to analyse blood (and other biological fluids) to reveal genomics and other omics data from a non-invasive sample

Liquid Biopsy

Samples are blood or other body fluids

Minimally invasive

Use Cases: Early disease detection, minimal residual disease, treatment selection and disease treatment monitoring

Easily repeatable at various time points before, during and after treatment

Tissue Biopsy

Samples are tissue surgically removed from a specific body site

Invasive procedure involving surgical biopsy

Use Cases: Initial diagnosis, histological classification, and genomic profiling

Limited by the invasiness, not suitable for frequent sampling

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Liquid Biopsy Analytes: Differing Insights, Complexity and Utility

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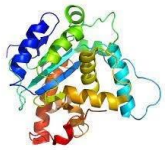
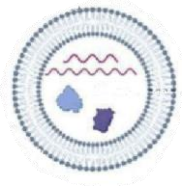
Analytes		Description
	**CTC**	Tumor Cells shed from primary tumors. Perhaps a/the mechanism of metastasis. Once the primary hope for the clinic but for now, scarcity and multiple steps for analysis moved CTCs to primarily research.
	**cfDNA**	Short fragments of DNA (120–220 bp long) continuously shed from both normal and tumor cells (e.g. during apoptosis/necrosis). Easier to analyze than CTCs; increasing speed and reducing cost of NGS has enabled cfDNA to become the most utilized clinical liquid biopsy analyte yielding chromosome disruption, mutation, and CpG methylation insight
	**cfRNA**	“Coding” mRNA transcripts (aka transcriptomics) used by all cells as the template for protein translation and generally shorter lived than DNA in circulation “Non-coding” RNA which is both a direct molecular actor (e.g. in the ribosome) and a primary mechanism of gene regulation.
	**Protein**	Proteins carry out nearly all activities within and between cells. Their presence enables tissue/cell tumor detection and reveals tumor growth pathways (presence of oncogenes and/or disabled tumor suppressor proteins). They can be identified/quantified by antibodies that they bind (ImmunoAssays) or directly by mass spectrometry.
	**EV**	Extracellular vesicles – small membrane-bound vesicles released by cells (including cancer cells) to communicate with other cells. Contain a chemical cocktail that includes RNA, DNA, and proteins, they can be isolated using antibodies and may be a driving factor in growth and metastasis

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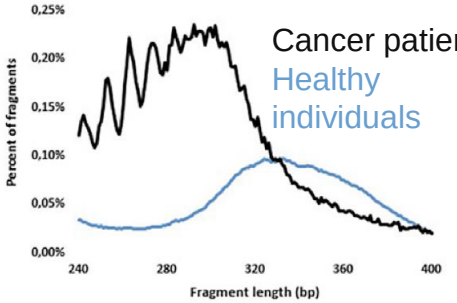
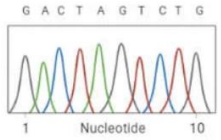
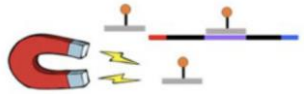
Liquid Biopsy: Technology making NGS even more useful

Technology	Description
Fragmentomics 	Fragmentomics is a new field studying cfDNA, especially regarding cancer screening. When cfDNA is detected in the blood, the DNA molecule is highly fragmented with a characteristic size profile. Cancer patient cfDNA shows subtle but reliable differences relative to healthy subjects (i.e., in individuals with cancer, cfDNA differences include a higher number of fragments below 150 bp)[1]. DELFI (DNA evaluation of fragments for early interception) is the first test to employ fragmentomics, harnessing information regarding fragment length in 5 Mb windows along the genome in bins from the ratio of short (100–150 bp) over long (151–220 bp) fragments.
Methylation Sequencing 	Methylation patterns in cfDNA have emerged as a promising genomic feature for detecting the presence of cancer and determining its origin. There are several types of methylation analysis in cancer cfDNA using NGS technology including bisulfite sequencing, affinity purification for methylated DNA and methylation sensitive restriction enzyme sequencing (MRE-seq)[2].
Ultra-Deep Sequencing 	Ultra-deep sequencing involves sequencing cfDNA at extremely high depths, which improves the detection of low-frequency mutations. This is particularly useful for identifying MRD and for monitoring cancer recurrence[3,4].
Hybrid Capture-Based 	Hybrid capture technology enriches specific regions of the genome before sequencing, improving the detection of mutations, copy number variations (CNVs), and structural variants in cfDNA. This method is especially useful for comprehensive genomic profiling of cancers[5].

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1. A.R. Thierry *et al*., Cell Genomics, January 2023, 2. H.J. Kwon *et al*., Sci Rep. August 2023, 3. T. Moser *et al*., Trends in Genetics (Cell Press), February 2023, 4. SEQC2 Oncopanel Sequencing Working Group, Nature, April 2022, 5. J. Zheng *et al*., The Lancet, May 2024

Liquid Biopsy: Three Major Segments – Oncology Focus

Highlights

Early Disease Detection

- **What**: Detects cancer in early stages with hope of improving patient outcomes by enabling timely intervention. Two segments: Single-cancer and Multi-Cancer Early Detection (MCED)
- **Market**: Multiple single cancer and MCED tests available, mostly Lab Developed Tests (LDTs)

Treatment Selection / Treatment Response / Companion Diagnostics

- **What**: Detects potential genomic causes for variances in treatment response and disease progression
- **Market**: Most mature segment, with numerous FDA-approved tests focused in oncology

Minimal Residual Disease (MRD)

- **What**: Detects presence of cancer cells to evaluate treatment effectiveness and / or disease recurrence
- **Market**: Multiple commercial products, mostly LDTs

How

Next-generation sequencing (NGS) serves as the predominant technology:

- covers large numbers of gene targets and variant types

- provides the sensitivity needed to detect low-abundance variants accurately with high sequencing depth

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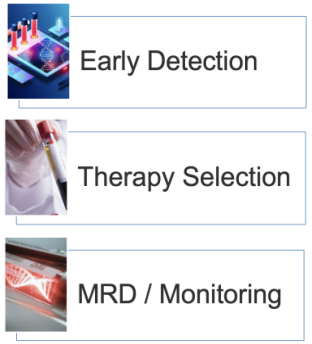
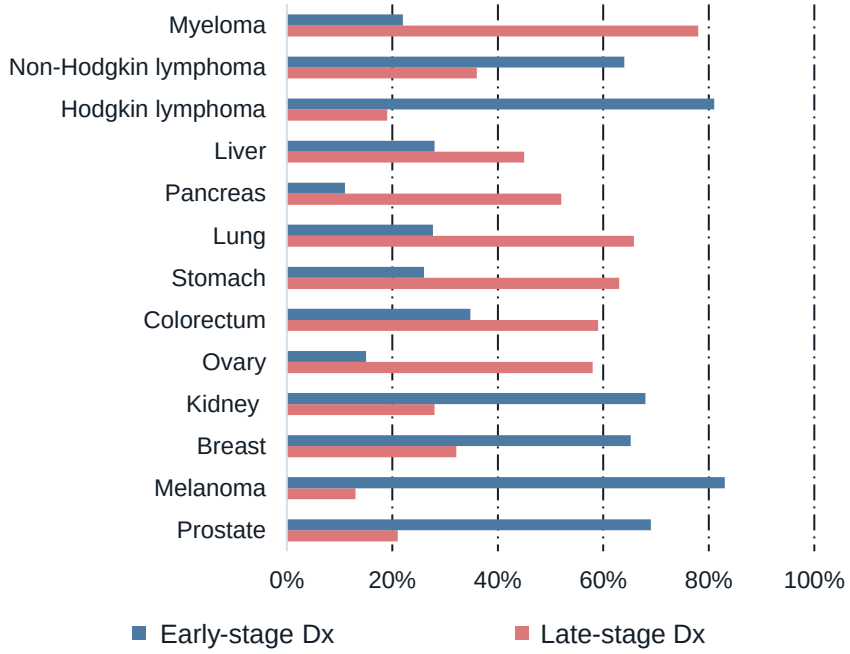
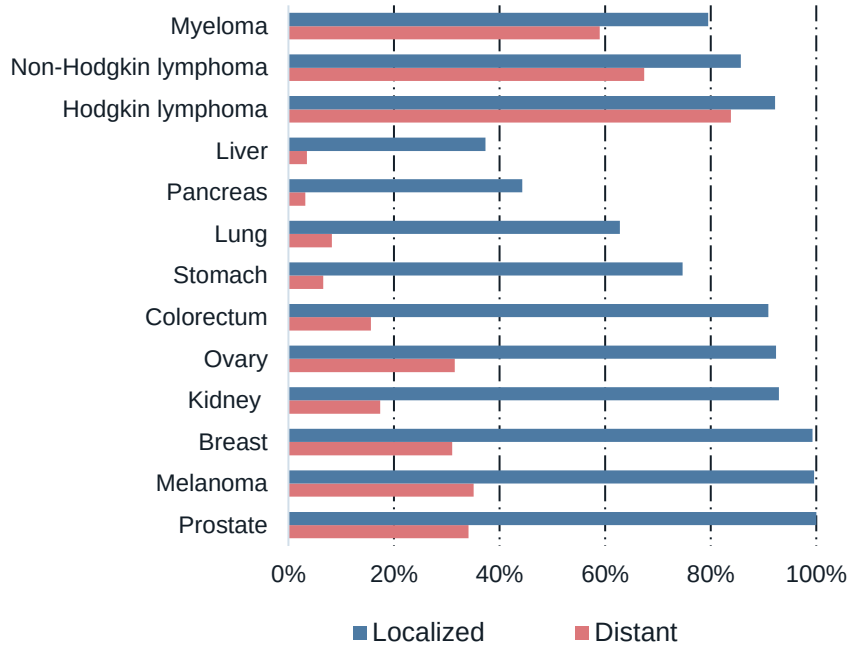


Opportunity: Detect Cancer Earlier for Longer Survival

Proportion of early-stage *versus* late-stage diagnoses for different cancer types[1]



5 year relative survival by stage, 2013 - 2019[2]



Today, many cancers are not found early

86%[3] of cancers not found through recommended screening

OBSERVATION

Early cancer diagnosis boosts likelihood of treatment success. Fewer options exist as cancer progresses creating need for effective early diagnostic solutions

Early Detection

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Sources: 1. CDC, SEER, ACS (2019-2021) 2. American Cancer Society: Cancer Statistics Center, June 2024 3. NORC at the University of Chicago (5-year survival rate),

Single Cancer Early Detection vs. MCED Tests

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Single Cancer Early-Detection Tests

- Designed to *prioritize sensitivity* to ensure that tests have a low false negative rate. Tests, however, often resulting in high false positive rates (10% or higher)[1]
- Used for individuals at high risk or showing symptoms of a particular cancer – most often where a standard screening methods exists but compliance is low. New tests plan to target average-risk populations.
- US commercially available tests:
  - Colon: Epi - proColon2.0: FDA approved test
  - Colon: Guardant Health - Shield: FDA approved test
  - Lung: Delfi - FirstLook: LDT
  - Prostate: MdxHealth - Select: LDT
  - Pancreas: Immunovia – IMMRay PanCd: LDT

Multi-Cancer Early Detection Tests (MCED)

- Designed to *prioritize specificity* at the expense of sensitivity resulting in a very low false positive rates (<1%). Sensitivity varies across cancer types and drops significantly for Stage 1 detection reaching only 16.8%[2]
- Used for individuals at average risk to complement other screening methods as well as detection for cancers without standard screening methods
- US commercially available tests:
  - Grail - Galleri: LDT
  - 20/20 Gene Systems - OneTest: LDT
  - Early Dx - CancerRadar: LDT
  - Singlera Genomics - PanSeer: LDT

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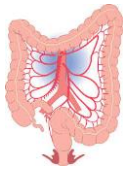
1. Blood Profiling Atlas in Cancer (BLOODPAC) Consortium, 2023; Press Release; 2. Klein et al., Annals of Oncology, 2021

Select Liquid Biopsy Tests for Single Cancers

Cancer Type	Company	Test	Sample	Technology	Commercial status
Colorectal 	Epigenomics (*Germany*)	epi pro colon® Colon Cancer Screening Blood Test	Blood	Methylated Septin 9 DNA	FDA approved CE marked
	Guardant Health (*USA*)	shield™	Blood	Multimodal Approach (genomics, epigenomics and proteomics)	LDT FDA approved
	CellMax Life (*USA*)	FirstSight™	Blood	CTCs	Breakthrough device designation from FDA
	Freenome (*USA*)	Freenome multiomics	Blood	Multimodal Approach (genomics, transcriptomics and proteomics)	LDT Undergoing clinical validation to seek FDA approval
Glioblastoma 	Datar Cancer Genetics (*UK*)	TriNetra™ Glio	Blood	CTCs	Breakthrough device designation from FDA
Ovarian 	Mercy BioAnalytics (*USA*)	Mercy Halo™	Blood	Extracellular vesicles	Breakthrough device designation from FDA

Early Detection

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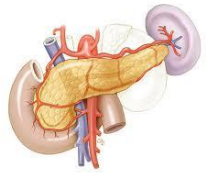
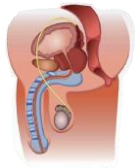
Select Liquid Biopsy Tests for Single Cancers (continued)

Cancer Type	Company	Test	Sample	Technology	Commercial status
Lung 	DELFI (*USA*)	FirstLook By DELFI Diagnostics	Blood	DNA fragment patterns (fragmentomics)	LDT Breakthrough device designation from FDA
Pancreas 	Immunovia (*Sweden*)	IMMray® PanCan-d	Blood	NASolCD44 and total protein Expression (29 biomarkers)	LDT
Prostate 	Mdxhealth (*USA*)	SelectMDx™ for Prostate Cancer	Urine	Measurement of two cancer-related mRNAs (HOXC6/DLX1)	LDT CE marked
	Datar Cancer Genetics (*UK*)	trublood® the no risk biopsy	Blood	CTCs	Breakthrough device designation from FDA CE marked
Breast 	Datar Cancer Genetics (*UK*)	Trucheck™ Breast Cancer	Blood	CTCs	Breakthrough device designation from FDA CE marked


Early Detection

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Select Multi-Cancer Early Detection (MCED) Tests

Test Name	Company	# of Cancers	Sample	Biomarker	Commercial status
Galleri®	Grail (*USA*)	27*	Whole Blood	Methylation patterns of cfDNA	LDT Breakthrough device designation from FDA
Trucheck™ Intelli	Datar Cancer Genetics (*UK*)	27*	Whole Blood	CTCs & C-ETACs (circulating ensembles of tumor associated cells)	LDT CE marked
CancerRadar	EarlyDx (*USA*)	4	Whole Blood	cfMethyl-Seq Assay. Genome-wide methylation profiling of cfDNA	LDT CE marked
oneTEST for early detection of cancers	20/20 Gene Systems (*USA*)	20	Whole Blood	Measurement of different tumor antigens	LDT
Panseer	Singlera Genomics (*USA*)	5**	Plasma	ctDNA methylation haplotypes	LDT
cancerguard™	Exact Sciences (*USA*)	8***	Whole Blood	DNA and protein biomarkers	Under development

* Galleri's description of 50 cancers and Trucheck's of 70 cancer types including multiple histopathological subtypes
** Zhang, W., et al. (2020). "Early detection of multiple cancers using a blood test" Nature Communications
*** Cohen, J.D., et al. (2018). "Detection and localization of surgically resectable cancers with a multi-analyte blood test" Science

Early Detection

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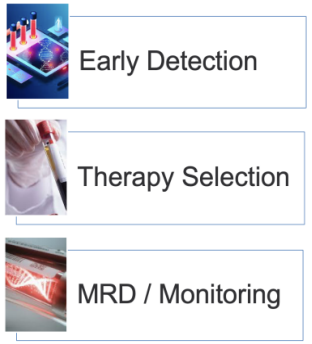
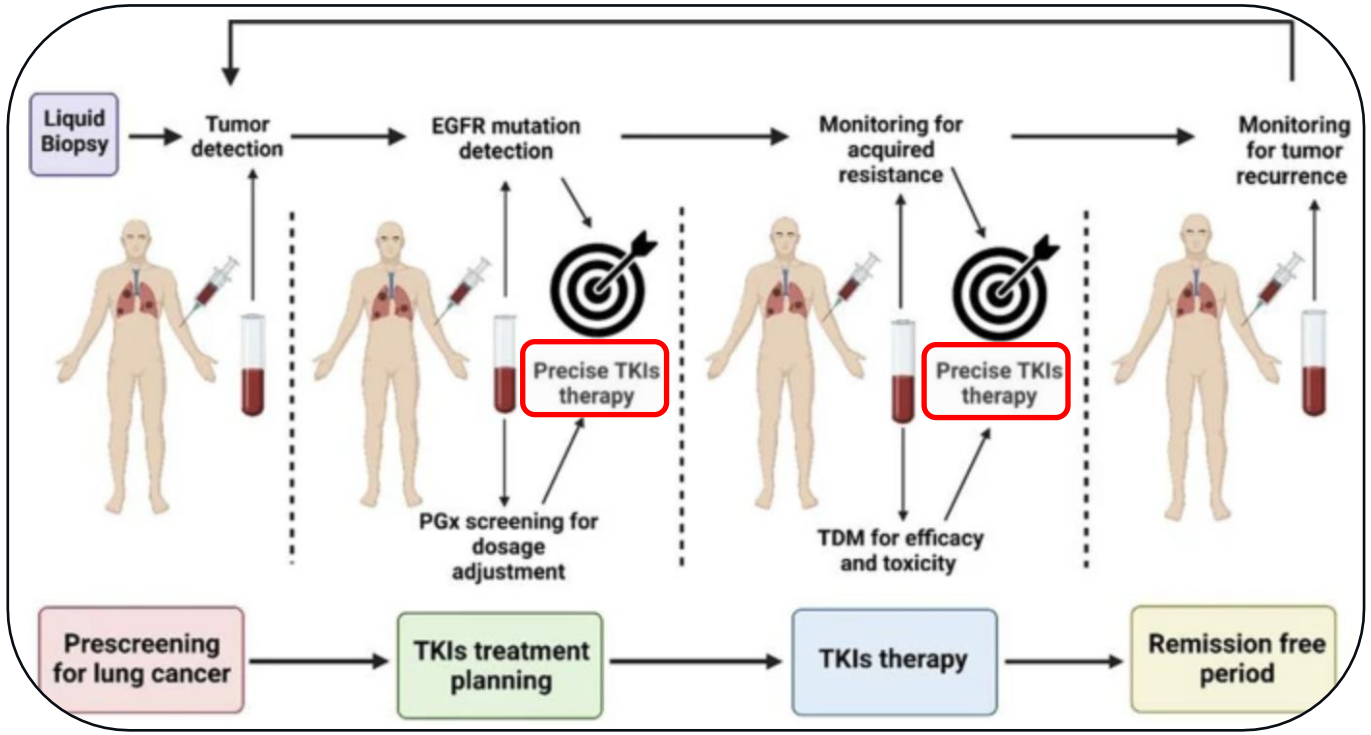


Opportunity: Personalized Medicine / Choosing the best treatment



Serial molecular profiling by liquid biopsy and pharmacogenomics across various phases of lung cancer

- Liquid Biopsy tests help to find the best treatment especially when:
  - No tissue is accessible at diagnosis
  - No tissue is accessible at clinical relapse
  - Tumors contain high levels of genetic and/or phenotypic heterogeneity
  - Multiple different treatments are available
- Advances in isolation methods and omics technologies are expanding applications and enhancing treatment personalization

OBSERVATION

Opportunity for diagnostic and therapeutic companies to partner early in the product research process (for both test and drug) to design more effective drugs with targeted patient populations and efficient clinical trials

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Adapted from Kolesar *et al*., Mol Cancer. 2022

Therapy Selection

FDA Approved Companion Diagnostic Liquid Biopsy Tests

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Date	Diagnostic Name (Manufacturer)	Cancer Type	Sample	Gene (ctDNA)	Companion Drugs
09/2024	**FoundationOne Liquid CDx (Foundation Medicine)**	mCRPC	Blood	*BRCA1, BRCA2*	Olaparib
04/2023	**xT CDx (Tempus AI)**	CRC	Blood	*KRAS* and *NRAS*	Erbitux, Vectibix
12/2022	**Agilent Resolution ctDx FIRST assay (Resolution Bioscience)**	NSCLC	Blood	*KRAS*	Krazati
08/2020	**Guardant360 CDx (Guardant Health)**	NSCLC Breast cancer	Blood	*EGFR (HER1)* *ESR1*	Tagrisso, Rybrevant, Lumakras, Enhertu, Orserdu
08/2020	**FoundationOne Liquid CDx (Foundation Medicine)**	NSCLC mCRPC Solid Tumors Ovarian Cancer Breast Cancer mCRC	Blood	*EGFR (HER1)* *BRCA1* and *BRCA2* *NTRK1-3 fusions* *BRCA1* and *BRCA2* *PIK3CA* *BRAF*	See (1) Rubraca, Lynparza Rozlytrek Rubraca Piqray
05/2019	**Therascreen PIK3CA RGQ (QIAGEN GmbH)**	Breast cancer	Plasma	*PIK3CA*	Piqray
06/2016	**Cbas EGFR Mutation Test v2 (Roche Molecular Systems)**	NSCLC	Plasma	*EGFR (HER1)*	Tagrisso, Iressa, Tarceva, Gilotrif

Source: US FDA - List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)
(1): Exkivity, Iressa, Tagrisso, Tarceva, Tabrecta, Rozlytrek, Alecensa

Therapy Selection

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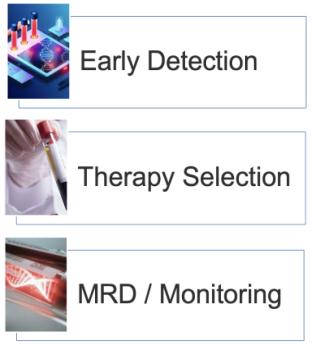
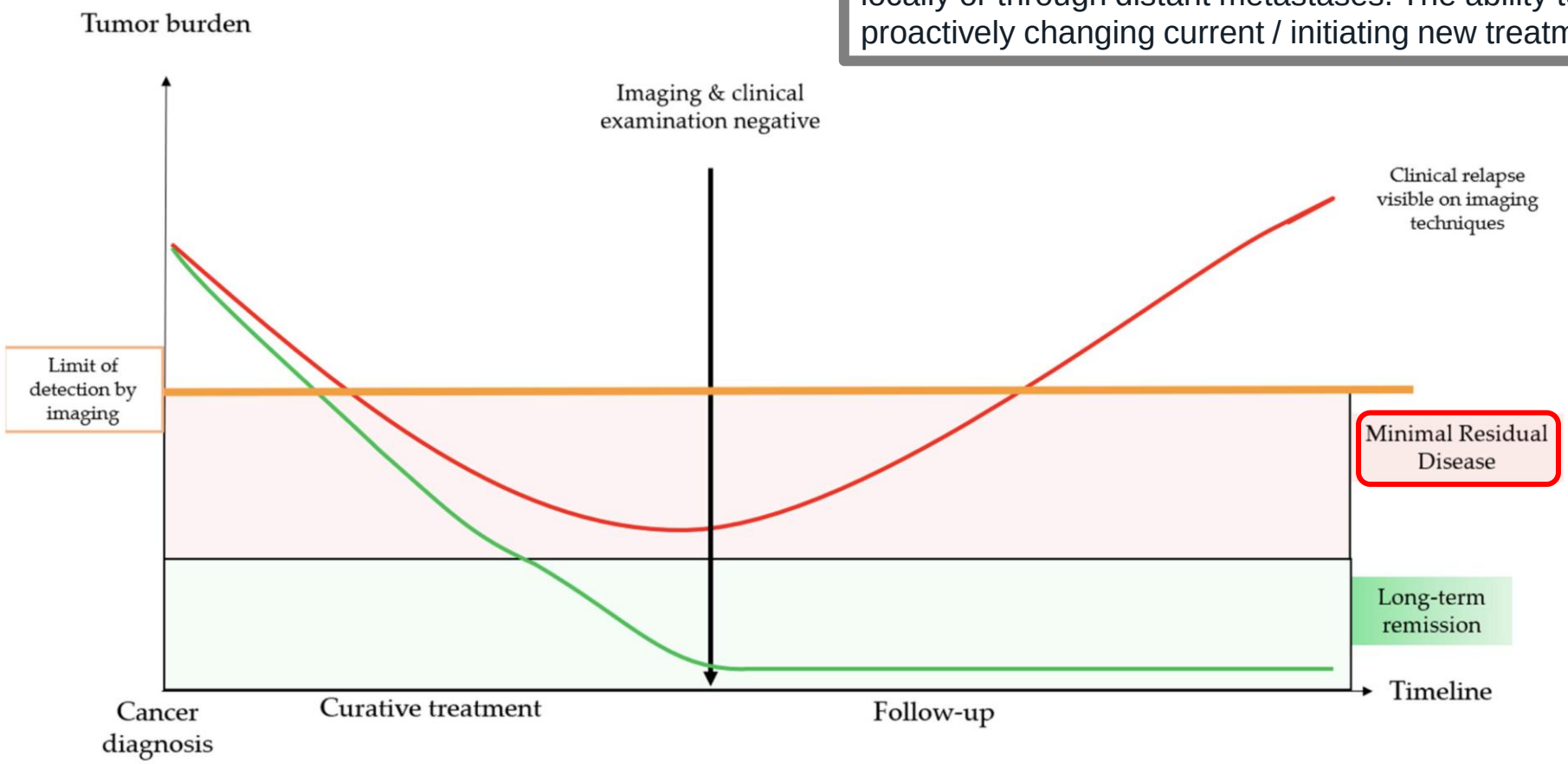


The Opportunity: Detect Cancer Recurrence earlier

OBSERVATION

If tumor cells remain in the patient's body after treatment, they may induce relapse, either locally or through distant metastases. The ability to detect MRD may prove beneficial to proactively changing current / initiating new treatments to effectively treat the cancer



Minimal Residual Disease (MRD), also known as Molecular Residual Disease, is defined as the tumor mass remaining after chemotherapy or hematopoietic stem-cells transplantation

Adapted from Honore et al., Cancers, 2021

MRD / Monitoring

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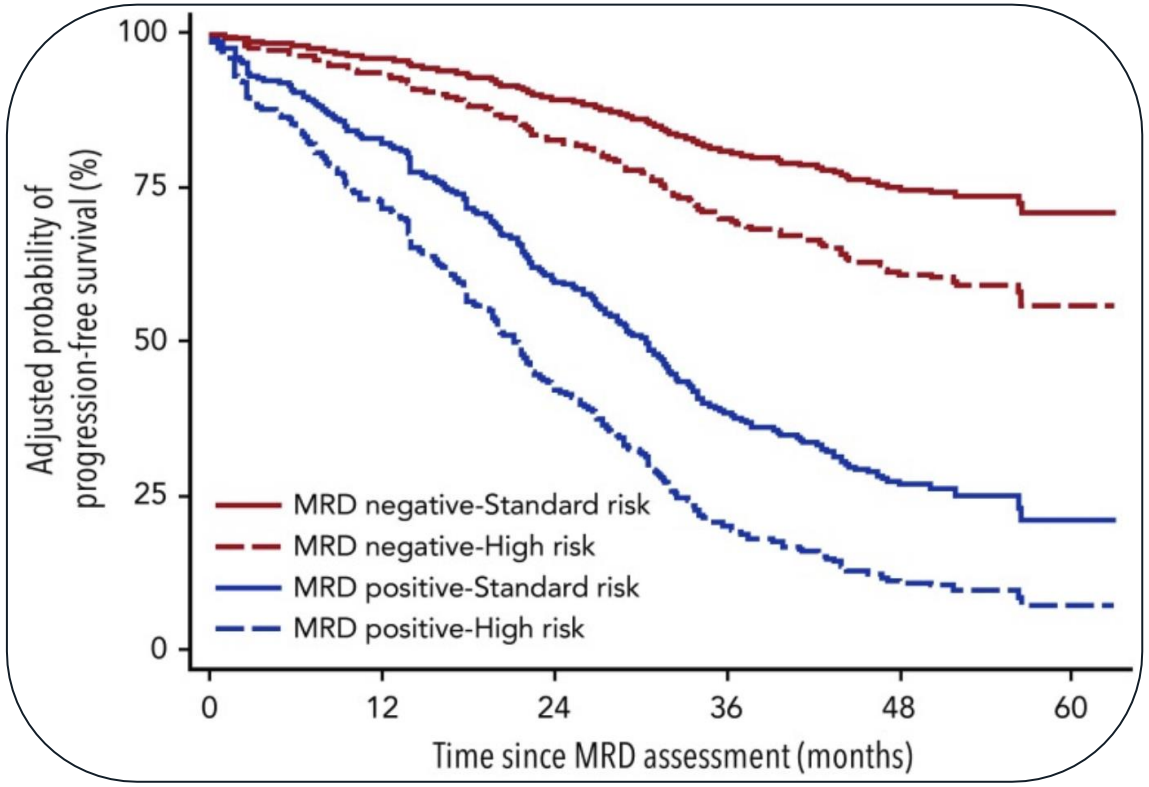
Case Study: MRD negativity as a Key Prognostic Factor in Multiple Myeloma (MM)



Progression-free survival (PFS): MRD status, as determined by NGS, is a prognostic biomarker in multiple myeloma[1]

- Patients with MM who are eligible for transplantation and have undetectable MRD by NGS during maintenance therapy have excellent outcomes with an unprecedented reduction in the risk of progression
- Because the benefit of undetectable MRD is independent of treatment, MRD could potentially be used as a clinical and surrogate end point
- Risk is dynamic. Patients with high-risk disease at diagnosis may enjoy progression-free survival (PFS) similar to that of standard-risk patients upon the achievement of MRD negativity
- "*We do expect MRD testing to move into NCCN guidelines (more of a 'when', not 'if')*" Mark Massaro, BTIG

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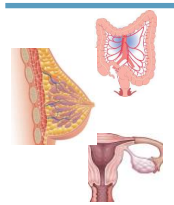
1. Perrot *et al.*, Blood, Dec. 2018

Select MRD Liquid Biopsy Tests

Cancer Type	Company	Test	Sample	Biomarker	Commercial status
 **CLL – Chronic Lymphocytic Leukemia**	Adaptive Biotechnologies (*USA*)	ClonoSEQ®	Blood	ctDNA using NGS	FDA approved
 **Colorectal (stage I-IV)** **Breast (stage II-IV)** **Ovarian (stage II-IV)**	Natera (*USA*)	Signatera	Blood	ctDNA using multiplex-PCR NGS	FDA Breakthrough Device Designation
 **Breast**	Inivata (*USA*)	RaDaR	Blood	ctDNA using multiplex-PCR NGS	FDA Breakthrough Device Designation CE marked
 **Colorectal**  **Bladder**	Foundation Medicine (*USA*)	FoundationOne Tracker	Blood	ctDNA using multiplex-PCR NGS	FDA Breakthrough Device Designation
 **Colorectal**	Clinical Genomics (*USA*)	Colvera	Blood	DNA methylation of BCAT1 and IKZF1 genes	LDT
 **Bladder**	Nucleix (*Israel*)	Bladder EpiCheck	Urine	Multiplex DNA methylation-based PCR test	CE marked

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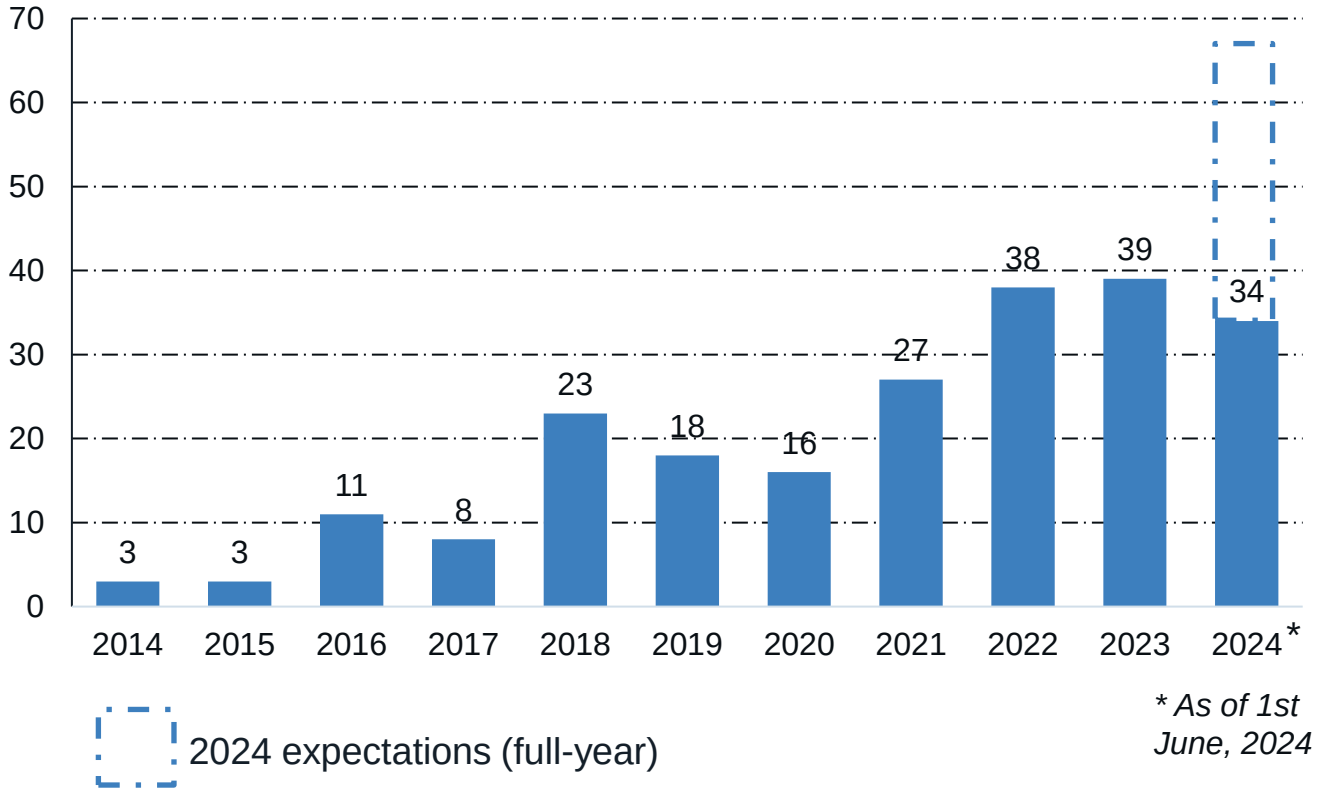
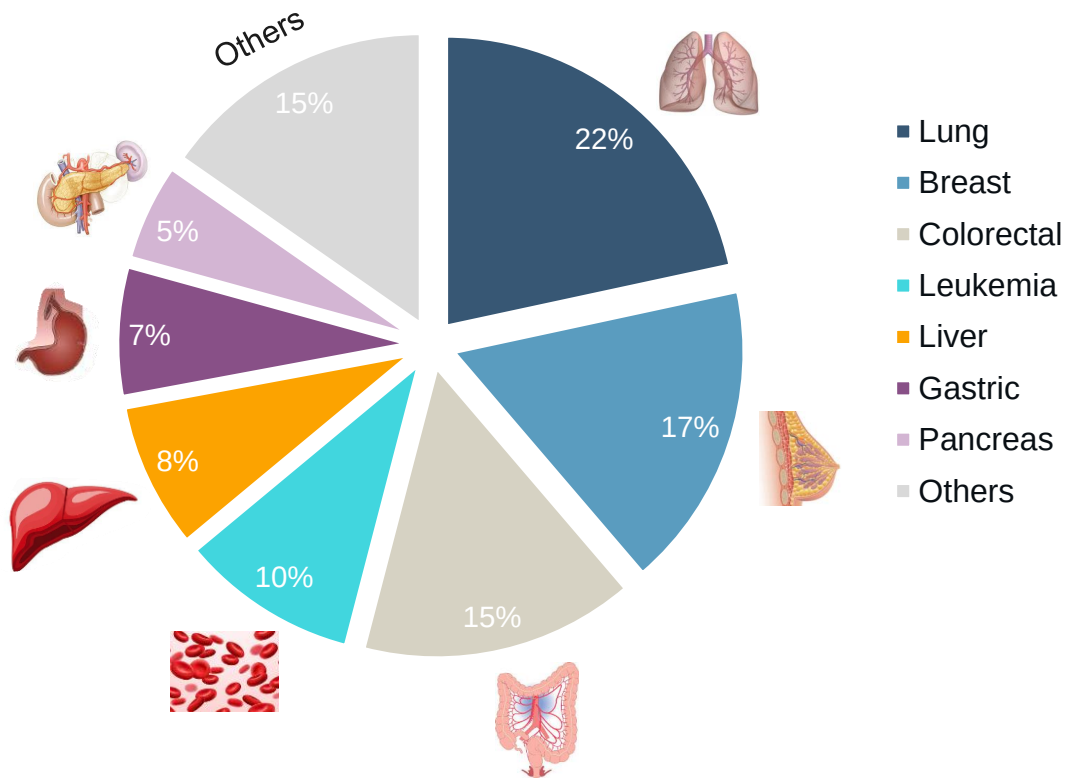
MRD Clinical Trials Still Small but on the Rise

Cancer Treatment Clinical Trials using MRD as a Measure[1]



** As of 1st June, 2024*

MRD clinical trials by organ: 2014-2023



MRD / Monitoring

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Source: ClinicalTrials.gov, 1. Including primary and secondary endpoints

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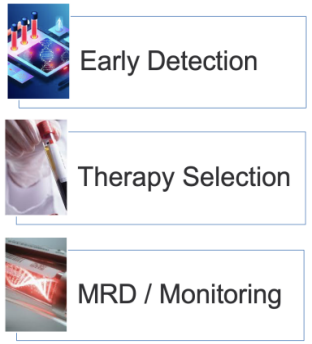
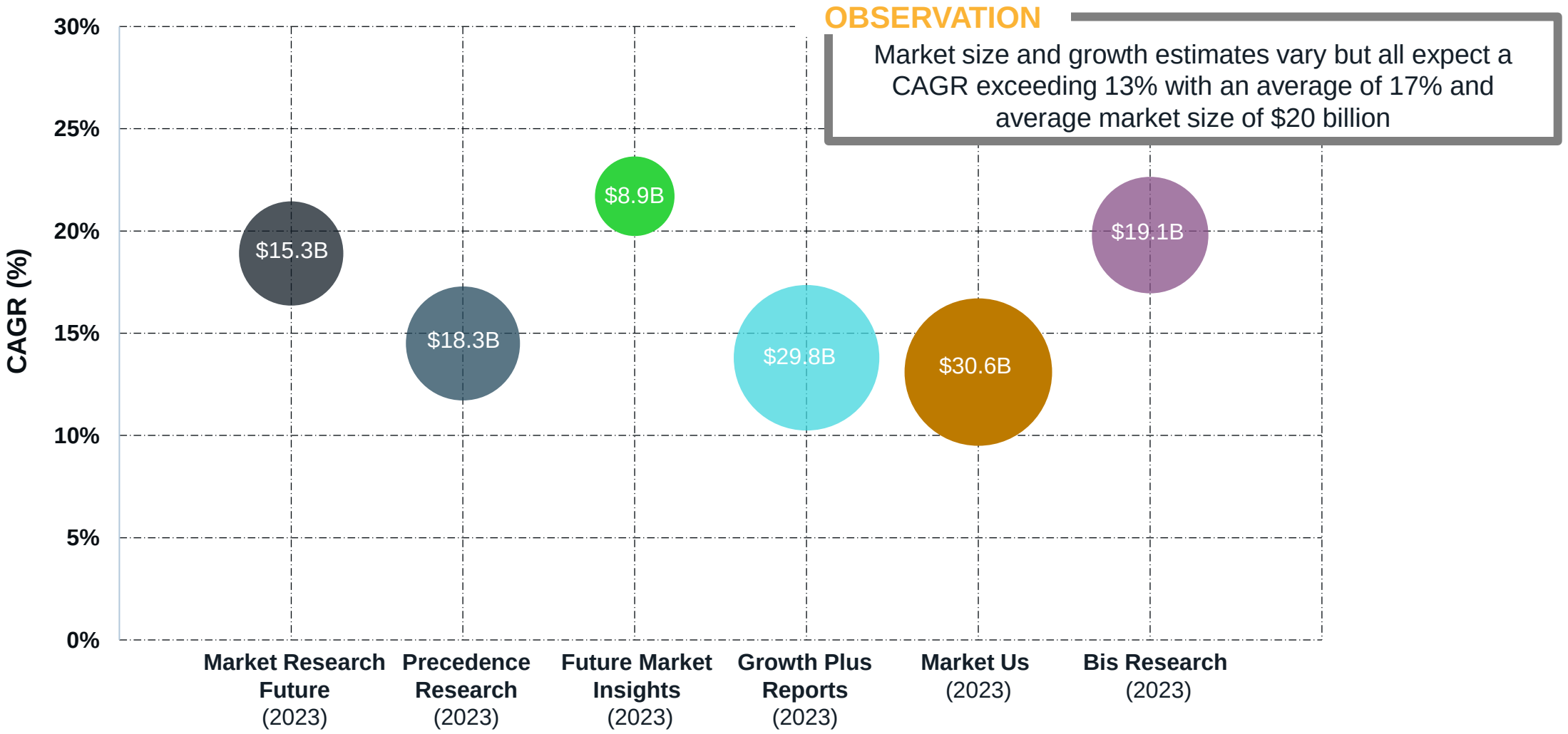
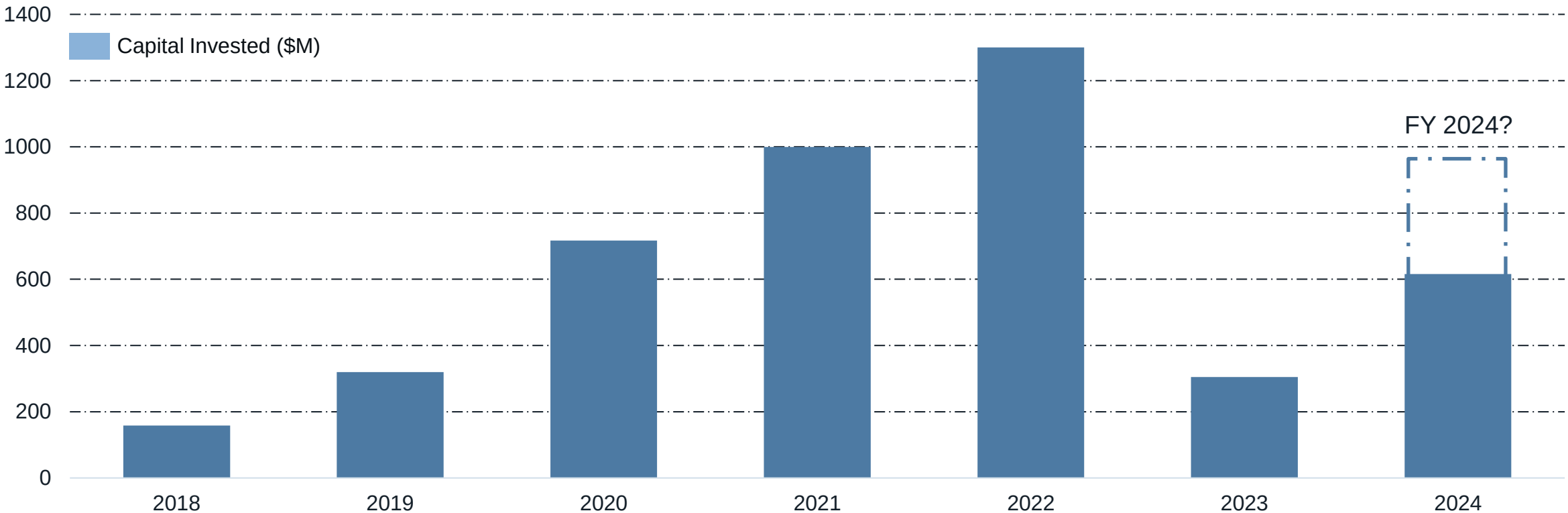


Liquid Biopsy Market Trends Forecast a Decade of Steady Growth (2023 - 2032)



Liquid Biopsy Venture Capital & Private Equity Investments 2018 – 08/2024



2018	2019	2020	2021	2022	2023	2024
Genecast 臻和 $32M Series C	Freenome $160M Series B	Freenome $270M Series C	Freenome $300M Series D	Freenome $1100M Series E	Universal DX Diagnostics $79M Series B	Freenome $254M Series E
Pillar Biosciences $16M Series B	VisionGate $37M Series A	Genecast 臻和 $181M Series E	Delfi $100M Series A	Delfi $225M Series B	Precede Biosciences $57M Series A	Billion to One $130M Series D
Predicine $15M Series A	RareCyte $22M Series F	Pillar Biosciences $135M Series C	ClearNote $85M Series C	Billion to One $198M Series C	adela $48M Series A	stilla® $27M Series C

Largest Three Deals

Source: Pitchbook (01-01-2018 to 31-08-2024)

Five Most Active Investors by Number of Deals (2018 – 08/2024)

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Venture Firms

illumina VENTURES™

andreessen horowitz

CIVILIZATION VENTURES

anri

DCVC

Corporate Venture Firms

AMGEN® Ventures

GV

KAISER PERMANENTE® VENTURES

labcorp

Roche

Governmental

NIH National Institutes of Health

Department of Health & Human Services · USA

Scottish Enterprise

Horizon 2020 European Union Funding for Research & Innovation

NIH National Cancer Institute

Source: Pitchbook (01/01/18 to 12/31/23)

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Illumina Ventures: Advancing Liquid Biopsy Innovations

DELFI

Non-invasive early cancer detection
Analyzing plasma-derived cell-free DNA fragmentation patterns

TAGOMICS

Disease diagnosis through comprehensive multiomic profiling
Multiomic biomarkers from a DNA sample using a streamlined workflow

Serimmune

NGS-based universal serology
Mapping human immunity through antibody detection

Precede Biosciences

Liquid biopsy delivering tissue transcriptional biology
Harnessing the full power of gene regulatory elements for precision medicine

STILLA

Simple, rapid, multiplexed digital PCR
Cost effective assays for genomic markers
Technology can be used for liquid biopsy tests

PREDICTA BIOSCIENCES

Utilizing AI and multi-omics to develop personalized diagnosis and treatment options

illumina VENTURES LABS™ Stealth Company

Machine Learning applied to NASH / MASH detection

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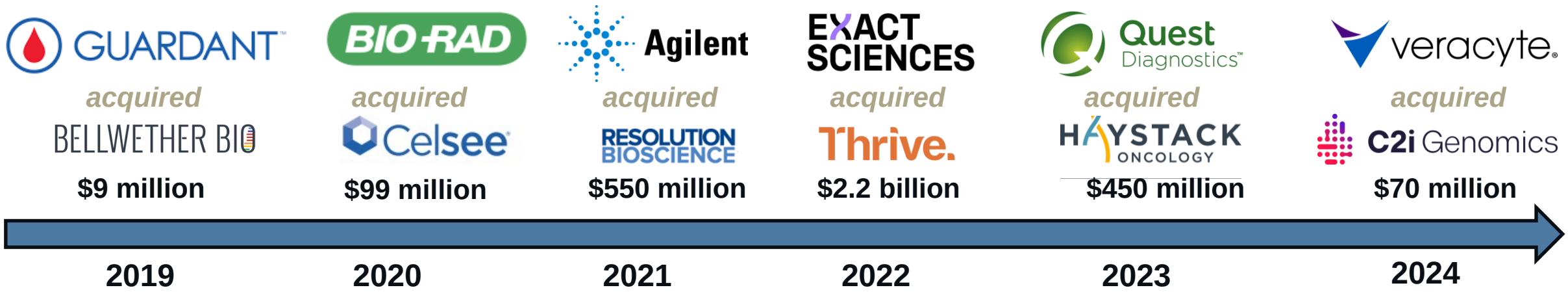
Liquid Biopsy M&A Activities

NEO GENOMICS acquired Inivata — $390 million

illumina acquired GRAIL — $8 billion



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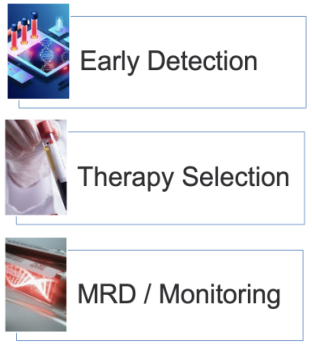
Source: Pitchbook 2019 – August 2024

Liquid Biopsy: Agenda

- Liquid Biopsy Overview
- Current Products and Research
  - Early Cancer Detection: Single-Cancer and Multi-Cancer
  - Therapy Selection and Response
  - MRD / Monitoring
- Market and Financials
- **Looking forward: Opportunities**



Strategic partnerships to address challenges

Diverse Clinical Data Generation

- *June 15, 2023: Freenome partnered with Walgreens to reduce clinical recruitment challenges and gain access to a diverse trial population*

Workflow Integration

- *April 4, 2022: Guardant partnered with EPIC to include its tests in EPIC's AURA electronic ordering/reporting platform to overcome integration challenges associated with traditional paper/fax ordering/reporting*

Reimbursement and Reach

- *May 16, 2024: GRAIL partnered with Savings Bank Mutual Life Insurance Company of Massachusetts (SBLI) to offer its test to members to address adoption and coverage challenges associated with traditional commercialization*

Logistics Support

- *February 13, 2023: Cleveland Dx partnered with Quest Diagnostics to offer its test through Quest to tap into a national logistics and phlebotomy network rather than internally developing infrastructure*

OBSERVATION

It is crucial for liquid biopsy companies to enter strategic partnerships to address highlighted challenges including data generation in specific patient populations, clinical integration, reimbursement, and commercial reach

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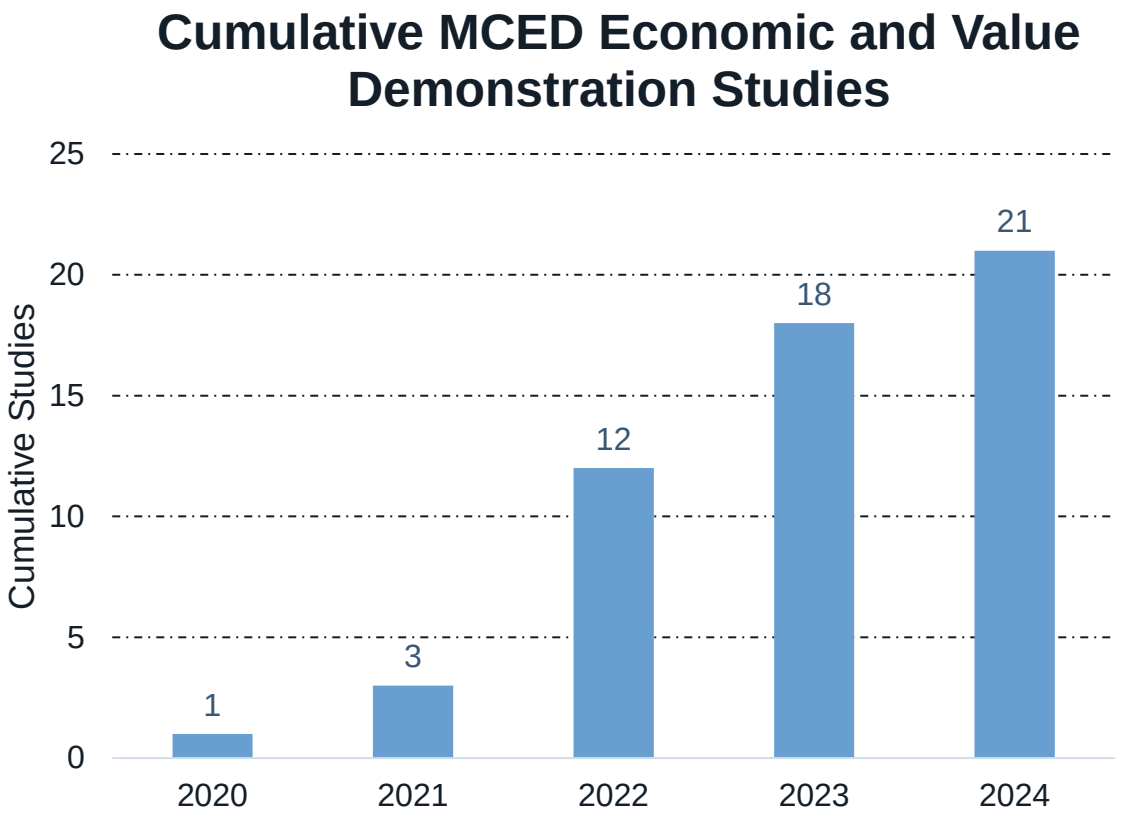
Source: Company press releases. Health Advances analysis.

MCED Economic Studies



Highlights

- Publications in peer reviewed journals and conference posters highlight key considerations required to assess the value of Multi-Cancer Early Detection (MCED) tests in cancer care
- Studies suggest that a MCED test with high specificity could potentially improve long-term health outcomes and reduce the cost of care for patients, particularly for cancers without clear screening paradigms
- However, further studies are needed to confirm cost-neutrality or savings for broad payer coverage

OBSERVATION

Given the level of reimbursement barriers surrounding MCED tests, developers have wisely invested in HEOR studies over the past few years to combat the concerns around cost effectiveness

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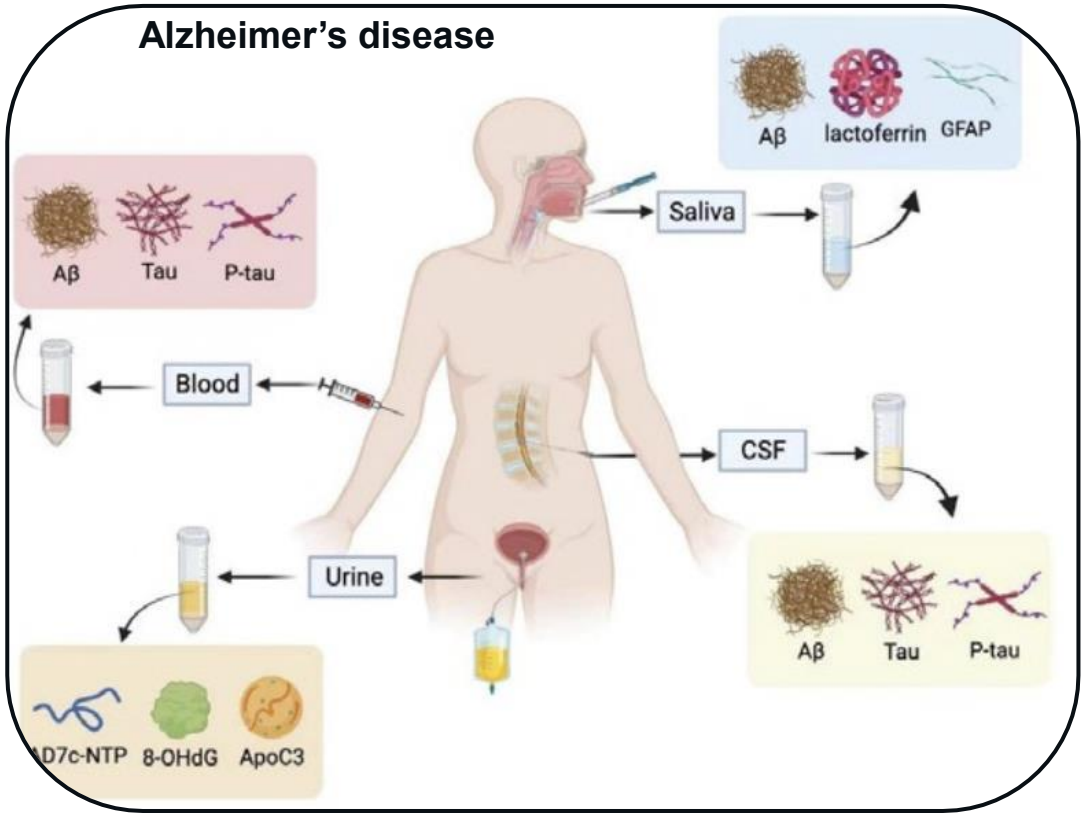
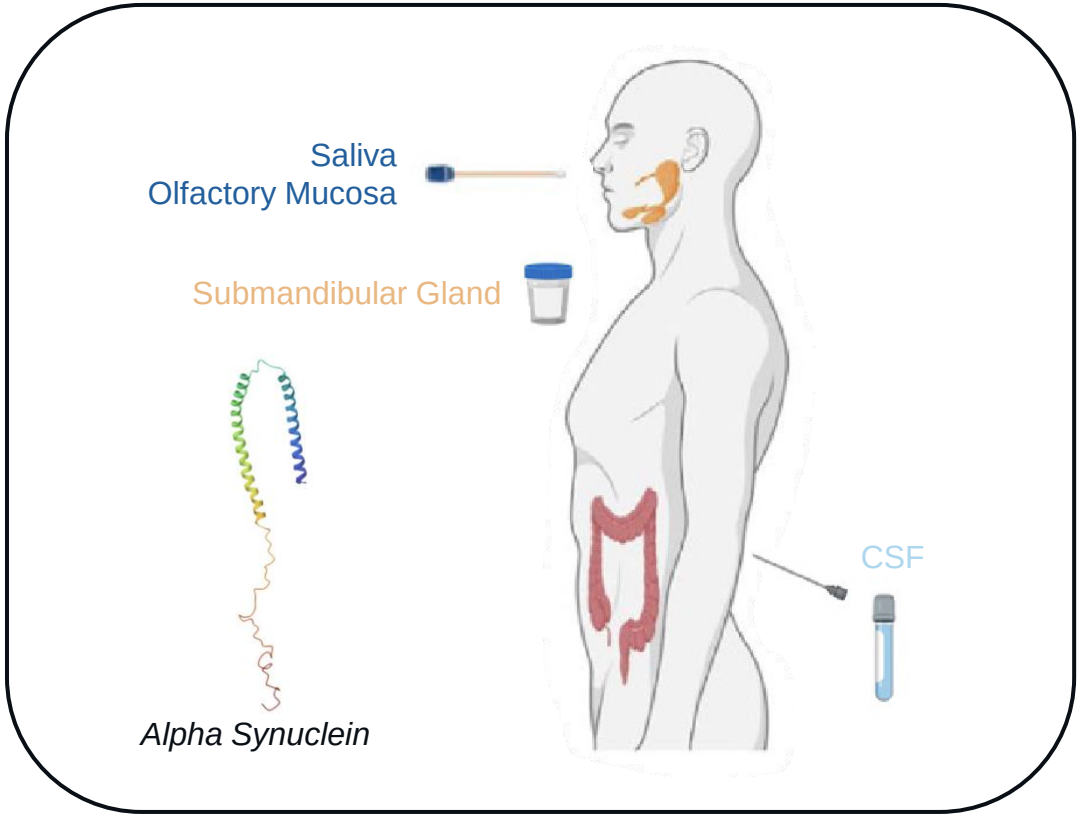
Source: PubMed, search terms (Value, HEOR, Payer) and MCED. Health Advances Analysis.

Beyond Oncology: Liquid Biopsy in Neurological Disorders



In the CSF and blood, Aβ, T-tau, and P-tau are used for the early diagnosis of Alzheimer's disease. Biomarkers in the urine are AD7c-NTP, 8-OHdG, and ApoC3, and salivary biomarkers are Aβ, Lactoferrin, and GFAP.



Alpha Synuclein assessments in different fluids for Parkinson's disease diagnostics.

OBSERVATION

The rapid and non-invasive nature of liquid biopsy assays could greatly enhance early detection and surveillance of neurological disorders

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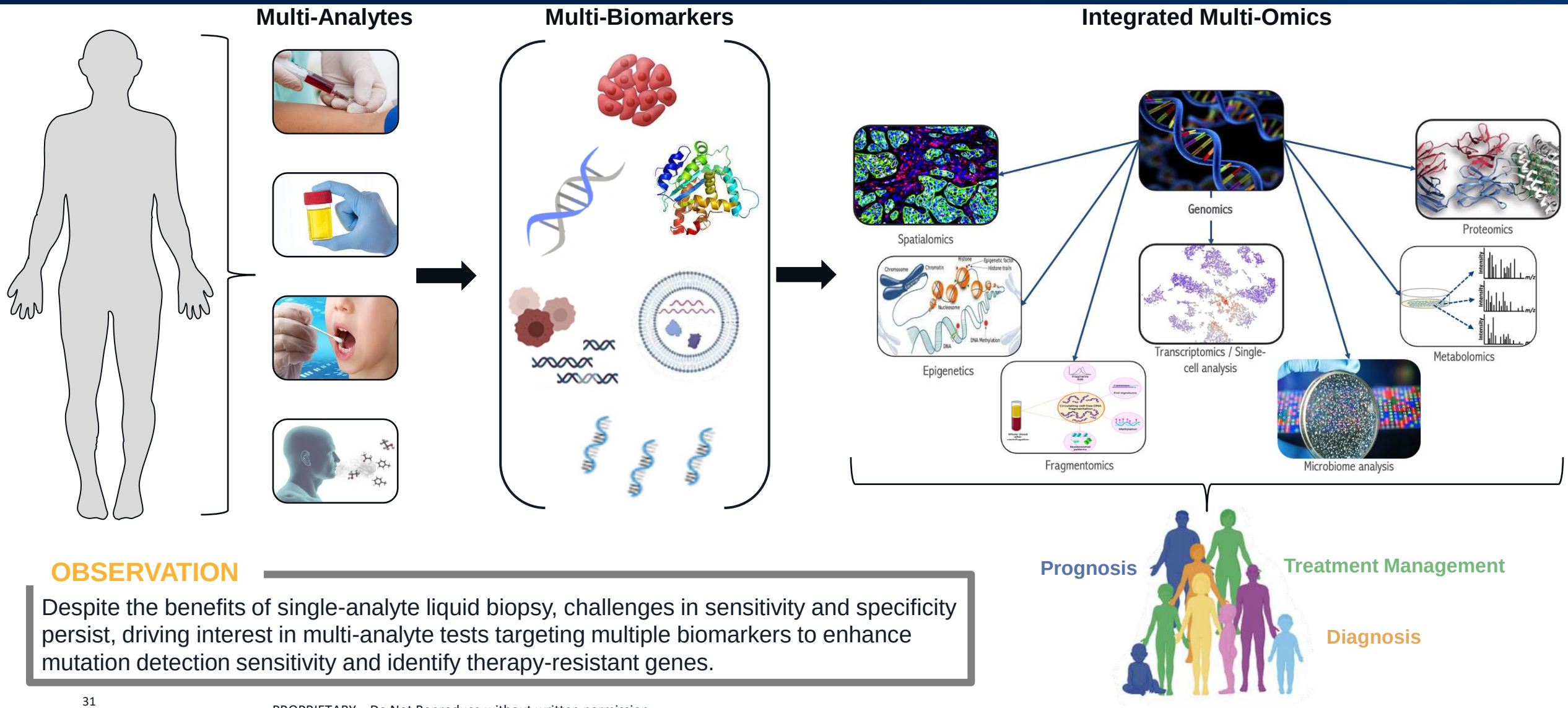
Sourced from Gong et al, 2022, Coughlin et al, 2023

Integrated Multi-Omics



OBSERVATION

Despite the benefits of single-analyte liquid biopsy, challenges in sensitivity and specificity persist, driving interest in multi-analyte tests targeting multiple biomarkers to enhance mutation detection sensitivity and identify therapy-resistant genes.

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AI/ML: Making Large Data Sets Usable

Liquid Biopsy - Integrated Multi-Omics data

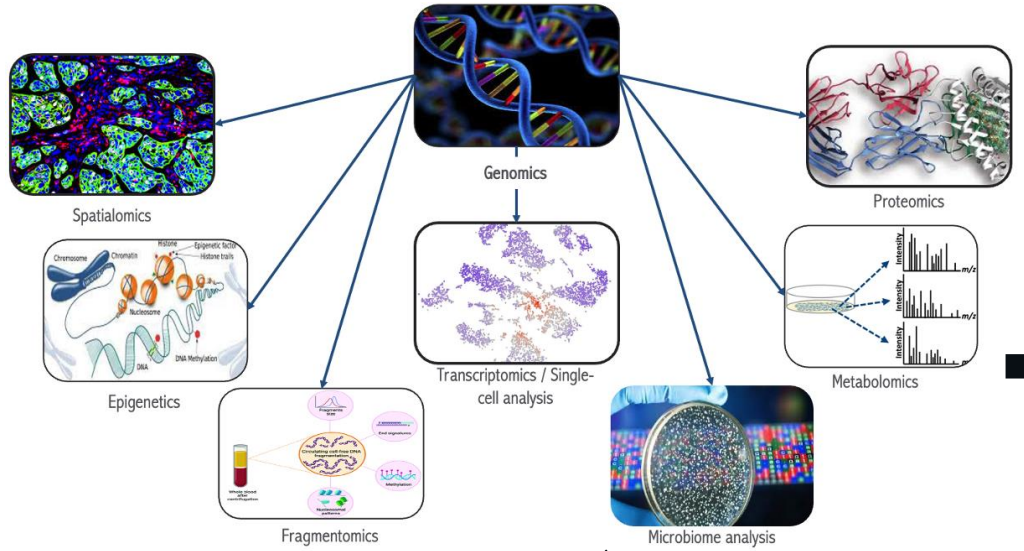
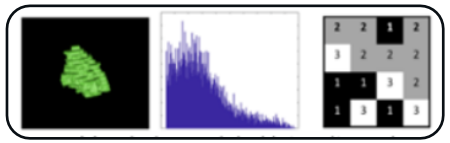
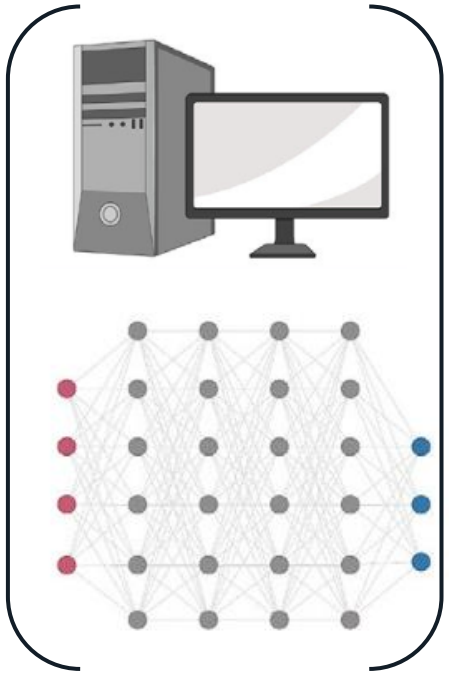


Image Acquisition



Advanced imaging Techniques

AI / ML methods



Data Analysis and Interpretation

- Complex patterns identification
- Molecular profiling

Enhanced Sensitivity and Specificity

- Increased in detecting low-frequency mutations
- Feature Selection (methylation patterns)

Early Detection and Prognostication

- Predictive Modelling
- Risk Stratification

Integration with Clinical Data

- Comprehensive Analysis
- Real-time Monitoring

Multimodal Biomarkers

- Multianalyte Analysis
- Cross-validation

Personalized Treatment Guidance

- Therapeutic Target Identification
- Response Monitoring

OBSERVATION

AI / ML will serve a critical function in tackling Liquid Biopsy limitations, bolstering analytical phases and aiding result interpretation through AI-driven clinical decision support systems

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Expanding Beyond Blood into other Biofluids

Blood

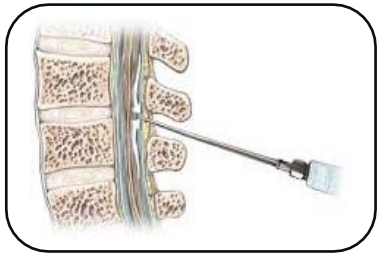
Future Potential Biofluids for Liquid Biopsies

Breast milk
e.g., Breast cancer

Urine
e.g., Prostate Cancer

Saliva
e.g., Oral cancer

Seminal fluid
e.g., Prostate cancer



Cerebrospinal fluid (CSF)
e.g., Brain cancer

Sweat
e.g., Lung cancer

Synovial fluid
e.g., Rheumatoid arthritis

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