Institut für Gewebemedizin und Pathologie

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Liquid Biopsy in Cancer Patients – Now and Tomorrow

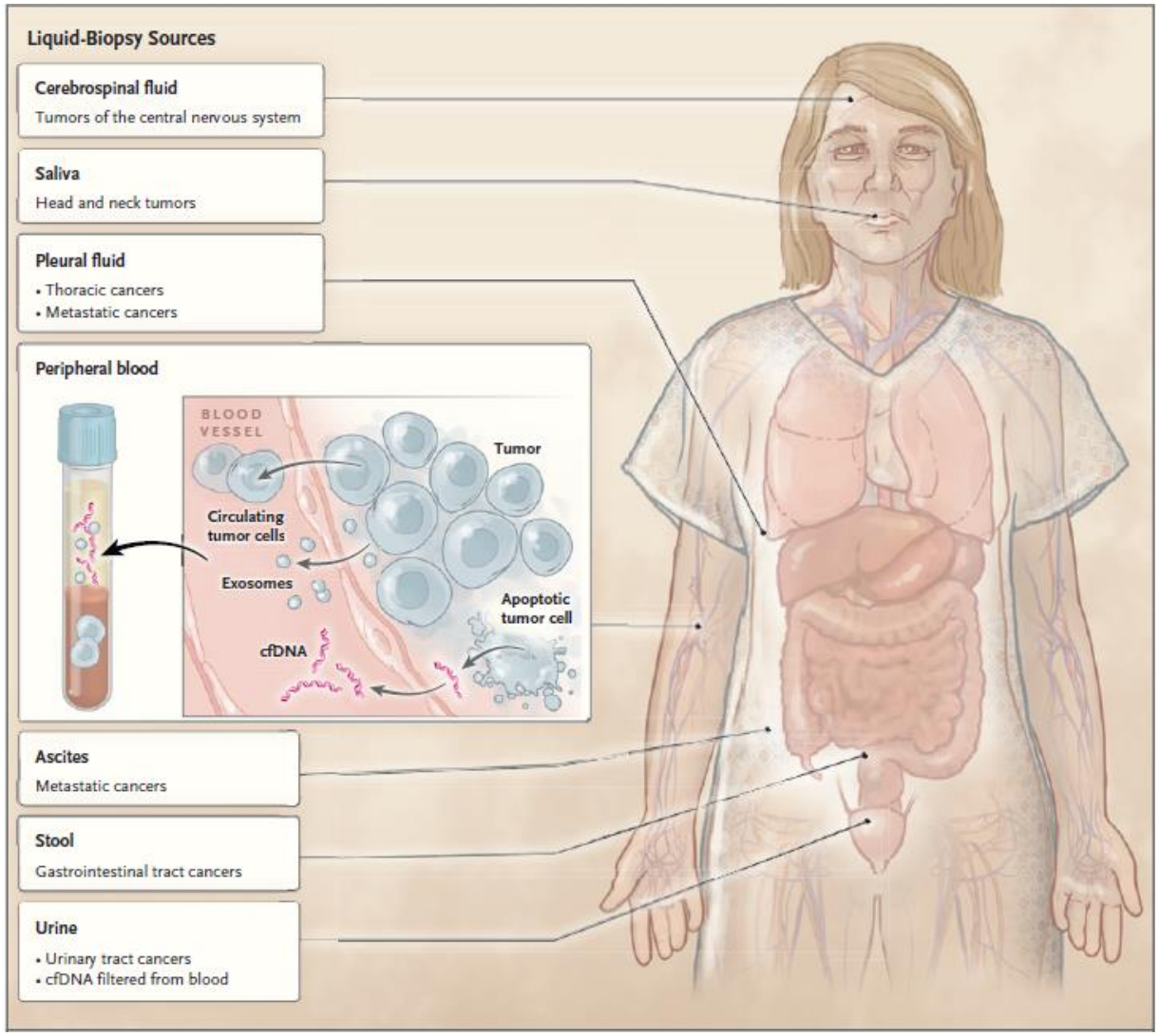
PD Dr. med. Heather Dawson

Molecular Diagnostics 2025, Zurich

Disclosures

Advisory activities for Takeda, Astellas and MSD

What is a liquid biopsy?

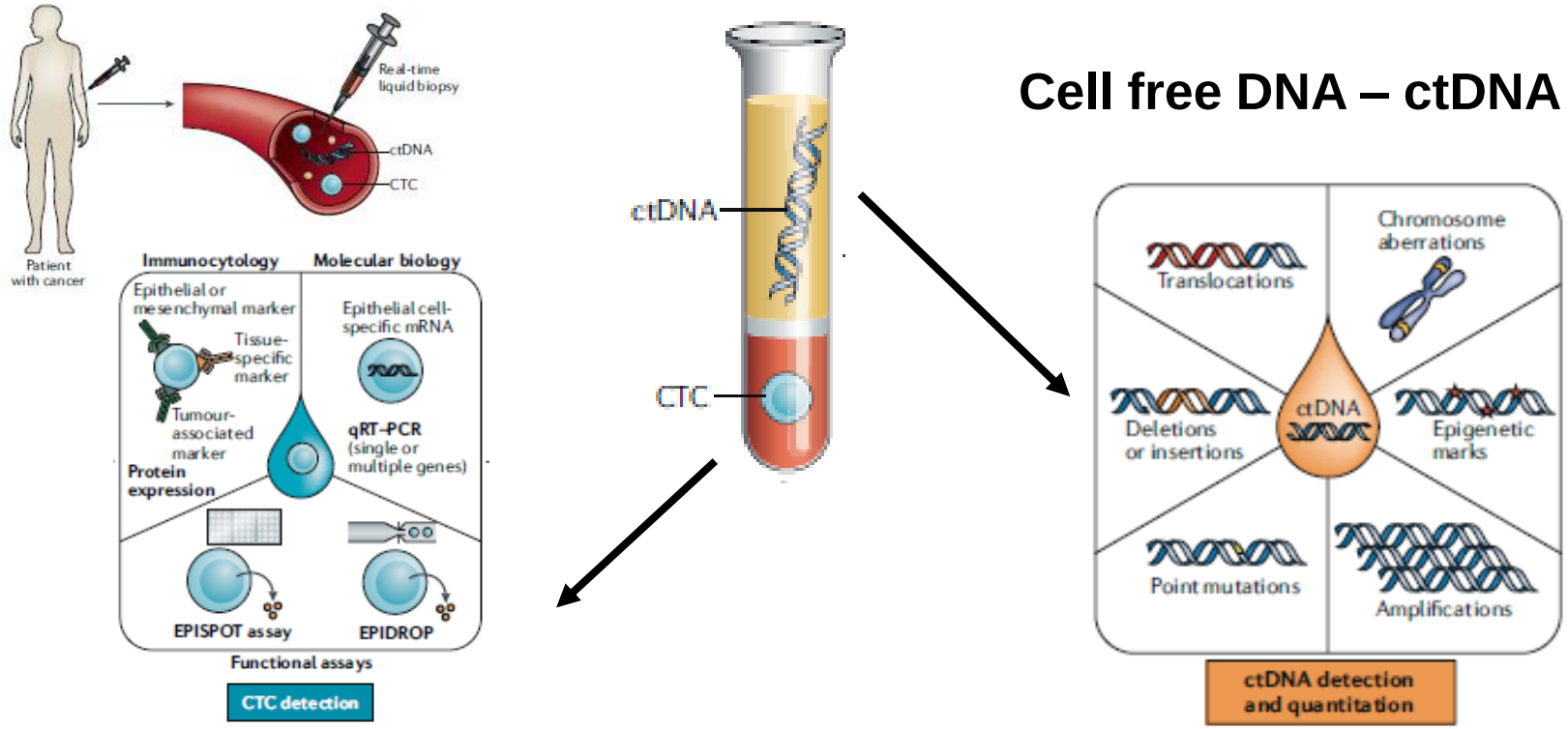


Any body fluid

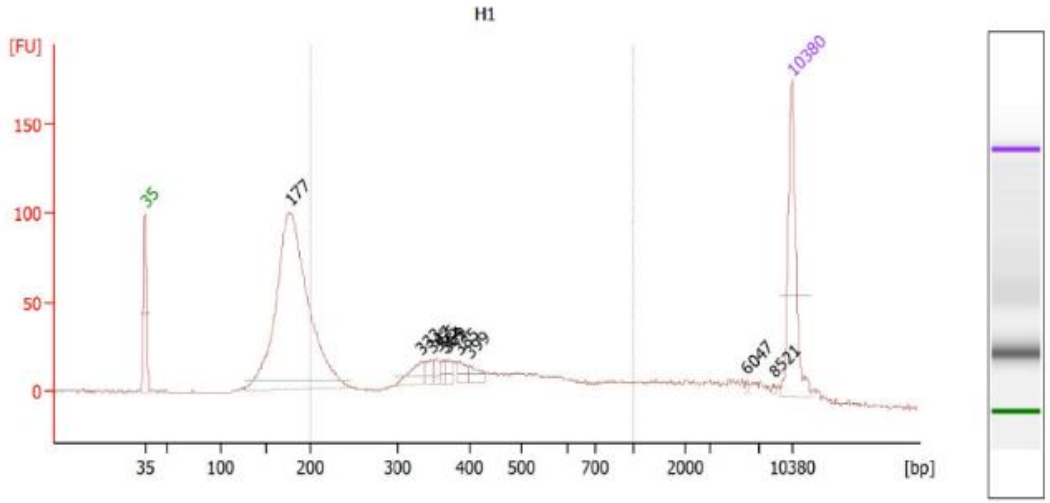
- Blood → solid & hematological malignancies
- Cerebrospinal fluid → brain tumors
- Saliva → Oral tumors
- Bile → Tumors of the bile ducts
- Urine → Urinary tract tumors

Corcoran et al, NEJM 2018

What can we analyze in a blood liquid biopsy?

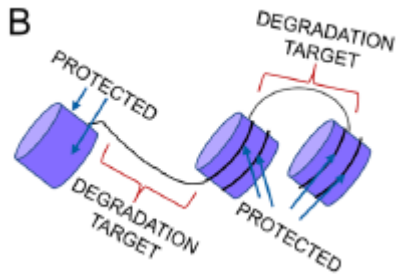


cfDNA vs ctDNA



Cell free DNA (cfDNA)

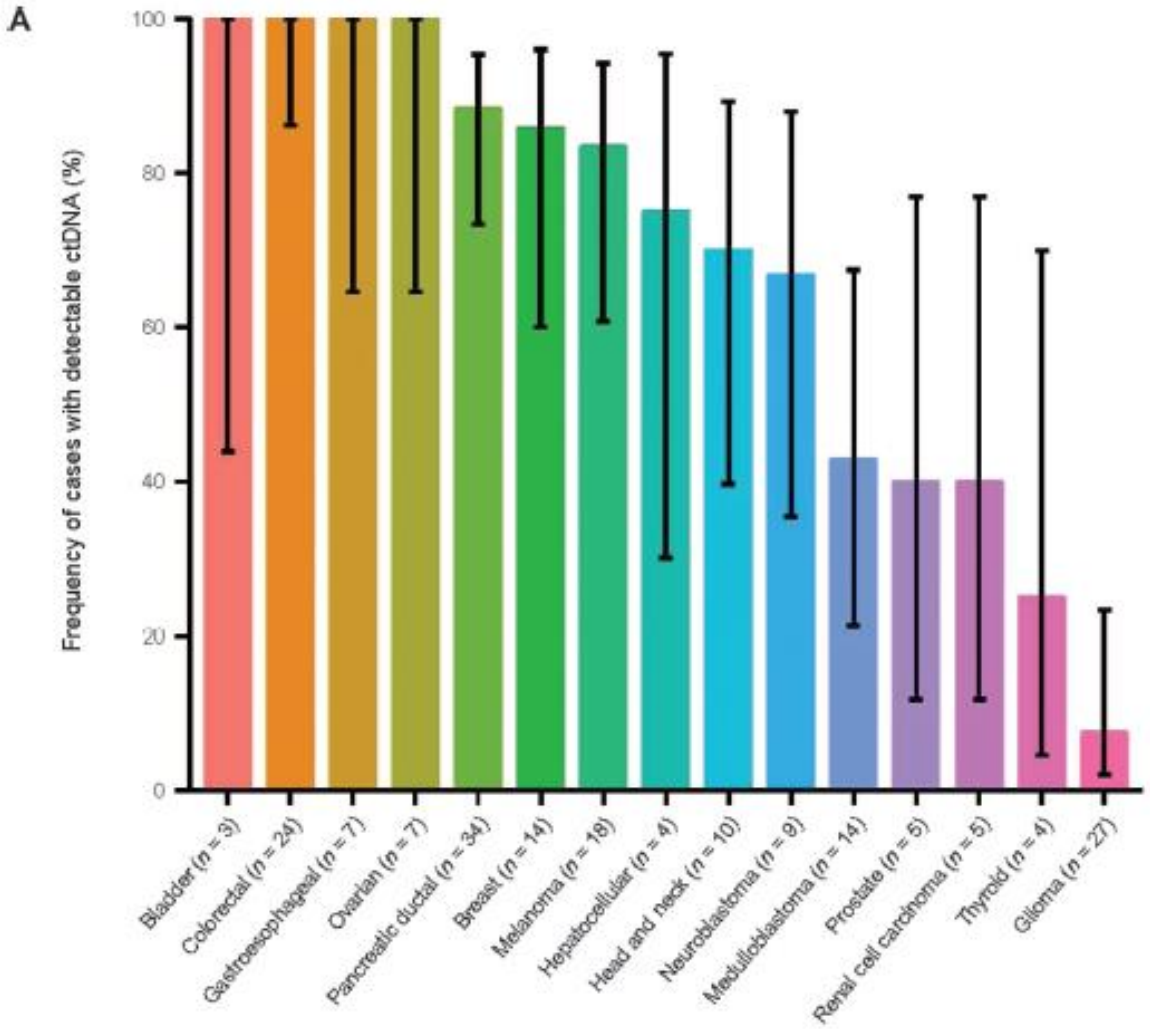
- Apoptosis or necrosis
- Double strand DNA 150-200 bp (length of DNA wrapped around a nucleosome and a linker fragment)
- Half life < 1h, degraded by enzymes and eliminated mainly by liver
- 1-10 ng/ml, increased with infection, injury and in tumors

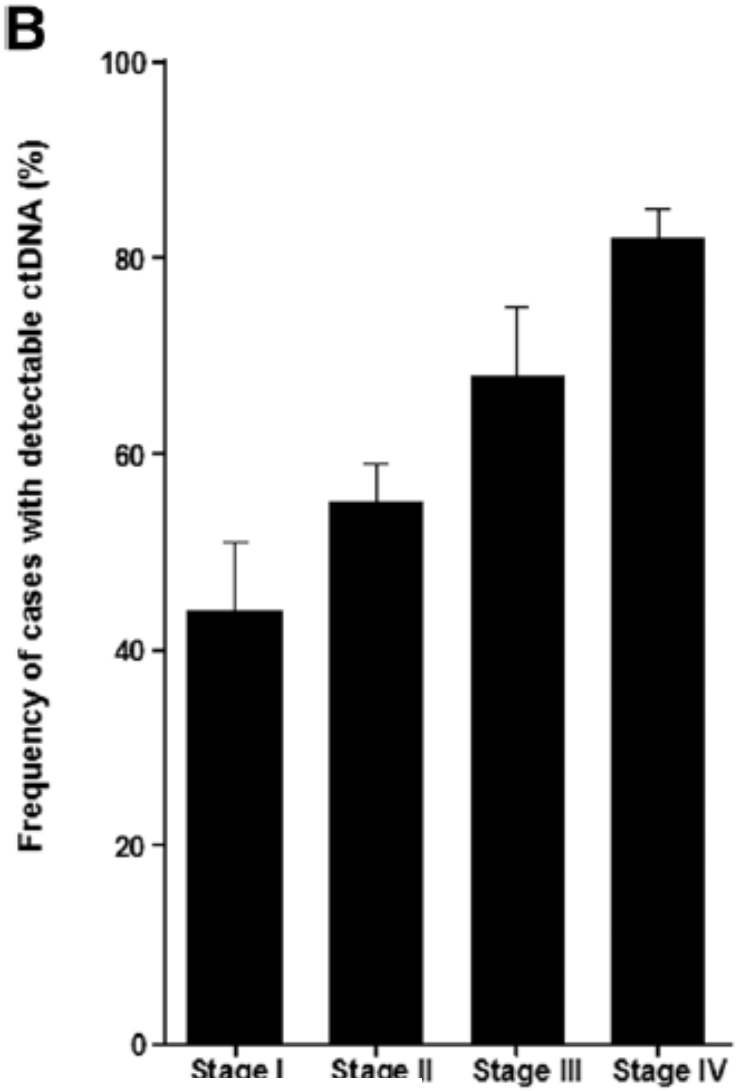


Circulating tumor DNA (ctDNA) is the fraction of cfDNA released by cancer cells

Hasenleitner et al, Molecular Cancer 2022

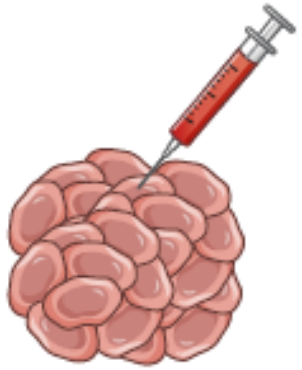
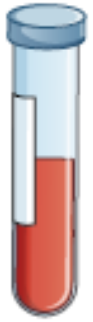
Dectection of ctDNA depends on tumor type and stage



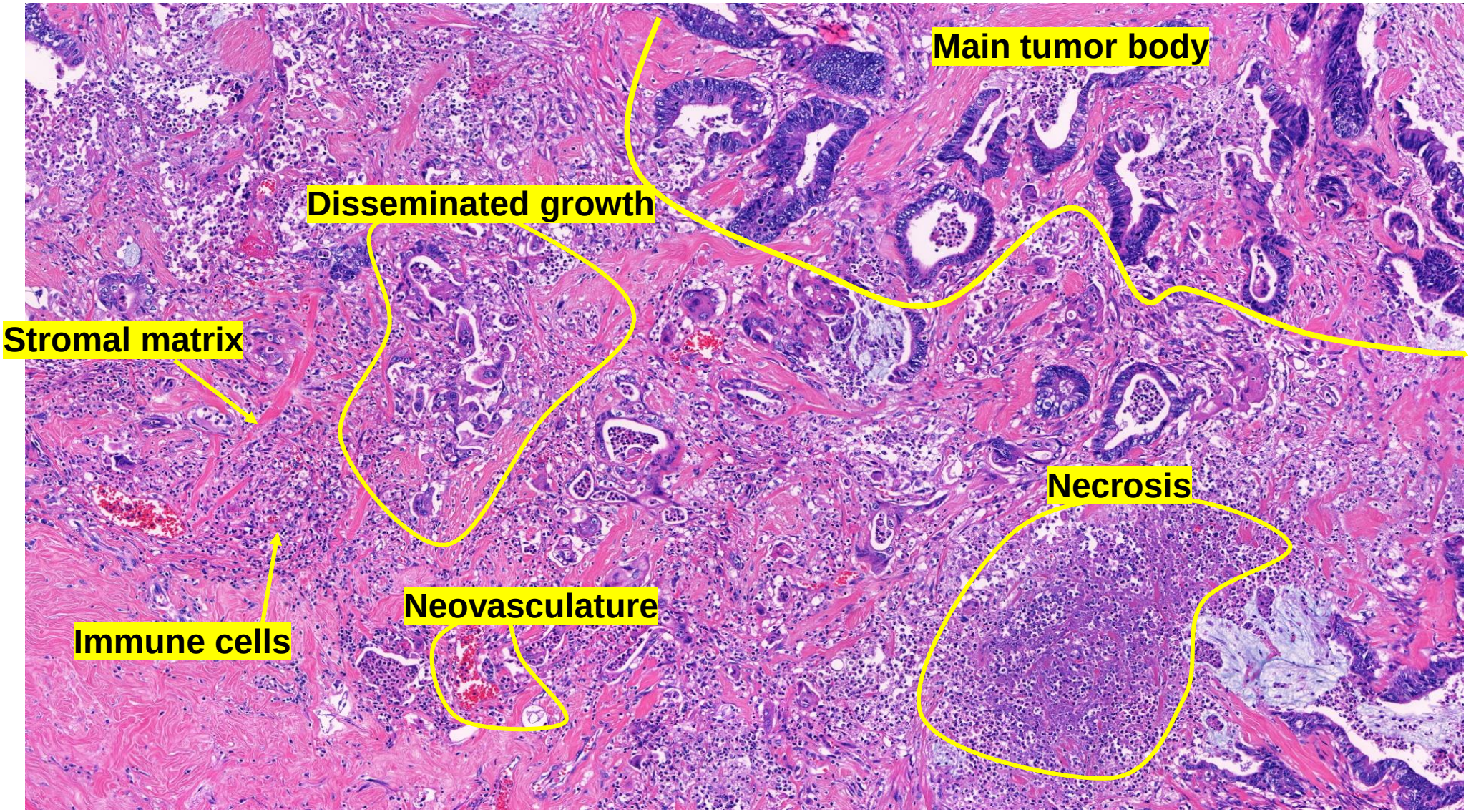


Bettegowda et al, Sci Translat Med, 2014

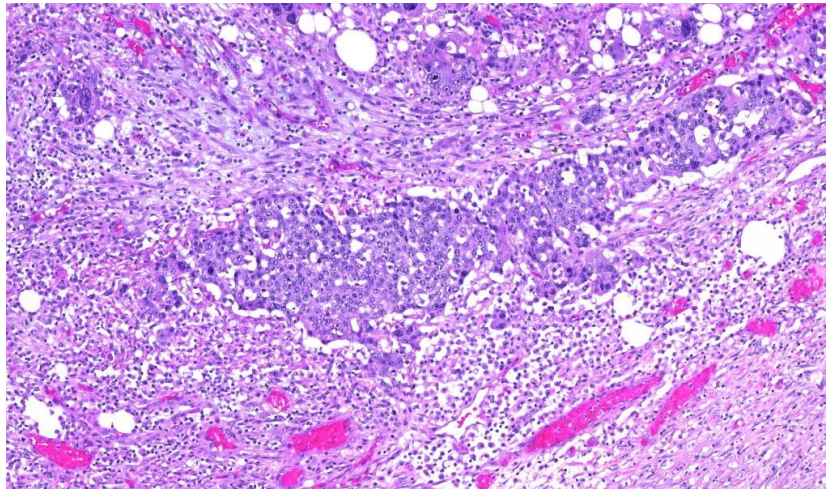
Liquid biopsy vs tissue biopsy

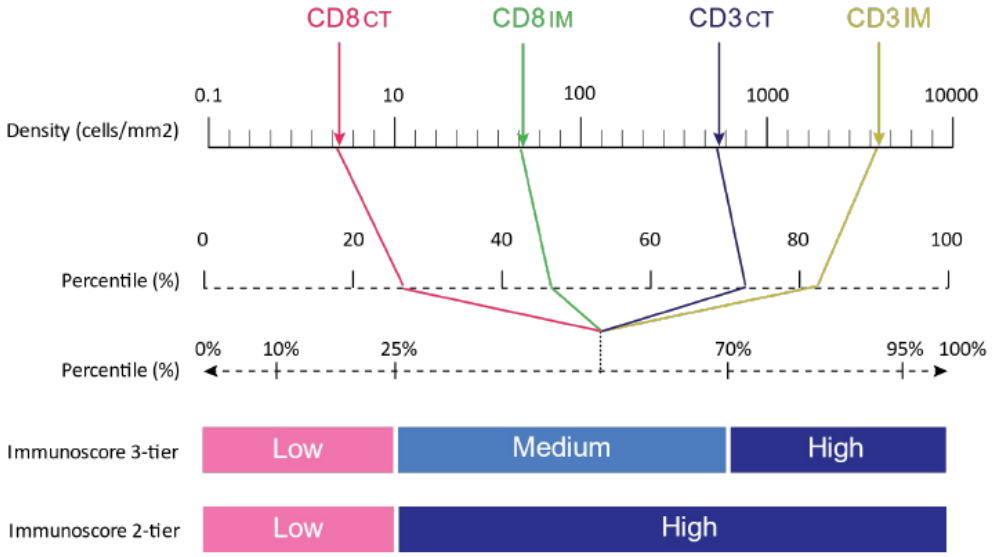
		At metastatic diagnosis	After subsequent lines of therapy
Tumor biopsy 	Advantages	• Key pathological information • Ability to assess non-DNA biomarkers (protein, RNA, etc)	• Important for research and discovery • Critical if assessment of non-DNA biomarkers needed
	Disadvantages	• Longer turnaround time for sequencing limits first-line precision-therapy selection • Limited tissue quantities can constrain breadth of testing or cause assay failure	• Requires repeat invasive procedure • Longer turnaround time for sequencing results may hinder rapid selection of therapy
Liquid-biopsy cfDNA 	Advantages	• High concordance with tissue biopsy • Ready sample availability • Rapid turnaround to facilitate first-line precision-oncology therapies • Baseline for subsequent liquid biopsy	• Non-invasive, easy to obtain serial samples • Captures heterogeneous resistance alterations • Rapid turnaround can enhance clinical-trial enrollment
	Disadvantages	• Parallel assessment with tumor testing increases cost • Cannot assess non-DNA biomarkers	• Cannot assess non-DNA biomarkers

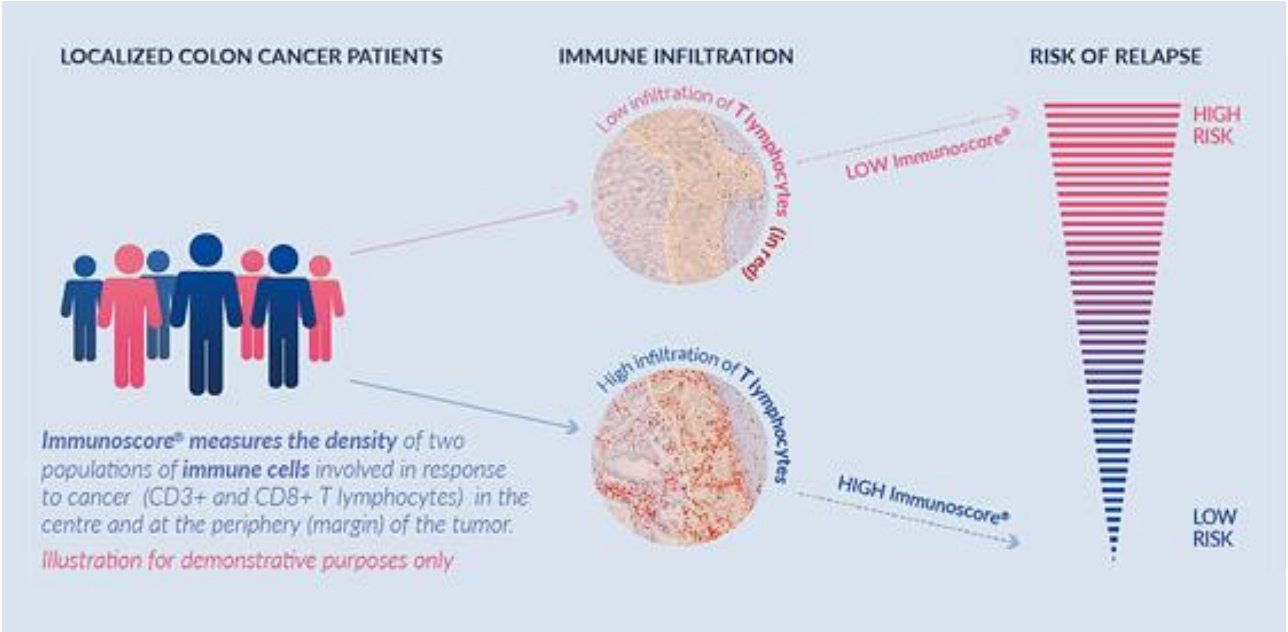
Tissue biopsy as a comparison…



Immunoscore: Example of tissue-based biomarker of the host reaction







Computational analysis of CD3 and CD8 T-cells in the tumor center and at the tumor front

Outperforms traditional factors (stage) in predicting cancer reccurrence

Angell et al, CCR 2020

Which kind of tests can be performed?

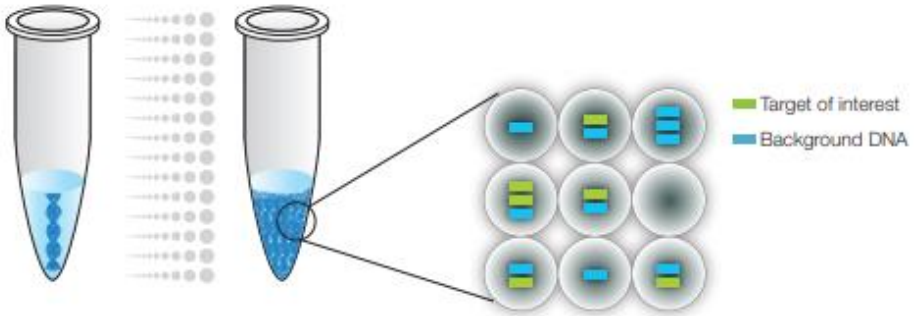


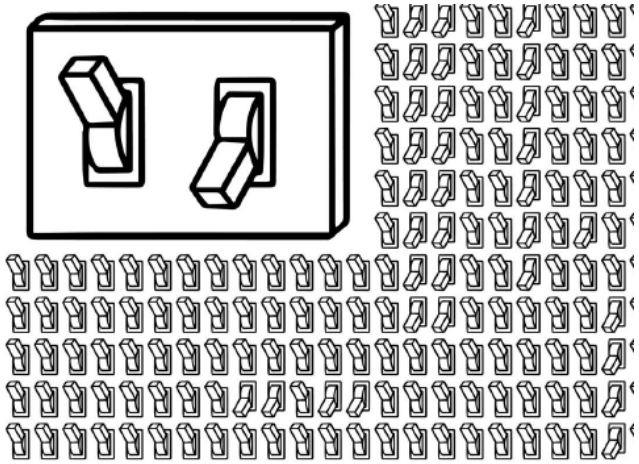
Fig. 1.3. In ddPCR, a single PCR sample is partitioned into 20,000 droplets.

Digital PCR

Second generation
(next generation sequencing)

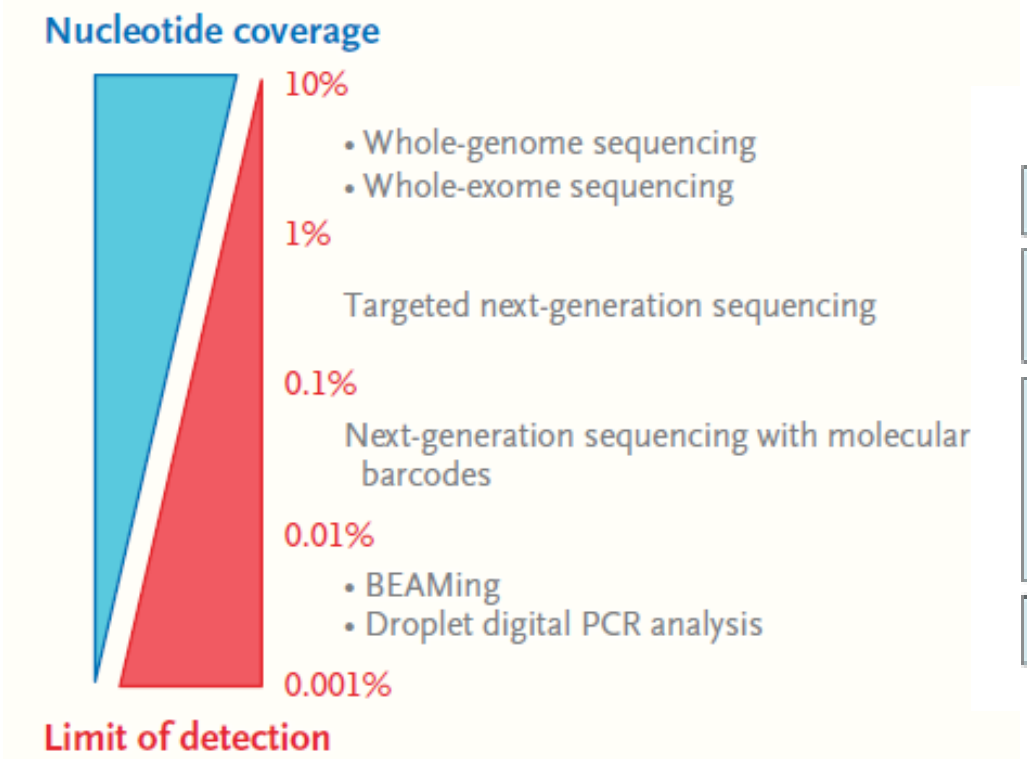
454, Solexa, Ion Torrent, Illumina

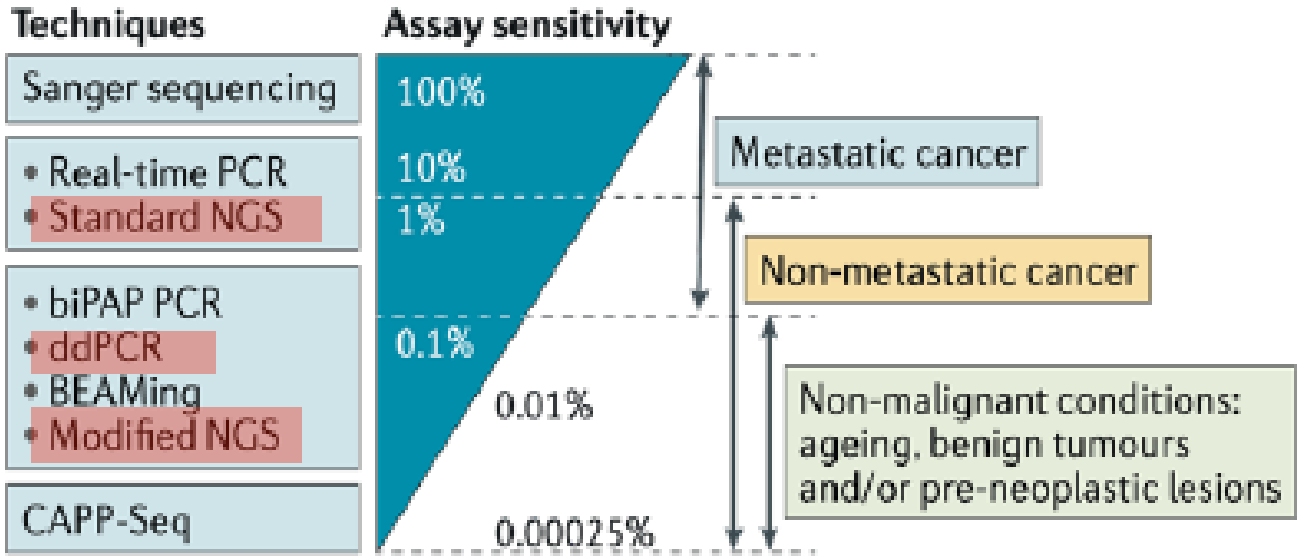
High throughput from the parallelization of sequencing reactions



Epigenetic profiling

Tradeoffs depending on assay

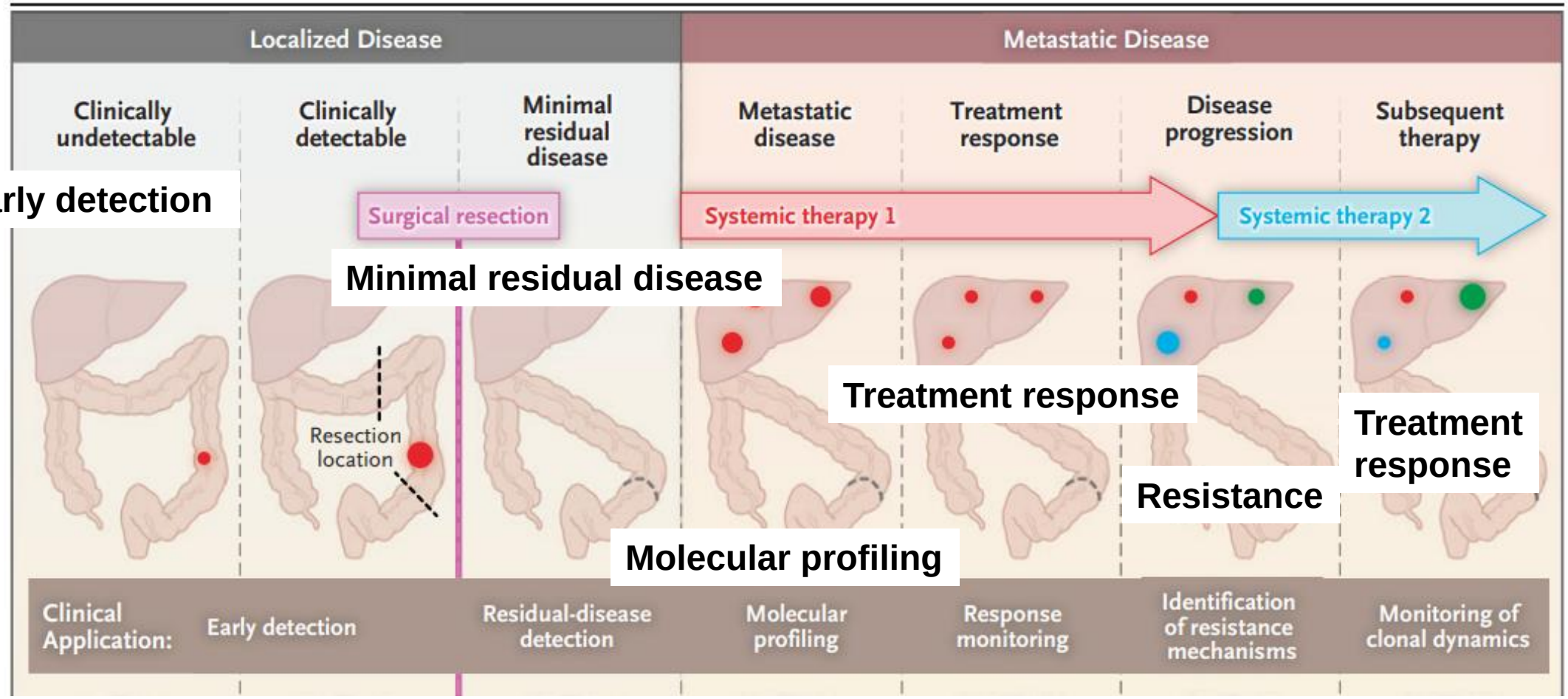




Traditional assays compromise between coverage and limit of detection (LOD)
Modified NGS assays increases sensitivity and fulfil requirements for genomic profiling

Corcoran et al, NEJM 2018

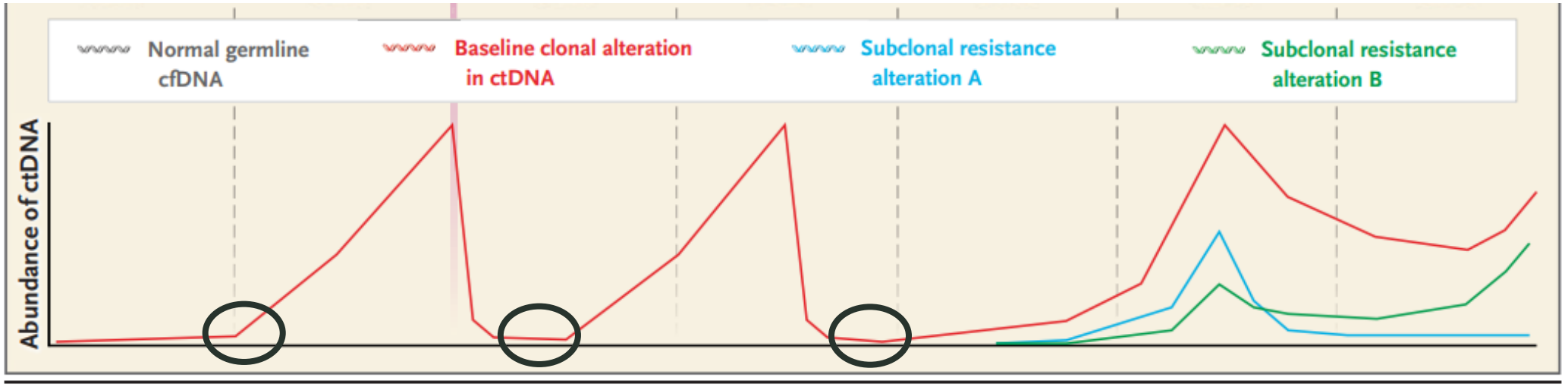
What clinical questions can be answered by liquid biopsies in cancer patients?



Corcoran et al, NEJM 2018

The fraction of ctDNA is variable across disease evolution

Early detection **Minimal residual disease** **Response to therapy**



Highly sensitive assays are required!

Corcoran et al, NEJM 2018

Confounder: Clonal hematopoiesis/CHIP

Non-tumor somatic mutations in hematopoietic cells leading to clonal expansion during ageing process

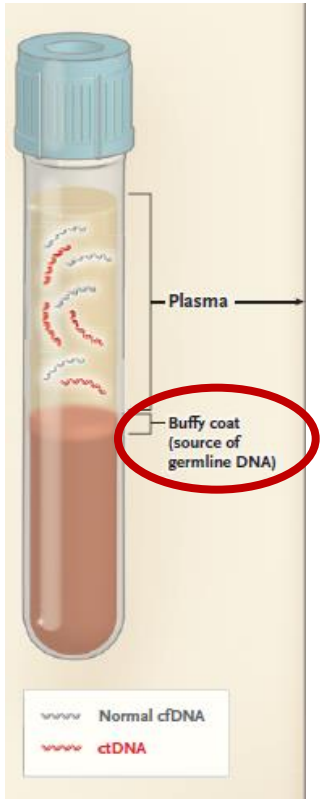
Frequent finding

CH ("clonal hematopoiesis"): clonal outgrowth of hematopoietic cells

CHIP ("clonal hematopoiesis of indeterminate potential"): mutations with at least of 2% VAF in driver genes in white blood cells known to be associated with hematological malignanices

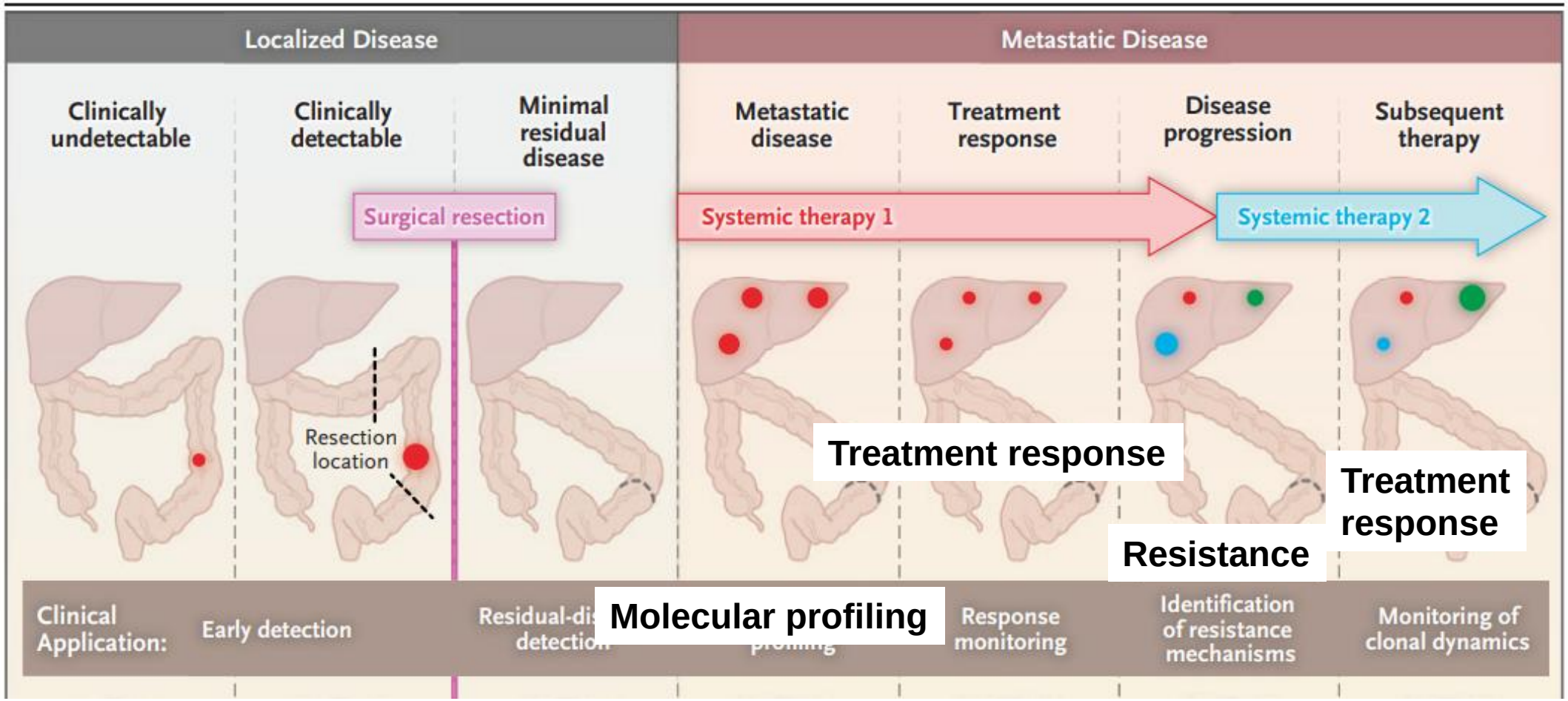
Increased risk of cardiovascular diseases and hematological malignancies

DNMT3A*, *TET2*, *ASXL1*,*JAK2*, *PPM1D*, *TP53*, *IDH2*, *SF3B1*, *SRSF



Solution: Paired sequencing with buffy coat/tumor informed assay

Liquid biopsies today



Corcoran et al, NEJM 2018

Current recommendations for liquid biopsies

Table 2. Tumour-specific table for advanced cancer genotyping

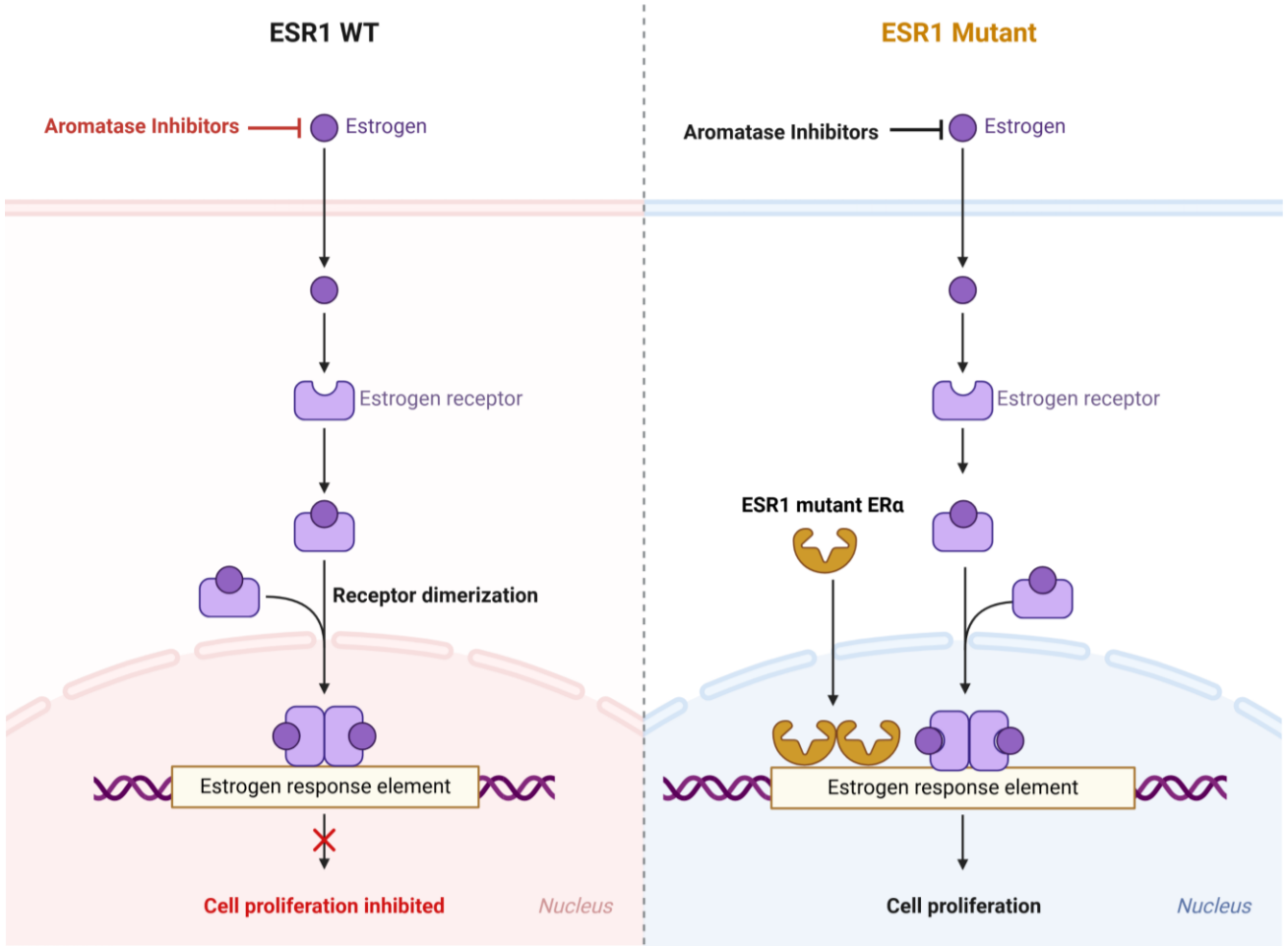
Tumour type	Indications	ESCAT tier and level of evidence	Recommendation
Non-small-cell lung cancer	*EGFR* (for common, uncommon, exon 20 insertions, T790M and other resistance mutations e.g. C797X).	IA[120]	ctDNA genotyping recommended in treatment-naive cancer patients and resistance upon prior TKIs. Caution should be kept as ctDNA assays will miss histological trans-differentiation. ctDNA testing may not have adequate sensitivity to detect *MET* true high copy number gain as resistance mechanism to osimertinib or lorlatinib. Amplification and fusion detection is suboptimal with ctDNA assays, and should be repeated in tissue where possible.
	ALK (for fusions and acquired resistance kinase domain mutations).	IA[121-125]	
	MET (for exon 14 splice site mutations, and acquired resistance mutations)	IB[126,127]	
	KRAS (for G12C and non-tier 1 other *KRAS* mutations)	IB[128]	
	BRAF (for V600E)	IB[129,130]	
	RET (for fusions and acquired resistance kinase domain mutations)	IB[131]	
	ROS1 (for fusions and acquired resistance kinase domain mutations)	IB[132,133]	
	NTRK 1/2/3 (for fusions and acquired resistance mutations)	IC[134]	
	MET (for high-level copy number gain/amplification)	IIA[135]	
	ERBB2 (for exon 20 insertions and transmembrane mutations, and amplification)	IIB[136-138]	
	BRAF (for non-V600E class I-III mutations)	IIB[139]	

General indication for liquid biopsies: '*if tumor tissue not available*'

Constraints of inadaequate biopsies for molecular testing (lung, pancreas)

Pascual et al, Ann Oncol 2022

New application for ESR1 testing in breast cancer



Activating ESR1 mutations are found in 20-40% in patients with metastatic breast cancer (mBC) who have previously received endocrine therapy

The selective estrogen receptor degrader Elacestrant available since 6/2024 in Switzerland for patients with progressive metastatic breast cancer with ESR1 mutations

Clinical trial patients selected by liquid biopsy → Swissmedic recommendation

Biorender.org

Workflow liquid biopsies at CGL

Illumina TSO500 ctDNA V2, 1x/week

1. **Prepare cfDNA**
   Hands-on: 10 minutes
   Total: 10 minutes
   Input: 20 ng
   Reagents: RSB
2. **Perform End Repair**
   Hands-on: 10 minutes
   Total: 50 minutes
   Reagents: ERP6
3. **Perform A-Tailing**
   Hands-on: 5 minutes
   Total: 40 minutes
   Reagents: ATL4
4. **Ligate Adapters**
   Hands-on: 10 minutes
   Total: 30 minutes
   Reagents: LIGX, UMI DIA
5. **Clean Up Ligation**
   Hands-on: 20 minutes
   Total: 30 minutes
   Reagents: EPM4, IPB, RSB, UD Index Plate, 80% EtOH
6. **Index PCR**
   Hands-on: 5 minutes
   Total: 50 minutes
7. **Hybridize Probes**
   Hands-on: 10 minutes
   Total: 85 minutes
   Reagents: EHB2, OPD2, NHB2
8. **Capture Hybridized Probes**
   Hands-on: 45 minutes
   Total: 80 minutes
   Reagents: EEW, EPM4, RSB, SMB3
9. **Amplify Enriched Library**
   Hands-on: 5 minutes
   Total: 45 minutes
   Reagents: PPC3
10. **Clean Up Amplified Enriched Library**
    Hands-on: 20 minutes
    Total: 30 minutes
    Reagents: IPB, RSB, 80% EtOH
11. **Bead-Based Normalization**
    Hands-on: 30 minutes
    Total: 40 minutes
    Reagents: EE1, HP3, LNA1, LNB1, LNS1, LNW1, RSB

Table 2: TruSight Oncology ctDNA v2 performance[a]

Parameter	Specification
Limit of detection (LOD)	0.2% VAF for SNVs 0.5% VAF for MNVs and indels 0.5% VAF for gene rearrangements ≥ 1.3-fold change for gene amplifications ≤ 0.6-fold change for gene deletions ≥ 0.3% tumor fraction for MSI
Analytical sensitivity (at LOD)	≥ 90% (at LOD of 0.2% VAF for SNVs) ≥ 95% (at LOD of 0.2% VAF for SNV hotspots) ≥ 95% (at LOD of 0.5% VAF for all other variant types)
Analytical specificity	≥ 99.999%

Sequencing on Novaseq 6000 S2 flow cell

DRAGEN bioinformatic pipeline

Example from diagnostics: mBC

Classification	Gen	AF	cDNA	Protein	RD	Co...	T...
SNV Missense	**PIK3CA**	AF: 0.0402	c.3140A>G	p.H1047R	2489	☑	T1
SNV Missense	**ESR1**	AF: 0.0358	c.1610A>C	p.Y537S	3157	☑	T1
SNV Frame_Shift_...	**PTEN**	AF: 0.024	c.955_958del	p.T319*	1872	☑	T2
SNV Missense	**TP53**	AF: 0.0028	c.473G>A	p.R158H	3225	☐	T2
SNV Nonsense	**MAP2K4**	AF: 0.048	c.841C>T	p.R281*	2269	☐	T2

20ng input (recommended amount)

DNA Total reads (>400 Mio.):	850'321'324
DNA Exon coverage, median (>1300):	2568
DNA Sequenzen mit ≥1000-x Coverage (>80%):	97.6
DNA Insert size, median (bp):	172
DNA Aligned reads (%):	99.6
DNA HRD ≥50-x Coverage (>50%):	---
max. somatic allele frequency:	*0.024*
DNA Mean family size	*7.8*

Not just ESR1, but additional possible targets (PIK3CA, PTEN)

Fusion detection

Fusions more difficult to detect at high sensitivity when large intronic regions are involved (NTRK3, FGFR1) that contain highly repetitive sequences

RNA could be a possible solution and also give information on gene expression, but extremely low amount and degrades quickly

All fusions detected in reference materials at 0.5% VAF → LOD of our assay
EML4::ALK, CD74::ROS1, NCOA4::RET

Fusion	EML4::ALK	AF: 0.003015
Fusion	CD74::GO...	AF: 0.003423
Fusion	NCOA4::RET	AF: 0.002606

IGMP Institut für Gewebemedizin und Pathologie

Colorectal cancer (CRC) – important facts

In western countries, 1 in 23 men and 1 in 25 women will be diagnosed with CRC in their lifetime.

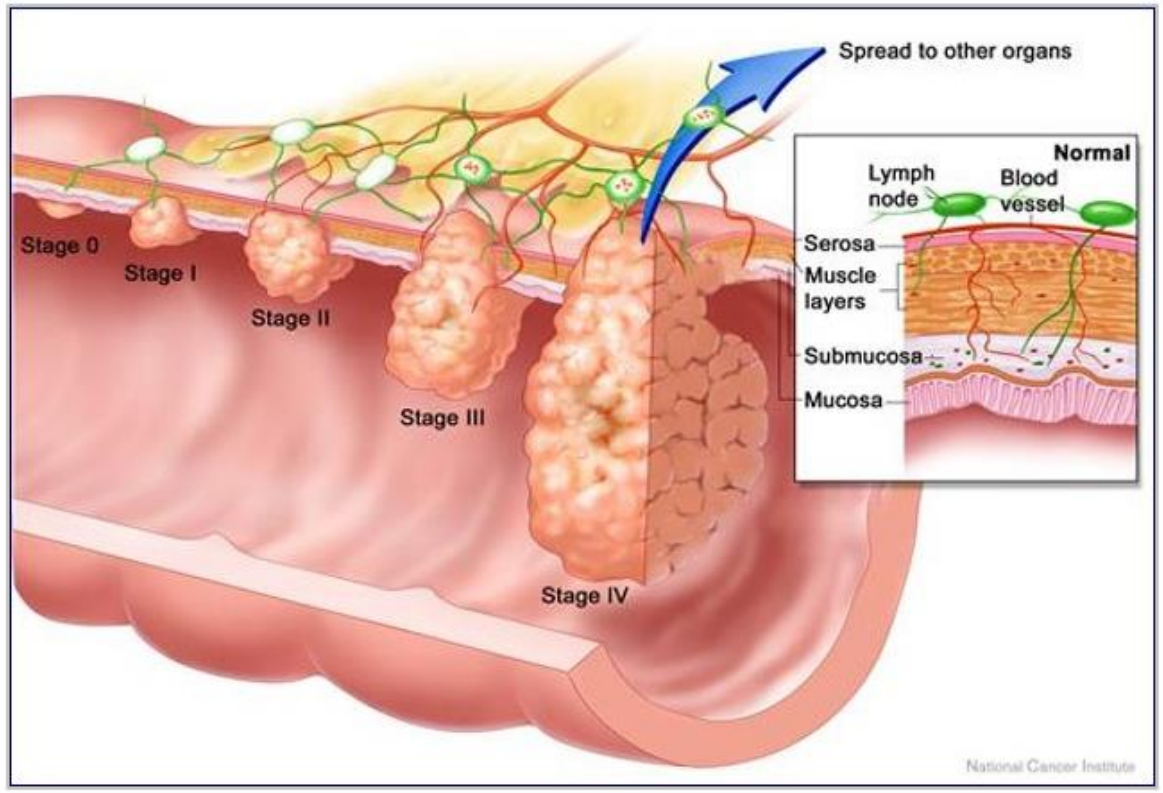
CRC is the 2nd most common cancer in non-smokers (male and female).

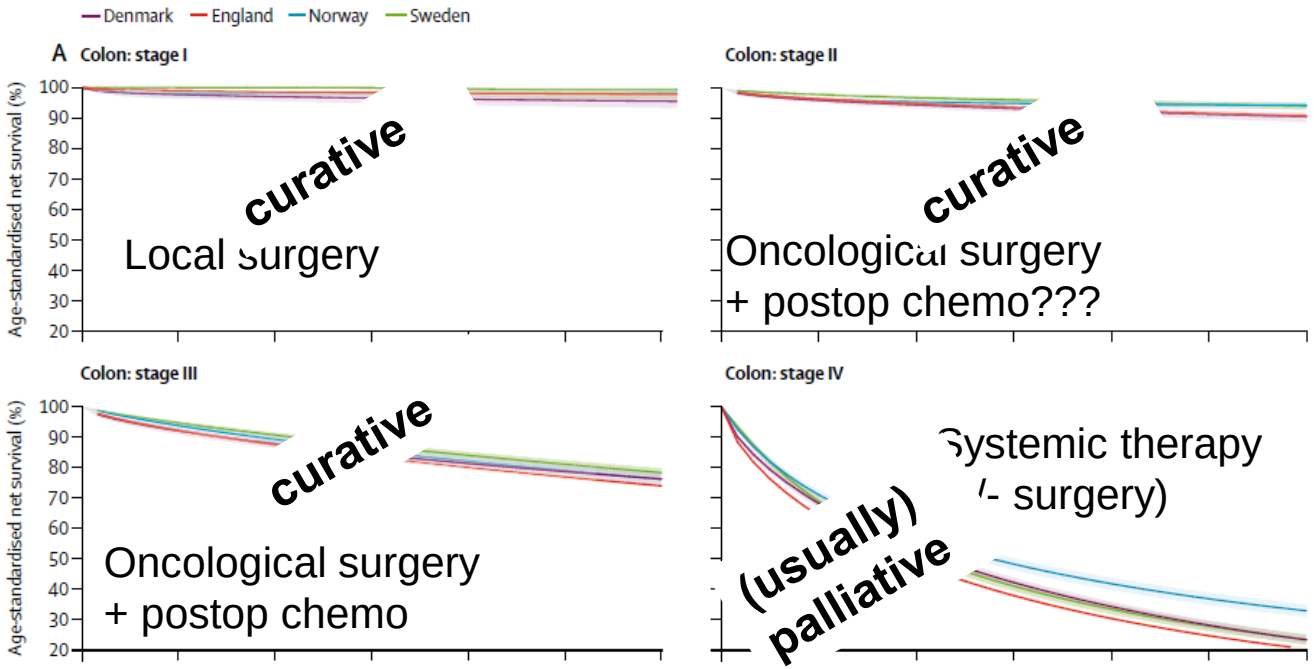
Mortality is still just over 40% in Switzerland

Early stage CRC is usually cured by surgery

SEER, darmkrebs.ch, krebsliga.ch, swisscancerscreening.ch

CRC – prognosis and therapy is stage dependent

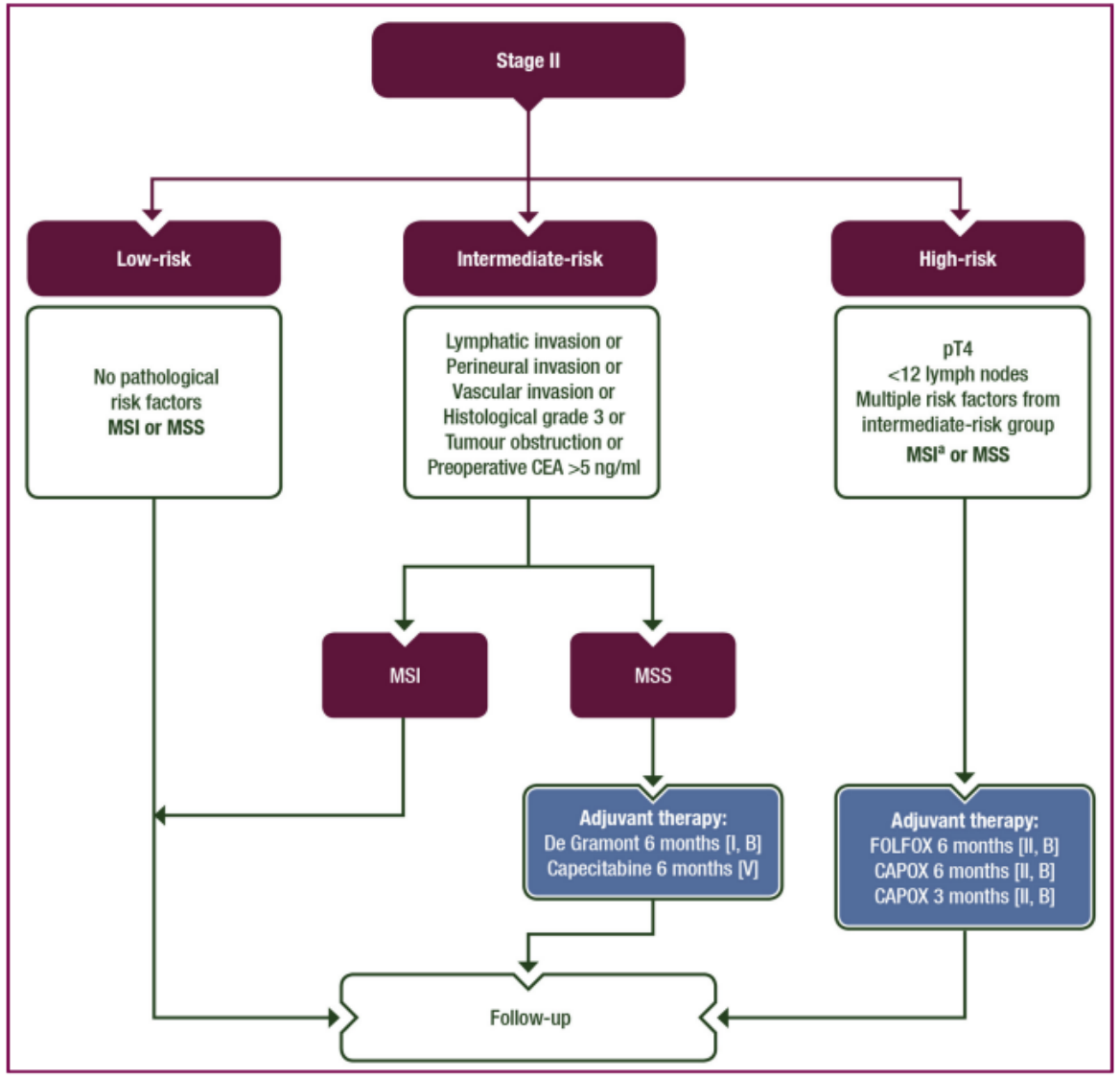




+/- even distribution among stages in CH

NICER data, NCI

Treatment guidelines in Stage II CRC



Postoperative treatment mainly determined by histopathological features:

Local tumor stage (pT3 vs pT4)

Vascular invasion

Tumor grade

Microsatellite stability (MSI)

Number of detected lymph nodes

With the exception of MSI, none of these features are actually predictive of chemotherapy benefit, yet +/- 1/3 of patients will qualify for adjucant chemotherapy

Adjuvant chemotherapy

FOLFOX:

Folinic acid (leucovorin)

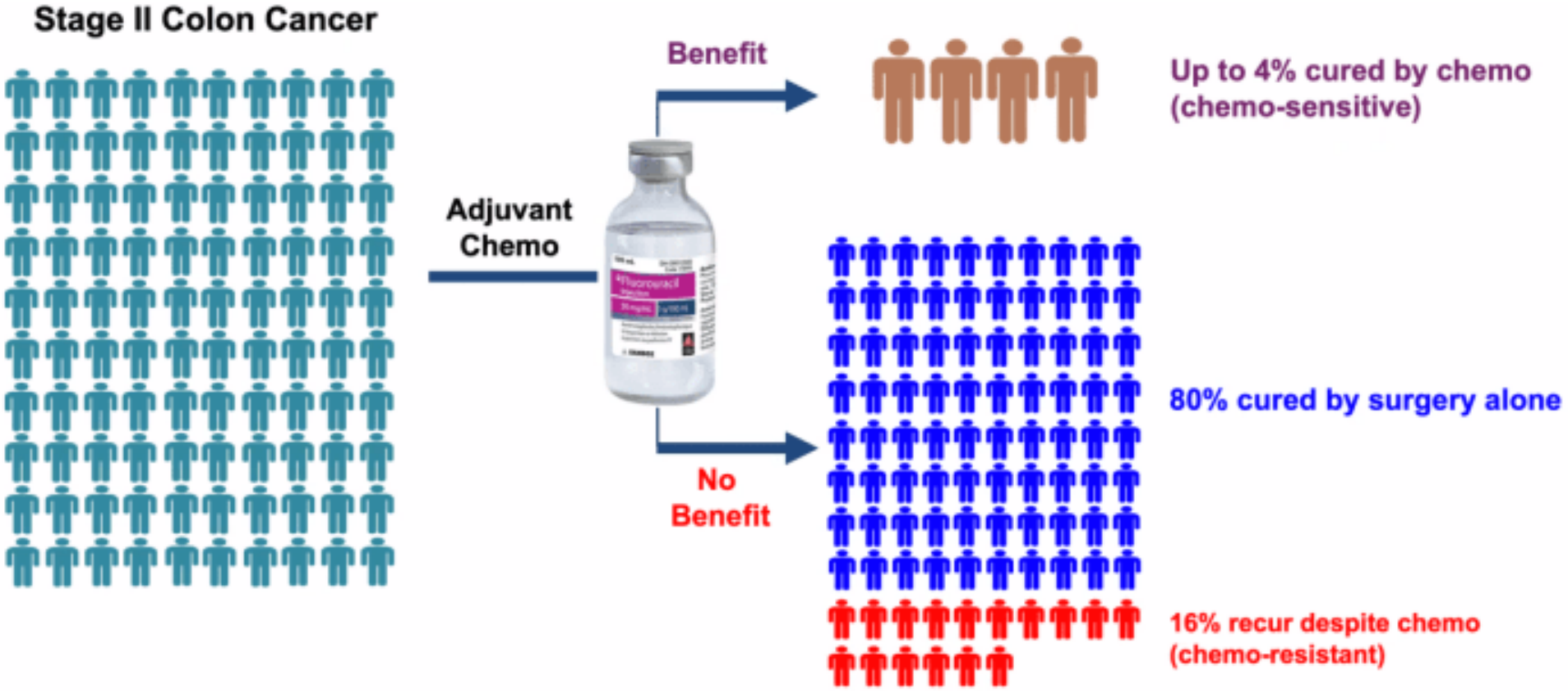
5-FU/capecitabine and

oxaliplatin

5-FU/capecitabine are fluoropyrimidines and require DYPD pharmacogenetic analysis/ DPD deficiency testing prior to treatment (side effects can be lethal without DPD)

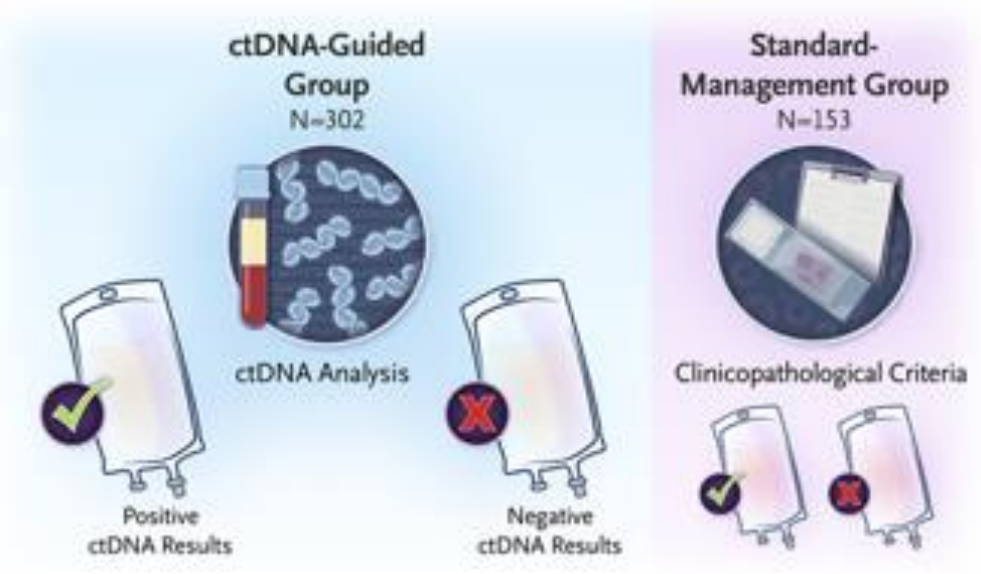
Oxaliplatin can cause long-term side effects (neuropathy)

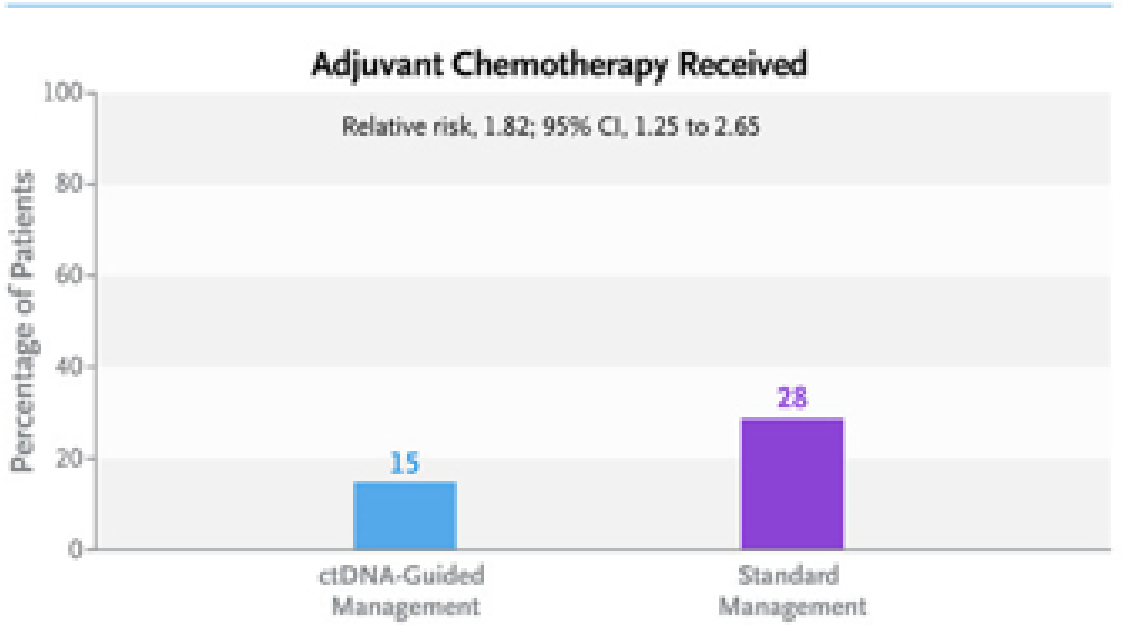
Chemo in stage II CRC: Treat many to save a few



De-escalation strategies are needed in stage II CRC!

Seminal phase 2 clinical trial for stage II CRC





DYNAMIC Trial: By using liquid biopsies for MRD ONLY to guide patient management in stage II colon cancer, ACT can be reduced by 50% without affecting mortality

Tie et al, NEJM 2022

De-escalation in stage III CRC???

Adjuvant chemotherapy for patients with stage III colon cancer has led to 3x better survival and has thus been standard of care since early 2000s

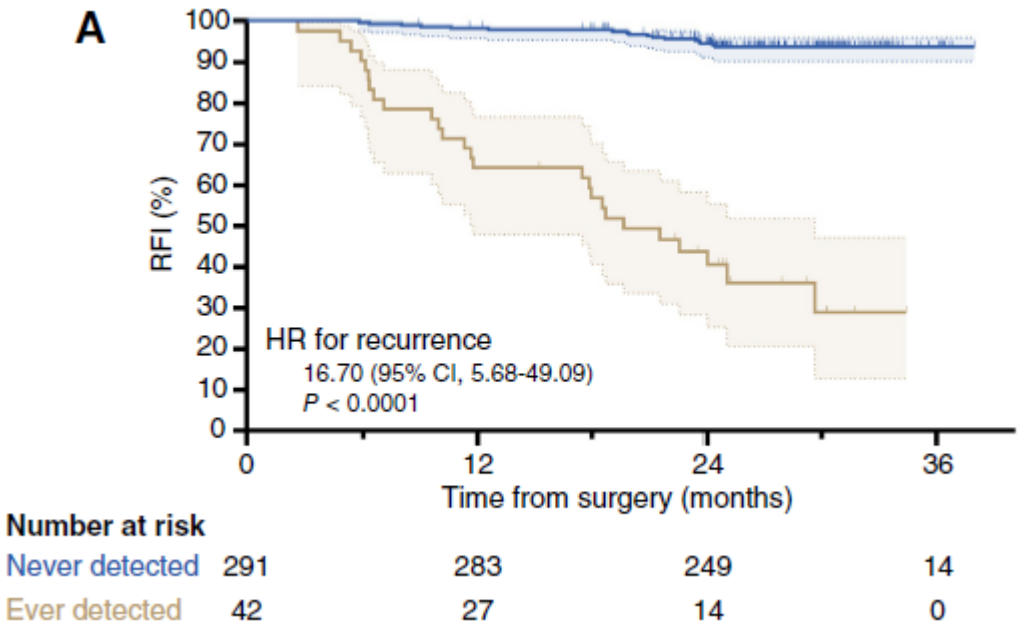
Actual benefit estimated to be in 30%, with 50% being cured by surgery and 20% recurring despite treatment

IDEA trial supports non-inferiority of reduction from 6 months to 3 months in stage III patients with lower substage

Biomarker studies have still not led to practice changers

Taieb et al, Cancers 2020

ctDNA is highly predictive of reccurrence



Lead time to clinical recurrence (CT, CEA)

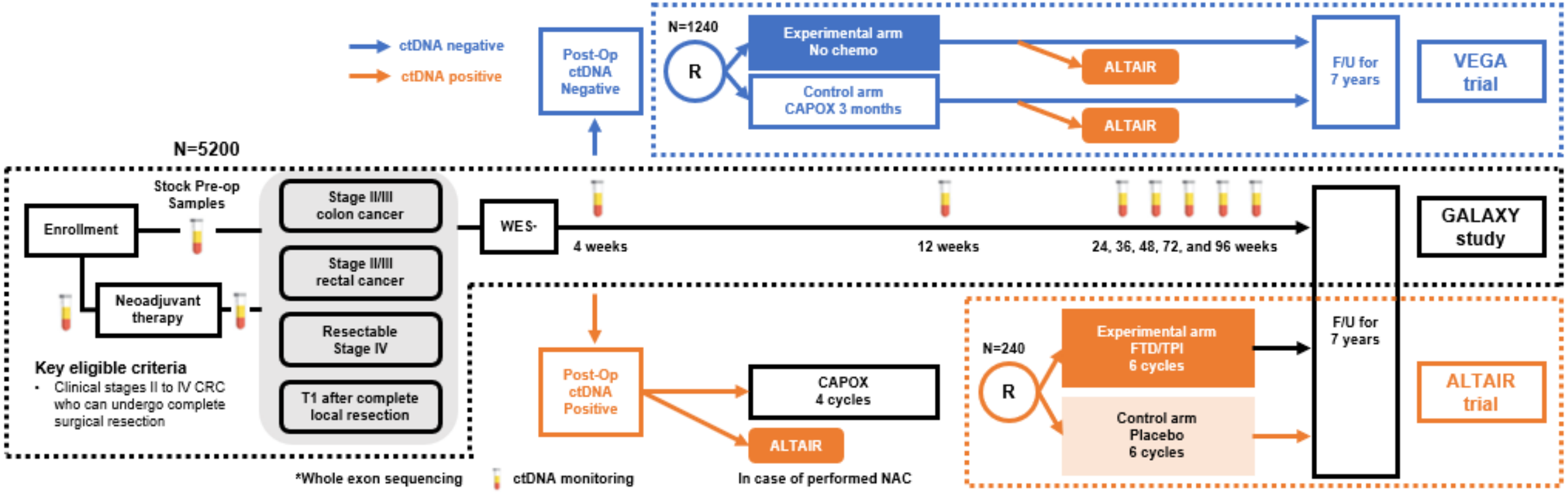
+/- 9 months

How often to test patients?

How to treat patients with molecular reccurrence?

Nakamura et al. CCR 2024

CIRCULATE trial design



Initiated in 2020

Kotani et al, Nat Med 2023

Some big questions arising from CIRCULATE

What is the role of pathologists and tissue-based factors?

What is the role of conventional follow-up?

Who will be performing all of these tests?

How often is serial testing really necessary?

Limits of detection

The limit of detection is determined by the input DNA and sequencing depth

We cannot compensate low input DNA with deeper sequencing!

20ng cfDNA → ca. 6000 genomic copies

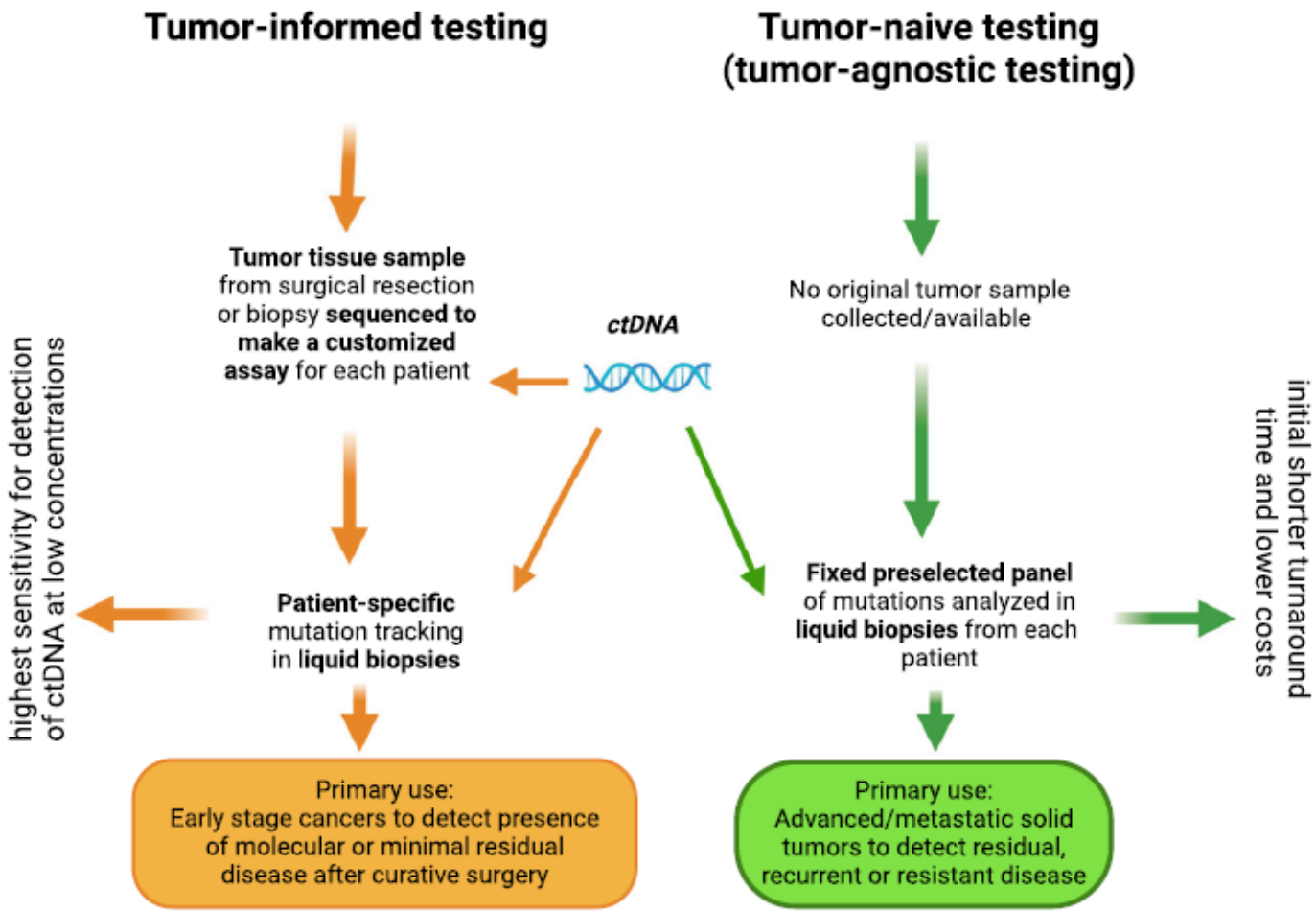
1% mutated DNA → 60 mutated molecules

0,1% mutated DNA → 6 mutated molecules

0,01% mutated DNA → 0,6 mutated molecules (?)

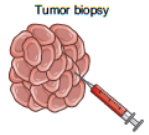
Assays used for MRD in clinical trials 0.01-0.02%

Tumor informed vs. tumor-agnostic assay

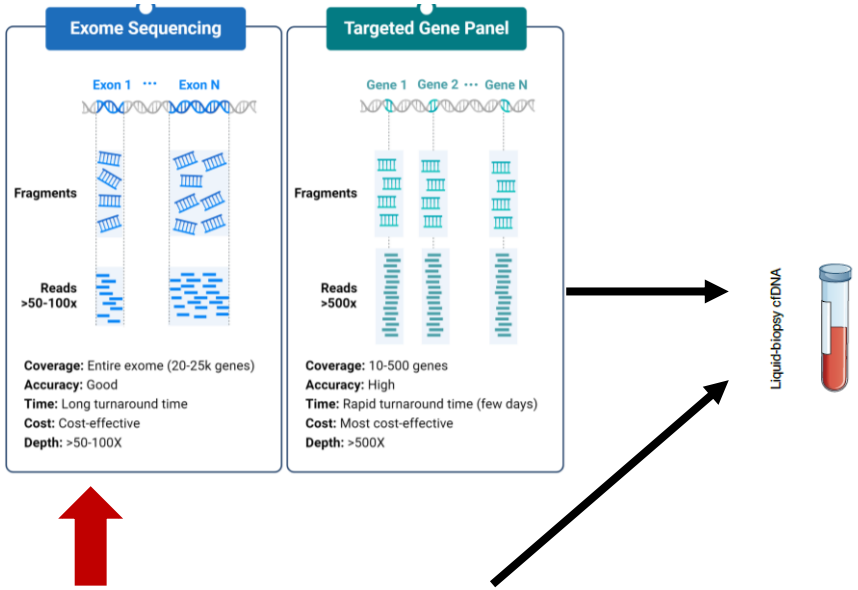


The difference between genomic profiling and MRD

MRD – is ctDNA present?

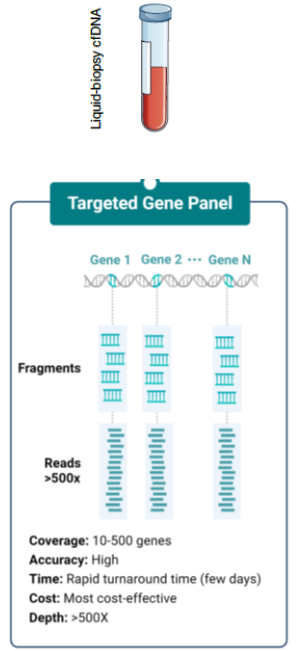


Identify mutations that will be present in a liquid biopsy



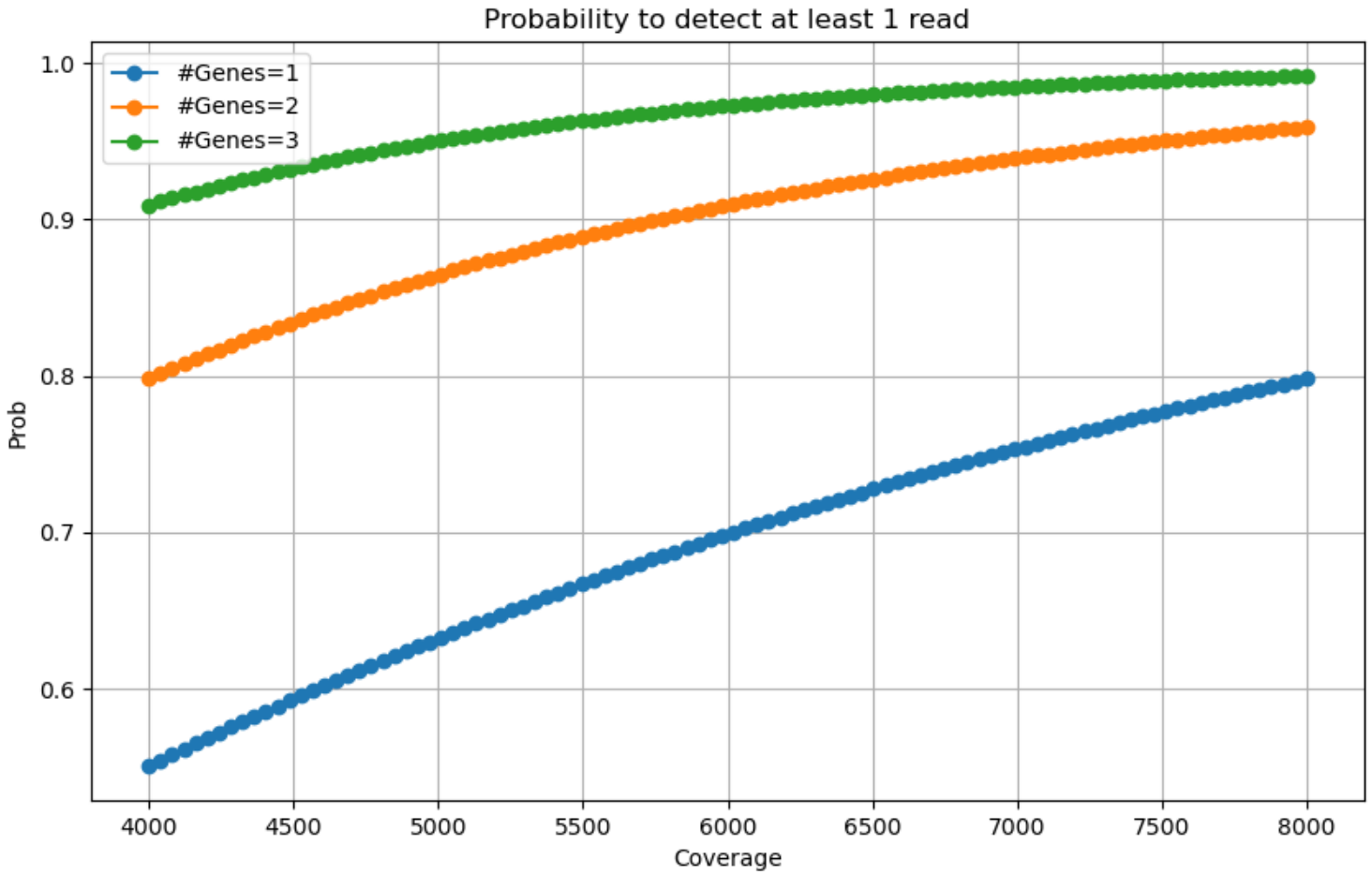
Create panel for deeper sequencing in liquid biopsy

Are actionable targets/resistance mechanisms present?



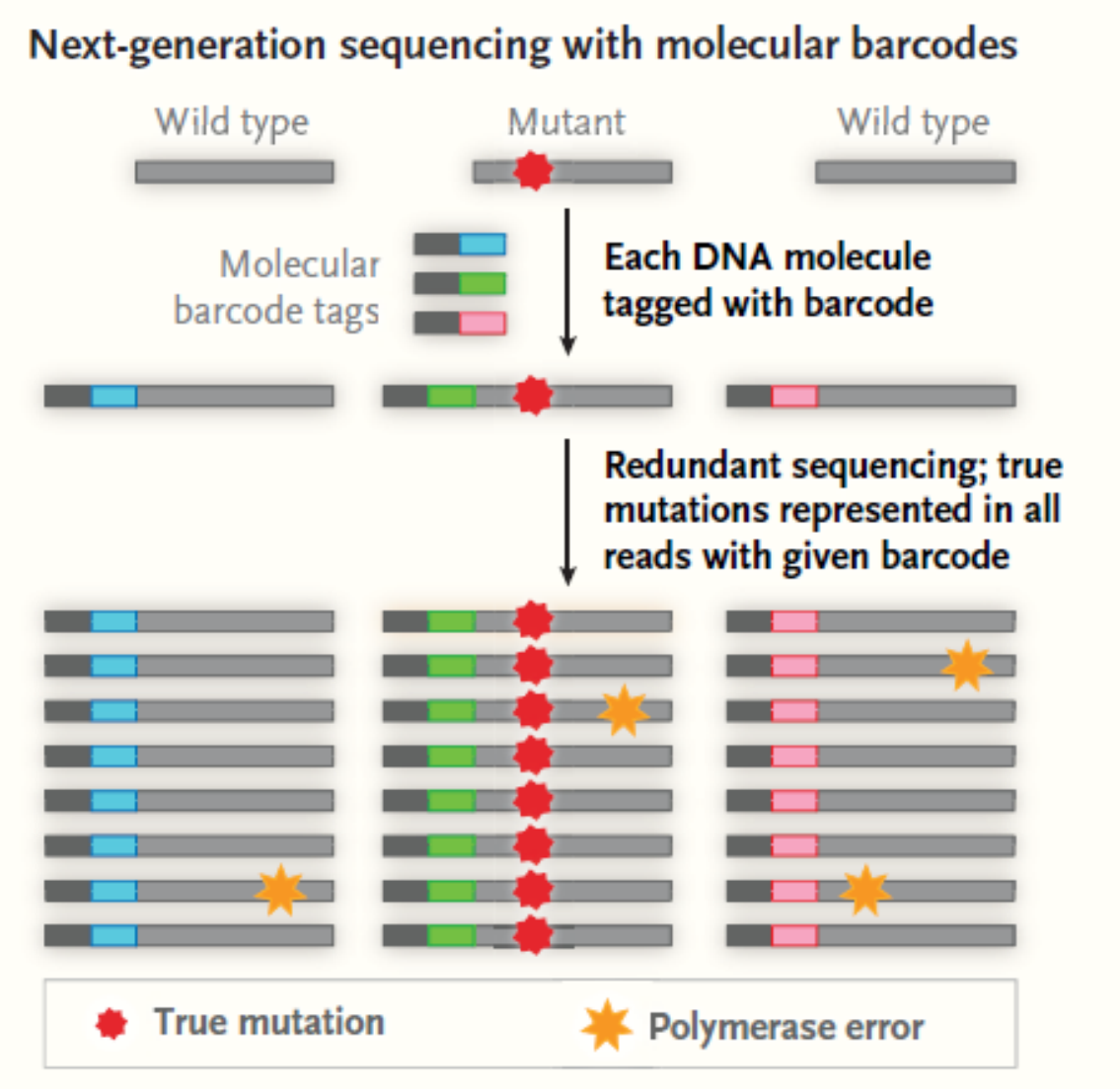
Panel broad enough to cover all relevant targets but small enough to be cost-effective at high sequencing depth

Not all mutations need to be detected for MRD – more targets increase sensitvity



Courtesy of M. Maiolo, CGL/IGMP

UMIs- a ‘must’ for ctDNA with NGS



Corcoran et al, NEJM 2018

The path to the clinic

DYNAMIC
CIRCULATE (Japan, USA)

Costs
Billability?
Availability?

Sequencing techniques

International Liquid Biopsy Standardization Alliance

Gold standard

False-negative and false-positive results

Negative result	Positive result
True negative: no ctDNA present	True postive: ctDNA present
False negative: low ctDNA fraction prevents detection of the variant → Assay sensitivity? → Input DNA	False positive: Background ‘noise’ mistaken for ctDNA

Risk: Patients are wrongly denied treatment

Longitudinal testing with appropriate panel

Risk: Patients receive unnecessary treatment

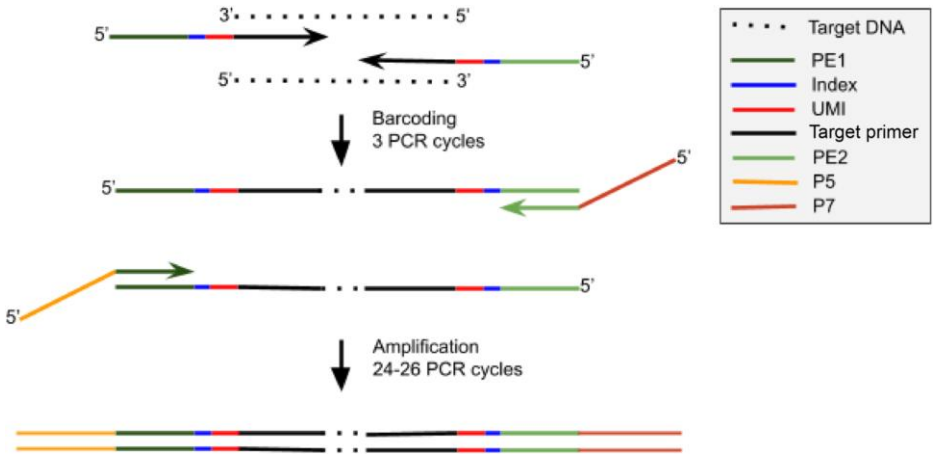
Tumor-informed approach:
Multiple targets present in liquid biopsy increases specificity

Tests on the market: examples

In-house solutions	End-to-end commercial products
Illumina's TruSight Oncology 500 for ctDNA	Signatera (Natera)
AVENIO Panels (Roche)	Guardant360® CDx Health
Oncomine™ Pan-Cancer Cell-Free Assay (ThermoFisher)	FoundationOne ® Liquid CDx (Foundation Medicine)
MSK-ACCESS® powered with SOPHiA DDM	Grail, Galleri, Methylation Cancer of Origin

Most clinical trials use end-to-end commercial products

Improving techniques: efficient library prep

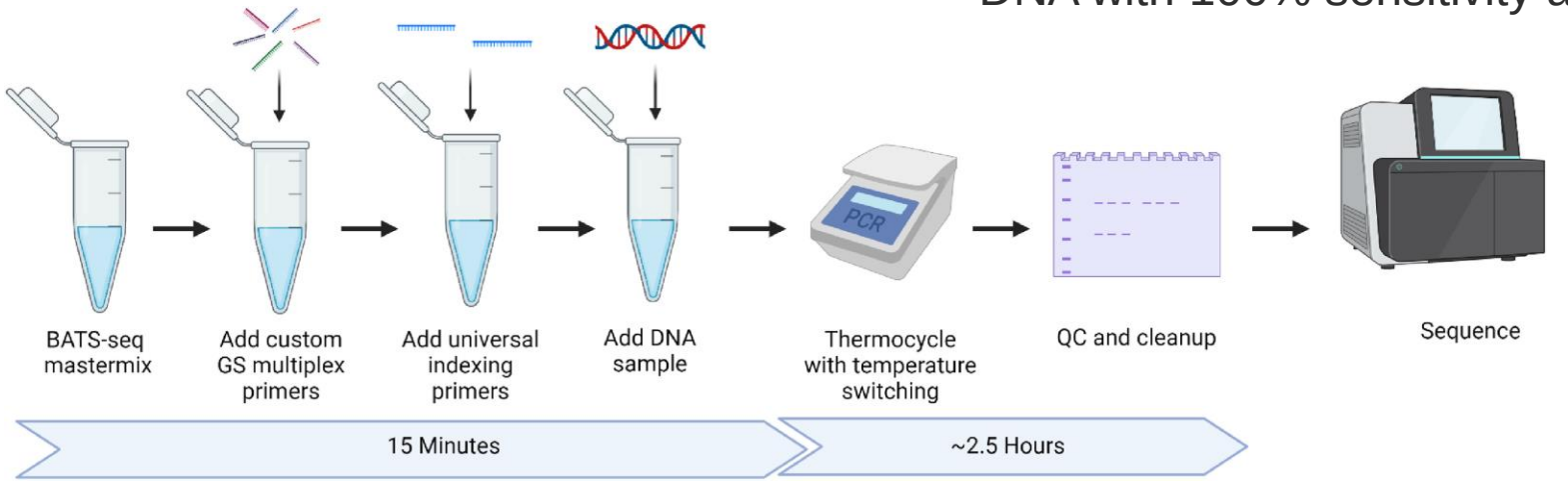


STAB-seq:
Single Tube switched temperature Amplicon Barcoding

Produces barcoded and indexed sequencing libraries in a single-tube process

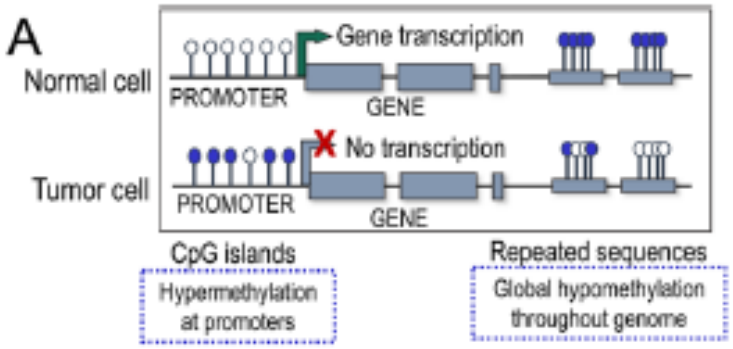
1-2 mutant copies of DNA with >95% specificity

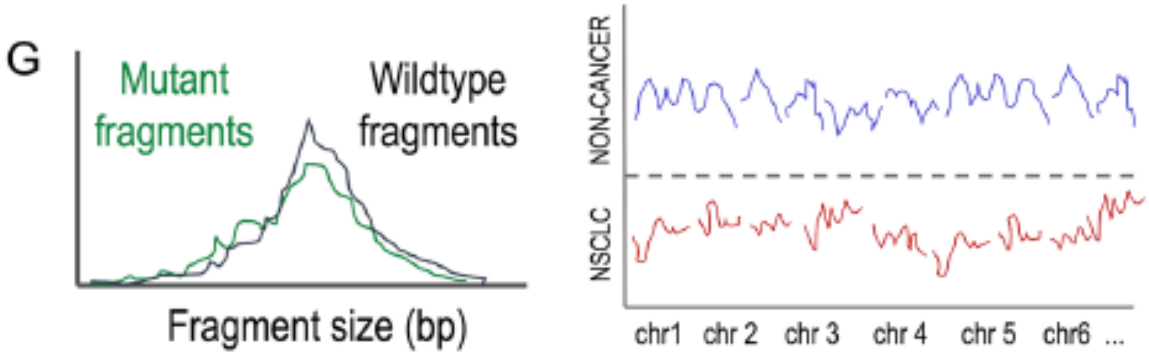
Eight-plex STAB-seq panel detects VAF of 0.03% in 10ng DNA with 100% sensitivity and 95% specificity



Godfrey et al, J Mol Diagn 2025

Improving techniques: incorporating additional features



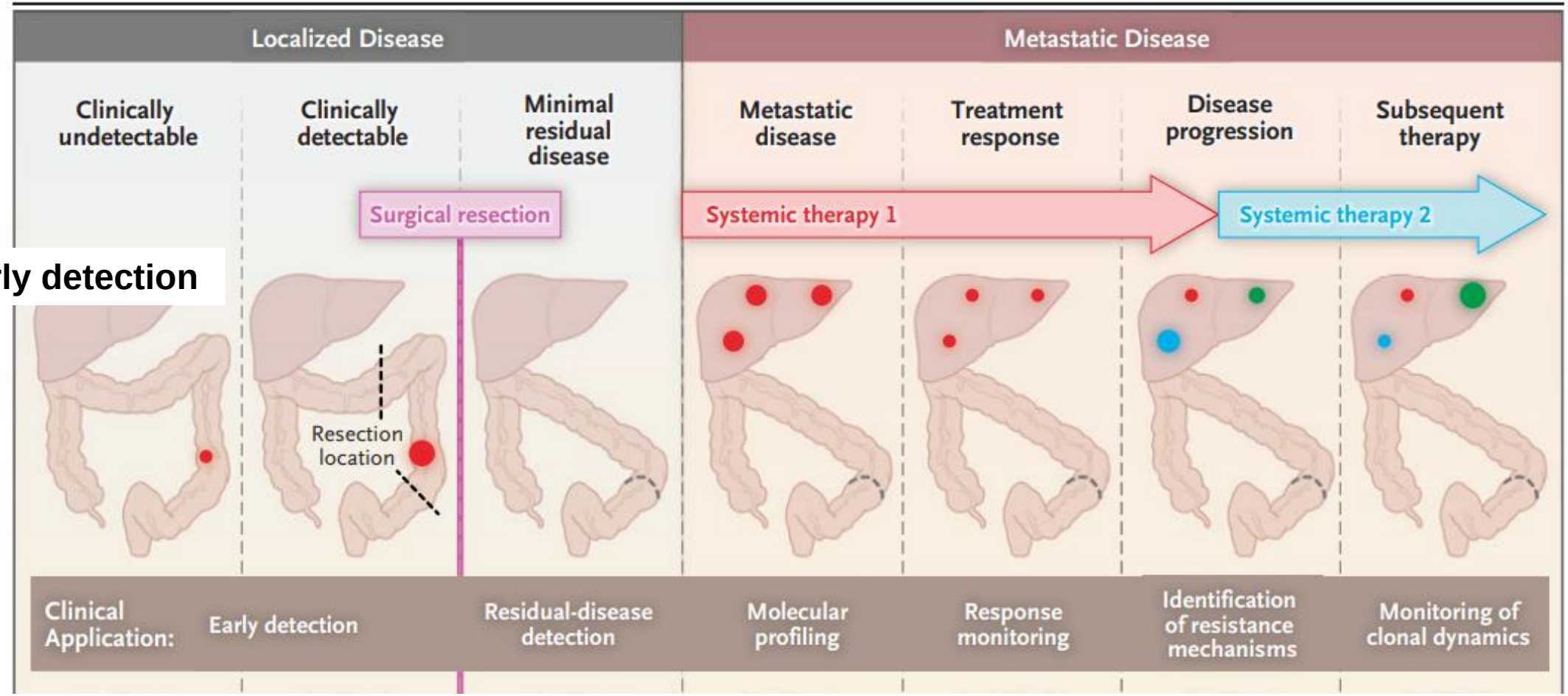


Methylation signatures

DNA fragment size distributions (fragmentome)

How to move forward with increasing sensitivity when the bar for therapy has been set by clinical trials?

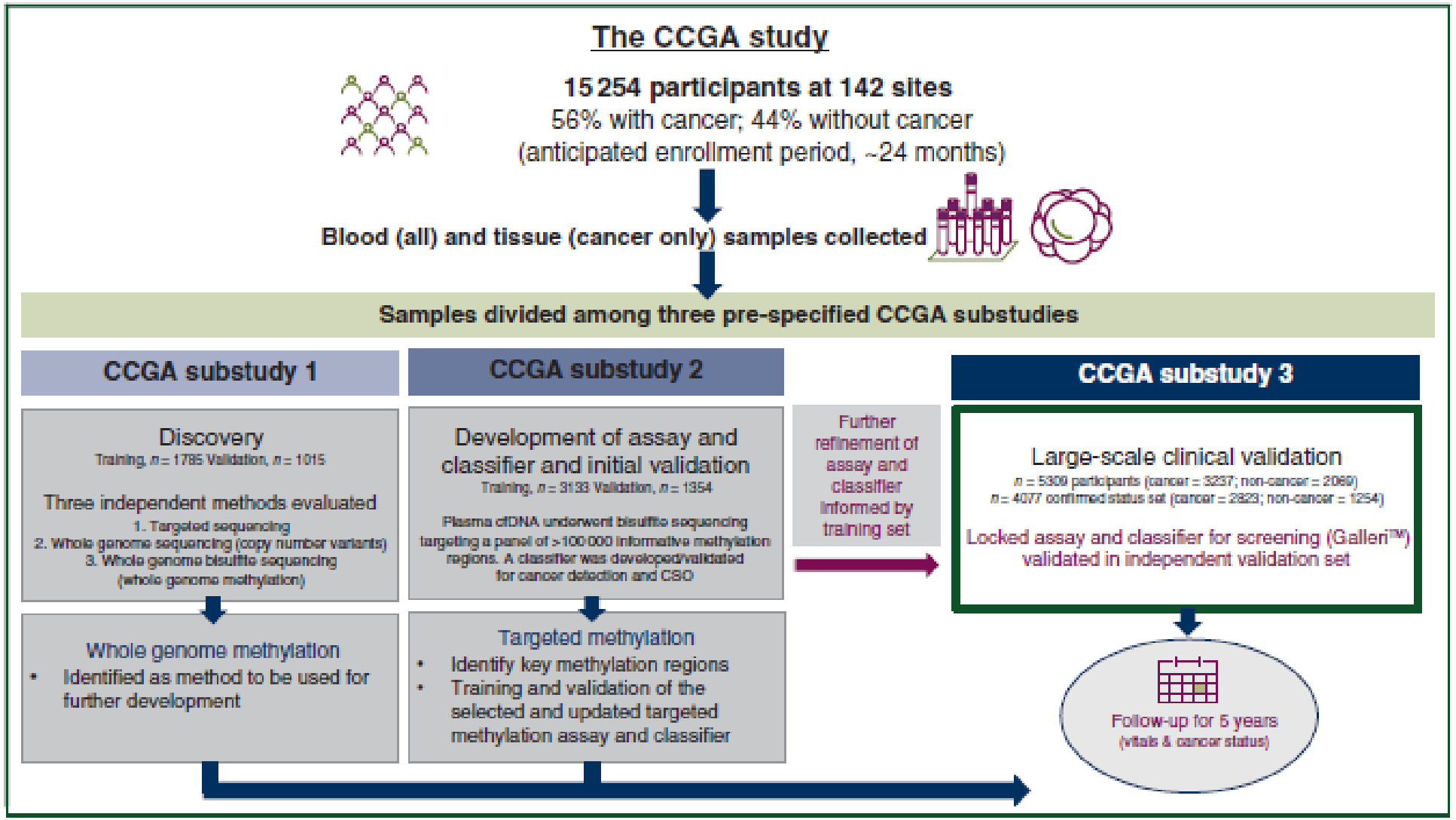
Future perspective: early cancer detection



Early detection

Challenge: highly sensitive, tumor-agnostic test

Multi-cancer early detection test



Klein et al. Ann Oncol 2021

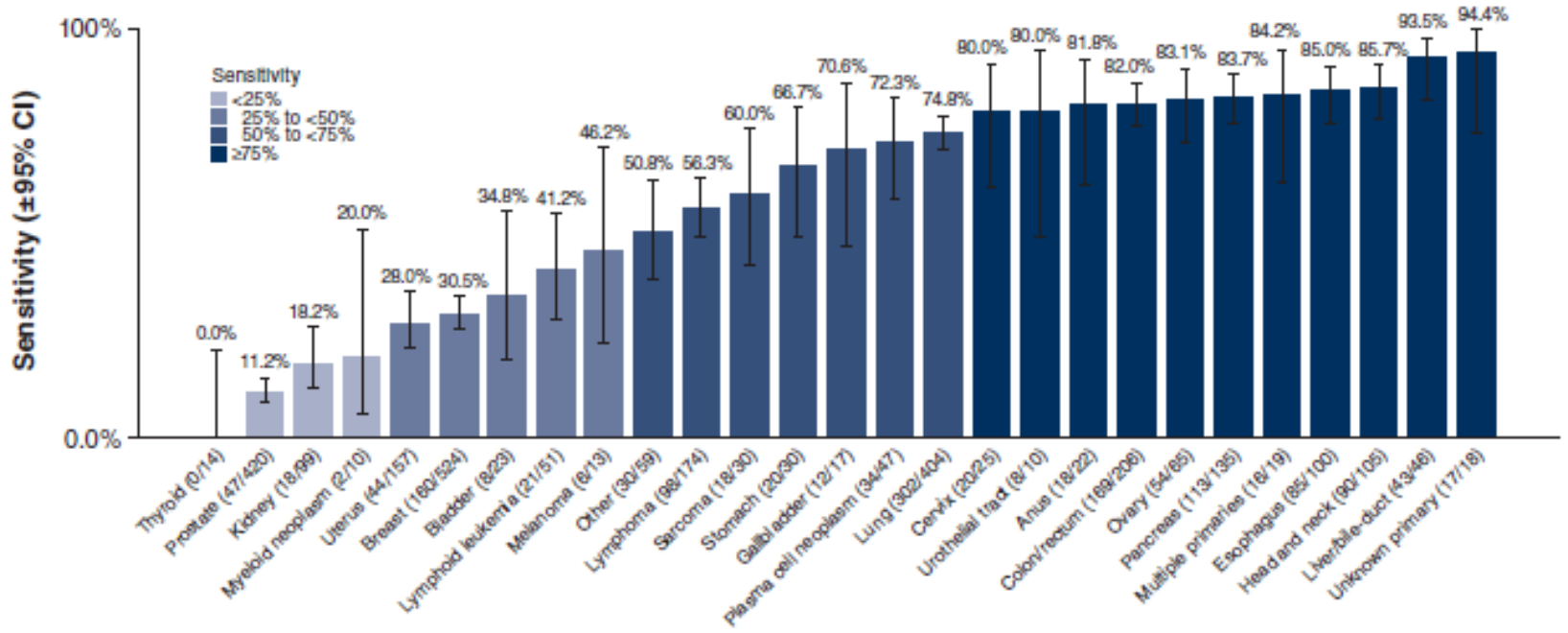
How well do tests work – and need to work?

A

	Cancer	Non-cancer	Total
	2823	1254	4077
Test positive	1453	6	1459
Test negative	1370	1248	2618
	Sensitivity = 1453/2823 51.5% (49.6%-53.3%)	Specificity = 1248/1254 99.5% (99.0%-99.8%)	

Two-sided 95% Wilson confidence intervals were calculated.

B



Klein et al. Ann Oncol 2021

Same test used in the PATHFINDER trial

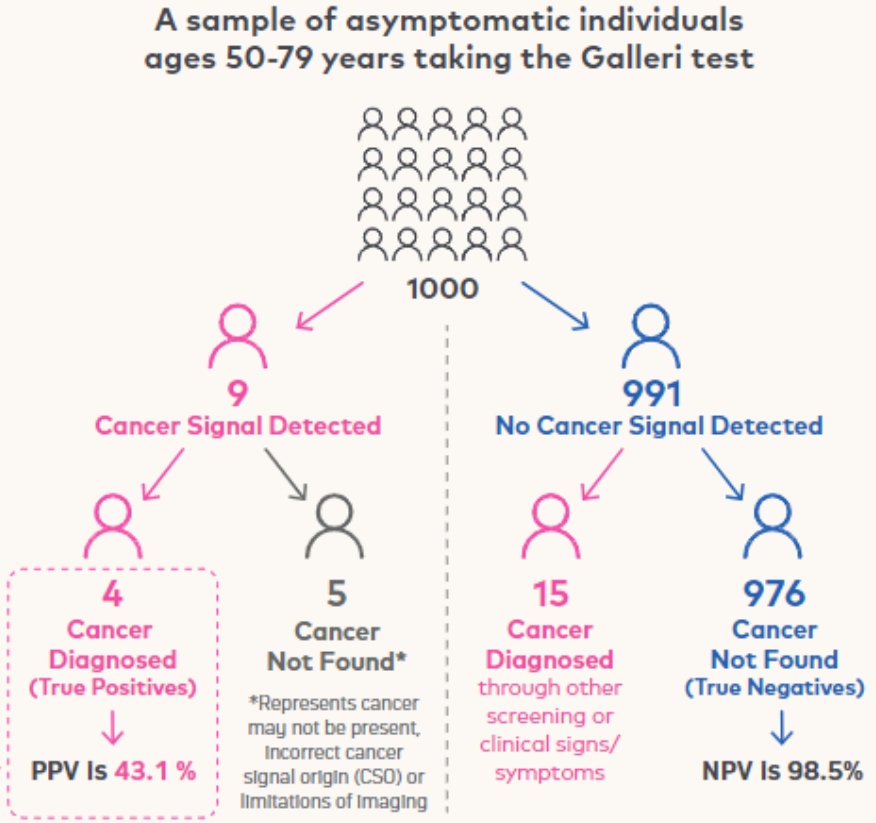
What Is PPV?

PPV represents the number of individuals who receive a Cancer Signal Detected test result who had cancer diagnosed. It can help guide the discussion on the implications and appropriate next steps.

What Does That Mean?

Galleri's PPV is 43.1% meaning that **about 4 out of 10** individuals with a "Cancer Signal Detected" result are expected to have a **confirmed cancer diagnosis** following diagnostic work-up.

PPV for the Galleri Test is: 43.1%



In the PATHFINDER study[1], out of 6,578 participants tested with Galleri, 58 had a Cancer Signal Detected result. Of these, 25 had a cancer diagnosed. 25/58 = 43.1%

https://view-su2.highspot.com/viewer/64540f014a3315d6aab0e252

More open questions

What happens with ‘excess’ information?

Can results be misconstrued (for example, BRAF mutations in a tumor-agnostic assay)

How to handle ambiguous results?

What is the acceptance in the general population?

Summary

Liquid biopsies exist in different platforms

Main indication ‘if tissue not available’ and resistance alterations in certain scenarios

MRD testing only performed in select cases, generally with commercial assays

Discussions are needed to on how to handle expected demand for MRD testing

MRD testing expected to play a large role in clinical management decisions and monitoring

Potential to be new standard of care for monitoring

Increasing sensitivity by multi-modal tests

Early pan-cancer testing

Thank you for your attention!

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