Liquid biopsies in clinical trials

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Disclosures

Employment Relationships:

- University Health Network: Staff Radiation Oncologist – Clinician-Scientist
- Adela: Co-Founder and Chief Innovation Officer

Consulting or Advisory Board/Speaker/Faculty/Research/Honoraria:

- EMD Serono: Advisory Board

Ownerships/Investments/Intellectual Property:

- Adela: Licensed patents, stock
- Roche: Licensed patent

Learning Objectives

- To discuss the role for circulating tumour DNA as a key correlative component of clinical trials
- To recognize the distinct scenarios where liquid biopsy can inform on patient outcomes or trial endpoints
- To assess the limitations of current liquid biopsy tests, and opportunities for future improvements

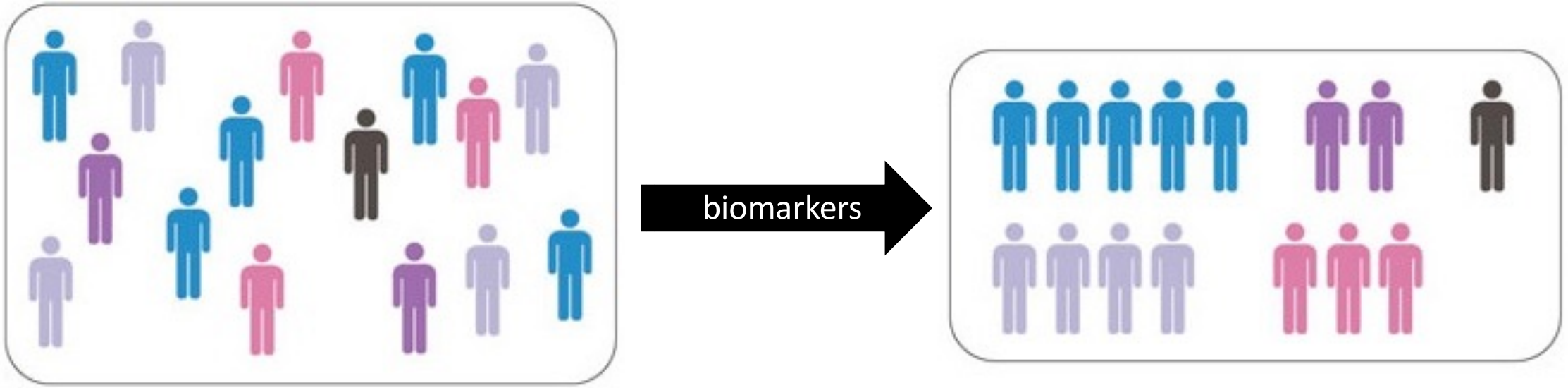
Lecture Overview

- Summary of liquid biopsy in oncology
- Types of ctDNA tests and practical considerations
- Evidence for ctDNA tests as tools to guide therapy

Lecture Overview

- **Summary of liquid biopsy in oncology**
- Types of ctDNA tests
- Evidence for ctDNA tests as tools to guide therapy

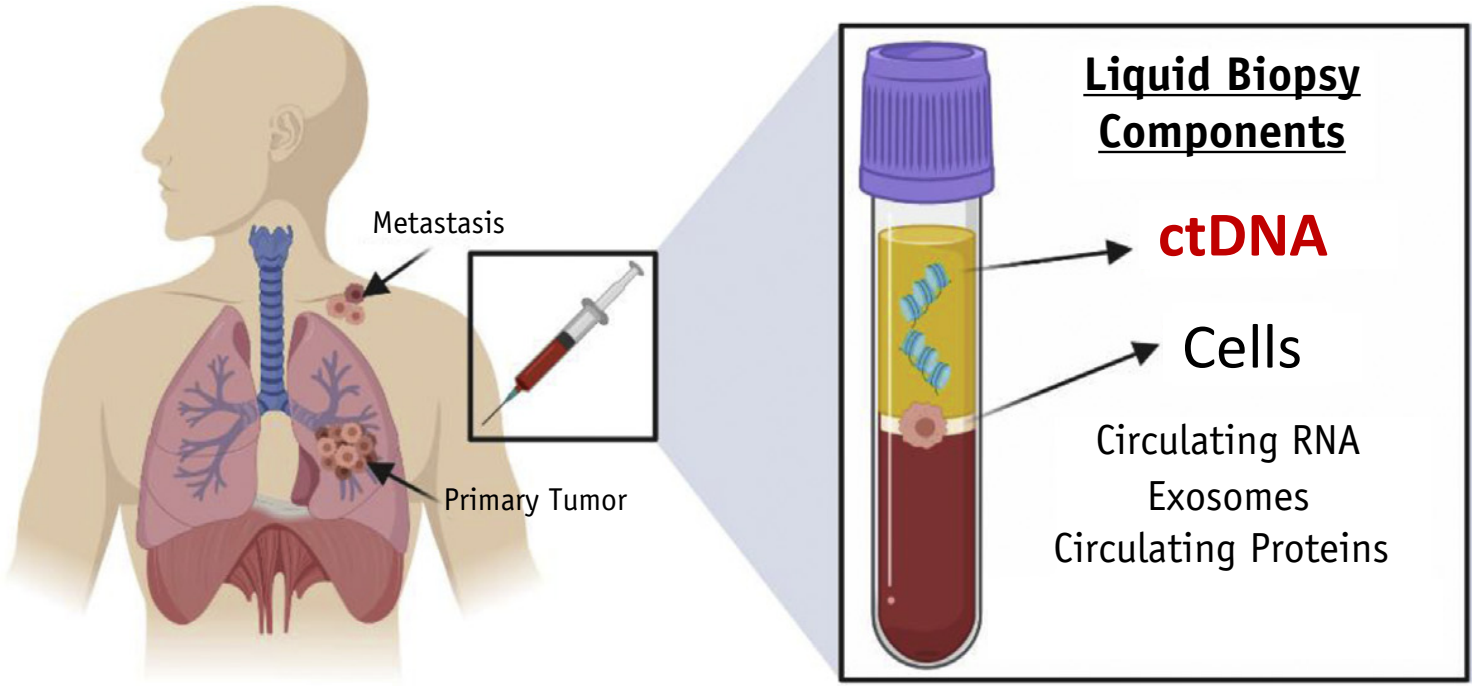
How can biomarkers guide personalized treatment regimens?

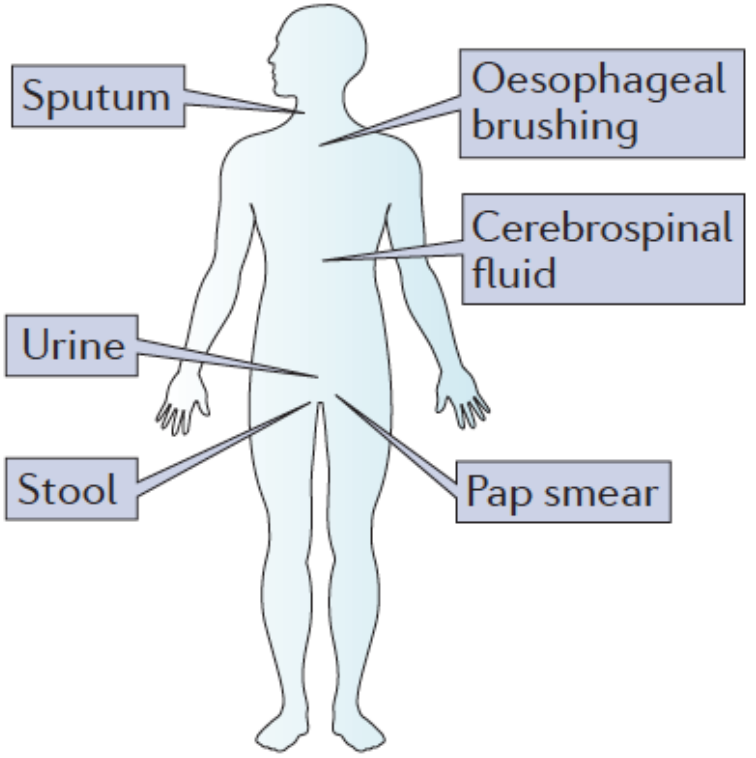


- Risk stratification / prognostication
- Prediction of response or toxicity
- Dynamic monitoring of response
- Surrogate endpoint for clinical trials

Baumann, Nature Rev Cancer 2016

Circulating tumor DNA (ctDNA): Cancer DNA in the bloodstream

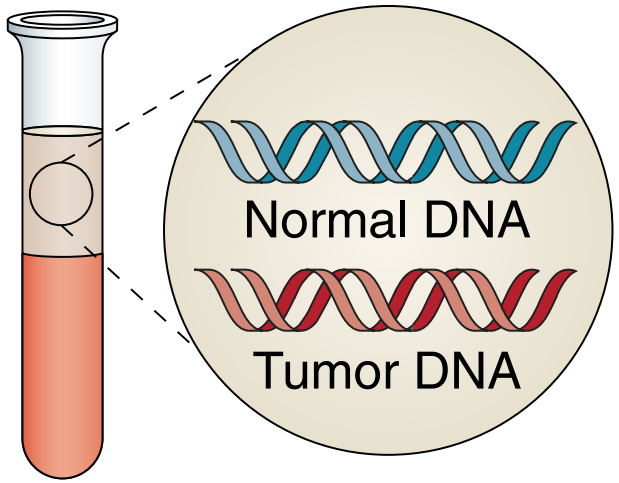




De Michino, Lok, Bratman et al. IJROBP 2020; 107(5),873-886

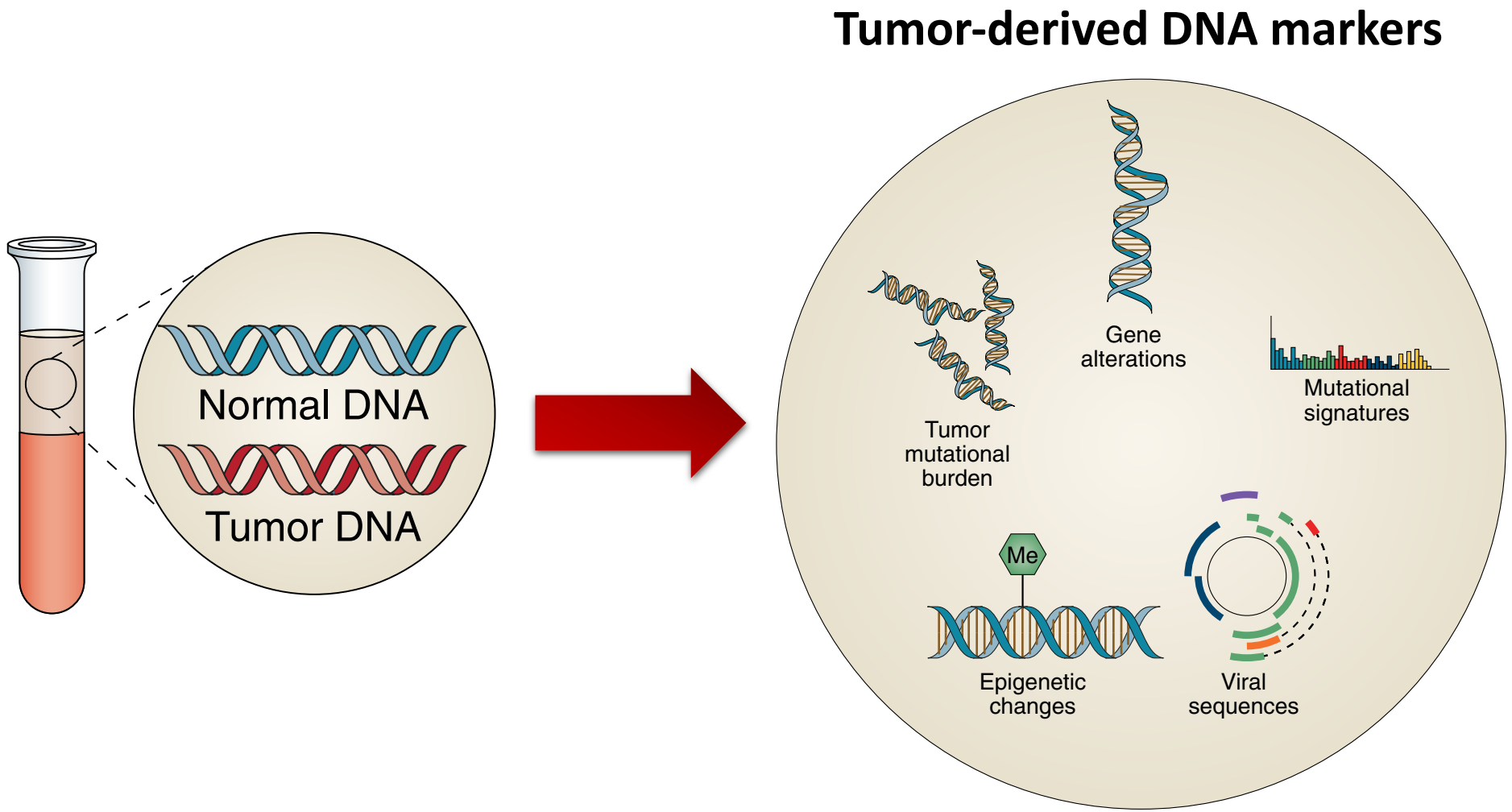
Wan…Rosenfeld et al. Nat Rev Cancer 2017

ctDNA: Finding a needle in the haystack of normal non-cancer-derived cell-free DNA



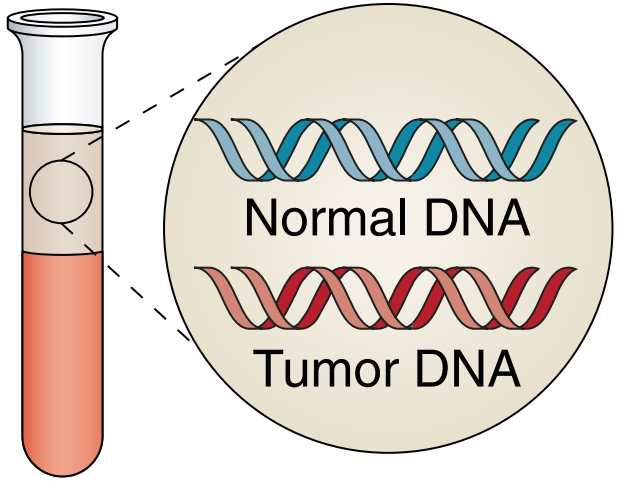
- Low fractional abundance
- Limited amount of blood

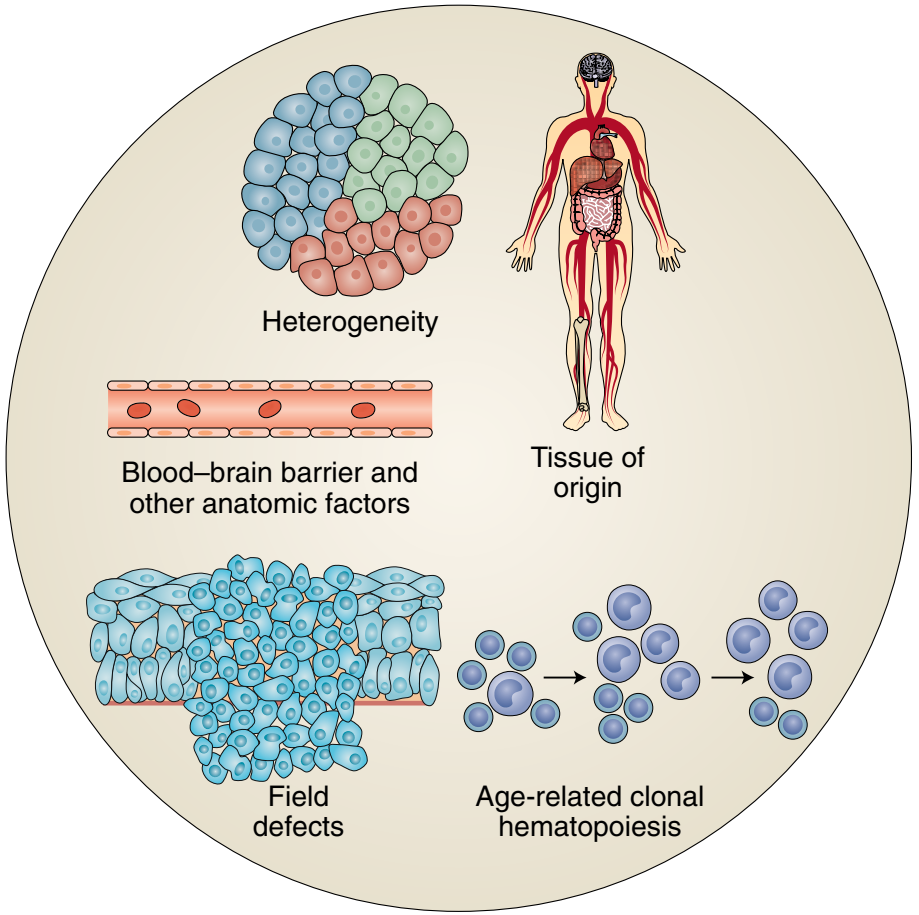
ctDNA: Distinguishing signal from noise



Cescon, Bratman, Chan, Siu. Nature Cancer 2020

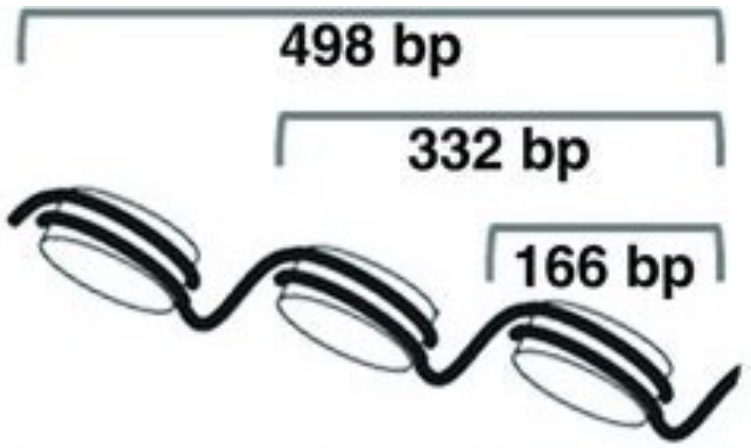
ctDNA: Distinguishing signal from noise



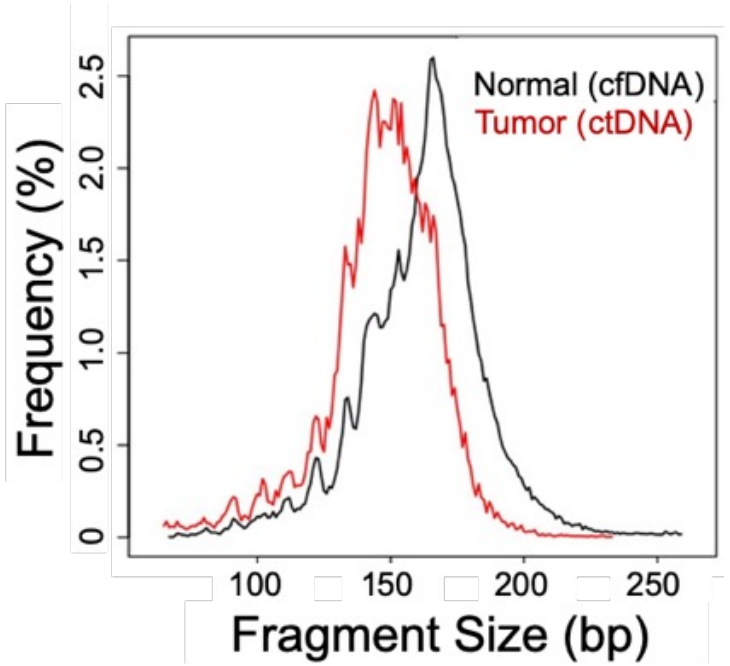


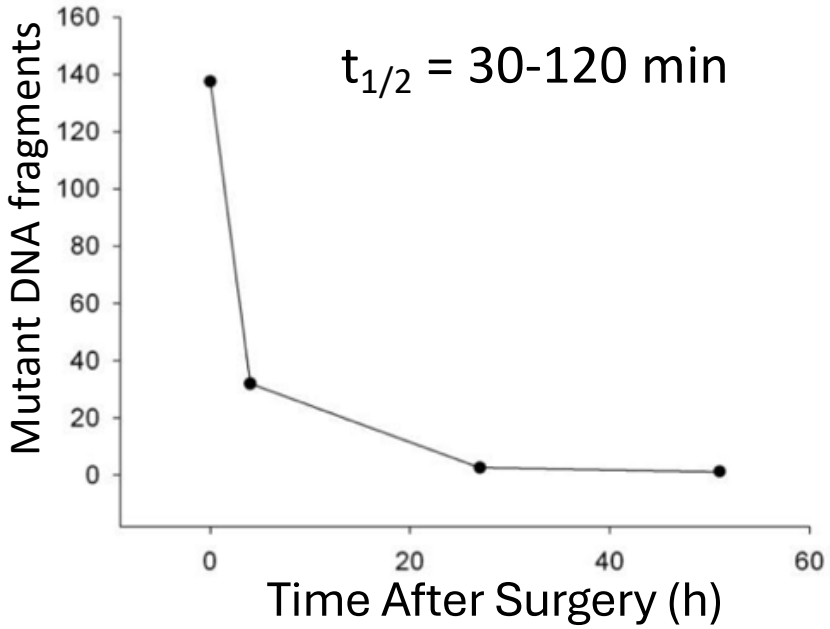
Cescon, Bratman, Chan, Siu. Nature Cancer 2020

ctDNA is associated with nucleosome complexes and has a characteristic size distribution and short half life



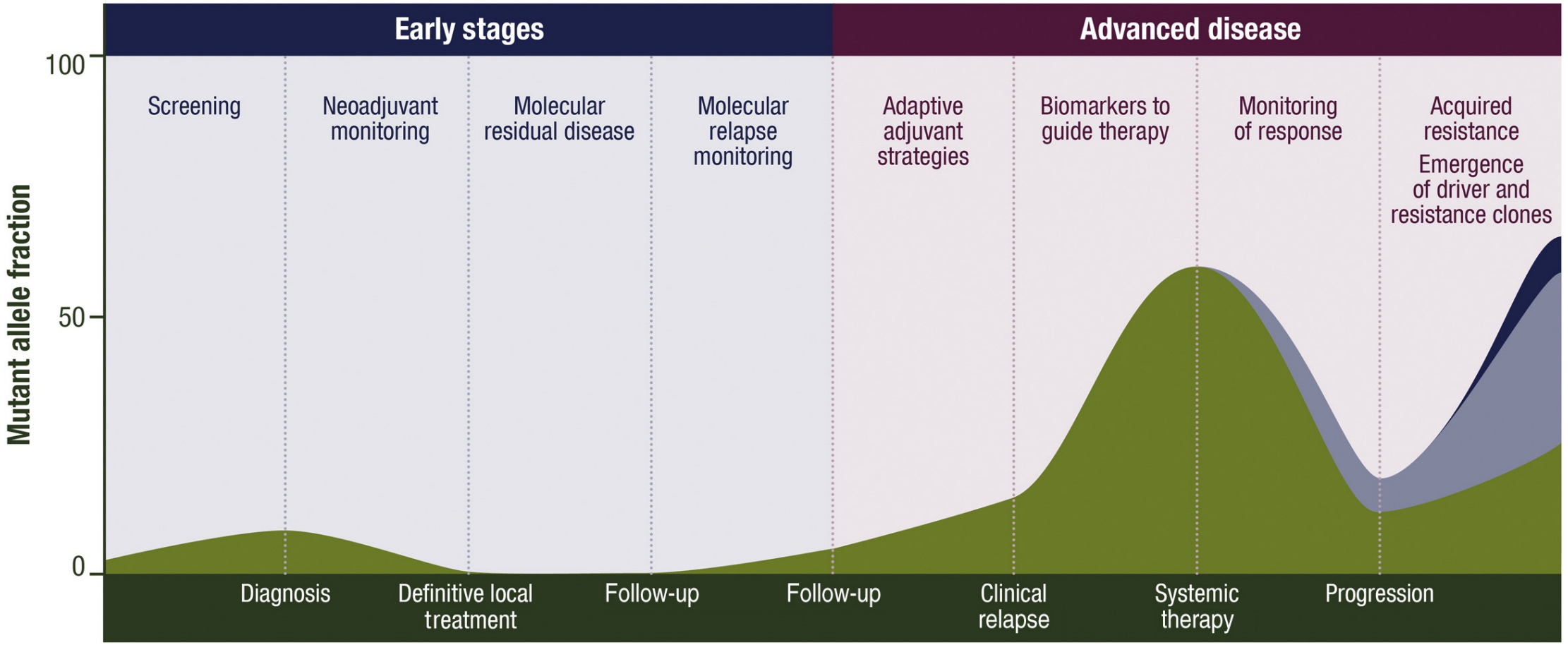
ctDNA within nucleosomes





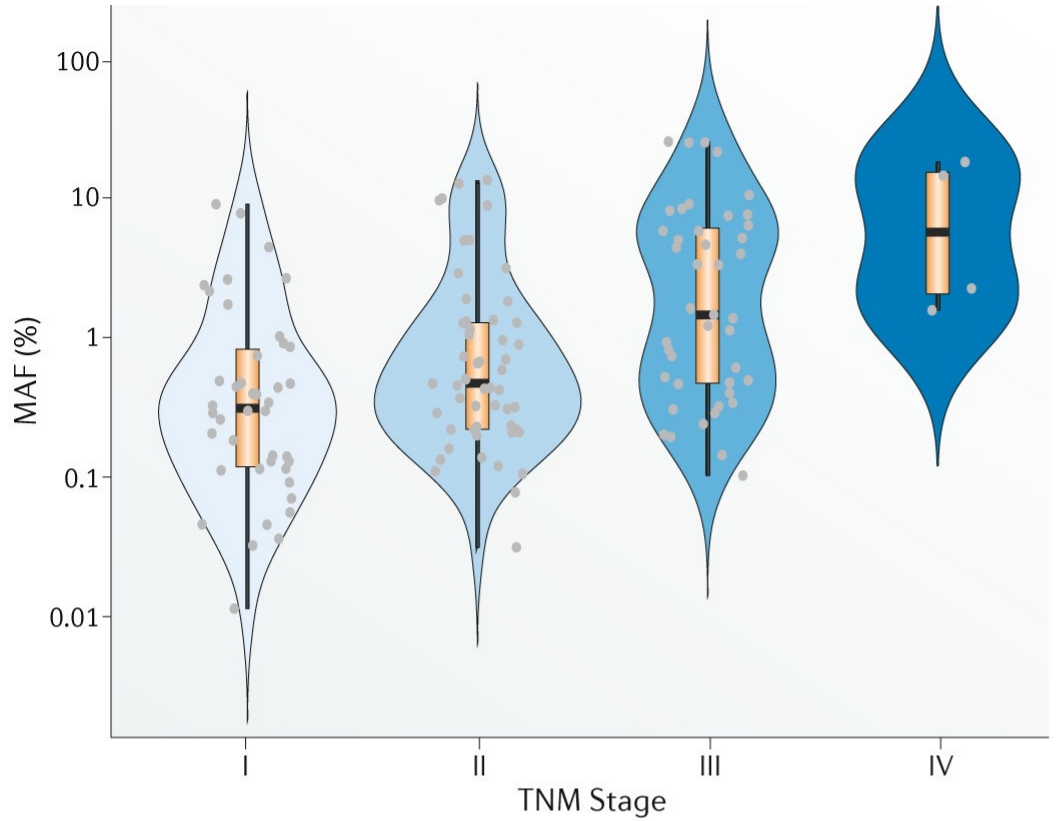
Heitzer, Ulz, Geigl, Clin Chem 2015
Leung, Han...Bratman, Clin Cancer Res 2021
Diehl...Diaz et al. Nat Med 2008

Levels of ctDNA vary across distinct phases of the cancer care continuum



Pascual, Turner et al. Annals Oncol 2022

Clinical factors influence ctDNA detectability & abundance

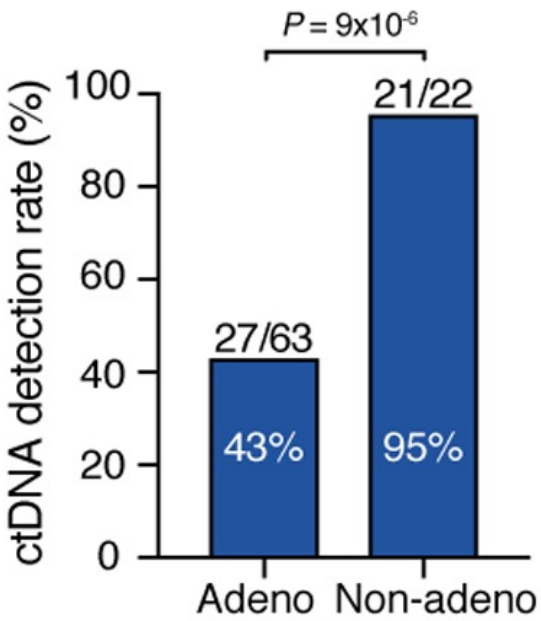


- Stage (M > N > T)
- Tumor volume (weak)
- Viability or necrosis
- Vascularization
- Blood brain barrier

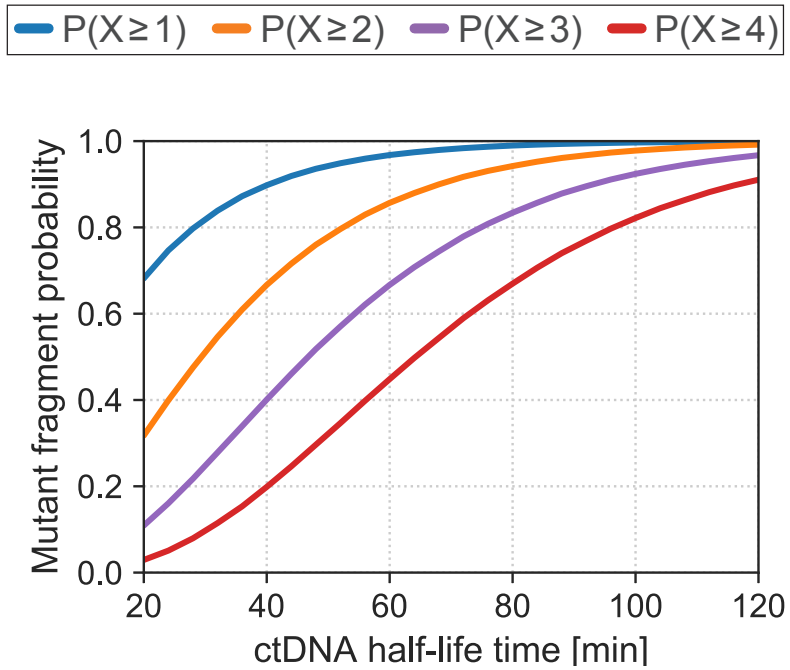
Abbosh, Birkbak, Swanton. Nat Rev Clin Oncol 2018

Impact of tumor biology & host factors on ctDNA detectability

Tumor histology

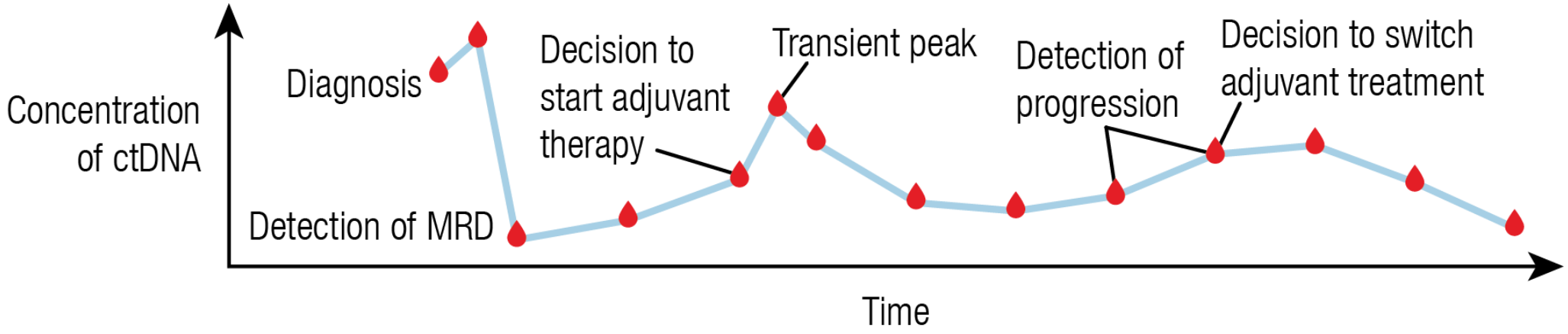


ctDNA clearance rate

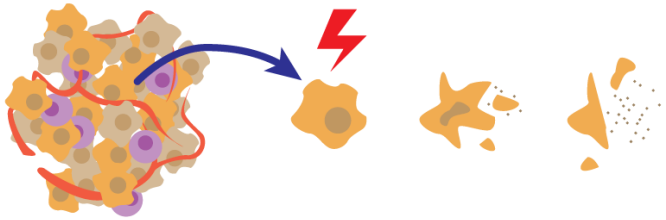


Chabon, Diehn, et al. Nature 2020
Avanzini, Reiter, et al. Sci Adv 2020

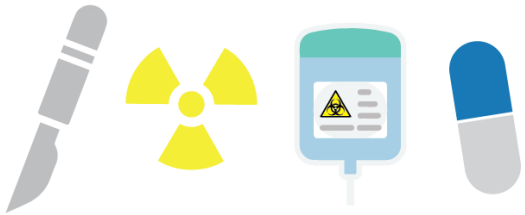
Clinical actionability of on-treatment ctDNA kinetics



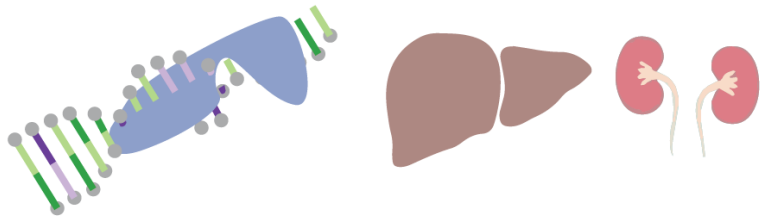
Factors affecting ctDNA kinetics



ctDNA Release Kinetics



Effects of Treatment



Degradation & Clearance Kinetics

Sanz Garcia, Zhao, Bratman, Siu, et al. Science Advances, 2022

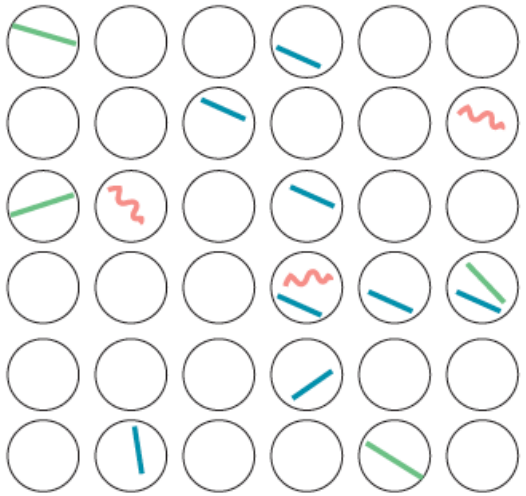
Lecture Overview

- Summary of liquid biopsy in oncology
- **Types of ctDNA tests and practical considerations**
- Evidence for ctDNA tests as tools to guide therapy

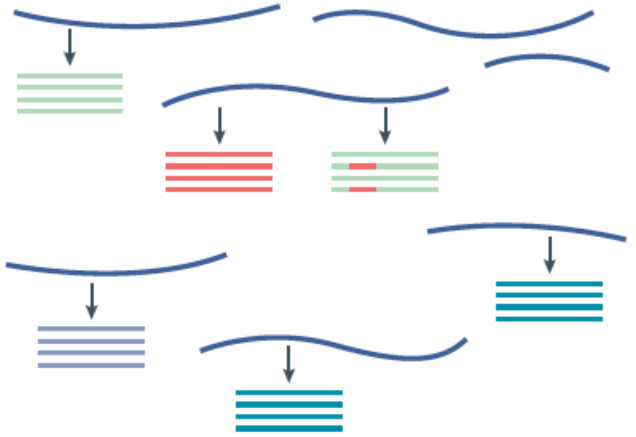
Methods for ctDNA Detection & Analysis

- Targeting of **genetic or epigenetic aberrations** within cell-free DNA
- Accounting for tumor-derived **signal** as well as technical and biological **noise**

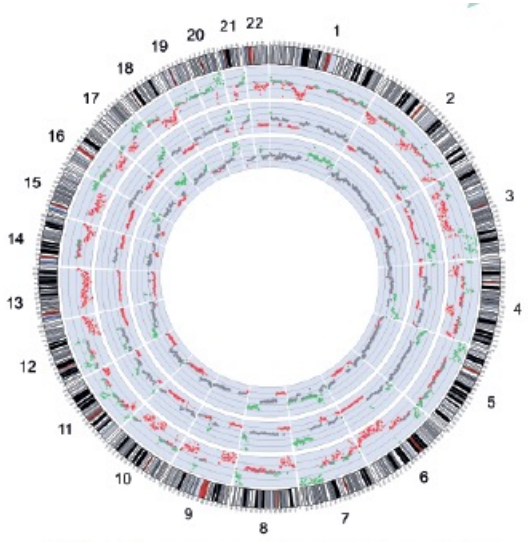
Single-locus assays



Targeted sequencing



Genome-wide



Wan...Rosenfeld et al. Nat Rev Cancer 2017

Methods for ctDNA Detection & Analysis

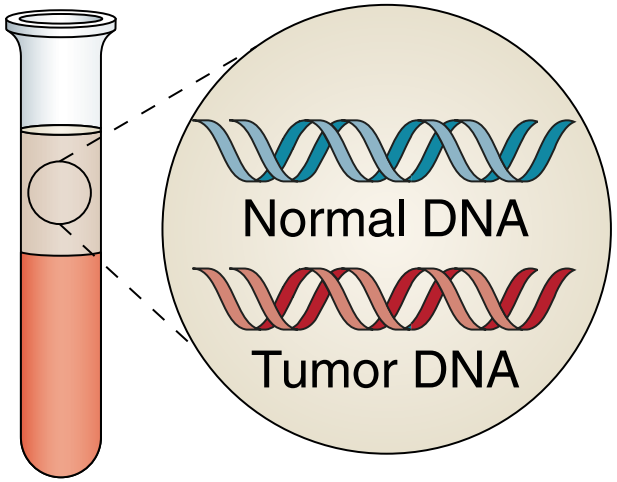
Polymerase chain reaction (PCR)

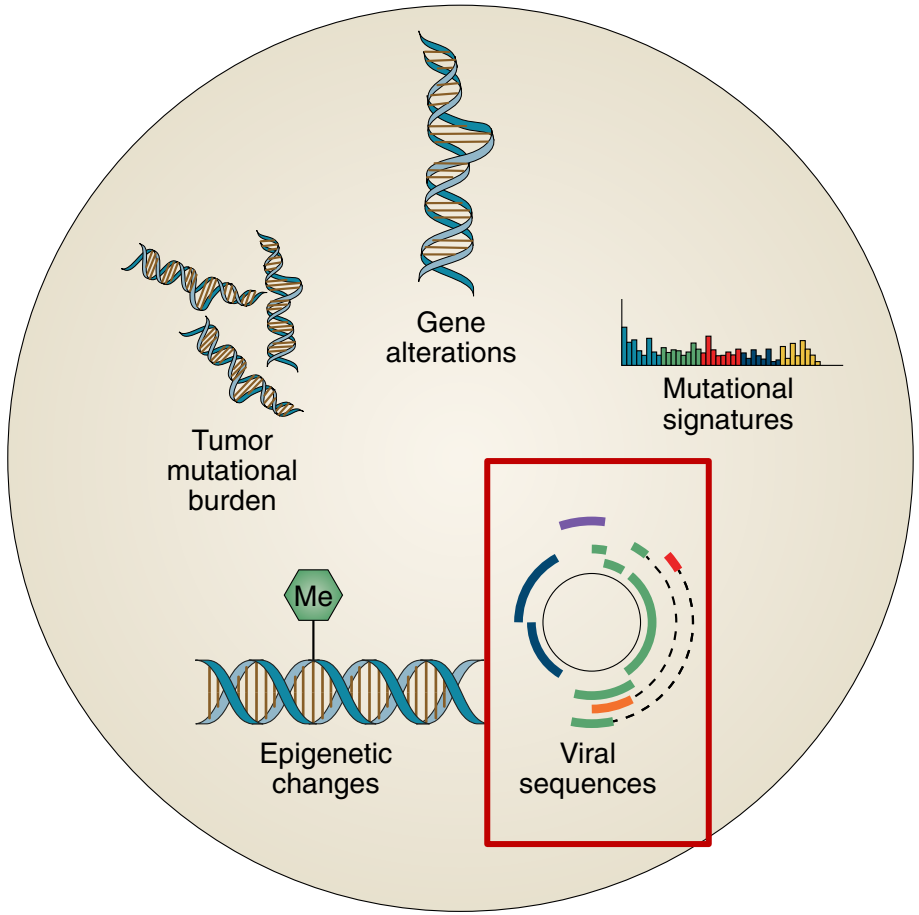
- Interrogates few targets at a time
- High analytical sensitivity
- Low cost & complexity
- Rapid procedures
- Mature technology

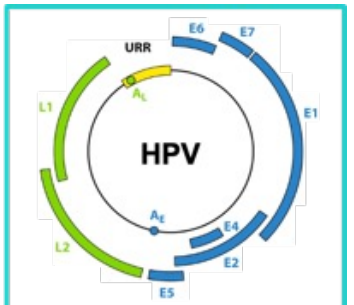
Sequencing

- Can interrogates many targets simultaneously
- Analytical sensitivity is highly platform-dependent
- High cost & complexity
- Longer time-to-data
- Emerging technologies

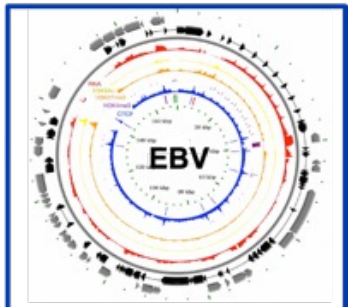
Plasma viral DNA is the archetypal ctDNA biomarker







- Oropharynx Ca
- Cervix Ca
- Anogenital Ca

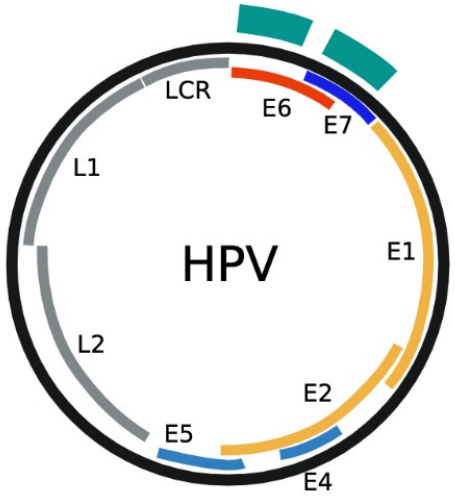


- Nasopharynx
- Gastric Ca
- Lymphomas

Cescon, Bratman, Chan, Siu. Nature Cancer 2020

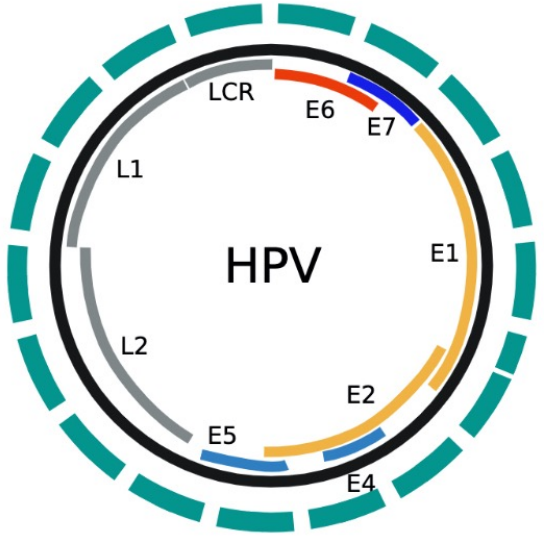
Classes of viral ctDNA detection methodologies

PCR



Covers limited number of loci from one or multiple HPV genotypes

Sequencing

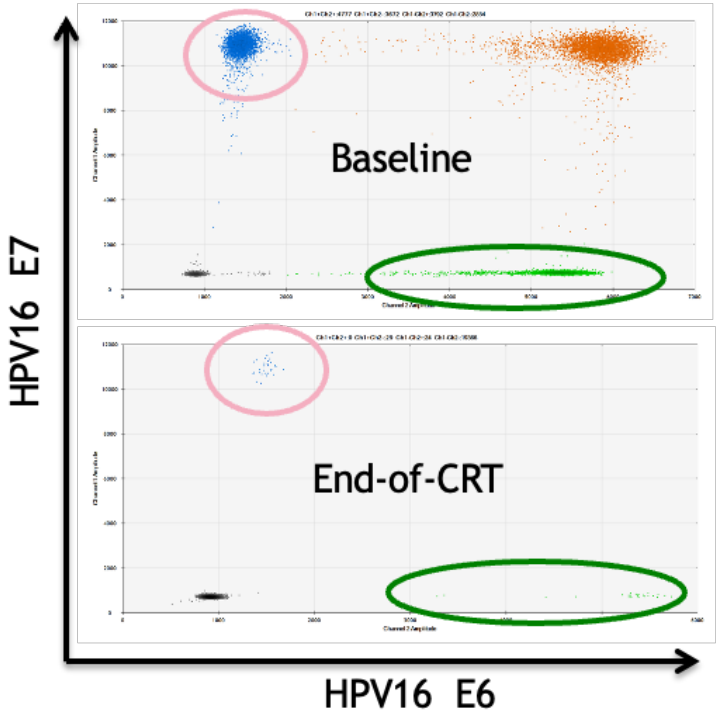


Allows full genome coverage of multiple HPV genotypes

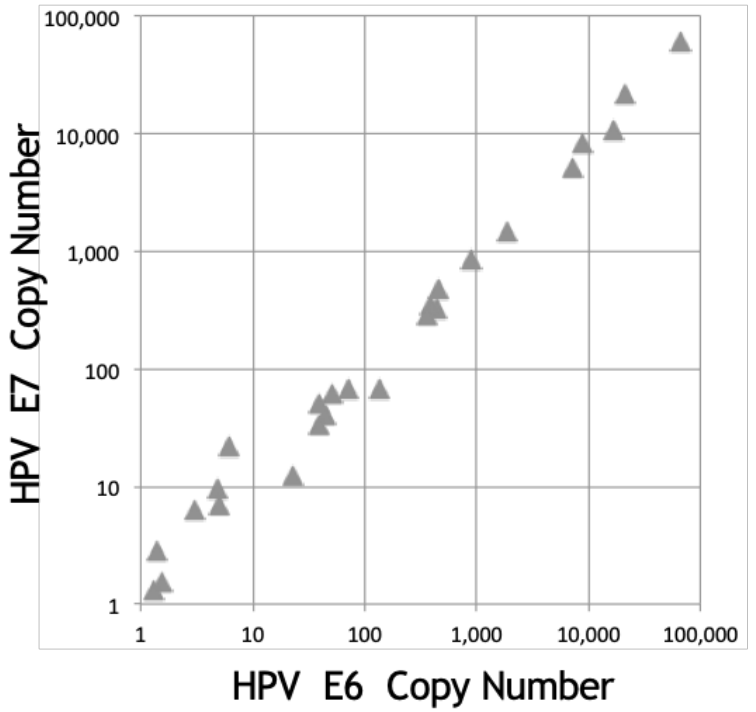
Han, Leung, Bratman et al. JCO Precis Oncol 2018
Leung, Han, Bratman et al. CCR 2021

Digital PCR for HPV ctDNA is limited to 1-2 markers

Representative patient

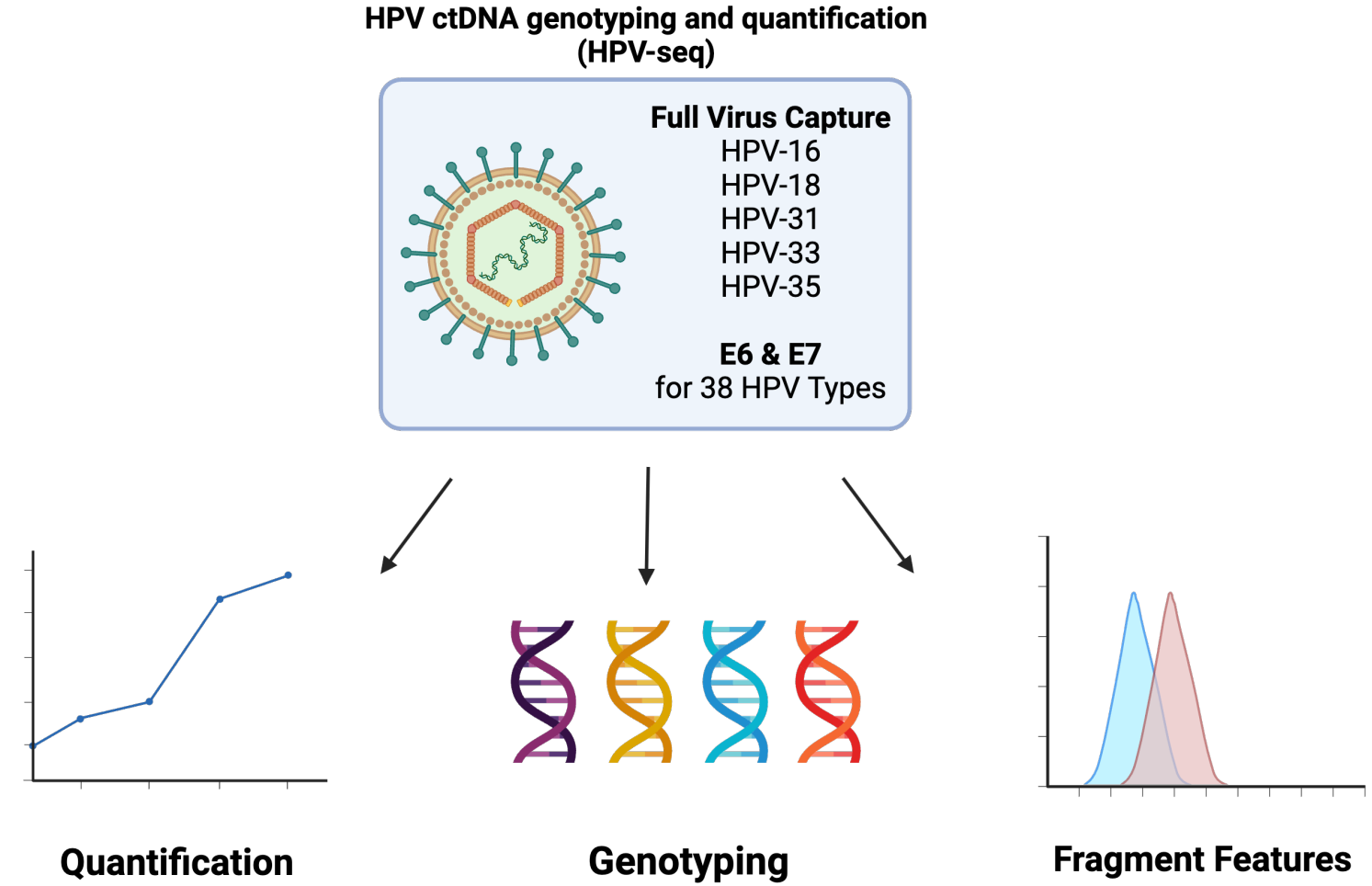


Concordance between E6 and E7



Han, Leung, Bratman, et al. JCO PO 2018

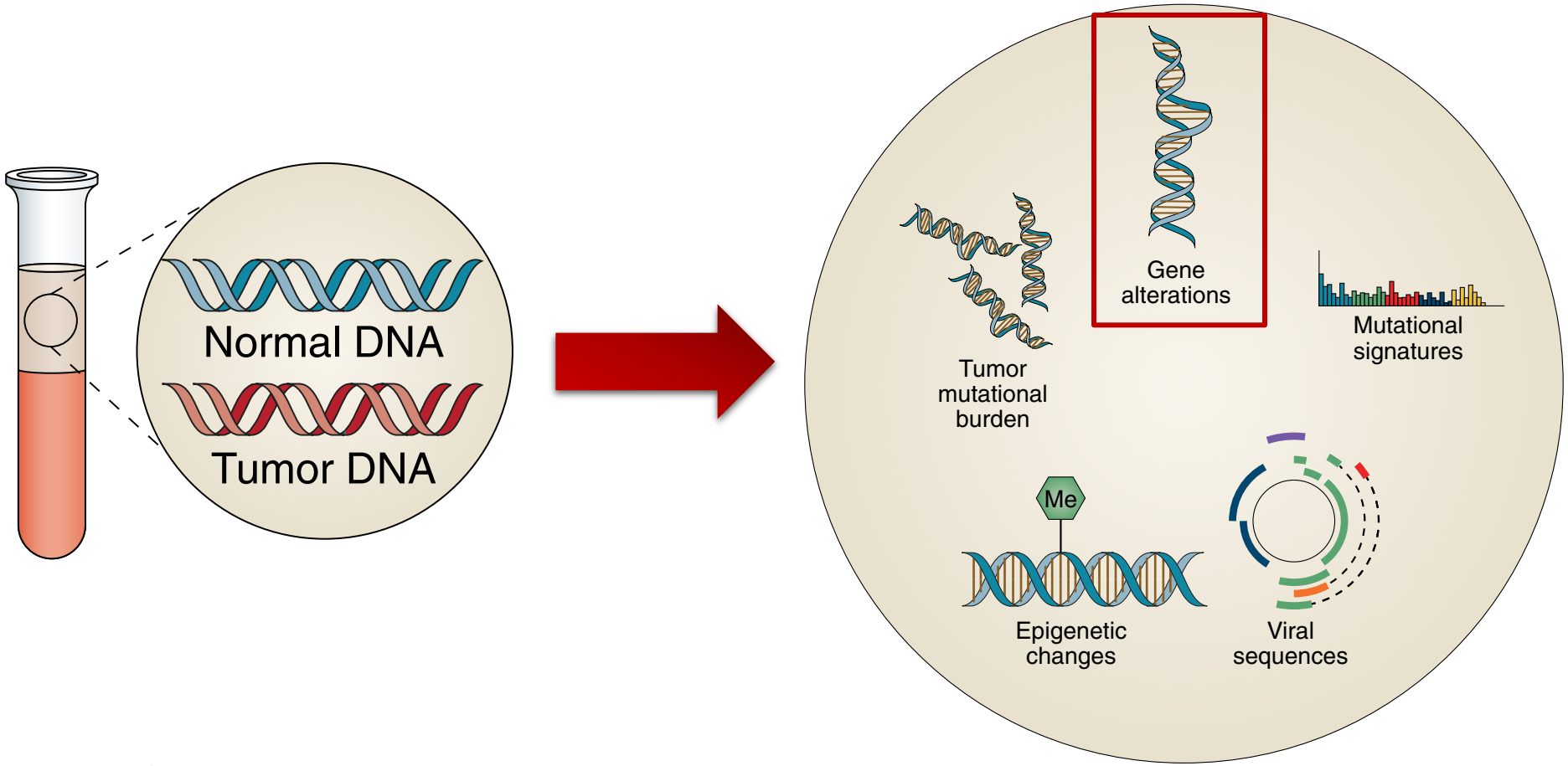
HPV sequencing (HPV-seq) allows for deeper characterization of HPV ctDNA



Leung, Han, Bratman, et al. CCR 2021

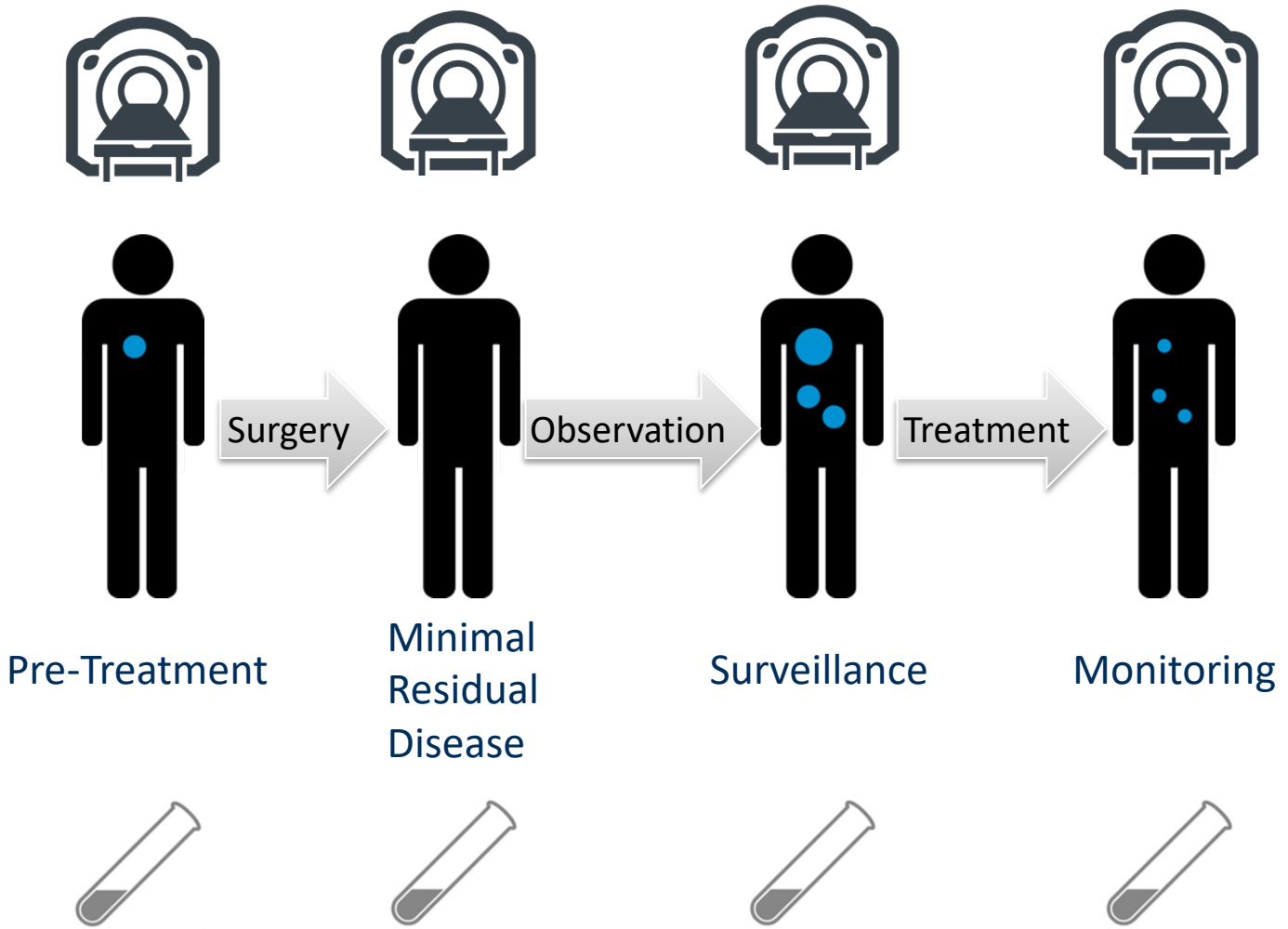
Han, Leung, Bratman et al. JCO 2024

Somatic genetic mutations represent the most common targets of commercial ctDNA assays



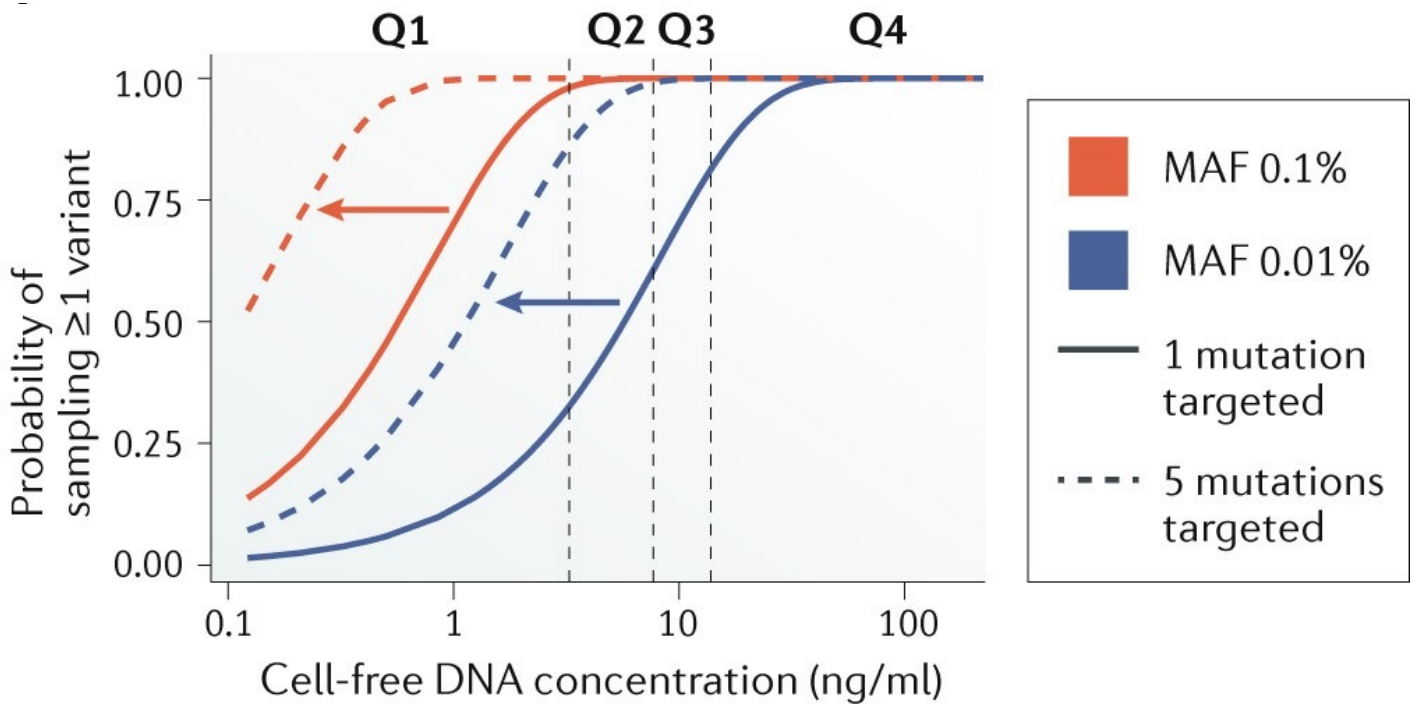
Cescon, Bratman, Chan, Siu. Nature Cancer 2020

Features & practical considerations for ctDNA-based MRD (molecular residual disease) & surveillance/monitoring assay



- Sensitivity and specificity for cancer
- Quantitative ctDNA assessment
- Precedes detection by standard imaging
- Requirement of tumor tissue analysis
- Off-the-shelf vs personalized “bespoke”
- Turn-around-time to receive results

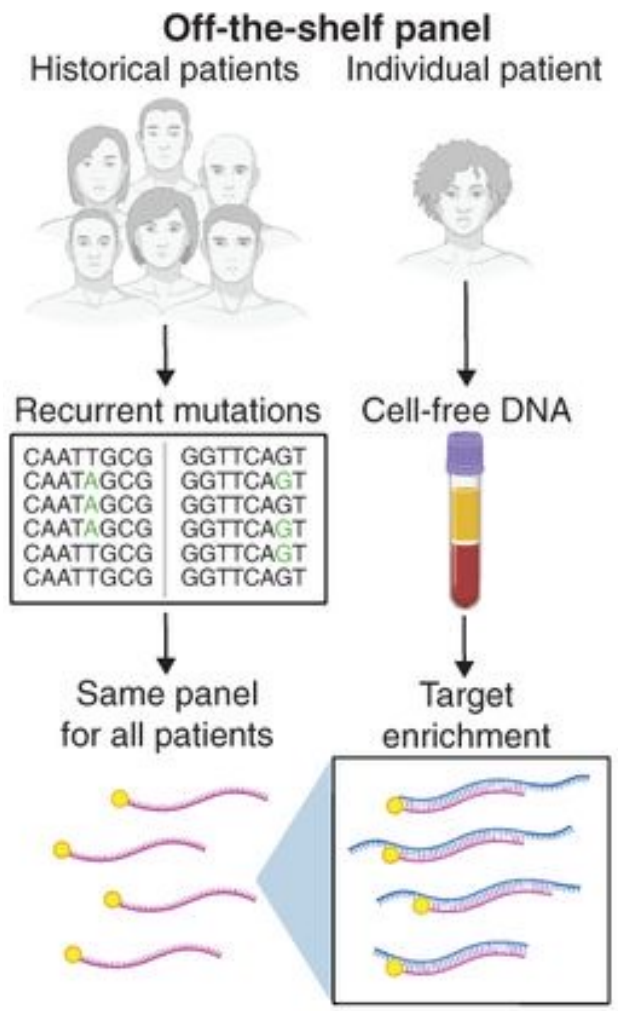
Features & practical considerations for ctDNA-based MRD (molecular residual disease) & surveillance/monitoring assay

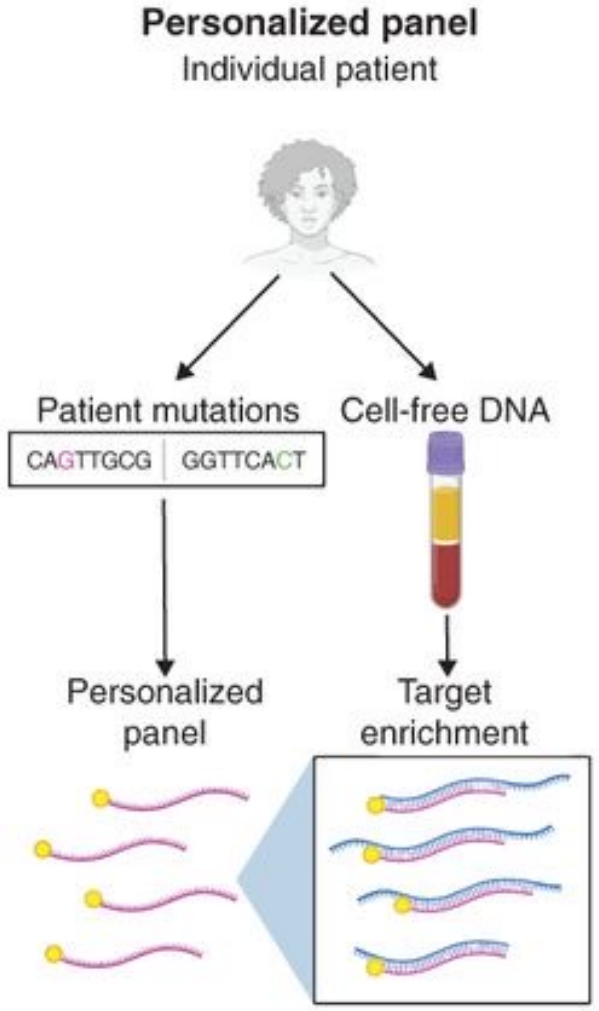


- Mutant allele fraction (MAF)
- Number of mutations targeted
- Amount of cell-free DNA

Abbosh, Birkbak, Swanton. Nat Rev Clin Oncol 2018

Features & practical considerations for ctDNA-based MRD (molecular residual disease) & surveillance/monitoring assay





Moding, Diehn, et al. Cancer Discov. 2021;11(12):2968-2986.

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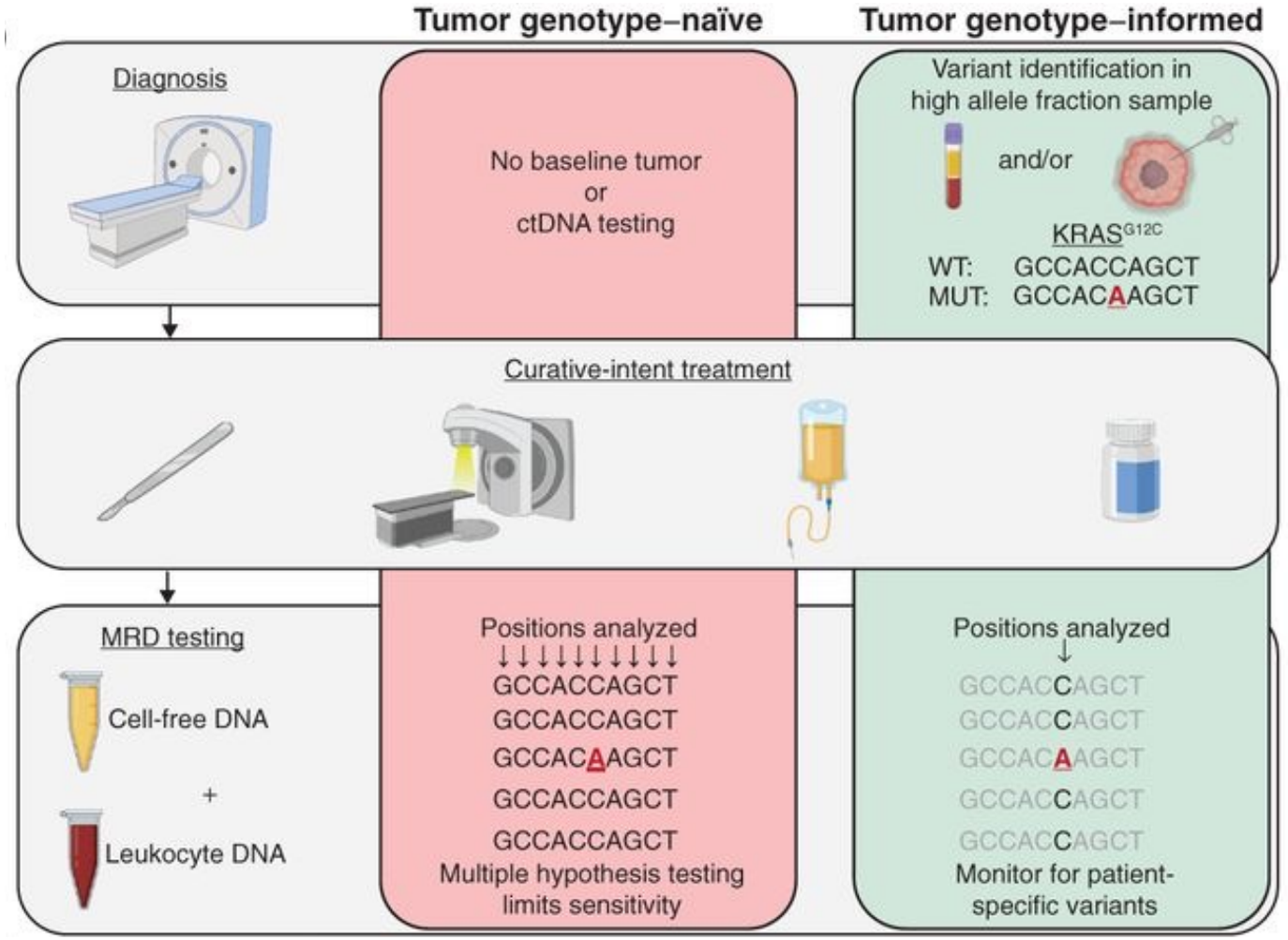
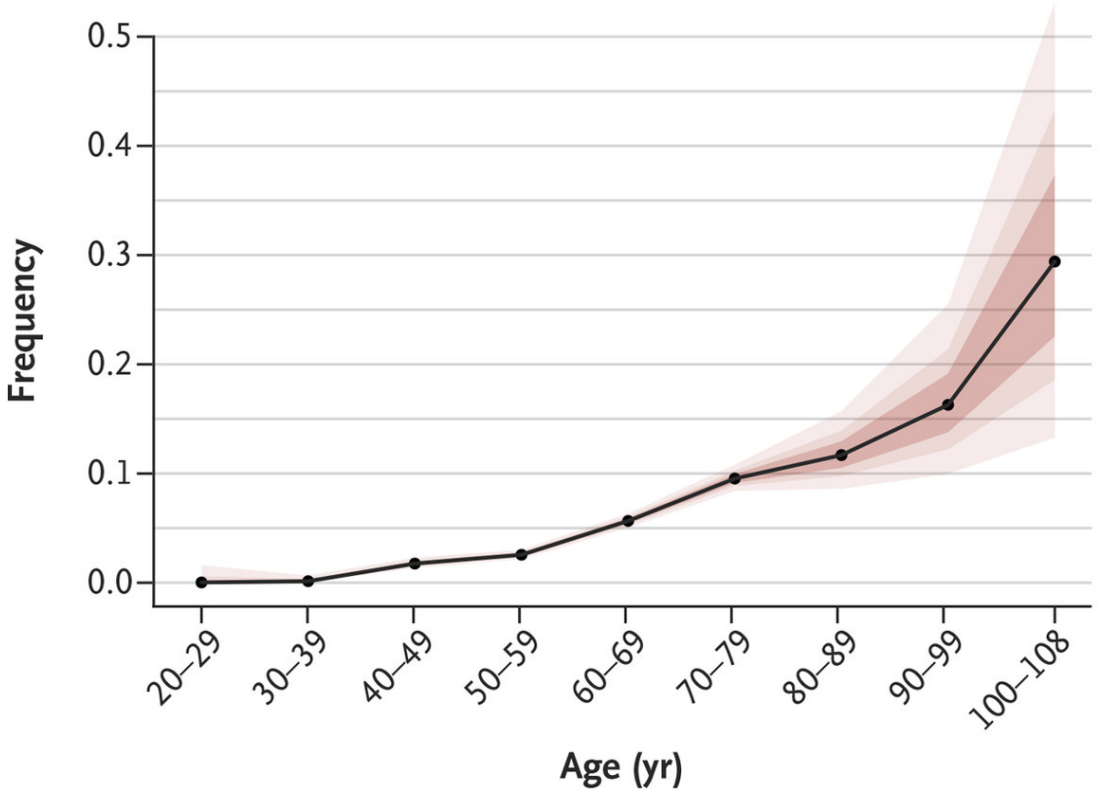


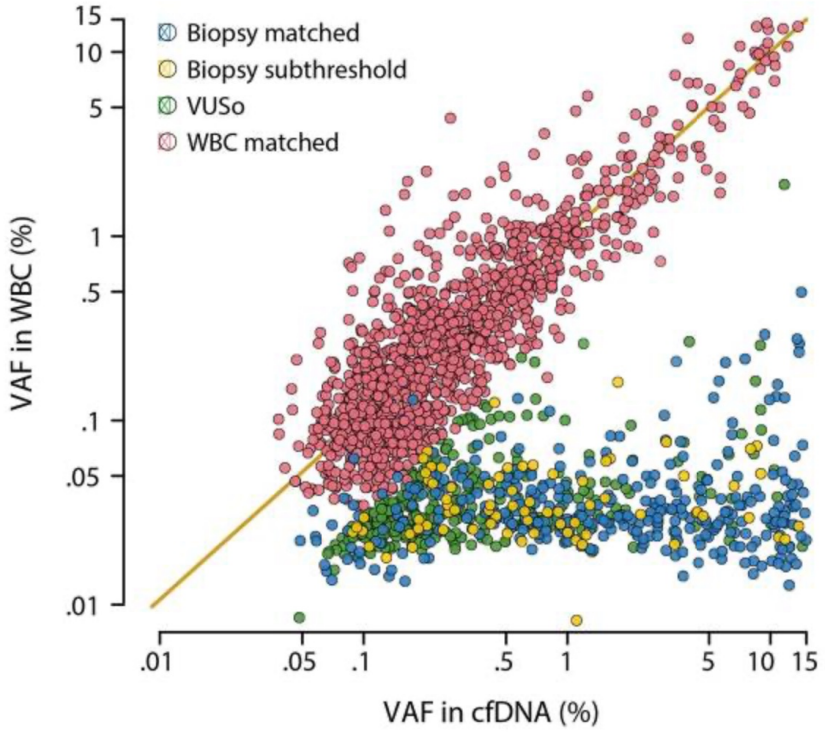
Moding, Diehn, et al. Cancer Discov. 2021;11(12):2968-2986.

Need to account for biological sources of false-positive signal within circulating cell-free DNA

Age-related clonal hematopoiesis

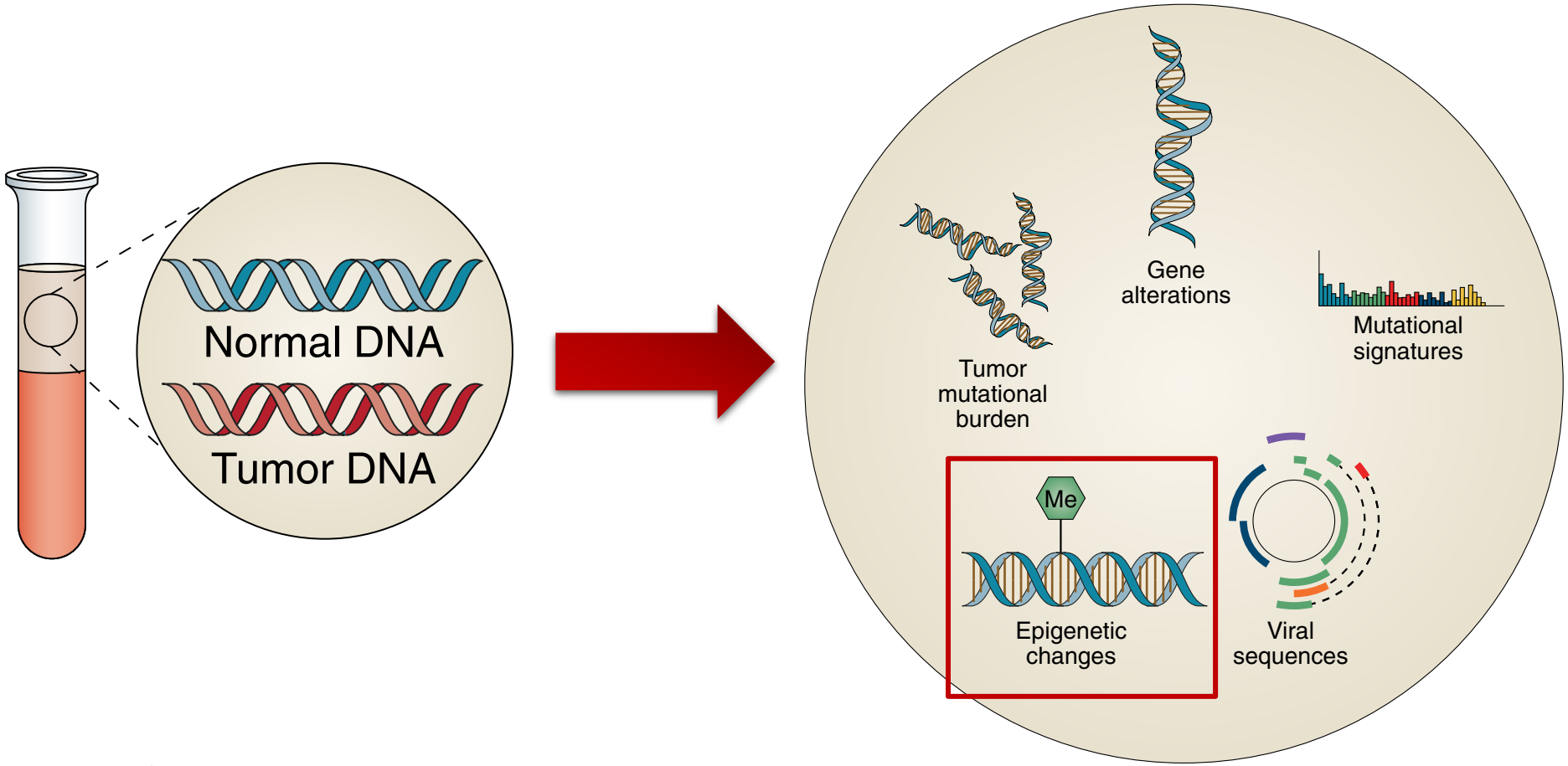


WBC mutations in cell-free DNA



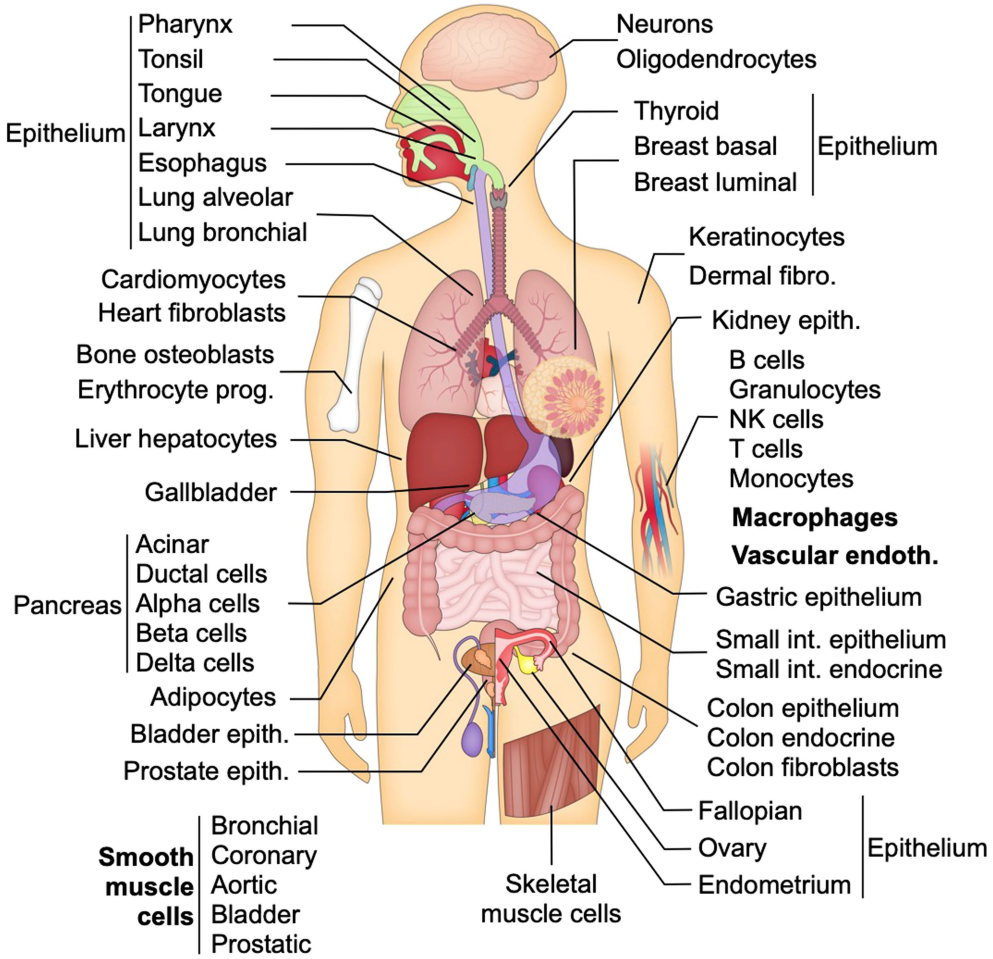
Jaiswal et al. NEJM. 2014;371(26):2488-98
Razavi, et al. Nature Med 2019;25(12):1928-1937

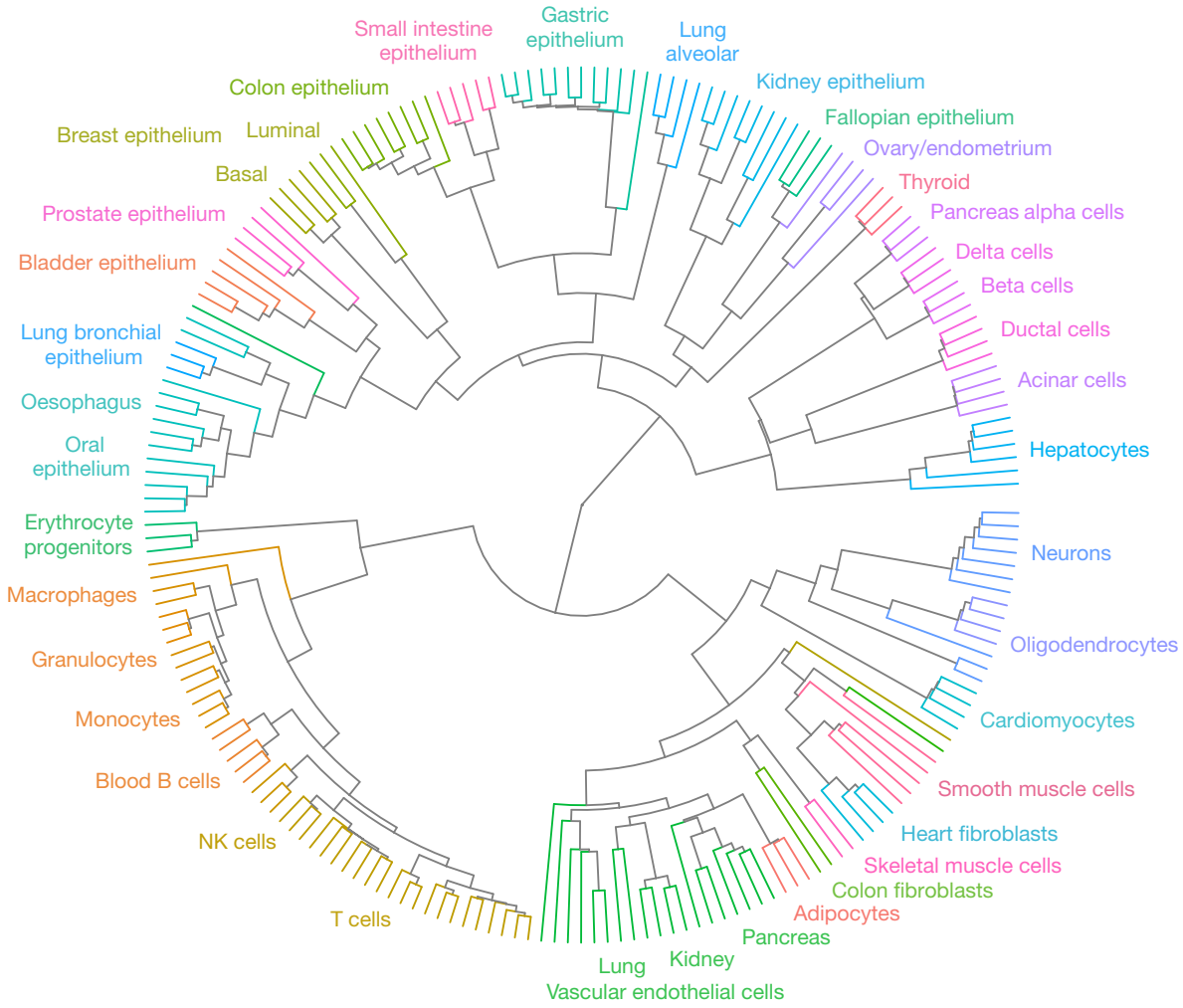
Epigenetic changes represent another promising class of biomarkers detectable within cell-free DNA



Cescon, Bratman, Chan, Siu. Nature Cancer 2020

DNA methylation patterns are specific to cell-of-origin

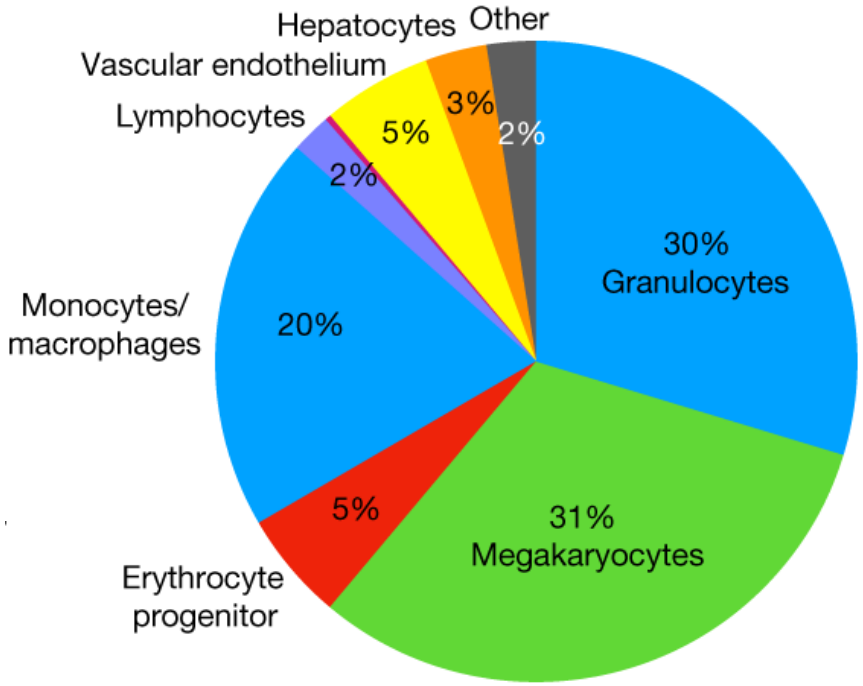




Loyfer et al, Nature, 2023; doi: 10.1038/s41586-022-05580-6

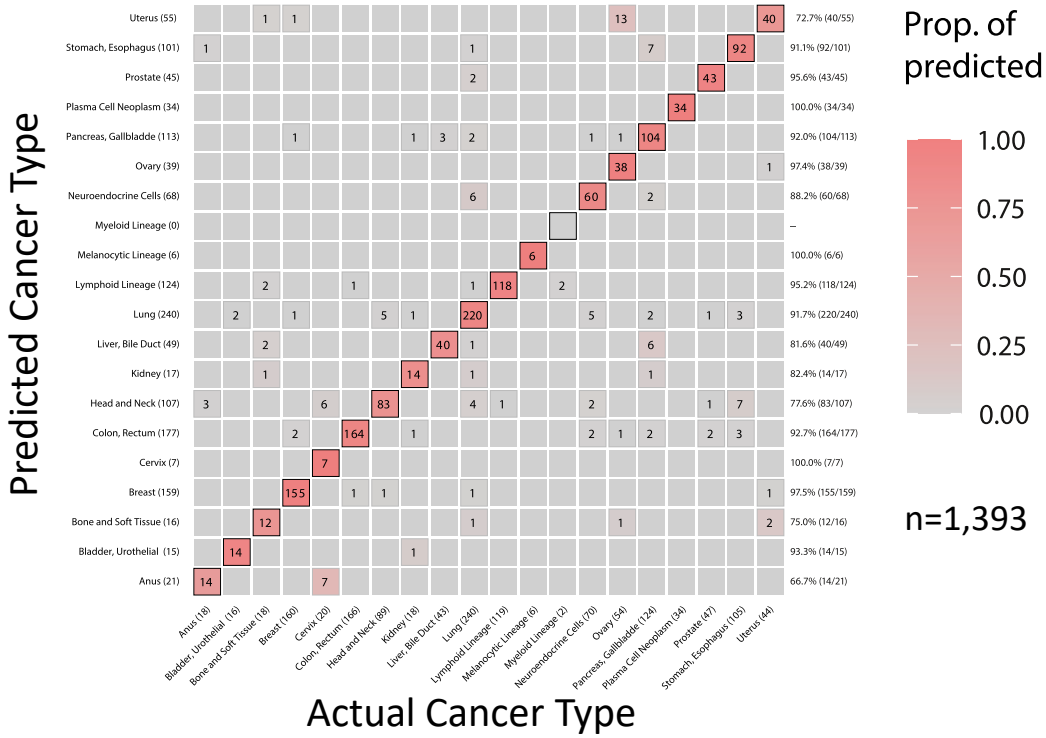
Cell-free DNA tissue-of-origin can be determined from analyzing DNA methylation patterns

Non-Cancer Tissues



Loyfer et al. Nature (2023)

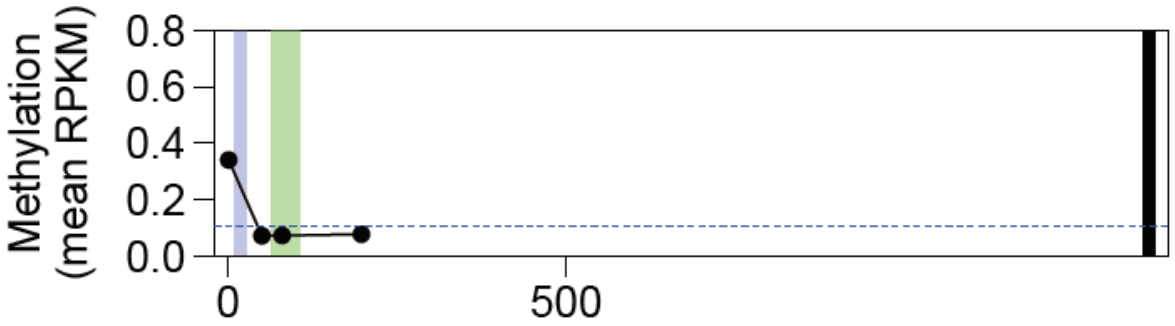
Cancer Type



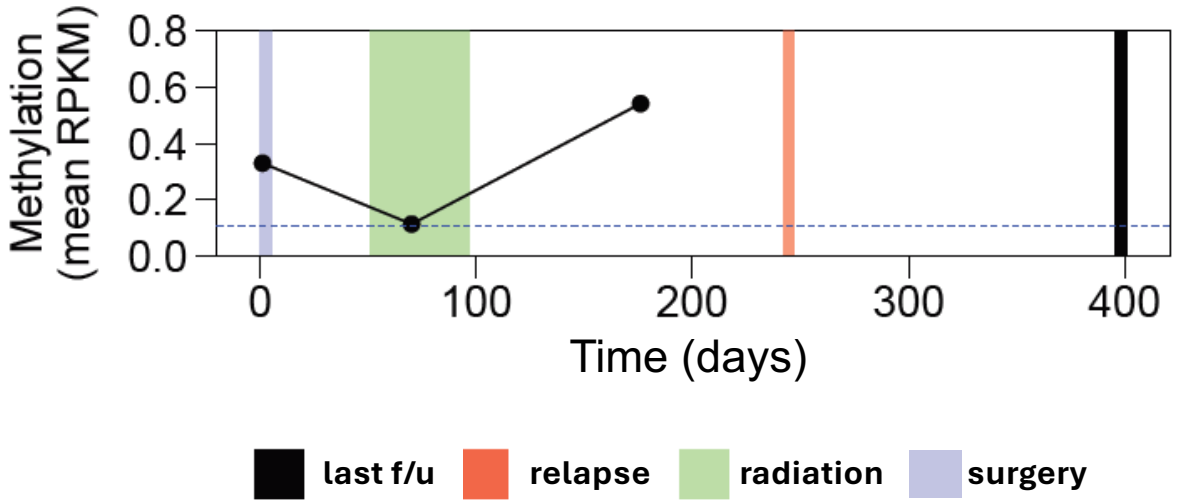
Klein, et al. Annals Oncol 2021

Methylated ctDNA for Tissue-agnostic MRD Detection

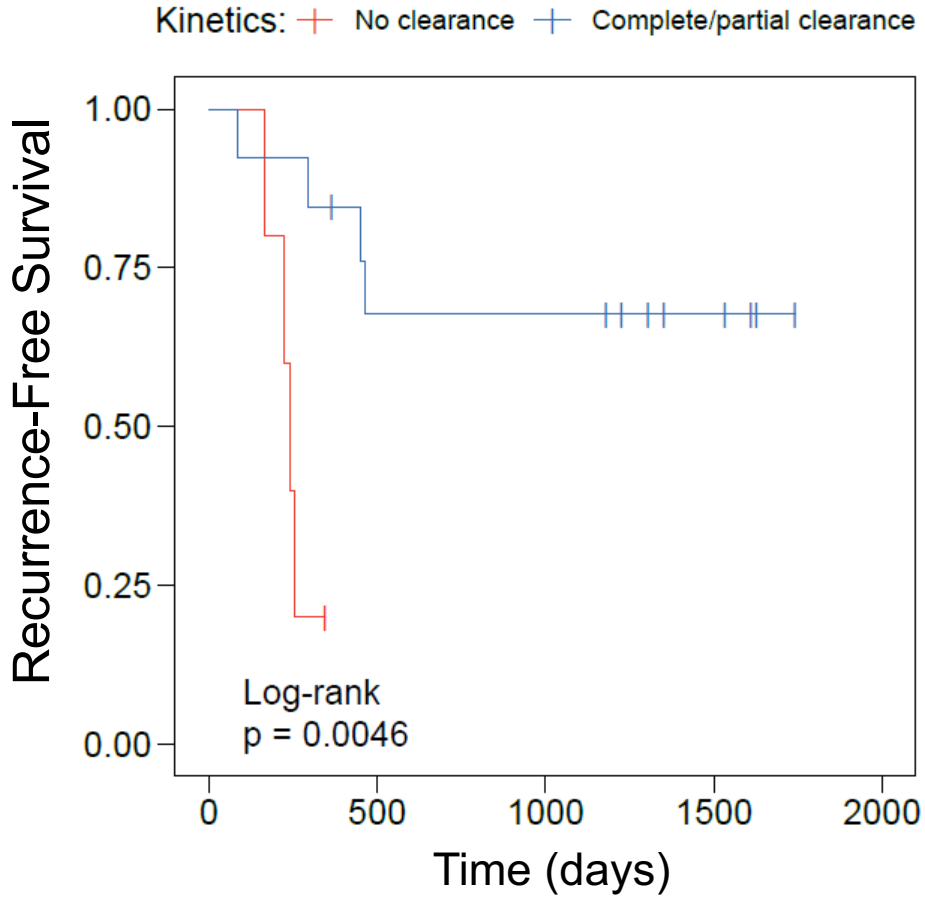
Complete Response → Remission



Partial Response → Progression



MRD+ HNSC Patients Have Rapid Relapse



Burgener, De Carvalho, Bratman, et al. Clin Cancer Res 2021

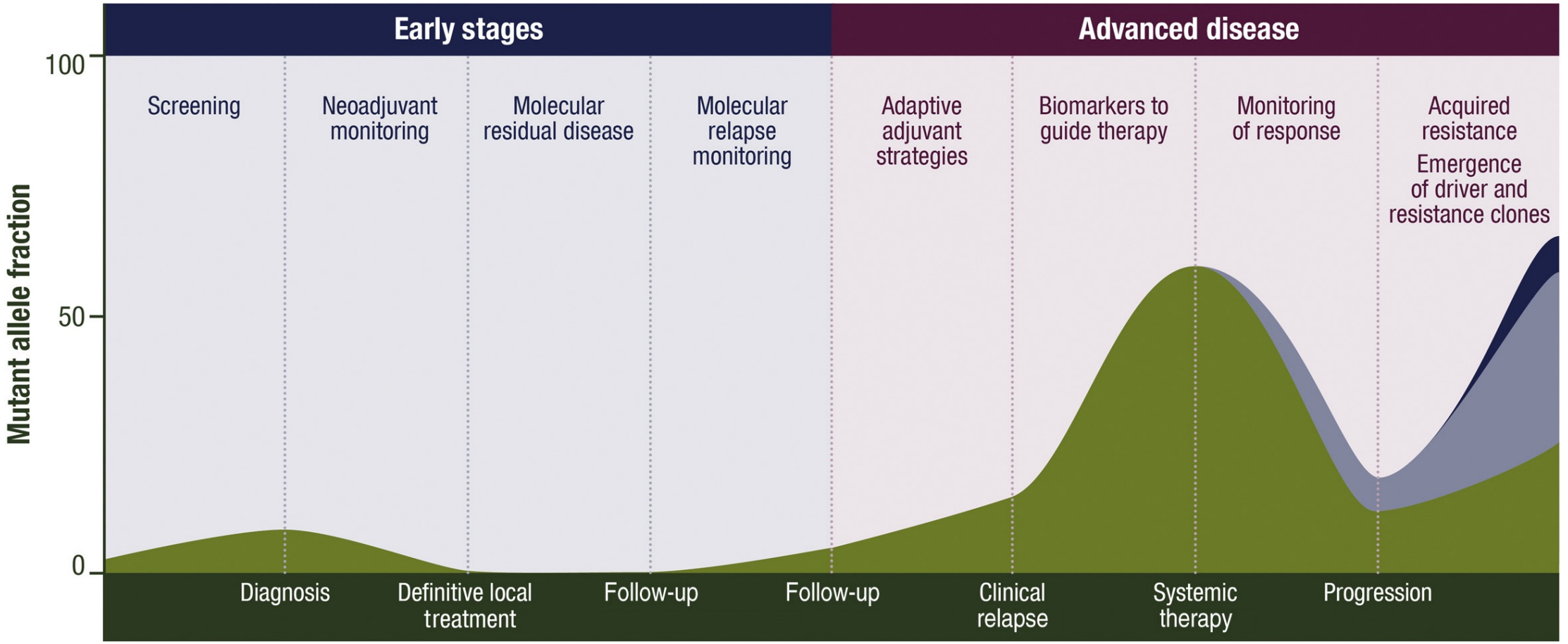
How could cell-free DNA ***epigenetic*** profiling potentially impact clinical decisions?

- Enable **tissue-agnostic detection** in screening or MRD settings
- Distinguish between **distinct cancer types** in unknown primary or early detection settings
- Uncover mechanisms of **therapeutic resistance** such as histologic transformation
- Reveal **tissue damage** that reflects treatment toxicity

Lecture Overview

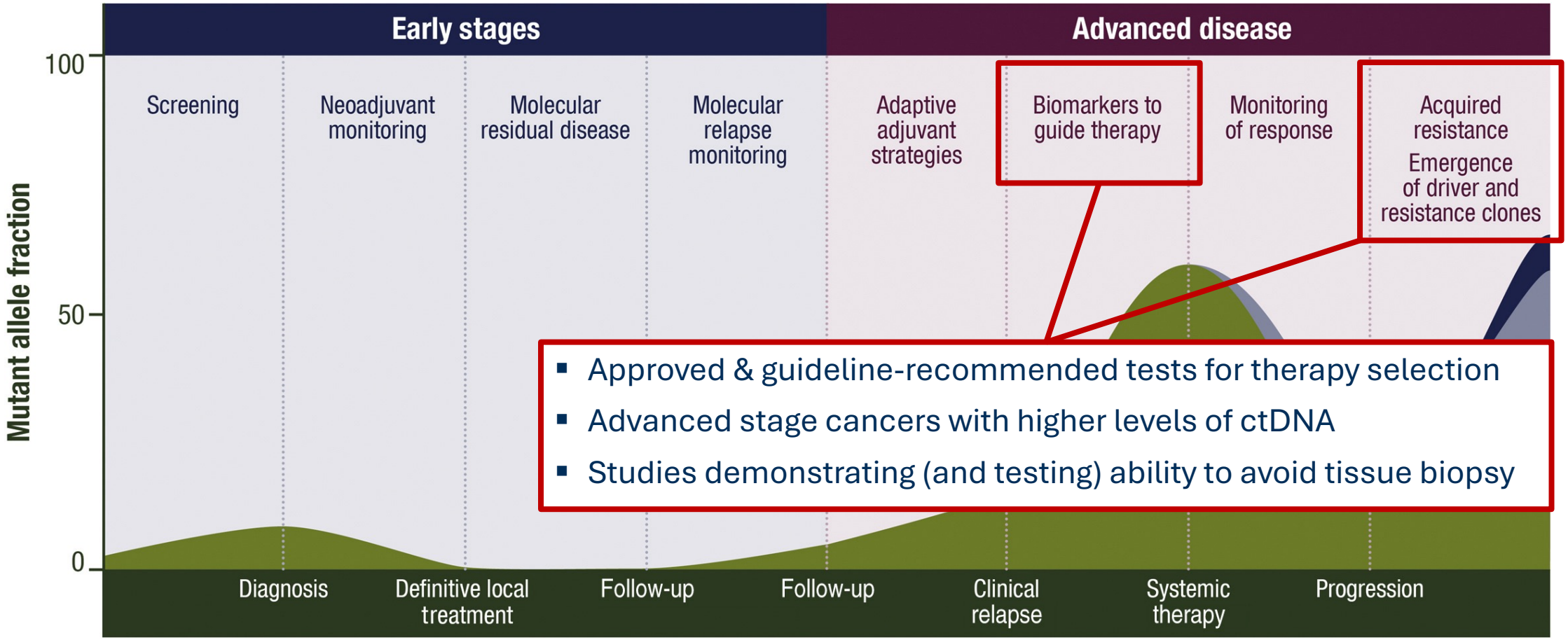
- Summary of liquid biopsy in oncology
- Types of ctDNA tests and practical considerations
- **Evidence for ctDNA tests as tools to guide therapy**

Could ctDNA have utility across the cancer care continuum?



Pascual, Turner et al. Annals Oncol 2022

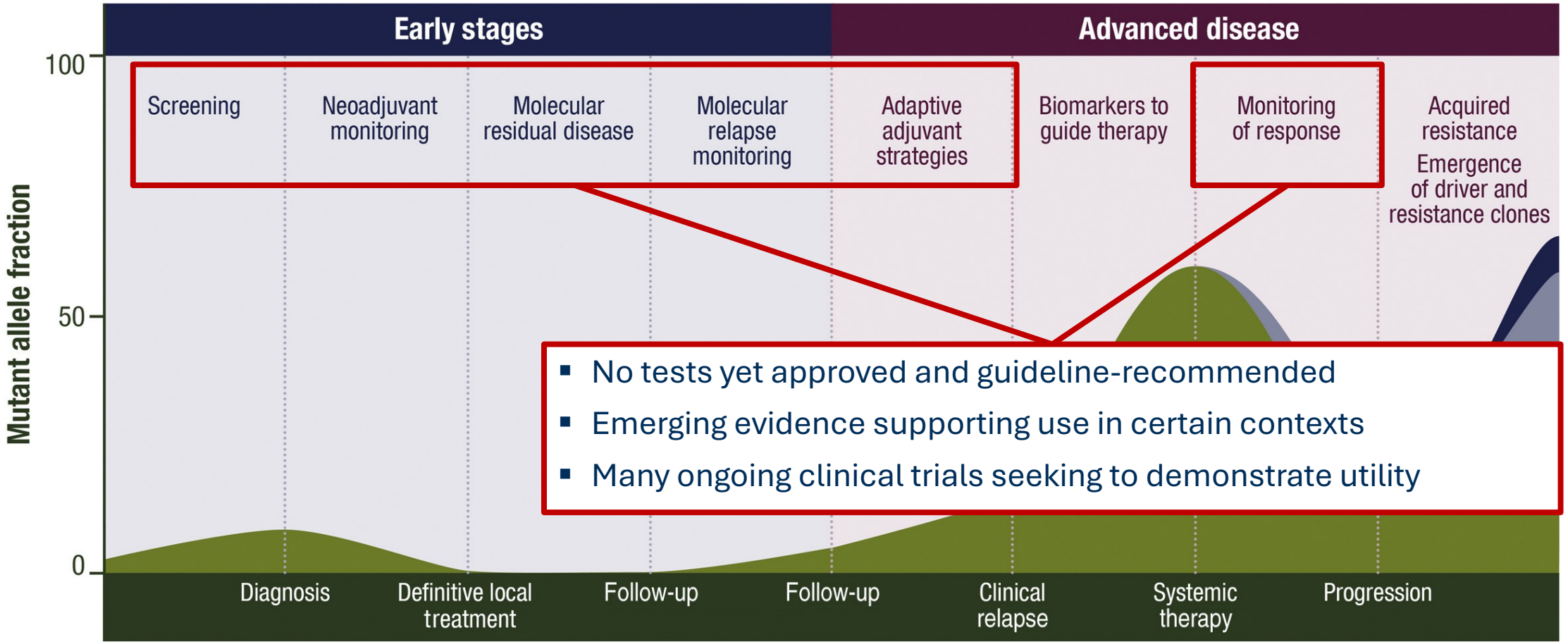
Could ctDNA have utility across the cancer care continuum?



- Approved & guideline-recommended tests for therapy selection
- Advanced stage cancers with higher levels of ctDNA
- Studies demonstrating (and testing) ability to avoid tissue biopsy

Pascual, Turner et al. Annals Oncol 2022

Could ctDNA have utility across the cancer care continuum?



- No tests yet approved and guideline-recommended
- Emerging evidence supporting use in certain contexts
- Many ongoing clinical trials seeking to demonstrate utility

Pascual, Turner et al. Annals Oncol 2022

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

SPECIAL ARTICLE

ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group

J. Pascual[1], G. Attard[2], F.-C. Bidard[3,4], G. Curigliano[5,6], L. De Mattos-Arruda[7,8], M. Diehn[9], A. Italiano[10,11,12], J. Lindberg[13], J. D. Merker[14], C. Montagut[15], N. Normanno[16], K. Pantel[17], G. Pentheroudakis[18], S. Popat[19,20], J. S. Reis-Filho[21], J. Tie[22,23], J. Seoane[24,25], N. Tarazona[26,27], T. Yoshino[28] & N. C. Turner[19,20*]

UNIVERSITY OF TORONTO FACULTY OF MEDICINE

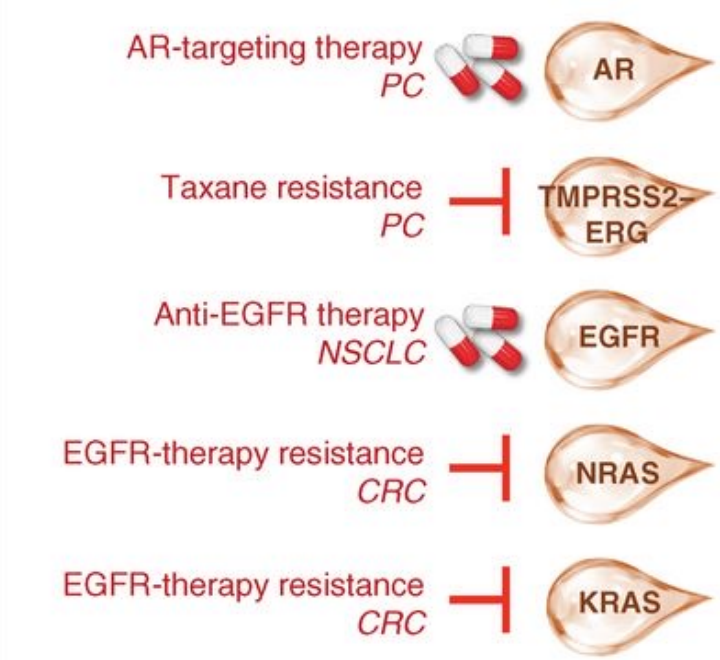
UHN Princess Margaret Cancer Centre

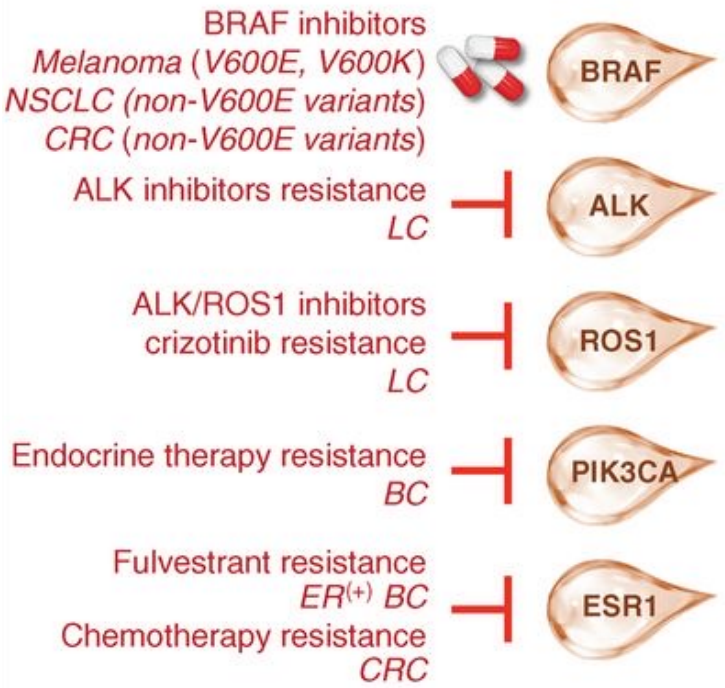
ESMO recommendations on the use of ctDNA

- For advanced cancers, validated and adequately sensitive **ctDNA assays have utility in identifying actionable mutations to direct targeted therapy**, and may be used in routine clinical practice, provided the limitations of the assays are taken into account
- For early-stage cancers, detection of MRD has high evidence of clinical validity in anticipating future relapse, but **MRD detection cannot be recommended** in routine clinical practice due to lack of clinical utility studies
- Additional potential applications of ctDNA assays are **not recommended** for routine practice

Pascual, Turner et al. Annals Oncol 2022

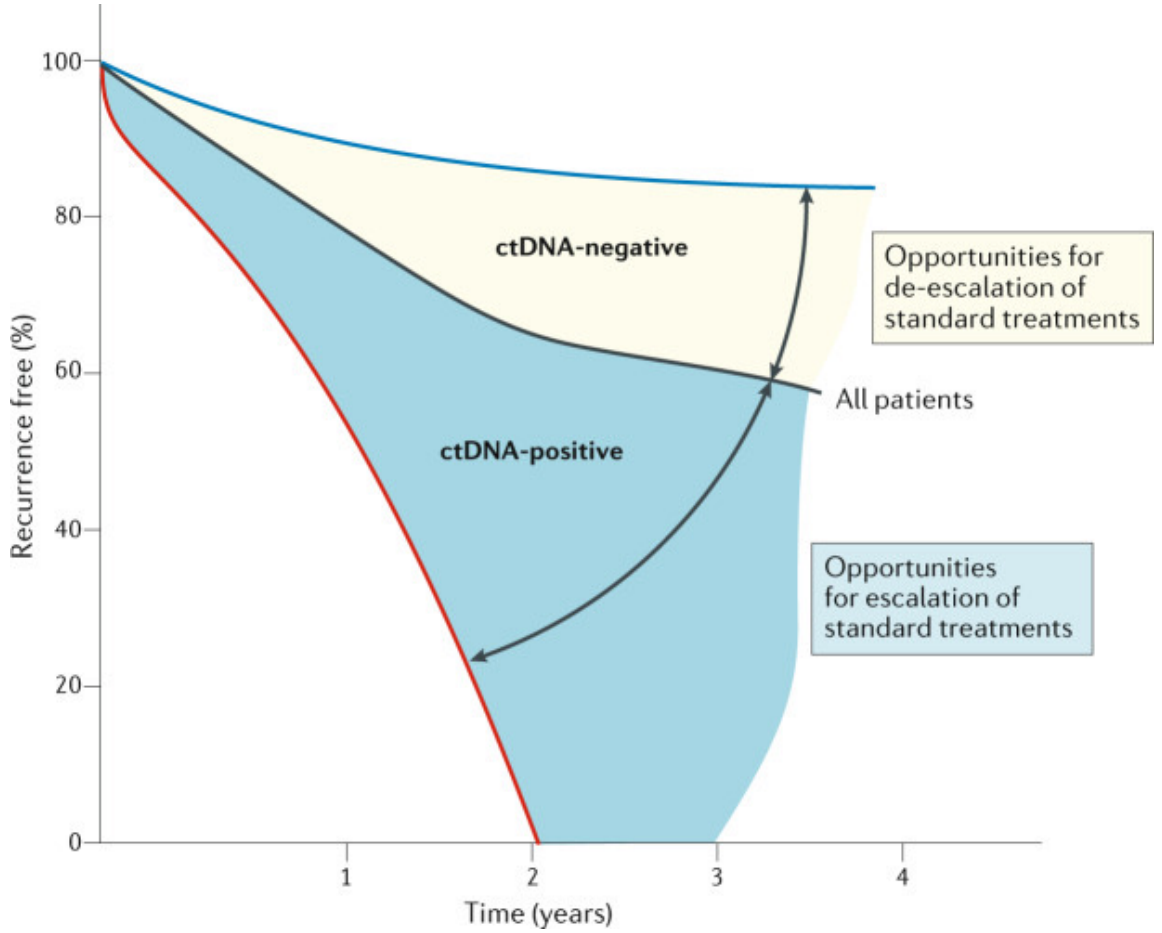
Therapeutic targets and resistance mechanisms potentially detectable within ctDNA





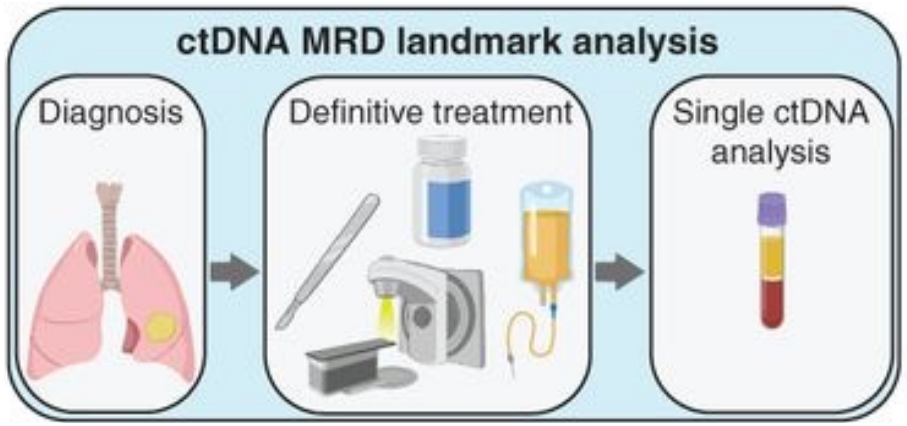
Alix-Panabières and Pantel, Cancer Discov. 2021;11(4):858-873.

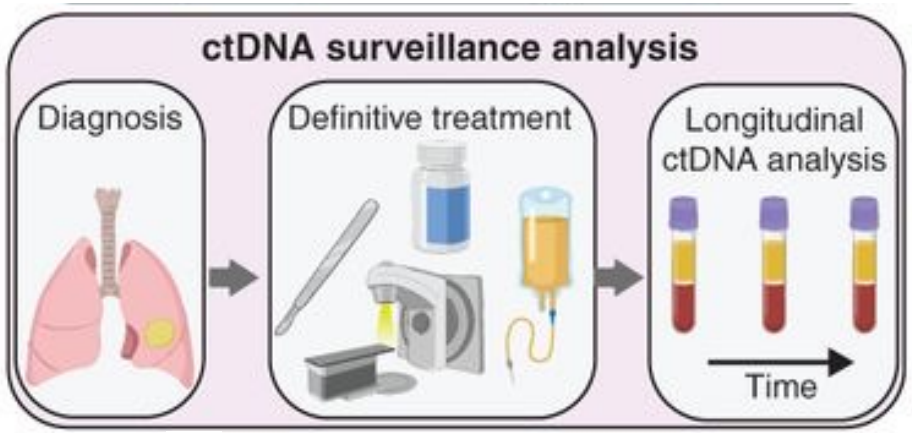
Potential applications of post-treatment ctDNA-based MRD in tailoring the aggressiveness of adjuvant therapy

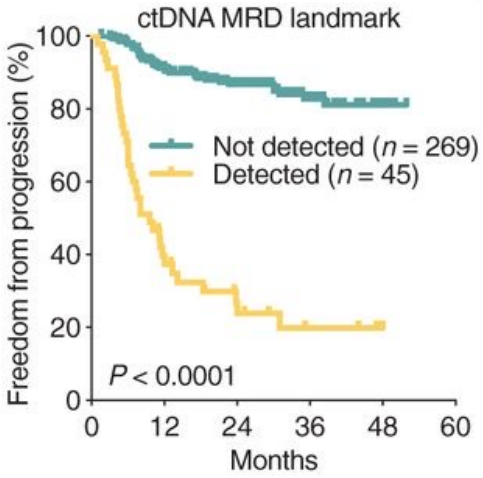


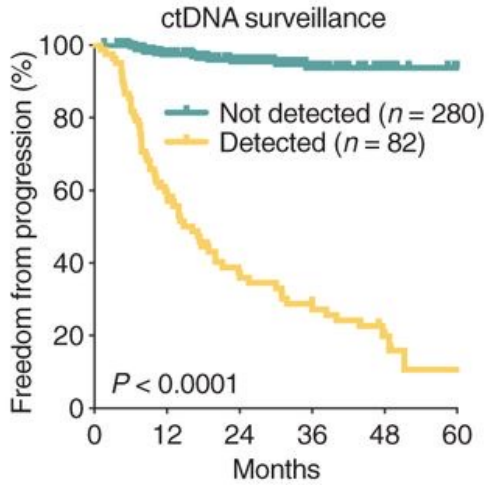
Dasari et al. Nat Rev Clin Oncol 2020

Assay characteristics impact accuracy for MRD detection; Longitudinal ctDNA analysis boosts sensitivity & NPV







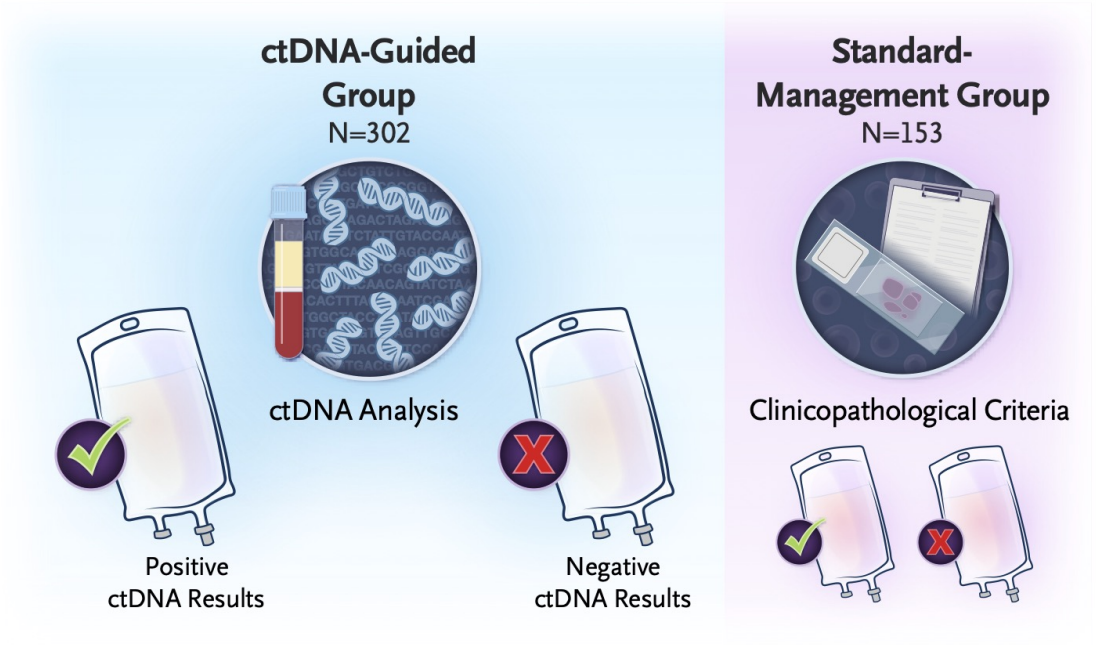


Moding, Diehn, et al. Cancer Discov. 2021;11(12):2968-2986.

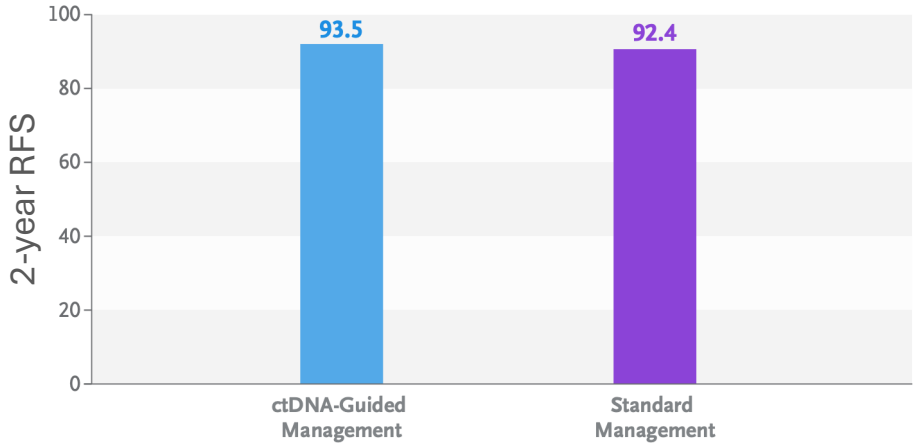
Emerging clinical utility of ctDNA-based MRD-guided cancer therapy

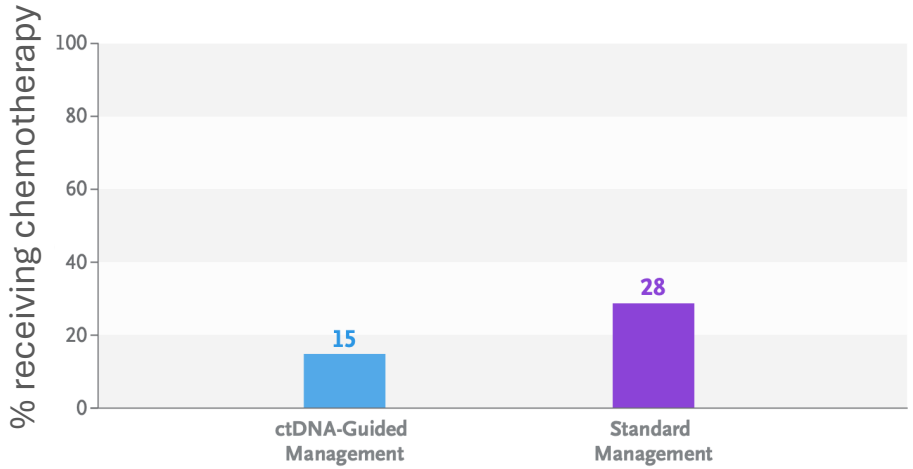
Tie, et al. NEJM 2022. doi: 10.1056/NEJMoa2200075

ctDNA-guided approach in stage II colon cancer reduced adjuvant chemotherapy use without compromising RFS



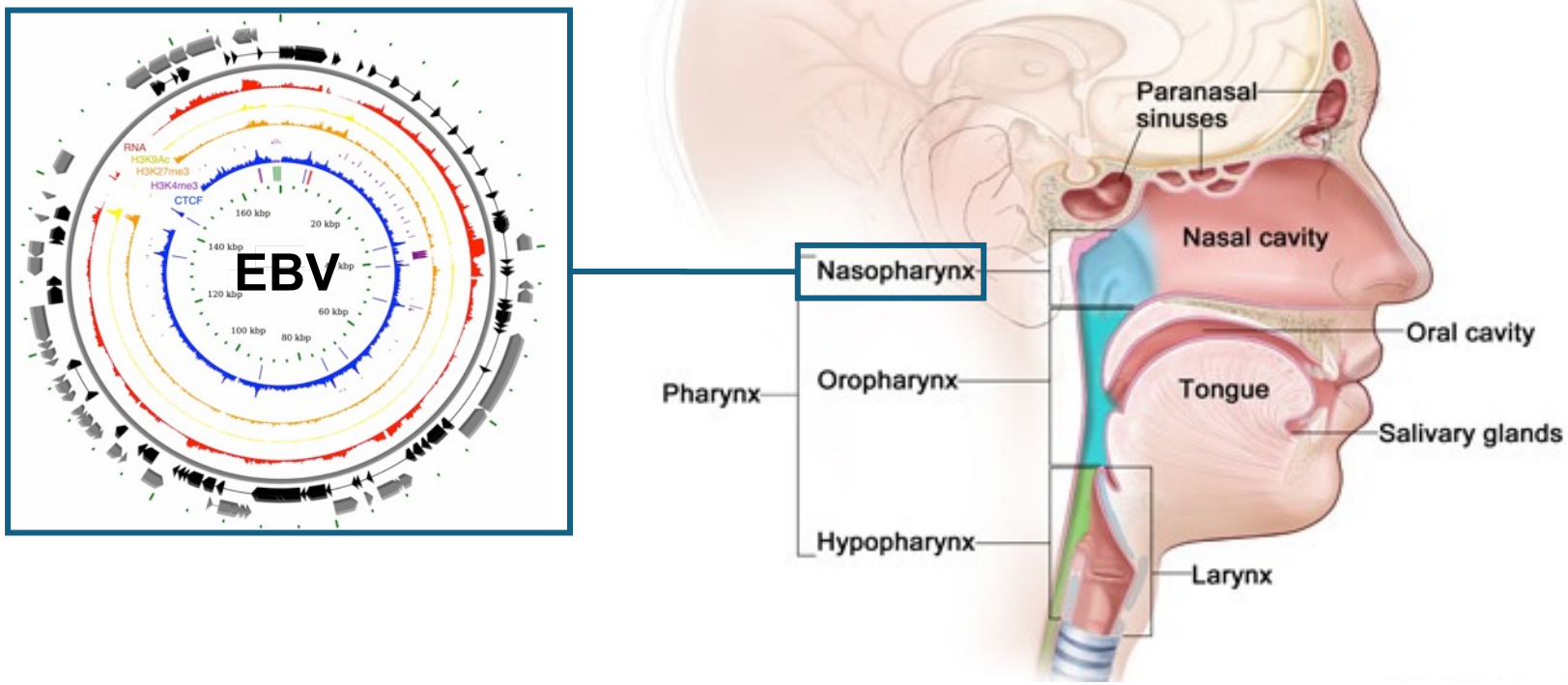
- Phase 2 RCT with noninferiority design
- Primary endpoint: 2-yr RFS
- Noninferiority margin: −8.5%





Tie, et al. NEJM 2022. doi: 10.1056/NEJMoa2200075

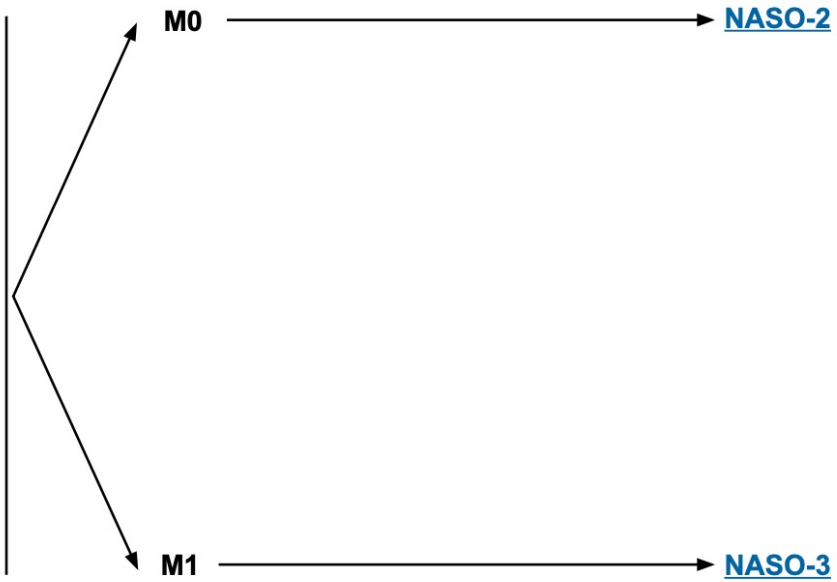
Lessons from studies on plasma Epstein-Barr Virus (EBV) in endemic nasopharynx cancer patients



WORKUP

- H&P[a,b] including a complete head and neck exam; mirror examination as clinically indicated
- Nasopharyngeal fiberoptic examination
- Biopsy of primary site or FNA of the neck[c]
- MRI with and without contrast of skull base to clavicle ± CT of skull base/neck with contrast to evaluate skull base erosion
- Imaging for distant metastases with FDG-PET/CT and/or chest CT with contrast[d]
- Consider Epstein-Barr virus (EBV)/DNA testing[e]
- As clinically indicated:
  - Dental/prosthodontic evaluation[f]
  - Nutrition, speech and swallowing evaluations/therapy[g]
  - Audiogram
  - Consider ophthalmologic and endocrine evaluation
  - Smoking cessation counseling[a]
  - Fertility/reproductive counseling[h]
  - Screening for hepatitis B
- Multidisciplinary consultation as clinically indicated

CLINICAL STAGING



[a] H&P should include documentation and quantification (pack years smoked) of tobacco use history, as well as alcohol use and counseling. All patients who currently smoke should be advised to quit smoking, and those who formerly smoked should be advised to remain abstinent from smoking. For additional cessation support, refer to the Patient/Provider Smoking Cessation and Treatment Resources in the NCCN Guidelines for Smoking Cessation.

[b] Screen for depression (NCCN Guidelines for Distress Management).

[c] Image-guided (US or CT) needle biopsy of cystic neck nodes may offer better diagnostic yield than FNA by palpation alone for initial diagnosis in this setting. For unresectable or metastatic disease where there is a plan for systemic therapy, a core biopsy would allow for ancillary immune-genomic testing.

[d] Principles of Imaging (IMG-A).

[e] For nonkeratinizing or undifferentiated histology, consider testing for EBV in tumor and blood. Common means for detecting EBV in pathologic specimens include ISH for EBV-encoded RNA (EBER) or immunohistochemical staining for latent membrane protein (LMP). The EBV DNA load within the serum or plasma may be quantified using PCR targeting genomic sequences of the EBV DNA such as BamHI-W, Epstein-Barr virus nuclear antigen (EBNA), or LMP; these tests vary in their sensitivity. The EBV DNA load may reflect prognosis and change in response to therapy.

[f] Principles of Dental Evaluation and Management (DENT-A).

[g] Principles of Nutrition: Management and Supportive Care (NUTR-A).

[h] See fertility and reproductive endocrine considerations in the NCCN Guidelines for Adolescent and Young Adult (AYA) Oncology.

Plasma EBV DNA detectability and levels are prognostic in patients with EBV-associated nasopharynx cancer

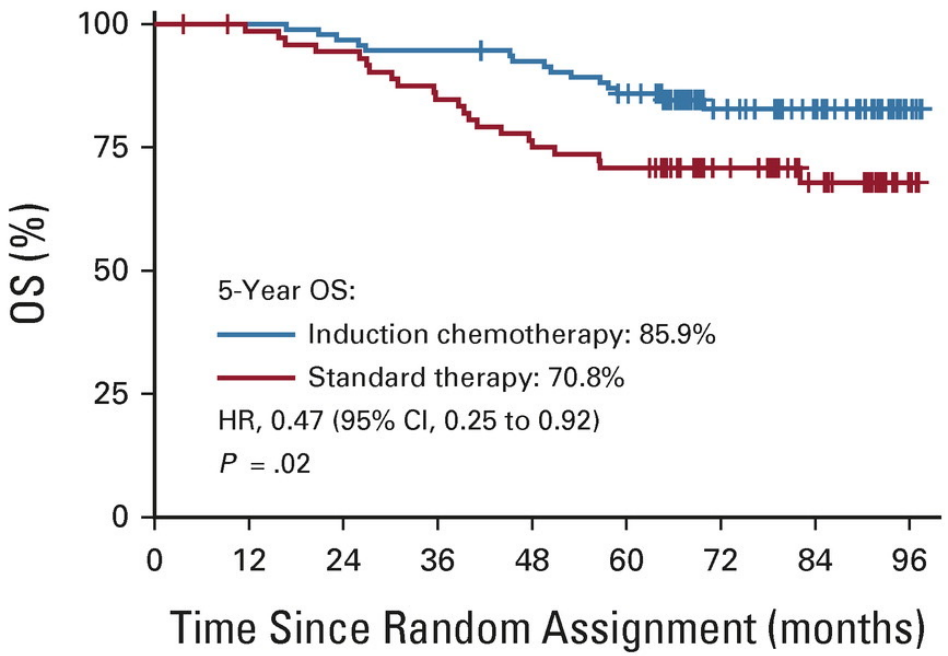
Outcomes	Studies No.	Hazard radio, 95%CI	Z-value	P-value
Pre-DNA				
OS	13	2.81, 2.44–3.24	14.31	<0.00001
PFS	6	2.74, 2.37–3.18	13.33	<0.00001
DMFS	6	3.89, 3.39–4.47	19.27	<0.00001
LRFS	6	2.02, 1.52–2.70	4.82	<0.00001
Post-DNA				
OS	6	4.26, 3.26–5.57	10.58	<0.00001
PFS	2	5.21, 3.29–8.27	7.01	<0.00001
DMFS	2	7.54, 3.39–16.77	4.95	<0.00001
LRFS	4	7.51, 5.11–11.02	10.30	<0.00001
Mid-DNA				
OS	1	3.29, 1.37–7.89	2.67	0.0077
PFS	1	4.05, 1.89–8.67	3.60	0.0003
DMFS	1	12.02, 2.78–51.93	3.33	0.0009
LRFS	1	2.05, 0.79–5.31	1.48	0.1378

- EBV DNA levels in plasma pre-, mid-, and post-treatment are prognostic
- Stronger association with **distant metastasis** than locoregional recurrence
- Results become more strongly prognostic **during and after treatment**

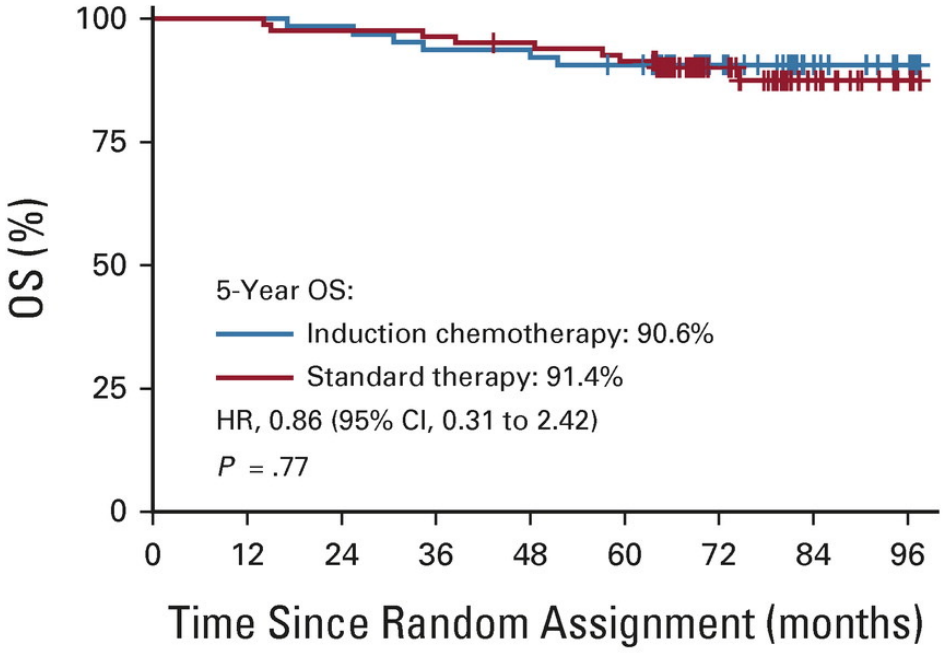
Zhang W, Chen Y, Chen L, et al. Medicine (Baltimore) 2015;94:e845.

Potential utility: Benefit of neoadjuvant chemotherapy restricted to patients with high pre-treatment plasma EBV DNA levels

≥4,000 copies/mL



<4,000 copies/mL

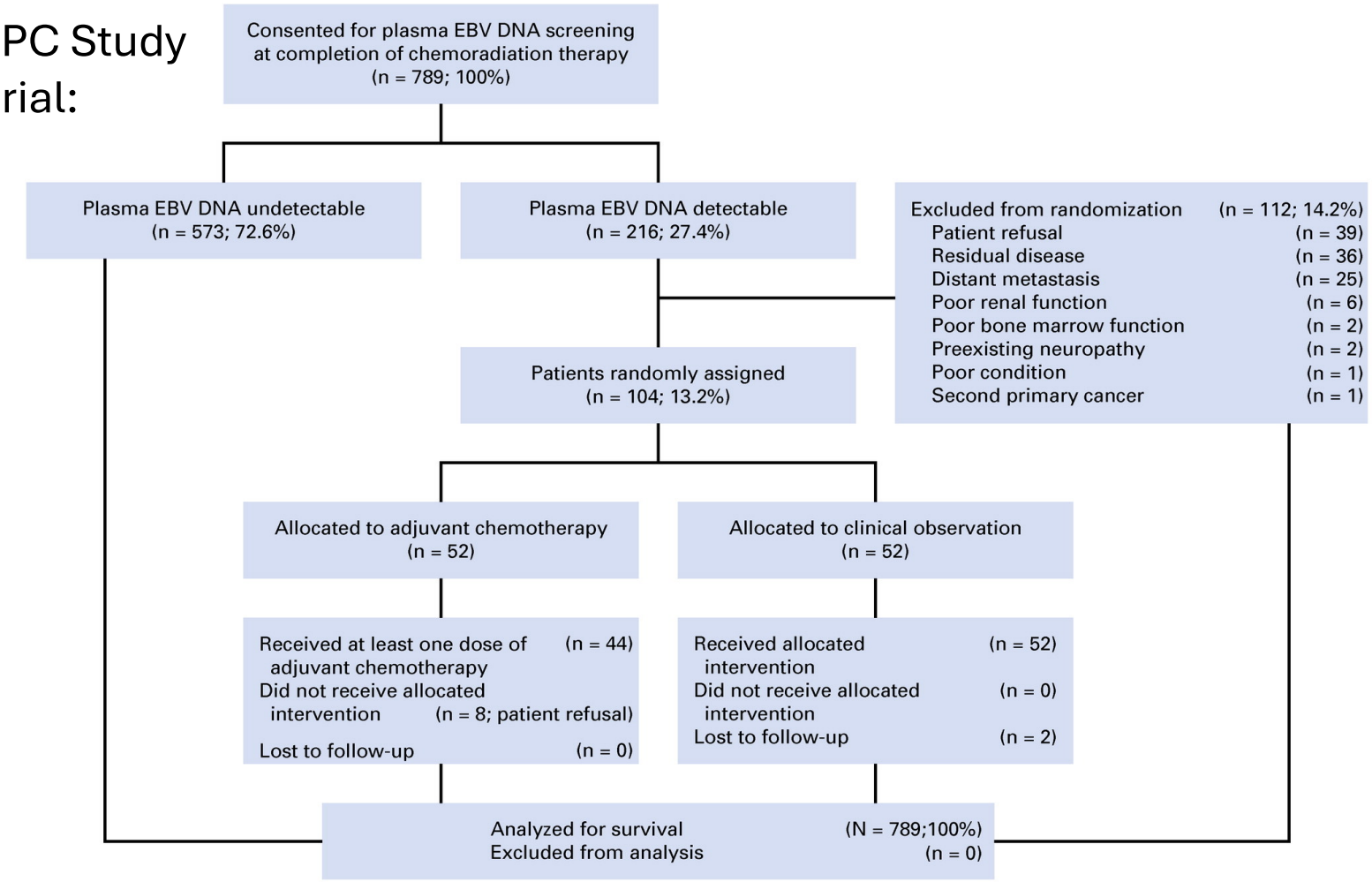


This exploratory analysis of a phase 3 trial should be confirmed in future prospective studies

Zhang Y, et al. J Clin Oncol 2022. 40(22):2420-2425

A cautionary tale: Randomized trial testing utility of post-treatment detectable plasma EBV DNA to escalate adjuvant chemotherapy

Hong Kong NPC Study Group 0502 trial:



Chan ATC et al. J Clin Oncol 2018 doi: 10.1200/JCO.2018.77.7847

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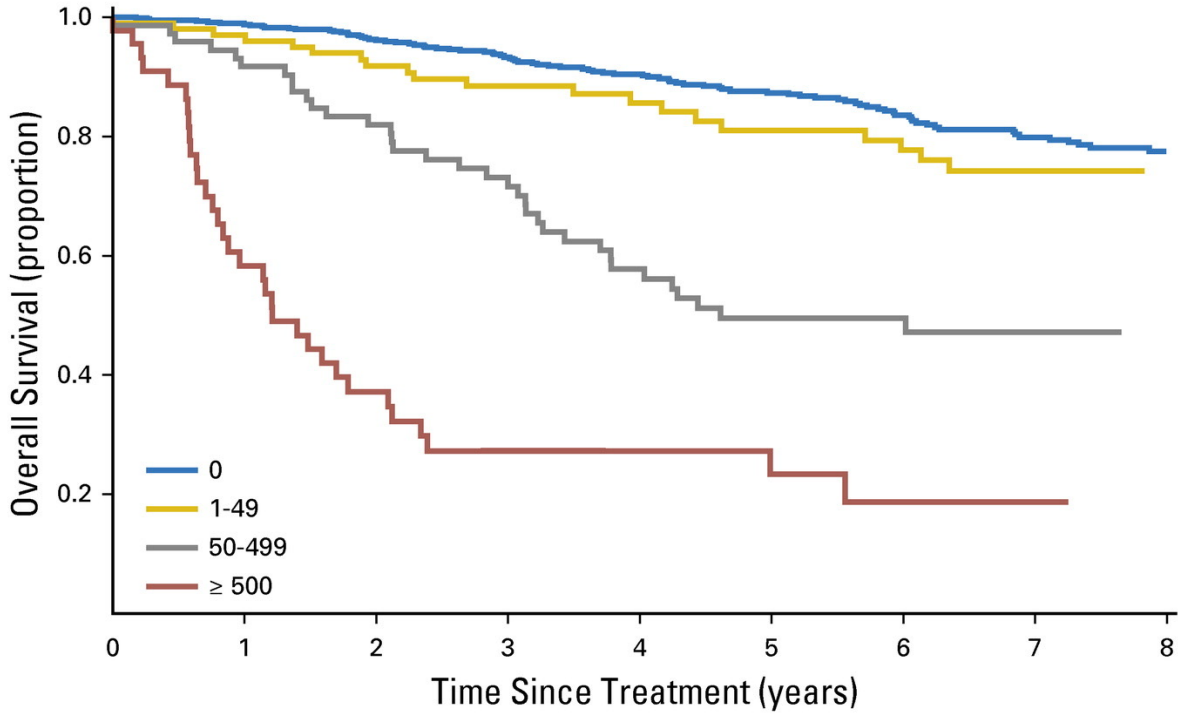
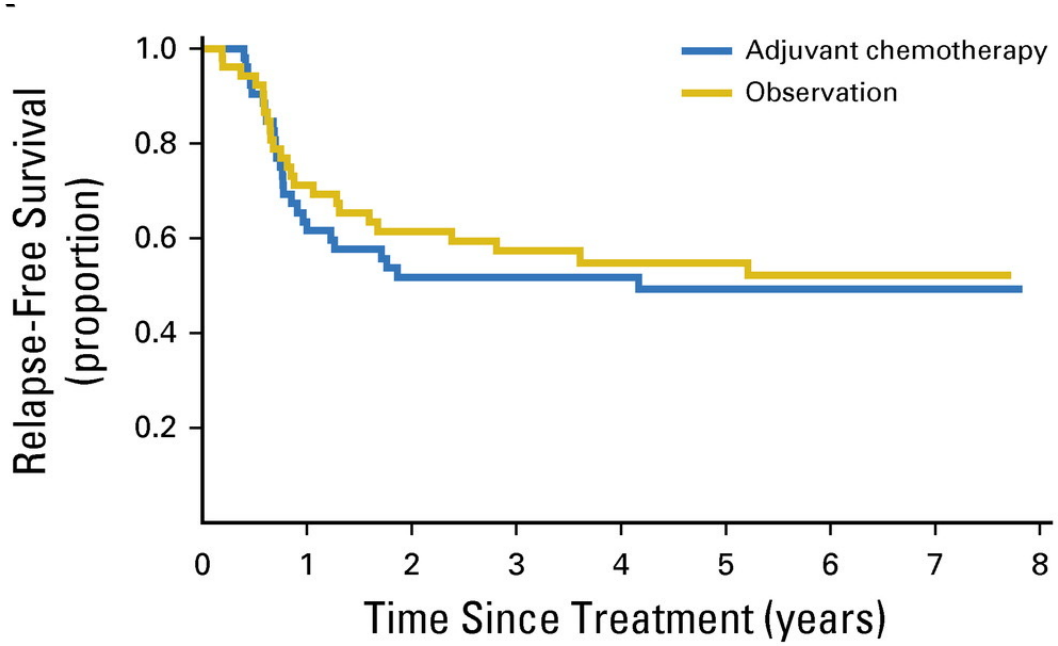


- Validated prognostic value of plasma EBV DNA following chemoRT

Chan ATC et al. J Clin Oncol 2018 doi: 10.1200/JCO.2018.77.7847

A cautionary tale: Randomized trial testing utility of post-treatment detectable plasma EBV DNA to escalate adjuvant chemotherapy



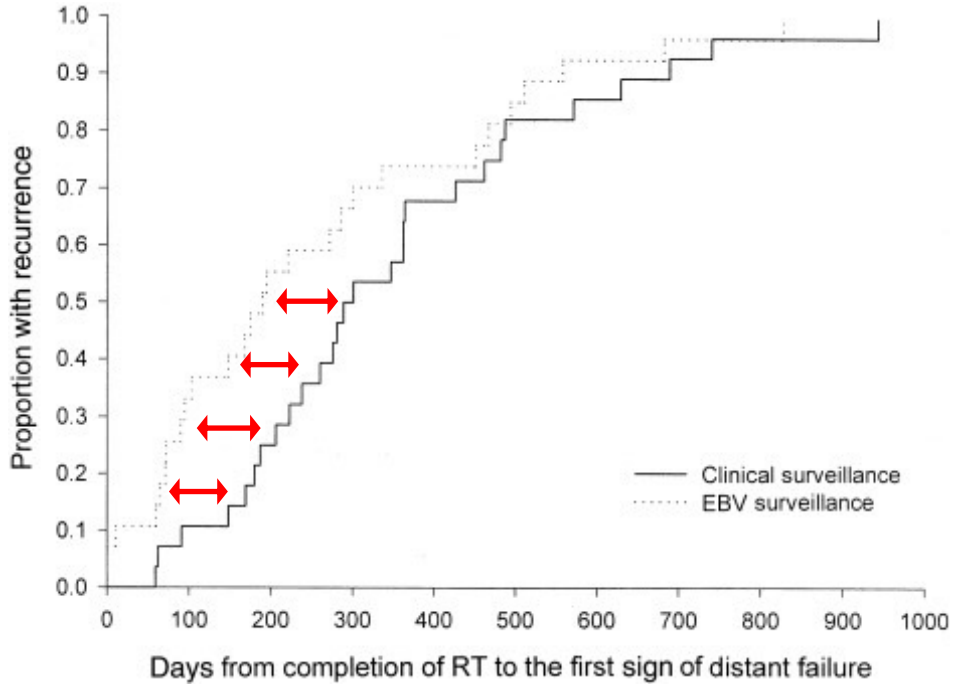
- Validated prognostic value of plasma EBV DNA following chemoRT
- No benefit to adjuvant chemotherapy in MRD+ patients following chemoRT
- Role of resistance to prior agents?
- Need new treatment options for MRD+ patients

NRG-HN001 randomized phase 2 study is current testing alternative adjuvant chemotherapy in MRD+ patients

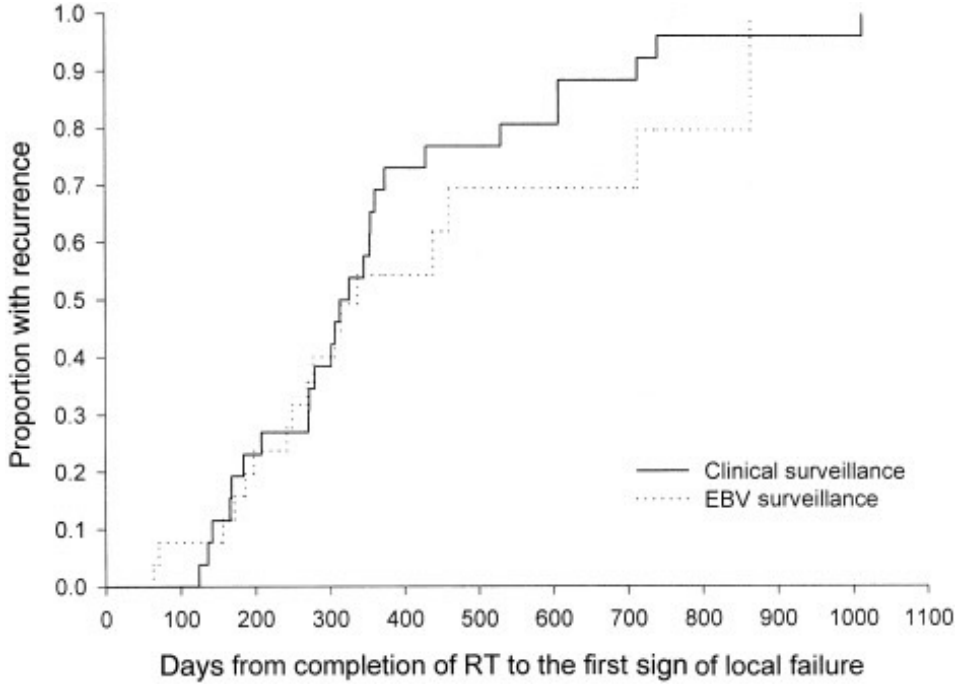
Chan ATC et al. J Clin Oncol 2018 doi: 10.1200/JCO.2018.77.7847

Plasma EBV DNA surveillance detects distant but not locoregional recurrence with an average of ~6 months lead time

Distant Recurrence



Locoregional Recurrence



Hong et al. Cancer; 2004, 100(7):1429-1437

Take Home Messages

- ctDNA is increasingly utilized in clinical practice and trials, particularly for noninvasive genotyping of advanced disease
- Many early-stage cancers and post-treatment recurrences are below detection limits of available assays
- ctDNA testing can enable serially updated prognostication
- ctDNA-based MRD detection has potential clinical utility, but rigorous prospective studies are needed