ctDNA/Liquid Biopsy:

call it what you want

Are you ready for it?

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DISCLOSURES

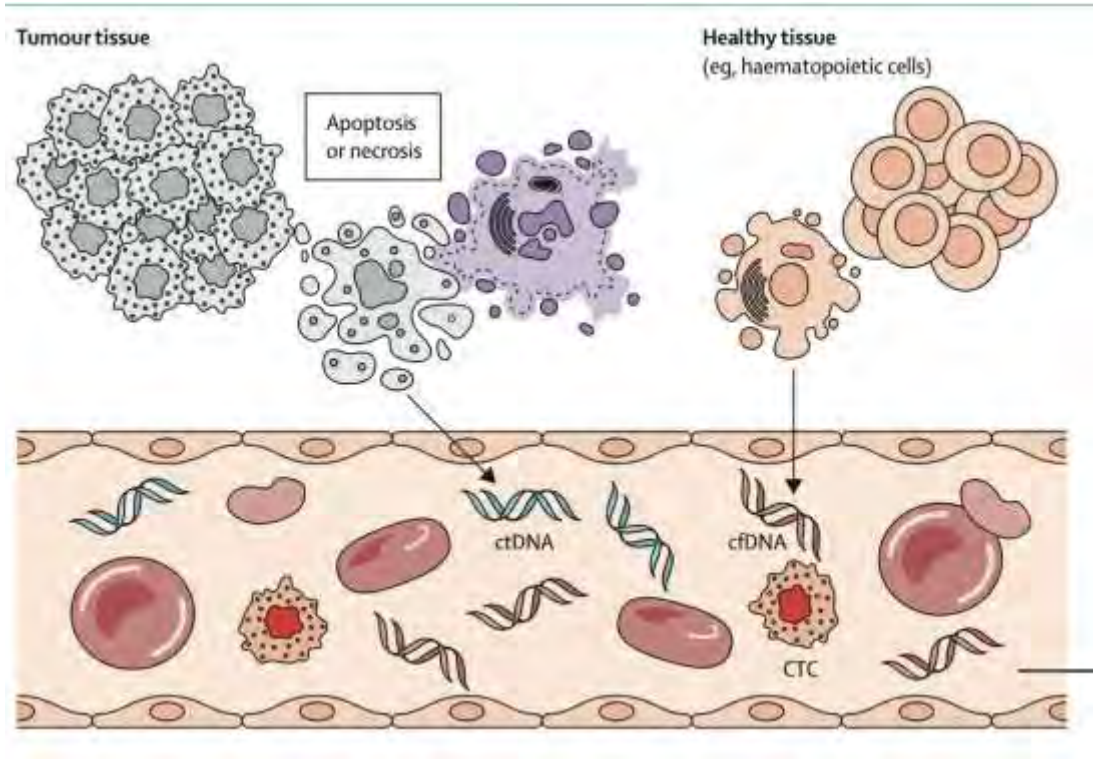
- Pfizer – Consultant
- AstraZeneca – Research support (institution)
- Daichii-Sankyo – Advisory board
- Cardiff Oncology – Research support (institution)

LEARNING OBJECTIVES

- What is ctDNA?
- How can we measure ctDNA
- How can we use ctDNA to help patients with GI cancers?
  - *Will not address ctDNA for early detection/screening today*

What is circulating tumor DNA (ctDNA)?

- Fragments of DNA are released into circulation by diseased and normal cells following cell death
- Most cell-free DNA (cfDNA) originates from hematopoietic cells in a healthy adult
- Fragments of DNA found in the cell-free component of whole blood
  - Released by diseased and normal cells
- ctDNA = fragments of tumor DNA released into circulation
- Half life of cfDNA = 2 hours
  - Half life of CEA: 3-5d, CA19-9: 1-3d



Loft M et al. Lancet Gastroenterol Hepatol, 2023.

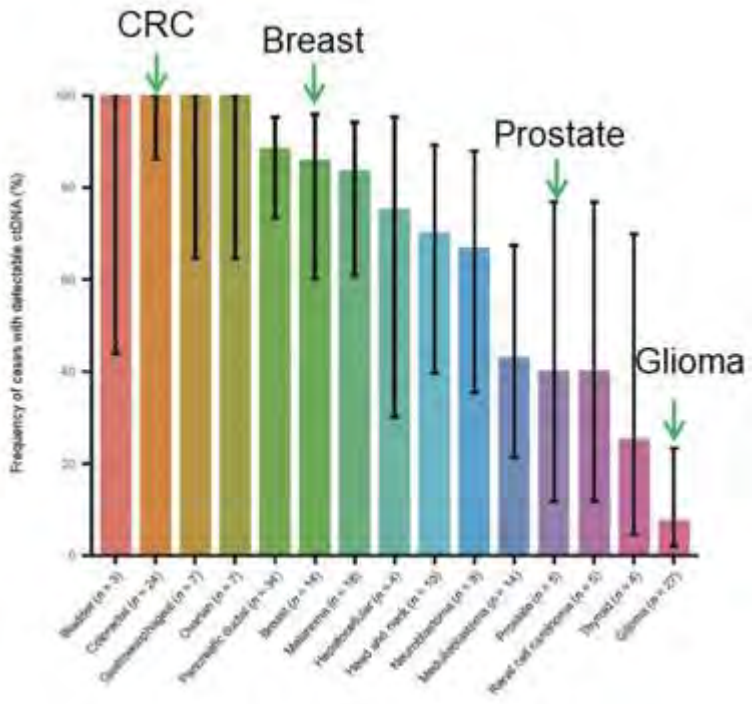
ctDNA: A Needle in the Haystack

- Even in patients with metastatic cancer, only a fraction of cfDNA originates from tumor
- ~1-5 mutant tumor DNA fragments per 10,000 “normal” DNA fragments (bone marrow, skin, GI tract)
- Presents a technical challenge

Gastroenterol Hepatol, 2023.

ctDNA shedding varies by tumor type

Variable detection across tumor types (metastatic)

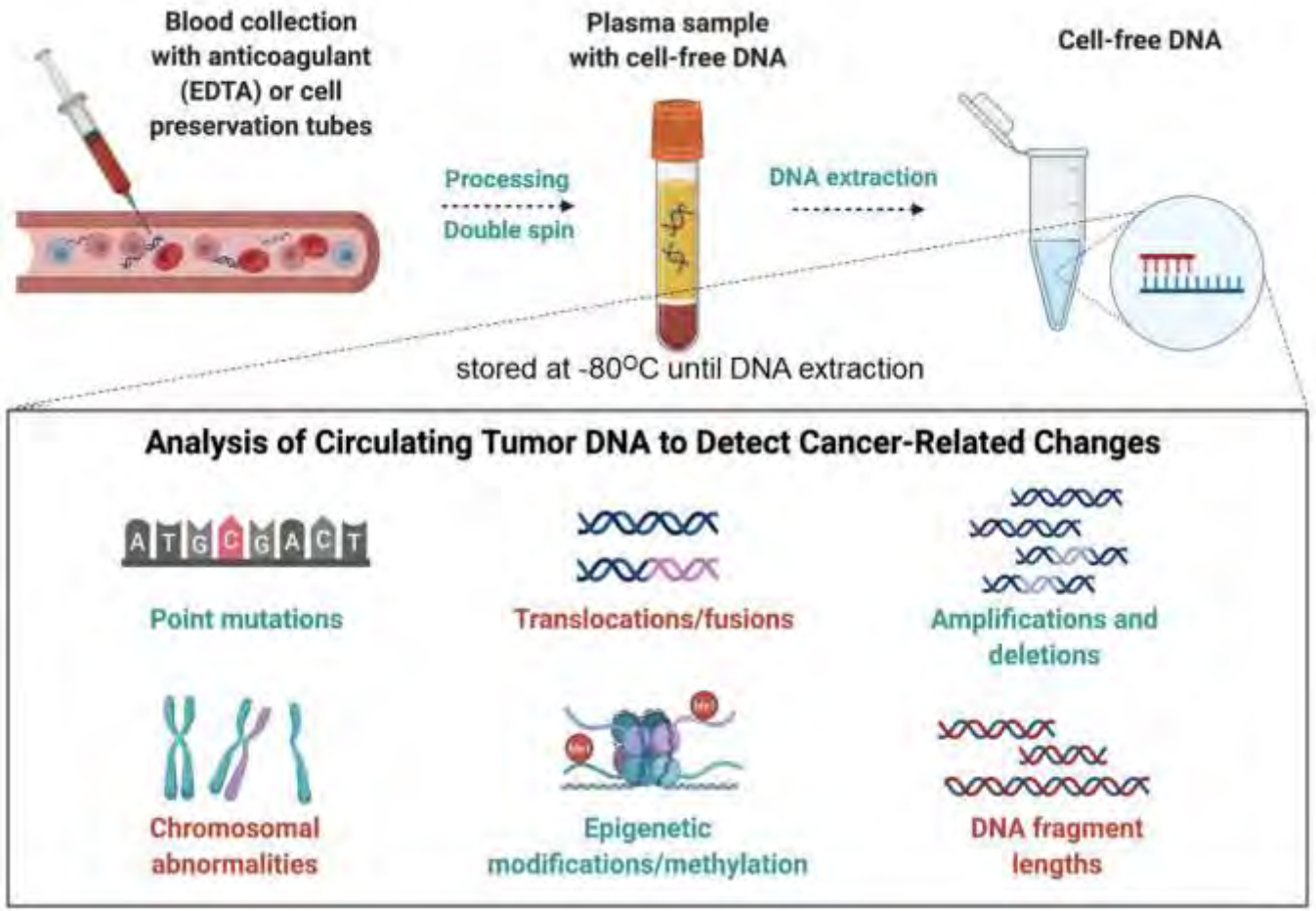


Factors affecting ctDNA concentrations and detection:

- Metastatic disease sites (liver > lung, peritoneum, and bone)*
- Cancer burden
- Disease status (eg, before or after surgery, responding or stable *vs* progressive disease)
- Types of variant (clonal > sub-clonal)*
- Timing of blood sampling in relation to local or systemic treatment
- Non-tumour-related factors (eg, inflammation, infection, and exercise)

Loft M et al. Lancet Gastroenterol Hepatol, 2023.
Bettegowda et al. *Science Translational Medicine.* 2014

Detecting Cancer-Specific Genomic Changes in ctDNA



Loft M et al. Lancet Gastroenterol Hepatol, 2023.

ctDNA Analysis: Tumor-Naïve or Tumor-Informed

	Tumor-Naïve	Tumor-Informed
Method	Detects mutations from plasma using a panel	Identifies mutations in the tumor, uses a personalized assay to detect mutations in plasma
Advantage	Does not require tumor tissue; quick	Greater sensitivity, specificity
Disadvantage	Lower sensitivity Greater risk of false positive	Requires tumor tissue, longer turnaround for first test
Applications	Cancer screening/Early detection Genotyping Detect emergent mutations (resistance)	Minimal residual disease detection Surveillance Response monitoring

Detecting Cancer-Specific Genomic Changes

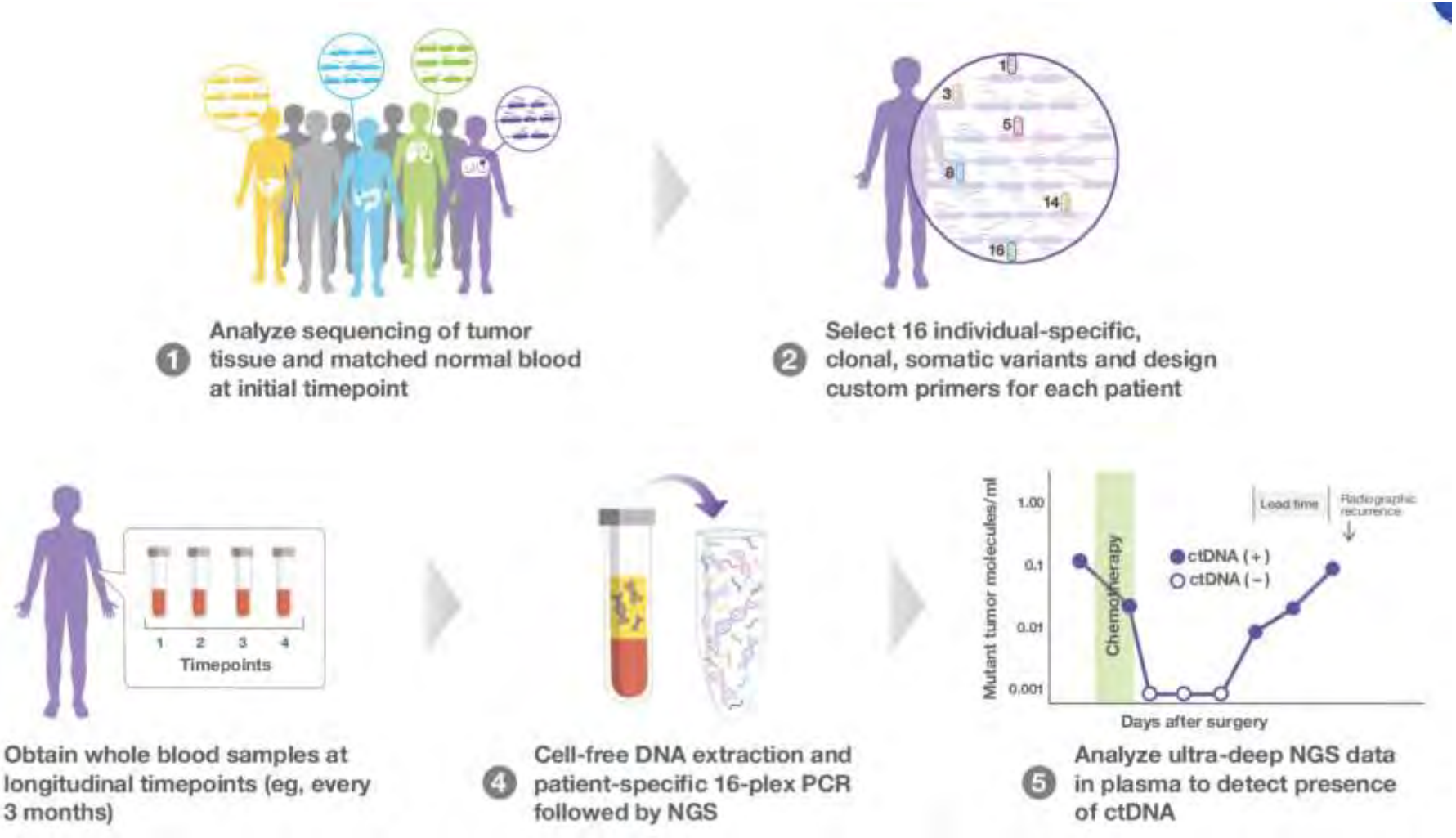
Tumor biopsy

- Invasive
- Serial testing requires repeat procedure
- Findings represent only the tissue sampled

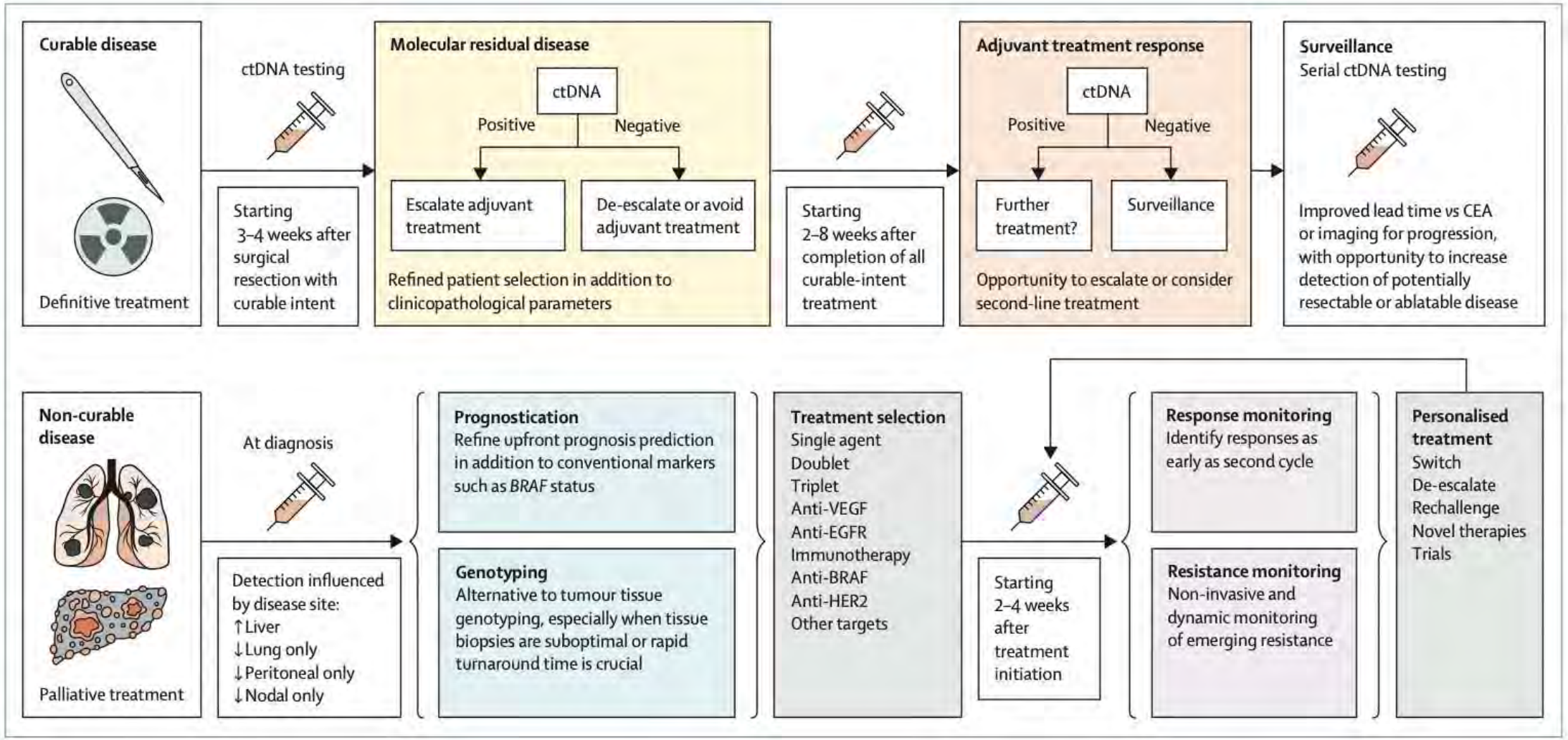
ctDNA

- Ease of serial testing
- Captures genomic information from all sites of disease
  - Tumor heterogeneity
  - Emergent resistance mutations

Sample Workflow: Tumor-Informed Assay (Signatera)

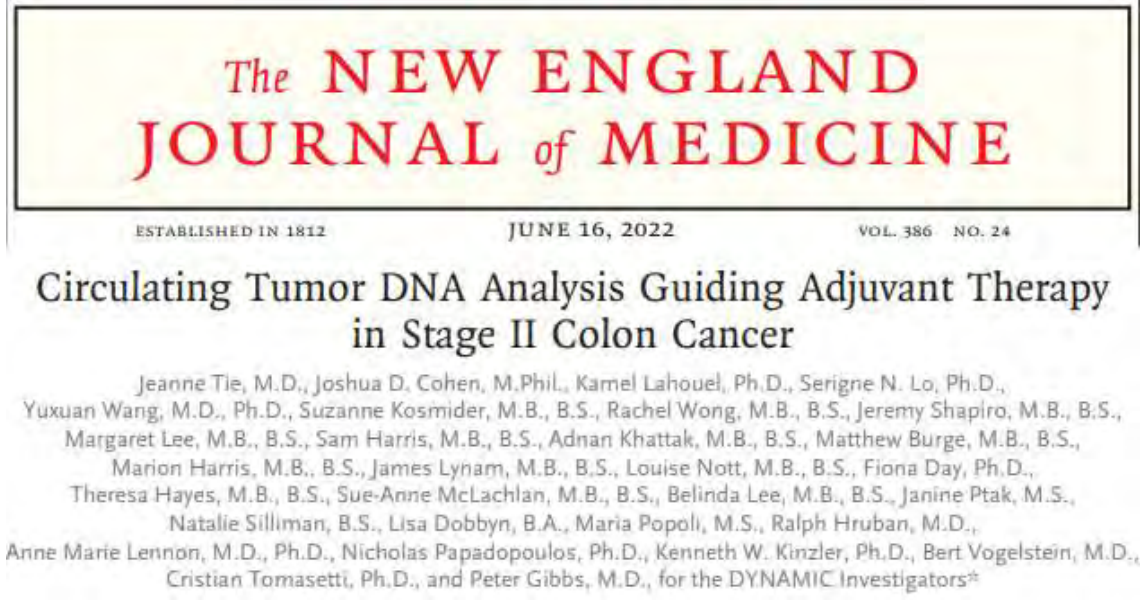


Potential Clinical Applications for ctDNA Example: Colorectal Cancer



Loft M et al. Lancet Gastroenterol Hepatol, 2023.

DYNAMIC trial



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Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer

Jeanne Tie, M.D., Joshua D. Cohen, M.Phil., Kamel Lahouel, Ph.D., Serigne N. Lo, Ph.D., Yuxuan Wang, M.D., Ph.D., Suzanne Kosmider, M.B., B.S., Rachel Wong, M.B., B.S., Jeremy Shapiro, M.B., B.S., Margaret Lee, M.B., B.S., Sam Harris, M.B., B.S., Adnan Khattak, M.B., B.S., Matthew Burge, M.B., B.S., Marion Harris, M.B., B.S., James Lynam, M.B., B.S., Louise Nott, M.B., B.S., Fiona Day, Ph.D., Theresa Hayes, M.B., B.S., Sue-Anne McLachlan, M.B., B.S., Belinda Lee, M.B., B.S., Janine Ptak, M.S., Natalie Silliman, B.S., Lisa Dobbyn, B.A., Maria Popoli, M.S., Ralph Hruban, M.D., Anne Marie Lennon, M.D., Ph.D., Nicholas Papadopoulos, Ph.D., Kenneth W. Kinzler, Ph.D., Bert Vogelstein, M.D., Cristian Tomasetti, Ph.D., and Peter Gibbs, M.D., for the DYNAMIC Investigators*

- Across virtually all solid tumors, detection of ctDNA following definitive surgery predicts a very high risk of recurrence (>80%) without further treatment.
  - ?Benefit of adjuvant therapy
- DYNAMIC study
  - Randomized Ph2 study designed to test a ctDNA guided adjuvant strategy

DYNAMIC Study Design

ACTRN12615000381583

Stage II Colon Cancer

- R0 resection
- ECOG 0 – 2
- Staging CT within 8 weeks
- Provision of adequate tumor tissue within 4 weeks post-op
- No synchronous colorectal cancer

Plasma Collections
Week 4 + 7 post-op

R
2:1

ctDNA-Guided Management

- **ctDNA-Positive → Adjuvant Chemo**
  (oxaliplatin-based or single agent FP)
- **ctDNA-Negative → Observation**

ctDNA-Positive = Positive result at week 4 and/or 7

Standard Management

Adjuvant treatment decisions based on conventional clinico-pathologic criteria

Endpoints

Primary
- RFS rate at 2 years

Key Secondary
- Proportion receiving adjuvant chemo

Secondary
- RFS by ctDNA status for ctDNA-guided arm
- TTR
- OS

Stratification Factors
- T stage (T3 vs T4)
- Type of participating center (metropolitan vs regional)

Surveillance:
- CEA → 3-monthly for 24M, then 6-monthly for 36M
- CT C/A/P → 6-monthly for 24M, then at 36M

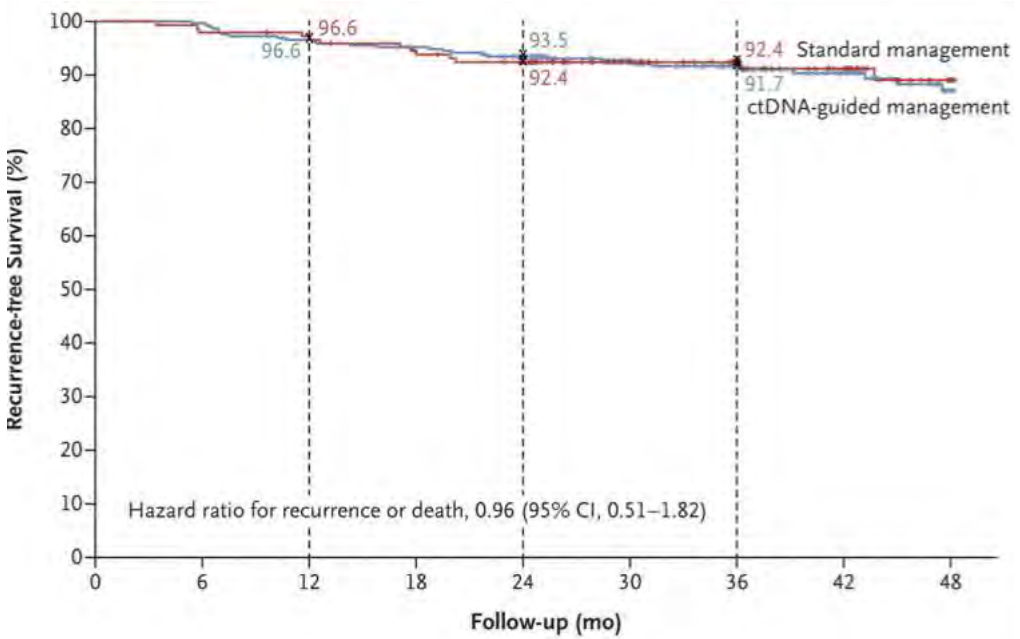
Tie J et al. ASCO Annual Meeting, 2022.

DYNAMIC trial

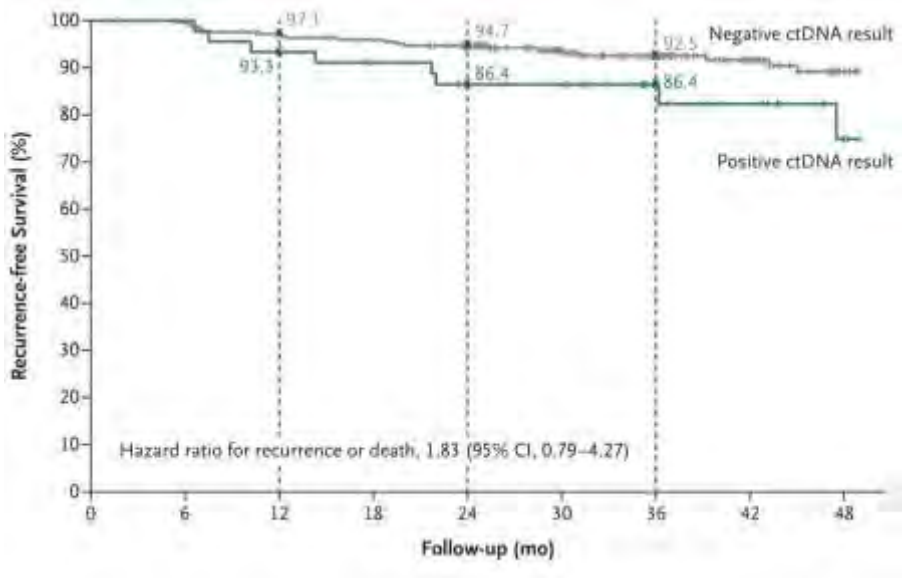
- Less patients in the ctDNA guided group received adjuvant therapy
  - 15% vs. 28% (RR 1.82, 95% CI 1.25-2.65)
- Noninferiority of ctDNA guided management was confirmed in the ITT population
- 3y DFS: 7% ctDNA- vs. 14% ctDNA+
  - HR 2.45; 95% CI 1.00-5.99

- **CtDNA negative patients have a low recurrence risk without adjuvant chemotherapy.**
  - **3y RFS 92.5%; 97% in clinical low risk**

Recurrence-free survival by treatment arm



Recurrence-free survival by ctDNA status



Tie J et al. NEJM, 2022.

NCT 04068103

RECRUITING

Circulating Tumor DNA Testing in Predicting Treatment for Patients With Stage IIA Colon Cancer After Surgery

Principal Investigator: Van K. Morris, MD – NRG Oncology

Phase II/III

Resected Low-Risk Stage IIA Colon Adenocarcinoma Suitable for Surveillance

1,408 Adults

Liquid Biopsy

ARM 1

Active surveillance

Blood stored and tested for ctDNA later

ARM 2

Blood tested for ctDNA at baseline

ctDNA detected

ctDNA not detected

Group 1

Combination chemotherapy

Group 2

Active surveillance

Designed to test whether ctDNA guided adjuvant approach improves outcomes in stage IIA colon cancer

OUTCOMES

PRIMARY

Clearance of ctDNA in ctDNA positive patients in Arms 1and 2 after 6 months (Phase II)

Recurrence-free survival in ctDNA positive patients in Arms 1and 2 for up to 3 years (Phase III)

SECONDARY

Recurrence-free survival for all included in study

Overall survival

Compliance with chemotherapy and/or active surveillance

Incidence of ctDNA in blood after resection

Study halted due to greater number of anticipated false positive ctDNA results

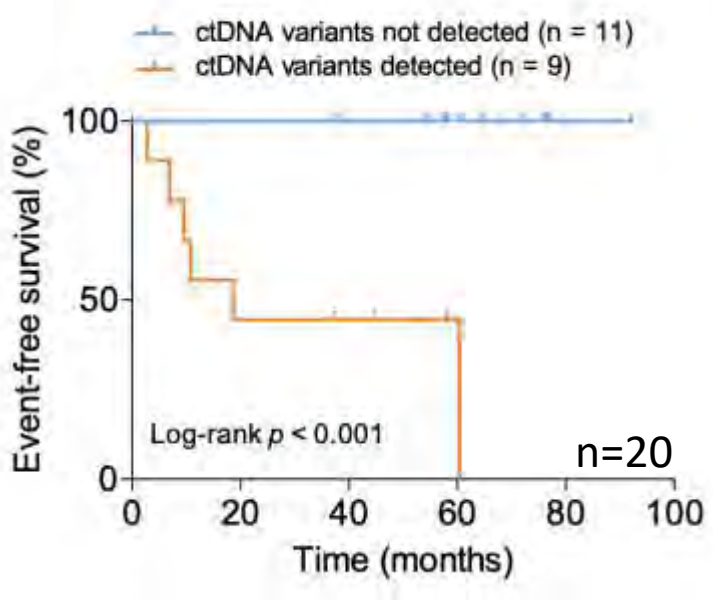
Morris et al. *Ann Surg Oncol.*
Ongoing Clinical Trials in Surgical Oncology Series

ANNALS OF SURGICAL ONCOLOGY

Following gastrectomy ctDNA is associated with recurrence risk

CRITICS: Post preoperative chemo and gastrectomy

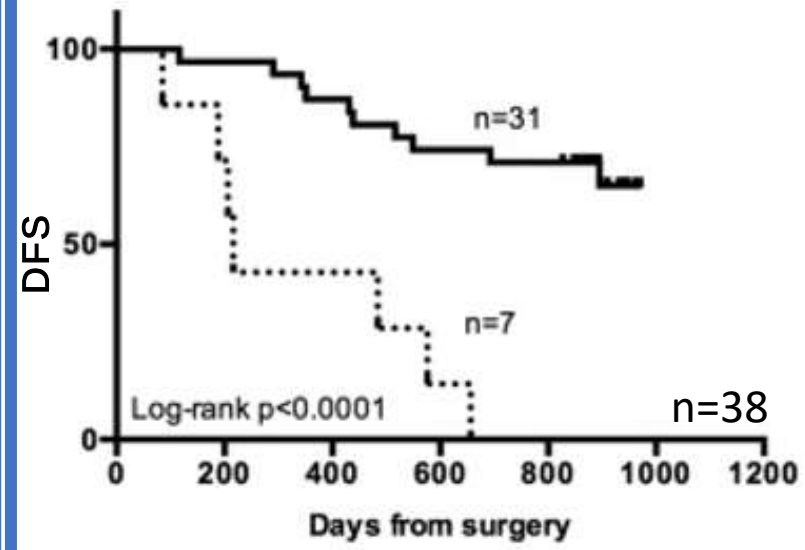
- Post-hoc subset analysis
- Postoperative ctDNA is associated with disease recurrence HR=21.8, p<0.001
- Median DFS 18.7mo vs. median not reached



Leal A et al. Nature Communications, 2020.

Post-gastrectomy (up-front):

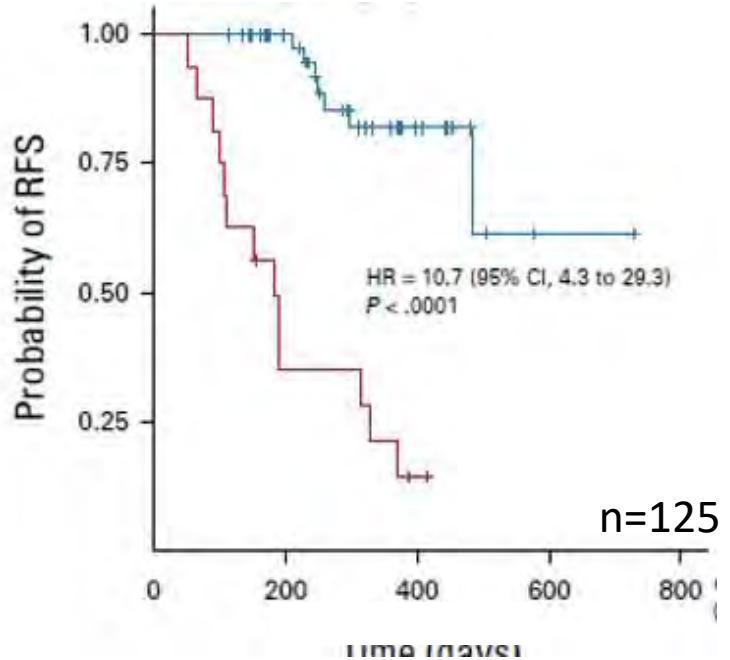
- Prospective cohort study
- Post-operative ctDNA is associated with disease recurrence (HR=6.56, p<0.0001)



Yang J et al. Cell Death Dis, 2020.

Esophagogastric cancer, post-resection (variable preoperative tx)

- Retrospective, real-world analysis
- Signatera assay
- Post-operative ctDNA is associated with recurrence-free survival (HR 10.7, p<0.0001)

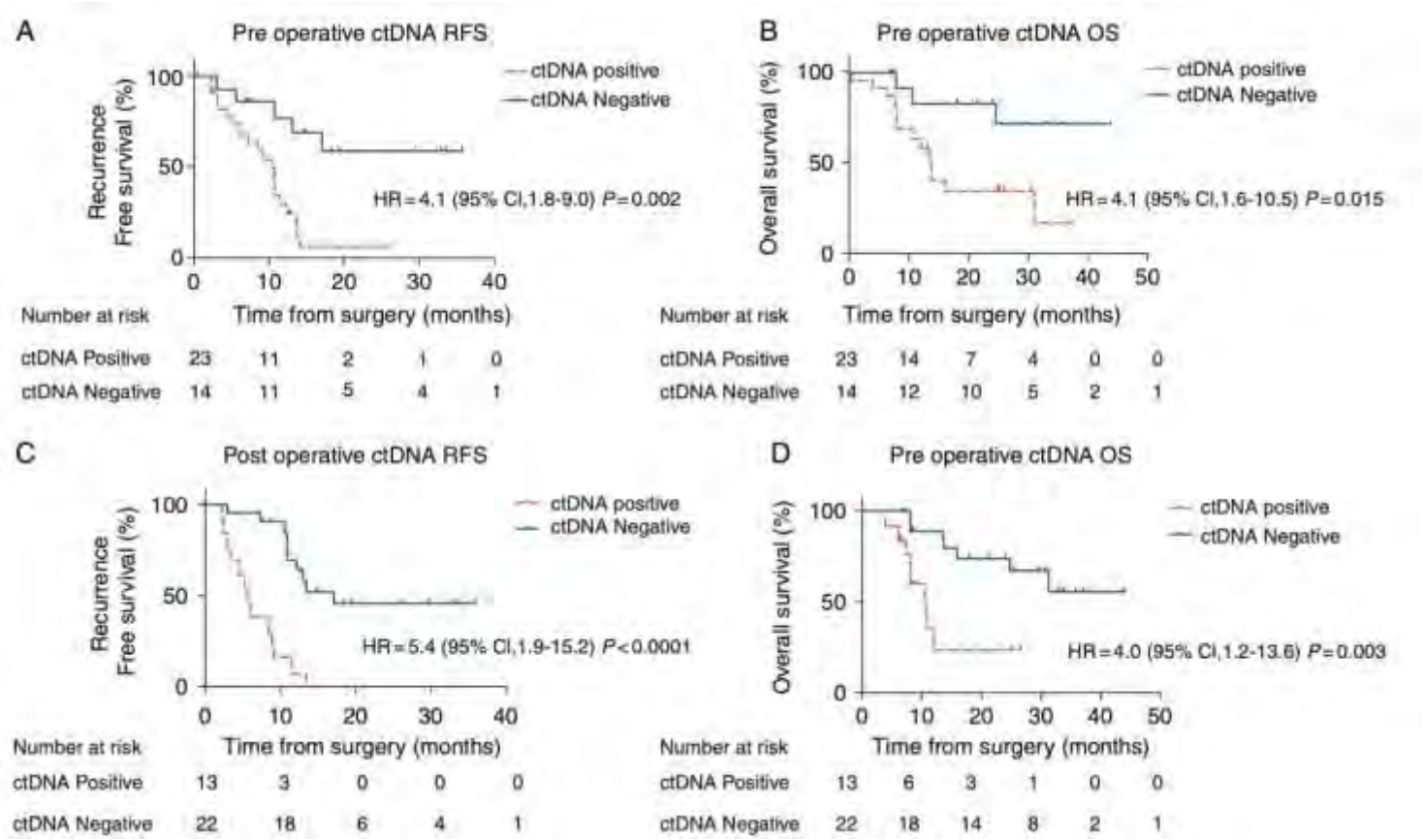


Huffman BW et al. JCO Precision Oncology, 2022.

Prior to and following resection of pancreatic adenocarcinoma, detection of ctDNA is associated with recurrence, survival

- Assay: *tumor informed, detection of mutant KRAS*
  - *KRAS* mutation detected in 38/42 resected tumors
  - Same *KRAS* mutation detected in plasma in 62% (23/37) pre-operative samples and 37% (13/35) post-operative cases

ctDNA detection in the pre- and post- operative setting was associated with shorter recurrence-free survival and overall survival



Lee B et al. *Annals of Oncology*, 2019.

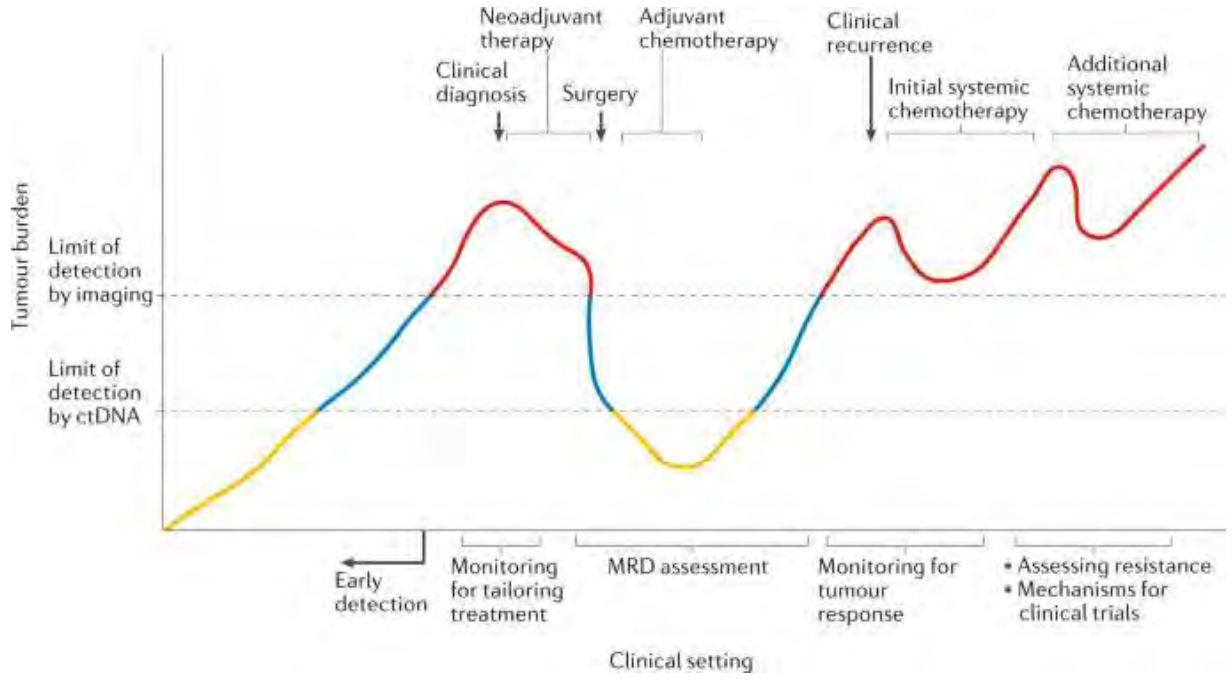
ctDNA has significant potential as a surveillance tool

Across multiple disease types, ctDNA recurrence (molecular recurrence) precedes radiographic recurrence

- Lead time varies

Acting on ctDNA by initiating systemic therapy has not been rigorously tested in prospective, randomized trials

- Risk of more time on treatment without meaningful benefit



Dasari A et al. Nature Reviews Clinical Oncology, 2020.

Potential Pitfalls with ctDNA for Genomic Analysis

False negatives – ctDNA does not detect a variant present in the tumor

- **Insufficient ctDNA present in the sample – active therapy/tumor response**
  - **Optimal timing: diagnosis, disease progression**
- **Insufficient assay sensitivity**
- **Low shed due to tumor type, disease site (ie. Peritoneum, bone)**

False positives – ctDNA detects a variant not present in the tumor

- Sequencing error
- CHIP (clonal hematopoiesis of indeterminate potential)
  - Increases with prior cancer treatment, smoking, age
  - Minimize with paired WBC DNA (buffy coat) sequencing or tumor-informed approach

Potential Clinical Applications for ctDNA

Early detection/screening

Localized disease

- Detection of minimal residual disease after definitive therapy
- Selection of patients for watch & wait approach
- Response to adjuvant therapy
- Genotyping & treatment selection
- Surveillance

Advanced disease

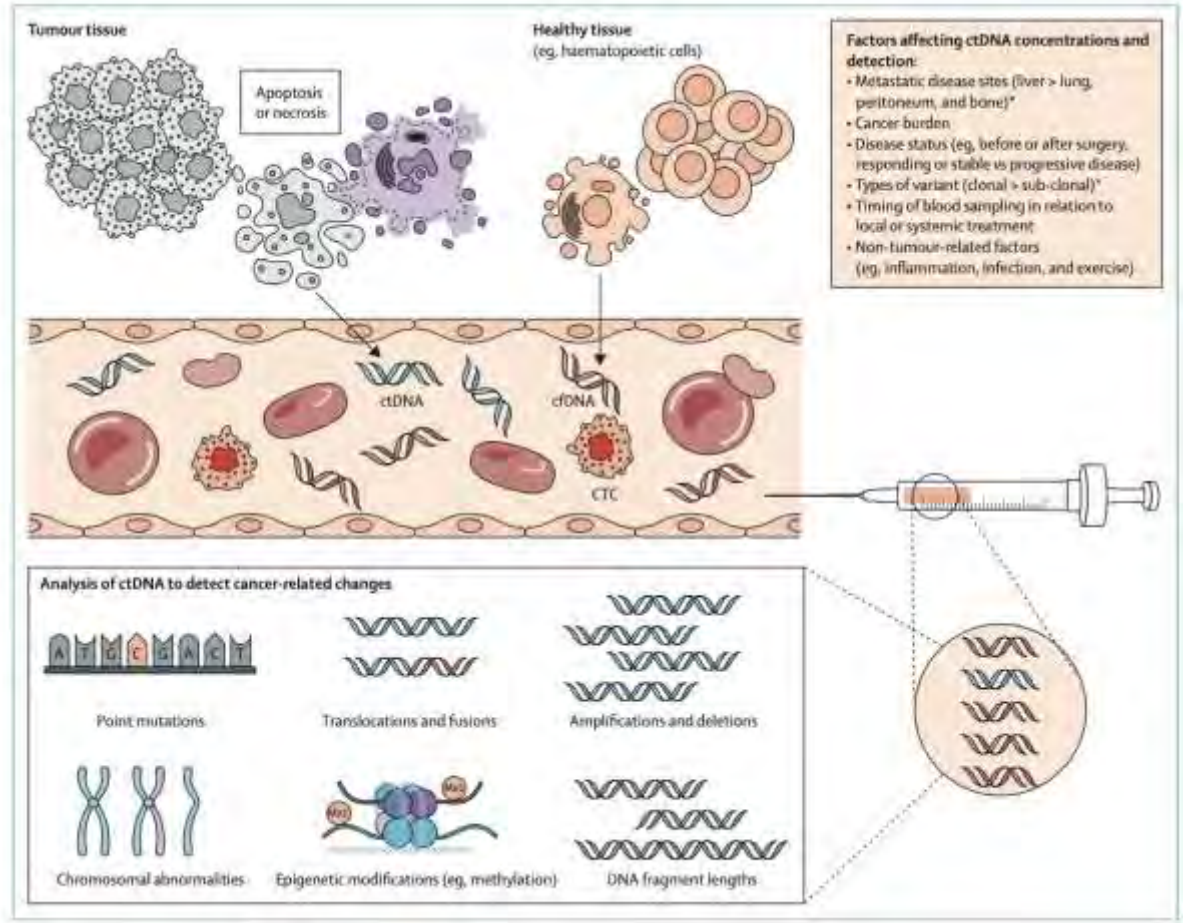
- Prognostication in metastatic disease
- Genotyping & treatment selection
- Response monitoring
- Resistance monitoring
- Guide intensification, de-escalation, rechallenge

Enthusiasm for using ctDNA to individualize a patient's treatment strategy is ubiquitous. **Ongoing studies will address whether ctDNA-guided approaches benefit patients in various treatment paradigms.**

Clinical application of circulating tumour DNA in colorectal cancer

Matthew Loft, Yat Hang To, Peter Gibbs, Jeanne Tie

Liquid biopsies that detect circulating tumour DNA (ctDNA) have the potential to revolutionise the personalised management of colorectal cancer. For patients with early-stage disease, emerging clinical applications include the assessment of molecular residual disease after surgery, the monitoring of adjuvant chemotherapy efficacy, and early detection of recurrence during surveillance. In the advanced disease setting, data highlight the potential of ctDNA levels as a prognostic marker and as an early indicator of treatment response. ctDNA assessment can complement standard tissue-based testing for molecular characterisation, with the added ability to monitor emerging mutations under the selective pressure of targeted therapy. Here we provide an overview of the evidence supporting the use of ctDNA in colorectal cancer, the studies underway to address some of the outstanding questions, and the barriers to widespread clinical uptake.



Review article: Lancet Gastroenterology and Hepatology – 2023

ctDNA: Ready for Prime Time?
Not universally…

- ctDNA holds significant potential to change treatment paradigms in GI oncology.

Entering the ctDNA era.

- Further evidence is needed prove ctDNA-guided strategies help patients. Using ctDNA to guide systemic therapy in the absence of radiographic recurrence lead to MORE treatment without clinical benefit.

Play stupid games, win stupid prizes.

- The role of ctDNA-guided strategies will be further defined by multiple ongoing randomized clinical trials.

Let the games begin.