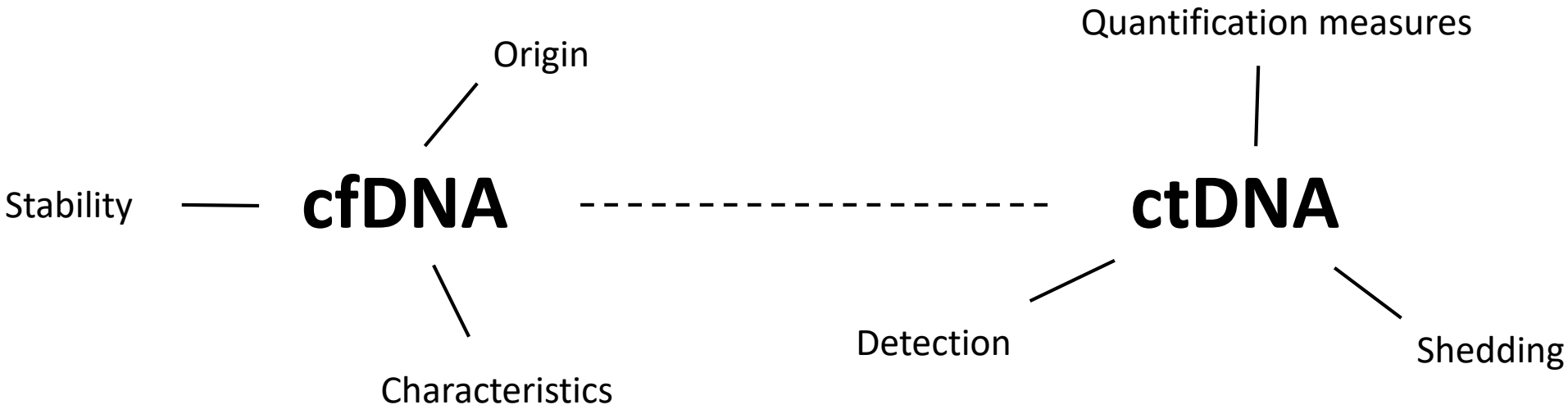
The biology of circulating cell free- and tumor DNA

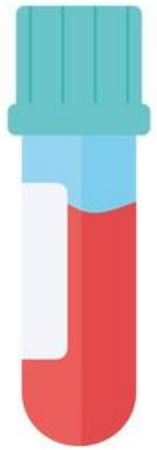
March 28th 2022, Aarhus, Denmark

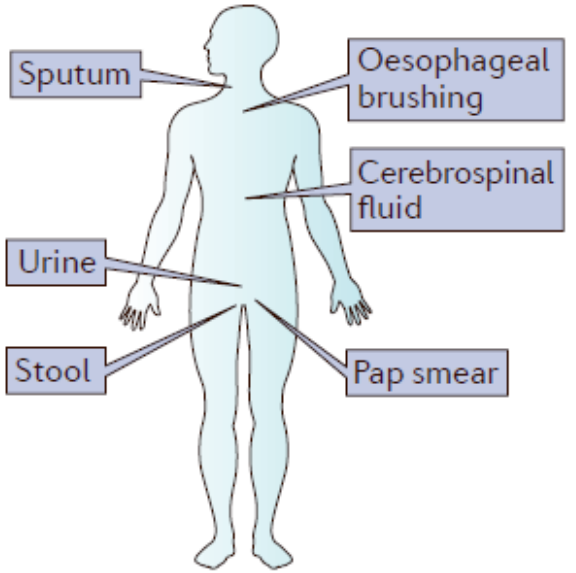
Emil Christensen, PhD

Agenda


Origin
Quantification measures
Stability
cfDNA
ctDNA
Characteristics
Detection
Shedding

Where do we find cfDNA?

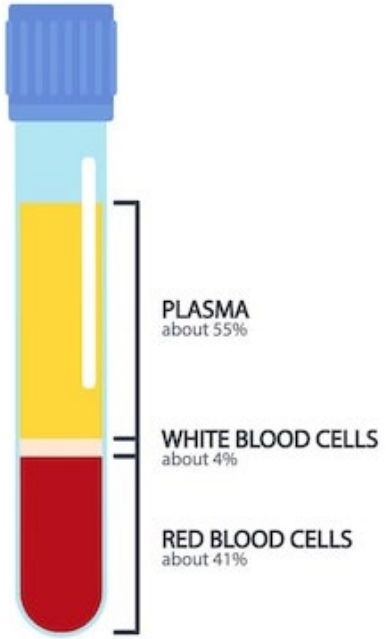




Wan et al., Nature Reviews Cancer, 2017

A new and promising field

- cfDNA was discovered as fragmented DNA in the non-celluar compartment of a blood sample by Mandel and Métais in 1948[1]
- In 1977, it was discovered the level of cfDNA was elevated in cancer patients[2]
- ctDNA, cfDNA fragments originating from tumor cells, was identified in 1989[3]

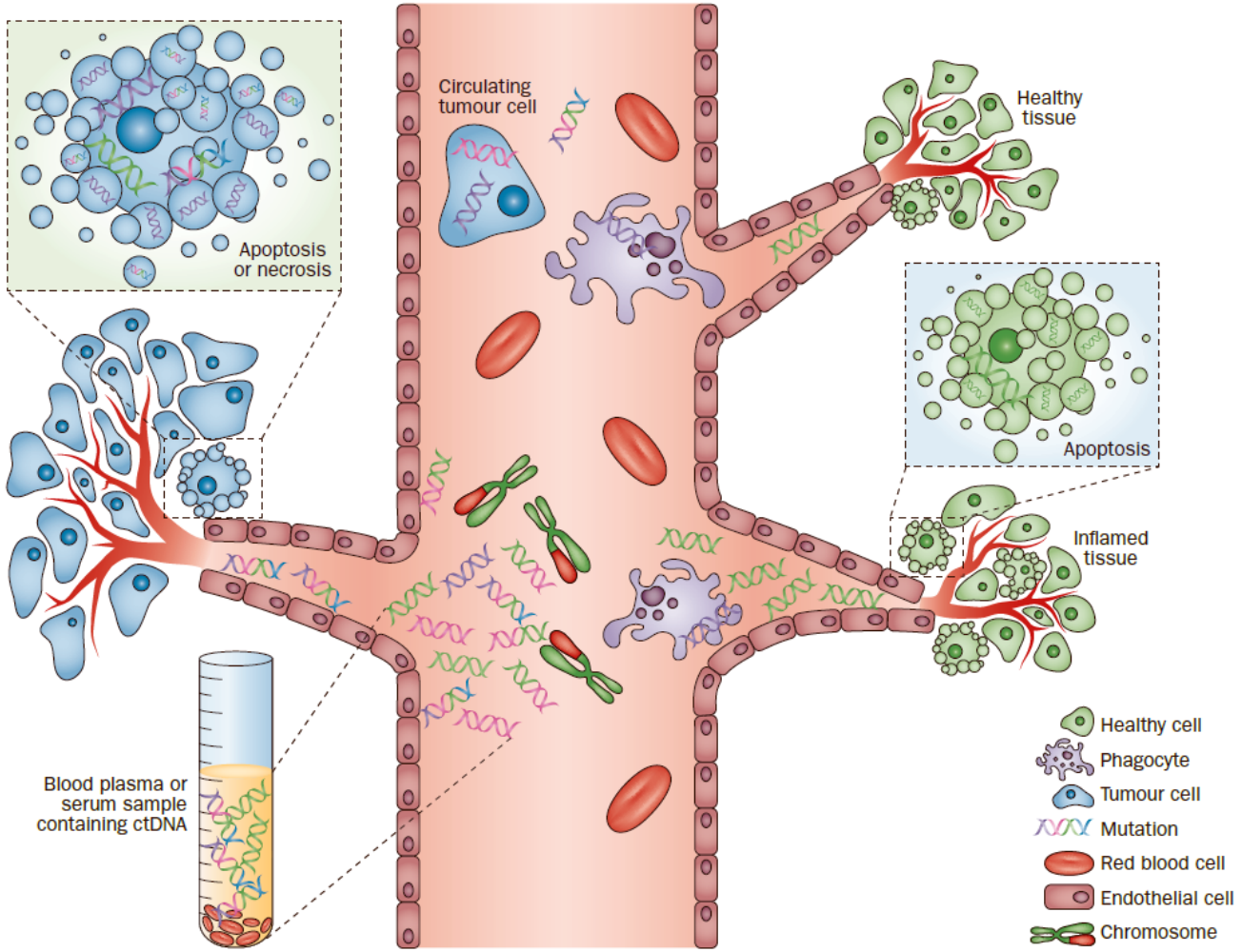


A slightly old and promising field… Why has it been so long in the making?

1 Thierry et al., Cancer Metastasis Reviews, 2016
2 Leon et al., Cancer Research, 1977
3 Stroun et al., Oncology, 1989

How did it get there?

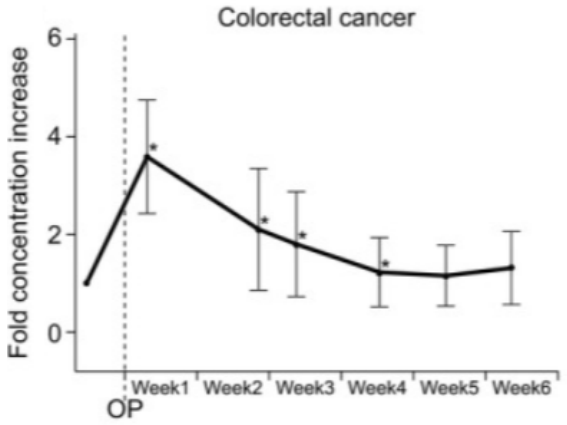
- Cells release cfDNA
  - Apoptosis
  - Necrosis
  - Active secretion

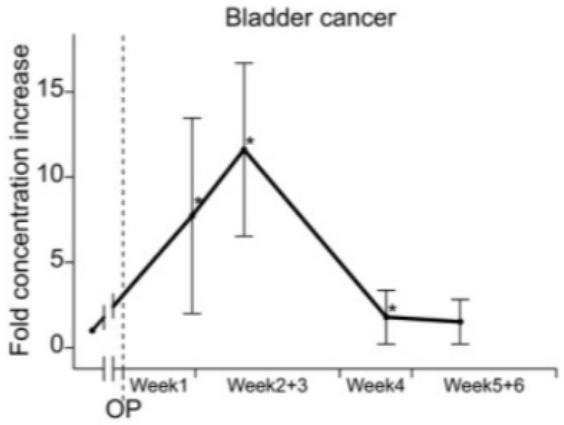


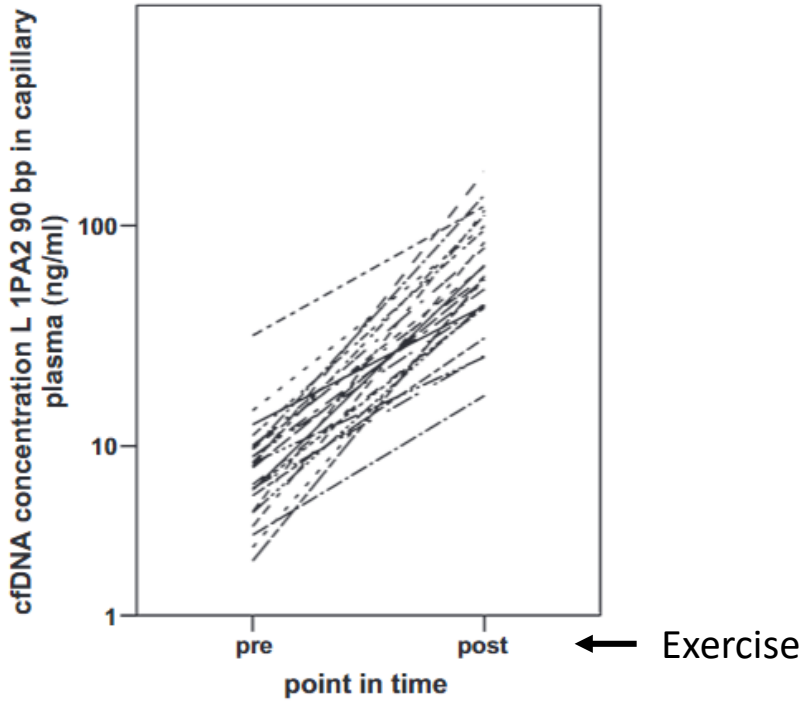
Crowley et al., Nature Reviews Clinical Oncology, 2013
Corcoran et al., New England Journal of Medicine, 2018

What affects the release of cfDNA?

- Cancer
- Exercise[1]
- Inflammation[2]
- Surgery[3]



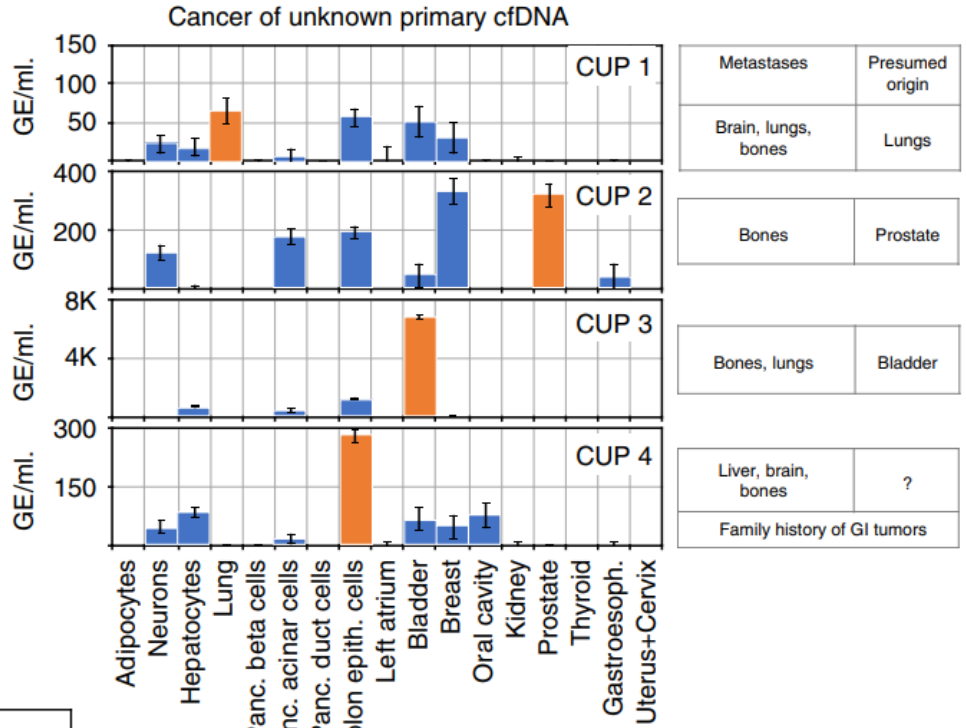


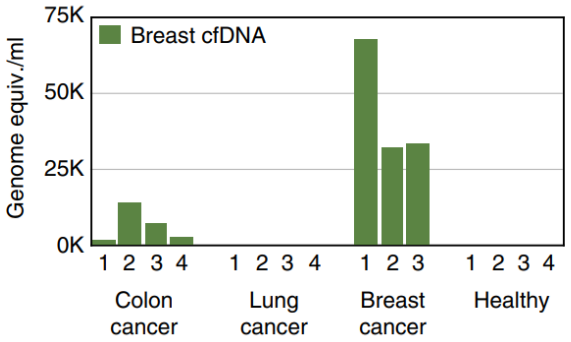


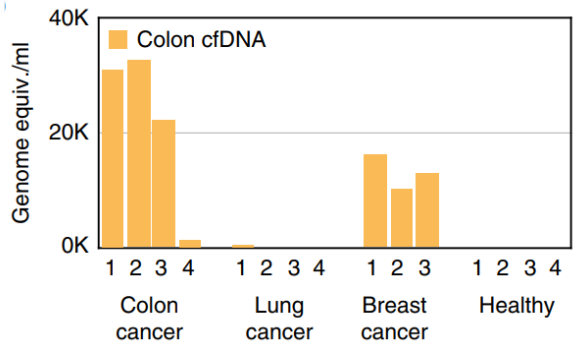
1 Breitbach et al., Journal of Applied Physiology, 2014
2 Frank et al., Biological Research for Nursing, 2016
3 Henriksen et al., Molecular Oncology, 2020

cfDNA contribution from various tissues

- Tissue-specific methylation patterns can estimate the tissue contribution of cfDNA in various settings
- Baseline cfDNA composition (healthy donors)
  - 55% white blood cells
  - 30% erythrocyte progenitors
  - 10% vascular endothelial cells
- Patients with sepsis
  - Vast majority from immune cells
- Patients with cancer



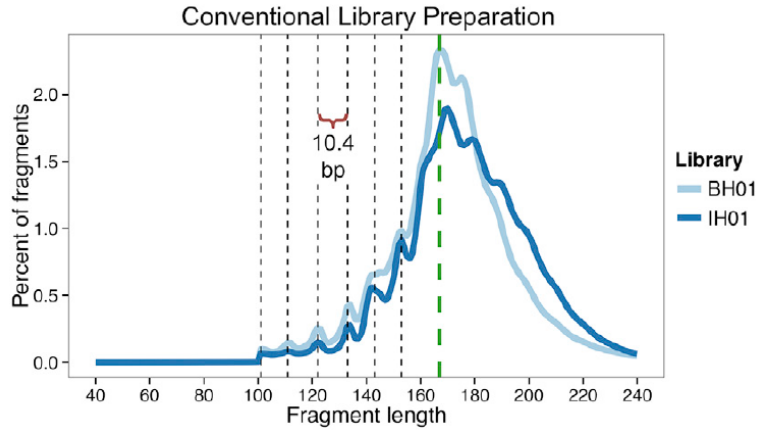


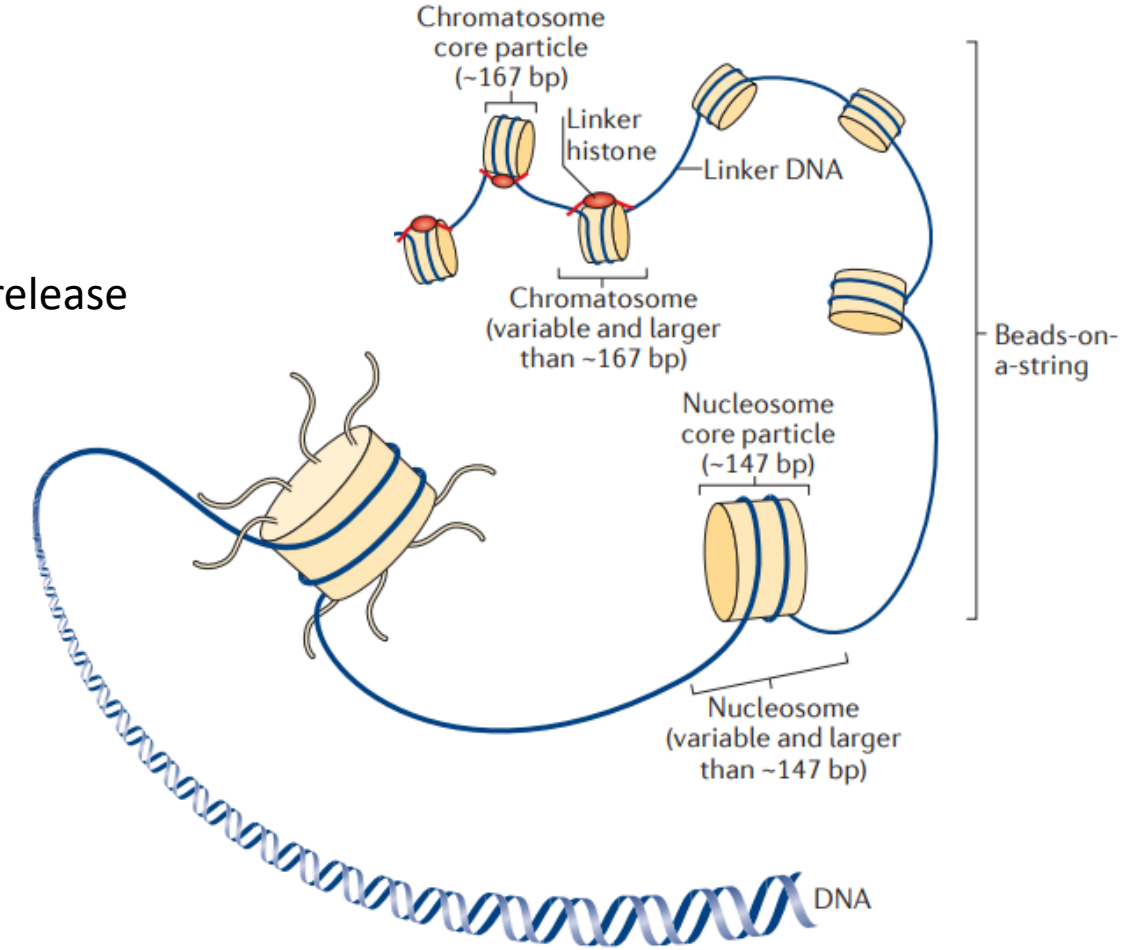


Moss et al., Nature Communications, 2018

What characterizes cfDNA?

- Fragments of approx. 167 bp[1]
  - Chromatosome
  - Protected from nucleases by association with proteins
  - Aligns with apoptosis being the main driver of cfDNA release





1 Snyder et al., Cell, 2016
2 Fyodorov et al., Nature Reviews Molecular Cell Biology, 2018

What characterizes cfDNA?

- Larger fragments have been observed[1]
  - Necrotic release
- Implications for analyses
  - Highly fragmented
  - Low yield compared to tissue extractions

1 Jarh et al., Cancer Research, 2001

What is the stability of cfDNA?

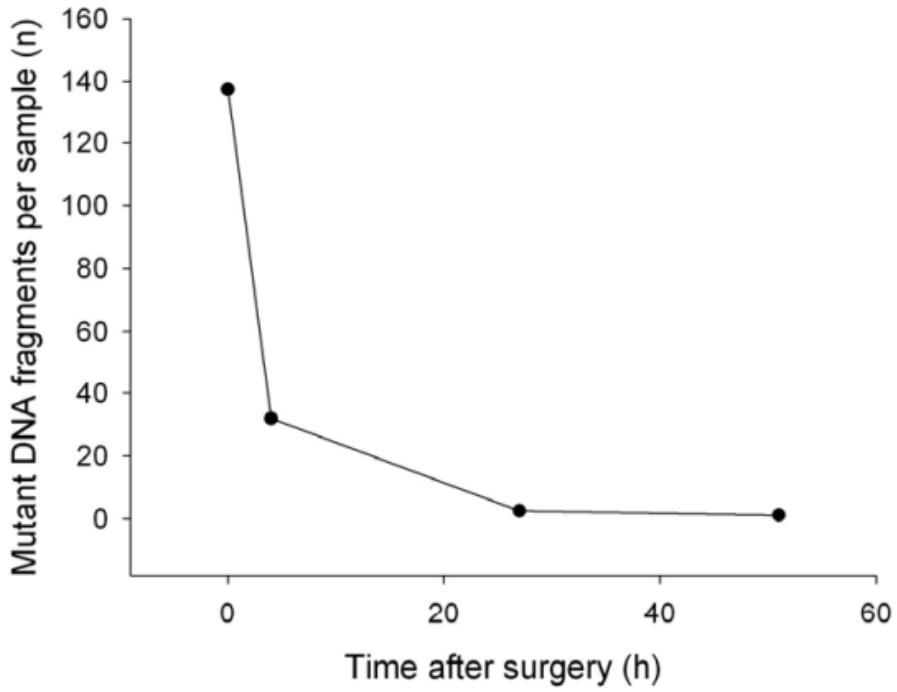
cfDNA degradation > < cfDNA release

- Degradation
  - Nucleases[1]
  - Renal clearance into the urine
    - Female, male blood transfusion[2]
    - Tumor derived DNA detected in urine from patients with lung cancer[3]
  - Uptake by liver and spleen -> degradation by macrophages[4]

1 Lo, American Journal of Human Genetics, 1999
2 Botezatu et al., Clinical Chemistry, 2000
3 Reckamp et al., Journal of Thoracic Oncology, 2016
4 Diehl et al., PNAS, 2005
5 Yu et al., Clinical Chemistry, 2013

What is the stability of cfDNA?

- Half-life of cfDNA
  - Fetal DNA in pregnant women
    - Rapid phase of <1 hour and a slow phase of approx. 13 hours[5]
  - 1-2 hours in cancer patients[6,7]



Single patient
Fit a model -> 114 minutes

6 Corcoran et al., New England Journal of Medicine, 2018
7 Diehl et al., Nature Medicine, 2008

ctDNA

- ctDNA = cfDNA fragments originating from tumor cells
- Foundation of ctDNA as a biomarker
  - All cells release DNA
  - Half-life of a few hours

What can we learn about the tumor from it?

How do we detect ctDNA?

- How do we distinguish ctDNA fragments?
  - Tumor-specific genetic or epigenetic alterations, estimation of copy number alterations

ACGTACGTACGTACGT

ACGTACGTATGTACGT

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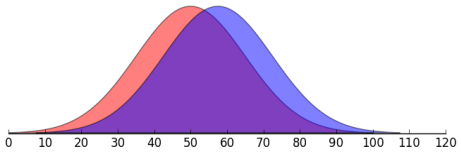
- Needle in a haystack issue
- Shorter fragment lengths of ctDNA compared to cfDNA from healthy cells[1]
  - Sample-level estimate

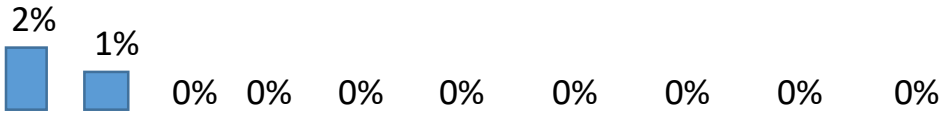


1 Underhill et al., PLOS Genetics, 2016

Quantification measures of ctDNA

- A number of ways across publications
  - Variant allele frequency (VAF)
  - Adjust for amount of cfDNA
    - #Mutated copies
    - 20% VAF based on 10,000 copies = 2,000 mutated copies
  - Adjust for plasma volume
    - Copies/mL plasma
  - Average across assays
    - Consider a ctDNA test including 10 assays

20%



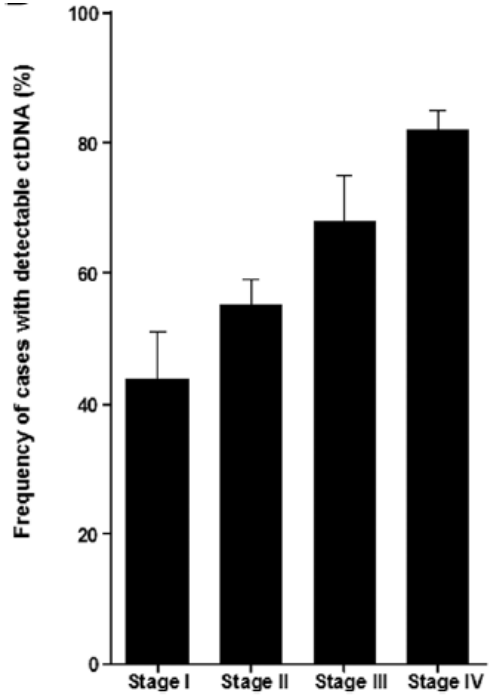
Sample VAF = 1.5%

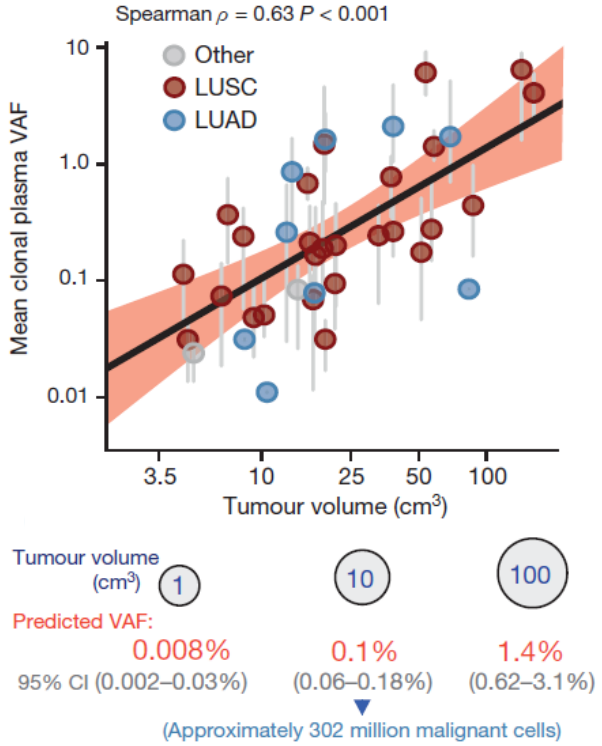
Sample VAF = 0.3%

Are all assays representative and can 0% be considered just below the detection level?

What affects the level of ctDNA?

- ctDNA fraction can range from <0.1% to >90%[1,2]
- Tumor stage
- Tumor size
- Across cancer types[3]
- Tumor features associated with shedding
  - Higher expression of (bladder cancer)[4]
    - Cell-cycle
    - Keratin genes
  - In lung cancer[5]
    - High proliferation index
    - Lymphovascular invasion
    - Non-adenocarcinoma histology





1 Diehl et al., Nature Medicine, 2008
2 Bettegowda et al., Science Translational Medicine, 2014
3 Zill et al., Clinical Cancer Research, 2018
4 Powles et al., Nature, 2021
5 Abbosh et al., Nature, 2017

Key points

- cfDNA is continually shed into the circulation
- cfDNA is highly fragmented, but protected by nucleosomes
- cfDNA has a half life of approx. 1-2 hours
- Various physiological conditions affects the level of cfDNA
- For ctDNA analysis, effective distinction of ctDNA from wild-type cfDNA is critical
- ctDNA is technically challenging to assess
- ctDNA release is affected by various tumor characteristics

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