Introduction to liquid biopsies

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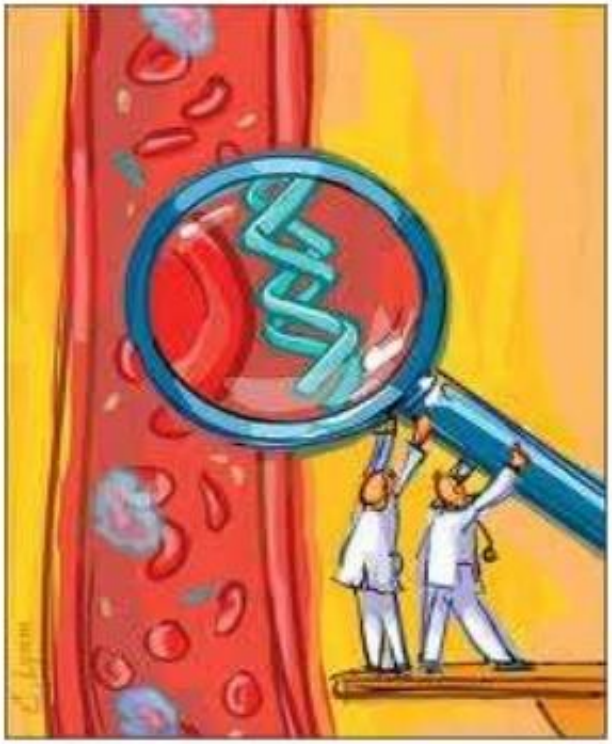
What is cell free DNA?

Cell Free DNA



Investigations:

- **Cancer** ***(circulating tumor DNA – ctDNA)***
- Myocardial infarction
- Pro-inflammatory diseases (cirrhosis, hepatitis, sistemic lupus erythematosus, rheumatoid arthritis)
- Chronic kidney disease
- Traumatic brain injury
- Exercise
- Prenatal screening "NIPT" *(cell free fetal DNA - cffDNA)*

↑↑↑ *cfDNA concentration in blood*

Diaz and Bardelli, J Clin Oncol 32:579-586

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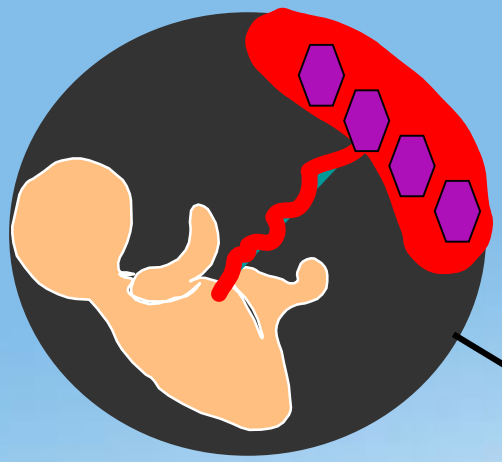
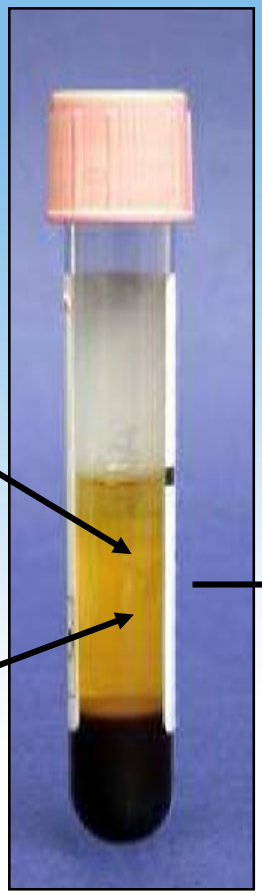
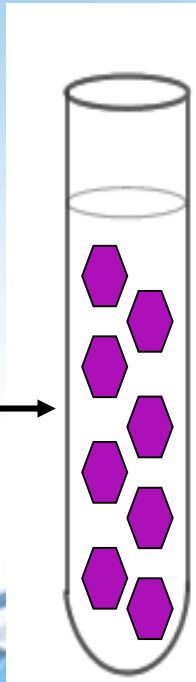
Non-Invasive Prenatal Testing (NIPT)

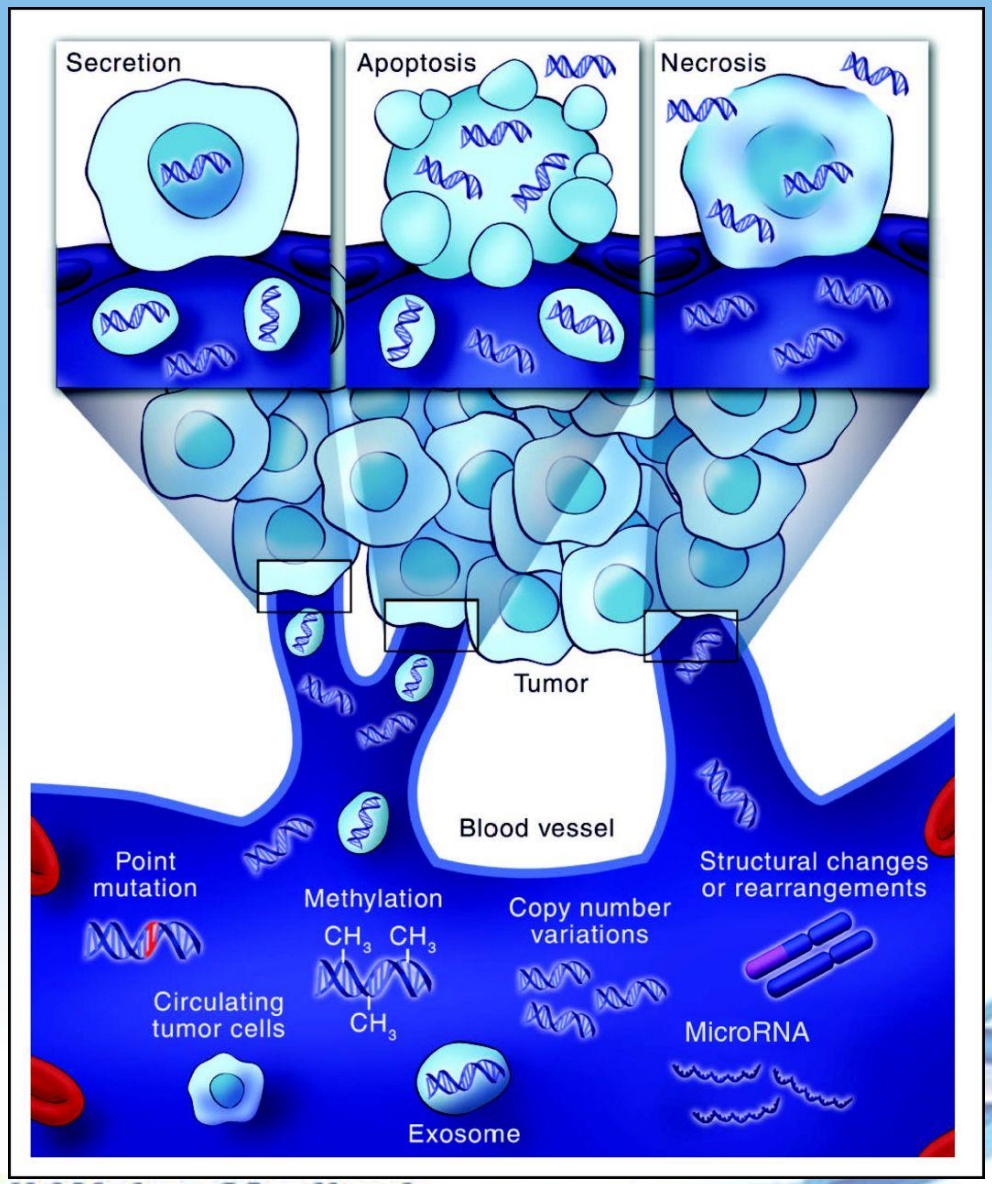




Extract DNA



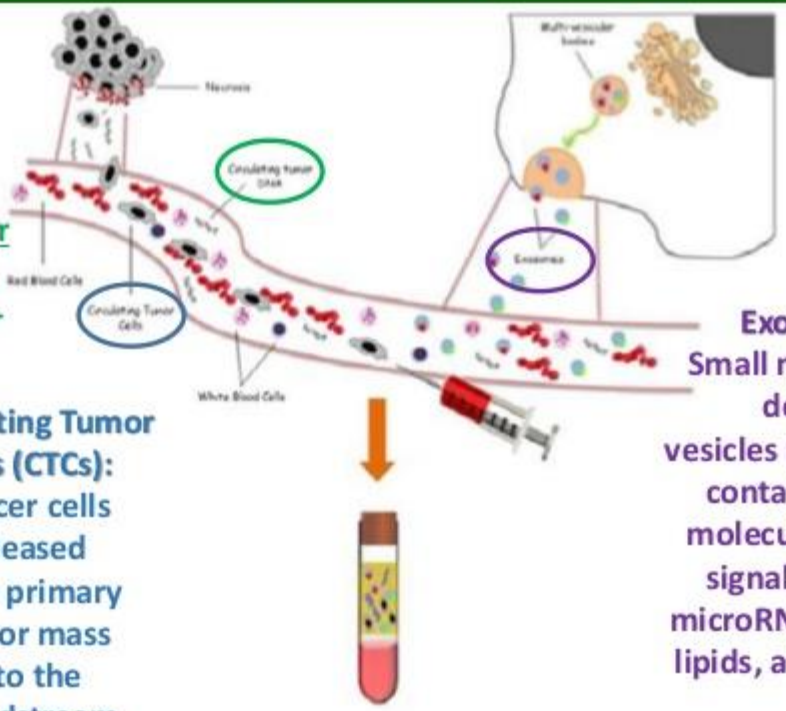
Genetic alterations detectable in circulating cell-free tumor DNA. Tumor cells release small fragments of cell-free DNA into circulation by multiple mechanisms.



Luis A. Diaz Jr, and Alberto Bardelli JCO 2014;32:579-586

Liquid biopsy

Circulating Nucleic Acids: Mainly ctDNA (circulating tumor DNA), miRNAs, mRNA, long non-coding RNA



Circulating Tumor Cells (CTCs): Cancer cells released from primary tumor mass into the bloodstream

Exosomes: Small membrane-derived vesicles (40–100 nm) contain various molecules such as signal proteins, microRNAs, mRNAs, lipids, and exoDNA.

C. Ralfo et al. / Biochimica et Biophysica Acta 1846 (2014) 539–546

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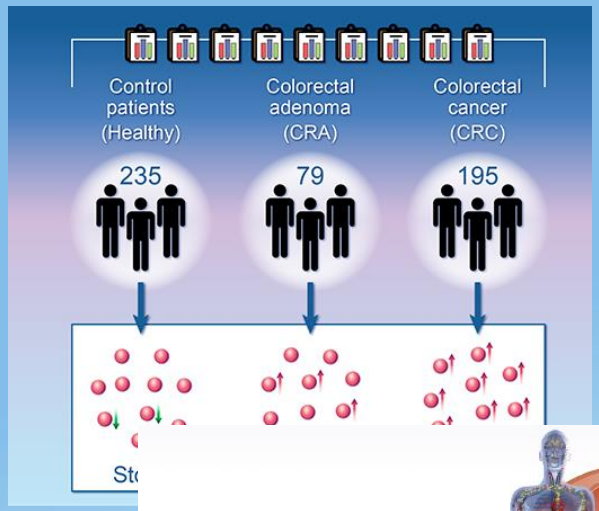
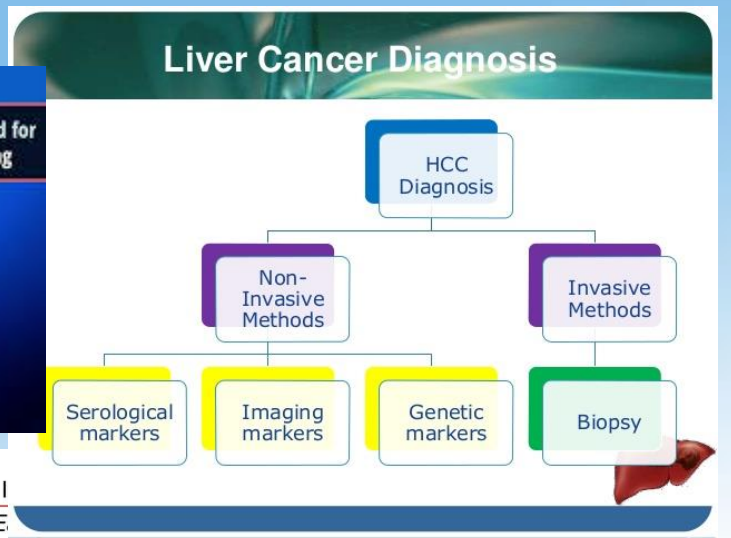
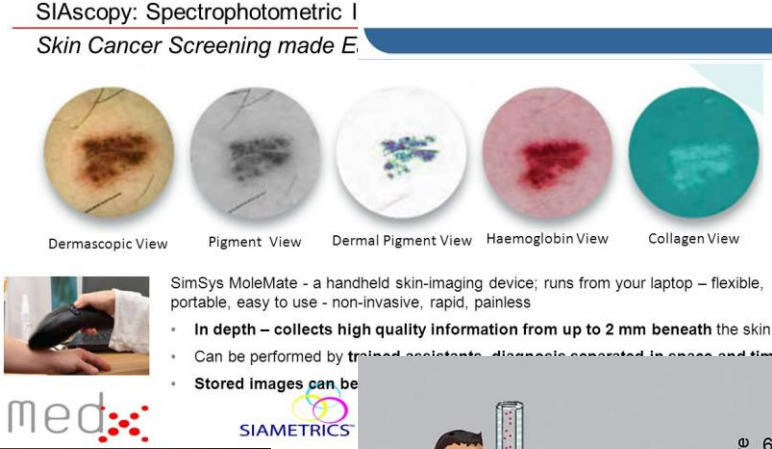
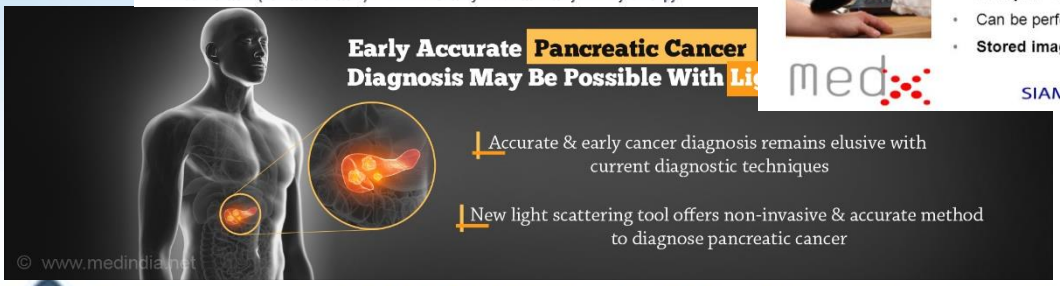
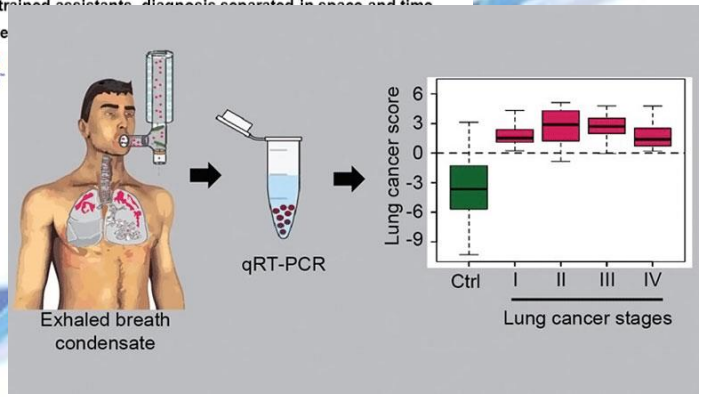
Opportunities of other non-invasive biopsies….











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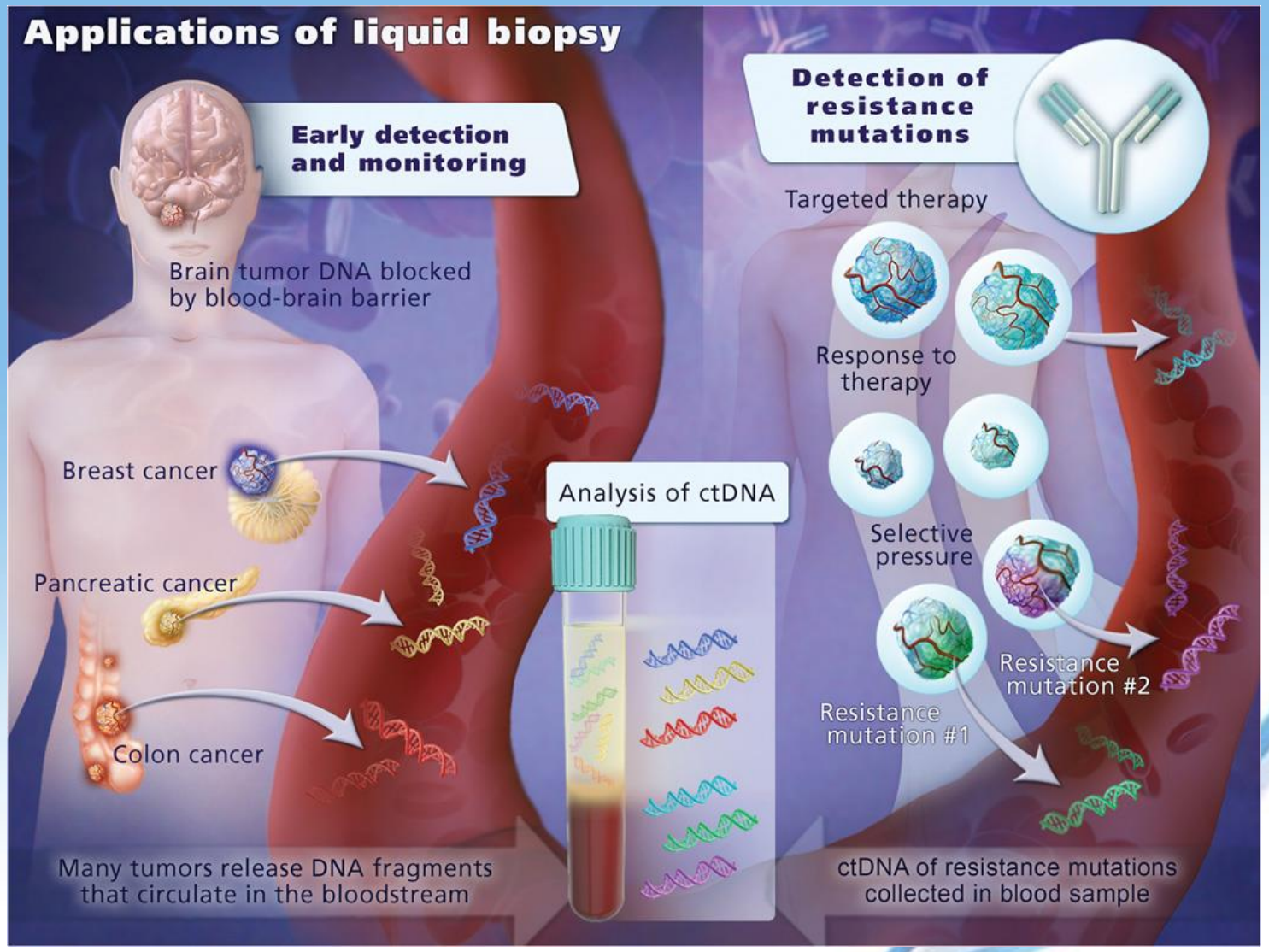
Potential applications of ctDNA testing



Chetan Bettegowda et al., Sci Transl Med 2014;6:224ra24

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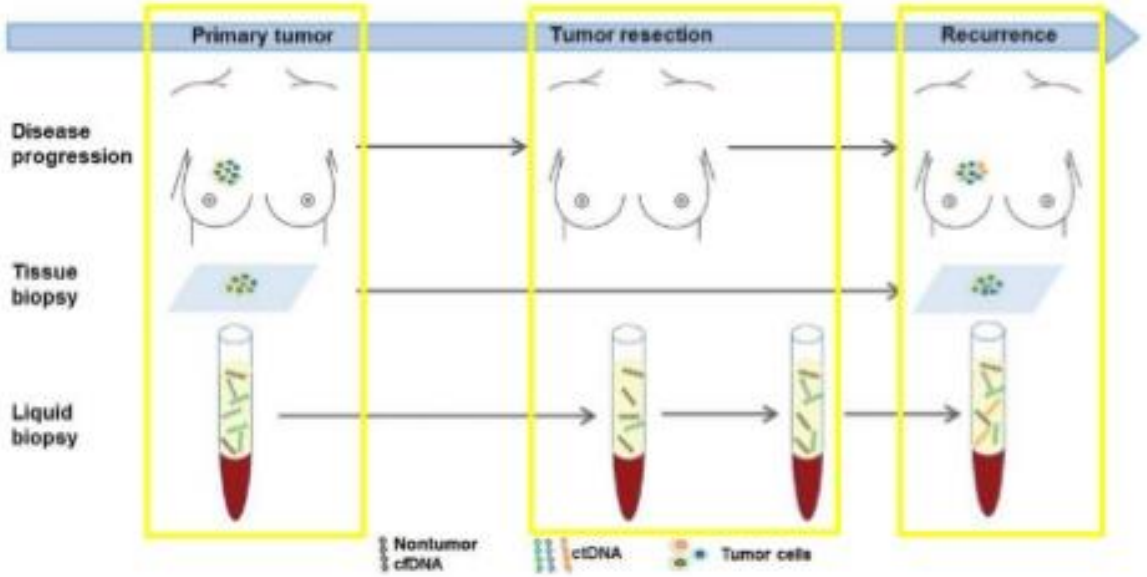
Liquid Biopsy – Clinical Applications

The improved sensitivity and specificity of ddPCR present the opportunity of using blood:



1. For mutations detection in patients with early-stage breast cancer

2. For minimal residual disease may help guide individualized decisions about adjuvant systemic therapies

3. For surveillance of patients with a high risk for cancer recurrence

Beaver et al, *Clin Cancer Res*; 20(10) May 15, 2014; Heitzer et al, *Clinical Chemistry* 61:1 (2015)

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GRAIL

Science Careers About Clinical Studies News Join the Team

Publications

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FFPE

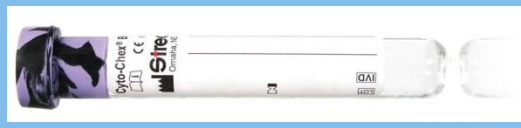
Pros

- The presence of tumour is known
- Easily stored at room temp

Cons

- Histopathology and macrodissected
- Downstream problems with quality of DNA
- Invasive biopsy procedure
- No tumour sample available
- One fixed time point

ctDNA



Pros

- Extracted from blood in house
- No need for invasive biopsy
- Sampling longitudinally and heterogeneity
- Detection at low levels in the blood

Cons

- Short half life
- Uncertain how much tumour DNA is circulating – **FALSE NEGATIVES**
- Very low concentrations from extraction

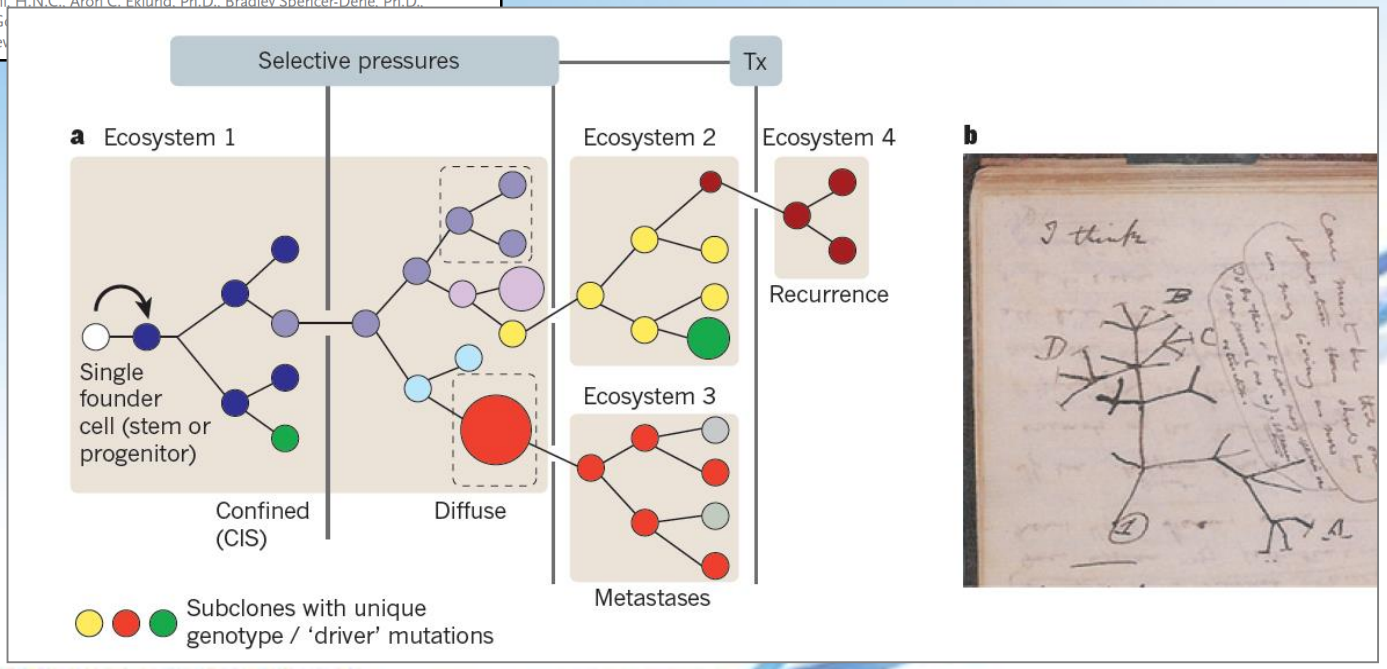
Tumour heterogeneity and evolution

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

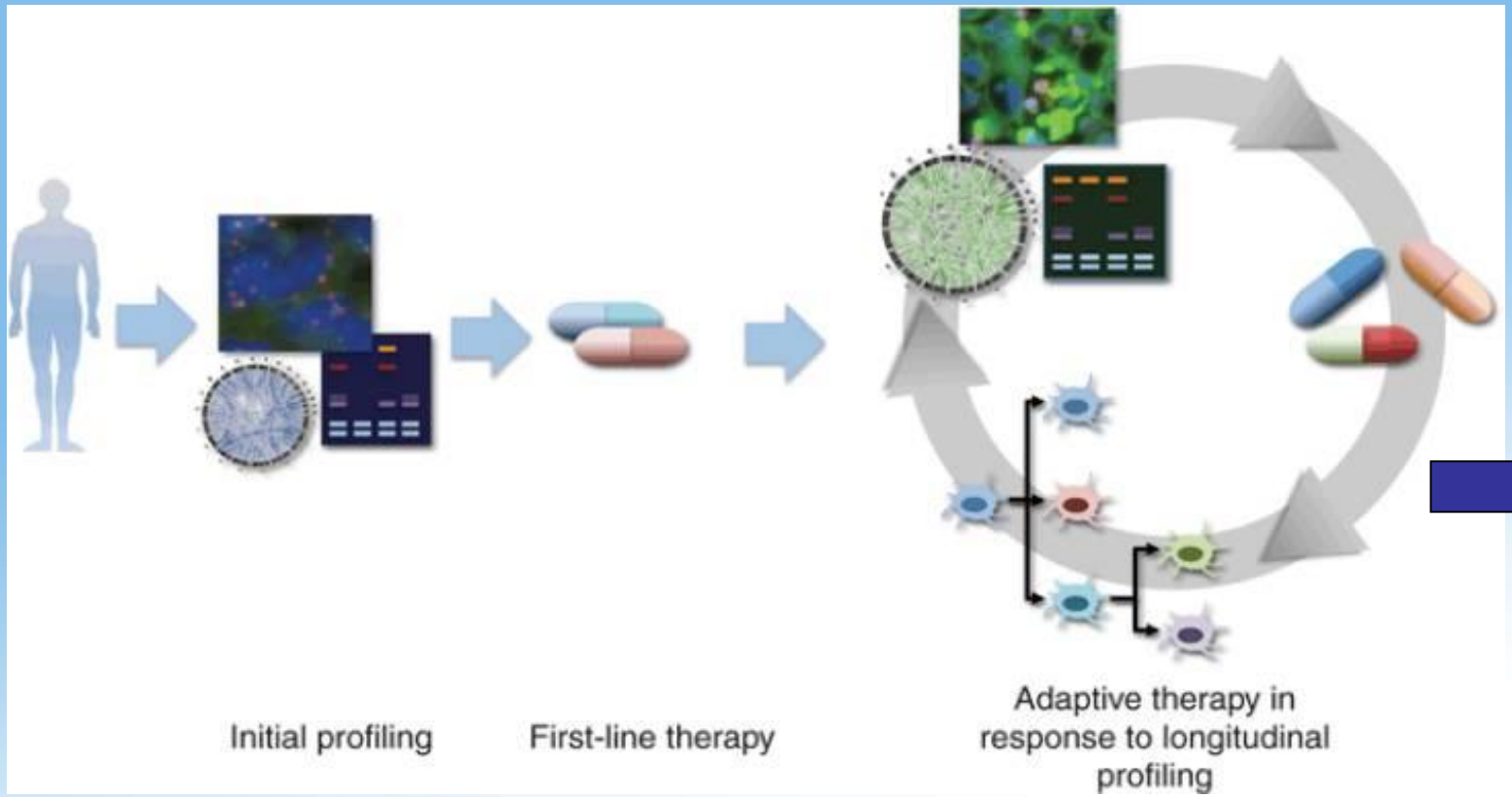
Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

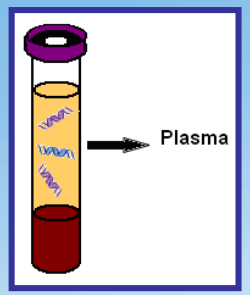
Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc., Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc., Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., G
Julian Downward, Ph.D., P. Andrew

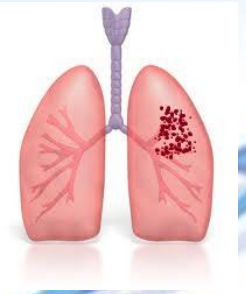


Greaves M, Maley CC. *Nature.* 2012;481:306–313
Gerlinger M *et al*. *N Engl J Med*. 2012;366:883–892

Imaginative thinking…..Longitudinal sampling







From Gonzalez et al. Clin Pharmacol Ther. Mar 2013; 93(3): 252–259

Tumours change at the molecular level, ctDNA analysis:

- **Determines molecular mechanisms of relapse and resistance**
- **Identifies potential new treatment targets**

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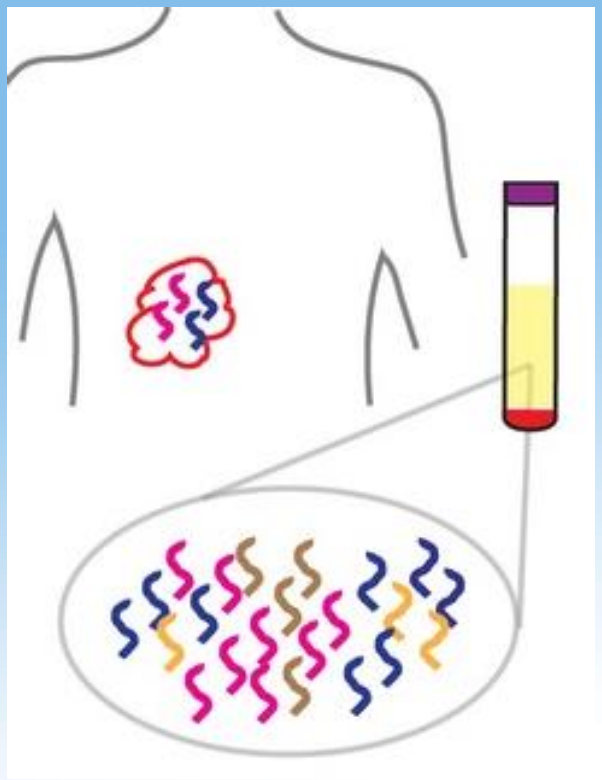
Limitations

- Technical
  - Sampling and logistics
  - Methodology selection and sensitivity
- Biological

Sample logistics



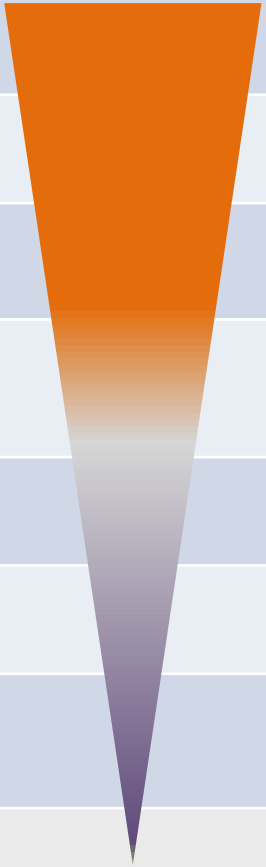
A simple blood test.....?

- Degradation of normal WBCs causes “contamination” / reduces sensitivity
- Sample handling can also preserve /degrade (tube type, temp, time...)

Education of healthcare professionals essential

What detection method?

Technique		Sensitivity	Optimal Application
Sanger sequencing		>10%	Tumour tissue
Pyrosequencing		10%	Tumour tissue
COLD-PCR and Pyro		2%	Tumour tissue
Next-generation Sequencing		2%	Tumour tissue
Q-PCR		1%	Tumour tissue
ARMS		0.10%	Tumour tissue
COBAS, Therascreen (adapted for ctDNA)		0.10%	ctDNA
ddPCR, BEAMing		0.01%	ctDNA



Adapted from Diaz and Bardelli, 2014 Journal of Clincial Oncology 32

Biology

Reasons for discordance:

- Tumour volume
- Metastatic disease
- Treatment
- Tumour type
- Necrosis
- Heterogeneity
- Time between original biopsy and longitudinal analysis

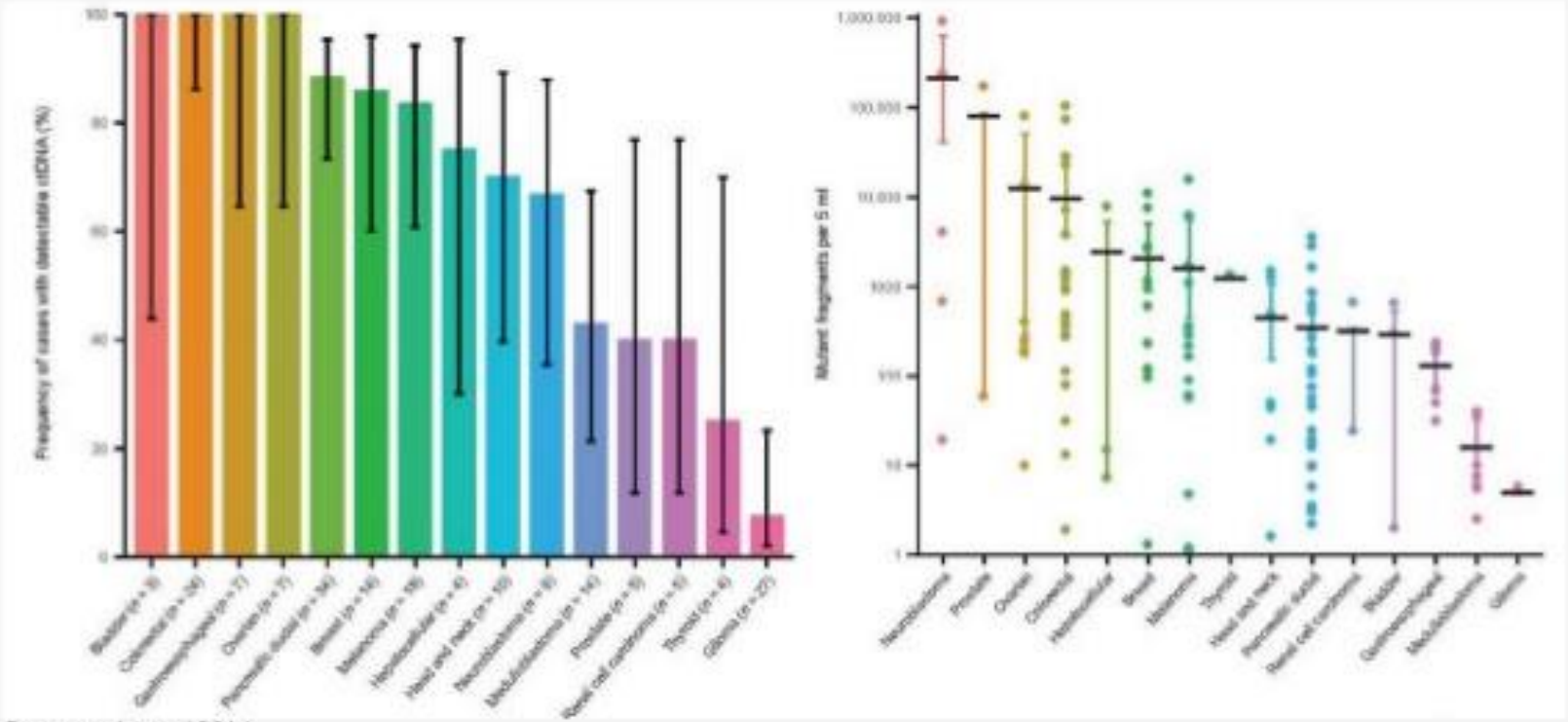
Liquid biopsy for cancer detection

The opportunity

Circulating tumour DNA (ctDNA) is easy accessible and can be detected in most metastatic cancers

The challenge

ctDNA is often only present at low levels



Bettegowda et al 2014.

Clinical utility

- What is the clinical question?
- What is the value of ctDNA analysis?
- Is there approval for ctDNA analysis?
- Clinical validation?
- Negative or positive predictive value?
- How will a normal result be reported?

Algorithms for testing

Diagnosis with NSCLC

FFPE sample analysed

EGFR mutation present

EGFR mutation absent

No tissue available

ctDNA analysis

EGFR mutation present

EGFR mutation absent

Progression on TKI

ctDNA analysis

EGFR T790M mutation detected

No EGFR T790M mutation detected

T790M testing of tissue biopsy

Clinical reporting
e.g. T790M plasma testing

1^0 Sensitizing mutation	T790M mutation	Interpretation
+	+	T790M positive: start treatment with 3° generation TKI
+	-	T790M negative: tissue biopsy recommended
-	+	T790M positive?: confirm with an orthogonal technique
-	-	Non informative: tissue biopsy strongly recommended

Quality assurance organisations

www.iqnpath.org

- Many opportunities of ctDNA analysis
- Limitations (technical and biological) must be considered

- Application to many tumour types and molecular abnormalities
- Early diagnosis and monitoring
- Future opportunities of alternate circulating biomarkers, and other "non-invasive" sampling methods