Circulating Cell-free DNA: Screening for Fetal Aneuploidy and Beyond

Owen Phillips, MD, MPH

Disclosures

- None

Objectives

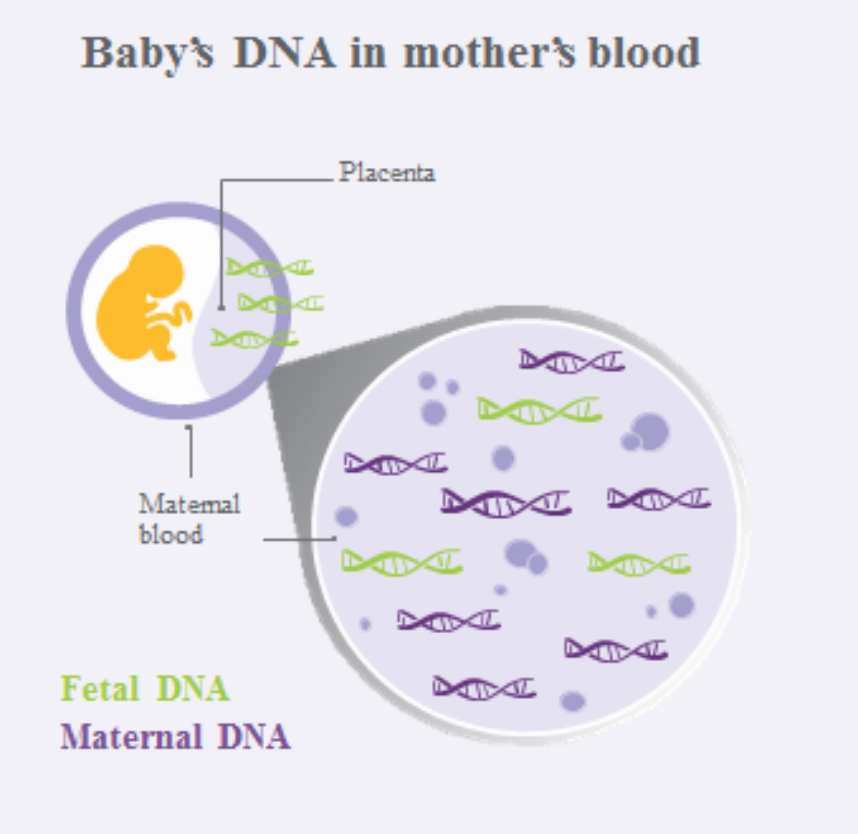
- Review the biology and technology behind cfDNA testing

- Discuss prenatal uses of cfDNA
  - Aneuploidy
  - Single gene prenatal testing
  - RH disease

- Discuss non-OB uses of cfDNA
  - Cancer
  - Transplant medicine
  - Infectious disease

What is Cell Free DNA?

- "Fetal DNA" released into maternal bloodstream as small fragments (150–200bp)
  - Maternal blood contains both placental and maternal cfDNA
  - 2–20% of total cfDNA in maternal circulation is fetal/placental in origin
- Fetal cfDNA reliably detected after 10+ weeks gestation
  - Undetectable within hours postpartum



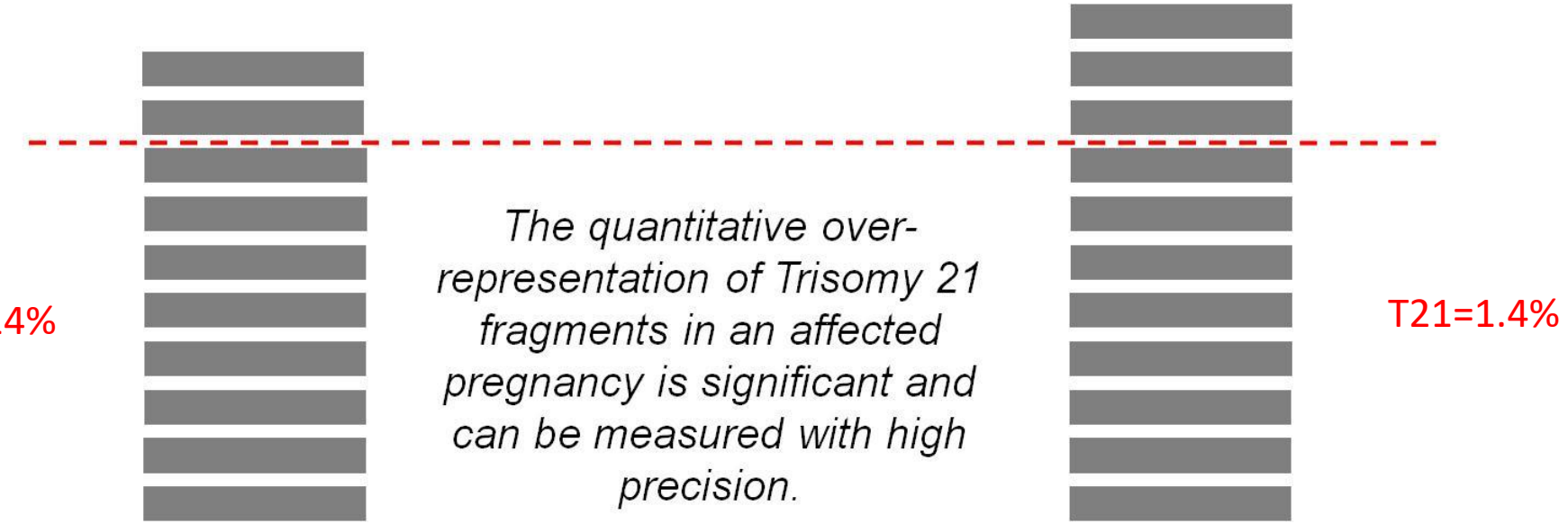
Two technologies (ccfNA, ccfDNA, cfDNA, NIPT, NIPS)

1. Massively parallel sequencing (the first and still used by *Labcorp* and *Billion2One)*

2. SNP based (Natera's platform)

Principles of Fetal Trisomy 21 Detection Using DNA Sequencing

DNA MPGS does not differentiate which fragments come from the mother and which from the fetus.



Natera

- SNP- based

SNP: single nucleotide polymorphisms

- The variations in non-coding regions that are highly likely to be variable between individuals
- “highly informative”: can distinguish between sources of DNA

- Can distinguish among maternal and fetal DNA

SNP-based cfDNA technology can predict aneuploidy by directly analyzing fetal DNA

And can detect:

- Polyploidy
- Zygosity of twins
- Vanishing twin

High detection rate for aneuploidy, regardless of platform

	Number of cases Detected	Performance
Trisomy 21	210/212	Sensitivity 99.1% Specificity 99.9%
Trisomy 18	59/59	Sensitivity 99.9% Specificity 99.6%
Trisomy 13	11/12	Sensitivity 91.7% Specificity 99.7%

Palomaki GE, *et al*.. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13, as well as Down syndrome: An international collaborative study. *Genet Med.* 2012 Jan 26. doi: 10.1038/gim.2011.73.

Understanding ‘positive predictive value’

- What are the odds that a positive result means the fetus is actually affected.

- Since there are false positives, the PPV depends on the prevalence of the disease in the population. Low prevalence, result more likely to be a false positive.

- In younger women, a positive result is more likely to be a false positive than in older women because fewer cases

PPV differs by age
PPV: the chance that a positive result means that the fetus is affected

			Age 25	Age 40
	Sensitivity	Specificity	PPV (%)	PPV (%)
Trisomy 21	99.3	99.8	33	87
Trisomy 18	97.4	99.8	13	68
Trisomy 13	91.6	99.9	9	57

Find a PPV calculator you like and use it

https://www.perinatalquality.org/Vendors/NSGC/NIPT/

UNC created an online calculator (www.mombaby.org/NIPS)

Takes in consideration the lab you use

Sex chromosome abnormalities

- High detection rate
- 47,XXY: more common than thought
- 45,X: High false positive rate. Mosaicism from placenta. Need confirmation with amniocentesis (not CVS)

Microdeletions: Opt-In Testing

Overall sensitivity of 91.6% and specificity of 99.84%

	Min. Region Size	Sensitivity	Specificity
22q11.2 deletion syndrome[3]	2.7 Mb	87.5%	99.8%
1p36 deletion syndrome[3]	5 Mb	*	99.9%
Angelman/Prader-Willi syndrome[3]	5.8 Mb	*	99.7%
Cri-du-chat syndrome[3]	9.8 Mb	100%	99.8%
Wolf-Hirschhorn syndrome[3]	3.6 Mb	100%	99.6%

**No sensitivity estimates were performed for sample sizes <2. Titration of fragmented genomic DNA derived from cell lines containing either a 1p36 or 15q11.2 deletion demonstrated a linear dose response and confirmed the assay's ability to measure copy number changes at those loci.* [3]

References
3. Data on file, Illumina, Inc.

Microdeletion detection by NIPT

- ACOG recommends against screening
- ACMGG recommends screening for del 22q11.2
- Most common (1/2000): del 22q11.2
  - Kids may have heart defect
  - Individuals may have just a psychiatric disorder
  - How do you counsel for that?

  - Rare disorders: PPV an issue 5.3%

What's NEW:
Fetal single gene defects: (sickle cell, CF, SMA)

- Standard care-
  - a patient identified as a carrier of an autosomal recessive disorder.
  - Partner tested. Both parents must be carriers to have an affected child
  - If both are carriers, risk of affected pregnancy is 1 in 4 (25%)
  - CVS or amniocentesis to make a diagnosis.
  - Results may take 3-4 weeks
  - Nearly 100% accurate

Cell free technology for fetal disease

Early case reported

Skeletal dysplasia on US (2013): Usually de novo mutations (no parent carries the gene)

- Differential Campomelic dwarfism and Thanatophoric dwarfism
- Because mother was not affected, she did not carry gene. If gene found, it would have to come from fetus

- ccfNA found known variant in gene (FGFR3) for Thanatophoric dwarfism

Autosomal recessive disease different

- Mother a carrier
- Father a carrier
- Fetus could be homozygous normal, a carrier or carry 2 abnormal genes and have the disease.

- So to predict fetal status, a dosage algorithm would be required

- 1 copy: Fetus unaffected (this copy came from mom)
- 2 copies: Fetus a carrier
- 3 copies: Fetus is affected (one from mom, 2 in fetus)

sgNIPT: single gene noninvasive prenatal testing

The possibility that we could bypass maternal genetic screening and just go to fetal screening for 4, or 50, or 100+ disorders with cfDNA.

Advantages

- ‘Vast majority’ of carrier screening is done during pregnancy- then there is urgency
- Fewer than half of partners complete the recommended screening
- 10% paternal mis-identification
- Results 9-16 days

Natera (*Fetal Focus*) using their SNP technology

- Can tell maternal genes, from fetal
- Can give a high risk, low risk prediction
- Recommend CVS/Amnio to confirm high risk results

EXPAND study. Fetal Focus test

SMFM Feb. 2026

- Maternal blood. cfDNA. Sequencing genes of interest. Dosage analysis.
- CF, SMA, hemoglobinopathies (sickle cell and thalassemias)
- 90 pregnant carriers
- Detection rate for affected fetuses was 91% (10/11)
- PPV was 53% in this study cohort.

42,000 patients; 7500 carriers (17.92%) Billion2One *Unity* (using counting technique)

CF, HBB (sickle S, C, B thalassemia), HBA (alpha thal), SMA

TABLE 2 Clinical analytics of the 528 sgNIPT with known neonatal/fetal outcomes.

	Screen positive sgNIPT >1 in 100	Screen negative sgNIPT ≤1 in 100	Total
Affected	24	1	25
Unaffected	24	479	503
Total	48	480	528
			95% CI
Sensitivity	96.00%		79.65%–99.90%
Specificity	95.23%		92.98%–96.92%
PPV	50.00%		35.23%–64.77%
NPV	99.79%		98.84%–99.99%

ACOG Practice Advisory: Cell-free DNA to Screen for Single-Gene Disorders (Feb, 2019)

- “not been sufficient data to provide information regarding accuracy and positive and negative predictive value in the general population.

- For this reason, single-gene cell-free DNA screening is not currently recommended in pregnancy.”

Clinical care is off and running

1. Moving toward in utero treatments for SMA, CF based on early diagnosis with sgNIPT

2. Natera has expanded their panel to 24 different genetic disorders

Use with RH disease or other antibody disorders

- Mom is RH negative- meaning she lacks the gene that codes for the RHD antigen
- If the fetus carries the gene, the fetus is RH positive.

= Risk for alloimmunization and hemolytic disease of the newborn

- ccfNA technology to detect the fetal RHD gene
- If negative, no need for Rhogam
- If present, Rhogam as usual

- Other antibody syndromes also (Duffy, Kidd, E, etc.)
- Becoming standard in European nations

ACOG: Practice Bulletin No. 181: Prevention of Rh D Alloimmunization (August, 2017)

- Concerns have been noted because of the rate of inconclusive results (range 2–6%), which are influenced by race
- Four cost analyses from North America and Europe have shown no economic benefit at current test-cost levels
- Noninvasive assessment of fetal Rh D status is not recommended for routine use at present
- Rhogam shortages?

Preeclampsia?

- Detection of placental markers in cfNA
- An attempt to predict risk prior to symptoms
- Clinical relevance?

cfNA technology: Everywhere!

- OBGYN research led to OBGYN being the first or early adopters
  - Ultrasound
  - Laser
  - Laparoscopy
  - Vaccines for cancer
  - Robot

In cancer care

- Bianchi, et al. (2015)

- NIPT for fetal aneuploidy (125,426 cases). Doctor calls about patients. Reported abnormal results. Fetus normal. Cancer in mom.

- Odd trisomies (often more than one) detected (8 cases). Fetus normal

- Occult maternal cancers: non-Hodgkin's lymphoma X 3, CRC X2, leukemia, neuroendocrine cancer

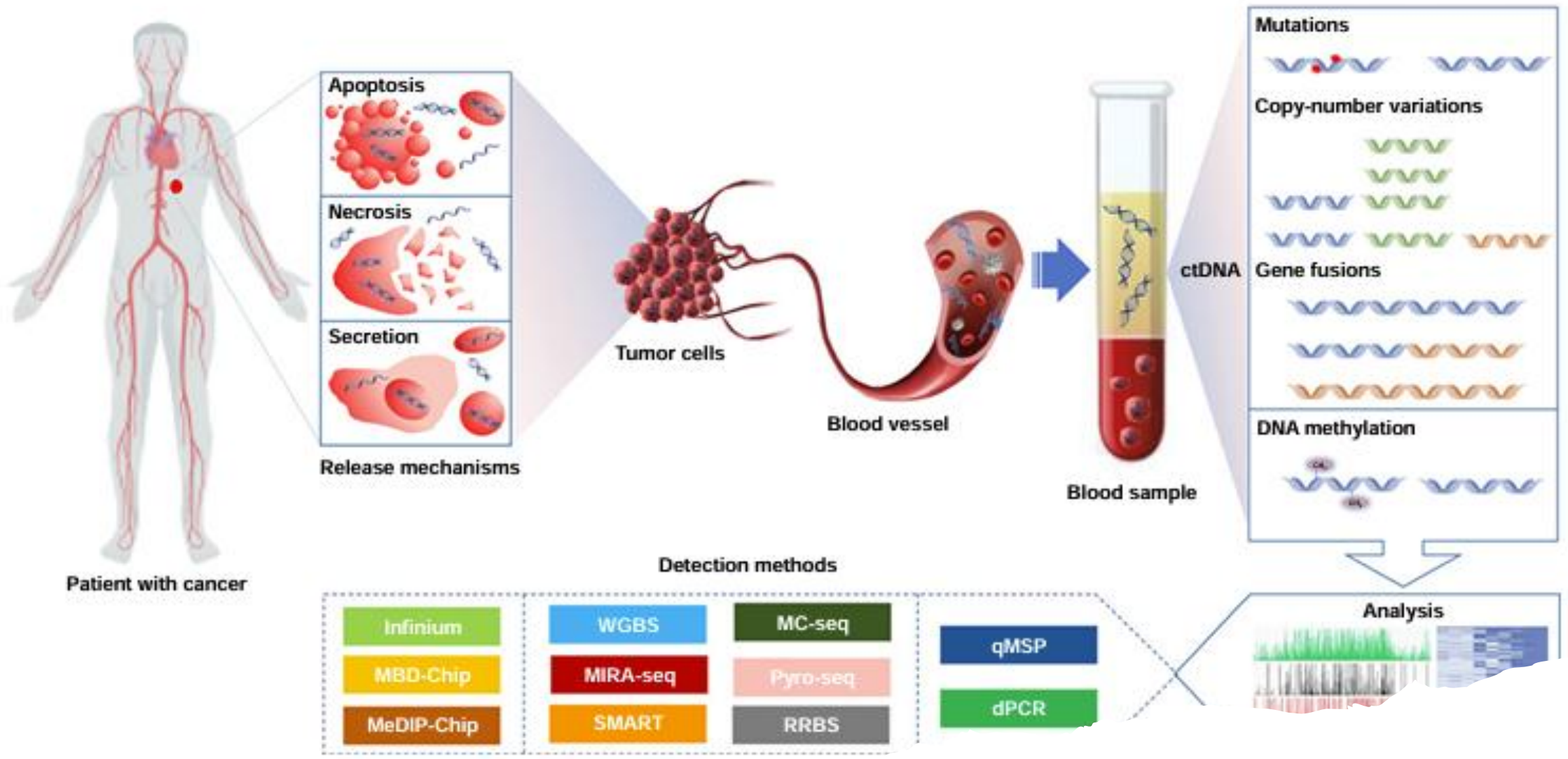
- Apoptosis of cancer cells spilling ccfNa into maternal circulation

Liquid biopsies for malignancy

- cfNA- studying DNA to find patient-specific alterations

- Capable of identifying tumor burden
- Assessing treatment response
- Defining resistance to chemo
- Assessing difficult-to-biopsy cancers

- Early cancer detection?



Roy, D. TrendsinCancer,February2020,Vol.6,No.2

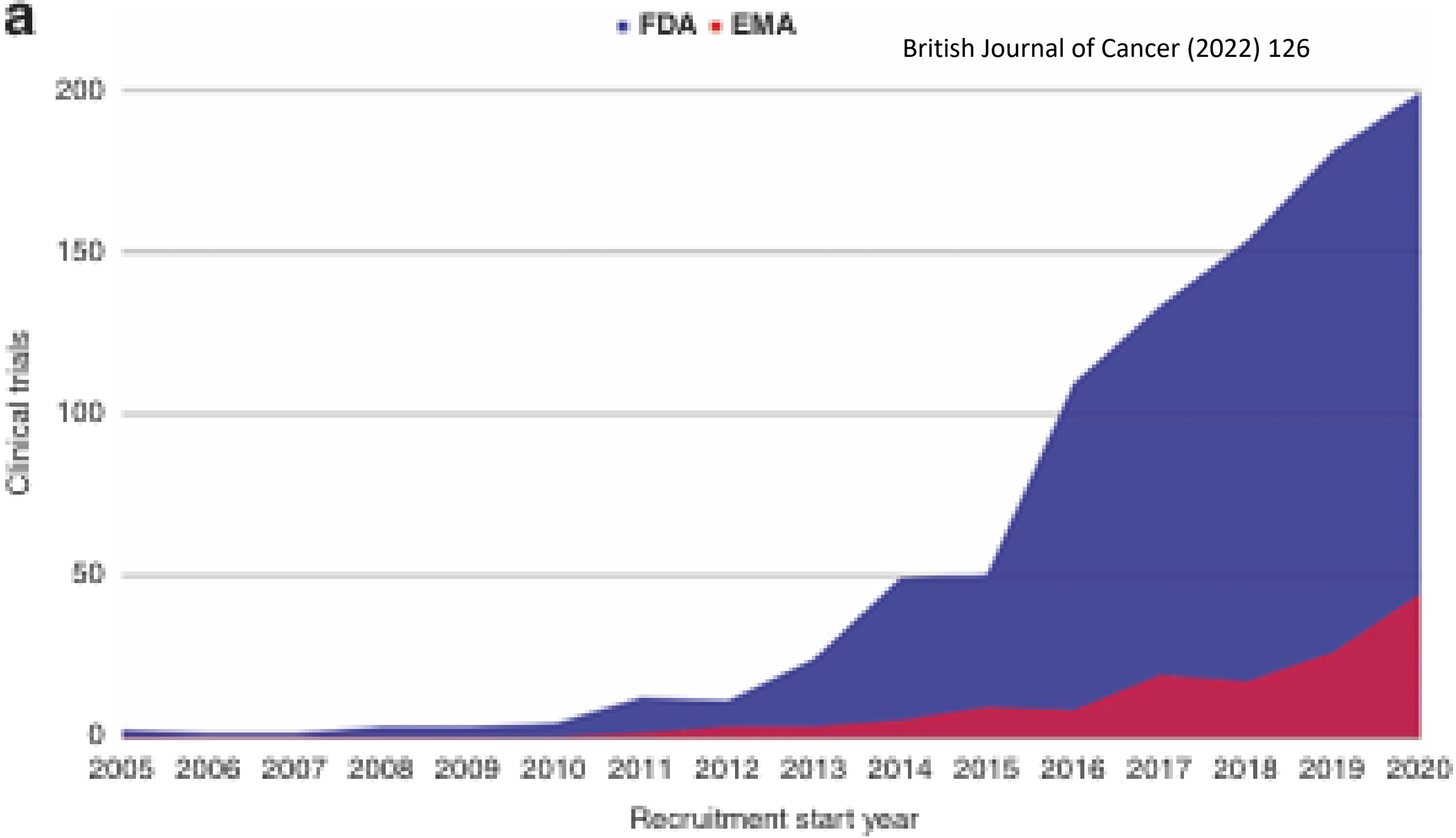
Chung, et al. NEJM 2024: Screening for colorectal cancer using cell-free DNA

- Retrospective analysis: 58 patients with cancer

cfNA: Identified 11 out of 17 (65%) with stage I disease

all individuals with stage II disease (n = 14), all with stage III disease (n = 17), and all stage IV disease (n = 10)

A false-positive rate of 10.1%

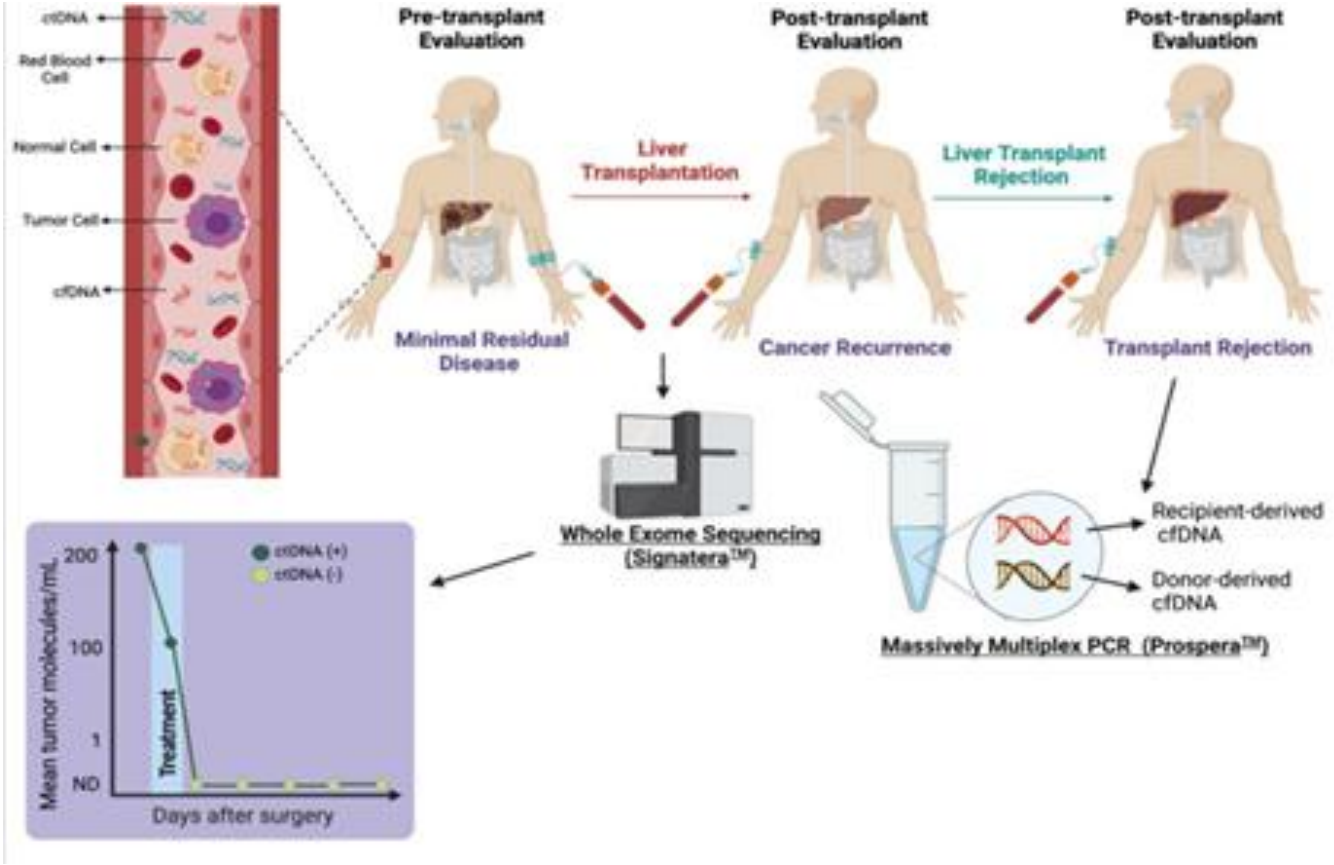


British Journal of Cancer (2022) 126

Transplant medicine

- cfNA: Apoptosis, necrosis, diseased tissue

- the concentration of organ <u>donor</u> DNA in recipient plasma is a potential marker for rejection.



Reddy, T: Cancers 2022.14,743

Heart transplant patients (2024)

- Routine surveillance biopsy Endomyocardial biopsy (EMB) is traditionally considered the gold standard for surveillance and detection of acute organ rejection

- Donor-derived cfDNA measured in 152 patients
- “High-validity screen”
- Detection of acute rejection- 76%

- NPV: (negative test is a true negative) 97%. Avoiding EMB

Infections

- 60% of cultures in hospitalized patients suspected to have an infection fail to identify an organism
- Microbial cell-free DNA using next-generation sequencing (NGS) technologies (each organism has a distinct DNA fingerprint)
- 93% sensitivity versus 76% in conventional testing (cultures)

  (Yu, et al. 2020)
- Negative testing can result in de-escalation of antimicrobials

NGS of cfDNA for infections: Has real value in detecting ‘challenging opportunistic infections’

* Toxoplasmosis, Pneumocystis, Legionella, Nocardia

• Turn around time: 1-2 days versus 1-2 weeks

Problem: FPR for identifying normal flora

Missed cases of HSV, mycobacterium, mold infections

No resultant sensitivities

But did change management in 59% of cases

Summary: a blood test

- New prenatal ccfDNA applications

- New applications in other medical fields

- Advances

Laboratory techniques (in the hands of companies)

Primary detection of cancers

Infection diagnosis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