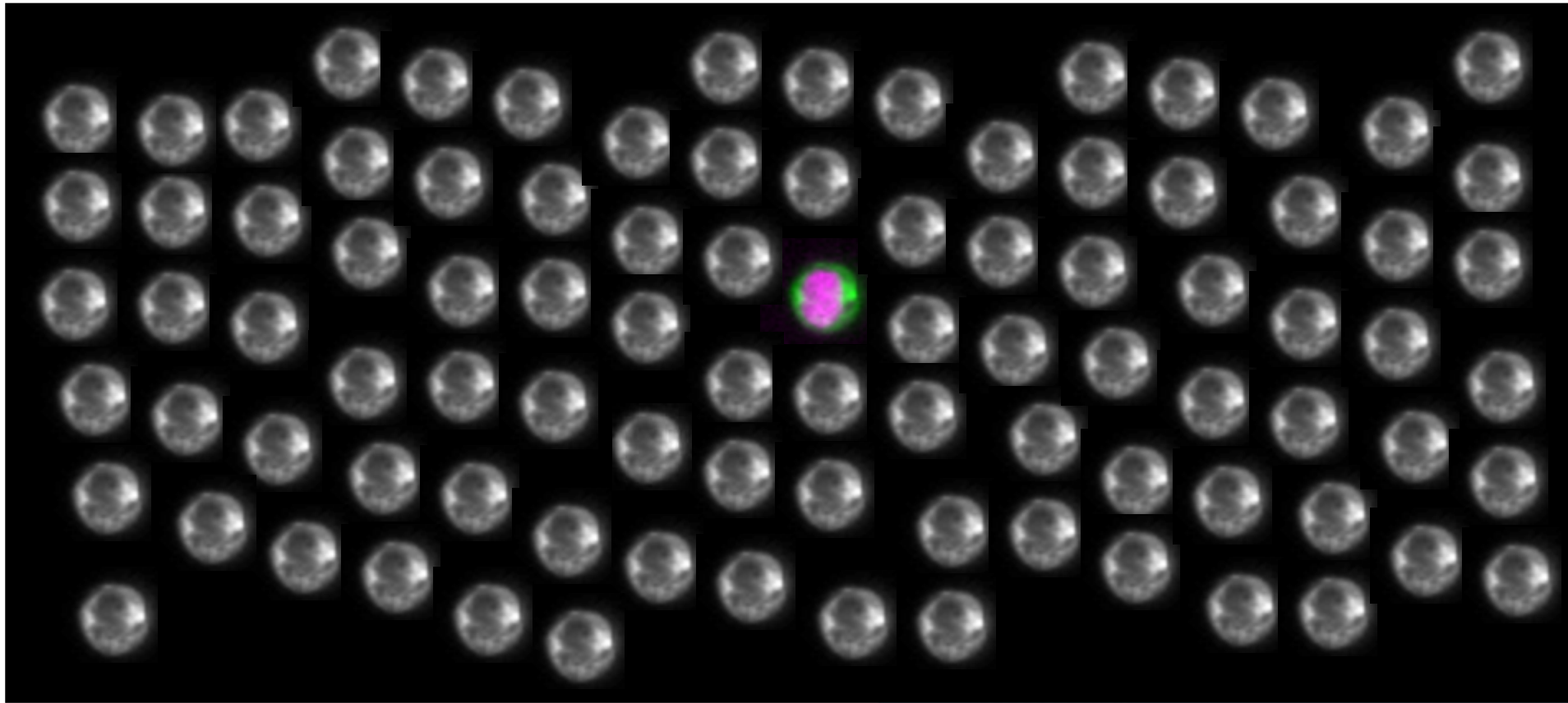# Liquid biopsies (vloeibare biopten)
De rol in primaire diagnose en resistentie analyse

John Martens, Interne Oncologie, Erasmus MC Cancer Institute, RDAM
NVvO Basiscursus Oncologie 2021

Disclosure of speaker's interests

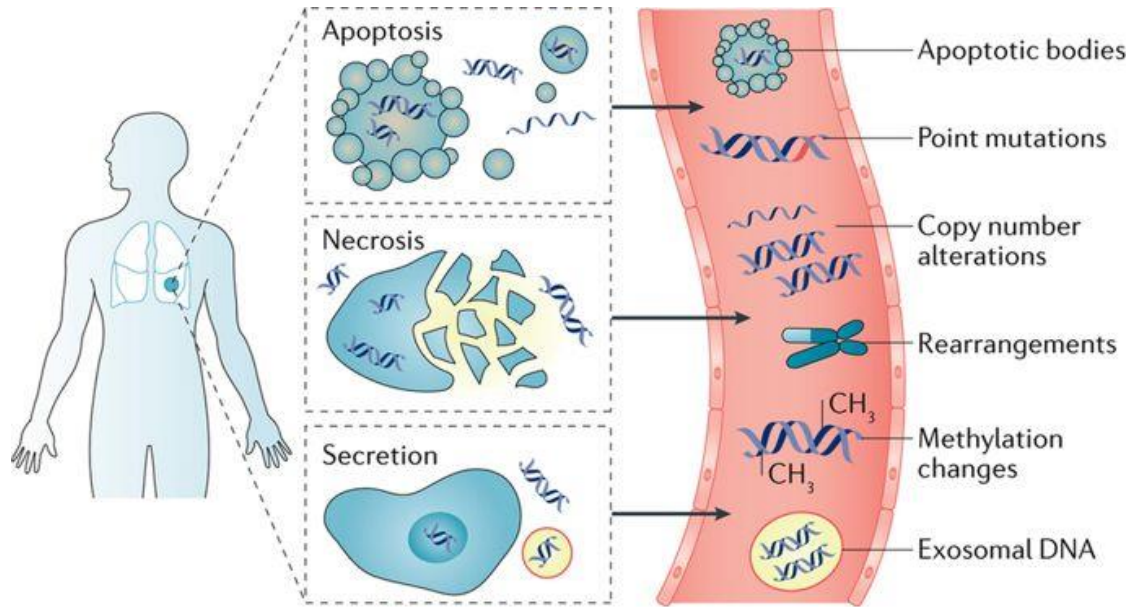| Conflict of interests	
Relations relevant for the work presented at the meeting	**Company names**

● Sponsorship or research funds
 	
● Payment or other (financial) remuneration
 	
● IP ownership
	● Menarini (owner of Cellsearch)
	   (Provides cofunding NWO funded research project)
	
	● Roche diagnostics
	   (I have given advice for the introduction of PIK3CA diagnostics in NL)
	
	● Our departments owns a patent on the ARv7 assay (presented here)

Diagnostiek vindt vaak plaats op primair tumorweefsel, maar dat heeft beperkingen!!

- Patienten overlijden t.g.v. uitzaaiingen

- Primaire tumoren en metastasen verschillen van elkaar
  - Genetisch
    - ESR1 mutaties na AI
    - KRAS pathway mutaties na Cetuximab
    - Secondaire mutaties na o.a.
      - c-KIT/ABL oiv van gleevec in GIST/CML
      - EGFR oiv erlotinib/gefitinib in longkanker
  - Epigenetisch
    - ER/HER2 status bij progressie
    - Transdifferentiatie in prostaat kanker
    - Inductie van EMT, stemness, senescence oiv (chemo)therapy

wtESR1
Remming van aromatase

mtESR1

Gevoelige tumor

Resistante tumorsubklonen

Zijn vloeibare biopten een oplossing?

Soorten en maten van vloeibare biopten

Wan et al. Nat. Rev. Can, 2017;17:223–238

Bronnen

- Bloed
- Urine
- Speeksel
- Liquor
- Nipple fluid
- Seminal fluid,
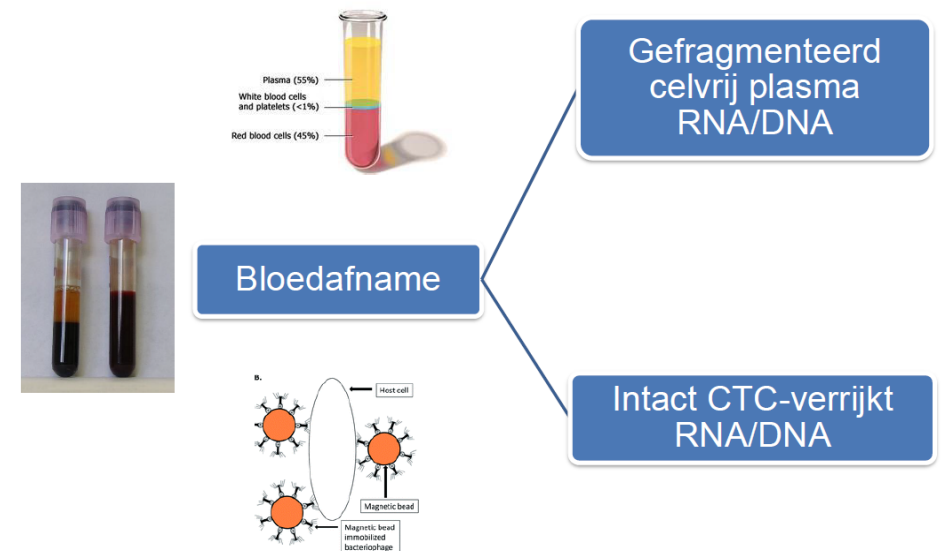- etc.

Analytes

- Circulerende tumorcellen (CTCs)
- Circulerend celvrij DNA (cfDNA)
- Exosomen
- Circulerende metabolieten
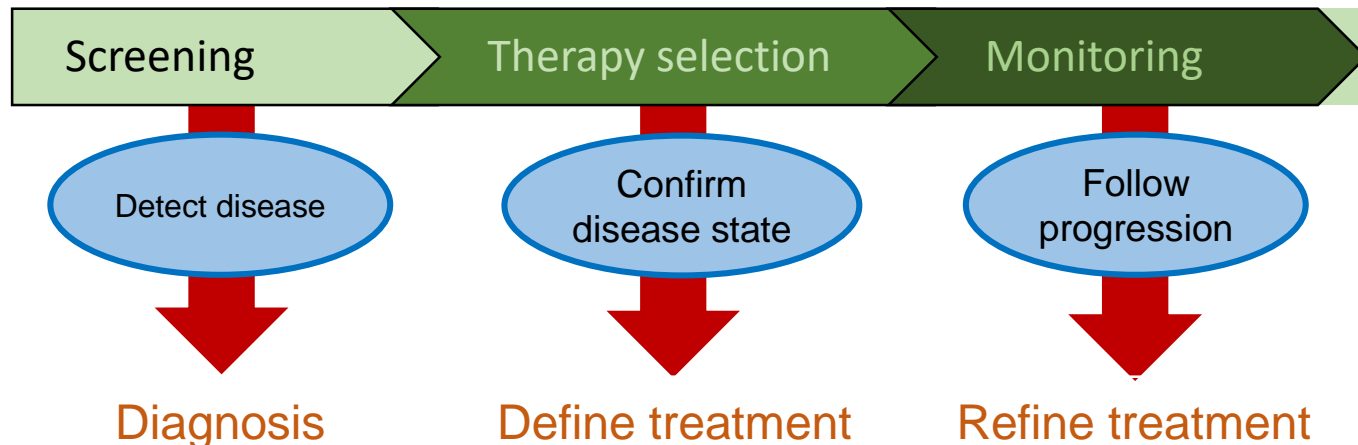- Circulerende eiwitten (b.v. PSA, CEA)

Toepassingen van vloeibare biopten

- Predictieve/prognostische biomarkers
- Detectie van aanwezige ziekte (minimal residual disease)
  - Disease monitoring
  - Minimal disease monitoring
  - Screening
- Identificatie van zich ontwikkelende resistante klonen
- Inzage in tumorheterogeniteit

Screening	Therapy selection	Monitoring
Detect disease	Confirm disease state	Follow progression
Diagnosis	Define treatment	Refine treatment

Liquid Biopsies: Genotyping Circulating Tumor DNA. LA Diaz Jr et al. Journal of Clinical Oncology. 2014

Predictie

Liquid biopsy: CTC onderzoek

Circulating Tumor Cell
EpCAM +
Cytokeratin (CK) +
DAPI (nucleus) +
CD45 -

Leukocyte contamination
CD45 +
DAPI +

Circulating tumor cellen

enuration

mRNA profile

Single cell genomics

Organoids

Christofanilli et al. N Engl J Med 2004; 351: 781-791

>18 Months

~10.1 Months

Logrank   < 0.0001
Cox Hazards Ratio = 4.3865
chi-square = 31.73
(p-value < 0.0001)

# of Patients
(Median Survival Time)
< 5 CTCs at Baseline   90 (> 80 Weeks)
≥ 5 CTCs at Baseline   87 (43.3 Weeks)

%Probability of Survival

Time from Baseline (Weeks)
[~4.3 Weeks / Month]

Toepassing als predictieve biomarker:
ARv7 status in CTC (of Exosomes) in CRPC

Enrich for CTCs	Isolate RNA	prepare cDNA	Pre-amplify cDNA for targets of interest	Evaluate overall quality of RNA with reference gene data	Evaluate presence of epithelial signal	Evaluate AR-V7 data (2 PI's)	Disseminate AR-V7 data
Within 24 hrs; 1 day	Once a week, 2 days hands-on time					1 day	Within 11 days

AR Proteins

AR (variant 1) — NTD — DBD — Hinge — LBD

AR3/AR-V7 — NTD — DBD — U

- AR-WT
- AR-V1, V3, V7, V9
- KLK3 (PSA)
- TMPRSS2:ERG

- PTPRC (CD45)
- HMBS, HPRT, GUSB
- EPCAM, KRT19

ARv7 status predicts ADT failure

A Enzalutamide-Treated Patients

AR-V7 positive   AR-V7 negative

Best PSA Response (% change)

But not response to cabazitaxel

A

AR-V7 absent   AR-V7 present

Fold change in PSA at week 12

Patient

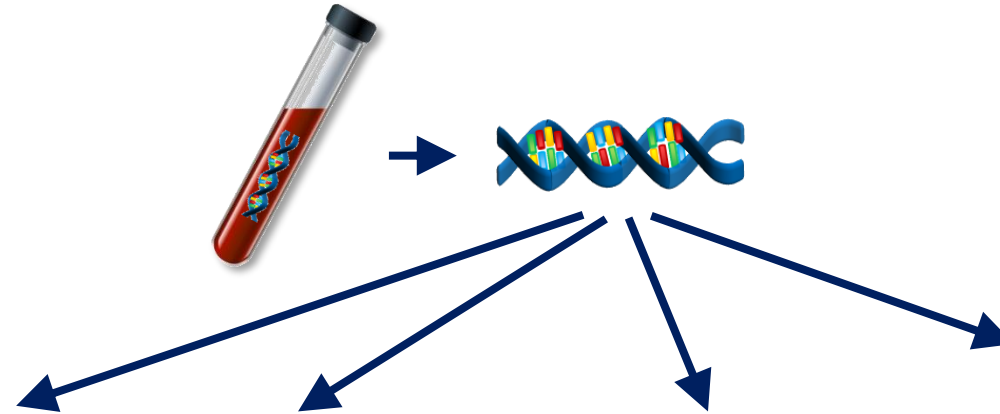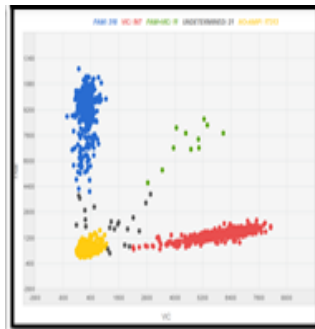| CTC count																									
Baseline	198	152	147	83	423	26	107	121	90	56	50	43	1025	61	321	30	375	567	288	26	53	120	13	100	154	94
After cycle 2	60	256	80	76	NA	4	179	123	36	NA	27	2	400	19	56	2	278	2266	275	3	32	10	11	4	8	0
AR-V7 status	-	-	+	+		-	+	+	+		-	-	+	-	-	+	+	+	+	-	-	+	+	+	+	+

Antonarakis, NEJM 2014, 371
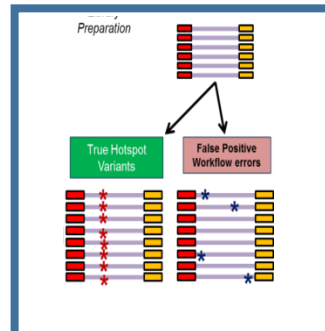
Onstenk Eur Urol. 2015 68:939-45

cfDNA analyse assays
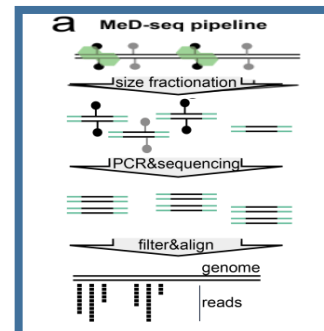

CelvrijDNA (cfDNA/ctDNA)
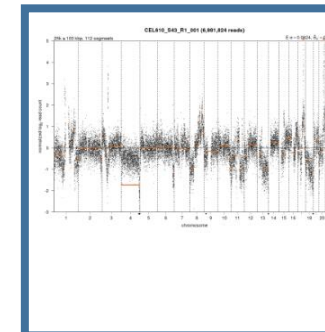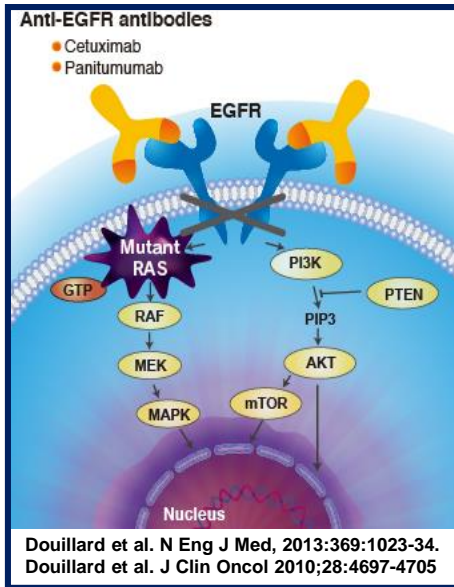
dPCR   UMI NGS   MeD-seq   NPT/Fastseq

Doelgerichte sequentie analyse van ctDNA (Oncomine™)

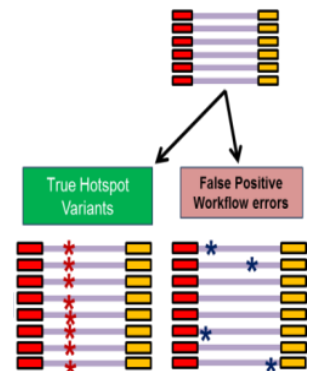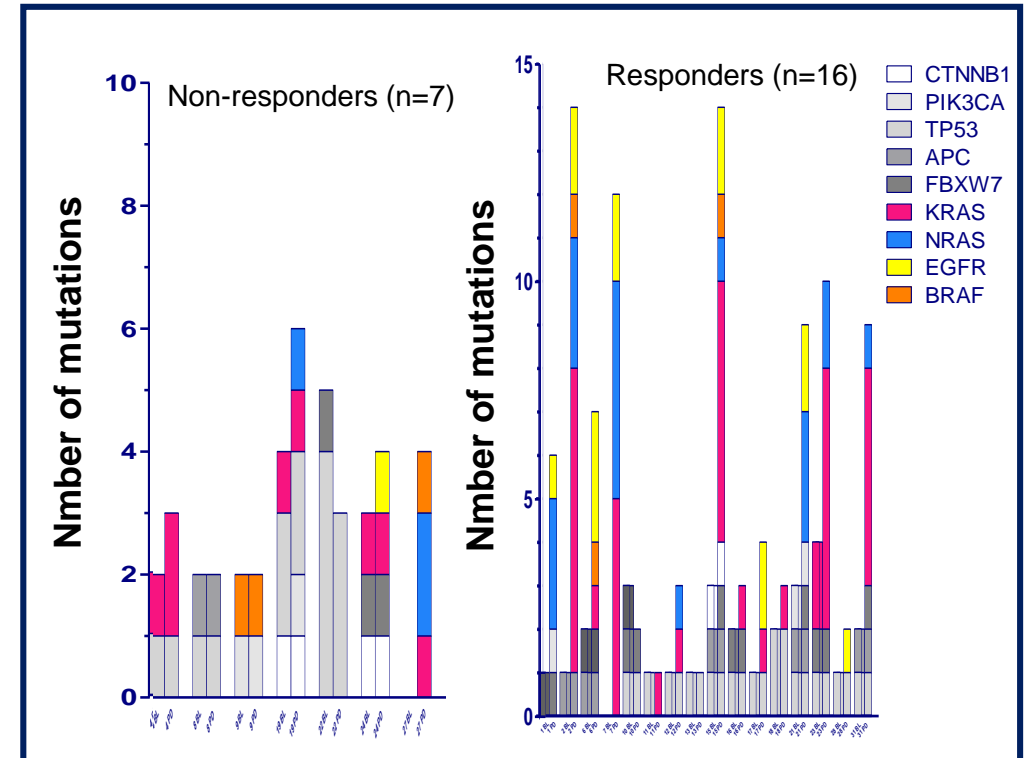## Resistantie mutatie detection in ctDNA (CRC-IMPACT)

Anti-EGFR antibodies
- Cetuximab
- Panitumumab

Douillard et al. N Eng J Med, 2013:369:1023-34.
Douillard et al. J Clin Oncol 2010;28:4697-4705

Cetuximab blocks EGFR
- EGFR-MAPK drives CRC
- 3rd line monotherapy in mCRC
- WT for KRAS/NRAS/BRAF
- Only 40-45% benefit
- Drug is expensive

Intrinsic resistance (40% of cases)
- KRAS (codon 12,13)
- KRAS (codon 61, 117, 146) } 17% of cases
- NRAS (codon 12, 13, 61)

CRC cfDNA assay
AKT1 (1)
APC (36)
BRAF (3)
CTNNB1 (6)
EGFR (10)
FBXW7 (8)
GNAS (5)
HER2 (9)
KRAS (13)
MAP2K1 (10)
NRAS (22)
PIK3CA (14)
SMAD4 (8)
TP53 (97)

True Hotspot Variants / **False Positive Workflow errors**

UMI NGS

Is serial cfDNA analysis useful to understand therapy failure?

Non-responders (n=7) — Number of mutations

Responders (n=16) — Number of mutations

Legend: CTNNB1, PIK3CA, TP53, APC, FBXW7, KRAS, NRAS, EGFR, BRAF

van Helden EJ, Mol Oncol. 2019 Nov;13(11):2361-2374

Conclusion of this study
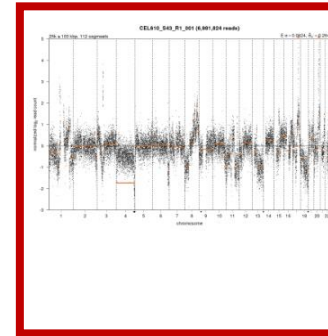
ctDNA is a predictive marker:

- ~ 40% of non-responders identified at baseline
- (presence of KRAS/BRAF mutations)

--> Potential to avoid "unnecessary toxicity" and to save costs

ctDNA gives insight into acquired resistance to anti-EGFR MoAbs:

- RAS/BRAF and EGFR mutations are significantly enriched at PD
- Resistance is multi-clonal riven by multiple mechanisms

-> might allow to detect progression at an early stage?
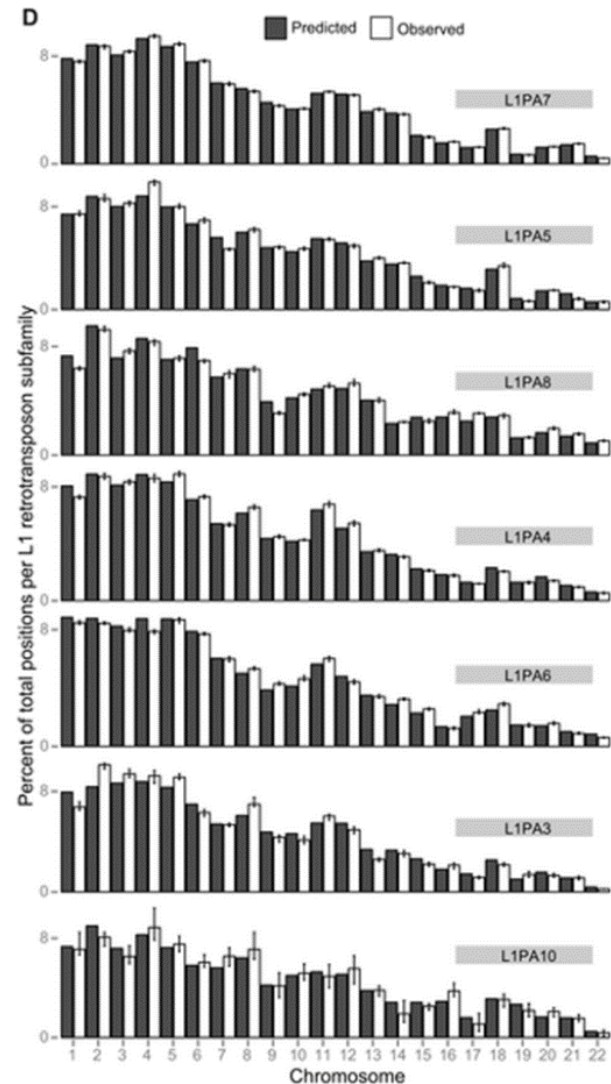
Prognose en response predictie

NIPT/Fastseq

CelvrijDNA (cfDNA/ctDNA)

Fast Aneuploidy Screening Test Sequencing System (FAST-SeqS)

~20.000 unieke LINE1-elementen in ons genoom

- Doelgerichte analyse van Line-1 elementen
  → Lage resolutie CNV profielen
- Snel (2 dagen) en goedkoop (~30 euros)
- Gevoeligheid ≥5-10% van het cfDNA = ctDNA

Since ~2017

1st PCR – LINE-1 specific PRIMER SEQUENCES

Index primer sequence (F)   LINE-1 specific sequence
LINE1_F TCTTTCCCTACACGACGCTCTTCCGATCT ACACAGGGAGGGGAACAT

Index primer sequence (R)   LINE-1 specific sequence
LINE1_R GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TTGCCATGGTGGTTTGCT

2nd PCR - Index PCR (single index) PRIMER SEQUENCES

F AATGATACGGCGACCACCGAGATCTACACT CTTTCCCTACACGACGCTCTTCCGATCT

Index
R CAAGCAGAAGACGGCATACGAGAT CGTGAT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Data analysis

1. Trimming of reads (remove primer/adapter sequences)
2. Mapping reads onto genome
3. Count number of reads per chromosome arm
4. Calculate Z-score per arm and genome wide

$$Z\text{-score} = \frac{count\ sample - avg\ count\ normal\ controls*}{stdev\ normal\ controls*}$$

*Data from 15 female and 15 male controls from Graz

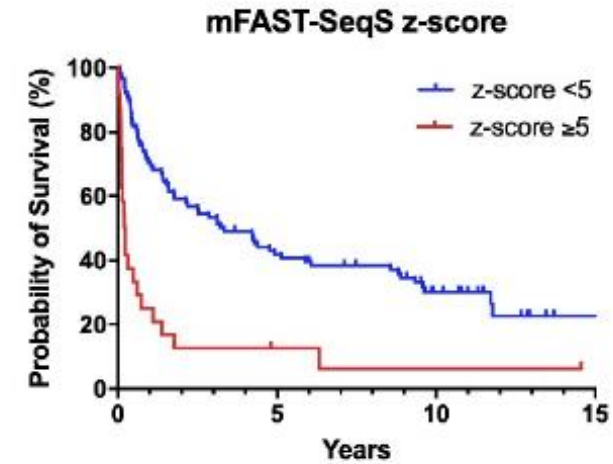gZ-score uses sum of squares of samples and controls

Genoom-brede Z score (gZ-score) >5:
100% sens. & 80% spec.
→ Genoeg ctDNA voor lpWGS

Belic J, Clin Chem 2015;61:838–49.

Enkele klinische toepassingen:

- Op cfDNA uit liquor (mBC)
    - Positief 19 vd 35 patienten (54%)
    - Predictief voor OS

    Angus et al Clin. Canc. Res. 2021

- Op cfDNA uit plasmsa (mBlCa; RESPONDER)
    - Positive 15 out of 41 (36%) patients
    - (before start with pembrolizumab (PD1-i) treatment)
    - Predicted response to Pembrolizumab

    Mendelaar, Wilting, Lolkema et al in prep

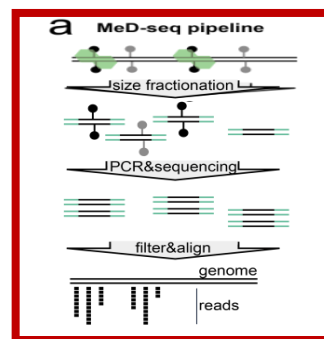- Op cfDNA van CRPC (on going)

    Isebia, Jansen, Wilting, Lolkema et al on going

- Fast-SeqS: is veelbelovende prognostische marker en bruikbaar voor therapie response monitoring

mFAST-SeqS z-score

Response	gZ-score >5	gZ-score <=5	total
PD	11	9	20
SD/PR	3	18	21
Total	14	27	41

p = 0.006 (Chi-Square)

Monitoring (screening)

CelvrijDNA (cfDNA/ctDNA)

MeD-seq

Monitoring disease using MeD-seq

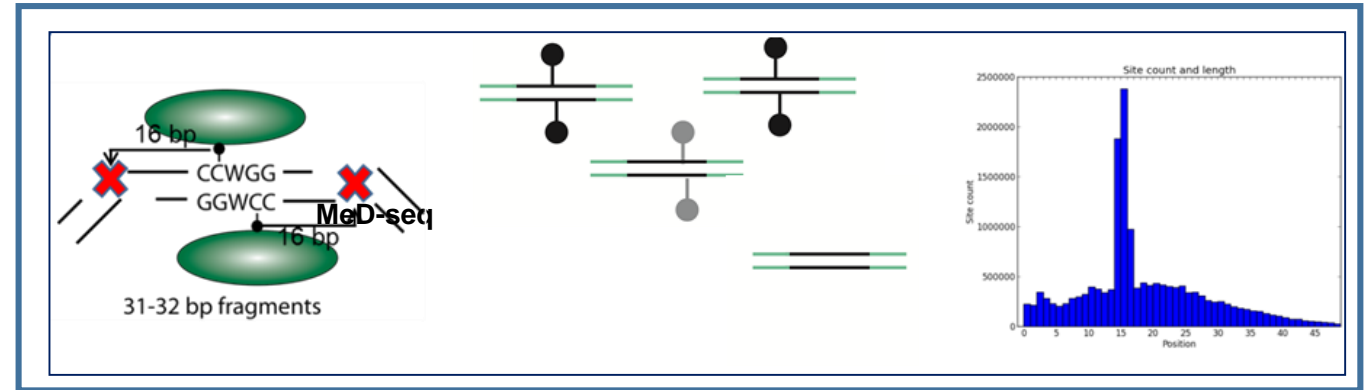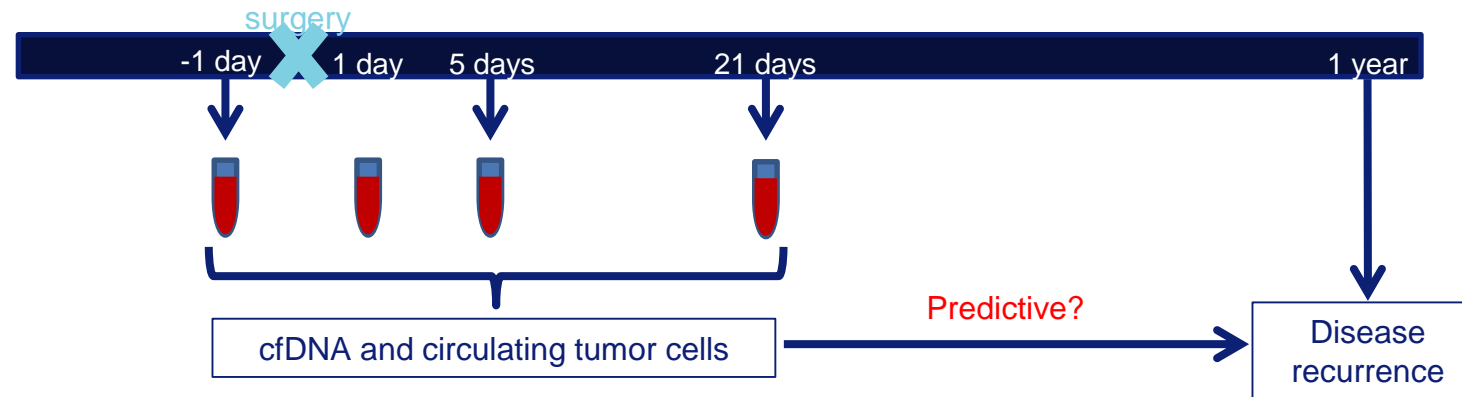A universal marker for disease monitoring?

Since ~2016

Methylation dependent restriction (LpnPI) followed by NGS

- mCRC/mBC compared to HBD.
- cfDNA isolated
- Subjected to MeD-seq

Deger, Gribnau & Wilting et al 2022

MIRACLE:

surgery

-1 day   1 day   5 days   21 days   1 year

cfDNA and circulating tumor cells

Predictive?

Disease recurrence

240 patients with colorectal liver metastases (CRLM)

Monitoring disease using MeD-seq
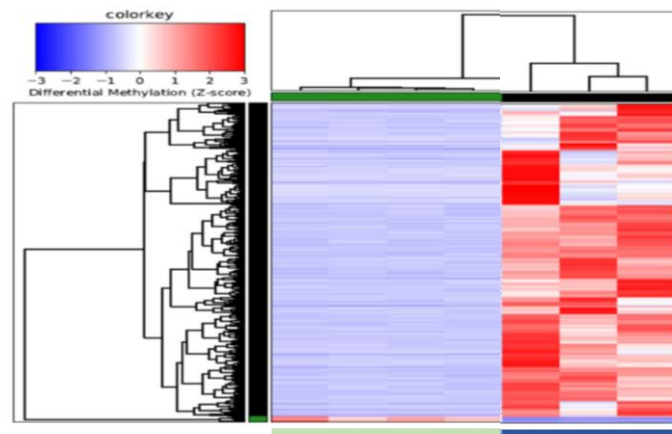
A universal marker for disease monitoring?

- mCRC/mBC compared to HBD.
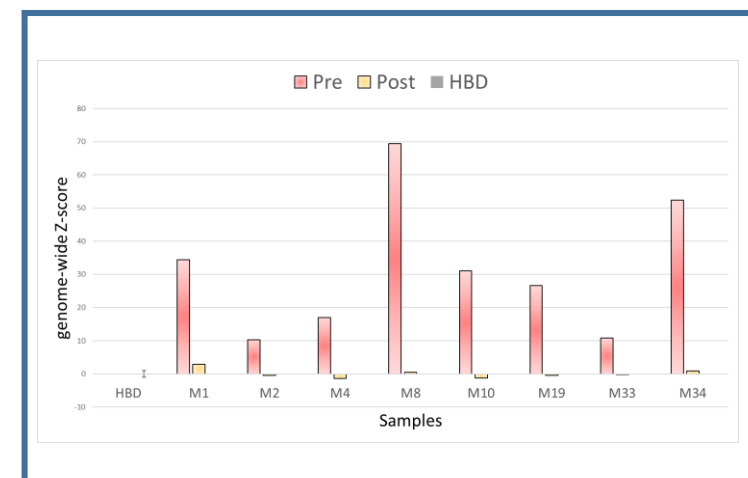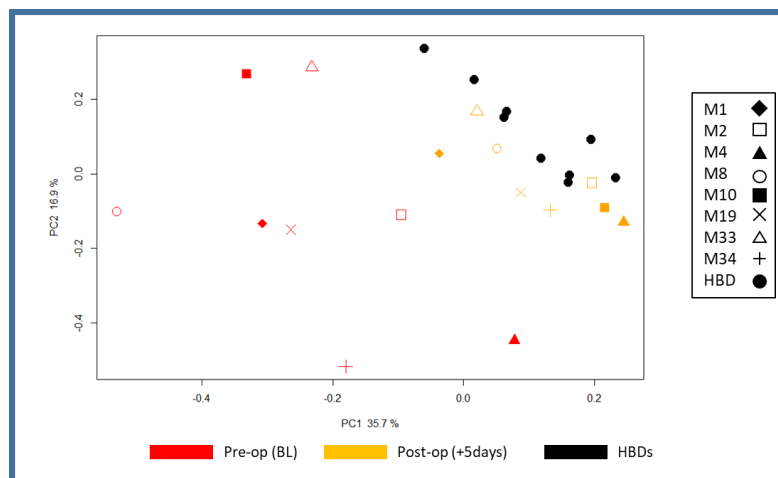- cfDNA isolated
- Subjected to MeD-seq

Methylation dependent restriction (LpnPI) followed by NGS

Deger, Gribnau & Wilting et al 2022
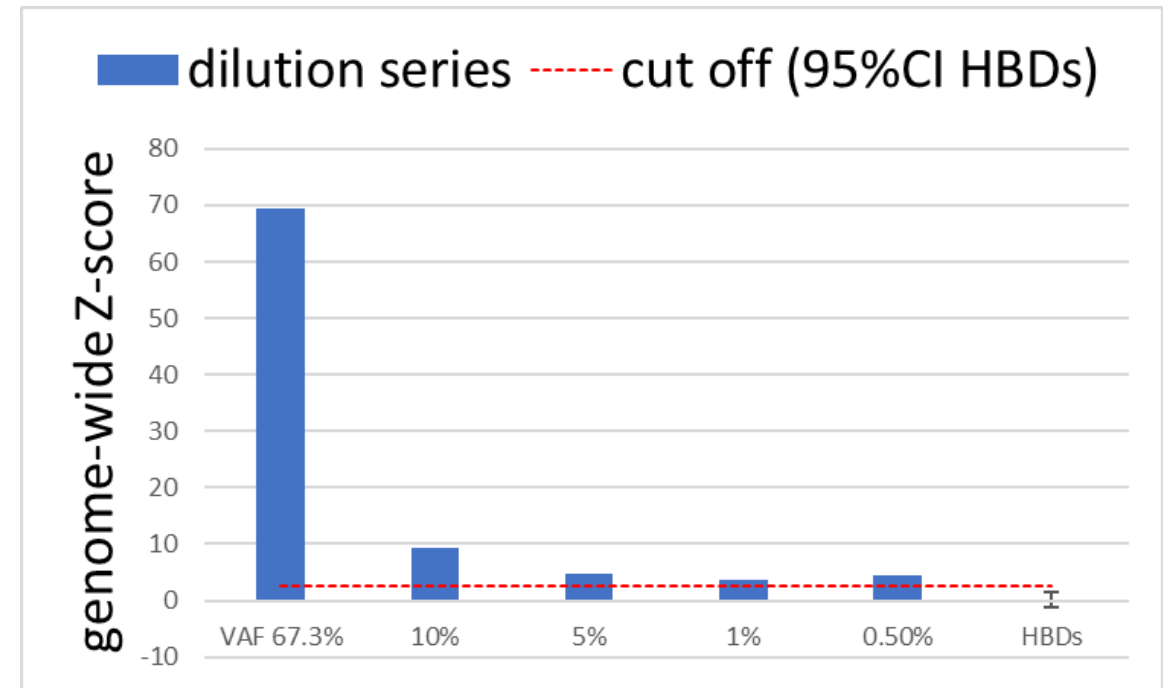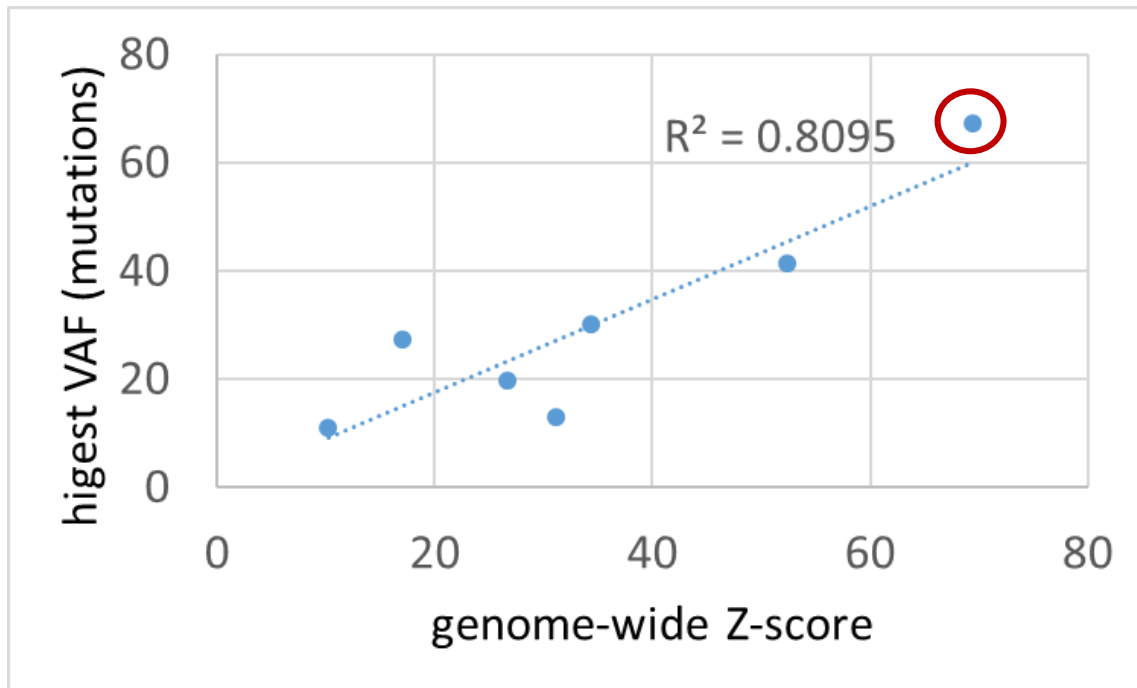
Differentiele DMR analyse

Controls v CRC

genome-wide Z-score (total deviation in MeD-seq profile from HBDs) reflects level of ctDNA in the blood

How sensitive is this assay?

Genome-wide Z-score remains elevated down to 0.5%
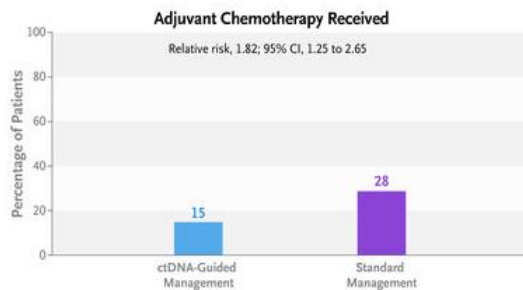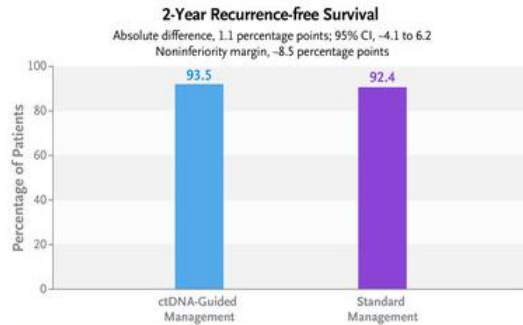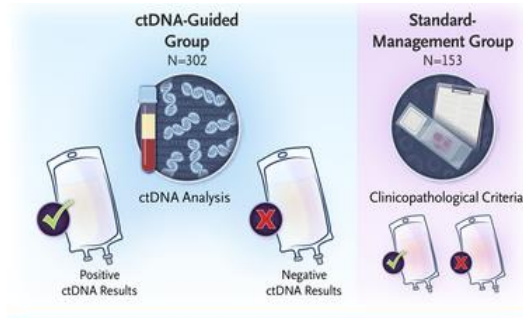(upper limit of 95% CI of controls)

Samenvatting van onderwerp:
Vloeibare biopten (CTCs/ctDNA) als diagnosticum

- Er is grote behoefte naar prognostische en predicatieve markers,

- Dergelijke markers willen we het liefst regelmatig meten tijdens het ziekteproces,

- Vloeibare biopten bieden een uniek middel om middels een simpele, niet-invasieve bloedafname,

1. de oorsprong van de primaire tumor te bepalen (gen expressie/methylering)

2. de prognose te betalen (CTC aantallen/FASTseq Z score)

3. Therapie response te voorspellen (ARv7)

4. DLA kan de CTC opbrengst verhogen → single CTC profilering/heterogeniteit onderzoek haalbaar

5. Response op therapie te monitoren (ctDNA VAF)

6. Optreden van resistentie monitoren (KRAS/ERS1) → in de kliniek voor longkanker

7. Universeel ziekte te monitoren (mbv MeD-seq of FASTseq)

8. Er zijn methoden in opkomst met zelfs potentie voor screening (CancerSeek; Fragmentomics)

Klinische significantie?

ctDNA guiding adj. chemotherapy in Stage II CRC
(DYNAMIC study: a ctDNA-guided trial)

BACKGROUND
- Adj chemo in stage II CRC is debated.
- cDNA predicts very poor survival

Primary objective: Do ctDNA-positive patients benetif from adj chemo.

METHODS
- Stage II CRC were randomised (2:1 ratio) to have treatment
- ctDNA-guided or standard-of-care features-guided
- Primary end point was RFS at 2 YRS.

RESULTS
- 455 ps randomized (302 ctDNA-guided; 153 standard).
- Median follow-up 37 months.
- Fewer pats adj chemo in ctDNA group (15% vs. 28%; RR 1.82; 95% CI, 1.25 to 2.65).
- 2-yr RFS in ctDNA-guided management was noninferior (93.5% versus 92.4%)
- 3-yr RFS was 86.4% in ctDNA-pos. (adj chemo) versus 92.5% in untreated ctDNA-neg

CONCLUSIONS
A ctDNA-guided treatment in stage II CRC can reduce adj. chemo use not affecting RFS

Tie, J. et al. 2022. N Engl J Med; 386:2261-2272

Translational Cancer Genomics
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