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# Liquid Biopsy in der Krebsfrüherkennung

Elke Holinski-Feder

2024

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## Genetische Veränderungen auf dem Weg zur Tumorzelle
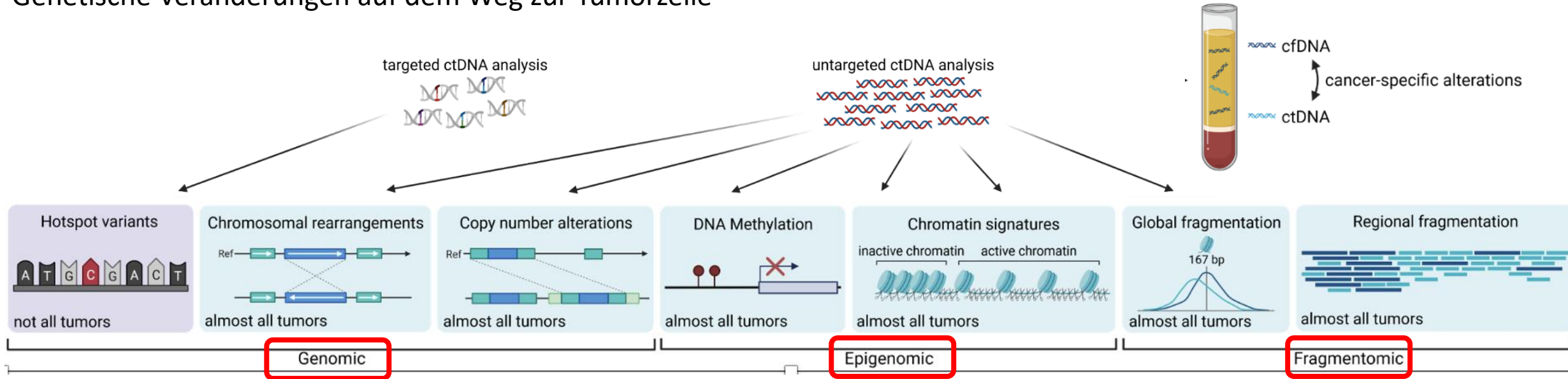


SNV
Spezifische
Driver -
Varianten

SV
Struktur-
Varianten
Dosisneutral
Translokationen
Inversionen

CNV
Deletionen
Duplikationen
von dosis-
sensitiven
Genen

Methylation
An- und Ab-
schalten von
Genen durch
Methylierung
von
Promoter-
regionen

Chromatin-
Veränderungen
Zugänglichkeit
von regulatorischen
Sequenzen
An- und Abschalten
Von Genen

Fragmentierung
Global über das gesamte Genom
Regional in spezifischen Bereichen
Mit tumorrelevanten Genen

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# A Cell-free DNA Blood-Based Test for Colorectal Cancer Screening

Daniel C Chung [1], Darrell M Gray 2nd [1], Harminder Singh [1], Rachel B Issaka [1],
Victoria M Raymond [1], Craig Eagle [1], Sylvia Hu [1], Darya I Chudova [1], AmirAli Talasaz [1],
Joel K Greenson [1], Frank A Sinicrope [1], Samir Gupta [1], William M Grady [1]

Affiliations  + expand
PMID: 38477985   DOI: 10.1056/NEJMoa2304714

**Conclusions:** In an average-risk screening population, this cfDNA blood-based test had 83% sensitivity for colorectal cancer, 90% specificity for advanced neoplasia, and 13% sensitivity for advanced precancerous lesions. (Funded by Guardant Health; ECLIPSE ClinicalTrials.gov number, NCT04136002.).

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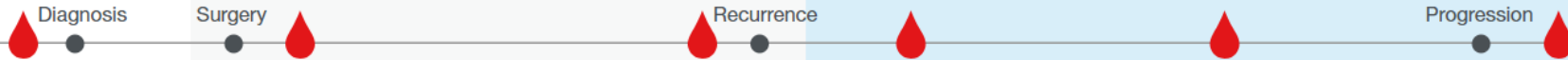Medizinisch Genetisches Zentrum

**SCREENING AND EARLY DETECTION***

**EARLY-STAGE CANCER***
Residual disease detection and recurrence surveillance (commercially available since 2021[3])

**ADVANCED CANCER***
Treatment selection and optimization (commercially available since 2014[3,4†])

Patient Timeline[‡]  Diagnosis  Surgery  Recurrence  Progression

shield™

GUARDANT**REVEAL**

FDA APPROVED
GUARDANT**360**CDx

GUARDANT**360**Response

GUARDANT**360**

GUARDANT**360**TissueNext

— Laboratory-developed test
— FDA-approved test

**ECLIPSE** STUDY
A registrational study validated Shield for CRC screening in over 10,000 average-risk patients.[3,5]

*200,000 patients tested & 260+ peer-reviewed publications[3]*

**National Cancer Institute and National Comprehensive Cancer Network cancer centers use Guardant Health's existing blood-based tests to inform treatment decisions for patients with early- and late-stage cancer[3]**

**Varianten, Fragmentierung, Methylierung**
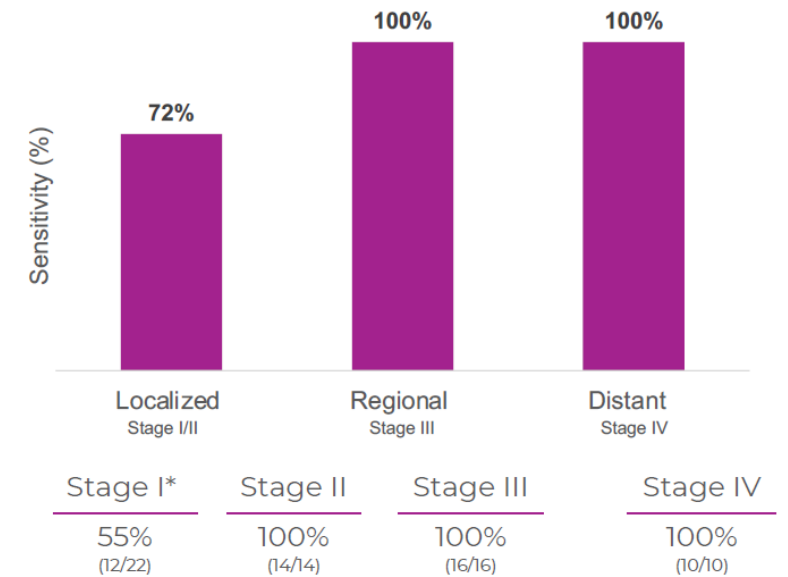**Keine Preisangaben**

**Varianten NGS-Panel , Methylierung**
**3500 Dollar**

**Varianten NGS-Panel**
**5000 Dollar**

A multimodal approach for accurate and early detection of colorectal cancer

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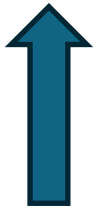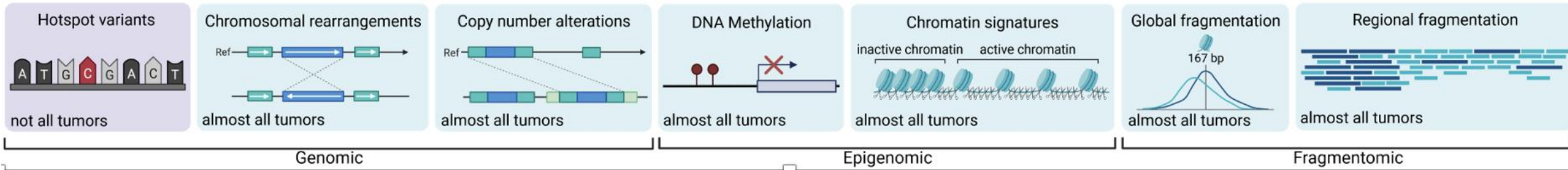
| Variable | Most Advanced Finding on Colonoscopy | cfDNA Blood-Based Test | |
|---|---|---|---|
| | | Positive Test | Sensitivity (95% CI) |
| | no. | no. | % |
| Colorectal cancer | | | |
| Any | 65 | 54 | 83.1 (72.2–90.3) |
| Stage I, II, or III* | 48 | 42 | 87.5 (75.3–94.1) |
| Advanced precancerous lesions† | 1116 | 147 | 13.2 (11.3–15.3) |
| | | | Specificity (95% CI) |
| Nonadvanced adenomas, nonneoplastic findings, and negative colonoscopy | 6680 | 698 | 89.6 (88.8–90.3) |
| Nonneoplastic findings and negative colonoscopy | 4514 | 457 | 89.9 (89.0–90.7) |

## Stage I – III Sensitivity: 81%#



Sensitivity (%)

72% — Localized Stage I/II
100% — Regional Stage III
100% — Distant Stage IV

| Stage I* | Stage II | Stage III | Stage IV |
|---|---|---|---|
| 55% (12/22) | 100% (14/14) | 100% (16/16) | 100% (10/10) |

*Assumes 5 incompletely staged malignant polyps are Stage I disease (1/5 detected)
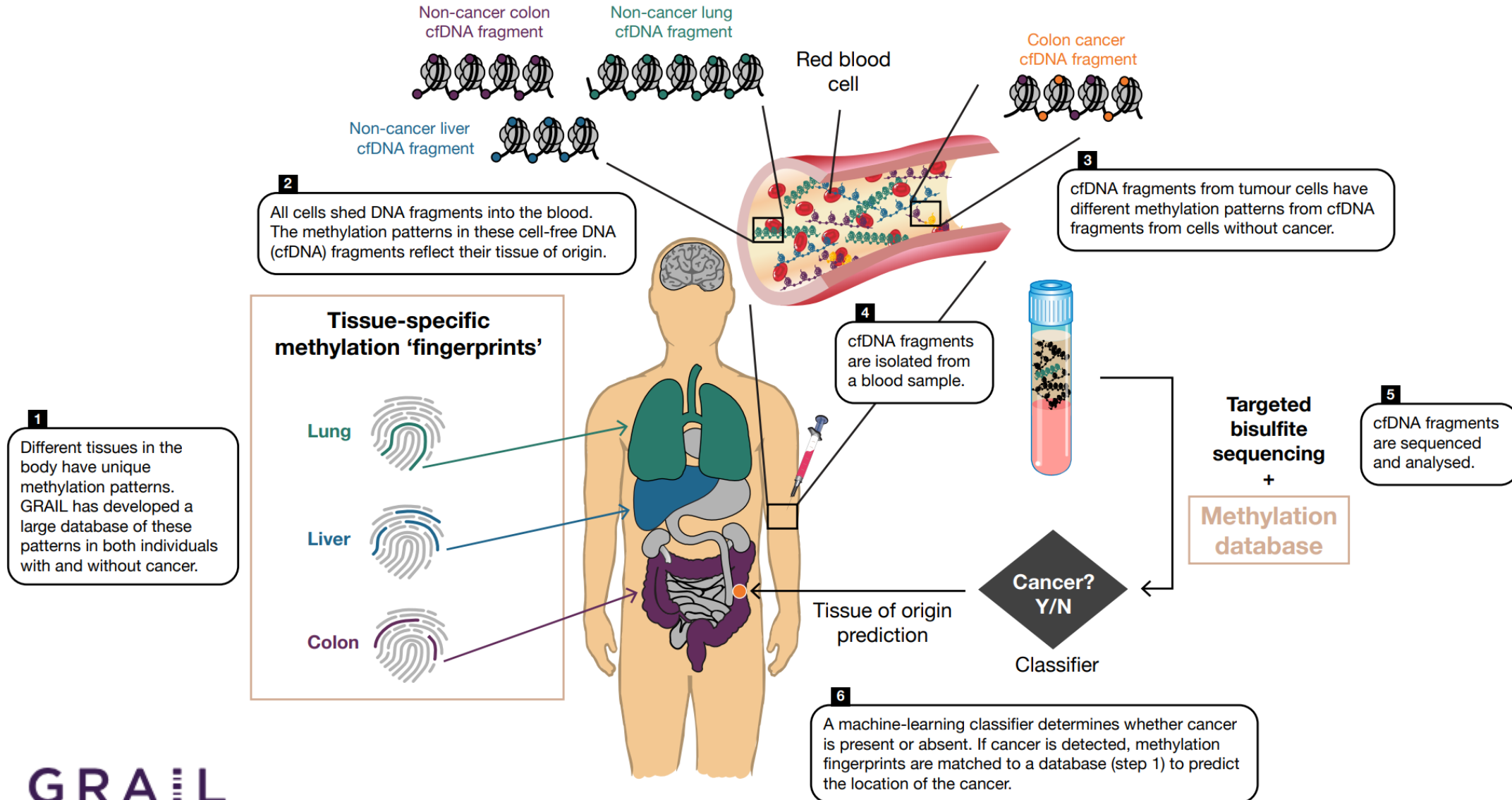
**Guardant Health Shield:**
- screening black box,
- vermutlich nicht gemomweit,
- binäres Ergebnis, ctDNA da oder nicht da

Proben müssen in die USA geschickt werden, IGEL

Vermutlich regionale Sequenzierung

**What else?**

- Grail Illumina = Methylierungsmuster
- EPINUC = Modifikation von Nukleosomen
- LIFE-CNA = LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration

# GRAIL - Multicancer early detection test performance
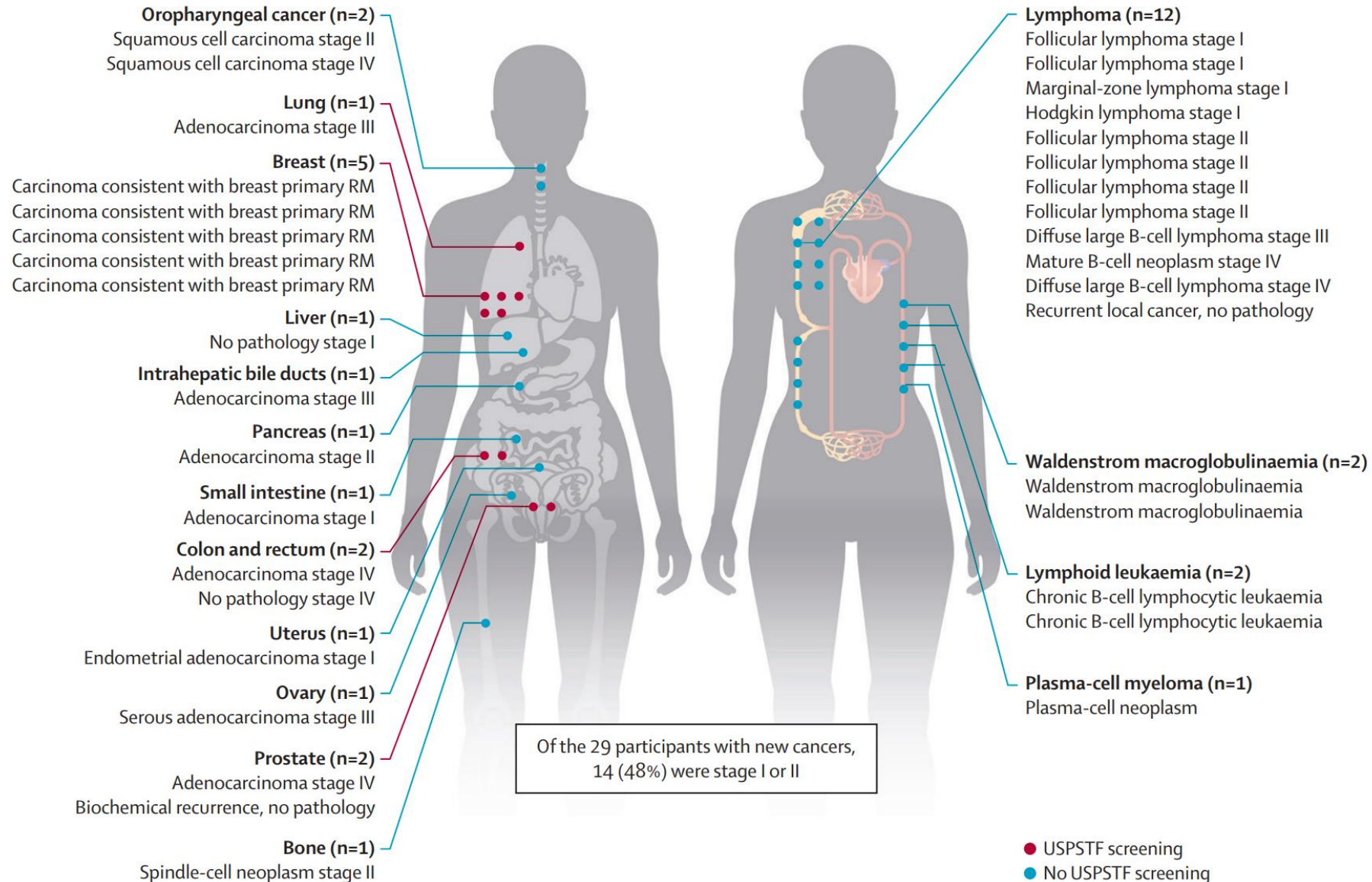
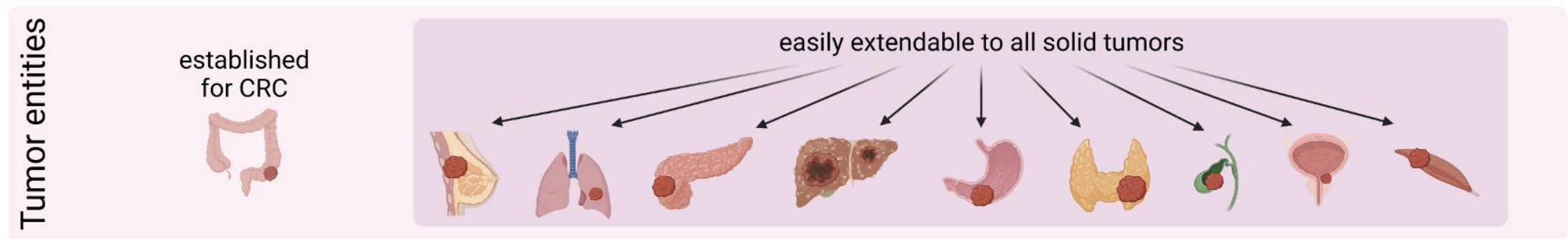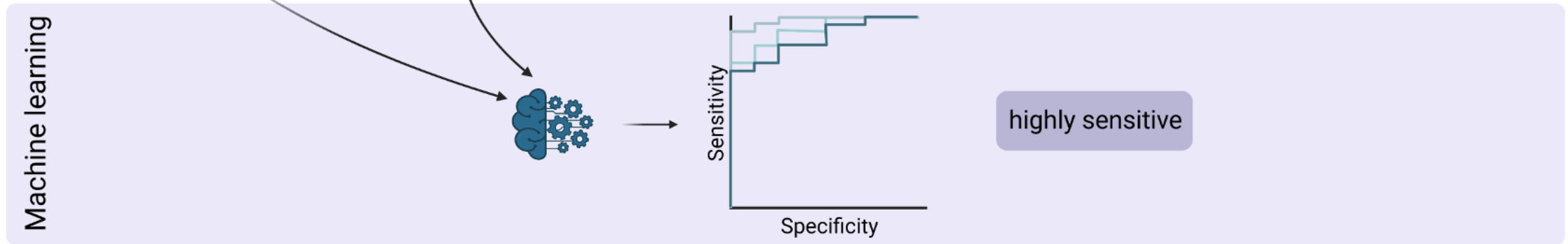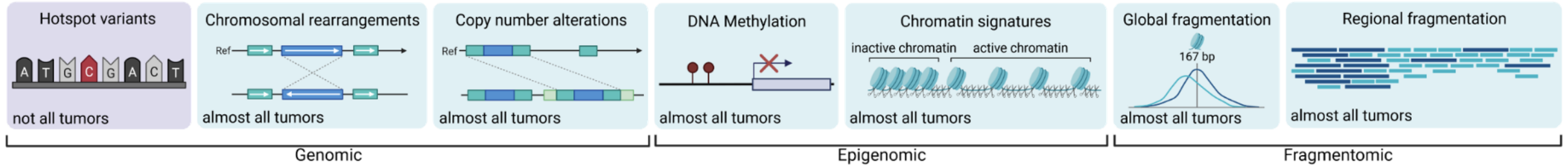| | Age ≥50 years with additional cancer risk (n=3681) | Age ≥50 years without additional cancer risk (n=2940) | Total (n=6621) |
|---|---|---|---|
| **Resolution** | | | |
| All | 56 (1·5%) | 36 (1·2%) | 92 (1.4%) |
| True positive | 24 (0·7%) | 11 (0·4%) | 35 (0·5%) |
| False positive | 32 (0·9%) | 25 (0·9%) | 57 (0·9%) |
| Positive predictive value | 24/56; 43% (30·8–55·9) | 11/36; 31% (18·0–46·9) | 35/92; 38% (28·8–48·3) |
| Negative predictive value | 3449/3502; 98·5% (98·0–98·8) | 2786/2819; 98·8% (98·4–99·2) | 6235/6321; 98·6% (98·3–98·9) |
| Specificity | 3449/3480; 99·1% (98·7–99·4) | 2786/2810; 99·1% (98·7–99·4) | 6235/6290; 99·1% (98·9–99·3) |
| Yield rate | 24/3681; 0·65% (0·41–0·92) | 11/2940; 0·37% (0·17–0·61) | 35/6621; 0·53% (0·36–0·71) |
| Number needed to screen | 3681/24; 153 (108–245) | 2940/11; 267 (163–588) | 6621/35; 189 (141–276) |
| **Predicted origin accuracy*** | | | |
| First CSO correct | 20/23; 87% (67·9–95·5) | 9/11; 82% (52·3–94·9) | 29/34; 85% (69·9–93·6) |
| First or second CSO correct | 23/23; 100% (85·7–100) | 10/11; 91% (62·3–99·5) | 33/34; 97% (85·1–99·8) |

Data are n (%), n/N, or % (95% CI). CSO=cancer signal origin. *Excludes one participant with indeterminate CSO from the true-positive set.

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**Oropharyngeal cancer (n=2)**
Squamous cell carcinoma stage II
Squamous cell carcinoma stage IV

**Lung (n=1)**
Adenocarcinoma stage III

**Breast (n=5)**
Carcinoma consistent with breast primary RM
Carcinoma consistent with breast primary RM
Carcinoma consistent with breast primary RM
Carcinoma consistent with breast primary RM
Carcinoma consistent with breast primary RM

**Liver (n=1)**
No pathology stage I

**Intrahepatic bile ducts (n=1)**
Adenocarcinoma stage III

**Pancreas (n=1)**
Adenocarcinoma stage II

**Small intestine (n=1)**
Adenocarcinoma stage I

**Colon and rectum (n=2)**
Adenocarcinoma stage IV
No pathology stage IV

**Uterus (n=1)**
Endometrial adenocarcinoma stage I

**Ovary (n=1)**
Serous adenocarcinoma stage III

**Prostate (n=2)**
Adenocarcinoma stage IV
Biochemical recurrence, no pathology

**Bone (n=1)**
Spindle-cell neoplasm stage II

**Lymphoma (n=12)**
Follicular lymphoma stage I
Follicular lymphoma stage I
Marginal-zone lymphoma stage I
Hodgkin lymphoma stage I
Follicular lymphoma stage II
Follicular lymphoma stage II
Follicular lymphoma stage II
Follicular lymphoma stage II
Diffuse large B-cell lymphoma stage III
Mature B-cell neoplasm stage IV
Diffuse large B-cell lymphoma stage IV
Recurrent local cancer, no pathology

**Waldenstrom macroglobulinaemia (n=2)**
Waldenstrom macroglobulinaemia
Waldenstrom macroglobulinaemia

**Lymphoid leukaemia (n=2)**
Chronic B-cell lymphocytic leukaemia
Chronic B-cell lymphocytic leukaemia

**Plasma-cell myeloma (n=1)**
Plasma-cell neoplasm

Of the 29 participants with new cancers,
14 (48%) were stage I or II

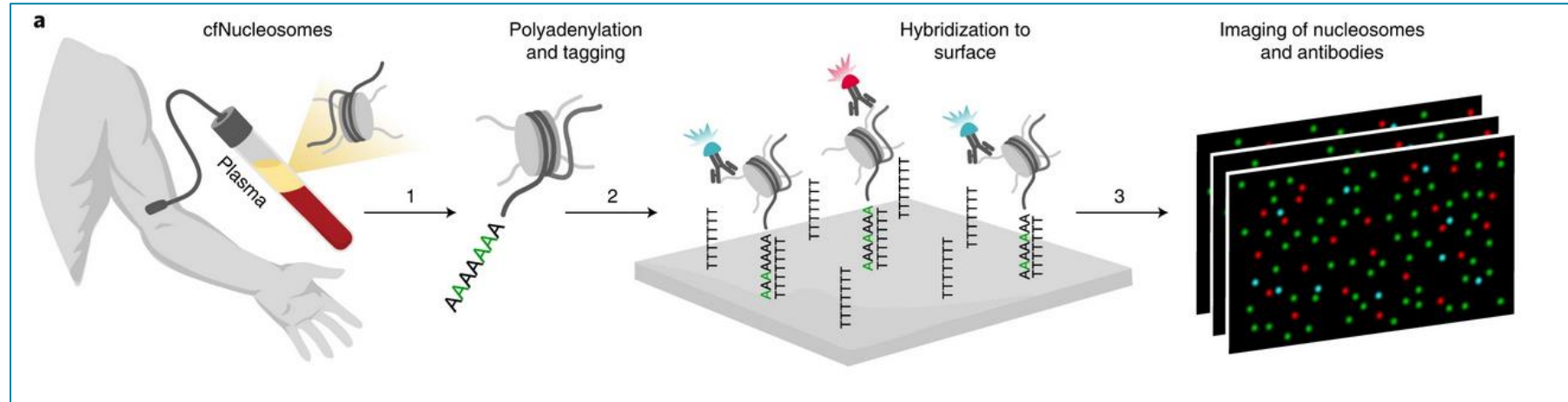● USPSTF screening
● No USPSTF screening

# Analysis of circulating tumor DNA - LIFE-CNA - CRC

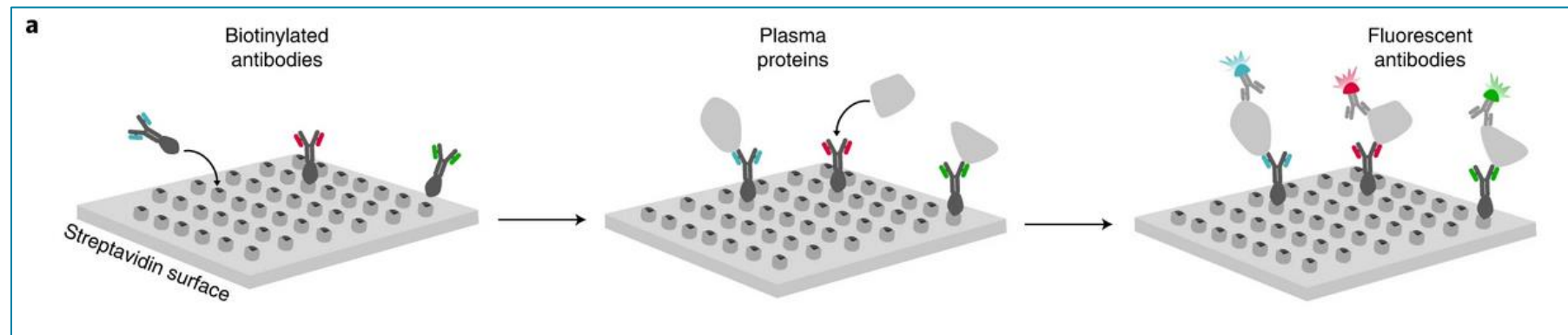*LIFE-CNA = LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration analysis*

cf Nucleosomes +PTMs
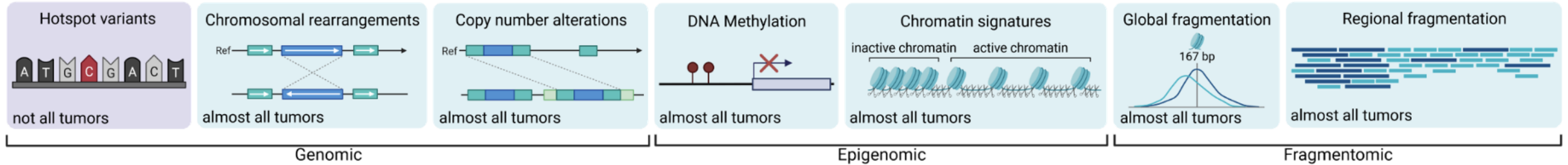


multiplexed protein biomarkers

EPINUC-Seq
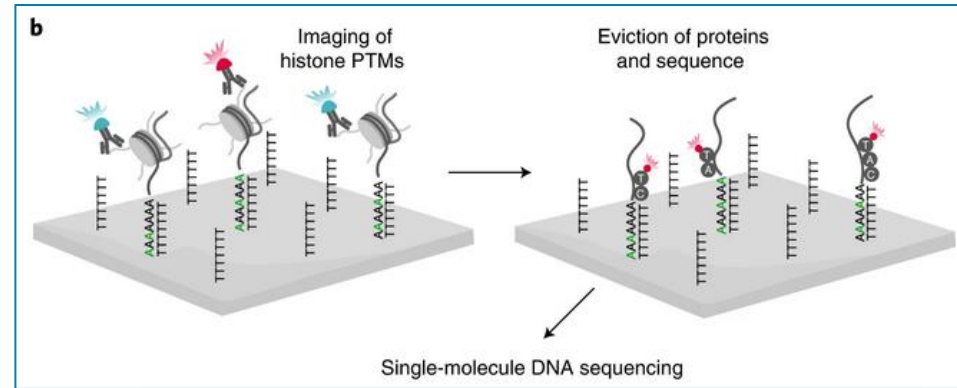


Proof of principle
→ sequences map to regions specific in tissue of origin
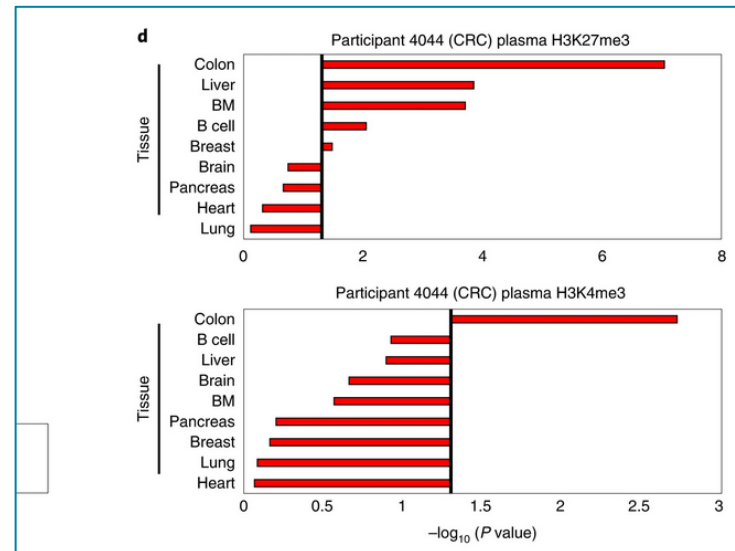
# Analysis of circulating tumor DNA - LIFE-CNA - CRC

*LIFE-CNA = LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration analysis*

**RESEARCH**                                                        **Open Access**

## Somatic copy number alteration and fragmentation analysis in circulating tumor DNA for cancer screening and treatment monitoring in colorectal cancer patients

Ariane Hallermayr[1,2,3], Tobias Wohlfrom[1], Verena Steinke-Lange[1,4], Anna Benet-Pagès[1,5], Florentine Scharf[1], Ellen Heitzer[6,7,8], Ulrich Mansmann[3], Christopher Haberl[9], Maike de Wit[10,11], Holger Vogelsang[12], Markus Rentsch[13,14], Elke Holinski-Feder[1,4] and Julia M. A. Pickl[1,4*]

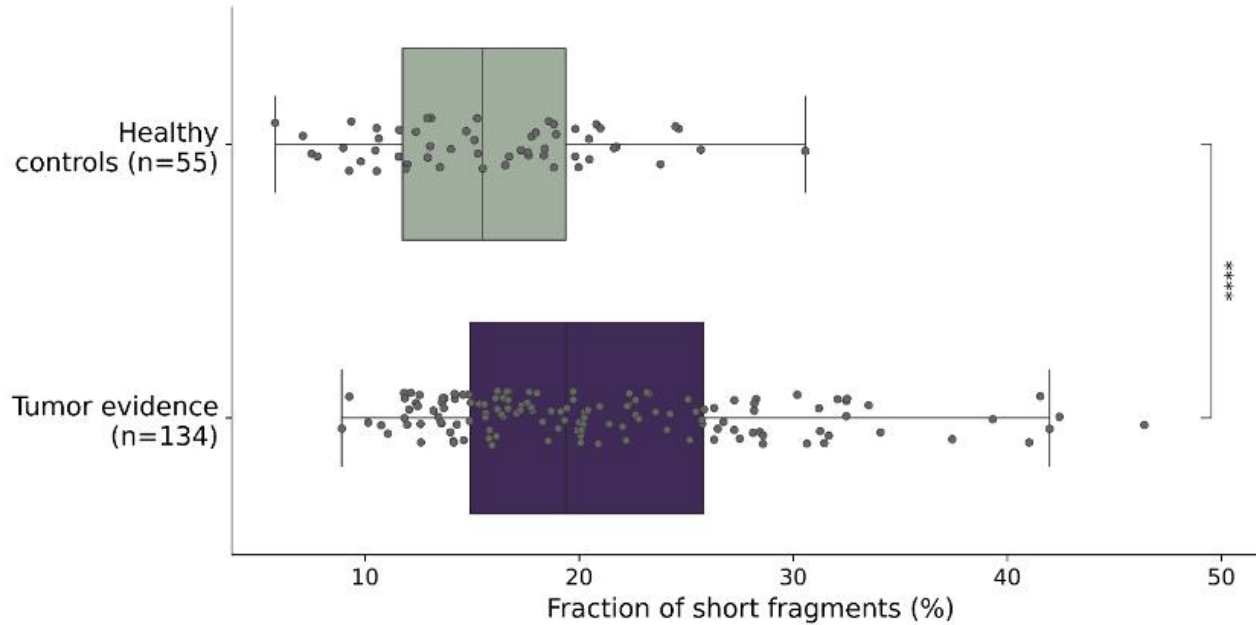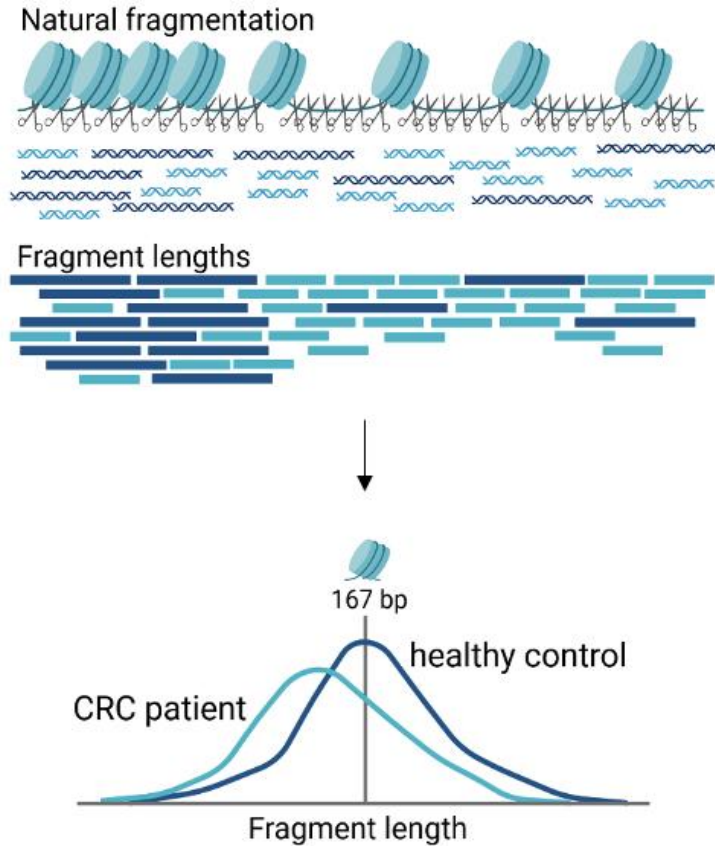*ctDNA detection at diagnosis:*

*16 patients*

**Localized CRC (UICC I-III)**

LIFE-CNA              81% LIFE-CNA + ML:          93%

**Metastatic CRC (UICC IV)**

LIFE-CNA              94% LIFE-CNA + ML:          94%

created with BioRender.com

Natural fragmentation

Fragment lengths

167 bp

healthy control

CRC patient

Fragment length

Healthy controls (n=55)

Tumor evidence (n=134)

Fraction of short fragments (%)

Higher fraction of short fragments (90 to 150 bp) in CRC patients compared to healthy controls

Hallermayr et al. *J Hematol Oncol*. 2022
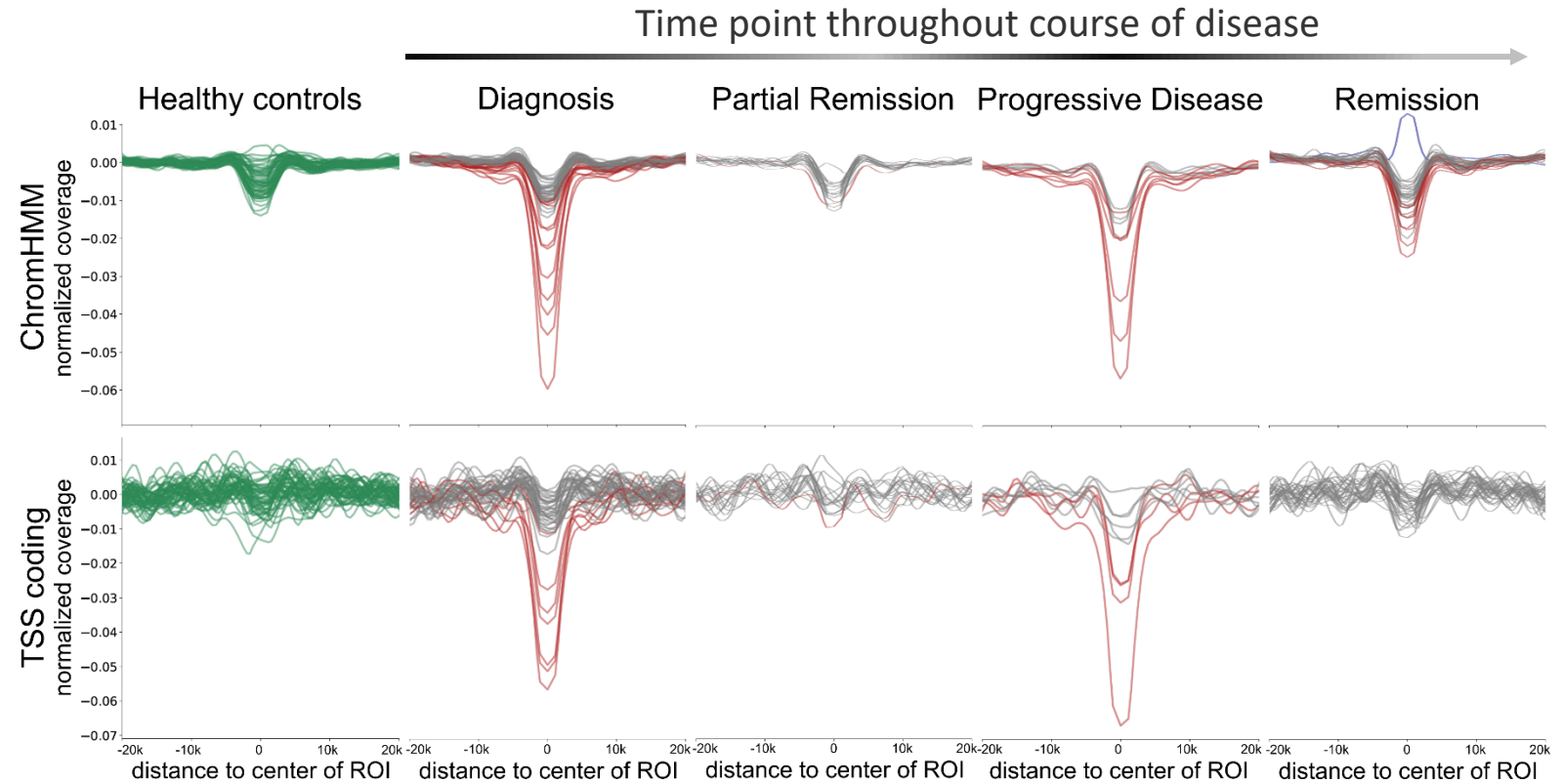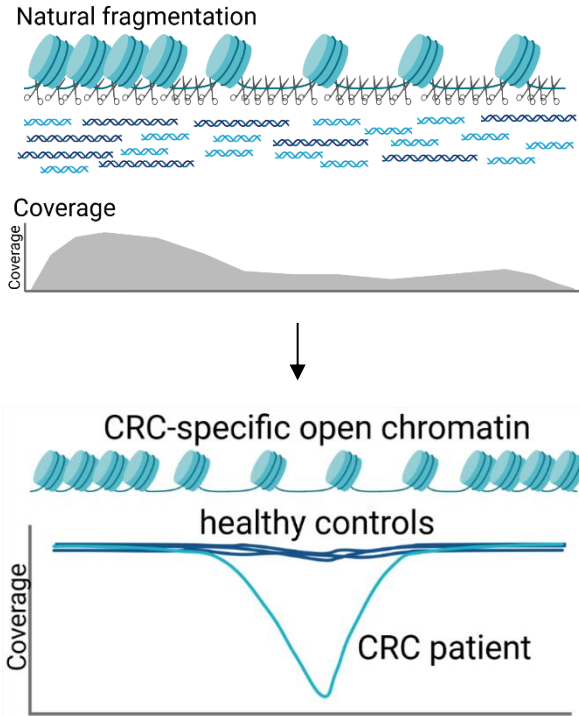
Regional cfDNA Fragmentation as indicator for ctDNA
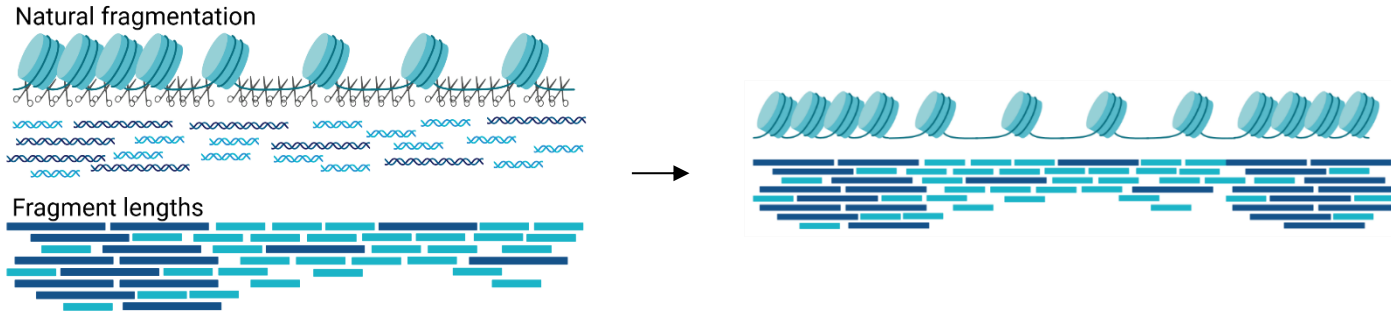
Significant differences in S/L ratio between healthy controls and CRC patient samples with tumor evidence

CRC specific fragmentation profile
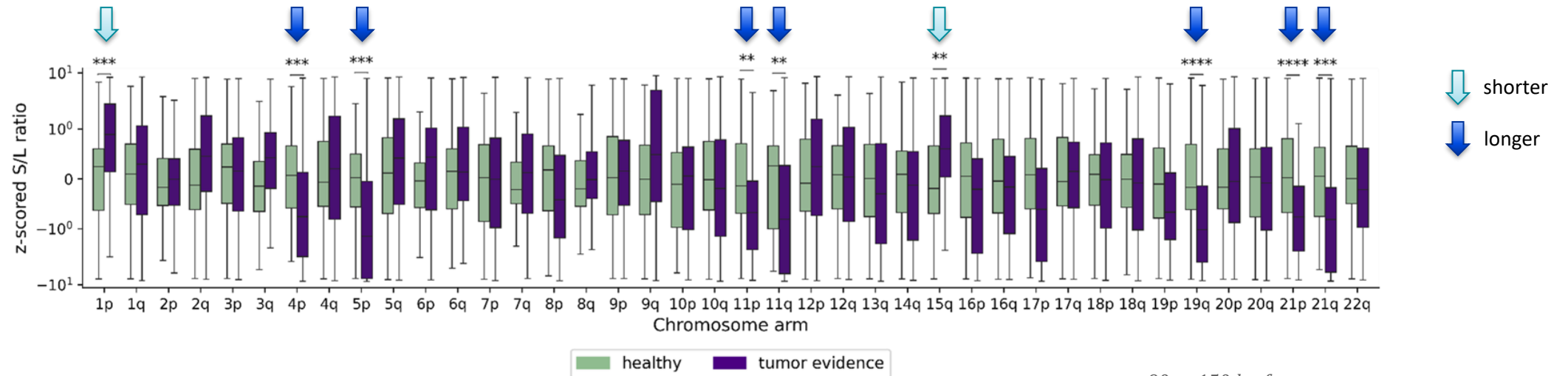
$$S/L{-}ratio = \frac{90 - 150\ bp\ fragments}{151 - 220\ bp\ fragments}$$

Hallermayr et al. *J Hematol Oncol* . 2022

# CRC specific chromatin signatures



Natural fragmentation

Coverage

CRC-specific open chromatin

healthy controls

CRC patient

Coverage

Time point throughout course of disease

Healthy controls | Diagnosis | Partial Remission | Progressive Disease | Remission

ChromHMM normalized coverage

TSS coding normalized coverage

distance to center of ROI

— healthy controls
— no difference to controls
— significantly stronger coverage drop
— significantly weaker coverage drop

Detection of ctDNA based on active chromatin in samples with clinically evident tumor burden

TSS = transcriptional start sites
ChromHMM = chromatin-state discovery and characterization tool

Hallermayr et al. *J Hematol Oncol* . 2022

Early detection of precancerous lesion is difficult
Early detection and disease monitorin of cancer is feasable in the near future

Available now
Guardant Health
- Guardant 360 Tumor advanced cancer: diagnosis and monitoring
- Guardant Reveal early stage cancer: diagnosis and monitoring
Under develpment
- Guardant Shield screening and early detection
- Grail screening and early detection
- LIFE-CNA