



Thomas Karn, Goethe University Frankfurt

Circulating tumor DNA and liquid biopsies: current challenges

Conflict of Interest

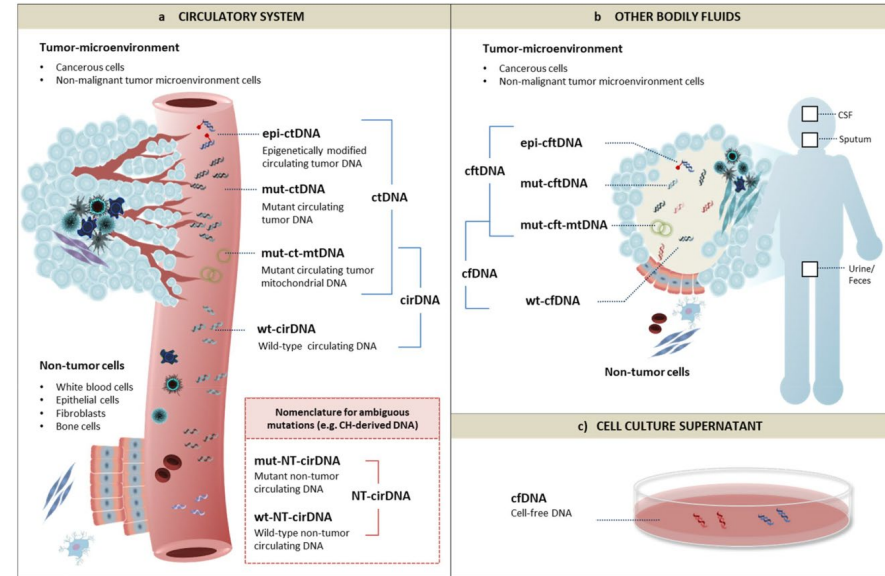
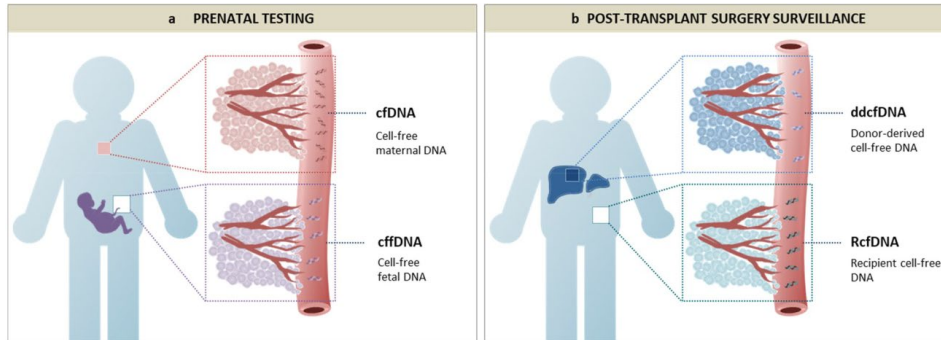
- None

- Besonderheiten von ctDNA/Liquid Biopsy vs. tissue
- Prinzipielle Anwendungen
- Kritische Punkte
- Aktuelle Beispiele
- Neue Entwicklungen

- Schon immer angestrebt
- Tumor Marker
- DTC im Knochenmark → CTC
- ctDNA

Cell free DNA

- cfDNA, circDNA,
- fetal: cffDNA, donor: ddcfDNA
- tumor: ctDNA

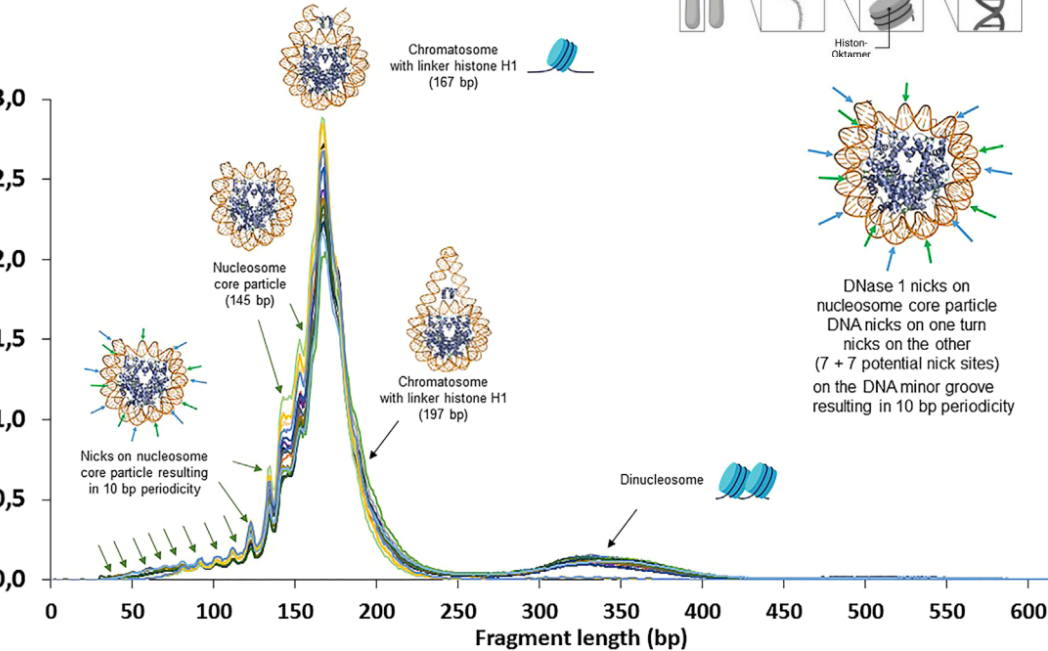
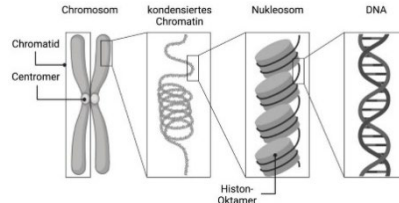


Cell free cfDNA vs. circulating tumor ctDNA

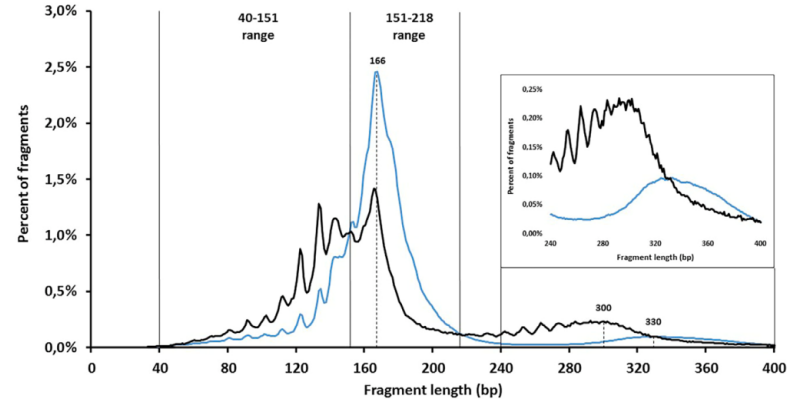
- cfDNA hauptsächlich aus hämatopoetischen Zellen
- Meist durch Zelltod (Nekrose, Phagozytose, Apoptose)
- Halbwertszeit 15 min – 2,5 h (Clearing durch Niere, Leber, Blutkomponenten etc.)
- ctDNA nur sehr geringer Anteil von cell free DNA, abhängig vom Tumorstadium

Wie sieht cell-free DNA aus?

Healthy controls:



“Fragmentomics” Cancer vs. control:



- Sequenzierung und Detektion von Mutationen:
- Tumor-agnostisch
- Tumor-informed

Agnostisch: „Täter-Informationen“

- Nur Probe vom Tatort
- Kategorisierung möglicher Täter
- Bsp.
 - Geschlecht
 - Blutgruppe
 - Merkmale



Informed: „Täter-Überführung“

- Vergleichsproben von Tatverdächtigen
- Ausschluss / Überführung



Tumor-agnostic: Liquid biopsy

- Mutationstest (Einzelgene / Panel)
- Profiling
- Zweck: Stratifikation zur Therapie-Entscheidung
- Resistenzmarker / Targets
- **Bsp.:**
 - ESR1mut (Elacestrant)
 - PIK3CAmut (Apelisib, Inavolisib)
 - BRCA-Reversion
 - ErbB2mut, AKT...

Tumor-informed: Minimal Residual Disease (MRD)

- Therapie-Monitoring
- Early Detection von Rezidiv
- Zweck: Therapie-Änderung (?)
- Spezifischen Test anhand Primärtumor
- **Vorgehen:**
 - Genomsequenzierung Primärtumor
 - Identifikation klonaler Mutationen
 - Etablierung eines individuellen Tests mit hoher Sensitivität und Spezifität (z.B. Rearrangements)

Limitationen:

Tumor-agnostic: Liquid biopsy

- Nur prädefinierte Mutationen
- Begrenzte Anzahl
- Niedrigere Sensitivität

Tumor-informed: MRD

- Genomsequenzierung nur am Primärtumor
- Keine Information über zusätzliche Mutationen im Verlauf wie Resistenzmutationen in Metastasen (ESR1 etc.)

Beispiele:

Tumor-agnostic: Liquid biopsy

- Lokale Pathologie, ddPCR, Panel-Sequencing
- Guardant360
- FoundationOne Liquid CDx
- Sysmex-Inostics
- Plasma-SeqSensei
- ...

Tumor-informed: MRD

- Signatera
- RaDaR

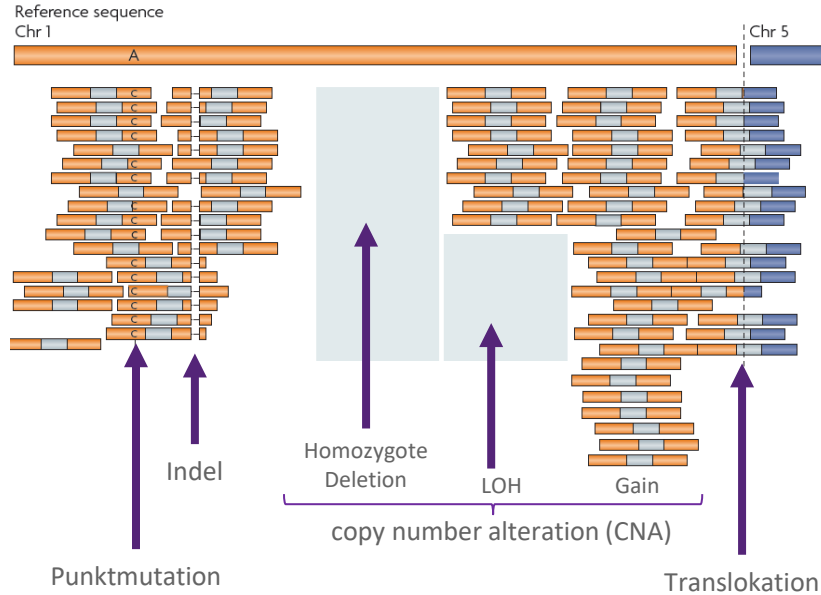
Generelle Limitationen:

Generelle Schwierigkeiten bei ctDNA- im Vergleich zur Gewebe- Untersuchung:

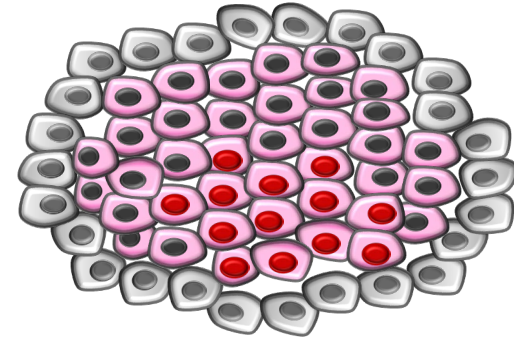
- Keine Information über Zellmorphologie
- Fehlendes Tumor-Microenvironment
- Schwierigkeiten bzgl. Variant Allele Frequency (VAF)
- CHIP

Variant Allele Frequencies (VAF)

NGS: Quantitative Häufigkeiten

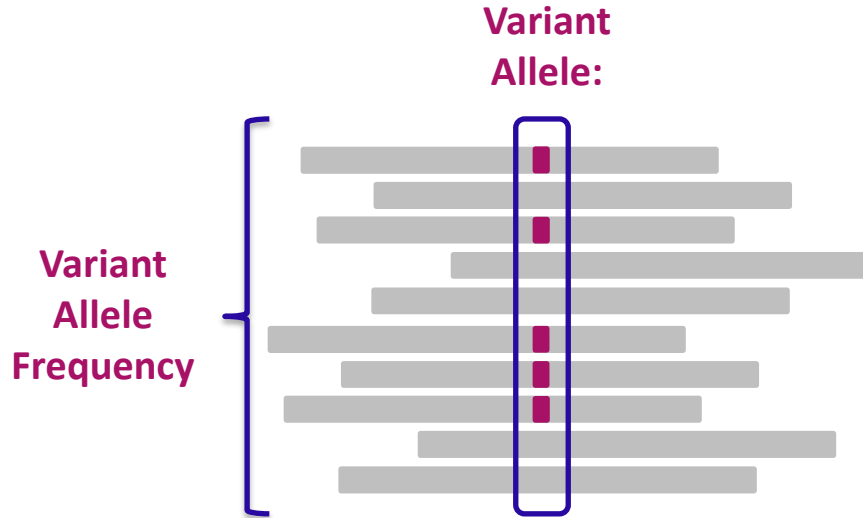


Tissue sample:

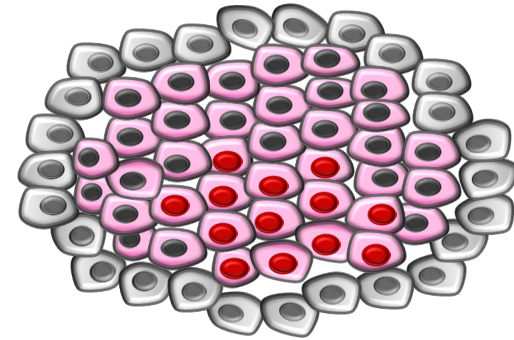


Meyerson et al. Nat Rev Genet 2010

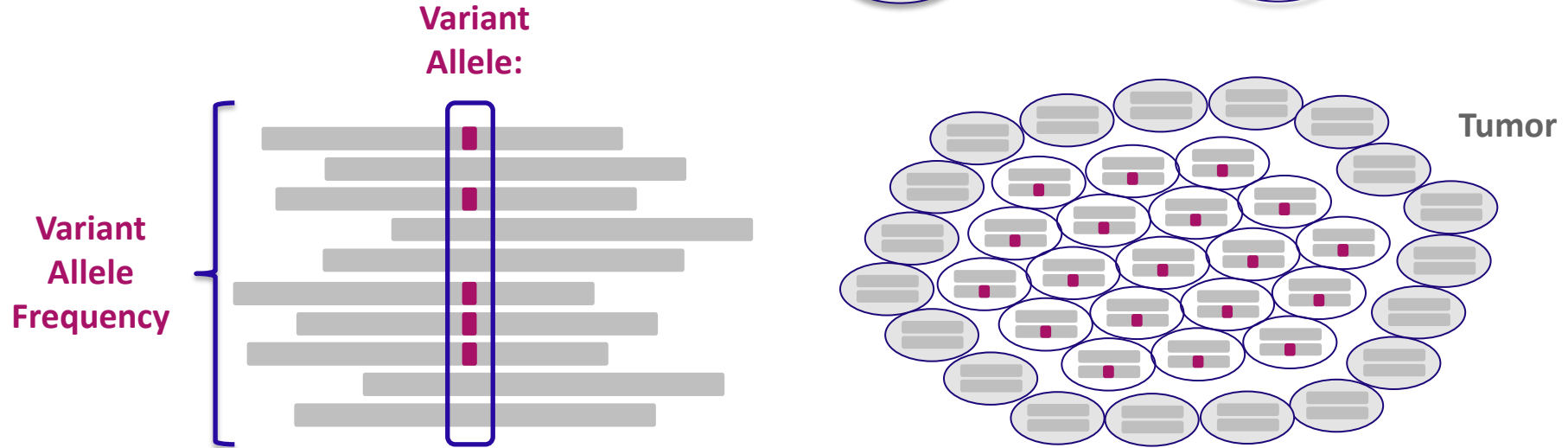
Variant Allele Frequencies (VAF)



Tissue sample:



Variant Allele Frequencies (VAF)



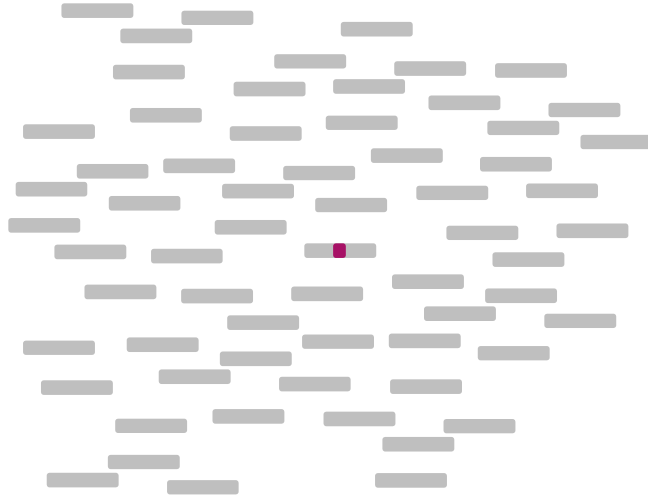
Variant Allele Frequencies (VAF)



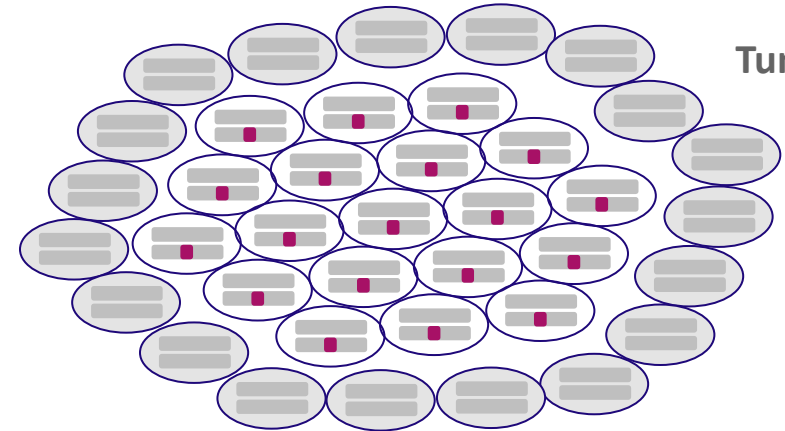
Normal cell



Cancer cell

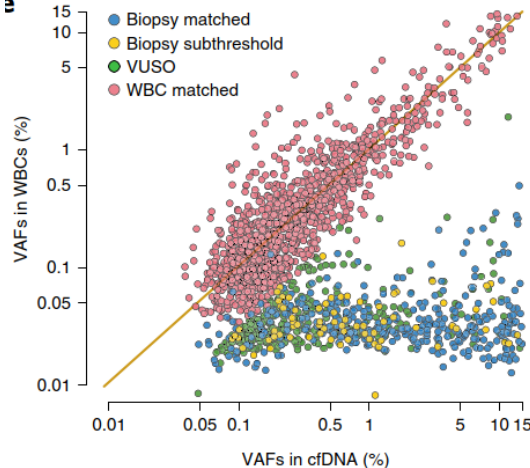


Cell free DNA



Tumor

- Clonal Hematopoiesis of Indeterminate Potential (CHIP)
- Mutationen in WBC, zunehmend mit Alter, Rauchen, Chemo
- Kann zu falsch-positiven ctDNA-Befunden und Behandlung führen



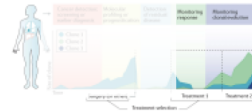
Study	Cancer	Study Size	Panel Size	CHIP Prevalence	Genes With CHIP & Tumor-Related Mutations
Leal et al.	Gastric	50	58 Genes	52%	<i>ATM, ERBB4, KRAS, JAK2, EGFR, PIK3R1, TP53, APC</i>
Ococks et al.	Esophageal	97	77 Genes	23%	<i>TP53, KRAS, ABL1, EGFR, PDGFRA, RB1</i>
Jensen et al.	Prostate	69	~350 Genes	19%	<i>ATM, BRCA2, CHEK2, ASXL1, DNMT3A, PTEN, TET2, TP53</i>
Chan et al.	Colorectal	39	52 Genes	17%	<i>TP53</i>

Tumor-agnostic: Liquid biopsy

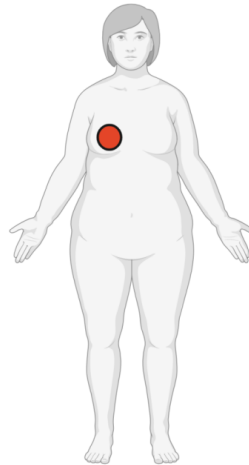
Tumor-agnostic: Liquid biopsy

- LODs 0.1% - 1%
- Nachweis von Resistenz-Mutationen (oder neuen Targets) in Metastasen (ESR1mut, PIK3CAmut, BRCA1rev, ErbB2mut, AKT)
- Vorteil gegenüber Gewebebiopsie: Kann Heterogenität der Erkrankung abbilden

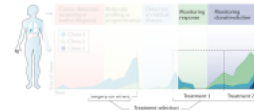
Heterogeneity – tumor tissue vs ctDNA



Surgery
Radiotherapy
Aromatase inhibitor



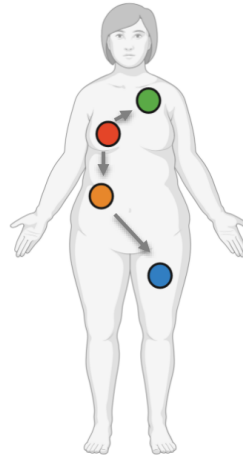
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Heterogeneity – tumor tissue vs ctDNA

Local and metastatic relapse

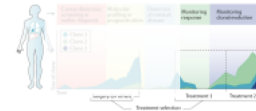
- Lung
- Liver
- Bone



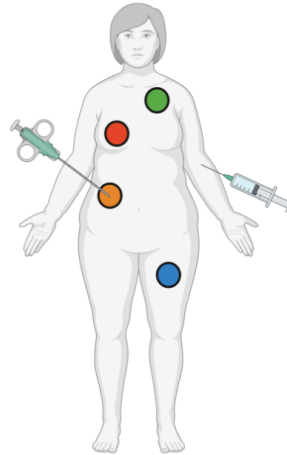
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Tumor-agnostic: Liquid biopsy

Heterogeneity – tumor tissue vs ctDNA



ESR1
wild type



ESR1
mutant

*25-50% of patients
treated with aromatase
inhibitor*



Fribbens et al 2016, *JCO*
Bidard et al 2022, *Lancet Oncology*
Liu et al 2022, *JCO PO*

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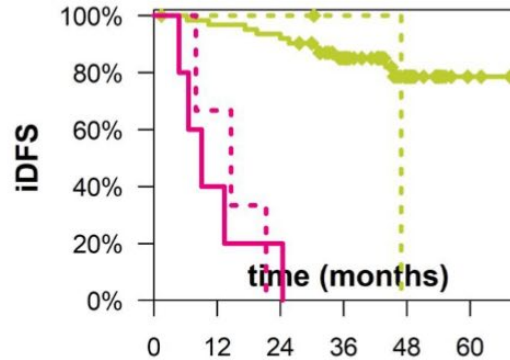
Tumor-informed: Minimal Residual Disease

- Vollständige Genomsequenzierung des Primärtumors
- Spezifische Assay-Entwicklung für individuellen Tumor
- “LODs down to 0.001%” (RaDaR)
- “Sensitivity >99% and Specificity >99.5% with VAF 0.01%” (Signatera)
- Therapie-Monitoring durch Nachweis von MRD

Tumor-informed ctDNA in Penelope-B

Results – ctDNA dynamics

Invasive disease free survival



— all undetected
- - - becoming undetected
- - - becoming detected
— all detected

63	60	58	43	17	2
2	2	2	1	0	0
3	2	0	0	0	0
5	2	1	0	0	0

iDFS by ctDNA dynamic groups

Patients with undetected baseline ctDNA, who become positive during treatment have poor outcome

Both patients who became undetected were on palbociclib

Analysis limited by small groups

Excludes 5 patients with a baseline sample and no subsequent samples

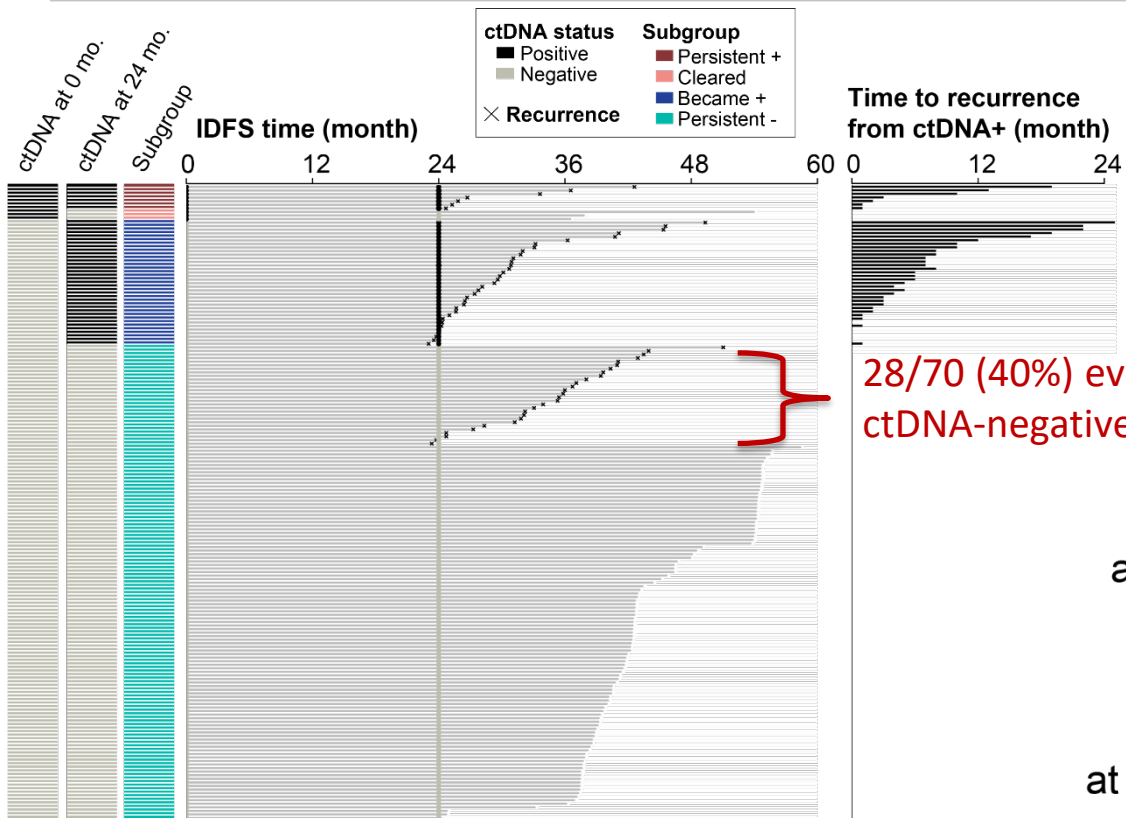
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BIG
Breast International Group

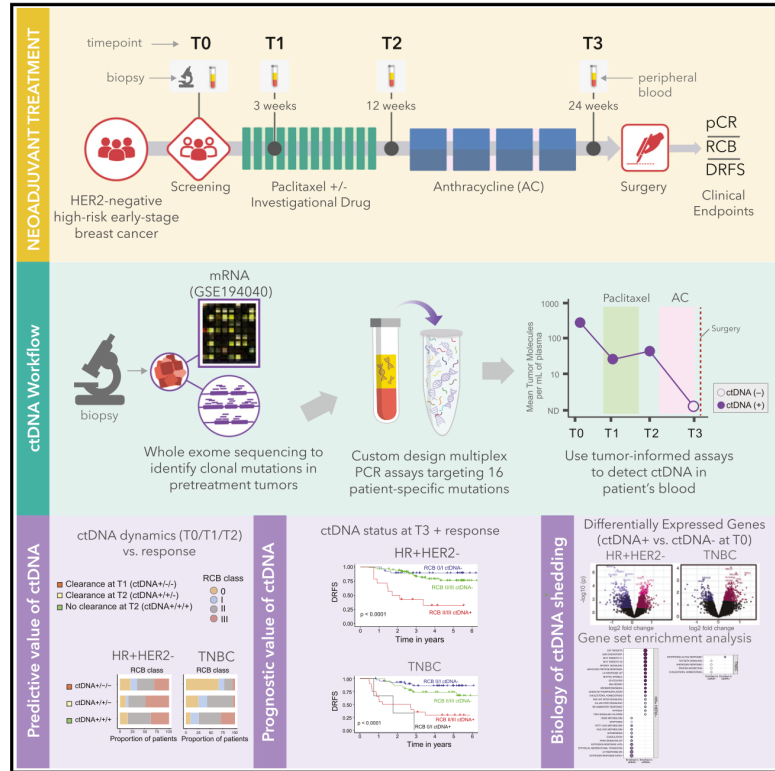
Tumor informed: Prognostic MonarchE



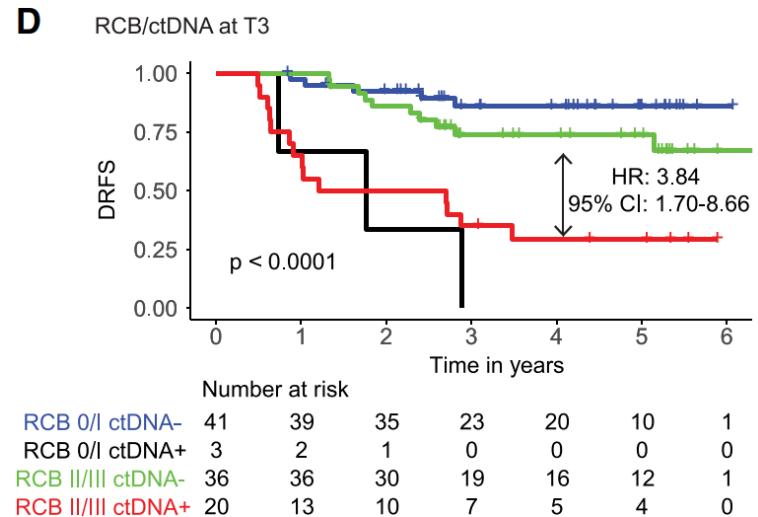
- N=178 cases with plasma samples + enriched for (n=70) iDFS events
- ctDNA pre & post 2yr abemaciclib

		Recurrence		
		Y	N	
ctDNA at 0 month	+	7	3	PPV = 70%
	-	63	105	NPV = 63%
		Y	N	
ctDNA at 24 months	+	42	0	PPV = 100%
	-	28	108	NPV = 79%

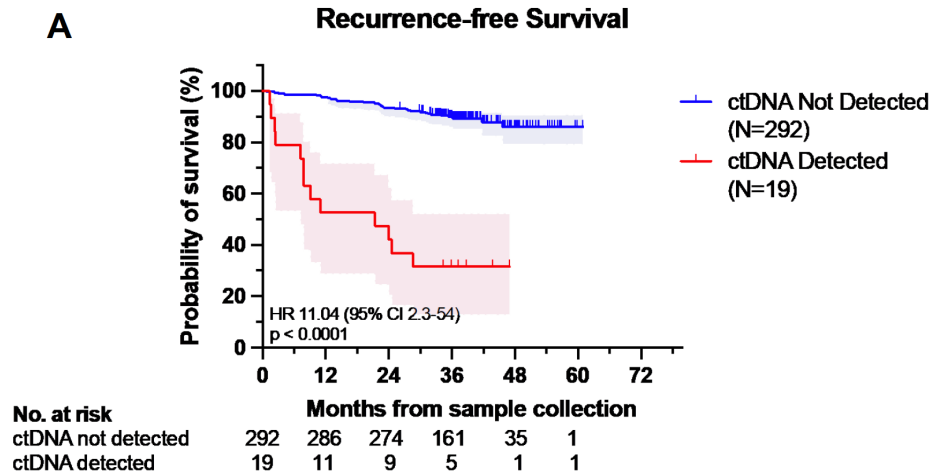
Tumor informed: Monitoring in ISPY-2



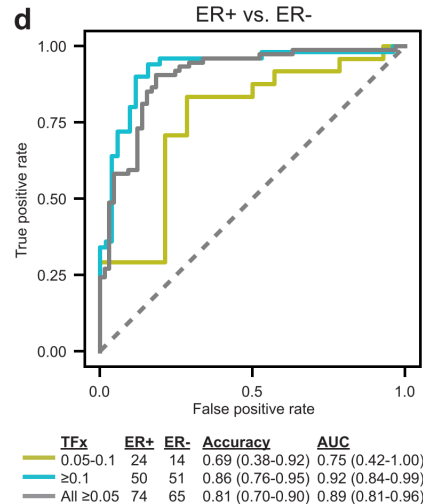
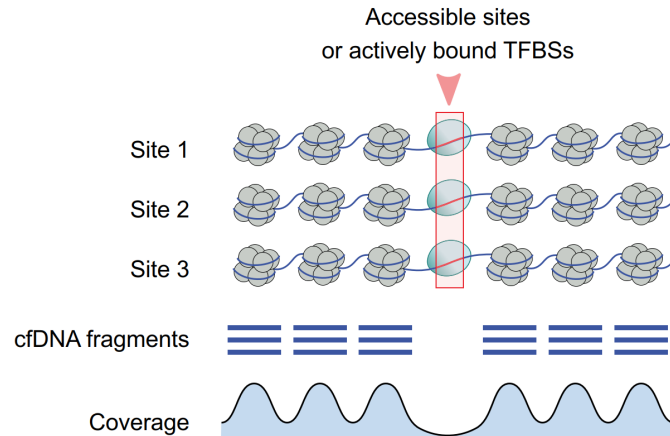
■ ctDNA clearance added prognostic value to RCB after NACT



- Signatures from non-methylated + methylated cfDNA (GuardantReveal)
- Prognostischer Wert in SUCCESS-A Studie



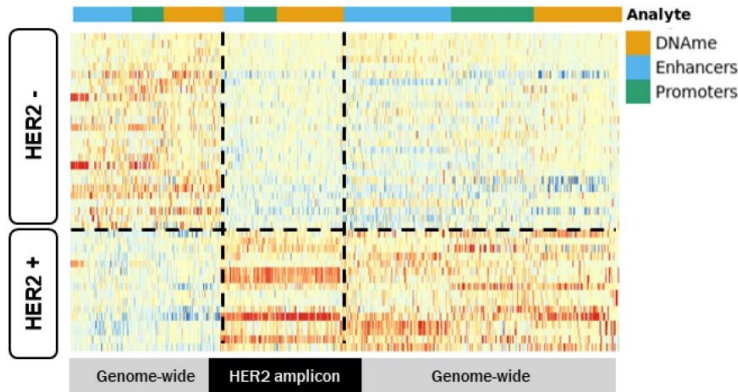
Fragmentomics and nucleosomal occupancy



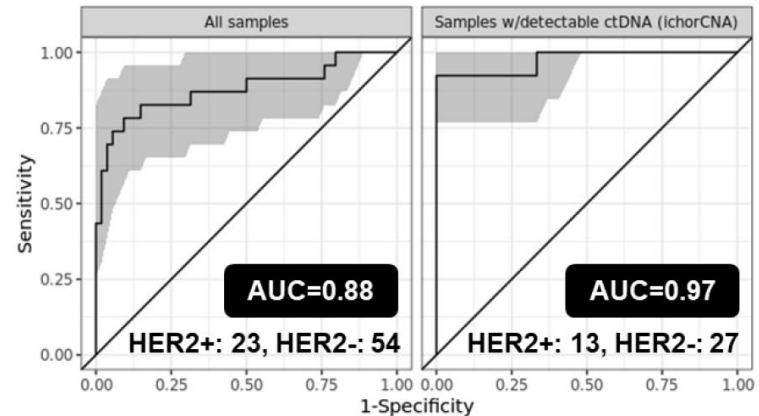
Novel approaches:

- Epigenomic profiling (histone modifications and DNA methylation)

HER2 +/- differential loci detected at amplicon and genome-wide



Robust cross-validated HER2 classifier



Prime Time?

	Tumor-agnostic ctDNA Liquid biopsy	Tumor-informed ctDNA MRD-detection
Analytical validity	✓	✓
Clinical validity	✓	✓
Clinical utility	✓	?



DARE trial, NCT04567420



www.survive-studie.de

■ ctDNA:

- Leichter zugänglich
- Größere subklonale Repräsentation
- Besonders geeignet für neu auftretende Resistenzmechanismen (ESR1, BRCA1/2)
- Mögliche neue Ansätze aus Fragmentomics oder Nucleosomal Occupancy
- Prognostische Bedeutung
- Weitere interventionelle Studien erforderlich, um klinischen Nutzen zu zeigen

■ Gewebe-Biopsie:

- Goldstandard für Evidenz zur Behandlung
- Liefert Zellmorphologie und Tumor-Microenvironment
- Besser geeignet für Copy-Number-Alterations und Structural Variants

Heilung durch Innovation, Kompetenz
und Partnerschaft – führend in der
Brustkrebs-Forschung

