Circulerende tumorcellen: methodologie en toekomst

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CTC geschiedenis

1869
Ashworth
"cells in the blood of the **same size and appearance** as those of the patient's multiple malignant skin tumors"

1906
Schleip
"cells (in the blood) **varying from the normal** in a case of gastric carcinoma"
Ward
"numerous **large** cells, presumed to be tumor, in the peripheral blood from a patient with gastric cancer **a few hours before death**"

1919
Marcus
"**abnormal** cells in the blood taken from the finger of a patient with bronchogenic carcinoma **five hours prior to death**"

Vanaf 1921
Studies van cohortes van patiënten: filtratie (1959) – sedimentatie (1960) - immunomagnetische scheiding (1998)

Roberts S et al. AMA Arch Surg 1958; 76(3): 334-346

waarom CTC-bepalingen?

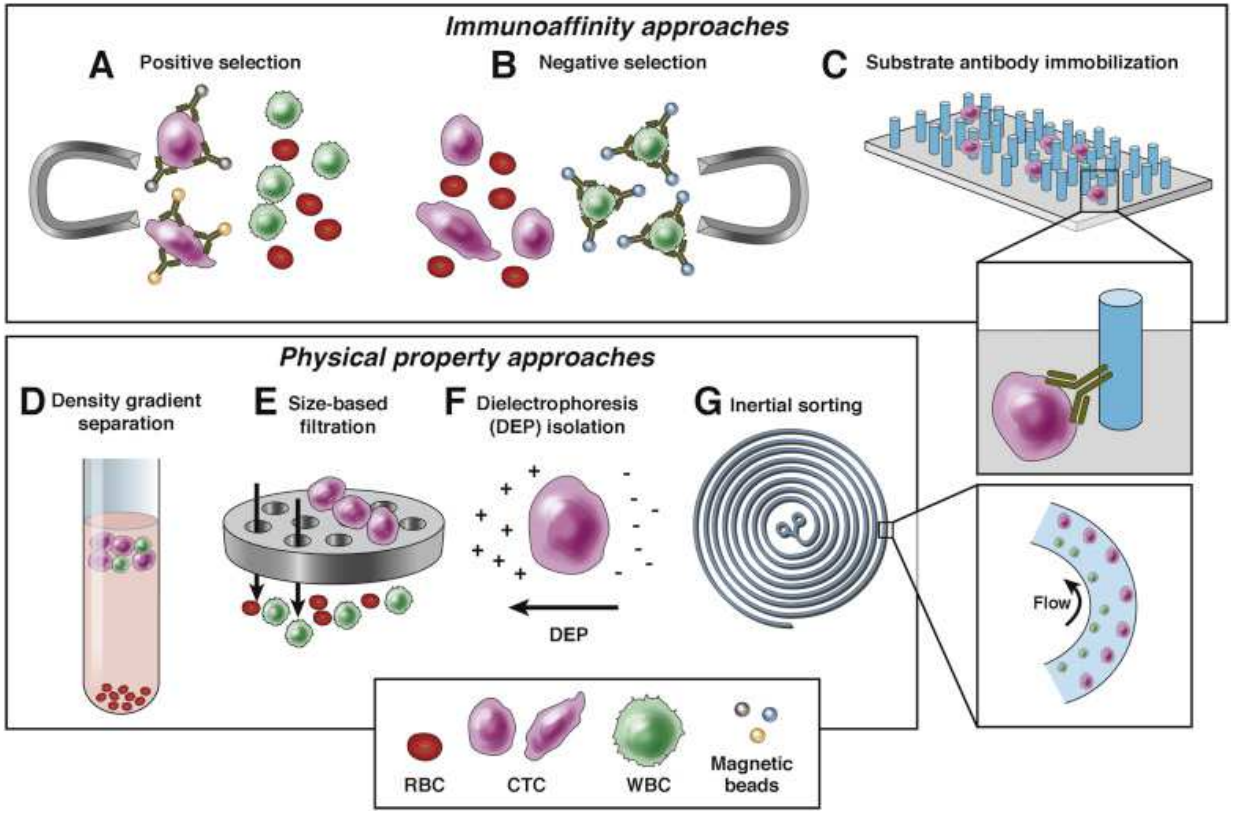
- prognose
- real-time monitoring van recidieven en therapie-effect
- identificatie nieuwe therapeutische doelwitten
- ontdekken van resistentie-mechanismen
- inzichten tumorprogressie en -metastasering

informatie

- detectie
- telling
- karakterisering
  - moleculair
  - functioneel

aandachtspunten

- opbrengst en zuiverheid
- identiteit CTC
- clusters

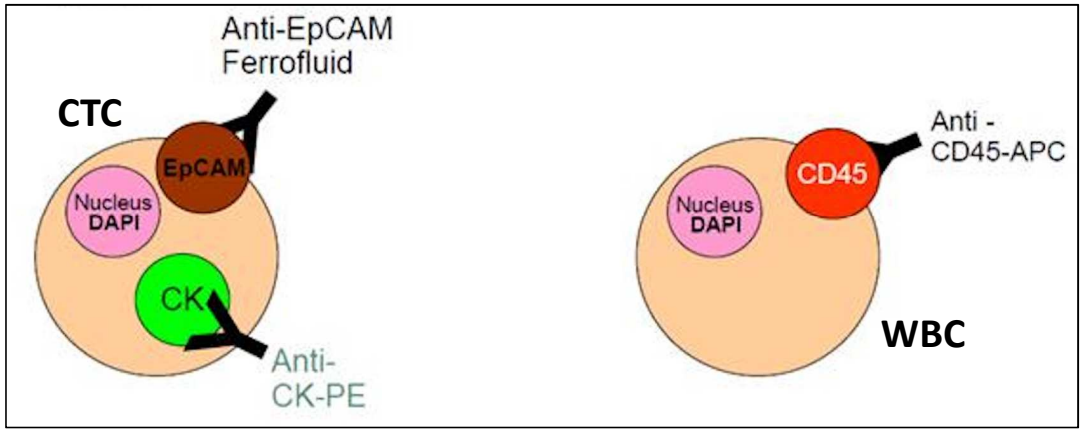


"antilichaam tegen CTC-antigeen"

"biofysische eigenschappen CTC"

"microscopische beeldanalyse van CTC
= minder stringente aanrijking"
(bijv. RareCyte)

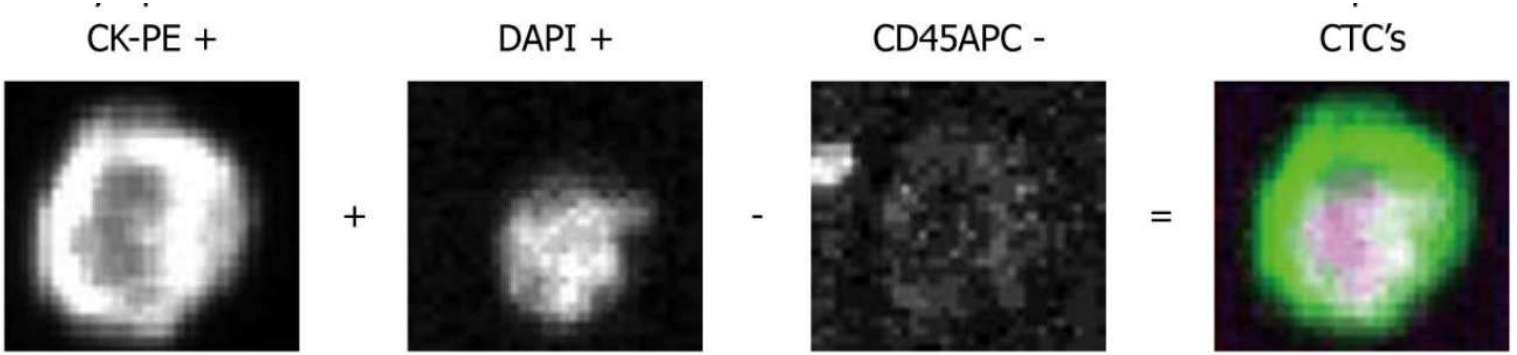
"antigeen-antilichaaminteractie" - **CellSearch**



- **EpCAM-positief** → aanrijking door magneet

- **Cytokeratine-positief**
- **CD45-negatief**
- **gekernd** (DAPI)

} microscopische beeldanalyse



Biomarker	Expression rate	Drug	Description
Markers for CTCs			
EpCAM	37-42.3%	Panorex, MT201, MT101, ING-1	• FDA-approved CellSearch™ system depends on EpCAM-specific capturing of CTCs in various cancers [21, 30, 47, 49, 125, 127, 258-262] • Loss of EpCAM on CTCs as a result of dynamic phenotypic changes during EMT[11, 35, 150, 151, 263]
CD44	35.2%	Pan-CD44 antibody H90	• CD44 expression in CTCs of HNSCC, breast, gastric and endometrial cancer patients [46, 264-268]
ALDH1	17.7-80%	ATRA, DEAB	• ALDH1 expression in CTCs of breast, non-small cell lung and endometrial cancer patients [36, 264, 266, 267, 269]
CD133	83%	CART133 chimeric antigen receptor (CAR) T cells	• Expression of the cancer stem cell marker CD133 in CTCs of metastatic breast, colon, colorectal, renal cell, hepatocellular and non-small cell lung cancer patients [266, 269-275]
FGF2	n.a.	Dovitinib, Pentraxin-3	• Frequent secretion of FGF2 by CTCs in pM1-staged prostate cancer [276]
KRT7, KRT18, KRT19	46.9%	Anti-KRT19 antibody HPA002465	• KRT7, 18 and 19 expression in CTCs from ovarian, gastric and gastroesophageal cancer patients [277, 278] • Used for therapy monitoring of advanced NSCLC and breast cancer[279]
c-Met+/CD47+	0.8-33.3%	Hu5F9-GA, ARG 197	• CD44/c-Met/CD47 CTCs from breast cancer patients display metastatic potential [46] • c-Met+/CD47+ CTCs as novel independent prognosticator of OS in luminal breast cancer [154, 236] • c-Met as a capture antigen for CTCs and as a therapeutic target [237, 238, 280] • CD47 expression on CTCs of colorectal cancer [239, 281]
HER2	7.9-35.9%	Herceptin, Pertuzumab, Lapatinib, Trastuzumab-mertansine (T-DM1)	• HER2 expression on CTC of metastatic breast, non-small cell lung, gastric, gastrointestinal, ovarian cancer [12, 13, 36, 119, 178, 189, 282] • Anti-HER2 therapy to address HER2-positive CTCs [283] • HER2 is part of the signature of breast cancer CTCs competent for brain metastases [284]
EGFR	18-56%	Cetuximab, Afatinib, Erlotinib, Gefitinib, Panitumumab	• EGFR expression on CTCs of colorectal, prostate, non-small cell lung, gastric, head and neck, and breast cancer [32, 36, 121, 210, 283, 285-288] • Treatment resistance T790M EGFR mutation in CTCs of non-small cell lung cancer [289] • Lapatinib treatment of metastatic breast cancer patients with EGFR-positive CTCs [290] • EGFR is part of the signature of breast cancer CTCs competent for brain metastases [284]

epitheliale differentiatiemerkers

stamcelmerkers

prognostische, predictieve merkers

therapeutische doelwitten

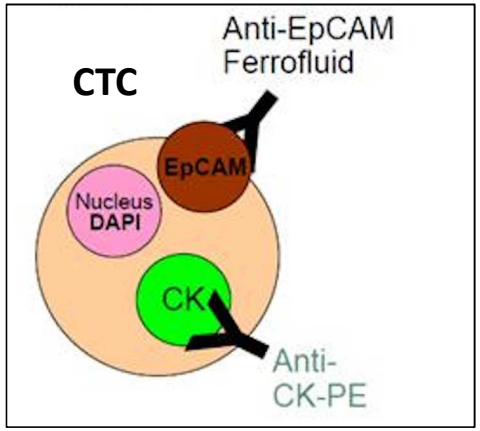
Biomarker	Expression rate	Drug	Description
Markers for CTCs			
MUC1/16	28.1-90%	ASI402	• Expression of mucin 1 and 16 in CTCs from ovarian cancer patients [277]
HPSE	n.a.	PI-88	• Breast cancer CTCs express heparanase [291] • HER2/EGFR/HPSE/Notch1-positive breast cancer CTCs have brain metastastic potential [284]
Androgen receptor	16.3-18%	Bicalutamide, Flutamide	• Nuclear expression of androgen receptor splice variant 7 protein in CTCs of metastatic castration-resistant prostate cancer is a treatment-specific biomarker that is associated with superior survival on taxane therapy over ARS-directed therapy [288, 292]
Telomerase	n.a.	Imetelstat	• Telomerase activity on CTC of metastatic prostate cancer is a prognostic marker [293] • Telomease-sensitive adenovirus as diagnostic and therapeutic tool against CTCs in various cancer [294, 295]
Vimentin	32.3%	Withaferin-A, Silibirin, Quercetin	• Decrease OS of castration-resistant prostate cancer patients with vimentin/ki-67-positive CTCs [296]
Ki-67	20.8-45.1%	n.a.	• Ki67 expression in CTCs of metastatic breast cancer [297, 298]
M-30	10-76.63%	M30 CytoDeath™ ELISA	• Apoptosis-related fragment of keratin 8 generated by caspases • Metastatic disease is associated with lower numbers of apoptotic CTCs [299]
TWIST1	n.a.	Curcumin, SFN, Quercetin, CADPE, Moscatilin, NAC, BMP7, Claudins	• TWIST1 is expressed in CTCs of breast cancer patients along with further EMT and stem cell markers [269]
uPAR	n.a.	PAI-1, anti-uPAR antibody 10G7, WX-UK1, Mesupron	• Expression of uPAR on subsets of CTCs in metastasized breast cancer [300] • Co-amplification of HER2 and uPAR in CTCs of breast cancer [301]

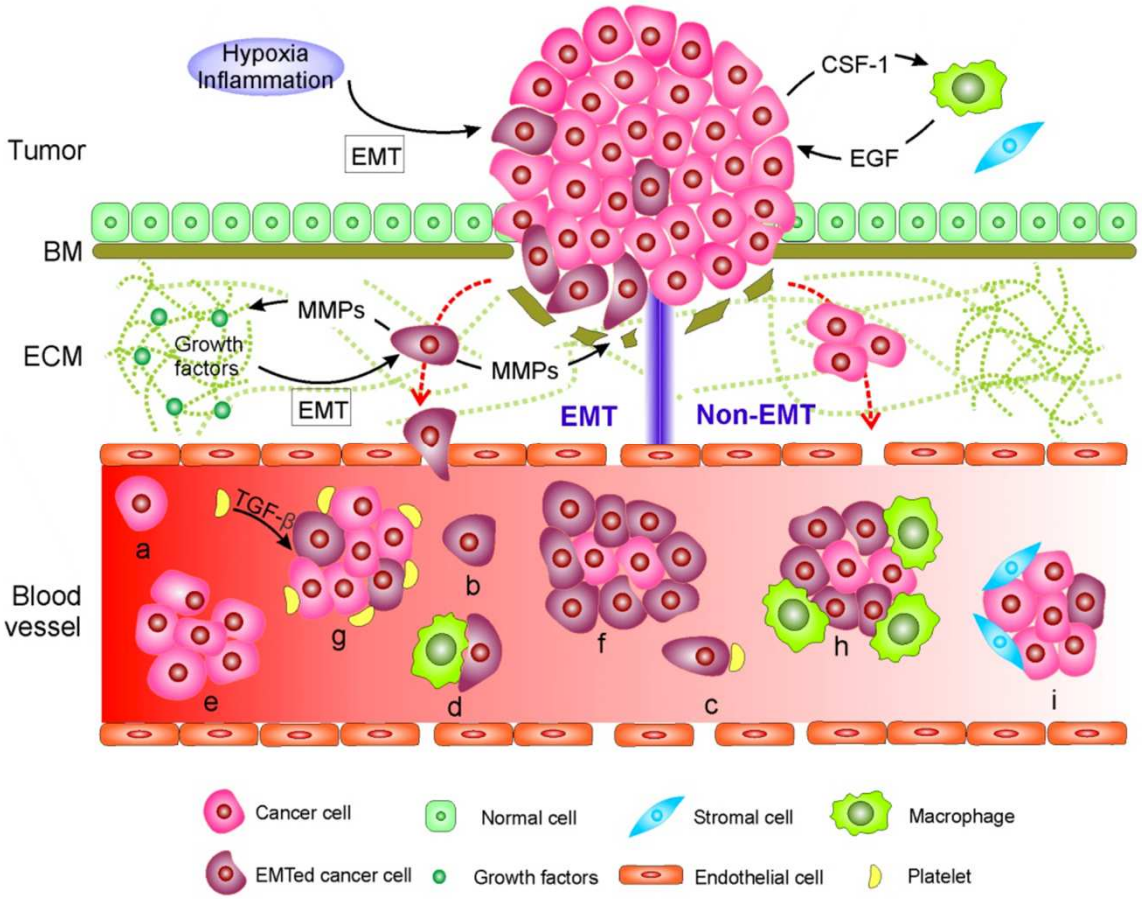
mesenchymale merkers

proliferatiemerker

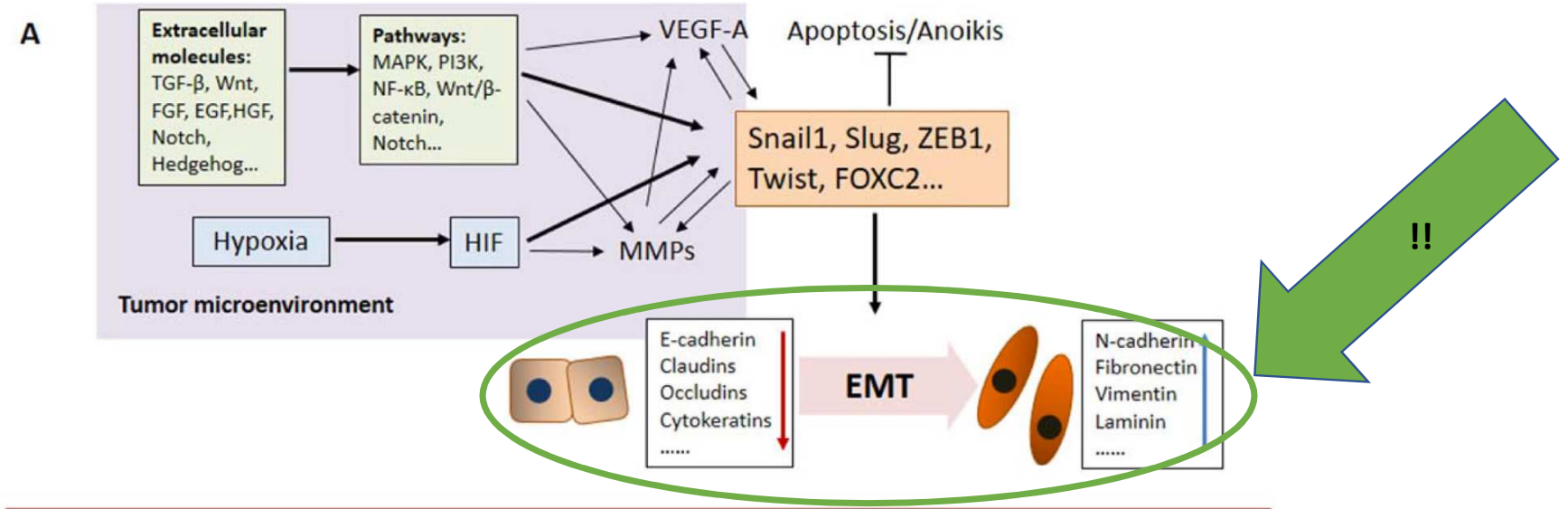
apoptosemerker

“aandachtspunt” – CTC identiteit en epitheliaal-mesenchymale transitie (EMT)

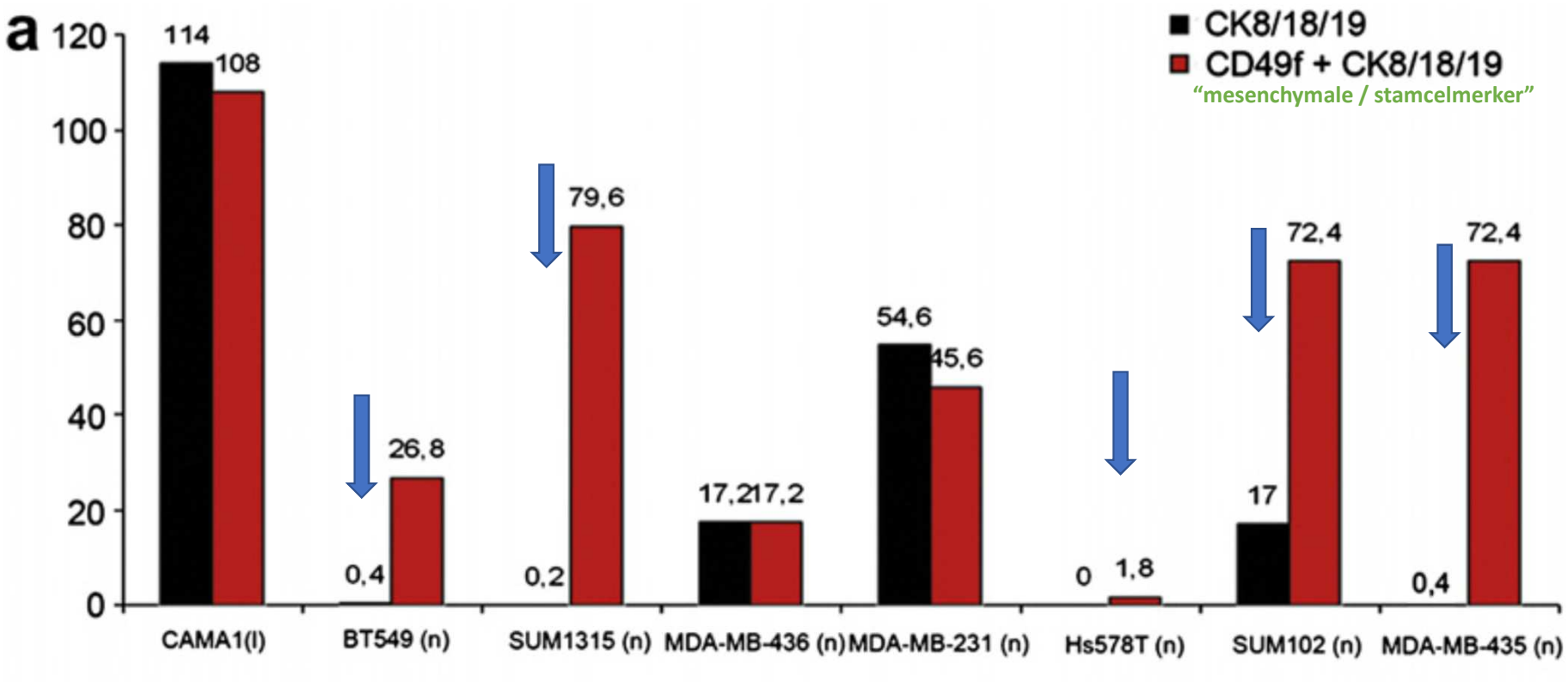




Rol van EMT in aanmaak van CTCs en EMT signaalwegen = **CTC merker shift!**



CTC-opbrengst luminaal-type (epitheliaal) versus normaal-type (EMT) borstcarcinoom



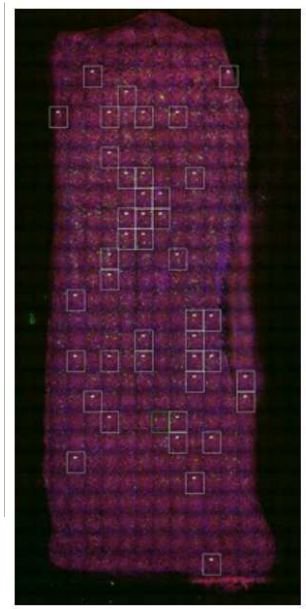
"aandachtspunt" – CTC identiteit versus EMT

“epitheliale-merker onafhankelijke CTC-detectie” - **RareCyte**

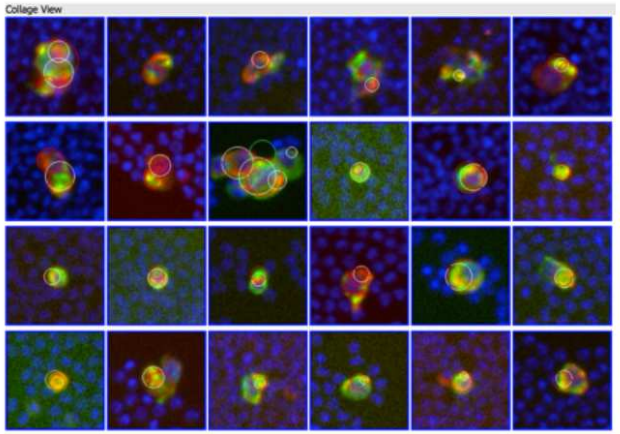
Algorithmic identification of target cells with reviewer confirmation (395 parameters)

HistoGeneX

Liesbet Vervoort



Whole slide “CyteMap”



Collage of identified target cells

“aandachtspunt” – CTC identiteit versus EMT

"epitheliale-merker onafhankelijke CTC-detectie" - **RareCyte**

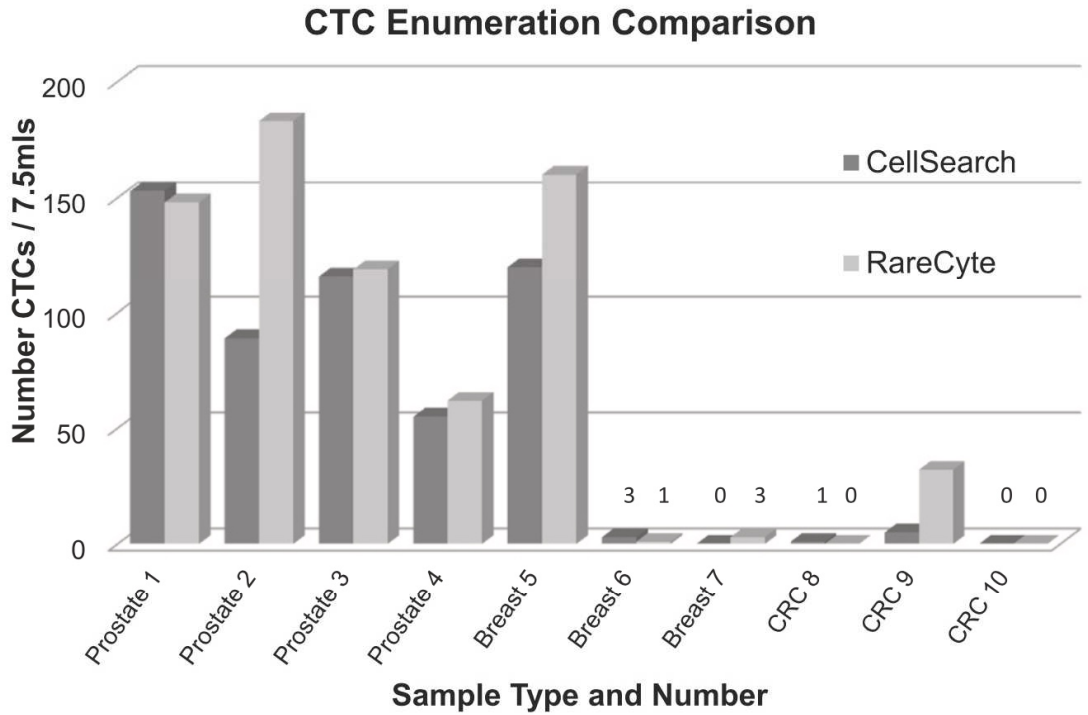
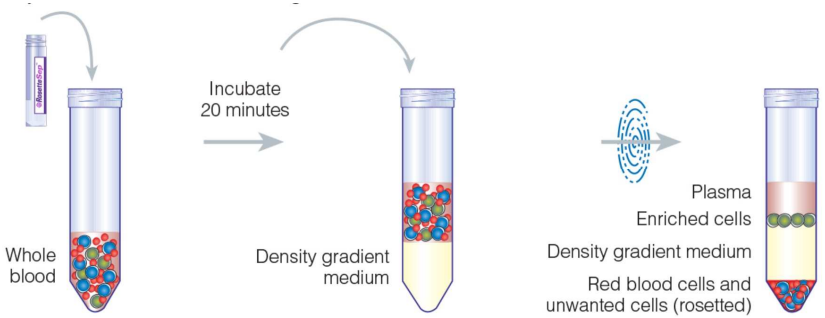
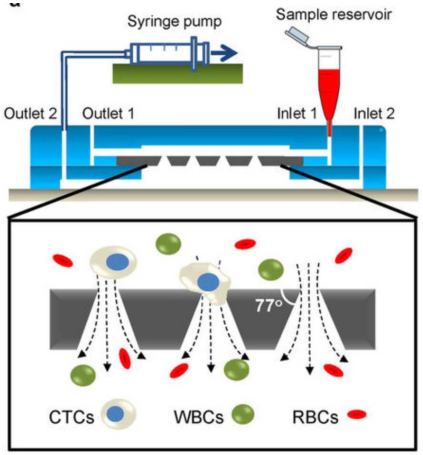


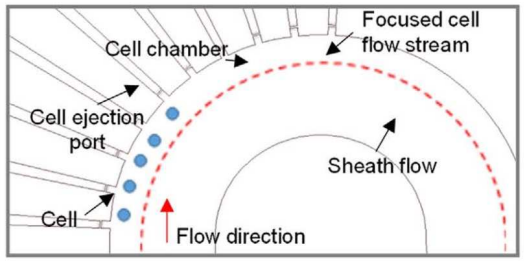
Figure 6 Comparison of CTC counts between AccuCyte – CyteFinder and CellSearch. 10 paired blood samples from patients with advanced prostate, breast or colorectal (CRC) cancer were processed independently using AccuCyte – CyteFinder or CellSearch systems to identify CTCs. In 3 samples counts equivalent (samples 1,3 and 4), in 3 samples AccuCyte – CyteFinder identified appreciably more CTCs than CellSearch (samples 2, 5 and 9), and in the remaining 4 samples numbers were very low by each method (3 CTCs or less).

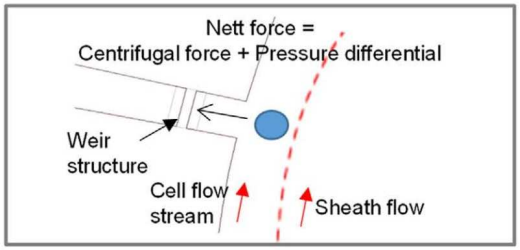
"aandachtspunt" – CTC identiteit versus EMT

CTC aanrijking op basis van fysische eigenschappen









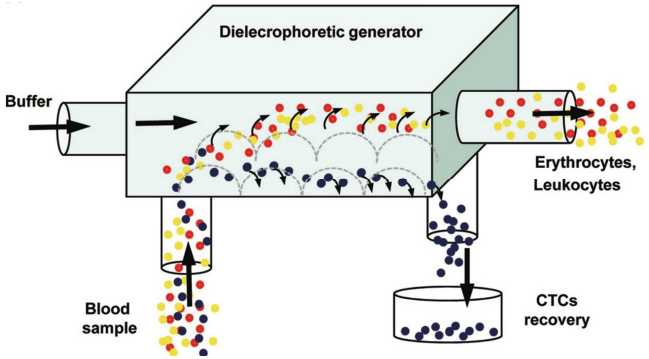


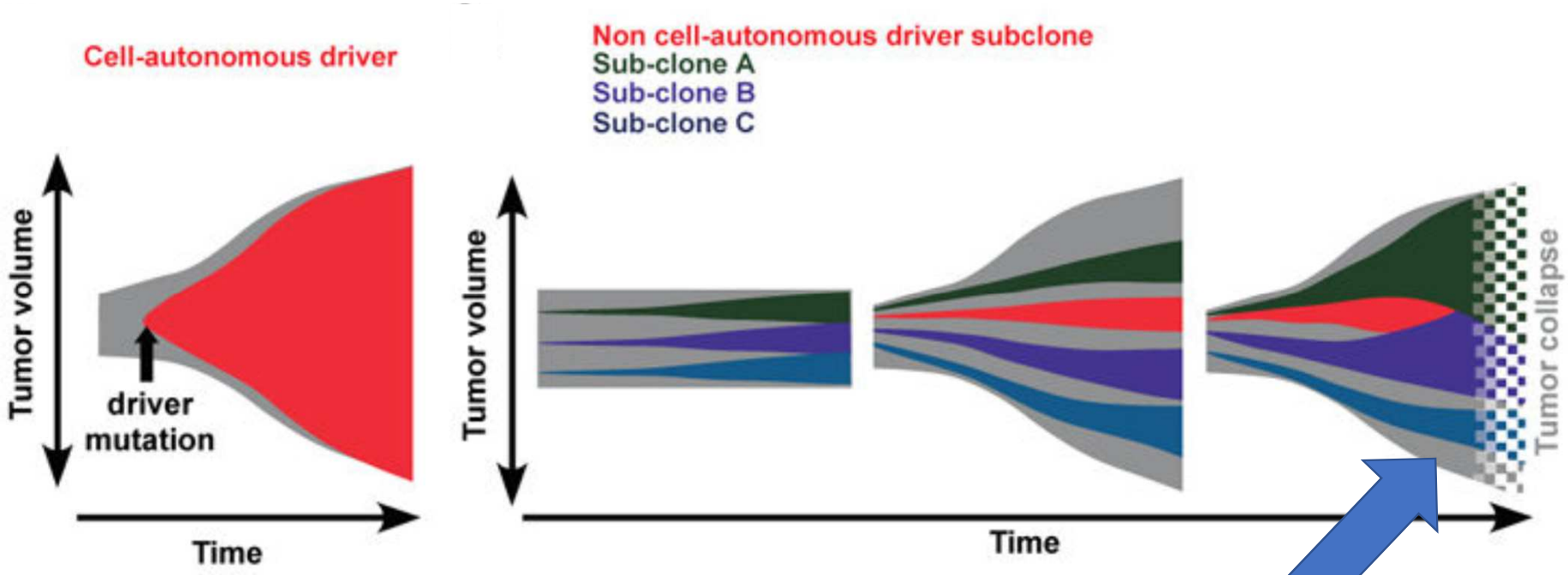
Table 1. Major advantages and disadvantages of CTC enrichment methods.

Method	Advantages	Disadvantages
Density gradient centrifugation	Inexpensive Reliable	Loss of large CTCs and cell aggregates Low purity Additional enrichment techniques required
Microfiltration	Rapid processing of large volumes High efficiency	Low purity Membrane clogging Different size of CTCs Difficult to detach CTCs from the filter
Microfluidics	Excellent purity High capture rates Little cell disturbance	Long, time-consuming process Sample preprocessing requirement to reduce volume
Dielectrophoresis	Single cell isolation High cell viability High efficiency	Limited volume Low purity in some devices Cell electrical properties can be affected during the procedure Large number of parameters must be controlled simultaneously
Immunoaffinity-based methods	High recovery High purity rates High cell viability using negative selection	Lack of cancer-specific markers Heterogeneous expression of markers in cells Problems with the antibody affinity or specificity

combinaties!

“aandachtspunt” – tumorheterogeniteit – supportieve kloon-concept (Polyak)

CTC-opbrengst zo hoog mogelijk om alle subklonen te kunnen capteren!



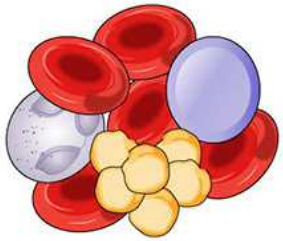
autonome – dominante kloon
= grote populatie!

multiple elkaar ondersteunende klonen:
niet dominant = kleine populaties!

Conclusies en perspectieven (1)

- circulerende tumorcellen: **heterogene populatie** (merkers, clusters, grootte, …) <> CellSearch FDA clearance
    - **directe cytologische beeldanalyse** (onafhankelijk van proteine-expressie of fysische eigenschappen van CTC): RareCyte
- **moleculaire karakterisering van enkelvoudige CTCs**: CellSearch – DepArray of RareCyte – CytePicker combinaties
- detectie van **EMT-CTCs en TICs** (tumor-initiërende cellen: 'self-renewal' – 'stamcel'
- detectie van **circulerende stromale cellen** zoals CAFs ('cancer-associated fibroblasts')
- detectie van **CTC-clusters** (hybride EMT – non-EMT CTCs, apoptose-resistent, metastase-efficiënt)
- klinische implementering: **high-throughput** platformen van CTC aanrijking en moleculaire karakterisering
- **vroege detectie** van CTCs: COPD-patiënten – screening tool

Conclusies en perspectieven (2)



CTC isolation and enrichment

- Cell size
- Immunomagentic method
- Electric method
- 2nd and 3rd generation chips
- direct imaging methods

→

CTC analysis

- Enumeration
- Molecular characterization
- *In vitro* culturing
- Xenograft models (functional assays)

→

Precision medicine

- Diagnostics (e.g. early disease detection, recurrence detection)
- Prognosis
- Treatment selection
- Monitoring of treatment

trials > 'clinical benefit'
high-throughput
automatisatie