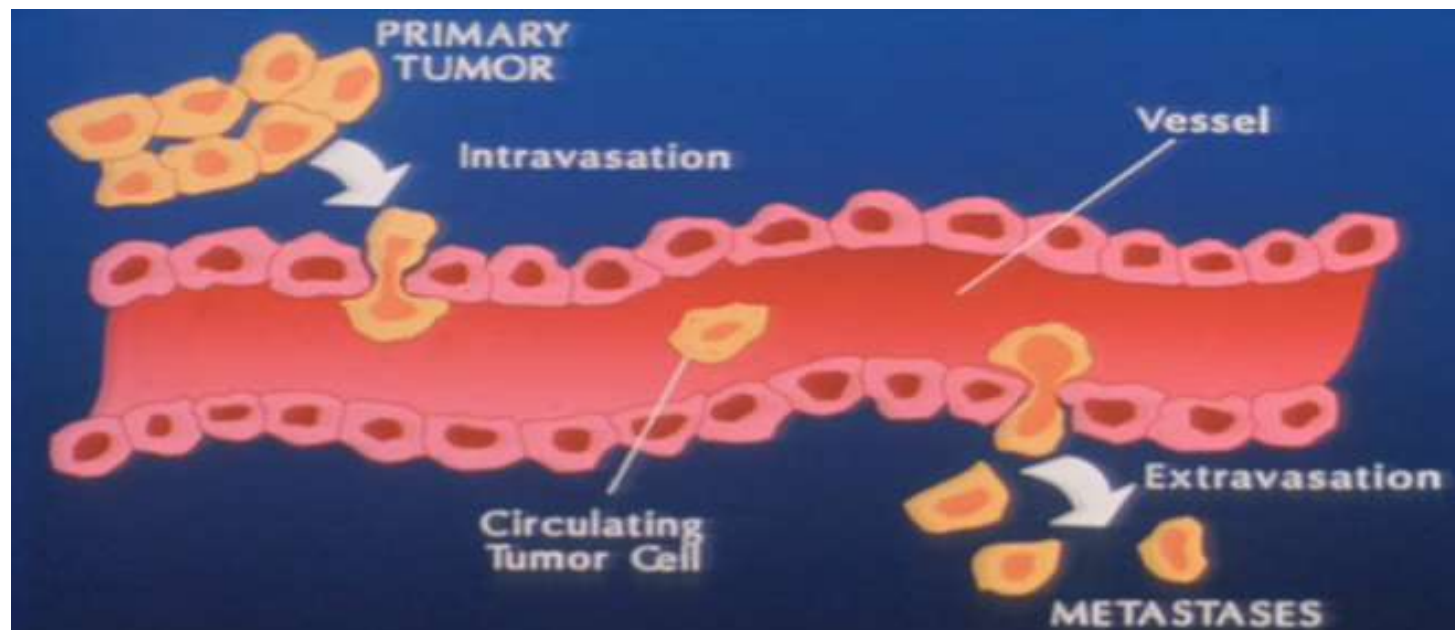


Since the detection of circulating tumor cells to the liquid biopsy of subcellular markers

16.9.2016

Petra Tesařová



V roce 2013 bylo v ČR nově diagnostikováno 81 541 pacientů se zhoubným novotvarem.

V roce 2013 zemřelo 26 944 osob v souvislosti se zhoubným novotvarem.

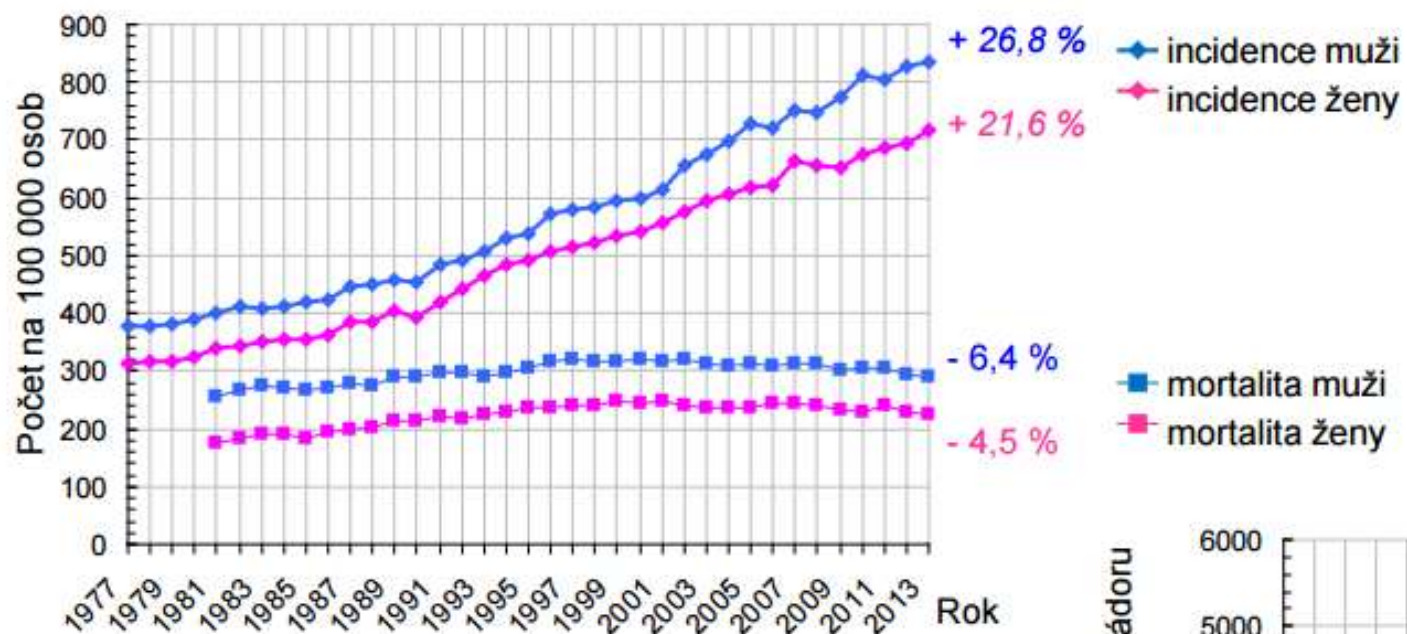
Celkem k 31.12.2013 v ČR žilo 518 667 osob se zhoubným novotvarem nebo s minulostí tohoto onemocnění.

Národní onkologický registr – zhoubné novotvary (C00–C97)

	2006	2007	2008	2009	2010	2011	2012	2013	Průměrná meziroční změna 2009–2013
Incidence	68 766	73 003	73 221	74 803	78 014	78 172	79 841	81 541	+2,2 %
Mortalita	28 393	28 579	28 692	27 950	27 881	28 467	27 470	26 944	-1,2 %
Prevalence	379 650	398 700	417 282	436 912	457 614	476 867	497 415	518 667	+4,4 %

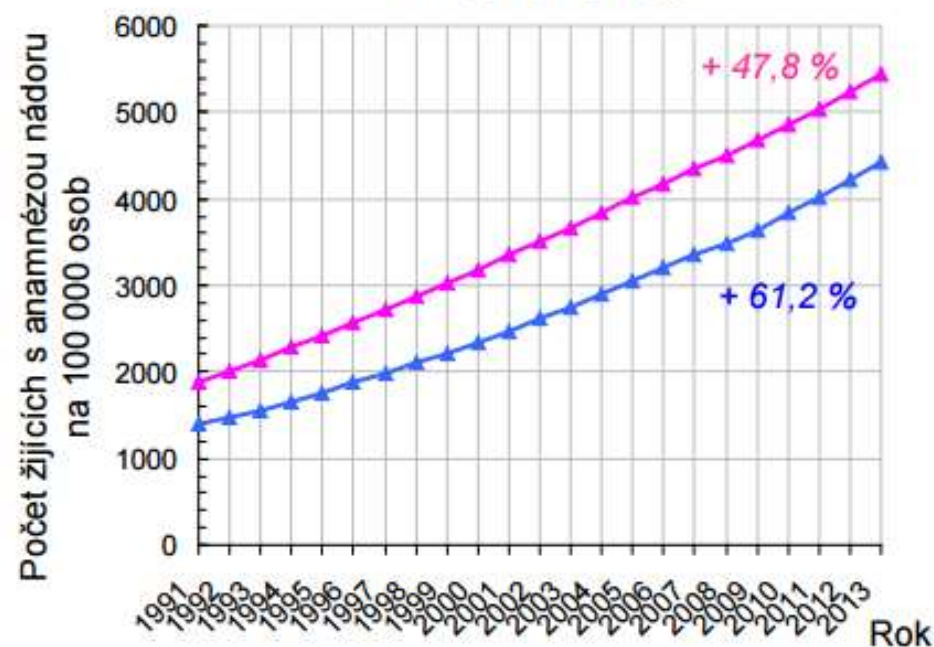
Zdroj: Národní onkologický registr, ÚZIS ČR

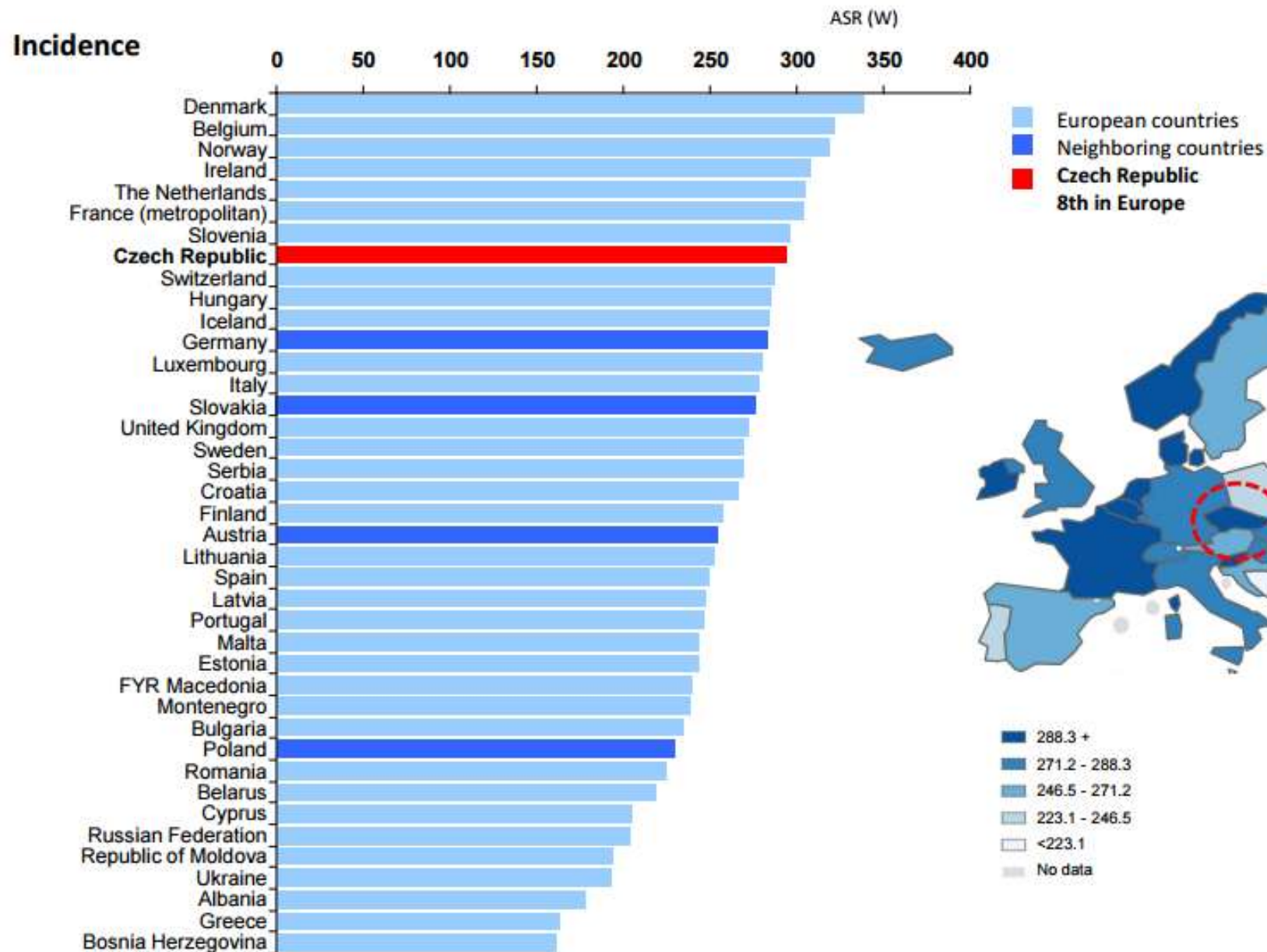
Incidence a mortalita



9 W š OE v OE ½ • š μ β - ů i i š OE } I Ç i i i

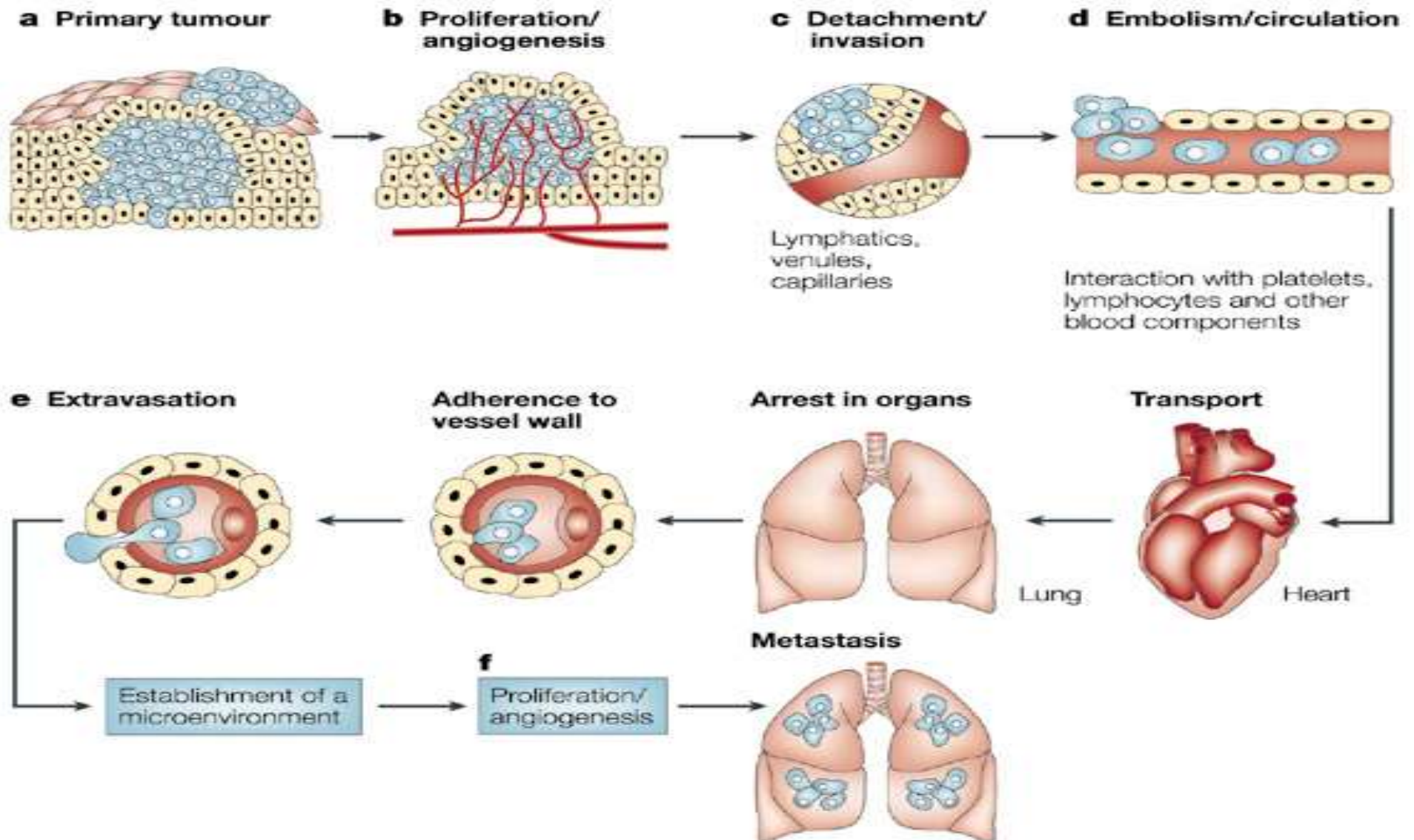
Prevalence





Source: Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: <http://globocan.iarc.fr>, 2/4/2015

Malignant tumor is able to metastasize



Obtaining tumor tissue for molecular testing can be challenging

Can be invasive

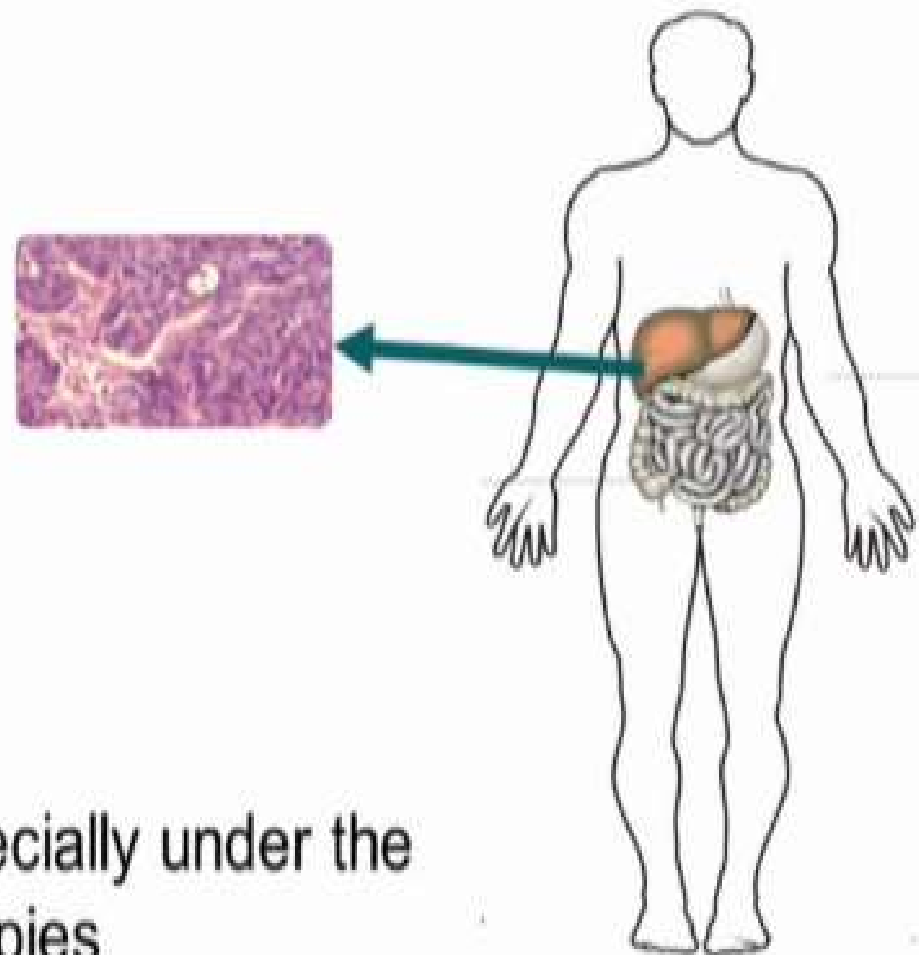
- May be high risk, unpleasant

Potential selection bias

- Intra-tumoral heterogeneity

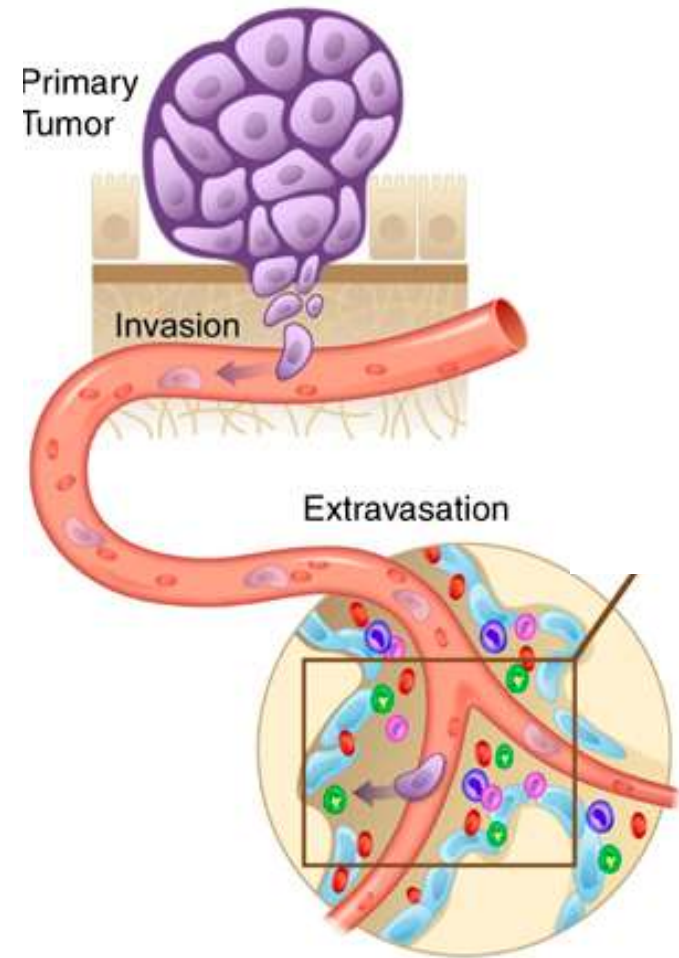
Evolving mutation profile?

- Primary versus metastases?
- Initial versus post therapy – especially under the selective pressure of targeted therapies



CTC detection goals

- ❑ The prognosis of the disease
- ❑ Biology of metastasis
- ❑ Monitoring of therapy
- ❑ Non-invasive biopsy replacement
- ❑ Detection of early stage malignancy-screening
- ❑ Prediction and tailoring treatment
- ❑ Resistance and sensitivity to treatment
- ❑

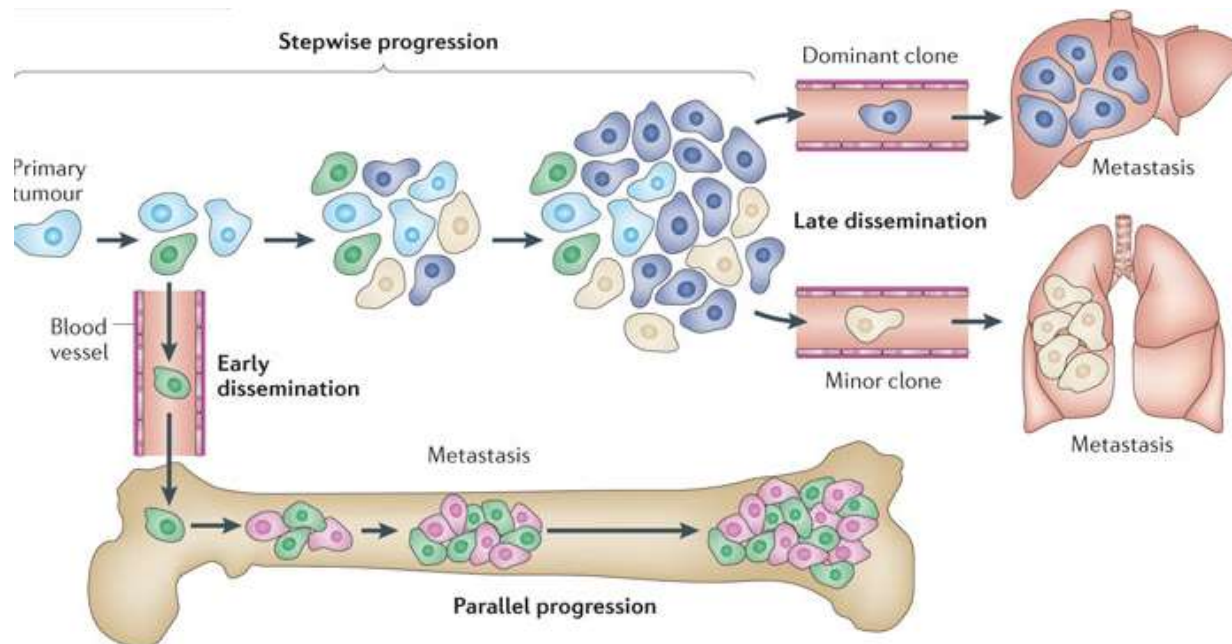


The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites

Y Shiozawa, A M Havens, K J Pienta and R S Taichman

Thomas Ashworth 1896-Melbourne

- Poprvé pozoroval nádorovou buňku v krvi a řekl : “may tend to throw some light upon the mode of origin of multiple tumours existing in the same person”.



Development of technical conditions

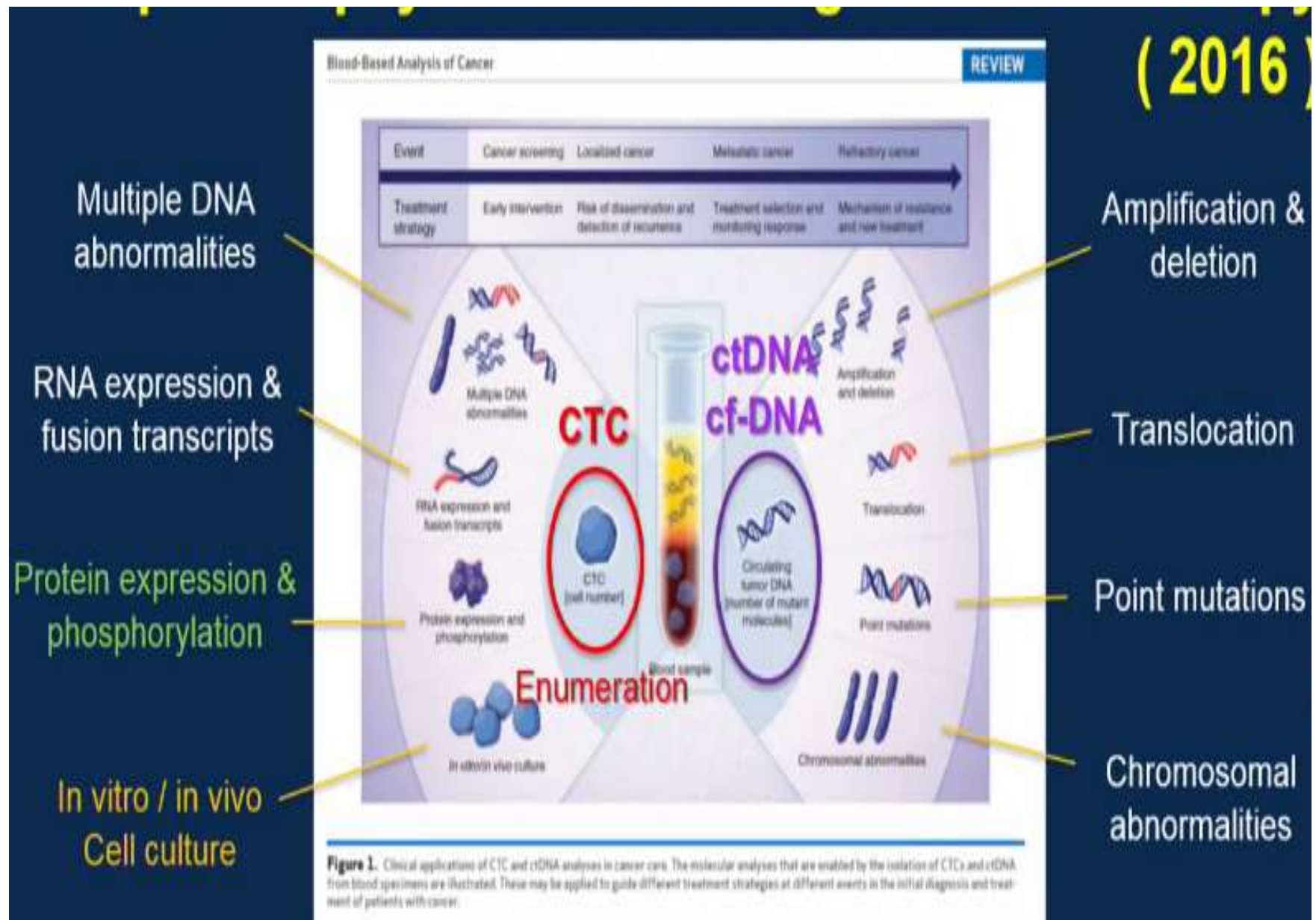
“Liquid Biopsy” in Cancer Diagnosis and Therapy (1965)



“Filtration of cancer cells by means of a plastic sieve. The holes have been etched to a diameter of 5 microns; holes of this size allow blood cells to pass through,
..... but **catch most cancer cells.**”

R. L. Fleischer, P. B. Price, R. M. Walker, *Science* 149, 383 (1965).

What we can find out from CTC and cf DNA?

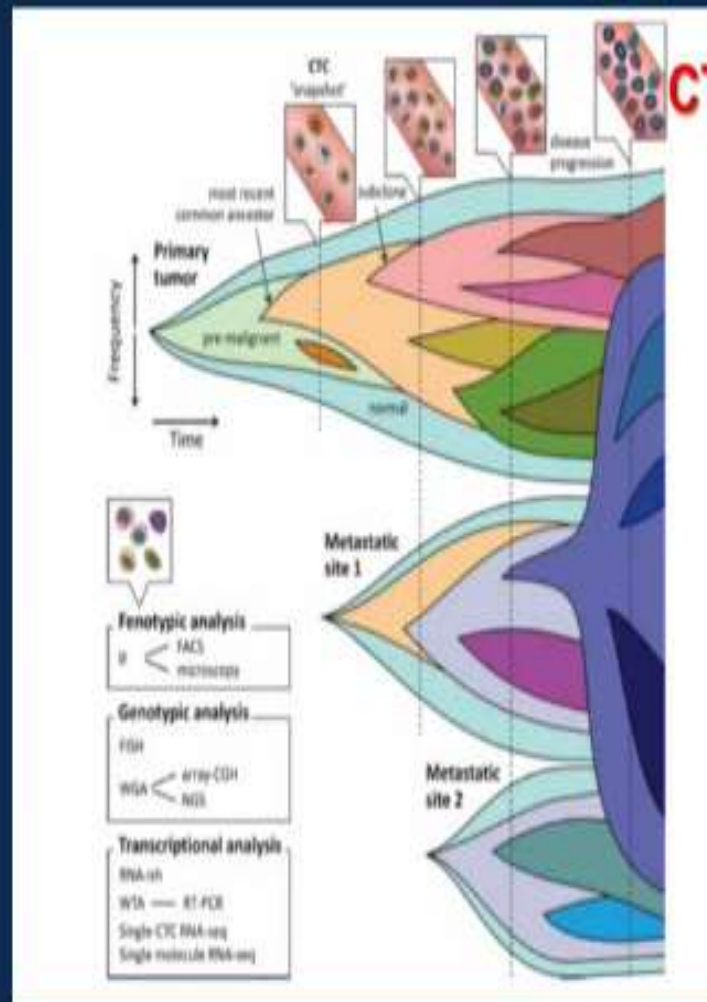


CTC- snapshot of developing cancer

CTC's sampling can serve as a "Snapshot" of the overall tumor bulk -

Primary tumor & Metastases,
At different Time-points:

1. Enumeration
2. Molecular-Genomic analysis



CTC's - ↑ / ↓ subclones

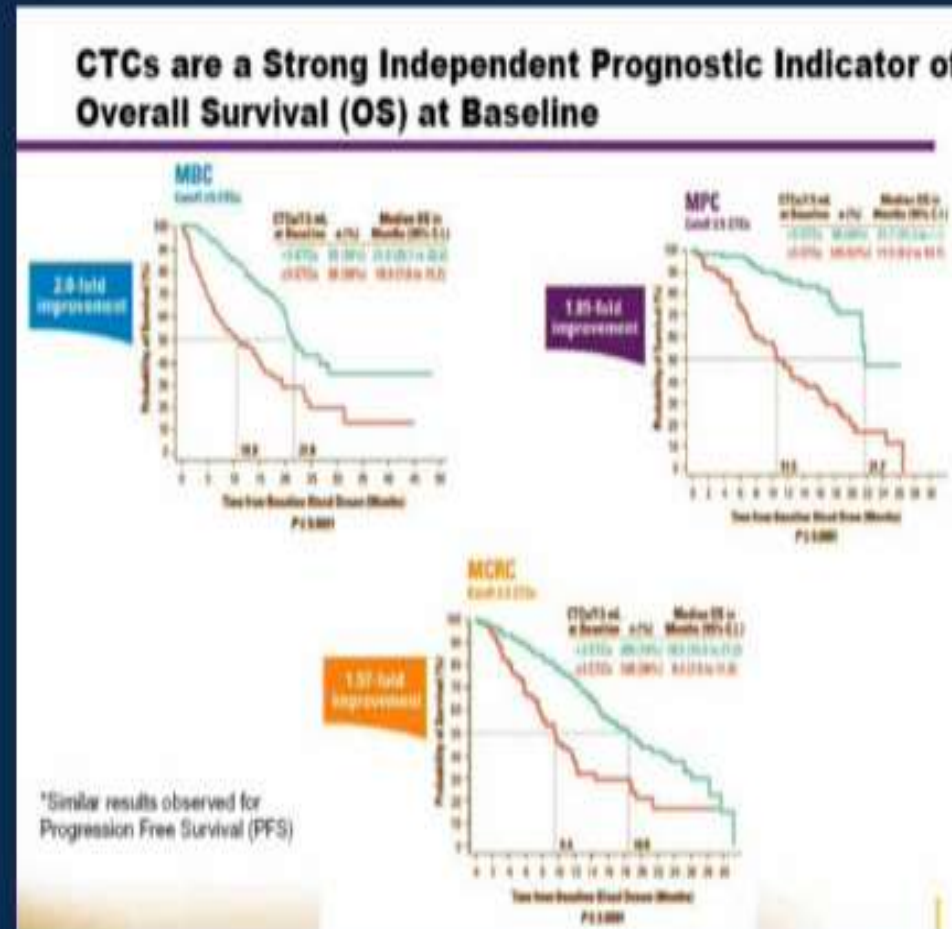
Clonal evolution depicted as emergence of clone after acquisition of driver mutations.

CTC is an independent prognostic factor for OS

Breast

Prostate

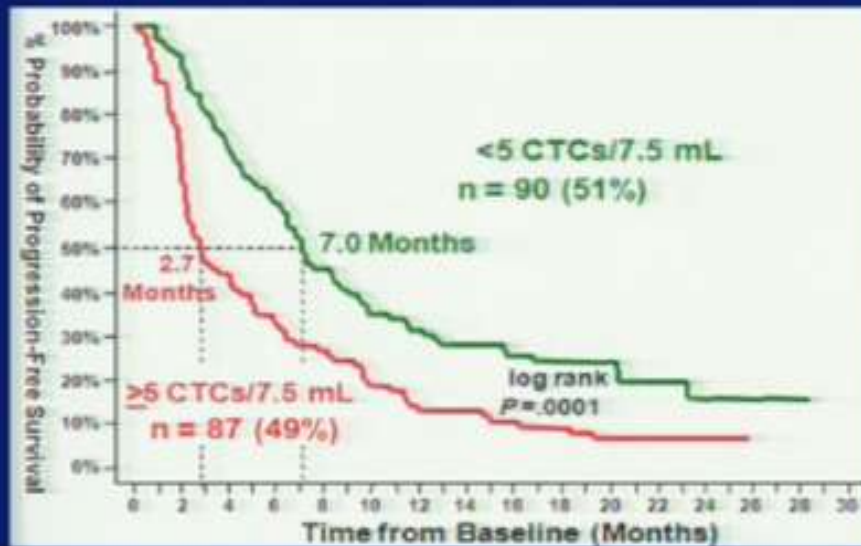
Colorectal



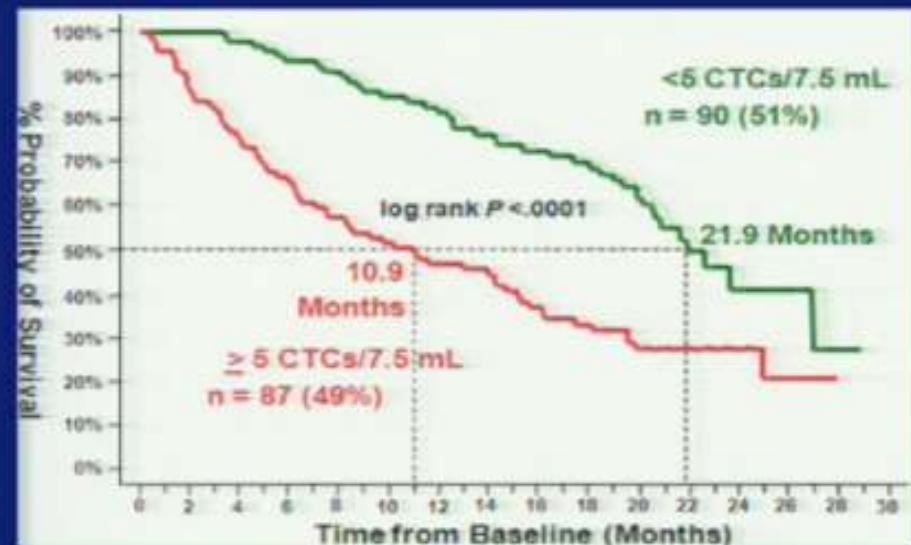
Racila, E., Euhus, D., Weiss, A. J., et al. (1998) *PNAS* 95, 4589; Cristofanilli, M., Budd, G. T., Ellis, M. J., et al. (2004) *NEJM* 351, 781; Budd, G. T., Cristofanilli, M., Ellis, M. J., et al. (2006) *Clin Cancer Res* 12, 6403; Cohen, S. J., Punt, C. J., Iannotti, N., et al. (2009) *Ann Oncol* 20, 1223; Scher, H. I., Jia, X., de Bono, J. S., et al. (2009) *Lancet Oncol* 10, 233

Prognostic significance of CTC in BC

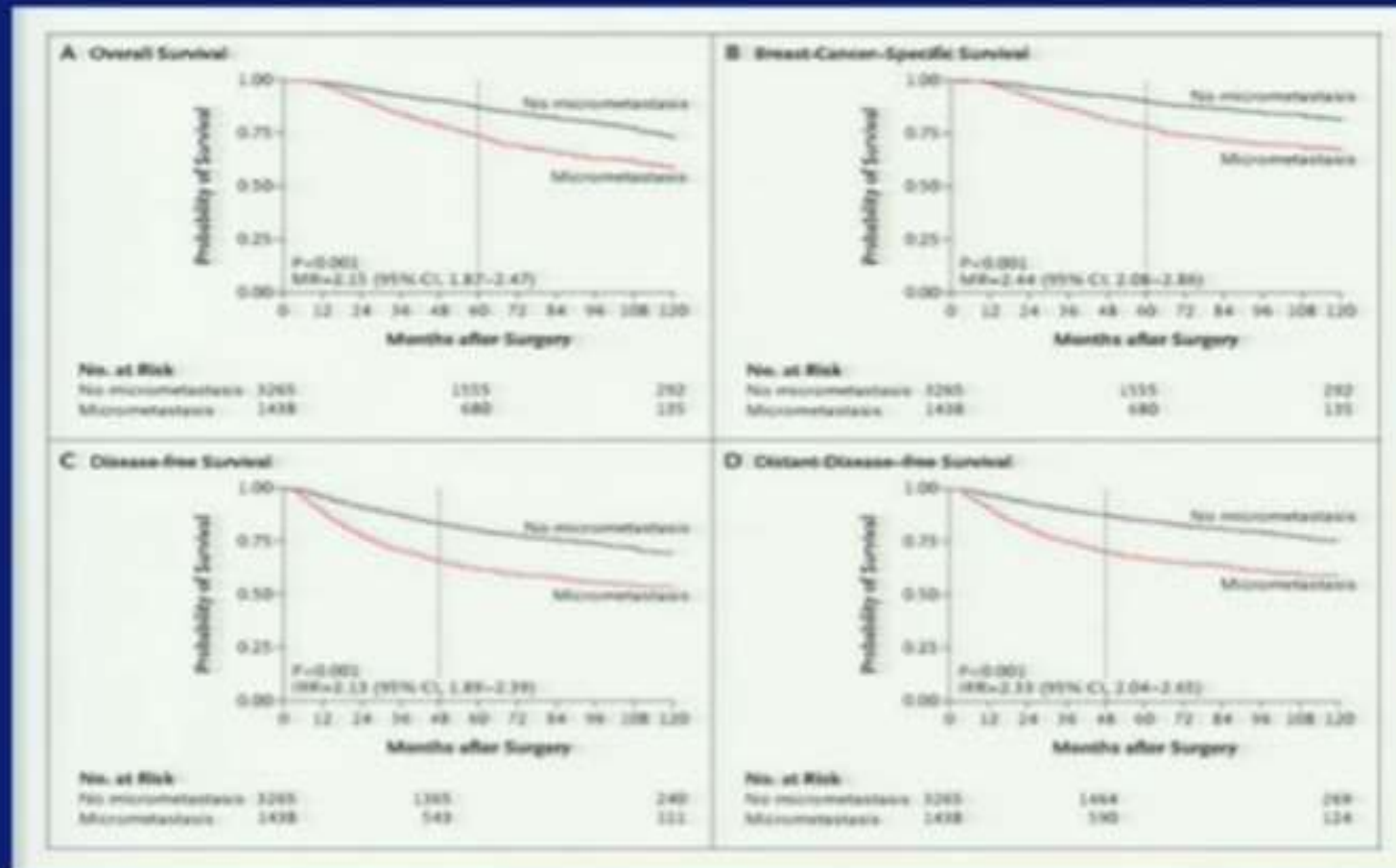
Progression-Free Survival



Overall Survival

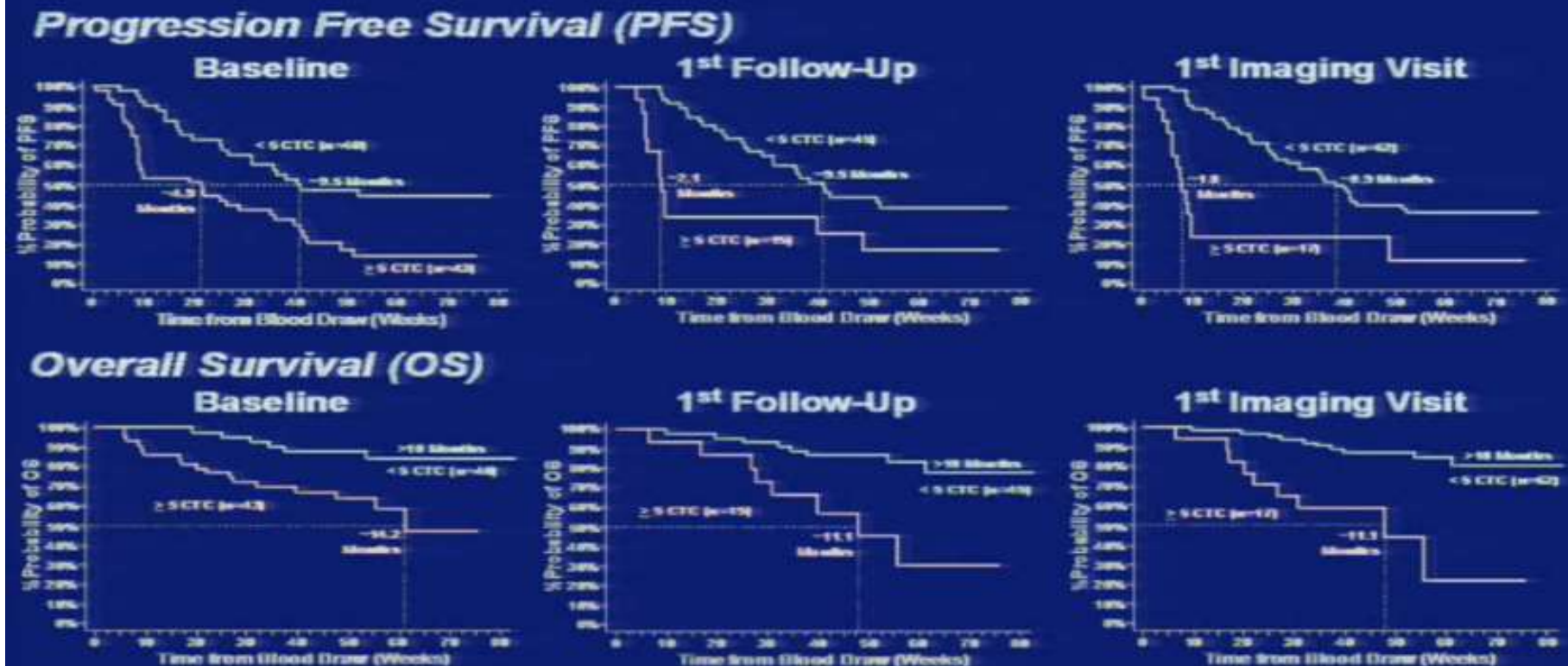


DTC- prognostic factor in BC



The prognosis of the disease course

CTCs Predict Outcome on 1st Line Rx

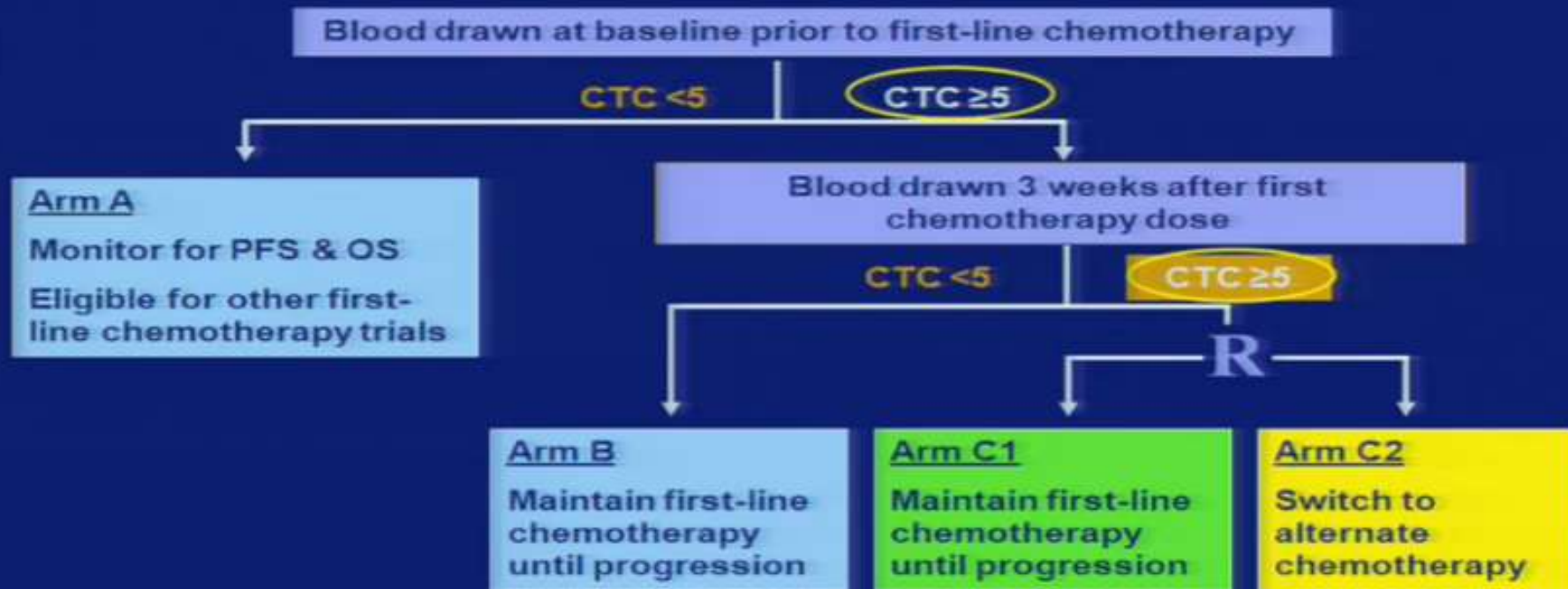


Cristofanilli M, et al. *J Clin Oncol*. 2005;23:1420-1430.

CTC are included in clinical trials

SWOG S0500:

Randomized Ph III Trial to Test the Strategy of Changing Vs Maintaining Therapy for MBC Pts who Have Elevated CTC Levels at First F/U Assessment



Smerage J, Hayes D *et al.*

Different methods of testing

Antibody-capture →

High-throughput imaging →

Physical properties →

Functional characteristics →

Leukocyte depletion →

Table 1. Technologies for isolation of CTCs

Underlying technology	Rationale	Representative platforms	Selected references
Antibody capture	Selection for EpCAM on tumor cells	Veridex/CellSearch Magsweeper Microfluidic CTC-Chip	54-56 57 59, 60, 110, 111
High-throughput imaging	Scanning of cells on slide	Epic	26, 38-41
Physical properties	Differential size, density, others	Physical filter Density gradient Dielectric Photoacoustic Microfluidic	33-37 42 43, 44 45, 46 47
Functional characteristics	Protein secretion, migratory properties	EPISPOT secretion assay Invasion assay	48-50 51-53
Leukocyte depletion	Negative depletion of leukocytes	Batch cell lysis Microfluidic CTC-iChip	112-114 28

CTC ASCO 2016

CTC – Enumeration and Molecular-Genomic Assay

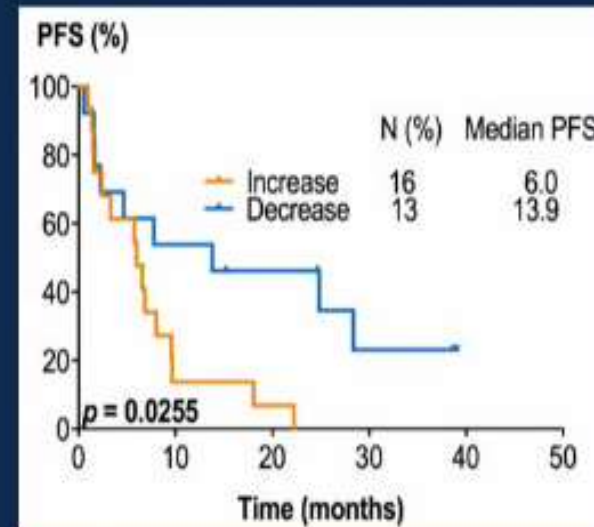
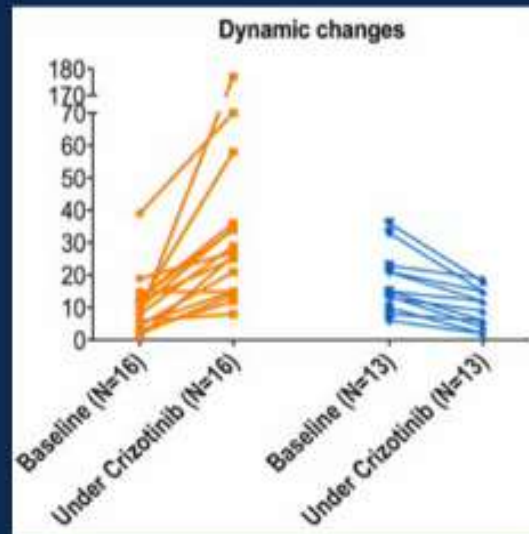
	Gene	Cancer Model	CTC Method	Enumeration	Time-points	Molecular Assay	Genomic Assay	Clinical Outcome
Abstract 11515 Jordi Remon	ALK-amp	ALK+ Lung	ISSET (size-based) Filtration	+	Baseline; 1-3 mo on ... Crizotinib	ALK-FISH IF: DAPI/CD45	-	Dynamic changes of CTCs w/ ALK copies gain correlated with PFS
Abstract 11516 Amir Goldkorn SWOG-S1216	Androgen-pathway genes	Mets-Hormone Sensitive Prostate Cancer (mHSPC) - Phase 3 Trial	CellSearch DEP (Dielectrophoresis)-Array	+	Baseline; Progression on ... Androgen-Deprivation therapy:- Bicalutamide or Orteronel	Multiplexed-QPCR (androgen-pathway genes)	Targeted NGS (androgen-pathway genes; Ion Torrent); RNA-Seq	Baseline CTC# (first 225 prospective samples) asso w/ worse PSA, bony mets and disease severity
Abstract 11517 Satoshi Matsusaka	EGFR; EMT markers	Mets-CRC	CellSearch	+	Baseline; Day 21; Progression on ... Regorafenib	mRNA: EGFR, EpCAM, CEA, EMT markers	-	Induced exp EGFR may be molecular escape mechanism

Lung cancer with ALK mutations

Abstract 11515: ALK-amplified circulating tumor cells as a surrogate marker for crizotinib benefit in ALK-rearranged NSCLC patients. Emma Paillet, [Jordi Remon](#), et al.

Results:

- PFS is correlated with dynamic changes of CTCs with amplification /gain of *ALK* copies



- Dynamic changes of CTCs with gain of *ALK*-native copies is correlated with PFS, potentially allowing us to predict patients at risk of early resistance under crizotinib therapy.

Prostate cancer - prognosis

Abstract 11516: Circulating tumor cells (CTCs) in SWOG S1216: A phase 3 multi-center trial in metastatic hormone sensitive prostate cancer (mHSPC).

Amir Goldkorn, et al., SWOG Genitourinary Committee

Results:

11/2014 - 10/2015, CTCs detected in 78 of 211 evaluable samples (37%)

Median CTC count for patients with detectable CTCs was **2** / 7.5 ml blood, and median for the entire cohort was 0 (range 0 - 4000).

Presence of Baseline CTCs was associated with ...

higher PSA ($p=0.03$), **bony metastases** ($p=0.05$), presence of **extensive disease** ($p<0.001$), and a trend toward worse performance status ($p=0.06$).

EGFR positive CTC - resistance to regorafenib

Abstract 11517: Epidermal growth factor receptor mRNA expression in CTCs as potential mechanisms of molecular escape from regorafenib therapy.

Satoshi Matsusaka, et al.

Time schedule



EGFR up-regulation compared to baseline value

	Day 21	PD	Day21 or/and PD
Increased ≥ 0.01 ng/ul	14	24	32
Increased < 0.01 ng/ul	36	17	18
%	28 ($P^*=0.60$)	59 ($P^*=0.041$)	64 ($P^*=0.004$)

- CTC measurement may be useful as a surrogate marker for Regorafenib in CRC.
- Induced expression of EGFR mRNA may be a molecular drug-escape mechanism.
- Therefore, combination of Regorafenib with anti-EGFR mAbs treatment will have synergistic effects.

AR-V7 antigen a hormonální rezistence u ca prostaty

- Androgen receptor isoform encoded by splice variant 7 lacks the ligand-binding domain, which is the target of enzalutamide and abiraterone
- 62 prospectively enrolled pts with castrate resistant prostate cancer¹

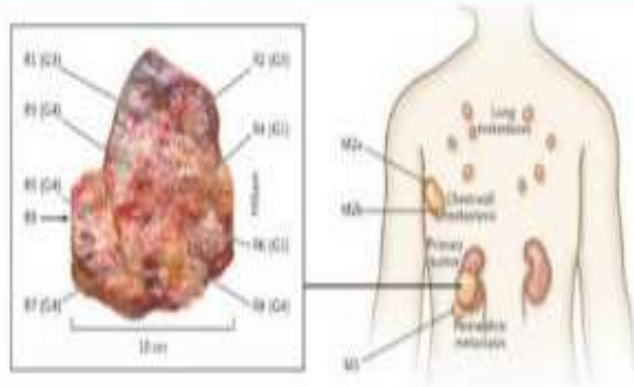
	<u>Enzalutamide</u>		<u>Abiraterone</u>	
• ARV7 detectable patients				
– PSA response	0% vs 53%	p=0.004	0% vs 68%	p=0.004
– Overall survival	5.5 vs NR mths	p=0.002	10.6 vs NR mths	p =0.006

¹Antonarakis ES, et al. *NEJM* 2014

Problems: Heterogeneity of tumor

- How are CTCs representative of the tumor bulks?

Tumor Heterogeneity Presents Significant Challenge to Precision Cancer Medicine



Biopsies obtained from multiple tumor sites demonstrate marked molecular heterogeneity--with no two sites being identical.

Gelberman M, et al. PLoS ONE 2013

Tumor Heterogeneity

REVIEW

doi:10.1016/j.ccr.2013.05.001

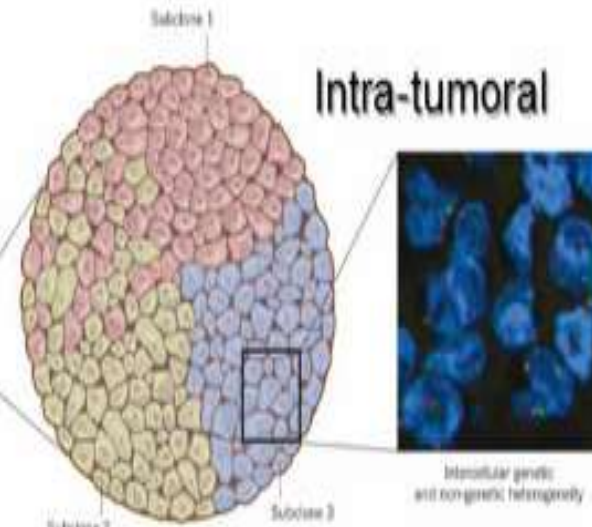
The causes and consequences of genetic heterogeneity in cancer evolution

Sharma S, Bertram J, Roberts S, et al. Cancer Cell 2013

Inter-tumoral



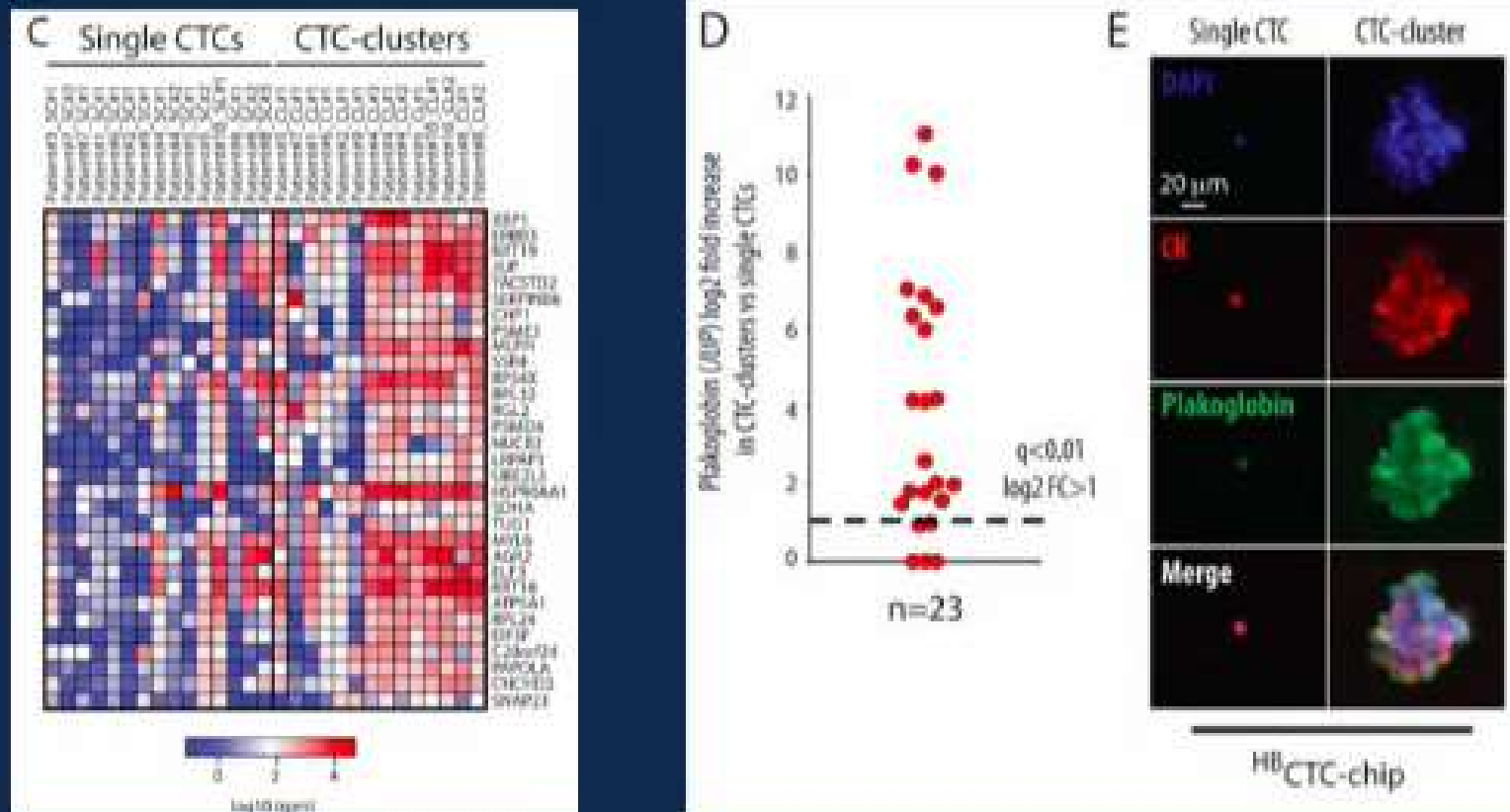
Intra-tumoral



Heterogeneity

It is difficult to detect clusters of CTC

Aceta N et al., CTC clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014;158(5):1110-22.

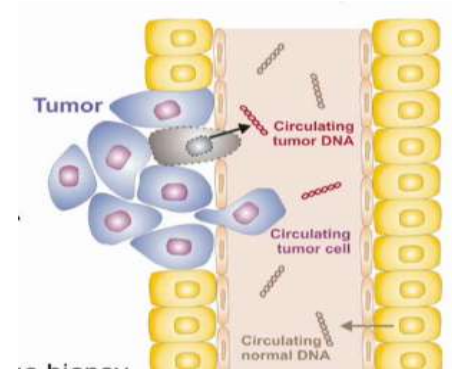


Use of CT in oncology- pros and cons

- ❑ Early detection of CTC reduces deaths ZN colorectal, breas, lung ca
- ❑ Safety, Simplicity, Reproducibility,
- ❑ use of a broad spectrum of tumors

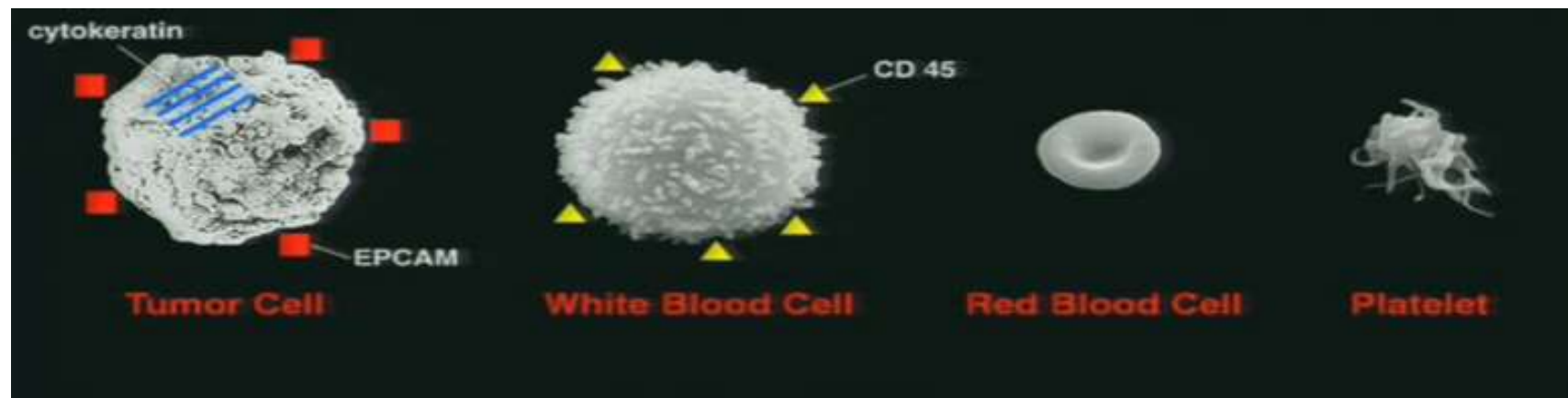
BUT:

- ❑ False positivity and negativity
- ❑ tumor heterogeneity
- ❑ not early detection of cancer
- ❑ Developments and changes, of the primary tumor vs. Metastasis, before and after treatment

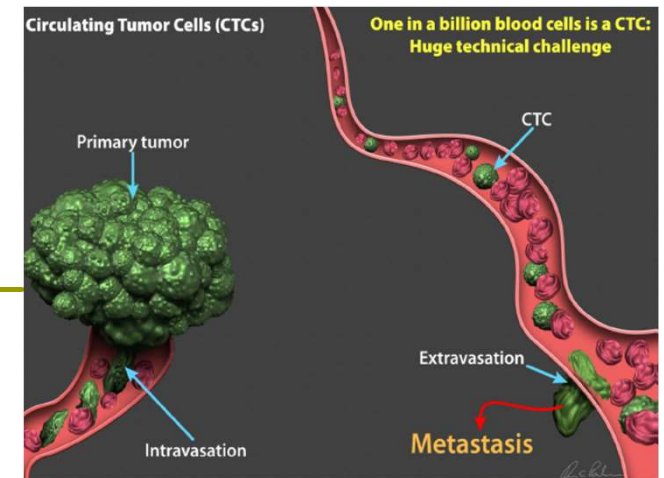


Přitažlivost CTC

- ❑ Bezpečnost
- ❑ Jednoduchost
- ❑ Reprodukovatelnost
- ❑ využití u širokého spektra nádorů
- ❑ Dobrá compliance
- ❑ Odběr kdekoliv, zpracování v centru



Biologie CTC

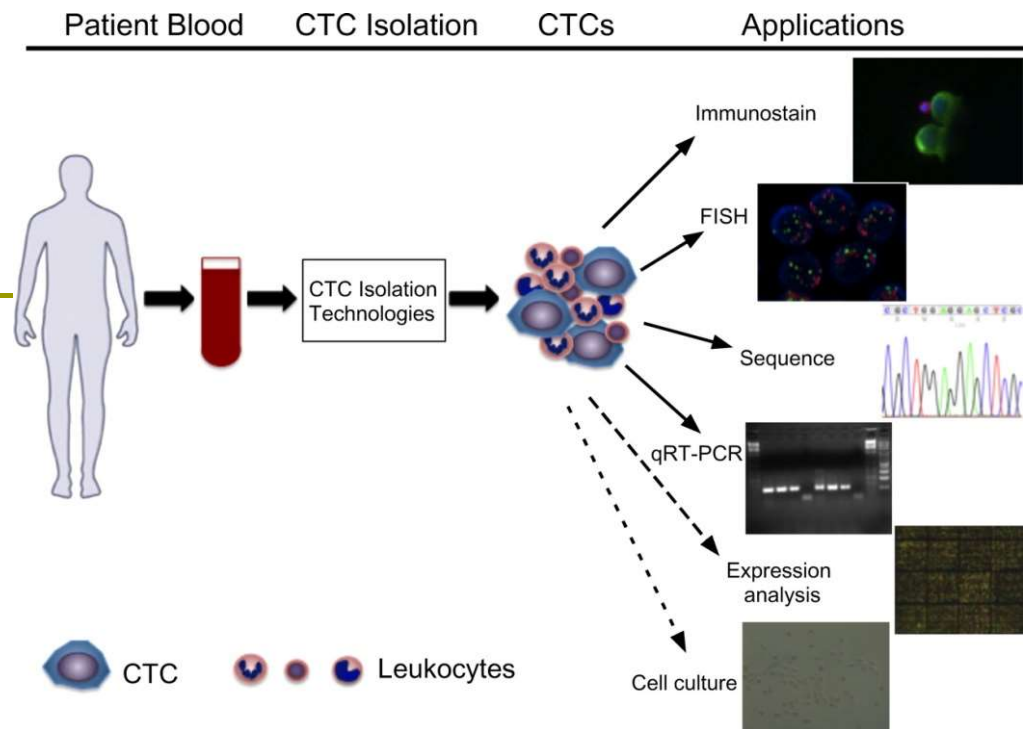


- ❑ 1 CTC na miliardu zdravých
- ❑ Krátké přežití v cirkulaci <24 hodin
- ❑ **Obrovská variabilita:**
- ❑ Poškozené, intaktní, apoptotické CTC
- ❑ Samotné CTC vs. klastry
- ❑ Rozdílné Ki 67
- ❑ Detekce abnormalit DNA, RNA, proteinů, exprese povrchových markerů

Neumíme zachytit

- Klastry,
- Kmenové buňky
- CTC v EMT
- Anaplastické buňky

- **Princip zachytu** : na základě fyzikálních nebo biologických vlastností CTC
- Pozitivní selekce-povrchové markery
- Negativní selekce. Deplece WBC



Applications of liquid biopsy

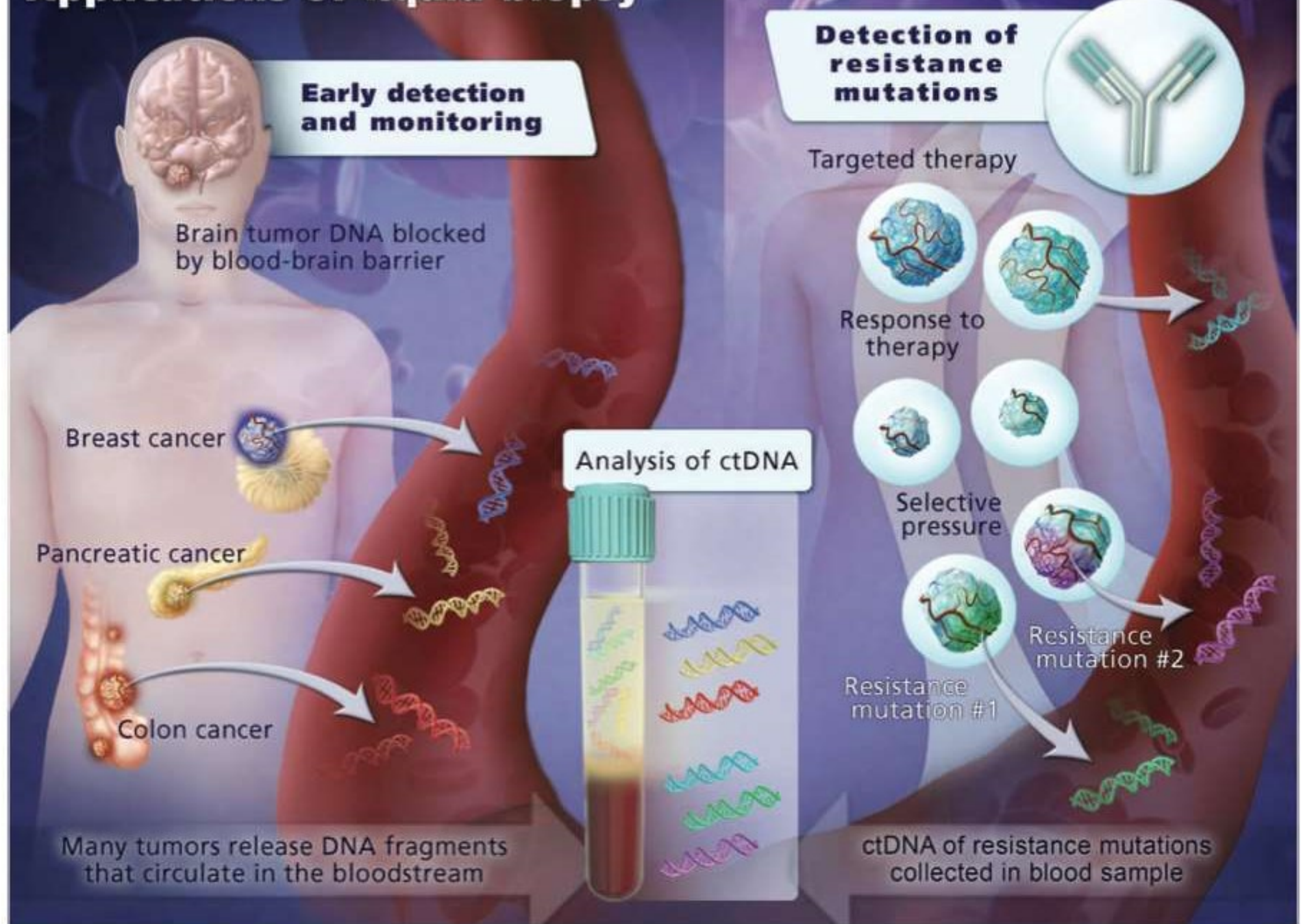
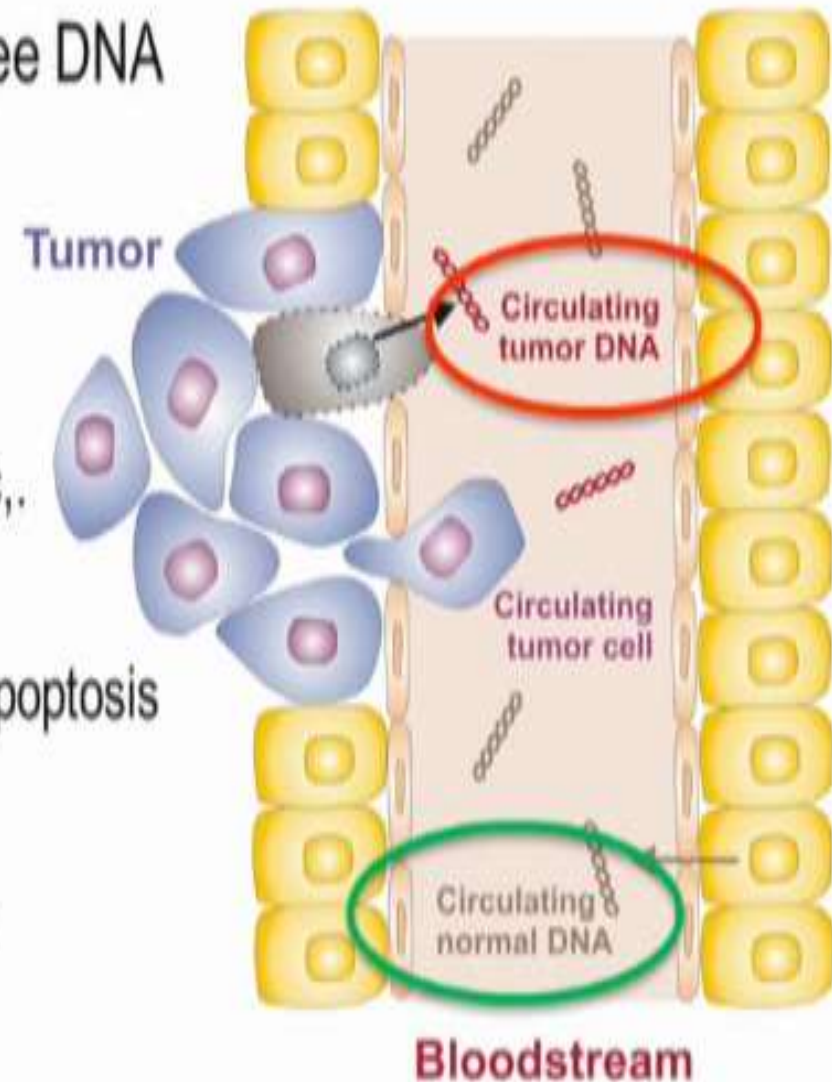


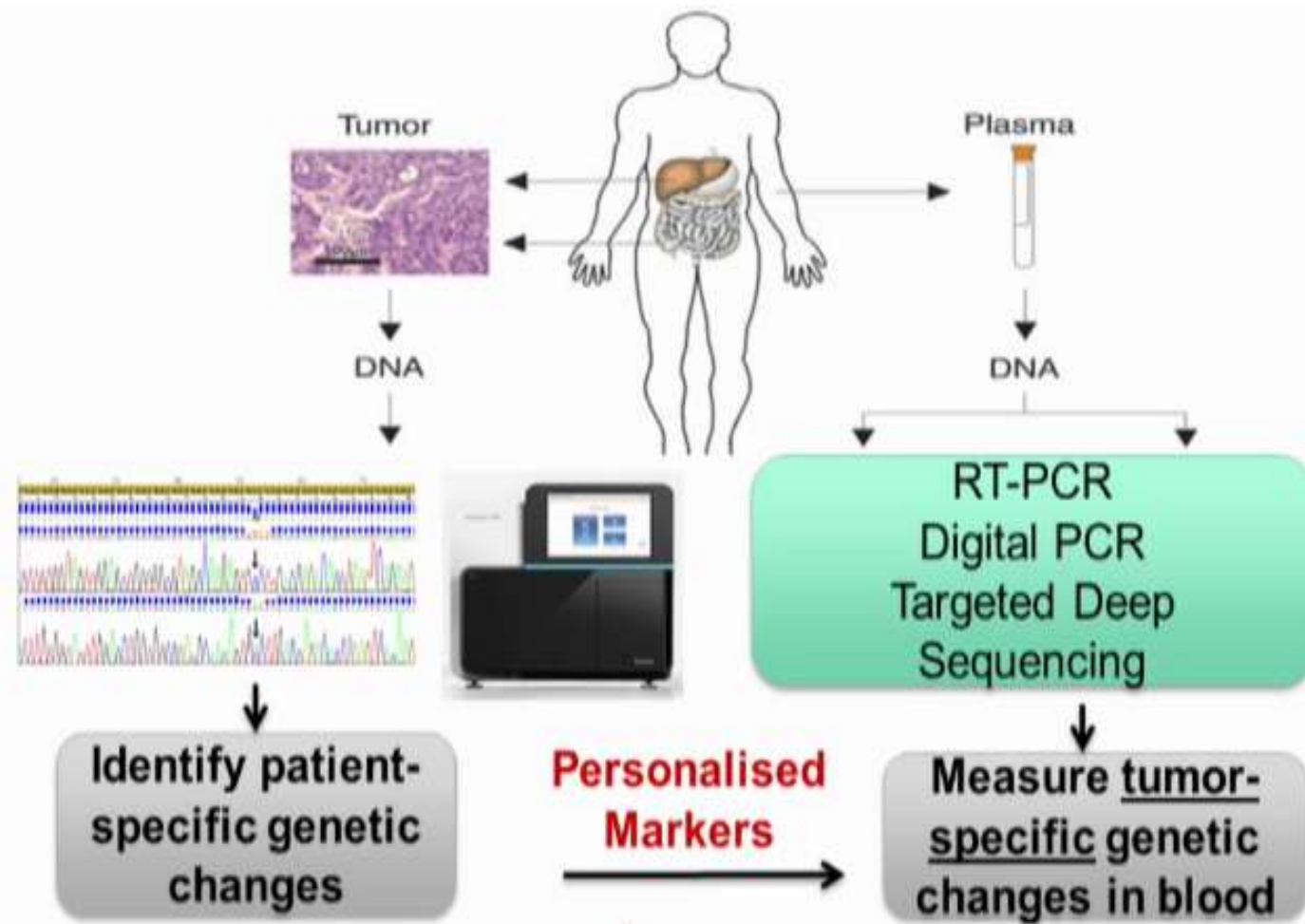
Fig. 1. Potential applications of ctDNA.

Biology of circulating tumor DNA (ctDNA)

- Large amounts of fragmented cell free DNA are present in the circulation
- Normal DNA – WBC, GI tract,....
- Increased with inflammation, trauma, etc.,
- Tumor DNA - released via necrosis or apoptosis
- 0.01 – 60 % of total cell free DNA
- Markers: point mutations, translocations, deletions, amplifications, methylation
- Half-life ~ 2hrs



Detection of ctDNA



Peter Gibbs MBBS, FRACP, MD ASCO 2015

CTC's vs ctDNA - Cancer screening potential

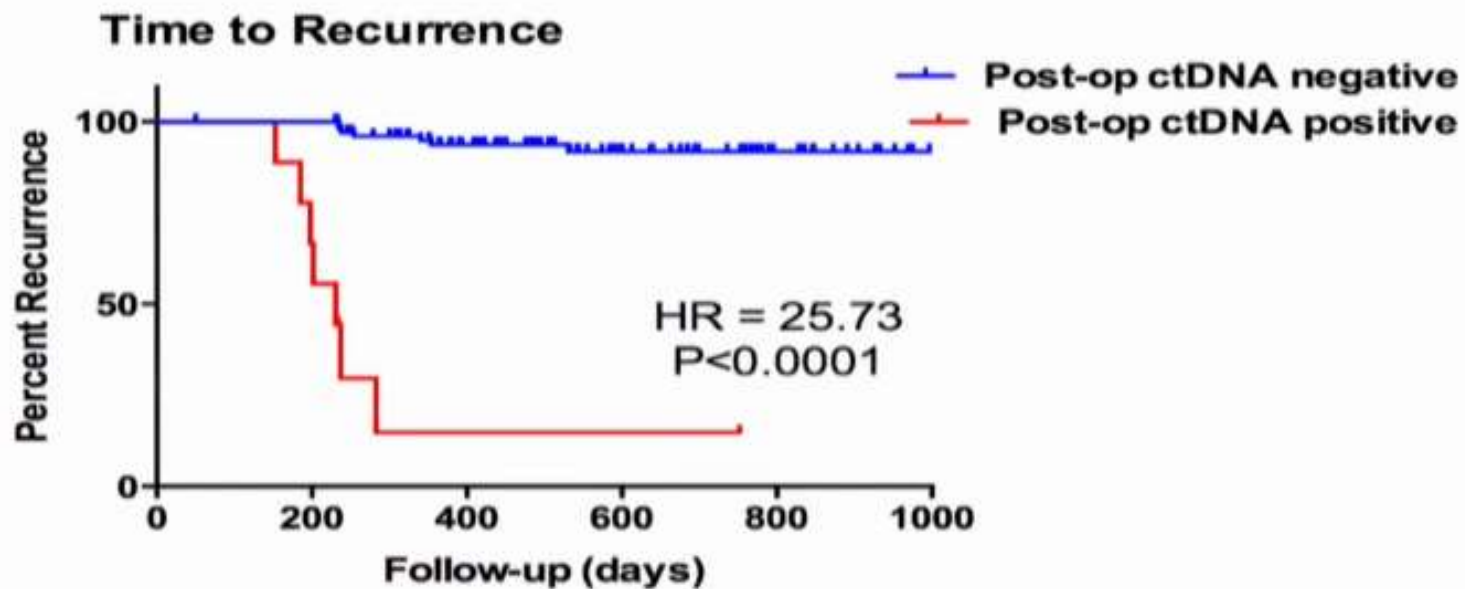
- Lessons learnt (advanced disease)
 - ctDNA has greater sensitivity
 - Detected in > 90% of many common cancers¹
 - Detected where CTCs not detected^{1,2}
 - Greater dynamic range
 - > 50 fold ctDNA fragments vs CTCs²
- ctDNA appears to have greater specificity (advanced and MRD)
 - "CTC's" detected in a small % of normals
 - ctDNA detected in 0% of normals in small series
 - Based on defined thresholds
- The majority of cancers have 2 or more point mutations

¹Bettegowda et al. *Science Translational Med* 2014, ²Dawson SJ, et al *NEJM* 2013

CTC vs ctDNA in MRD detection

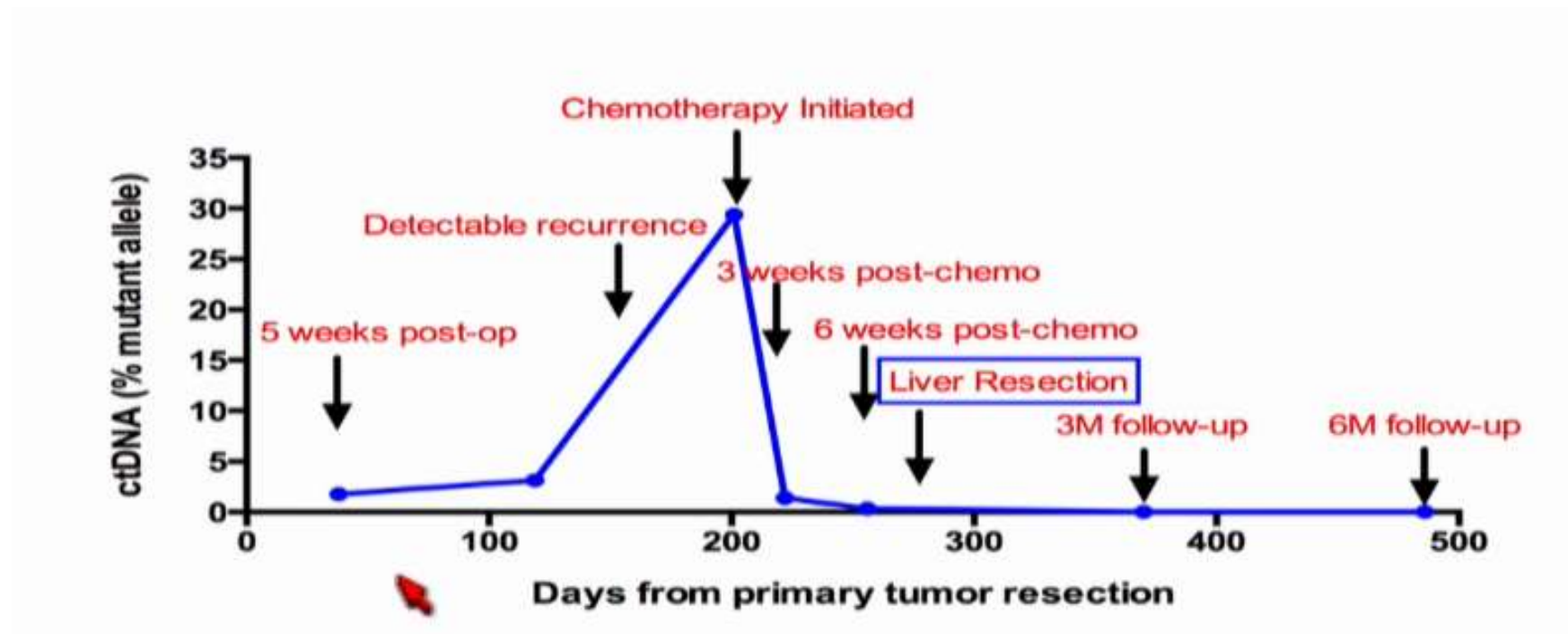
- ❑ **CTC** prognostic significance in some large studies, HR relatively low?
- ❑ impact on individual tailoring of treatment among patients?
- ❑ **ctDNA** prognostic significance in small trials in colorectal cancer
- ❑ impressive HR
- ❑ Treatment MD promising marker (prognosis and response)

The presence of ctDNA patients with CC st. II after surgery (n-112)



¹Tie J, et al. ASCO 2014

Monitoring of disease progression using ctDNA



Ct DNA in the early stages of tumor

	Colorectal (both series)	Breast ¹ (PI3K)
Tumor with mutation	60.3 – 63% (total n = 80)	93.3% (n=15)
Tumor without mutation	N/A	0% (n=14)

1. Beaver JA, et al. *Clin Cancer Res* 2013

Early stage CRC detection of CT DNA

Clinicopathologic features		ctDNA Positive (n=41)	ctDNA Negative (n=27)	P
Age, median (range)		66 (33-85)	68 (40-87)	0.849
Sex	Male	31 (66%)	16 (34%)	0.186
	Female	10 (48%)	11 (52%)	
Tumor Site	Right colon	7 (44%)	9 (56%)	0.087
	Left colon	11 (52%)	10 (48%)	
	Rectum	23 (74%)	8 (26%)	
Stage	I	6 (46%)	7 (54%)	0.070
	II	10 (56%)	8 (44%)	
	III	5 (42%)	7 (58%)	
	LARC*	20 (80%)	5 (20%)	
Differentiation	Well-mod	34 (59%)	24 (41%)	0.729
	Poor	7 (70%)	3 (30%)	
CEA at diagnosis [^]	Normal	28 (64%)	16 (36%)	1
	Elevated	6 (60%)	4 (40%)	
Recurrence	No	36 (57%)	27 (43%)	0.149
	Yes ^o	5 (100%)	0 (0%)	

- Molecular profiling of the colorectal cancer – mutation identified
- ctDNA detectable in 41 of 68 (60.3%) patients
- High detection rate (46%) for stage I cancers
- Similar for right vs left

¹Tie J, et al. ASCO GI 2014

SLIDES ARE THE

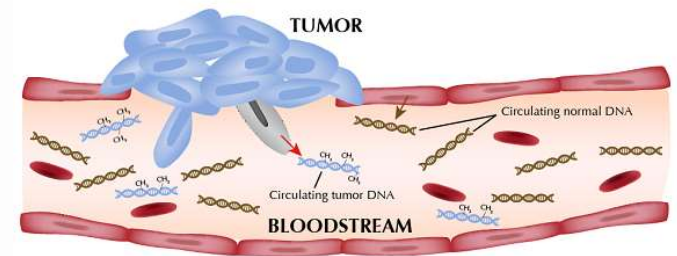
PRESENTED AT:

ASCO Annual '15 Meeting

The challenge is screening - ctDNA

Small burden of tumor, it is not certain place, it is not clear specific mutation

- Sample = Liquid papanicoloau smears
 - Somatic mutations accumulate in the cervix, being shed from endometrial or ovarian cancer
 - Prototype test based on 12 frequently mutated genes
- 14 patients (endometrial cancer = 12, ovarian cancer = 2)
 - Identified 1-5 mutations in all 14 cases
- 14 normals
 - No mutations identified
- Small series, but
 - High sensitivity and specificity (100% for both)



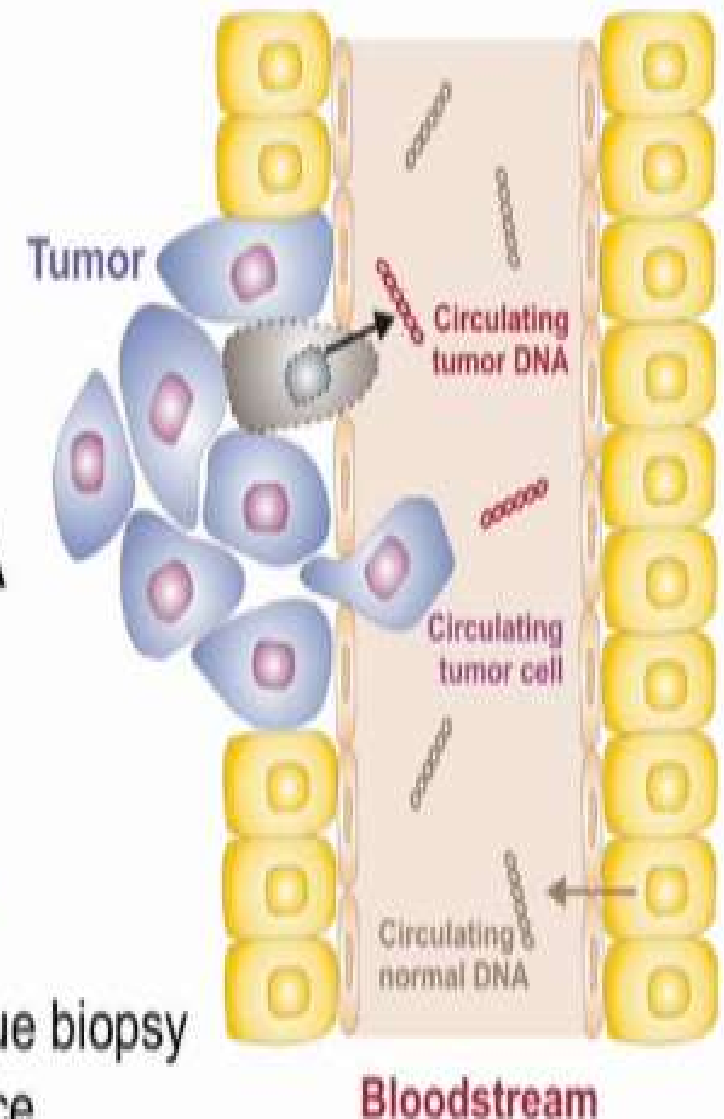
¹Kinde I, et al. *Sci Trans Med* 5,167ra4,2014

Early detection of ctDNA

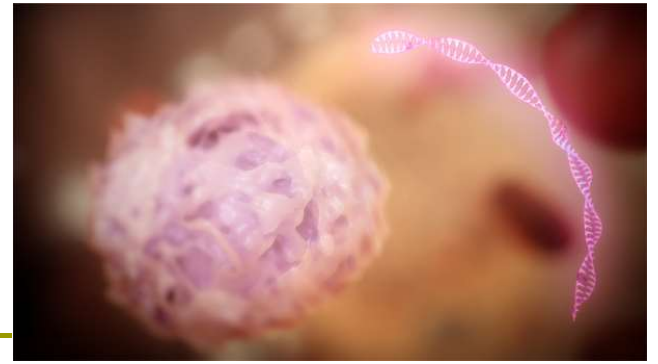
- ❑ it works well for: breast, lung and colorectal cancer
- ❑ > 50% of tumors of early stage has a positive ctDNA
- ❑ Ovary, pancreas, stomach, esophagus
...

Circulating Tumor Cells (CTC's) & Circulating Tumor DNA (ctDNA or cfDNA)

- “Liquid biopsy” - examining tumor material released into the circulation
- Yield varies by tumor stage and type
- Clinical utility demonstrated- CTCs > ctDNA
 - Prognosis in advanced disease
 - Assessment of tumor bulk
 - Early assessment of response
 - Molecular characterisation
 - To tailor initial treatment, alternative to tissue biopsy
 - To tailor later treatment, emerging resistance



Future place for ctDNA



- ❑ Supplement of **routine screening**
- ❑ **Instead** of the previous imaging screening
- ❑ **Early detection** of generalized tumor or tumor of unknown origin
- ❑ **BUT:**
- ❑ Must be validated **specificity and sensitivity**
- ❑ It is necessary to verify **the impact on overall survival**

Prospects for the future? Probably complementarity!

