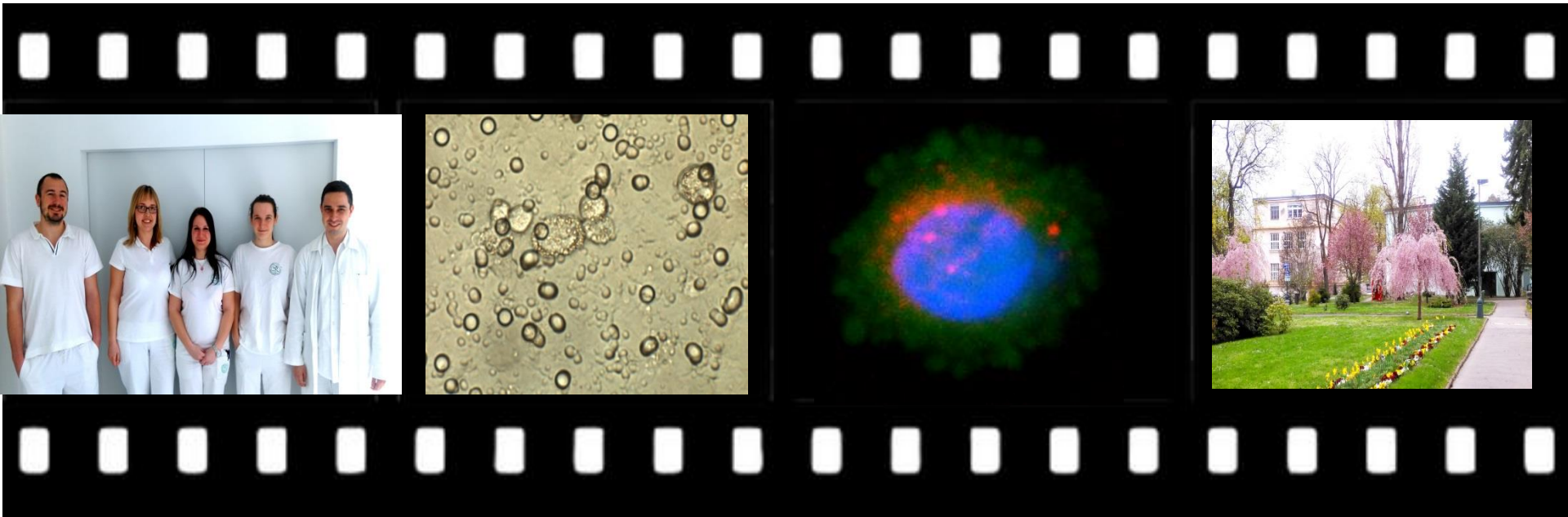




DEPARTMENT OF LABORATORY DIAGNOSTICS, FNKV, PRAQUE

presents



CIRCULATING TUMOR CELLS IN SOLID TUMORS

Liquid biopsy

- Liquid biopsy – a noninvasive blood test detecting circulating tumor cells (**CTCs**) and tumor DNA fragments (**cfDNA - ctDNA**) shed to the circulation from a primary tumor or metastasis

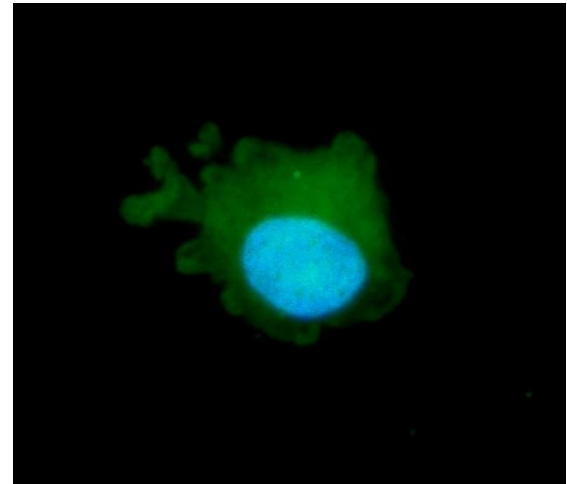
Will the promise of liquid biopsies be a clinical reality? It is hard for me to not to be excited about the benefits they can offer to patients...



Giuseppe Curigliano
Affiliation: European
Institute of Oncology,
Milan, Italy

Circulating Tumor Cells (CTCs)

- Diagnostic
- Prognostic
- Predictive
- Therapy monitoring



Circulating Tumor Cells (CTCs)

- Cost- effective way to tailor the therapy
- Increased therapy efficiency
- Limited drug side-effects
- New druggable biomarkers detection
- CTCs = immediate **real – time** information on tumor development

What is the problem now ?

CTC clinical studies

ClinicalTrials.gov

A service of the U.S. National Institutes of Health

Example: "Heart attack" AND "Los Angeles"

Search for studies:

Search

[Advanced Search](#) | [Help](#) | [Studies by Topic](#) | [Glossary](#)

[Find Studies](#) ▾

[About Clinical Studies](#) ▾

[Submit Studies](#) ▾

[Resources](#) ▾

[About This Site](#) ▾

[Home](#) > [Find Studies](#) > Search Results

[Text Size](#) ▾

868 studies found for: circulating tumor cells

Found 868 studies with search of: circulating tumor cells

Recognized Terms and Synonyms:

circulating tumor cells: 405 studies

CTC clinical studies

Scientific Rationale – Update June 2016

The NCCN Clinical Practice Guidelines on Breast Cancer (Version 2.2016) notes: “The clinical use of circulating tumor cells (CTC) in metastatic breast cancer is not yet included in these guidelines for disease assessment and monitoring. Patients with persistently increased CTC after 3 weeks of first-line chemotherapy have a poor progression free survival (PFS) and overall survival (OS). In spite of its prognostic ability, CTC count has failed to show a predictive value”.

Why predictive value of CTCs could not be shown so far ?

Cancer Metastasis Rev (2015) 34:527–545
DOI 10.1007/s10555-015-9582-0

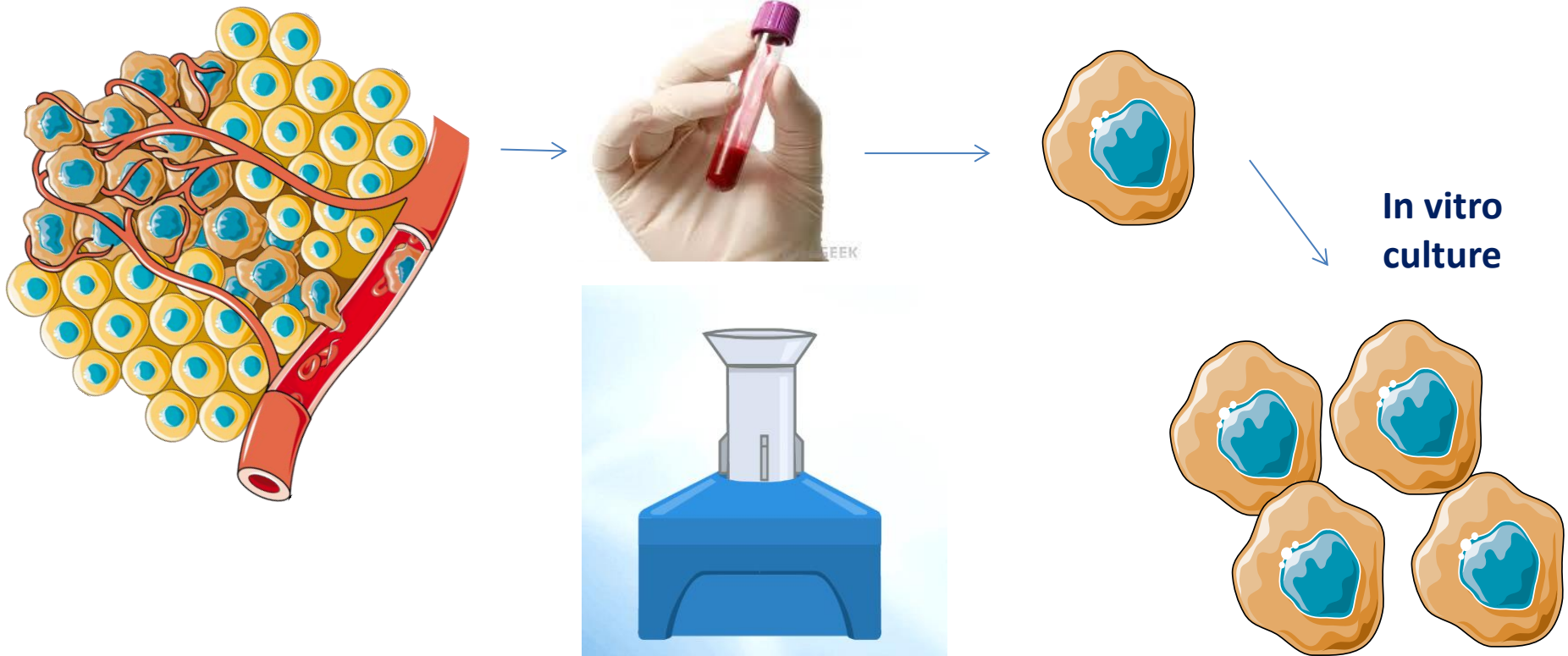


CLINICAL

Diversity of assessing circulating tumor cells (CTCs) emphasizes need for standardization: a CTC Guide to design and report trials

S. Bünger¹ • M. Zimmermann¹ • J. K. Habermann¹

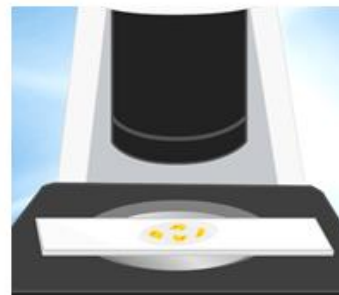
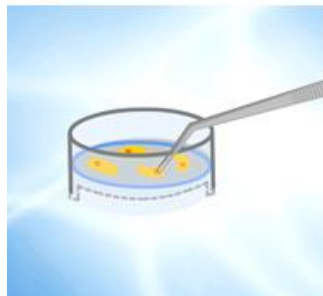
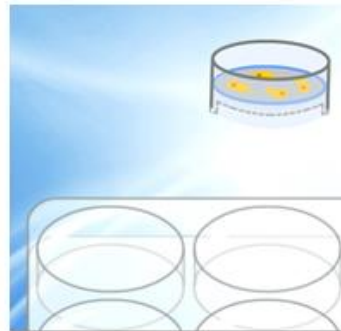
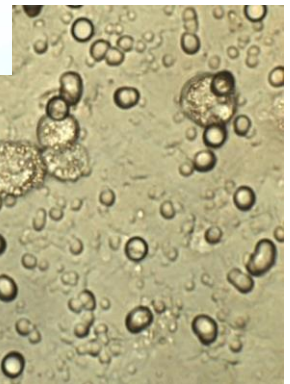
Liquid Biopsy



- MetaCell® platform = making CTC separation simple
- getting viable CTCs to propagate – no sample amount limitation for downstream application !!!!!

MetaCell[®] platform

....a tube size-based exclusion CTC



STORAGE !!!



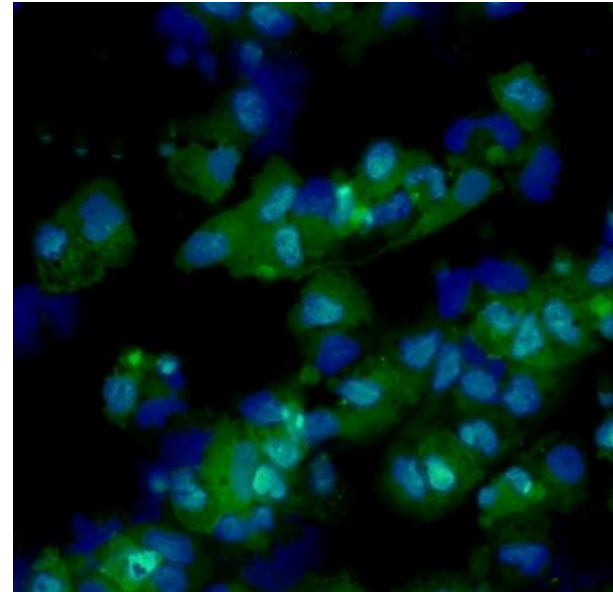
CTC diagnostics in daily routine

1. CYTOMORPHOLOGY

- A. Without cultivation
- B. After in vitro culture

2. GENE expression testing

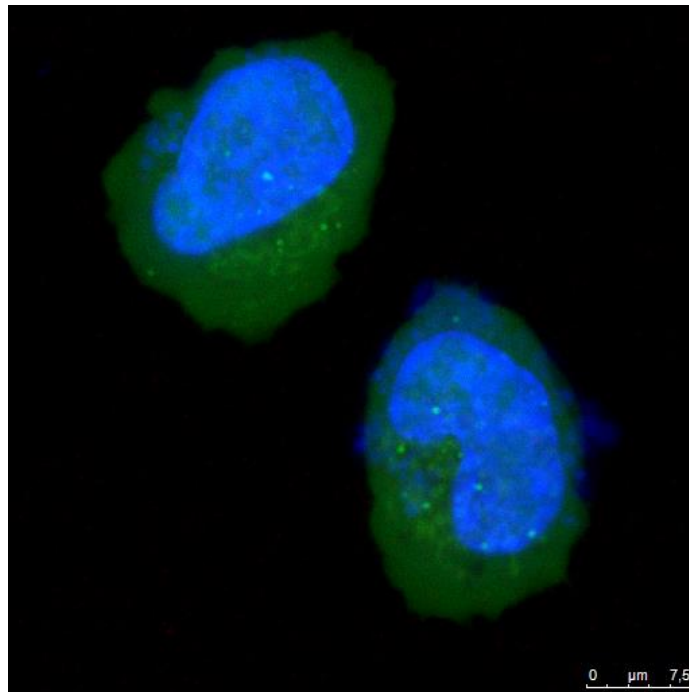
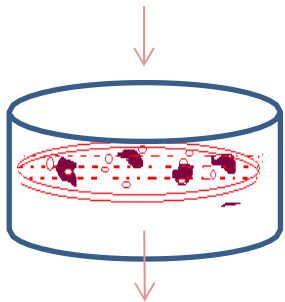
- A. TUMOR ASSOCIATED GENES
- B. CHEMOREZISTANCE ASSOC.GENES



CTC identification process



Viable Nuclear Stain
(NucBlue™) + vital
cytoplasm stain



Proliferation

Plasticity

Cytomorphological evaluation
(Live)

DNA

Mutational analysis

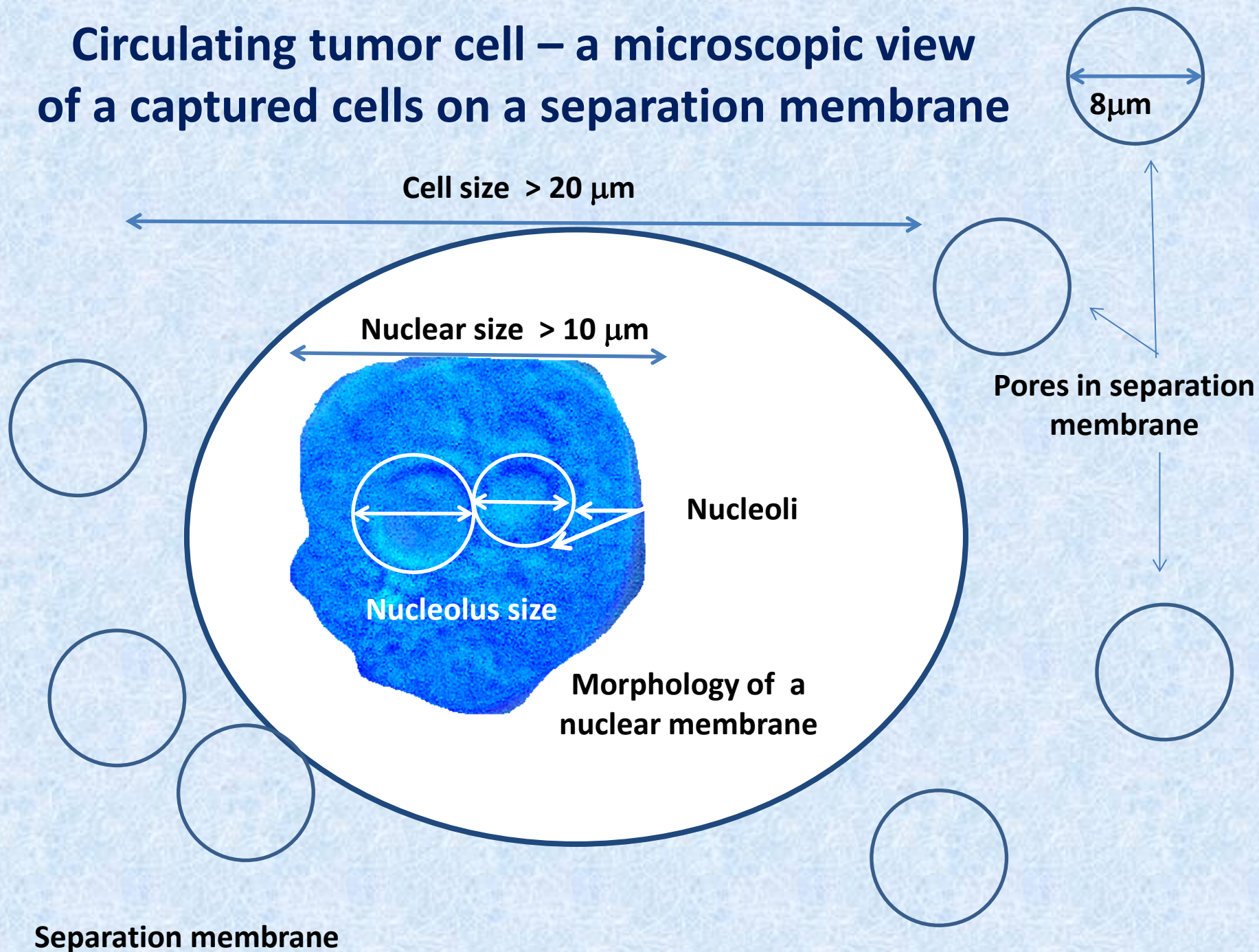
Methylation analysis

RNA

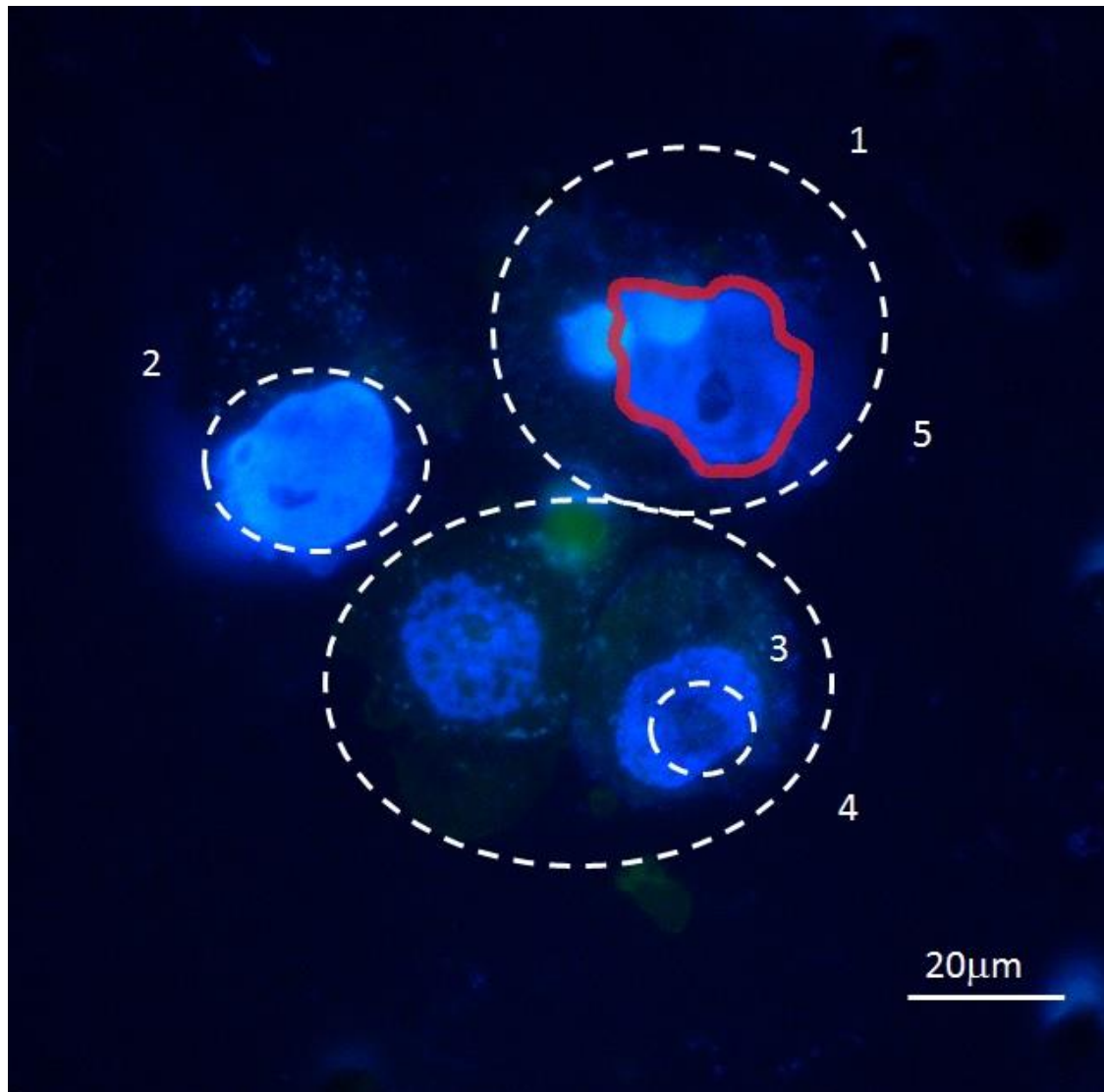
Multimarker
Gene expression
profiling

High variability and multiple
marker combinations

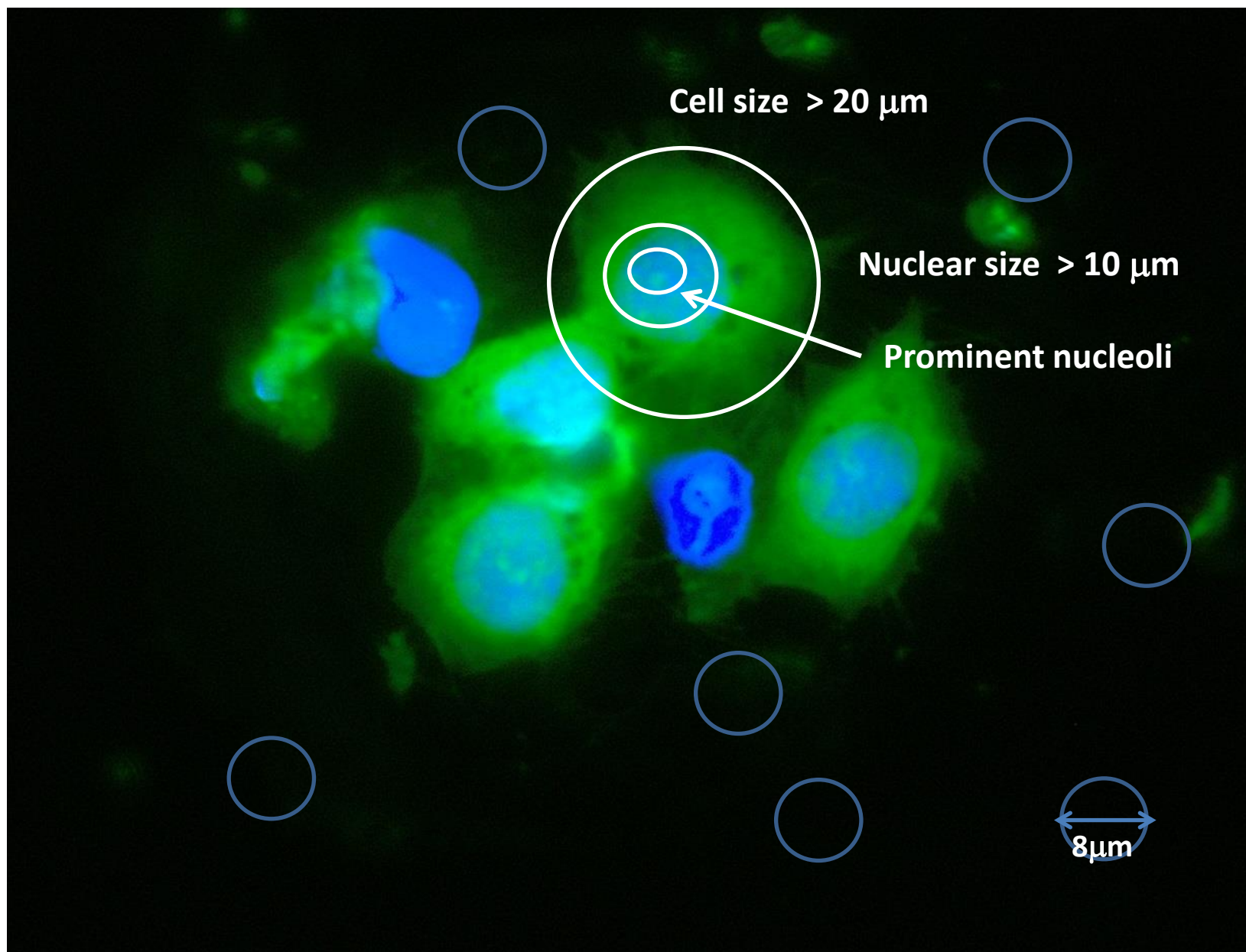
Circulating tumor cell – a microscopic view of a captured cells on a separation membrane



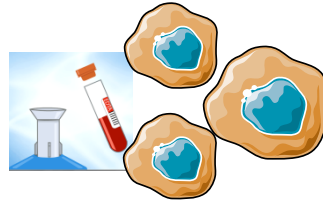
Captured cells on a separation membrane – CYTOPATHOLOGY CRITERIA (vital fl.stain)



1. Cell size
2. Nuclear size
3. Prominent nucleoli
4. Cells making 2D sheets - epithelial like
5. Irregular nuclear membrane
6. Nuclear / cytoplasmatic ratio
7. Forming of 3D sheets
8. Invading the membrane
9. Proliferation



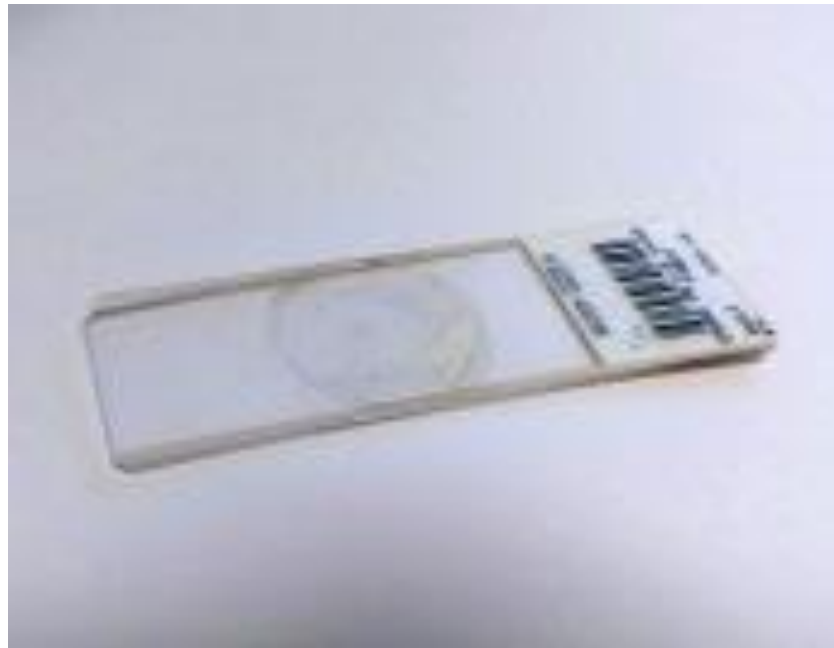
How to standardize the testing outcomes ?



**Standardized
outcomes of
cytopathological
testing for all
tumor types**

Blood withdrawal (EDTA)

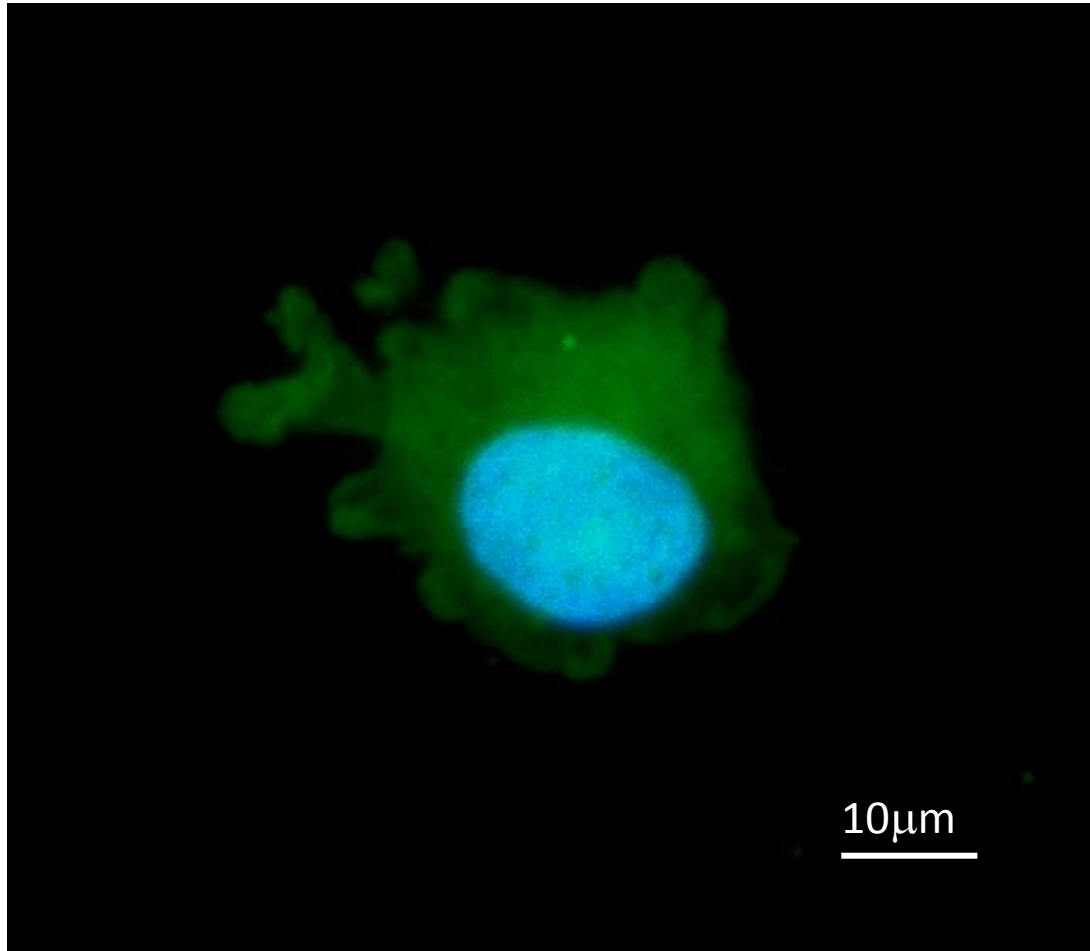
CTC isolation Automatization of IHC analysis(Ventana)



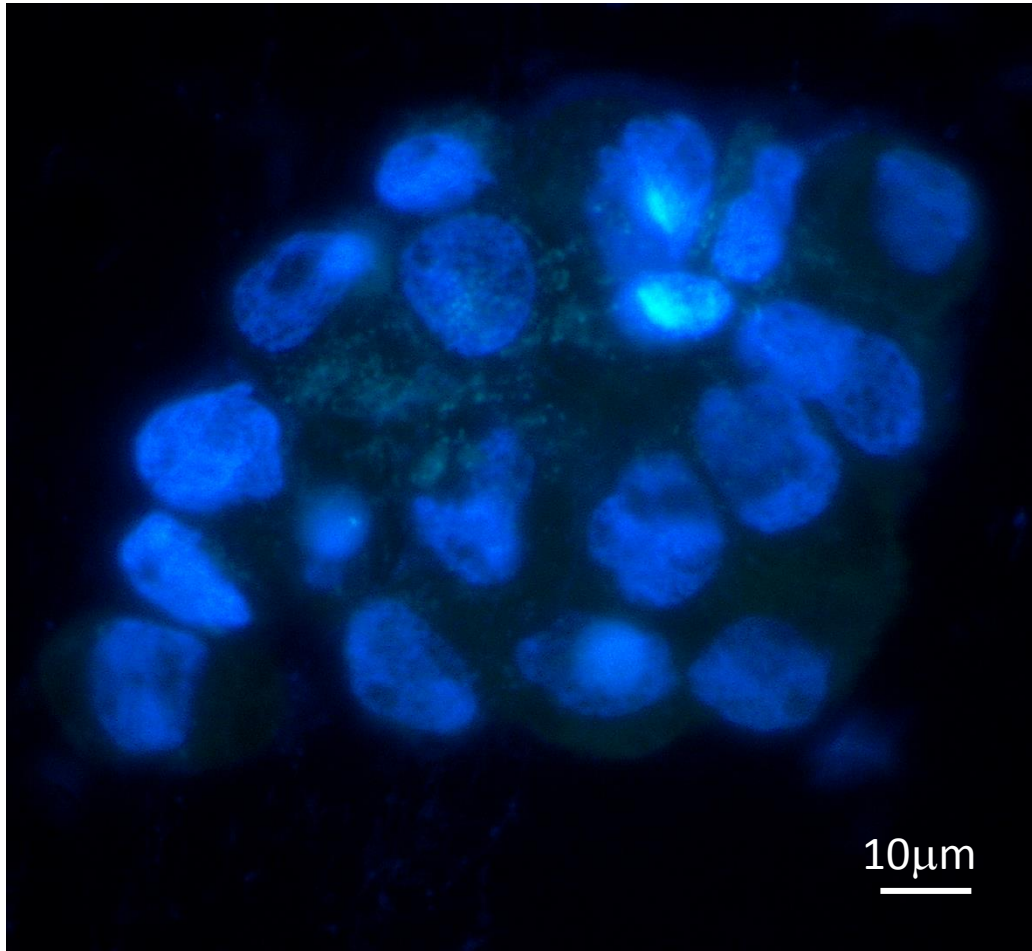
VENTANA®



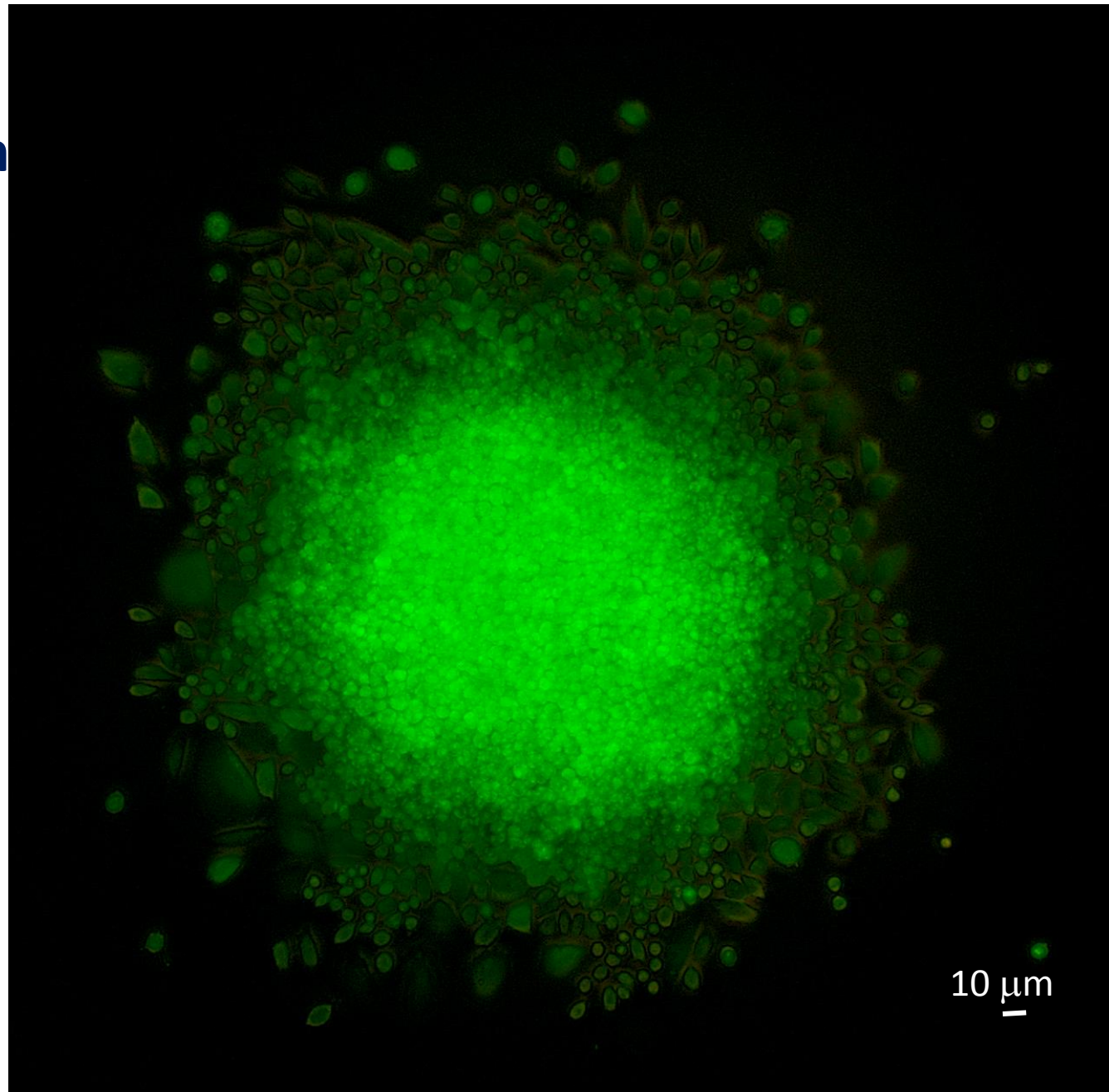
Clear cell carcinoma CTC



CTCs – ovarian cancer (BRCA 1- mutated)



**CTC – *in vitro*
culture of ovarian
cancer**



CTC diagnostics from 1 → 2

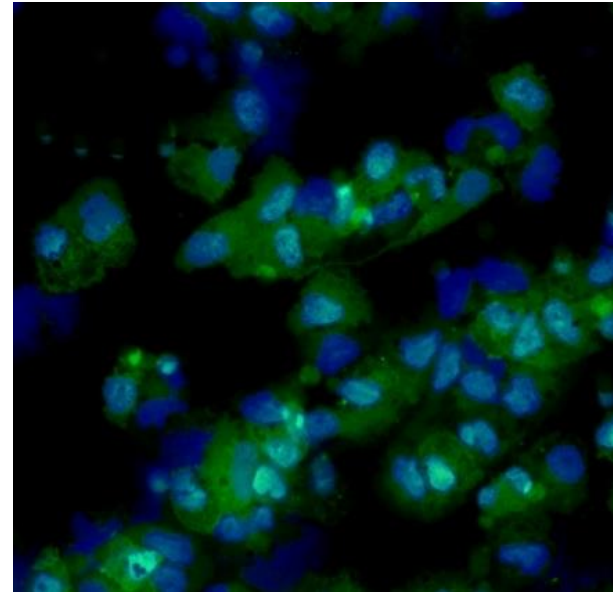


1. CYTOMORPHOLOGY

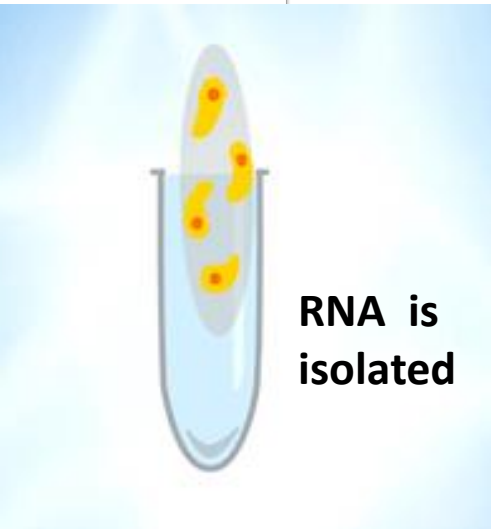
- A. Without cultivation
- B. After in vitro culture

2. GENE expression testing

- A. TUMOR ASSOCIATED GENES
- B. CHEMOREZISTANCE ASSOC.GENES
- C. RNA in vivo detection



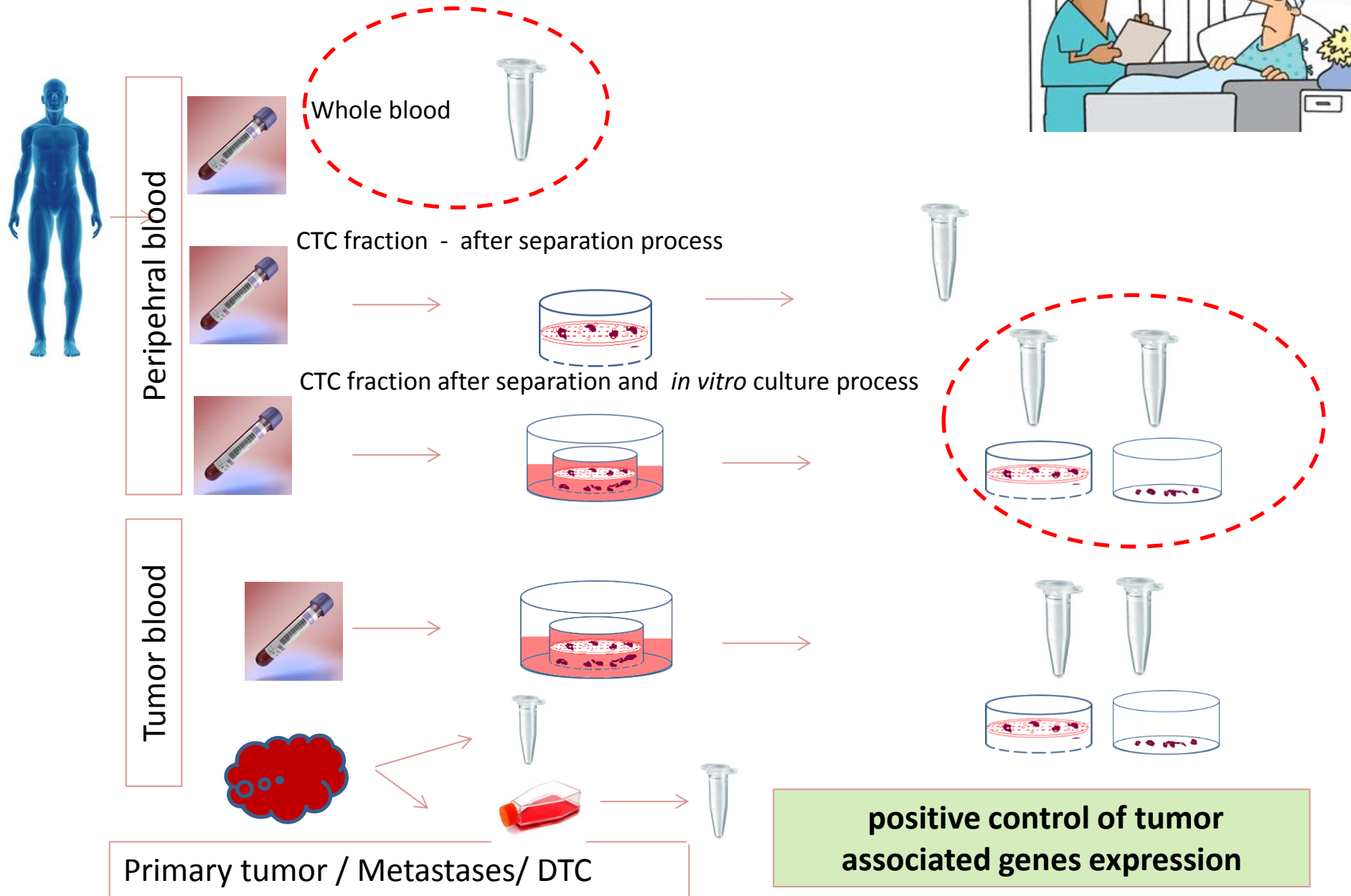
Gene expression analysis



RNA is isolated

Gene expression analysis of CTC- enriched fraction based on qPCR													
Tumor as.genes		T	↑		Tumor as.genes		T	↑		Chemoresistance	T	↑	
1	ACTB	X			14	CHGA				27	MRP1	X	
2	CD45	X			15	MLANA				28	MRP2	X	
3	CD68	X			16	S100B				29	MRP4	X	
4	EPCAM	X			17	VIM	X			30	MRP5	X	
5	MUC1	X			18	KLK3/PSA				31	MRP7	X	
6	TTF1	X			19	WT1				32	MDR1	X	
7	KRT5	X			20	CD56				33	ERCC1	X	
8	KRT6	X			21	CD57				34	RRM1	X	
9	KRT7				22	PSMA				35	RRM2	X	
10	KRT18	X			23	ALDH	X			36	dCK		
11	KRT19	X			24	CD24	X			37	hENT		
12	KRT20				25	CD44	X			38	HPRT		
13	EGFR				26	tp63							
		T			Genes tested by qPCR								
		↑			Genes with relatively elevated gene expression tested by qPCR								
		!			Consider therapy change								

Gene expression analysis - Dx.



Gene expression analysis data presentation

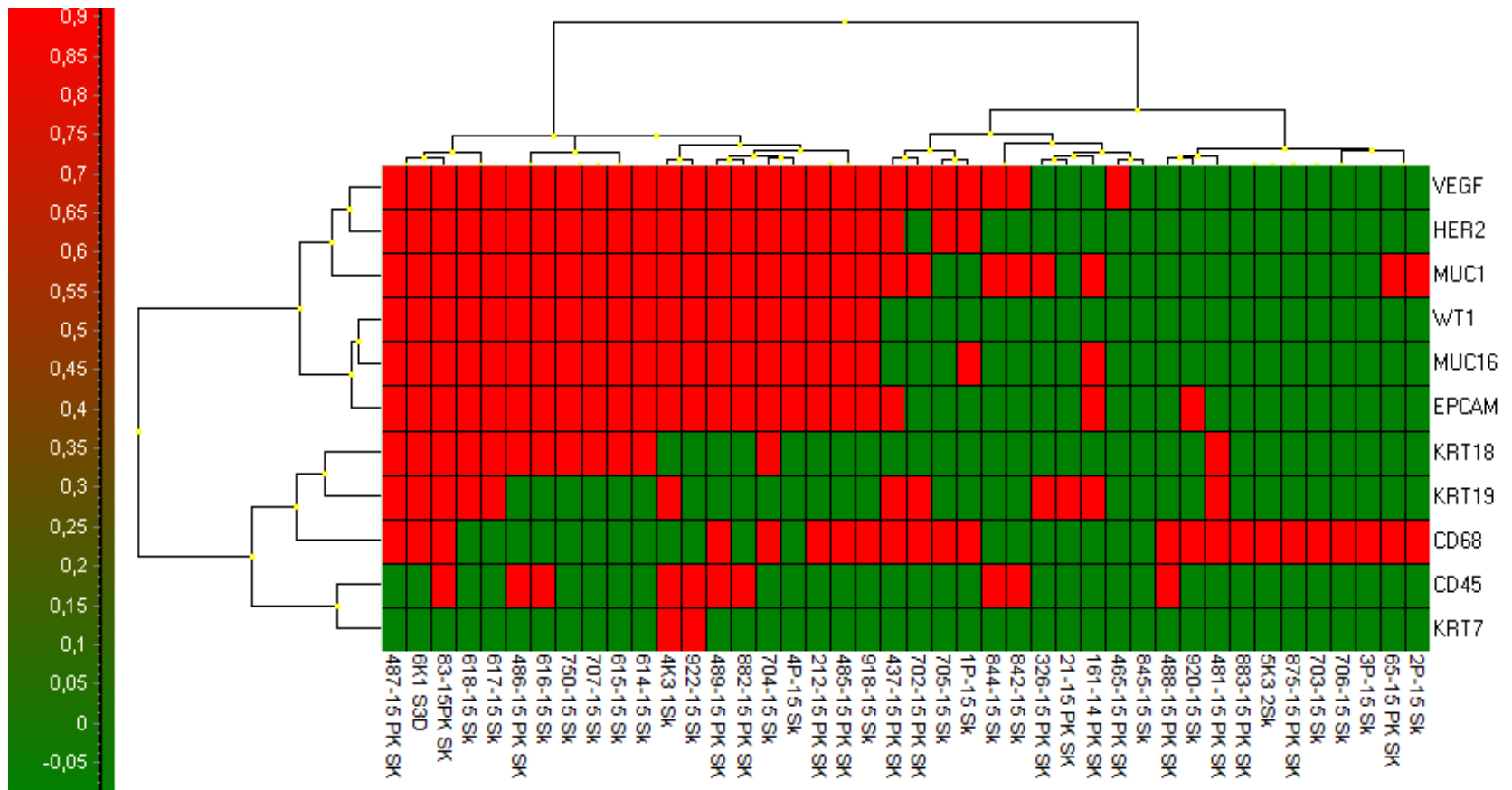
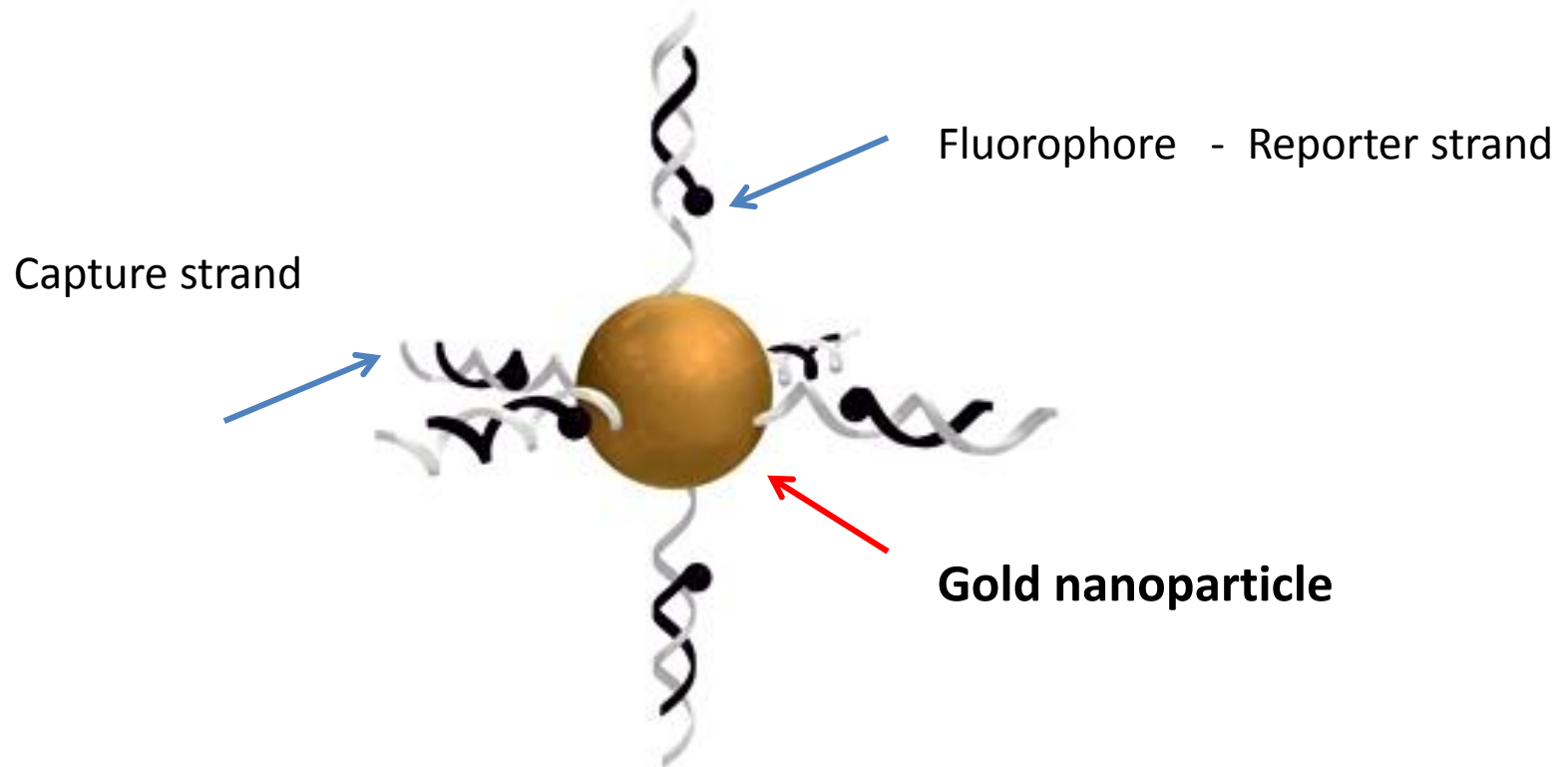
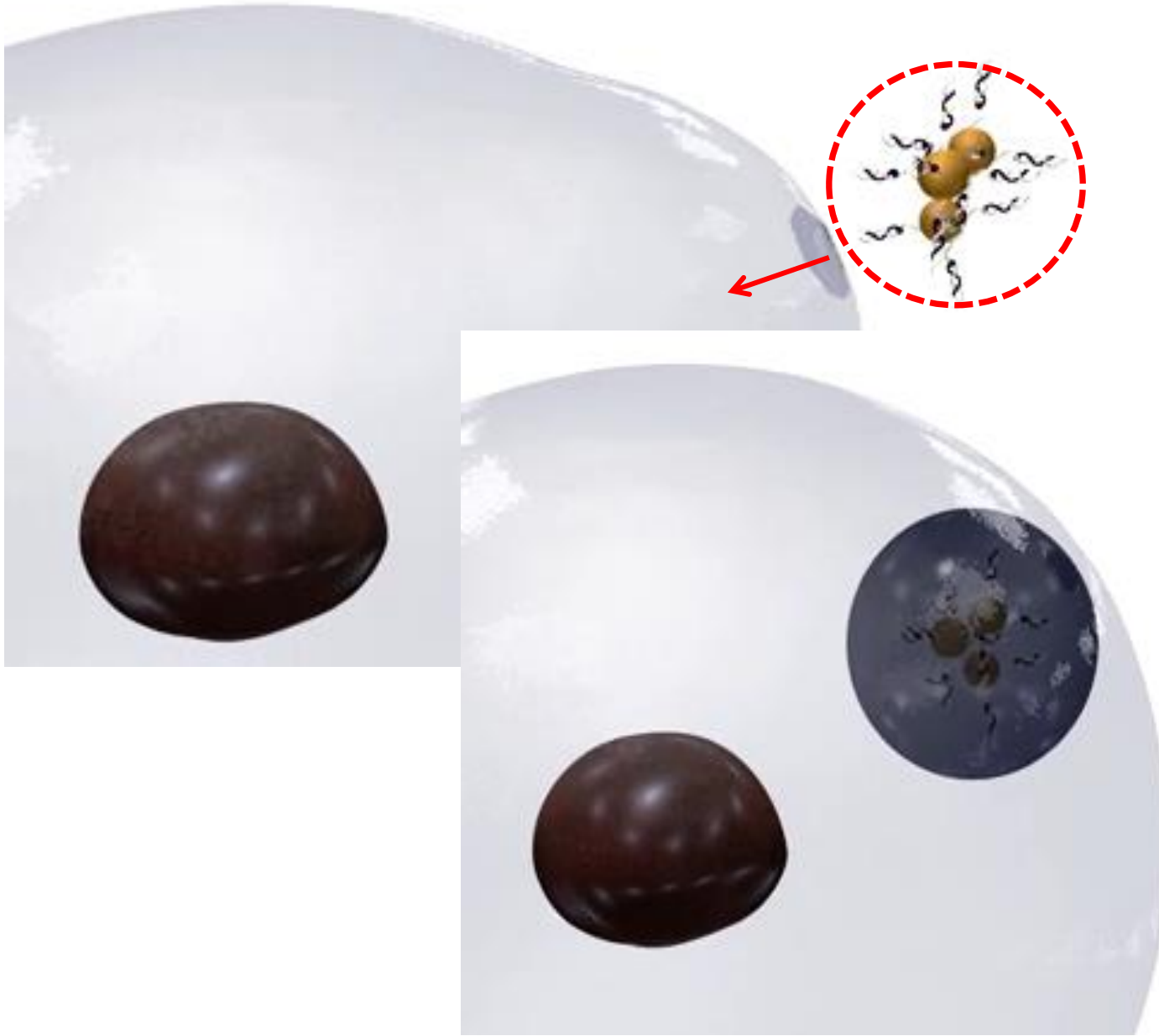


Fig. **Cluster analysis of gene expression data** for all CTC- membrane fractions (PK- SK) is clearly identifying CTC- positive and CTC negative group of patients with ovarian cancer (higher expression is shown in red). (Published data)

RNA in vivo detection - SmartFlare™ (an alternative to qPCR testing)



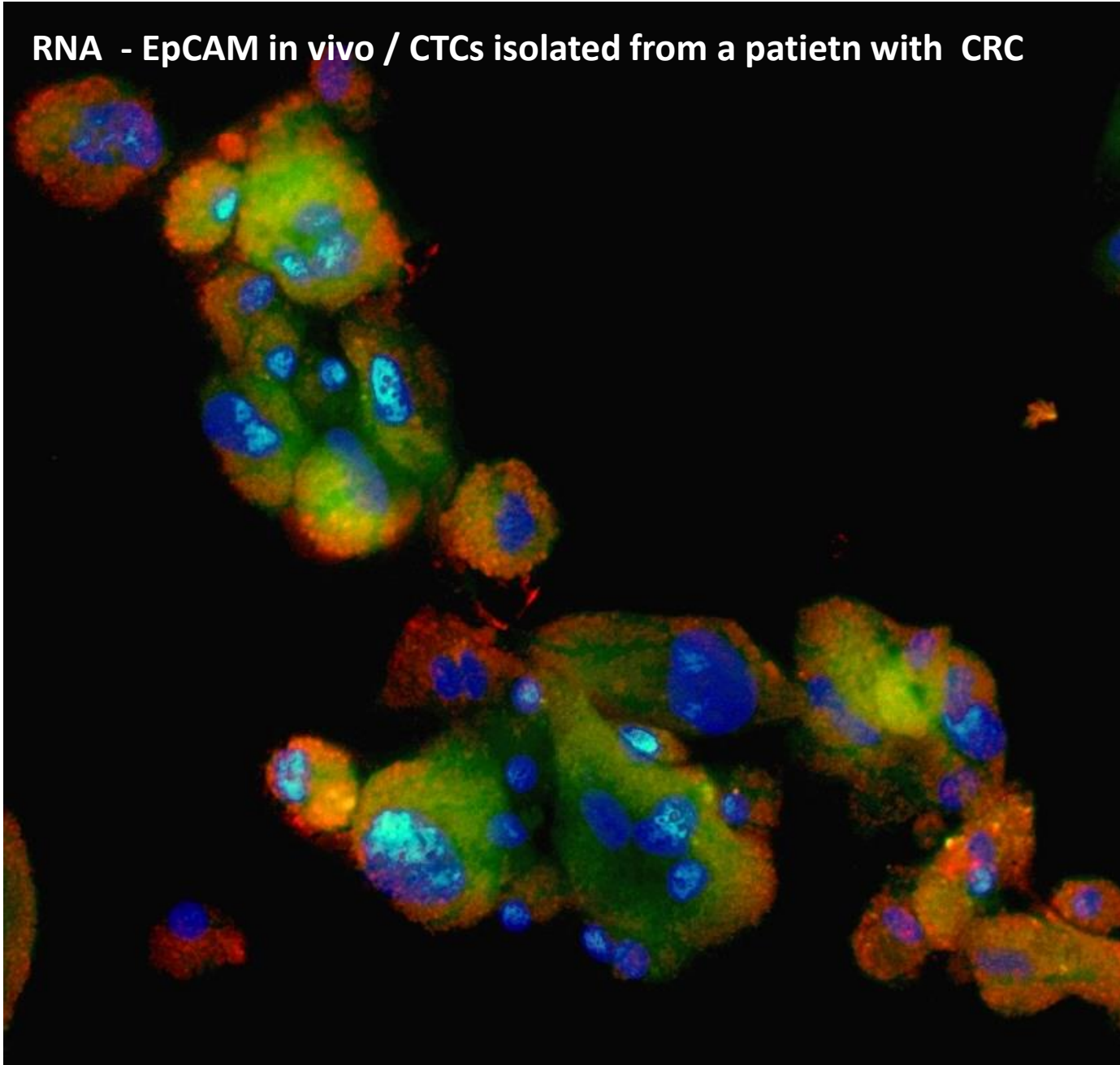
Import via endocytosis



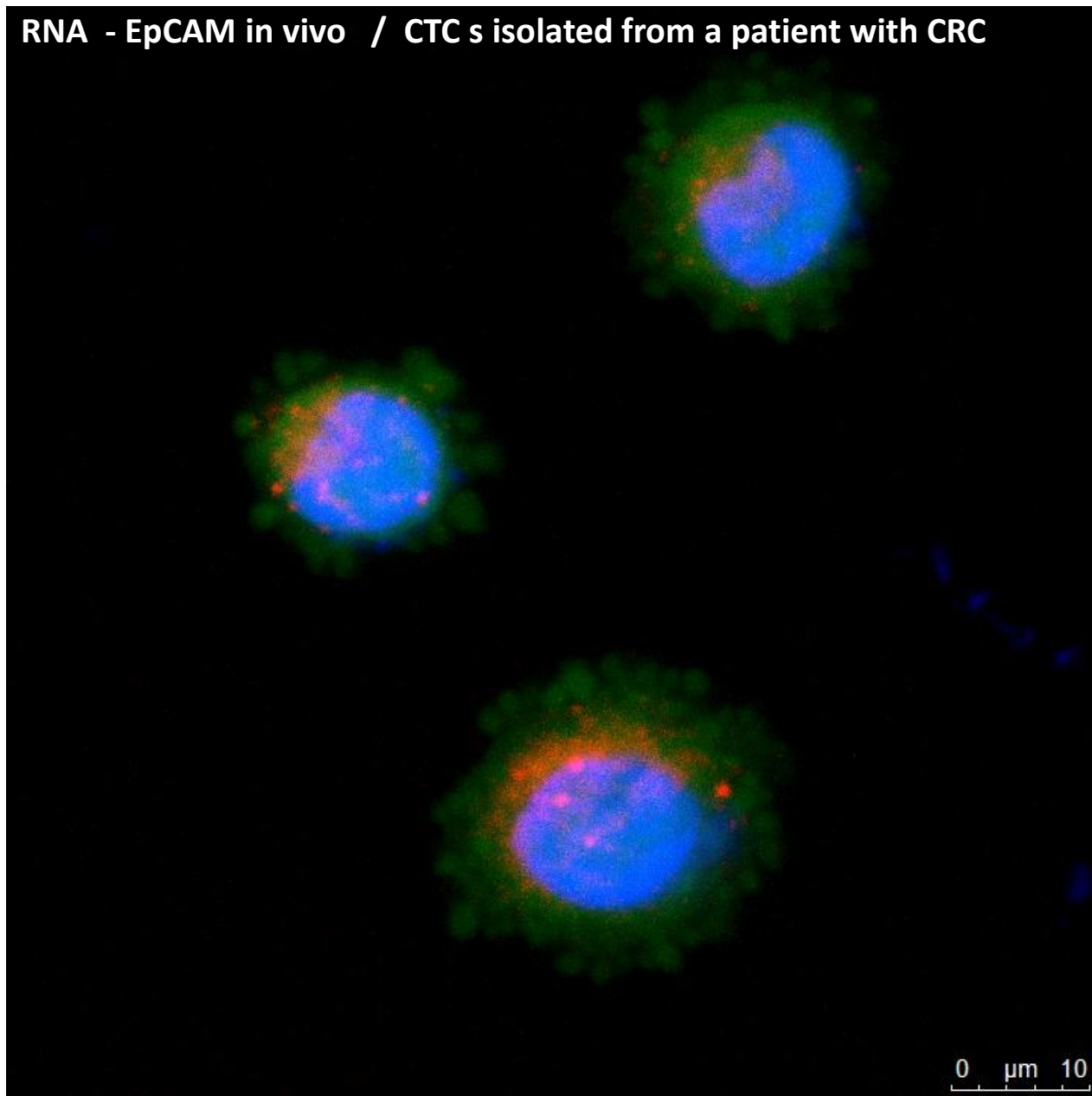
Export via exosomes



RNA - EpCAM in vivo / CTCs isolated from a patient with CRC



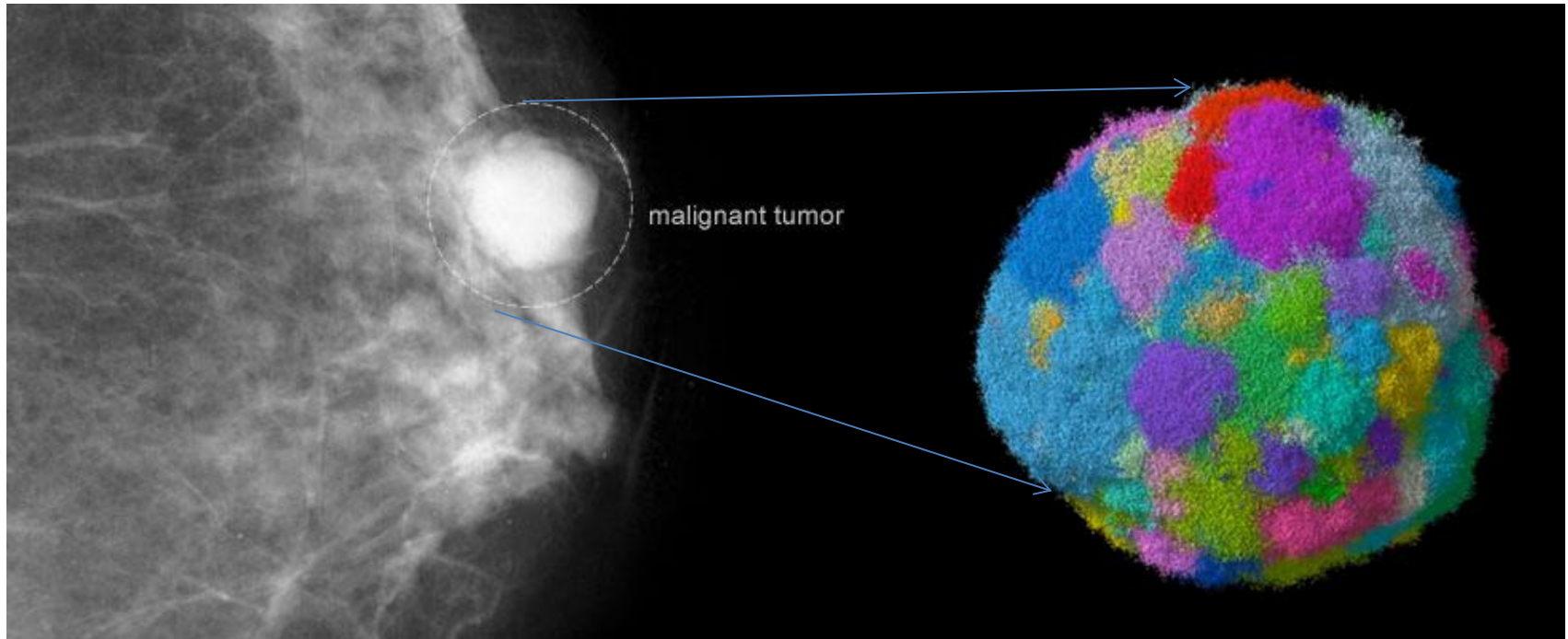
RNA - EpCAM in vivo / CTC s isolated from a patient with CRC



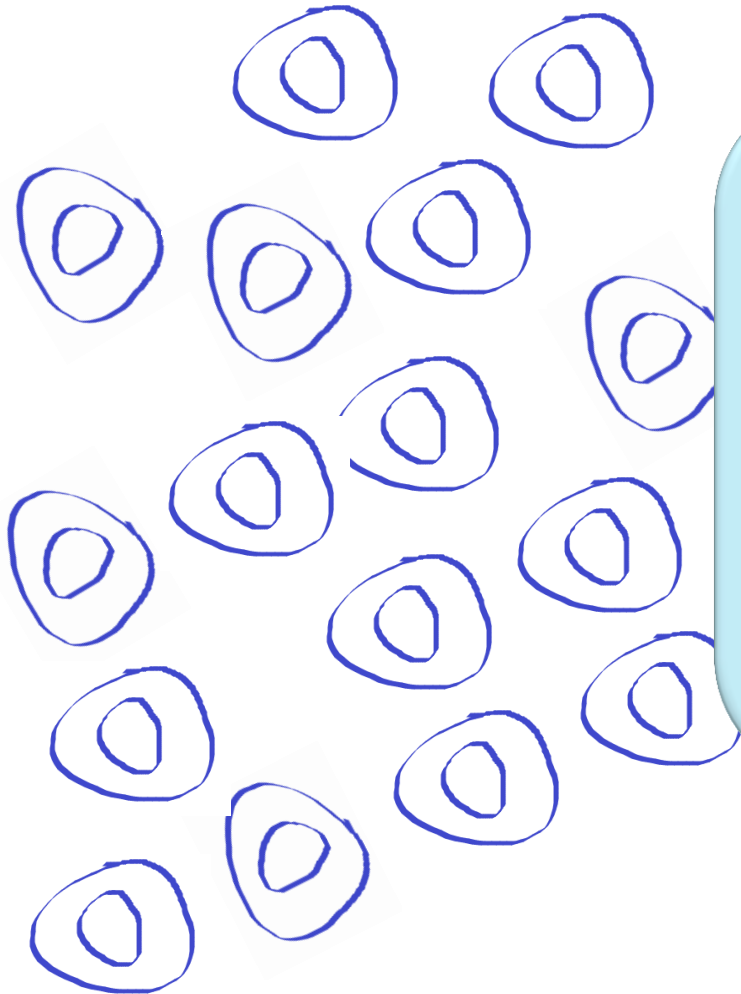
1. Do CTCs reflect tumor heterogeneity ? .

**2. Chemoresistance testing
on the CTC - level ?**

Do CTCs reflect tumor heterogeneity ?



Does the CTC- count matter ?



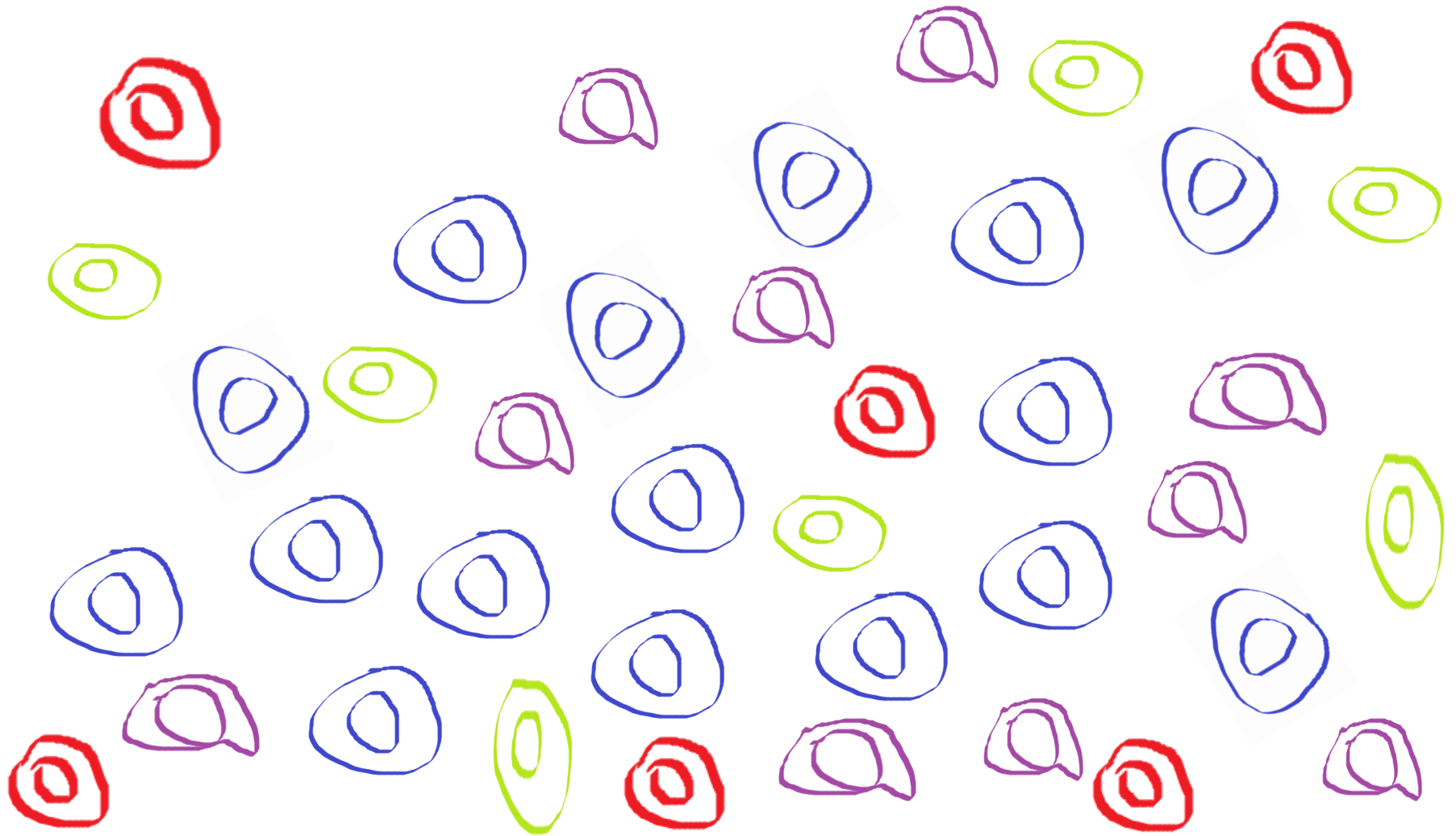
**Clinical studies (based on FDA-
approved Cellsearch protocol) →
CTC- count matters !!!**

**Patients with elevated CTC- numbers
are doing worse (OS, DFS)**

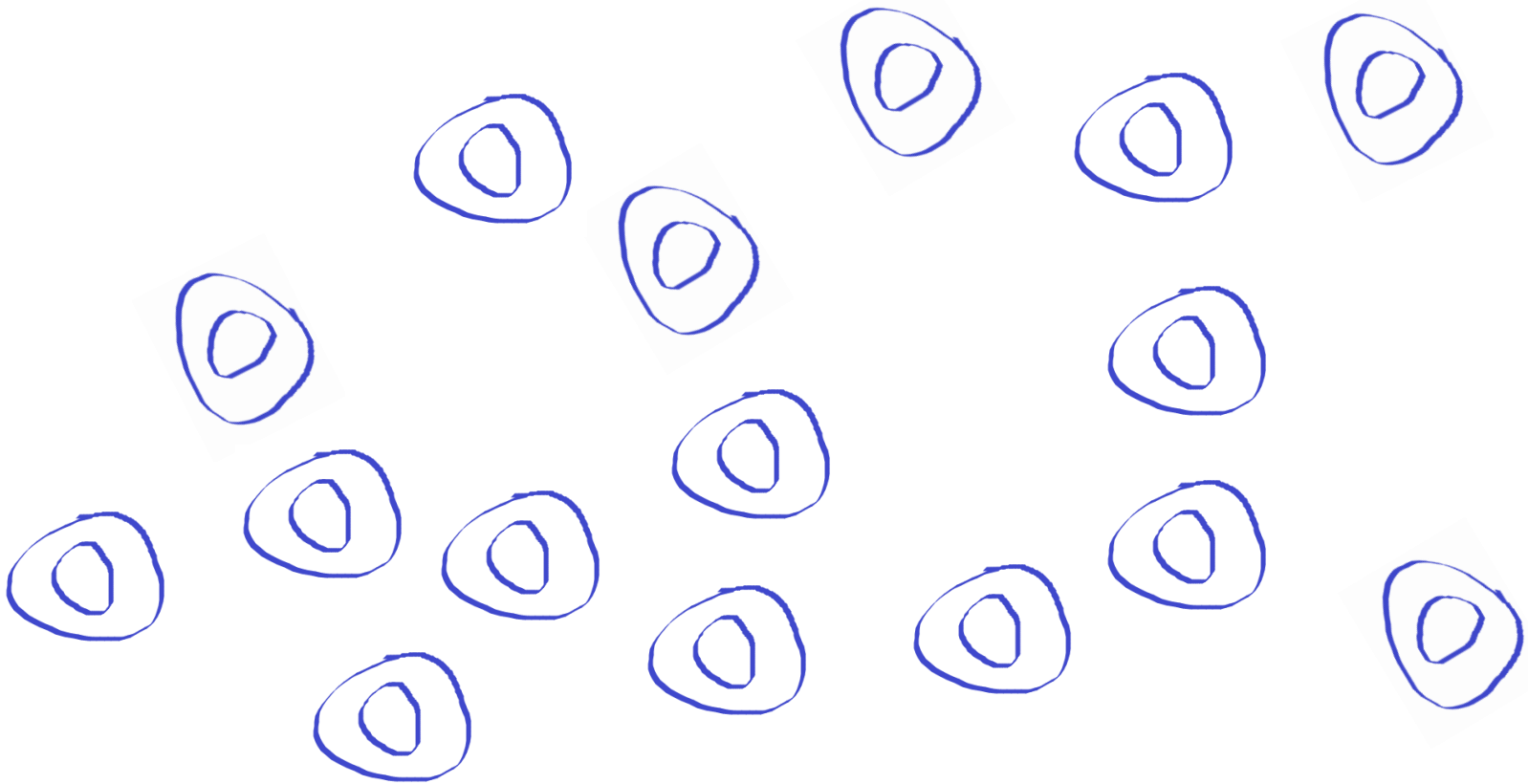


*Could the treatment be
predicted by CTC - count ?*

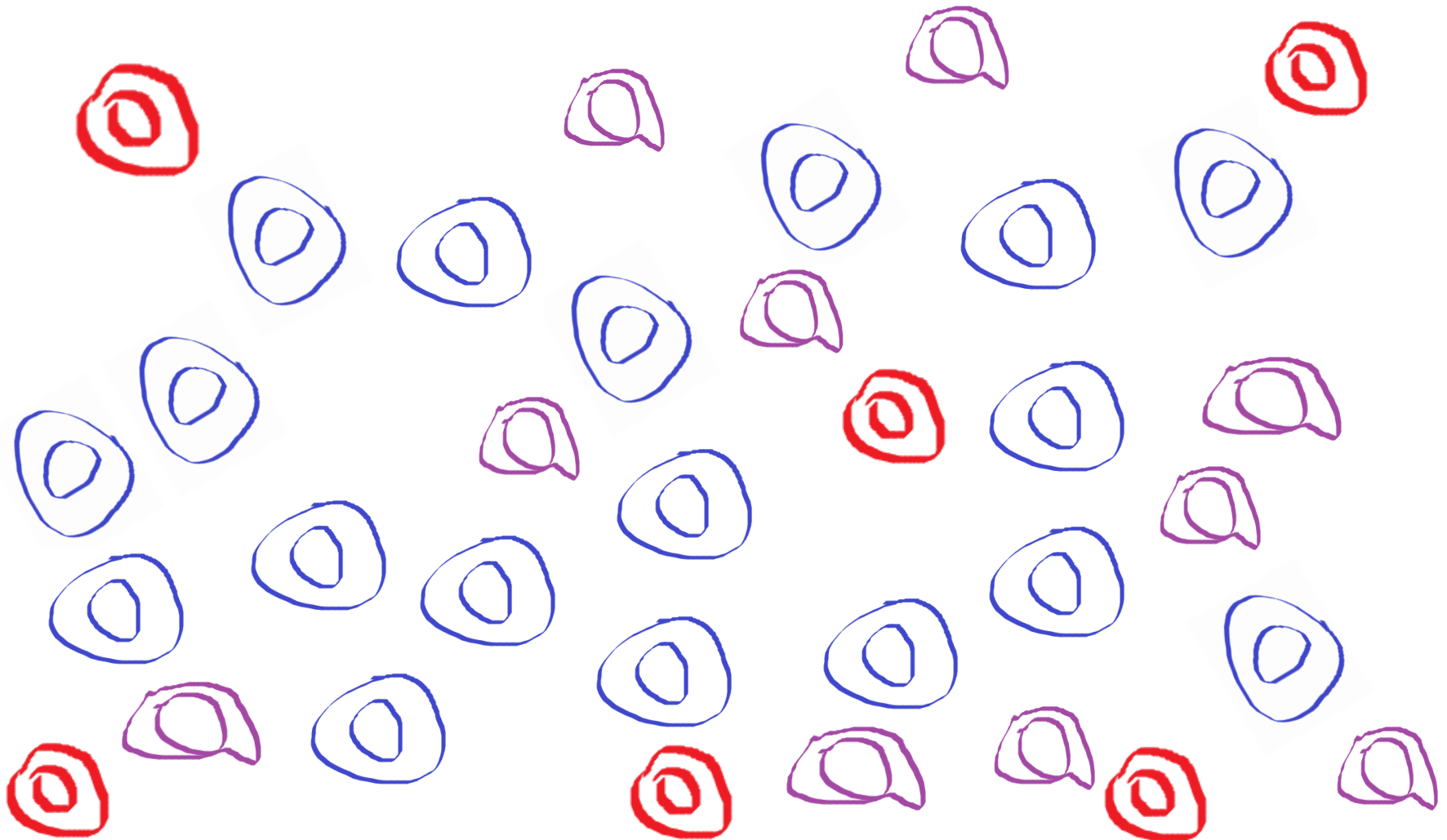
CTC- character matters ...



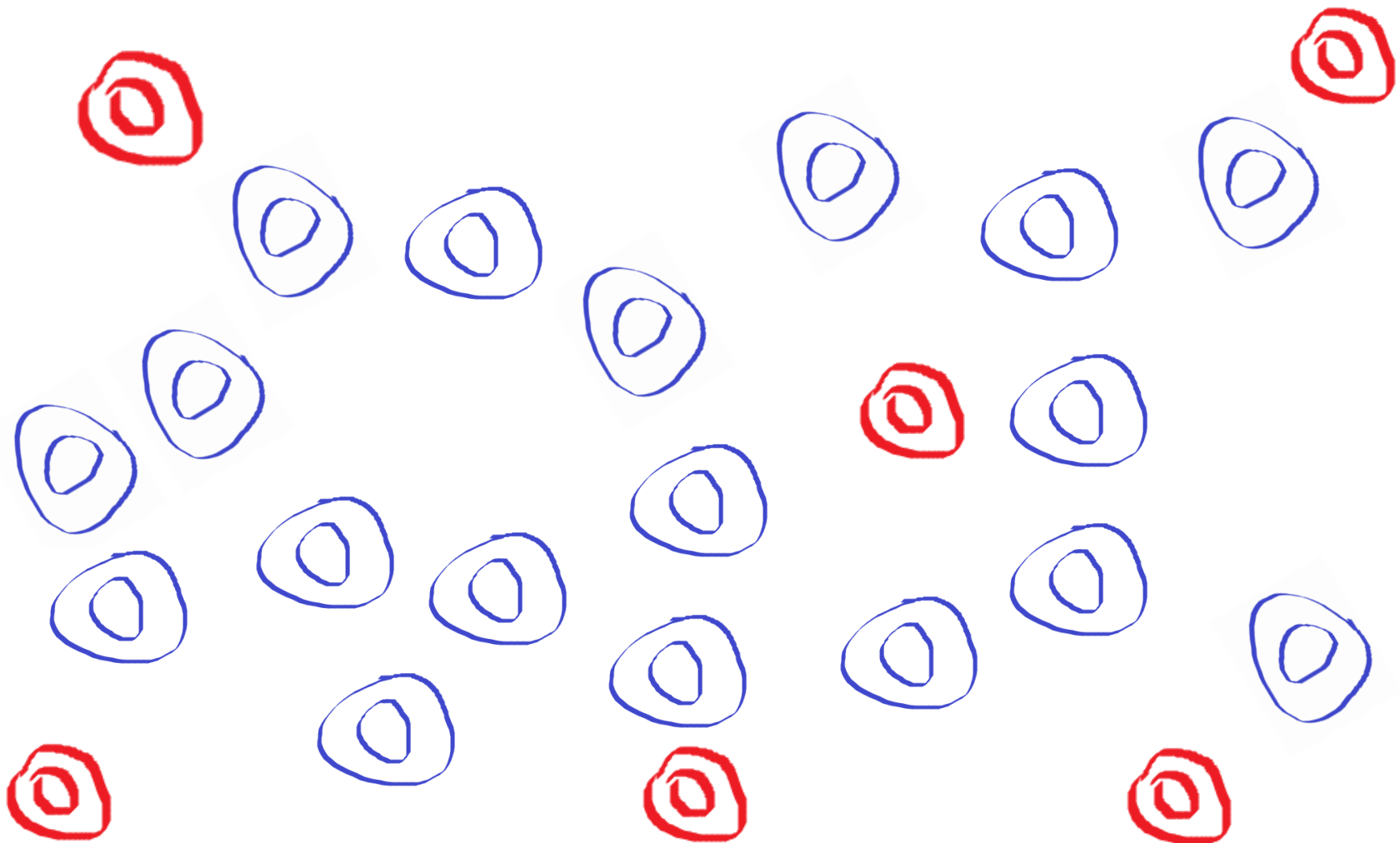
What if only EpCAM + cells are captured ... ???



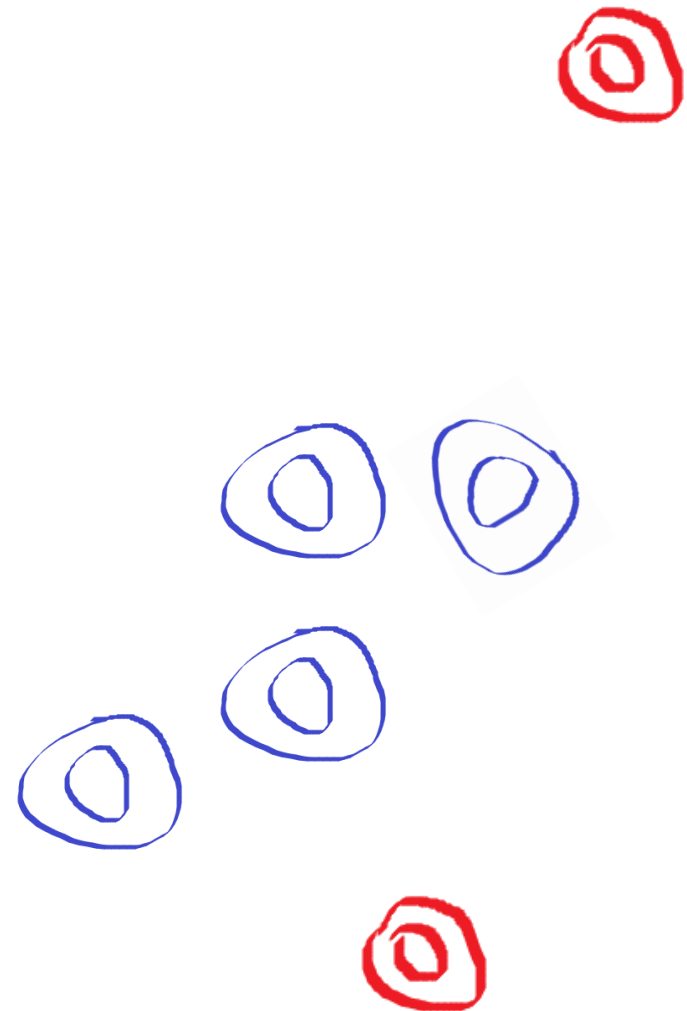
What if only some cell populations are eradicated by administered chemotherapy ... ?



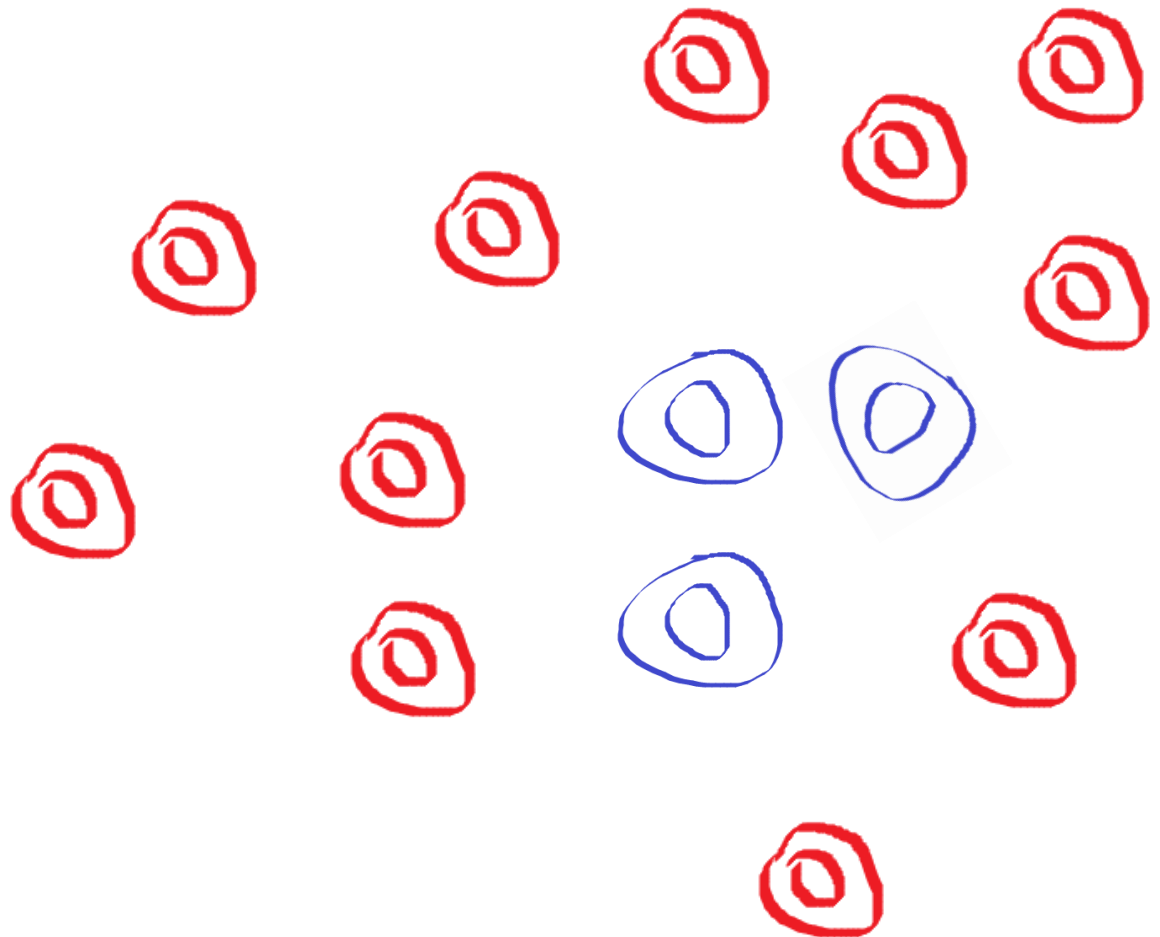
What if only some cell populations are eradicated by administered chemotherapy ... ?



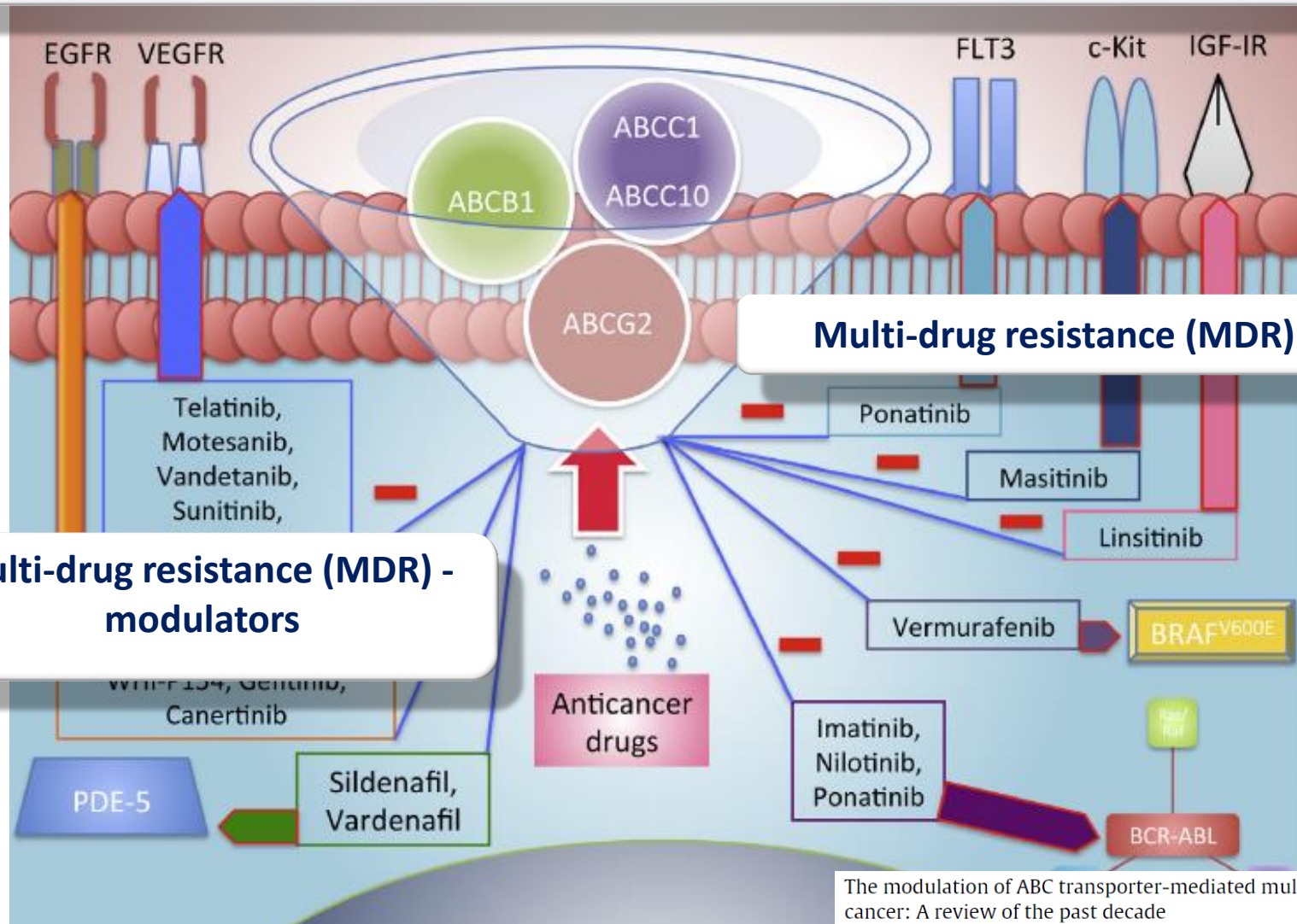
What cells do survive the treatment ?



They may change and give the origin to new dangerous populations ..



Specification of chemoresistance on the CTC level



The modulation of ABC transporter-mediated multidrug resistance in cancer: A review of the past decade

Rishil J. Kathawala, Pranav Gupta, Charles R. Ashby Jr. **, Zhe-Sheng Chen*

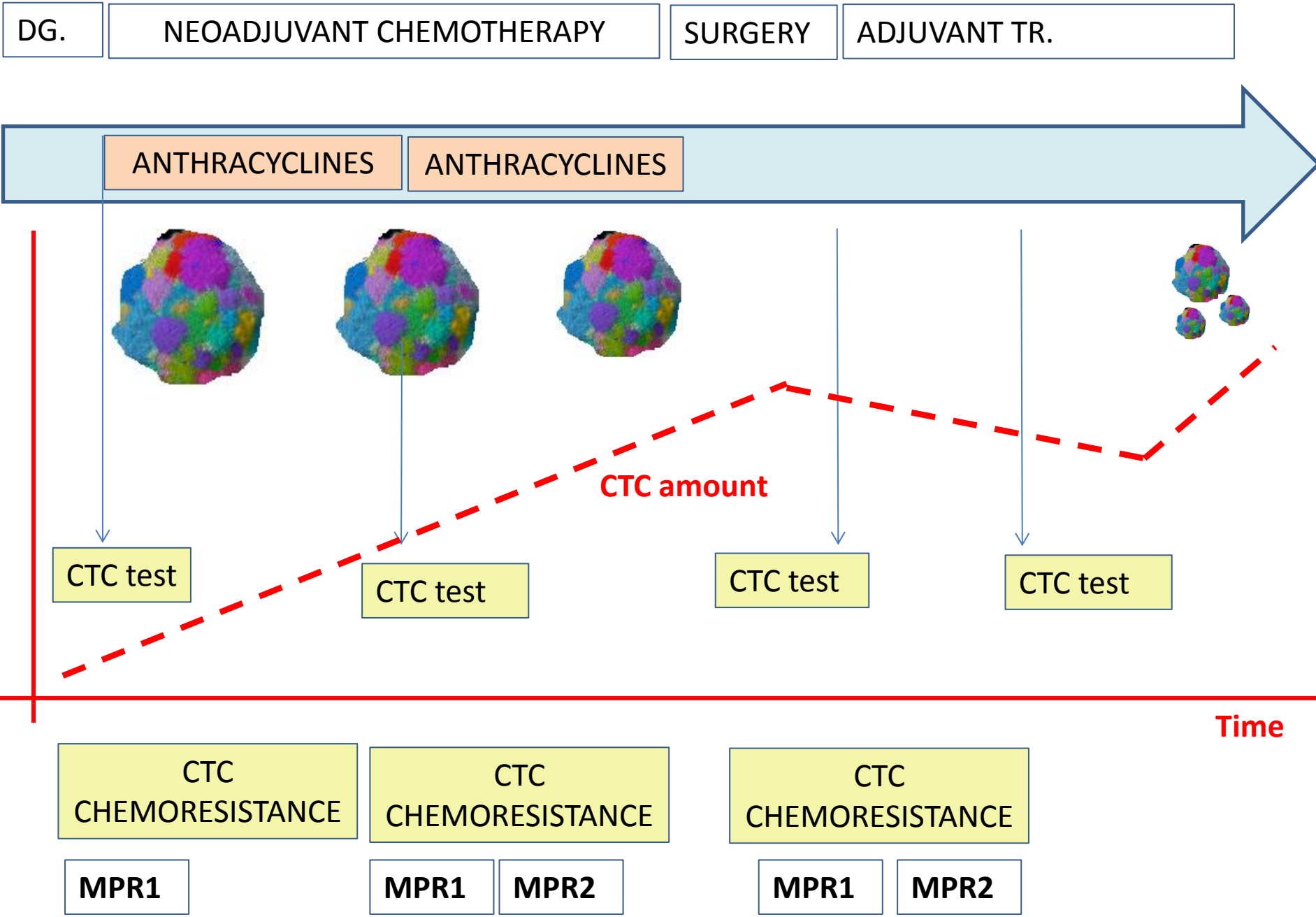
Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY, USA

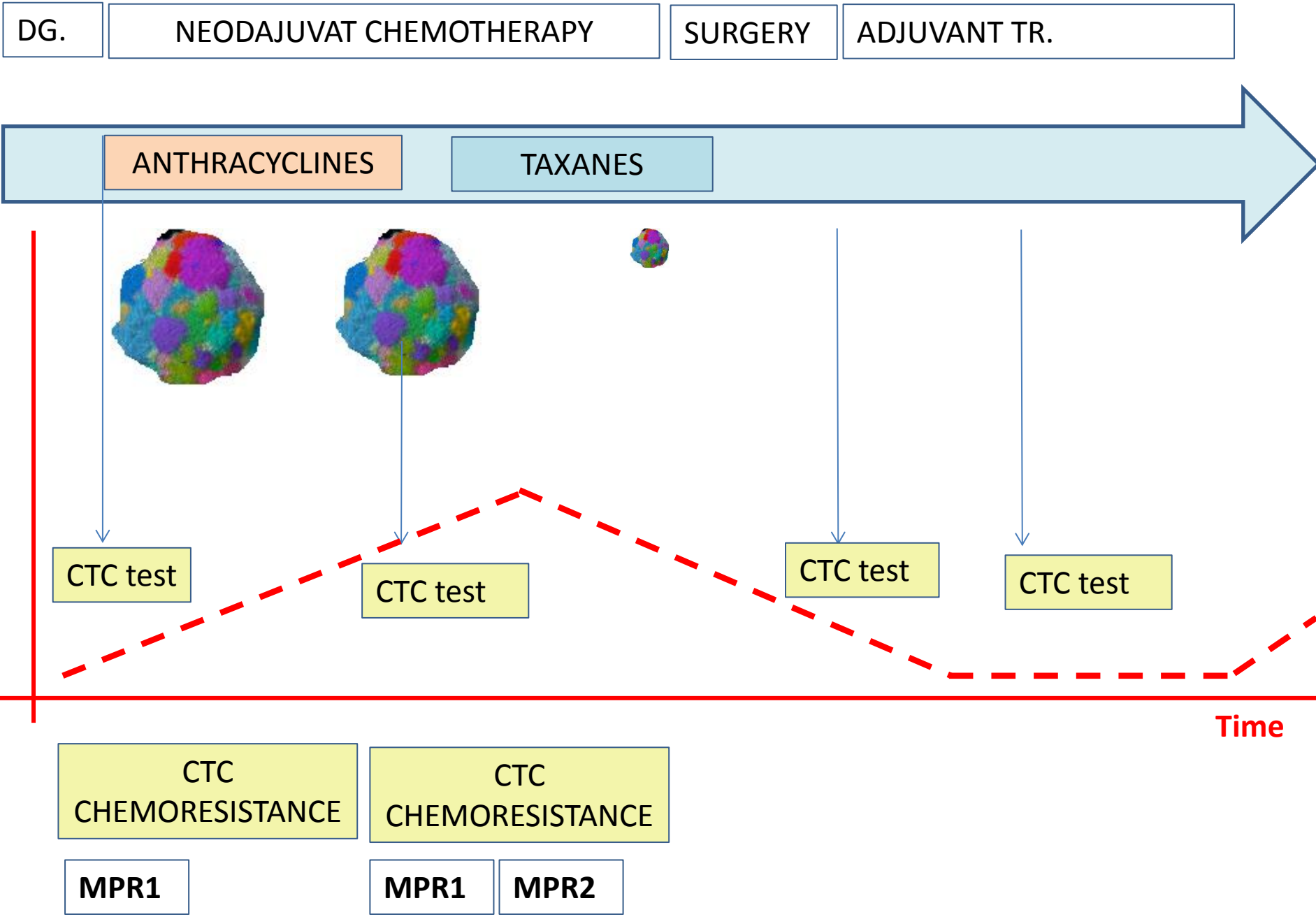


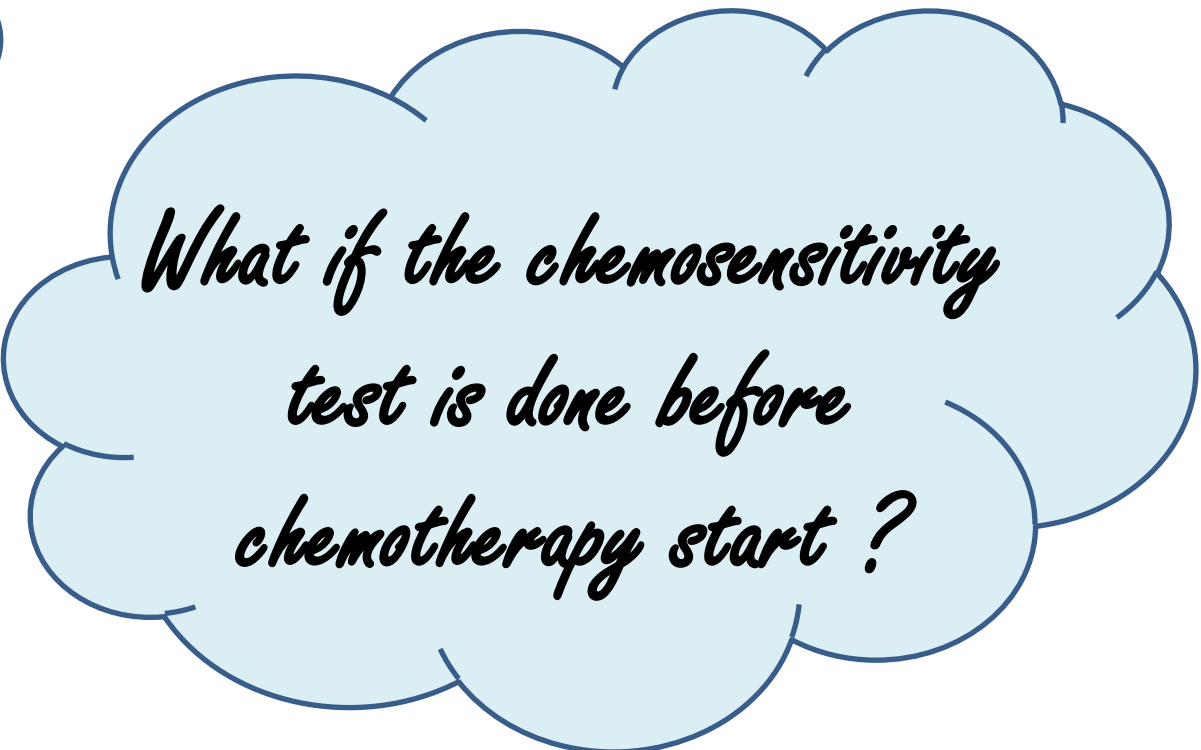
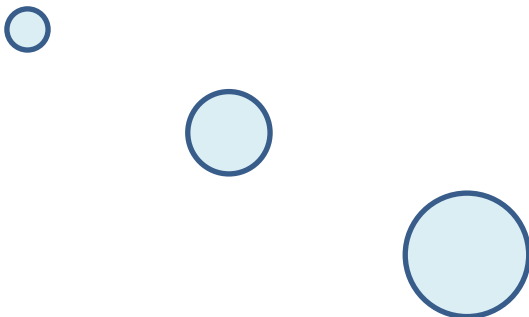
STUDY 2014- 2016 : Breast cancer treatment response monitoring in neoadjuvant setting by means of CTCs molecular analysis .

**Oncology clinic, VFN, Prague
Dept. Laboratory diagnostics, FNKV,
Prague**

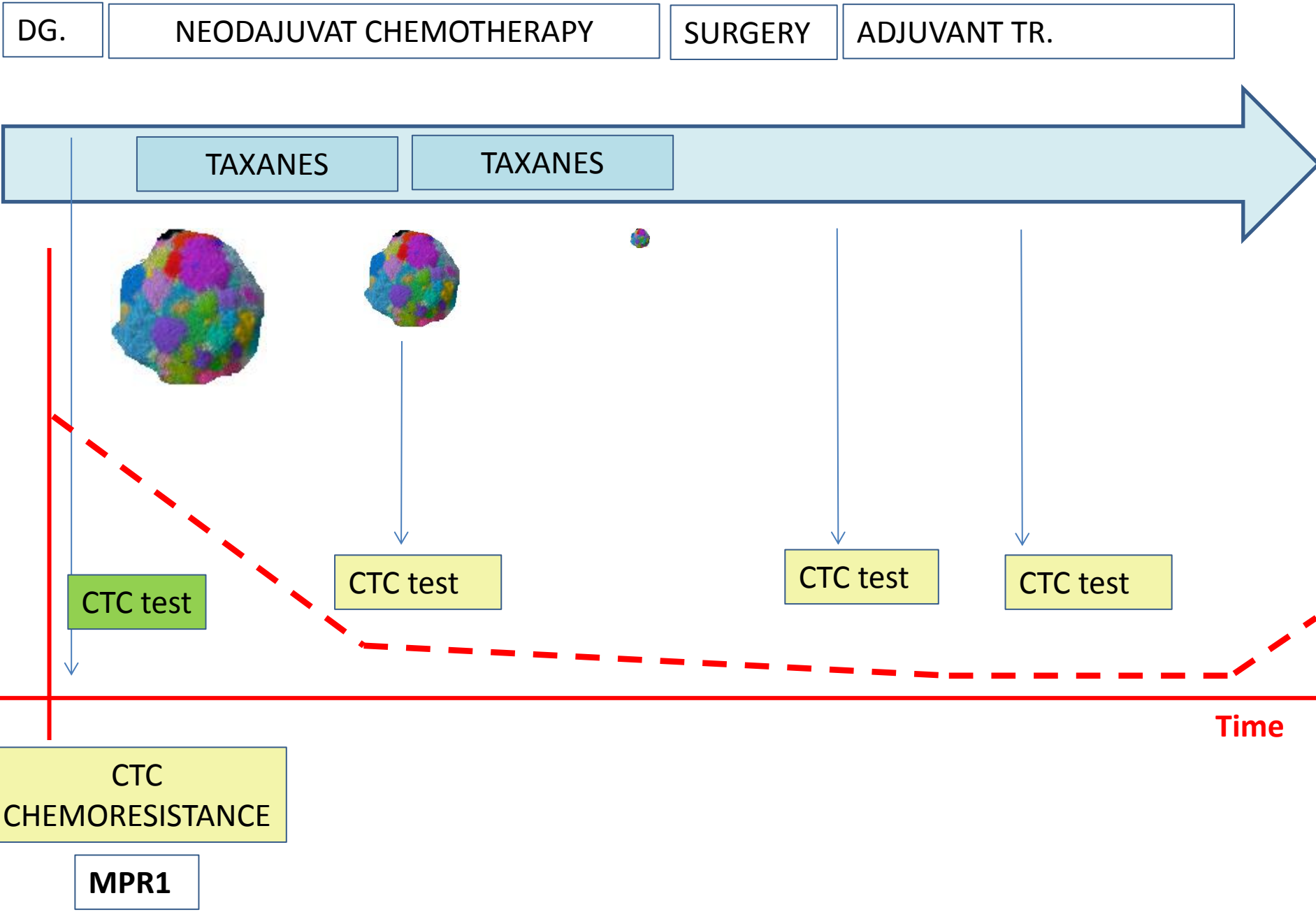
**Dr. Zuzana Bielčíková
Ing. Anna Jakabová**







*What if the chemosensitivity
test is done before
chemotherapy start ?*



BC NEO- STUDY



- 20 BC patients / undergoing neoadjuvant treatment were tested within the study protocol
- 7/ 20 BC patients were having triple negative histology
- Several blood withdrawals were taken and analyzed (min. 4)
- The clinical response has been monitored in parallel with CTC examinations
- The patients were treated according the standard guidelines

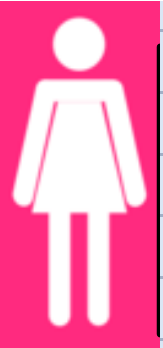
CTC- character matters ...



Date	Timing of CTC test	MRP1	MRP2	MRP4	MRP5	MRP7	MDR1	ERCC1
18.9.2014	before 1.FEC							
13.11.2014	before 1.DH							
28.1.2015	before 3.D							
2.3.2015	after surgery							
25.5.2015	after radiotherapy							
24.8.2015	monitoring							
26.4.2016	monitoring							

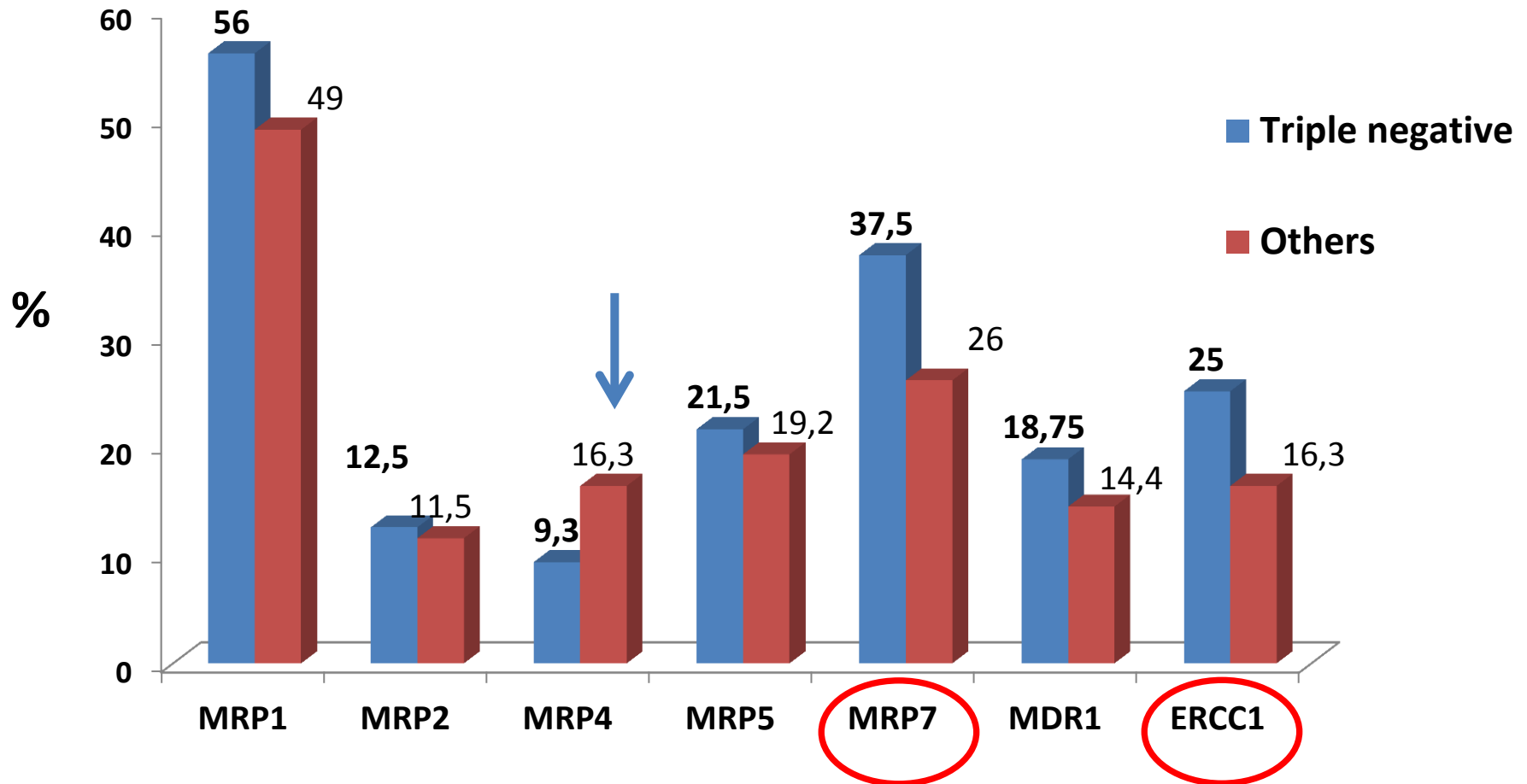


Date	Timing of CTC test	MRP1	MRP2	MRP4	MRP5	MRP7	MDR1	ERCC1
15.1.2015	before 1.AC							
26.3.2015	before 1.D							
28.5.2015	before 4.D							
21.10.2015	after radiotherapy							
27.1.2016	monitoring							
19.5.2016	monitoring							



Date	Timing of CTC test	MRP1	MRP2	MRP4	MRP5	MRP7	MDR1	ERCC1
30.7.2015	before 1.FEC							
14.9.2015	before 3.FEC							
5.1.2016	before radiotherapy							
10.5.2016	after radiotherapy							

Relative gene expression elevation of the genes associated with chemoresistance in CTC enriched samples



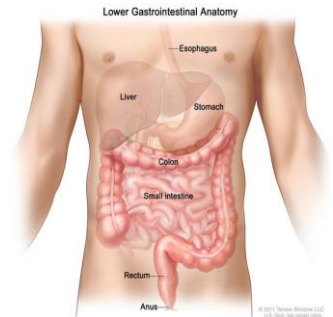
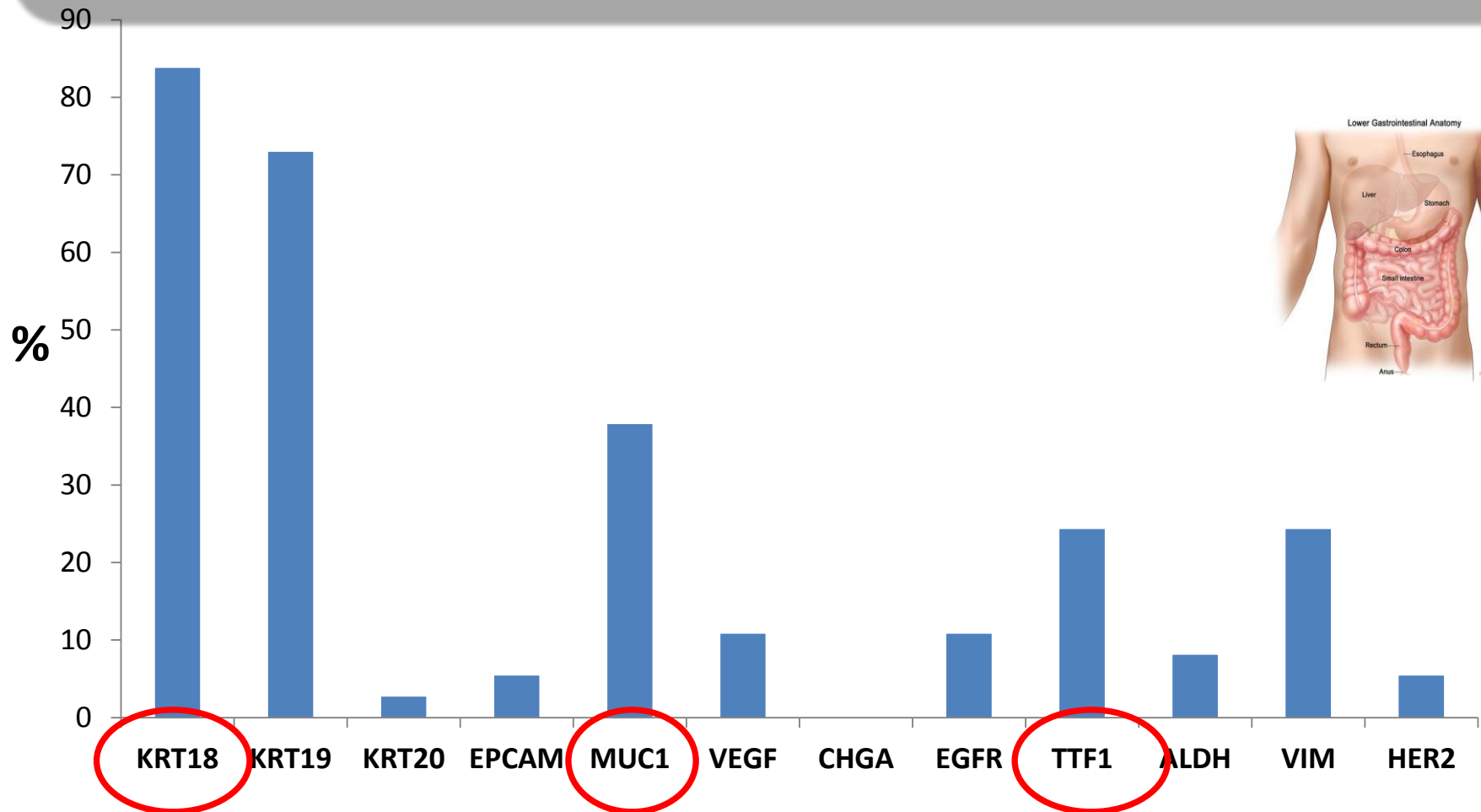
STUDY 2013- 2016 : CTC in GIT tumors



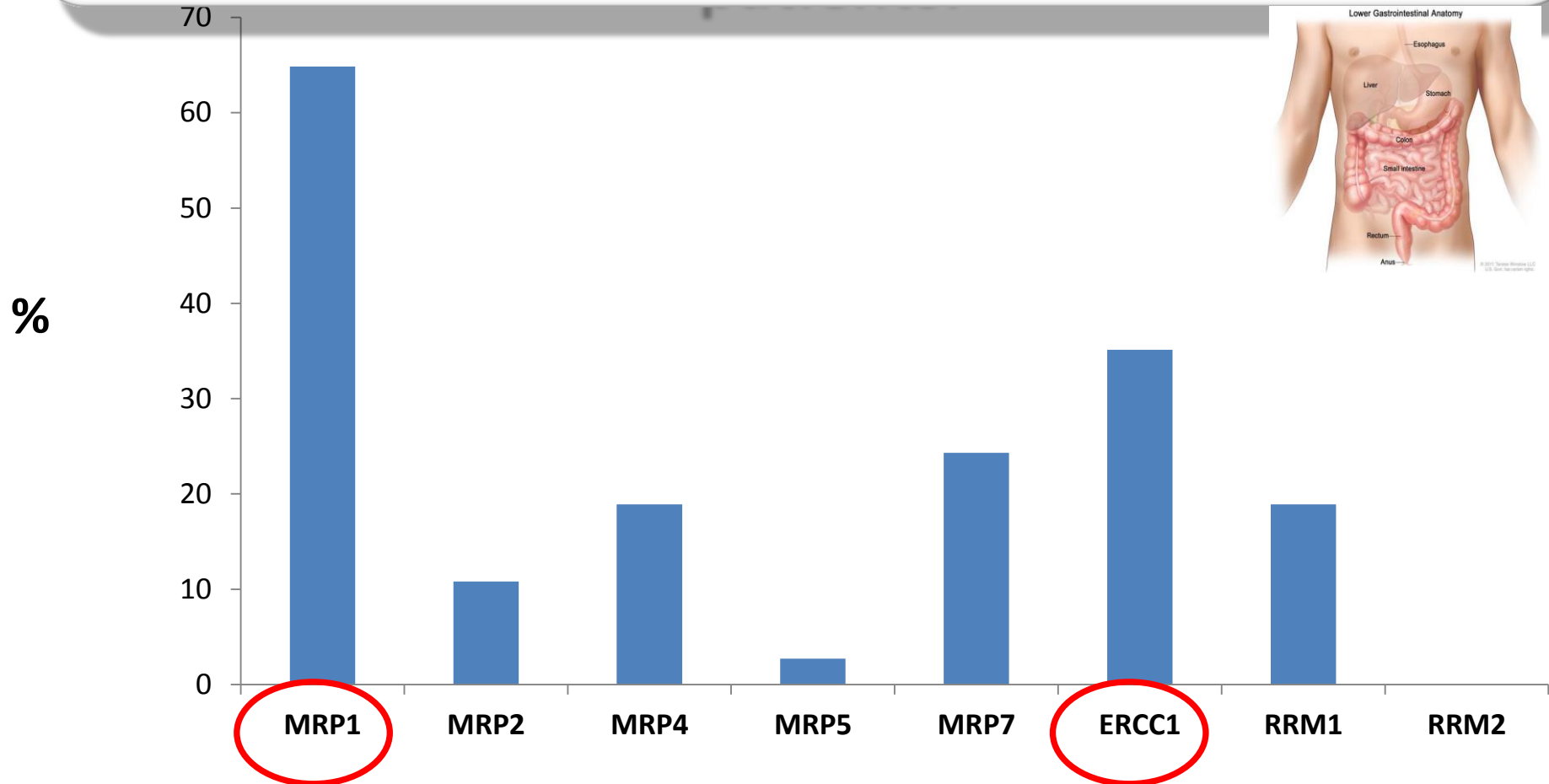
Dept. Laboratory Diagnostics, FNKV, Prague

Ing. Michael Pinkas

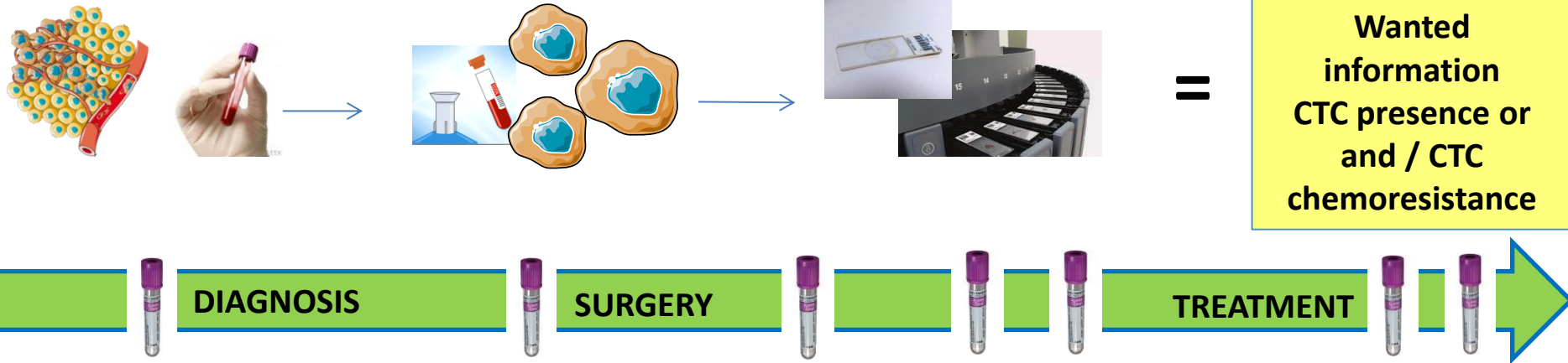
Elevated gene expression of tumor associated genes in CTC – fractions in CRC patients.



Elevated genes expression of chemoresistance associated genes in CTC – fractions in CRC patients.



Timing of CTC – testing during oncological treatment



- **Confirmation for tumors of unknown origin based on CTC qPCR / Immunohistochemistry**
- Additional dg. Information

- CTC before surgery - prognostic information
- CTC after surgery – predictive information for ongoing oncological treatment
- STAGING ?
- Mutational analysis based on DNA from CTC for targeted therapy (eg. KRAS, HER2)

- Treatment monitoring
- Early relaps detection
- **Chemoresistance testing during applied therapy (adjuvant, neoadjuvatn, palliative)**
- **Identification of new target molecules on separated CTC**

Acknowledgement

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P. Svobodová

P. Eliášová

M. Čegan

K. Trhanová

E. Kučera

J. Pavlásek

Cooperating partners



Masarykova nemocnice
v Ústí nad Labem,
příspěvková organizace



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KLINICKÉ
A EXPERIMENTÁLNÍ
MEDICÍNY



tataabiocenter



Fraunhofer
ISC

