Liquid Biopsies: CTCs and cell-free DNA for monitoring NSCLC

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15-18 April 2015, Geneva, Switzerland

Organisers

IASLC
International Association for the Study of Lung Cancer

ESMO
GOOD SCIENCE BETTER MEDICINE BEST PRACTICE
European Society for Medical Oncology

Partners

ESTRO
European Society for RADIOTHERAPY & ONCOLOGY

European Society of Thoracic Surgeons · 1993 ·

etop
Information | Research

Disclosure slide

- Commercial research Support: Janssen and Astellas
- Honoraria: Merck, Pfizer, Astra Zeneca

The Liquid Biopsy

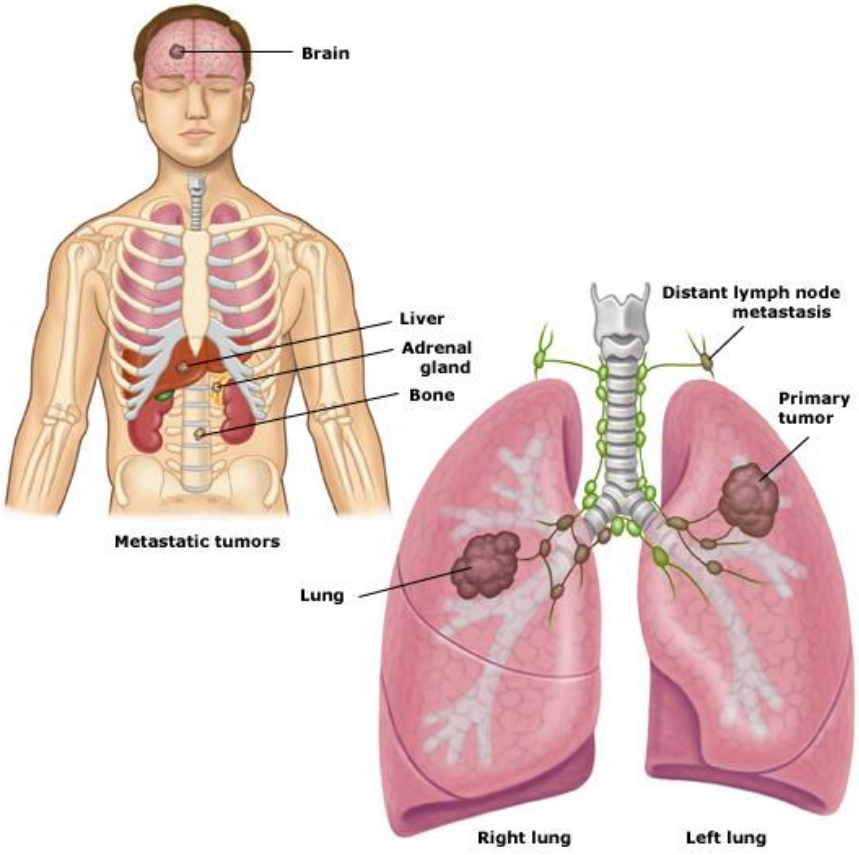
Narrow Definition: A blood test that is associated with cytopathological assessment of CTCs

Broader Definition: ctDNA, ctRNA and Exosomes

Appeal of the “Liquid Biopsy”

Diagnosis, Prognosis, Theranostics, Prediction, Biology

The Liquid Biopsy v Targeted Tissue Biopsy



Heterogeneity:
Liquid Biopsy as a Gestalt
Accessibility:
Blood, serum, plasma, urine, pleural fluid etc.
Temporal Heterogeneity of Disease:
Serial Access

Image from PixShark.com

Promise of CTCs

- Tumour Staging
- Real time markers of disease progression and survival
- Guide Therapy
- Indicate therapy effectiveness
- Clues to drug resistance
- Surrogate endpoints
- Treatment targets

CTCs

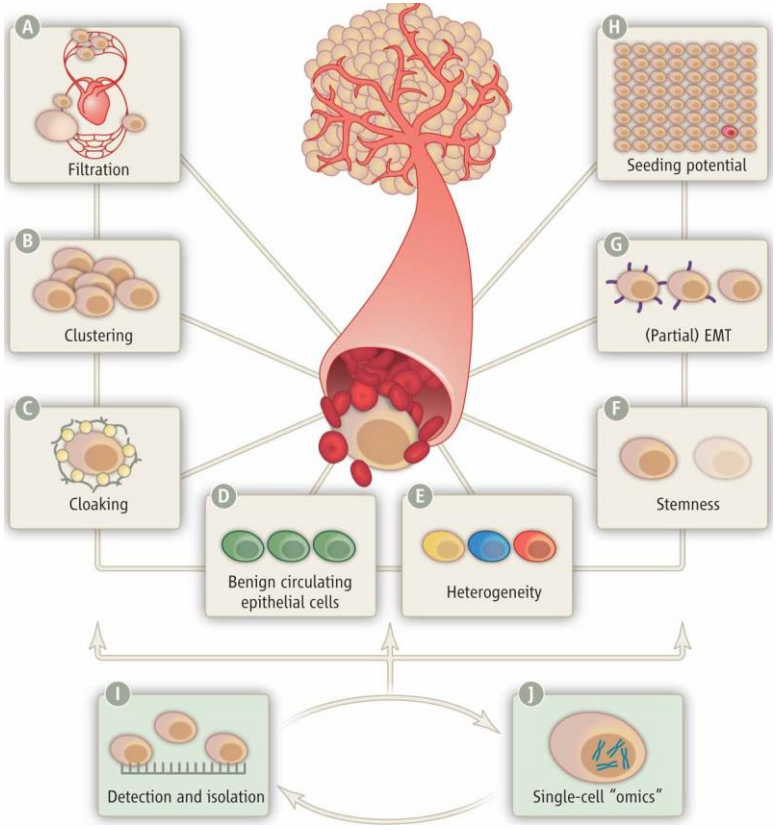
- 1mL blood:
  - 1 million WBCs
  - 1 Billion RBCs
  - A few CTCs

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

Blood-Based Analyses of Cancer: Circulating Tumor Cells and Circulating Tumor DNA. Haber and Velculescu. Cancer Discovery 2014

Hurdles and solutions in CTC research



Biophysical factors that may diminish the detection of CTCs

(A) Filtration of large CTCs in smaller capillaries

(B) Clustering of tumor cells that lodge in capillaries

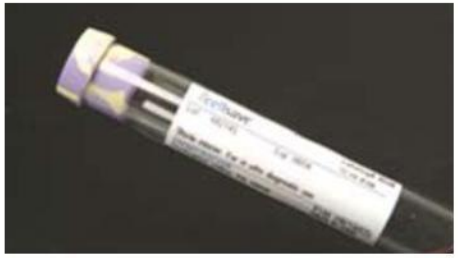
(C) Cloaking of CTCs by platelets or coagulation factors

Vicki Plaks et al. Science 2013;341:1186-1188

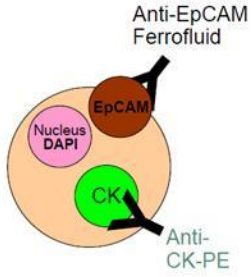
Science
AAAS

Technologies: CellSearch

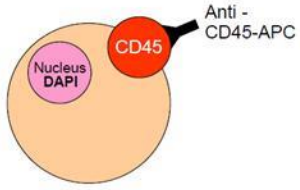
1. Blood drawn into special tube (called cellsave); to patient, this is like any other blood draw. Sample stable for 96 hours for analysis or transport.



2. Blood exposed to antibodies conjugated to ferrofluid, as well as antibodies to DAPI and CD45.



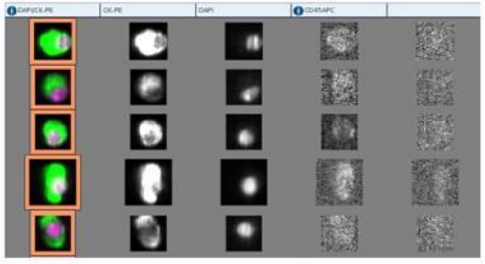
Circulating Tumor Cell



Leukocyte

3. Most nucleated cells in blood are white blood cells. CTCs are enriched by magnet then identified by being EpCAM +, CK +, and CD45-. They can now be counted or analyzed.

CTC's
CK-PE+/DAPI+/CD45-APC-

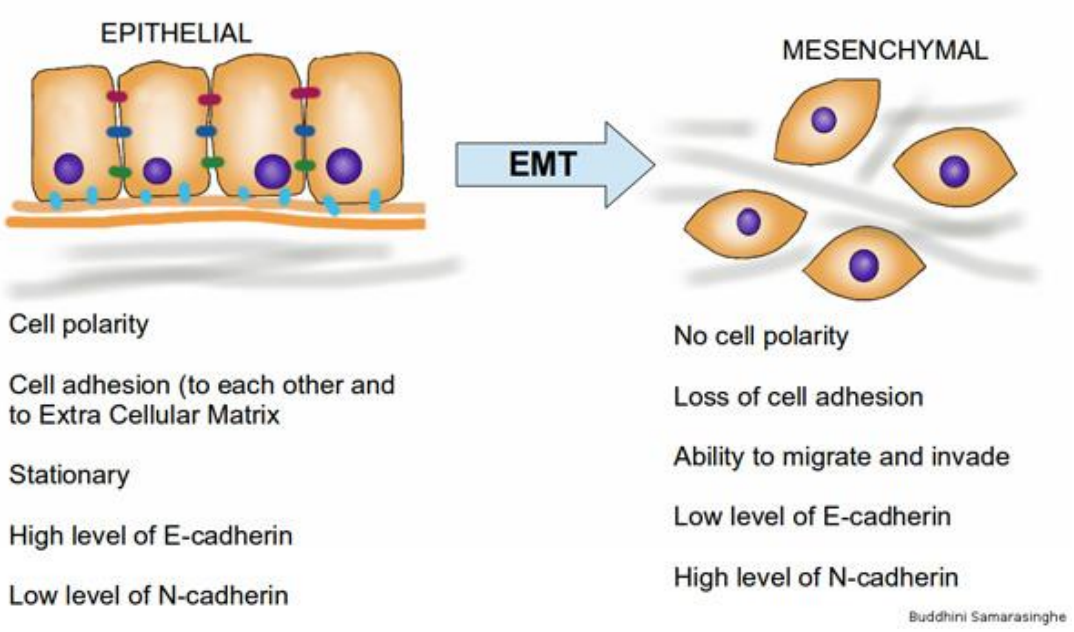


Leucocytes
CK-PE-/DAPI+/CD45-APC+

Slide created for GRACE with images at veridex.com

The problem of differential antigen expression on CTCs

- EpCAM
- EMT, EMT, EMT, EMT!



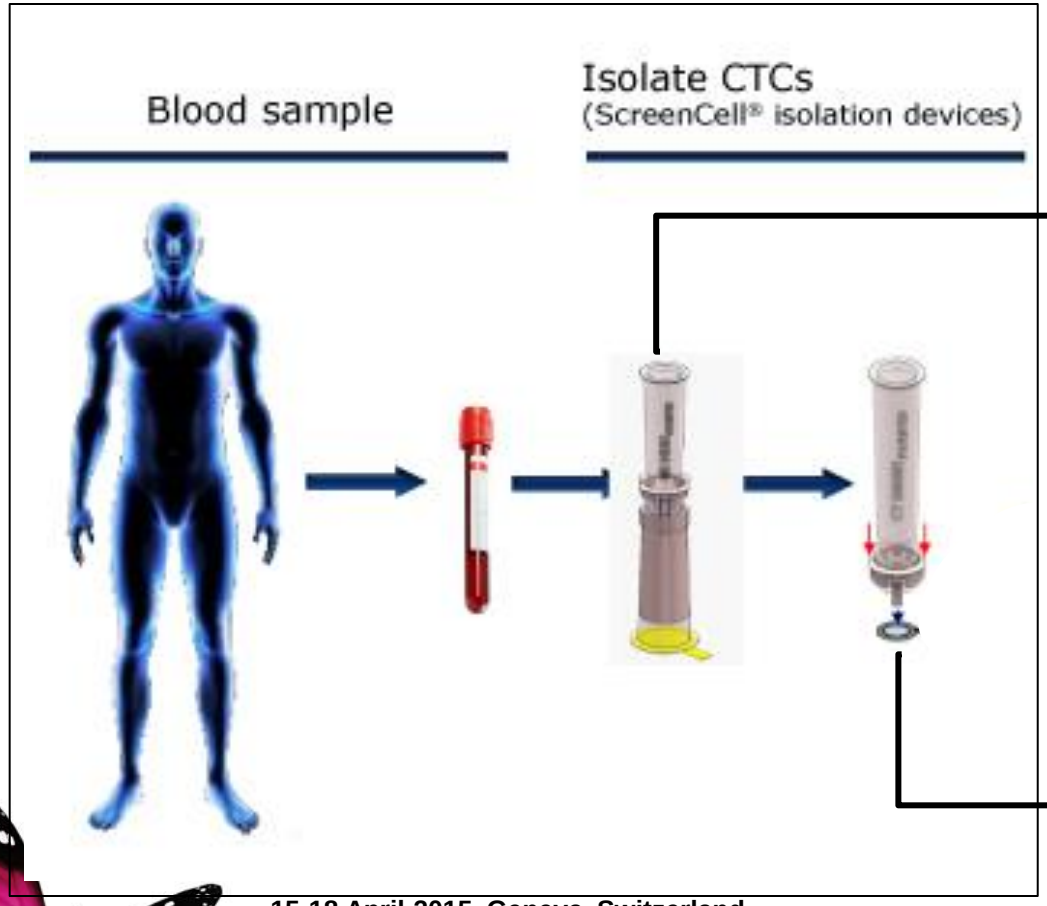
Problems

Sensitivity
Specificity
Cost
Feasibility

Technologies

- ISET: Isolation by size of epithelial tumour cells
  - Rarecells
- ScreenCell
  - Polycarbonate filtration membrane
  - 8 micron
- Cell Sieve

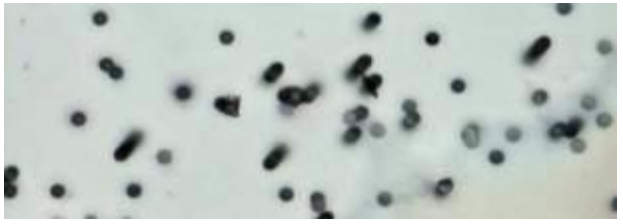
ScreenCell CTC Isolation Devices



CELL CULTURE

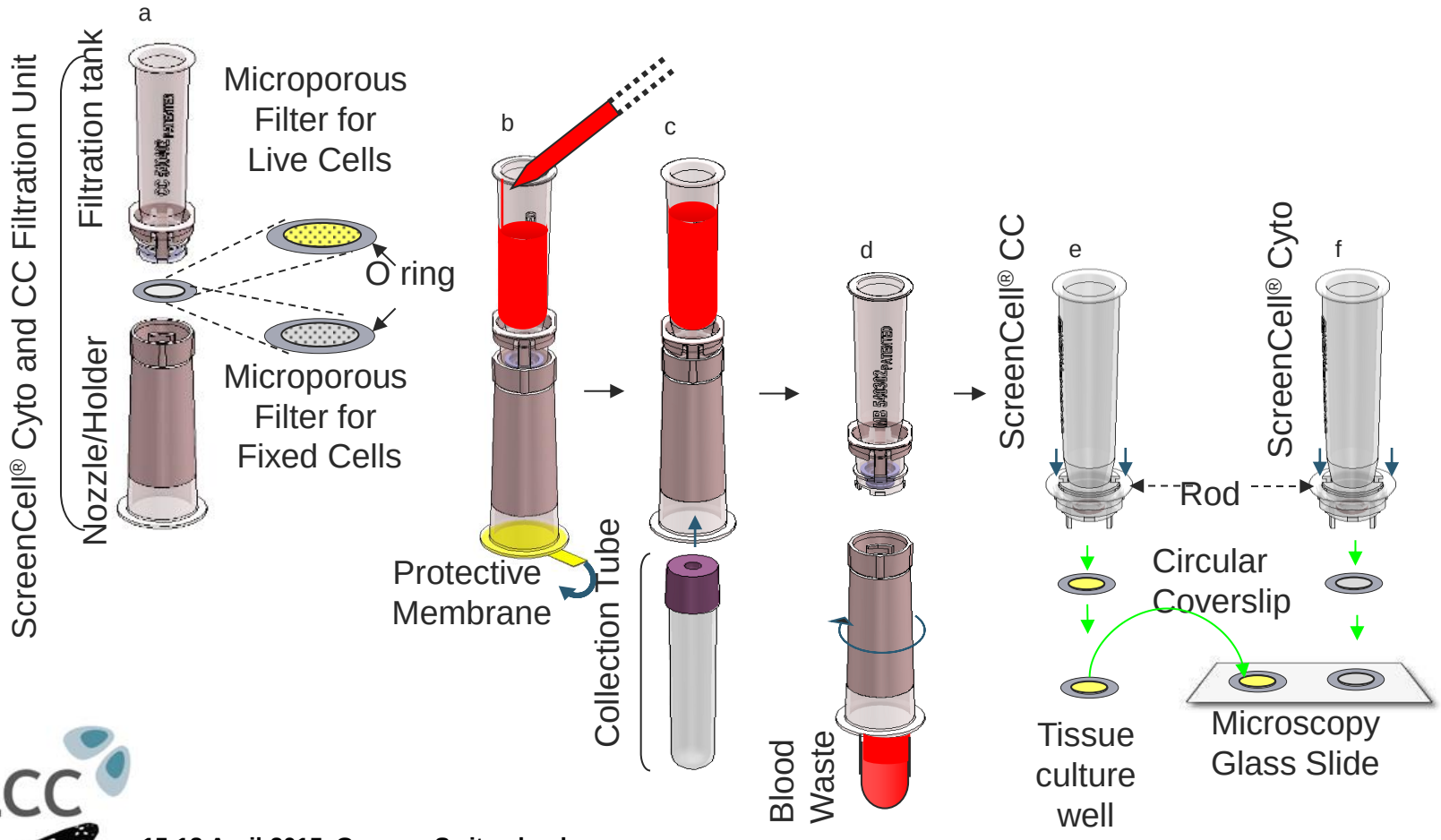
MOLECULAR BIOLOGY

CYTOLOGY

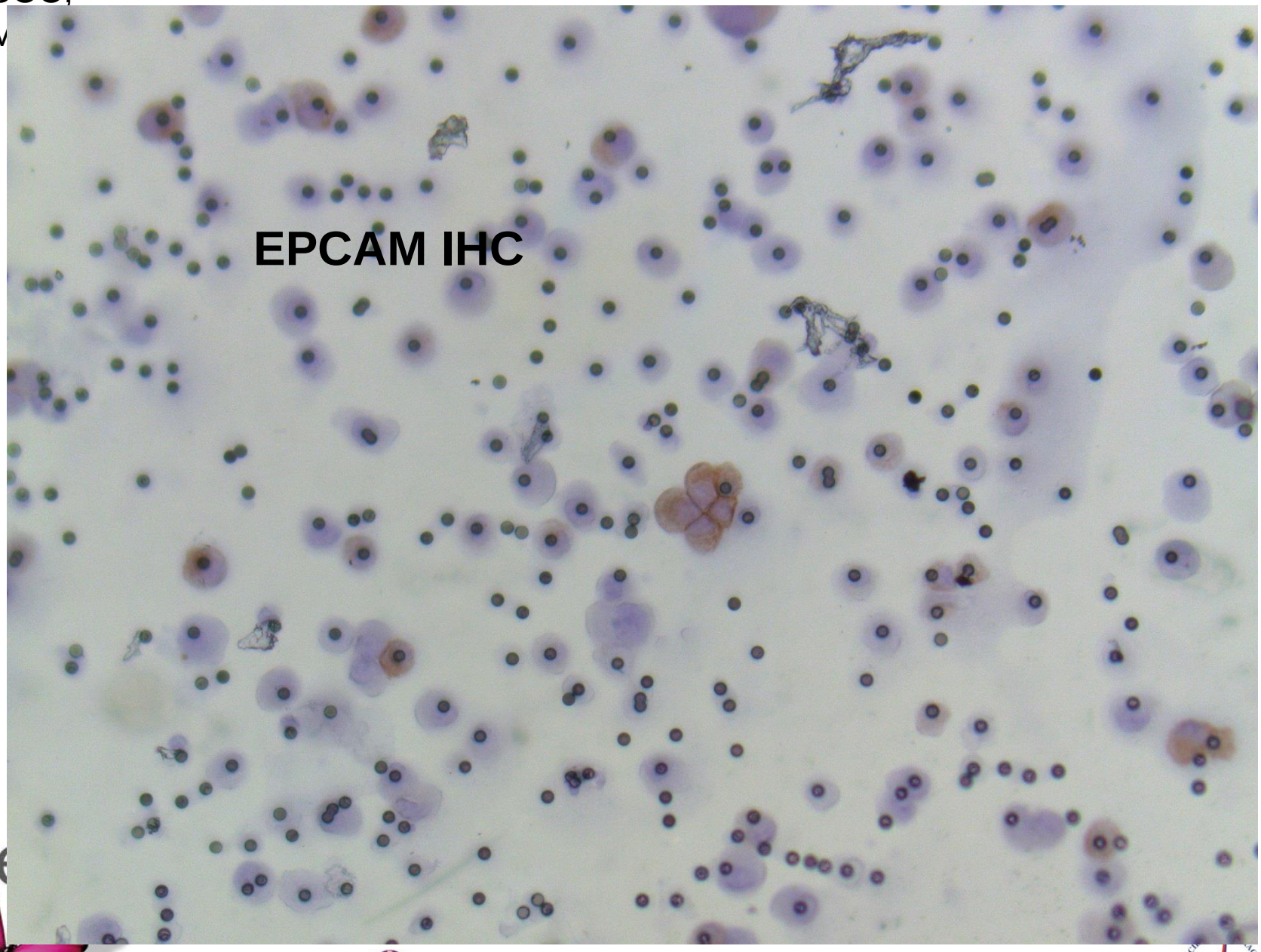


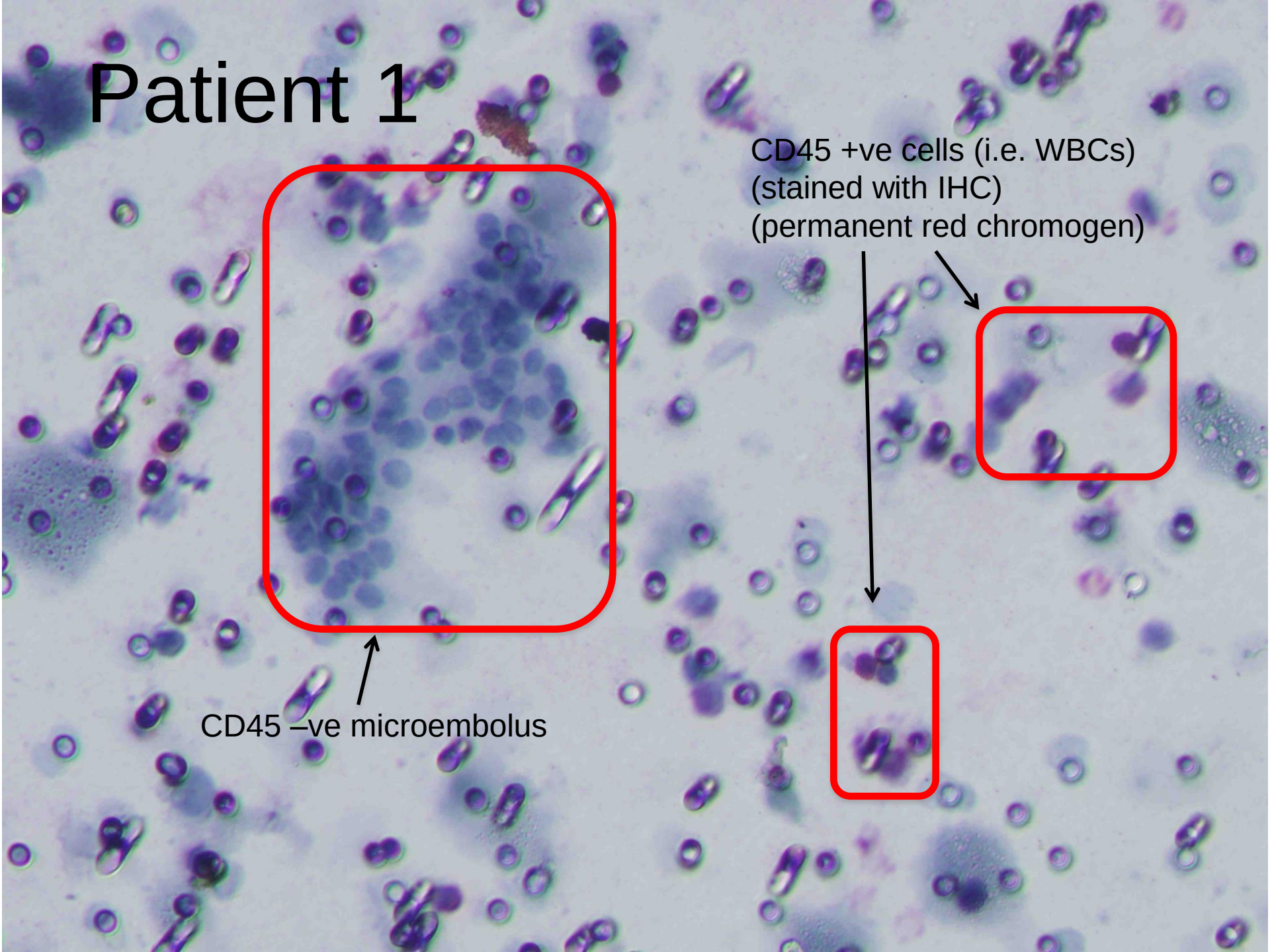
Organisers

Partners

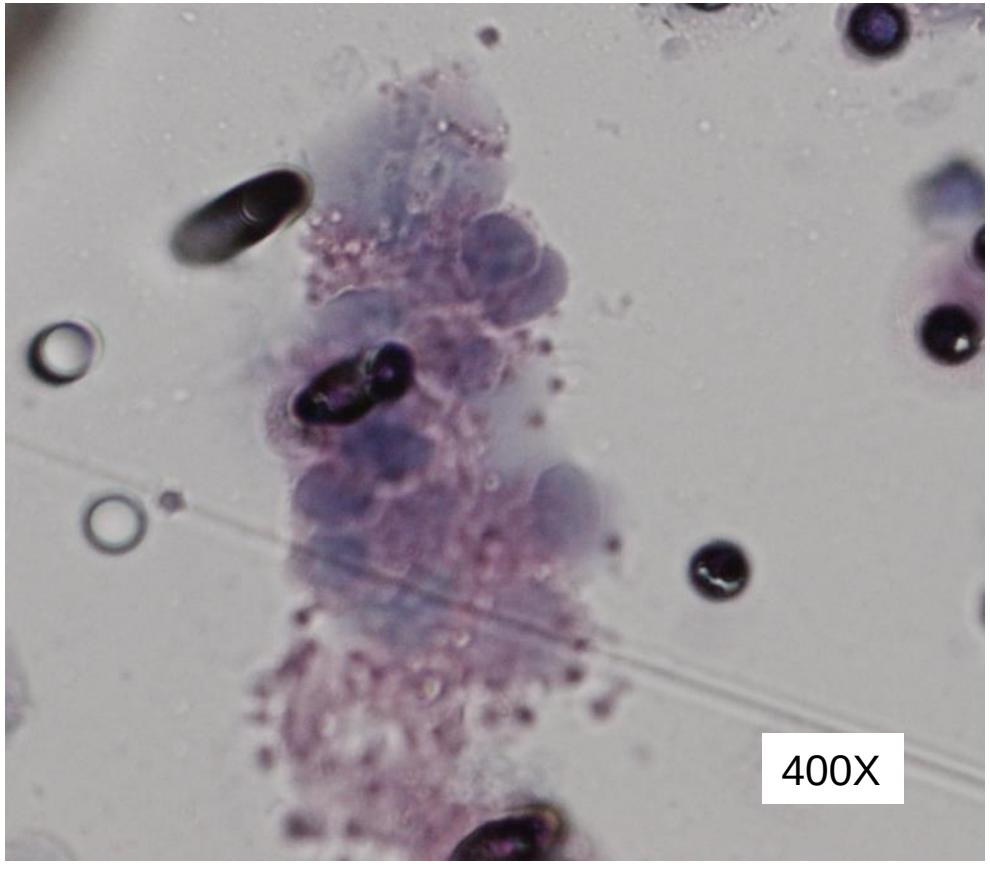

ScreenCell® Cyto and CC Filtration Unit
Filtration tank
Nozzle/Holder
a
Microporous Filter for Live Cells
O ring
Microporous Filter for Fixed Cells
b
c
Protective Membrane
Collection Tube
d
Blood Waste
ScreenCell® CC
e
ScreenCell® Cyto
f
Rod
Circular Coverslip
Tissue culture well
Microscopy Glass Slide

SCC,
M




Patient 1
CD45 +ve cells (i.e. WBCs)
(stained with IHC)
(permanent red chromogen)
CD45 –ve microembolus

Circulating Tumour Microemboli with platelet cloaks: Avoidance of Immune Editing



Still No Game Changing Technology

- Technical Challenges
- ☐Identifying few CTC among millions of white blood cells and billion of red blood cells
- ☐Enrichment step often needed
- ☐Limitation in the blood volume that can be analysed
- ☐Distinguishing CTCs from non-tumour epithelial cells
- ☐Sensitive and specific methods are needed

NSCLC: Where are we with CTCs?

- **Diagnostics:**
  - Tanaka et al, 2009, Clin Cancer Res: 150 Patients, CellSearch
  - CTCs were detected both in cancer patients (30.6%) and healthy controls (12%)!!!!!!
  - Predicted distal metastases
- **Prognostics (Mainly Enumeration)**
- Early stage Lung Cancer:
  - Pre-Operative detection of CTCs (ISET method)
    - An independent new prognostic biomarker
    - Shorter Disease Free Survival
    - Shorter Overall Survival (Hofman et al, Clin Cancer Research 201; Int J Cancer 2011)
- Late stage Lung Cancer
  - Metastatic NSCLC with <5 CTCs per 7.5ml blood better OS*
  - Normanno et al 2014: SCLC

- *Krebs et al JCO 2011

NSCLC: Where are we with CTCs?

- **Response to Treatment**

Hirose *et al*

- 33 metastatic NSCLC patients treated with Gemcitabine and Carboplatin
- No prediction of chemo response between CTC +/- patients
- However, PFS higher in CTC+ patients
- A useful predictive marker for effectiveness of cytotoxic chemotherapy

Punnoose *et al*

- Phase II multicentre study of patients with advanced NSCLC treated with erlotinib and pertuzumab
- Decreased CTC count correlated with PFS (p=0.05)
- Correlation of CTC count with PET and CT (p=0.009)
  - Provides a rationale for defining clinical response and monitoring treatment efficacy with CTCs

NSCLC: Where are we with CTCs?

- **CTC Characterization:**
  - The Holy Grail
  - But low sensitivity for mutational assays
    - 1 of 8 EGFR Mutations known in the primary detected in the CTCs Punnoose et al
    - Marchetti et al PLOS one 2014 (CellSearch + NGS)
  - FISH, Array CGH, Proteomics all very demanding
  - Katz et al Clin Cancer Res 2010: Circulating Genetically Abnormal cells (CACs). Much higher yields
    - MRD, Diagnosis of indeterminate lung lesions

Historical Context Circulating DNA

Proofs of concept

- 1977 – cancer patients have increased levels of total plasma DNA
- 1991 – p53 mut found in urine from bladder cancer patients
- 1997 – KRAS mut identified in plasma of colorectal cancer patients

Era of ctDNA specific technology development

- 2003 – BEAMing for digital evaluation of point mutations
- 2008 – Shotgun sequencing of cell free DNA
- 2011 – Safe-SeqS for digital sequencing of small amplicon panels
- 2013 – Exome sequencing of cell free DNA

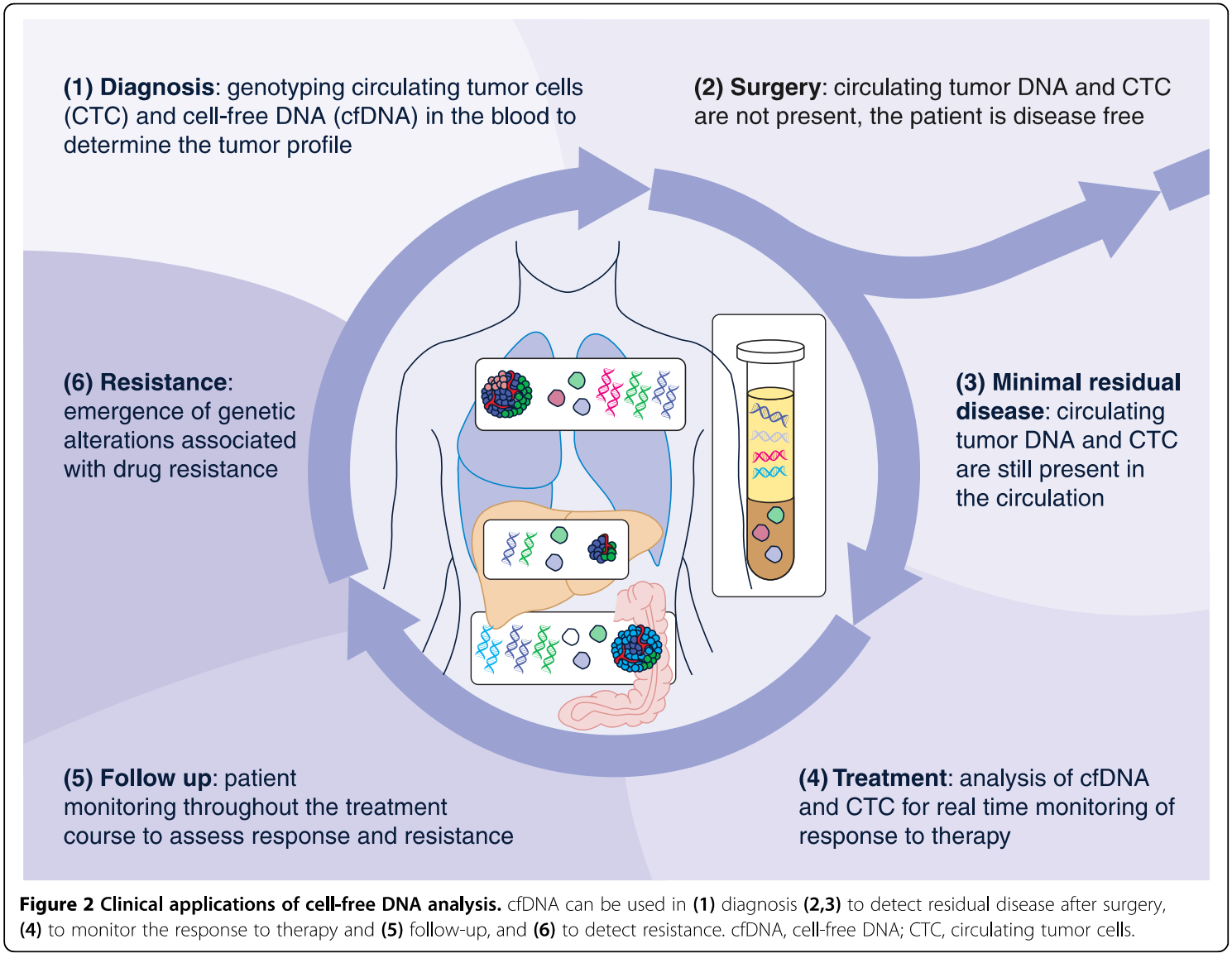


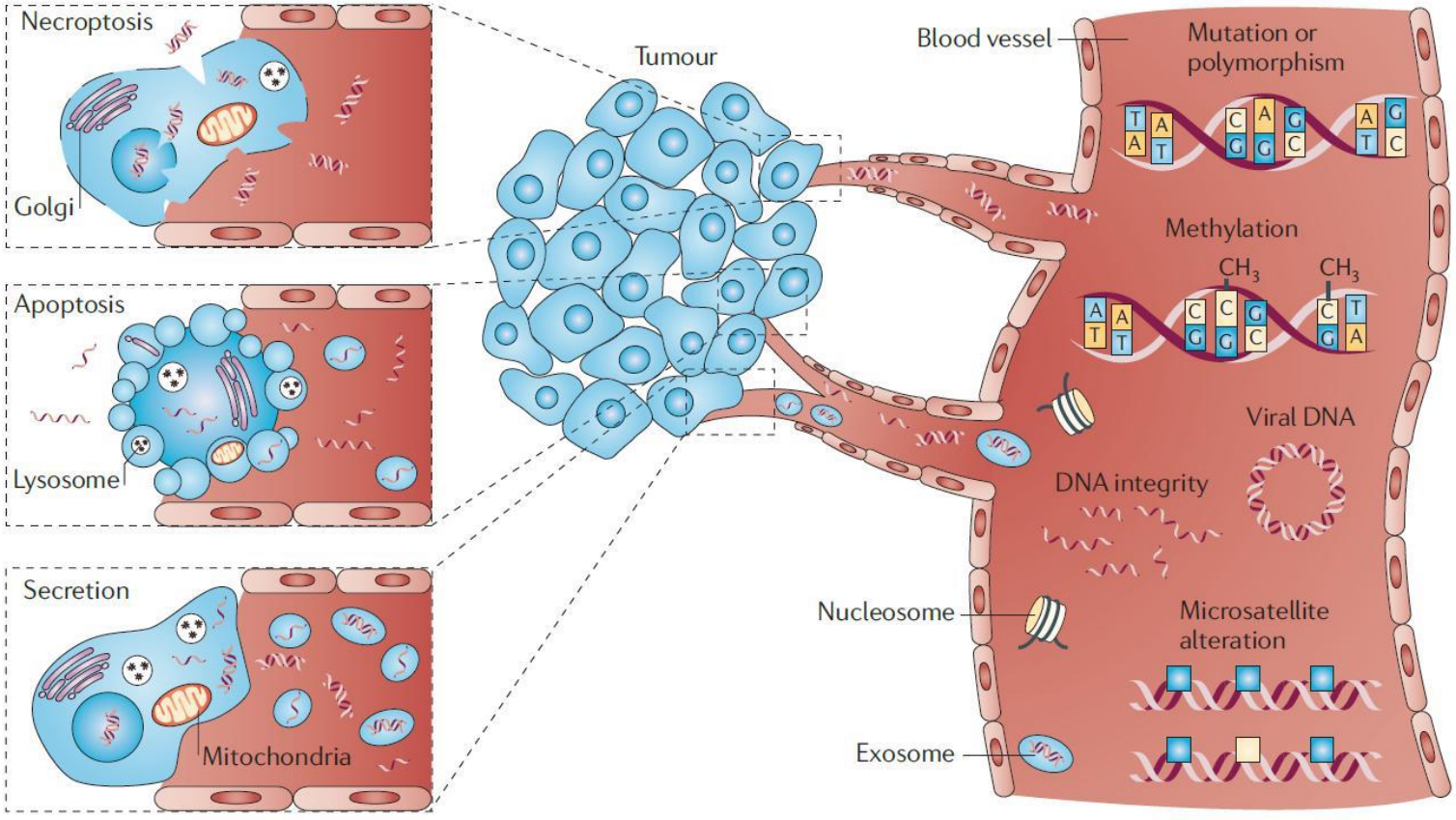
Figure 2 Clinical applications of cell-free DNA analysis. cfDNA can be used in **(1)** diagnosis **(2,3)** to detect residual disease after surgery, **(4)** to monitor the response to therapy and **(5)** follow-up, and **(6)** to detect resistance. cfDNA, cell-free DNA; CTC, circulating tumor cells.

Siravegna and Bardelli, 2014

ctDNA, cfDNA, fcNA

- Terminology
- Circulating tumour DNA (ctDNA)
  - Misnomer
- Relatively abundant
  - cfDNA 25ng/mL
  - It has been estimated that for a patient with a tumor that weighs 100g ($3x10^{10}$ tumour cells) up to 3.3% of tumour DNA may enter the blood every day
  - DNA from apoptotic cells == 185-200bp (Nucleosome protected). Indeed Long DNA fragments may be suggestive of malignancy

Blood Nucleic Acids



Schwarzenbach H, Hoon DS, Pantel K Nat Rev Cancer. 2011, Jun;11(6):426-37

Now feasible to perform whole exome NGS on cfDNA

LETTER

doi:10.1038/nature12065

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA

Muhammed Murtaza[1]*, Sarah-Jane Dawson[1,2]*, Dana W. Y. Tsui[1]*, Davina Gale[1], Tim Forshew[1], Anna M. Piskorz[1], Christine Parkinson[1,2], Suet-Feung Chin[1], Zoya Kingsbury[3], Alvin S. C. Wong[4], Francesco Marass[1], Sean Humphray[3], James Hadfield[1], David Bentley[3], Tan Min Chin[4,5], James D. Brenton[1,2,6], Carlos Caldas[1,2,6] & Nitzan Rosenfeld[1]

- Full exome sequencing
- Multiple and Sequential
- Selection Pressure of therapy
- Emergence of T790M in response to Erlotinib

NSCLC: Where are we with cfDNA?

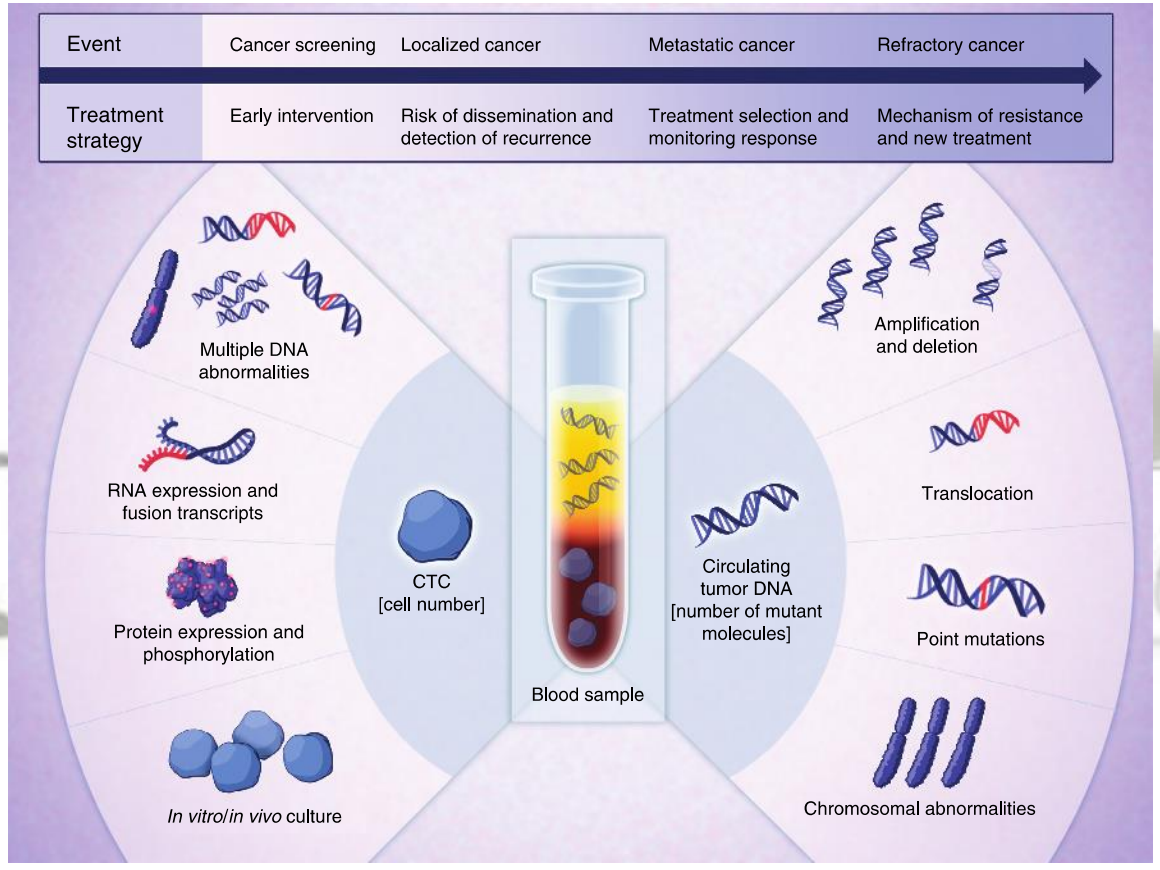
- Increased in Lung Ca Patients (Sozzi, JCO 2003)
  - But also after exercise, liver disease, DM, CVS, non-neoplastic lung disease
- cfDNA associated with tumour burden (PET/CT)
- Associated with Tumour Size, stage and mets +/-
- fcDNA associated with poor Prognosis
  - KRAS mutation detected in fcDNA shorter OS and PFS

NSCLC: Where are we with fcNA?

- Genotyping
- EGFR
  - Diagnostics
  - Predictive decisions
  - T790M (Oxnard et al)

Debate: CTCs versus ctDNA

CTCs



Complementary and synergistic to Each Other

15-18 April 2015, Geneva, Switzerland

Blood-Based Analyses of Cancer: Circulating Tumor Cells and Circulating Tumor DNA
Haber and Velculescu. Cancer Discovery 2014

Organisers

Partners

miRNA and Exosomes

- Exosomes:
  - Membrane bound vesicles
  - Role in cell-to-cell communication
  - Horizontal transfer of genetic information
  - Protein, RNA, miRNA
  - Exosomal miRNA particularly promising

Conclusions

- CTCs, ctDNA, Exosomes are synergistic substrates for a wide and complementary array of potential clinical applications
  - Tumour Genotyping
    - EGFR, EML4/ALK, c-Met amplification, Her 2 mutation, BRAF
  - Identifying and Monitoring acquired resistance
    - T790M
    - NSCLC to SCLC
  - Surrogates of Drug Response
  - Detecting Early relapse
  - Novel Therapeutic Targets (Met amplification)
  - Early Detection (miRNA/Smokers)

What needs to be done?

- Optimize diverse technologies
- Standardize approaches
- Translate to the Molecular Diagnostic Laboratory (The Every day Lab)
- Multicentre clinical Trials

Thank you