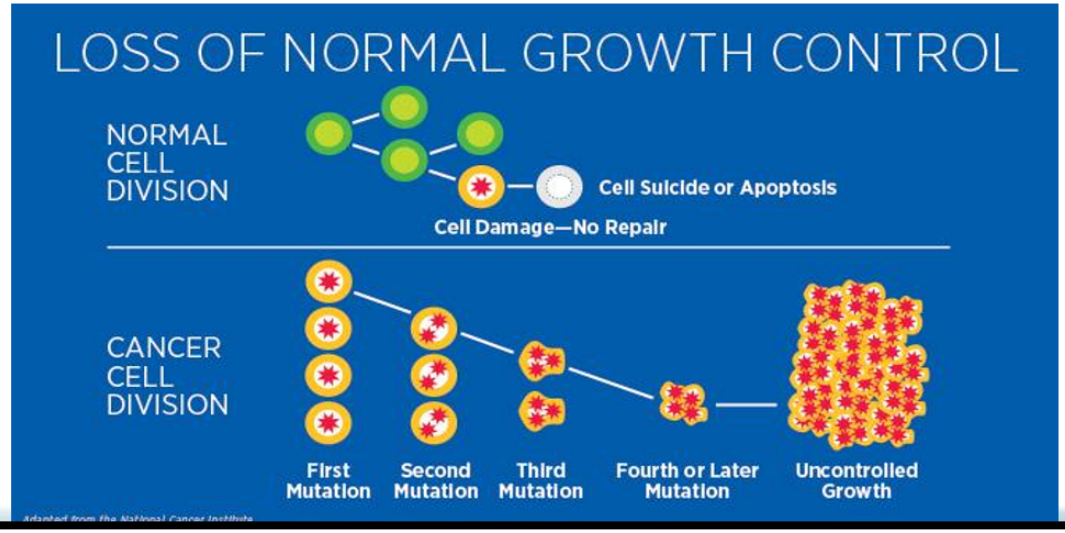
LİQUİD BİOPSY
Cancer Screening - Patient Stratification - Monitoring

Dr. Ferda Alpaslan Pınarlı

Dışkapı Yıldırım Beyazıt Eğitim Araştırma Hastanesi Kök Hücre ve Genetik Tanı Merkezi

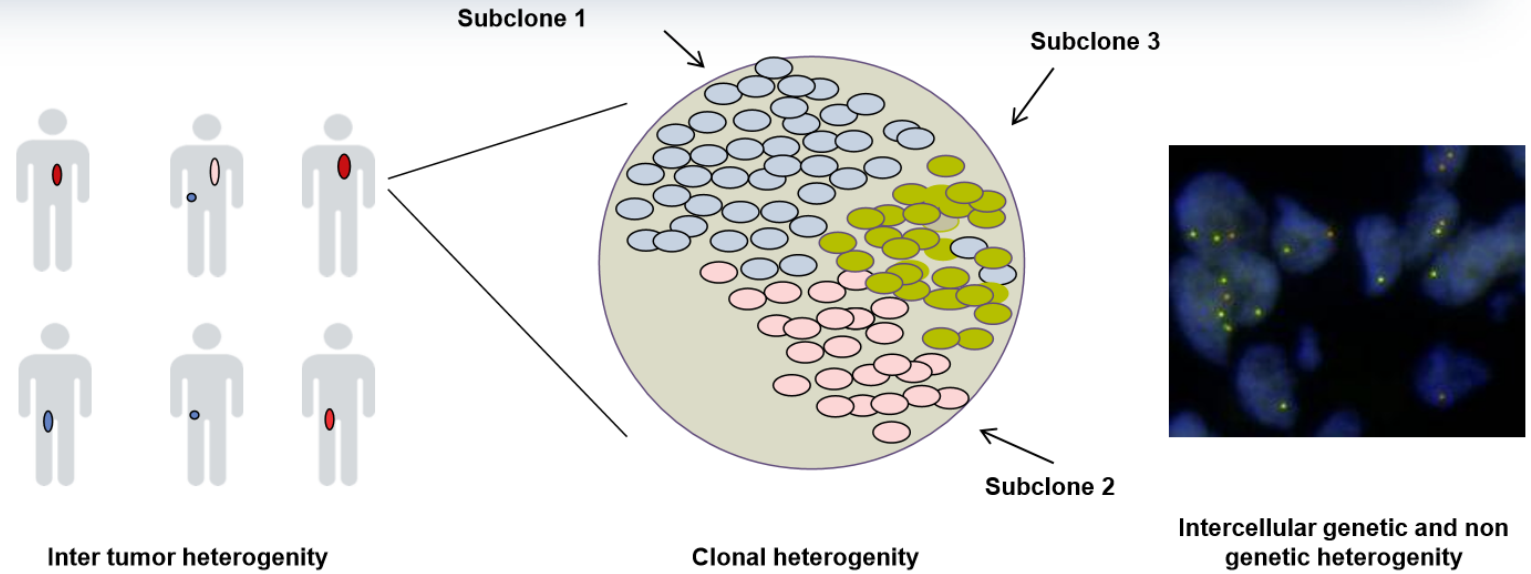
What is Cancer?

- Uncontrolled cell growth/proliferation
- Gain of function of oncogenes, loss of function of tumour suppressor genes
- Historically cellular phenotype / characterisation
- Tests for expression of protein biomarkers
- Cancer a molecular disease and requires characterising on genetic level, (hotspot, CNV, fusion, genetic collapse)

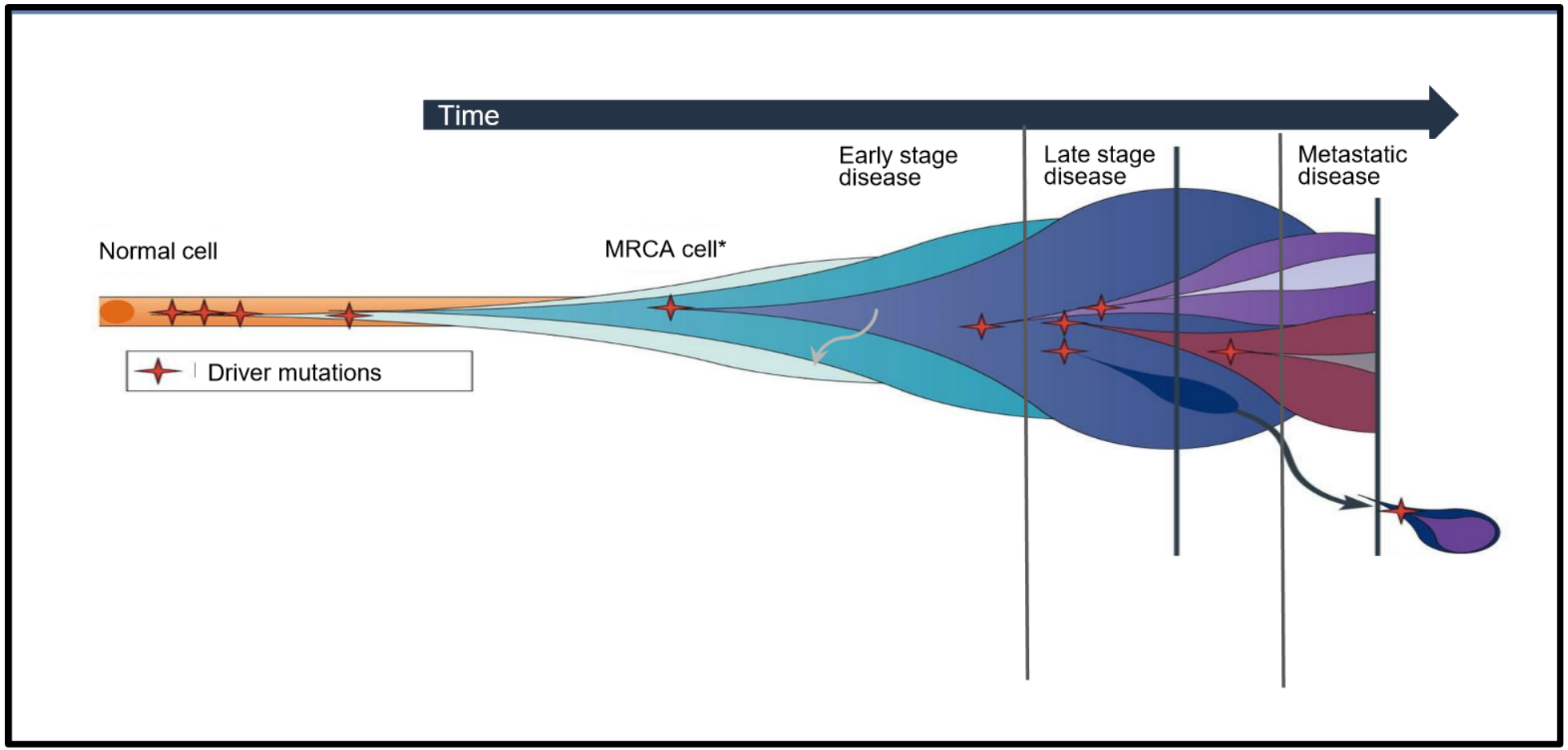


Tumor Heterogenity

- Molecular properties differ between samples and within a tumor
  - Intertumor heterogenity
  - Intratumor heterogenity



Tumor Cell Evolution



Analysis of Tissue Samples

Today's Challenges

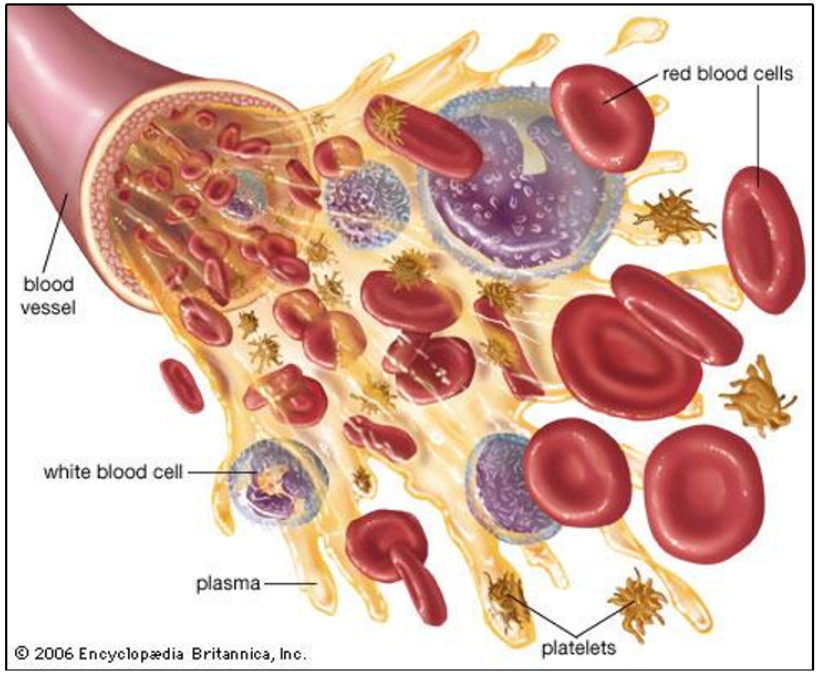
- No information about tumor metastases
- Can be technically difficult and invasive to obtain samples
- Tissue samples and imaging tests are expensive to obtain
- DNA from FFPE tissue samples can be degraded
- Tumor heterogeneity and tumor sampling limit assessment of the full picture

A single tissue sample is limited; a more-comprehensive genomic picture of overall tumour content is needed.[1]

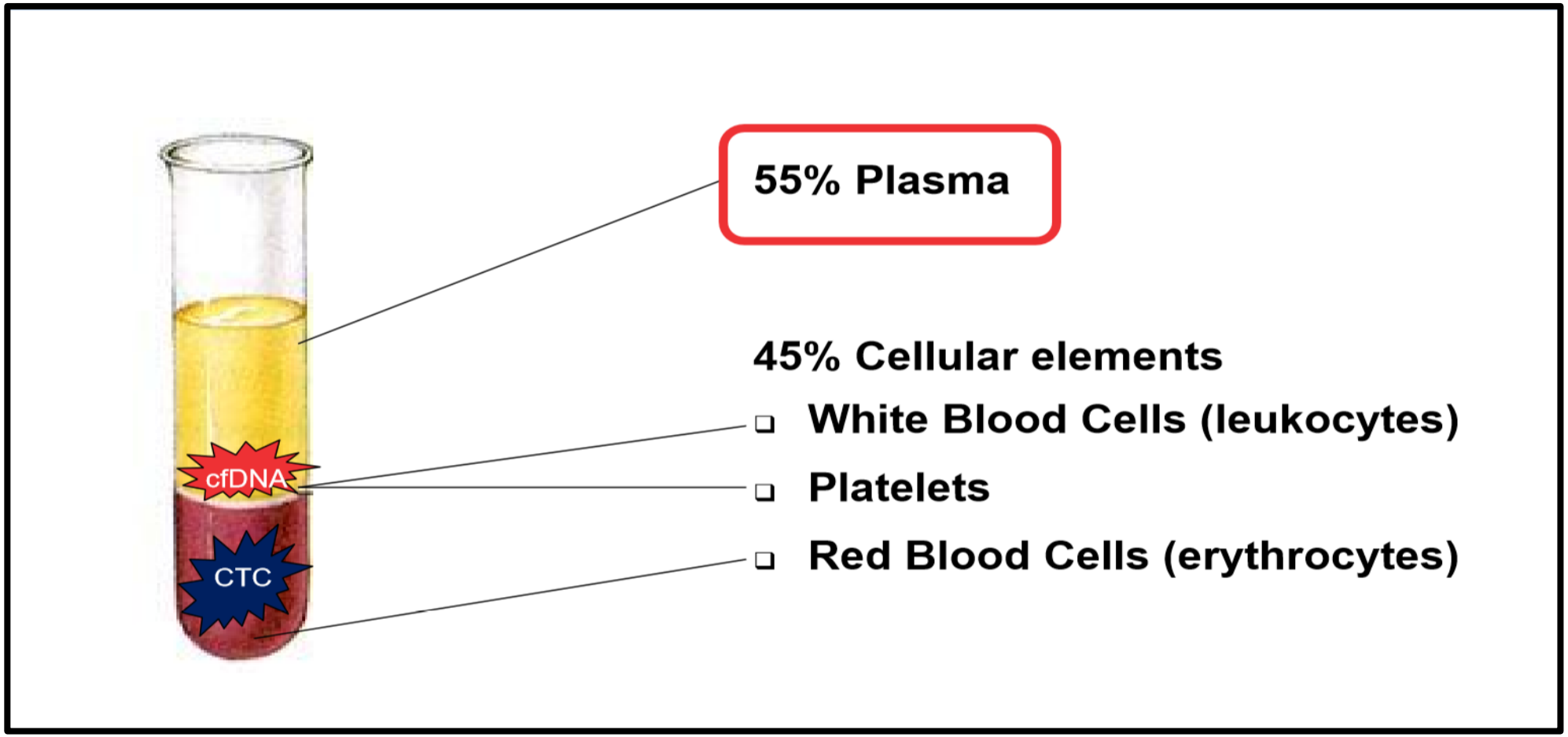
[1]Krebs MG, et al. (2014) Molecular analysis of circulating tumour cells—biology and biomarkers. *Nat Rev Clin Oncol* 11: 129–144.

This is a miracle: BLOOD

- Components of the Blood
- Function of Components
- Terminology



Three Templates from One Blood Sample

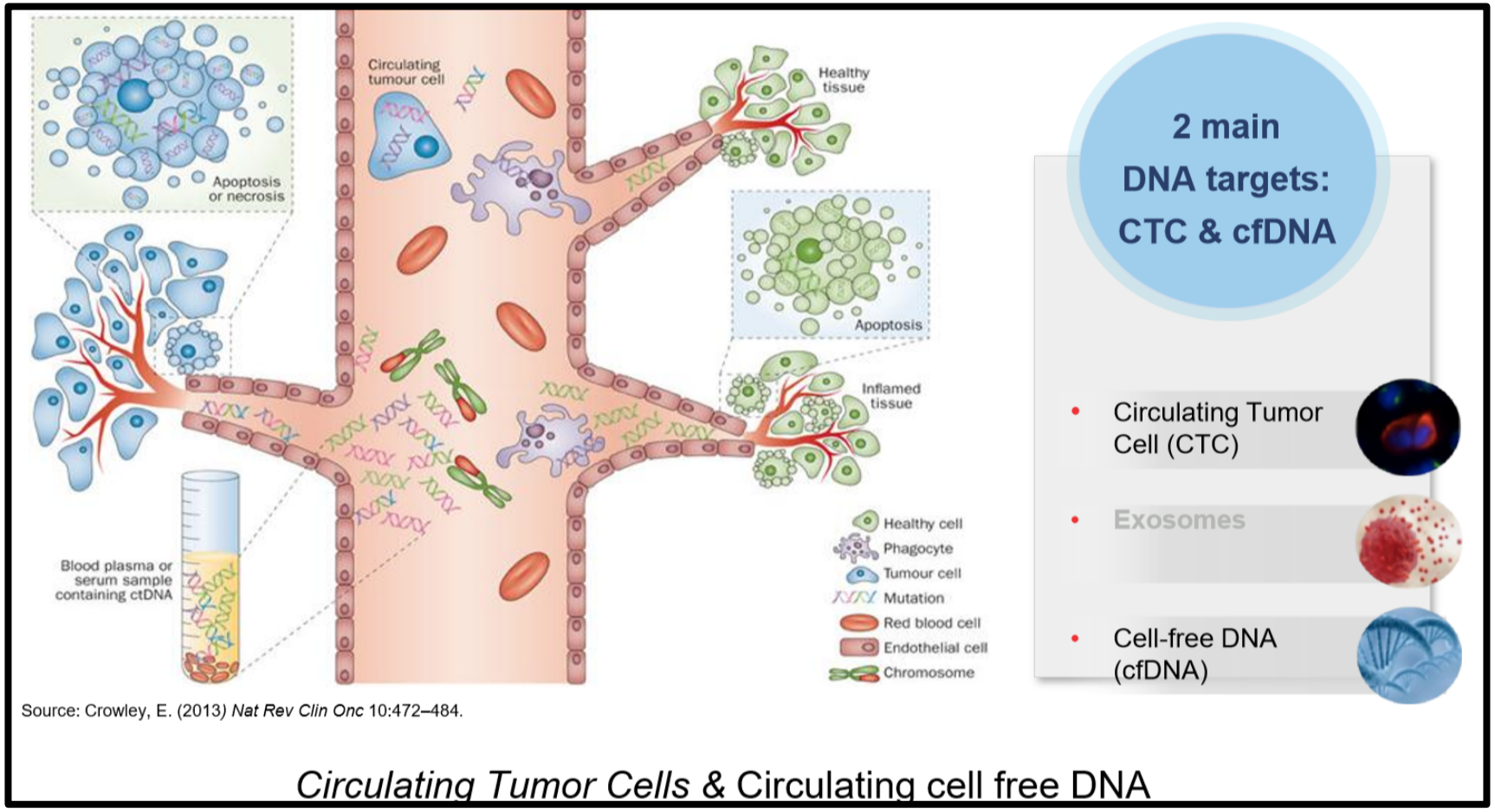


What is Liquid Biopsy?

"Liquid biopsy" (with a space) is a scientific/industry term referring to the use of liquid biological samples (as opposed to tissue biopsy) for analysis of tumour biomarkers

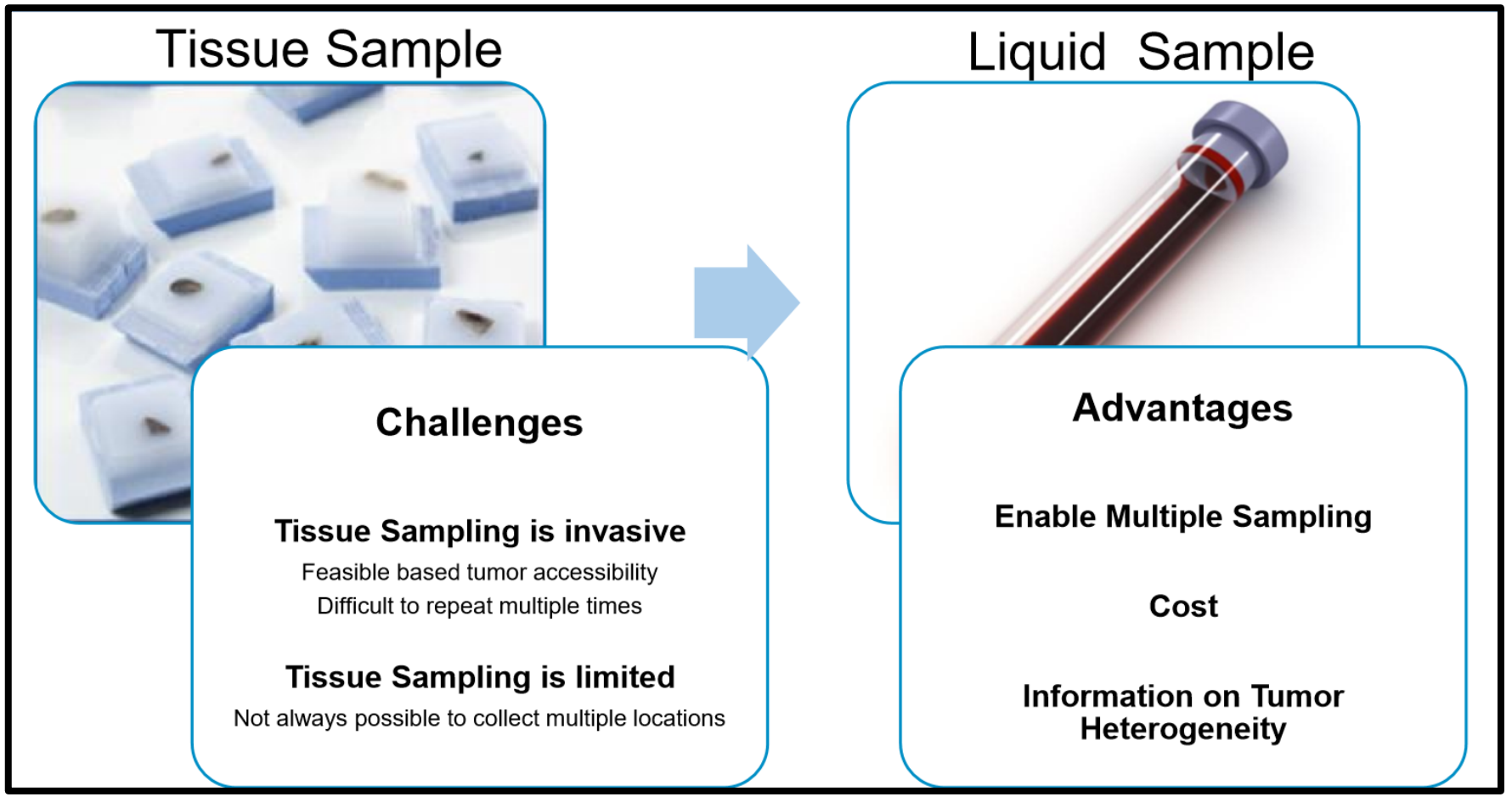
- There are three main targets/templates
  - Circulating Tumor Cell (CTC)
  - Cell-free DNA (cfDNA) $\longrightarrow$ circulating tumor DNA (ctDNA)
  - Exosomes & Cell-free RNA (cfRNA)

There are multiple potential sources of tumor DNA in blood



Circulating Tumor Cells & Circulating cell free DNA

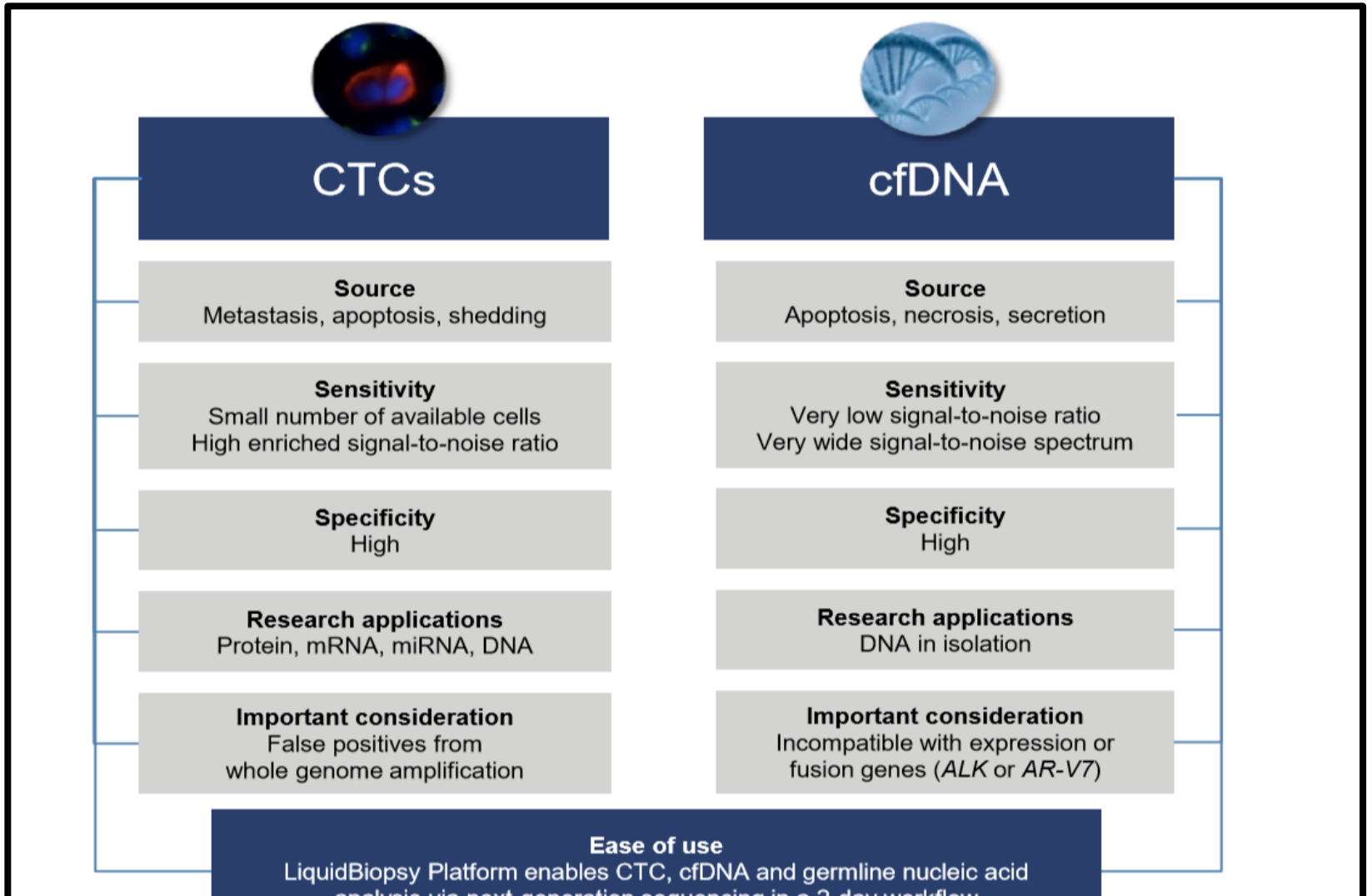
Tissue Sampling - Liquid Biopsy



Peripheral Monitoring in Oncology

- **Peripheral monitoring is a noninvasive research method that detects circulating tumor cells (CTCs) and/or cell-free DNA (cfDNA) fragments of tumor DNA that are shed into the bloodstream from primary and metastatic tumor sites**

Two Templates Reflect Different Processes in Disease Biology

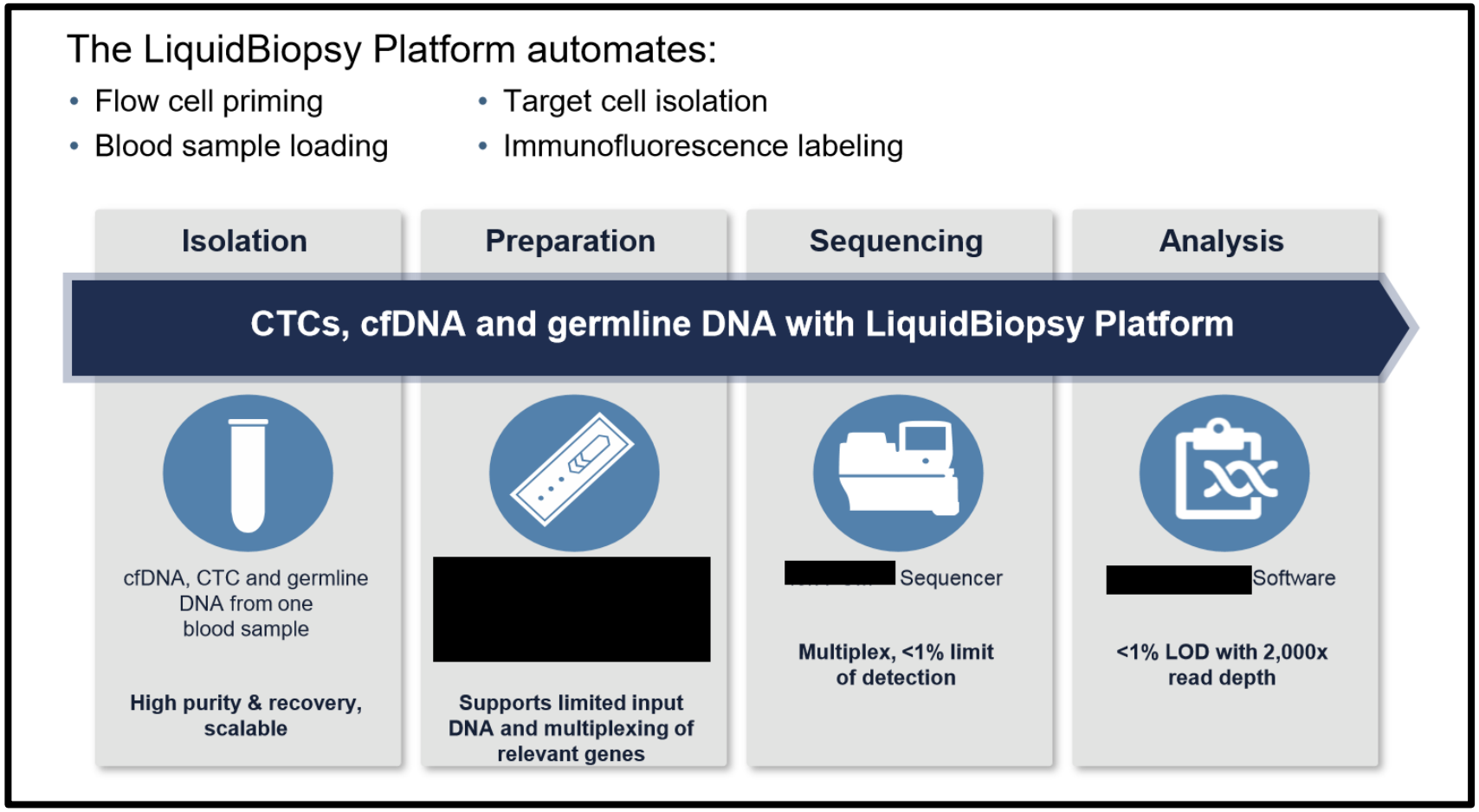


Technology

- A variety of platforms have been developed to enumerate and purify CTCs.
- Only the CellSearch platform is FDA approved.
- Various properties have been exploited to purify CTCs such as their protein expression, size, mass or their viscoelastic or electrophoretic properties

Automated Workflow- Sample to Genomic Data

Liquid Biopsy Workflow — Single Sample to Genomic Data from Three Different Templates



PLOS | ONE

RESEARCH ARTICLE

Exome Sequencing of Cell-Free DNA from Metastatic Cancer Patients Identifies Clinically Actionable Mutations Distinct from Primary Disease

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OPEN ACCESS

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Abstract

The identification of the molecular drivers of cancer by sequencing is the backbone of precision medicine and the basis of personalized therapy; however, biopsies of primary tumors provide only a snapshot of the evolution of the disease and may miss potential therapeutic targets, especially in the metastatic setting. A liquid biopsy, in the form of cell-free DNA (cfDNA) sequencing, has the potential to capture the inter- and intra-tumoral heterogeneity

The Aim:
Molecular Characterization of A Patient's Tumor

- Molecular characterization of a patient's tumor to guide treatment decisions is increasingly being applied in clinical care and can have a significant impact on disease outcome
- These molecular analyses, including mutation characterization, are typically performed on tissue acquired through a biopsy at diagnosis
- However, **tumors are highly heterogeneous** and **sampling in its entirety is challenging**

- Furthermore, **tumors evolve over time** and can alter their molecular genotype, making clinical decisions based on historical biopsy data suboptimal
- **Personalized medicine for cancer patients aims to tailor** the best treatment options for the individual at diagnosis and during treatment
- To fully enable personalized medicine:
- **easily accessible**
- **minimally invasive way to determine**
- **follow the molecular makeup of a patient's tumor longitudinally**

- The identification of the molecular drivers of cancer by sequencing is the backbone of precision medicine and the **basis of personalized therapy**
- However, **biopsies of primary tumors provide only a snap shot of the evolution of the disease** and may miss potential therapeutic targets, **especially in the metastatic setting**
- **A liquid biopsy,** in the form of cell-free DNA (cfDNA) sequencing, has the potential to **capture the inter-and intra-tumoral heterogeneity present in metastatic disease, and, through serial blood draws, track the evolution of the tumor genome.**

- A liquid biopsy can be used for molecular characterization of the tumor and its non-invasive nature allows **repeat sampling to monitor genetic changes over time** without the need for a tissue biopsy

The identification of emerging mutations may allow therapies to be started or stopped

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10th ISMRC International Symposium on Minimal Residual Cancer:
Liquid Biopsy in Cancer Diagnostics and Treatment

March 19-21 2016 in Hamburg, Germany

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Which cells are resistant to the cancer treatment? Circulating Tumor Cells (CTCs) or Cancer Stem Cells (CSCs)

Ersan B.[1], Arkan T.K.[1], Oz B.E.[1], Ercan E.[1], Altundag M.K.[2], Alparslan Pinarli F.[3], Guler G.[4], Ozensoy Guler O.[1], Carhan A.[1], Simsek E.[1]

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Cancer is the second leading cause of death worldwide (1) and the majority of deaths associated with cancer are due to the metastasis of the original tumor cells (2). Breast cancer is the most common cancer among women worldwide (3) and circulating tumor cells (CTCs) play a critical role in the metastatic spread of breast cancer (4). Among CTC subpopulations, there are several phenotypes: epithelial, epithelial-mesenchymal, mesenchymal and mesenchymal stem cells (5). Cancer stem cells (CSCs) constitute a subpopulation of the CTCs (6). Epithelial-to- mesenchymal transition (EMT) can cause loss of EpCAM and CK expression, which can cause CTCs to change into CSCs (4). Thus, CTCs (7) and CSCs (8) can lead to an aggressive phenotype, metastasis, and resistance to current therapies. The aim of our study was to determine whether the amounts of CTCs and CSCs are different after the treatment. With this purpose, we classified breast cancer patients according to their tumor node metastasis (TNM) stages as well as cancer types and investigated to show which type of cancer cells is more resistant to cancer treatment. For this reason, the peripheral blood (7.5 ml) samples were collected from breast cancer patients and healthy volunteers. For the negative selection of our study

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A novel strategy for determining circulating tumour cells in lung cancer

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Circulating tumor cells (CTCs) play a crucial role in the metastatic spread of carcinoma. Therefore, CTC has been interest of subject in the past few decades in terms of prognosis and response to the therapy in several cancer. Since lung cancer is the leading cause of cancer-related death worldwide, with poor prognosis that commonly metastasis before disease diagnosis. In this preliminary study, our goal is to detect CTC enumeration in lung cancer with an alternative method. This method consists of a ficoll density gradient centrifugation and a magnetic bead separation technique by CD45 negative depletion method. After having the final solution, we determined CTCs with the expression of anti-epithelial cell adhesion molecule (EpCAM) and cytokeratins (CK7,8,14,15,16,19) by multi-parameter flow cytometry. According to these results, we defined CTCs in lung cancer patients by this novel strategy, and it may lead us for using this method as diagnostic, prognostic, and predictive biomarkers in cancer treatment process as alternatively.

Keywords: Lung Cancer, Circulating Tumor Cells, Flow Cytometry

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A noninvasive method for bladder cancer: CTC detection with flow cytometry

Ercan E.[1], Ersan B.[1], Arkan T.K.[1], Oz B.E.[1], Canda A.E.[2,3], Alparslan Pinarli F.[4], Simsek E.[1], Ozensoy Guler O.[1], Carhan A.[1]

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Cancer is a group of diseases described by unregulated cell growth and the invasion and spread of cells from the location of origin, or primary location, to other locations in the body (1). Bladder cancer is one of the most common malignancies of the urinar system (2) and causes for 15,500 deaths every year. The worldwide age standardised rate (ASR) is 10.1 per 100 000 for men and 2.5 per 100 000 for women. The global world mortality rate is 4 per 100 000 among men and 1.1 per 100 000 among women (3). Bladder cancer is the ninth most common cancer and 430,000 new cases diagnosed in 2012 (4). Malignant cells spread out from primary tumors and become invasive. These cells may then intravasate into the blood or lymphatic circulation and later extravasate from the circulation and build a secondary tumor in another organ far from the primary tumor (5). Systemic progress consists when disseminated bladder cancer cells spread into the blood or lymph nodes and cause metastasic lesions. However, it is difficult to diagnose metastatic disease before a surgical procedure (2).Most cancer deaths are dependent on metastasis to distant organs due to disease

HOTSPOT CANCER PANEL

ABL1, EGFR, GNAS, KRAS, PTPN11, BRAF, FGFR1, JAK2, NPM1, SMO, CDH1, FGFR2, JAK3, NRAS, SRC, CDKN2A, FGFR3, IDH2, PDGFRA, STK11, CSF1R, FLT3, KDR, PIK3CA, TP53, CTNNB1

AKT1,ERBB2, GNAQ,MET, RB1, ALK, ERBB4,HNF1A, MLH1, RET, APC, EZH2, HRAS, MPL, SMAD4, ATM, FBXW7, IDH1, NOTCH1, SMARCB1, GNA11, KIT, PTEN, VHL

Familial Cancer Panel

NBN, BARD1, CDH1, MRE11A, ATM,PTEN,

STK11, RAD51C,ALB2, BRIP1, MSH6, RAD51, CHEK2, TP53

IF YOU WANT TO SEE
YOU SHOULD LOOK AT
TO THE CORRECT
PLACE