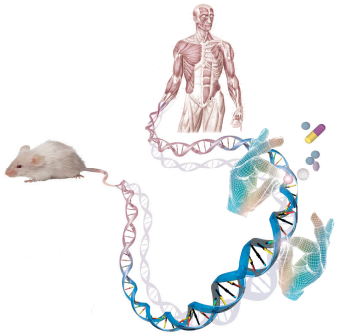


Nuevas técnicas para el estudio del perfil genómico. *Biopsias Líquidas*

Isidro Sánchez-García

isg@usal.es

16 Congreso Asociación Castellano-Leonesa Oncología
6 y 7 de Mayo de 2016
Segovia

Research on circulating tumor cells (**CTCs**) is a very dynamic field with more than 17,000 articles listed in PubMed as of December 2015.

More recently, the term "**liquid biopsy**" has also been adopted for the analysis of circulating cell-free tumor DNA (**ctDNA**) released from apoptotic or necrotic tumor cells.

The application of CTCs and ctDNA for the early detection of cancer is of **high public interest**

Redefining cancer and cure.

Outline

1- **The Liquid Biopsy Concept**

2- **The Biology behind the Liquid Biopsy Concept** (CTCs/ctDNA)

3- **Technologies for CTC and ctDNA Detection**

4- **The clinical applications of CTCs and ctDNA as liquid biopsy in patients with cancer**

5- **Conclusions**

The Liquid Biopsy Concept

Recent technological advances have enabled the detection and detailed characterization of circulating tumor cells (**CTC**) and circulating tumor DNA (**ctDNA**) in blood samples from patients with cancer.

Often referred to as a “liquid biopsy” CTCs and ctDNA are expected to provide **real-time monitoring** of **tumor evolution** and **therapy efficacy**, with the potential for improved cancer diagnosis and treatment.

Recently, it has been described that **tumor-associated blood platelets** provide specific information on the location and molecular composition of the primary tumor

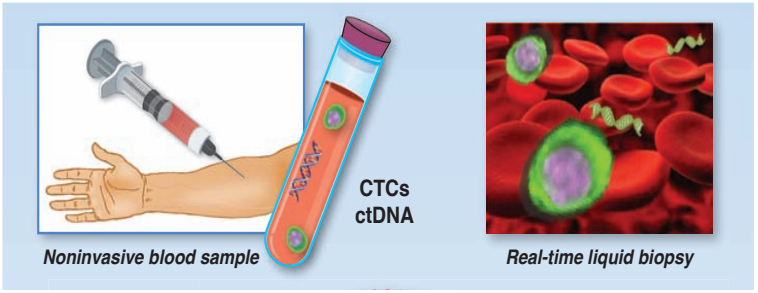
Tumor-Educated Platelets as Liquid Biopsy in Cancer Patients

Simon A. Joosse[1] and Klaus Pantel[1,*]
[1]Department of Tumor Biology, Center of Experimental Medicine, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
*Correspondence: pantel@uke.de
http://dx.doi.org/10.1016/j.ccell.2015.10.007

Real-time monitoring of changes in cells or cell products released from malignant lesions into the blood has opened new diagnostic avenues (“liquid biopsy”). In this issue of *Cancer Cell*, Best and colleagues describe that tumor-associated blood platelets provide specific information on the location and molecular composition of the primary tumor.

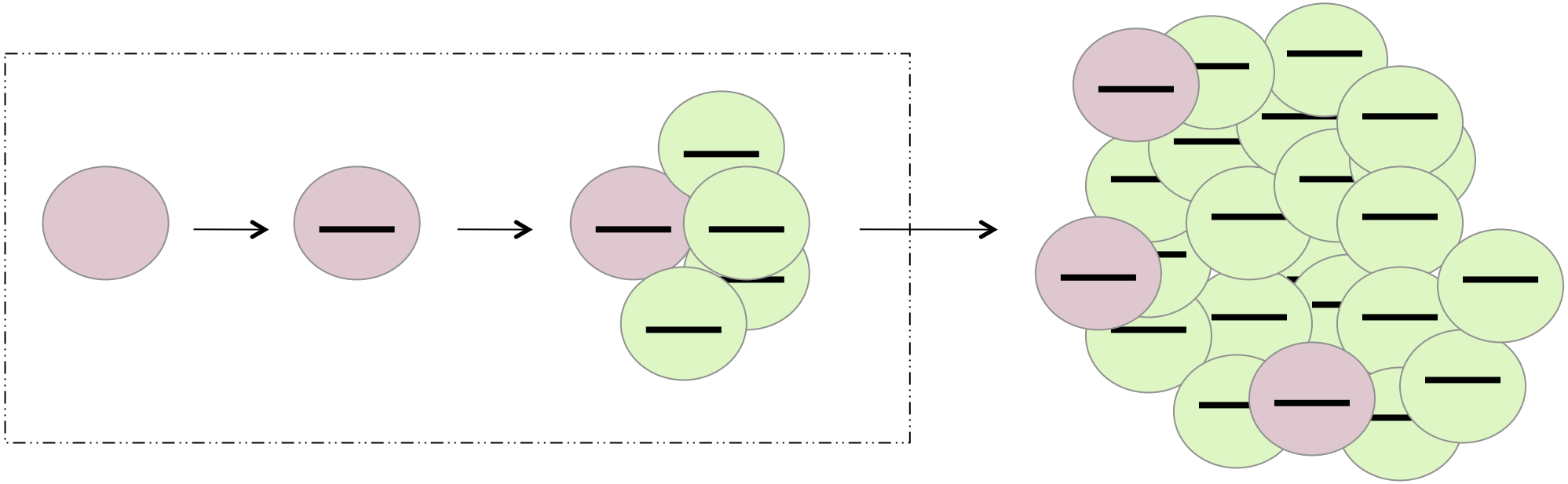
The peripheral blood of a cancer patient is a pool of cells and/or cell products derived from the primary tumor and different metastatic sites, including circulating tumor cells (CTCs) and stromal cells of the tumor microenvironment (e.g., macrophages) as well as tumor-derived DNA, RNA, and proteins. The analysis of these blood components can, therefore, provide a comprehensive real-time picture of the tumor-associated changes in an individual cancer patient (Figure 1). This information can be used for screening and early detection of cancer, estimation of the risk for metastatic relapse or progression (prognostic information), stratification and real-time monitoring of treatment response, identification of therapeutic targets and resistance mechanisms (biological therapies), and a better understanding of the biology of metastatic development.

In contrast to tissue biopsies, blood samples can be obtained easily and repeatedly from cancer patients. In the last decade, many reports have focused on CTCs and circulating nucleic acids (in particular, tumor-derived DNA fragments and various



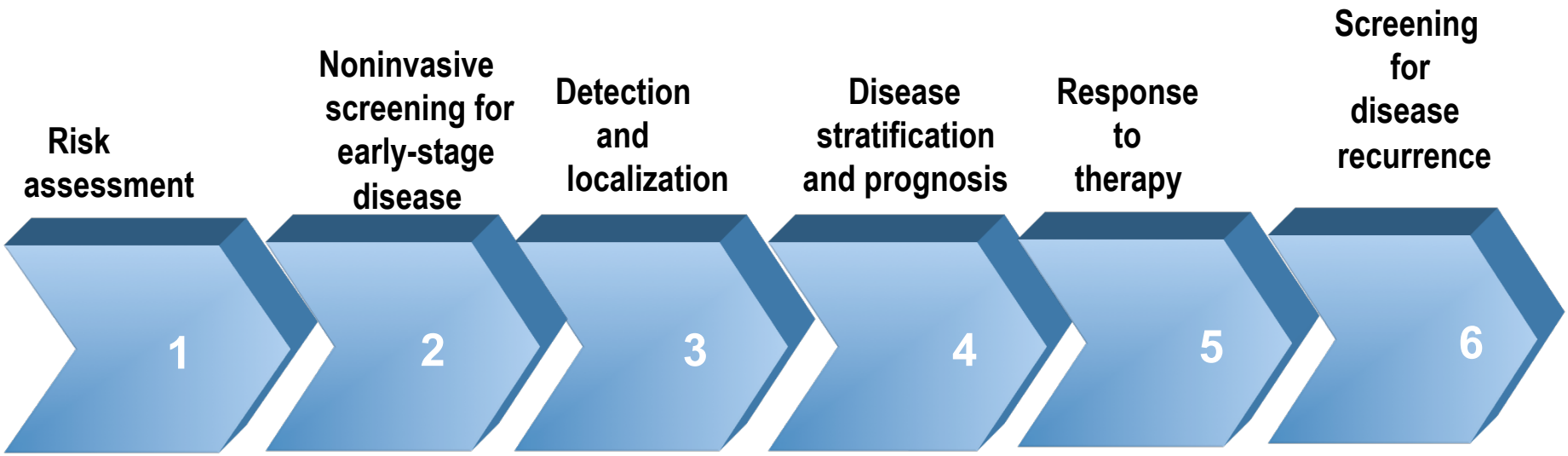
¿Do we need the liquid biposy?

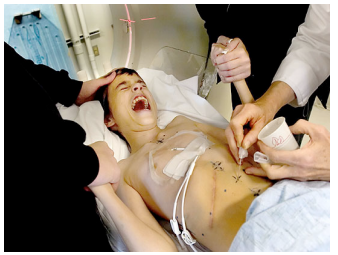
Early stages in cancer

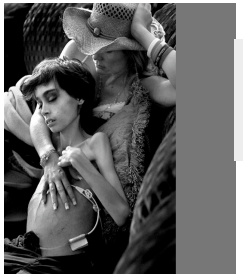


Clinical malignant tumor mass
"**billion-cell threshold**"
(Oncology remission means 0 ---- 10^9 cells)

Oncology and therapy



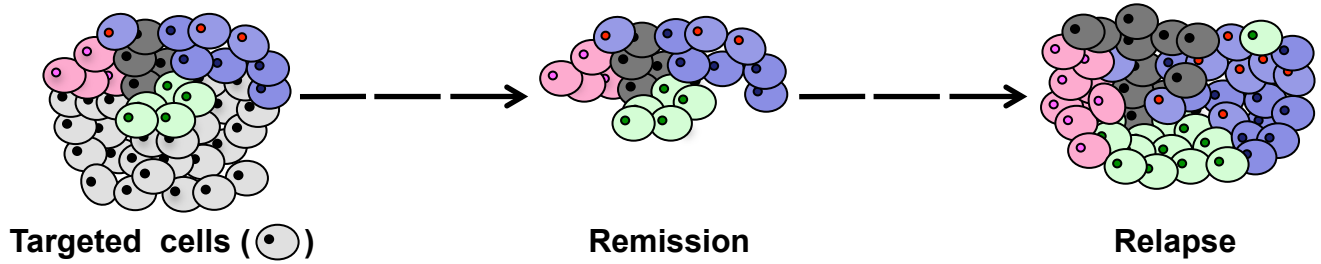




Derek
2005-2007

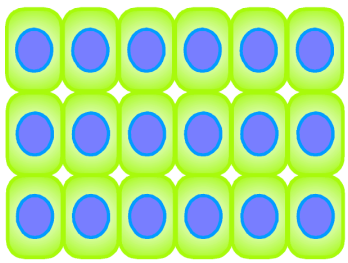
The identical twin on the right was given various treatments as a child for acute lymphoblastic leukaemia.

Interpatient and, more importantly, intrapatient heterogeneity



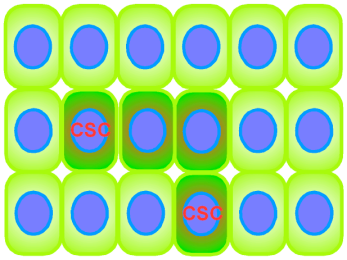
spatial heterogeneity

Temporal heterogeneity



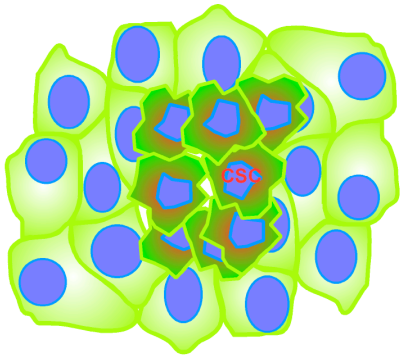
Normal Tissue

First Genetic/Epigenetic Alterations



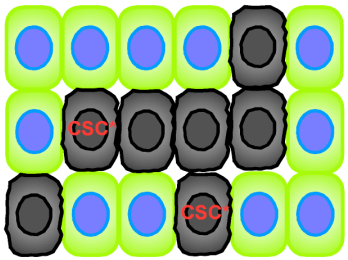
Primary Cancer With Heterogeneous Cells
Primary CSCs

Dissemination
Metastasis



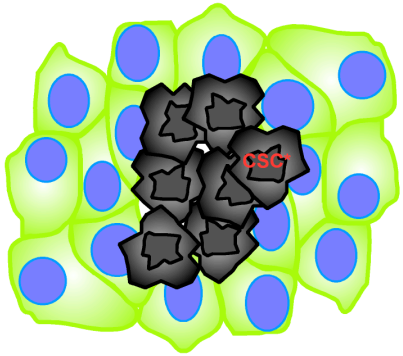
New Environment, New Signals
Different CSCs

PROGRESSION
Secondary Genetic/Epigenetic Alterations as a Result of Disease Progression or Radio/ChemoTherapy

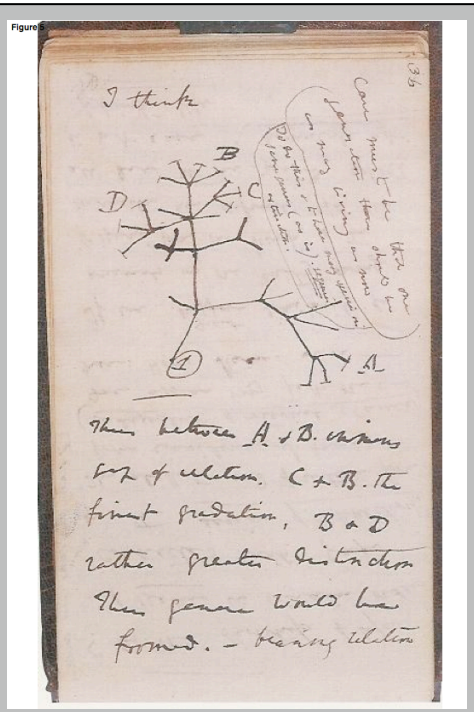


Secondary Cancer
Within the Same Tissue
New CSCs with Different Properties

Dissemination
Metastasis



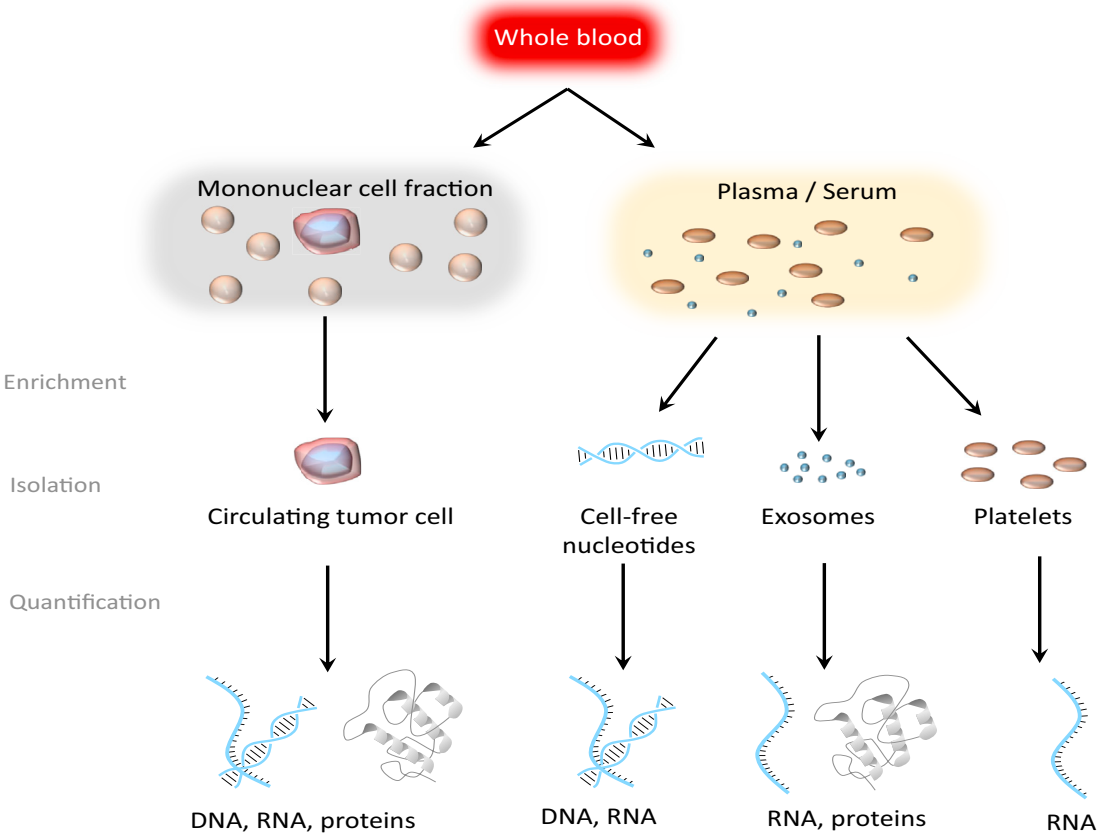
New Environment, New Signals
Different CSCs

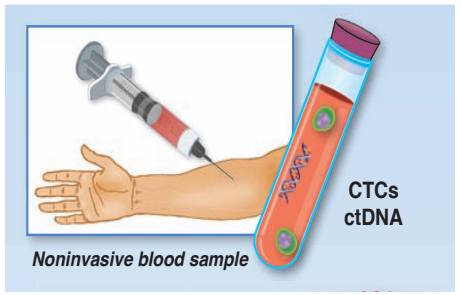


Evolutionary speciation or ancestral tree, from Charles Darwin's 1837 Transmutation notebook B

Semin Cancer Biol. 2010

Sources of Liquid Biopsy



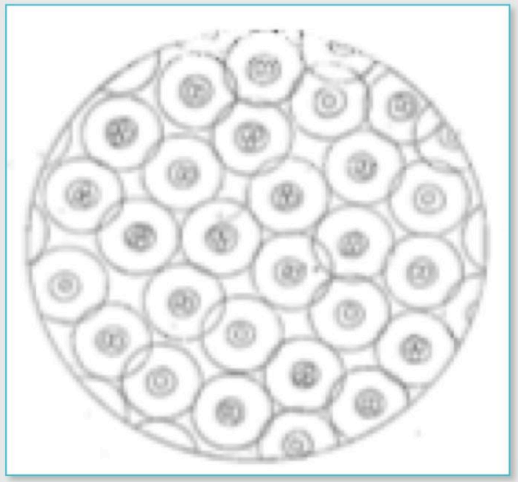


Blood can be separated into different fractions in order to enrich for tumor-associated biomarkers.
From the mononuclear cell fraction, CTCs may provide genomic, transcriptomic, and proteomic information on the tumor.
From plasma o serum, cell-free nucleotides and exosomes can be further used to interrogate cancer-secreted bioparticles.
Tumor educated platelets (TEPs) carry additional information on the location of the tumor in their mRNA.

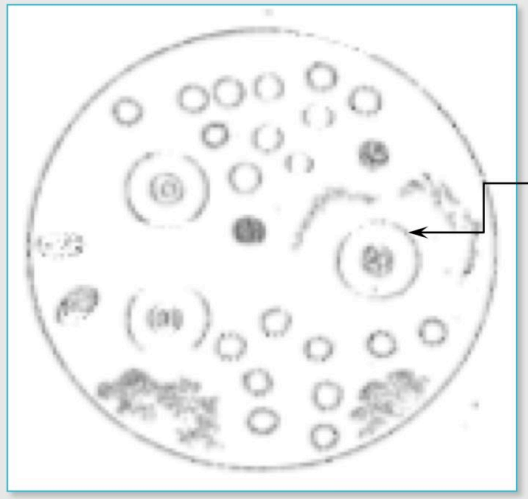
The Biology behind the Liquid Biopsy Concept (CTCs)

Ashworth (1869)

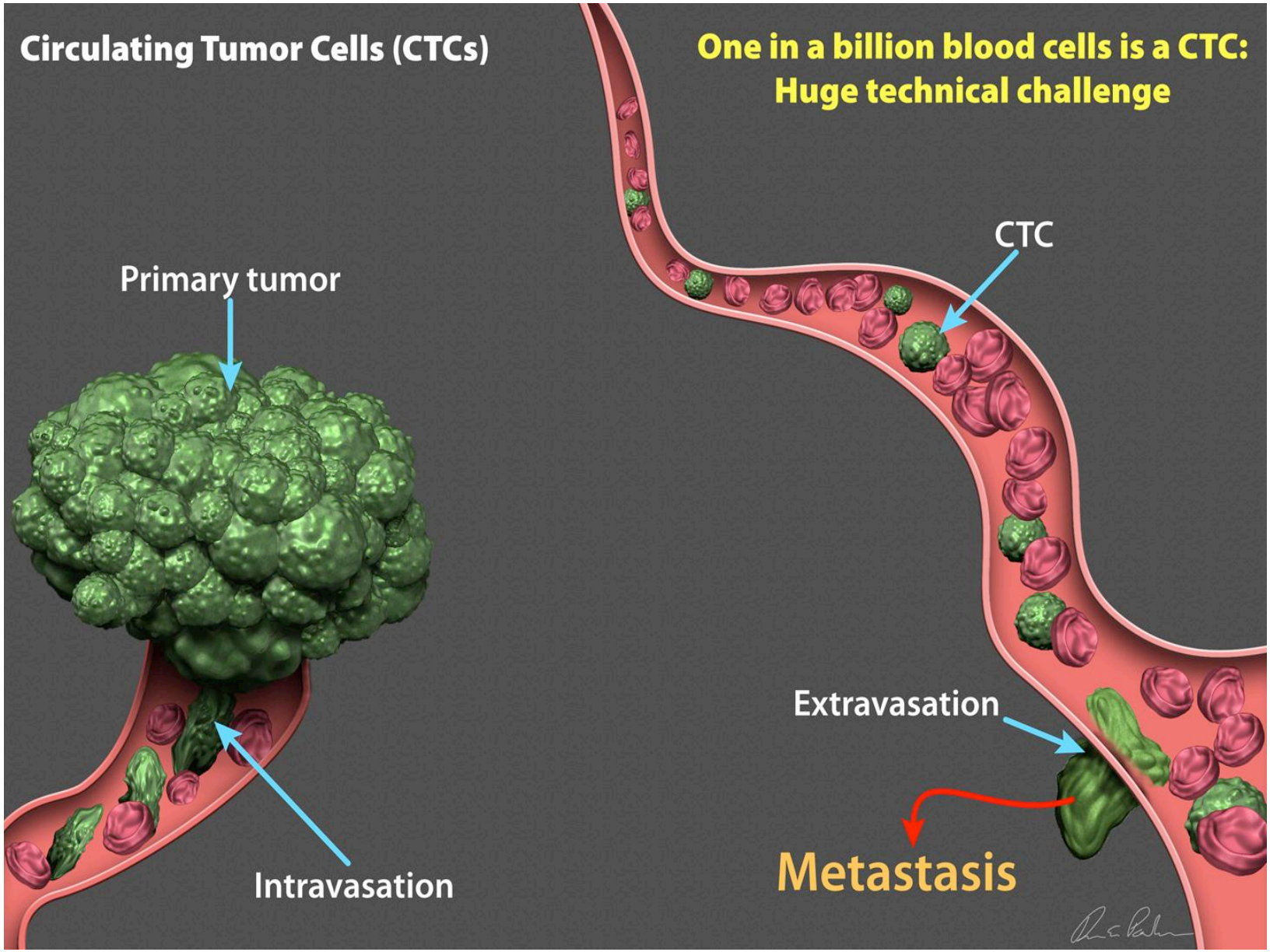
Primary Tumor



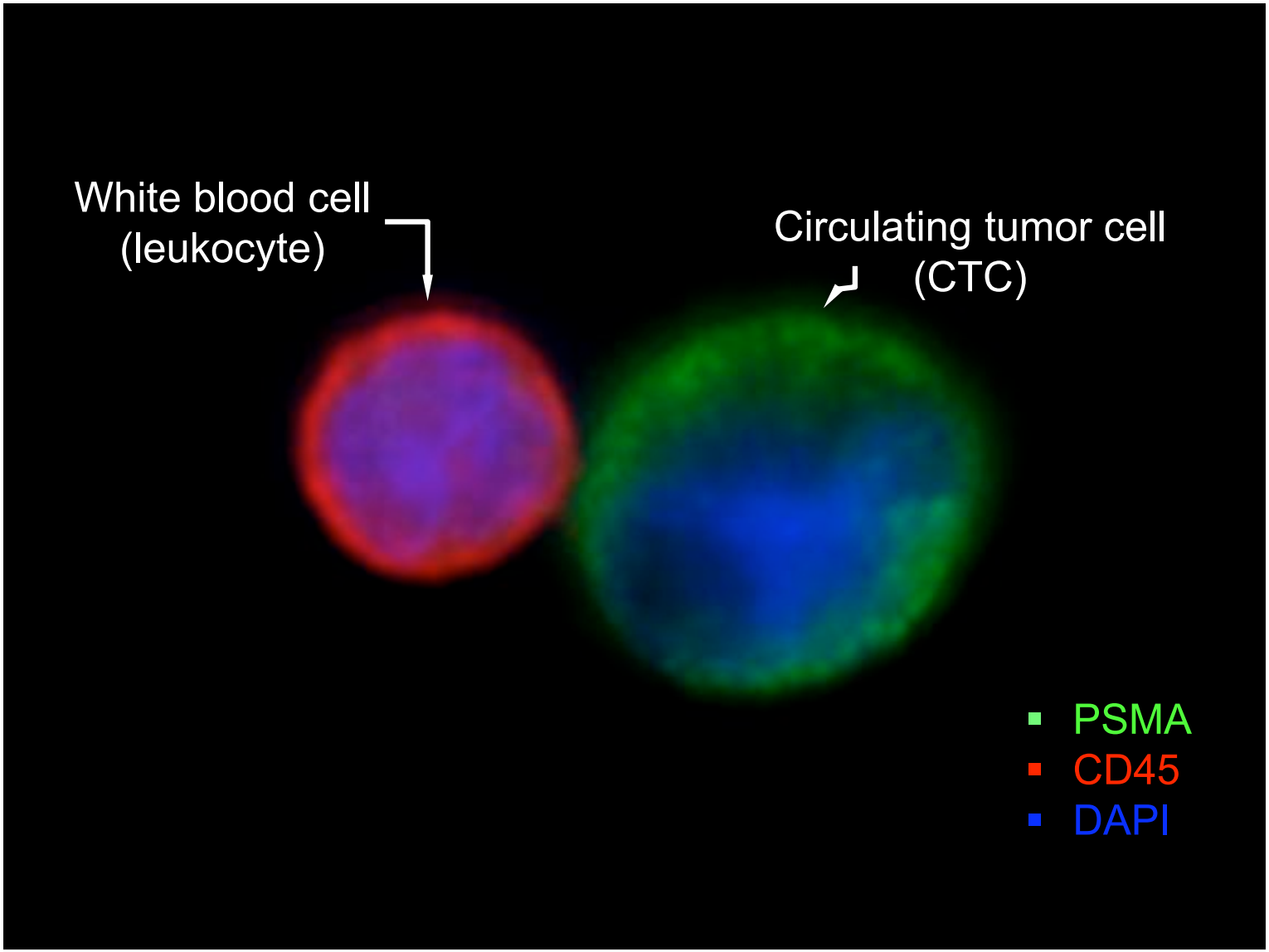
Peripheral Blood



The Biology behind the Liquid Biopsy Concept (CTCs)

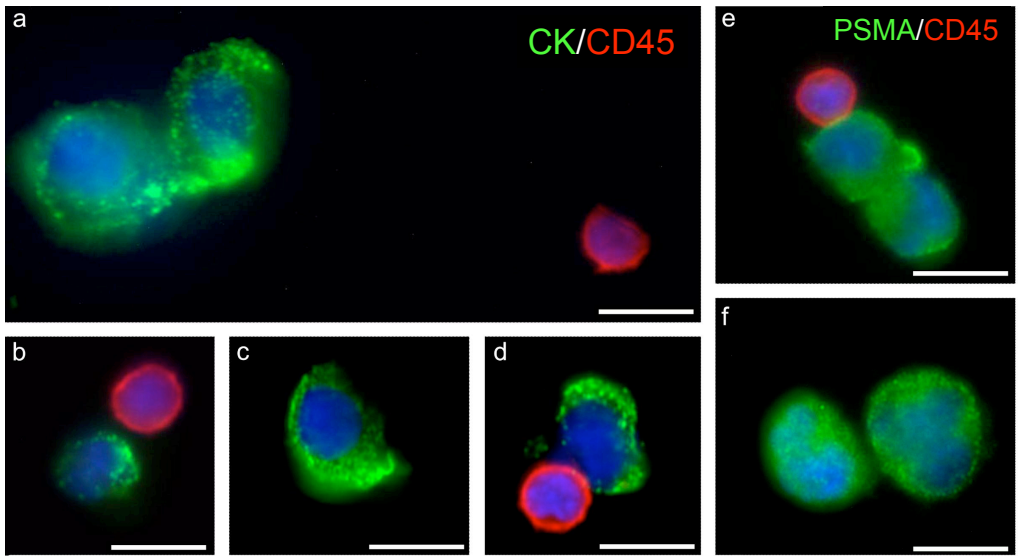


The Biology behind the Liquid Biopsy Concept (CTCs)



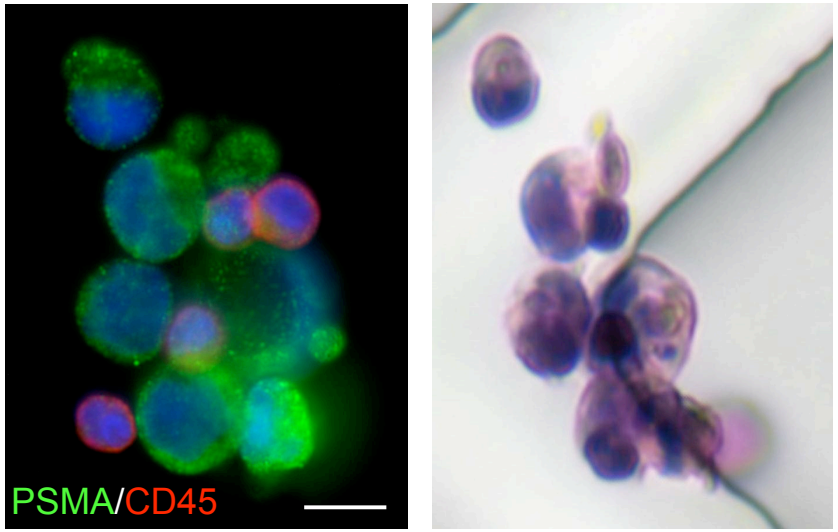
The Biology behind the Liquid Biopsy Concept (CTCs)

Patient CTCs



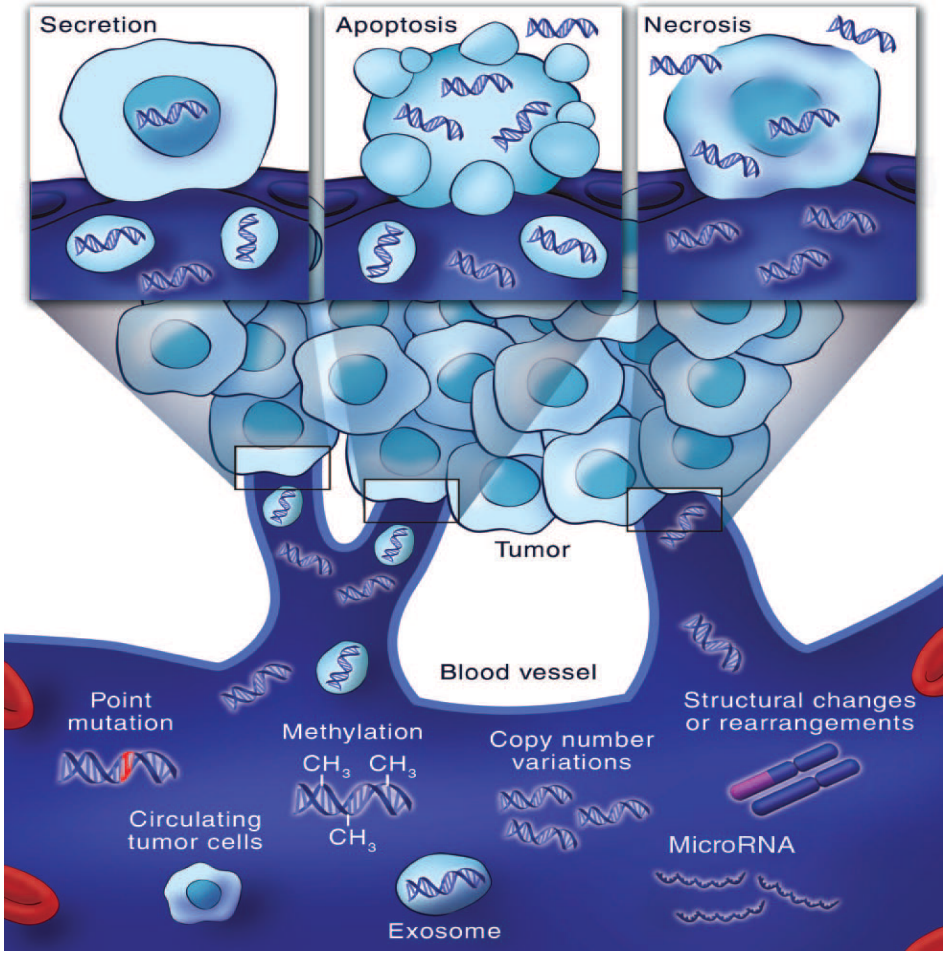
Stott et al. ***PNAS*** 2010

CTC Clusters: Prostate Cancer



Stott et al. ***PNAS*** 2010

The Biology behind the Liquid Biopsy Concept (ctDNA)



Diaz and Bardelli, 2014 Journal of Clincial Oncology 32

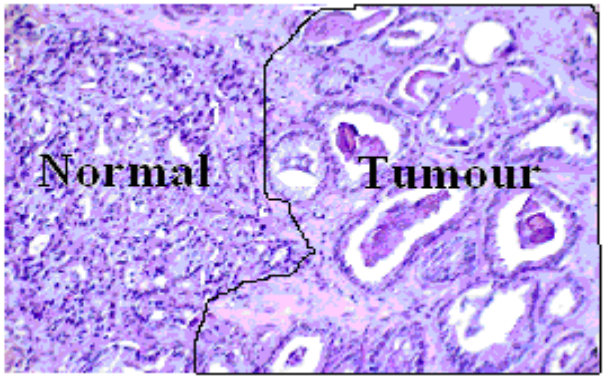
- ctDNA is tumour DNA that has been shed into the bloodstream
- ctDNA can be present in 0.01% - >90% of the total Cell Free DNA (cfDNA)
- The amount of ctDNA is related to the tumour burden and varies between patients with different clinical presentations

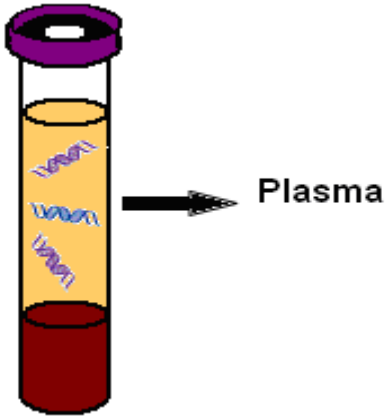
The Biology behind the Liquid Biopsy Concept (ctDNA)

FFPE versus ctDNA

- **FFPE Samples**
- Tumour DNA extracted from fixed biopsy samples or tumour resections
- Problems with quality of DNA due to fixation
- Mixture of normal and tumour DNA
- Long time to process by histopathologists.
- Macrodissected to enrich tumour content
- Some patients have no tumour sample available
- The sample represents the tumour at one fixed time point

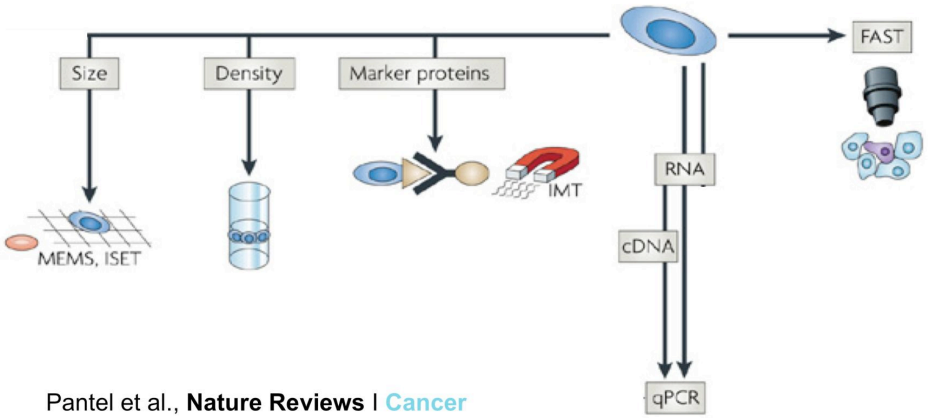
- **ctDNA Samples**
- ctDNA shed directly from tumour
- Extracted from the plasma component of whole blood
- Large fragment sizes possible
- Small quantities extracted ~ 30ng/ 5ml plasma
- Separate out plasma within a few hours of receipt of blood sample.
- Serial samples can be taken at various time points during the patient's treatment





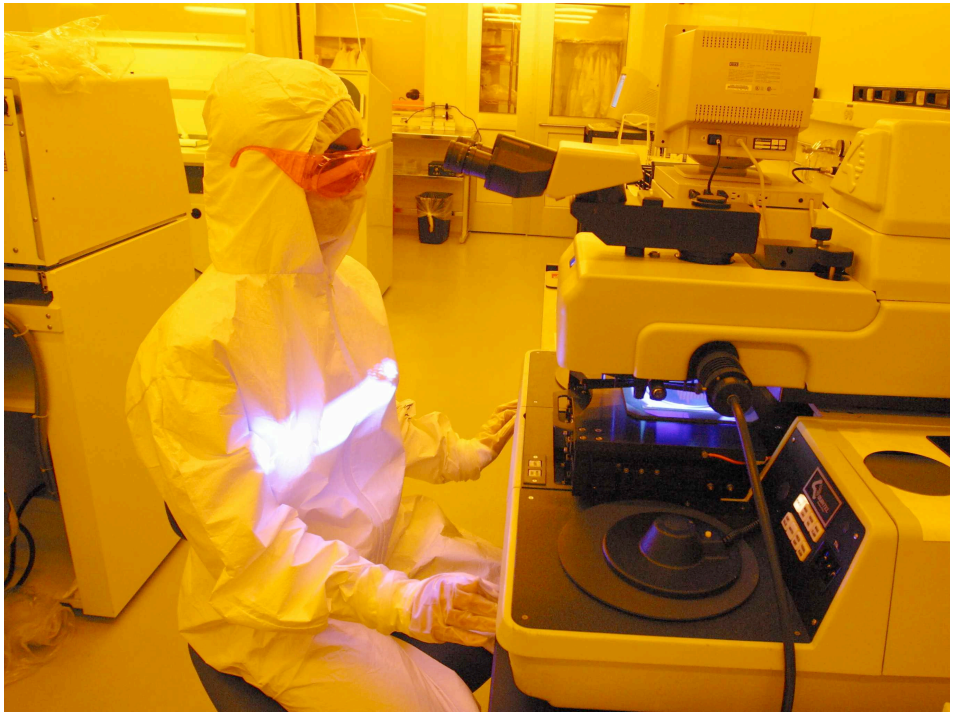
Technologies for CTC and ctDNA Detection

Methods for CTC detection



Pantel et al., **Nature Reviews** | Cancer

CTCs are present in only **20-50%** of metastatic cancers with frequencies in the range of **1 CTC per 7.5 mL** whole blood

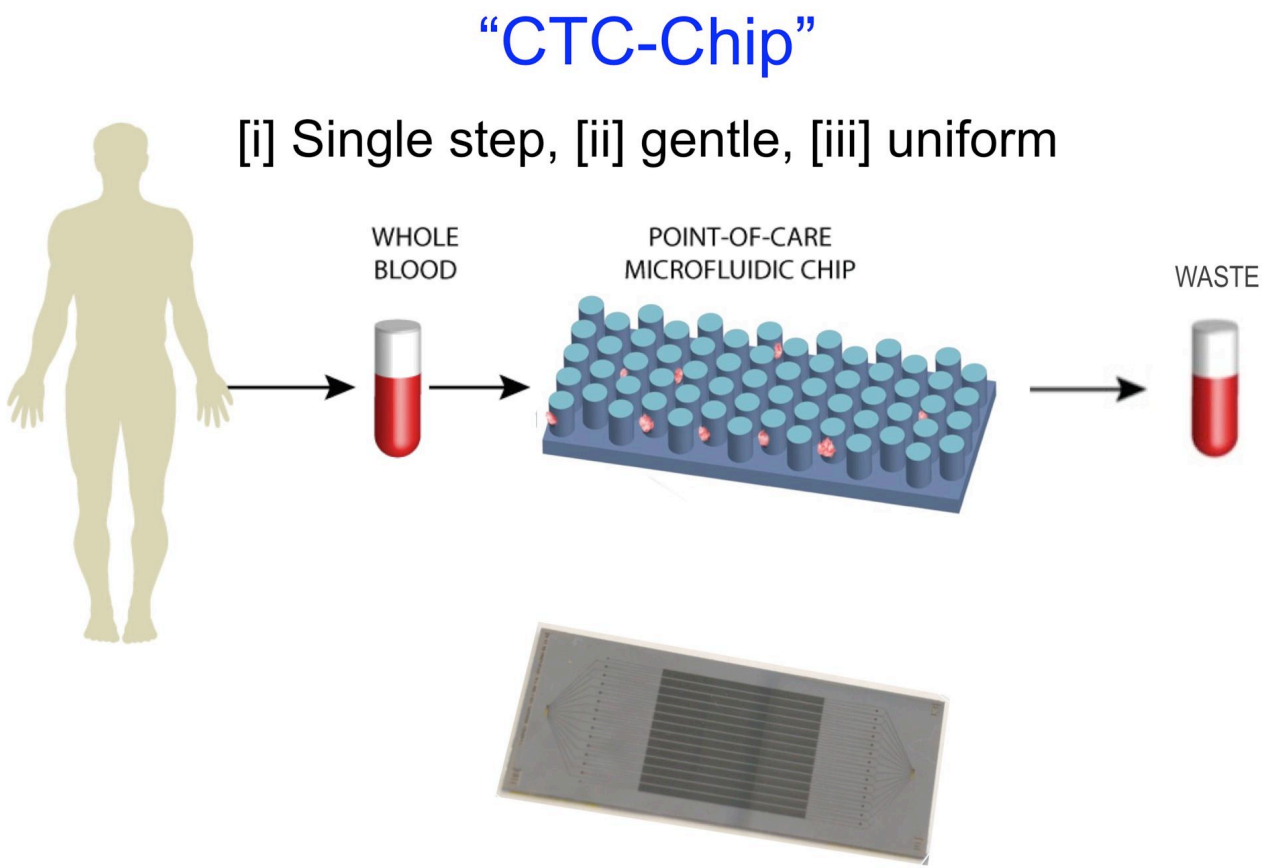


- **Label dependent methods** (positive enrichment with cell markers: cytokeratine+/CD45- cells but this procedure does not detect cells suffering EMT)

- **Label independent methods** (based on negative selection, size, etc)

The only system currently approved by FDA
is
CELL SEARCH (Jansen Diagnostics)

Technologies for CTC and ctDNA Detection



Nagrath et al, ***Nature*** 2007

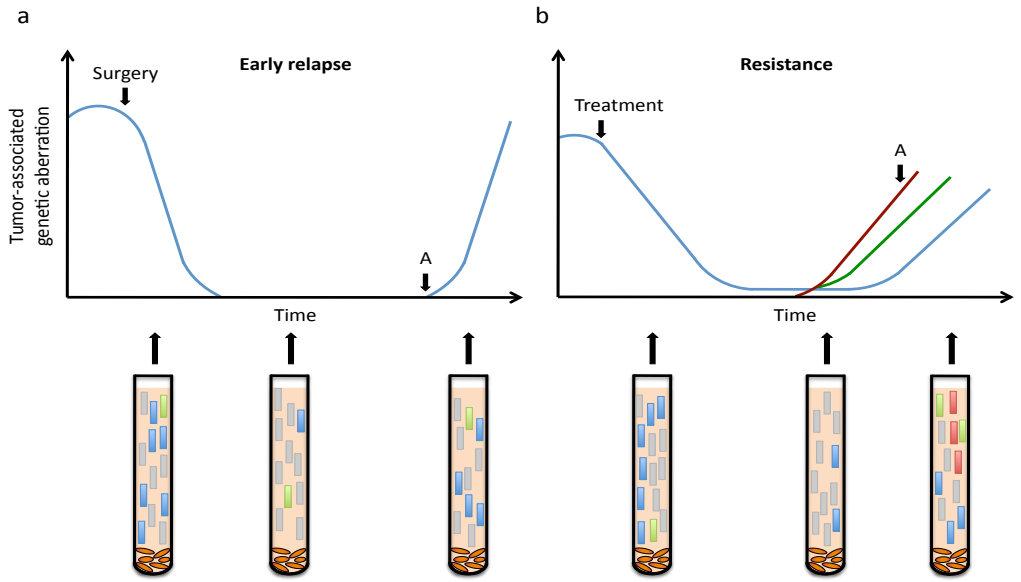
Circulating Tumor Cells (CTCs)

•CTCs can be found in the bloodstream of patients with cancer as single cells or as cell clusters.

• CTCs levels seem to have clinical associations with survival and response to therapy (**CTC enumeration**).

•CTCs could derive from primary tumor and/or distant metastasis.

• CTCs could contain “culpruit cells” which are responsible for seeding (**CTC characterization**).



PROSTATE CANCER

RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance

David T. Miyamoto,[1,2*] Yu Zheng,[1,3*] Ben S. Wittner,[1,4] Richard J. Lee,[1,4] Huili Zhu,[1] Katherine T. Broderick,[1] Rushil Desai,[1] Douglas B. Fox,[1] Brian W. Brannigan,[1] Julie Trautwein,[1] Kshitij S. Arora,[1,5] Niyati Desai,[1,5] Douglas M. Dahl,[1,6] Lecia V. Sequist,[1,4] Matthew R. Smith,[1,4] Ravi Kapur,[7] Chin-Lee Wu,[1,5] Toshi Shioda,[1] Sridhar Ramaswamy,[1,4] David T. Ting,[1,4] Mehmet Toner,[7] Shyamala Maheswaran,[1,8]† Daniel A. Haber[1,3,4]†

Prostate cancer is initially responsive to androgen deprivation, but the effectiveness of androgen receptor (AR) inhibitors in recurrent disease is variable. Biopsy of bone metastases is challenging; hence, sampling circulating tumor cells (CTCs) may reveal drug-resistance mechanisms. We established single-cell RNA-sequencing (RNA-Seq) profiles of 77 intact CTCs isolated from 13 patients (mean six CTCs per patient), by using microfluidic enrichment. Single CTCs from each individual display considerable heterogeneity, including expression of *AR* gene mutations and splicing variants. Retrospective analysis of CTCs from patients progressing under treatment with an AR inhibitor, compared with untreated cases, indicates activation of noncanonical Wnt signaling ($P = 0.0064$). Ectopic expression of Wnt5a in prostate cancer cells attenuates the antiproliferative effect of AR inhibition, whereas its suppression in drug-resistant cells restores partial sensitivity, a correlation also evident in an established mouse model. Thus, single-cell analysis of prostate CTCs reveals heterogeneity in signaling pathways that could contribute to treatment failure.

After the initial response of metastatic prostate cancer to androgen deprivation therapy (ADT), it invariably recurs as castration-resistant disease (*1*). Second-line inhibitors of the androgen receptor (AR) have been shown to increase overall survival in castration-resistant prostate cancer (CRPC), consistent with the reactivation of AR signaling in the tumor, but responses are heterogeneous and often short-lived, and resistance to therapy is a pressing clinical problem (*1*). In other types of cancer, molecular analyses of serial biopsies have enabled the study of acquired drug-resistance mechanisms, intratumor heterogeneity, and tumor evolution in response to therapy (*2*)—an approach that is restricted by the predominance of bone metastases in prostate cancer (*3*, *4*). Thus, isolation of circulating tumor cells (CTCs) may enable noninvasive monitoring, as patients initially respond and subsequently become refractory to therapies targeting the AR pathway (*5*). Here, we established single-cell RNA-sequencing (RNA-Seq) profiles of CTCs, individually isolated after microfluidic enrichment from blood specimens of men with prostate cancer, to address their heterogeneity within and across different patients and their differences from primary tumor specimens. Retrospective analyses of clinical and molecular data were then performed to identify potentially clinically relevant mechanisms of acquired drug resistance.

Building on earlier approaches for capturing and scoring CTCs (*3*), highly efficient microfluidic technologies enable molecular analyses (*6*–*9*). We applied the CTC-iChip to magnetically deplete normal hematopoietic cells from whole-blood specimens (*10*). Untagged and unfixed CTCs were identified by cell surface staining for epithelial and mesenchymal markers [epithelial cell adhesion molecule (EpCAM) and cadherin-11 (CDH11), respectively], and absent staining for the common leukocyte marker CD45. These labeled CTCs were then individually micromanipulated (fig. S1, A and B). A total of 221 single-candidate prostate CTCs were isolated from 18 patients with metastatic prostate cancer and 4 patients with localized prostate cancer (fig. S1C and table S1). Of these, 133 cells (60%) had RNA of sufficient quality for amplification and next-generation RNA sequencing, and 122 (55%) had >100,000 uniquely aligned sequencing reads (*11*) (figs. S1C and S2A). Although many cancer cells in the circulation appear to undergo apoptosis, the presence of intact RNA identifies the subset enriched for viable cells. In addition to candidate CTCs, we also obtained comprehensive transcriptomes for bulk primary prostate cancers from a separate cohort of 12 patients (macrodissected for >70% tumor content) (table S2), 30 single cells derived from four different prostate cancer cell lines, and five patient-derived leukocyte controls (fig. S1C). The leukocytes were readily distinguished by their expression of hematopoietic lineage markers and served to exclude any CTCs with potentially contaminating signals. Strict expression thresholds were used to define lineage-confirmed CTCs, scored by prostate lineage-specific genes (*PSA*, *PSMA*, *AMACR*, and *AR*) and standard epithelial markers (*KRT7*, *KRT8*, *KRT18*, *KRT19*, and *EPCAM*) (*11*) (fig. S2B). Given the presence of leukocyte transcripts suggestive of cellular contamination or misidentification during selection, 28 cells were excluded, and, given low expression of both prostate lineage-specific genes and standard epithelial markers, 17 cells were excluded. The remaining 77 cells (from 13 patients; average of six CTCs per patient) were defined as categorical CTCs (fig. S1C and table S1).

Unsupervised hierarchical clustering analysis of single prostate CTCs, primary tumor samples, and cancer cell lines resulted in their organization into distinct clusters (Fig. 1A). Single CTCs from an individual patient showed considerably greater intercellular heterogeneity in their transcriptional profiles than single cells from prostate cancer cell lines (Fig. 1, B and C) (mean correlation coefficient 0.10 versus 0.44, $P < 1 \times 10^{-20}$), but they strongly clustered according to patient of origin, which indicated higher diversity in CTCs from different patients (Fig. 1C and fig. S2C) (mean correlation coefficient 0.10 for CTCs within patient versus 0.0014 for CTCs between patients, $P = 2.0 \times 10^{-11}$).

We examined gene markers of prostate lineage, epithelial, mesenchymal, and stem cell fates, and cellular proliferation (Fig. 2A). Epithelial markers were abundantly expressed [>10 reads per million (rpm)] by nearly all CTCs analyzed (92%), whereas mesenchymal genes were not up-regulated compared with primary tumors or prostate cancer–derived cell lines. Among robustly expressed transcripts were putative stem cell markers (*12*), including *ALDH7A1*, *CD44*, and *KLF4*, present in 60% of CTCs. In addition, 47% of CTCs expressed markers of cell proliferation. We performed differential gene expression analysis to identify genes that are up-regulated in prostate CTCs compared with primary tumor samples. A total of 711 genes were highly expressed in CTCs compared with primary tumors; the most enriched were (i) the molecular chaperone *HSP90AA1*, which regulates the activation and stability of AR, among other functions (*13*), and (ii) the noncoding RNA transcript *MALAT1*, which has been implicated in alternative mRNA splicing and transcriptional control of gene expression (*14*) (Fig. 2B, fig. S4A, and table S3) [false discovery rate (FDR) < 0.1, and fold change > 2]. We used the Pathway Interaction Database (PID) (*15*) to identify key molecular pathways up-regulated in

[1]Massachusetts General Cancer Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [2]Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [3]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA. [4]Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [5]Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [6]Department of Urology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [7]Center for Bioengineering in Medicine, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA. [8]Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.
*These authors contributed equally to this work. †Corresponding author. E-mail: haber@helix.mgh.harvard.edu (D.H.); smaheswaran@mgh.harvard.edu (S.M.)

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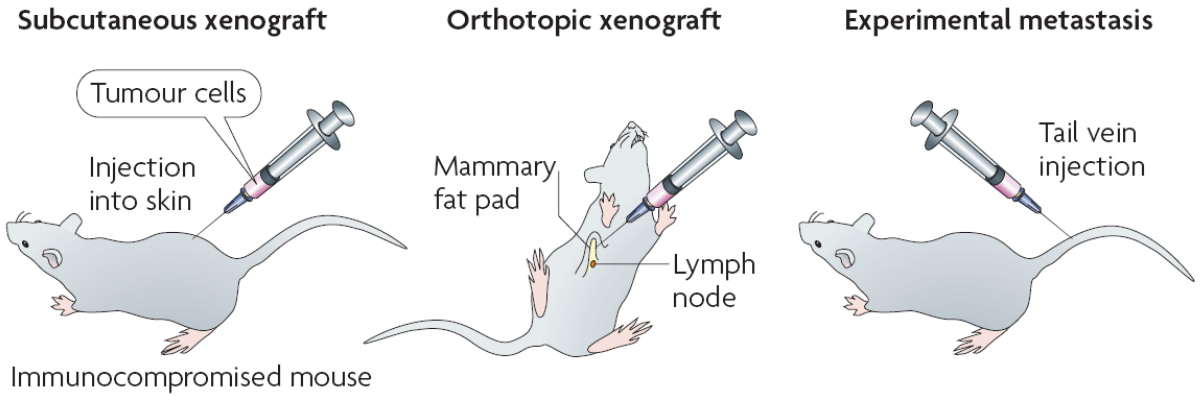
Circulating Tumor Cells (CTCs): in vitro and in vivo models for drug response

•**CTCs from patients have been propagated *in vitro*** by multiple groups:

- short term cultures (28 days or less).

- long-term cultures (6-24 months)

• **Mouse xenografts generated directly from CTCs** (patients specific models for guiding treatment).



Limitations:

- Do not recapitulate the tumor cell heterogeneity.

- Do not recapitulate tumor-host interactions (important role in drug resistance, etc).

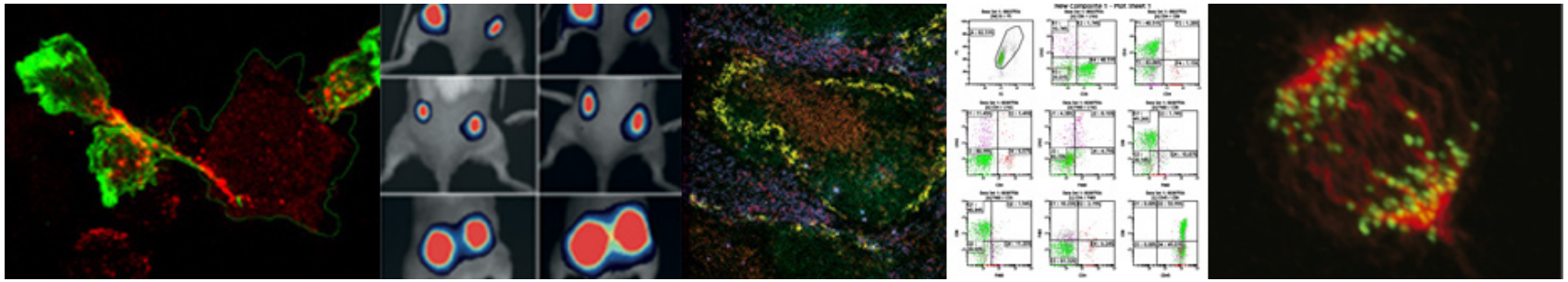
Circulating Tumor Cells (CTCs) and associated cells: propensity for metastatic colonization

-An EMT phenotype is associated with poor prognosis.

-Clusters of CTCs seem to be associated with progression.

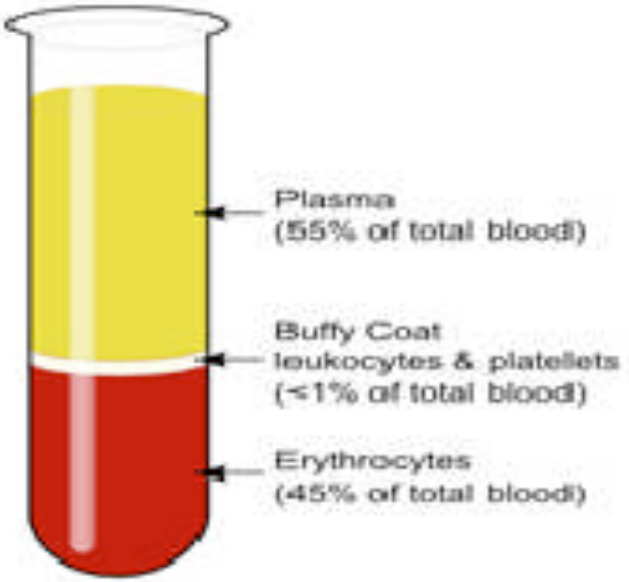
-CTCs close to **circulating Tumor-derived stromal cells** seem to have survival advantage.

-**Cancer-associated macrophage-like cells** facilitate distant CTC colonization and neovascularization.

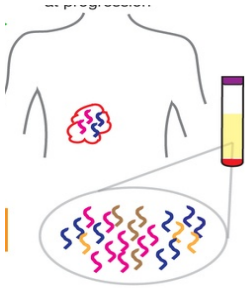


Technologies for ctDNA Collection

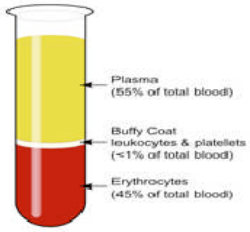
- ctDNA has a very short half life ranging from 15 minutes to several hours
- It is stable in plasma at -80ºc
- Blood can be sampled in ETDA tubes but the plasma has to isolated and stored at -80ºc within one hour of collection
- Preservative tubes can be used to stabilise the cfDNA in blood for up to 4 days at room temperature.



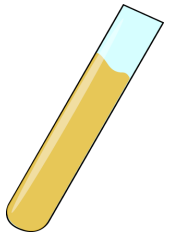
ctDNA Workflow



Blood sample taken in Cell Save preservative tubes



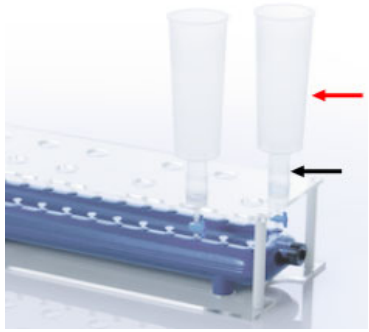
Sample arrives in lab and spun to isolate the plasma



Plasma is stored at -80ºc

Sample is extracted on the same day as the downstream process set up due to ctDNA instability

ctDNA is extracted from the plasma using the QIAamp Circulating Nucleic Acid on the QIAVac system



Set up:
Pyrosequencing
Next-generation sequencing
Quantative PCR
BEAMing
Digital PCR

Technologies for ctDNA Collection: Problems

- Due to the unstable nature of ctDNA the sample is has to be collected and processed correctly
- Only get 30ng of cfDNA per 5ml plasma extraction
- The amount of ctDNA is related to the tumour burden and varies between patients
- Difficult to discriminate ctDNA from normal cfDNA
- The technique used must be sensitive enough to pick up the low level variants

Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

Diaz and Bardelli, 2014 Journal of Clincial Oncology 32

ctDNA: considerations for sensitive detection of ctDNA

•As with CTCs, proposed clinical applications of ctDNA are:

- Surrogate of tumor burden: Profiling and quantitation of noninvasive characterization of tumor molecular features .

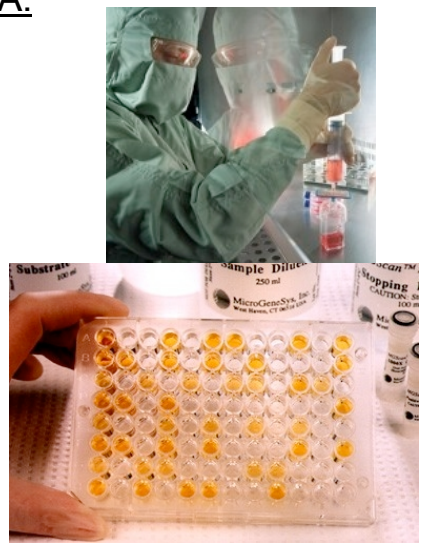
Clinical utility will depend on:

-Analytic sensitivity (reliable detection of ctDNA when it is present).

-Clinical sensitivity (the proportion of patients from whom ctDNA should be detectable).

Detection of ctDNA is further challenged by the high background levels of circulating wild-type DNA.

ctDNA detection platforms are based on digital PCR and NGS



An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

Aaron M Newman[1,2,7], Scott V Bratman[1,3,7], Jacqueline To[3], Jacob F Wynne[3], Neville C W Eclov[3], Leslie A Modlin[3], Chih Long Liu[1,2], Joel W Neal[2], Heather A Wakelee[2], Robert E Merritt[4], Joseph B Shrager[4], Billy W Loo Jr[3], Ash A Alizadeh[1,2,5] & Maximilian Diehn[1,3,6]

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Circulating tumor DNA (ctDNA) is a promising biomarker for noninvasive assessment of cancer burden, but existing ctDNA detection methods have insufficient sensitivity or patient coverage for broad clinical applicability. Here we introduce cancer personalized profiling by deep sequencing (CAPP-Seq), an economical and ultrasensitive method for quantifying ctDNA. We implemented CAPP-Seq for non–small-cell lung cancer (NSCLC) with a design covering multiple classes of somatic alterations that identified mutations in >95% of tumors. We detected ctDNA in 100% of patients with stage II–IV NSCLC and in 50% of patients with stage I, with 96% specificity for mutant allele fractions down to ~0.02%. Levels of ctDNA were highly correlated with tumor volume and distinguished between residual disease and treatment-related imaging changes, and measurement of ctDNA levels allowed for earlier response assessment than radiographic approaches. Finally, we evaluated biopsy-free tumor screening and genotyping with CAPP-Seq. We envision that CAPP-Seq could be routinely applied clinically to detect and monitor diverse malignancies, thus facilitating personalized cancer therapy.

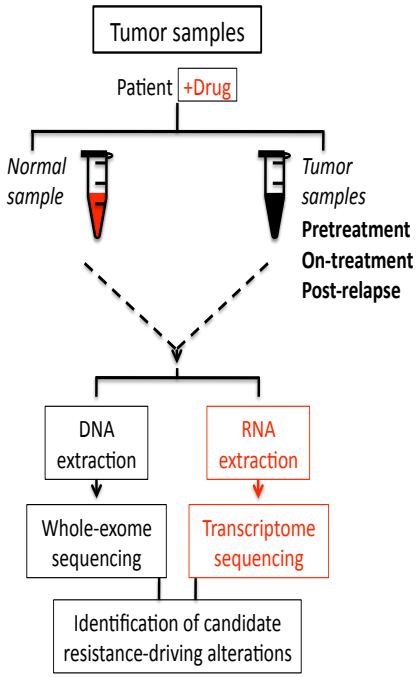
Analysis of ctDNA has the potential to revolutionize detection and monitoring of tumors. Noninvasive access to cancer-derived DNA is particularly attractive for solid tumors, which cannot be repeatedly sampled without invasive procedures. In NSCLC, PCR-based assays have been used to detect recurrent point mutations in genes such as *KRAS* (encoding kirsten rat sarcoma viral oncogene homolog) or *EGFR* (encoding epidermal growth factor receptor) in plasma DNA[1–4], but the majority of patients lack mutations in these genes. Recently, approaches employing massively parallel sequencing have been used to detect ctDNA[5–12]. However, the methods reported to date have been limited by modest sensitivity[13], applicability to only a minority of patients, the need for patient-specific optimization and/or cost. To overcome these limitations, we developed a new strategy for analysis of ctDNA. Our approach, called CAPP-Seq, combines optimized library preparation methods for low DNA input masses with a multiphase bioinformatics approach to design a 'selector' consisting of biotinylated DNA oligonucleotides that target recurrently mutated regions in the cancer of interest. To monitor ctDNA, the selector is applied to tumor DNA to identify a patient's cancer-specific genetic aberrations and then directly to circulating DNA to quantify them (**Fig. 1a**). Here we demonstrate the technical performance and explore the clinical utility of CAPP-Seq in patients with early- and advanced-stage NSCLC.

RESULTS

Design of a CAPP-Seq selector for NSCLC

For the initial implementation of CAPP-Seq, we focused on NSCLC, although our approach is generalizable to any cancer for which recurrent mutations have been identified. To design a selector for NSCLC (**Fig. 1b**, **Supplementary Table 1** and Online Methods), we began by including exons covering recurrent mutations in potential driver genes from the Catalogue of Somatic Mutations in Cancer (COSMIC)[14] and other sources[15,16]. Next, using whole-exome sequencing (WES) data from 407 patients with NSCLC profiled by The Cancer Genome Atlas (TCGA), we applied an iterative algorithm to maximize the number of missense mutations per patient while minimizing selector size (**Supplementary Fig. 1** and **Supplementary Table 1**).

Approximately 8% of NSCLCs harbor rearrangements involving the receptor tyrosine kinase genes *ALK* (encoding anaplastic lymphoma receptor tyrosine kinase), *ROS1* (encoding c-ros oncogene 1 tyrosine kinase) or *RET* proto-oncogene[17–21]. To utilize the low false detection rate inherent in the unique junctional sequences of structural rearrangements[5,6], we included the introns and exons spanning recurrent fusion breakpoints in these genes in the final design phase (**Fig. 1b**). To detect fusions in tumor and plasma DNA, we developed a breakpoint-mapping algorithm optimized for ultradeep coverage data (**Supplementary Methods**). Application of this algorithm to next-generation sequencing (NGS) data from two NSCLC cell lines known to harbor fusions with previously uncharacterized



[1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, California, USA. [2]Division of Oncology, Department of Medicine, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [3]Department of Radiation Oncology, Stanford University, Stanford, California, USA. [4]Division of Thoracic Surgery, Department of Cardiothoracic Surgery, Stanford School of Medicine, Stanford University, Stanford, California, USA. [5]Division of Hematology, Department of Medicine, Stanford Cancer Institute, Stanford University, Stanford, California, USA. [6]Stanford Cancer Institute, Stanford University, Stanford, California, USA. [7]These authors contributed equally to this work. Correspondence should be addressed to M.D. (diehn@stanford.edu) or A.A.A. (arasha@stanford.edu).

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ctDNA: sources of variability

Inefficient ctDNA recovery during sample preparation.

Intrinsic error rates for PCR.

Sequencing which exceed the lower range of ctDNA abundance.

Biases in enrichment of genomic regions for analysis.

Detection methods for 160-180 bp fragments (nucleosomal pattern), but fragments could be lower than 100-60bp.

Tumor heterogeneity is the most challenging source of variability (both spatial and temporal).

For broad applicability, ctDNA detection platforms should not only have high analytic sensitivity but also sufficient genomic coverage to identify a tumor with multiple molecular markers and to anticipate molecular alterations expected with tumor evolution.

ctDNA detection with state-of-the-art techniques remains consistently lower for early-stage-disease than for metastatic disease

nature COMMUNICATIONS

ARTICLE

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Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer

Muhammed Murtaza[1,2,3,4,*], Sarah-Jane Dawson[1,5,6,*], Katherine Pogrebniak[1,2], Oscar M. Rueda[1,2], Elena Provenzano[5,7], John Grant[7], Suet-Feung Chin[1,2], Dana W.Y. Tsui[1], Francesco Marass[1,2], Davina Gale[1], H. Raza Ali[1,2,5,7], Pankti Shah[3], Tania Contente-Cuomo[3], Hossein Farahani[8], Karey Shumansky[8], Zoya Kingsbury[9], Sean Humphray[9], David Bentley[9], Sohrab P. Shah[8], Matthew Wallis[5,10], Nitzan Rosenfeld[1,2,**] & Carlos Caldas[1,2,5,**]

Circulating tumour DNA analysis can be used to track tumour burden and analyse cancer genomes non-invasively but the extent to which it represents metastatic heterogeneity is unknown. Here we follow a patient with metastatic ER-positive and HER2-positive breast cancer receiving two lines of targeted therapy over 3 years. We characterize genomic architecture and infer clonal evolution in eight tumour biopsies and nine plasma samples collected over 1,193 days of clinical follow-up using exome and targeted amplicon sequencing. Mutation levels in the plasma samples reflect the clonal hierarchy inferred from sequencing of tumour biopsies. Serial changes in circulating levels of sub-clonal private mutations correlate with different treatment responses between metastatic sites. This comparison of biopsy and plasma samples in a single patient with metastatic breast cancer shows that circulating tumour DNA can allow real-time sampling of multifocal clonal evolution.

The clinical applications of CTCs and ctDNA as liquid biopsy in cancer patients

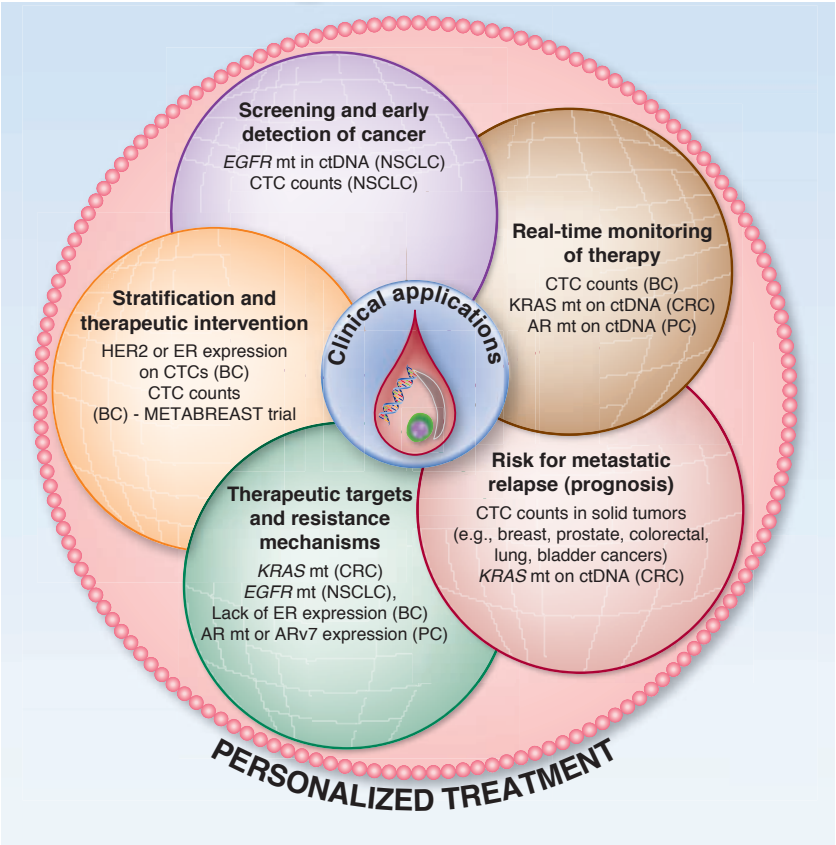
-Screening and Early Detection of Cancer (CTC/ctDNA)

- Estimation of the Risk for Metastatic Relapse (Prognostic Information)

- Identification of Therapeutic Targets and Resistance Mechanisms

- Real-time Monitoring of Therapies

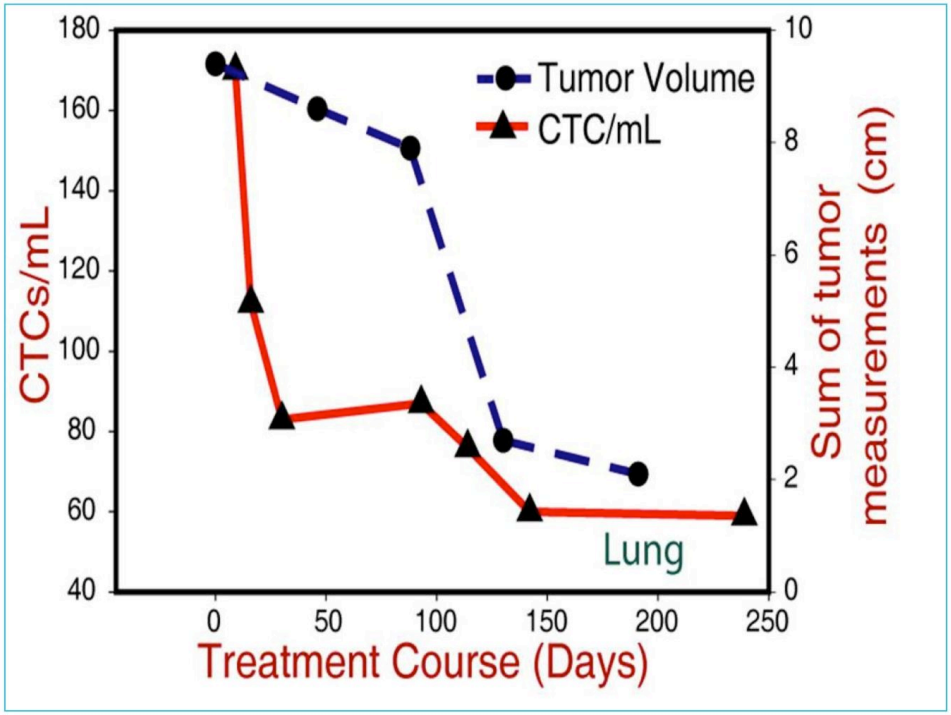
- Stratification and Therapeutic Intervention based on Liquid Biopsy



The clinical applications of CTCs and ctDNA as liquid biopsy in cancer patients

Dynamic Range of CTC Enumeration

CTC Numbers Track with Disease Course

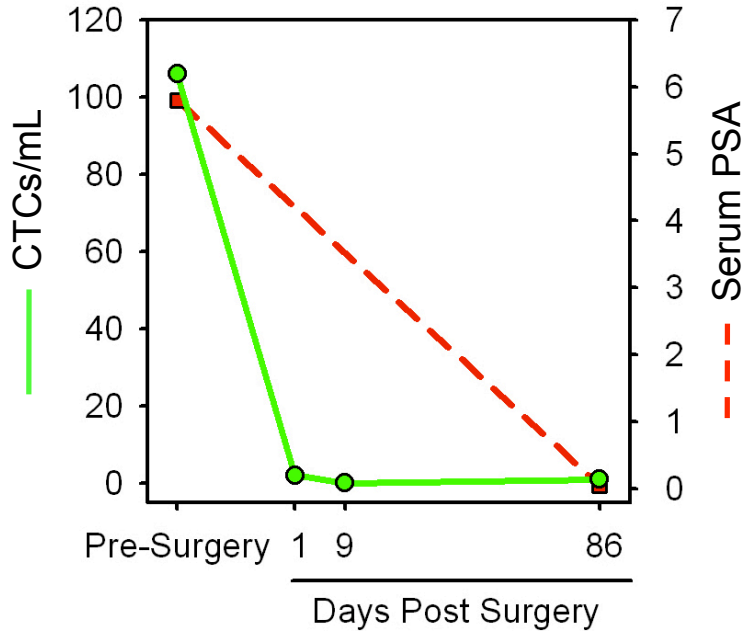


Lung Cancer Patient Responding

Nagrath et al, ***Nature*** 2007

The clinical applications of CTCs and ctDNA as liquid biopsy in cancer patients

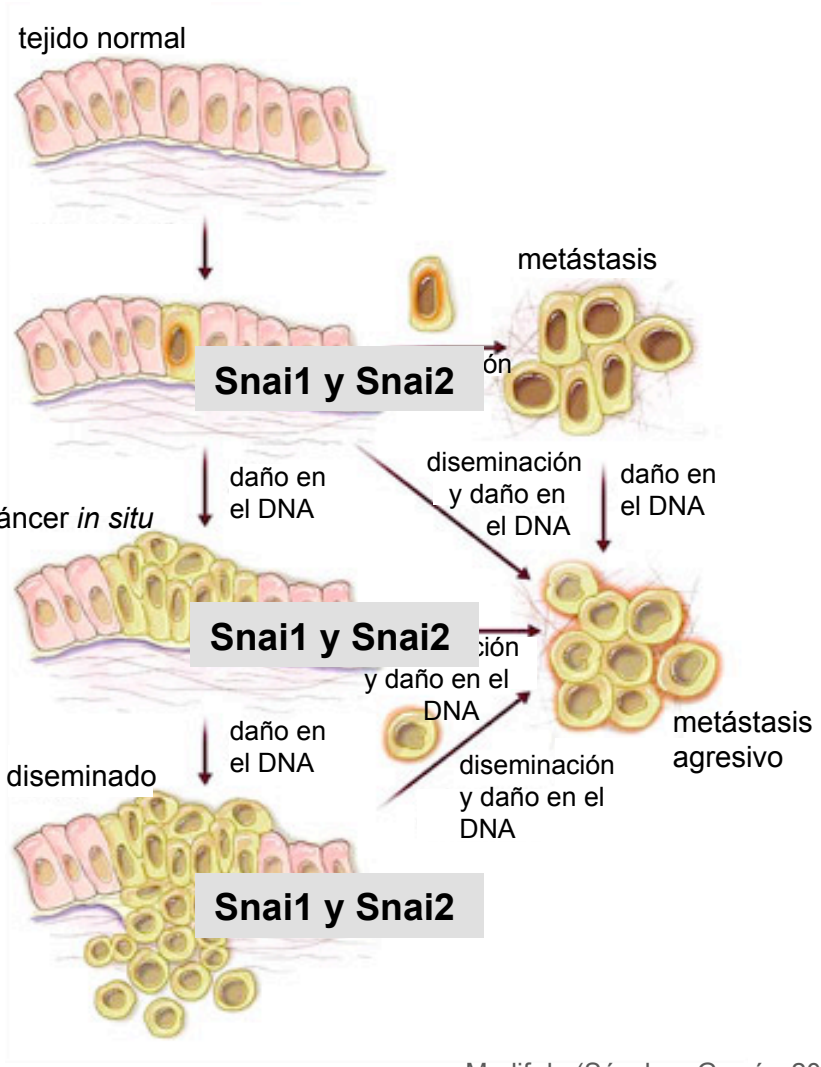
Early Detection in Prostate Cancer



40% (8/20) of patients with **localized** disease have CTCs

Stott et al. ***Science Trans Med*** 2010

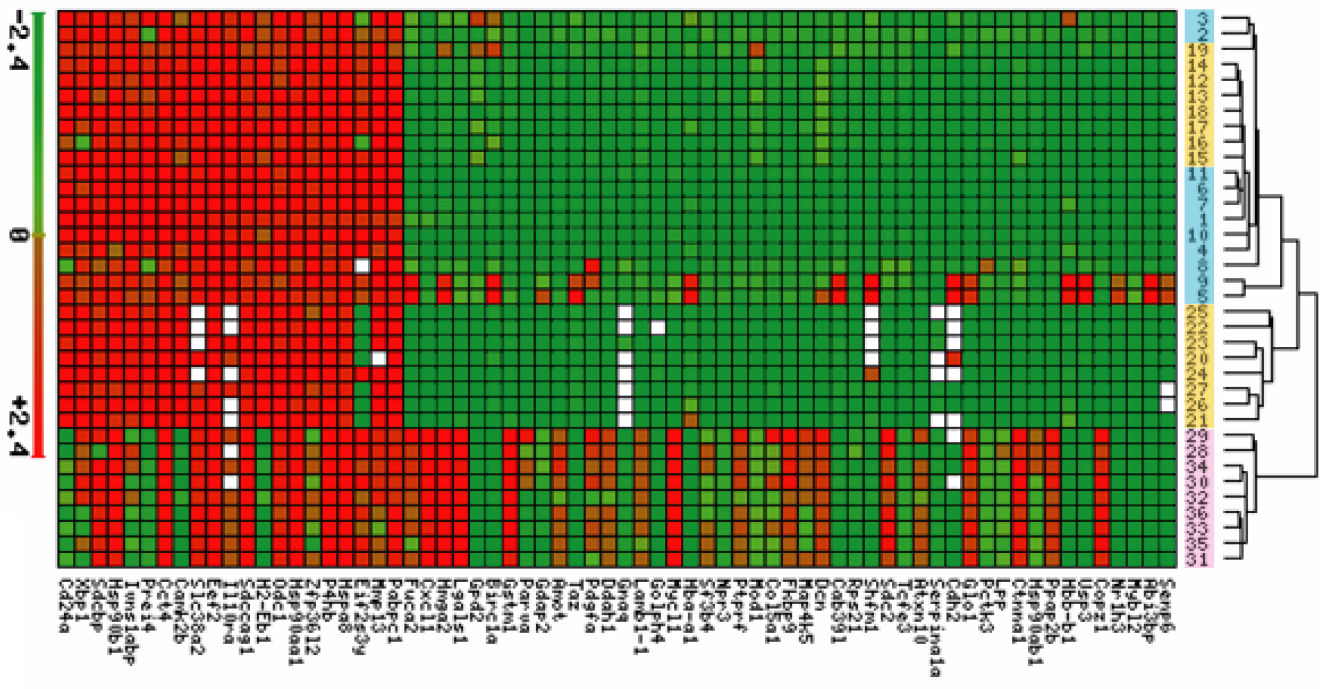
Modelo alternativo del desarrollo del cáncer



Modif de (Sánchez-García, 2009)

N Engl J Med. 2009 Jan 15;360(3):297-9.

CSC maintenance

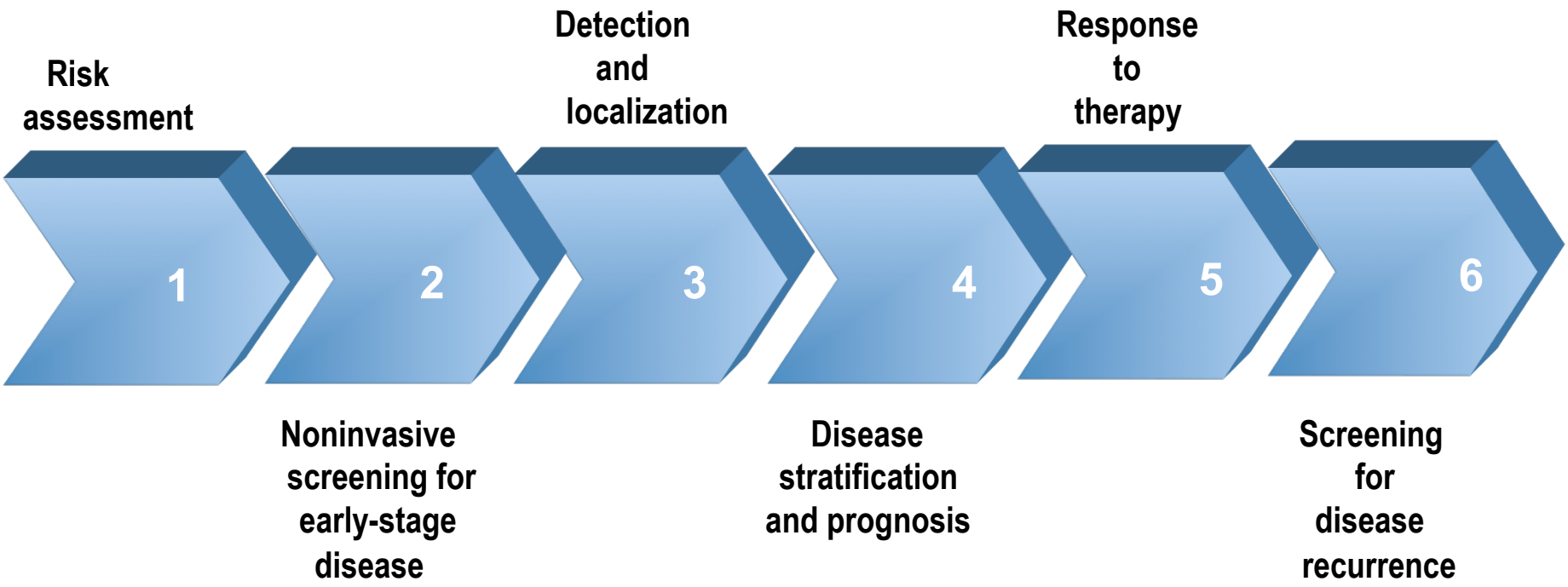


1- Slug
2- Ovo family

3- Bcl-2/Apoptosis
4- Telomerase

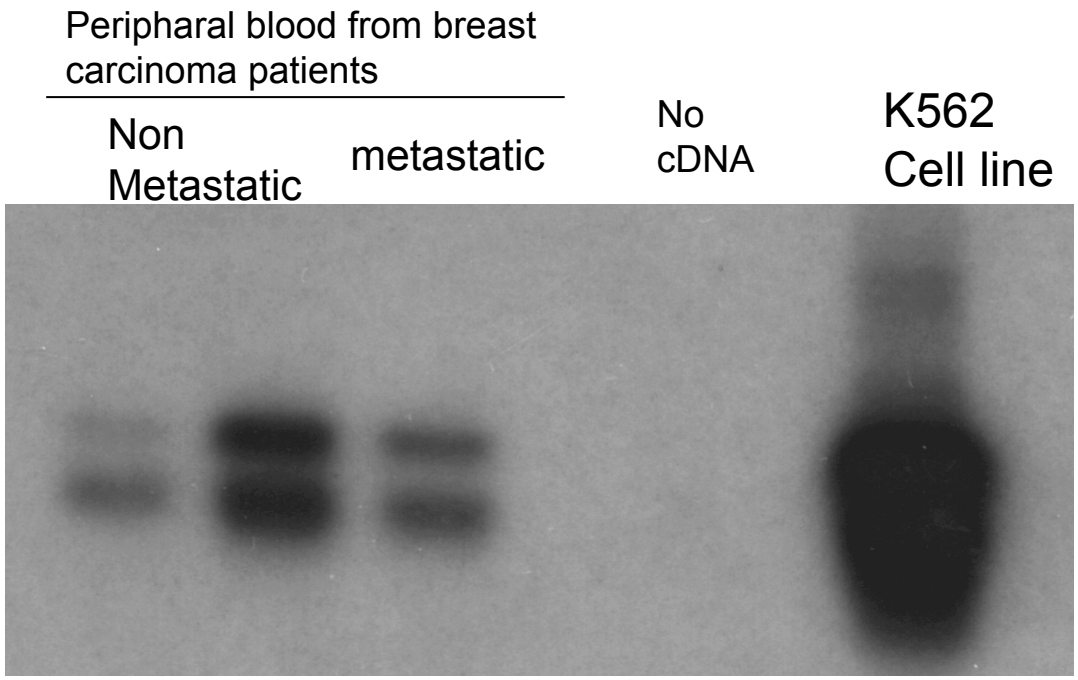
5- Genetic approach to identify CSC maintenance parameters

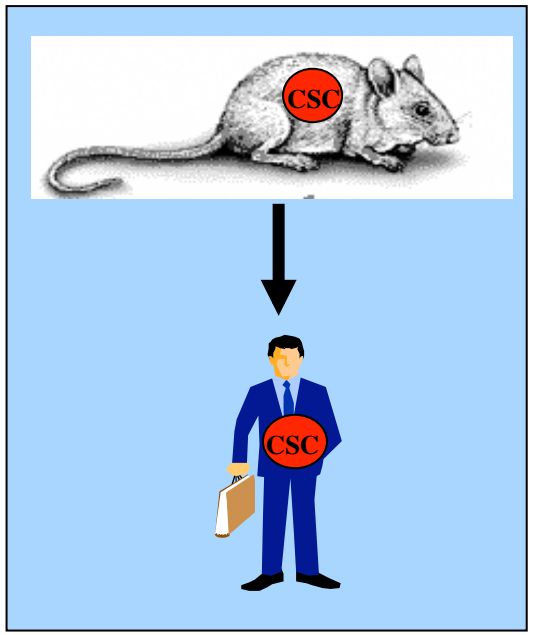
Translating in vivo diagnostics into clinical reality: *cancer biomarkers*



The identification of disease or disease stage-specific biomarkers will facilitate preclinical candidate selection and accelerate clinical development by improving patient selection and providing methods for early assessment of clinical efficacy....

Specific Biomarkers, useful for clinical applications, have been identified from our Stem models





1. Biomarker is positive in breast cancer patients with clinically defined metastatic cancer.
2. Biomarker was detected in a significant percentage of patients clinically defined as non metastatic; these patients were later found to have developed metastases.
3. Further trials on going in patients with lung, ovarian and colon carcinomas.

70 metastatic breast carcinoma patients: survival study

RISK OF PROGRESSION: MULTIVARIANT ANALYSIS

	p-valor	R.R.	I.C. 95,0% TO R.R.	
			Inferior	Superior
SLUG	,016	,310	,119	,805
previous treatments	,005	0,453	,182	,856
N. metastasis*ln(ILG)	,050	,102	,010	,999

THE RISK OF PROGRESSION IS:

3,226 (1/0,310) times higher in SLUG positive-patients
2,2 times higher in patients with previous treatments
10 times higher in patients with 3 or more metastasis.

CTCs and ctDNA: Future considerations for these complementary approaches

-A major reason for treatment failures is our inability to monitor tumor evolution and adapt treatment accordingly.

-Identifying tumor recurrence at an earlier time point does not improve clinical outcome if an effective therapy is not selected or available.

-Liquid biopsy technologies are potentially important advances in this regard.

-Plasma ctDNA assays may prove more usefyl for monitoring disease burden and limited molecular profiling.

-Once increased disease burden is recognized, then CTC analysis for comprehensive characterization of tumor DNA/RNA/protein levels, including their co-localization, may help to optimize therapy selection.

- Ex vivo advantages of CTCs (drug sensitivity evaluation, etc).

CTCs and ctDNA: significant challenges remain

- Before using these tools into routine clinical practice will neccessitate rigorous demonstration of **analytic validity** and, most importantly, **clinical utility**.

- In population screening, there are 11-19% of patients with benign inflammatory conditions (Crohn disease) with small numbers of **benign circulating epithelial cells detectable** (source of false-positive CTC result).

-Another risk is the **detection of clinically irrelevant molecular changes** due to the high sensitivity of the methods (large annotated databases will be needed to distinguish potentially important genomic aberrations from noise).

-Moreover, **only clinical studies will provide evidence** about whether a **genomic aberration detected in blood** can **predict benefit** from a specific targeted agent.

-Although most efforts are currently focused on testing liquid biopsy in the metastatic setting, **we expect that future studies will evaluate its role in the early disease setting or even as a potential tool to assist early cancer diagnosis.**

IBMCC (CSIC/USAL)
Salamanca
Inés González Herrero
Carolina Vicente Dueñas
Isabel Romero Camarero
Lucía Ruíz-Roca
Idoia García Ramírez
Alberto Martín Lorenzo
Guillermo Rodríguez Hernández
Lucía Villamañán de Santiago
Isidro Sánchez-García

Universidad Salamanca
Hospital Clínico Univ.
Teresa Flores
Oscar Blanco
MD Ludeña
Juan Jesús Cruz
Gonzalo Varela
Concha Roman
Fco Javier Garcia Criado
Rafael Jiménez
Alberto Orfao

Lymphoma

U. Miami-USA
Izidore Lossos

Stanford-USA
Ash Alizadeh

Cornell Institute (NY)
Ari Melnick

IMP Vienna
Meinrad Busslinger

CIMA- Pamplona
Jose A. Martinez-Climent

CBMSO Madrid
César Cobaleda

Transgenic Unit
CNB-CBMSO
Belén Pintado
Verónica Domínguez

Leukemia/BCR-ABL

IMB- Salamanca
Dionisio Martín-Zanca

CBMSO Madrid
César Cobaleda

Imperial-London
Cristina Lo Celso

Sanger Institute-Cambridge
Natalie Conte
Allan Bradley

IBMCC-Salamanca
Alberto Orfao

Lung & Breast

CNIO Madrid
Mariano Barbacid

USC-Santiago Compostela
Angel Carracedo

IBMCC-Salamanca
Jesus Perez-Losada

Multyple myeloma

IBMCC-Salamanca
JF San Miguel/Norma

Pediatric leukemia/lymphoma

Med.Uni-Duesseldorf
Arndt Borkhardt

Junta de Castilla y León

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José Carreras Leukämie-Stiftung
"Unser Ziel ist klar: Leukämie muss heilbar werden. Immer und bei jedem." José Carreras

Arimmora FP7-ENV-2011

EuroSyStem

Seventh Framework Programme

DECIDE

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