THE “GREEK” TEST

Contribution of Liquid biopsy in cancer diagnosis, prognosis and treatment: techniques and methods

AUTHOR-PRESENTER: DR. IOANNIS PAPASOTIRIOU

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INTRODUCTION

(WHO WE ARE-WHAT WE OFFER)

THE “GREEK” TEST

- **Clinical Services**

1. Chemosensitivity testing
2. Detection , quantification and immunophenotyping CTCs (MRD test)

- **Research activities**

1. Evaluation of substances candidates for drugs “whanabe”.
2. Detection of new targets for new therapeutic approaches
3. Basic research in molecular Oncology

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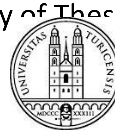
Personal Scientific Background

(IOANNIS PAPASOTIRIOU)

- DOB: 1973 in Munich Germany
- Primary degree: Medical diploma- Aristoteles University of Thessaloniki Greece



Universität
Zürich UZH

- First Specialty: Human Genetik-UZH

- Secondary Specialty: Haematologie Onkologie-MLU/UKH Halle/Saale

- MSc: Molecular Biology and Genetics in Medicine-Westminster University-UK

- MSc: Molecular Oncology-University of Nottingham

- PhD/MD: Validation of Valdetatin in thyroid human cancer cell lines (***ASTRA ZENECA***)

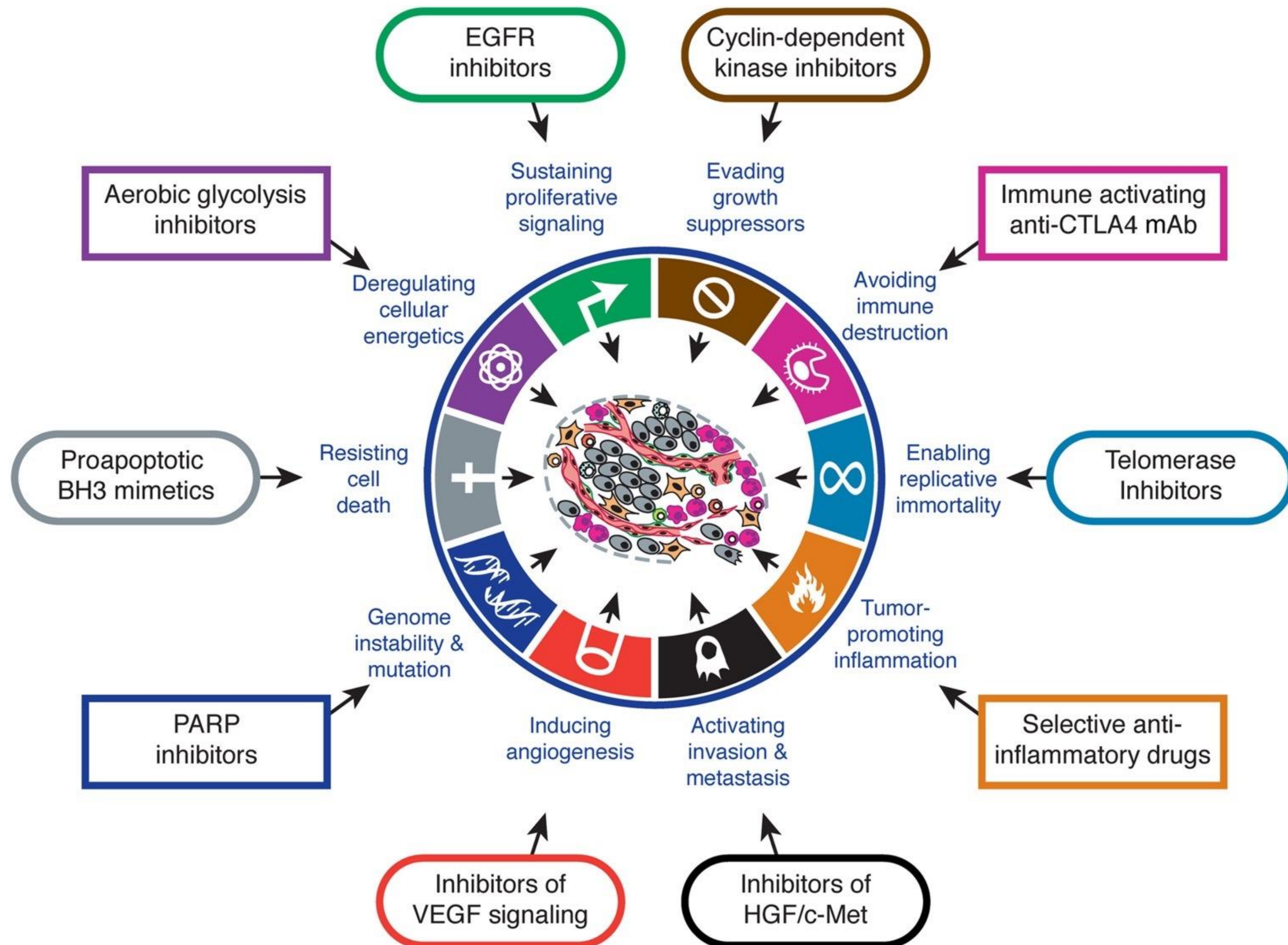
- International certified Cytometris (ISAC, ICCE)-2016

- Certified Qualified Person/Responsible person (QP) according to European pharmacopoeia for inspecting and enforce EU GMP, GLP, GCP (Registered in Germany and in Greece)

Structure of this presentation

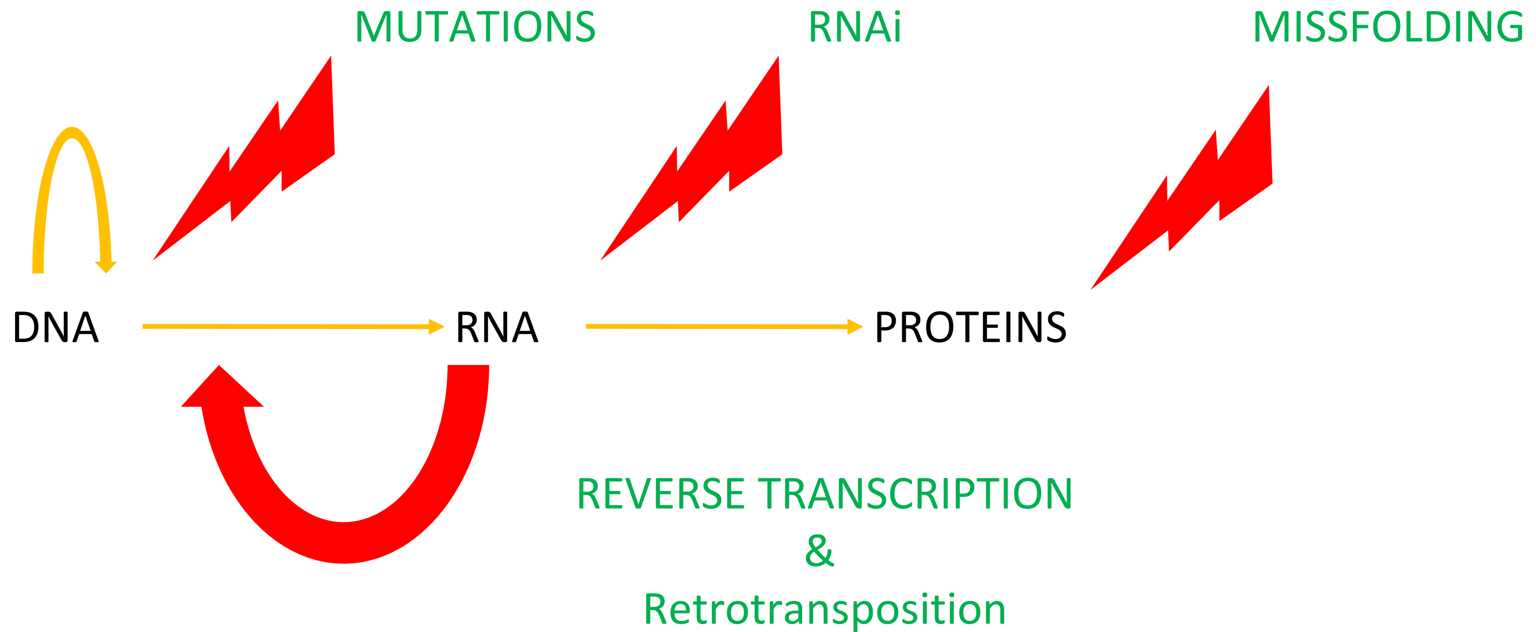
- Cancer physiology
- Definitions
- Diagnosis
- Treatment decision
- Statistics
- “Gaps”
- CTCs and CSCs
- Cf (tumor)DNA
- Definitions
- Technical issues
- Utility
- Clinical application
- Future perspective

Cancer Hallmarks



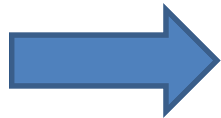
Weinberg et al (20014)

DOGMA OF BIOLOGY



CARCINOGENESIS STEPS

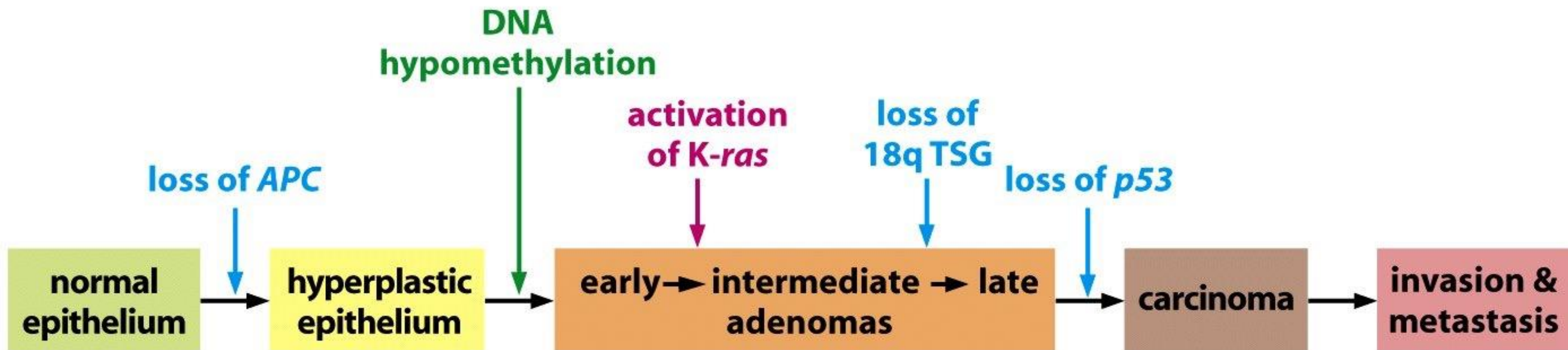
INITIATION
- •Viral interference
- •Chemical interference
- •Radiation influence



PROMOTION
- •Cell cycle instability
- •DNA repair aberration
- •Apoptosis instabiliity

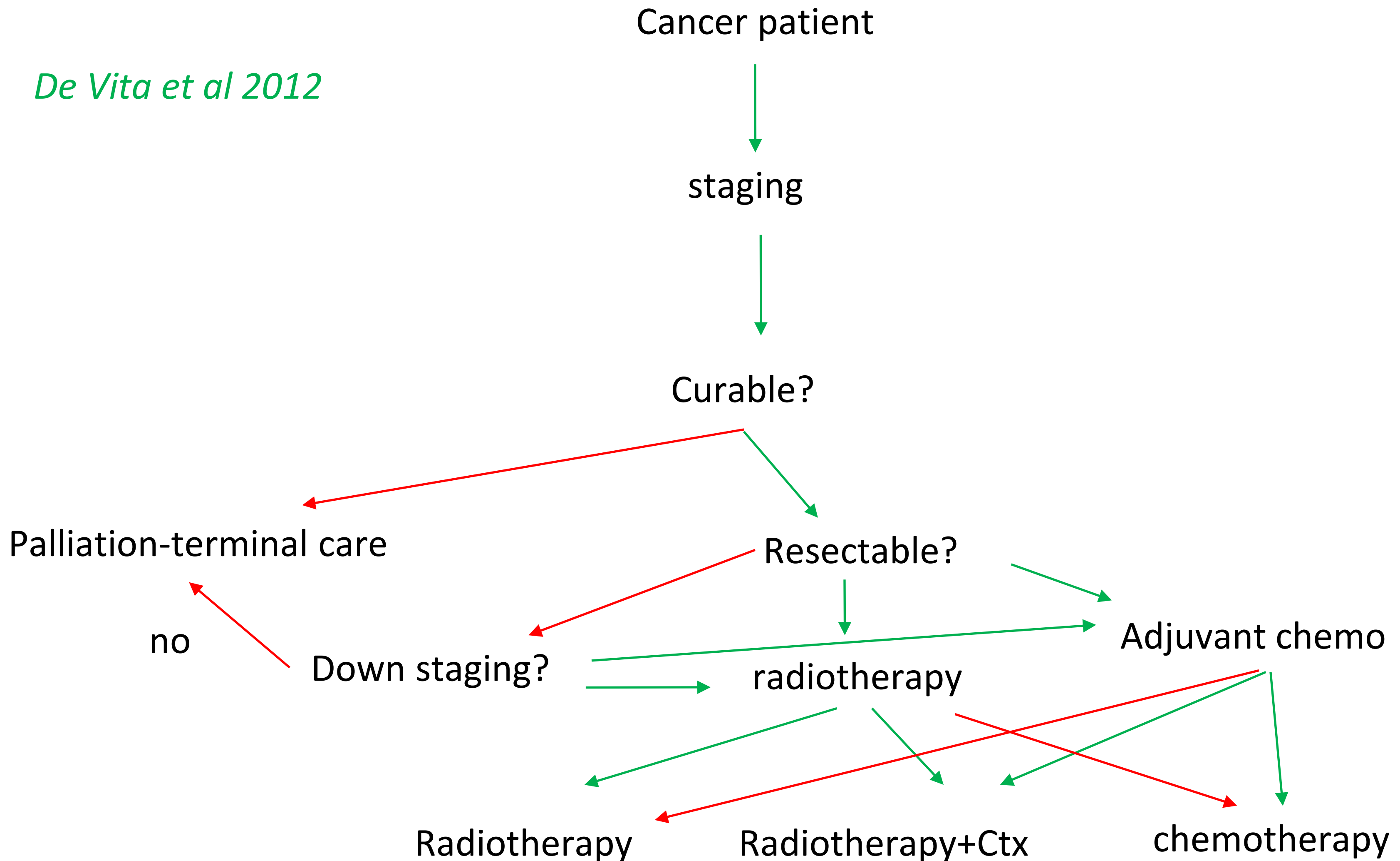
PROGRESS
- •Invasion
- •Neo-angiogenesis
- •EMT and MET

VOGELSTEIN MODEL OF DEVELOPING COLON CANCER

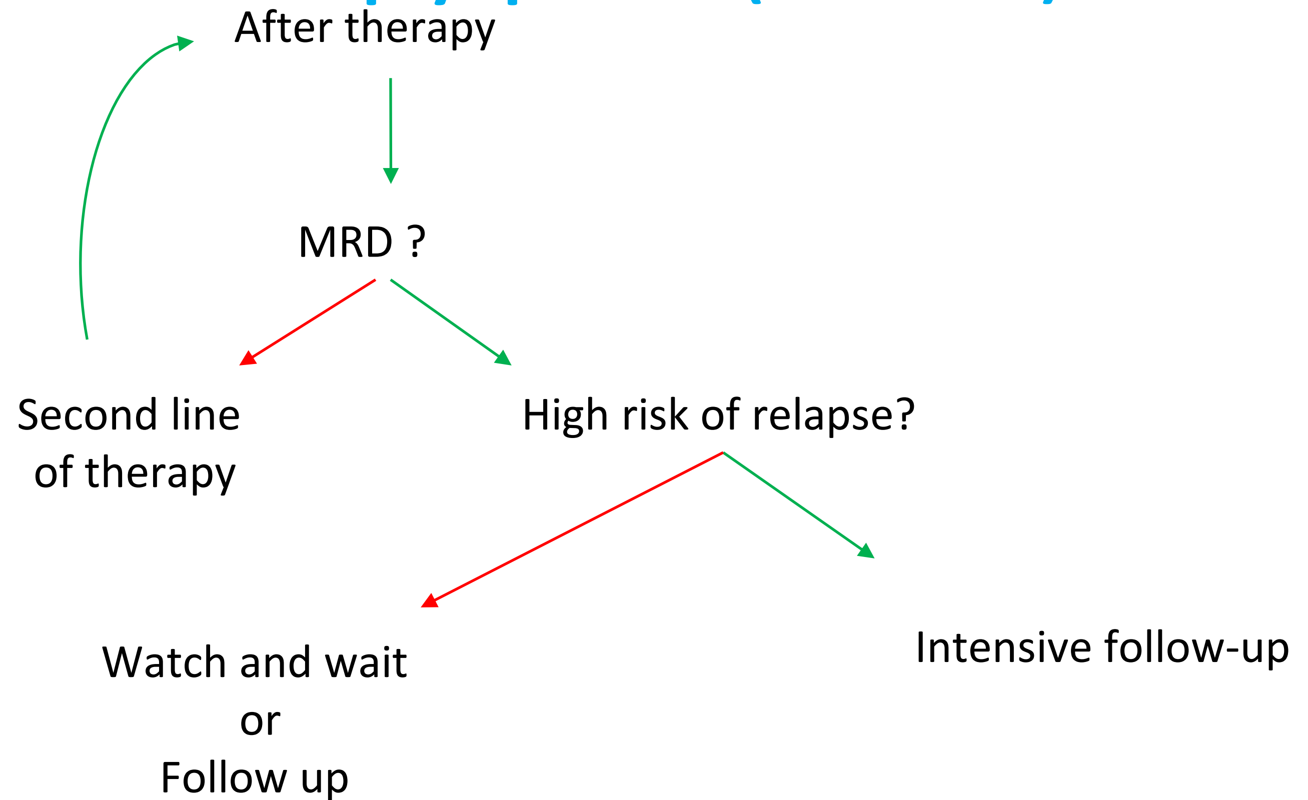


Present therapeutic concept

Cancer therapy plan (so far)



Cancer therapy plan (so far)



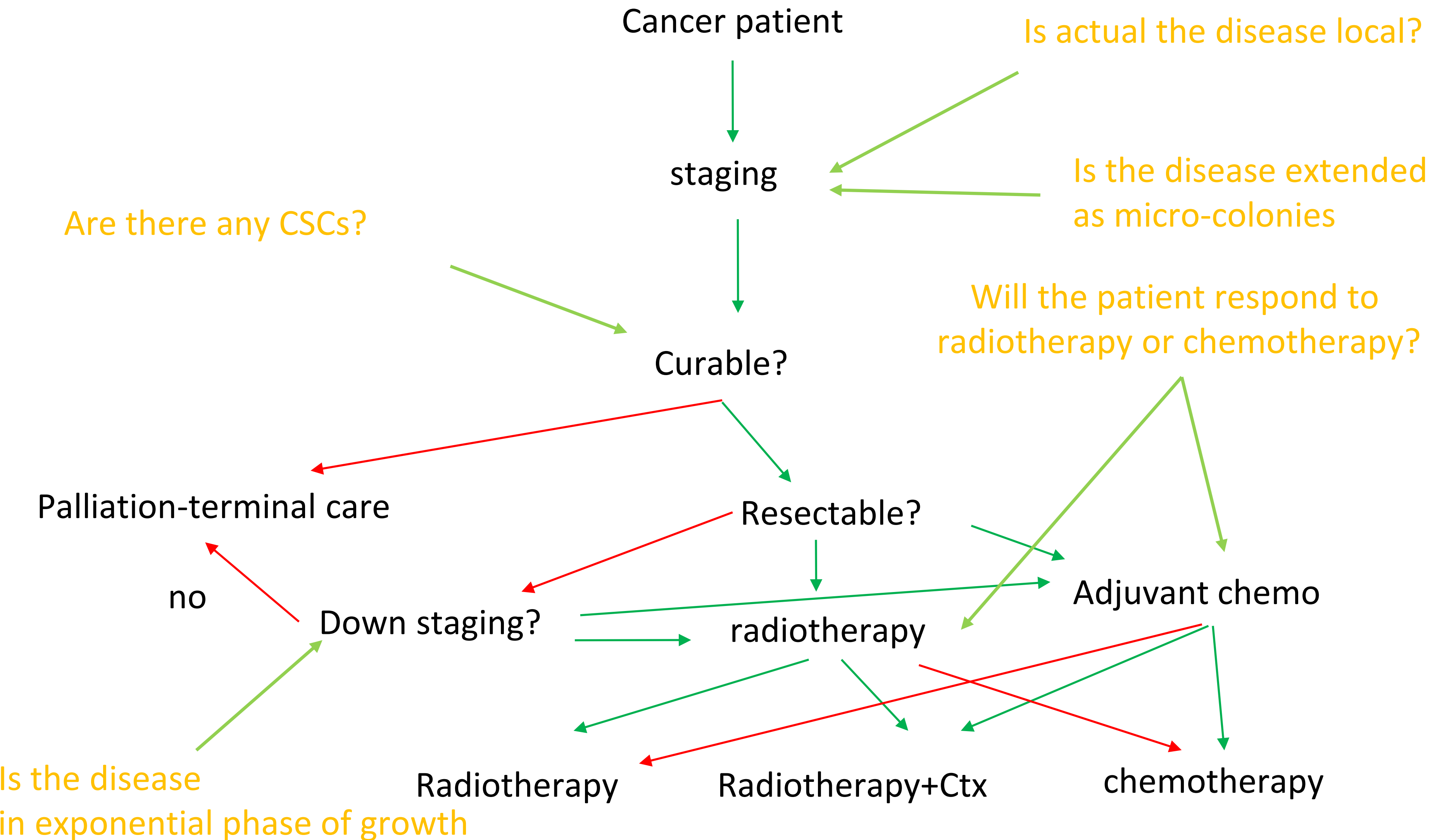
Rate of success

- For Adjuvant chemotherapy the success rate for the 5 major types of malignancy varies from 2.1% to 2.3% in 5 years.

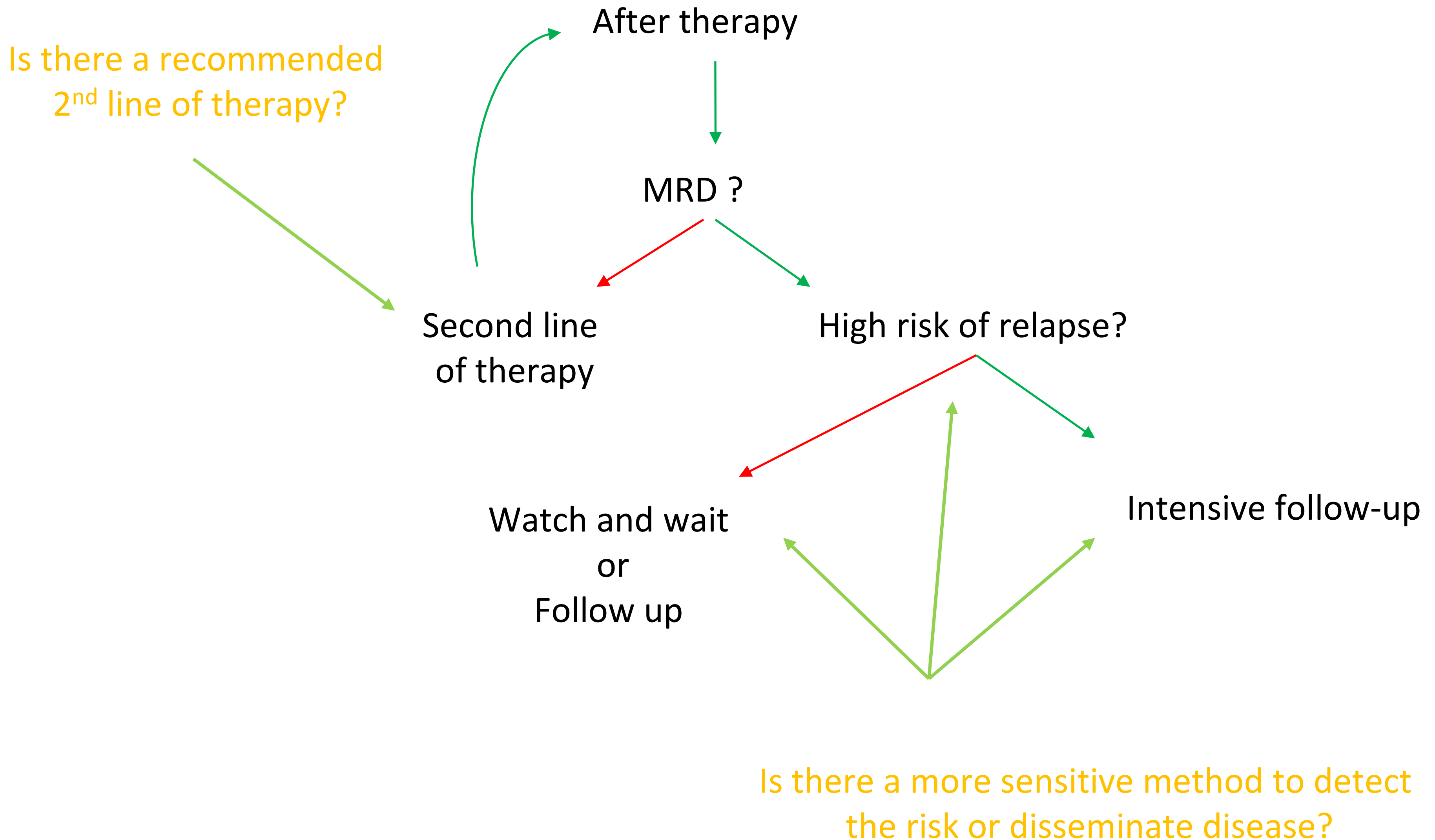
Royal North Shore Hospital Clin Oncol (R Coll Radiol) 2005 Jun;17(4):294

- For curative stage of disease the success rate varies between 5 to 7.5% for the same 5 types of malignancies.

Dead-End in empirical treatment



Dead-End in empirical treatment



Reasons and causes

1. Lack of sensitive methods to detect the MRD
2. Lack to discriminate the actual important cell from the irrelevant
3. Lack to detect and control the genetic instability of malignant cells.
4. Lack to distinguish which cells may shift to the driving entity and which may not.

WHAT PERSONALIZED MEDICINE STANDS FOR TODAY

- The stratification of patients to different therapeutic protocols based on biomarkers.

EXAMPLE

K-ras Wild/mutated type selection of patients to be treated wit elrotinib (Terceva) or gefitinib (Iressa) when EGFr+ve

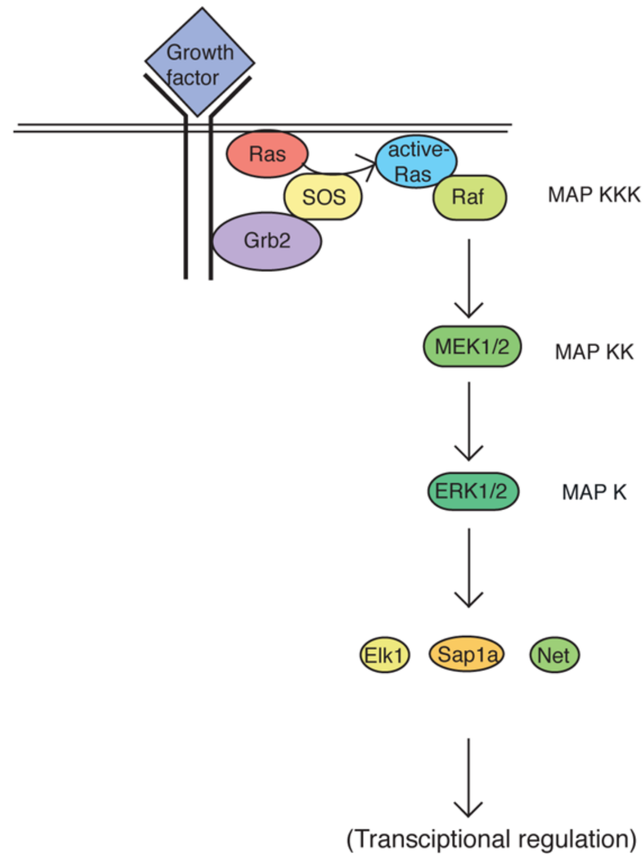
How many biomarkers are used in clinical practice ?

- Haematology:
  1. Brc-abl
  2. Flit-3
  3. CD33
  4. CD52, CD20

- Solid Tumors:
  1. ALK
  2. K-ras, N-ras
  3. BRCA1, BRCA2
  4. EGF-r
  5. VEGF

How reliable the biomarkers can be?

- Example

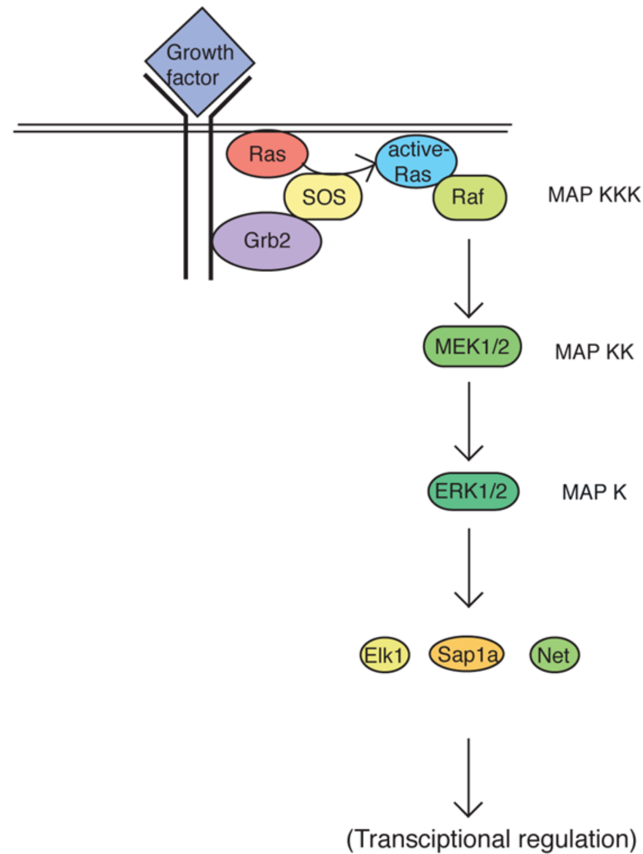


Summary of Map kinase pathway

How reliable the biomarkers can be?

- Assumption

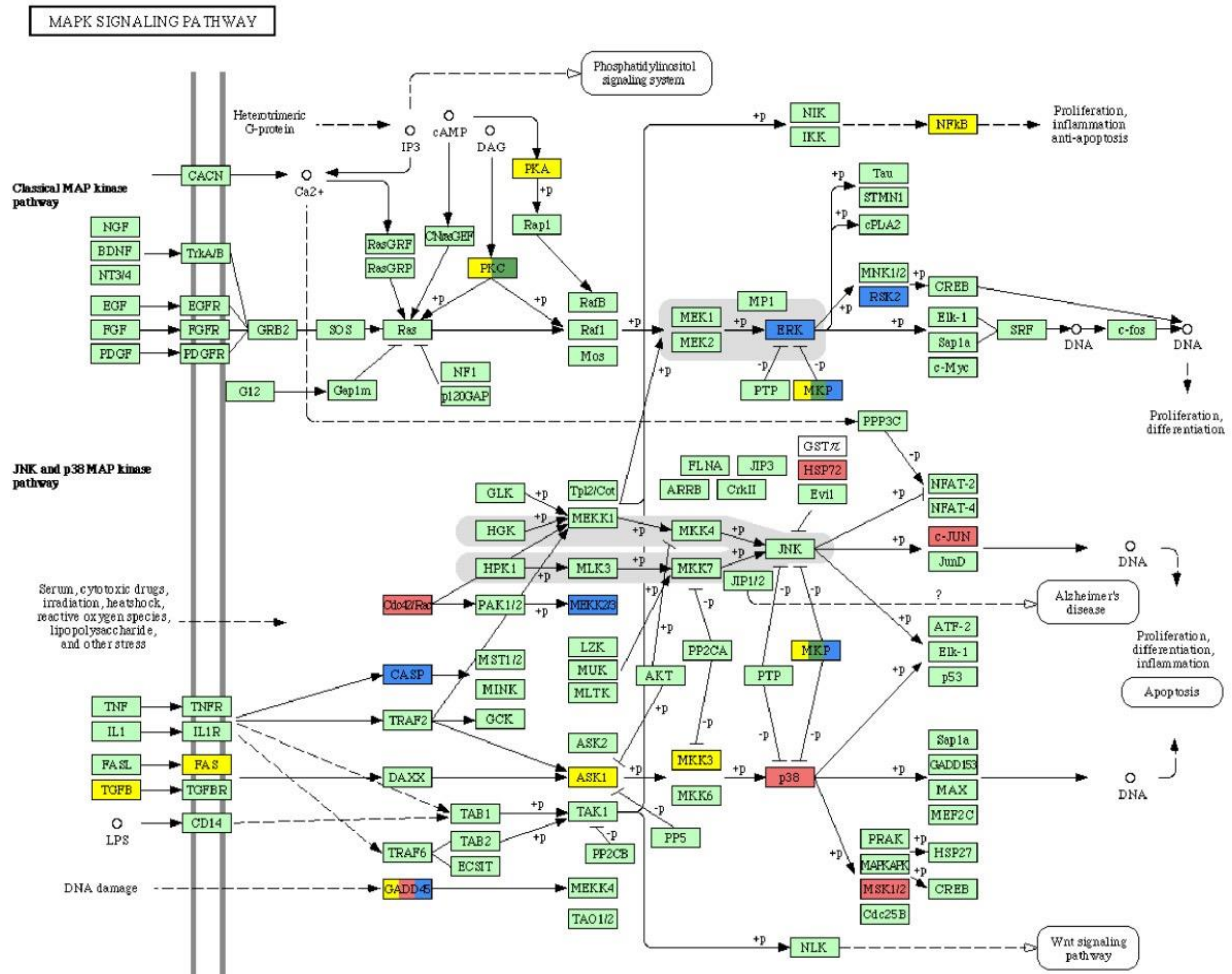
(The cascade is linear)



Summary of Map kinase pathway

How reliable the biomarkers can be?

- In reality (cross talking)



How we detect our biomarkers?

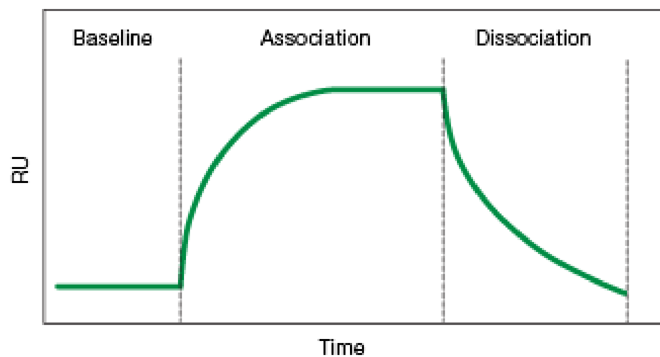
1. Mainly with Genetic techniques (NGS, PCR etc)

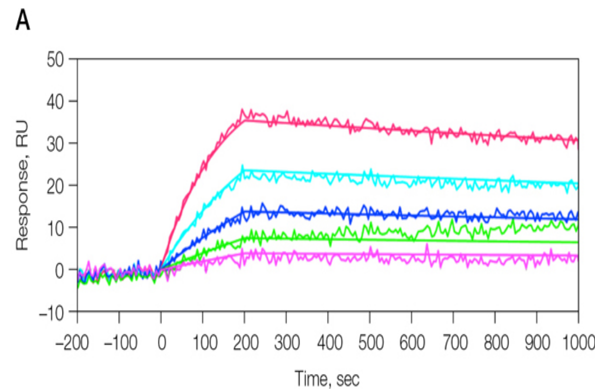
ISSUES

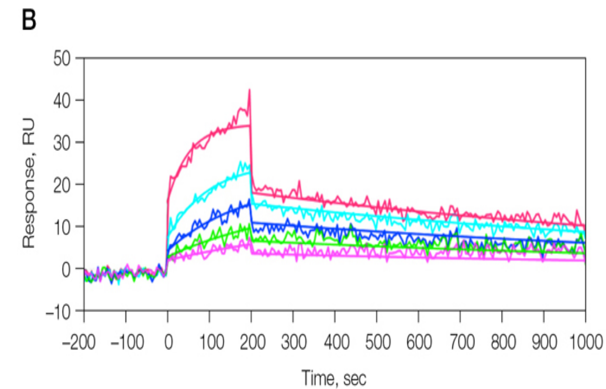
- We do not know whether the referred sequence is expressed
- We do not know the influence of the genetic background to the cellular phenotype.

What we need to consider for applied true personalized approach

- Pharmacology
  - What the drug do to the disease (PD)
  - What the body do the drug (PK)







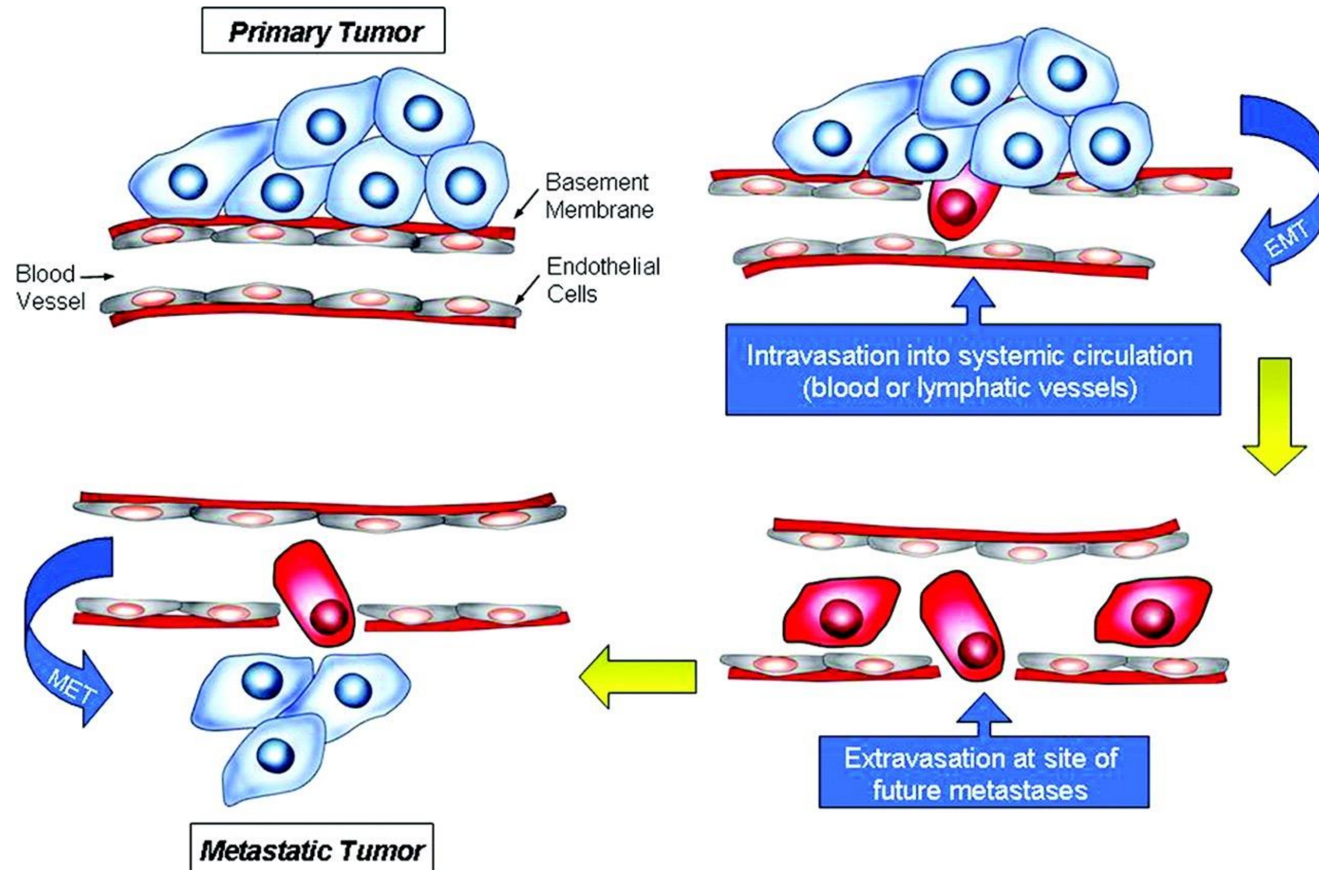
What we need to consider for applied true personalized approach

- Precise information with downstream reflect or outcome
- Multimodal data not only in a genomic level but also in :
  1. Epigenetic (gene expression)
  2. Proteomics
  3. Glucoproteomics

What we need to consider for applied true personalized approach

- Translational medicine (from bed to bed)
- Multi-level of scientist and clinician with both fields background (scientist need to be trained in clinical issues and clinicians in scientific assays and methodologies)
- Pharmacology methodologies and knowledge need to be very close to clinicians

Heterogeneity of CTCs (EMT-MET)



Analyzing the proper sample

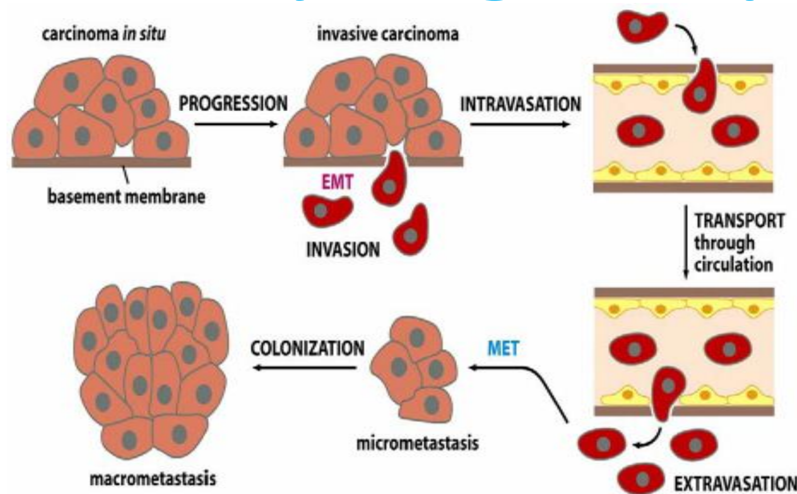
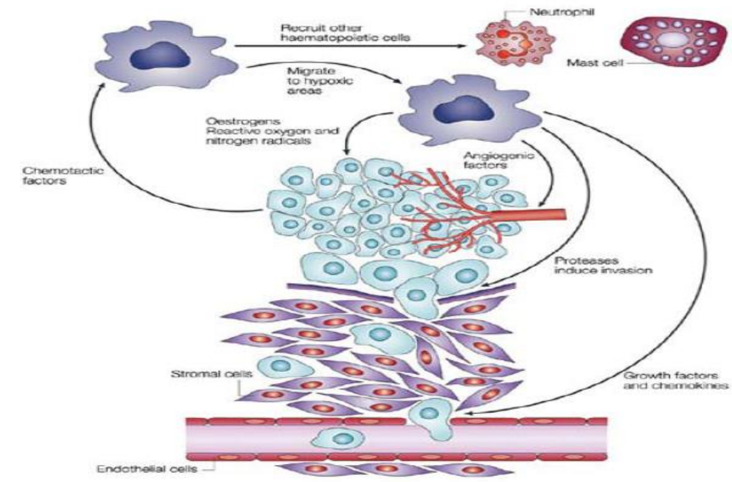


Figure 14-17b The Biology of Cancer (© Garland Science 2007)

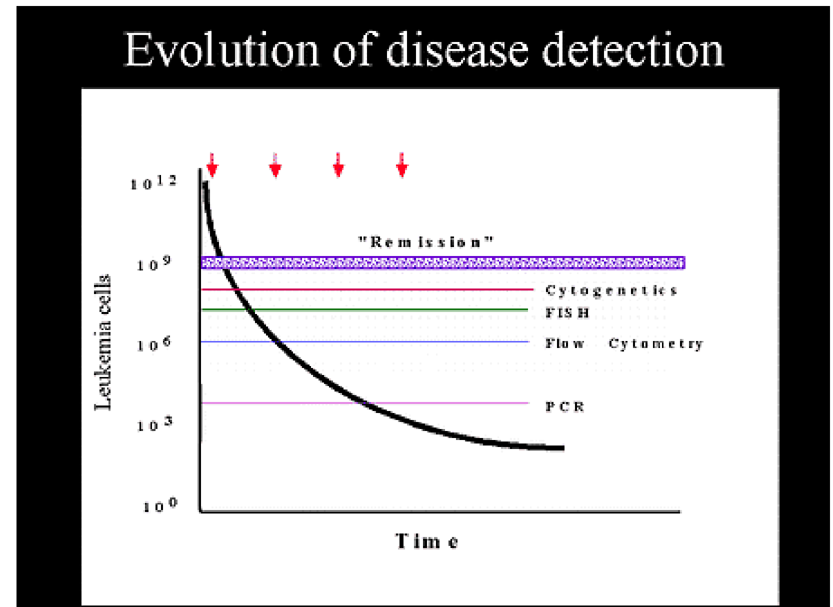


- Facts that are well established and proved:
  1. A tumor consist from the cancer cells and the stroma
  2. The stroma cells composed from fibroblast, lymphocytes, endothelial cells etc
  3. The cancer population is heterogeneous and composed from subpopulations with different features and aggressive behavior.
  4. One of the subpopulation is the progenitor of a tumor and the generator of metastases. This population is known as Cancer Stem Cell like cells
  5. This subpopulation has the ability to invade the surrounding organs, enter the circulation (blood vessel or lymphatics) and engraft to distant organs in order to generate metastases and relapses.

Do we use a right non invasive diagnostic for prevention?

- Methods
  - X ray
  - MRI
  - PET/CT or PET/MRI
  - CT
  - U/S (Echo)

- Limitations



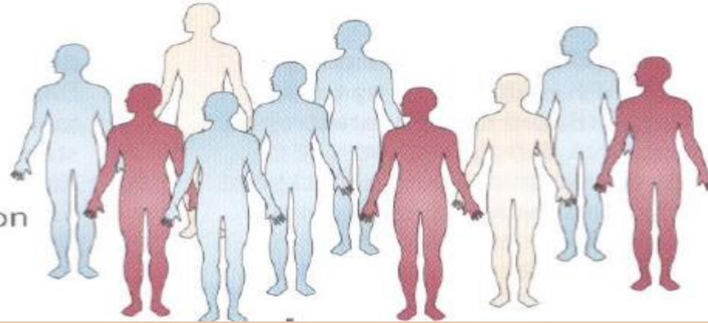
Minimal invasive method: BIOPSY

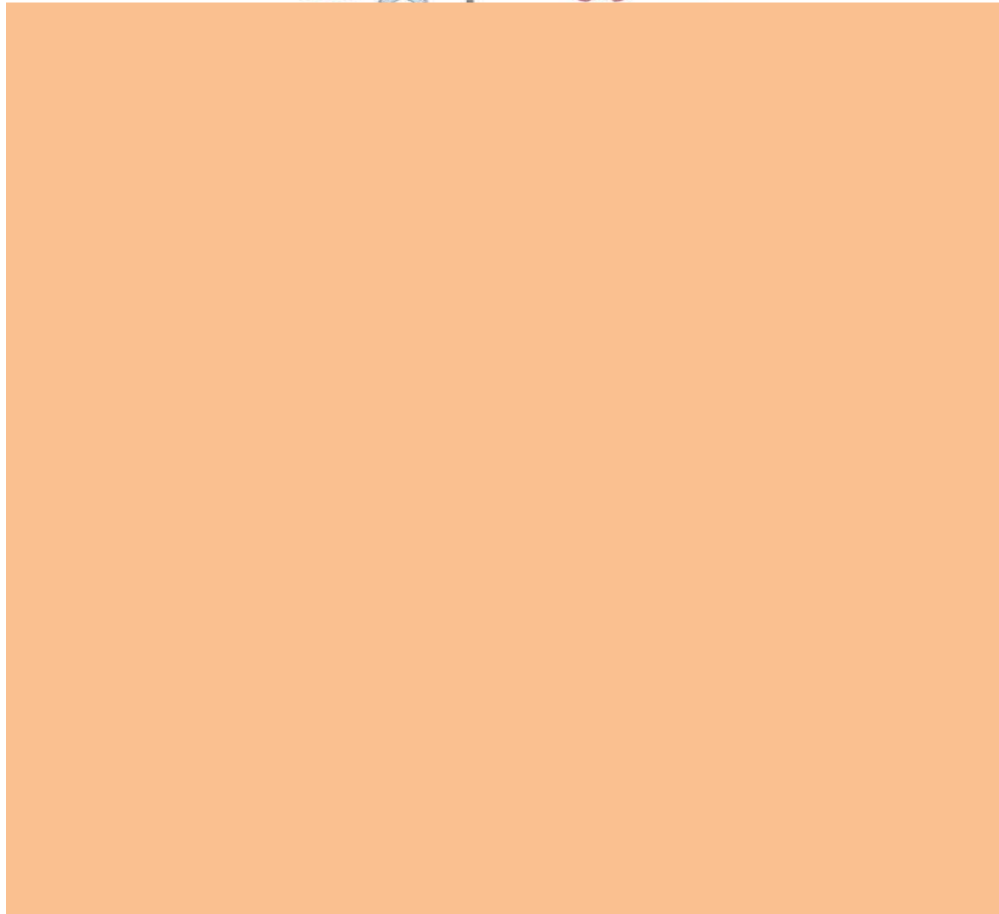
Are we focused to a wrong type of cancer cells?

- The tumor consist from in-homogenous population of cancer cells
- Few sub-clones are able to metastasize and generate metastases
- The CTCs are cancer cells that have perform in majority the EMT
- CTCs are still in-homogenous but with bigger proportion of cancer cells with metastatic features
- CSCs are a subset of CTCs that may generate relapses

The reason of heterogeneity and plasticity of the disease lead us to the personalized approach as therapeutic concept

Nature
Cancer review





Empirical vs Personalized treatment Pros & Cons

How personalized treatment rise the last years.

1. Need of pharma industry of select the patients where their product will be successful.
2. Need to medical practitioners to identify candidates that will develop severe side effects from a medication.

How Personalize treatment is feasible:

1. Identify each case metabolic abilities (normal, accumulator, rapid metabolizer)-Pharmacokinetic
2. Identify for each case the cellular genetic and protein profile of their abnormal cells that causes the disease.

CTCs & CSCs

Katoih et al 2015

Tumor Physiology (CTCs)

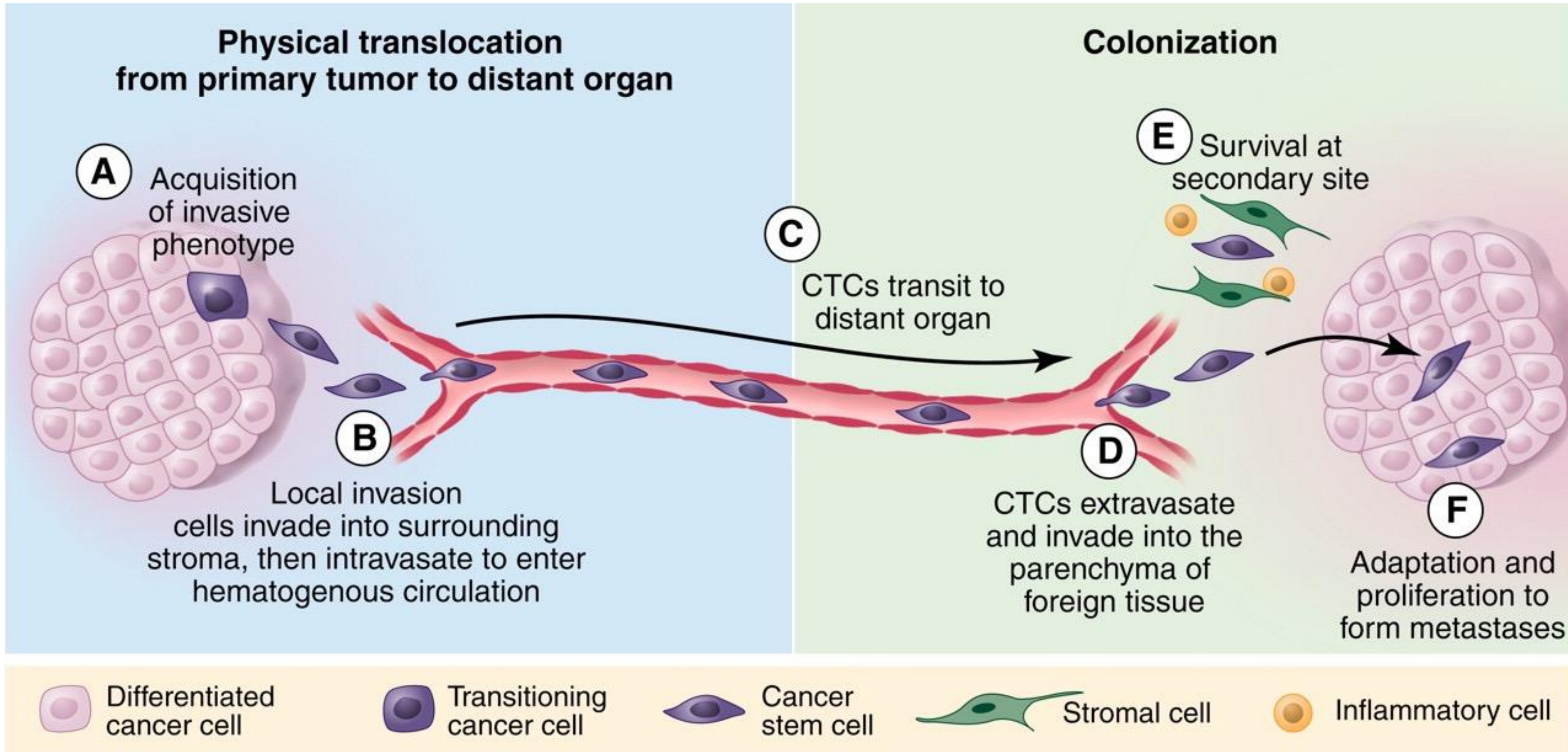
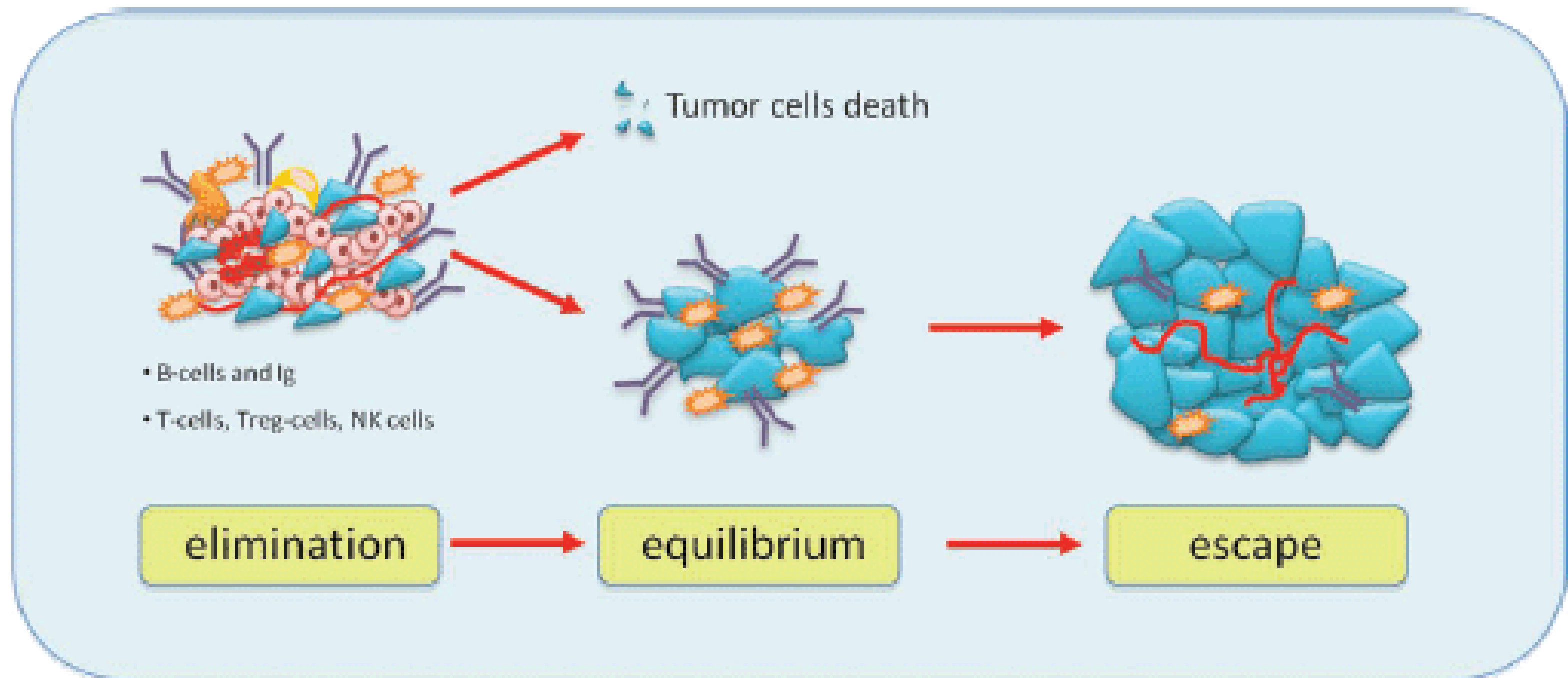


Figure from Chaffer, C. L. and Weinberg

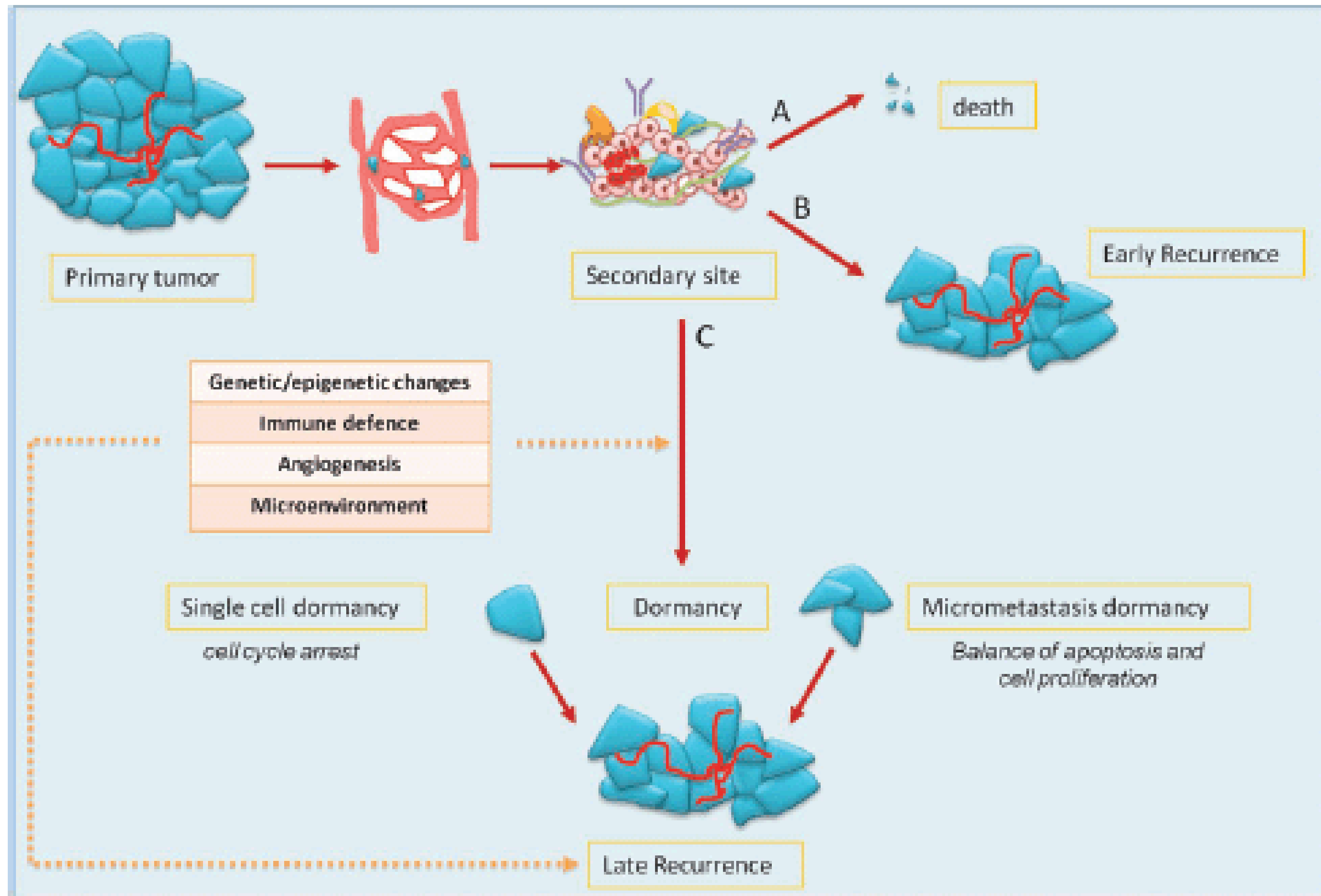
Tumor Physiology



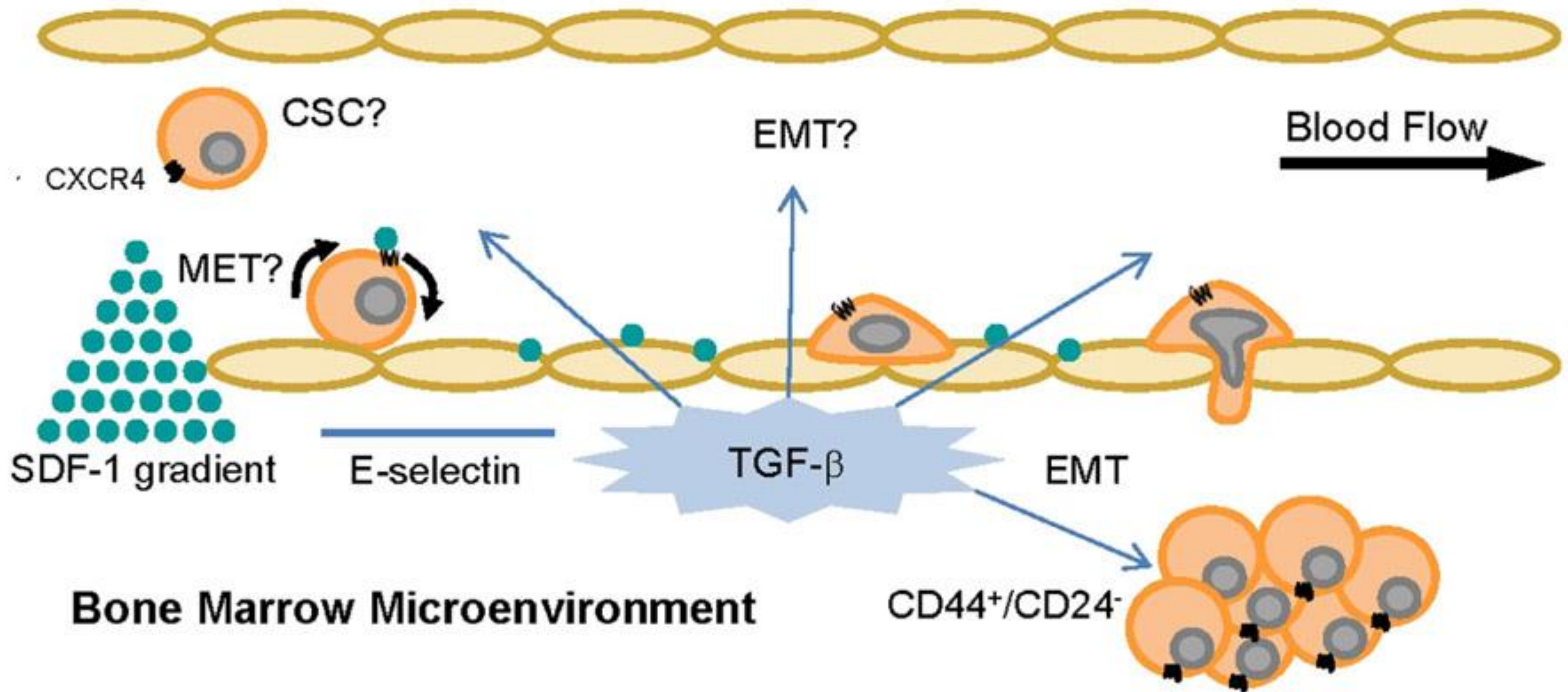
Gelao et al 2013

Tumor Physiology (CTCs)

Gelao et al 2013



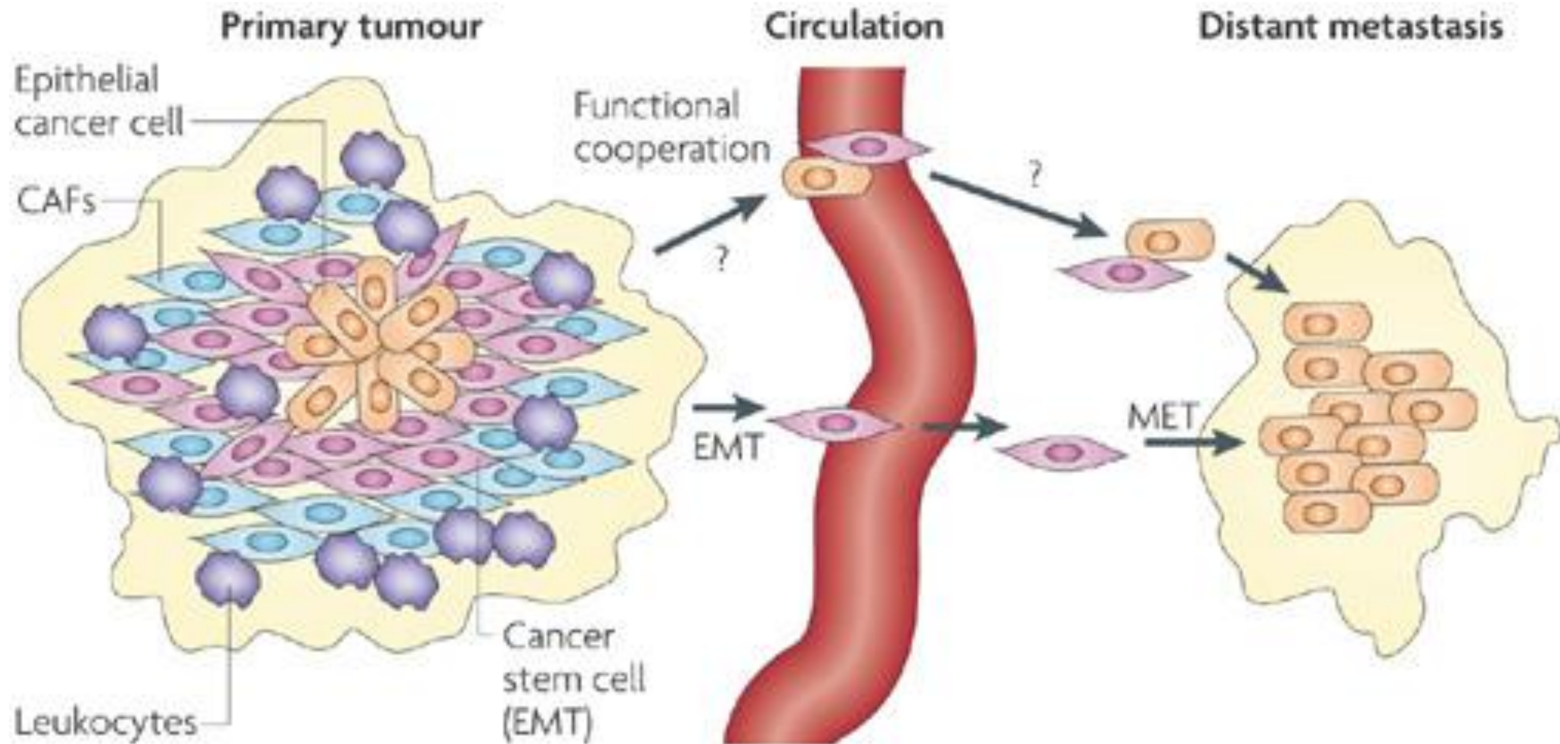
Tumor Physiology



Mueller et al 2005

Tumor physiology

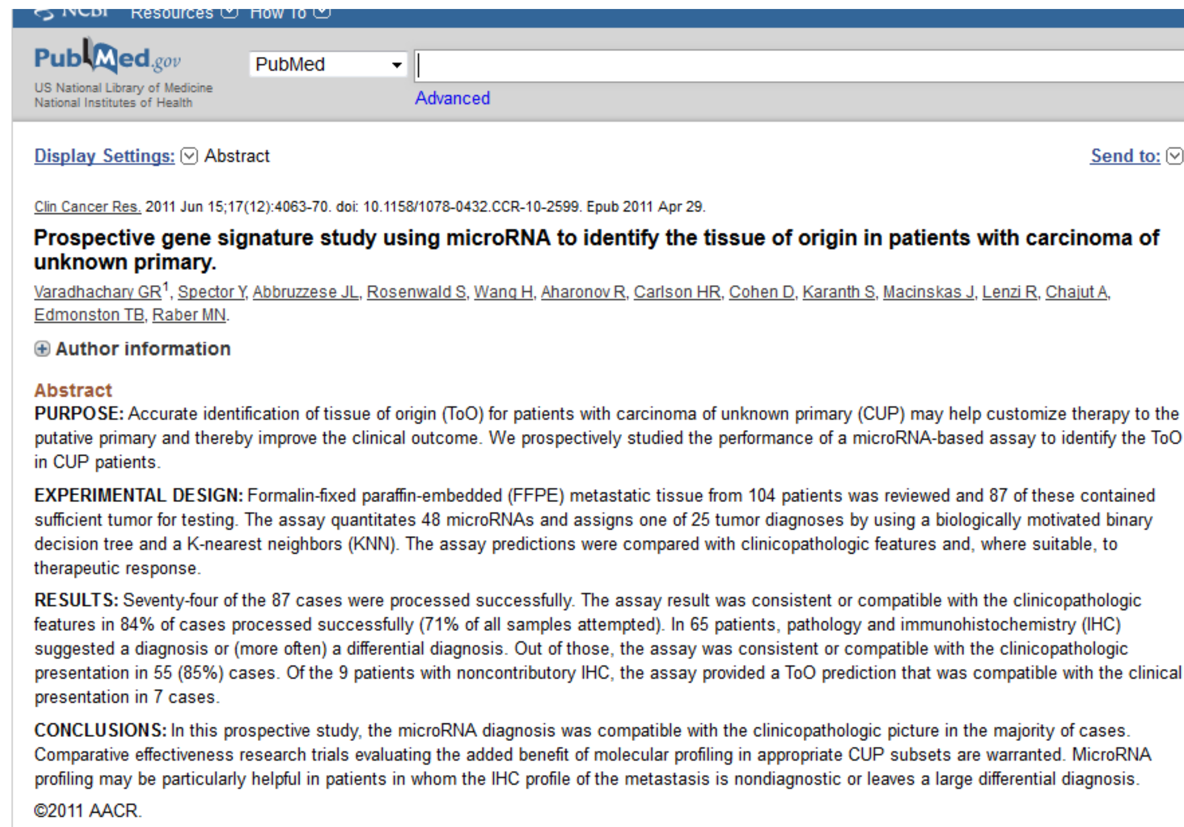
(heterogeneity-pleomorphy)



Comparison between primary and metastases

- GENOMIC LEVEL

The gene mutations signature is similar between primary and metastatic tumors.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Clin Cancer Res. 2011 Jun 15;17(12):4063-70. doi: 10.1158/1078-0432.CCR-10-2599. Epub 2011 Apr 29.

Prospective gene signature study using microRNA to identify the tissue of origin in patients with carcinoma of unknown primary.

Varadhachary GR[1], Spector Y, Abbruzzese JL, Rosenwald S, Wang H, Aharonov R, Carlson HR, Cohen D, Karanth S, Macinskas J, Lenzi R, Chajut A, Edmonston TB, Raber MN.

Author information

Abstract

PURPOSE: Accurate identification of tissue of origin (ToO) for patients with carcinoma of unknown primary (CUP) may help customize therapy to the putative primary and thereby improve the clinical outcome. We prospectively studied the performance of a microRNA-based assay to identify the ToO in CUP patients.

EXPERIMENTAL DESIGN: Formalin-fixed paraffin-embedded (FFPE) metastatic tissue from 104 patients was reviewed and 87 of these contained sufficient tumor for testing. The assay quantitates 48 microRNAs and assigns one of 25 tumor diagnoses by using a biologically motivated binary decision tree and a K-nearest neighbors (KNN). The assay predictions were compared with clinicopathologic features and, where suitable, to therapeutic response.

RESULTS: Seventy-four of the 87 cases were processed successfully. The assay result was consistent or compatible with the clinicopathologic features in 84% of cases processed successfully (71% of all samples attempted). In 65 patients, pathology and immunohistochemistry (IHC) suggested a diagnosis or (more often) a differential diagnosis. Out of those, the assay was consistent or compatible with the clinicopathologic presentation in 55 (85%) cases. Of the 9 patients with noncontributory IHC, the assay provided a ToO prediction that was compatible with the clinical presentation in 7 cases.

CONCLUSIONS: In this prospective study, the microRNA diagnosis was compatible with the clinicopathologic picture in the majority of cases. Comparative effectiveness research trials evaluating the added benefit of molecular profiling in appropriate CUP subsets are warranted. MicroRNA profiling may be particularly helpful in patients in whom the IHC profile of the metastasis is nondiagnostic or leaves a large differential diagnosis.

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Comparison between primary and metastases

- EPIGENETIC AND BIOMARKERS LEVEL

The gene expression profile alters between primary and metastatic tumors.

Difference of Biomarker Expression Among Primary Tumor and Brain Metastasis: a Report of Immunohistochemical Profiles of Resected Brain Metastases from Breast Cancer

Chikako Shimizu, MD

Breast and Medical Oncology Division
National Cancer Center Hospital
Japan

(clone D5/16B4; Dako) were performed using the streptavidin-biotin method and were considered positive if 10% or more of the nuclei in the invasive component of the tumor were stained. The HER2/neu status, as assessed using Herceptest (Dako), was scored on a scale of 0 to 3+, according to the Dako scoring system. HER2/neu-positive was defined by HER2/neu 3+ or HER2/neu 2+ and fluorescence in situ hybridization-positive.

The median age at the time of the diagnosis of brain metastasis was 53 years old (range, 39 to 78 years). The median time to brain metastasis from the time of breast cancer diagnosis was 2.9 years (range, 0 to 23.1 years). Seven of the patients had received no systemic therapy prior to brain tumor resection. Among 22 patients who had a prior history of receiving systemic therapy, eight had received trastuzumab-containing chemotherapy.

The proportion of ER-, PgR-, HER2/neu-positive tumors in 24 primary lesions were 12.5%, 8.3%, 37.5%, respectively. The proportion of ER-, PgR-, HER2/neu-, and CK5/6-positive tumors among the brain metastases were 13.8%, 6.9%, 37.9%, and 24.1%, respectively. The immunohistochemical profiles including ER, PgR, and HER2/neu of the primary tumor and the brain metastasis differed in 7 patients (29.2%, N=7/24) [see Figure 2]. Among eight patients who had been previously treated with trastuzuamab, two had HER2/neu negative brain metastases.

Future Tasks

The results of the above-described study suggest that distant metastases are not necessarily biologically similar to the primary tumors. The difference of the biomarker expression between the primary tumors and brain metastases may be due to modification by systemic treatments or change along with disease progression.

Re-assessment of the immunohistochemical status of the brain metastasis, if possible, may be useful to optimize treatment in the future. Although biomarker studies of brain metastases are very difficult to carry out because only a limited number of patients undergo surgery for brain metastases, in order to develop biologically rational treatments, further studies to elucidate the mechanism and biology of brain metastases are warranted.

References

Sorlie T, Perou CM, Tibishirani R, Aas T, Geisler S, Johnsen H, et al. Proc Natl Acad Sci U S A 2001; 98: 10869-10874.

Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A et al. Proc Natl Acad Sci U S A 2003; 100: 8418-8423.

Gaedcke J, Traub F, Milde S, Wilkens L, Stan A, Ostertag H, et al. Predominance of basal type and HER2-/neu type in brain metastasis from breast cancer. Modern Pathology 2007; 20: 864-870.

Yonemori K, Tsuta K, Shimizu C, Hatanaka Y, Hashizume K, Ono M, et al. Immunohistochemical profiles of brain metastases from breast cancer. J Neurooncol 2008 Jul 23 [e-pub ahead of print].

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Gynecol Oncol. 2011 Aug;122(2):356-60. doi: 10.1016/j.ygyno.2011.04.039. Epub 2011 May 24.

Comparison of estrogen and progesterone receptor status of circulating tumor cells and the primary tumor in metastatic breast cancer patients.

Aktas B[1], Müller V, Tewes M, Zeitz J, Kasimir-Bauer S, Loehberg CR, Rack B, Schneeweiss A, Fehm T.

Author information

Abstract

OBJECTIVES: The expression of predictive markers including the estrogen (ER) and progesterone receptor (PR) expression can change during the course of the disease. Therefore, reassessment of these markers at the time of disease progression might help to optimize treatment decisions. Metastatic tissue may be difficult to obtain for repeated analysis. In this context, characterization of circulating tumor cells (CTCs) could be of relevance. It was the purpose of the present study (1) to reevaluate the ER/PR expression by CTCs and (2) to compare the hormone receptor status expression profile of CTCs with the primary tumor.

METHODS: We evaluated 193 blood samples from metastatic breast cancer patients at the time of first diagnosis of metastatic disease or disease progression. All samples underwent immunomagnetic enrichment using the AdnaTest BreastCancerSelect (AdnaGen AG, Germany) within 4h after blood withdrawal followed by RNA isolation and subsequent gene expression analysis by reverse transcription and Multiplex-PCR in separated tumor cells using the AdnaTest BreastCancerDetect. CTCs were analyzed for the three breast cancer-associated markers: EpCAM, Muc-1, Her-2 and actin as an internal PCR control. Expression of the ER and PR was assessed in an additional RT-PCR. The analysis of PCR products was performed by capillary electrophoresis on the Agilent Bioanalyzer 2100.

RESULTS: The overall detection rate for CTCs was 45% (87/193 patients) with the expression rates of 71% for EpCAM (62/87 patients), 73% for MUC1 (64/87 patients), 48% for HER2 (42/87 patients), 19% for ER (17/87 patients) and 10% for PR (9/87 patients), respectively. Comparisons with the primary tumor were only performed in CTC+ patients (n=87). In 48/62 (77%) patients with ER+ tumors, CTCs were ER- and 46/53 (87%) patients with PR+ tumors did not express PR on CTCs. Primary tumors and CTCs displayed a concordant ER and PR status in only 41% (p=0.260) and 45% (p=0.274) of cases, respectively.

CONCLUSION: Most of the CTCs were ER/PR-negative despite the presence of an ER/PR- positive primary tumor. The predictive value of hormone receptor status expression profile of CTCs for palliative endocrine therapy has to be prospectively evaluated. STATEMENT: We recently demonstrated in more than 400 primary breast cancer patients that the expression profile between CTCs and the primary tumor with regard to ER/PR/HER2 positivity differs. The concordance rate between ER, PR and HER2 status of CTCs and the primary tumor was 29%, 25% and 53%, respectively (Fehm T et al., Breast Cancer Res Aug 10 2009, 11(4) pR59). Based on these results we studied blood samples of 193 metastatic breast cancer patients participating in the German DETECT study (1) to reevaluate the ER/PR expression by CTCs and (2) to compare the hormone receptor status expression profile of CTCs with the primary. As already shown for primary breast cancer, most of the CTCs were ER/PR-negative despite the presence of an ER/PR- positive primary tumor. In the metastatic setting the phenotype of CTC reflects the phenotype of metastatic disease. Therefore palliative treatment selected based on the expression profile may not be effective since the phenotype has changed during disease progression. To our knowledge, this study is one of the biggest to compare hormonal receptor expression on CTC and the primary tumor. We hope that our manuscript is suitable for publication in Gynecologic Oncology.

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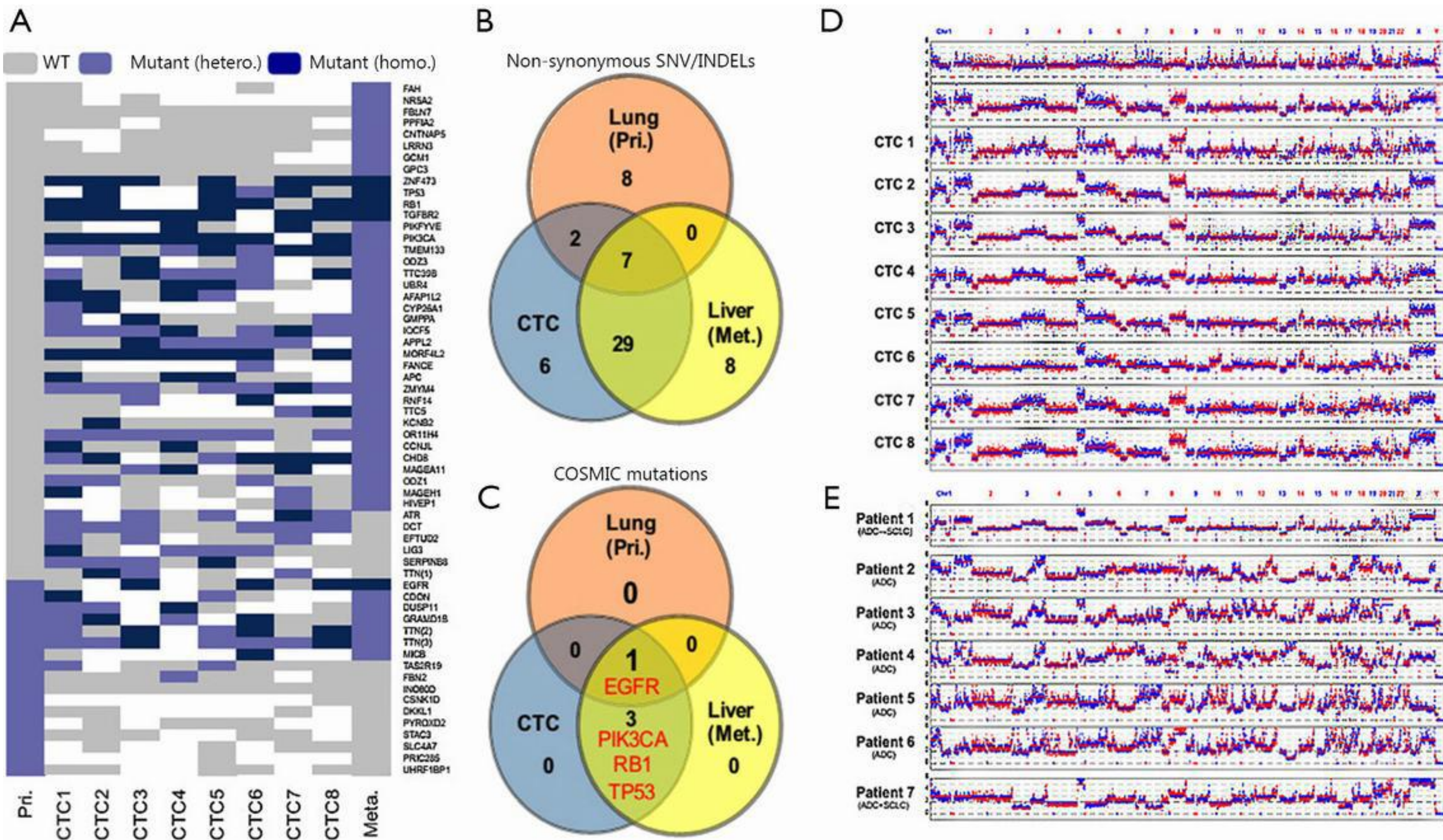
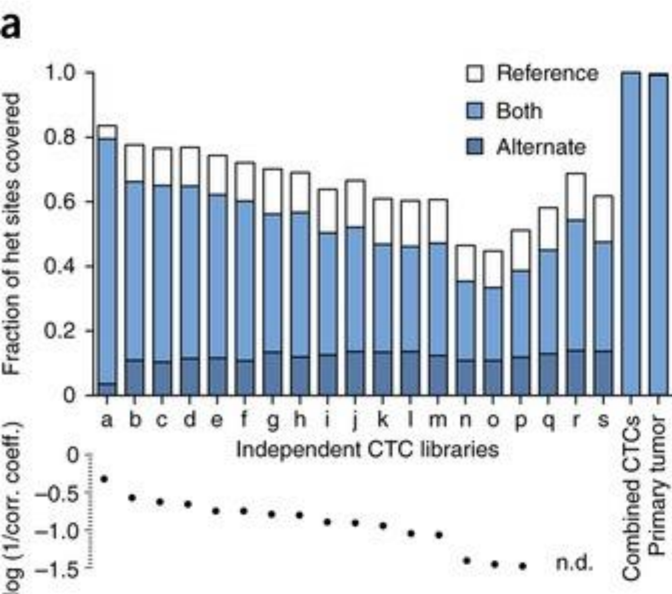
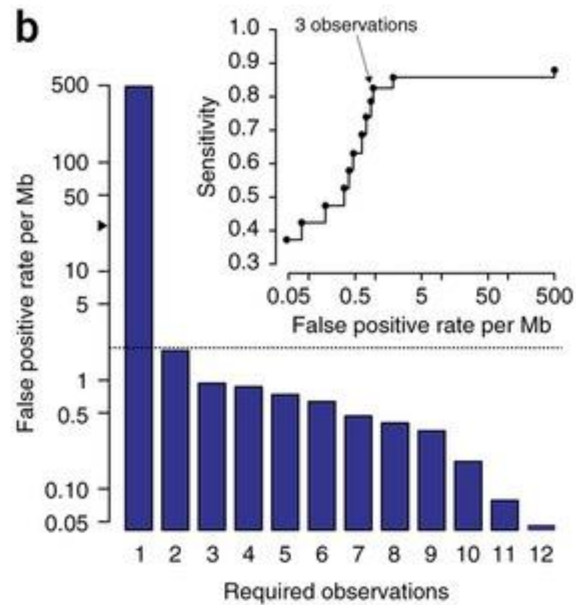
Relevance between primary, CTCs and metastases

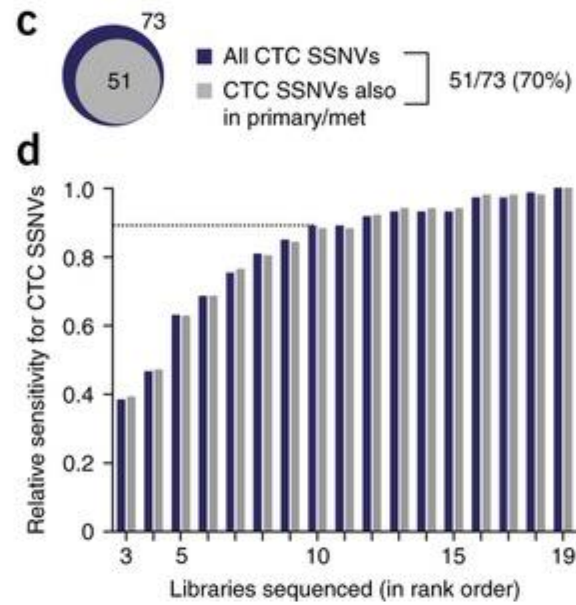


Xi-Xi Chen et al, Ann of Transl. medicine, 2015

Relevance between primary, CTCs and metastases



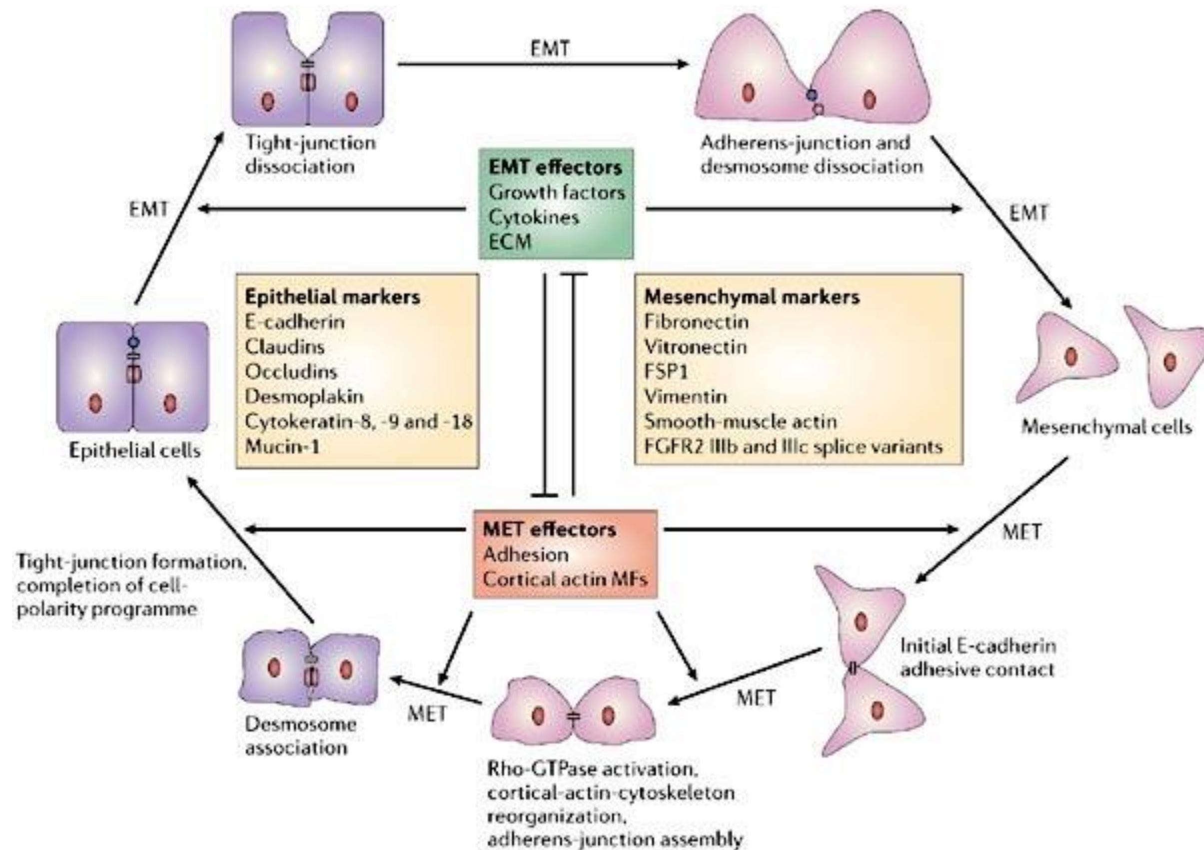




Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer

Lohr et al, 2014, Biotechnology, Research, Letters

Heterogeneity of CTCs (EMT-MET)



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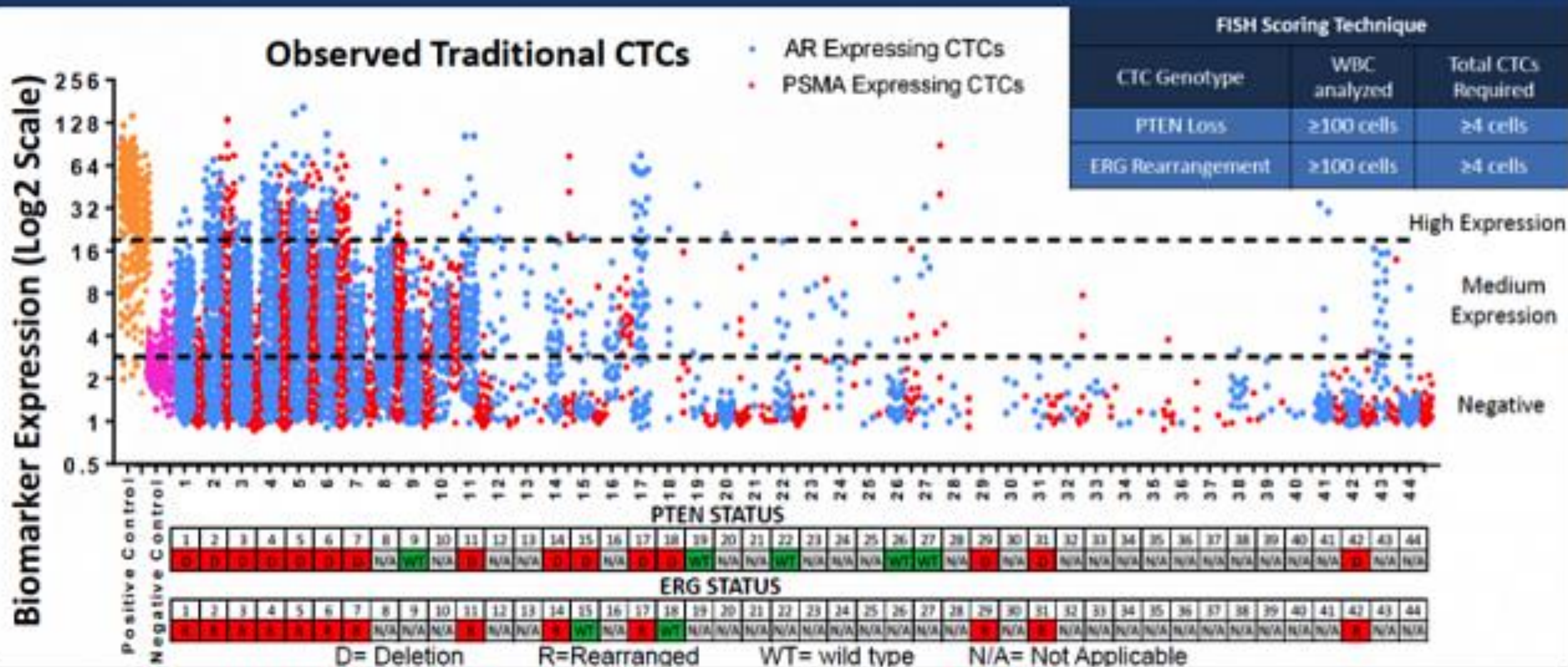
Heterogeneity of CTCs (EMT-MET)



PRACTICAL ISSUES WHAT PROHIBIT THE “JUMP” FROM BENCH TO BED
-TRANSLATIONAL MEDICINE-

(CLINICAL REALITY)

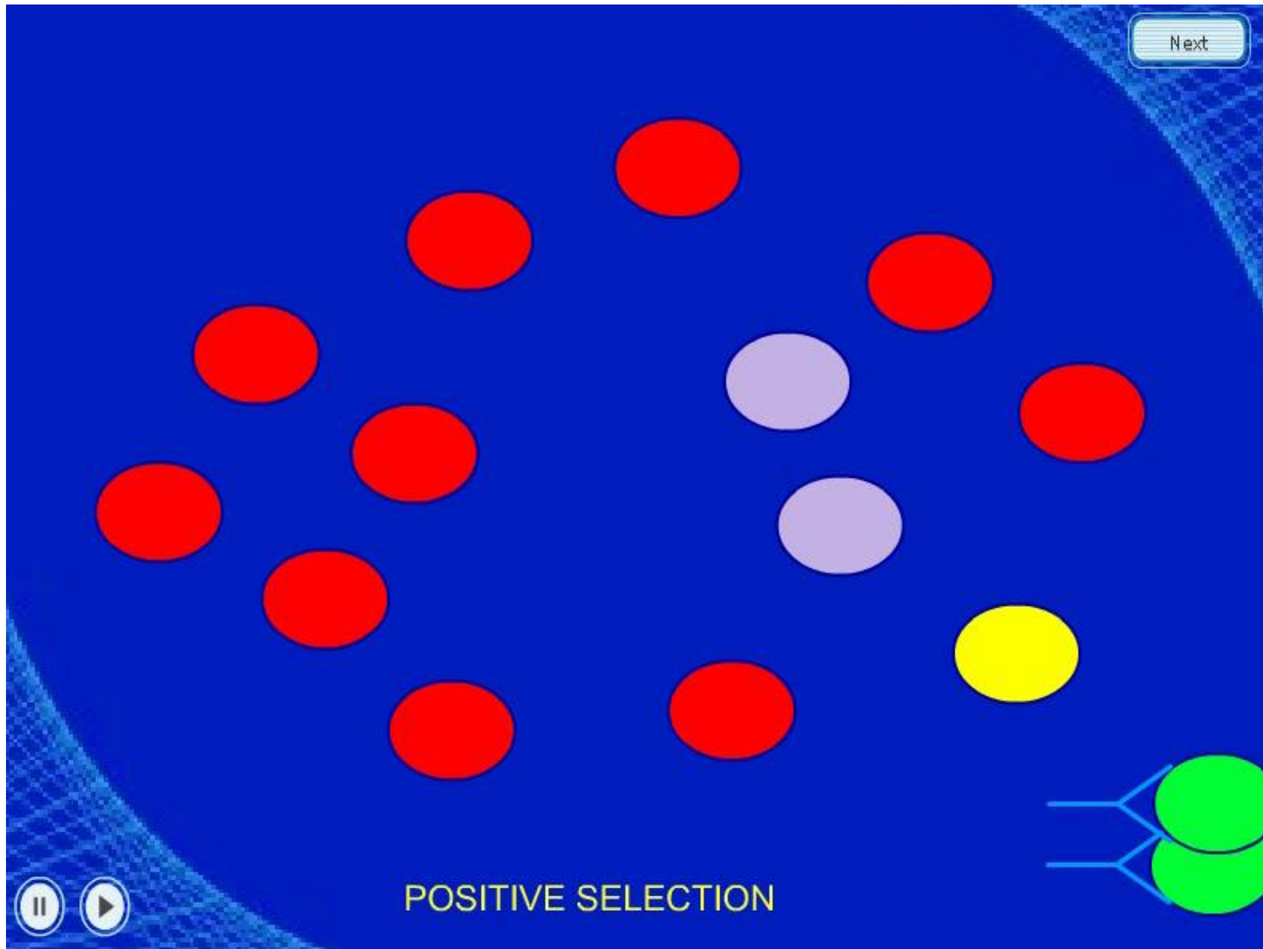
How can be found a needle in a hay-stack?

Average No of CTCs in blood sample is
10-30cell/50.000 events
(RBC and platelets have been subtracted)

What we need to preserve during detection and isolation of CTCs

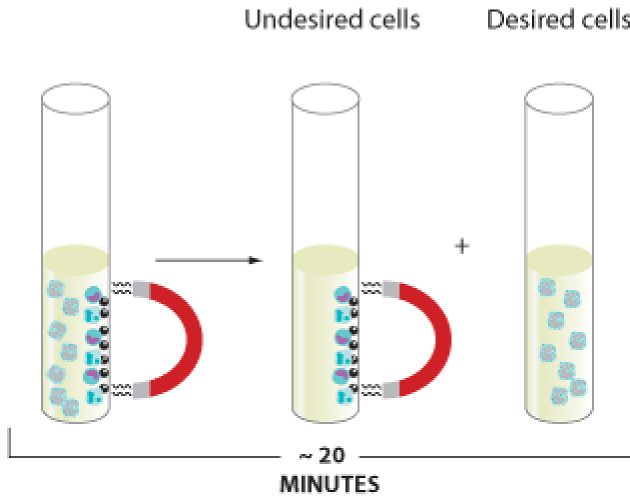
1. High purity of CTCs
2. Viable CTCs
3. Isolate all subsets of CTCs
4. Detect the disease relevant CTCs
5. Detect CTCs subset with stemness properties
6. Pin point the subclasses of CTCs with plasticity properties (EMT-MET)

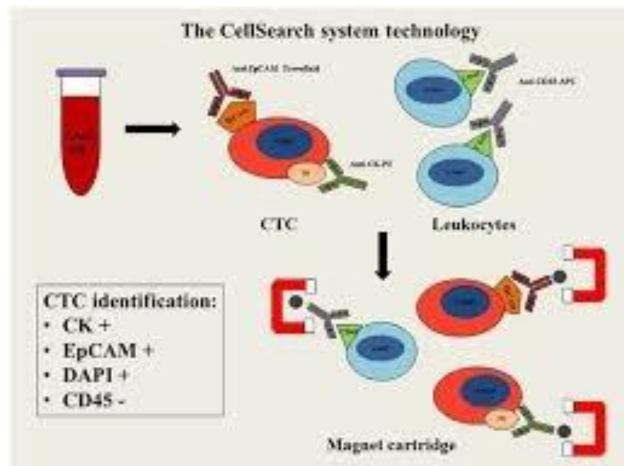
CHOOSING THE RIGHT METHOD

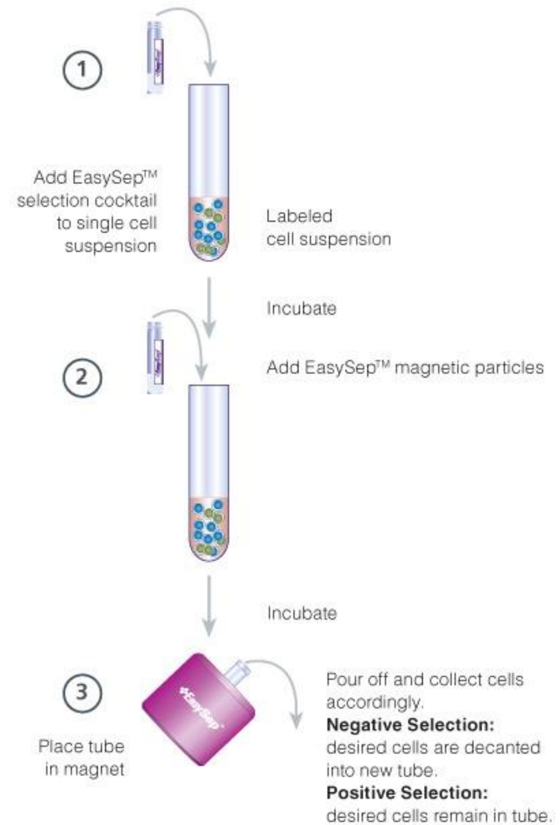


CHOOSING THE RIGHT METHOD

BEAD BASED METHOD

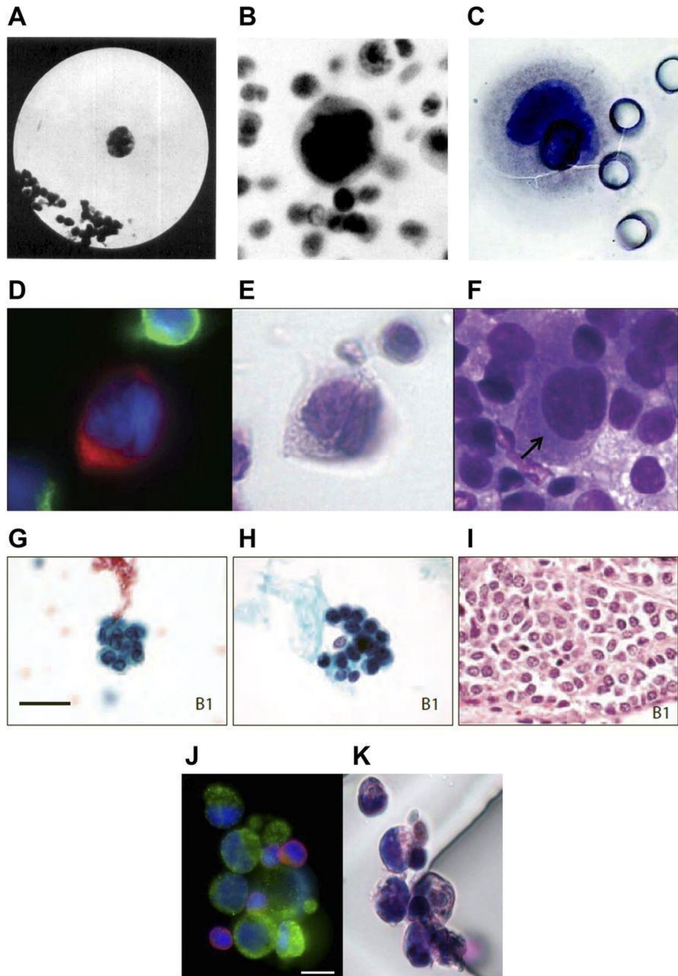






CHOOSING THE RIGHT METHOD

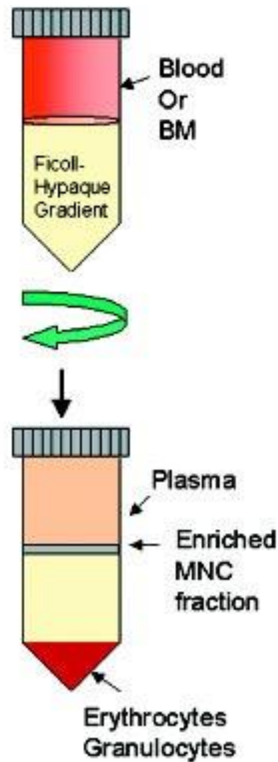
MICROSCOPY BASED METHOD

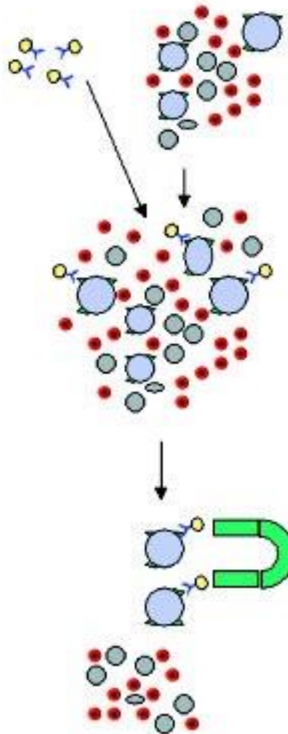


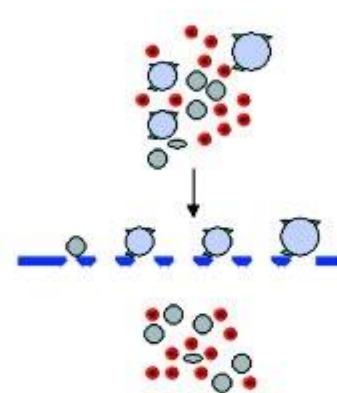
- FIXATION OF THE SAMPLE
- POSITIVE SELECTION METHOD
- DAMAGE OF SAMPLE DURING PROCESS

CHOOSING THE RIGHT METHOD

GRADIENT BASED METHOD

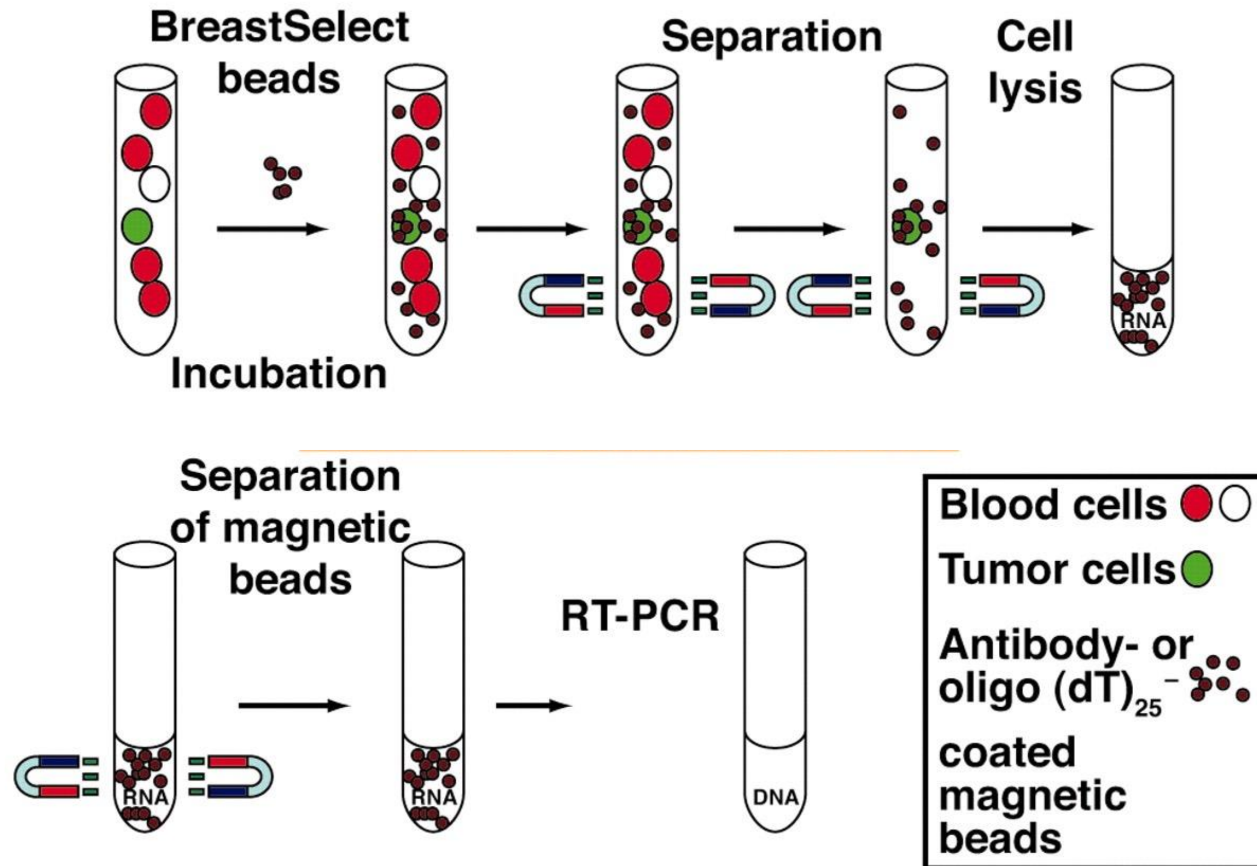






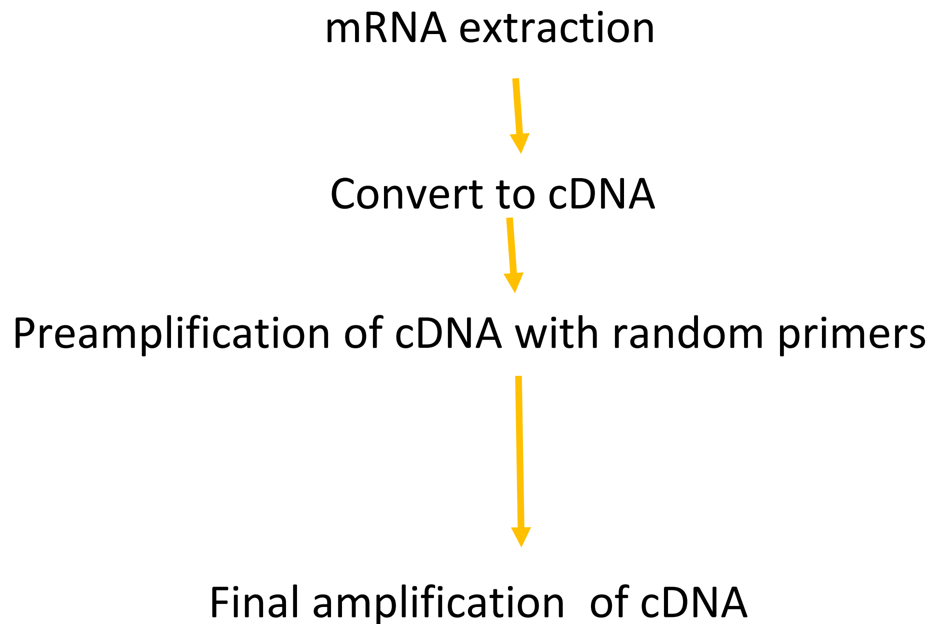
CHOOSING THE RIGHT METHOD

PCR BASED METHODS



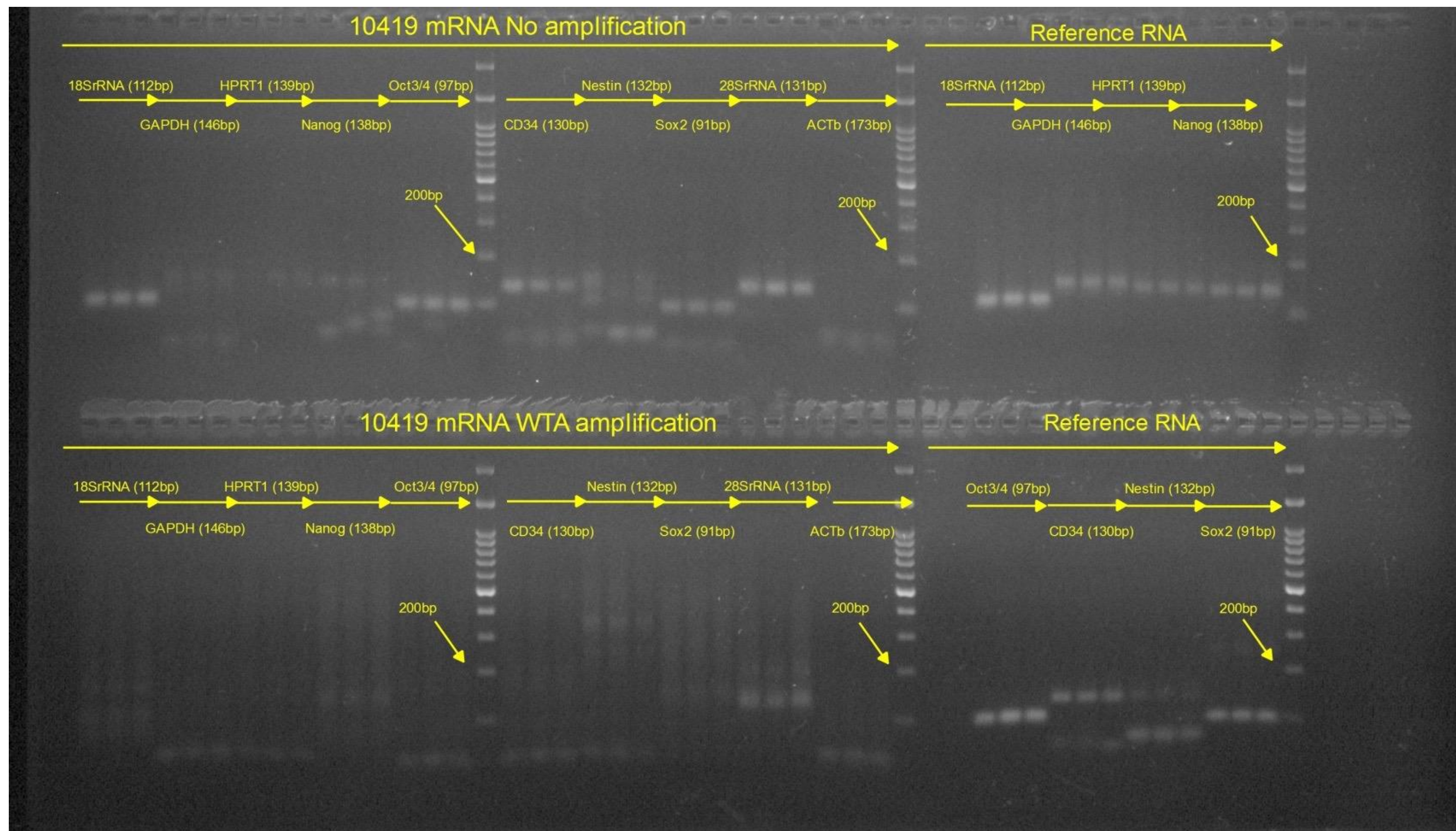
More things to consider

- qPCR (all genome)



More things to consider

- qPCR or real time PCR (all genome expression amplification)



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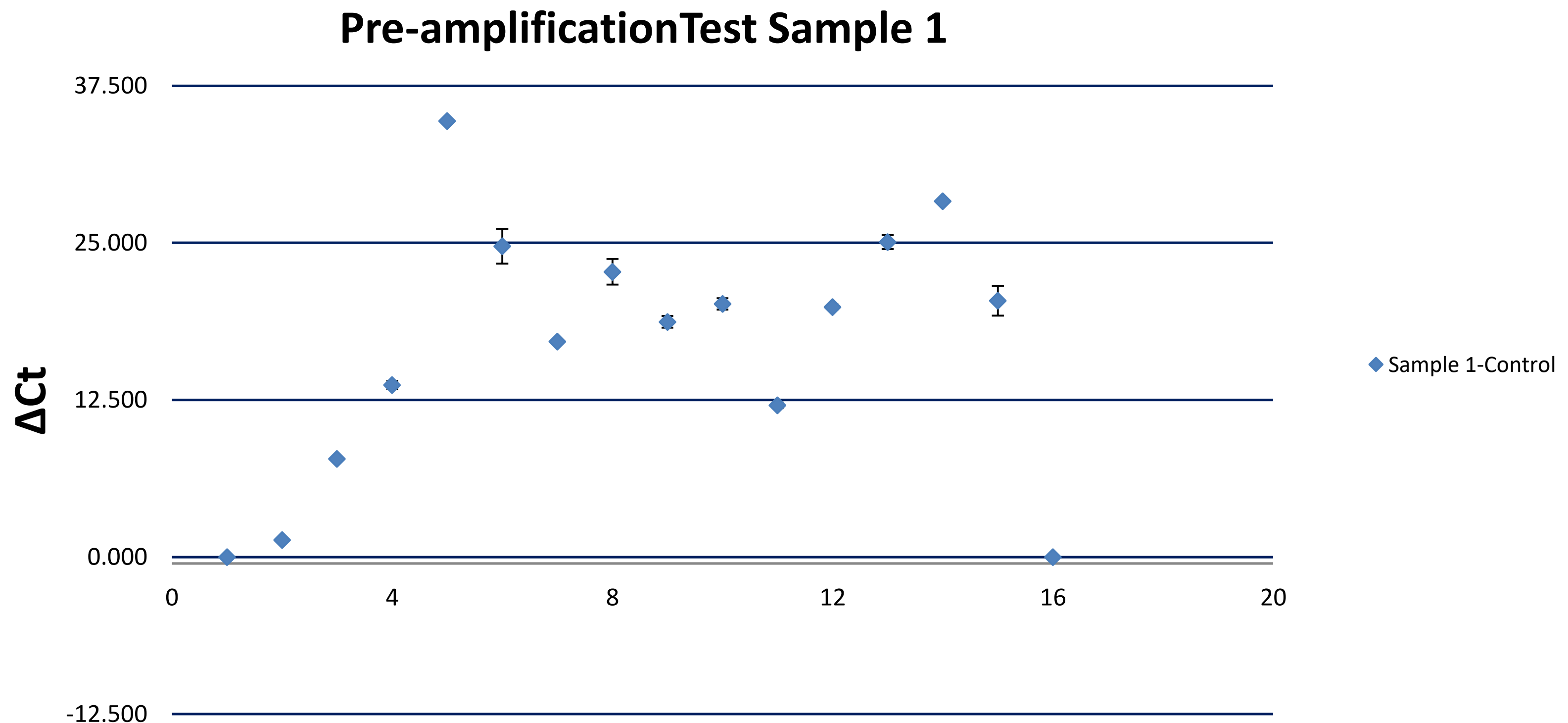
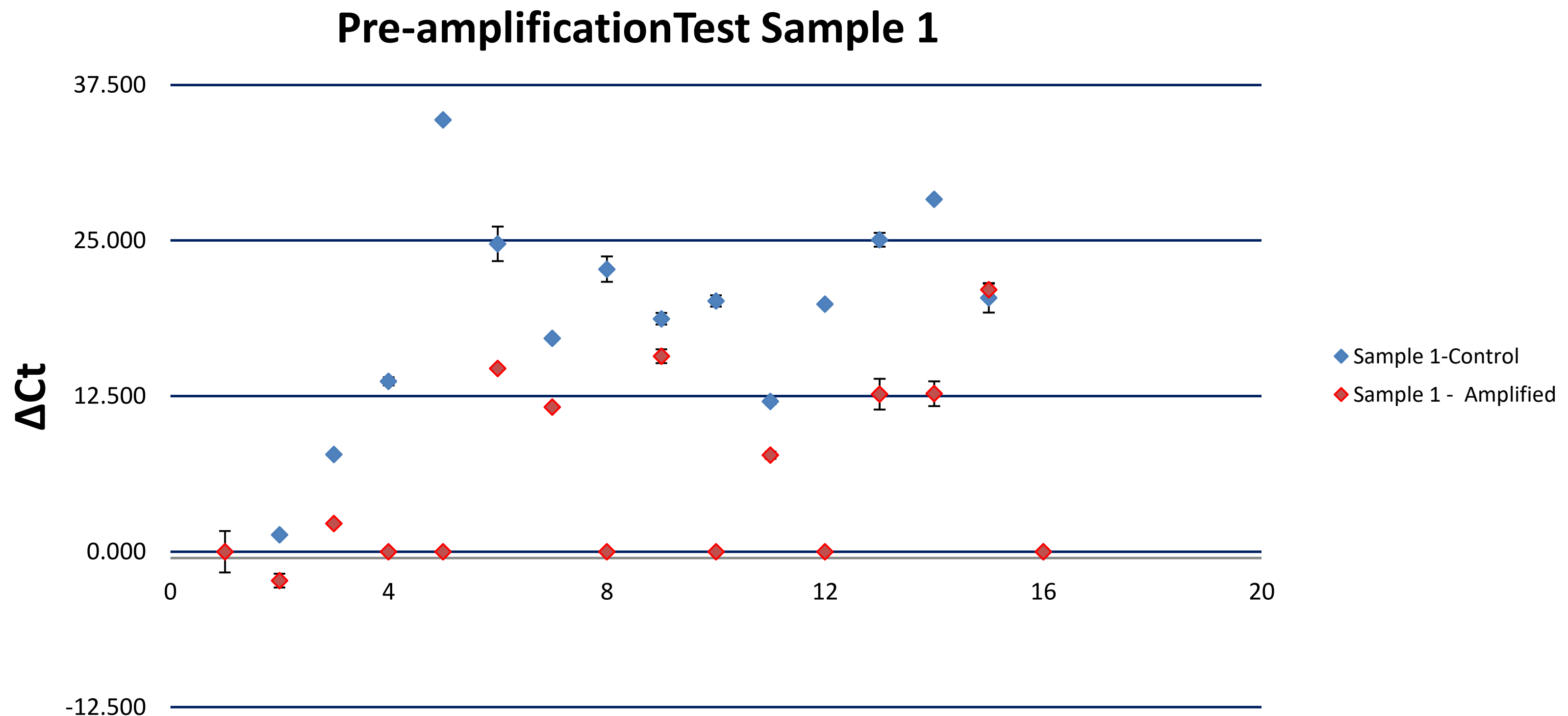


More things to consider

- qPCR or real time PCR (all genome expression amplification)



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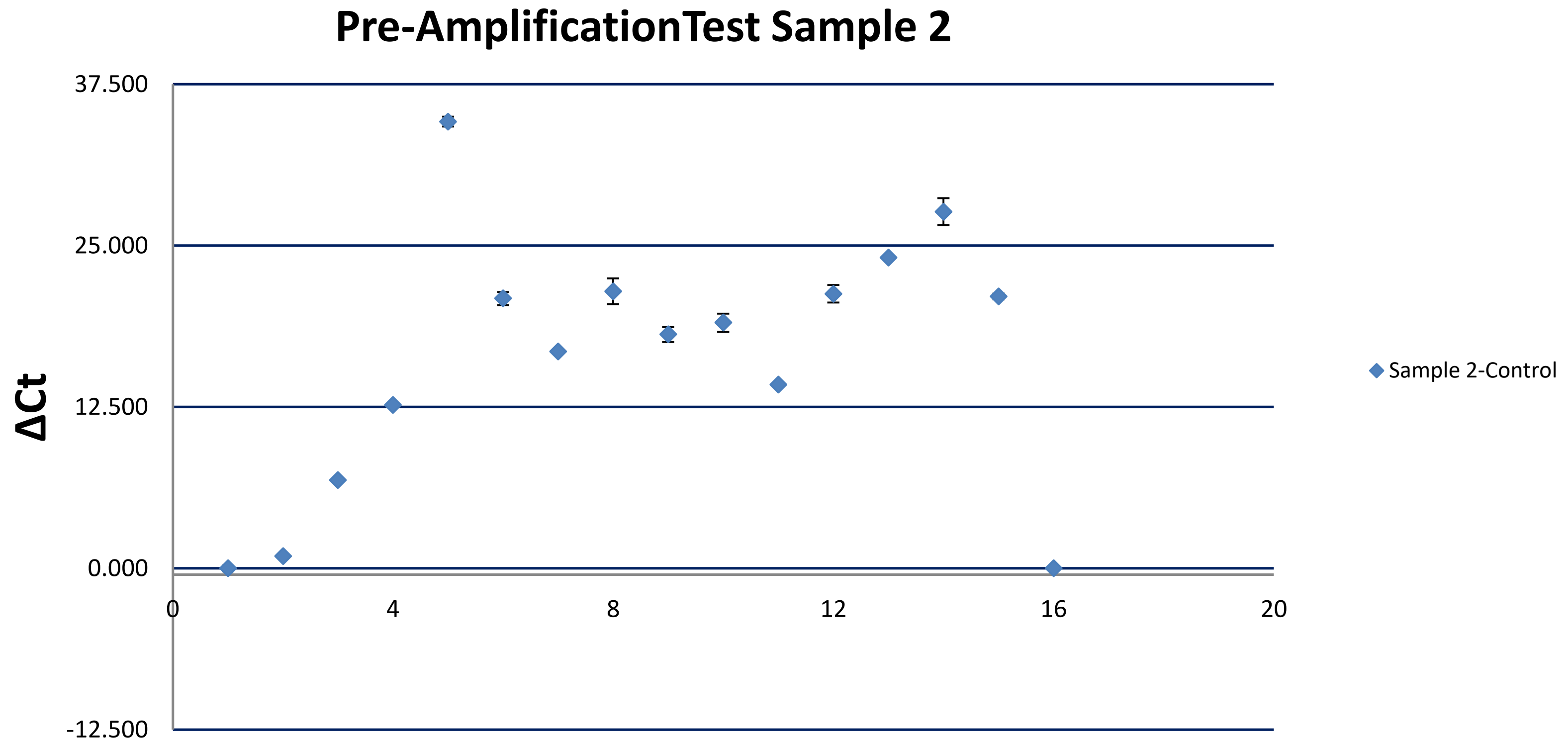

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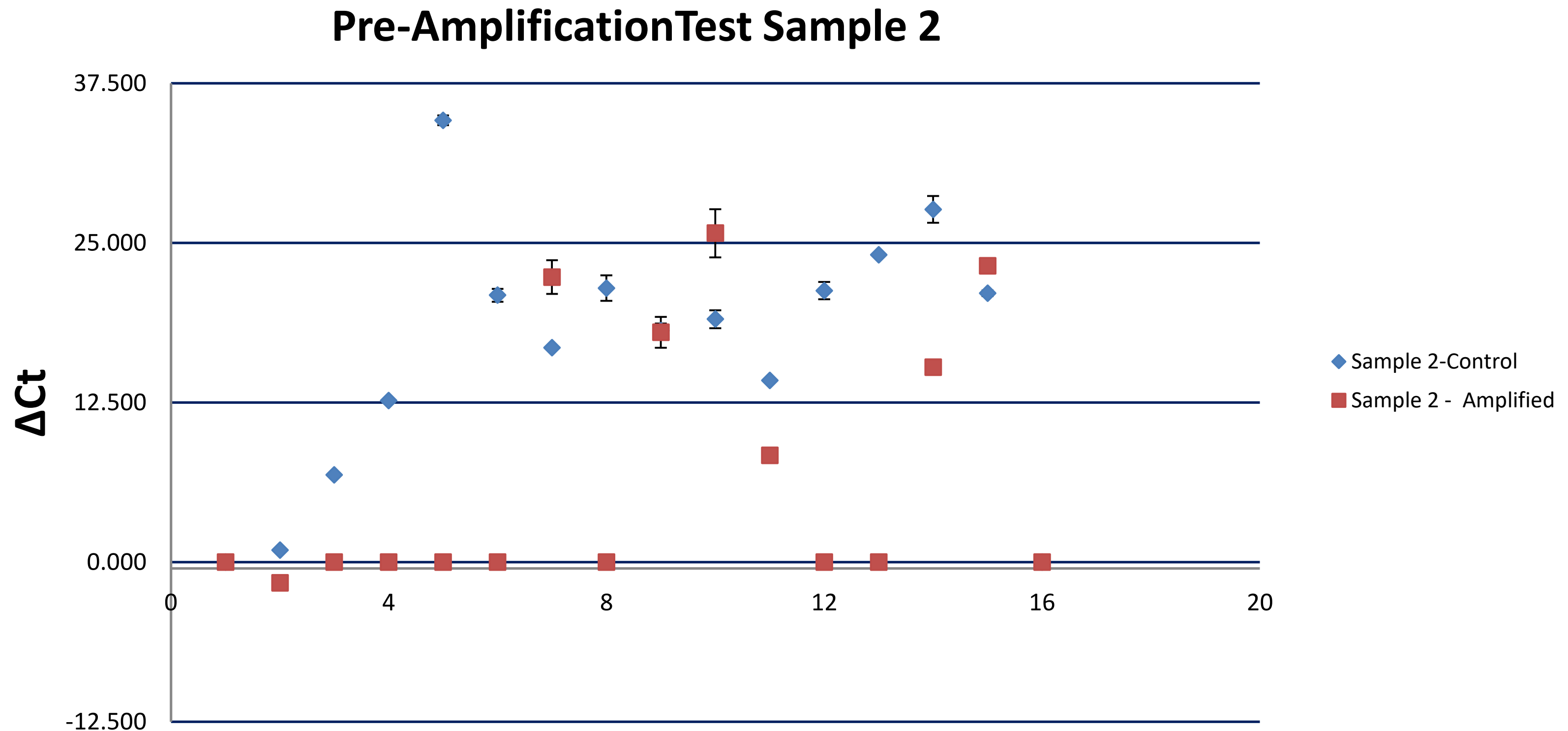


More things to consider

- We may consider to epithelial carcinomas that a common marker will allow us to detect CTCs (like CKs or EpCam). Is this correct?

*3368523

Medical Status

PRESENT DIAGNOSE OF MALIGNANCY (CANCER): CANCER MARKER POSITIVE

STAGE: 0 DATE OF PRIMARY DIAGNOSIS: / 7 / 14

Medical Record

(COMMENTS / ADDITIONAL INFORMATION):

Oncotrace -ve, positive CK, CTC positive

Genetic Pedigree & Family History

paternal bowel cancer, high risk personal

Primary Diagnose

SURGERIES (including dates): –

CHEMOTHERAPIES (including dates): –

RADIOTHERAPIES (including dates): –

BIOLOGICAL THERAPIES (including dates): –

VAT NUMBER (EEA - EU):

NATIONAL INSURANCE NUMBER (GB):

UMSATZSTEUERIDENTIFIKATION NUMMER (D):

CODICI FISCALE (IT):

SOCIAL SECURITY NUMBER (USA):

Cancellation policy:

In case of canceling the analysis order or sample sending the policy will be as follow:

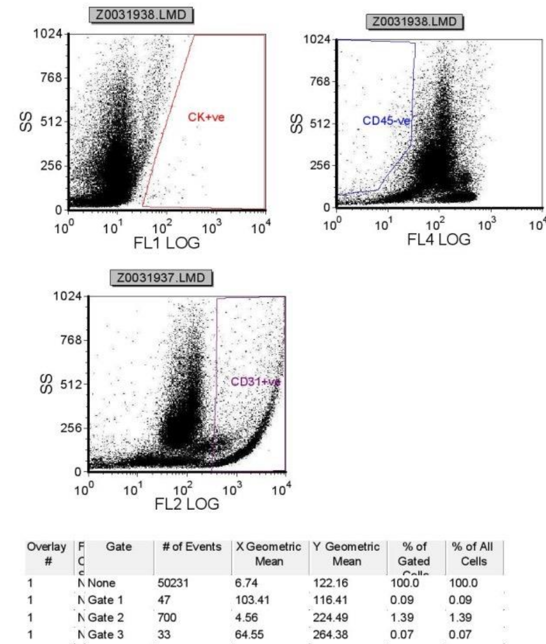
a. In case of canceling during the first two days after shipping (sending) date, there will be charging only 425 Euros.
b. In case of canceling between third date of shipping and 24 hours before the end of analysis, there will be charging of 50% of the total price.
c. In case of canceling 24 hours before the final results there will be no refund.

Declaration – TERMS & CONDITIONS

Samples shipment by Client to R.G.C.C. International GmbH and subcontractor and the delivery thereof means total acceptance of the terms and conditions written below.

1) "Client" means the shipper who seeks for R.G.C.C.s laboratory services.
2) "Sample" or "Samples" means the blood sample drawing from patient which have been put in provided vials with preservative liquid or tissue sample that have been piut in provided vials with specific preservative liquid
3) The Client certifies that the samples have been collected under the consent of the patient and according to the existing laws and medical regulations.

R.G.C.C. International GmbH Page 2 of 3



Overlay #	F C S	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	N	None	50231	6.74	122.16	100.0	100.0
1	N	Gate 1	47	103.41	116.41	0.09	0.09
1	N	Gate 2	700	4.56	224.49	1.39	1.39
1	N	Gate 3	33	64.55	264.38	0.07	0.07

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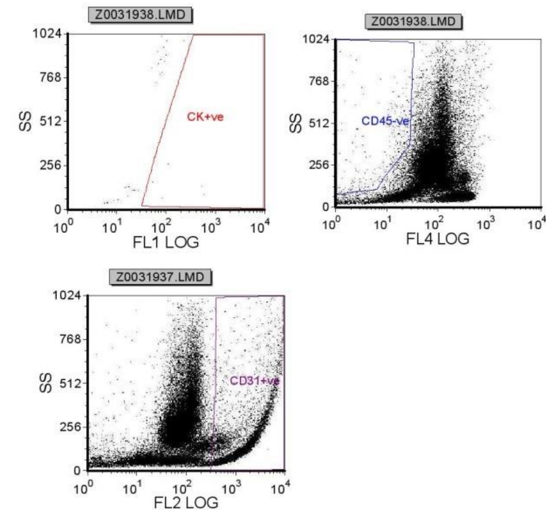
R.G.C.C. International GmbH Page 2 of 3



Overlay #	F	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	N	None	33	64.55	264.38	100.0	0.07
1	N	Gate 1	6	221.67	147.27	18.18	0.01
1	N	Gate 2	0	0.0	0.0	0.0	0.0
1	N	Gate 3	33	64.55	264.38	100.0	0.07

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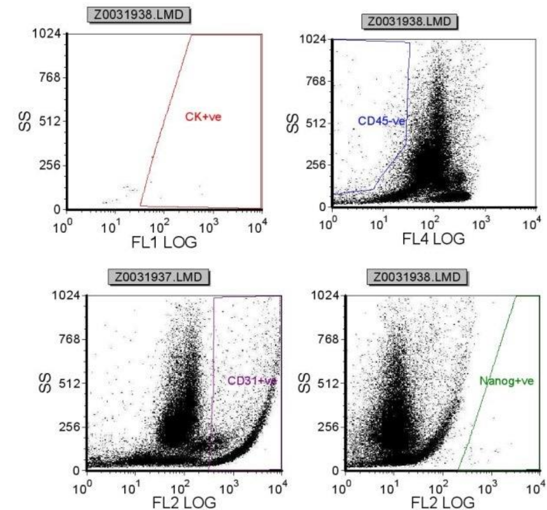
R.G.C.C. International GmbH

Page 2 of 3



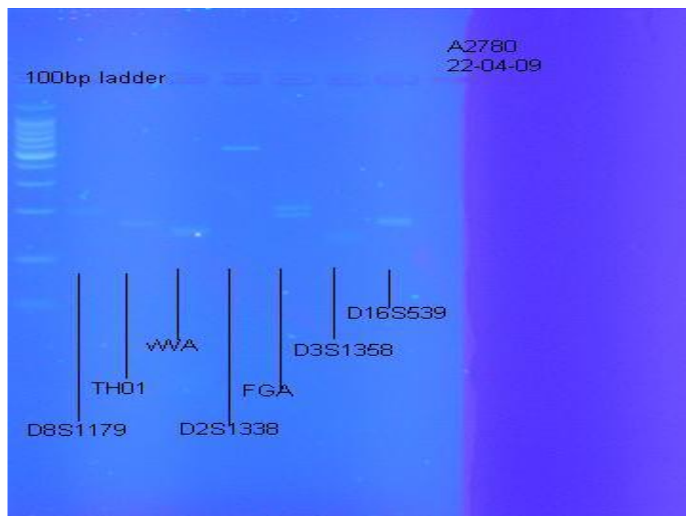
Overlay #	F C S	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	N	None	21	63.29	130.41	100.0	0.04
1	N	Gate 2	0	0.0	0.0	0.0	0.0
1	N	Gate 1	0	0.0	0.0	0.0	0.0
1	N	Gate 3	19	52.16	116.66	90.48	0.04
1	N	Gate 4	21	63.29	130.41	100.0	0.04

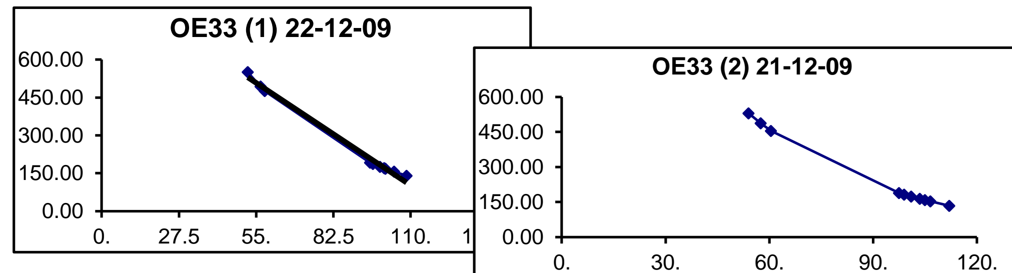
TECHNICAL ISSUE

- Since cancer cells are genetically unstable how is it possible to expand them without deviated severely from clinical reality?

Short Tandem Repeats (STRs)

Short Tandem Repeats are short sequences of DNA (2-16 base pairs), that are repeated numerous times. The repeated sequences are directly adjacent to each other and typically are in the non-coding "intron" region. The polymorphisms in STRs are due to the different number of copies of the repeat element that can occur in a population of individuals. By identifying the repeats of a specific location in the genome, can be created a genetic profile of an individual STRs loci amplified with polymerase chain reaction (PCR), without the problem of differential amplification





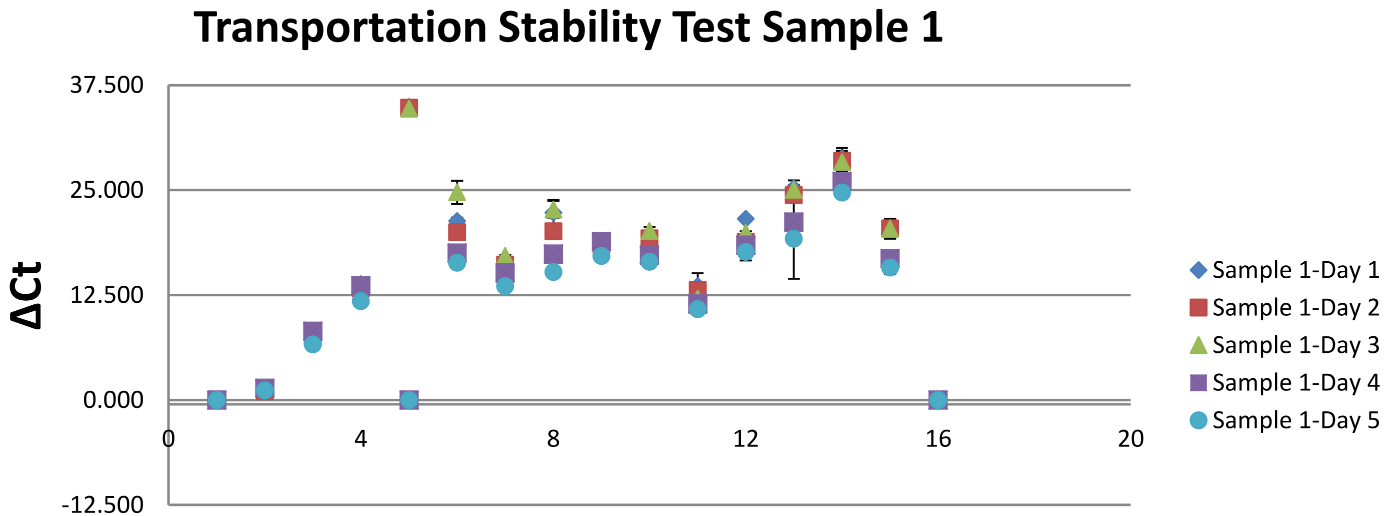
TECHNICAL ISSUE 2

- How stable the sample is during transportation under the parameter of time?

Short Tandem Repeats (STRs)

Samples have been tested on epigenetics as well as according to immunophenotype using the following techniques:

1. Real time PCR



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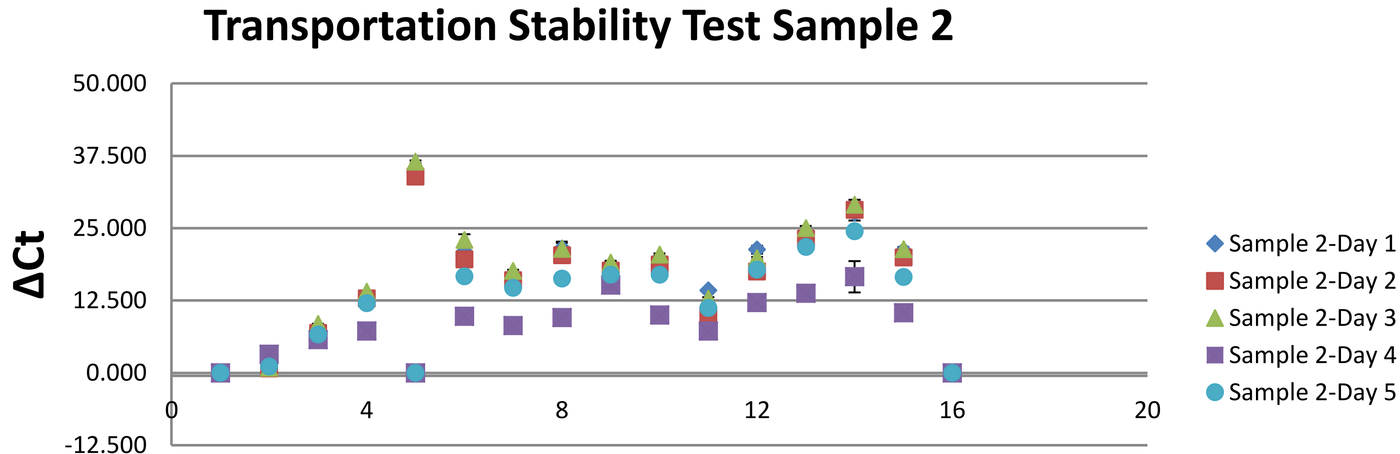

TECHNICAL ISSUE 2

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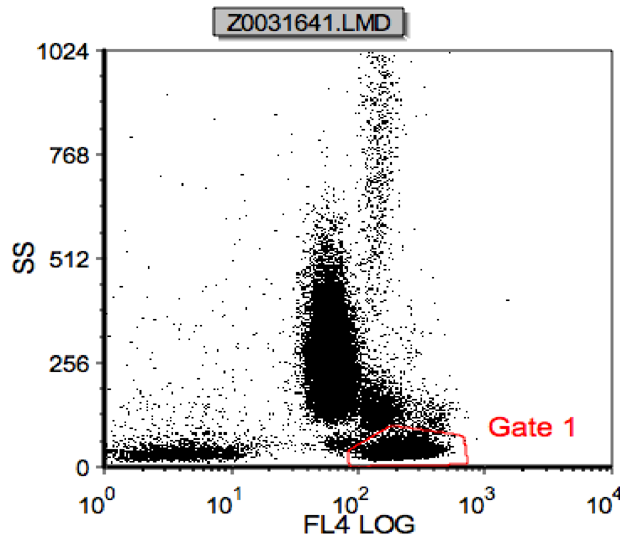
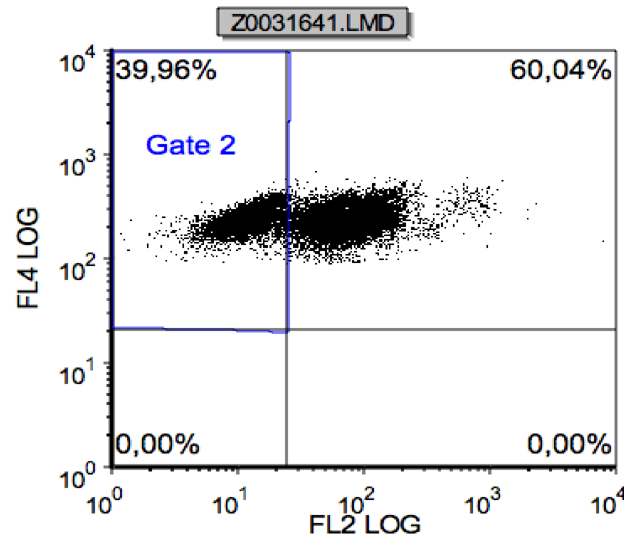
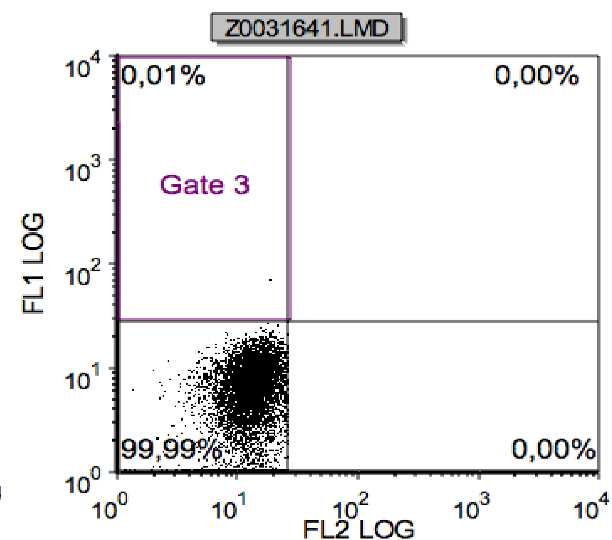
Short Tandem Repeats (STRs)

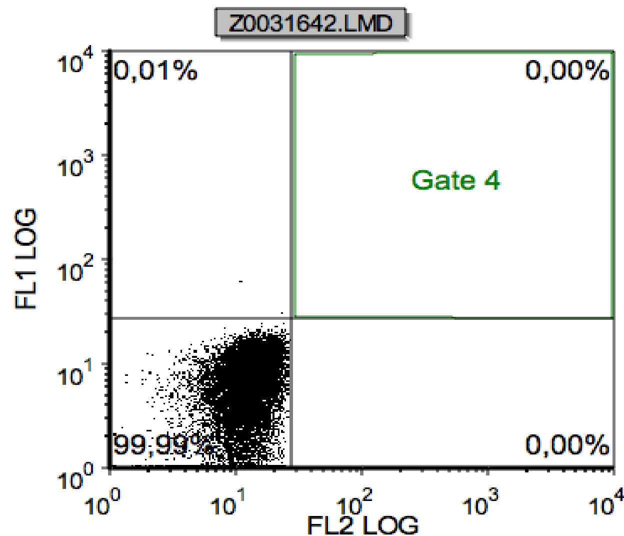
Samples have been tested on epigenetic as well as according to immunophenotype using the following techniques:

1. Flow cytometry









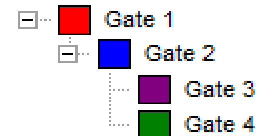
Sample 1 Day 0 (sample collection)

Gate 1: lymphocytes

Gate 2: CD31 negative cells

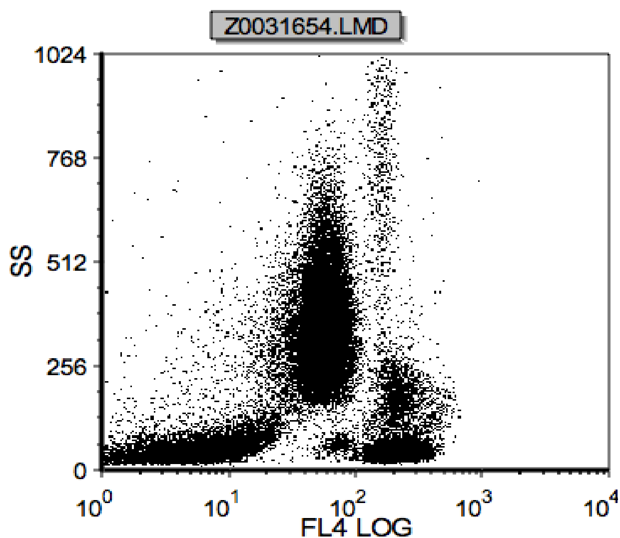
Gate 3: CK positive cells

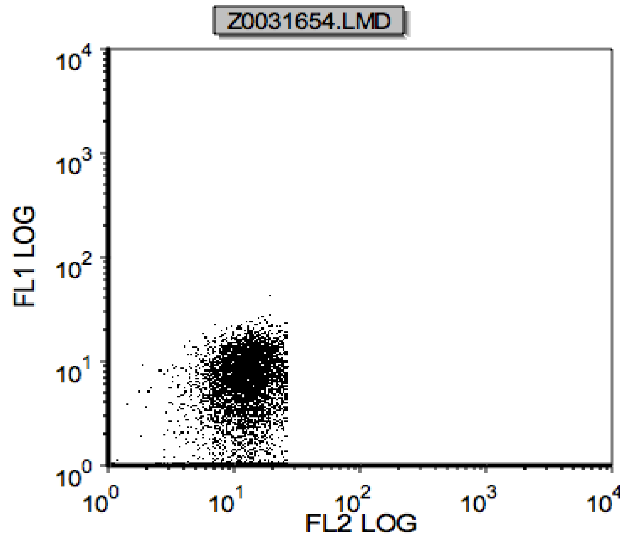
Gate 4: cMet positive cells

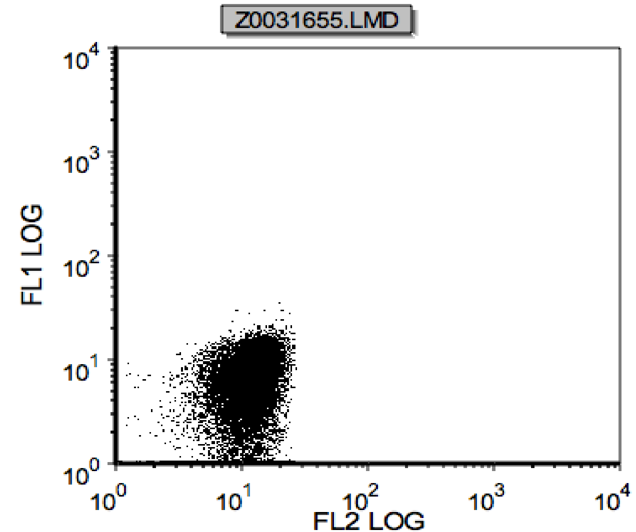


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031642.LMD	None	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 1	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 2	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 3	1	10,84	59,35	0,01	0,0
1	Z0031642.LMD	Gate 4	0	0,0	0,0	0,0	0,0

Sample 1 Day 1

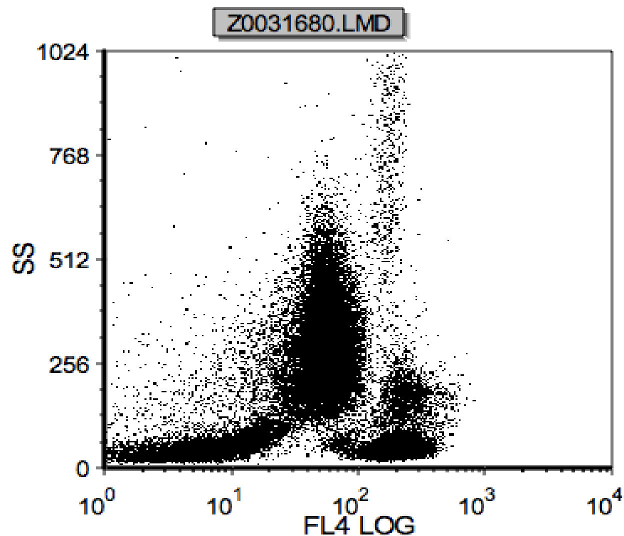


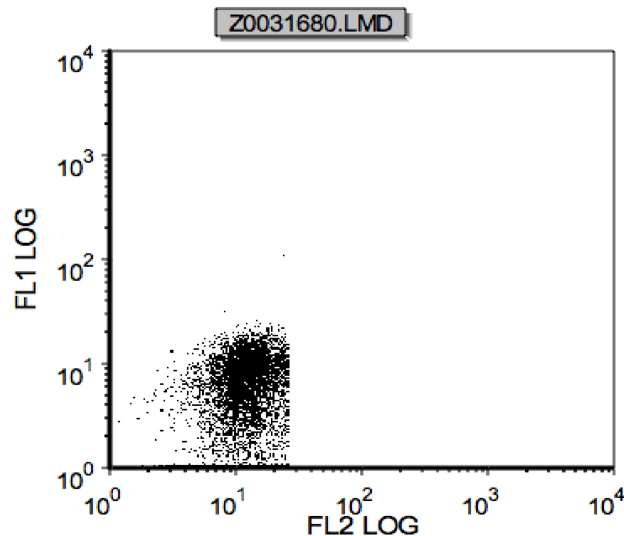


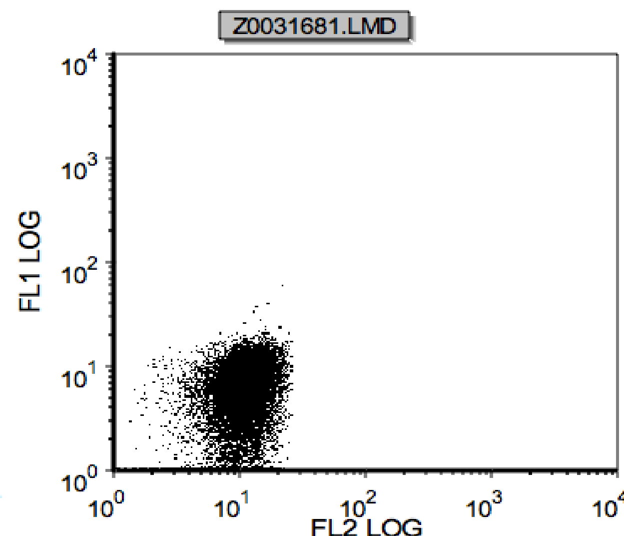


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031654.LMD	None	50889	42,85	0,0	100,0	100,0
1	Z0031654.LMD	Gate 1	12953	213,69	36,56	25,45	25,45
1	Z0031654.LMD	Gate 2	5627	213,34	34,34	11,06	11,06
1	Z0031654.LMD	Gate 3	1	248,05	37,0	0,0	0,0
1	Z0031654.LMD	Gate 4	0	0,0	0,0	0,0	0,0

Sample 1 Day 2

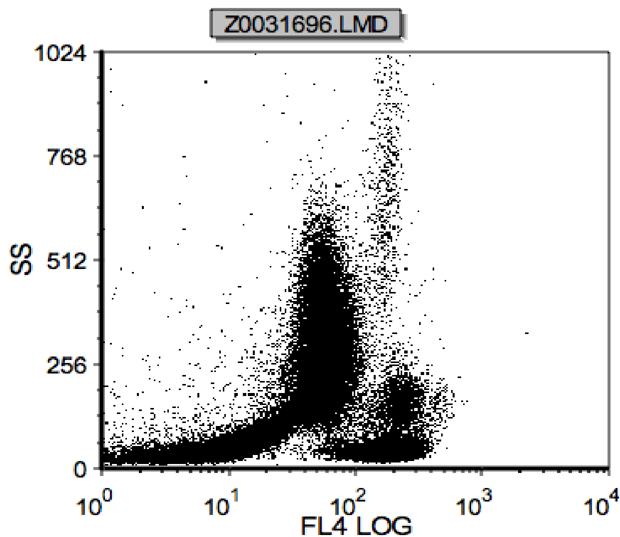


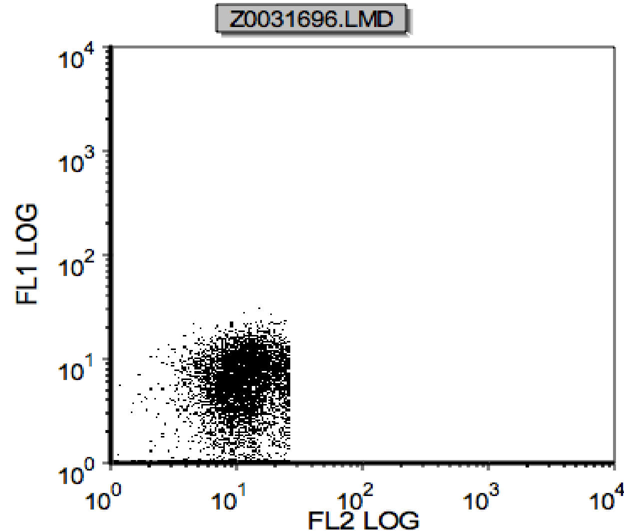


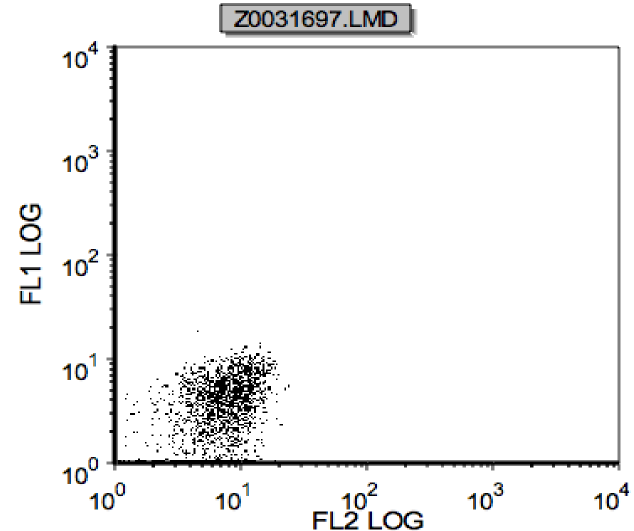


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031680.LMD	None	50278	46,87	105,34	100,0	100,0
1	Z0031680.LMD	Gate 1	13076	203,12	37,87	26,01	26,01
1	Z0031680.LMD	Gate 2	5729	204,05	37,15	11,39	11,39
1	Z0031680.LMD	Gate 3	2	196,32	28,98	0,0	0,0
1	Z0031680.LMD	Gate 4	0	0,0	0,0	0,0	0,0

Sample 1 Day 3

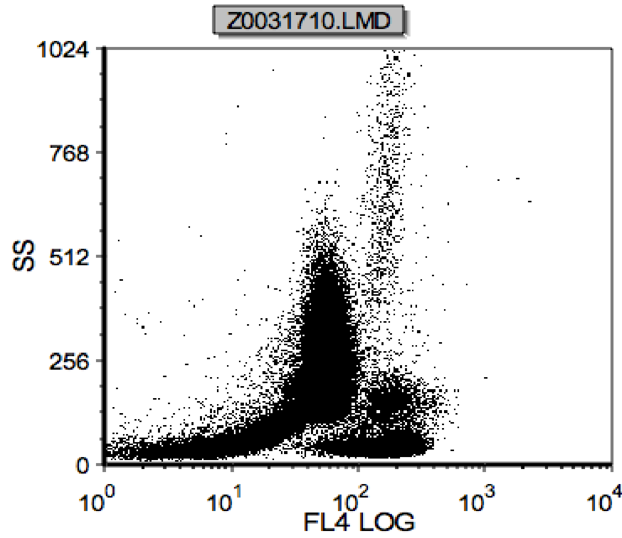


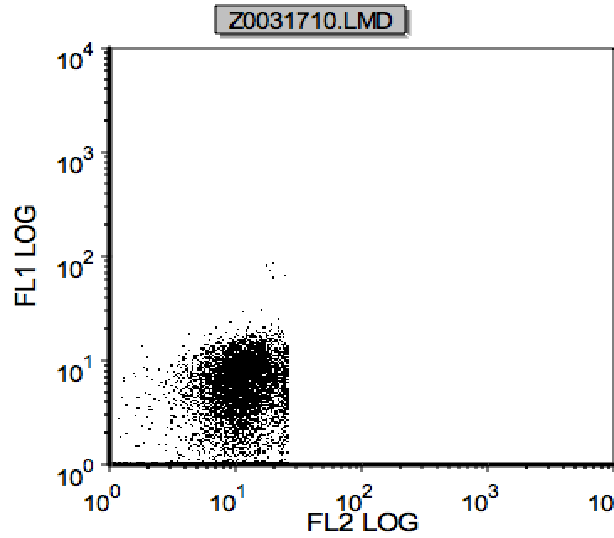


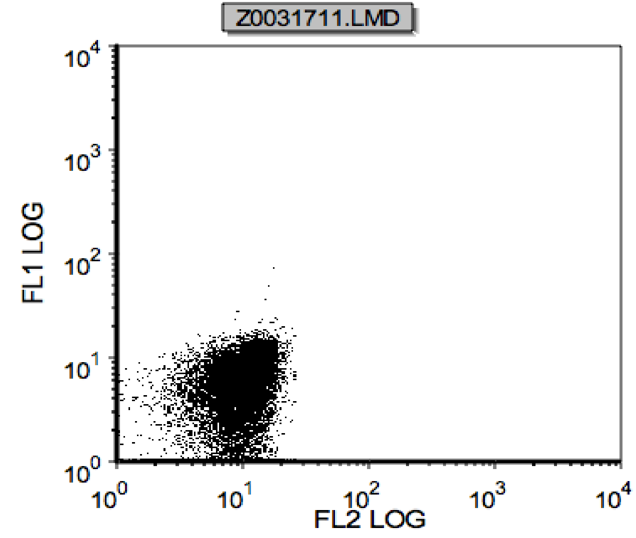


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031696.LMD	None	56573	38,43	0,0	100,0	100,0
1	Z0031696.LMD	Gate 1	13561	181,42	35,14	23,97	23,97
1	Z0031696.LMD	Gate 2	6424	184,41	34,3	11,36	11,36
1	Z0031696.LMD	Gate 3	0	0,0	0,0	0,0	0,0
1	Z0031696.LMD	Gate 4	0	0,0	0,0	0,0	0,0

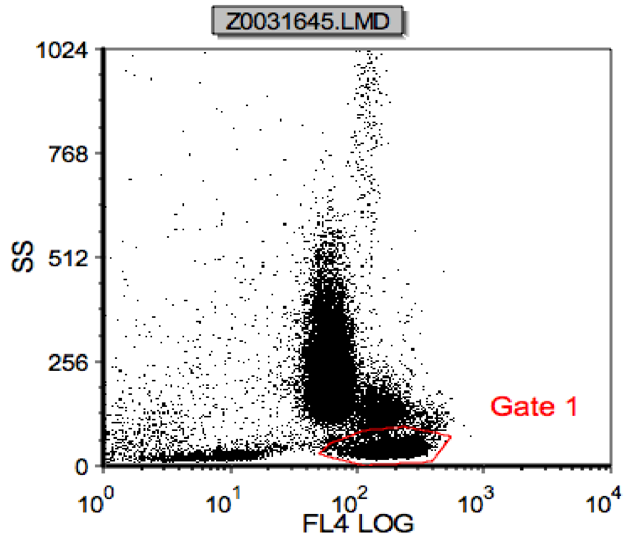
Sample 1 Day 4

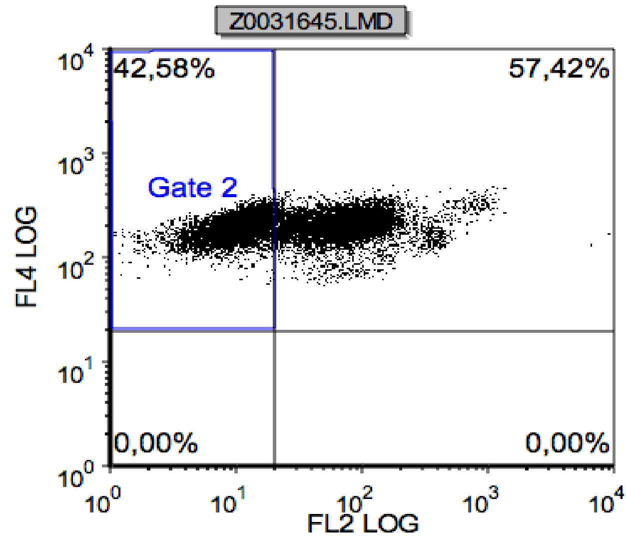


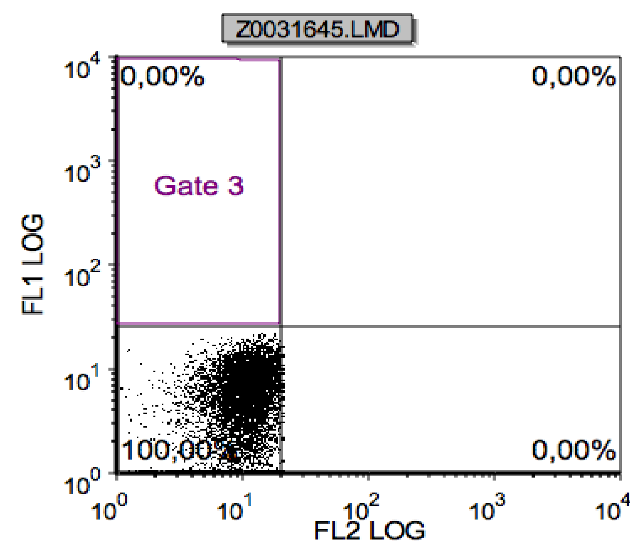


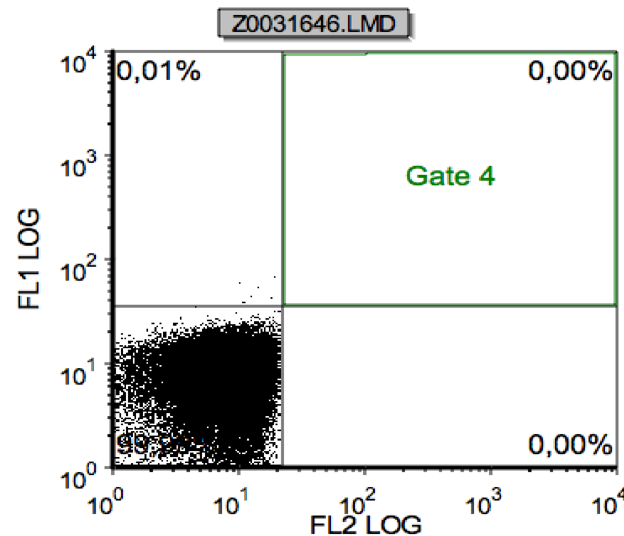


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031710.LMD	None	58035	45,72	0,0	100,0	100,0
1	Z0031710.LMD	Gate 1	13910	169,33	35,48	23,97	23,97
1	Z0031710.LMD	Gate 2	6839	176,43	34,92	11,78	11,78
1	Z0031710.LMD	Gate 3	7	191,1	38,12	0,01	0,01
1	Z0031710.LMD	Gate 4	0	0,0	0,0	0,0	0,0









Sample 2 Day 0 (sample collection)

Gate 1: Lymphocytes

Gate 2: CD31 negative cells

Gate 3: CK positive cells

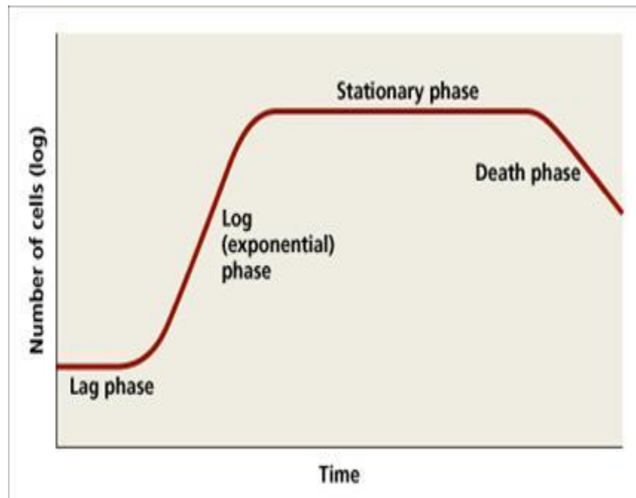
Gate 4: cMet positive cells

Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031646.LMD	None	45557	7,06	5,39	100,0	87,6
1	Z0031646.LMD	Gate 1	20981	10,12	4,17	46,05	40,34
1	Z0031646.LMD	Gate 2	45557	7,06	5,39	100,0	87,6
1	Z0031646.LMD	Gate 3	13	11,9	36,22	0,03	0,02
1	Z0031646.LMD	Gate 4	0	0,0	0,0	0,0	0,0

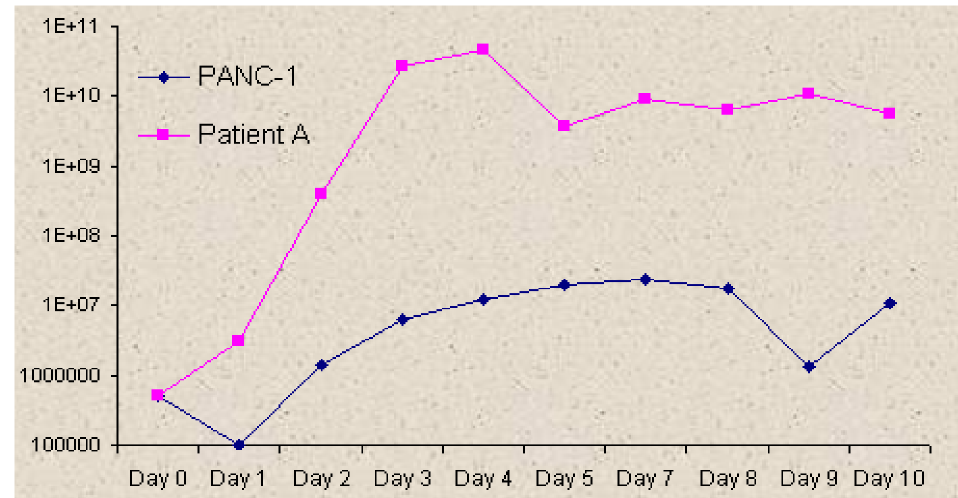
STEMNESS PHENOTYPE

Growth curve analysis

Useful model in order to study the cancer cell's growth rate over a period of time



A typical growth curve



Growth curve analysis for pancreatic cancer cells.

RELEVANT ARTICLE FOR EXPAND CTCs & CSCs

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112 *Current Stem Cell Research & Therapy*, 2014, *9*, 112-116

Comparison of the Growth Curves of Cancer Cells and Cancer Stem Cells

Maria Toloudi[1], Eleni Ioannou[1], Marina Chatziioannou[1], Panagiotis Apostolou[1], Christos Kiritsis[2], Stella Manta[2], Dimitrios Komiotis[2] and Ioannis Papasotiriou[1,*]

[1]*Research Genetic Cancer Centre Ltd (R.G.C.C. Ltd), 115 M. Alexandrou str., Filotas 53070, Florina, Greece;* [2]*Department of Biochemistry & Biotechnology, University of Thessaly, 26 Ploutonos str., Larissa 41221, Greece*

Abstract: A fundamental problem in cancer research is identification of the cells responsible for tumor formation. The latest field of cancer research has revealed the existence and role of cancer stem cells (CSCs). These findings support the idea that malignancies originate from a small fraction of cancer cells that show self-renewal and multi- or pluripotency. Identification of this CSC population has important implications for the management of cancer patients, including diagnostic and predictive laboratory assays as well as novel therapeutic strategies that specifically target CSCs. In this study, we investigated the growth rates of CSC populations for comparison with cancer cell lines. To construct the growth curves, blood-derived CSCs were isolated from patients with breast, colon, or lung cancer and cultured *in vitro*. Quantitative real-time PCR was then performed to identify CSCs in the samples. We found that CSCs did not follow the common pattern of a typical growth curve of mammalian cells in contrast to the cancer cell lines. This observation of rapidly growing CSCs indicates their involvement in tumor formation.

Keywords: Cancer stem cells, growth curves, Nanog, Oct3/4, Sox2.

Published: January 2011
Vol. 4, No. 1, 2011

Correlation between Cancer Stem Cells and Circulating Tumor Cells and Their Value

Maria Toloudi, Panagiotis Apostolou, Marina Chatziioannou, Ioannis Papasotiriou

Research Genetic Cancer Center (R.G.C.C. Ltd.), Filotas, Greece

Case Rep Oncol 2011;4:44-54

114 *Current Stem Cell Research & Therapy*, 2014, *Vol. 9, No. 2*

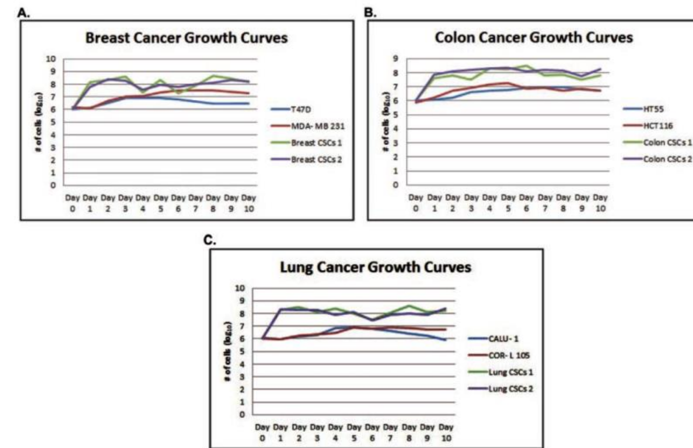
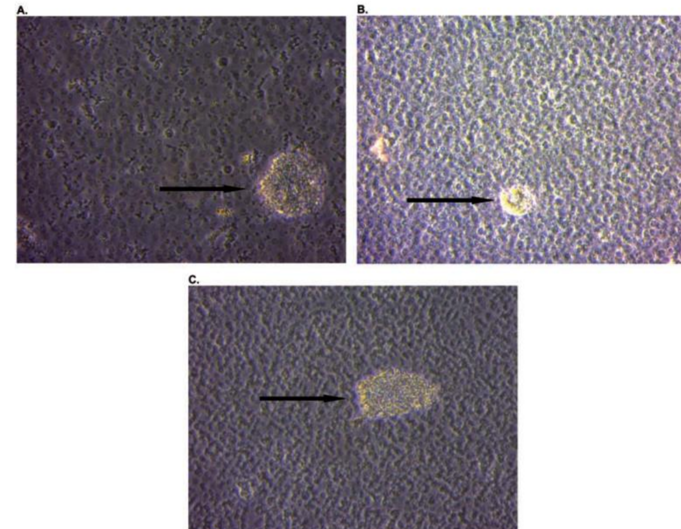


Fig. (1). Growth curves of each cancer type.

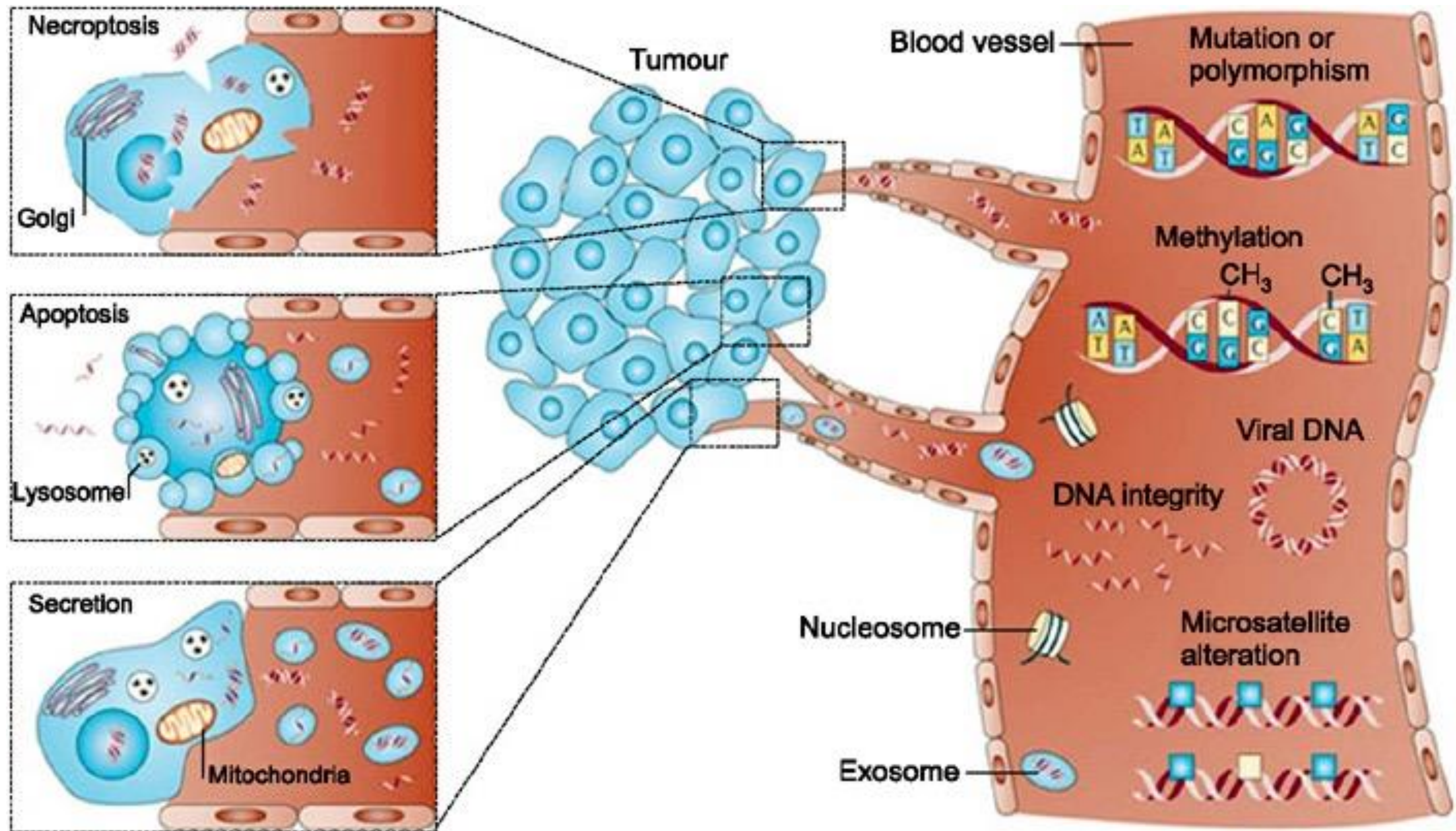


CHOOSING THE RIGHT METHOD

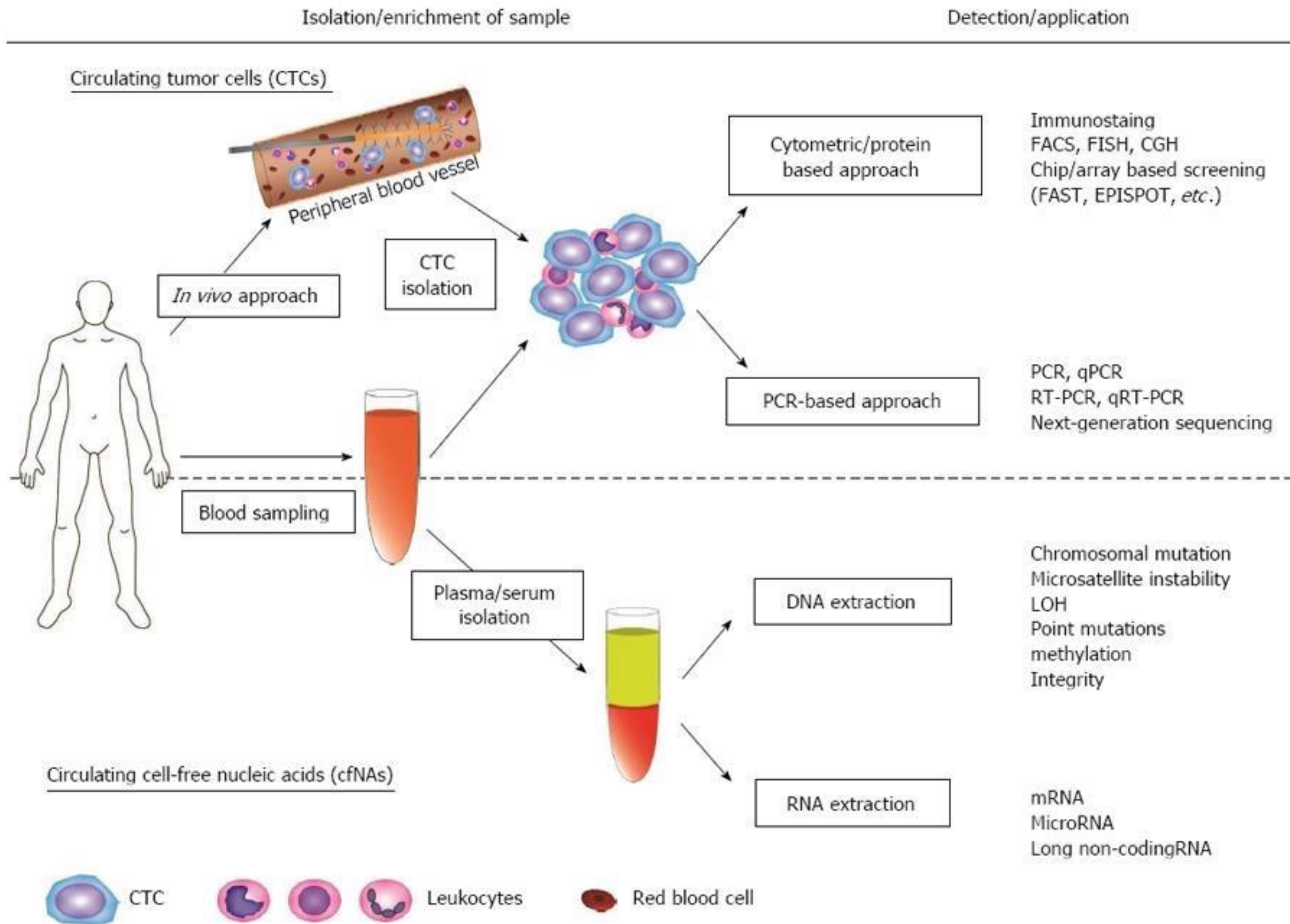
Method	Mechanism	Volume of blood used (ml)	Capture rate	References
Density gradient centrifugation	Differential migration of CTCs during centrifugation	Variable	70%	Rosenberg et al., 2002; Gertler et al., 2003; Kuhn and Bethel, 2012
Size-dependent selection	Separation based on cell diameter	6–7.5	90%	Vona et al., 2000; Lin et al., 2010; Farace et al., 2011
Immunomagnetic bead-based capture (CellSearch)	Positive selection using EpCAM coated magnetic beads	7.5	85%	Tibbe et al., 2002; Allard et al., 2004; Balic et al., 2005
Antibody-based negative selection	Depletion of normal blood cells using CD-45 coated magnetic beads	2.5 ml	52–88.4%	Wang et al., 2000; Zigeuner et al., 2000, 2003; Jatana et al., 2010; Liu et al., 2011; Schmidt et al., 2004
Flow cytometry	Cell sorting using fluorescently labeled epithelial antigens	NA	NA	Racila et al., 1998; He et al., 2008; Wu et al., 2011
Microfluidic device	Positive selection of CTCs using antibodies attached to microfluidic device	1–5.1	60–91.8%	Nagrath et al., 2007; Gleghorn et al., 2010; Stott et al., 2010a,b; Mayer et al., 2011; Kirby et al., 2012; Santana et al., 2012

Dimond et al, Frontiers of Oncology, 2012

CIRCULATING TUMOR DNA



CTCs vs cftDNA



CHOOSING THE RIGHT METHOD

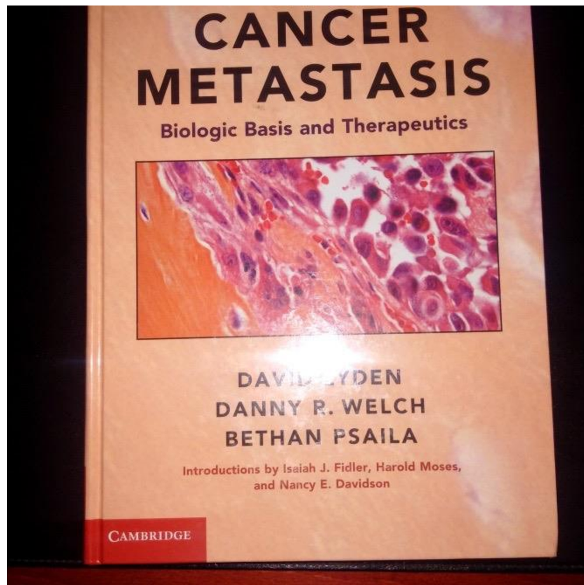
	CTCs	ctDNA
Concentration	1-30cells/ml of blood	180ng/ml cfDNA(0.01%-1% of ctDNA in cfDNA)-
Isolation/detection	-by biological properties (eg. immunoaffinity of antibody and cell surface antigen), - by physical properties (eg. CTCs' density, size and surface charge) - directly analyzing CTCs in the blood (-)Rare 'events' – isolation technically challenging, profiling may be more costly if necessary to also profile blood background (-) Sampling bias of captured cells – affinity based, size based selection (-)Single-cell/ low cell number sequencing challenging (heterogeneity observed could be biological or technical bias)	By commercial kit (for isolation of cfDNA) +Technically easier to isolate than CTCs + DNA is more stable than cells or RNA (-)Not all DNA mutations are expressed (-) Limitation of available material (NGS detection of mutations < 1% AF challenging) (-)Blood cell death under therapy could spike ctDNA fraction (not reflecting cancer cell death) (-) Source not clear – lytic, apoptotic tumor cells or are they derived from CTCs (-)Large background of 'normal' cfDNA (detected in healthy volunteers)
Characterization	Phenotypic and genotypic analysis (FISH, target PCR, DNA sequencing, RT-PCR and RNA-seq)- Despite their heterogeneity CTCs strongly express EpCam and cytokeratins	Only genotypic analysis (droplet digital PCR (ddPCR),BEAMing Safe-Seq, Tamseq)- need for significant protocol optimization and known mutational targets for analysis.
Applications	-Early diagnosis -Tumor progression in all stages -drug susceptibility test -studying CTCs gives informations on therapeutic targets and resistance mechanisms at the protein, RNA, and genome levels.	-tumor's grade, stage, -estimate tumor progression in late stage cancer

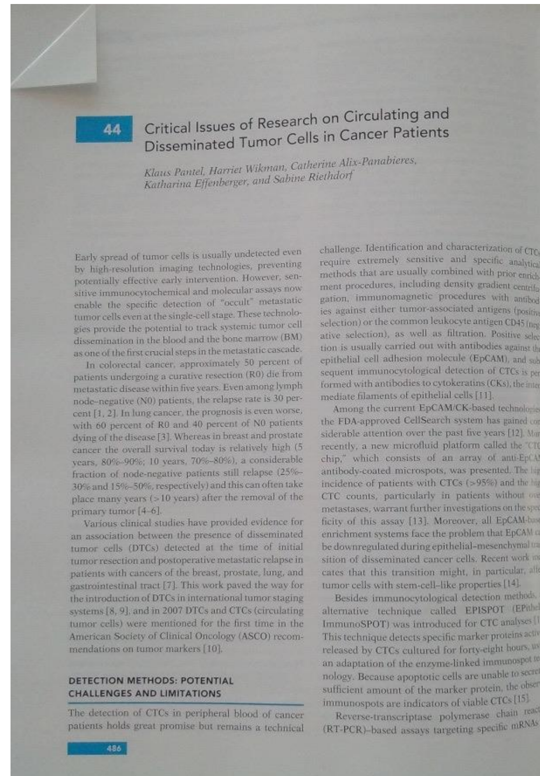
CHOOSING THE RIGHT METHOD

	CTCs	ctDNA
Test index	**-Count** **-Marker proteins** **-Mutation** **-DNA methylation** **-RNA expression profile**	**-Concentration** **-DNA integrity** **-Microsatellite alterations** **-Mutation** **-DNA methylation**
Specificity	-high specificity	**-Low specificity because of cfDNA from normal tissues** **-False negative(low level of mutated DNA in the whole DNA extract)and false positive results**
FDA approval	**Counts of CTCs have been approved from FDA in prostate colorectal and metastatic breast cancer**	**Not applicable**

LEARN BY EXAMPLES

- Search the literature
- Search for sensitivity & Specificity





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Critical Issues of Research on Circulating and Disseminated Tumor Cells in Cancer Patients

Klaus Pantel, Harriet Wikman, Catherine Alix-Panabieres, Katharina Effenberger, and Sabine Riethdorf

Early spread of tumor cells is usually undetected even by high-resolution imaging technologies, preventing potentially effective early intervention. However, sensitive immunocytochemical and molecular assays now enable the specific detection of "occult" metastatic tumor cells even at the single-cell stage. These technologies provide the potential to track systemic tumor cell dissemination in the blood and the bone marrow (BM) as one of the first crucial steps in the metastatic cascade.

In colorectal cancer, approximately 50 percent of patients undergoing a curative resection (R0) die from metastatic disease within five years. Even among lymph node–negative (N0) patients, the relapse rate is 30 percent [1, 2]. In lung cancer, the prognosis is even worse, with 60 percent of R0 and 40 percent of N0 patients dying of the disease [3]. Whereas in breast and prostate cancer the overall survival today is relatively high (5 years, 80%–90%; 10 years, 70%–80%), a considerable fraction of node-negative patients still relapse (25%–30% and 15%–50%, respectively) and this can often take place many years (>10 years) after the removal of the primary tumor [4–6].

Various clinical studies have provided evidence for an association between the presence of disseminated tumor cells (DTCs) detected at the time of initial tumor resection and postoperative metastatic relapse in patients with cancers of the breast, prostate, lung, and gastrointestinal tract [7]. This work paved the way for the introduction of DTCs in international tumor staging systems [8, 9], and in 2007 DTCs and CTCs (circulating tumor cells) were mentioned for the first time in the American Society of Clinical Oncology (ASCO) recommendations on tumor markers [10].

DETECTION METHODS: POTENTIAL CHALLENGES AND LIMITATIONS

The detection of CTCs in peripheral blood of cancer patients holds great promise but remains a technical challenge. Identification and characterization of CTCs require extremely sensitive and specific analytical methods that are usually combined with prior enrichment procedures, including density gradient centrifugation, immunomagnetic procedures with antibodies against either tumor-associated antigens (positive selection) or the common leukocyte antigen CD45 (negative selection), as well as filtration. Positive selection is usually carried out with antibodies against the epithelial cell adhesion molecule (EpCAM), and subsequent immunocytological detection of CTCs is performed with antibodies to cytokeratins (CKs), the intermediate filaments of epithelial cells [11].

Among the current EpCAM/CK-based technologies, the FDA-approved CellSearch system has gained considerable attention over the past five years [12]. More recently, a new microfluid platform called the "CTC-chip," which consists of an array of anti-EpCAM antibody-coated microspots, was presented. The high incidence of patients with CTCs (>95%) and the high CTC counts, particularly in patients without overt metastases, warrant further investigations on the specificity of this assay [13]. Moreover, all EpCAM-based enrichment systems face the problem that EpCAM can be downregulated during epithelial–mesenchymal transition of disseminated cancer cells. Recent work indicates that this transition might, in particular, affect tumor cells with stem-cell–like properties [14].

Besides immunocytological detection methods, an alternative technique called EPISPOT (EPithelial ImmunoSPOT) was introduced for CTC analyses [11]. This technique detects specific marker proteins actively released by CTCs cultured for forty-eight hours, using an adaptation of the enzyme-linked immunospot technology. Because apoptotic cells are unable to secrete a sufficient amount of the marker protein, the observed immunospots are indicators of viable CTCs [15].

Reverse-transcriptase polymerase chain reaction (RT-PCR)–based assays targeting specific mRNAs are

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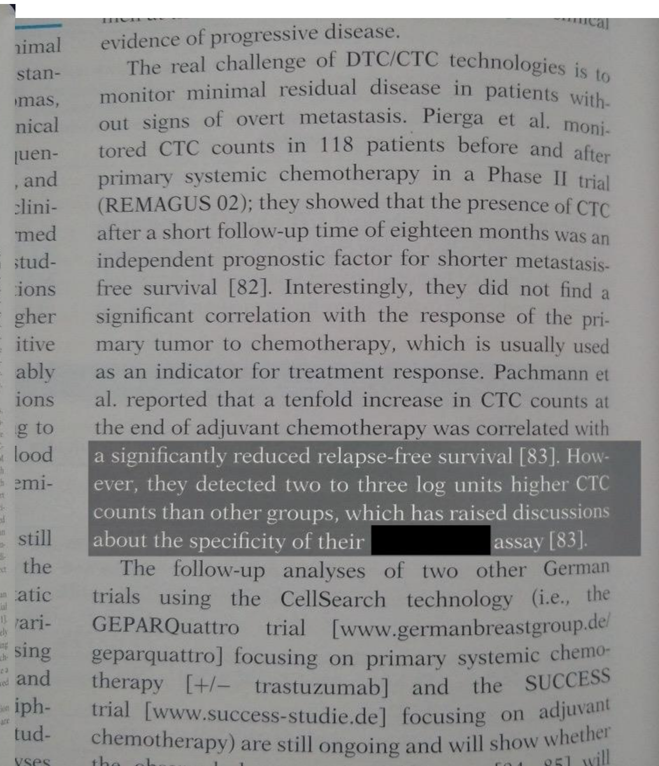


evidence of progressive disease.

The real challenge of DTC/CTC technologies is to monitor minimal residual disease in patients without signs of overt metastasis. Pierga et al. monitored CTC counts in 118 patients before and after primary systemic chemotherapy in a Phase II trial (REMAGUS 02); they showed that the presence of CTC after a short follow-up time of eighteen months was an independent prognostic factor for shorter metastasis-free survival [82]. Interestingly, they did not find a significant correlation with the response of the primary tumor to chemotherapy, which is usually used as an indicator for treatment response. Pachmann et al. reported that a tenfold increase in CTC counts at the end of adjuvant chemotherapy was correlated with a significantly reduced relapse-free survival [83]. However, they detected two to three log units higher CTC counts than other groups, which has raised discussions about the specificity of their [redacted] assay [83].

The follow-up analyses of two other German trials using the CellSearch technology (i.e., the GEPARQuattro trial [www.germanbreastgroup.de/geparquattro] focusing on primary systemic chemotherapy [+/– trastuzumab] and the SUCCESS trial [www.success-studie.de] focusing on adjuvant chemotherapy) are still ongoing and will show whether

Learn by examples

Individualised diagnosis & therapies
based on molecular medicine

Patient — DOB 01/01/1959
4 Budd Street, Brighton, VIC 3186, Australia

Practitioner — Peter Eng
24-26 Armstrong Street, Middle Park, VIC 3206, Australia

Request — Final , CTC Count , Androgen Receptor , Ki67 ,

The Genostics Summary Letter is based on data received from the Molecular Medicine Laboratory which has carried out the testing. Please refer to the original results which are attached to this email. The Genostics Summary Letter and the original results are not a prescription for medical treatment. The results and expert opinion however can be taken into account when deciding on medical management.

Results	
CTC	500 cells/ml of HEA positive cells Slight to Moderately elevated PSA=46.7%, AR=58.5%, Ki67=82%, Tu Spheres = 60 %

FINAL REPORT

Circulating Tumour Cells have been detected.
The number is elevated at 500 cells/ml.
CTCs are defined here by positive Epithelial Cell Antigen (HEA) and reported as cell numbers/ml
The detection level is 10 cells / ml.
Additional identifiers of cell fragmentation are noted. See report and comments by Prof K Pachmann

The cells are identified by Laserscanning Microfluorimetry.
Maintrac Testing is reported by Laboratory Pachmann, Bayreuth, Germany. The method is accredited to the standard of ISO 15189 (DAkkS)
NATA (the Australian Accrediation Authority) and DAKKS (the German Accrediation Authority) are both signatories to ILAC (the International Laboratory Accreditation Cooperation) and their MRA (Mutual Recognition Arrangement) for further info: www.ilac.org/ilac-mra-and-signatories

Ki67 as a marker of activity within the cell growth phase was positive in 82 % of identified CTCs.
PSA expression was noted on 46.6 % of CTCs
Androgen Receptor expression was noted on 58.5 % of CTCs

As per request, testing for the in-vitro development for Tumour Sphere Units has been initiated.
60 % of the identified CTCs developed Tumour Spheres in laboratory conditions.
Further diagnostic testing is advisable
A follow upseries of CTC tests is recommended

Sincerely

Literature and publications are available on request.

Labor Dr. med. Ulrich Pachmann, Kurpromenade 2, 95448 Bayreuth

Bayreuth, 08.01.2016

Your patient:
Born: **01.01.1959**

Your request from: 07.12.2015
Our Lab number: T525732

Final Report to the Partial - Report on diagnostic findings on Circulating Tumor Cel... m 14.12. 2015

Dear Dr.

Many thanks for sending your examination request regarding the detection of circulating tumor cells.

Diagnosis: Screening – ISET +

The automated microfluorimetric image analysis of the **epithelial cell antigen (HEA)**-positive cells with visual control (MAINTRAC) from **1 ml EDTA blood** resulted in following findings (detection limit is at 10 cells/ml):

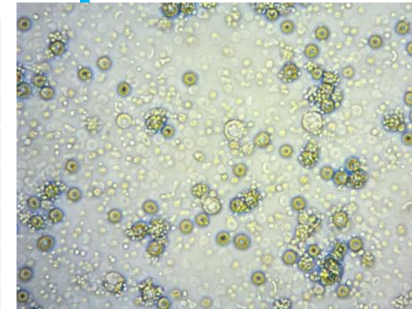
	Number of epithelial cell antigen (HEA)-positive cells			
Examination parameter	In the sample (1ml)	In circulation (5l) (in millions)	In addit. examination: % of HEA-pos. cells	Cell fragments
HEA	**500**	**2,50**		numerous
PSA	**233**	**1,17**	**46,7%**	
AR	**292**	**1,46**	**58,5%**	
Ki67	**410**	**2,05**	**82,0%**	
Spheroid-forming Cells			**60%**	

The material you sent for examination could be thoroughly evaluated.
We found a **slightly to moderately increased number of epithelial cell antigen (HEA)-positive circulating in the blood.**
About a half of the cells express the **Prostate-specific-Antigen** and more than half express the **Androgen receptor,**
A large part of the cells is in the **growth phase** of the cell cycle (**Ki67-Index 82%**).
In addition, there were numerous specific cell fragments detected.
Specific cell fragments occur, for example, as part of an immune response and indicate damaged cells.

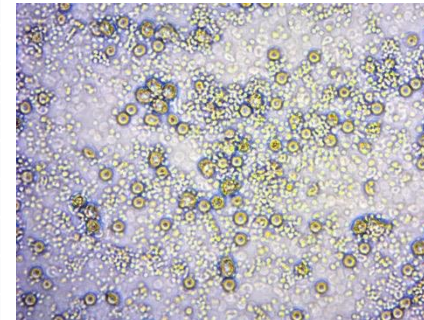
During cultivation of epithelial-antigen positive cells we found growth of Spheroid-forming Cells. The impact of this finding is not clear yet.

Learn by examples

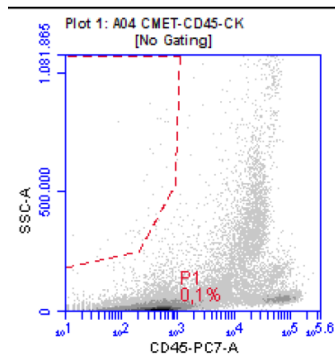
CD45 positive cells (Hematologic origin cells)		CD45 negative cells (non Hematologic origin)	
CD15	NEGATIVE	CD34	NEGATIVE
CD30	NEGATIVE	CD99	NEGATIVE
BCR-ABL	NEGATIVE	EpCam	POSITIVE
CD34	NEGATIVE	VHL mut.	NEGATIVE
CD19	NEGATIVE	**CD133**	**NEGATIVE**
		Nanog	**NEGATIVE**
		Okt-4	**NEGATIVE**
		Sox-2	**NEGATIVE**
		PSMA	NEGATIVE
		c-MET	**NEGATIVE**
		CD31	POSITIVE
		CD19	NEGATIVE
		MUC-1	NEGATIVE
		CD44	**NEGATIVE**
		PAN-CK	POSITIVE

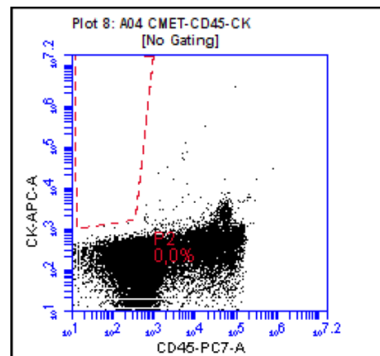


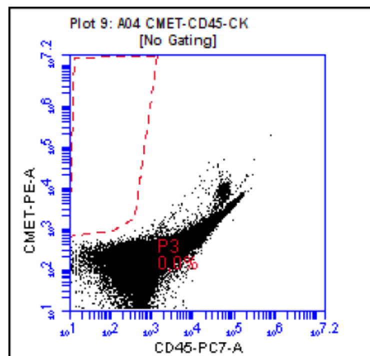
Day 0



Day 1







Plot 1: A04 CMET-CD45-CK	Count
All	50.000
P1	36

Plot 8: A04 CMET-CD45-CK	Count
All	50.000
P2	0

Plot 9: A04 CMET-CD45-CK	Count
All	50.000
P3	0

Learn by examples

Department of Cancer Imaging
Peter MacCallum Cancer Centre
St Andrews Place
East Melbourne, VIC, Australia 3002
Ph: +61 3 9656 1026 Fx: +61 3 9656 1406
Email: imaging@petermac.org

Exam Date: Tuesday, 08 March 2016
Reported Date: Tuesday, 08 March 2016

Fax: 96829218

RE:

DOB: 01/01/1959 **PMCC UR#:** 161161229

DR PETER ENG

PETx - Ga68 PSMA

Clinical notes: CTC positive, PSA 46.7. ?malignant

Radiotracer: Ga-68 prostate-specific membrane antigen (PSMA) ligand

PET/CT technique: Scanning was performed encompassing the vertex to upper thighs on a PET/CT scanner (Biograph 64). A contemporaneous low dose non-contrast multislice CT scan was performed for anatomic correlation and attenuation correction. Uptake time=82minutes..

Findings:
Primary tumour: None. There is no focal PSMA uptake in the prostate. Mild focal calcification in the prostate may be indicative of prior inflammation / prostatitis.

Nodal metastases: None.

Distant metastases: None.

Further findings: The distribution of radiotracer elsewhere is physiologic.

Conclusion: There is no PET/CT evidence of PSMA avid malignancy. Focal calcification in the prostate may represent prior inflammation / prostatitis as a possible explanation of elevated PSA level.

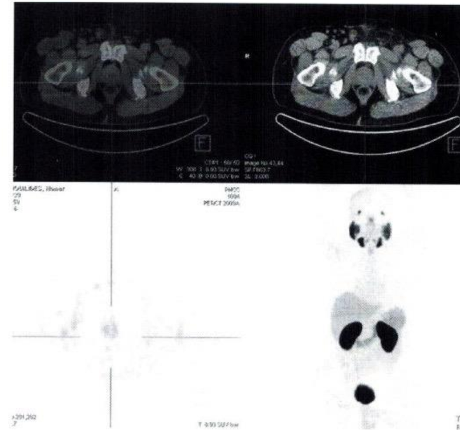
Yours sincerely,

Maria Boya
Nuclear Medicine Observership.

DR. DAVID PATTISON, MBBS (Hons), MPH, FRACP, FAANMS
Nuclear Medicine Physician

CC: Dr S Smith

Performed: 08/03/2016 Series: 1 Page: 1 of 2
Authorised: 08/03/2016



Performed: 08/03/2016 Series: 1 Page: 2 of 2
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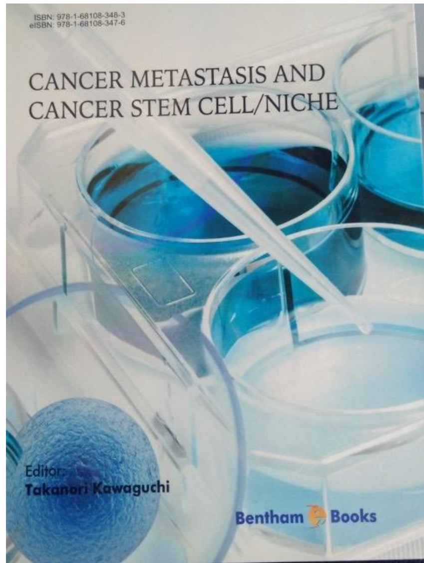
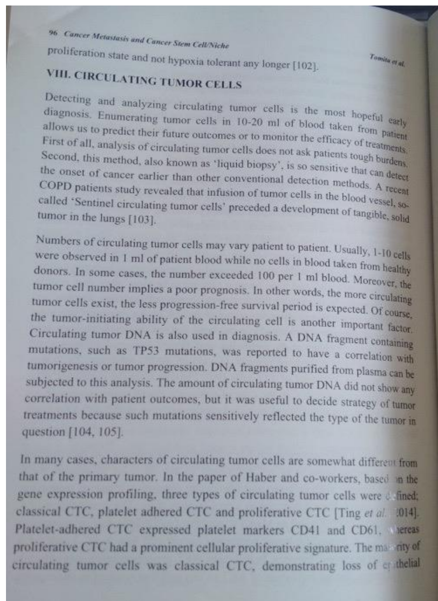
Supportive Literature





proliferation state and not hypoxia tolerant any longer [102].

VIII. CIRCULATING TUMOR CELLS

Detecting and analyzing circulating tumor cells is the most hopeful early diagnosis. Enumerating tumor cells in 10-20 ml of blood taken from patient allows us to predict their future outcomes or to monitor the efficacy of treatments. First of all, analysis of circulating tumor cells does not ask patients tough burdens. Second, this method, also known as 'liquid biopsy', is so sensitive that can detect the onset of cancer earlier than other conventional detection methods. A recent COPD patients study revealed that infusion of tumor cells in the blood vessel, so-called 'Sentinel circulating tumor cells' preceded a development of tangible, solid tumor in the lungs [103].

Numbers of circulating tumor cells may vary patient to patient. Usually, 1-10 cells were observed in 1 ml of patient blood while no cells in blood taken from healthy donors. In some cases, the number exceeded 100 per 1 ml blood. Moreover, the tumor cell number implies a poor prognosis. In other words, the more circulating tumor cells exist, the less progression-free survival period is expected. Of course, the tumor-initiating ability of the circulating cell is another important factor. Circulating tumor DNA is also used in diagnosis. A DNA fragment containing mutations, such as TP53 mutations, was reported to have a correlation with tumorigenesis or tumor progression. DNA fragments purified from plasma can be subjected to this analysis. The amount of circulating tumor DNA did not show any correlation with patient outcomes, but it was useful to decide strategy of tumor treatments because such mutations sensitively reflected the type of the tumor in question [104, 105].

In many cases, characters of circulating tumor cells are somewhat different from that of the primary tumor. In the paper of Haber and co-workers, based on the gene expression profiling, three types of circulating tumor cells were defined; classical CTC, platelet adhered CTC and proliferative CTC [Ting *et al.* 2014]. Platelet-adhered CTC expressed platelet markers CD41 and CD61, whereas proliferative CTC had a prominent cellular proliferative signature. The majority of circulating tumor cells was classical CTC, demonstrating loss of epithelial

markers (E-cadherin and MUC1) and enrichment of a stem cell marker, Aldh1a1. Comparison of gene expression patterns between primary tumor (mouse pancreatic ductal adenocarcinoma) and circulating tumor cells revealed that three extracellular matrix genes (Decorin, KLF4, IGFBP5) were remarkably upregulated in classical CTCs [106].

Considering the origin of circulating tumor cells, two types of tumor cells may exist in the bloodstream. One is an epithelial cell phenotype, holding epithelial cell adhesion molecule (EpCAM) expression. This type of cells are supposed to be pushed out passively (Passive intravasation). The other type is a mesenchymal phenotype due to EMT in the primary tumor site, having an invasion activity to attain intravasation by oneself alone (Active intravasation). These facts indicate that the circulating tumor cell is heterogeneous and not always EpCAM positive.

According to the circulating tumor cell detection system, provided images clearly showed that the majority of the cells were single cells, but clusters did also exist. Tumor cell clusters and the stromal cells (such as tumor-associated macrophages) are also possible because there are many immune cells, probably the tumor-friendly type of cells around the primary tumor. The tumor cell clusters were constituted of 2-50 tumor cells [107]. Clustered tumor cells are more likely to survive so that the metastasis rate of the clustered tumor cells is higher than that of single tumor cells.

There are two types of the detection system, label-dependent and label-independent method. In the label dependent circulating tumor cell enrichment, EpCAM/or MUC1 expressing cells are captured. For instance, FDA approved CellSearch equips magnetic beads coated with anti-EpCAM. Its capture rates are estimated roughly 70-80%, depending on tumor type. Pre-treatment with anti-CD45 coated magnetic beads as a negative depletion step may be applied to enhance the processing speed, recovery rate and purity of tumor cells. In the label-independent enrichment, cells are fractionated based on their physical properties, such as density, size, invasive capacity, or surface charge. By using these devices, circulating tumor cells initially scattered at the ratio of one in 10^6-10^8 blood cells was enriched to be one in 10^2-10^3 blood cells. Isolated circulating tumor cells were subjected to further analysis, including PCR and protein secretion [108].

SEARCH FOR LOW LIMIT OF DETECTION METHODS (LoD)

MRD in AML Clinical Practice

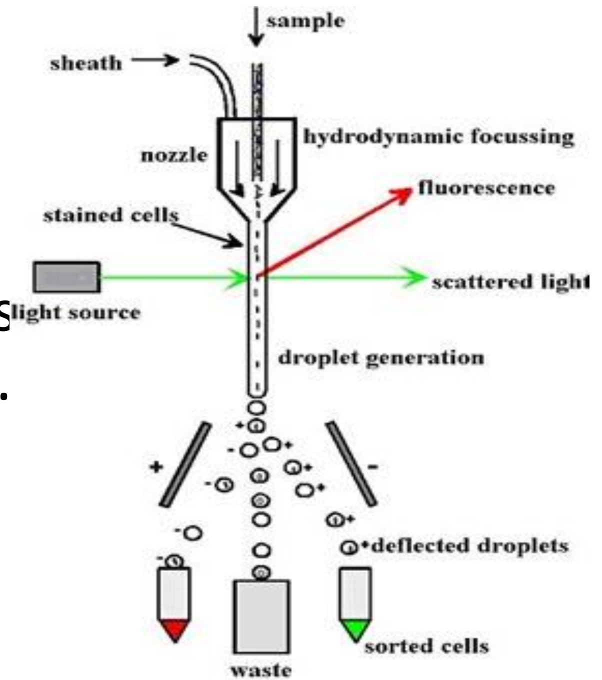
Table 1. Methods for Minimal Residual Disease Assessment

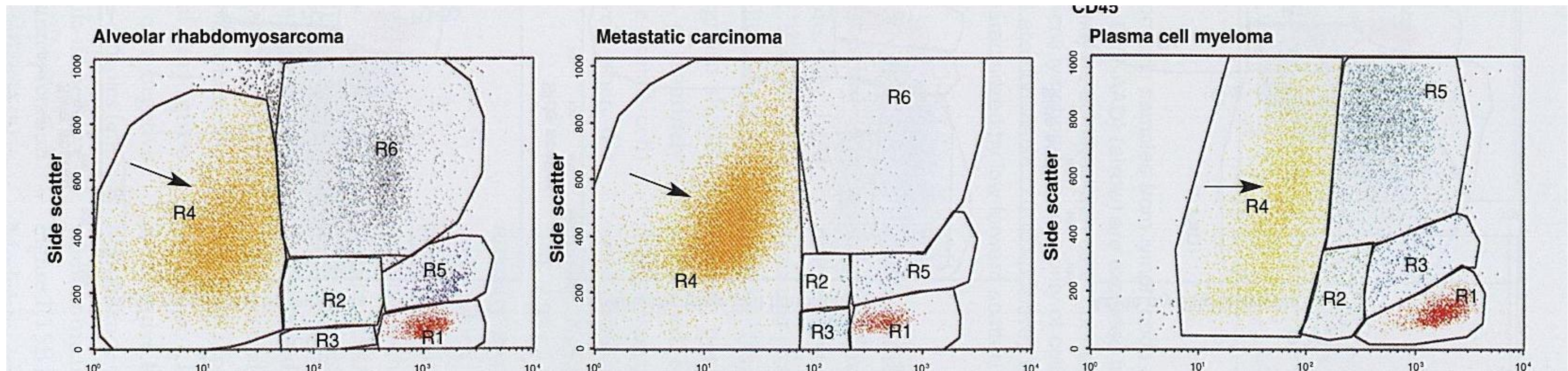
Method	Target	Sensitivity	Pros and Cons
Cytogenetics	Aberrant karyotype	1:20	Widely available; not applicable to normal karyotype AML; not sensitive
Quantitative RT-PCR	Fusion gene transcripts (eg, *PML-RARA*, *RUNX1-RUNX1T1* and *CBFB-MYH11*); recurrent gene mutations (eg, *NPM1*); overexpressed genes (eg, *WT1*)	1:10,000 to 1:100,000	Only 50%-80% of patients have appropriate target; use of RT-PCR most established for clinical care (APL and CBF AML)
Multiparameter flow cytometry	Leukemia-associated immunophenotype	1:10,000 to 1:1,000,000	Broadly applicable; technically challenging
Next-generation sequencing	Recurrent myeloid gene mutations (eg, *NPM1*, *RUNX1*, *IDH1/2*, etc)	Not well defined	New; role needs to be defined

Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CBF, core-binding factor; RT-PCR, reverse-transcribed polymerase chain reaction.

FLOW CYTOMETRY and CTCs

- Using parameters like FS, SS and fluorescence we can detect multiple antigens inside each cell.
- There are two approaches to detect CTCs positive selection and negative selection.
- FC can provide information about quantity and quality of CTCs





CHOOSING THE RIGHT METHOD

COMPARATIVE METHODS

	Beads Based Method	PCR Based Method	R.G.C.C.	Microscopy Based Method	Gradient
Method of isolation	Magnetic Beads (antibodies with iron particles	PCR based method which need to destroy the cells in order to identify one maker (mainly panCK or Epcam)	Flow cytometric sorting with interrogation in droplets in ratio of drop-let per cell (1:1)	Immobilizing cells on a slide and staning	The cells are isolated based on size
Purity of CTCs	Enrichment method and not isolation method	There are no cells any more	***Purity is higher than 97-99% (isolation method)***	The CTCs are simply stained not isolated	It is an enrichment method
Viability of the isolated cells	70-85%	No cells	***Viability >99%***	NO vialable cells remain	Questionable
Quality of CTCs for further analysis	Inappropriate for further molecular analysis due to lymphocyte contamination	Limited for further molecular amalysis	Appropriate for further molecular analysis since there is no noise	The CTCs are no longer viable	Not recommented for further studies
Selection of CTCs	Based mainly in positive selection of CTCs in a few number of markers	Based on positive selection	Based on negative and positive selection in order to identify and secondly immunophenotyping CTCs	Posible selection method	Based on size
Further abilities			Identification of hetero-geneity of CTCs	The dentification of heteroge-nete depends of the selected markers	Identification of hetero-geneity of CTCs
Additional features	Method only to enu-merate CTCs	Method to enumerate CTCs and identify only very limited features of CTCs	Method which allows to perform gene expression assays and determine features vital for therapy scheduling	A method for detection and enumeration only	

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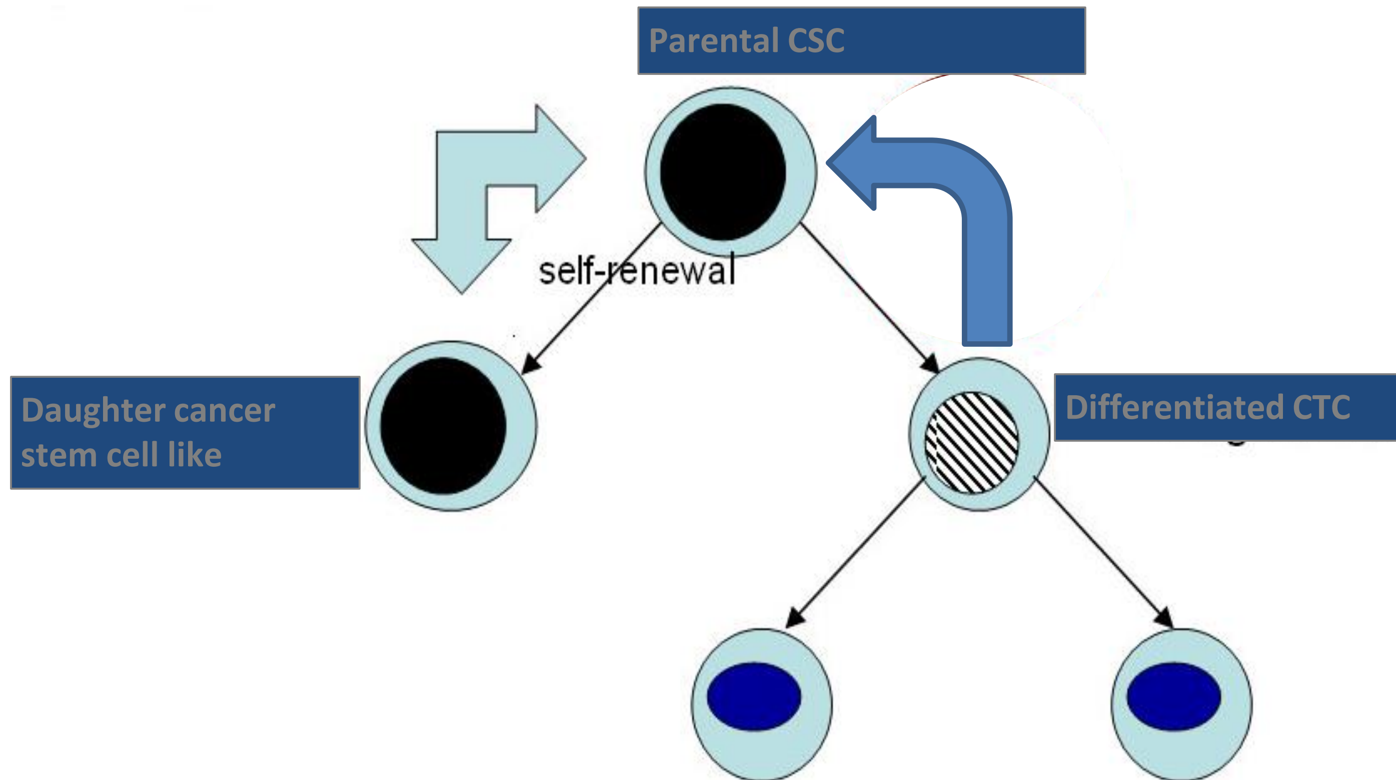
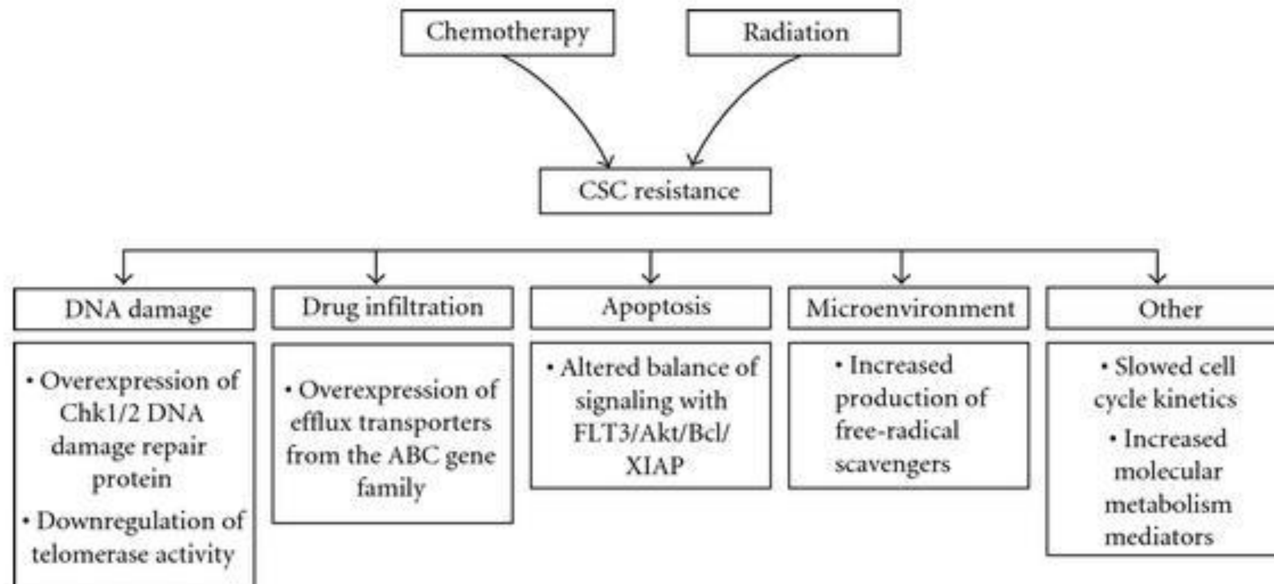
CTCs & CANCER STEM CELL LIKE OR TUMOR INITIATING CELLS

SELF – RENEWAL(CSCs)



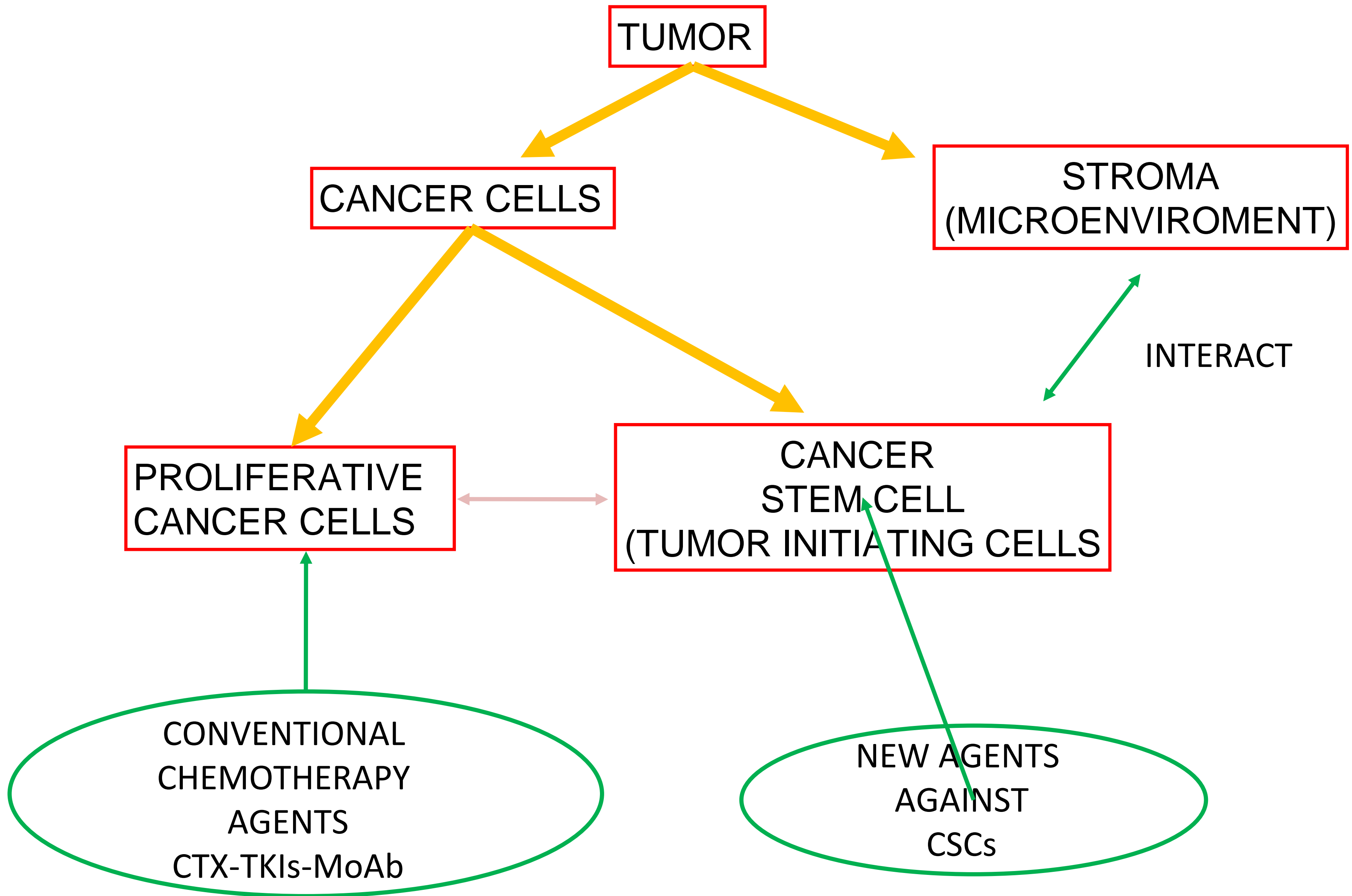
http://njms.umdnj.edu/gsbs/stemcell/scofthemonth/scofthemonth2/braincancerstemcellsci.htm

Special hallmarks of CSCs
CSC RESISTANCE



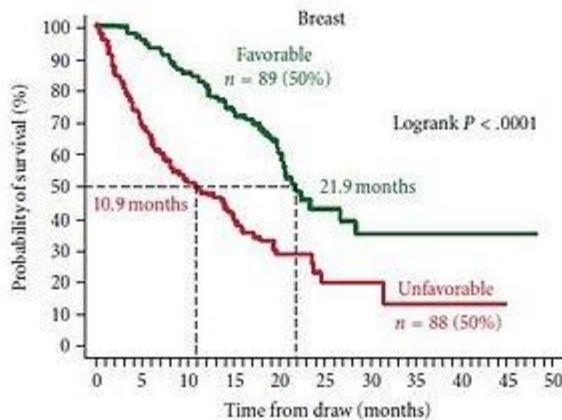
Journal of Oncology, vol 2011 , Article ID 941876, doi:10.1155/2011/941876

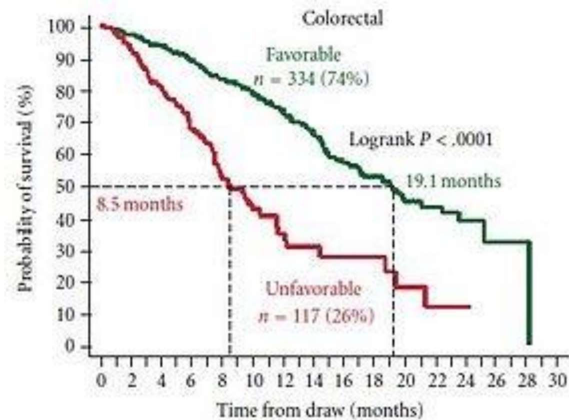
Proposed algorithm of treatment

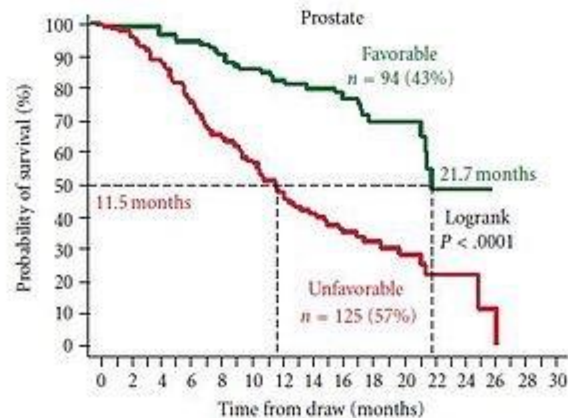


AT THE END

- Parameters of clinical value: RR, OS, DFS



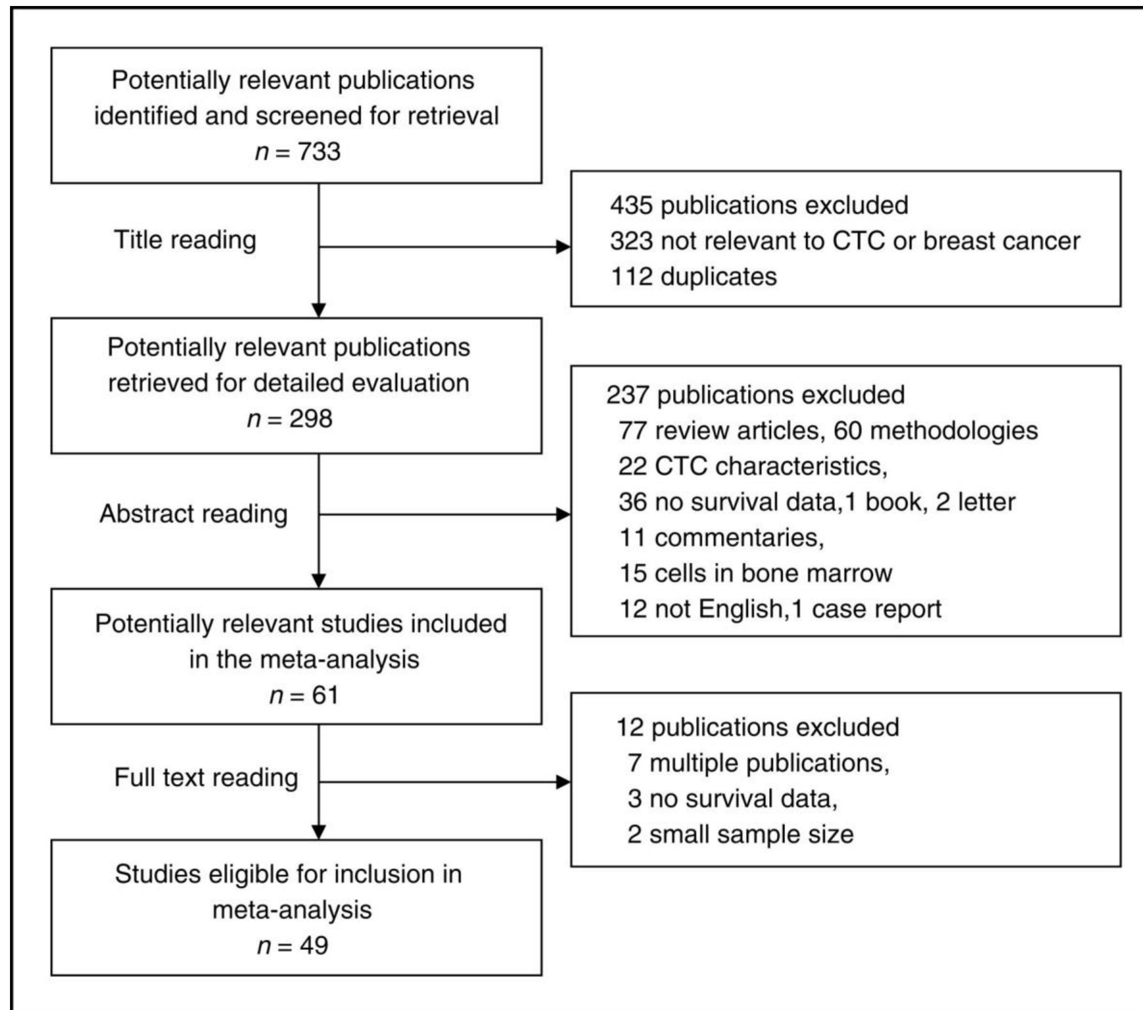




FUTUTE PERSPECTIVES

- Identify patterns of mechanism on CTCs
- Understand plasticity
- Pin point “drugable” targets
- Design tailor made therapies based on markers and molecular patterns
- Change the therapeutic concept based on understanding cancer biology

FUTUTE PERSPECTIVES



Meta-Analysis of the Prognostic Value of Circulating Tumor Cells in Breast Cancer
Zhang L et al. Clin Cancer Res 2012;18:5701-5710

Conclusion

- Liquid biopsy offers the ability for prognosis, diagnosis and treatment decision tools.
- Not all methods of liquid biopsy covers all aspects
- Be careful about parameters like LoD, LoQ, Specificity and Sensitivity of the method.
- Challenge the method about the accuracy and the clinical relevance.

QUESTIONS?

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THANK YOU FOR YOUR TIME.
Visit our web site
www.rgcc-group.com

Since the data and information are large and further questions and definition may be generated, we strongly recommend to visit RGCC group website or contact us or our distributors where more information and definition can be obtained in order to help therapist to understand what is feasible in a laboratory field and what is applicable to clinical use.

In case also of additional questions please do not hesitate to come in direct contact with RGCC International GmbH for any inquiry. The previous direct email address is specifically for this purpose.

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