R.G.C.C. International GmbH

PERSONALIZE MEDICINE IN ONCOLOGY

SIMILARITIES AND DIFFERENCES BETWEEN CTC AND CANCER CELLS: MECHANISMS AND PATHWAYS

AUTHOR-PRESENTER: DR. IOANNIS PAPASOTIRIOU

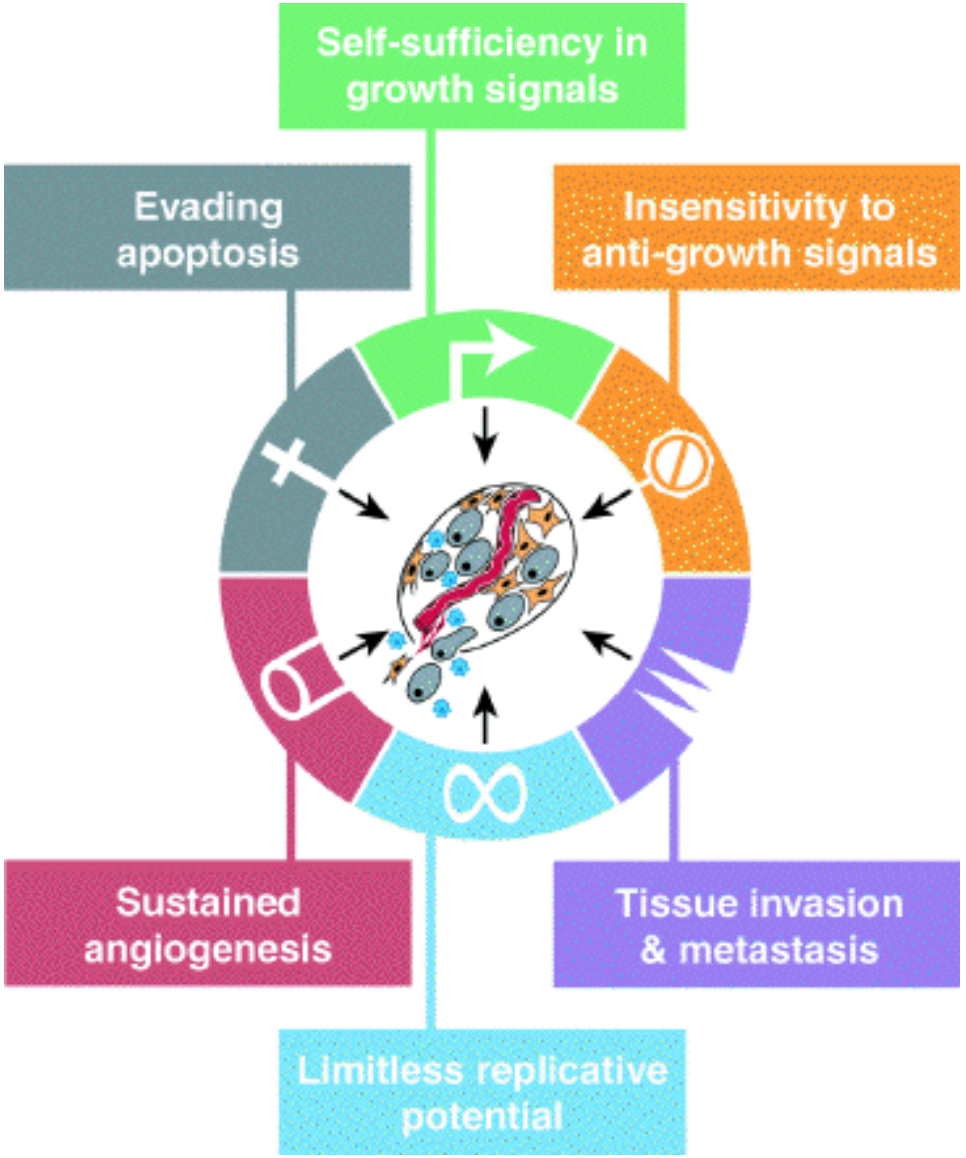
THIS PRESENTATION CONSIDERED RGCC LTD INTELECTUAL PROPERTY AND ATHE USE OF A PART OR ALL RESENTATION FOR GENERATION OF ANOTHER MATERIAL OR ADVERTISEMENTPRIOR RGCC's CONSENT, WILL BE AVIOLATION OF LAW

SCIENTIFIC BACKGROUND

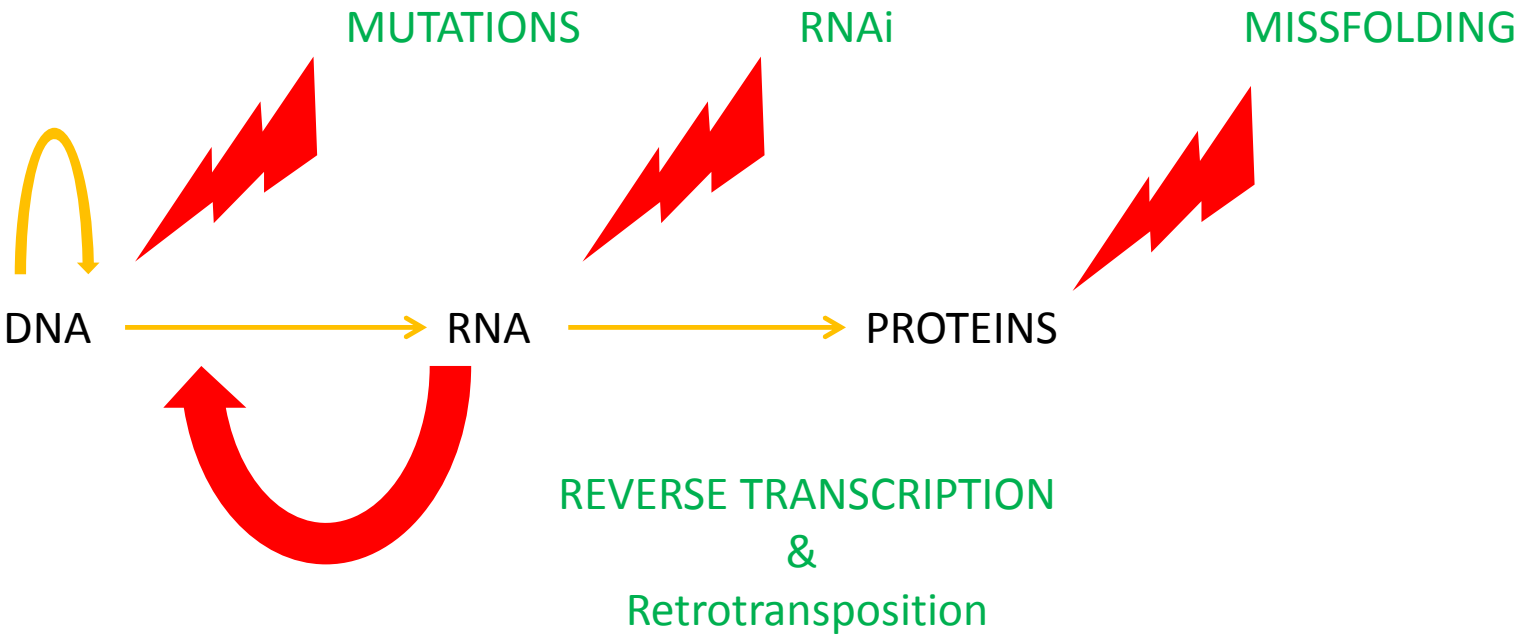
(HOW CANCER IS PROGRESSING
AND
WHICH IS THE RESPONSIBLE ENTITY WHICH CONNECTS THE DOTS)

Weinberg et al (2004)

Cancer Hallmarks



DOGMA OF BIOLOGY



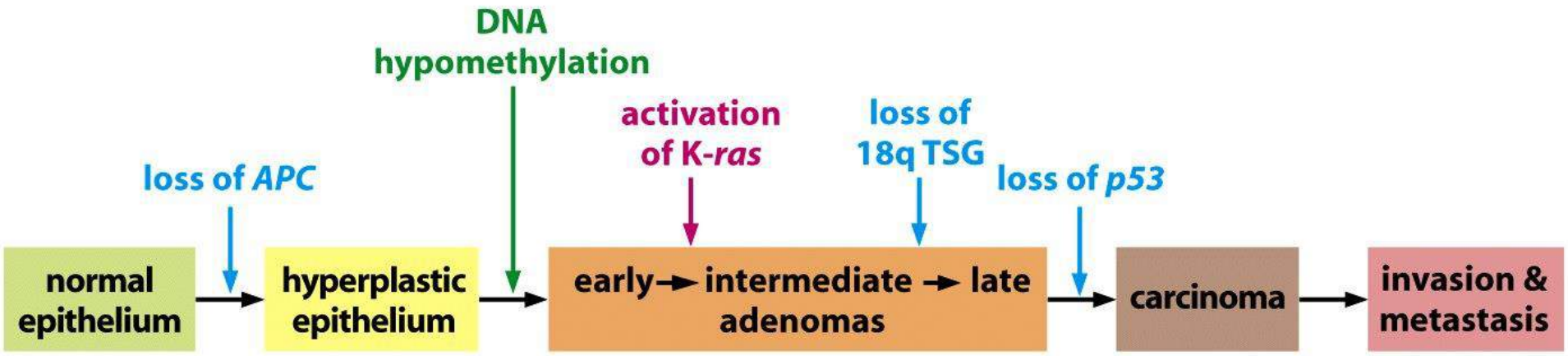
CARCINOGENESIS STEPS

INITIATION
- Viral interference
- Chemical interference
- Radiation influence

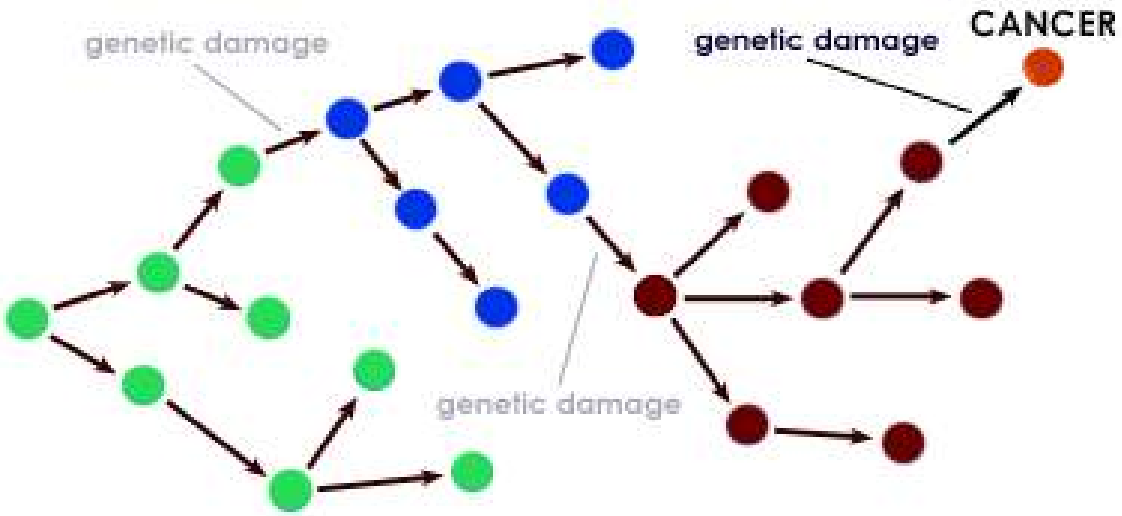
PROMOTION
- Cell cycle instability
- DNA repair aberration
- Apoptosis instabiliity

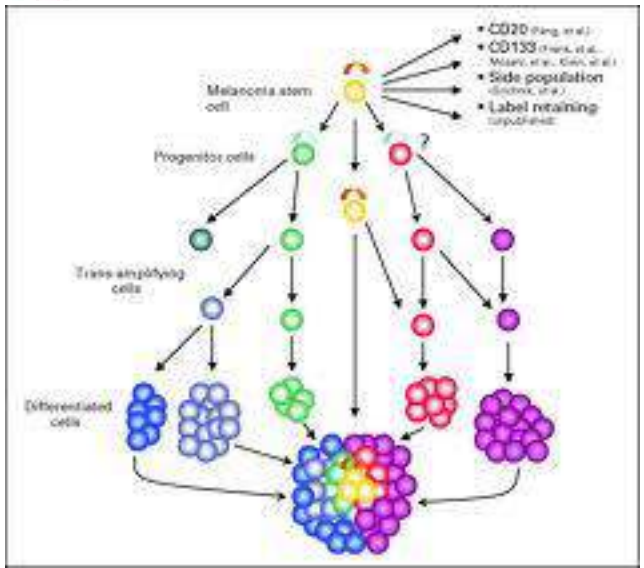
PROGRESS
- Invasion
- Neo-angiogenesis
- EMT and MET

VOGELSTEIN MODEL OF DEVELOPING COLON CANCER



Tumor heterogeneity and plasticity: a critical problem

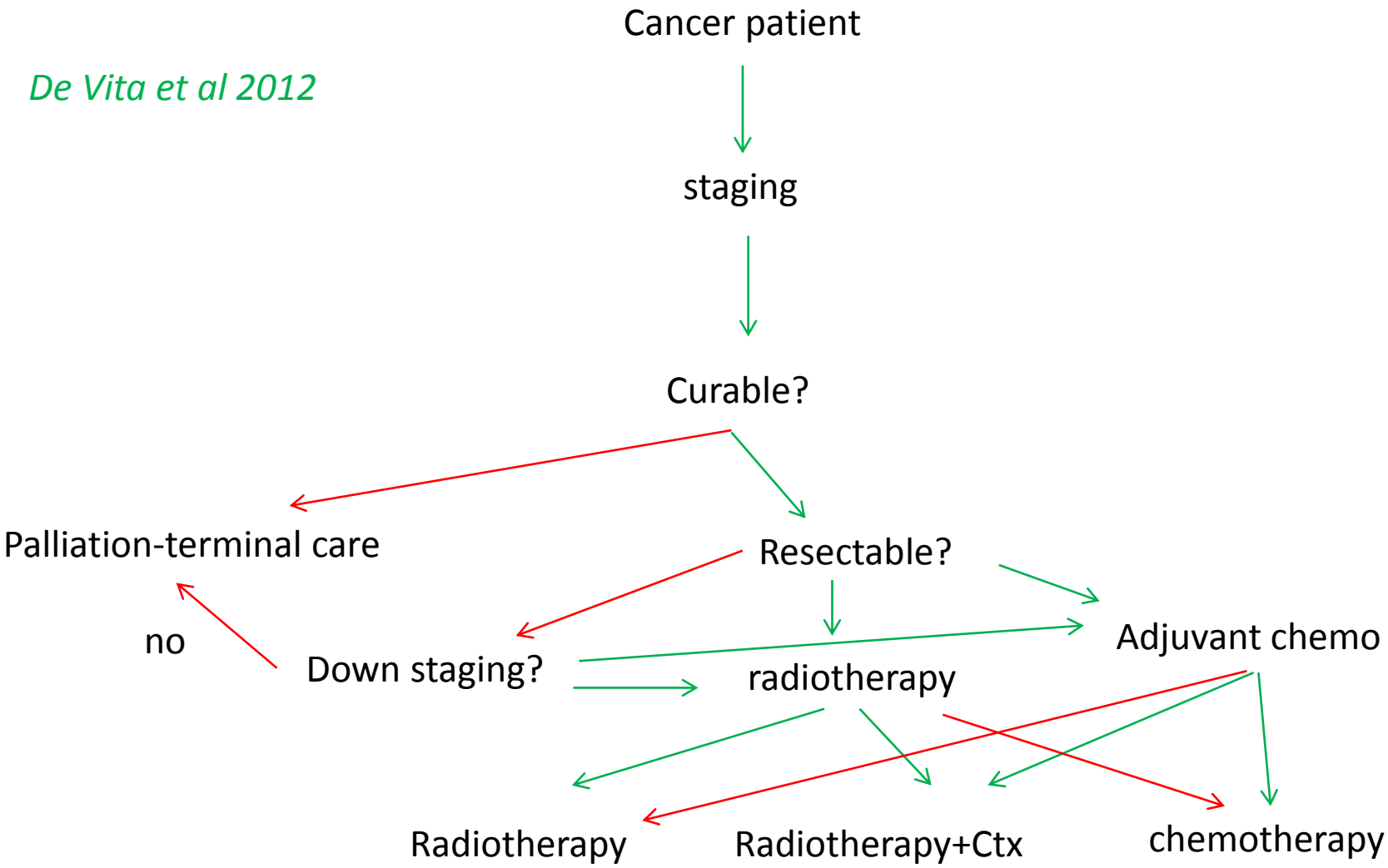




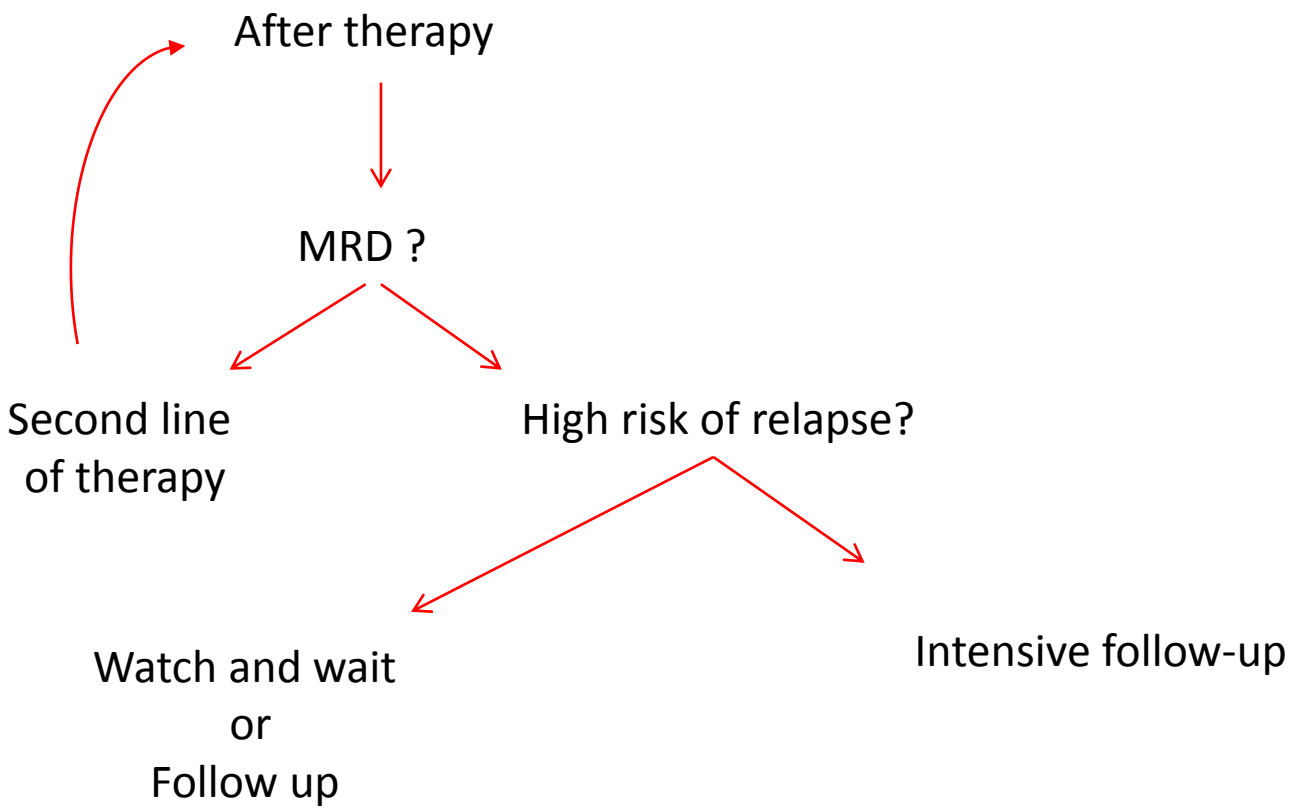
Present therapeutic concept

Cancer therapy plan (so far)

De Vita et al 2012



Cancer therapy plan (so far)



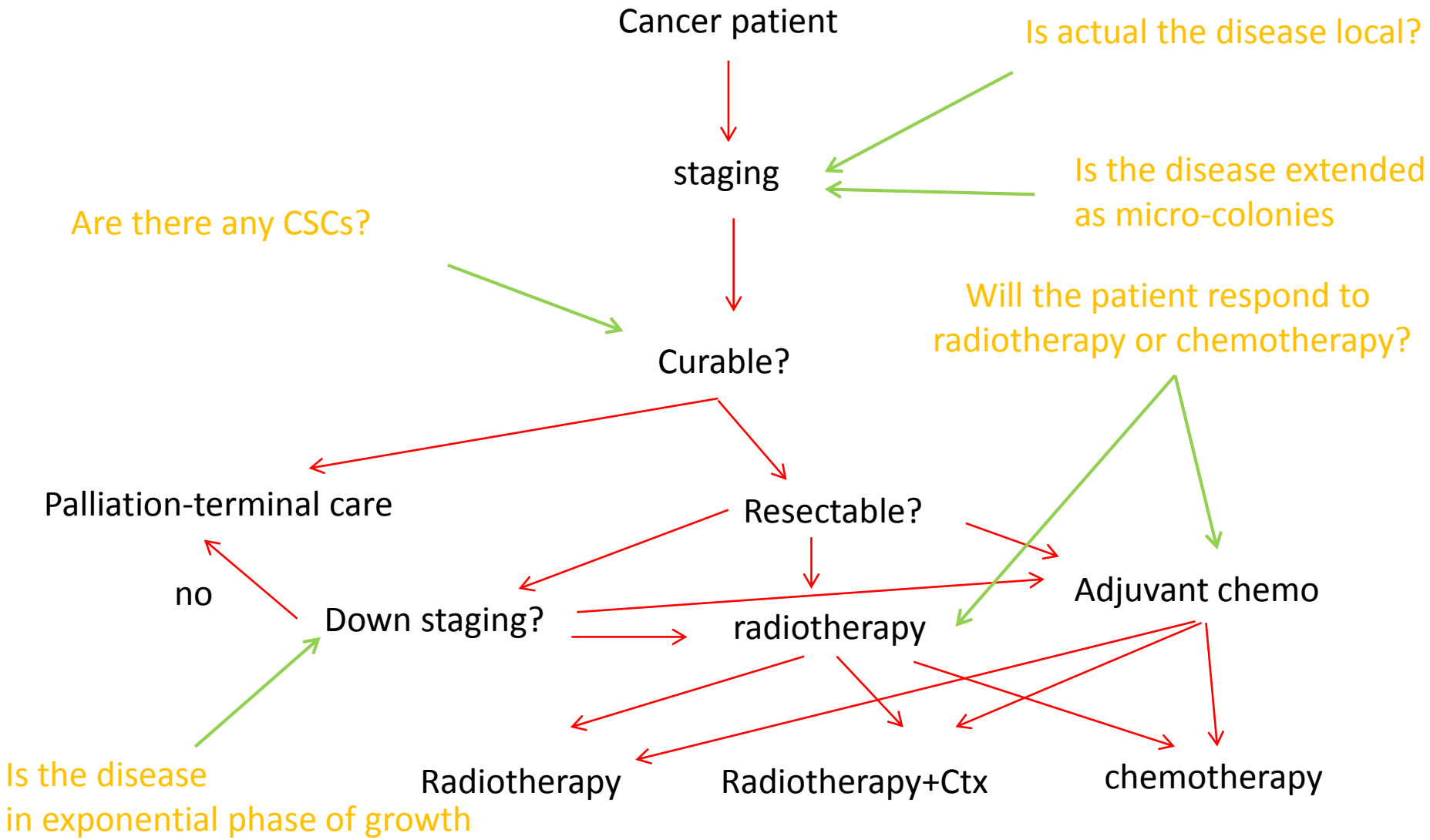
Rate of success

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

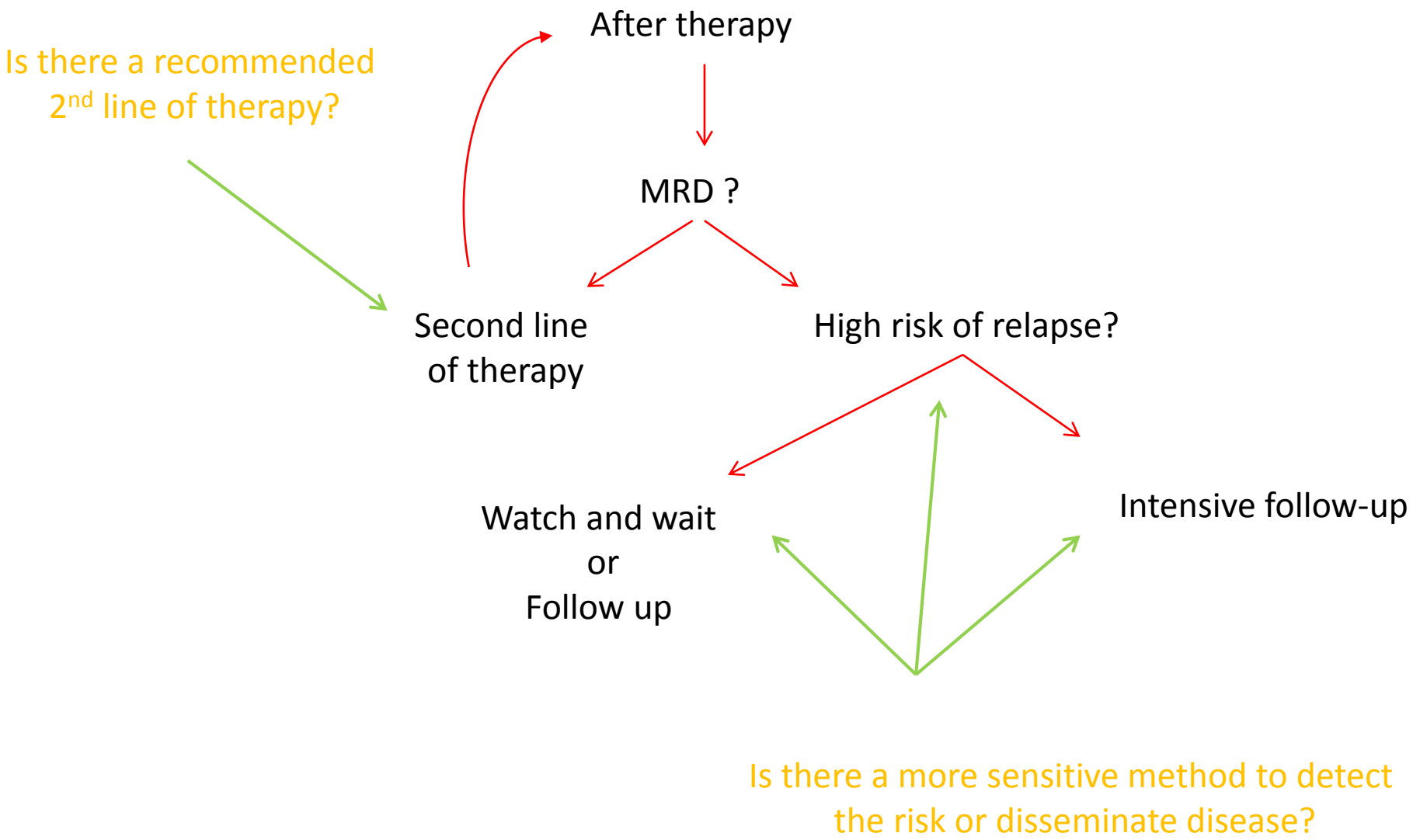
  Royal North Shore Hospital Clin Oncol (R Coll Radiol) 2005 Jun;17(4):294

- 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



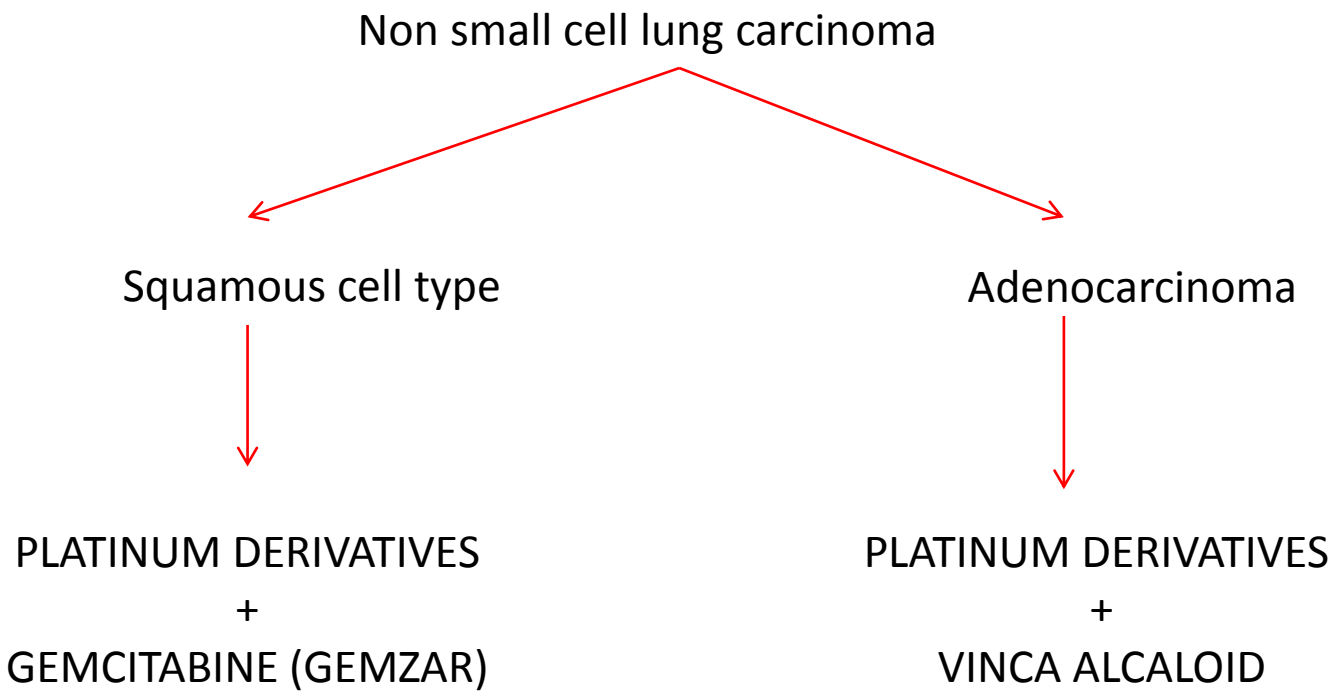
Dead-End in empirical treatment



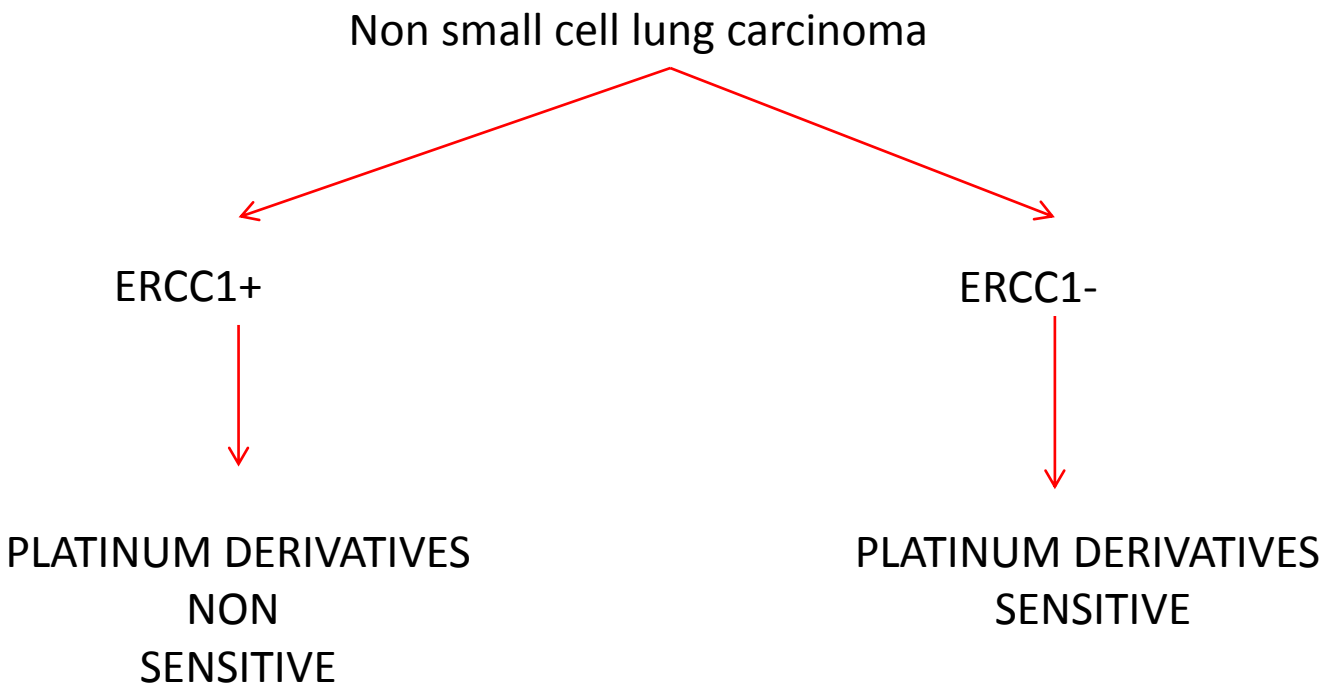
Reasons and causes

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.

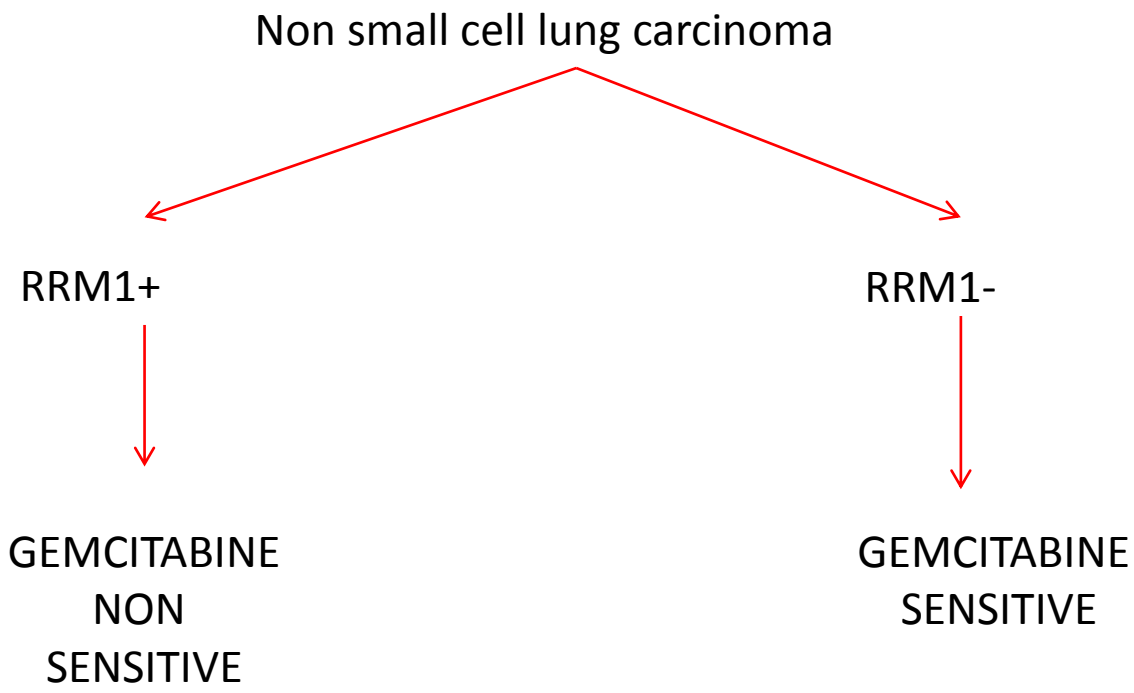
Examples


Non small cell lung carcinoma
Squamous cell type
Adenocarcinoma
PLATINUM DERIVATIVES
+
GEMCITABINE (GEMZAR)
PLATINUM DERIVATIVES
+
VINCA ALCALOID

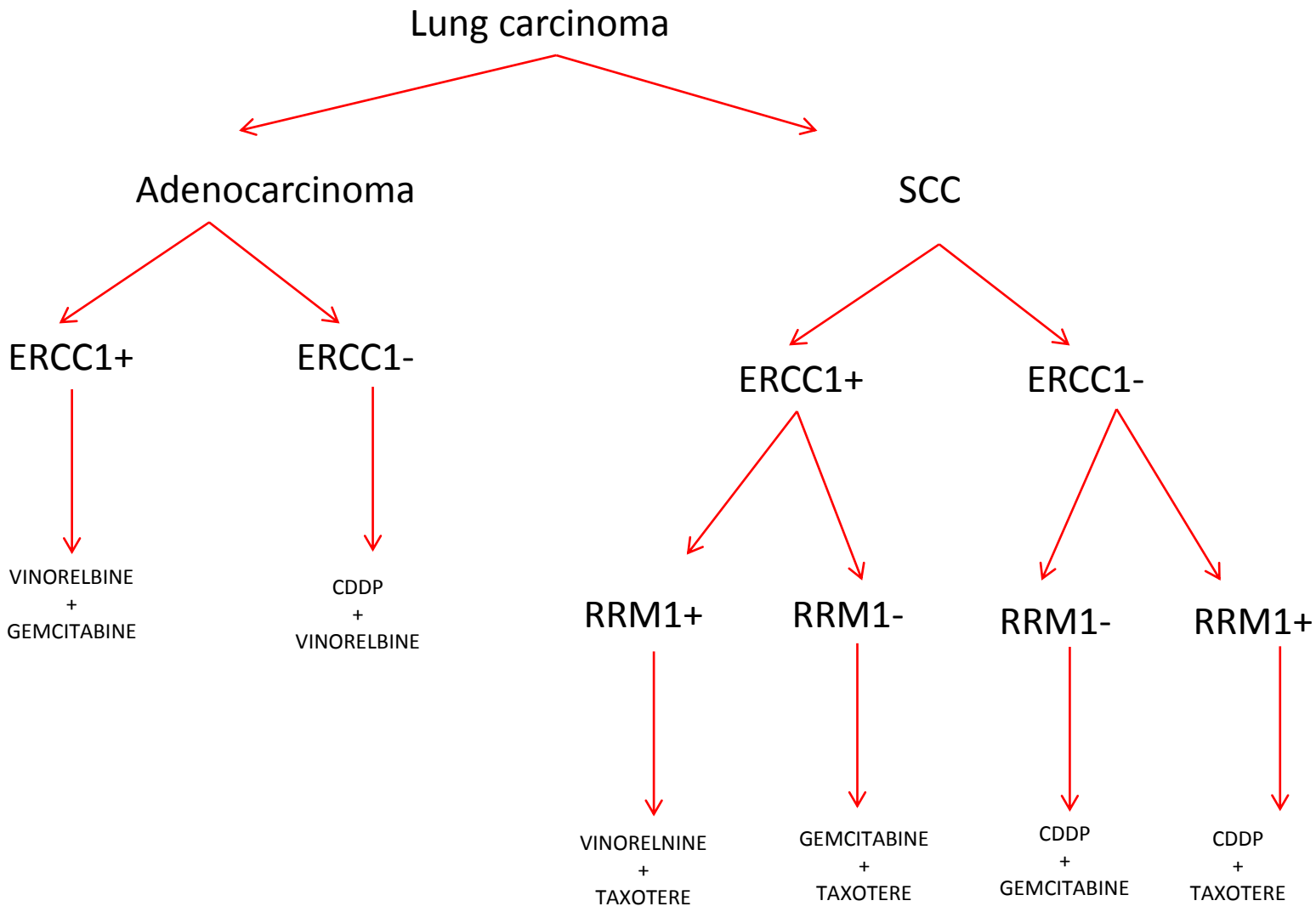
Examples


Non small cell lung carcinoma
ERCC1+
ERCC1-
PLATINUM DERIVATIVES
NON
SENSITIVE
PLATINUM DERIVATIVES
SENSITIVE

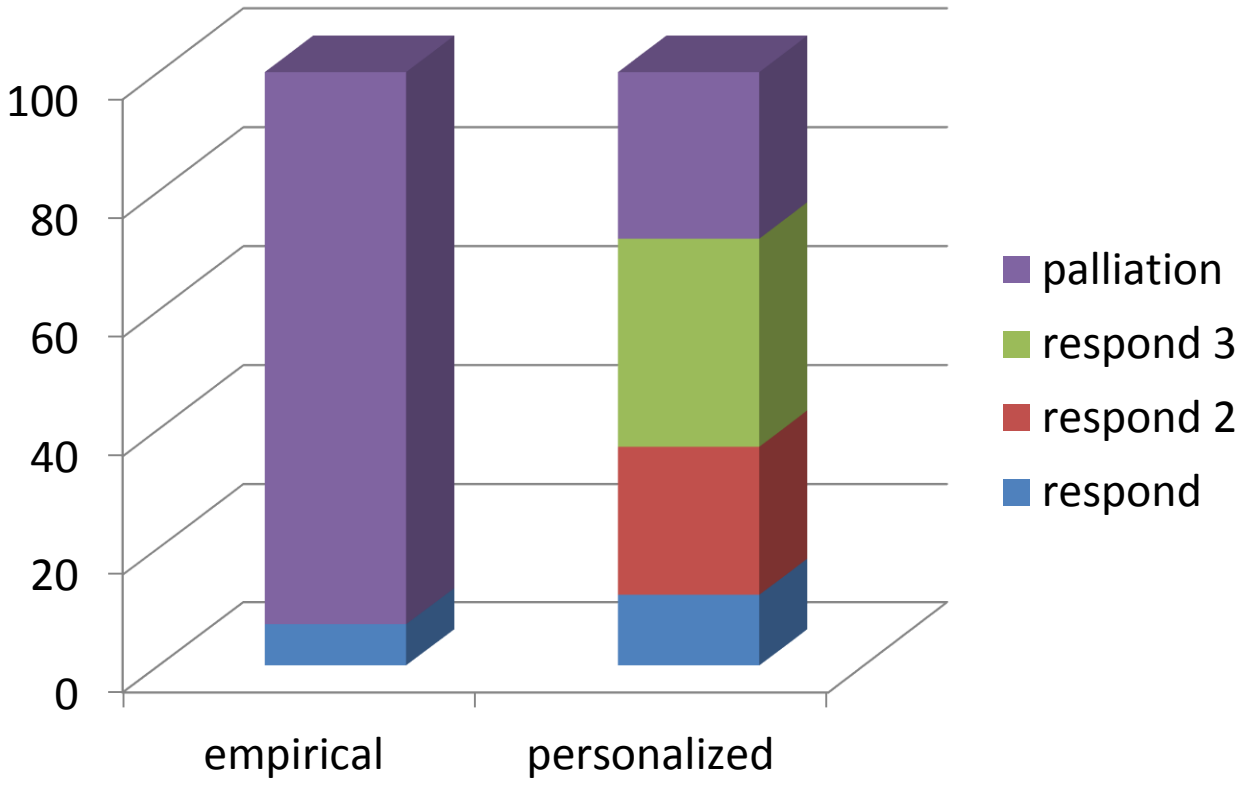
Examples


Non small cell lung carcinoma
RRM1+
RRM1-
GEMCITABINE NON SENSITIVE
GEMCITABINE SENSITIVE

Examples


Lung carcinoma
Adenocarcinoma
SCC
ERCC1+
ERCC1-
ERCC1+
ERCC1-
VINORELBINE
+
GEMCITABINE
CDDP
+
VINORELBINE
RRM1+
RRM1-
RRM1-
RRM1+
VINORELNINE
+
TAXOTERE
GEMCITABINE
+
TAXOTERE
CDDP
+
GEMCITABINE
CDDP
+
TAXOTERE

Response rates



Analyzing the proper sample

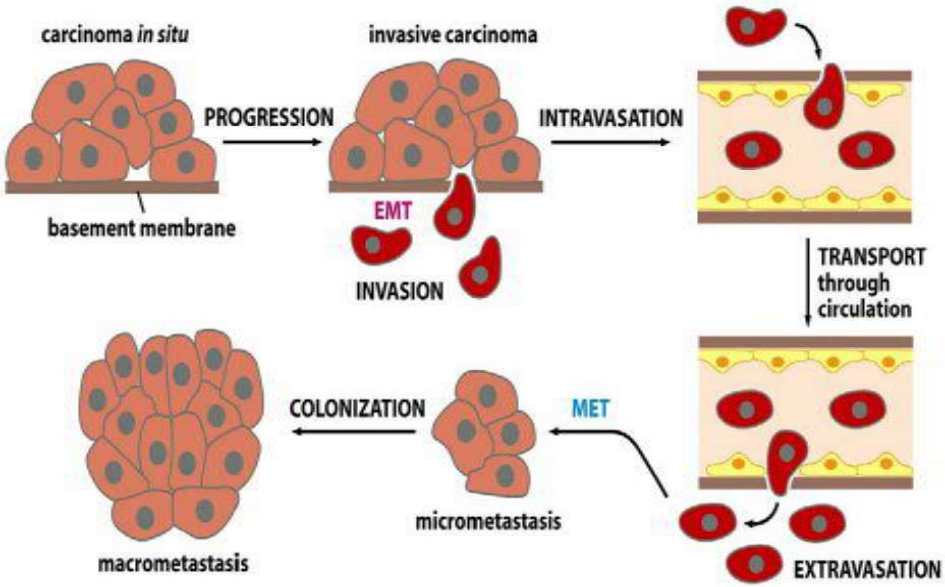
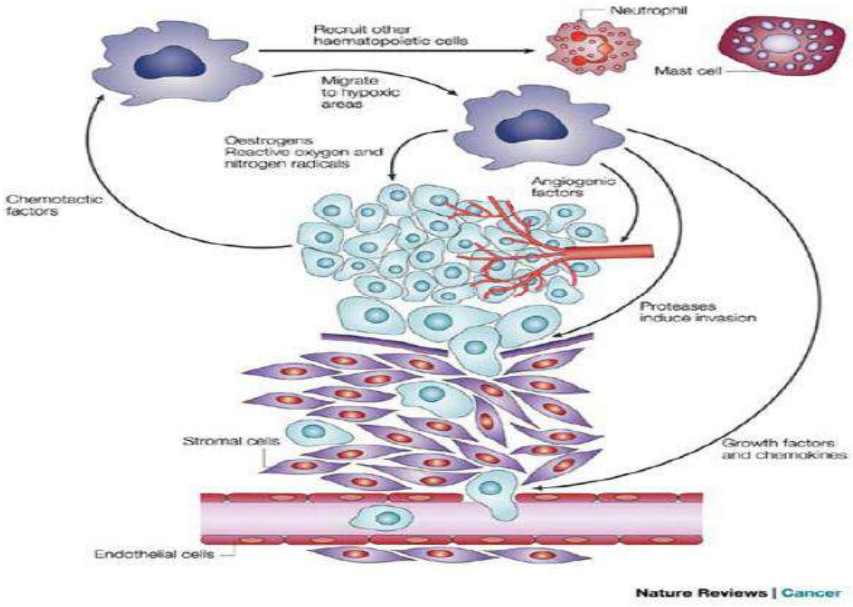


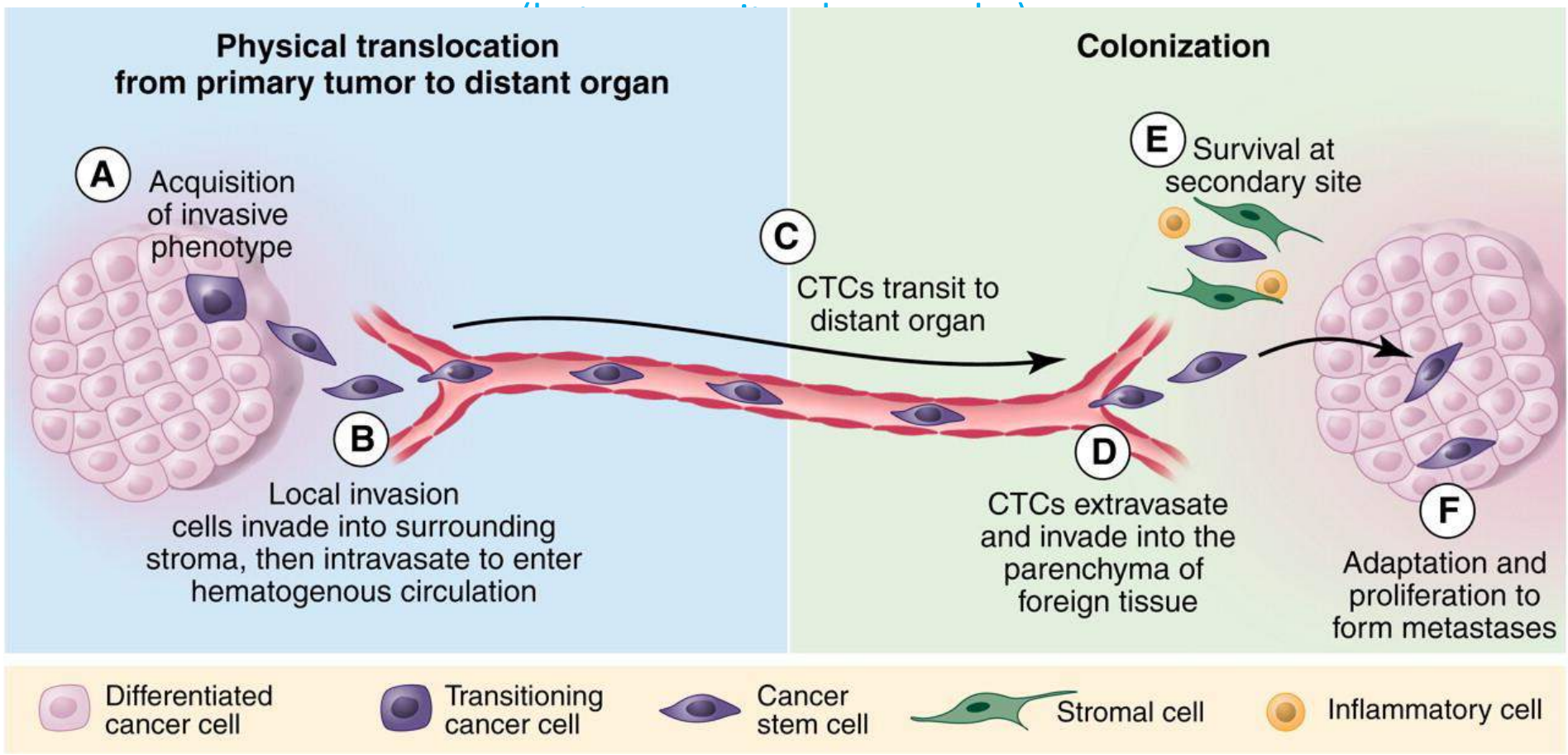
Figure 14-17b The Biology of Cancer (© Garland Science 2007)



Nature Reviews | Cancer

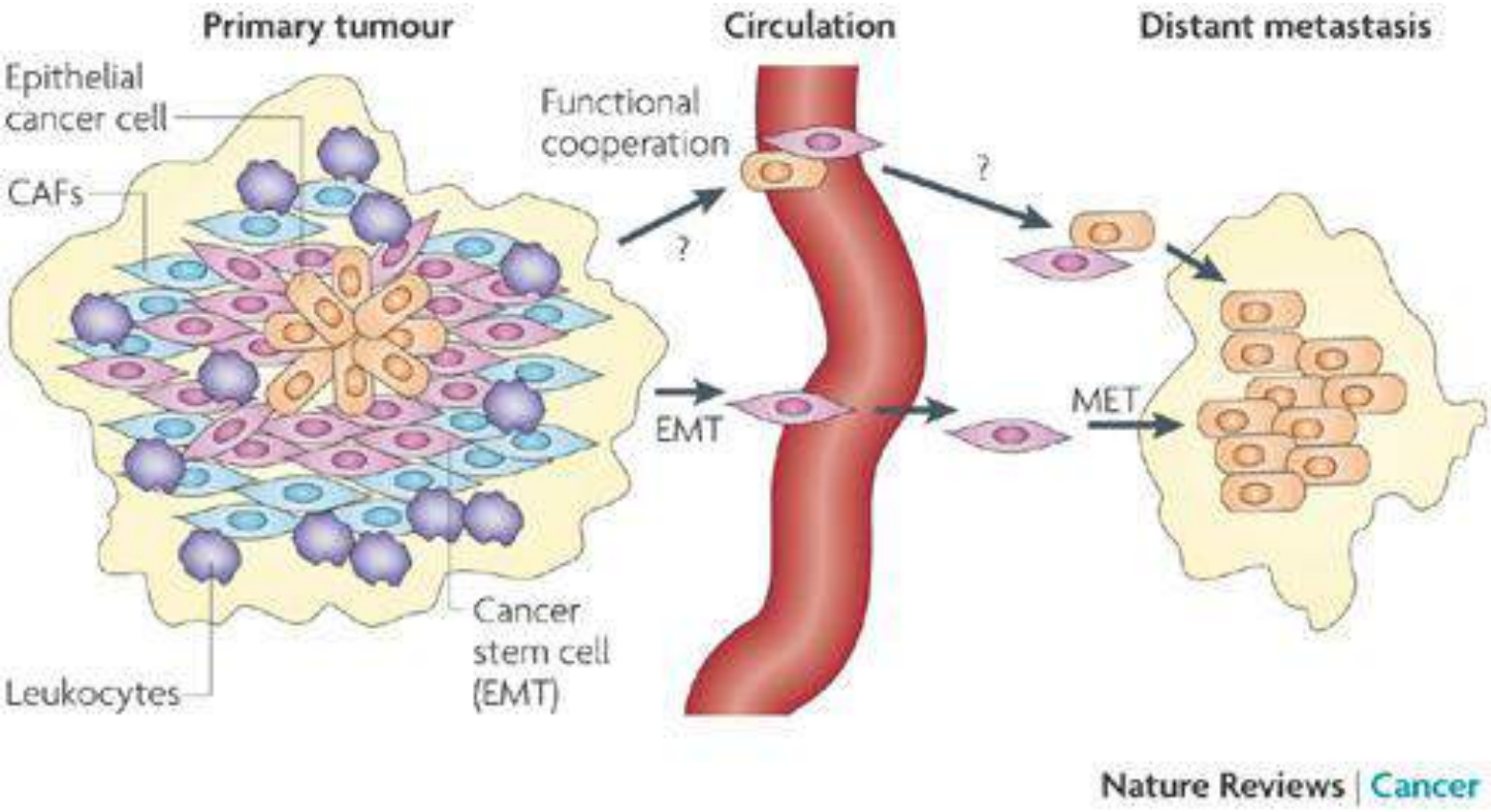
- Facts that are well established and proved:
  1. A tumor consist from the cancer cells and the stroma
  2. The stroma cells composed from fibroblast, lymphocytes, endothelial cells etc
  3. The cancer population is heterogeneous and composed from subpopulations with different features and aggressive 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.

Tumor physiology

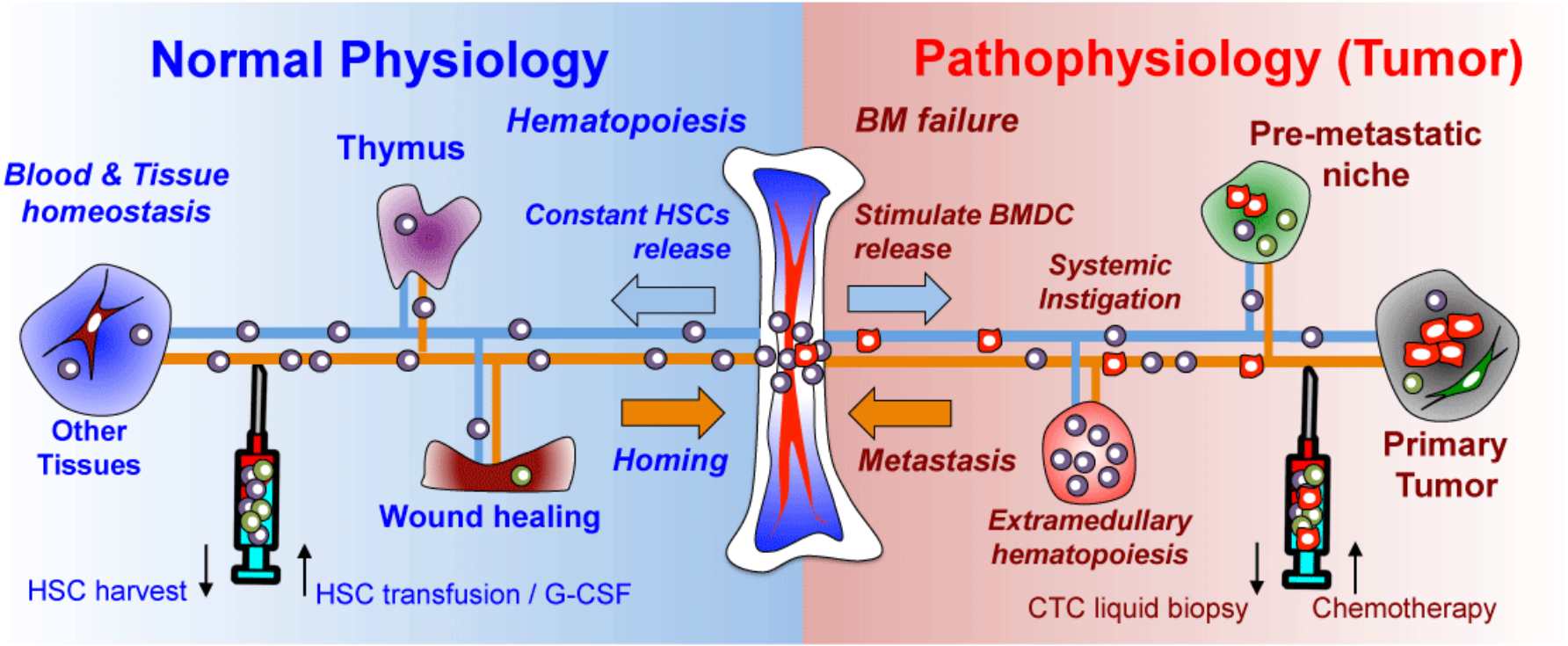


Tumor physiology

(heterogeneity-pleomorphy)



Tumor physiology (heterogeneity-pleomorphy)



Comparison between primary and metastases

- GENOMIC LEVEL

The gene mutations signature is similar between primary and metastatic tumors.



NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Display Settings: Abstract

Send to:

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.

©2011 AACR.

Comparison between primary and metastases

- EPIGENETIC AND BIOMARKERS LEVEL

The gene expression profile alters between primary and metastatic tumors.

Biomarkers Article

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, Tibshirani 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].

NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Display Settings: Abstract

Send to:

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.

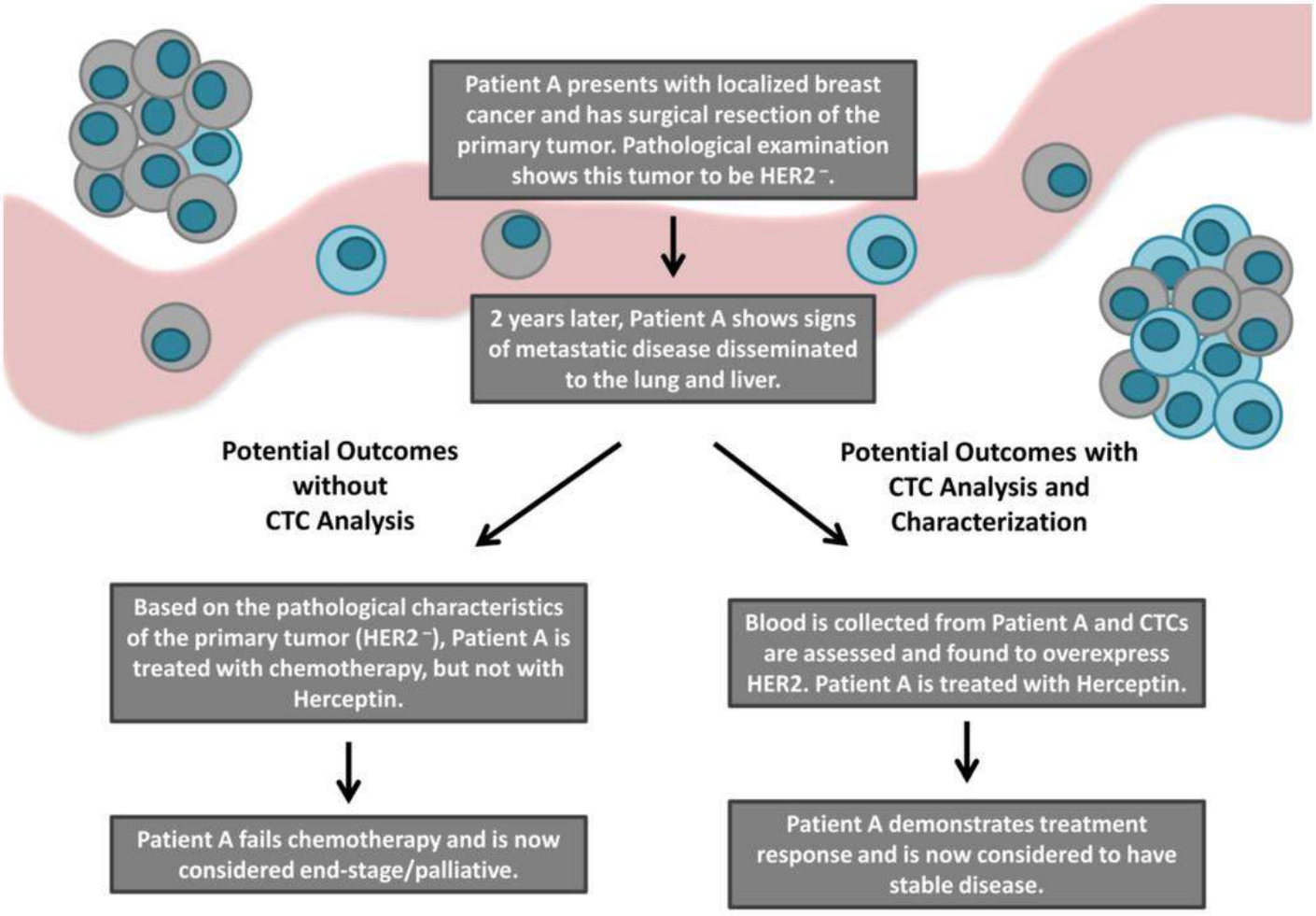
METHODS: We evaluated 193 blood samples from metastatic breast cancer patients at the time of first diagnosis of metastatic disease or disease progression. All samples underwent immunomagnetic enrichment using the AdnaTest BreastCancerSelect (AdnaGen AG, Germany) within 4h after blood withdrawal followed 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.

Copyright © 2011 Elsevier Inc. All rights reserved.

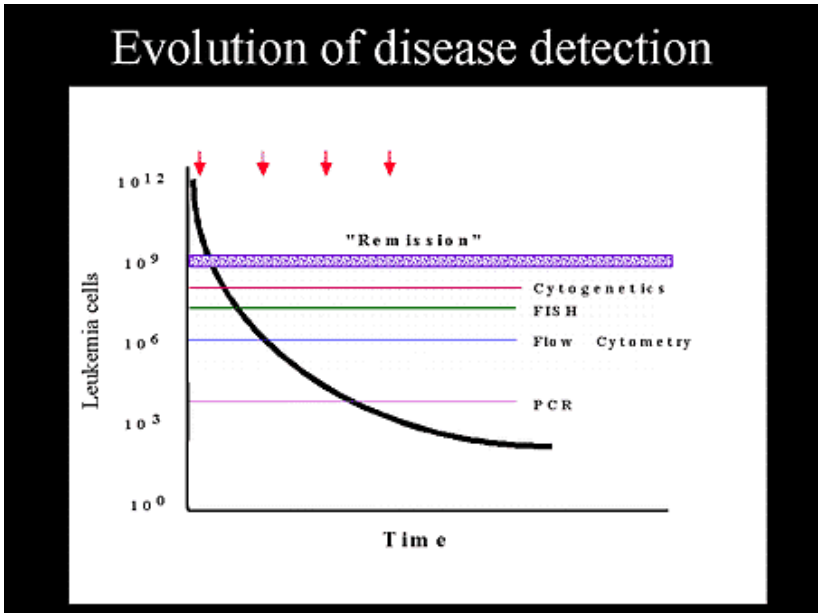
Possible scenario



Do we use a right non invasive diagnostic for prevention?

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

- Limitations

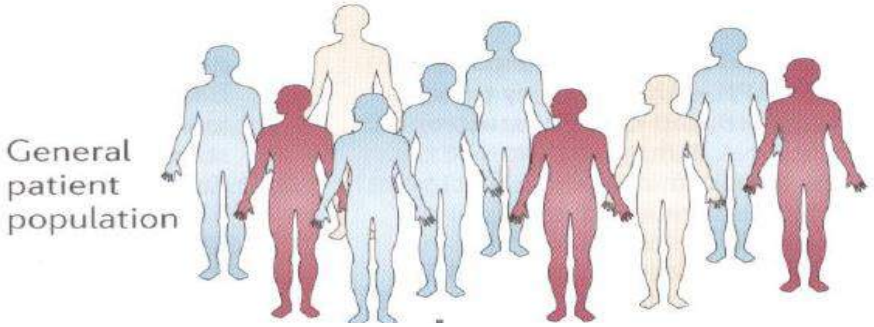


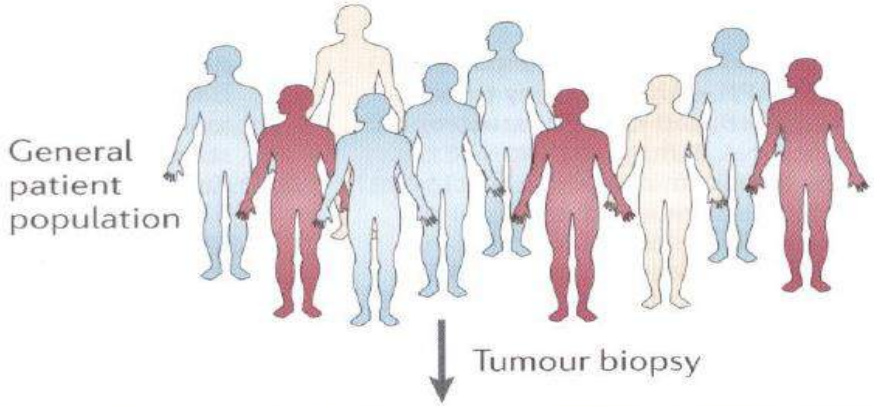
Minimal invasive method: BIOPSY

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

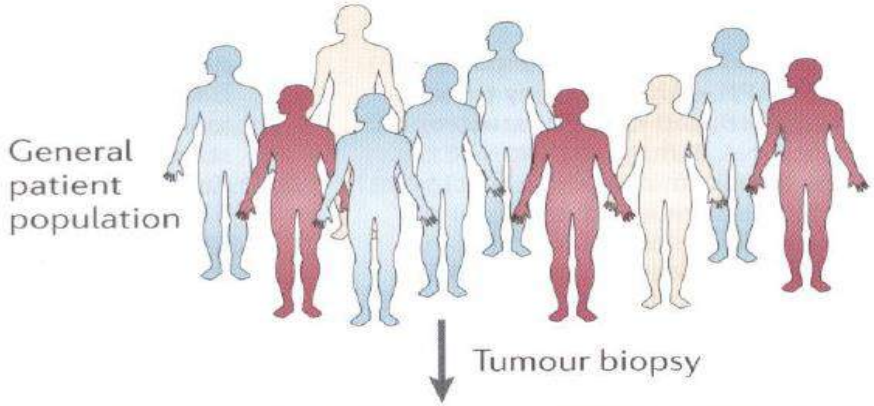

General
patient
population



ANALYSIS ON SEVERAL LEVELS

- GENOMIC
- EPIGENETIC
- PROTEOMIC

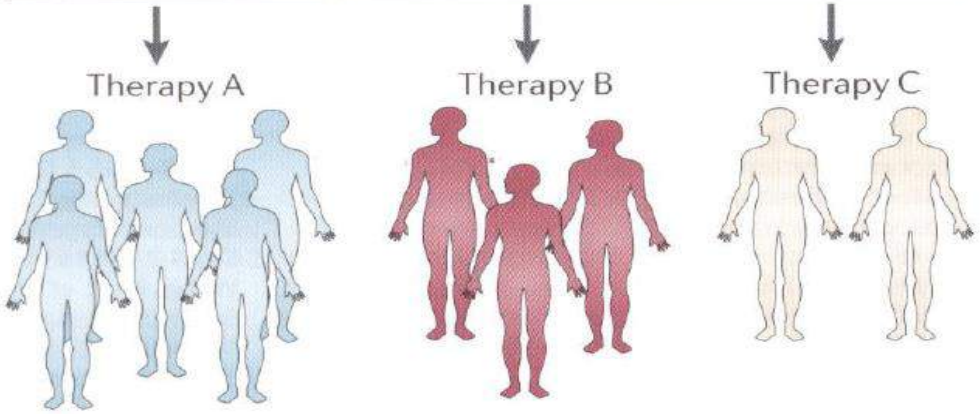
MULTIPLE PLATFORM NEED TO BE APPLIED IN ORDER TO STRATIFY PATIENTS AND DEVELOP THERAPEUTIC OPTIONS UNDER MORE PERSONALIZED PERSPECTIVE



ANALYSIS ON SEVERAL LEVELS

- GENOMIC
- EPIGENETIC
- PROTEOMIC

MULTIPLE PLATFORM NEED TO BE APPLIED IN ORDER TO STRATIFY PATIENTS AND DEVELOP THERAPEUTIC OPTIONS UNDER MORE PERSONALIZED PERSPECTIVE



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.

Empirical vs Personalized treatment Pros & Cons

	Empirical lines of therapy	Personalized plan of treatment
Advantages	•Low cost (in short term) •Fast applicable •Applicable to masses •No need of higher training of physician in PK and PD	•High rate of success •Avoid of side effect & toxicities •Long term cost effective •Shortening of hospital admission and residence of a patient
Disadvantages	•High rate of failure •No participation of each case individuality •After last recommended line, no other option	•Higher cost than empirical •Need previously a series of analysis to be performed

CTCs & CSCs

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

(CLINICAL REALITY)

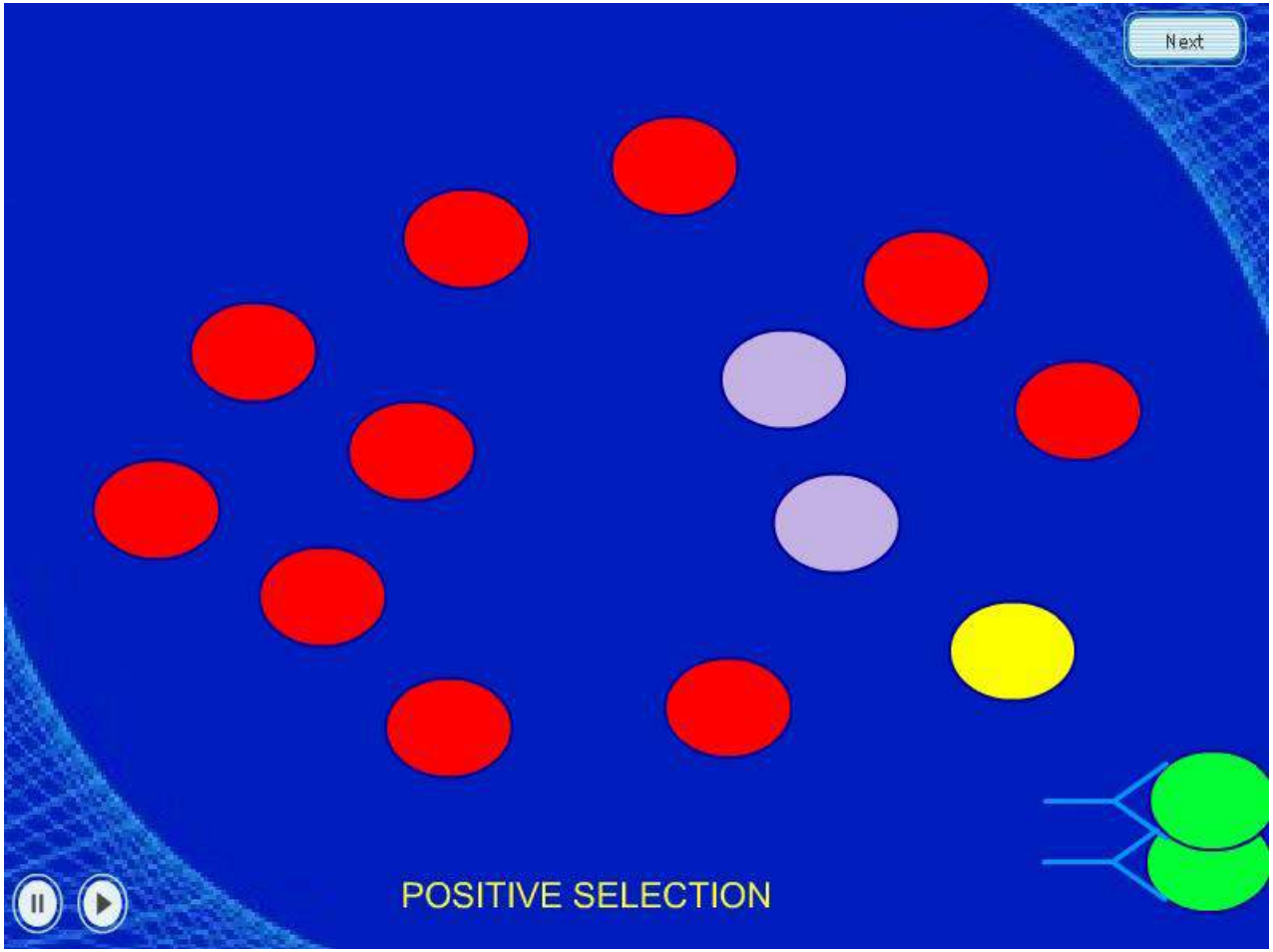
How can be found a needle in a hay-stack?

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

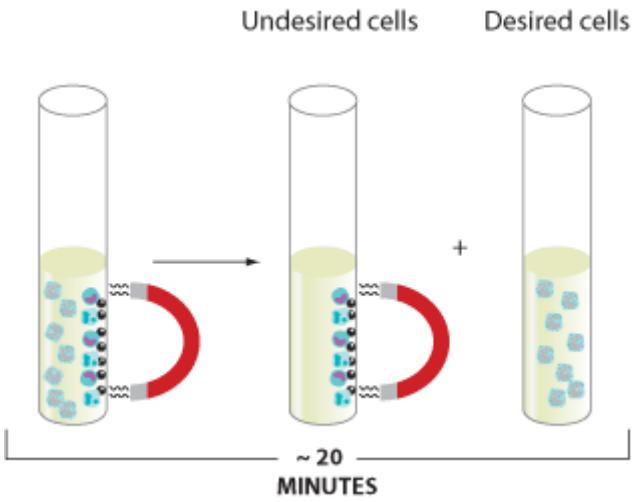
1. High purity of CTCs
2. Viable CTCs
3. Isolate all subsets of CTCs
4. Detect the disease relevant CTCs
5. Detect CTCs subset with stemness properties
6. Pin point the subclasses of CTCs with plasticity properties (EMT-MET)

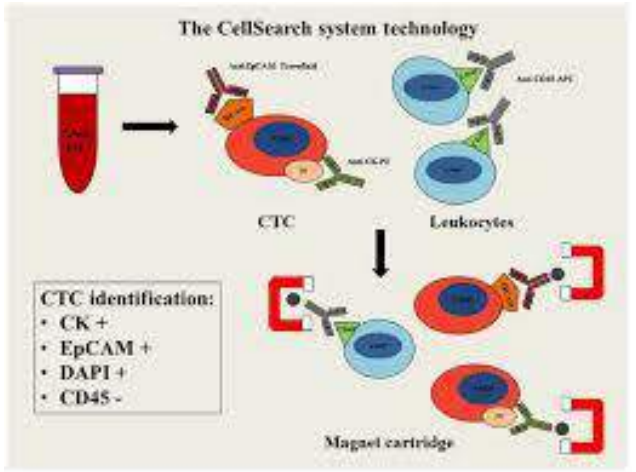
CHOOSING THE RIGHT METHOD

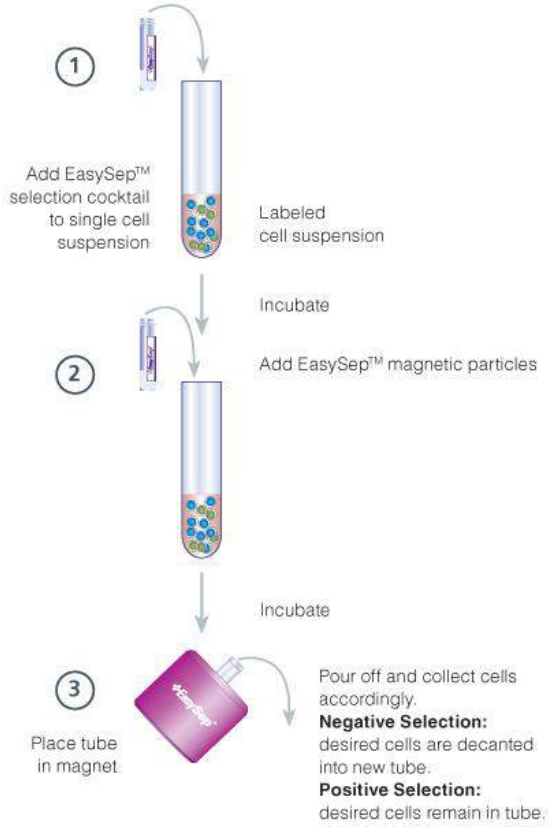


CHOOSING THE RIGHT METHOD

BEAD BASED METHOD

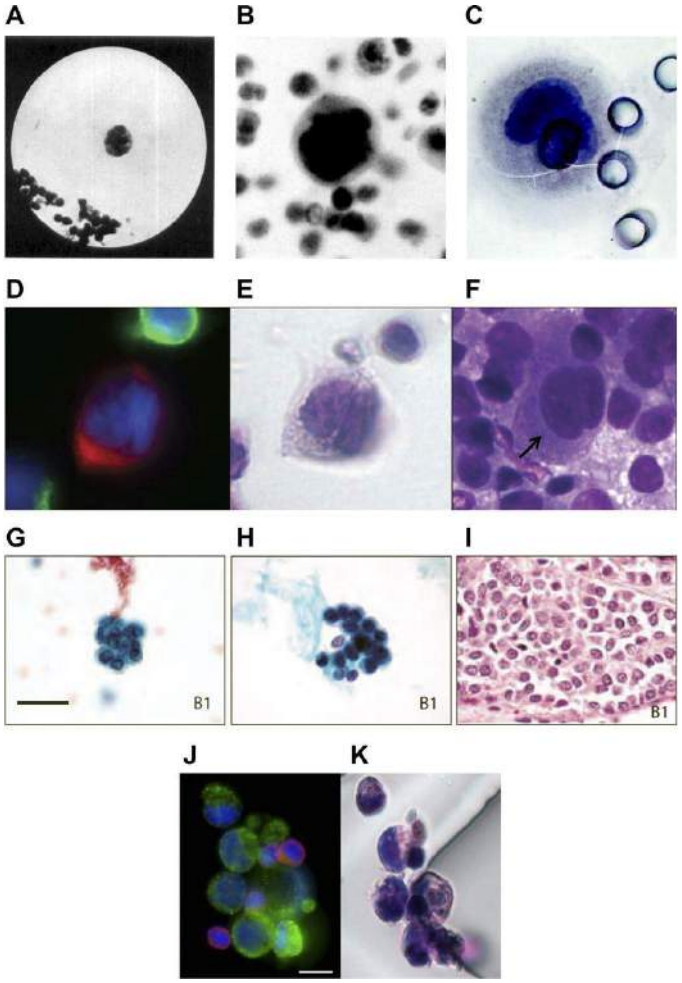






CHOOSING THE RIGHT METHOD

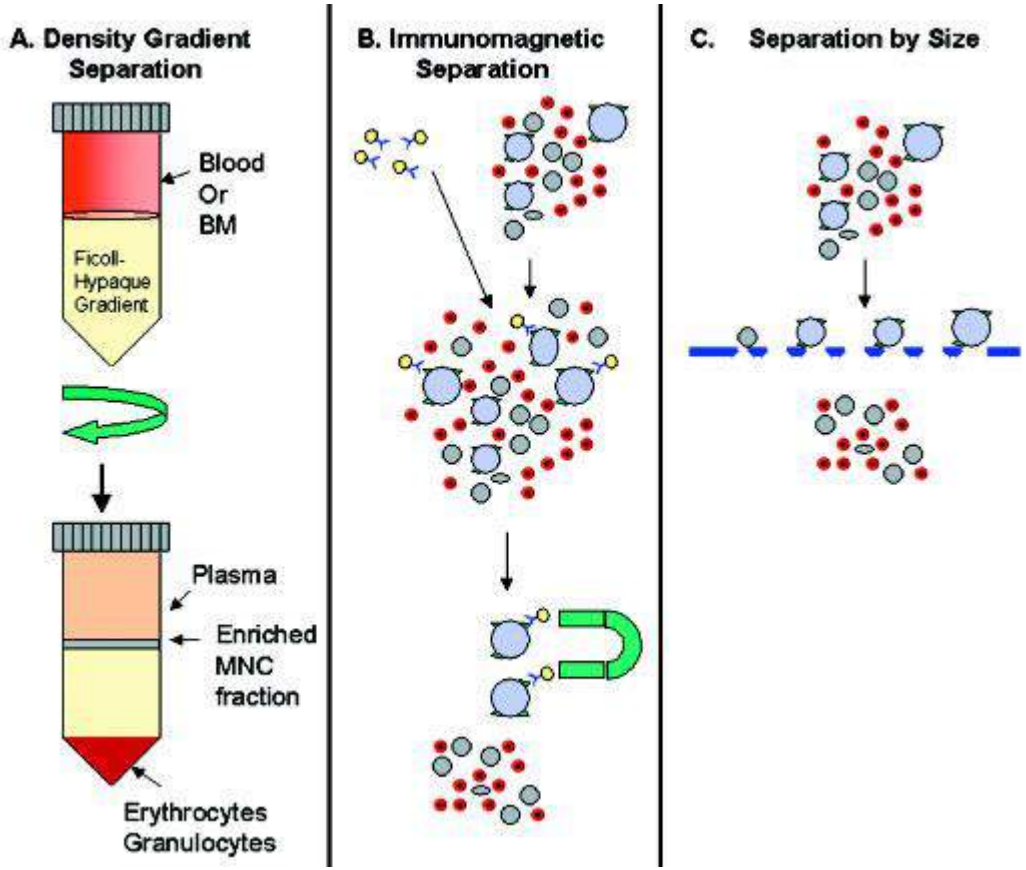
MICROSCOPY BASED METHOD



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

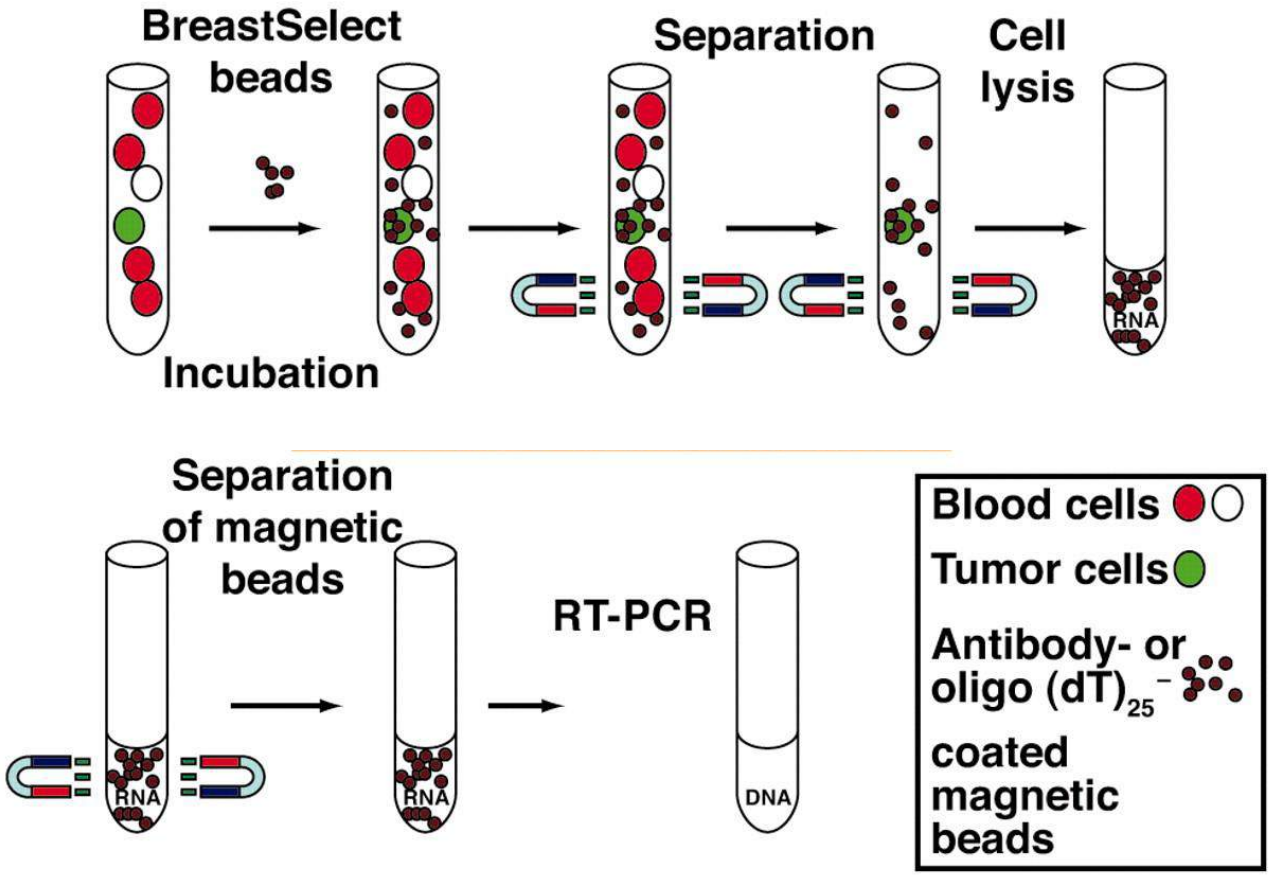
CHOOSING THE RIGHT METHOD

GRADIENT BASED METHOD



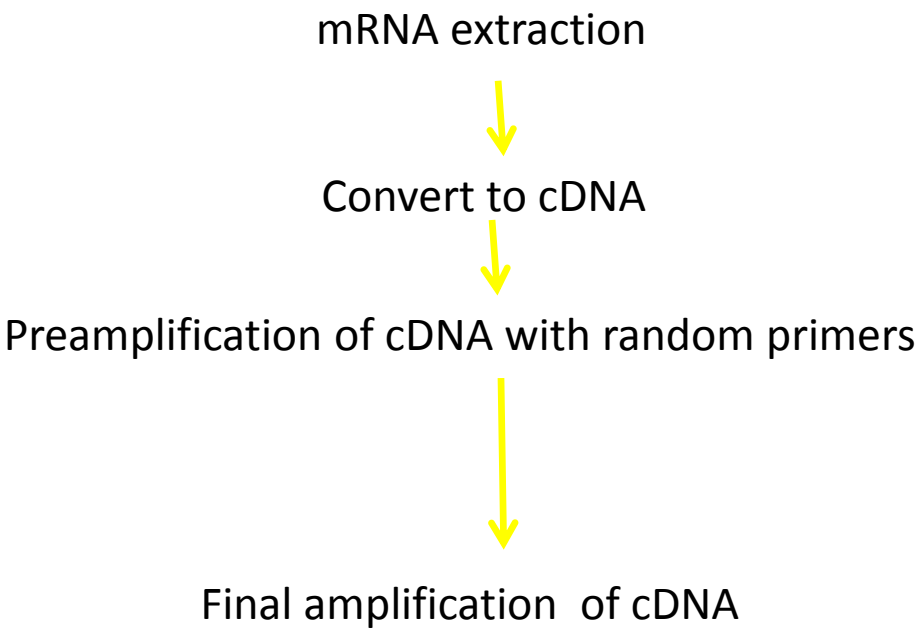
CHOOSING THE RIGHT METHOD

PCR BASED METHODS



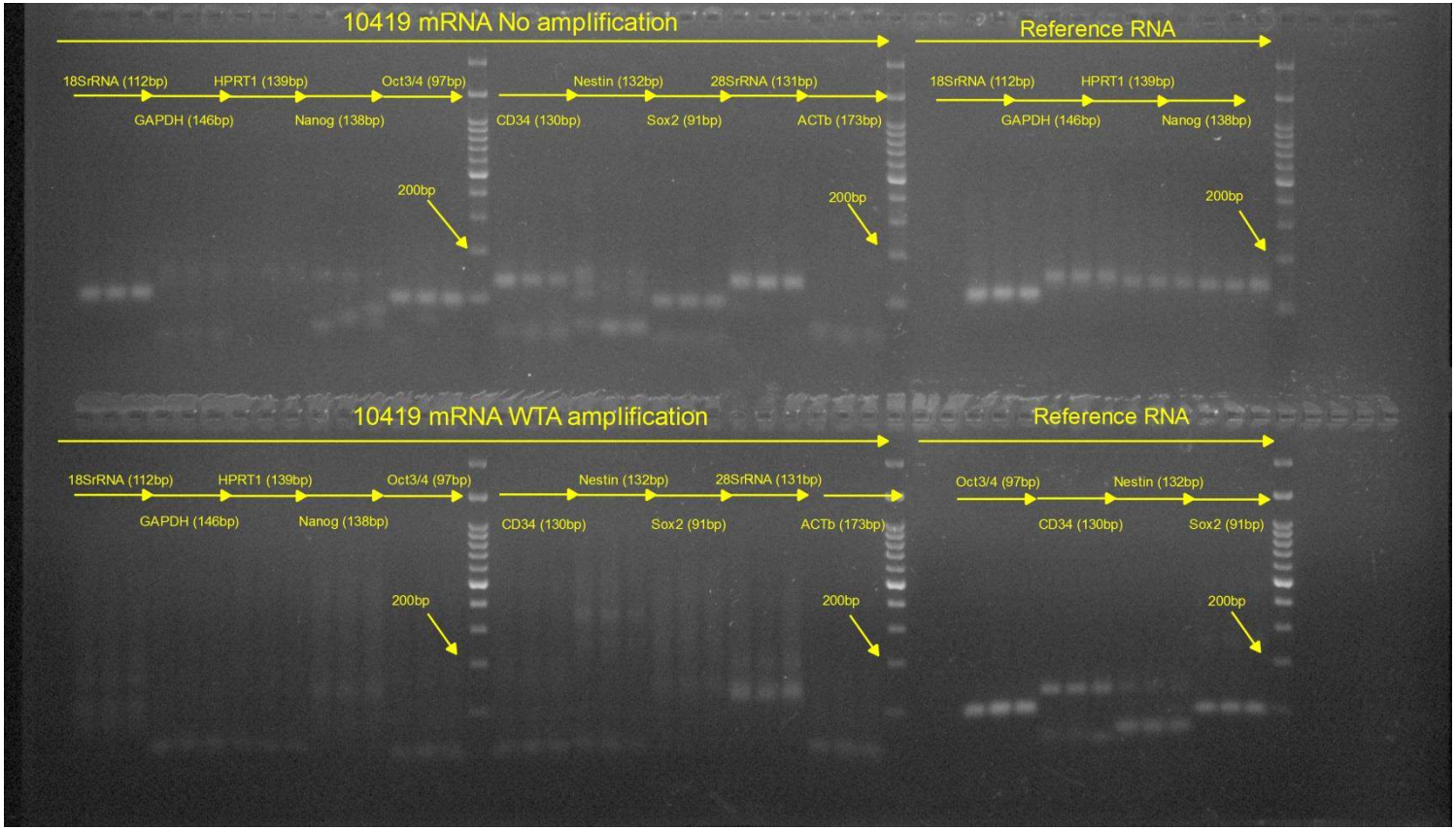
More things to consider

- qPCR (all genome)



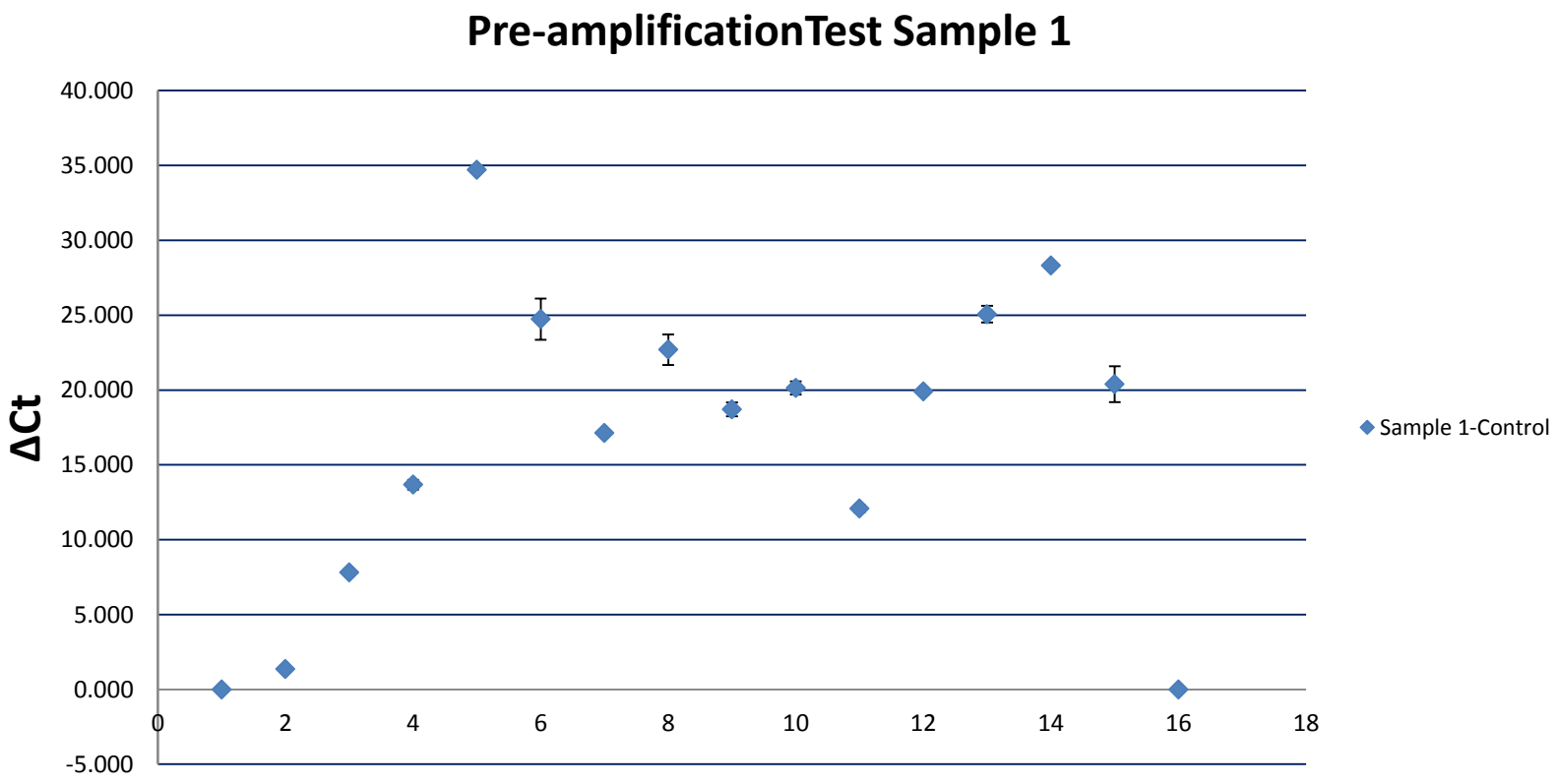
More things to consider

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



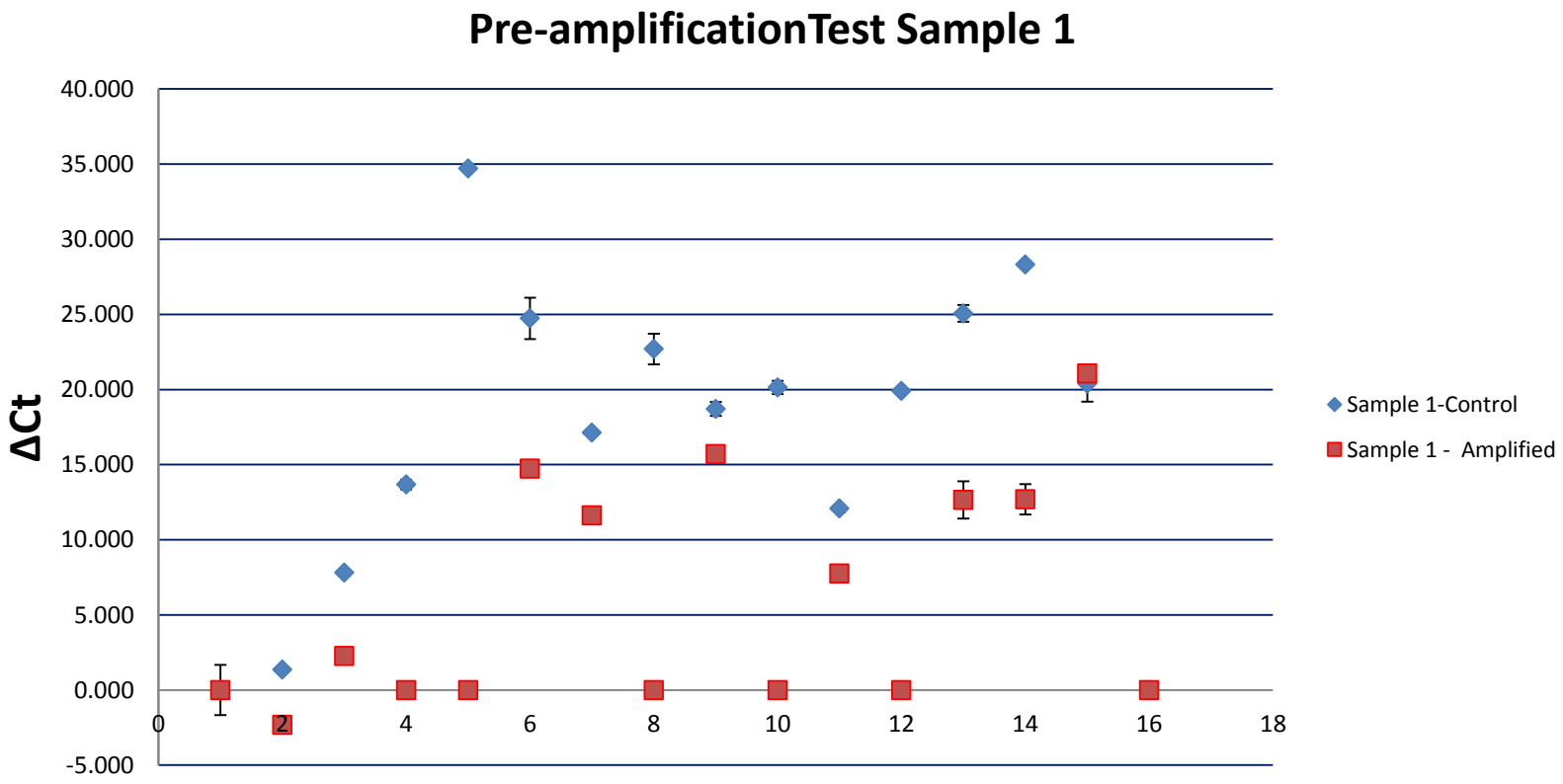
More things to consider

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



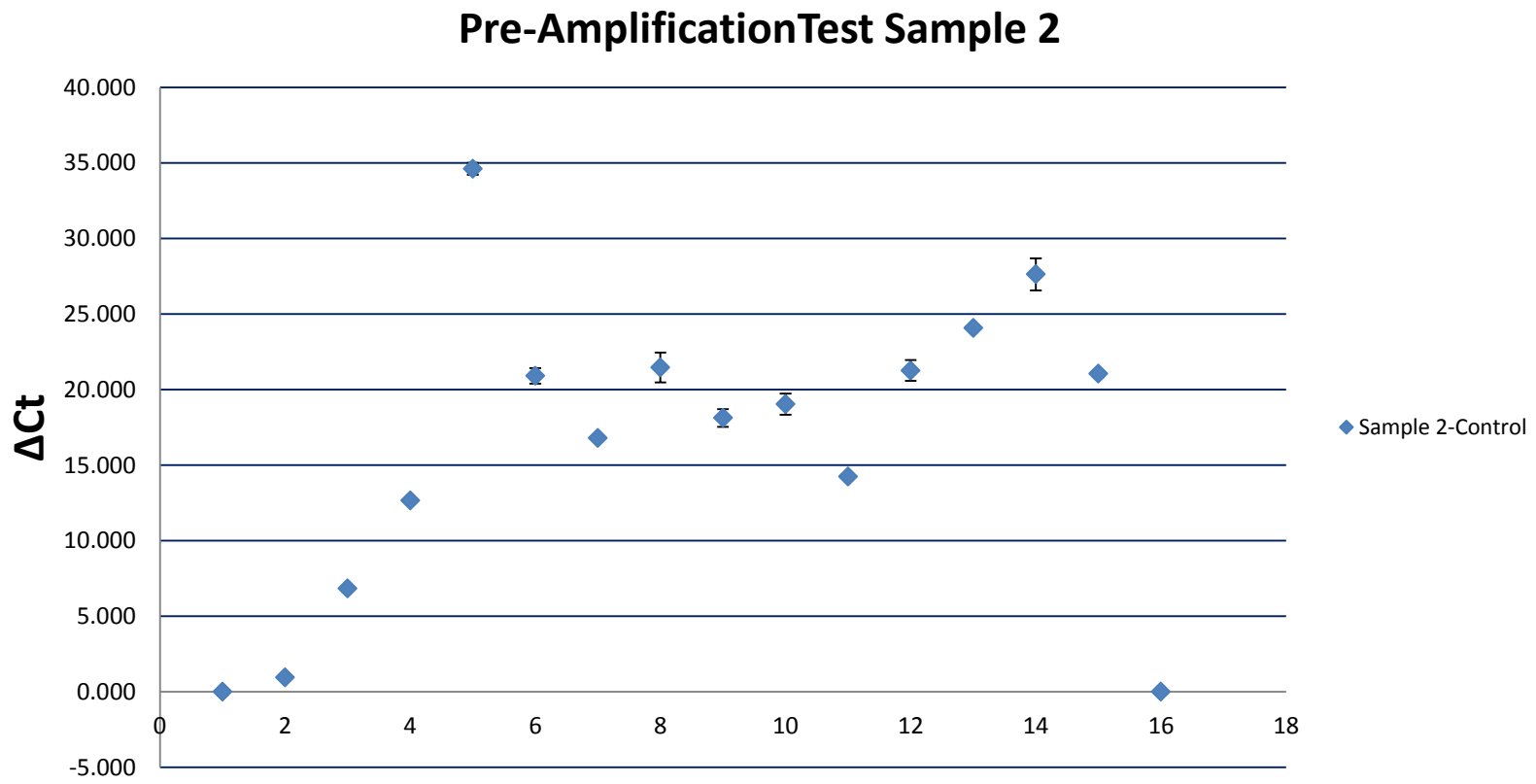
More things to consider

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



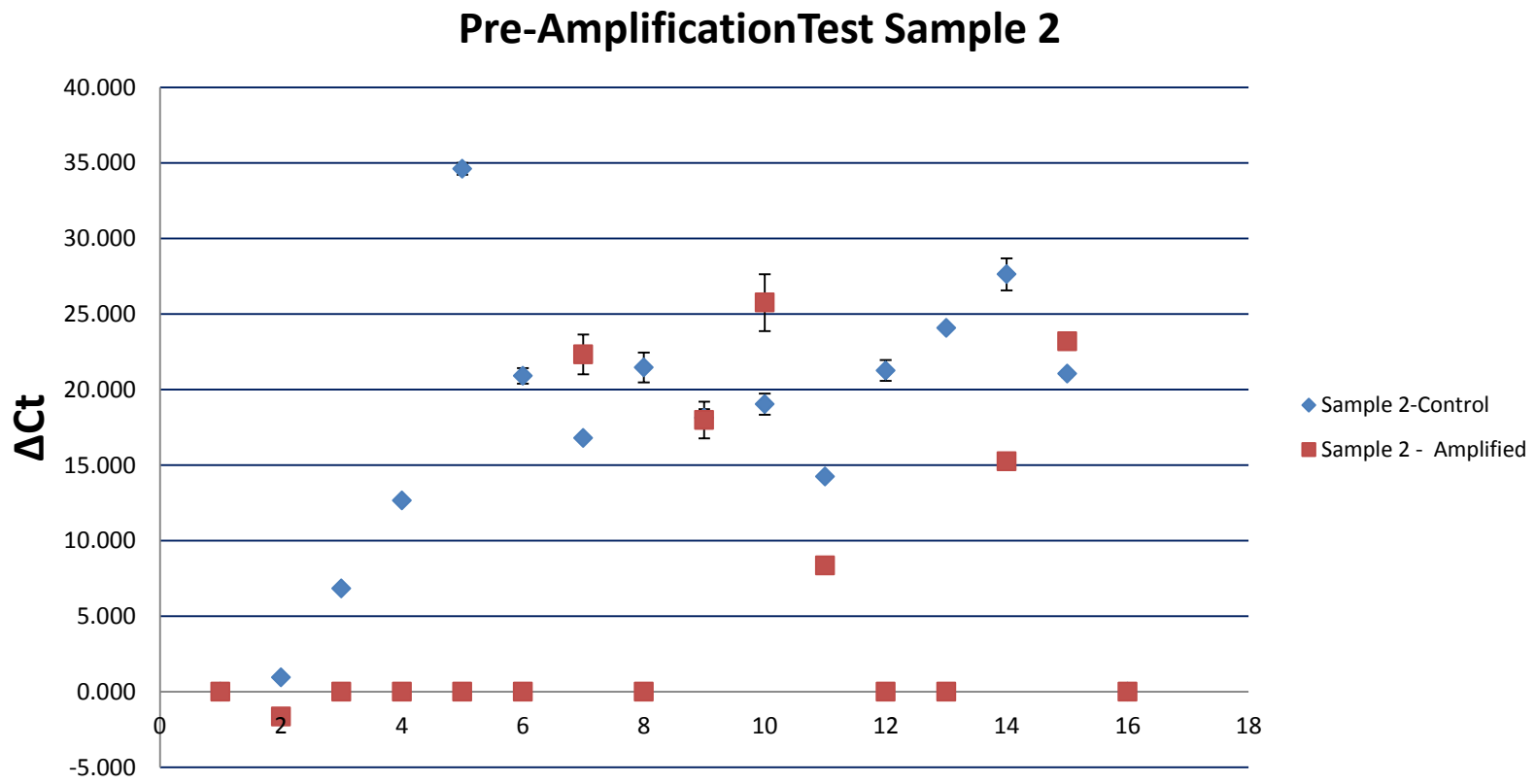
More things to consider

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



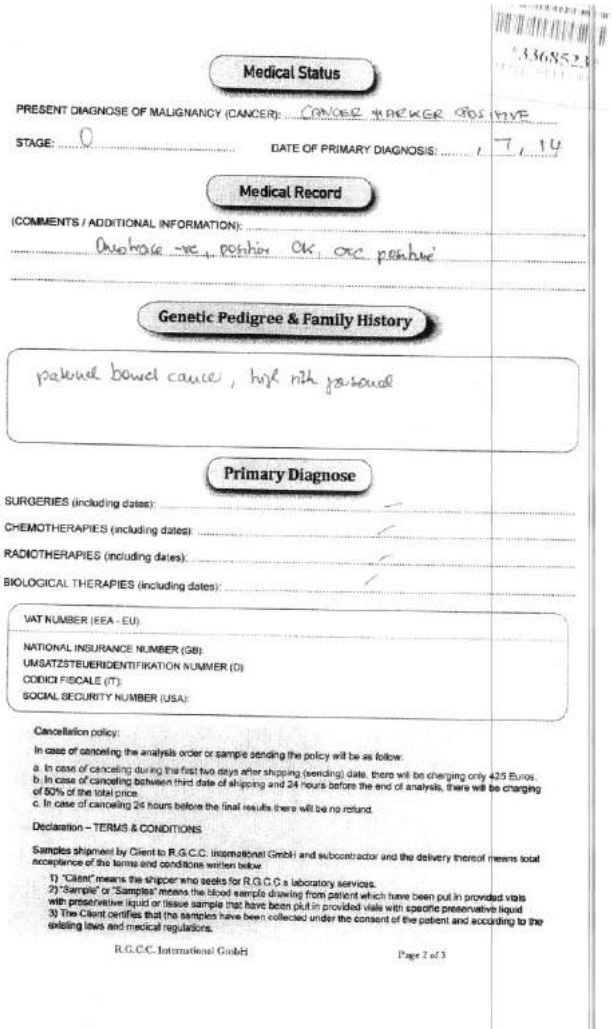
More things to consider

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



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?



Medical Status

PRESENT DIAGNOSE OF MALIGNANCY (CANCER): CANCER MARKER POSITIVE

STAGE: 0 DATE OF PRIMARY DIAGNOSIS: 1.7.14

Medical Record

(COMMENTS / ADDITIONAL INFORMATION):

Dysplasia -ve, position CK, CEC positive

Genetic Pedigree & Family History

paternal bowel cancer, high mh jealousal

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:

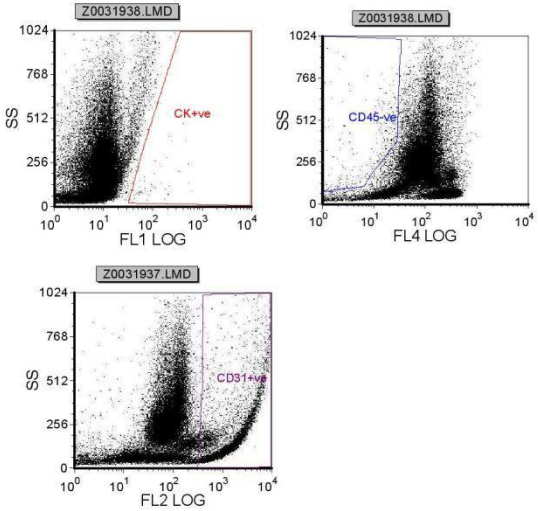
a. In case of canceling during the first two days after shipping (sending) date, there will be charging only 425 Euros.
b. In case of canceling between third date of shipping and 24 hours before the end of analysis, there will be charging of 50% of the total price.
c. In case of canceling 24 hours before 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 put 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.

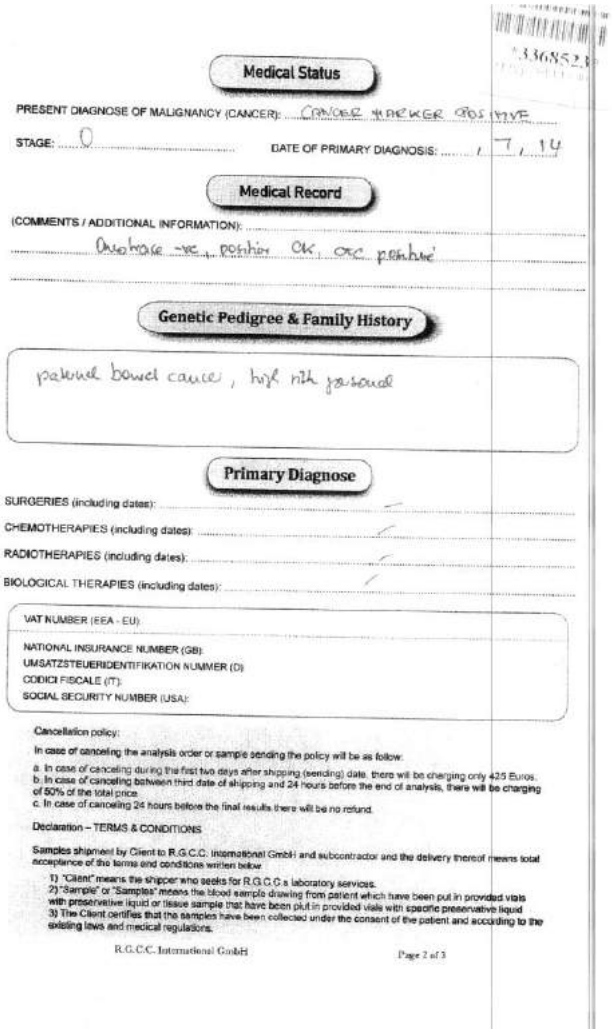
R.G.C.C. International GmbH Page 2 of 3



Overlay #	F C e	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

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?



Medical Status

PRESENT DIAGNOSE OF MALIGNANCY (CANCER): CANCER MARKER POSITIVE

STAGE: 0 DATE OF PRIMARY DIAGNOSIS: 1.7.14

Medical Record

(COMMENTS / ADDITIONAL INFORMATION):

Oncomarker -ve, position CK, CEC positive

Genetic Pedigree & Family History

paternal bowel cancer, high mth grandad

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:

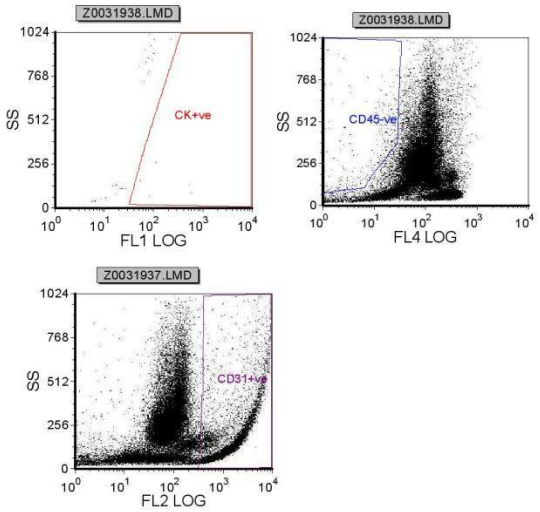
a. In case of canceling during the first two days after shipping (sending) date, there will be charging only 425 Euros.
b. In case of canceling between third date of shipping and 24 hours before the end of analysis, there will be charging of 50% of the total price.
c. In case of canceling 24 hours before 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 put 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.

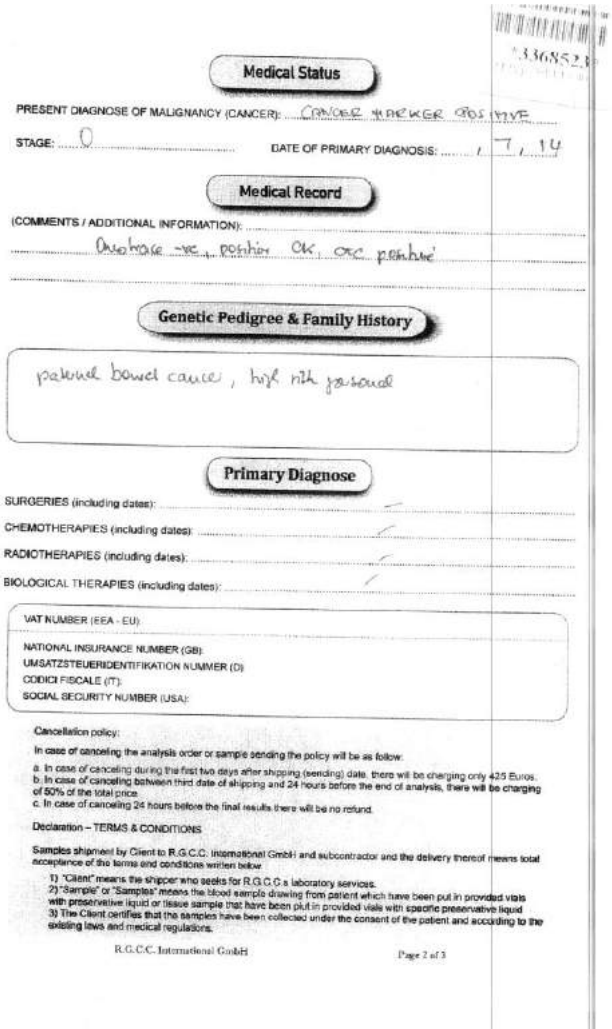
R.G.C.C. International GmbH Page 2 of 3



Overlay #	F C	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated	% 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

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?



Medical Status

PRESENT DIAGNOSE OF MALIGNANCY (CANCER): CANCER MARKER POSITIVE

STAGE: 0 DATE OF PRIMARY DIAGNOSIS: 1.7.14

Medical Record

(COMMENTS / ADDITIONAL INFORMATION):

Dysplasie -ve, position CK, CEC positive

Genetic Pedigree & Family History

paternal bowel cancer, high mth jaundice

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.

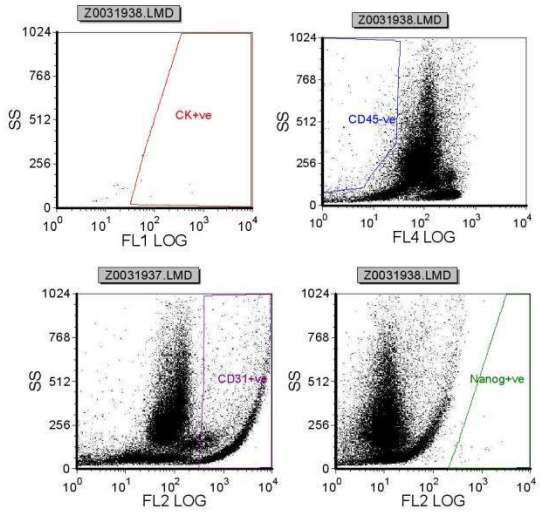
Declaration – TERMS & CONDITIONS

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 put 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

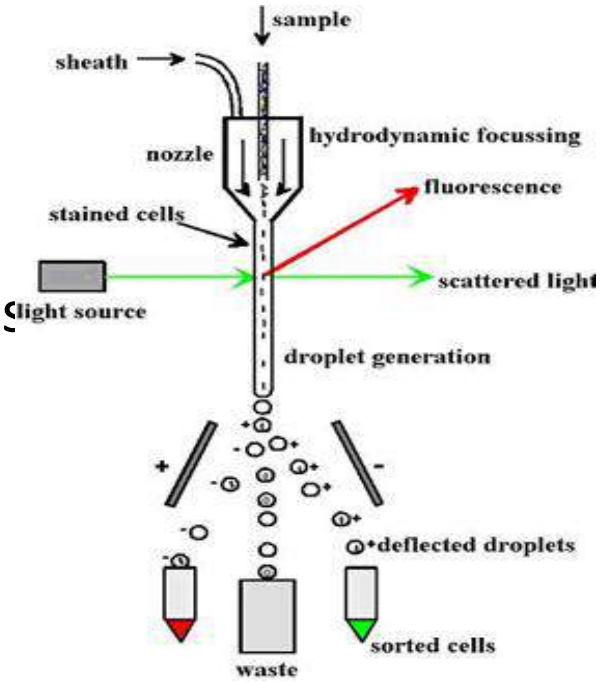
Page 2 of 3

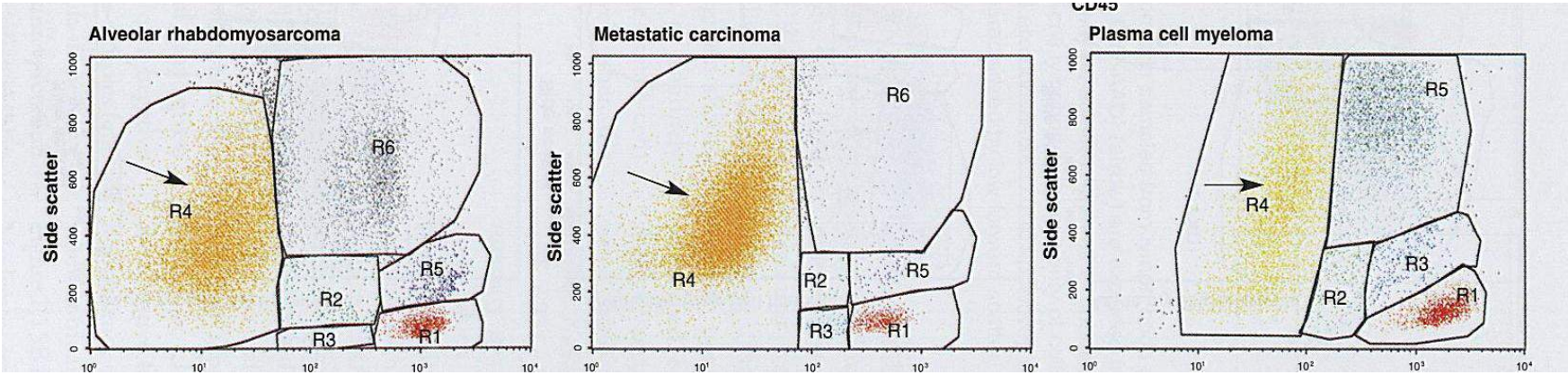


Overlay #	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	None	21	63.29	130.41	100.0	0.04
1	Gate 2	0	0.0	0.0	0.0	0.0
1	Gate 1	0	0.0	0.0	0.0	0.0
1	Gate 3	19	52.16	116.66	90.48	0.04
1	Gate 4	21	63.29	130.41	100.0	0.04

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





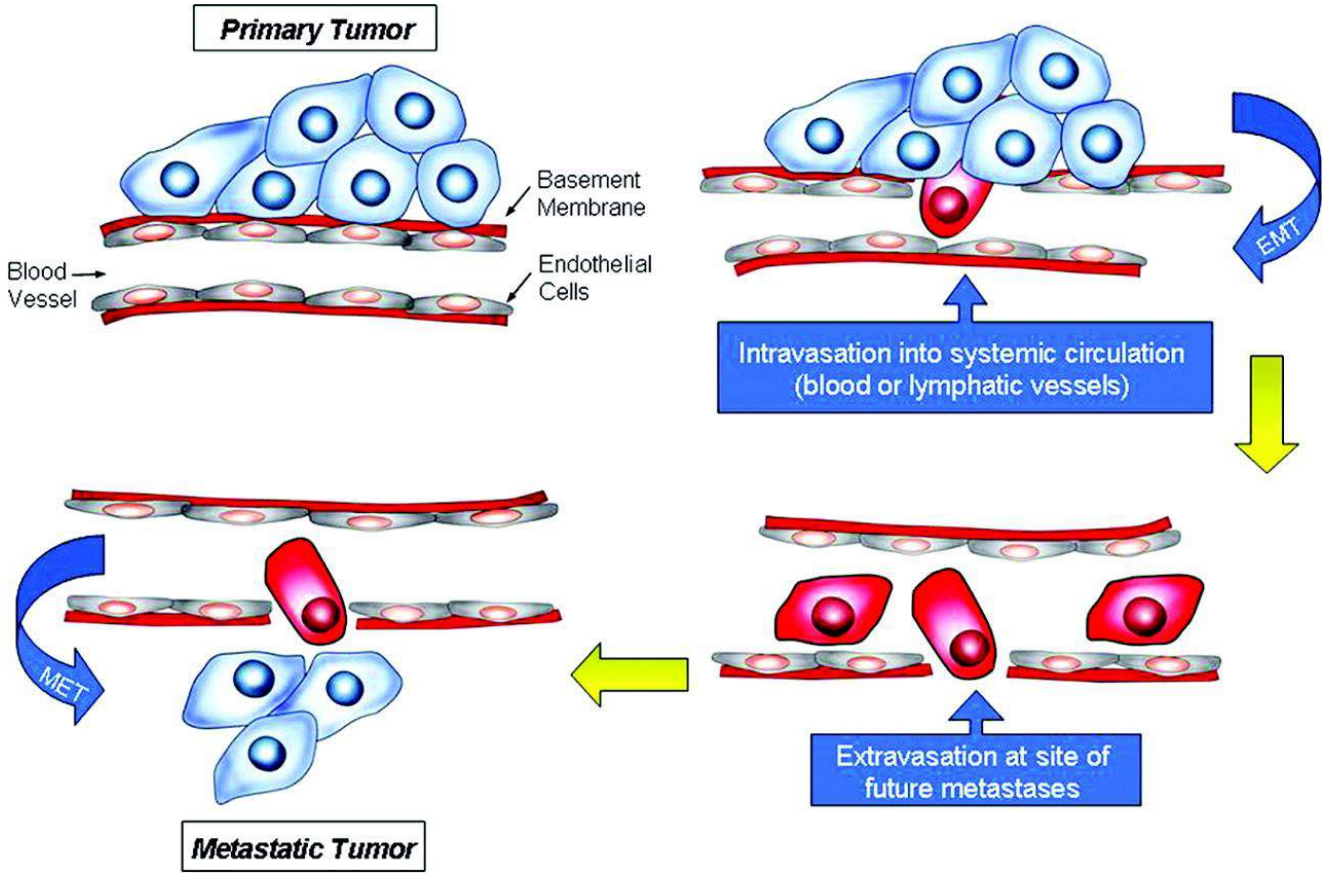
CHOOSING THE RIGHT METHOD

COMPARATIVE METHODS

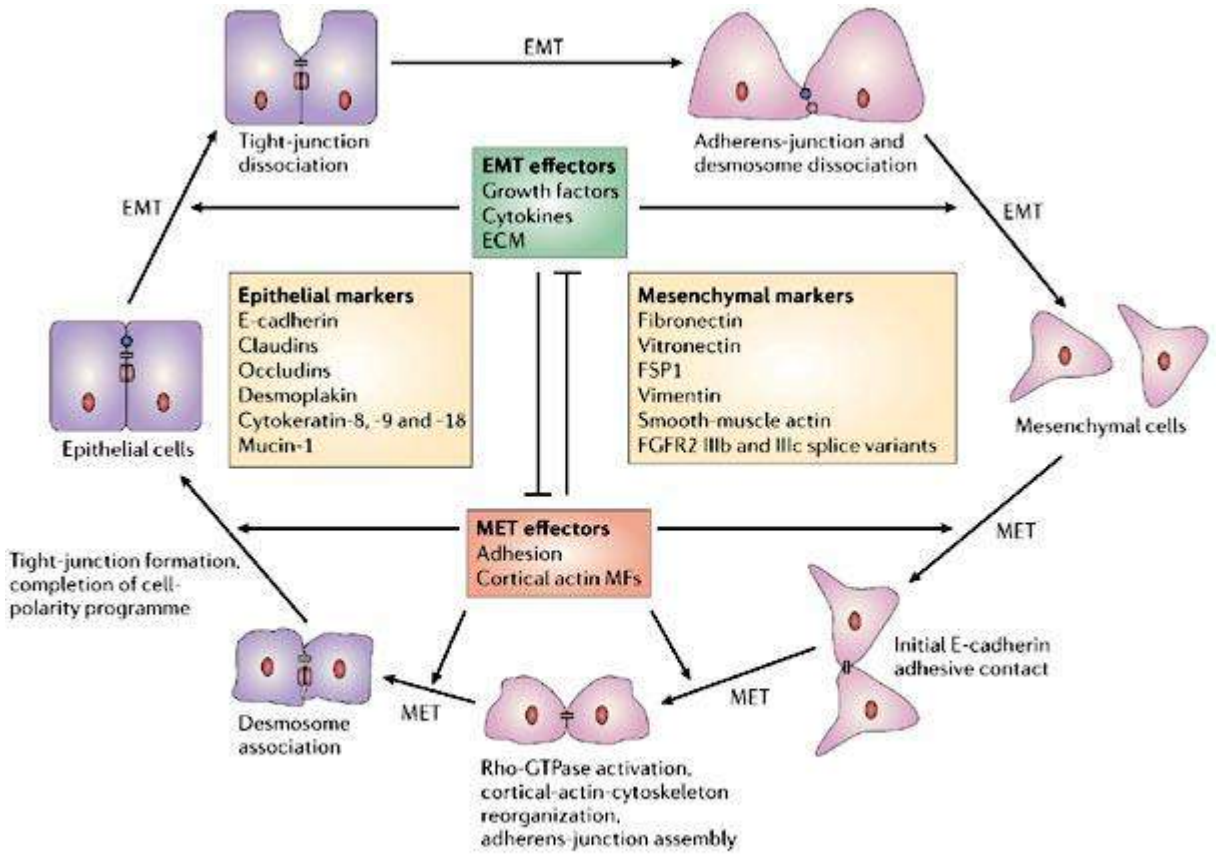
	Beads Based Method	PCR Based Method	R.G.C.C. Ltd
Method of Isolation	Magnetic Beads (antibodies with iron particles)	PCR based method which need to destroy the cells in order to identify one marker (mainly panCK or Epcam)	Flow cytometric sorting with interrogation in droplets in ratio of droplet per cell (1:1)
Purity of CTCs	Enrichment method and not isolation method	There are no cells any more	Purity is higher than 97 to 99% (isolation method)
Viability of the isolated cells	70-85%	No cells	Viability >99%
Quality of CTCs for further analysis	Inappropriate for further molecular analysis due to lymphocyte contamination	Limited for further molecular analysis	Appropriate for further molecular analysis since there is no noise
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
Further abilities			Identification of heterogeneity of CTCs
Additional features	Method only to enumerate 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.

R.G.C.C. LTD
M. Alexandrou 115 Str
53070, Filotas
Phone: 0030- 2463 0 42264, Fax: 0030-2463 0 42265

Heterogeneity of CTCs (EMT-MET)

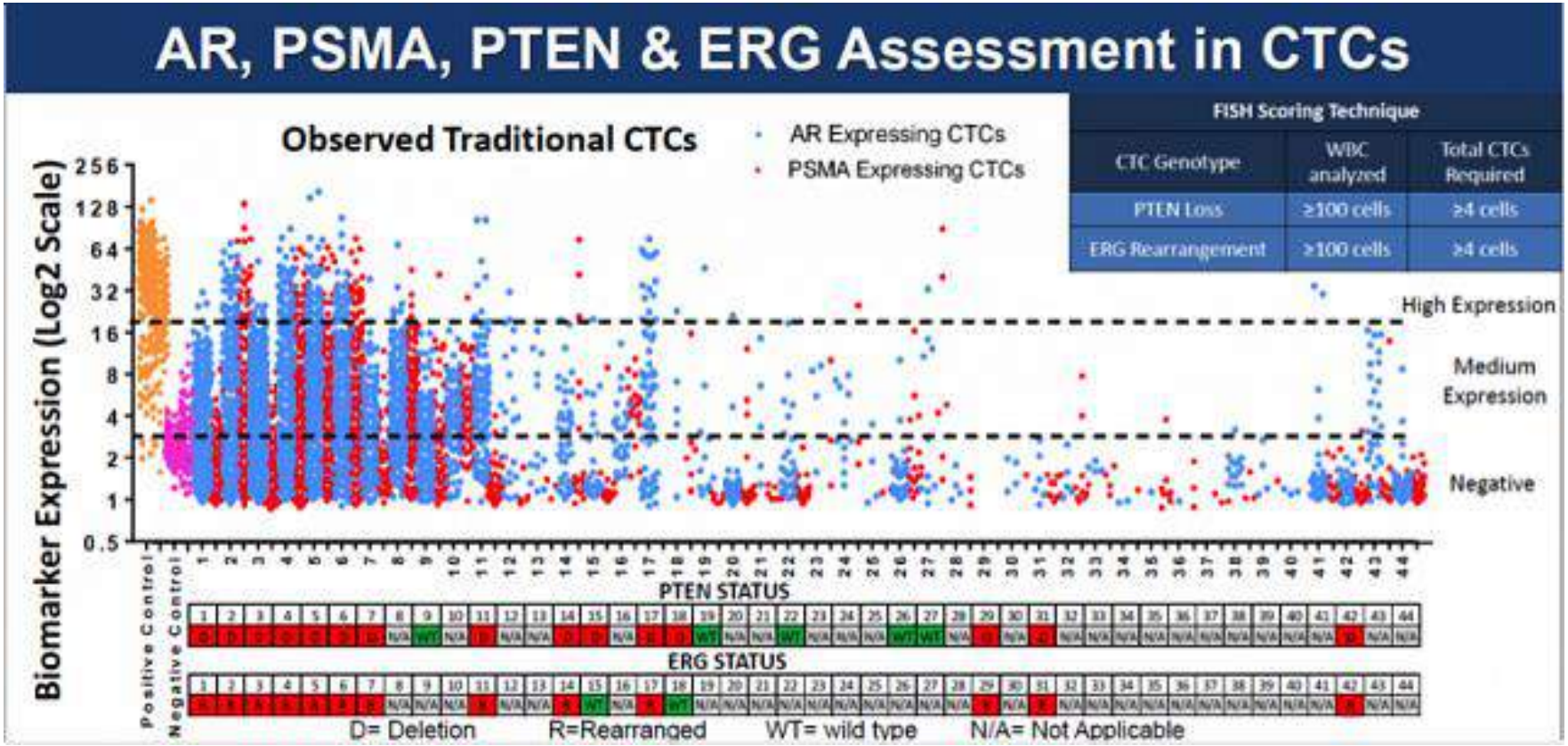


Heterogeneity of CTCs (EMT-MET)



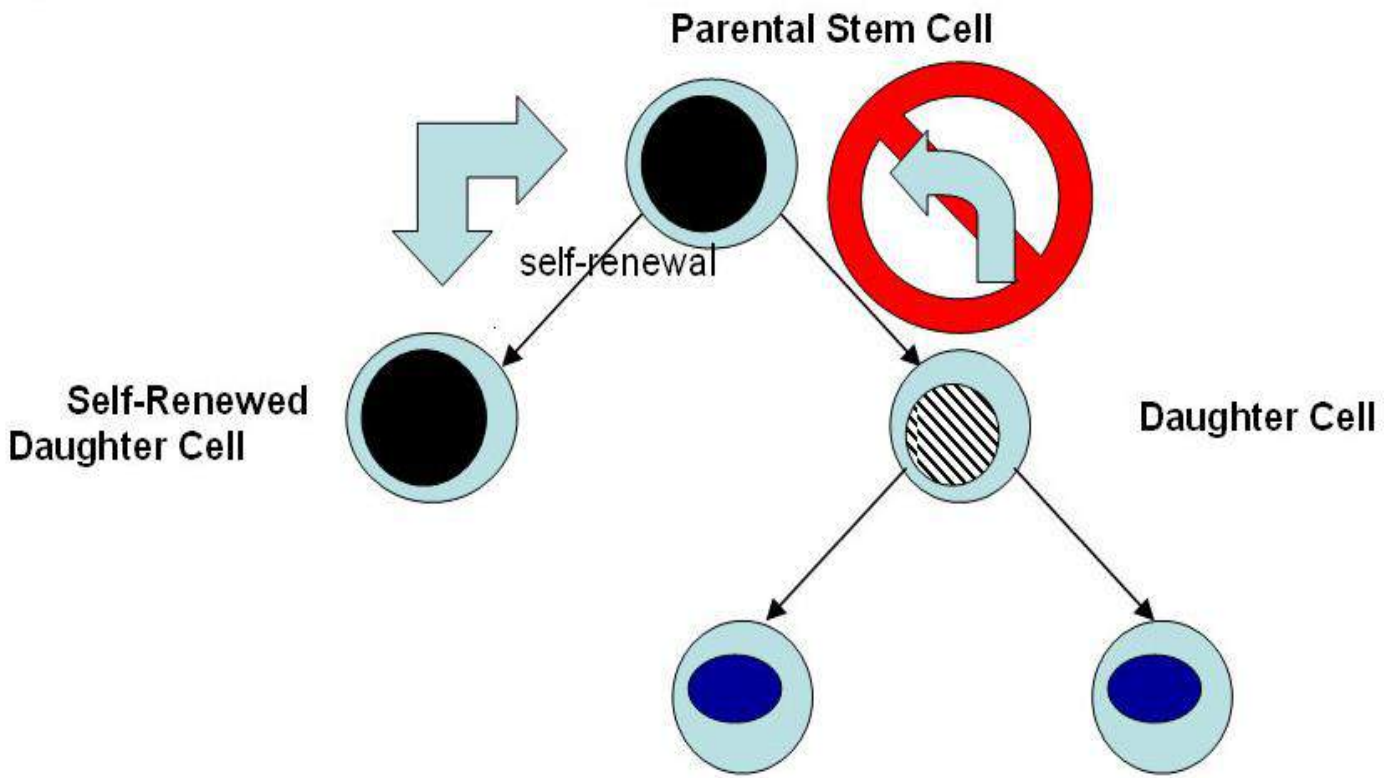
Copyright © 2006 Nature Publishing Group
Nature Reviews | Molecular Cell Biology

Heterogeneity of CTCs (EMT-MET)



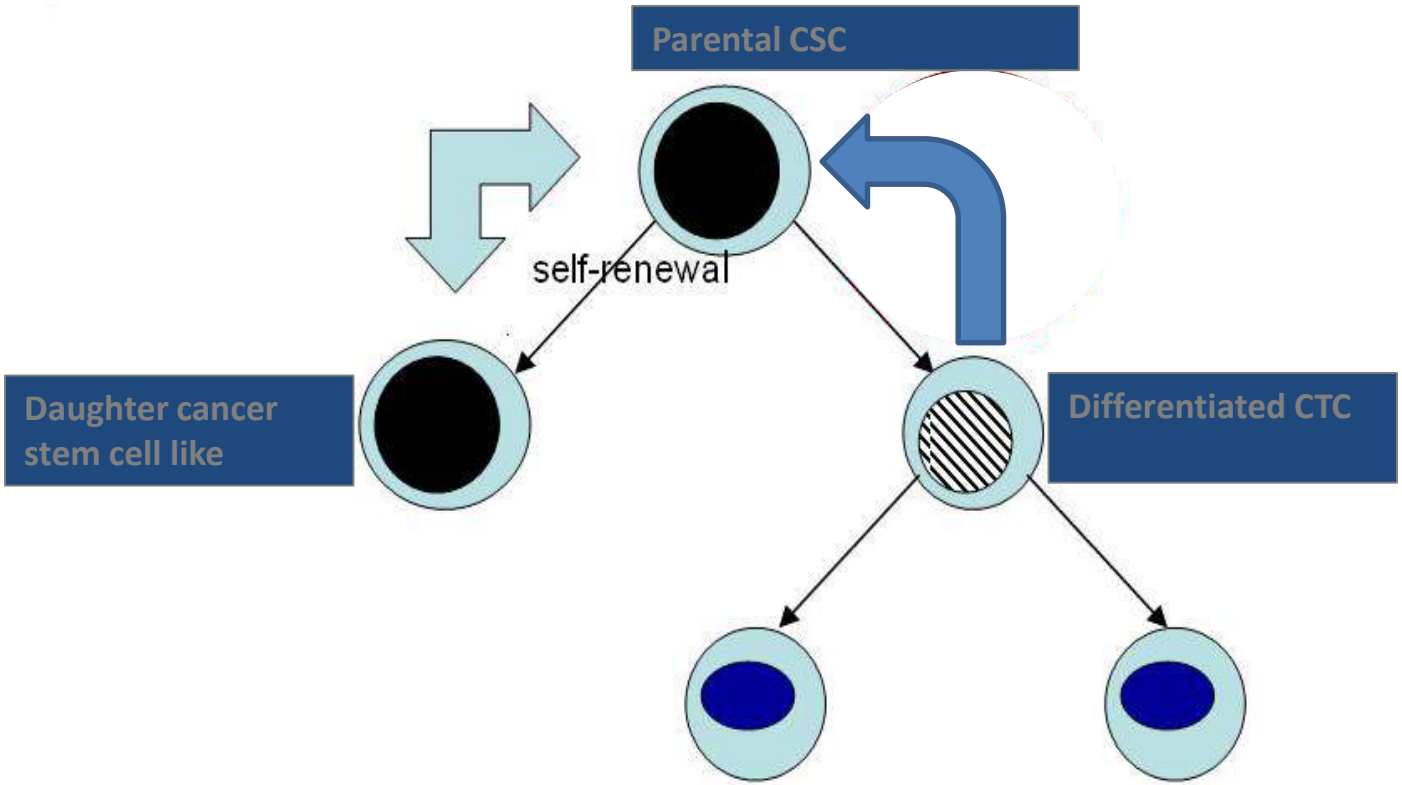
CTCs & CANCER STEM CELL LIKE OR TUMOR INITIATING CELLS

SELF – RENEWAL(CCSs)



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

SELF – RENEWAL(CCSs)



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

RELEVANCE BETWEEN TUMOR INITIATING CELLS AND TUMORIGENICITY

NIH Public Access
Author Manuscript
Stem Cells. Author manuscript; available in PMC 2013 October 01.

Published in final edited form as:
Stem Cells. 2012 October ; 30(10): 2065–2075. doi:10.1002/stem.1139.

Phosphosulindac (OXT-328) selectively targets breast cancer stem cells *in vitro* and in human breast cancer xenografts

Caihua Zhu[1], Ka-Wing Cheng[1], Nengtai Ouyang[1], Liqun Huang[1], Yu Sun[1], Panayiotis P. Constantinides[2], and Basil Rigas[1,*]

[1]Division of Cancer Prevention, Department of Medicine, Stony Brook University, Stony Brook, New York

[2]Medicon Pharmaceuticals, Inc., Stony Brook, New York

Abstract

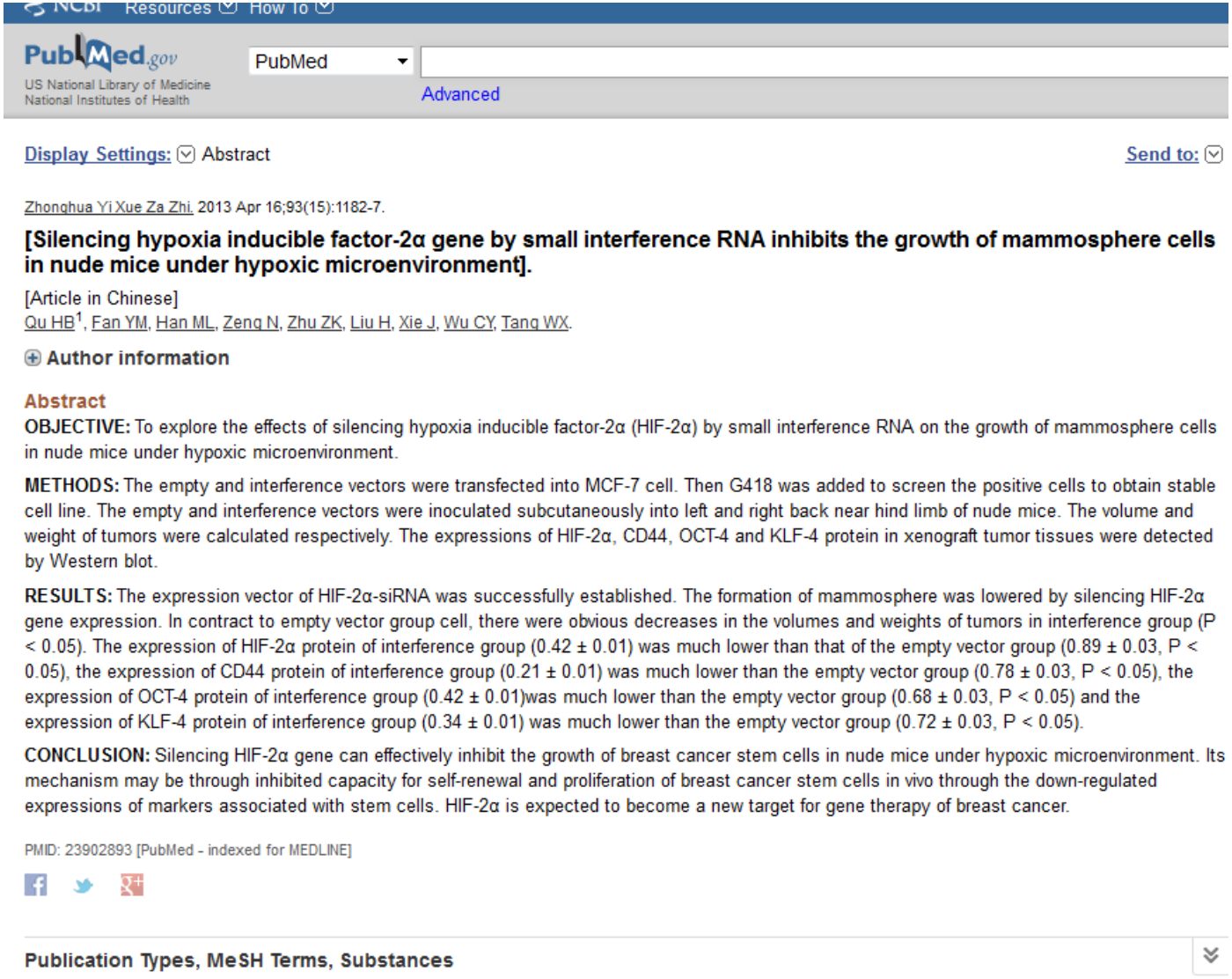
Pharmacological targeting of breast cancer stem cells (CSCs) is highly promising for the treatment of breast cancer, as the small population of CSCs appears responsible for tumor initiation and progression and also for resistance to conventional treatment. Here we report that the novel phosphosulindac (OXT-328, PS) selectively and effectively eliminates breast CSCs both *in vitro* and *in vivo*. PS reduced cell proliferaition and induced apoptosis in various breast CSCs. Breast CSCs are resistant to conventional cancer drugs but are sensitive to PS. Long-term treatment with PS of mixtures of cultured breast CSCs with breast cancer cells preferentially eliminated the CSCs. PS impaired the ability of CSCs to form mammospheres and markedly suppressed the expression of CSC-related genes. More importantly, PS prevented by half ($p=0.06$) the formation of tumors initiated by CSCs in immunodeficient mice, and inhibited by 83% ($p<0.05$) the growth of already formed breast cancer xenografts, reducing the proportion of CSCs in them. PS suppressed the Wnt/β-catenin pathway by stimulating the degradation of β-catenin and its relocalization to the cell membrane; and also blocked the epithelial-mesenchymal transition (EMT) and the generation of breast CSCs. These results indicate that PS has a strong inhibitory effect against breast cancer, acting, at least in part, by targeting CSCs through a signaling mechanism involving Wnt signaling.

Keywords

CSCs; phosphosulindac; Wnt/β-catenin; EMT; breast cancer

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH

RELEVANCE BETWEEN TUMOR INITIATING CELLS AND TUMORIGENICITY



NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Display Settings: Abstract

Send to:

Zhonghua Yi Xue Za Zhi. 2013 Apr 16;93(15):1182-7.

[Silencing hypoxia inducible factor-2α gene by small interference RNA inhibits the growth of mammosphere cells in nude mice under hypoxic microenvironment].

[Article in Chinese]

Qu HB[1], Fan YM, Han ML, Zeng N, Zhu ZK, Liu H, Xie J, Wu CY, Tang WX.

Author information

Abstract

OBJECTIVE: To explore the effects of silencing hypoxia inducible factor-2α (HIF-2α) by small interference RNA on the growth of mammosphere cells in nude mice under hypoxic microenvironment.

METHODS: The empty and interference vectors were transfected into MCF-7 cell. Then G418 was added to screen the positive cells to obtain stable cell line. The empty and interference vectors were inoculated subcutaneously into left and right back near hind limb of nude mice. The volume and weight of tumors were calculated respectively. The expressions of HIF-2α, CD44, OCT-4 and KLF-4 protein in xenograft tumor tissues were detected by Western blot.

RESULTS: The expression vector of HIF-2α-siRNA was successfully established. The formation of mammosphere was lowered by silencing HIF-2α gene expression. In contract to empty vector group cell, there were obvious decreases in the volumes and weights of tumors in interference group ($P < 0.05$). The expression of HIF-2α protein of interference group (0.42 ± 0.01) was much lower than that of the empty vector group (0.89 ± 0.03, $P < 0.05$), the expression of CD44 protein of interference group (0.21 ± 0.01) was much lower than the empty vector group (0.78 ± 0.03, $P < 0.05$), the expression of OCT-4 protein of interference group (0.42 ± 0.01)was much lower than the empty vector group (0.68 ± 0.03, $P < 0.05$) and the expression of KLF-4 protein of interference group (0.34 ± 0.01) was much lower than the empty vector group (0.72 ± 0.03, $P < 0.05$).

CONCLUSION: Silencing HIF-2α gene can effectively inhibit the growth of breast cancer stem cells in nude mice under hypoxic microenvironment. Its mechanism may be through inhibited capacity for self-renewal and proliferation of breast cancer stem cells in vivo through the down-regulated expressions of markers associated with stem cells. HIF-2α is expected to become a new target for gene therapy of breast cancer.

PMID: 23902893 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

RELEVANCE BETWEEN TUMOR INITIATING CELLS AND TUMORIGENICITY

NCBI Resources How To

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed

Advanced

Display Settings: Abstract

Send to:

Breast Cancer Res. 2013 Nov 14;15(6):R109. doi: 10.1186/bcr3576.

A mammosphere formation RNAi screen reveals that ATG4A promotes a breast cancer stem-like phenotype.

Wolf J, Dewi DL, Fredebohm J, Müller-Decker K, Flechtenmacher C, Hoheisel JD, Boettcher M.

Abstract

INTRODUCTION: Breast cancer stem cells are suspected to be responsible for tumour recurrence, metastasis formation as well as chemoresistance. Consequently, great efforts have been made to understand the molecular mechanisms underlying cancer stem cell maintenance. In order to study these rare cells in-vitro, they are typically enriched via mammosphere culture. Here we developed a mammosphere-based negative selection shRNAi screening system suitable to analyse the involvement of thousands of genes in the survival of cells with cancer stem cell properties.

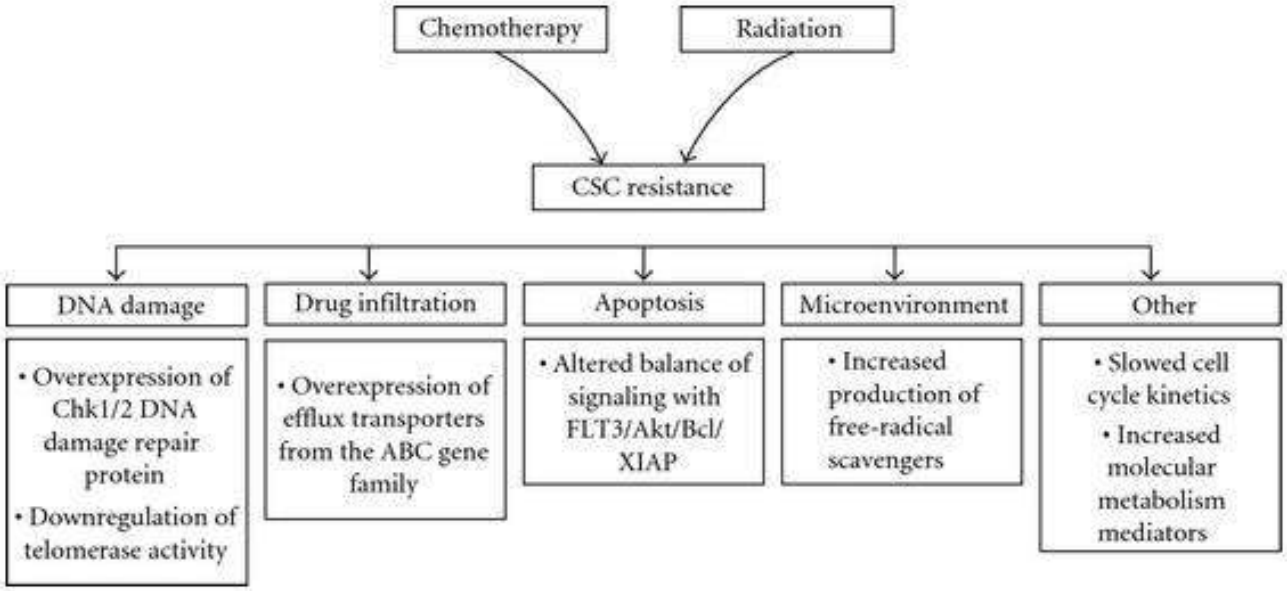
METHODS: We describe a sub-population expressing the stem-like marker CD44(+)/CD24(-/low) in SUM149 that were enriched in mammospheres. To identify genes functionally involved in the maintenance of the sub-population with cancer stem cell properties, we targeted over 5000 genes by RNAi and tested their ability to grow as mammospheres. The identified candidate ATG4A was validated in mammosphere and soft agar colony formation assays. Further, we evaluated the influence of ATG4A expression on the sub-population expressing the stem-like marker CD44(+)/CD24(low). Next, the tumorigenic potential of SUM149 after up- or down-regulation of ATG4A was examined by xenograft experiments.

RESULTS: Using this method, Jak-STAT as well as cytokine signalling were identified to be involved in mammosphere formation. Furthermore, the autophagy regulator ATG4A was found to be essential for the maintenance of a sub-population with cancer stem cell properties and to regulate breast cancer cell tumourigenicity in vivo.

CONCLUSION: In summary, we present a high-throughput screening system to identify genes involved in cancer stem cell maintenance and demonstrate its utility by means of ATG4A.

Special hallmarks of CSCs

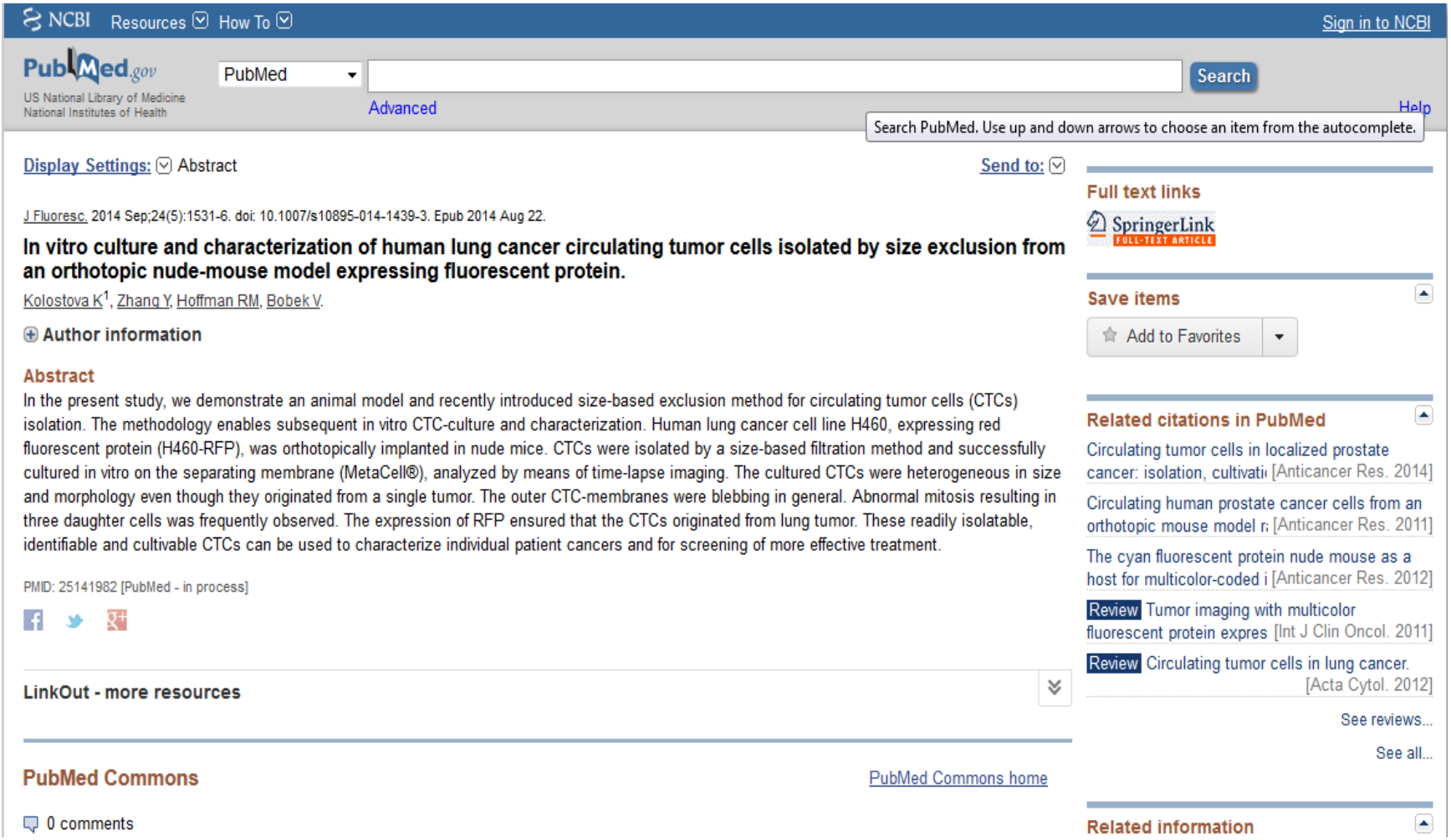
CSC RESISTANCE



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

Can these cells be expanded?

Literature



NCBI Resources How To Sign in to NCBI

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed Search

Advanced Help

Search PubMed. Use up and down arrows to choose an item from the autocomplete.

Display Settings: Abstract Send to:

J Fluoresc. 2014 Sep;24(5):1531-6. doi: 10.1007/s10895-014-1439-3. Epub 2014 Aug 22.

In vitro culture and characterization of human lung cancer circulating tumor cells isolated by size exclusion from an orthotopic nude-mouse model expressing fluorescent protein.

Kolostova K[1], Zhang Y, Hoffman RM, Bobek V.

Author information

Abstract

In the present study, we demonstrate an animal model and recently introduced size-based exclusion method for circulating tumor cells (CTCs) isolation. The methodology enables subsequent in vitro CTC-culture and characterization. Human lung cancer cell line H460, expressing red fluorescent protein (H460-RFP), was orthotopically implanted in nude mice. CTCs were isolated by a size-based filtration method and successfully cultured in vitro on the separating membrane (MetaCell®), analyzed by means of time-lapse imaging. The cultured CTCs were heterogeneous in size and morphology even though they originated from a single tumor. The outer CTC-membranes were blebbing in general. Abnormal mitosis resulting in three daughter cells was frequently observed. The expression of RFP ensured that the CTCs originated from lung tumor. These readily isolatable, identifiable and cultivable CTCs can be used to characterize individual patient cancers and for screening of more effective treatment.

PMID: 25141982 [PubMed - in process]

LinkOut - more resources

PubMed Commons PubMed Commons home

0 comments

Full text links

SpringerLink FULL-TEXT ARTICLE

Save items

Add to Favorites

Related citations in PubMed

Circulating tumor cells in localized prostate cancer: isolation, cultivati [Anticancer Res. 2014]

Circulating human prostate cancer cells from an orthotopic mouse model r [Anticancer Res. 2011]

The cyan fluorescent protein nude mouse as a host for multicolor-coded i [Anticancer Res. 2012]

Review Tumor imaging with multicolor fluorescent protein expres [Int J Clin Oncol. 2011]

Review Circulating tumor cells in lung cancer. [Acta Cytol. 2012]

See reviews...

See all...

Related information

Literature



NCBI Resources How To Sign in to NCBI

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed Search
Advanced Help

Display Settings: Abstract Send to:

Science. 2014 Jul 11;345(6193):216-20. doi: 10.1126/science.1253533.

Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility.

Yu M[1], Bardia A[2], Aceto N[3], Bersani F[3], Madden MW[3], Donaldson MC[3], Desai R[3], Zhu H[3], Comaills V[3], Zheng Z[4], Wittner BS[3], Stojanov P[5], Brachtel E[6], Sgroi D[7], Kapur R[8], Shioda T[2], Ting DT[2], Ramaswamy S[2], Getz G[9], Iafrate AJ[7], Benes C[2], Toner M[10], Maheswaran S[11], Haber DA[12].

Author information

Abstract

Circulating tumor cells (CTCs) are present at low concentrations in the peripheral blood of patients with solid tumors. It has been proposed that the isolation, ex vivo culture, and characterization of CTCs may provide an opportunity to noninvasively monitor the changing patterns of drug susceptibility in individual patients as their tumors acquire new mutations. In a proof-of-concept study, we established CTC cultures from six patients with estrogen receptor-positive breast cancer. Three of five CTC lines tested were tumorigenic in mice. Genome sequencing of the CTC lines revealed preexisting mutations in the PIK3CA gene and newly acquired mutations in the estrogen receptor gene (ESR1), PIK3CA gene, and fibroblast growth factor receptor gene (FGFR2), among others. Drug sensitivity testing of CTC lines with multiple mutations revealed potential new therapeutic targets. With optimization of CTC culture conditions, this strategy may help identify the best therapies for individual cancer patients over the course of their disease.

Copyright © 2014, American Association for the Advancement of Science.

PMID: 25013076 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances, Secondary Source ID, Grant Support

LinkOut - more resources

PubMed Commons PubMed Commons home

0 comments

Full text links

Science

Save items

Add to Favorites

Related citations in PubMed

Analysing the mutational status of PIK3CA in circulating tumor cells from me [Mol Oncol. 2013]

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of p [Nature. 2013]

Isolation of breast cancer and gastric cancer circulating tumor cells by use of [Lab Chip. 2014]

Review Circulating tumor cells in breast cancer: detection systems, molecular ([Clin Chem. 2011]

Review Towards a personalized breast cancer treatment approach guid [Cancer Treat Rev. 2013]

See reviews...

See all...

Related information

Related Citations

GEO DataSets

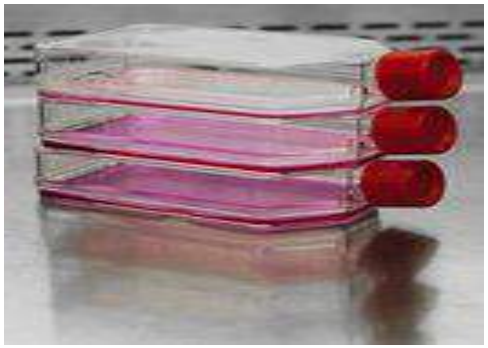
MedGen

CTCs expansion

- Proper culture media
- Treat the cells as Cell with stemness properties
- Require of the proper environmental conditions
- Require proper supplements (StemPro base media etc)

Cultivation of CSCs

- Isolation from blood or tissue samples
- Flasks which are treated with specialized extra cellular matrix which provides the foundation for three-dimensional (3D) culture studies
- Appropriate growth medium containing growth factors and ingredients



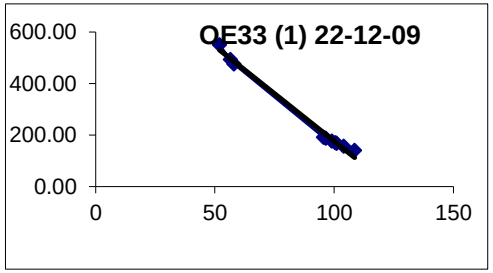
TECHNICAL ISSUE 1

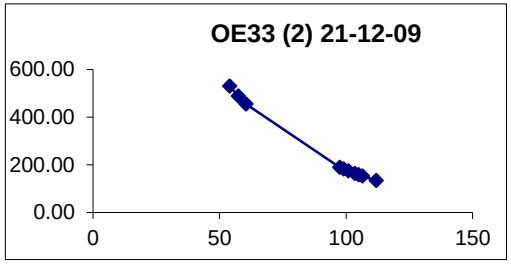
- Since cancer cells are genetically unstable how is it possible to expand them without deviated severely from clinical reality?

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







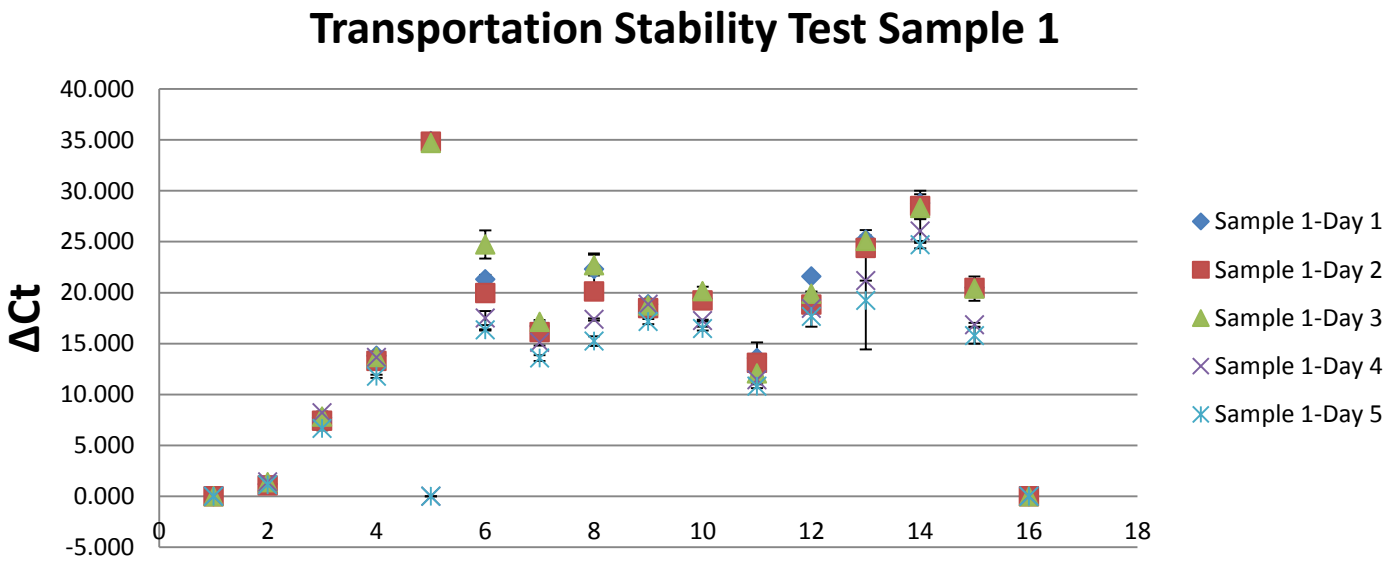
TECHNICAL ISSUE 2

- 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



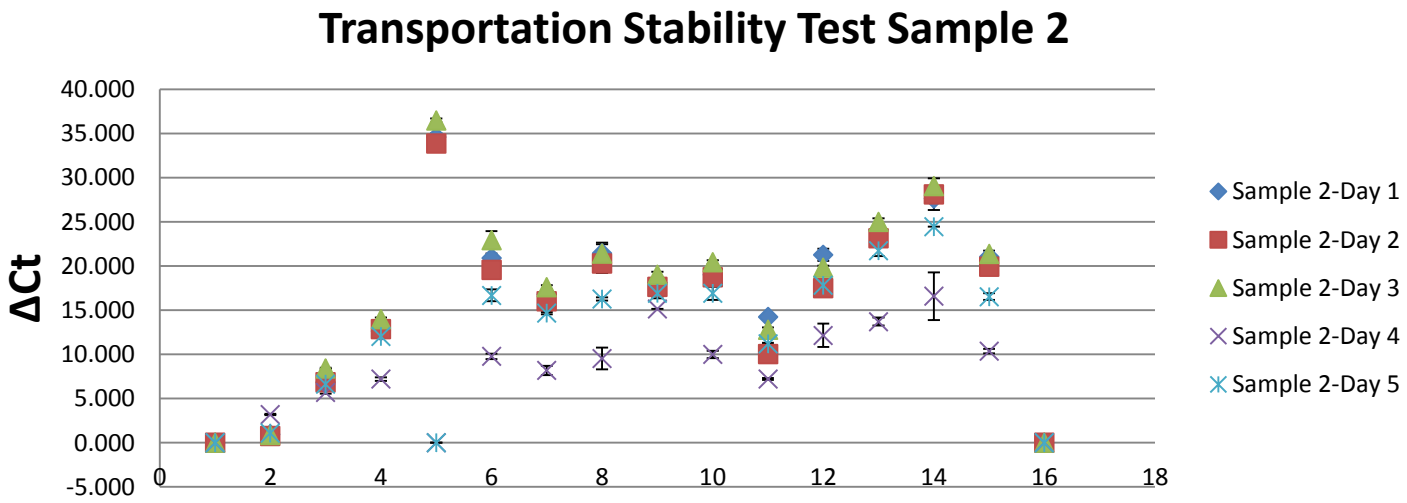
TECHNICAL ISSUE 2

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

Short Tandem Repeats (STRs)

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

1. Real time PCR



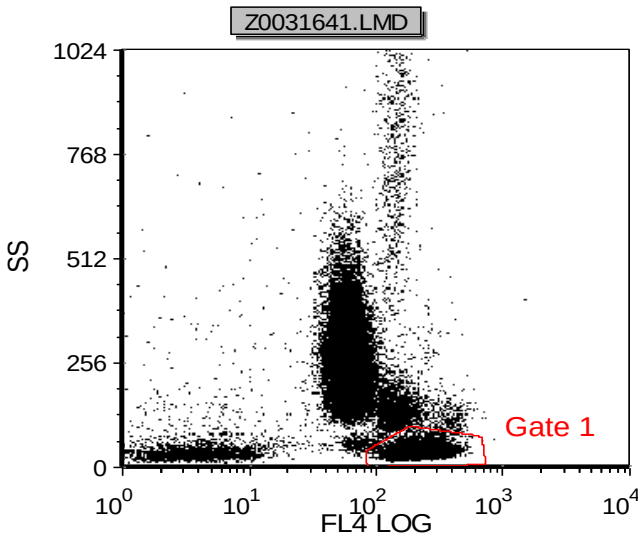
TECHNICAL ISSUE 2

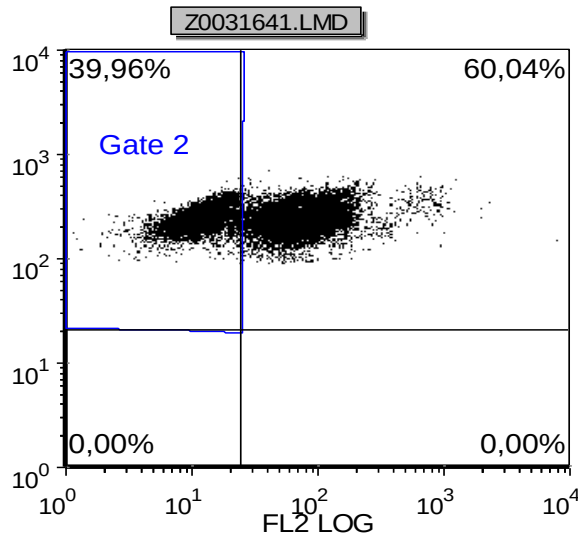
- How stable the sample is during transportation under the parameter of time?

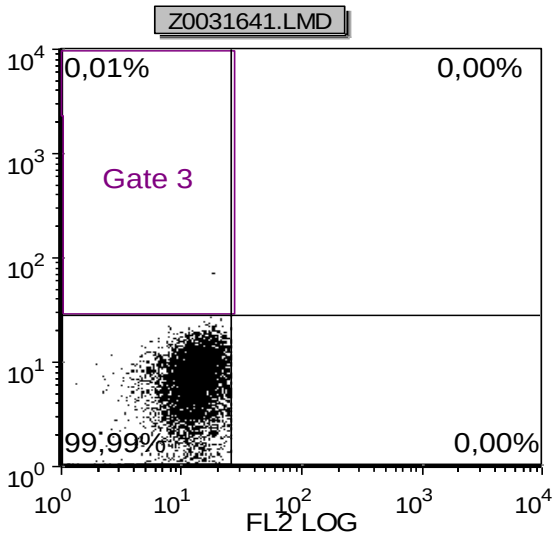
Short Tandem Repeats (STRs)

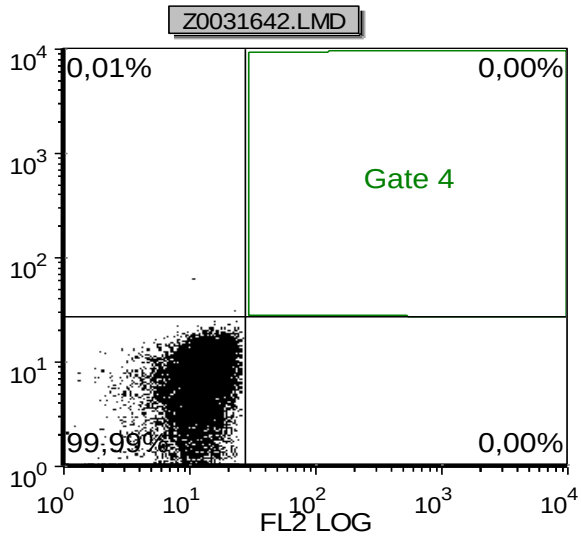
Samples have been tested on epigenetic as well as according to immunophenotype using the following techniques:

1. Flow cytometry







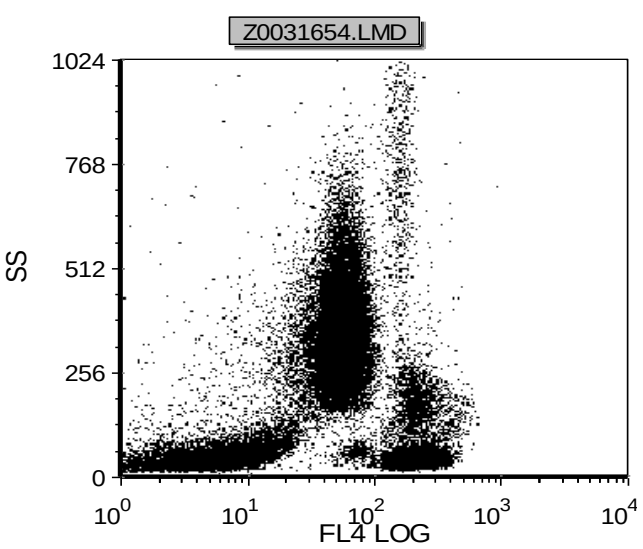


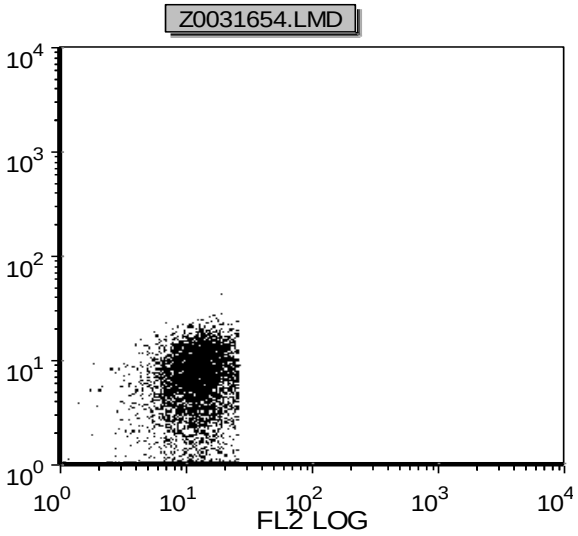
Sample 1 Day 0 (sample collection)
Gate 1: lymphocytes
Gate 2: CD31 negative cells
Gate 3: CK positive cells
Gate 4: cMet positive cells

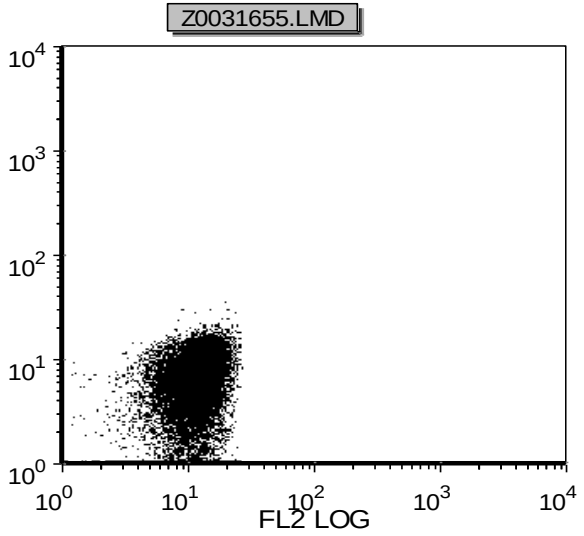


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031642.LMD	None	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 1	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 2	17570	11,78	4,81	100,0	34,28
1	Z0031642.LMD	Gate 3	1	10,84	59,35	0,01	0,0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

Sample 1 Day 1

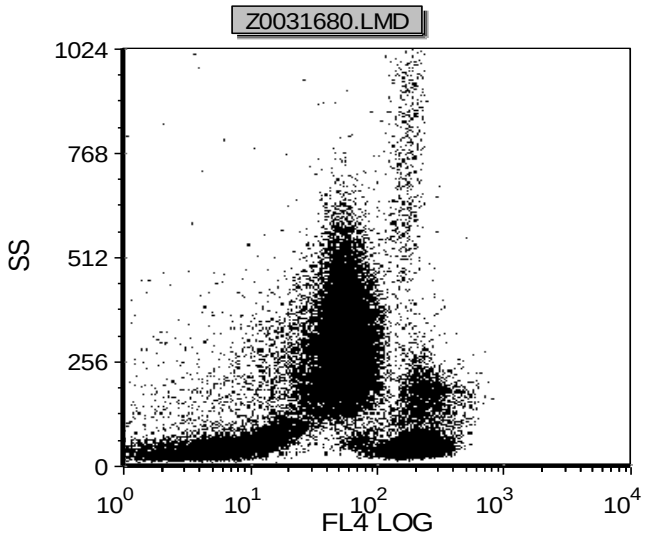


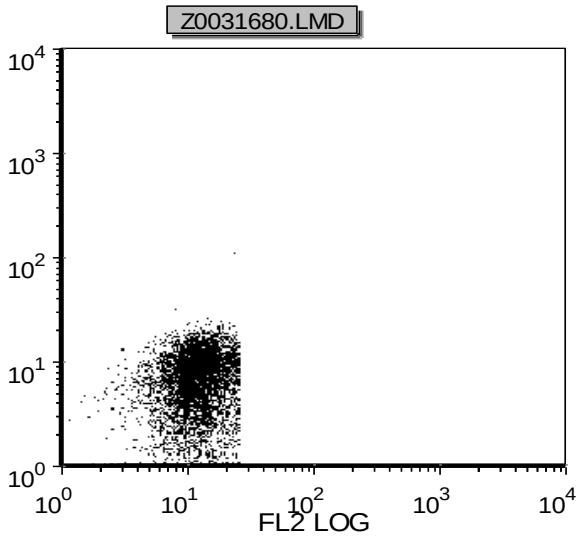


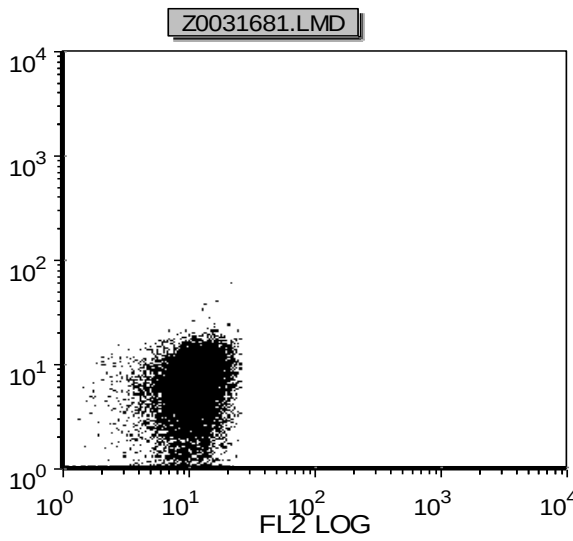


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031654.LMD	None	50889	42,85	0,0	100,0	100,0
1	Z0031654.LMD	Gate 1	12953	213,69	36,56	25,45	25,45
1	Z0031654.LMD	Gate 2	5627	213,34	34,34	11,06	11,06
1	Z0031654.LMD	Gate 3	1	248,05	37,0	0,0	0,0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

Sample 1 Day 2

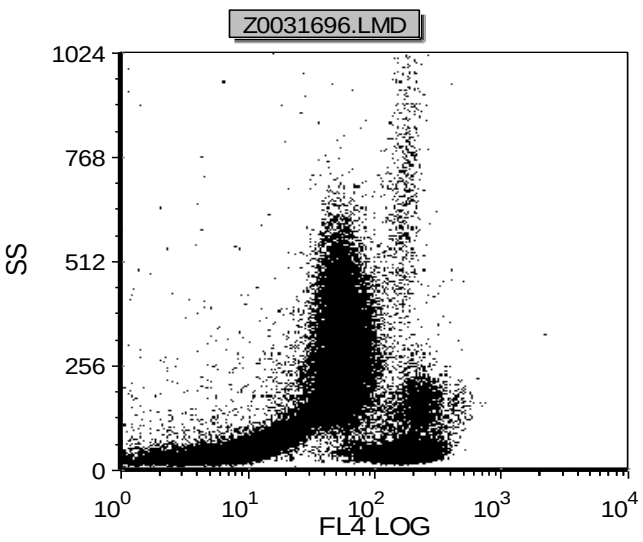


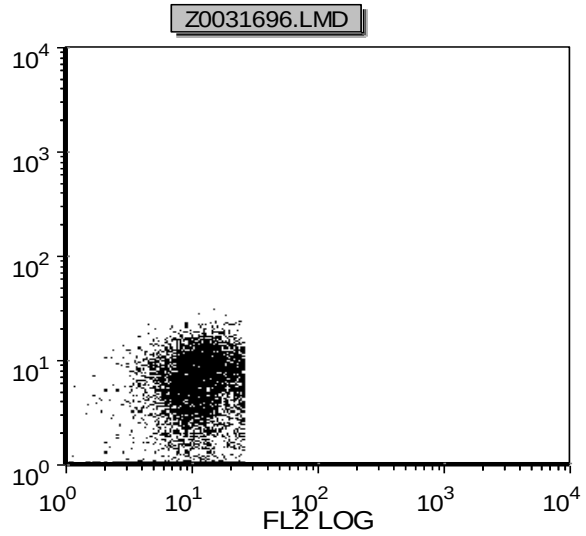


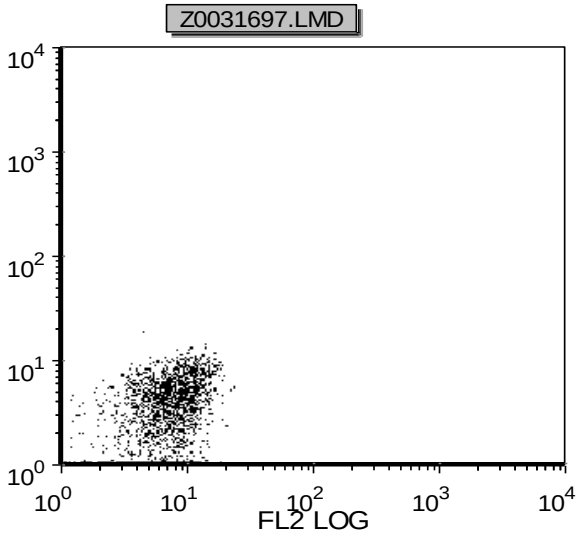


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031680.LMD	None	50278	46,87	105,34	100,0	100,0
1	Z0031680.LMD	Gate 1	13076	203,12	37,87	26,01	26,01
1	Z0031680.LMD	Gate 2	5729	204,05	37,15	11,39	11,39
1	Z0031680.LMD	Gate 3	2	196,32	28,98	0,0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

Sample 1 Day 3

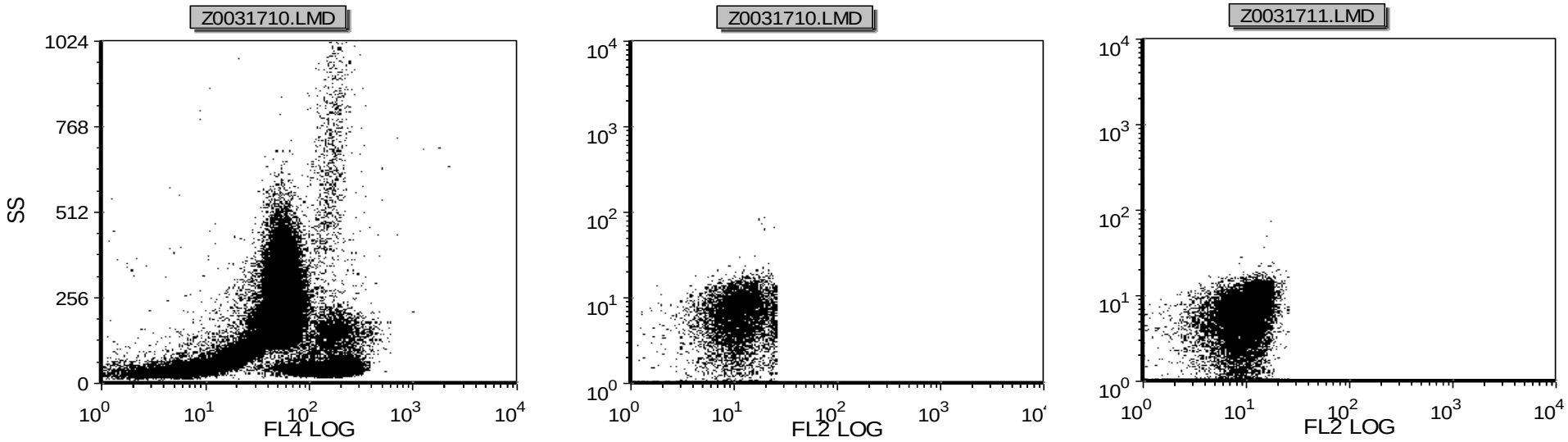




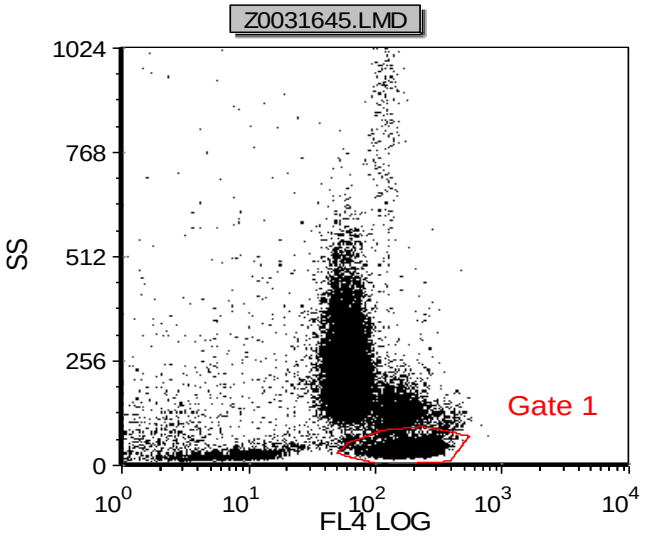


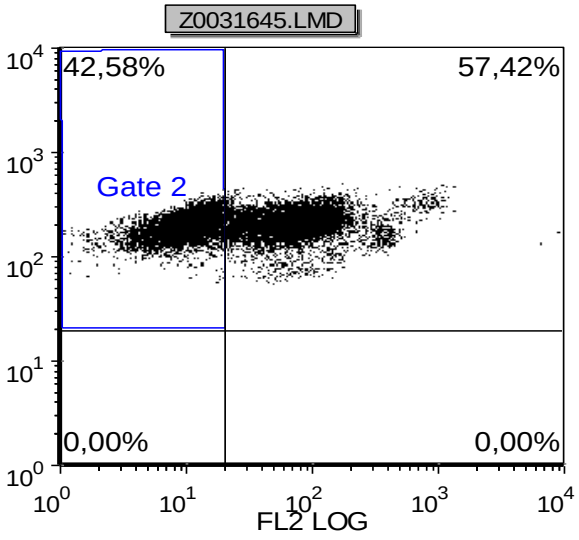
Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031696.LMD	None	56573	38,43	0,0	100,0	100,0
1	Z0031696.LMD	Gate 1	13561	181,42	35,14	23,97	23,97
1	Z0031696.LMD	Gate 2	6424	184,41	34,3	11,36	11,36
1	Z0031696.LMD	Gate 3	0	0,0	0,0	0,0	0,0
1	Z0031696.LMD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

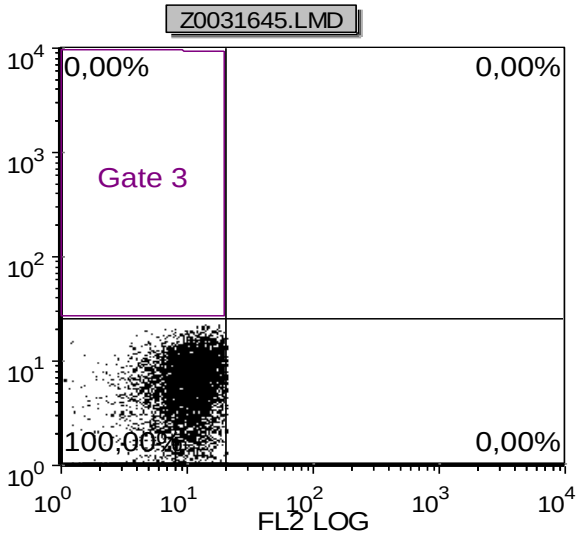
Sample 1 Day 4

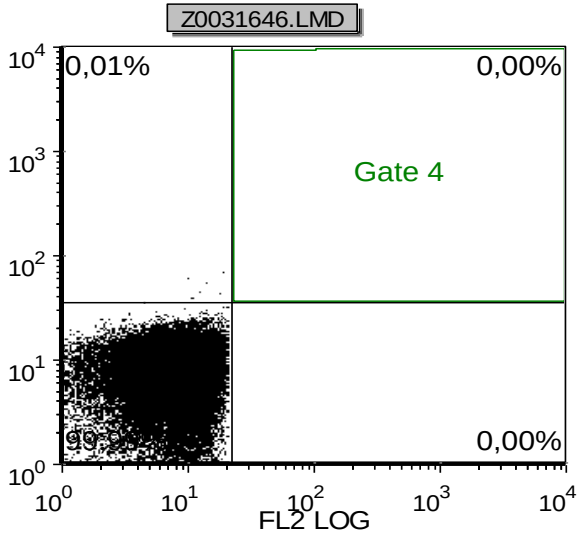


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031710.LMD	None	58035	45,72	0,0	100,0	100,0
1	Z0031710.LMD	Gate 1	13910	169,33	35,48	23,97	23,97
1	Z0031710.LMD	Gate 2	6839	176,43	34,92	11,78	11,78
1	Z0031710.LMD	Gate 3	7	191,1	38,12	0,01	0,01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







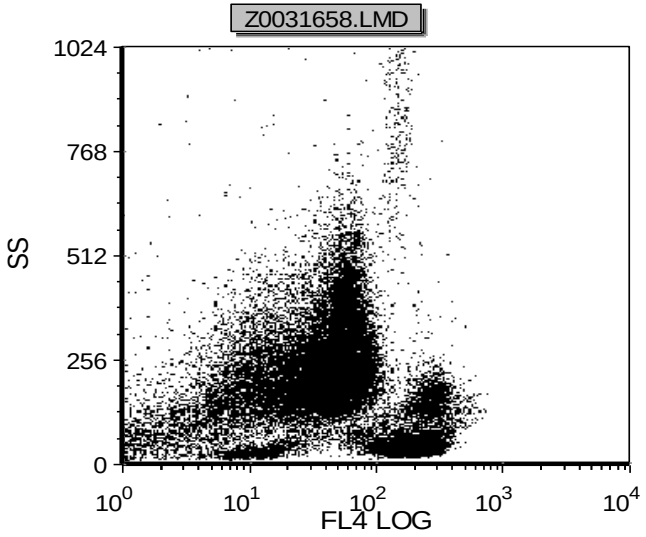


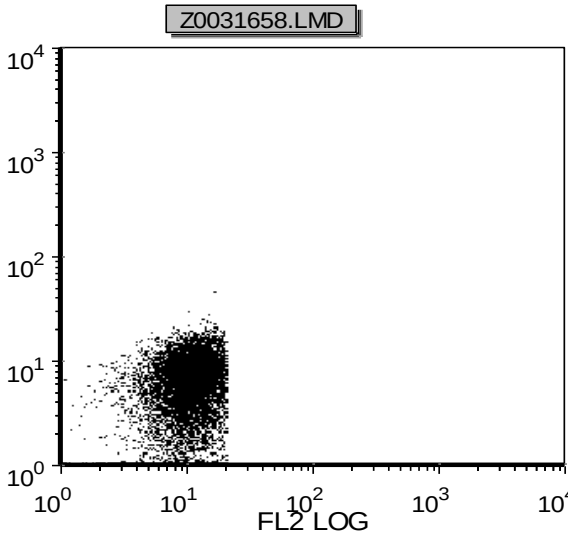
Sample 2 Day 0 (sample collection)
Gate 1: Lymphocytes
Gate 2: CD31 negative cells
Gate 3: CK positive cells
Gate 4: cMet positive cells

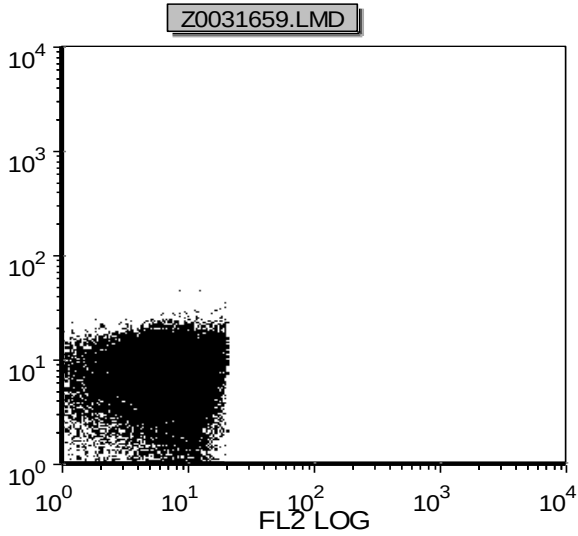


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031646.LMD	None	45557	7,06	5,39	100,0	87,6
1	Z0031646.LMD	Gate 1	20981	10,12	4,17	46,05	40,34
1	Z0031646.LMD	Gate 2	45557	7,06	5,39	100,0	87,6
1	Z0031646.LMD	Gate 3	13	11,9	36,22	0,03	0,02
1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

Sample 2 Day 1

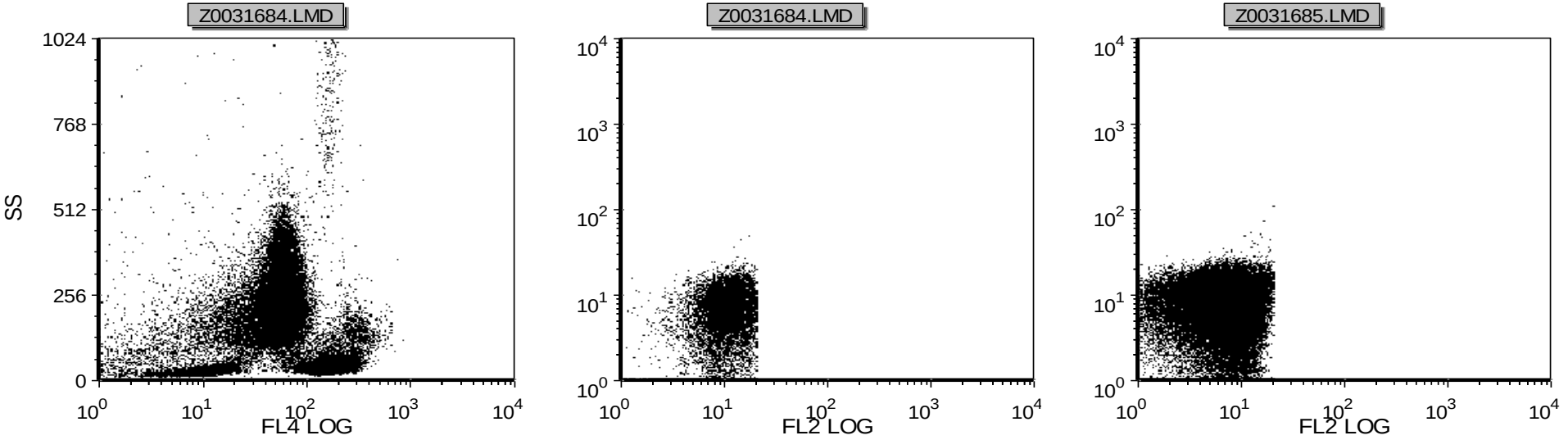






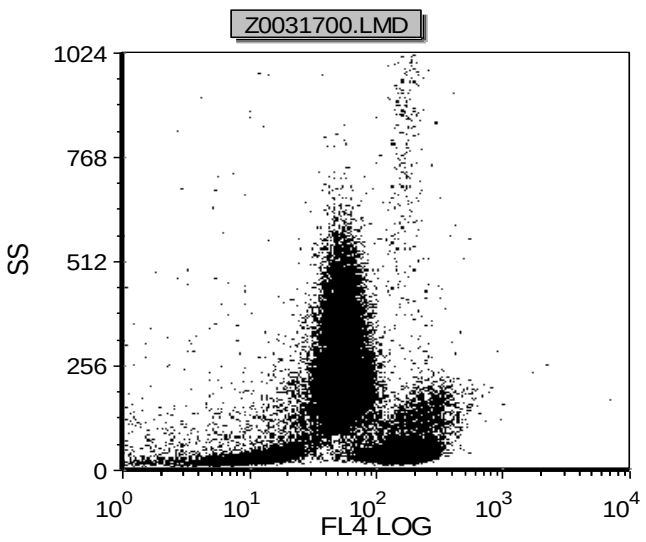
Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031658.LMD	None	8769	10,0	4,31	100,0	17,39
1	Z0031658.LMD	Gate 1	8748	10,0	4,3	99,76	17,35
1	Z0031658.LMD	Gate 2	8769	10,0	4,31	100,0	17,39
1	Z0031658.LMD	Gate 3	2	13,22	35,71	0,02	0,0
1	Z0031658.LMD	Gate 4	0	0,0	0,0	0,0	0,0

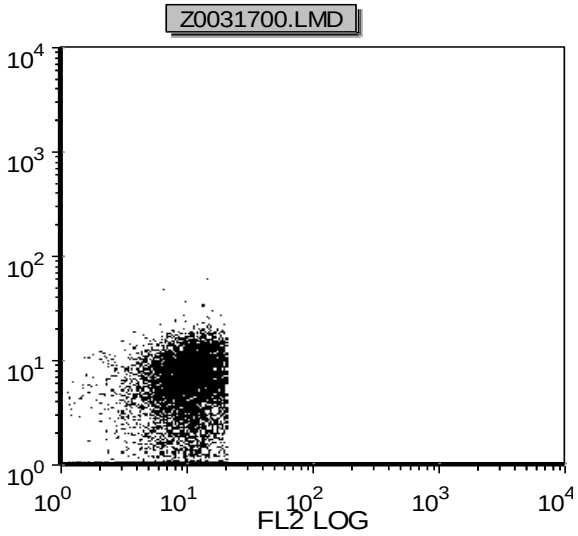
Sample 2 Day 2

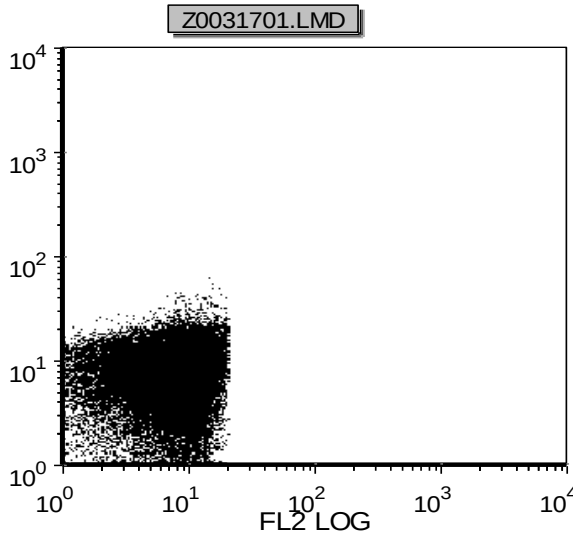


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031684.LMD	None	50963	55,81	84,1	100,0	100,0
1	Z0031684.LMD	Gate 1	19926	178,95	36,43	39,1	39,1
1	Z0031684.LMD	Gate 2	9093	177,43	36,07	17,84	17,84
1	Z0031684.LMD	Gate 3	3	164,0	42,22	0,01	0,01
1	Z0031684.LMD	Gate 4	0	0,0	0,0	0,0	0,0

Sample 2 Day 3

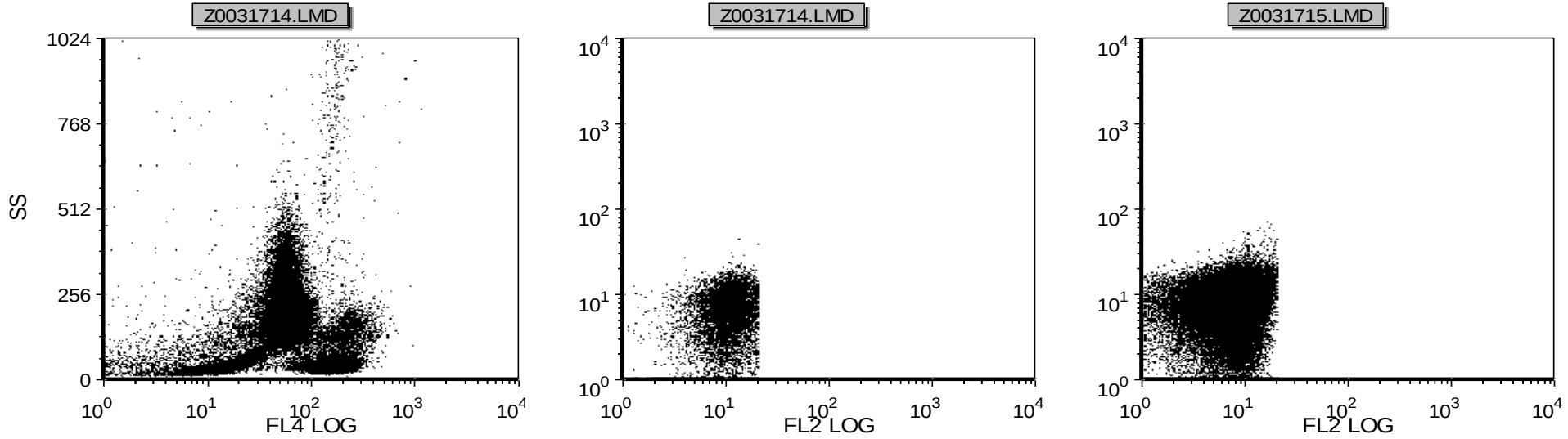






Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031700.LMD	None	50336	51,85	0,0	100,0	100,0
1	Z0031700.LMD	Gate 1	18283	167,1	34,88	36,32	36,32
1	Z0031700.LMD	Gate 2	8419	166,04	34,88	16,73	16,73
1	Z0031700.LMD	Gate 3	5	127,26	38,07	0,01	0,01
1	Z0031700.LMD	Gate 4	0	0,0	0,0	0,0	0,0

Sample 2 Day 4

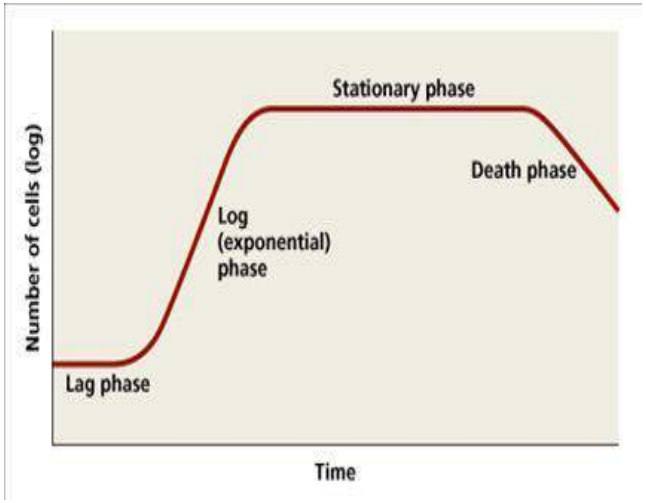


Overlay #	FCS Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of Gated Cells	% of All Cells
1	Z0031714.LMD	None	50295	47,43	0,0	100,0	100,0
1	Z0031714.LMD	Gate 1	17596	169,61	36,25	34,99	34,99
1	Z0031714.LMD	Gate 2	8150	170,23	36,47	16,2	16,2
1	Z0031714.LMD	Gate 3	3	204,12	68,94	0,01	0,01
1	Z0031714.LMD	Gate 4	0	0,0	0,0	0,0	0,0

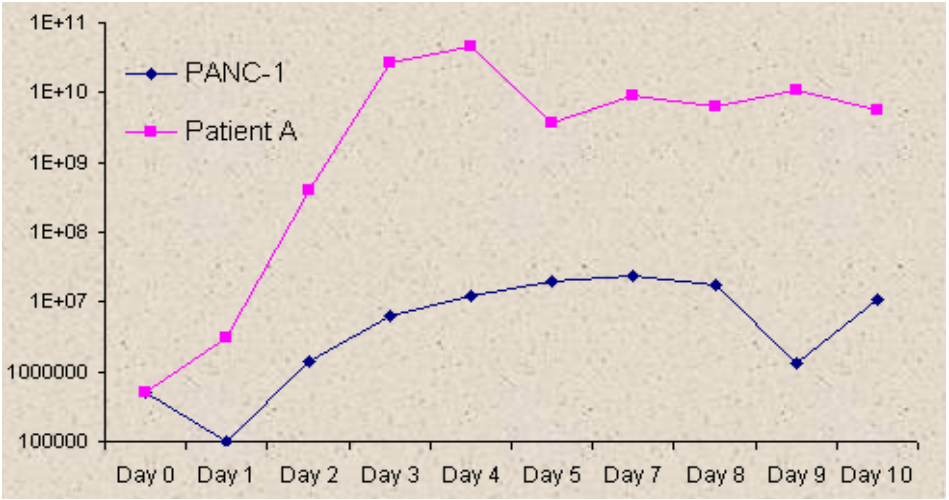
STEMNESS PHENOTYPE

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

Send Orders for Reprints to reprints@benthamscience.net

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

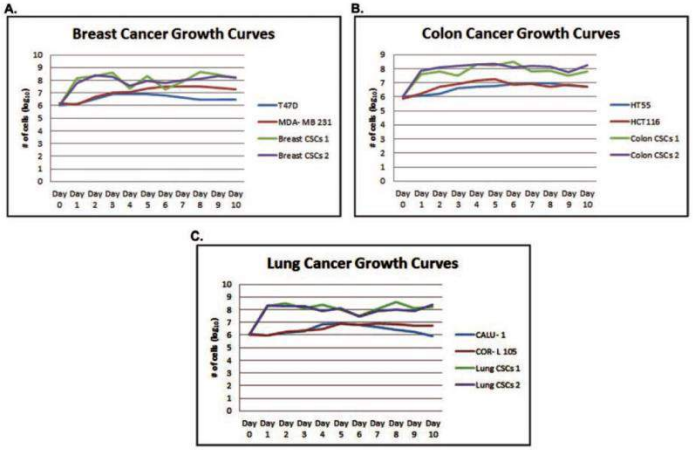
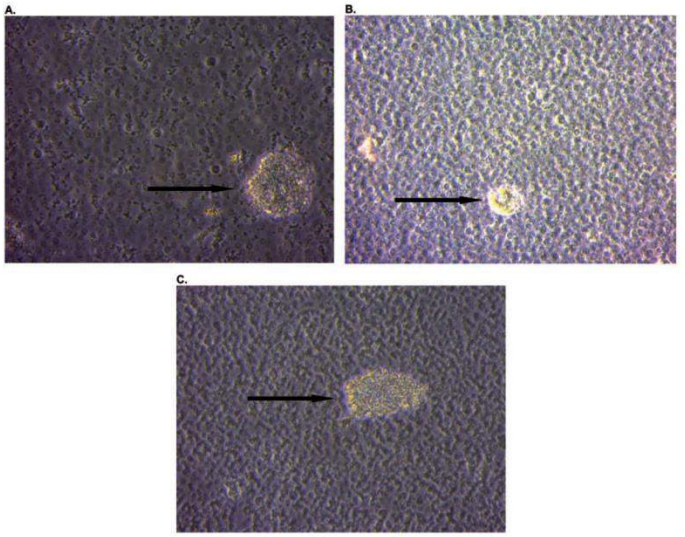


Fig. (1). Growth curves of each cancer type.



CSCs molecular markers

- **Nanog gene** :

  Pluripotency promoting gene, self renewal, block differentiation, embryonic stem cell marker

- **Oct3/4 gene** :

  (Octamer-binding protein 3/4) Key regulator of self renewal, embryonic stem cell marker, pluripotent marker

- **Nestin & Sox-2 gene :**

  Pluripotent and early neural stem cell marker

Publication

Send Orders of Reprints at reprints@benthamscience.org

Current Stem Cell Research & Therapy, 2012, *7*, 00-00

Cancer Stem Cells Stemness Transcription Factors Expression Correlates with Breast Cancer Disease Stage

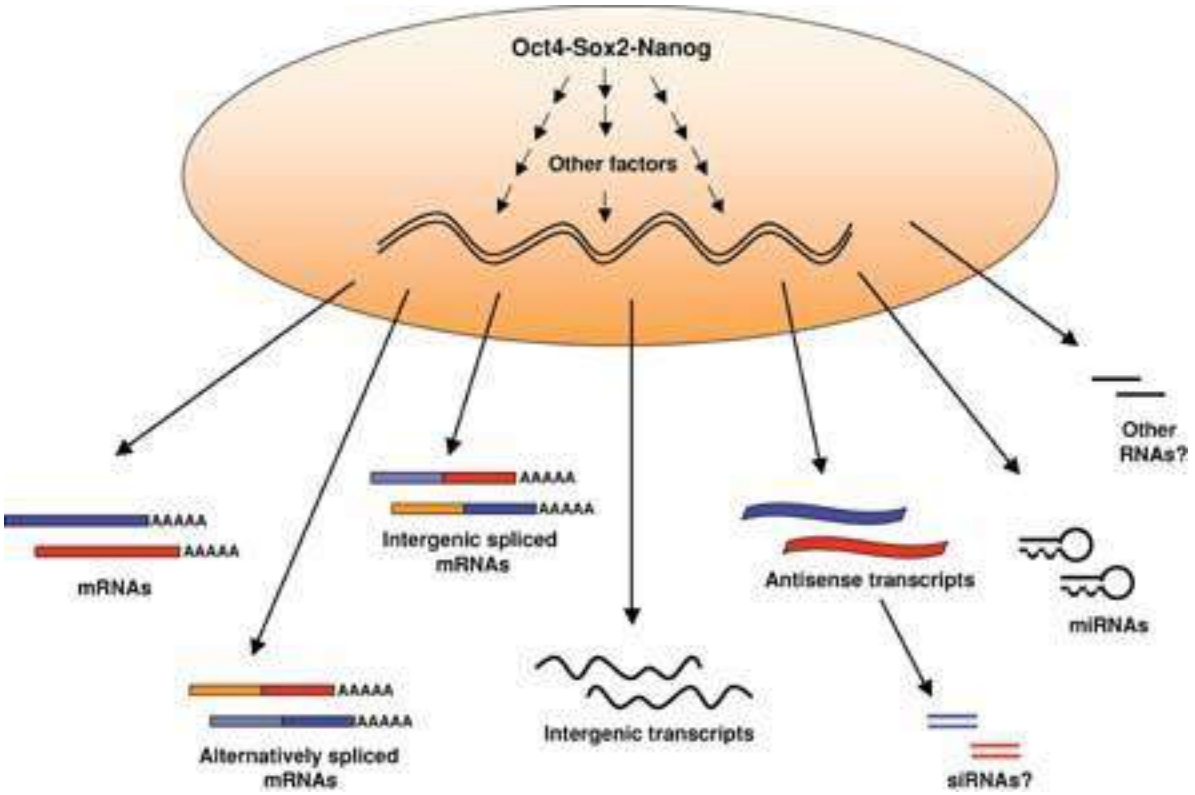
Panagiotis Apostolou[1], Maria Toloudi[1], Marina Chatziioannou[1], Eleni Ioannou[1] and Ioannis Papasotiriou*[,1]

[1]*Research Genetic Cancer Centre Ltd (R.G.C.C. Ltd), 115 M.Alexandrou Str, 53070 Filotas, Florina, Greece*

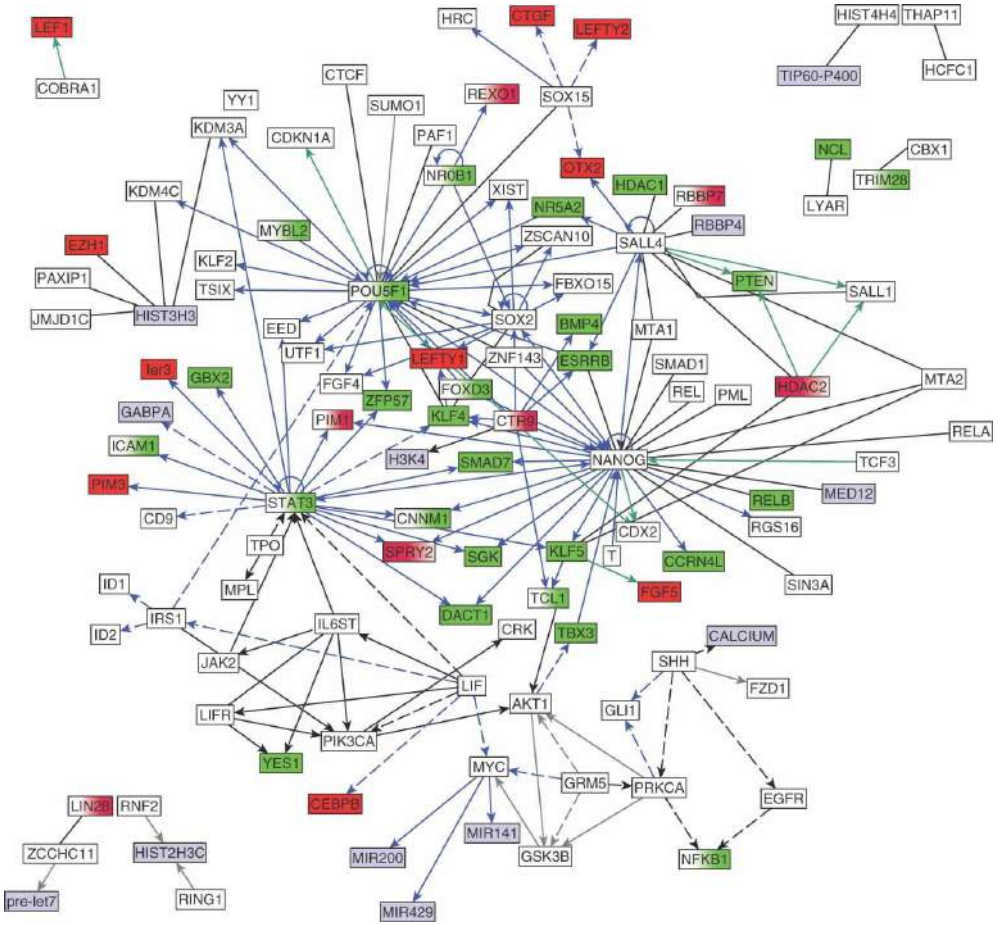
Abstract: Cancer stem cell-like cells (CSCs) are cancer cells that have the ability of self-renewal and differentiation into multiple malignant cell types (hierarchy). Thus, can cause relapses and metastasis. CSCs' phenotype is defined by special transcription factors such as Nanog, Oct3/4, Sox2, Nestin, and CD34. The present study aims to determine the change in gene expression of the above markers in correlation with the stage of the disease in breast cancer patients. Initially, whole blood samples from patients with breast cancer were collected, followed by the isolation and culture of circulating tumor cells (CTCs). This was followed by the quantification of CSCs from the above cultures. CSCs' molecular analysis was performed with qPCR, with the use of gene specific primers. At the same time of the analysis, the clinical assessments of the patients were requested from their physicians. The results indicated a linear relationship between the gene expression of stemness markers and the stage of the disease, as well as specific expression patterns by stage. It seems that these genes have an important role in the progression of the disease, thus they might be target for new treatment approaches.

Keywords: Breast cancer, cancer stem cells, Nanog, Sox2, Oct3/4, CD34, Nestin.

What the stemness driving molecule induce



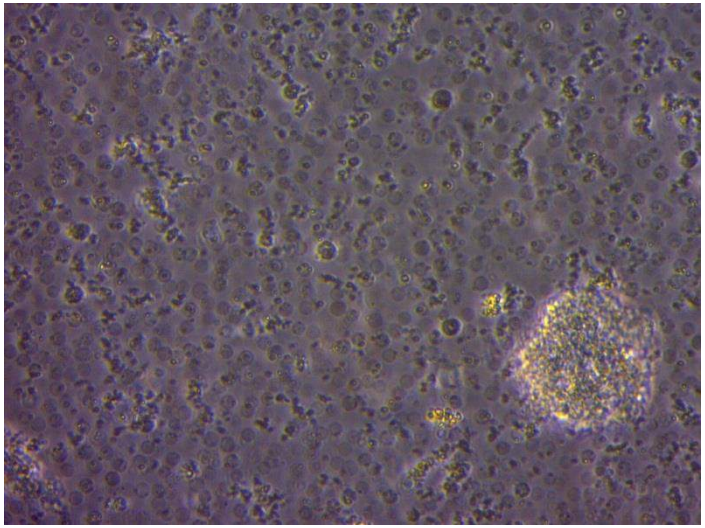
Intrinsic regulation of stemness (spontaneous dedifferentiation)

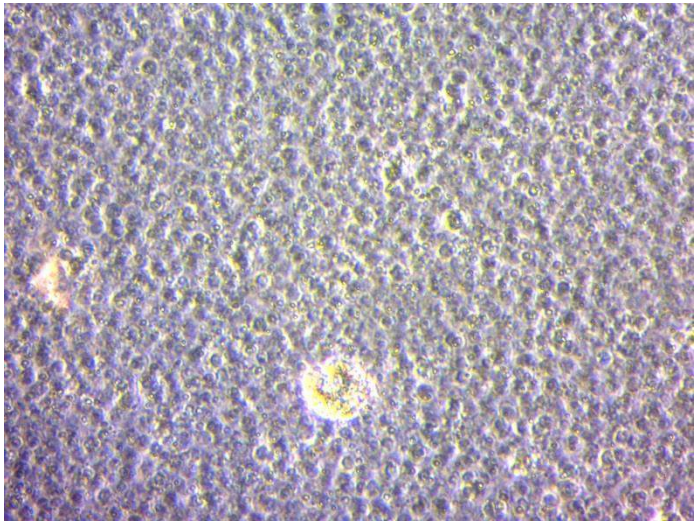


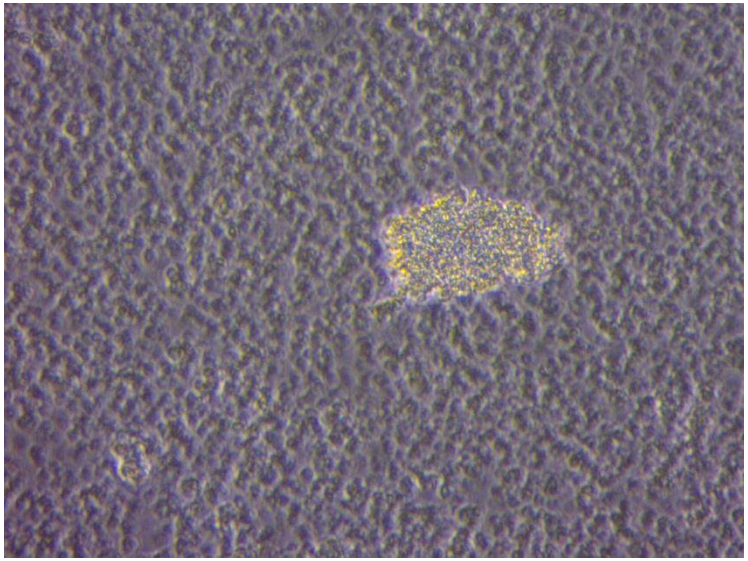
1. Internal induction of transcription factors line Sox-Oct-4 (epigenetic factors)

2. Transposition factors (L1 retrotransposones non LTR is related with stemness phenotype and CSCs)

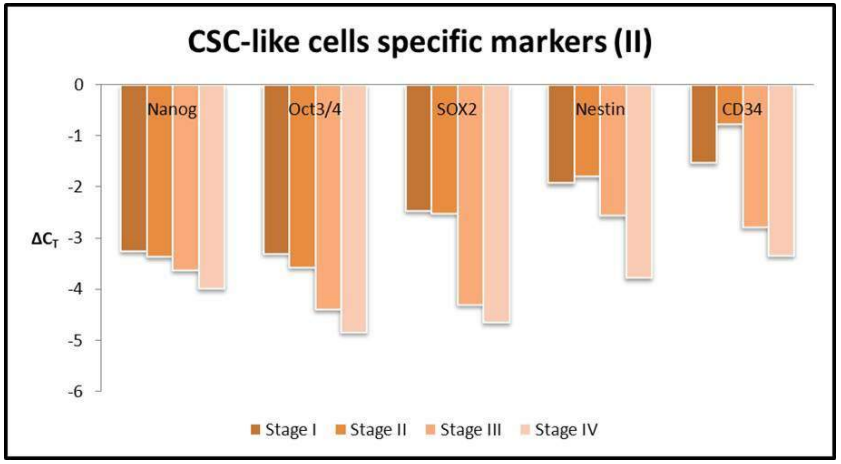
How CSCs can appeared in a culture

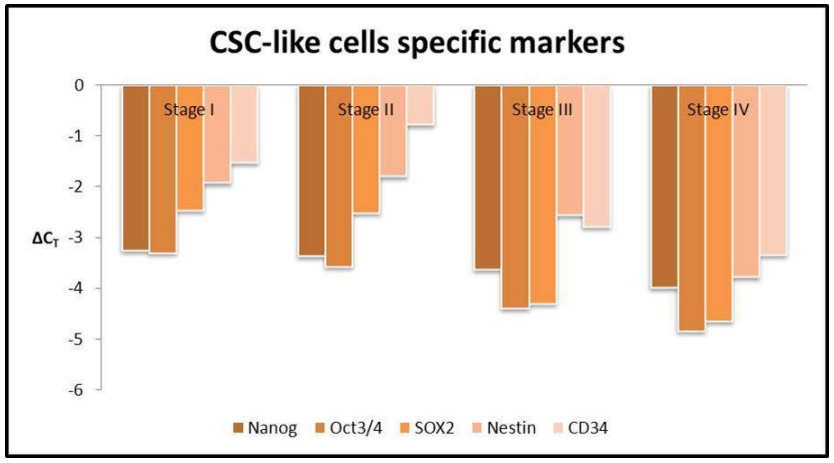






CSCs and clinical reality





There is an obvious trent of incresing expression of CSCs markers inside the population of CTCs in linear relation with stage and progress of the disease

Publication

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

Publication

Send Orders of Reprints at reprints@benthamscience.org

Current Stem Cell Research & Therapy, 2012, *7*, 00-00

Cancer Stem Cells Stemness Transcription Factors Expression Correlates with Breast Cancer Disease Stage

Panagiotis Apostolou[1], Maria Toloudi[1], Marina Chatziioannou[1], Eleni Ioannou[1] and Ioannis Papasotiriou*[,1]

[1]*Research Genetic Cancer Centre Ltd (R.G.C.C. Ltd), 115 M.Alexandrou Str, 53070 Filotas, Florina, Greece*

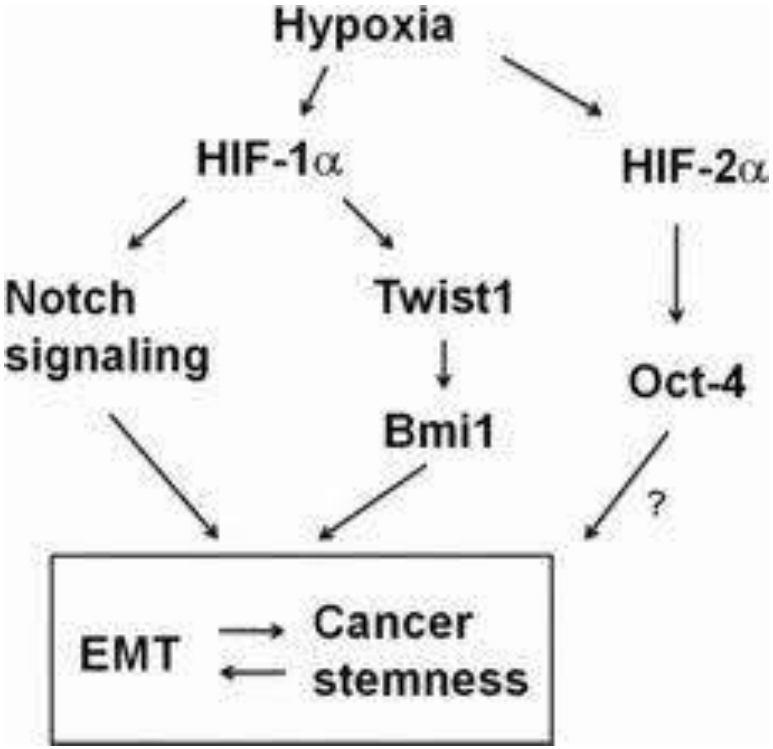
Abstract: Cancer stem cell-like cells (CSCs) are cancer cells that have the ability of self-renewal and differentiation into multiple malignant cell types (hierarchy). Thus, can cause relapses and metastasis. CSCs' phenotype is defined by special transcription factors such as Nanog, Oct3/4, Sox2, Nestin, and CD34. The present study aims to determine the change in gene expression of the above markers in correlation with the stage of the disease in breast cancer patients. Initially, whole blood samples from patients with breast cancer were collected, followed by the isolation and culture of circulating tumor cells (CTCs). This was followed by the quantification of CSCs from the above cultures. CSCs' molecular analysis was performed with qPCR, with the use of gene specific primers. At the same time of the analysis, the clinical assessments of the patients were requested from their physicians. The results indicated a linear relationship between the gene expression of stemness markers and the stage of the disease, as well as specific expression patterns by stage. It seems that these genes have an important role in the progression of the disease, thus they might be target for new treatment approaches.

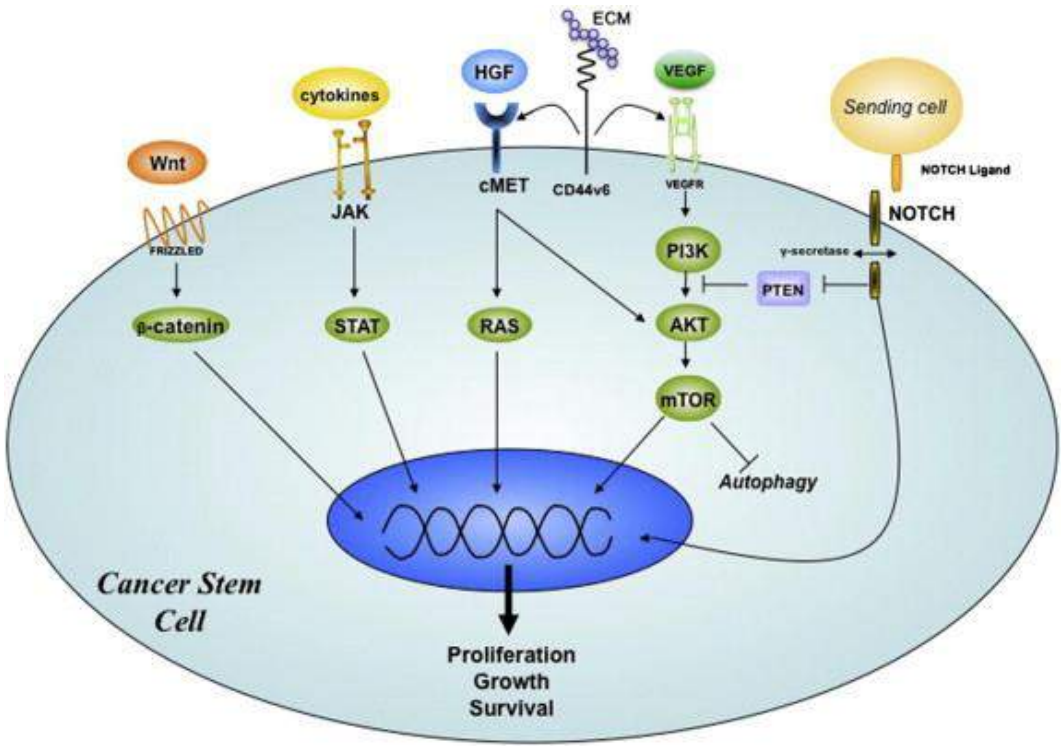
Keywords: Breast cancer, cancer stem cells, Nanog, Sox2, Oct3/4, CD34, Nestin.

How epigenetics implicated in CSCs phenotype development

- The stemness phenotype is triggered and “be revived’
- Mechanism of reactivation:
  - Methylations/hypemethylation of genes that involved in stemness cascade (nanog, Sox-2,Oct-3/4, twist, snail etc)
  - Genetic rearrangent/instabiity (transpositions etc)
  - Gene expression regulation though micro-RNA

CSCs-EMT and MET





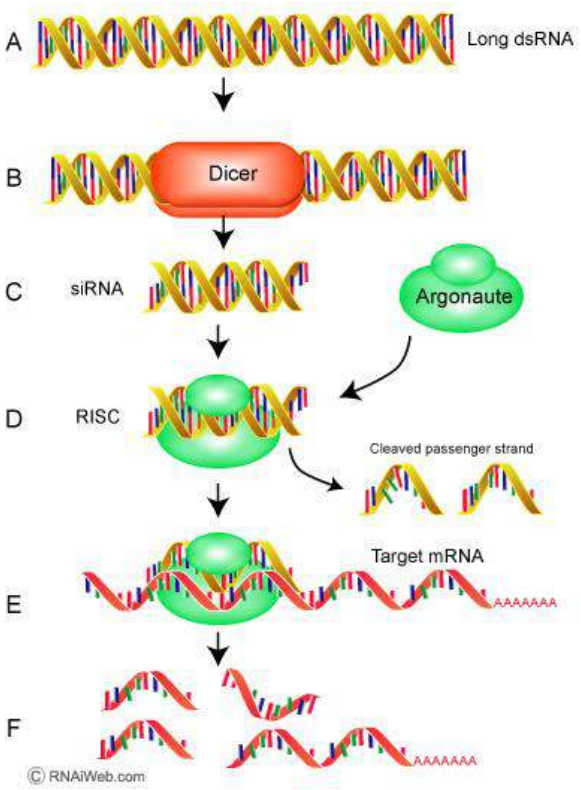
How epigenetics implicated in CSCs phenotype development

- The stemness phenotype is triggered and “be revived’
- Mechanism of reactivation:
  - Methylations/hypemethylation of genes that involved in stemness cascade (nanog, Sox-2,Oct-3/4, twist, snail etc)
  - Genetic rearrangent/instabiity (transpositions etc)
  - Gene expression regulation though micro-RNA

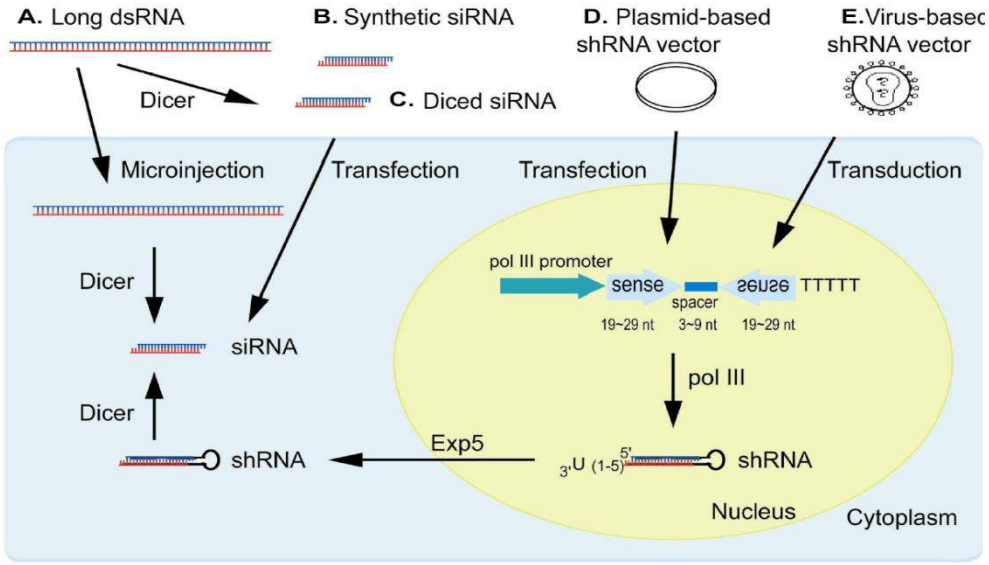
DNA hypermethylation & hyperacetylation

- Involes enzymes which methylate/acetylate parts of genome and shift the expression rate of genes.
- The field is already under extensive research
- Hypermethylation is not clearly related with stemness (multiple other co-founders are involved)
- New agents have been generated as inhibitors of hypemethylation/hyperacetylation of DNA (Vorinostat-HDAC inhibitor)

Micro-RNA (RNAi) and CSCs phenotype



- Several patterns have been identifies but very few related with stemness
- So far there is no drugable target for these patterns and mechanisms.



Transposable genetic elements

- Transposones (cut paste)-Class II
  - Transposase
  - Resolvase
  - Gene of interest
- Retrotransposones (copy paste)-Class I
  - LTR retrotransposon
  - Non LTR
    - LINE (they include reverse transcriptase)
    - SINE

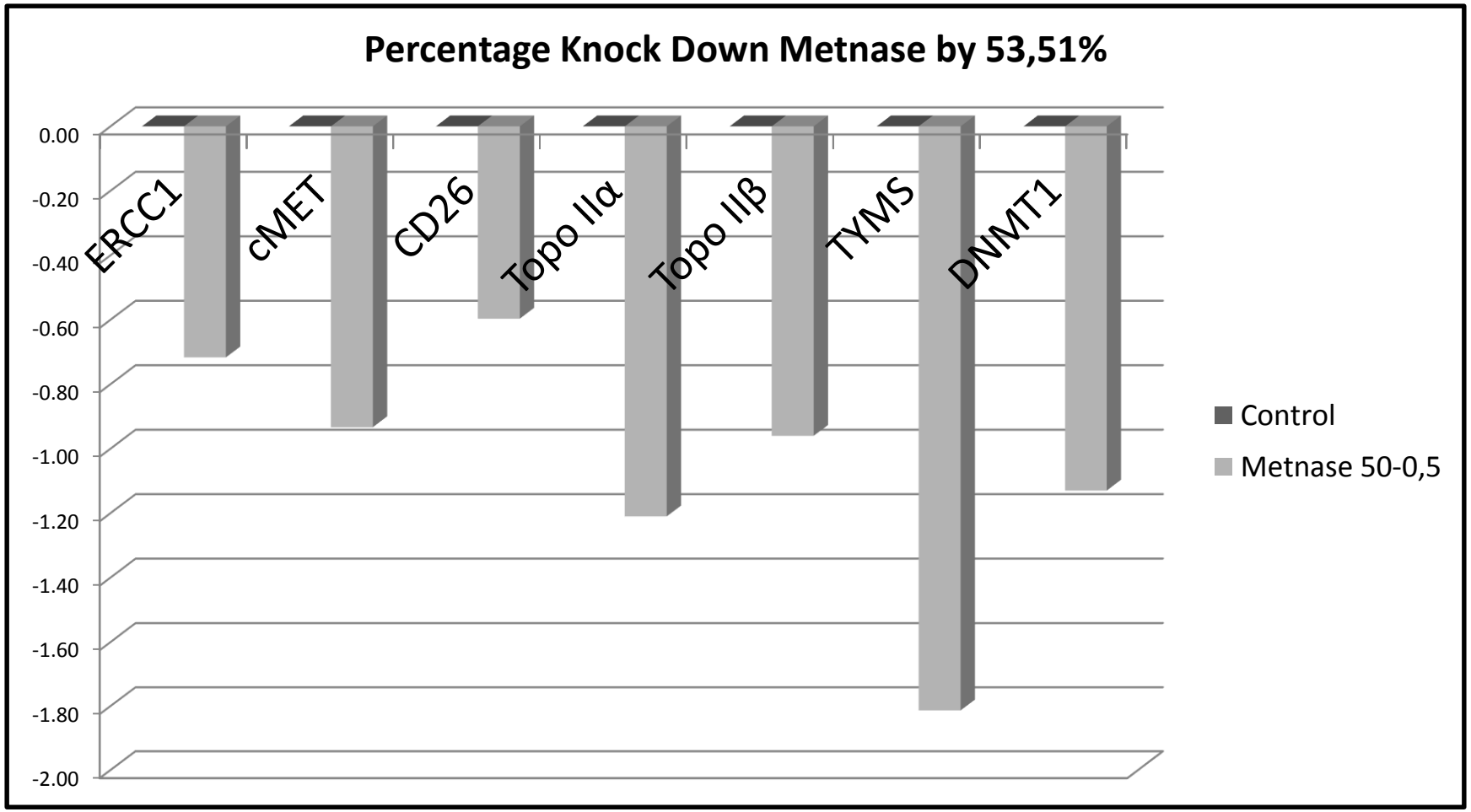
Relation between transposable genetic elements and cancer

- Responsible to resistance phenotype development (ERCC-1 and platinum derivatives resistance)
- Relation between SETmar and toposimerase II inhibitor resistance in breast carcinoma
- Microsatelite instability (MLH1, MSH) and resistant phenotype in colon cancer
- Responsible for genetic instability and rearrangements in all genome

Transposable genetic elements and CSCs phenotype

- Expression of L1 in CSCs
- Expression of SETMAR in CSCs
- Relation between METnase in CSCs and MET transition process
- Resistance of CSCs and transposase activity

Experimental evidence (Knock down METnase)



Experimental evidence (Knock down METnase)



Study of the interaction among Notch pathway receptors, correlation with stemness, as well as their interaction with CD44, dipeptidyl peptidase-IV, hepatocyte growth factor receptor and the SETMAR transferase, in colon cancer stem cells.

Apostolou P, Toloudi M, Ioannou E, Kourtidou E, Chatziioannou M, Kopic A, Komiotis D, Kiritsis C, Manta S, Papasotiriou I.

Research Genetic Cancer Centre Ltd (R.G.C.C. Ltd) , Florina , Greece .

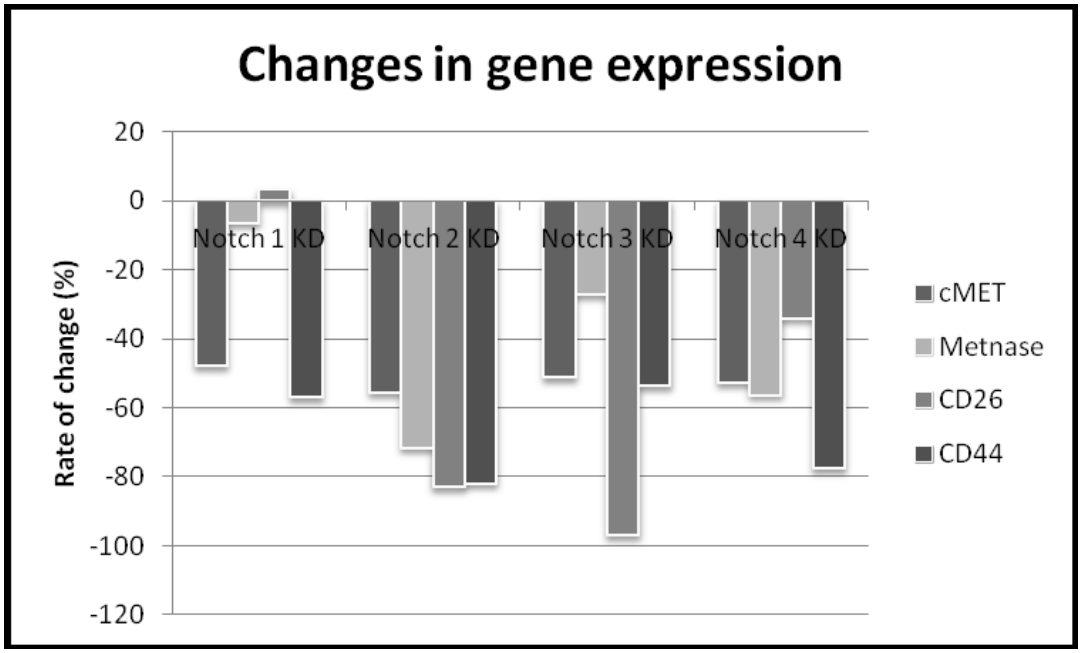
Abstract

Abstract Context: The Notch signaling pathway is one of the most important pathways during normal development and implicated in self-renewal of adult stem cells and differentiation of progenitor cells. Abnormal expression of Notch receptors has been associated with many epithelial metaplastic and neoplastic lesions. Objective-materials and methods: In this particular study, it was determined the relative gene expression of Notch receptors after knockdown experiments in colon cancer stem cells (CSCs) and the gene expression changes in stemness transcription factors (Oct4, Sox2, Nanog), as well in dipeptidylpeptidase-4, CD44 antigen, Met proto-oncogene and in Metnase transposase. Results: In control CSCs Notch-2 had the higher expression, followed by Notch-1, Notch-3. Notch-4 demonstrated the lower gene expression among the receptors. The suppression of Notch-1 led to increased expression of Oct4 and Sox2, but in decreased gene expression of cMET, Setmar and CD44. The CD26 expression remained unchanged. The knockdown of Notch-2 led to decreased expression of all transcription factors. Notch-3 down regulation caused increased Oct4 gene expression, but decreased levels for the rest of the genes. Finally, the suppression of Notch-4 had the same effect as in receptor Notch-3. Discussion and conclusion: The above experimental data suggest the possible interaction between the four different receptors of Notch signaling pathway. The expression of CD26, cMET and N-methyltransferase Setmar was also changed. Finally, the stemness phenotype was changed in a different way each time, according to the receptor that was down regulated. All Notch receptors and particularly Notch-2 seem to play an important role in cancer stem cells.

Not all tranpsosases are present in cells with “Stemness” properties

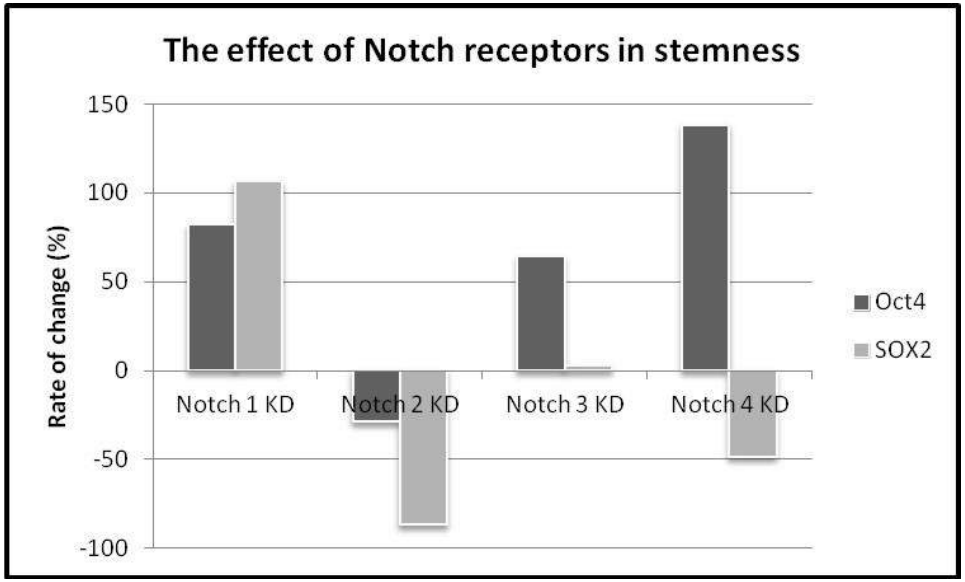
Experiments

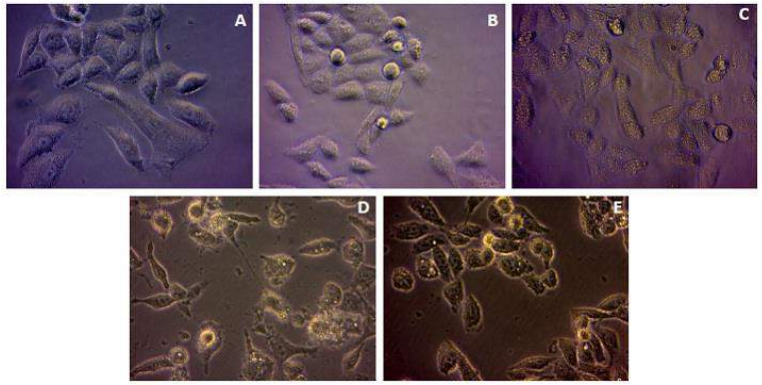
Gene	siRNA	Sequence (5'-3')
Notch-1	Sense	UAGUAGGGGAAGAUCAUCUUU
Antisense		AGAUGAUCUUCCCCUACUAUU
Notch-2	Sense	UAAAUUUGGAUAGGACUGAUU
Antisense		UCAGUCCUAUCCAAAUUUAUU
Notch-3	Sense	UUAUUGGCUCCAUUUUUGAUU
Antisense		UCAAAAAUGGAGCCAAUAAUU
Notch-4	Sense	UAAAUAGCGAUAGCAGUGGUU
Antisense		CCACUGCUAUCGCUAUUUAUU



Experiments

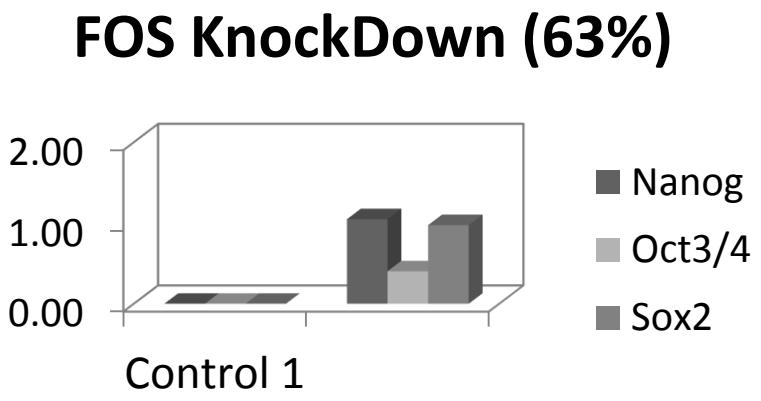
Notch Knockdown				
Cell Line	**Notch-1**	**Notch-2**	**Notch-3**	**Notch-4**
Colon CSCS	**92%**	**95%**	**92%**	**88%**

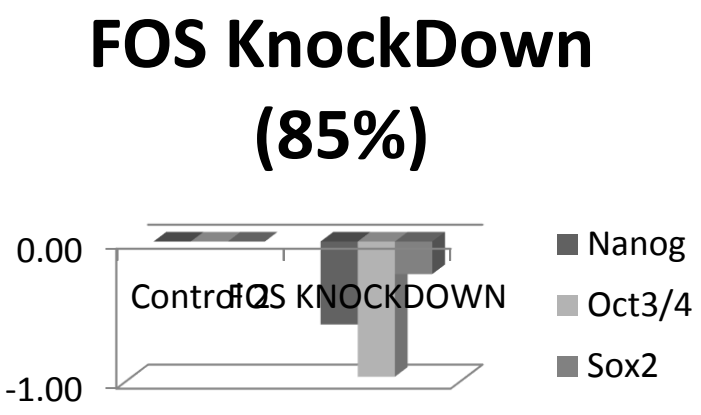




Experiments (controversies)

Another pathway that may trigger the stemness is the heterodimmer AP-1 which is formed by c-FOS and c-JUN.





Experiments (intrinsic pathways)

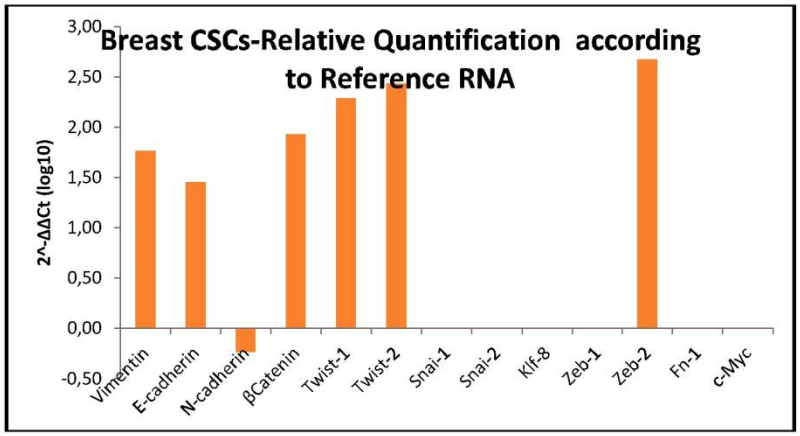


Figure 13: Breast CSCs (18SrRNA)

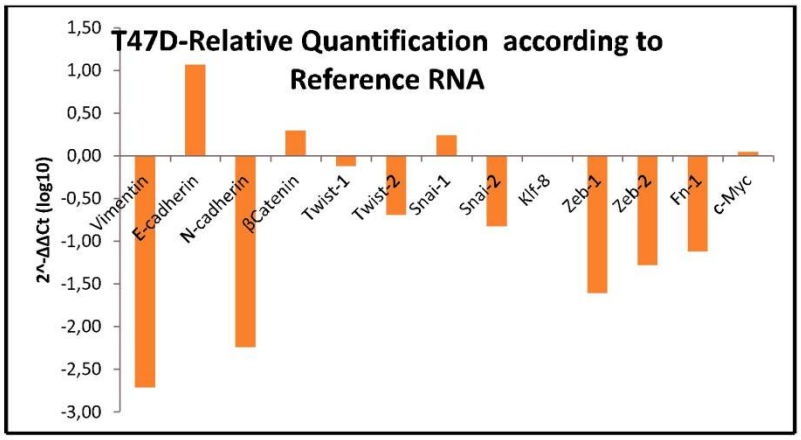


Figure 14: T47D (18SrRNA)

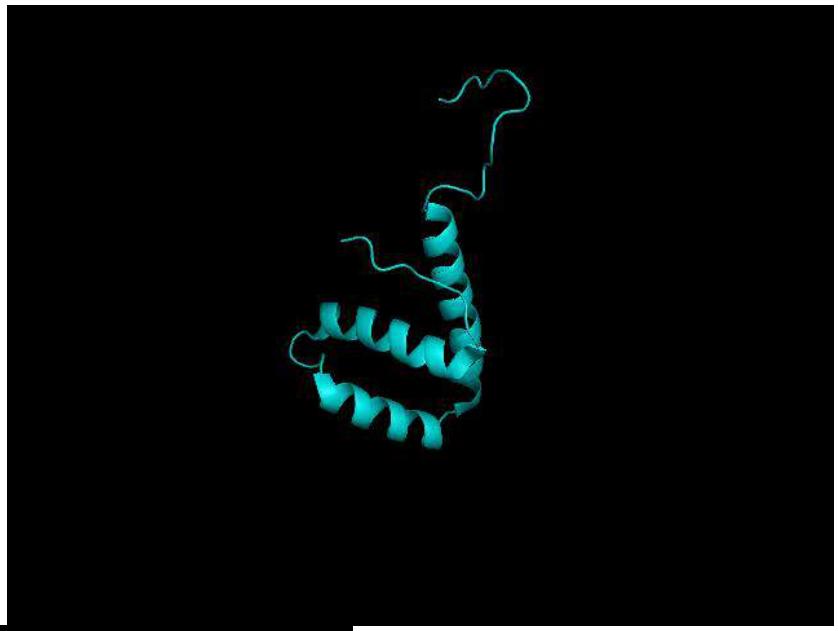
Possible involvement of MET process as well as plasticity

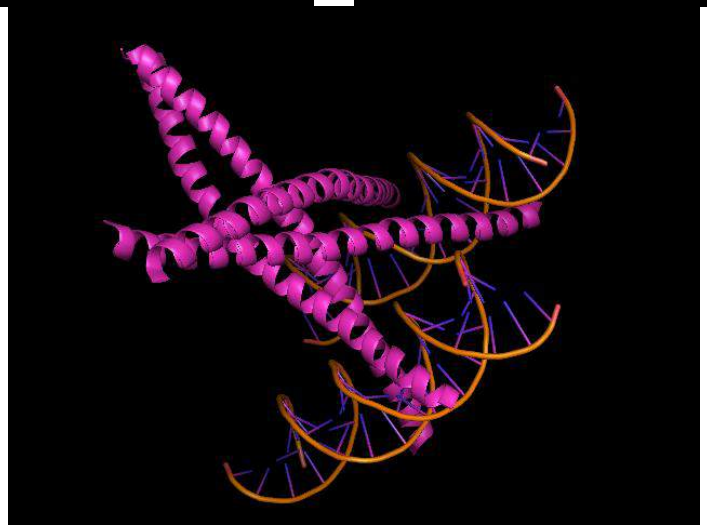
Outcome of the experiments

- Microenvironment play's important role in stemness development
- During progression many types of CSCs may be generated
- There are several pathways with multiple "cross talking" to promote stemness phenotype

Experiments II (Sox-2 or 17 & Oct-4)

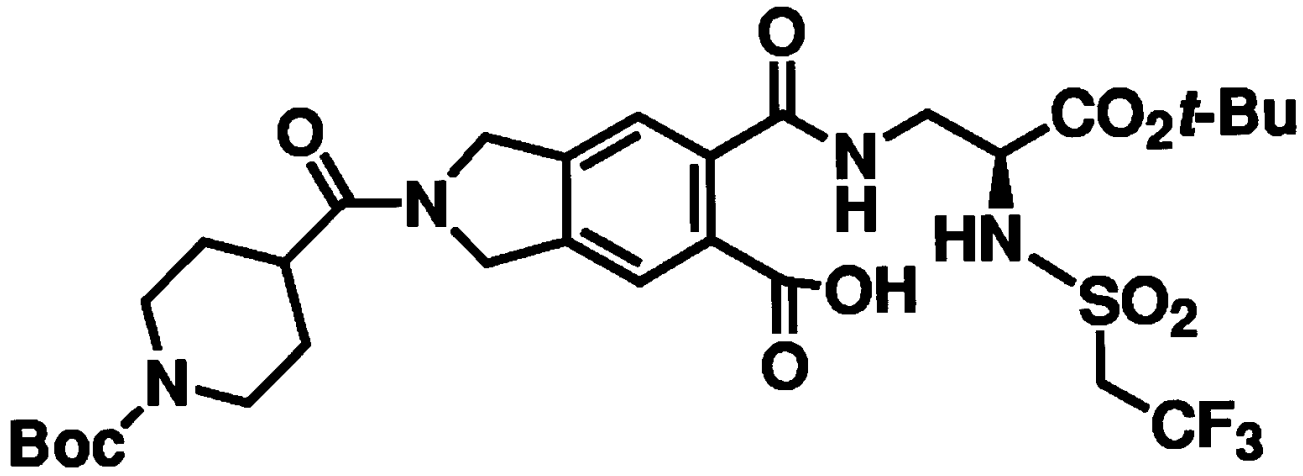






Experiment II continue

- Leading compound for leucin Zipper transcription factor Sox-Oct-4 inhibitor



IA4B6

Vogt et all 2002

Exctrinsic mechanism promoting the progression of stemness

ASCO University
LIFELONG LEARNING

HOME ASCO-SEP MOC COURSE CATALOG CERTIFICATES TRAINING PROGRAMS MEETING LIBRARY BOOKSTORE CAREER CENTE

Home » Meeting Library » Abstracts » 2014 ASCO Annual Meeting

printer-friendly version

TMX2: A potential biomarker or a predictive marker in breast cancer?

Subcategory:
Genomic and Epigenomic Biomarkers

Category:
Tumor Biology

Meeting:
2014 ASCO Annual Meeting

Session Type and Session Title:
This abstract will not be presented at the 2014 ASCO Annual Meeting but has been published in conjunction with the meeting.

Abstract Number:
e22077

Citation:
J Clin Oncol 32, 2014 (suppl; abstr e22077)

Author(s):
Ioannis Papasotiriou, Panagiotis Apostolou, Maria Toloudi, Marina Chatziioannou, Georgia Mimikakou, Eleni Kourtidou, Ioanna Vlachou; Research Genetic Cancer Centre, Ltd, Filotas, Greece

REALITY vs LAB BENCH

- We must not forget that clinical reality is different than a controlled environment of a lab.
- Complexity of cancer may generate options of escape in clinical practice.
- PD and PK may play equally important role.
- Toxicity may also generate barriers to an option.

LIMITATIONS-BARRIERS

- *Clinically*
  1. CTCs are still inhomogenous
  2. Shifting of CTCs to CSCs phenotype (plasticity).
  3. Relevant subpopulation that drives the disease
  4. In advance stage even the CSCs are heterogeneous and which CSCs initiate relapse.
  5. Still many factors that shift CTCs to CSCs and backwards are unknown

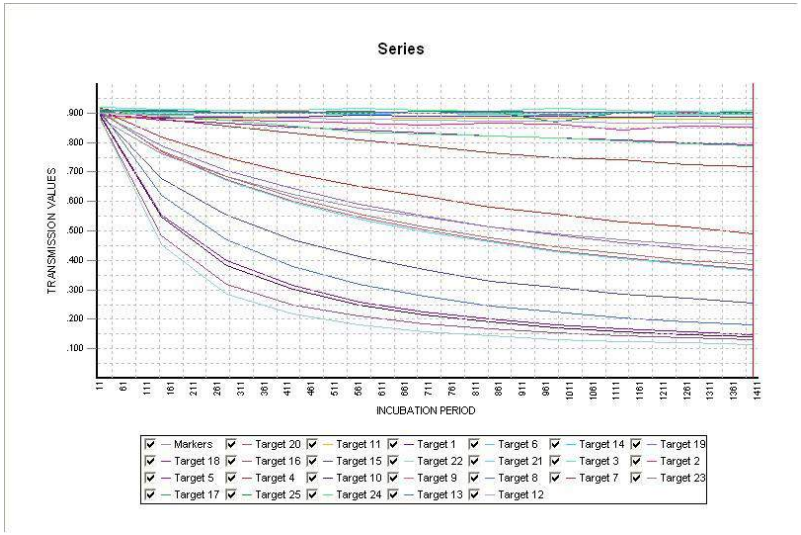
- *Research field*
  1. Plasticity of the CTCs and CSCs
  2. Multiple mechanism that drives the genetic instability
  3. Dependency of stemness from multiple pathways.
  4. What triggers the CSCs to exponential phase of growth, is still unknown.

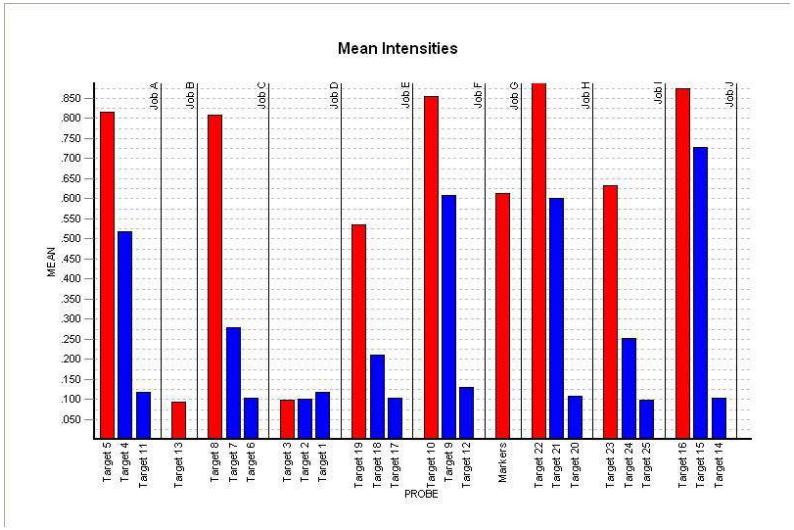
POINT THAT NEED TO BE CONSIDERED

- Cancer Stem cell like (Tumor initiating cells) model is not applicable in all types of malignancies (melanoma for example)

Is it possible to analyze the gene expression profile of CTCs?

Data derived from micro arrays

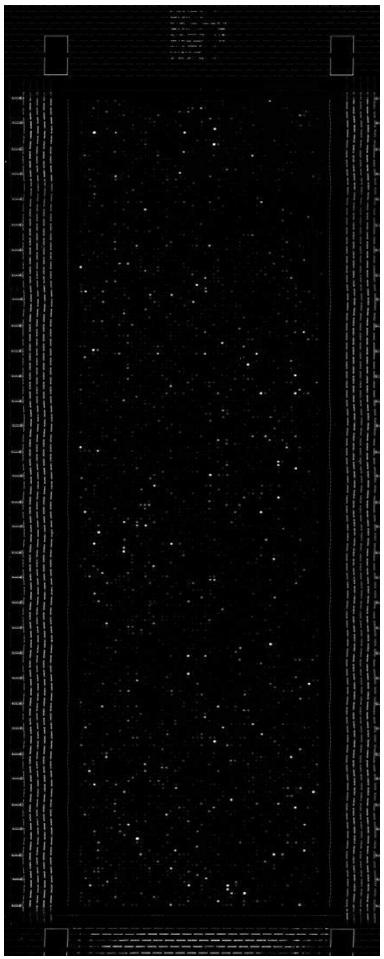


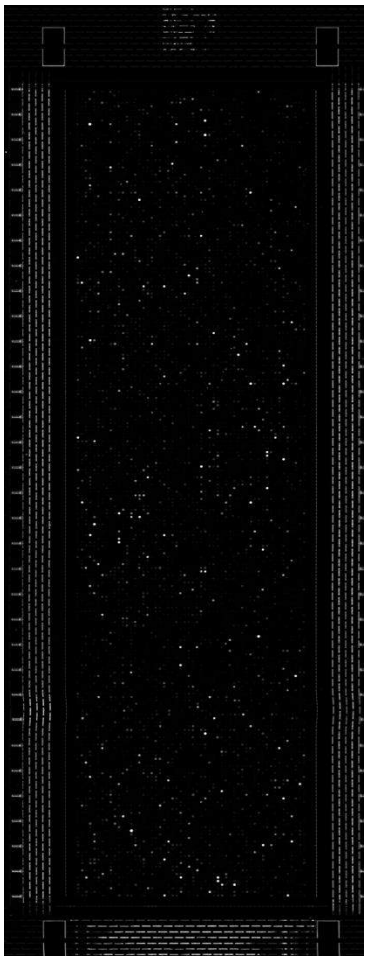


- The information are normalized through a series of endogenous genes (housekeeping)
- The hybridization is always take place with orthotopic use of appropriate normal cell of tissue of origin.
- The non parametric analysis (ANOVA) may allow reveal relevance between genes, types of cancer or even repeatable patterns.

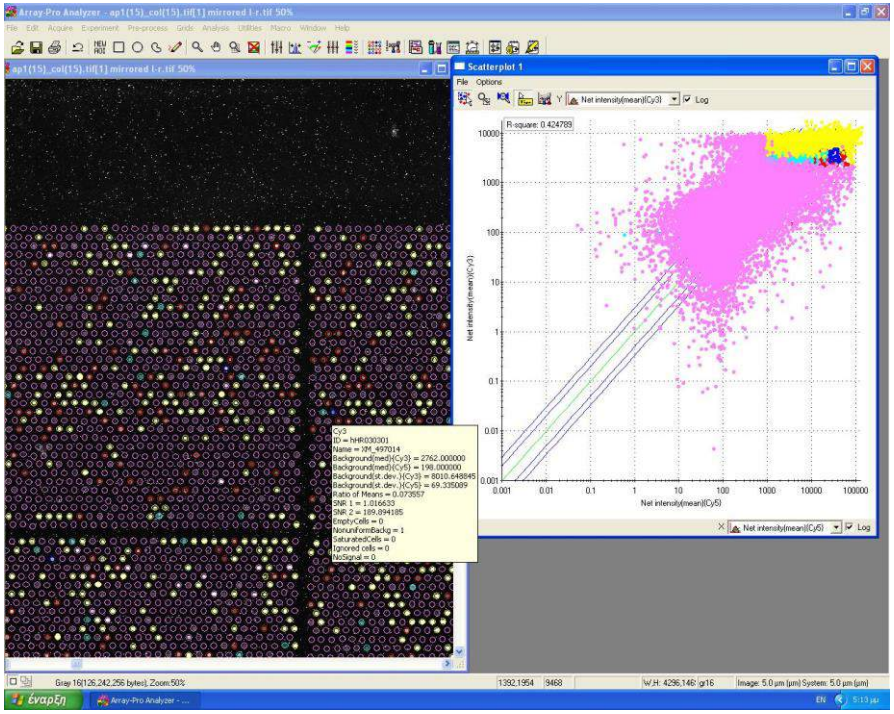
Micro-Arrays analysis

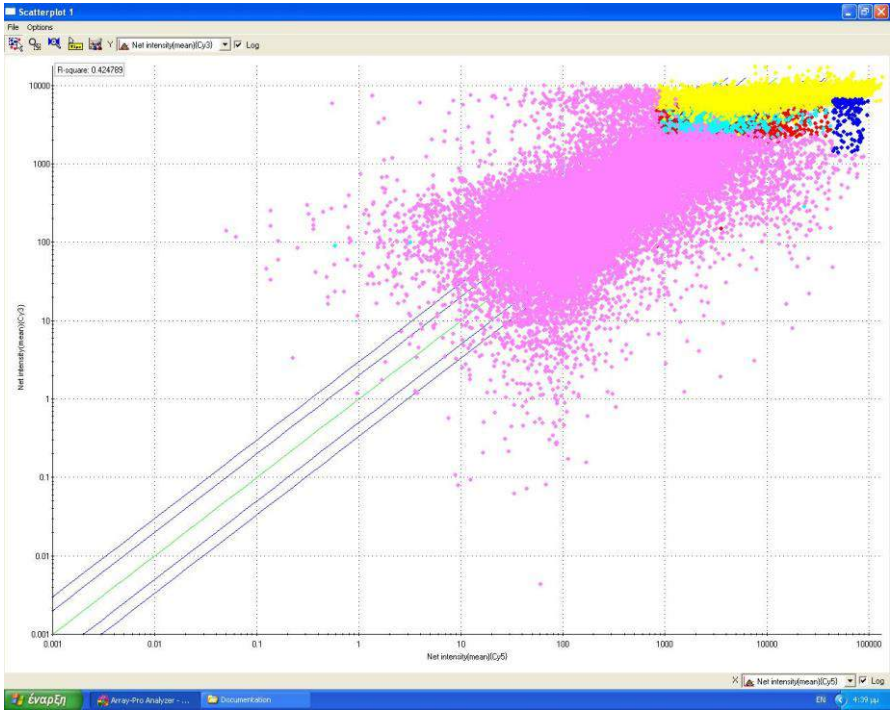
- **TODAY IT IS POSSIBLE TO DETECT ALL GENOME TRANSCRIPTOMICS WITH 12K SPOT SLIDES UP TO 90K SPOTSLIDES (hyperdense micro-array slide)**



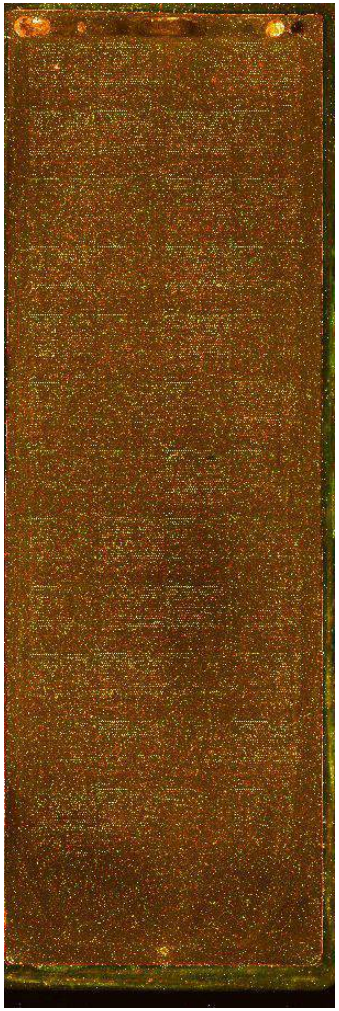


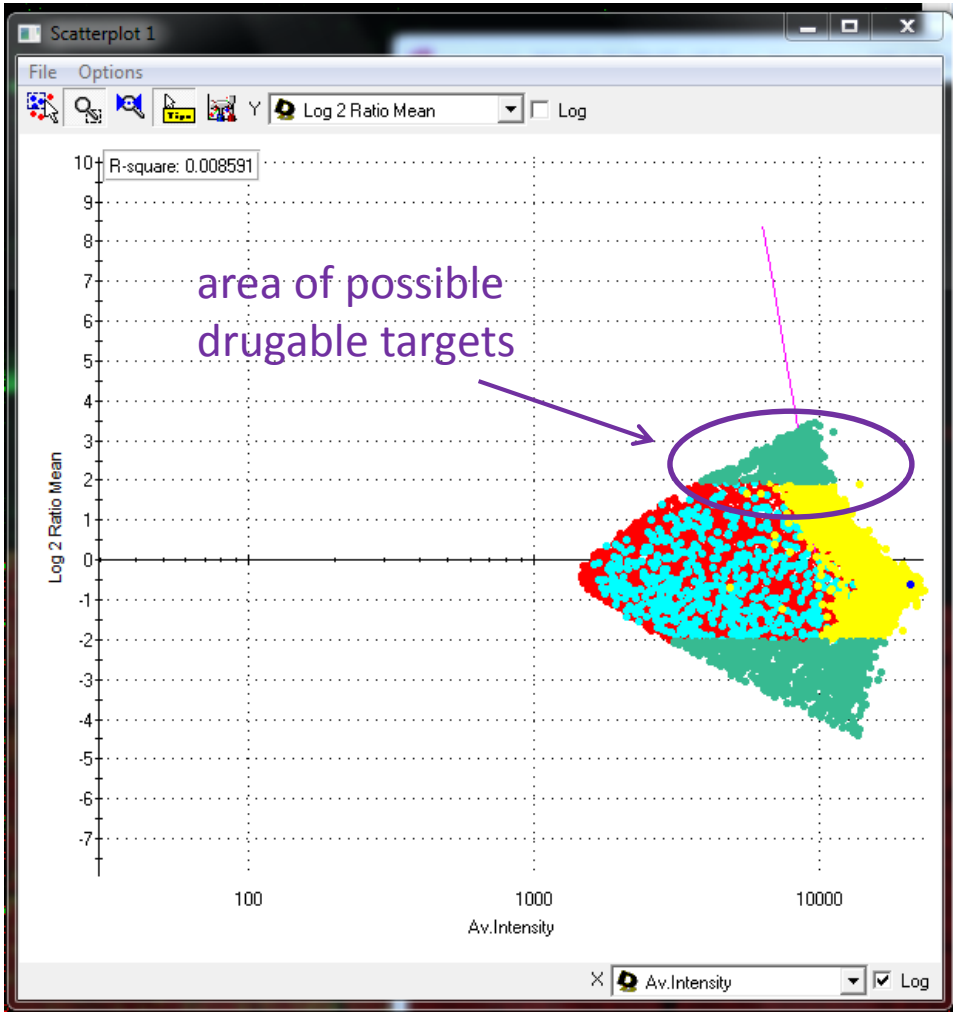
Analysis of epigenetic data





Experiments
(expression patterns in CSCs)





- Hybridization image Cy5-cDNA with Cy5 refrence mRNA

Chemosensitivity testing

- All assays started from the need to define and point out recommended agents with anti-cancer activity
- It combines molecular as well as cellular approach which all validated by a viability assays (there is not always linear relation between gene expression profile, protein production and cellular activity)

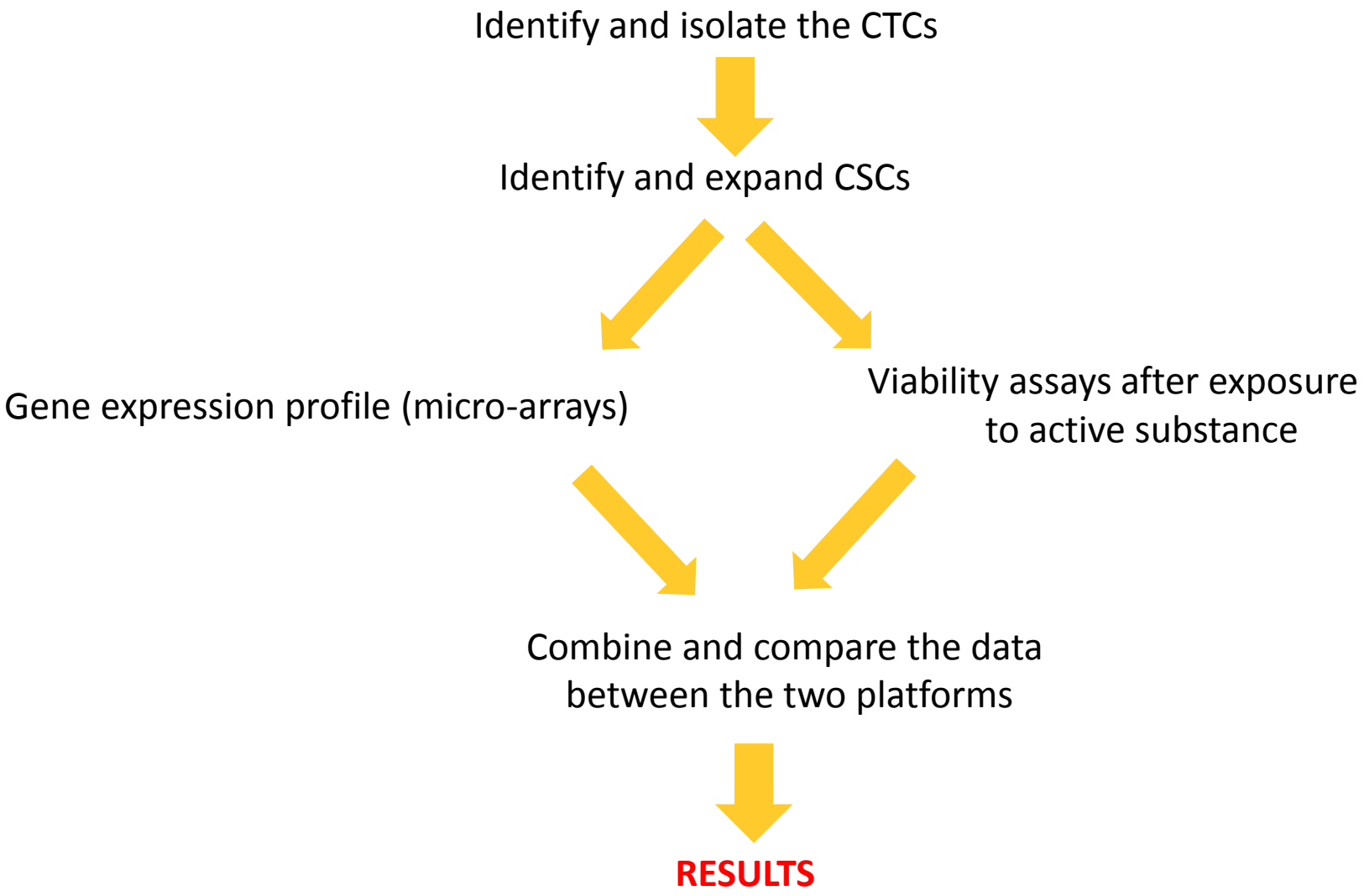
Golden rule: Always double platform

- It is known that gene expression profile has not always linear relation with protein expression rate (post transcription processes, glucosilation etc)

- A drug to have an effect needs to be distributed to the intracellular area (membrane permeability).

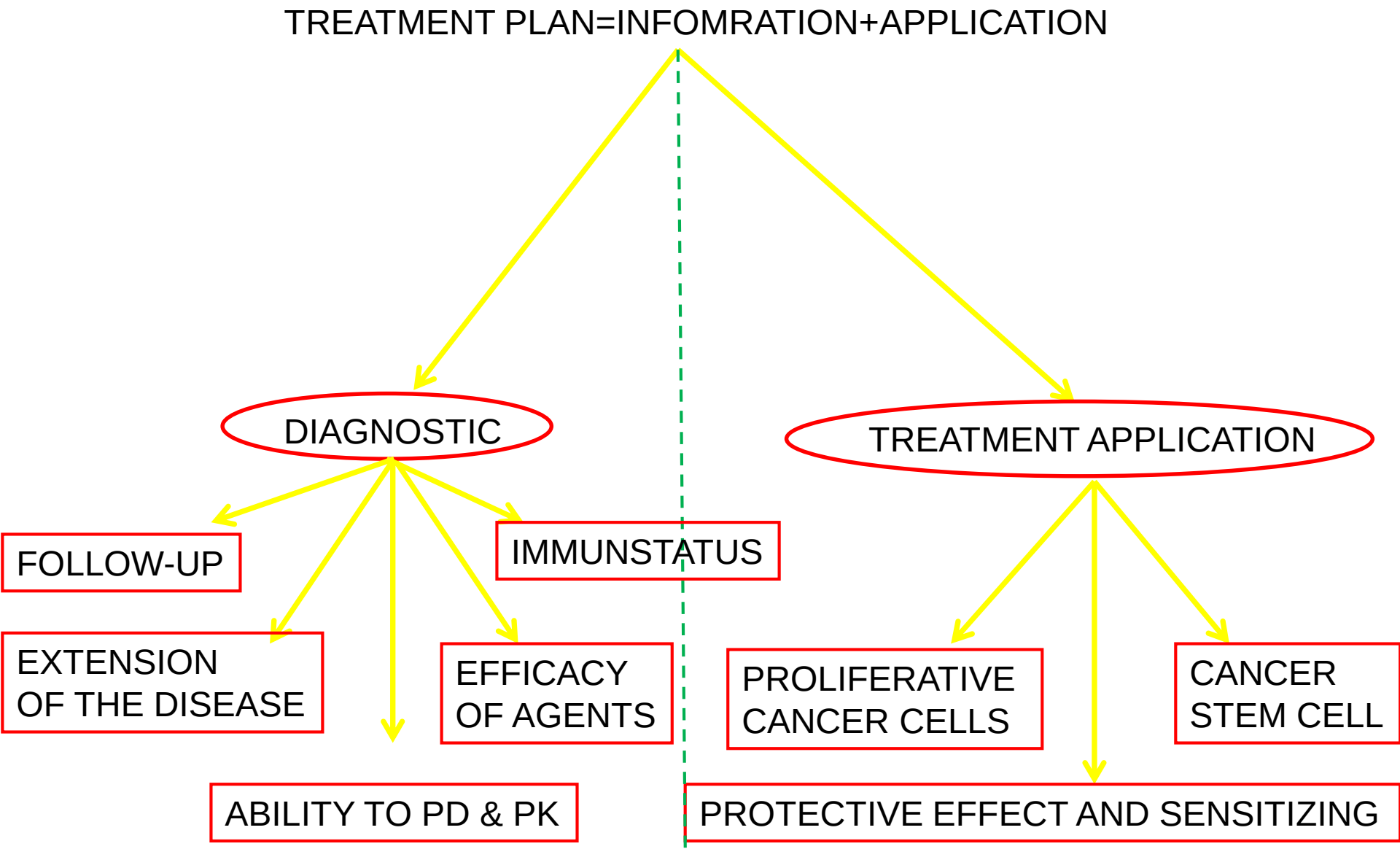
- In order to confirm the information that the expression micro-array generates, the cancer cells are exposed to the active substance of each drug and the cytotoxicity/viability is explored

Steps of the process

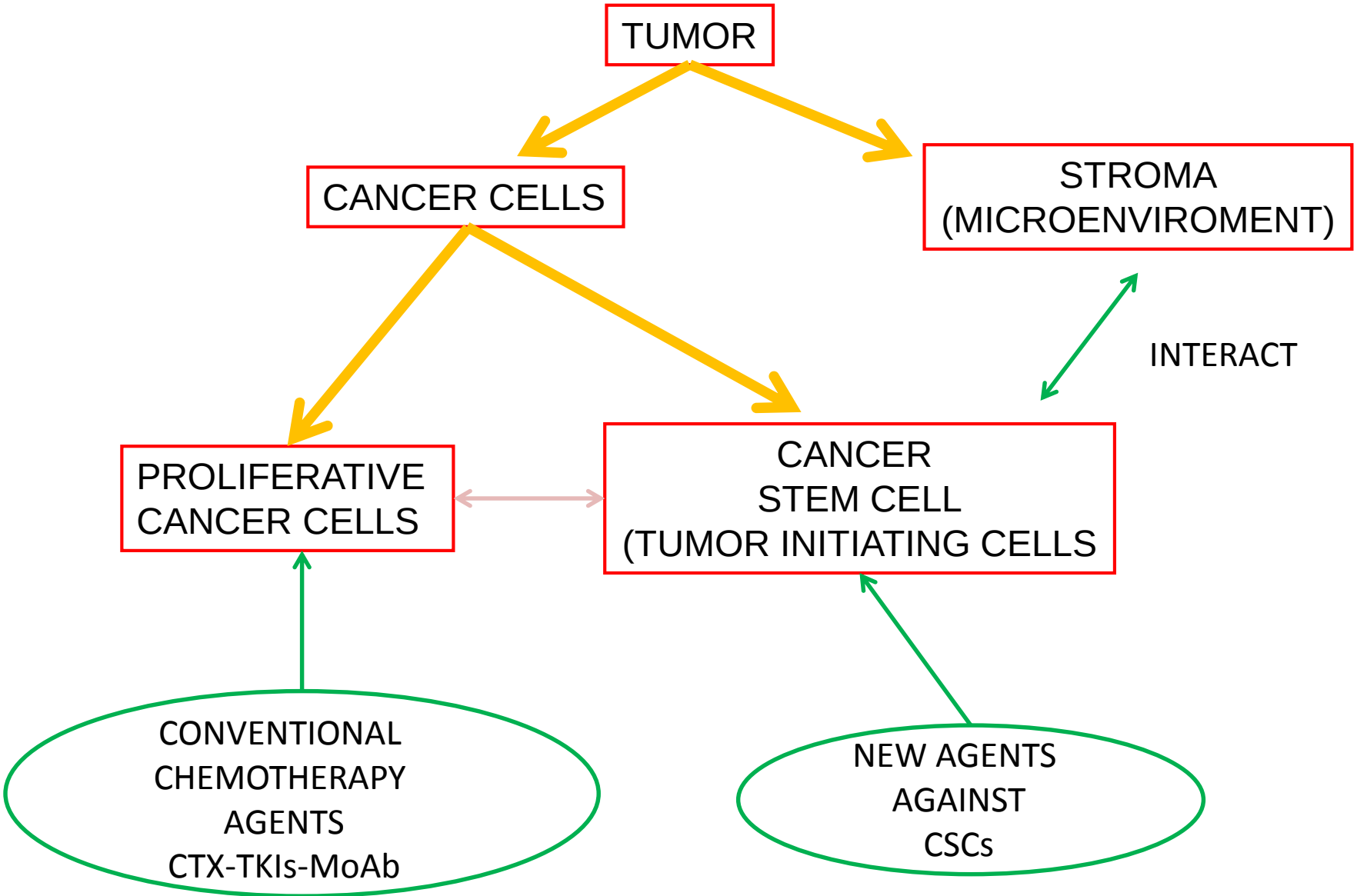


Should we start thinking broadening our way of treating cancer?

THEORETICAL PLAN OF TREATMENT

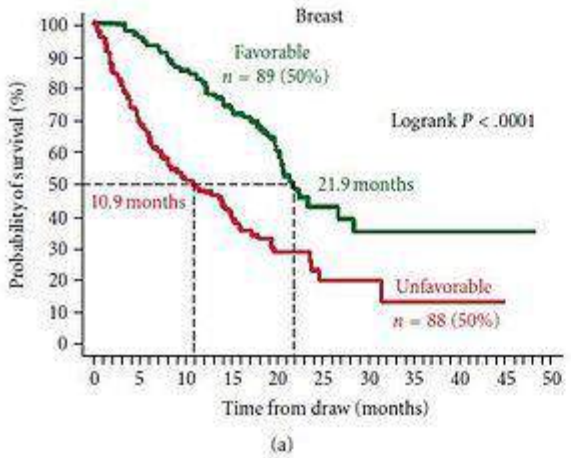


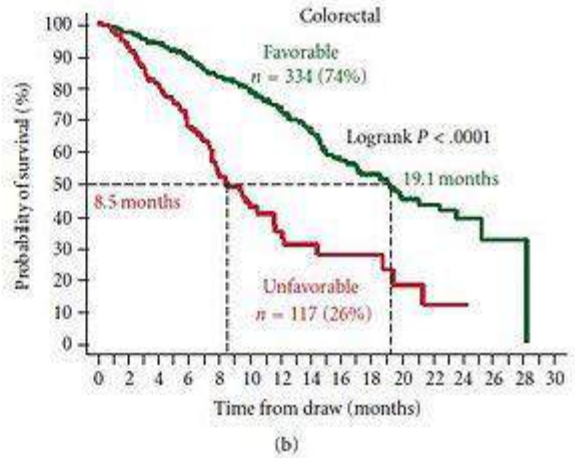
Proposed algorithm of treatment

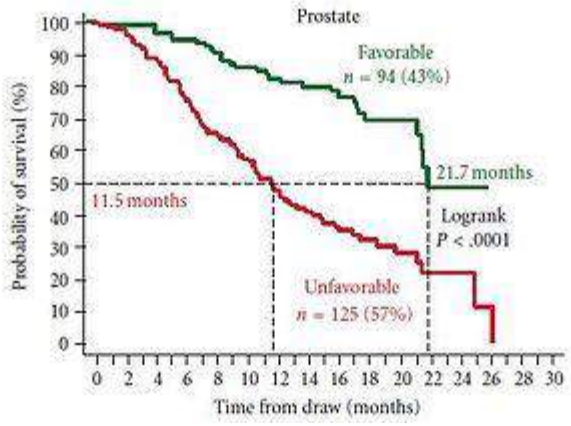


AT THE END

- Parameters of clinical value: RR, OS, DFS



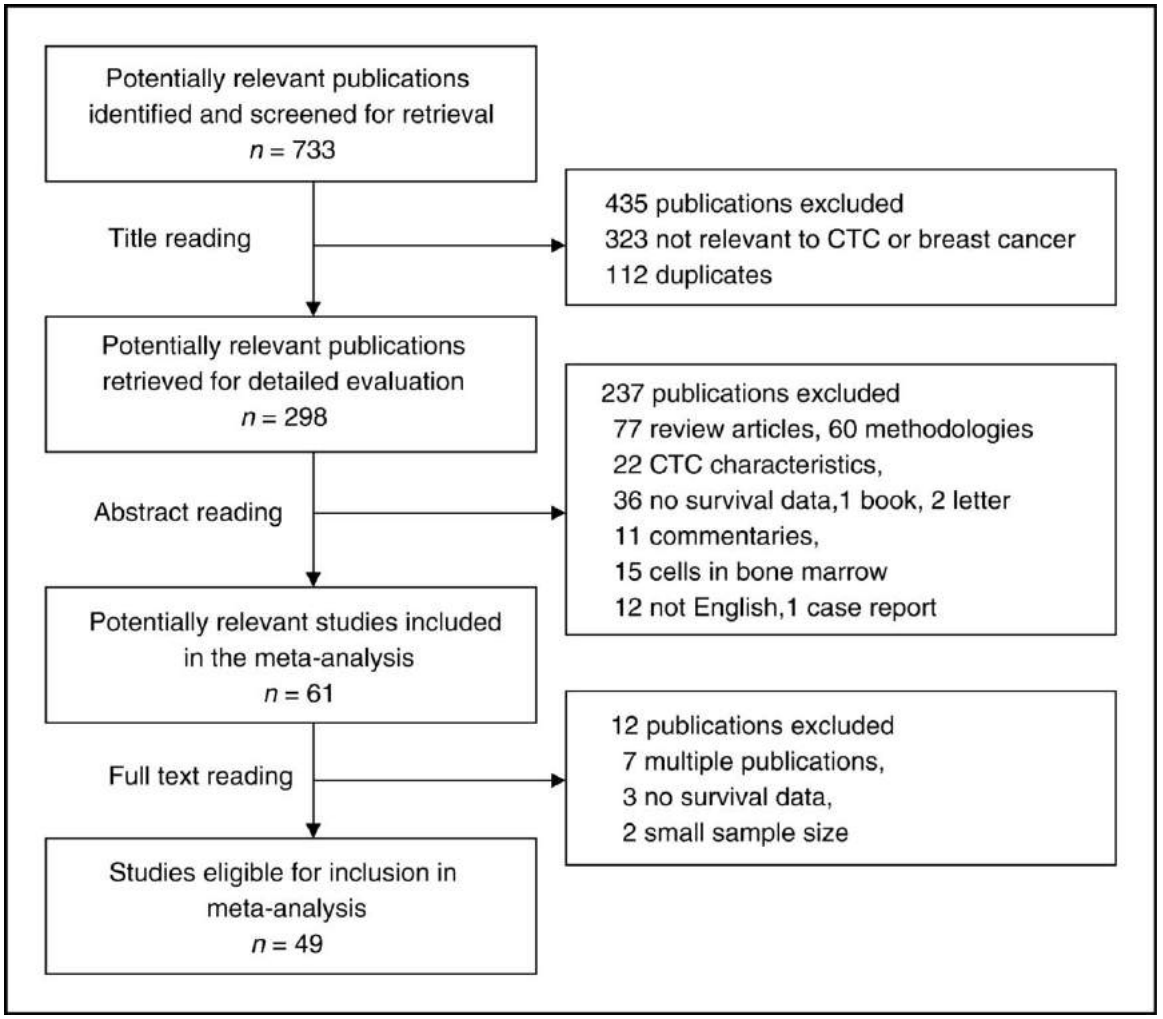




FUTUTE PERSPECTIVES

- 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

- Personalized treatment becomes the optimal strategy for effective cancer treatment
- CTCs have more similarities with the metastatic points as well as with the primary.
- CTCs are heterogenous population but their proportion in cancer stem cells is higher than the primary tumor.
- CSCs is the responsible type of cancer cells that have the ability to regrow a tumor.
- CTCs can become the proper sample to detect and isolate the CSCs.
- Today there are proper methodologies and techniques to analyze the CTCs and the CSCs in order to harvest clinically relevant data.

IN SUMMARY

- CTCs & CSCs HAVE SIGNIFICANT DIAGNOSTIC AND PREDICITVE VALUE BUT…

1. A COMBINATION OF ASSAYS ARE REQUIRED FOR DETECTING THE RARE EVENT OF CTCs
2. AN ACCURATE SERIES OF METHODS ARE REQUIRED TO DETECT THE CLINICALY RELEVANT SUBCLONES OF CANCER CELLS.
3. MORE RELEVANT INFORMATION OF PD BUT ALSO PK ARE REQUIRED.
4. MORE FLEXIBLE TEST ARE REQUIRED TO FOLLOW UP CANCER PATIENTS.

QUESTIONS?

questions@rgcc-international.com

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

Since the data and information is large and further questions and definition may generated, we strongly recommend to obtain the DVD presentation of RGCC International GmbH where more information and definition are there in order to help therapist to understand what is feasible in laboratory reality 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.

THIS PRESENTATION CONSIDERED RGCC GROUP INTELECTUAL PROPERTY AND ATHE USE OF A PART OR ALL RESENTATION FOR GENERATION OF ANOTHER MATERIAL OR ADVERTISEMENTPRIOR RGCC's CONCENT, WILL BE AVIOLATION OF A LAW.