Epithelial Mesenchymal Transition (EMT) and CTCs

Massimo Cristofanilli, M.D., F.A.C.P.
Professor of Medical Oncology
Deputy Director of Translational Research
Director of the Jefferson Breast Cancer Center
Thomas Jefferson University-Kimmel Cancer Center, Philadelphia, USA

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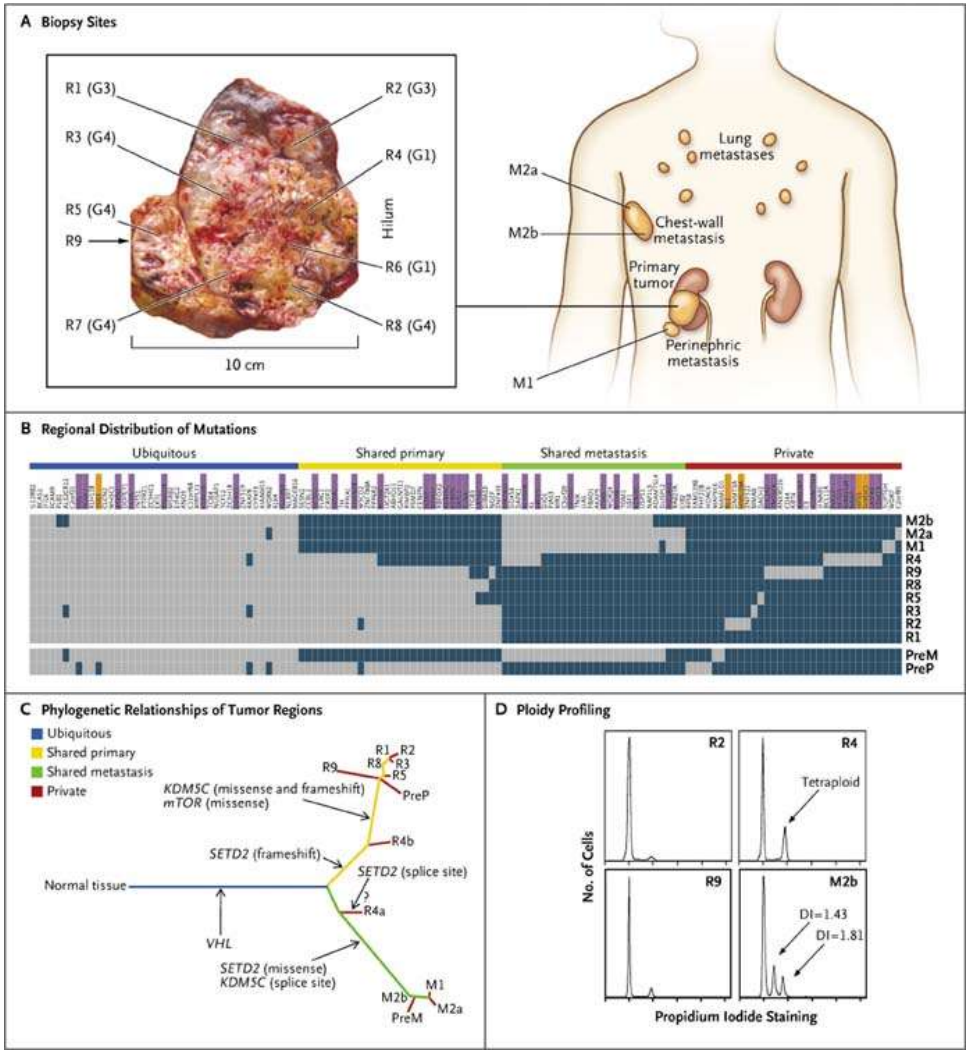
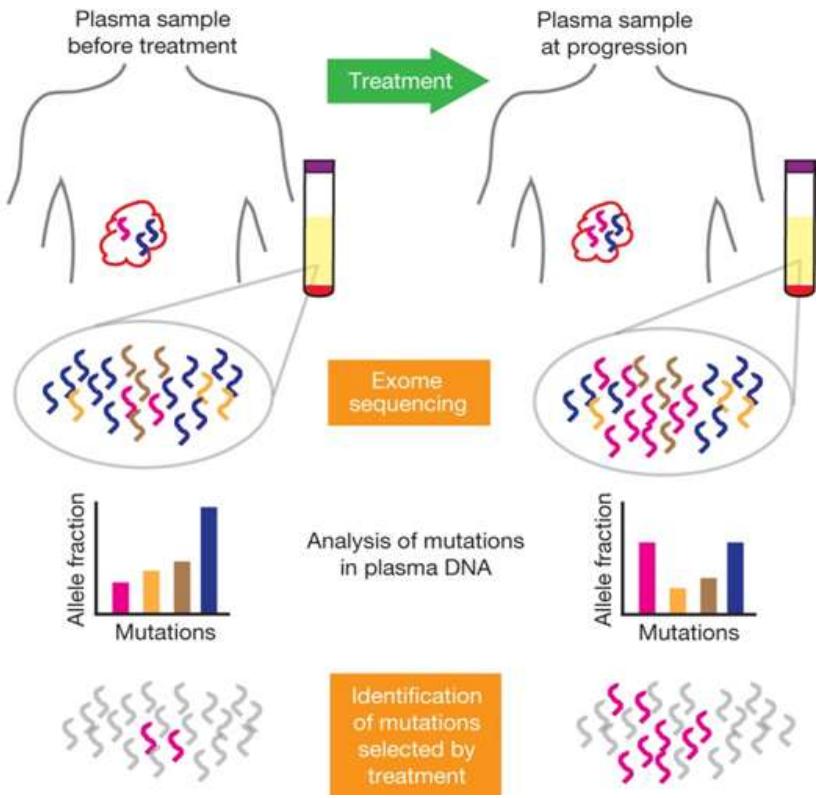
Paris, September 26, 2013

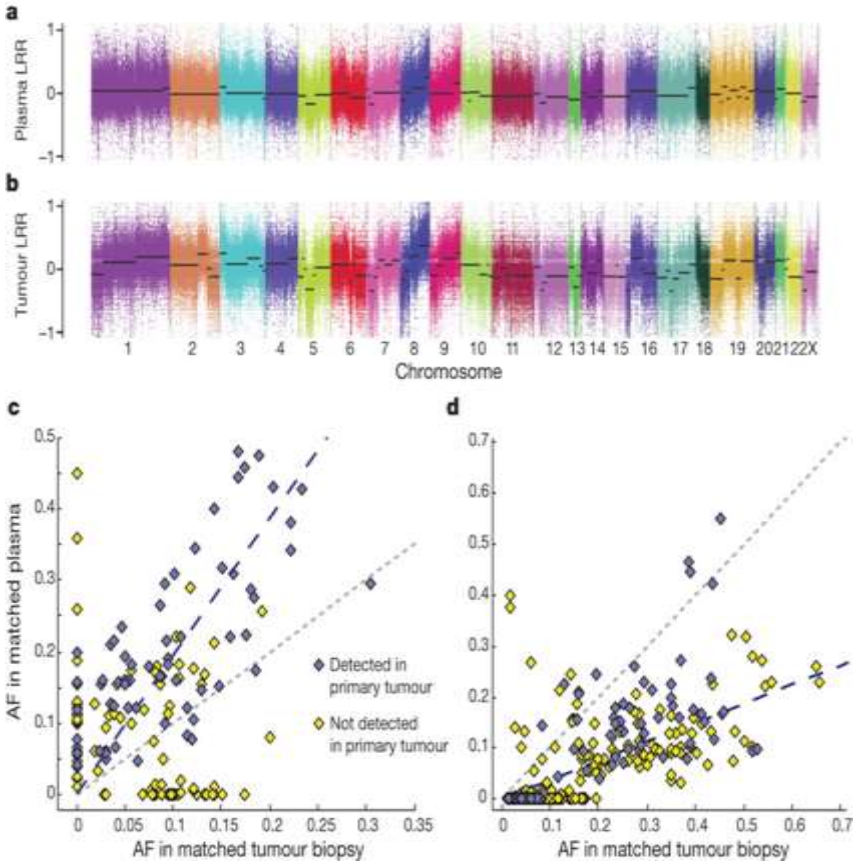
Genetic Intratumor Heterogeneity



Gerlinger M et al. N Engl J Med 2012;366:883-892

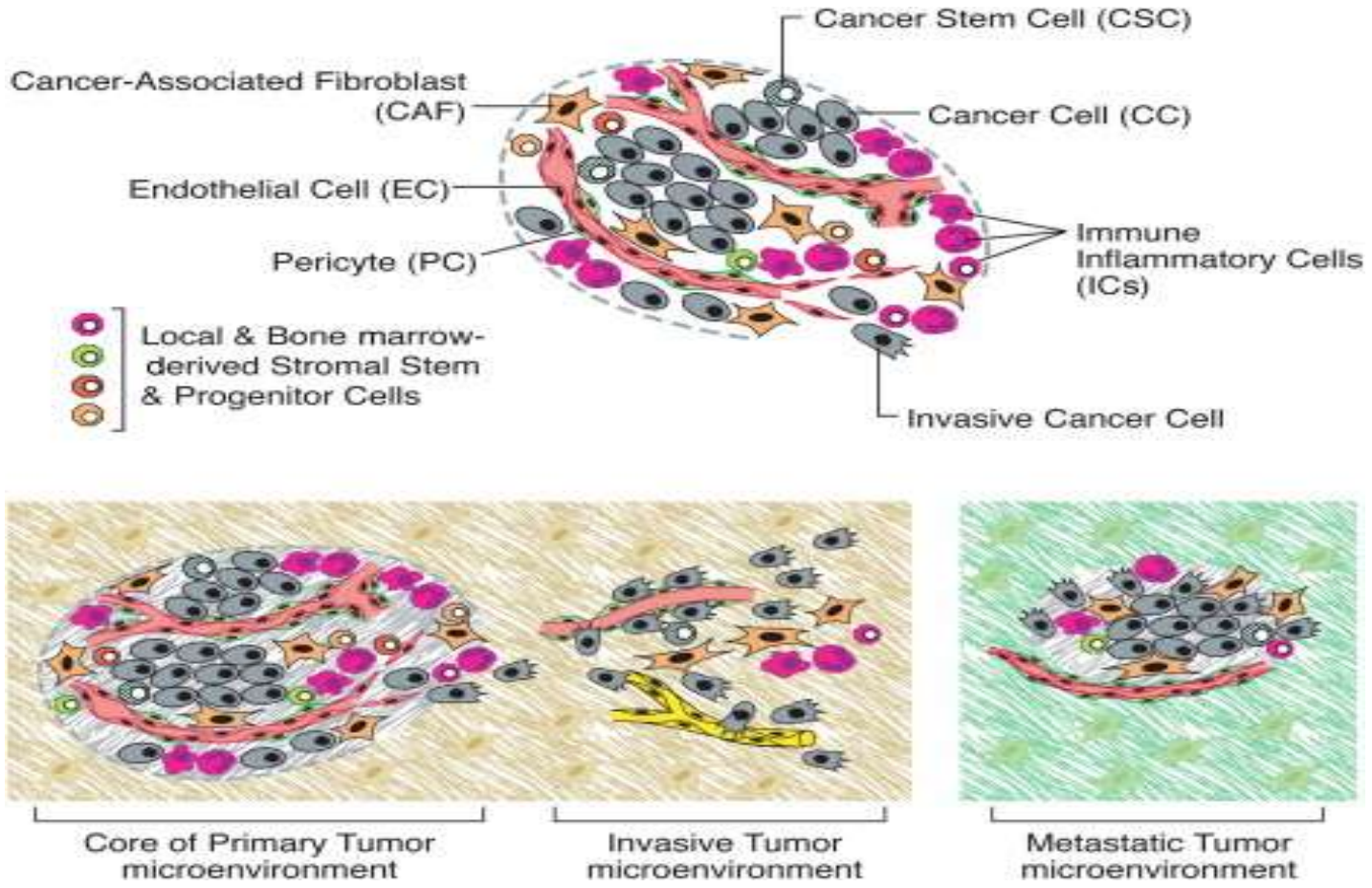
Peripheral Genetic Heterogeneity





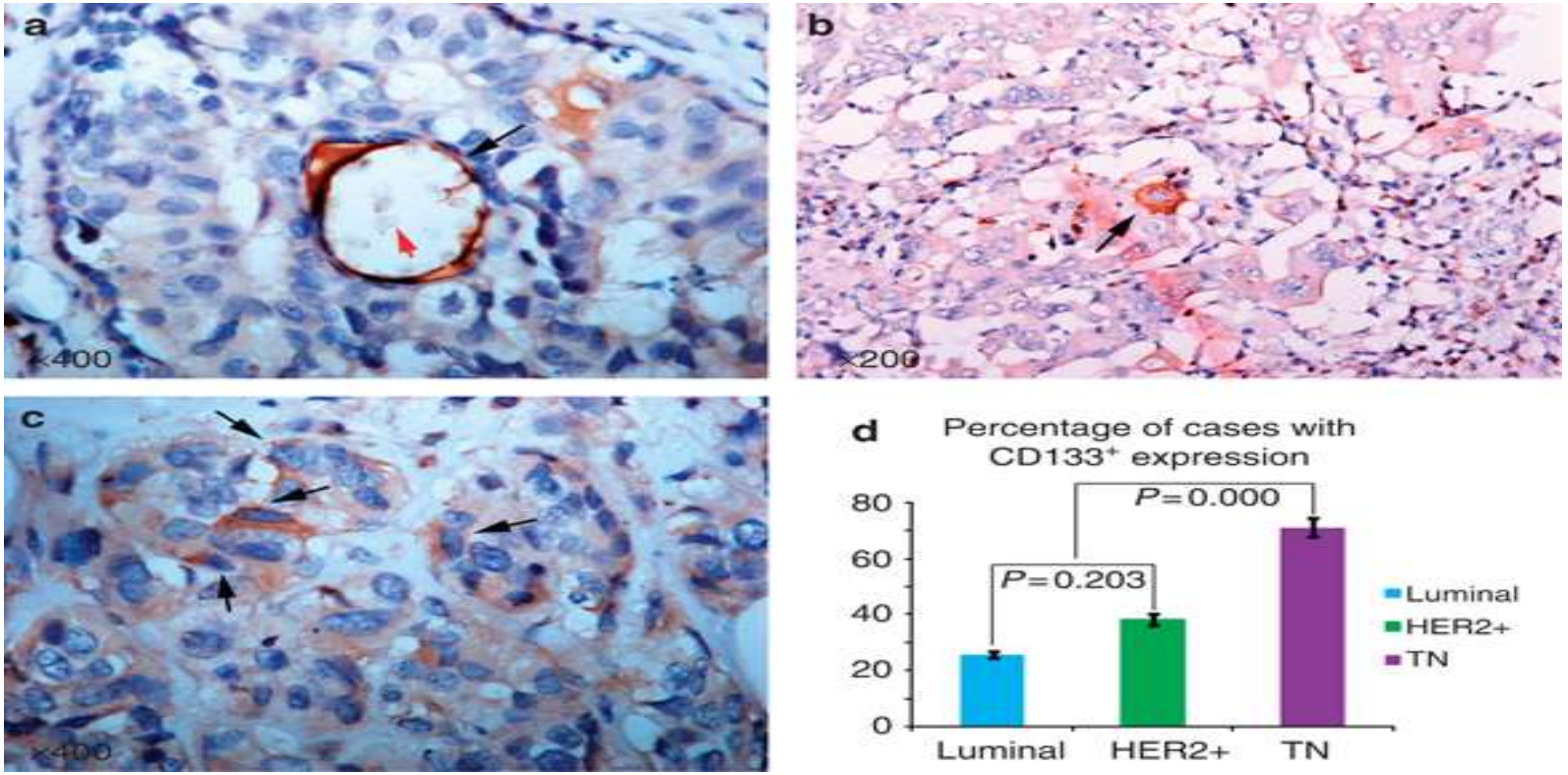
M Murtaza *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12065

Phenotypic Intratumoral Heterogeneity



Douglas Hanahan , Robert A. Weinberg, Cell Volume 144, Issue 5 2011 646 - 674

CD133 and disease subtype



CD133 expression breast cancer specimens by immunohistochemistry

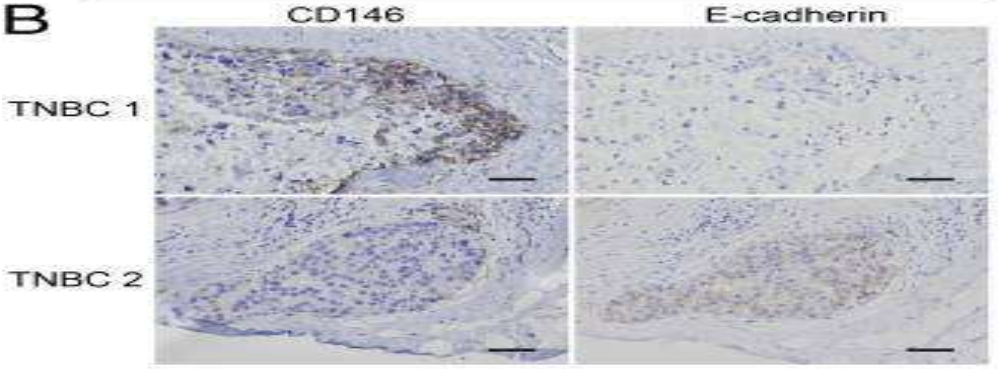
Liu TJ, Oncogene. 2012 Apr 2. doi:

CD146 and disease subtypes

A

Tumor type	CD146 positive	*P*
Estrogen receptor		< 0.001
Negative	125/228 (54.4%)	
Positive	54/274 (19.6%)	
Progesterone receptor		<0.001
Negative	139/226 (61.5%)	
Positive	93/276 (33.7%)	
ERBB/HER2		<0.001
Negative	149/373 (39.9%)	
Positive	28/129 (21.7%)	
Triple-negative (TNBC)		<0.001
Yes	102/148 (68.9%)	
No	75/354 (21.1%)	

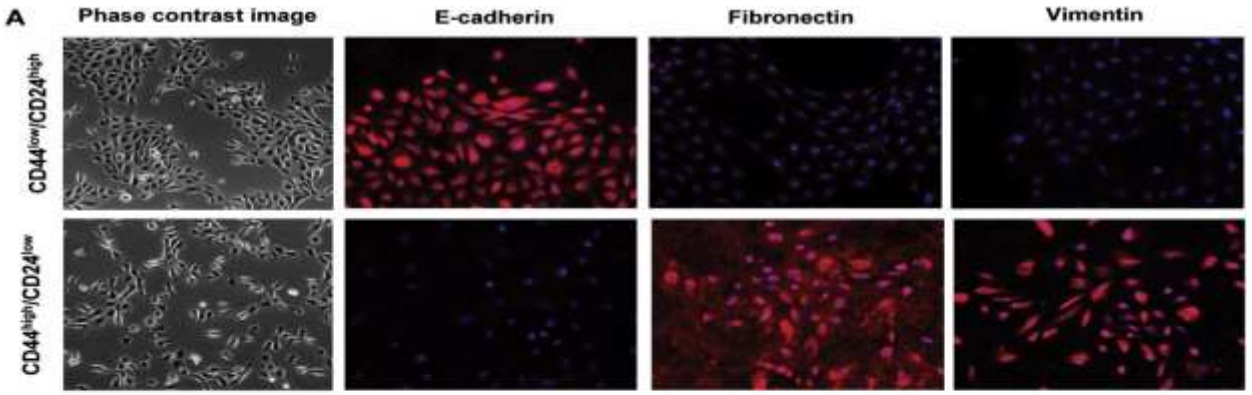
B



	CD146 expression		
	-	+	*P*-value
E-cadherin			
-	3 (3.3%)	36 (40.0%)	
+	14 (15.6%)	37 (41.1%)	*P*=0.018

Zheng Q, Proc Natl Acad Sci U S A. 2012 Jan 24;109(4):1127-32.

The epithelial-mesenchymal transition generates cells with properties of stem cells



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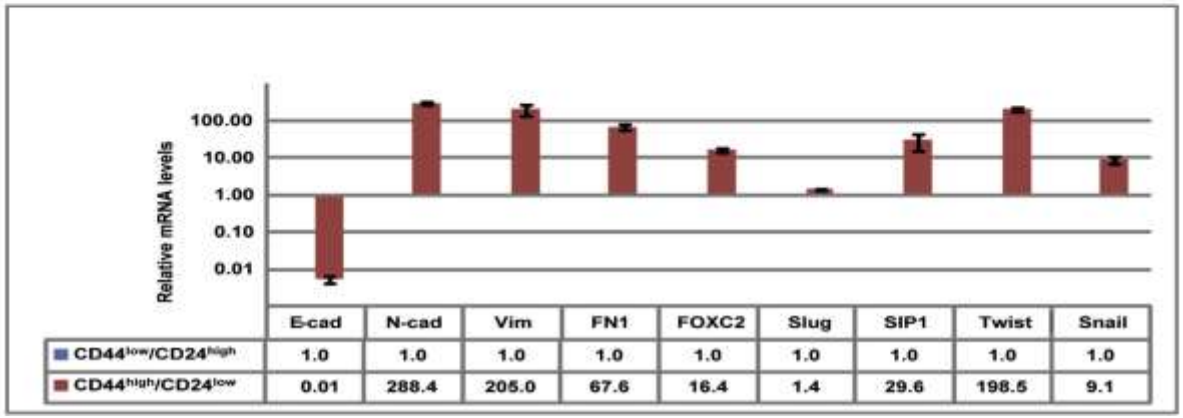
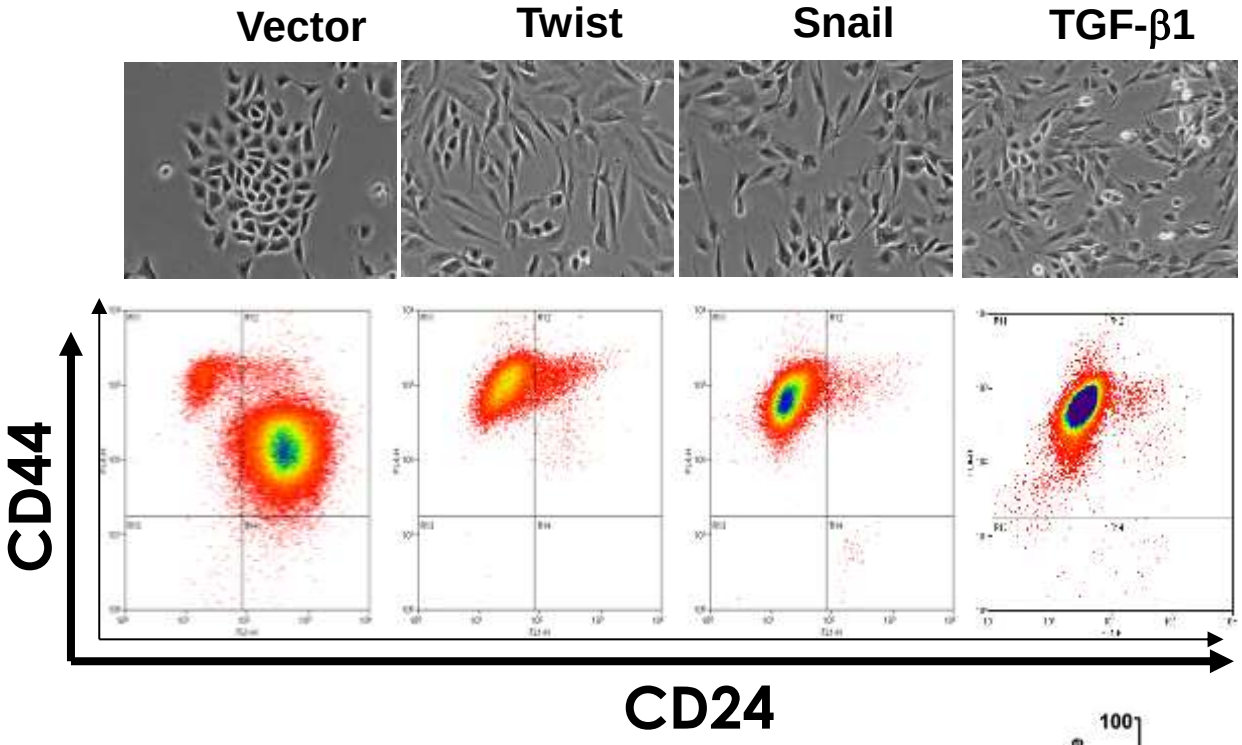
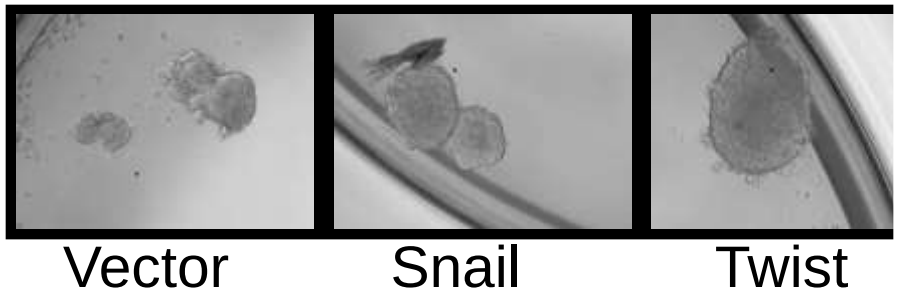


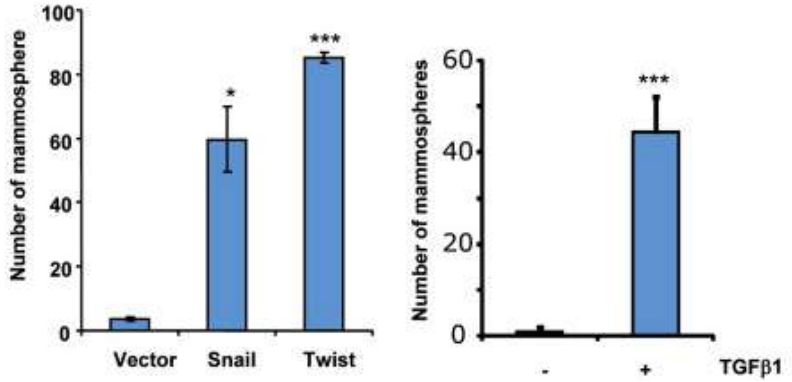
	E-cad	N-cad	Vim	FN1	FOXC2	Slug	SIP1	Twist	Snail
CD44low/CD24high	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CD44high/CD24low	0.01	288.4	205.0	67.6	16.4	1.4	29.6	198.5	9.1

Mani SA et al, Cell 133(4):704-15,2008

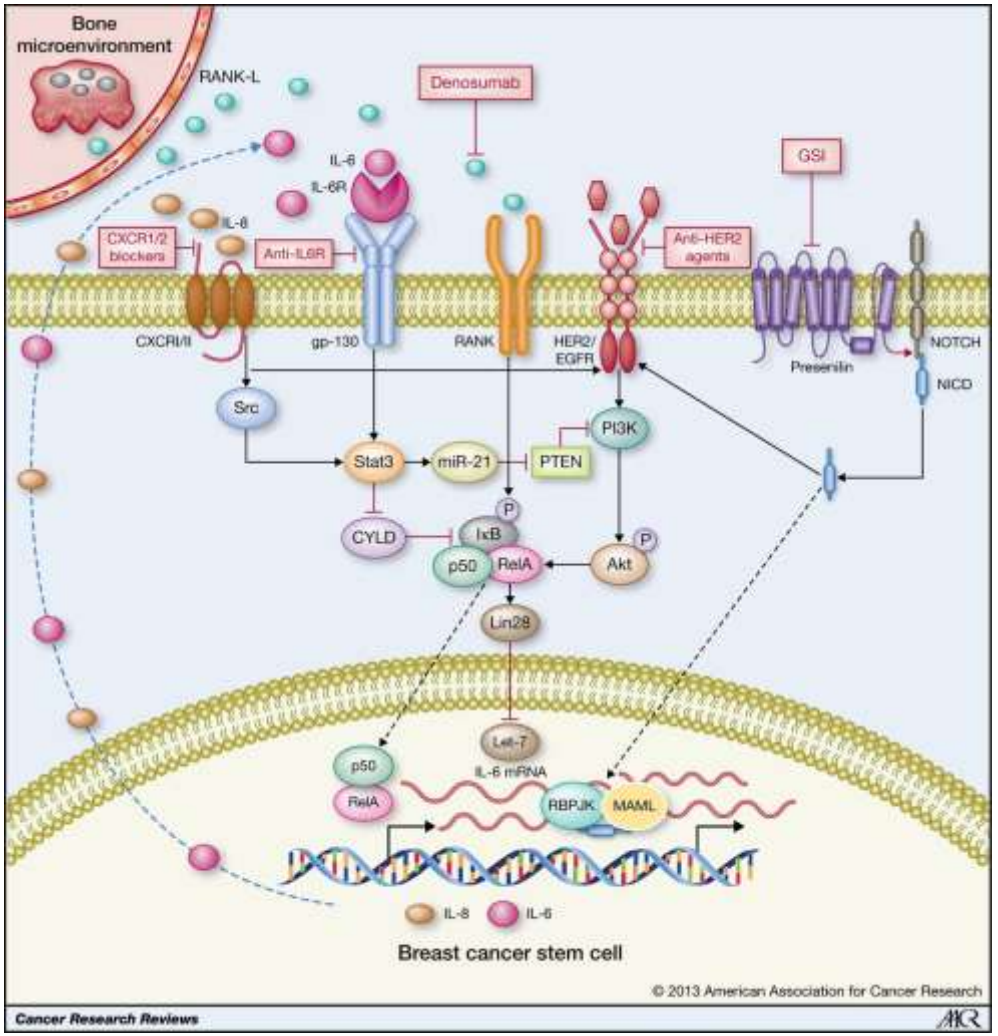
Induction of EMT generates cells with stem cell properties





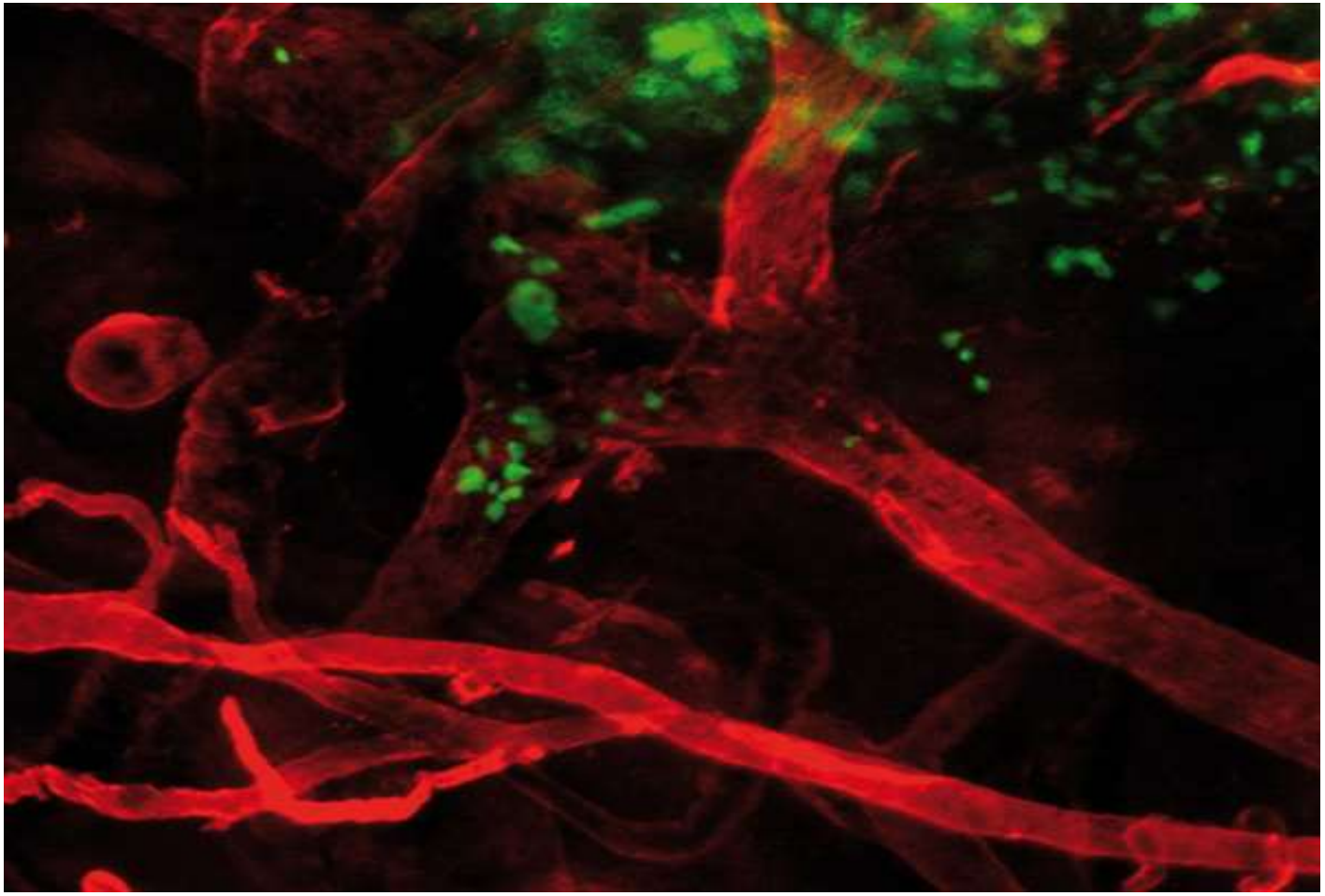


Pathways regulating breast CSCs.



Korkaya H , and Wicha M S Cancer Res 2013;73:3489-3493

Understanding Metastasis



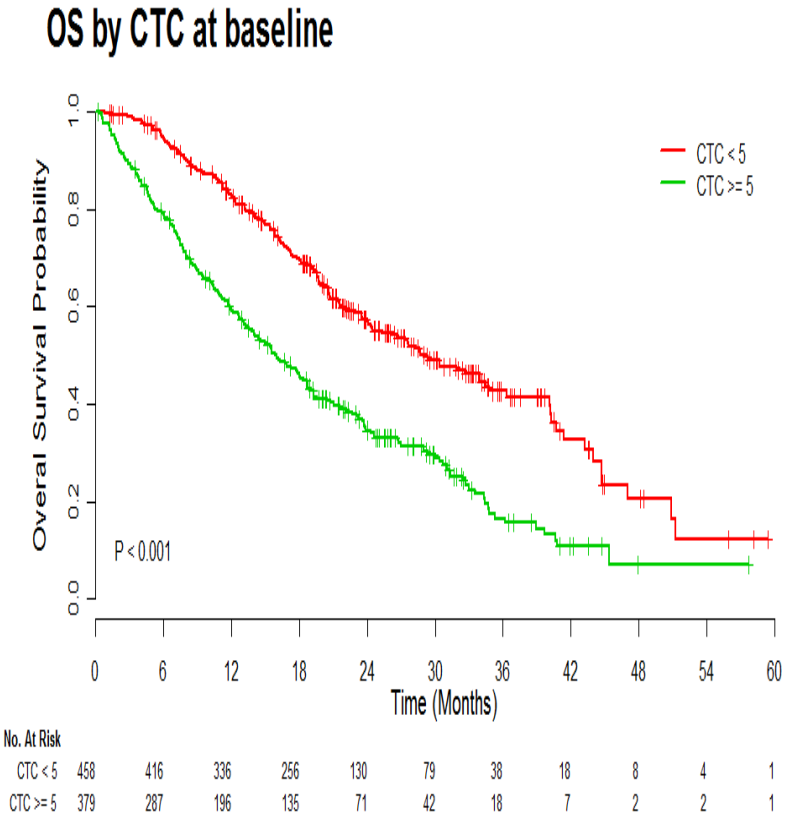
Marx V, Nature, 494: 133–138

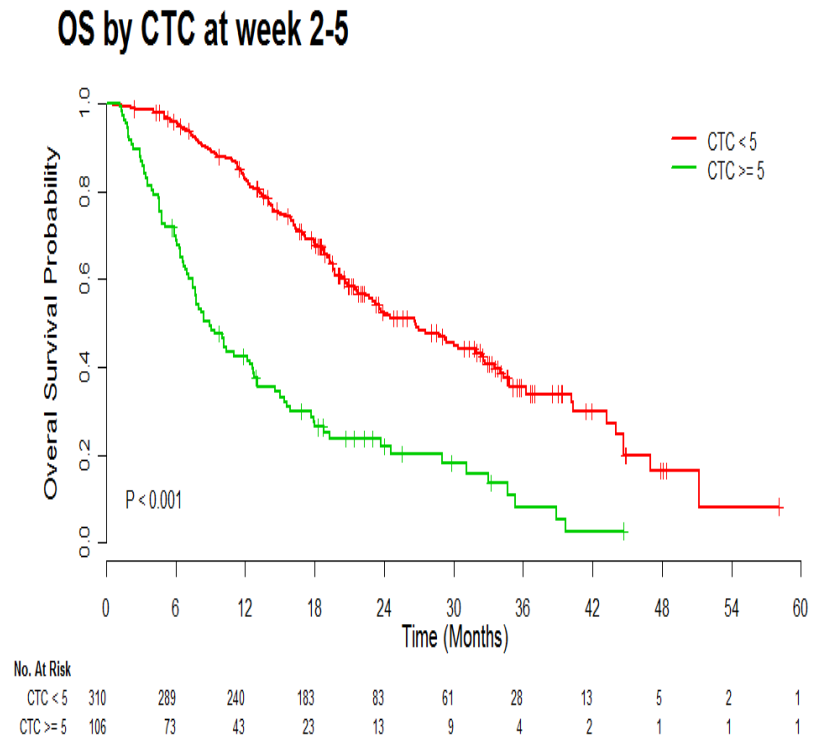
Challenges

- Genetic heterogeneity represents a challenge for the effective application of molecular targeted therapies
- Phenotypic heterogeneity represents a challenge to develop therapies that reduce tumor burden AND the development of metastases
- The phenotypic plasticity of cancer cells suggests that CTCs represent an ideal tool/model to study the metastatic process

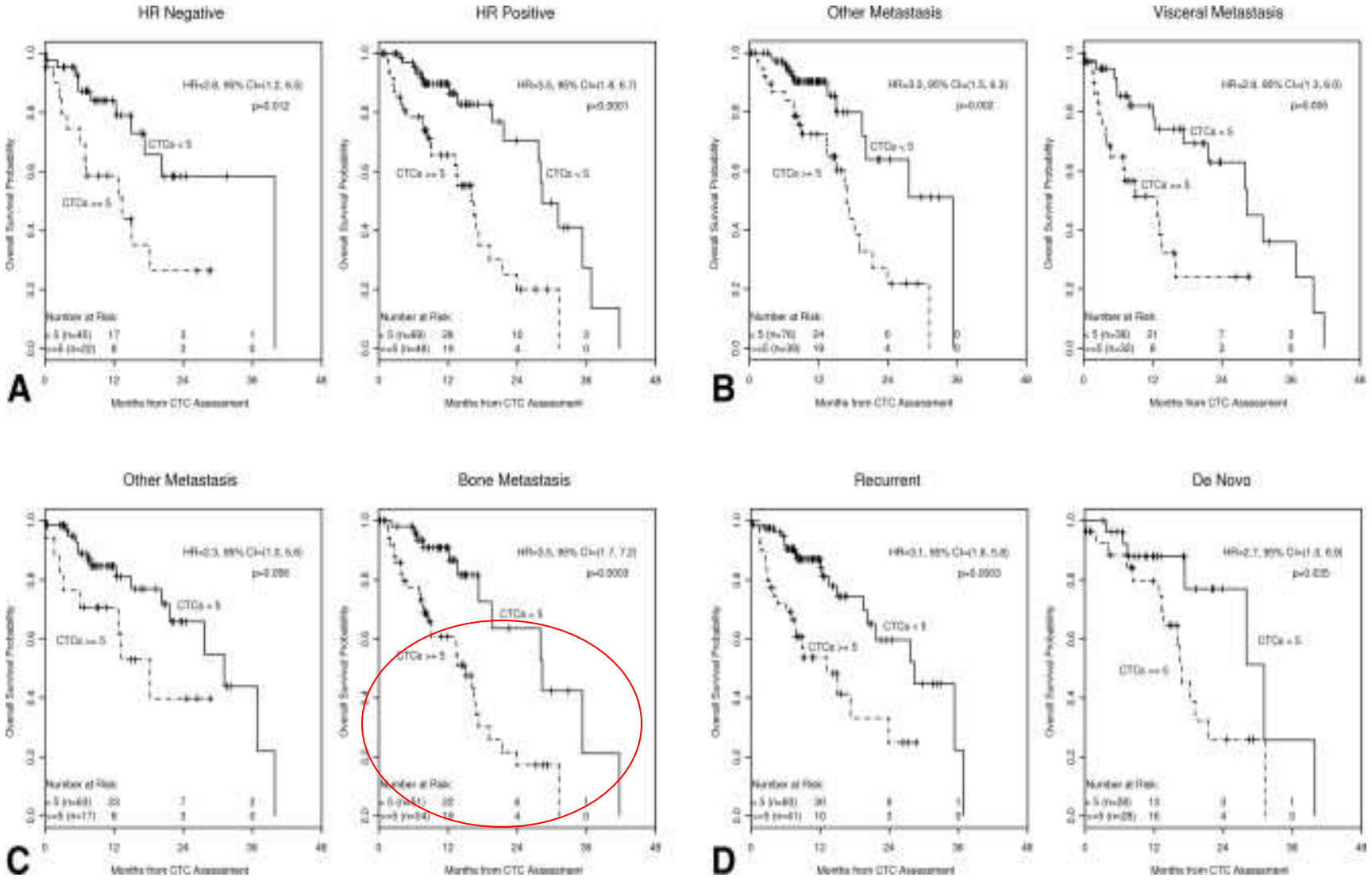
CTCs enumeration, disease progression, seeding and metastases

Pooled analysis: Validation Prognostic Value Baseline CTCs in MBC



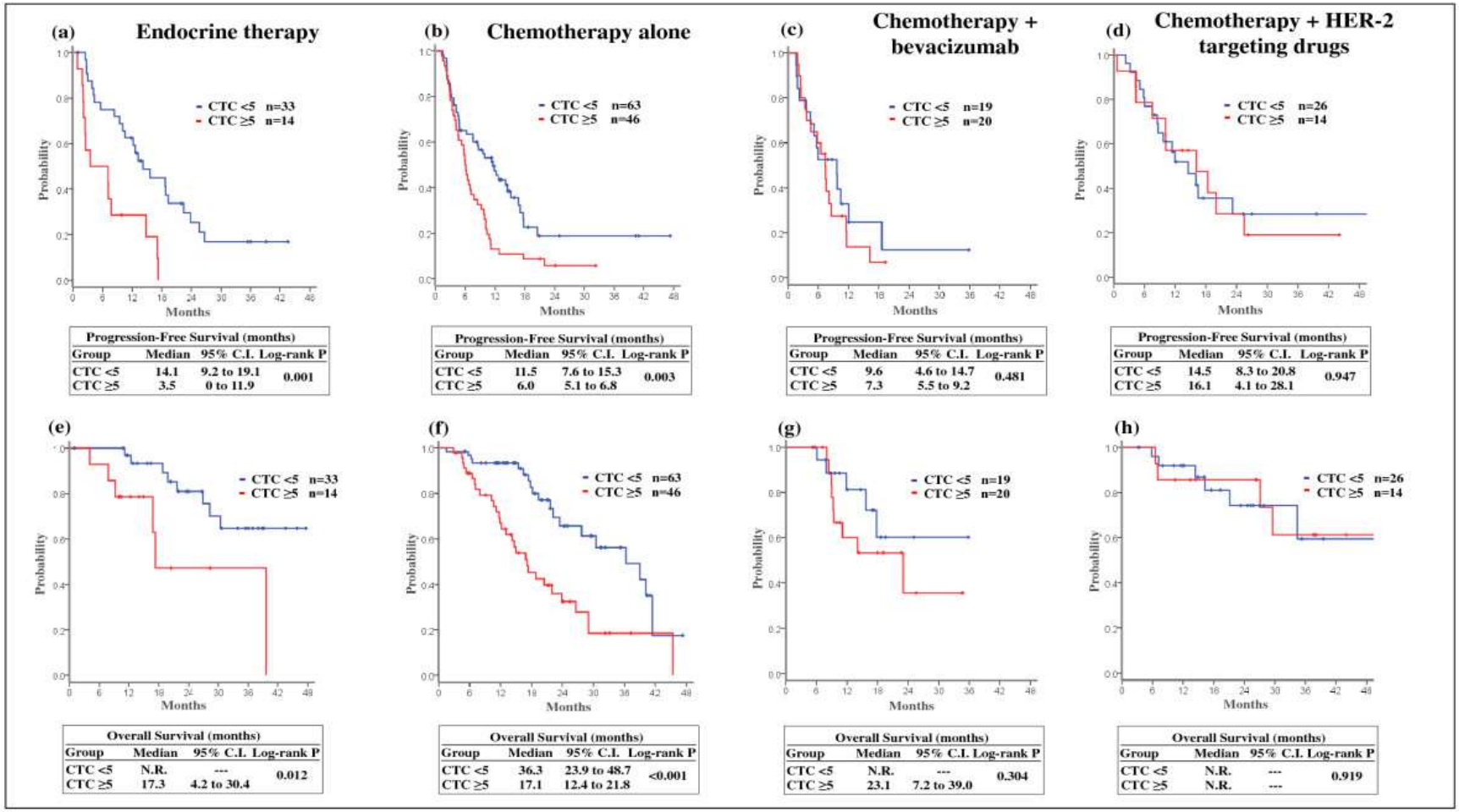


Liu MC, et al, ASCO 2011



Dawood S et Cancer, 113(9):2422-30, 2008

CTCs and First-line Systemic Therapy



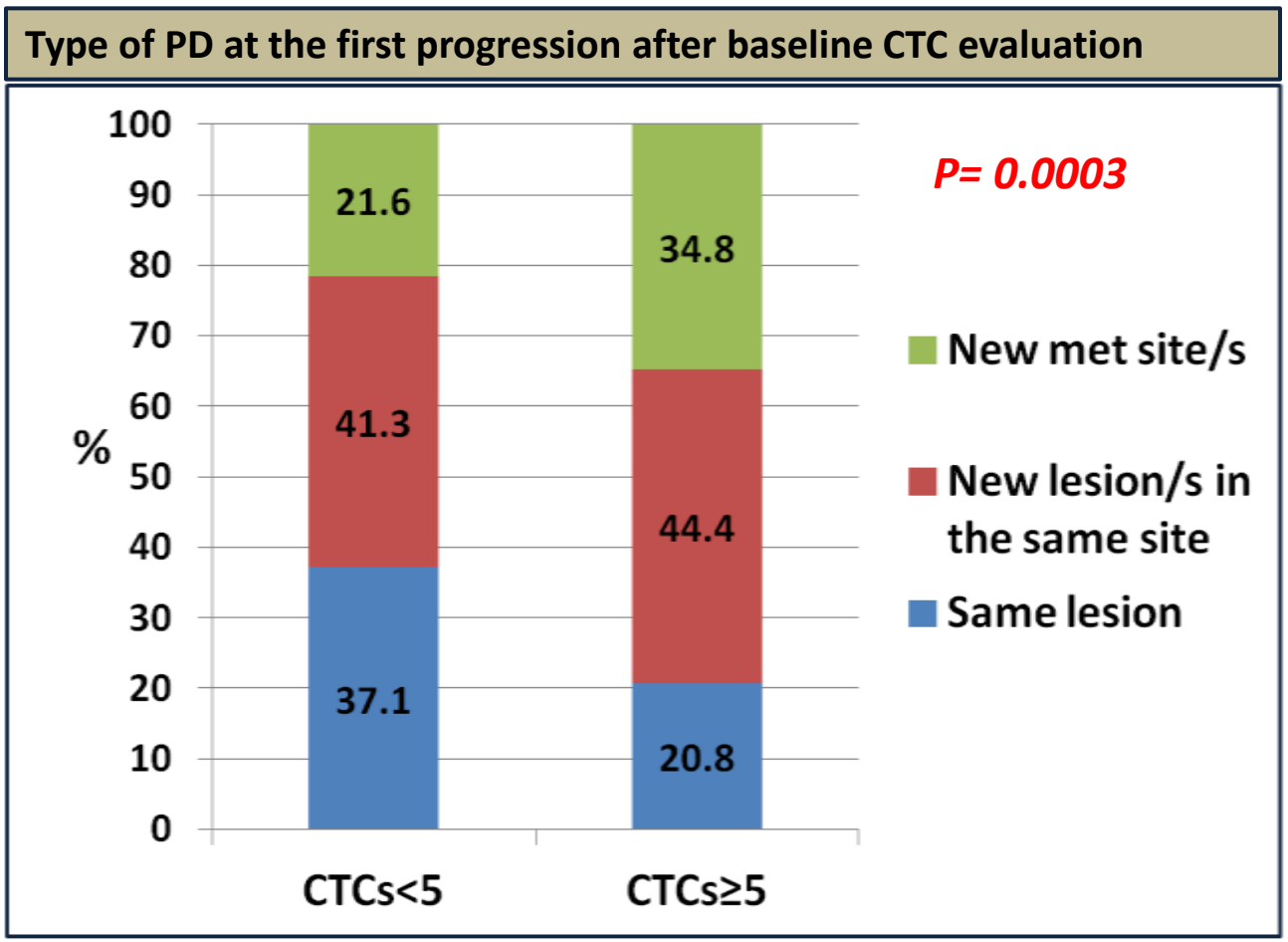
Overall population

Patients with documented PD

N=442

CTC<5 n= 264
CTC≥5 n= 178

Type of PD at the first progression after baseline CTC evaluation



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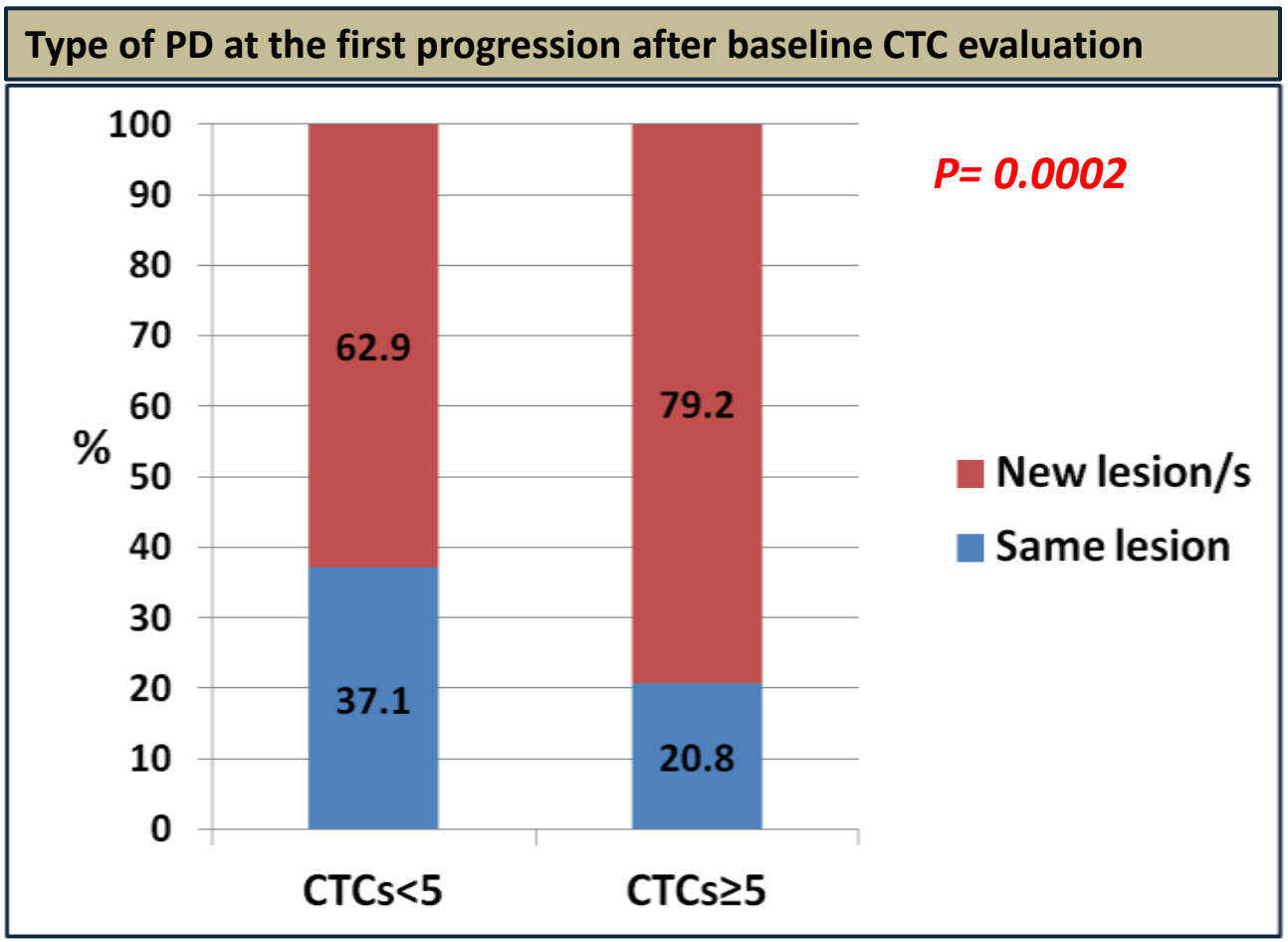

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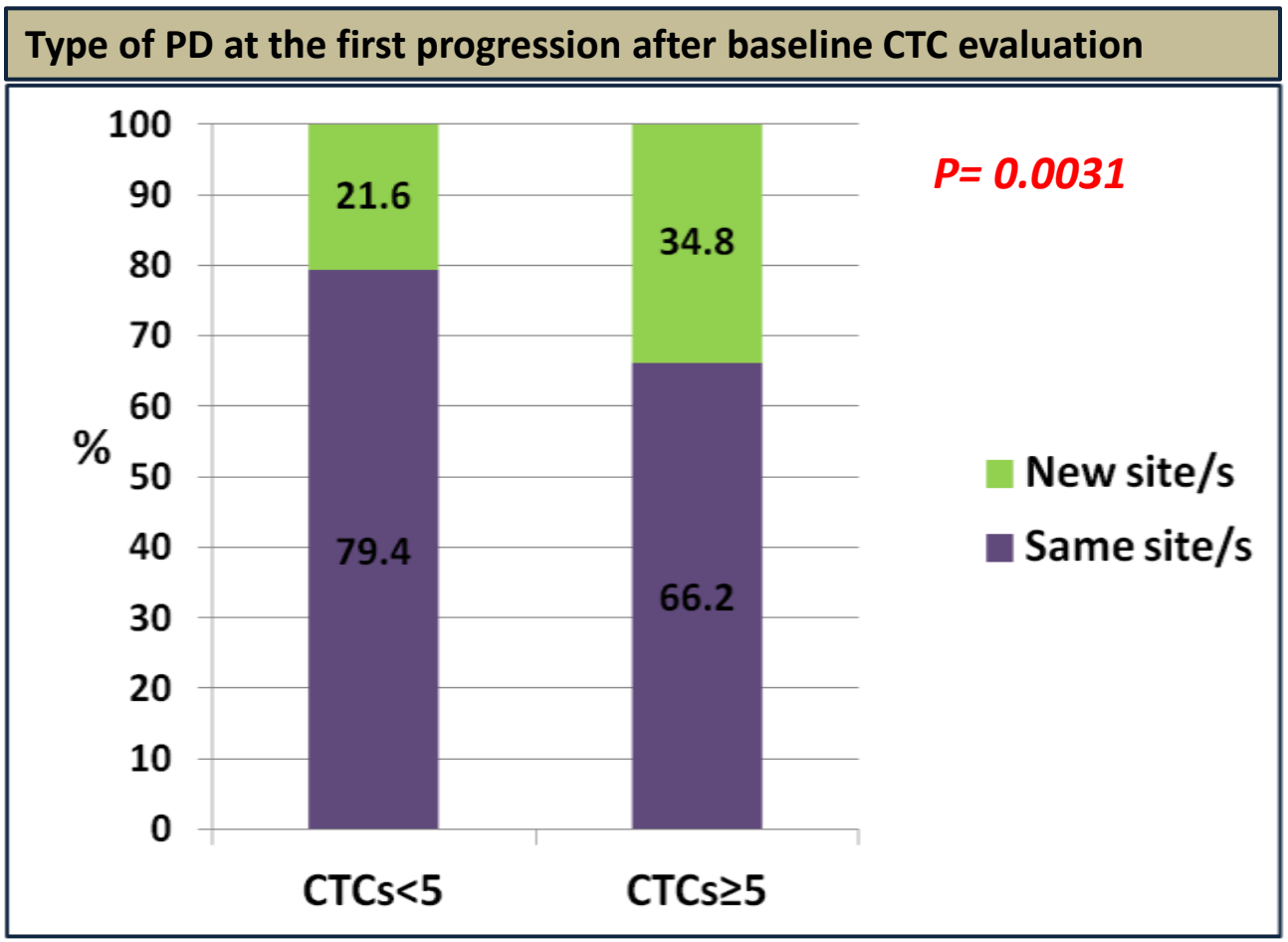
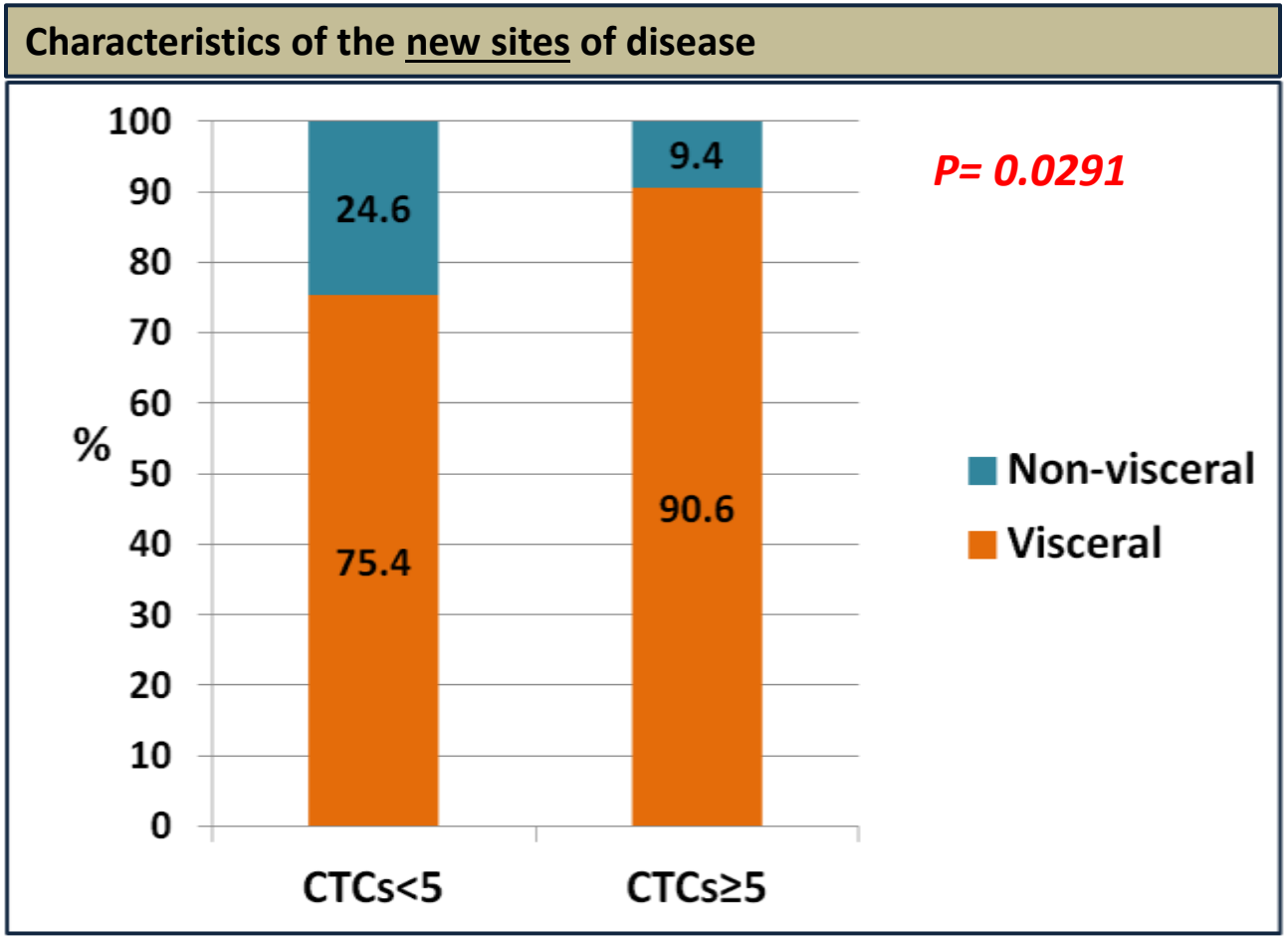


Overall population

Patients with PD in a new site

N=121

CTC<5 n= 57
CTC≥5 n= 64



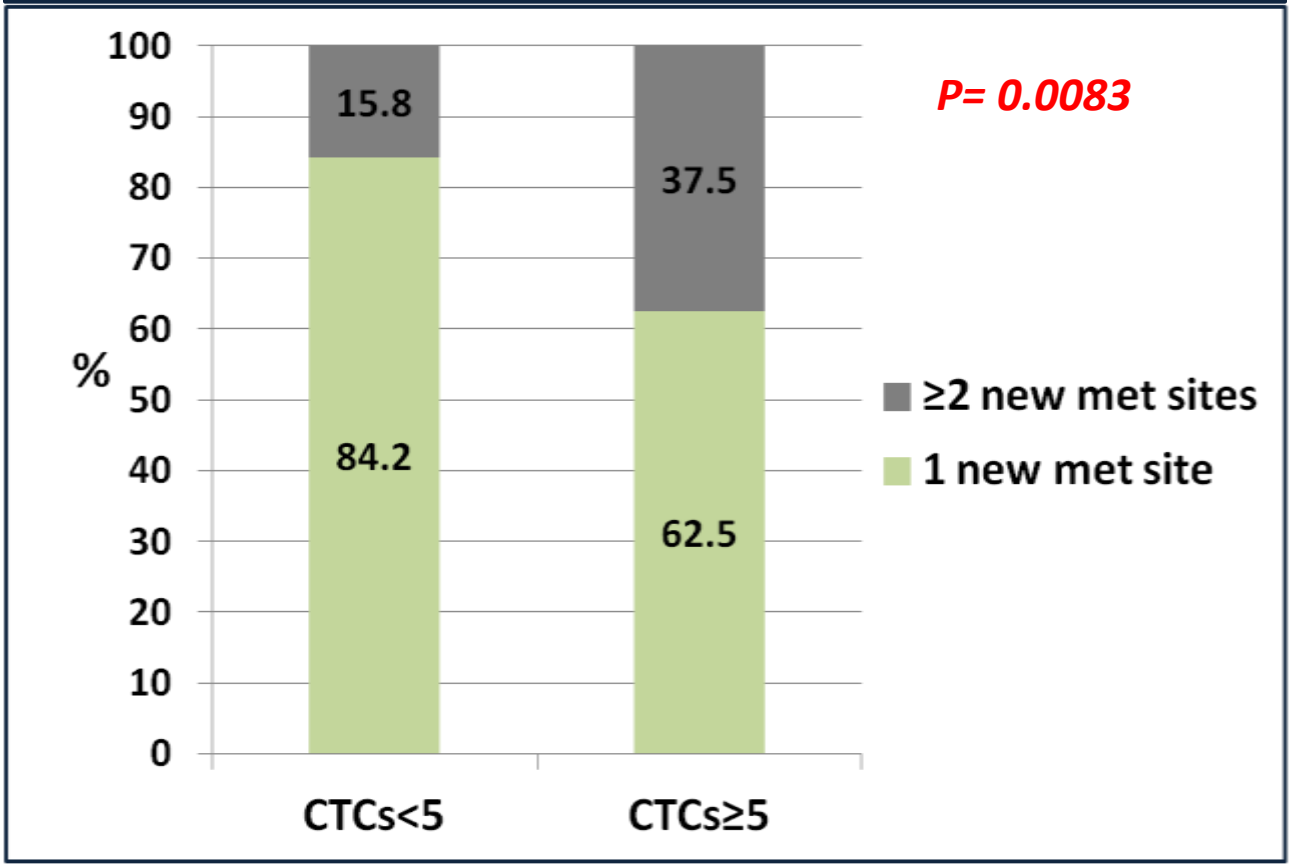
Overall population

Patients with PD in a new site

N=121

CTC<5 n= 57
CTC≥5 n= 64

Number of new metastatic sites developed by those patients who progressed in new met site/s at first PD



Overall population

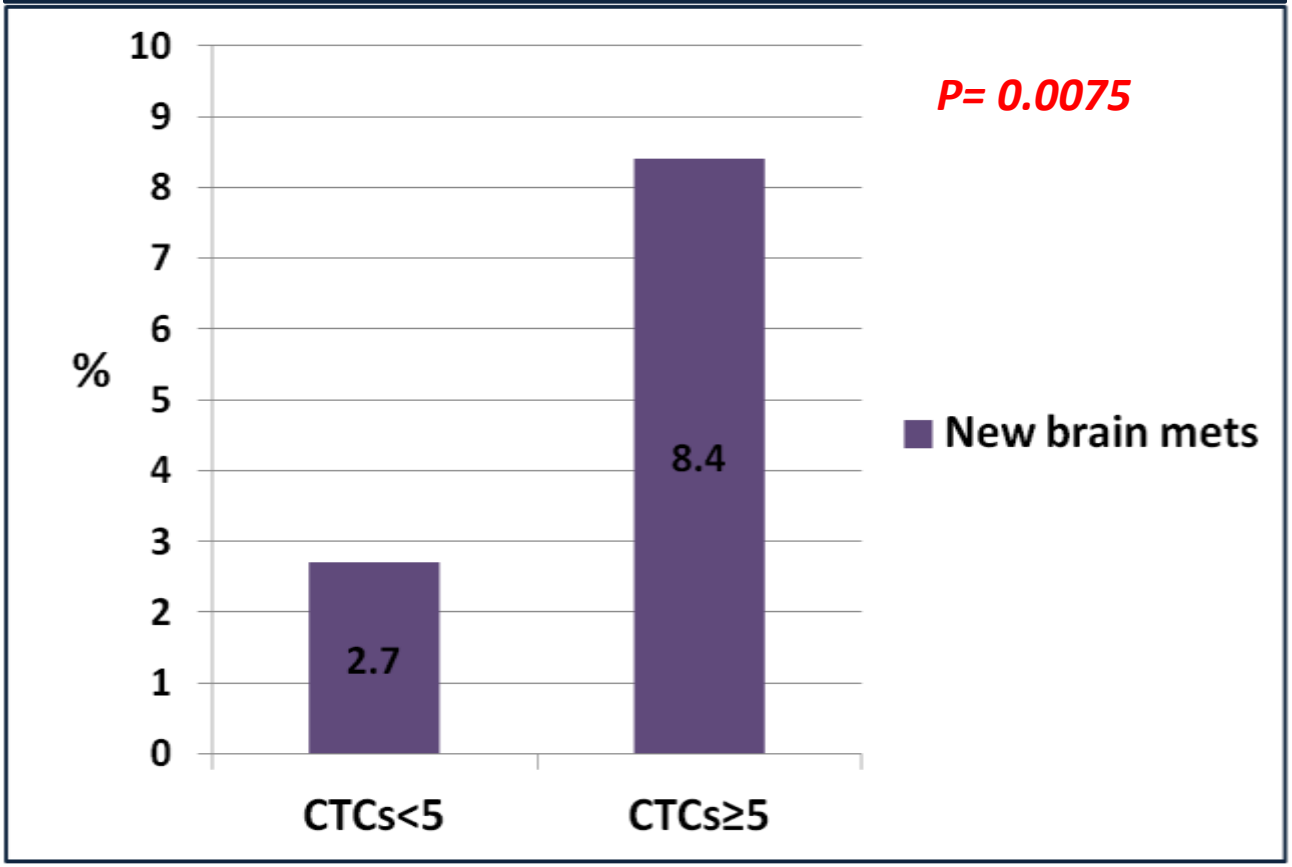
Patients with documented PD

N=442

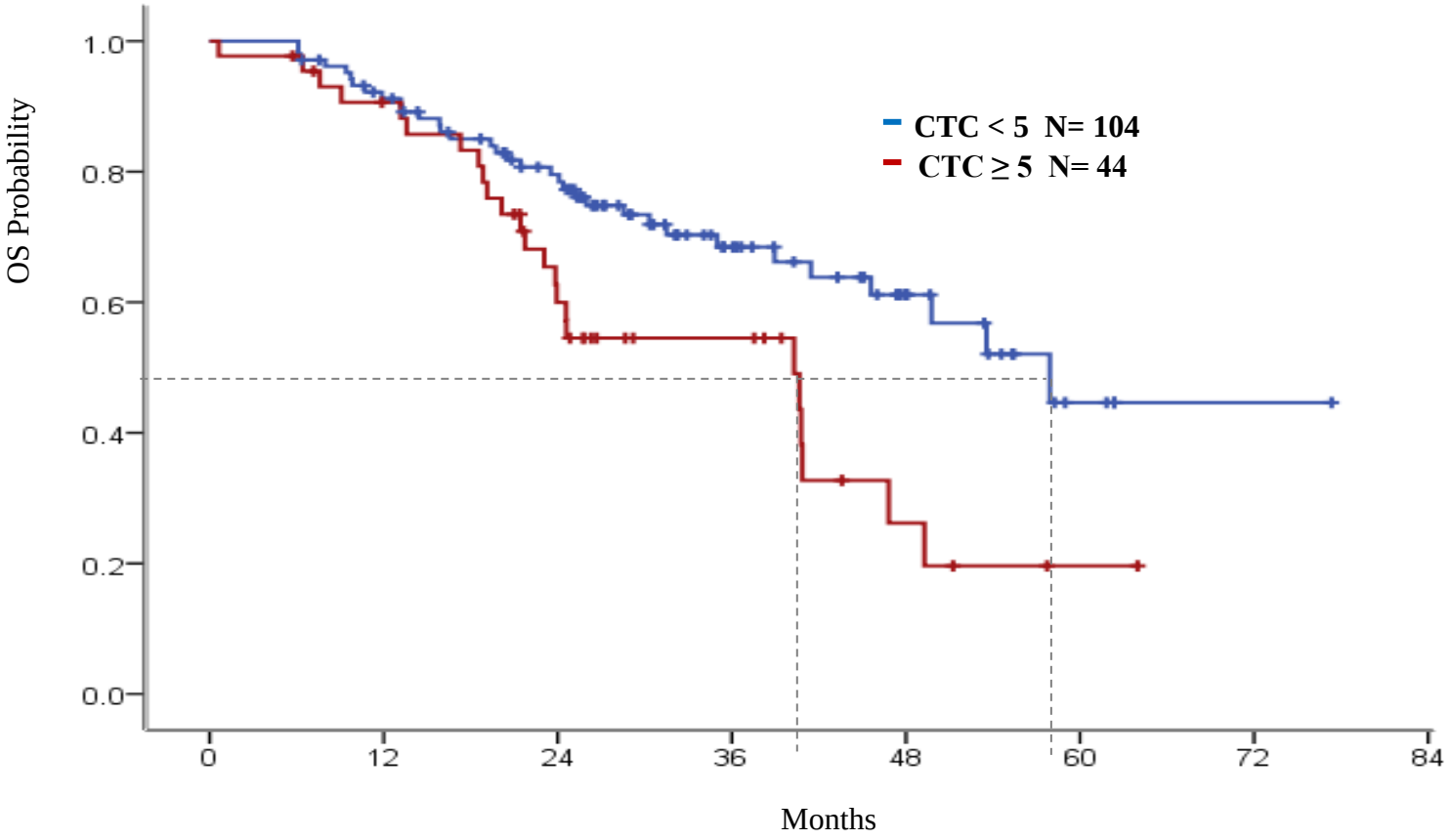
CTC<5 n= 264

CTC≥5 n= 178

Percent of pts developing new brain mets at the first PD after baseline CTC evaluation (among all pts who had PD, n=442)



Estimated OS in patients with oligometastatic disease according to CTC baseline value

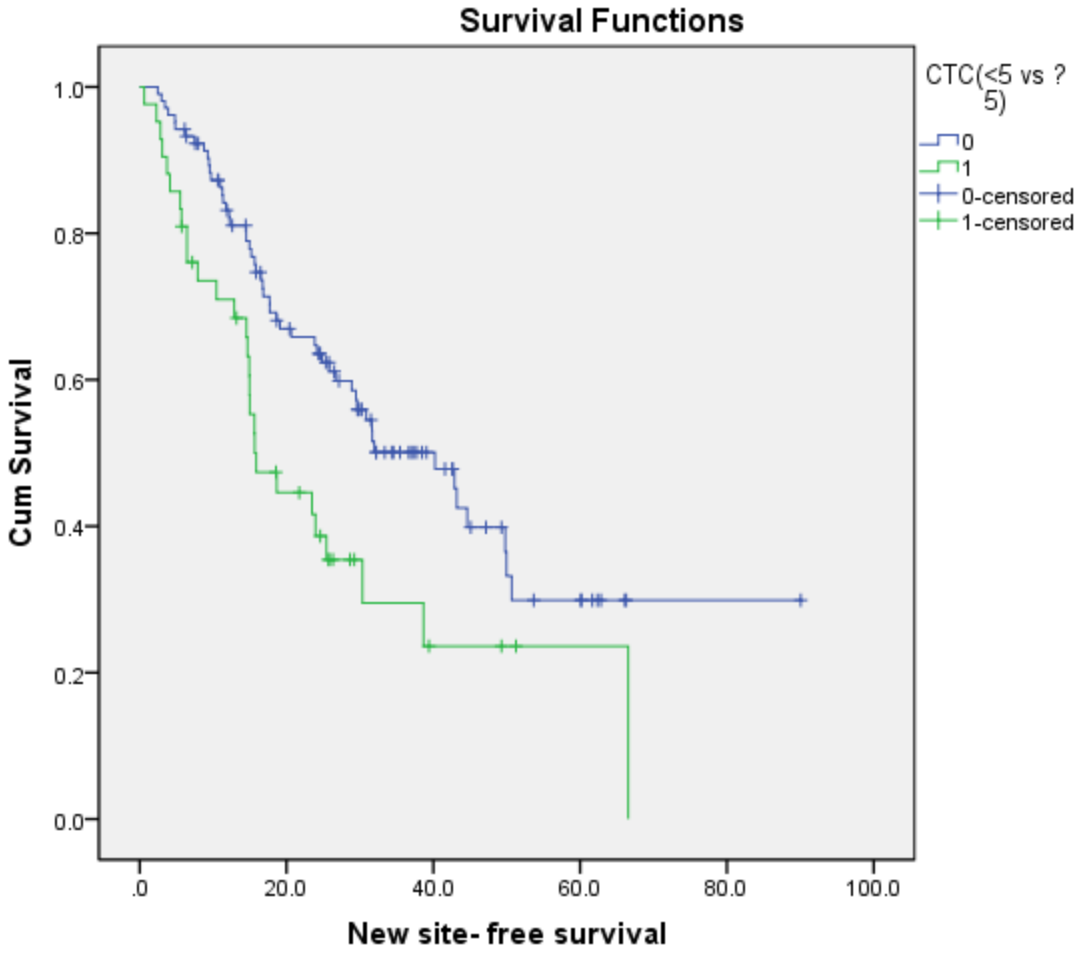


Overall Survival

Group	Median	95% C.I.	Log-rank *P*
CTC <5	57.9	45.2 to 70.6	0.006
CTC ≥5	40.3	19.8 to 60.8	

Oligometastatic Patients n= 146

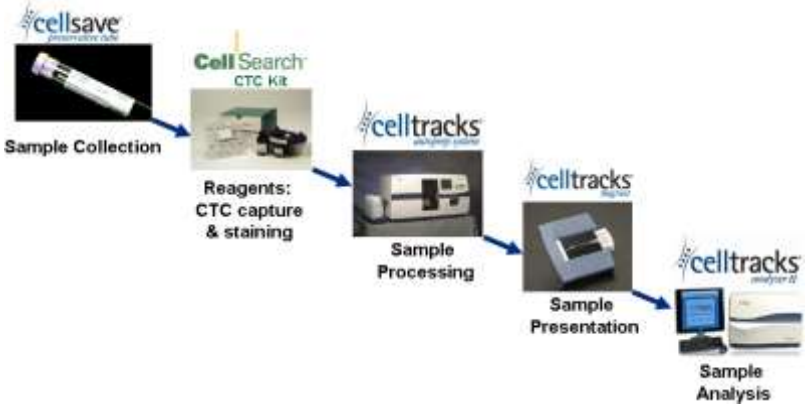
Time To New metastatic Sites (TTNS)

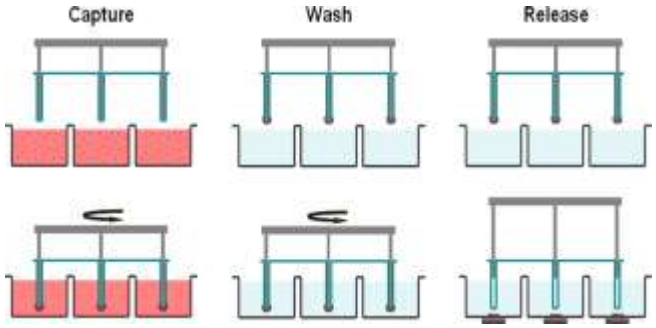


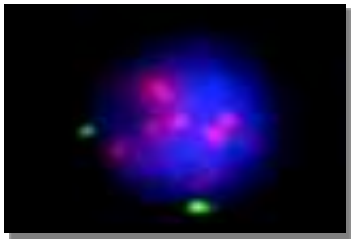
Comparison of Survival Curves	
Log-rank (Mantel-Cox) Test	
Chi square	*7.875*
df	*1*
P value	***0.005***
P value summary	***
Are the survival curves sig different?	*Yes*
Median survival (months)	
CTC<5	***40.230***
CTC>=5	***15.888***

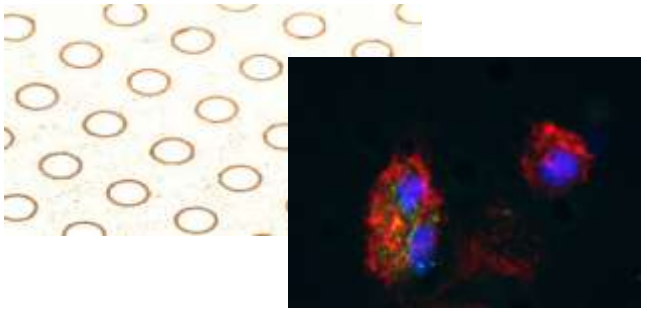
CTCs and stem cells/EMT

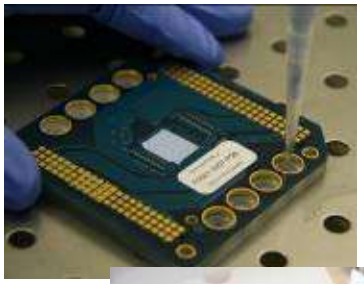
Molecular Characterization



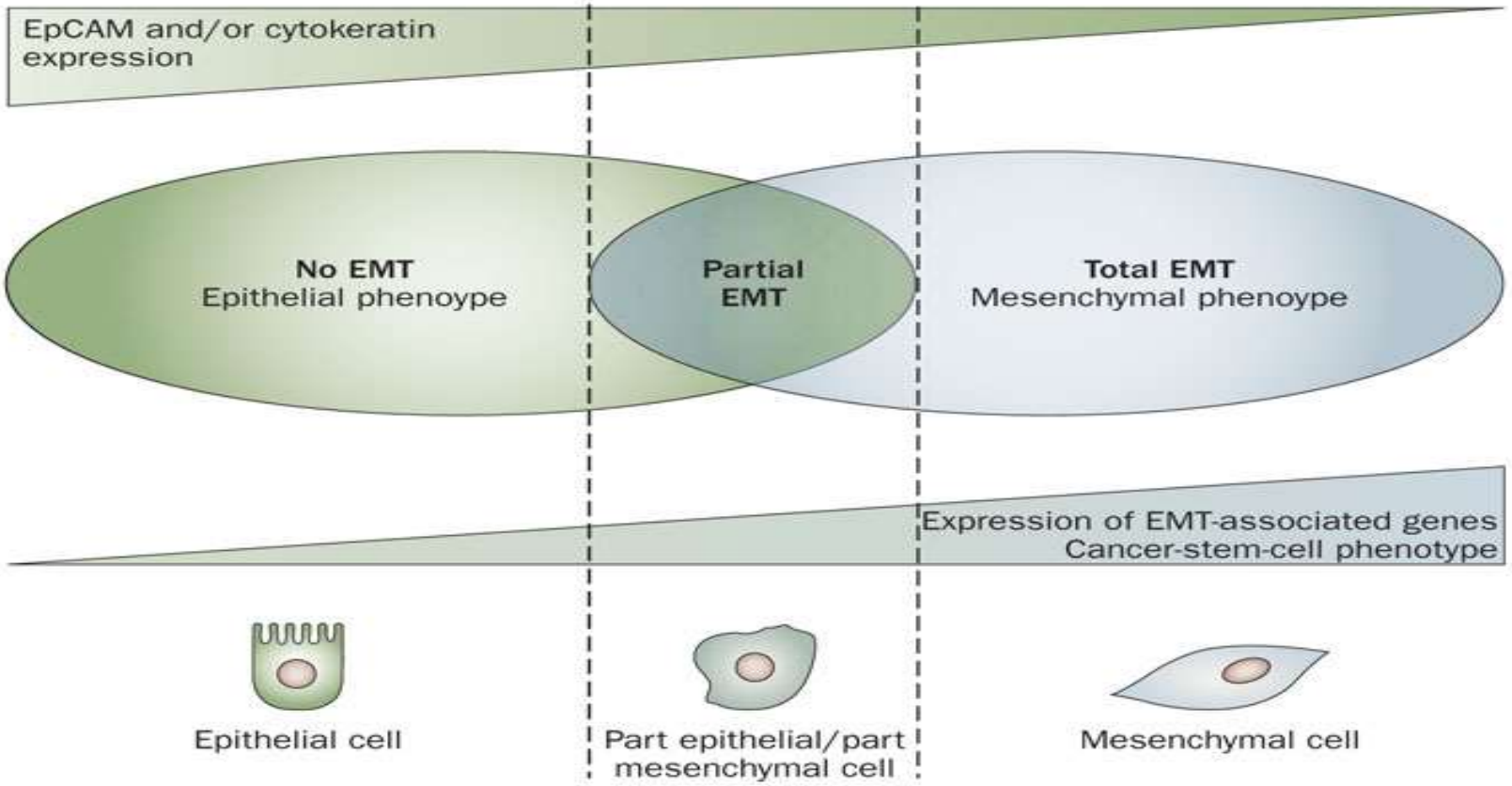






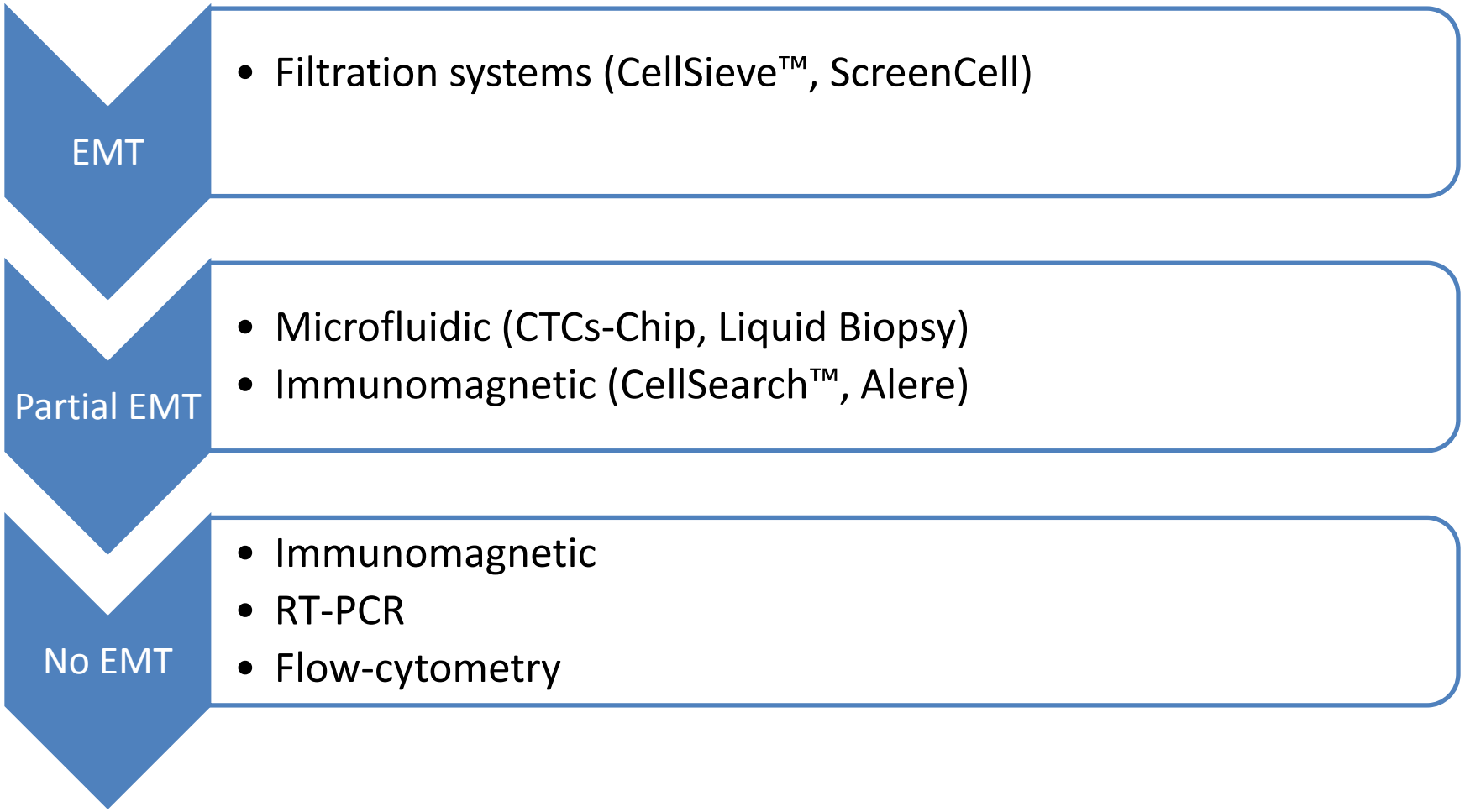


Heterogeneity of CTC



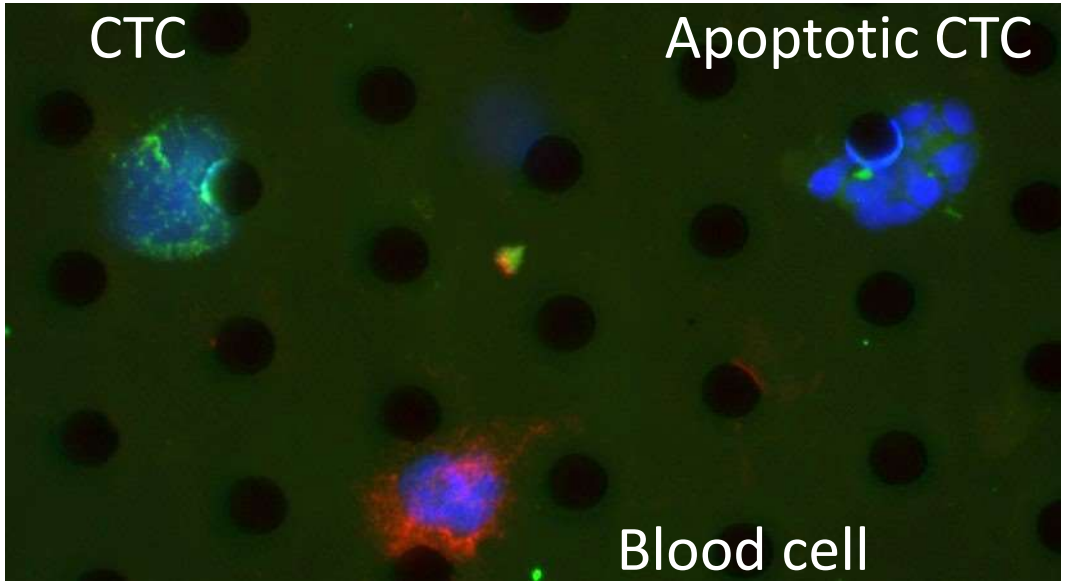
Mego, M., Mani S. A. & Cristofanilli, M. (2010), *Nat. Rev. Clin. Oncol.*

Detection methods for EMT



CellSieve™ Assay

- **Enumeration assay protocol**
  - Filtration of 7.5 ml whole blood in 90 sec
  - Fixation, permeabilization & staining
  - Mount on slide for cell counting
- **Staining**
  - Nucleus (blue)
  - CK 8, 18, 19 (green)
  - EpCAM (orange)
  - CD45 (red)

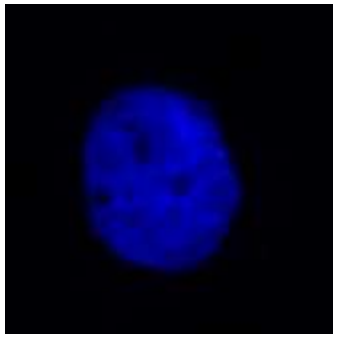


Patient Sample

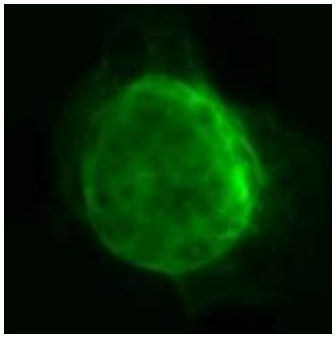
- **Stage IV breast cancer patient**

	CellSieve™		CellSearch®
	EpCAM (-)	EpCAM (+)	
CTC Count	16	1	1

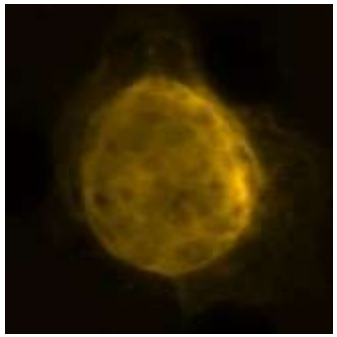
Many CTCs don't express EpCAM



Nucleus



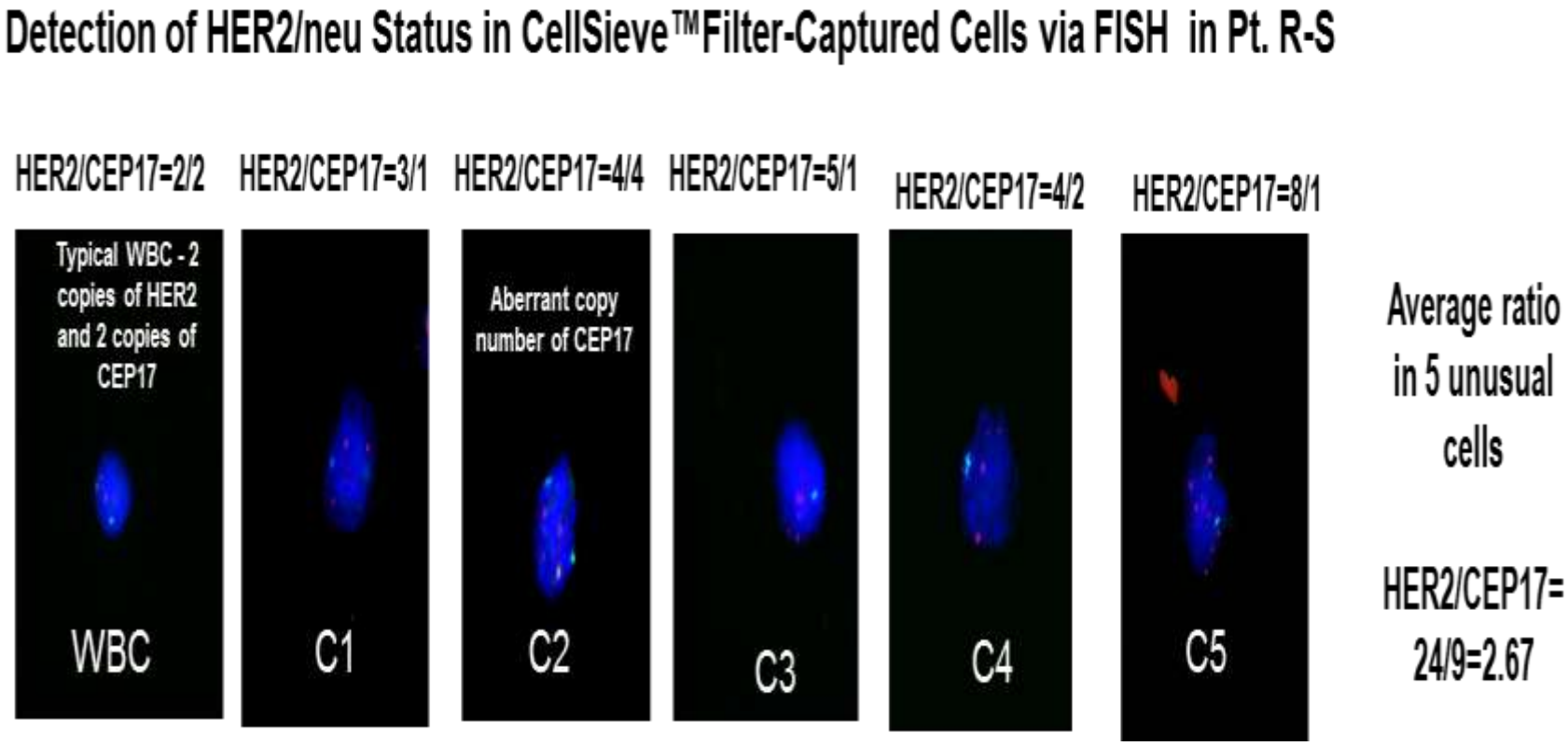
CK 8, 18, 19



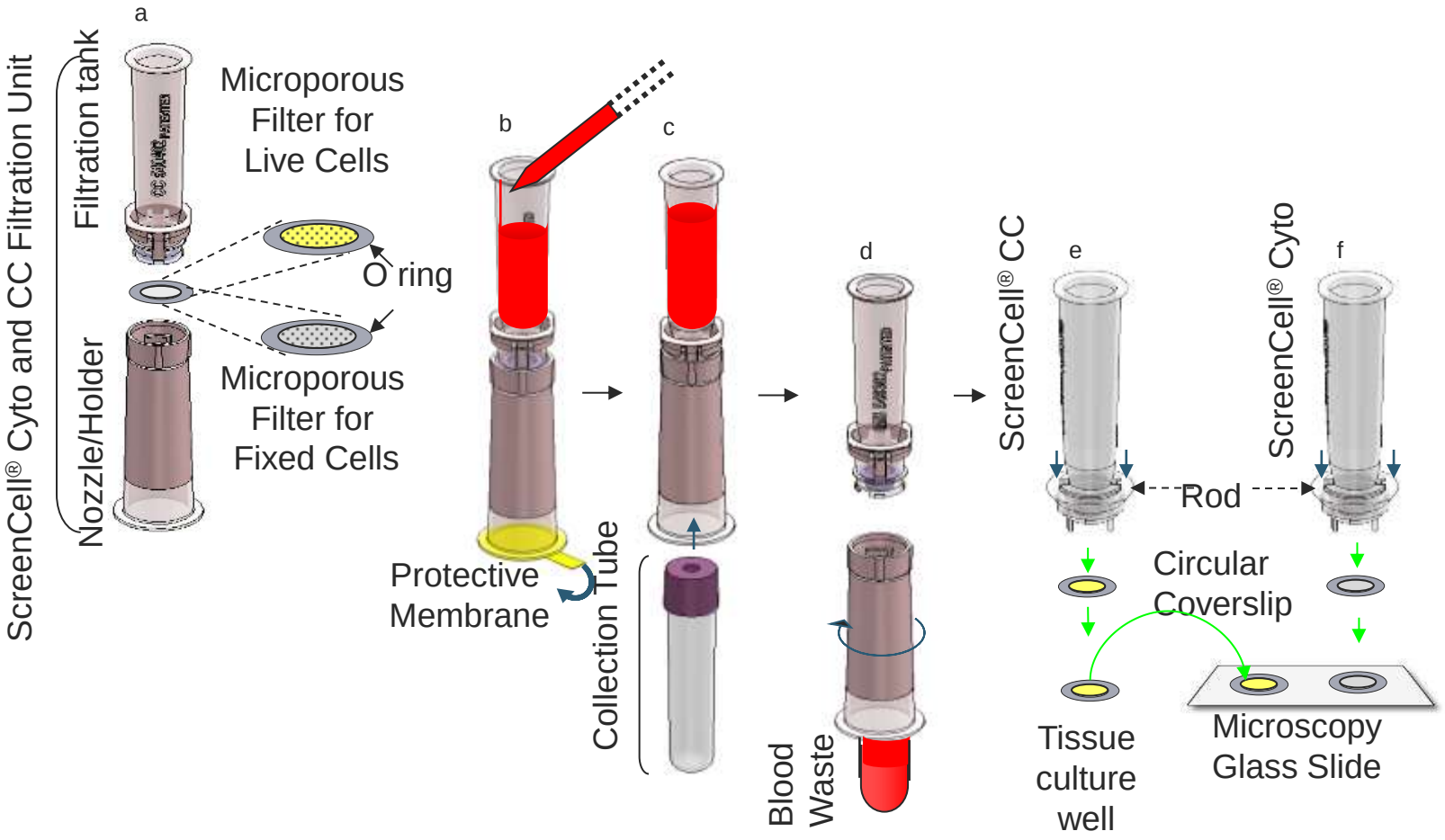
EpCAM

CD45

Biomarkers in EpCAM - cells

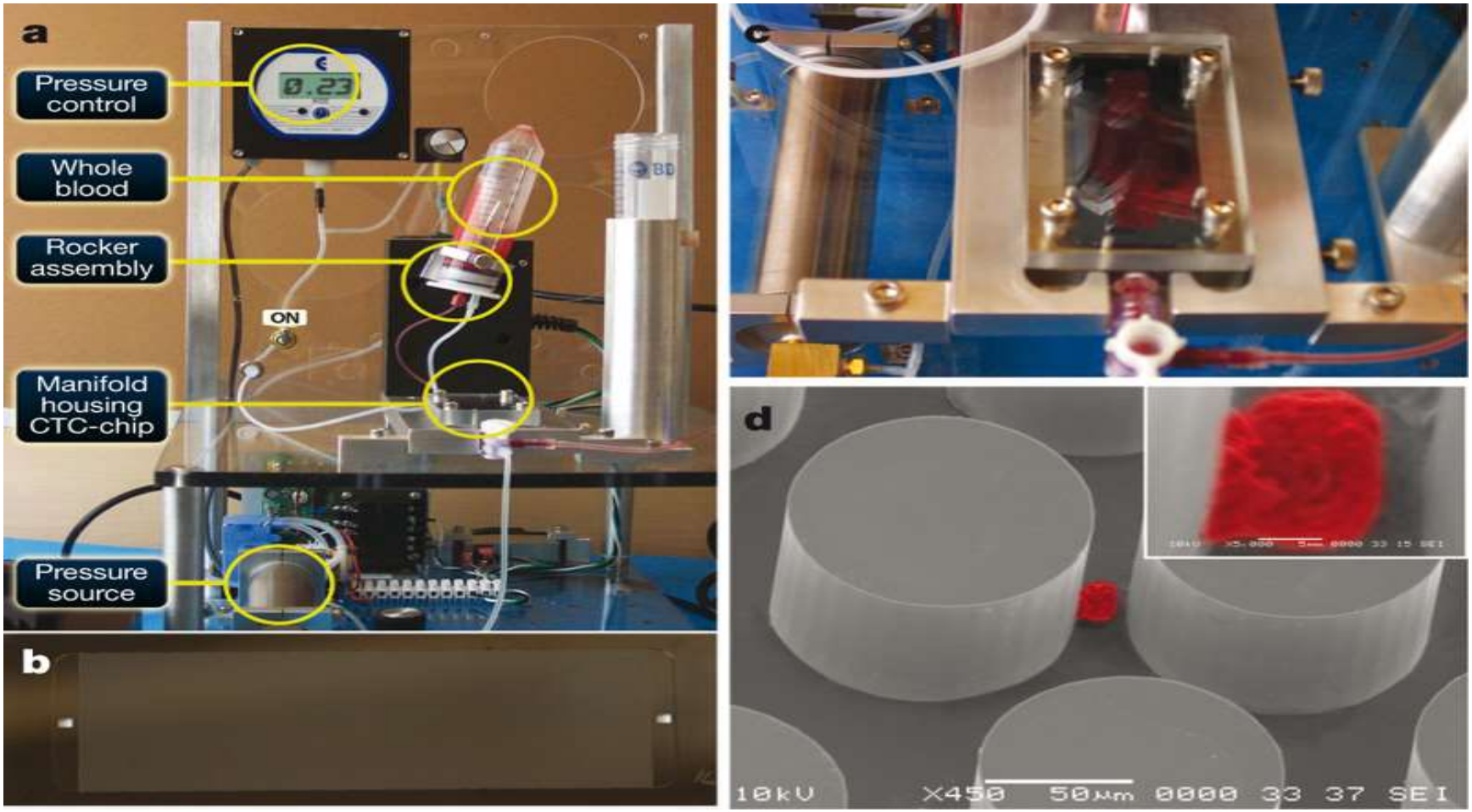


ScreenCell® Devices for cytology and Cell Culture



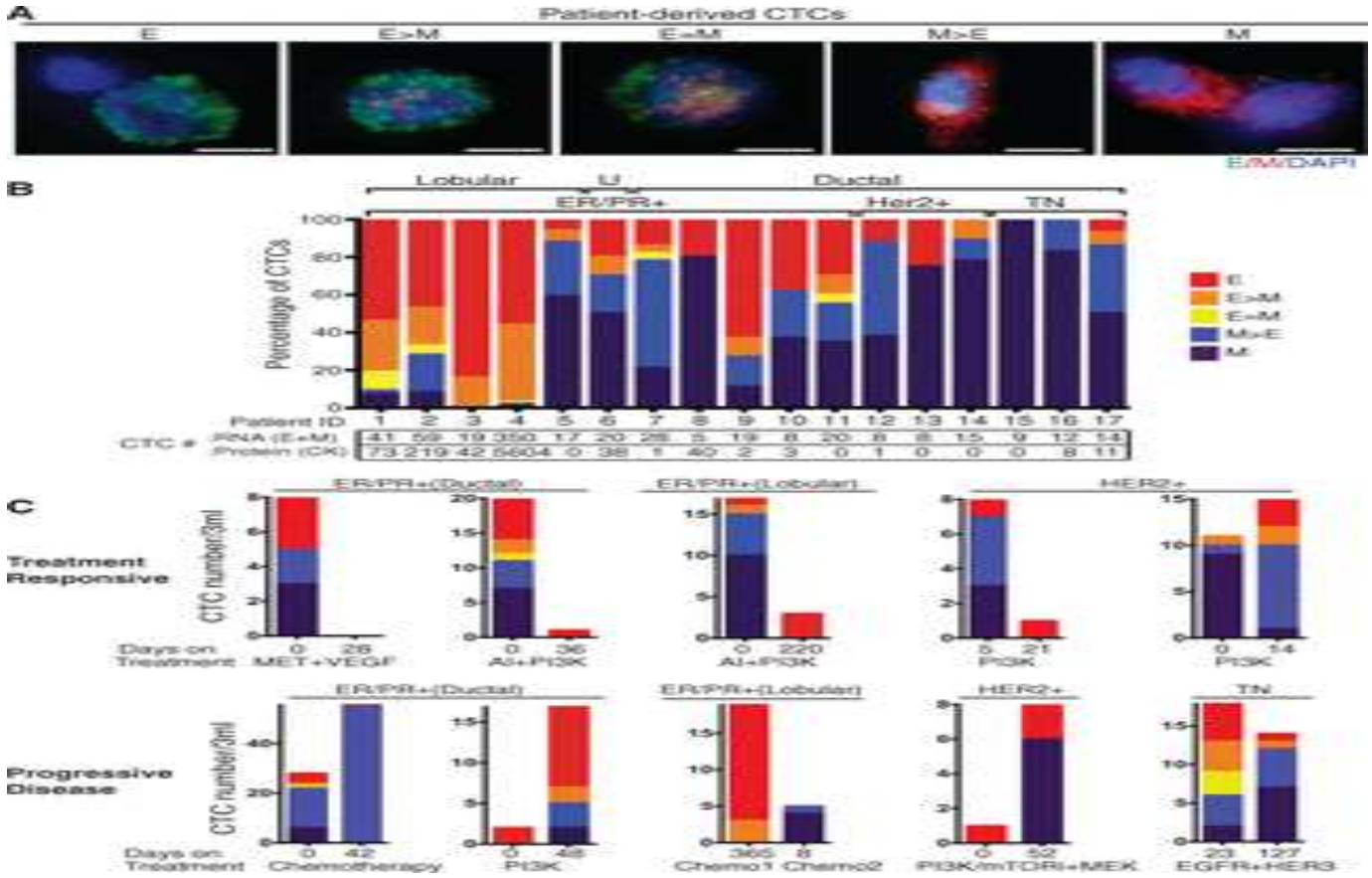
Copyright ScreenCell ©

Isolation of rare circulating tumour cells in cancer patients by microchip technology



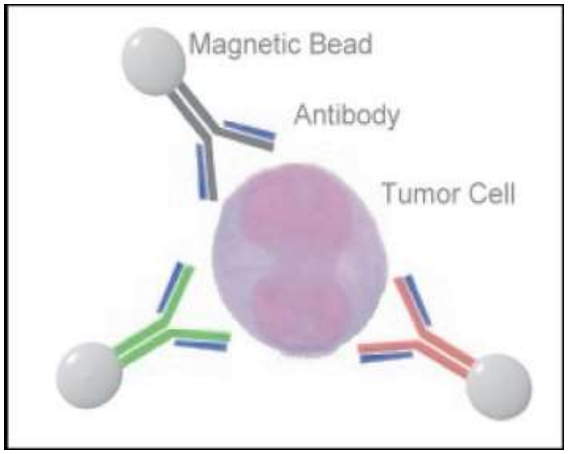
Nagrath S. et al, Nature 450(7173):1235-9, 2007

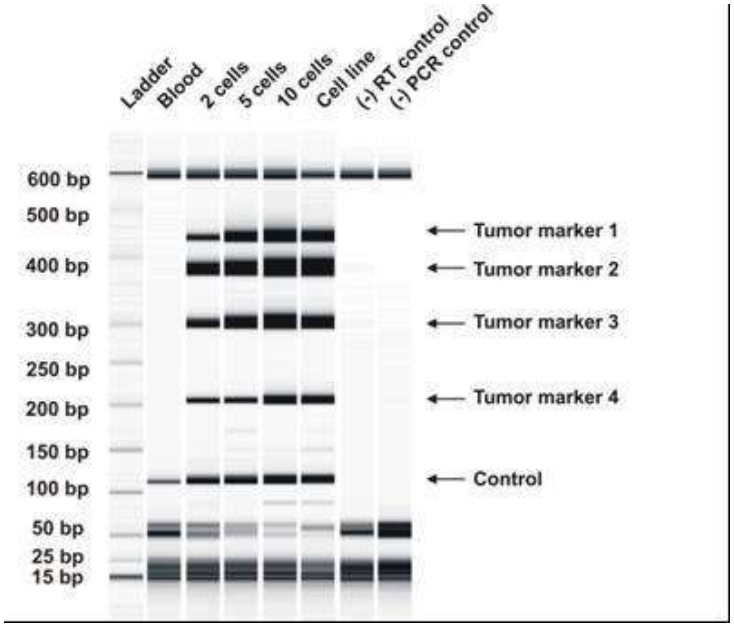
Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition



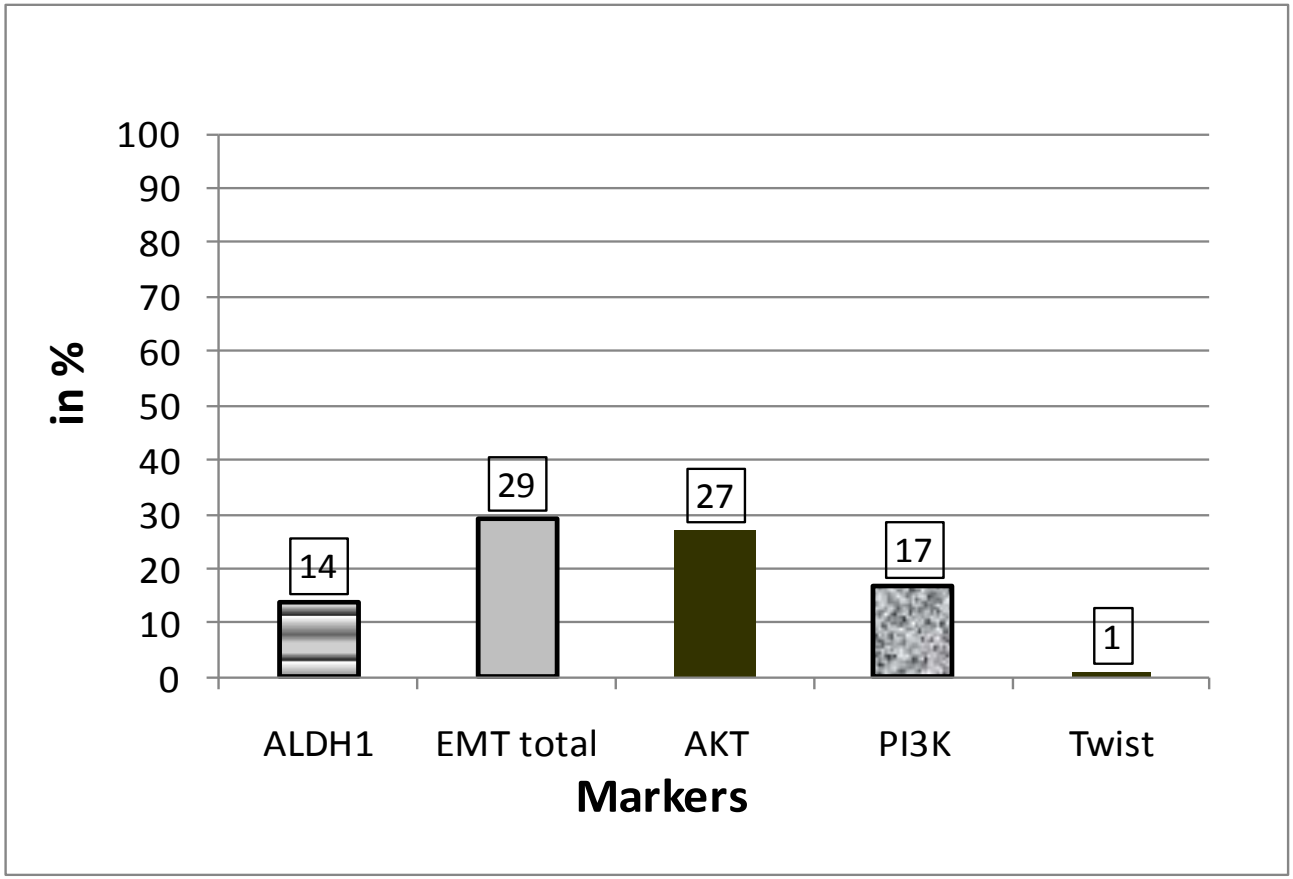
Yu M, Science, *339 ; 6119:580-584, 2013*

AdnaBreast DetectTest





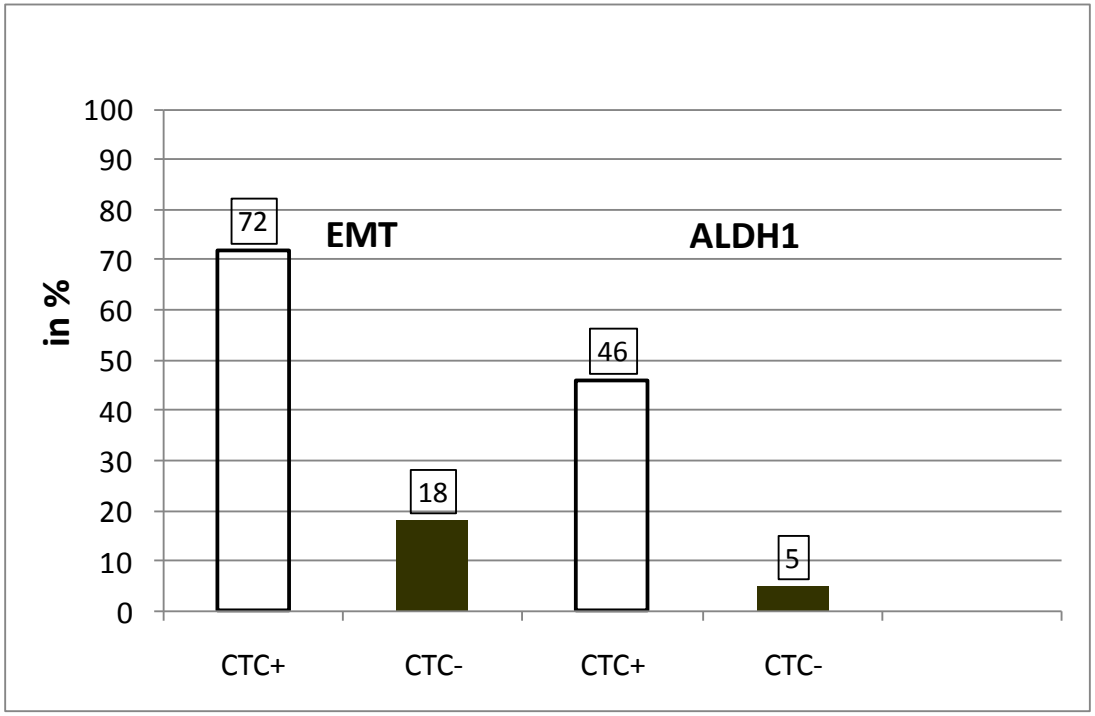
Expression of EMT-markers and ALDH1.



At least one of the EMT markers was expressed in 29% and ALDH1 was present in 14% of the samples, respectively.

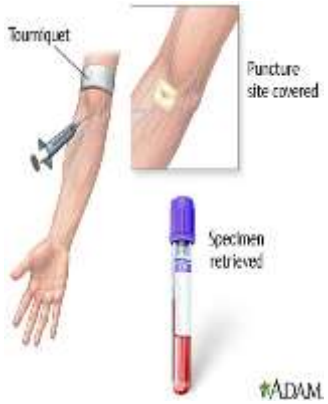
Kasimir-Bauer S, Hoffmann O, Wallwiener D, Kimmig R, Fehm T. Breast Cancer Res. 2012 Jan 20;14(1):R15.

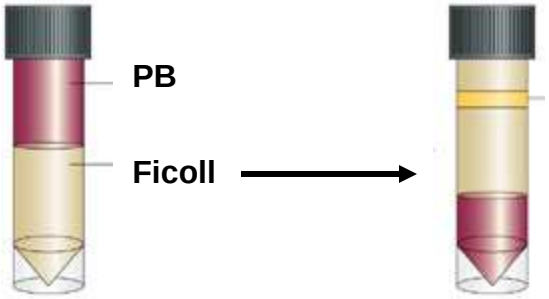
Expression of EMT-markers and ALDH1 in CTC+ and CTC- patients



In the CTC+ group, 66 of 92 patients (72%) were positive for at least one of the EMT markers and 42 of 92 patients (46%) were positive for ALDH1, respectively. In the CTC- group, the percentages were 18% (63 of 354 patients) and 5% (19/353 patients).

Detection of CTC with EMT





PBMC

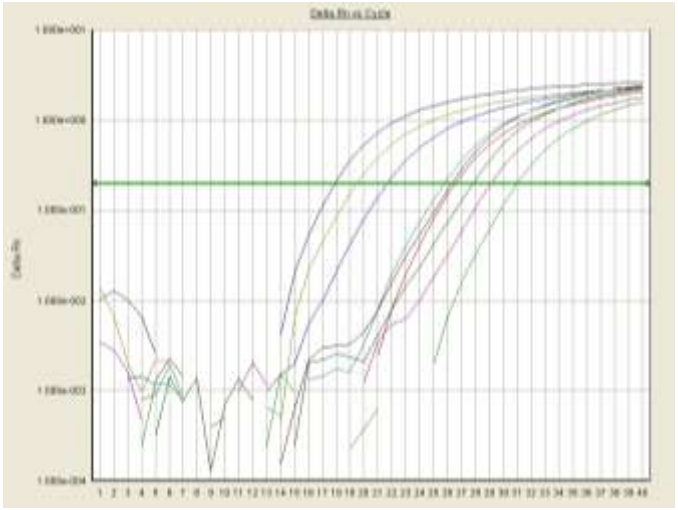
CD326/CD45 selection

unselected

CD326-
CD45

CD326-
CD45 -

Quantitative RT-PCR dissociation curve



RNA isolation, reverse transcription, quantitative RT-PCR for EMT related genes **(TWIST,SNAIL,SLUG, FOXC2, ZEB2)** and EpCAM

Lab Study 2008-0079

To assess CTCs, EMT-CTCs and circulating cancer stem cells in HER2^{+} breast cancer patients to identify potential targets

Characteristics	N	%
Overall	30	100
HER2+	28	93.3
HER2-	2	6.7
ER/PR+	17	56.7
ER/PR-	13	43.3
1st line	10	33.3
≥ 2nd line	20	66.7
>30x10^{6} PBMCs	17	56.7
<30x10^{6} PBMCs	13	43.3
CTCs <5 (CTC 0)	19 (13)	63.3 (43.3)
CTCs ≥5	4	13.4
CellSearch not performed	7	23.3

Giordano A et al, Mol Cancer Ther. 2012 Sep 12

Cell Fraction Analyses

EpCAM+ CTCs

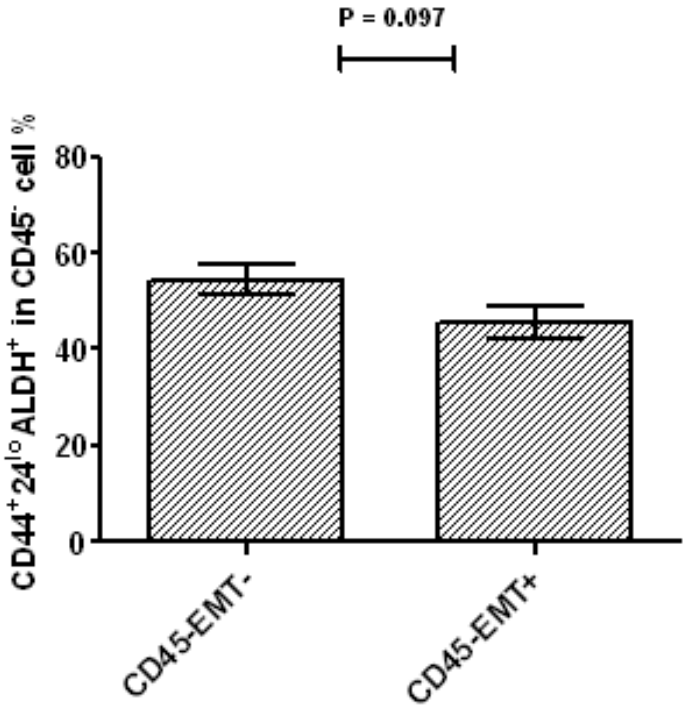
CD45+ WBC

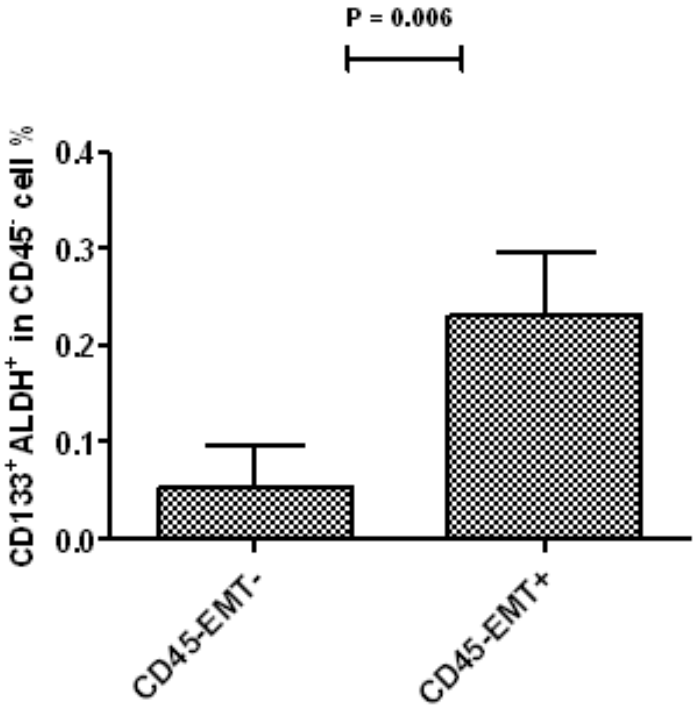
CD326-/CD45- EMT-CTCs

- EMT-Transcription factors (EMT-TF) Gene expression analysis by RT–qPCR (Mego, Reuben 2011)
  - TWIST1, SNAIL1, Slug, ZEB1, FOXC2, TG2 (GAPDH)

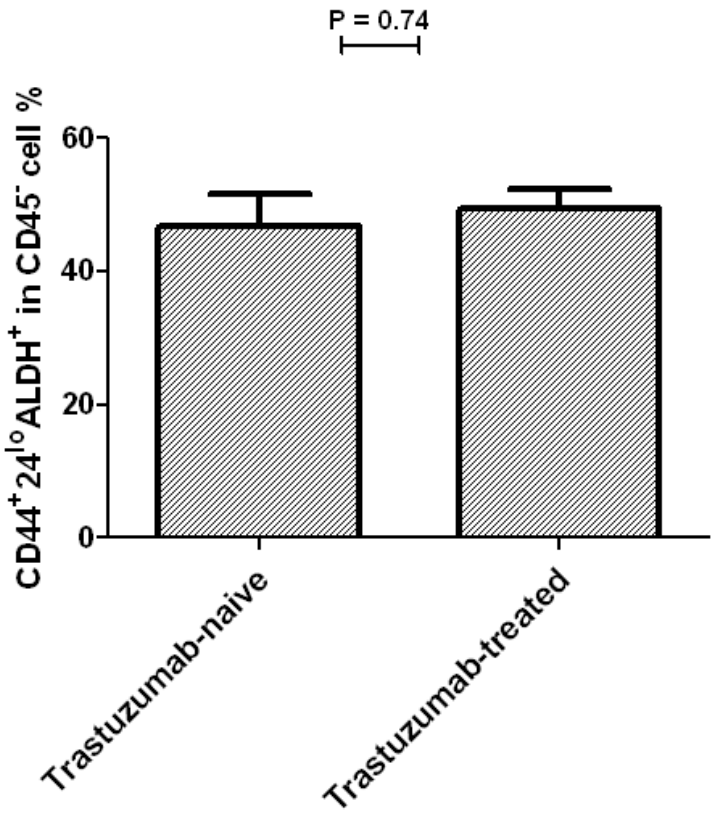
- Stem cell markers by FACS (Reuben et al., 2011)
  - ALDH activity (Aldefluor)
  - CD44, CD24, CD133
  - CD326, CD45

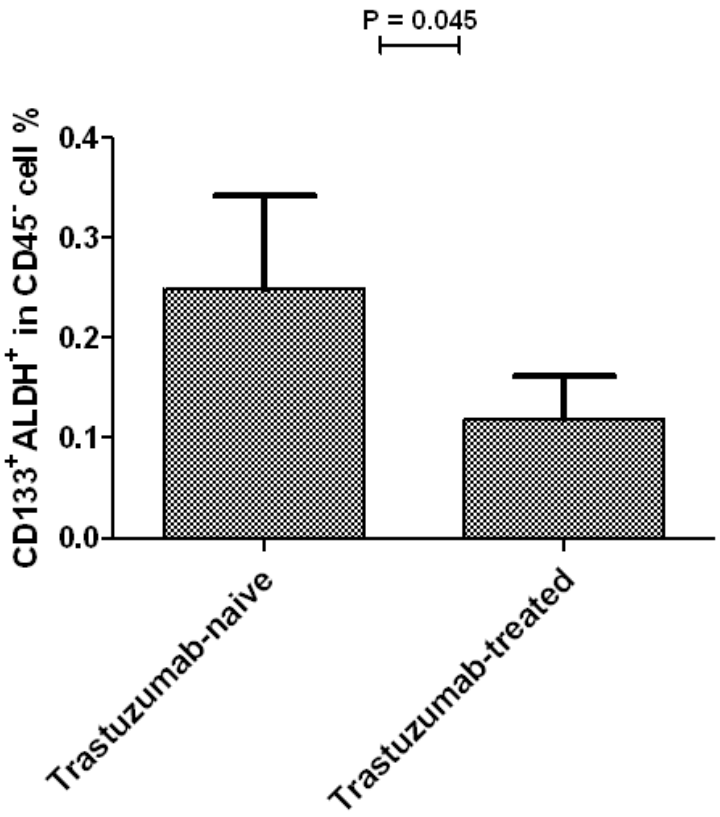
CD45^{-} EMT-CTCs are ALDH^{+} CD133^{+} CSCs and Not ALDH^{+} CD44^{+}CD24^{-} CSCs



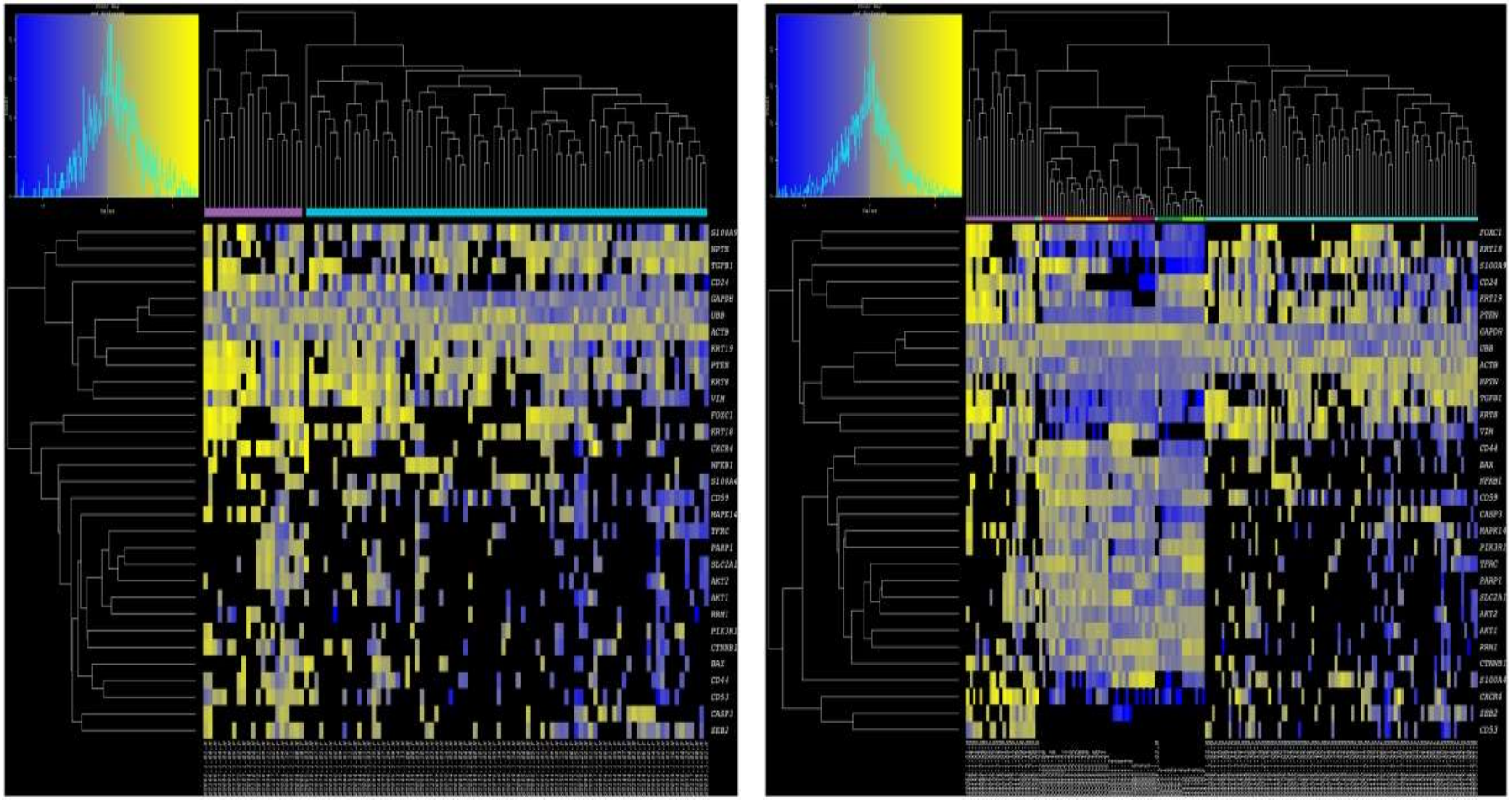


Treatment Naïve HER2+ patients have more ALDH+ CD133^{+} CSC than CD44^{+} CD24^{-} CSCs



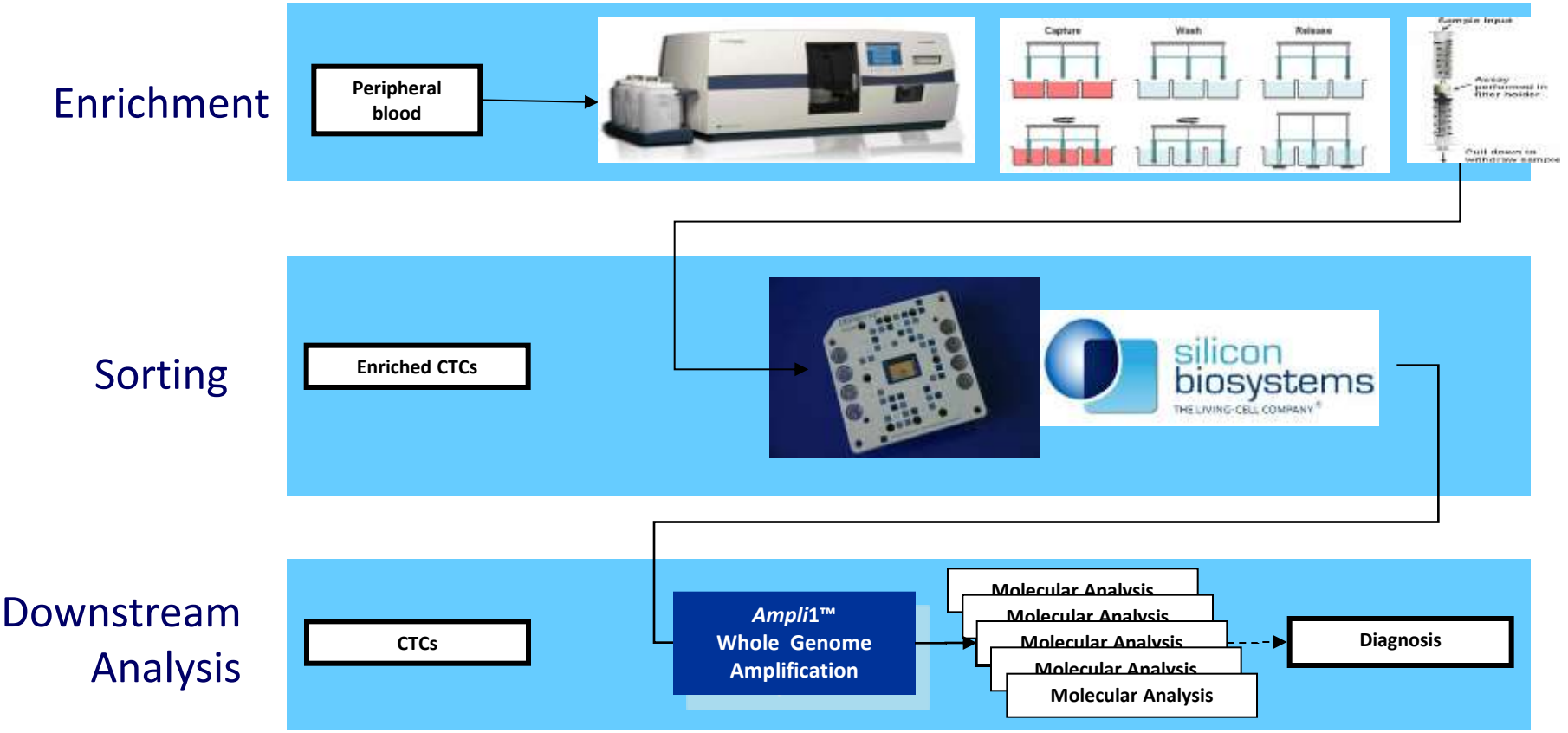


Single cell profiling of circulating tumor cells: transcriptional heterogeneity and diversity from breast cancer cell lines



Powell AA, PLoS One. 2012;7(5): e33788

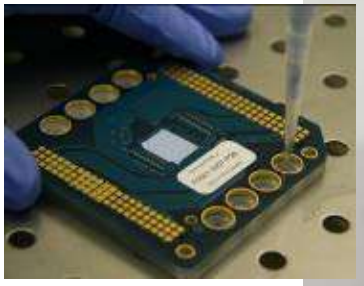
DEPArray Delivers Pure Single Tumor Cells from CellSearch™ or Another Sample Enrichment



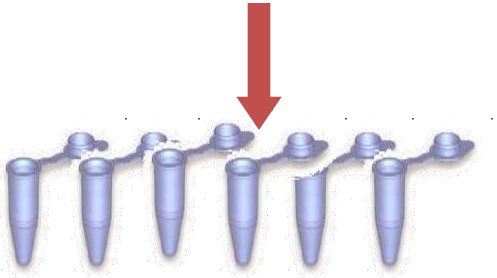
DEPArray- Samples Processing Flow

Cartridge

The sample is extracted from the cartridge and washed twice in SB manipulation buffer



The sample is loaded into DEPArray™



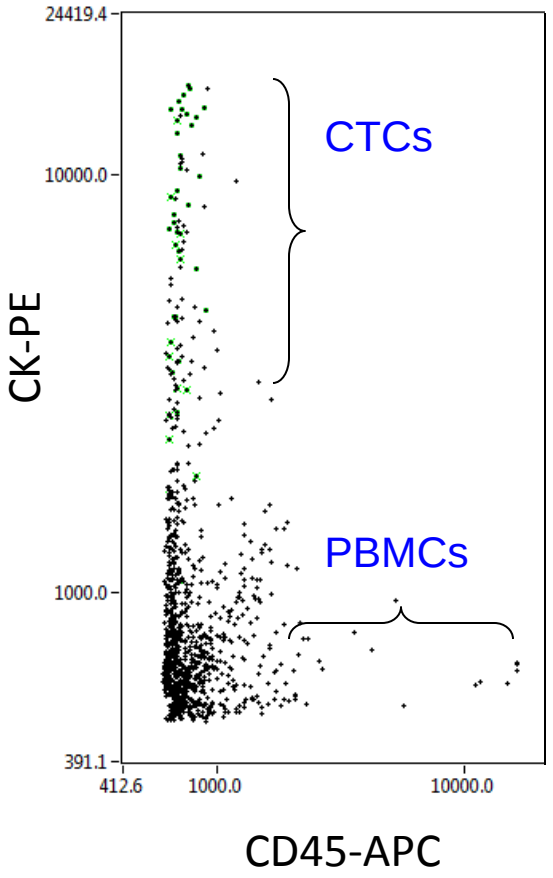
Singles CTCs are recovered in different tubes

Ampli1™ Whole Genome Amplification

Downstream Molecular Analysis

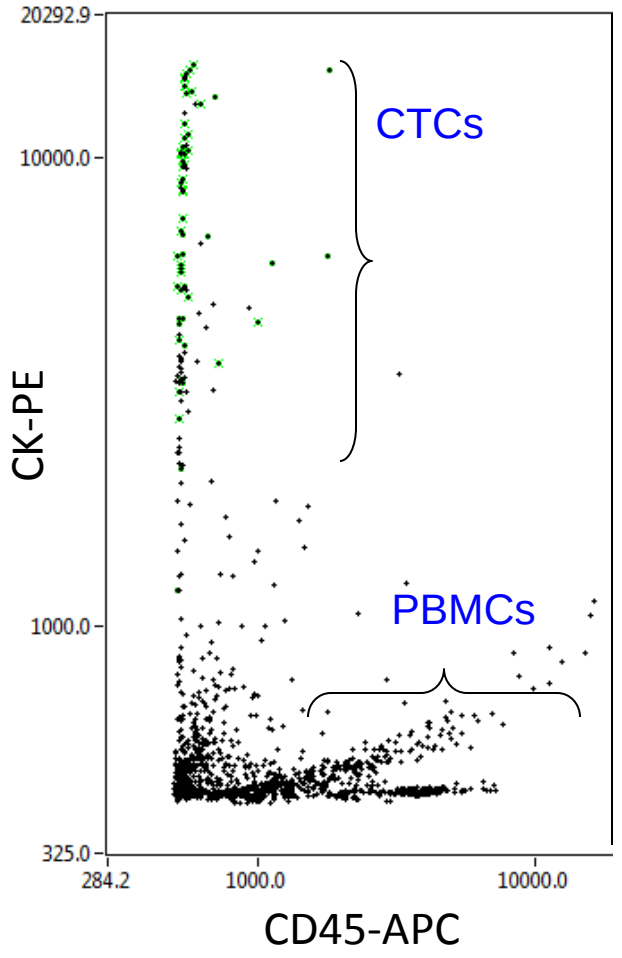
Fluorescently-Labeled CR-009 Cells on DEPArray Scatter Plot

- Sample was enriched for CTCs using the Veridex Cell Search™ system.
- Sample was found to have 432 CTCs:
  251 Her2/Neu + (59%)
  181 Her2/Neu – (41%)
- Her2/Neu positive and negative cells were recovered using the DEPArray and are being used for genetic analysis.
- PBMCs were recovered using the DEPArray and will be used as controls for mutational studies.



Fluorescently-Labeled KMO-015 Cells on DEPArray Scatter Plot

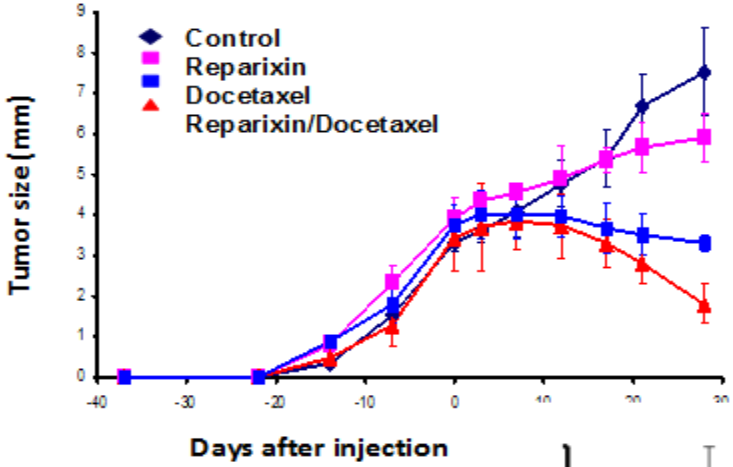
- CTCs in peripheral blood were enriched using the Veridex CellSearch™ system.
- Sample was found to have 175 CTCs:
  50 Her2/Neu +
  125 Her2/Neu –
- Her2/Neu positive and negative cells were recovered using the DEPArray and are being used for genetic analysis.
- PBMCs were recovered using the DEPArray and will be used as controls for mutational studies.

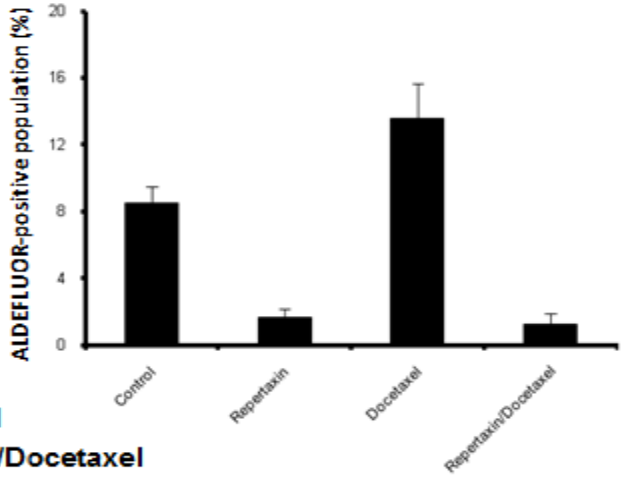


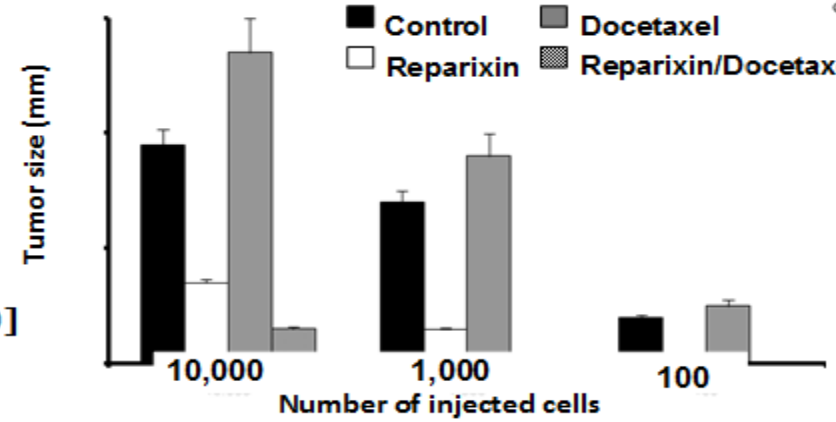
CTC and CSCs-directed therapies

CXCR1 and Cancer Stem Cells

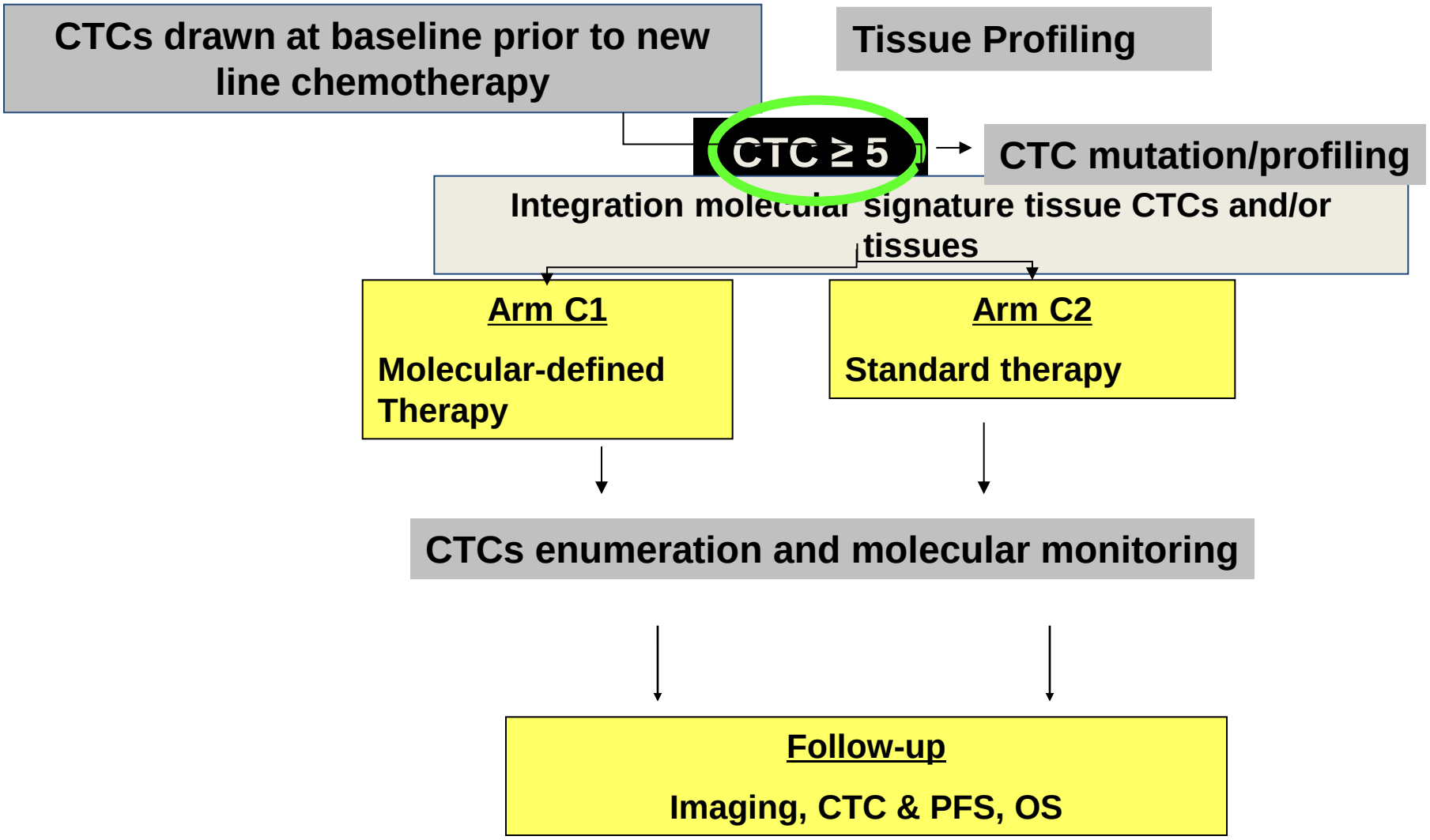
Reparixin Targets CSCs







[Ginestier, JCI 2010]


CTCs drawn at baseline prior to new line chemotherapy
Tissue Profiling
CTC ≥ 5
CTC mutation/profiling
Integration molecular signature tissue CTCs and/or tissues
Arm C1
Molecular-defined Therapy
Arm C2
Standard therapy
CTCs enumeration and molecular monitoring
Follow-up
Imaging, CTC & PFS, OS

Blood-based monitoring in advanced malignancies

- CTCs

  - Prognostic and predictive value of enumeration (breast, prostate, colon)
  - Biomarkers expression (ER, PR, HER-2)/Genomics
  - Heterogeneity and EMT
  - Treatment resistance and stem cells
  - **Detection dependent on enrichment methods**

- ctDNA

  - identification of specific tumor-related mutations (e.g. TP53, PI3KCA, KRAS)
  - Monitoring of tumor load (response)
  - **Detected in all patients**

Conclusions
The Liquid Phase of Solid Tumors

- What to detect ?
  - **CTCs**, cfDNA and **ctDNA**
  - **Prognostic and predictive value**
  - **Tumor heterogeneity and single mutations**
- How to measure ?
  - CellSearch™, Microfludics, label-free (filtration systems)
  - DNA extraction from plasma followed by sequencing (e.g. digital PCR confirmed by Sanger sequencing; Safe-SeqS)
- When to measure ?
  - Baseline and monitoring in advanced solid tumors
- Why to use it ?
  - *real time* monitoring of advanced disease

Acknowledgments

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