National Cancer Institute, Slovakia

Translational Research Unit

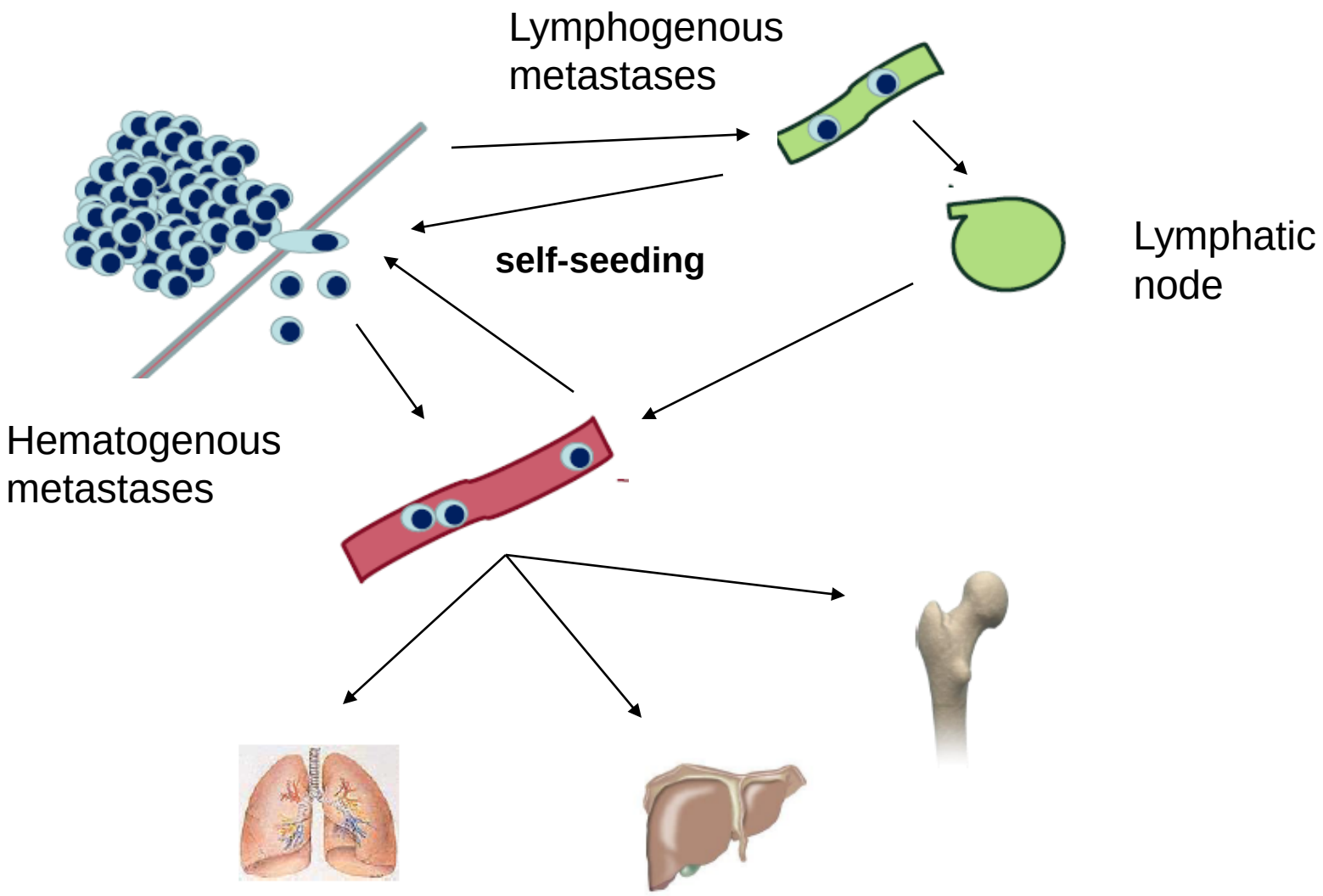
Circulating tumor cells as biomarker for hormonal treatment in breast and prostate cancer

Michal Mego

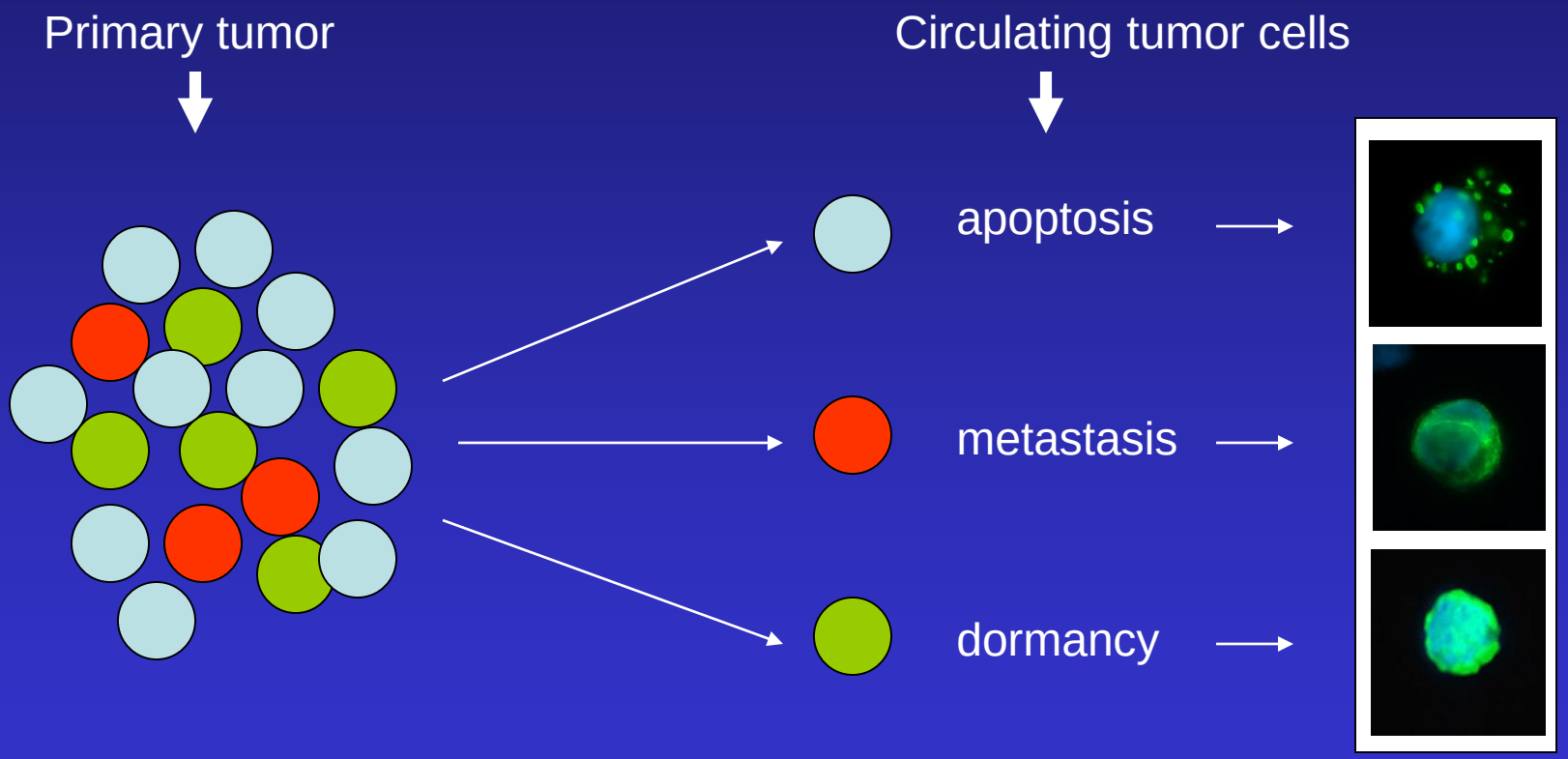
2nd Department of Oncology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Tarin D. Semin Cancer Biol. 2010

Metastatic cascade

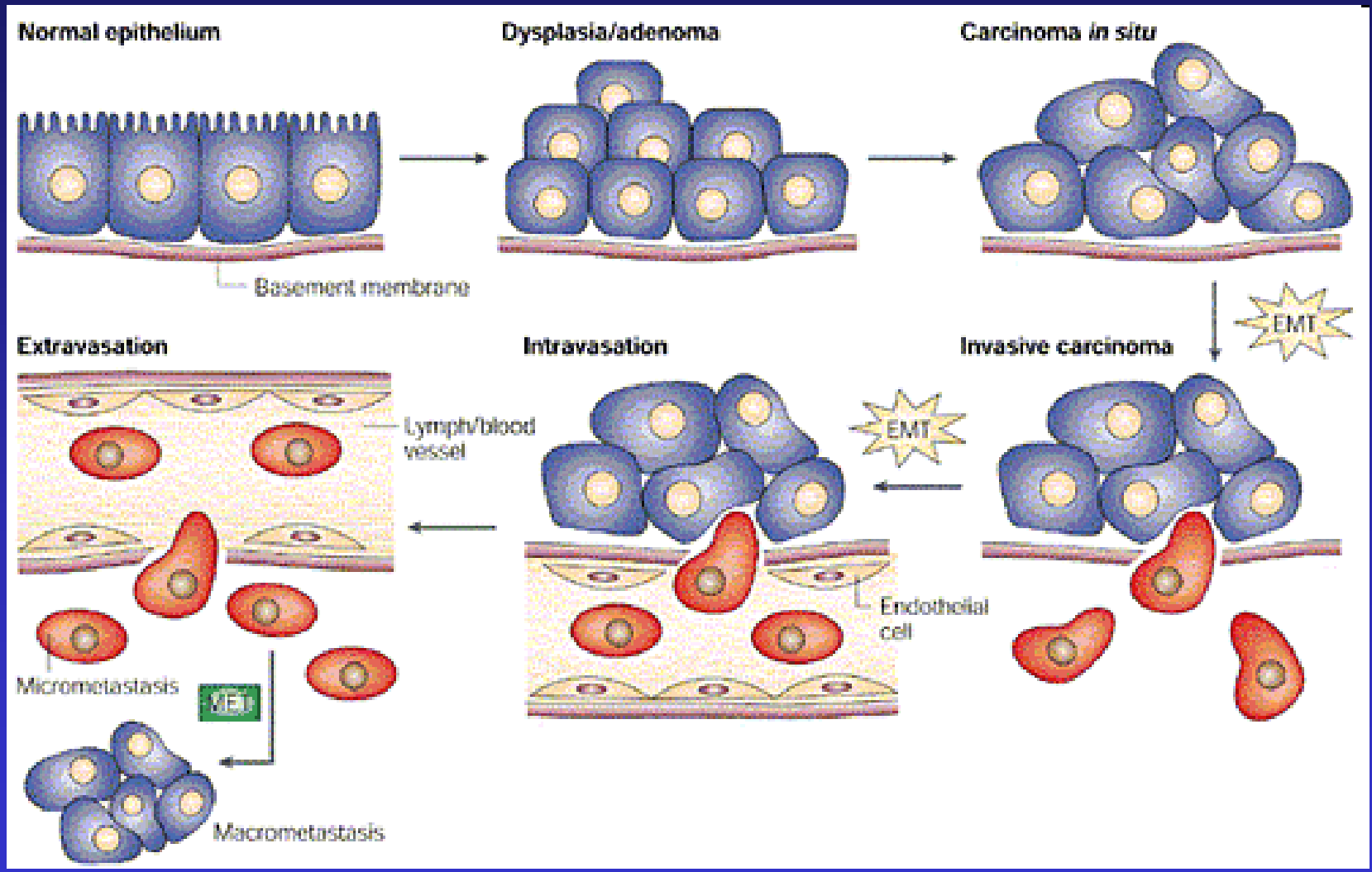


Circulating tumor cells



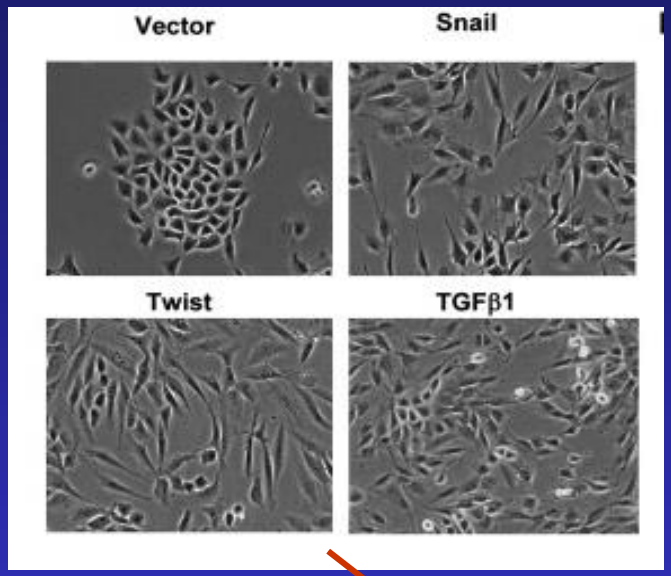
Fehm, Uhr, et al. Cytotherapy 2005

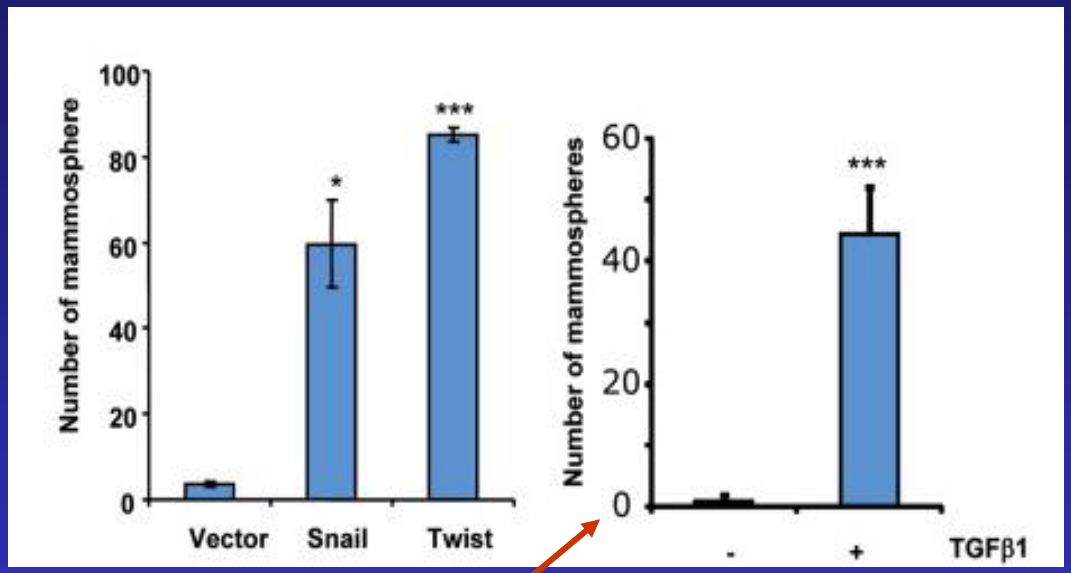
EMT and tumor dissemination

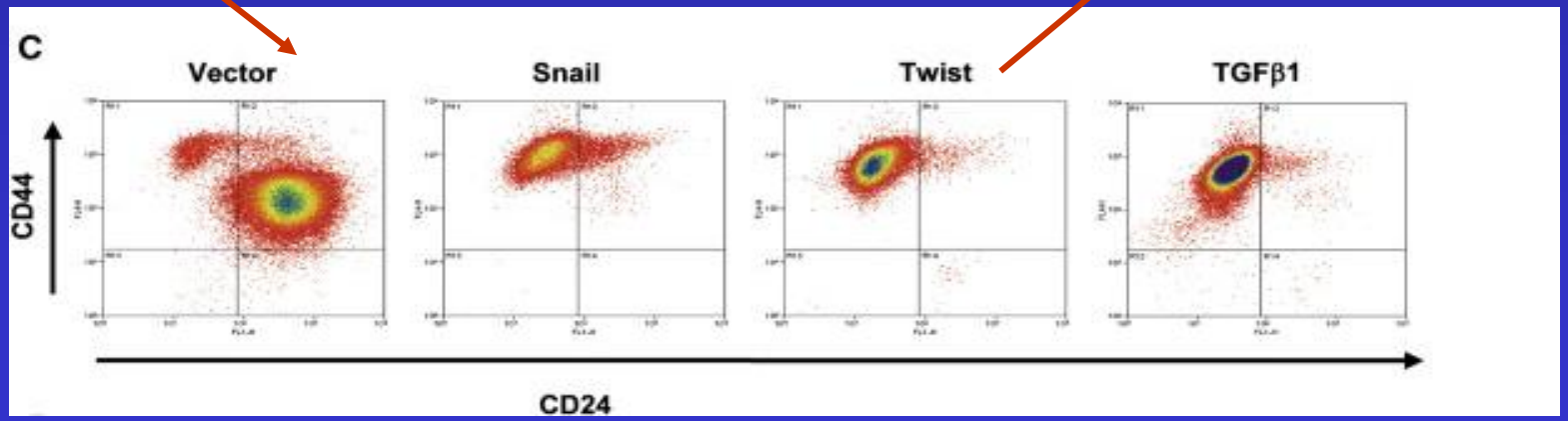


http://www.uchsc.edu/cdb/People/Faculty/images/PrekerisFigure3-rev.gif

EMT generates cells with stem cell like properties

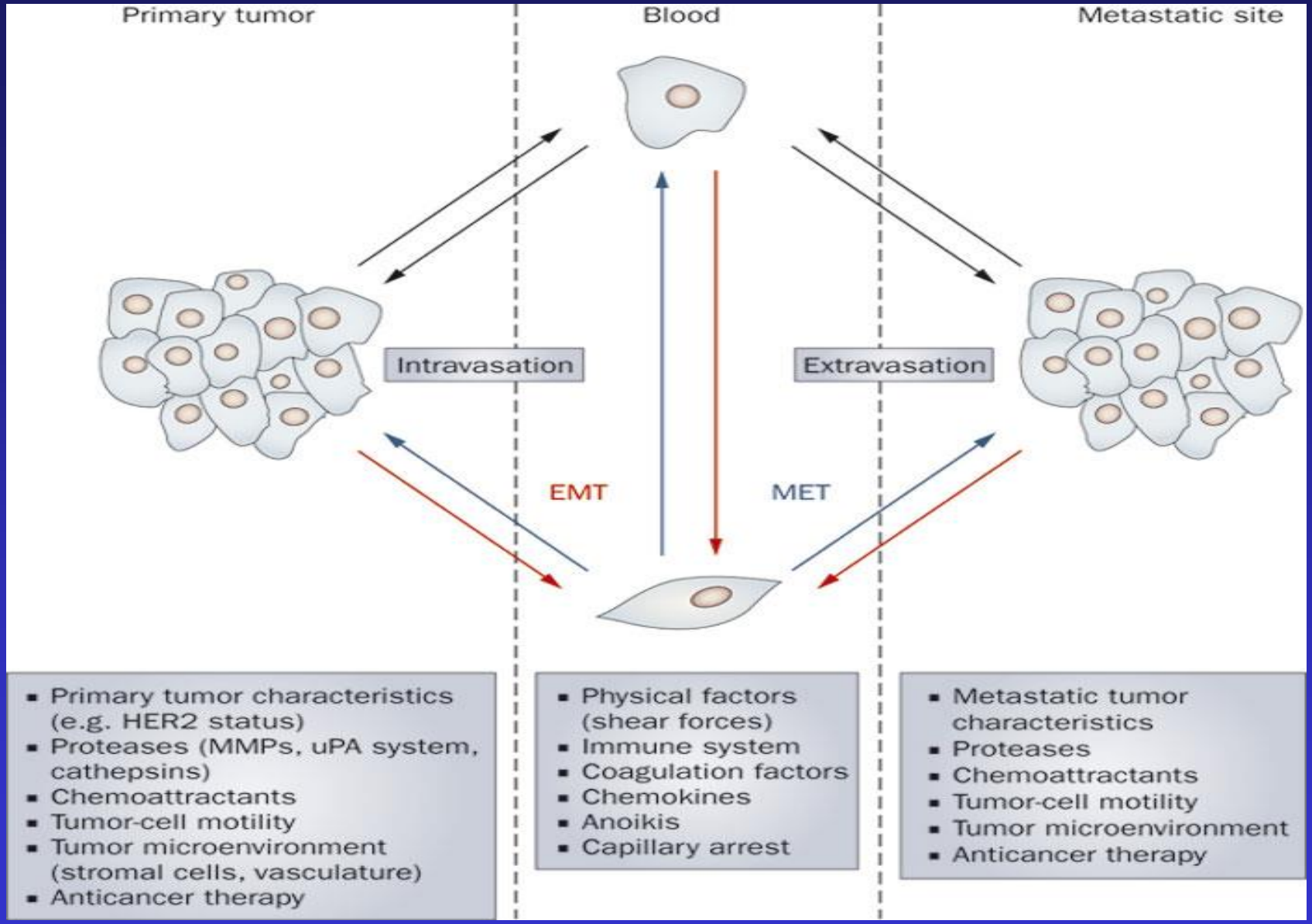






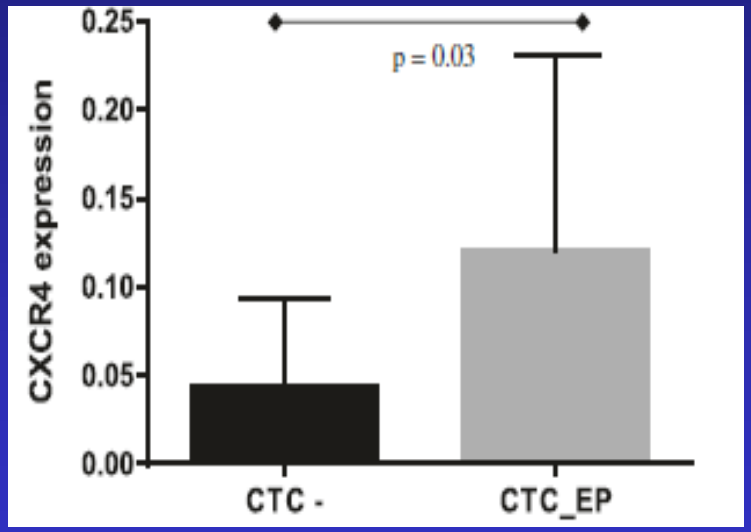
Mani et al, Cell, 2008

Factors affecting CTCs

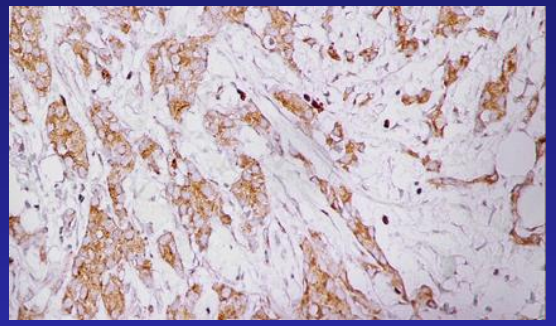


Mego M *et al. Nat Rev Clin Oncol, 2010*

SDF-1/CXCR4 axis is involved in CTC migration



CXCR4 is overexpressed in epithelial CTC*



Circulating Tumor Cells	Variables	*P* Value
CTC EP*	Positive SOCS1 expression	<.001
CTC EMT†	Grade 3	.036
	Positive APC expression	.063
CTC any‡	Positive CXCL12 expression	.025
	Positive SOCS1 expression	<.001

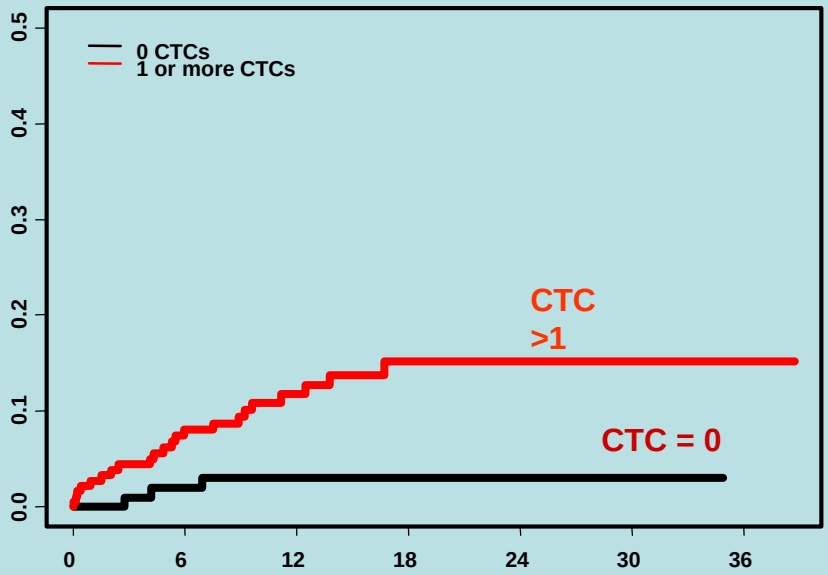
SDF-1 (CXL12) in primary tumor correlated with CTC in peripheral blood **

* Mego et al., BMC Cancer, 2016

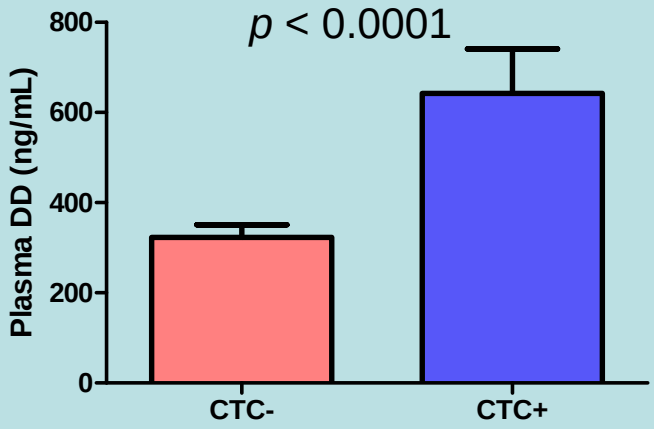
** Smolkova, Mego et al., Trans Oncol, 2016

CTC are associated with activation of coagulation in breast cancer patients

Risk of venous thromboembolism (VTE)



Circulating tumor cells and plasma d-dimer (DD)

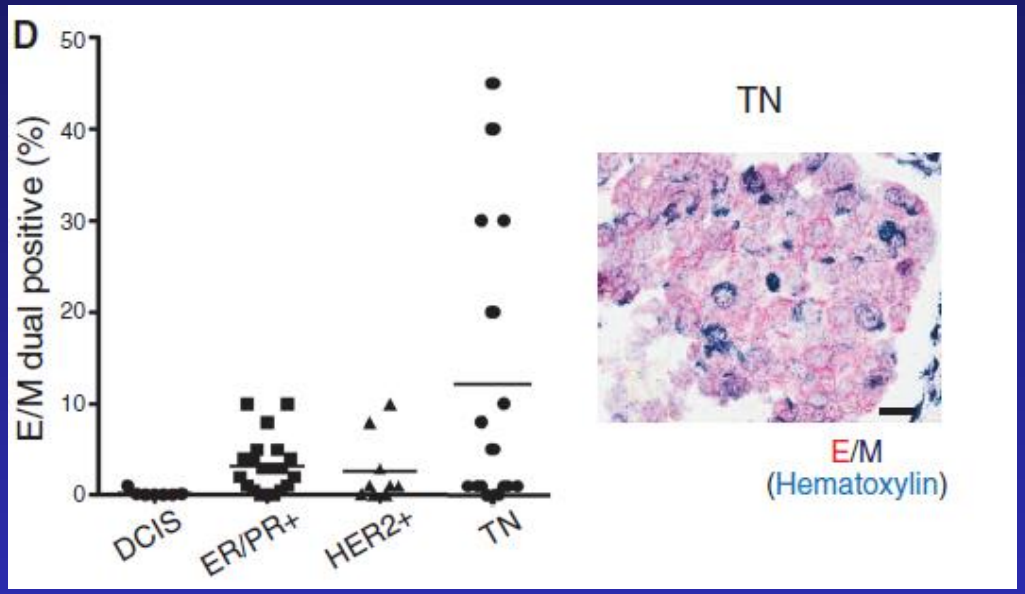


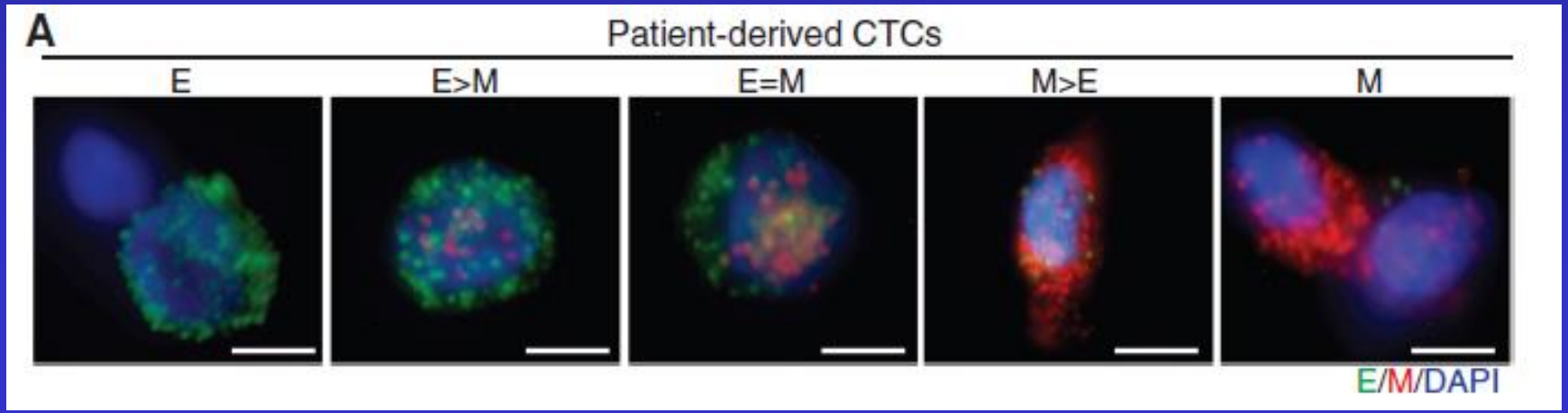
Risk of VTE:	**CTC+**		**CTC-**
	11.7%	vs.	3.0%*, p = 0.003
	9.0%	vs.	0%**

* Mego et al., Br J Cancer, 2009, Mego et al., Breast J, 2014

** Mego et al., Thromb Hemostasis, 2014

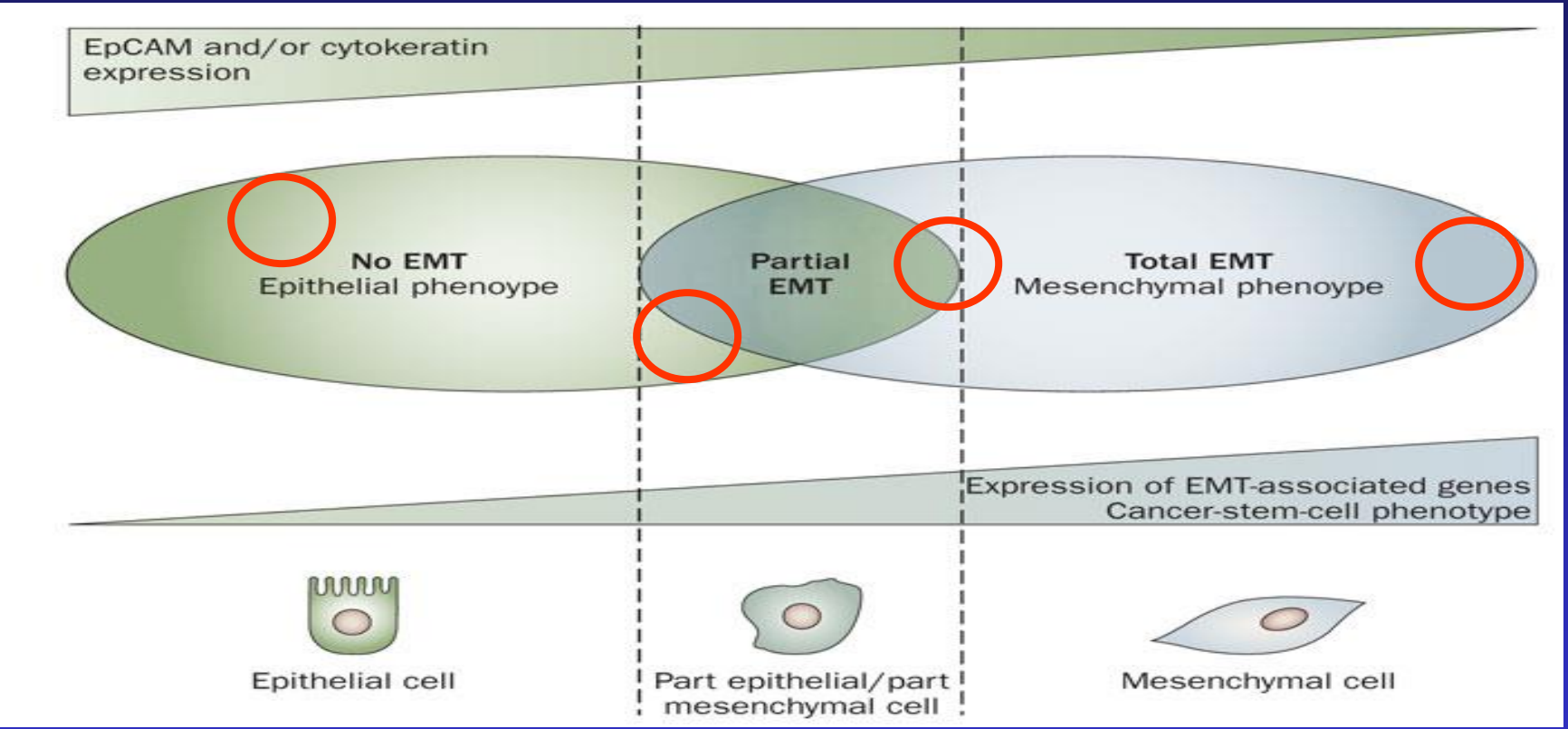
Phenotypic heterogeneity of CTC





Yu *et al. Science, 2013*

Phenotypic heterogeneity of CTC and its clinical relevance

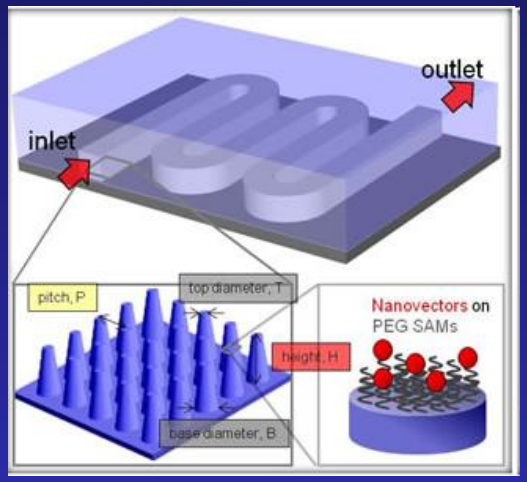


- Different methods detect variout subpopulations of CTC with different biological properties and different clinical significance
- The clinical significance of CTC always interpreted within the context of the detection method used

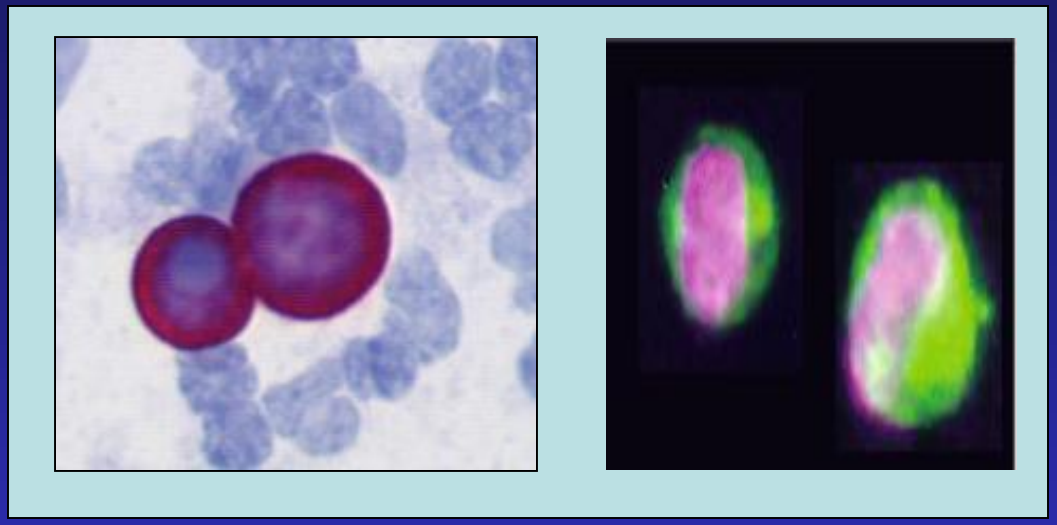
Mego M *et al. Nat Rev Clin Oncol, 2010*

Different method for CTC detection

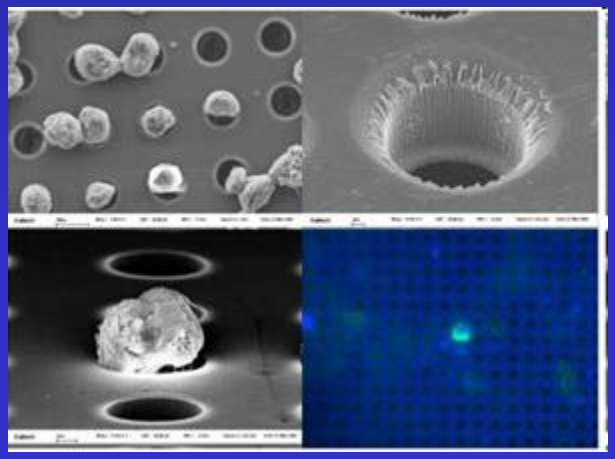
Microfluidic platforms



Imunophenotypisation

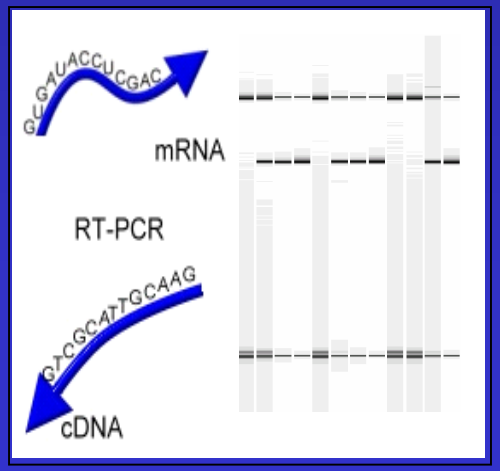


Filtration methods



Epispot assay

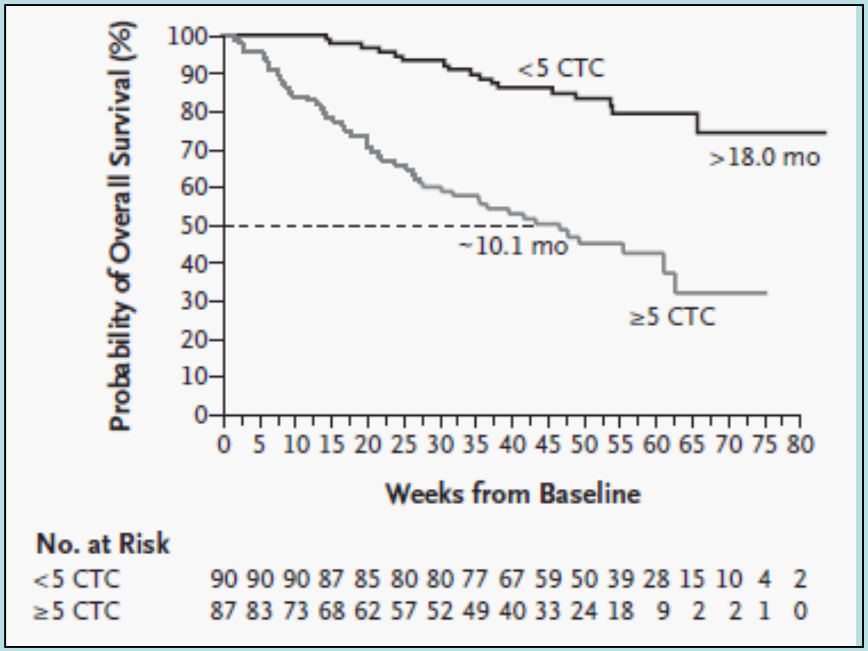
Molecular biology - PCR



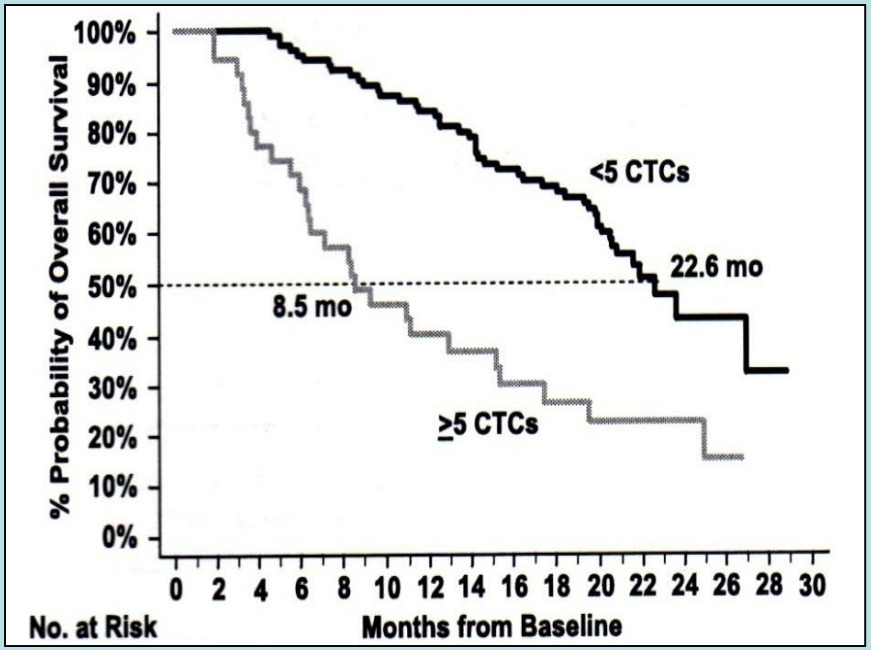
Clinical validity of CTC in breast and prostate cancer

CTC prognostic value in metastatic breast cancer

Baseline CTC

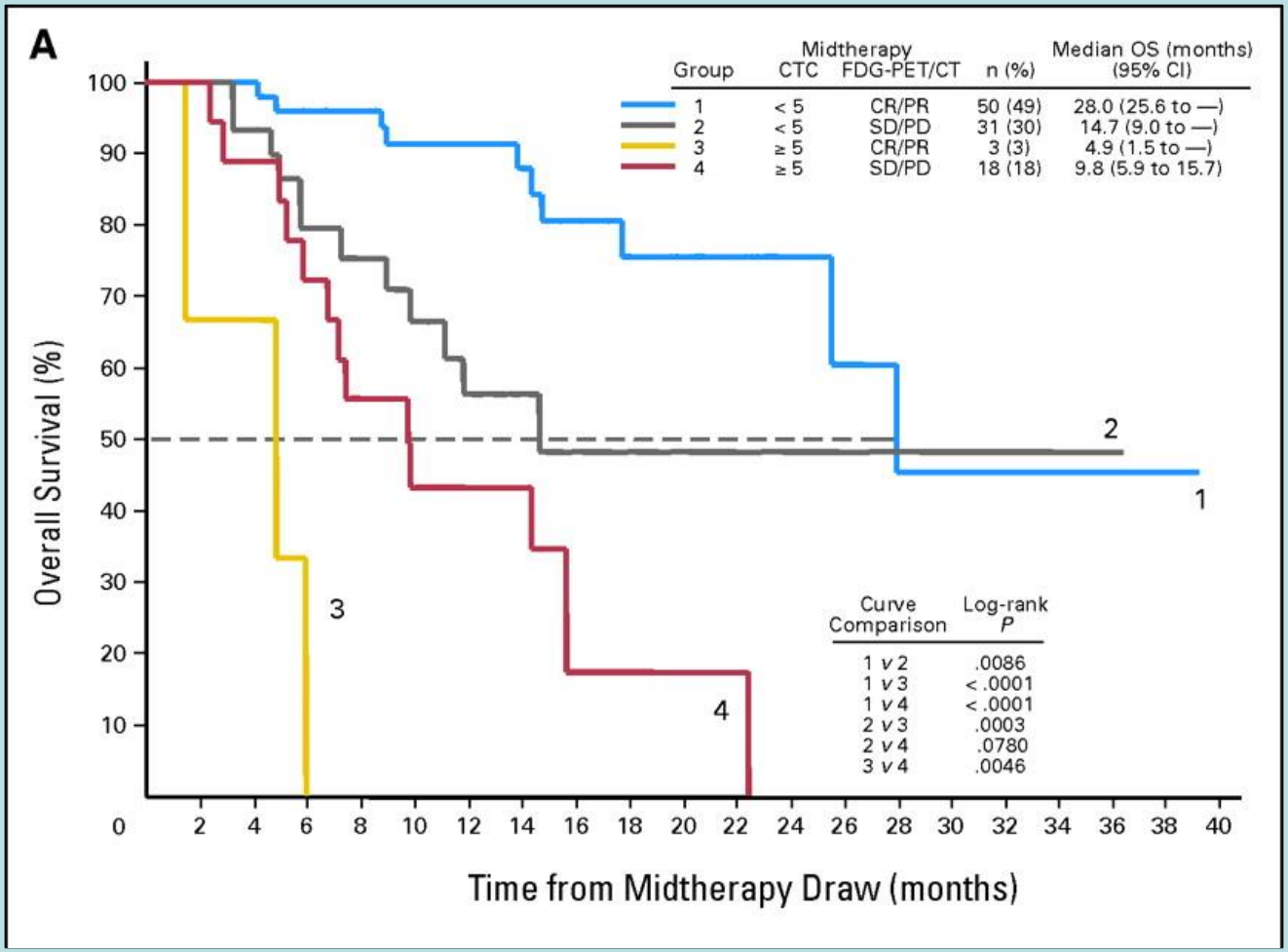


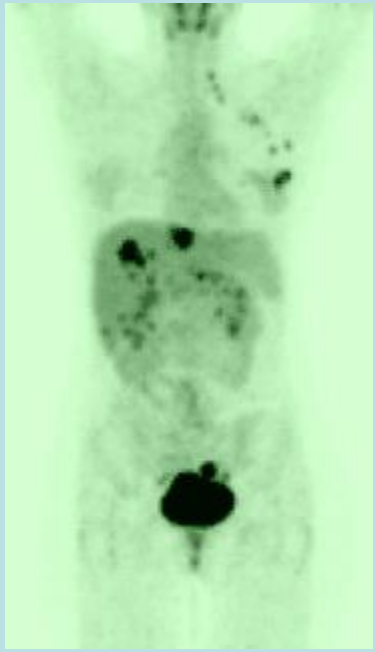
CTC at first follow-up



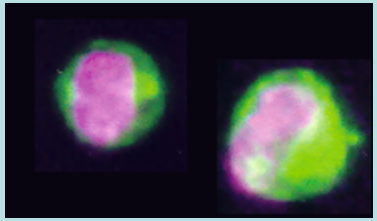
Cristofanilli et al., NEJM 2004
Budd et al. Clin Cancer Res 2006

Predictive value of CTC is superior to functional imaging by PET





vs.



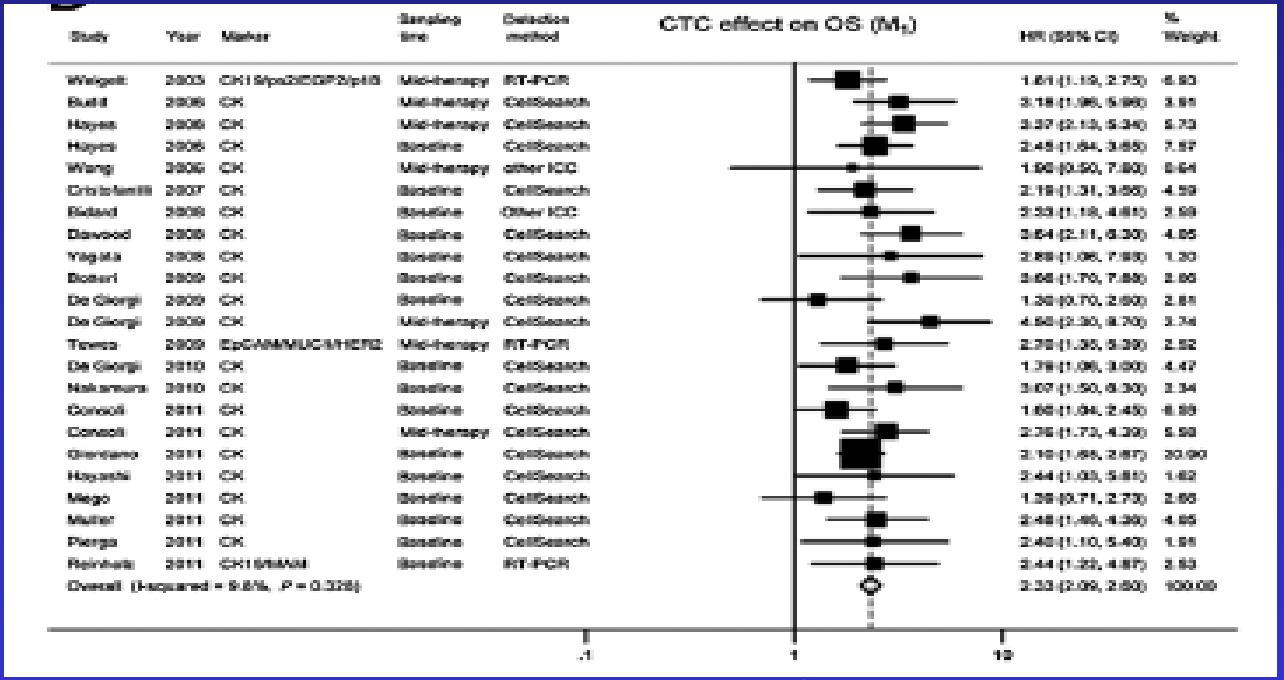
De Giorgi, U. et al. J Clin Oncol; 27:3303-3311, 2009

Meta-Analysis of the Prognostic Value of Circulating Tumor Cells in Breast Cancer

Liling Zhang[1], Sabine Riethdorf[2], Gang Wu[1], Tao Wang[1], Kunyu Yang[1], Gang Peng[1], Junli Liu[1], and Klaus Pantel[2]

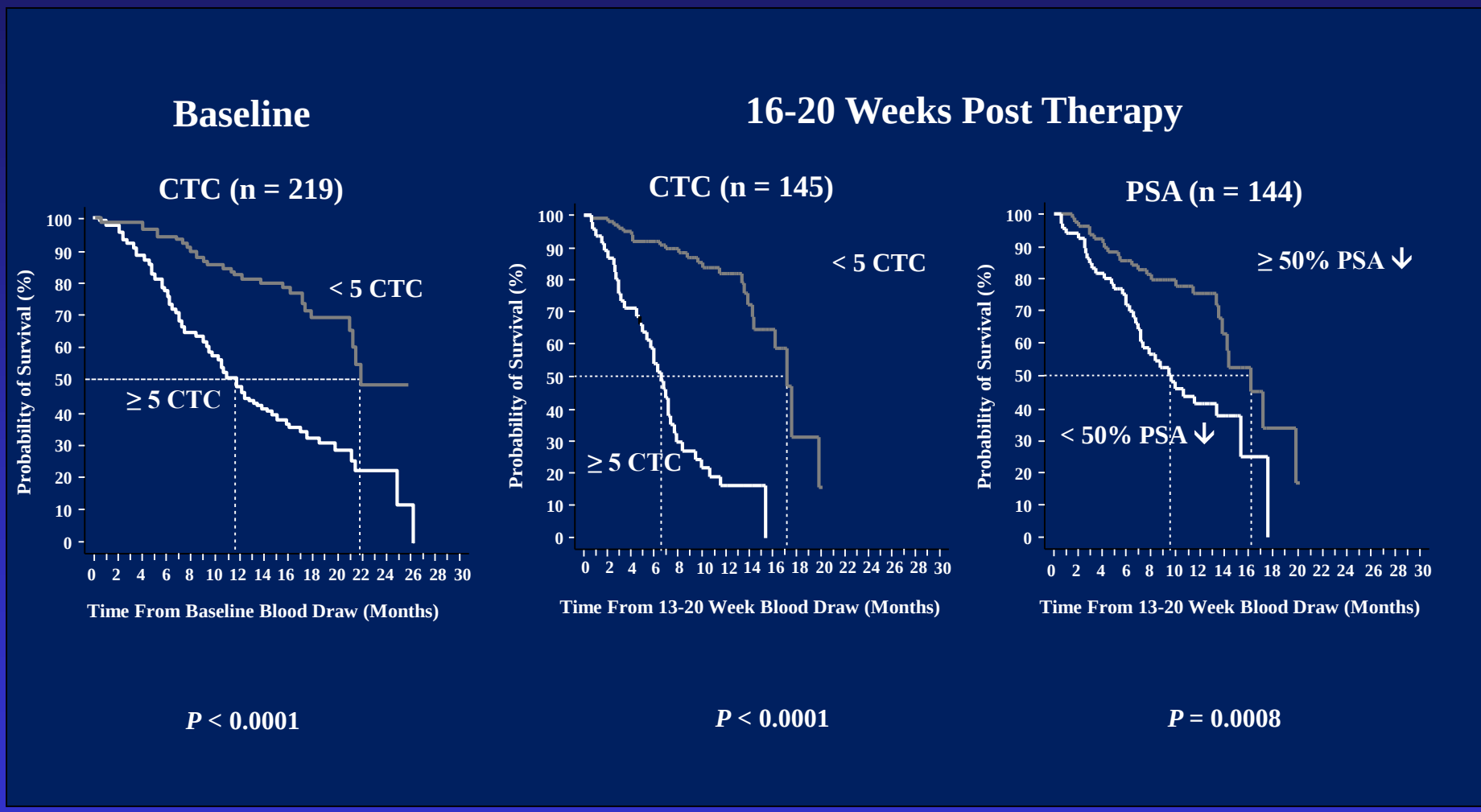
Clin Cancer Res; 18(20) October 15, 2012

Overall survival (OS): Hazard ratio = 2.33 95% CI (2.09 – 2.60)



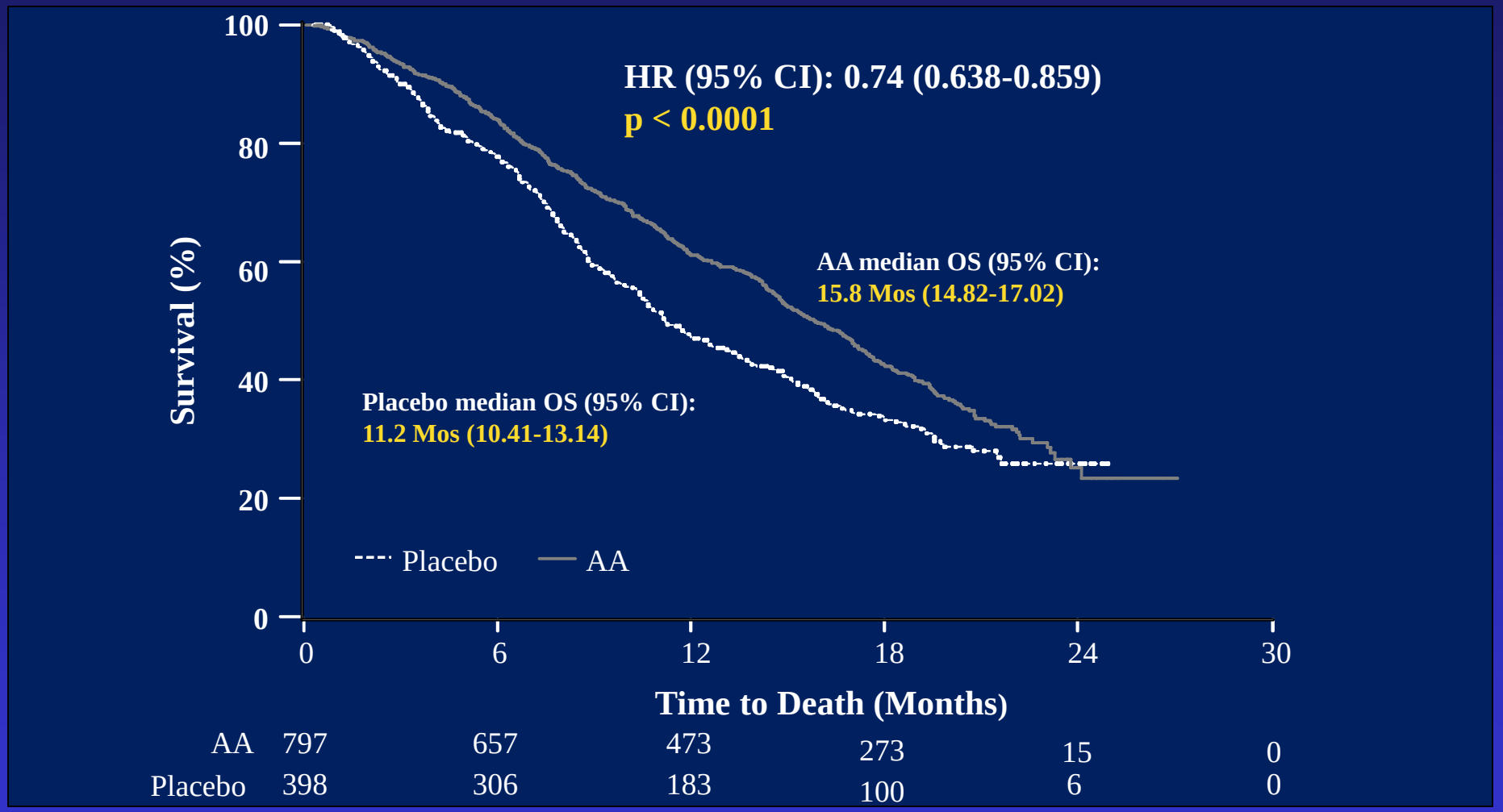
CTC + longer OS CTC + shorter OS

CTC are prognostic in metastatic castration-refractory prostate cancer



de Bono JS, et al. *Clin Cancer Res*. 2008
Scher H, et al. *Lancet Oncology*. 2009

COU-AA-301: AA (abiraterone) vs. Placebo in docetaxel pretreated patients



Scher H, ASCO, 2011

The Biomarker Panel Associated with Survival

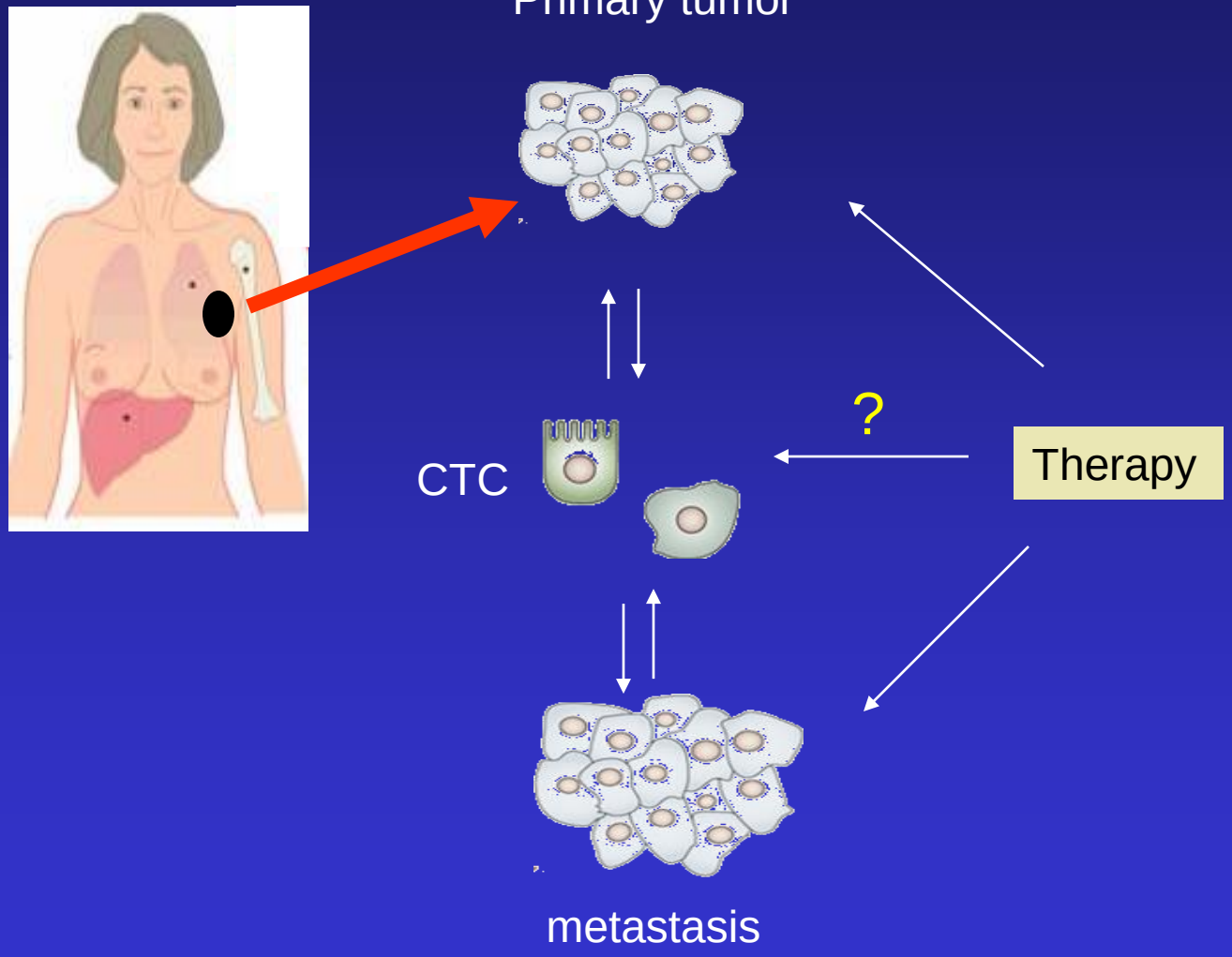
Baseline CTC ≥ 5		
	Week 12 (n = 321, CPE = 0.71 [SE = 0.014])	
Model Factors	HR (95% CI)	p Value
Treatment	1.030 (0.773, 1.372)	0.8371
LDH_FC	**1.247 (1.048, 1.483)**	**0.0127**
LDH_BL	3.044 (2.282, 4.056)	<0.0001
CTC Conversion	0.390 (0.289, 0.527)	<0.0001
CTC_BL	**1.143 (0.988, 1.323)**	**0.0729**

Low risk	CTC < 5 cells/7.5mL
Intermediate risk	CTC ≥ 5 cells/7.5mL; LDH < 250 U/L
High risk	CTC ≥ 5 cells/7.5mL; LDH > 250 U/L

Scher H, ASCO, 2011

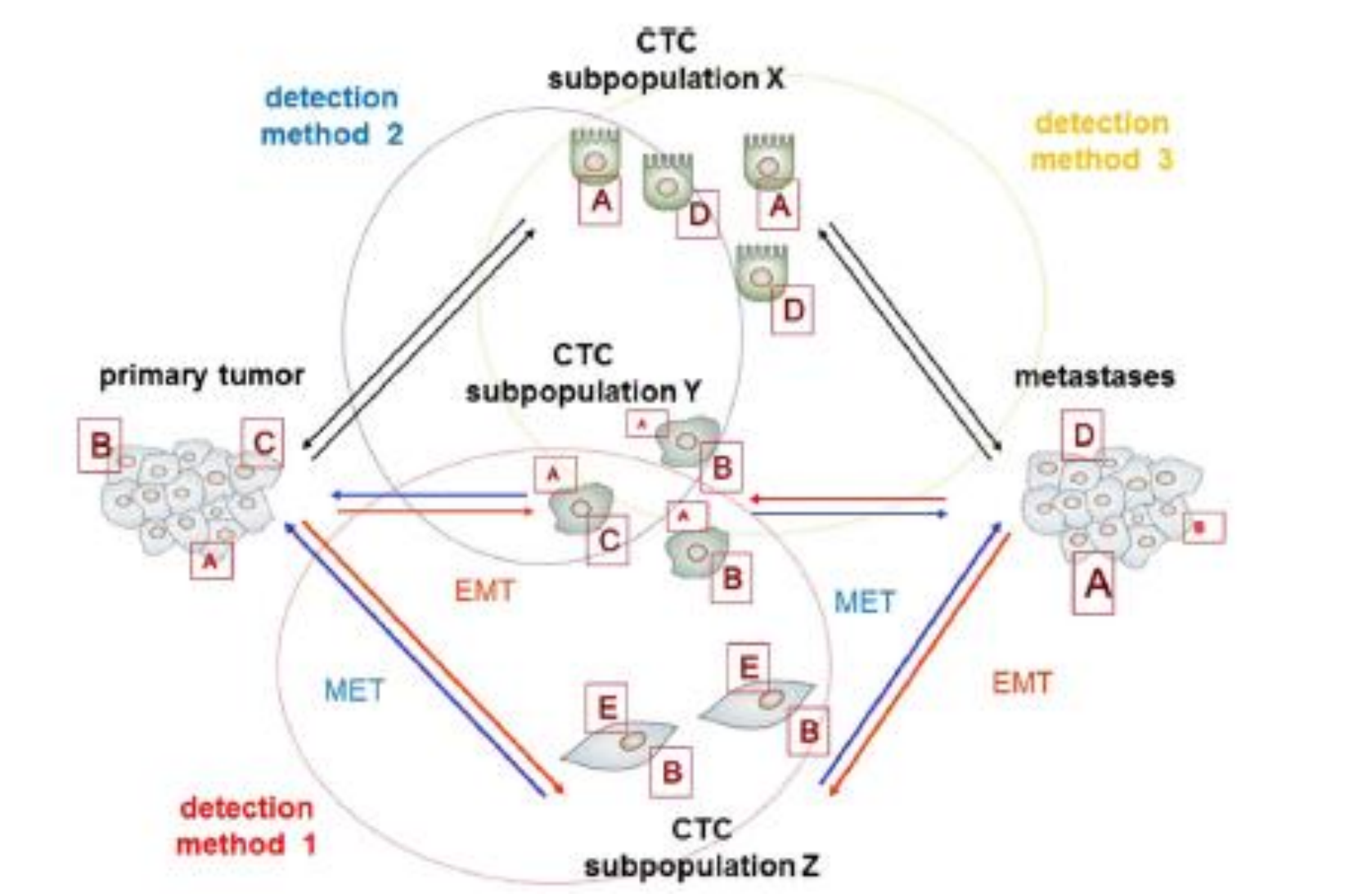
Clinical utility of CTC

Clinical utility of CTC



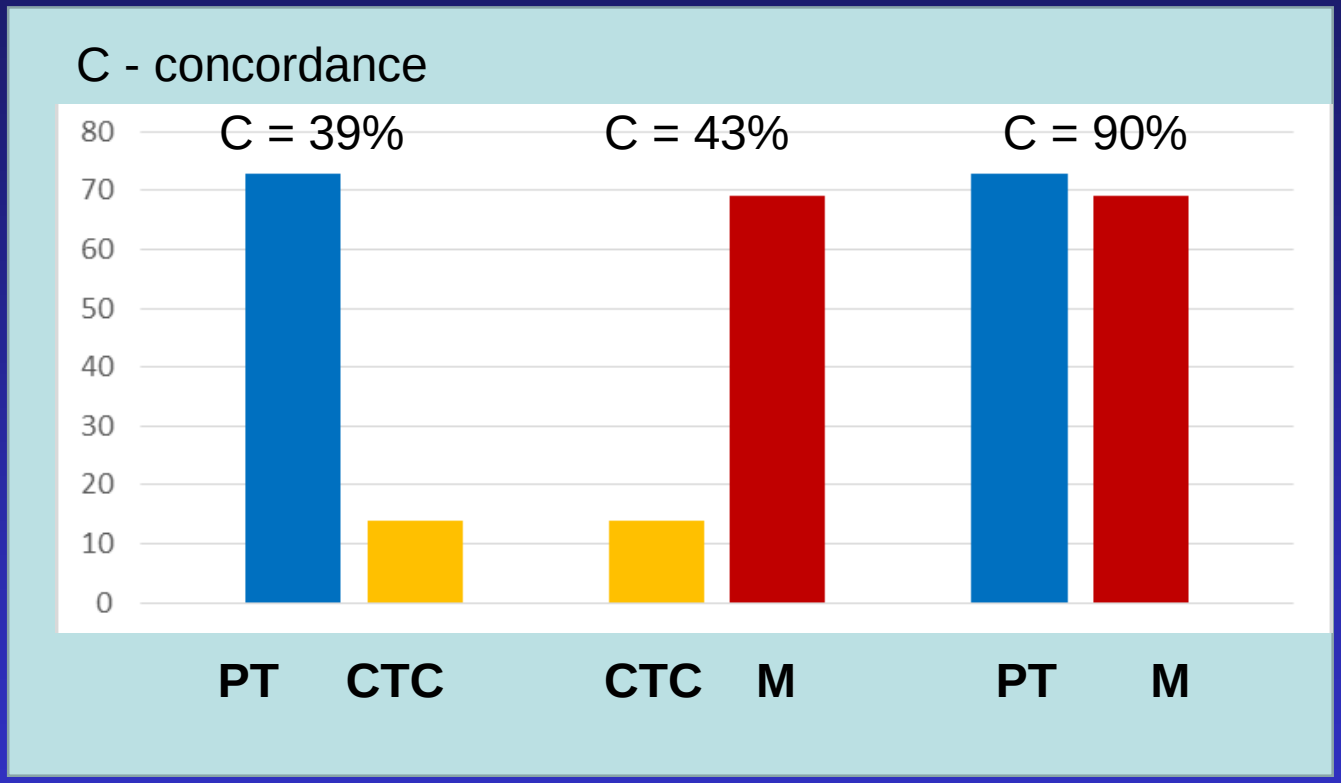
- currently now select treatment based on the characteristics of the primary tumor patient
- CTC phenotype is in part the same as the phenotype of the primary tumor
- CTC heterogeneity
- intratumoral heterogeneity

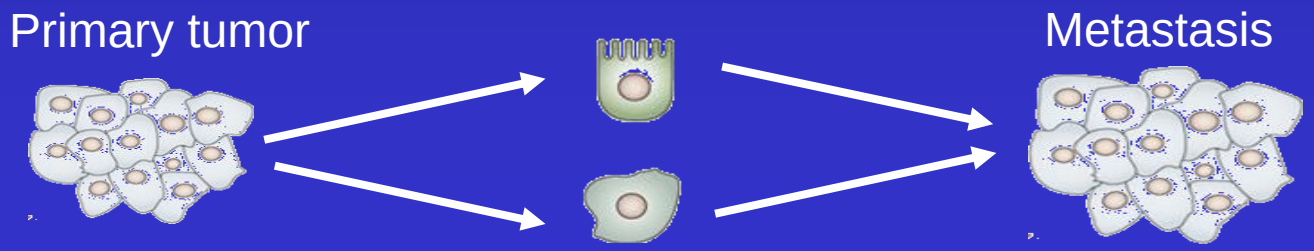
Treatment selection based on CTC profiling



Mego and Reuben, Curr Breast Cancer Rep. 2014

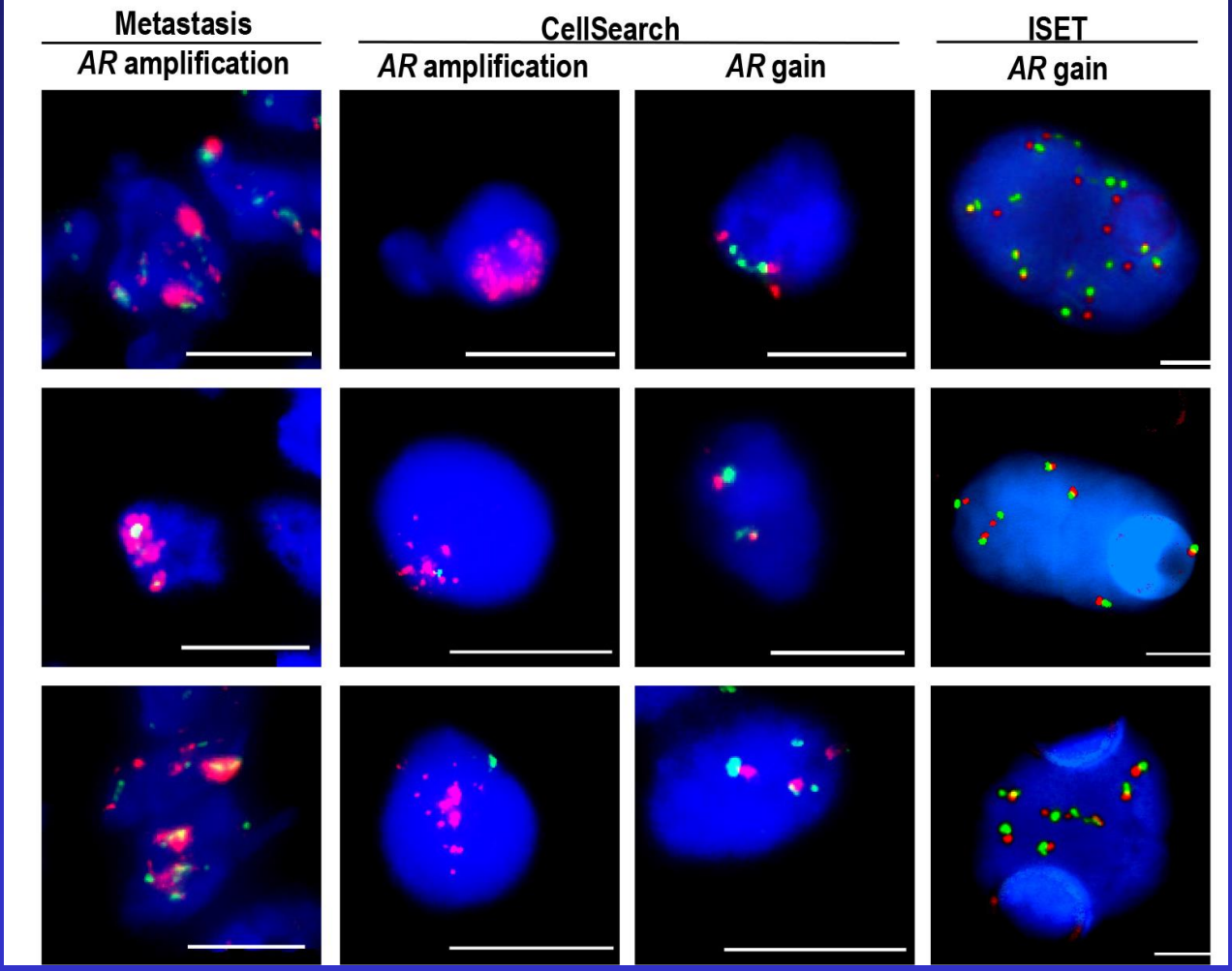
Estrogen receptors in primary tumor (PT), metastases (M) and CTC in breast cancer





Aktas et al, BMC Cancer 2016

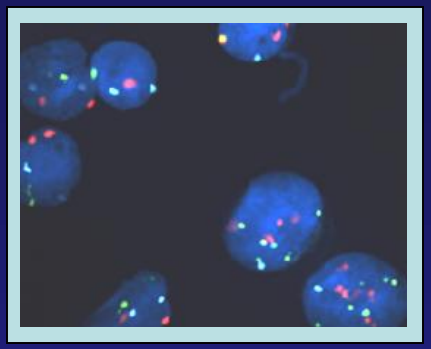
Detection of *AR* amplification and gain of copies in metastasis and CTCs isolated by ISET filtration and CellSearch



Massard et al. Oncotarget, 2016

CTC – biopsy in real time

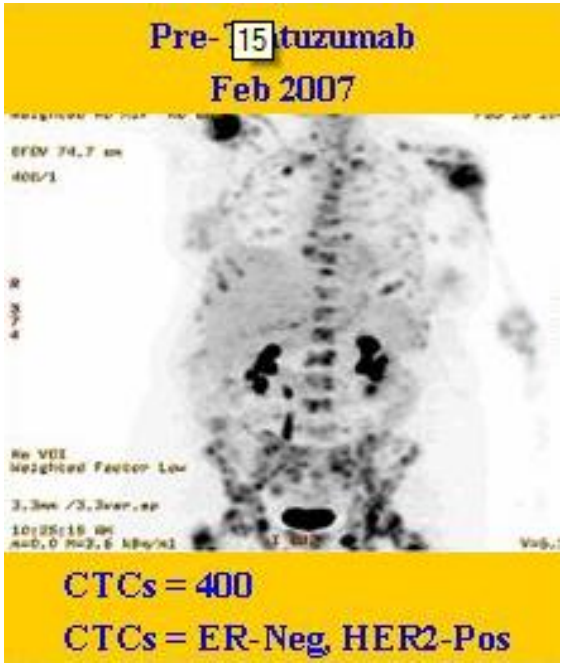
FISH HER2/Neu →



- Primary and Metastatic Tumors
  - ER-Pos
  - HER-2 Neg (FISH)
- Pretreatment CTCs >900
- Endocrine Therapies
- 4th Line CHT
- Post-therapy and at time of PET/CT scan (Feb 2007)
  - CTCs = 400

ER-Neg

HER-2 Pos (PCR)



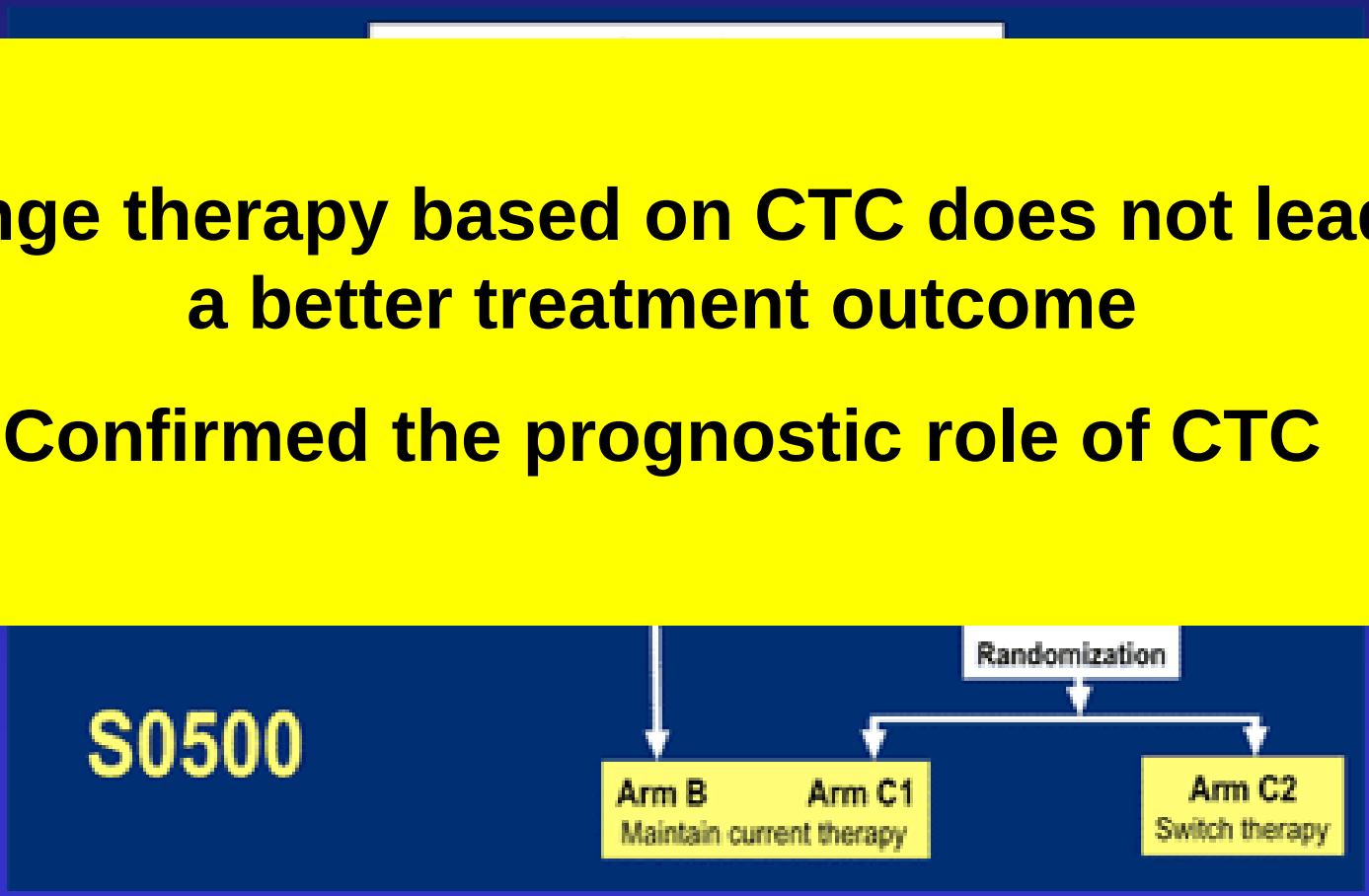
Reuben et al. 2007, ASCO

Clinical utility

SWOG S0500 – CTC as a treatment selection factor in metastatic breast cancer

Change therapy based on CTC does not lead to a better treatment outcome

Confirmed the prognostic role of CTC



Smerage, J ClinOncol. 2014

Clinical utility in ER+ breast cancer - COMETI P2

CTC – Endocrine therapeutic index (ETI)

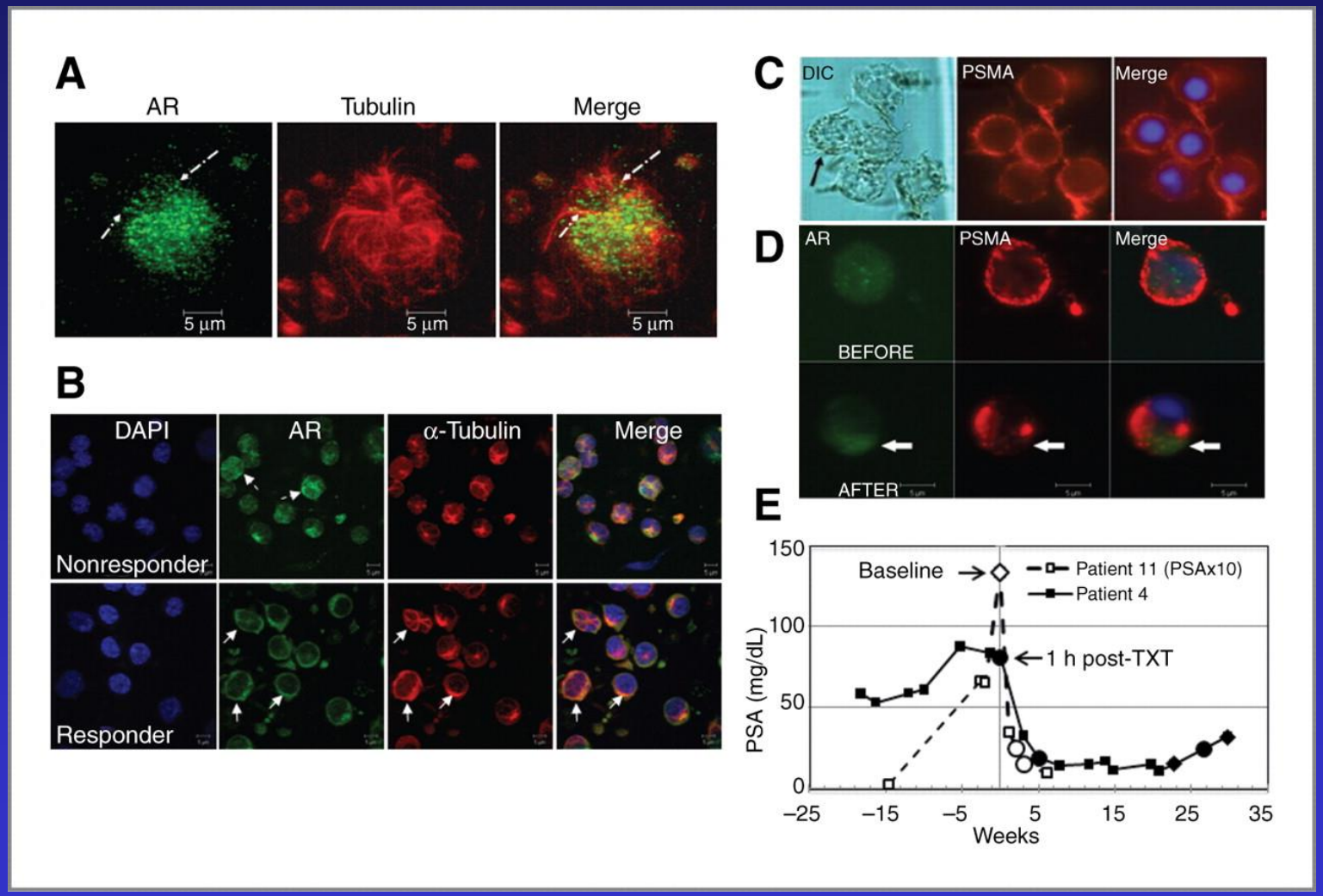
- CTC count
- ER
- Bcl2
- KI67
- HER2

Treatment selection: endocrine therapy vs. chemotherapy based on CTC-ETI

ClinicalTrials.gov Identifier: NCT01701050

Paoletti et al., CCR, 2014

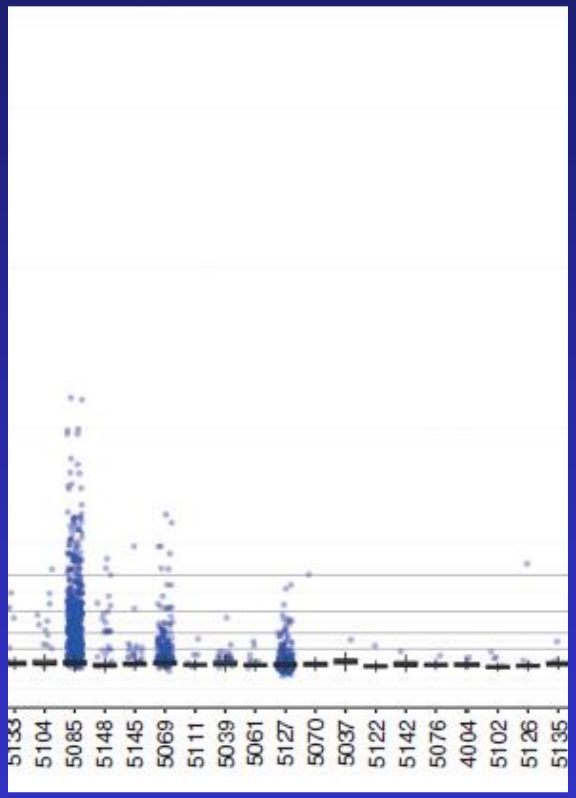
Clinical response to taxane-based chemotherapy correlates with AR cytoplasmic sequestration in CTCs.



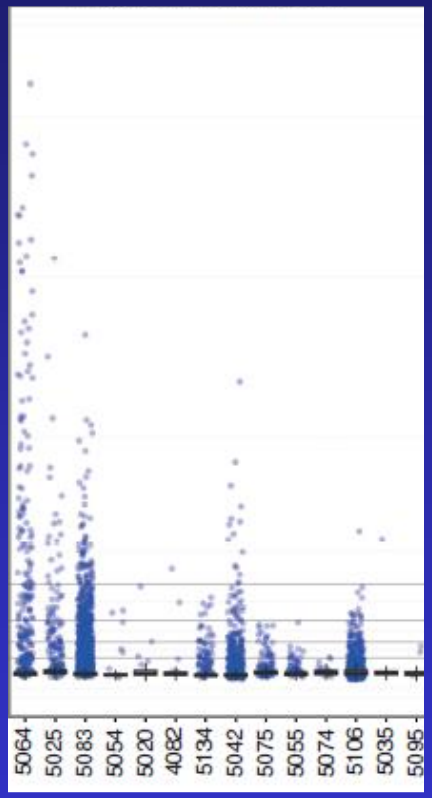
Medha S. Darshan et al. Cancer Res 2011;71:6019-6029

Clinical response to Abi/Enza correlates with AR expression intensity in CTCs.

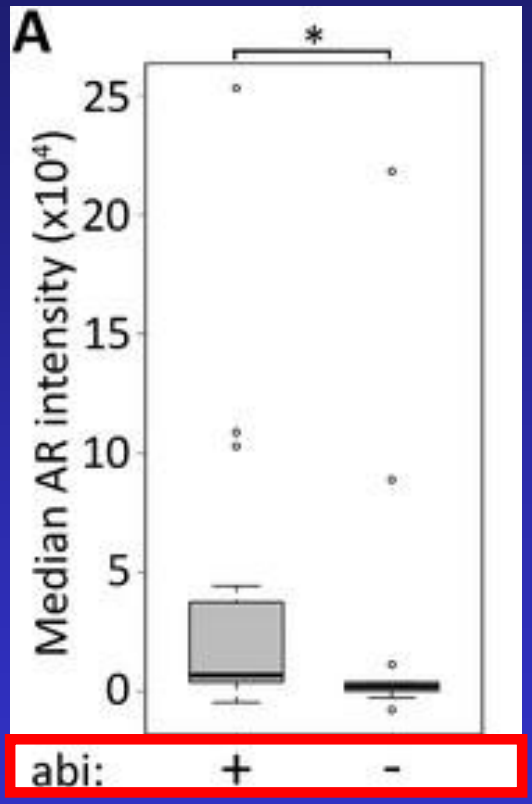
AR expression measured on individual CTC



Abi/Enza naïve patients

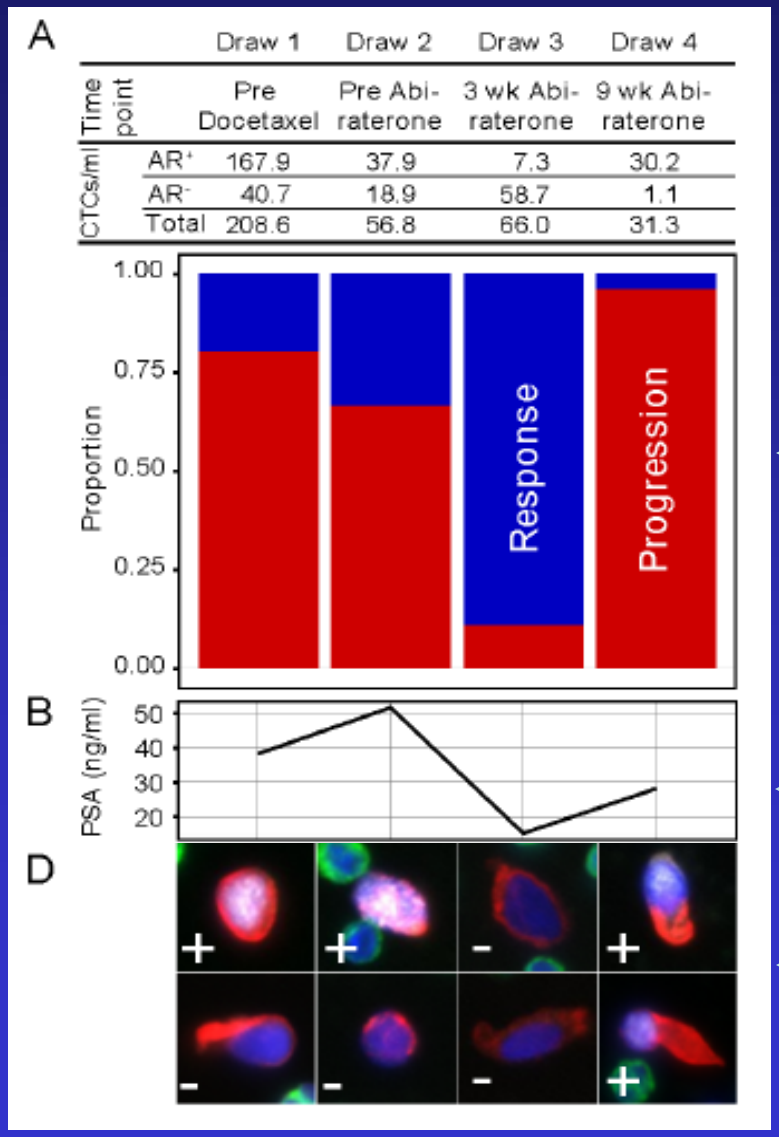


Abi/Enza resistant patients



Crespo et al. Br J Cancer 2015
Reyes et al. J Trans Med 2014

Phenotypic changes of CTC in response to treatment pressure to Abiraterone



Androgen receptor (AR) on CTC

CTC AR +

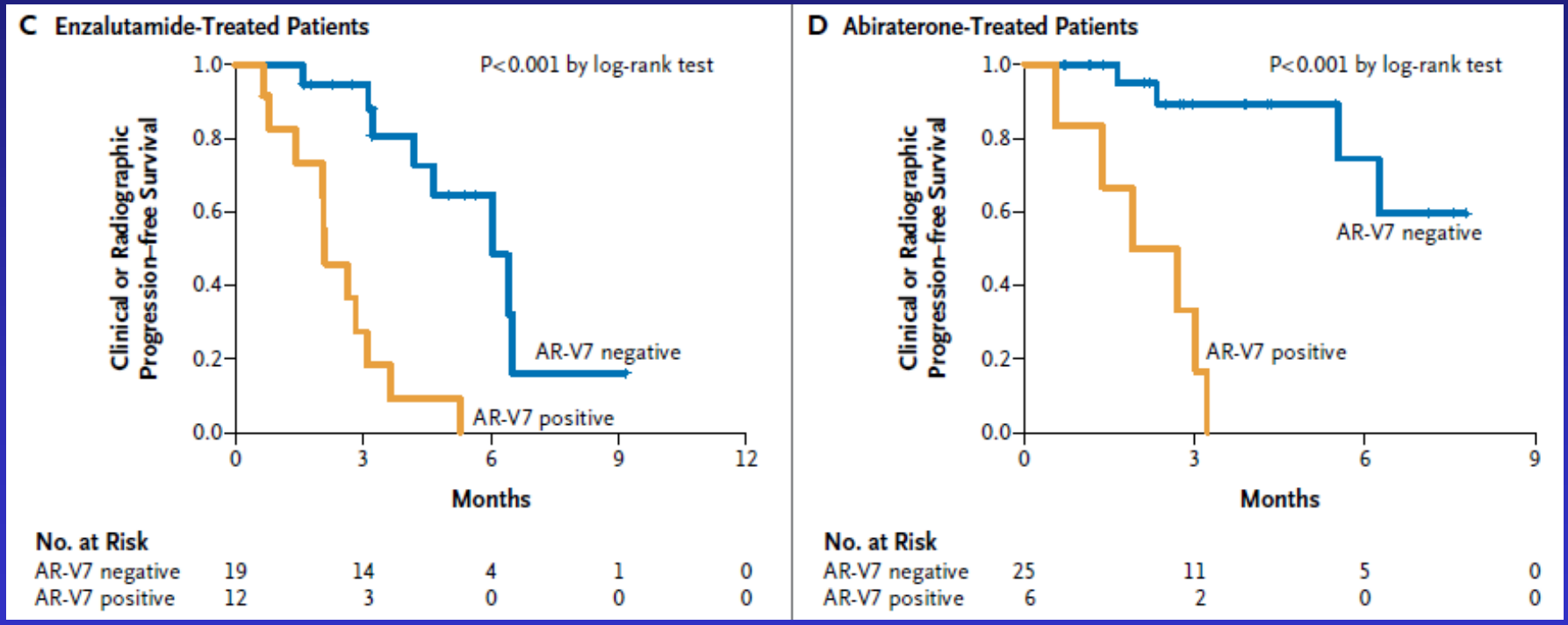
CTC AR -

PSA changes

Phenotypic changes

Dago et all, PLOS ONE, 2014

Androgen Receptor Splice Variant 7 on CTC predict efficacy of Abi/Enza in mCRPC



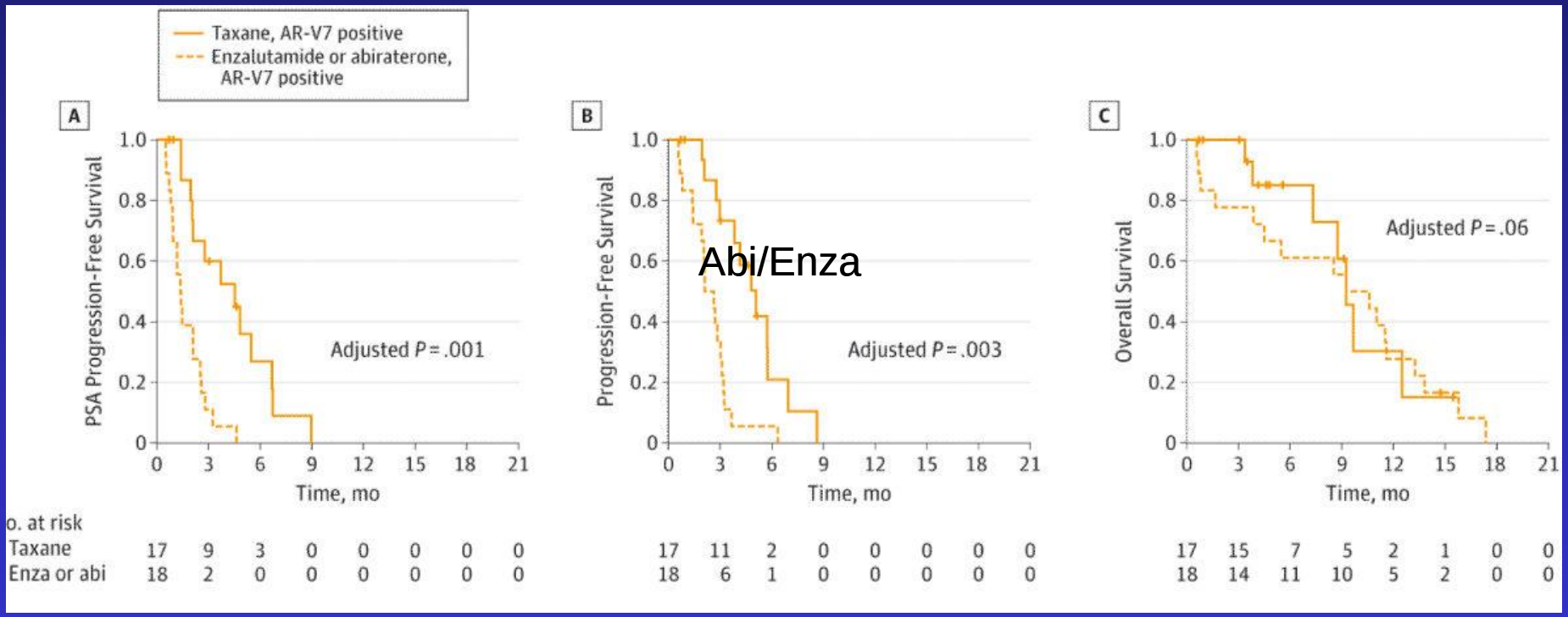
Antonarakis et al., N Engl J Med. 2014

Androgen Receptor Splice Variant 7 on CTC predict efficacy of Abi/Enza in mCRPC

N (%)	Overall (N = 202)			
	CTC neg n = 53 (26.2%)	CTC+/ ARV7 n = 113 (56.0%)	CTC+/ ARV7+ n = 36 (17.8%)	P-value
PSA response	75%	52%	14%	< .001
PSA-PFS (mo)	11.3	6.2	2.1	< .001
PFS (mo)	13.9	7.7	3.1	< .001
OS (mo)	28.7	29.5	11.2	< .001

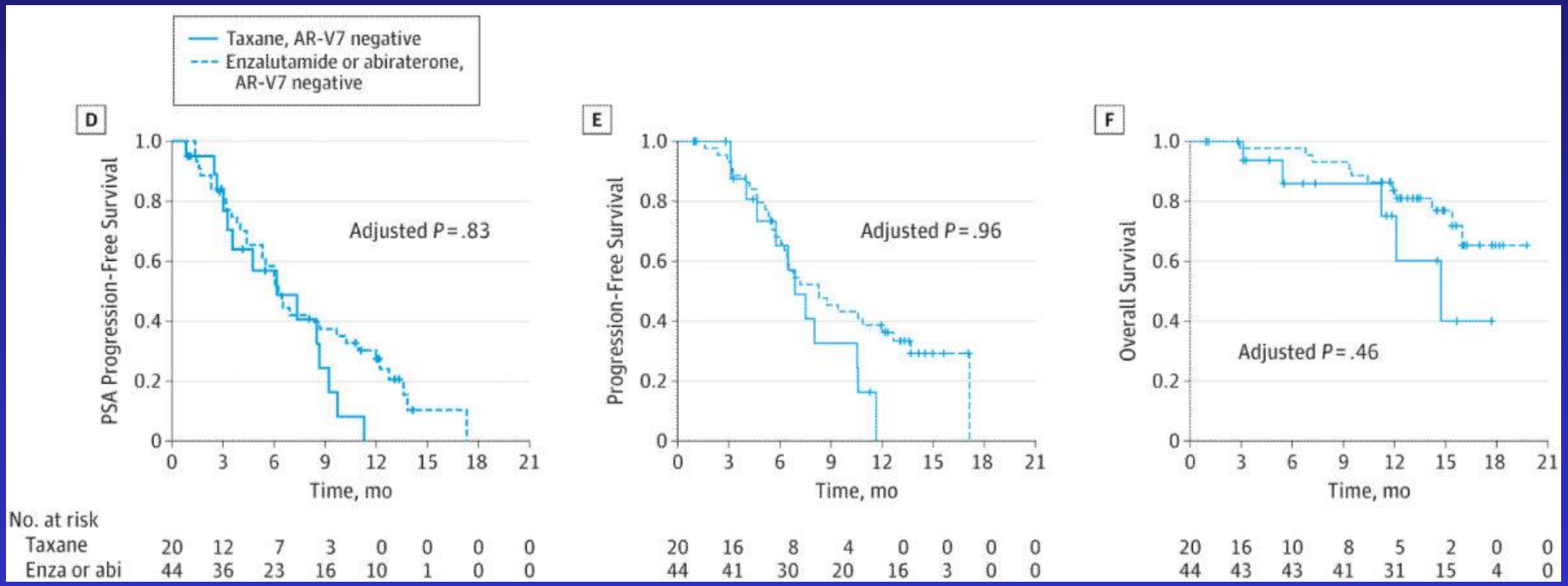
Antonarakis et al., J Clin Oncol 34, 2016 (suppl; abstr 5012)

Androgen Receptor Splice Variant 7 and efficacy of Abi/Enza vs. taxane based therapy



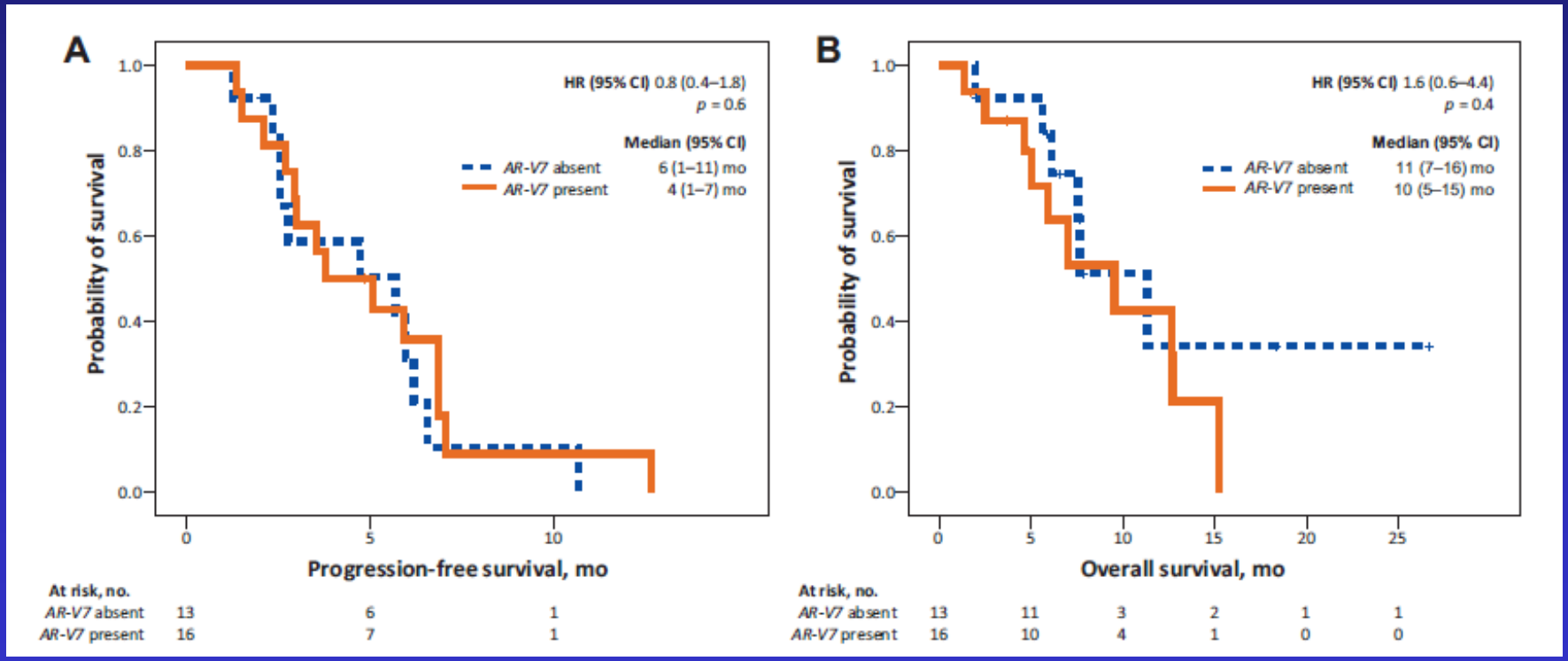
Antonakrakis et al. JAMA Oncol. 2015

Androgen Receptor Splice Variant 7 and efficacy of Abi/Enza vs. taxane based therapy



Antonakrakis et al. JAMA Oncol. 2015

Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells.



Onstenk et al., Eur Urol, 2015

Conclusions

- CTC showed consisted prognostic value in metastatic breast and prostate cancer
- Different methods detect various subpopulations of CTC with different biological properties and different clinical significance
- The clinical significance of CTC always interpreted within the context of the detection method used
- Molecular characterization of CTC is promising tool for treatment selection

National Cancer Institute, Bratislava, Slovakia

Thank you for your attention

NATIONAL CANCER INSTITUTE
the art of healing

Thank you for your attention

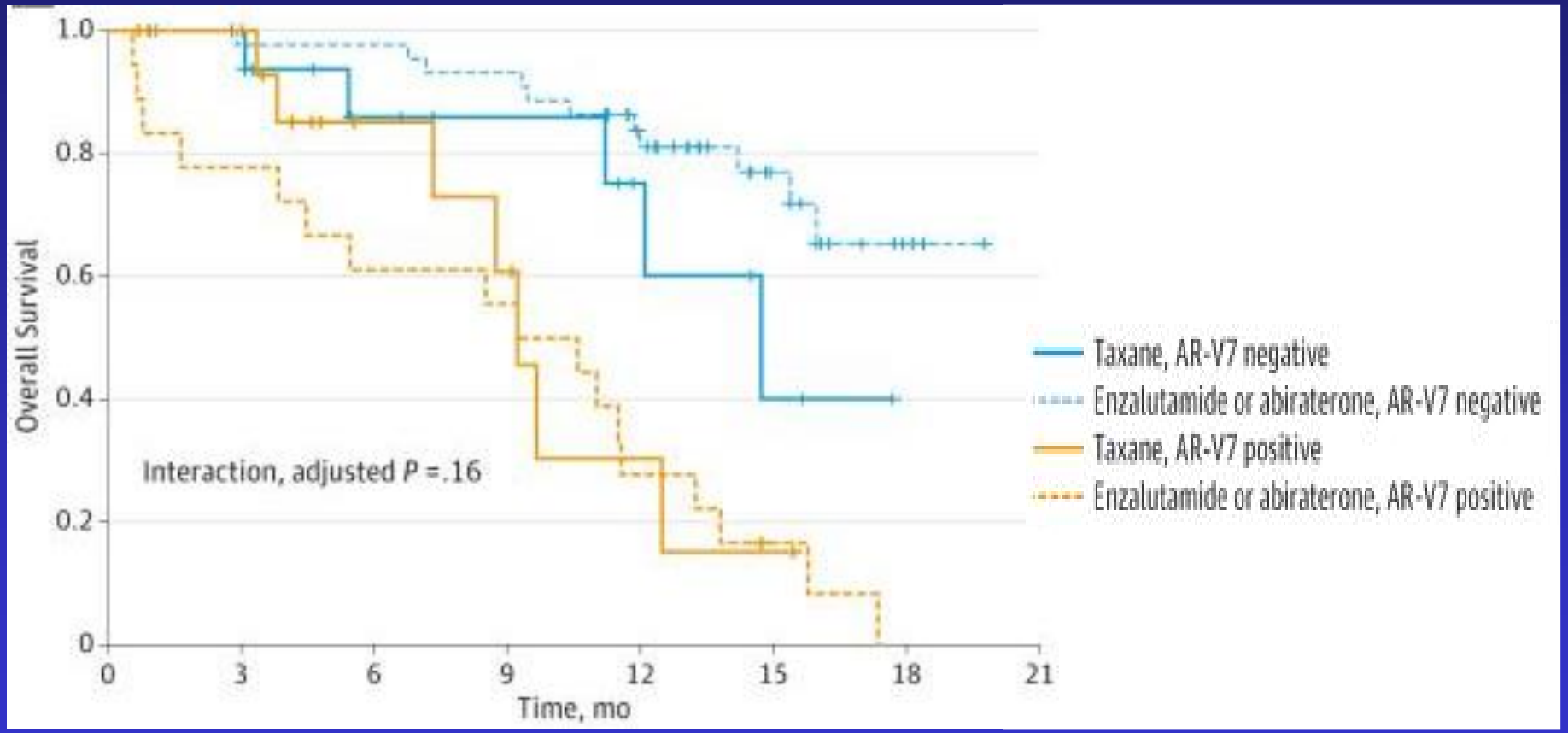
Conversion Rates From Unfavorable (≥ 5 CTC) to Favorable (< 5 CTC) Were Significantly Higher With Abiraterone Acetate Relative to Placebo

	Week 4		Week 8		Week 12	
No. of patients with baseline CTC ≥ 5 and a postbaseline CTC value	422		374		330	
Conversion status	AA (n = 272)	Placebo (n = 150)	AA (n = 245)	Placebo (n = 129)	AA (n = 217)	Placebo (n = 113)
Conversion	42%	14%	50%	17%	48%	17%
(n)	(113)	(21)	(123)	(22)	(105)	(19)
P value	< 0.0001		< 0.0001		< 0.0001	

***P* value from chi-square statistic.**

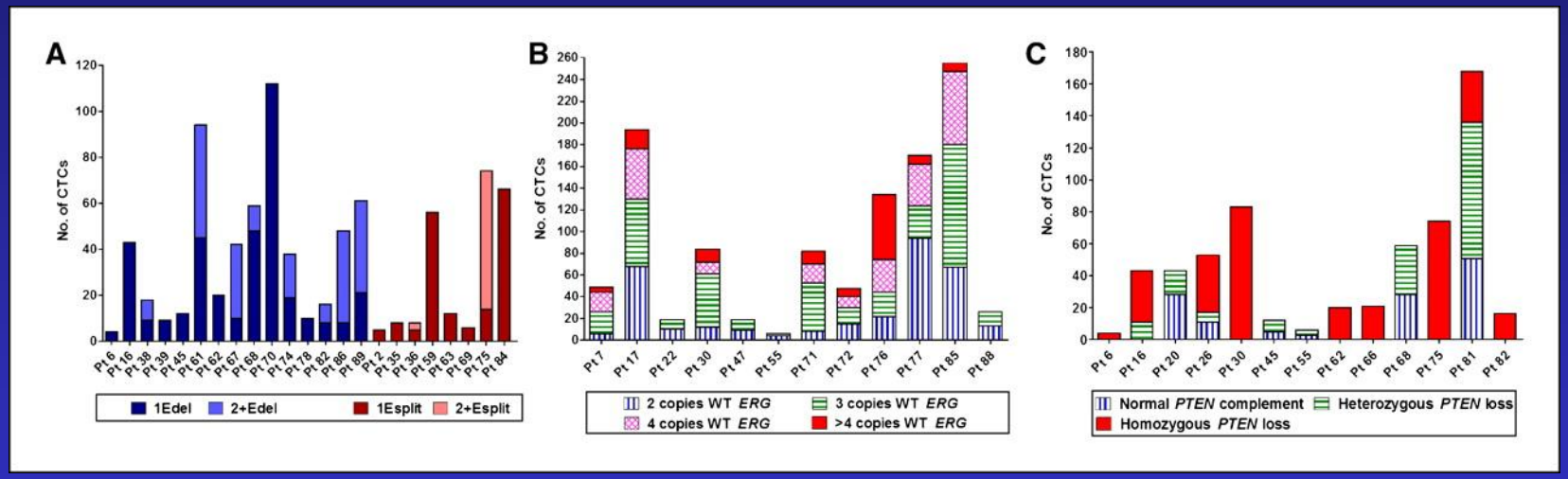
Scher H, ASCO, 2011

Androgen Receptor Splice Variant 7 and Efficacy of Taxane Chemotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer.



Antonakrakis et al. JAMA Oncol. 2015

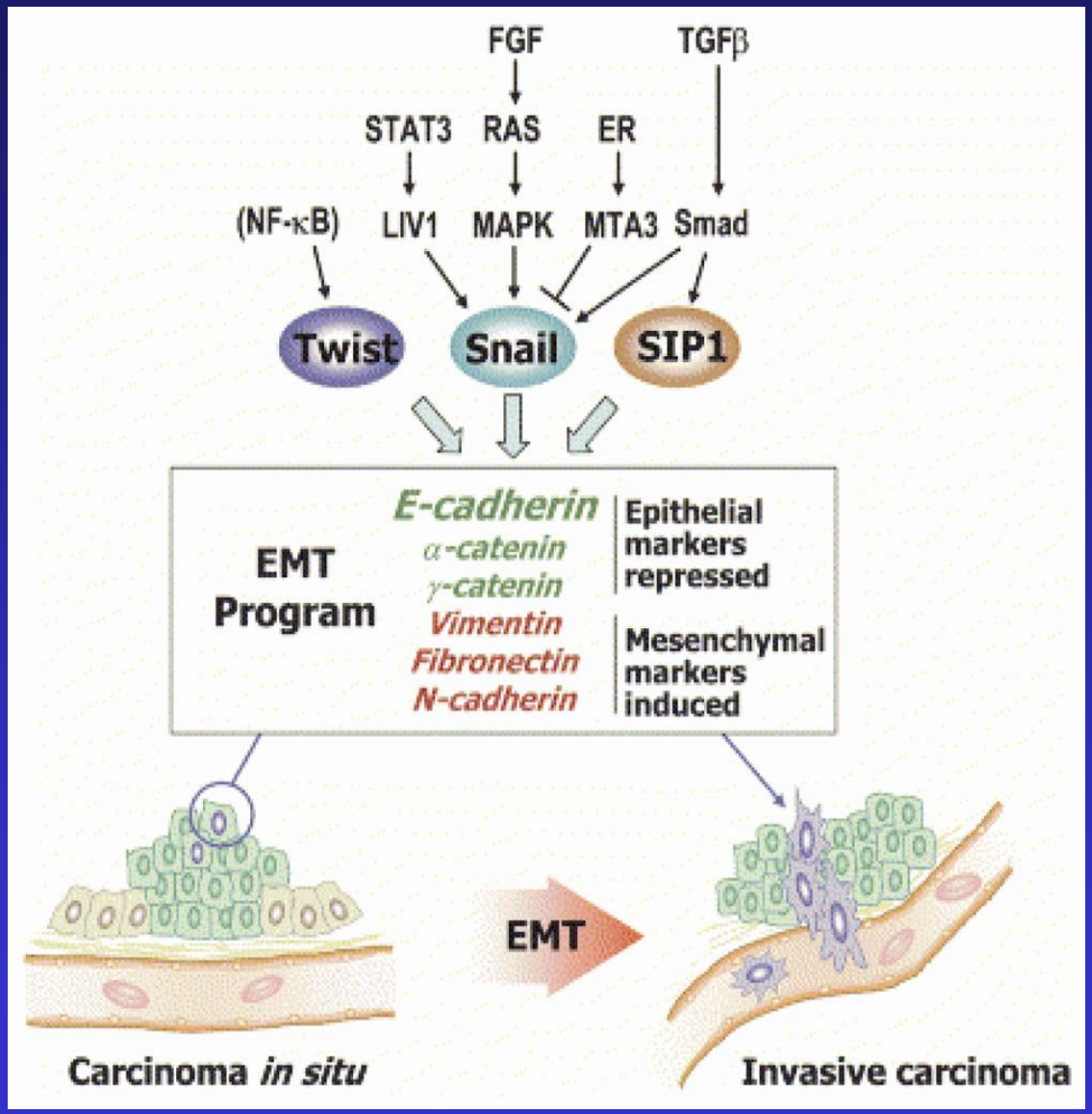
Homogeneity of ERG gene rearrangement and heterogeneity of PTEN loss and AR copy number gain.



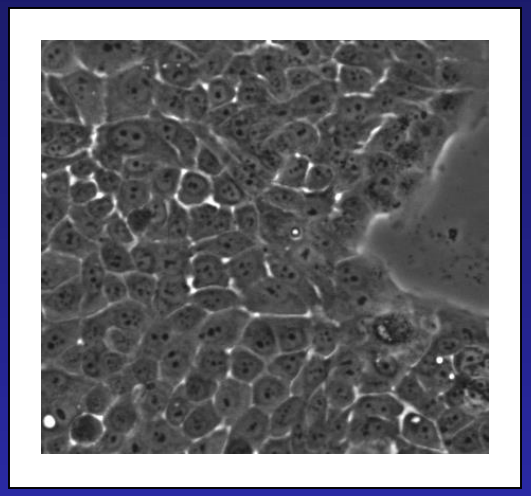
Gerhardt Attard et al. Cancer Res 2009;69:2912-2918

AACR American Association for Cancer Research
Cancer Research

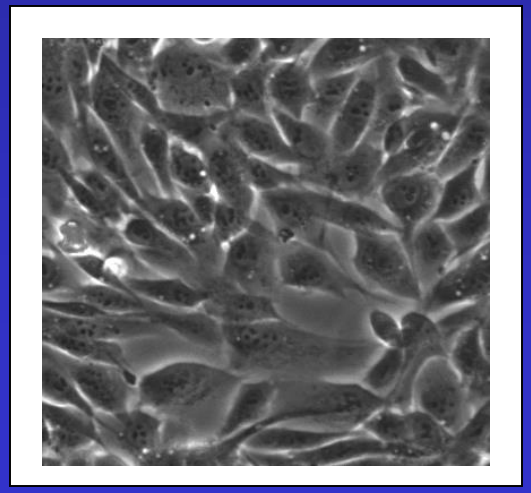
Epithelial-mesenychmal transition (EMT)



Epithelial cells



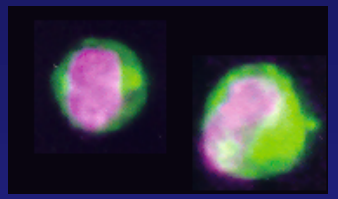
Epithelial cells after EMT



Zvaifler *Arthritis Research & Therapy* 2006 **8**:210

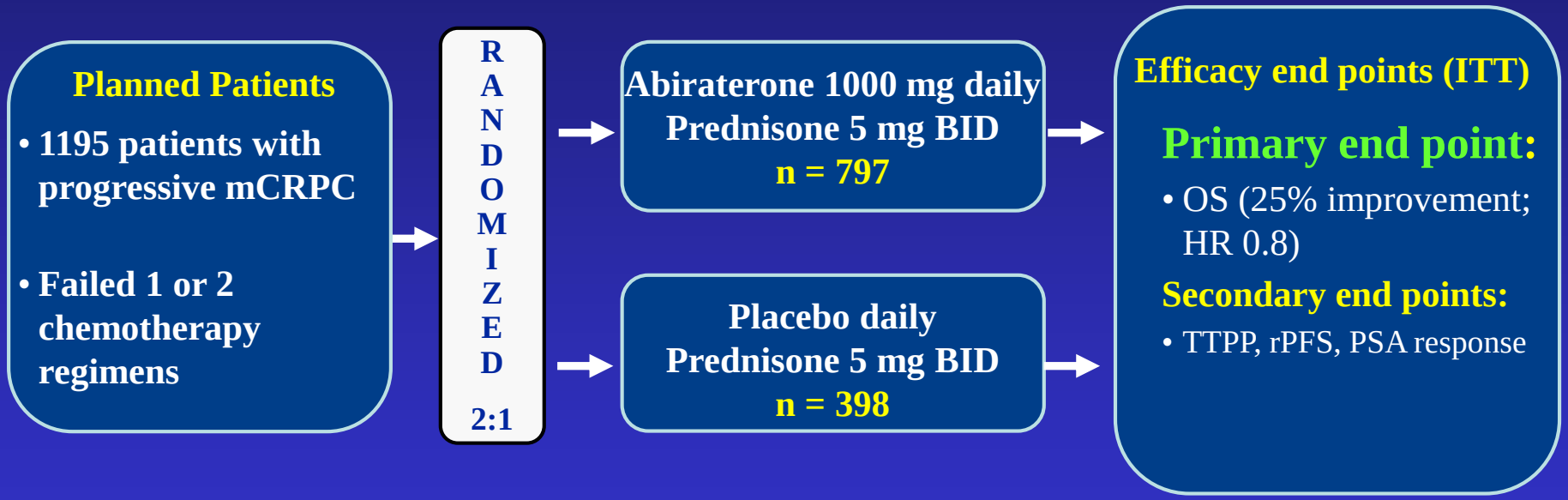
CTC – CellSearch™ (Veridex)





- IHC based method
- EpCAM enrichment
- CTC count is not related to tumor burden or serum tumor markers – actively released CTC ?
- prognostic factor for PFS and OS in MBC patients (Cristofanilli, 2004)
- Superior prognostic value compared to functional imaging (De Giorgi, 2009)

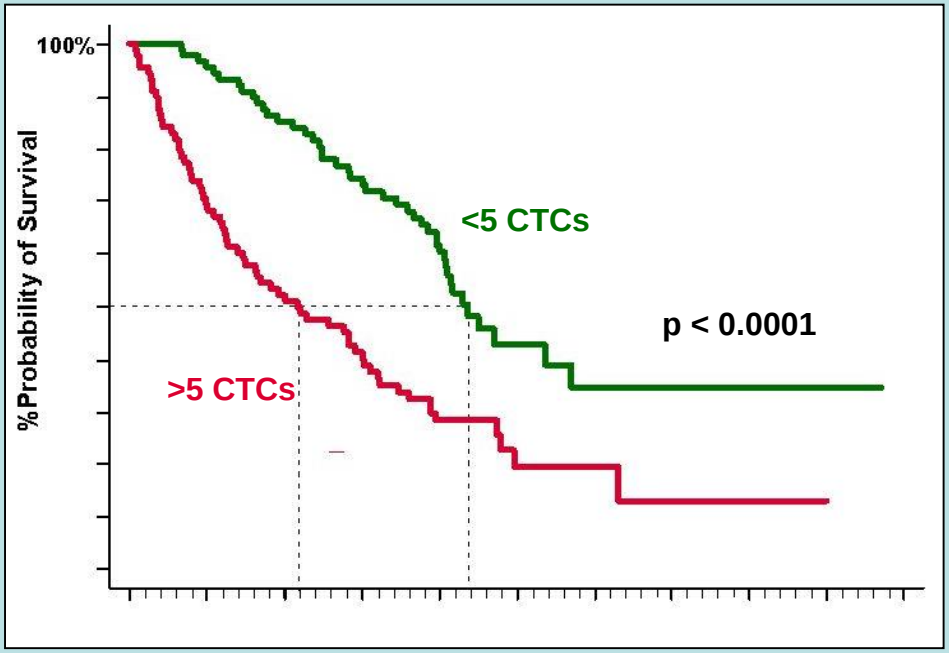
COU-AA-301: A Phase 3 Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial Designed to Show an Improvement in Overall Survival



Scher H, ASCO, 2011

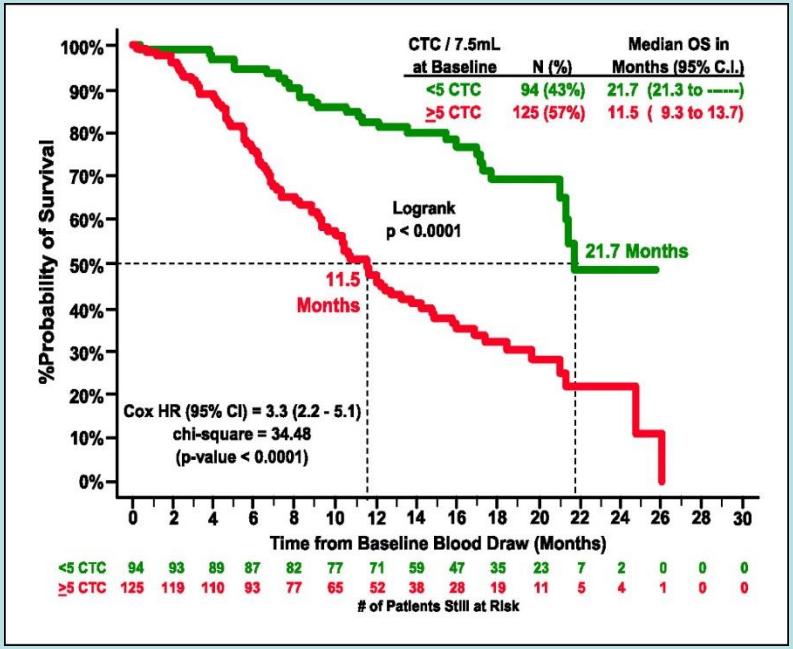
Clinical validity of CTC

Breast cancer



Cristofanilli et al., NEJM 2004

Prostate cancer



De Bono et al., CCR, 2008