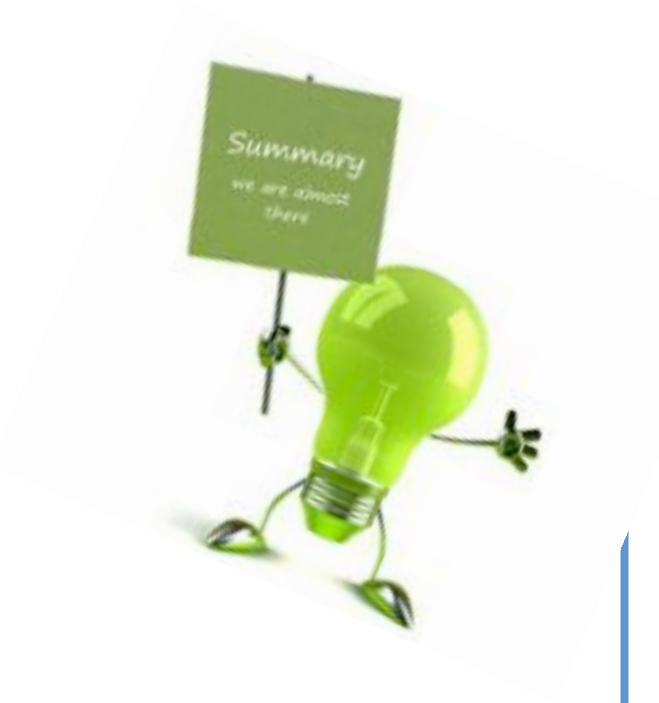
“Lo studio delle cellule tumorali circolanti: esperienze e controversie“

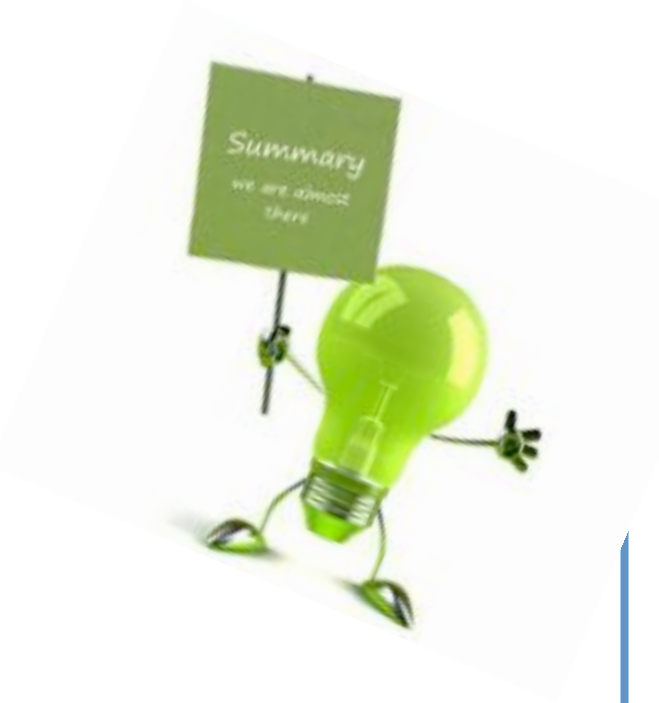
Michela Buglione di Monale
Cattedra di Radioterapia – Università degli Studi di Brescia



- ✓ **the concept behind CTC**

- ✓ **experiences and perspectives:**
  - **breast cancer**
  - **prostate cancer**
  - **head and neck cancer**

- ✓ **controversies**



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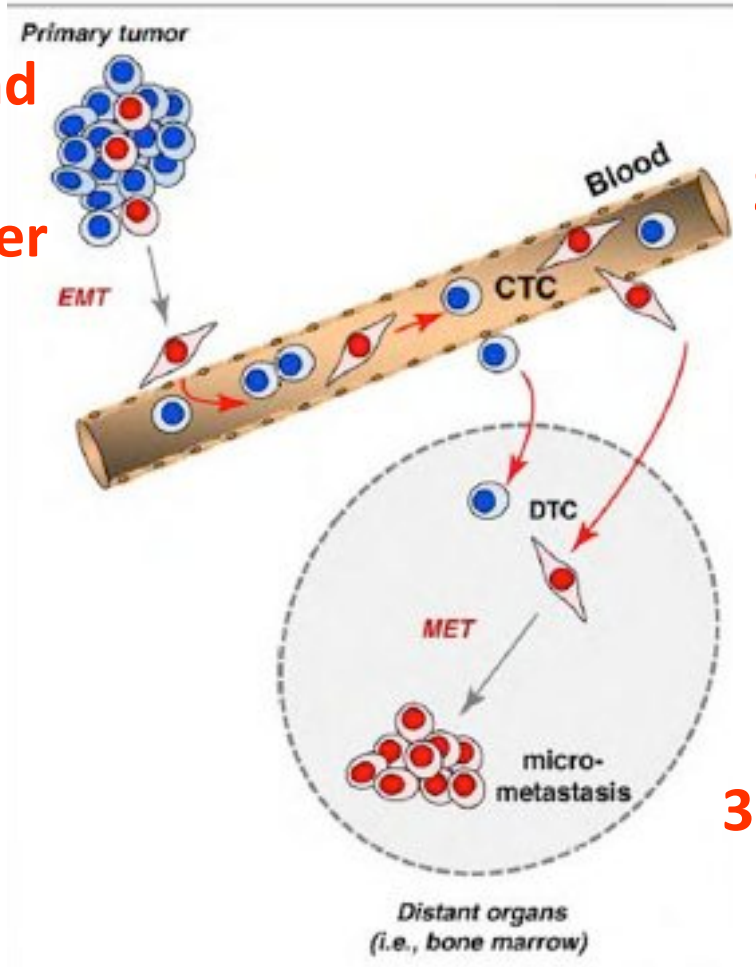
- ✓ controversies

Disseminated Tumor Cells (DTCs) and Circulating Tumor Cells (CTC)

1: Proliferating and non-proliferating cancer cells (Cancer Stem Cells)



2: Circulating TCs (CTC)

3: Disseminated TCs (DTC)

Pantel K. Trend in Mol Med 2010

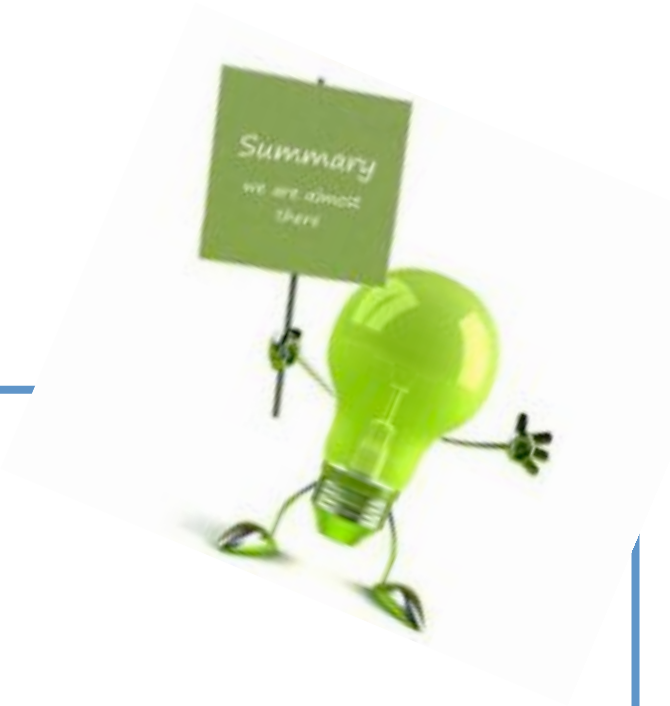
Four "broad areas":

1. stratification of patients with early disease

2. clinical subdivision of patients with advanced cancer into different prognostic groups

3. as an intermediate endpoint ('surrogate') of survival for therapeutic efficacy studies

4. molecular sub-classification of advanced cancer patients



- ✓ **the CTC: the concept behind CTC**
- ✓ **experiences and perspectives:**
  - **breast cancer**
  - prostate cancer
  - head and neck cancer
- ✓ controversies

breast cancer

✓**DTC in the bone marrow (BM) and CTC in blood are considered a valuable surrogate marker of Minimal Residual Disease (MRD)**

✓ **DTC presence in the BM at the time of diagnosis independently predicts poor clinical outcome**

✓**Is it possible to enhance the prognostic value of CTC in early and advanced breast cancer in order to avoid BM biopsy?**

Adjuvant setting Early breast cancer

✓ **Reports on the prevalence of CTC detection and their prognostic impact are incoherent**

Author	Year	*N*	Method	Positivity rate (%)	Follow-up (months)	Prognostic relevance
Franken *et al* [26]	2012	404	CellSearch	19[4]	48	DFS[5], BCSS[6]
Molloy *et al* [27]	2011	733	RT-PCR	8	91	DFS, BCSS
Rack *et al* [28]	2010	2,026	CellSearch	22[1]	35	DFS, OS
Rack *et al* [29]	2010	1,489	CellSearch	9[4]	32	DFS[2], OS[1]
Bidard *et al* [30]	2010	115	CellSearch	23	36	DFS, OS
Daskalaki *et al* [31]	2009	165	RT-PCR	55[1], 52[2]	59	OS[1]
Pierga *et al* [32]	2008	118	CellSearch	23[1], 17[2]	18	DFS[3]
Xenidis *et al* [33]	2006	167	RT-PCR	22	32	DFS, OS
Benoy *et al* [34]	2006	116	RT-PCR	12–14	26	None
Ntoulia *et al* [35]	2006	101	RT-PCR	14	24	DFS
Nieto *et al* [36]	2004	242	ICC	7	84	DFS, OS
Zach *et al* [37]	2002	218	RT-PCR	2	>12	DFS
Stathopoulou *et al* [38]	2002	148	RT-PCR	30	28	DFS, OS

[1]Before chemotherapy.
[2]After chemotherapy.
[3]Combined positivity before and/or after neoadjuvant chemotherapy.
[4]At least one CTC.
[5]Multivariate analysis.
[6]Univariate analysis.
ICC: Immunocytochemistry.

Range 9-23% Cell search
8-55% RT-PCR

N Krawczyk, M Banys, A Hartkopf et al *e*cancermedicalscience 2013, 7:352

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CTC + →
DFS HR 1.88
OS HR 1.91

N Krawczyk, M Banys, A Hartkopf et al *e*cancermedicalscience 2013, 7:352

Neo-Adjuvant setting breast cancer

✓Patients can have a relapse despite pathological complete remission

✓Most of the studies indicate that CTC/DTC detection after neo-adjuvant systemic treatment is independent from the primary tumor response and not associated to any clinico-pathological characteristics of breast cancer

✓…DTC detection after neo-adjuvant treatment is prognostic of survival, the CTC status has no impact on prognosis…

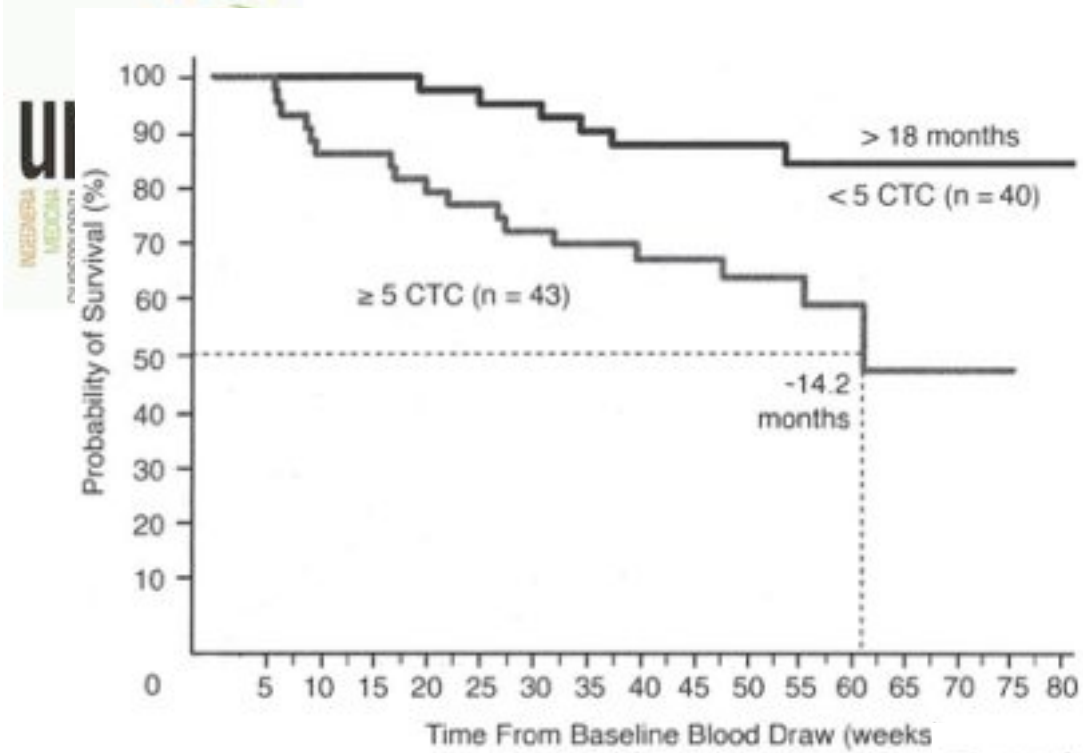
N Krawczyk, M Banys, A Hartkopf et al *e*cancermedicalscience 2013, 7:352

Advanced breast cancer

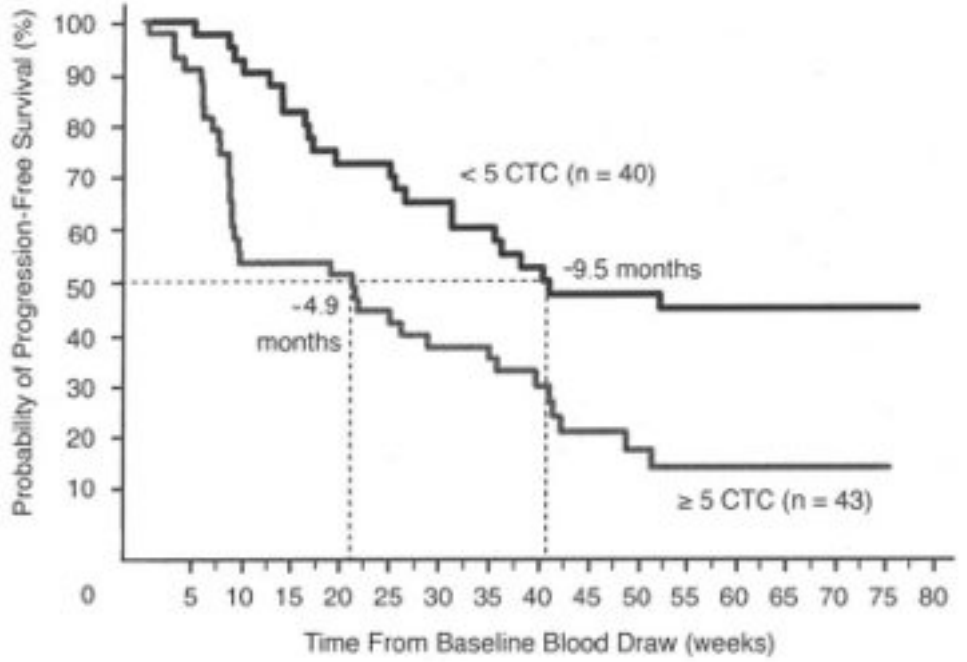
- ✓40-80% of metastatic breast cancer present with CTC in PB
- ✓Cut-off >=5 CTC
- ✓Prognostic significance of CTC's has been demonstrated

- ✓CTC dynamics during chemotherapy may serve as a new therapy monitoring tool

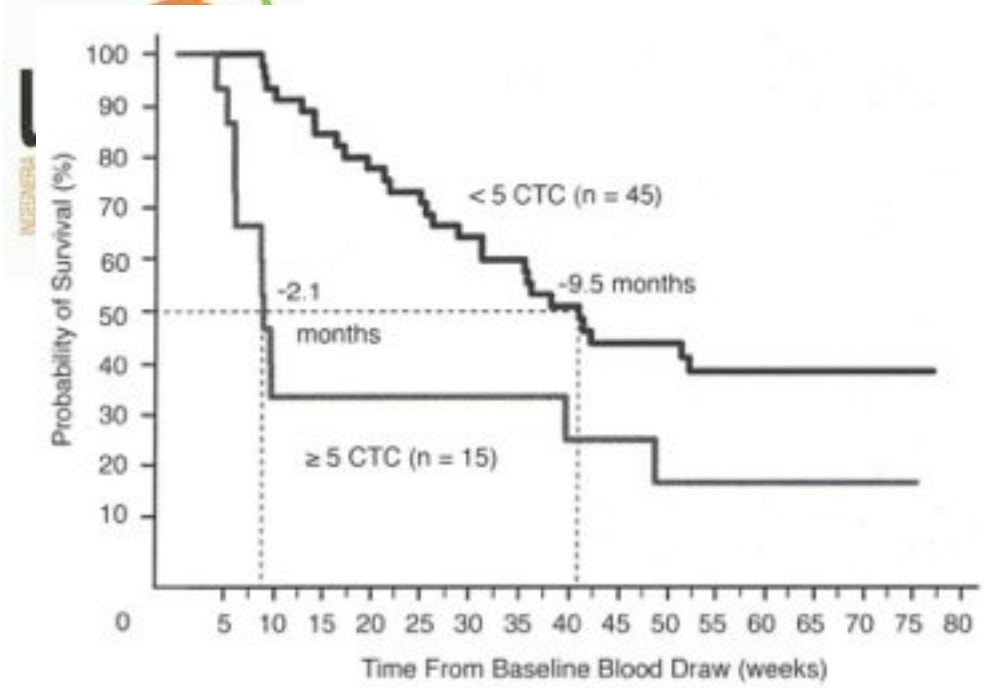
N Krawczyk, M Banys, A Hartkopf et al *e*cancermedicalscience 2013, 7:352



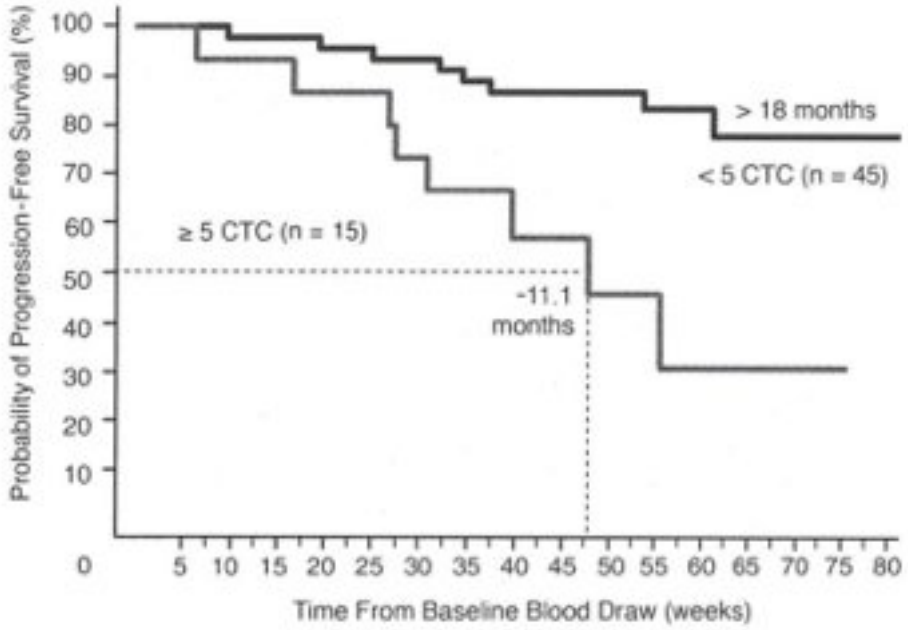
baseline



M Cristofanilli, DF Hayes , GT Budd et al *JCO 23(7): 1420, 2005*



1° follow-up



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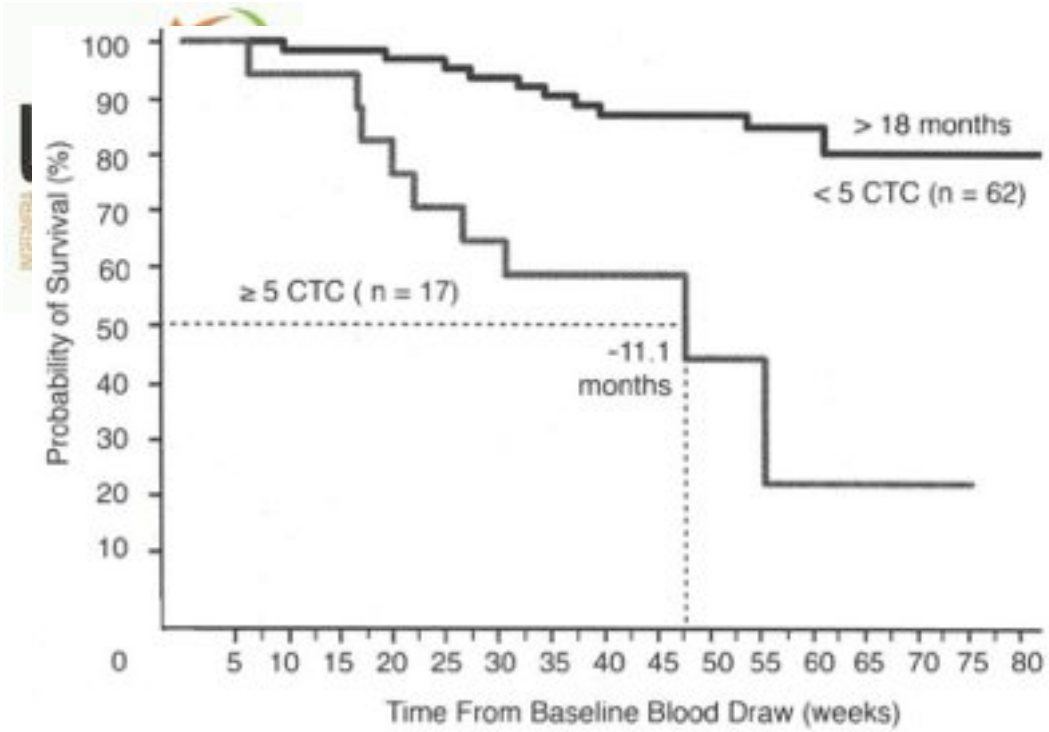
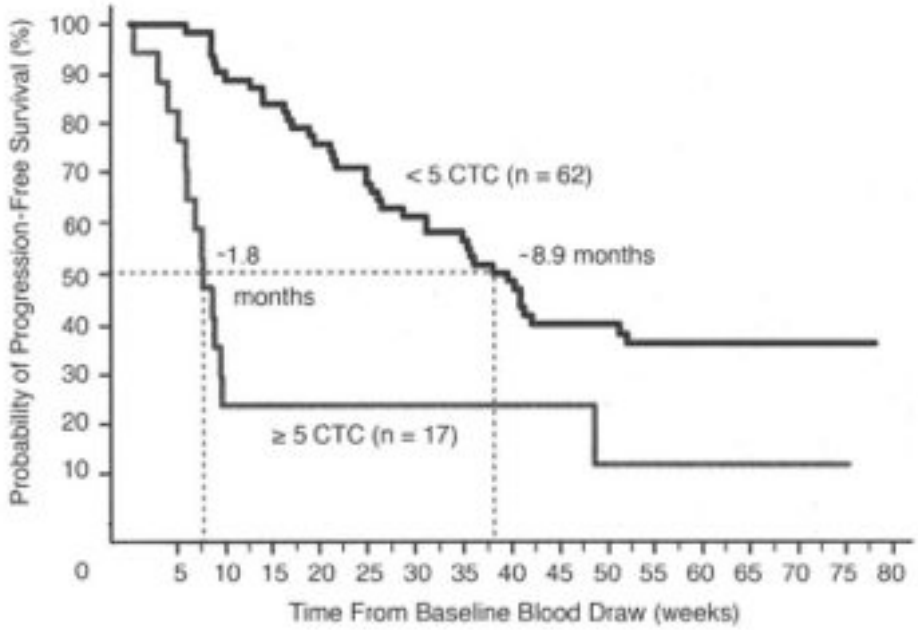


restaging



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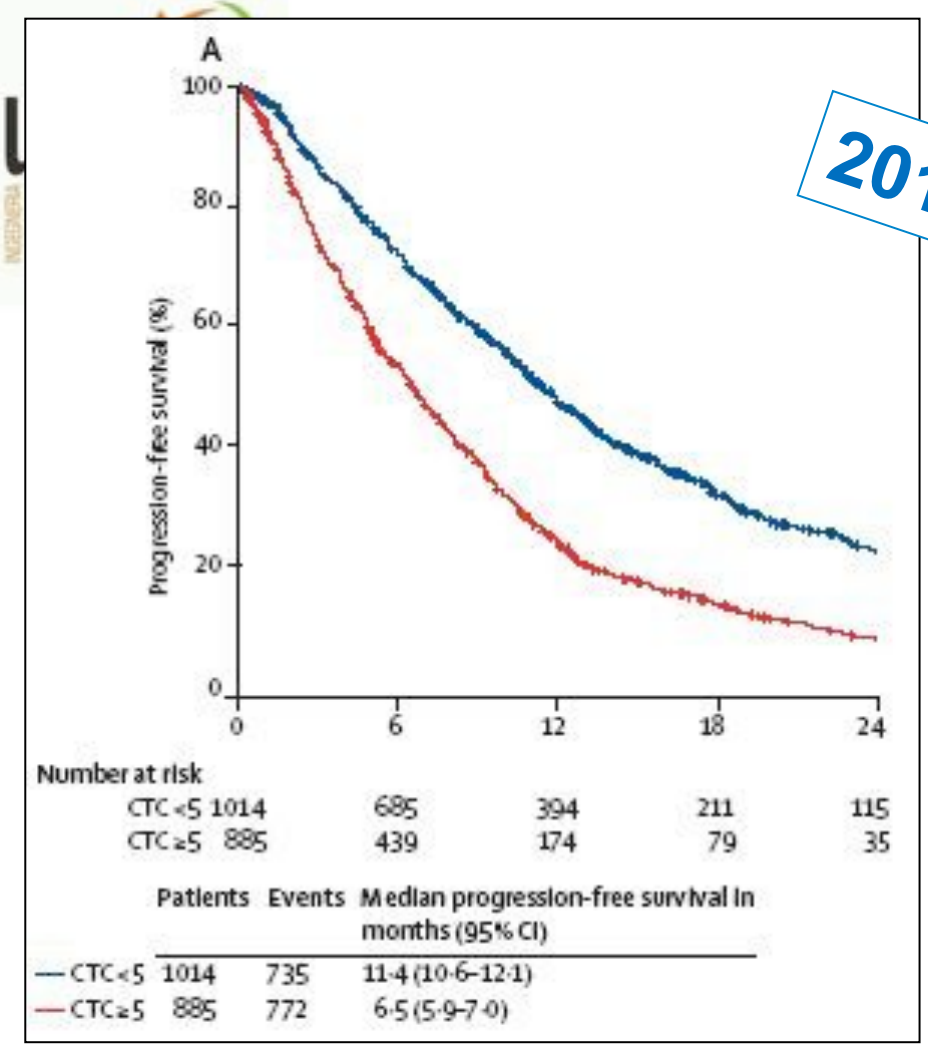
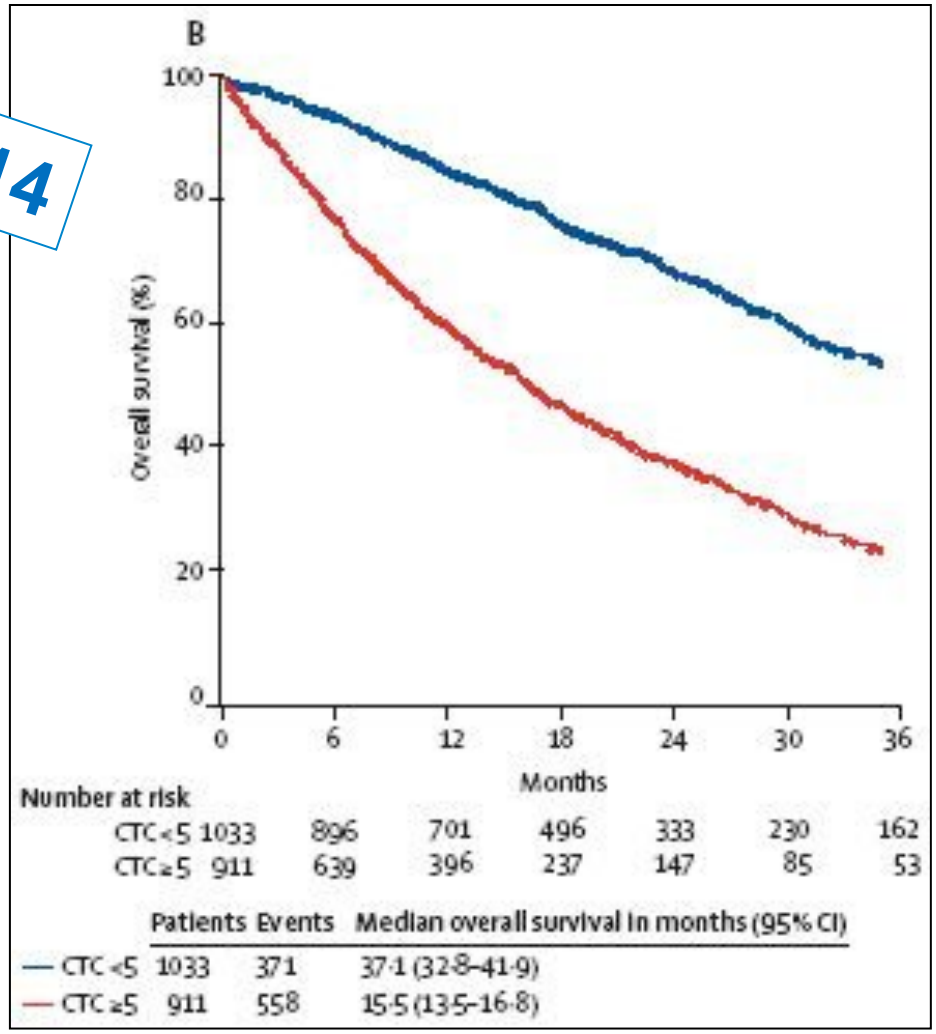


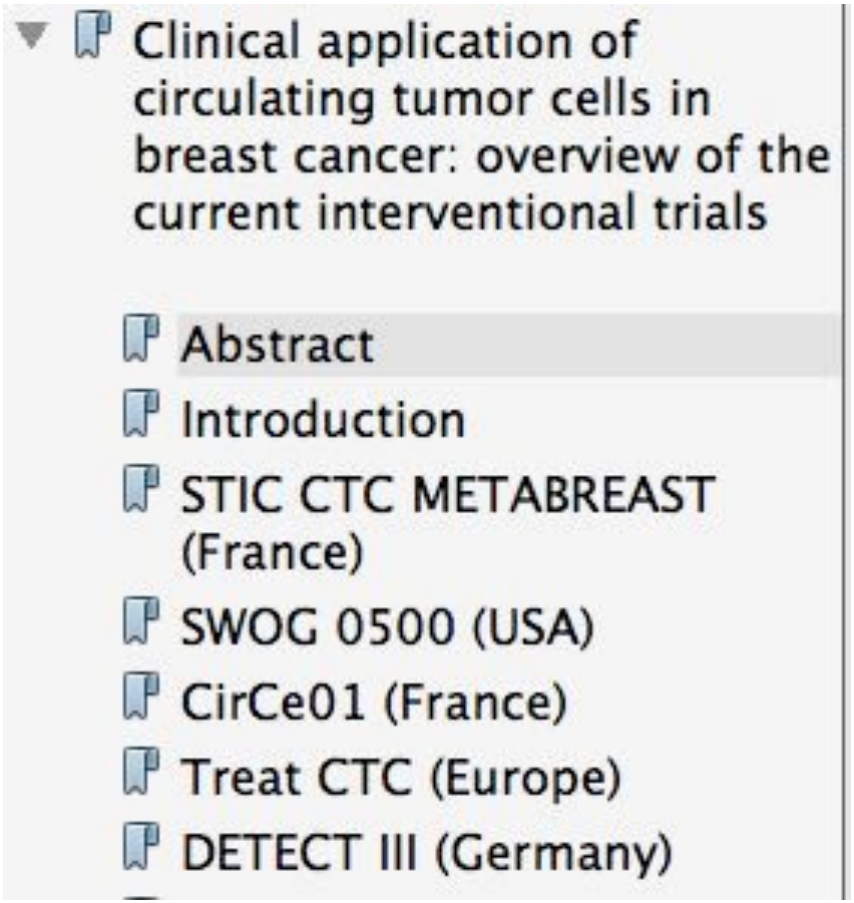
2014



CTC count also improves prognosis definition of metastatic breast cancer when added to existing clinico-pathological predictive models, whereas serum tumor markers do not (CEA, Ca15.3)

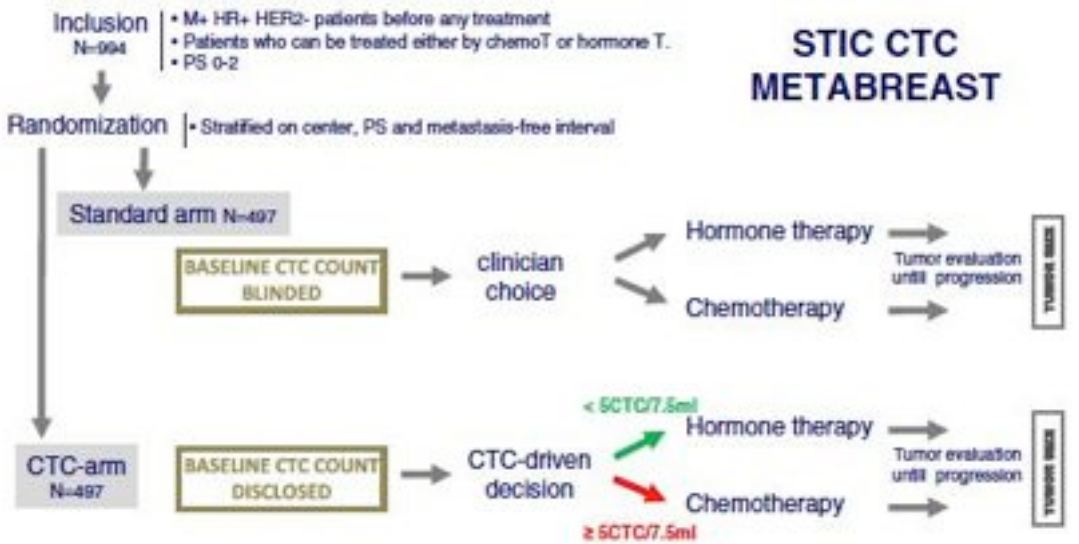
FC Bidard, DJ Peeters, T Fehm et al ***Lancet Oncol 15: 406, 2014***

Future clinical perspective: breast cancer



FC Bidard, T Fehm, M Ignatidianis et al *Cancer Metastasis Rev 32:179; 2013*

- Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials
  - Abstract
  - Introduction
  - STIC CTC METABREAST (France)
  - SWOG 0500 (USA)
  - CirCe01 (France)
  - Treat CTC (Europe)
  - DETECT III (Germany)



M1 ER+ pz; CTC-guided hormone therapy vs chemotherapy decision in M1 patients

FC Bidard, T Fehm, M Ignatidianis et al *Cancer Metastasis Rev 32:179; 2013*

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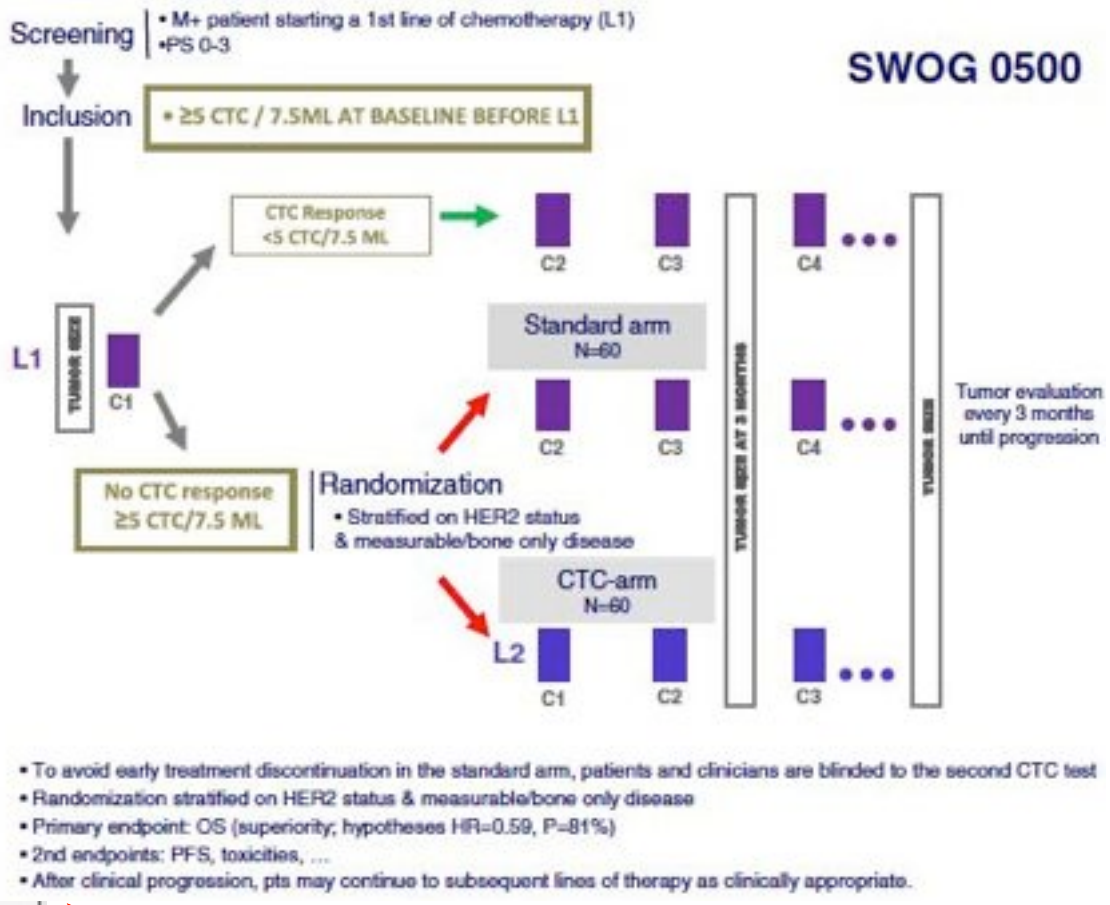
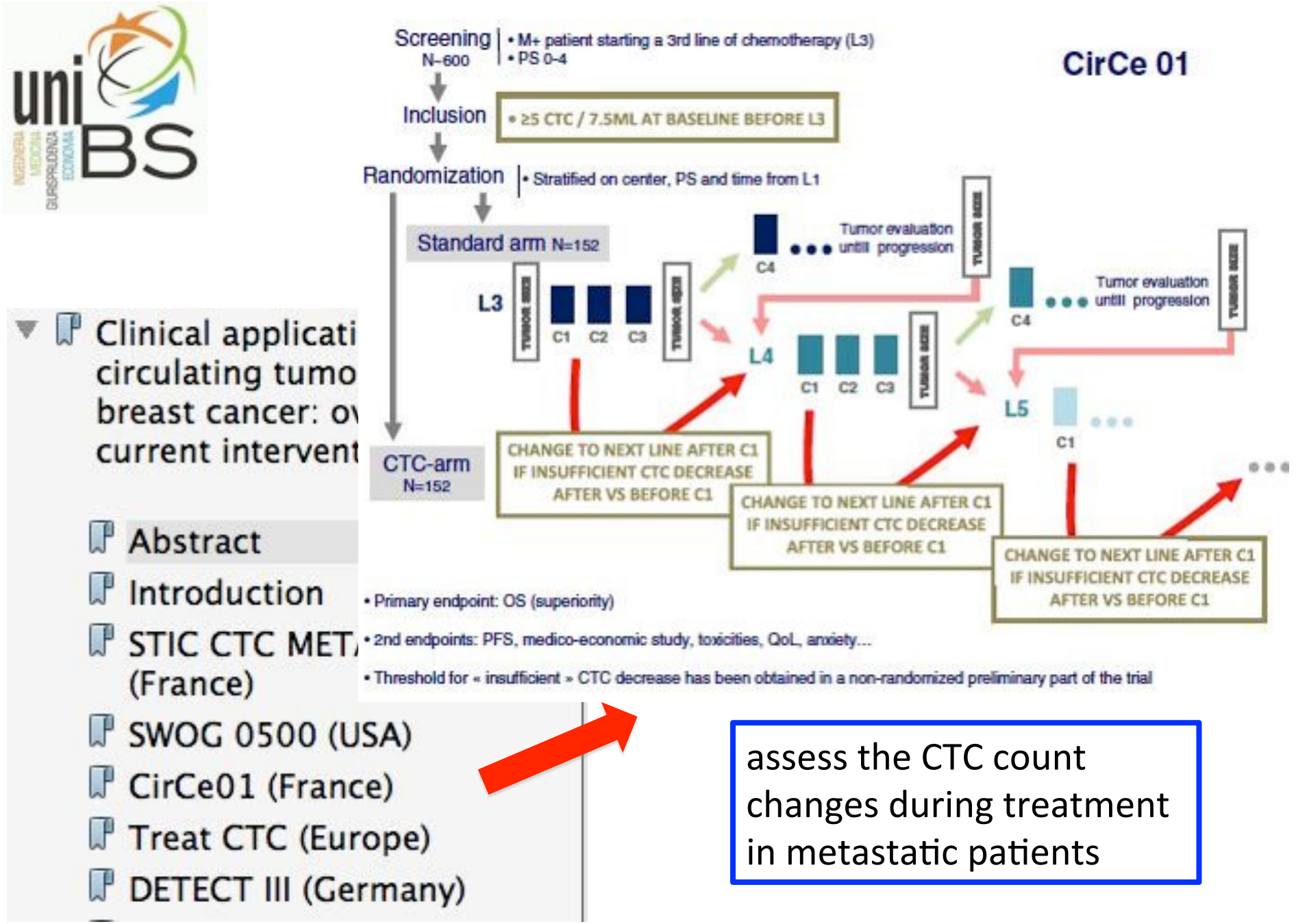


assess the CTC count changes during treatment in metastatic patients

FC Bidard, T Fehm, M Ignatidianis et al *Cancer Metastasis Rev 32:179; 2013*



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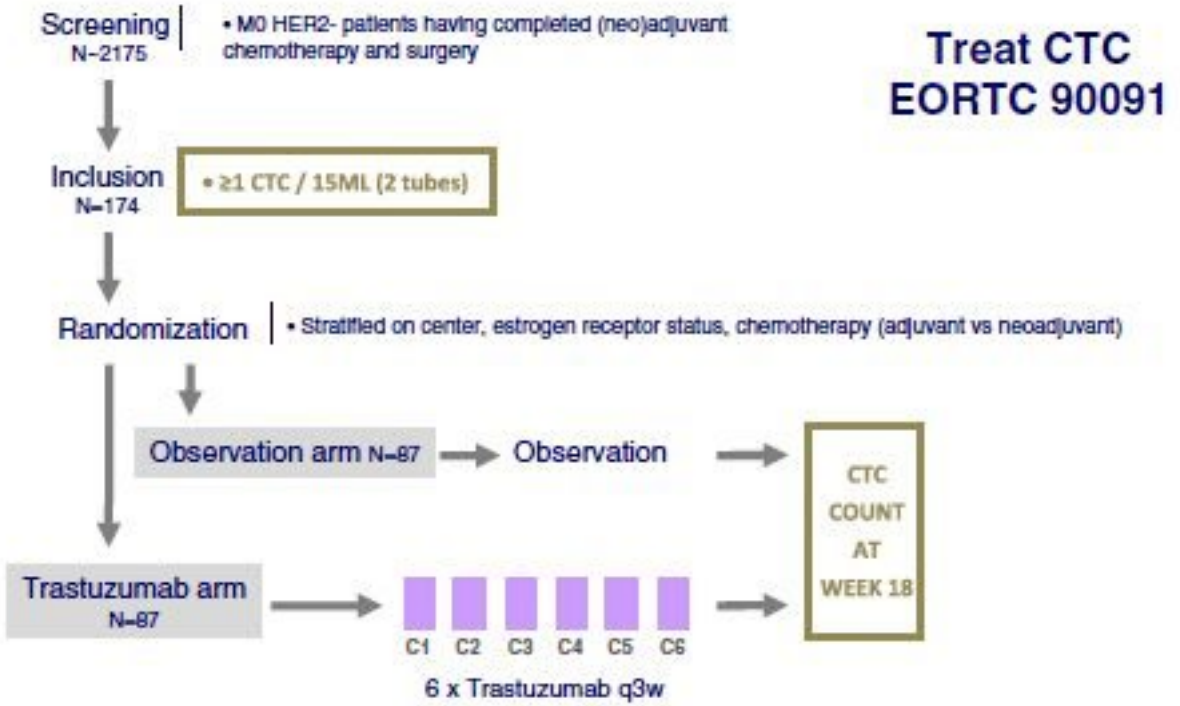
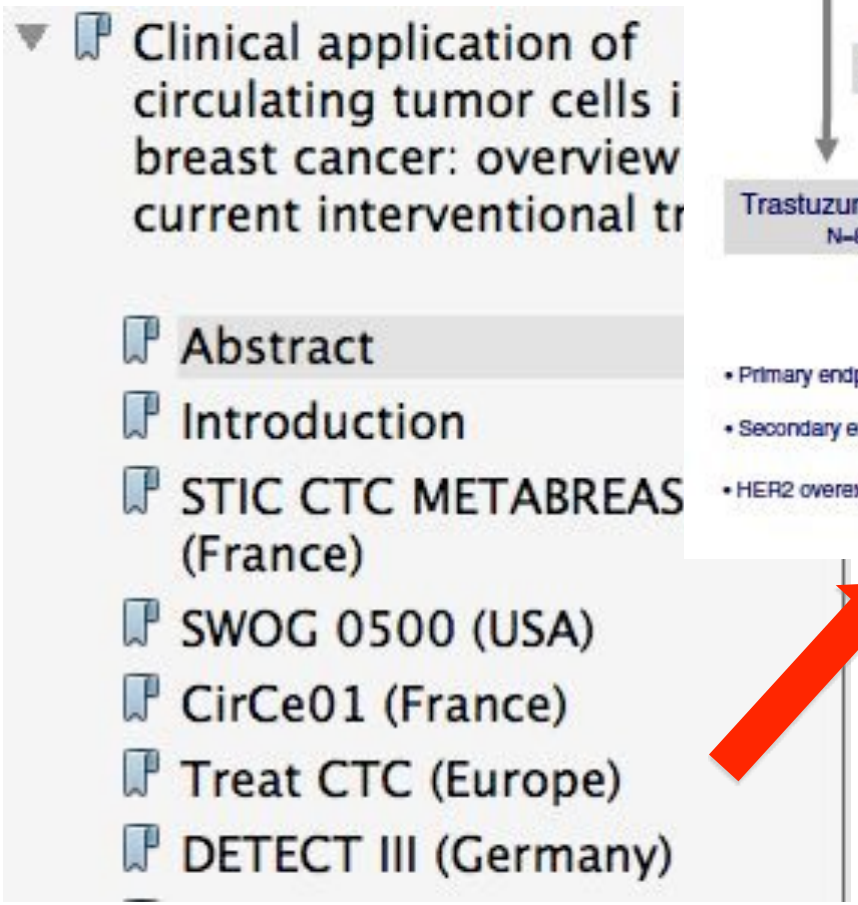
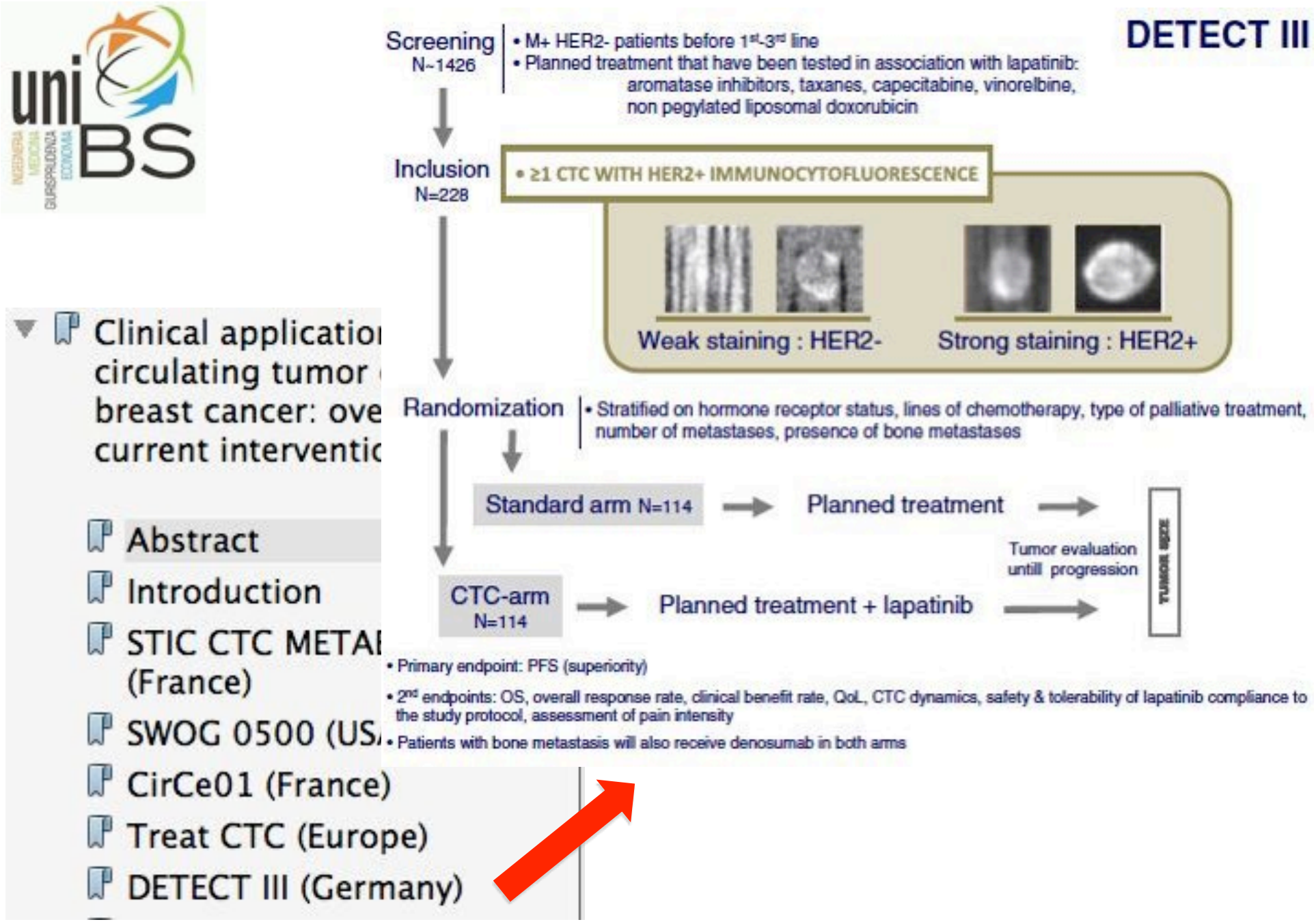




cM0(i+) patients, assess the use of anti-HER2 treatments in HER2 negative breast cancer patients selected on the basis of CTC detection/characterization

FC Bidard, T Fehm, M Ignatidianis et al *Cancer Metastasis Rev 32:179; 2013*



FC Bidard, T Fehm, M Ignatidianis et al *Cancer Metastasis Rev 32:179; 2013*

Future clinical perspective: breast cancer

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Waiting for the results

Future biological perspective: breast cancer

✓ **Identify CTC subpopulations**

- *Stem like CTC*:

  stem cells signature ($CD44^{+}/CD24^{-/low}$) → poor prognosis

Problem: many of these stem like CTC do not have EpCAM expression but show evidence of EMT and would be missed by most epithelial platforms

- *Mesenchymal CTC*:

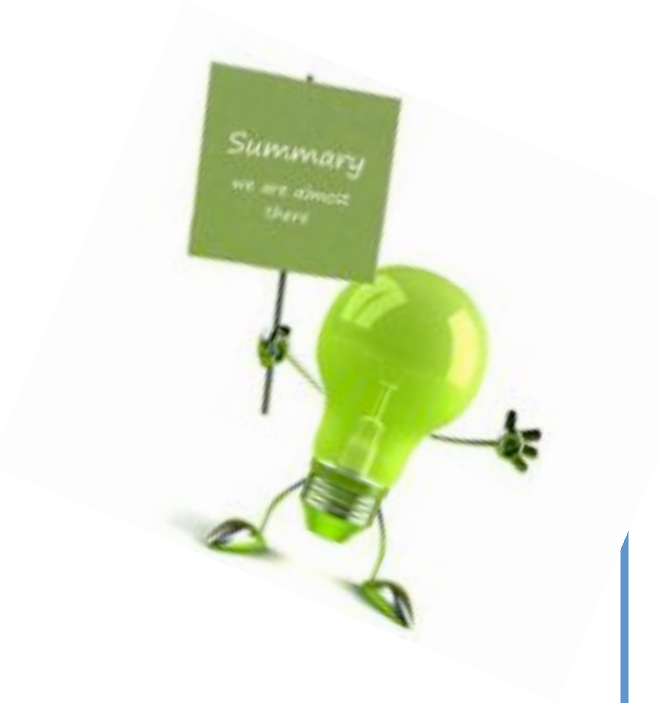
relevancy of EMT subpopulation to cancer progression, invasion and metastases

mRNA in situ hybridization to detect the presence of transcripts associated with either an epithelial or mesenchymal state → this status increase with resistance to target therapy

- Relationship between *cluster formation* and stemness, EMT, tumor progression

✓ ***CTC culture*** → …..determination of sensitivity to CT*;* xenotransplantation

TW Friedlander *Pharmacology &Therapeutics 2014*



- ✓ **the CTC: the concept behind CTC**

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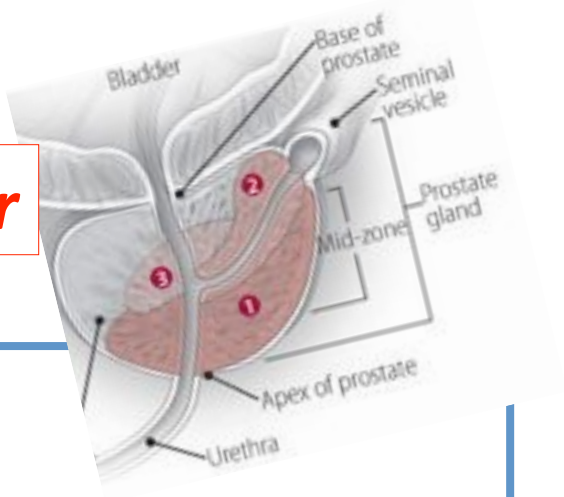
  - **head and neck cancer**

- ✓ **controversies**

prostate cancer



✓ **PSA is routinely used as serum marker**

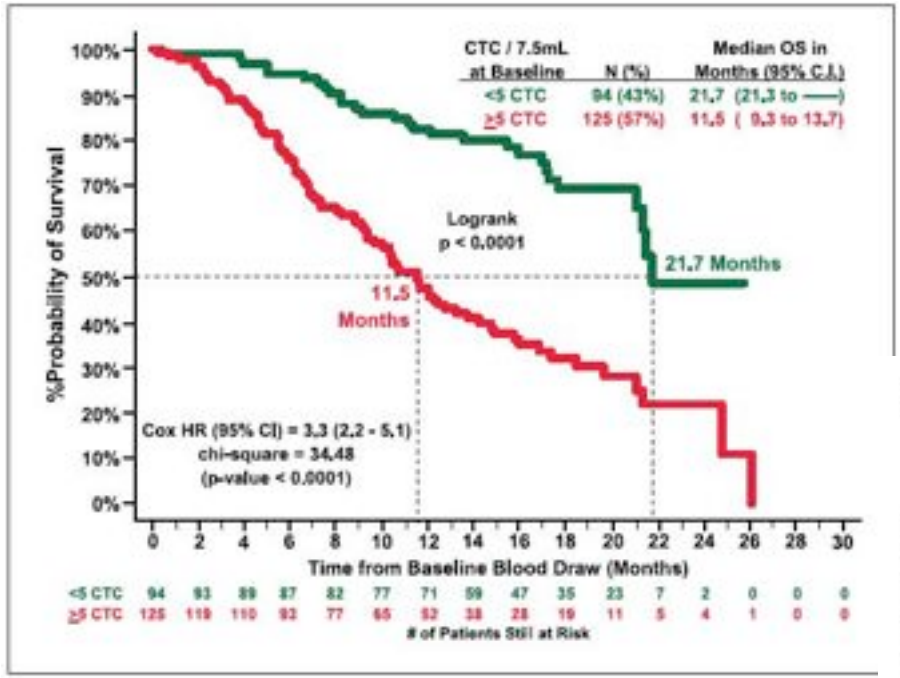
but

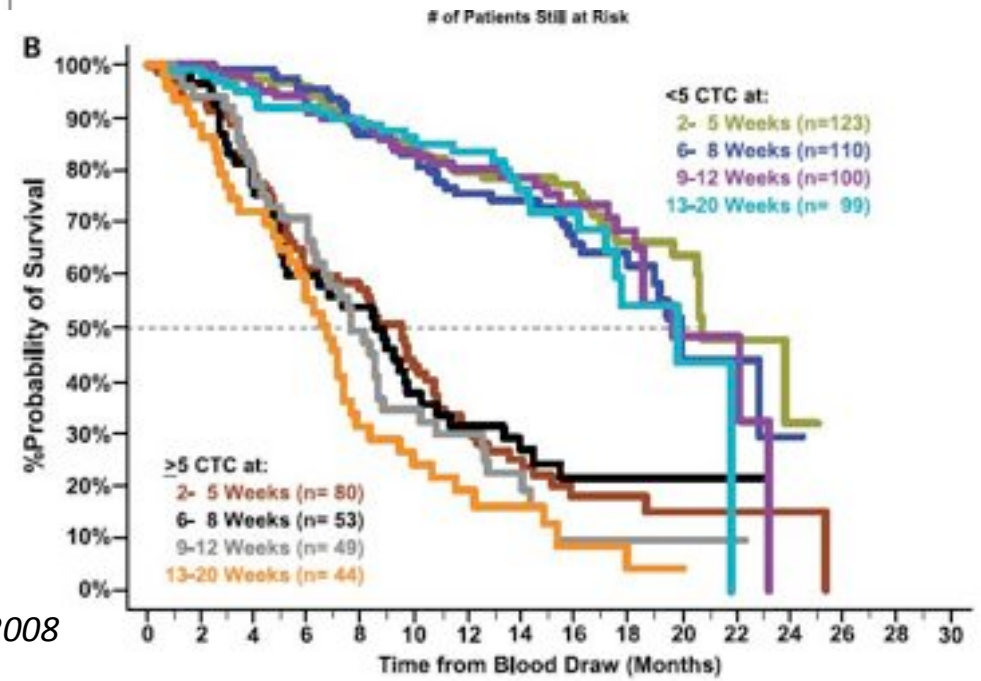
✓ **low positive predictive value in localized stage disease**
✓ **it is unable to differentiate stage**

✓**It is incoherent with imaging results in metastatic disease during treatment monitoring**
✓ **is weakly related with survival in castration-resistant PC with bone metastases, so it is not adequate to guide treatment in the first 12 weeks**

Metastatic castration-resistant prostate cancer

✓ **CTC can be used as a surrogate of survival ?**





JS de Bono, HI Scher et al *Clinical Cancer Res 14 (19): 6302, 2008*

Metastatic castration-resistent prostate cancer

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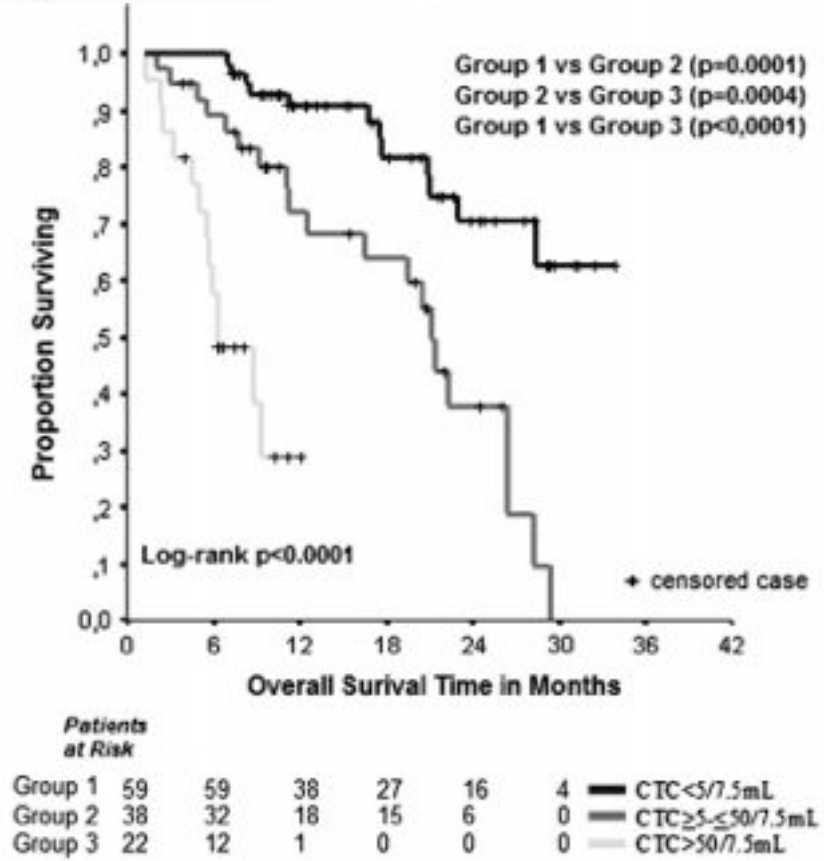

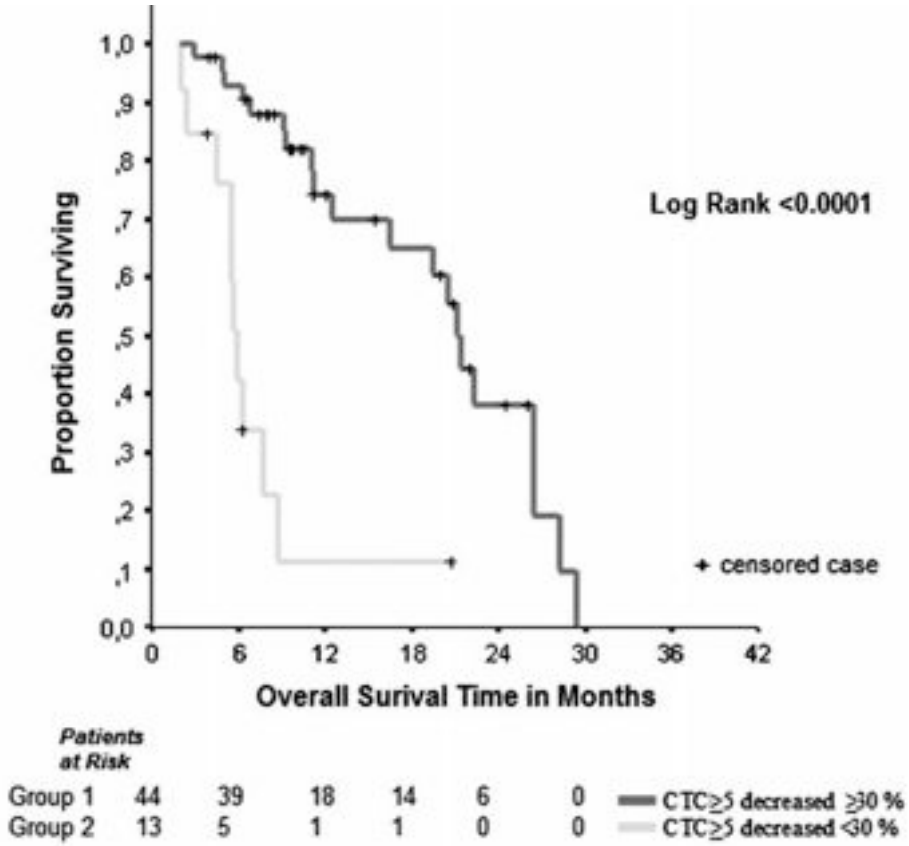

D Olmos, HT Arkenau et al *Annals Oncol: 20: 27, 2009*

CTC's as prognostic/predictive factor

Prostate M -

Prostate biopsy neg
PSA 2.5-10 ng/ml

EpCam linked method

Localized prostat cancer → radical prostatectomy			
CTC	**Caso**	**Controllo**	**p**
Pos	20/97 (21%)	5/25 (20%)	0.946
Neg	77/97 (79%)		
Pos→Pos	3/20 (16%)		0.51
Neg→Pos	8/77 (10%)		
Pos→ Neg	18/20		

✓No correlation with factors related to disease (GS, T stage)

JW Davis et al J of Urology 2008 179:2187

CTC's as prognostic/predictive factor

Prostate M -

EpCam linked method

Prostate biopsy neg PSA 2.5-10 ng/ml

Localized prostat cancer → radical prostatectomy			
CTC	Caso	Controllo	p
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Neg	77/97 (79%)		
			0.51
Po	/20		

Fizazi K, Morat L, Chauveinc L, Prapotnich D, De Crevoisier RD and Escudier B: High detection rate of circulating tumor cells in blood of patients with prostate cancer using telomerase activity. Ann Oncol 2007; **18:** 518.

55/70 (79%)

from 20 to 100 CTCs/ ml

✓No correlation

Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L et al: Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 2007; **450:** 1235.

CTC in different stages of prostate cancer

✓ 20 Locally advanced prostate cancer (no RT o OT)	✓ before radical prostatectomy
✓ 40 castration resistant metastatic (PSA raising or M1 during OT) → docetaxel	✓ before the first cycle of docetaxel
✓ 15 taxane refractory	✓ at diagnosis of progression

M Thalgott, B.Rack et al *J Cancer Res Clin Oncol 139: 755, 2013*

CTC in different stages of prostate cancer

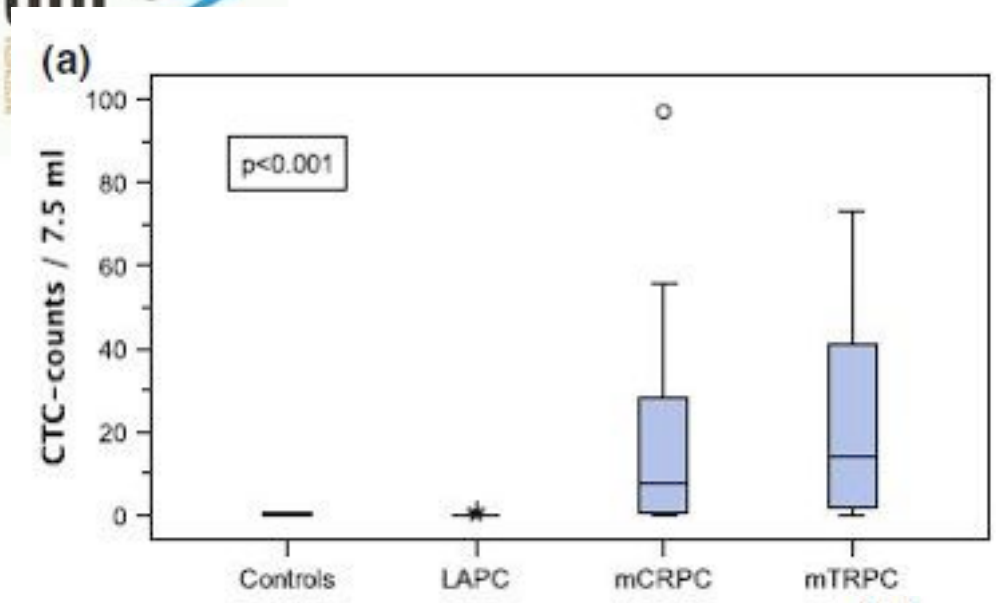
✓ **20 Locally advanced prostate cancer**

Median risk of tumor recurrence of 90 % (range 45–95 %) within 5 years according to the Kattan nomogram.

Despite an elevated preoperative median PSA level of **21 ng/ml** and a tumor stage **of >=cT3a in 95 % of LAPC cases**, **only one subject (5 %) presented a characteristic CTC**. Hence, LAPC patients displayed no difference in CTC counts compared to controls (p = 0.66).

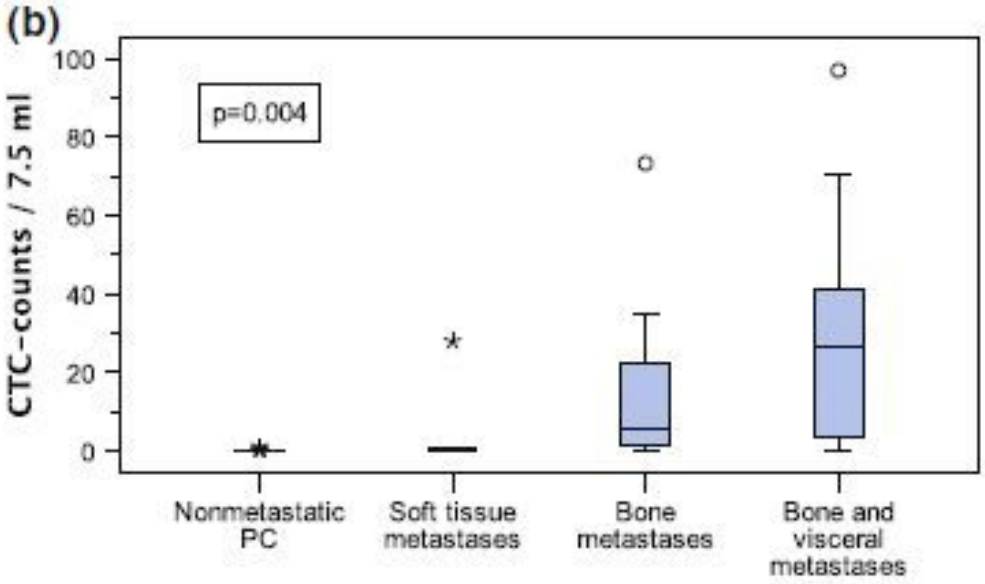
M Thalgott, B.Rack et al *J Cancer Res Clin Oncol 139: 755, 2013*

CTC in different stages of prostate cancer



Metastatic : CR or TR

CTC counts showed no correlation with bone tumor burden, but a significant association with PSA-DT (p=0.01)



M Thalgott, B.Rack et al *J Cancer Res Clin Oncol 139: 755, 2013*

CTC in different stages of prostate cancer

Metastatic : CR or TR

✓CTC counts showed no correlation with bone tumor burden

✓significant association with PSA-DT (p=0.01) (first report)

confirmed the association with
✓LDH (p=<0.001)
✓ ALP (p=<0.001)
✓ low Hb (p=0.004)

M Thalgott, B.Rack et al *J Cancer Res Clin Oncol 139: 755, 2013*

CTC in different stages of prostate cancer

What about biochemical recurrence?

RUOLO DELLE CELLULE TUMORALI CIRCOLANTI NEL
CARCINOMA PROSTATICO IN PROGRESSIONE SIEROLOGICA

STUDIO OSSERVAZIONALE PROSPETTICO

U.O. Oncologia Medica, Spedali Civili di Brescia, Università degli Studi di Brescia.

U.O. Radioterapia, Spedali Civili di Brescia, Università degli Studi di Brescia

U.O. Urologia, Spedali Civili di Brescia, Università degli Studi di Brescia

Laboratorio manipolazione e Criopreservazione Cellule Staminali, Servizio di Immunoematologia e Medicina Trasfusionale, Spedali Civili di Brescia

CTC in different stages of prostate cancer

What about biochemical recurrence?

Obiettivo primario:

Obiettivo primario è valutare, nei pazienti con carcinoma prostatico in progressione biochimica, il ruolo prognostico e predittivo delle cellule tumorali circolanti, cioè l'impatto della presenza e del numero di CTC sulla sopravvivenza globale, sulla mortalità tumore specifica e sulla probabilità di sviluppare una malattia metastatica.

Obiettivi secondari

Obiettivi secondari sono la valutazione del ruolo dei cambiamenti delle CTC come segno di risposta alla terapia e il ruolo delle CTC durante terapia e in occasione della ripresa del PSA, come marcatore predittivo di malattia metastatica.

Future perspective: prostate cancer

1. CHANGES IN CTC COUNTS AS SURROGATE OF SURVIVAL IN CRPC

- biomarker is evaluated in therapies that ***provide survival benefit***
- the treatment has ***an effect on the proposed biomarker***
- the biomarker has an effect on the clinical endpoint
- the ***full effect of treatment on the endpoint is captured by the biomarker***
- these criteria must be met in a number of large prospective trials, and a meta analytic approach must prove surrogacy at the trial level as well as at individual level

Future perspective: prostate cancer

2. MOLECULAR CHARACTERIZATION OF CTC

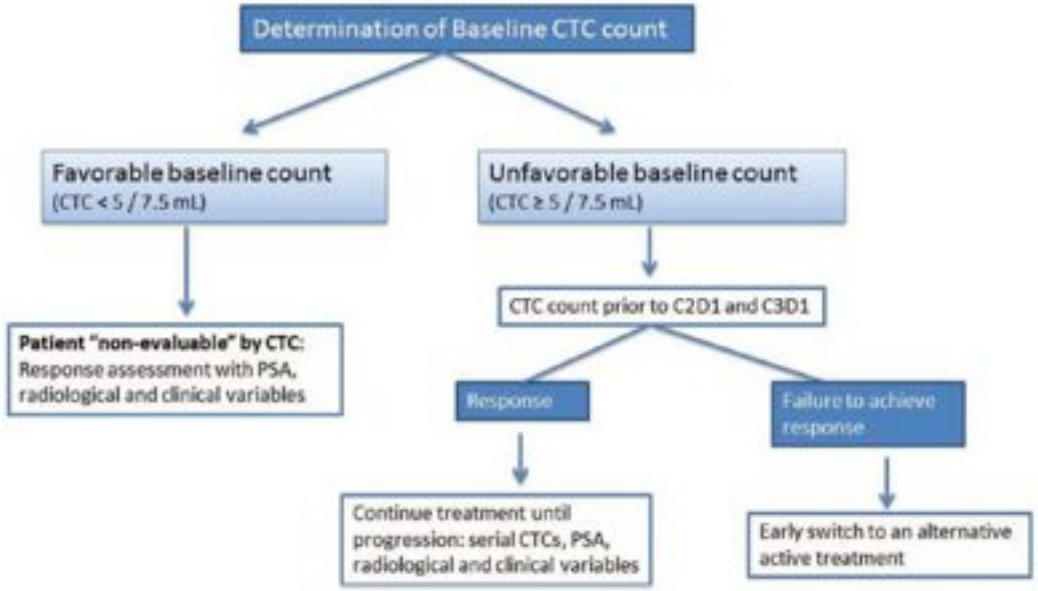
- **Qualitative assessment of CTC at genomic and proteomic levels could provide an insight into biologic processes of the disease** and could have applications in diagnostic, staging, biomarker discovery, and treatment individualization

- **CTC could permit longitudinal analyses by collecting sequential samples over time to assess the effect of treatments in tumor evolution**.

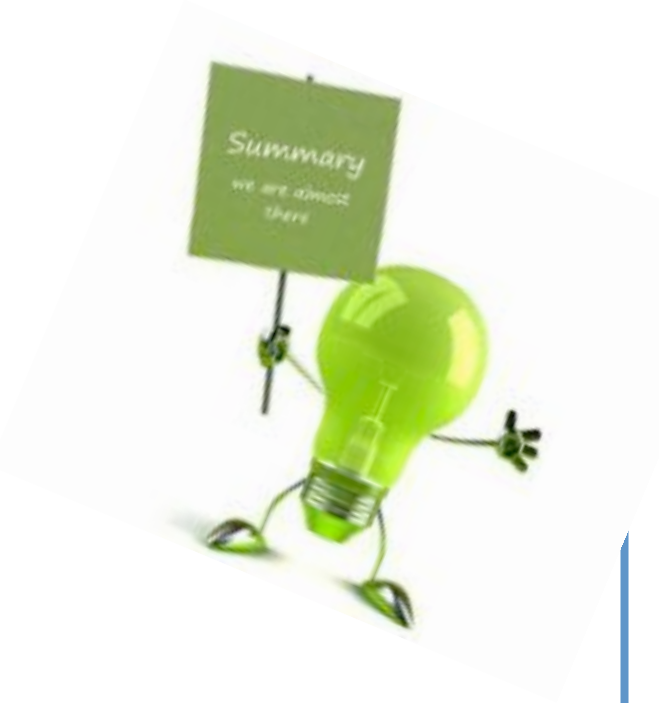
- molecular characterization of CTCs in parallel to **new drug development** should bring advances in the current lack of biomarker driven individualization of treatment (eg: erythroblast transformation-specific–related gene (*ERG)-based translocations, PTEN, PI3K/AKT)*

Future perspective: prostate cancer

4. PROPOSAL OF "CLINICAL DECISION MAKING " ALGORITM"



D Lorente, J Mateo, JS de Bono *asco.org/edbook | 2014 ASCO EDUCATIONAL BOOK e197*

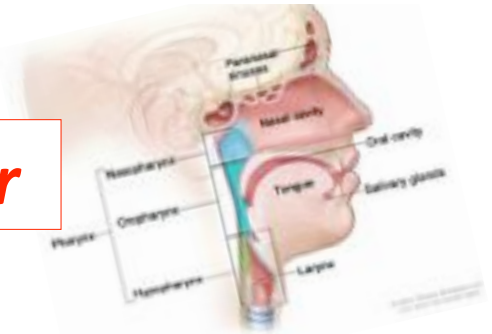


- ✓ **the CTC: the concept behind CTC**

- ✓ **experiences and perspectives:**
  - **breast cancer**
  - **prostate cancer**
  - **head and neck cancer**

- ✓ **controversies**

Head and neck cancer



✓ **patients treated locally advanced head and neck tumors (LAHNC) frequently show recurrence after complete response (CR) (20-30%);**
✓**10% of newly diagnosed patient already present with distant metastases**

✓ **the main already defined prognostic system is the tumor-node-metastasis (TNM) schema**
✓ **other biological prognostic factors are EGFR (epidermal growth factor receptor) or HPV (human papilloma virus) positivity**

Great demand for a less invasive method to determine the state of disease

✓**identification of CTC in the blood could ameliorate the prognostic profile**

Correlation with clinico-pathological features

- **T → data about primary site are not comparable in the different studies[1]**
- **T1-4 → trend to an increase in the number of CTC[1] along with increasing T stage**

- **N → the frequency of CTC is significantly increased in cases with worse lymph-nodal status [2]**

- **Stage → 18% pos in stage IV vs 6% in other stages[1]**

- **As a prognostic marker → to define the risk profile of each single patient → better DFS in patients without CTC (p=0.04)[3]**

1) **M Buglione, S Grisanti, C Almici et al** *Eur J Cancer 48:3019, 2012*
2) **T Hristozova, Konschak R et al** *Ann Oncol 22(8): 1878, 2011*
3) **Jatana KR, P Balasubramanian, JC Lang et al** *Arch Otolaryngol Head Neck Surg 136: 1274, 2010*

Correlation to the treatment

• **response → Partial or complete response in patients with decreased number of CTC or negative for CTC (p=0.017)**[(1)]

correlation biology/treatment → Before treatment, CTC were detected in 9 /31 patients (29%). EGFR was detected in 55% of the CTC + cases. CTC detection was not influenced by induction CHT, but an observed RT-induced increase in CTC numbers was less pronounced when radiotherapy was combined with cetuximab compared to its combination with cisplatin/ 5-fluorouracil. The former treatment regimen was also more effective in reducing pEGFR expression in CTCs. [(2)]

1) **M Buglione, S Grisanti, C Almici et al** *Eur J Cancer 48:3019, 2012*
2) **I Tinhofer, T Hristozova, C Stromberger** *IJROBP 83(5): e685, 2012*

Conclusions…..and controversies

✓Breast cancer

- Prognostic and predictive value of CTC is well defined in metastatic breast cancer
- it is not still clear their utility in non metastatic patients
- clinical prospective application under investigation

✓Prostate cancer

- Clear data in metastatic disease
- Poor data in locally advanced disease
- Clinical prospective application not evident

✓H&N cancer

- Preliminary data on locally advanced disease
- No published data on metastatic disease
- Very far from clinical application

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The future: Not only IDENTIFY CTC but CHARACTERIZE them

Much remain to be learned about CTC'S and their clinical potential as biomarkers and therapeutic target

✓ **CTC** are larger than the blood vessels… the site of blood collection for CTC detection may be critical

✓ lymphatic spread is still poorly understood but could also be a route for tumor cell dissemination

✓Not all CTC are clinically relevant (can be present in benign infiammatory disese)

Plaks et al *Science 341: 341, 2013*

Much remain to be learned about CTC'S and their clinical potential as biomarkers and therapeutic target

- ✓ CTC with high metastatic potential might be CSC's
- ✓ Ongoing discussion on whether tumor cells undergo EMT during dissemination resulting in a more mesenchymal or more stem like phenotype
- ✓ That some CTC's are undetectable and not all detected CTC's have metastatic potential indicate that enumeration is not a good marker for disease staging and prognosis
- ✓ It is important to define the genomic profile of CTC's
- ✓ **The potential clinical value of CTC's is clear but elucidating CTC biology is indispensable.**

Plaks et al *Science 341: 341, 2013*

Thank you for your attention