Cellules malignes circulantes : signification et conduite à tenir

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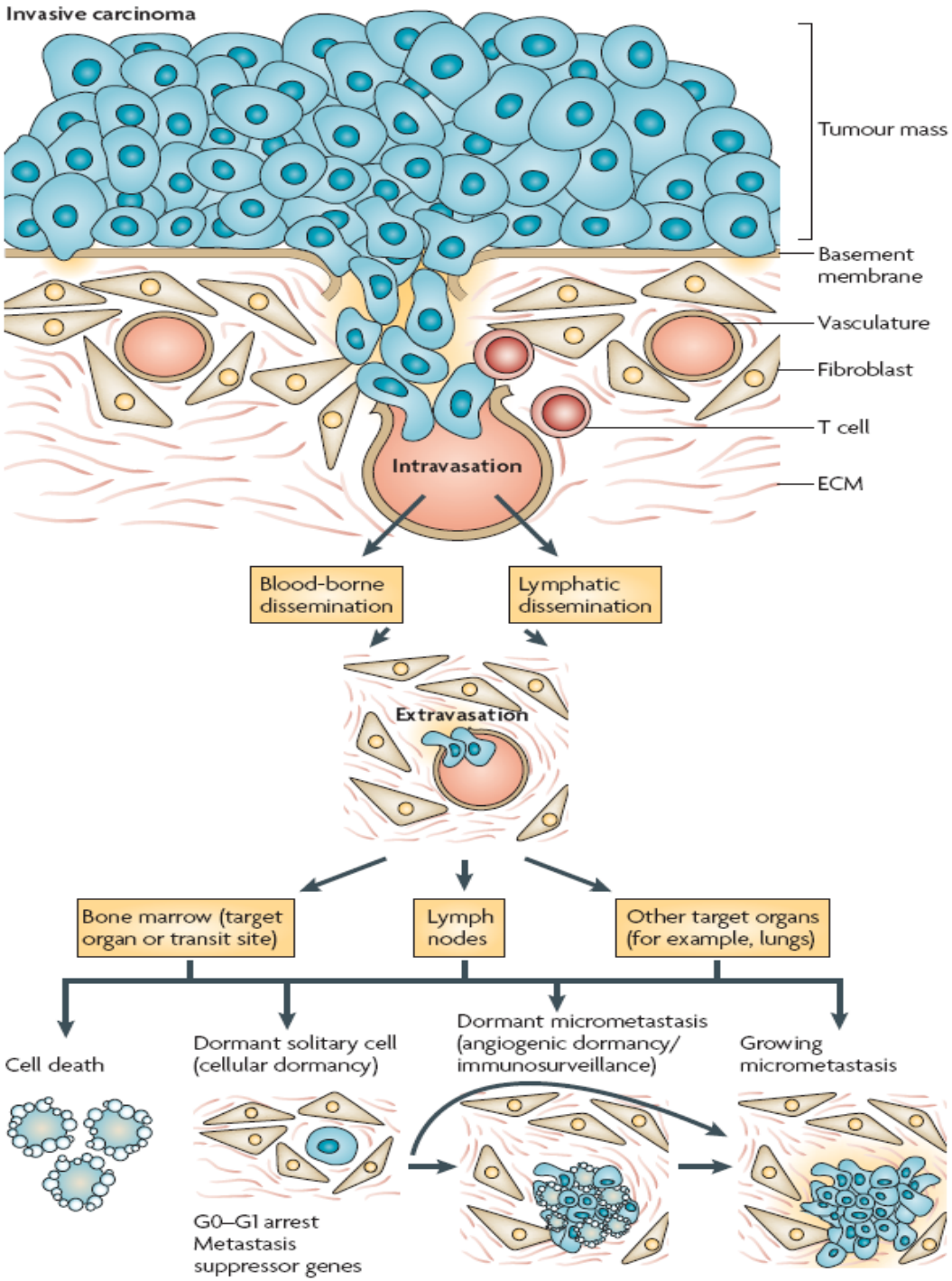
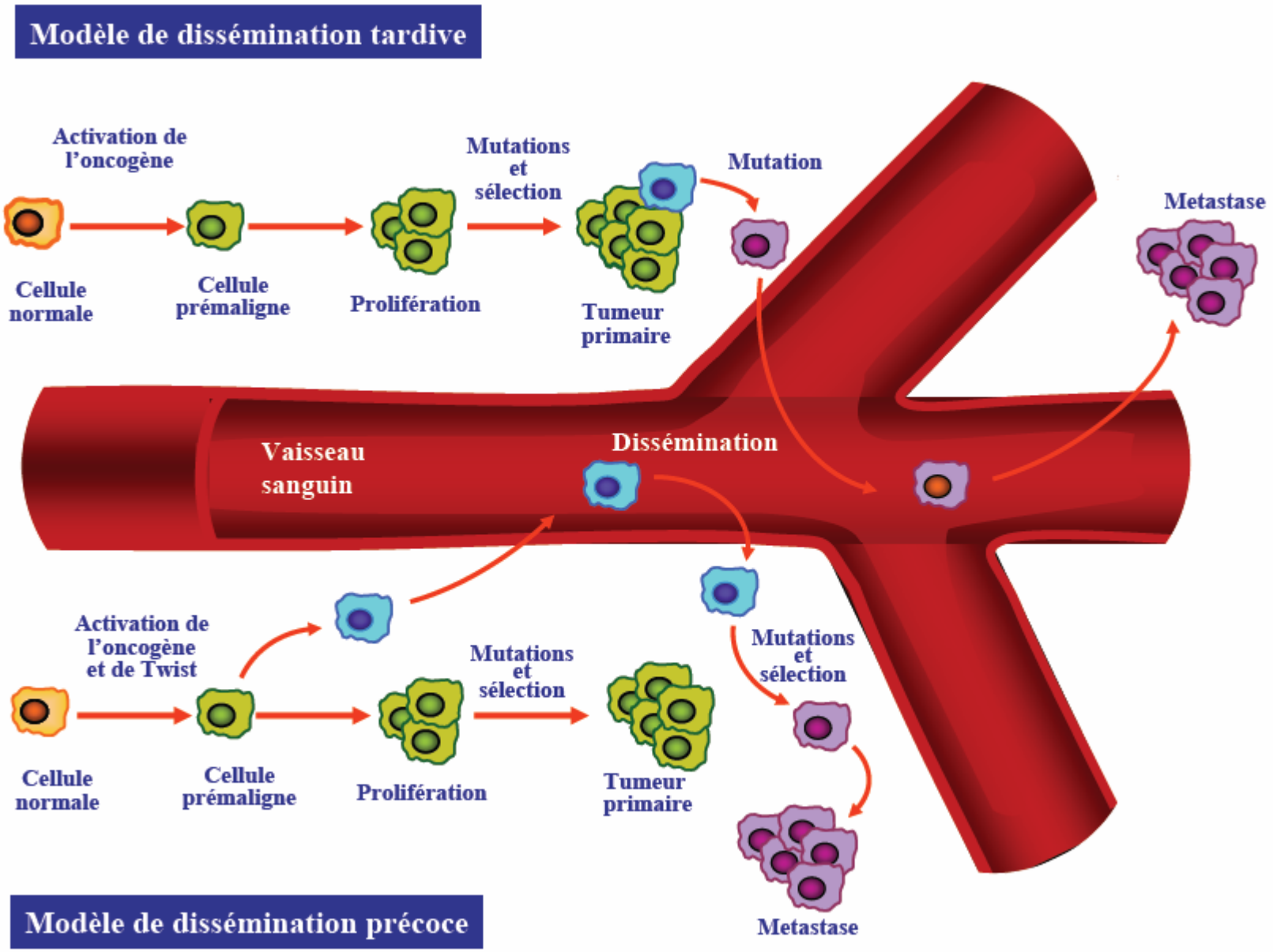


Dissémination métastatique

La dormance

Models, mechanisms and clinical evidence for cancer dormancy

Julio A. Aguirre-Ghiso, Nature Cancer Reviews, 2007



Endométriose?

Klein, C. Science 2008

Détection des cellules tumorales isolées

- **Sites de détection**
  - Moelle osseuse (Disseminated Tumor Cell, DTC)
  - Sang périphérique (Circulating Tumor Cell, CTC)
  - Ganglion (Sentinelle)pNi+
- **Méthodes de détection**
  - Immunocytochimie ICC
  - Sélection Immunomagnétique IMS
  - RT-PCR

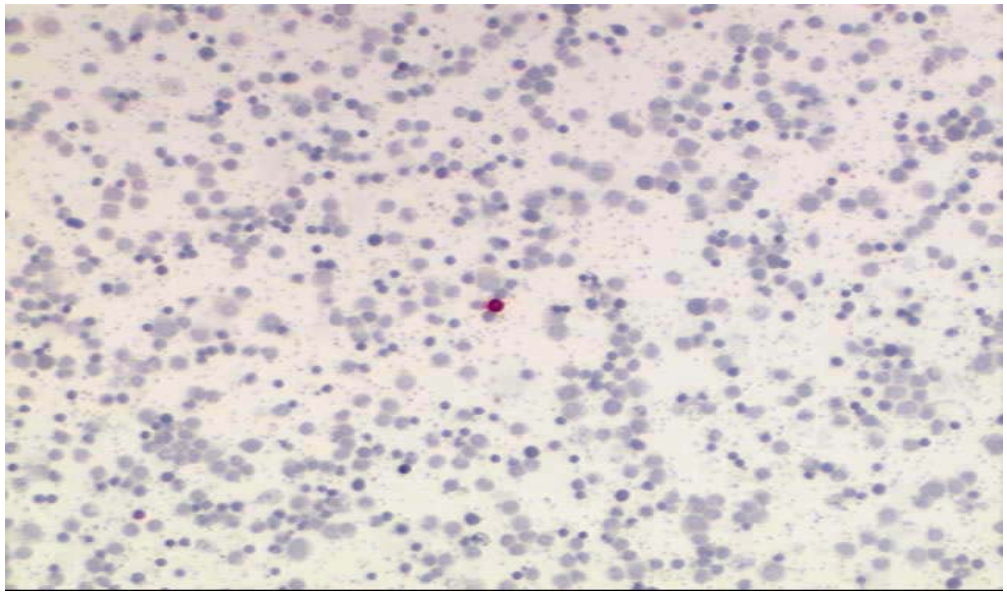
Disseminated Tumor Cells (moelle osseuse)

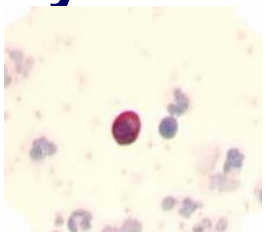
Ficoll

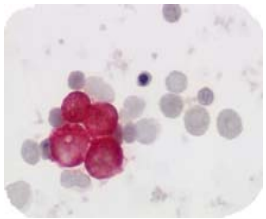
Cytospins

Marquage « épithélial » (A45B/B3 Ab)

Analyse morphologique



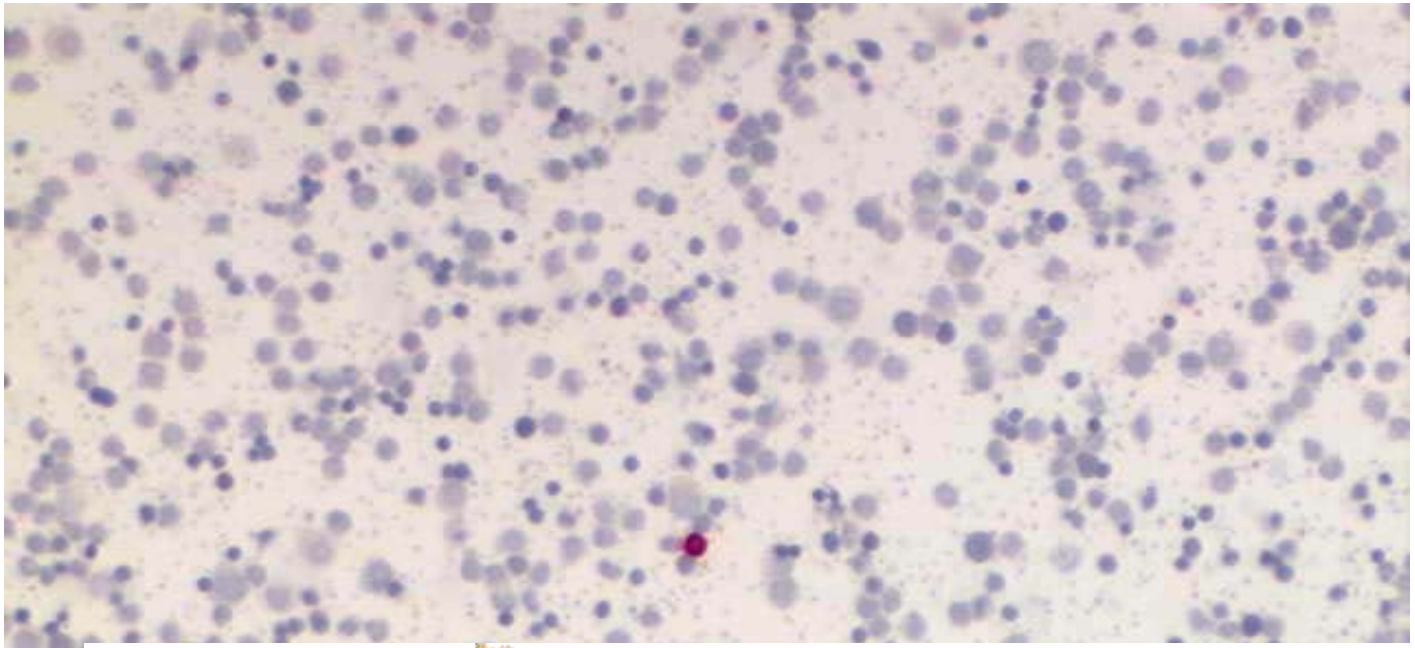


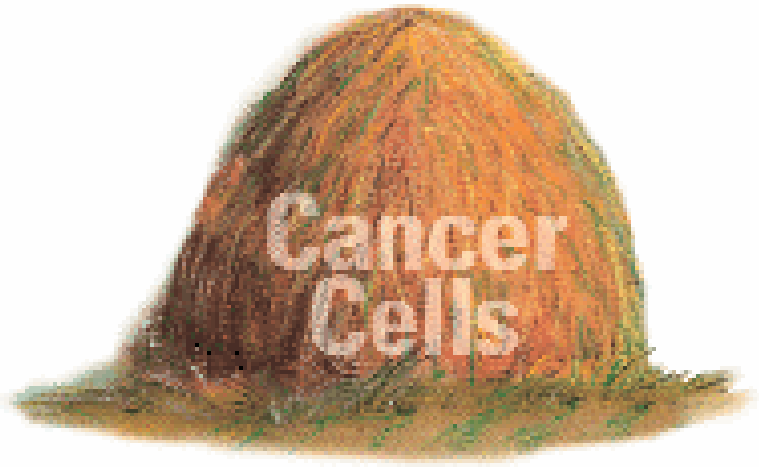


European consensus conference for DTC analysis

Borgen *et al*, Cytotherapy 1999

Cellule Cytokératine + isolée dans la moelle osseuse

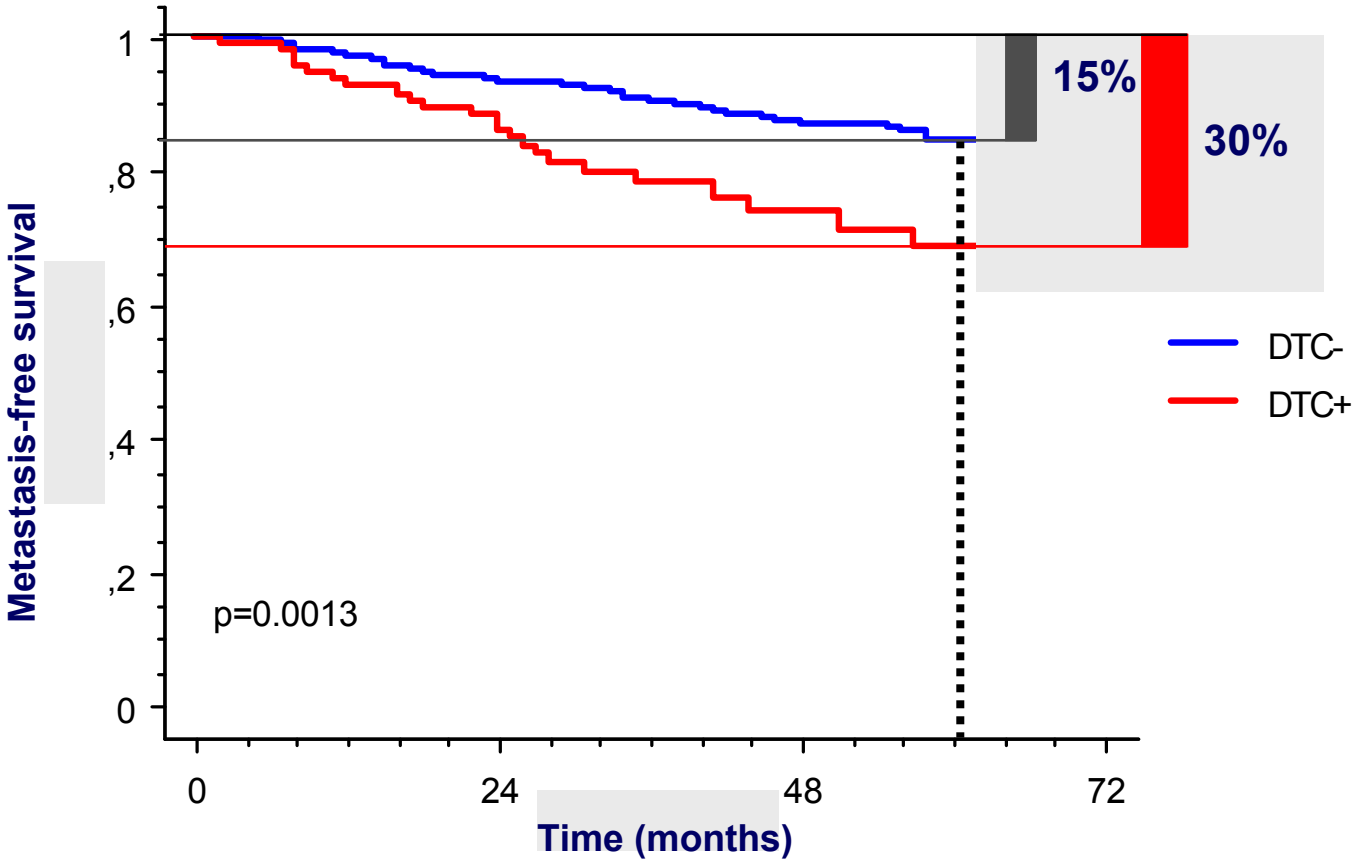




Disseminated Tumor Cells: Distant Metastase Free Survival

Adjuvant

N 621 pts

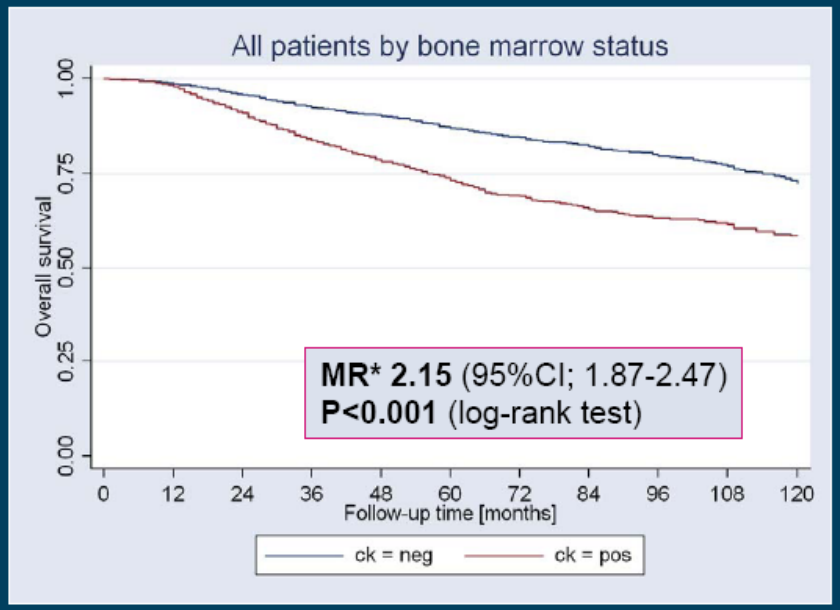


15.1% positivity rate

Bidard FC (…) Pierga JY, Clin cancer Res 2008

DTCs, cancers du sein M0

Pooled Analysis of ITC in Bone Marrow at Primary Diagnosis (n=4.703)



Median follow-up 62 months

Braun *et al*, NEJM, 2005

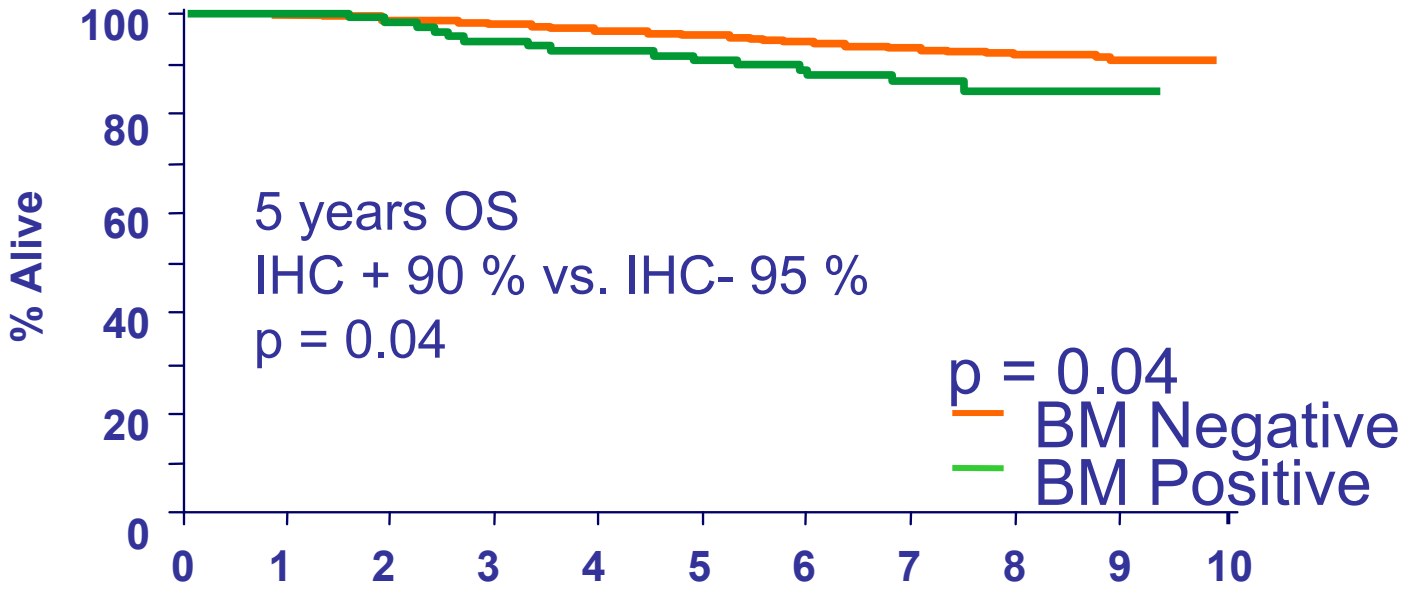
➔ **DTC = facteur pronostique indépendant**

➔ **Niveau de Preuve 1**

(mais ce n'est pas un test parfait !)

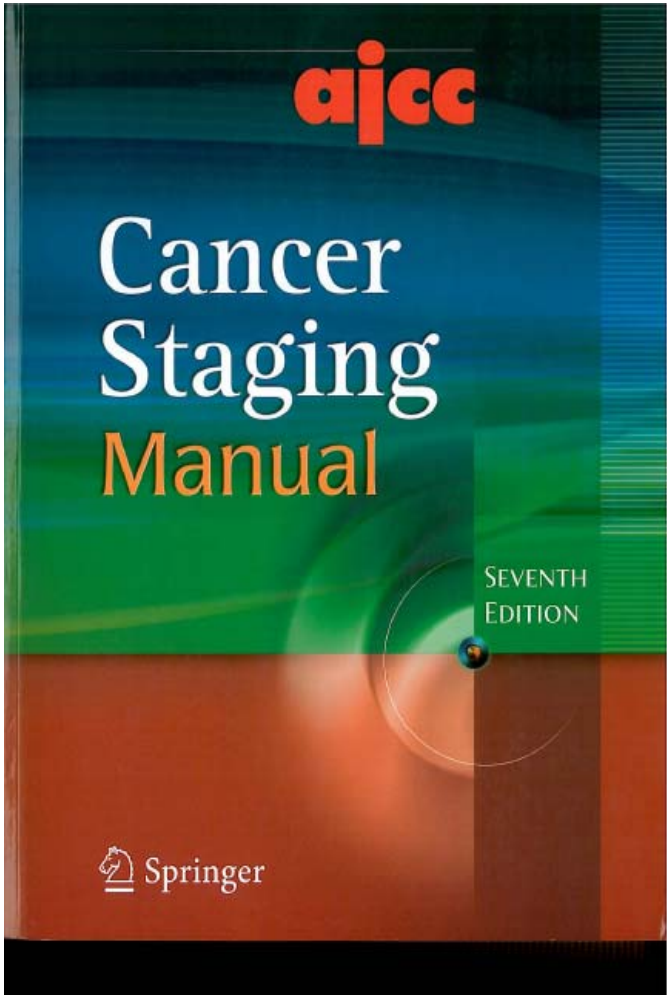
Essai ACOSOG Z0010
Survie globale pN0 N =3902

	Univariate		Multivariate	
Parameter	**HR (CI_{95})**	**p**	**HR (CI_{95})**	**p**
Sentinel Node **- IHC negative** **- IHC positive**	1.00 0.90 (0,59-1,39)	0.64	1.00 0.88 (0.45-1.71)	0.70
Bone Marrow (BM) **- IHC negative** **- IHC positive**	1.00 1.94 (1.02-3.64)	0.04	1.00 1.83 (0,79-4.26)	0.15



Giuliano AE, JAMA 2011;306(4):385-93

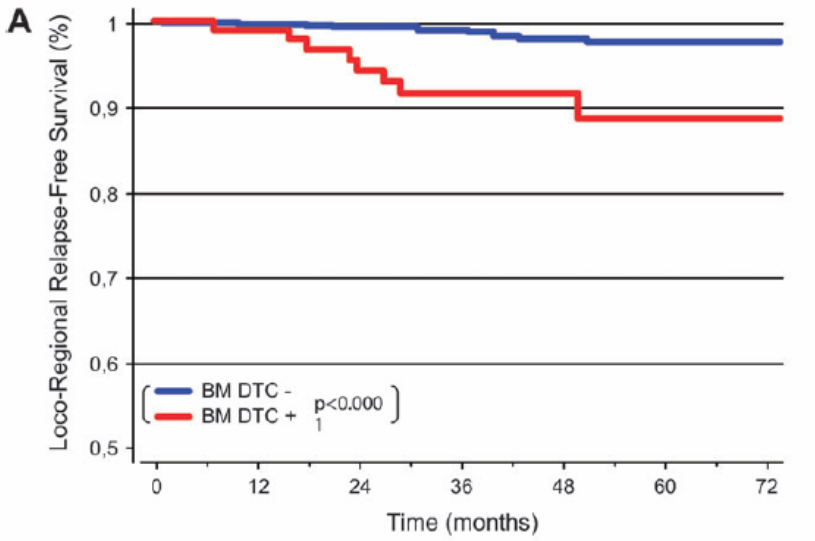
2010: DTC & CTC → nouvelle classe cM0(i+) !!!



Distant Metastases (M)	
M0	No clinical or radiographic evidence of distant metastases
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm

Recirculation des DTC vers le site initial ?

Les DTC dans la moelle sont associées à un + fort risque de récidive loco-régionale

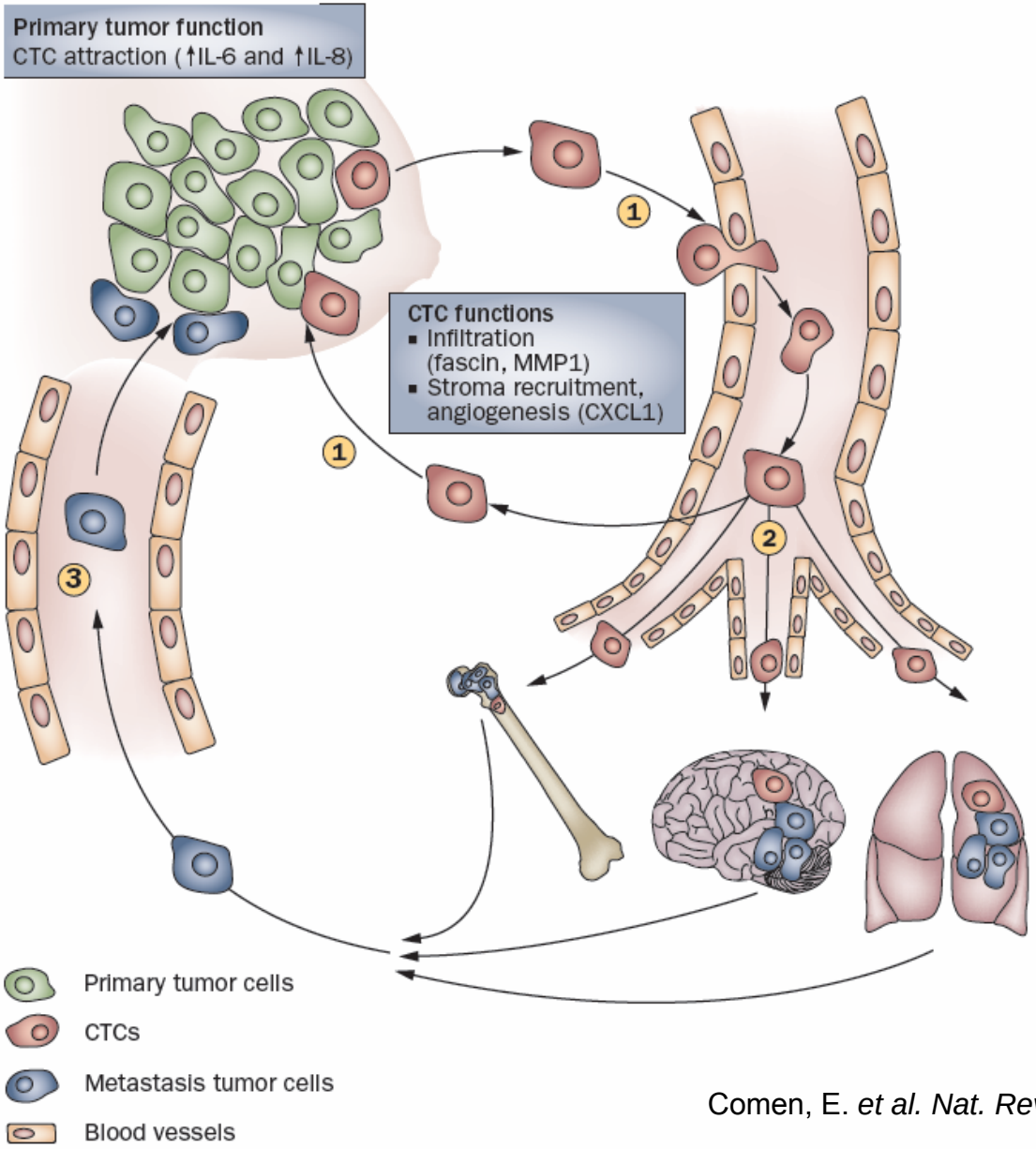


BM DTC-	527	516	471	391	282	206	97
BM DTC+	94	91	76	46	33	28	18

Bidard et al, Clin Cancer Res 2008

Bidard et al, Ann Oncol 2009

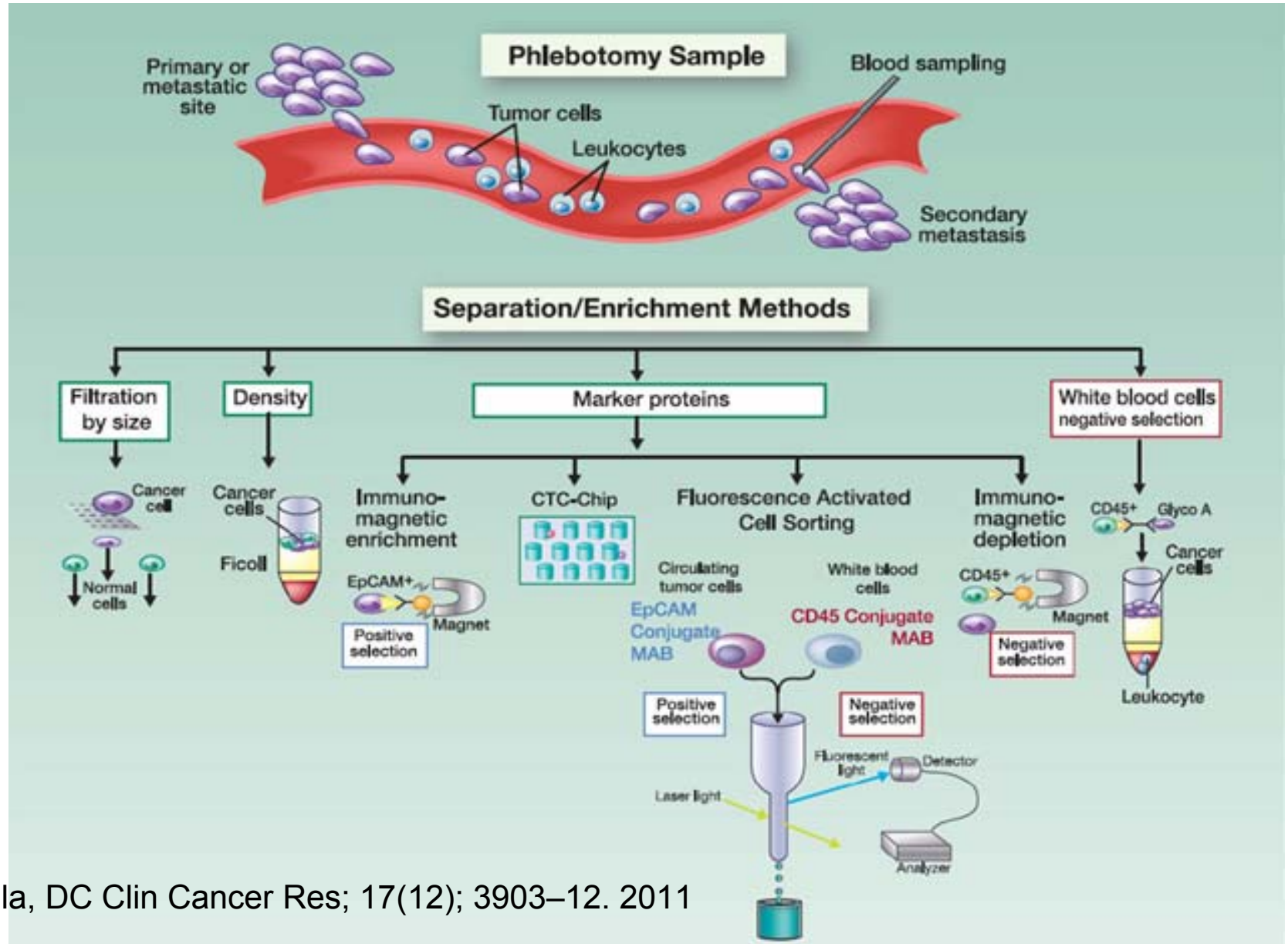
Self-Seeding: auto-ensemencement?



Kim & Massagué, Cell Dec 2009

Comen, E. *et al. Nat. Rev. Clin. Oncol. 8, 369–377 (2011)*

Techniques de détection des CTC dans le sang (> 50 in 2012)



Danila, DC Clin Cancer Res; 17(12); 3903–12. 2011

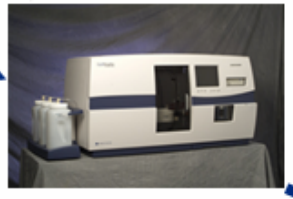
Détection des cellules tumorales circulantes

VERIDEX: CellSearch™ System

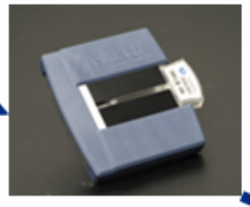
❶ Selection positive EPCAM
❷ Staining : CK 8/18/19, CD45, DAPI
❸ others: HER2/Neu, MUC1, EGF-R)

Sample Collection

Reagents:
CTC capture
& staining

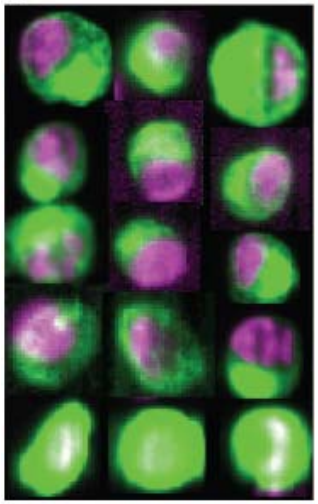


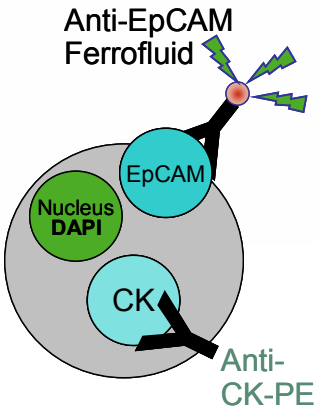
Sample
Processing



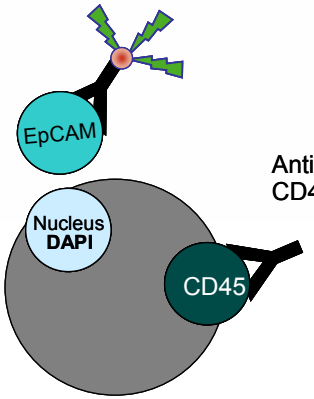
Sample
Presentation

Sample
Analysis



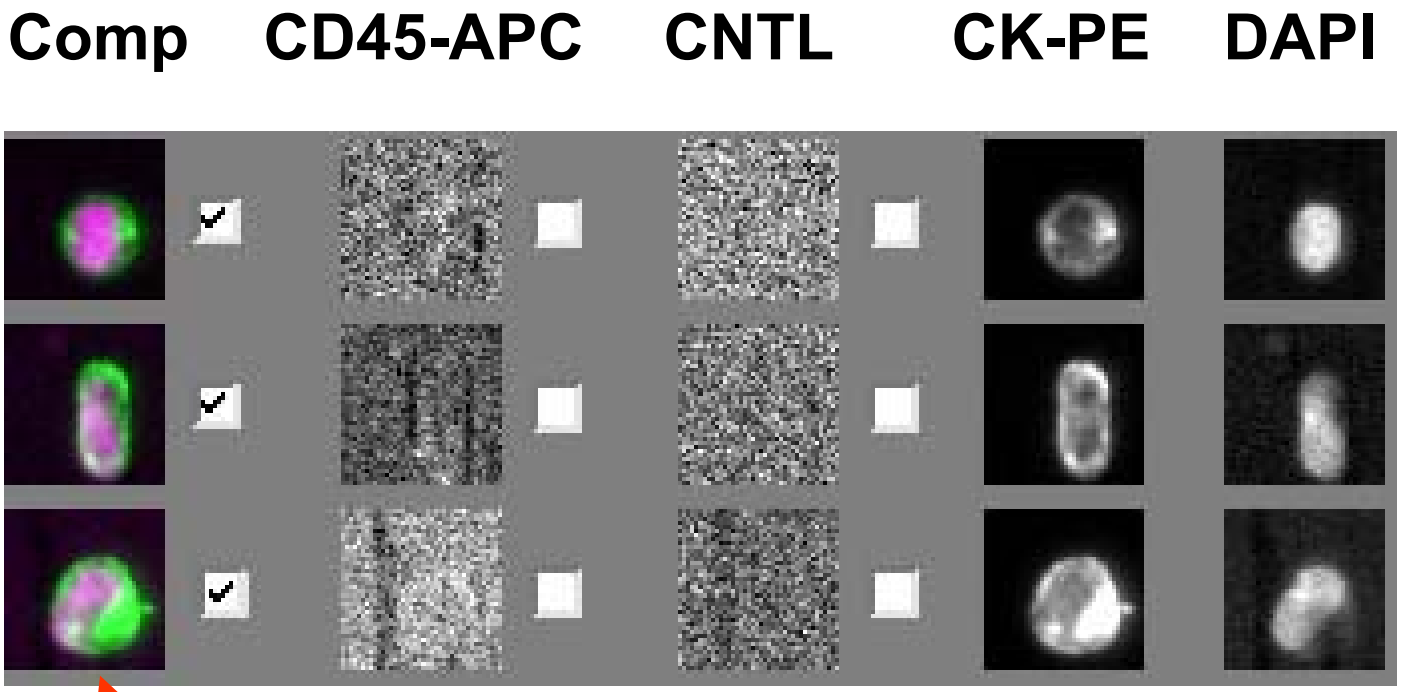


Epithelial
Cell



Leukocyte

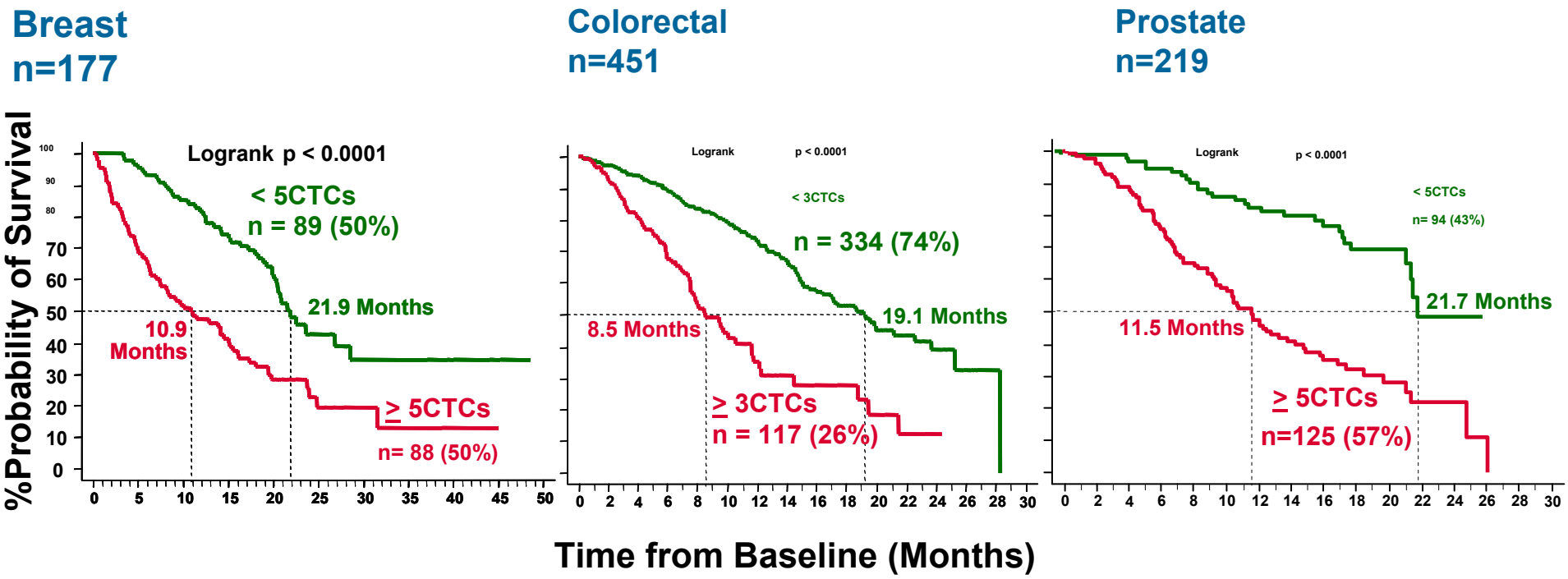
CellSpotter™ Cell Analysis



Intact Tumor Cells

CTCs with Cellsearch Before Therapy: Predicting OS at metastatic stage

Metastatic Carcinomas

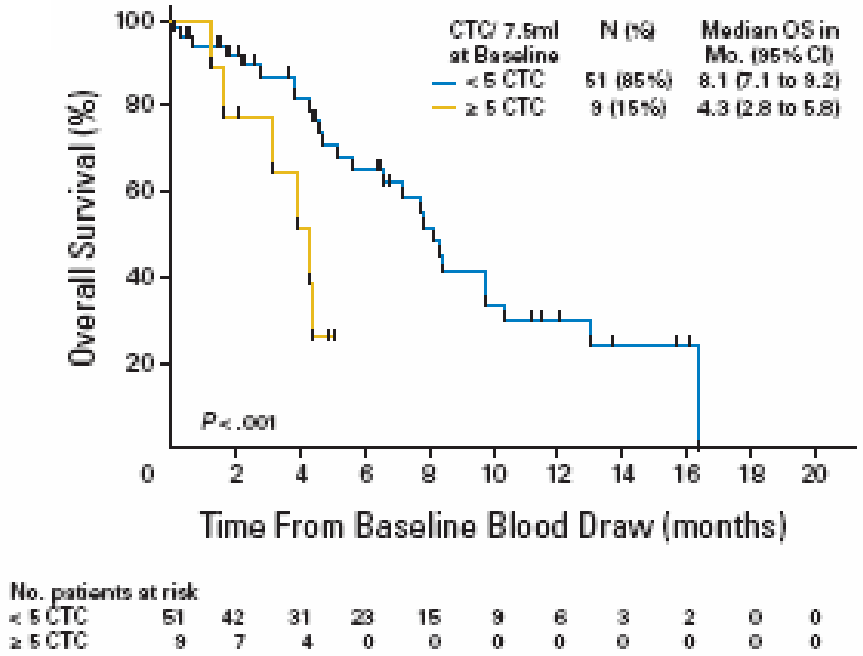


Cristofanilli et al
NEJM 2004
JCO 2005

Cohen et al JCO2008
Tol et al, Ann Oncol 2010

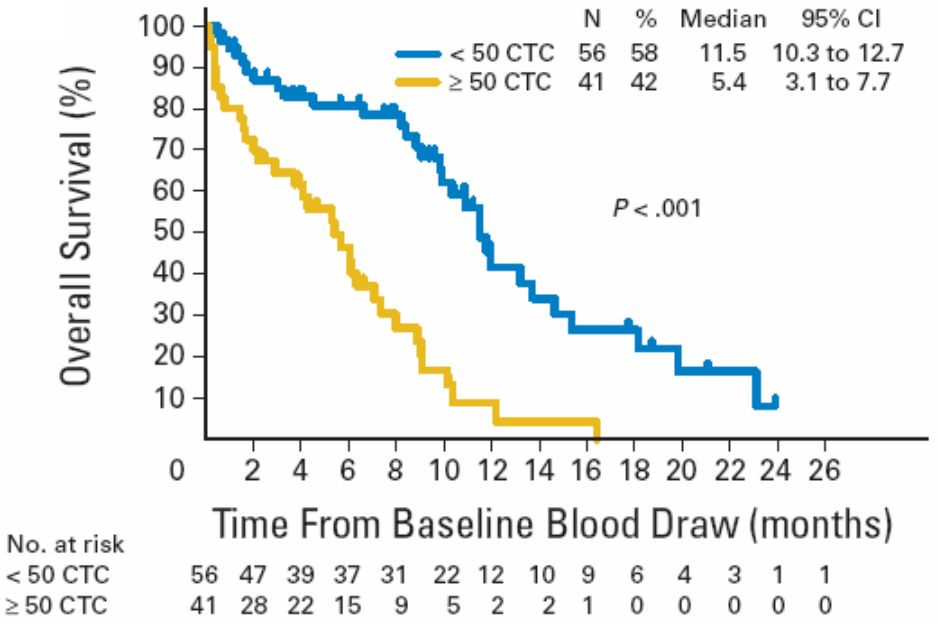
De Bono et al CCR 2008

Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non–Small-Cell Lung Cancer



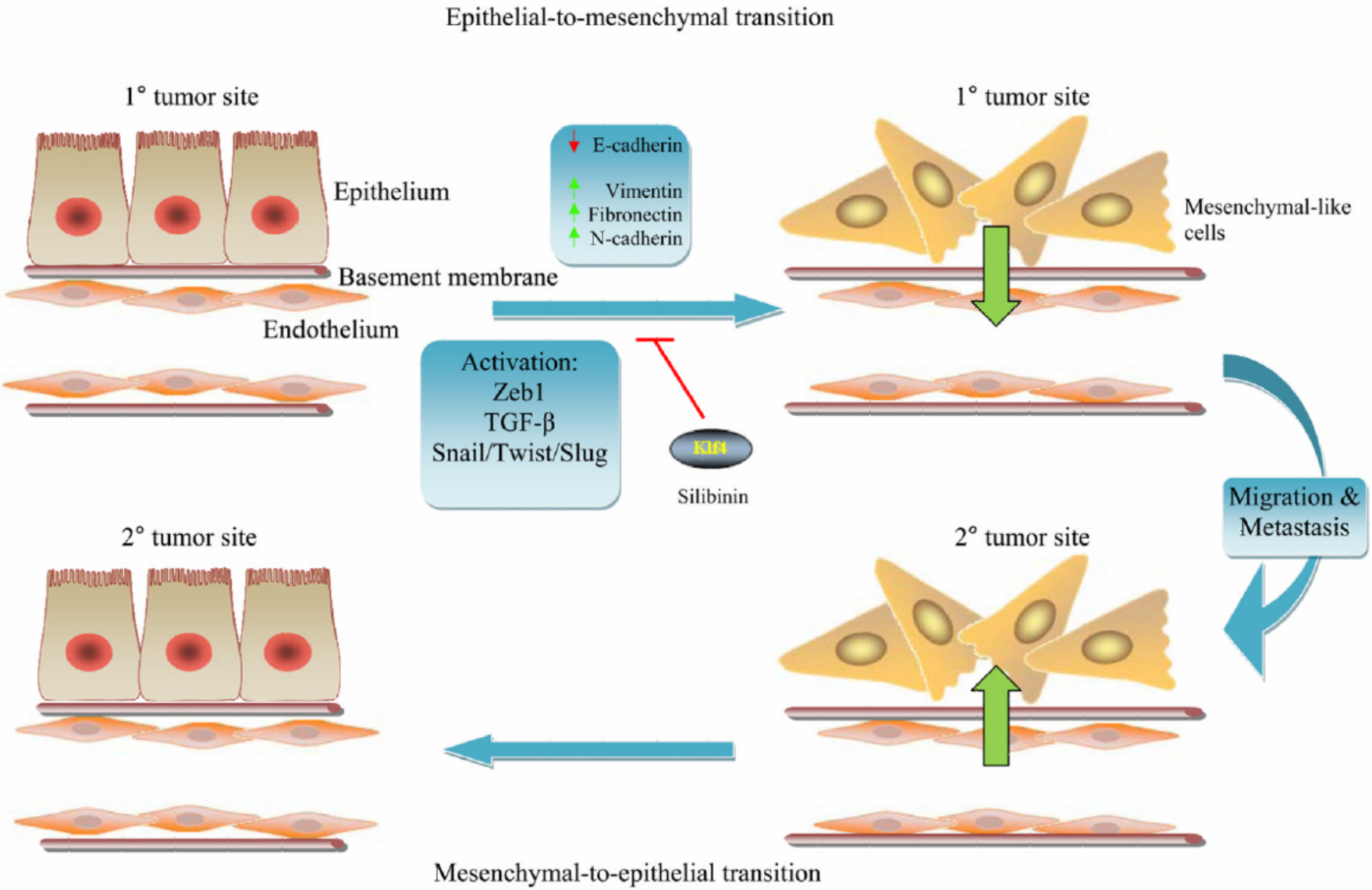
MG Krebs *J Clin Oncol 29. © 2011*

Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Small-Cell Lung Cancer



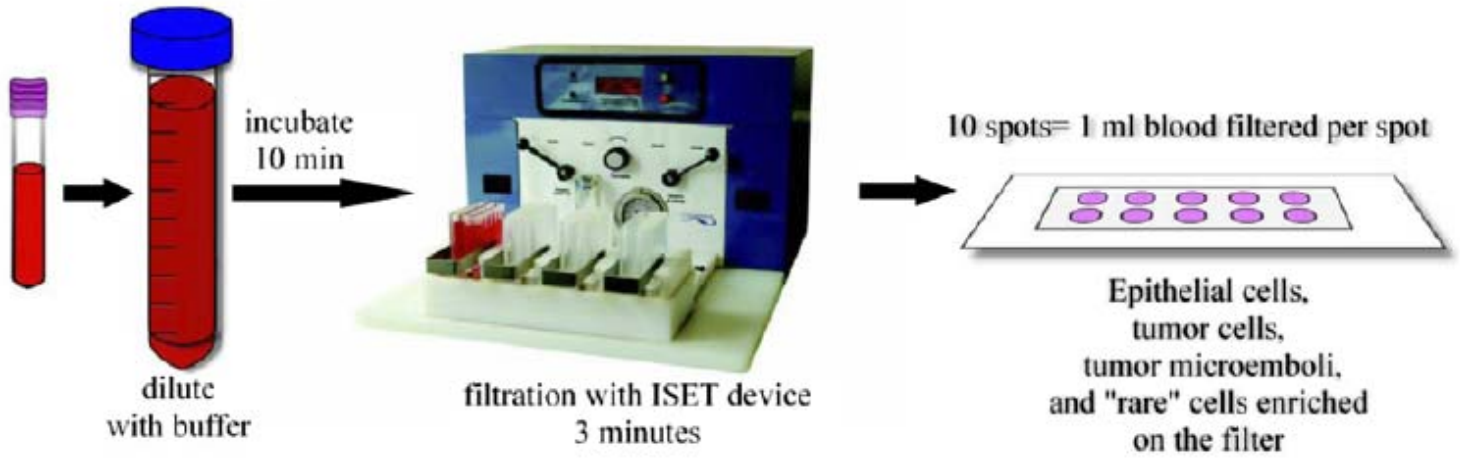
Jian-Mei Hou, J Clin Oncol 30. © 2012

La transition épithélium- mésenchyme (EMT)



Patel et al Oncol Rev. 2011 June 1; 5(2): 93–102

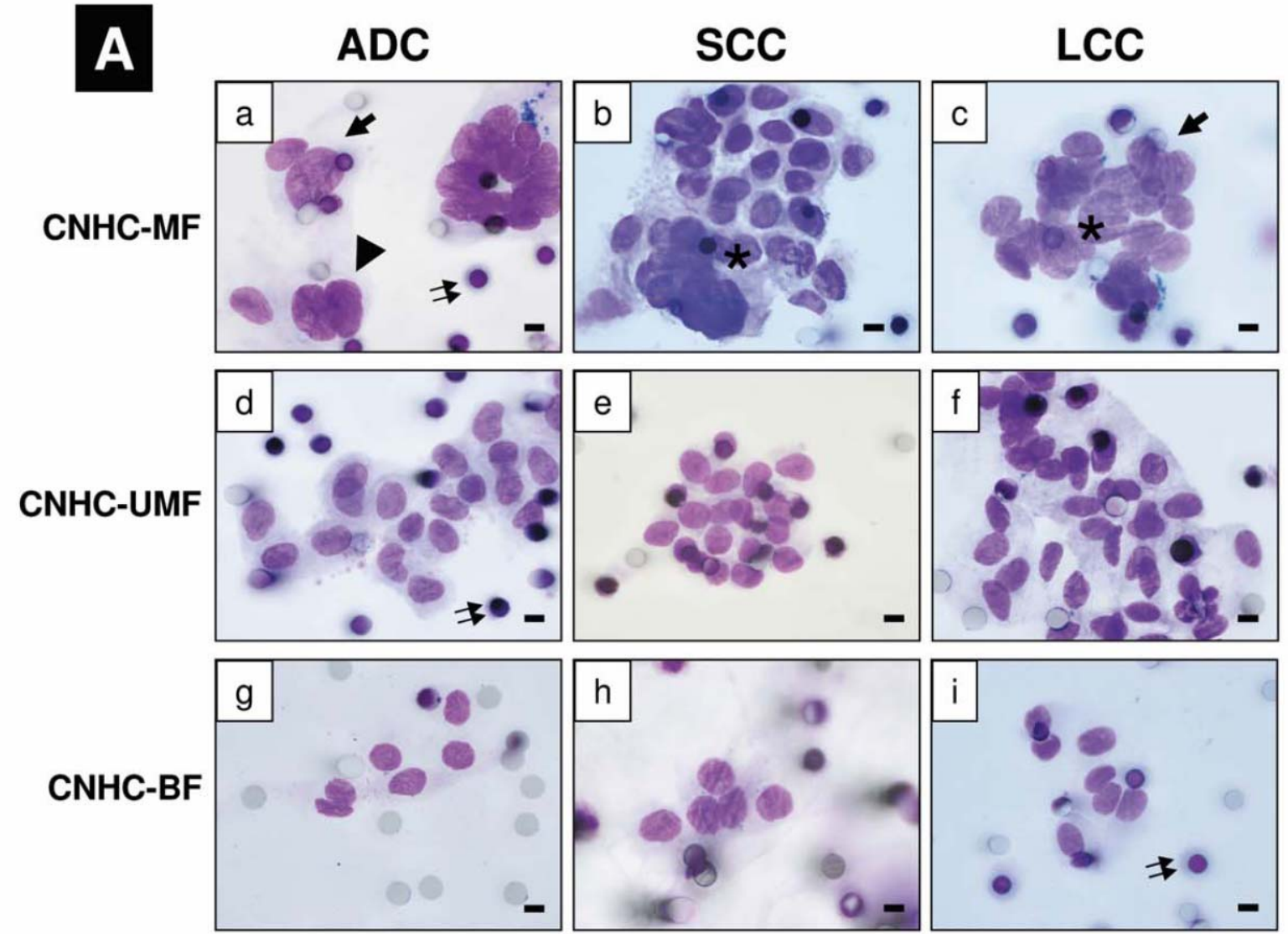
ISET^{TM} :Isolation by Size Epithelial Tumor cells



Paterlini-Brechot et . Cancer Letters 2006
Letters 2007

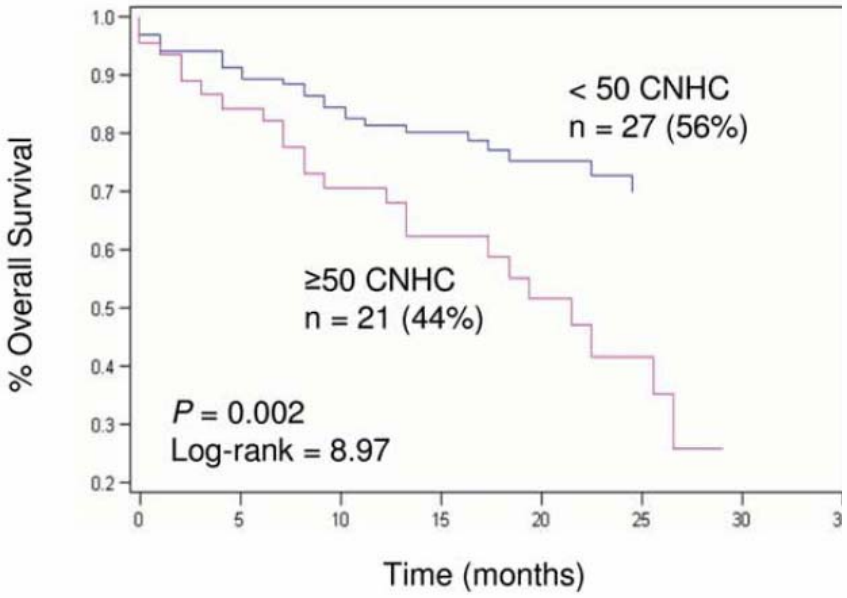
Circulating tumorous cells in patients with hepatocellular carcinoma. Clinical impact and future directions. Semin Cancer Biol. 2000 Jun;10(3):241-9.**Paterlini-Brechot P, Vona G, Brechot C.**

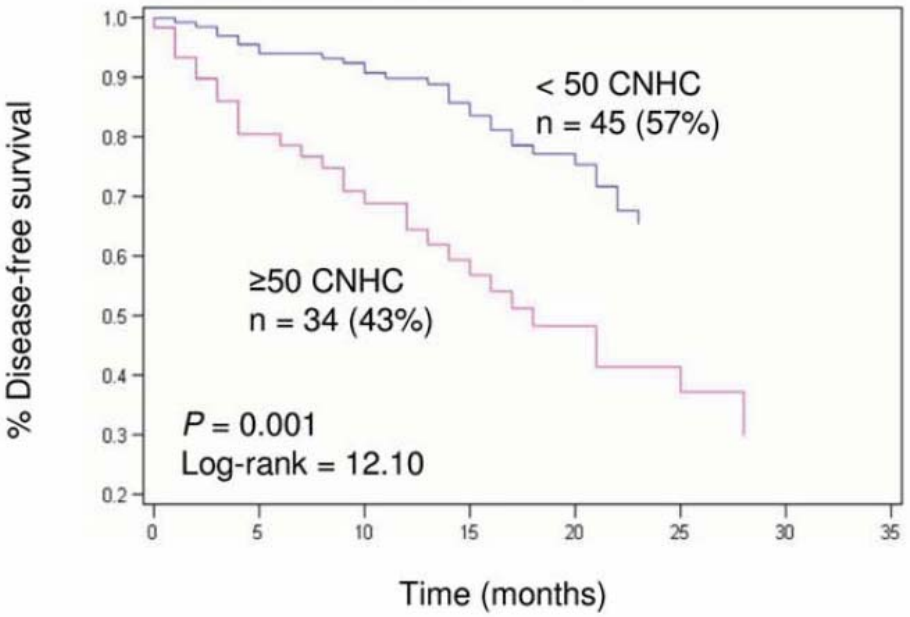
Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker



Hofman, V. Clin Cancer Res 2011

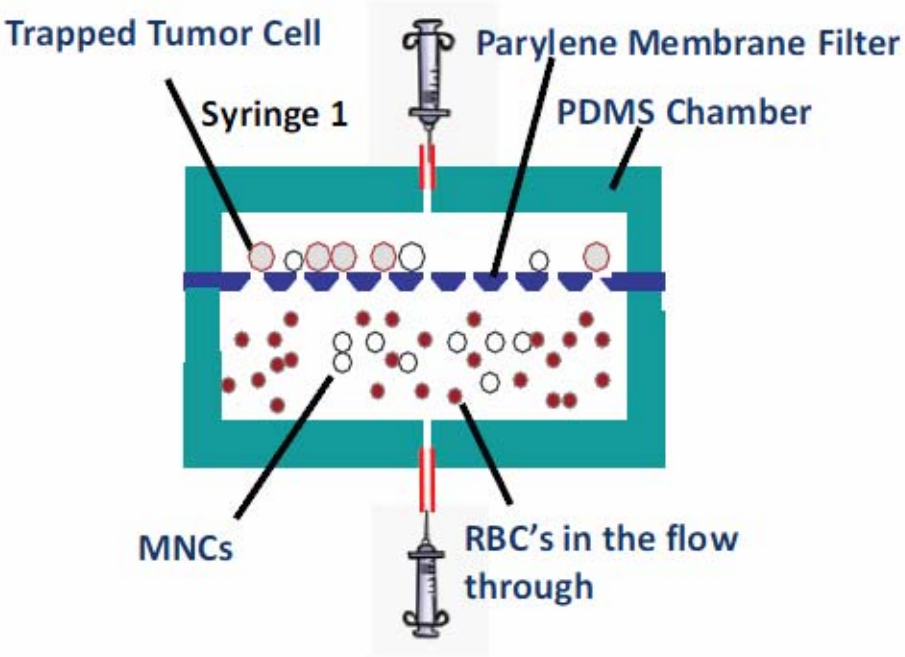
Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker



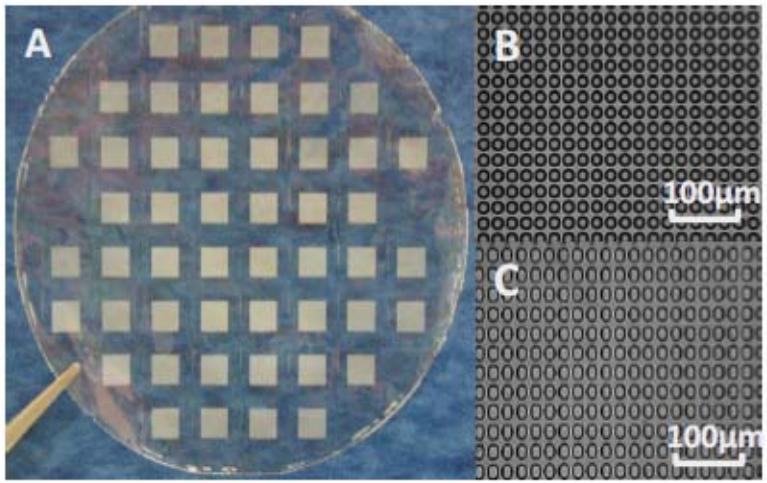


Hofman, V. Clin Cancer Res 2011

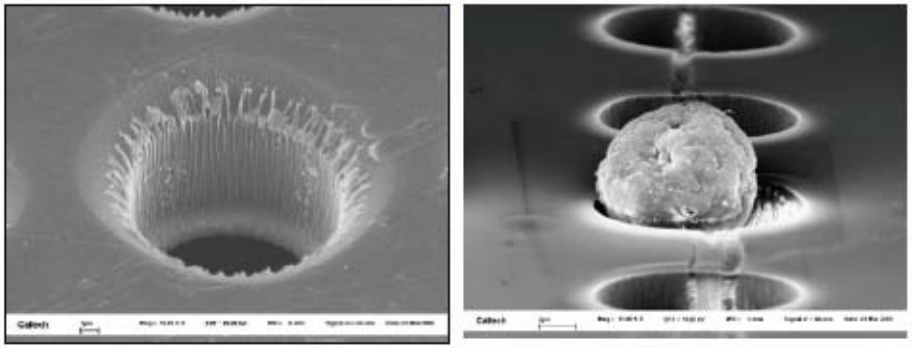
Microfluidics Based Cell Separation



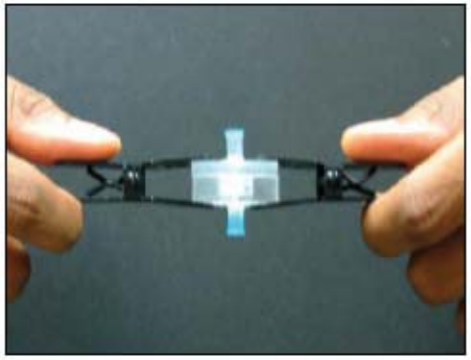
Parylene Filter Membrane



SEM of Cell Captured on filter



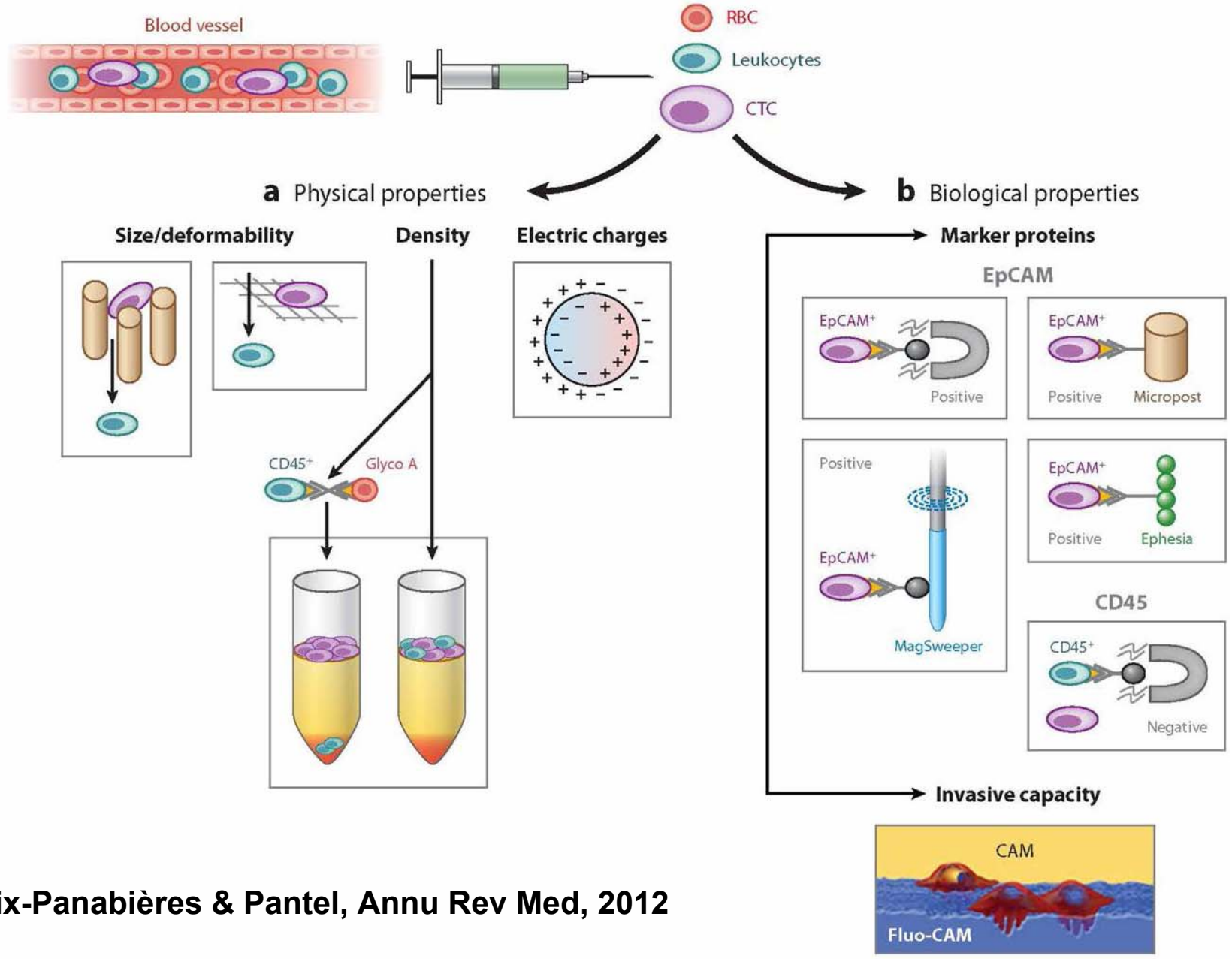
Assembled Filter Device



Automated Flow Device

R.Cote

Détection dans le sang

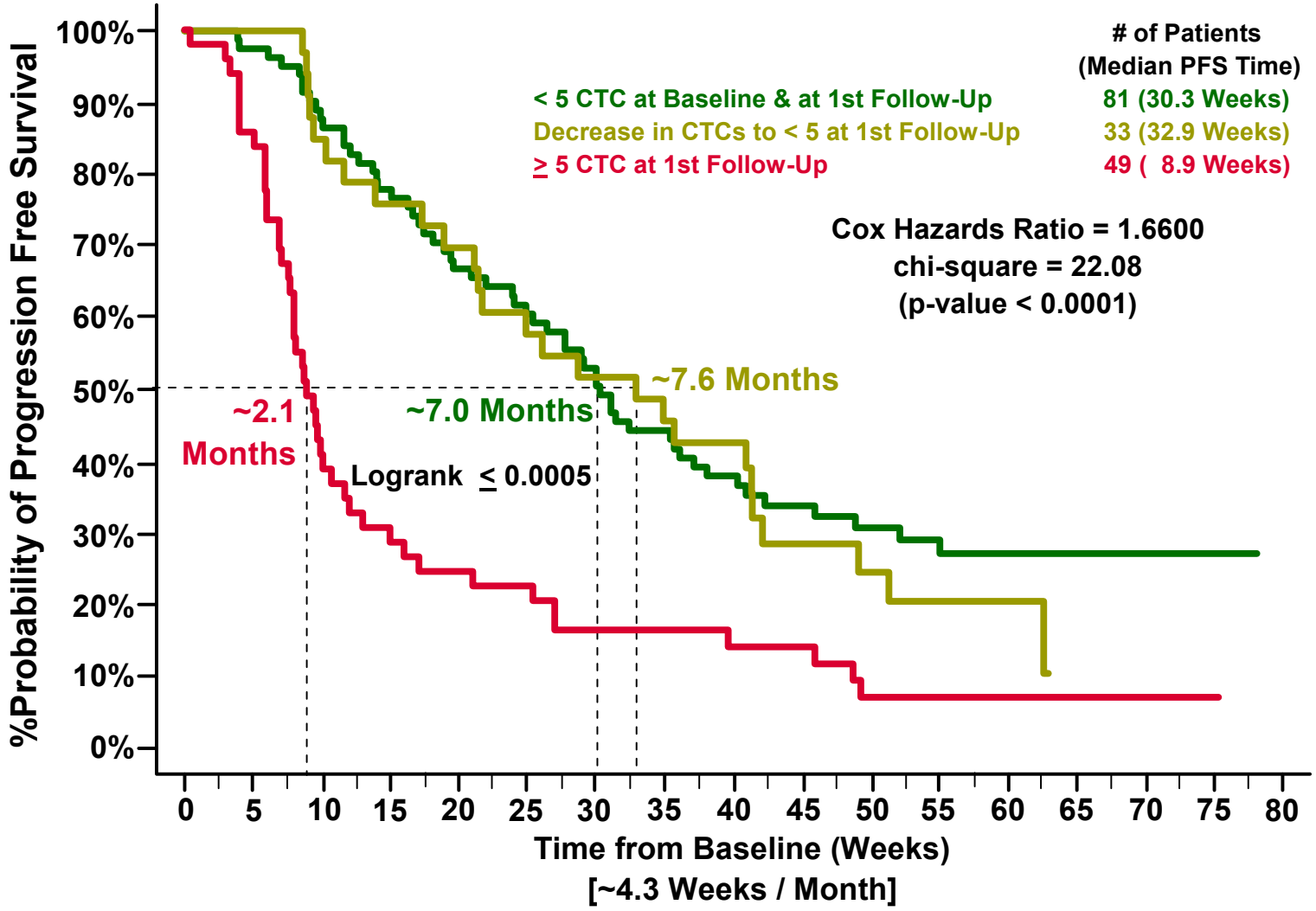


Alix-Panabières & Pantel, Annu Rev Med, 2012

Suivi dans le cancer du sein

In metastatic patients

A Decrease in CTC Numbers (<5) Predicts Therapeutic Effectiveness at First Follow-up



Cristofanilli NEJM 2004

CTCs in metastatic breast cancer patients (MBC)

Publication			Treatment			Outcome	
Author	Year	N=	% 1st line	chemo	chemo + targeted therapy	baseline count	changes under treatment
Cristofanilli	2004	177	47%	67%		PFS, OS	PFS, OS

FDA clearance for the management of chemotherapy in MBC patients

Only small observational studies reported since 2004

Nolé	2008	80	41%	NA		PFS	PFS
Dawood	2008	185	100%	NA		OS	
Liu	2009	74	28%	28%	25%	PFS	Response PFS
Nakamura	2010	107	38%	53%	23%	OS	Response
Bidard	2010	67	100%	0%	100%	PFS	Not Significant

IC 2006-04 study (NCT00898014)

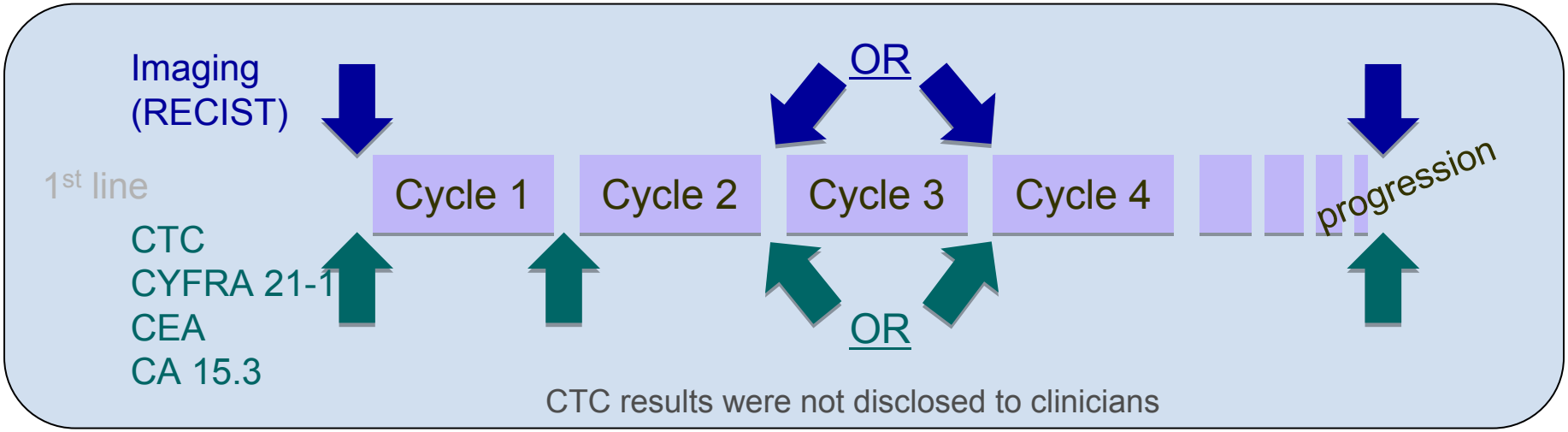
Inclusion criteria

M+ breast cancer patients prior to the first line of chemotherapy +/- targeted therapy (clinician's choice)

Method

CellSearch® technique (Veridex, NJ), 1 CellSave tube, 4 points during follow-up

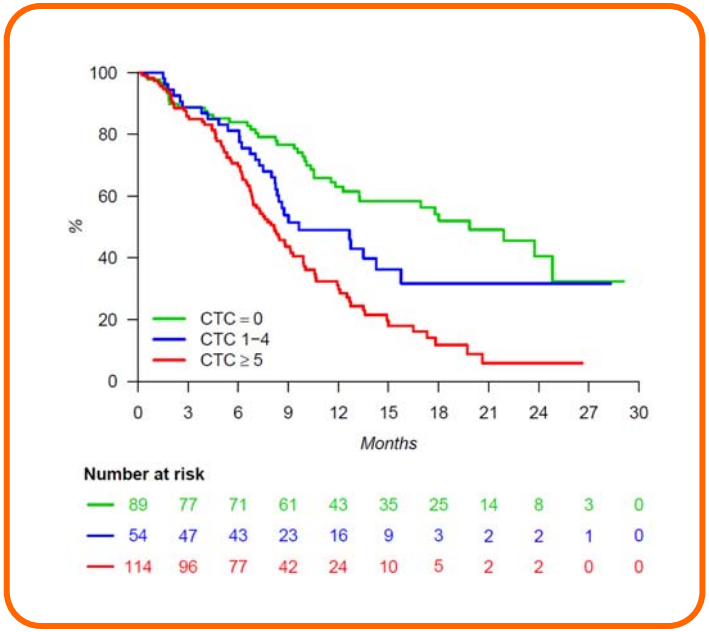
Study Design



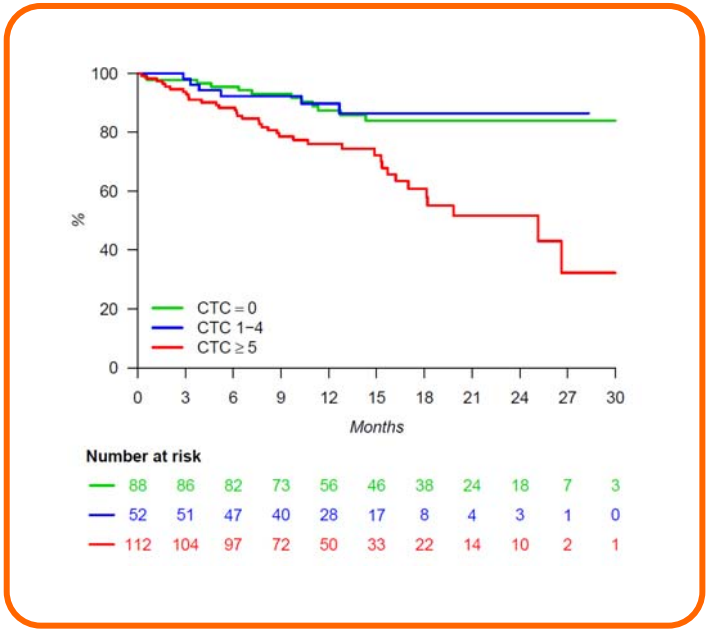
Pierga et al Ann Oncol 2011

CTC count at baseline

Univariate analyses



PFS p <0.0001



OS p =0.0001

CTC count at baseline

Multivariate analyses

PFS

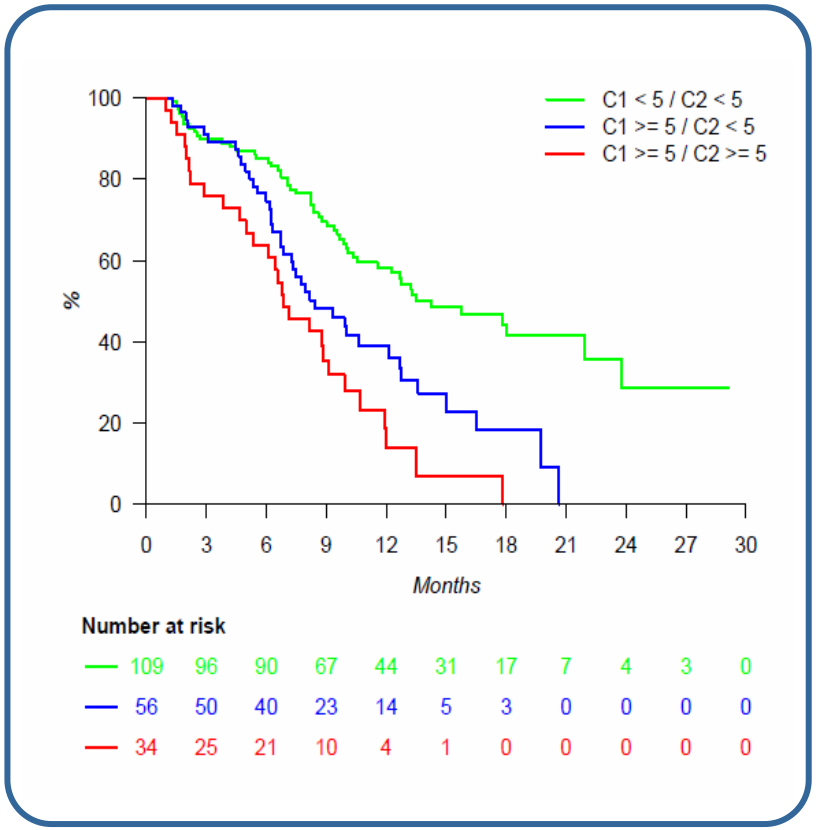
Prognostic factors	RR	CI(0.95)	P value
CTC ≥5	**1.90**	**1.3-2.9**	**0.002**
CEA >N	2.02	1.3-3.1	0.002
Triple neg.	4.16	2.4-7.0	<0.0001
PS > 0	2.22	1.4-3.4	0.0002

OS

Prognostic factors	RR	CI(0.95)	P value
Triple negative	4.31	2.1-8.9	0.0001
PS > 0	2.67	1.1-6.5	0.02
CTC ≥5	**2.41**	**1.1-5.4**	**0.02**

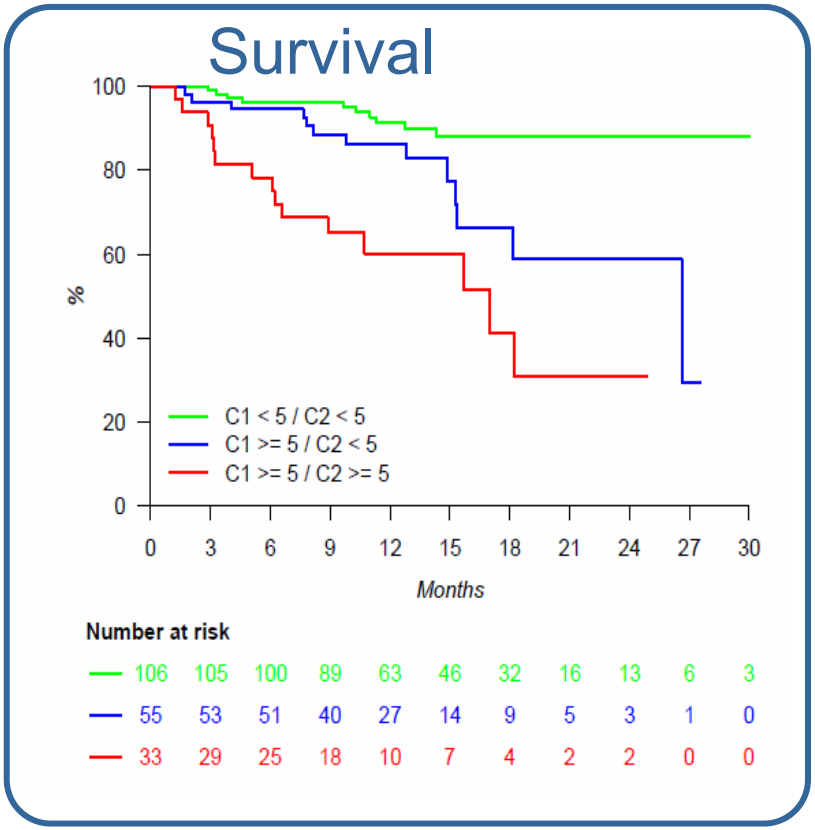
CTC count changes under treatment

Progression-Free Survival



p<0.0001

Overall Survival



p<0.0001

CTC count changes under treatment

	Nb pts with ≥5 CTC at baseline	% with ≥5 CTC before C2	% with ≥ 5 CTC before C3/4
Chemo. alone	N= 39	47%	38%
Chemo. + bevacizumab	N= 60	36%	23%
Chemo. + anti-HER2 therapy	N= 15	17%	0%
			p = 0.04

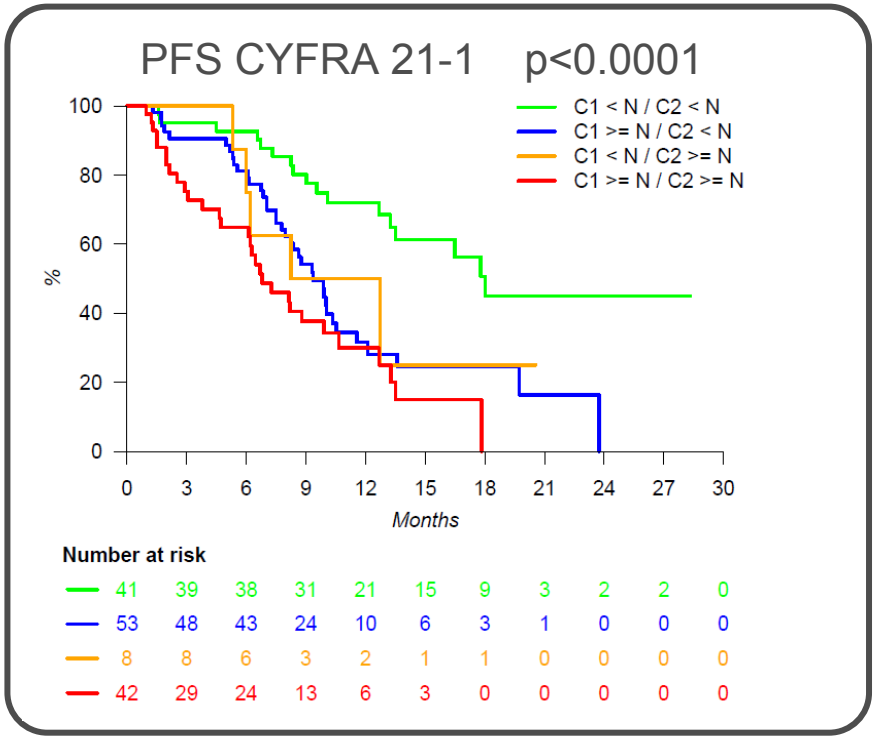
Comparison with serum markers

CYFRA 21-1 : cytokeratin 19 fragments

mAb derived from MCF7 breast cancer cell lines

commonly elevated in MBC (>70%)

« CTC-like » curves were obtained between baseline & C2 ➔



Similar results with

CEA p<0.0001

CA15.3 p<0.0001

Head to head comparison of CTC *vs* serum markers for PFS prediction showed no significant difference *(ROC comparison & Concordance index)*

But the study was not statistically powered for this secondary endpoint

LANDSCAPE: a FNCLCC phase II study with lapatinib and capecitabine in patients with brain metastases from HER2-positive metastatic breast cancer before whole brain radiotherapy

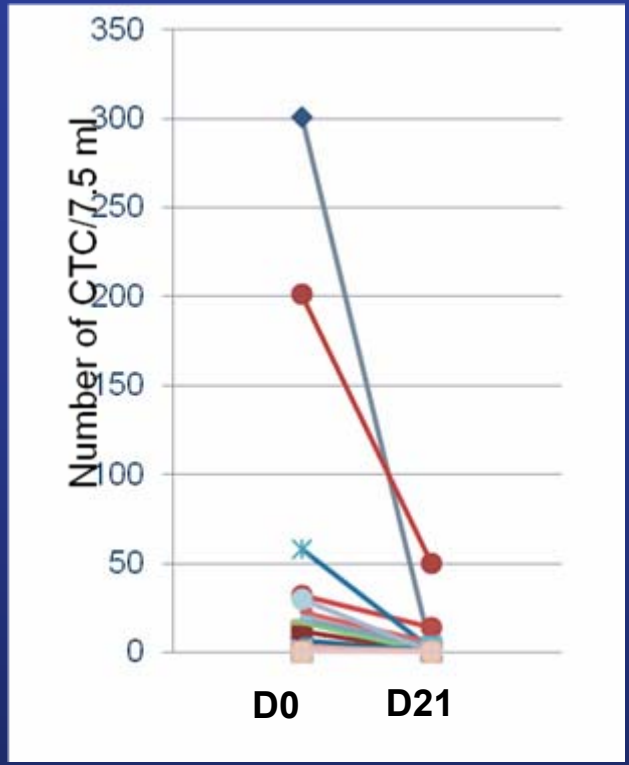
Thomas BACHELOT, Gilles ROMIEU, Mario CAMPONE, Véronique DIERAS, Claire CROPET, Florence DALENC, Marta JIMENEZ, Emilie LE RHUN, Jean-Yves PIERGA, Anthony GONCALVES, Marianne LEHEURTEUR, Julien DOMONT, Maya GUTIERREZ, Hervé CURE, Jean-Marc FERRERO, **Catherine LABBE- DEVILLIERS**

CTC analysis

CTC/7.5ml at baseline and changes under treatment

Date of sampling	≥1 (%)	≥ 5 (%)
Baseline (n=41)	20 (48.8)*	9 (22)
Day 21 (n=38)	7 (18.4)*	3 (7.9)

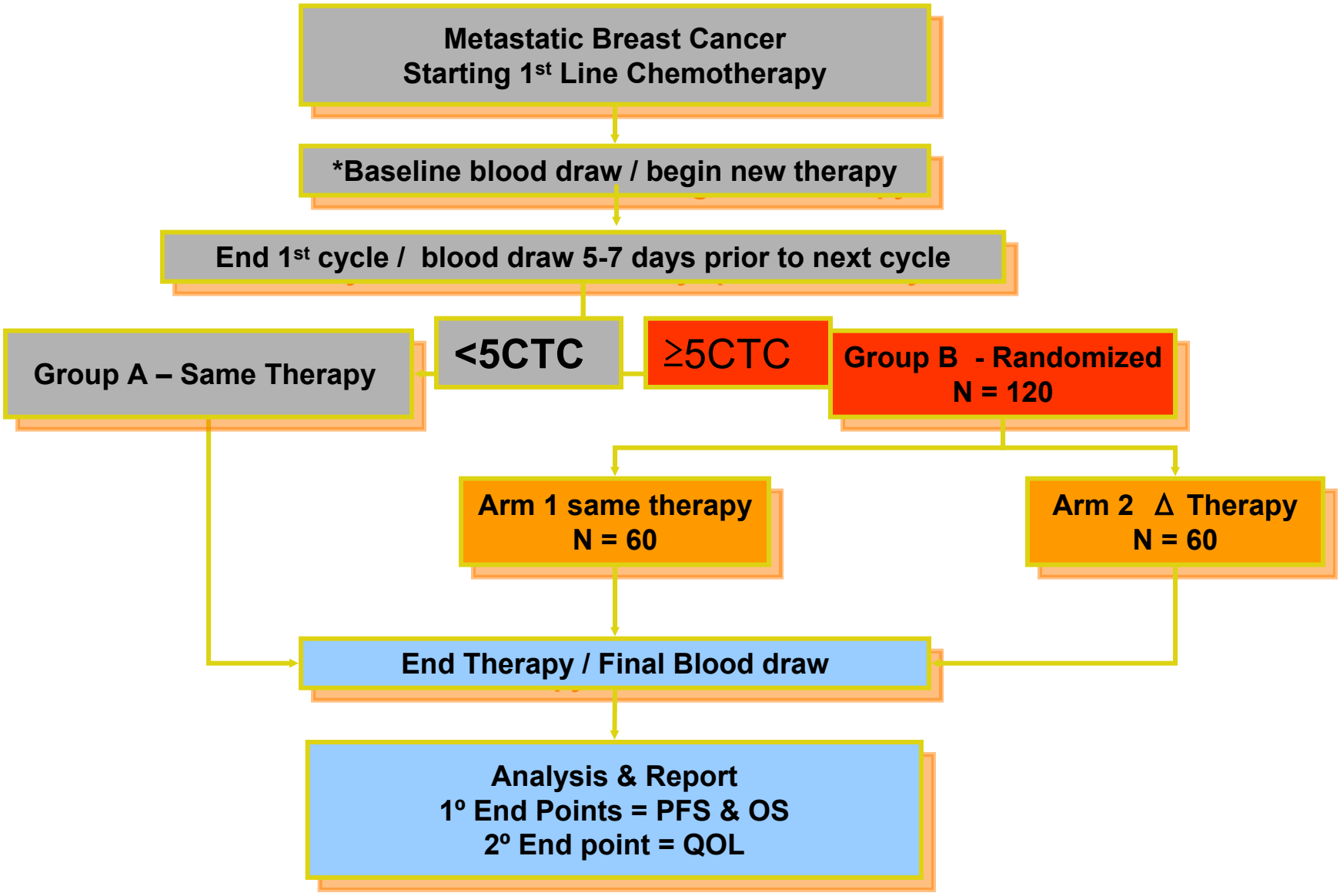
*p=0.006



Correlation with CNS-OR, (n=40)

Date of sampling	CTC Status	CNS-OR (%)	p
Baseline (n=41)	0 at baseline	17 / 21 (81)	NS
	≥ 1 at baseline	11 / 19 (57.9)	
Day 21 (n=38)	0 at day 21	25 / 31 (80.6)	0.03
	≥ 1 at day 21	2 / 6 (33.3)	

SWOG 0500

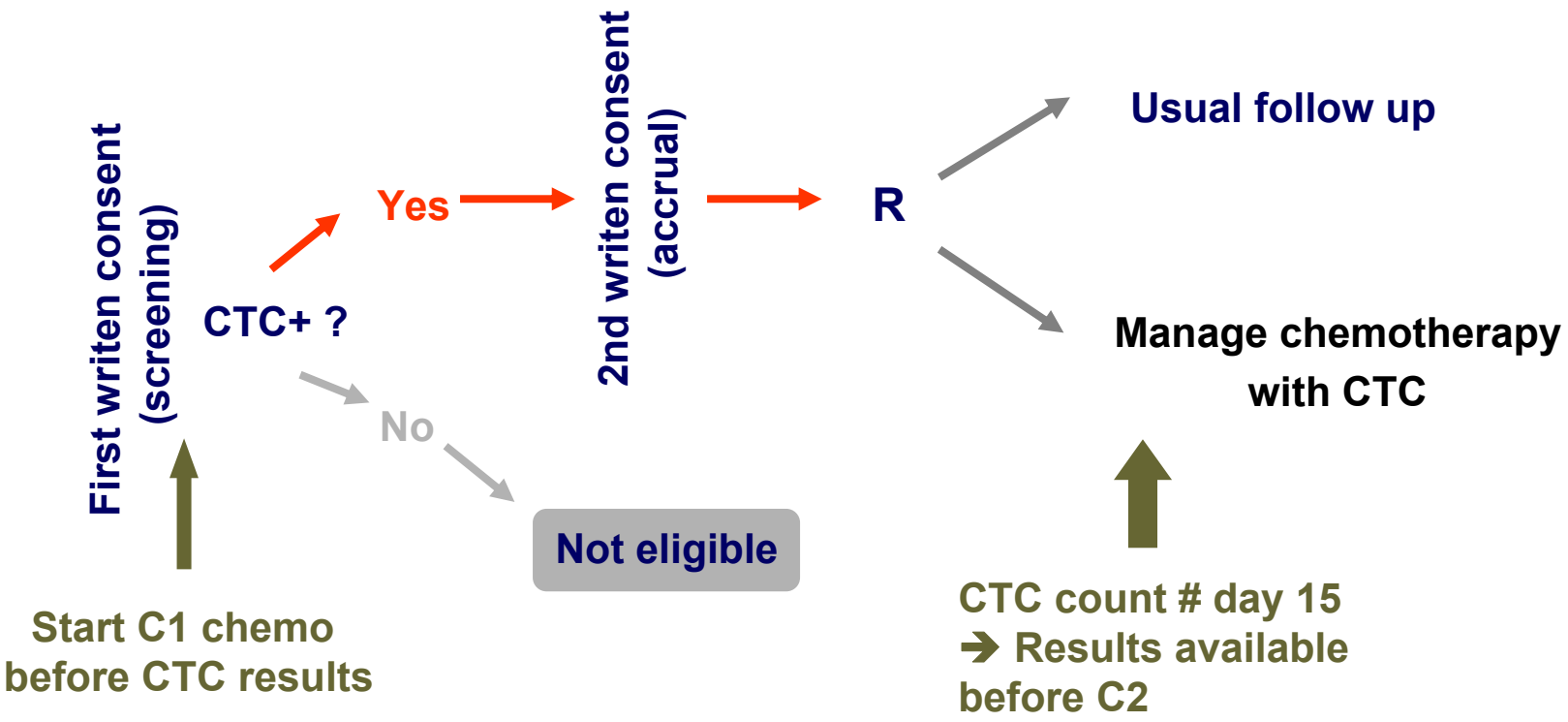

Metastatic Breast Cancer
Starting 1st Line Chemotherapy
*Baseline blood draw / begin new therapy
End 1st cycle / blood draw 5-7 days prior to next cycle
Group A – Same Therapy
<5CTC
≥5CTC
Group B - Randomized
N = 120
Arm 1 same therapy
N = 60
Arm 2 Δ Therapy
N = 60
End Therapy / Final Blood draw
Analysis & Report
1º End Points = PFS & OS
2º End point = QOL

CirCe01

Observationnal phase ➔ threshold finding

Interventional phase : 304 randomized patients

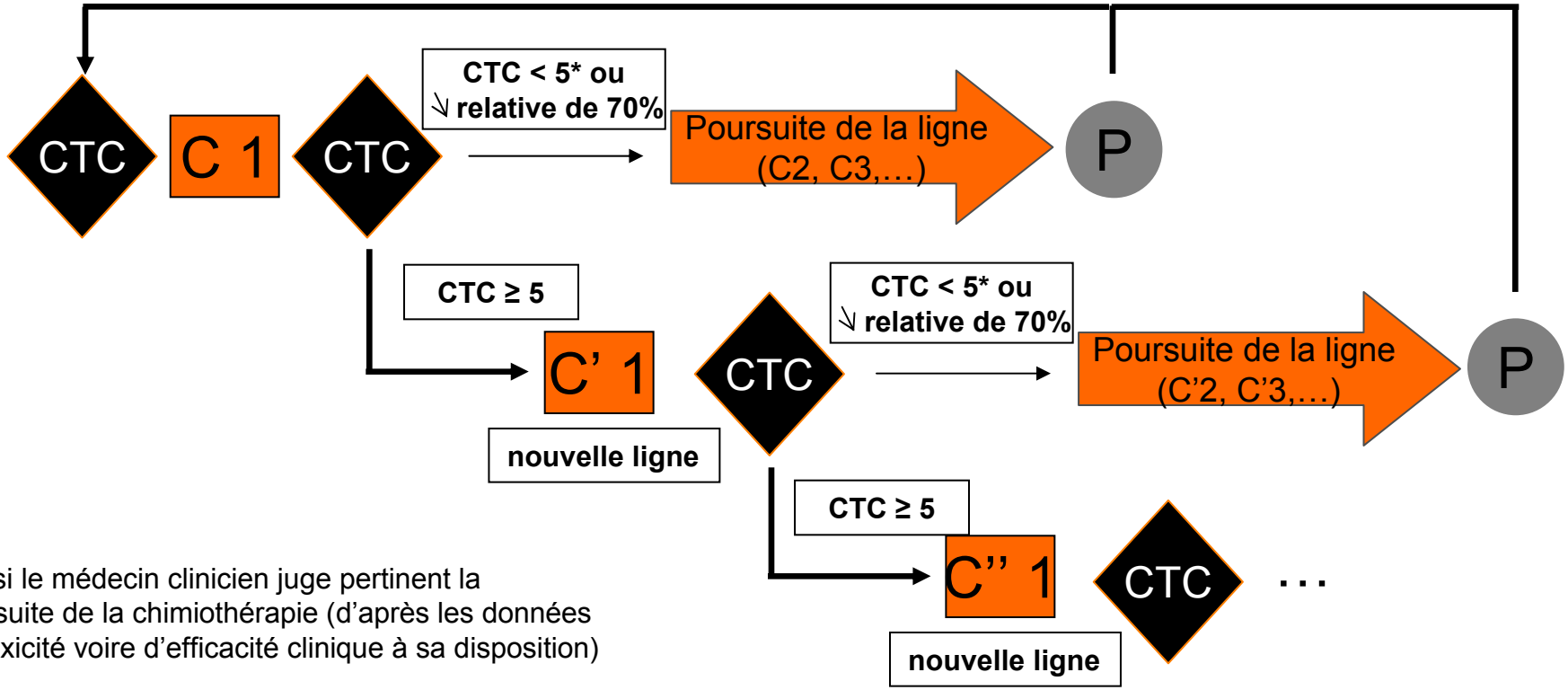
- 3rd & subsequent lines of chemo will be managed by CTC in patients randomized in the CTC arm
- CTC will be studied before/after the first cycle of each new chemo (spontaneous resistance) but not on the following cycles (usual follow-up)



5 – Déroulement de l'étude : Phase Interventionnelle (1)

5.3 – Suivi en cours d'essai :

5.3.3 – Phase **Interventionnelle** : Bras A uniquement



*ET si le médecin clinicien juge pertinent la poursuite de la chimiothérapie (d'après les données de toxicité voire d'efficacité clinique à sa disposition)

STIC CTC METABREAST

Cancer du sein M+ RH+

pouvant recevoir hormonoT ou chimioT.

Randomisation

stratification par centre & PS

N=1000

Bras standard

Critères habituels

non-péjoratifs → **HormonoT.**

péjoratifs → **ChimioT.**

Bras CTC

Mesure des CTC

<5 CTC/7.5ml → **HormonoT.**

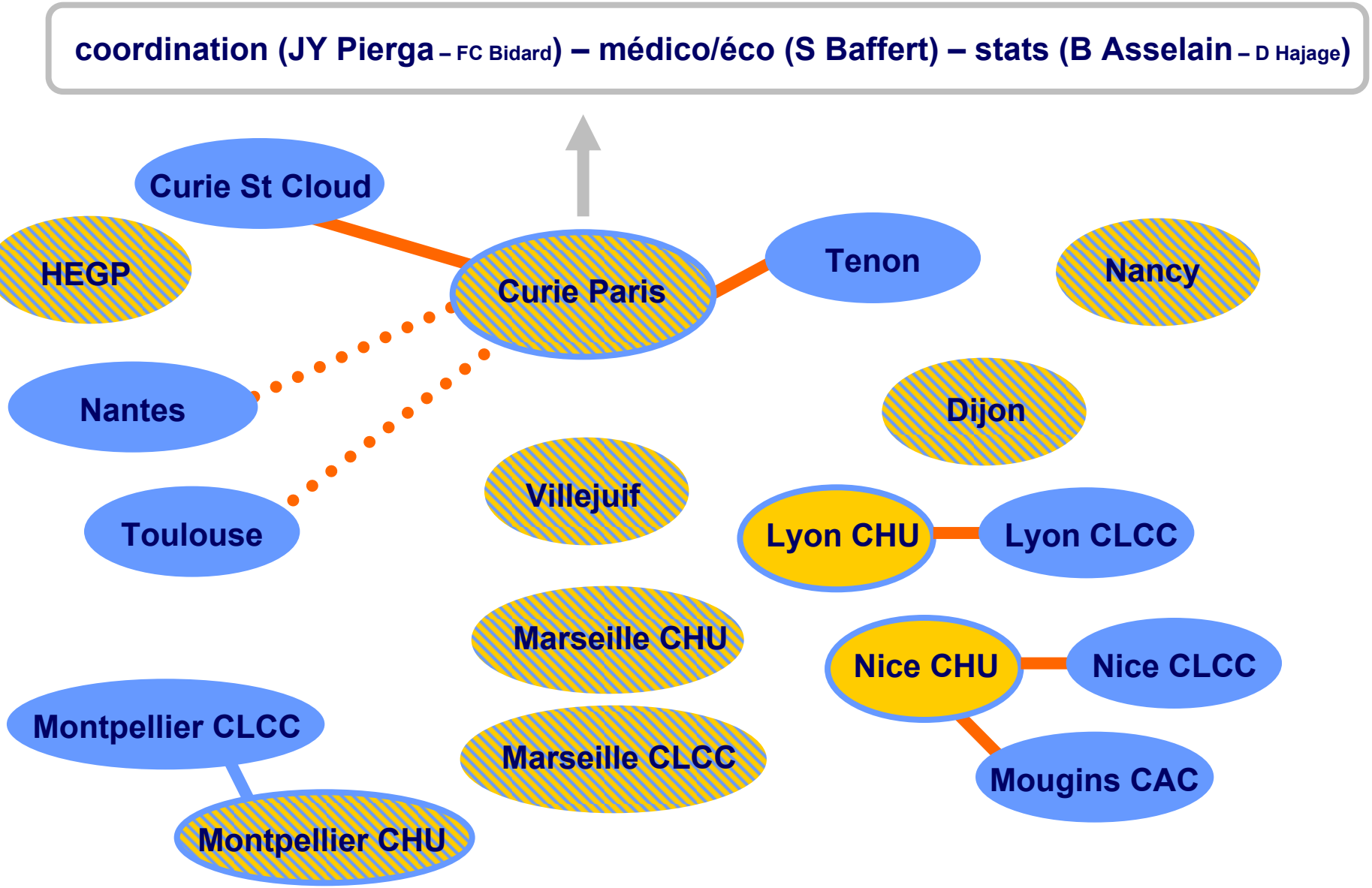
≥5 CTC/7.5ml → **ChimioT.**

Comparaison

Médicale : Survie sans progression, qualité de vie, toxicité, survie globale

Economique : coûts différentiels par année de vie sans progression,
coûts globaux de prise en charge

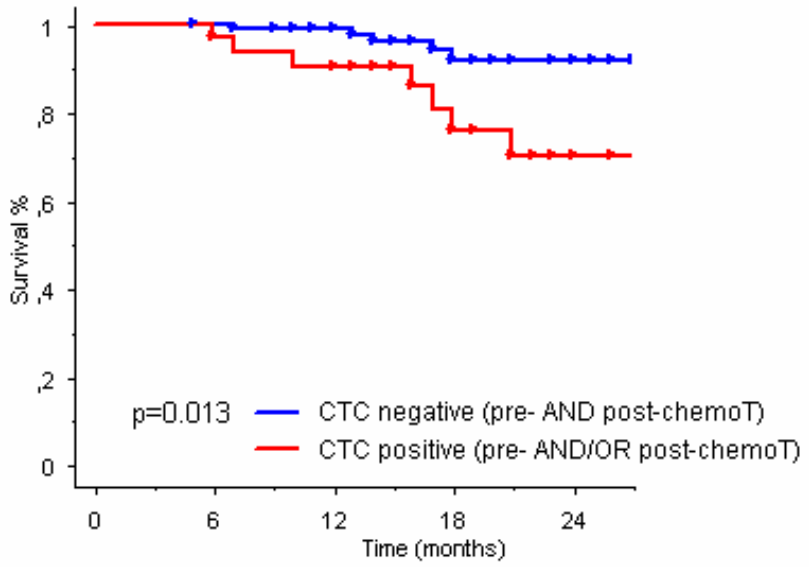
STIC CTC METABREAST



Situation adjuvante et néoadjuvante

Monitoring Circulating Tumor Cells (CTCs) during neoadjuvant chemotherapy for large operable and locally advanced breast cancer in a randomized phase II trial N=118

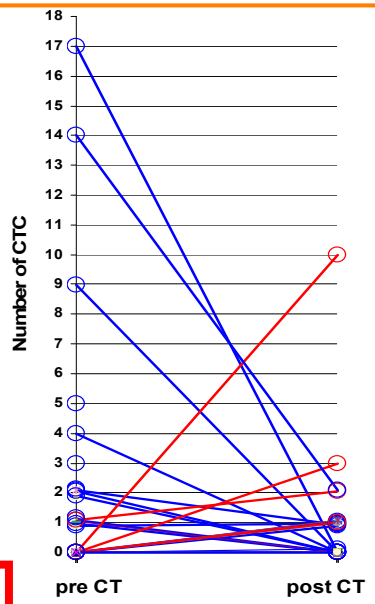
23% cases ≥1CTC



CTC-	86	84	77	43	17
CTC+	32	31	27	15	8

Remagus 02 Clinical Trial

CTC detection before and after neoadjuvant chemotherapy



No correlation with pCR !

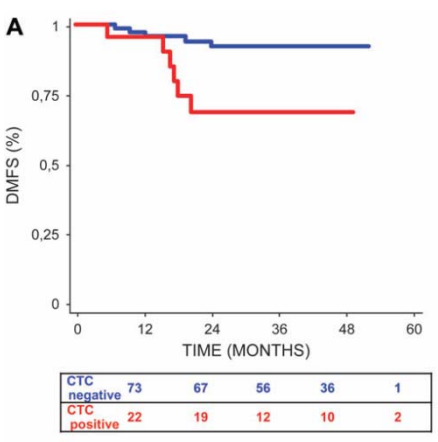
Pierga JY, *et al* Clin Cancer Res 2008

REMAGUS02: French neoadjuvant study

Confirmée ultérieurement par l'étude Geparquattro allemande

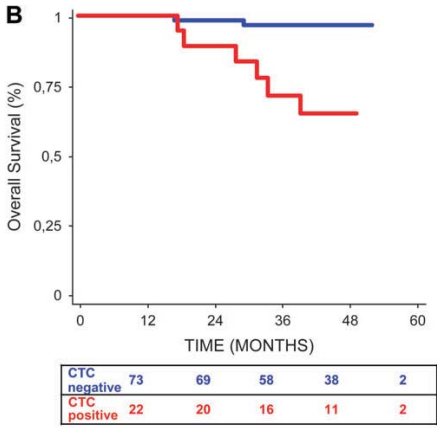
In both studies, although low detection rates & short follow-up, CTC detection was independently correlated with DMFS and OS

DMFS

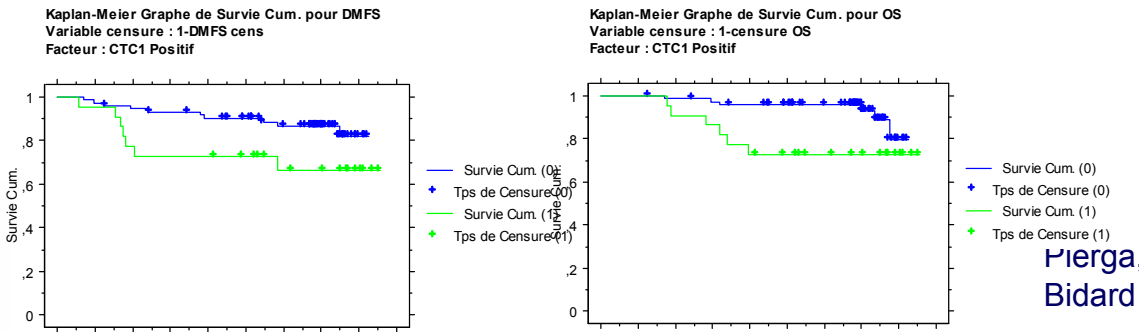


P=0.01 RR=5

OS



P=0.007 RR=9



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Pierga, Bidard (…). Clin Cancer Res 2008
Bidard (..) Pierga, Ann Oncol 2010

Results : Multivariate analysis including pCR

Significant prognostic factors (Metastasis-free survival) Median

	p	RR	95%CI
Triple negative .	0.002	9.5	[2.4-37]
CTC (preCT)	0.01	5.0	[1.4-17]
Tumor size	0.04	3.8	[1.07-13]

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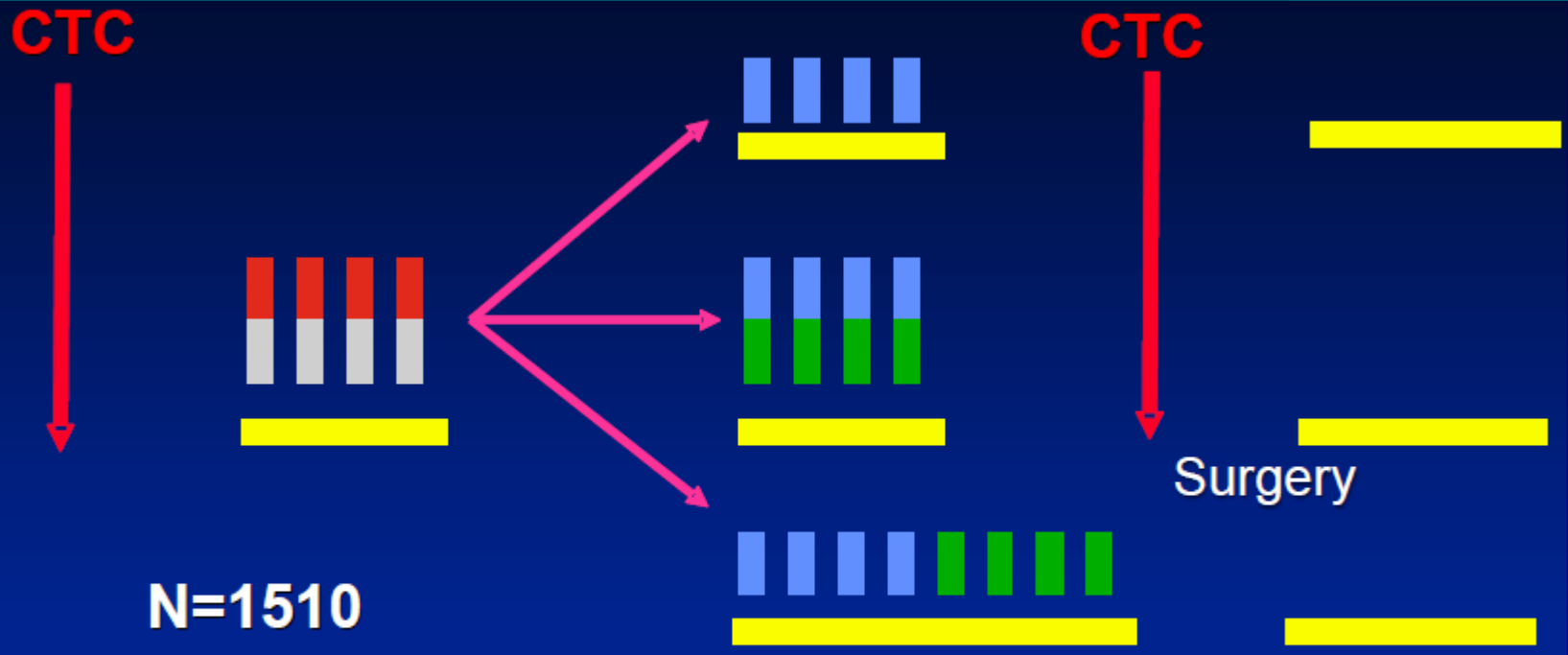
Bidard *et al*, Ann Oncol 2011

GeparQuattro Study design



Epirubicin 90 mg/m²
Cyclophosphamide 600 mg/m²
Docetaxel 100 mg/m² (A) 75 mg/m² (B,C)
Capecitabine 1800 mg/m²
Her2 pos: Trastuzumab 6 mg/kg q3w 1y

www.germanbreastgroup.de

Universitätsklinikum Hamburg-Eppendorf

Geparquattro-Study: Detection of CTC before and after primary systemic therapy (PST) in breast cancer patients

Time point	n patients	n CTC-positive pats*	CTC/7.5 mL Mean (Median)	Range CTC/7.5 mL
Before PST	216	48 (22.2%)	6.8 (1)	1-200
After PST	208	22 (10.6%)	1.9 (2)	1-5

*detected with the CellSearch™ System (FDA-approved)

No correlation with pCR

Universitätsklinikum Hamburg-Eppendorf

Riethdorf/Pantel *et al.*, CCR, 2010

Perspectives
CTC and CEC in inflammatory breast cancer

« BEVERLY 1 »

(BEVacizumab hER-2 negative infLammatorY)

- Promotion : FNCLCC (soutien Roche)
- Coordonator : Pr C. Tarpin
- 100 included

« BEVERLY 2 »

(BEVacizumab hER-2 positive infLammatorY)

- Promotion : Roche
- Coordonator : Pr P. Viens
- 50 included

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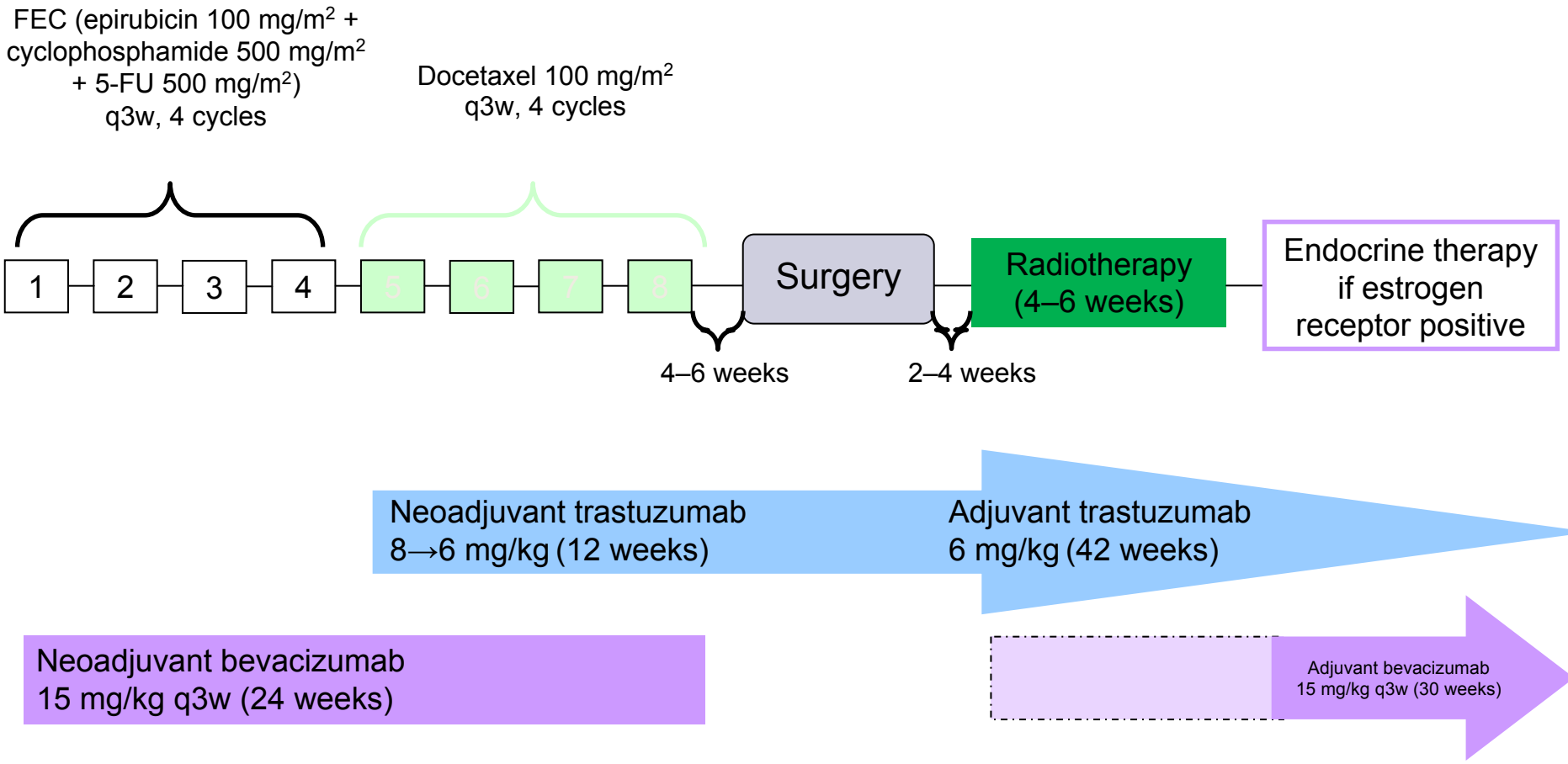
Treatment schedule in BEVERLY 2



Patients are followed until 5 years after enrollment of the last patient.

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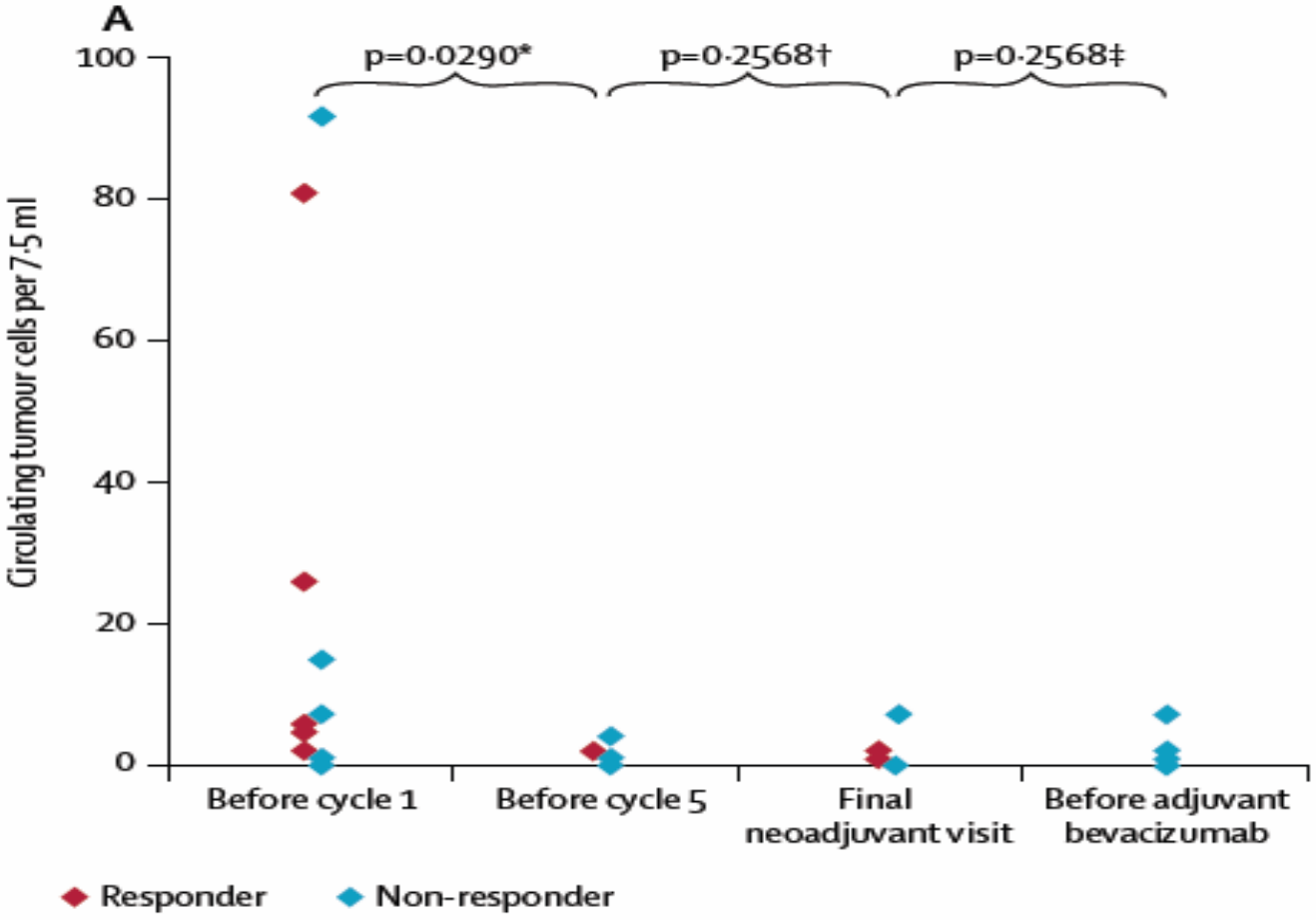
Change in CTC count during therapy, by patient



Pierga et al, Lancet Oncol 2012

CTC in the adjuvant setting

Prevalence of CTCs in peripheral blood in early breast cancer

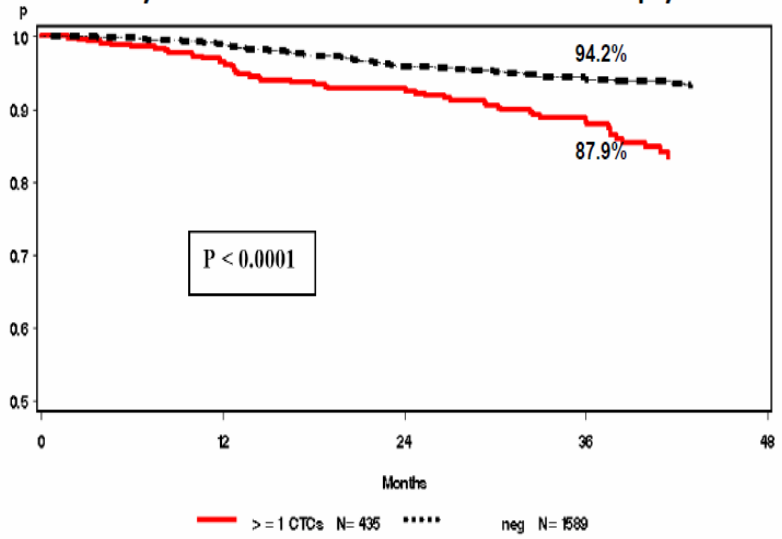
	No CTCs in blood	CTCs in blood
Breast Cancer	1591	435
Patients Stage I – III	78.5%	21.5%

Rack B. SABCS 2010

Impact pronostique des CTC avant et après une chimiothérapie adjuvante dans le cancer du sein : SUCCESS trial *(Rack et al, SABCS2010)*

San Antonio Breast Cancer Symposium – Cancer Therapy and Research Center at UT Health Schience Center – December 8 – 12, 2010

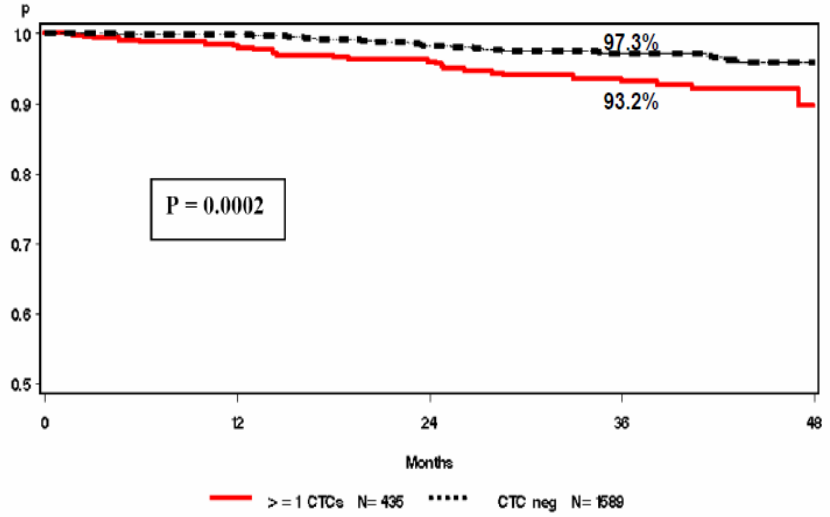
Distant Disease-free Survival by CTCs before chemotherapy



	CTC+	CTC-
Recurrences	41 / 436	72 / 1589
Mean Survival Time	38.6 mon	41.5 mon

San Antonio Breast Cancer Symposium – Cancer Therapy and Research Center at UT Health Schience Center – December 8 – 12, 2010

Overall Survival by CTCs before chemotherapy



	CTC+	CTC-
Recurrences	23 / 436	33 / 1589

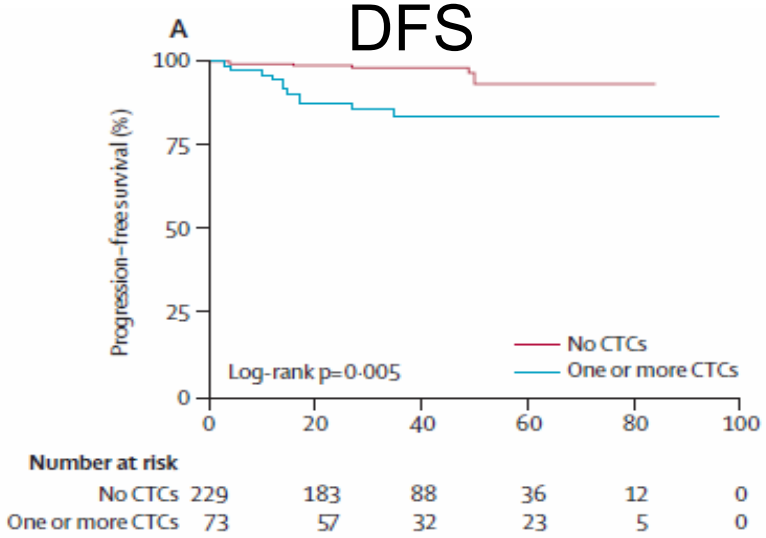
SUCCESS LMU KLINIKUM

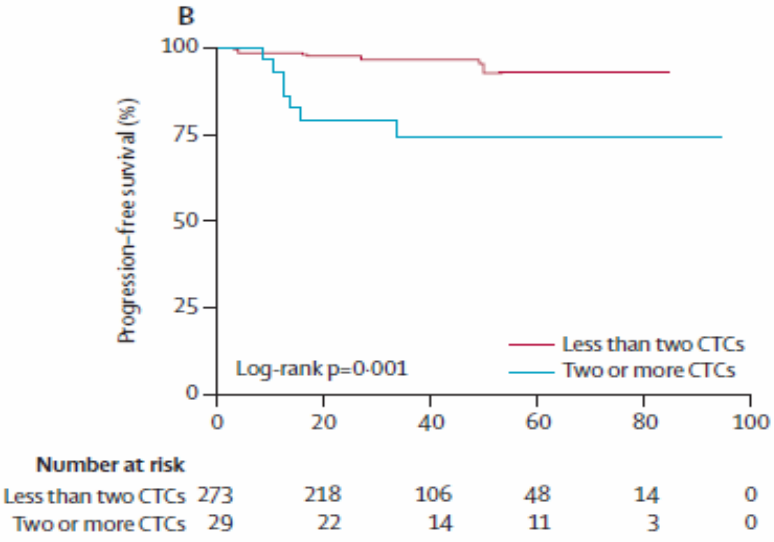
SUCCESS LMU KLINIKUM

Median follow-up 36 months

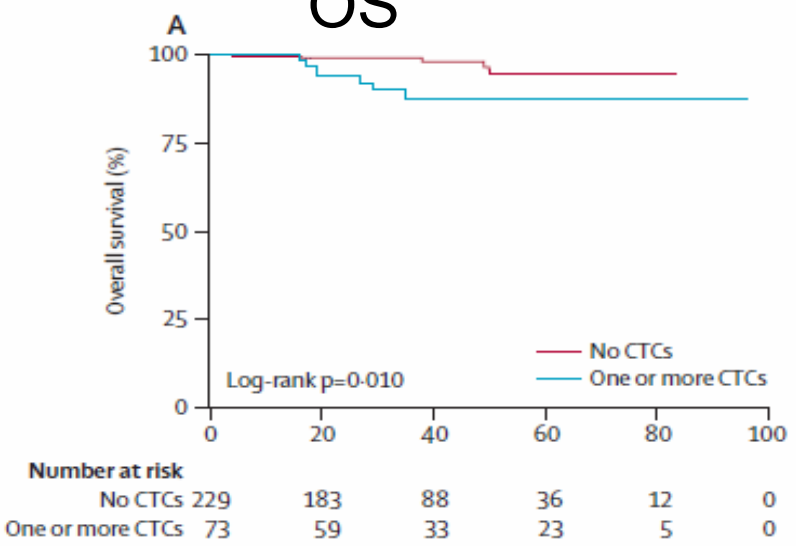
Circulating tumour cells in non-metastatic breast cancer: a prospective study N=302

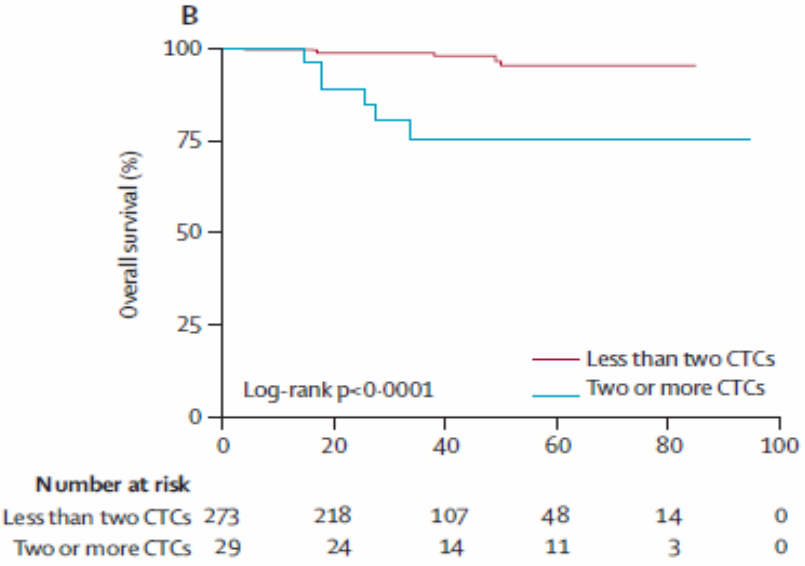
DFS





OS





A Lucci Lancet Oncol, June 2012

Role de la moelle osseuse: lieu de transit place ou réservoir de DTC et CTC?

OPEN ACCESS Freely available online

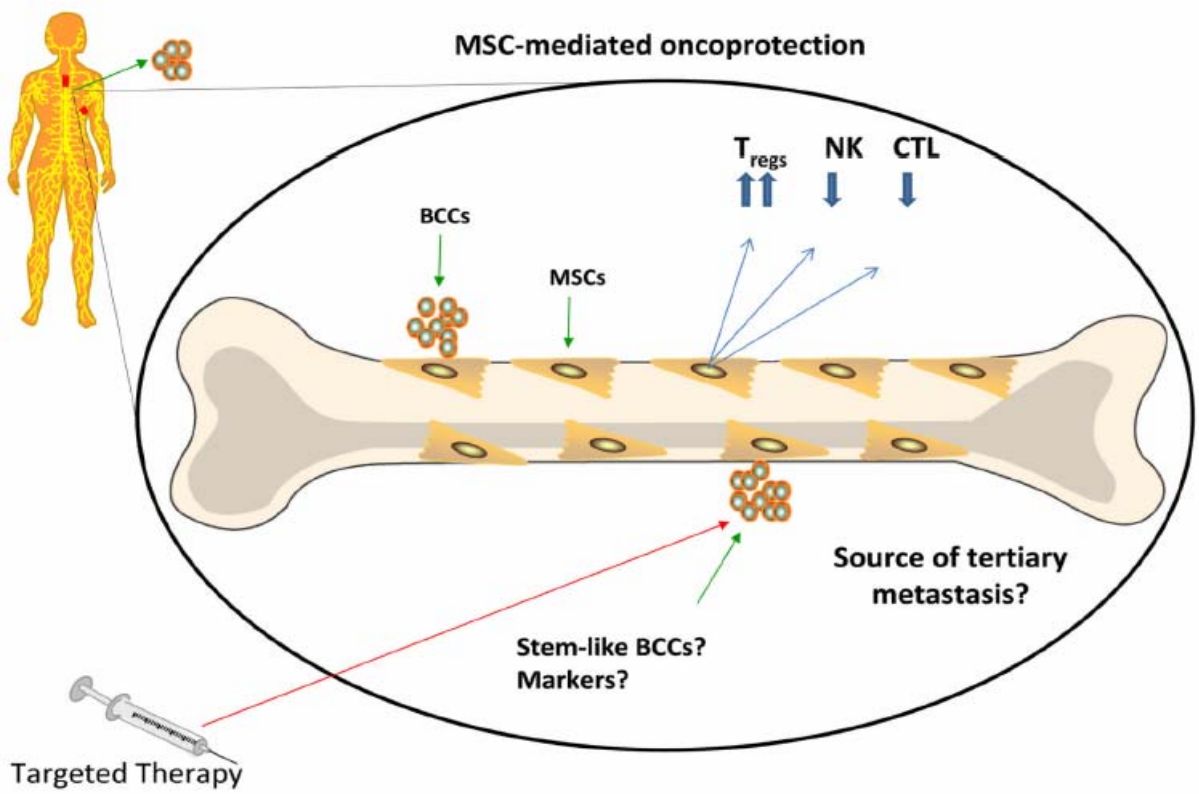
PLoS one

Mesenchymal Stem Cells in Early Entry of Breast Cancer into Bone Marrow

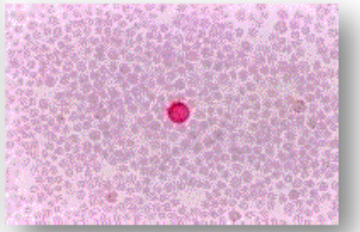
June 2008 | Volume 3 | Issue 6 | e2563

Kelly E. Corcoran[1], Katarzyna
Venkatesh Srinivas[1], Kathryn

- Through Tac1-mediated regulation of SDF-1a and CXCR4.

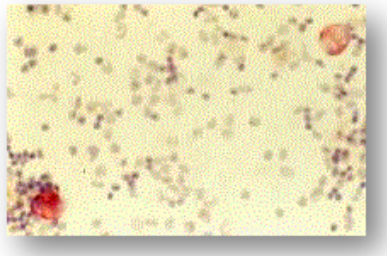


Prévention par clodronate



Etude prospective (1990-1996)
302 patientes

Cancer du sein (T1-T4)
avec µmétastases
médullaires



Chirurgie, RT, CT et HT selon les recommandations AGO

Randomisation

Clodronate 1600 mg/j PO
pendant 2 ans

Contrôle

Diel IJ *et al*, N Engl J Med, 1998;339:357-63

original article

Annals of Oncology 19: 2007–2011, 2008
doi:10.1093/annonc/mdn429
Published online 29 July 2008

Adjuvant oral clodronate improves the overall survival of primary breast cancer patients with micrometastases to the bone marrow—a long-term follow-up

I. J. Diel[1,2*], A. Jaschke[1], E. F. Solomayer[3], C. Gollan[1], G. Bastert[1], C. Sohn[1] & F. Schuetz[1]

[1]*Department of Gynecology and Obstetrics, University of Heidelberg, Heidelberg, Germany;* [2]*Institute for Gynecological Oncology, Mannheim, Germany;* [3]*Department of Obstetrics and Gynecology, University of Tübingen, Germany*

Received 27 April 2008; revised 18 June 2008; accepted 19 June 2008

	(*n* = 157)		
Median 36-month follow-up [9]			
Distant metastases	21 (13.4)	42 (29.0)	<0.001
Bone metastases	12 (7.6)	25 (17.2)	0.003
Visceral metastases	13 (8.3)	27 (18.6)	0.003
Deaths	6 (3.8)	22 (15.2)	0.001
Median 55-month follow-up [10]			
Distant metastases	32 (20.4)	51 (35.2)	0.022
Bone metastases	20 (12.7)	34 (23.4)	0.044
Visceral metastases	24 (15.3)	37 (25.5)	0.091
Deaths	13 (8.3)	32 (22.1)	0.002
Median 103-month (8.5-year) follow-up			
Distant metastases	61 (38.9)	57 (39.3)	0.816
Bone metastases	37 (23.6)	38 (26.2)	0.770
Visceral metastases	33 (21.0)	32 (22.1)	0.222
Deaths	32 (20.4)	59 (40.7)	0.049

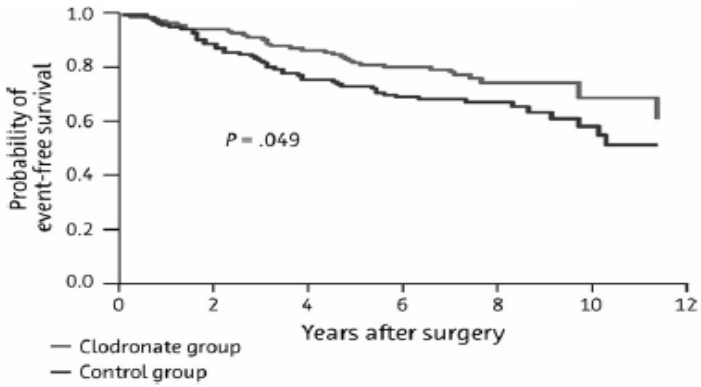


Figure 3. Kaplan–Meier curve of overall survival among patients treated with oral clodronate compared with standard follow-up therapy ($N = 209$).

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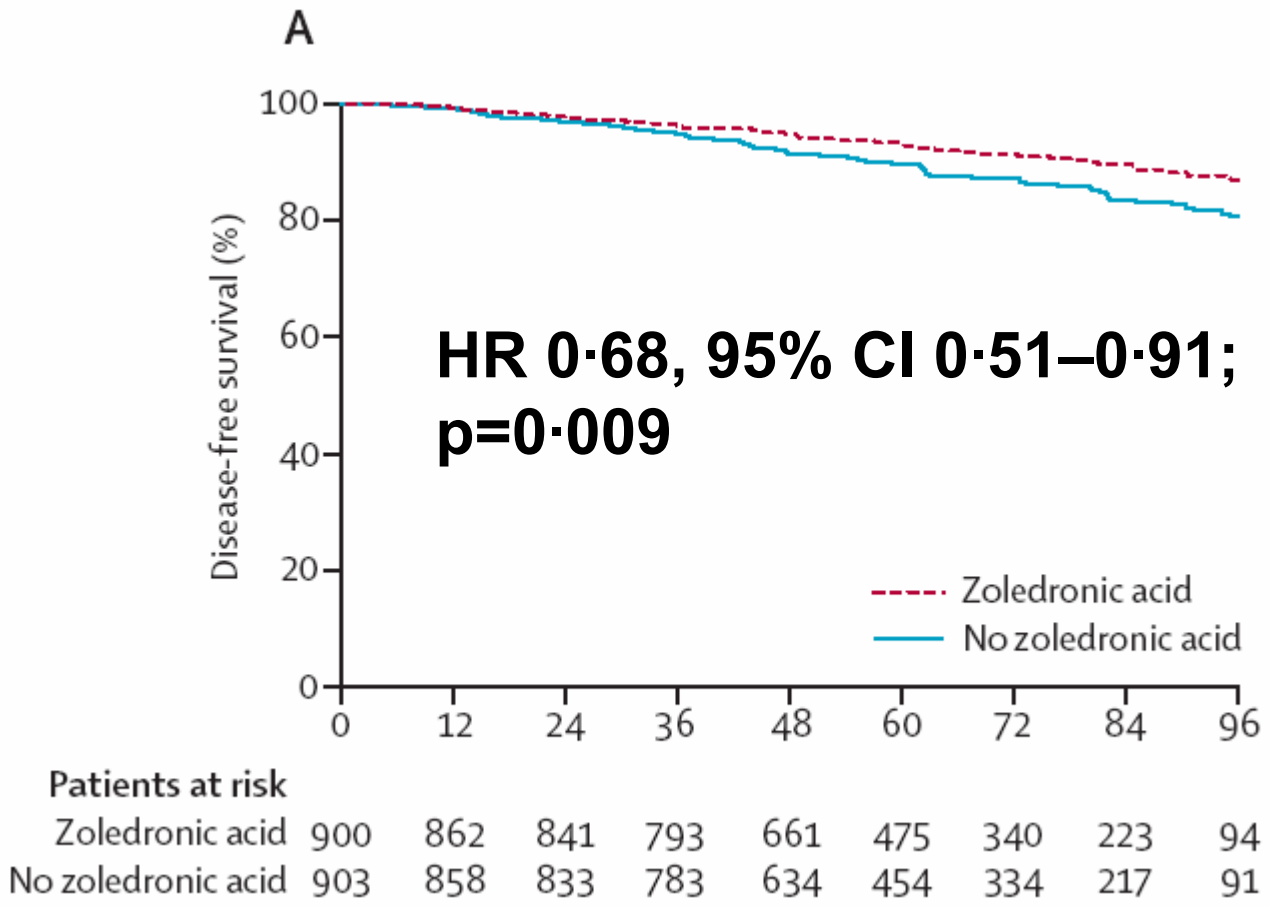
Adjuvant zoledronate

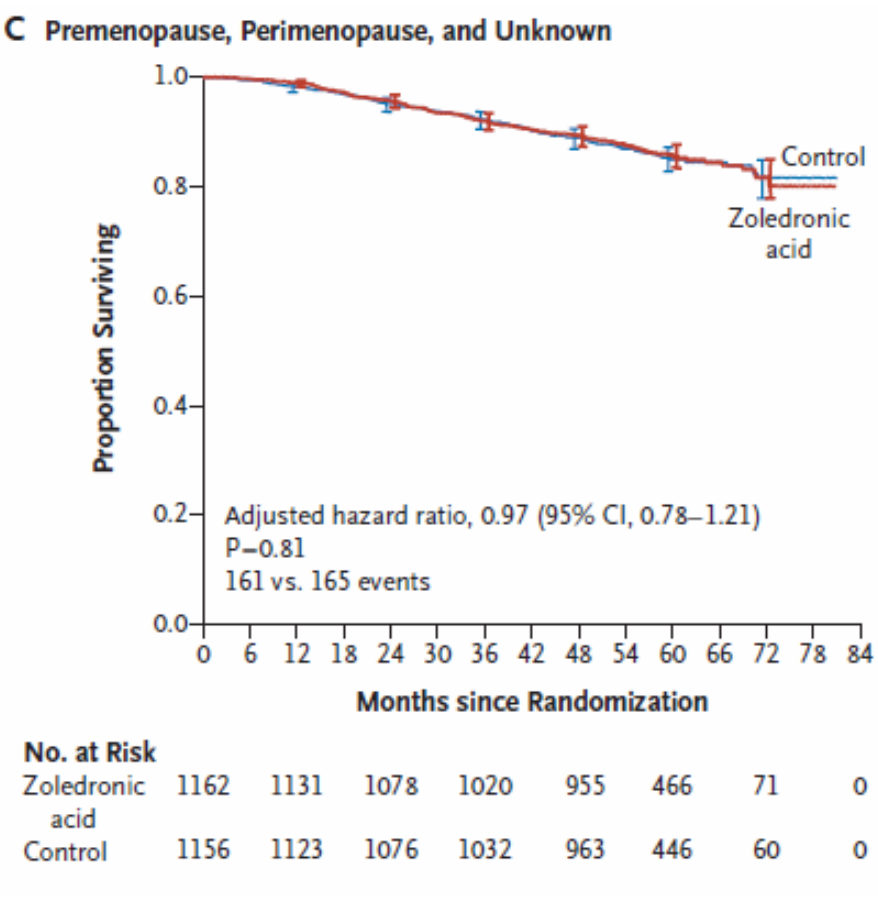
median follow-up of 62 months (range 0–114·4 months)

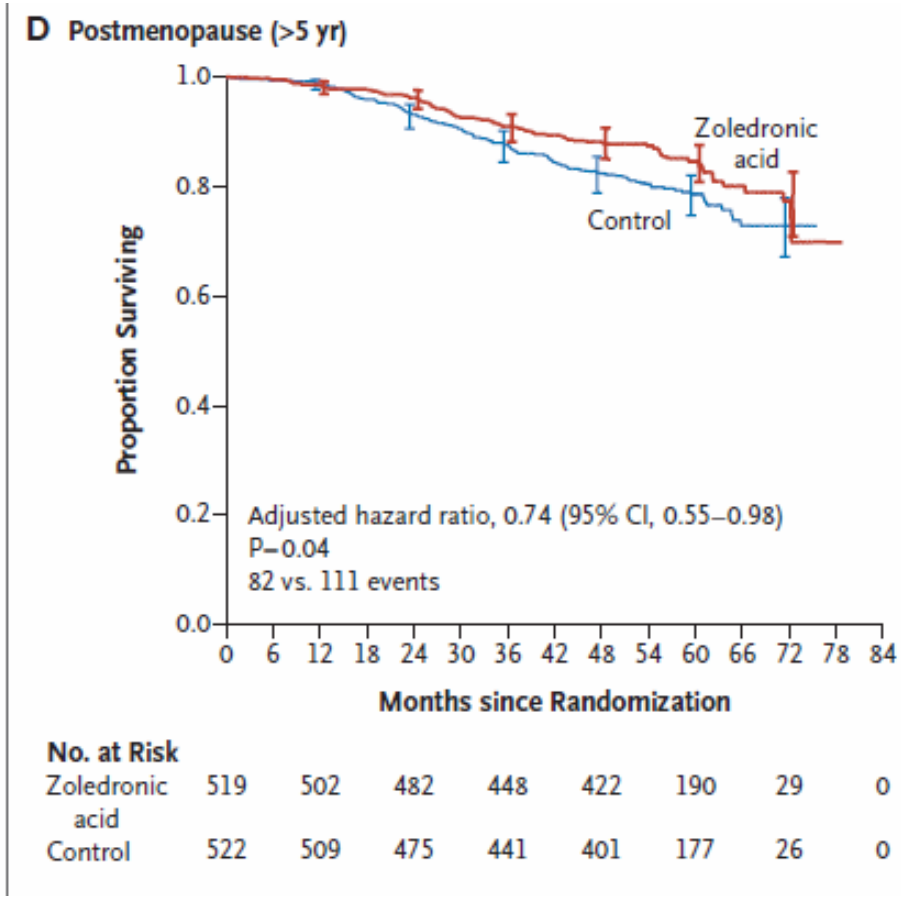
ABCSG-12



Gnant M, Lancet Oncol 2011;12:631-41

AZURE trial





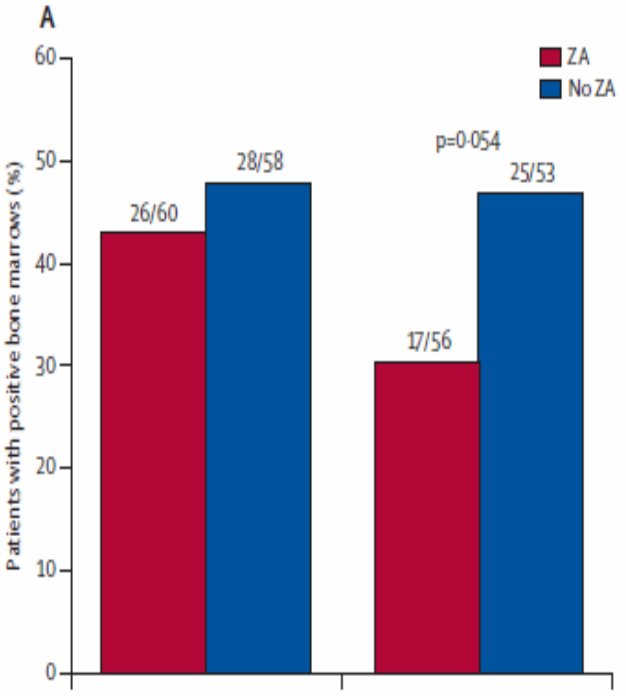
Coleman RE, N Engl J Med 2011

Effect of zoledronic acid on disseminated tumour cells in women with locally advanced breast cancer: an open label, randomised, phase 2 trial

Rebecca Aft, Michael Naughton, Kathryn Trinkaus, Mark Watson, Lourdes Ylagan, Mariana Chavez-MacGregor, Jing Zhai, Sacha Kuo, William Shannon, Kathryn Diemer, Virginia Herrmann, Jill Dietz, Amjad Ali, Matthew Ellis, Peter Weiss, Timothy Eberlein, Cynthia Ma, Paula M Fracasso, Imran Zoberi, Marie Taylor, William Gillanders, Timothy Pluard, Joanne Mortimer, Katherine Weilbaecher

120 patients recevant de la CT ± ZOL toutes les 3 semaines pendant 1 an
Chez les patients DTC-free at baseline, 79% des pts recevant ZOL restent DTC free à 3 mois vs. 51% sans ZOL

A 1 an, 38% des pts ZOL sont DTC-free vs. 25% des pts sans ZOL

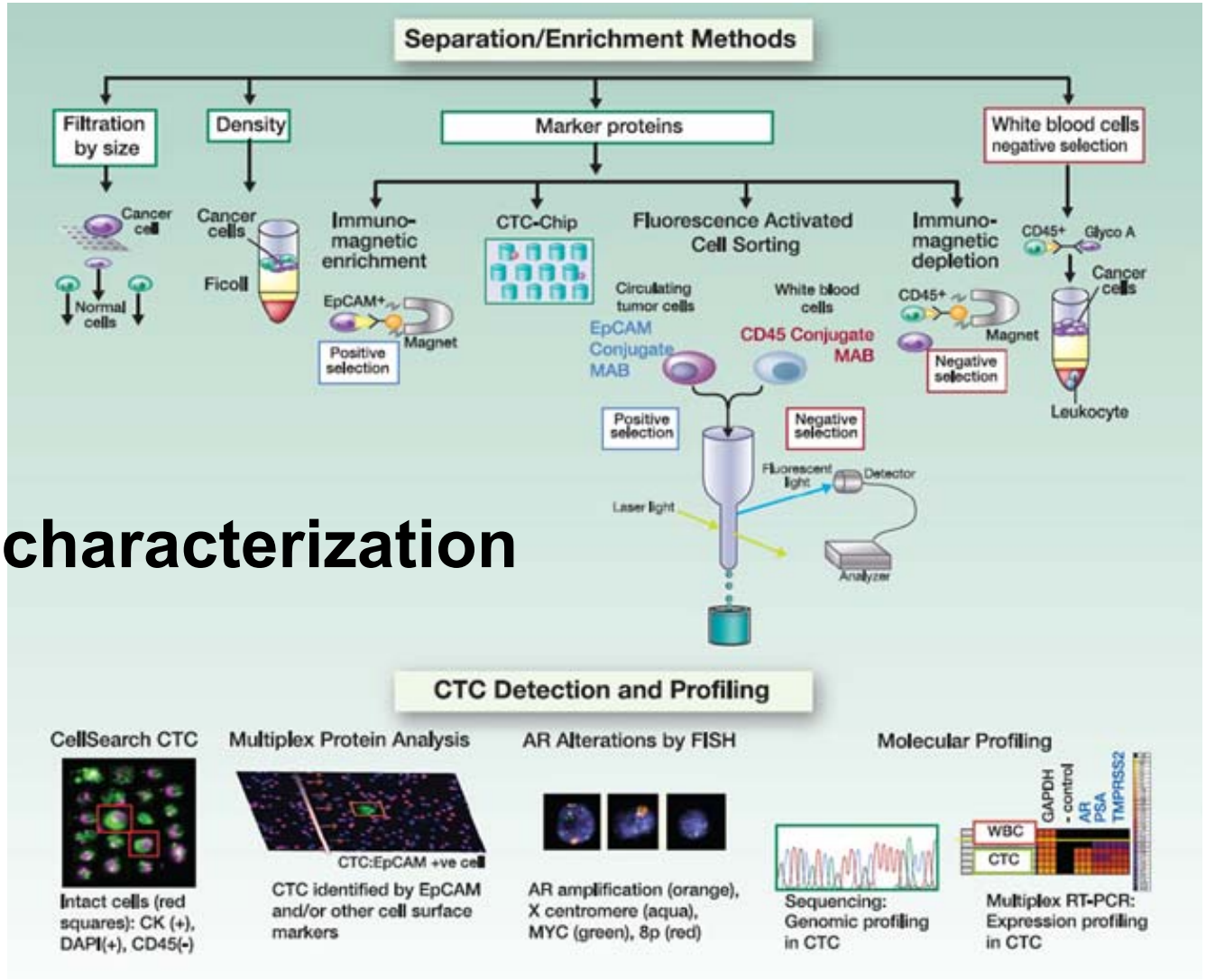


www.thelancet.com/oncology Published online April 1, 2010 DOI:10.1016/S1470-2045(10)70054-1

La biopsie liquide

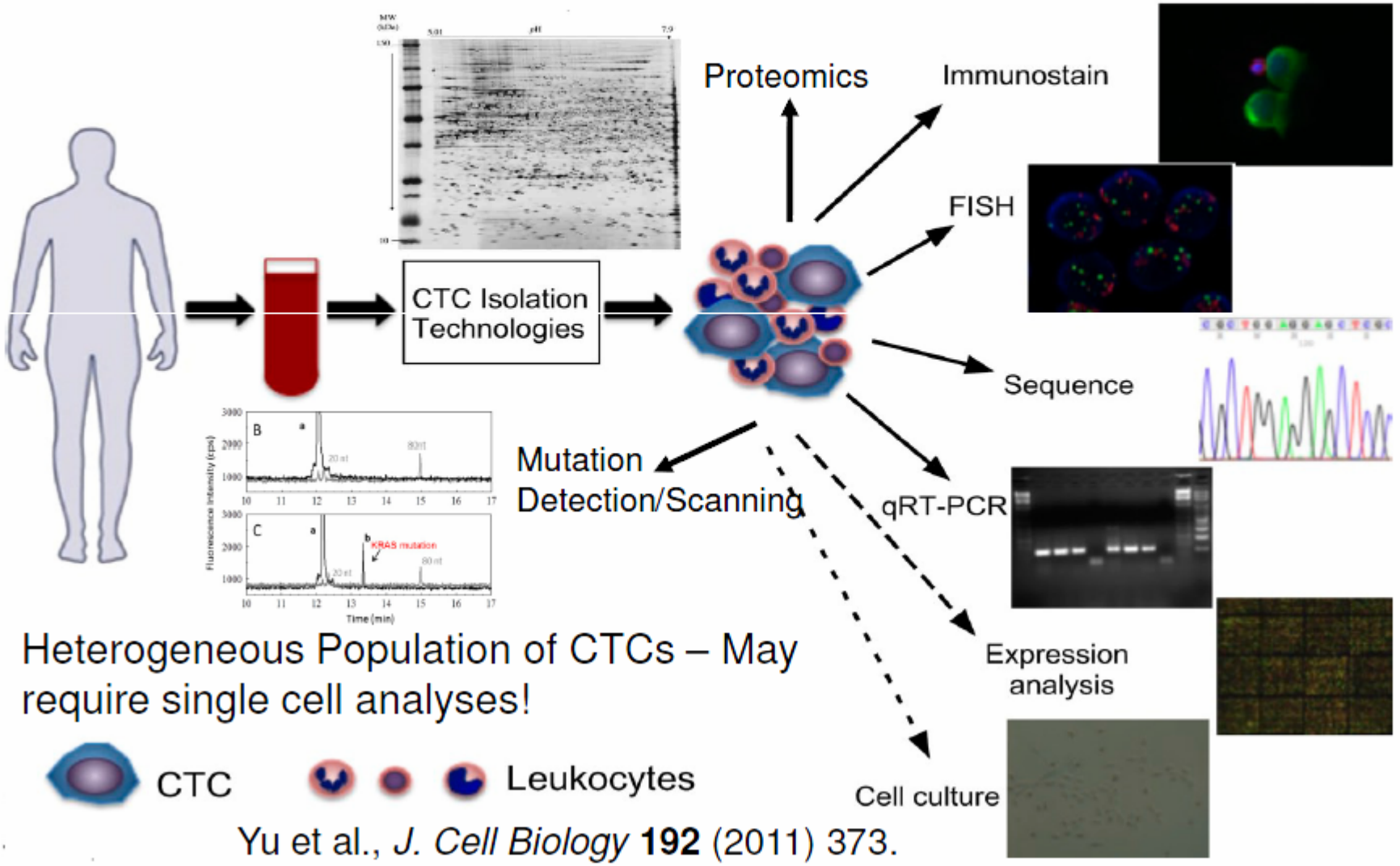
Increasing number of detection techniques (> 50 in 2012)

CTC characterization



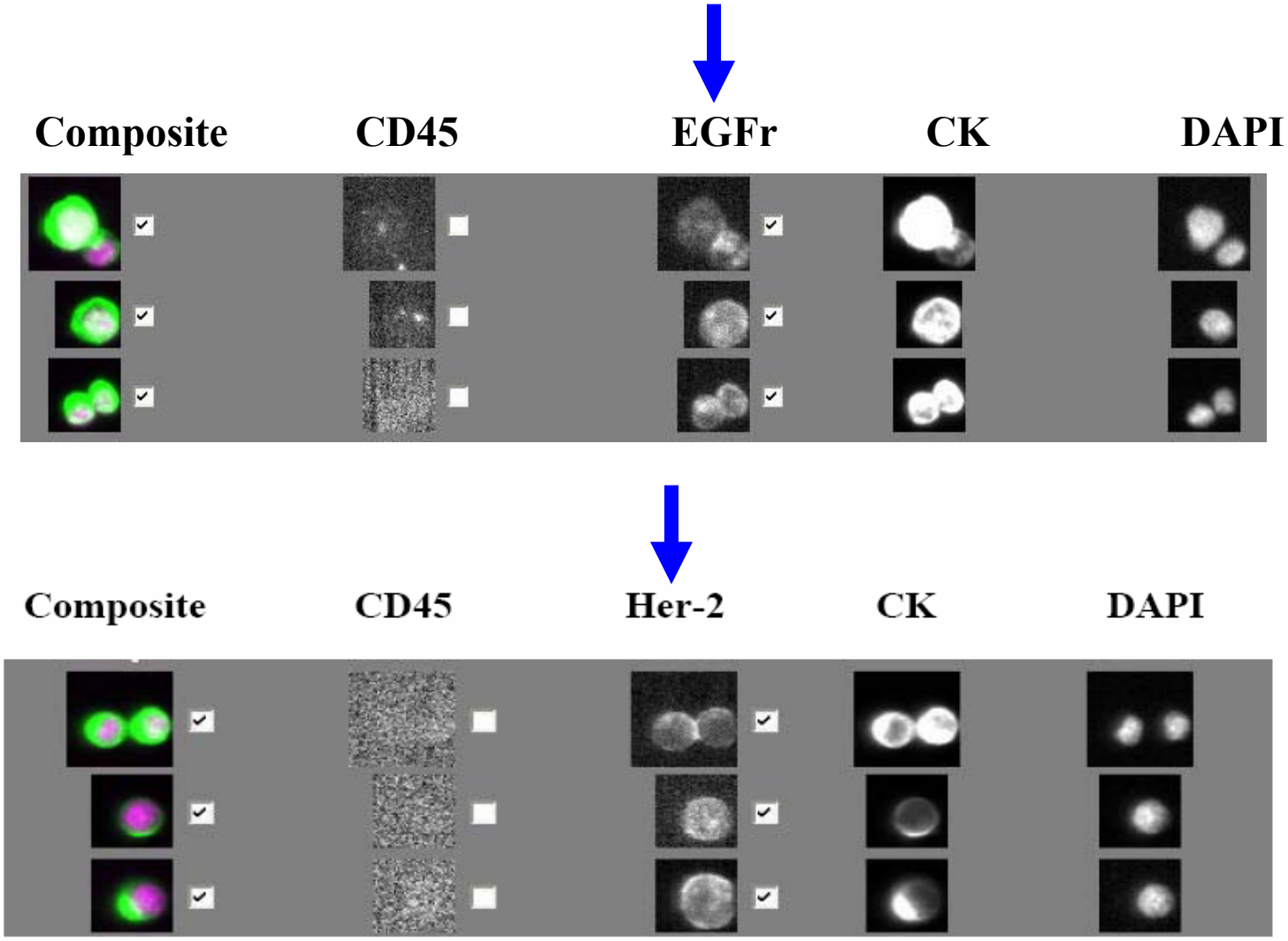
Danila, DC Clin Cancer Res; 17(12); 3903–12. 2011

Dealing with Mass-Limited Samples – Don't Just Count, Analyze



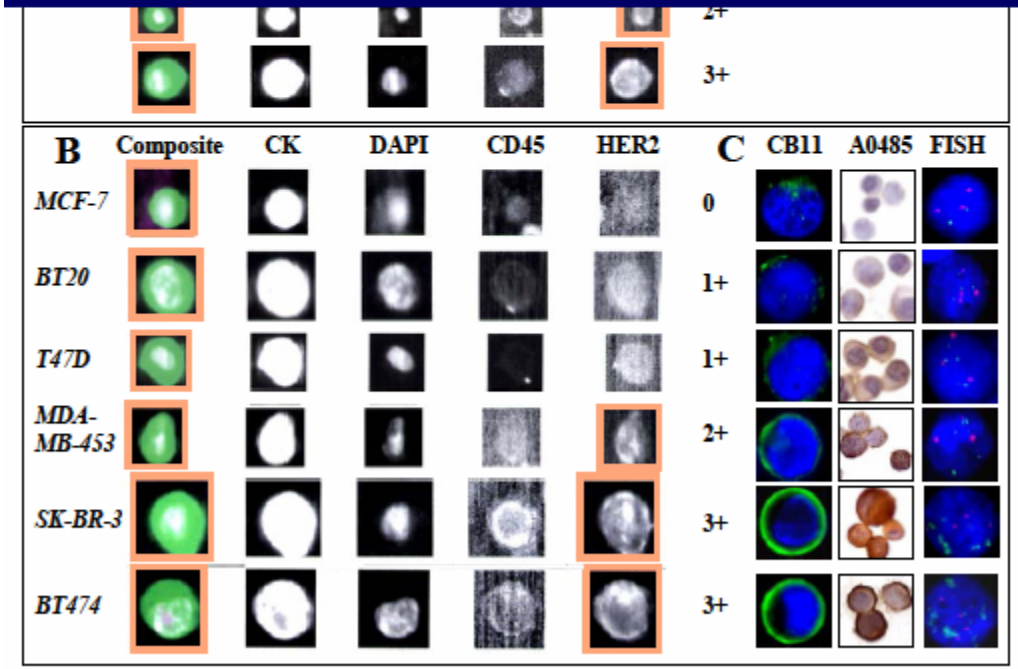
Yu et al., *J. Cell Biology* **192** (2011) 373.

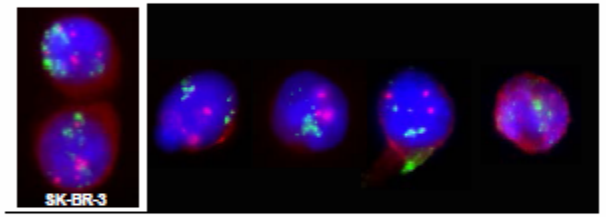
Her2Neu expression on CTC
Open Channel: Drug Targeting



Detection of therapeutic target molecules on CTC
Example: HER2 in breast cancer

Potential benefit from anti-HER2 therapy (e.g., trastuzumab) also in patients with „HER2-negative“ tumors (Paik *et al.*, NEJM 2008)

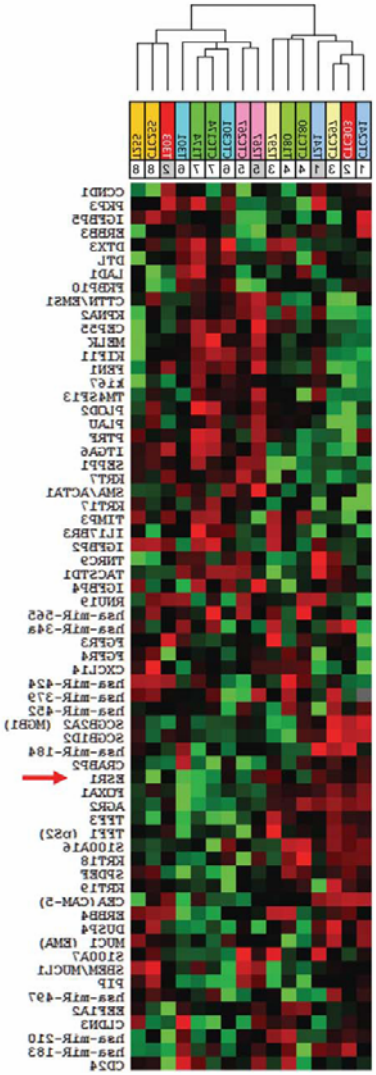


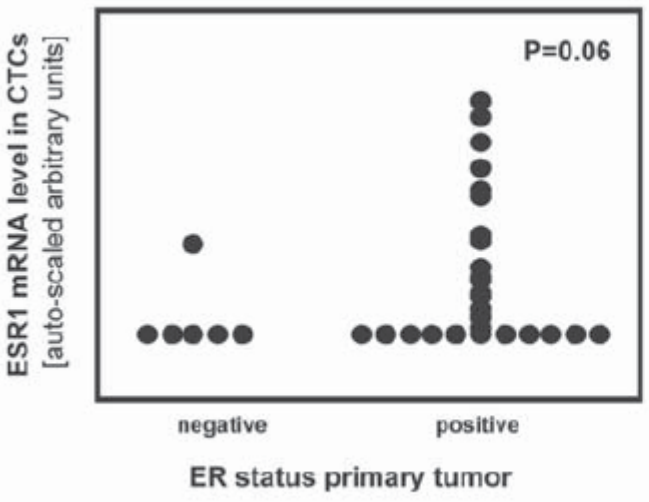


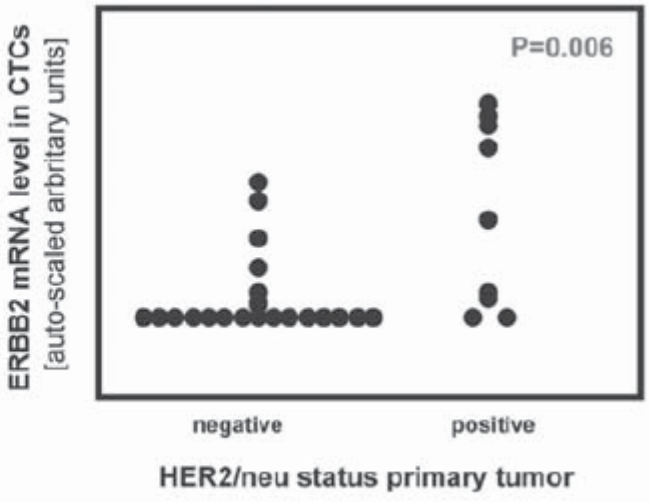
- **HER2-pos. CTC in pats w HER2-neg. primary tumors**
- **HER2-neg. & HER2-pos. CTC after trastuzumab**

Riethdorf/Pantel et al., Clinical Cancer Res 2010; Fehm/Pantel et al., Breast Cancer Res Treat 2010
Ignatiadis/Sotiriou et al, PlosONE, 2011

mRNA and microRNA expression profiles in circulating tumor cells and primary tumors of metastatic breast cancer patients

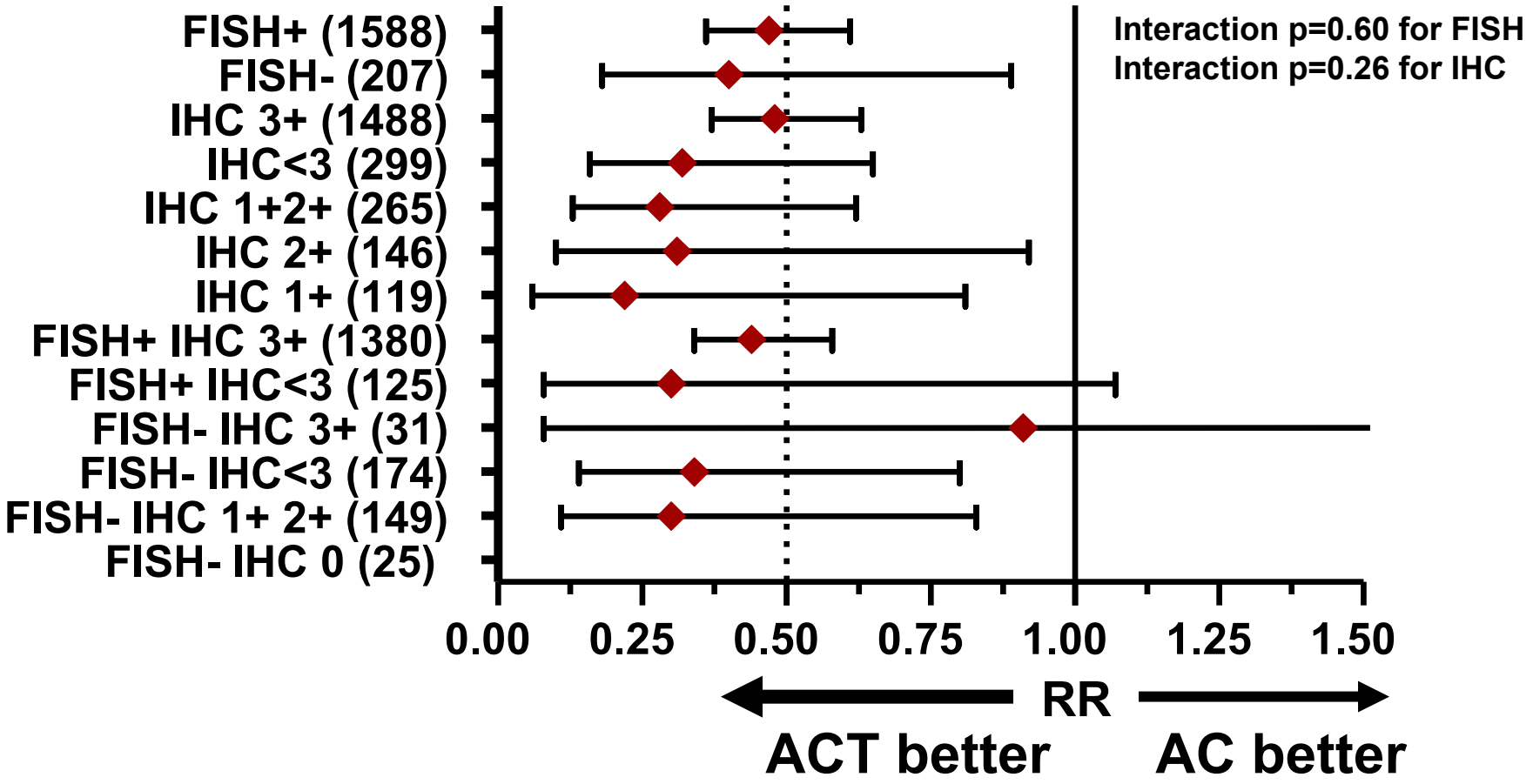






A Sieuwerts Clin Cancer Res, May 2011

RR of ACTH/ACT for DFS in B31



Paik S, Kim C, Wolmark N. NEJM 2008

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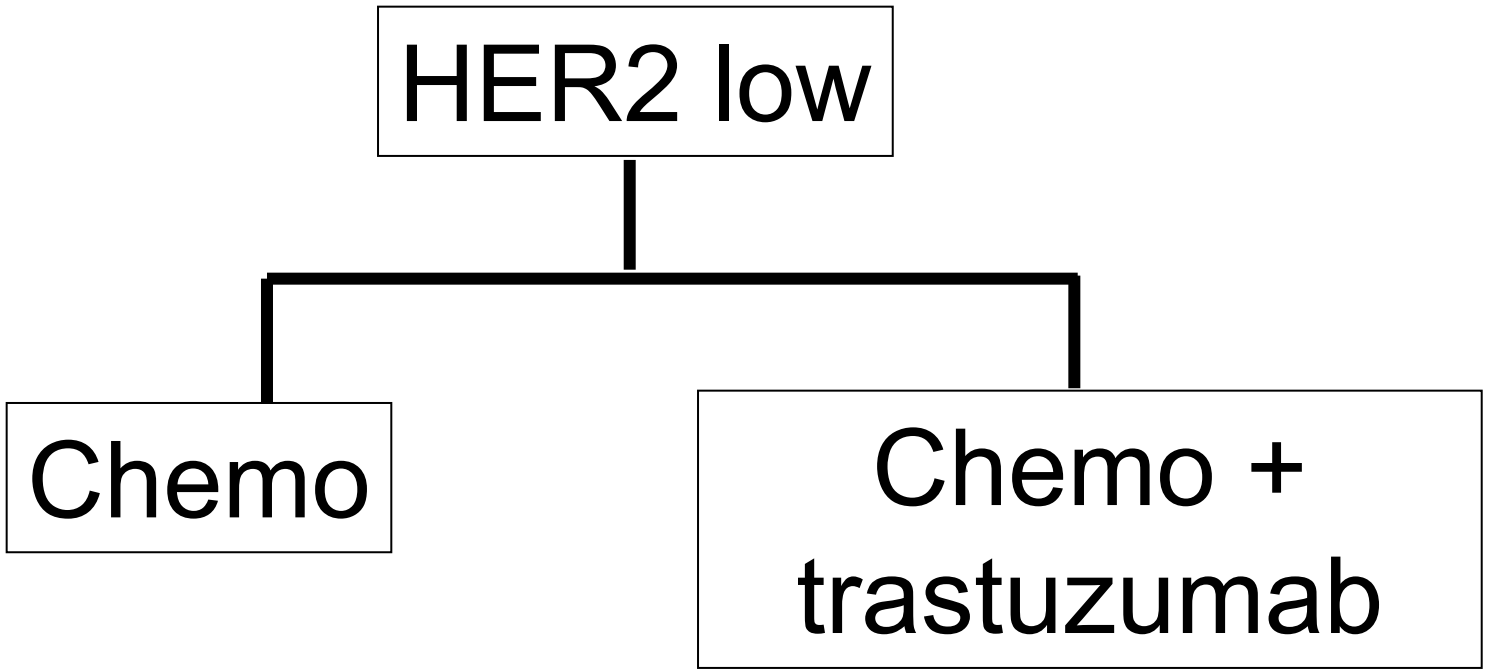
HER2 low trial concept NSABP B47



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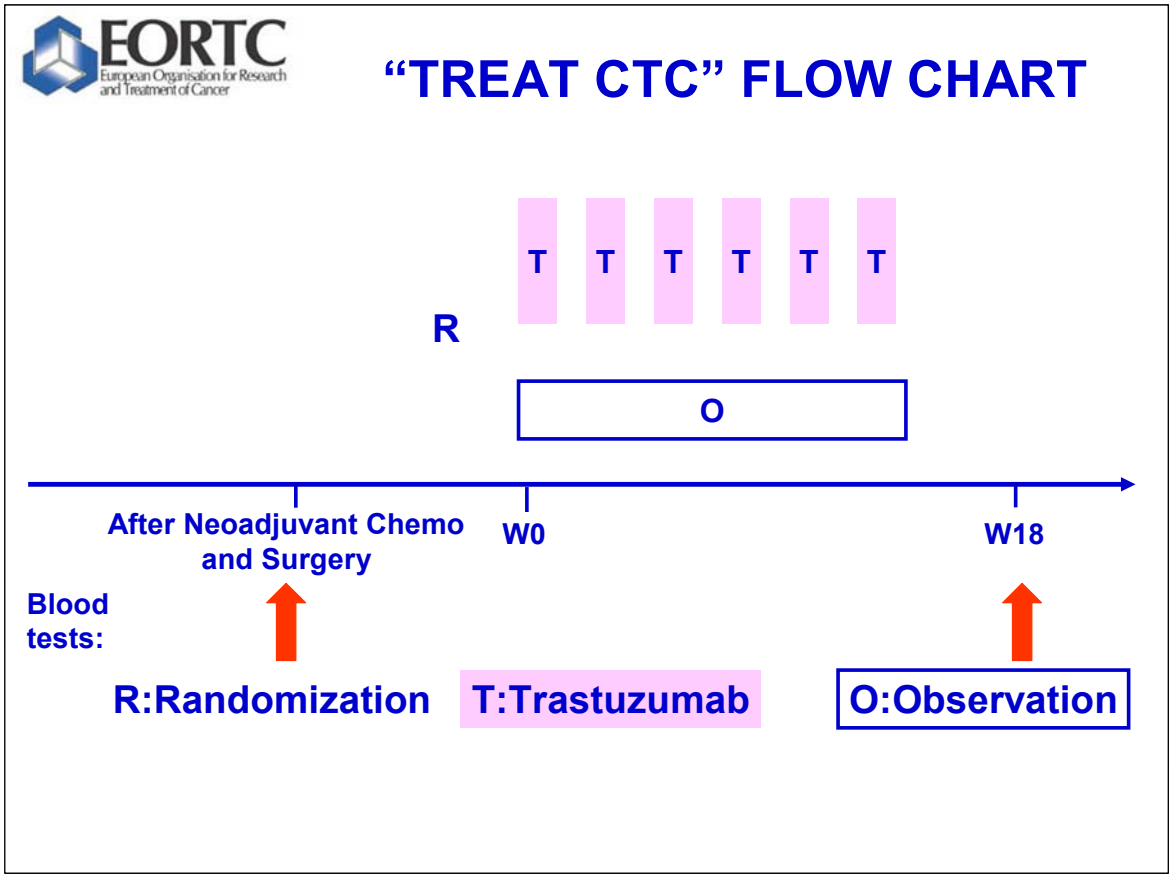
cM0(i+) : TREAT-CTC trial M. Ignatiadis PI



Start in 2012 (?)

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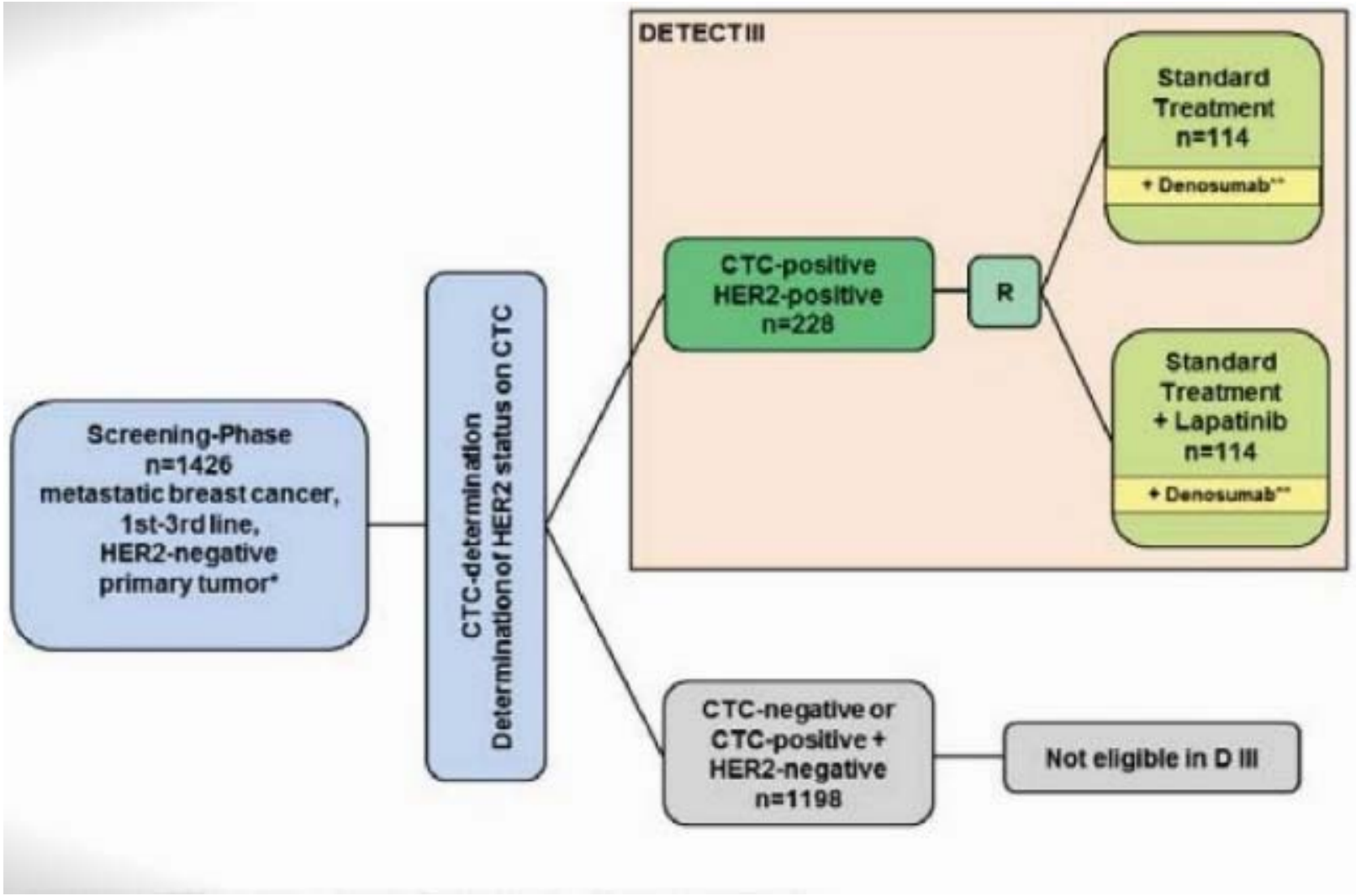
Essai DETECT 3

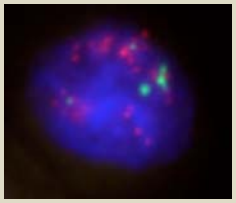


CirCe XXX1

Screening by FISH
N~400

- M+ HER2- patients before 2nd line
- Mesurable disease

≥1 and <5 CTC
WITH HER2/CEP17≥2.2
➔ Cohort « L » (low)



≥5 CTC
WITH HER2/CEP17≥2.2
➔ Cohort « H » (high)

No CTC detected or
no HER2 amplification
➔ Not included

1st step (N=14): 7 patients « L » + 7 patients « H » treated by anti-HER2 drug

N= 0 /14 response(s)

Inefficacy in the whole population

N= 1-2 /14 response(s)

N ≥3 /14 responses

With ≥1 response in each group

Efficacy in the whole population

With no response in H group

Efficacy in the L population

With no response in L group

Efficacy in the H population

2nd step (N=14): Add 7 patients « L » + 7 patients « H » treated by anti-HER2 drug

N ≤3 /28 response(s)

Inefficacy in the whole population

N ≥4 /28 responses

With ≥1 response in each group

Efficacy in the whole population

With no response in H group

Efficacy in the L population

With no response in L group

Efficacy in the H population

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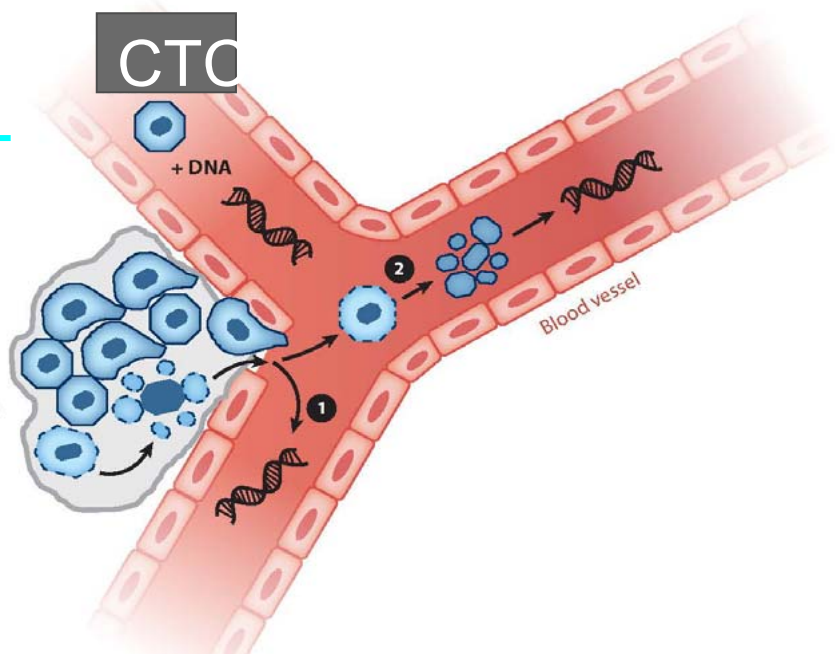
Bidard FC, Fehm T, Ignatiadis M, Smerage JB et al, Cancer Met Rev, 2013 Jan, *in press*

Autres marqueurs circulants

Detection de l'ADN libre dans le serum



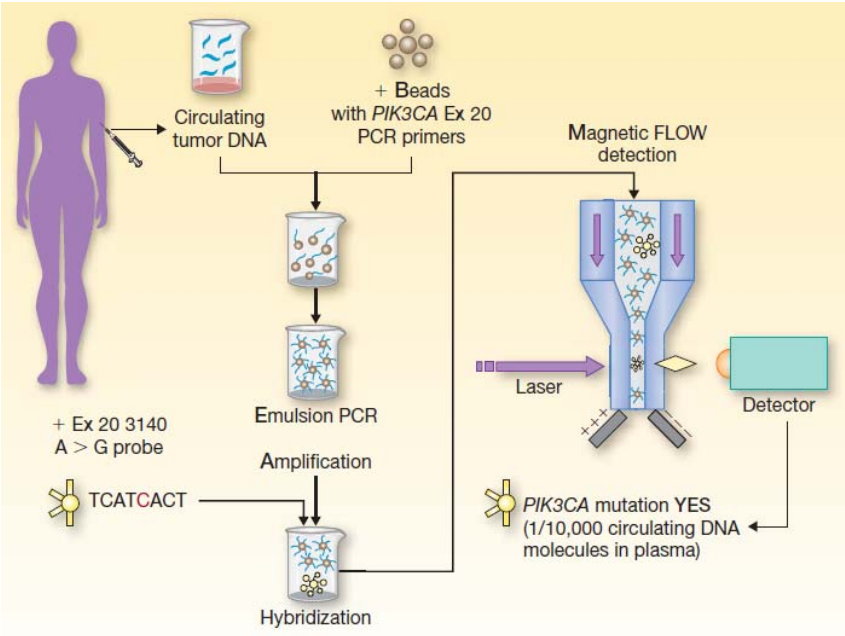
It has been estimated that for a patient with a tumor that weighs 100 g, which corresponds to 3×10^{10} tumor cells, up to 3.3% of tumor DNA may enter the blood every day

Schwarzenbach/Hoon/Pantel, *Nat Rev Cancer,* 2011
Alix-Panabières/Schwarzenbach/Pantel, *Annu Rev Med*, 2012

BEAMing Up Personalized Medicine: Mutation Detection In Blood

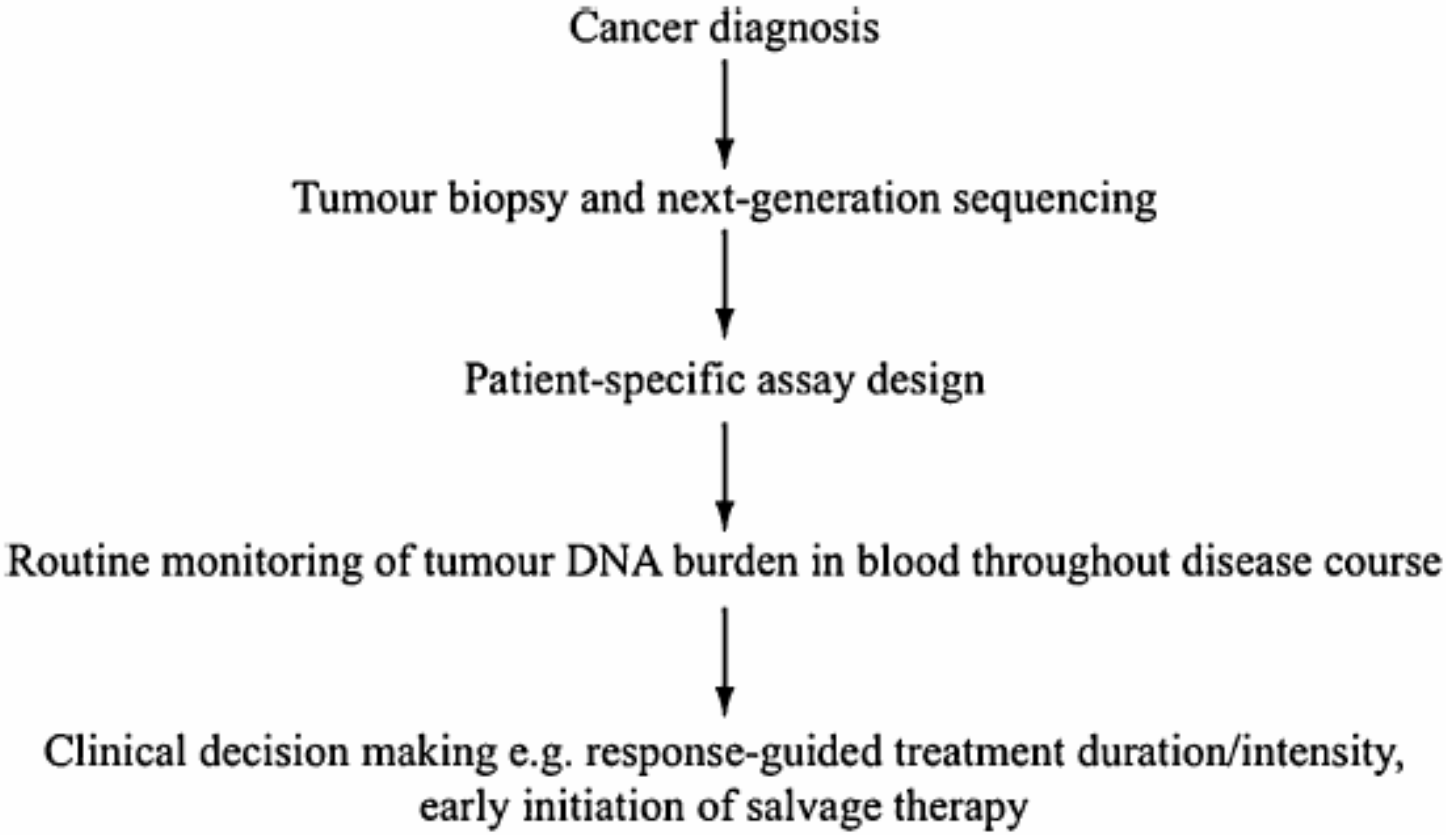
100% agreement in *PIK3CA mutation* status determined by BEAMing detection using free circulating tumor DNA (ctDNA) from peripheral blood samples in a prospective study of metastatic breast cancer patients when compared to standard sequencing performed on the same tumor samples

- Change in mutation status in 27,5% of the patient during follow-up



Richardson AL CCR May 2012, Higgins MJ CCR 2012

Circulating DNA and Next-Generation Sequencing



The disseminated laboratory of DTC/CTC:

Medical Oncology
Pr JY Pierga
Dr FC Bidard
& others

Pathology
Dr A Vincent -Salomon
Dr X Sastre-Garau
O Mariani
& others

Surgery
Dr Séverine Alran
Dr Pascale Mariani
& others

Hematology
Dr C Mathiot
I Vaucher
M Khazour

CNRS UMR 168
Jean-Louis Viovy
Julien Autebert
Benoit Coudert

Statistics
Dr B Asselain
Dr D Hajage
E Rolland

CNRS UMR 168
F Amblard

Radiotherapy
Dr Youlia Kirova

UGEC
P. Tresca
S Armanet & coll

Immunology
Dr O Lantz
M Milder

Inserm U 830
Dr MH Stern

Translationnal Research dpt
S Roman-Roman
C Decraene
A Almeida

LIP
Dr Didier Decaudin

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MD, Ph.D, University Medical Centre
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