

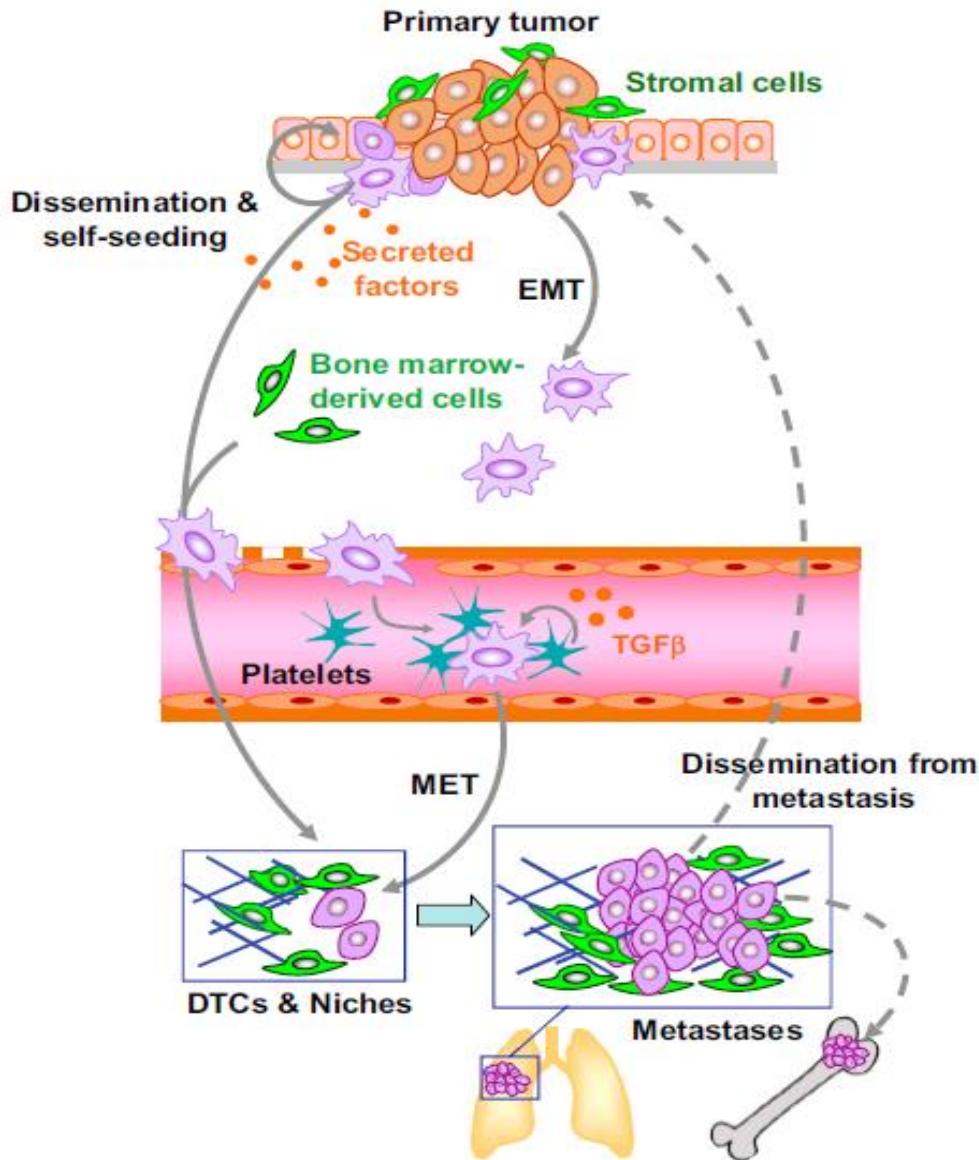
# **Personnaliser les traitements médicaux en situation non métastatique:**

**La présence de CTC et/ou d'ADN  
circulant change-t-elle la décision?**

**Jean-Yves Pierga**

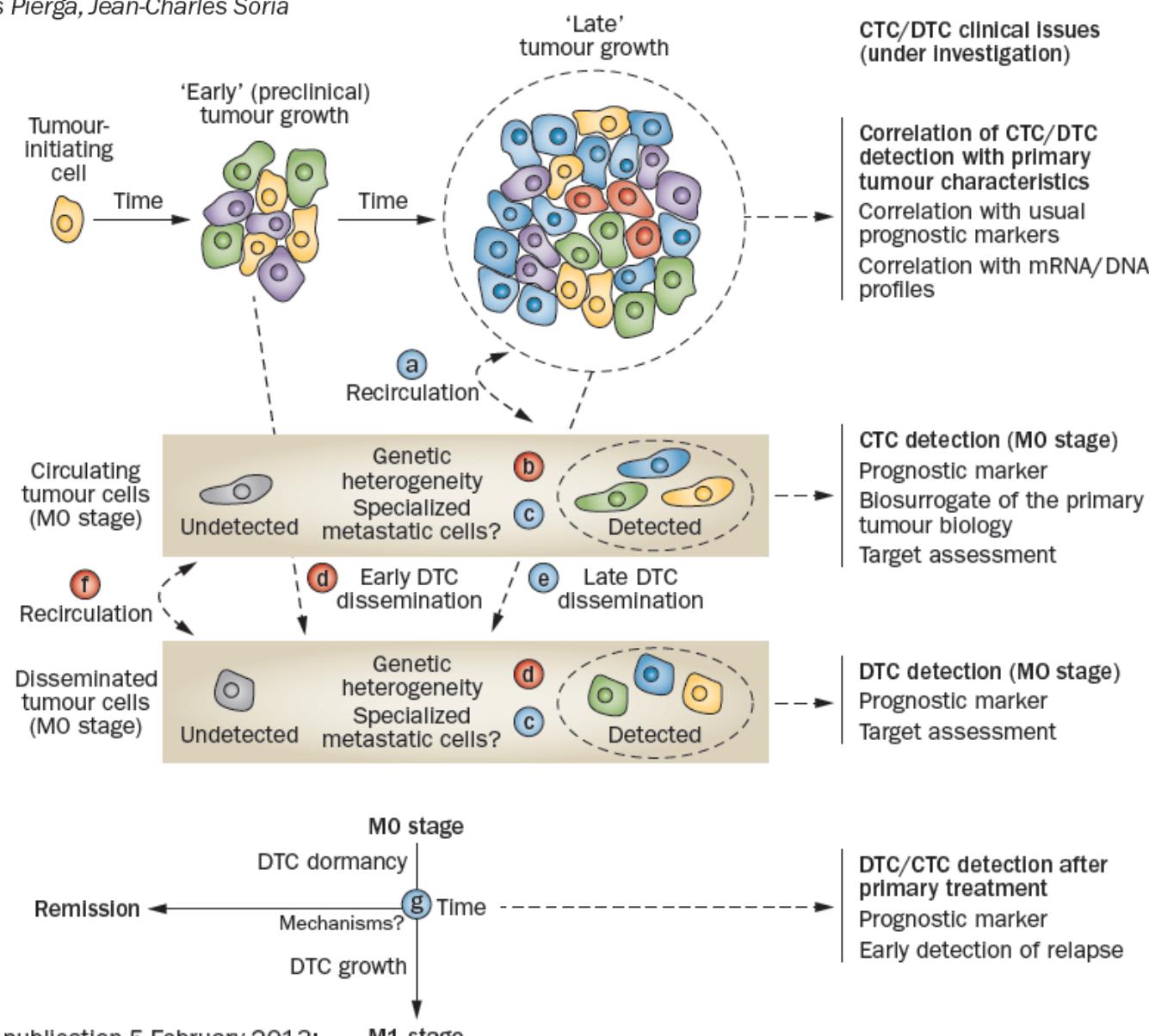
Département d'Oncologie médicale  
Institut Curie

# Etapes de la dissémination métastatique et de la survie des cellules tumorales circulantes CTC



# Translating metastasis-related biomarkers to the clinic—progress and pitfalls

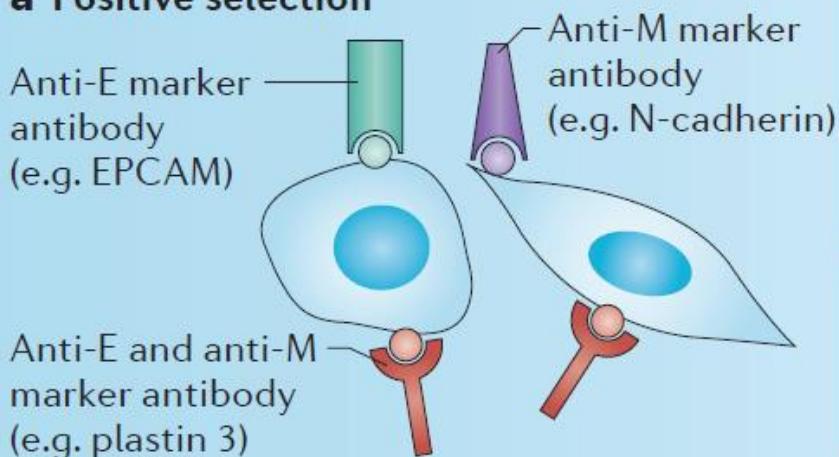
François-Clément Bidard, Jean-Yves Pierga, Jean-Charles Soria  
and Jean Paul Thiery



# Multiples techniques de détection des CTC

## Biological properties

### a Positive selection



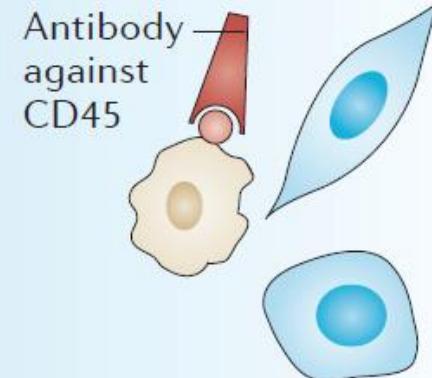
### Ex vivo

- CellSearch® system
- MagSweeper™
- EPHESIA CTC-chip
- CTC-chip
- Velcro-like device

### In vivo

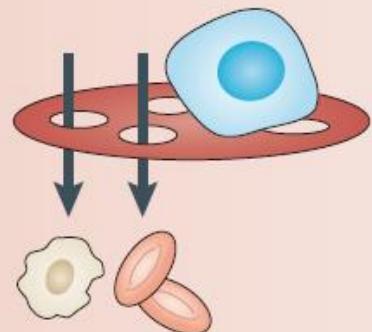
- CellCollector®
- Photoacoustic nanodetector

### b Negative selection

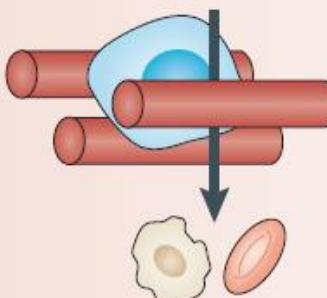


## Physical properties

### c Filtration



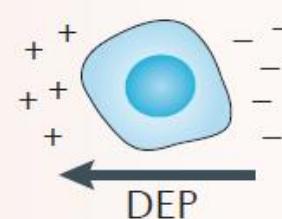
### d Chip



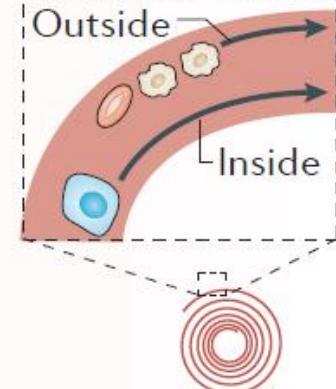
### e Ficoll gradient



### f Electric field



### g Single spiral microchannel



# Détection des cellules tumorales circulantes

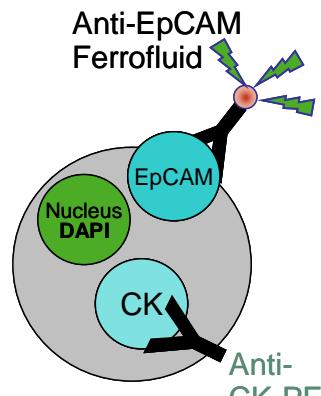
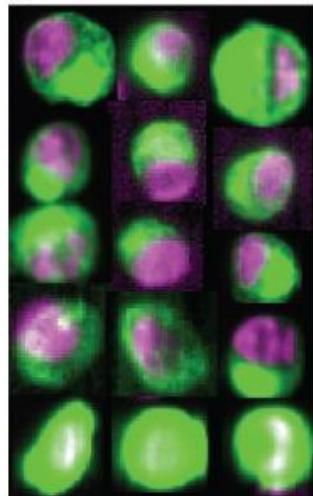
## CellSearch™ System FDA cleared



Sample Collection



Reagents:  
CTC capture  
& staining



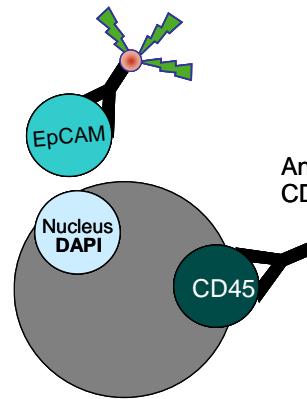
Epithelial  
Cell



Sample  
Processing



Sample  
Presentation



Leukocyte

- ① Selection positive EPCAM
- ② Staining : CK 8/18/19, CD45, DAPI
- ③ others: HER2/Neu, MUC1, EGF-R)

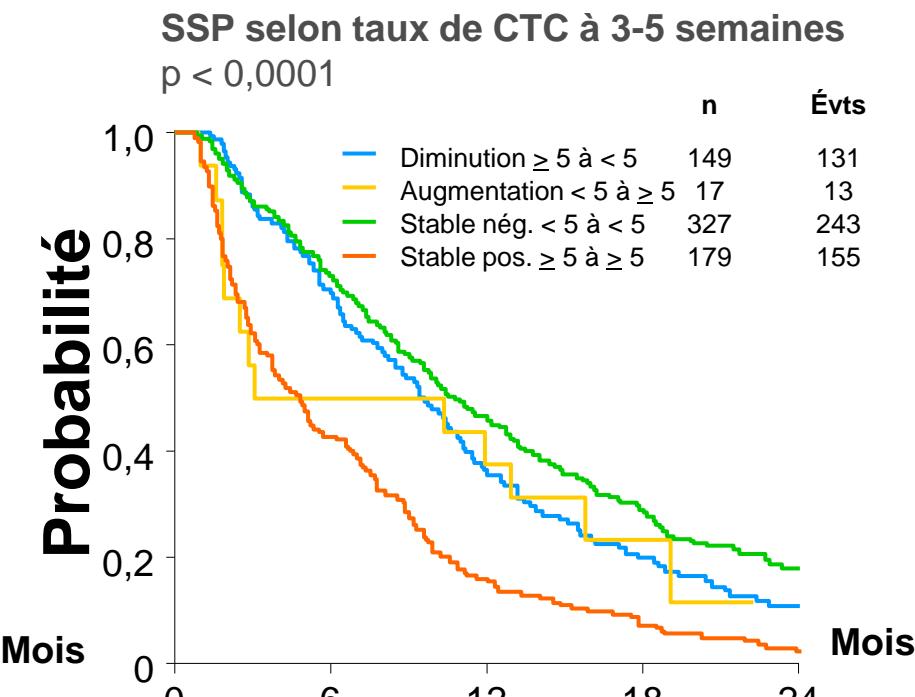
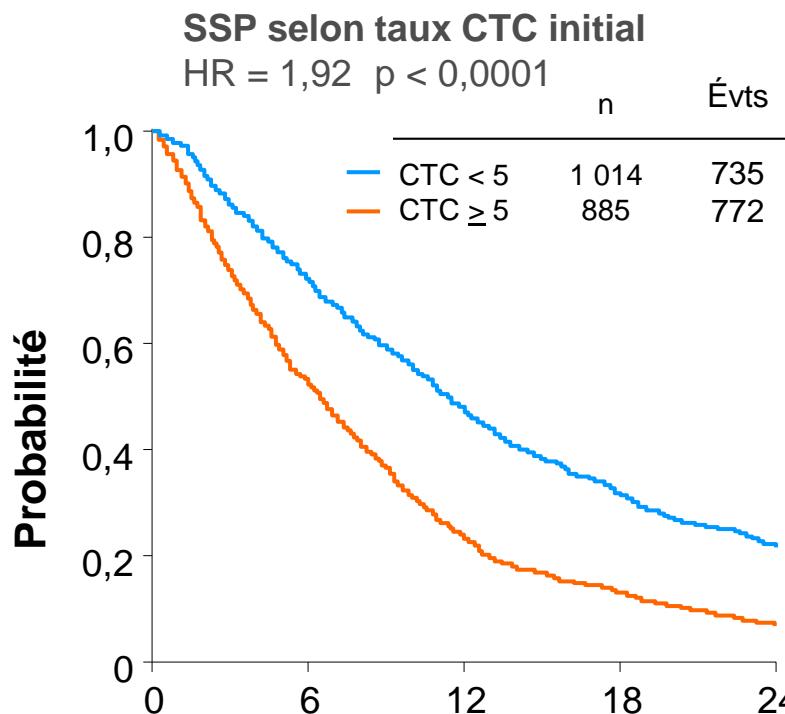


Sample  
Analysis

# Résultats de la méta-analyse portant sur 1 944 patientes métastatiques

$\geq 5$  CTC chez 47 % des patientes initialement →

45 % des patientes : diminution  $< 5$  CTC  
3-5 semaines après (courbe bleue)  
55 % des patientes : avec encore  $\geq 5$  CTC  
(courbe rouge)



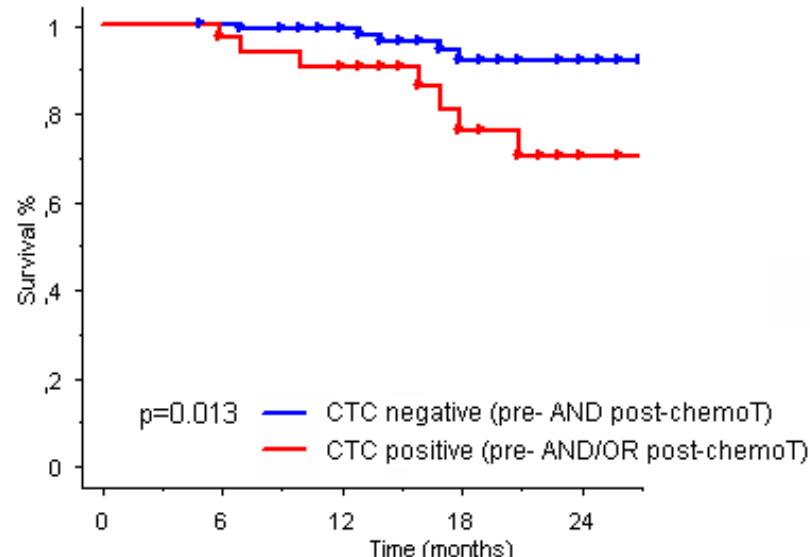
- Résultats identiques pour la survie globale
- Supériorité des CTC sur les marqueurs sériques tumoraux

# 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

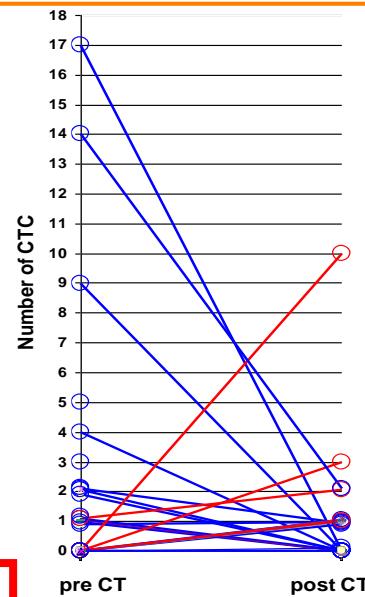
23% cases  $\geq$ 1CTC

Remagus 02 Clinical Trial



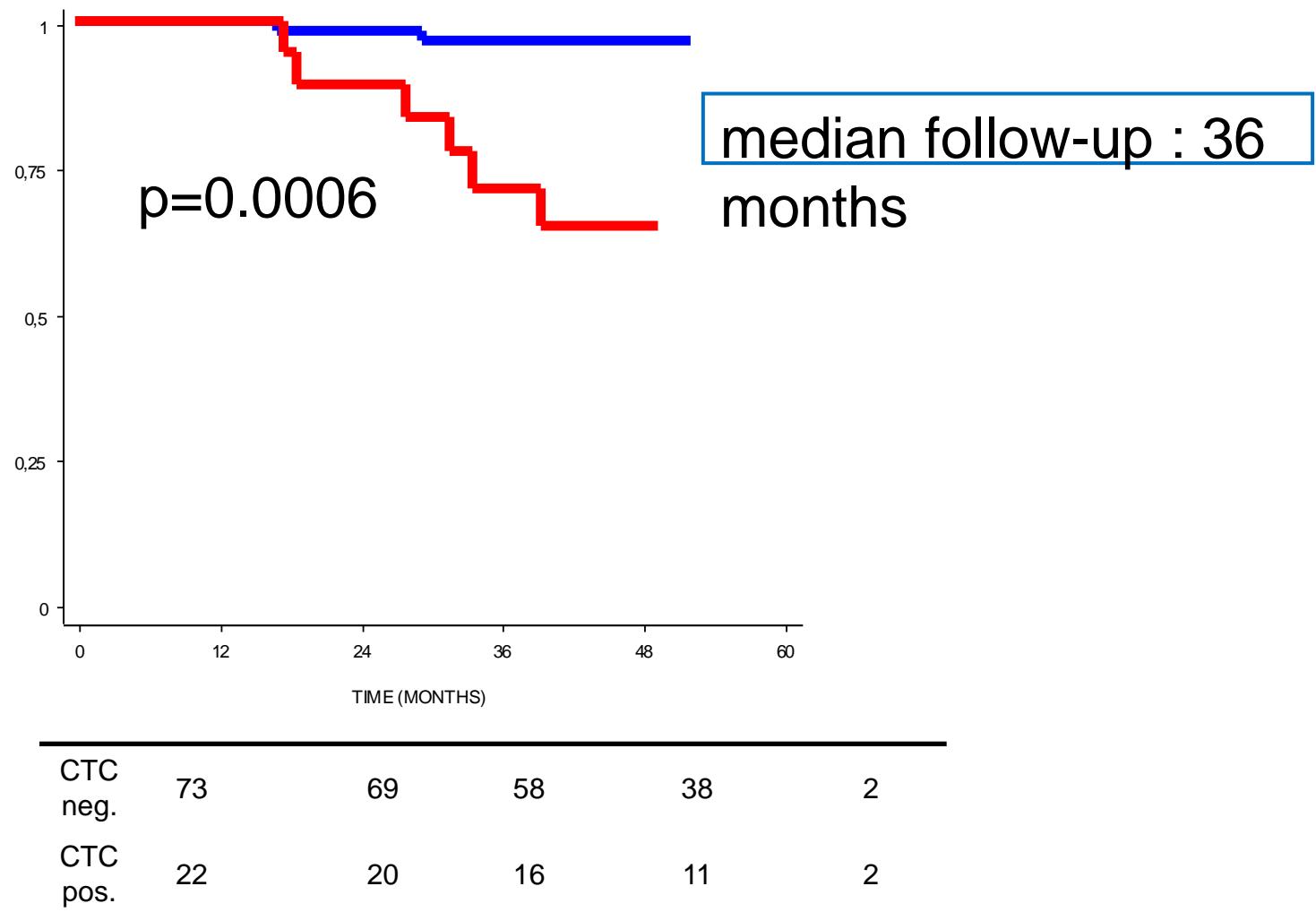
CTC-	86	84	77	43	17
CTC+	32	31	27	15	8

CTC detection before and after neoadjuvant chemotherapy



No correlation  
with pCR !

## Survie globale CTC pre-chemo N= 97



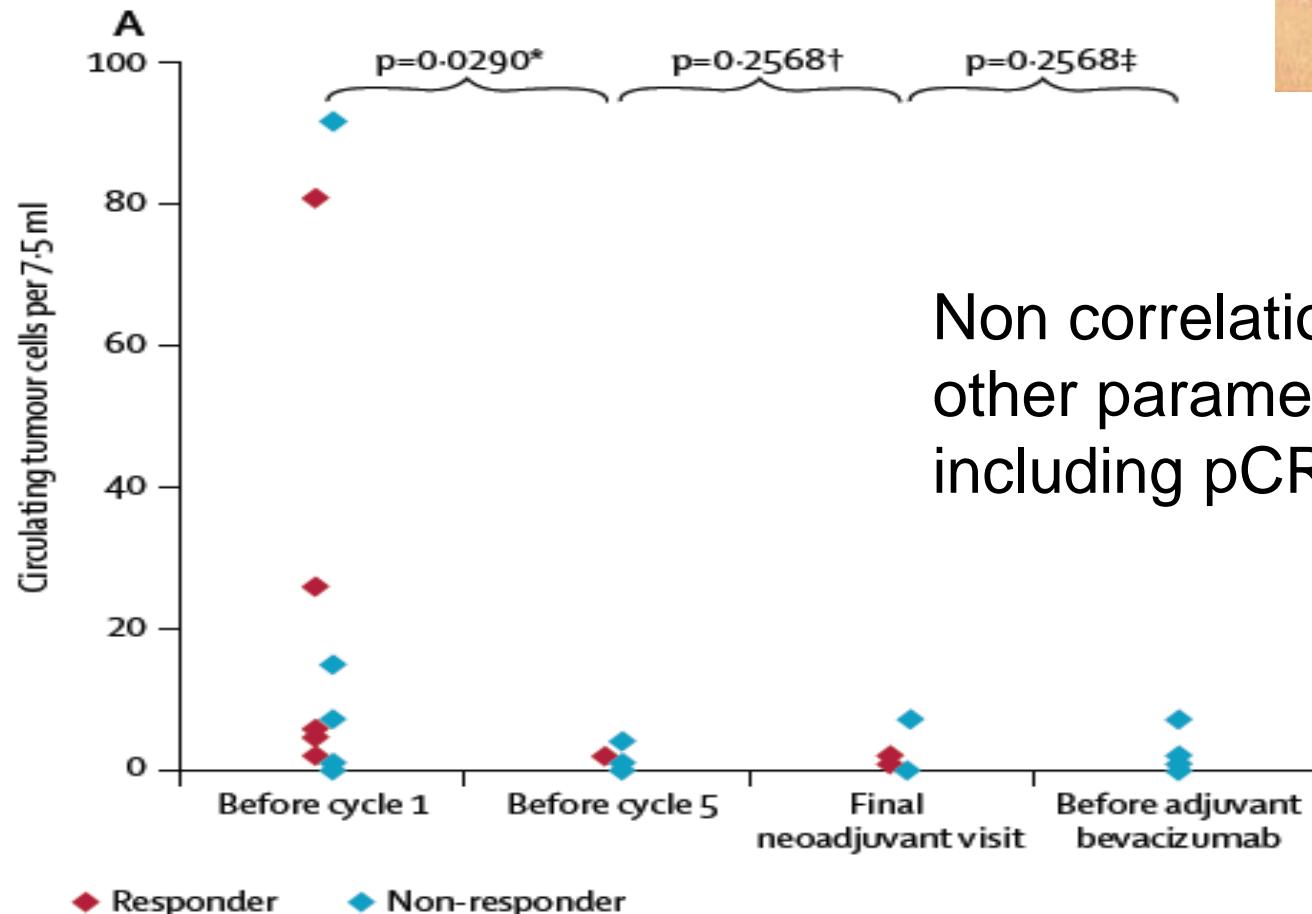
BEVERLY 2

Inflammatory Breast cancer HER2+

N=52 detection rate of CTC= 35%

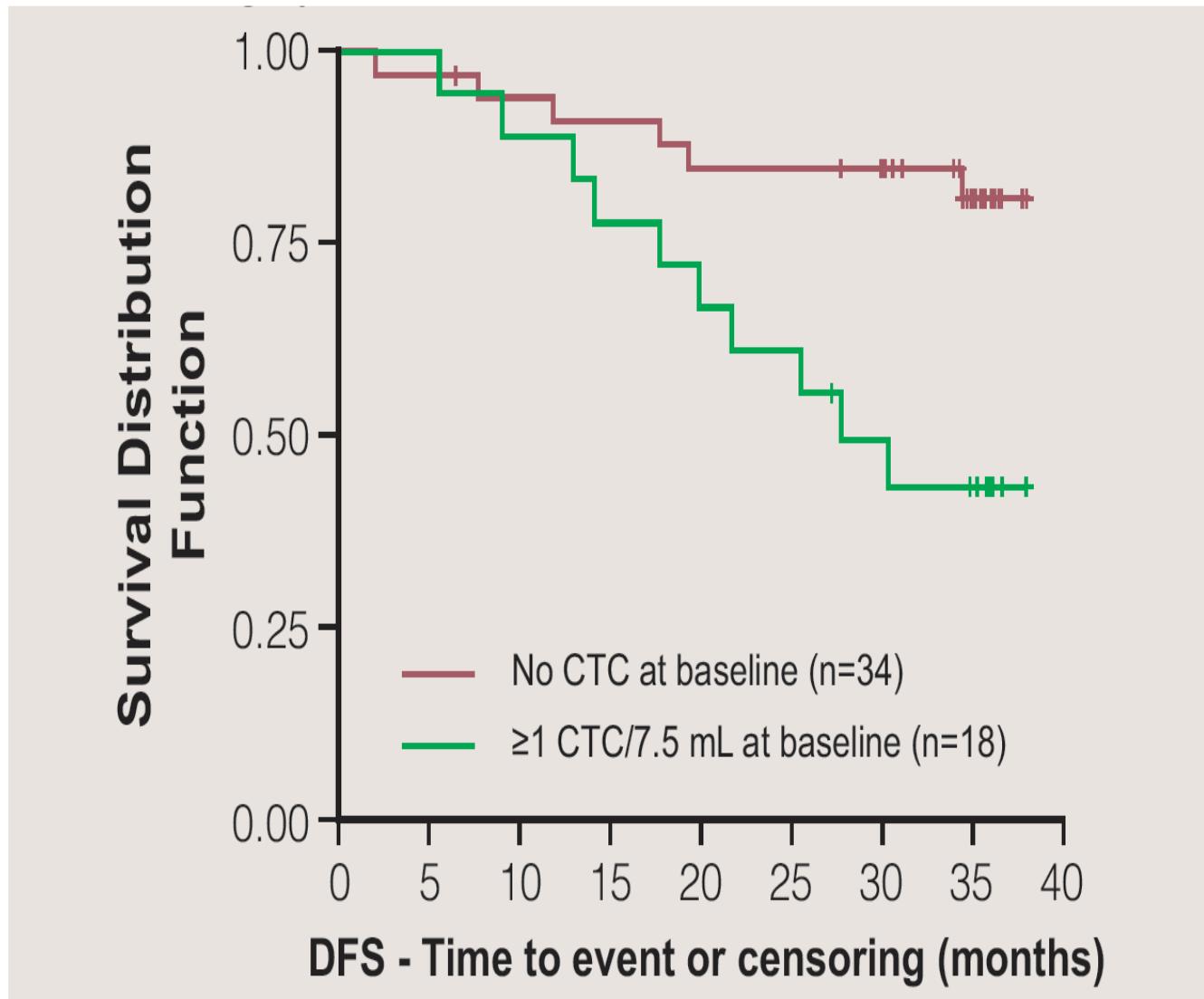


## Change in CTC count during therapy, by patient



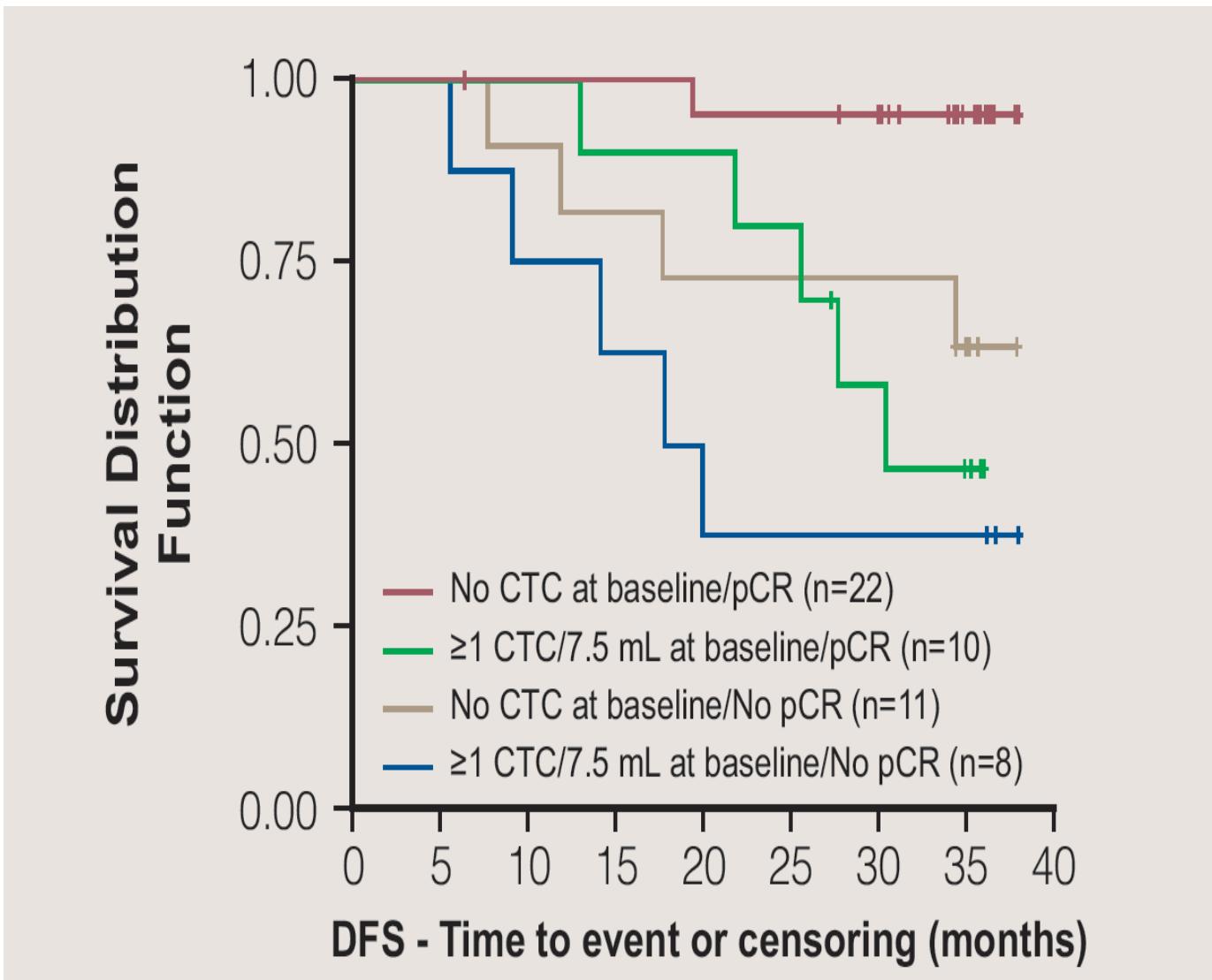
## BEVERLY 2

### DFS according to baseline CTCs category



# BEVERLY2

## DFS according to baseline CTCs category and pCR status



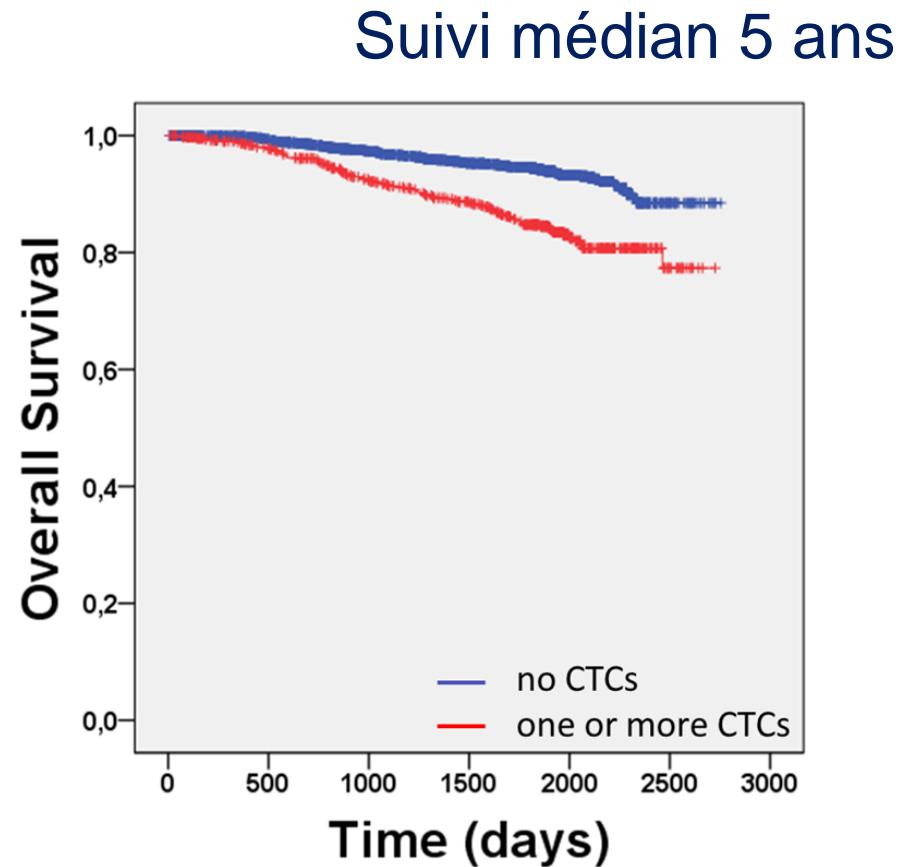
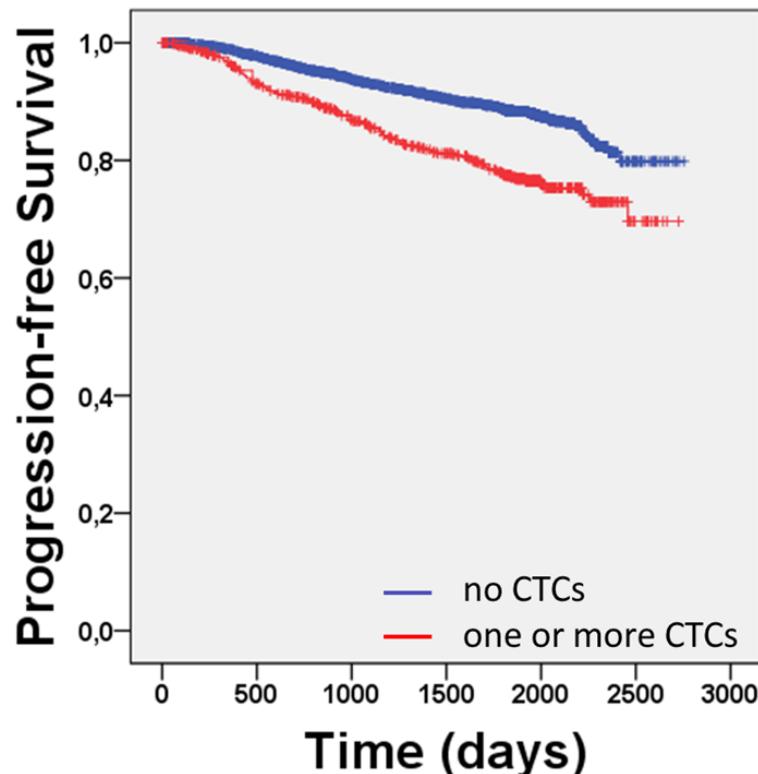
# CTC dans le cancer du sein en adjuvant

$\geq 1$  CTC = facteur pronostique DFS & OS

Méta-analyse européenne

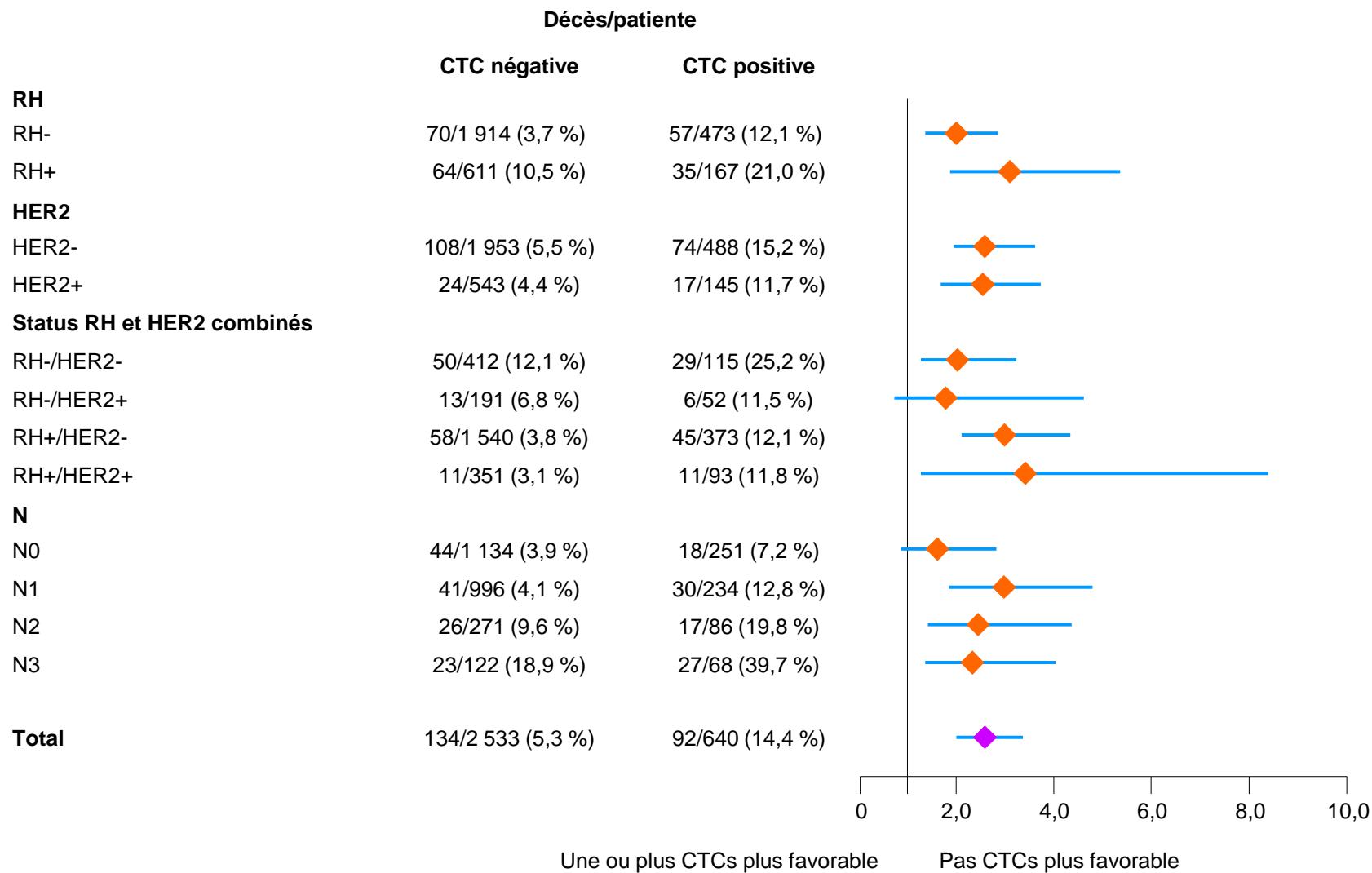
3172 dossiers individuels, stade I-III 5 centres, 5 pays

→ 20% de patientes CTC+

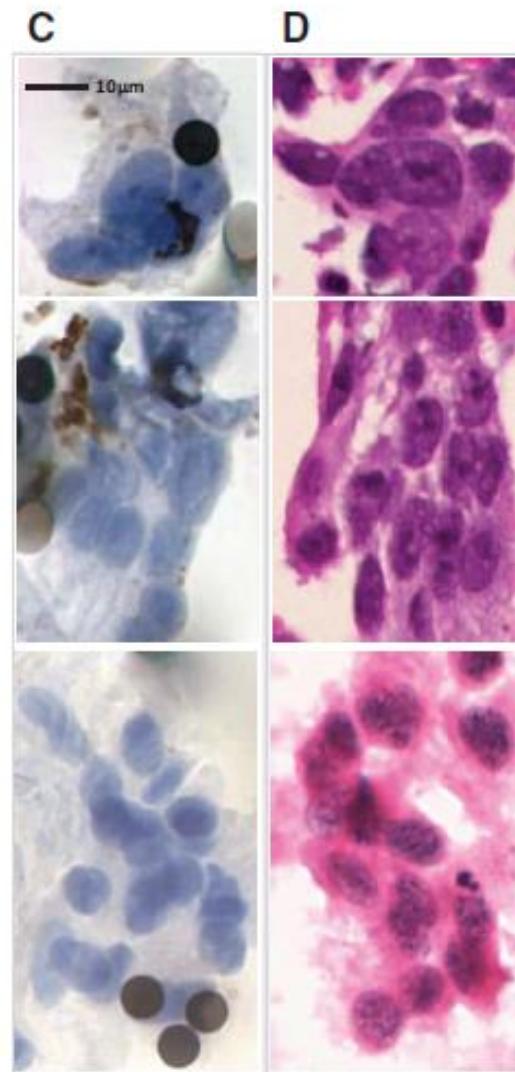
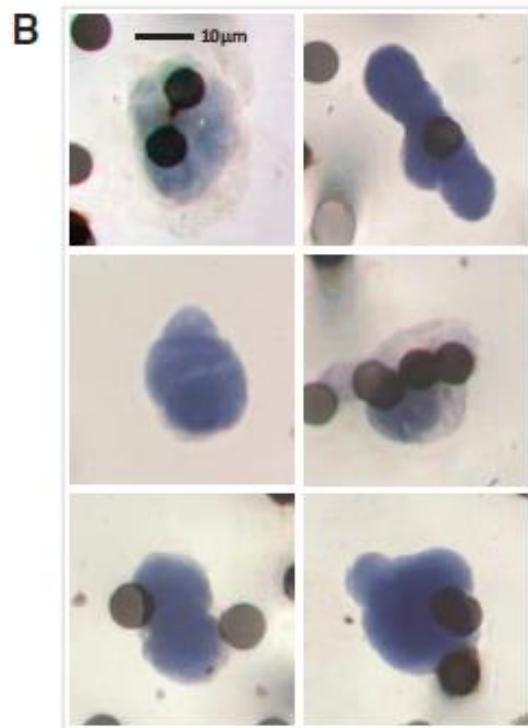
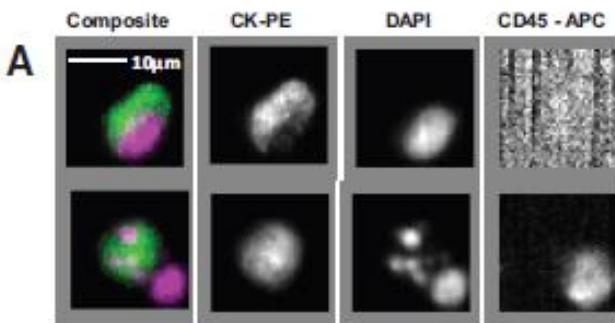


# Effet pronostic des cellules tumorales circulantes

## Résultat de la méta-analyse des études en adjuvant



# Circulating tumor cells (CTCs) and circulating tumor microemboli (CTM) from patients with non-small cell lung cancer (NSCLC) using CellSearch and ISET in comparison to primary tumor biopsies.



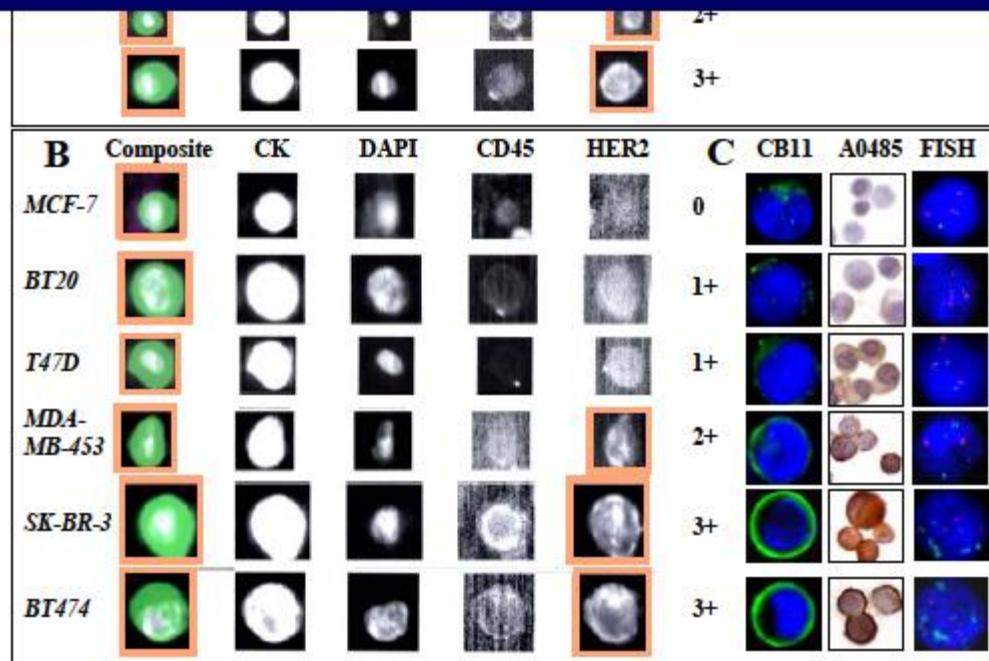
CTCs were detected using ISET in 32 of 40 (80%) patients compared with 9 of 40 (23%) patients using CellSearch.

A subpopulation of CTCs isolated by ISET did not express epithelial markers. Circulating tumor microemboli (CTM, clusters of 3 CTCs) were observed in 43% patients using ISET but were undetectable by CellSearch.

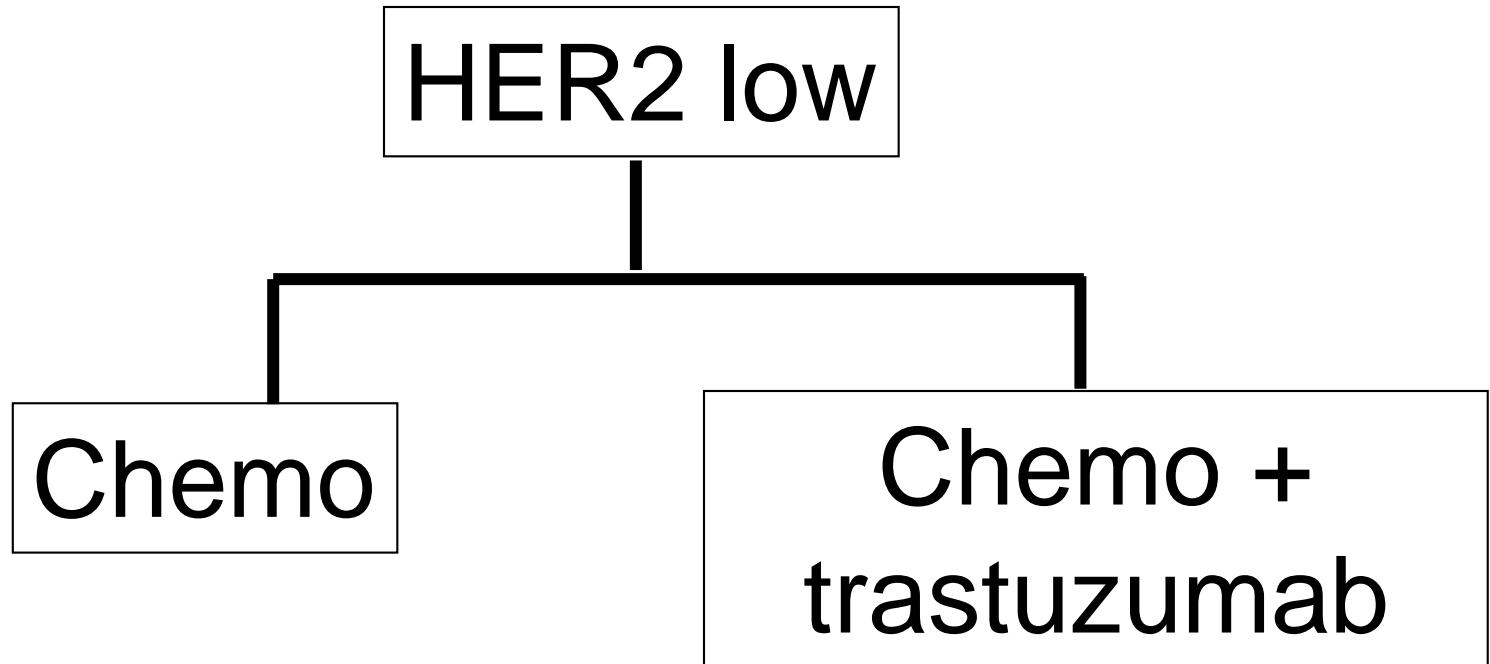
# Detection of therapeutic target molecules on CTC

## 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)

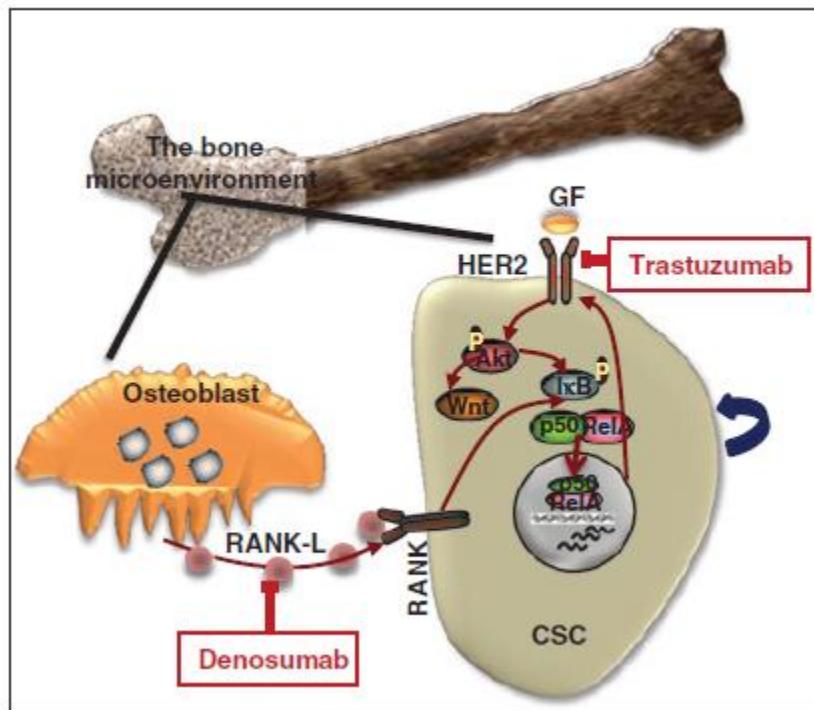


# **HER2 low trial concept NSABP B47**



# HER2 Drives Luminal Breast Cancer Stem Cells

A



B

Breast tumors	Metastatic-advanced clinical endpoint tumor regression	Micrometastatic-adjuvant clinical endpoint reduction in tumor recurrence
HER2+++ (amplified)	Yes	Yes
HER2++ or ++ (non-amplified)	No	Yes
Basal HER2- ER-	No	No

Below each row, there are two small images: the top one showing green fluorescence and the bottom one showing green fluorescence with yellow arrows pointing to specific cells.

Ithimakin S et al, Cancer Res; 2013, 73(5); 1635–45.

EORTC 90091-10093:

Trastuzumab in HER2-negative Early breast cancer as Adjuvant Treatment for Circulating Tumor Cells (CTC) (Treat CTC trial)

**Study coordinator:**

Dr Michail Ignatiadis

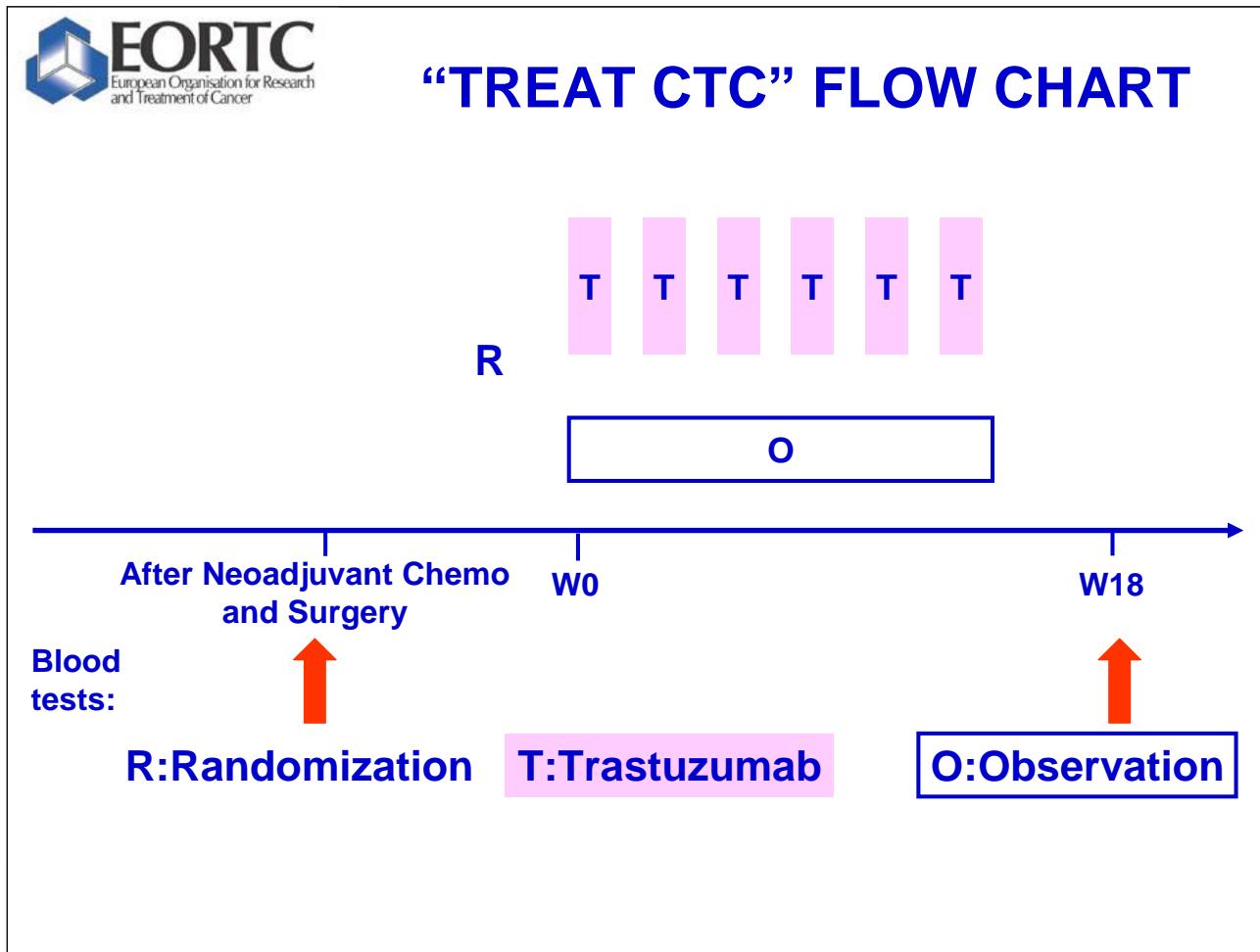
**Study co-coordinators:**

Dr Martine Piccart, Dr Jean-Yves Pierga,

Dr Brigitte Rack, Dr Christos Sotiriou

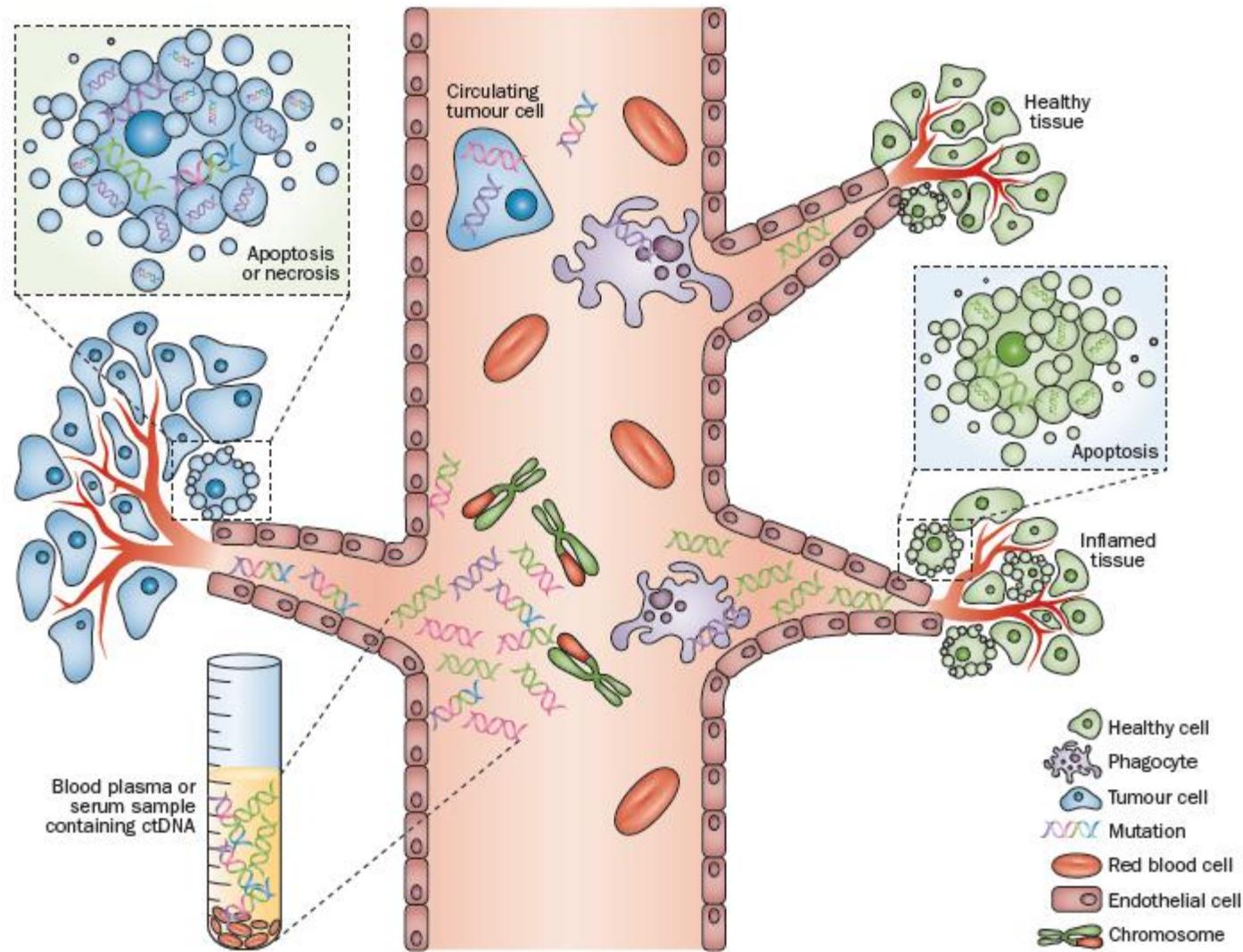
Slides version 1.0, dated 24/05/2013

Protocol version 2.0, dated 02/02/2012



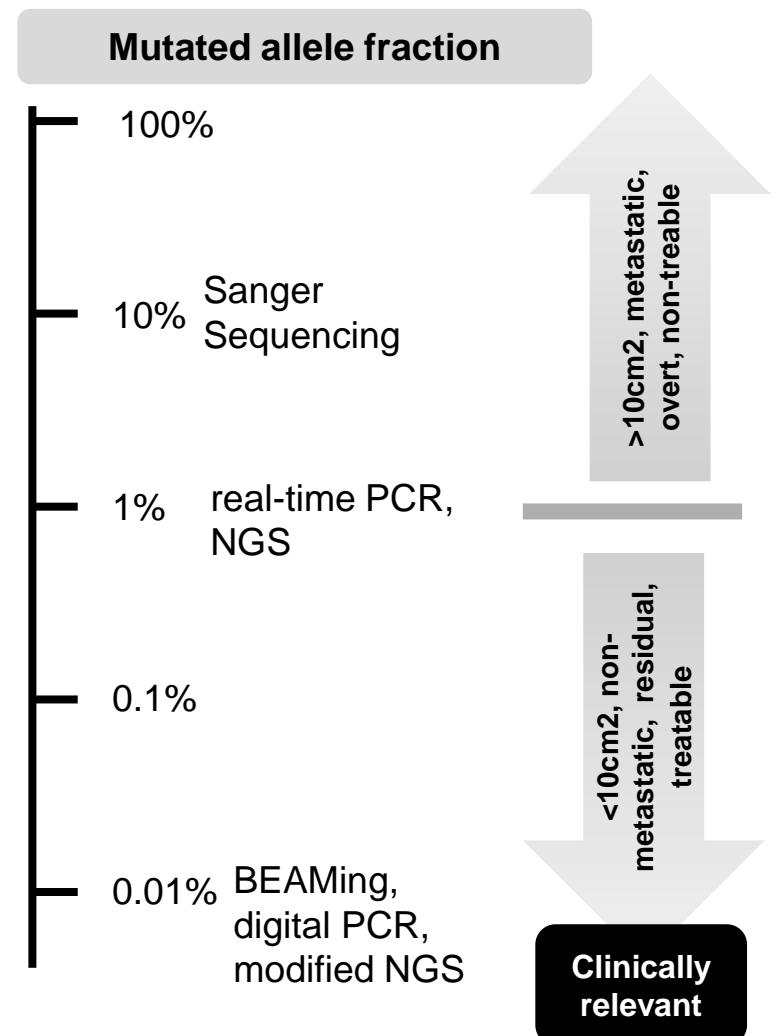
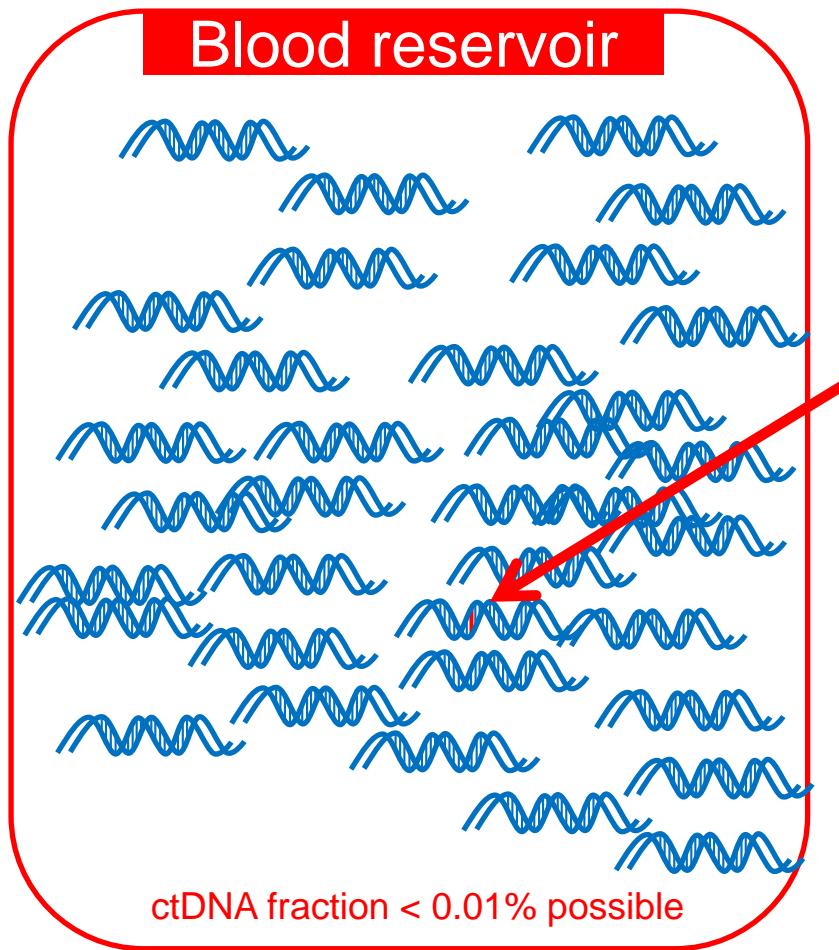
Start in 2013, 2014 in France

# Release and extraction of cfDNA from the blood



# ADN tumoral circulant

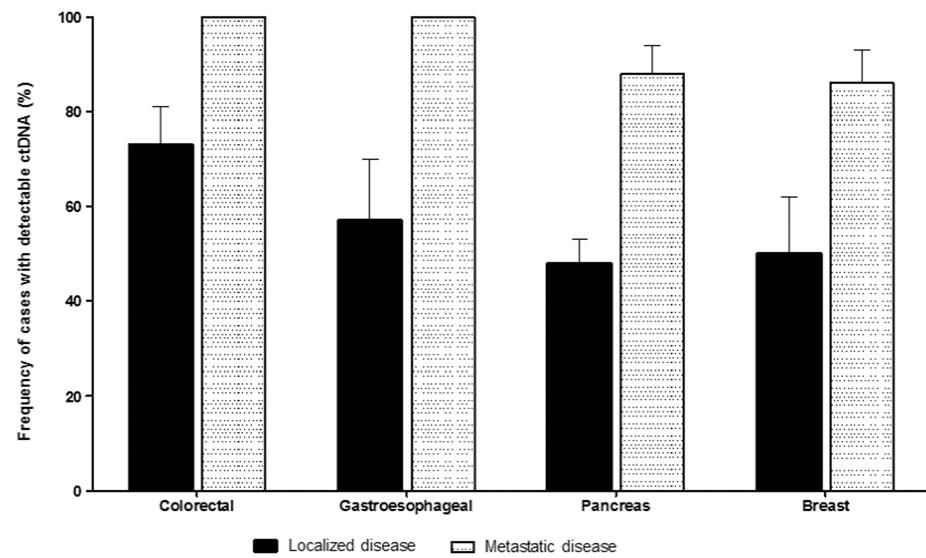
## Sensibilité / Spécificité



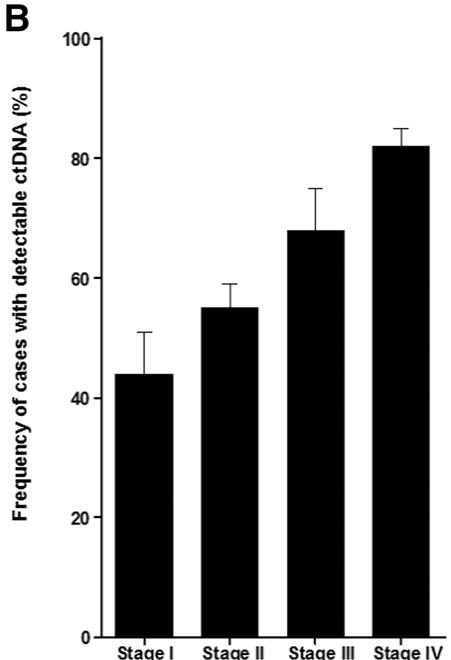
Multiplex: plusieurs mutations à la fois

# ctDNA in localized and non localized malignancies.

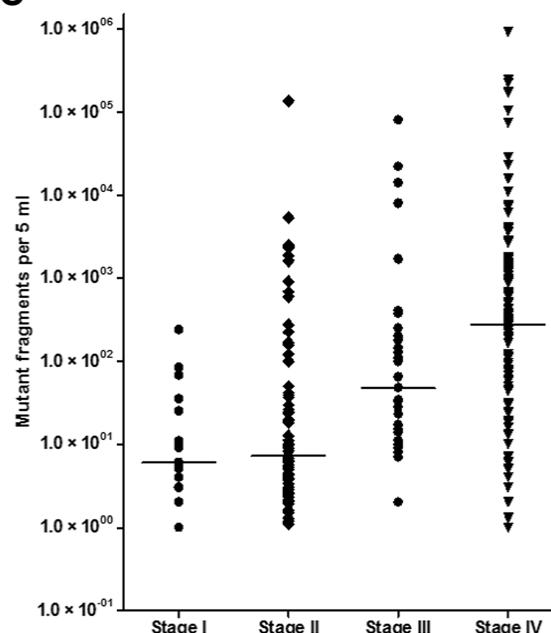
A



B



C



(A) Fraction of patients with detectable ctDNA in localized (stages I to III) and metastatic (stage IV) colorectal, gastroesophageal, pancreatic, and breast cancers. (B) Fraction of patients with detectable ctDNA. (C) Quantification of mutant fragments in cancer cases categorized by stage.

## Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status.

- In the phase IV, open-label, single-arm study NCT01203917, first-line gefitinib 250 mg/d Caucasian patients with epidermal growth factor receptor (EGFR) mutation-positive non-small-cell lung cancer

		Plasma 1 <i>EGFR</i> Mutation Status, <i>n</i>		Total
		Positive	Negative	
Tumor <i>EGFR</i> mutation status, <i>n</i> <sup>a</sup>				
Positive	69	36		105
Negative	1	546		547
Total	70	582		652
		95% Confidence Interval		
		<i>n</i>	Rate, %	
Concordance	652	94.3	92.3–96.0	
Sensitivity	105	65.7	55.8–74.7	
Specificity	547	99.8	99.0–100.0	
Positive-predictive value	70	98.6	92.3–100.0	
Negative-predictive value	582	93.8	91.5–95.6	

Oct 2014: modification of gefitinib approval on ctDNA by EMEA

# De novo mutation detection from ctDNA correlates with variants detected on metastasis of patients with any kind of refractory cancer from the SHIVA trial

1571PD

Jean-Yves Pierga<sup>1,4,7</sup>, Charles Decraene<sup>1,2</sup>, Virginie Bernard<sup>3</sup>, Maud Kamal<sup>4</sup>, Anthony Blin<sup>3</sup>, Thomas Rio Frio<sup>3</sup>, Nicolas Servant<sup>3</sup>, François-Clément Bidard<sup>1,4</sup>, Xavier Paoletti<sup>5</sup>, Marc-Henri Stern<sup>6</sup>, Ronald Lebofsky<sup>1</sup>, Christophe Le Tourneau<sup>4,5</sup>

<sup>1</sup> Circulating Cancer Biomarkers Lab, SiRIC, Translational Research Department,

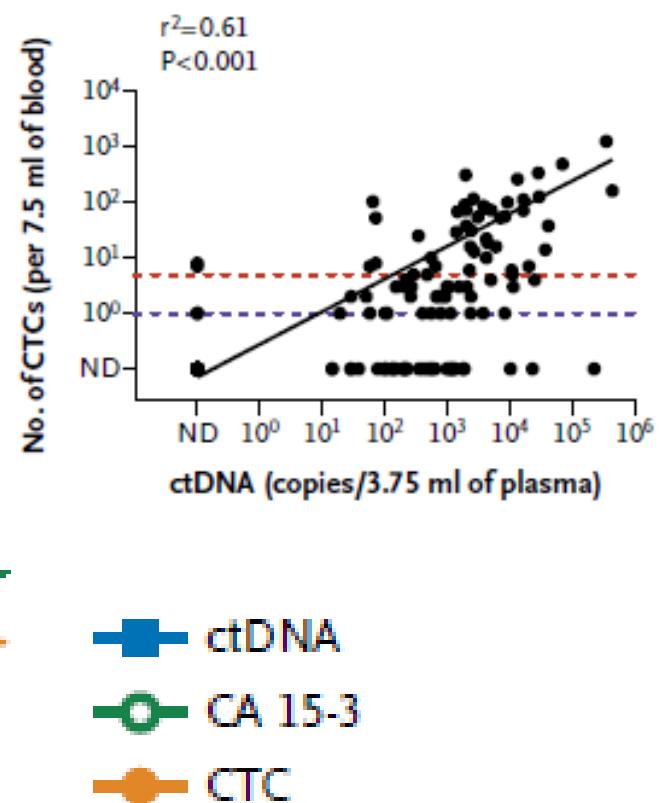
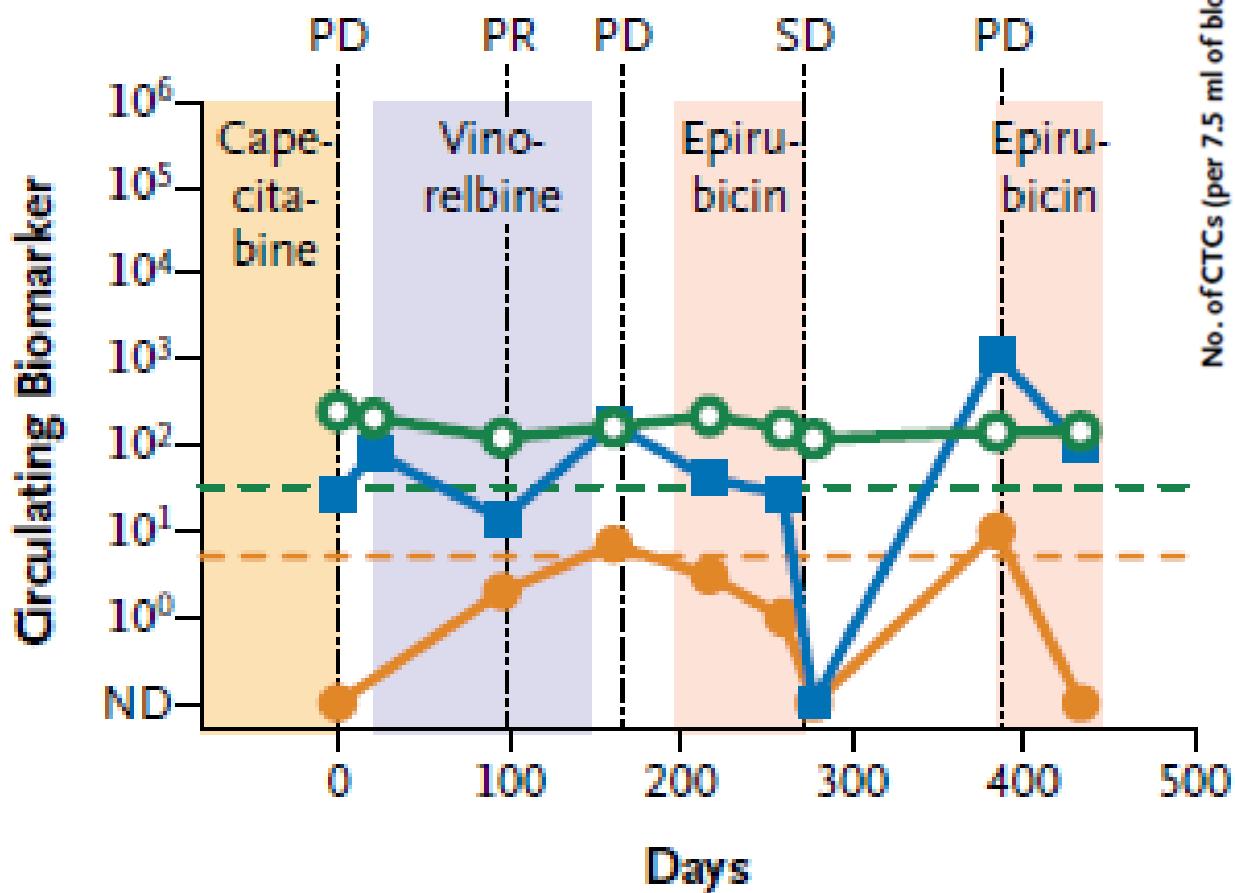
<sup>2</sup> CNRS UMR144, <sup>3</sup> ICGex NGS platform, <sup>4</sup> Department of Medical Oncology, <sup>5</sup> INSERM U900, <sup>6</sup>INSERM U830,

Institut Curie, Paris, France

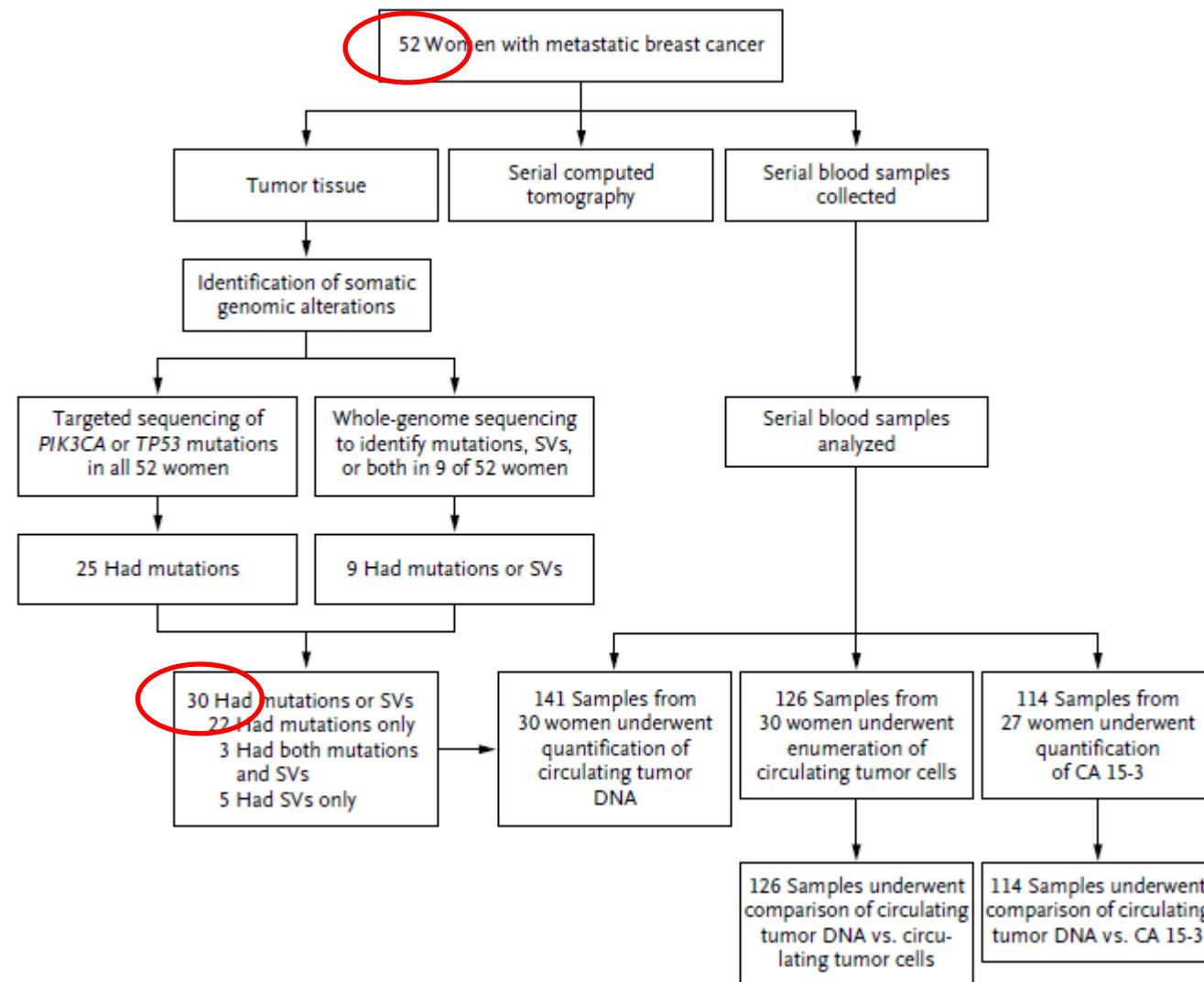
<sup>7</sup> Université Paris Descartes, Paris, France

# Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

## A Patient 17



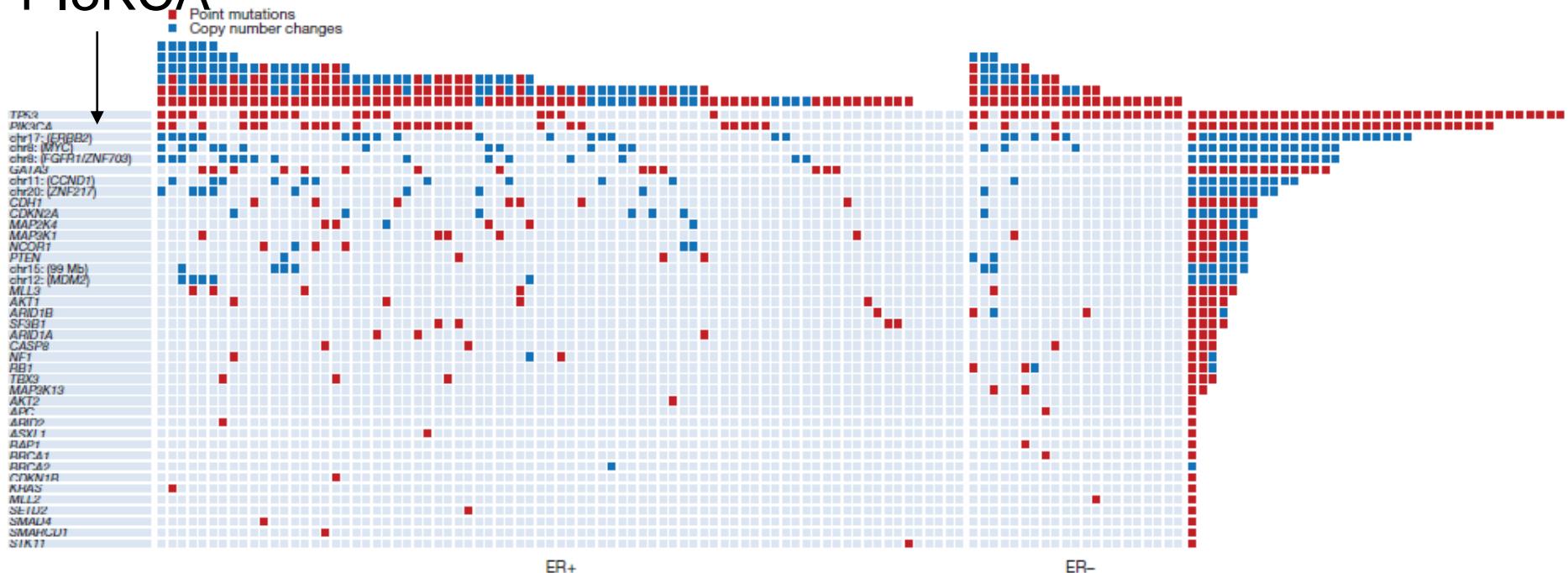
# Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer



# Genomic segmentation of breast cancer

TP53

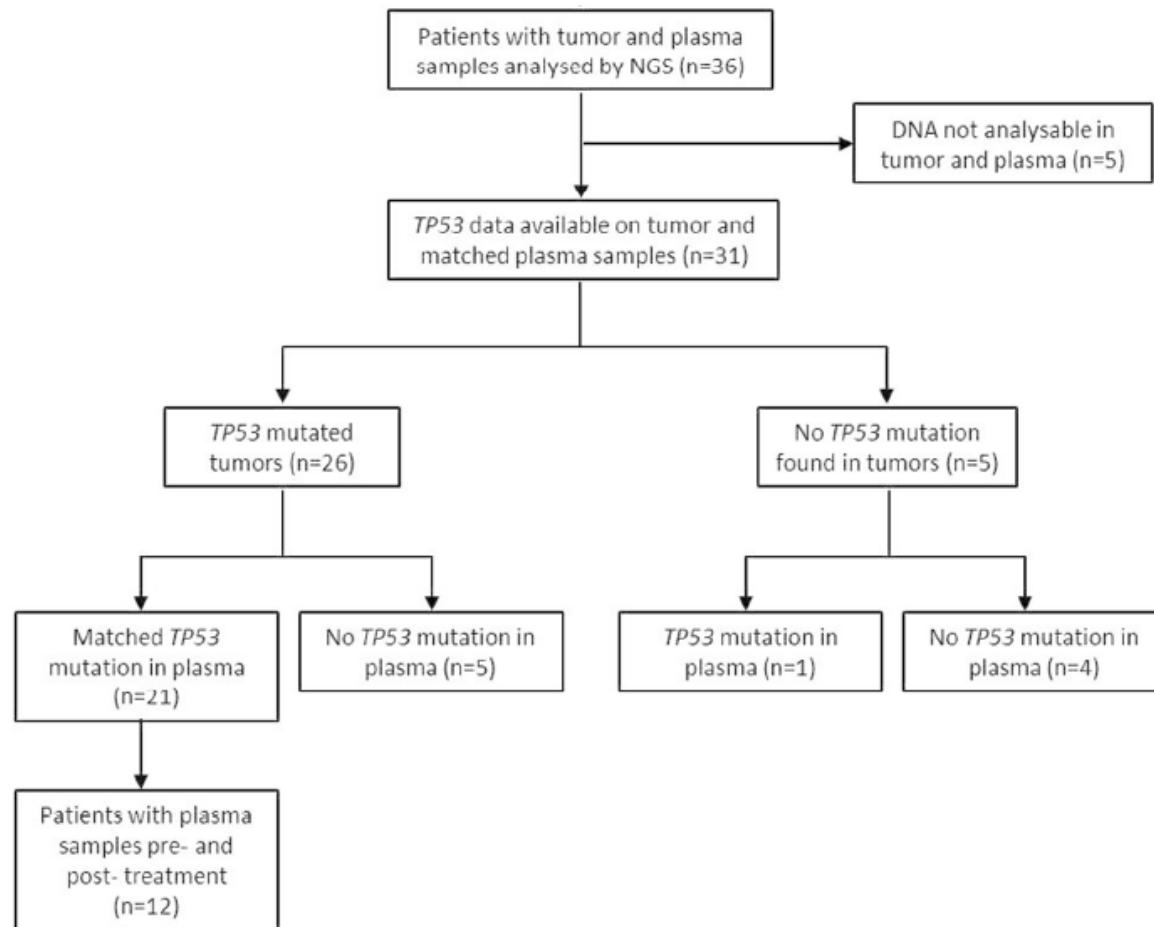
PI3KCA



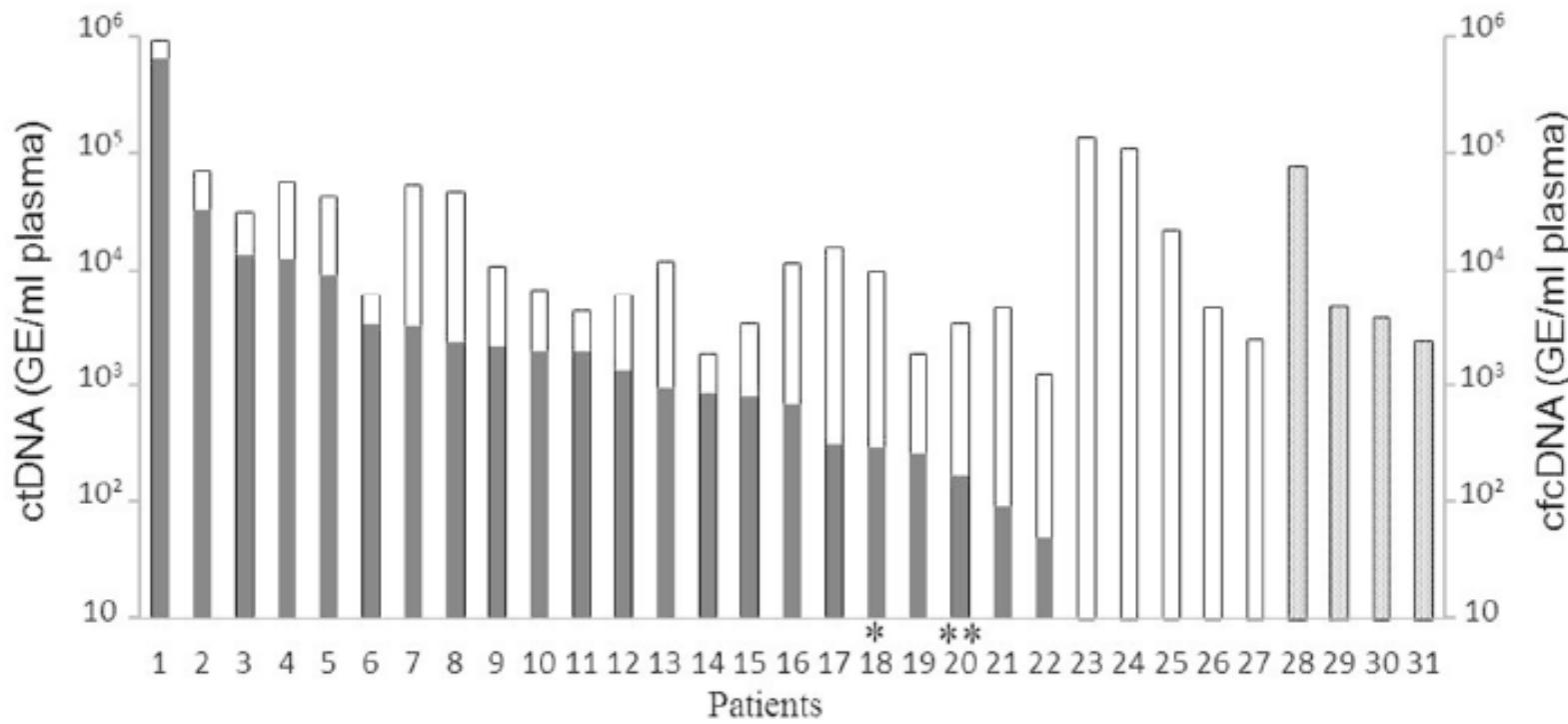
**Breast cancer disease includes a large number of RARE genomic segments**

**Treatment should include specific agent for each segment**

# Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients



# Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients



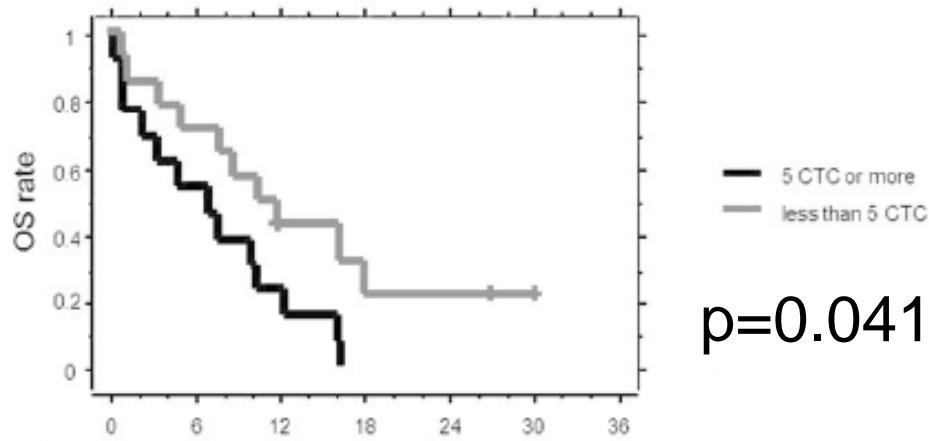
cfDNA concentration, filled bar:

ctDNA concentration, dotted bar:

cfDNA concentration in patients with  
wild-type *TP53* in both tumor  
and plasma.

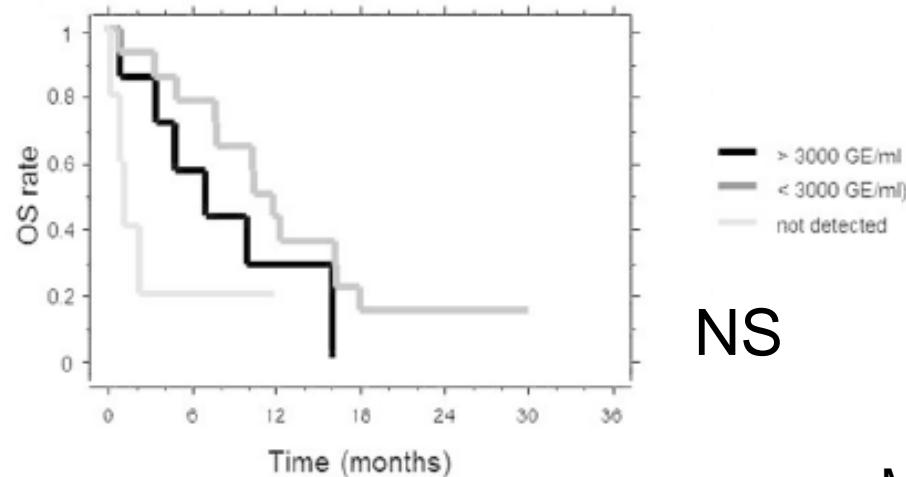
Madic J Int J Cancer 2014 in press

# Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients



CTC detected with  
CellSearch

p=0.041



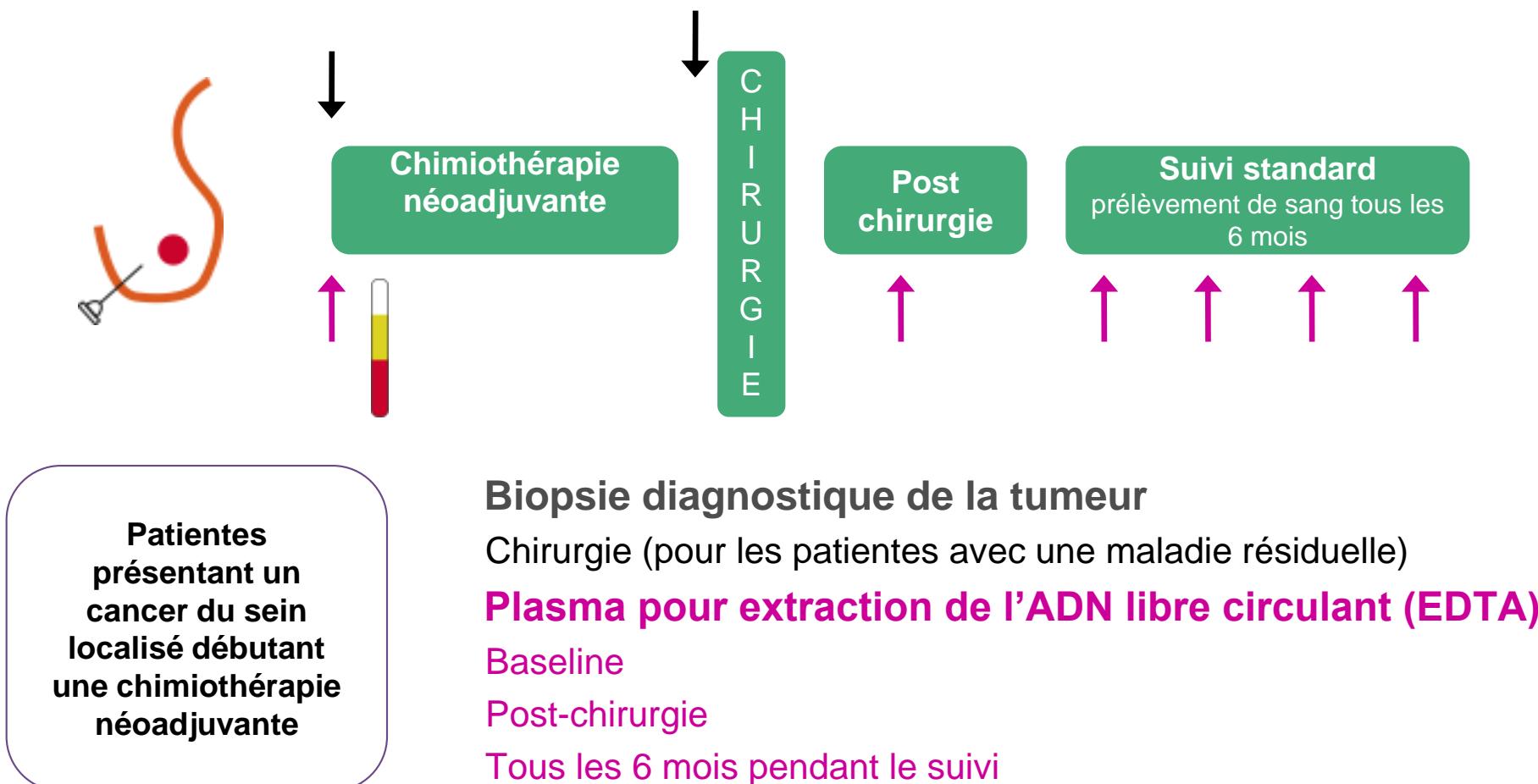
ctDNA TP53 mutation

NS

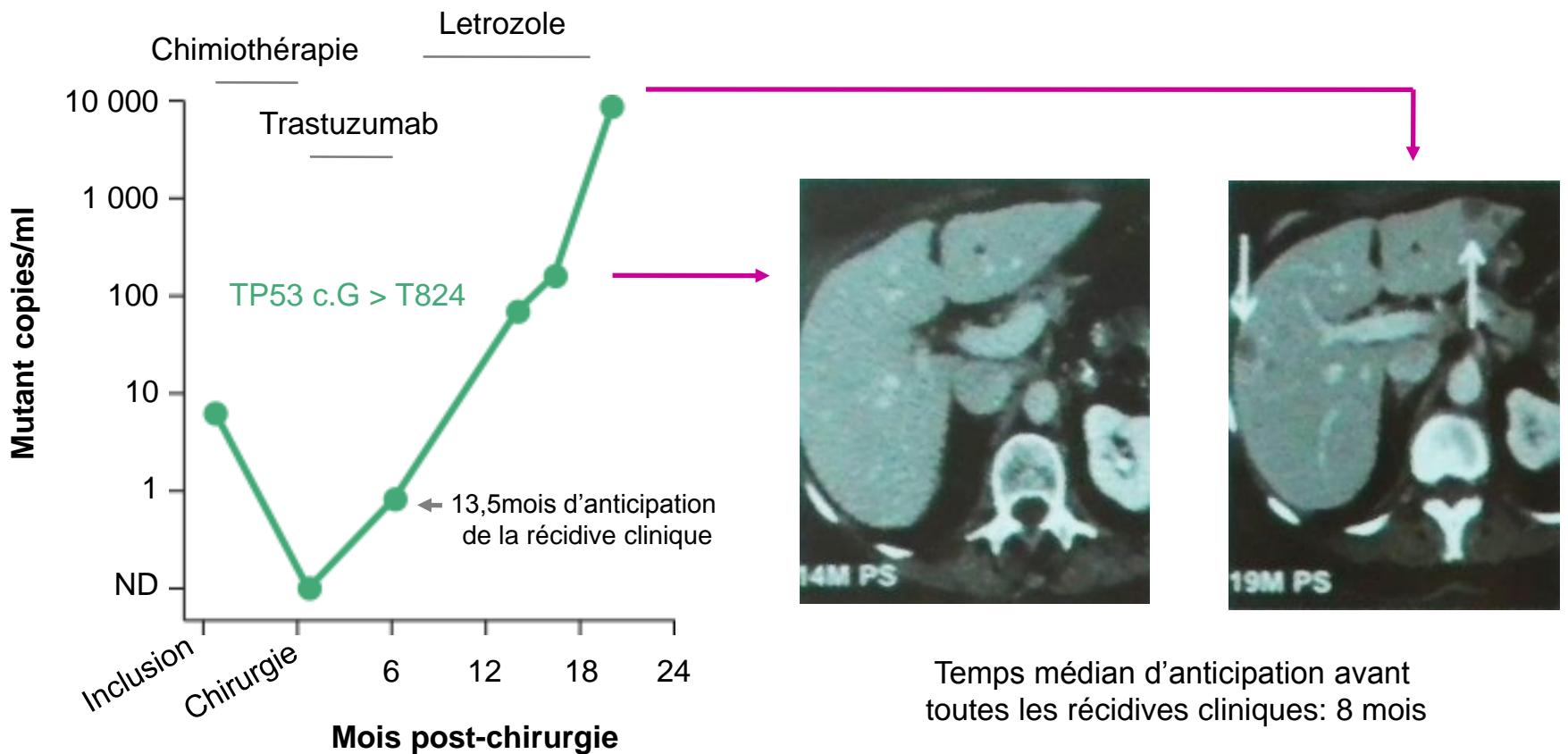
Madic J Int J Cancer 2014 in press

# Détection de mutation spécifique de tumeur dans l'ADN tumoral circulant (ADNct): prédition de la récidive après chimiothérapie première

## Schéma de l'étude

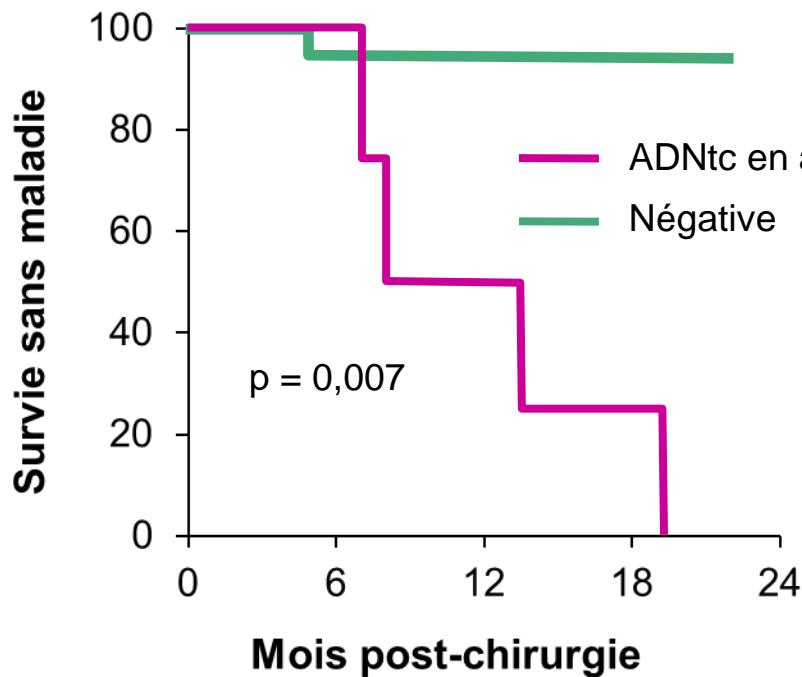


# Anticipation avant la récidive clinique



Cancer du sein RO +ve HER2 + en réponse complète histologique mammaire et ganglionnaire après chimiothérapie néoadjuvante et trastuzumab

# Prédiction des récidives précoces – détection des mutations



La détection dynamique de l'apparition de mutation est hautement prédictive de récidives précoces

Augmentation de l'ADNtc après chirurgie

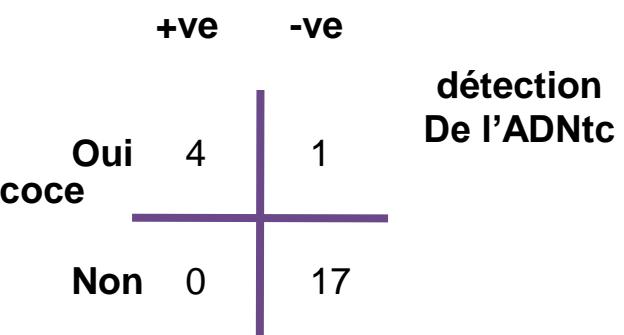
Rechute précoce

Néoadjuvant chimiothérapie

CHIRURGIE

Post chirurgie

Standard follow-up  
blood samples every 6 mois





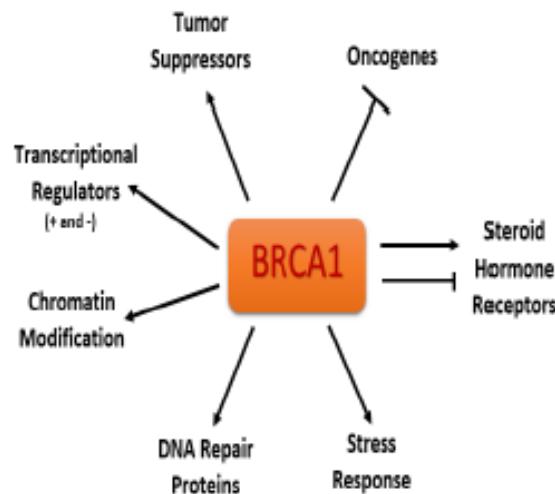
# CirCA 01 IC 2014-05



*« Détection d'ADN tumoral circulant et suivi des patientes porteuses d'une mutation BRCA1. »*

## 1 – Rationnel (2/2)

- La population ciblée sera les femmes porteuses d'une mutation constitutionnelle BRCA1.
- L'intérêt de ce modèle vient du fait que l'inactivation de *BRCA1* dans les cellules tumorales va de pair avec la perte de fonction de *TP53*, donc (*quasiment*) tous les cancers liés à *BRCA1* sont aussi mutés pour *TP53* et donc potentiellement détectables par ctDNA ciblant *TP53*.
- Chez une patiente porteuse d'une mutation constitutionnelle *BRCA1*, une rechute d'un cancer précédemment connu présentera la même mutation de *TP53*, un nouveau cancer aura très probablement une mutation distincte de *TP53*.



# Biopsie liquide

« surrogate » de la biopsie solide +/-  
information supplémentaire ?

- ▶ Cellules Tumorales Circulantes (CTC)
- ▶ ADN tumoral circulant (ctDNA)

▶ Autres :

miRNA, RNA

cellules endothéliales

marqueurs sériques...



Bidard et al, Sci Transl Med 2013

Différences : processus biologiques de « relargage »  
différents → ≠ signification

Similarités : cellules et ADN très rares, « perdues » au milieu  
de cellules / ADN normal

## Conclusions

### Détection des CTC

Marqueur pronostique reproductible dans plusieurs pathologie métastatique (Sein, prostate, etc...) Niveau de preuve I

L'utilité clinique reste à démontrer pour passage en routine en particulier en situation adjuvante

### Intérêt en clinique de la « biopsie clinique »

ctDNA: Corrélation avec le statut de la maladie métastatique

Evaluation itérative en cours de traitement

Démonstration à faire en situation non métastatique

# Circulating Biomarkers Lab

Medical Oncology

Dr FC Bidard

Dr V Diéras  
& others

**CNRS UMR 168**

JL Viovy  
S Descroix  
B Coudert

**Statistics**

Dr B Asselain  
C Mauborgne  
F Berger



I Vaucher  
A Rampanou  
J Madic  
A Saliou  
M Milder



**Inserm U 830**

Dr MH Stern

**Immunology**

Dr O Lantz

Translationnal

Research dpt

C Decraene

S Roman-Roman

**UGEC**

C Simondi  
S Armanet  
S Pelissier  
P Tresca

**Pathology**

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

**Grant supports**

PIC Curie – DOM – ERCs – INCA/DHOS – PHRC  
– La Ligue – Roche – Jansen diagnostics