

miRNA, Régulations post-transcriptionnelles et Cancer

*Stéphan
VAGNER*

Inserm

Equipe 5
INSERM U563/CPTP

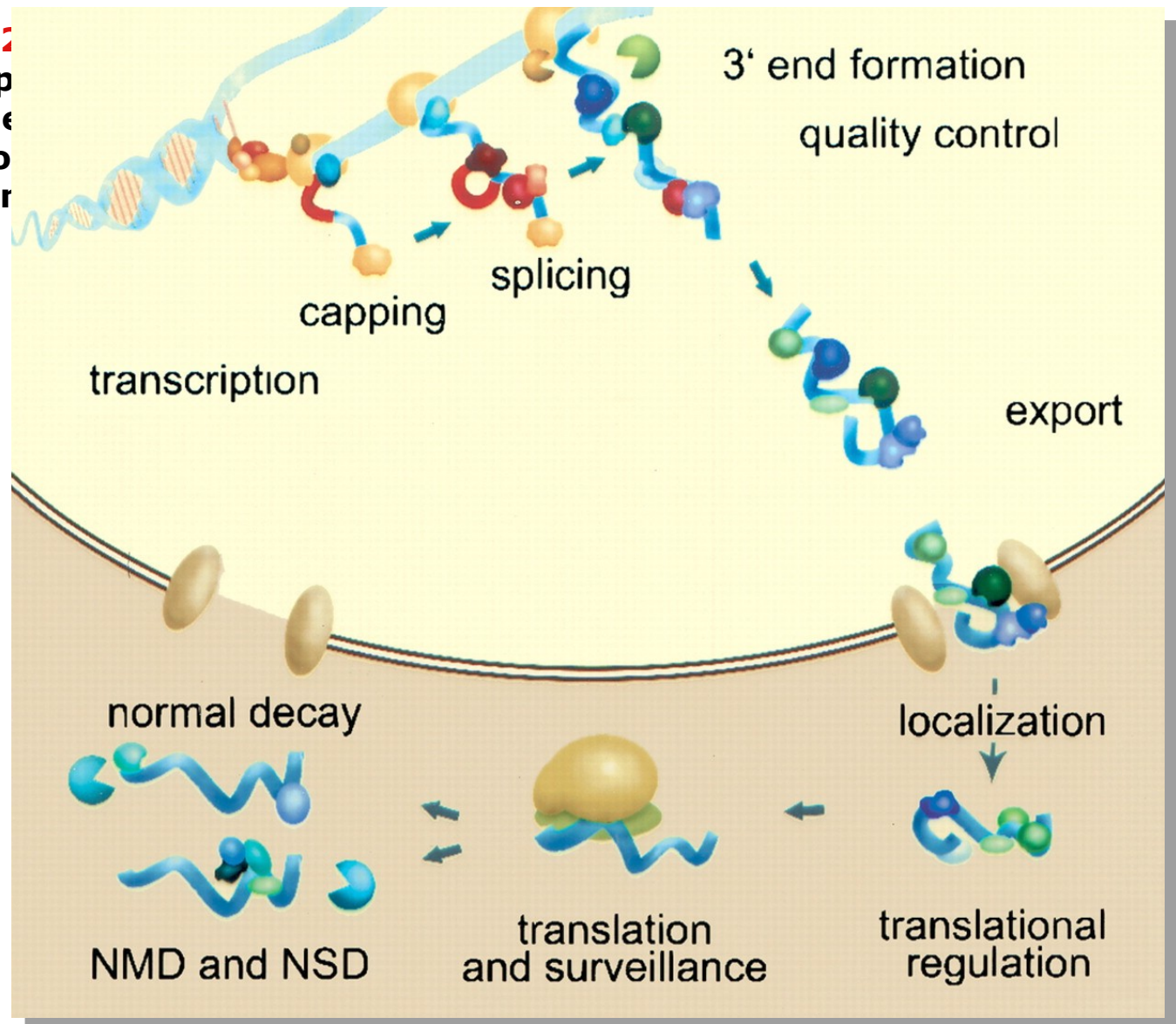


INSERM U981/IGR

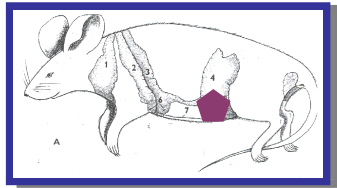
Post-transcriptional control of gene expression

Early step
in a gene
expression
program

« Putting translation
before
transcription »,
Cancer Cell, 2004

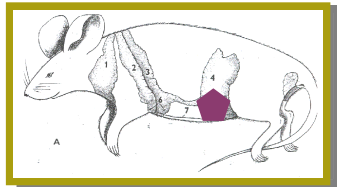


A Mouse Mammary Tumor Model of Metastasis Development



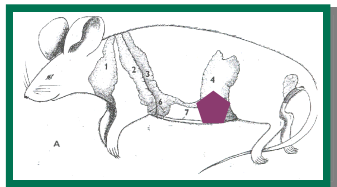
67NR

No
metastasis



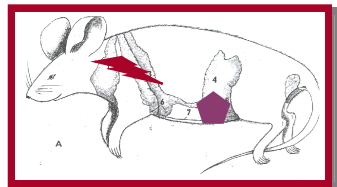
168FARN

Micrometastasis
Lymph node



4T07

Micrometastasis
Lung



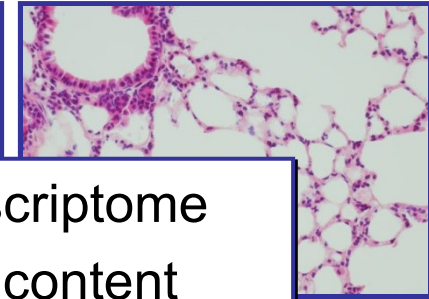
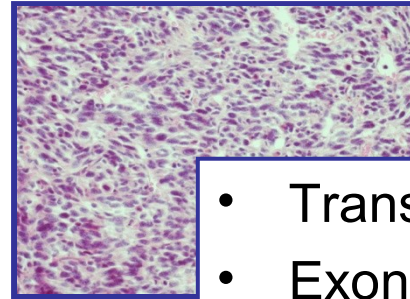
4T1

Metastasis
Lung

BALB/c

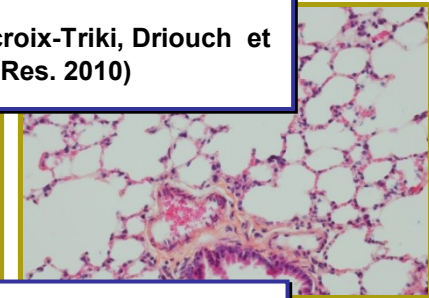
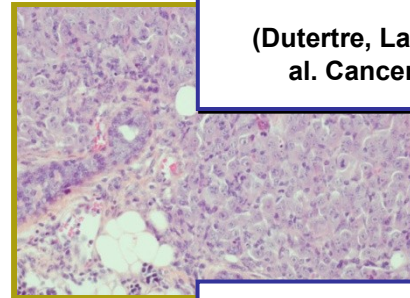
Primary tumor

Lung

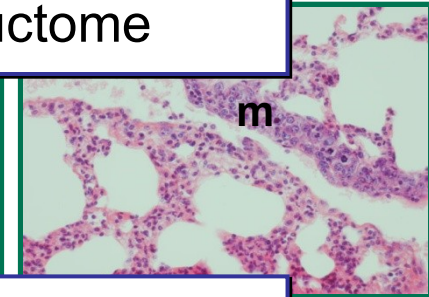
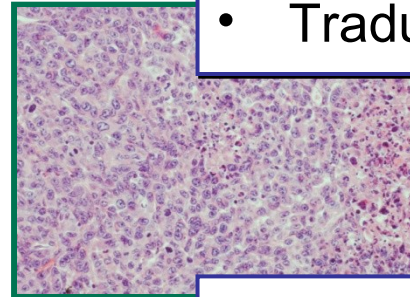


- Transcriptome
- Exon content

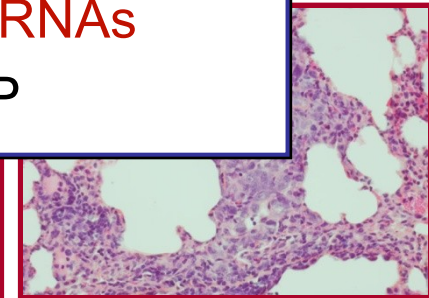
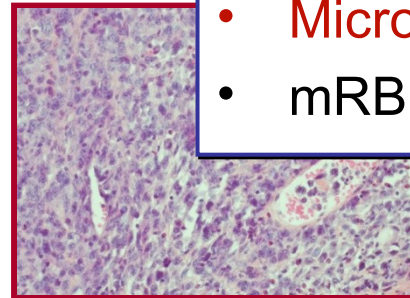
(Dutertre, Lacroix-Triki, Driouch et al. Cancer Res. 2010)



- Traductome

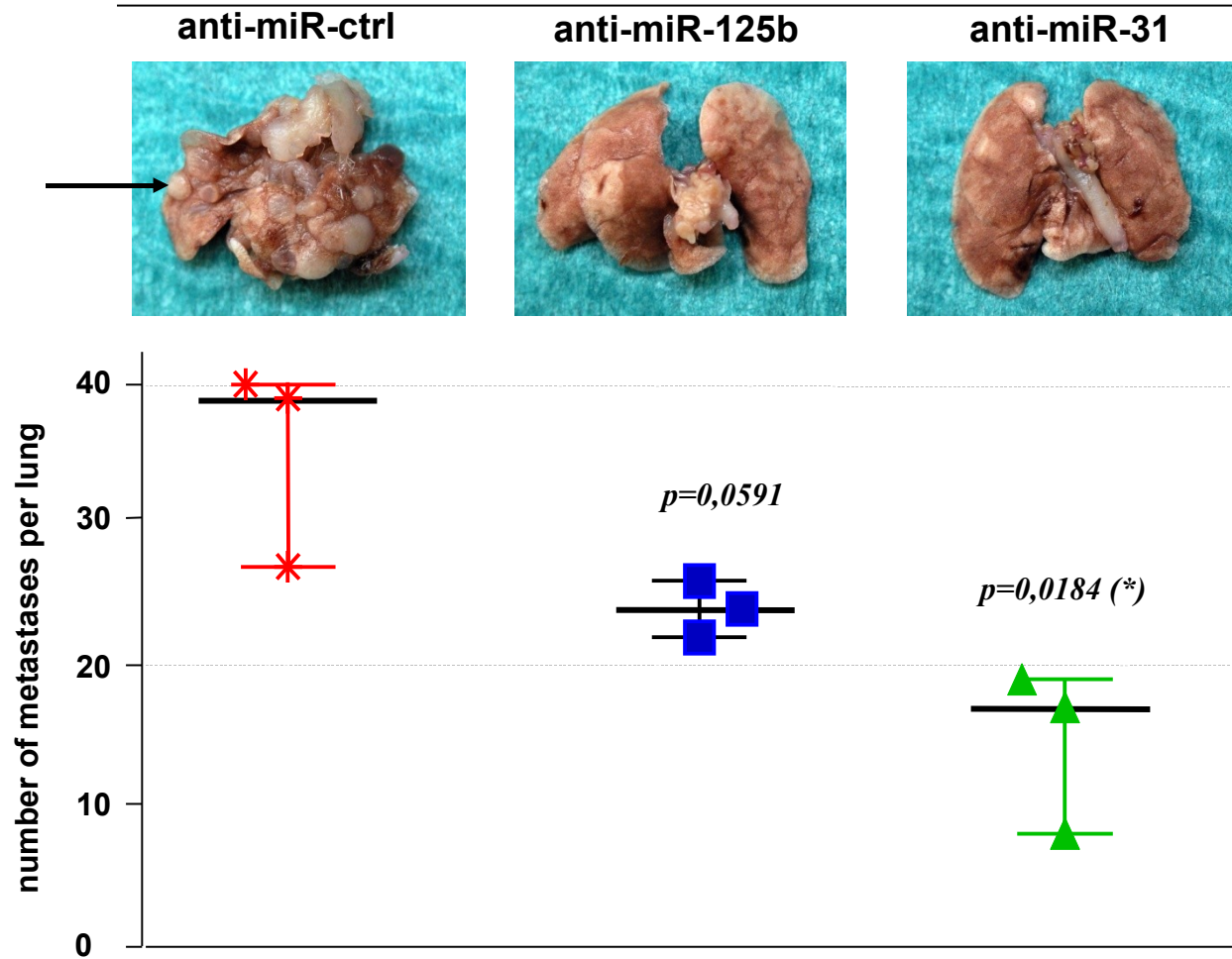


- MicroRNAs
- mRBP



In vivo metastasis formation (extravasation)

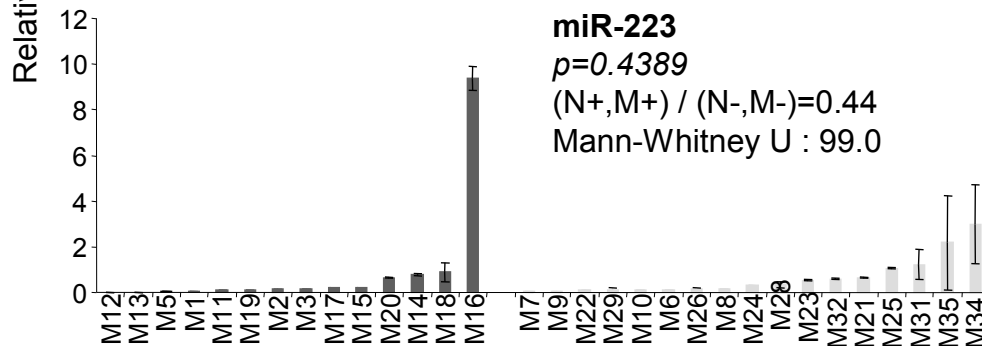
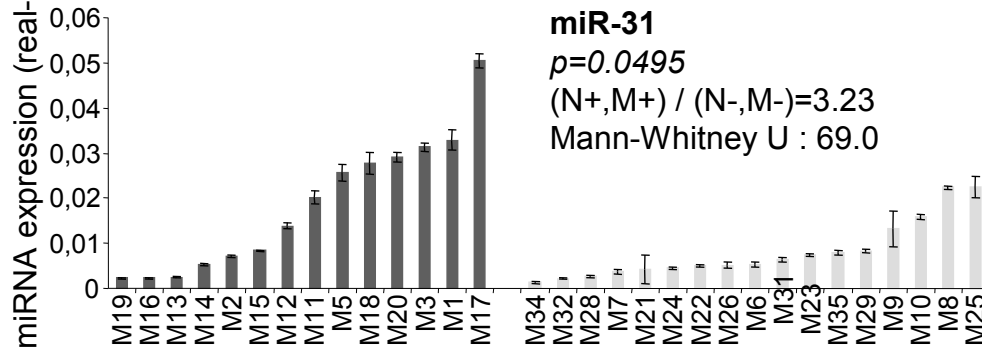
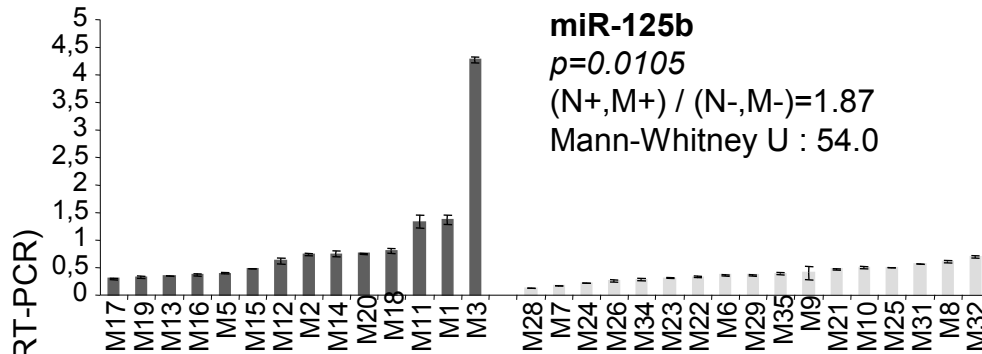
4T1



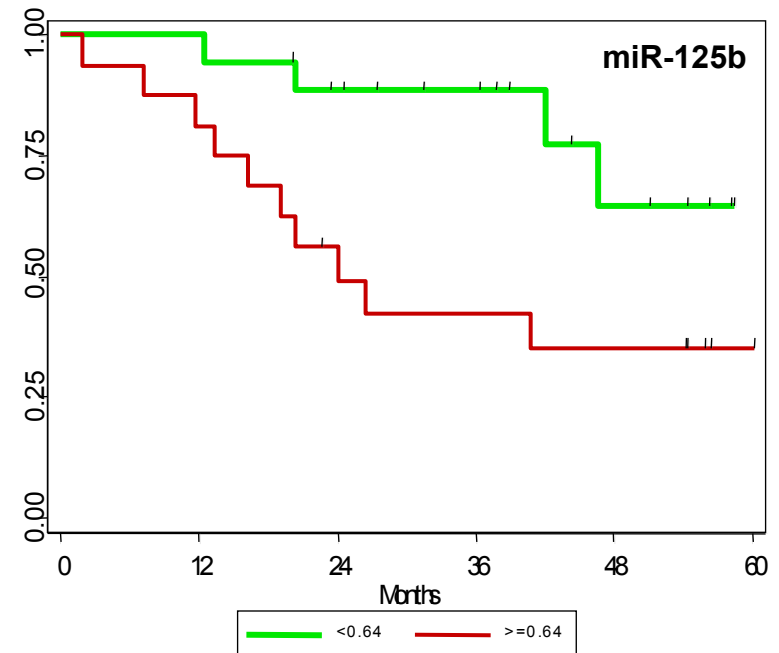
Expression in breast tumors (ICR n=34 serie)

(N+,M+)

(N-,M-)

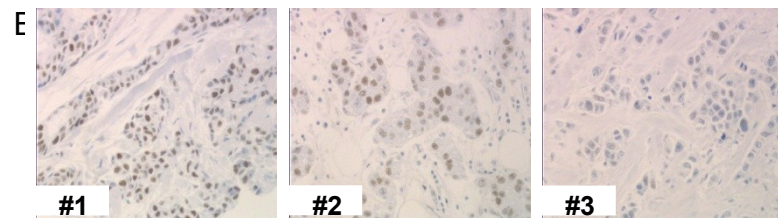
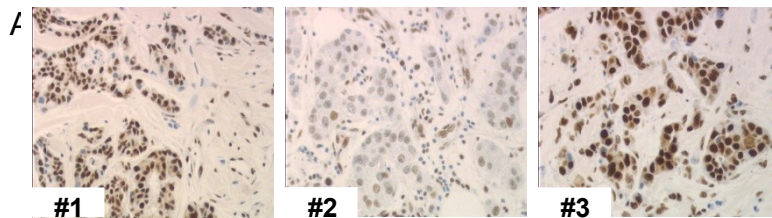
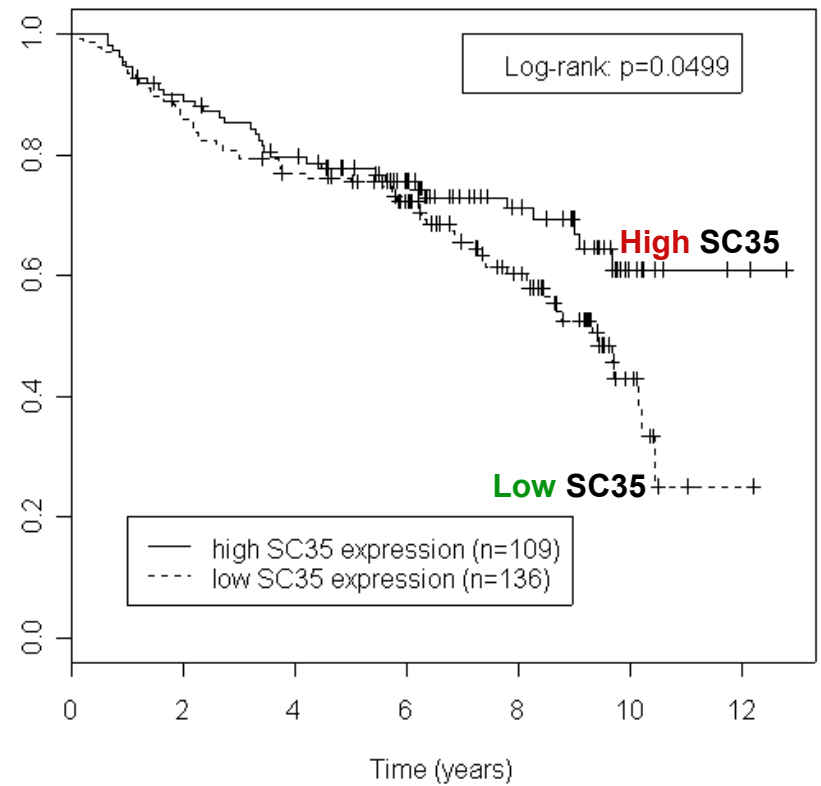
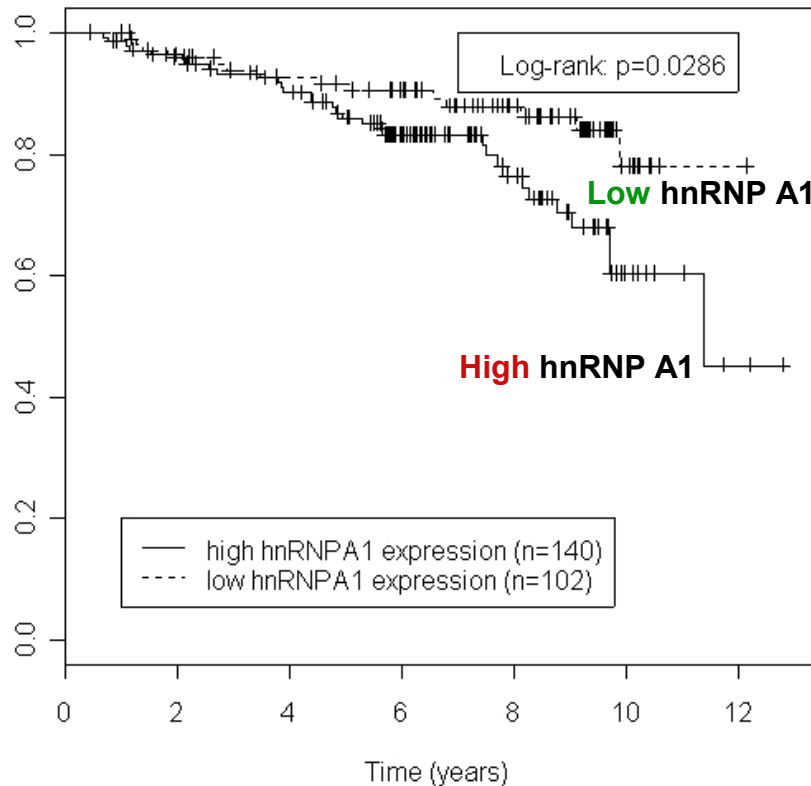


DFS (Disease-free Survival)



mRNA Binding Protein (2/6) Expression is Associated with Metastatic Relapse

**Metastatic relapse probability (Kaplan-Meier)/
Tissue microarray: 277 human breast tumors specimen (ICR)**



PHÉNOTYPAGE MOLÉCULAIRE DES CANCERS DU SEIN EN COURS DE GROSSESSE

Dr Bénédicte Lesieur

Dr Alice Trefoux, Dr Martine Antoine, Dr Lise Selleret, Dr Sonia
Zilberman,

Pr Roman Rouzier

Hôpital TENON, Paris UPRES EA 4053 Paris 6

Etude cas-témoin

□ Etude rétrospective

- } 121 patientes âgées de < 43 ans
- } carcinomes invasifs
- } 3 groupes
 - CSAG : n = 17 (15 %)
 - nullipares : n = 32 (26 %)
 - CSnAG : n = 72 (59 %)

□ Données cliniques

- } histologie et immunohistochimie de routine
- } type de traitement
- } survie

□ Réalisation d'un tissu microarray (TMA)

- } panel de 17 marqueurs : RH, cytokines, marqueurs de prolifération
- } marquage HLA-G (Ac primaire MEM-G /02 : IgG1)
- } analyse de l'informativité du TMA et des marqueurs utilisés

R é s u l t a t s c l i n i q u e s

	CSAG	CSnAG		Nullipares	
Taille au diagnostic					
T 0-T 1	5 (29 %)	25 (35 %)	N S	15 (47 %)	N S
T 2-T 4	12 (71 %)	42 (65 %)		17 (53 %)	
Taille histologique (mm)	20 (6 - 100)	22 (0 -120)	N S	20 (4 -180)	N S
Grade SBR					
1-2	5 (29 %)	41 (57 %)	P=0,041	17 (53 %)	N S
3	12 (71 %)	31 (43 %)		15 (47 %)	
RE					
Positifs	6 (35 %)	61 (85 %)	P<0,01	22 (69 %)	P=0,024
Négatifs	11 (65 %)	11 (15 %)		10 (31 %)	
Her2 +	5 (29 %)	9 (12 %)	P = 0,08	10 (31 %)	N S
Triple Négatif	9 (53 %)	68 (11 %)	P<0,01	6 (19 %)	P=0,01
TTT Radical	13 (76 %)	41 (57 %)	N S	18 (55 %)	N S

Niveau d'expression des marqueurs sur T M A

	CSAG	CSnAG		Nullipares	
	n = 25	n = 58		n = 29	
RE	24,1	60,1	0,003	49,5	NS
RP	16,8	43,2	0,021	31,8	NS
Bcl2	35,6	55,1	NS	51,1	NS
EGFR	22,4	3,6	0,015	19,6	NS
CK5	0,7	0,6	NS	0,3	NS
CK18	60,2	79,8	0,033	74,3	NS
E C adhérinte	47,2	52,7	NS	54,1	NS
P C adhérinte	30,1	20,4	NS	30,3	NS
p53	24,9	13,8	NS	18,9	NS
p63	2,1	2,5	NS	1,6	NS
C-kit	19,1	10,7	NS	13,6	NS
Vimentine	16,6	7,8	0,028	13,4	NS
Cox2	14,5	15,8	NS	19,7	NS
Prolactine R	10,5	49,5	<0,001	38,3	0,024

Conclusion

- Les CSAG ne semblent pas présenter un phénotype moléculaire spécifique
- Nécessité d'étude de profils d'expression génique sur des populations larges
- Utilité du réseau CALG afin de colliger les cas et pouvoir mener une étude de puissance suffisante
- Limites ++ des modèles animaux
 - } souris mutées: prédisposition à un sous-type moléculaire

C A L G : C a n c e r s A s s o c i é s à L a G r o s s e s s e

- **Coordination d'un réseau national dans le cadre de l'appel d'offre INCA: Structuration nationale pour la prise en charge des cancers rares de l'adulte**
- **Réseau national en structuration :
INCA/CNGOF**
- **But :**
 - } aider à une prise en charge homogène et optimale des patientes sur le territoire national
 - } soutenir les travaux cognitifs de recherche biologique autour de la thématique des cancers associés à la grossesse (pharmacocinétique, oncogenèse, registres)

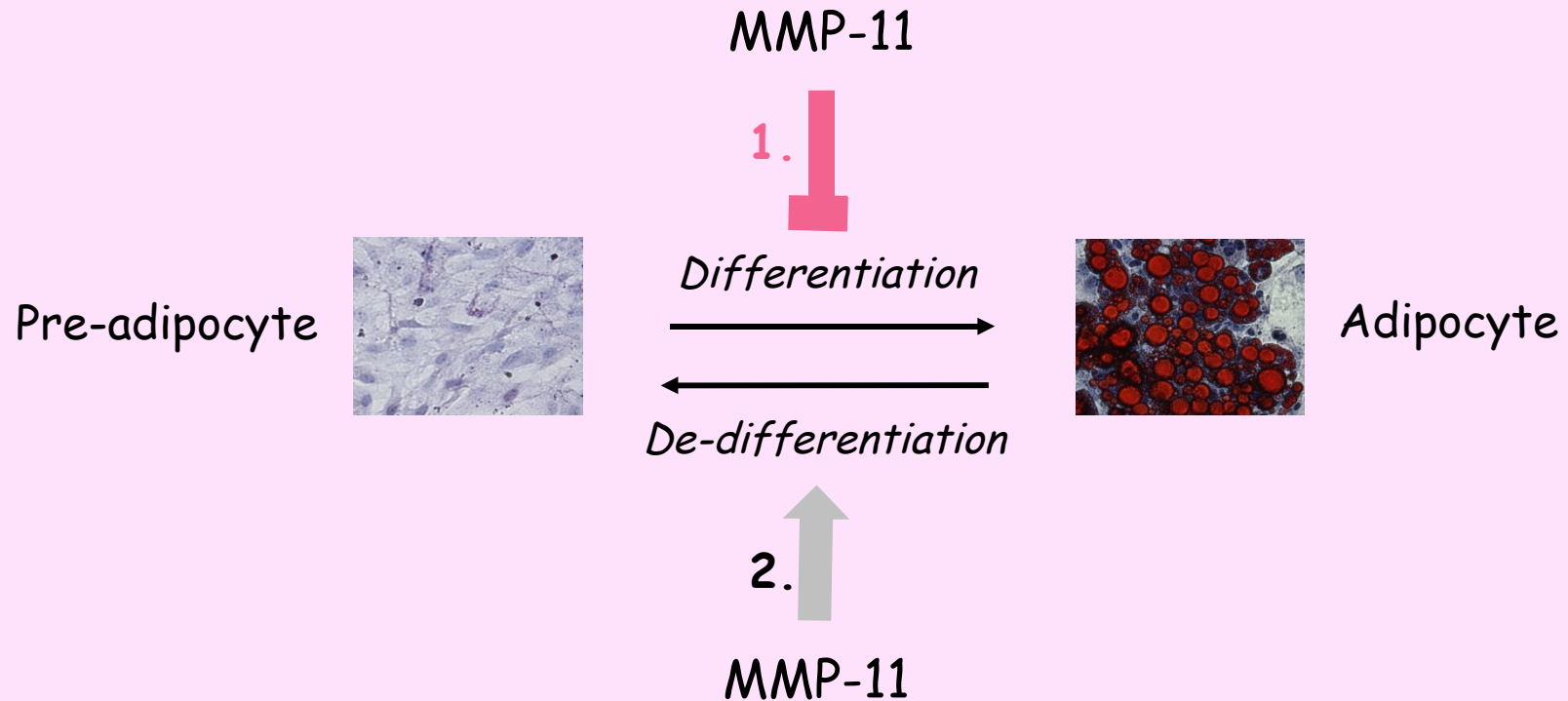
**Cancer Cell - Adipocyte Crosstalk:
Role of Matrix Metalloproteinase-11
(MMP-11)**

Emilie BUACHE

*32èmes Journées de la Société Française
de Sénologie et de Pathologie Mammaire*

- Strasbourg (03/05 novembre 2010) -

Conclusion : MMP-11 negatively regulates fat homeostasis

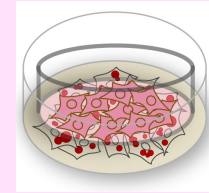
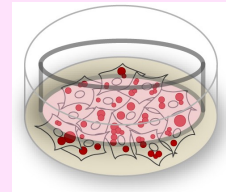
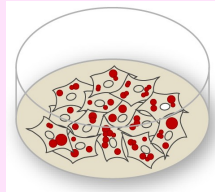


1. By lowering pre-adipocyte differentiation
2. By favoring adipocyte de-differentiation

The Co-culture with Breast Cancer Cells leads to a Delipidation of Adipocytes

Oil Red Staining

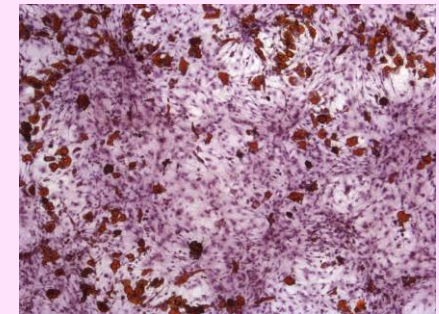
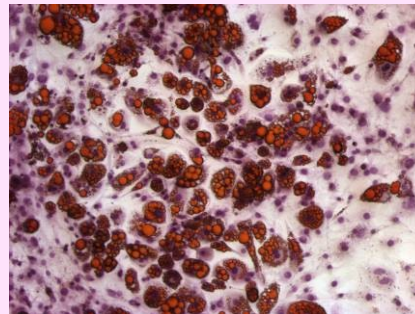
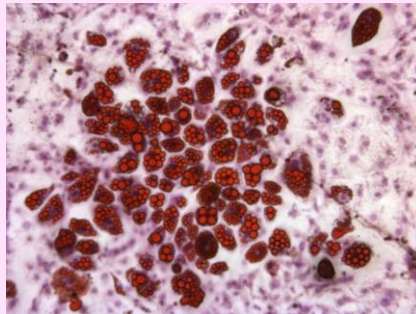
*Differentiation status
of 3T3L1 cells*



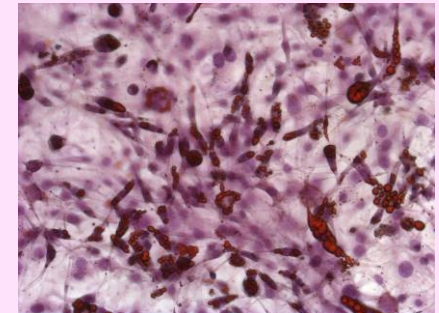
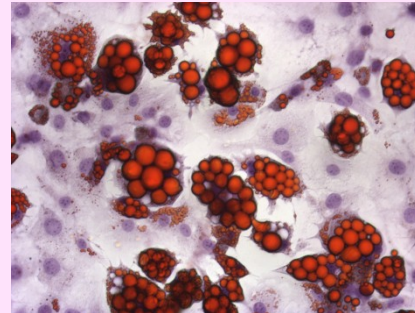
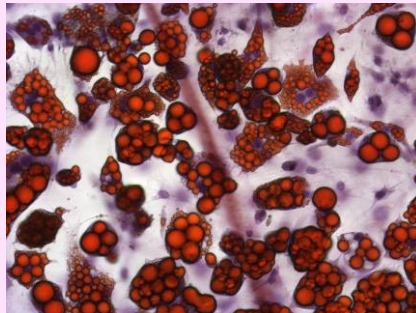
Numerous Lipid Droplets

**Reduced
Number and Size
of Adipocytes**

X 10



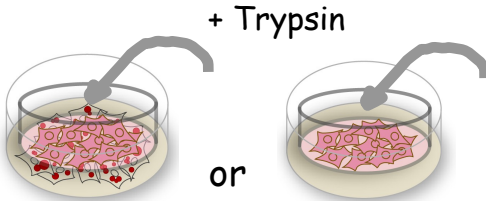
X 20



The Co-Culture leads to an Increase of Cancer Cell Migration & Invasion

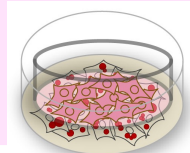
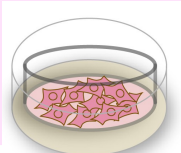
Protocol

Co-culture step

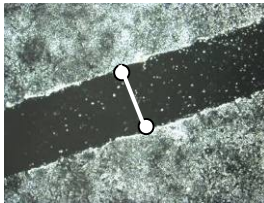


Migration
& Invasion
Assays

Breast Cancer
Cells



$t = 0$



$t = 15h$



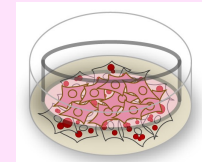
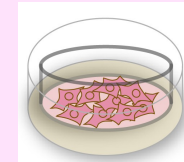
Wound Healing Assay

6 well plates
15h culture

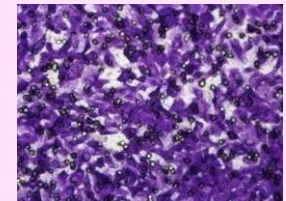
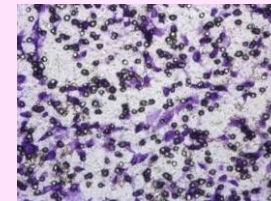


Boyden Chamber Assay

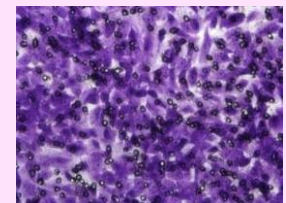
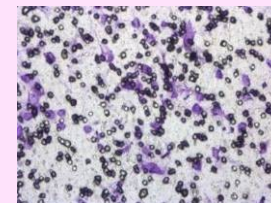
Porous filter 8 μm
+/- **Matrigel Coating (10 μg)**
100 000 cancer cells
24h culture
Crystal violet staining



- Matrigel
= **Migration**

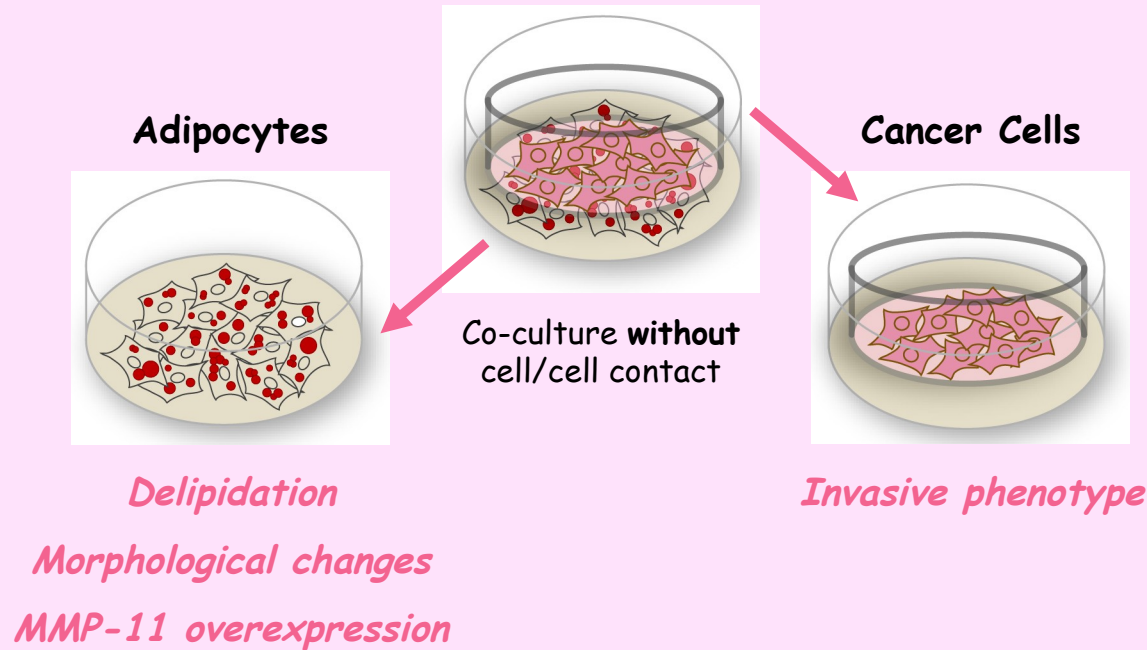


+ Matrigel
= **Invasion**



Conclusions & Perspectives

 Real bidirectional crosstalk between adipocytes & cancer Cells



 For the Future...

Molecular Mechanisms behind MMP-11 Function

1) **Unbiased** approach (Proteomic & Transcriptomic levels)

2) **Targeted** approach

3) **In vivo** analysis of the identified molecules at invasive front of human breast tumor

Société Française de Sénologie et de Pathologie Mammaire

.....www.senologie.com



Tissu adipeux, lipidome et cancer du sein de l'alimentation au microenvironnement tumoral

Philippe Bougnoux

INSERM U921
"Nutrition, Croissance et Cancer"
Centre Henry S. Kaplan
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Tours

bougnoux@med.univ-tours.fr

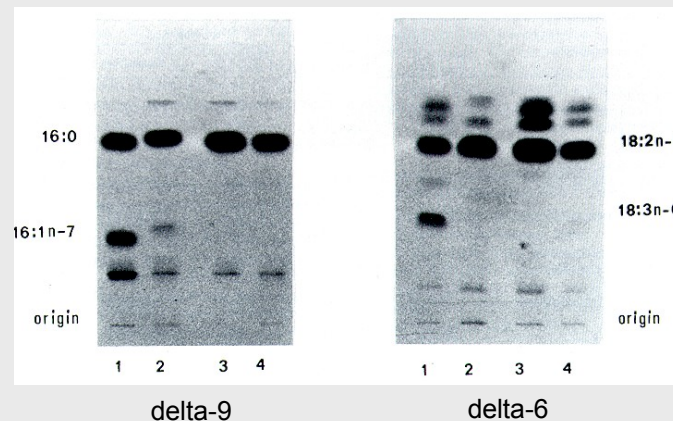
What are the determinants of tumor lipid profile ?

Fatty acid	carcinoma	non-tumor breast tissue	p value *
	Mean†(n=53)	Mean (n=26)	
Saturates			
16:0 (palmitic acid)	18.7	19.3	NS &
18:0 (stearic acid)	15.9	15.9	NS &
total saturates	36.2	37.9	NS &
Monounsaturates			
16:1 (palmitoleic acid)	2.1	1.2	0.0009
18:1 (oleic acid)	22.1	17.9	0.0001
total monounsaturates	27.5	22.6	0.0001
Polyunsaturates n-6			
18:2 n-6 (linoleic acid)	8.7	9.6	0.04
20:4 n-6 (arachidonic acid)	9.2	10.2	NS &
total n-6	20.5	21.8	NS &
Polyunsaturates n-3			
18:3 n-3 (α -linolenic acid)	0.1	0.4	0.0001
22:5 n-3 (docosapentaenoic acid)	0.6	0.6	NS &
22:6 n-3 (docosahexaenoic acid, DHA)	1.3	1.2	NS &
total n-3	2.2	2.6	NS &

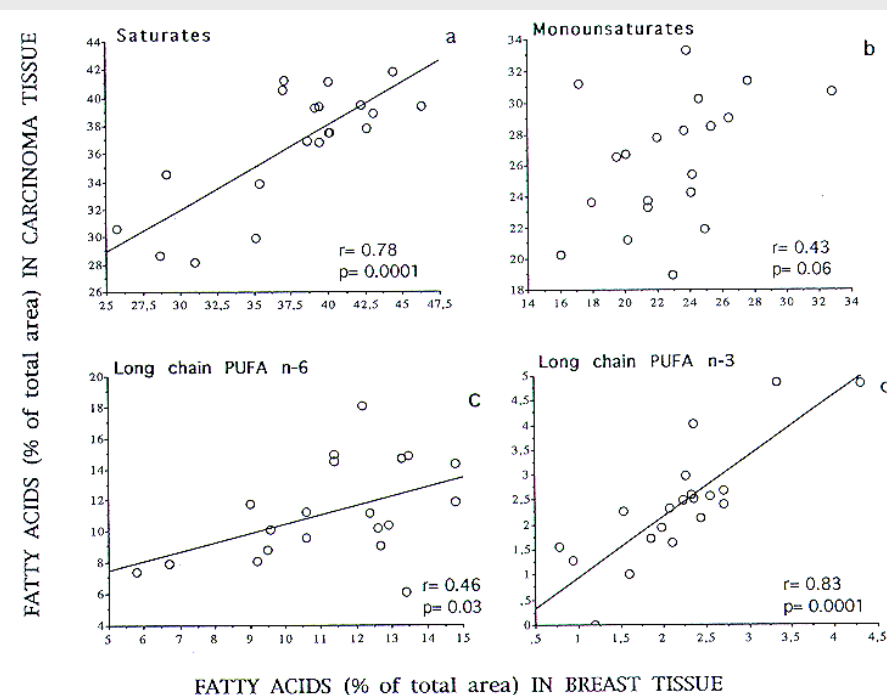
† % of total fatty acids, aldehydes 12%, NI 2% * Mann-Whitney test; &NS : not significant

Lipid profile of carcinoma is close –but distinct – to that of non tumor, epithelial breast tissue

Chajes et al, Int. J. Cancer, 1995



No detectable desaturase activity in the carcinoma tissues



Carcinoma and non tumor fatty acid levels are correlated

The similar fatty acid composition indicates that a common causal factor may be operating. Diet is an obvious candidate.

Case-control study on adipose tissue

- 241 patients with invasive, non metastatic breast cancer (**cases**)
- 88 patients with benign, non proliferative tumor (**controls**)
- Adipose breast tissue sampled at diagnosis during surgery
- fatty acid composition of adipose tissue
- Multivariate analysis

Estimated relative risk of breast cancer by levels of n-3 and n-6 fatty acid in adipose tissue (n = 329)

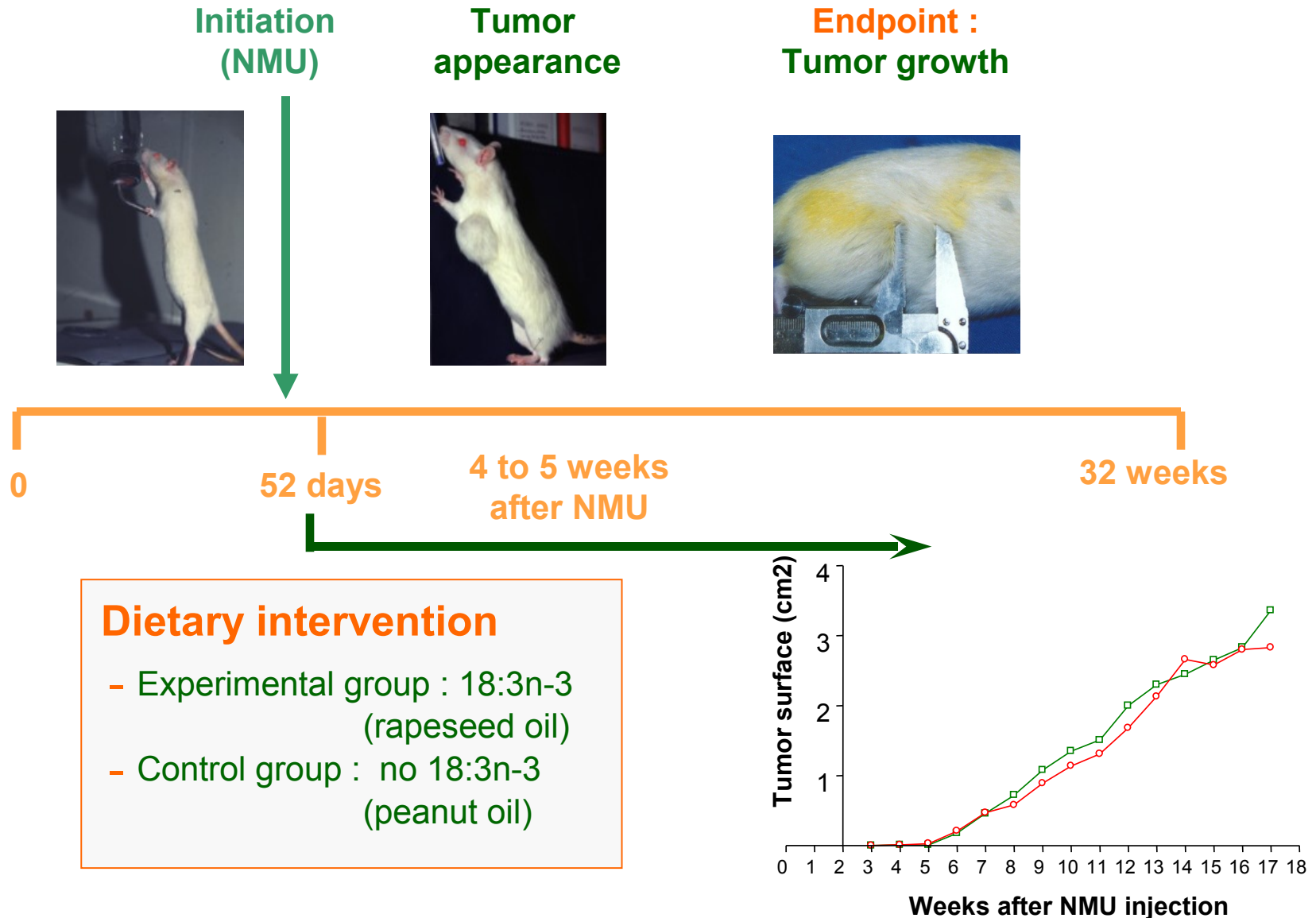
Fatty acids	Odds ratio (95% CI)			P for trend
	1st Tertile (low)	2 nd Tertile	3 rd Tertile (high)	
18:2 n-6c	1.00	1.60 (0.76-3.36)	2.31 (1.15-4.67)	0.06
18:3 n-3	1.00	0.97 (0.50-1.90)	0.39 (0.19-0.78)	0.01
22:6 n-3	1.00	0.84 (0.40-1.75)	0.31 (0.13-0.75)	0.016
Total n-3	1.00	0.91 (0.45-1.87)	0.40 (0.17-0.94)	0.001
Ratios				
18:3n-3/18:2n-6	1.00	0.89 (0.46-1.75)	0.41 (0.20-0.81)	0.0004
Long chain n3/total n-6	1.00	0.48 (0.23-0.97)	0.33 (0.17-0.66)	0.0002

Adjusted for age, height, BMI, menopause (pre- and post) and menopausal status-BMI interaction.
Test for trend performed by using the means within each category in the logistic regression model.

Maillard V. et al, Int J Cancer, 2002

Adipose tissue lipids predict breast cancer risk:
Omega-3 PUFA (ALA and DHA) in adipose tissue are inversely
associated with the risk of breast cancer **causality ?**

Dietary intervention study in the rat



No effect of the 18:3 dietary intervention on tumor growth

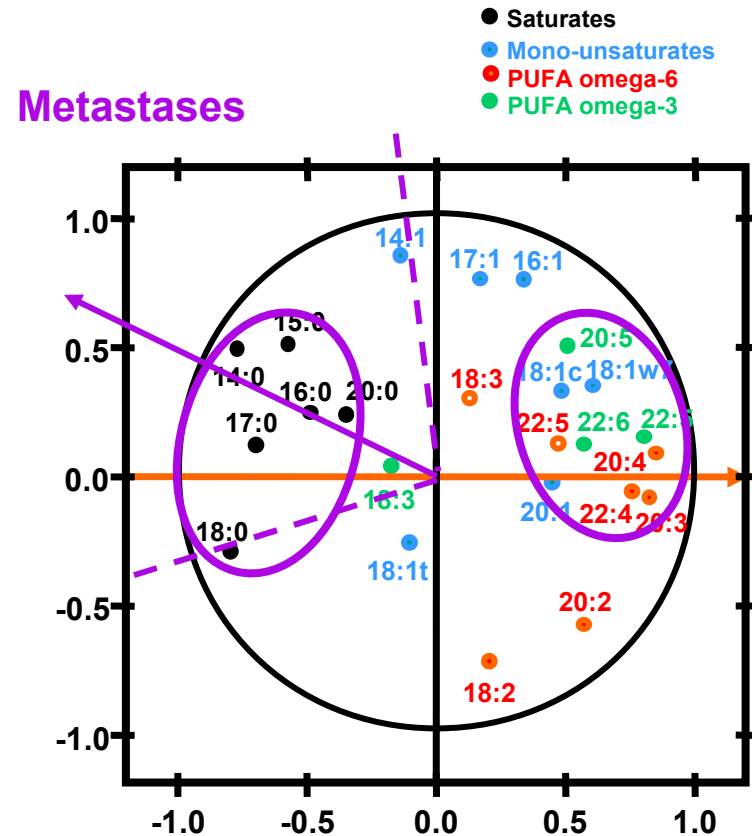
Lipidomic profile and the risk of metastases

Multivariate analysis of prognostic factors

Prognostic factor		Relative Risk	CI 95%	p
Tumor size (mm)	< 20	1.0		
	20 – 50	2.7	(1.4 – 5.6)	0.005
	> 50	2.4	(1.5 – 3.9)	< 0.001
Positive nodes	0	1.0		
	1 - 4	0.7	(0.4 – 1.3)	ns
	> 5	3.3	(1.8 – 6.0)	< 0.001
1st principal component		0.9	(0.8 – 0.97)	0.014

The first principal component is an independent covariate associated with a low risk of metastases

A protective profile combines low levels of saturates with high levels of long chain polyunsaturates



Modification of the lipidome through decreased dietary ω -6 / ω -3 ratio (DHA)



Sprague-Dawley rats

- Initiation (NMU)
- 7 % peanut/rapeseed
- 2 nutritional groups (supplement)
 - saturates (8 % palm oil)
 - DHA (8 % DHASCO)

Adipose tissue sampled at autopsy

Fatty acids analyzed by GC

Fatty acids (% area)	Adipose tissue TGL	
	Control (n=7)	DHA- supplemented (n=6)
	Mean (%)	Mean (%)
Saturates		
16:0	22.9	20.8
18:0	2.8	3.4
Total	27.0	28.9
Monounsaturates		
18:1n-9c	52.7	43.8
Total	57.9	48.9
n-6 PUFA		
18:2n-6c	12.2	11.1
Total	12.7	11.3
n-3 PUFA		
18:3n-3	0.6	0.8
22:6n-3	0.1	8.2
Total	0.7	9.3

***18:3 or DHA supplementation
do not bring about an
adequate coordinate change
in the lipid profile***

Lipidome

Stromal cells

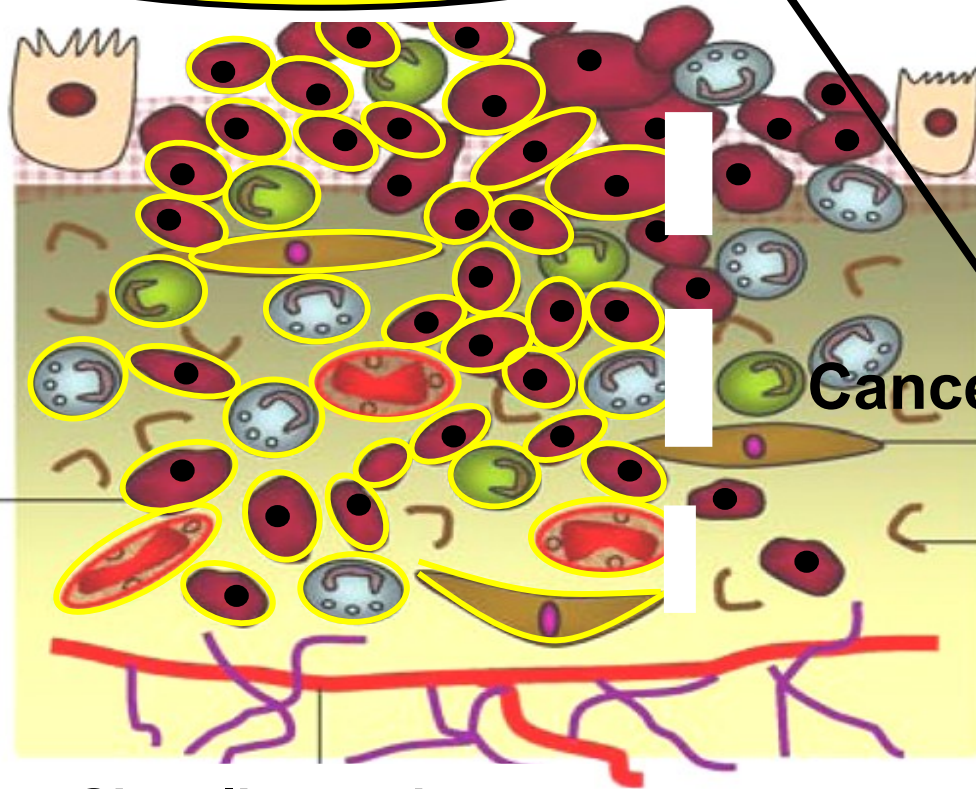
Cancer cells

Myofibroblasts

Invasive
tumor cells

ECM
fragment

Signaling pathways
Proliferation
Death
Migration
Angiogenesis

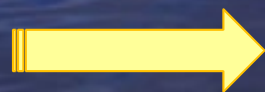


Détection des CTC

Challenge:

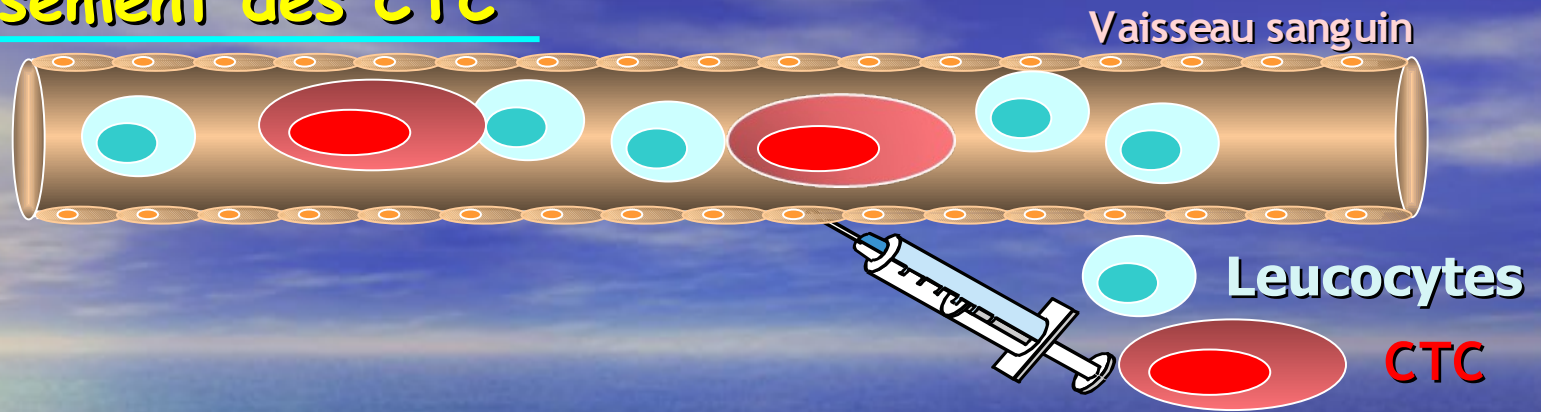
Détecter *une* cellule tumorale

parmi 10 – 10 cellules



EVENEMENTS RARES !

Enrichissement des CTC

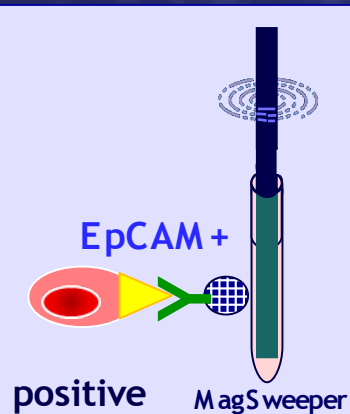
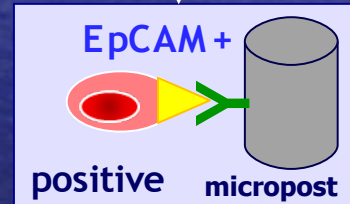
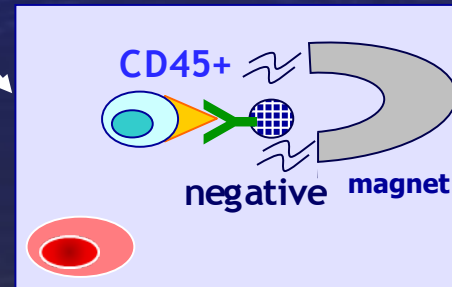
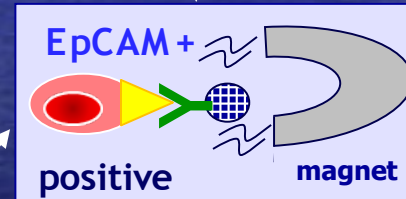
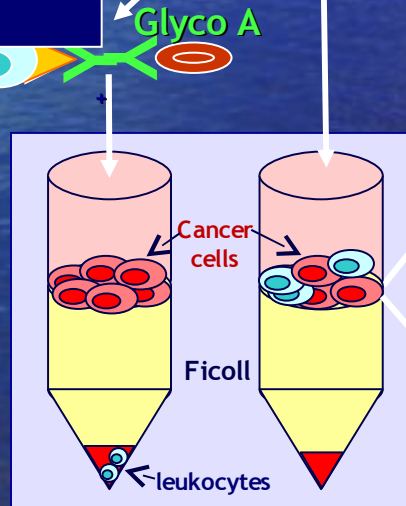
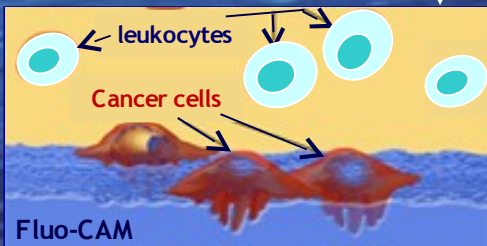
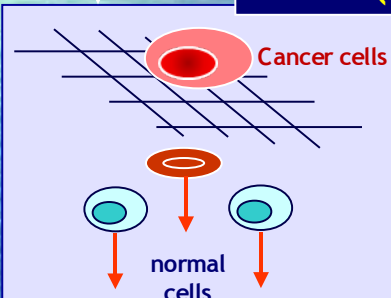


Taille

Collagen Adhesion Matrix (CAM)

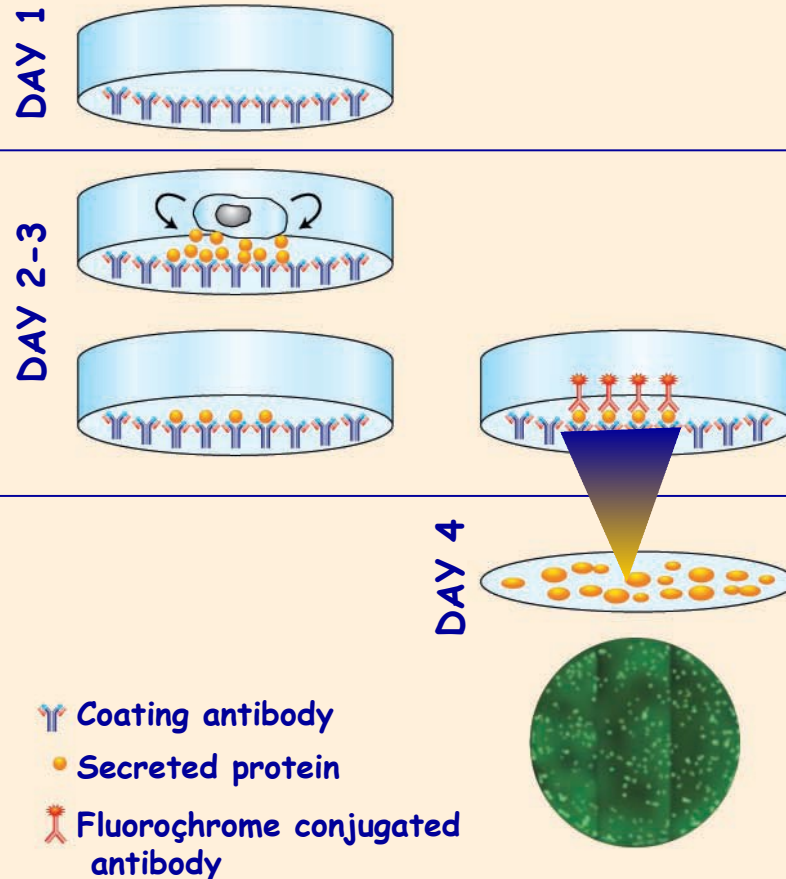
Densité

Marqueurs protéiques



EPISPOT : Culture cellulaire & Détection de CTC/DTC vivantes sécrétrices de protéines

Alix-Panabières, Riethdorf & Pantel, Clin Cancer Res, 2008



Alix-Panabières et al., Clin Cancer Res, 2008

Cancer du sein: Technique double CK19/MUC1-EPISPOT

Moelle osseuse

Patients	Patients positifs (%)
M (n = 20)	90
M (n = 37)	54
Groupe Control (n = 10)	0

(p = 0.01)

Potentiel Biologique particulier

*Gudjonsson *et al.* (Gene & Dev. 2002)
ont suggéré que les cellules MUC1 /

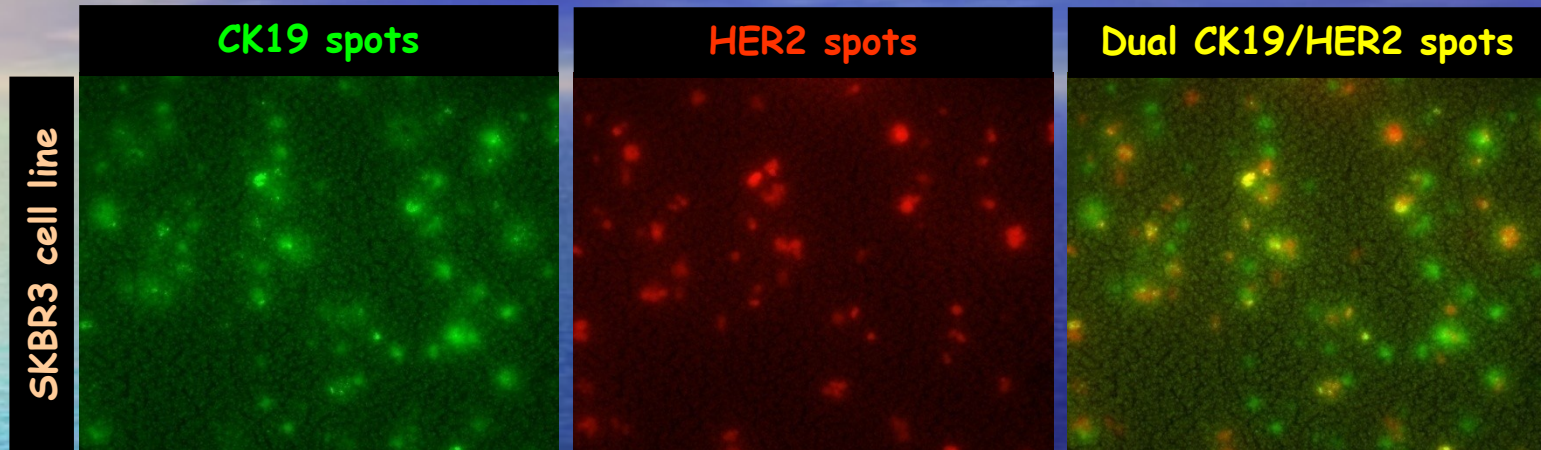
CK19 pourraient avoir des

	CK19 /MUC1 +	+	-	CK19 /MUC1 +
M	Total nDTC	2011	1395	198
M	Total nDTC	90	597	2

présentes de 'cellules souches' dans un tissu mammaire sain.

Breast Cancer: CK19/HER2-EPISPOT assay

CK19/HER2-EPISPOT assay



CTC vivantes peuvent sécréter Her2.

Événement rare chez des patientes en stade avancé du cancer du sein

4/91 (4.4%)

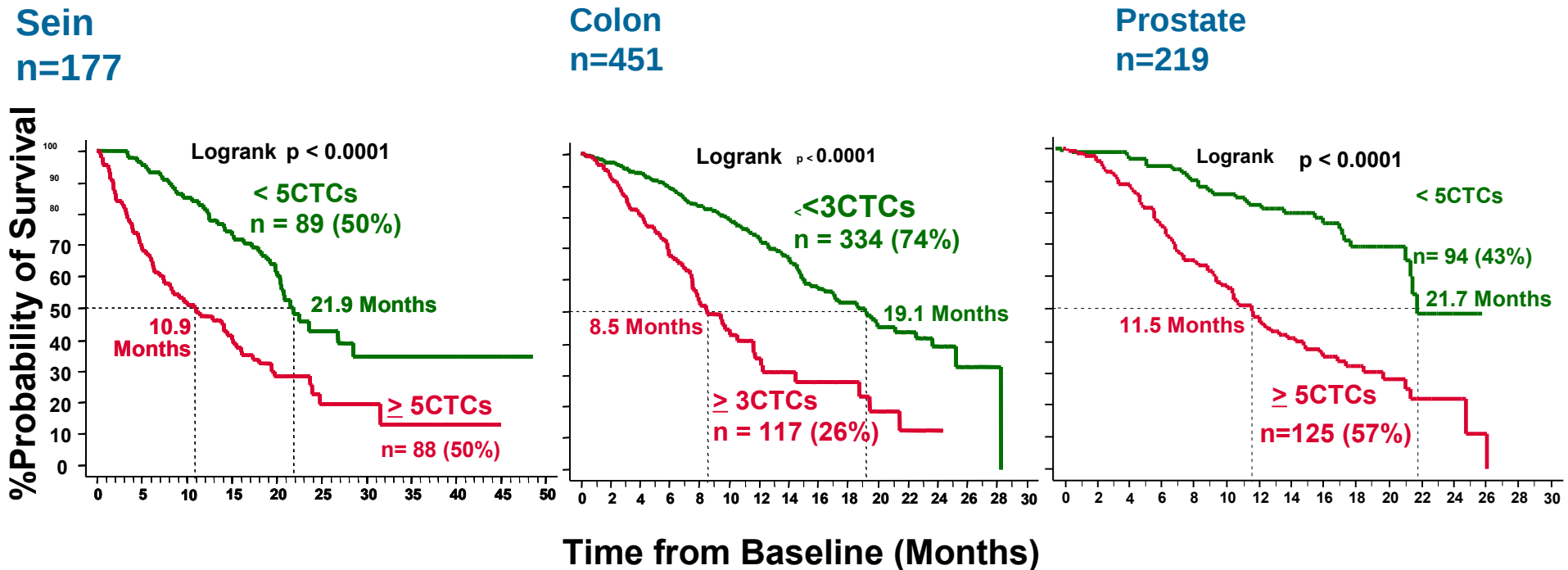
Cellules tumorales circulantes: intérêt pronostique

Jean-Yves Pierga

Département d'Oncologie médicale
Institut Curie

Technique Cellsearch

Taux de CTC avant traitement: facteur pronostique pour la survie globale au stade métastatique



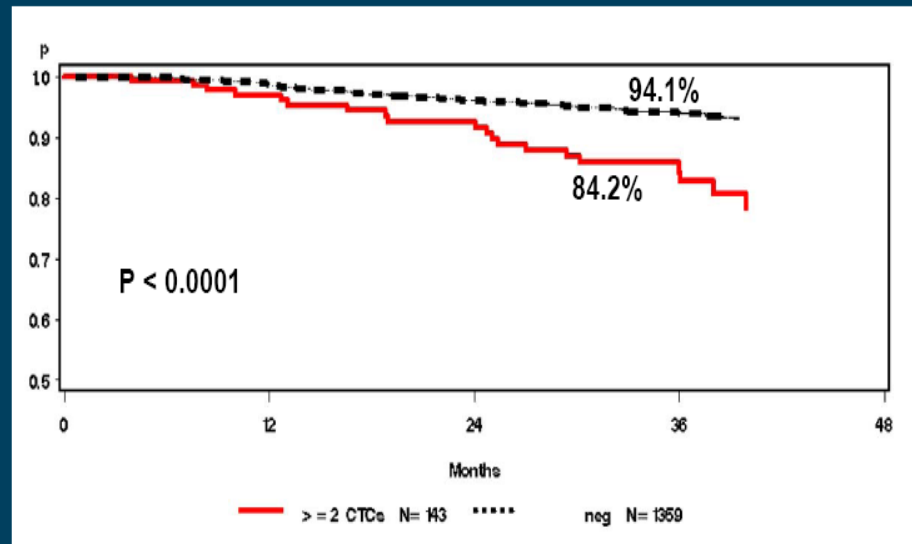
Cristofanilli et al
NEJM August 2004
JCO March 2005

Cohen et al
JCO July 2008
Cohen, Ann Oncol 2009

De Bono et al
CCR October 2008

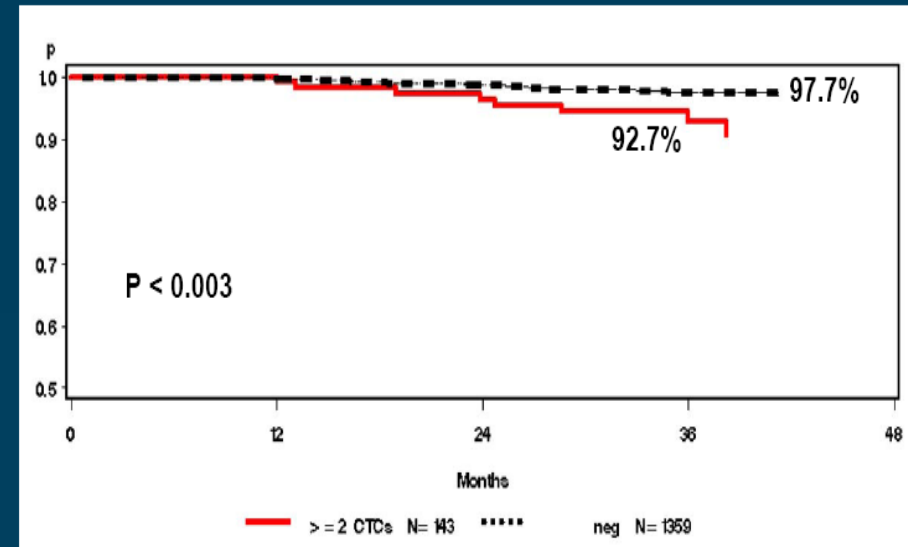
Impact pronostique des CTC avant et après une chimiothérapie adjuvante dans le cancer du sein : SUCCESS trial (*Rack et al, ASCO 2010*)

CTCs before Chemotherapy Disease-Free Survival



	CTC+	CTC-
Recurrences	20 / 143	67 / 1359
Mean Survival Time	36.8 mon	38.1 mon

CTCs before Chemotherapy Overall Survival



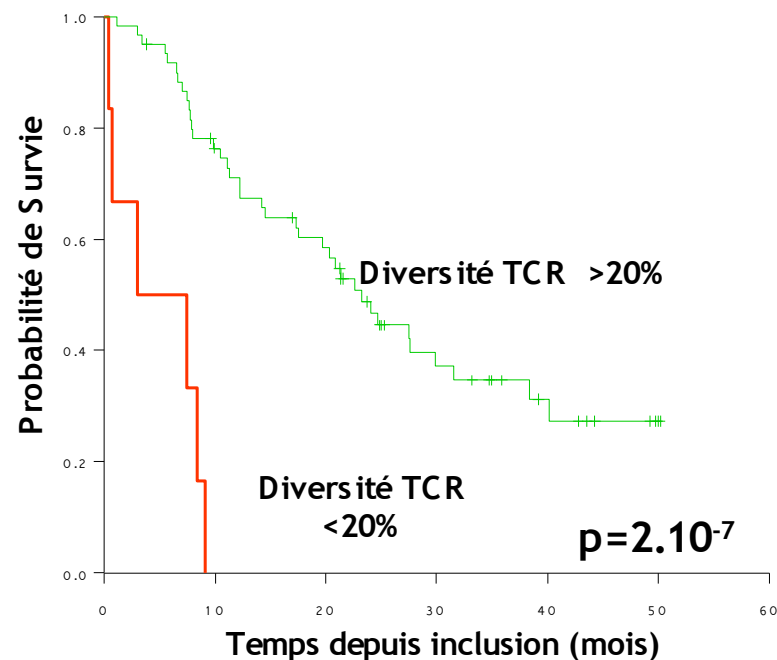
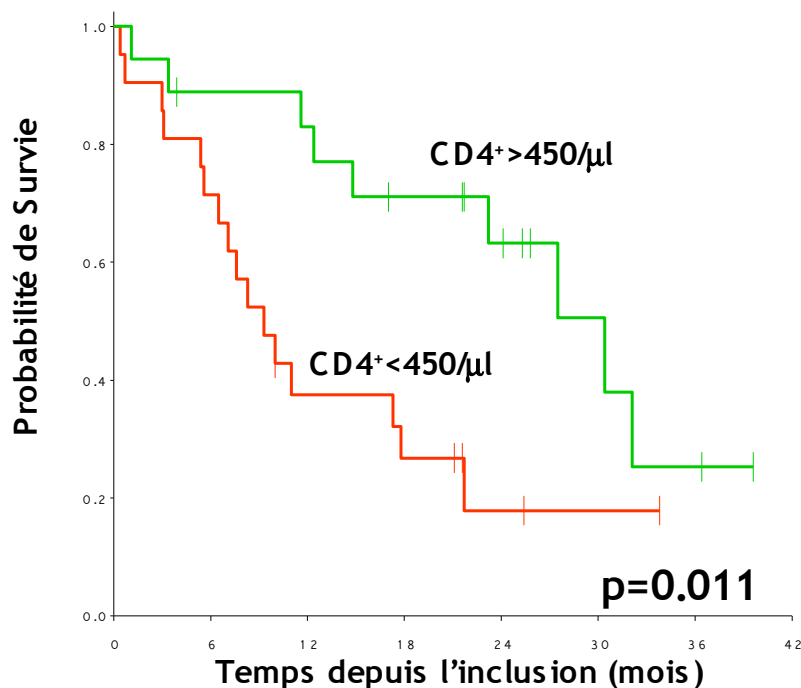
	CTC+	CTC-
Deaths	8 / 143	25 / 1359
Mean Survival Time	37.2 mon	40.8 mon

33 -Hormonothérapie adjuvante Median follow-up 32 months

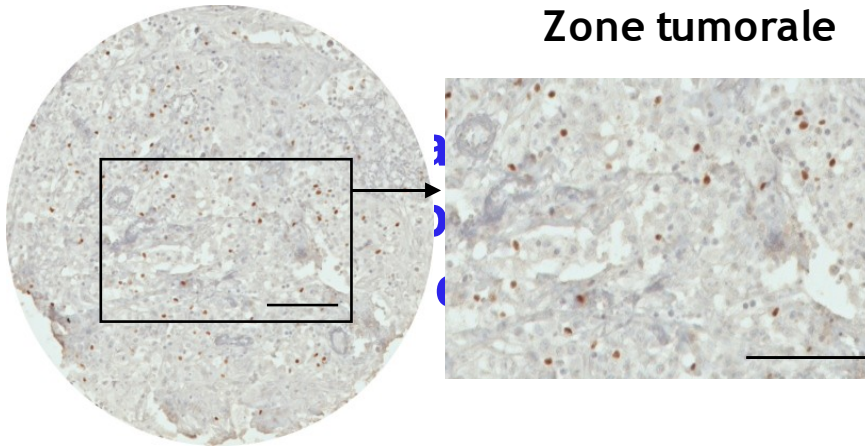


La diversité du répertoire TCR est hautement prédictive de la survie globale chez les patientes en rechute d'un cancer du SEIN

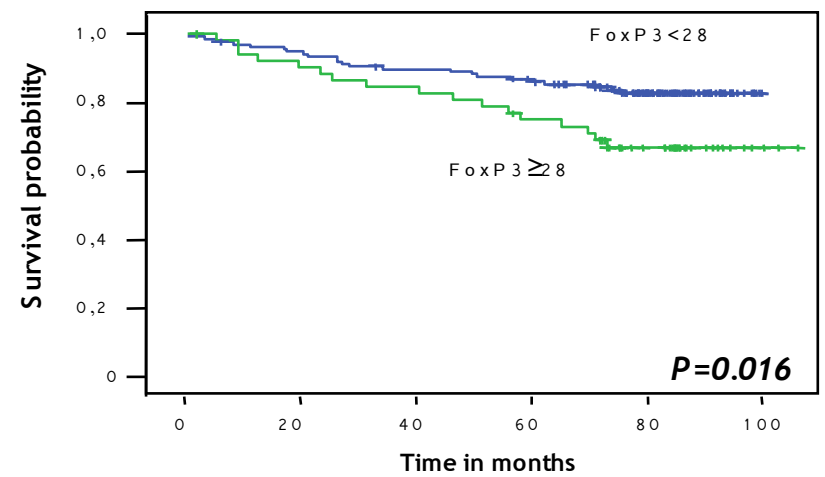
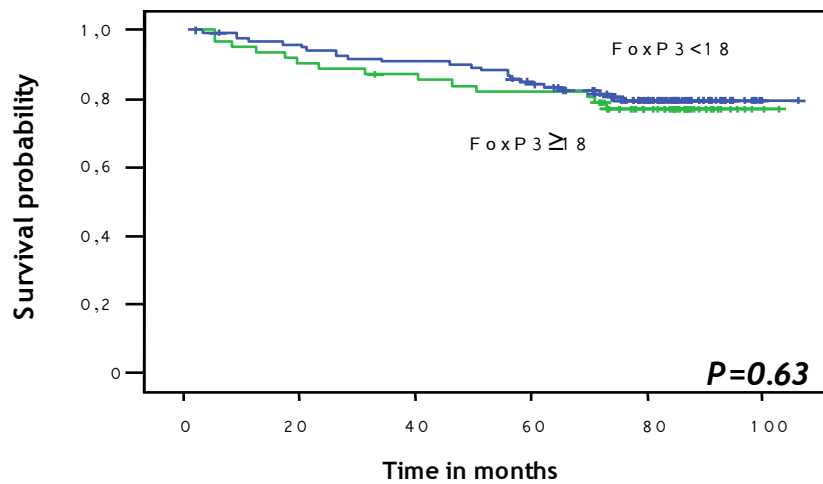
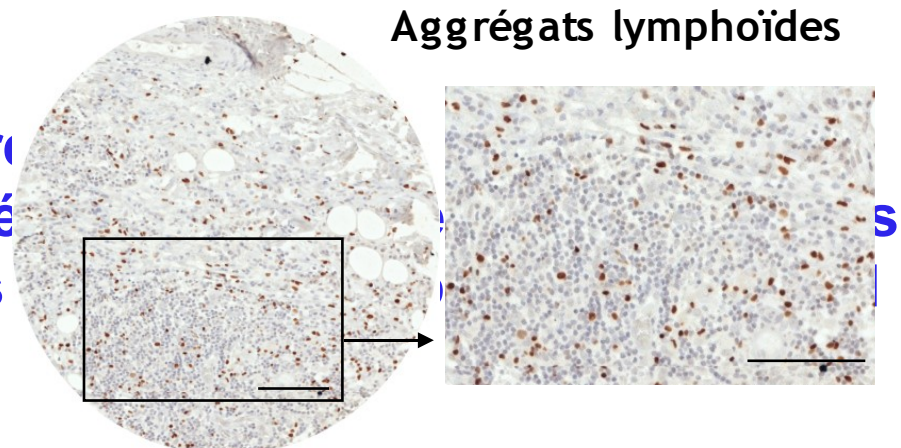
Diversité du répertoire TCR (N=66)



Zone tumorale

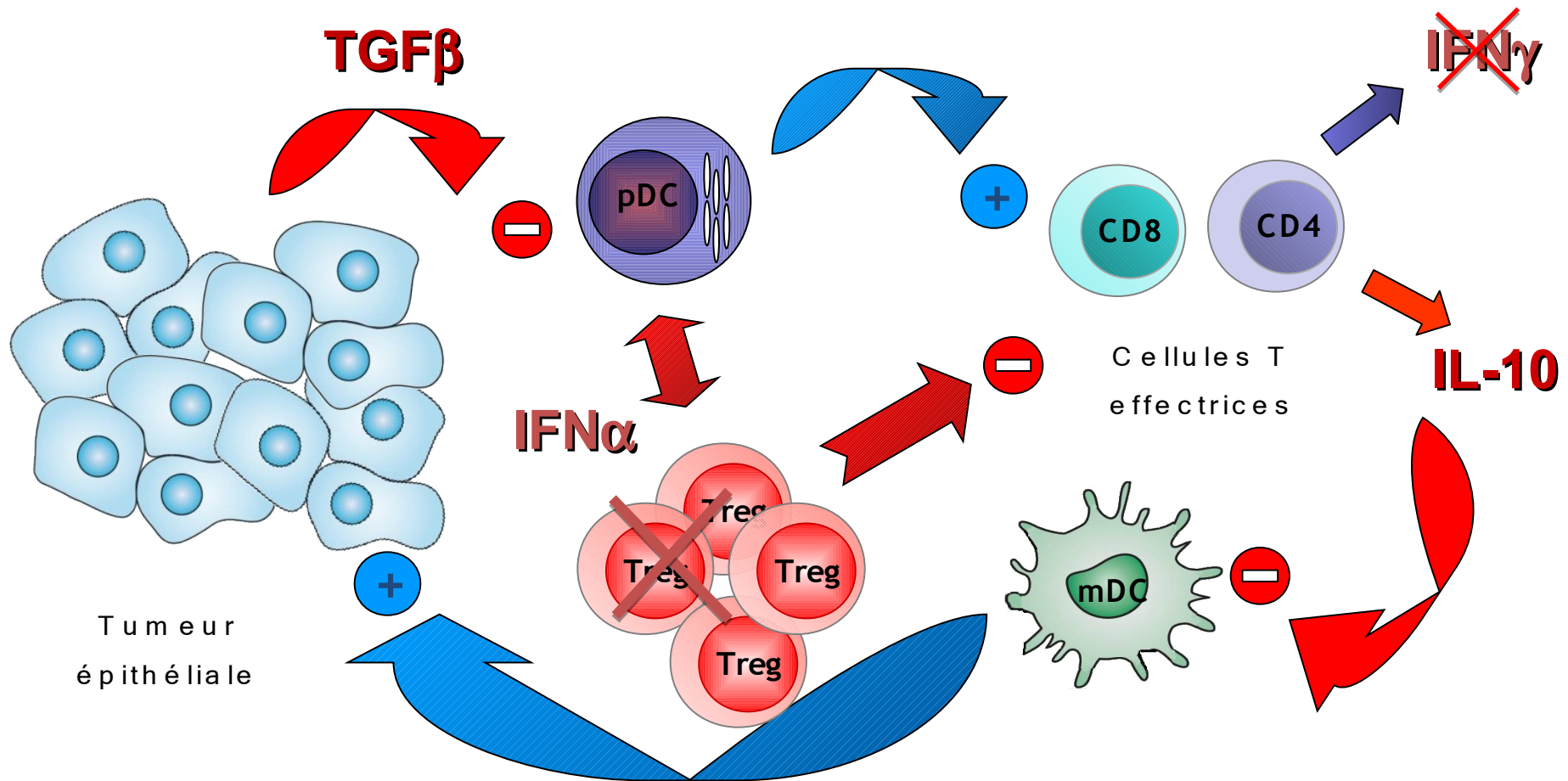


Aggrégats lymphoïdes





Les pDC sont au centre du réseau immuno-suppresseur dans l'environnement tumoral du cancer du SEIN



Ménétrier-Caux C, Cancer Research 2009

Sisirak V, Faget J, Bendriss-Vermare N soumis pour publication

Faget J, Ménétrier-Caux C en préparation

pDC : DC plasmacytoïdes

mDC : DC myéloïdes