Prognostic and predictive significance of T cell infiltration in breast cancer

Francois Ghiringhelli and Sylvain Ladoire

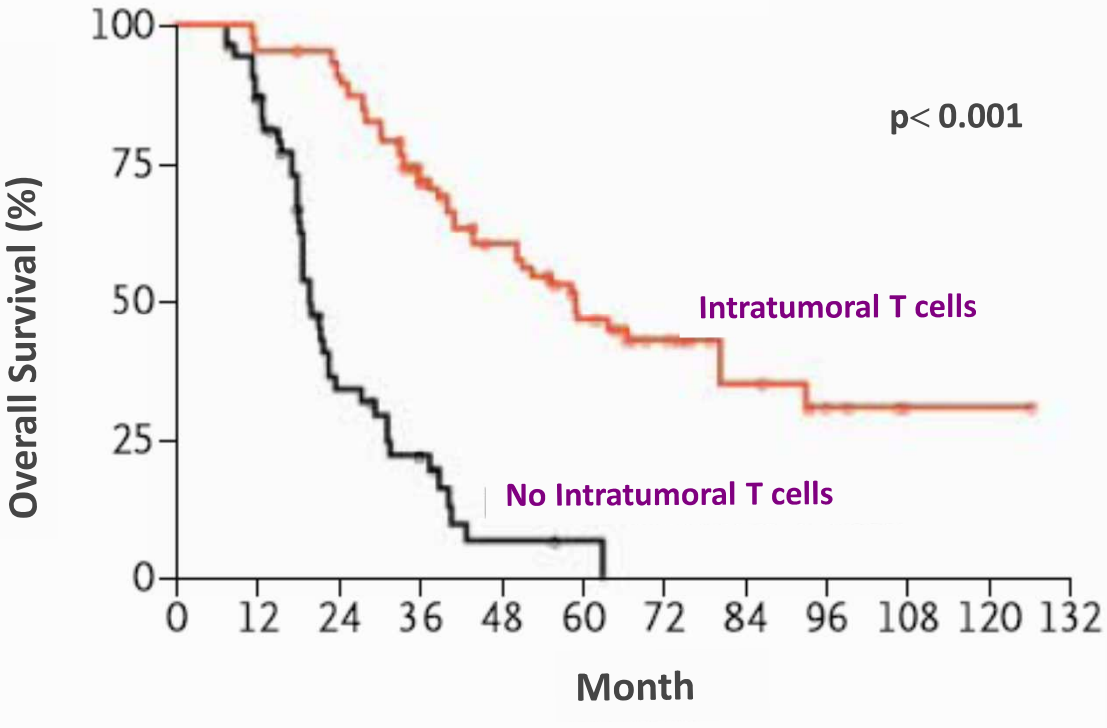
AVENIR Team UMR866 INSERM Dijon

T cells and cancer

The NEW ENGLAND JOURNAL of MEDICINE

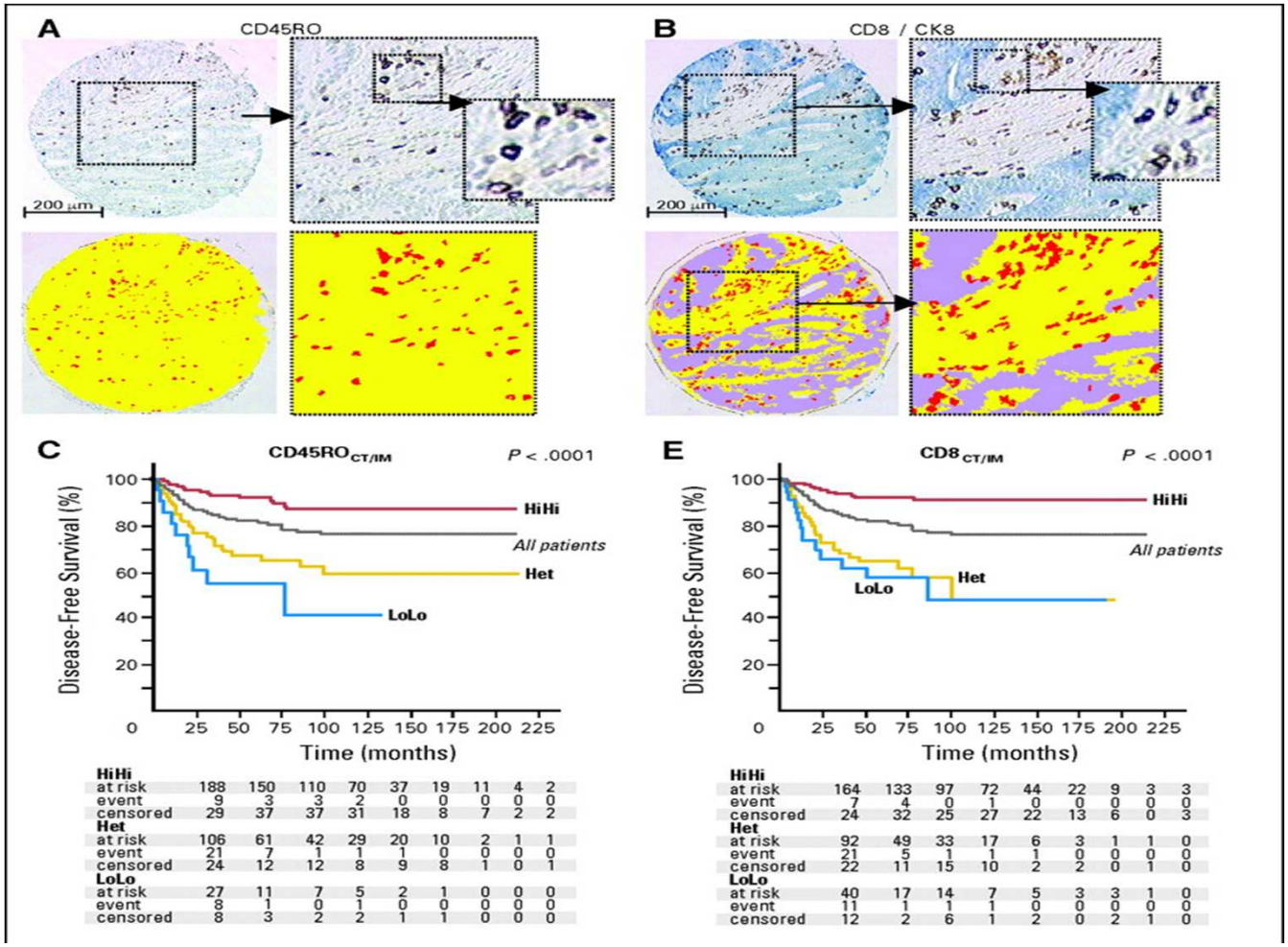
Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.



N Engl J Med 2003;348:203-13

CD45RO and CD8 immunostaining of colorectal cancer and prognosis.



Pagès F et al. JCO 2009;27:5944-5951

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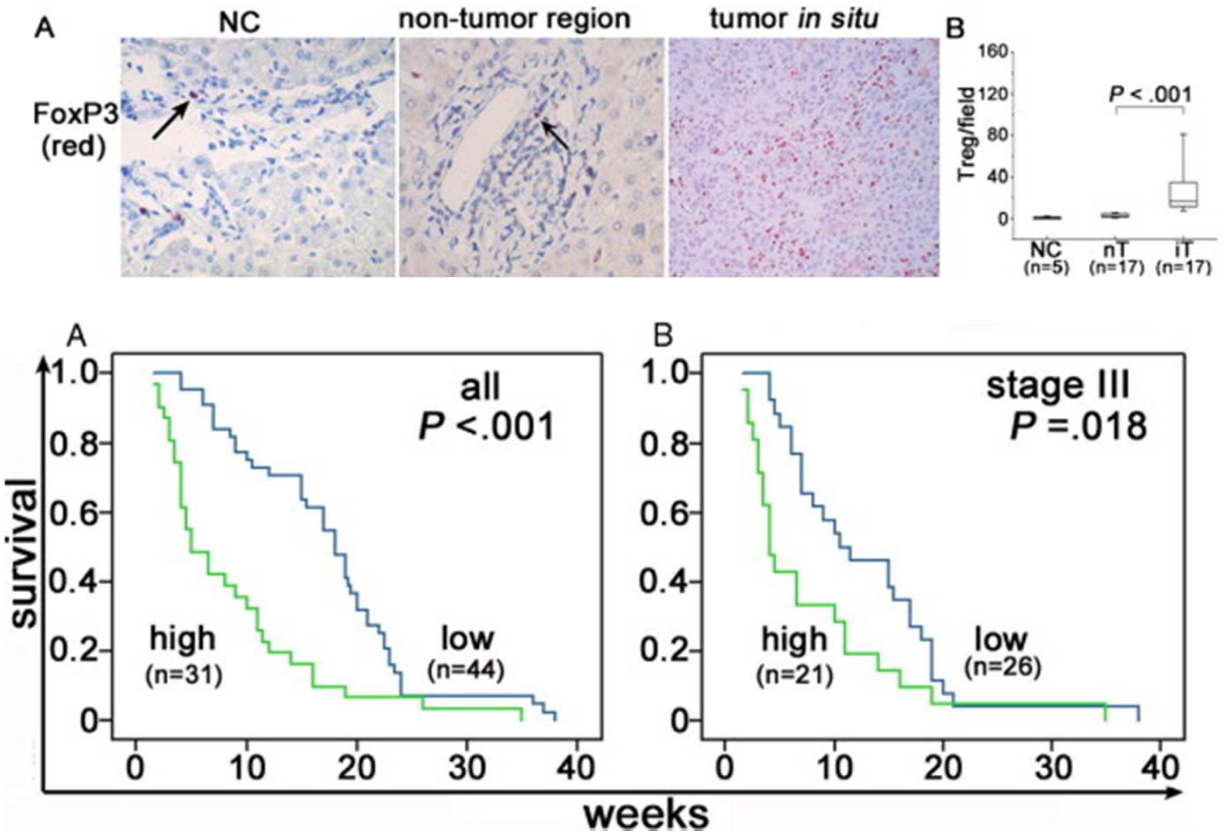
Treg cells and cancer

Foxp3 expression is often associated with poor prognosis

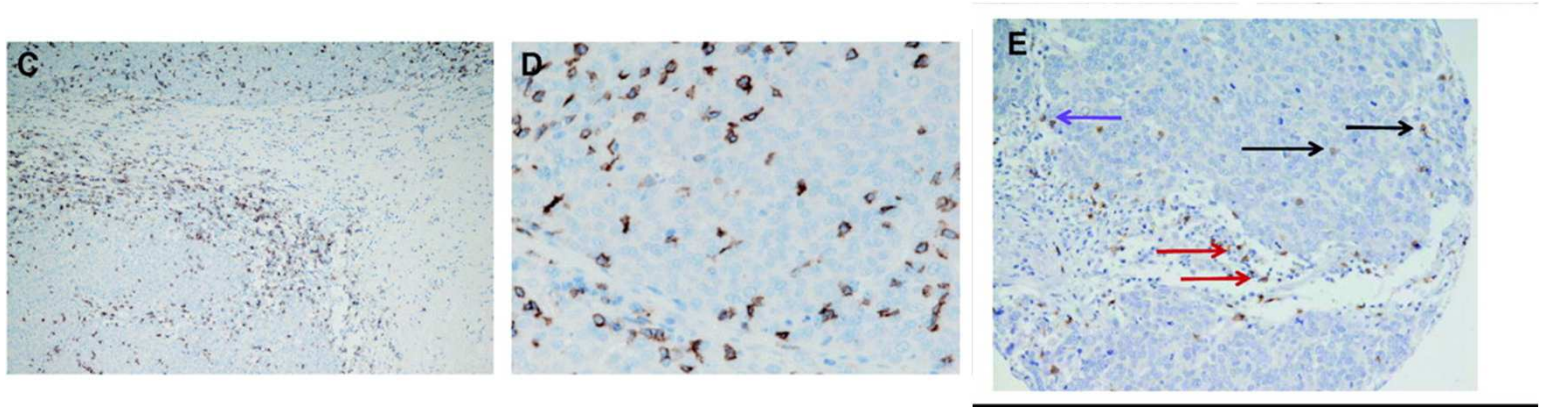


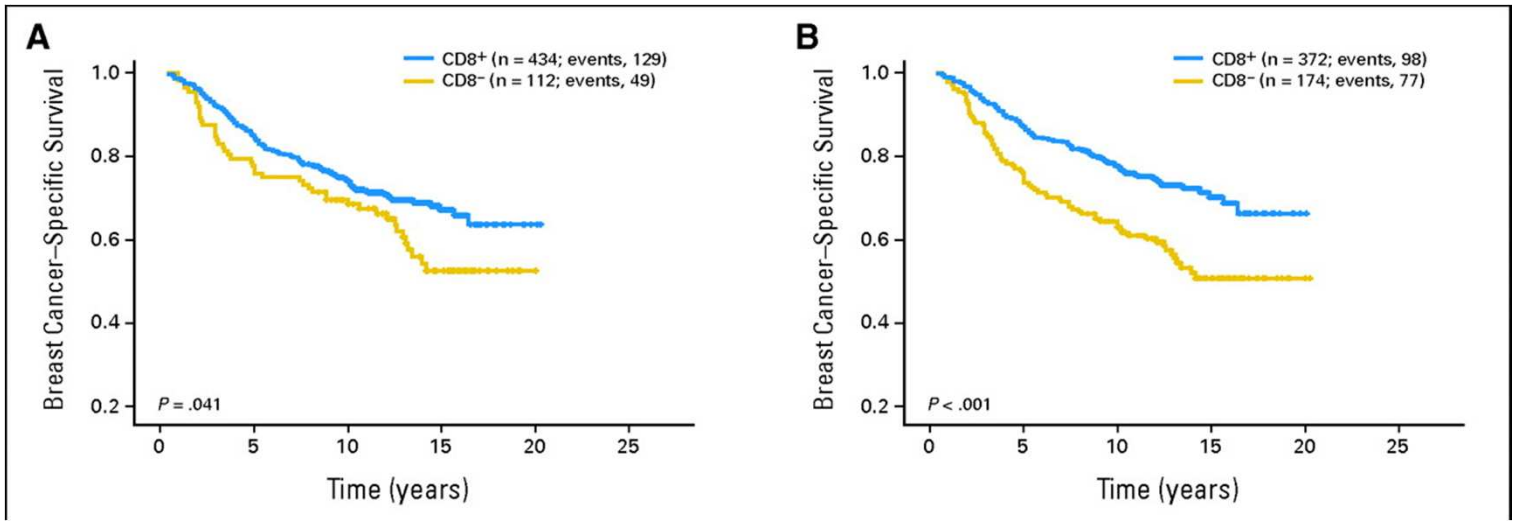
FU X et al. Gastroenterology. 2007 Jun;132(7):2328-39.

Gastroenterology

The case of localized breast cancer :

Role of CD8





Mahmoud S M et al. JCO 2011;29:1949-1955

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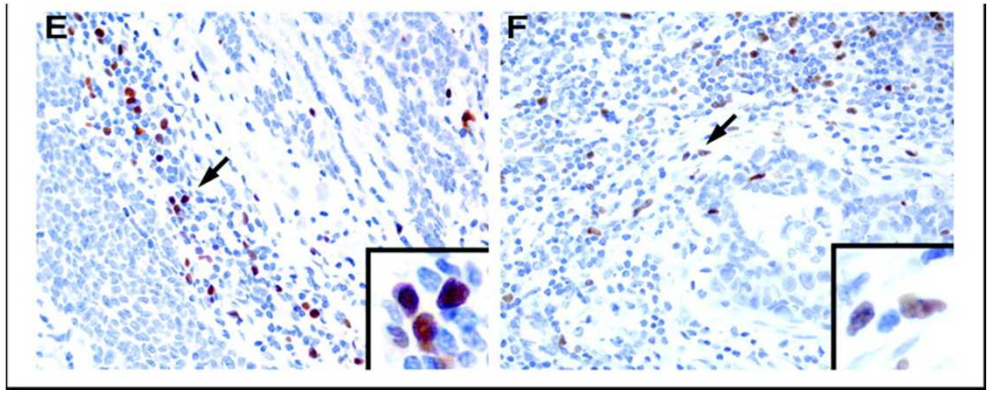
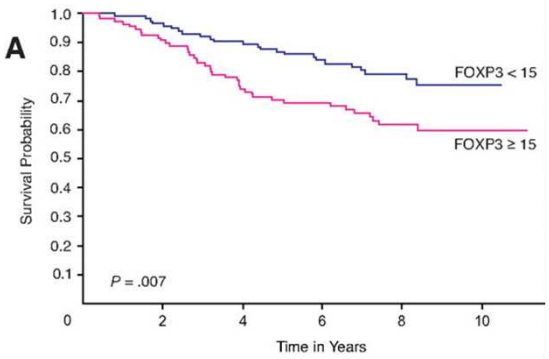
The case of localized breast cancer :

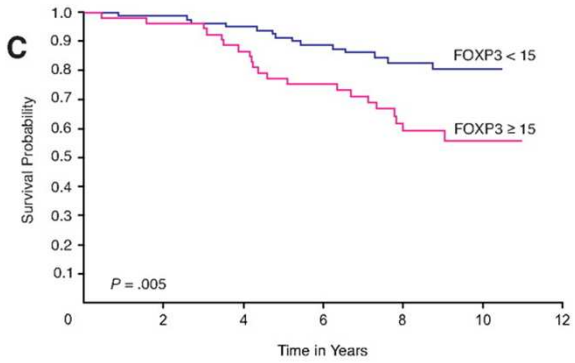
Role of regulatory T cells in subgroup of breast cancer



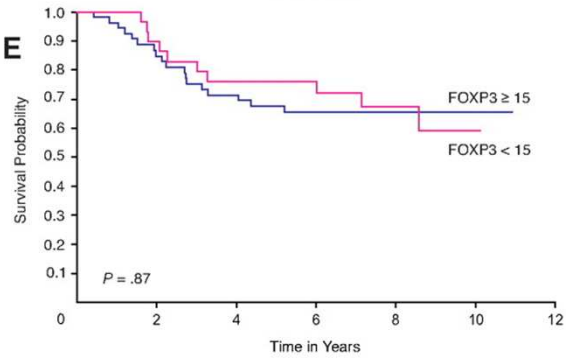
All patients



Hormon receptor +



Hormon receptor -



Bates G J et al. JCO 2006;24:5373-5380

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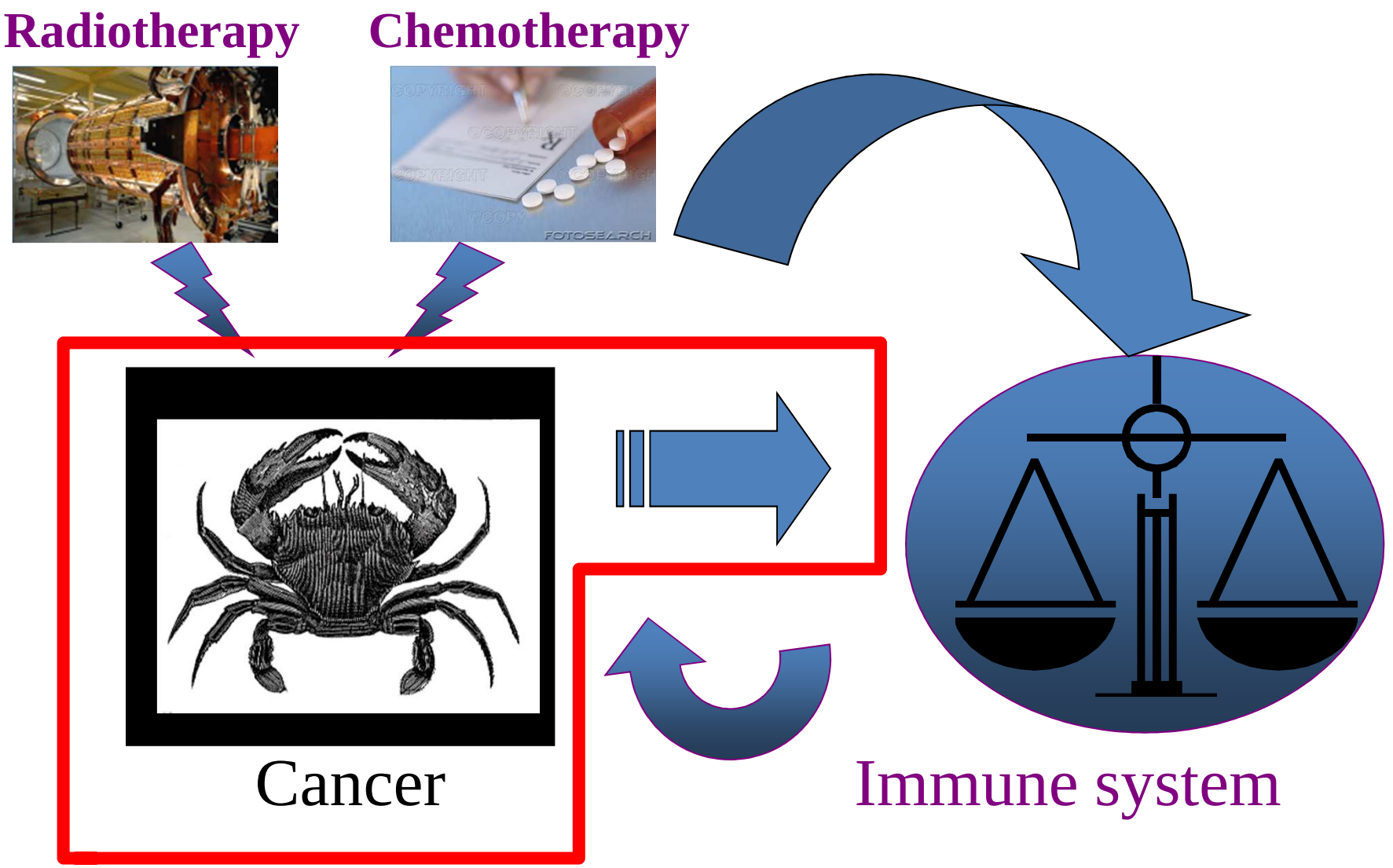
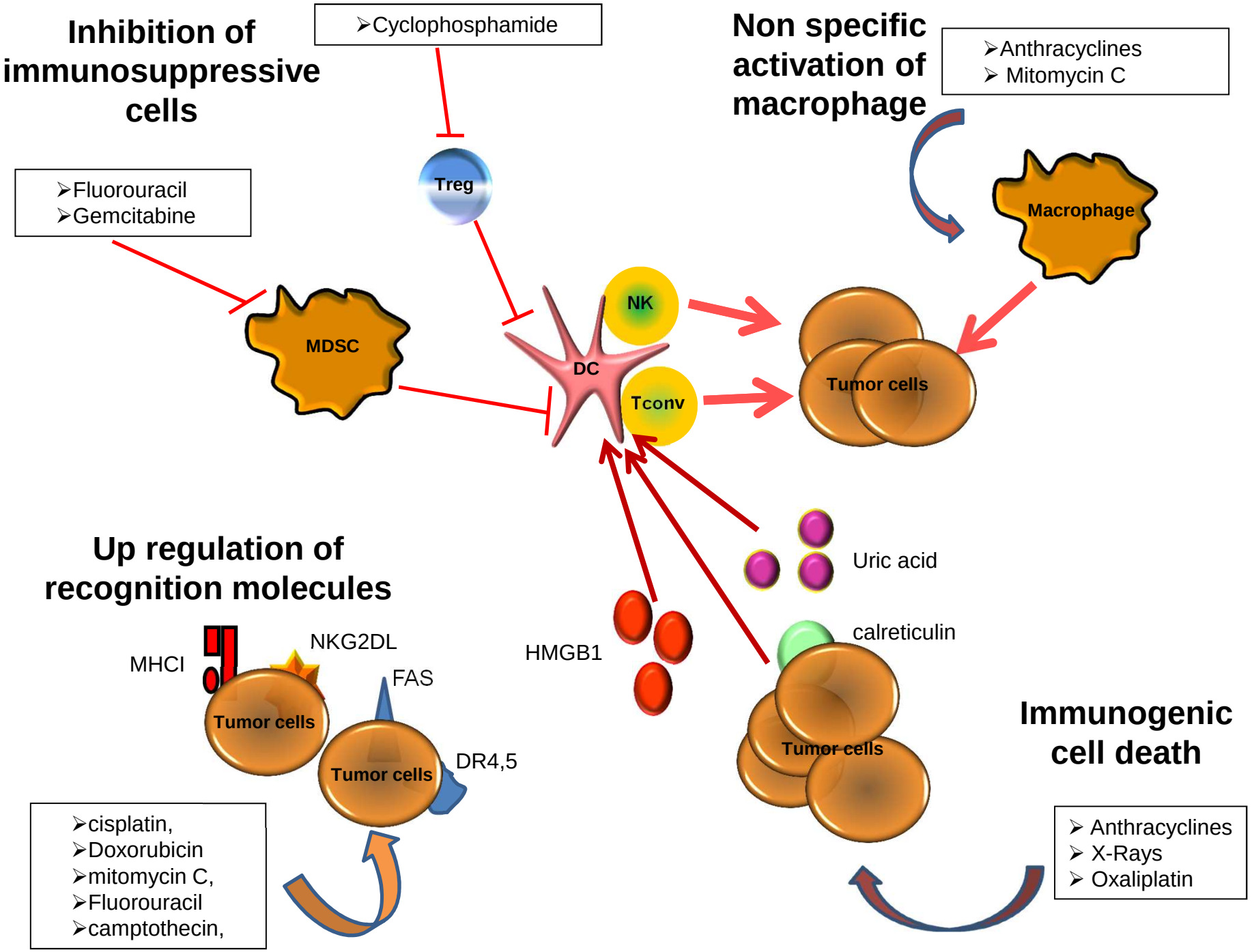
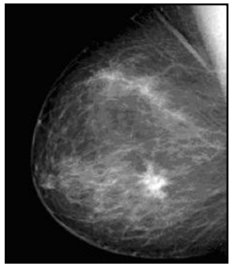
All these studies do not take in account the role of further therapies!!!



Zitvogel, Ghiringhelli and Kroemer Nature Review Immunology 2008


Inhibition of immunosuppressive cells
➢Cyclophosphamide
➢Fluorouracil
➢Gemcitabine
Treg
MDSC
NK
DC
Tconv
Non specific activation of macrophage
➢Anthracyclines
➢ Mitomycin C
Macrophage
Tumor cells
Uric acid
calreticulin
HMGB1
Up regulation of recognition molecules
MHCI
NKG2DL
FAS
DR4,5
Tumor cells
Tumor cells
Tumor cells
➢cisplatin,
➢Doxorubicin
➢mitomycin C,
➢Fluorouracil
➢camptothecin,
Immunogenic cell death
➢ Anthracyclines
➢ X-Rays
➢ Oxaliplatin

Neoadjuvant treatment of breast cancer



Diagnostic → Chemotherapy → Surgery → Evolution

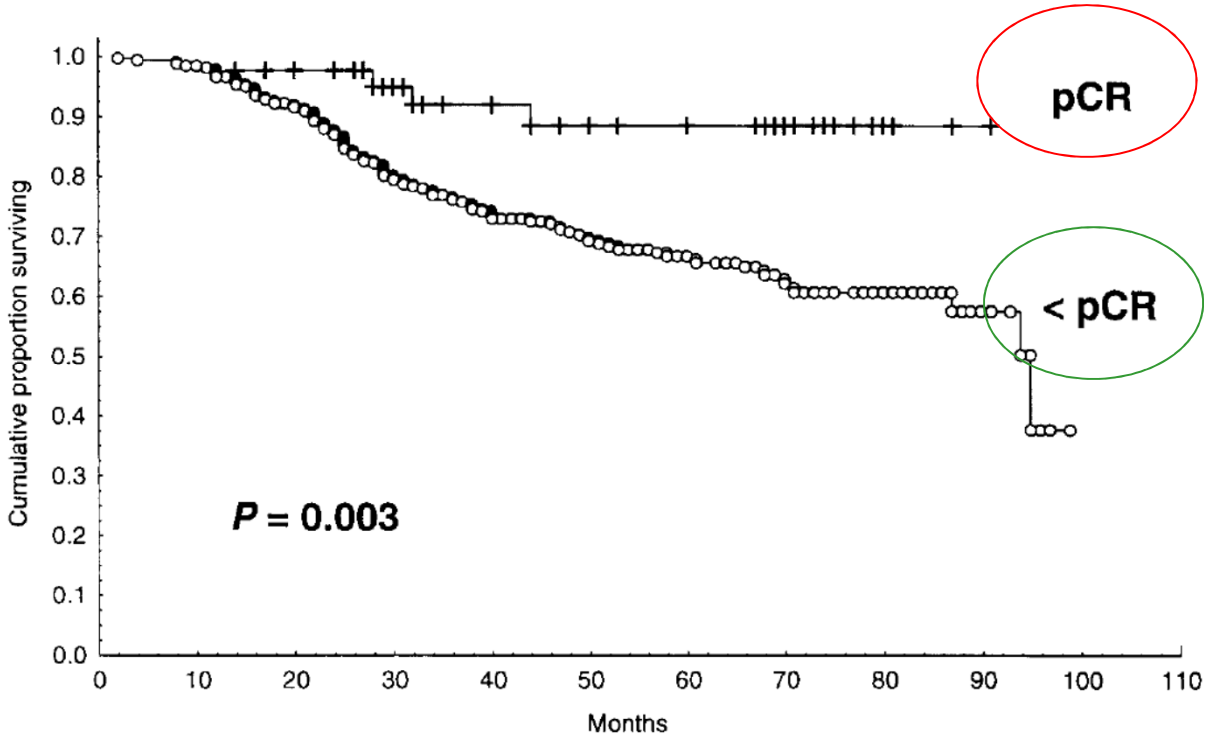
Samples | Samples | Samples

Tumor growth

Histological response

Surrogate markers of response to chemotherapy

Prognostic factor for patients treated by neoadjuvant chemotherapy



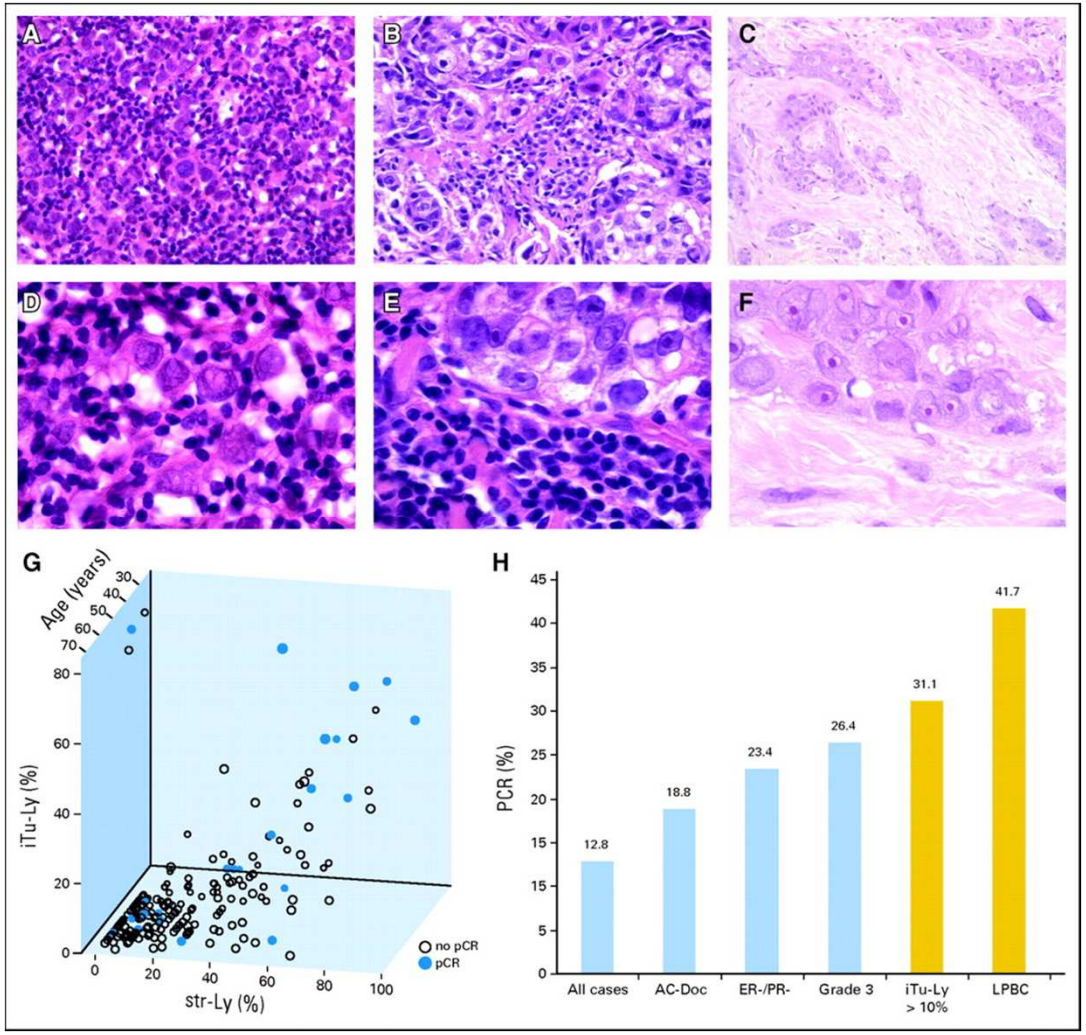
Relapse with Pcr

No prognostic factor in this group

Complete histological response is a good marker of chemosensitivity and survival

Kuerer et al. *J Clin Oncol* 1999

Potential association between leucocyte infiltrate before treatment and response.



Denkert C et al. JCO 2010;28:105-113

JOURNAL OF CLINICAL ONCOLOGY

Pathologic Complete Response to Neoadjuvant Chemotherapy of Breast Carcinoma Is Associated with the Disappearance of Tumor-Infiltrating Foxp3$^+$ Regulatory T Cells

Sylvain Ladoire,[1,2] Laurent Arnould,[1] Lionel Apetoh,[3] Bruno Coudert,[1] Francois Martin,[2] Bruno Chauffert,[1,2] Pierre Fumoleau,[1] and François Ghiringhelli[1,2,3]

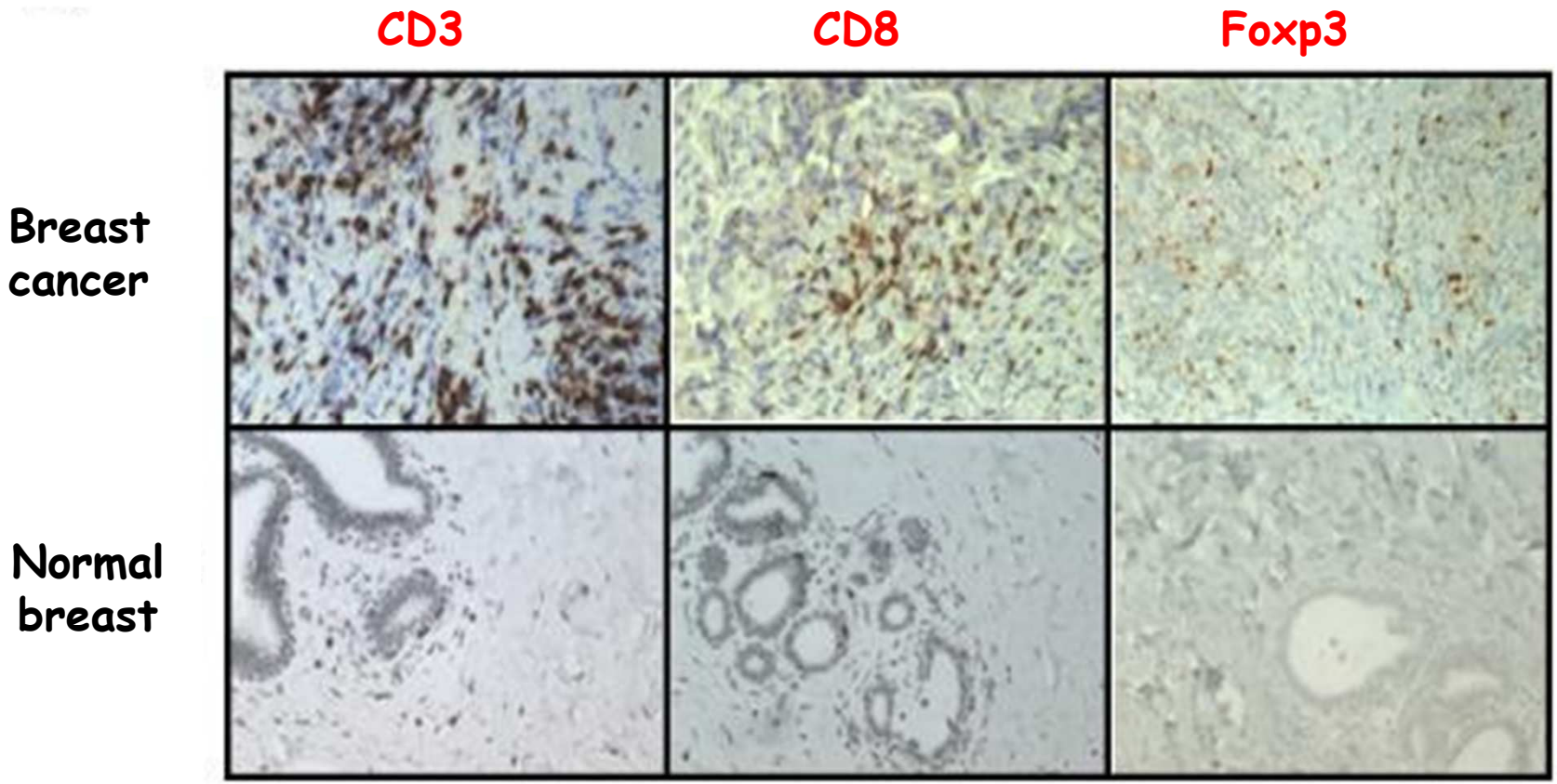
Clin Cancer Res 2008;14:2413-2420

Patients

Total	56
Age	
mean (±SD)	49.9±8
median (range)	51.5 (30-67)
Tumor size	
mean(±SD)	4.4±1,7
median (range)	4 (1-8)
T stage	
T1	1
T2	33
T3	17
T4	5
N stage	
N0	17
N1	31
N2	6
N3	2

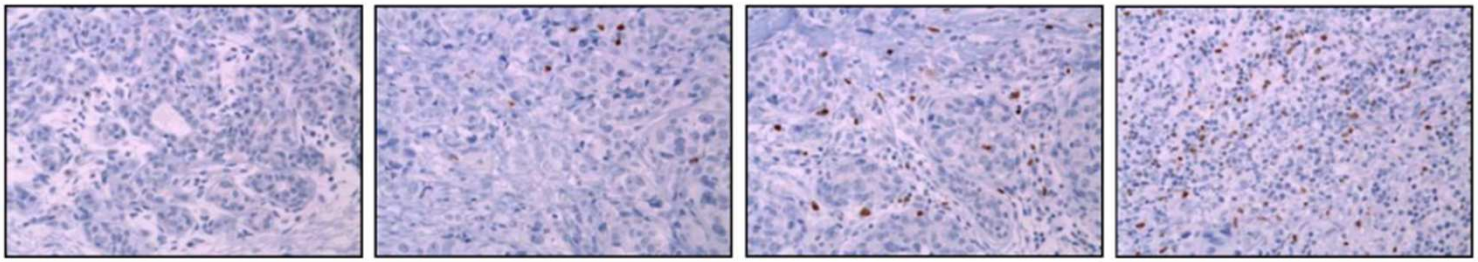
SBR	
I	4
II	33
III	19
HER2 +++	21
oestrogen	
Positifs	37
Negatifs	19
progesteron	
Positifs	27
Negatifs	29
chemotherapy	
Anthracyclines	26
Anthracyclines + taxane	11
Trastuzumab + taxane	20
Response	
PCR	12
No PCR	44

Breast cancer are invaded by leucocytes



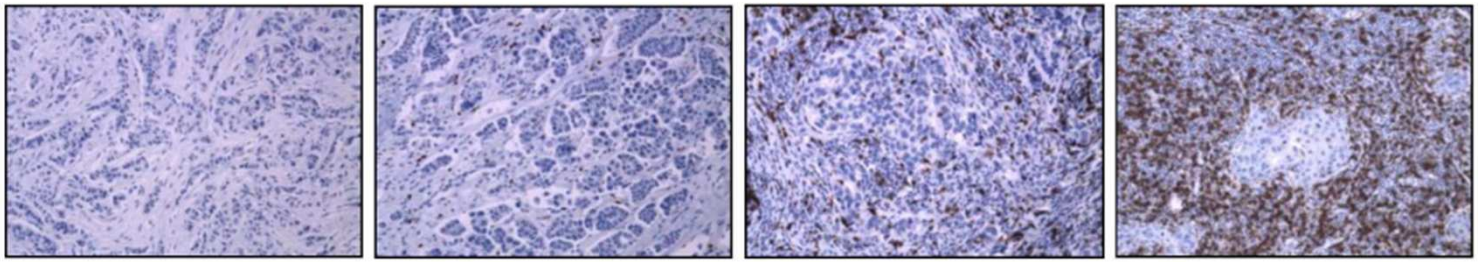
Semi-quantitative evaluation of CD8+ and Foxp3+infiltrates

Foxp3 infiltrates



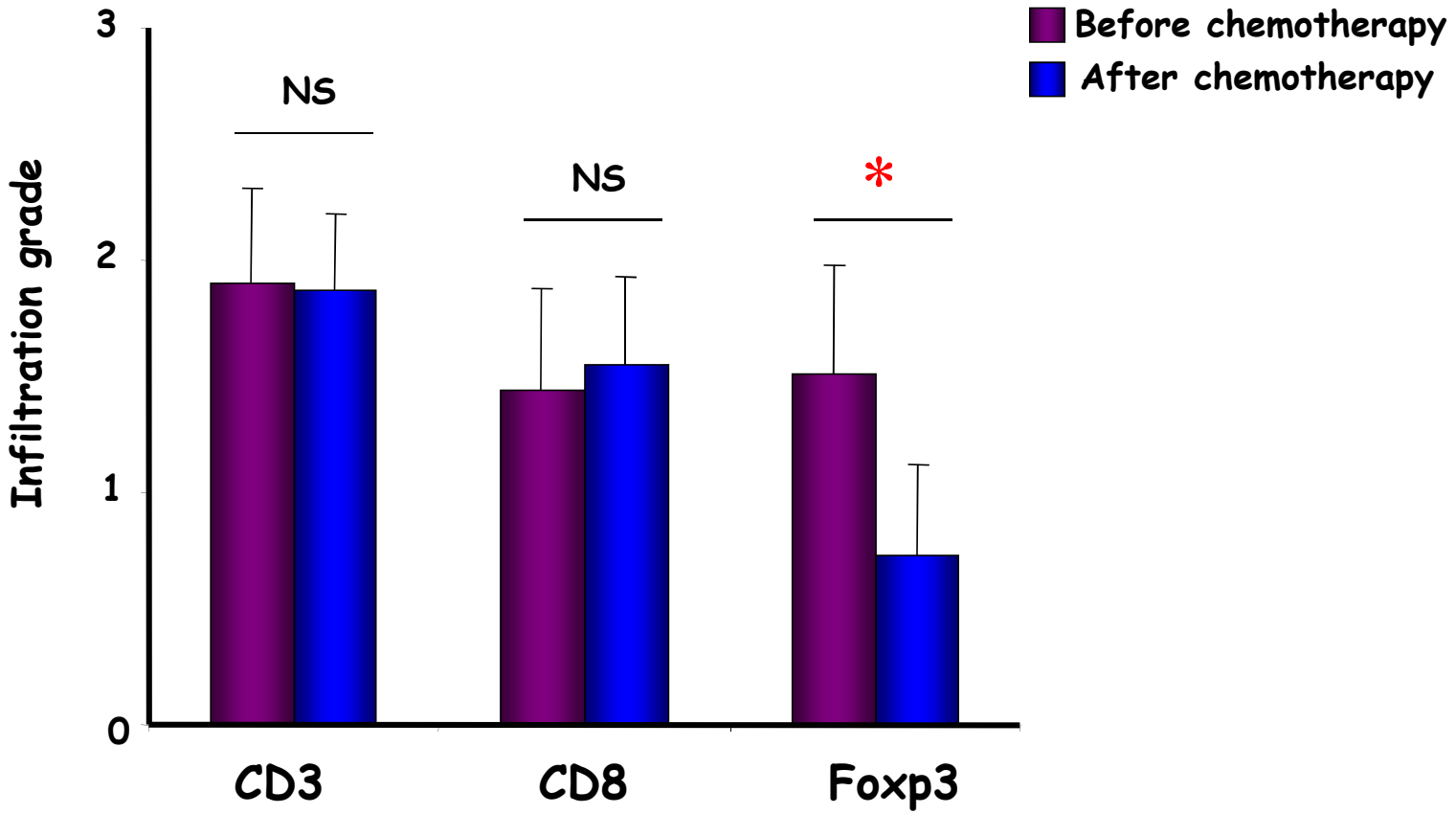
Grade 0 Grade 1 Grade 2 Grade 3

CD8 infiltrates



Grade 0 Grade 1 Grade 2 Grade 3

Evolution of CD8+ and Foxp3+ infiltrates



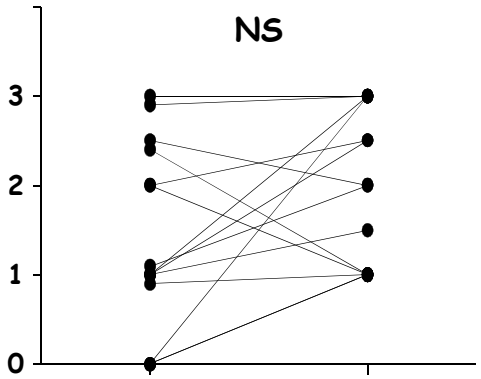
* p = 0,01

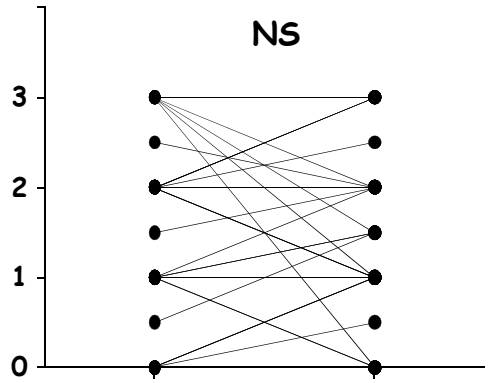
Evolution of lymphocytes infiltrates and tumor response

PCR

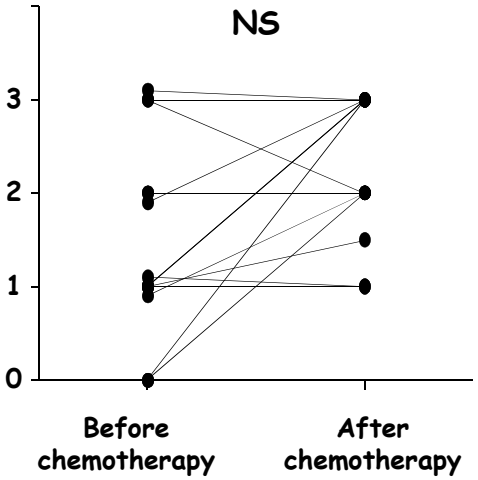
Non PCR

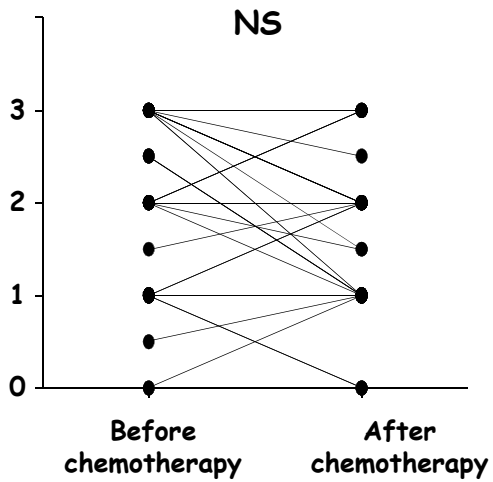
CD8





CD3

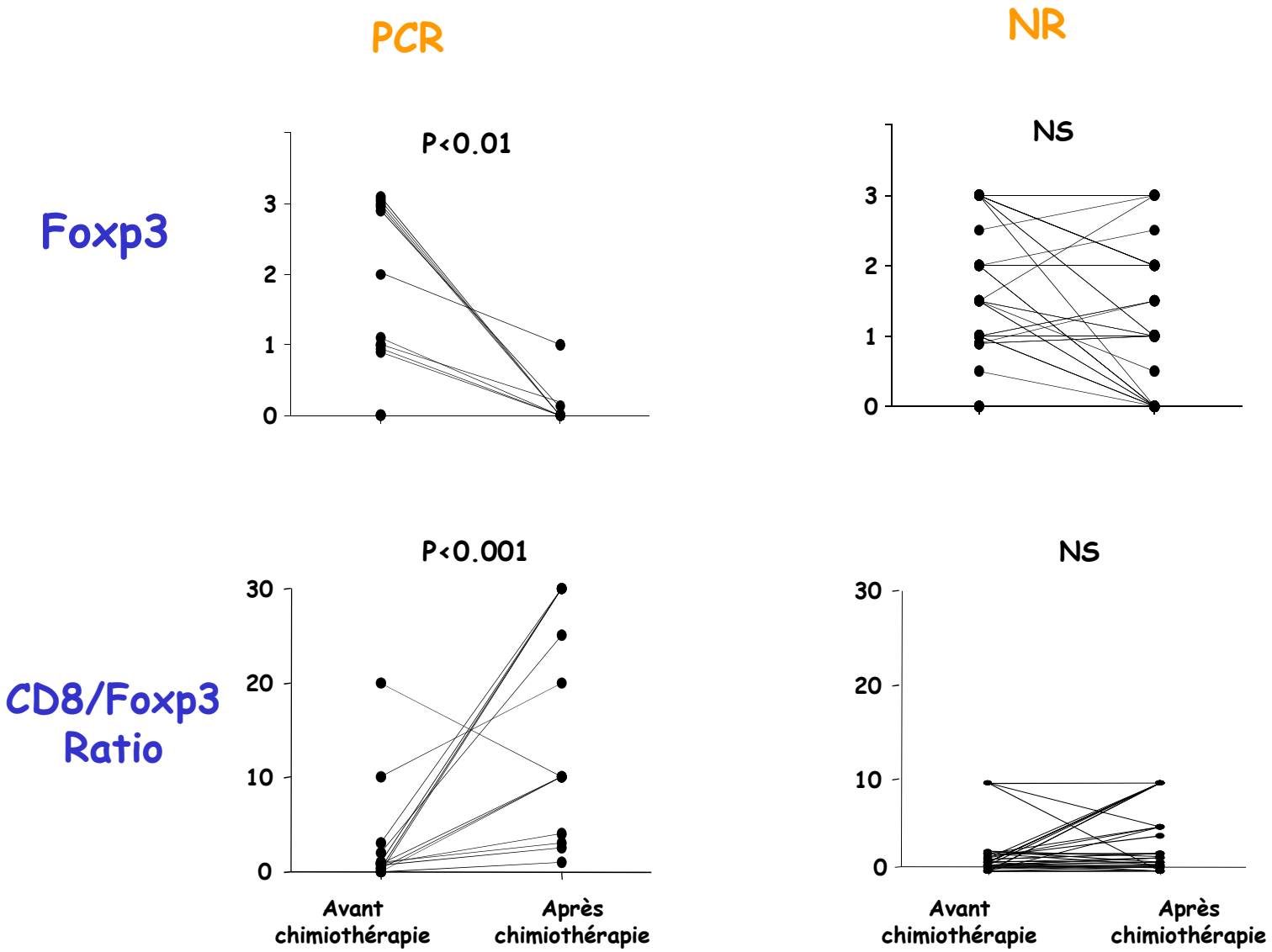




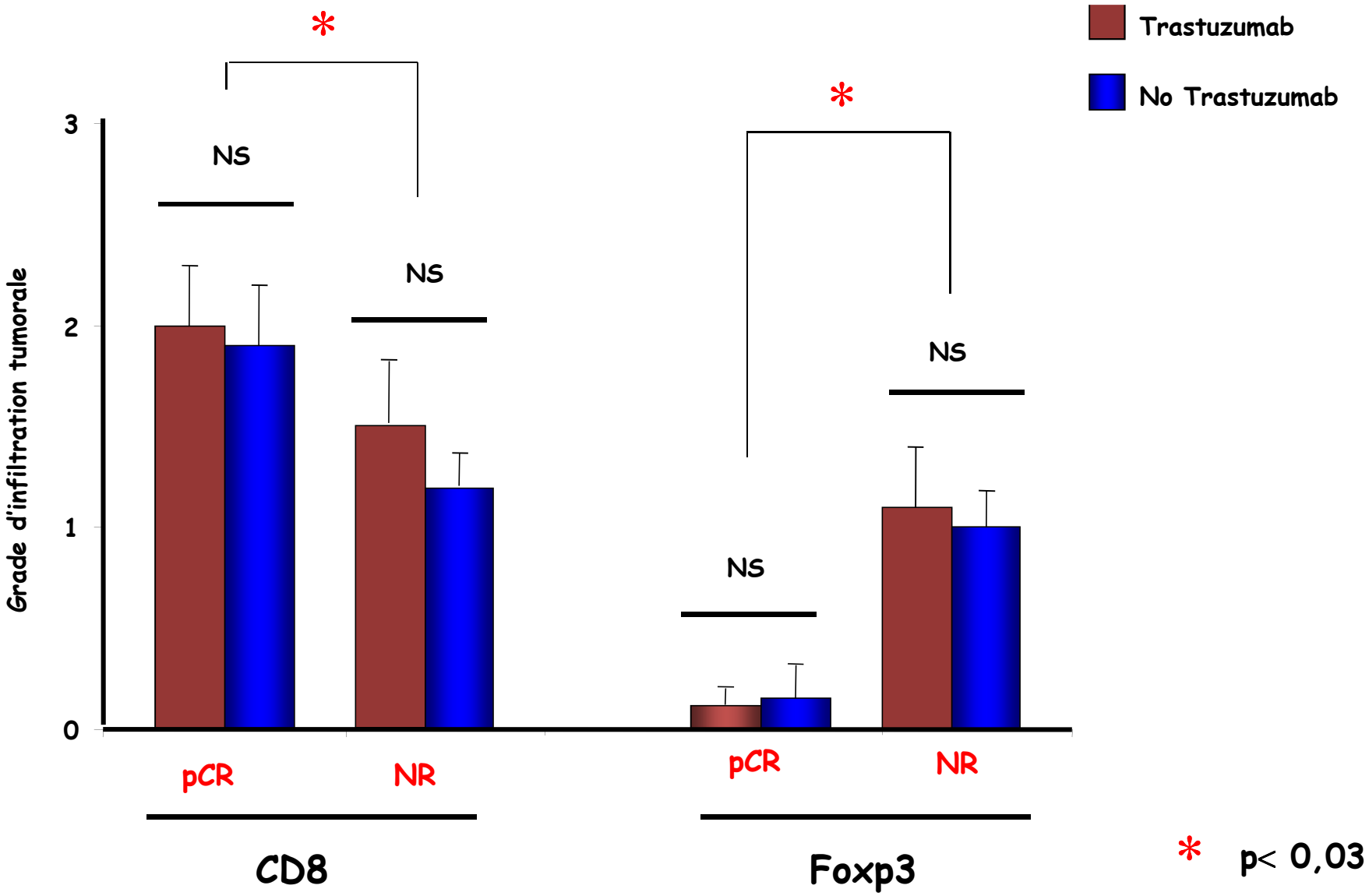
* $p < 0,05$

RCH vs NR

Evolution of lymphocyte infiltrate and clinical response



Does Trastuzumab influence lymphocytes infiltrates ?



* p< 0,03

Factors associated with PCR

Classical factors

	variable	OR (95% IC)	p
T	I+II vs III + IV	2.3 (0.5-8.2)	0.3
N	0 vs +	1.2 (0.3-4.4)	0.9
SBR	I+ II vs III	6.2 (1.6-23.2)	**0.01**
ER	0 vs +	0.66 (0.17-2.5)	0.56
PR	0 vs +	7.2 (0.8-31)	0.07
HER	0 vs +	8 (2-32)	**0.007**
Chemotherapy	Anthracycline Anthracycline + taxane		**0.001**
	Trastuzumab + taxane		

Factors associated with PCR

Immune factors

Comparison of lymphocytes infiltrates between PCR and Non PCR						
population	Before chemotherapy			After Chemotherapy		
	PCR	NR	p	PCR	NR	p
CD3	1.7± 1.1	1.9± 1	0.603	2.3± 0.7	1.8± 0.8	0.072
CD8	2± 0.7	1,4± 1	**0.037**	2± 0.8	1.4± 0.9	**0.026**
Foxp3	1.6± 1.4	1.5± 1	0.783	0.08± 0.2	0.90± 1	**0.003**

Foxp3 0 + CD8 ≥ 2

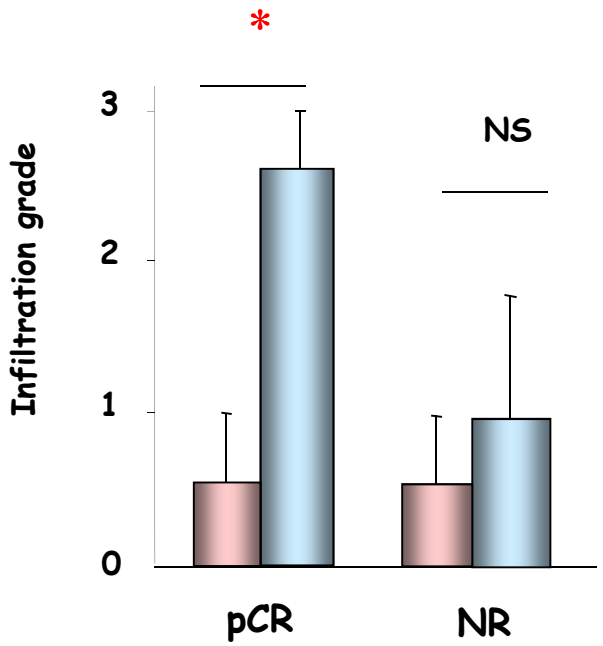
PCR: Se 75%

Spe 93%

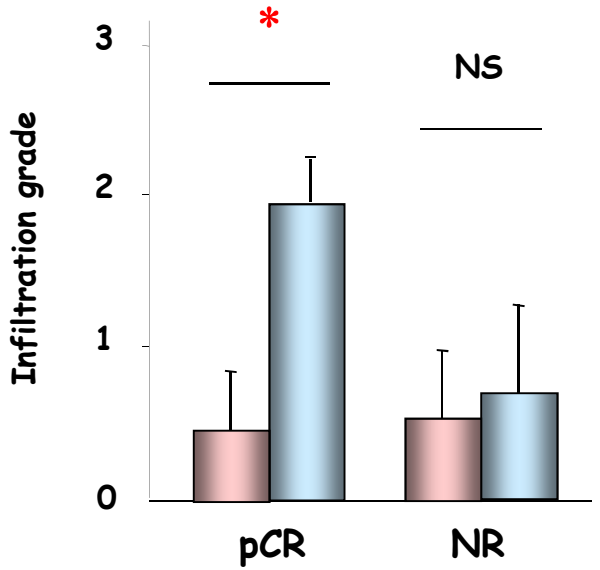
Multivariate analysis for PCR			
	variable	OR (95% IC)	p
Tumor grade	I+ II vs III	19.9 (1.3 to 315.1)	**0.03**
Trastuzumab	0 vs +	18.9 (1.2 to 308,7)	**0.04**
Combined immune factor	Foxp3 0 + CD8≥2 vs Foxp3>1 ou CD8<1	99.9 (5.8 to 1727,9)	**0.0015**

Evaluation of cytotoxic response

TiA1



Granzyme B



Before chemotherapy

After chemotherapy

* $p < 0,05$

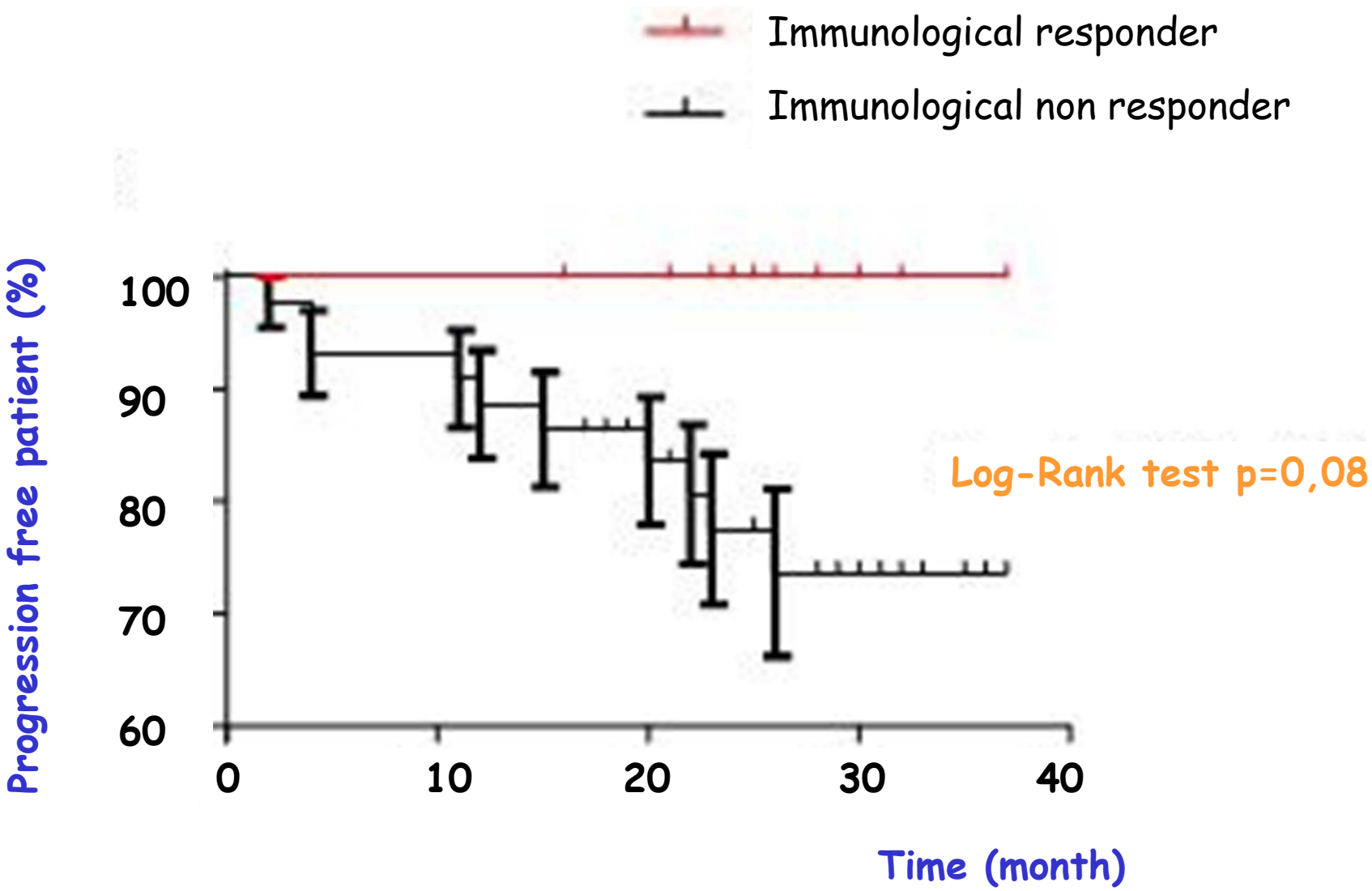
Conclusion

. A High CD8/Foxp3 ratio after chemotherapy is associated with PCR

. Only Foxp3 subpopulation seems modify by chemotherapy

. Capacity to dichotomise PCR and none PCR is independent of chemotherapy regimen

Progression free survival and immune response



Does the prognostic role of this immunological score could be validated in an independent cohort?

ORIGINAL PAPER

In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival

Sylvain Ladoire,[1,2†] Grégoire Mignot,[2†] Sandrine Dabakuyo,[3] Laurent Arnould,[4] Lionel Apetoh,[2] Cedric Rébé,[2] Bruno Coudert,[1] Francois Martin,[2] Marie Hélène Bizollon,[5] André Vanoli,[6] Charles Coutant,[7] Pierre Fumoleau,[1] Franck Bonnetain[3] and François Ghiringhelli[1,2*]

[1] *Department of Medical Oncology, Georges François Leclerc Center, Dijon, France*
[2] *Institut National de la Santé et de la Recherche Médicale, Avenir Team INSERM, CRI-866 University of Burgundy, Dijon, France*
[3] *Biostatistics and Epidemiological Unit, EA 4184, Georges François Leclerc Centre, Dijon, France*
[4] *Department of Pathology and Biology of Tumours, Georges François Leclerc Centre, Dijon, France*
[5] *Department of Pathology, Chalons sur Saone, France*
[6] *Department of Medical Oncology, Sainte Marie Private Hospital, Chalon sur Saone, France*
[7] *Department of Gynaecology and Obstetrics, Hôpital Tenon, Assistance Publique des Hôpitaux de Paris Paris, France*

Journal of Pathology
J Pathol (2011)

HER2 overexpression patients

111 patients treated by neoadjuvant chemotherapyy for HER2+++ breast cancer

Chemo with or without trastuzumab

Study of immune infiltrats CD8/Foxp3 and histological response (pAJCC)

Relapse free survival (RFS) and overall survival (OS)

Patient and tumor characteristics (n=111)

	N	%
Age		
<50	61	55
≥50	50	45
AJCC initial stage		
0/I/IIA	23	21
IIB/IIIA/IIIB	88	79
Axillary nodal status		
Positive	81	73
Negative	30	27
Tumor grade		
I+II	60	54
III	51	46
Estrogen receptor		
Positive	53	48
Negative	58	52
Chemotherapy		
Trastuzumab-Docetaxel	63	57
Anthracycline	48	43
Pathological complete response		
Yes	33	30
No	78	70
AJCC pathological stage		
0/I/IIA	65	58
IIB/IIIA	46	42
CD8 infiltration		
high	91	82
low	20	18
Foxp3 infiltration		
high	43	39
low	68	61
CD8/Foxp3 ratio		
favorable	55	49
unfavorable	56	51

Prognostic factor associated to RFS

Multivariate analysis for factors associated with RFS (bootstrap 1000 replications)

	Multivariate HR	95%CI	p	Bootstrapping 95%CI and p value	
AJCC pathological stage					
0/I/IIA	1				
IIB/IIIA	2.56	[1.20-5.48]	**0.016**	[1.14-5.72]	0.016
CD8/Foxp3 ratio after CT					
favorable	1				
unfavorable	3.35	[1.54-7.27]	**0.002**	[1.30-8.62]	0.002
Chemotherapy					
Trastuzumab-Docetaxel	1				
Anthracycline	1.45	[0.71-2.95]	**0.313**	[0.71-2.94]	0.313

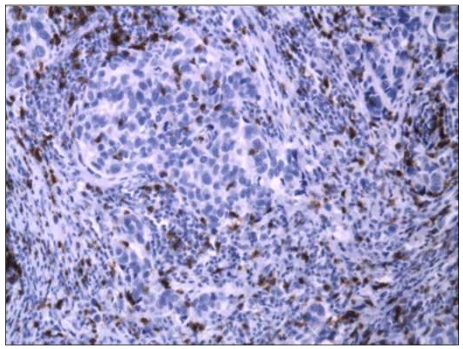
Prognostic factors associated with OS

Multivariate analysis for factors associated with OS (bootstrap 1000 replications)

	Multivariate HR	95%CI	p	Bootstrapping 95%CI and p value	
AJCC pathological stage					
0/I/IIA	1				
IIB/IIIA	3.15	[1.14-8.70]	**0.027**	[0.005-2136]	0.730
CD8/Foxp3 ratio after CT					
favorable	1				
unfavorable	2.97	[1.19-7.43]	**0.020**	[0.21-41.37]	0.417
Chemotherapy					
Trastuzumab-Docetaxel	1				
Anthracycline	7.74	[1.74-34.33]	**0.007**	[5^{E}-10-1.20^{E}11]	0.864

Pathologic-Immunologic (*PathIm*) Score

Tumor and nodal categories	Stage
T0N0 (including residual ductal carcinoma in situ)	0
T1N0	I
T0-1N1; T2N0	IIA
T2N1; T3N0	IIB
T0-3N2; T3N1	IIIA
Any T4	IIIB
Any N3	IIIC

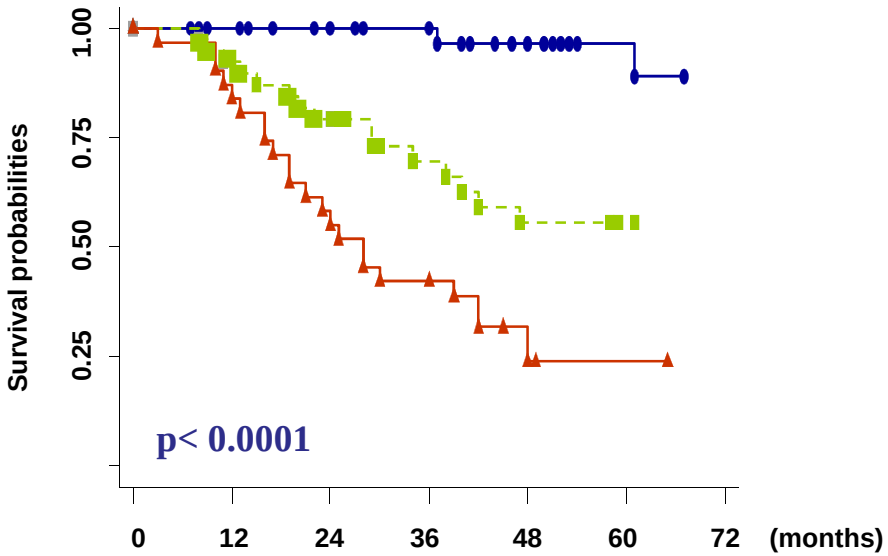


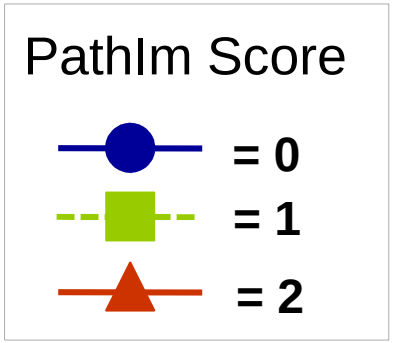
AJCC pathologic stage (0: favourable; 1: unfavourable)	**CD8/Foxp3 infiltrates** (0: favourable; 1: unfavourable)	**PathIm score**
0 (≤ IIA)	**0** (CD8 high + Foxp3 low)	**0**
1 (>IIA)	**0** (CD8 high + Foxp3 low)	**1**
0 (≤ IIA)	**1** (CD8 low and/or Foxp3 high)	**1**
1 (>IIA)	**1** (CD8 low and/or Foxp3 high)	**2**

Pathologic-Immunologic score (PathIm score)

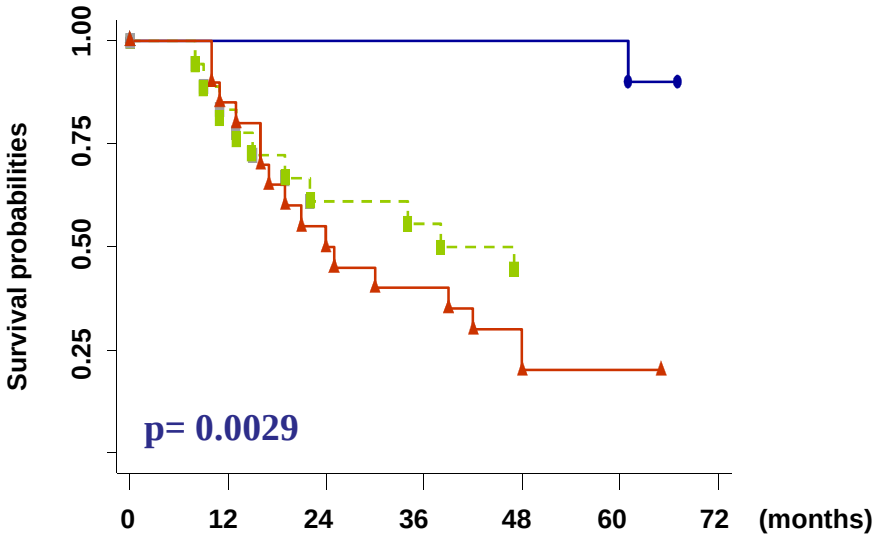
RFS

All Patients

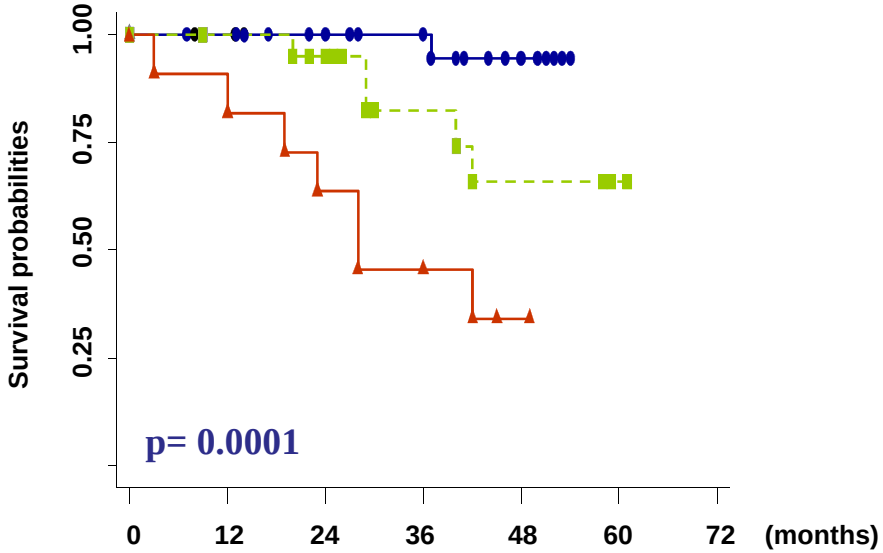




Anthracyclines

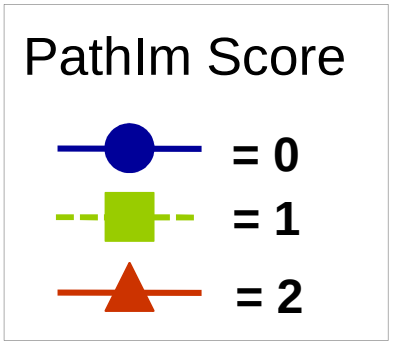


Trastuzumab-Taxanes

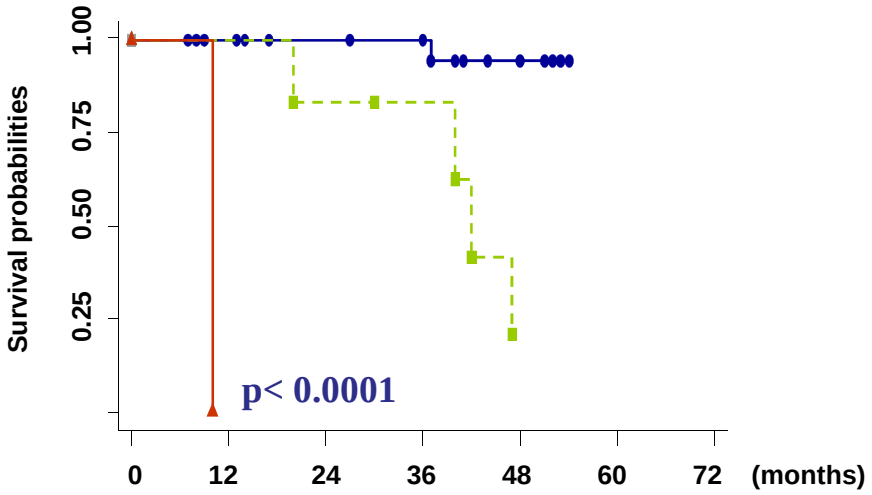


Ladoire et al. SABCS 2010 Abstr 07-05

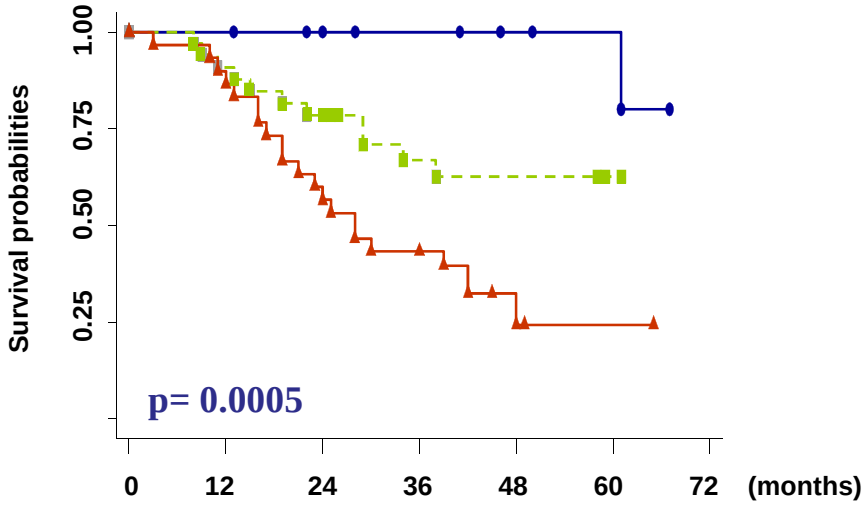
RFS



Pcr patients



Non Pcr patients



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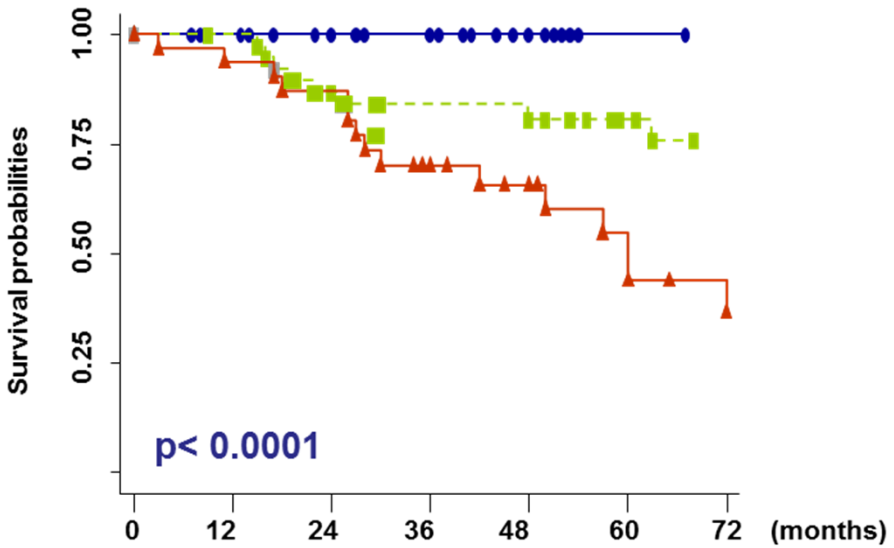
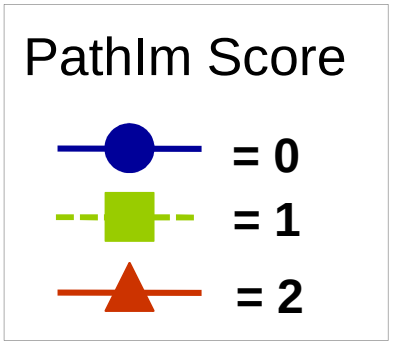
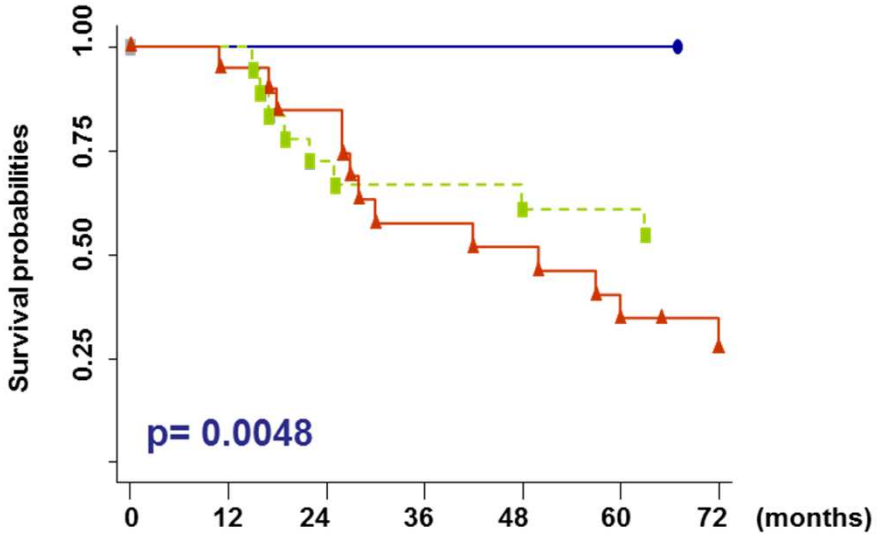
OS

All patients

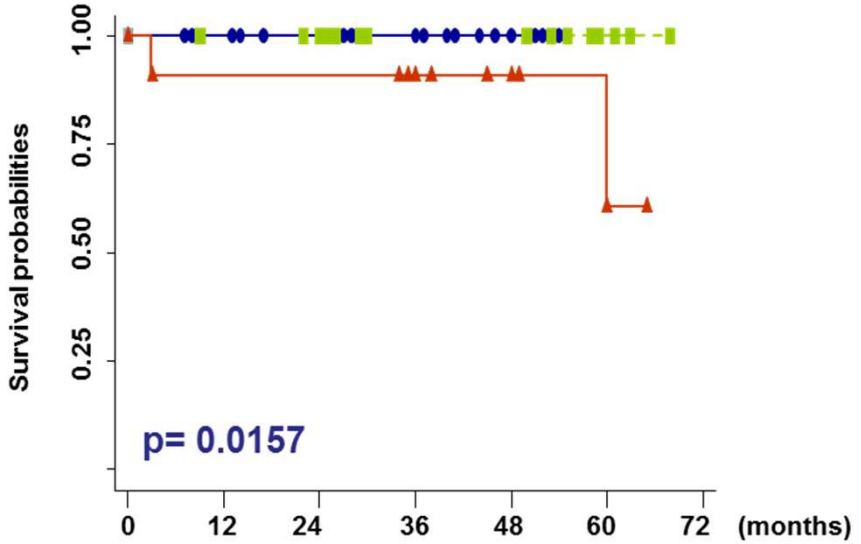




Anthracyclines

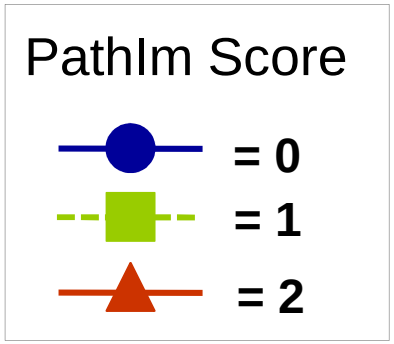


Trastuzumab-Taxanes

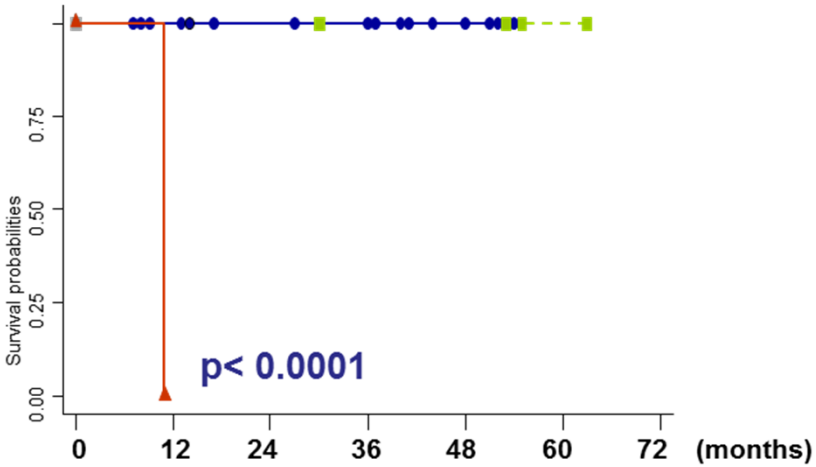


Ladoire et al. SABCS 2010 Abstr PD 07-05

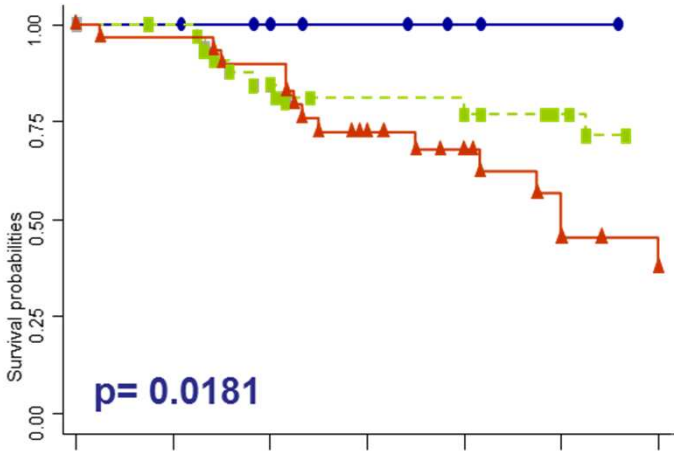
OS



Patients with PCR



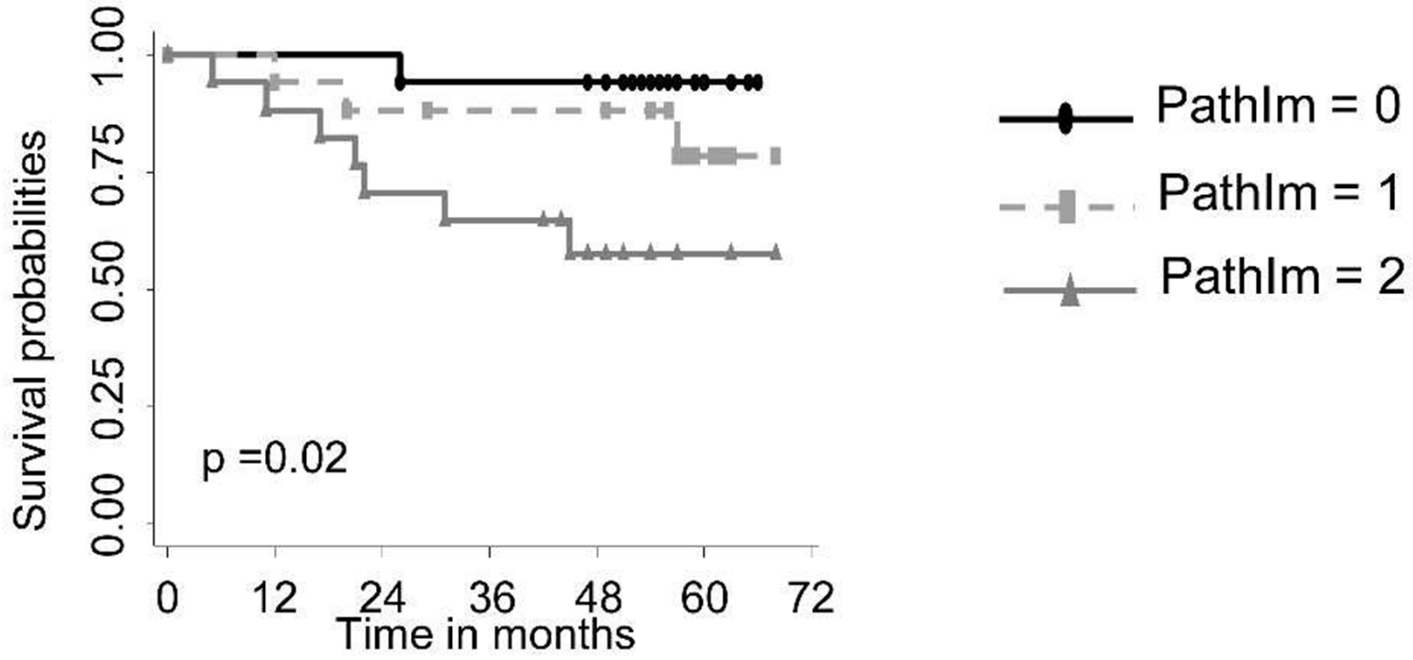
Patients without PCR



Ladoire et al. SABCS 2010 Abstr PD 07-05

An effect dependent of molecular subtype ?

External validation cohort in HER2 - patients



Conclusion:

The CD8 "high" and Foxp3 "low" score is a predictive factor of relapse free survival in patients treated with neoadjuvant chemotherapy

This effect is observed in patients treated with traztuzumab or anthracycline

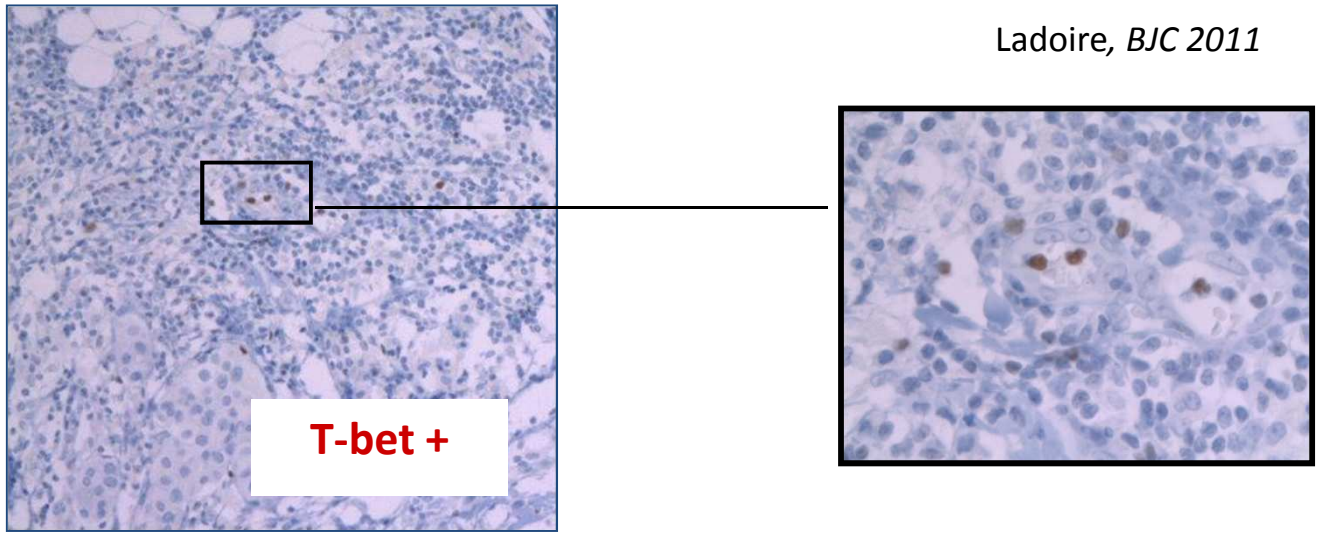
The Pathimm score using immunological and pathological assessment determine prognostic either in HER+ and – tumors

This score must be validated in a prospective cohort

PRIMUNEO (PHRC 2011)

The role of Th1 cells

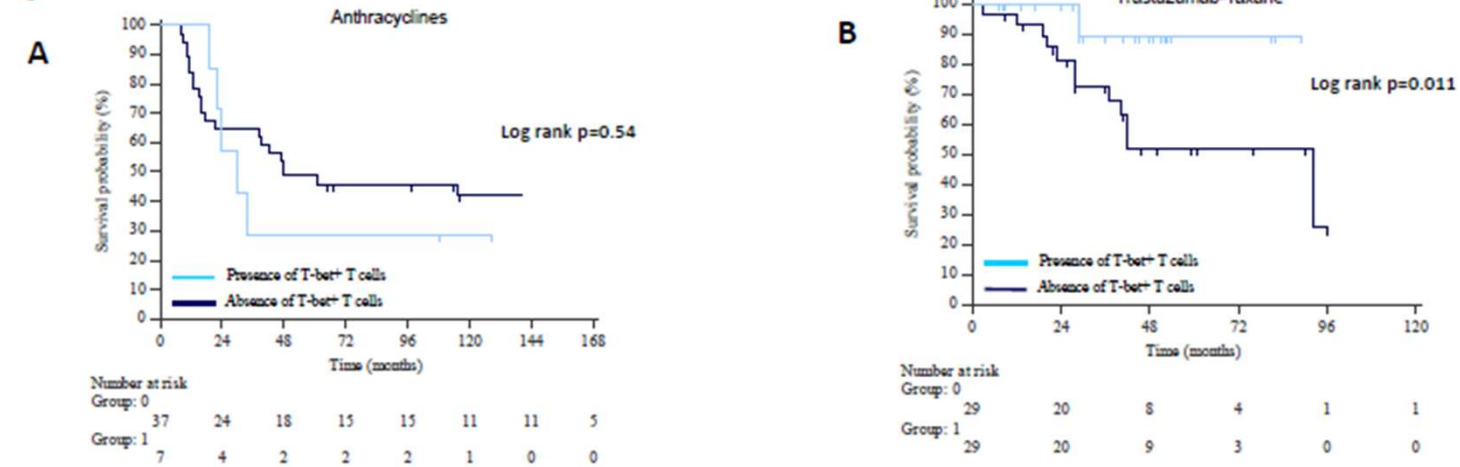
. **Interaction between immune system and chemotherapy the role of CD4 T cells?**

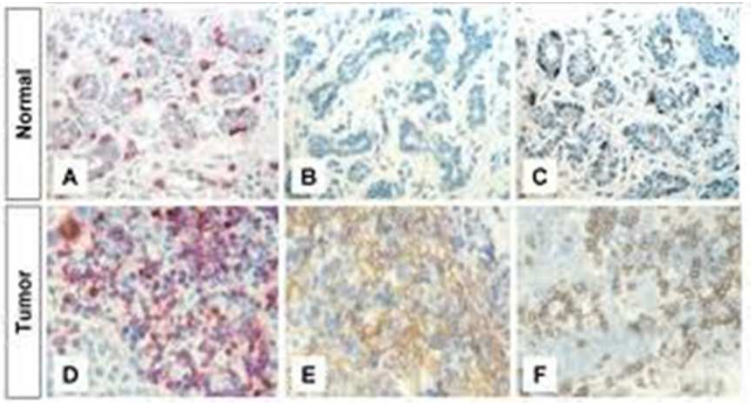


Ladoire, *BJC 2011*

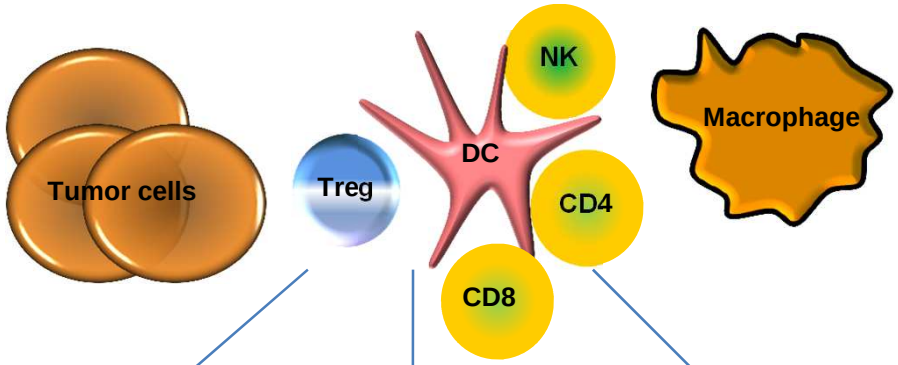
. **Which chemotherapy for which immunological effect**

Figure 2:





Chemotherapy



Immune system

Treg depletion

CD8 recruitment and activation

CD4 polarization

Modification of cancer prognosis

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