

I have no relationships to disclose

Wolf Herman Fridman



INSERM U872
Cordeliers Research Centre
UPMC-P6 and Université Paris Descartes
Paris, France



HEGP “Georges Pompidou
European Hospital”
Paris, France

Contrasted prognostic impact of tumor infiltration by various subsets of immune cells

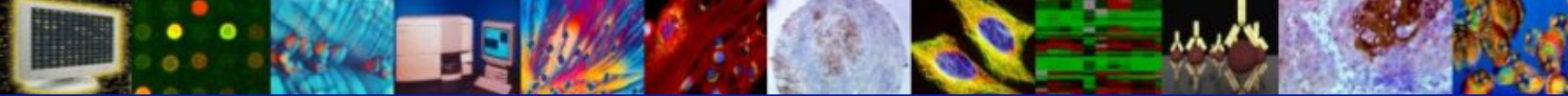
Wolf Herman Fridman



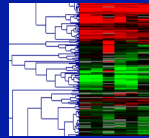
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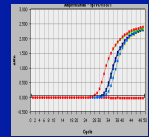
HEGP “Georges Pompidou
European Hospital”
Paris, France



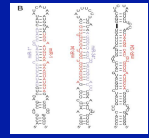
Integrative Cancer Immunology approach



DNA MicroArrays (n=54000)



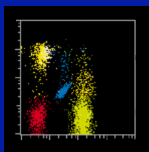
Low Density Arrays (LDA) (n=384)



microRNA expression (n=384)



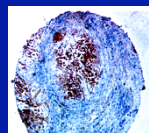
Seldi-ToF
Antibody arrays (proteomic)



FACS Phenotype Data (n=820)



FACS Functional Data (n=980)



Tissue MicroArrays (TMA)
of Colorectal Tumors (n=750)

Database



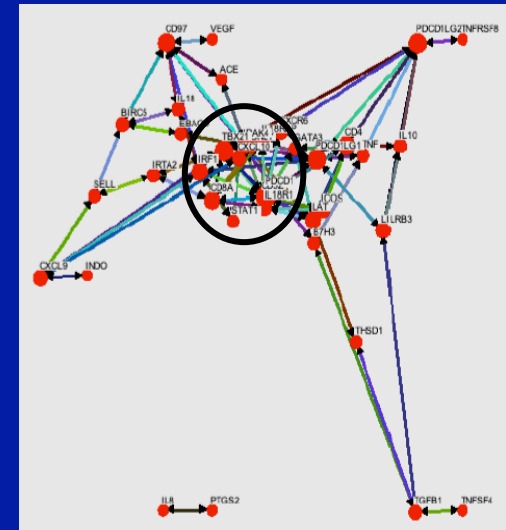
1012 CRC



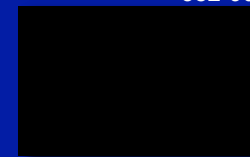
> 1 000 000 parameters

CNIL
agreement

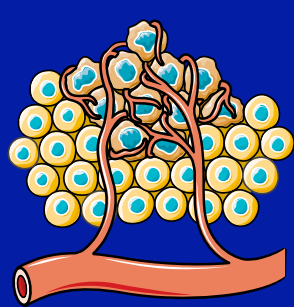
Bioinformatics programs
Statistic and Networks



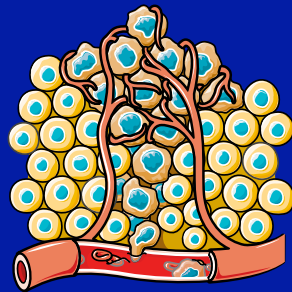
**ARACNe (algorithm for the
reconstruction
of accurate cellular networks)**
Basso K, et al. Nat Genet. 2005 (4):
382-90.



The process of metastasis

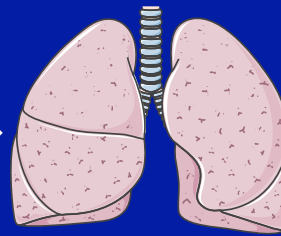


Primary tumor

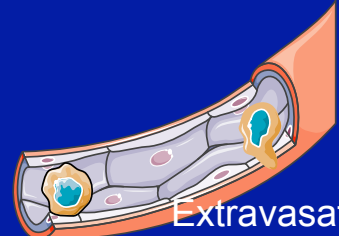


Detachment/invasion

Transport

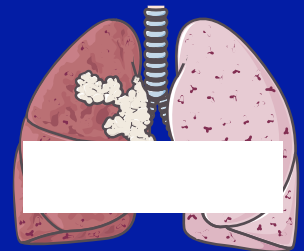


Arrest in organs



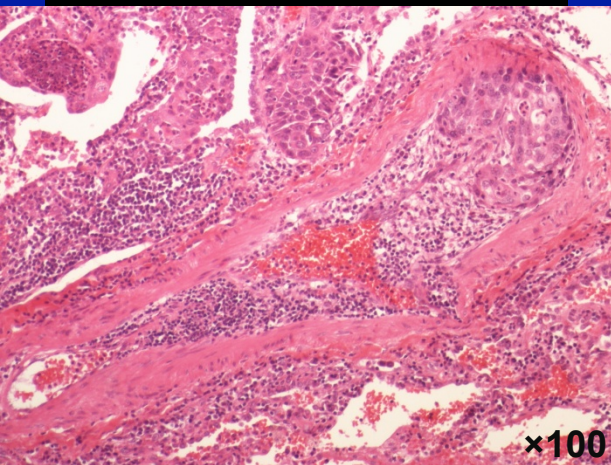
Adherence

Extravasation

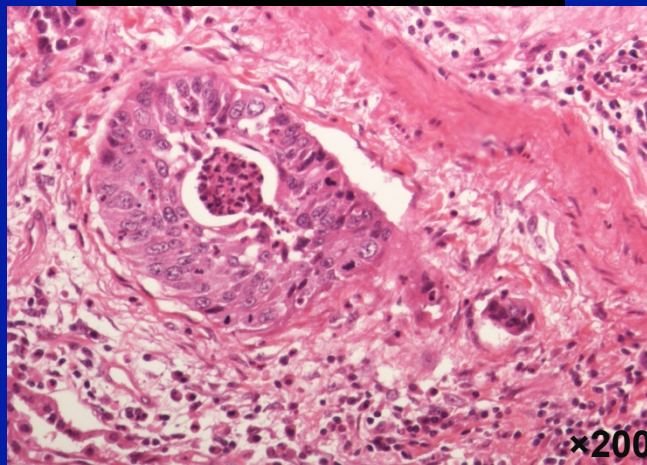


Establishment of a microenvironment

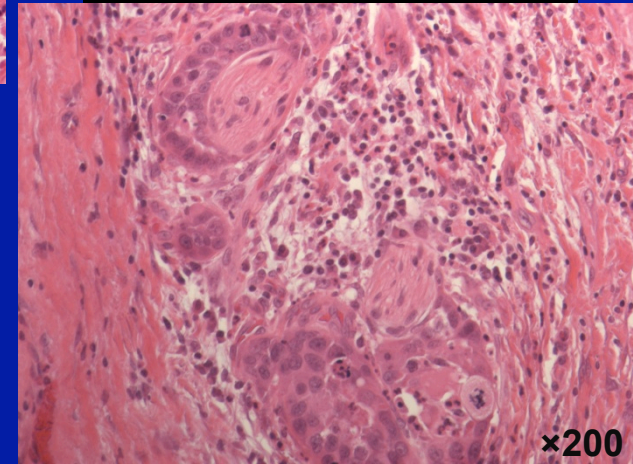
Blood stream tumor emboli



Lymphatic tumor emboli

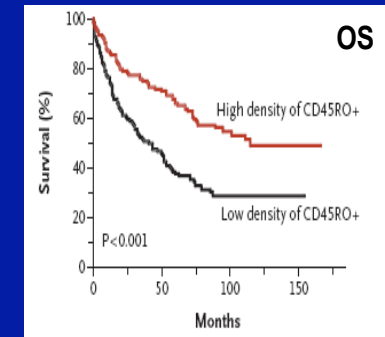
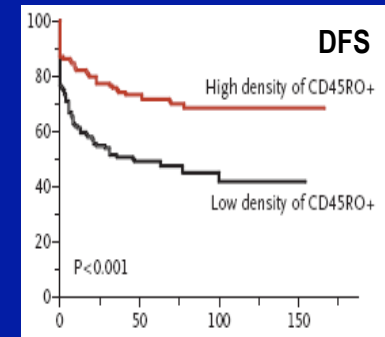
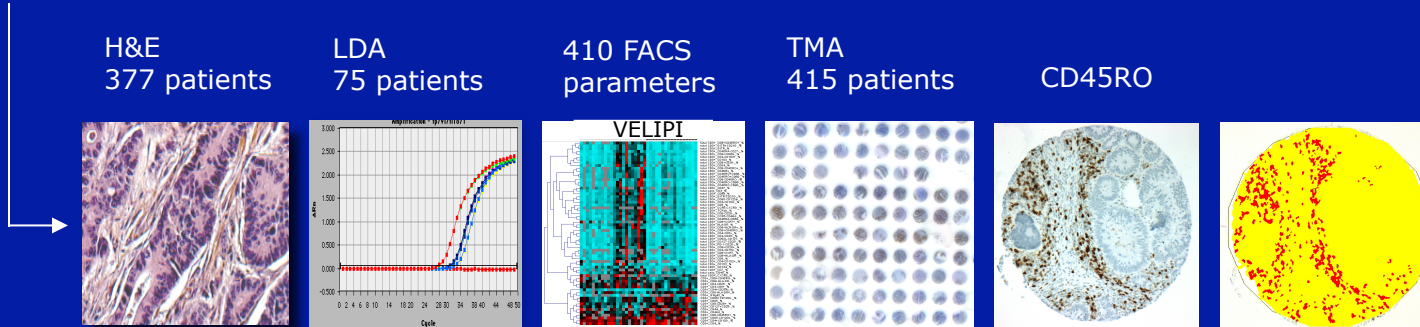


Peri-neural tumor emboli

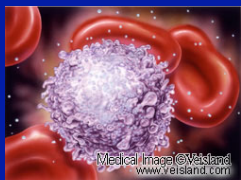


-> Global analysis of tumor microenvironment

VELIPI is prognostic
959 colorectal cancer patients



A high density of **Memory T cells** (in particular, T_{EM}) in colorectal carcinoma correlates with the absence of metastatic spread, and an improved clinical outcome



Memory T cells found in the tumor
are maintained in the body for long periods

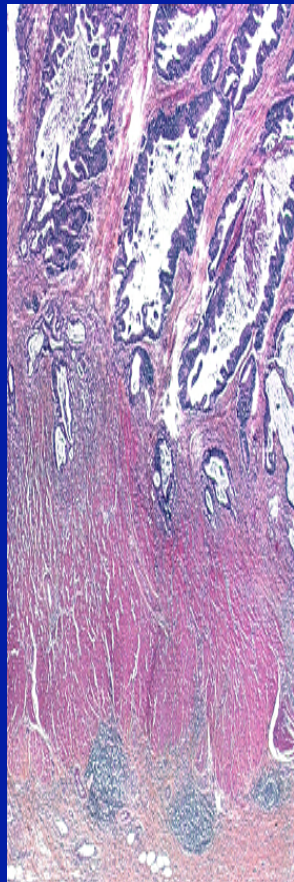
- > could control the tumor cells that disseminate
- > prevent relapse

Pagès F et al, New Engl J Med ,
353, 2654-66, 2005

Immune cells are present in the center of tumors, in their invasive margin and in adjacent lymphoid islets

(colorectal, lung, prostate, breast, kidney, bladder, ovarian, melanoma,.....)

ex : colorectal cancer

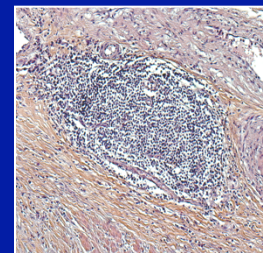
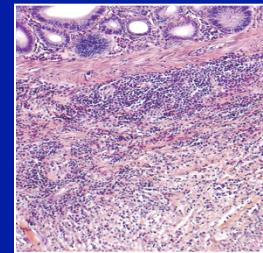
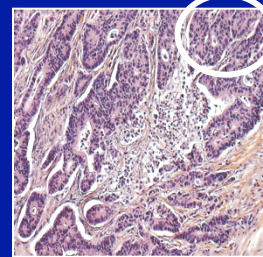


H&E sections

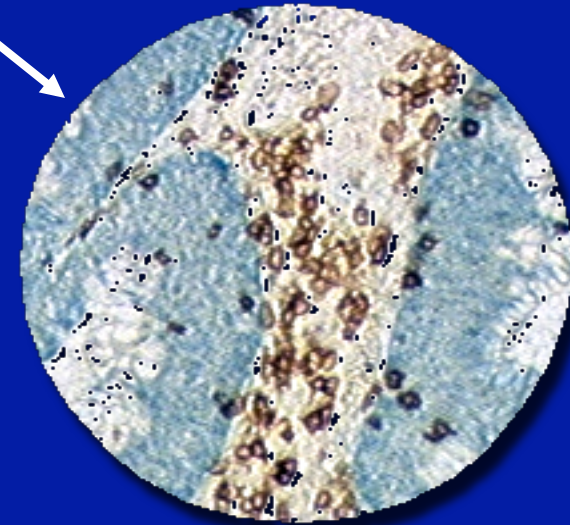
center

invasive margin

at distance



H&E sections

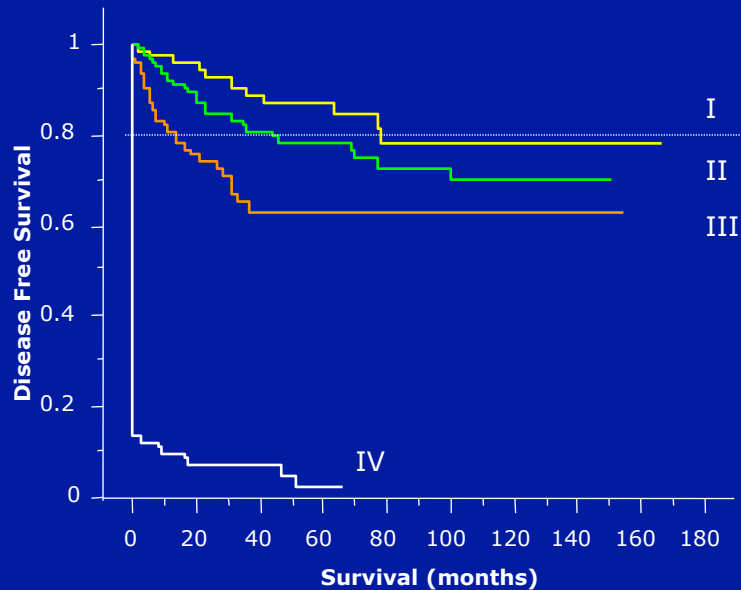


CD3 T cells (brown)
tumor cells (blue)

The adaptive immune response more than tumor stage predicts clinical outcome (959 patients)

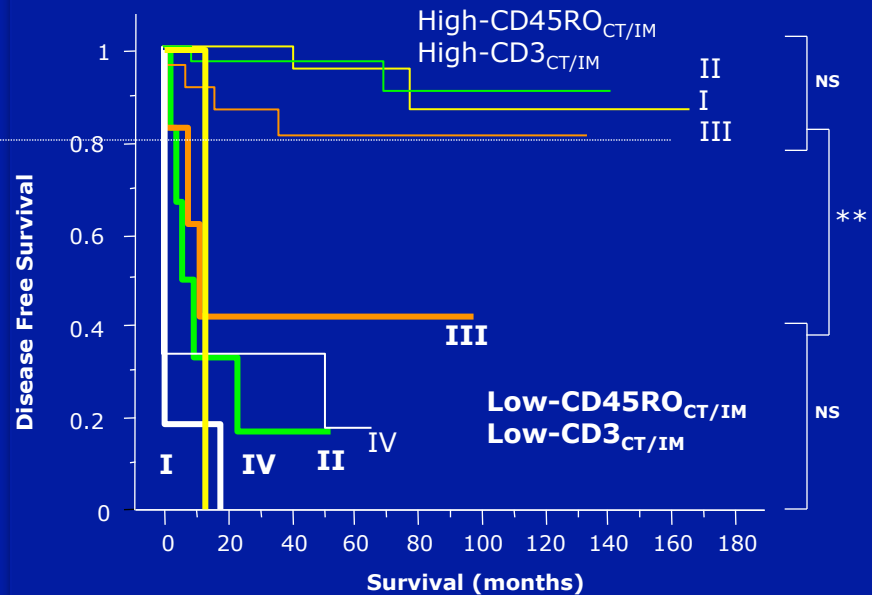
Tumor Histopathologic Findings

UICC-TNM, (Dukes' staging)
Current prognosis classification



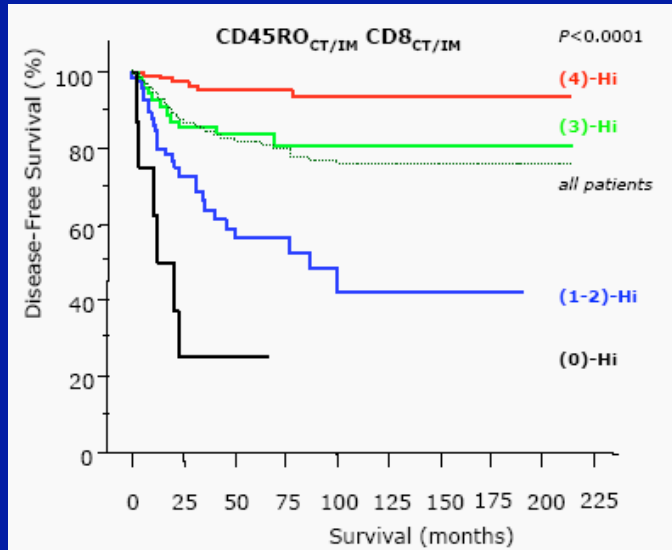
Immune cells analysis

CD3_{CT}/CD3_{IM} evaluation
plus
CD45RO_{CT}/CD45RO_{IM} evaluation



The local immune score identifies a population of high risk-patients with non-metastatic cancer (stage I-III)

stage I-II colorectal cancers (282 patients)



stage I-III colorectal cancers

COX analysis for DFS	HR	Log Rank P-values
Tumor (T) stage	1.24	0.29
N Stage	1.31	0.17
Gender	1.47	0.18
Number of total lymph nodes	1.13	0.68
Histological grade	0.69	0.29
Mucinous Colloid	1.29	0.47
Occlusion	1.03	0.94
Perforation	4.03	0.0084
Immune Score	0.65	0.0003

AJCC/UICC- TNM classification and the Immune score

COX analysis	DFS			OS			DSS		
	HR	P-value		HR	P-value		HR	P-value	
AJCC/UICC-TNM	1.38	0.09	ns	1.18	0.29	ns	1.43	0.10	ns
Immune Score	0.64	<0.0001		0.71	<0.0001		0.63	<0.0001	

-> Validation in 2 independent cohorts of colorectal cancer patients

(Pagès F et al, J Clin Oncol 2009; Mlecnik B et al, J Clin Oncol 2011)

Conclusions

- 1) The adaptive immune reaction at the tumor site plays a role in preventing tumor recurrence
- 2) The beneficial effect of the adaptive immunity may persist throughout tumor progression (stage II, III)
- 3) These data provide strong evidence of the importance of the natural anti-tumor adaptive immunity in human cancer
- 4) The local immune score identifies a population of patients with localized cancer (stage I- II) who deserve adjuvant therapy

Galon J et al., *Science*, 313(5795):1960-4, 2006

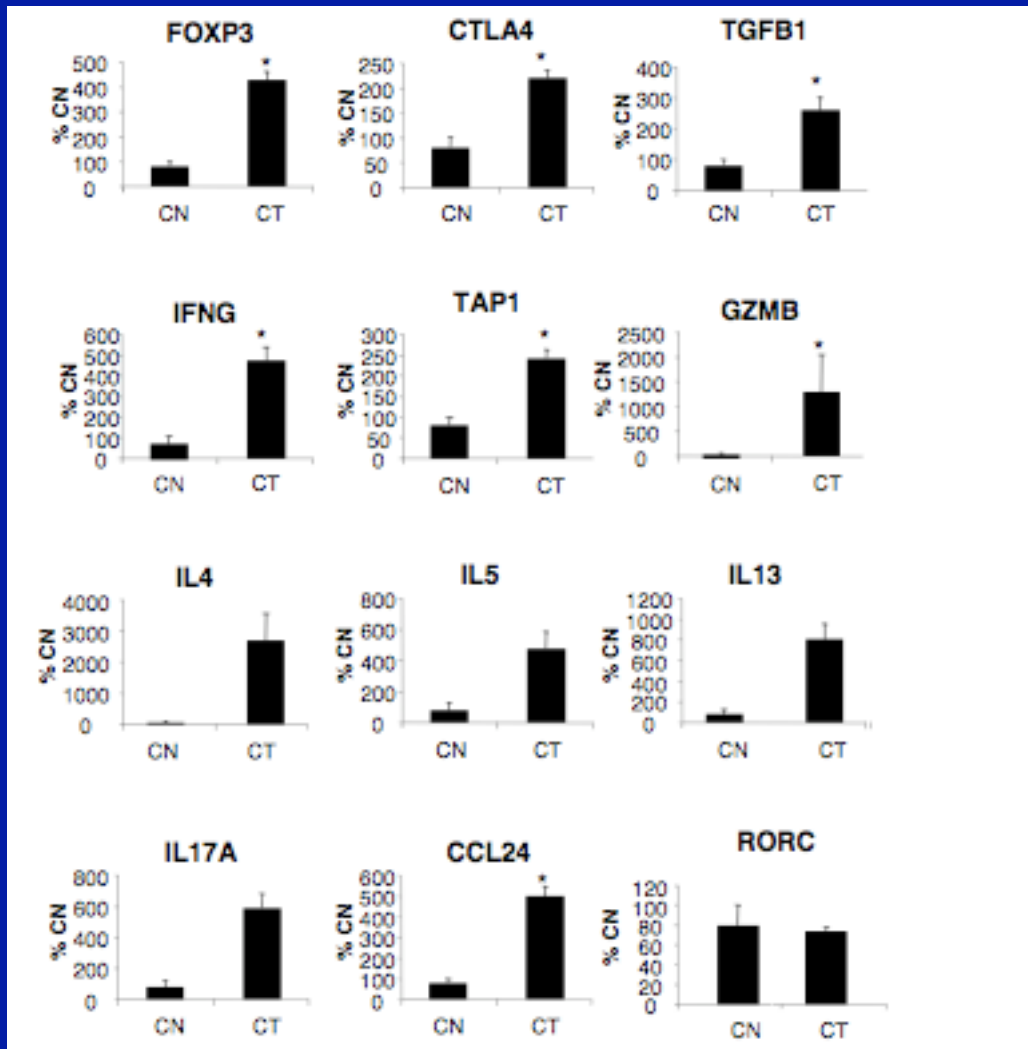
Pages et al., *J. Clin. Oncol.*, 27, 5944-51, 2009

B. Mlecnik and al, *J Clin Oncol.*, 29, 610-618, 2011

Can an immune gene expression
pattern predict disease control?

Genes overexpressed in the tumors

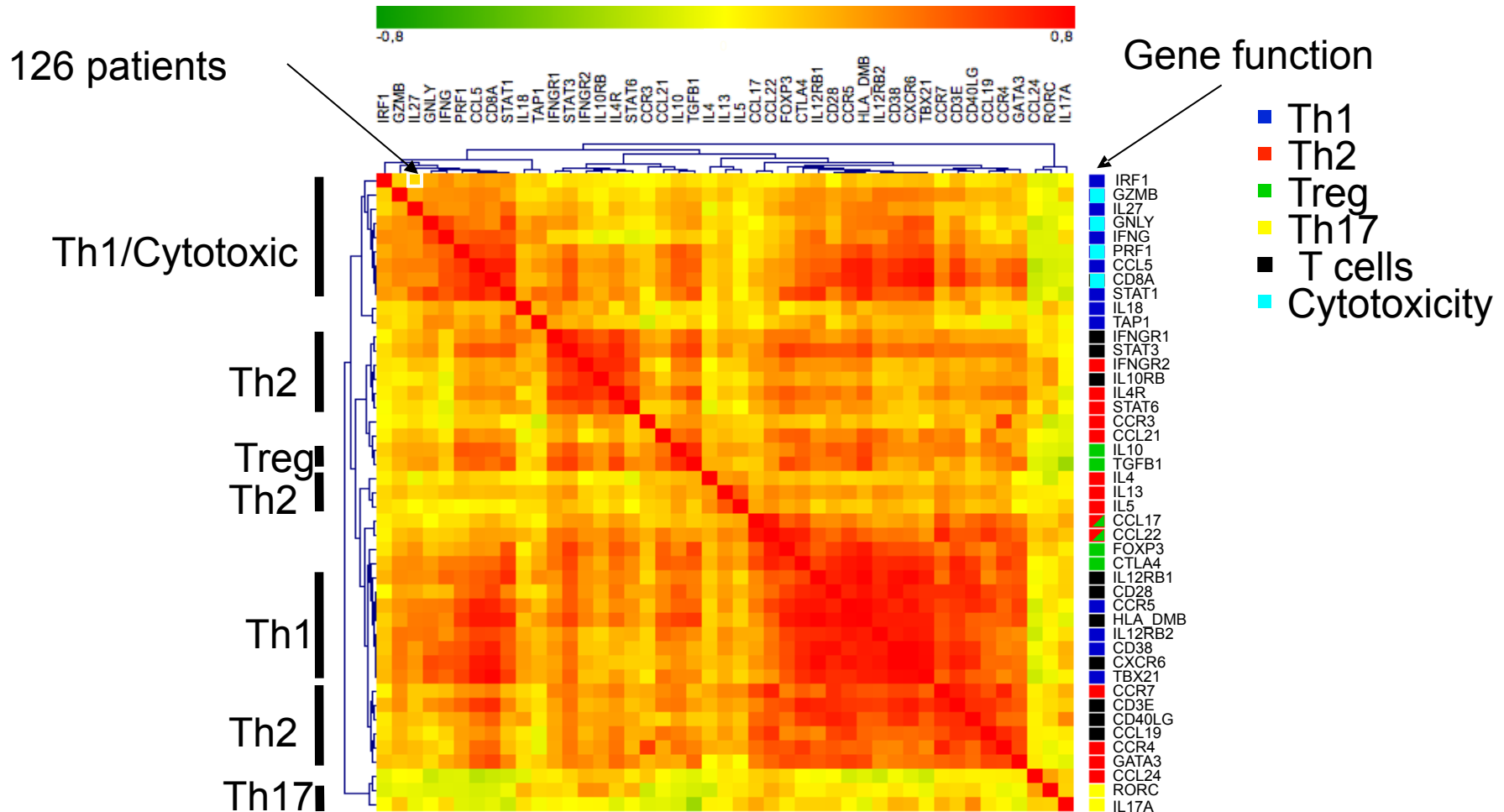
Immunosuppression



Inflammation

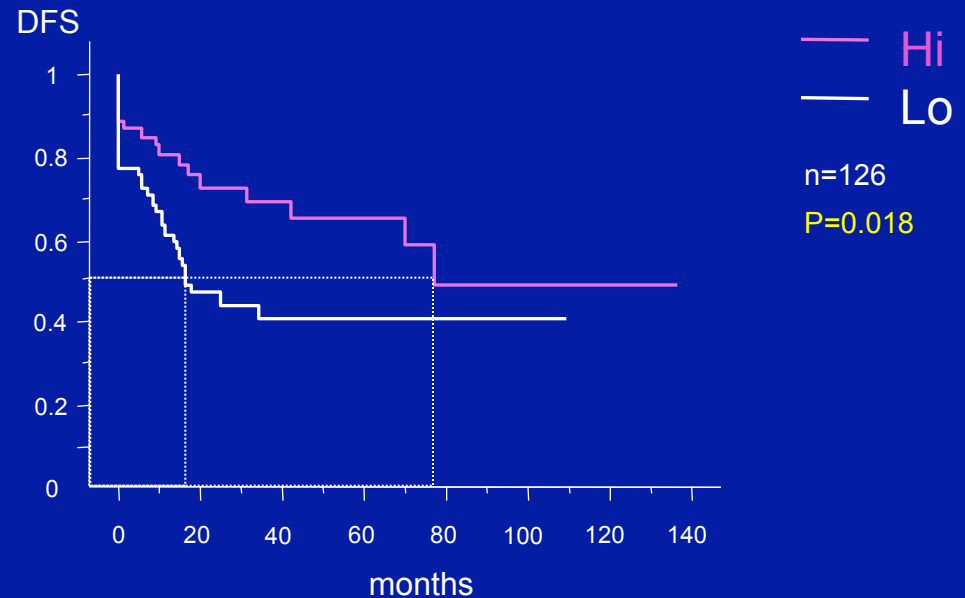
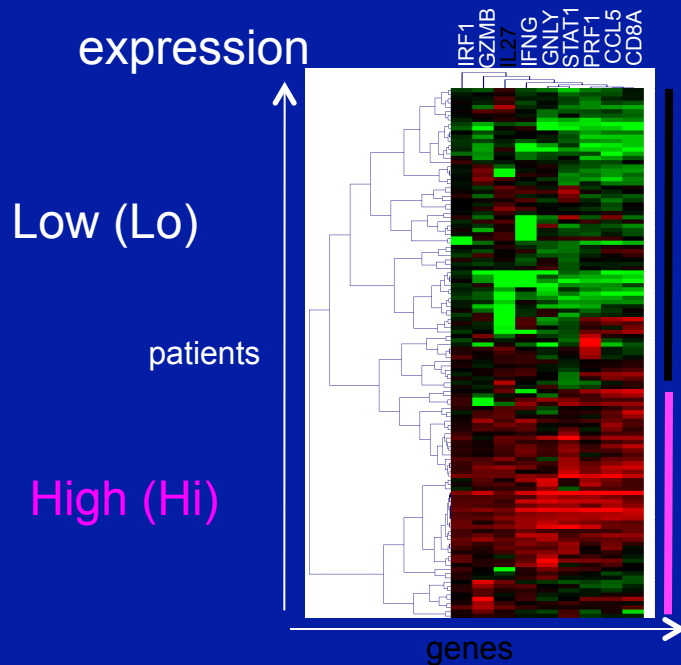
mRNA correlation matrix

M. Tosolini et al., Cancer Res. , 714, 1263-71, 2011



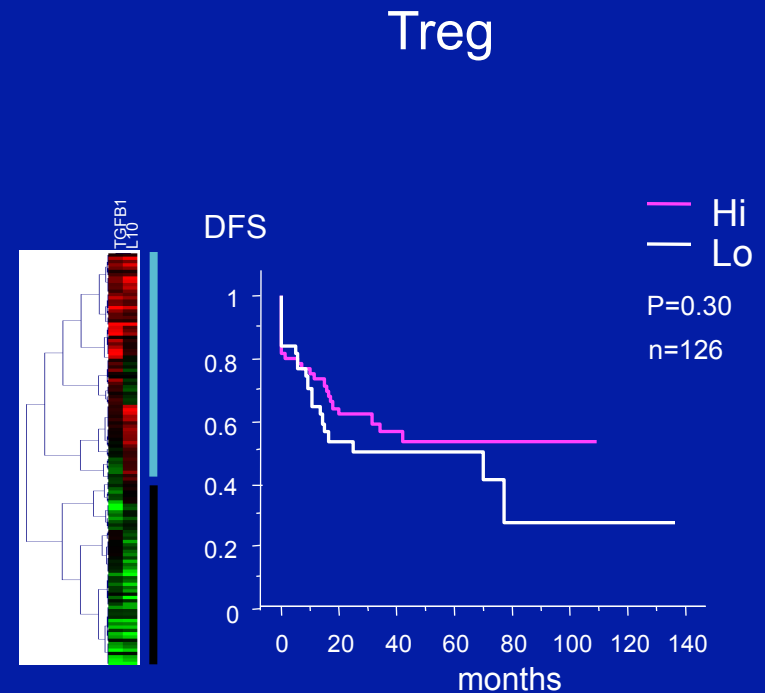
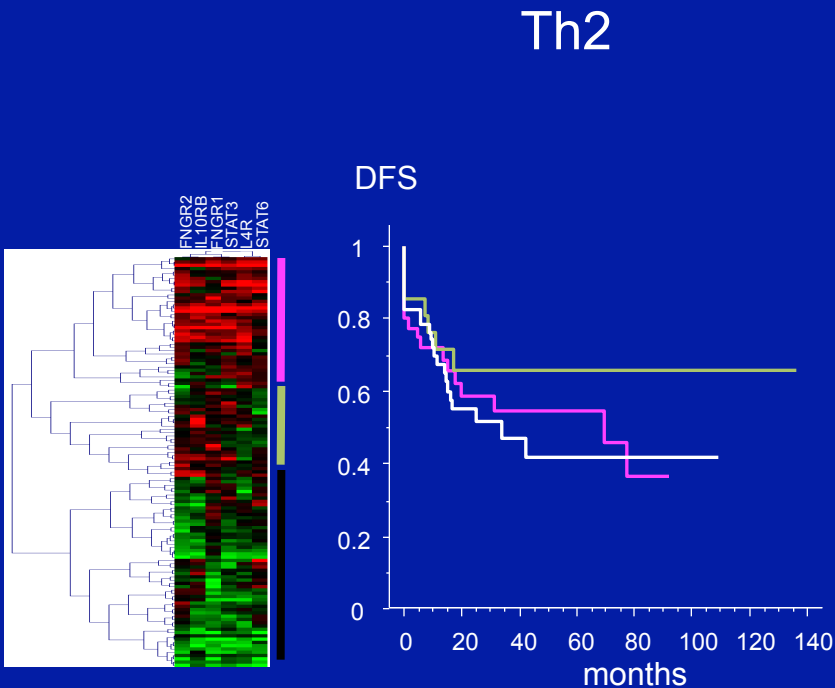
Th1/cytotoxic cluster

Th1



Patients presenting with high expression of Th1/cytotoxic genes in their primary tumor have a better disease free survival (M. Tosolini et al, Cancer Research, 71, 1263-71, 2011)

Th2 and T reg clusters



No association of Th2 and Treg clusters with clinical outcome
(M. Tosolini et al, Cancer Research,71, 1263-71, 2011)

Th17 cluster

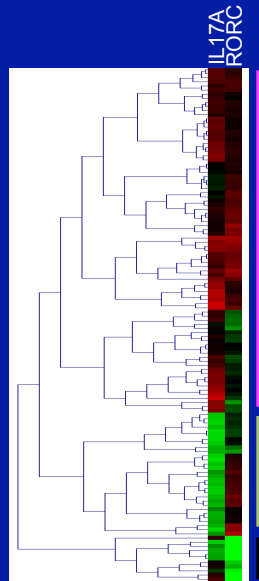
Th17

expression

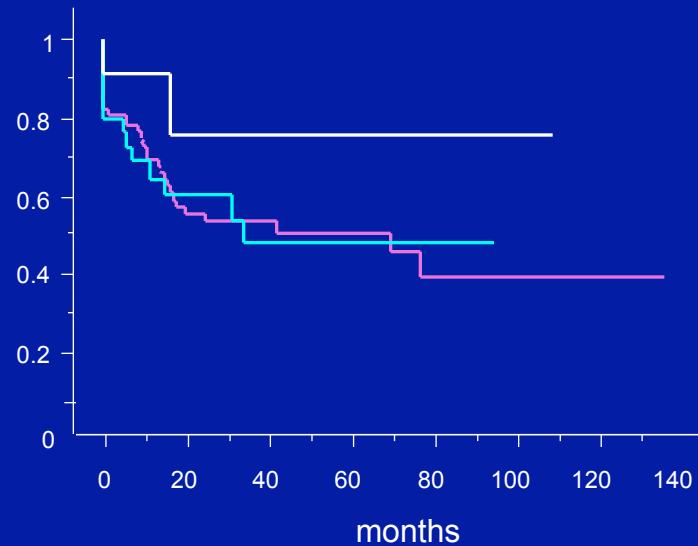
High (Hi)

Heterogeneous (het)

Low (Lo)

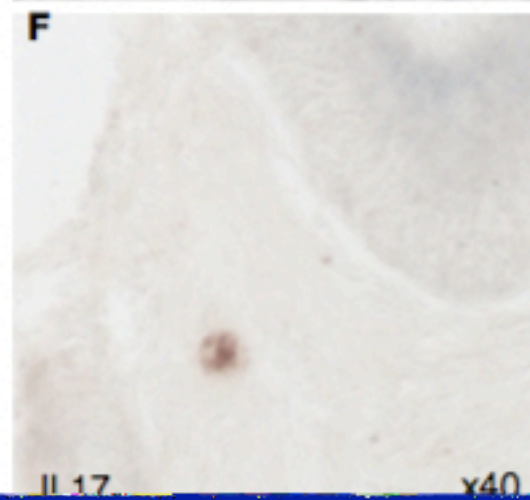
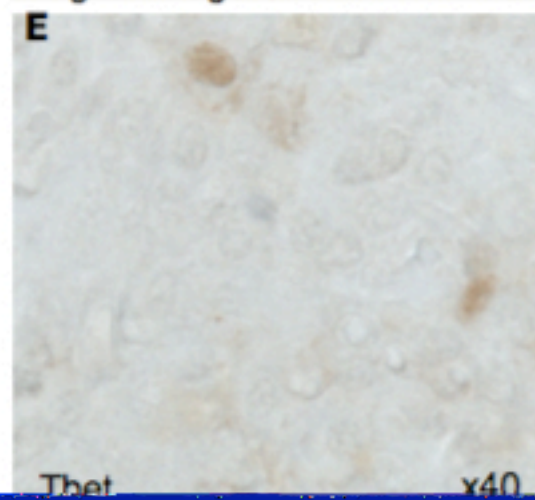
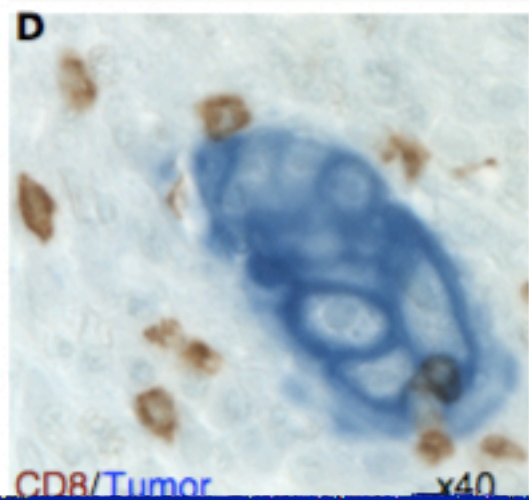
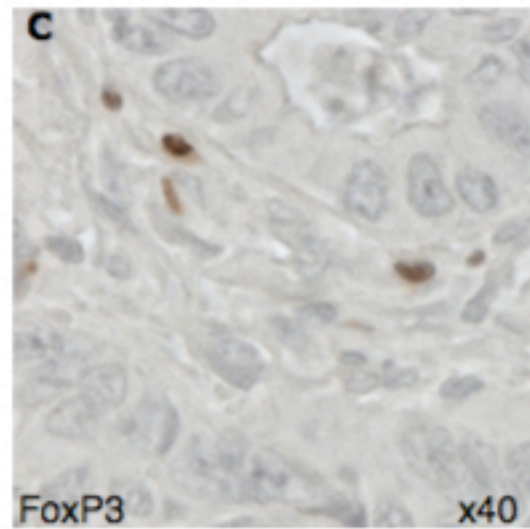
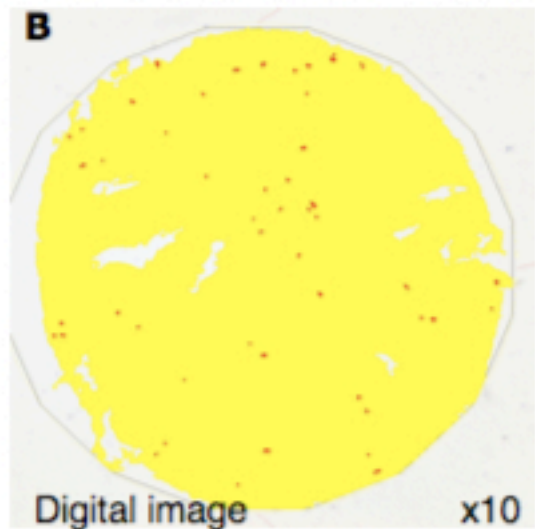
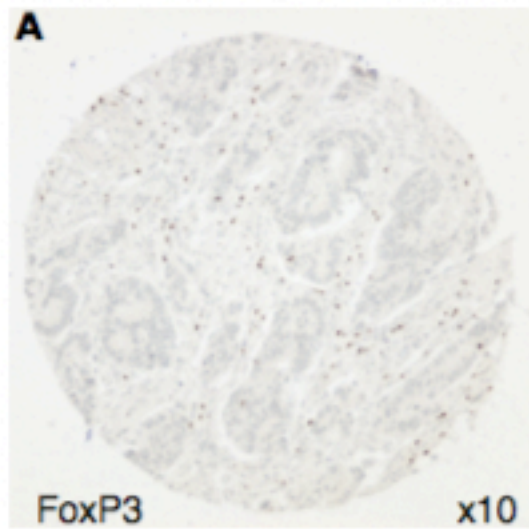


DFS



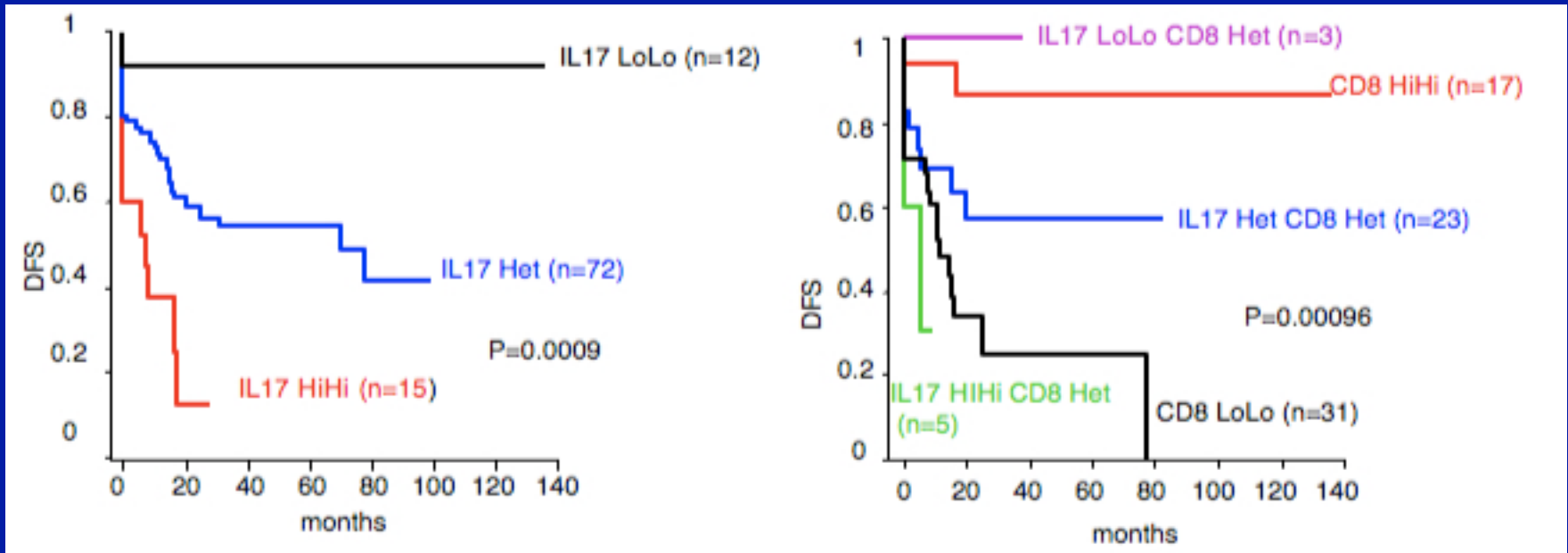
— Hi
— het
— Lo
n=126
P=0.17

Patients presenting with low expression of genes from the Th17 cluster in their primary tumor have a better disease free survival (M. Tosolini et al, Cancer Research, 71, 1263-78, 2011)



Impact of the densities of IL-17 and CD8 positive cells on Disease Free Survival

(M. Tosolini et al, Cancer Research,71, 1263-71, 2011)



In silico prediction

- Search on 730 000 proteins
- 180 fully sequenced organisms
- 19 millions references PubMed (littérature)

• conserved genomic neighborhood

• phylogenetic profile

• protein-protein interaction

• functional genomics

• littérature co-occurrence

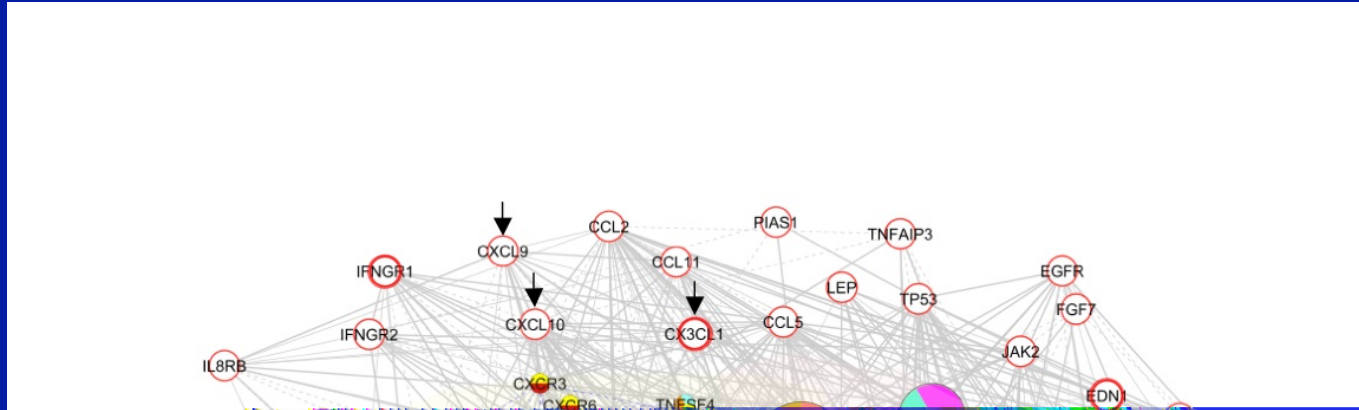
-> prediction score *in silico*

Mlecnik, B. et al. **Gastroenterology** 2010;138, 1429-40

Bindea G, et al. **Bioinformatics** 2009;25:1091-3

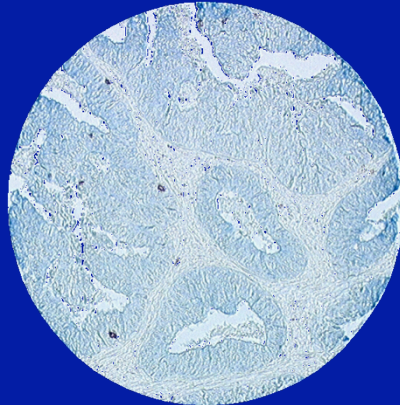
von Mering C, et al. **Nucleic Acids Res** 2005;33:D433-7

Top predictive genes associated with tumor recurrence (experimental data + in silico information)



Experimental validation of in silico predictions

In situ TMA

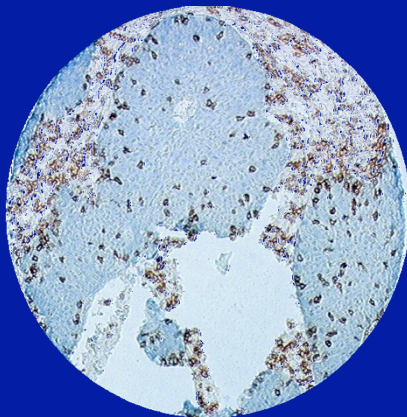


CX3CL1-Lo
CXCL9-Lo
CXCL10-Lo

Validation

Flow cytometry

Immunohistochemistry



CX3CL1-Hi
CXCL9-Hi
CXCL10-Hi

tumor (blue)
CD8 (brown)

High expression of CX3CL1, CXCL10 and CXCL9
correlate with high densities of CD3 T cells.

CX3CL1 correlates with Th1 and CTL

CXCL10 and CXCL9 correlate with memory T cells

High expression of CX3CL1, CXCL10 and CXCL9
correlate with good clinical outcome

CONCLUSIONS

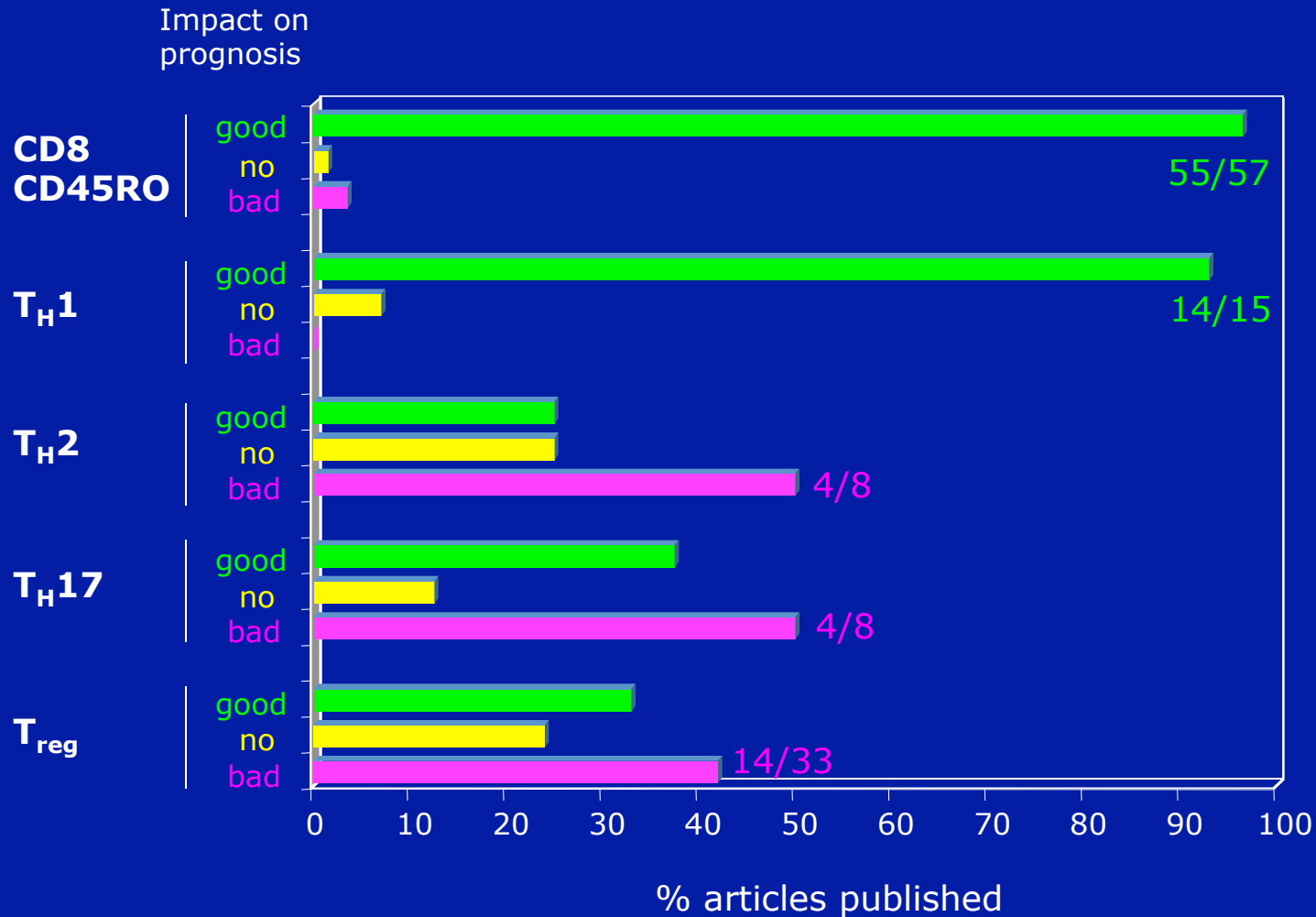
The immune pattern (high infiltration of T memory cells with Th1 and cytotoxic orientation) is the strongest prognostic factor for cancer recurrence in human.

A coordinated immune reaction keeps potentially metastatic cells on hold as long as other microenvironmental factors (neovascularization, tissue destruction) do not allow tumor cell immigration.

CX3CL1, CXCL9 , CXCL10 produced in the tumor appear to be essential chemokines to shape an efficient immune reaction, associated with specific T cell populations.

B. Mlecnik et al., Gastroenterology, 138, 1429-40, 2010

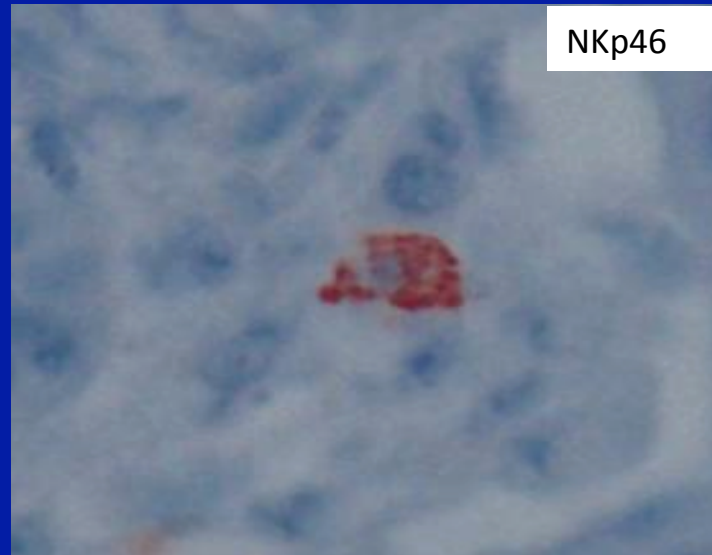
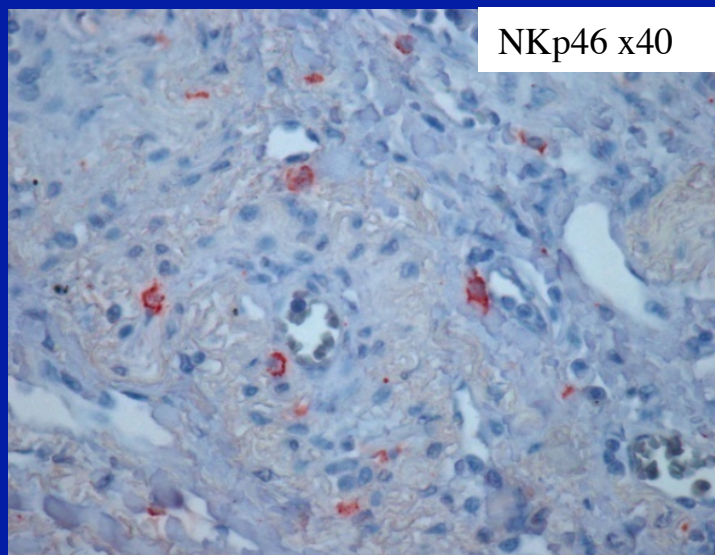
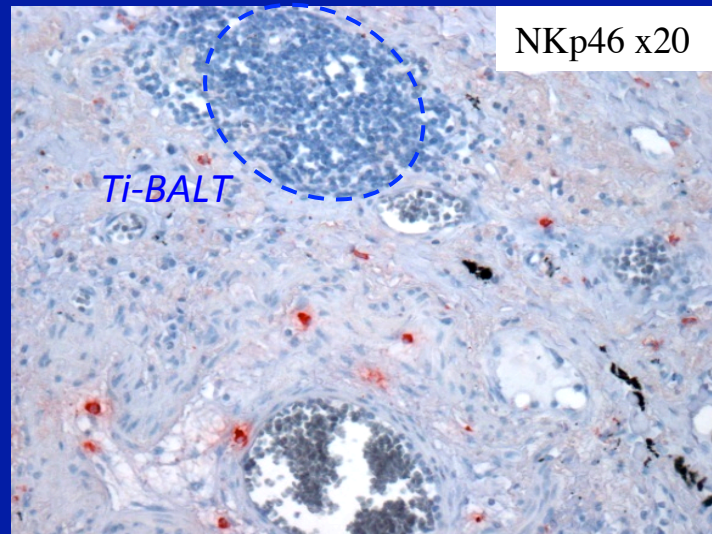
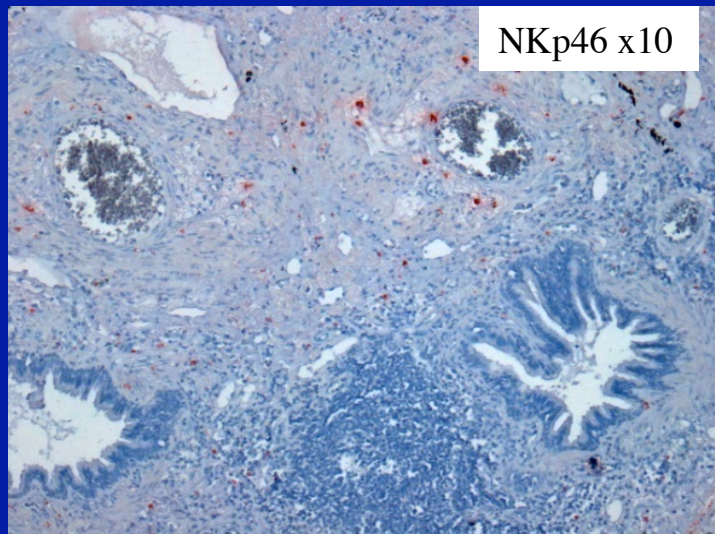
Meta-analysis of 121 published articles studying the impact of cytotoxic T cells, memory T cells, and T-helper subpopulations with regards to prognosis of patients with cancer (20 cancer types analyzed)



What is the function of NK cells which do not show up as a prognostic factor?

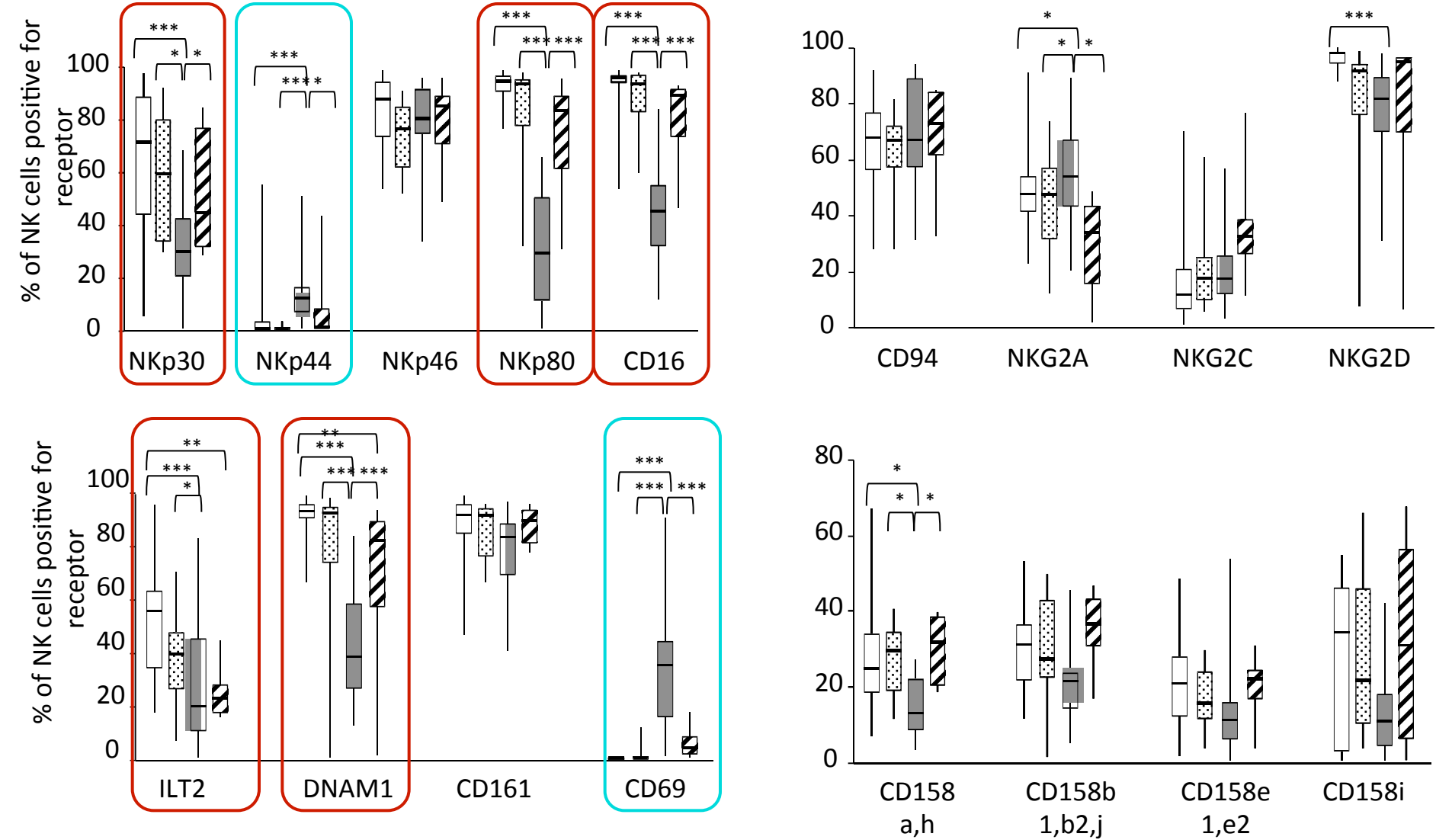
Study in Non Small Lung Cancer

Presence of NKp46+ Cells in NSCLC



Adenocarcinoma

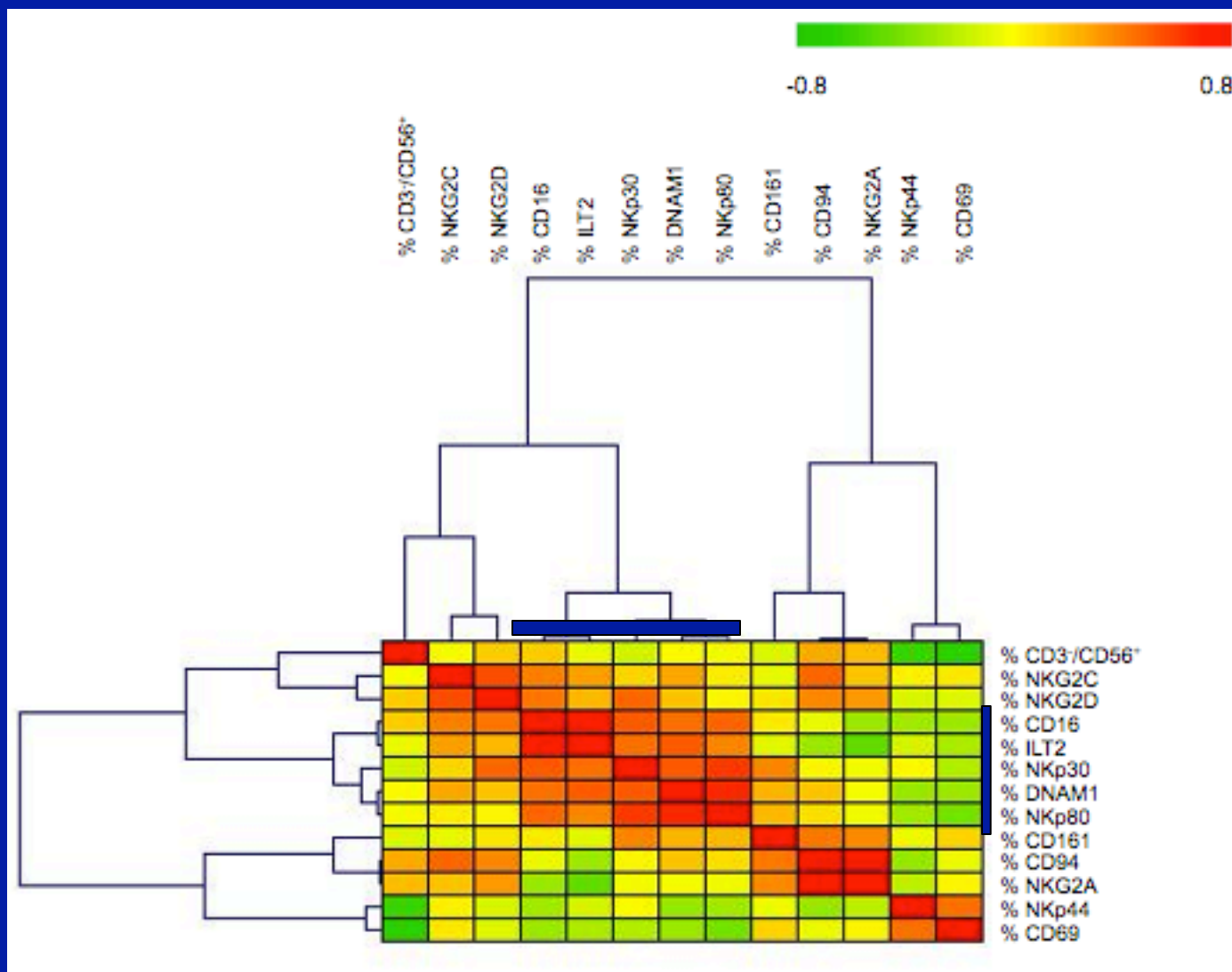
Intratumoral NK cells display a profound down regulation in 5 NK R



NK cells from blood of healthy donors (n=30)
 NK cells from blood of patients (n=10)

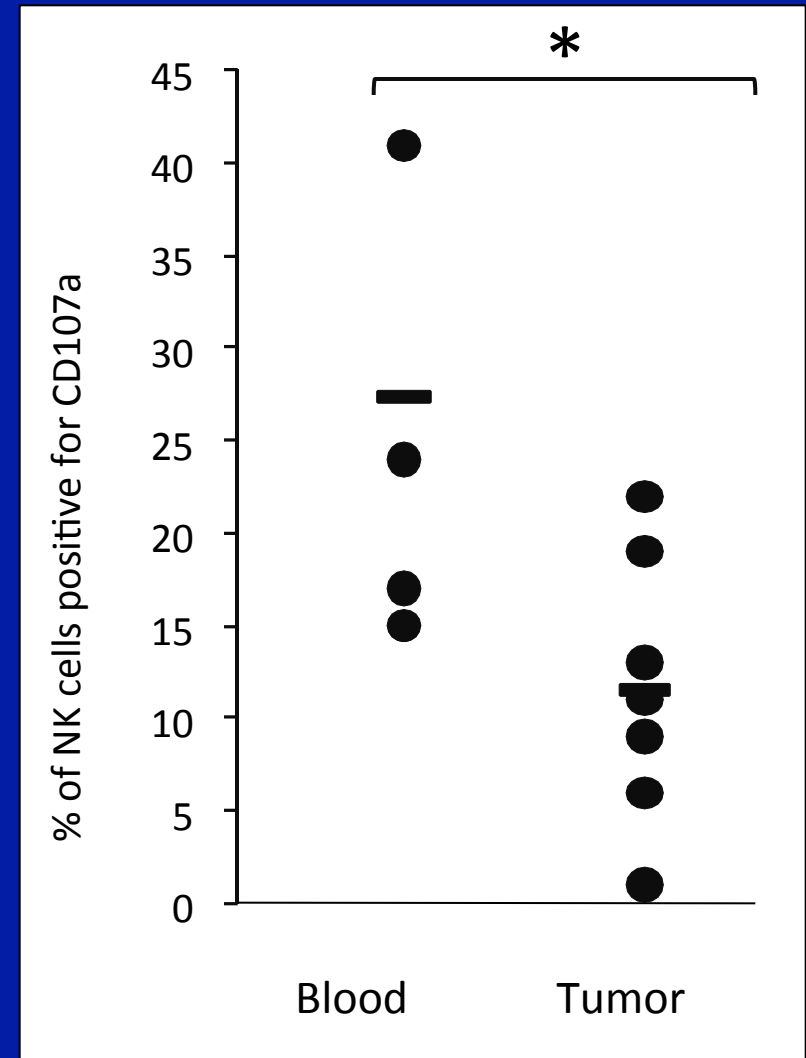
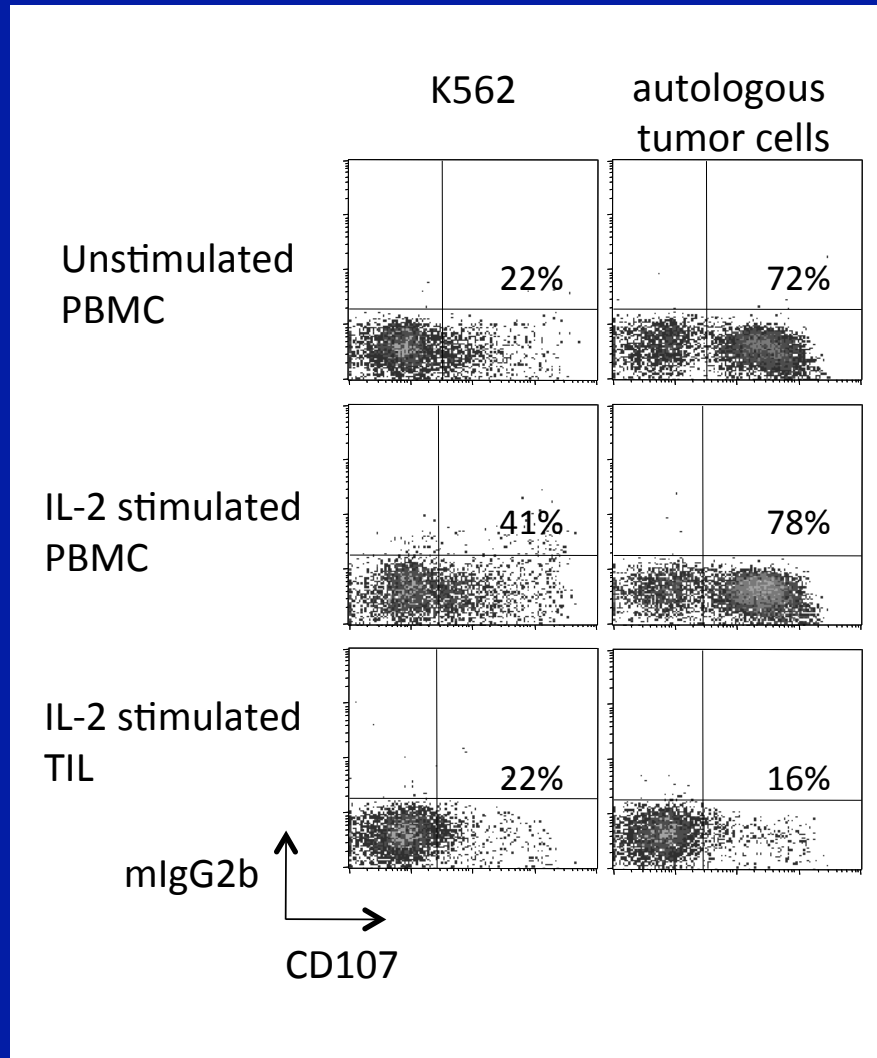
Intratumoral NK cells (n=30)
 NK cells from non tumor tissue (n=10)

Co-modulation of a cluster of 5 NK R in NSCLC in the 30 patients

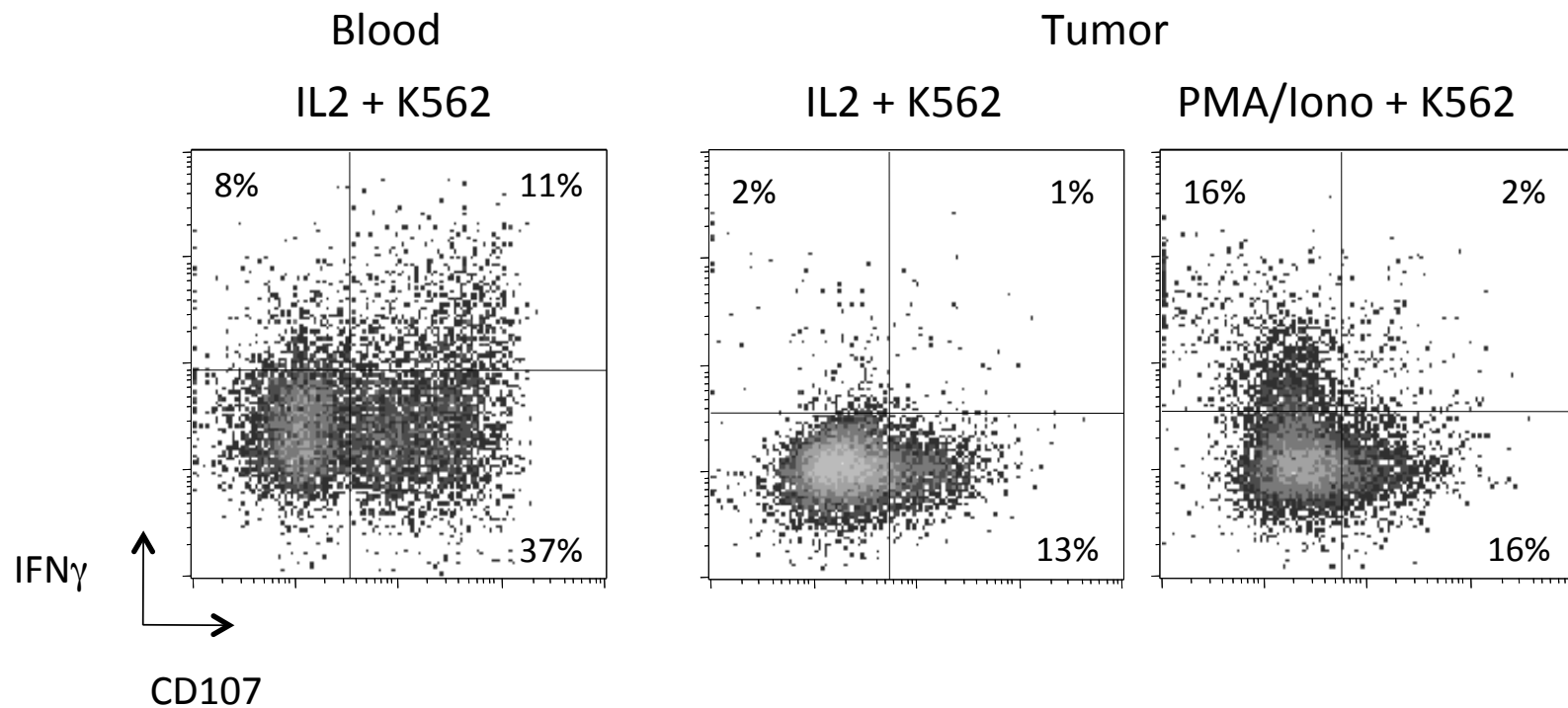


Pairwise comparisons of the markers : Pearson correlation coefficient (r) measures and related P values

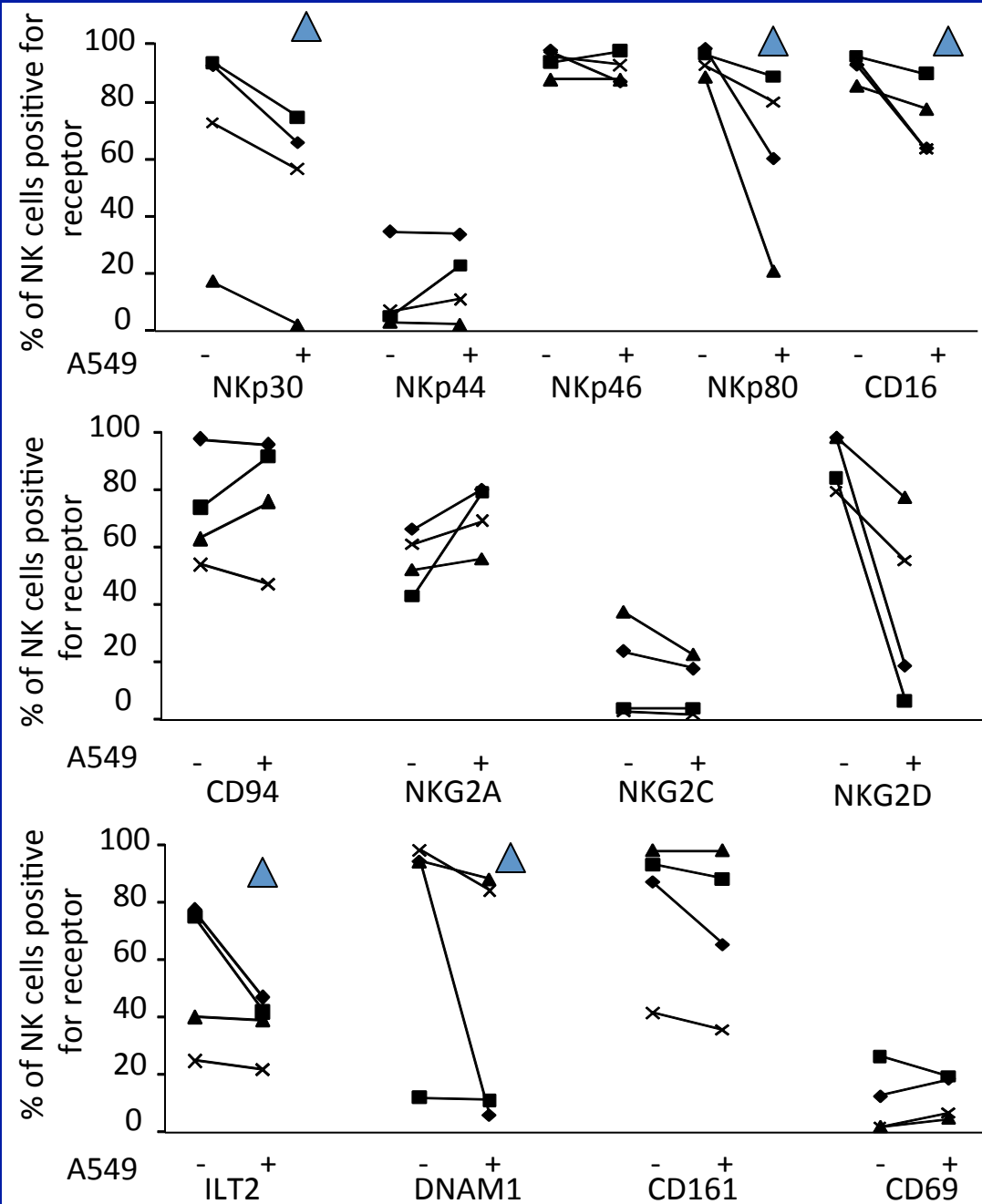
Impaired CD107a degranulation by intratumoral NK cells



Impaired IFN γ secretion by intratumoral NK cells



The impaired
NK phenotype
is induced
by the tumor
(5 days coculture)

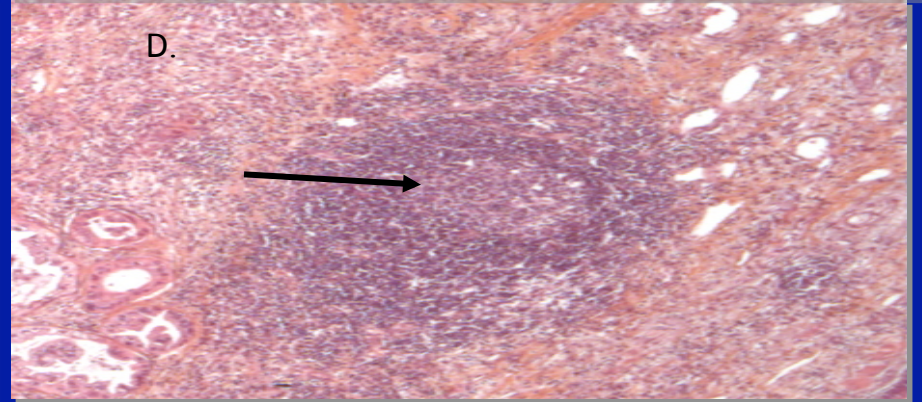
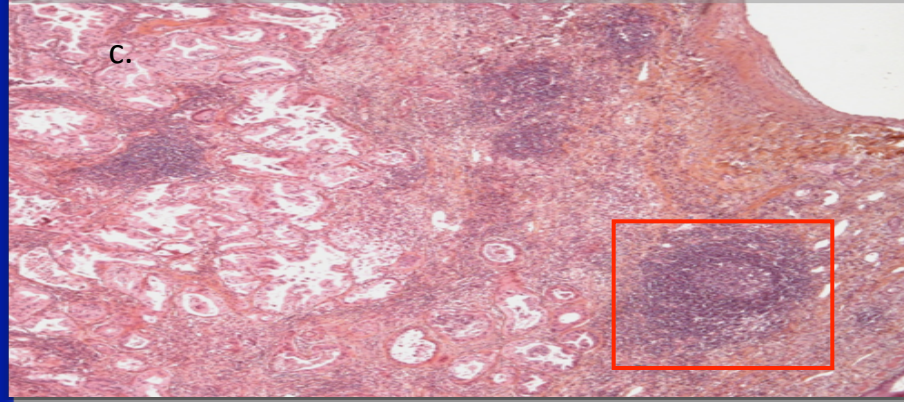
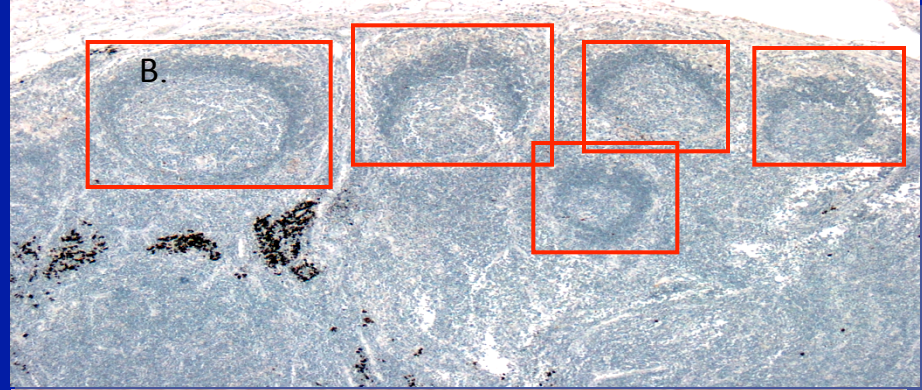
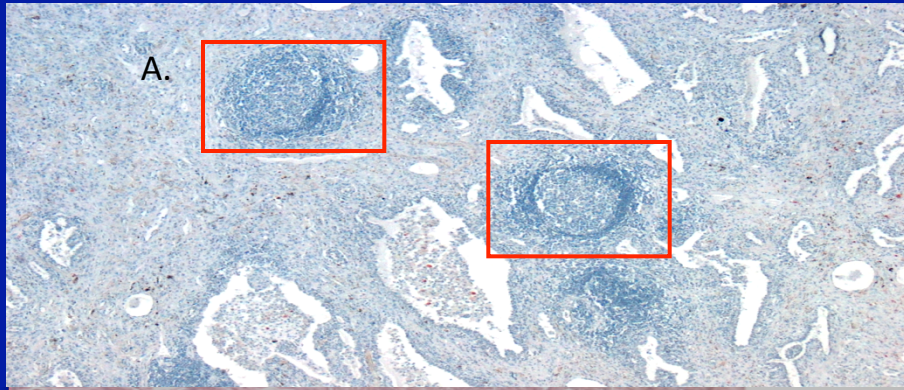


Conclusions

- NK cells are present in NSCLC, with higher density in stroma of tumor than in non tumoral area
- Intratumoral NK cells display a unique phenotype with a drastic down-regulation of a cluster of 5 NK Cell R
- The 5 NK Cell R are co-modulated
- Intratumoral NK cells display an impaired CD107a degranulation and IFN γ secretion
- The reduced expression of NK cell R is induced by the tumor
- S Platanova et al., Cancer Res., 71, 5412-5422, 2011

Where could the immune response be shaped?

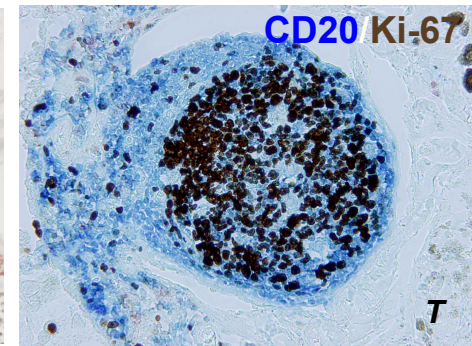
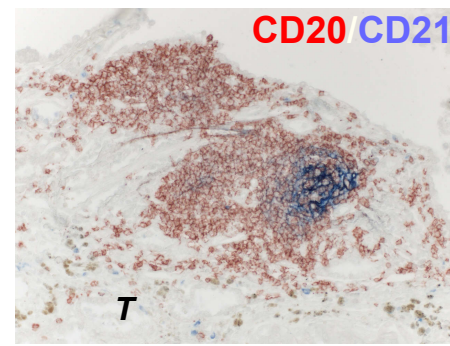
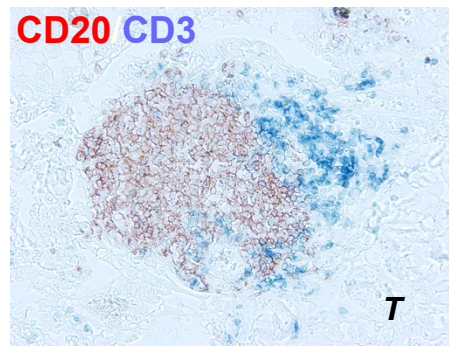
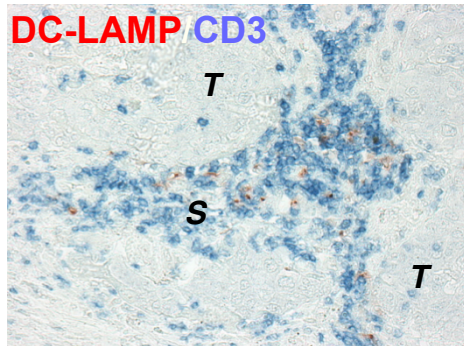
Lymphoid Structures in NSCLC (Ti-BALT) and in lymph node



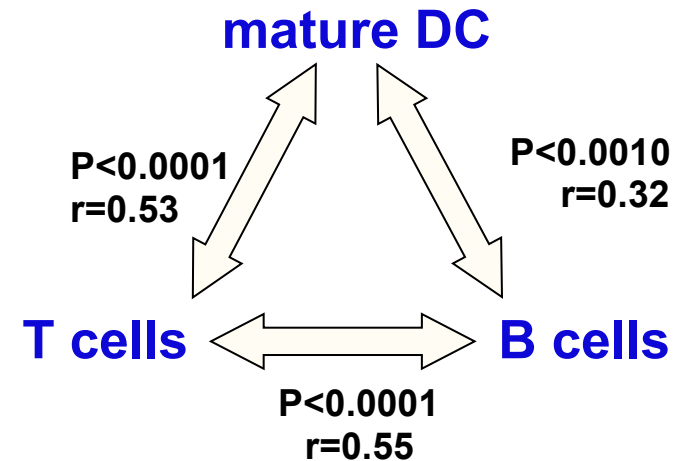
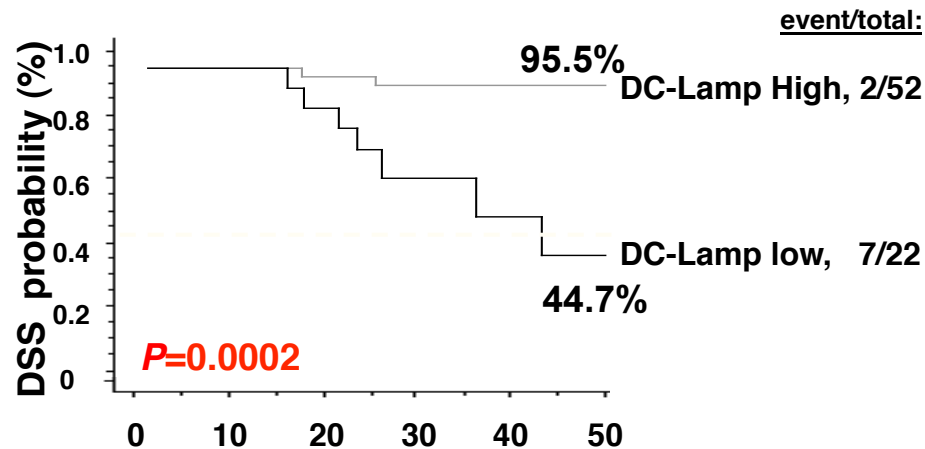
Hematoxylin staining of tumor (A) and lymph node (B)

Hematoxylin and Eosin staining of tumor (C: X100, D: X200)

Long-term survival for patients with lung cancer with intra-tumoral lymphoid structures



T: tumor nest , S: stroma reaction



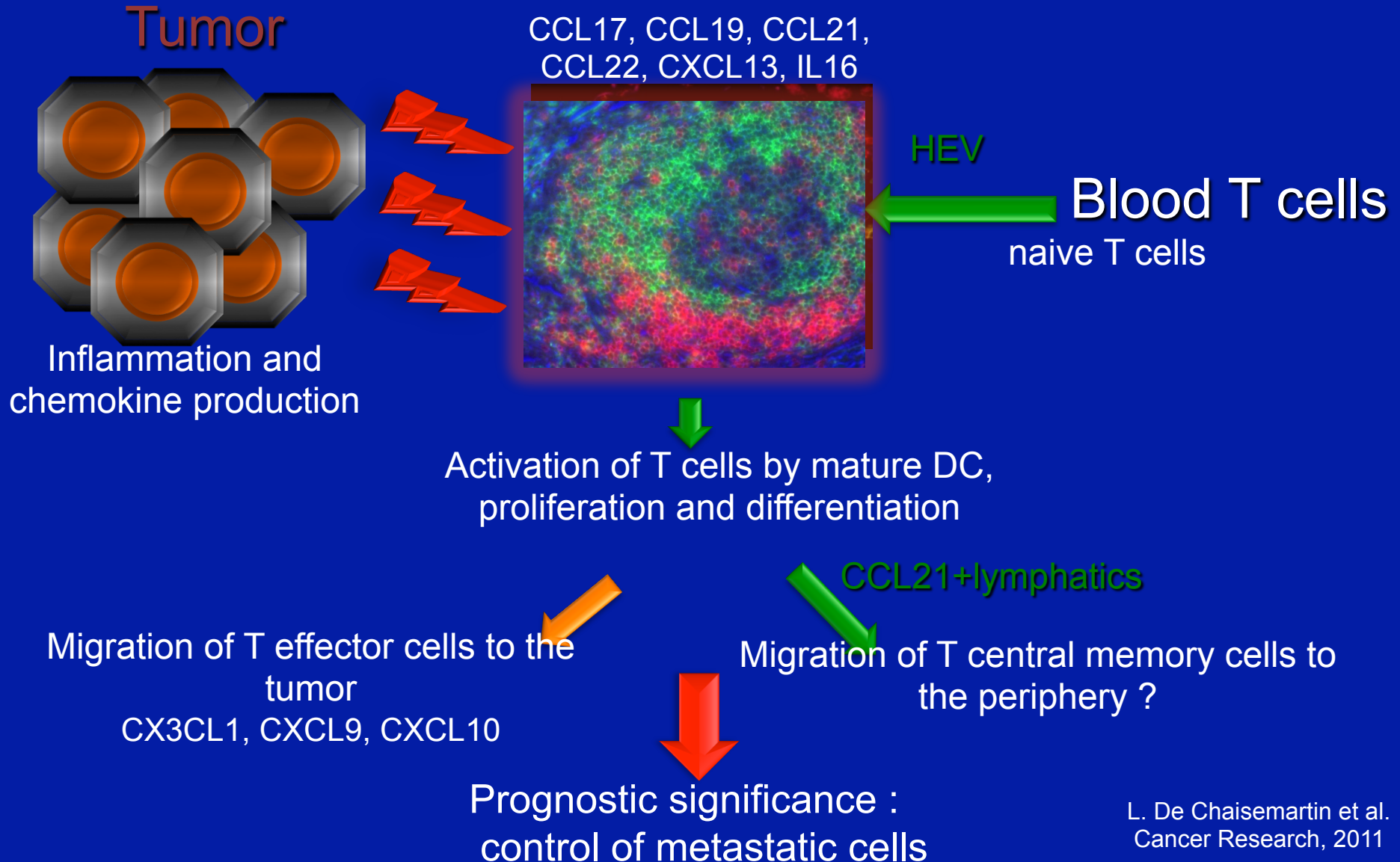
The immune pattern (high or low density of mature DC, memory T and B cells, Th1 and CTL) is independent of:

- Age
- Gender
- Smoking history
- Tumor histological type
- Tumor stage and differentiation

-High numbers of mature DC correlate with high numbers of T effector/memory, B memory cells and favorable prognosis

M.C.Dieu-Nosjean et al., J.Clin.Oncol.,26, 4410-17, 2008

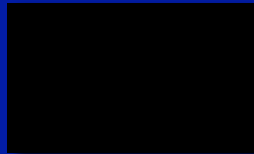
Tumor-induced TLS as a conductor of local and systemic anti-tumoral responses



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Participants in lung cancer studies

- **Cordeliers Research Centre
(Paris) INSERM U872,**

- M.-C. Dieu-Nosjean
- L. de Chaisemartin
- J. Goc
- D.Damotte
- C.Sautès- Fridman

- **Institut Mutualiste
Montsouris (Paris)**

- P. Validire

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- **Hôtel Dieu (Paris)**

- D. Damotte
- M. Alifano
- P.Magdalenat