Induction of Tertiary Lymphoid Structures, a new strategy of the immune system to fight against tumor

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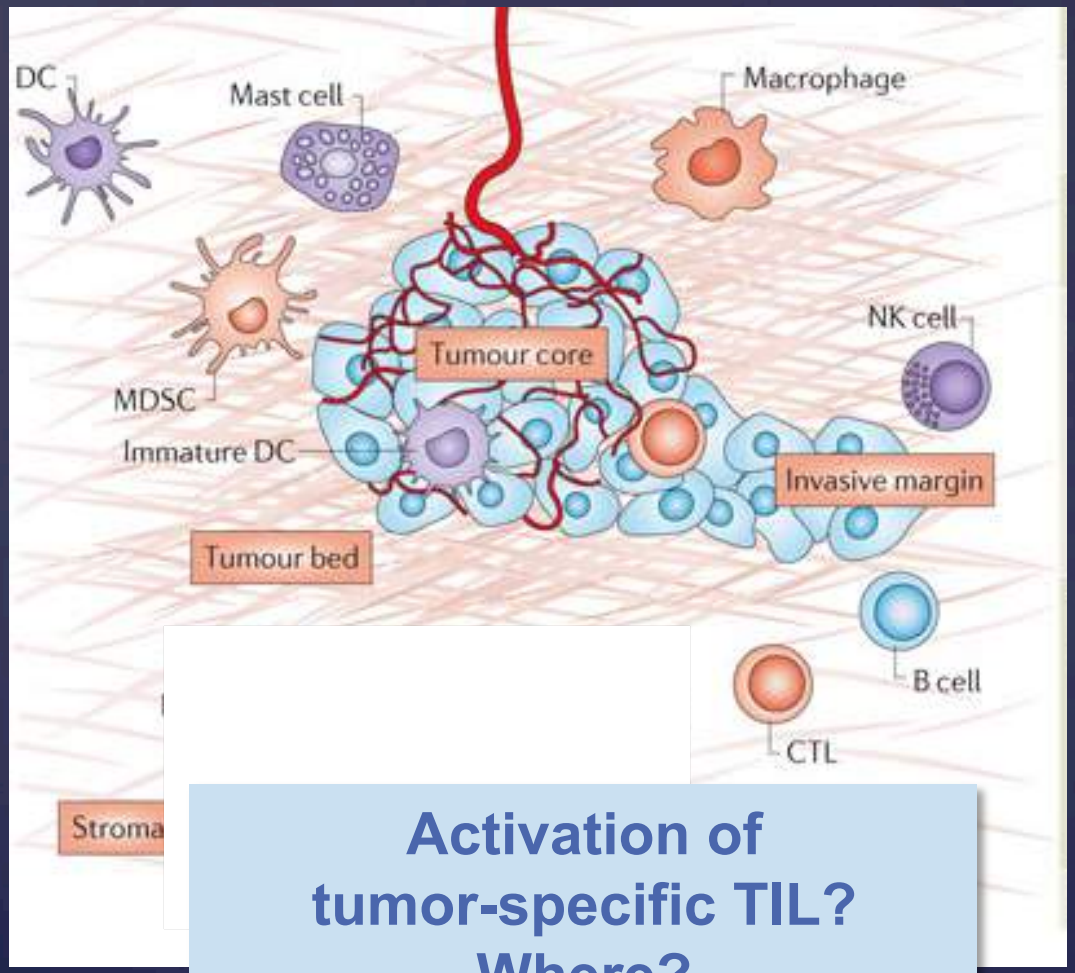
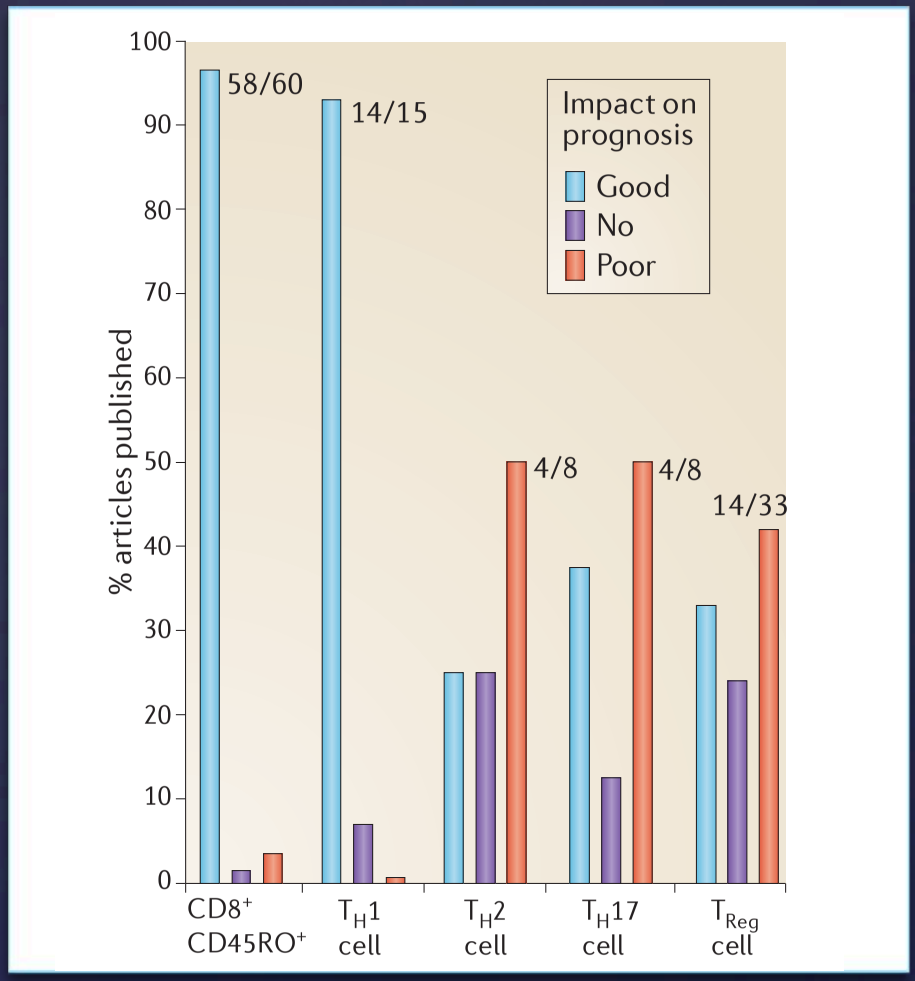
Complexity of the tumor microenvironment and heterogeneity between patients



Activation of tumor-specific TIL? Where?

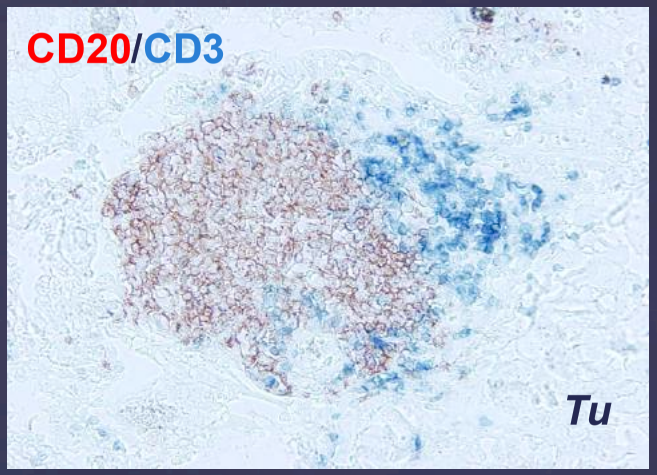


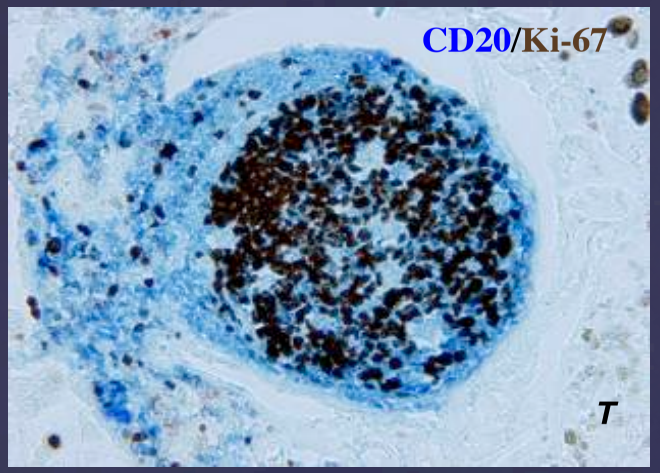
124 published articles, analysis of 20 cancer types

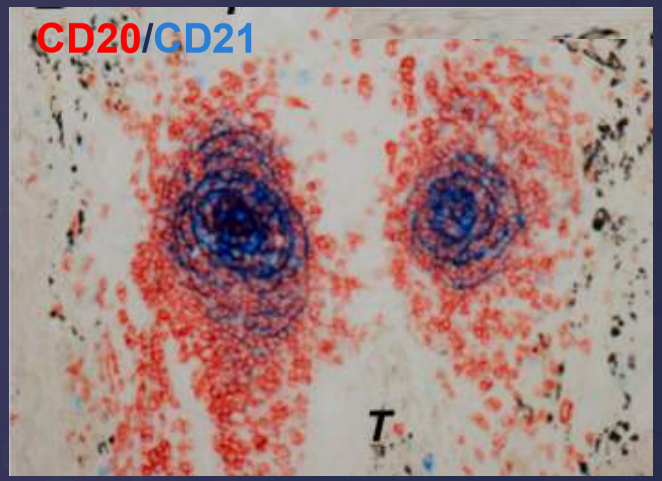
Adapted from Fridman *et al.*, Nature Rev. Cancer, 2012

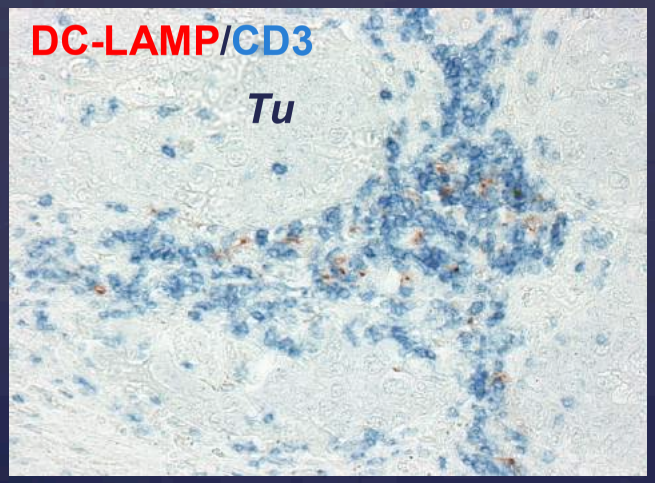
Presence of Tertiary Lymphoid Structures (TLS) in some tumors of NSCLC patients

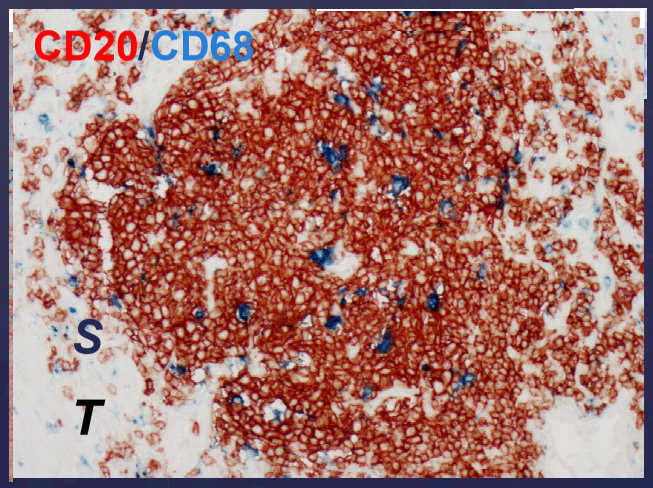
- Hallmark of TLS = mature DC (DC-Lamp)

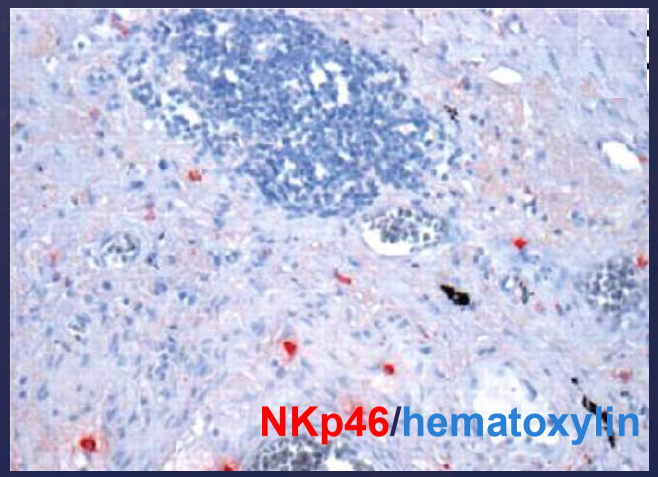












Tu: tumor nest, S: stroma

Dieu-Nosjean *et al.*, J Clin Oncol, 2008
Platonova *et al.*, Cancer Res., 2011
De Chaisemartin *et al.*, Cancer Res., 2011

Definition of TLS

- Structural analogy with secondary lymphoid organs
  (spleen, lymph nodes, MALTs)

- Detected in non-lymphoid organs
  (lung, pancreas, liver, articulations, thyroid, kidney, …)

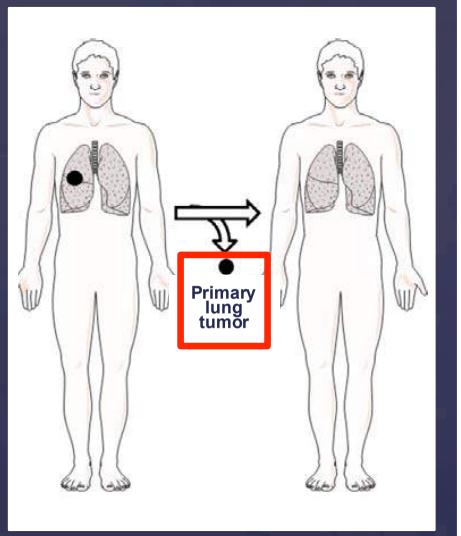
- Induced in response to chronic inflammation
  (auto-immunity, chronic allograft rejection, infection, …)

- Disappear after removal of the inflammation
  (lung, pancreas, liver, articulations, thyroid, kidney, …)

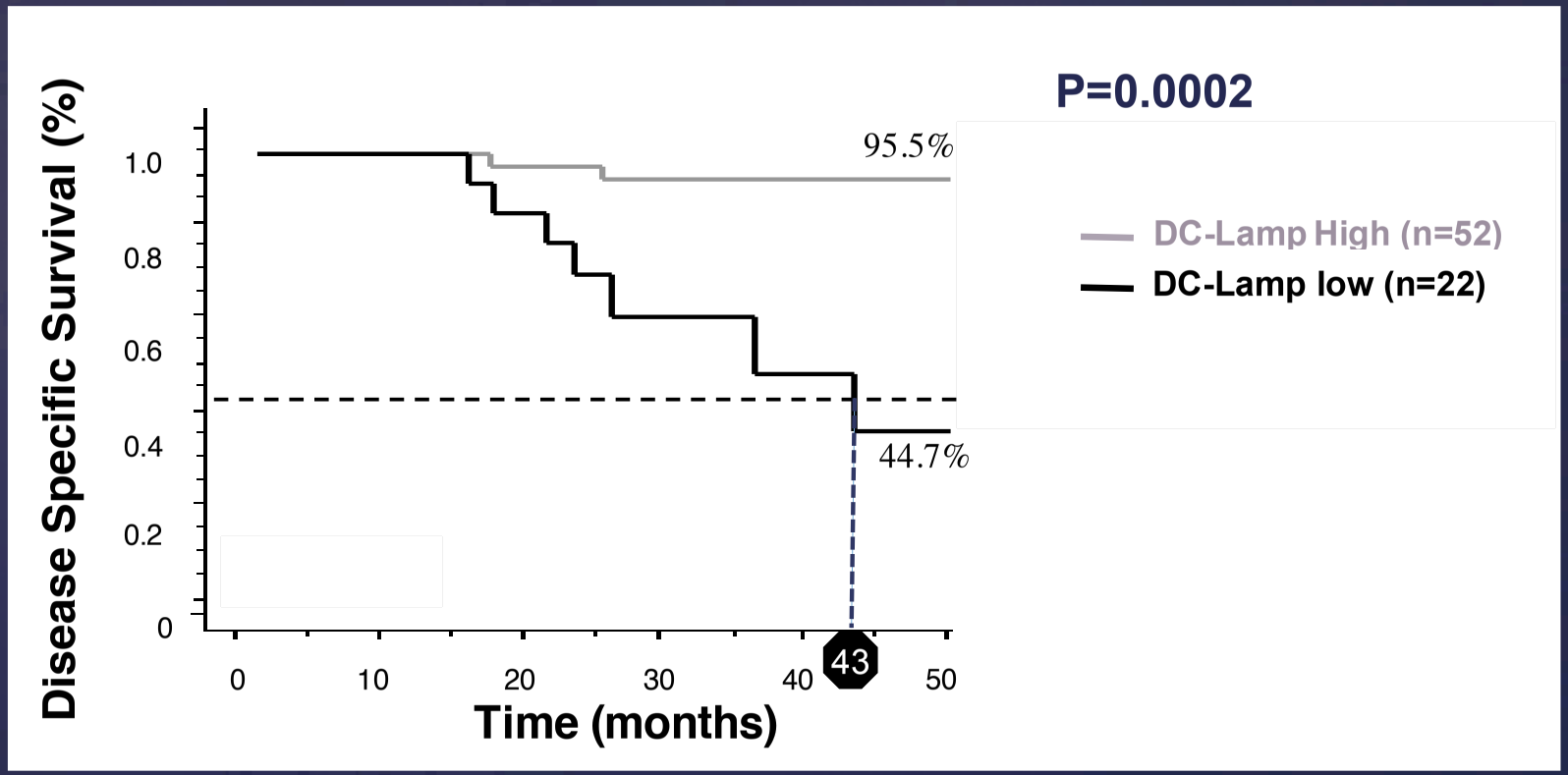
- Dual role:
  deleterious (autoimmunity, transplantation, ...) *versus* protective role (microbial infections)

Cancer patients?

High density of mature DC is associated with a favorable clinical outcome in early-stage lung cancer patients



N=74 patients with primary early-stage NSCLC



Kaplan-Meier method, Log-Rank test

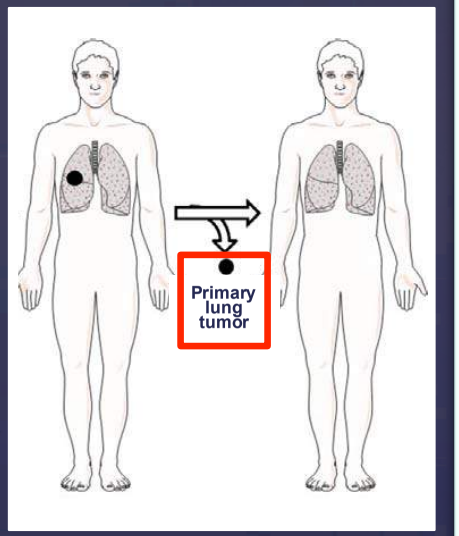
Fridman *et al.*, Cancer Met. Rev., 2011

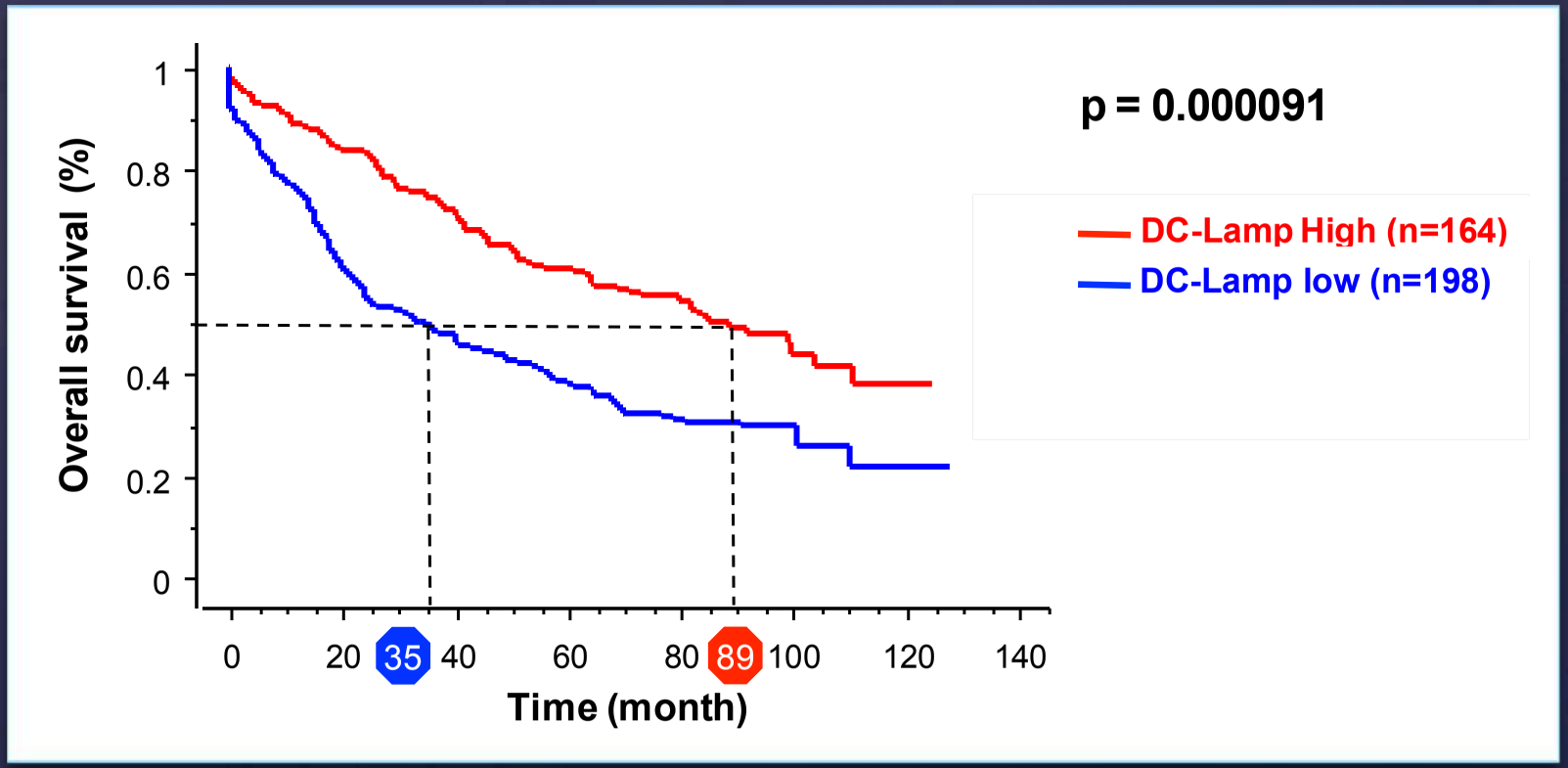
Same result with OS, DFS

Dieu-Nosjean *et al.*, J. Clin. Oncol., 2008

High density of mature DC is associated with a favorable clinical outcome in patients with all stage lung cancer

N=372 patients with primary NSCLC (stages I to IIIa, wo neo-adj chemotherapy)



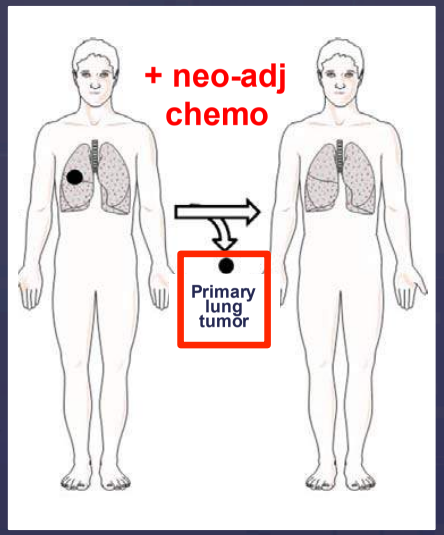


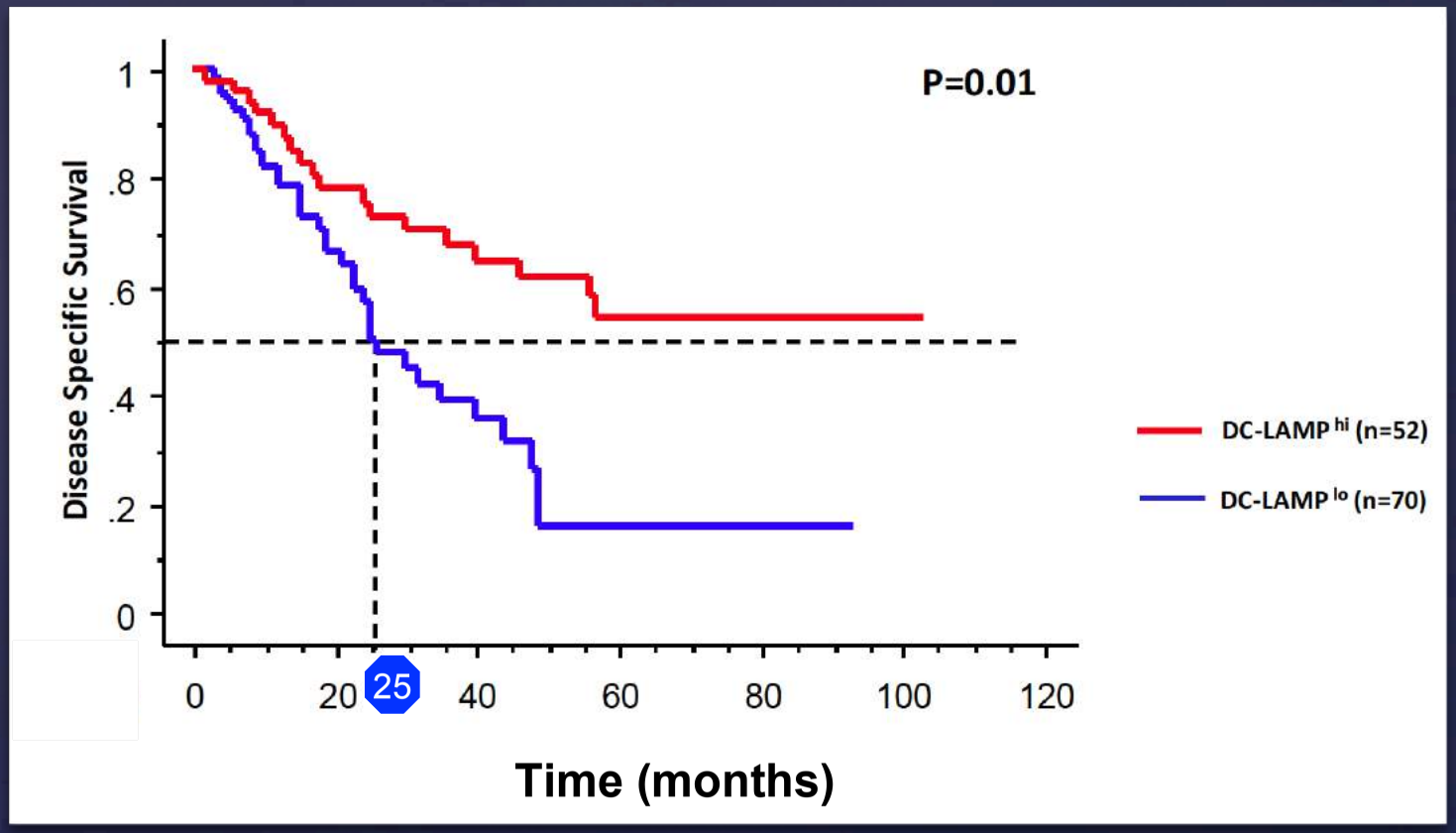
Kaplan-Meier method, Log-Rank test

Goc *et al.*, Cancer Res., 2014

High density of mature DC is associated with a good outcome in advanced NSCLC + neo-adj chemotherapy

N=122 NSCLC patients treated by neo-adjuvant chemotherapy (stage IIIB)



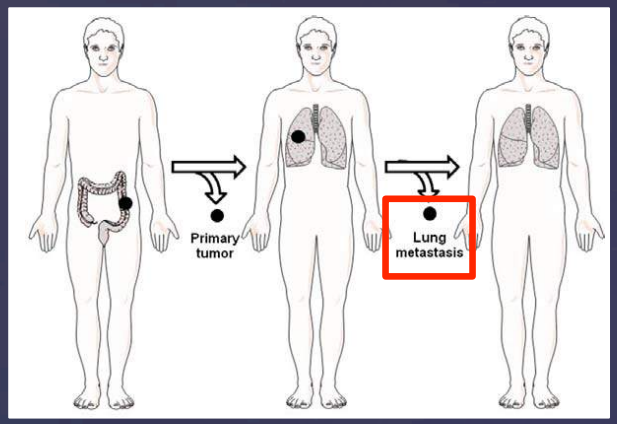


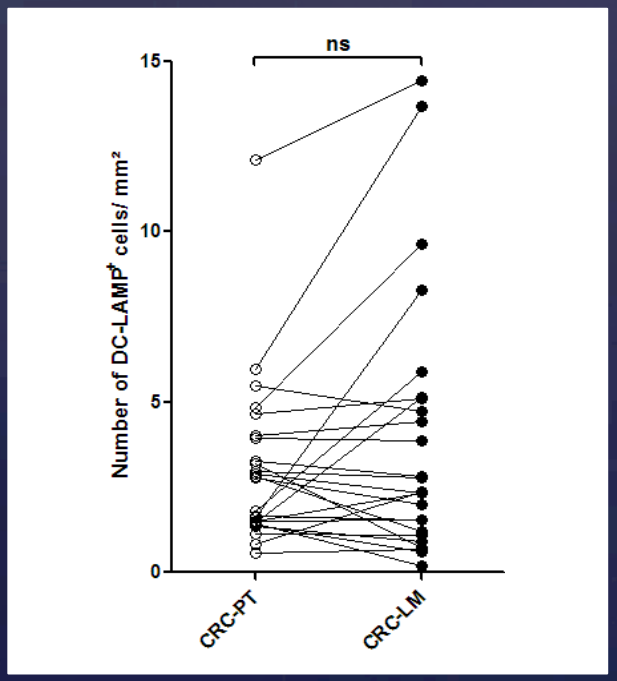
Kaplan-Meier method, Log-Rank test

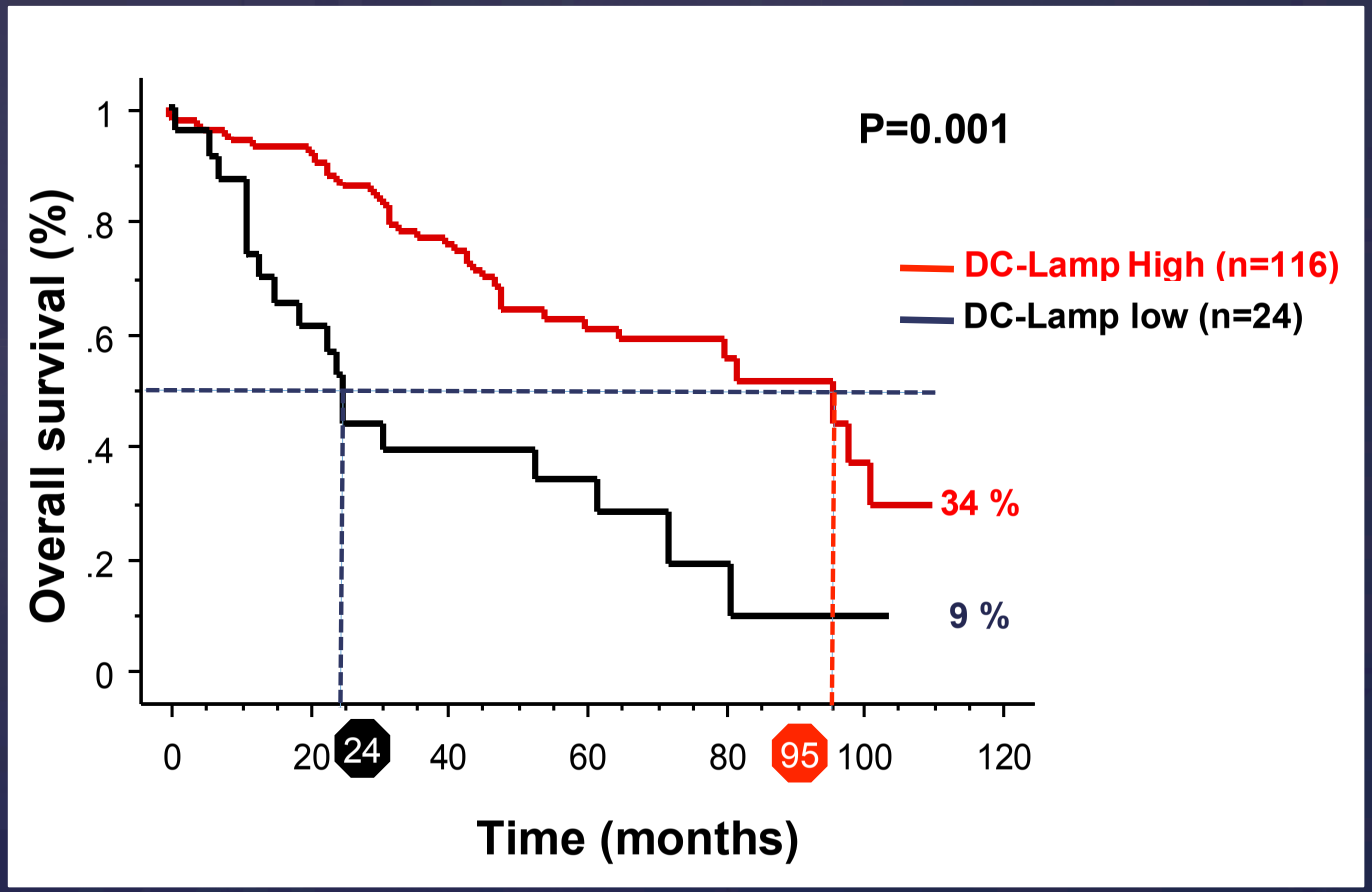
Remark *et al*., unpublished data

High density of mature DC is associated with a favorable clinical outcome even in metastatic patients

N=140 patients with colorectal carcinoma lung metastasis





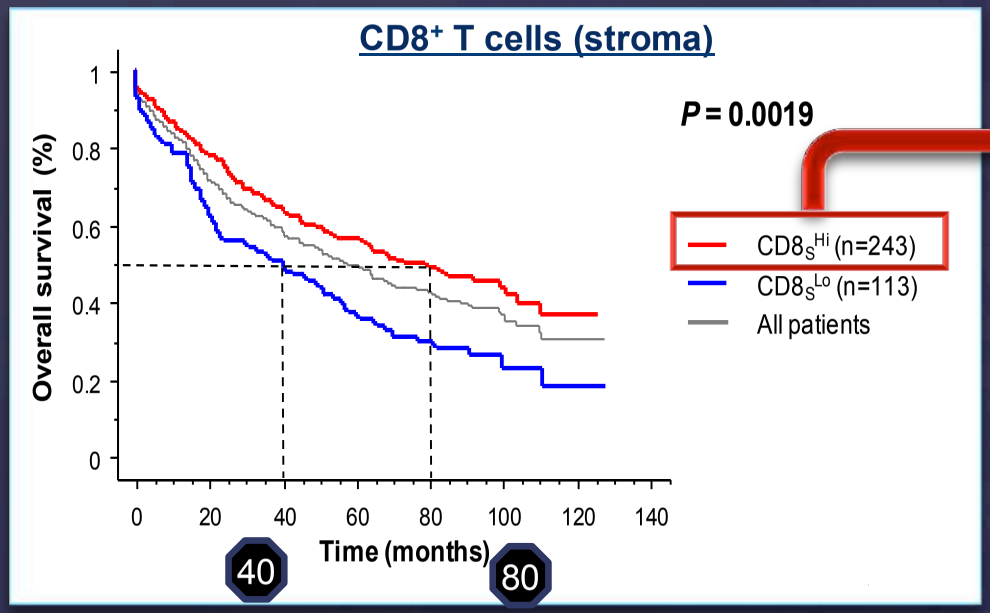


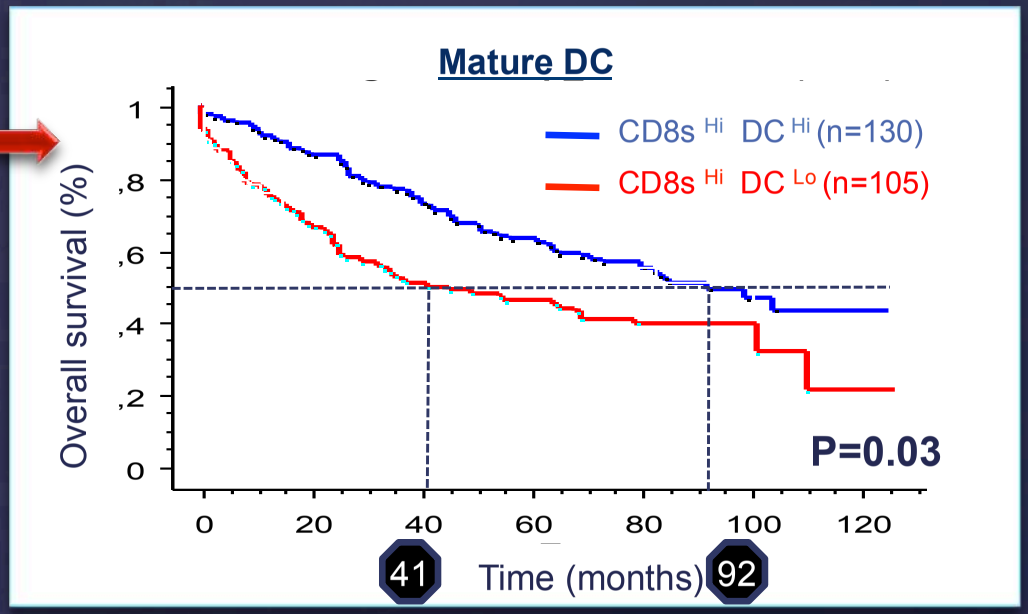
Kaplan-Meier method, Log-Rank test

Remark *et al*., Clin. Cancer Res., 2013

Presence of mature DC is required to license the positive prognostic value of infiltrating CD8+ T cells

N=356 patients with primary NSCLC (stages I to IIIa, wo neo-adj chemotherapy)



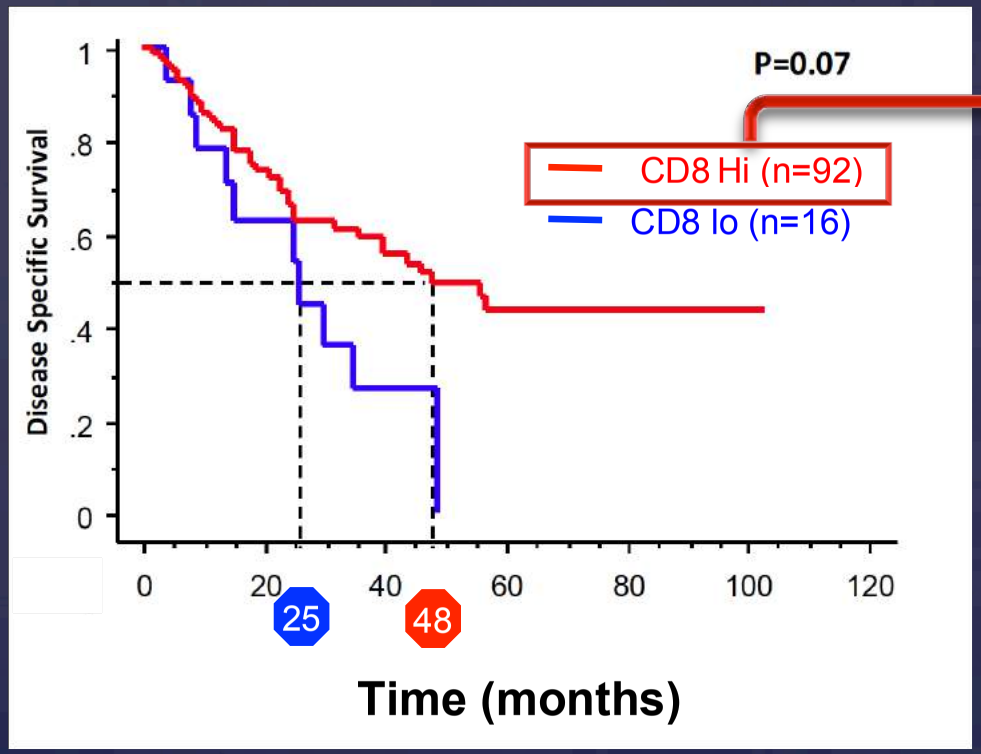


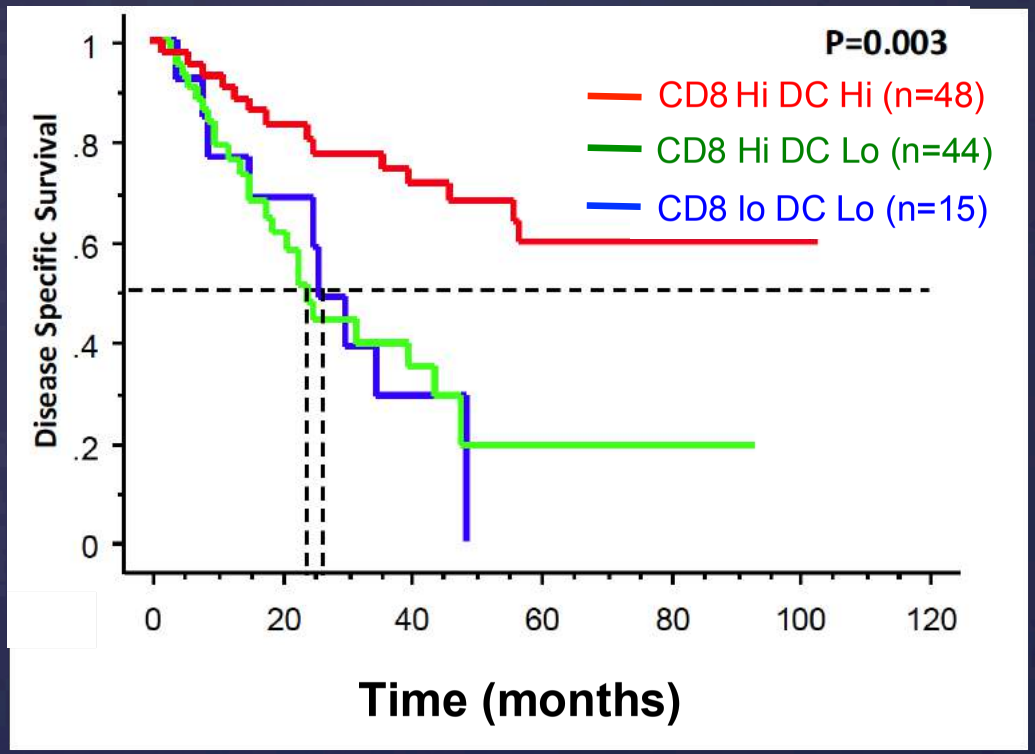
Kaplan-Meier method, Log-Rank test

Goc *et al.*, OncoImmunol., 2013
Goc *et al.*, Cancer Res., 2014

Presence of mature DC is required to license the positive prognostic value of infiltrating CD8$^+$ T cells

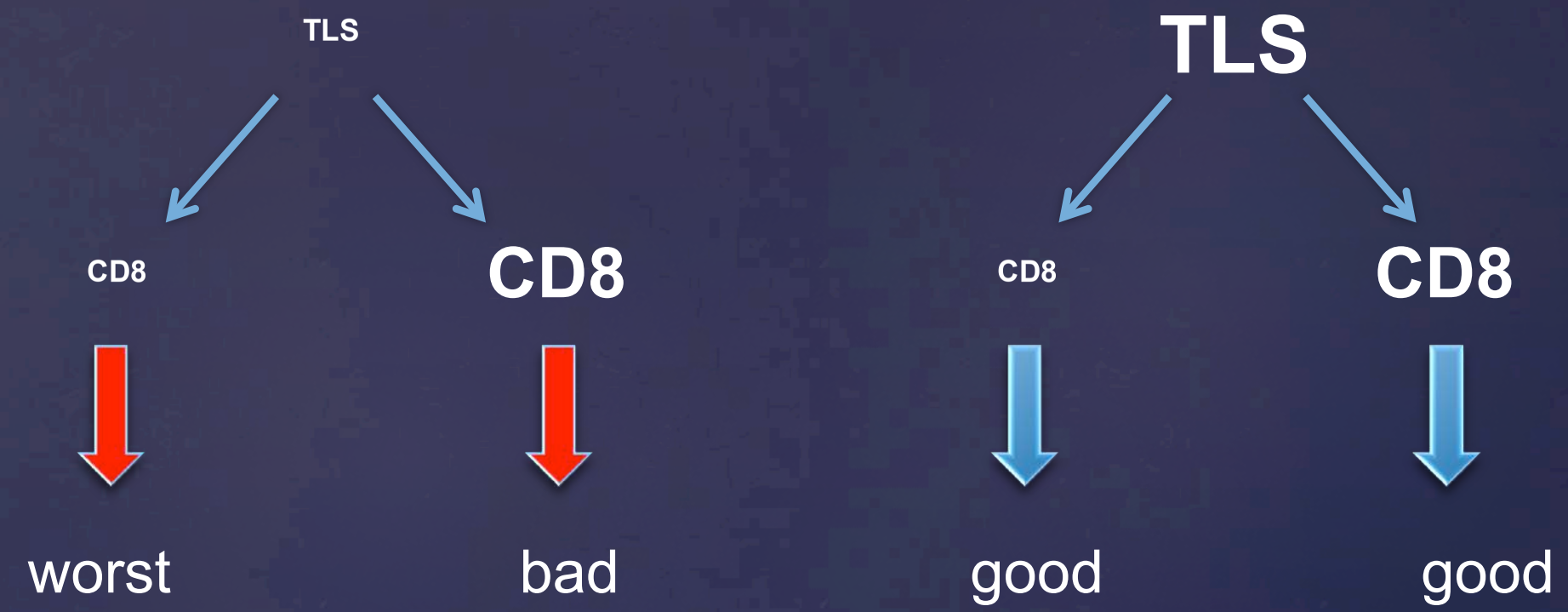
N=108 patients with advanced NSCLC (stage IIIb, + neo-adj chemotherapy)





Kaplan-Meier method, Log-Rank test

Remark *et al*., unpublished data

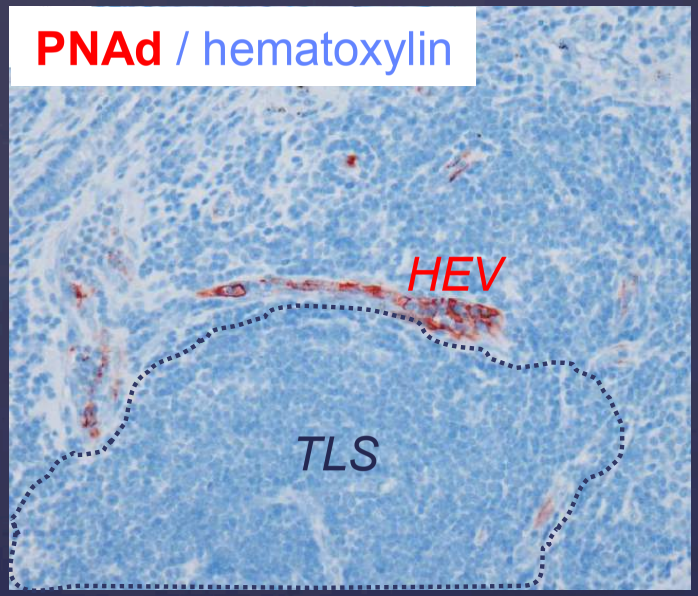


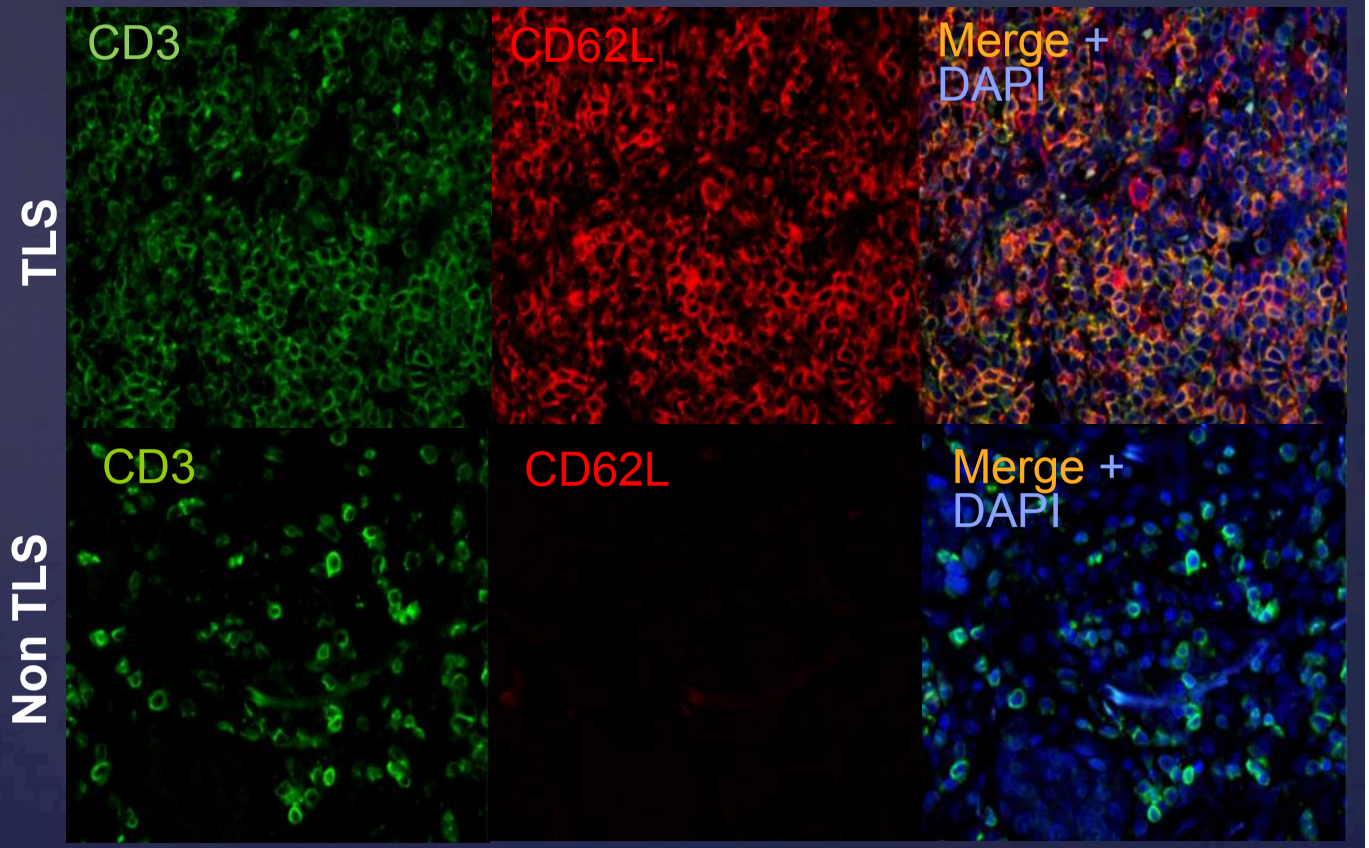
No local education of specific T cells
Priming in lymph node only

Local education of specific T cells (TLS)
± priming in lymph node

TLS demonstrate vascular features specialized in lymphocyte recruitment

- CD62L is a specific marker of TLS T cells



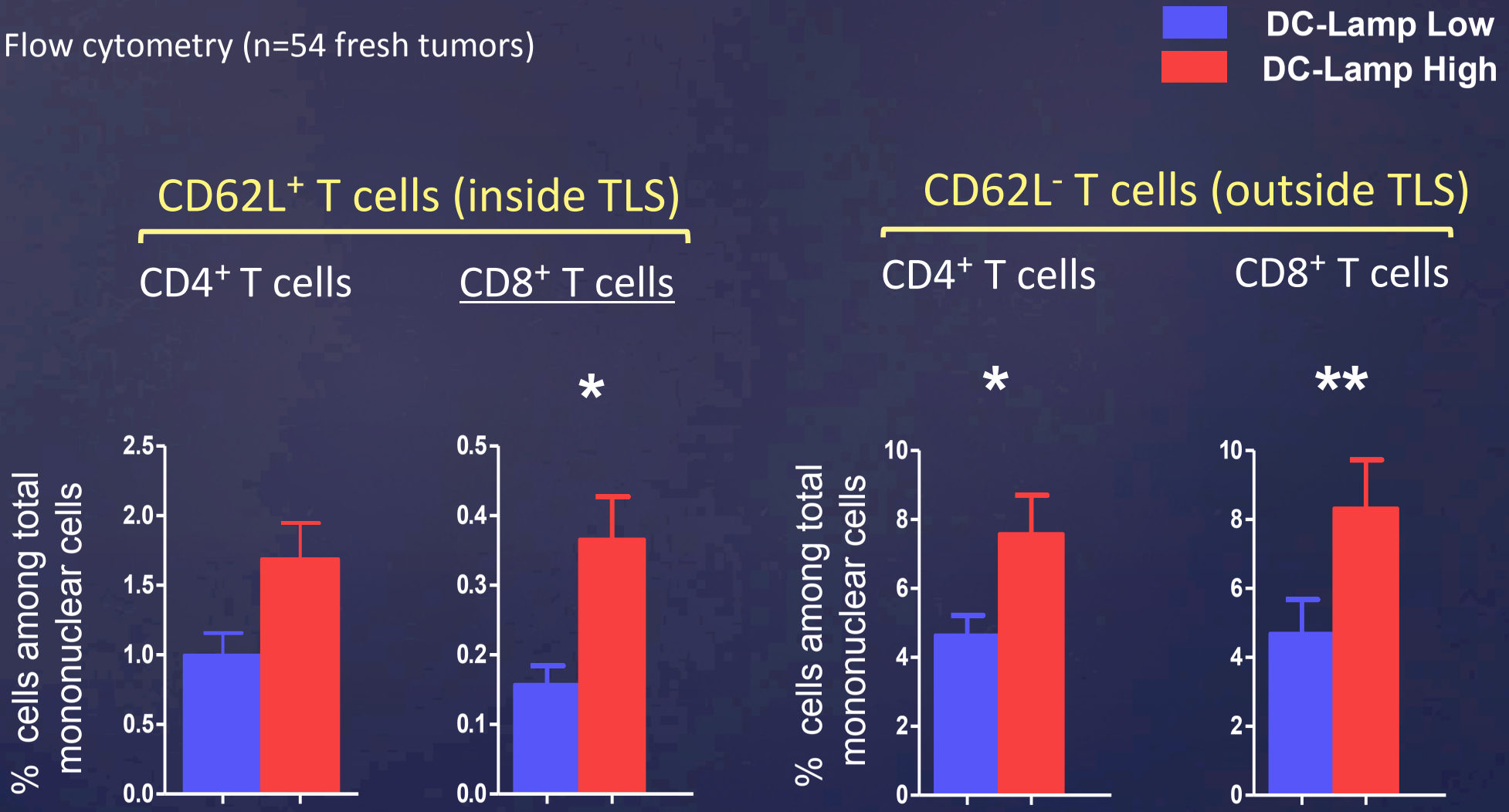


x400

De Chaisemartin *et al.*, Cancer Res., 2011

More CD4^{+} and CD8^{+} T cells in both TLS and non-TLS areas in DC-Lamp^{Hi} *versus* DC-Lamp^{Lo} tumors

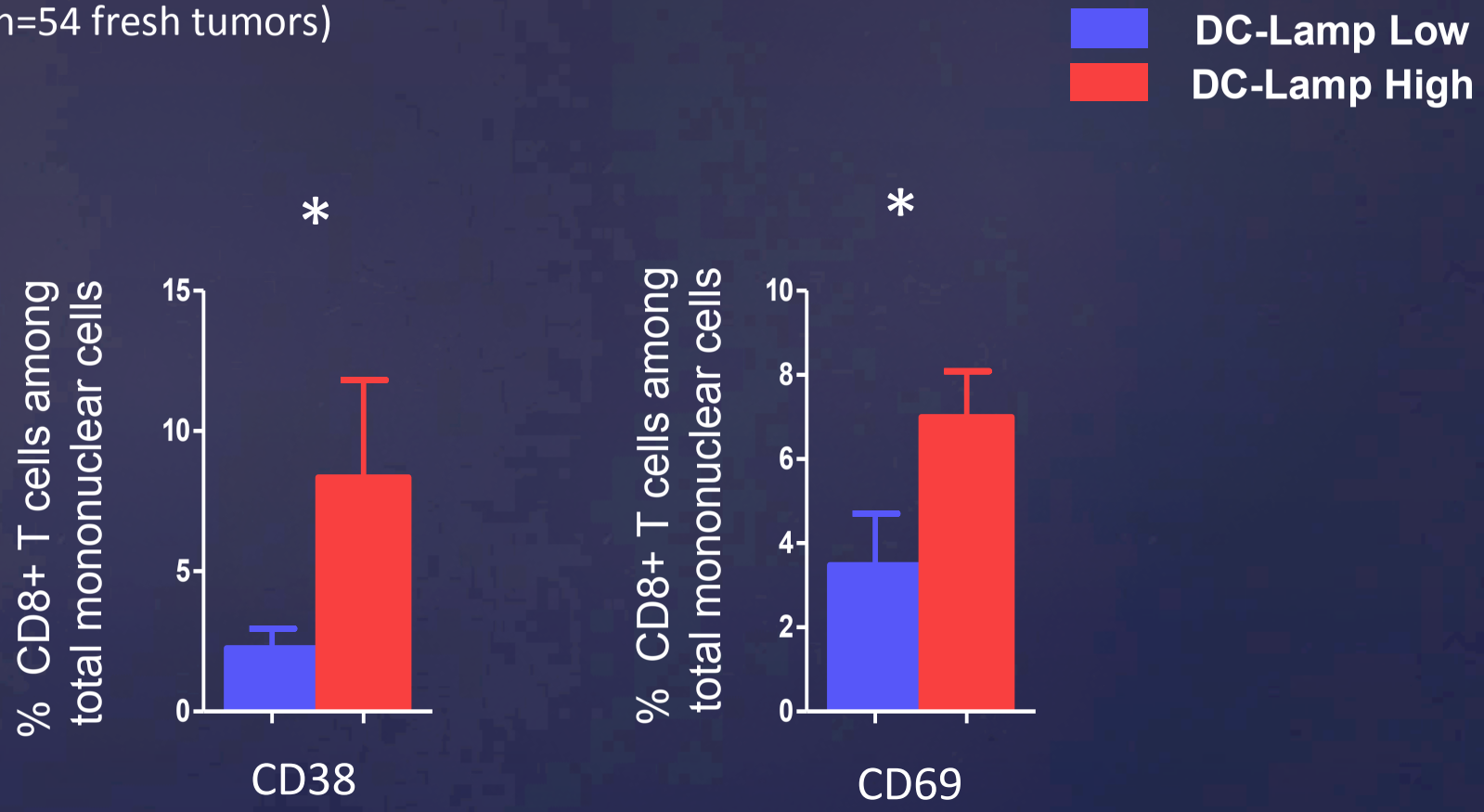
Flow cytometry (n=54 fresh tumors)



Goc *et al.*, Cancer Res., 2014

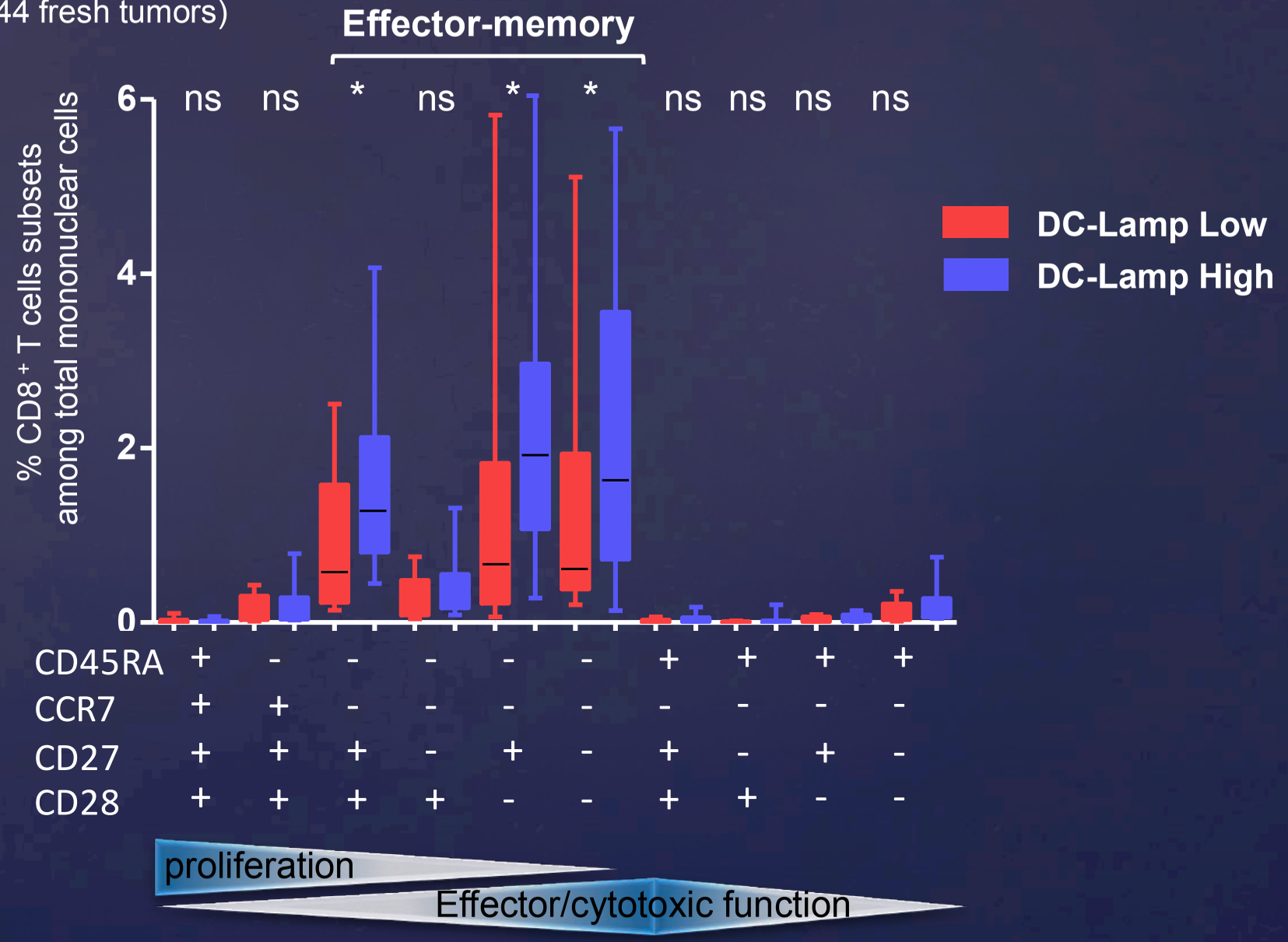
Increased proportion of activated CD8^{+} T cells among DC Lamp^{Hi} *versus* DC Lamp^{Lo} tumors

Flow cytometry (n=54 fresh tumors)



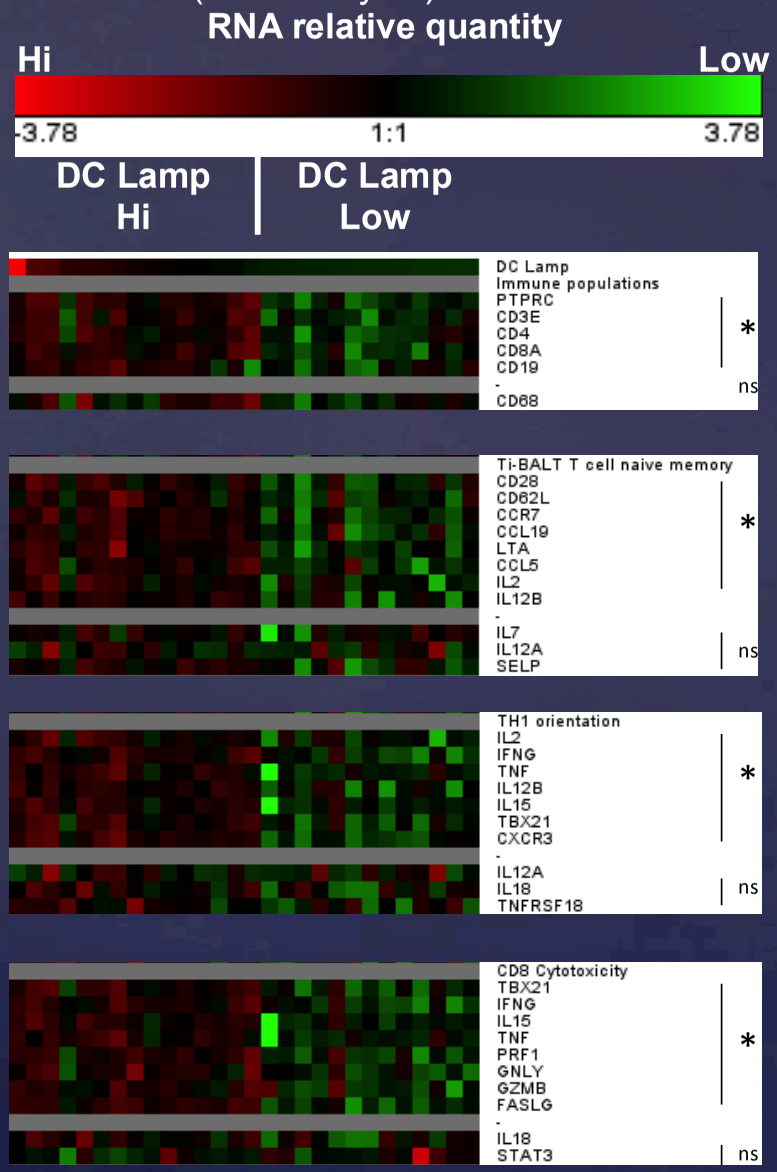
More effector-memory CD8^{+} T cells in DC-Lamp^{Hi} *versus* DC-Lamp^{Lo} tumors

Flow cytometry (n=44 fresh tumors)



Th1, cytotoxic and activation genes are overexpressed in DC-Lamp^{Hi} *versus* DC-lamp^{Lo} tumors

n = 28 tumors (LDA analysis)

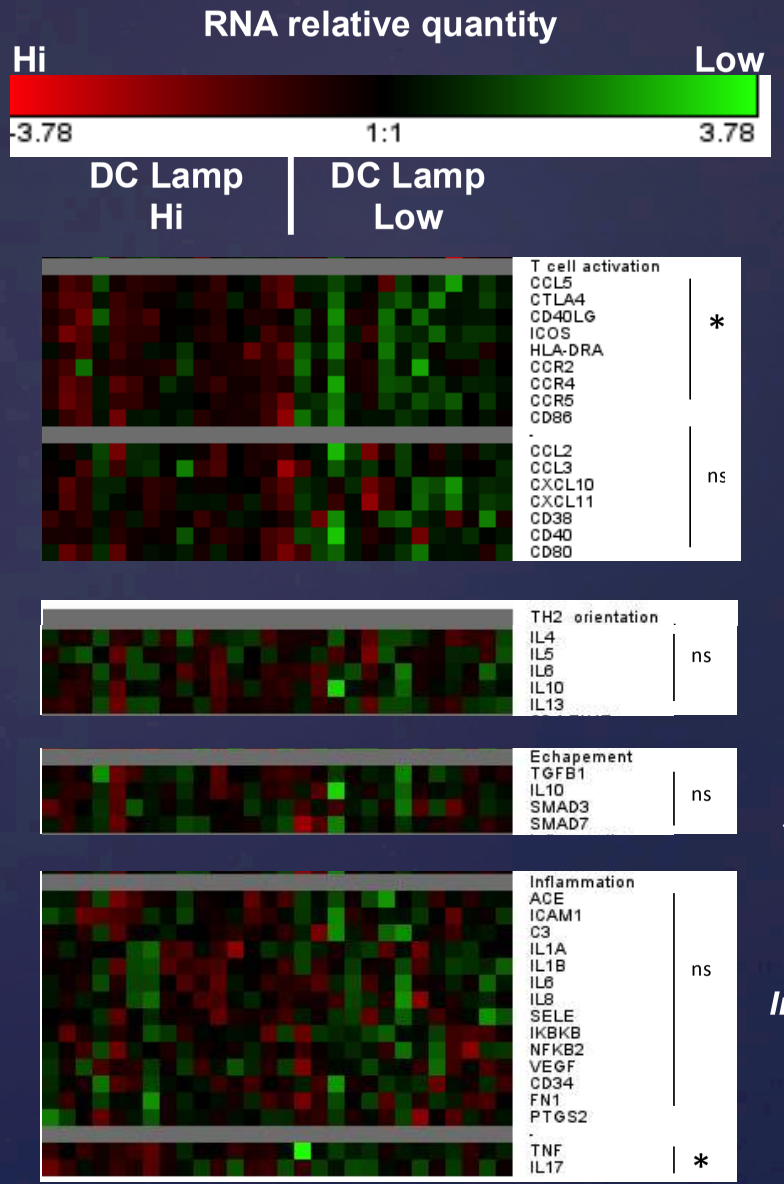


Immune populations

TLS

Th1 orientation

CD8 cytotoxicity



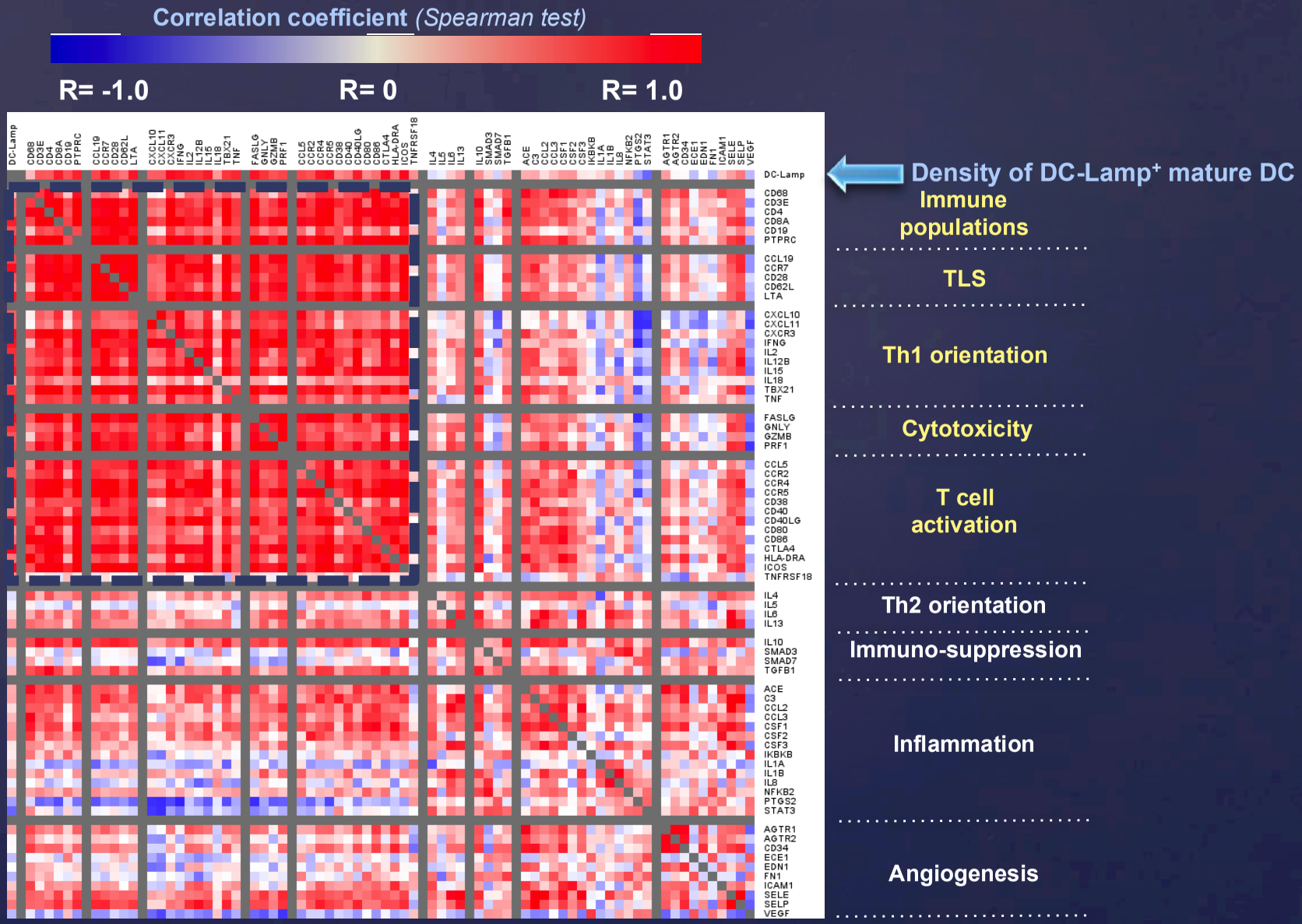
T cell activation

Th2 orientation

Immuno-suppression

Inflammation

Th1, cytotoxicity and activation genes are co-regulated, and associated with high density of mature DC



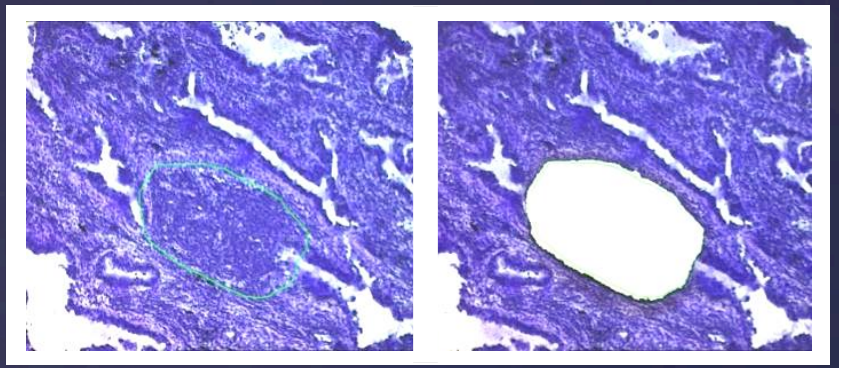
Correlation matrix using hierarchical clustering

Gene signature in TLS *versus* tumor nests

Frozen tumor

Selection of tumors with high density of TLS (DC-Lamp^{Hi} group)

ex. TLS

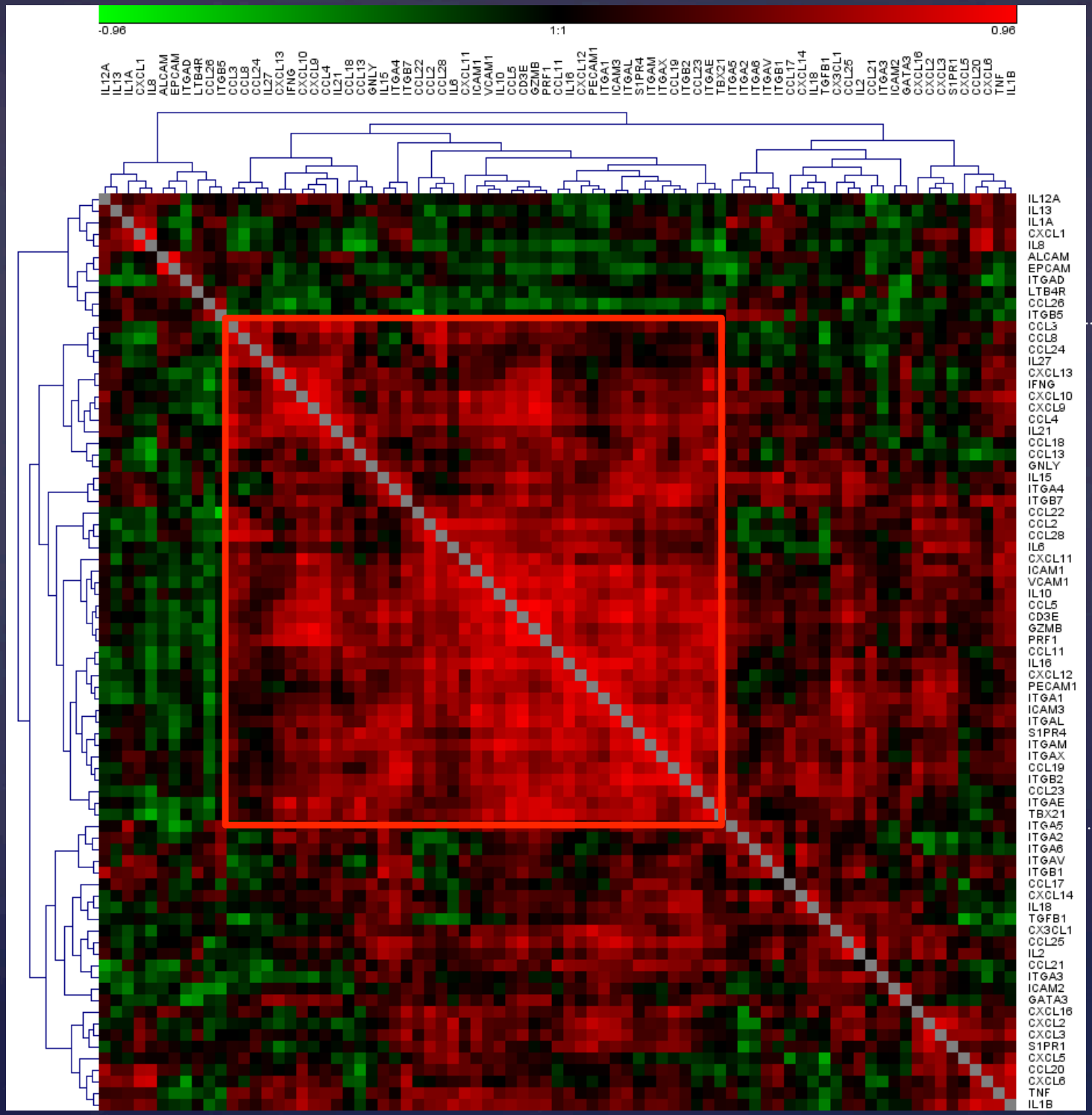


laser capture microdissection of TLS and tumor nests
(n=13 frozen tumors)

RNA extraction and validation

Gene expression *(TaqMan LDA)*

Immune signature in tumor nests of DC-Lamp^{High} tumors



Correlation matrix using hierarchical clustering

Th1 + EM T cells + cytotoxicity

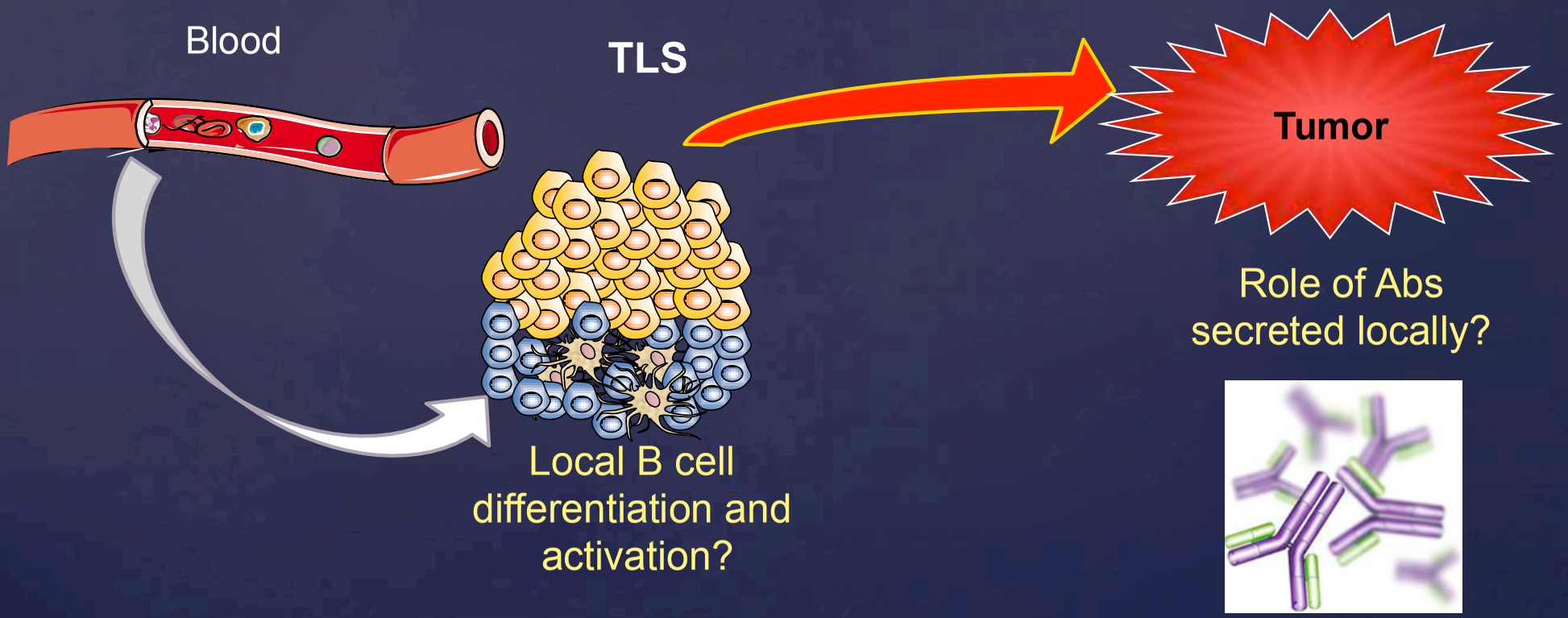
n = 13 tumors

Conclusion

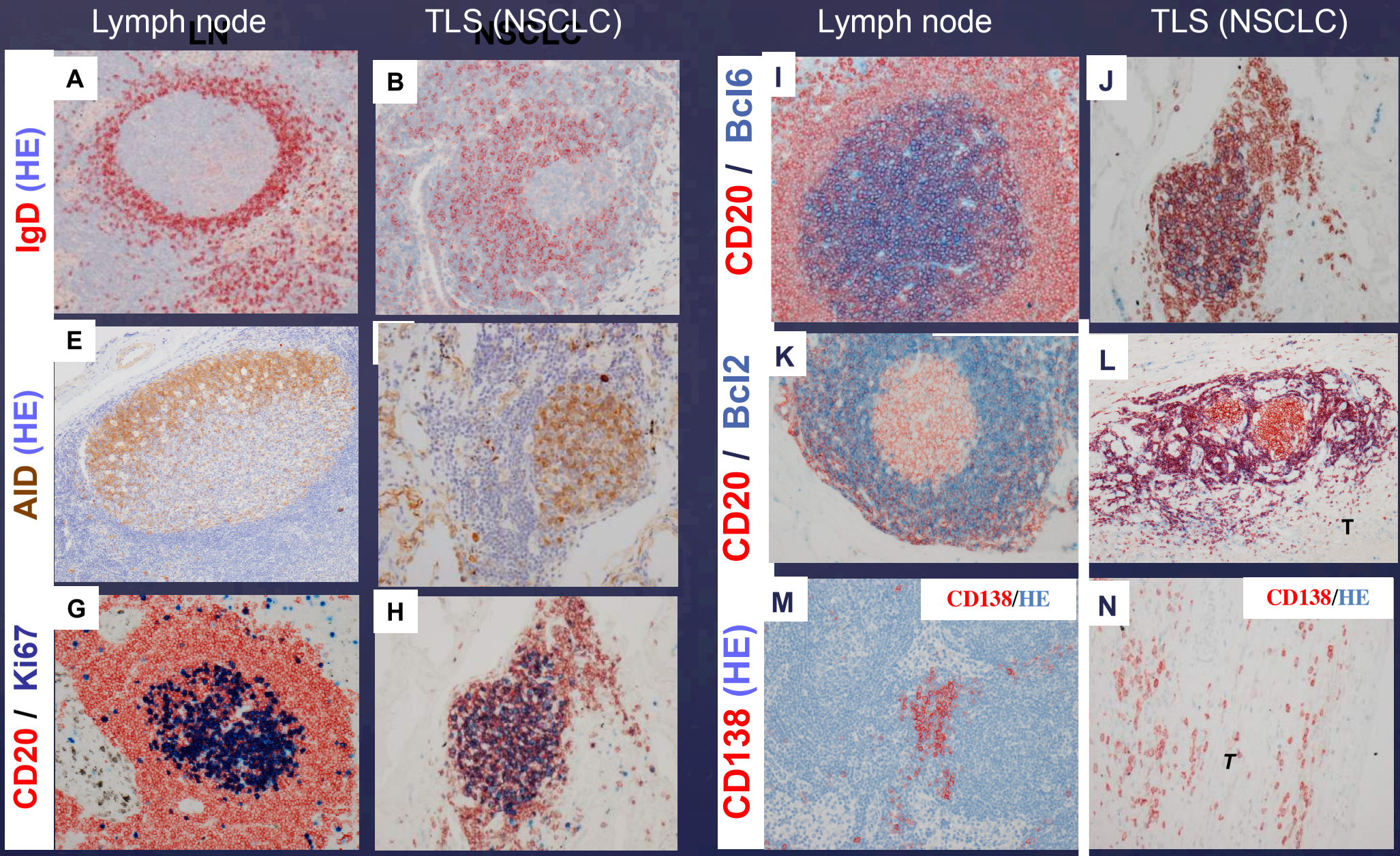
- TLS in lung cancer
  - induced by the tumor microenvironment
  - same organization as canonical lymphoid organs
- TLS-DC can orchestrate the local immune contexture
  - effector-memory phenotype
  - Th1 polarization, activation & cytotoxic markers
  - Coordination of the local immune reaction
  - Imprinting of the behaviour of tumor-infiltrating CD8+ T cells
- An important benefit for lung cancer patients
  - from early to late-stage of disease
  - identification of patients with high risk of relapse

TLS is also composed of a B-cell rich area

Role of TIL-B cells? Are they effector cells (Abs) and/or APC?



Presence of fully reactive germinal centers in TLS as observed in canonical secondary lymphoid organ

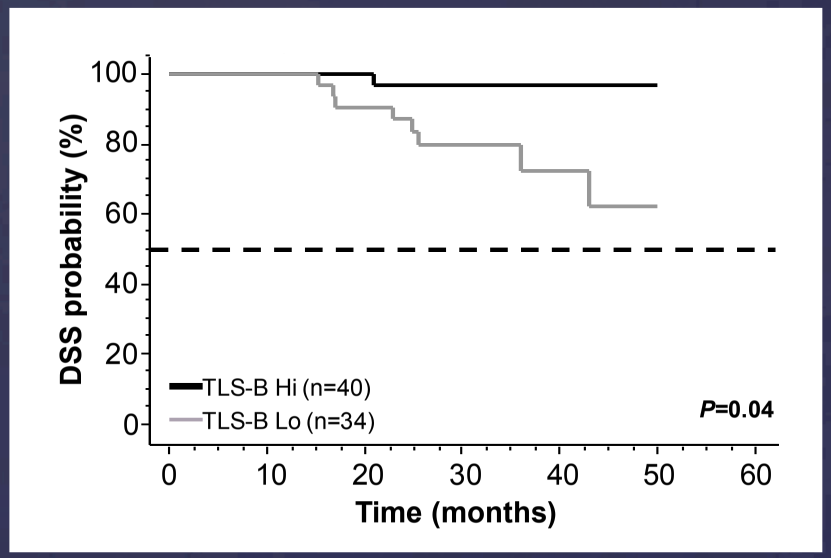


HE: hematoxylin; T: tumor nest
AID: class switch recombination + somatic hypermutation

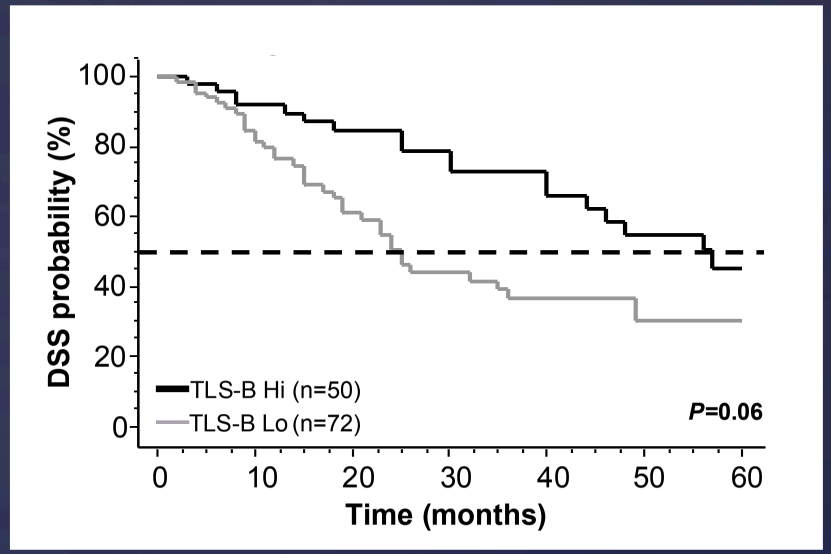
Germain *et al.*, Am J Respir Crit Care Med, 2014

High density of TLS-B cells is associated with a favorable clinical outcome

(74 early-stage NSCLC)

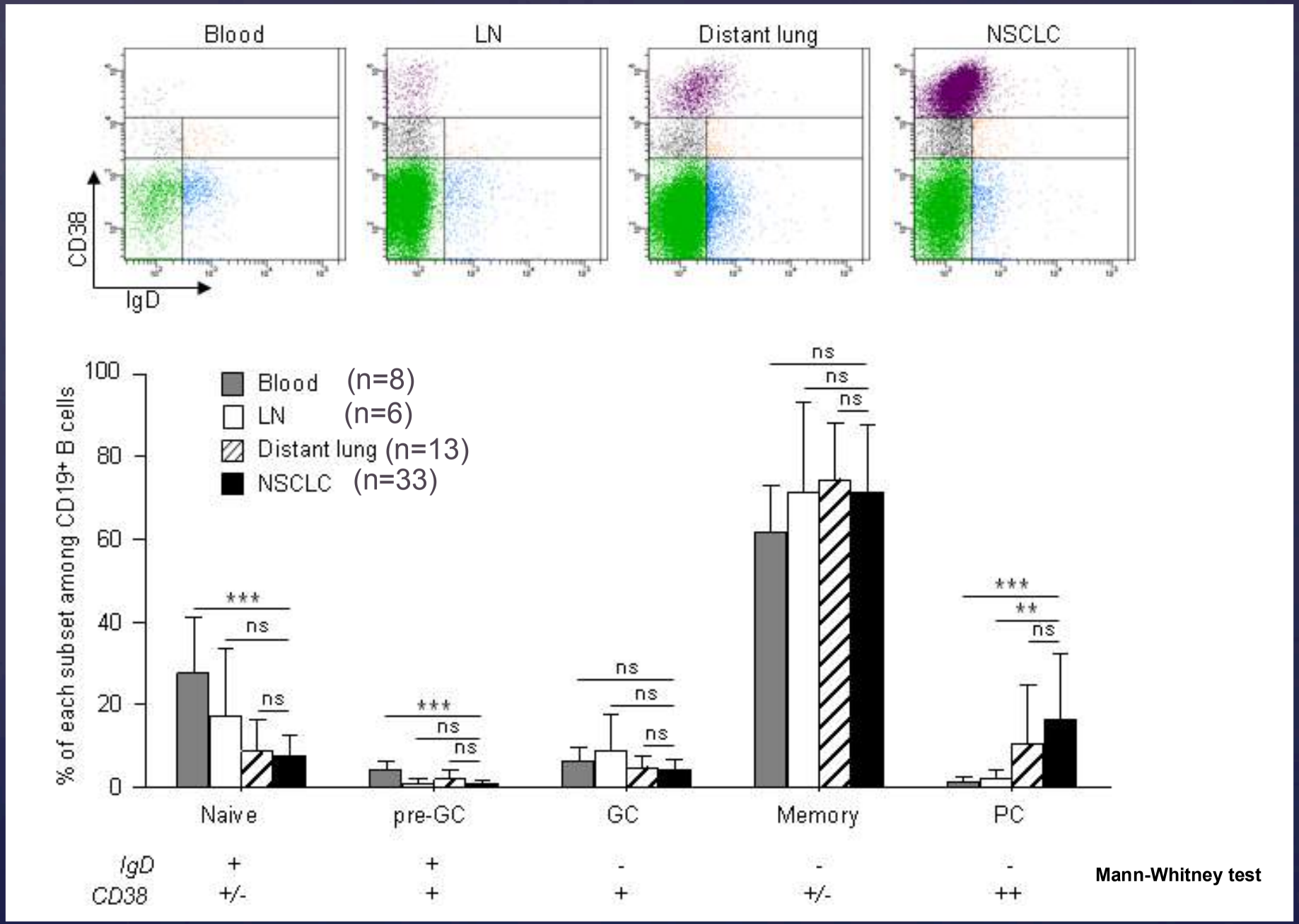


(122 advanced-stage NSCLC)

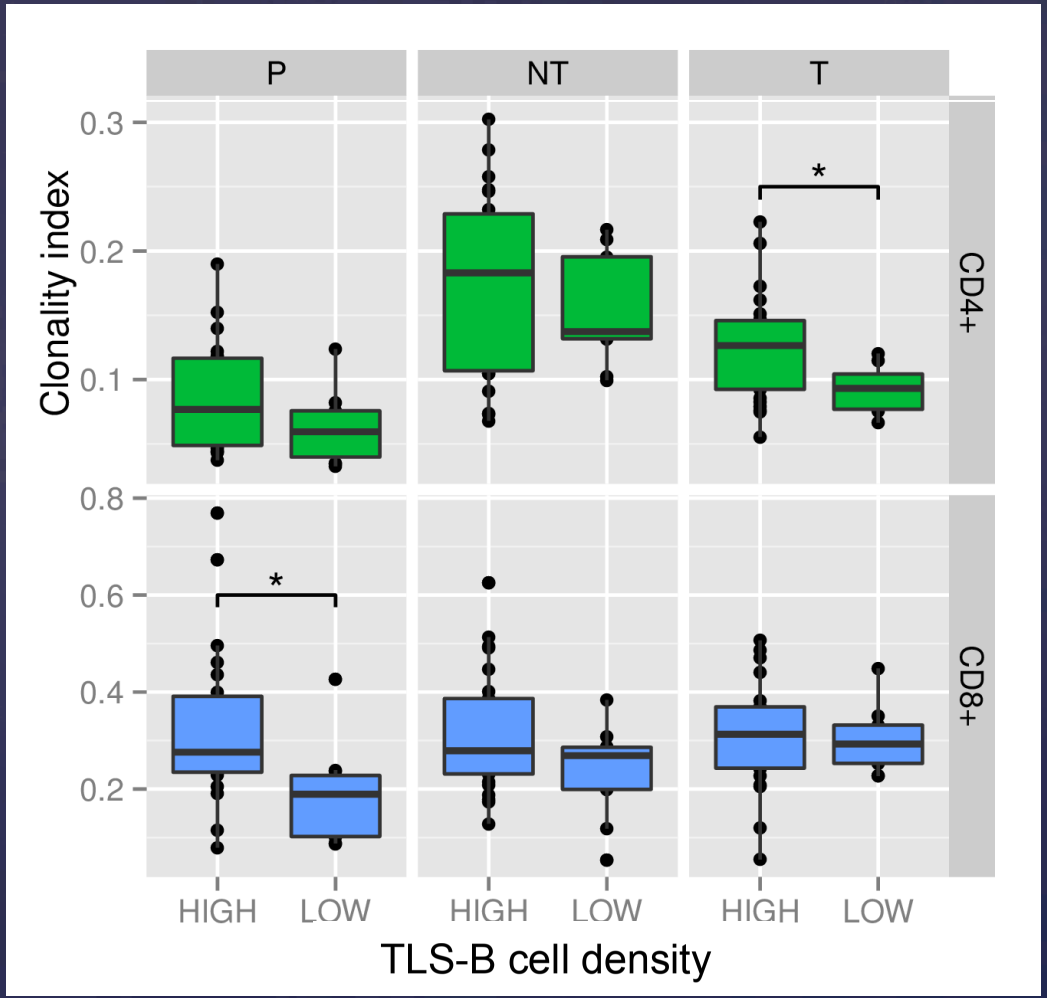


TNM stage and TLS-B are an independent prognostic factor for survival
(both cohorts of NSCLC patients)

Memory B cells and PC are the major B-cell subsets in NSCLC as opposed to LN and blood



Higher clonality of peripheral CD8^{+} and intratumoral CD4^{+} T cells in TLS-B^{high} vs TLS-B^{low} tumors suggesting an APC function of B cells in TLS



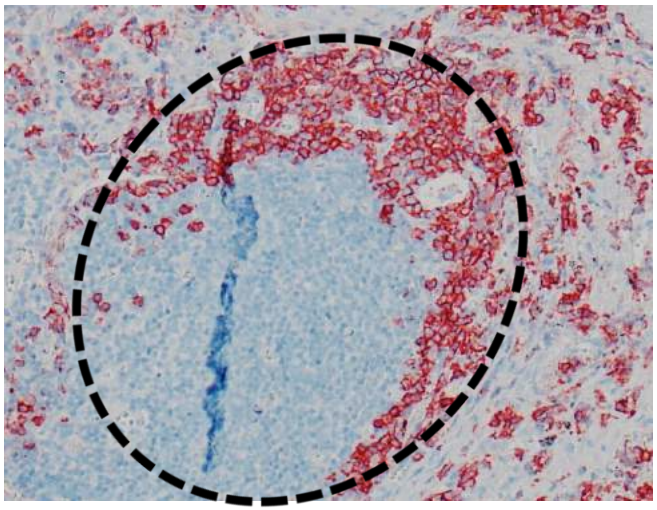
Clonal expansion of CD4^{+} T cells in the tumor

Clonal expansion of peripheral CD8^{+} T cells

Zhu *et al.*, OncoImmunology, 2015

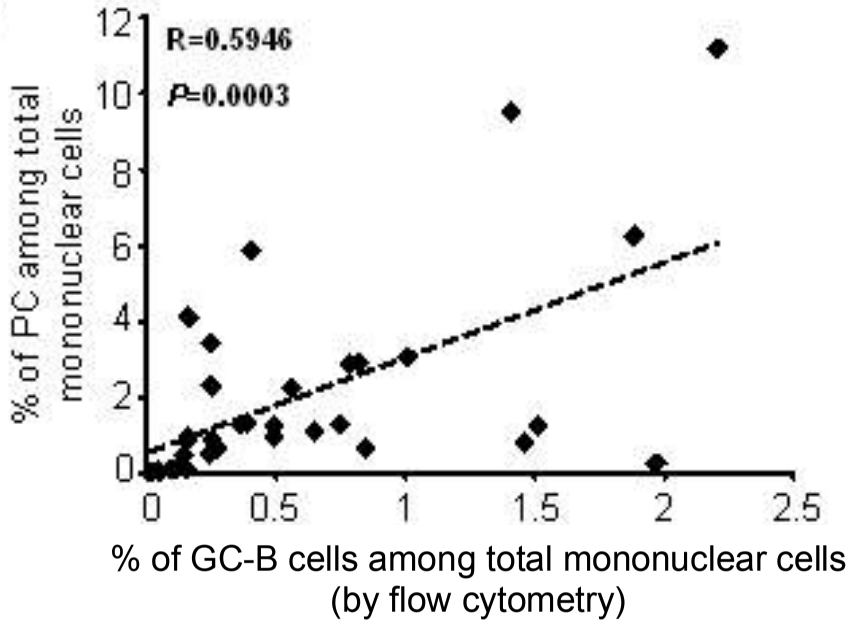
Presence of newly differentiated plasma cells in TLS

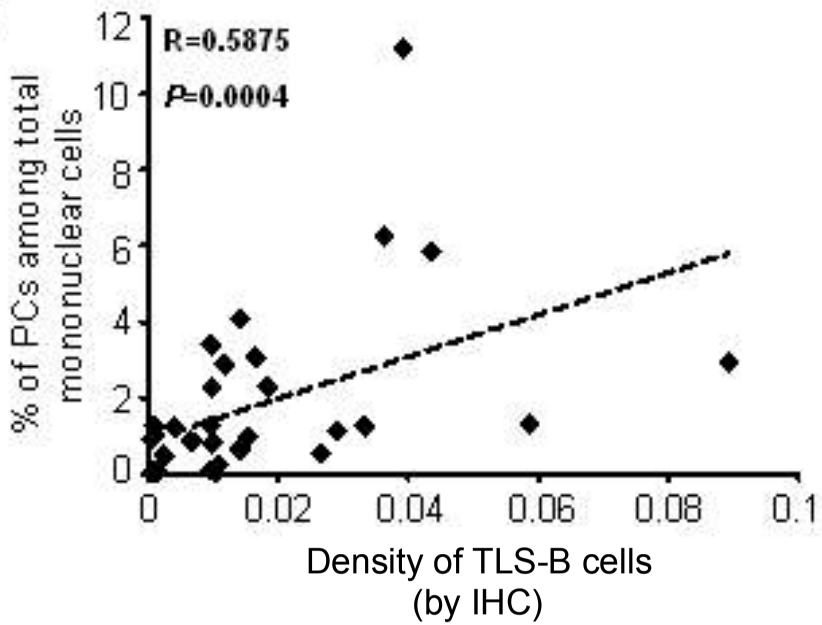
TLS



CD138 (HE)

n=32 tumors





Spearman test

Half of the tumors tested are infiltrated by B cells which secrete Abs specific to at least 1 tumor antigen

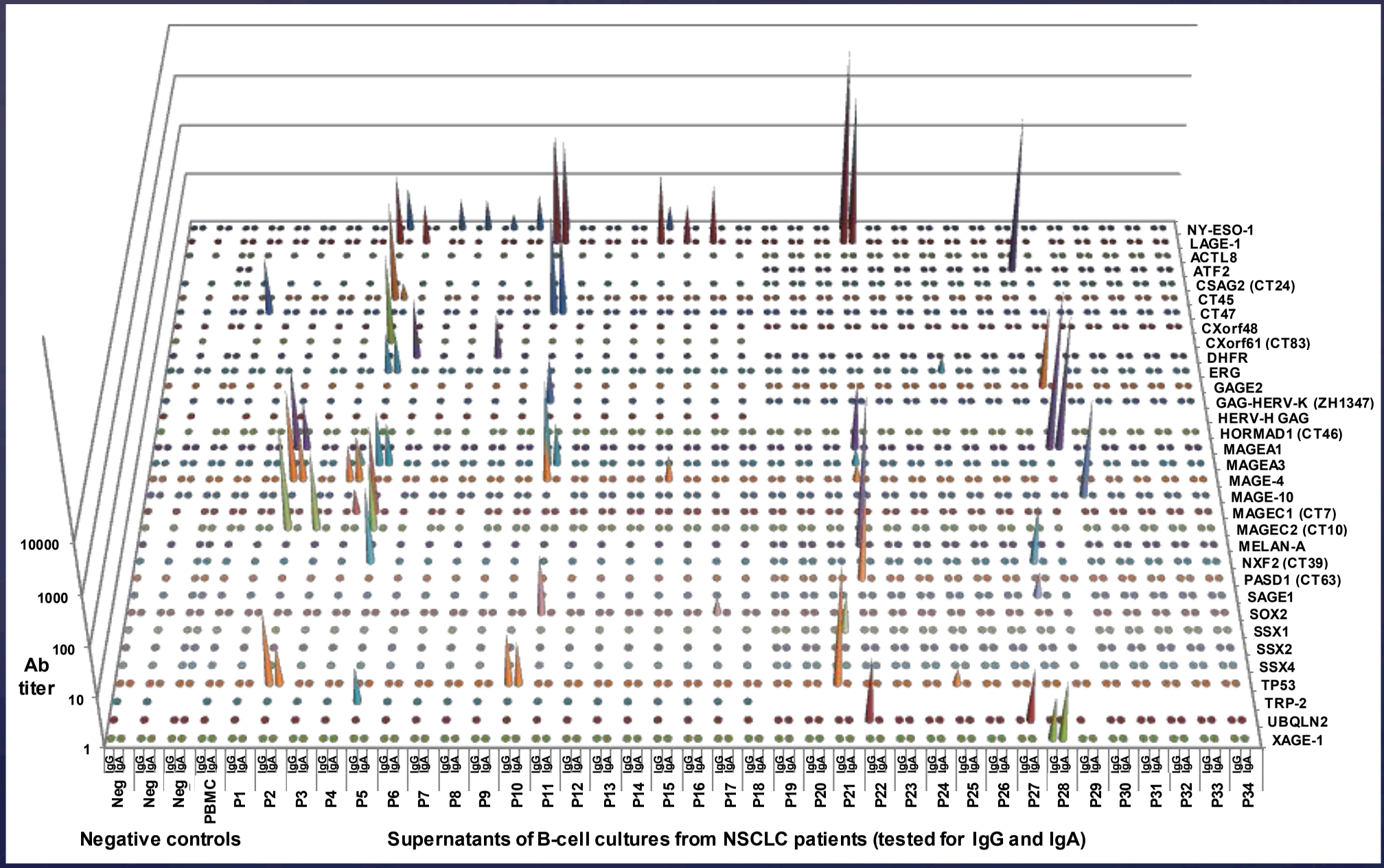
(collaboration : Dr. S. Gnjatic, Mount Sinai)

Culture of B cells sorted from tumors (n=34 patients)
IgG detection in cultured B-cell supernatant of TAAs (36 TAAs tested by ELISA)

Ag/Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	out of 15
LAGE-1				++	+			+++	++	+	++		++++			7
CT10	+++	++		++												3
P53	++						+					+++				3
MAGEA1	++												++	++++		3
MAGE-4	+++							+								2
MAGEA3				+				++								2
DHFR					+	+										2
NY-ESO-1								+++					++++			2
NXF2 (CT39)				++											(+)	2
CT47								+++								1
CT45				++												1
ACTL8			++													1
CT7				++												1
CXorf61 (CT83)				++												1
SOX2								++								1
ERG				+												1
ZH1347								+								1
TRP-2				+												1
GAGE2								+								1
MELAN-A													++++			1
XAGE-1															(+)	1
PASD1 (CT63)													++(+)			1
SAGE1															(+)	1

Germain *et al*., Am J Respir Crit Care Med, 2014

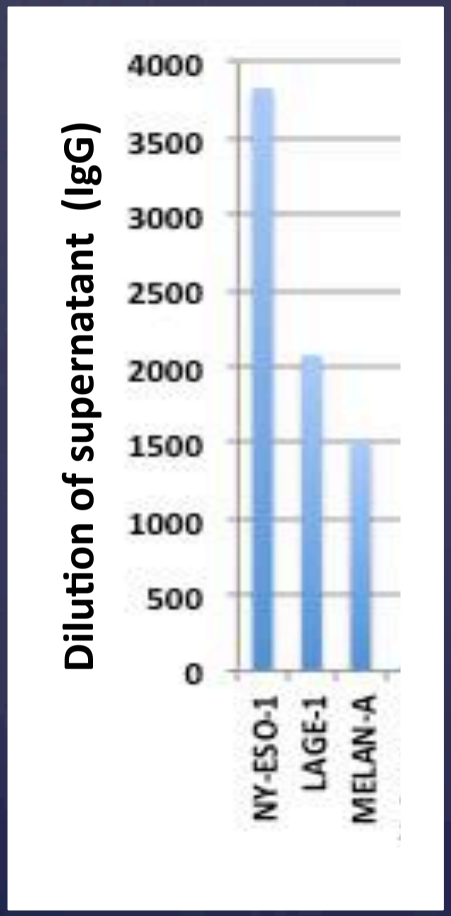
IgG and IgA secreted by intra-tumoral B cells recognize TAA

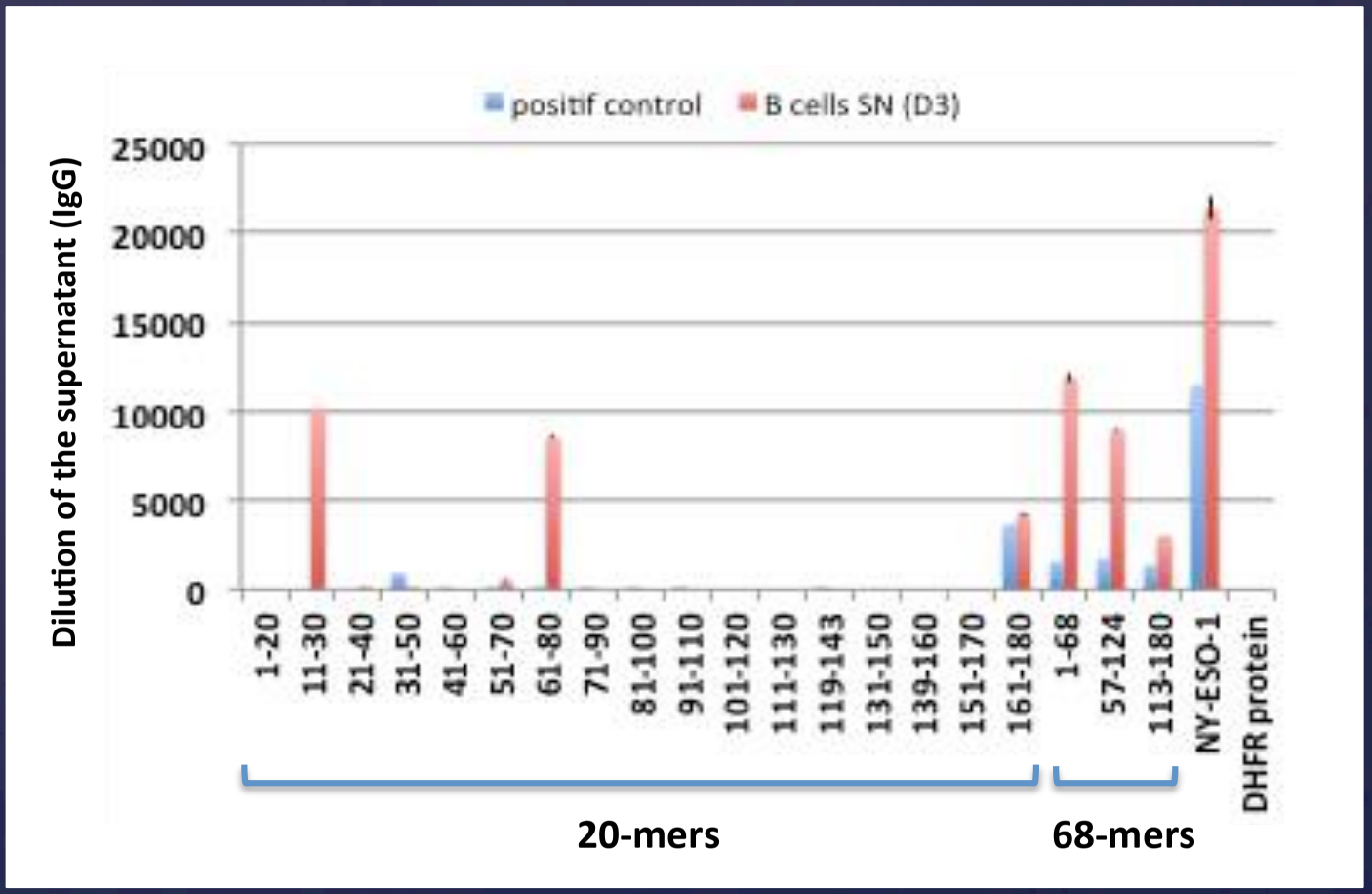


Polyclonal IgG response to NY-ESO-1 which targets 3 main regions of the protein

(collaboration : Dr. S. Gnjatic, USA)

Epitope mapping using overlapping 20-mers, 68-mers and full length NY-ESO-1 on patient P22

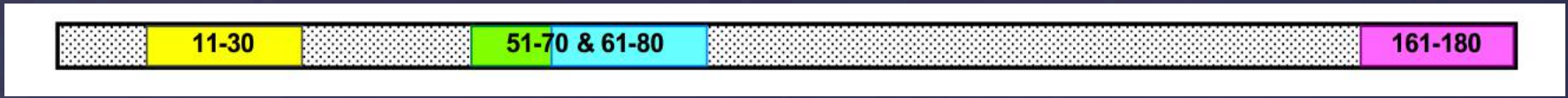


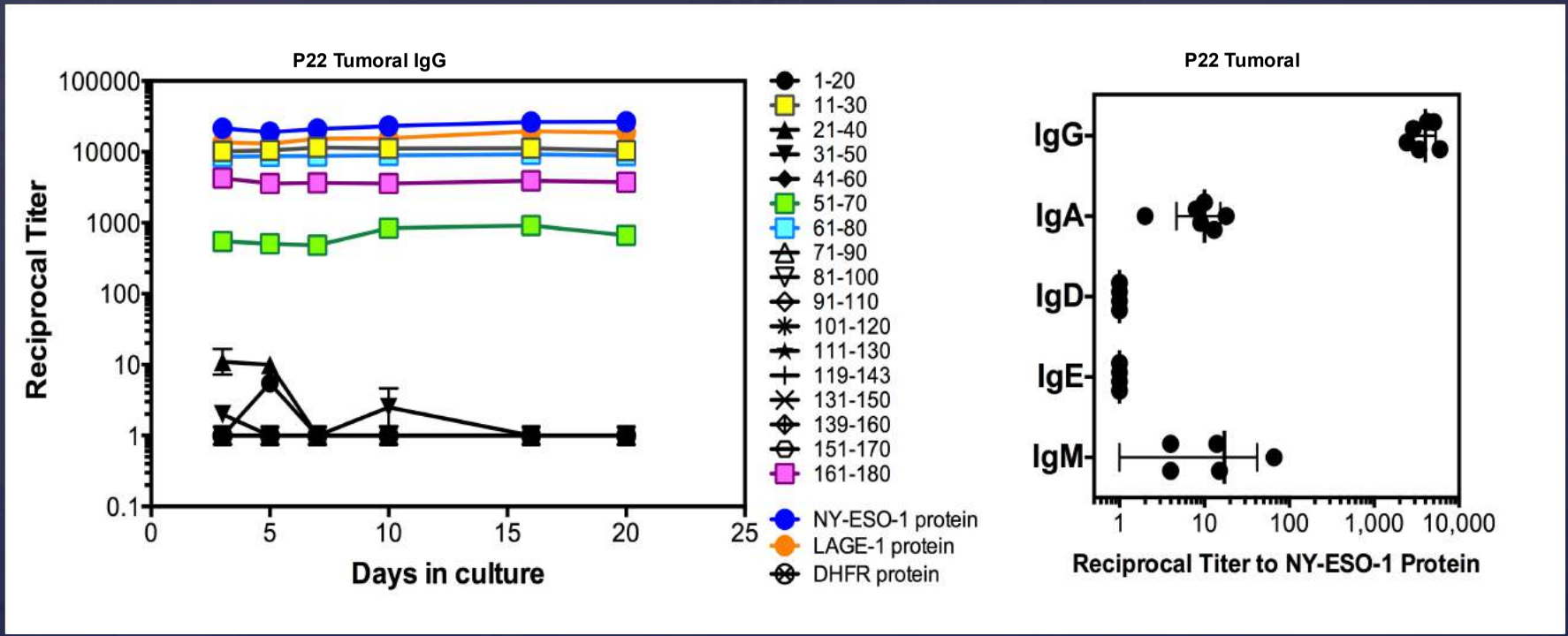


- Same results for p53 in P21 (polyclonal response)

Polyclonal IgG response to NY-ESO-1 which targets 3 main regions of the protein

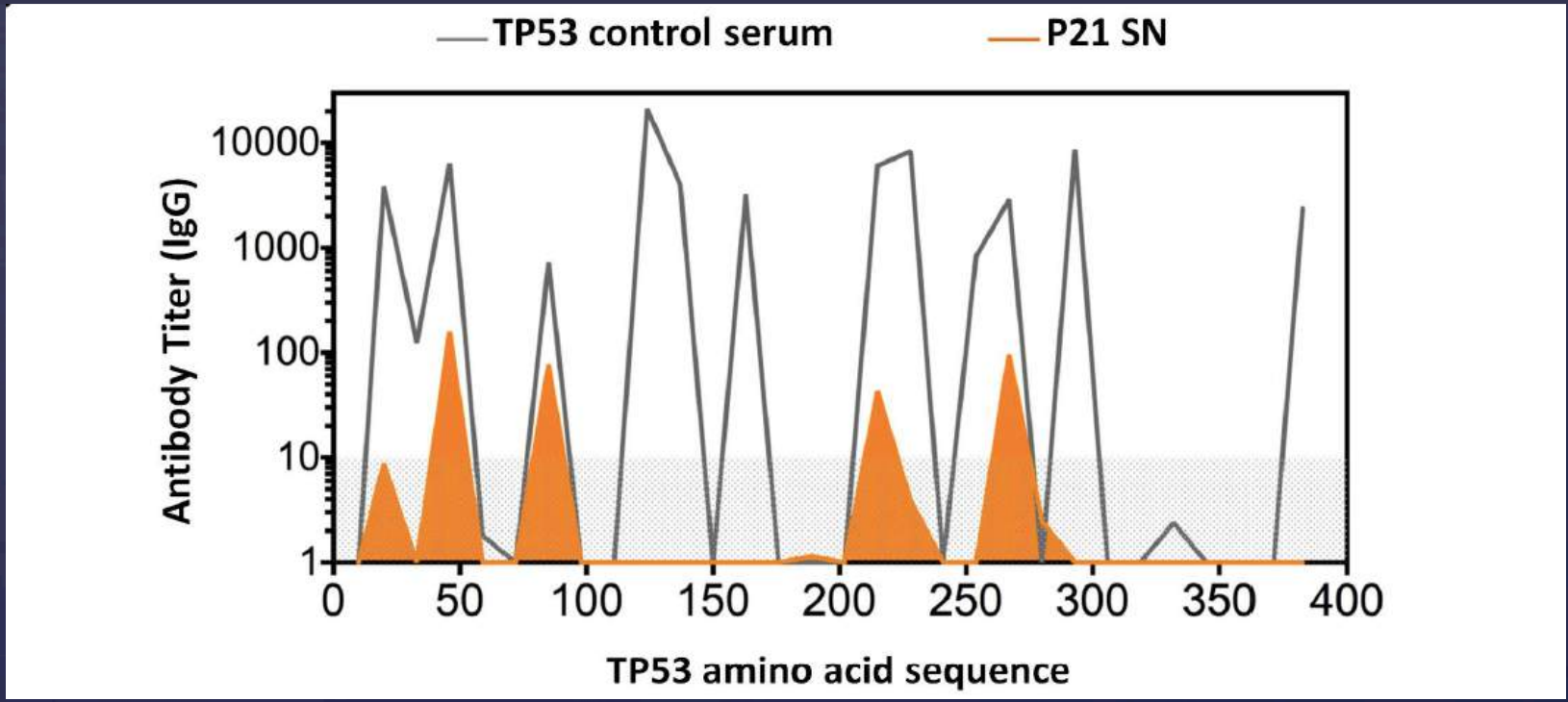
(collaboration : Dr. S. Gnjatic, USA)





Polyclonal IgG response to p53
which targets 4 main regions of the protein

(collaboration : Dr. S. Gnjatic, USA)



Conclusion

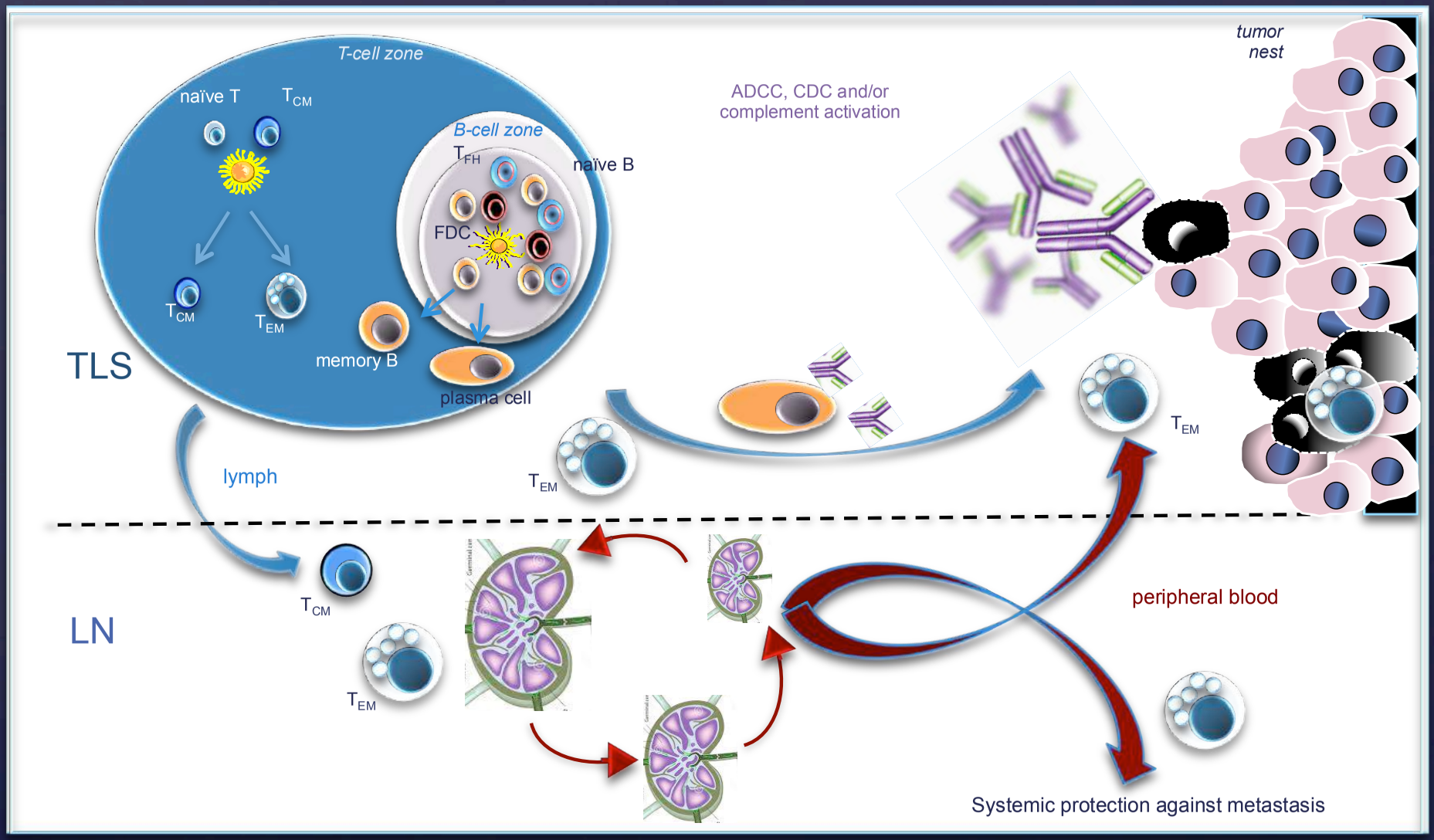
- ✓ TLS-B cells display the same organization as in SLO
- ✓ Density of TLS-B cells is highly predictive of survival
  - ✓ To allow the identification of patients with high risk of relapse
- ✓ B cell follicle is correlated with the development of humoral response against tumor-associated antigens
- ✓ Clonal expansion of peripheral $CD8^+$ and intratumoral $CD4^+$ T cells is associated with increased TLS-B cell density

TLS as a powerful biomarker in human cancers

	Criteria	Cancer type	Stages of the disease	No. of patients	TLS detection by IHC	TLS detection by gene expression	Prognostic value
Naïve cancer patients	Primary tumors	Breast carcinoma	I to III	146	PNAd	-	Positive
			I to III	146	DC-Lamp	-	Positive
			I to III	794	-	T_{FH}, Th1, CXCL13	Positive
		Breast carcinoma (triple negative)	I to III	769	HES (+TIL)	-	Positive
		Colorectal cancer	I to IV	350	HES	-	Positive
			ND	25	DC-Lamp	-	Positive
			I to IV	40	CD3, CD83	-	Positive
			II	185	CD3	-	Positive
			III	166	CD3	-	No value
			0 to IV-A	21	-	12-chemokine genes	Positive
			I to IV	125	-	CXCL13 and CD20	Positive
		Gastric cancer	all without chemo	82	CD20	-	Positive
			I to III	365	-	both Th1 and B	Positive
		NSCLC	I to II	74	DC-Lamp	-	Positive
			I to IV	362	DC-Lamp	-	Positive
			III with neo-adj. chemo	122	DC-Lamp	-	Positive
			III with neo-adj. chemo	122	DC-Lamp, CD20	-	Positive
			III with neo-adj. chemo	122	CD20	-	Positive
		Melanoma	I-A to III-A	82	DC-Lamp	-	Positive
			IV	21	-	12-chemokine genes	Positive
		Merkel cell carcinoma	I to IV	21	CD4, CD8, CD20, S100	-	Positive
		Oral SCC	all	80	CD3, CD20, CD21, BCL6, PNAd	-	Positive
		Pancreatic cancer	all	308+226	HE	-	Positive
		RCC	all	135	DC-Lamp	-	Positive
	Metastatic tumors	Colorectal cancer (liver)	all	14+51	CD20	-	Positive
		Colorectal cancer (lung)	ND	140	DC-Lamp	-	Positive
Vaccinated cancer patients	HPV DNA vaccine	CIN	CIN2/3	12	CD3, CD20, PNAd	-	TLS neogenesis
	G-VAX	PDAC	ND	54	CD3, CD20, CD21, CD68, CD83, CD163, DC-Lamp, CCL21, FoxP3, Podoplanin	-	TLS neogenesis

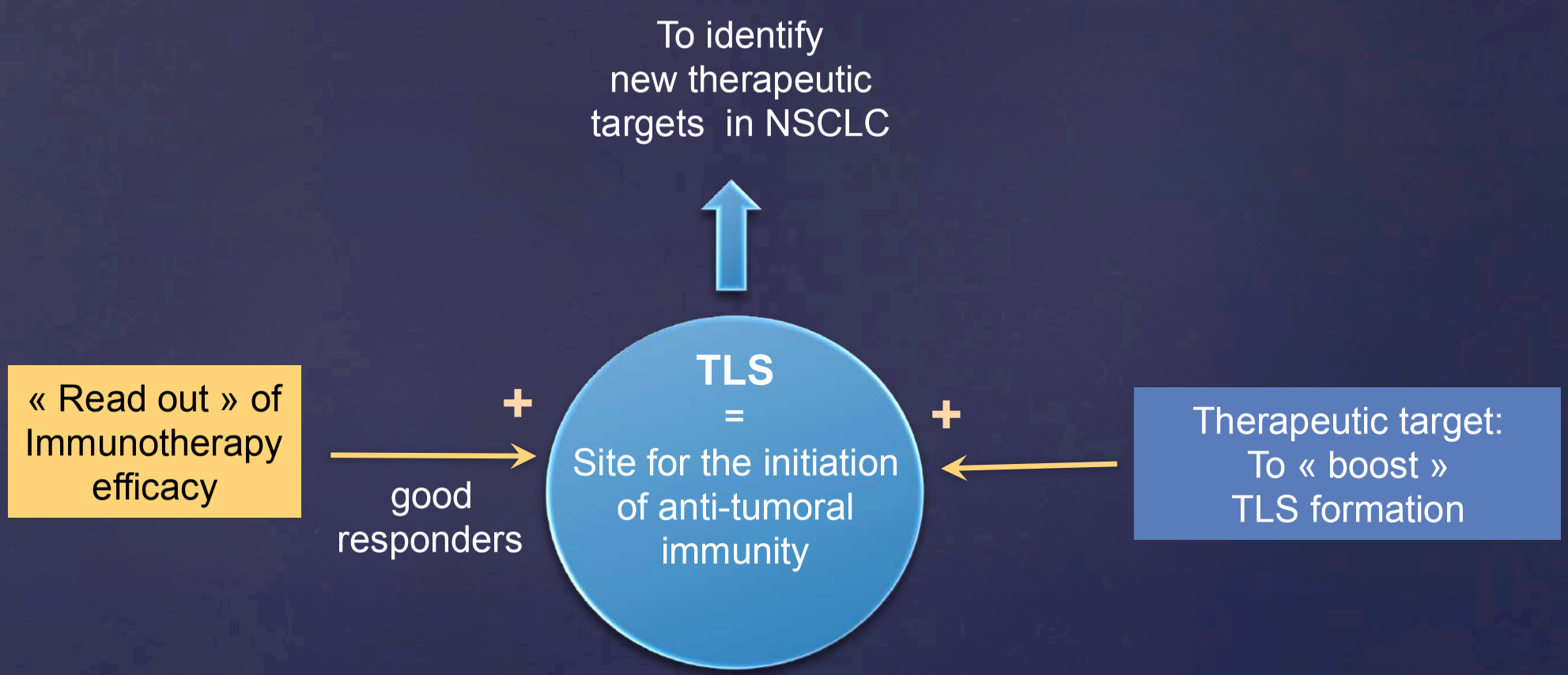
Dieu-Nosjean *et al*., Immunol. Rev, 2016

Model:
role of TLS in the initiation of protective immune responses against tumor and metastasis



=> Efficient immune response is shaped in tumor-associated TLS

TLS and Immunotherapy



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