

Con il Patrocinio di



NSCLC avanzato: quali novità nel 2018?



NEGRAR
30 Ottobre 2018

Centro Formazione
IRCCS Ospedale Sacro Cuore Don Calabria

Immunoterapia: l'Anatomo Patologo incontra l'Oncologo...

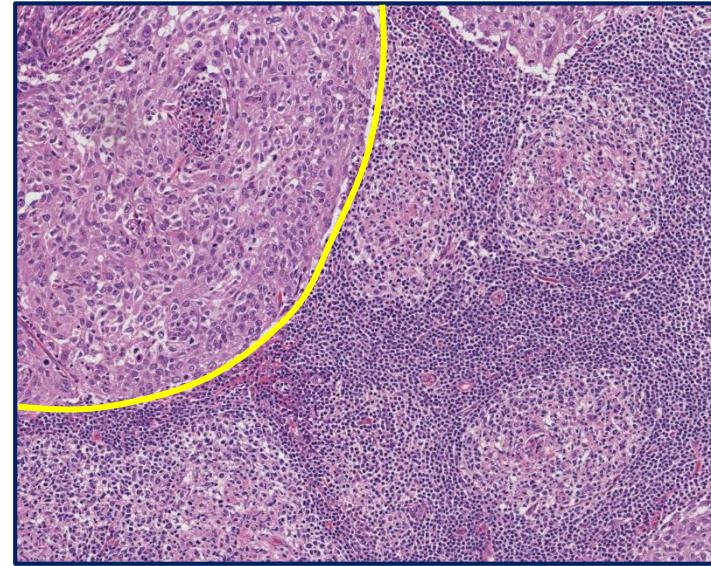
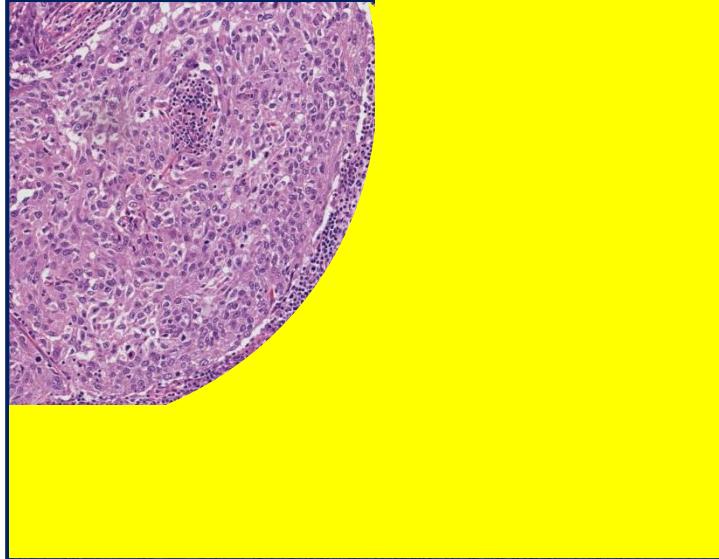
TILs

Giuseppe Bogina

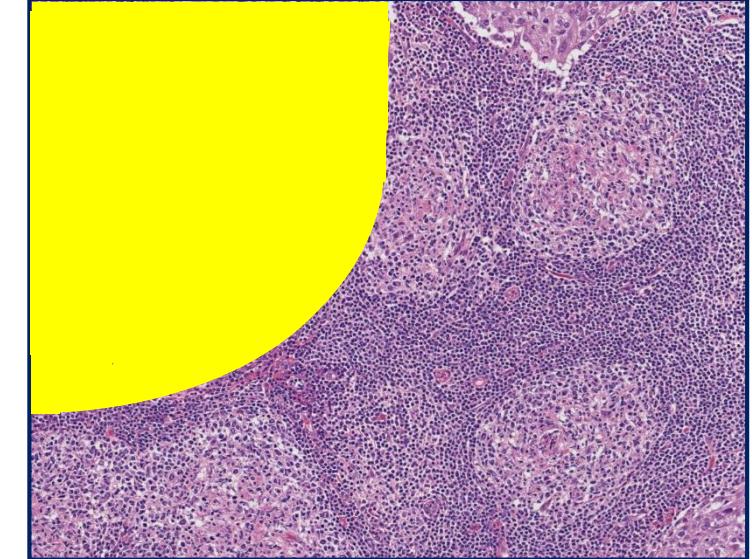
giuseppe.bogina@sacrocuore.it

RIVOLUZIONE IN ONCOLOGIA

Visione Tumore-centrica



Visione Microambiente-centrica



RIVOLUZIONE IN ONCOLOGIA

PubMed Advanced Search Builder

tumor infiltrating lymphocytes[Title]

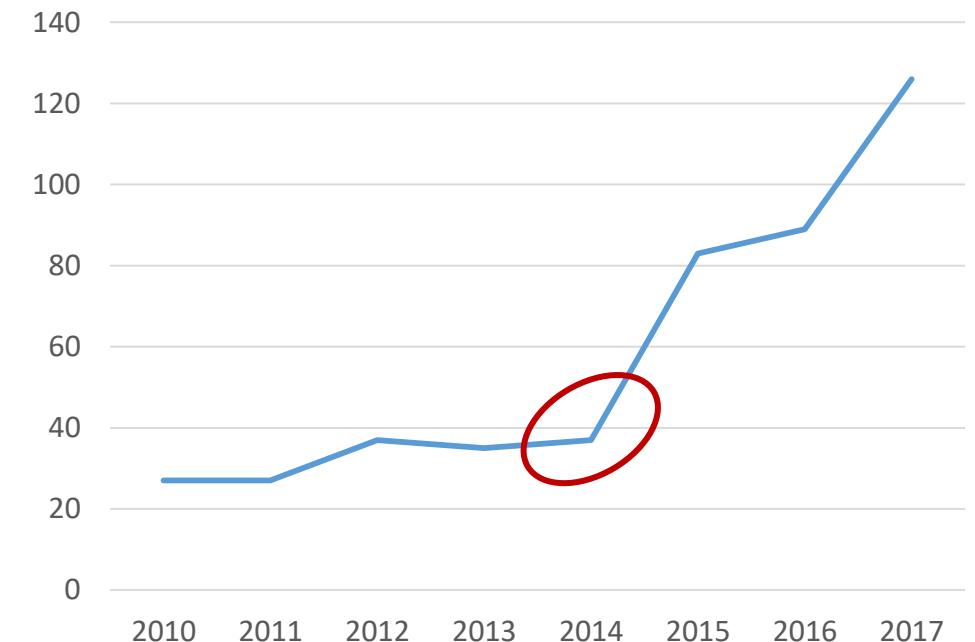
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Builder

Title tumor infiltrating lymphocytes

AND Date - Publication YYYY/MM/DD to present [Show index list](#)

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PubMed Advanced Search Builder

immunotherapy[Title]

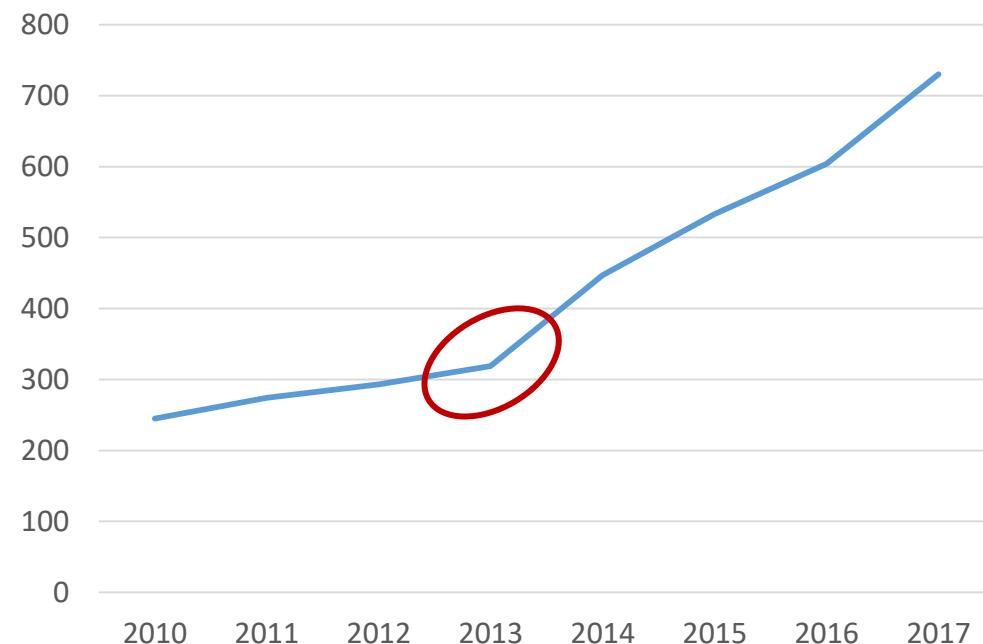
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Title immunotherapy

AND Date - Publication YYYY/MM/DD to present [Show index list](#)

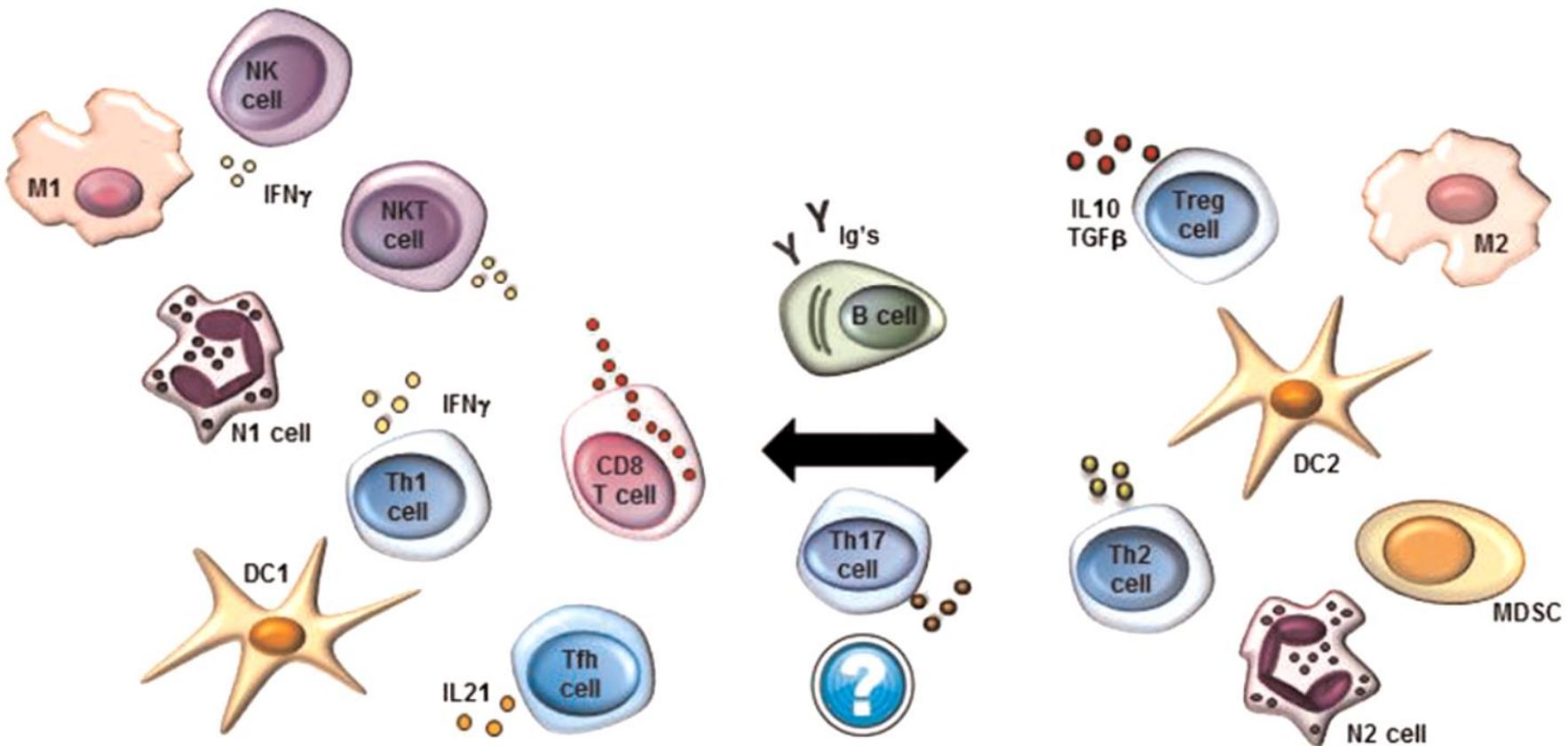
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TILs



TILs



CD20: linfociti B

CD3: linfociti T

CD8: linfociti T citotossici

CD4: linfociti T helper

FOXP3: linfociti Treg

CD68: istiociti

Th = helper CD4 $^{+}$ T

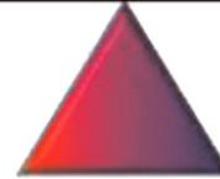
M = macrofage

N = neutrofili

DC = dendritični ćelijski

MDSC = myeloid suppressor

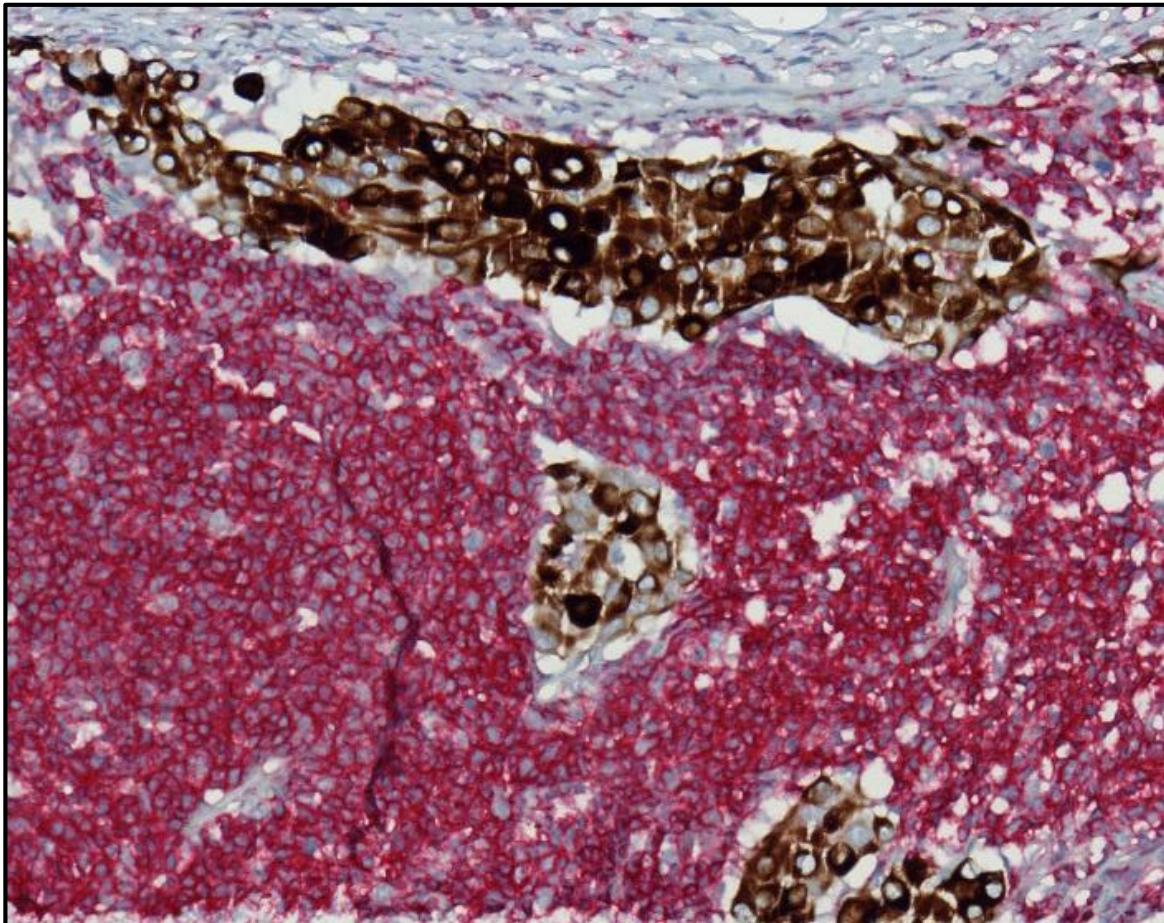
Tumor Suppression



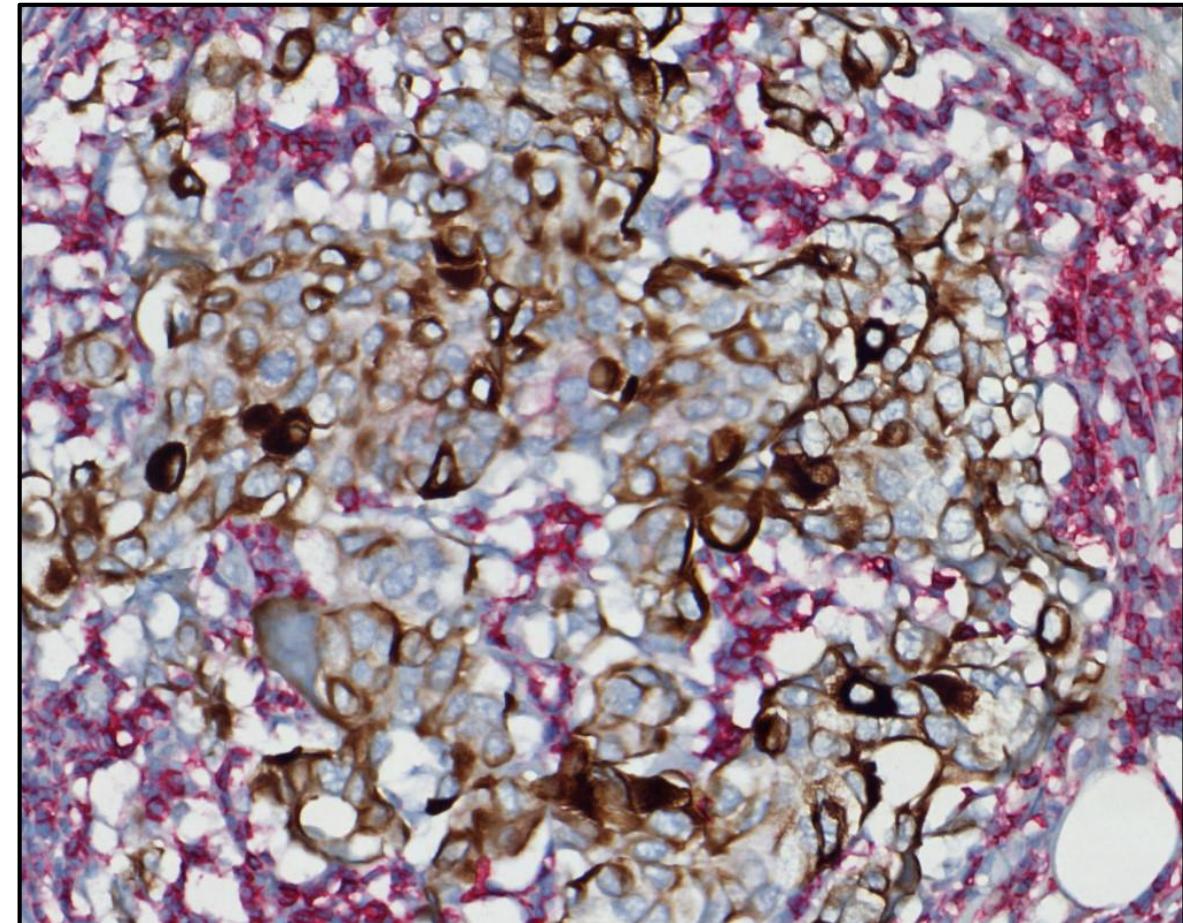
Tumor Progression



TILs



STROMAL TILs

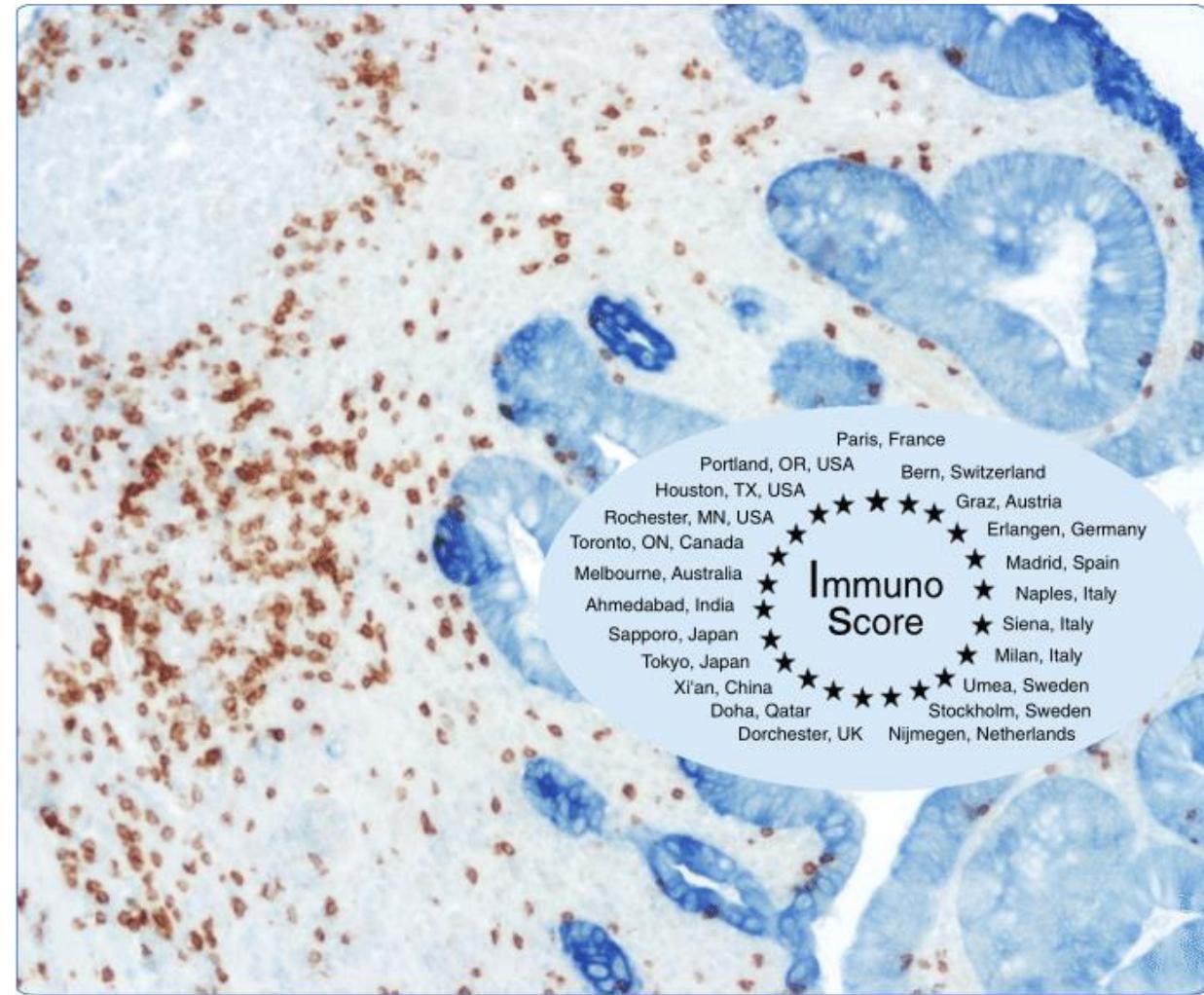
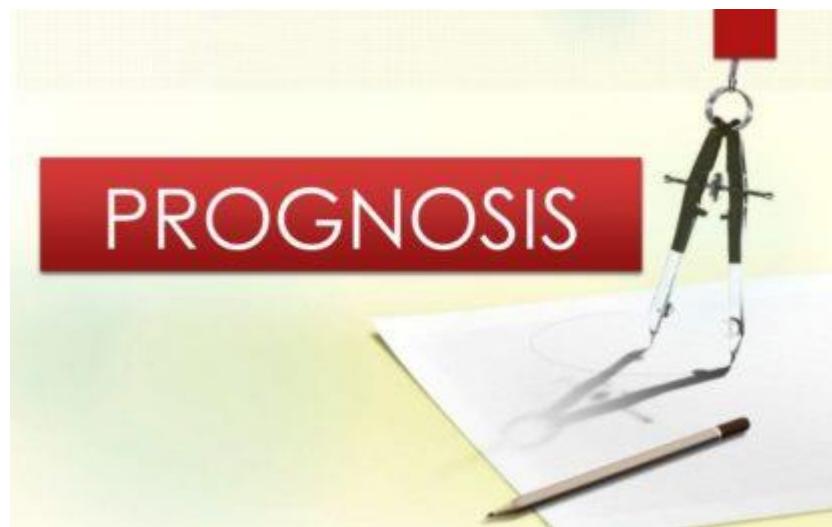


INTRATUMORAL TILs

TILs

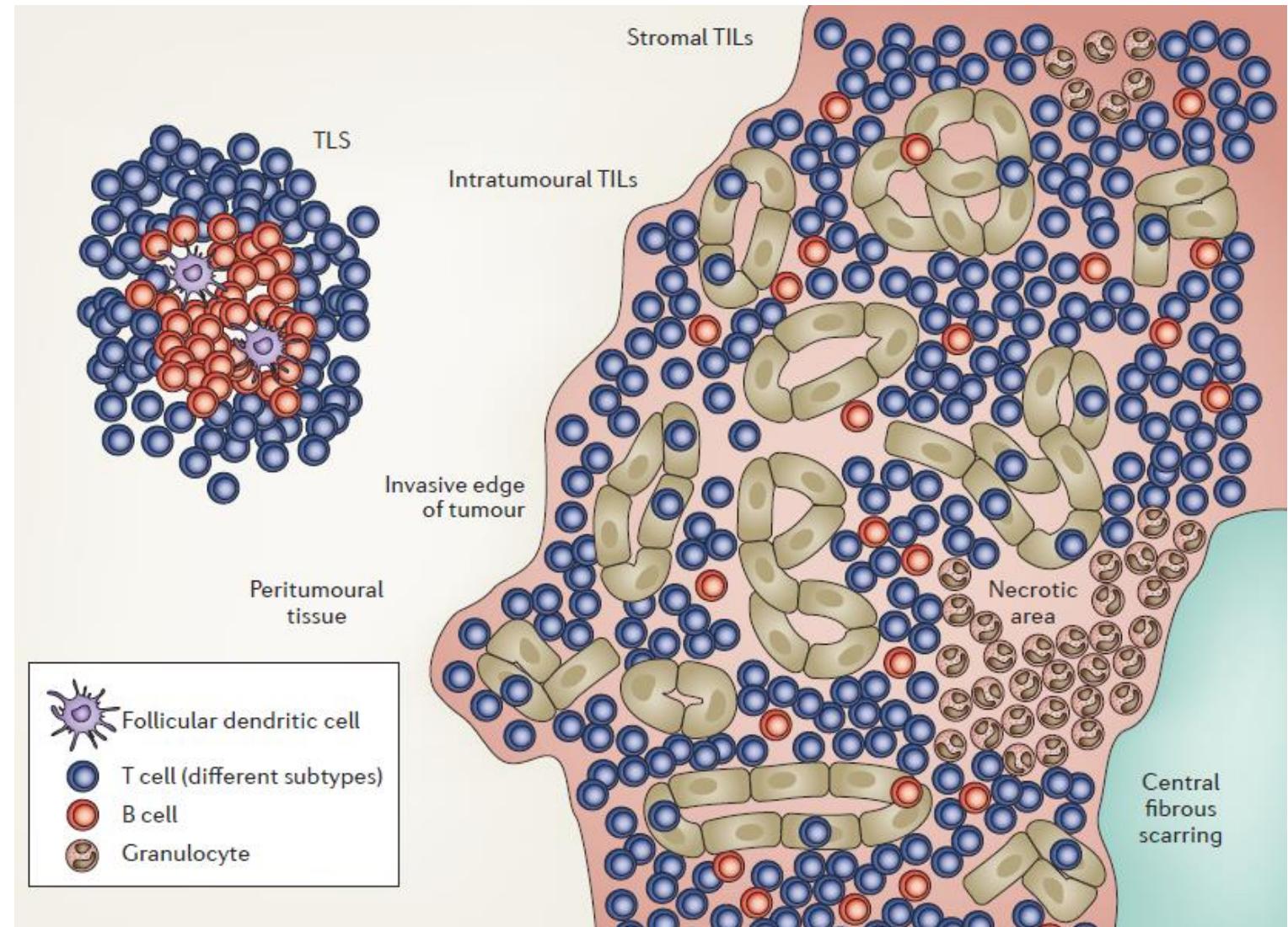


Cancer classification usign the Immunoscore: A worldwide task force



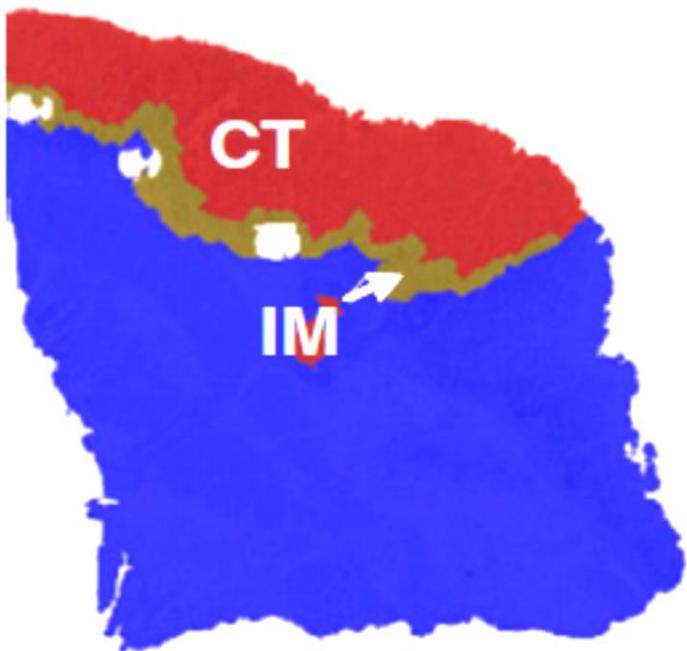
TILs MAMMELLA

The evaluation of TILs
in breast cancer:
recommendations by
an International TILs
Working Group 2014

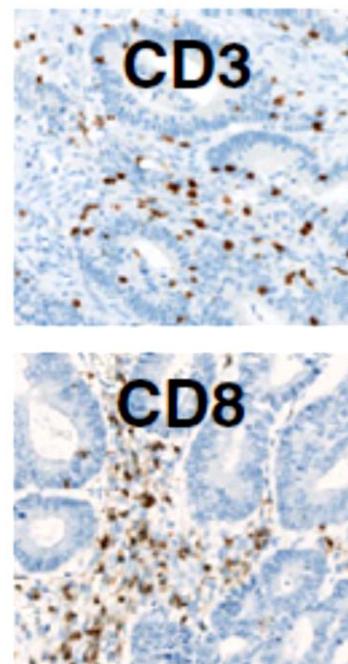


TILs COLON

Tumor regions (CT & IM)



Immunostainings



Immunoscore (CT + IM)



TILs POLMONE ?

..... However, the prognostic value of TILs in lung cancer is still controversial, varying with the distribution site and cell types.

Cellular Physiology and Biochemistry 2015

TILs may be a potential prognostic biomarker in lung cancer; however, its role is still controversial, probably due to both variable composition and heterogeneous distribution.

Expert Opinion on Biologica Therapy 2017

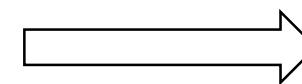
TILs POLMONE



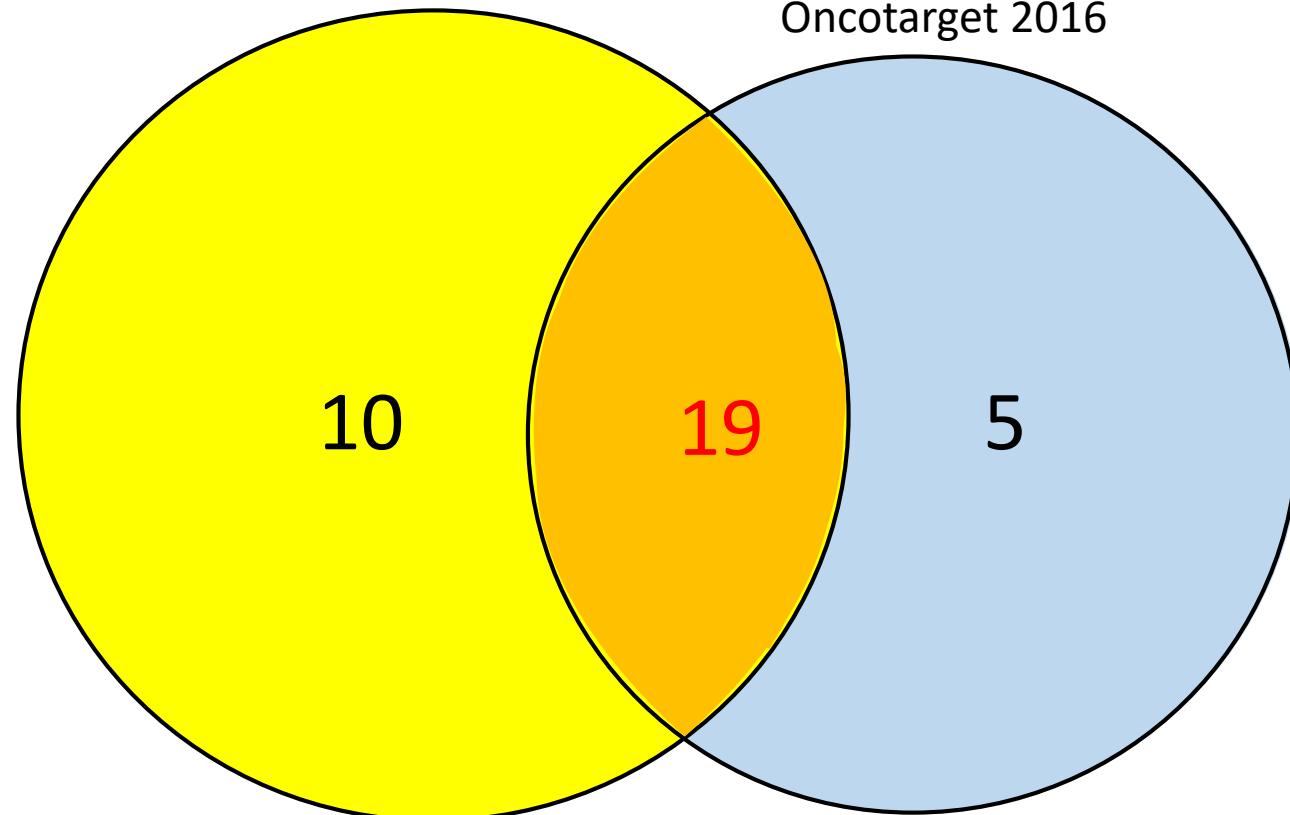
TILs POLMONE

Cell Physiol Biochem 2016

Oncotarget 2016



METANALISI



$$= 34$$

+4

$$= 38$$

Perchè?



TILs POLMONE

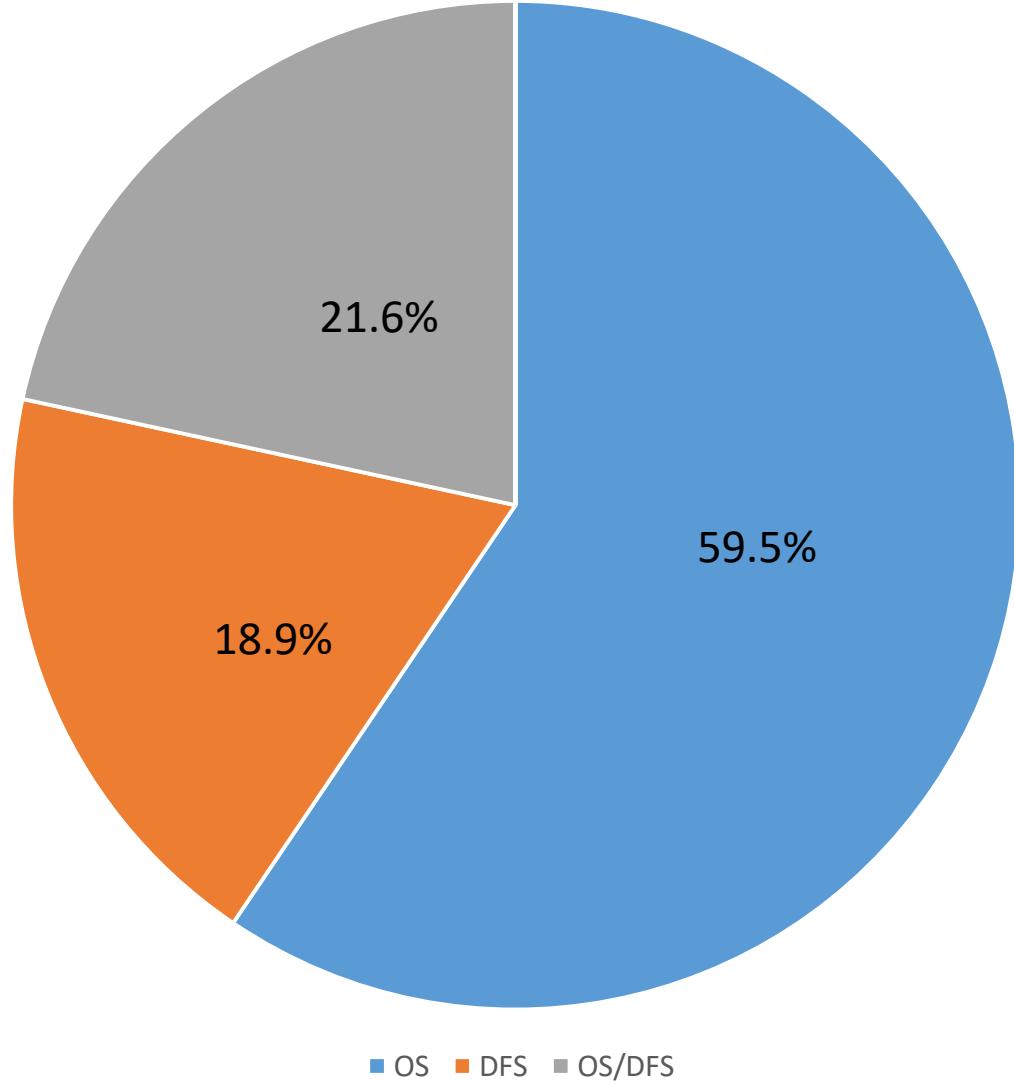
PROGNOSI

Perchè?



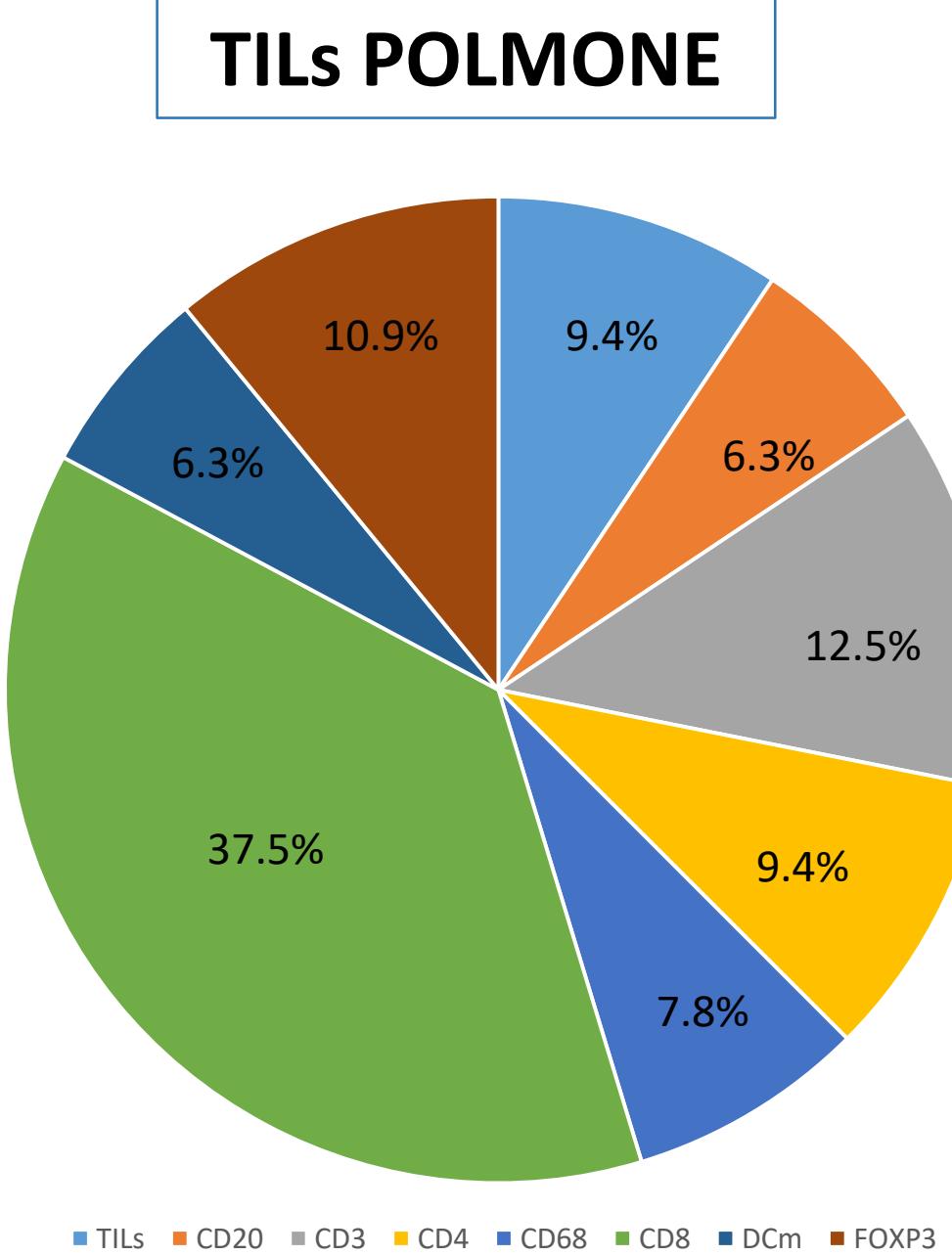
TILs POLMONE

PROGNOSI





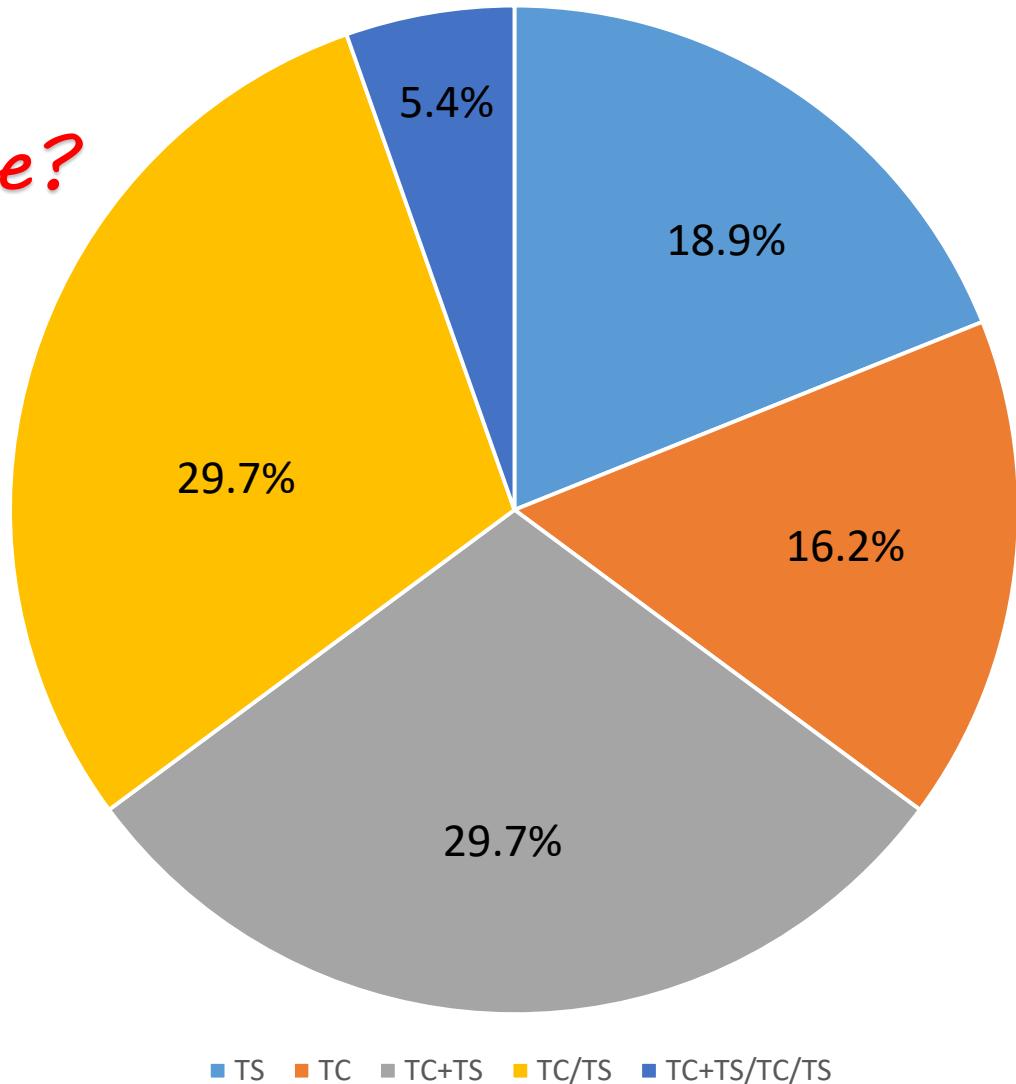
POPOLAZIONE CELLULARE



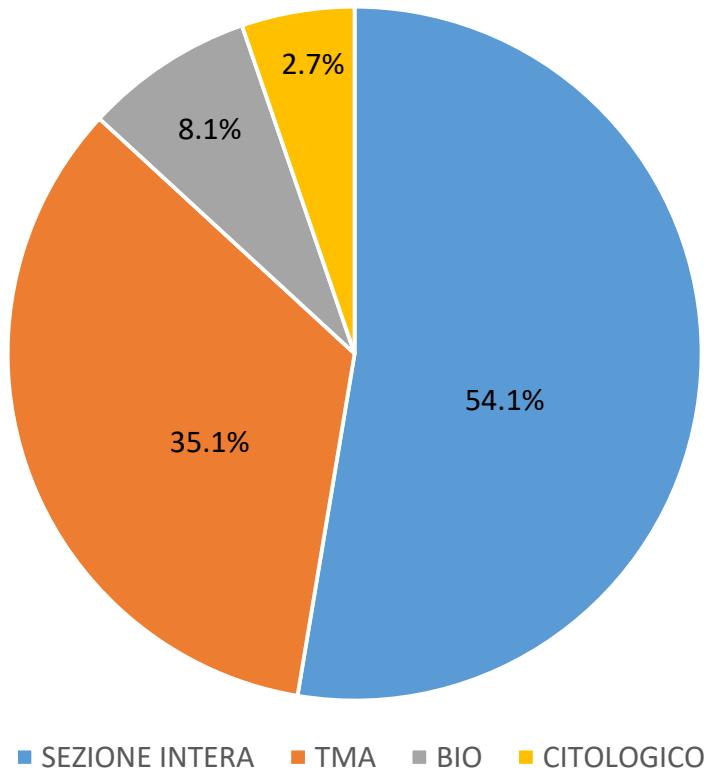


TILs POLMONE

SEDE



MATERIALE



■ TS ■ TC ■ TC+TS ■ TC/TS ■ TC+TS/TC/TS

■ SEZIONE INTERA ■ TMA ■ BIO ■ CITOLOGICO

Dove?



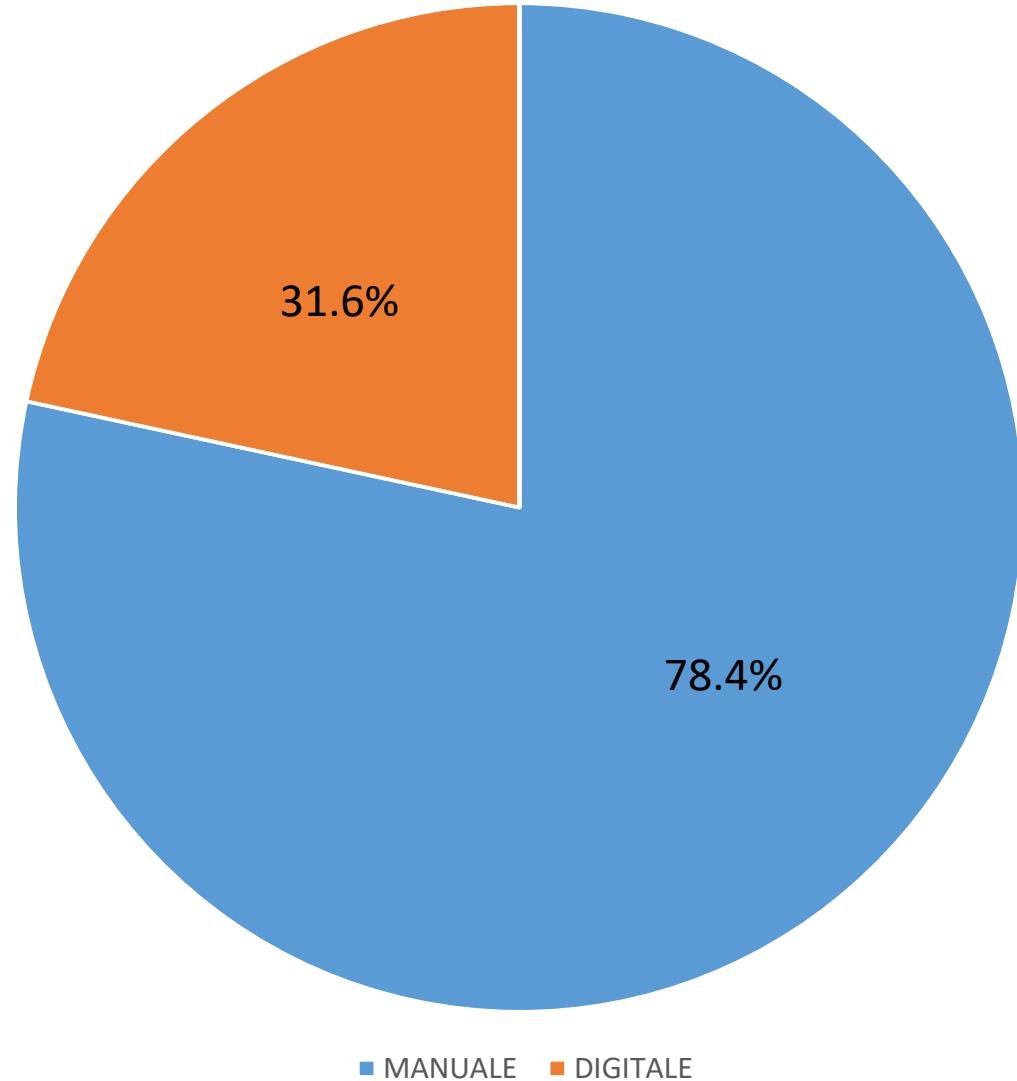
TILs POLMONE

MODALITA'



TILs POLMONE

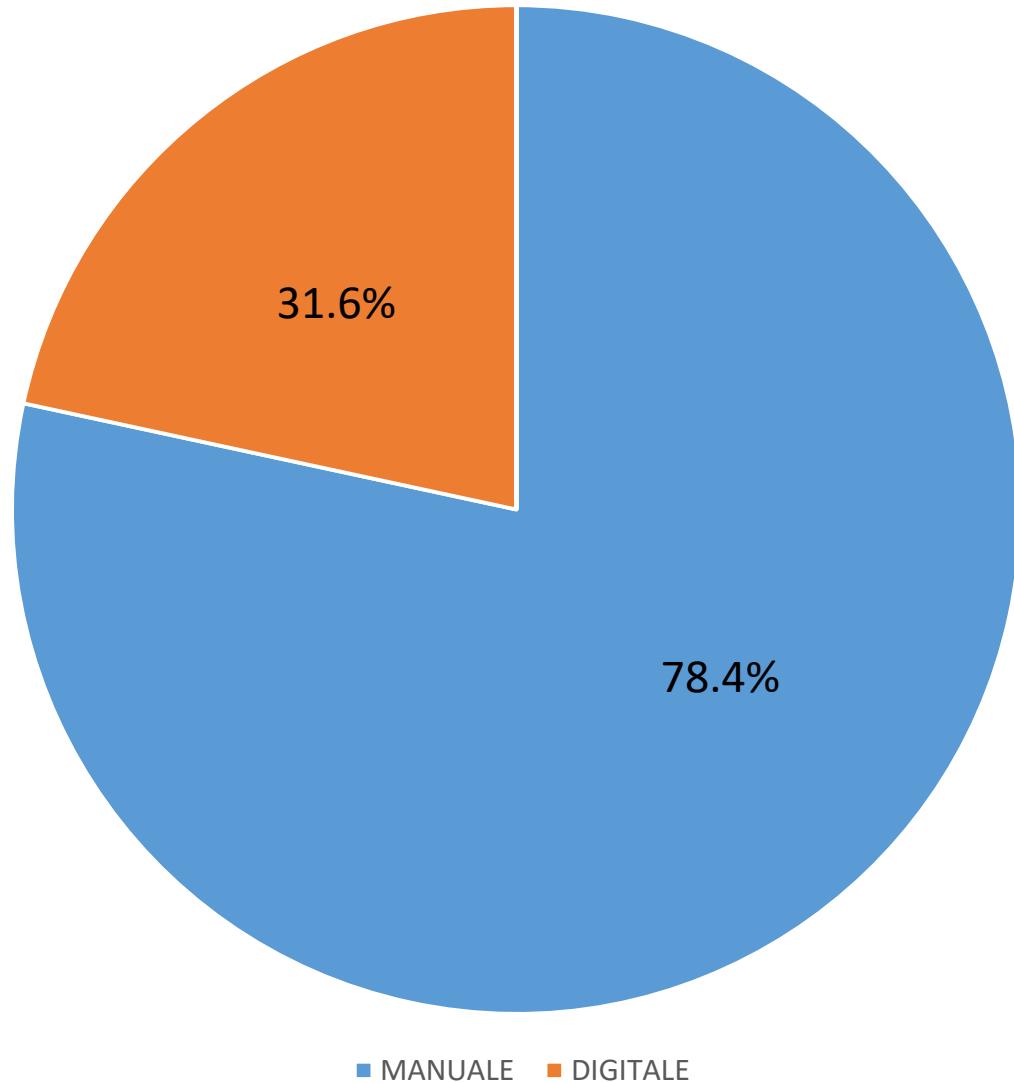
MODALITA'



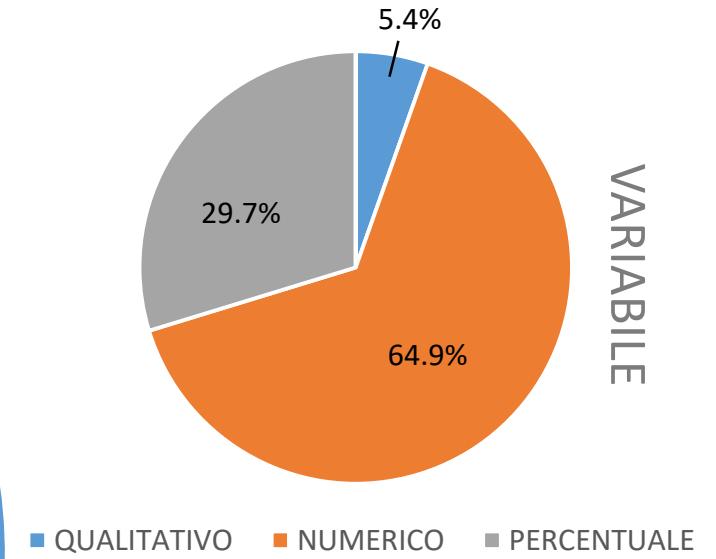


TILs POLMONE

MODALITA'



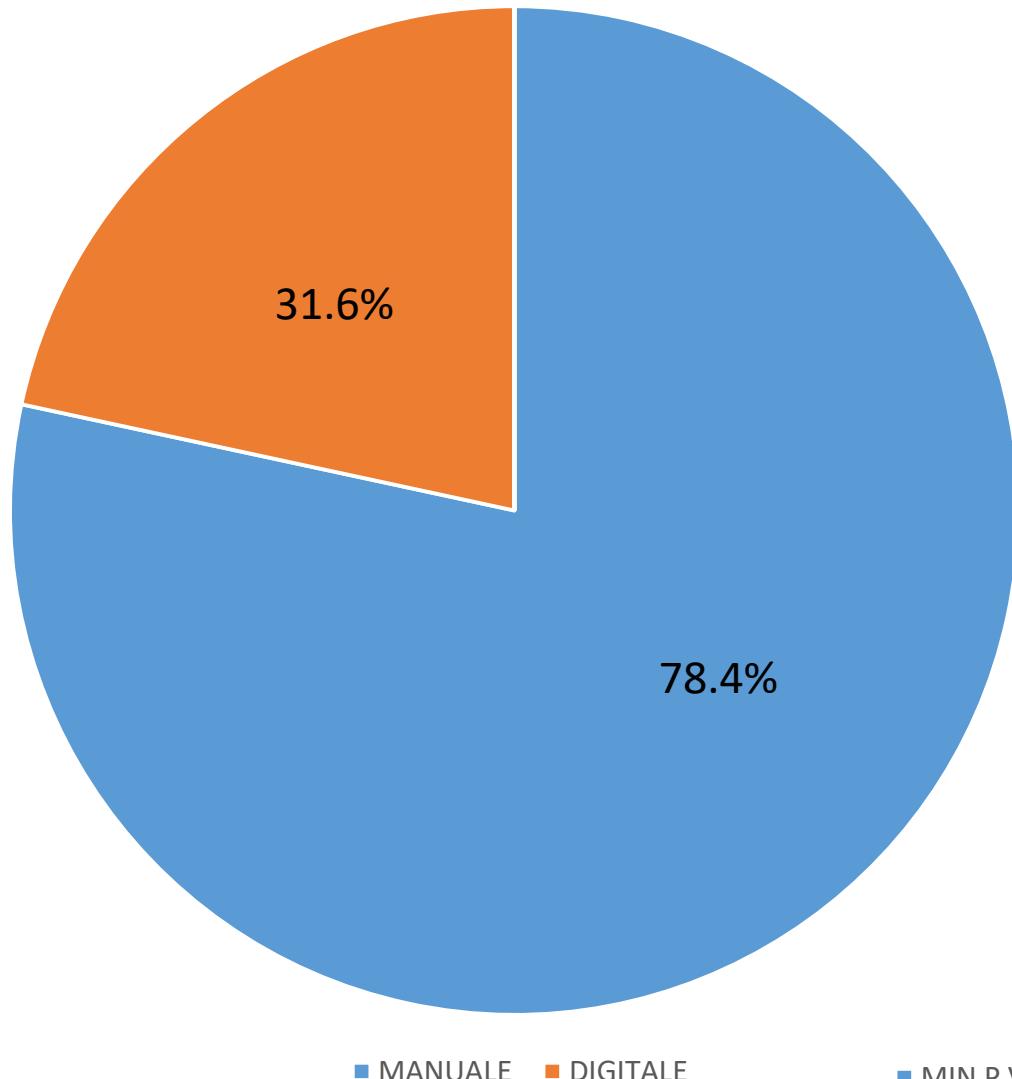
VARIABILE



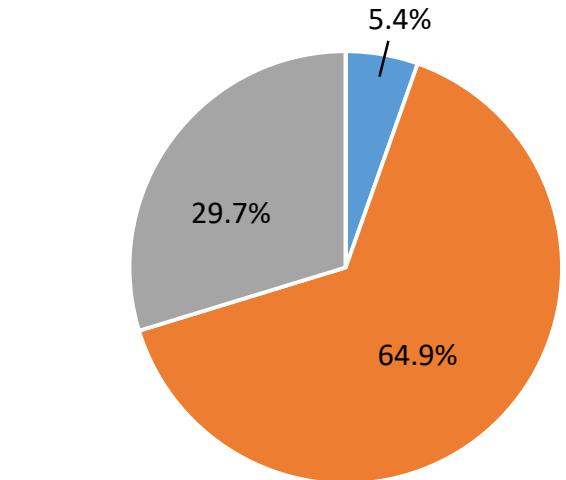


TILs POLMONE

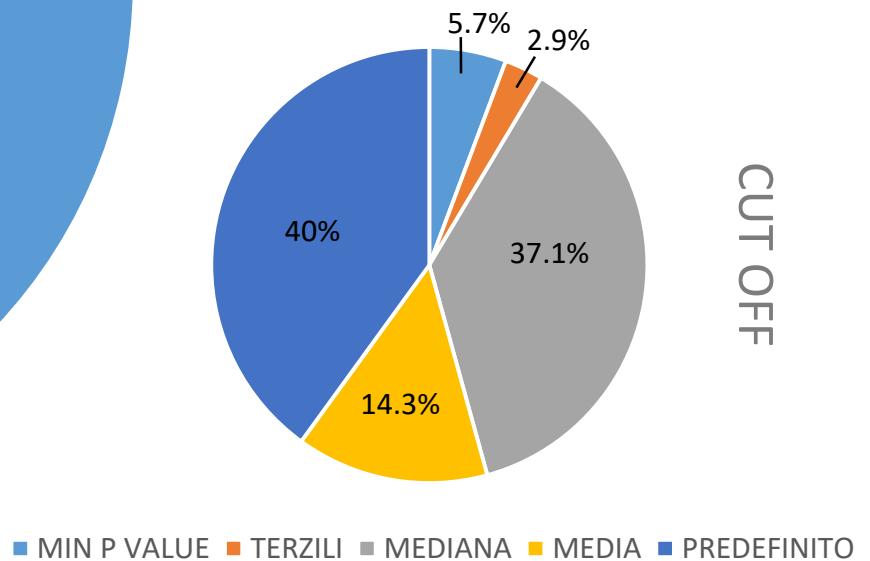
MODALITA'



VARIABILE



CUT OFF





TILs POLMONE

Cosa? (8) x *Dove?* (3x4) x *Come?* (2x2x5) = **2880**

Popolazione

Materiale
Sede

Valutazione
Metodo
Cut off

TILs POLMONE



Sacro Cuore
Don Calabria

Negrar - Verona

IRCCS Ospedale
Sacro Cuore Don Calabria
PRESIDIO OSPEDALIERO ACCREDITATO - REGIONE VENETO

TILs POLMONE

Subset
TILs
CD3
CD8
CD4
FOXP3

TILs POLMONE

Subset	Distribution
TILs	TC+TS
CD3	
CD8	TC
CD4	
FOXP3	TS

TILs POLMONE

Subset	Distribution	Outcome
TILs	TC+TS	OS
CD3		
CD8	TC	
CD4		
FOXP3	TS	DFS

TILs POLMONE

Subset	Distribution	Outcome	N °of Studies	N °of Patients	HR (95% I.C.)	P Value	Heterogeneity I^2 (%)	P						
TILs	TC+TS	OS												
CD3														
CD8		TC												
CD4														
FOXP3		TS												

TILs POLMONE



Subset	Distribution	Outcome	N °of Studies	N °of Patients	HR (95% I.C.)	P Value	Heterogeneity I^2 (%)	P						
TILs	TC+TS	OS	≥ 2	≥ 200	≤ 0.8	< 0.05	$< 50\%$	< 0.05						
CD3														
CD8		TC												
CD4														
FOXP3		TS			≥ 1.25									

TILs POLMONE

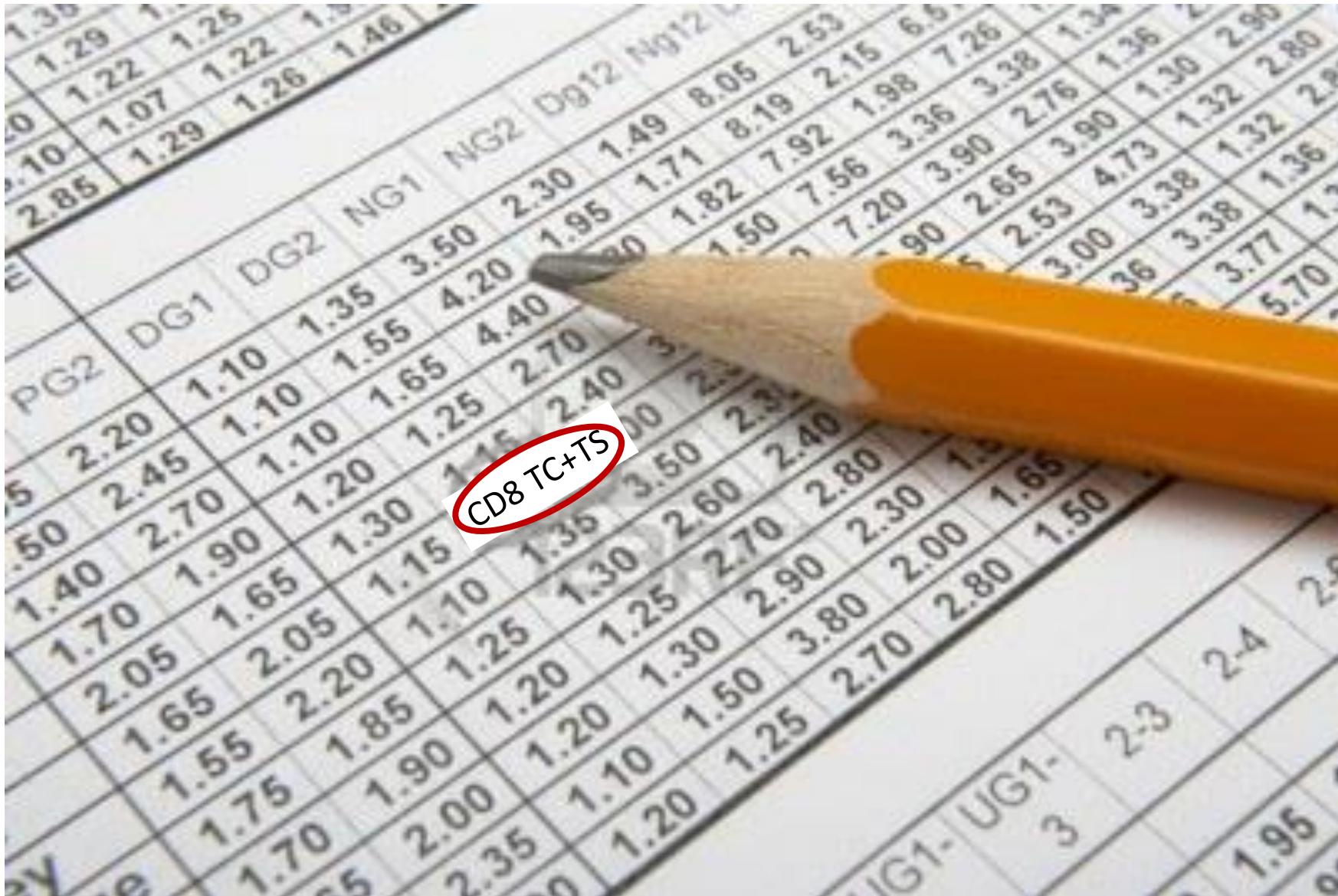
Subset	Distribution	Outcome	N °of Studies	N °of Patients	HR (95% I.C.)	P Value	Heterogeneity I^2 (%)	P			
TILs	Both	OS	≥ 2	≥ 200	≤ 0.8	< 0.05	< 50%	< 0.05			
CD3											
CD8											
CD4		TN	DFS		≥ 1.25						
FOXP3											

TILs TC+TS DFS

CD8 TC+TS OS/DFS

FOXP3 TS OS/DFS

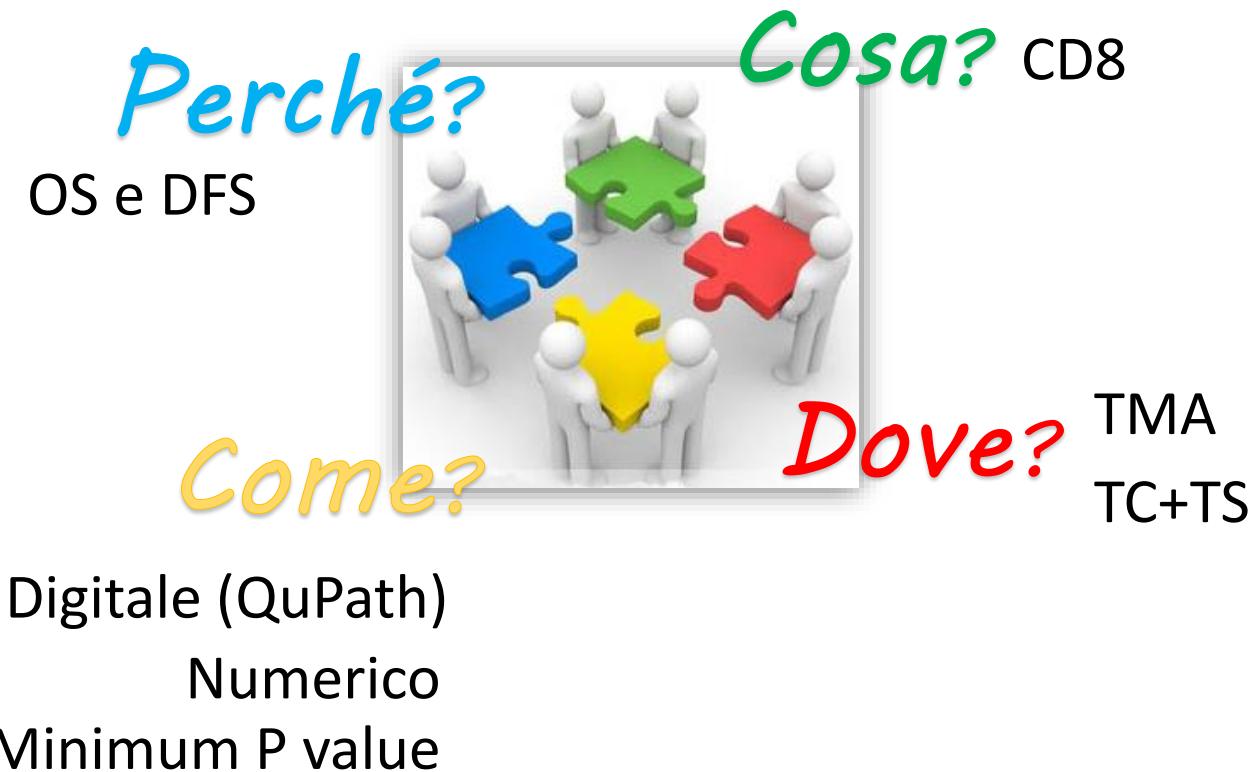
TILs POLMONE



TILs POLMONE



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PRESIDIO OSPEDALIERO ACCREDITATO - REGIONE VENETO



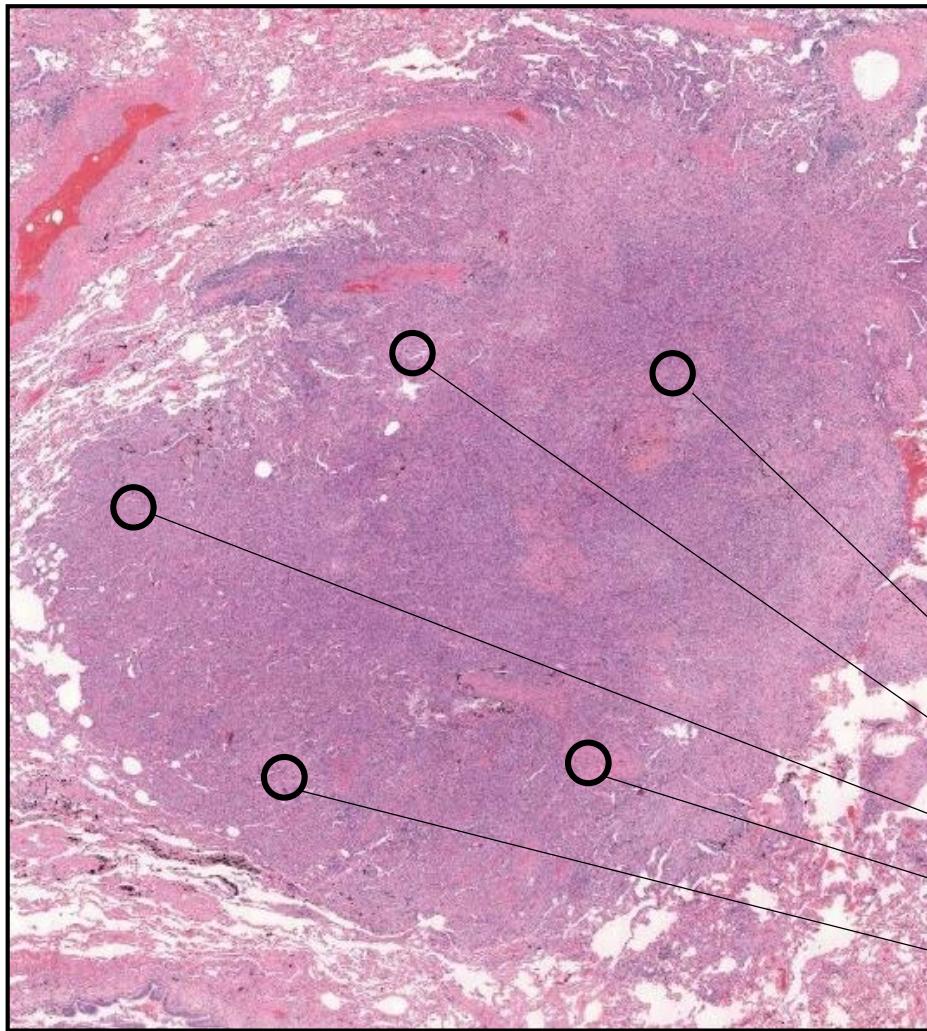
CD8 POLMONE



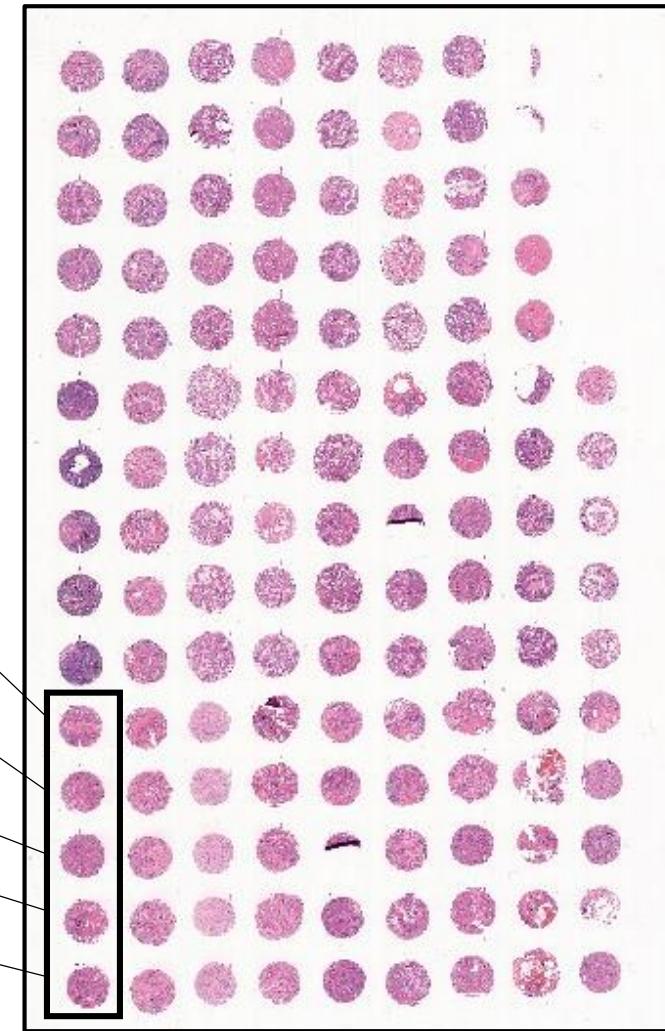
Caratteristiche Cliniche

ETA': <ul style="list-style-type: none">➤ Mediana➤ Range	70 anni 40-86 anni
SESSO: <ul style="list-style-type: none">➤ Maschi➤ Femmine	167 (70%) 73 (30%)
ISTOTIPO: <ul style="list-style-type: none">➤ Adenocarcinoma➤ Carcinoma squamoso➤ LCC	163 (68%) 61 (25%) 16 (7%)
TNM: <ul style="list-style-type: none">➤ I➤ II-III	153 (64%) 87 (36%)
FOLLOW-UP: <ul style="list-style-type: none">➤ Mediano➤ Range	33 mesi 5-179 mesi

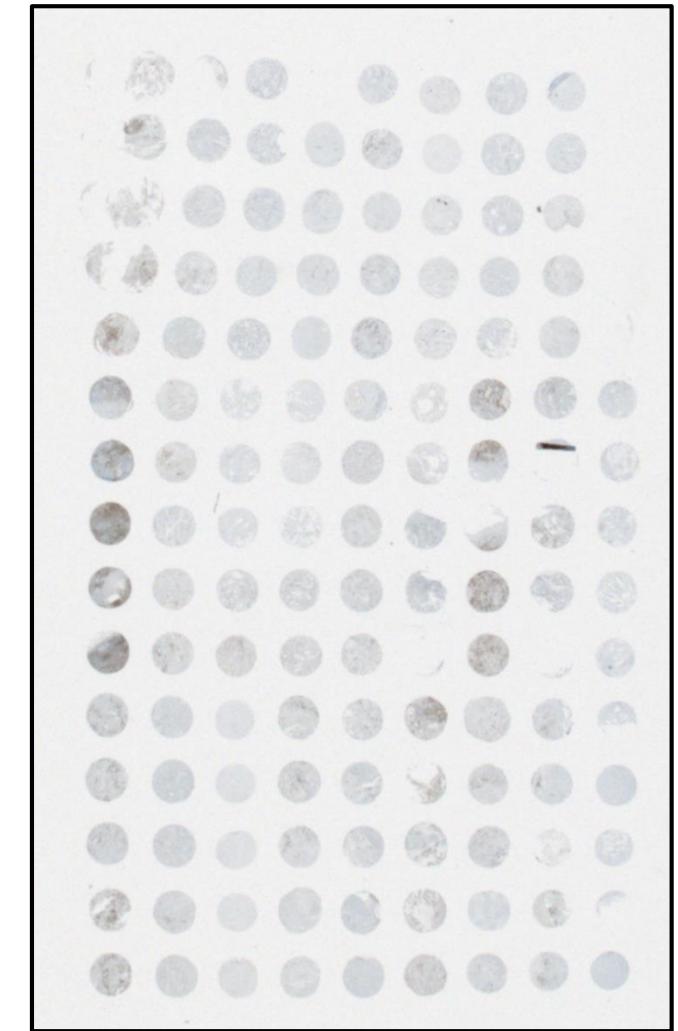
CD8 POLMONE



HE



CD8



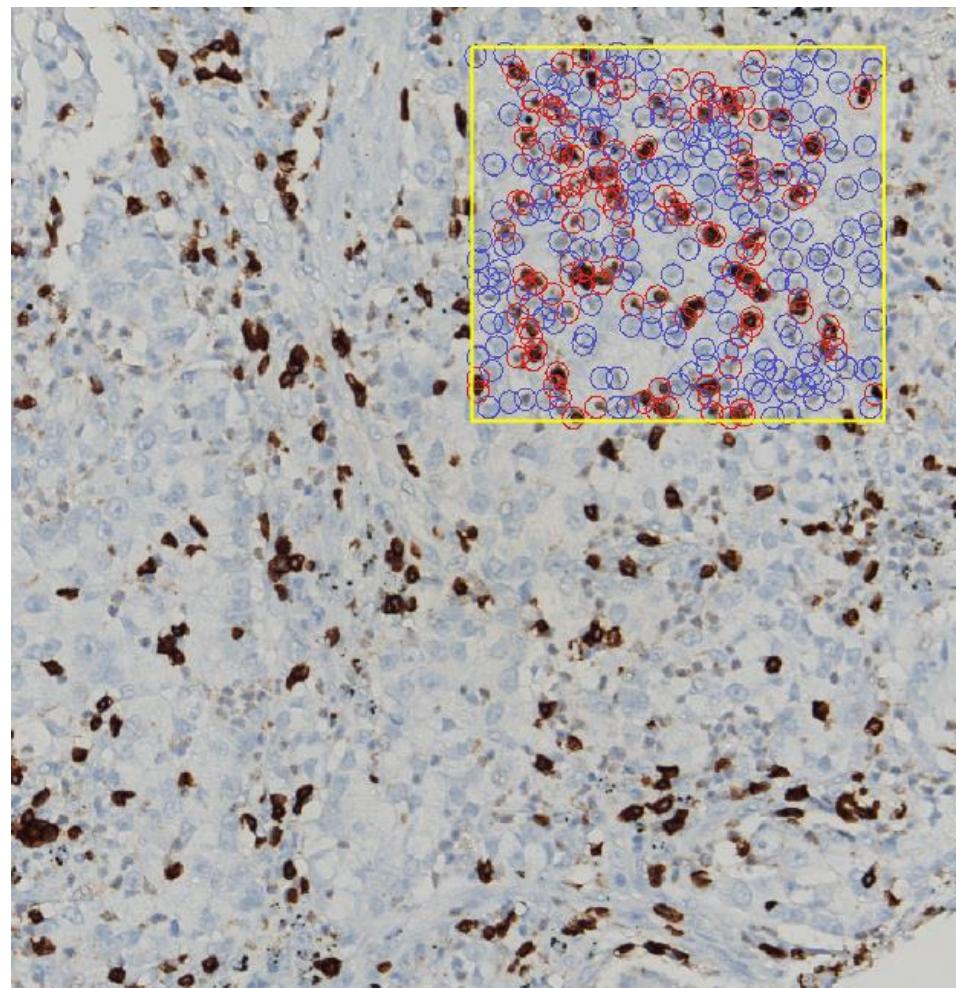
CD8 POLMONE



“QuPath: Open source software for digital pathology image analysis”

Sci Rep. 2017

Key	Value
Name	PathAnnotationObject
Class	
ROI	Rectangle
Centroid X μm	6678.7431
Centroid Y μm	10169.406
Num Negative	197
Num Positive	125
Positive %	38.8199
Num Positive per mm^2	2904.1566
Area μm^2	43041.7566
Perimeter μm	830.8696

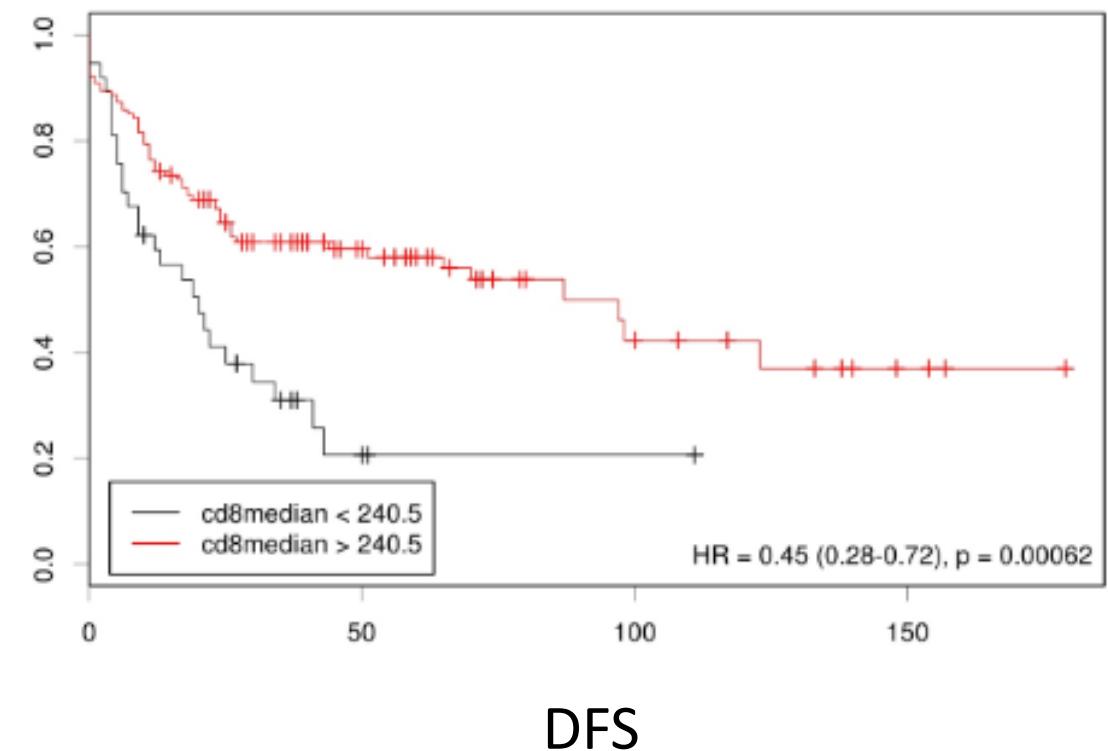
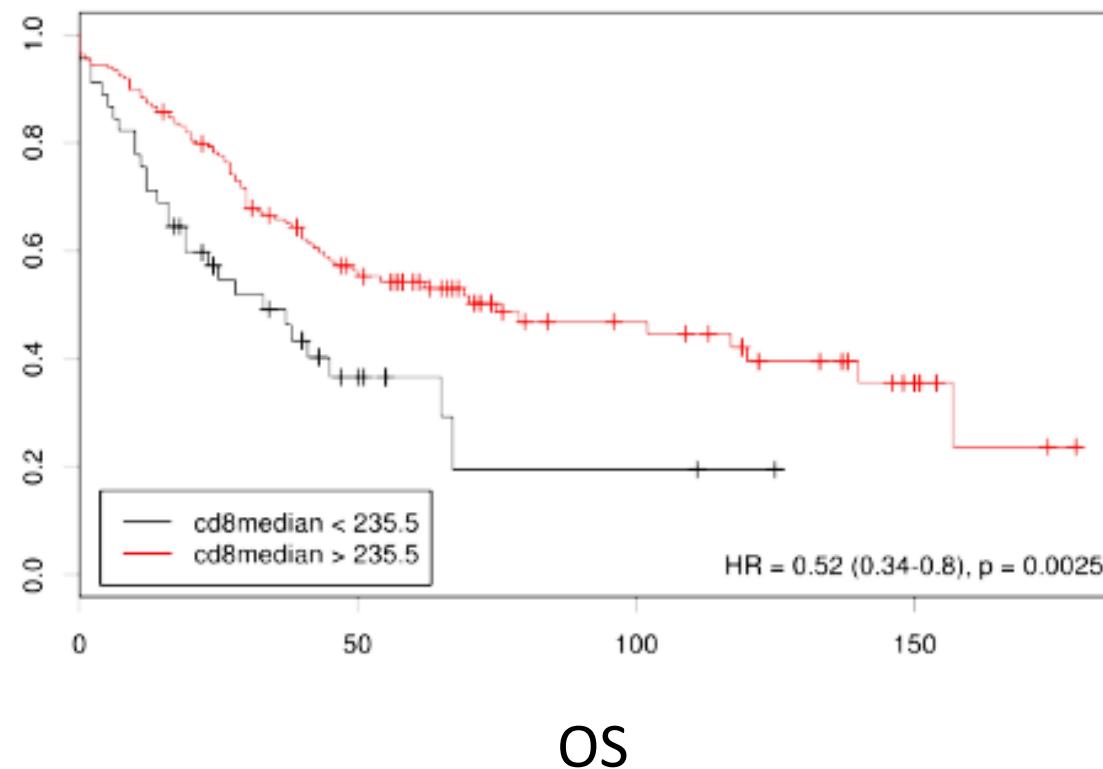


CD8 POLMONE

Minimum P value...

“Cutoff Finder: A Comprehensive and Straightforward Web Application Enabling Rapid Biomarker Cutoff Optimization”

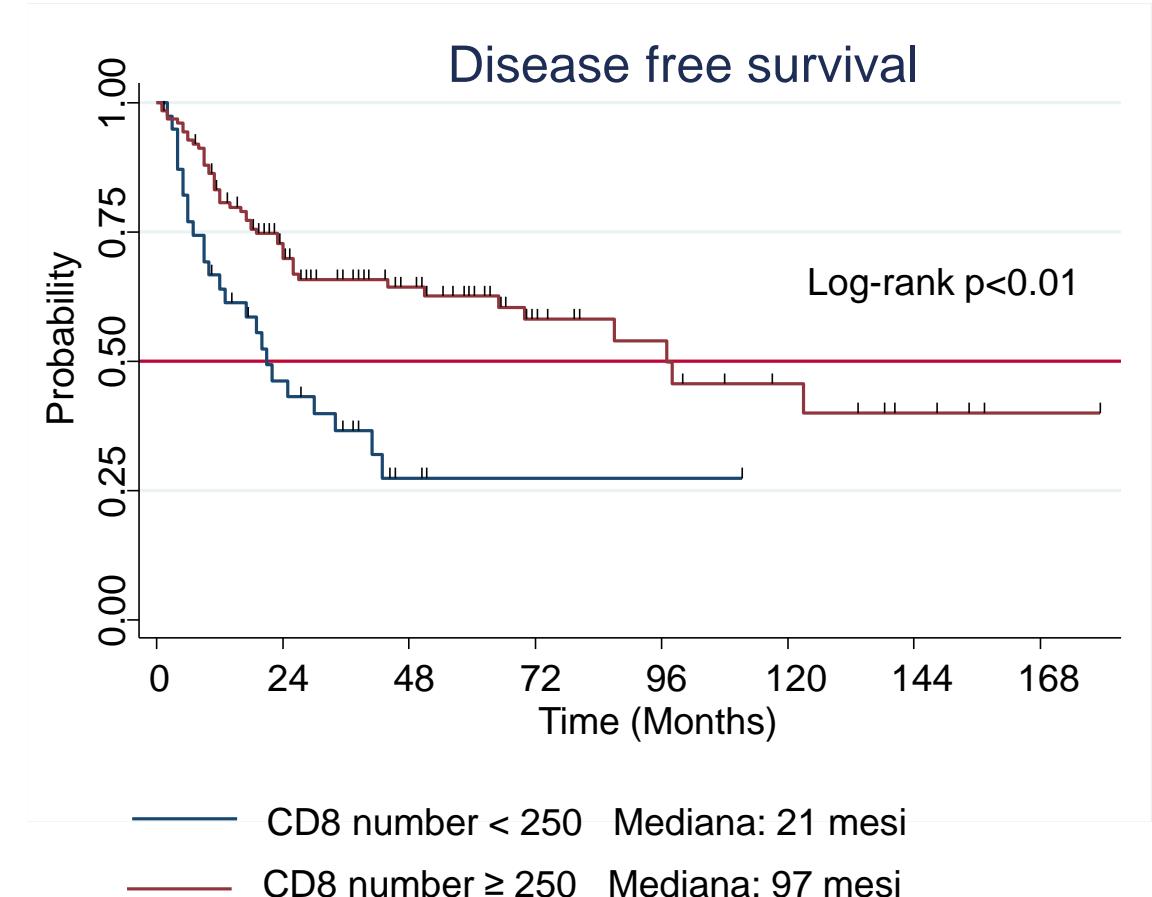
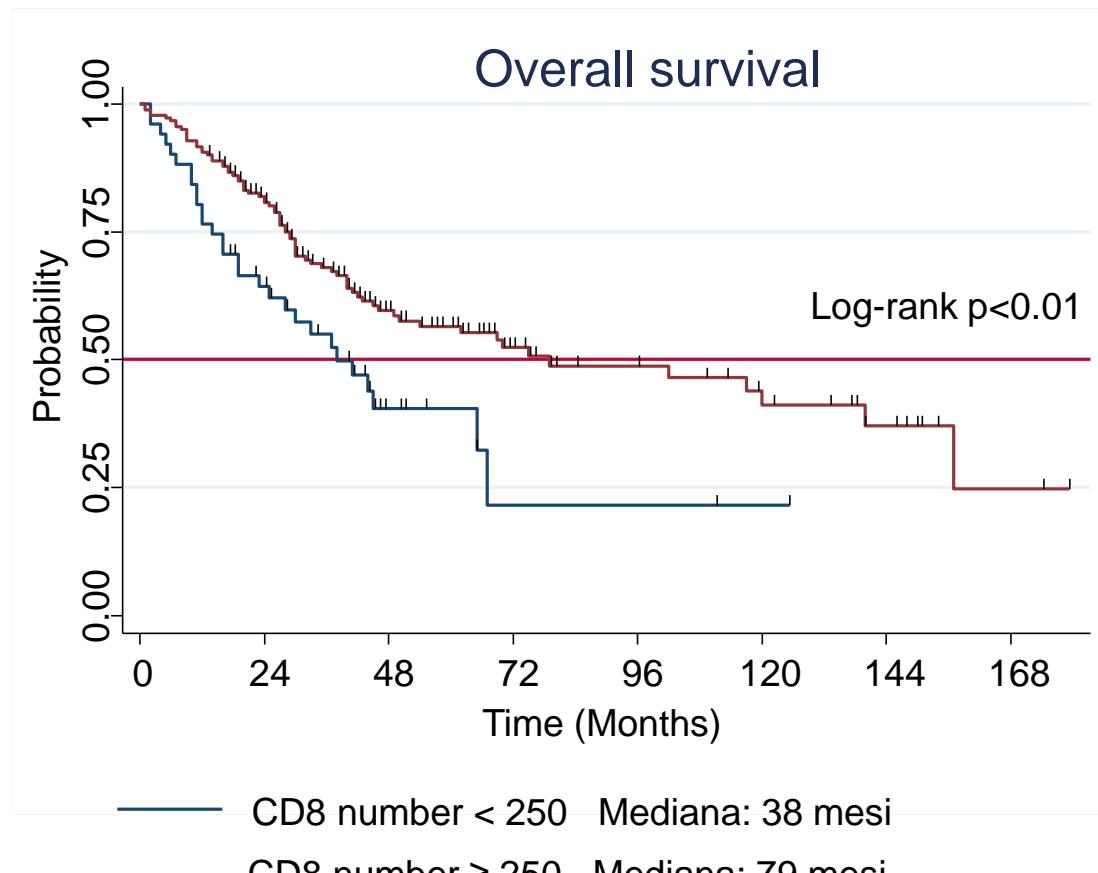
Plos One 2012



CD8 POLMONE

	< 250	≥ 250	P
ETA'			
≤ 70 anni	24.4%	75.6%	
> 70 anni	19.7%	80.3%	0.37
SESSO:			
Maschi	20.4%	79.6%	
Femmine	24.6%	75.4%	0.45
ISTOTIPO:			
Adenocarcinoma	19%	81%	
Carcinoma squamoso	27.9%	72.1%	
LCC	25%	75%	0.34
TNM:			
I	17%	83%	
II-III	30%	70%	0.02

CD8 POLMONE



CD8 POLMONE

OS

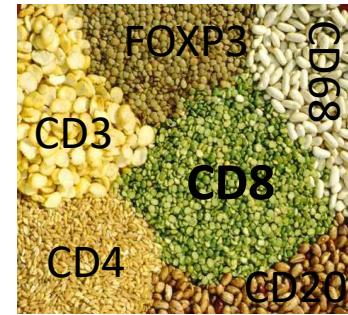
DFS

	HR	95% CI	P	HR	95% CI	P
ETA'						
≤ 70 anni	1			1		
> 70 anni	1.07	0.72-1.60	0.73	0.83	0.38-1.78	0.63
SESSO:						
Maschi	1			1		
Femmine	0.52	0.31-0.85	0.01	1.01	0.45-2.29	0.97
ISTOTIPO:						
Adenocarcinoma	1			1		
Carcinoma squamoso	1.14	1.61-3.36	0.56	1.19	0.49-2.85	0.70
LCC	2.40	1.22-4.72	0.01	0.85	0.11-6.39	0.87
TNM:						
I	1			1		
II-III	1.9	1.31-2.94	0.001	2.49	1.13-5.48	0.02
CD8:						
< 250	1			1		
≥ 250	0.56	0.36-0.89	0.01	0.36	0.16-0.79	0.01

TILs POLMONE

Conclusioni:

- Eterogeneità
 - Intrinseca
 - Estrinseca
- CD8 *in* TC+TS
- CD8 *in* TC+TS: Fattore prognostico indipendente
 - *Digital*
 - *Cut-Off: 250 x mm²*





Grazie

26 pts: - 19 CHT 1 Linea

- 2 AntiEGFR 1 Linea (4 erano EGFR mutati????)
- 5 Immunoterapia 1 Linea

Tutti i 26 pts trattati con nivolumab

	Number of patients	Response rate (%)	p value	Odds ratio	CI 95% lower	CI 95% upper
CD8+—number of lymphocytes per mm ² of tumor—NSCLC						
< 886	12	16.7	0.046	1.0	—	—
886–1899	5	60.0	0.017	7.5	1.43	39.41
1900 +	9	22.2	0.65	1.43	0.31	6.7
CD8+/CD4+ ratio—NSCLC						
< 2	15	13.3	0.046	1.0	—	—
2–2.69	4	50.0	0.035	6.5	1.14	37.05
2.7 +	7	42.9	0.038	4.88	1.1	21.69

Multivariata? PD-L1? Stratificazione tra 1 e 2 Linea?

Table 3. Relationship between pretreatment microenvironmental parameters in the tumor specimen obtained closest to treatment initiation, and clinical response to anti-PD-1^a

Pathologic parameter (number of patients analyzed)	All patients (%)	Objective response ^b			Clinical benefit ^c		
		Non (%)	Yes (%)	P value ^d	Non (%)	Yes (%)	P value ^d
Tumor PD-L1 expression (n = 41)^e							
Absent	18 (44)	17 (94)	1 (6)	0.025	17 (94)	1 (6)	0.005
Present	23 (56)	14 (61)	9 (39)		12 (52)	11 (48)	
Immune cell infiltrate PD-L1 expression (n = 41)^e							
Absent	18 (44)	16 (89)	2 (11)	0.142	16 (89)	2 (11)	0.038
Present	23 (56)	15 (65)	8 (35)		13 (57)	10 (43)	
Immune infiltrate score (n = 41)^f							
Absent	9 (22)	8 (89)	1 (11)	0.410	8 (89)	1 (11)	0.240
Present	32 (78)	23 (72)	9 (28)		21 (66)	11 (34)	
TIL PD-1 expression (n = 37)^g							
Absent	21 (57)	18 (86)	3 (14)	0.067	17 (81)	4 (19)	0.077
Present	16 (43)	9 (56)	7 (44)		8 (50)	8 (50)	
Immune cell or tumor cell PD-L2 expression (n = 13)^e							
Absent	9 (69)	7 (78)	2 (22)	1.000	6 (67)	3 (33)	0.497
Present	4 (31)	4 (100)	0 (0)		4 (100)	0 (0)	
CD4:CD8 (n = 29)							
CD4 ≥ CD8	13 (45)	10 (77)	3 (23)	0.454	9 (69)	4 (31)	0.702
CD4 < CD8	16 (55)	10 (63)	6 (37)		9 (56)	7 (44)	
CD20⁺ B cells (n = 30)							
Absent	19 (63)	16 (84)	3 (16)	1.000	16 (84)	3 (16)	0.372
Present ^f	11 (37)	9 (82)	2 (18)		7 (64)	4 (36)	
Lymphoid aggregates (n = 41)							
Absent	35 (85)	26 (74)	9 (26)	1.000	25 (71)	10 (29)	1.000
Present	6 (15)	5 (83)	1 (17)		4 (67)	2 (33)	
Necrosis (n = 41)							
Absent	27 (66)	19 (70)	8 (30)	0.447	19 (70)	8 (30)	1.000
Present	14 (34)	12 (86)	2 (14)		10 (71)	4 (29)	
Small sample (n = 41)							
No	27 (66)	20 (74)	7 (26)	1.000	18 (67)	9 (33)	0.494
Yes	14 (34)	11 (79)	3 (21)		11 (79)	3 (21)	
Interval between specimen procurement and treatment initiation (n = 41)							
<1 year	16 (39)	10 (63)	6 (37)	0.15	9 (56)	7 (44)	0.16
≥1 year	25 (61)	21 (84)	4 (16)		20 (80)	5 (20)	

Sudio su 41 tumori di cui 12 NSCLC