ImmunoScore/Immuneprofiling in Melanoma

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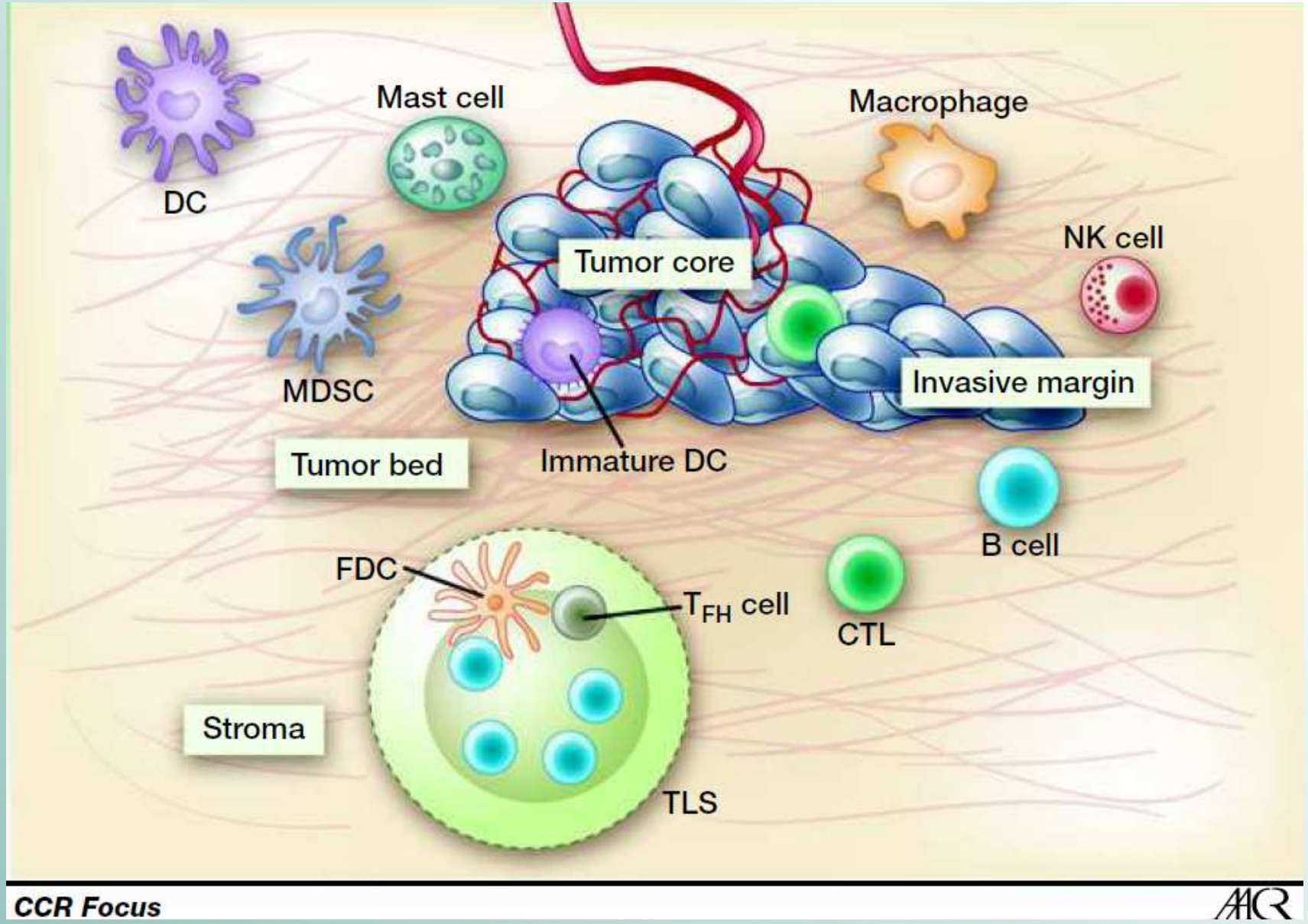
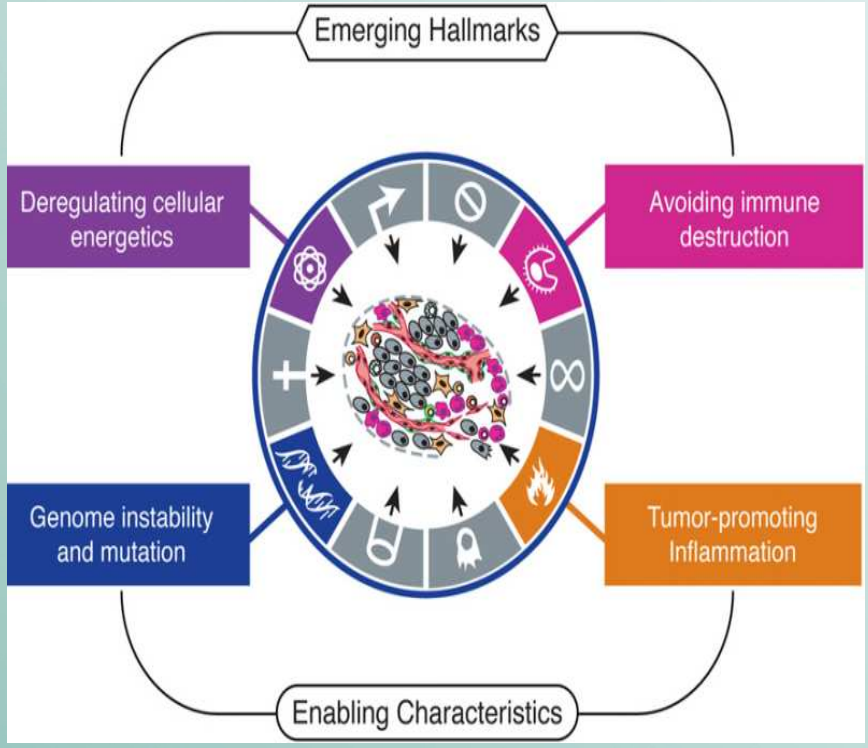
SITC 2015

Tumor microenviroment



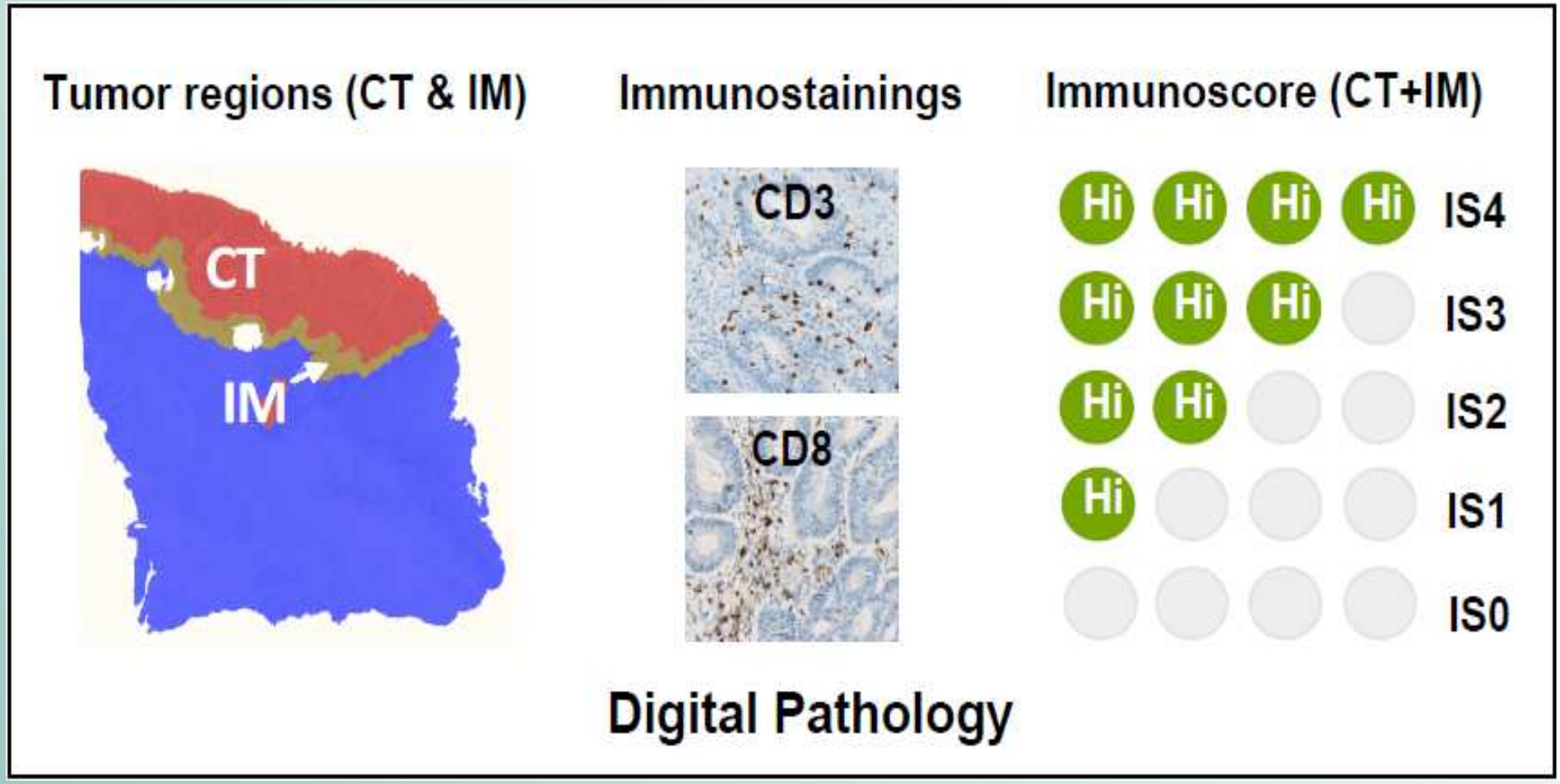
Importance of the Immune contexture

In fact the Immune contexture influence multiple aspects of tumor development and present multiple opportunities to gage this response in order to make the best treatment decisions:



- **Prognostic Value: The presence of the right immune effector cells is correlated with better prognosis and survival (staging criteria?)**
- **Immunotherapy: Manipulate the patient's own immune system to respond to the tumor (Ipi, etc…)**
- **Chemotherapy: Response to CDX may also be immune-related**

The Immunoscore in colorectal cancer



- Great tool for **Staging**

Courtesy of Jerome Galon

ImmunoScore and Immunoprofiling

	Immunoscore	Immunoprofiling
	Prognostic/Predictive(?)	Prognostic/Predictive(?)
Number of immune markers	**2-4**	**1 – Several**
Immunoscore markers	CD3/CD8	Immune gene signatures Multiplex assays CD137, Galectin1, LAG-3, OX40, PD-L1, TIM3, etc.
Immunoscore-like markers	CD3/CD8/CD20/FoxP3 CD3/CD8/CD45RO CD4/CD8/CD68 CD3/CD8/CD20, CD3/GZMB CD8/FoxP3 CD8/IL17 (others)	
Possible application	• Staging in **Melanoma,** Breast cancer, Ovarian cancer, NSCLC, Prostate cancer, Pancreatic cancer, Head & Neck cancer (to be defined).	• Prognostic assay • Predictive assay • Personalized immune-treatment

Ascierto PA, et al. *J Transl Med*. 2013.

Melanoma ImmunoScore projects

- Melanoma ImmunoScore in Lymph Nodes

- MISIPI study: Melanoma ImmunoScore evaluation in patients treated with Ipilimumab

- Melanoma ImmunoScore: Discovery of predictive biomarker for IPILIMUMAB response

Melanoma ImmunoScore in Lymph Nodes

Why the Lymph Nodes Represent an Interesting Model for the ImmunoScore

1. In many cases, the metastatic lymph nodes from lymphectomy is the only available tissue, Lymph nodes are more accessible vs visceral metastases;
2. More patients with lymph node+ (stage III), high risk of distant metastases;
3. Previous works evidenced the importance of the Lymphnodes and associated microenvironment for melanoma progression and outcome:
   - Cochrane AJ et al. Sentinel lymph nodes show profound downregulation of antigen-presenting cells of the paracortex: implications for tumor biology and treatment. Mod Pathol 2001 .
   - Cochrane AJ et al. Prediction of metastatic melanoma in nonsentinel nodes and clinical outcome based on the primary melanoma and the sentinel node Mod Pathol 2004.
   - Mohos et al. Immune cell profile of sentinel lymph nodes in patients with malignant melanoma - FOXP3+ cell density in cases with positive sentinel node status is associated with unfavorable clinical outcome. *JTM* 2013.

The evaluation of TME in the lymph nodes could be important for better classify patient and for predict clinical outcome;

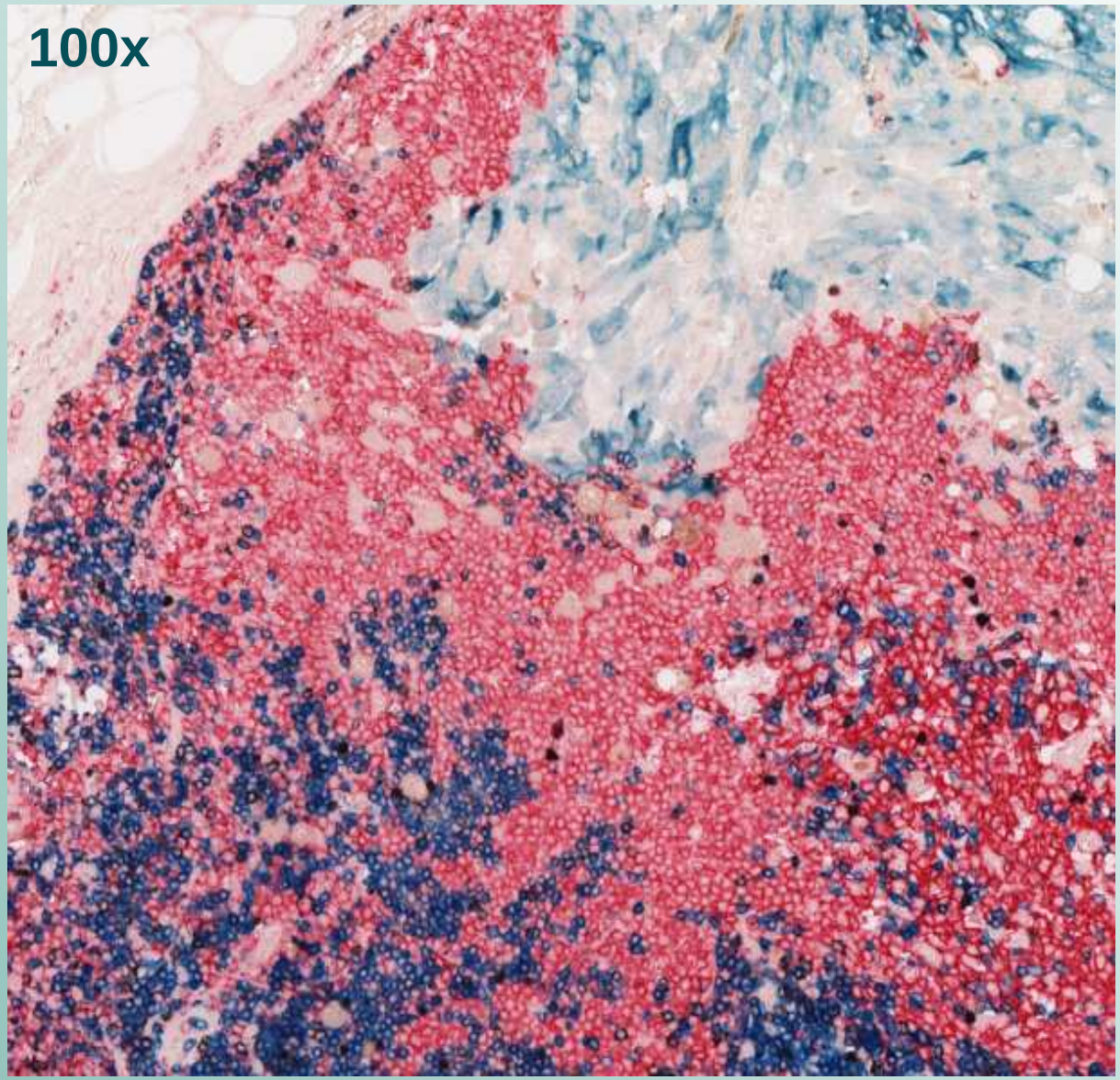
Melanoma ImmunoScore in Lymph Nodes metastasis

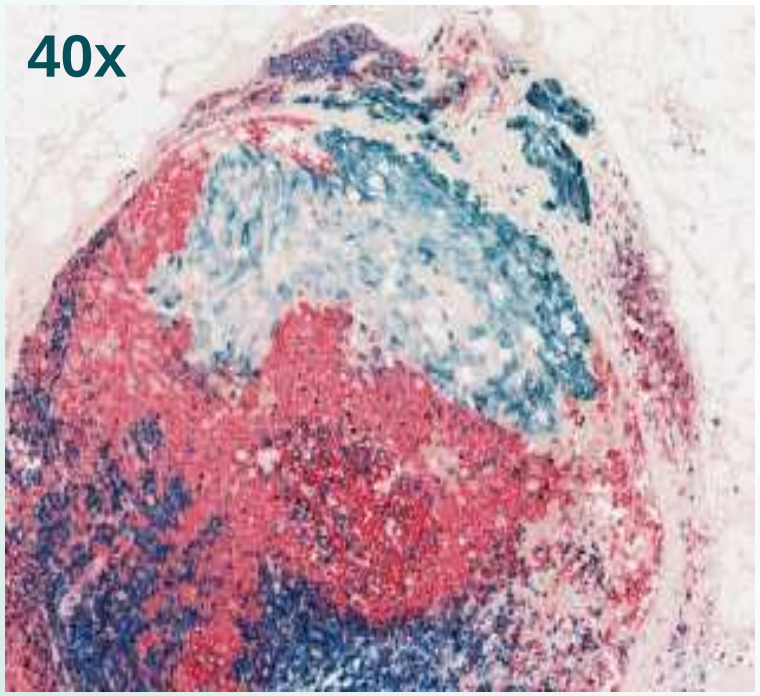
AIM

To explore evidence of correlation between the immuno infiltrate in Lymphnodes and patients outcome;

- **On lymphadenectomies from 34 Stage III melanoma patients, for a total of 277 lymph-nodes;**
- **Analyzing 5 immuno markers (FoxP3, CD3, CD8, CD20, S100) expression;**
- **Firstly, evaluating manualy a multiplex IHC stained slides with the 5 markers all together;**
- **After the development of an algorithm, evaluating single stained IHC slide for the 5 marker, in an automatic way.**

Melanoma ImmunoScore in Lymph Nodes metastasis





FoxP3 – DAB (brown)
CD8 – Gray/black
CD3 – Blue
CD20 – Red/magenta
S100 – Green

Capone et al. SITC 2014.

Concerns on ImmunoScore in Lymph Nodes

1. The lymph nodes are the “house” of immune cells, constitutively rich in CD3 and CD20 lymphocytes;

2. The evaluation of the periphery of the tumor is particularly complex (it might be defined as 0,4 mm deep from the tumor but difficult to apply to lymph nodes).

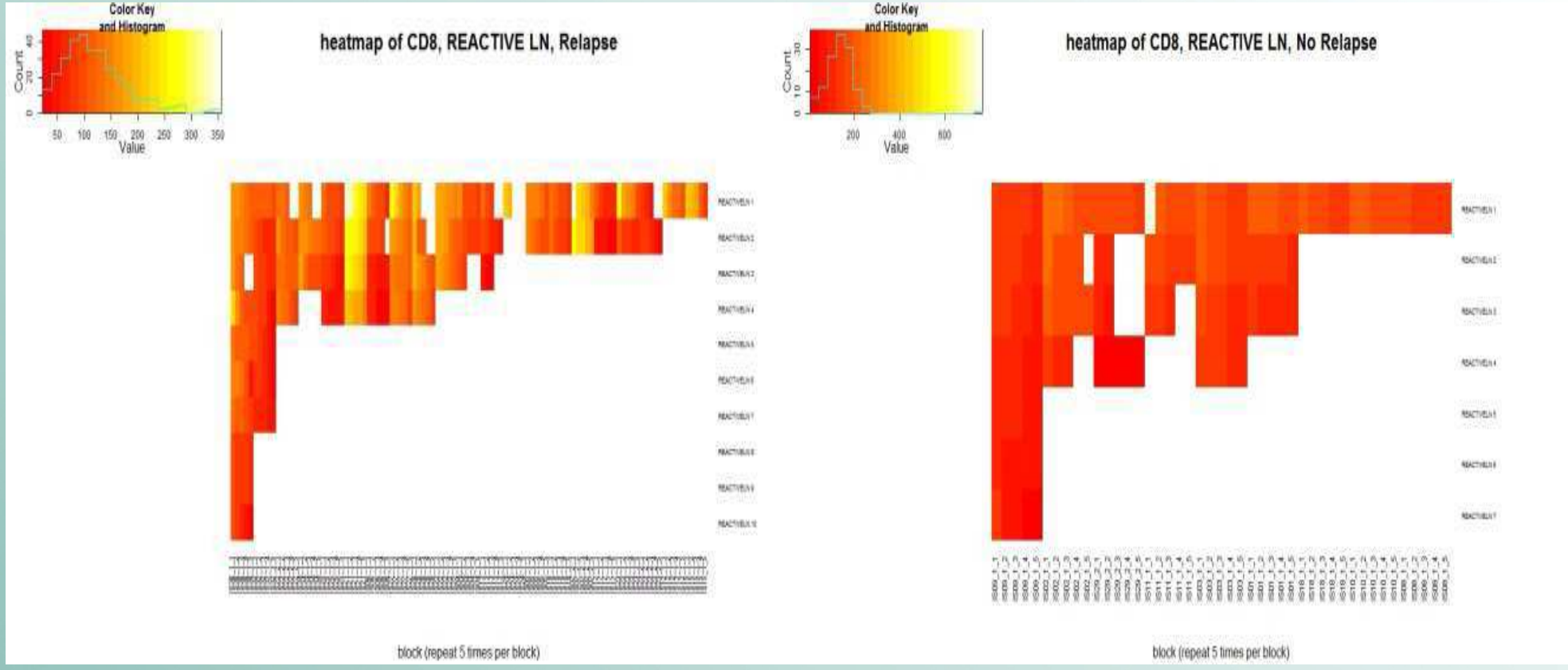
3. Lymph node metastases may be different in terms of immune infiltration compared to other metastatic lesions.

We have walked over!!

Data Analysis *(manual counting in multiplex slides)*

For each patient, we analyzed the cell counts for all markers (summarized as median expression across the different sampled nodes of the same), and the obtained values were then compared between relapse and no relapse groups.

In the reactive lymph nodes, there are no clear differences in expression levels between relapse and no relapse groups, except for CD8, where there are more patients with high expressing cells in the relapse group.

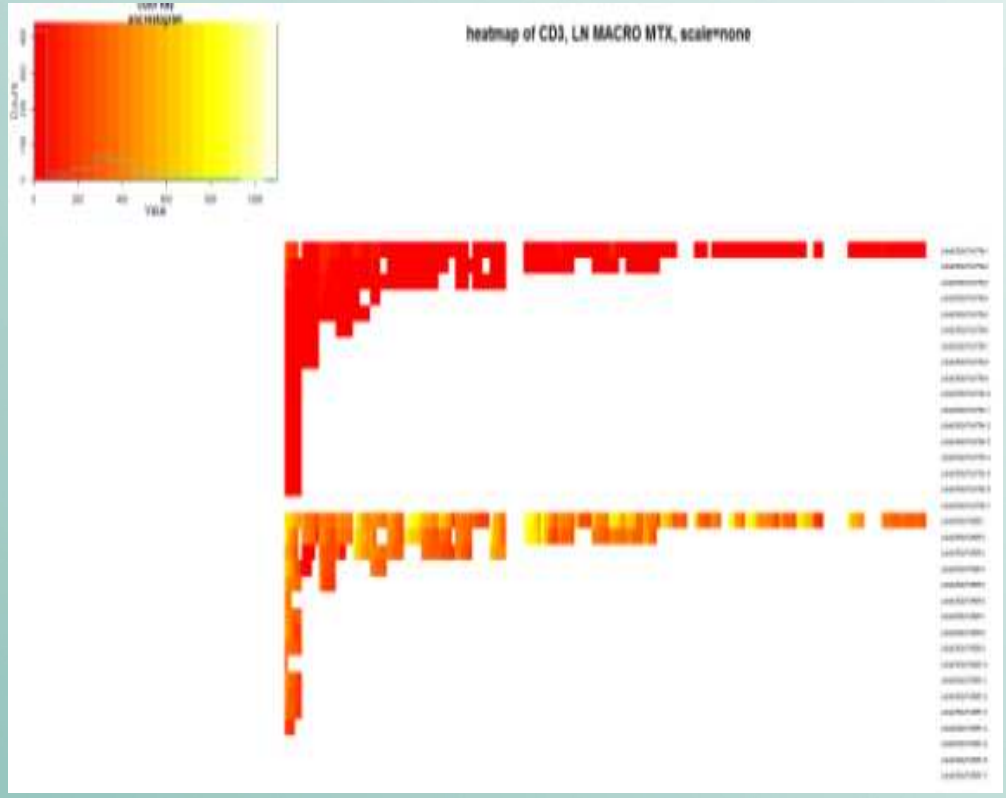


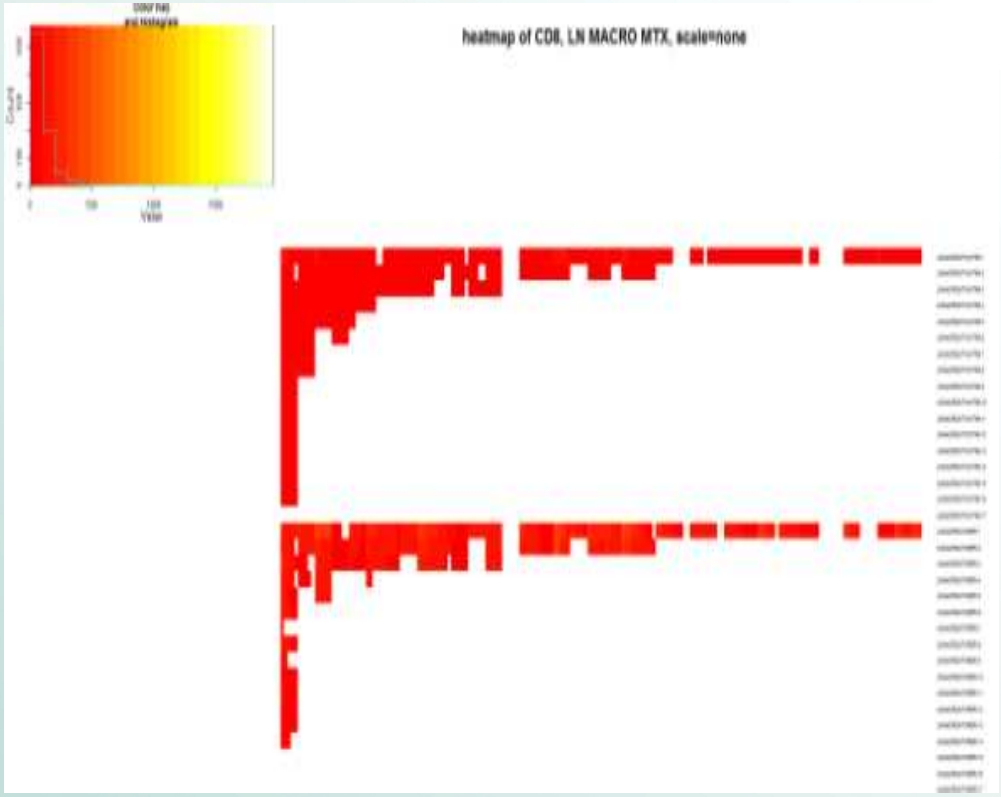
Capone et al. SITC 2014.

Data Analysis *(manual counting in multiplex slides)*

In metastatic LNs there were significant differences in the peri /intra tumoral ratio for both CD3 and CD8, with the ratio being higher in no relapse patients compared to relapse patients for both proteins.

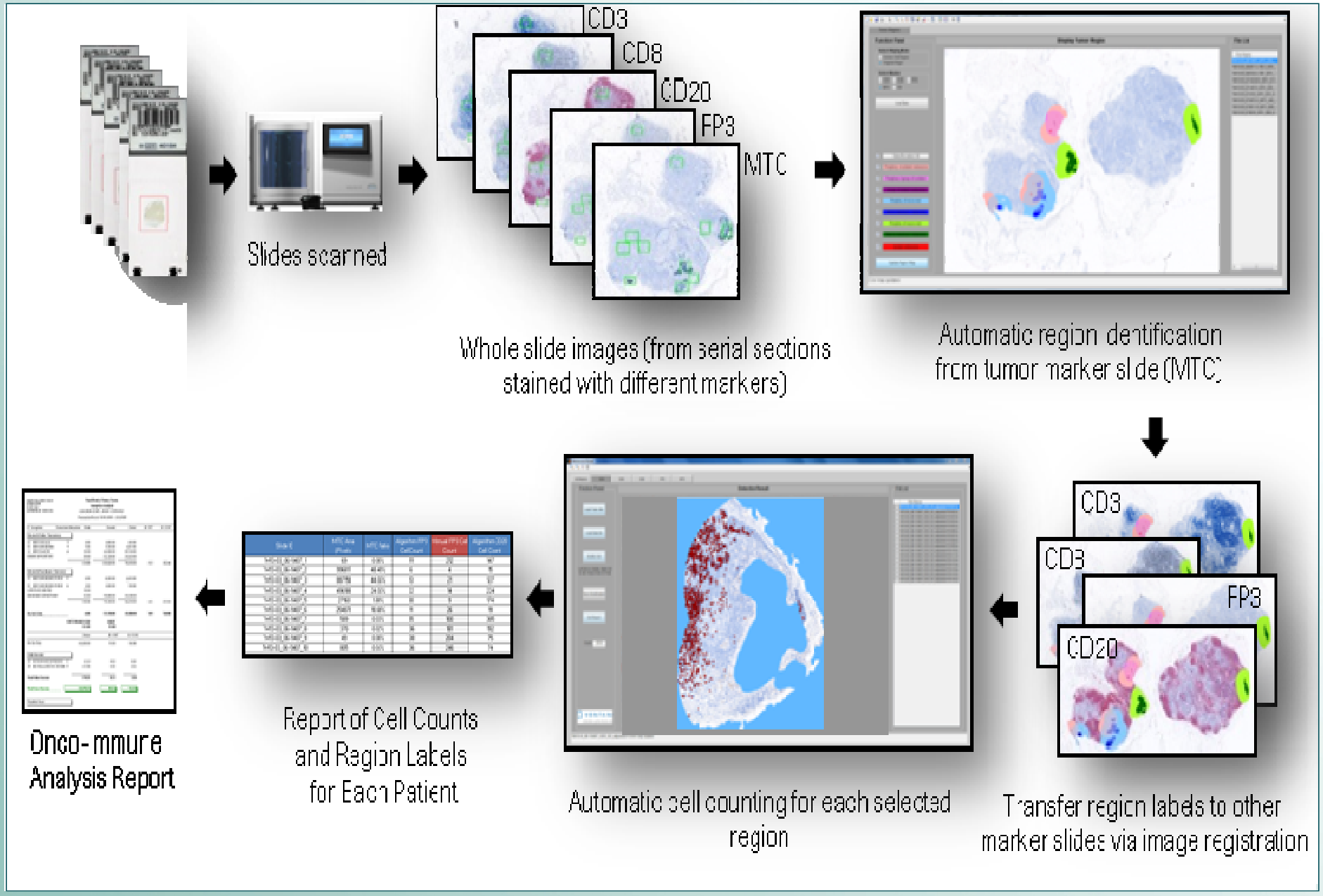
Similar differences were seen in FoxP3 and CD20, although power to detect statistically significant was low given our small sample size.





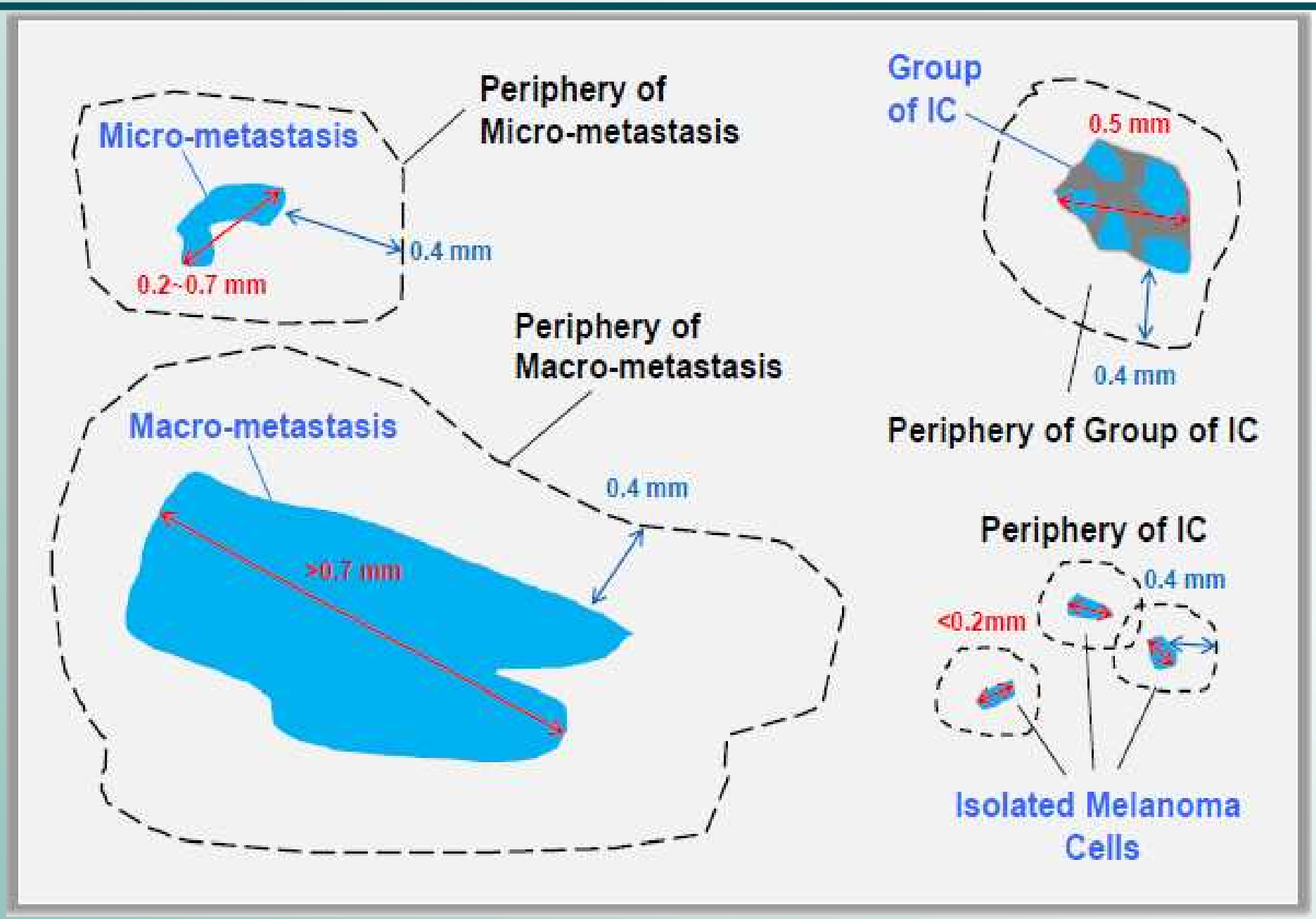
All these data have been useful for to develop the algorithm.

The Algorithm



Capone et al. SITC 2014.

Definition for the Algorithm



Capone et al. SITC 2014.

Data Analysis *(automatic counting in single staining slides)*

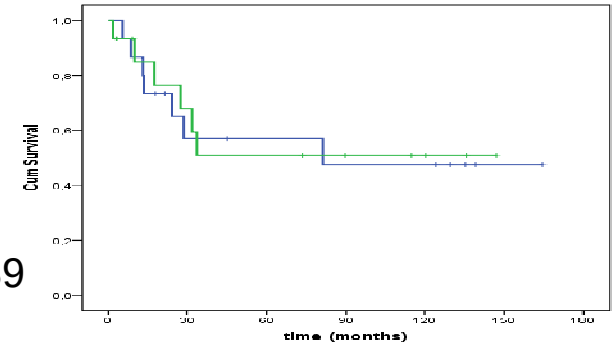
- **Such analysis was performed on a first cohort of 30 patients**
- **For each factor patients were divided in two groups: below median value (low-blu line) and over median value (high-green line);**
- **Correlate markers expression with survival;**
- **For patients with more than 1 slide evaluation the mean for each parameter was calculated;**
- **The value 0.0 was considered as a value and not a missing data.**
- **Overall survival was measured from dissection date. P value refers to the log-rank test;**

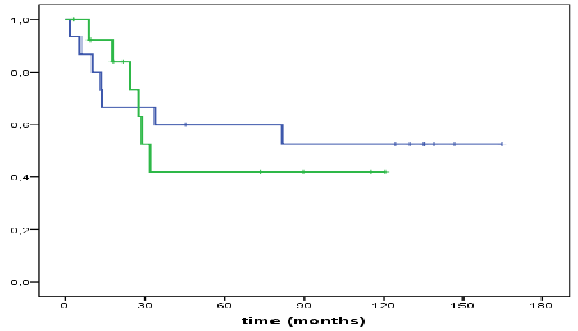
intratumoral

peritumoral

CD3

P=0.89

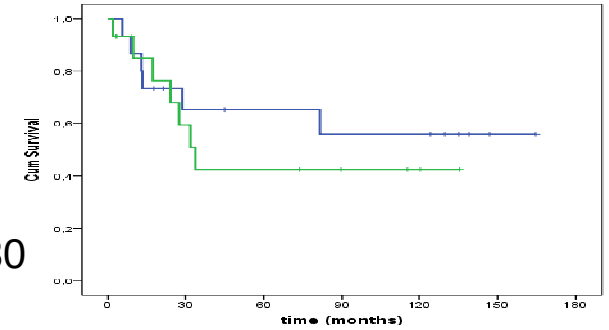


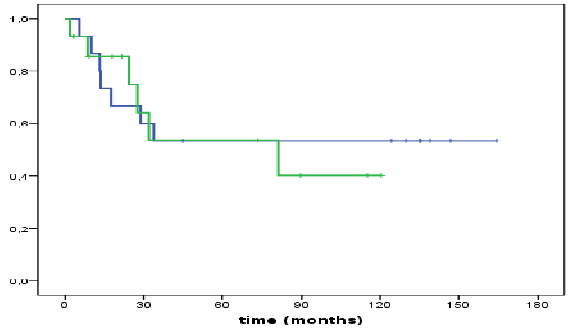


P=0.54

CD8

P=0.80

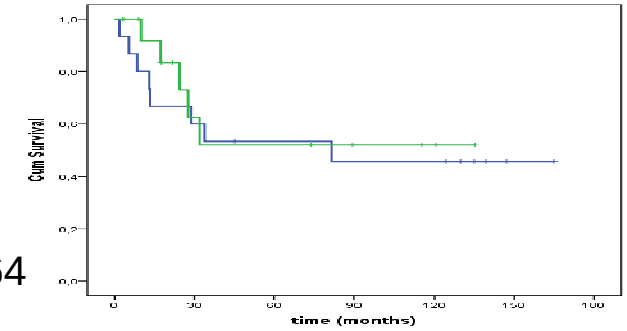


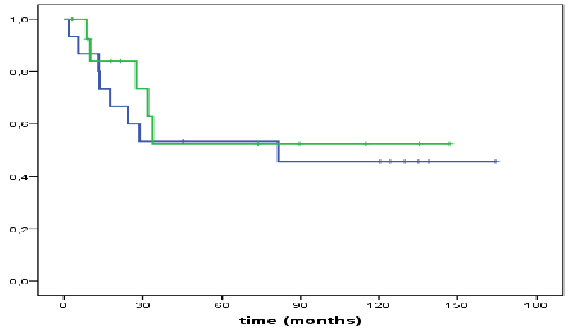


P=0.80

CD20

P=0.64

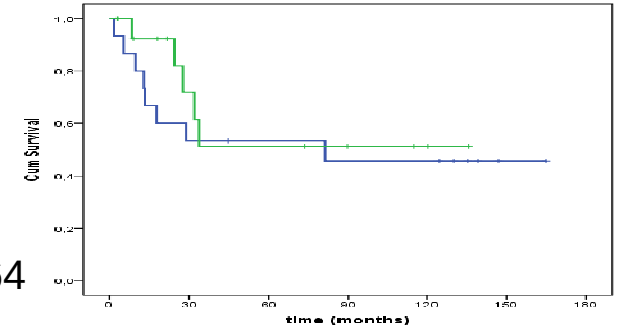


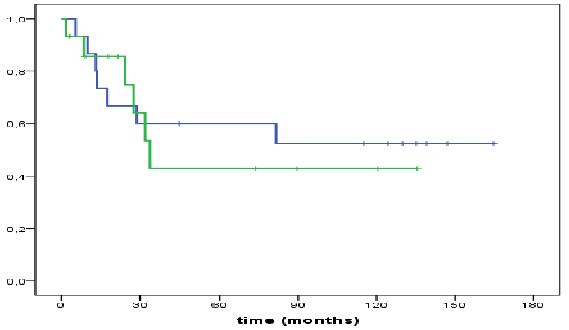


P=0.61

FP3

P=0.64



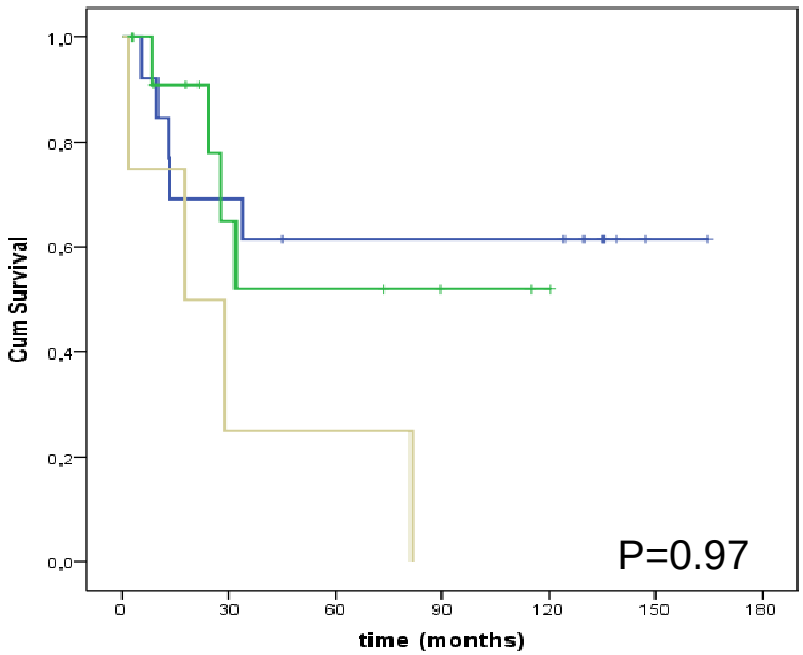


P=0.76

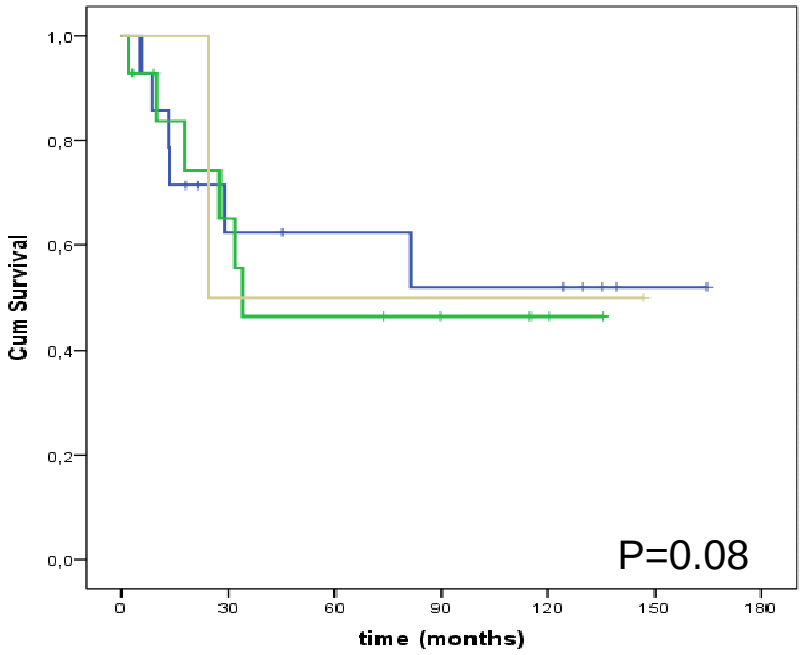
High

Low

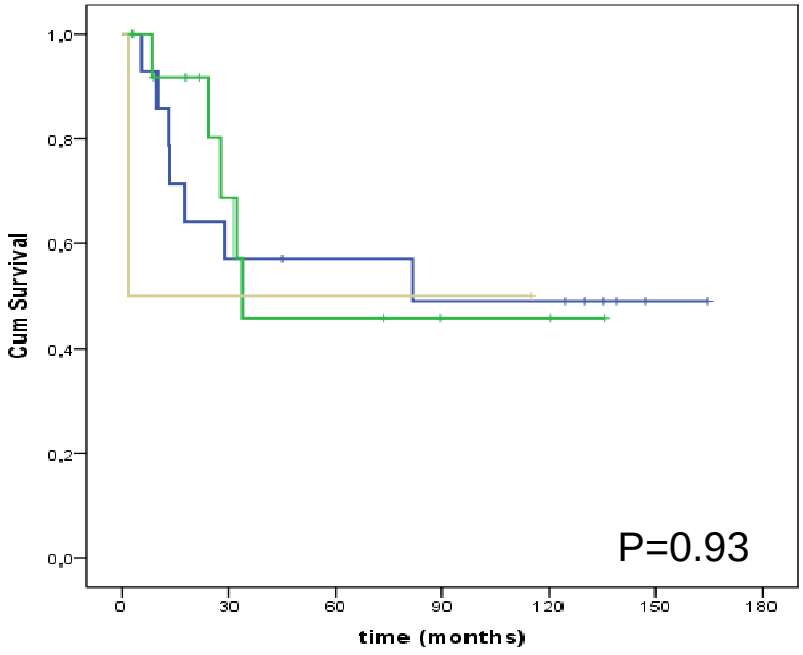
CD3 intra and peri



CD8 intra and peri

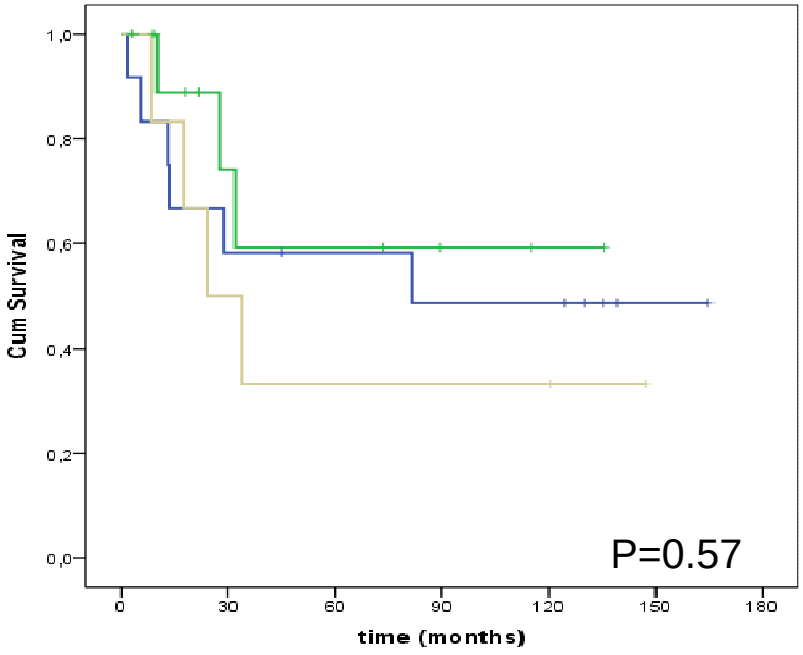


FP3 intra and peri

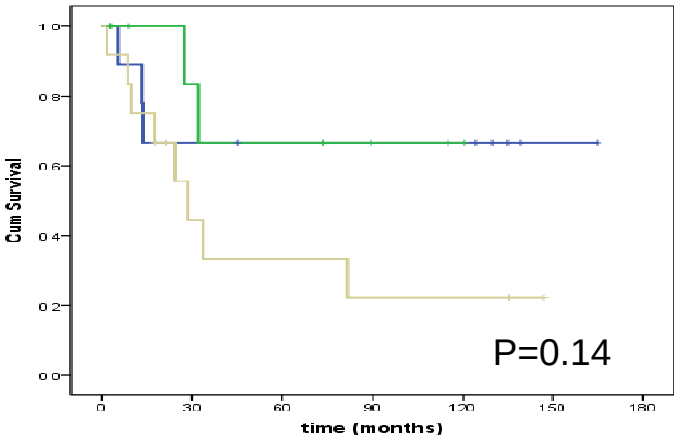


both_high
both_low
discordant

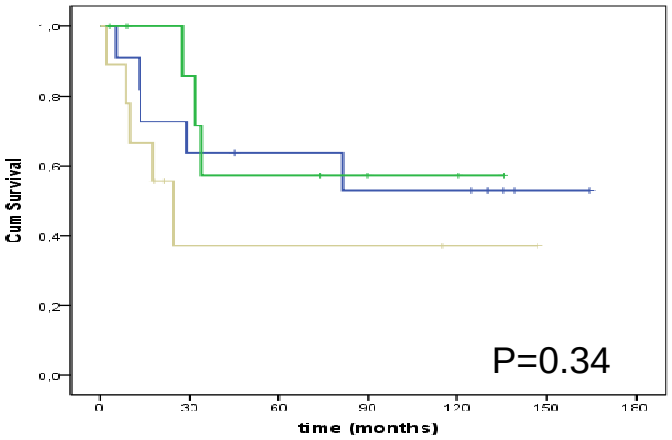
CD20 intra and peri



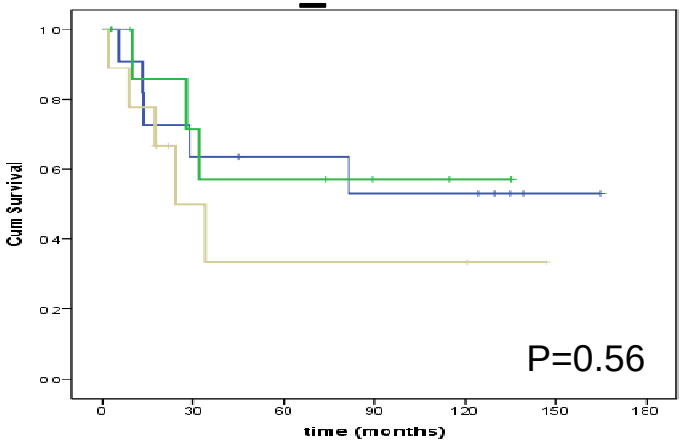
CD3_CD8



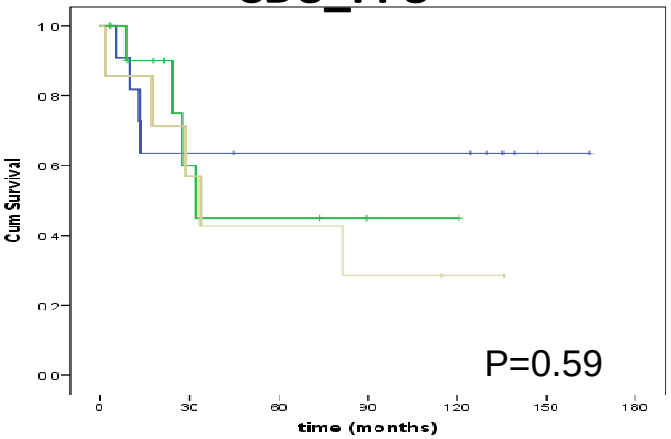
CD3_FP3



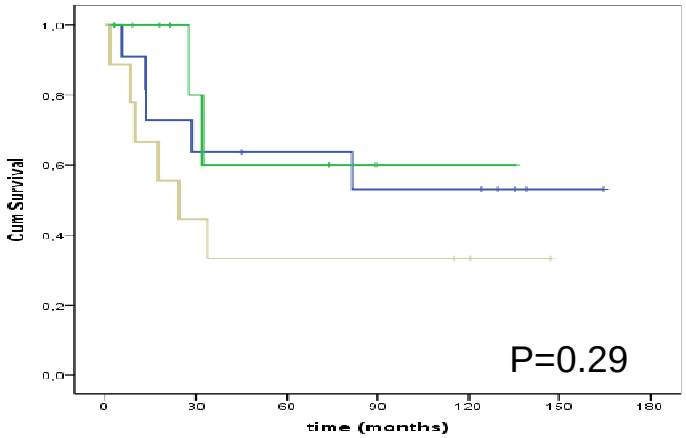
CD3_CD20



CD8_FP3



CD20_FP3

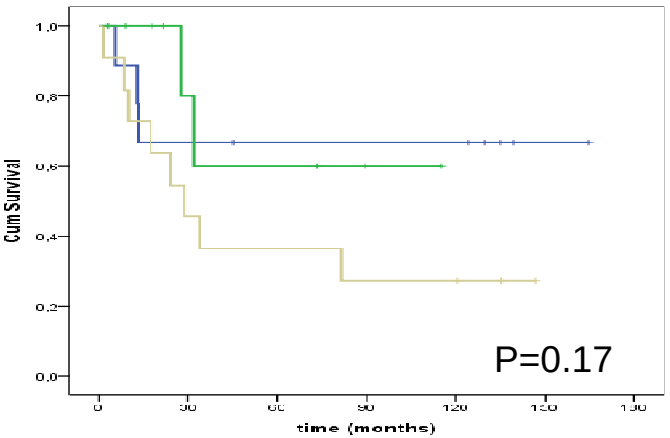


all_high

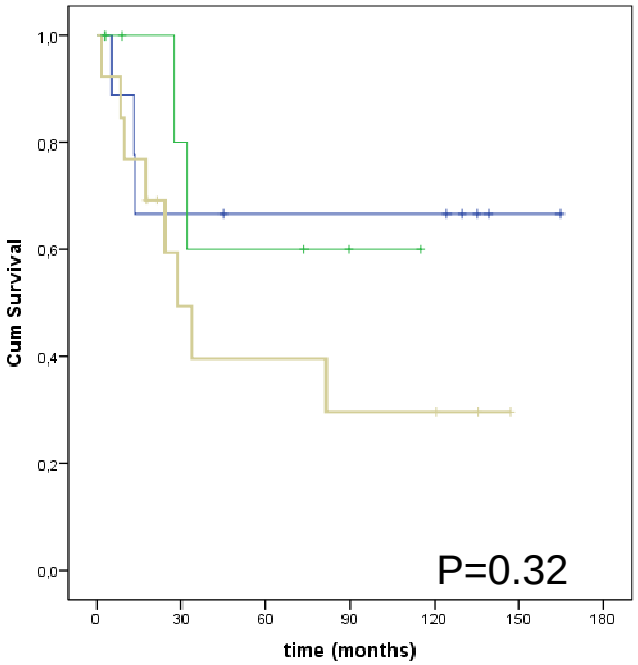
all_low

mixed

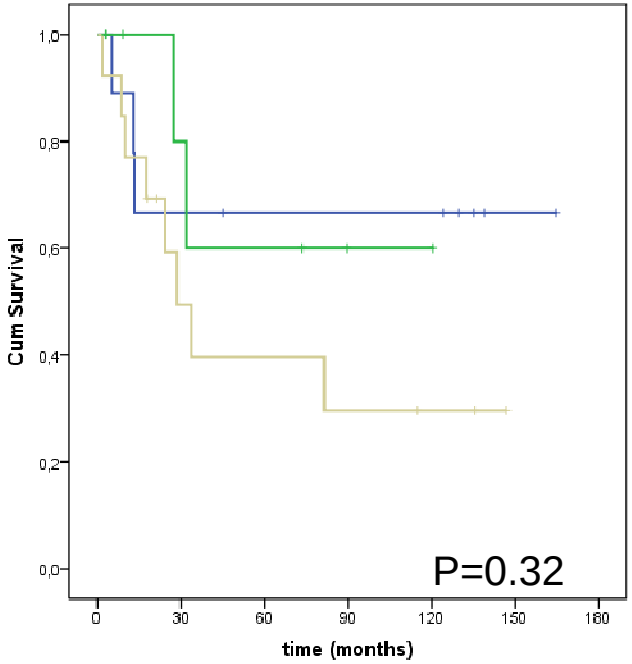
CD20_CD8



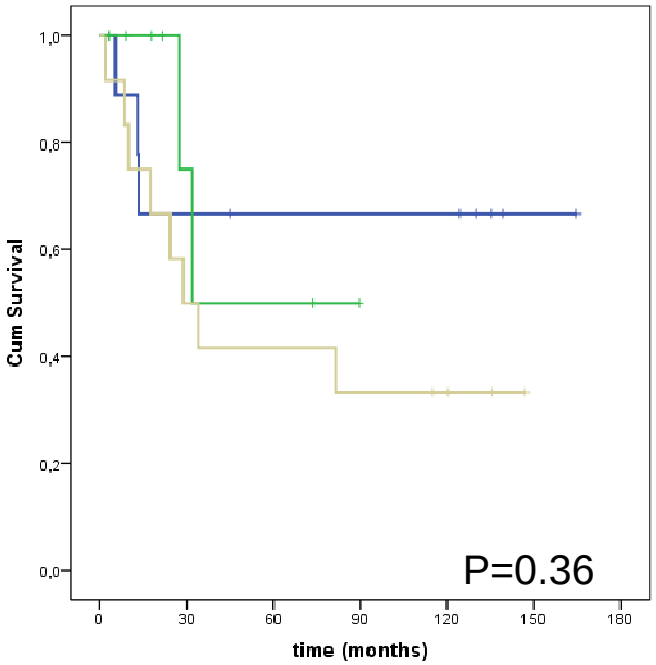
CD20_CD8_CD3



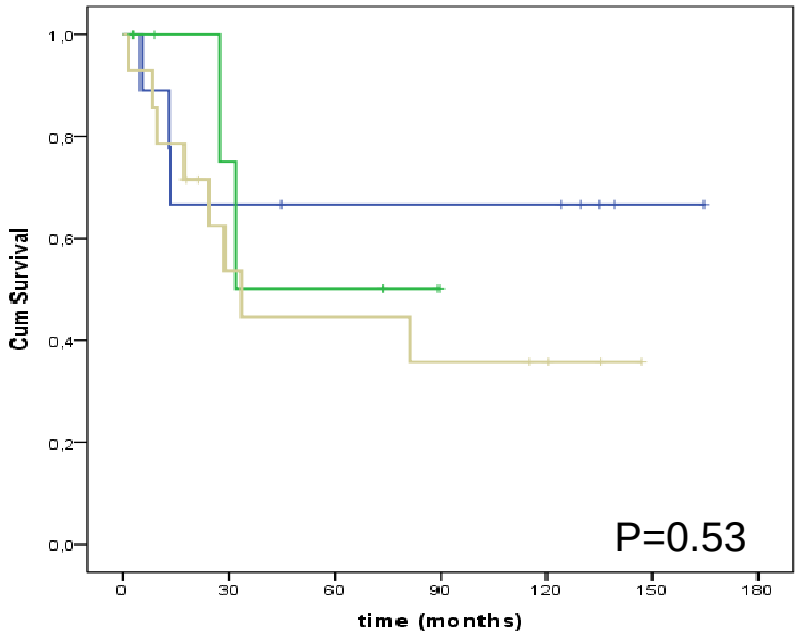
CD3_CD8_FP3



CD20_CD8_FP3



CD20_CD8_FP3_CD3



all_high

all_low

mixed

Results and Comments

Any evidence of correlations between infiltrate and outcome.

Manual count:

- **There are differences in expression levels in the reactive lymph nodes for CD8, high in the relapse group.**
- **There were significant differences in the peri /intra ratio for both CD3 and CD8, with the ratio being higher in no relapse patients**

Automatic count:

- **Initial analysis performed in a small cohort of heterogeneous patients (macrometastases, micrometastases, isolated tumoral cells, etc.), power to detect statistically significant differences low.**

We need

- **to evaluate a larger and homogenous cohort of patients**
- **to explore other markers.**

But…

The Algorithm is ready!

MISIPI Study: Melanoma ImmunoScore evaluation in patients treated with Ipilimumab

MISIPI Study

AIM

To evaluate the possible prognostic and predictive value of the immunoscore in patients receiving ipilimumab.

- **Immunescore based on the analysis of CD3, CD8, CD68, CD163 and FoxP3 may yield a new approach to classify, prognosticate, and potentially predict response to Ipilimumab for metastatic melanoma patients.**

MISIPI Study

- **200 FFPE samples from 150 metastatic melanoma patients treated with Ipilimumab;**

- **characterized the immune infiltrate and the density of different cellular immune population using the immunohistochemically detected expression: CD3, CD8, CD68, FoxP3 and CD163;**

- **Correlate marker expression profile with clinical outcome;**

…Study ongoing…

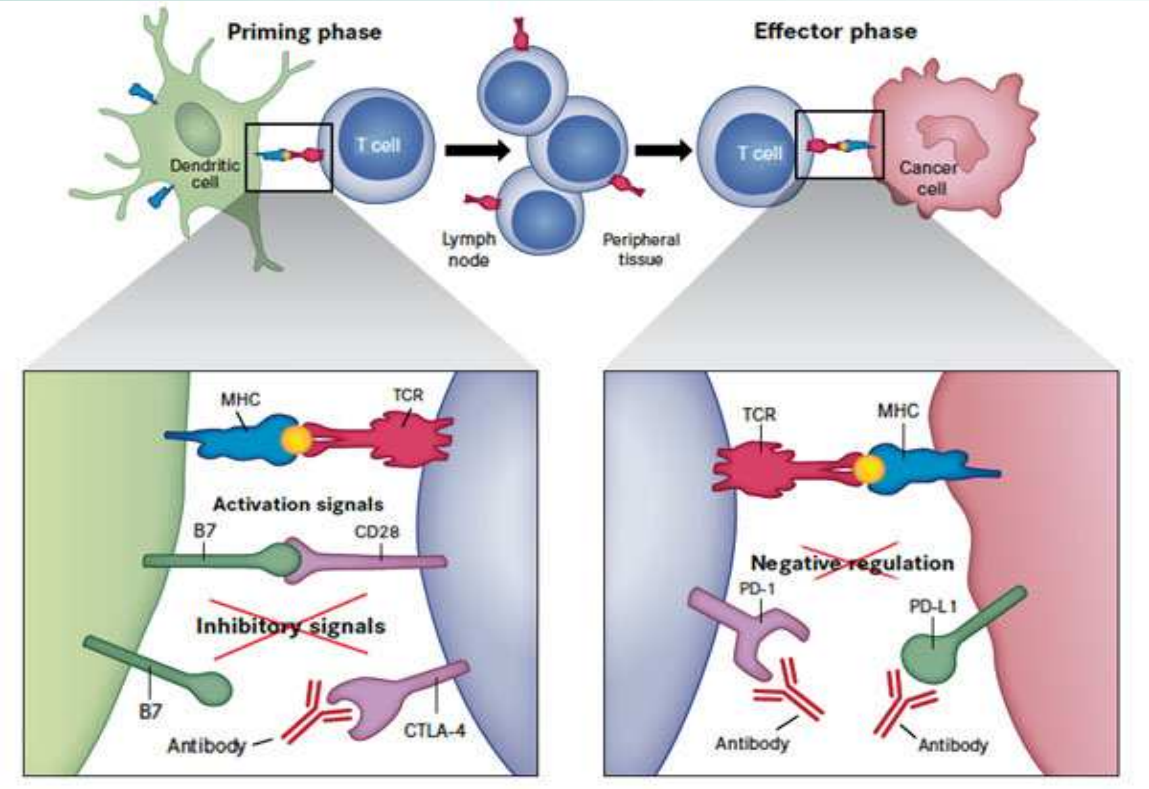
Meanwhile, evaluation of possible correlation between PD-L1 expression and patient outcome.

Bifulco C. et al. *Journal of Translational Medicine* 2014, 12(Suppl 1):P11.

PD-L1 Expression and Ipilimumab

AIM:

To evaluate correlation between baseline levels of PD-L1 (through DAKO assay) and outcome prediction in melanoma patients treated with Ipilimumab.



CTLA-4 and PD-L1 play different but complementary roles in the regulation of adaptive immunity!

Patients characteristics

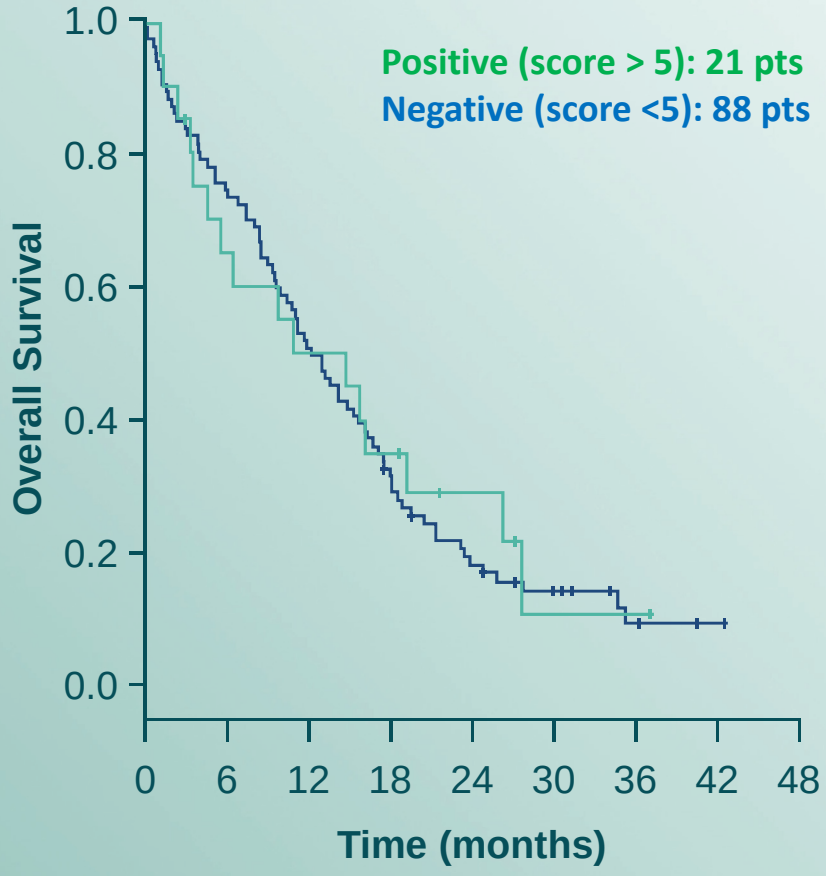
Median age, years (range)	61 years (25-90)
Male/female	57 (50%) 57 (50%)
Stage IV Melanoma	114/114 pts
BRAF status	
mutated	37 pts (32.5%)
WT	66 pts (57.9%)
Unknown	11 pts (9.6%)
Number of previous therapy, n(%)	
0	15 (13.1%)
1	83 (72.8%)
2	16 (14.1%)
≥3	0
Previous therapy type*	
CDDP+TMZ	24
Dacarbazine	23
Fotemustine	9
Temozolamide	8
MAGE A3 (PRAME)	4
MEK 162	6
Dabrafenib	5
Vemurafenib	25
Electrochemotherapy (Bleomicin + Electroporation)	6
Other (L19-IL2, Paclitaxel, Allovectin, CBDCA + DTC, etc)	10

*More than 114 therapies because some patients performed more than 1 line of treatment

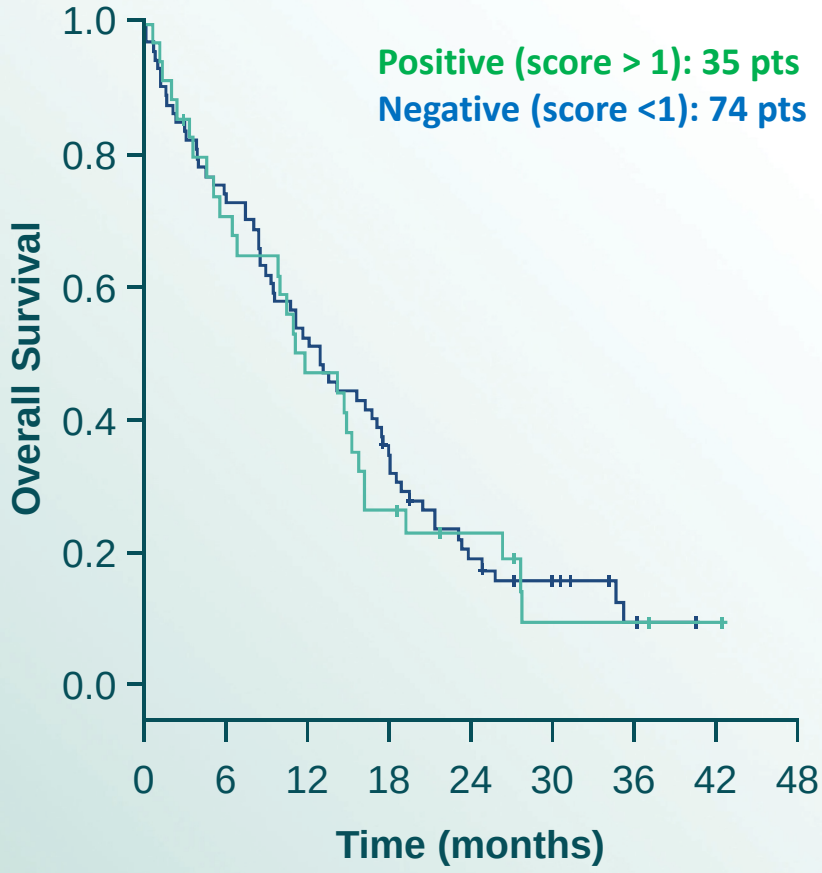
Ipilimumab Response

Patients evaluable	109 Pts
Ipilimumab cycles (median, range)	(4, 1 – 4)
4	75 (65.8%)
3 (2 stopped for toxicity)	16 (14.0%)
2	14 (12.3%)
1 (not evaluable – rapid progressors)	9 (7.9%)
Immuno-Related Disease Control Rate	25 (23%)
Immune-related DCR	
Complete response	11 (10.1%)
Partial response	4 (3.7%)
Stable disease	10 (9.2%)
Progression of disease	84 (77%)

PD-L1 status and Survival



PD-L1 status using a 5% tumor cell expression cut-off



PD-L1 status using a 1% tumor cell expression cut-off

PD-L1 status and IPILIMUMAB Response

IPI_response	Score <5	Score>5
CR	9 (10.2)	2 (9.5)
PR	2 (2.3)	2 (9.5)
SD	10 (11.4)	0
PD	67 (76.1)	17 (81.0)

P=0.18

IPI_response	Score <1	Score>1
CR	8 (10.8)	3 (8.6)
PR	2 (2.7)	2 (5.7)
SD	9 (12.2)	1 (2.9)
PD	55 (74.3)	29 (82.9)

P=0.37

Results and Comments

<u>PD-L1 was not a predictive biomarker for overall survival of melanoma patients treated with Ipilimumab</u>;

PD-L1 status cannot used to select patients for ipilimumab.

- PD-L1+ expression on tumor cells has been associated with favorable response rates in studies of nivolumab. (Topalian SL, N Engl J Med, 2012)
- PD-L1–negative/indeterminate expression had an improvement of overall survival with nivolumab compared with dacarbazine. (Michael A. Postow, The ASCO Post 2015)

<u>PD-L1 not a good predictive biomarker for overall survival and for patients selection for Nivolumab</u>:

Our findings are enhanced!!

Results and Comments

Probably due to heterogeneous expression of PD-L1 between patients and within individual patients (Madore J, et al. Pigment Cell Melanoma Res. 2015);

It is possible that a model with more than one immunologic factor, may help us to correlate PD-L1 and some aspects of melanoma disease.

We have an ongoing project where we correlate PD-L1 status and Mutational status with outcome of melanoma patients…interesting initial results!!!

Melanoma ImmunoScore: Discovery of predictive biomarker for IPILIMUMAB response

ISTITUTO NAZIONALE TUMORI
RCCS – Fondazione Pascale

DEFINIENS®
the tissue phenomics company

Discovery of predictive biomarker for IPILIMUMAB response

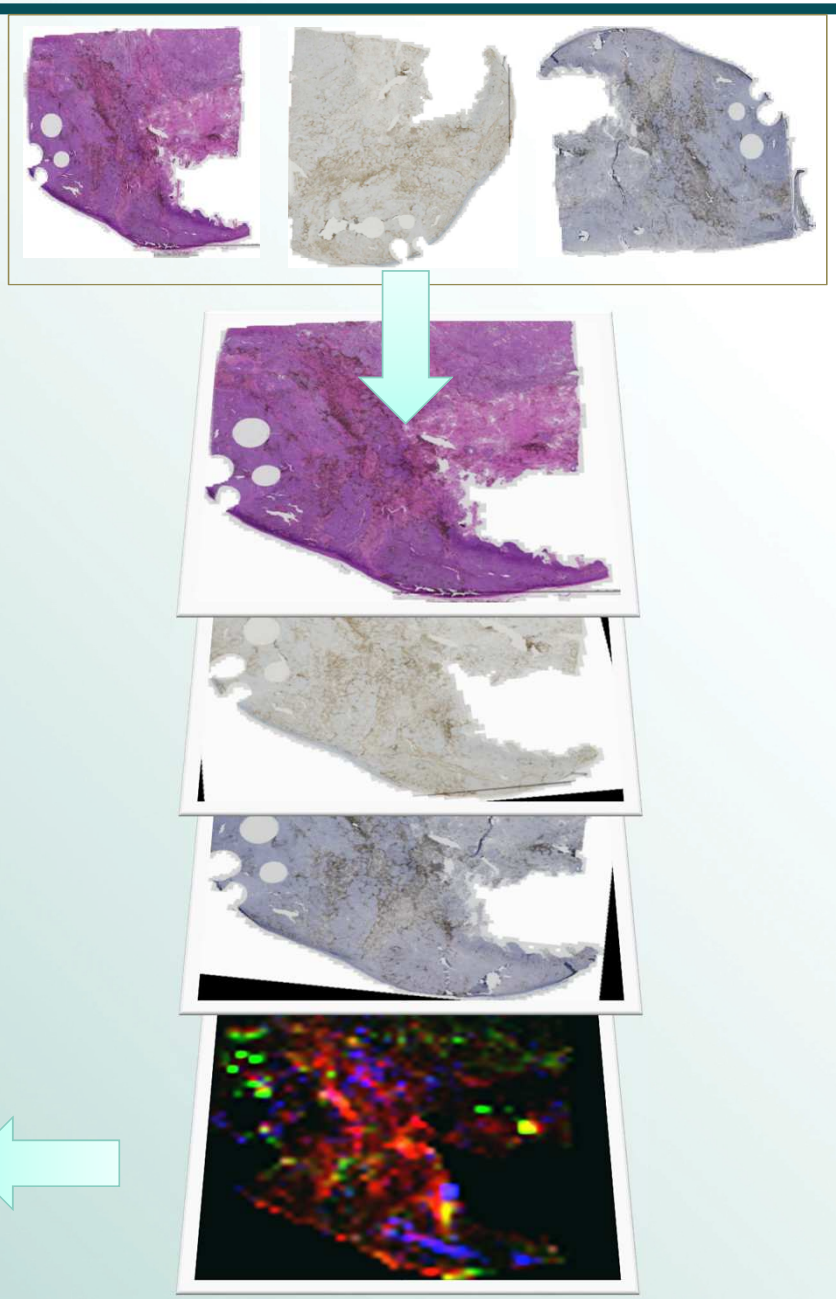
AIM:
To evaluate correlation between expression levels of CD3, FoxP3 and outcome prediction in melanoma patients treated with Ipilimumab.

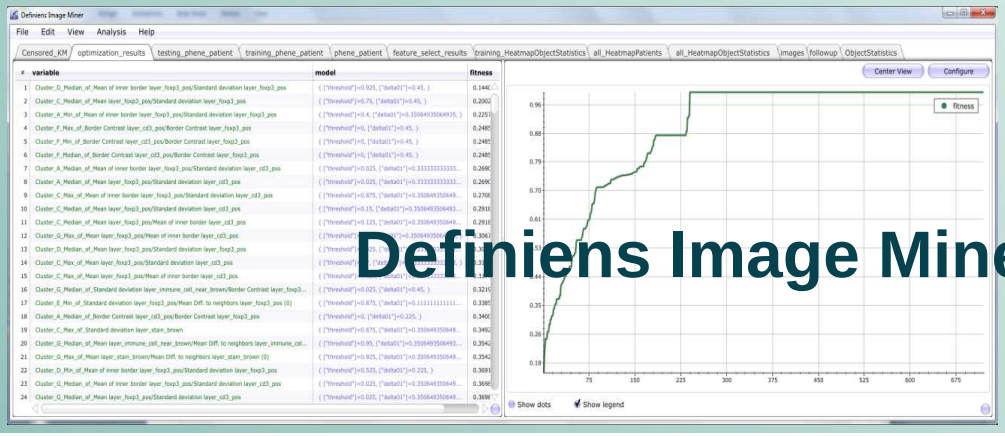
Patients evaluated	**31**
Immune-related DCR	**16**
Complete response	**11**
Partial response	**1**
Stable disease	**4**
Progression of disease	**15**

Analysis was performed on serial IHC stained section with:
H&E (morphology, tumor cells)
CD3 (T cells)
FoxP3 (regulatory T cells, Tregs)

Image Analysis and Data Mining Workflow

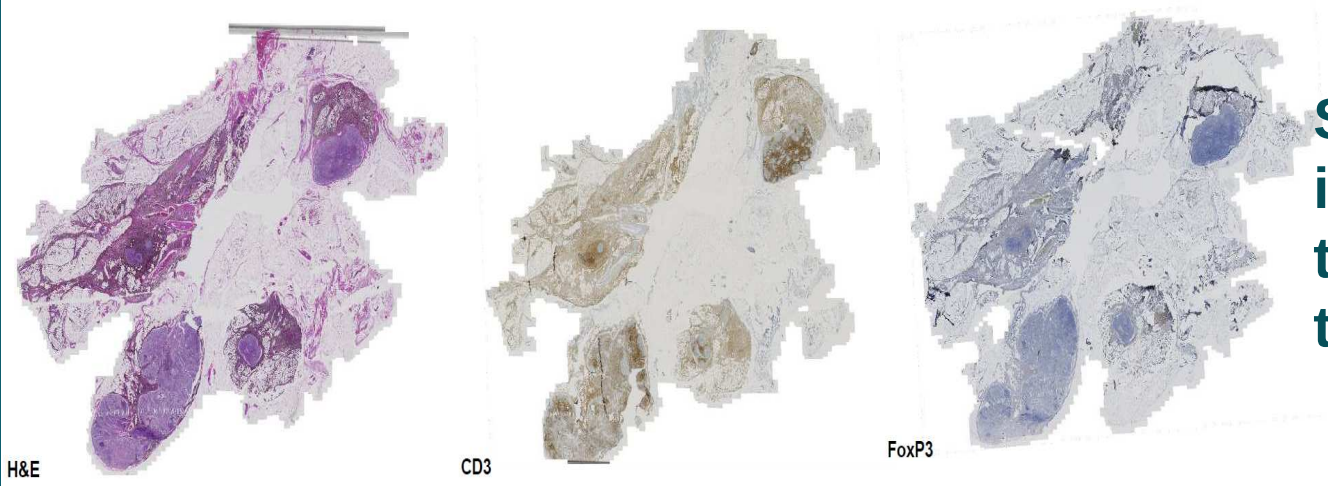
- Automated alignment of H&E, CD3 and FoxP3 tissue sections;
- Detection of tumor cells, $CD3^+$ and $FoxP3^+$ cells;
- Produce an image overlapping signals;
- On tumor cells regions (red) make automated measurement of $CD3^+$ (green) and $FoxP3^+$ (blue) evaluating center and border;
- Image analysis results was delivered in a scoresheet for the most predictive factors.



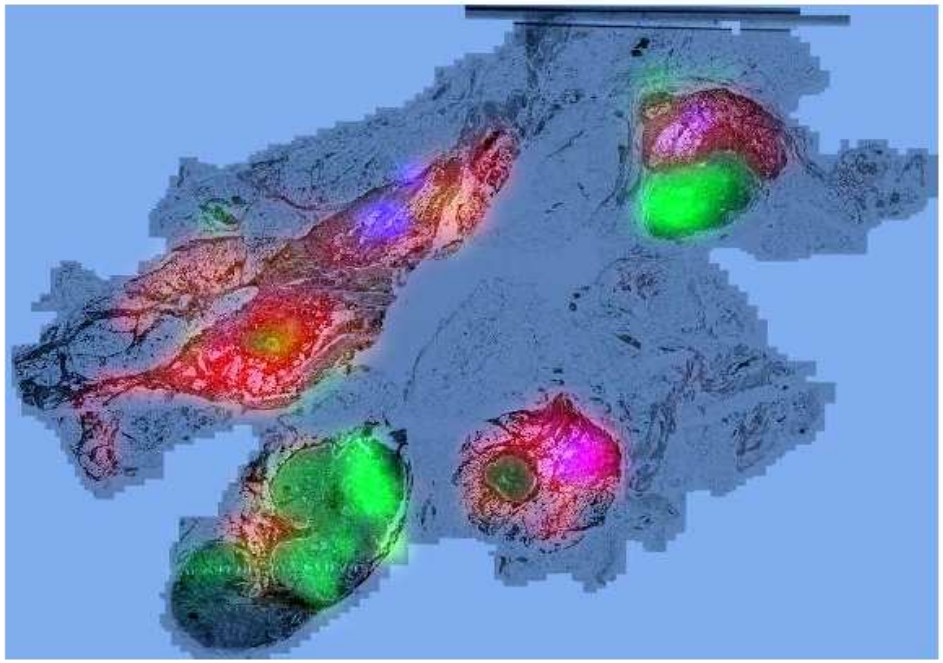


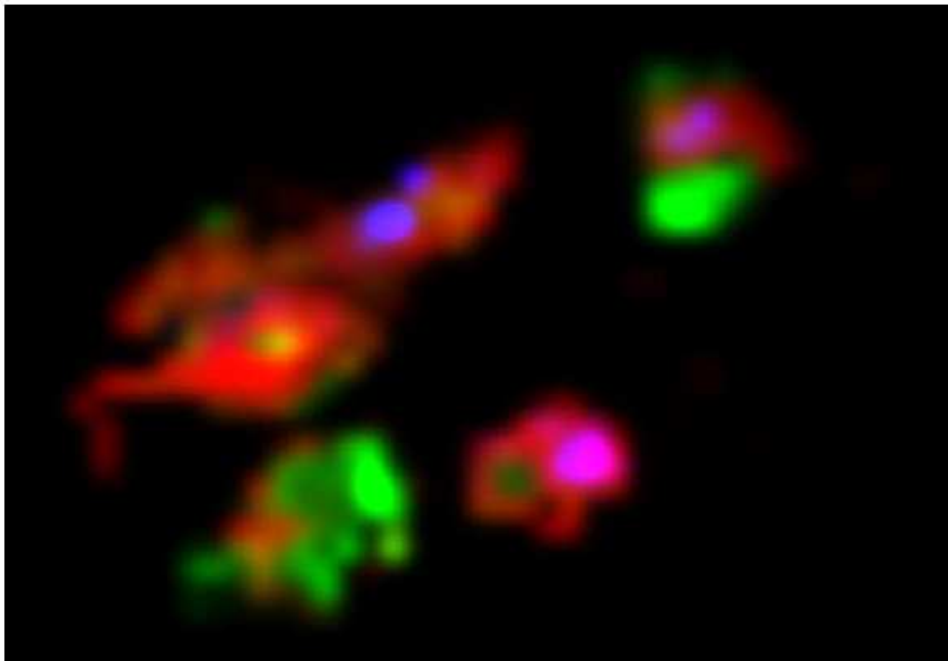
Definiens Image Miner®

Automated Alignment of Serial Sections for Co-Expression Analysis



Software works on an image taken by overlapping the signals obtained from the different slides.





Tumor cell: red **CD3:green** **FP3: blue**

Results and Comments

First results indicate that:

- **Tumor cells-FoxP3 spatial relations are the most predictive factors: IPILIMUMAB Therapy benefit if FoxP3+$_{TB}$ > 20% FoxP3+$_{TC}$;**
- **CD3/FoxP3 ratio on tumor margin seems to be a promising additional factor;**
- **CD3 alone doesn't provide predictive information.**

These early results need further validation with larger cohorts and other biomarkers :

- **CD8 (ready, ongoing a re-analysis of the results including any added data derived from CD8 analysis)**
- **CD45RO, CD163, CD68 and other markers.**

The Next Steps

Additional investigations with immuno markers predictive for outcome and for response to the therapies remain of great interest.

To evaluate the functionality of the different immune cells in the tumor microenviroment may be a good future prospective.

TO BE CONTINUED…

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