Transcriptional Signatures of Immune Surveillance and a Favorable Tumor Immune Micro-environment

SITC Cancer Immune Responsiveness Workshop
September 4, 2019
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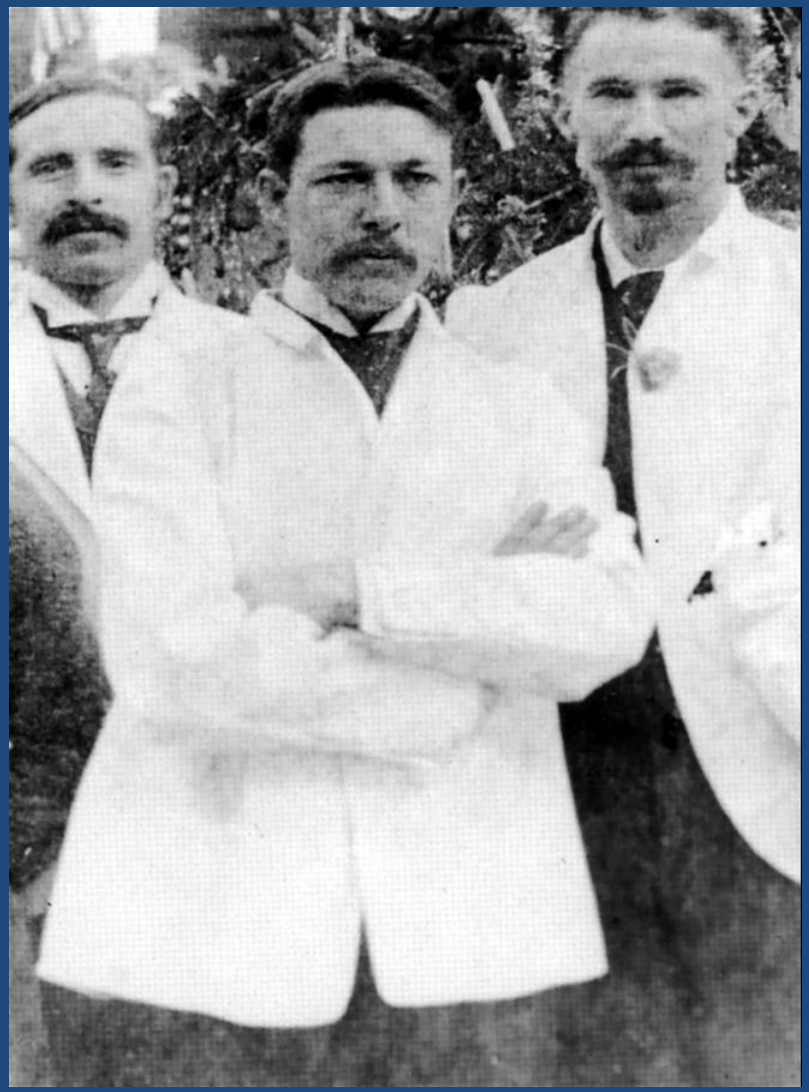
Disclosures

- Research Funding from Amgen
- Research materials provided by NanoString and Perkin Elmer (now Akoya)

Talk Outline

1. Introduction
2. Transcriptional Signature of Immune Surveillance
3. Cellular Composition of the tumor immune micro-environment (TiME) in melanoma
4. Methods to study the TME in formalin fixed archival tissues

The long history of cancer immunotherapy



New York Times - July 29, 1908

ERYSIPELAS GERMS AS CURE FOR CANCER

Dr. Coley's Remedy of Mixed Toxins Makes One Disease Cast Out the Other.

MANY CASES CURED HERE

Physician Has Used the Cure for 15 Years and Treated 430 Cases— Probably 150 Sure Cures.

Following news from St. Louis that two men have been cured of cancer in the City Hospital there by the use of a fluid discovered by Dr. William B. Coley of New York, it came out yester-

Chemotherapy- still "20th century medicine"

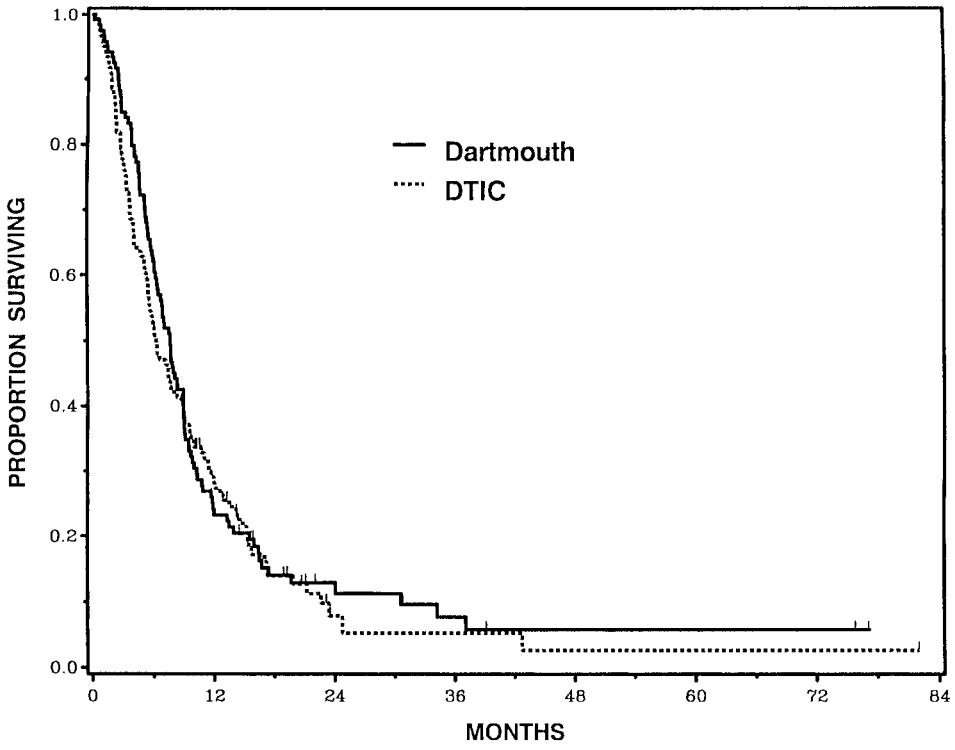
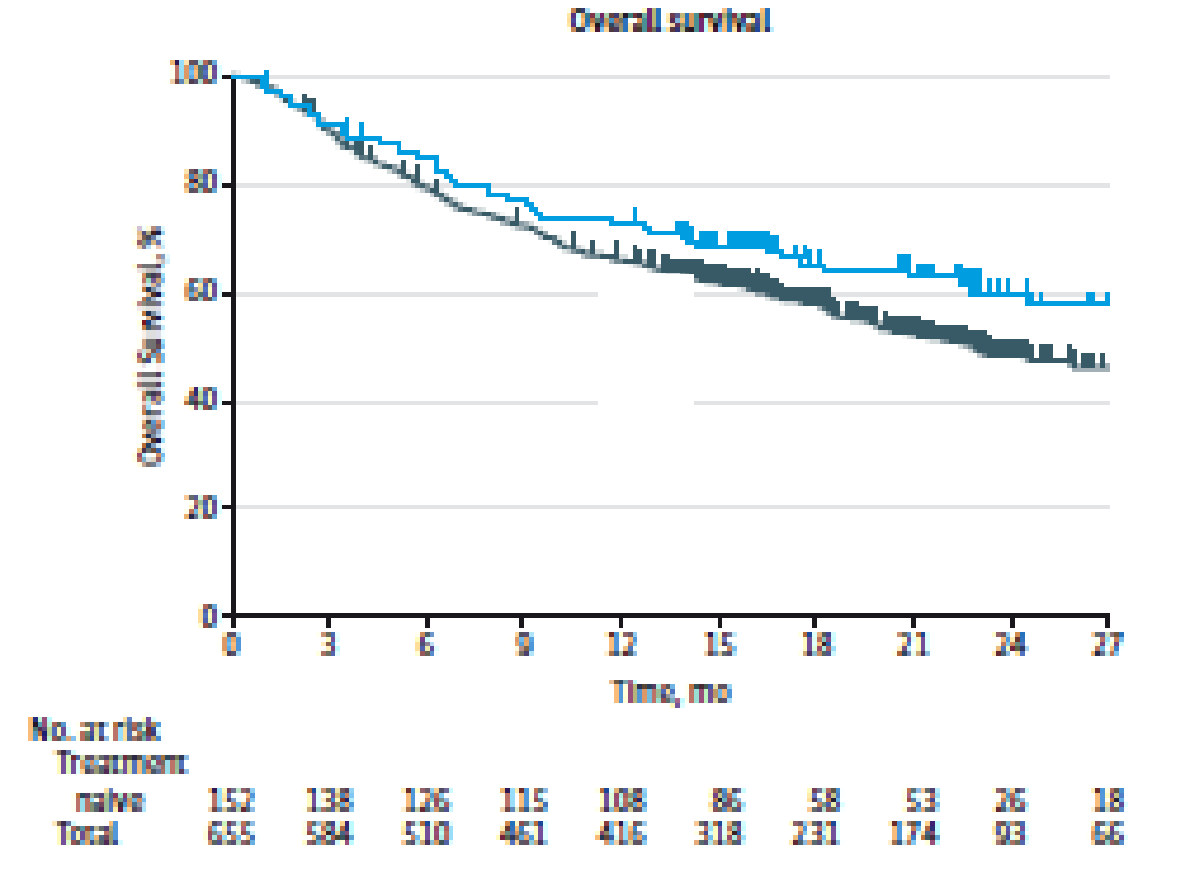


Fig 1. Kaplan-Meier survival plot of all 240 patients randomized based on intent to treat. Median survival time on Dartmouth regimen was 7.7 months (95% CI, 6.3 to 8.9 months) versus 6.3 months (95% CI, 5.4 to 8.7 months) on dacarbazine ($P = .52$). Tick marks represent censored patients.

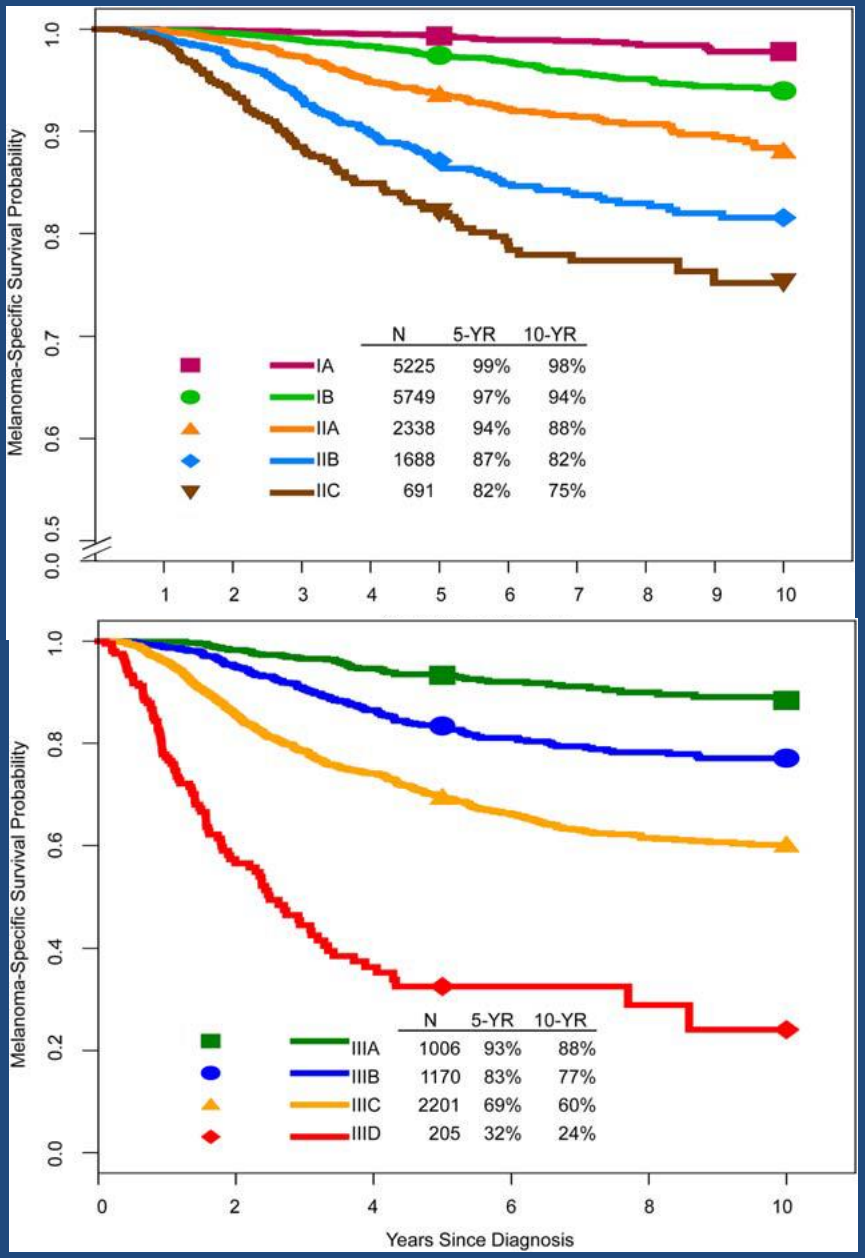
Chapman et al, "Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in metastatic melanoma," *Journal of Clinical Oncology,* 1999.

Pembrolizumab: Survival from Multiple Trials in Treatment Naïve (Light Blue) and Pre-Treated (Dark Blue) Patients



Schadendorf D et al. *J Clin Oncol.* 2015;33:1889-1894.

The clinical problem of stage II-III resectable melanoma



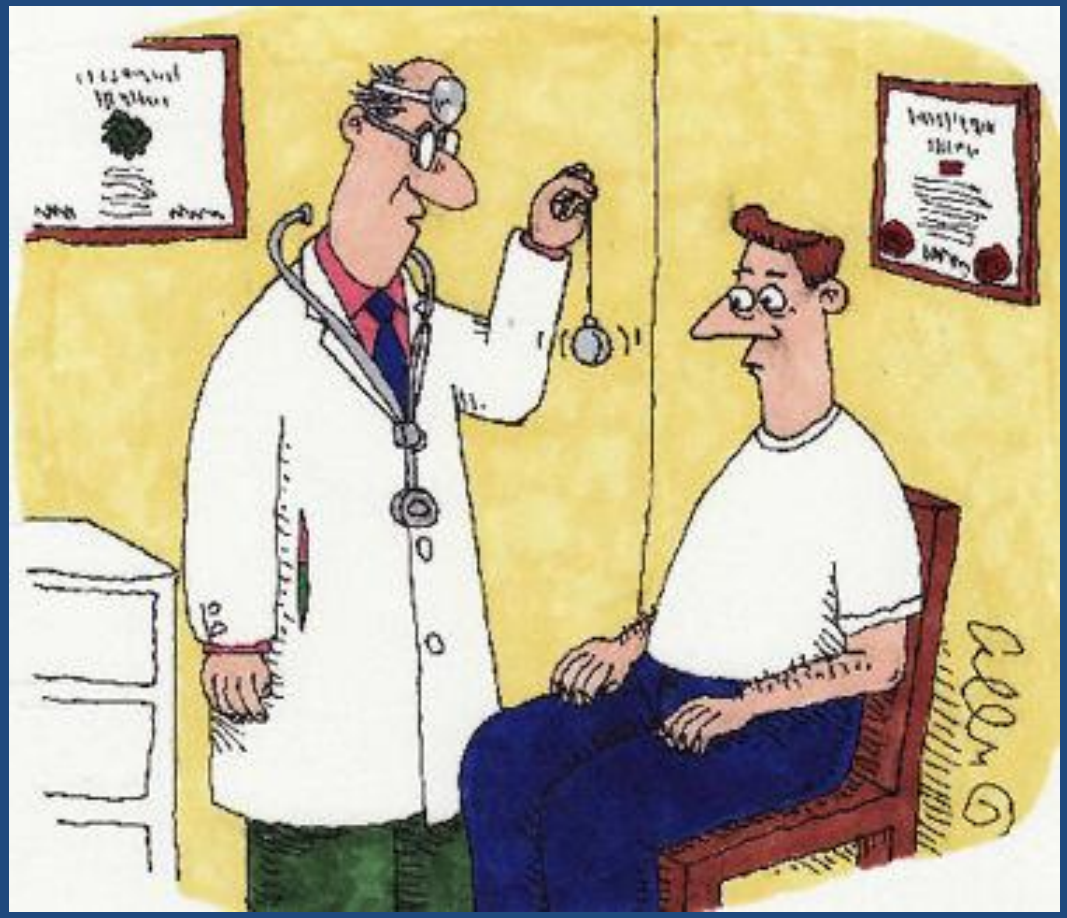
What do we tell the patient?

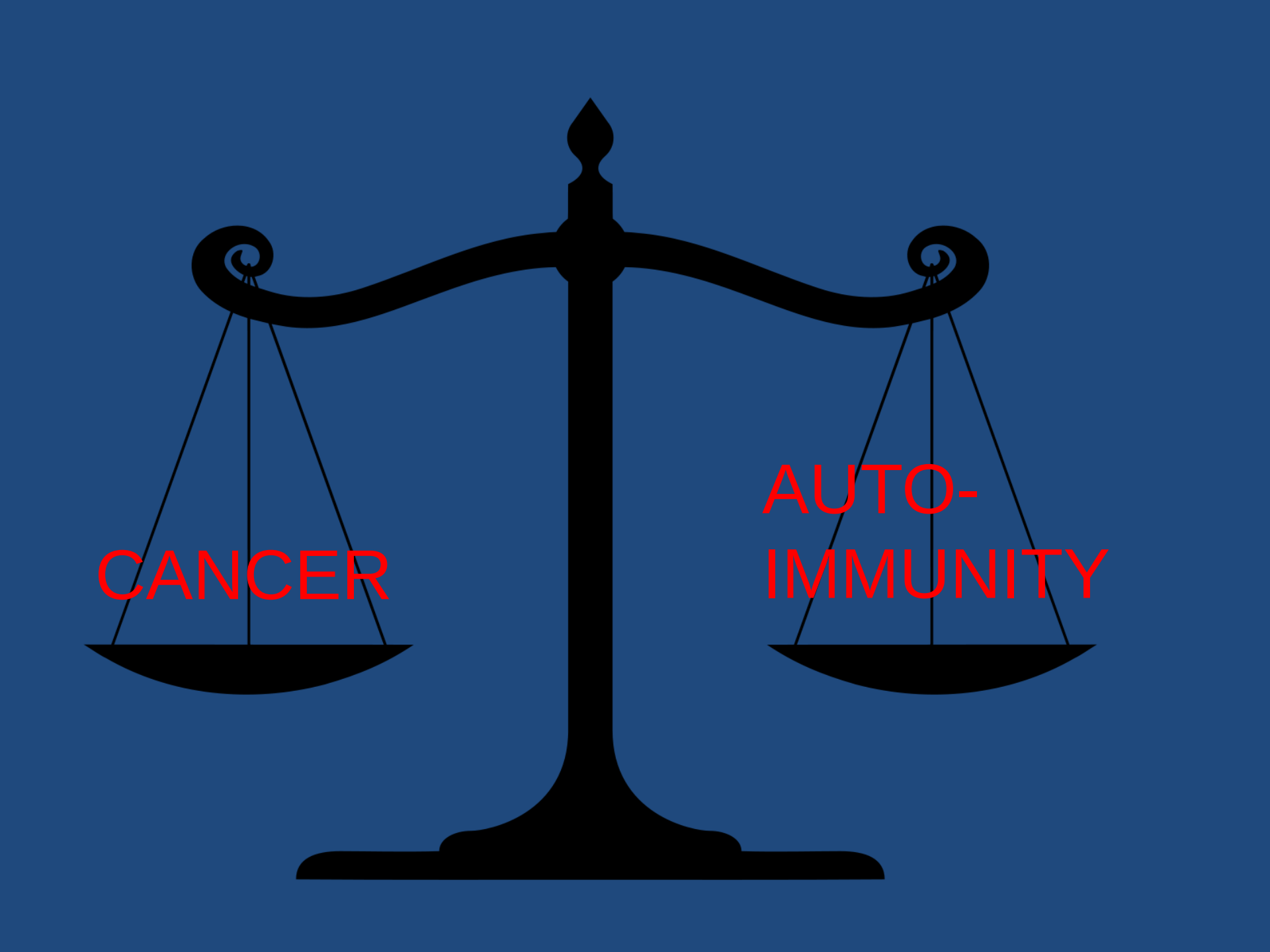
Should she take interferon or an experimental treatment?

Should she get scans? How often?

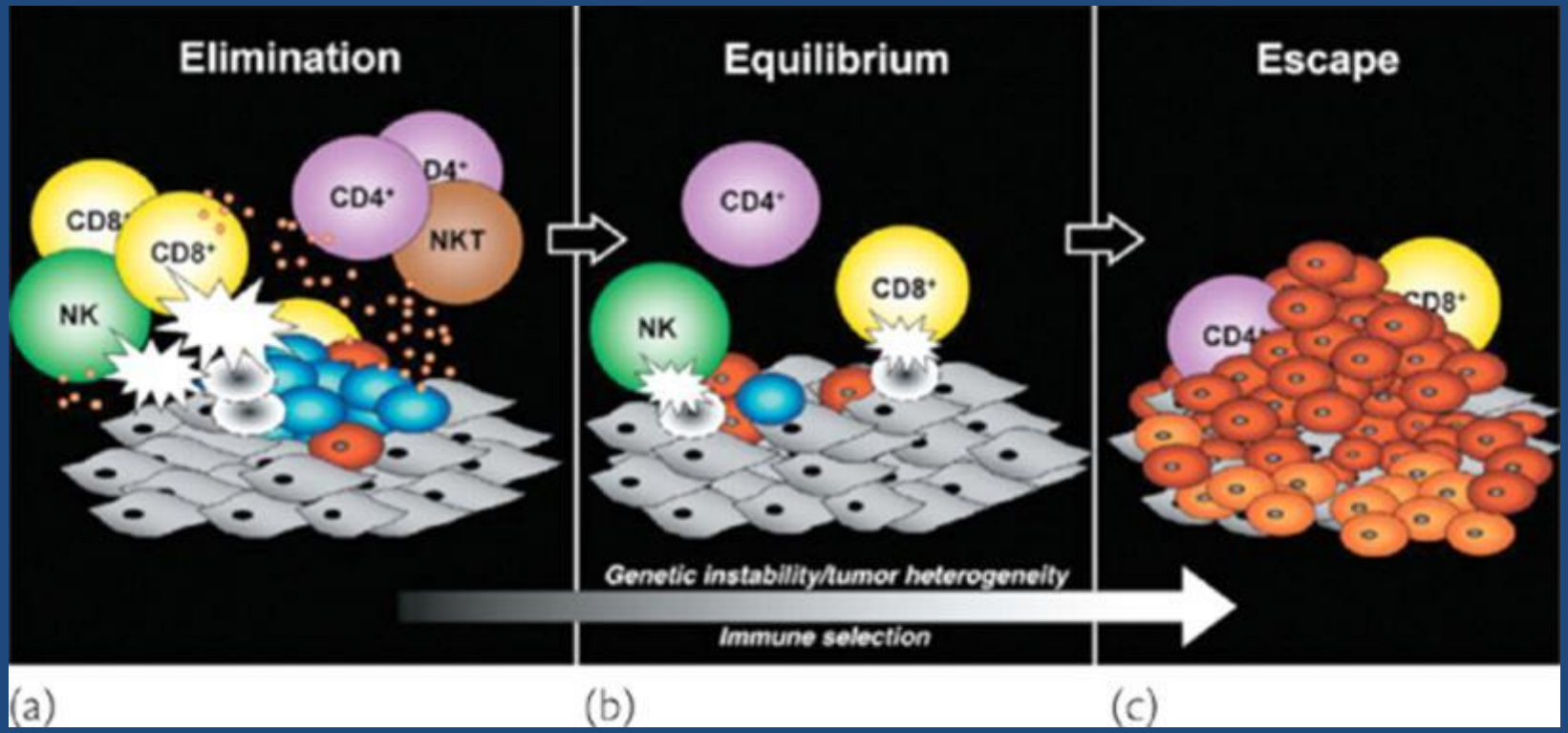
Gershenwald et al, "Melanoma Staging: Evidence-Based Changes in the AJCC 8th Edition Cancer Staging Manuel," 2017

“Is your immune system working?”



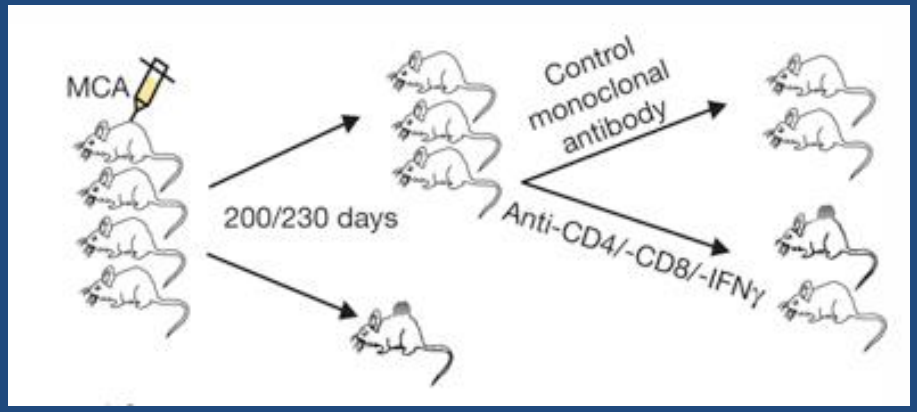

CANCER
AUTO-
IMMUNITY

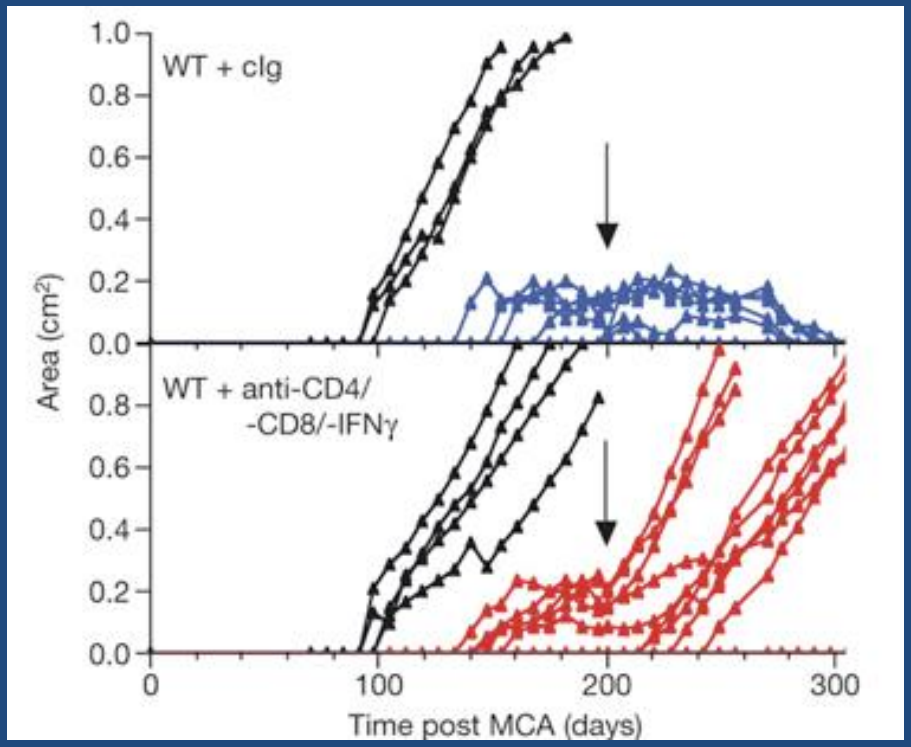
Hypothesis- One of the factors increasing risk of micrometastasis is Weakened Immunosurveillance



Can we identify patients who have weaker immune surveillance and require additional immunotherapy after surgery?

Immune surveillance controls dormant tumors





Koebel et al. Nature;450:903, 2007

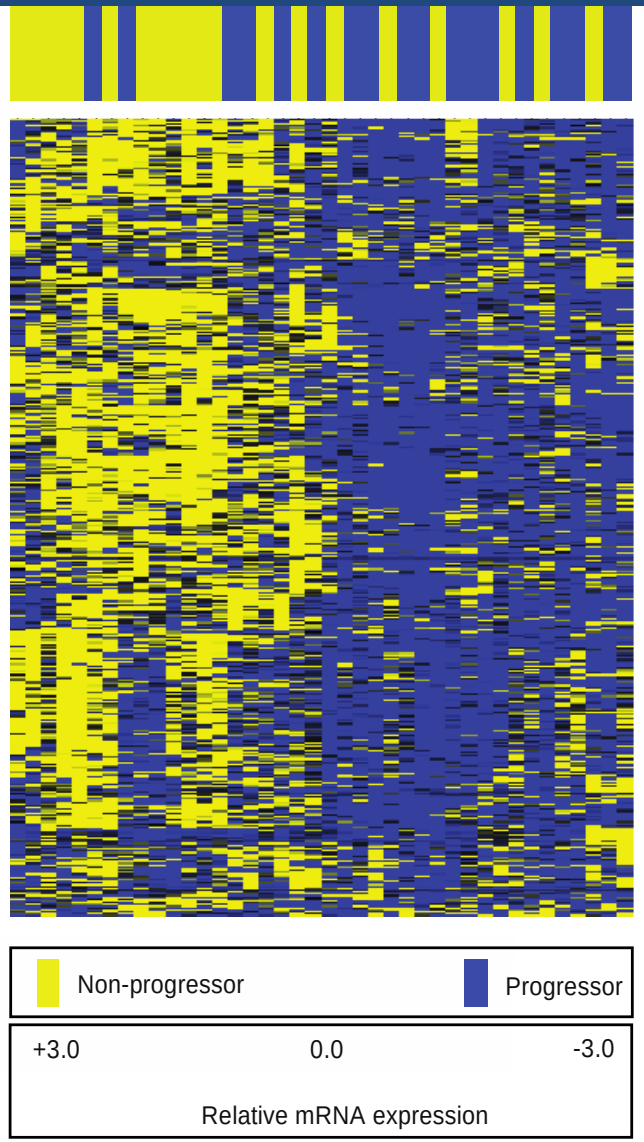
Transcriptional Signature of Immune Surveillance- Melanoma Immune Panel (MIP)

mRNA copy number for 446 genes in a training set of primary tumors from patients with resectable stage II-III melanoma

Characteristics of the Training Set

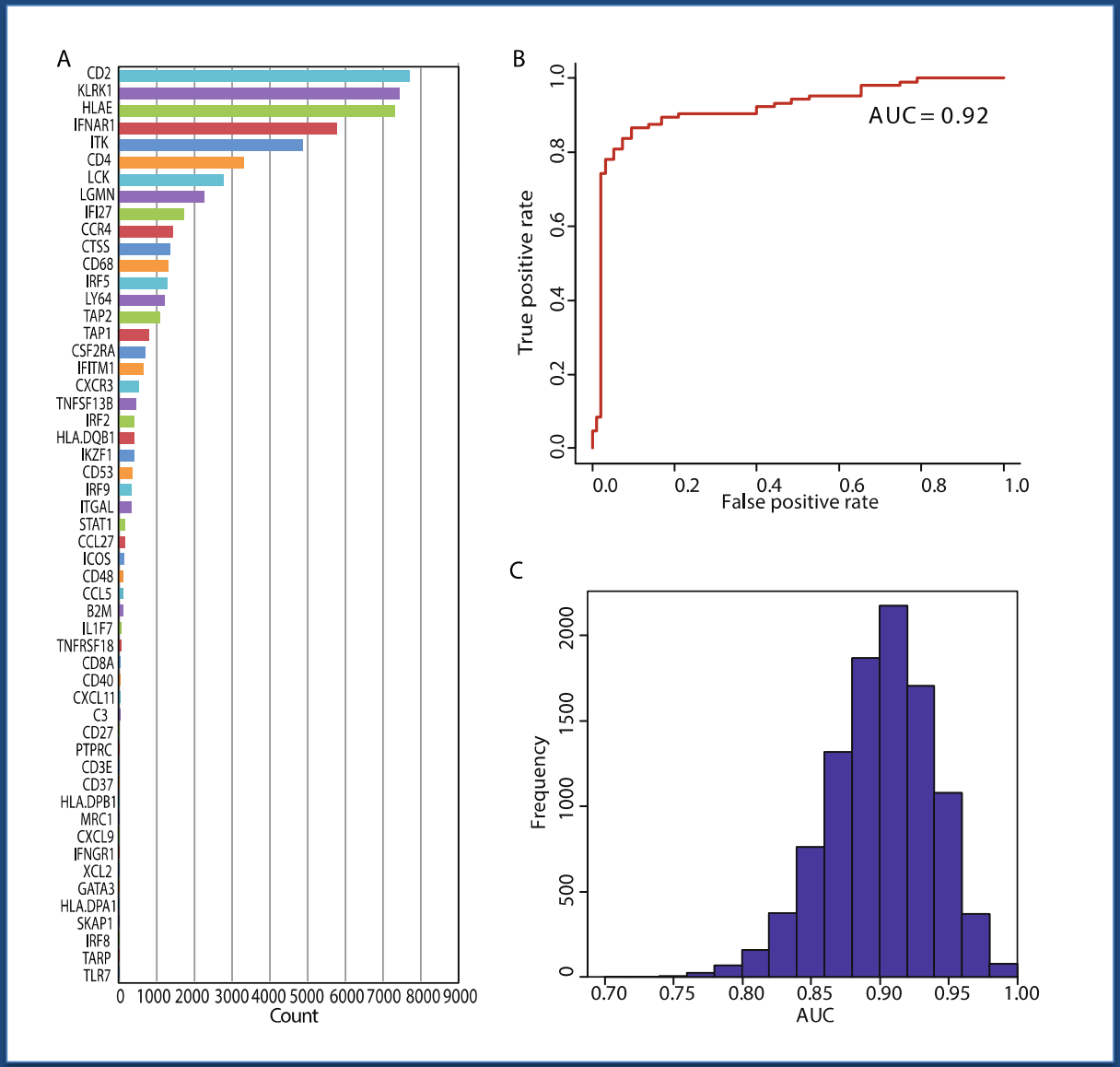
Characteristics	Training Set (N=40)
Clinical characteristics	
Gender	
Male--no.(%)	28 (70)
Female--no.(%)	12 (30)
Age median (range) --no.	67 (29-87)
Location of tumor	
Trunk--no.(%)	24 (60)
Extremity--no.(%)	16 (40)
Stage	
II--no. (%)	18 (45)
III--no. (%)	22 (55)
Pathological characteristics	
Depth (mm) -- median (range)*	2.7 (1.2 -13)
Ulceration	
Absent--no.(%)	21 (52)
Present--no.(%)	19 (48)
Tumor-infiltrating lymphocytes	
Absent--no.(%)	7 (17)
Non-brisk--no. (%)	28 (70)
Brisk--no.(%)	5 (13)
Mitoses --median (range)	6.5 (0-26)
Patient outcome	
Disease progression-- no. (%)	21 (52)
Died from melanoma--no.(%)	17 (43)
Patient Follow-up (months)	
Time to death--median (range)	19 (6-81)
Time to censoring – median(range)	61 (25-130)

* Depth is available for 39 patients.



Sivendran et al, *JID* 2014

Defining Melanoma Immune Profile- a 53 immune gene panel predictive of non-progression



Sivendran et al, *JID* 2014

Screen of publicly available primary melanoma expression data (GEO) identifies subnetwork of 758 gene module with immune function containing 42 out of the 53-gene immune panel

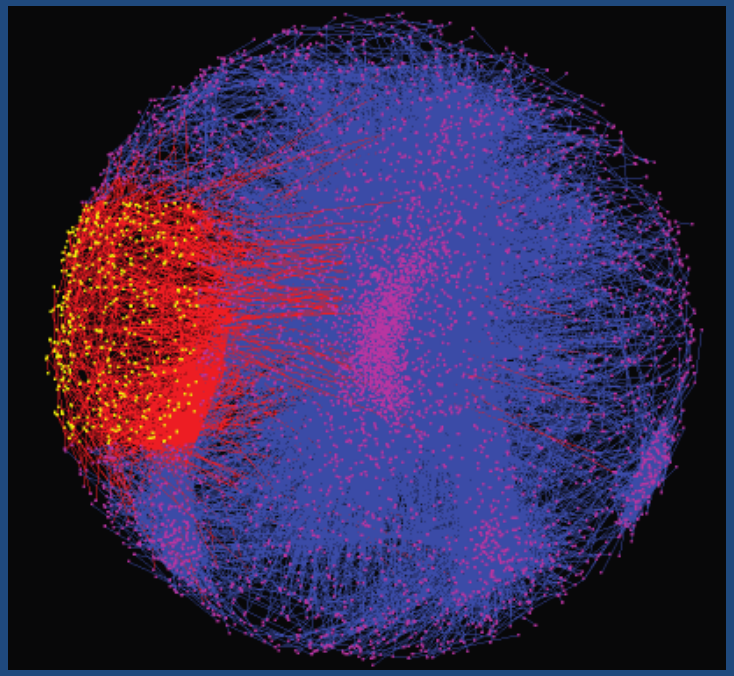


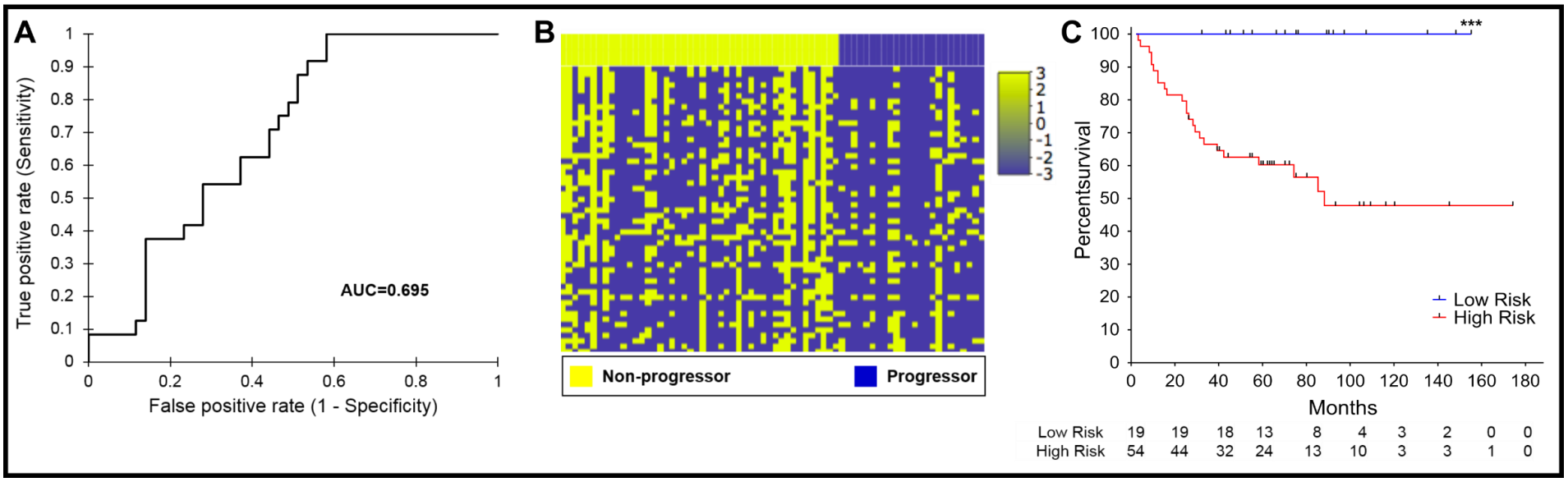
Table S6. Relative mRNA counts for top 25 hub genes comparing progressors and non-progressors in the training set.

Gene Symbol	Out-Degree	Log2 Fold Change	T-Statistic	P Value	FDR
CCR5	12	-0.5754	1.9699	0.0593	0.1598
CD8A	12	-0.9392	2.5076	0.0182	0.1021
CD3D	11	NT*	NT	NT	NT
CD8B	11	NT	NT	NT	NT
IKZF1	11	-0.5553	2.3094	0.0265	0.1044
BTK	10	-0.4292	2.3713	0.0234	0.1044
LCK	10	-0.9079	2.9279	0.0063	0.1021
CD3E	9	-0.6031	2.192	0.0357	0.1205
CD53	9	-0.5731	2.425	0.0222	0.1044
COPS2	9	NT	NT	NT	NT
CXCL9	9	-0.7667	1.9471	0.0592	0.1589
EGFR	9	NT	NT	NT	NT
EPS8L1	9	NT	NT	NT	NT
FGFR3	9	NT	NT	NT	NT
GPS1	9	NT	NT	NT	NT
MS4A1	9	NT	NT	NT	NT
PPP3CB	9	NT	NT	NT	NT
CD1A	8	-0.1511	0.5498	0.5857	0.6981
CD2	8	-0.9678	3.4058	0.0020	0.0852
CD58	8	-0.0554	0.3335	0.7406	0.8211
CD81	8	NT	NT	NT	NT
IL2RB	8	NT	NT	NT	NT
NCOR2	8	NT	NT	NT	NT
PRKD2	8	NT	NT	NT	NT
TNFRSF1B	8	NT	NT	NT	NT

* Not tested. Data is only available for those hub genes selected as part of the 446 gene panel.

Sivendran et al, *JID* 2014

Confirming MIP in a third independent patient population



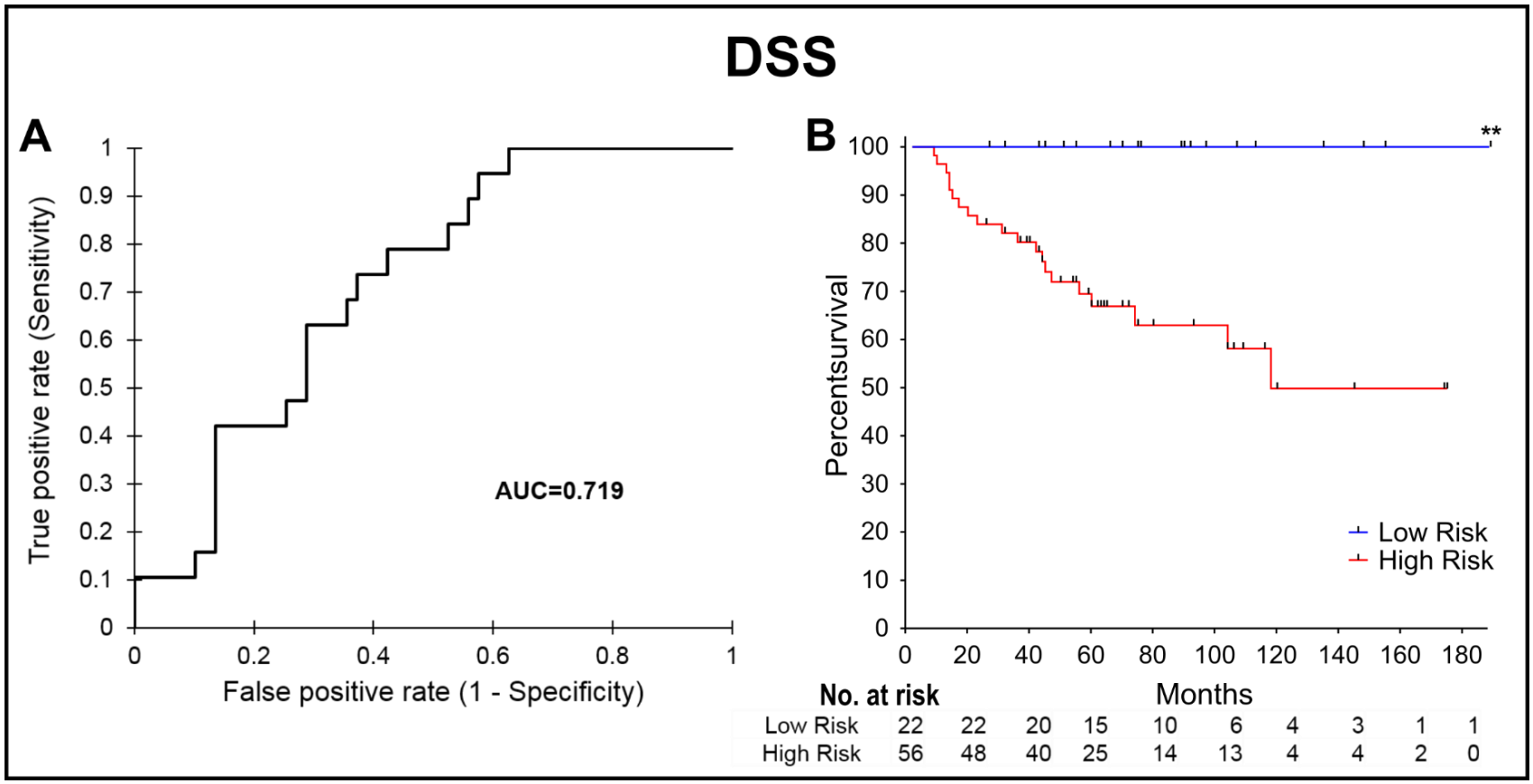
Receiver Operating Characteristic (ROC) curve analysis \(AUC)=0.695, p=0.008).

Heat map Yellow indicates higher expression and blue indicates lower expression of each gene in the color scale.

Kaplan-Meier (KM) curve for Distant Metastasis-Free Interval (DMFI) (p=0.0009)

Gartrell et al, *CCR* 2019

Confirming MIP in a third independent patient population

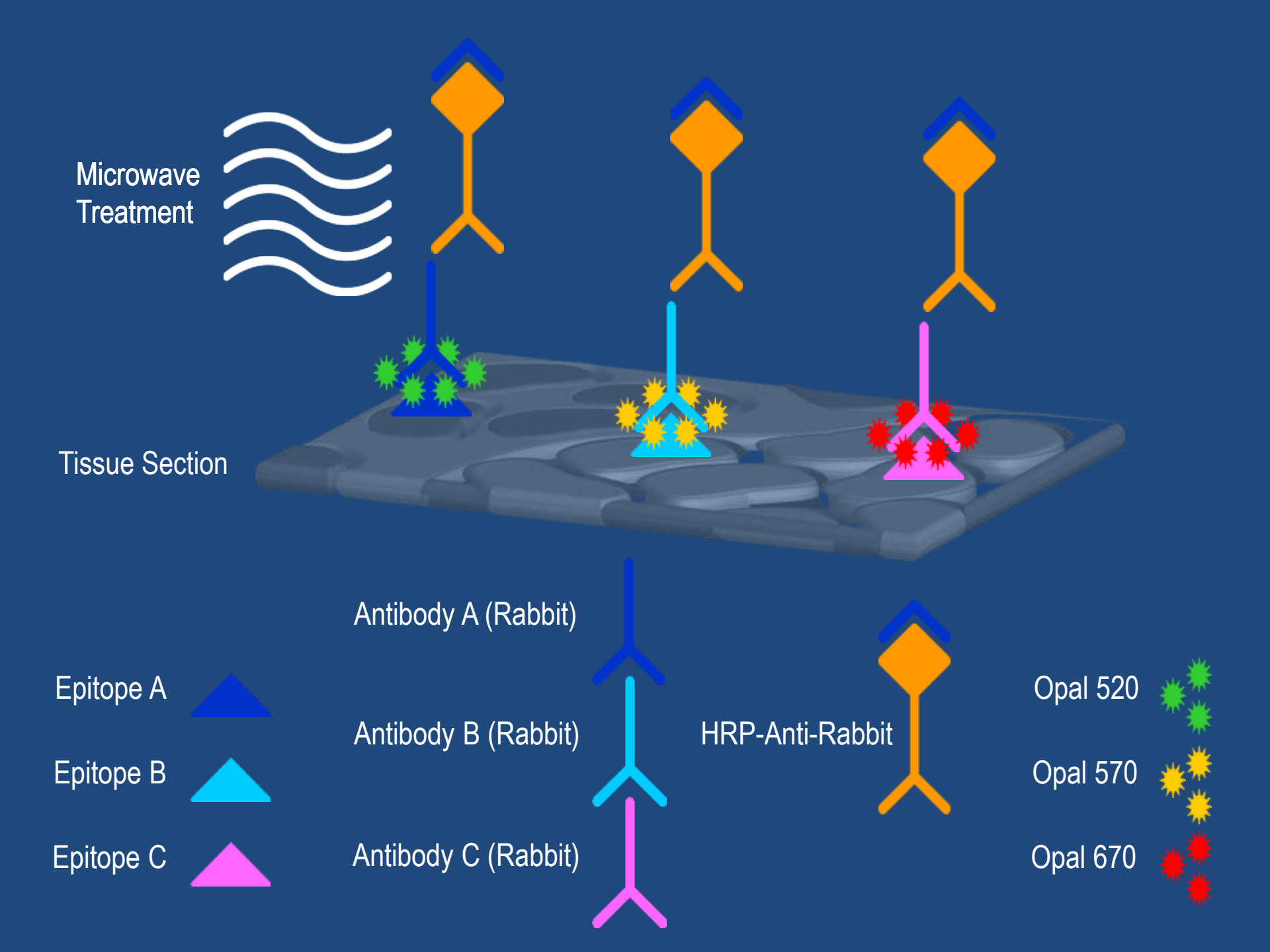


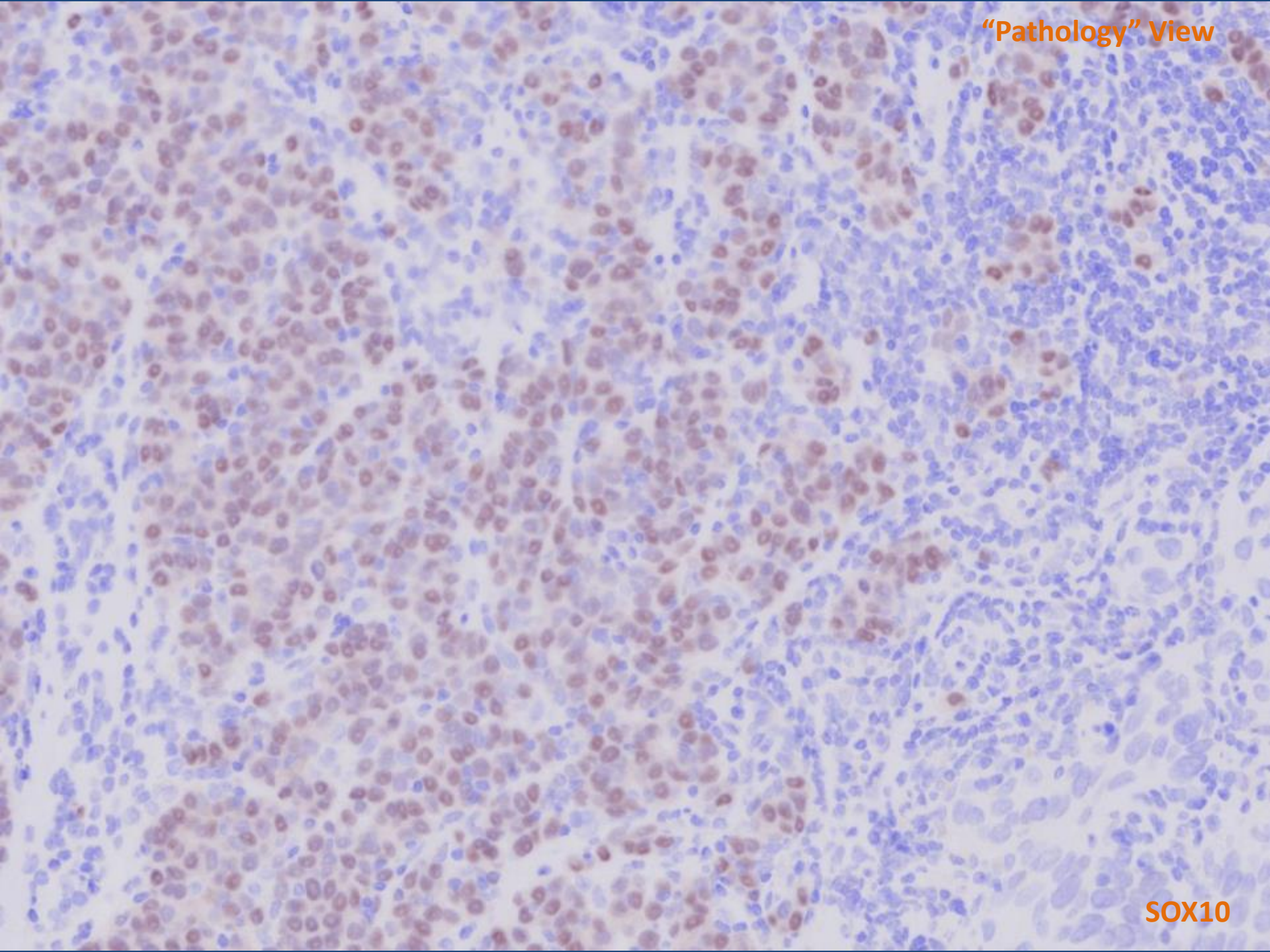
AUC curve for DSS, (n=78, AUC=0.719, p=0.004).

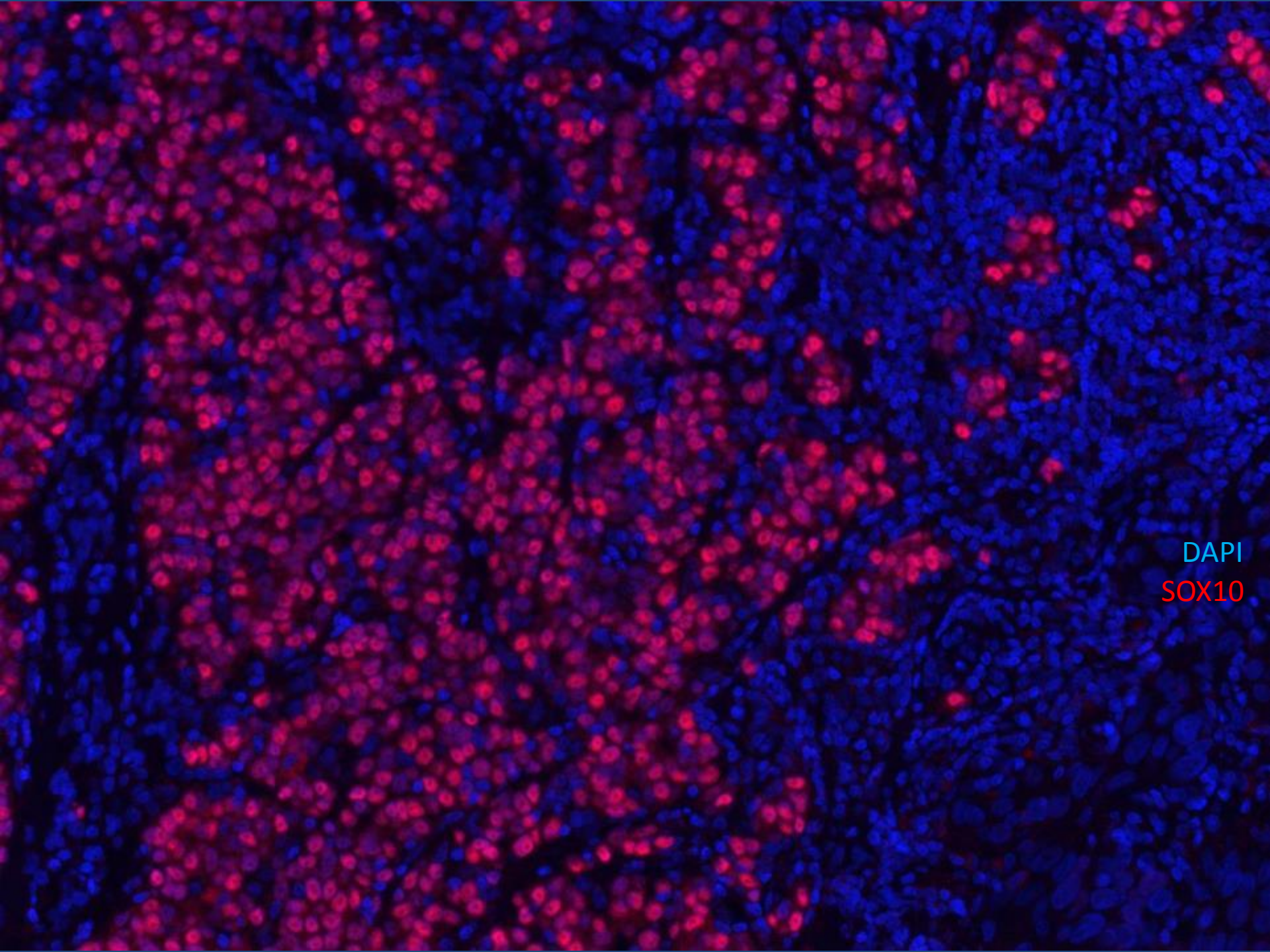
KM curve for DSS (p=0.003).

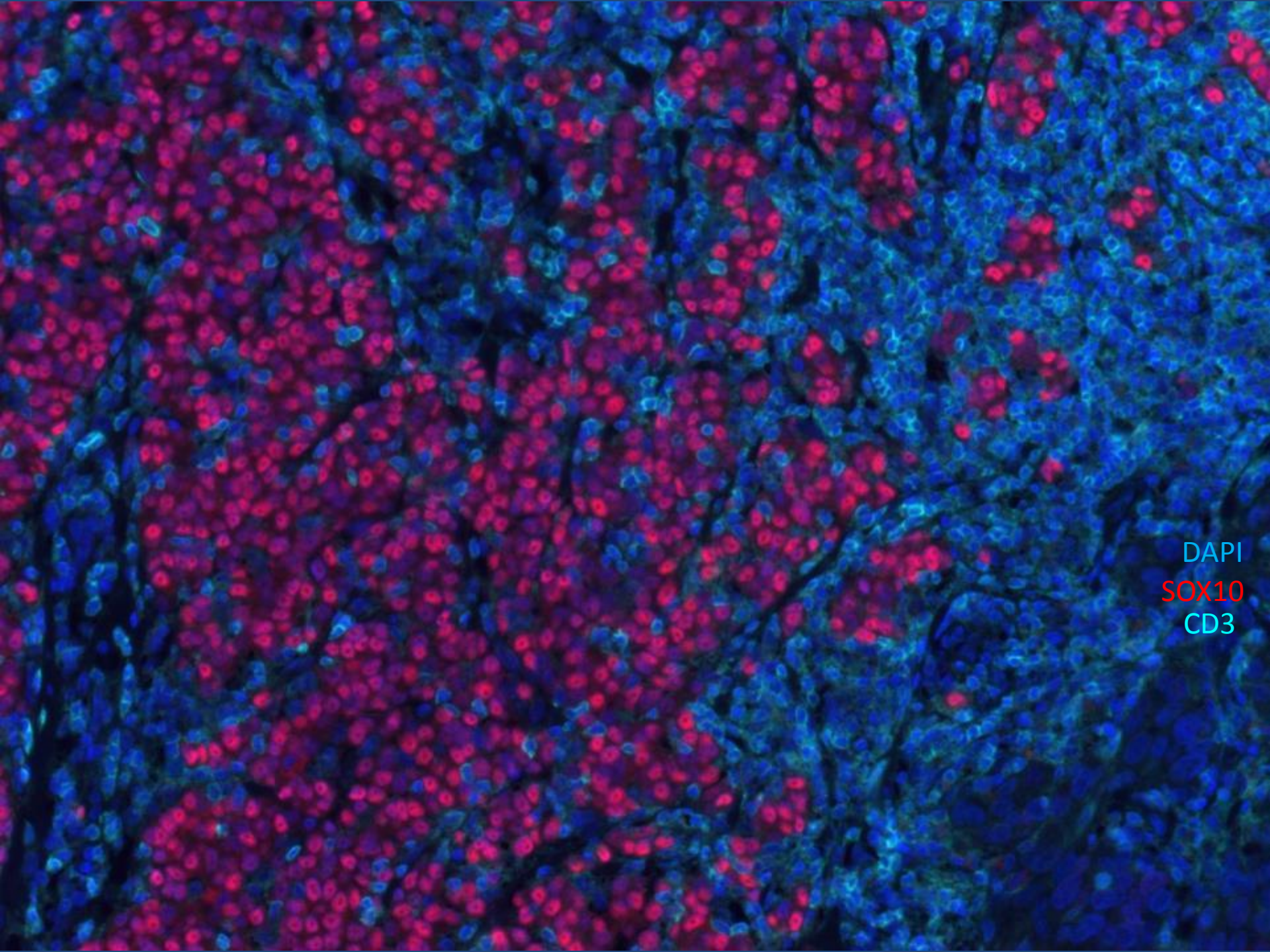
Gartrell et al, *CCR* 2019

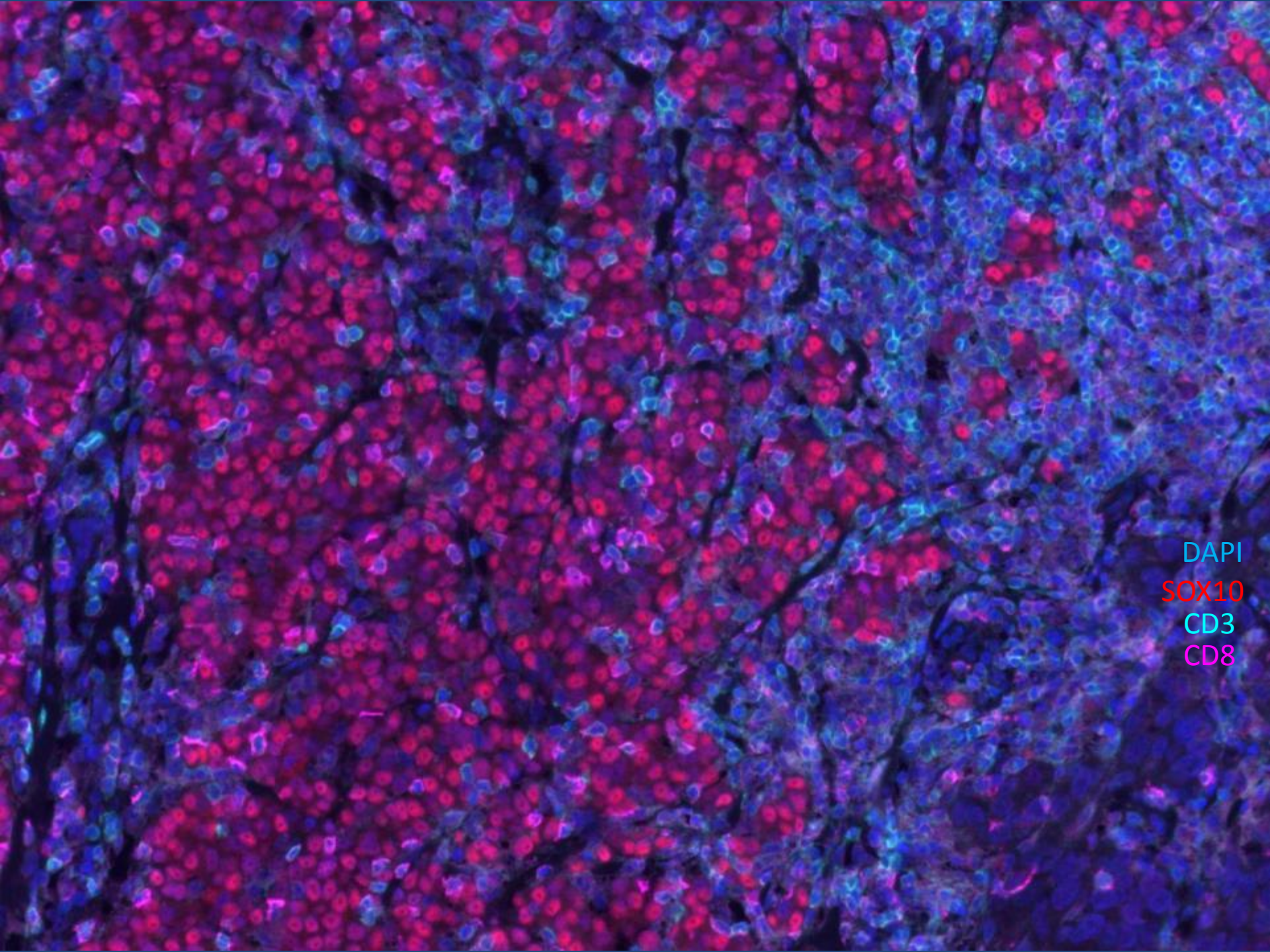
Cellular Composition of the TiME in melanoma

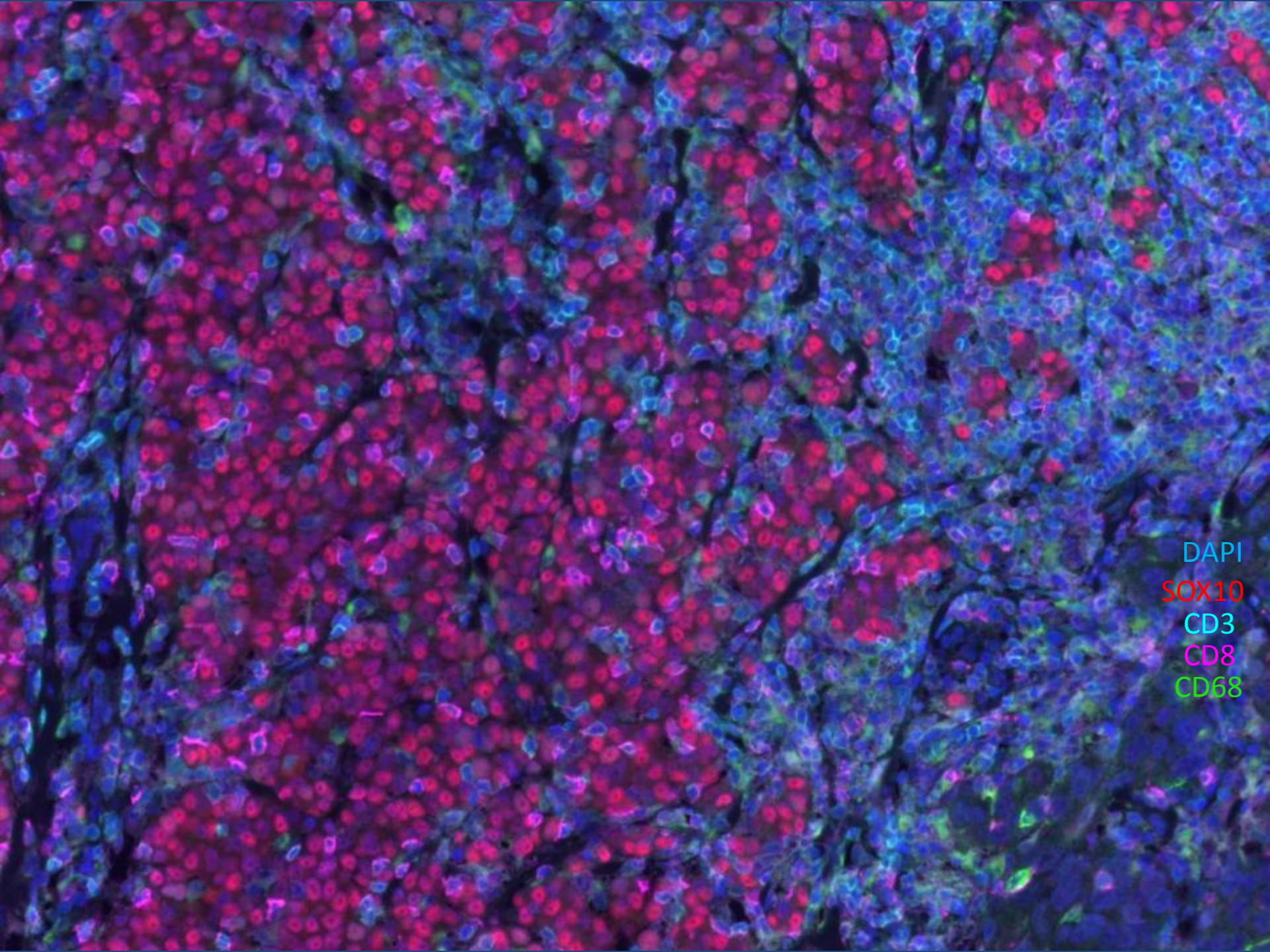

Microwave Treatment
Tissue Section
Antibody A (Rabbit)
Antibody B (Rabbit)
Antibody C (Rabbit)
HRP-Anti-Rabbit
Epitope A
Epitope B
Epitope C
Opal 520
Opal 570
Opal 670

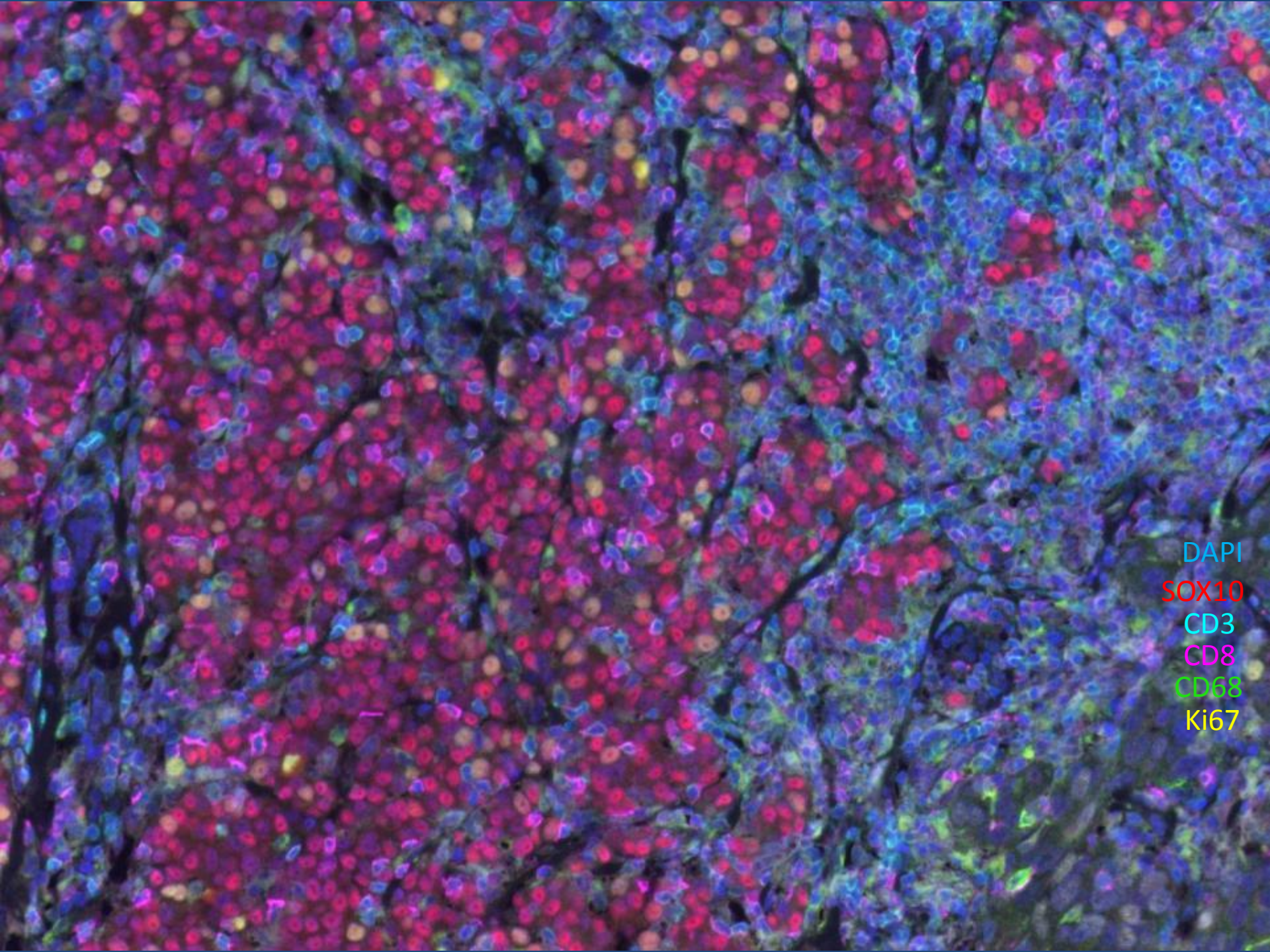

"Pathology" View
SOX10

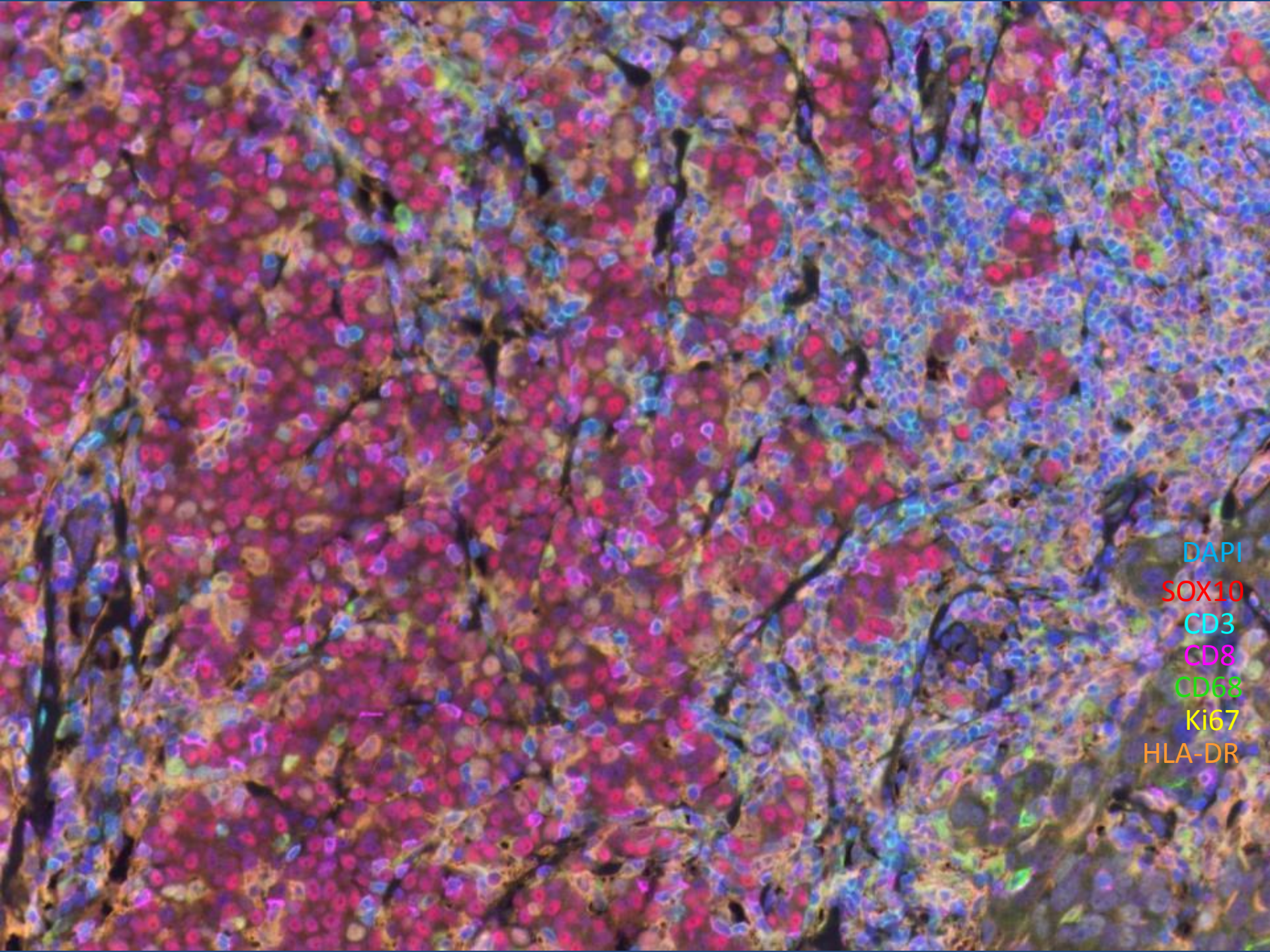

DAPI
SOX10

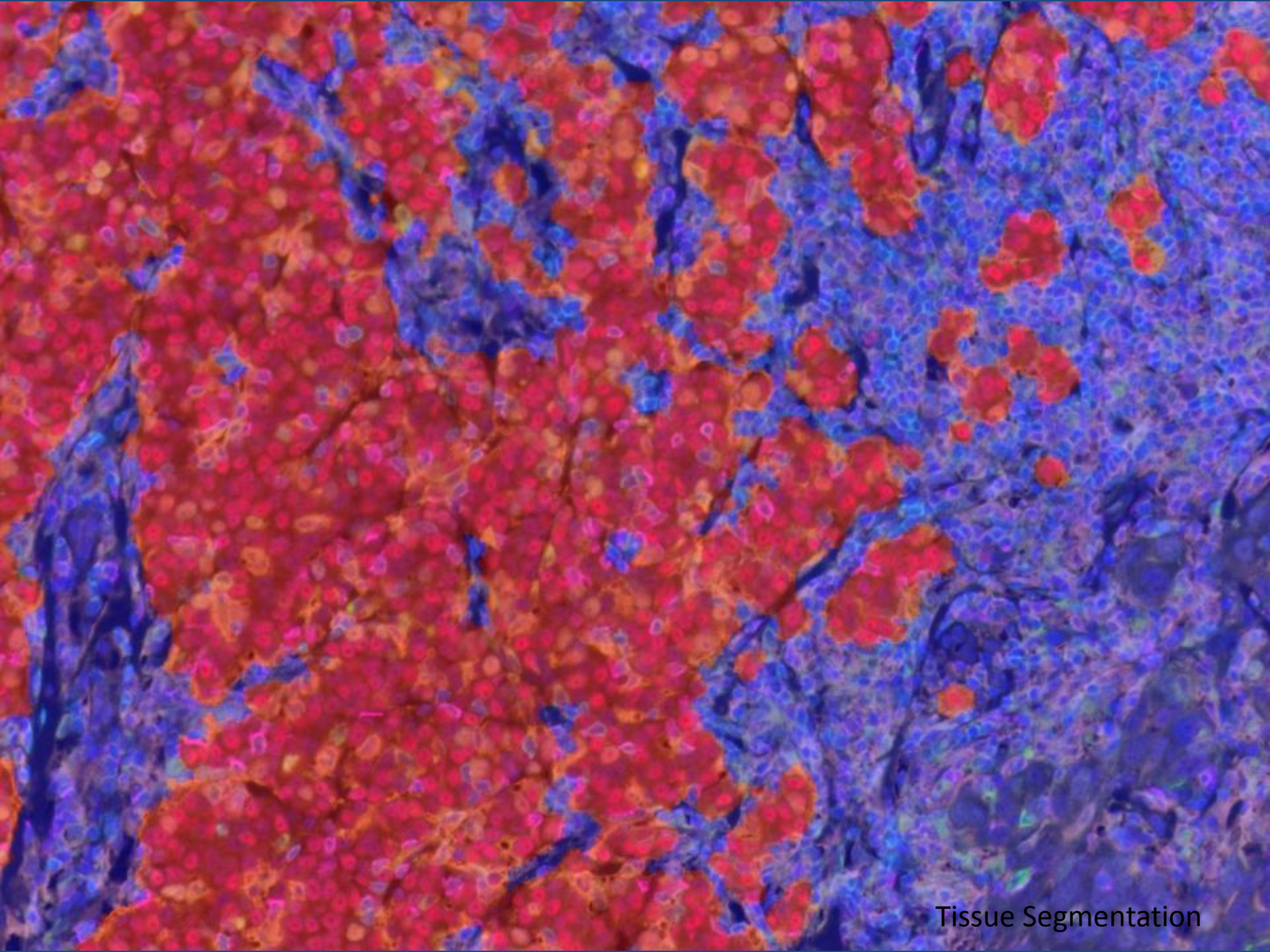

DAPI
SOX10
CD3

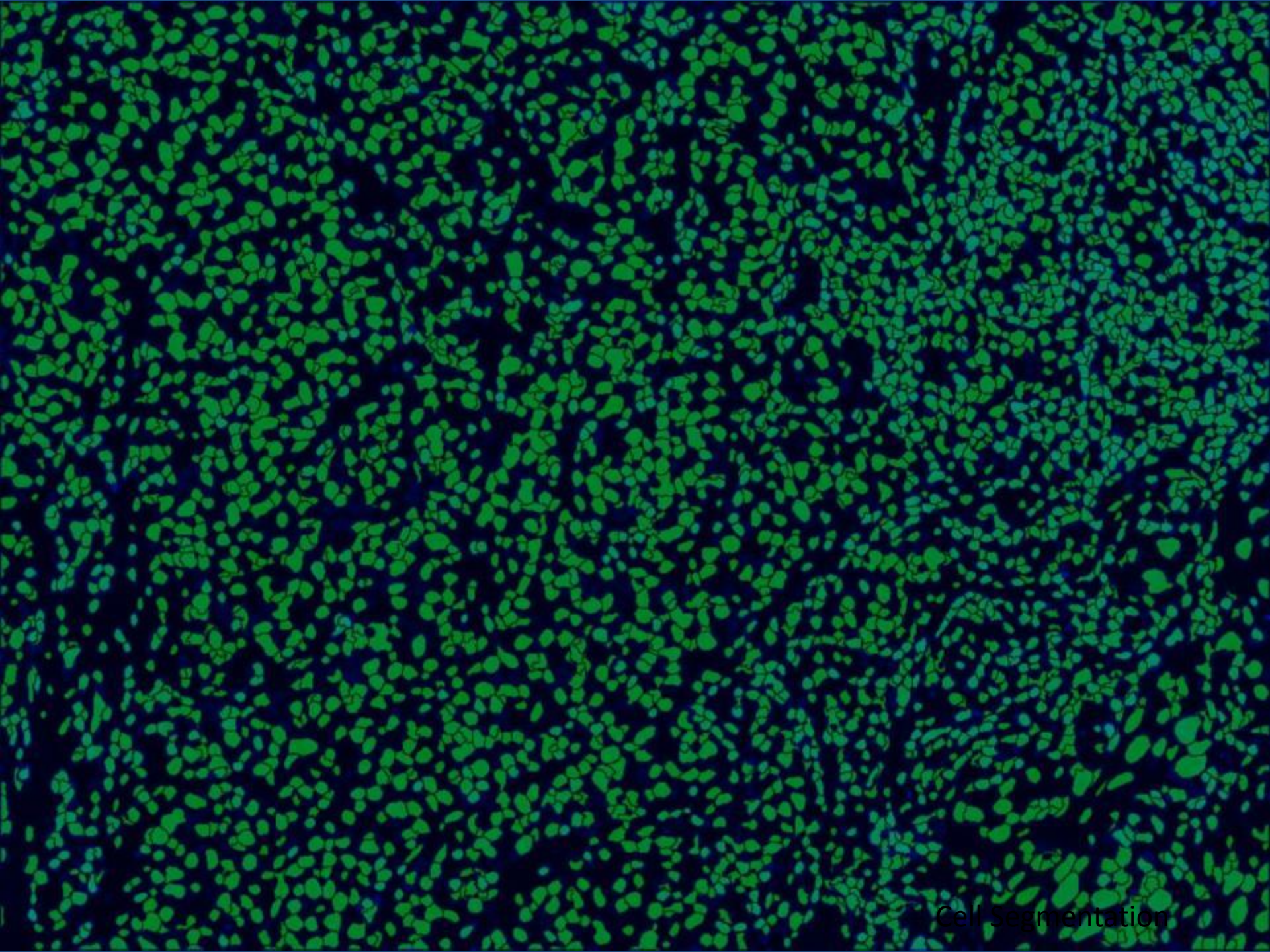

DAPI
SOX10
CD3
CD8

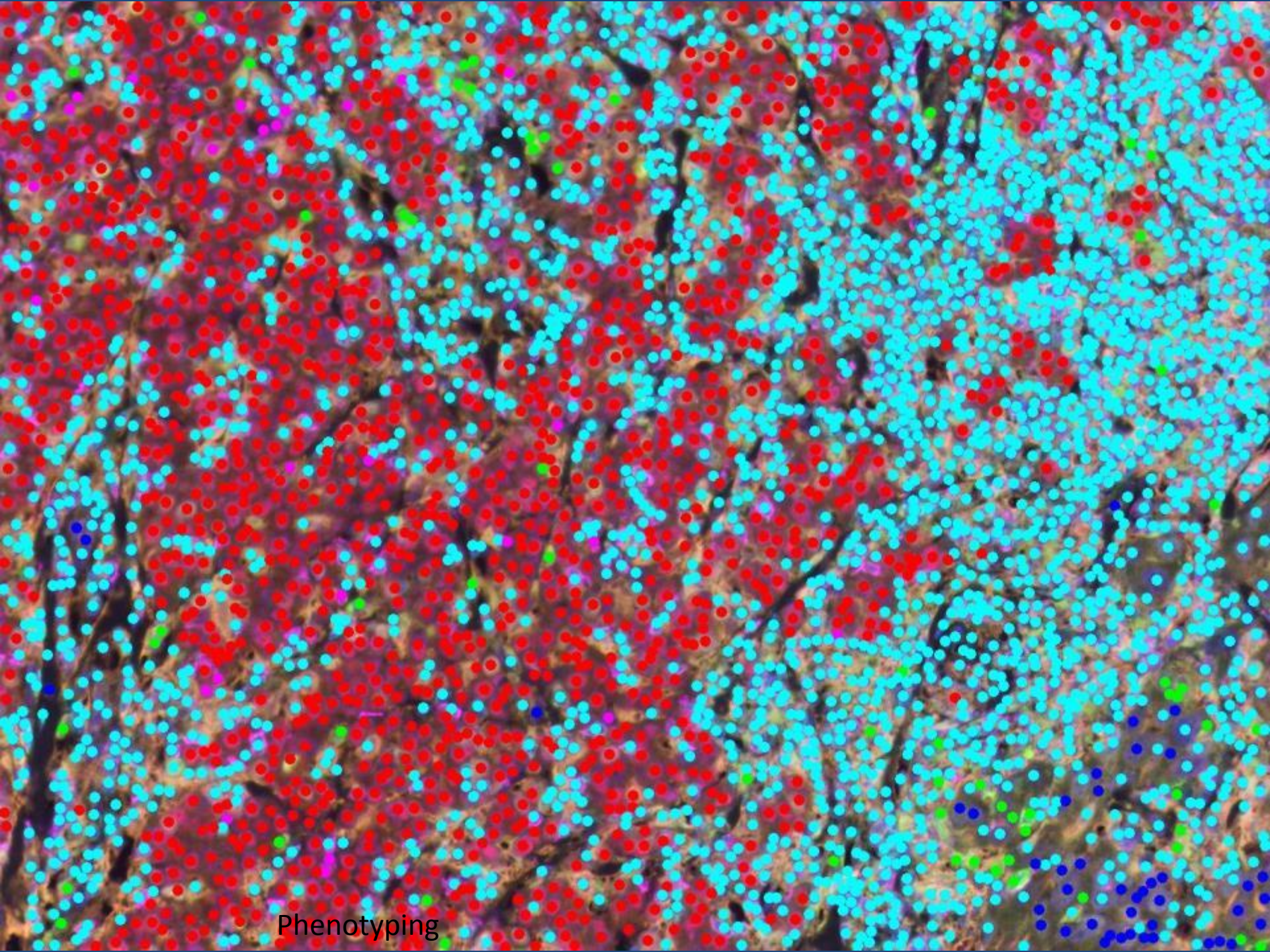

DAPI
SOX10
CD3
CD8
CD68


DAPI
SOX10
CD3
CD8
CD68
Ki67


DAPI
SOX10
CD3
CD8
CD68
Ki67
HLA-DR


Tissue Segmentation


Cell Segmentation


Phenotyping

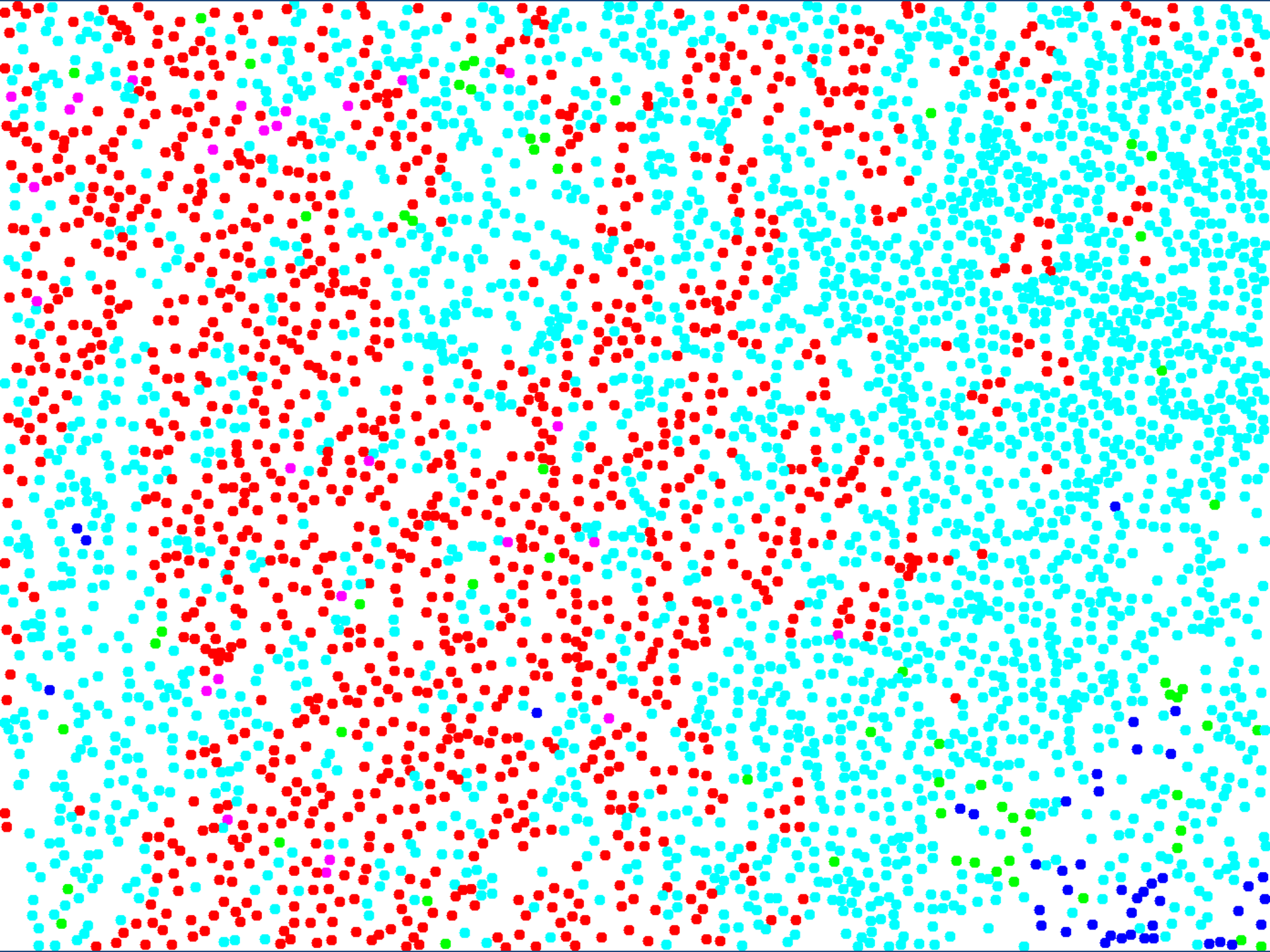
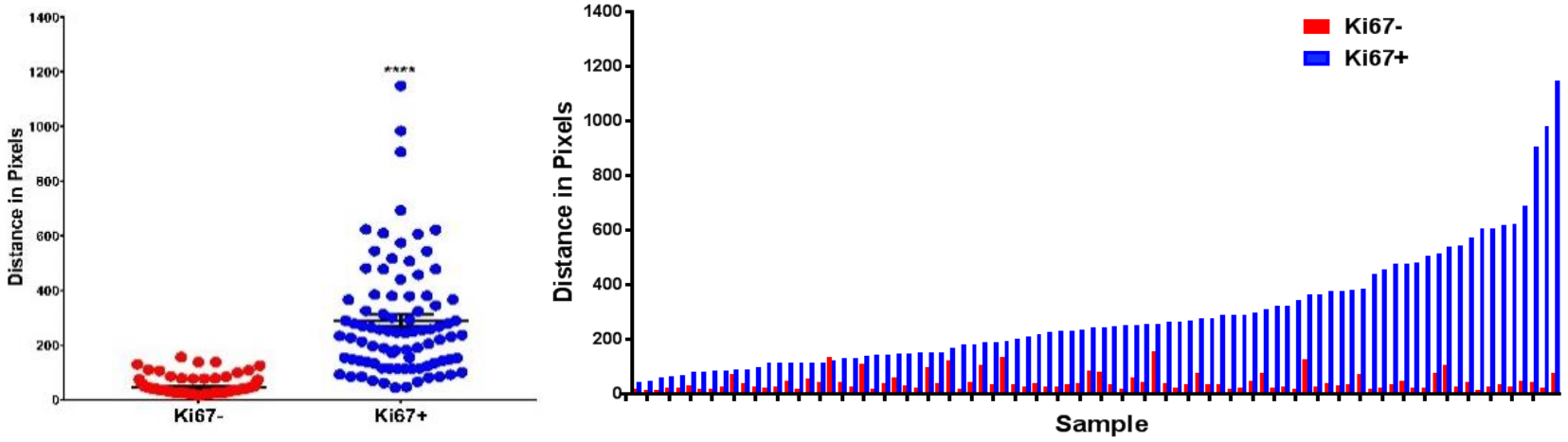


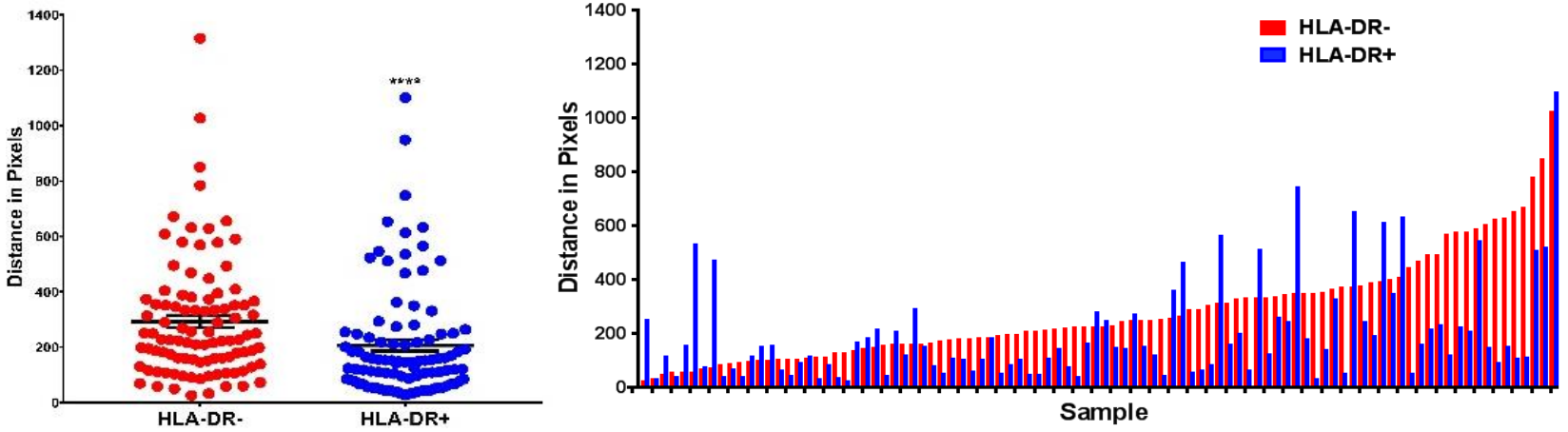
Table 1: Melanoma Patient Characteristics (n=104)	
Clinical Characteristics	
Gender	
Male, no. (%)	75 (72.1)
Female, no. (%)	29 (27.9)
Age	
Median, no., (range)	74.5 (22-96)
Location of Tumor	
Trunk, no. (%)	61 (58.7)
Extremity, no. (%)	41(39.4)
Unknown, no. (%)	2 (1.9)
Stage	
II, no. (%)	91 (87.5)
III, no. (%)	13 (12.5)
Pathological Characteristics	
Depth (mm)	
Median, no. (range)	2.5 (0.6-26)
Ulceration	
Absent, no. (%)	36 (34.6)
Present, no. (%)	65 (62.5)
Unknown, no. (%)	3 (2.9)
TILs	
Absent, no. (%)	2 (1.9)
Non-Brisk, no. (%)	59 (56.8)
Brisk, no. (%)	33 (31.7)
Unknown, no. (%)	10 (9.6)
Outcome Characteristics	
Patient Follow Up (months)	
Median, no. (range)	45 (4-173)
Overall Survival (months)	
Alive (at least 2 years), no. (%)	31 (29.8)
Dead, no. (%)	73 (70.2)
Disease Specific Survival (DSS) (months)	
Alive without melanoma, no. (%)	42 (40.4)
Dead with melanoma, no. (%)	22 (21.2)
Unknown, no. (%)	40 (38.4)

Representing Biology with Distance

Distance of CD3+CD8+ to SOX10+ Tumor

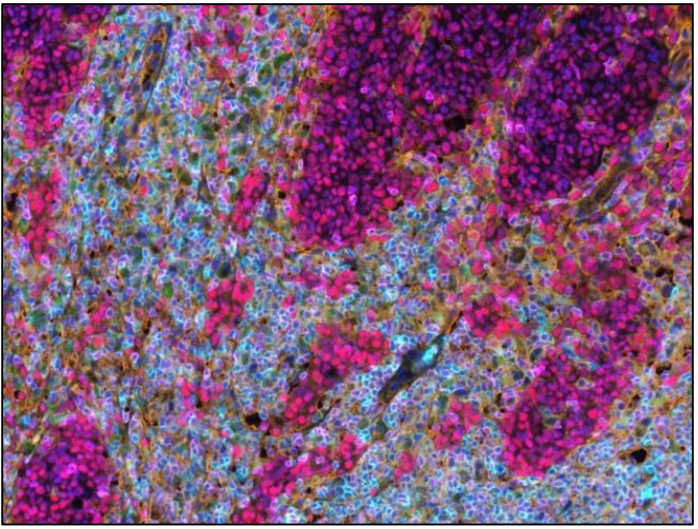


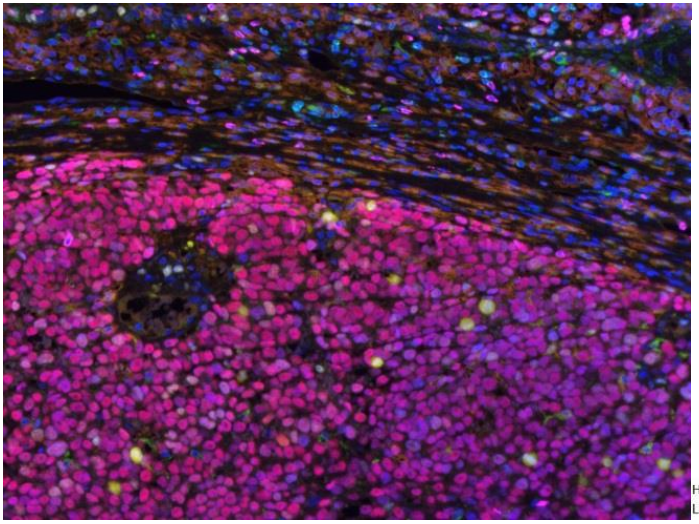
Distance of CD3+CD8+ to CD68+ Macrophages

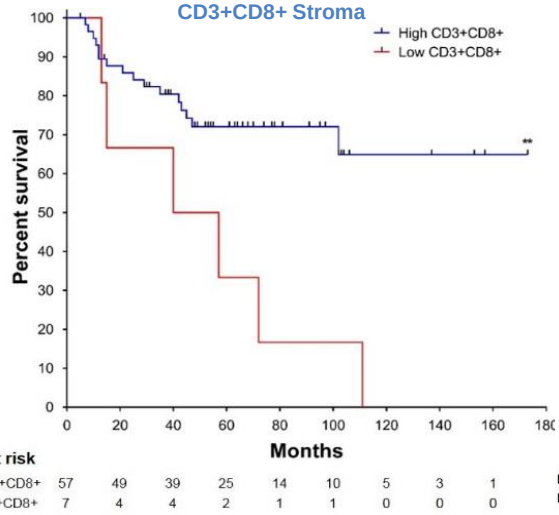


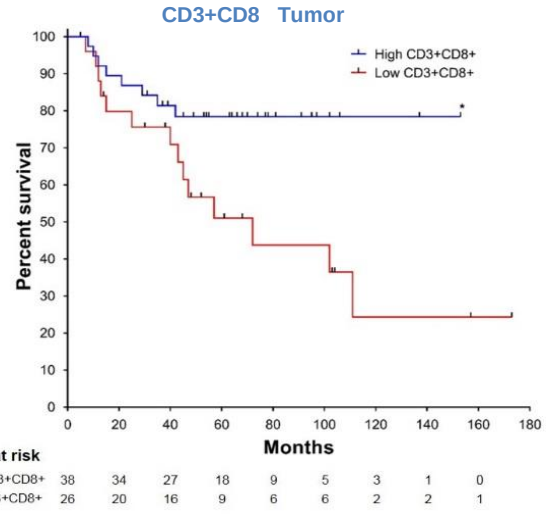
Gartrell RD, Marks DK, Hart T, et al: Characterizing the tumor microenvironment (TME) in primary melanomas using multiplex immunohistochemistry (mIHC). Journal of Clinical Oncology 35:9580-9580, 2017

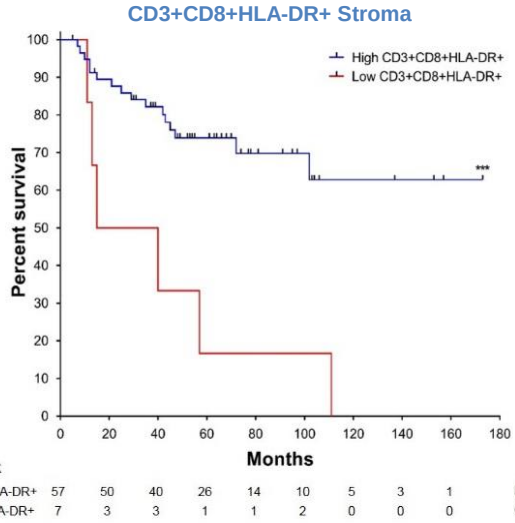
CD3+CD8+ CTLs Correlate with DSS

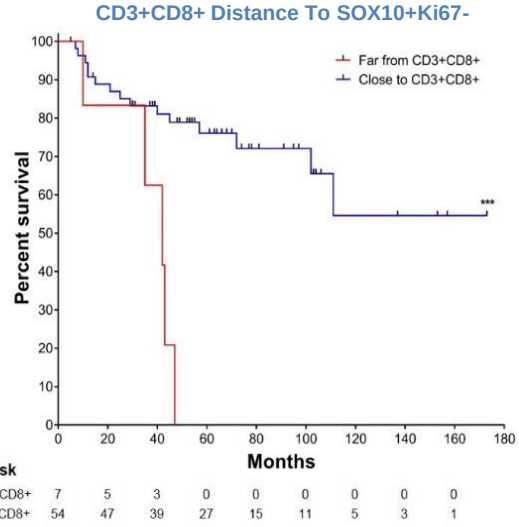






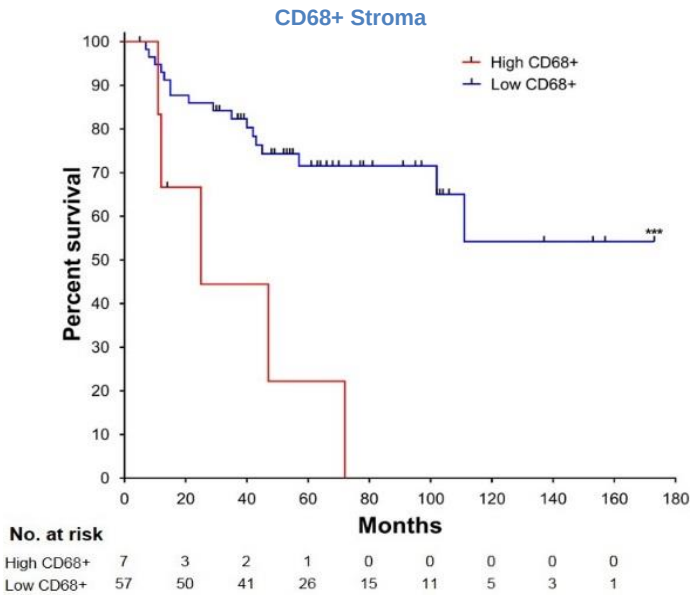


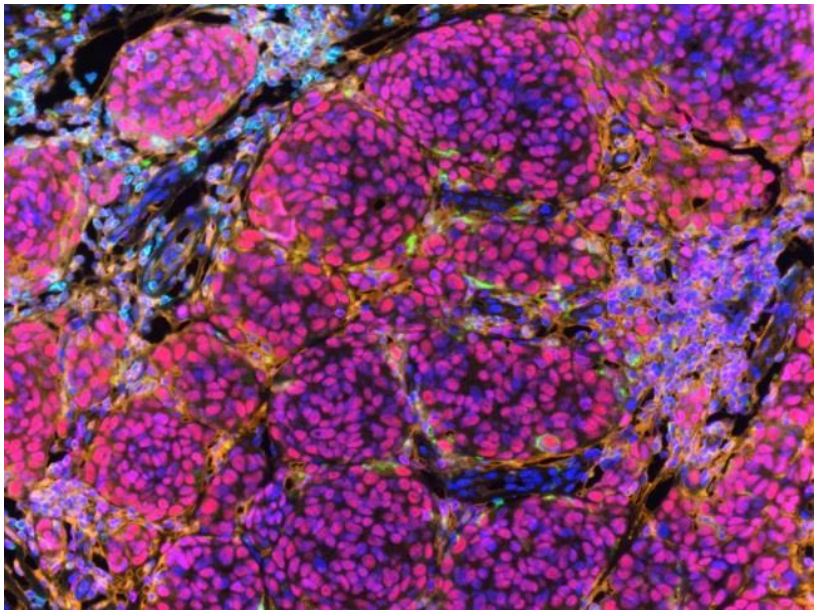


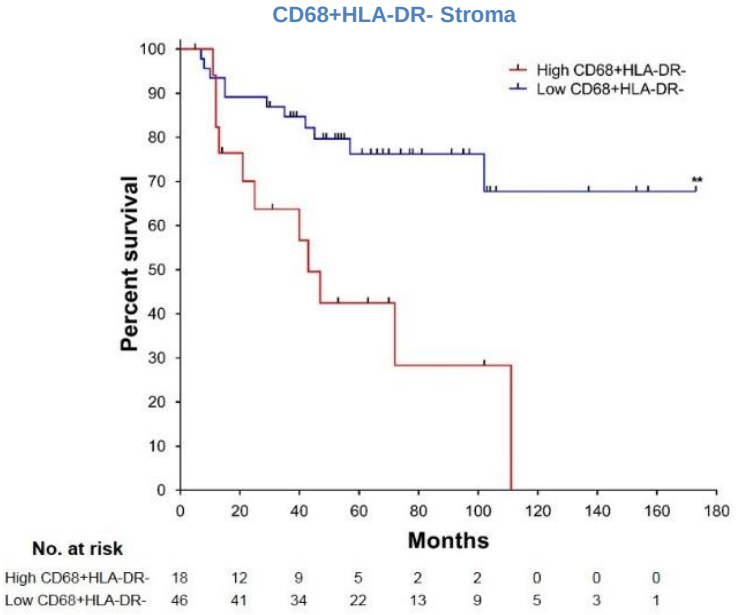


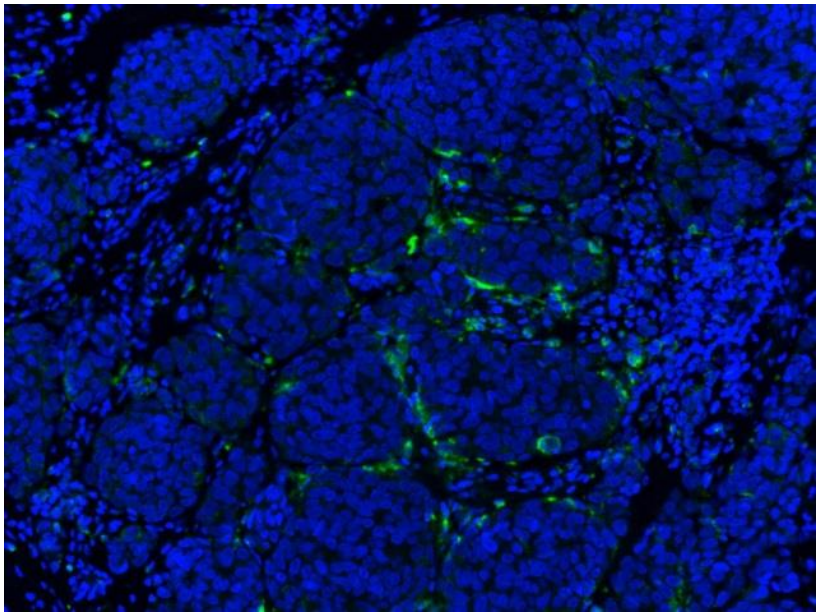
Gartrell RD, Marks DK, Hart T, et al: Characterizing the tumor microenvironment (TME) in primary melanomas using multiplex immunohistochemistry (mIHC). Journal of Clinical Oncology 35:9580-9580, 2017

Density of CD68+ Macrophages Correlate with DSS









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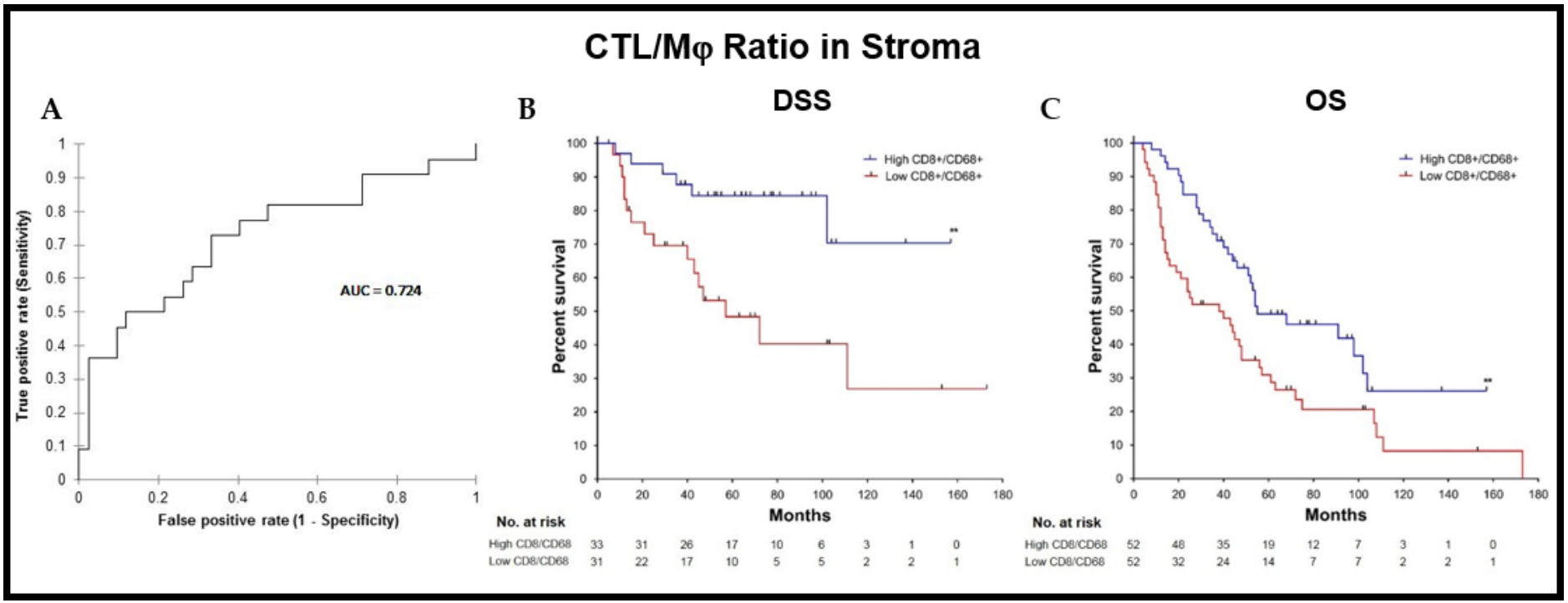
Ratio of CD8+/CD68+ as Biomarker



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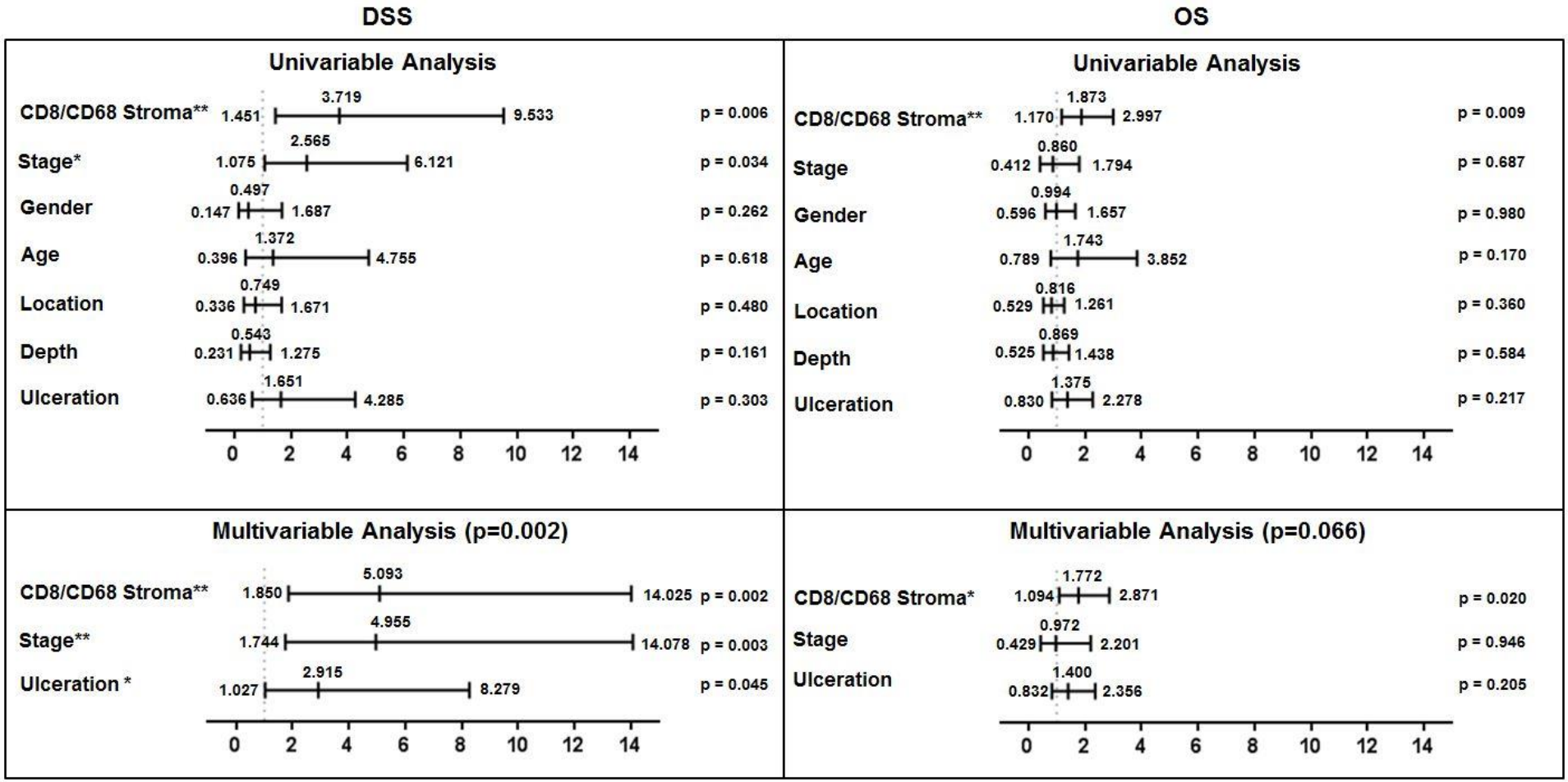
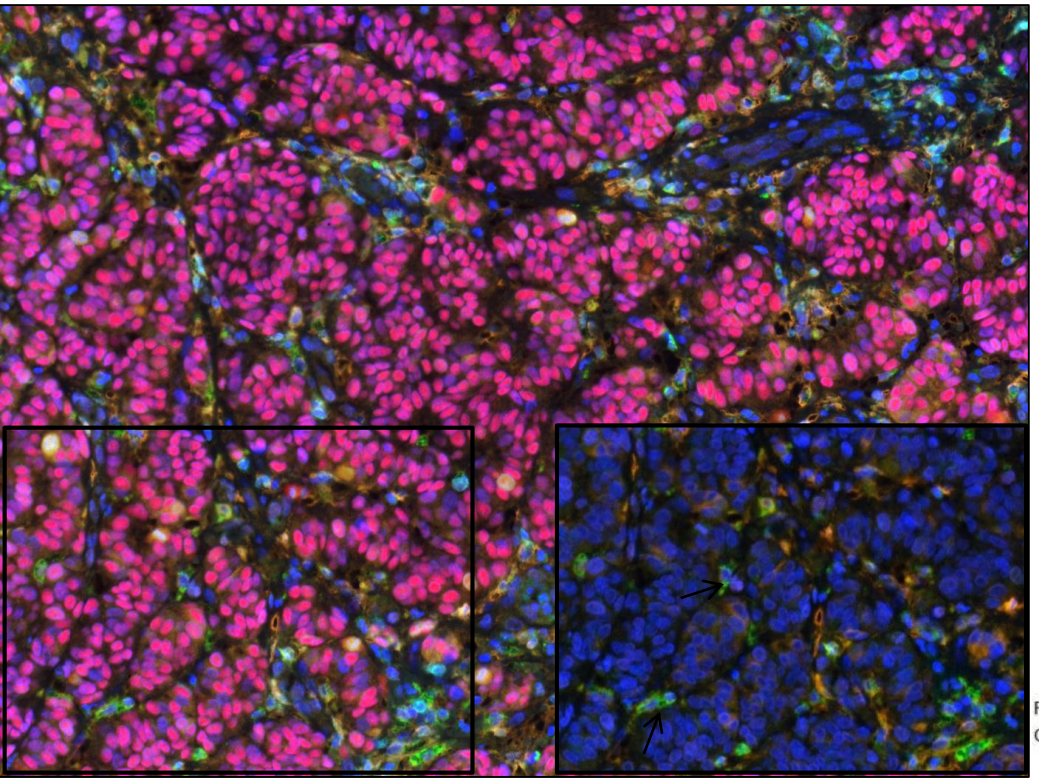
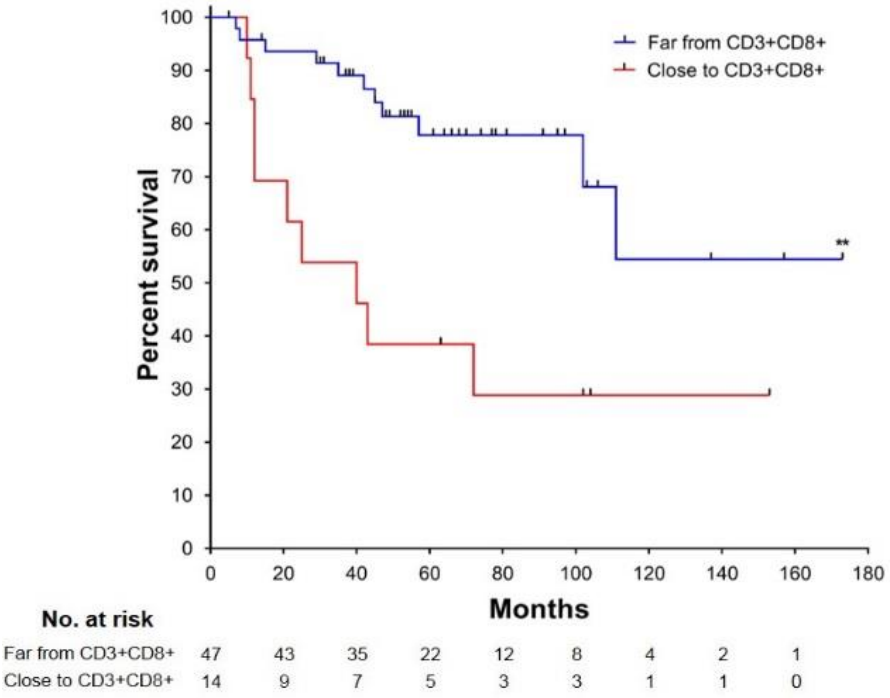


CD3+CD8+ Distance to CD68+HLA-DR- Macrophages Correlates with DSS





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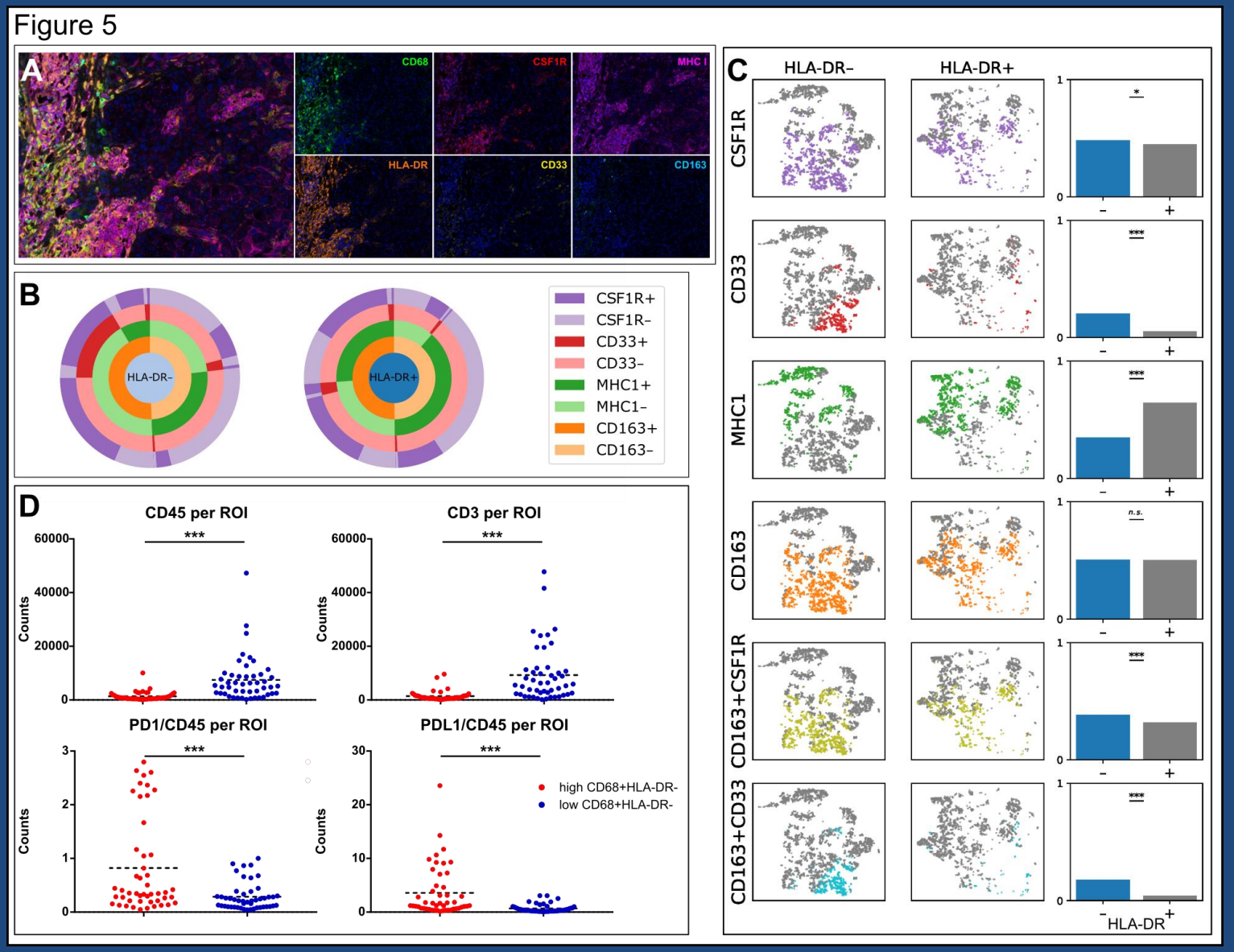
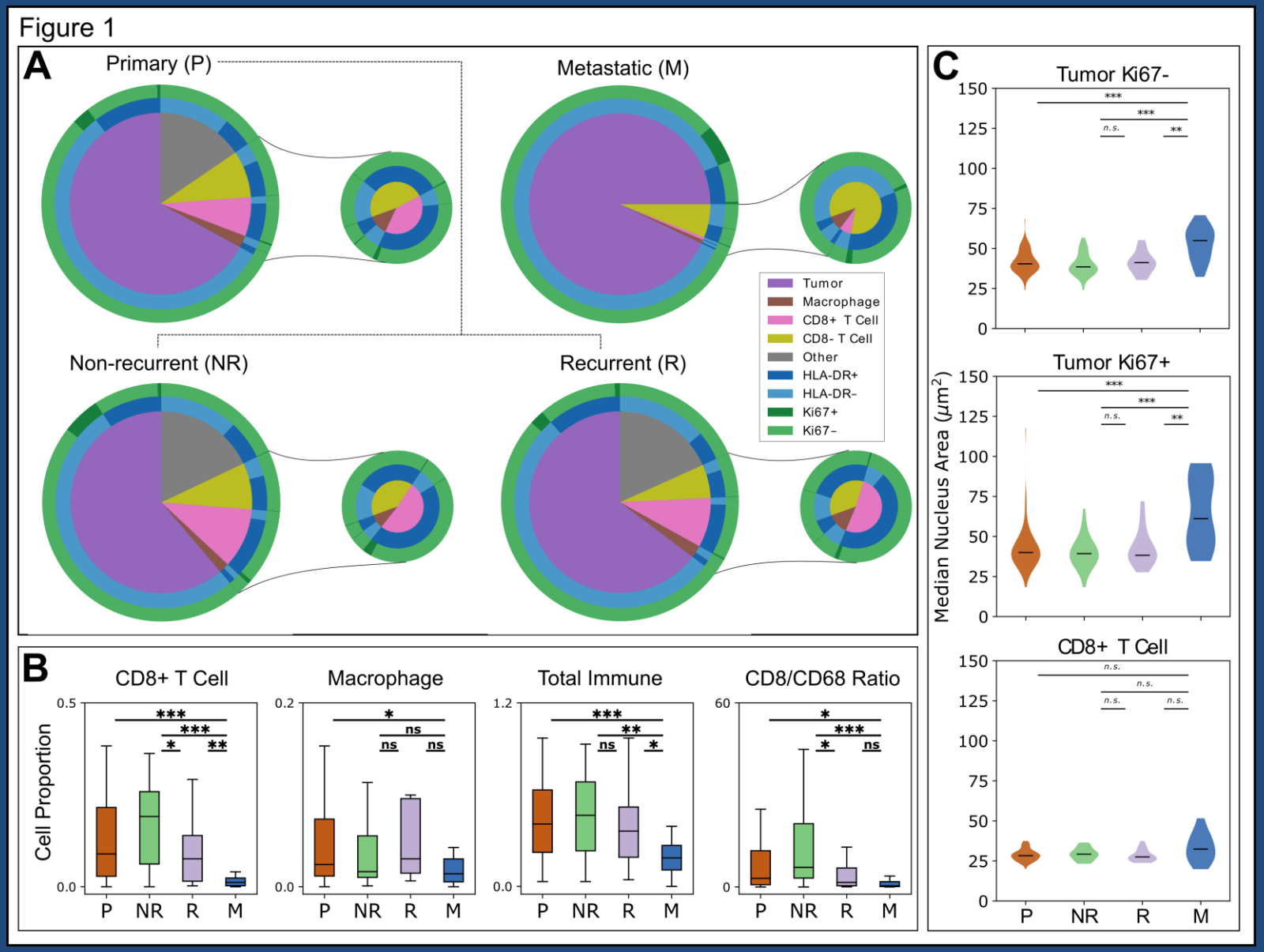
Characterization of HLA-DR- Macrophages

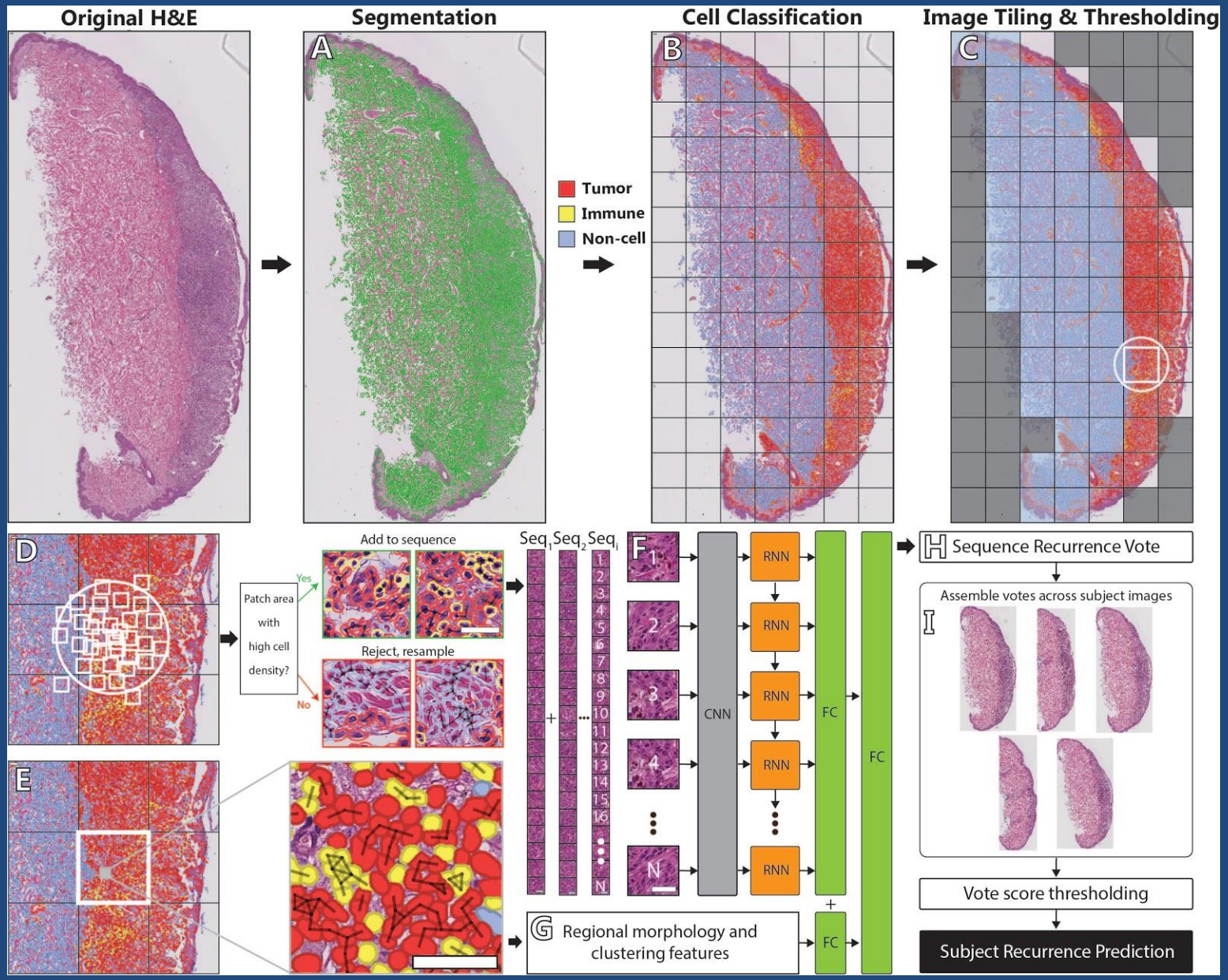


Comparison of Primary to Metastatic Tumors



Novel Methods to Study the TiME-Image Analysis

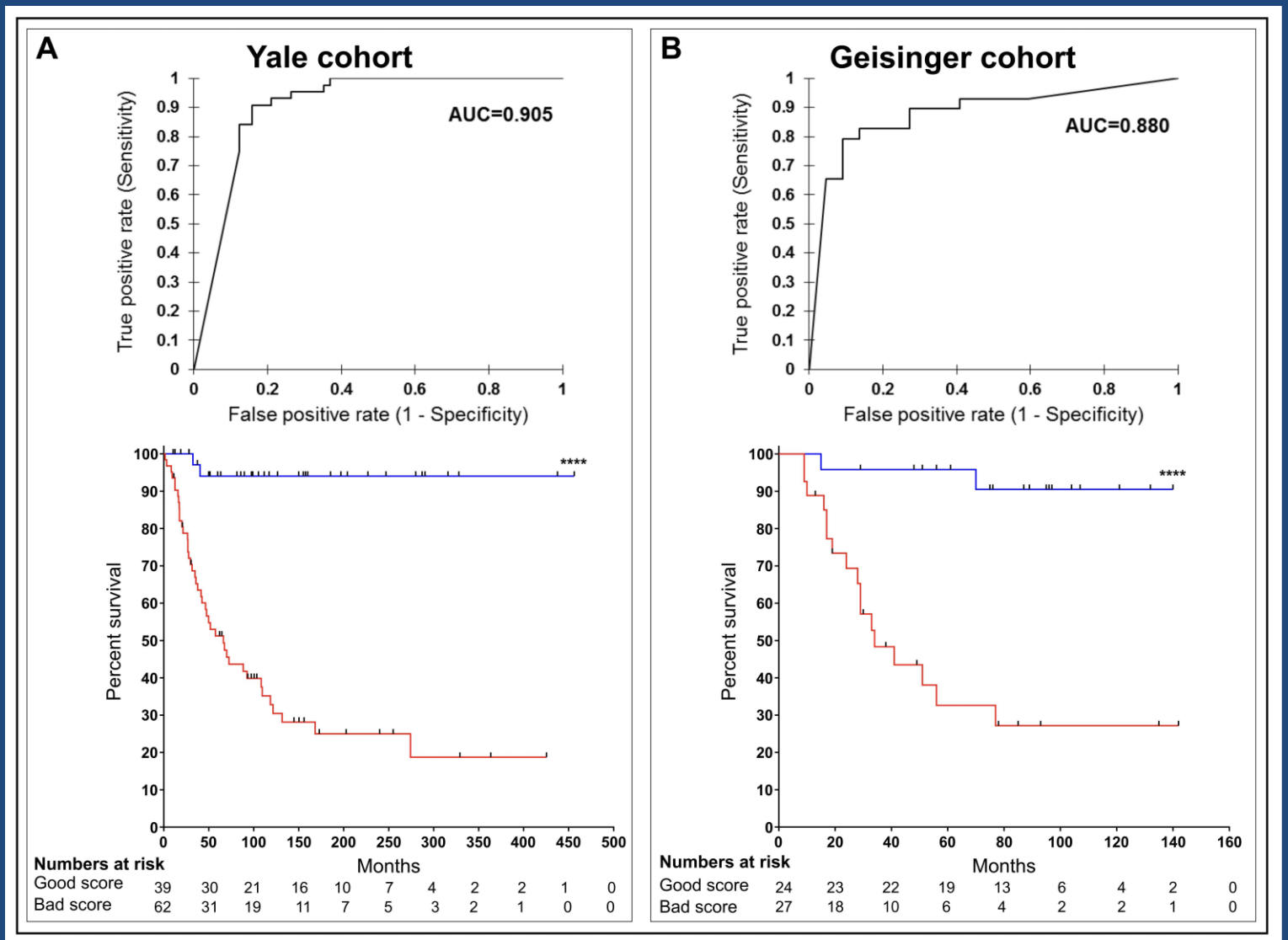
And onto image analysis applying AI to tiles selected using QuPath



Univariable Cox Training, Yale, and GHS

	Training set				*YUMC*				*GHS*		
	Hazard ratio	**95% CI**	***P***		**Hazard ratio**	**95% CI**	***P***		**Hazard ratio**	**95% CI**	***P***
Score	N/A	N/A	N/A	**Score******	55.023	9.486 to 319.157	< 0.0001	**Score*****	20.135	3.865 to 104.882	<0.001
Stage***	3.374	1.826 to 6.234	<0.001	**Stage**	N/A	N/A	N/A	**Stage**	1.472	0.579 to 3.743	0.417
Gender	0.665	0.285 to 1.554	0.347	**Gender**	0.597	0.331 to 1.078	0.087	**Gender**	1.695	0.681 to 4.222	0.257
Age	1.009	0.983 to 1.036	0.449	**Age**	1.016	0.994 to 1.038	0.165	**Age**	1.024	0.990 to 1.059	0.163
Depth***	1.087	1.039 to 1.138	<0.001	**Depth****	1.203	1.048 to 1.381	0.009	**Depth**	1.153	0.998 to 1.331	0.053
Ulceration*	2.605	1.209 to 5.611	0.014	**Ulceration**	1.412	0.777 to 2.568	0.258	**Ulceration**	2.696	0.969 to 7.498	0.057

Supplementary table 3. Univariable Cox regression analysis in training cohort. Univariable Cox analysis of disease-specific survival.


A
Yale cohort
AUC=0.905
True positive rate (Sensitivity)
False positive rate (1 - Specificity)
Percent survival
****
Months
Numbers at risk
Good score 39 30 21 16 10 7 4 2 2 1 0
Bad score 62 31 19 11 7 5 3 2 1 0 0
B
Geisinger cohort
AUC=0.880
True positive rate (Sensitivity)
False positive rate (1 - Specificity)
Percent survival
****
Months
Numbers at risk
Good score 24 23 22 19 13 6 4 2 0
Bad score 27 18 10 6 4 2 2 1 0

Conclusions

- GOAL is to provide better guidance to patients with early stage melanoma and clinicians taking care of them
- The tumor immune micro-environment has a large impact on patient outcomes
- Using FFPE specimens, quantification of RNA transcripts of immune genes (Nanostring) and precise measurement of densities and spatial localization of cells (qmIF) provide biomarkers of clinical utility
- More accurate immune biomarkers allow for streamlined clinical trial design and a more personalized approach to each patient based on their immune parameters.
- Multiple methods are under development (RNA, protein, digital image analysis based) and testing is ongoing on E1697

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QmIF Collaborator
Edward Stack PhD (Jounce)