A Novel Gene Expression Signature with Pathologic Correlates of Immune Reactivity

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Disclosure
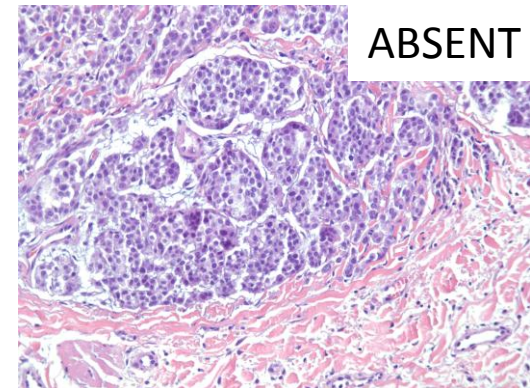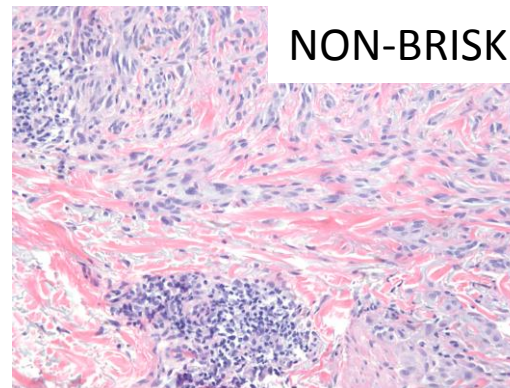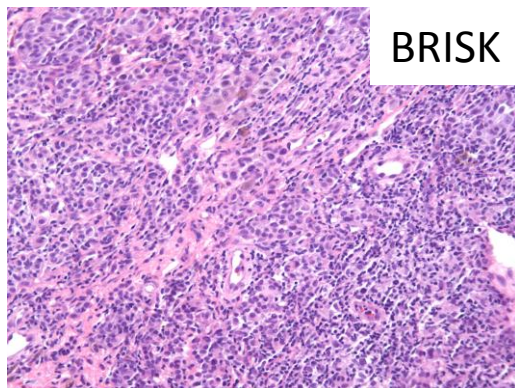
- Consultant: Myriad Corporation

Acknowledgement

- Jim Mule, PhD, for kindly providing slides used in this presentation

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Overview

- Immune markers of prognosis in melanoma
  - Primary melanoma
  - Metastatic melanoma
- Methods of assessment
- Clinical applications

Immune infiltrates in primary melanoma

- Three-tier classification of tumor-infiltrating lymphocytes (TIL's)

- Brisk TIL's independently predict survival

- Brisk TIL's inversely correlate with sentinel node involvement

BRISK   NON-BRISK   ABSENT

Azimi F, Scolyer RA, Rumcheva P. J Clin Oncol 2012 Jul 20;30(21)2678-83
Taylor RC, Patel A, Panageas KS et al. J Clin Oncol 2007 Mar 1;25(7):869-75
Thomas NE, Busam KJ, From L. et al. J Clin Oncol 2013 Nov 20; 31(33): 4252-9

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Immune infiltrates in metastatic melanoma

- Brisk TIL's (CD3, CD8) in lymph node metastasis predictive of overall survival
- Brisk TIL's (CD4) in lymph node and distant metastasis predictive of response to interferon-α (n=20)

Mihm MC Jr, Clemente CG, Cascinelli N. LabInvest 1996;74:43–7.
Bogunovic D, O'Neill DW, Belitskaya-Levy I, et al . Proc Natl Acad Sci U S A 2009;106:20429–34.
Hakansson A, Gustafsson B, Krysander L, Hakansson L. Br J Cancer 1996;74:670–6.

# Immune infiltrates predictive of therapeutic response

- High baseline expression of genes for Foxp3 and indoleamine 2,3 dioxygenase and increase in TILs at week 4 predict response to anti-CTLA-4 therapy

- Tumor expression of PD-L1 predicts response to anti-PD1 therapy

Hamid O, Schmidt H, Nissan A, et al. J Transl Med 2011, 9:204.
Topalian SL, Hodi FS, Brahmer JR, et al. N Engl J Med2012, 366:2443–2454.

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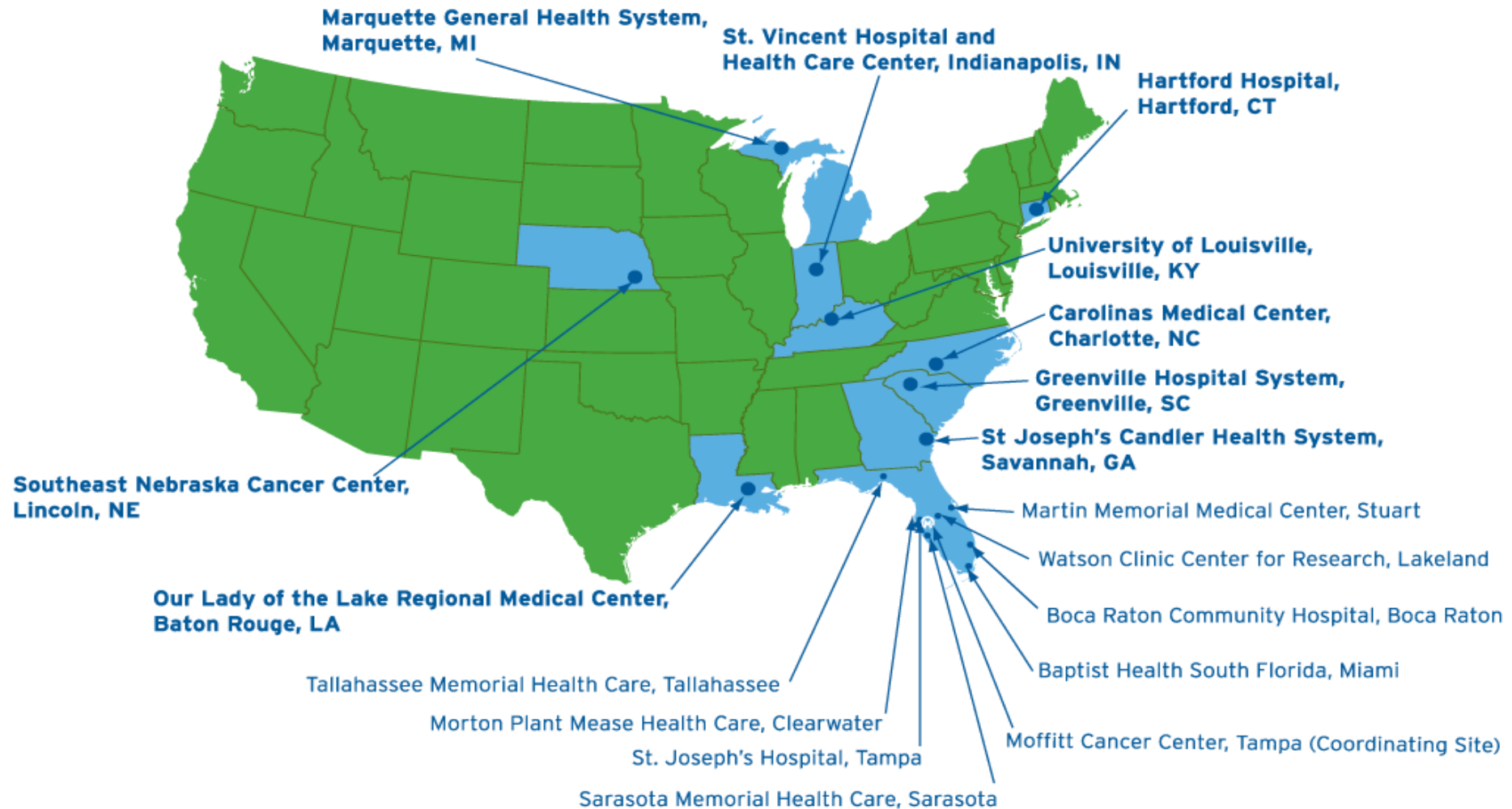# Prediction of response to anti-PD-1

- 46 patients from phase I study
- Response predicted by:
  - Higher pre-treatment CD8/PD-1/PD-L1 density at invasive margin and in tumor
  - More PD-1 and PDL-1 cells in proximity
  - Greater increase in CD8+ during therapy
  - Responders more likely to express Stat1 in CD8+ areas
- CD8+ density at invasive margin best predictor

Tumeh et al. Nature 515: 27 November 2014, 568-572
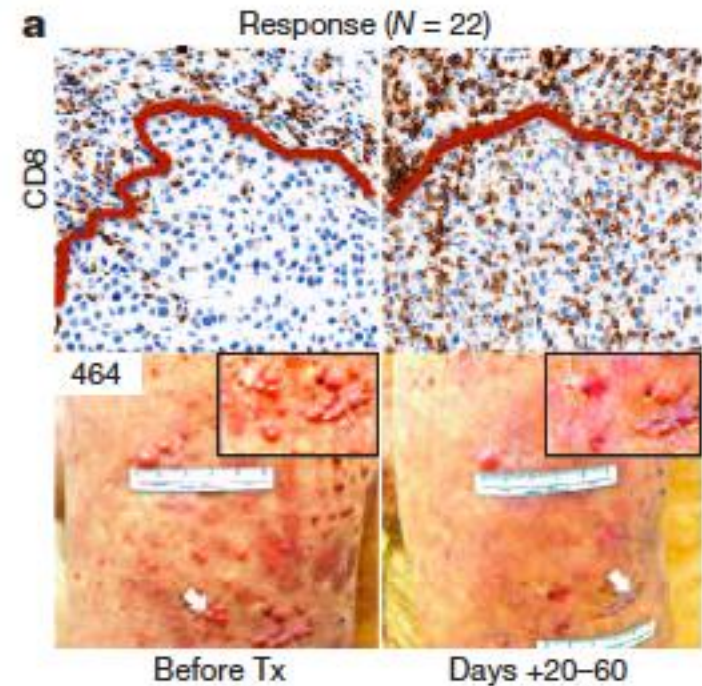
Utilization of Tissue Bank and Gene Expression Profiling for Discovery and Validation of Immune Gene-related Signatures

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Total Cancer Care Initiative

Total Cancer Care™ Consortium

Marquette General Health System, Marquette, MI

St. Vincent Hospital and Health Care Center, Indianapolis, IN

Hartford Hospital, Hartford, CT

University of Louisville, Louisville, KY

Carolinas Medical Center, Charlotte, NC

Greenville Hospital System, Greenville, SC

St Joseph's Candler Health System, Savannah, GA

Southeast Nebraska Cancer Center, Lincoln, NE

Martin Memorial Medical Center, Stuart

Watson Clinic Center for Research, Lakeland

Boca Raton Community Hospital, Boca Raton

Our Lady of the Lake Regional Medical Center, Baton Rouge, LA

Baptist Health South Florida, Miami

Tallahassee Memorial Health Care, Tallahassee

Morton Plant Mease Health Care, Clearwater

Moffitt Cancer Center, Tampa (Coordinating Site)

St. Joseph's Hospital, Tampa

Sarasota Memorial Health Care, Sarasota

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Total Cancer Care features

- Lifetime prospective patient followup

- Single-site biorepository with capability to hold 120,000 snap-frozen (15 minutes) tissues

- Specimens assessed by pathologist for tumor %, necrosis

- Data warehouse stores clinical, pathologic, cancer registry, and molecular data

Total Cancer Care to Date

443,000 Patients
Community Sites and Moffitt

105,000
TCC Consented Patients
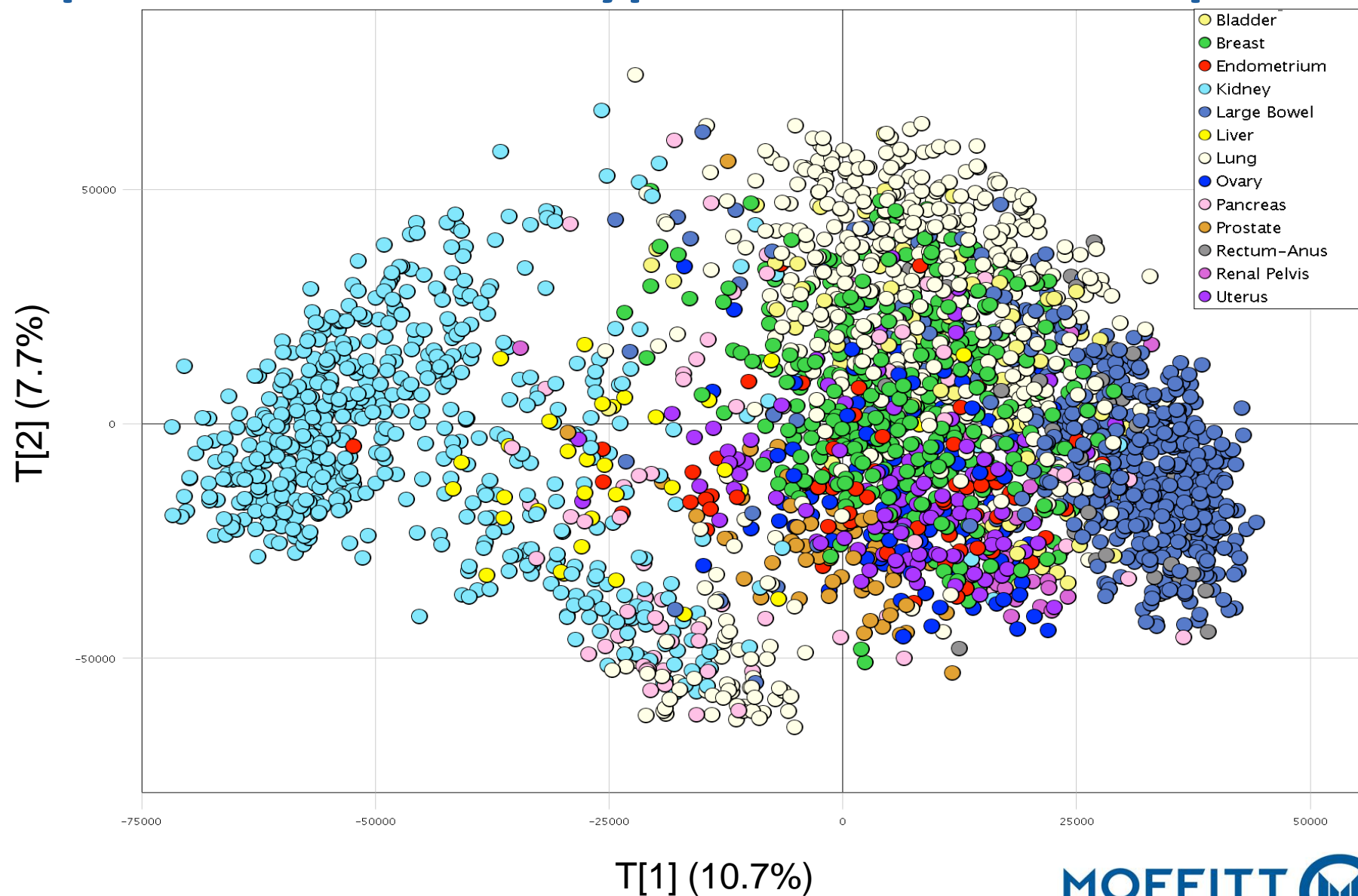MCC (60%)
Sites (40%)

35,356
Tumors Collected
MCC (43%)
Sites (57%)

16,279
Gene Expression Profiles

As of May 2, 2014

Data Generated from Specimens	
CEL Files (Gene Expression Data)	16,279 files
Targeted Exome Sequencing	4,016 samples
Whole Exome Sequencing (Ovary, Lung, Colon, Myeloma)	574 samples
Whole Genome Sequencing (Melanoma)	13 samples with normal pairs
SNP/CNV (Lung, Breast, Colon)	559 samples
RNA Sequencing (Breast, Myeloma)	430 samples

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Separation of tissue types in first PCA component

Legend:
- Bladder
- Breast
- Endometrium
- Kidney
- Large Bowel
- Liver
- Lung
- Ovary
- Pancreas
- Prostate
- Rectum–Anus
- Renal Pelvis
- Uterus

T[2] (7.7%)

T[1] (10.7%)

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Study site does not affect the gene expression profile
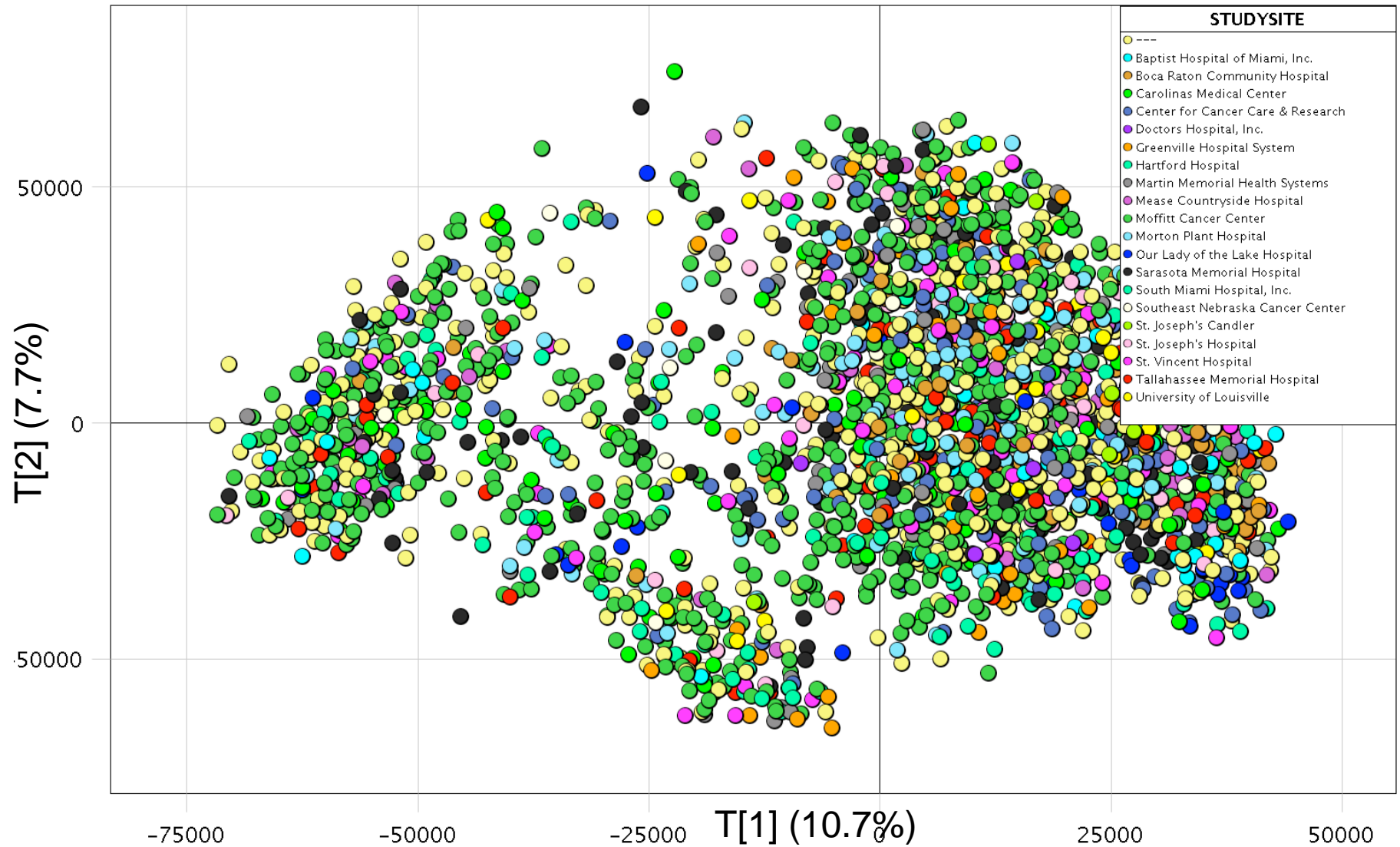
Source of Immune Gene-related Signatures

- Solid tumor microarrays

- Peripheral blood microarrays

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Discovery of Immune Gene-related Signatures using TCC: genomic, pathology, clinical data

- 20,155 unique genes on the chip
- 338 colon tumors analyzed from Moffitt TCC repository
- ~50 metagene groups (bioinformatics in cooperation with industry) - one was denoted "T cell activation", with 97 unique immune gene symbols with overwhelming enrichment for immune-related and inflammation-related genes
- Sections of selected paraffin blocks from highest and lowest signatures evaluated for the presence or absence of immune cell infiltration composition and patterns of inflammation
- Correlated with annotated clinical data

Metagene Grouping: "T Cell Activation" Genes
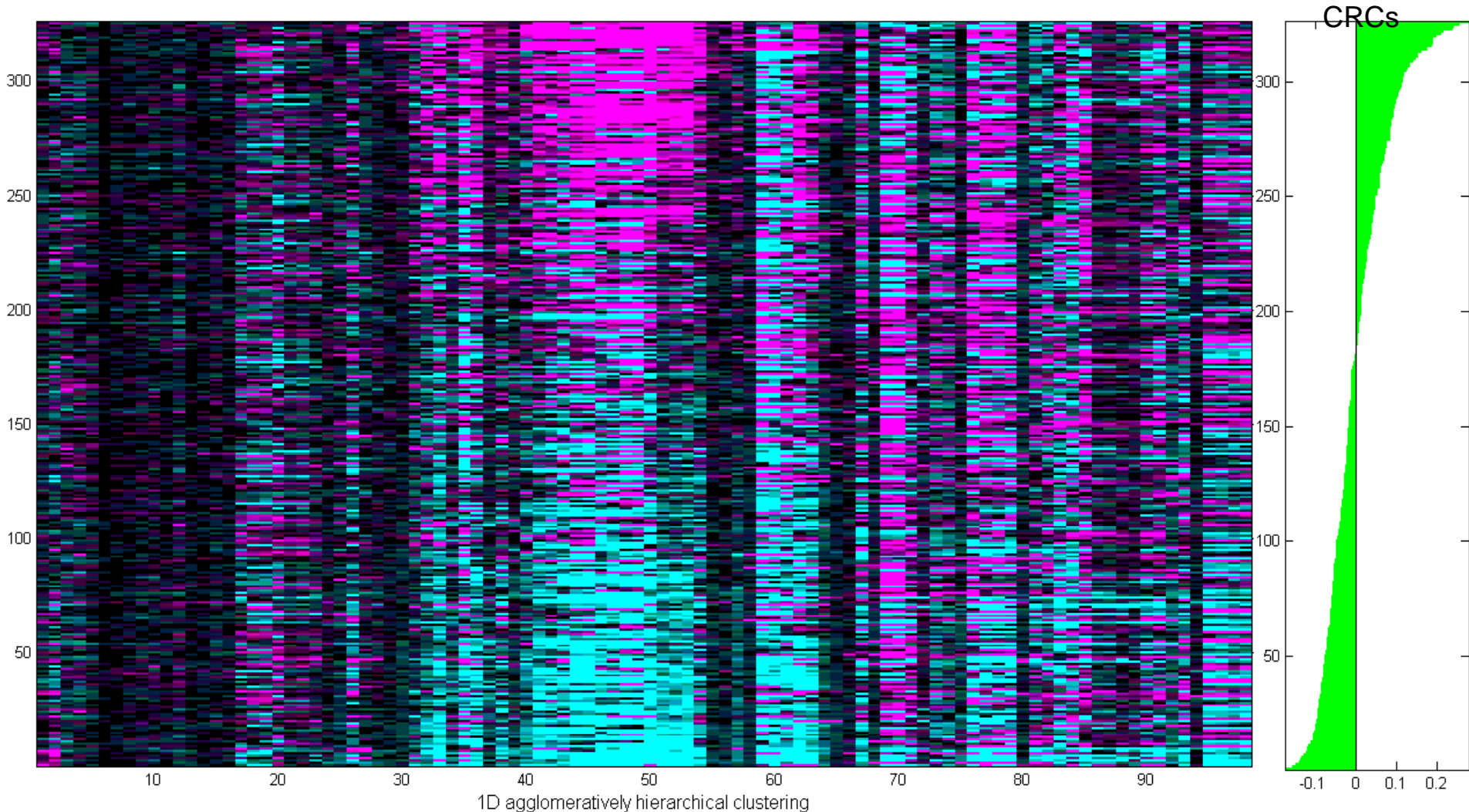
12 Chemokines Uncovered in the Metagene Group "T Cell Activation"
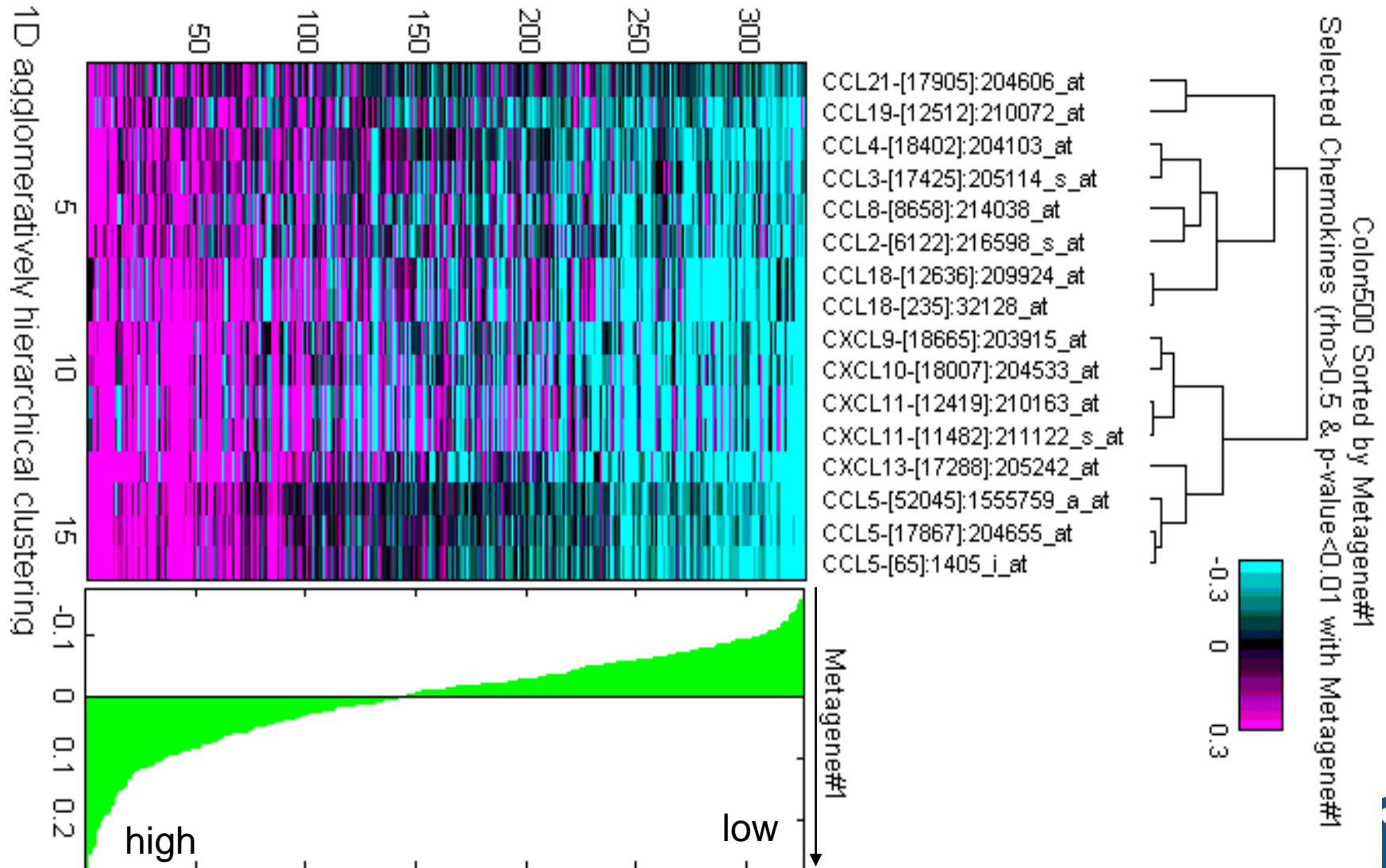
Biologic Features of the Chemokine Signature

- CXCL13, CCL19, CCL21: essential in coordinated involvement in LTi cell homing and lymph node development
- CXCL13: within B cell follicles and highly selective for B cell attraction
- CCL19 and CCL21: critical in normal lymphocyte homing of T (and dendritic) cells in secondary lymph nodes
- CXCL9, 10, and 11: all related T cell attractants
- CCL2, 3, 4, 5, 8, and 18: powerful chemoattractants, with some individual degrees of differences, for monocytes, dendritic cells, T cells, B cells, and NK cells (including naïve and/or activated)

- Notably absent: CCL1, CCL20, and CCL22 - selectively recruit and/or maintain T regulatory cells

Variation in lymphoid infiltrates observed in human colorectal cancer
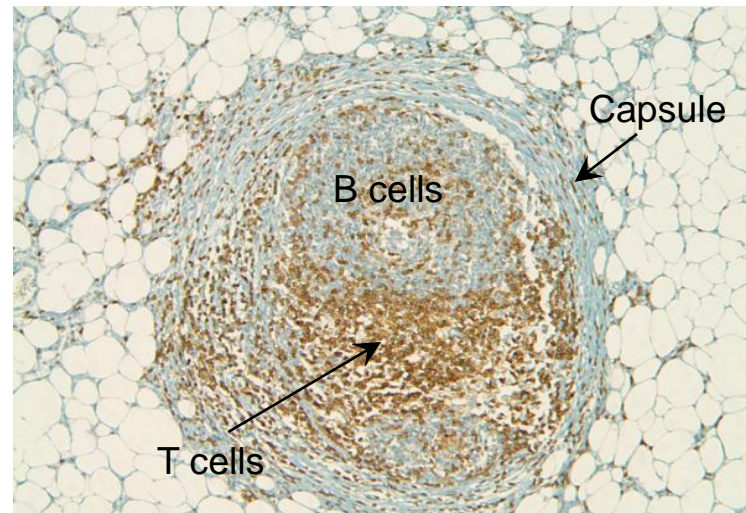
Gene Signature Negative

Gene Signature Positive

None

B cells
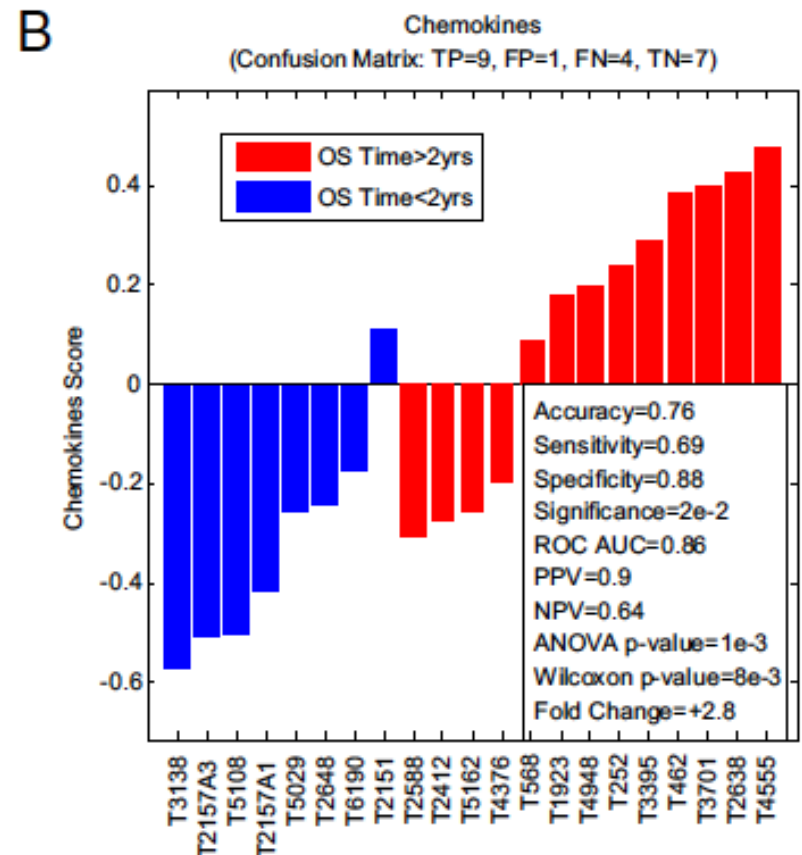
T cells

Ectopic follicle

Dispersed

Capsule

B cells

T cells
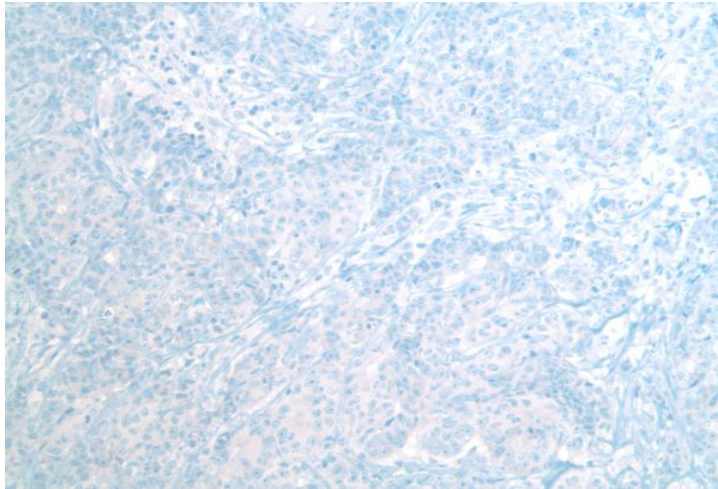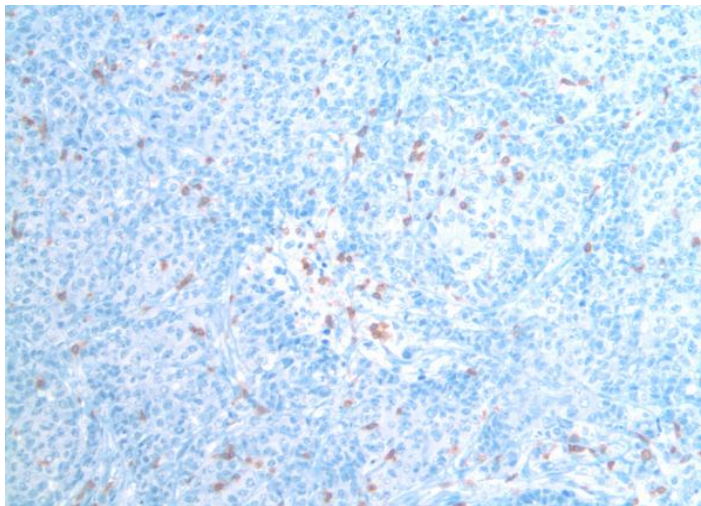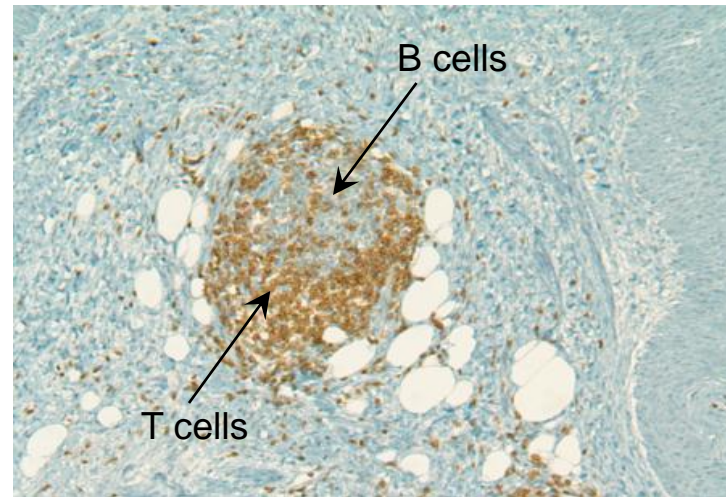
Ectopic follicle with capsule

12-Chemokine GES Identifies Primary CRC Patients with Better Overall Survival

- No association with patient age, gender, tumor stage, location, differentiation, or MSI/MSS status

- But direct correlation with presence of ELNs and patient survival: >4 years versus <18 months (p < 0.0004) for signature positive v negative tumors

B

Chemokines
(Confusion Matrix: TP=9, FP=1, FN=4, TN=7)

OS Time>2yrs
OS Time<2yrs

Chemokines Score

Accuracy=0.76
Sensitivity=0.69
Specificity=0.88
Significance=2e-2
ROC AUC=0.86
PPV=0.9
NPV=0.64
ANOVA p-value=1e-3
Wilcoxon p-value=8e-3
Fold Change=+2.8

Coppola D, Mulé JJ. J Clin Oncol. 2008 Sep 20;26(27):4369-70
Coppola et al., Nebozyhn N, Khalil F et al. Am J Pathol. 2011 Jul;179(1):37-45

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Interrogation of a 12-Chemokine Gene Expression Signature across 14,492 Solid Tumors of Differing Histology: Principal Component Analysis

- TCC Samples
- Primary & Metastatic Lesions
- PCA score as a measure of chemokine signal
- Top 10% of samples were selected as high
- High expression tissues includes Skin

*mainly ascites/effusions
**insufficient tissue/quality issues

Tissue Type	Total # CEL files	# Above 90th percentile	% Above 90th percentile	exact_test p value
Oral Cavity	98	25	25.51	8.08E-06
Cervix	75	19	25.33	0.000106163
Tongue	32	8	25	0.011540008
Skin	569	115	20.21	1.11E-13
Lung	2708	488	18.02	3.63E-47
Soft Tissue	97	14	14.43	0.170421294
Bladder	212	27	12.74	0.20279835
Larynx	56	7	12.5	0.501205795
Breast	3705	401	10.82	0.052757354
Stomach	133	13	9.77	1
Large Bowel	2111	169	8.01	0.000837406
Kidney	850	61	7.18	0.003839317
Thyroid	71	5	7.04	0.550835068
Esophagus	90	6	6.67	0.377460472
Rectum-Anus	188	10	5.32	0.027468551
Liver	116	5	4.31	0.041696746
Endometrium	333	14	4.2	0.000131198
Small Intestine	52	2	3.85	0.167045365
Uterus	377	13	3.45	2.00E-06
Pancreas	468	15	3.21	2.70E-08
Ovary*	670	21	3.13	8.78E-12
Renal Pelvis	62	1	1.61	0.01881245
Brain	438	4	0.91	2.30E-15
Prostate**	981	5	0.51	9.64E-39
Total	14492	1448		

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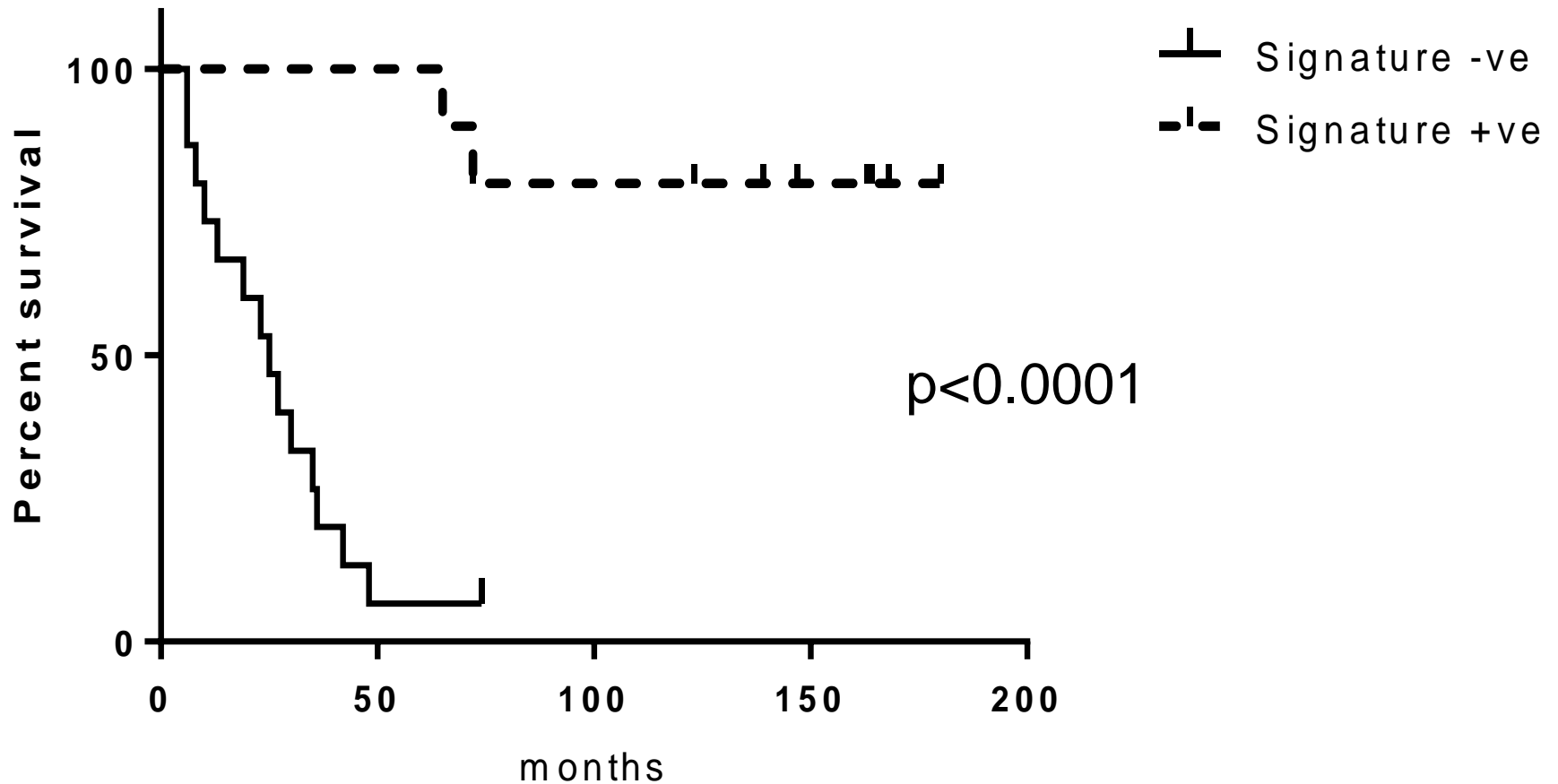# 12-Chemokine Gene Expression Signature (GES): 120 Stage IV Non-Lymph Node Melanoma Metastases
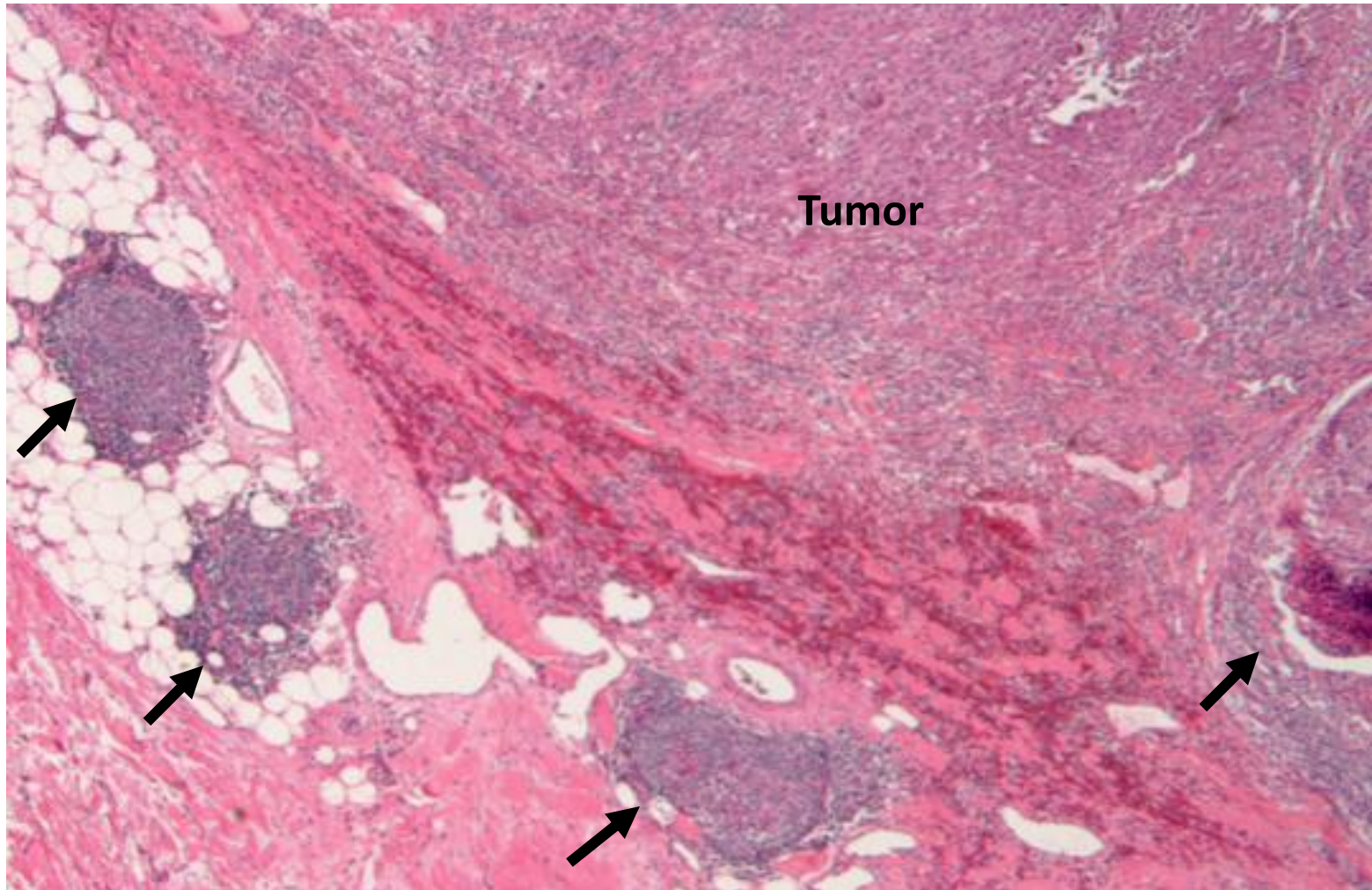
Messina et al. *Nature – Sci. Rep.* (nature.com)
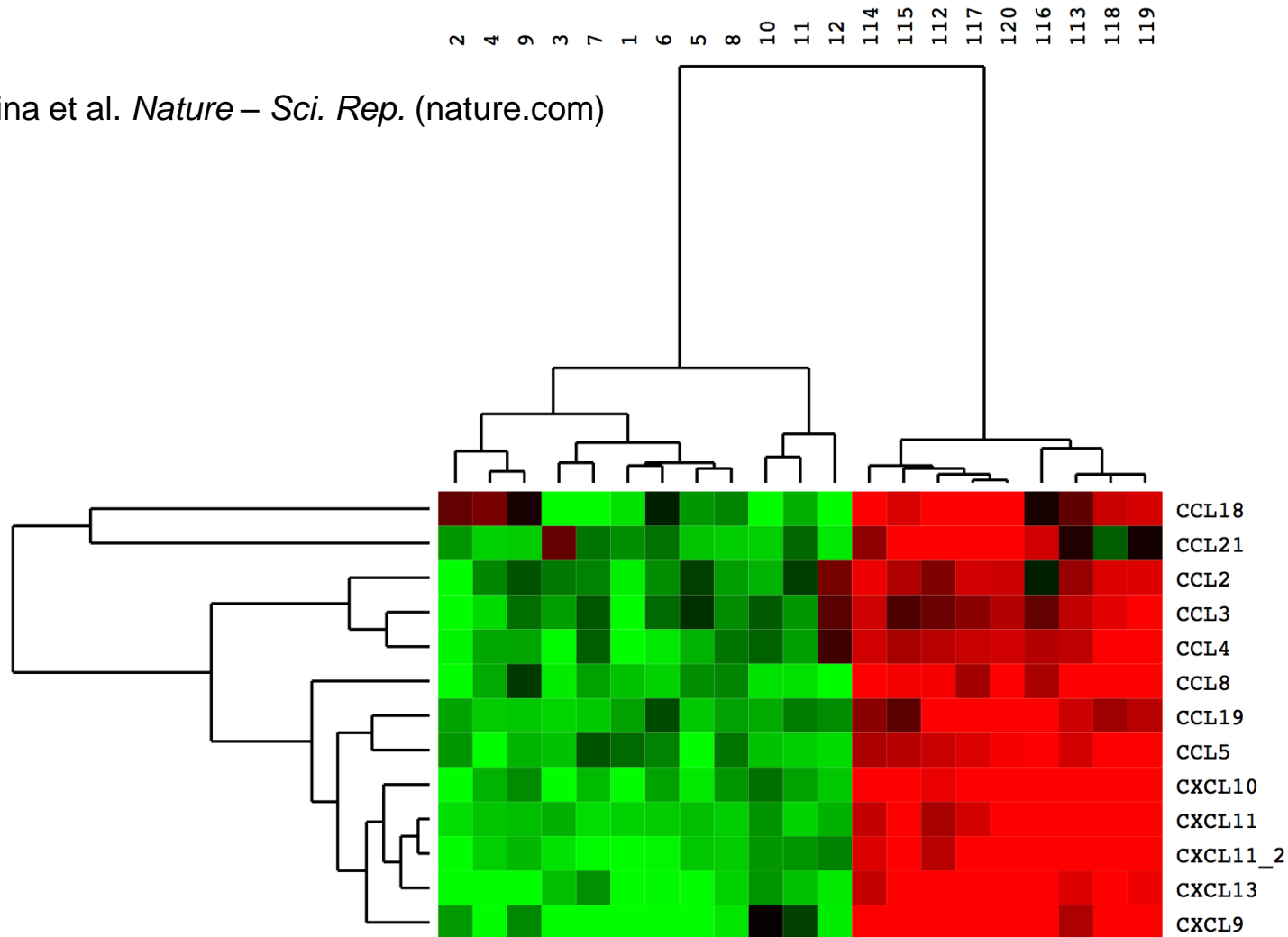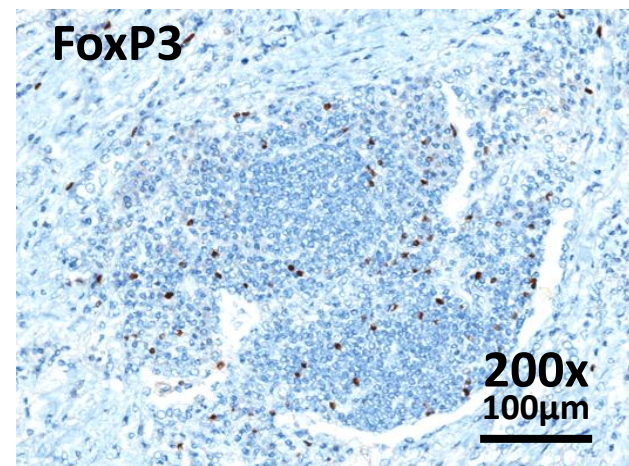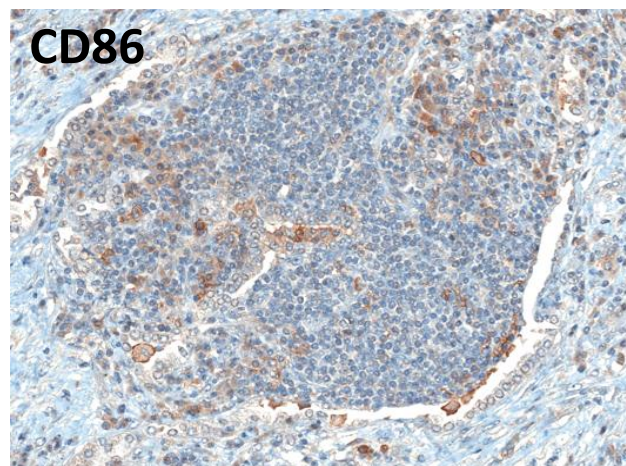

Green negative for GES

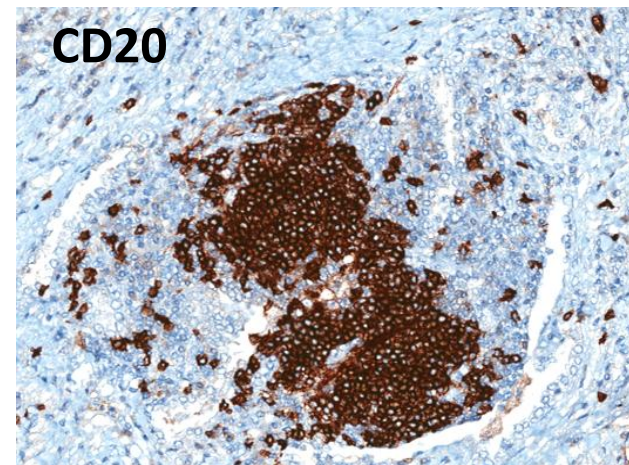Red positive for GES

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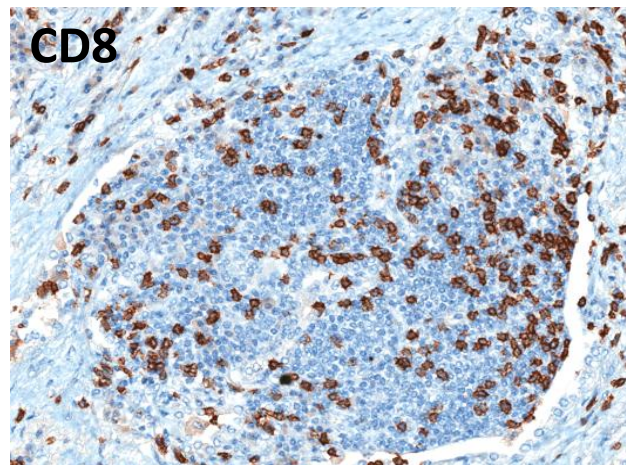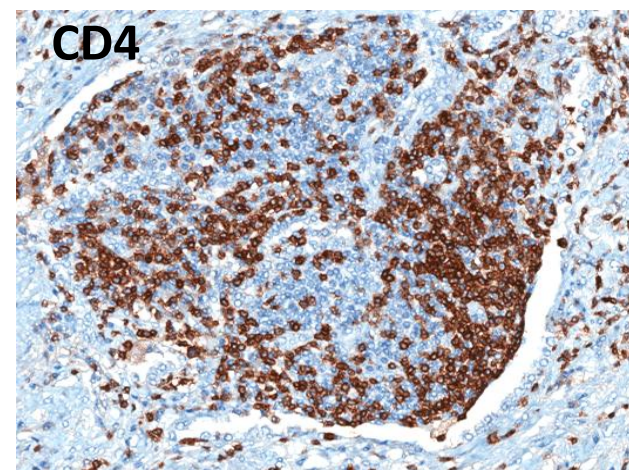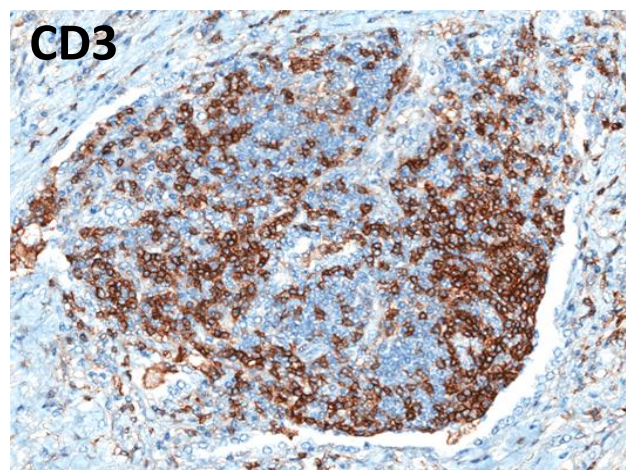
12-Chemokine GES Identifies Stage IV Melanoma Patients with Better Overall Survival

12-Chemokine GES Identifies Ectopic Lymph Node Structures in Melanoma

12-Chemokine GES can identify clinical responders in vaccine-treated metastatic melanoma

- Phase II vaccine study in 75 pts. with nonresectable MAGE-A3–positive stage III or IV M1a metastatic melanoma

- Response defined as CR, PR (Recist) or SD

- Using cel data, Moffitt GES accurately identified the clinical responders in 20/22 (91%) cases

- Now working with industry to evaluate OS in both melanoma and non-small cell lung cancer patients

Ulloa-Montoya F et al. *J. Clin. Oncol.* 31:2388-2395, 2013

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Evaluation of the 12-chemokine GES in FFPE: a multiplexed quantitative hybridization-based gene expression assay
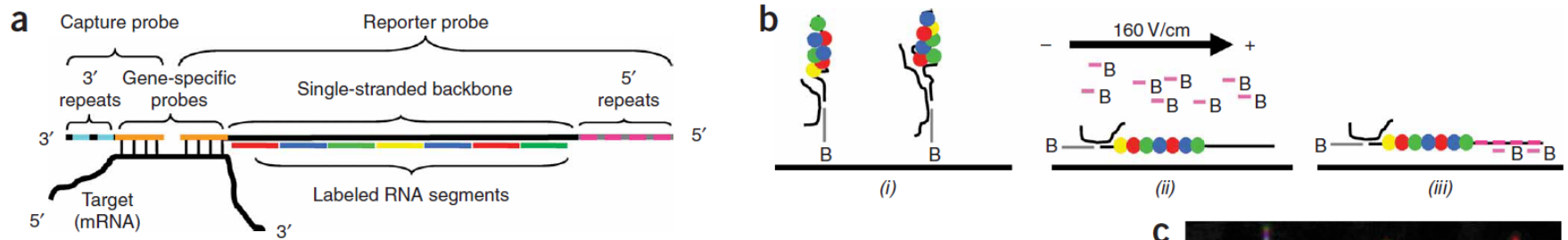
*Panel comprises the 12 chemokine genes; CTLA-4, PD-1, PD-L1, and LAG3 genes, as well as reference genes EIF2B5, GSK3A, MYLK2, RPL10L, SRSF4, THEM4
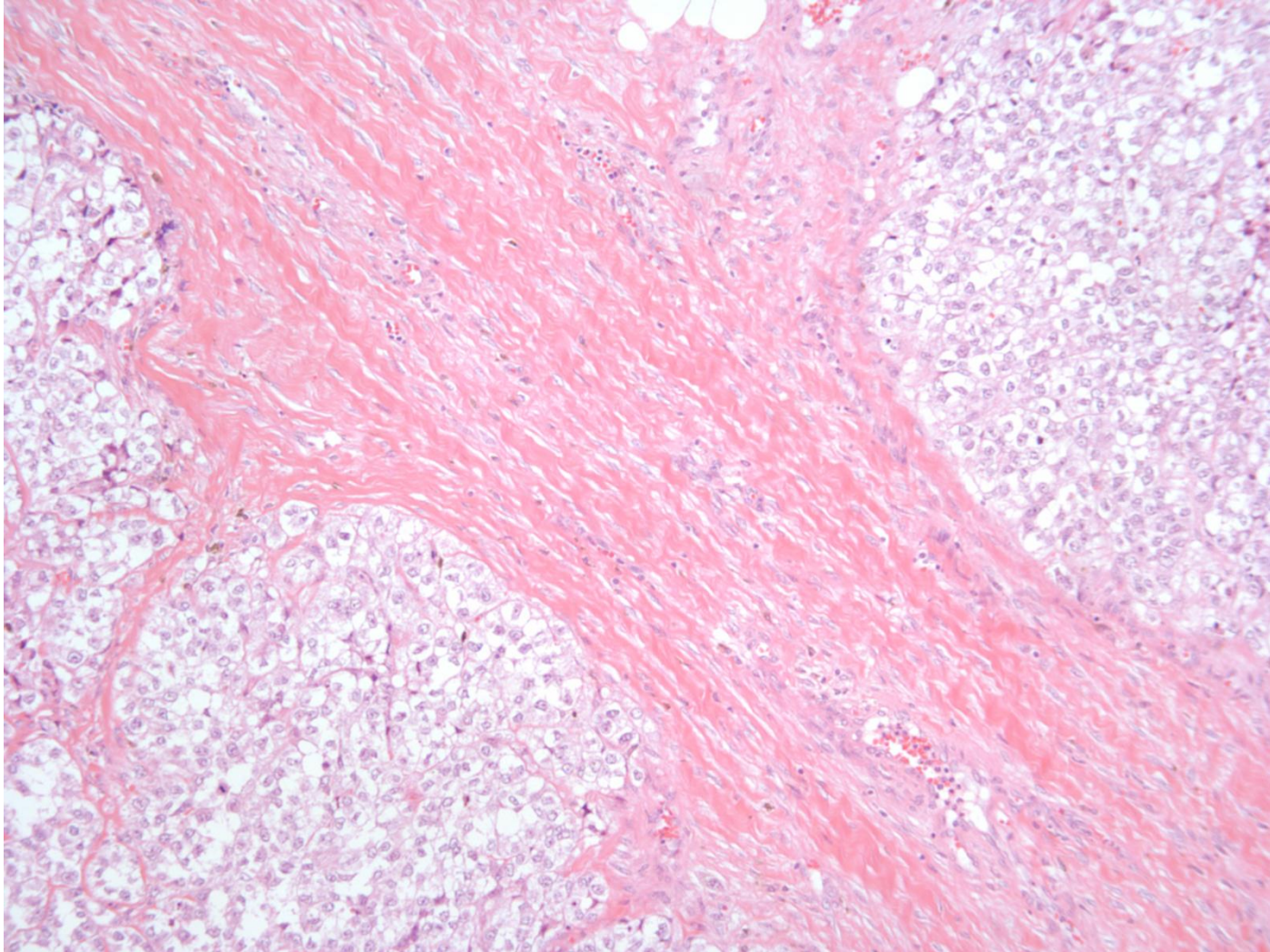*Correctly identified the ten specimens with the highest and lowest 12-chemokine GES
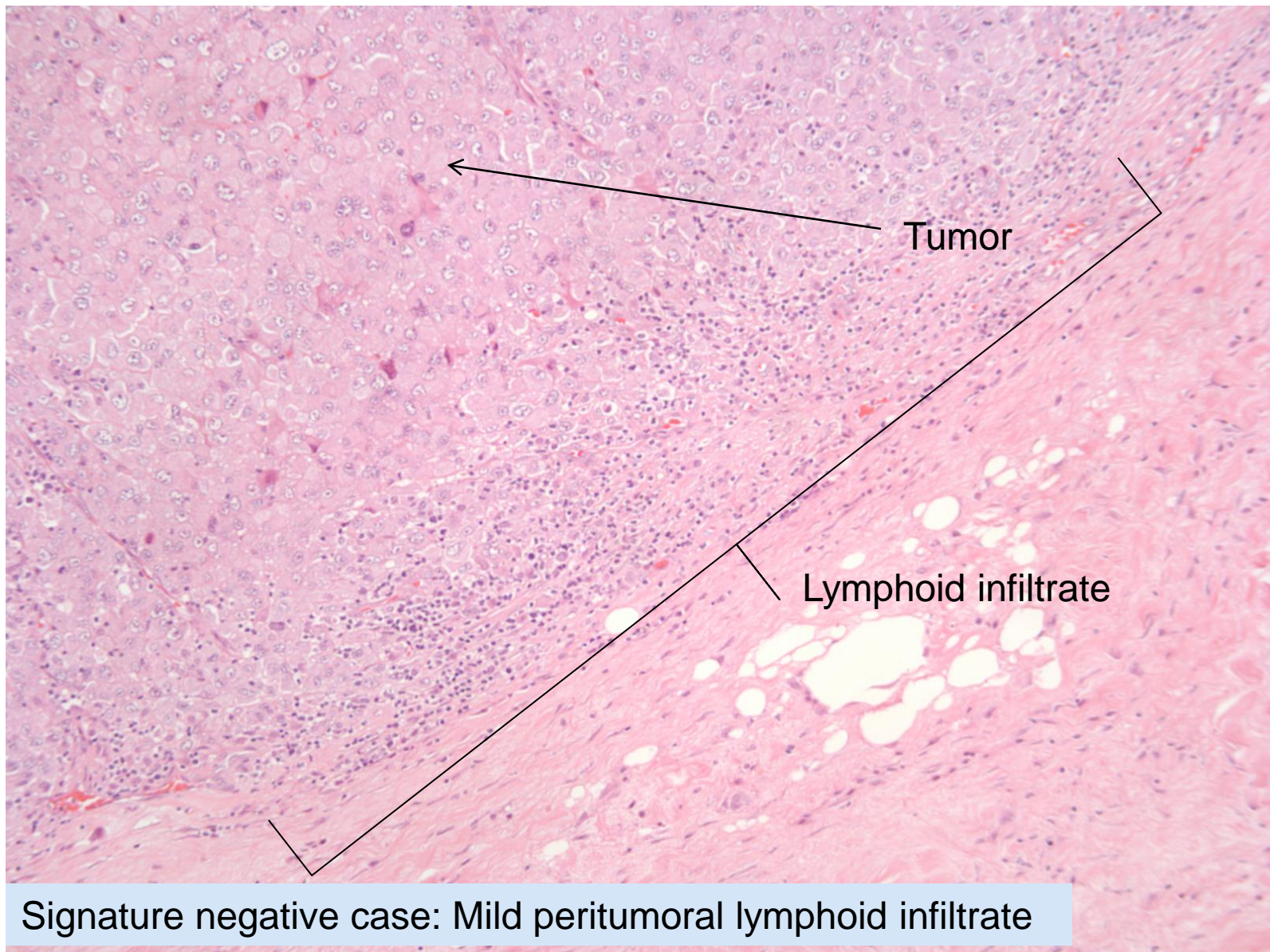
Evaluation of GES on validation cohort

- Tissue from 62 melanoma patients (10-12 paraffin slides per case) for RNA extraction, H&E, IHC staining and analysis

- Cases are from MDACC's tumor infiltrating lymphocyte trial(s)*

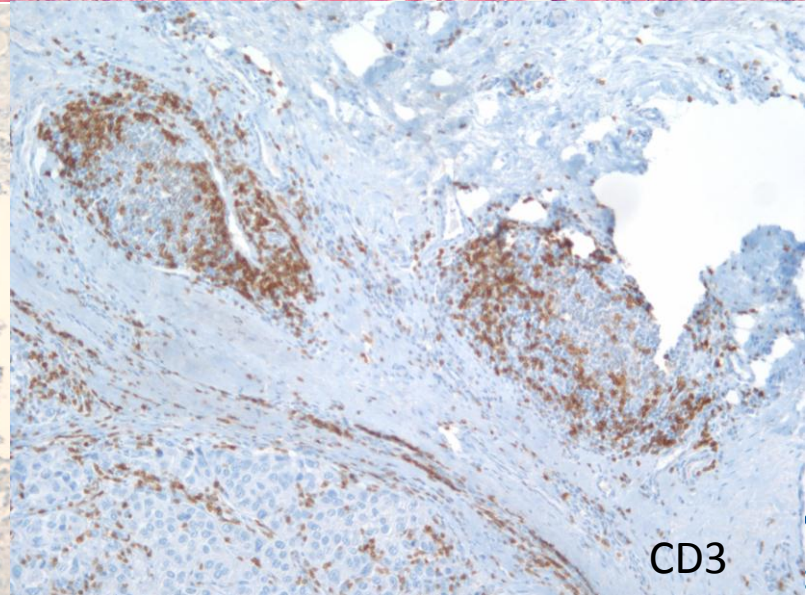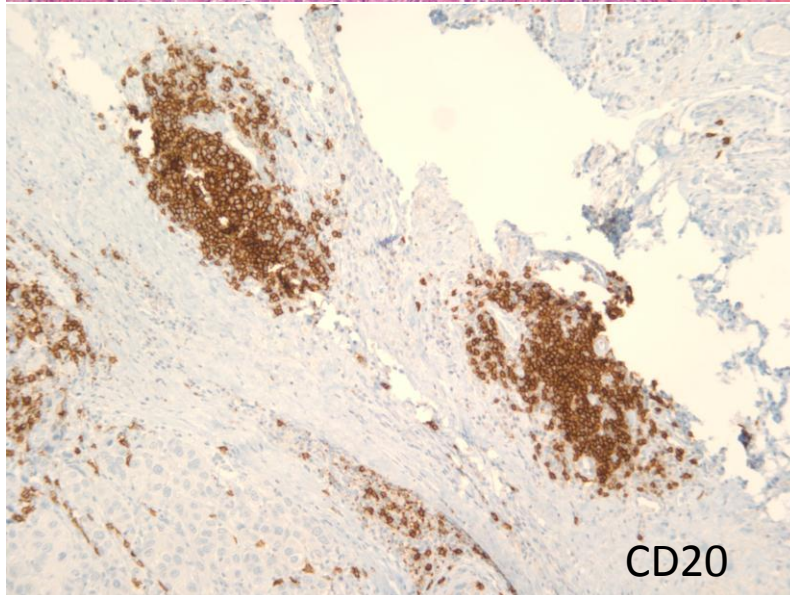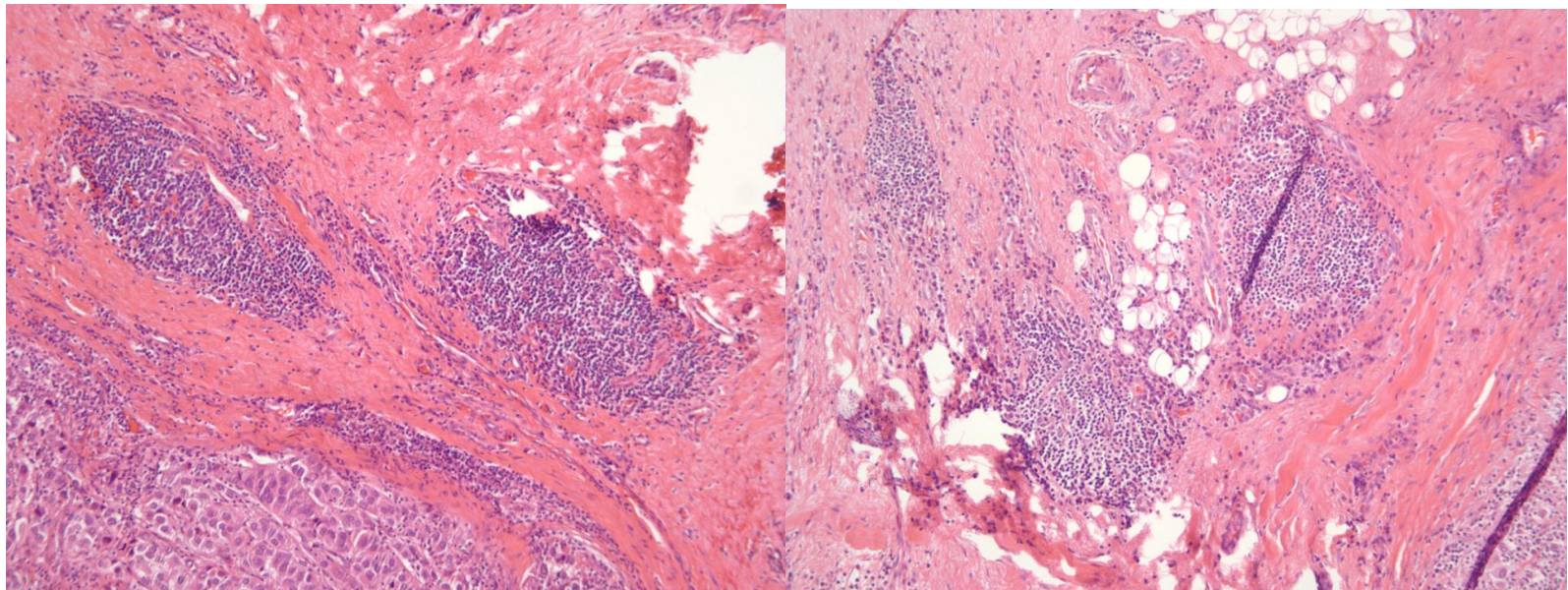- 4/62 cases failed FFPE assay due to extracted RNA quality issues

*courtesy of Dr. Laszlo Radvanyi

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Signature negative case

Tumor

Lymphoid infiltrate

Signature negative case: Mild peritumoral lymphoid infiltrate

# Signature positive case

CD20

CD3

Implications/Future Studies

- Correlation of gene-related signature to response to immunotherapy

- Focus on response to nivolumab, ipilimumab, and anti-PD-L1 monoclonal antibody (MPDL3280A)

- Potential for selection of cancer patients for immunotherapy interventions based on a particular immune gene-related signature

Additional Studies

- Employing laser capture microdissection with DNA and RNA isolation and sequencing methodologies to address questions of clonality and functional relationships of the resident B cells and T cells within the tumor microenvironment, including in depth T cell receptor and immunoglobulin transcript repertoire analyses

Laser Capture Microdissection Studies
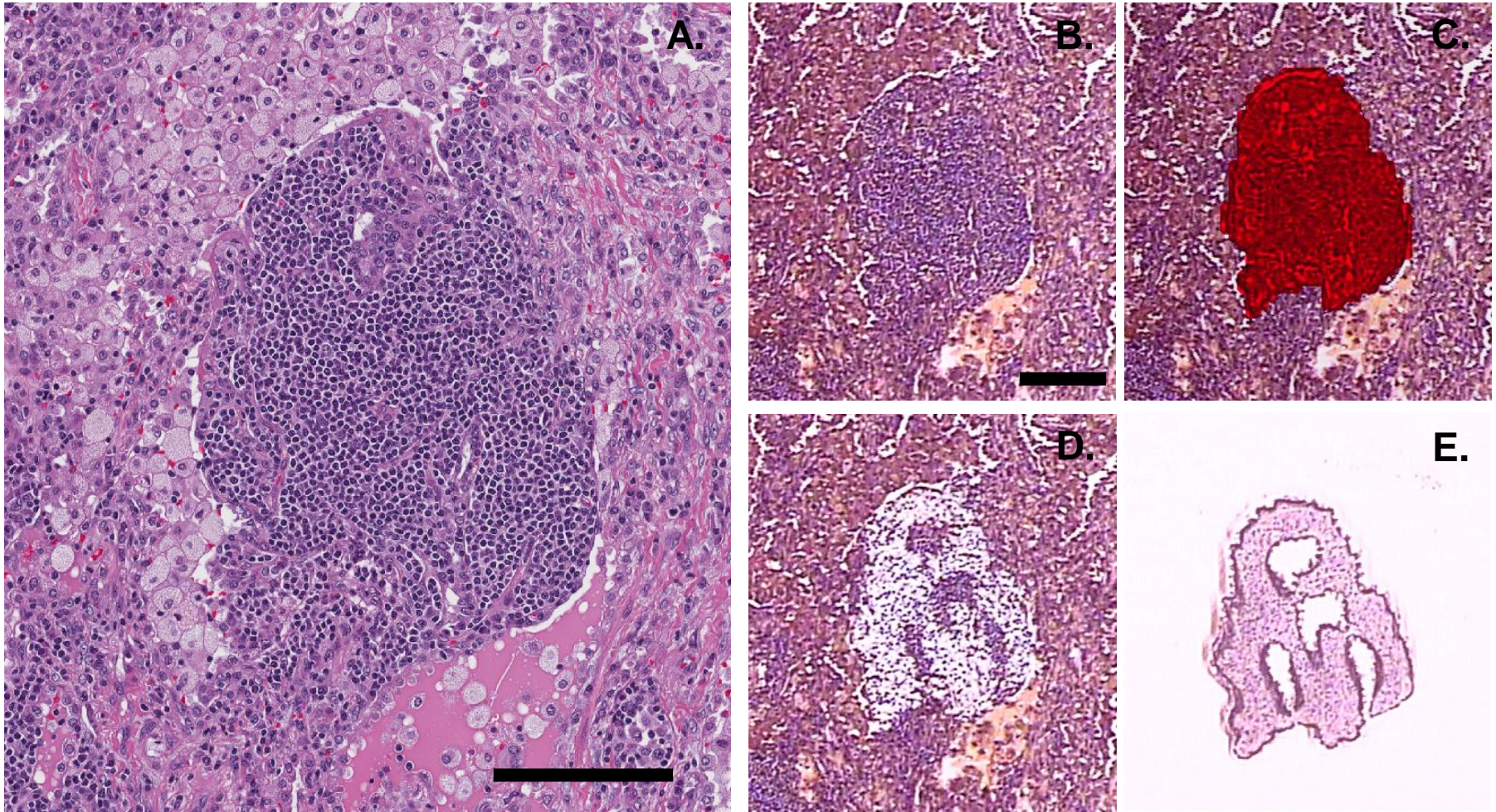
Ectopic lymph nodes in melanoma metastasis to the lung; A) stained with H&E at 200x [scale = 200µm]; B) sample prepared for laser capture microdissection (w/o coverglass) [scale = 100 µm]; C) targeted tissue (red); D) tissue sections post LCM documenting the missing material; E) the LCM cap containing the captured material. Note: The missing targeted material in the cap is due to a phenomena described as polymer depletion and is normal, but should be documented as a % efficiency of the capture (~80% efficient).

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