Immune Changes in Tumor-Draining Lymph Nodes as Novel Biomarkers

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TDLNs in Breast Cancer

- Tumor invasion of TDLNs is a key determinant of clinical outcome in breast and other cancers

- Lymph nodes are immune organs!

- Are immune cell populations (T cells, B cells, and dendritic cells) altered in sentinel and axillary lymph nodes?
- If so, do immune changes in a LN reflect tumor invasion?
- Do immune changes predict clinical outcome?
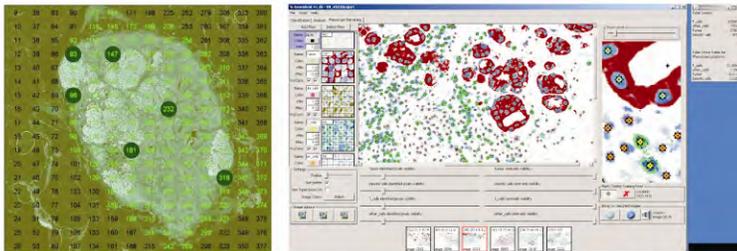
Integrated image analysis approach

1.
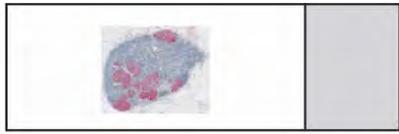
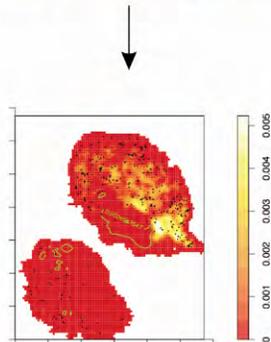Multicolor staining of tissue sections

2.

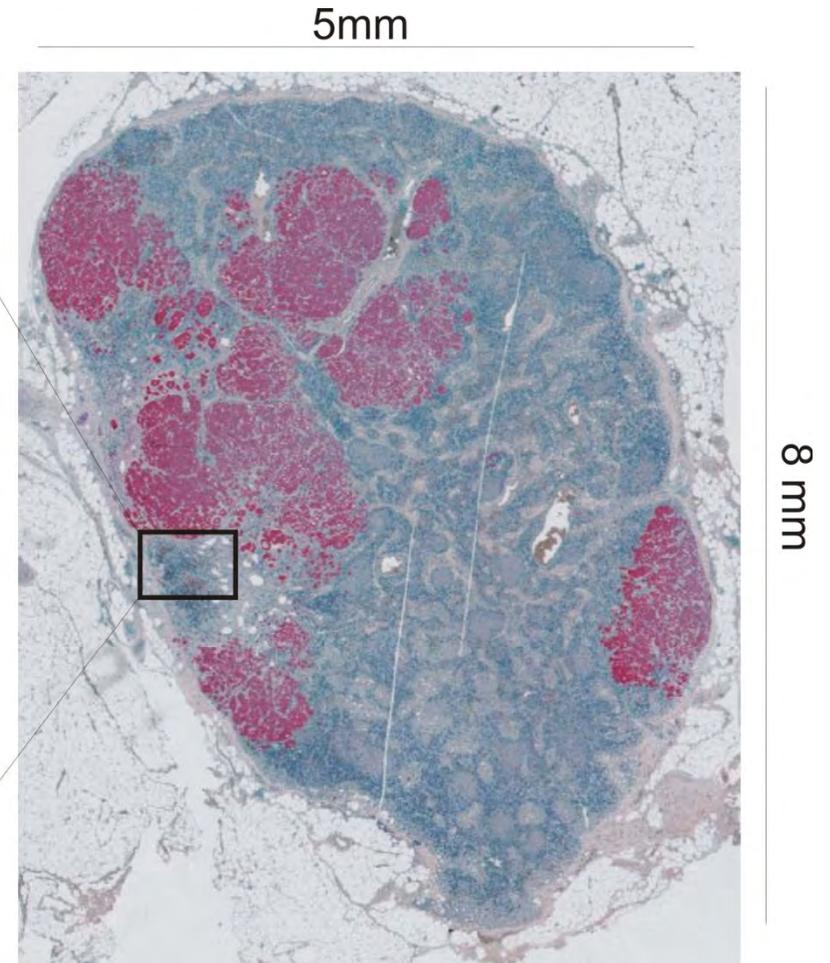High-res spectral imaging & automated scanning of the whole tissue section
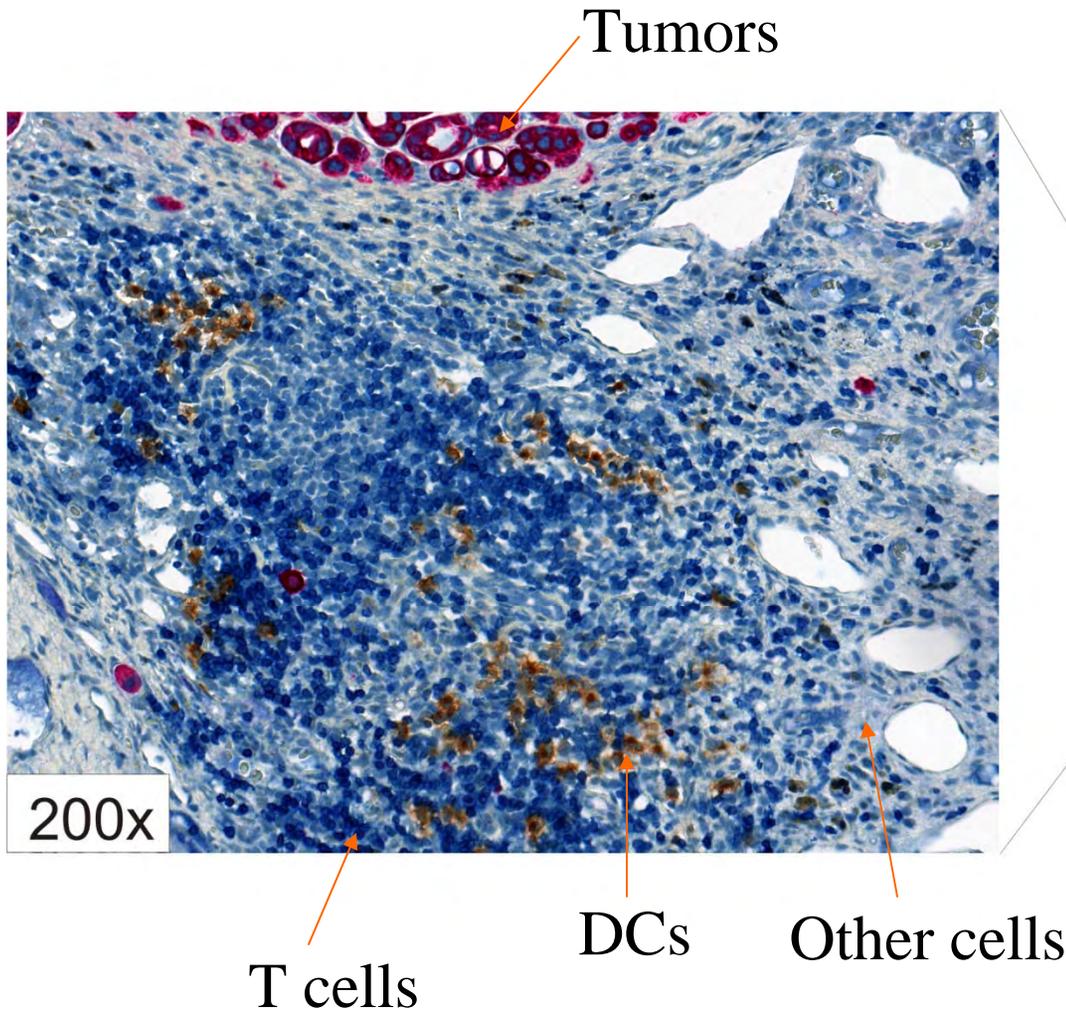
3.

Machine-learning-based cell identification by GemIdent
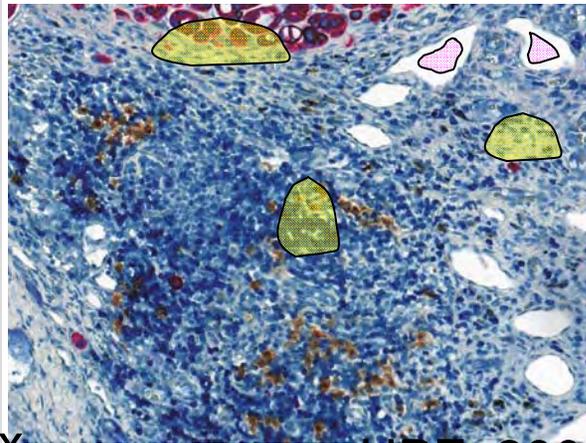→ number of cells and Cartesian coordinates

4.

Quantitative and spatial statistical analyses

Multicolor staining

Tumors

T cells

DCs

Other cells

5mm

8 mm

200x

Imaging & scanning by Vectra™

LPF: 40x

HPF: 200x

Build a classifier:
enable the program to recognize tissue vs. non-tissue (empty spaces, bubbles, fats, etc).
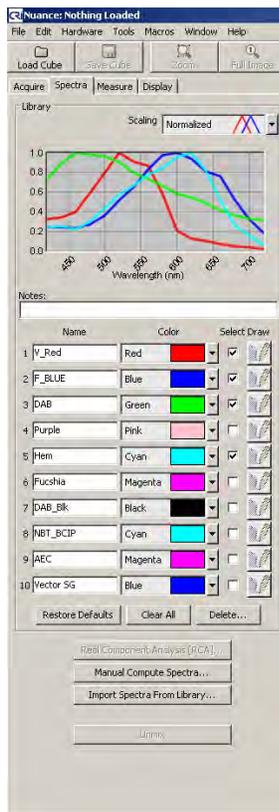
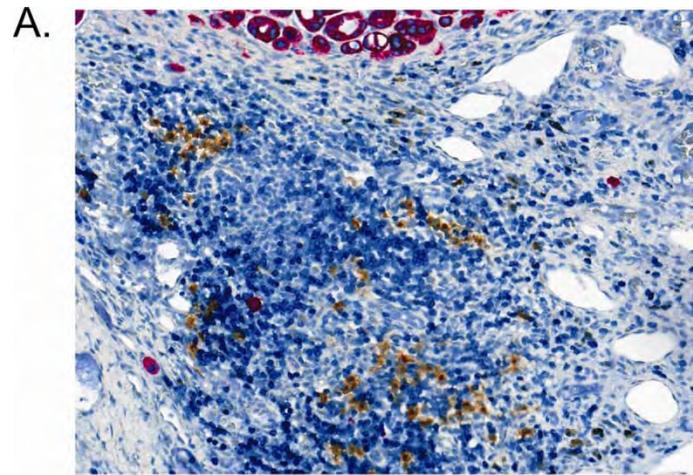🟡 = tissue

🟣 = non-tissue

Build a spectral library:
enable the program to unmix signals from different chromogens
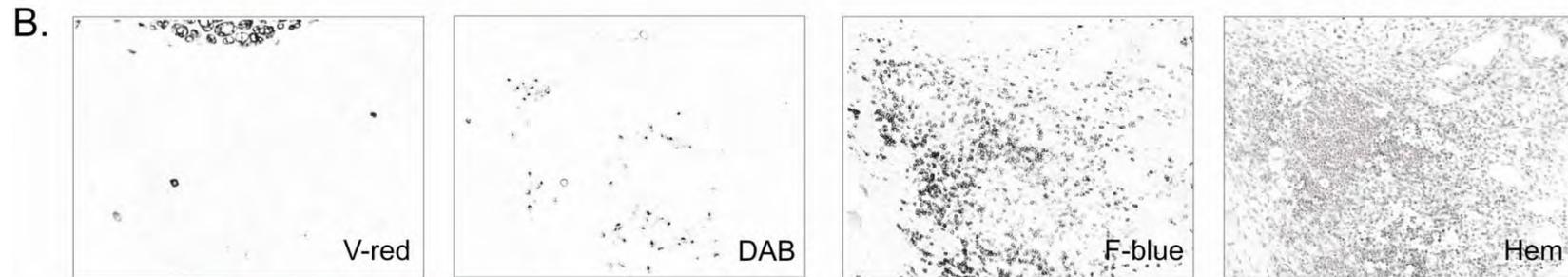
Set up the scan:
- Organize slides in cassettes
- Set up autofocus
- Take brightfield reference images
- Determine imaging area
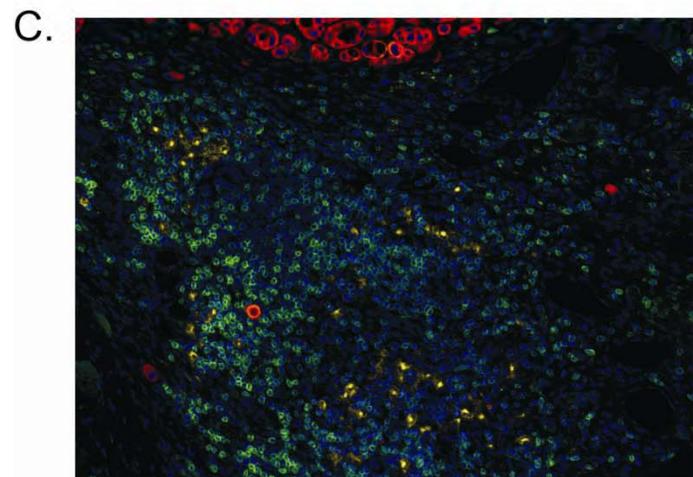- Determine threshold for tissue finding (for both LPF and HPF imaging)

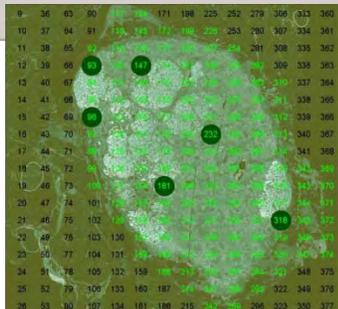# Chromogen unmixing and image reconstruction by Vectra^{TM}

A.

Original image

B.

V-red DAB F-blue Hem

Unmixed images

C.

Re-constructed composite image

GemIdent analysis

Whole image overview

Maps of each cell type
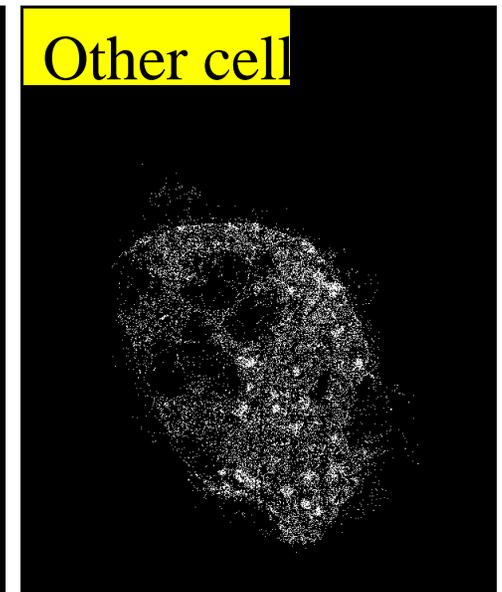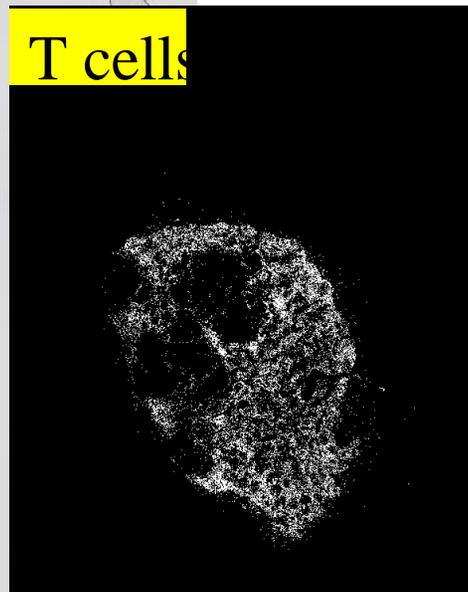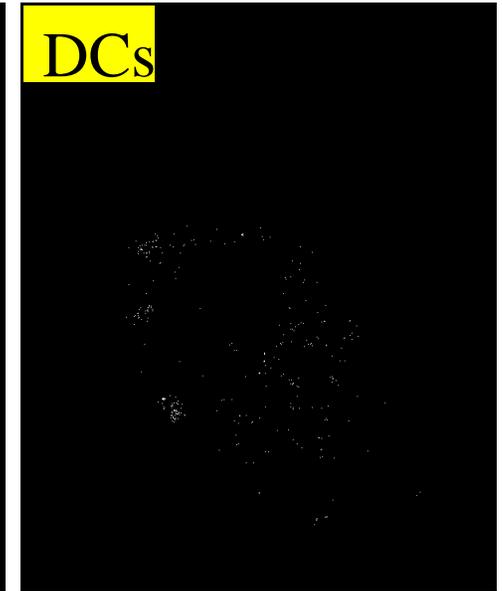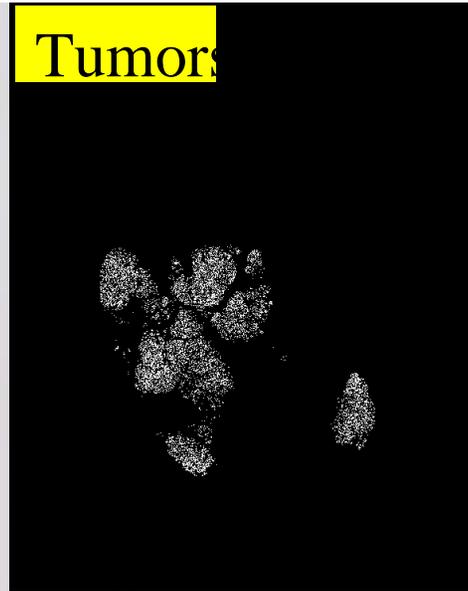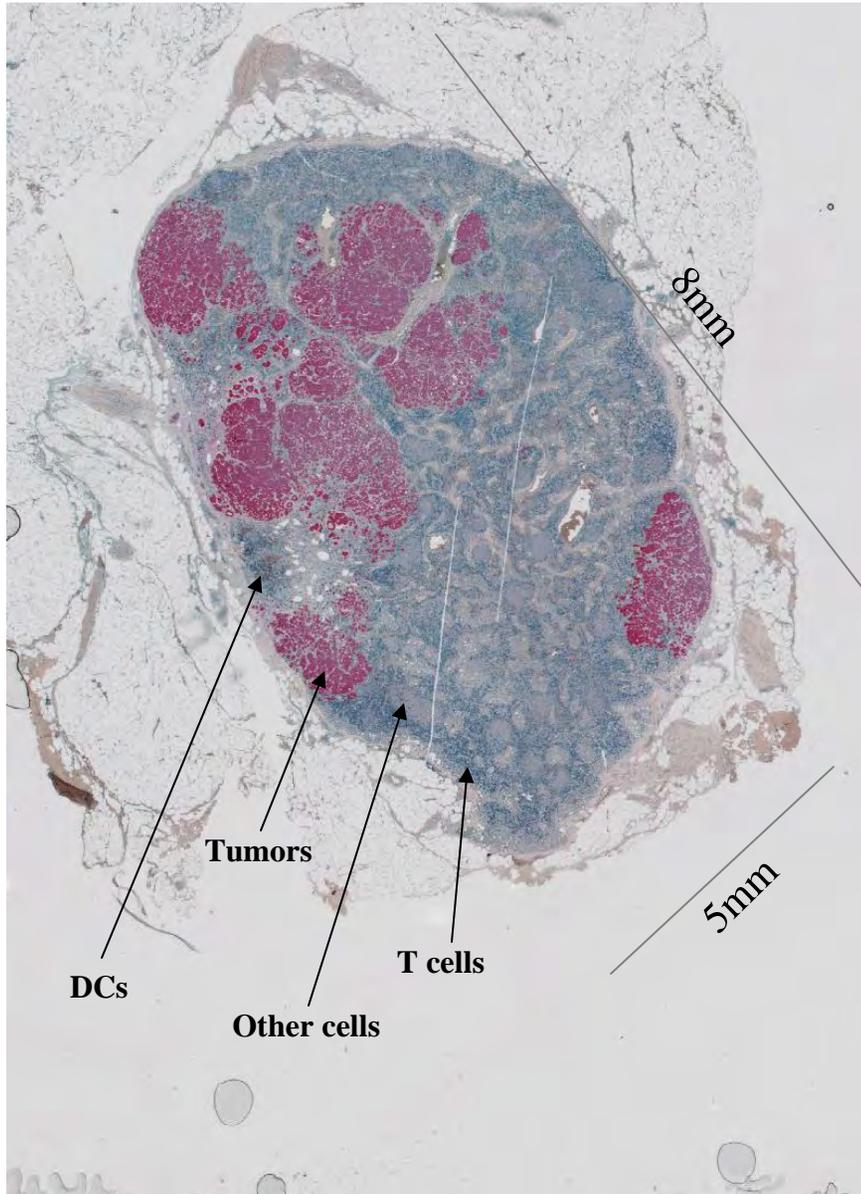
Analysis of Results

- Number and proportion of each cell type
- Spatial statistics: architectural pattern analysis of immune cells and tumor cells

Relationship with >5-year clinical outcome & parameters

Proof of Concept: Breast Cancer

– Total 77 stage IIA, IIB,IIIA breast cancer patients analyzed

– All patients had positive SLN biopsy and thus had ALN dissection

– Significant numbers of recurrences within 5 years allowing correlation with clinical outcome

– 10 non-cancer LNs analyzed as controls

Kohrt et al, PLoS Med 2005

IHC analysis of TDLN in breast CA

SLN – CD8 | **ALN – CD8** | **Control – CD8**

25 µm
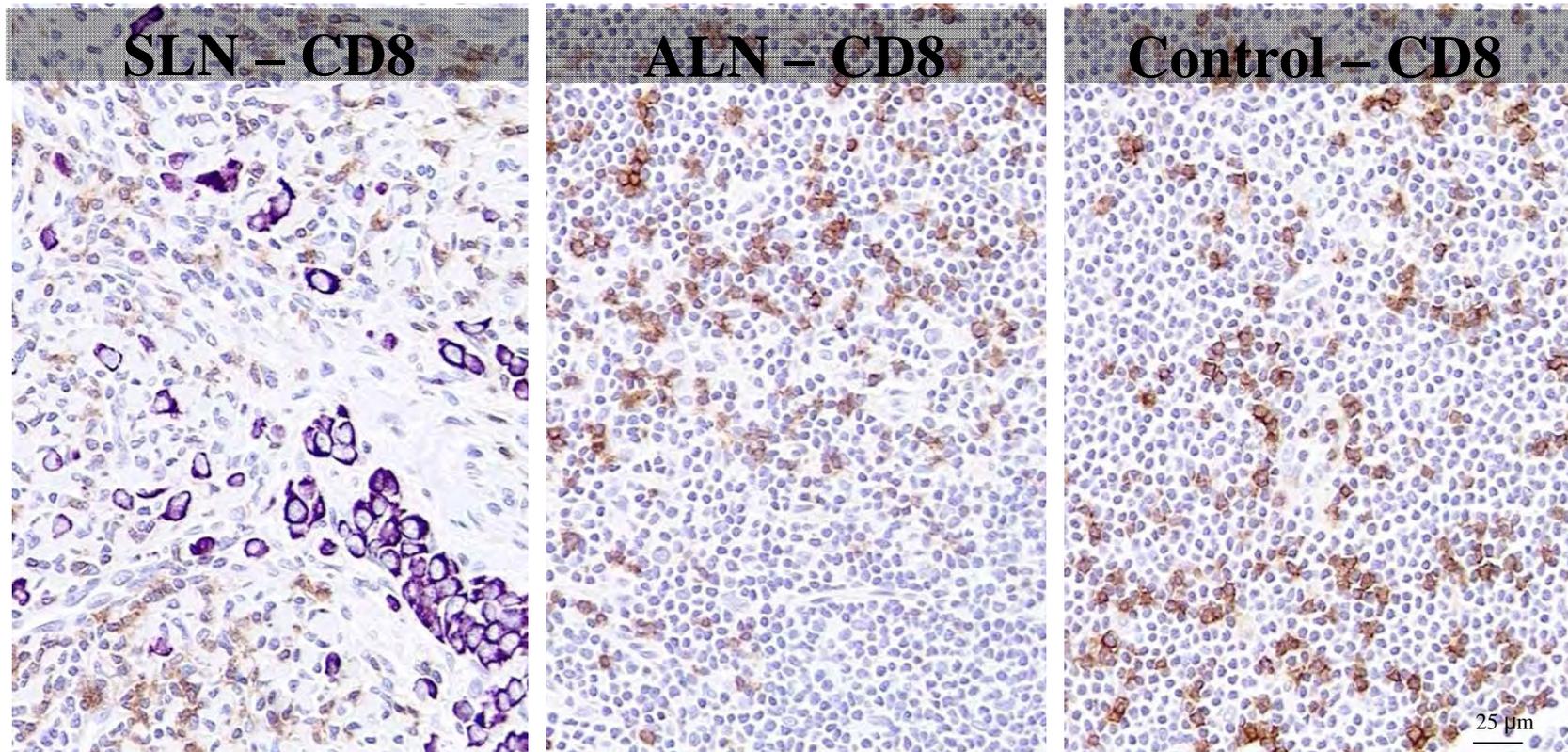
Breast cancer cells:
AE1/AE3

Immune cells:

– CD4 'helper' T cells

– CD8 'cytotoxic' T cells

– CD1a dendritic cells - 'antigen presentation'

# Immune Alterations Correlates with Tumor involvement of TDLN

Immune Profile	Tumor-free lymph node % (mean ± SE)	Tumor-involved lymph node % (mean ± SE)	Wilcoxon rank sum test *P*-value*
Lymph Node Predictors of Lymph Node Metastases			
CD4%	17.85±2.19	2.11±0.35	<0.001
CD8%	7.93±0.99	7.52±0.71	0.682
CD1a%	3.59±0.56	0.26±0.06	<0.001
CD4/CD8 Ratio	2.47±0.28	0.34±0.08	<0.001
P-values adjusted for multiple comparisons.			

TDLN Immune Status Correlates with DFS
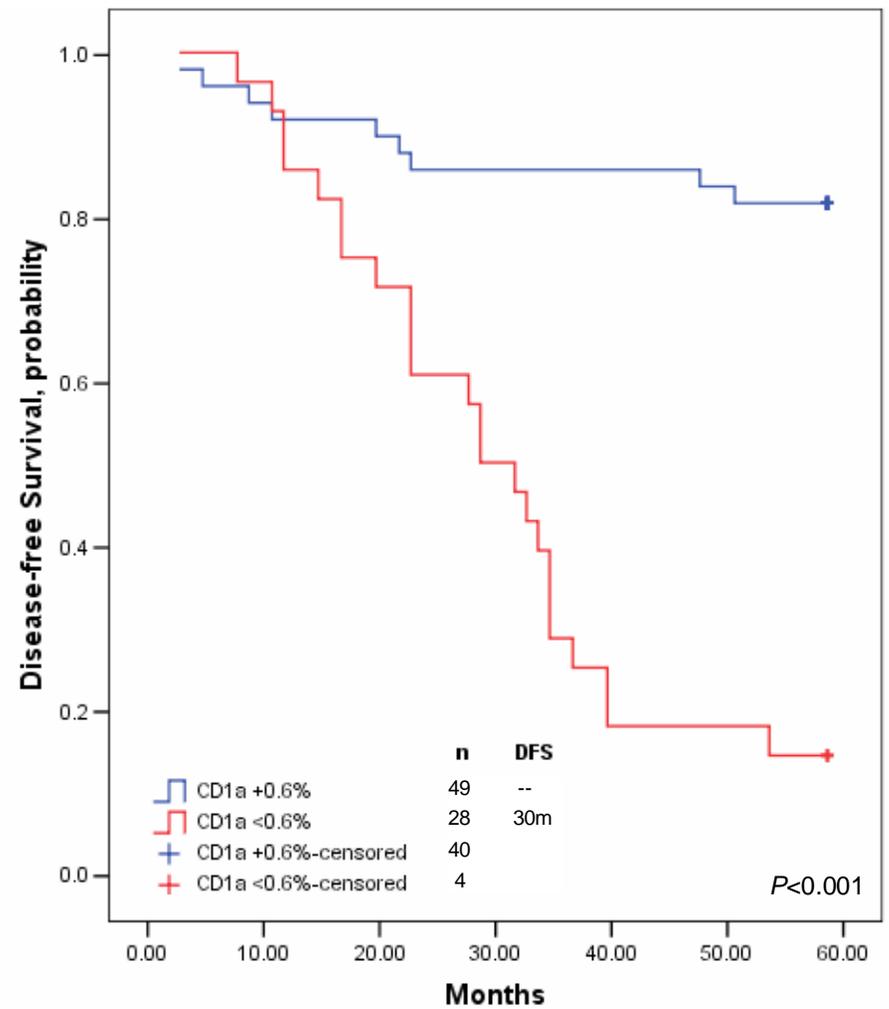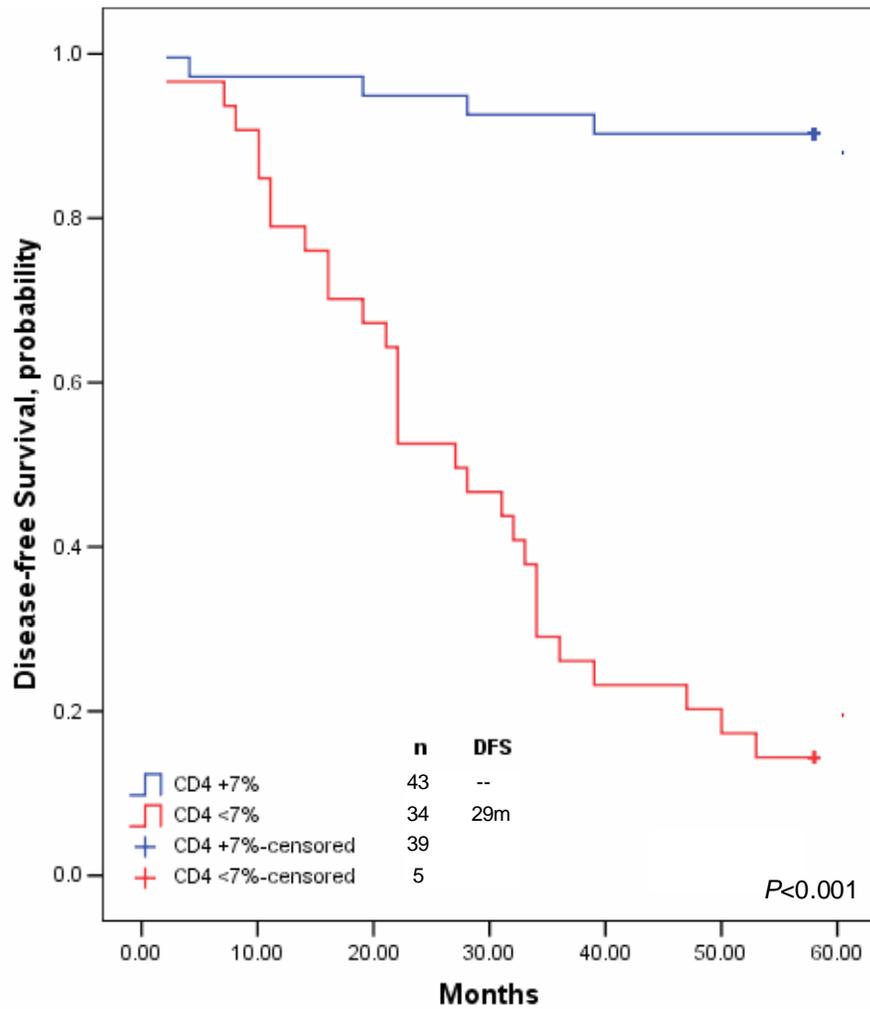
DFS Stratified by Tumor Stage (T1) and TDLN Immune Profile
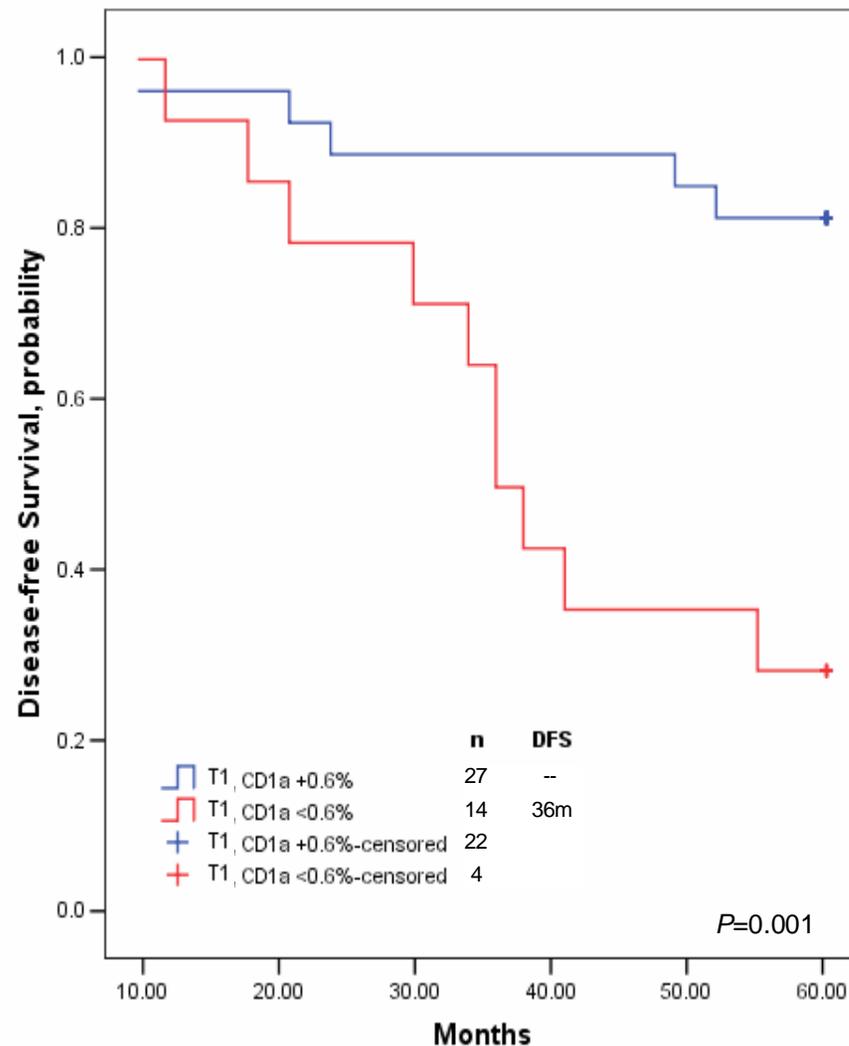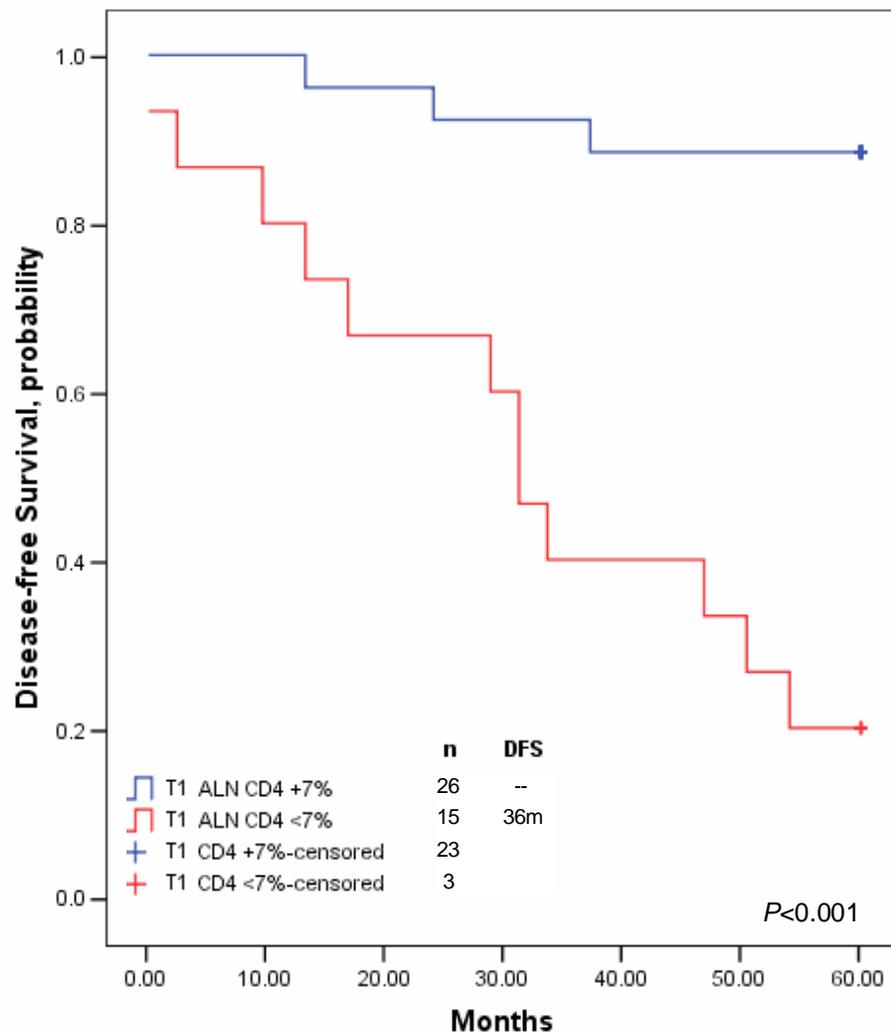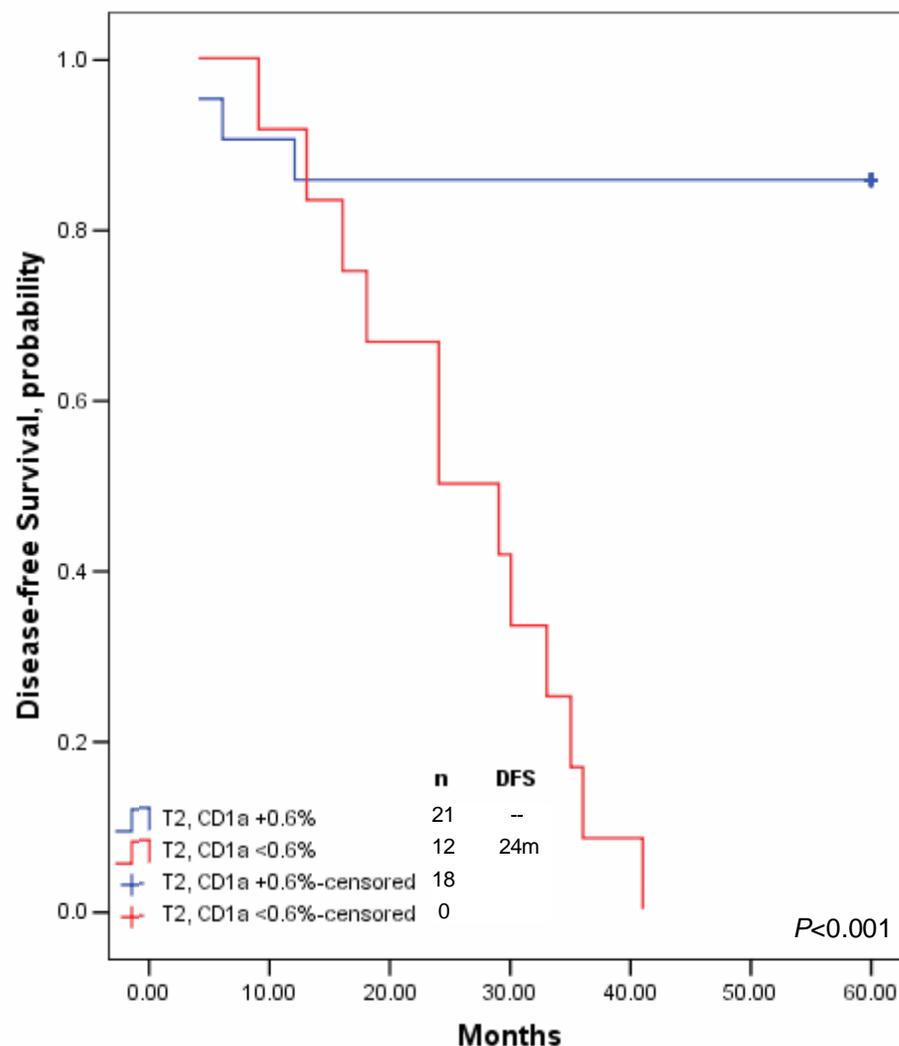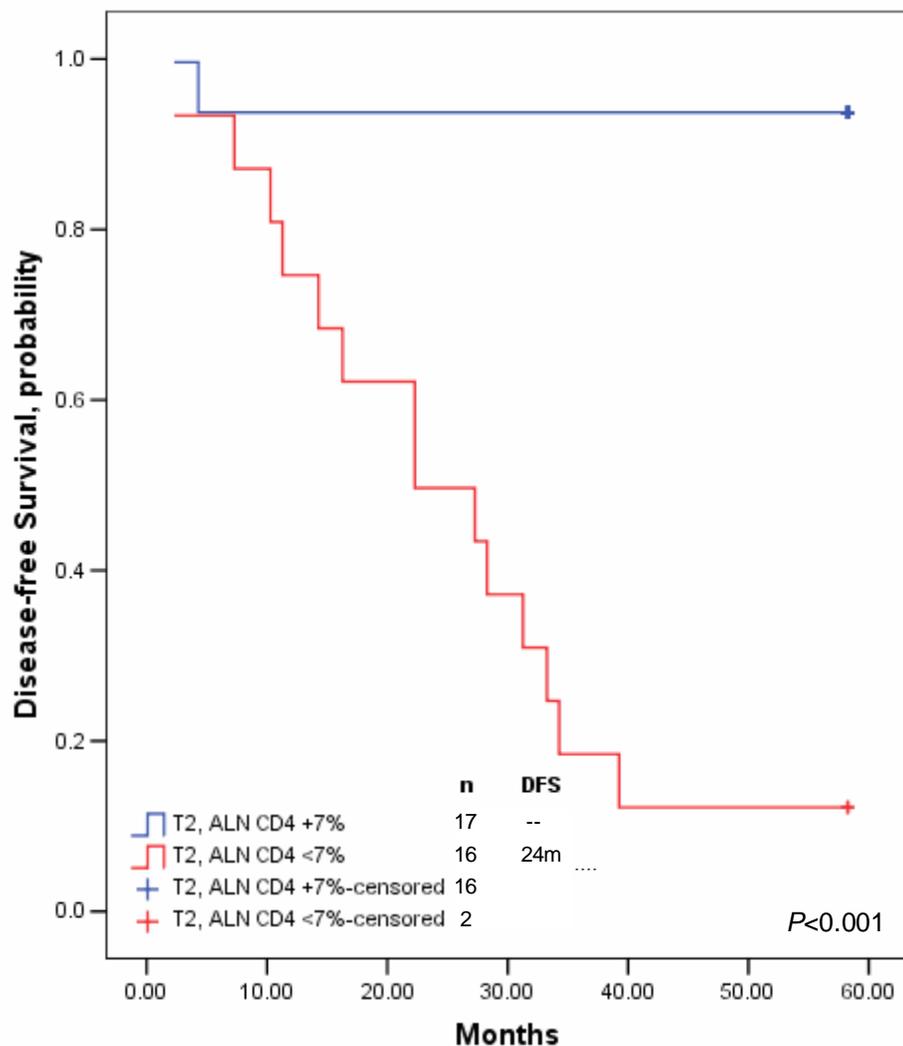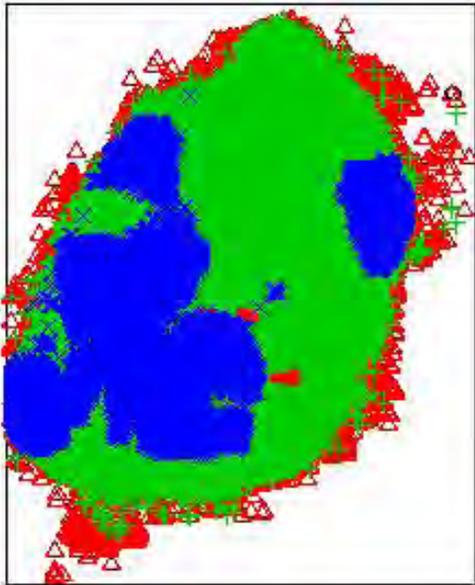
DFS Stratified by Tumor Stage (T2) and TDLN Immune Profile

Beyond numbers: spatial patterns

Identified Cells
Black=Dendritic, Red=Others, Green=Tcells, Blue=Tumo

- Different cell phenotypes constitute a "marked point process"

- Goal: to quantify spatial characteristics in order to understand cellular interaction

- Preliminary Findings: T and B cells from TDLN and healthy LN have different spatial distribution patterns [PLoS ONE 5(8):e12420, 2010]

Dendritic Cell Clustering and Relapse
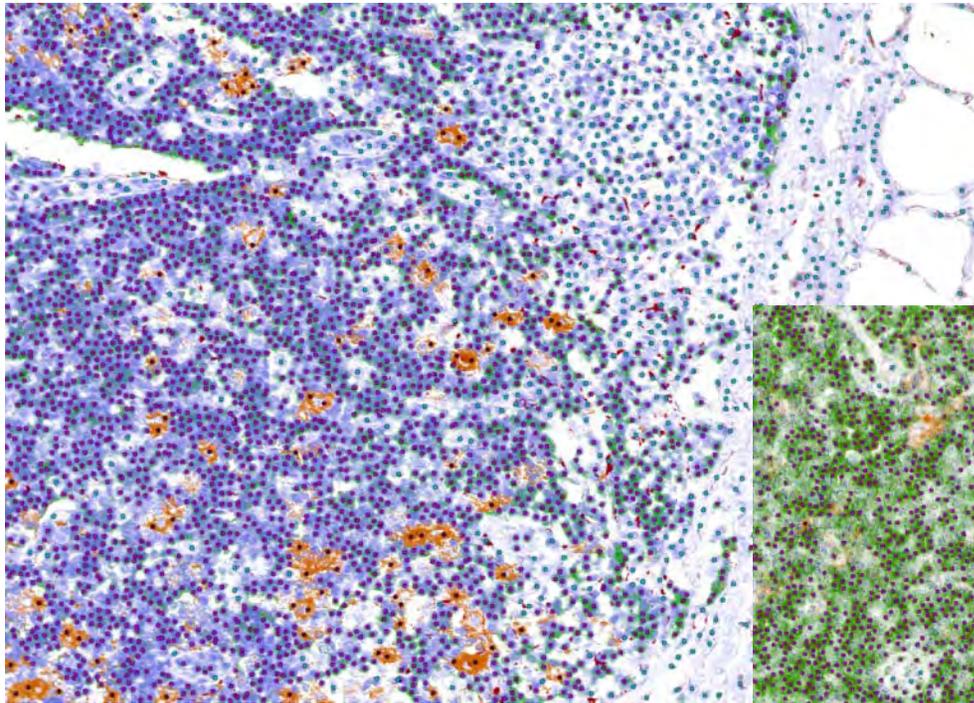
Disease Free

Relapsed

Summary

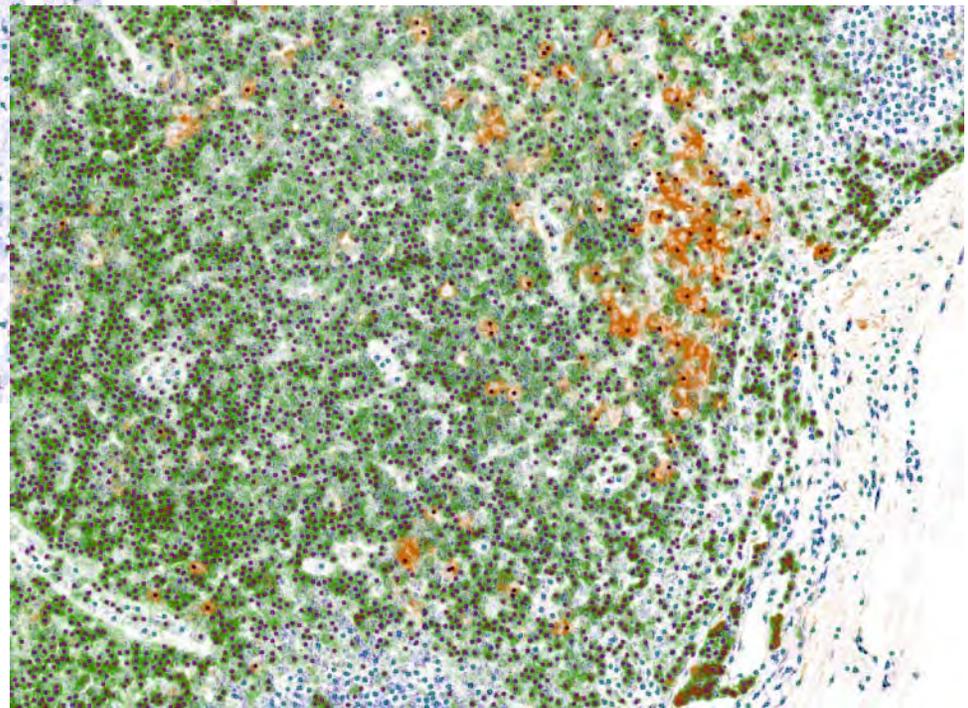- Numerical and spatial changes arise in immune cells in TDLNs
- Some of these changes appear to predict clinical outcome
- Quantitative, spatial analysis tools for histology have been developed for high throughput analysis
- Immune cells in TDLNs provide novel biomarkers for cancer
- Proof-of-concept in breast cancer – extend to melanoma, GI cancer

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The following relationships exist related to this presentation:
None