

# Immune Changes in Tumor- Draining Lymph Nodes as Novel Biomarkers

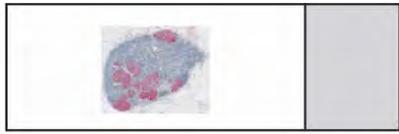
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# TDLNs in Breast Cancer

- 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.



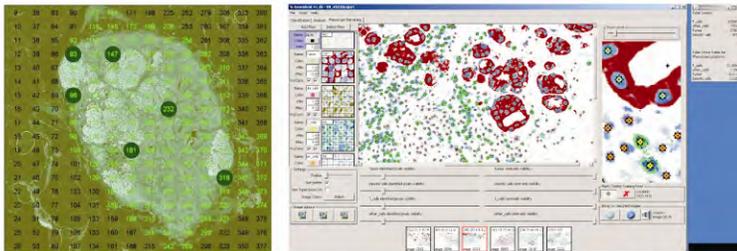
Multicolor staining of tissue sections

2.



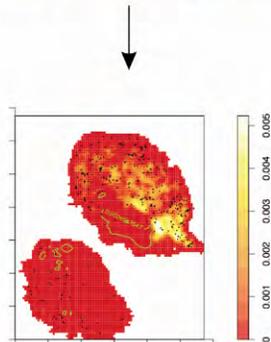
High-res spectral imaging & automated scanning of the whole tissue section

3.



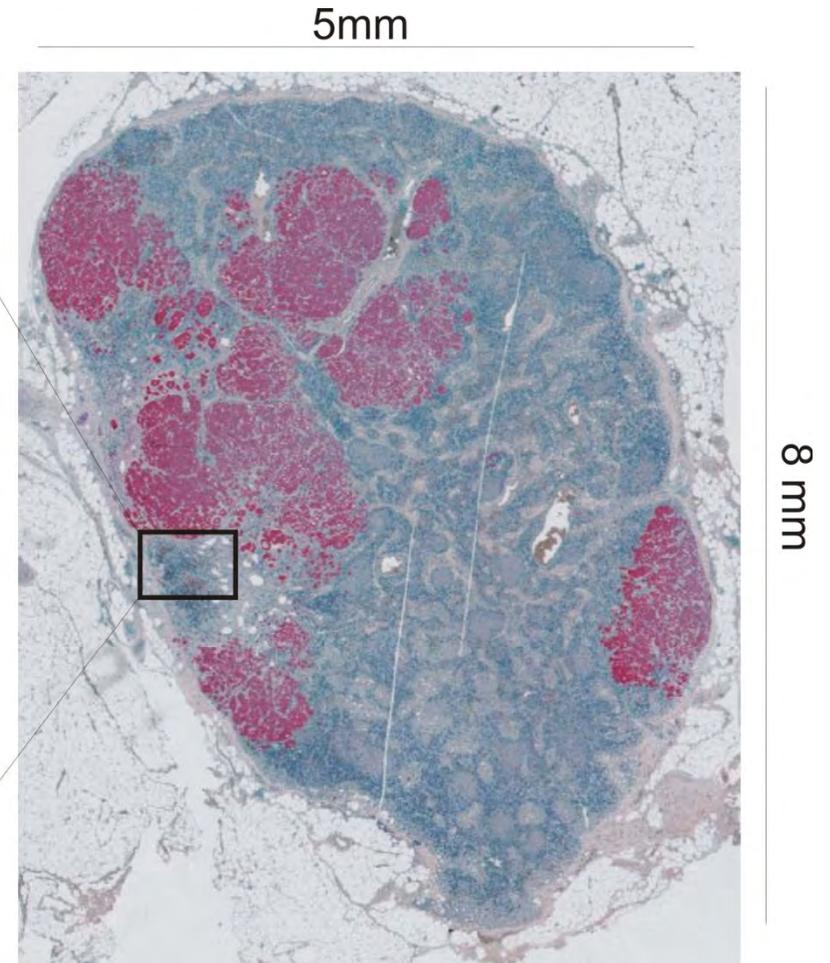
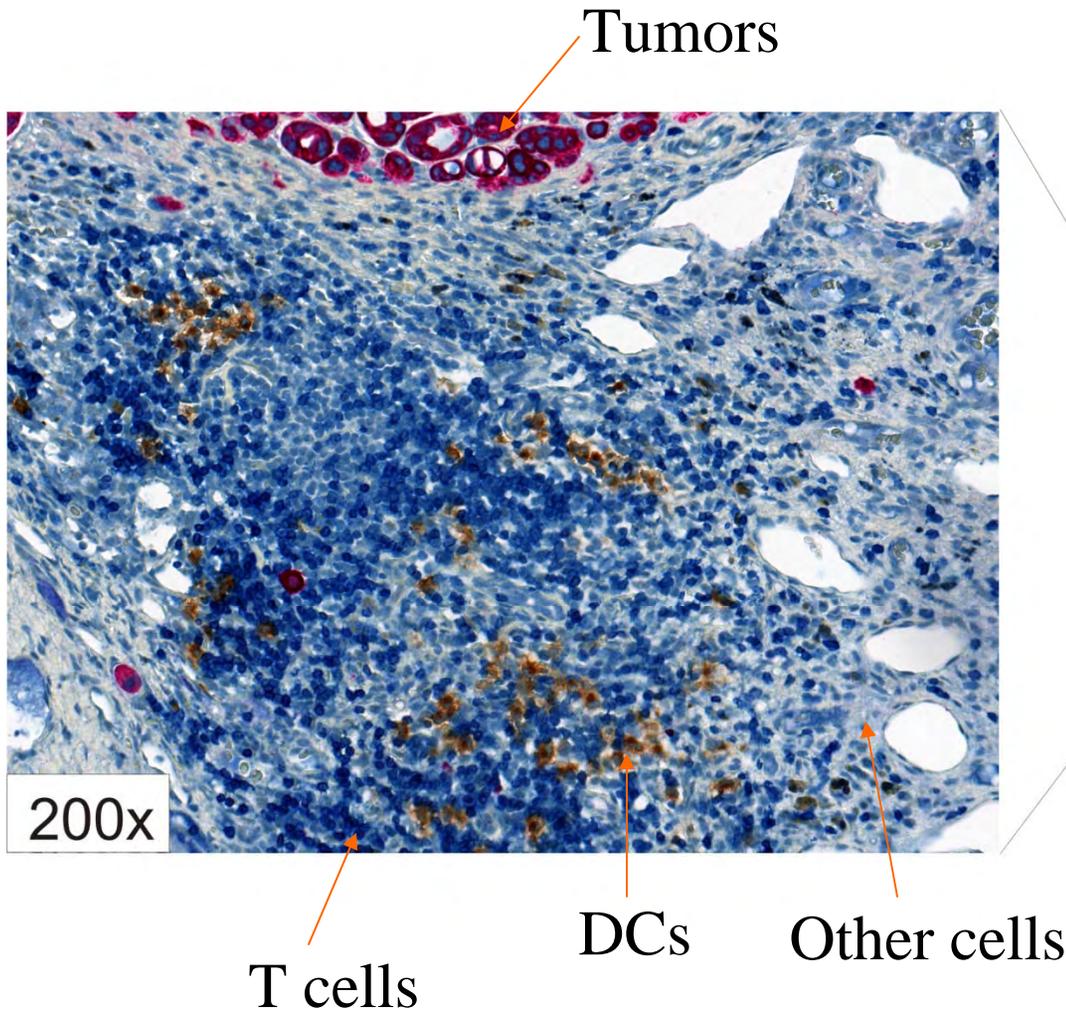
Machine-learning-based cell identification by GemIdent  
→ number of cells and Cartesian coordinates

4.

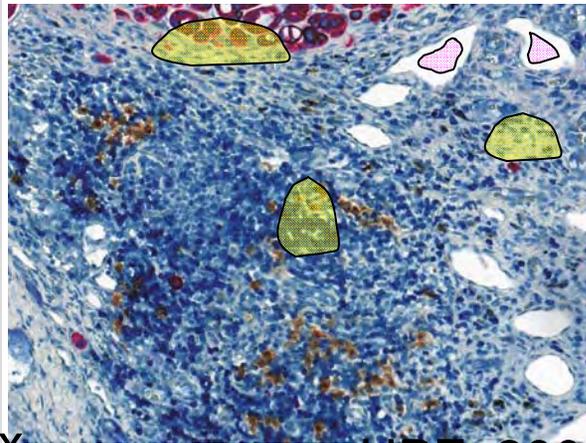


Quantitative and spatial statistical analyses

# Multicolor staining



# Imaging & scanning by Vectra™



## Build a classifier:

enable the program to recognize tissue vs. non-tissue (empty spaces, bubbles, fats, etc).

 = tissue

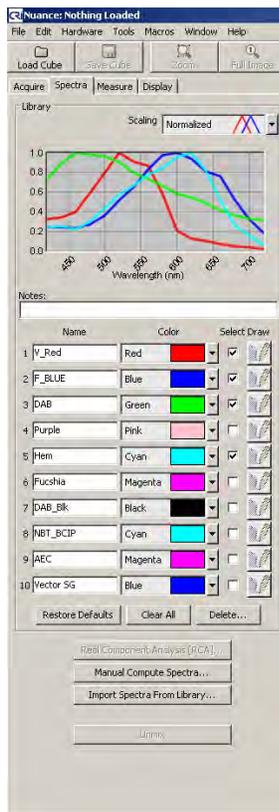
 = non-tissue

## Build a spectral library:

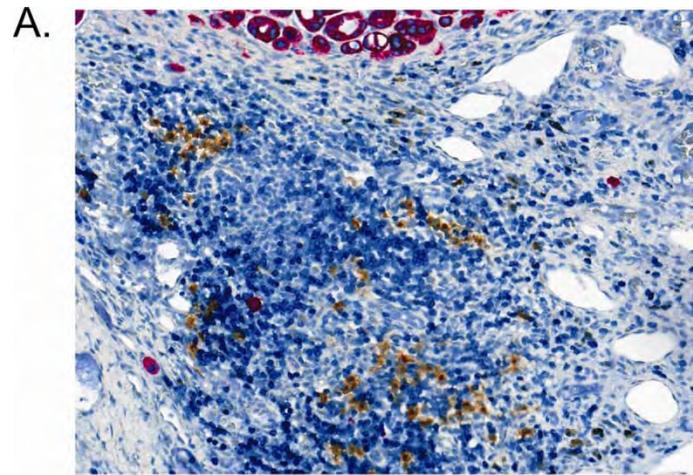
enable the program to unmix signals from different chromogens

## Set up the scan:

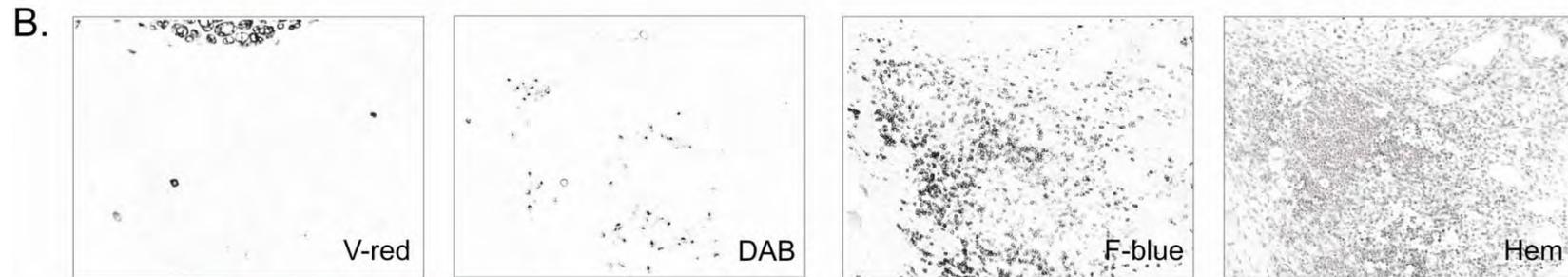
- Organize slides in cassettes
- Set up autofocus
- Take brightfield reference images
- Determine imaging area
- Determine threshold for tissue finding (for both LPF and HPF imaging)



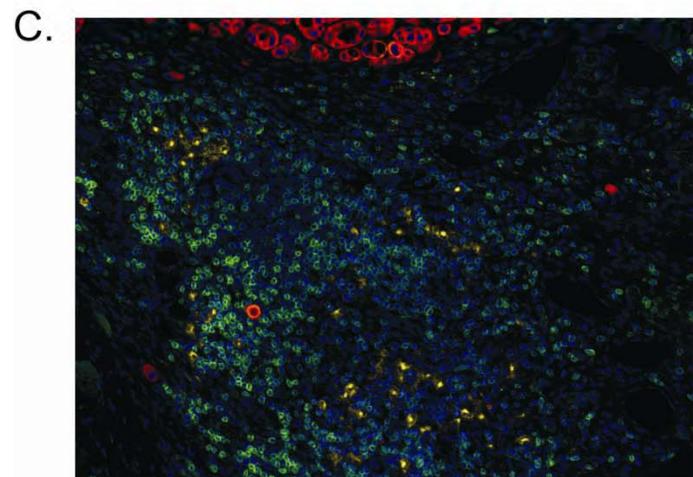
# Chromogen unmixing and image reconstruction by Vectra™



Original image



Unmixed images



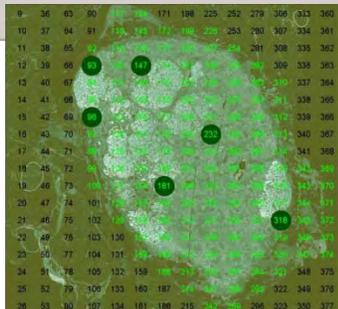
Re-constructed composite image

# GemIdent analysis

The screenshot displays the GemIdent v1.1b software interface. The main window shows a histology image with various cell types identified and colored: Tumor (red), T\_cells (yellow), other\_cells (blue), and Denritic cells (green). The interface includes several control panels:

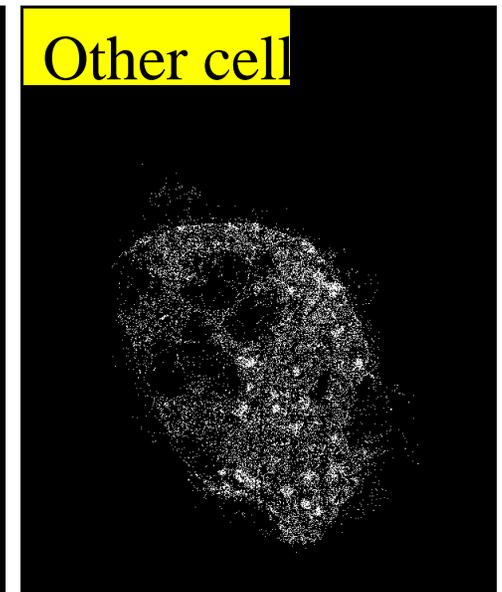
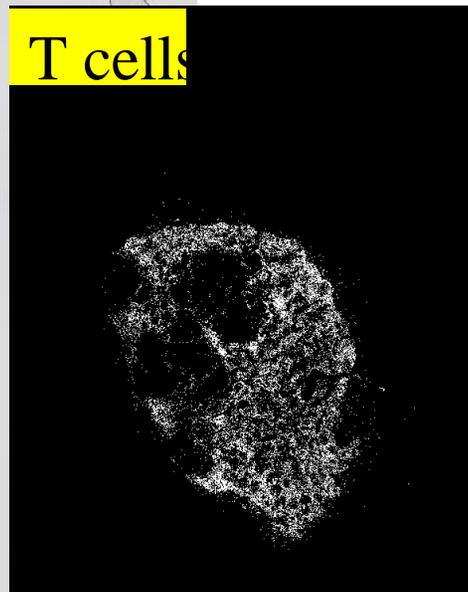
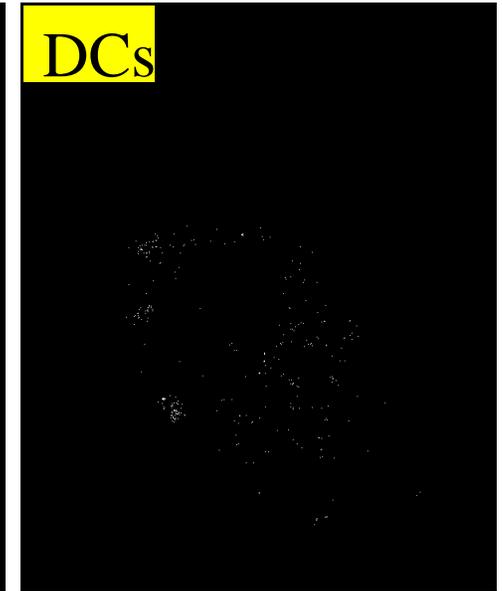
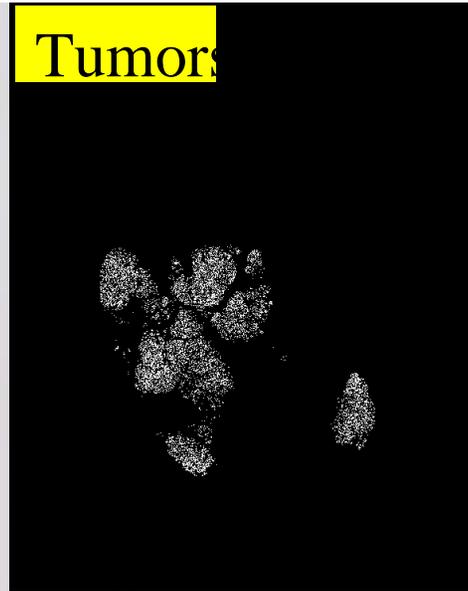
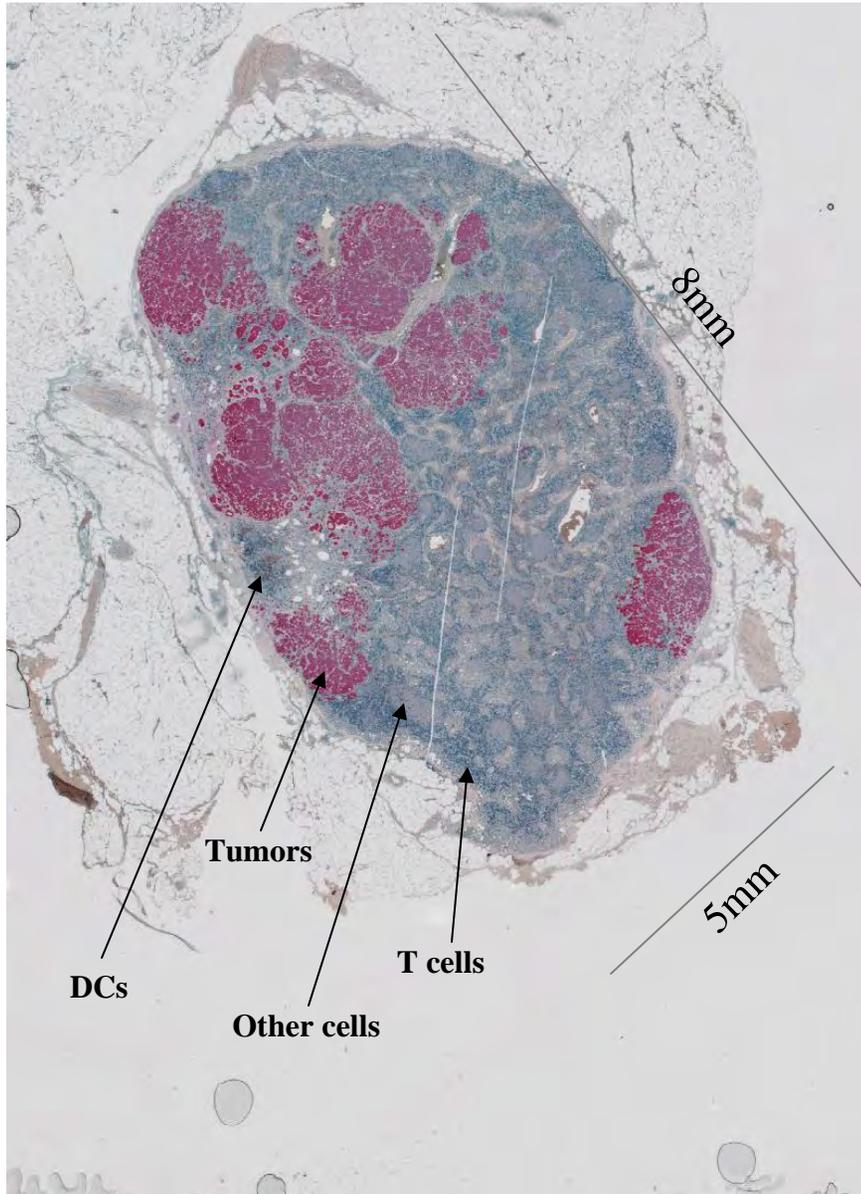
- Classification Panel:** Lists identified phenotypes with their names, counts, and colors.
 

Name	Count	Color
NON	962	Black
Tumor	107	Red
ibc cells	32	Magenta
T_cells	69	Yellow
er_cells	56	Orange
- Settings Panel:** Includes sliders for visibility of identified pixels and centroids for Tumor, Denritic cells, T\_cells, and other\_cells. It also has checkboxes for 'See points' and 'See TypeI Errors (4)', and an 'Image Colors: Adjust' button.
- Image options Panel:** Contains icons for zooming and image navigation.
- Zoom Level:** A slider and a zoomed-in view of a specific region.
- Mark / Delete Training Point:** A panel with a location coordinate (923,410) and a 'Boost on classified Images' section.
- Stage Selection:** A row of six thumbnail images labeled 'stage 0131', 'stage 0096', 'stage 0318', 'stage 0093', 'stage 0147', and 'stage 0232'. The 'stage 0318' thumbnail is highlighted with a red border.



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

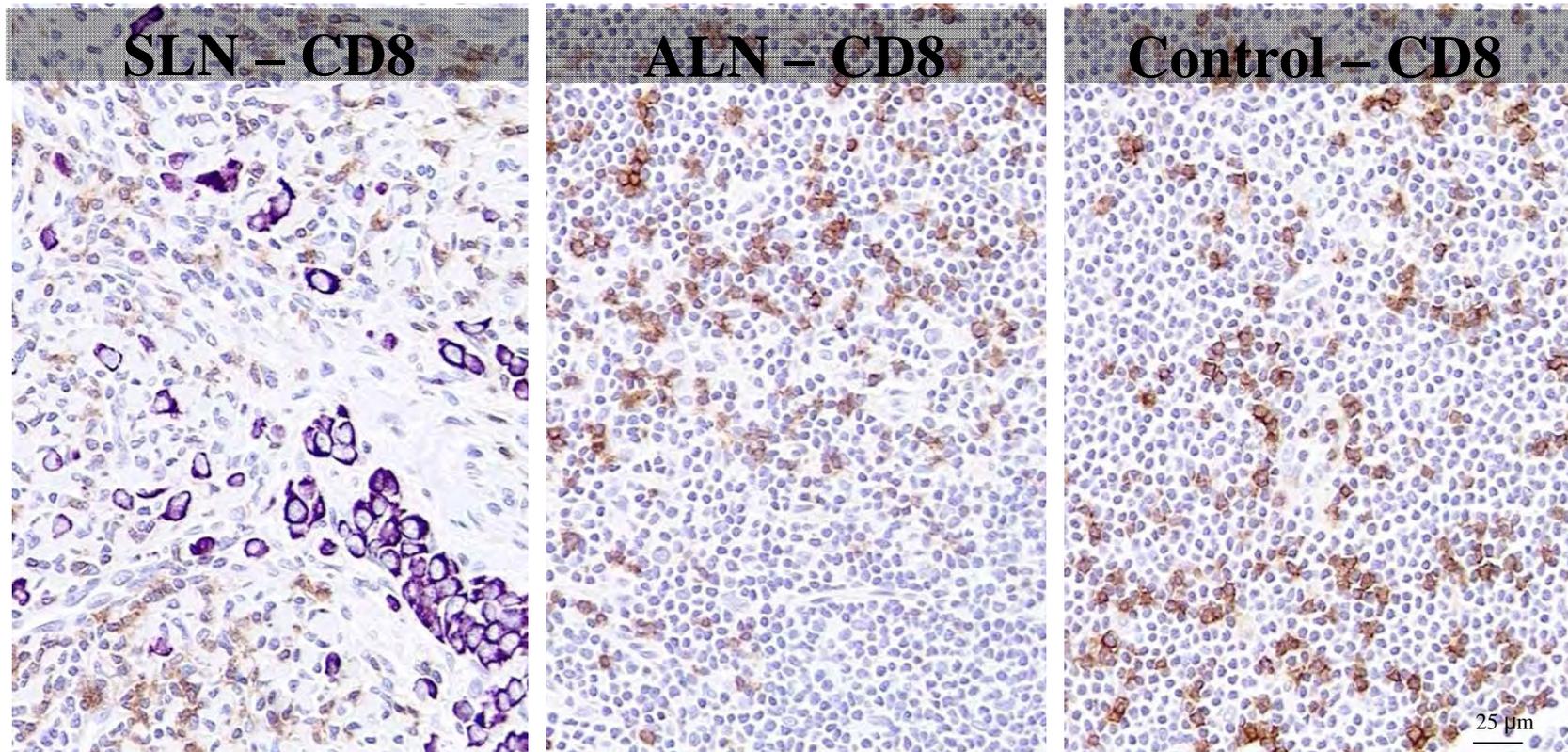


Relationship with >5-year clinical outcome & parameters

## 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

# IHC analysis of TDLN in breast CA



Breast cancer cells:  
AE1/AE3

Immune cells:

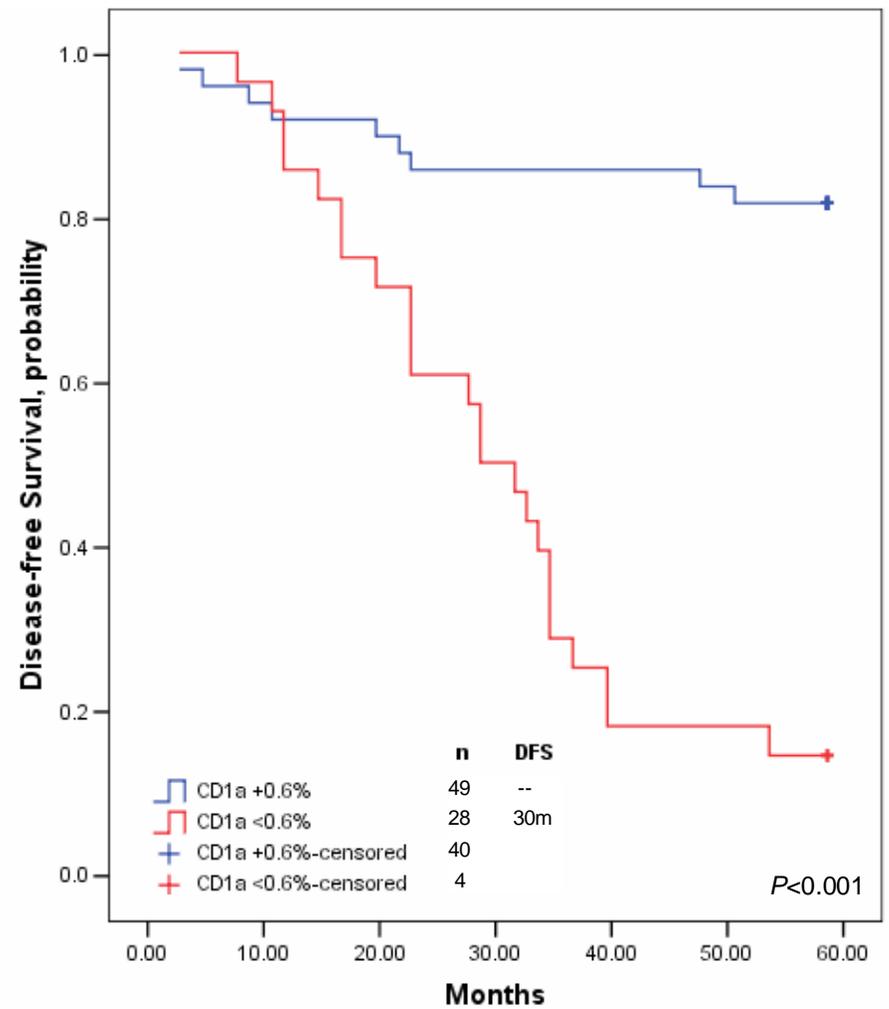
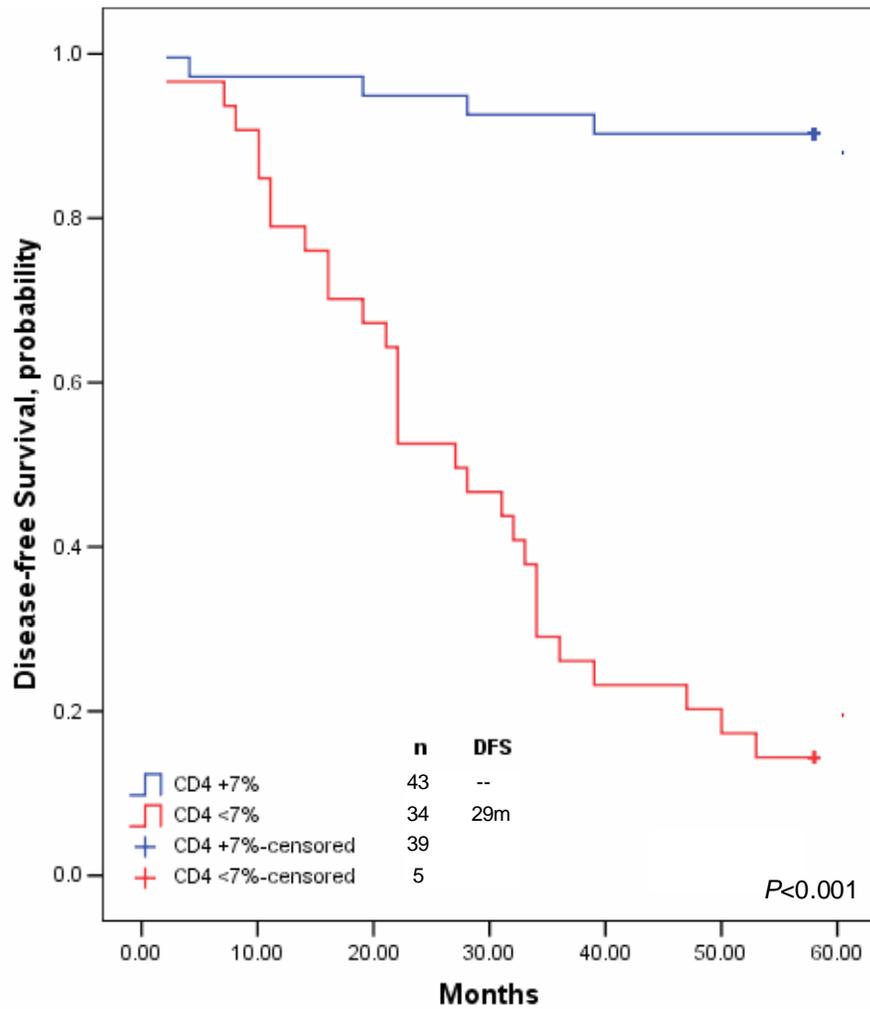
- CD4 'helper' T cells
- CD8 'cytotoxic' T cells
- CD1a dendritic cells - 'antigen presentation'

# Immune Alterations Correlates with Tumor involvement of TDLN

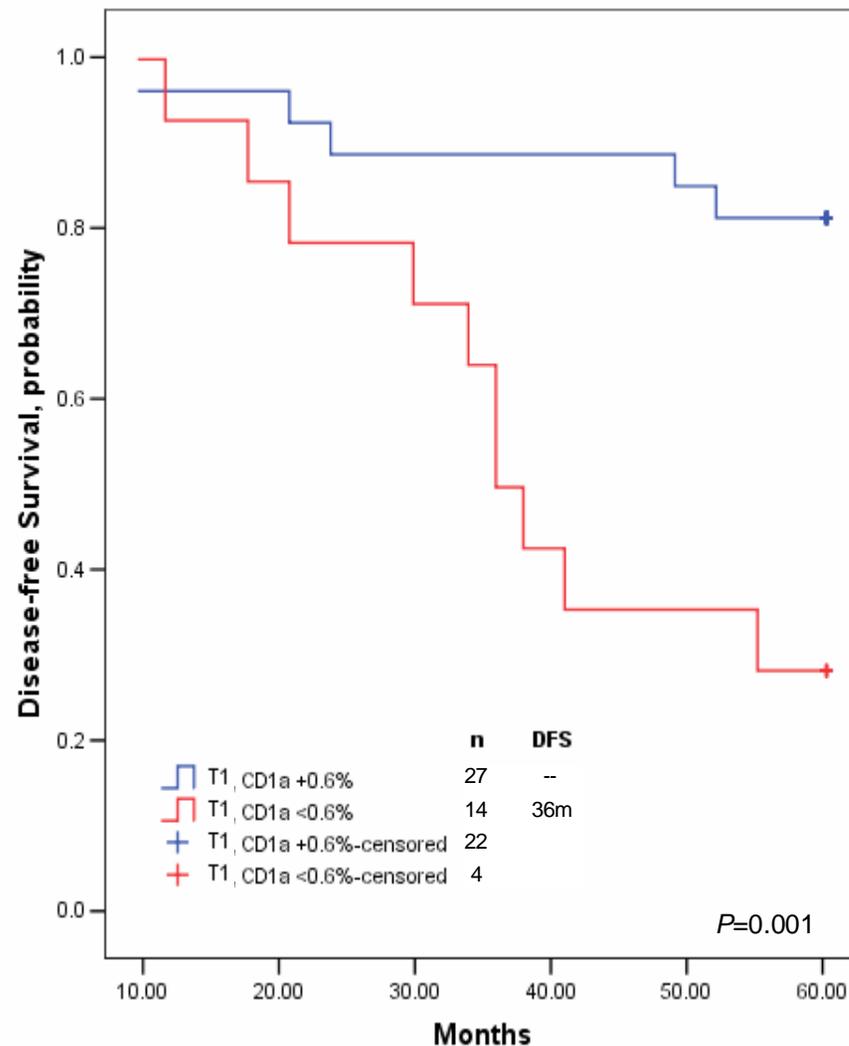
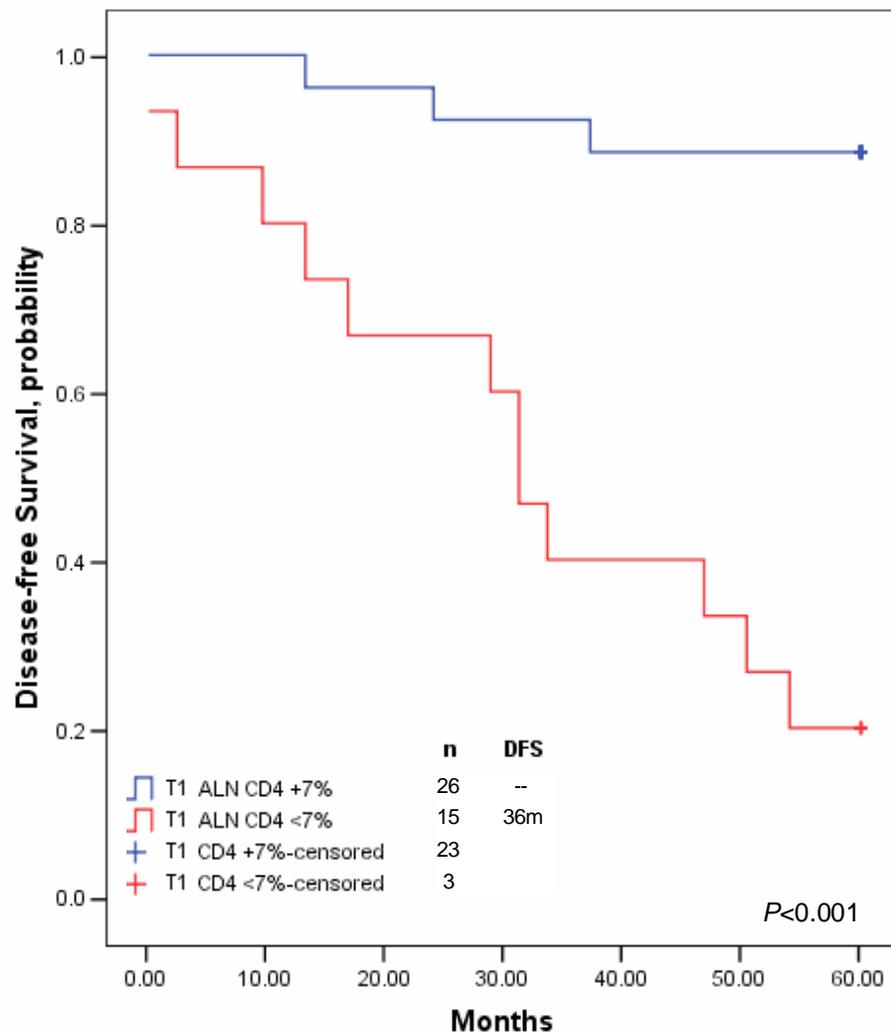
Immune Profile	Tumor-free lymph node % (mean ± SE)	Tumor-involved lymph node % (mean ± SE)	Wilcoxon rank sum test <i>P</i> -value*
<b>Lymph Node Predictors of Lymph Node Metastases</b>			
CD4%	<b>17.85±2.19</b>	<b>2.11±0.35</b>	<b>&lt;0.001</b>
CD8%	7.93±0.99	7.52±0.71	0.682
CD1a%	<b>3.59±0.56</b>	<b>0.26±0.06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

*\*P-values adjusted for multiple comparisons.*

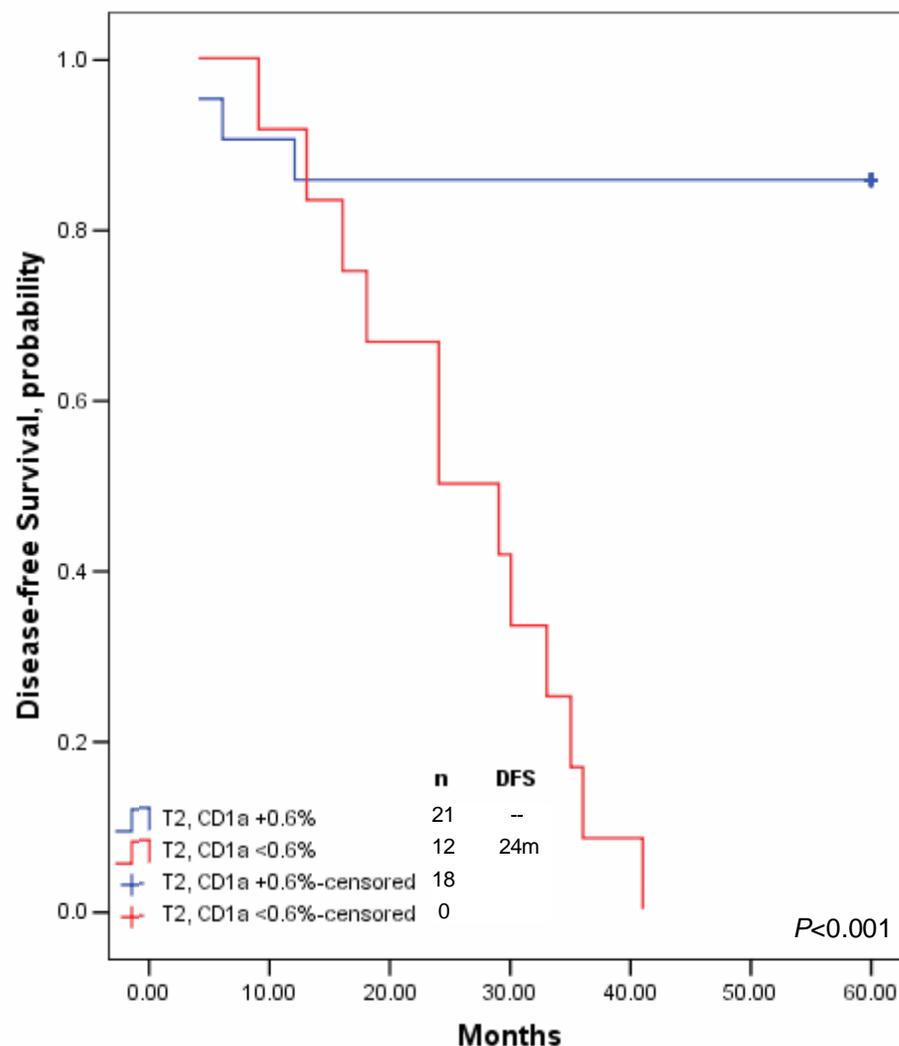
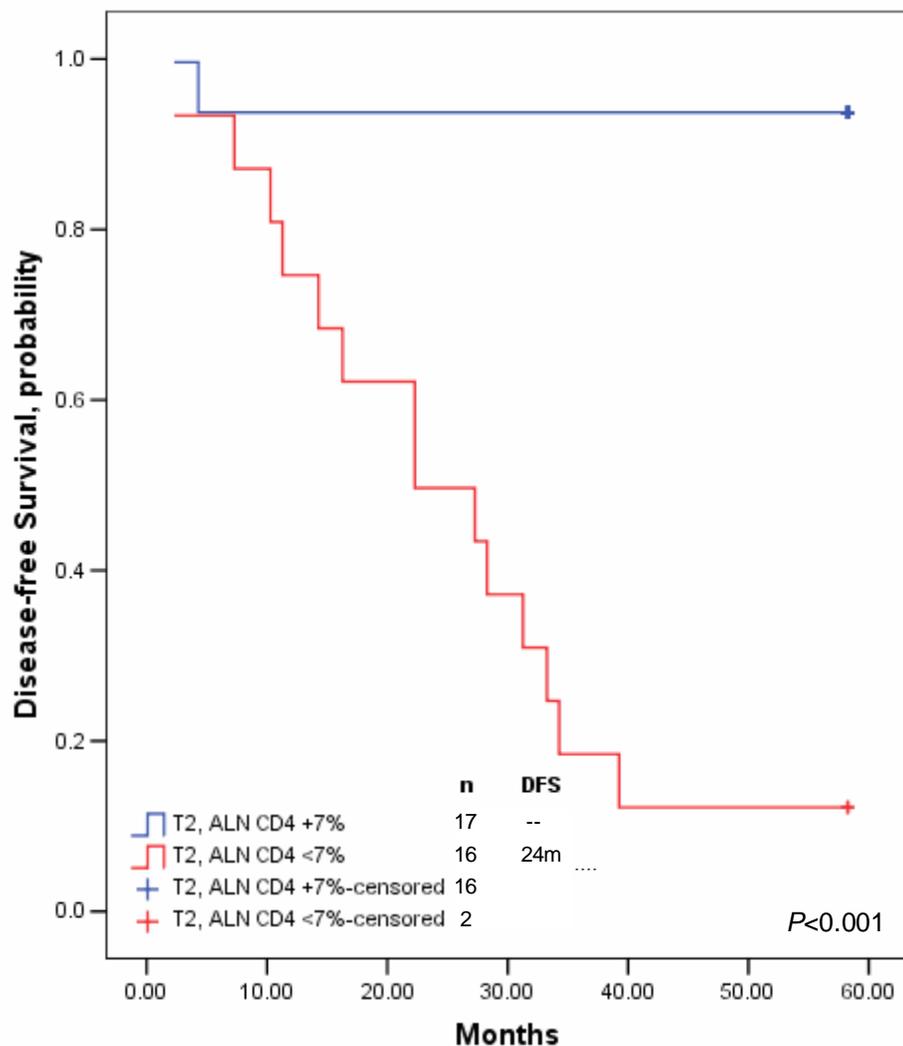
# TDLN Immune Status Correlates with DFS



# DFS Stratified by Tumor Stage (T1) and TDLN Immune Profile

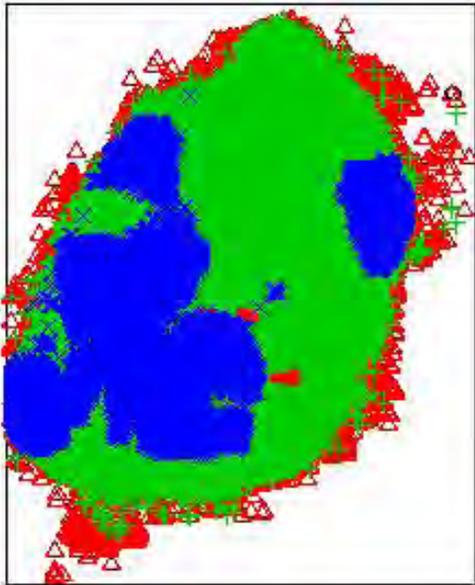


# DFS Stratified by Tumor Stage (T2) and TDLN Immune Profile



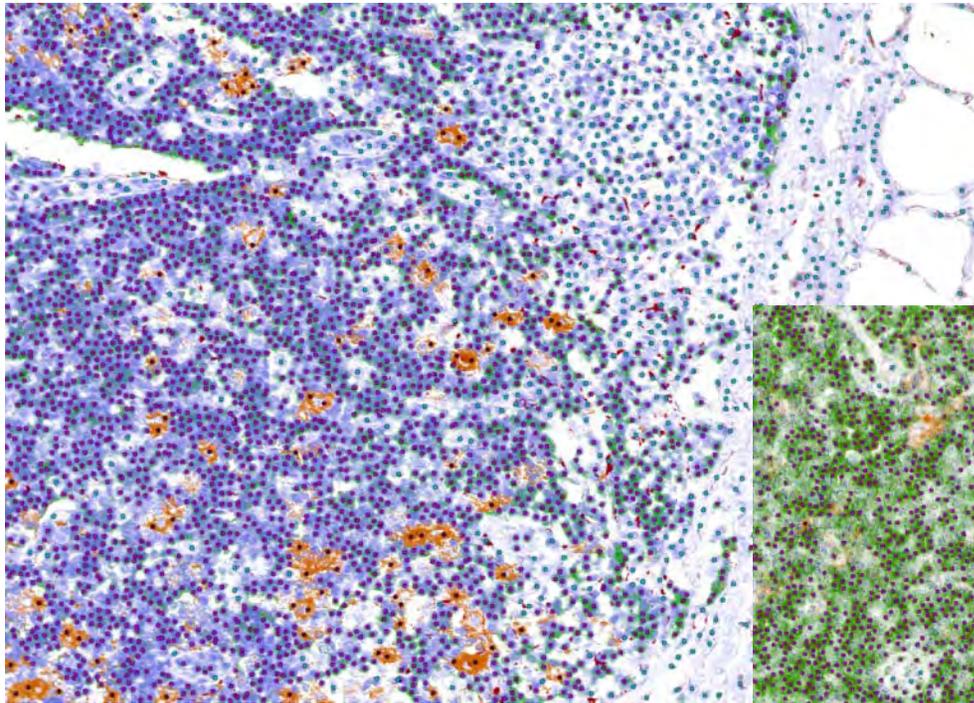
# Beyond numbers: spatial patterns

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



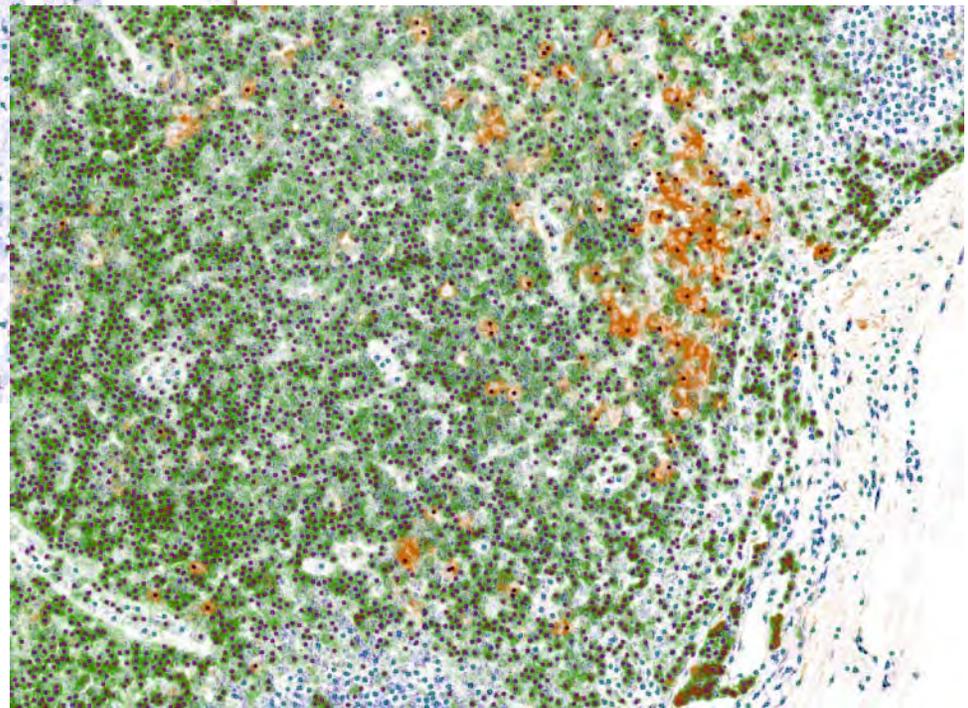
- Different cell phenotypes constitute a “marked point process”
- Goal: to quantify spatial characteristics in order to understand cellular interaction
- Preliminary Findings: T and B cells from TDLN and healthy LN have different spatial distribution patterns [PLoS ONE 5(8):e12420, 2010]

# Dendritic Cell Clustering and Relapse



Relapsed

Disease Free



# 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

# Acknowledgments

Holbrook Kohrt, MD  
Francesca Setiadi, PhD  
Valeria Carcamo-Cavazos  
Adam Kapelner  
Andrew Chang

Fred Dirbas, MD (Surg Onc)  
Erich Schwartz (Pathology)  
Susan Holmes, PhD (Statistics)

Notre Dame  
Danny Chen, PhD (Computer Science)  
Mark Alber, PhD (Math)

The following relationships exist related to this presentation:  
**None**