#### **Tumor Microenvironment**

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### **Tumor Microenvironment**

- It's not just about the cancer cells: importance of the tumor microenvironment
- It's not just about numbers: importance of spatial relationships between cells in tissue
- It's not just about the tumor: importance of tumor-draining lymph nodes (TDLNs)



#### Tumors are more than just cancer cells

- Around 50% of human tumors are >50% stroma
- Patients with 'stroma-rich' tumors have worse clinical outcome, which is an independent prognostic parameter in breast, colorectal, pancreatic, and other cancers
- Stromal cells provide growth and metabolic factors to cancer cells; also shown to protect cancer cells from a variety of anti-cancer drugs
- Intratumoral immune cells strongly shown to predict clinical outcome: Immunoscore



#### **Cancer Progression & Clinical Outcome**

#### **Cancer Cells**



#### 

#### Imaging with Vectra



Multi-modal high thoroughput pattern-recognition based image analysis technology for biomarker quantification and morphometric analysis.



#### Inform analysis

#### inForm Analysis Software Workflows







(green) areas

EXPORT DATA

PREPARE IMAGES: load and unmix

FIND TISSUE: draw tumor (red) and stroma FIND CELLS: based





SCORE: double positive (yellow) for membrane E-cadherin and cytoplasmic vimentin

FIND CELLS: based on counterstair

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Primary breast tumors 40x images

CD3 PanCK DAPI

#### Different immune patterns within tumors



#### **Immune-cancer** separation

Primary Breast tumor 200x image

CD3 CD20 CD123 CD33 CD56 PanCK DAPI



#### Immune-cancer co-mingling

Primary Breast tumor 200x image

CD3 CD20 CD123 CD33 CD56 PanCK DAPI



### Myeloid cells surround cancer cells

Primary Breast tumor 200x image

PanCK CD33 CD3 CD20 DAPI



### Tumor-Draining Lymph Nodes (TDLNs)



injected material is located visually and/or with a device that detects radioactivity (middle panel). The sentinel node(s) (the first lymph node(s) to take up the material) is (are) removed and checked for cancer cells (last panel).

NIH National Cancer Institute

## **TDLNs in Breast Cancer**

- Lymph Nodes are Immune Organs!
- Hypothesis:
  - Cancer alters the immune cells and function of draining lymph nodes
  - Extent of immune alterations in TDLNs may determine clinical outcome
- Questions:
  - Are immune cell populations (T cells, B cells, and dendritic cells) altered in TDLNs?
  - Does TDLN immune profile predict clinical outcome?

# Higher % CD4 T cells and CD1a DCs in TDLNs from Disease-Free vs. Relapsed Breast Cancer Patients



Kohrt et. al. 2005 PLoS Med

#### **TDLN Immune Profile Correlates with DFS**



Figure 3.

#### Anatomical Compartments of the Lymph Node



Janeway, C, et. al. Immunobiology. 6th edition. The Immune System in Health & Disease. Garland Science 2005.

### **Tissue Segmentation**



#### **Regional distributions of FoxP3+ cells within TDLNs**





Extrafollicular Region Foxp3+ Cells per Total T Cells

Follicular Region Foxp3+ Cells per Total T Cells







#### **Dendritic Cell Clustering in TDLNs**



#### Analyzing DC-T cell Interactions



- Step 1: identify DC clusters
- Step 2: for each T cell, look for its nearest DC cluster
- Step 3: if the distance exceeds the threshold, ignore it; otherwise, consider it as clustered

#### Decreased DC %, Maturity, and Clustering in Tumor-Invaded Lymph Nodes



**DC Clustering in TDLNs Correlates with Clinical Outcome** 





### Summary

- Tumors are complex, heterogeneous collections of cancer, stromal, and immune cells.
- Reciprocal interplay between these cell populations drive progression, metastasis, response to therapy, and clinical outcome.
- Numerical and spatial relationships between immune cells are altered within tumors and TDLNs that correlate with clinical outcome in cancer.
- Quantitative, spatial image analysis of tumors and TDLNs will be highly informative in understanding how therapies modulate the balance between cancer and host immune responses.

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In memory of Holbrook Kohrt, MD, PhD

