Surgical Pathology of SNB:

Comparator Test for Veridex GeneSearch™ BLN

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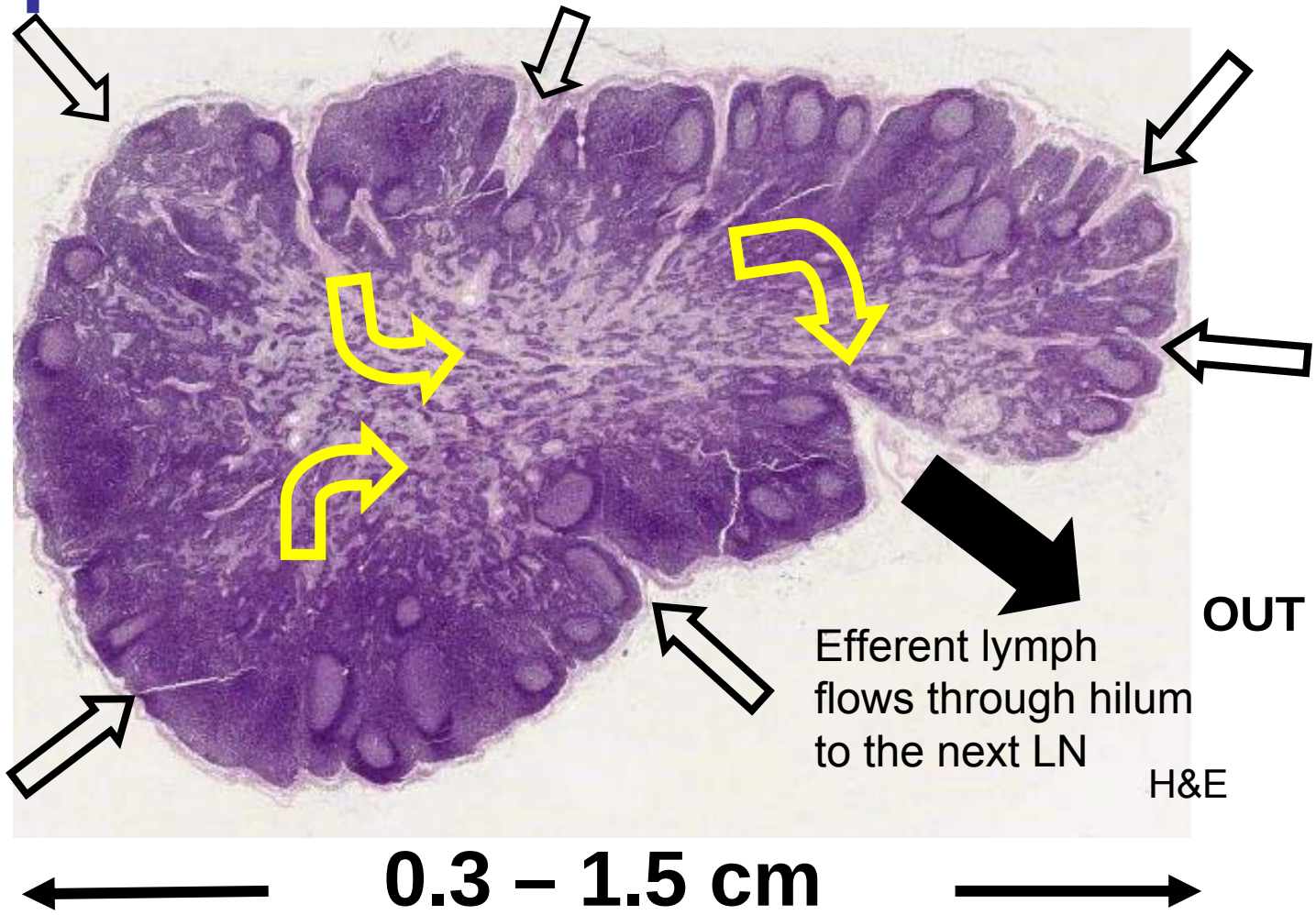
Sentinel Lymph Node: Circulation of Lymph Fluid from Outside into Node



Typical Lymph Node as found in the Axilla

Veridex Trial Comparator Dx = Clinical Sites' Existing Clinical & Surgical Pathology Practices

- FDA does ***not*** endorse any practice guideline
- We will use a 2005 multidisciplinary, evidence-based consensus guideline as **an example of current practices**
- *American Society for Clinical Oncology (**ASCO**) Guideline Recommendations for Sentinel Lymph Node Biopsy in Early Stage Breast Cancer 2005 JCO 2005;23(30):7703-20.*
  - ASCO incorporates CAP, ADASP, & NCCN (NIH) recommendations

American Society of Clinical Oncology Practice Guidelines 2005

- "***Each*** institution must establish a policy on intraoperative assessment or deferral to permanent sections" (no one size fits all…)
- "SNB procedure is very much a team effort with active skilled involvement of ***multiple disciplines*** including surgery, pathology, radiology, nuclear medicine, nursing and pharmacy among others."

➢ One must understand the strengths & limitations of each diagnostic method

Directions for Pathologist

- All submitted nodes should be
  - Counted and measured
  - Note coloration (blue dye)
  - Record the relative radioactivity uptake reported by the surgeon

ASCO Practice Guidelines for SNB 2005

Pathologic Evaluation of Sentinel Lymph Nodes (SLNs)

- Pathologists systematically ***quantify*** and characterize the tumor burden in each SLN
- Pathologic examination of axillary LNs is requirement for consistent, categoric reporting using the **AJCC/UICC TNM cancer staging system**
  - **"Gold Standard" for axillary lymph nodes is the complete ALND by permanent section H&E**
  - **N** of T**N**M requires resection & exam of at least the low axillary LNs (6 or more LNs)
    - If < 6 LNs = pN0
  - Sentinel LN = pNx(sn)

Am Society of Clinical Oncology Practice Guidelines 2005

Macrometastases [pN1 or >]

- Macrometastases ≥ **2 mm**
- Usually show histologic evidence of metastatic activity such as:
  - Proliferation
  - Stromal reaction
  - Penetration of vascular or lymphatic sinus walls
- **"If any node metastasis is larger than 2.0 mm, the total number of tumor positive nodes determines the N category"**

Micrometastases [pN1(mi)]

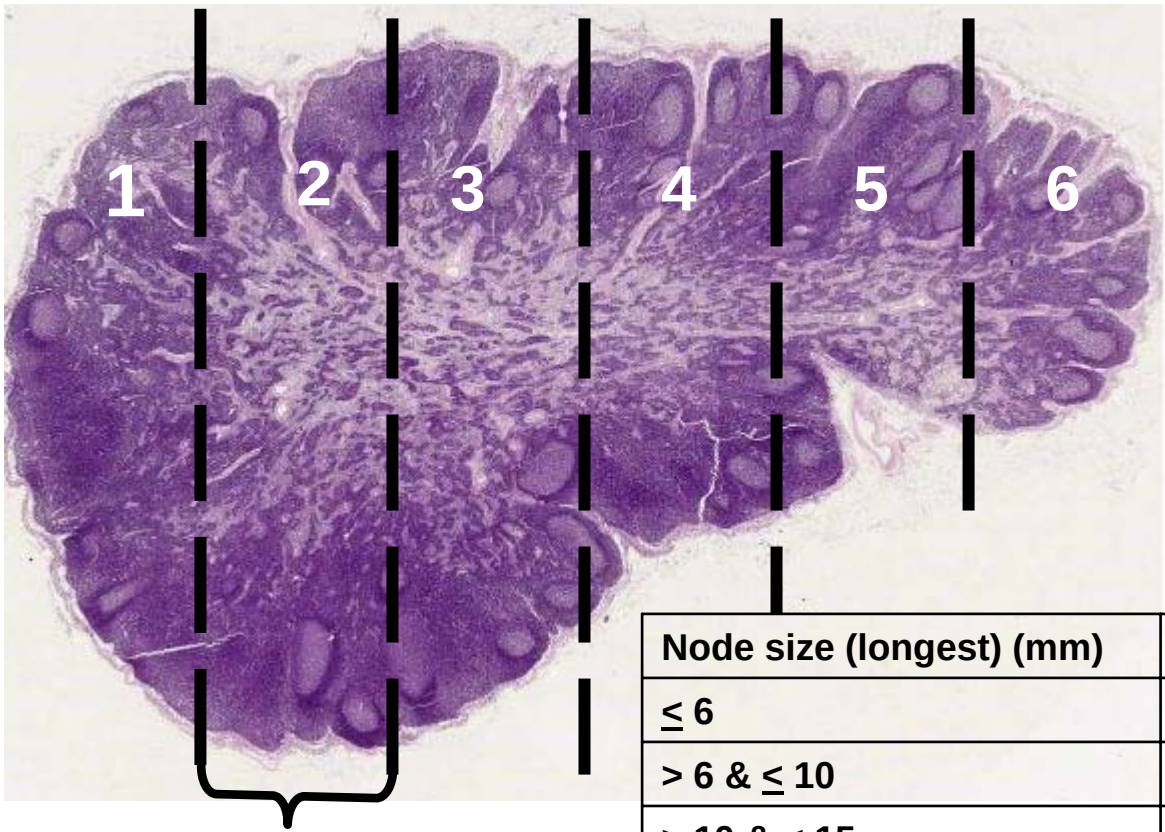
- **Micrometastases** = metastases **>0.2 mm** to **<2.0 mm**)
  - The lower limit accommodates the frequency of small tumor deposits identified in SLNs
  - **Micrometastases** are classified as pN1(mi)

Isolated Tumor Cells (ITC) [pN1(i)]

- Single tumor cells or small clusters of cells **< 0.2 mm** in greatest dimension
  - Usually detected by IHC or molecular methods, but may be verified on H&E (pN1(i))
- Do **NOT** show evidence of metastatic activity
- ITCs may be diffuse, multifocal, and single foci

AJCC/UICC TNM System for reporting SNB for Staging

Veridex Sectioning Plan for Sharing Alternating Slabs of SLN for Histology & BLN Test



Macrometastases > 2 mm diameter

1.5- 3 mm slabs

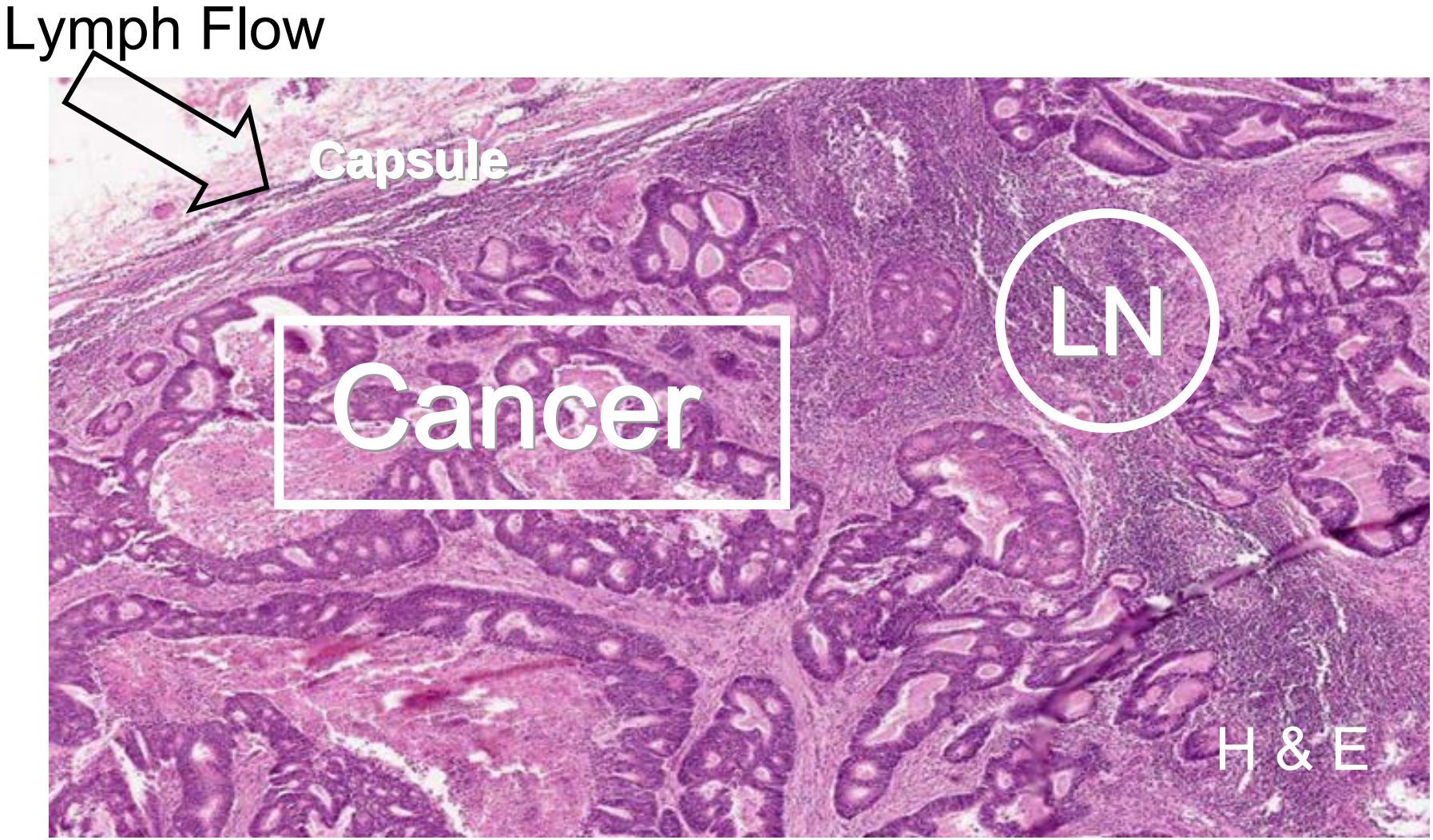
ASCO = 2 mm slabs

Node size (longest) (mm)	Total # slabs
≤ 6	2
> 6 & ≤ 10	4
> 10 & ≤ 15	6
> 15 & ≤ 20	8
> 20	10 or more

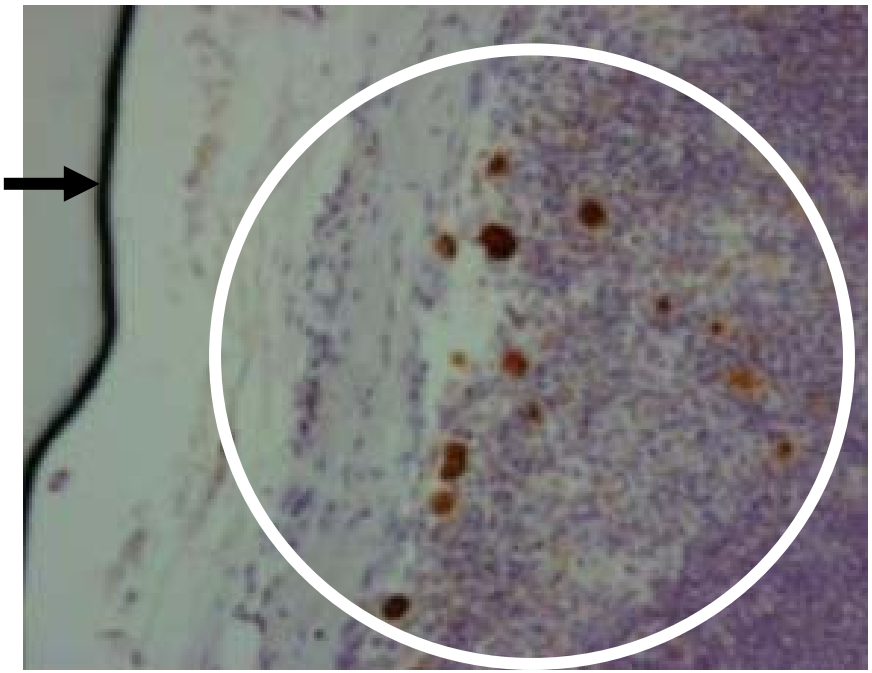
Protocol for Limited Step-Sectioning Sampling of Paraffin Block (permanent sections)

- Step-sections cut from the block (top level plus one or two sections at 200 to 500-µm intervals into the block) will detect:
- Virtually all ***macrometastases***
- Most ***micrometastases*** (>0.2 mm to 2.0 mm)
- In some patients, ***isolated tumor cells*** and clusters (<0.2 mm), particularly if IHC is utilized
- More yield than superficial serial sections that limit sampling to the upper levels of the block

Macrometastases in LN



Sentinel LN: Isolated Tumor Cells (ITCs) Immunohistochemistry



Approx. 15 tumor cells stained brown by IHC to cytokeratin

One node may have multiple collections of ITCs

Choices for Intra-operative Examination

1. Gross inspection of the cut faces of the node
2. Cytology of node imprints or cell smears
3. Frozen section histopathology

- Permanent section histopathology = Definitive pathologic diagnosis
- "Evaluation of the SLN is likely to be more accurate on the basis of paraffin sections"

ASCO Practice Guidelines for SNB 2005

Intra-operative Expected Results: As of 2005 Current Practice Guideline

- About 75% of pts considered for sentinel lymph node biopsy (SNB) have ***tumor free*** lymph nodes in permanent sections
- In the 25% of pts with positive nodes confirmed by permanent sections, some disease may **NOT** be detected intraoperatively because of intentional limits to FS sampling & the challenge of detecting micrometastases
- For every 100 patients to have SNB evaluation **intraoperatively**, 16 to 17 will have positive nodes and 8 to 9 will have **false negative** results (compared to the permanent sections)

Frozen Sections

- FS most desirable intraoperative assessment for ***some*** surgeon/pathologist teams
- More sensitive than cytology
- Quality of FS preparations is seldom as good as well-fixed tissue (FFPE) “permanent” sections
  - **Microscopic features** not as detailed
  - **Thorough sectioning** vs. risk significant **destruction** of potentially diagnostic tissue
  - Incomplete sections may **miss** the subcapsular area
  - Prior **freezing** may compromise the quality of paraffin section histology

Frozen Section Reporting

- **Negative or suspicious frozen section findings should be reported as "not diagnostic for tumor and deferred to paraffin section".**

ASCO Practice Guidelines for SNB 2005

IHC Analysis: Recommendations as of 2005

- **Insufficient evidence** to recommend that IHC to cytokeratin be performed routinely (for detection of micrometastases)
- Patient should be informed of the **uncertain significance** of any positive results, e.g., isolated tumor cells or micrometastases

ASCO Practice Guidelines for SNB 2005

Molecular Approaches: Recommendations as of 2005

- Highly sensitive, may permit evaluation of relatively large amounts of tissue
- Remains investigational
  - **Tissues examined are destroyed**, making it not possible to identify the cells that were the source of the augmented signal for tumor marker mRNA (micrometastases, ITCs vs macrometastases?)
  - Tissue potentially required for histologic diagnosis should **not** be utilized for investigational purposes until the diagnosis is secure.

ASCO Practice Guidelines for SNB 2005