

Assessment of Stromal Tumor Infiltrating Lymphocytes

Continuing Medical Education Activity

Course Structure

Part 1

- Introduction to stromal tumor infiltrating lymphocytes (sTILs)

Part 2

- Methodology for assessing density of stromal tumor infiltrating lymphocytes (sTILs) in breast cancer

Part 3

- Pitfalls and challenges in assessing sTILs

Part 4

- Independent reading

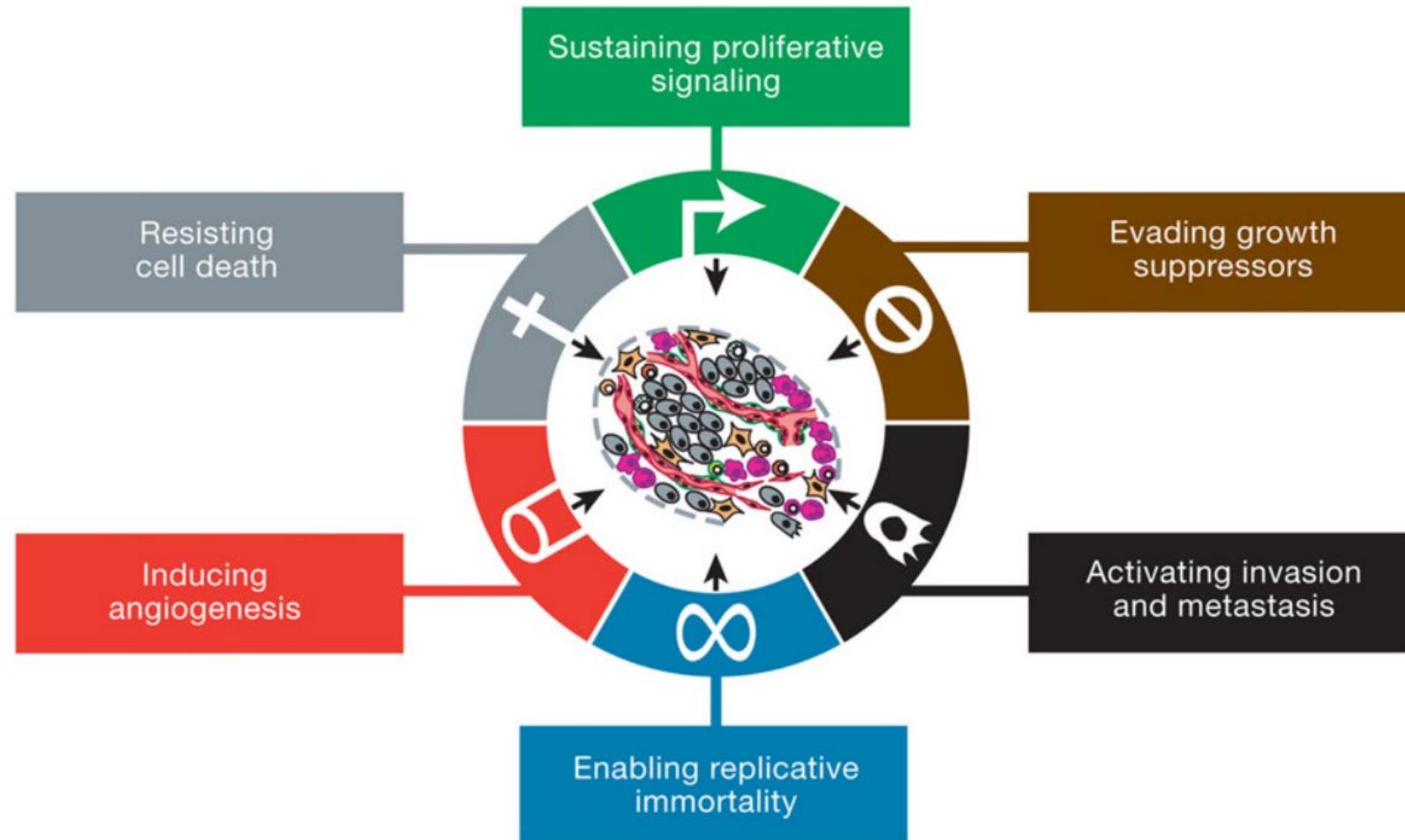
Part 1: Introduction to Stromal Tumor Infiltrating Lymphocytes

Learning Objectives:

- Role of immune cells in cancer
- Stromal Tumor Infiltrating Lymphocytes (sTILs) as a Prognostic Biomarker

Role of Immune Cells in Cancer

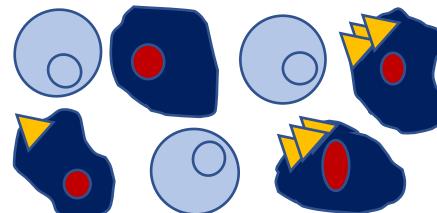
Hallmarks of cancer cells



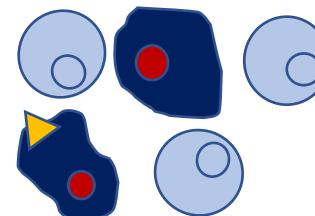


Immune System functions in Cancer

Precursors to malignancy



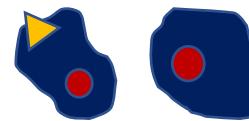
Elimination



Immunosurveillance

Cancer cell precursors are eliminated

Malignancy established



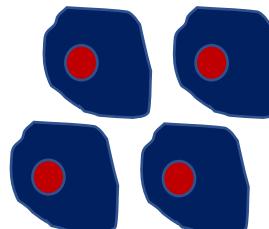
Equilibrium



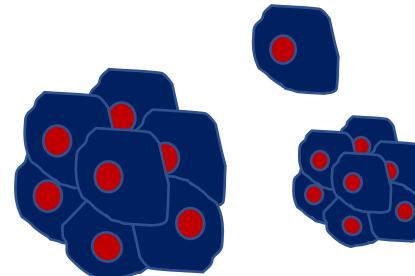
Immunoselection

Less immunogenic variants avoid being detected and survive

Malignancy grows



Escape

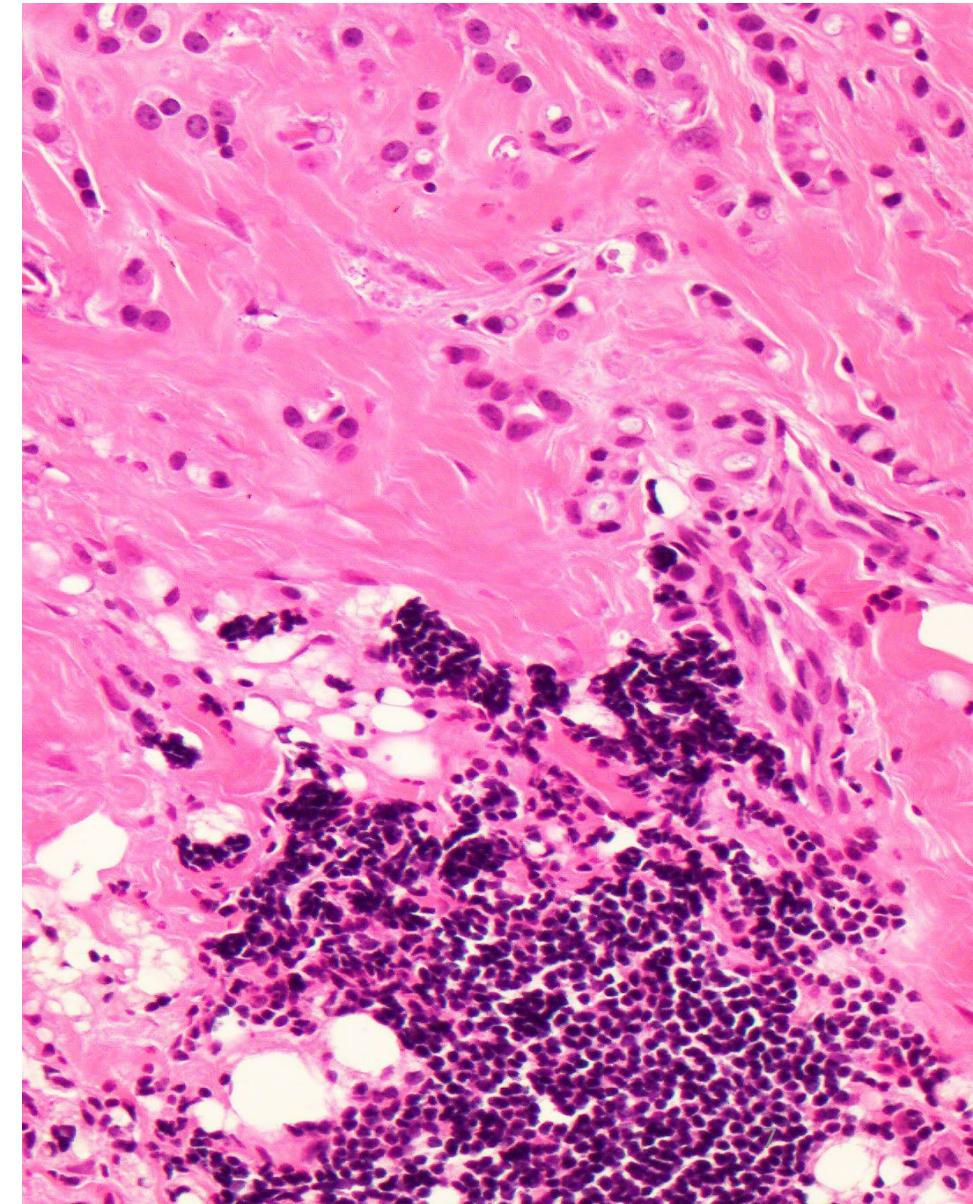


Immunosubversion

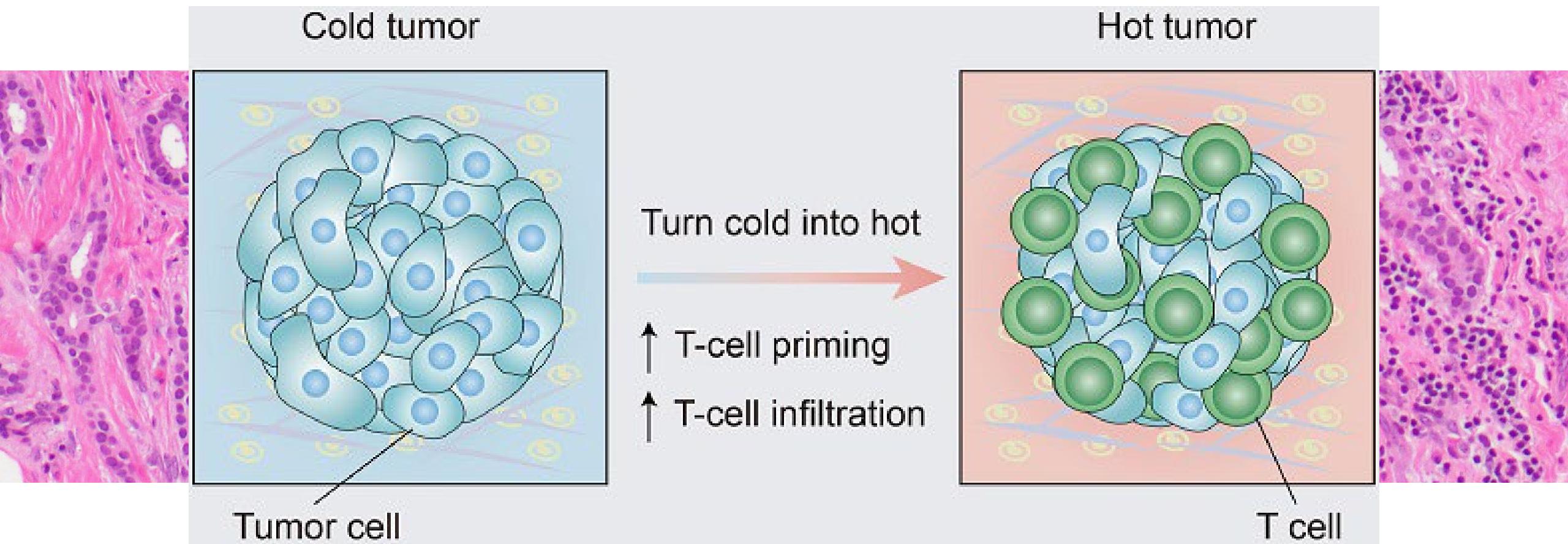
Malignant cells actively evade immune system

The “Immune Contexture”

- Immune cells play an important role in regulating cancer development
- The density, location and organization of the immune cells/tumor infiltrating lymphocytes (TILs) around cancer → **immune contexture**
 - Fridman et al., 2012; Bruni et al., 2020



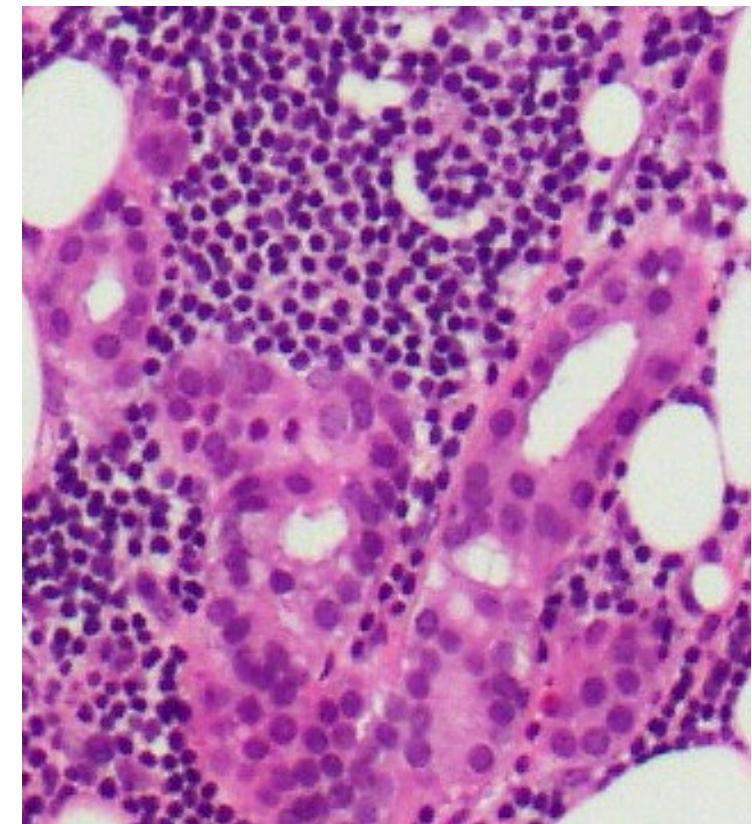
The Immune Response in Cancer



Liu YT, Sun ZJ. Turning cold tumors into hot tumors by improving T-cell infiltration. *Theranostics* 2021; 11(11):5365-5386. doi:10.7150/thno.58390. Available from <https://www.thno.org/v11p5365.htm> as an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>).

Tumor Infiltrating Lymphocytes (TILs) in Cancer

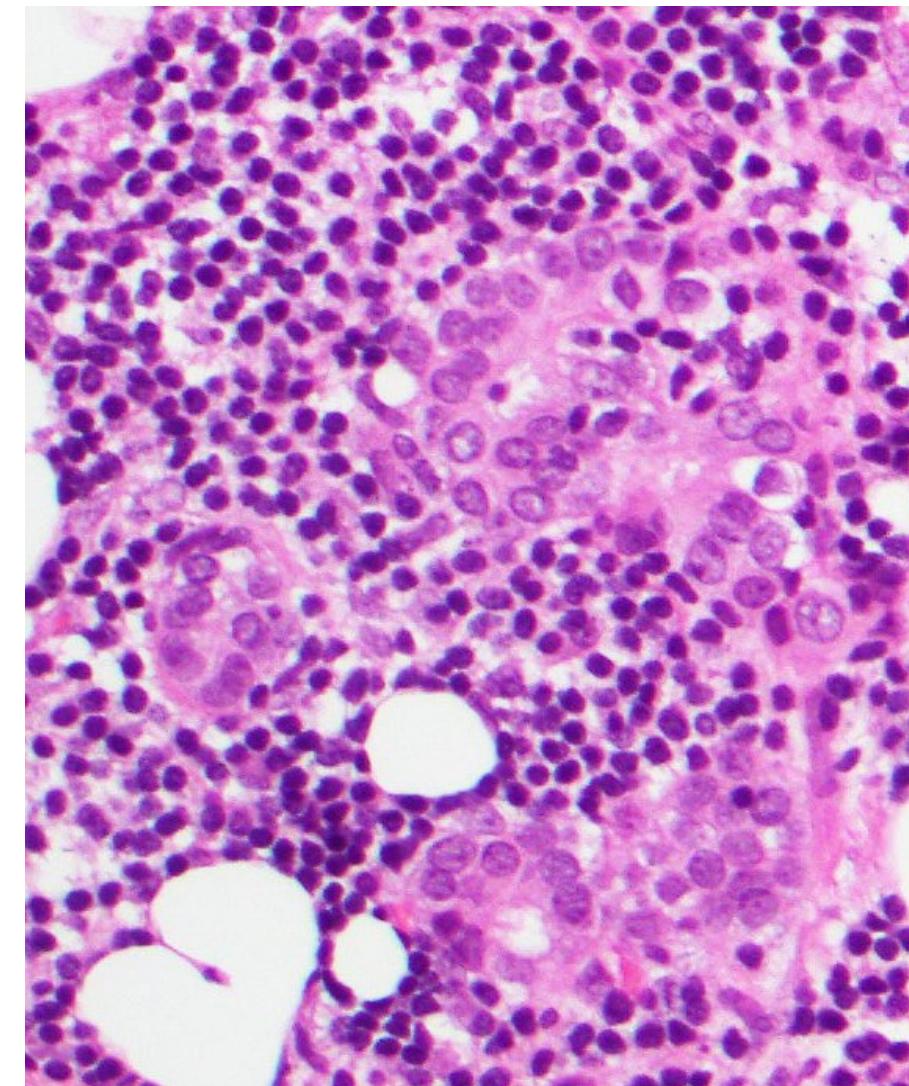
- TILs evaluation is useful in multiple solid tumors as a prognostic marker
- Frequency of TILs correlates with clinical outcomes



Hendry, et al. Assessing Tumor-Infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in Melanoma, Gastrointestinal Tract Carcinomas, Non-Small Cell Lung Carcinoma and Mesothelioma, Endometrial and Ovarian Carcinomas, Squamous Cell Carcinoma of the Head and Neck, Genitourinary Carcinomas, and Primary Brain Tumors. *Adv Anat Pathol.* 2017 Nov;24(6):311-335. doi: 10.1097/PAP.0000000000000161.

Tumor Infiltrating Lymphocytes in Cancer

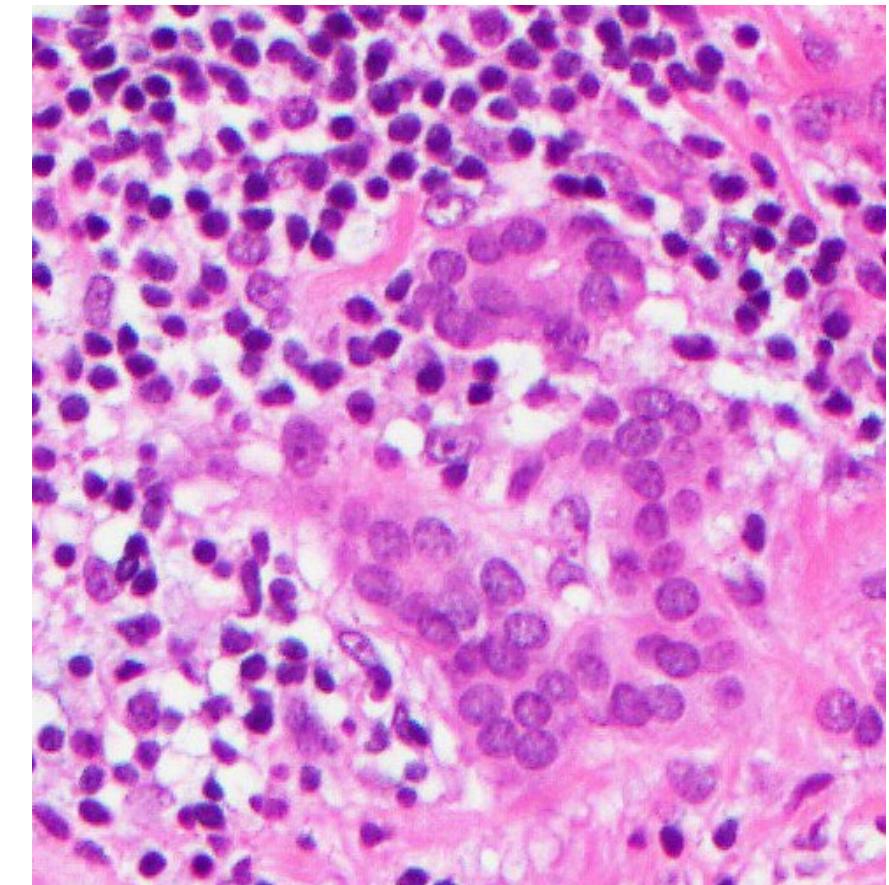
- TILs may be assessed in both intra-epithelial and stromal tumor compartments.
- Methodology for TIL assessment varies by tumor type



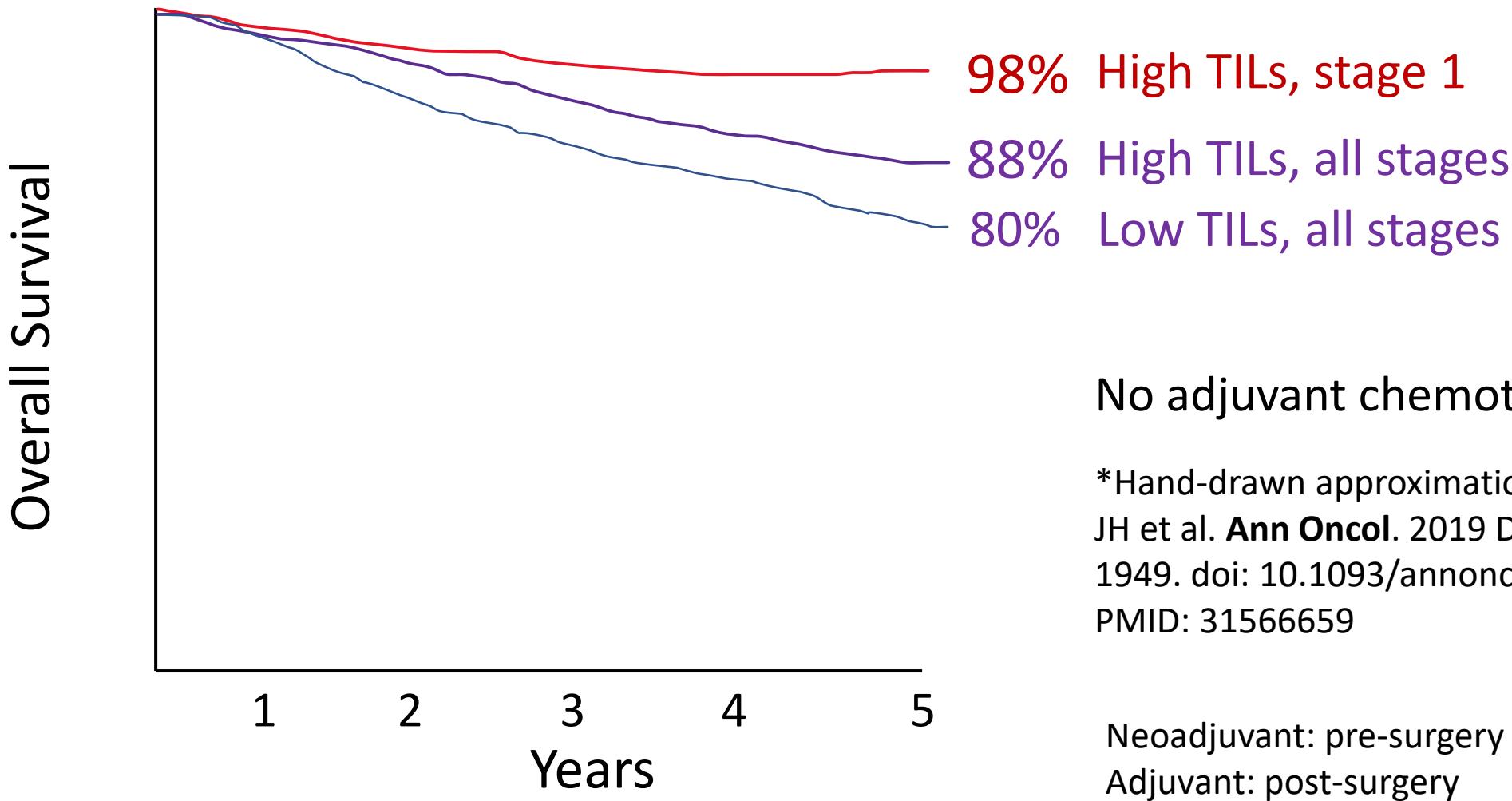
Stromal Tumor Infiltrating
Lymphocytes (sTILs) are a Prognostic
Biomarker

TILs Assessment in Breast Carcinoma

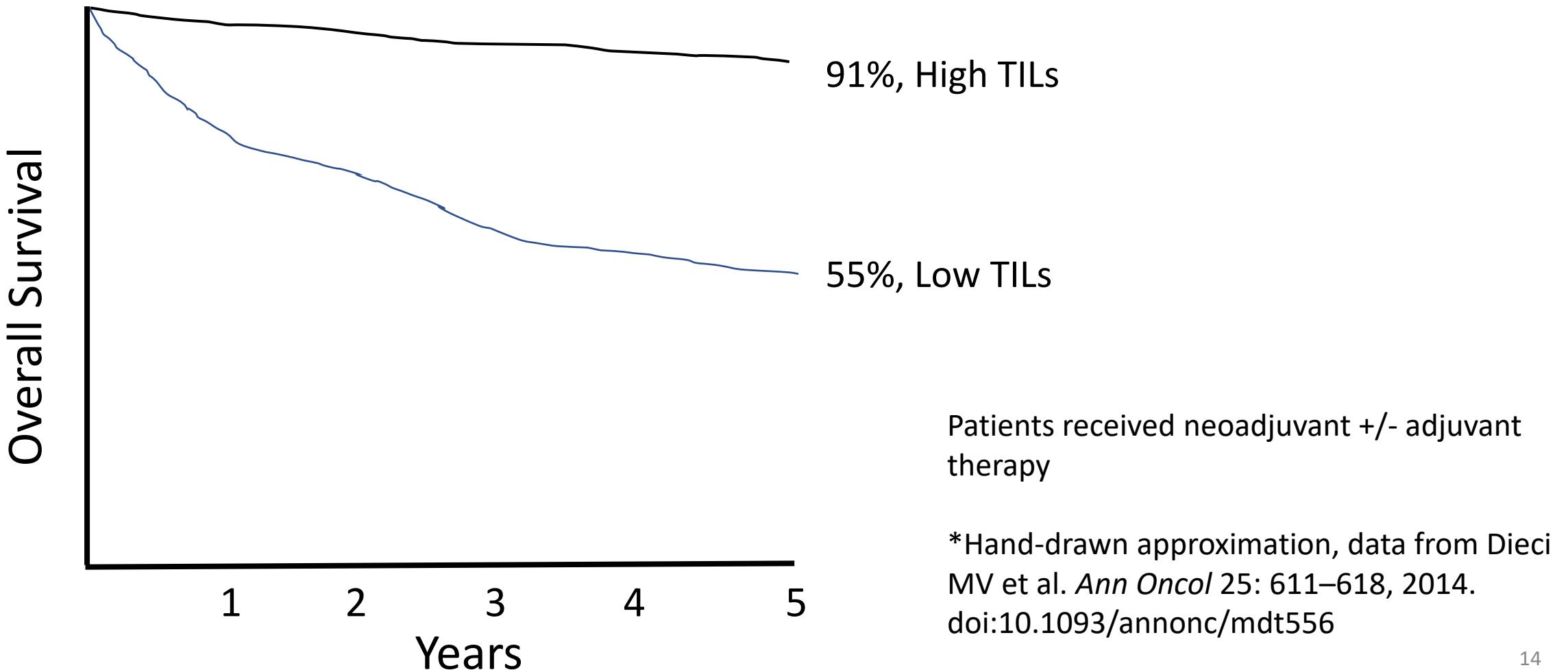
- Stromal TILs are preferred over intra-epithelial TILs for evaluation
 - Typically more numerous and less geographically heterogeneous
 - More reproducible using H&E sections
 - May be performed on core biopsies and excision specimens



Triple Negative Breast Cancers with high TILs ($>30\%$) have better outcome



Better outcome associated with presence of high TILs (>60%) in residual Triple Negative Breast Cancer after neoadjuvant therapy

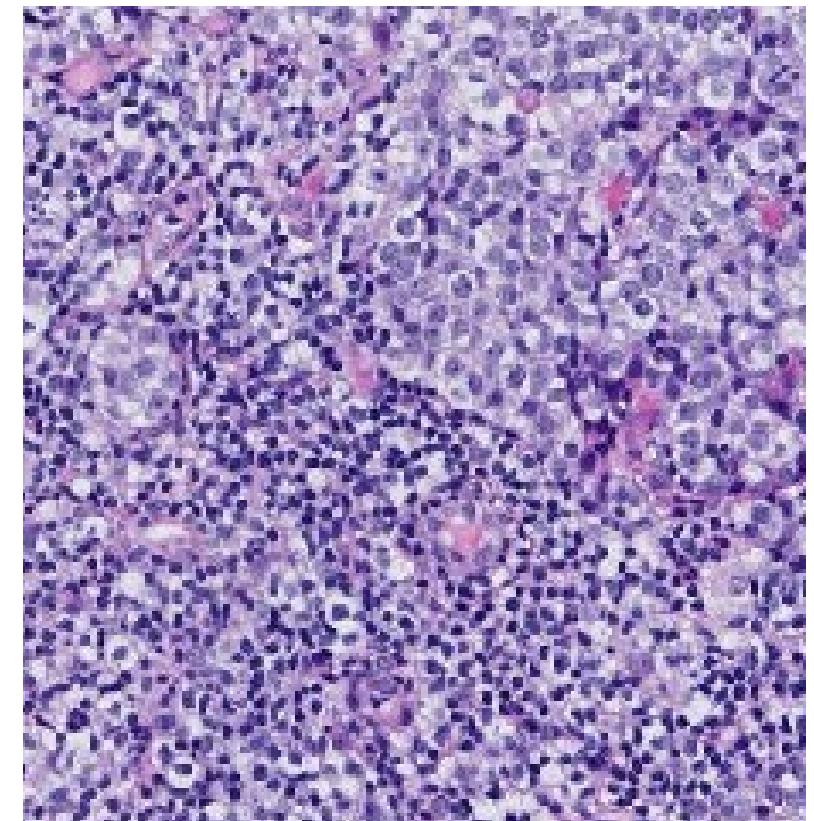


TILs are Prognostic in Breast Cancer

- High TILs associated with better prognosis in Triple Negative and HER2+ Breast Cancers
 - Loi 2013; Adams 2014; Loi 2014; Salgado 2015; Luen 2017
- Immune response is prognostic for Triple Negative and HER2+ Breast Cancers from gene expression datasets
 - Desmedt 2008; Bense 2017
- Presence of TILs on pre-therapeutic core biopsies is associated with favorable response to neoadjuvant chemotherapy
 - Denkert 2010; Issa-Nummer 2013; Ono 2012

Stromal TILs are Prognostic in Breast Cancer

- Tumors with high stromal TIL density (e.g. >50%) associated with best outcome
- Routine quantitative reporting of TILs is endorsed for Triple Negative Breast Cancer by international oncology and pathology organizations
 - St. Gallen 2019; ESMO 2019
- WHO 5th edition advocates considering TILs to stratify patients in clinical trials and prognostic studies



Additional resources

- Tutorial prepared by the International Working Group for TIL in breast cancer ([20200219 HTTdataCollectionWebinar TILsEvaluation – YouTube](#)).
- Slides available at: <https://www.tilsinbreastcancer.org/wp-content/uploads/2017/10/Tutorial-Website.pptx>
- What are TILs and why are they important? <https://www.tilsinbreastcancer.org/what-are-tils/>

Next: Stromal TIL Assessment in Breast Cancer (Video Tutorial)

Continue to Part 2 of 4

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Acknowledgements

- This work was supported by the FDA Office of Women's Health. This project was supported in part by an appointment (V.G.) to the ORISE Research Participation Program at the CDRH, U.S. Food and Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and FDA/Center.

Part 3: Challenges and Pitfalls in Breast Cancer Stromal TIL Assessment

Learning Objectives

- Review of Stromal TIL assessment in breast cancer
- Structures excluded from TIL assessment
- Pitfalls and challenges in TIL assessment

Review of Stromal TIL Assessment

- Stromal TIL density is the proportion of tumor-associated stromal area occupied by mononuclear inflammatory cells

$$\text{Stromal TIL Density (\%)} = \frac{\text{Area of tumoral stroma occupied by mononuclear inflammation}}{\text{Entire area of tumoral stroma}} \times 100$$

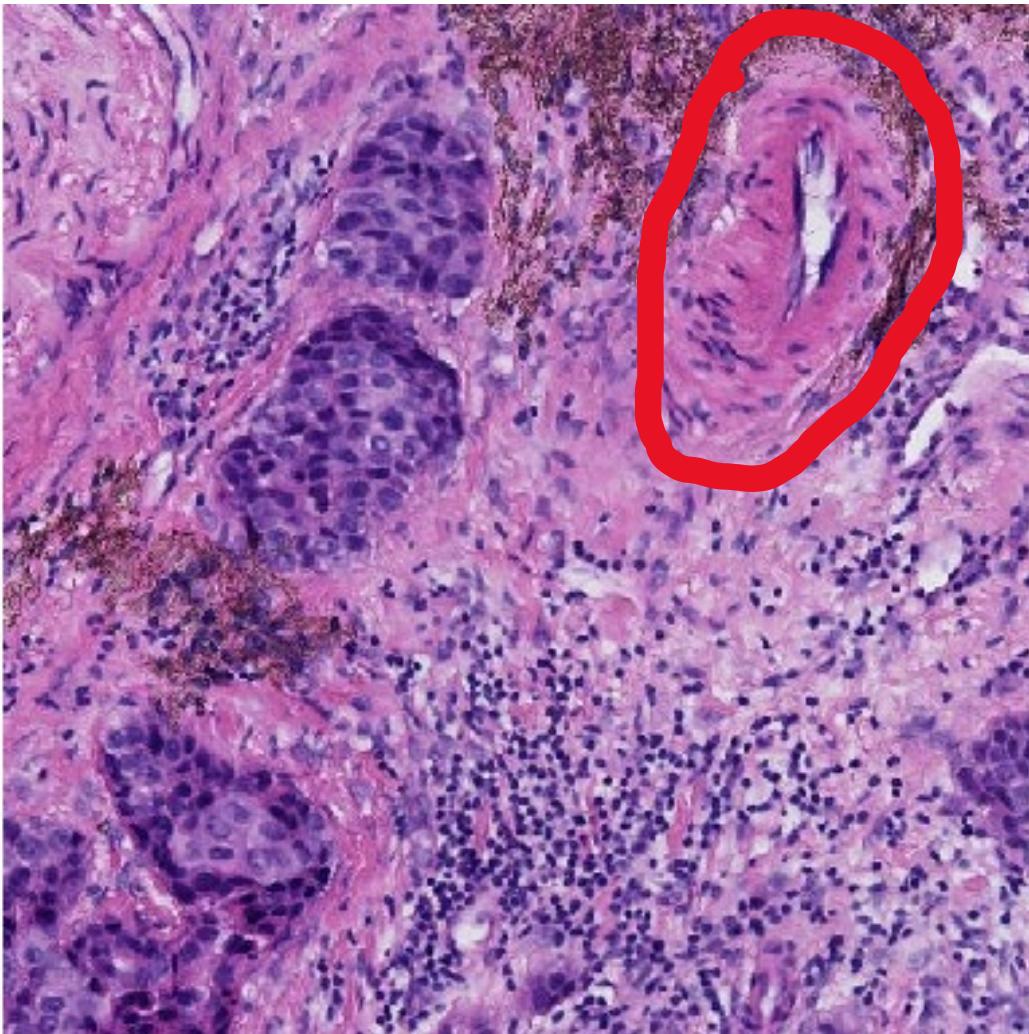
Review of Stromal TIL Assessment

- Assess stromal TIL density over entire invasive tumor
- Include the invasive tumor border
- Report the stromal TIL density averaged over entire tumor area
 - Do not focus on hotspots
- Only count plasma cells and lymphocytes

Structures excluded from stromal TIL assessment

- Thick-walled vessels
- Benign glandular elements
- Fat
- Carcinoma in situ
- Tertiary lymphoid structures

Thick-walled vessels
are not considered stroma

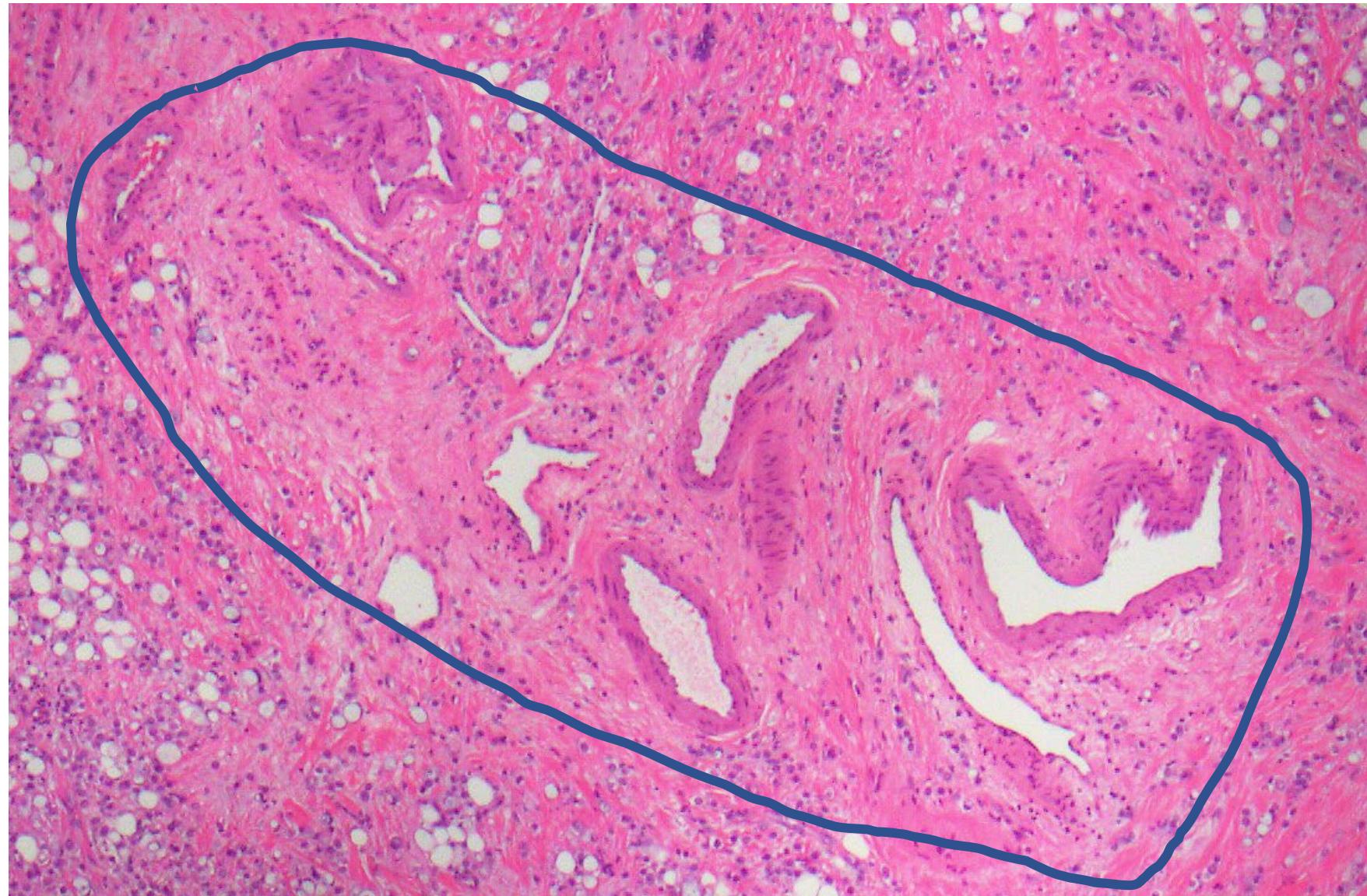


Area of tumoral stroma occupied by
mononuclear inflammation

Entire area of tumoral stroma

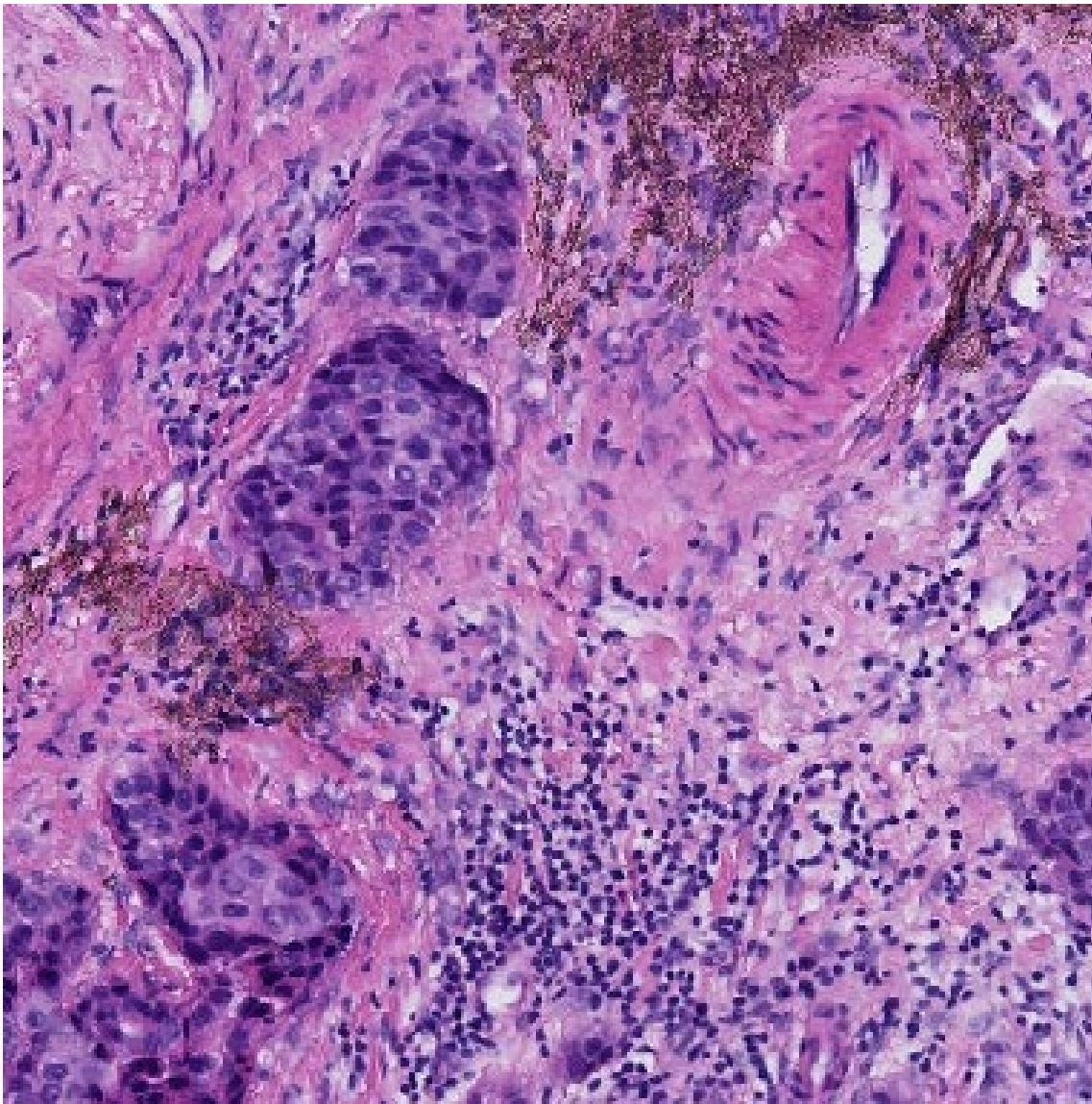
X 100

Thick-walled vessels
are not considered stroma





How much tumor-associated stroma is present?

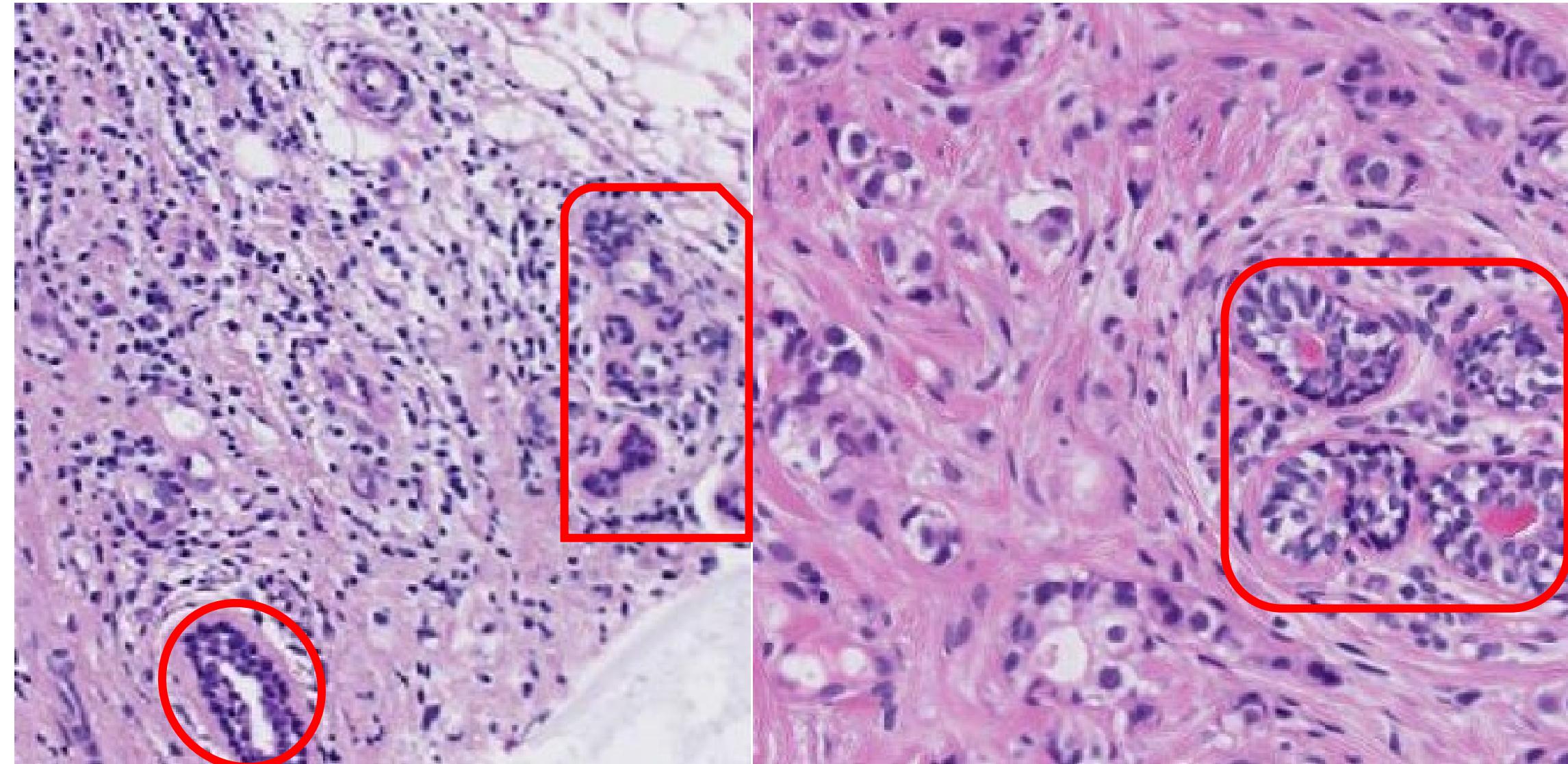


ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	75	30
Evaluable	35	60
Evaluable	86	15
Evaluable	75	30
Evaluable	70	25
Evaluable	70	20

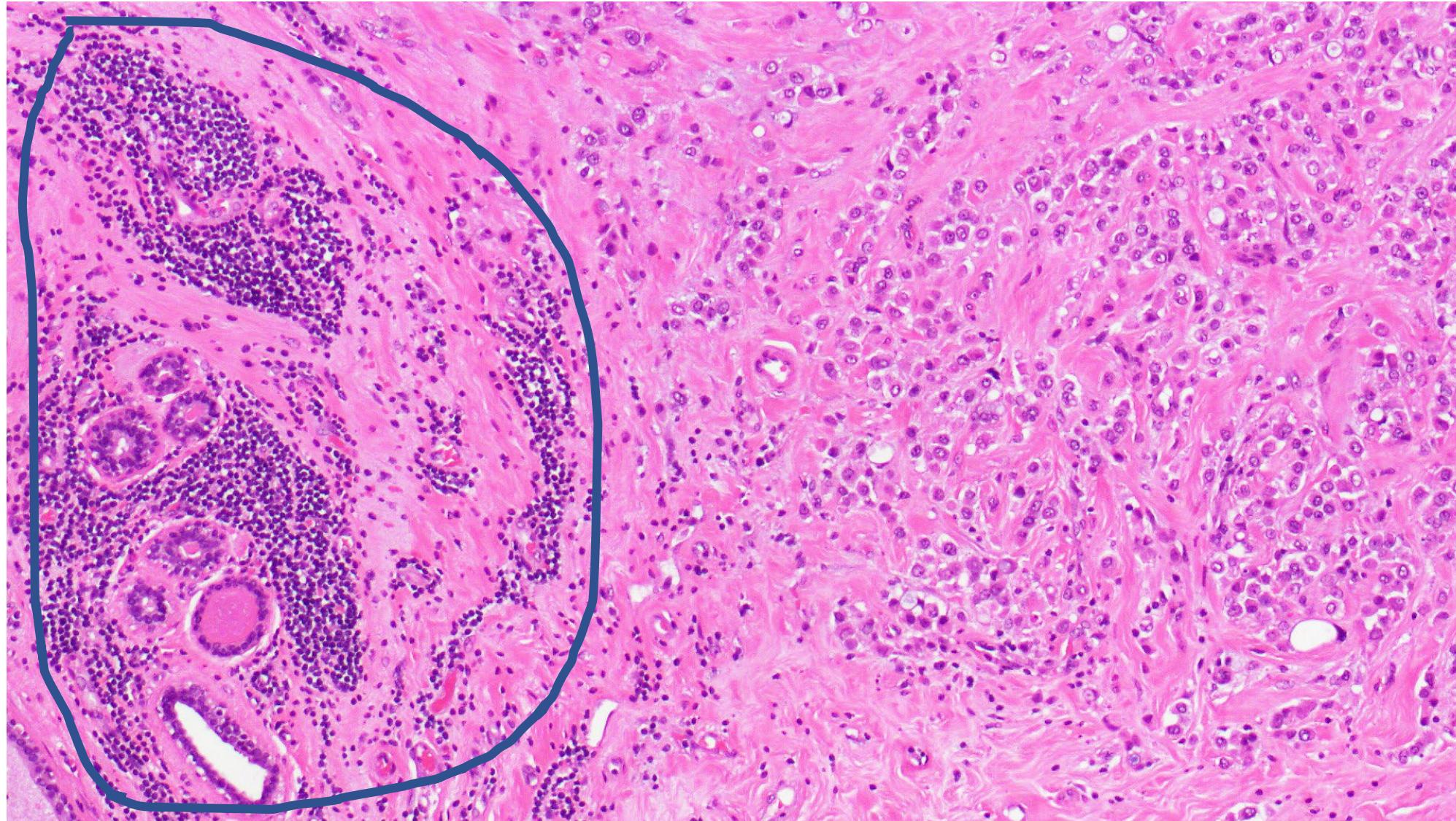
Mean Percent Tumor-Associated Stroma: 68.5

Mean sTILs Density: 30

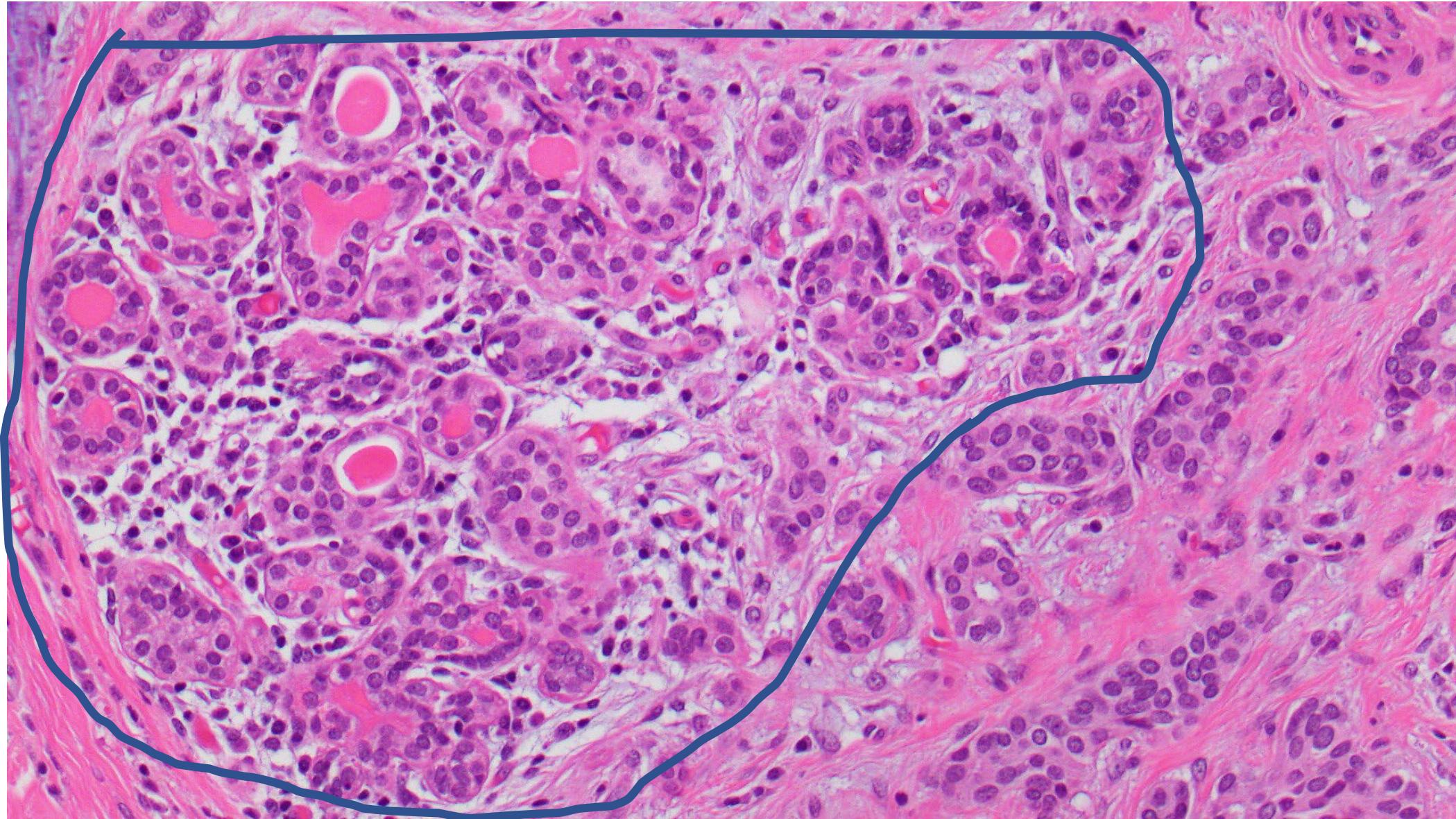
Benign ductal elements are not considered stroma



Lymphocytes around benign ducts
are not TILs

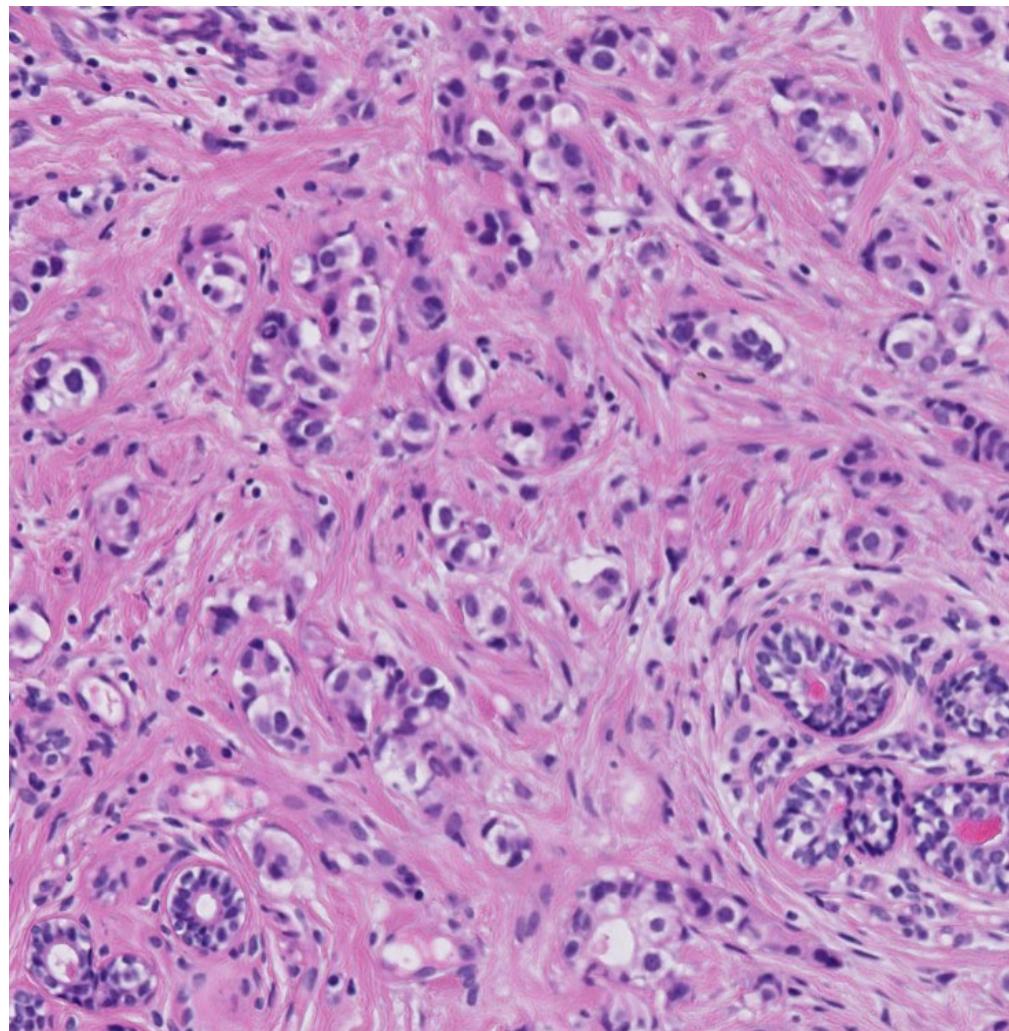


Lymphocytes around benign ducts
are not TILs





How much tumor-associated stroma is present?

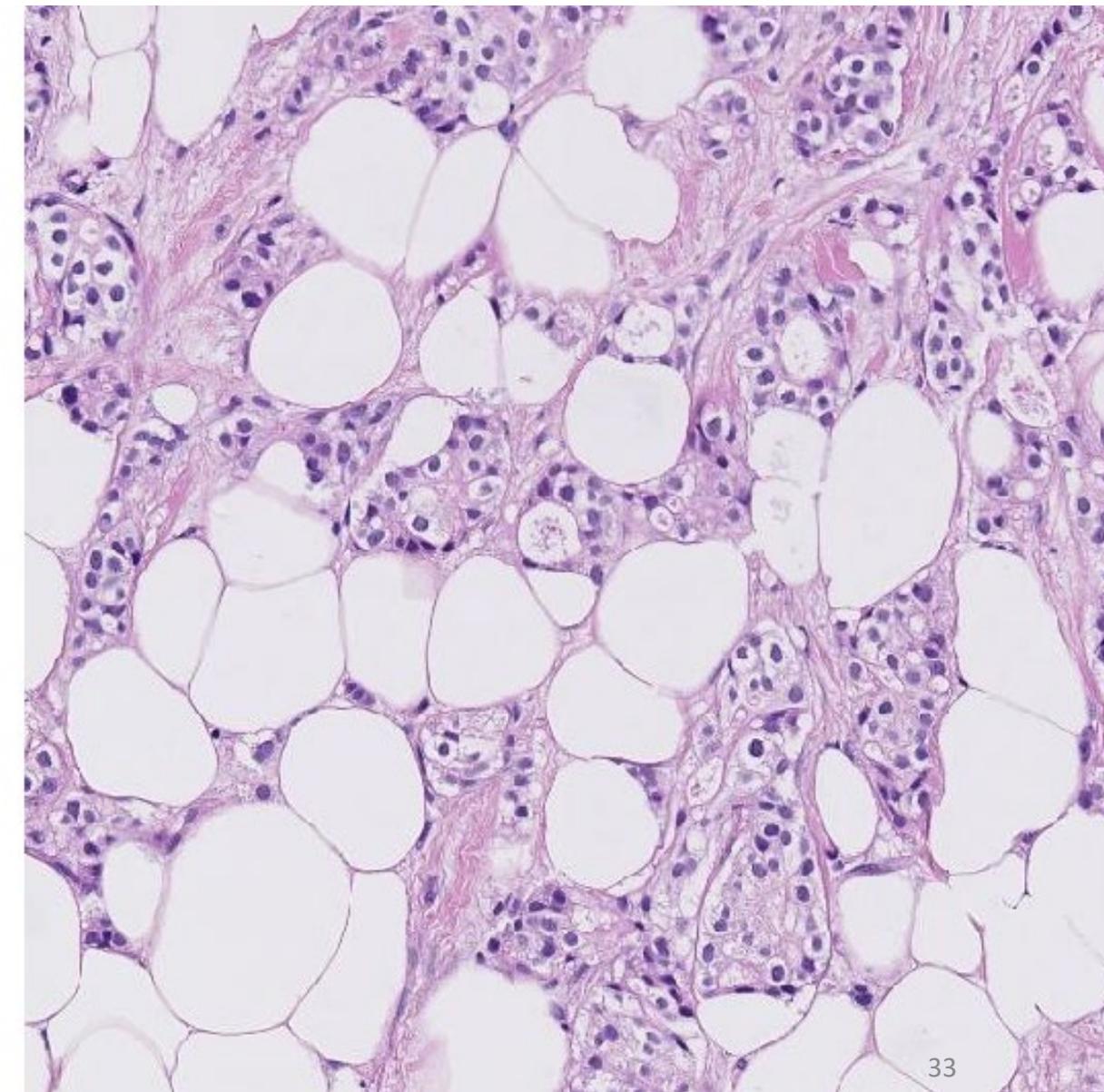
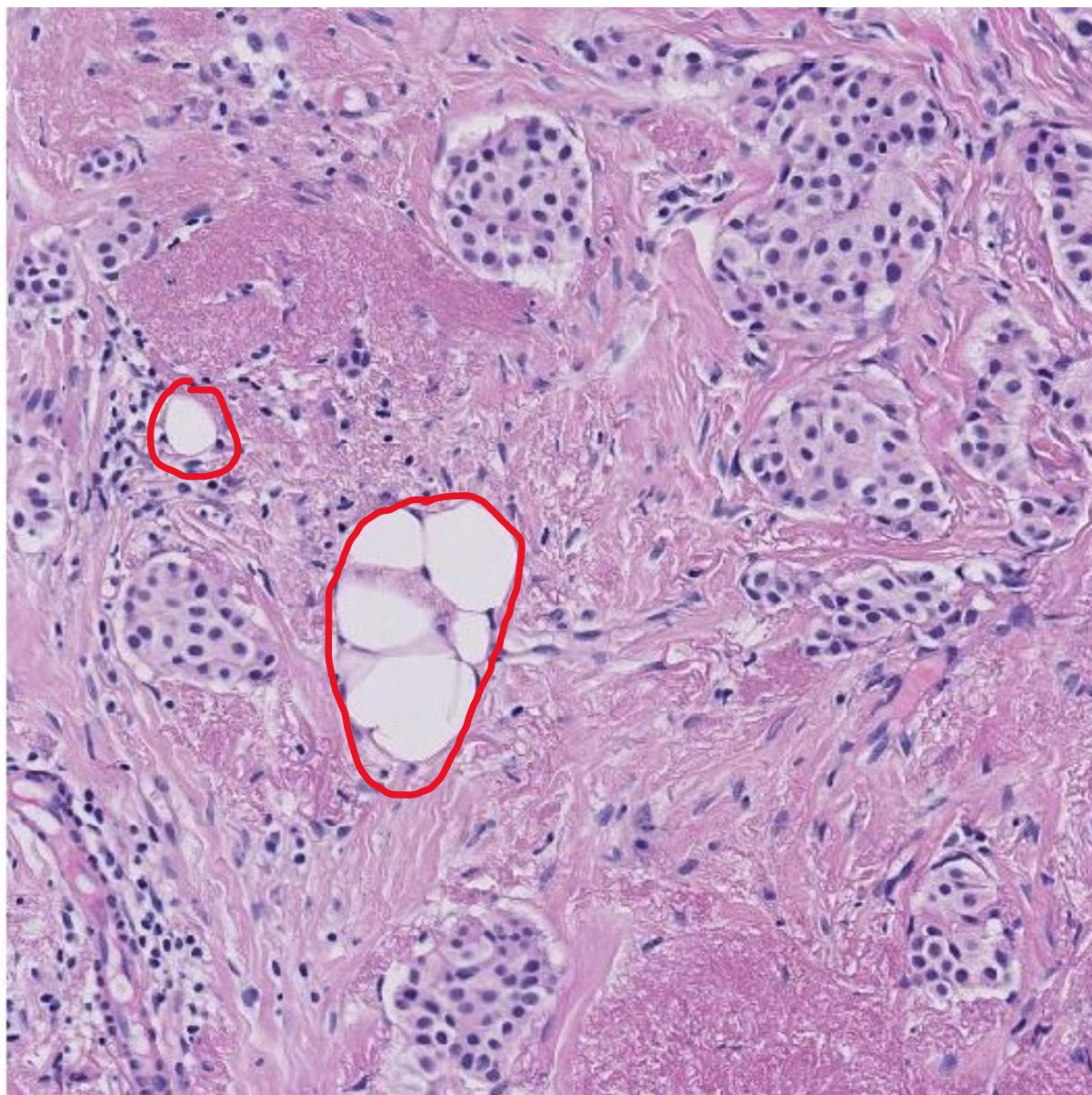


ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	53	3
Evaluable	70	10
Evaluable	60	10
Evaluable	60	0
Evaluable	70	3
Evaluable	80	3

Mean Percent Tumor-Associated Stroma: 65.5
Mean sTILs Density: 4.8

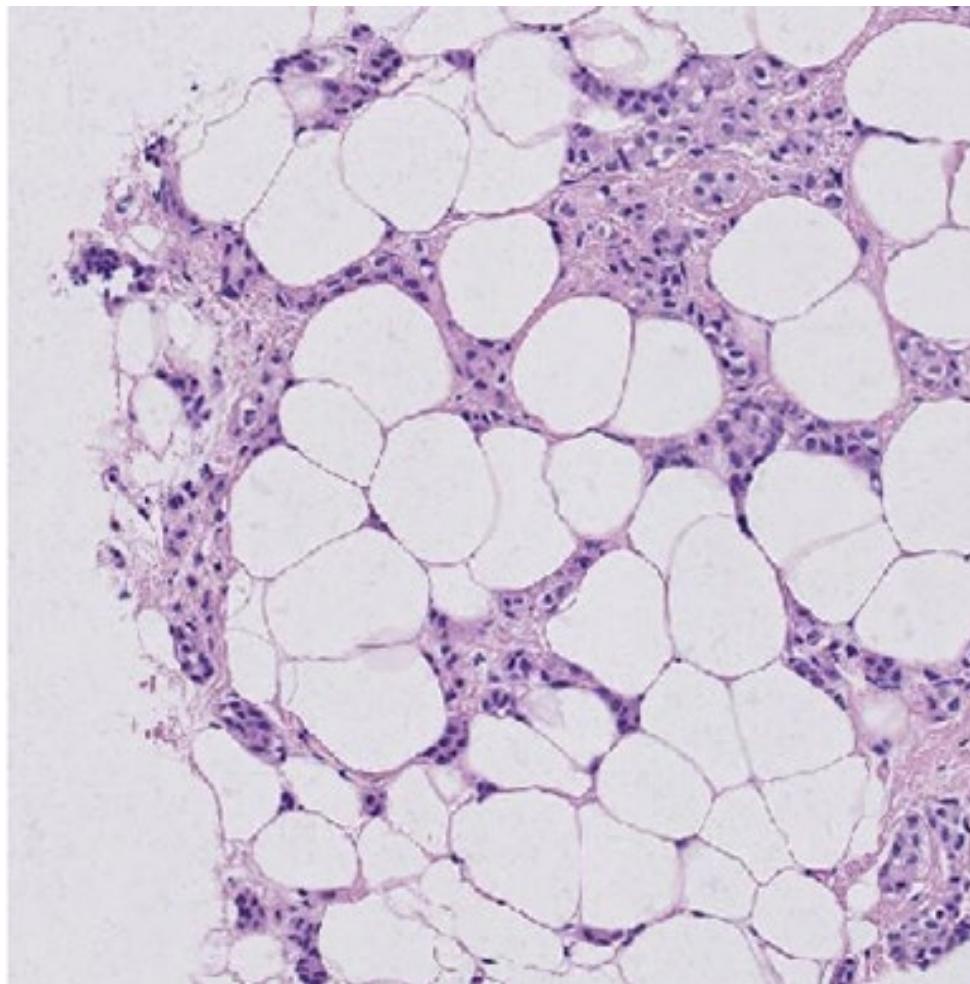


Adipose tissue is not considered stroma





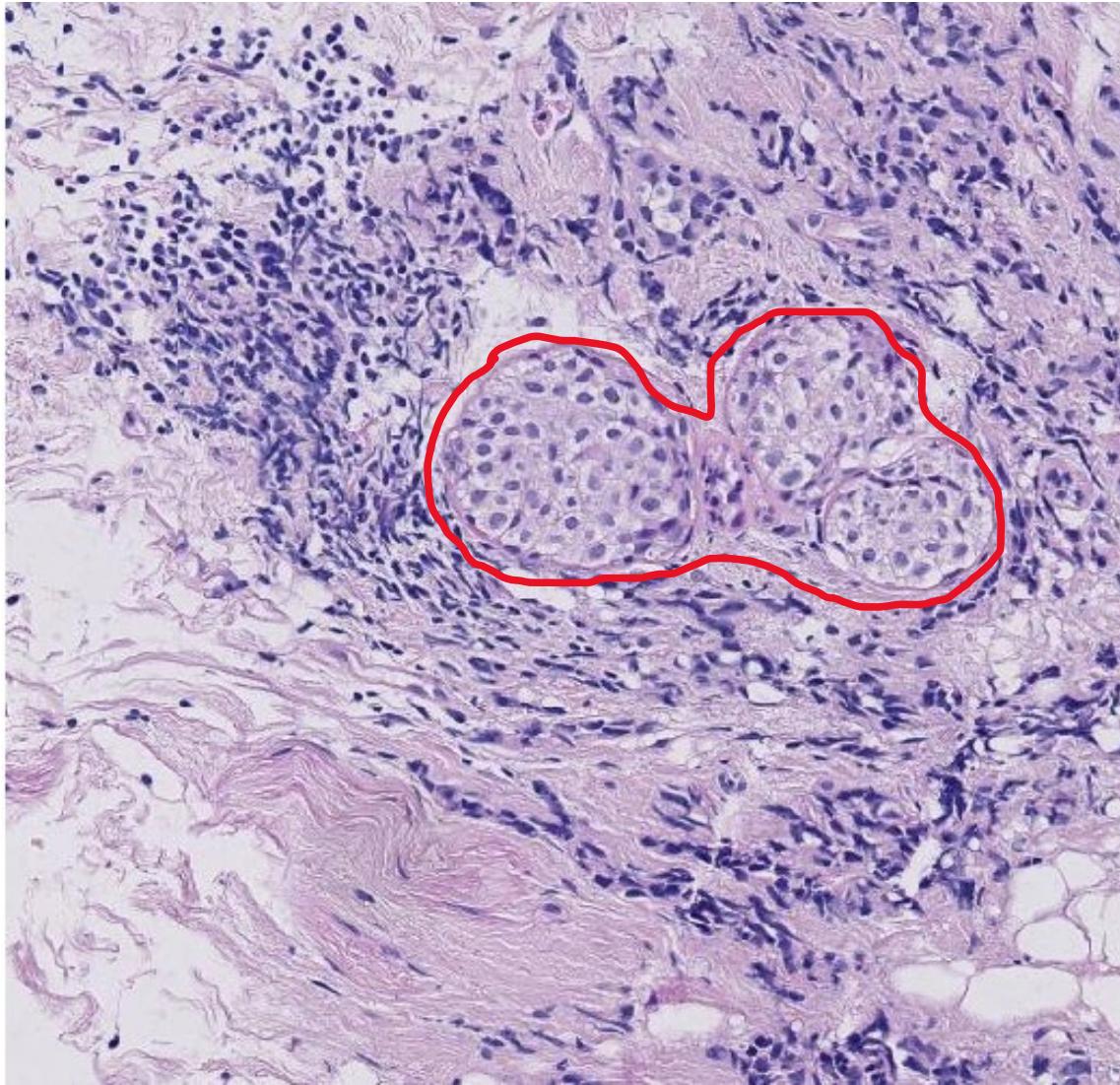
How much tumor associated stroma is present?



ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	10	0
Evaluable	5	1
Evaluable	14	4
Evaluable	20	0
Evaluable	40	0
Evaluable	50	2

Mean Percent Tumor-Associated Stroma: 23.2
Mean sTILs Density: 1.2

Carcinoma in situ is not stroma,
and any associated inflammation is not TIL

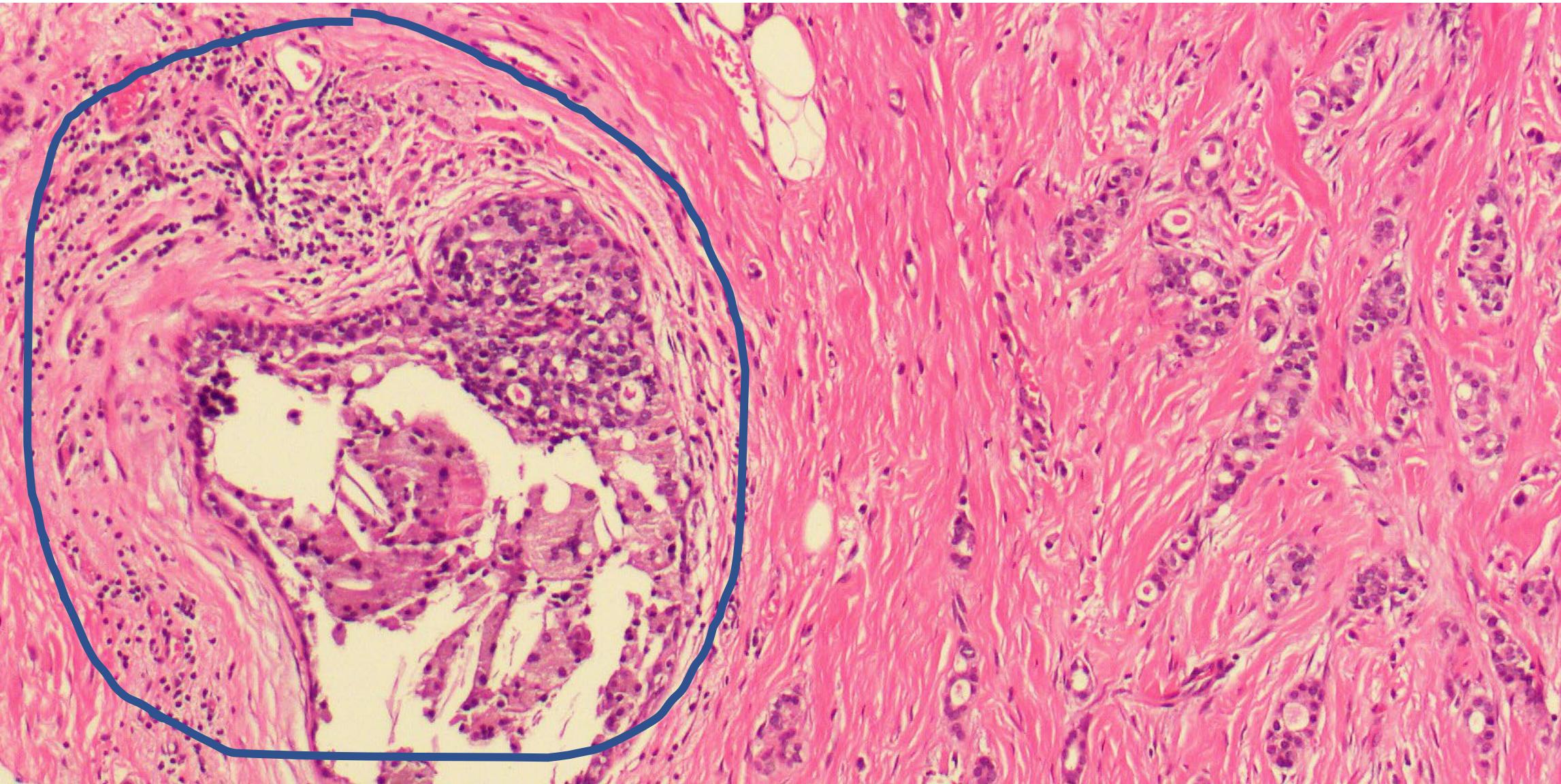


Area of tumoral stroma occupied by
mononuclear inflammation

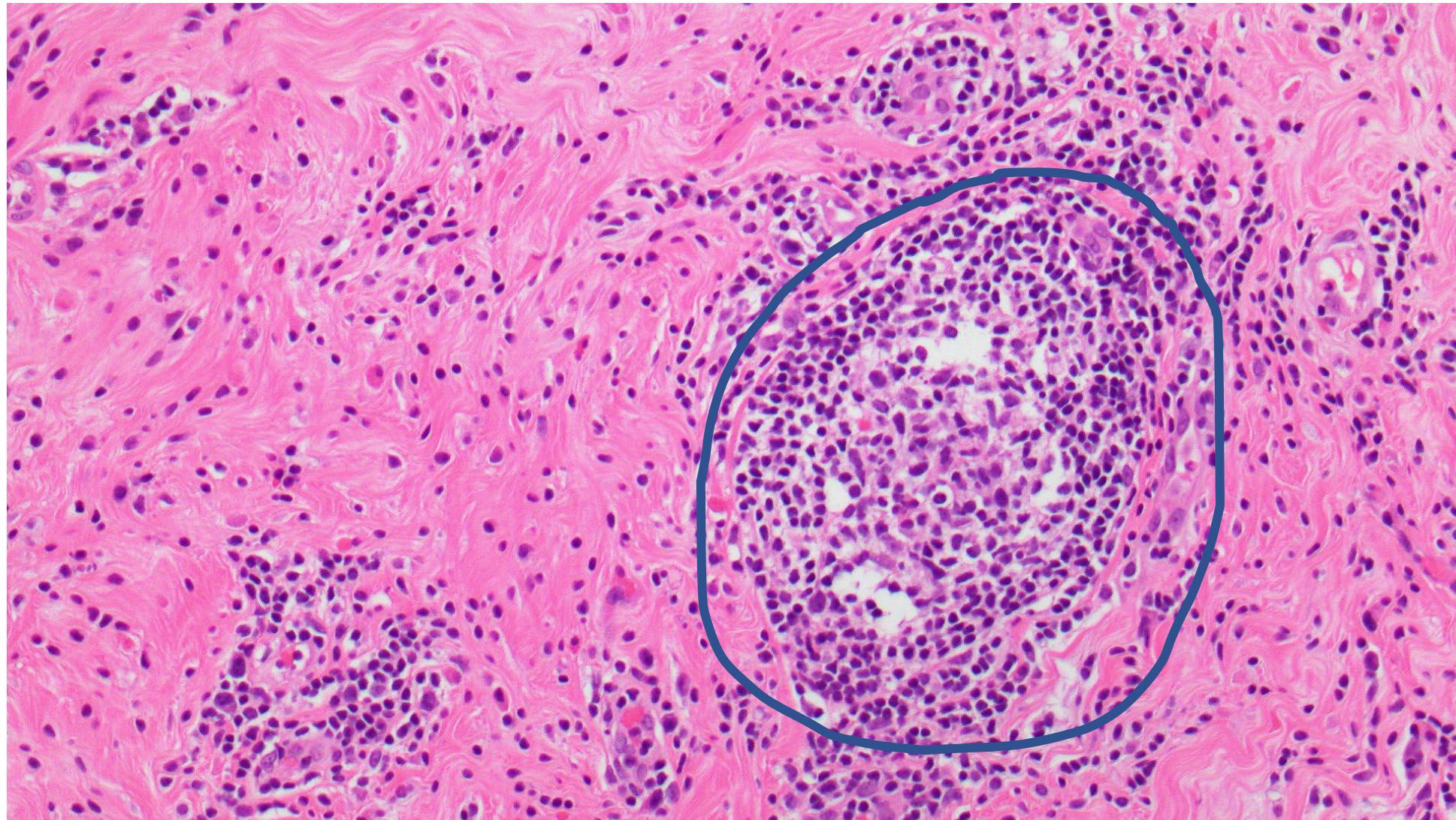
Entire area of tumoral stroma

X 100

Carcinoma in situ is not stroma,
and any associated inflammation is not TIL



Tertiary lymphoid structures
(i.e. ectopic lymph nodes) are not TILs



Mimics of TILs

- Degenerated cells with apoptotic/pyknotic nuclei
- Cells with perinuclear clearing
- Fibroblasts sectioned axially

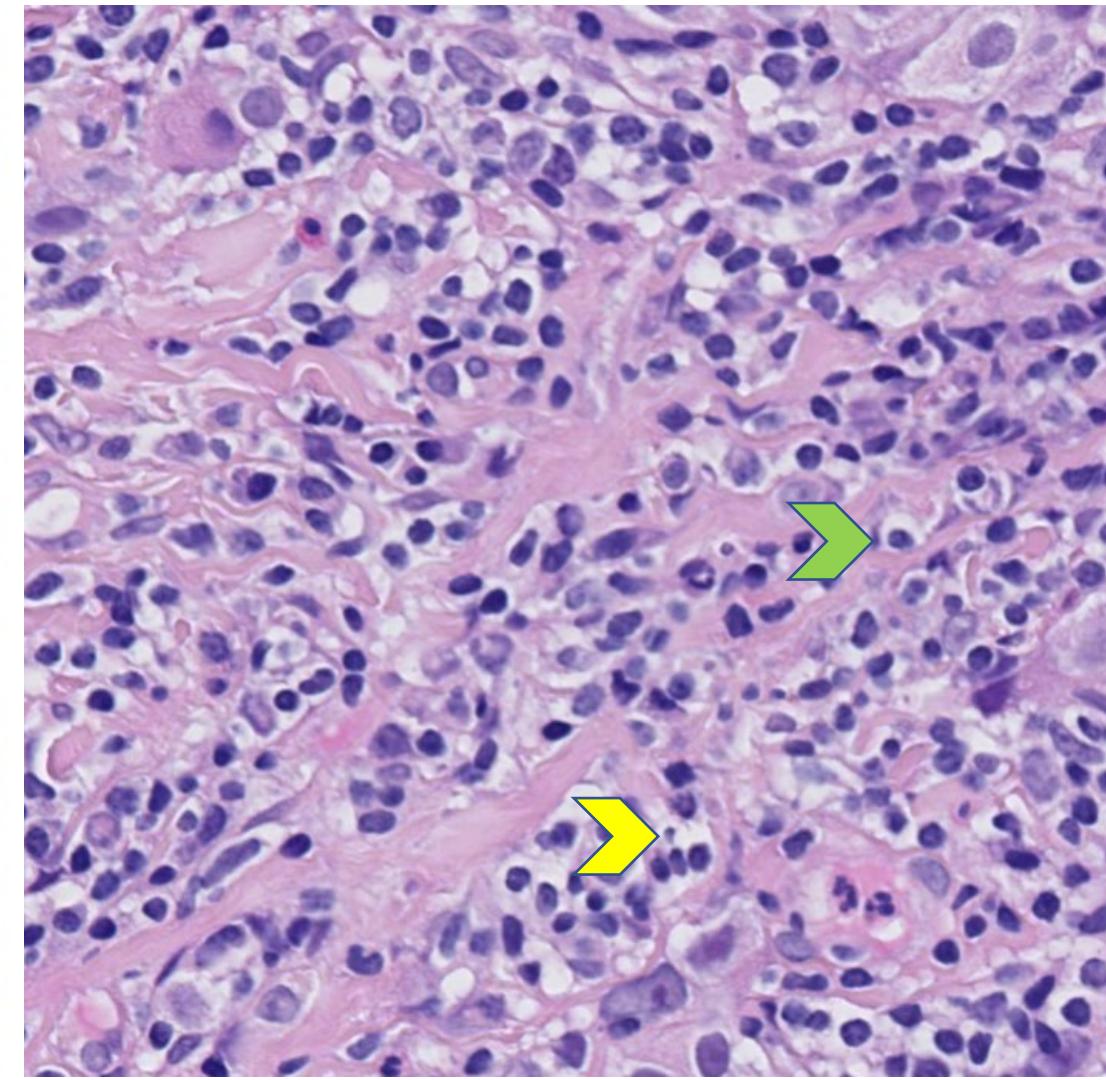
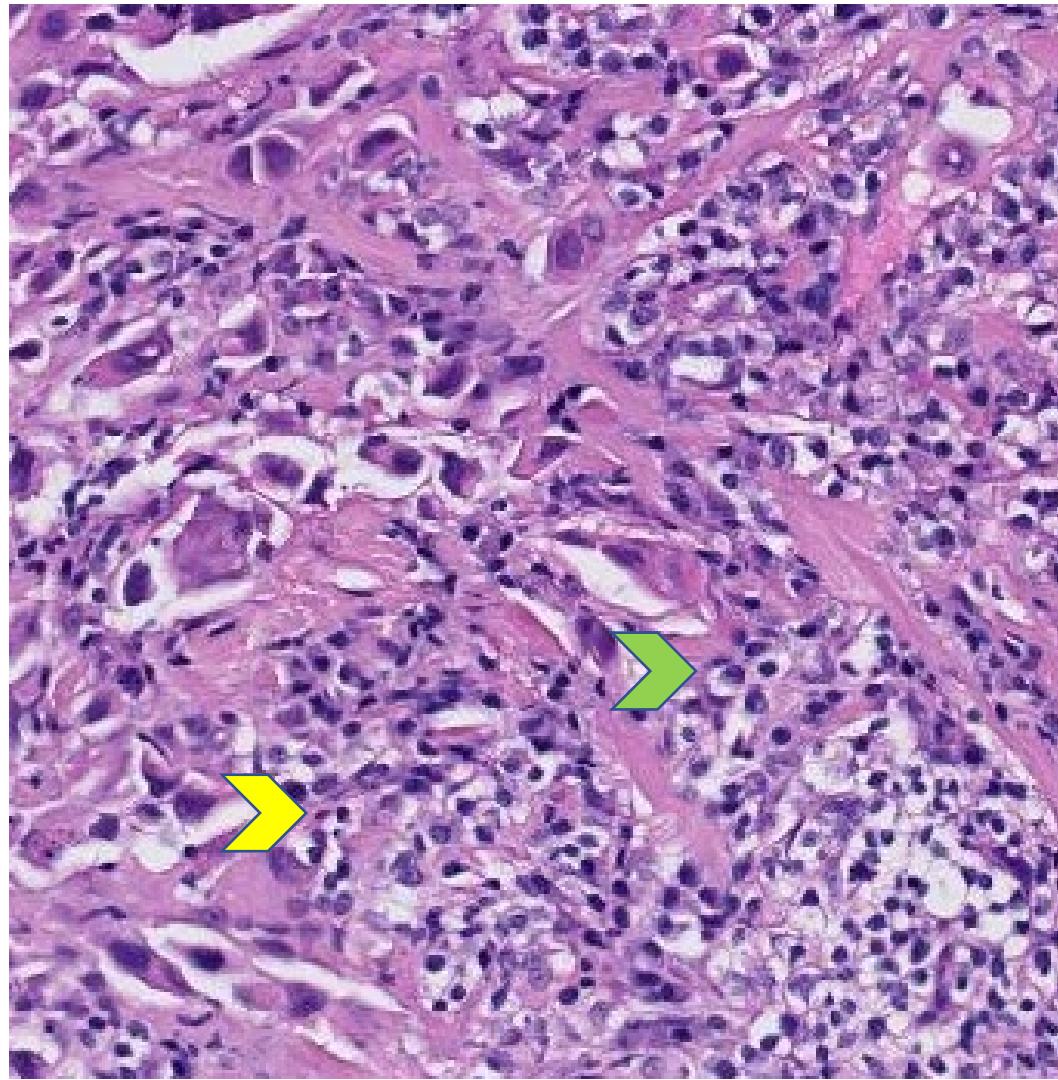
Area of tumoral stroma occupied by mononuclear inflammation

Entire area of tumoral stroma

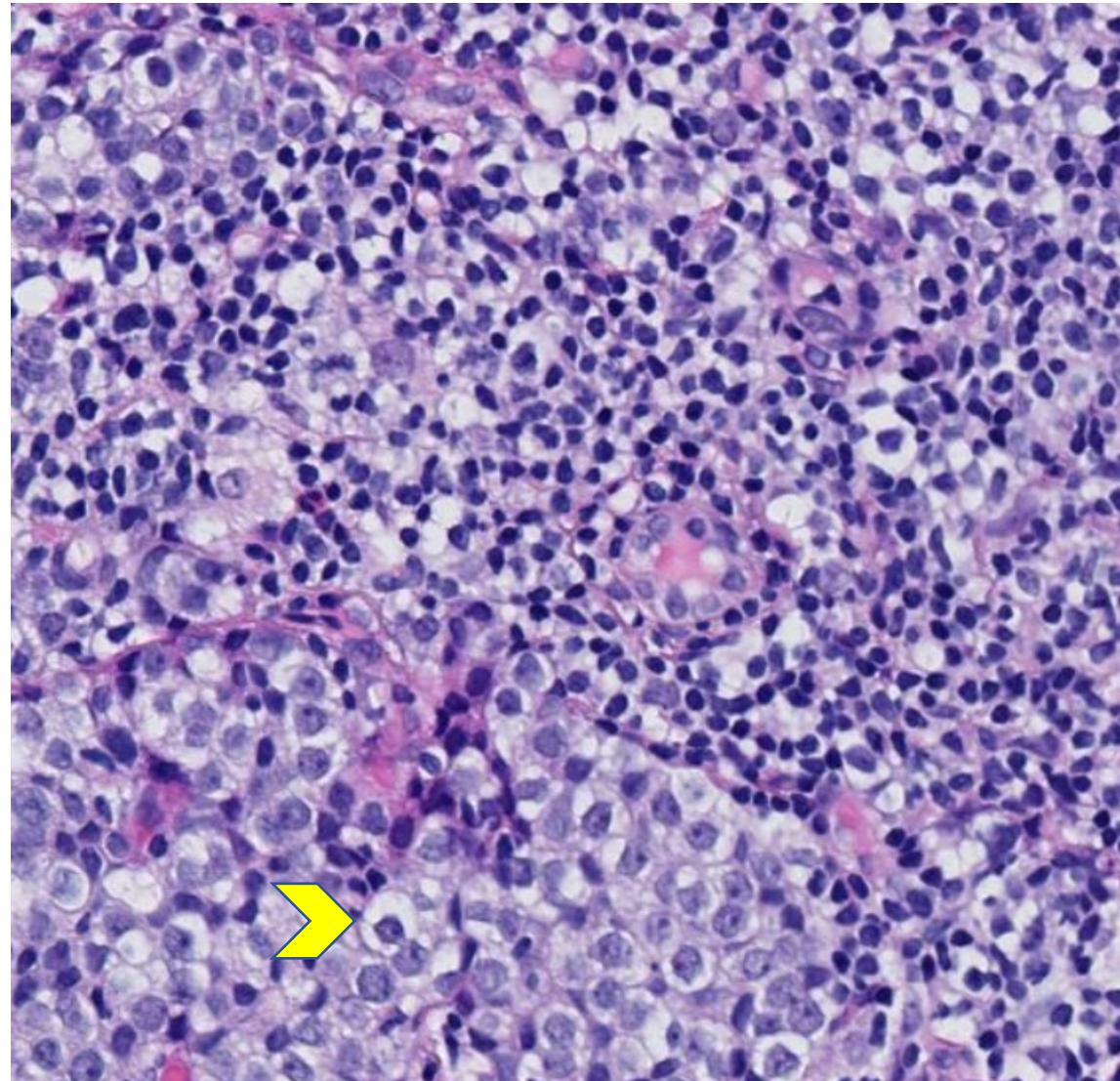
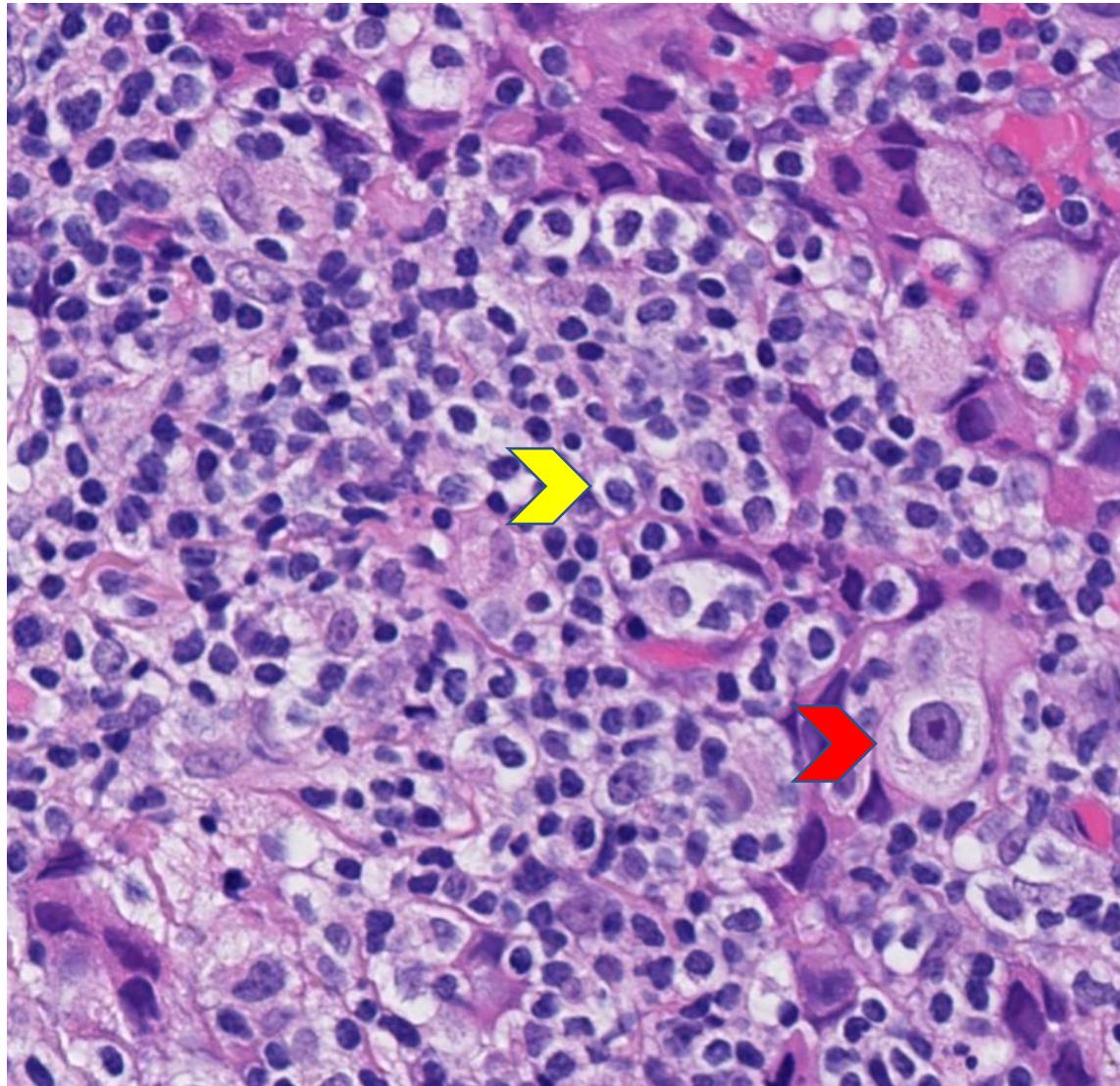
X 100



Apoptotic forms and perinuclear clearing

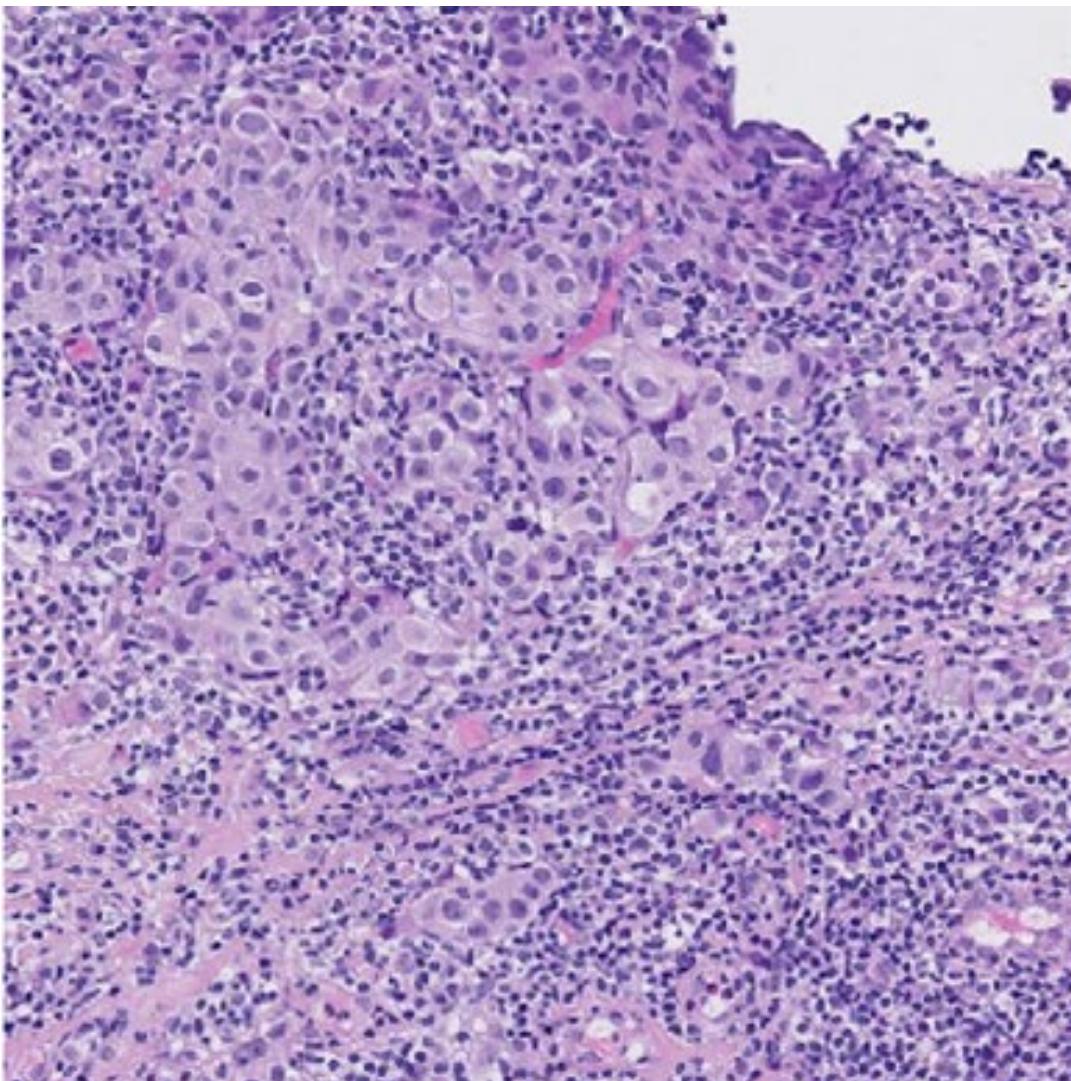


Cells with perinuclear clearing





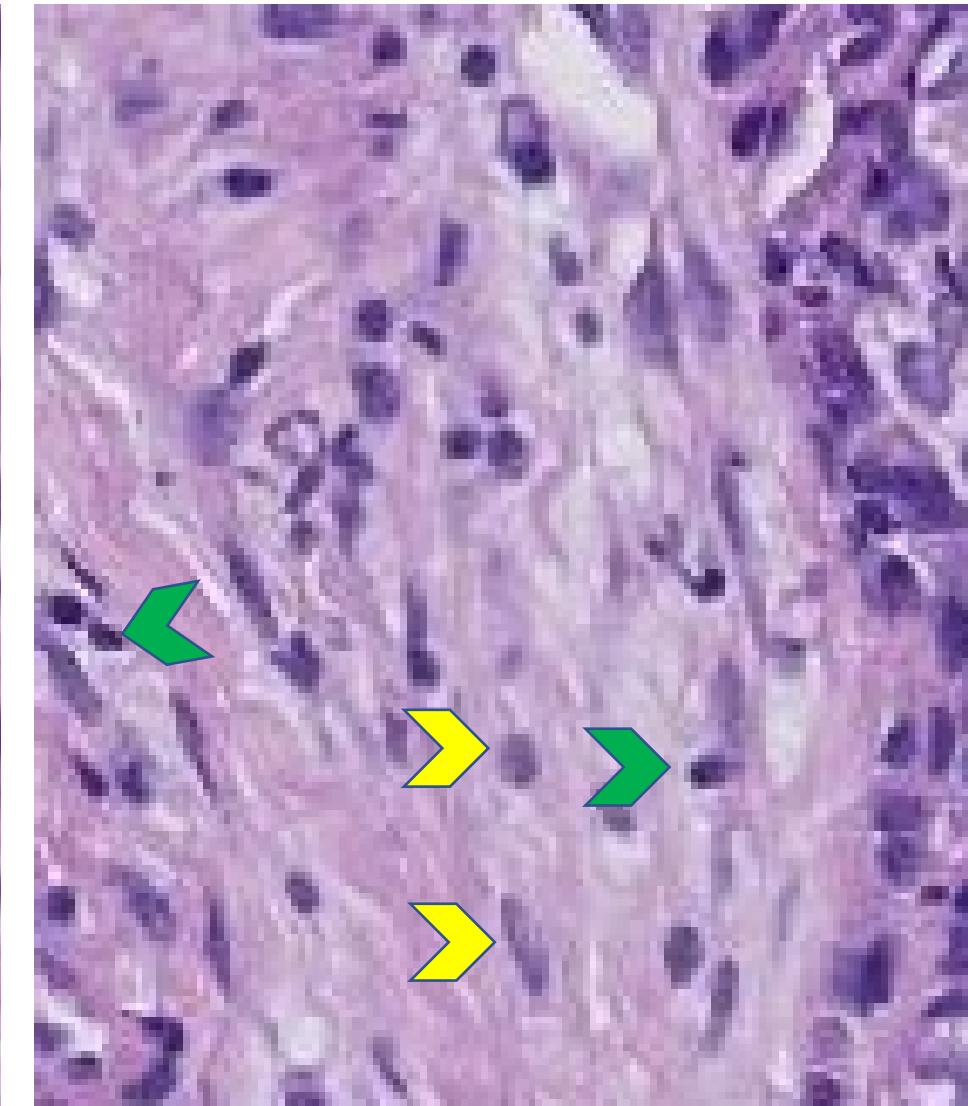
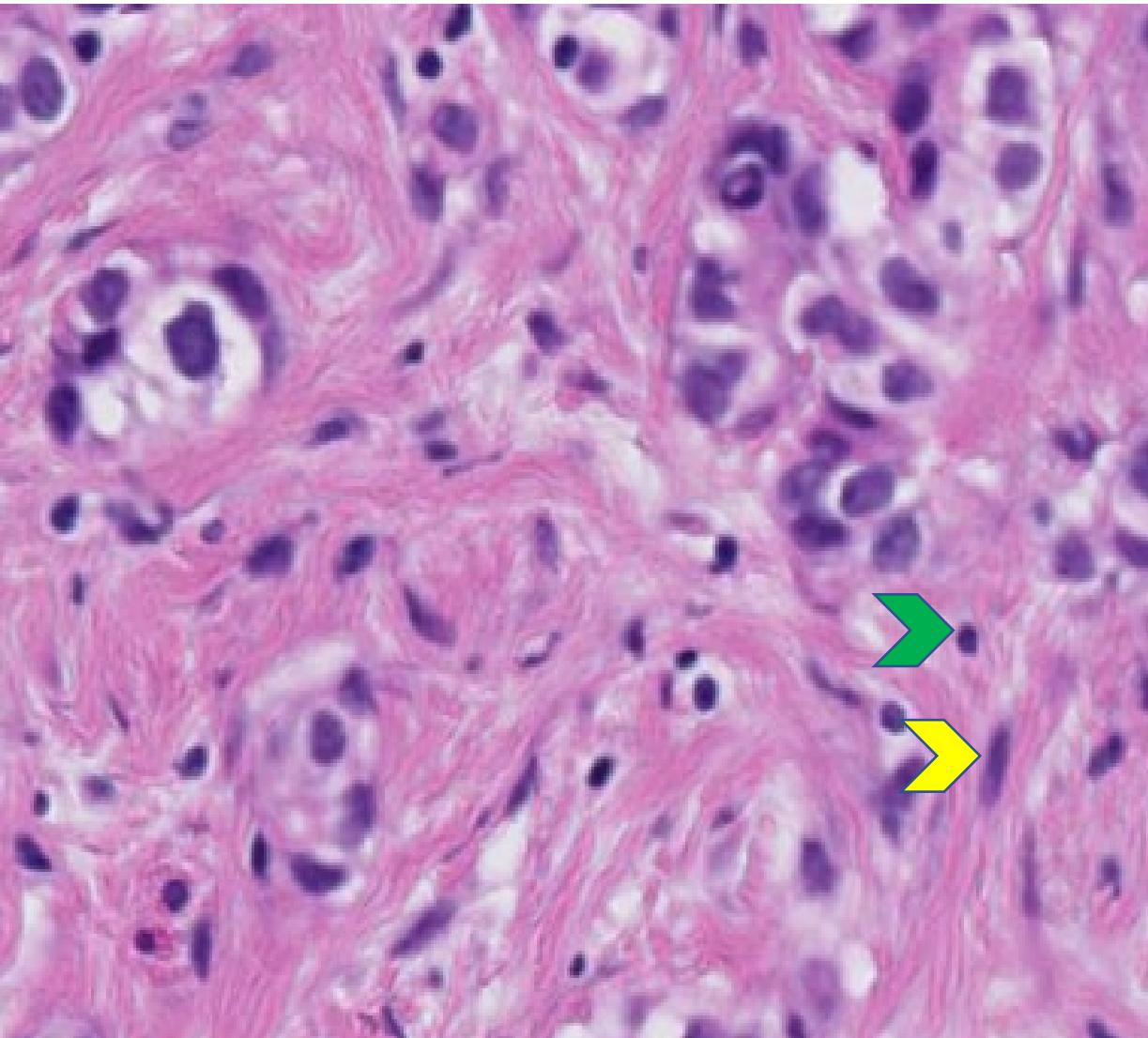
How much tumor-associated stroma is present?



ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	60	70
Evaluable	45	90
Evaluable	67	75
Evaluable	60	85
Evaluable	60	85
Evaluable	50	70

Mean Percent Tumor-Associated Stroma: 57
Mean sTILs Density: 79.2

Cross-sectionally cut fibroblasts
mimic lymphocytes



Avoid assessing stromal TIL density in:

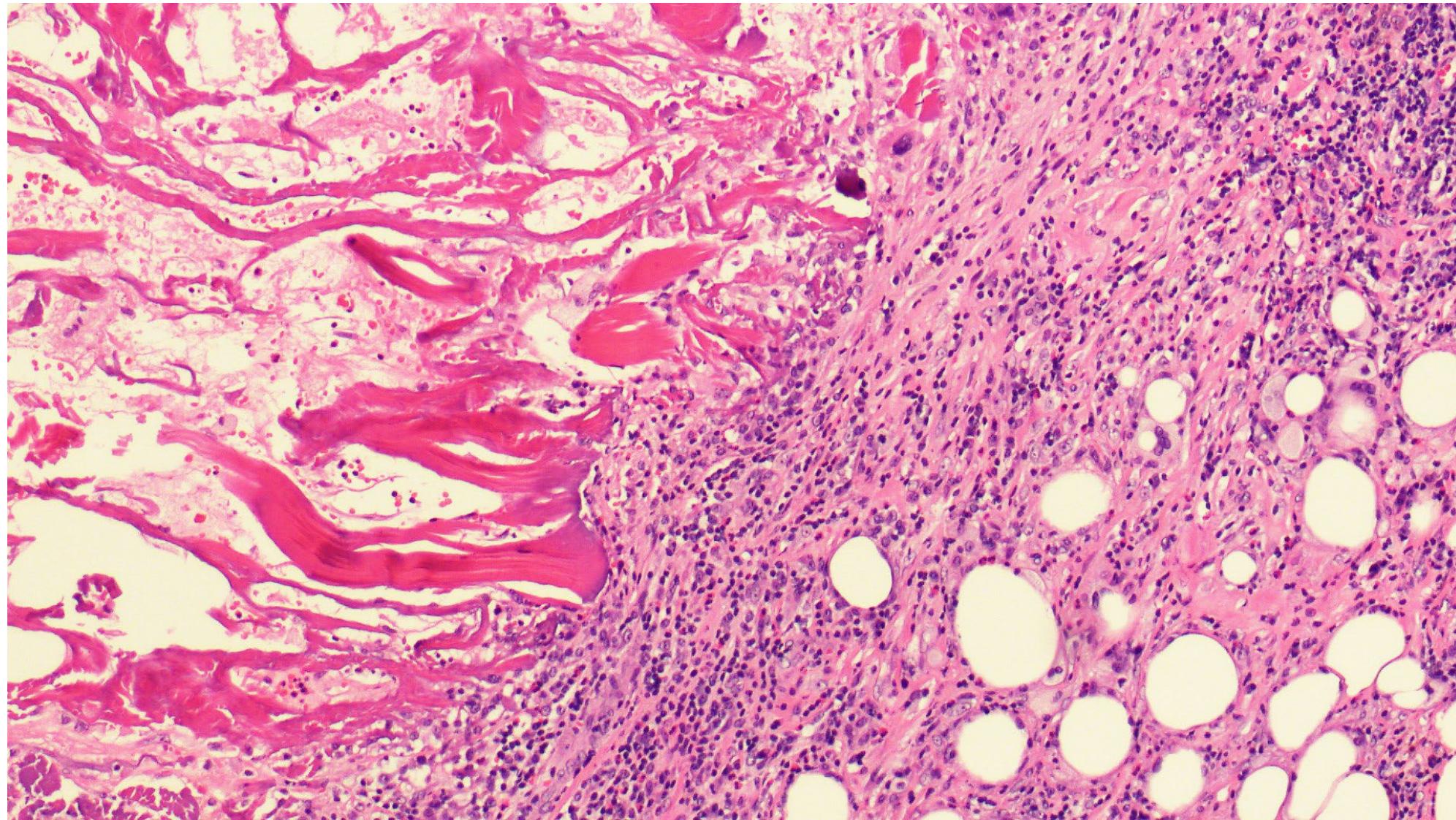
- Areas of inflammation associated with a biopsy site
- Areas showing crush artifact
- Necrotic areas
- Densely fibrotic/hyalinized/sclerotic areas

Area of tumoral stroma occupied by
mononuclear inflammation

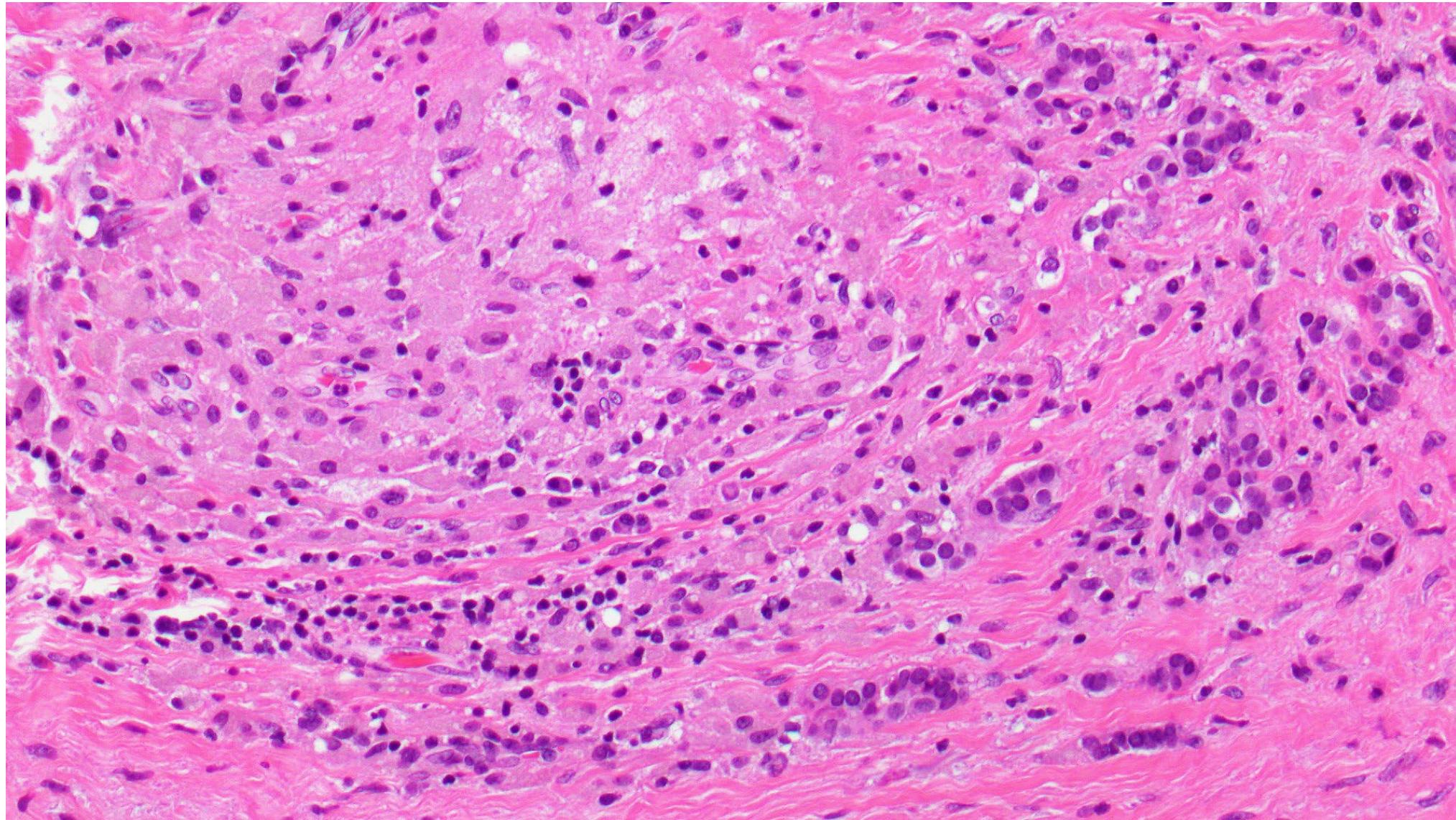
Entire area of tumoral stroma

X 100

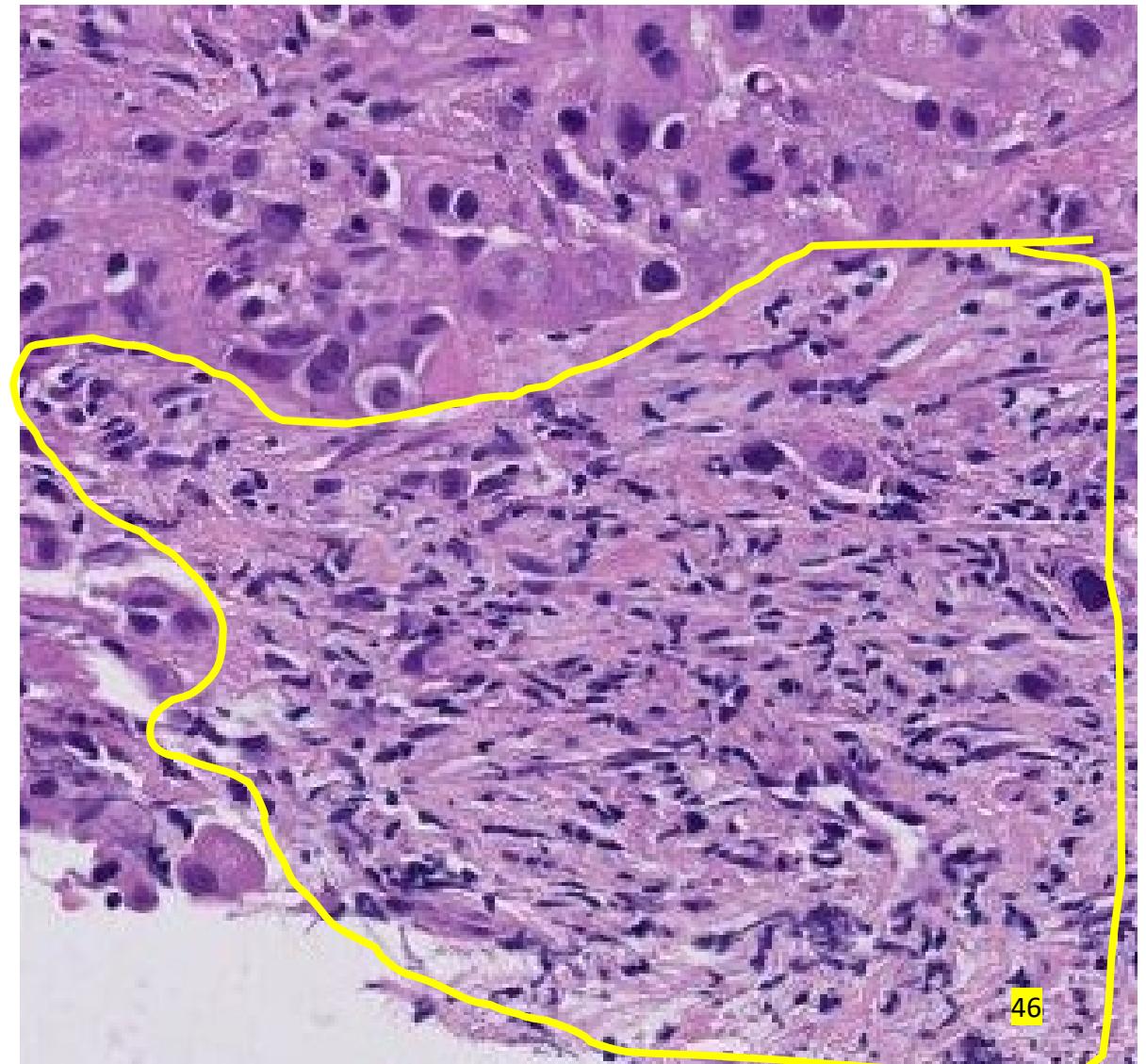
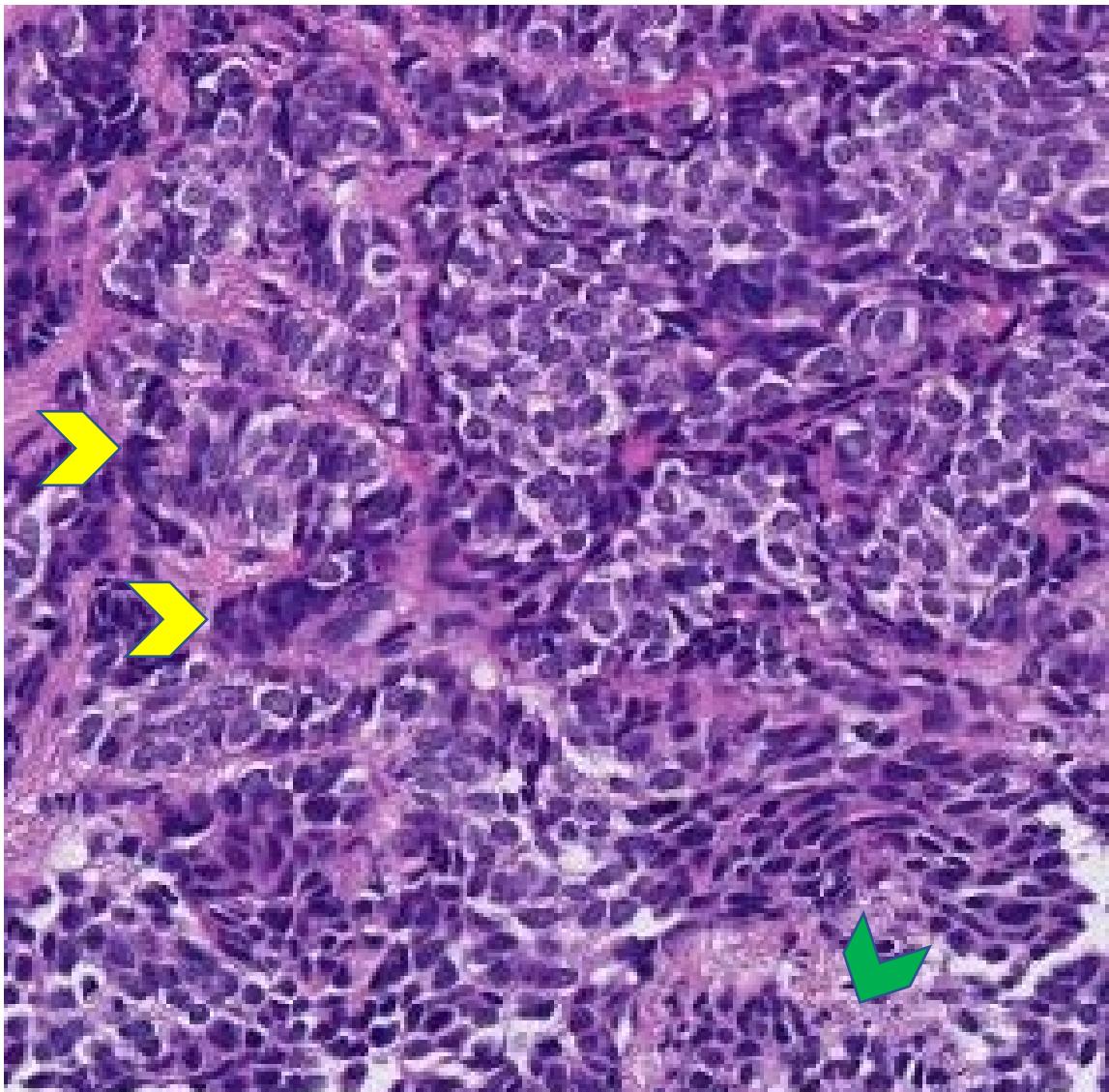
Biopsy site inflammation

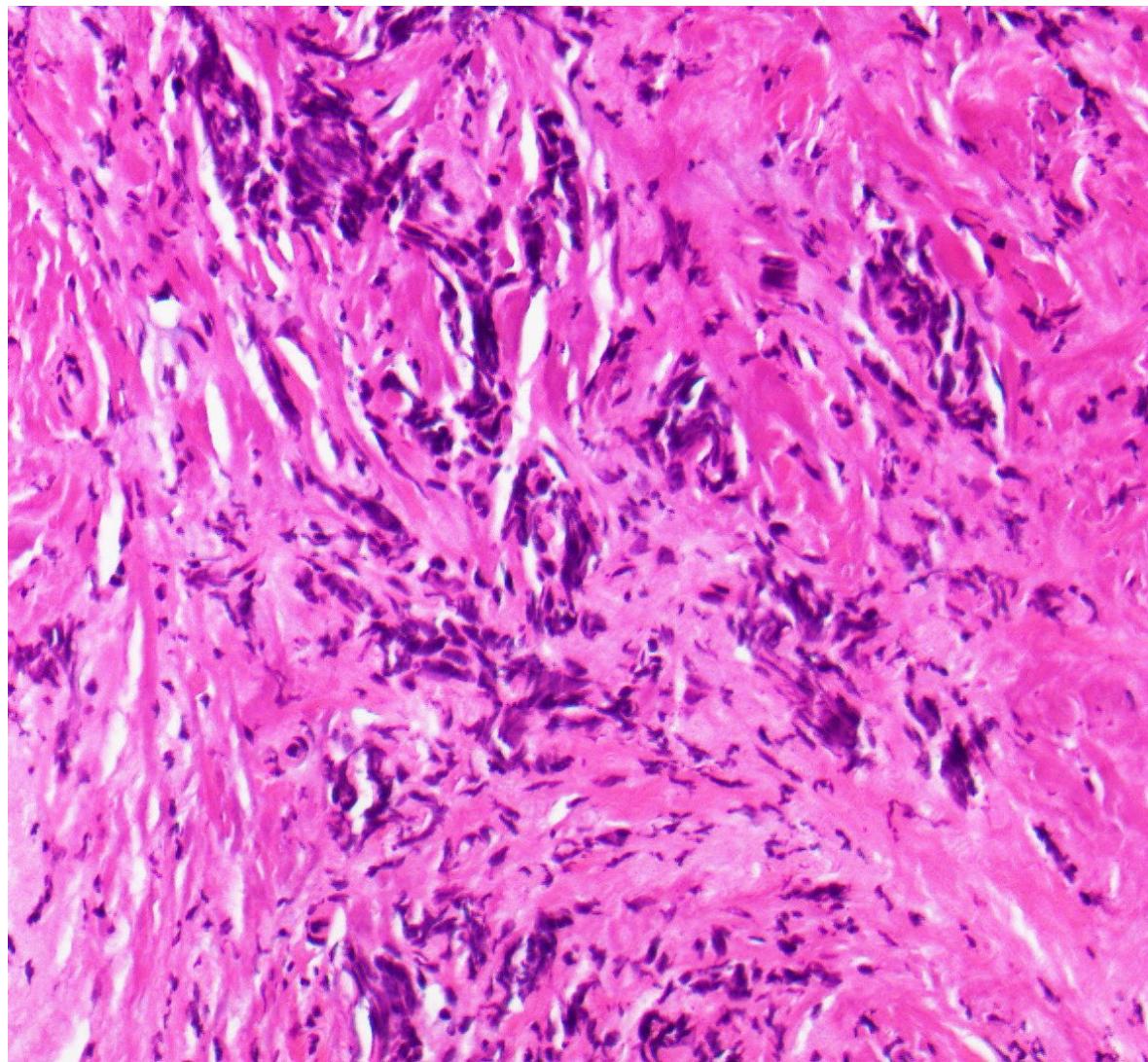


Biopsy site inflammation



Crush Artifact





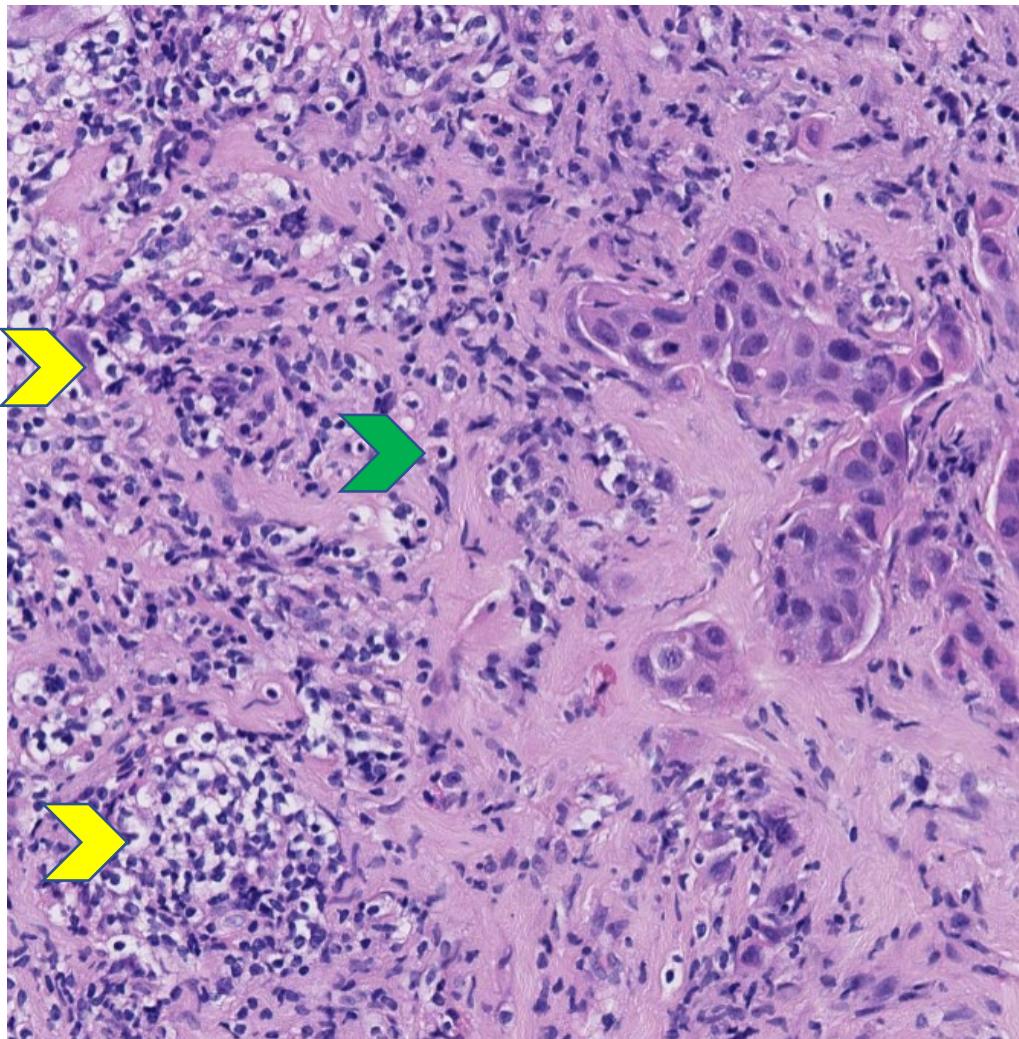
Severe crush artifact

Area of tumoral stroma occupied by mononuclear inflammation

X 100

Entire area of tumoral stroma

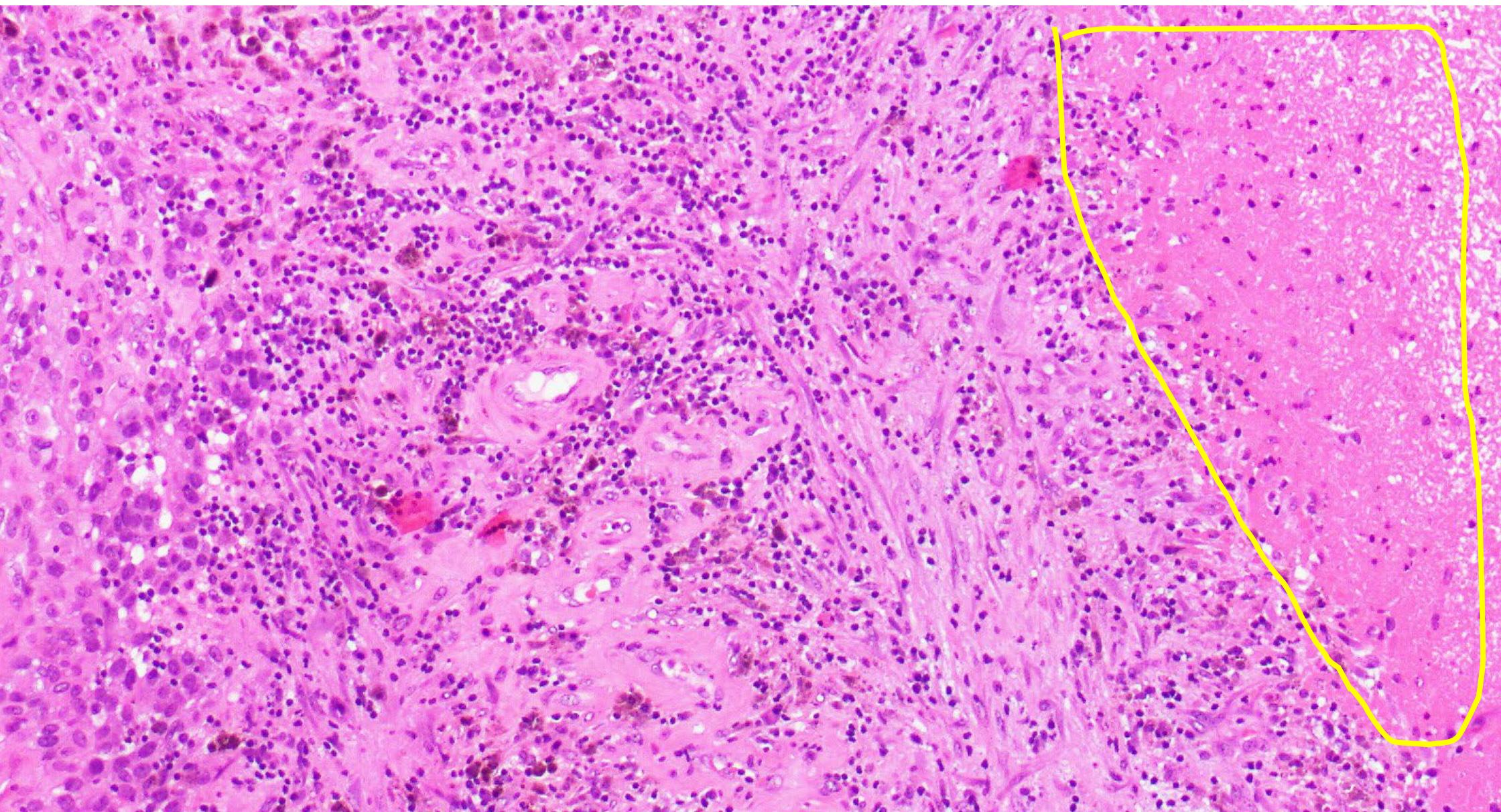
How much tumor-associated stroma is present?



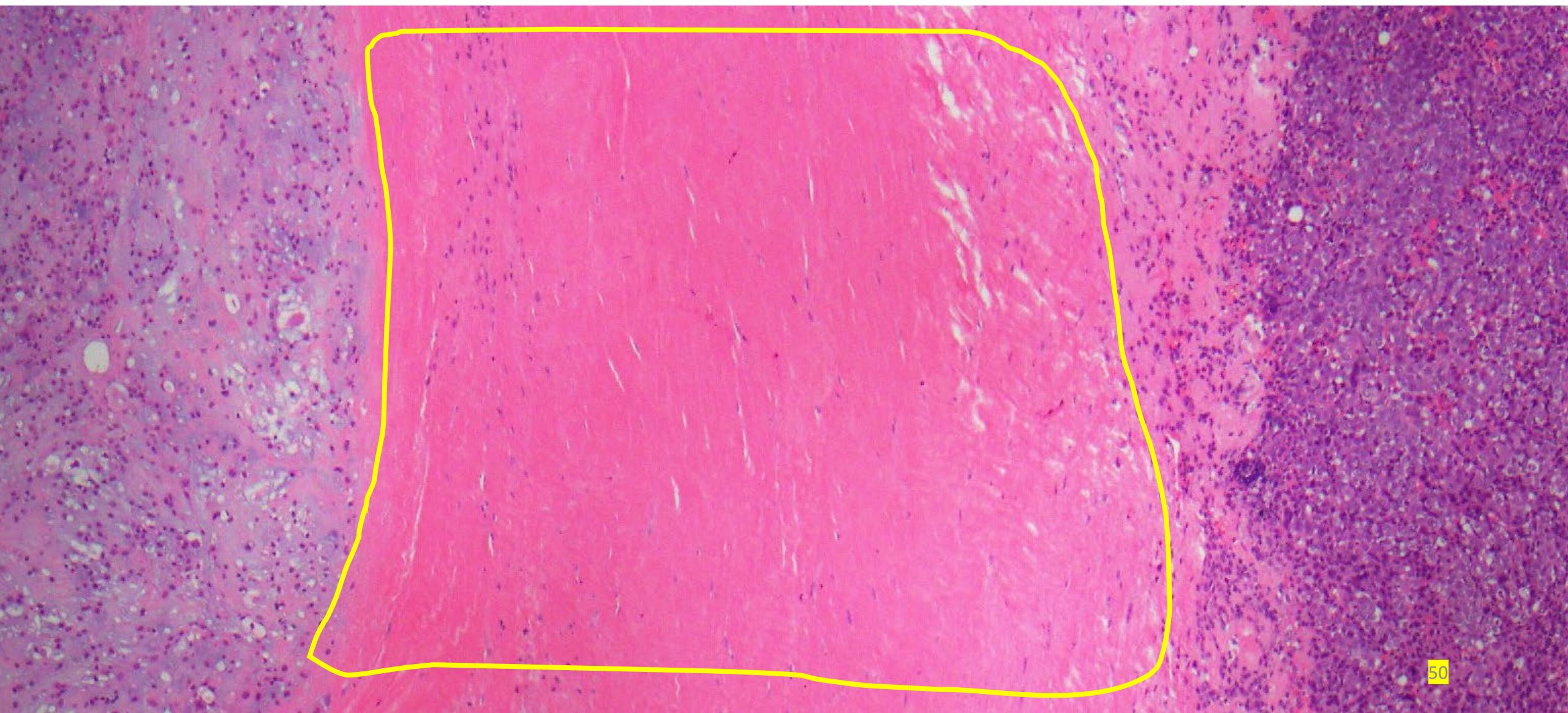
ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	85	40
Evaluable	80	35
Evaluable	86	61
Evaluable	85	65
Evaluable	80	50
Evaluable	85	30

Mean Percent Tumor-Associated Stroma: 83.5
Mean sTILs Density: 46.8

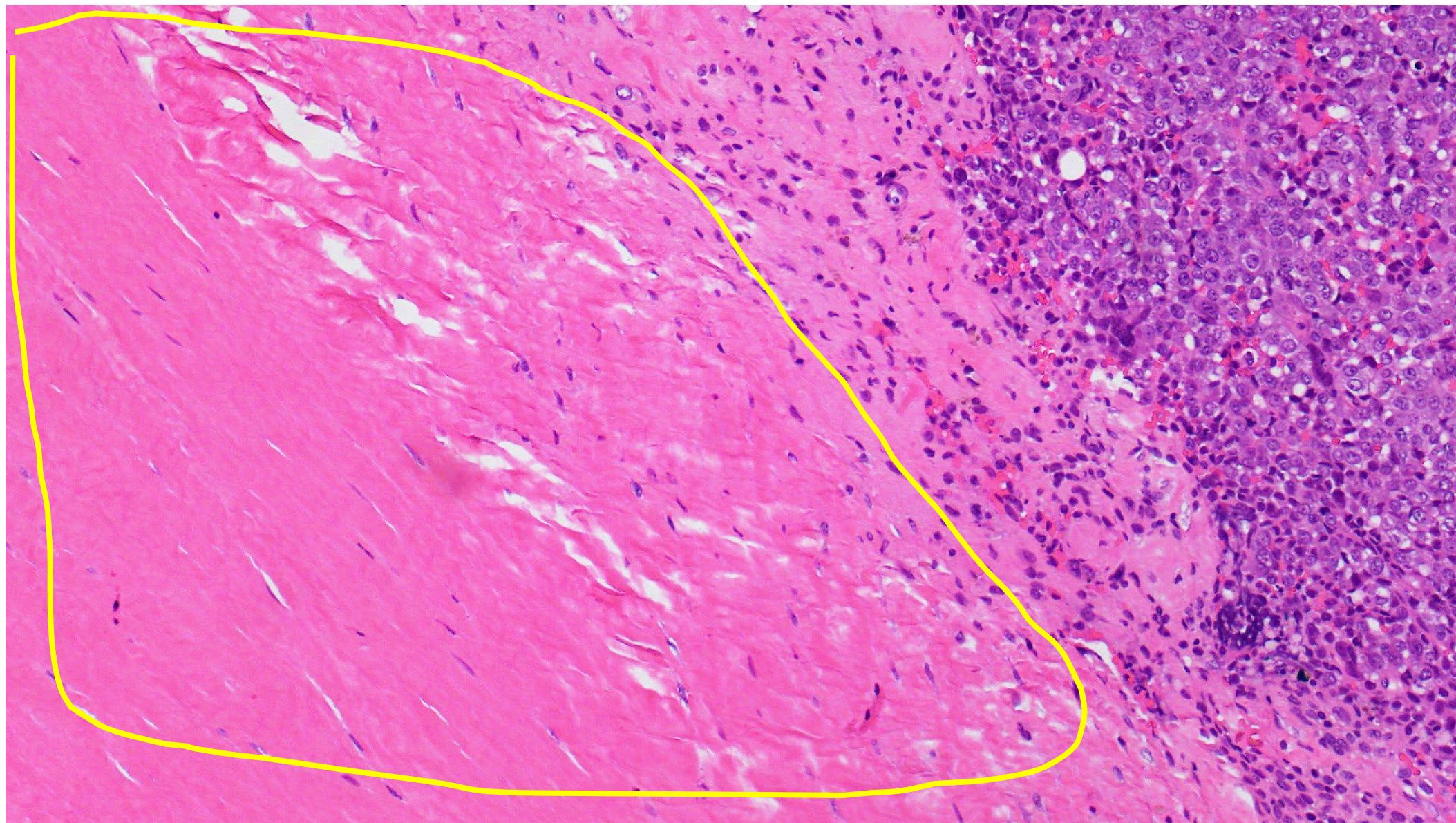
Necrosis



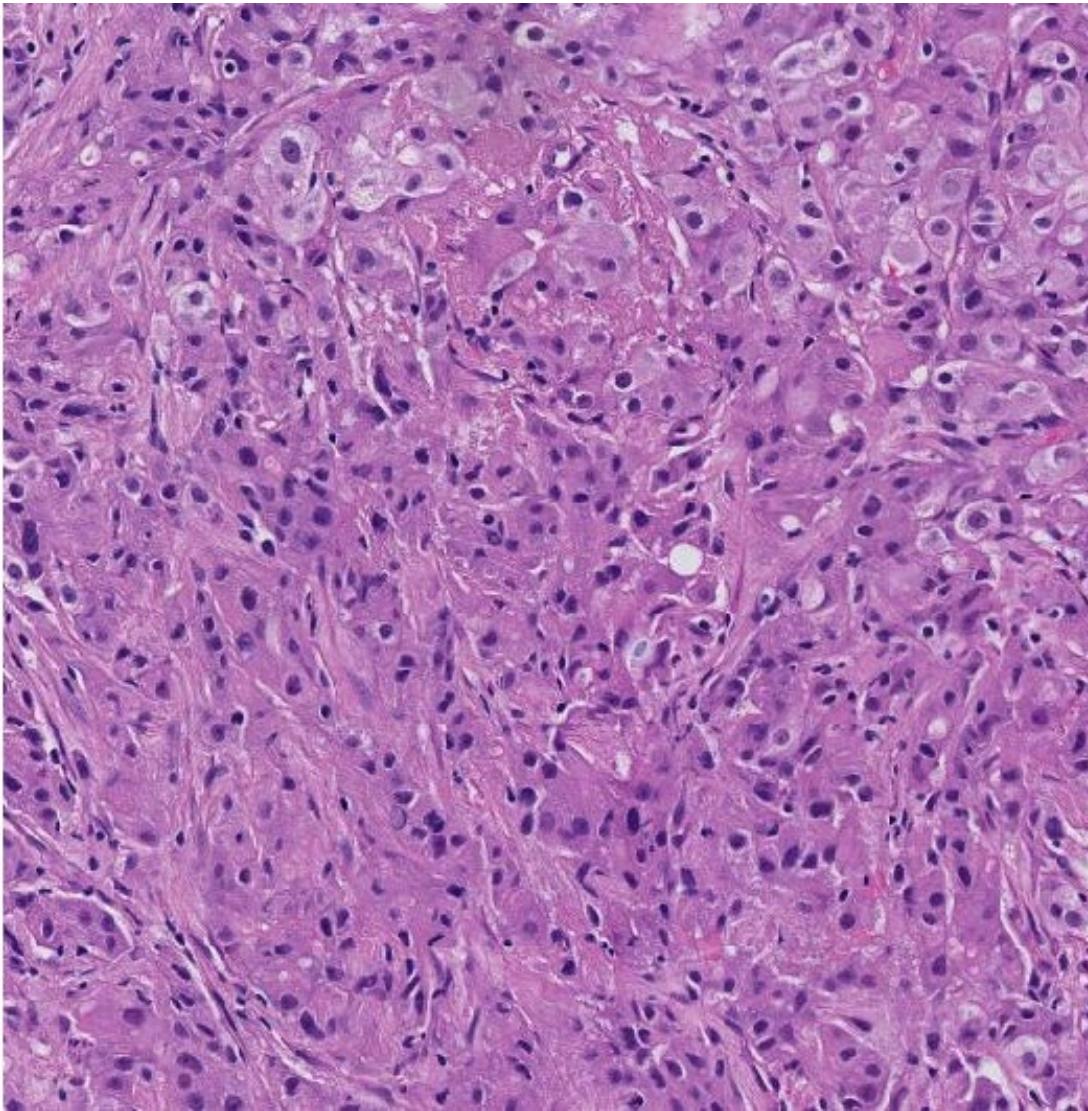
Dense fibrosis



Dense fibrosis



Challenge: Eosinophilia similar in tumor cells and stroma

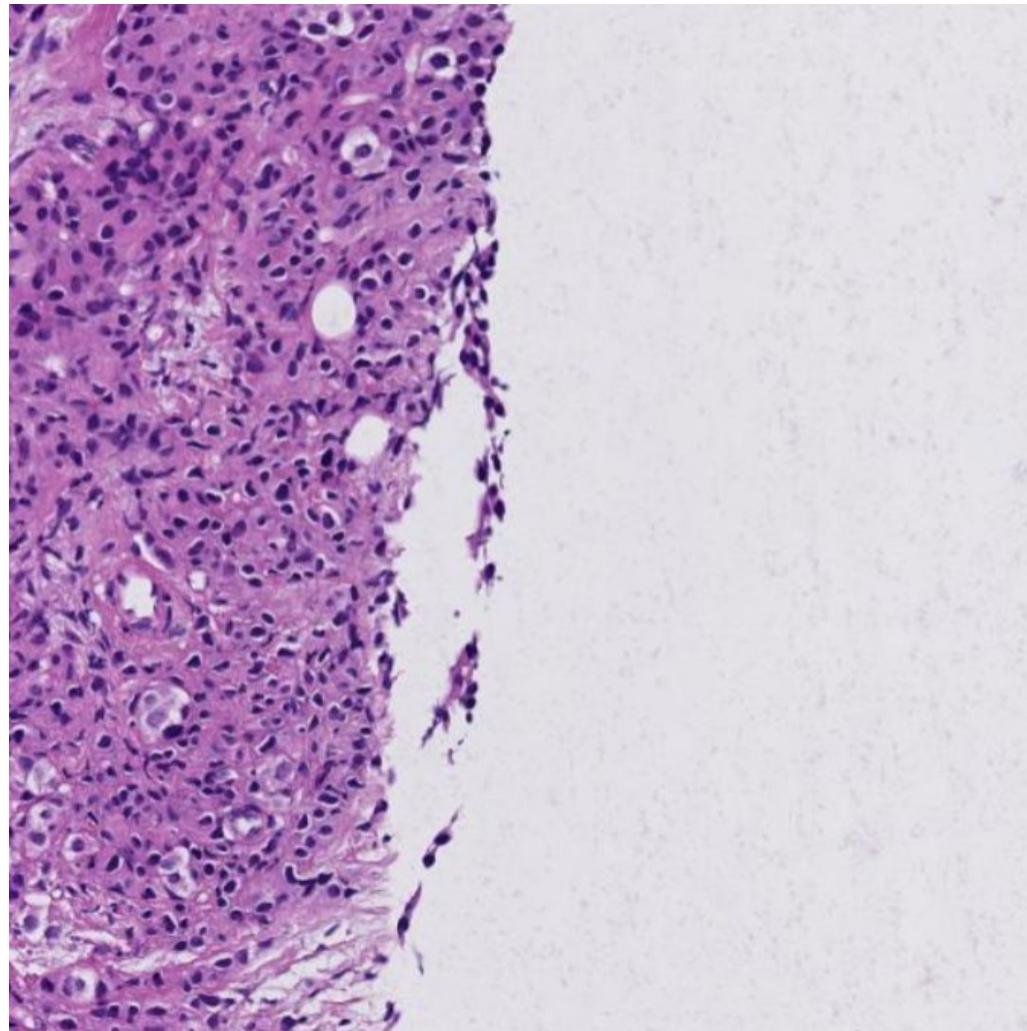


Area of tumoral stroma occupied by
mononuclear inflammation

X 100

Entire area of tumoral stroma

How much tumor-associated stroma is present?



ROI Type	Percent Tumor-Associated Stroma	sTILs Density
Evaluable	5	0
Evaluable	5	5
Evaluable	8	5
Evaluable	15	3
Evaluable	10	0
Evaluable	15	10

Mean Percent Tumor-Associated Stroma: 9.7
Mean sTILs Density: 3.8

Reporting stromal TIL Density

- Report stromal TIL density quantitatively, at least in 5-10% increments
 - Consensus threshold for low or high is under investigation
- Sample reporting language:

Average stromal tumor infiltrating lymphocyte density is approximately X %.

Note: Stromal TIL density has been shown to have significant clinical prognostic value in breast cancer.

Reference: Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015 Feb;26(2):259-71. doi: 10.1093/annonc/mdu450. Epub 2014 Sep 11. PMID: 25214542.

Additional resources

- Kos, Z., Roblin, E., Kim, R.S. *et al.* Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer. *npj Breast Cancer* **6**, 17 (2020). <https://doi.org/10.1038/s41523-020-0156-0>

Next: Independent Reading

Evaluation of tumor-infiltrating lymphocytes in breast cancer:
recommendations by the
International TILs Working Group

Continue to Part 4 of 4

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Acknowledgements

- This work was supported by the FDA Office of Women's Health. This project was supported in part by an appointment (V.G.) to the ORISE Research Participation Program at the CDRH, U.S. Food and Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and FDA/Center.