Part 1: Introduction to Stromal Tumor Infiltrating Lymphocytes

Slide 1. Title Page: Welcome to this continuing medical education activity about stromal tumor infiltrating lymphocytes in breast cancer.

Slide 2. This activity has 4 parts. In part 1, we will learn about the role the immune system plays in the development of cancer and the significance of stromal tumor infiltrating lymphocytes in breast cancer as a prognostic marker. Part 2 provides a video tutorial on the methodology for assessing the density of stromal tumor infiltrating lymphocytes, specifically in breast cancer. Part 3 addresses the pitfalls and challenges of assessing stromal tumor infiltrating lymphocytes in breast cancer. In Part 4, you will independently read the manuscript published by the international TILs working group that delineates recommendations for assessing stromal tumor infiltrating lymphocytes in breast cancer.

Slide 3. We will begin Part 1 now by introducing you to the subject of stromal tumor infiltrating lymphocytes. And we begin by discussing the role of immune cells in cancer.

Slide 4. Subtitle page: no narration.

Slide 5. There are several features that characterize cancer cells. Cellular growth and proliferation are dysregulated in cancer cells. In contrast to normal cells, cancer cells are much less reliant on external signals for multiplication. They are capable of self-sustained growth and proliferation: they may upregulate certain growth factor receptors, or even produce growth factors themselves that results in a self-stimulating positive feedback loop for proliferation. Simultaneously, they may become insensitive to growth inhibiting factors in their environment, for example through mutation or loss of certain cellular proteins. Cancer cells are also able to bypass the apoptosis pathway and evade programmed cell death. As a result, cancer cells have an infinite potential for replication. They can also stimulate formation of new blood vessels, invade adjacent tissues, and spread to other sites in the body.

Slide 6. As we know, cancer does not grow in a vacuum. In every patient, there is an immune system and its role includes detecting and eliminating abnormal cells, including pre-malignant cells or cancer cell precursors. This function is called Immunosurveillance. Once malignancy is established, the immune system seeks to destroy cancer cells. As cancer cells acquire additional mutations, they may be able to avoid detection by becoming less noticeable or immunogenic to the immune system, for example by decreasing cell surface expression of particular markers. This process allows some cancer cells to escape destruction by the immune system, and is called Immunoselection. Cancer cells may also acquire the ability to actively suppress threatening immune responses, such as by overexpressing certain proteins, in a process called immunosubversion.

Slide 7. Increasingly, we are becoming aware of the critical role that immune cells play in regulating cancer development. The layout of the immune cells in the environ of a tumor has been referred to as the “immune contexture,” and features include the density of the immune cells and how they are spatially organized around a tumor. Some recent research has focused specifically on the lymphocyte subgroup of the immune cells in tumor microenvironments. Lymphocytes in the tumor microenvironment are called “tumor infiltrating lymphocytes.” These lymphocytes may be intimately associated with the tumor cells themselves; in a carcinoma these would be called intra-epithelial tumor infiltrating lymphocytes. Tumor infiltrating lymphocytes may also be found in the stroma immediately adjacent to tumor cells; these are called “stromal tumor infiltrating lymphocytes.”

Slide 8. Not all tumors have infiltrating lymphocytes. But the immune cell microenvironment around a tumor is not a static feature. Pathways have been described for turning a “cold tumor” which has little or no associated immune cells, into a “hot tumor” which has attracted many immune cells to itself.

Slide 9. The presence or absence of tumor infiltrating lymphocytes (TILs) has been studied and this feature has been shown to have clinical prognostic utility in multiple solid tumors. The quantity of tumor infiltrating lymphocytes correlates with clinical outcomes.

Slide 10. TILs may be assessed in both the intra-epithelial and stromal compartments and the specific protocol for TIL assessment varies by tumor type.

Slide 11. Subtitle page: Stromal tumor infiltrating lymphocytes have been shown to be prognostic for breast cancer patient outcome.

Slide 12. When assessing TILs in breast cancer, we prefer to perform the assessment in the stromal compartment because stromal TILs are typically more numerous and less geographically heterogenous than intra-epithelial TILs. In addition, stromal TIL assessment is more reproducible between observers. Stromal TIL assessment for breast cancer can be performed on both core biopsies and excision specimens.

Slide 13. A study published in 2019 showed that patients with triple negative breast cancer with high TILs (which defined as 30% or greater in this study) had better overall survival (88%) compared with patients with low TILs (80%). Patients with stage 1 and high TILs had the best outcome, with overall survival of 98%. None of the patients in this study received adjuvant therapy.

Slide 14. A separate study showed that the presence of high TIL density in residual triple negative breast cancer that had been treated with neoadjuvant therapy correlated with a 91% overall survival, which was significantly better than the 55% overall survival for low TIL patients. Notably, the cut-off for defining high TIL density in this study was 60%, whereas the study we discussed on the previous slide used a 30% cutoff; currently there is no consensus on the specific quantitative threshold for what would qualify as high TIL density so you should report TIL density quantitatively in 5-10% increments.

Slide 15. The presence of high TIL density in breast cancer has been shown to be associated with better prognosis in breast cancer, specifically the triple negative and HER2+ subtypes. The prognostic value of TILs is maintained when reviewing gene expression data for immune response. Assessing TIL density has clinical utility when evaluated on core biopsies as well, so TIL assessment can be performed on small samples, not necessarily surgical excisions.

Slide 16. Again, high TILs in certain breast subtypes (TNBC, HER2+) are associated with the best clinical outcomes. And routine quantitative reporting of TILs is endorsed for TNBC by several international oncology and pathology organizations. The latest edition of the WHO blue book on breast tumors recognized the emerging importance of stromal TILs in characterizing breast cancer, and encourages using TILs to characterize cases being enrolled in clinical trials and prognostic studies.

Slide 17. A few additional resources for learning about TIL evaluation are listed here.

Slide 18. This concludes the first part of this continuing education activity. The next component involves viewing the video tutorial on performing TIL assessment in breast cancer.

End Transcript

New Transcript

Part 2: TILs Education: What are TILs and their Assessment

0:00
Dear viewer, thank you for watching this
0:02
video on TILs.
0:04
TILs are tumor infiltrating lymphocytes.
0:07
TILs are the immune cells that should
0:09
protect us against
0:11
infections in cancer. This video was
0:13
prepared by The International
0:15
Immuno-Oncology Biomarker Working Group.
0:19
Meet your companions in this story: the
0:21
pathologist,
0:23
the clinician, and the patient.
0:26
What do TILs look like? Well, TILs are
0:29
immune cells.
0:31
They give us an indication on how strong
0:33
immunity is
0:34
attacking the cancer cells. These tiny
0:37
little, small blue
0:39
cells are called lymphocytes and plasma
0:41
Cells.
0:42
They are the TILs. Cancer cells look
0:45
like this.

0:46
So, here we see plenty of immune cells
0:49
attacking the cancer.
0:52
This animation illustrates the location
0:54
of immune cells in the cancer.
0:56
The buildings represent the cancer cells;
0:59
the dots
1:00
are the immune cells trying to kill the
1:02
cancer.
1:04
On this image, you only see cancer cells.
1:07
There are no TILs, so the immune system
1:10
isn't trying to protect the patient.
1:12
The first publication on TILs was
1:14
exactly 100 years ago.
1:17
In 1920, two physicians from the Mayo
1:19
Clinic
1:20
Dr. Sistrunk, a surgeon, and Dr. MacCarty a
1:23
pathologist,
1:24
described the importance of the presence
1:27
of immune cells in breast cancer.
1:29
They established that "…patients with
1:31
glandular and with local lymphocytic
1:34
infiltration
1:35
lived 146 per cent longer than patients with
1:39
glandular

1:40
involvement without lymphocytic
1:42
Infiltration.
1:43
As general facts, they've given some clue
1:46
to the defensive mechanism of the body
1:48
against malignant neoplasms.” Regrettably,
1:52
the teals have been forgotten for a
1:54
century,
1:55
until their resuscitation today. TILs
1:58
have a level of evidence of Ib in
2:00
Triple Negative Breast Cancer
2:02
and HER2 positive Breast Cancer. This
2:05
evidence is much more substantial
2:07
than for some other biomarkers we used
2:10
for decades
2:11
in our breast cancer daily practice.
2:13
Assessment of TILs in breast cancer
2:16
using the internationally accepted
2:18
assessment guideline
2:19
will benefit both patients and future
2:21
cancer research.
2:23
Patients should feel empowered to
2:25
request testing
2:26
and ask for tools to be scored. Patients
2:29
will

2:29
immediately understand more about their
2:31
prognosis,
2:32
and this will help them with treatment
2:34
decisions and provide
2:36
longer-term reassurance. How should we
2:39
use the TILs?
2:41
TILs can be scored when making a
2:43
histological diagnosis of breast cancer.
2:46
TILs scored in daily practice provide
2:48
the oncologist with important
2:50
information
2:51
on the survival of their patients. TILs
2:54
inform patients on their cancer immunity
2:56
and help them understand their prognosis.
2:59
The day you need to identify patients
3:01
for immune therapy,
3:03
TILs can also help find those patients.
3:07
Now, let's take a closer look at the
3:09
guideline for the standardized
3:11
evaluation of tumor
3:12
infiltrating lymphocytes in breast
3:15
cancer.
3:16
This guideline consists of five steps
3:18
and is based on H&E

3:20
slides of core biopsies or full slides
3:23
of properly fixed tissues.
3:25
Step One: Define the area for TILs
3:28
evaluation.
3:30
Only TILs within the borders of the
3:32
invasive tumors are evaluated.
3:34
The invasive edge is included in the
3:37
evaluation
3:38
but not reported separately.
3:41
Immune infiltrates outside the tumor
3:43
borders are not included.
3:46
Large areas of central necrosis or
3:49
fibrosis
3:50
are not included in the evaluation.
3:55
Step Two: Focus on stromal TILs.
3:59
In the clinical setting, only stromal
4:01
TILs are relevant.
4:03
It's important to understand where to
4:05
score, namely in the stromal area.
4:09
Here's a tumor zone with both a stromal
4:11
as well as an
4:12
intratumoral or intraepithelial
4:14
compartment.
4:16
Scoring TILs in the intratumoral

4:18
compartment is poorly reproducible
4:20
between pathologists. That's why we score
4:23
TILs only
4:24
in the stromal area.
4:28
Scan the tumor at low magnification and
4:30
focus
4:31
on tumor stroma. The stroma contains
4:35
predominantly collagenous tissue,
4:37
with a few immune cells. All the stroma
4:40
contains many immune cells,
4:42
hence the collagenous tissue is difficult
4:45
to recognize.
4:49
Step Three: Determine the type of
4:51
inflammatory infiltrate.
4:54
Include only mononuclear cells,
4:56
Lymphocytes, and plasma cells,
4:58
not granulytes. Do not include immune
5:01
cells
5:02
in necrotic areas.
5:06
Step Four: As a first approach, include the
5:09
tumor in one of three groups
5:10
based on low magnification and assess
5:13
the percentage stromal TILs.
5:16
Group A: tumor with no minimal immune

5:19
cells,
5:20
0 to 10 percent stromal TILs. Group
5:23
B:
5:24
tumor with intermediate heterogeneous
5:26
Infiltrate,
5:27
10 to 40 percent stromal TILs. For this
5:30
intermediate group, evaluate different
5:32
areas at higher magnification
5:35
And, Group C: tumor with high immune
5:38
Infiltrate,
5:39
40 to 90 percent stromal TILs.
5:42
The denominator used to determine the
5:44
percentage stromal TILs is the area of
5:47
stromal tissue,
5:48
not the number of stromal cells.
5:53
Step Five: Report the percentage of TILs.
5:57
Report the average of the stromal
5:58
areas you see.
6:00
Don't focus on hot spots. On our website,
6:03
you'll find a set of reference images
6:05
that can help you define the stromal
6:07
TILs
6:08
for your case. Here are some examples.
6:11
There are no TILs on this image, so the

6:14
score is
6:14
0 percent. Here, the number of TILs is
6:18
still
6:18
very low, around 10 percent.
6:22
Now, we're up to around 50 percent.
6:26
And, on this final image, there are plenty
6:28
of immune cells, namely
6:30
more than 80 percent of TILs. These
6:34
are the five steps of our guideline for
6:36
the standardized evaluation of TILs.
6:39
We hope they come in handy in your daily
6:41
practice.
6:42
We are here to help, so if there are any
6:45
questions,
6:46
please contact us.
6:49
Be careful with the assessment of TILs,
6:52
there can be some pitfalls.
6:54
Visit our website and learn more about
6:56
them.
6:58
Also, have a look at the paper of Zuzana
7:00
Kos in Nature
7:02
npj Breast Cancer with all the details
7:05
hereof.
7:07
Nearly 25,000 people from 42 different

7:10
countries have visited our website.
7:12
Why don't you join the TILs Working Group?
7:15
Scoring TILs
7:16
is relatively easy and training tools
7:18
are freely available.
7:20
TILs informs patients on how immunity
7:22
looks like in cancer,
7:24
and it informs pathologists and
7:26
clinicians of the status of immunity
7:29
in the cancer of their patients. Finally,
7:32
it's all about collaboration if we want
7:34
to bring
7:35
new biomarkers into daily practice. Our
7:38
Working Group practices
7:39
what we preach, and we invite patients,
7:42
clinicians,
7:43
regulatory authorities, and industry to
7:46
be part of this.
7:47
If you want to know more about TILs,
7:49
please contact the International Immuno-
7:51
Oncology Biomarker Working Group.
7:54
Our work is supported with BCRF funds.
7:57
Thank you for your attention.

End Transcript

New Transcript

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

Slide 1. Title

Slide 2. This section will review how to calculate stromal TIL in breast cancer, and discuss structures that are excluded from the assessment, and challenges and pitfalls to assessing stromal TIL density in breast cancer.

Slide 3. To briefly review, stromal TIL density is defined as the proportion of tumoral stroma that is occupied by mononuclear inflammatory cells. Expressed as an equation, the definition of stromal TIL density, which is expressed as a percentage, is the area of tumoral stromal occupied by TILs over the entire area of tumor-associated stroma, multiplied by 100.

Slide 4. Stromal TILs in breast cancer are calculated over the entire invasive tumor, and includes the border of the invasive tumor. More likely than not, the TIL density will be heterogeneous over the entire tumor, and you should average the TIL density over the entire area of invasive tumoral stroma. Plasma cells and lymphocytes only are considered TILs.

Slide 5. There are some specific structures that you may encounter in your slide review that are to be excluded from calculating the stromal TIL density. These include thick-walled vessels, benign glandular elements, fat, CIS, and tertiary lymphoid structures.

Slide 6. This image contains a thick-walled vessel, which is circled. The area occupied by this thick-walled vessel does not count toward tumor-associated stroma, even though it is close to tumor and there are lymphocytes nearby. You would average TIL density over only the collagenous stroma in this image.

Slide 7. Here is an example of invasive lobular carcinoma that surrounded multiple thick-walled vessels. These vessels would not be counted as part of the tumor-associated stroma, and you would subtract these structures from the denominator of the TIL density equation.

Slide 8. This image shows invasive breast cancer adjacent to a thick-walled blood vessel. An expert panel determined that the proportion of tumor-associated stroma in this image ranged from 35% to 86%. The TIL density assessments provided by the panel ranged from 15% to 60%.

Slide 9. Benign ductal or glandular elements are also excluded from evaluation. Such structures are circled in these images. We can see there is a dual population of epithelial and myoepithelial cells in this duct (Left lower circled structure), which is surrounded by a basement membrane, features which allow us to recognize this as a benign duct. Similarly, basement membrane surrounds the duct profiles in this terminal duct lobular unit TDLU (center boxed structure) and this other TDLU on the right here (right outlined structure). There are nests of invasive carcinoma flanking these benign structures, but the benign structures themselves do not count toward tumor-associated stromal area.

Slide 10. In addition, any lymphocytes associated with the benign ducts do not count as TILs, so you would not count any of this inflammation in the numerator of the equation. In this

particular case, it is relatively easy to the border between the edge of the invasive carcinoma on the right and this area of benign ducts with heavy lymphocytic inflammation on the left. If this focus of inflamed benign ducts were an island embedded in the center of an invasive tumor, such as in this case…

Slide 11. …where we have a TDLU with associated lymphocytes and plasma cells, with abutting invasive carcinoma, this focus would then be excluded from the denominator as it is not an area of tumor-associated stroma, and the lymphocytic inflammation would be excluded from the numerator because the inflammation is not associated with tumor but rather with benign ducts.

Slide 12. This is an image of invasive breast cancer with an adjacent benign terminal ductal lobular unit. An expert panel of pathologists assessed the stromal TIL density, and their responses ranged from 0% to 10%, with an average of 4.8%%

Slide 13. Fat is excluded from the denominator in TIL assessment. Adipose tissue is an element normally found in breast tissue, and as pathologists we typically think of adipose tissue as a type of stroma. However, for the purposes of TIL assessment in breast cancer, adipocytes actually do not count as stroma. The idea behind this is that lymphocytes cannot occupy the same space as fat cells; lymphocytes can, however, overly fibrous tissue. In some cases, you will have a small amount of fat, such as in the image on the left. The area of tumor-associated stroma would include all the eosinophilic staining regions of pink collagen and elastic fibers. The small islands of fat would not count towards the area of tumor-associated stroma (the denominator), and we would calculate the TIL density based on only the pink staining regions of tumoral stroma. In other cases, such as on the right, invasive cancer can be seen infiltrating through large amounts of fat. Again, the adipocytes themselves do not count as tumoral stroma. The area of tumor-associated stroma we would be interested in assessing would be the fibrous collagen that is present between fat cells. When you see fat cells packed very closely together, there is essentially no stroma present for evaluation. You really need to see the eosinophilic fibers in order to count as stroma. We would average the TIL density over only eosinophilic stroma that is present between adipocytes.

Slide 14. This is an image of invasive breast cancer associated with adipocytes. Only the pink collagenous stroma may be considered in the denominator; the area occupied by adipocytes is excluded from evaluation. An expert panel of pathologists assessed the stromal TIL density, and their responses ranged from 0% to 4%, with an average of 1.2%. And that is reasonable since we do not see any significant number of lymphocytes in the stroma. However, it is notable that when the expert panel was asked how much area in this image was occupied by tumor-associated stroma, their answers ranged from 5% to 50%. It is easy to be tripped up by adipose tissue and even experts had a difficult time achieving consensus on the amount of evaluable stroma in this case.

Slide 15. This image is an example of carcinoma in situ, in the circle, which is flanked by invasive carcinoma. The area occupied by carcinoma in situ does not count toward the area of tumor-associated stroma. Any inflammation associated with the CIS would also be excluded from TIL assessment. This case in addition shows significant crush artifact, which limits our ability to be confident about identifying lymphocytes.

Slide 16. Here is an example of ductal carcinoma in situ (DCIS) involving a duct on the left, which is adjacent to invasive carcinoma on the right. The DCIS has associated stromal lymphocytes. But

the DCIS does not count as tumor-associated stroma, and the lymphoplasmacytic inflammation associated with the DCIS is not considered to be TILs.

Slide 17. Occasionally you will see lymphoplasmacytic inflammation that focally forms an aggregate and has a germinal center. This structure is a tertiary lymphoid structure (TLS), which is essentially an ectopic lymph node structure, and it is not considered to be TILs. So you would not count the lymphocytes in this TLS in the numerator of the TIL density equation.

Slide 18. Certain phenomena can mimic tumor infiltrating lymphocytes. These include degenerating cells with apoptotic or pyknotic nuclei, cells that exhibit perinuclear clearing, and fibroblasts that are sectioned axially.

Slide 19. Apoptotic forms are present in these 2 images as small dark black dots. These tell us there is cellular degeneration or necrosis occurring. It is possible some or all of these small dark forms are degenerated or necrotic tumor cells. These small dark forms come in a range of sizes, and when they are occasionally the same size that we would expect for lymphocytes, then it becomes difficult to distinguish apoptotic bodies from lymphocytes. These images show many cells with perinuclear clearing. Cells with perinuclear clearing may be interpreted macrophages, but alternatively they could be either apoptotic nuclei or lymphocyte nuclei with retraction artifact. Retraction artifact can occur with suboptimal tissue fixation. Areas of tumor like these 2 images contain a combination of apoptosis and suboptimal tissue preservation, and are not reliable for TIL assessment. Occasionally, tumor cells show cleared out cytoplasm, in which case that would be another possibility in the differential.

Slide 20. These are additional example images of cells with perinuclear clearing. On the left you can see the tumor cell shows cytoplasm with pale eosinophilia, which is similar to the cytoplasm associated with some of these adjacent smaller cells, which appear to be inflammatory. In this case, we can easily recognize this is a tumor cells because of its obvious nuclear pleomorphism. But if the tumor were low grade, it may become difficult to distinguish the tumor cells from a background of morphologically similar inflammatory cells. The differential is again between macrophages and lymphocytes that have perinuclear clearing due to suboptimal fixation. Similarly on the right, we have cells with perinuclear clearing; some have larger nuclei and appear to be cohesive or clustered, which allows us to recognize these are tumor cells and distinguish them from the adjacent inflammatory cells. As the tumor cells become blander, it can become more difficult to discern carcinoma from macrophages, for example. You can use immunohistochemistry such as keratin or CD8 staining to help define the populations you are evaluating.

Slide 21. This is an image of invasive breast cancer with a significant amount of cells showing perinuclear clearing. It is challenging to definitively classify some of the inflammatory cells as lymphocytes or macrophages. An expert panel assessed this case and provided TIL densities ranging from 70% to 90%, so they appeared to interpret most of the cells surrounding the carcinoma as lymphocytes.

Slide 22. Cross-sectionally cut fibroblast nuclei may mimic lymphocyte nuclei. On the left you see multiple bands of pink collagen fibers that are infiltrated by tumor cells. These smaller darker nuclei are likely to be lymphocytes (green arrowheads). If you notice, the lymphocyte nuclear diameter in this example is similar to the nuclear diameter along the short axis of a fibroblast (yellow arrowheads). So if this fibroblast nucleus, which is typically elongate, were sectioned at axially, it could appear to be a lymphocyte; it could be difficult to be certain how many of these

nuclei are truly lymphocytic. Fortunately, we could use immunohistochemistry to help us. In addition, lymphocyte nuclei are often a bit darker staining than fibroblast nuclei. Similarly on the right, we have bands of collagen containing a combination of fibroblasts and lymphocytes. From the side view, fibroblast nuclei are easily recognized with their elongate morphology (yellow arrowheads). The fibroblast nuclei in this case have a similar nuclear diameter to lymphocytes, which we see elsewhere, which are nice and round, and slightly darker in this case (green arrowheads). So cross-sectionally cut fibroblasts could be a potential challenge for interpretation.

Slide 23. You should avoid assessing stromal TIL density in areas of inflammation that are associated with a healing biopsy site, areas with crush artifact, and necrotic or densely hyalinized or sclerotic areas.

Slide 24. Previously biopsied breast tissue, such as shown here, typically shows chronic inflammation on the subsequent excision specimen with variable populations of lymphocytes, plasma cells, and macrophages that are associated with degenerating adipocytes.

Slide 25. Because the invasive tumor is the target of the biopsy, there is usually an intimate spatial relationship between the biopsy site and the invasive carcinoma. Lymphocytes can be in close proximity to nests of invasive tumor. However, if other features of biopsy site change are present (like here, we can see aggregates of macrophages related to a prior biopsy), then we would not consider this lymphoplasmacytic inflammation to be TILs.

Slide 26. Crush artifact is a challenging scenario to evaluate. On the left, the image contains crushed nuclei, frequently at the edges of tumor cell aggregates (examples are highlighted by yellow arrowheads). We also see some scattered apoptotic nuclear debris (green arrowhead). It would be difficult to feel confident to assess the nature of the crushed cells- whether they are tumor cells or inflammatory cells. The cells at the edges of aggregates appear crushed and are hard to appreciate. On the right, there is an area of eosinophilic tumor cells, and an adjacent (circled) area with cells with smaller nuclei infiltrating through the stroma. These are possibly lymphocytes but there is also a suggestion of apoptotic debris because we do see these very small dot-like forms that could be karyorrhexis. So it could be possible to overcount TILs in this example if we did not recognize that some of these are not lymphocytes but rather are apoptotic degenerated forms. In this case, there is also some clearing of the cytoplasm in the carcinoma cells. Fortunately we are able to recognize the carcinoma because of its clustered architecture. If the carcinoma cells were more discohesive, we may mistake some of them for macrophages, particularly if the cells are low nuclear grade.

Slide 27. Definitely avoid areas like this where there is severe crush artifact and the cells are essentially impossible to categorize.

Slide 28. In this image, of invasive breast cancer and associated stroma with inflammation, there is frequent perinuclear clearing (e.g. yellow arrowhead) and likely some scattered apoptotic forms (e.g. green arrowhead). An expert panel provided TIL densities ranging from 30% to 65%, with and average of 46.8%.

Slide 29. Do not evaluate areas with geographic tumor necrosis. Findings in necrotic zones (circled) like this do not count toward either the numerator or denominator of the TIL density equation. In this case there is viable appearing carcinoma along the left border of the image and a pink zone of geographic necrosis on the right. In between is an area containing lymphocytes.

This area in between counts as tumor-associated stroma, and the lymphocytes within it are considered TILs.

Slide 30. You can also get areas of dense fibrosis, for example if there is carcinoma arising within the fibrous scar of a prior surgical excision. This is a case of metaplastic carcinoma so this tumor has 2 morphologic appearances: on the left it has a myxoid appearance and on the right it is a highly cellular grade 3 invasive ductal carcinoma. Between these 2 areas of tumor is a strip of eosinophilic densely fibrotic tissue. This densely fibrotic zone would not be considered tumor-associated stroma. On closer inspection of the tumor area on the right side…

Slide 31. …we can see there is a little bit of tumor-associated stroma that would be counted in the denominator of the TIL density equation. We can morphologically appreciate that there is a difference in the very pink coloration of the densely collagenized tissue on the left which has extremely low cellularity, in contrast to the stroma immediately adjacent to the tumor, which has some small vessels within it, scattered infiltrating cells, a slightly less pink color and a slightly looser quality to the collagen fibers. We can quite easily draw a line distinguishing these 2 stromal areas. The area to the right of the line, immediately abutting the invasive carcinoma cells would be counted in the denominator as tumor-associated stroma, and any lymphocytes in this area would be assessed as TILs. The densely fibrotic area to the left of the line would not be counted as tumor-associated stroma, and would not be evaluated for TILs.

Slide 32. And finally, occasionally tumor cells exhibit eosinophilic cytoplasm that is very similar in color to the adjacent stroma. In such scenarios, it can be difficult to sort out where the tumor border with the stroma is. It would be challenging to assess the stromal compartment for TILs if you are not entirely sure which areas contain stroma. This case also happens to have scattered foci of nuclear degeneration and apoptosis.

Slide 33. This image shows invasive breast cancer with cytoplasmic eosinophilia that is difficult to differentiate from adjacent stroma. An expert panel assessed the amount of cancer-associated stroma to be between 5% and 15%, and the TIL density to range from 0% to 10%.

Slide 34. Stromal TIL density is reported as a continuous variable and usually in 5-10% increments. At this point, there is no consensus for specific cut-offs that would denote low, moderate, or high TIL density categories for breast cancer. That is still under investigation. As a result, for now it is best not to qualify the TIL density, but rather to report it out quantitatively. A sample of the language that we could use in a pathology report would be to state that the average stromal tumor infiltrating lymphocyte density is approximately X%, and to add a note or comment that stromal TIL density has been shown to have significant prognostic value in breast cancer with some references.

Slide 35. This paper by Kos et al is an additional resource on the topic of pitfalls in evaluating breast cancer stromal TILs.

Slide 36. The next component of this CME activity is to read this article by the international TILs working group.