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REVIEW

Training pathologists to assess stromal tumour-infiltrating lymphocytes in breast cancer synergises efforts in clinical care and scientific research

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Training pathologists to assess stromal tumour-infiltrating lymphocytes in breast cancer synergises efforts in clinical care and scientific research

A growing body of research supports stromal tumour-infiltrating lymphocyte (TIL) density in breast cancer to be a robust prognostic and predicive biomarker. The gold standard for stromal TIL density quantitation in breast cancer is pathologist visual assessment using haematoxylin and eosin-stained slides. Artificial intelligence/machine-learning algorithms are in development to automate the stromal TIL scoring process, and must be validated against a reference standard such as pathologist visual assessment. Visual TIL assessment may suffer from significant interobserver variability. To improve

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interobserver agreement, regulatory science experts at the US Food and Drug Administration partnered with academic pathologists internationally to create a freely available online continuing medical education (CME) course to train pathologists in assessing breast cancer stromal TILs using an interactive format with expert commentary. Here we describe and provide a user guide to this CME course, whose content was designed to improve pathologist accuracy in scoring breast cancer TILs. We also suggest subsequent steps to translate knowledge into clinical practice with proficiency testing.

Keywords: breast cancer, continuing medical education, FOAMed, pathology education, tumour infiltrating lymphocytes

Introduction

Cancer prognosis and response to immune checkpoint inhibitor therapies rely upon endogenous host effector T cells in the tumour microenvironment, which consist of heterogeneous immune cells that may augment or oppose cancer growth. These immune cells are known as tumour-infiltrating lymphocytes (TILs) and can be characterised quantitatively (e.g. 'hot' tumours show high numbers of TILs compared with 'cold' tumours) and geographically (e.g. TILs may be intimately associated with tumour cells and/or found in tumoral stroma). A growing body of evidence supports the TIL density within tumour-associated stroma (stromal TILs) as a robust prognostic and predictive biomarker in breast cancer. For example, high levels of stromal TILs in triple-negative breast cancers (TNBCs) and human epidermal growth factor 2-positive (HER2+) disease are associated with decreased risk of cancer recurrence and patient death.[1,2] High stromal TILs in TNBCs also correlate with increased response to neoadjuvant chemotherapy and immunotherapy.[3,4]

Assessing and reporting breast cancer TILs: current practice and future directions

The St Gallen Consensus, European Society of Medical Oncology and World Health Organisation (WHO) Blue Book on Breast Tumours all currently suggest that stromal TIL scores may be useful for prognostication.[5,6] However, routine reporting of stromal TILs has not yet been widely integrated into clinical guidelines by professional oncology and pathology organisations. Time lags between research data generation and medical guideline issuance are necessary to ensure that data are critically reviewed for patient safety and efficacy where, on average, the time lag has been estimated to be 17 years.[7] However, interventions occasionally enter real-world use before formal guidelines are issued, such as new targeted therapies and off-label drug uses.[8] A global survey conducted by the International Immuno-Oncology Biomarker Working Group (also known as the TILs-WG; www.tilsinbreastcancer.org) and the University of Leuven (Leuven, Belgium) found that among 168 respondents from more than 30 countries, more than 95% consider TILs a clinically valuable prognostic and/or predictive biomarker for breast cancer management. Nearly half of survey participants currently report TILs in breast cancer or plan to do so in the near future (see Supporting information, Data S1 and S2, Figures S1–S7). Anecdotally, patient awareness of TILs is also growing, with individual breast cancer patients requesting TIL quantitation. As TIL scoring can provide patients with valuable data regarding their risks of recurrence and long-term survival, it is unsurprising that some clinics have already instituted routine TIL reporting.[9]

Currently, stromal TIL density in breast cancer is determined by pathologist visual assessment.[10] Standard haematoxylin and eosin (H&E)-stained slides of tumour sections are reviewed to generate a score from 0 to 100%, equal to the area of tumoral stroma occupied by lymphocytic inflammation divided by the area of tumoral stroma. This may be performed on core biopsies or larger excisions, including specimens with residual disease after neoadjuvant chemotherapy or immunotherapy. No ancillary testing is involved, rendering TILs an inexpensive and potentially widely available biomarker. However, performing the stromal TIL visual assessment without direction has been shown to suffer from observer variability, as is typical for biomarkers interpreted by pathologists in routine practice.[11]

Pathologist agreement levels range from excellent to fair, depending upon whether stromal TILs are treated as a continuous or categorical variable, and if whole slides or specific fields of view are analysed.[12–17] Harmonisation of data collection and analysis

methods can help to clarify our understanding of observer agreement levels, especially if data are shared to allow meta-analyses, but pathologist education can also improve observer concordance. As the scientific and medical communities elucidate the clinical significance of stromal TILs as a biomarker, continuing to collect TIL data as a continuous variable per current recommendations will enable eventual optimisation of cut-point values for clinical decision making. The TILs-WG previously published freely available training materials, as well as guidance on potential pitfalls that complement their practice recommendations, to support standardised TIL assessment in breast cancer.[10,18] However, interobserver agreement may still be suboptimal even when applying these recommendations. For example, inter- and intraobserver variability may increase in difficult cases with heterogeneous immune infiltration or artefacts that mimic immune cells. In such situations clinical management of patients may be negatively impacted, especially if a quantitative cut-off is employed.[13] Suboptimal observer agreement may also negatively impact related research efforts, such as the creation of tools for identification and quantification of objects in digital images. Hence, the pathology community should not hesitate to employ standard training and implement new technologies that may improve the accuracy of TIL assessment where it is necessary.

There is significant research interest in developing artificial intelligence/machine-learning (AI/ML) algorithms to improve pathologist evaluation of tissues, including quantification of TILs. AI/ML utilisation could be particularly helpful for cases that are challenging to interpret. Such AI/ML algorithms must be validated against a reference standard, which is frequently determined by pathologist visual assessment. Recognising the challenges to create a validation data set and evaluate the performance of an AI/ML algorithm, we came together as a multidisciplinary collaborative group to explore data collection and analysis methods, including experts at the US Food and Drug Administration (FDA), academic medical centres (AMCs) and industry. We refer to our efforts as the High-Throughput Truthing (HTT) project, which aims to generate an AI/ML algorithm validation data set derived from pathologist annotations (project website – https://didsr.github.io/HTT.home/).[19] Such a validation data set could qualify as an FDA medical device development tool (MDDT) and would be a valuable public resource for others developing quality validation data sets.[20,21] A successfully validated algorithm could be more confidently adopted into clinical practice knowing how it performs on a reference data set. Subsequently, with appropriate validation, the data collection and analysis methods developed by the HTT project may be applied to other diseases, including non-breast malignancies, to evaluate TILs as well as other visually assessed variables such as mitotic figures, nuclear grade, and immunohistochemical and multiplex immunofluorescent staining.

FDA CME course in breast cancer TIL assessment: structure and user guide

Training is essential to ensure competence in scoring breast cancer stromal TILs. To optimise pathologist performance of this task, we created free open-access medical education (FOAMed) materials with multistakeholder input. The impacts of this education are potentially far-ranging and would include the ability to support the clinical value of stromal TILs as a biomarker, to enable enrollment of patients into clinical trials and to create high-quality data sets for validating AI/ML models.[22,23] This course, which launched online in March 2023, is the FDA's first-ever anatomical pathology-focused CME course and is freely available at https://ceportal.fda.gov/. The course structure comprises the following multimedia format: a series of three video lectures and an independent reading of the published TILs-WG recommendations (Figure 1). The three videos, respectively, introduce the foundational scientific observations that support stromal TILs as a biomarker in breast cancer, detail the methodology used for stromal TIL assessment and quantitation, and review pitfalls of TIL assessment with histological examples. In addition, we provide a visual quantitation 'cheat-sheet' to assist pathologists in calibrating their stromal TIL evaluation and additional detailed case material with expert commentary for participants who seek a deeper dive into high-complexity/challenging cases (Figure 2).[10] Completing the course enables participants to claim 3.0 AMA PRA category 1 CME credits. The lecture slides may be downloaded for learner reference, and the content may be used over time and accessed worldwide, allowing for asynchronous learning and user flexibility. Participants may also return to the course to revisit areas of interest or deficiencies and/or to refresh their knowledge, which may be especially helpful in clinical practice settings with low volume or diversity of breast cancer cases. Detailed instructions for enrolling in this course are provided in Supporting information, Figure S8.

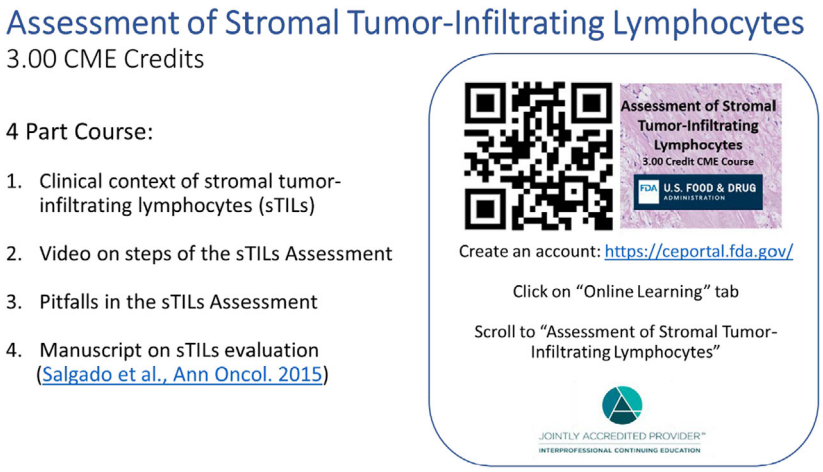
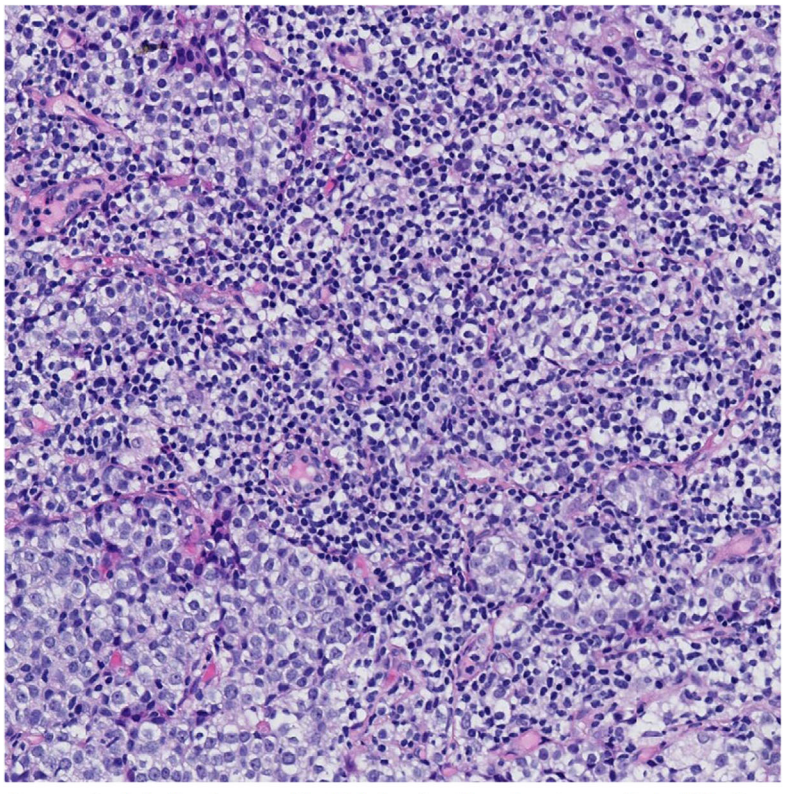


Figure 1. Structure of FDA continuing medical education course to train pathologists in breast cancer stromal TIL assessment.



Comments: A challenging case. The high density of lymphocytes results in difficulty determining whether the lymphocytes are located in stroma, or whether they infiltrate tumor cell nests. The presence of small blood vessels and small gaps between lymphocytes suggest the lymphocytes reside within stroma. Occasional tumor cells with small nuclei (possibly degenerating) may be confused for lymphocytes.

Figure 2. Example of case material provided to course participants, highlighting challenging scenarios for breast cancer stromal TIL assessment. Case-specific scores and comments from expert panellists provide additional guidance to learners.

This FDA CME course supports pathologists staying current on clinical concepts in breast cancer characterisation by addressing knowledge gaps. Reducing deficiencies in pathologist knowledge and familiarity regarding TIL reporting can improve the performance and frequency of pathologists scoring TILs. Specific

language is suggested for use in documenting the stromal TIL density in patient pathology reports, which can assist pathologists with implementation of a procedure that is not yet standard of care. As a free web-based course, it significantly reduces geographic and financial barriers to accessing professional educational resources and is accessible by the global pathology community. There has been a notable rise in the number of FOAMed offerings online, but many are of unclear quality.[24,25] This FDA CME course was developed in a highly collaborative fashion, involving pathologists from the FDA, six AMCs in the United States and Europe, industry and the TILs-WG. The participation of scientific experts and opinion leaders in the field of breast cancer ensures that the content is high-calibre, timely, evidence-based and presents a balanced perspective that avoids single-institution bias. This course was designed by and tailored to anatomical pathologists working in this arena and is endorsed by the International Society of Breast Pathology (personal communication).

Completion of CME coursework is the first step for successful conversion of acquired knowledge into everyday routine clinical practice as well as for clinical trial work, which requires documenting pathologist competence in assessing any biomarkers under evaluation. Completion of our CME course before embarking on clinical trial work involving TILs could serve this purpose. Repeated completion of the CME course during and after the trial could also provide documentation of competency maintenance. Such an approach would actively support local (i.e. non-centralised) laboratory biomarker assessment, which would directly increase the number and diversity of patients and healthcare centres that may participate in clinical trials. Using local testing for trial-inclusion of patients is an emerging new paradigm of future trials.

Next steps for pathologists: deploying knowledge into practice

Anatomical pathologists generally learn to exercise new skills by independently evaluating a case, and then comparing their findings with those of credentialled or expert pathologists who can then provide feedback. To model this learning framework in a web-based setting, participants would be well-positioned to take our feedback test and proficiency test (https://wolf.cci.emory.edu//camic/htt/login.html) after completing the CME course. While not required for CME credit, these tests allow pathologists opportunities to hone their skills. The tests are available through caMicroscope, an open-source, web-based digital whole-slide image viewer platform.[26,27] In both tests, pathologists are presented with a series of images showing regions of interest (ROI) derived from digitally scanned de-identified authentic patient breast cancer core needle biopsy samples. The cases in these tests are a 2:1 mix of cases that generated high and low pathologist variability, with a subset including pitfalls as determined in a pilot study and during expert panel sessions.[23] For each test, pathologists are asked to quantify the amount of tumoral stroma and stromal TIL density within the ROI. In the feedback test, participants score 36 ROIs and are informed how their evaluation compares to the performance of six experts (https://didsr.github.io/HTT.home/assets/pages/training-2023/feedbackRefDoc). In this way, participants can appreciate discrepancies between their score and the scores of experts while still able to review the ROI. Additional text curated by the experts is displayed that describes important points and pitfalls relevant to each ROI (Figure 3). This interactive format, where immediate feedback is given to learners, can help to identify strategies to help improve individual performance and agreement with expert interpretations. A similar methodology has been utilised in other CME courses developed by experts in breast pathology to educate pathologists in subject matter with lower interpathologist agreement. Such training sets were shown to improve diagnostic agreement between individual pathologists and consensus reference diagnoses for difficult areas in breast pathology.[28]

After completing the feedback test, participants may progress onto the proficiency test, where they evaluate another set of 36 ROIs in a similar fashion, but without receiving feedback from experts for comparison. After completing the feedback and proficiency tests, participants receive a performance test report for each which compares the participant's scores to experts (Figure 4 and Supporting information, Data S1). Participants pass these tests if their agreement with experts is as good as the agreement among experts in each case. Passing the proficiency test for breast cancer stromal TIL assessment should provide pathologists with the necessary self-confidence to deploy their knowledge into routine practice, complementing existing training tools freely available on the TILs-WG website. Conceptually similar proficiency testing in anatomical pathology already exists in the form of gynaecological Pap test evaluation, which is administered annually to ensure high-quality diagnostic performance in clinical practice.[29]

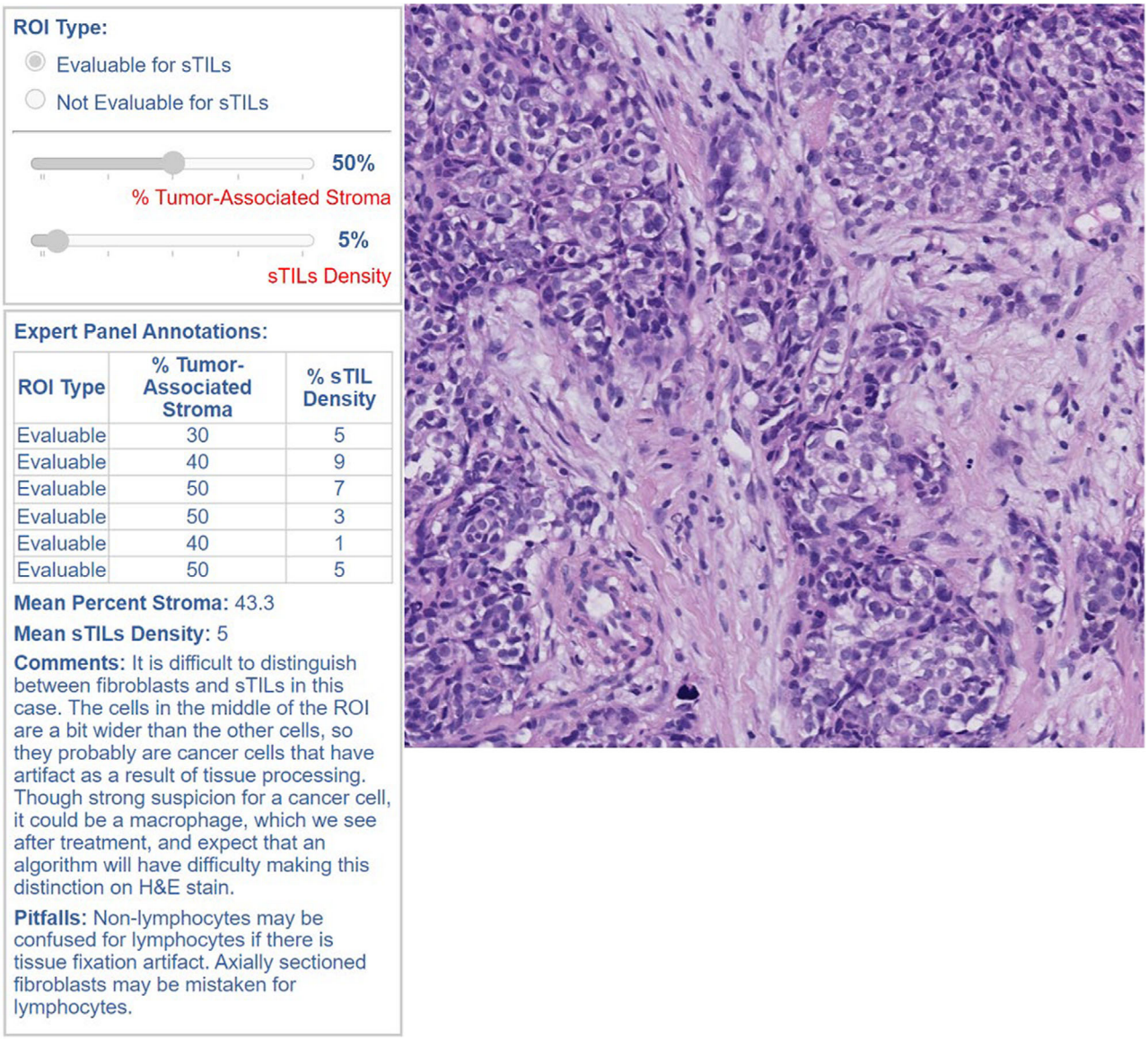


Figure 3. Sample feedback. Participants score ROIs for stromal TILs and percentage of tumoral stroma for the feedback test, and then compare their performance to that of six experts. Mean sTIL density and mean percentage of tumor-associated stroma are averages based on expert panel annotations. Additional commentary from experts highlights diagnostic issues relevant to each ROI. [Colour figure can be viewed at wileyonlinelibrary.com]

In addition to demonstrating competency in their ability to score stromal TILs in breast cancer, passing the proficiency test qualifies pathologists to participate in a pivotal study led by the HTT collaborators (https://didsr.github.io/HTT.home/assets/pages/whatIsHTT). In this currently ongoing study, participating pathologists will estimate the stromal TIL densities in ROIs derived from TNBC H&E biopsies. The ROI-based format was deliberately chosen for the feedback and proficiency tests as it dovetails with the pivotal study methodology to enable creation of a reference standard for validating AI/ML algorithms. The virtual nature of training in TIL assessment and data collection for the study supports pathologist engagement in biomedical research efforts regardless of their practice location or type. Encouraging more pathologists to contribute to biomedical research in this manner enhances the overall quality and representativeness of the collected data, which can accelerate clinical translation of scientific discoveries for patient benefit. Participation may be particularly meaningful for practitioners worldwide, who do not

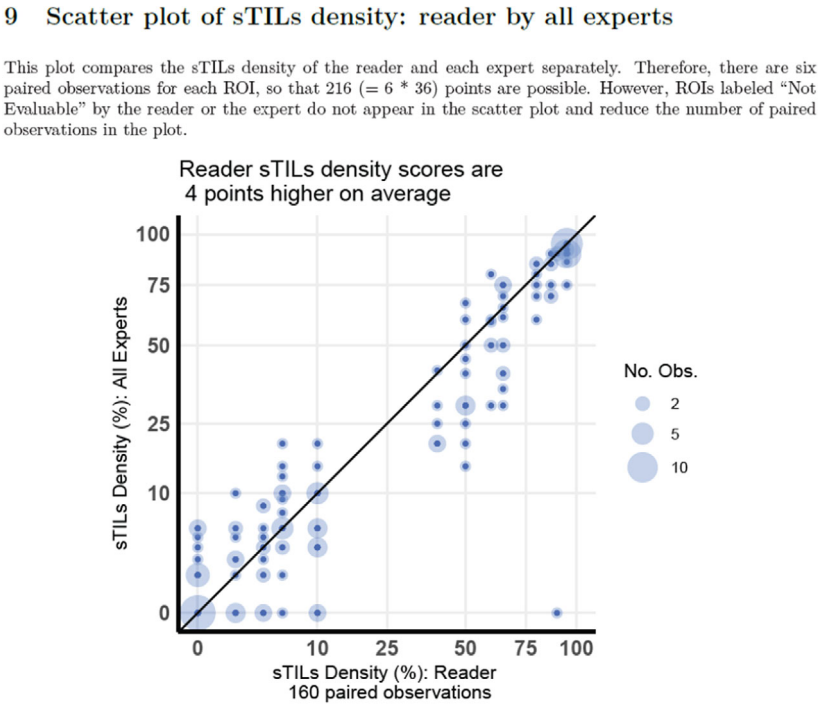


Figure 4. Sample graph comparing participant performance to the performance of experts for all regions of interest scored in the feedback test. See Supporting information, Data S1 for a full sample report. [Colour figure can be viewed at wileyonlinelibrary.com]

otherwise have easy access to such opportunities, while also inviting collaboration with pathologists working at the 'periphery' of the field, including those in community and non-academic hospitals.

Conclusions

Highly collaborative multi-institutional approaches such as this, involving trusted sources to create educational material based on the best available level of evidence, are more likely to engender broad-based acceptance from practising pathologists. In addition, barriers are lowered for training in and adoption of reporting new biomarkers such as stromal TILs by utilising a FOAMed format that is broadly accessible and inclusive. To date, this FDA CME course has enrolled participants from countries as diverse as Uganda and Thailand, demonstrating its rapid global reach. Stromal TIL assessment and analysis are increasingly incorporated into breast cancer clinical trial designs, and routine reporting may be incorporated into oncology and pathology clinical guidelines in the near future. Building local capacity at the level of individual pathologists will advance effective implementation of stromal TIL reporting into practice worldwide. This educational tool both enables interested pathologists to participate in related research and exemplifies ways to efficiently achieve high-quality healthcare and drive scientific progress. The FDA CME course, interactive training modules, proficiency testing and pivotal study resulted from an international partnership between healthcare providers and academic, regulatory and industry scientists that synergises professional education, research and regulatory aims while maintaining respect for patient safety and avoiding duplication of efforts. Innovative collaborative frameworks such as this may serve as a model for developing other biomarkers and are optimal for validating computational tools in digital pathology.

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Institutional review board statement (IRB): slides

This study was approved by the Ethics Commission of the Institute Jules Bordet (CE3049). Pathologist annotations: the FDA IRB determined that the research study was exempt from the requirements of 45 CFR part 46; 45 CFR 46.104(d) (2)(ii). Protocol number: 2019-CDRH-109.

Conflicts of interest

A.L.: consultant: Ultivue. R.S.: advisory board/consultancy: B.M.S., Exact Sciences, Roche, Owkin; research funding: Roche, Puma, Merck. K.R.M.B.: research funding: SimbioSys, Carevive. K.A.W.: employee/equity holder: Roche Diagnostics Solutions former employee/equity holder, Ultivue, Leica Biosystems/Danaher, Novartis. D.J.E.P.: consultant pathologist: CellCarta NV.

Data availability statement

Data reported in this study are available from the authors upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Respondents in this global survey about TILs (n = 168) were largely from the US and Europe. *TILs*, tumour infiltrating lymphocytes.

Figure S2. TILs in breast cancer are considered predictive, prognostic, or both by >95% of survey respondents. 149/161 respondents (92.5%) think future research on TILs as a biomarker in breast cancer and potentially other cancer types is worth pursuing. *TILs*, tumour infiltrating lymphocytes.

Figure S3. 45.2% of survey participants who deliver care integrate TILs into their daily practice. *TNBC*, triple negative breast cancer; *TILs*, tumour infiltrating lymphocytes.

Figure S4. 47.1% of oncologists reported requesting TILs reporting by pathologists. Of 46 oncologists who were not currently requesting TILs data, 24 (52.2%) indicated they planned to begin doing so in the future; 20 indicated an intention to start in either 2021 (n = 10) or 2022 (n = 10). Of the oncologists who did not ask for TILs information, a subset (n = 15) provided the following reasons: TIL data would not change treatment plans, absence of national or international guidelines endorsing TIL use, or lack of strong enough evidence for its use in clinical practice. When evaluating TILs in breast cancer, 94% of pathologists and scientists follow the recommendations of the TILs Working Group (Salgado et al 2015). *TILs*, tumour infiltrating lymphocytes.

Figure S5. Most oncologists consider the available evidence to be very strong in support of TILs either as a prognostic or predictive biomarker. *TILs*, tumour infiltrating lymphocytes.

Figure S6. Most oncologists choice of therapy is not influenced by TILs. *TILs*, tumour infiltrating lymphocytes.

Figure S7. Survey responses were varied as to specific TIL cut-off values to be utilised in chemotherapy de-escalation trials for patients with early-stage TNBC. However, clinical use of TILs was overall high, with 87% supporting development of a prospective phase II or III trial for chemotherapy de-escalation using TILs in early stage TNBC, and 84% supporting development of a prospective phase II or III trial for immunotherapy using TILs in early or late stage TNBC. Furthermore, 93% stated they would consider including patients in a de-escalation trial using TILs, 99% stated they would consider including patients in a clinical trial for immunotherapy benefits evaluation using TILs, and 46% stated they would include patients with low stage early TNBC high-TILs with a BRCA1/2 germline mutation in a chemotherapy de-escalation trial. *TNBC*, triple negative breast cancer; *TILs*, tumour infiltrating lymphocytes.

Figure S8. Detailed instructions for enrolling in FDA CME course for breast cancer stromal TIL assessment training. Step 1. Create account at ceportal.fda.gov. Step 2. From Dashboard, select 'Enduring Materials.' Step 3. Search for 'TILs' in Text Search box. Step 4. The 'TILs' search term will return 'Assessment of Stromal Tumor-Infiltrating Lymphocytes' CME course. Step 5. Click 'Enroll' to begin course.

Data S1. HTT test report.

Data S2. Supplementary material.