PD-L1 testing in triple-negative breast cancer: Post hoc IMpassion130 substudy evaluates PD-L1 IHC assay performance

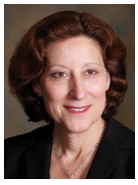
Sherrie Rice

April 2020—IMpassion130 was the first phase three trial to demonstrate a clinical benefit of cancer immunotherapy in patients with PD-L1-positive metastatic triple-negative breast cancer, and based on the data, atezolizumab plus *nab*-paclitaxel is approved for this indication.

In the trial, the Ventana SP142 PD-L1 assay with a one percent or greater cutoff was used to evaluate PD-L1 expression in immune cells. But questions remained about how to best identify patients who could benefit from the drug combination, Hope S. Rugo, MD, professor of medicine and director of breast oncology and clinical trials education at the University of California San Francisco Comprehensive Cancer Center, said in a CAP TODAY webinar last November.

To find answers, she and colleagues at institutions in the U.S. and abroad conducted a retrospective post hoc IMpassion130 analysis in which the Dako 22C3 and Ventana SP263 PD-L1 assays were studied for their analytic concordance with SP142. Their results were reported at the ESMO2019 conference last fall.

Dr. Rugo joined Gary Tozbikian, MD, assistant professor and director, Division of Breast Pathology, Department of Pathology, Ohio State University Wexner Medical Center, in the webinar, made possible with support from Genentech.



Dr. Rugo

“The data support that at this point other PD-L1 assays are not interchangeable with SP142,” said Dr. Tozbikian, who provided case examples to demonstrate how the stain is performed and interpreted in clinical practice.

For the post hoc analysis, Dr. Rugo and colleagues looked at PD-L1 by the three IHC assays. “These assays were performed using the respective package inserts,” Dr. Rugo said, “and each slide was read by a single pathologist out of a panel of eight pathologists, all of whom were trained and qualified to read all three assays.”

A positive result for SP142 and SP263 is defined as PD-L1 immune cell positivity in at least one percent of the tumor area. For the Dako 22C3 assay, a combined positive score, or CPS, is used. This includes the number of PD-L1-positive tumor cells, lymphocytes, and macrophages divided by the number of viable tumor cells and multiplied by 100.

“For the purpose of this retrospective post hoc analysis,” Dr. Rugo said, “we used our biomarker-evaluable population, or BEP. This population included 68 percent, or 614 patients, from the intention-to-treat IMpassion130 population who had adequate tissue available to perform this comparison using three different assays.”

In the biomarker-evaluable population, 46 percent of samples were PD-L1 immune-cell–positive versus 41 percent in the intention-to-treat analysis, and the improvement in progression-free survival in the biomarker-evaluable population with the addition of atezolizumab was slightly greater than in the overall population. All other evaluated baseline characteristics were balanced between the BEP and ITT.

The different assays identified different percentages of cases that were positive for PD-L1. More tumors were classified as positive for PD-L1 using the two alternative antibodies and assays (22C3, 81 percent; SP263, 75 percent) than SP142 (46 percent).

“Interestingly, almost all SP142-positive cases are captured by either the 22C3 CPS or the 22C3 assay using the respective cutoffs. Only one percent of tumor samples were positive only using SP142,” Dr. Rugo said.

This combination positivity leads to a suboptimal analytical concordance or overall percentage agreement of only 64 to 69 percent, she said. “An additional 36 percent and 30 percent of cases were positive only for 22C3 CPS or 263, respectively.”

Previous studies demonstrated a correlation between the percent of stromal tumor-infiltrating lymphocytes and clinical outcome in triple-negative breast cancer. In IMpassion130, patients whose tumors tested positive for PD-L1 immune cells using SP142 and one of the other two antibodies, classified as double-positive, had the highest percentage of TILs compared with those whose testing was positive by only one of the other two assays.

“The analysis found an absolute difference in progression-free survival for tumors that were SP142-positive—46 percent of our biomarker population—of 4.2 months, and an absolute difference in overall survival in this subset of our population reported in the overall intention-to-treat analysis of 9.4 months,” Dr. Rugo said.

In contrast, the absolute benefit using 22C3 CPS, which was 81 percent of the BEP, or SP263, which was 75 percent of the BEP, was only 2.1 or 2.2 months for progression-free survival and 2.4 or 3.3 months for overall survival, respectively.

They then looked at both of the combinations of the two different assays and clinical outcomes. Again, more patients had tumors positive for PD-L1 using 22C3 and SP263. “So we’re first looking at the 22C3 CPS of one or greater assay and at tumors that were double-positive for SP142 and 22C3, compared with tumors that were positive only for 22C3 and then the double-negatives.”

In the double-positive population (SP142 and 22C3), the absolute difference in progression-free survival is 4.4 months and overall survival 9.3 months, and this is similar to the finding for tumors positive by SP142 alone.

“In contrast, the differences narrow and become less clinically important in tumors that are single-positive for 22C3 at 1.7 months and essentially no difference for PFS and OS, respectively. And then, as expected based on our overall analysis of this sample and our previously reported data, there was no difference in patients who were double-negative for both assays,” Dr. Rugo said.

The difference in the SP142 and SP263 double-positives that represent a larger population, 45 percent, is 4.2 and 9.4 months for progression-free survival and overall survival, respectively. The difference essentially goes away for the tumors that are SP263-positive alone or, of course, double-negative.

Also studied was the source of tumor and its relationship to outcome in IMpassion130.

Overall, tissue obtained from the breast was more likely to be PD-L1–positive than tissue obtained from different metastatic sites, at 44 percent and 36 percent, respectively.

However, the median time of sample collection to randomization was only 61 days, suggesting that most primary tissue samples were obtained in the metastatic setting.

PD-L1 status also varied by the anatomic site from which tissue was obtained. “Interestingly, the least likely organ to have tissue that was positive for PD-L1 was liver,” Dr. Rugo said, “although this represented only five percent of the total tissues analyzed.” This difference in PD-L1 positivity has been shown in other studies and in other diseases, she said.

Regardless of the source of tissue, however, PD-L1 status predicted benefit from atezolizumab.

Dr. Rugo summed up the findings of the post hoc exploratory biomarker substudy of the IMpassion130 trial:

- Clinical activity was seen in the SP142 PD-L1 immune-cell–positive subgroup regardless of whether the sample came from the primary tissue or metastatic tissue. "Although it's important to keep in mind that the majority of tumor tissues that were used for PD-L1 testing were obtained within two months of study start," she said, "we don't know that there are significant differences between tumor tissue that was obtained a longer time from when you're intending to analyze and start new treatment."
- With overall percentage agreements of 64 percent for 22C3 and 69 percent for SP263, the analytic concordance was subpar at less than 90 percent, "and the assays are clearly not equivalent." "22C3 with a CPS of one or greater and SP263 with an immune cell count of one percent or greater for PD-L1 assays identified a much larger patient population, and the SP142 immune cell one percent or greater group is a subgroup of this larger population."
- The clinical benefit in 22C3-positive and SP263-positive subgroups was driven by the SP142-positive subgroup. This assay identifies patients with the smallest hazard ratio point estimates and the longest median progression-free and overall survival from atezolizumab and *nab*-paclitaxel.
- In the United States, the SP142 assay is the approved diagnostic test used to identify patients with metastatic triple-negative breast cancer most likely to benefit from the addition of atezolizumab to *nab*-paclitaxel.

When the FDA approves a companion diagnostic assay, Dr. Tozbikian said, it's approving the entire system as a device. For the SP142 assay, this includes not just the antibody but also the detection kit with the amplification step.

The staining instrument is the BenchMark Ultra, and it includes the specific staining protocol interpreted according to the interpretation guide. If any component is changed, the lab has to validate the changed system component.

The acceptable specimens for PD-L1 testing are formalin-fixed and paraffin-embedded, archival tissues or tissues obtained recently from resections, excisions, or biopsies, and they can come from primary or metastatic sites. To be considered adequate, they need to contain at least 50 viable tumor cells with associated stroma. "For that reason, cytology specimens are unacceptable. Likewise, any decalcified bony specimens are not acceptable due to lack of validation."

As for other breast biomarkers, tissues should be fixed in 10 percent neutral buffered formalin with a six- to 72-hour fixation range.

In Dr. Tozbikian's experience, preanalytics tend to be more of an issue for metastatic cases where a history of breast cancer or a breast cancer met may not be suspected and adherence to fixation requirements may not be 100 percent. "That is something to consider when you are doing PD-L1 testing and considering what specimens to

perform the test on," he said.

When possible, freshly cut unstained slides should be used for the SP142 test. "Try to avoid testing on any archived, precut unstained slides, especially those that have been stored for longer than two months, as these can show staining degradation due to cut sample instability." In some situations, older archived material may be all that is practically available, but in that case, he advises using freshly cut slides from those stored blocks.

'You will pick up many more positive cases by looking at the immune cell component.'
Gary Tozbikian, MD

The prevalence of PD-L1 expression in the immune cell or tumor cell component differs by tumor type. In urothelial carcinoma, non-small cell lung cancer, and triple-negative breast cancer, PD-L1 expression was found to be more prevalent in the immune cell component than the tumor cell component, according to data from the IMvigor210, POPLAR, and IMpassion130 trials, respectively.

For triple-negative breast cancers, 41 percent showed PD-L1 positivity in the immune cell component versus only nine percent in the tumor cell component. "So you will pick up many more positive cases by looking at the immune cell component," Dr. Tozbikian said.

The 41 percent positive rate is an important number to note, he said. "If you were to initiate testing at your lab and ask the question, 'When I perform this test using SP142 on triple-negative breast cancers, what positive rate should I expect to observe?,' the answer is 41 percent. This was the rate that was observed from the data in IMpassion130, and it is the best available current benchmark or positive rate to reference."

In the triple-negative breast cancers, when the tumor cells were positive, frequently the immune cell component was also positive. Lung carcinomas showed relatively less overlap. "When it comes to PD-L1 testing," Dr. Tozbikian said, "being aware of which cell component—tumor cell or immune cell or both—is relevant and used for scoring is important, especially since expression rates and scoring systems can differ by site of origin."

In triple-negative breast cancer, it is only staining in the immune cell component that counts toward scoring. "For the majority of cases, the tumor cell component will be negative. But in a minority of cases, you will observe tumor cell staining that could be present and could potentially increase the interpretive complexity of the case," Dr. Tozbikian said.

When positive, the immune cells tend to show a more punctate or granular pattern of staining, which appears on the cell membrane. The tumor cells when positive will show a more uniform, complete, honeycomb-like or circumferential staining pattern on the cell surface.

Any immune cell staining counts toward scoring—lymphocytes, macrophages, neutrophils, multinucleated giant

cells, and so on—"provided that these immune cells are within the tumor area and are not in necrotic areas," he said, adding that staining intensity is included in the scoring.

Generally two different patterns of immune cell staining will be seen: aggregate and single cell spread. And intratumoral heterogeneity or regional variation for PD-L1 expression is commonly encountered.

The scoring method for SP142 in triple-negative breast cancer uses a proportion of tumor area scoring system, which is what was used in the clinical trials. "It was the scoring system found to be more reproducible and best correlated with efficacy," Dr. Tozbikian said.

"You score in the immune cell component only. You calculate the proportion of the tumor area occupied by PD-L1-positive staining immune cells." The tumor area is defined as the tumor mass itself and includes the associated intratumoral stroma and immediate contiguous peritumoral stroma.

"To interpret the stain, you estimate the proportion of that total tumor area occupied by PD-L1–positive immune cells that are present, either infiltrating the tumor or in the intratumoral stroma or immediate contiguous peritumoral stroma." If positive immune cell staining occupies greater than or equal to one percent of the total tumor area, the result is considered positive. No staining or less than one percent area staining is negative. "Any tumor cell staining that you observe is ignored."

The report should specify the PD-L1 immunostain and antibody that was performed, he said, and give an overall result interpretation, positive or negative. It should also provide detail about the scoring system used. "For triple-negative breast cancer, it's a tumor area scoring system with a one percent cutoff scored in immune cells." Providing a raw score is optional. "In clinical practice, most positive results you see are going to be in the one to five percent positive range," he said.

Case No. 1 demonstrates the typical appearance of immune cell staining for PD-L1 SP142. Shown is a low-power view of a core needle biopsy with primary triple-negative breast cancer (**Fig. 1a**), and a higher power view (**Fig. 1b**). "This was an invasive ductal carcinoma that was triple negative. You can see a dense, inflammatory infiltrate adjacent to the tumor cell nests and the intratumoral stroma as well as infiltrating the tumor itself," he said.

Fig. 1c is the corresponding PD-L1 SP142, and PD-L1 expression can be seen in the immune cell component, mainly in the intratumoral stroma. The staining pattern in these immune cells is punctate and granular whereas the tumor cells are negative. "This is the most commonly encountered staining pattern," Dr. Tozbikian said.

He circled regions of the tumor area that are occupied by PD-L1-positive immune cells (**Fig. 1d**). To help estimate the proportion of tumor area involvement, he combined the positive regions (**Fig. 1e**).

Case 1 *All images provided courtesy of Gary Tozbikian, MD*

Fig. 1a IC staining characteristics

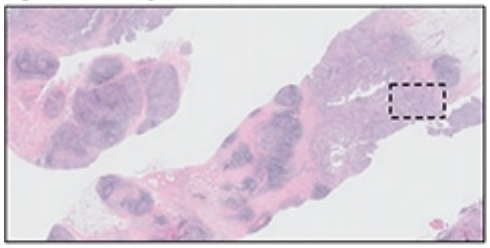


Fig. 1b IC staining characteristics

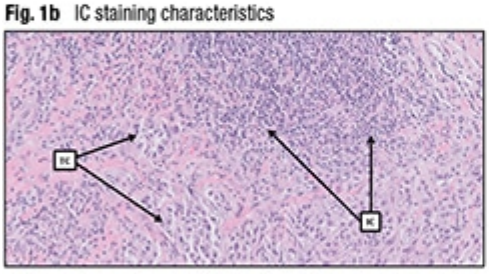


Fig. 1c PD-L1 (SP142) IC staining characteristics

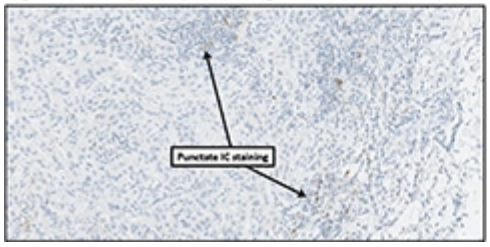


Fig. 1d PD-L1 (SP142) IC staining characteristics

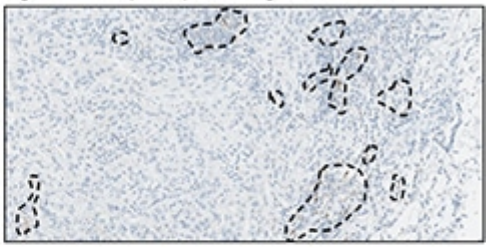


Fig. 1e PD-L1 (SP142) IC staining characteristics

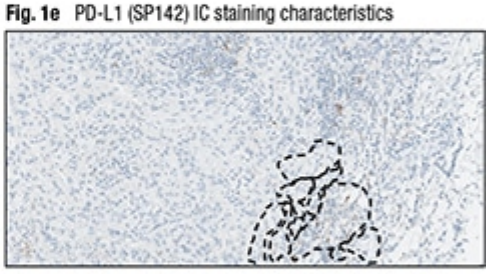
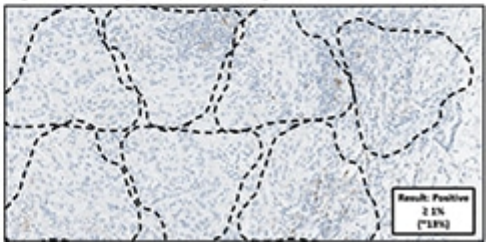
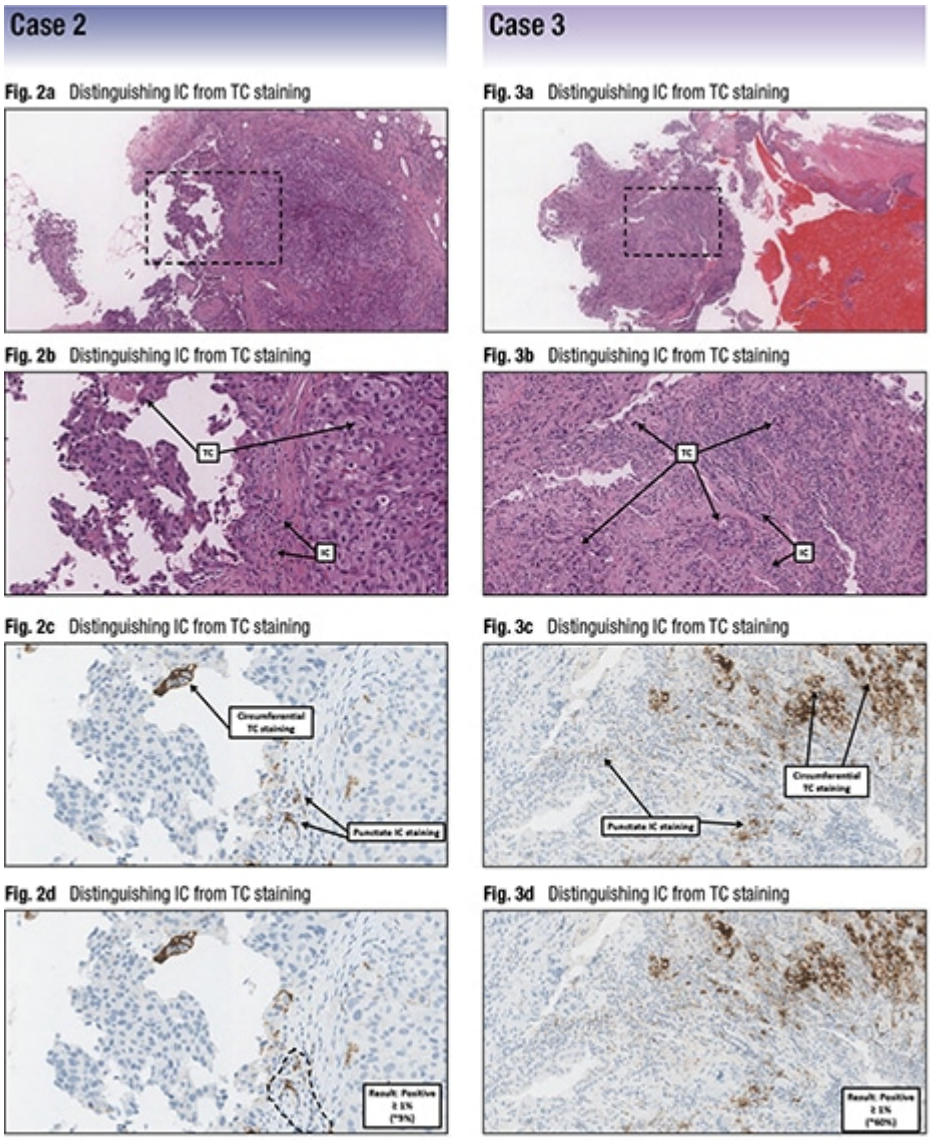


Fig. 1f PD-L1 (SP142) IC staining characteristics



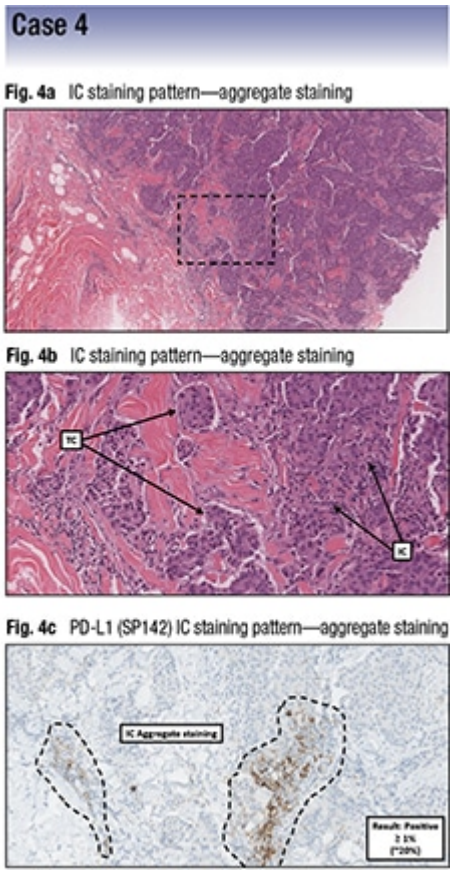
The next step is to estimate the combined area as a proportion of the total tumor area (**Fig. 1f**). “This positive area occupies approximately a little less than one-seventh of the total tumor area, at least in this high-power field. So assuming that the rest of the entire tumor looked like this, the result would be positive with about 13 percent of the total tumor area involved,” he said.



Case No. 2 (**Fig. 2a**) was taken from a case of a primary breast triple-negative invasive ductal carcinoma and highlights a tumor that will demonstrate both tumor cell and immune cell staining. “You can appreciate an inflammatory infiltrate involving the adjacent intratumoral stroma.” (**Fig. 2b**).

Fig. 2c is the corresponding PD-L1 immunostain. The majority of the tumor cells are PD-L1-negative, but at the top of the image there is a small cluster of several tumor cells that show PD-L1 expression. In comparison to the immune cells, these tumor cells show a more uniform, complete circumferential staining pattern on the cell surface. The immune cell staining shows a more punctate or granular staining pattern.

Scoring in one high-power field (**Fig. 2d**) reveals that about five percent of the tumor area is occupied by positive immune cells. “Assuming that the rest of the tumor showed a similar staining pattern, the final result would be positive,” he said.



Case No. 3 (**Fig. 3a**) came from a lung metastasis by triple-negative breast cancer, and again demonstrates the difference between tumor cell and immune cell staining. At higher power (**Fig. 3b**), large clusters of epithelioid cells can be seen, as can a dense, inflammatory lymphoplasmacytic infiltrate in the surrounding stroma.

Fig. 3c is the corresponding PD-L1 stain. The majority of the tumor cells are PD-L1-negative, but in the top and right side of the image are several tumor cells showing PD-L1 expression. “Again, in comparison to the immune cells, the tumor cells that are positive show a more uniform or complete circumferential staining pattern.”

“Ignoring the tumor cell staining, still a large proportion of the immune cells in this high-power field are positive.” (**Fig. 3d**). About 60 percent of the tumor area is involved by PD-L1-positive immune cells. To arrive at a final overall result, he said, the entire tumor area in a case has to be evaluated.

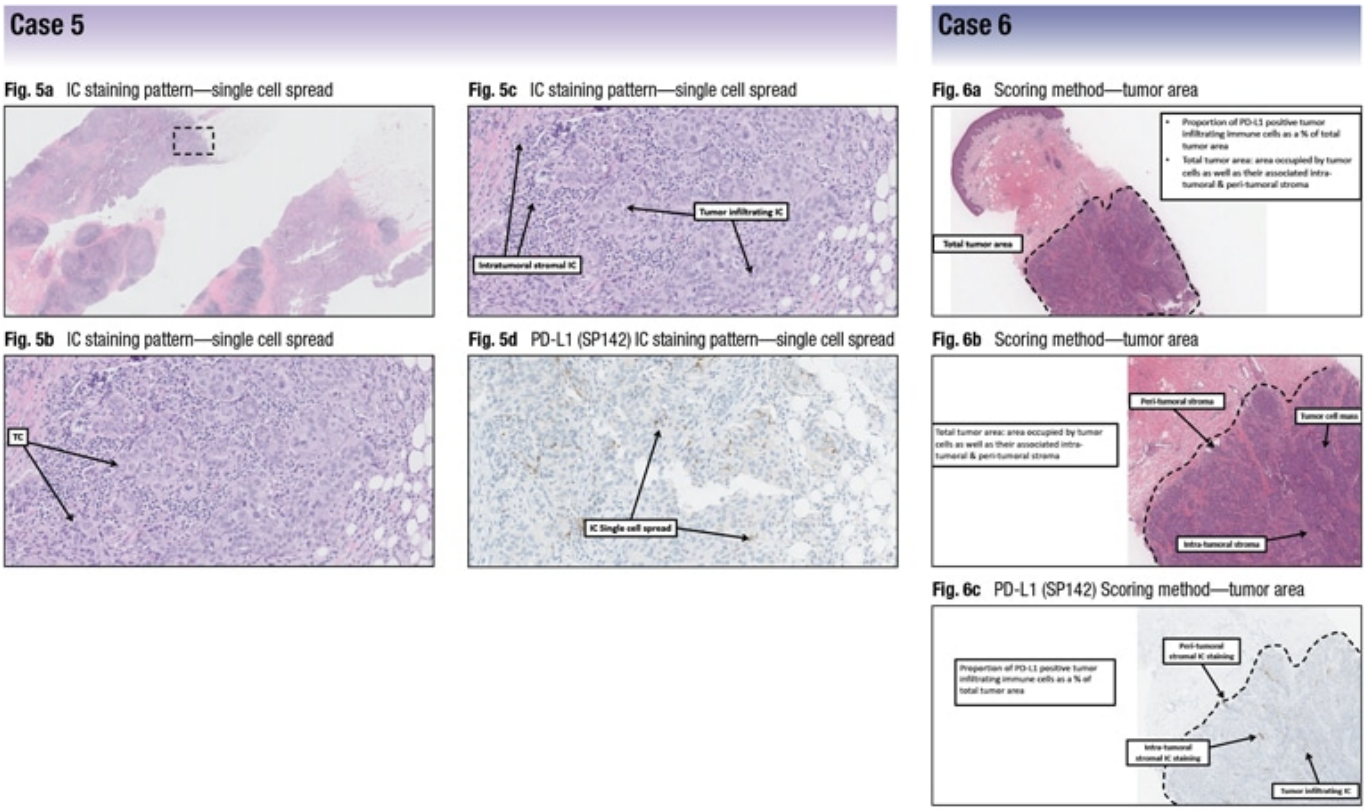
Case No. 4 (**Fig. 4**) demonstrates an aggregate staining pattern (the more common of the two patterns) in the immune cell. **Fig. 4a** is a skin recurrence by a triple-negative invasive ductal carcinoma. **Fig. 4b** is a higher-power view in which a dense, inflammatory cell infiltrate can be seen, mostly in the surrounding stroma but also infiltrating the tumor itself.

Fig. 4c is the corresponding PD-L1 immunostain. Positive PD-L1 immune cells can be seen with punctate expression, and the majority of the PD-L1 positivity is seen in aggregates or clusters of positive immune cells located in the intratumoral or peritumoral stroma. This is the aggregate staining pattern.

Case No. 5 (**Fig. 5**) highlights the single cell spread pattern. **Fig. 5a** is a low-power view of a corneal biopsy containing a primary triple-negative breast cancer. **Fig. 5b** is a high-power field. “This is an invasive ductal carcinoma. There is a dense inflammatory infiltrate surrounding the tumor nests. You can appreciate immune cells infiltrating as single cells admixed among the tumor cells and in the stroma.” (**Fig. 5c**).

Fig. 5d is the corresponding PD-L1 stain, where strong punctate staining can be seen in the immune cells, “but expressed in a more dispersed pattern seen in scattered single cells more diffusely in the TILs that are infiltrating among the tumor cell nests.” This is the single cell spread pattern.

In the same tumor both aggregate and single cell spread patterns of staining can be encountered. “Both of them count,” Dr. Tozbikian said.



Case No. 6 (**Fig. 6**) demonstrates how to define the total tumor area. **Fig. 6a** is a low-power view (tumor area circled) of a biopsy of a skin recurrence by triple-negative breast cancer. This is the region where PD-L1 is scored. **Fig. 6b** is the higher-power view; the entire region in the circle is considered the tumor area. It includes the tumor mass itself, the associated intratumoral stroma, as well as the immediate contiguous peritumoral stroma.

Fig. 6c is the corresponding PD-L1 stain. “You would include any immune cell staining within the circled tumor area to include any PD-L1-positive immune cells that are infiltrating the mass, immune cells that are positive in the intratumoral stroma, and at the periphery of the tumor any PD-L1-positive immune cells in the contiguous peritumoral stroma.”

Case 7

Fig. 7a Scoring method—tumor area (lymph node)

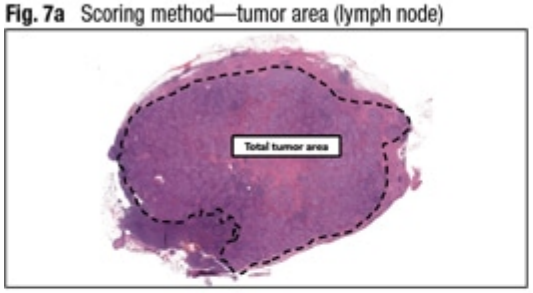


Fig. 7c Scoring method—tumor area (lymph node)

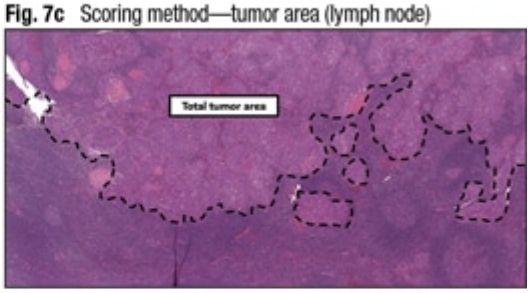


Fig. 7b Scoring method—tumor area (lymph node)

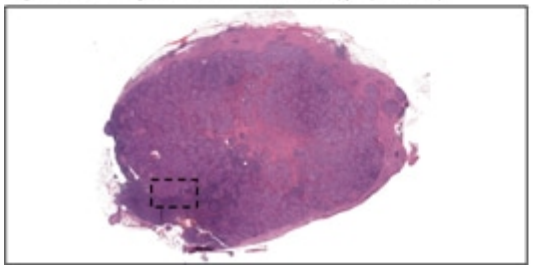
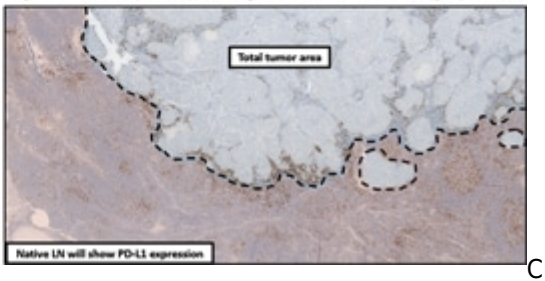


Fig. 7d PD-L1 (SP142) Scoring method—tumor area (lymph node)



Case No. 7 (**Fig. 7**) also demonstrates how to define the tumor area, but this case is a lymph node metastasis by triple-negative breast cancer. It can be seen in **Figs. 7a** and **7b** (low-power view of the lymph node) that the majority of the lymph node has been replaced by metastatic carcinoma. At the bottom left there is a portion of the lymph node that is still uninvolved by metastatic carcinoma. **Fig. 7c** is a medium-power view of the interface between the metastasis and the uninvolved lymph node. PD-L1 would be scored and assessed inside this circled tumor area.

Case 8

Fig. 8a Scoring method—intratumoral heterogeneity

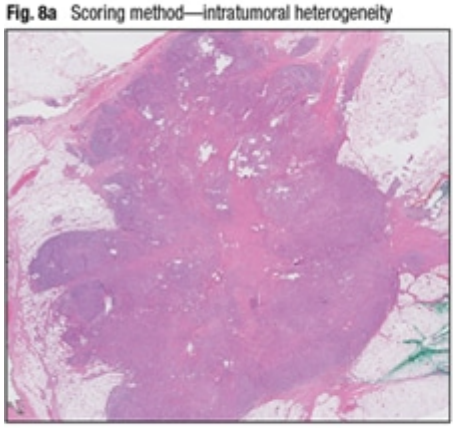


Fig. 8b PD-L1 (SP142) Scoring method–intratumoral heterogeneity

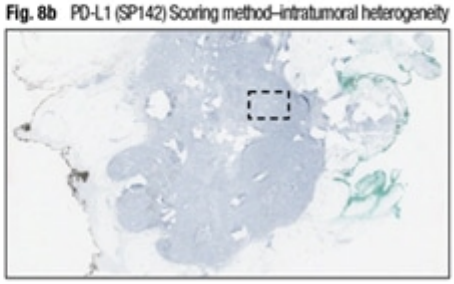
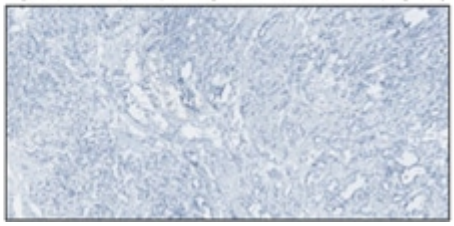


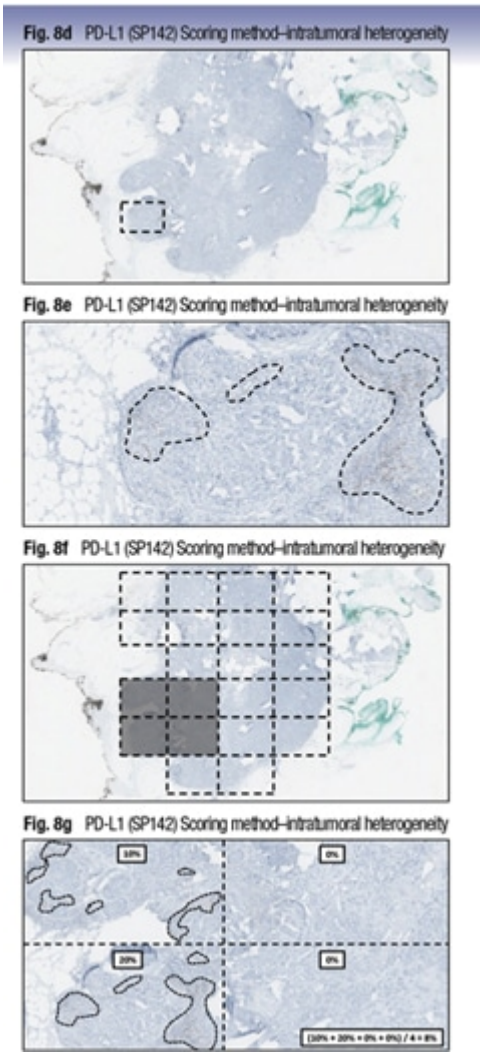
Fig. 8c PD-L1 (SP142) Scoring method–intratumoral heterogeneity



For lymph node metastases, the scoring method is identical to that of primary and other metastatic sites, he said. “But care should be taken to exclude scoring in the normal or uninvolved lymph node tissue that is not part of the tumor area because native lymph node tissue will show staining for PD-L1 SP142, particularly in lymph node germinal centers. So staining in the uninvolved or native lymph node should be excluded.”

Fig. 7d is the corresponding PD-L1 immunostain, where there is accentuated PD-L1–positive expression in the periphery of the tumor and immune cells, and within the metastatic deposit itself. PD-L1 would be scored within the tumor area, and then you would exclude any scoring in the normal, uninvolved lymph node tissue, as the native lymph node tissue will show expression for SP142, particularly in germinal centers.

Case No. 8 (**Fig. 8)** demonstrates intratumoral heterogeneity for PD-L1 expression. “In clinical practice, it is common to encounter tumors with regional variation for PD-L1 SP142 expression,” he said. For this reason, when assessing PD-L1, “make sure you scan the entire tumor area for PD-L1 expression, evaluating for the presence of intratumoral heterogeneity.”



Case No. 8 is taken from a primary triple-negative breast cancer. **Fig. 8a** is from an excision specimen, and **Fig. 8b** is the corresponding PD-L1 immunostain at scanning magnification. This tumor showed significant regional variation for PD-L1 expression. At higher power (**Fig. 8c**) there is virtually no PD-L1 expression in the immune cell component. If a different region of the tumor is examined (**Fig. 8d**), “it shows a different story,” Dr. Tozbikian said. On the bottom left side of the tumor at higher power (**Fig. 8e**), “you can appreciate the presence of PD-L1 immune cells. This is showing a more aggregate staining pattern in the intratumoral stroma. At least in this high-power field, the area involved by PD-L1-positive immune cells is greater than one percent of the tumor area. But you have to consider the entire tumor area when scoring.”

As a practical recommendation, in cases like this, one helpful approach is to do a semiquantitative assessment, he said. “It may be easier to break it up by scoring multiple fields at high power and then take an average.” In this case he used a grid to divide the tumor into separate smaller fields, which could then be assessed separately and from which an average could be taken (**Fig. 8f**).

“So let’s consider scoring in the bottom left side of the tumor. Going to a more medium-power view, I divided this portion of the tumor into a quadrant-like fashion to help make area quantification easier.” Significant intratumoral heterogeneity is seen (**Fig. 8g**). The two fields on the right show minimal staining for PD-L1 whereas the two fields on the left show aggregate staining in the immune cells in clusters within the intratumoral and peritumoral stroma, which he circled.

The next step would be to score each separate field. The two fields at right would receive a score of zero percent and on the left 10 and 20 percent. “And you would calculate an average of these: Ten percent plus 20 percent plus two fields with zero percent—the result would be about eight percent overall, at least in this field.”

This is done for the entire tumor and then an overall average is calculated. “I think this approach is useful when you have intratumoral heterogeneity.”

Sherrie Rice is editor of CAP TODAY. The full webinar is at www.captodayonline.com.