Evaluation of HiPath Pro™ for Analysis of PD-L1 (Programmed Death Ligand-1) Immunohistochemistry

Lija Joseph, M.D.[1], Chanan Sluszny, PhD[2]

[1] Medical Director, Department of Pathology and Laboratory Medicine, Lowell General Hospital
[2] Applications Specialist, Applied Spectral Imaging

SUMMARY

This white paper summarizes results of a comparison study between manual and computer-aided analysis, using the HiPath Pro system, for scoring of PD-L1 in non-small cell lung cancer (NSCLC) tissue samples.

SUMMARY OF FINDINGS

Concordance between HiPath Pro and manual analysis was 91% (32 out of 35 cases), when eliminating non-consistent sampling factors

Without elimination of sampling errors, concordance between HiPath Pro and manual analysis was 83% (29 out of 35 cases). It was found that the main reason for this discrepancy was undersampling in the manual analysis, mainly in the No expression cases

While a minimum of 100 viable tumor cells must be present for the specimen to be considered adequate for manual PD-L1 evaluation, in this study the average cell count per sample with HiPath Pro was 2,300. This improved the statistical significance and lead to higher confidence in scoring results

For the medium expression cases (1-49% positivity) HiPath Pro and manual scoring gave 100% concordance

The HiPath Pro system, with its membranous-IHC algorithm, proved to be accurate in assessment of PD-L1 expression. HiPath Pro overcomes manual sampling errors and insufficient cell-count and can be reliably used to assess PD-L1 expression.

BACKGROUND AND GOALS

Discovery of targeted therapy for oncology patients have significantly improved outcomes of cancer survival and prolonged response rates. Expression of the target proteins on cells aid in identifying specific tumors that are likely to respond to such agents.

Immunohistochemistry (IHC) is used for detection and scoring of PD-L1 expression in formalin-fixed, paraffin-embedded (FFPE) tissue samples. Results of IHC scoring are shown to predict the likelihood of response to treatment with PD-1/PD-L1 inhibitors and assist in appropriate patient selection for these drugs. Scoring criteria for PD-L1 depends on the specific antibody and sample type that is used and relies on the particular recommendations provided by assay manufacturers. For example, the Dako Agilent 22C3 PharmDx IHC assay has a three-class scale for scoring PD-L1: <1% No expression, 1-49% medium expression and ≥50% high expression (table 1, ref 1). Patients who are scored medium or high expression may be selected for treatment with Keytruda (Merck).

According to the current recommendations, all viable tumor cells shall be analyzed and a minimum of 100 tumor cells from a tissue sample is sufficient for scoring PD-L1. In cases where expression of PD-L1 is heterogeneous, scoring may be inaccurate, due to sampling limitations. With the advent of computer-aided platforms, which allow fast and accurate analysis of all tumor cells in the slide, accuracy may be improved dramatically.

In this study we compared results of manual and Computer Aided (CA) analysis of PD-L1 IHC expression, in NSCLC tissue samples, using the 22C3 pharmDx IHC assay from Dako Agilent. Our goal was to validate the use of HiPath Pro, with its current membranous IHC algorithm, for analysis and scoring of PD-L1 stained tissue samples. In particular, we wanted to understand how CA analysis can benefit the accuracy of PD-L1 analysis.

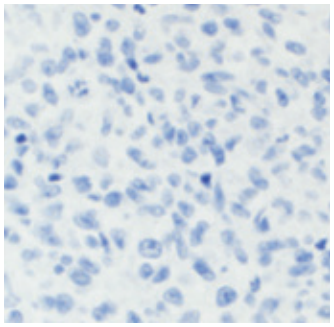
TABLE 1: Tumor Proportion Score (TPS), expression levels and staining characteristics of PD-L1 using the Dako Agilent 22C3 PharmDx IHC assay[1]

EXPRESSION LEVEL

No PD-L1 Expression

TPS

< 1%



STAINING PATTERN

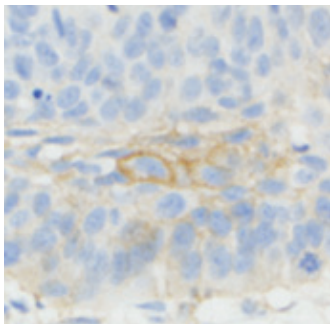
Partial or complete cell membrane staining (≥ 1+) in <1% of viable tumor cells

EXPRESSION LEVEL

Medium PD-L1 Expression

TPS

1-49%



STAINING PATTERN

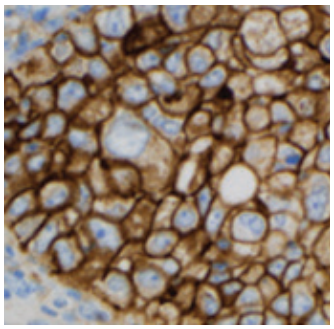
Partial or complete cell membrane staining (≥ 1+) in ≥1-49% of viable tumor cells

EXPRESSION LEVEL

High PD-L1 Expression

TPS

≥ 50%



STAINING PATTERN

Partial or complete cell membrane staining (≥ 1+) in ≥ 50% of viable tumor cells

[1]Dako PD-L1 IHC 22C3 PharmaDx Interpretation Manual

METHODOLOGY AND WORKFLOW

IRB exempt status of this study was requested and obtained through the Institutional Review Board of Lowell General Hospital.

Design: Fifty (50) non-small cell lung cancer (NSCLC) tissue slides, from different cases, were stained with Dako AutostainerLink 48, using the Monoclonal Rabbit Anti-PD-L1 antibody, clone 22C3 (DAKO).
Slides were divided into two groups.
The first group, composed of fifteen (15) slides, was used for training/optimization of the HiPath Pro membranous IHC algorithm, for analysis of PD-L1.

The second set, which is the study-group, included 35 slides. Slides were first evaluated by the expert pathologist, who manually counted and analyzed 100 tumor cells.
The same cases were then analyzed with the HiPath Pro system by the same pathologist.
The tumor cells were specifically evaluated for membranous staining as identified by the Dako training manual (online and handout) and graded as 1+, 2+ or 3+. Cytoplasmic staining was eliminated in this study.

RESULTS

The first 15 cases analyzed by HiPath Pro were used as the training set for optimization of the membranous IHC algorithm for analysis of PD-L1 samples. Image analysis settings were not altered once the training slides were evaluated. These settings were then used for analysis and scoring of the 35 PD-L1 slides of this study.
Manual scoring and HiPath Pro scoring for the 35 study cases of the study are shown, as a comparison matrix, in table 2.

TABLE 2: Scoring results for the 35 slides of the study

Manual (pathologist) / HiPath Pro	No Expression	Medium expression	High expression
No Expression	6	0	0
Medium expression	5	10	1
High expression	0	0	13

NOTE: scoring criteria is for Dako Agilent 22C3 PharmDx IHC assay, where PD-L1<1% is No expression, PD-L1 1-49% is medium expression and PD-L1≥50% is high expression.

Overall concordance between pathologist and HiPath Pro was 83% (29 out of 35 cases). Discordance was mainly in the border-line cases, where the pathologist scored No expression (lower than 1% positivity) and HiPath Pro scored medium expression (1-49% positivity). We found that the discrepancy for these cases was due mostly to sampling error of manual scoring, which is based on analysis of small regions within the tumor. When adding more tumor regions, by using HiPath Pro, such non-consistent sampling factors were eliminated and concordance improved to 91% (32 out of 35 cases).

As noted above, manual scoring is based on counting ≥100 viable tumor cells. The pathologist had stopped scoring after 100 representative cells were counted by the manual method. This in turn may lead to erroneous results, in particular in cases where the sample is heterogeneous. Figure 1 demonstrates the vital importance of image analysis for accurate scoring of such cases. The original image, shown on the left, appears as No expression. Indeed, this slide was scored as No expression by the pathologist. HiPath Pro image analysis, on the other hand, reveals two cells that are classified as positive for membranous staining (marked in yellow). The overall count for this slide was 1310 negative cells and 14 positive cells, with a calculated TPS of 1.06. According to the scoring recommendations for this kit, this slide is scored medium expression.

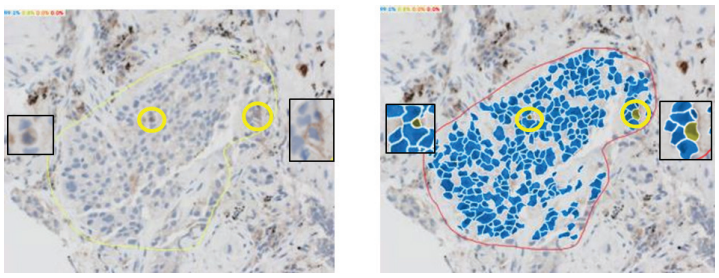


FIGURE 1: Original (left) and analyzed (right) case of PD-L1 demonstrating importance of computer-assisted image analysis for accurate scoring.

Figure 2 is another example of a slide that was scored as No expression by the pathologist, due to insufficient cell-count. In this case manual score by the pathologist was done on area A, which meets the 100 cells criteria and scored as No expression for PD-L1. By using HiPath Pro for analysis of this case, 10 frames were captured and analyzed, with a total of 6400 cells and a score of 8% positivity. This is medium expression for PD-L1.

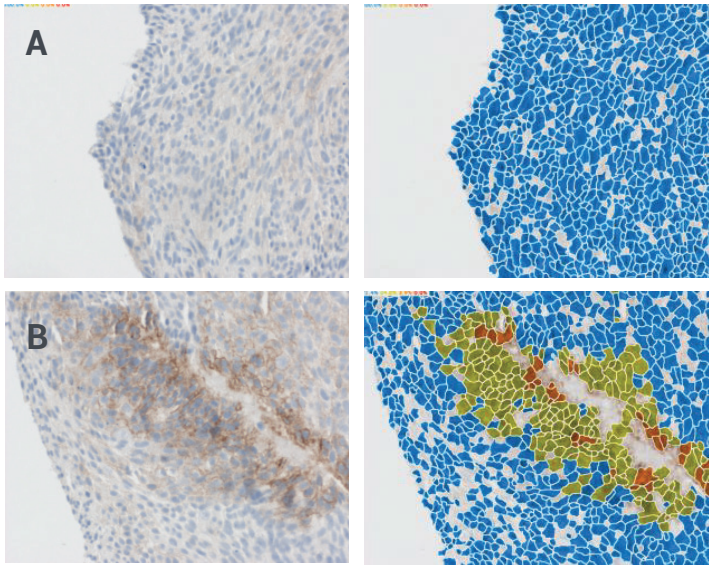


FIGURE 2: Two frames of a PD-L1 case that were captured and analyzed using HiPath Pro. Final score for this case was 8%, which is medium expression for PD-L1.

In addition to sampling errors, we found that interpretation and separation between membranous and other positive staining could affect scoring results. This happened only with No and medium-expression cases. For example, in Figure 3, a tumor region marked and scored by the pathologist as No expression, was classified as positive with HiPath Pro, showing positive (yellow-marked) tumor cells. In such cases, the pathologist was advised to adjust a slider scale, which is an integral part of the HiPath Pro software, so that the classification reflects his/her expert interpretation of membranous staining.

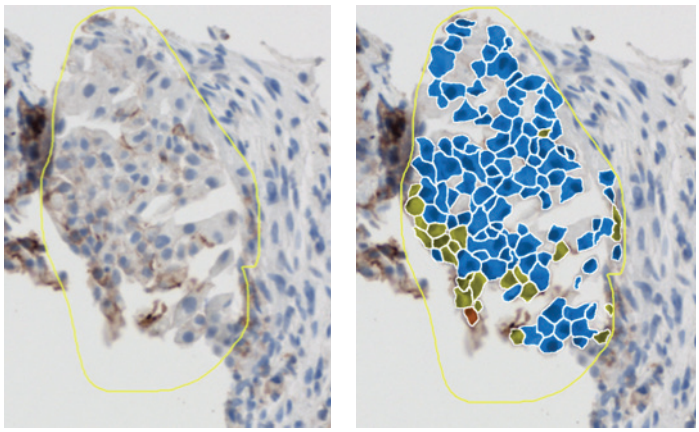


FIGURE 3: Tumor region scored as No expression and medium expression, by the pathologist and HiPath Pro, in accordance. On the right-hand image, blue overlay is for negative cells, yellow and orange markings are cells classified by HiPath Pro as positive for membranous stain.

CONCLUSIONS

HiPath Pro is a robust system to evaluate PD-L1 IHC staining. Contrary to manual scoring that stops after a certain number of cells, HiPath Pro analysis includes every tumor cell, thus providing a statistically-based, reliable result that reflects the overall expression of PD-L1 of a specific tissue sample. Moreover, standardization, which is based on using computer-aided analysis, would eliminate the variability of a manual count for different pathologists. Overall, HiPath Pro overcomes manual sampling errors and insufficient cell-count and can be reliably used to assess PD-L1 expression.