Hot Topic

PD-L1 Testing in Non-Small Cell Lung Cancer
Our speaker for this program is Dr. Anja Roden, an associate Professor of Laboratory Medicine and Pathology and a consultant in Anatomic Pathology at Mayo Clinic in Rochester, Minnesota.
Disclosures

- None

I have nothing to disclose.
As you view this presentation, consider the following important points regarding testing:

- How is the testing going to be used in your practice?
- When should the tests be used?
- How will results impact patient management?

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First, we will discuss the role of PD-1 – PD-L1 interaction in the immune system. We will then discuss the challenges of PD-L1 testing. We will end with take-home points.
PD-L1, or programmed death-ligand 1, is located on tumor cells but can also be found on some immune cells. On tumor cells, it will interact with PD-1, or programmed death protein 1, which is located on T cells, specifically regulatory T cells. This interaction will lead to the inhibition of T cells and, therefore, tumor cells can grow out.
PD-L1 in Immune System

- PD-L1: also known as CD274, B7-H1
- PD1 - PD-L1 interaction
  - inhibition of T cells
  - Tumor growth
- Anti-PD1 or anti-PD-L1 drugs
  - block PD1-PD-L1 interaction
  - boost host anti-tumor immune response
  - Inhibit tumor growth

The PD-1 – PD-L1 interaction, therefore, will lead to inhibition of T cells and the tumor can growth. Anti-PD-1 or anti-PD-L1 drugs have been developed to block the PD-1 – PD-L1 interaction and, therefore, to boost the host anti-tumor immune response and inhibit tumor growth.
In fact, in unselected non-small cell lung carcinomas, the response to anti-PD-1 and anti-PD-L1 agents has been about 20%. These tumors were treated as second- or higher-line therapy; that means that these patients had already another therapy such as surgery, chemotherapy, and/or radiation before. Furthermore, responses not only have been seen in lung adenocarcinomas but also in squamous cell carcinomas, tumors for which we currently have very, very limited targeted therapy available. PD-L1 – PD-1 blockade has also been shown to have durable responses in other metastasizing tumors, including melanomas, carcinomas of the urinary bladder, kidney, prostate, breast, colon, head and neck, and germinal cell tumors, and lymphomas.
What are the current challenges of PD-L1 testing in the Immunostains Lab? We don’t exactly know what will predict the response to anti-PD-1 or anti-PD-L1 inhibitors. Is that the PD-L1 expression on tumor cells, TILs or both? Or is there, maybe, another marker that helps us to predict this response? Multiple anti-PD-1 and anti-PD-L1 drugs have been currently approved for clinical trials or treatment. Also, drugs were tested in conjunction with different anti-PD-L1 clones by immunohistochemistry.
This table shows you the current anti-PD-1 or anti-PD-L1 inhibitors that are FDA-approved or in development in non-small cell lung carcinoma.

Pembrolizumab, which is FDA-approved for non-small cell lung carcinoma this requires a companion test that means the tissue has to be tested by immunohistochemistry, using the clone 22C3, using a Dako platform. In order to be eligible for the drug, 50 or more percent of tumor cells have to express PD-L1. Nivolumab, another inhibitor, is FDA-approved for squamous cell carcinoma and non-squamous cell carcinoma, non-small cell lung carcinoma. This has a complimentary test; that means that this test is highly recommended but not necessary in order to provide the drug. However, if performed, this test should use the clone 28-8 on a Dako platform. It also requires that one or more percent tumor cells should express PD-L1. Atezolizumab is in development but is tested with the clone SP142, using a Ventana platform, and it looks at tumor cells and/or tumor-infiltrating lymphocytes. Durvalumab, another agent, is also in development and is currently tested, using the PD-L1 clone, SP263, using a Ventana platform, and it is looking at a tumor cell expression of 25% or more.
What are other challenges? There is another clone, E1E3N, which is a research-use only. Also, evidence shows that there is a poor concordance between the expression of PD-L1, using clone SP142 and clone E1E3N. Furthermore, as you could see, clones require different staining platforms in the IHC lab. Also, different PD-L1 expression thresholds have been used by different studies for treatment, including over 1% through 50%.
When we validated several antibodies in our lab, we also found that different clones have different expressions. As you can see, the clone E1L3N and the clone SP263 appear to have a comparable staining in this tumor. However, the clone SP142 shows much less staining in the tumor cells.
Heterogeneity of PD-L1 Expression

• Within a single tumor (maybe focal or patchy)

Between independent primary NSCLC (agreement, 52.2%) \(^5\)

High level of agreement between intrapulmonary metastasis (88.9%) \(^5\)

• Sampling might be an issue

Furthermore, there is heterogeneity of PD-L1 expression within a single tumor. While in some tumors the expression is diffuse, it might be just focal or patchy. Literature has shown that there is heterogeneity in PD-L1 expression between independent primary non-small cell lung carcinoma with an agreement of only about 50%. However, there appears to be a high level of agreement of PD-L1 expression between intrapulmonary metastasis in up to 89%. Given all these facts, sampling might be an issue.
Here is an example of a malignant mesothelioma,
which shows quite heterogeneous expression.; And, you can imagine, depending on where the biopsy was performed, there might not be any PD-L1 expression in the tumor cells on a small biopsy.
PD-L1: Current Questions

- New biopsy for PD-L1 after adjuvant therapy?
- Does a lab need multiple platforms and clones to fulfill all clinical requirements?
- How should PD-L1 be reported?
- Which PD-L1 expression threshold is useful?
- Report PD-L1 expression on immune cells?
  - Currently no standard

There are still questions that have not been answered at this time. For instance, is a new biopsy required for PD-L1 testing after the patient underwent adjuvant therapy? Does a lab need multiple platforms and clones to fulfill all the clinical requirements? How should PD-L1 be reported? Which PD-L1 expression threshold is useful for treatment? Also, should PD-L1 expression be reported on immune cells? Currently, there is no standard in reporting of PD-L1 results.
Our approach includes the FDA-approved clone SP263 on a Ventana platform. We look for membranous staining on tumor cells. We do not report positive vs. negative, but we do report the percent of positive tumor cells and then leave it up to the clinician whether this percent of positive tumor cells qualifies the patient for treatment. We currently report whether immune cells are positive or negative for PD-L1. However, this information is not used by the clinician currently.
Here is an example of a non-small cell lung carcinoma.
You can appreciate that over 90% of tumor cells express PD-L1,
and show a nice membranous staining pattern. Some of these cells also show cytoplasmic staining.
We need to be aware that macrophages also express PD-L1, which sometimes can be a pitfall.
In this case, immune cells were also positive for PD-L1, but this staining is easily recognizable, since immune cells are smaller than tumor cells.
In summary, multiple clones are available for PD-L1 testing. Staining might not be concordant between these different clones. Furthermore, staining might be heterogeneous within a tumor and, therefore, sampling could be an issue. Clones require different staining platforms in the immunohistochemistry lab. Reporting currently is not standardized.
References

Questions or requests…
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