PD-L1 Testing for Non-Small Cell Lung Cancer: Belgian Guidelines

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SUMMARY

In recent years, the outcome of patients with non-small cell lung cancer (NSCLC) has improved thanks to the development of targeted therapies. Currently, the introduction of immunotherapy for lung cancer patients offers new treatment opportunities. The pathologist is now asked to provide the most accurate possible diagnosis in association with theranostic information in order to provide the best therapeutic option. For immunotherapy, programmed death receptor ligand 1 (PD-L1) status is, at the present, the required biomarker for patient stratification, at least in first line treatment. Different international societies have already underlined the importance of guidelines for managing samples of non-small cell lung cancer NSCLC. With the goal of adapting these international recommendations to the Belgian landscape, Belgian guidelines were published in 2016. This update integrates immunotherapy into the previously published guidelines. (BELG J MED ONCOL 2018;12(5):233-238)

INTRODUCTION

In 2016, Belgian guidelines were published for the optimal management of non-small cell lung cancer (NSCLC) samples. At that time, we stated that: "At the present moment, no consensus has emerged for PD-L1 immunohistochemistry (IHC) neither about the choice of the antibody nor for the scoring method, because different companies suggest different antibodies and different scoring systems. In addition, given the fact that some IHC negative patients might respond to immunotherapy, the working group has agreed that it is not possible to give general recommendations now."¹ However, with the publication of the results from the Blueprint studies, German and French reference ring trials and the reimbursement

of pembrolizumab – particularly in first line treatment, depending on programmed death receptor ligand 1 (PD-L1) expression – PD-L1 IHC is now part of the NSCLC workup.²⁻⁴ In anticipation, we also stated in the previous guidelines that: "The development of new targeted therapies with predictive biomarkers renders this a rapidly changing field, and these guidelines will very likely require updates reflecting changes in daily practice."¹ The present update has the goal of integrating PD-L1 testing into the previous guidelines.

TARGETING THE PD-1/PD-L1 AXIS

The human body is constantly exposed to a highly diverse world of pathogens (in particular, foreign proteins) every day.

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FIGURE 1. Non-small cell lung cancer staining with the PD-L1 immunohistochemistry 22C3 pharmDx.

A: Negative case without membranous staining of tumour cells and with staining of macrophages (original magnification X200).

B: Case with a TPS between 1 and 49% (original magnification X200).

C: Case with a TPS higher than 50% (original magnification X200).

Fortunately, several lines of defence exist in the human body. The first consists of the 'innate immune system', which consists of an immune response that is non-specific in nature, of limited duration and lacks immunological memory. The second line of defence is the so-called adaptive immune system, mediated by T cells and B cells.

The hallmarks of this system are the specificity of the immune response to antigenic stimulation and the ability to confer lasting immunological memory. Cancer cells (like viruses) express antigens that are 'foreign' to the body because of the presence of altered proteins. The dysregulated, uncontrolled cell division of cancer cells creates a milieu in which the product of normally silent genes may be expressed. Sometimes the encoded differentiation antigens are associated with an earlier development stage. These tumours derived from the same cell type and are often found to express such oncofetal antigens that are also expressed on embryonic cells. Examples are α -fetoprotein in hepatocellular carcinoma and carcinoembryonic antigen (CEA) in colorectal cancer. Special to mention is the case of MAGE-1, a gene encoding a melanoma antigen. MAGE-1 is not expressed in normal tissues, except for germ line cells in testis, and gives rise to antigen T-cell epitopes that, in the light of the absence of class I MHC on the testis cells, must be considered tumour specific.

DNA mutations lead to the formation of altered proteins, called neo-antigens. These can be detected by our immune system and can be targeted, particularly by CD8⁺ cytotox-ic T cells.

Activated T cells express the PD-1 (programmed cell death protein-1) receptor. In physiologic conditions, this PD-1 re-

ceptor expression modulates effector T-cell responses, either during migration to the site of inflammation or in the target tissue itself, in an autocrine and paracrine way. When attacking, T cells release several cytokines, in particular interferon gamma (IFN- γ). IFN- γ can induce the expression of two PD-1 ligands, PD-L1 and PD-L2 (programmed cell death ligand-1 and -2), on antigen-presenting cells. The interaction between PD-1 and its ligands down-regulates the activity of T cells. In particular, this includes inhibition of T-cell proliferation, survival and effector functions (cytokine release and cytotoxicity), and promotion of differentiation of CD4+ T cells into regulatory T cells (Tregs), which are also immunosuppressive. In the presence of chronic antigen exposure, PD-1 receptor expression can be excessive, leading to an 'exhausted phenotype' in which T cells become dysfunctional. Interestingly, tumour cells can be induced to express PD-L1 (and PD-L2) ligands under the influence of IFN- γ , in a similar way neutralising T-cell action by hijacking their abundantly present PD-1 receptor.

Current immunomodulatory anti-cancer drugs target the PD-1/PD-L1,2 axis. Nivolumab (Opdivo, BMS) and pembrolizumab (Keytruda, MSD) are highly specific antibodies that bind PD-1 and block the interaction between PD-1 with both PD-L1 and PD-L2, while atezolizumab (Tecentriq, Roche), avelumab (Bavencio, Pfizer) and durvalumab (Imfinzi, Astra Zeneca) target PD-L1.

By blocking the interaction between the PD-1 receptor and its ligands, CD8⁺ cytotoxic cells can be re-activated. Currently, nivolumab is reimbursed for second line treatment, regardless of the histological subtype or PD-L1 score. Pembrolizum-



TABLE 1. PD-L1 immunohistochemistry assays.				
Antibody	Platform	Associated drug	Positivity threshold	Minimum tumour cells
22C3 (Dako)	Link 48 Autostainer	pembrolizumab	First line: ≥50% tumour cells Second line: ≥1% tumour cells	100
28-8 (Dako)	Link 48 Autostainer	nivolumab	≥1% tumour cells	100
SP142 (Ventana)	Benchmark ULTRA	atezolizumab	>50% tumour cells or >10% immune cells	50
SP263 (Ventana)	Benchmark	durvalumab	≥25% tumour cells	100

ab can be used for first line treatment when PD-L1 expression in tumour cells is 50% or higher. Patients with lung cancer containing an EGFR activating mutation or an ALK/ROS1 rearrangement are currently not eligible, although some of these patients might be sensitive. Generally speaking, these tumours are characterised by a low mutational load, which makes response to current immunotherapy less likely. However, it seems that some of these tumours can also have a high mutational load, which could explain some rare responses in this category of NSCLC.

PD-L1 TESTING

One could easily think that PD-L1 is just one more predictive biomarker for NSCLC. However, PD-L1 testing is different for several reasons: (i) it is not a binary biomarker, such as EGFR mutation (present/absent), but rather a continuous biomarker with a range of expression levels; (ii) it is also a heterogeneous marker, with intra-tumoral and temporal variation of expression; (iii) different assays have been developed with different scoring criteria and different positivity thresholds; (iv) some patients with PD-L1-negative tumours might respond to immune checkpoint inhibitors; and (v) PD-L1 testing is also required for squamous cell carcinomas.⁵

In contrast to other predictive biomarkers, international guidelines from international societies for the implementation of PD-L1 testing in the pathology lab are lacking.^{1,6,7} In April 2017, the International Association for the Study of Lung Cancer (IASLC) published the IASLC atlas of PD-L1 IHC testing in lung cancer.⁵ The present guidelines are based on this guide, although many questions have not been answered at the present time, as already highlighted by other authors.⁸

OVERVIEW OF PD-L1 IHC

As for all other immunohistochemical tests, several factors can influence PD-L1 IHC results.

Pre-analytic factors:

Pre-analytic parameters are crucial for molecular and IHC testing. These parameters include time to fixation, fixation time and type of fixative. The standardisation of such factors remains difficult. However, the use of standardised procedures should minimise too large variations of these parameters. Recommendations about pre-analytic factors for molecular testing were given in the previous version of the guidelines, and overall, the tissue handling for PD-L1 testing should be the same as for other molecular biomarkers¹. For PD-L1 testing, there is, at present, no information about the effect of a delayed fixation.⁵ The time to fixation should be as short as possible (at most one hour) in order to avoid degradation of proteins or nucleic acids. Nevertheless, a fixation time of at least three hours is recommended.⁵ Decalcifying solutions must be avoided, because PD-L1 assays have not been validated on decalcified tissues. In case only bone metastases can be reached for biopsy, a bone marrow aspiration should be considered. If a biopsy is planned, information to the pathologist should be forwarded so that a 'light' version of a decalcification procedure should be applied (EDTA).

Analytic factors

Different assays have been separately developed, each in conjunction with a specific drug (*Table 1*). The 28-8 antibody (Agilent technologies/Dako) was developed in association with nivolumab; the 22C3 clone (Agilent technologies/Dako) in association with pembrolizumab (MSD); the SP142 assay (Ventana) in association with atezolizumab and the SP263 assay (Ventana) in association with durvalumab. Moreover, these assays have been developed on specific platforms: the Dako Autostainer Link 48 for the Dako clones and the Ventana platforms for the Ventana antibodies. This combination of drugs with specific assays poses challenges for the pathologists. How to implement PD-L1 testing? How to choose which assay to

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FIGURE 2. Algorithm for the optimisation of the management of non-small cell lung cancer samples.

develop? Depending on available drugs or on available immunostainers?

Different studies have evaluated the concordance between the different PD-L1 assays.²⁻⁴ The results show that 28-8, 22C3 and SP263 clones show comparable performance when they are used with the appropriate test kits. The SP142 assay appeared to stain less tumour cells as compared to other antibodies.

The Food and Drug Administration has approved the 22C3 pharmDx test as a companion diagnostic for pembrolizumab use, while the European Medicines Agency recommends a validated test (without antibody specification).

The panel agreed that the minimal requirement for using PD-L1 IHC in daily practice is that the technique and antibody used should be accredited (including participation in external quality assessment). However, some members of the panel recommended the use of a validated assay on the appropriate platform (i.e., 22C3 or 28-8 antibodies on the Dako

Autostainer Link 48 and SP263 on the Benchmark Ventana platform). Protocols for use of the aforementioned Dako antibodies on the Omnis platform are in development.

Post-analytic factors

PD-L1 scoring is different across the different validated assays. For the 28-8 and 22C3 assays, only linear membrane tumour cell staining should be evaluated (at any intensity, whether partial or circumferential) (*Figure 1*). Granular membranous staining is also taken into account. Moreover, the threshold to consider a case as PD-L1 positive is different across the different assays (i.e., 1%, 25% or 50% of tumour cells) (*Table 1*).

Immune cells such as lymphocytes or macrophages can express PD-L1. This expression can hamper interpretation. Especially, macrophages can have membranous staining and they can be falsely interpreted as positive tumour cells when they are close to PD-L1 negative tumour cells.⁵ There is a gen-



eral agreement and recommendation that the interpretation of PD-L1 IHC should be done by pathologists that have been trained to interpret this test.

WHEN IS PD-L1 TESTING INDICATED?

PD-L1 testing is recommended for all patients with NSCLC at an advanced stage (III or IV). PD-L1 testing is indicated for all NSCLC subtypes. The NCCN Panel recommends IHC testing for PD-L1 expression before first line treatment in patients with metastatic NSCLC with negative or unknown tests results for EGFR mutations, ALK rearrangements, and ROS1 rearrangements (*https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf*).

PD-L1 testing should be ordered, by clinicians, at the time of diagnosis for patients presenting with stage III or IV NS-CLC. Reflex testing for stage III/IV patients could be initiated by the pathologist in order to avoid delay between the diagnostic procedure and PD-L1 testing. For implementation of this reflex testing, good communication between the thoracic oncology team and the pathologist is crucial, because the pathologist needs to know the clinical stage. We propose that clinical stage, in addition to the minimum required clinical information defined in the previous Belgian guidelines, should be mentioned in the request.¹ This information is useful in helping the pathologist to determine priorities.

In addition, as for other testing, the pathologist should alert the clinician as soon as possible if the quality or the tumour content of the sample is insufficient to perform further testing. The minimum number of tumour cells, defined for the different assays, is 100 tumour cells for the 28-8 and 22C3 assays. The decision to test PD-L1 status at the time of diagnosis for patients with lower stage disease should be made locally in consultation with the thoracic oncology team and should be discussed at the multidisciplinary oncology meetings (COM/ MOC). Reflex testing for the lower stage patients could be initiated, especially in case of immunotherapy trial availability.

ROLE OF THE PATHOLOGIST IN THE SELECTION OF THE SAMPLE

In contrast to EGFR or ALK molecular testing, the influence of sample types or sites is less established. The Working Group proposes that the choice of which sample to test should be based on the sample characteristics: tumour content (in particular the number of tumour cells) and pre-analytic features. Samples can be resection specimens, biopsies and cytological samples. If resection and biopsy samples are available, we propose performing the test on the resection sample (on the block with the most tumour cells). Cytology is considered to be a powerful diagnostic method in the diagnosis of lung cancer and, frequently, a cytological sample is the only specimen available. Although PD-L1 assays have not been validated for cytological samples, studies on matched cytological and histological samples report that cytological samples can be used for PD-L1 testing.⁹

For patients with multiple apparently separate tumours, testing each tumour may be considered.

Archival samples can be used for PD-L1 testing. IASLC recommends that archival formalin-fixed paraffin embedded material may be used when not older than three years, based on findings by Midha *et al.*¹⁰

REPORTING

Guidelines for reporting the results of PD-L1 testing are based on the International Organisation for Standardization (ISO) 15189:2012 requirements for medical laboratories. Reports should include clear results. The protocols should contain the sample characteristics (including the identity of the block used for analysis), the name of the antibody clone, the platform used and the scoring criteria. The results section should mention whether the number of analysed cells is above the threshold. IASLC recommends reporting the extent of positive cells, at least in 10% increments.⁵ They base this recommendation on the fact that therapeutic response to immune checkpoint inhibitors is reported to be in proportion to PD-L1 expression. Especially, it should be mentioned whether <1% or $\ge 1\%$ of the tumour cells stains positively. If a result is inconclusive, whether due to assay failure or an insufficient specimen (less than 100 tumour cells) or another reason, the report should state the reason and should suggest testing a different specimen that is more likely to yield a definite result.

As for other biomarker testing, the Working Group recommends that the PD-L1 test result should be available within ten working days after sample reception in the testing laboratory, together with the results of other tests necessary for patient management.

QUALITY

The PD-L1 test procedure needs to be standardised and performed in labs that are accredited according to the ISO 15189:2012 and participate in internal and external quality controls as detailed in the nomenclature article 33bis. For successful patient treatment, it is of great importance that IHC test results are highly reliable and accurate. Participation in external quality assessment (EQA) allows rapid exposure of errors or deviations from the protocol. However, the development of different PD-L1 assays with different scoring criteria will complicate development of EQA.⁵ The European Society of Pathology has launched a pilot EQA in 2017 (*http://lung.eqascheme.org*). A Belgian ring trial has also been





KEY MESSAGES FOR CLINICAL PRACTICE

- 1. PD-L1 immunohistochemistry reflex testing is recommended for all types of stage III and stage IV non-small cell lung cancer patients.
- 2. PD-L1 immunohistochemistry testing for patients with lower stage disease should be discussed locally.
- 3. The interpretation of PD-L1 immunohistochemistry should be done by a pathologist, recognised by the Belgian Ministry of Health, who obtained an appropriate training certificate.
- 4. These guidelines will be updated in the future.

organised. Evaluation of the prevalence of positive cases in comparison to both the data reported in the literature and the expected frequency is another control method for detecting deviation from the protocol (i.e., for Ab 22C3, one third of the analysed cases should be negative, one third should show a low expression and one third a high expression using the cut-off of 50%). The pathologists involved in molecular testing must be qualified according to Belgian law and accordingly recognised by RIZIV/INAMI (Rijksinstituut voor ziekte- en invaliditeitsverzekering/Institut national d'assurance maladie-invalidité) and should have been trained in the interpretation of PD-L1 IHC testing.

CONCLUSIONS AND PERSPECTIVES

Introduction of immunotherapy has changed treatment and biomarker testing algorithms for NSCLC patients. The algorithm of the previous guidelines has been updated (Figure 2). At present, PD-L1 IHC is the required biomarker for patient stratification for the use of immune checkpoint inhibitors. However, there are still open questions regarding PD-L1 testing and for several points, new data are needed and may change the current recommendations. Moreover, new predictive biomarkers, such as mutational burden, are under investigation and may modify our current practice. It must be stressed that PD-L1 testing is considered as an 'enrichment factor', meaning that the higher the percentage of PD-L1 positive cells, the higher the likelihood for response to current immunotherapy, in particular, when looking at the \geq 50% cut-off. Hundreds of trials of combination therapy are ongoing such as the combination of immunotherapy with anti-oncogenic therapy, chemotherapy and radiotherapy. It is expected that, based on the results of these trials, current guidelines will be changed in the near future.

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