THE CHALLENGES OF USING PD-L1 AS A PREDICTIVE BIOMARKER AND THE THERAPEUTIC APPROACH IN NON-SMALL CELL LUNG CANCER IMMUNOTHERAPY

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Abstract

Lung cancer has gained worldwide a top place in the incidence and mortality related to malignancy. Out of the two different types of pulmonary neoplasms, the histological subtypes included in non-small cell category have received a particular interest because of the various therapeutic targets identified. In the recent years, due to its promising results, the development of immunotherapy is considered a major discovery and has become one of the main therapeutic options along with chemotherapy, radiotherapy and surgery. In order to determine the selection of patients who can obtain beneficial approach from immune checkpoint inhibitors treatment, programmed death-ligand 1 (PD-L1) expression evidenced by immunohistochemistry (IHC) testing has been implemented as a complementary or as a mandatory diagnostic tool in patients with advanced non-small cell lung cancer (NSCLC). The developing studies in this field were concentrated upon the importance of the expression of PD-L1 as a predictive biomarker for treatment response and patients selection validated into clinical daily practice. The aim of this review is to enlighten the factors which influence PD-L1 expression and the utility as biomarker in immunotherapy.

Rezumat

Cancerul bronhopulmonar a câștigat un loc fruntaș în topul incidenței și al mortalității de cauză malignă la nivel global. În cadrul cele două tipuri diferite de neoplasm pulmonar, subtipurile histologice care aparțin categoriei „fără celule mici” au dobândit un interes particular datorită numeroaselor ținte terapeutice identificate. În ultimii ani, conform rezultatelor promițătoare oferite, imunoterapia este considerată o descoperire importantă și a devenit una dintre principalele opțiuni terapeutice alături de chimioterapie, radioterapie și chirurgie. Cu scopul de a selecta pacienții care vor beneficia de tratament cu inhibitori ai punctelor de control imun, expresia ligandului morții celulare programate 1 (PD-L1) evidențiată prin imunohistochimie (IHC) a fost folosită ca metodă de diagnostic complementară sau condiționată la pacienții cu neoplasm bronhopulmonar fără celule mici. Studiile desfășurate în acest domeniu s-au concentrat asupra valorii expresiei PD-L1 ca și biomarker predictiv al răspunsului la tratament și al selectării pacienților, aplicabil în practica medicală zilnică. Scopul acestui review este de a evidenția factorii care influențează expresia PD-L1 și utilitatea sa ca biomarker în tratamentul cu inhibitori ai punctelor de control imun.

Keywords: programmed death-ligand 1 (PD-L1), biomarker, non-small cell lung cancer, immune checkpoint inhibitors (ICI)

Introduction

Pulmonary cancer is considered one of the most common causes of mortality in cancer patients in both genders. There are two main histological categories, with the predominance of non-small cell type (> 80%), also subdivided into non squamous (especially adenocarcinoma) and the small cell type (~ 20%) [21]. Unfortunately, at the great majority of patients, the

diagnosis is established in advanced stages and until the development of targeted therapy and immunotherapy, their prognosis and overall survival was decreased. In order to benefit from the latest treatment options, biomarkers are being tested for patients' selection and are directly correlated with a particular therapy. Immunotherapy is a major discovery in cancer treatment and one of the main topics of research in this field. The aim of this treatment method is to boost the

capacity of the immune system in order to detect and destroy cancer cells and in consequence to develop a long-term memory of the adaptive immune response. This immune response leads to durable tumour regression and possible cure. The main role of immune checkpoints is to maintain tolerance to self-antigens and down-regulate T cells effector function. The mechanism of action of the immune checkpoint inhibitors (ICI) is based on reversing the tumour immune escape by suppressing immune checkpoints, which activate effector-T cells and subsequently eliminate the tumour cells. Immunotherapy has been approved in advanced (metastatic) non-small cell lung cancer in 2015 by the Food and Drug Administration (FDA) with anti-PD1 antibody nivolumab followed by pembrolizumab and it has provided better survival outcome and different toxicity profile in comparison with classical chemotherapy. The efficacy of using immune checkpoint inhibitors is highlighted by the long-term survival population, defined as patients with advanced NSCLC who have survived more than 2 years after the initial diagnosis [21].

Programmed cell death-1 protein (PD-1) is a protein expressed on the immune cells which connects with PD-L1 protein expressed in cancer cells and determines the immune suppression of cancer cells, by blocking their attack from host immunity. PD-1 is expressed on T cells, B cells and NK (natural killer) cells, and by binding to its ligands PD-L1 and PD-L2 determines a temporary or permanent inhibition of the cytotoxic properties of CD8+ T cells. This PD1-PDL1 interaction is the physiological mechanism of control of the autoimmunity, but when PD-L1 is connected with tumour cells or immune cells, PD1 interaction with CD8+ T cells is suppressed and allows the tumour to escape the adaptive antitumor immune response. Immune checkpoint inhibitors (ICI) which target the axis PD-1/PD-L1 have been implemented with the purpose of restoring T cells toxicity. By binding to PD-1, PD-L1 inhibits immune-mediated tumour destruction and progression of malignancy. Despite the major interest and research in non-small cell lung cancer cells, PD-L1 expression can also be identified in other malignancies such as: melanoma, gastric cancer, hepatocarcinoma, renal cell carcinoma, haematological malignancies, etc. [11].

Nowadays cancer medicine, FDA has approved four molecules in non-small cell lung cancer immunotherapy. Nivolumab is a PD-1 immune checkpoint inhibitor antibody IgG4 fully human, recommended in the treatment of metastatic NSCLC which progressed after prior chemotherapy (platinum-based). Pembrolizumab is an IgG4 monoclonal antibody approved as first-line monotherapy for advanced or metastatic NSCLC with PD-L1 positive on tumour cells and without EGFR (epidermal growth factor receptor) or ALK (anaplastic lymphoma kinase) mutations and in second-line at PD-L1 positive ($\geq 1\%$) with disease progression

during or after platinum-containing chemotherapy. Also, it has been approved by FDA as first-line option in patients with metastatic squamous and non-squamous lung cancer in combination with chemotherapy. Atezolizumab is a human monoclonal antibody IgG1 approved recently as first-line treatment in metastatic non-squamous NSCLC patients without EGFR/ALK mutations, in combination with chemotherapy and biological agents. Also, it is recommended after/during progression of platinum-based chemotherapy. Durvalumab is an antibody IgG1 anti- PD-L1 with indication as consolidation therapy at stage III patients with unresectable disease, without progression after concurrent platinum-based chemoradiotherapy and requires PD-L1 levels of $> 1\%$ on tumour cells [8]. Avelumab is an antibody anti PD-L1 currently under research in NSCLC setting [11].

Materials and Methods

We have used PubMed as the main search engine. Using the terms “PD-L1” “expression” “biomarker” “non-small cell lung cancer” “immunotherapy” and additional filters as 10-years criteria, human species and English language we have identified a total number of 129 articles. In order to highlight the scientific research interest on this topic evidenced by the constant emerging new data from clinical trials and the gradual development of immunotherapy, we considered appropriate the use of 10-years filter.

We have used as inclusion criteria the articles which referred exclusively to the correlation between non-small cell lung cancer and PD-L1, factors which influence PD-L1 expression, the role of biomarker in this malignant setting, the accuracy and the limits as a prognostic factor in immunotherapy. The articles with no relevant clinical data on this topic, with referral to PD-L2 or with referral to other tumour sites treated with immune checkpoint inhibitors were excluded. After sorting the results using the previous terms, we have collected a total number of 33 articles which best matched our search.

Figure 1.
Steps and criteria for article selection

Results and Discussion

In order to evaluate the PD-L1 expression and prognostic value, immunohistochemistry (IHC) staining has been

implemented as complementary or mandatory diagnosis tool for the quantification of PD-L1. In spite of the utility of this method, PD-L1 expression has several limitations due to the method of testing, the cut-off value and the heterogeneity.

PD-L1 expression and IHC testing limitations

The testing method used to identify the expression of PD-L1 on the membrane of tumour cells is IHC staining which offers a percentage defined as the tumour proportion score (TPS) varying from 1% to 100% and every test sets an individual cut-off value. Each one of the approved drug molecules in this setting is accompanied by a different anti-PD-L1 IHC assay, whether it is complementary or mandatory and currently there are four standardized assays approved by FDA. The need of a standardized IHC assay for all the molecules has been intensively studied but there has not been so far implemented. Nivolumab is associated with 28-8 pharmDx assay and uses against PD-L1 a rabbit monoclonal primary antibody, in comparison with 22C3 Dako pharmDx which is used for pembrolizumab and includes a mouse monoclonal primary antibody. In SP263 Ventana method for durvalumab and SP142 Ventana assay for atezolizumab, the sections are stained with anti-PD-L1 rabbit monoclonal primary antibody [18].

The Blue Print Project compared the four PD-L1 IHC assays used in clinical trials, which are different due to the primary monoclonal antibody used, testing platform, detection system and scoring method, and has concluded that all the assays detected the immune cells (IC) but only three of them (28-8, 22C3 and SP 263) were similar in analysing the proportion of tumour cells (TC) [14]. This data emphasizes the similarities and differences among these assays without affecting their individual clinical specificity and sensitivity [25]. Although these IHC tests are commercially available, many countries have adopted their own laboratory developed IHC methods due to high expenses and the lack of standardization. The statistical data shows that 20 - 30% of the NSCLC patients present a PD-L1 value $\geq 50\%$ and low to moderate expressions (TPS/tumour proportion score, ranging from 1% to 49%) are identified at 30% of the patients [18].

In order to be adequate for PD-L1 quantification, a number of minimum 100 tumour cells from the histological sample is required. One of the most common methods used to obtain the sample specimen for analysing PD-L1 status is percutaneous image-guided (computer tomography) core-needle lung biopsy. Although, in some cases the material is insufficient, studies have proved that rebiopsy is considered a safe diagnostic tool with patient complication rates similar to the general population [31]. Because many patients have advanced and unresectable disease at presentation, therefore only small samples are available for testing, the quantification of PD-L1 on cytology specimens from thoracentesis, pericardiocentesis,

endobronchial ultrasound-guided (EBUS) fine-needle aspiration (FNA) is considered a feasible method, according to the literature. Also, this diagnostic tool (EBUS FNA) has similar results compared to surgical resected specimens and has minimal complications [13]. In addition, data shows that the cytological specimens obtained from EBUS FNA can also be used for other testing methods such as next generation sequencing (NGS) [4]. On the other hand, there are available literature data which reveal a discordance between PD-L1 expression from biopsy samples and their correspondent from the surgical specimen, which may alter the therapeutic decision. As a consequence, the authors suggest that this poor correlation leads to the necessity of repeated biopsies from the same tumour, but from different sites [15]. Also, in the resected specimens PD-L1 expression was higher on immune cells as compared to the positivity on the tumour cells.

The influence and interactions of oncogenic drivers and other biological markers with PD-L1 expression

The expression of PD-L1 can be influenced by a variety of factors and the correlation between oncogenic drivers and PD-L1 has been an actively researched domain. The literature data shows that epidermal growth factor receptor (EGFR) pathway induces PD-L1 protein activation, therefore in EGFR - mutant NSCLC patients a higher value of PD-L1 has been observed, in comparison with EGFR - wild type patients [3]. Also, the PD-L1 and EGFR positive patients treated with tyrosine kinase inhibitors (TKI) have a better response rate and longer time to progression, unlike the PD-L1 negative patients, due to the immunomodulatory effect induced by EGFR [23]. According to these results, preclinical research and clinical based evidence of the association between TKI and immunotherapy is considered a promising method, but precautions are needed because the risk of side-effects is increased. In contrast with these results, other studies reveal the opposite, that by using EGFR - TKI, PD-L1 is decreased and the benefit of using immunotherapy in pre-treated EGFR - mutant patients is less effective. As a conclusion on this topic, more data needs to be validated [30]. A higher PD-L1 value was correlated with mutant tumours which express the rearrangement of EML4-ALK (echinoderm microtubule - associated protein-like 4 in fusion with anaplastic lymphoma kinase) and KRAS (kirsten rat sarcoma viral oncogene homologue) [3]. Some data shows that patients with EGFR mutation and without KRAS mutation had an increased percentage of PD-L1 on tumour cells as compared to the patients without EGFR mutation and the presence of KRAS mutation. On the other hand, PD-L1 expression level on tumour infiltrating lymphocytes (TILs) was increased in patients without EGFR or KRAS mutations [16].

PD-L1 expression on T lymphocytes was investigated at patients treated with EGFR - TKI and concluded that disease progression is higher at patients with

PD-L1 positive on T cells following targeted therapy and is the most significant after one week of treatment. Also, PD-L1 positivity on peripheral T cells is described as a predictor of worse clinical outcome associated with declined progression free survival (PFS) and overall survival (OS) [20].

Lymphocyte activating 3 gene (LAG-3) is an immune checkpoint found on natural killer cells, tumour infiltrating lymphocytes (TILs), T and B-cells, which is identified more frequent in non - adenocarcinoma histological type. LAG3 has the role of inhibiting the activation of T-cell proliferation and homeostasis and it was associated with poor prognosis. LAG3 positivity was correlated with a higher value of PD-L1 on tumour cells and in terms of clinical outcome, it has a negative impact because it decreases recurrence-free survival rate and determines early postoperative recurrence [12]. A potential negative correlation of PD-L1 was identified with the epithelial- mesenchymal transition status (EMT), a key process in tumour progression which gives the cancer cells the ability to metastasize. EMT has the role to downregulate PD-L1 expression at patients following chemotherapy and may be useful to stratify the patients who could further benefit from immunotherapy [9].

Regarding the association between biological markers and PD-L1, the literature reveals that among the inflammatory markers such as neutrophil lymphocyte ratio (NLR), lymphocyte monocyte ratio (LMR) and platelet lymphocyte ratio (PLR), only C-reactive protein (CRP) level was associated with PD-L1 positivity. Also, it has been demonstrated that increased level of serum CRP is a predictive factor of the PD-L1 expression in adenocarcinoma and squamous cell carcinoma patients. Because of this association, CRP could be implemented as a predictive biomarker for immunotherapy efficacy, but further research is needed [2]. Another potential biological marker associated with PD-L1 positivity on both tumour and immune cells is the lymphocyte density. PD-L1 expression was correlated with advanced stages and lymphovascular invasion, but due to the intra-tumour heterogeneity, it has not gained a significant prognostic value and in some cases low lymphocyte density was observed even in tumours with PD-L1 positivity [6].

One of the major challenges in defining correctly PD-L1 expression is the intra-tumour heterogeneity and dynamic, which can lead to false results with clinical impact on subsequent therapy and it is one of the main reasons of the imperfection of PD-L1 as a biomarker. This tumour parameter can explain the failure of response to immune checkpoint inhibitors in PD-L1 positive patients, and in contrast the benefit of immunotherapy in PD-L1 negative patients [33]. PD-L1 expression is also influenced by the histological and morphological characteristics. Studies reveal that PD-L1 value is higher in pleomorphic carcinomas or in NSCLC with pleomorphic characteristics as

compared to lepidic components of adenocarcinoma. The correlation made by the IHC analysis of the tissue samples obtained from biopsy and surgical resection indicated that pleomorphic carcinoma was associated with poor prognosis. Also, it has been demonstrated the link between a higher value of PD-L1 positivity and solid components of adenocarcinoma [22]. On the other hand, another study referring to the histology evidences that high expression of PD-L1 is more frequent identified in the squamous subtype of NSCLC [19].

In the need of potential associated biomarkers, PD-L1 correlated with mRNA (micro ribonucleic acid) molecule expression has become of particular interest. The literature data shows that in patients with borderline positive PD-L1 levels on IHC, RNA *in situ* hybridization ISH technique can be complementary used to stratify the patients and it is associated with an improved outcome [29]. Also, mRNA expression levels can be associated with amplifications and deletions of PD-L1 but further investigation is needed [5].

Although the great majority of patients are diagnosed in advanced stages and PD-L1 expression has a therapeutic role in this category, literature data reveals that in a small percentage of early stage NSCLC there is a higher expressed PD-L1, mainly associated with young age and poorly differentiated histology. The clinical - pathological data are correlated with an improved outcome, potentially explained as an adaptive tumour response at the immune pressure of the host [7]. In contrast, some studies reveal that in early-stage NSCLC patients, PD-L1 expression is not appropriate for neither prognostic nor predictive factor of benefit from adjuvant chemotherapy, irrespective of the cut-off value [32]. Another category of patients associated with positive PD-L1 were those with completely resected/ surgically removed stage I non-small cell lung cancer. In this category, a negative correlation has been established because a high PD-L1 expression on tumour cells and tumour infiltrated macrophages had determined lower survival rates [28]. Also, PD-L1 is described as positive predictive biomarker in patients with squamous cell histology, an increased T size, lymph node metastasis and prior adjuvant therapy [24].

One of the controversial characteristics of the prognostic role of PD-L1 as a prognostic is the concordance of the expression levels between metastatic and primary tumours and a similitude was identified in primary adenocarcinoma and in the metastatic tumour, despite intra-tumour heterogeneity. Hence, PD-L1 expression in metastatic adenocarcinoma can predict PD-L1 positivity in the primary tumour site [17]. The role of tumour PD-L1 expression as a biomarker for survival outcomes in NSCLC patients who undergone immunotherapy is highlighted by the association between PD-L1 positive expression and improved progression-free survival and overall survival parameters [27].

Furthermore, in pre-treated NSCLC patients, tumour PD-L1 expression evidenced by IHC staining at a cut-off value of > 1% is correlated with improved response rate to immunotherapy, which suggests the role as predictive biomarker for clinical outcome [1]. In order to enhance to utility of PD-L1 as a prognostic factor of immune checkpoint inhibitors, PD-L1 expression in tumour tissue is correlated with the clinical response at immunotherapy, regardless of the cut-off value for positivity [10]. Despite the contradictory results obtained in preclinical and clinical research, PD-L1 will remain the main biomarker used in daily clinical practice for the selection of patients with immunotherapy [26].

Conclusions

In the rapidly evolving field of immunotherapy in NSCLC, the need of a biomarker implementation for patients' selection and prediction of response is crucial. As it was mentioned above, PD-L1 has been recognized as the only biomarker associated so far with NSCLC, although it has several imperfections. The increased number of studies in the recent years revealed the particular interest in improving the accuracy of PD-L1 expression and discovering potentially associated biomarkers. One of the unmet needs in this field is to harmonize the IHC assays and to identify the individual therapy-related PD-L1 testing cut-off value. Despite the biological and genetic factors mentioned above which affect its predictability, PD-L1 remains the only approved biomarker in NSCLC immunotherapy.

Conflict of interest

The authors declare no conflict of interest.

References

1. Aguiar PN Jr, De Mello RA, Hall P, Tadokoro H, Lima Lopes G, PD-L1 expression as a predictive biomarker in advanced non-small cell lung cancer: updated survival data. *Immunotherapy*, 2017; 9(6): 499-506.
2. Akamine T, Takada K, Toyokawa G, Kinoshita F, Matsubara T, Kozuma Y, Haratake N, Takamori S, Hirai F, Tagawa T, Okamoto T, Yoneshima Y, Okamoto I, Shimokawa M, Oda Y, Nakanishi Y, Maehara Y, Association of preoperative serum CRP with PD-L1 expression in 508 patients with non-small cell lung cancer: a comprehensive analysis of systemic inflammatory markers. *Surg Oncol.*, 2018; 27(1): 88-94.
3. Bassanelli M, Sioletic S, Martini M, Giacinti S, Viterbo A, Staddon A, Liberati F, Ceribelli A, Heterogeneity of PD-L1 expression and relationship with biology of NSCLC. *Anticancer Res.*, 2018; 38(7): 3789-3796.
4. Biswas A, Leon ME, Drew P, Fernandez-Bussy S, Furtado LV, Jantz MA, Mehta HJ, Clinical performance of endobronchial ultrasound-guided transbronchial needle aspiration for assessing programmed death ligand-1 expression in nonsmall cell lung cancer. *Diagn Cytopathol.*, 2018; 46(5): 378-383.
5. Budczies J, Bockmayr M, Denkert C, Klauschen F, Gröschel S, Darb-Esfahani S, Pfarr N, Leichsenring J, Onozato ML, Lennerz JK, Dietel M, Fröhling S, Schirmacher P, Iafrate AJ, Weichert W, Stenzinger A, Pan-Cancer analysis of copy number changes in programmed death-ligand 1 (PD-L1, CD 274)- association with gene expression, mutational load and survival. *Genes Chromosomes Cancer.*, 2016; 55(8): 626-639.
6. Casadevall D, Clavé S, Taus Á, Hardy-Werbin M, Rocha P, Lorenzo M, Menéndez S, Salido M, Albanell J, Pijuan L, Arriola E, Heterogeneity of tumor and immune cell PD-L1 expression and lymphocyte counts in surgical NSCLC samples. *Clin Lung Cancer.*, 2017; 18(6): 682-691.
7. Cooper WA, Tran T, Vilain RE, Madore J, Selinger CI, Kohonen-Corish M, Yip P, Yu B, O'Toole SA, McCaughan BC, Yearley JH, Horvath LG, Kao S, Boyer M, Scolyer RA, PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. *Lung Cancer*, 2015; 89(2): 181-188.
8. www.fda.gov.
9. Funaki S, Shintani Y, Kawamura T, Kanzaki R, Minami M, Okumura M, Chemotherapy enhances programmed cell death 1/ligand 1 expression via TGF- β induced epithelial mesenchymal transition in non-small cell lung cancer. *Oncol Rep.*, 2017; 38(4): 2277-2284.
10. Gandini S, Massi D, Mandalà M, PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: A systematic review of the literature and meta-analysis. *Crit Rev Oncol Hematol.*, 2016; 100: 88-98.
11. Gautam KV, Vennepureddy A, Ibrahim U, Safa F, Samra B, Atallah JP, Anti-PD-1/PD-L1 antibodies in non-small cell lung cancer: the era of immunotherapy. *Exp Rev Anticancer Ther.*, 2017; 17(1): 47-59.
12. He Y, Yu H, Rozeboom L, Rivard CJ, Ellison K, Dziadziuszko R, Suda K, Ren S, Wu C, Hou L, Zhou C, Hirsch FR, LAG-3 protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. *J Thorac Oncol.*, 2017; 12(5): 814-823.
13. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, Haghighi M, Fazlollahi L, Stoopler MB, Sonett JR, Sacher AG, Shu CA, Rizvi NA, Saqi A, PD-L1 expression in non-small cell lung carcinoma: Comparison among cytology, small biopsy, and surgical resection specimens. *Cancer Cytopathol.*, 2017; 152(12): 896-907.
14. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, Richardson W, Towne P, Hanks D, Vennapusa B, Mistry A, Kalamegham R, Averbuch S, Novotny J, Rubin E, Emancipator K, McCaffery I, Williams JA, Walker J, Longshore J, Tsao MS, Kerr KM, PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol.*, 2017; 12(2): 208-222.
15. Ilie M, Long-Mira E, Bence C, Butori C, Lassalle S, Bouhlef L, Fazzalari L, Zahaf K, Lallée S, Washetine K, Mouroux J, Vénissac N, Poudenx M, Otto J, Sabourin JC, Marquette CH, Hofman V, Hofman P, Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a

- potential issue for anti-PD-L1 therapeutic strategies. *Ann Oncol.*, 2016; 27(1): 147-153.
16. Ji M, Liu Y, Li Q, Li X, Ning Z, Zhao W, Shi H, Jiang J, Wu C, PD-1/PD-L1 expression in non-small-cell lung cancer and its correlation with EGFR/KRAS mutations. *Cancer Biol Ther.*, 2016; 17(4): 407-413.
 17. Kim S, Koh J, Kwon D, Keam B, Go H, Kim YA, Jeon YK, Chung DH, Comparative analysis of PD-L1 expression between primary and metastatic pulmonary adenocarcinomas. *Eur J Cancer.*, 2017; 75: 141-149.
 18. Lantuejoul S, Damotte D, Hofman V, Adam J, Programmed death ligand 1 immunohistochemistry in non-small cell lung carcinoma. *J Thorac Dis.*, 2019; 11(Suppl1): S89-S101.
 19. Mandarano M, Bellezza G, Belladonna ML, Van den Eynde BJ, Chiari R, Vannucci J, Mondanelli G, Ludovini V, Ferri I, Bianconi F, Del Sordo R, Cagini L, Albini E, Metro G, Puma F, Sidoni A, Assessment of TILs, IDO-1, and PD-L1 in resected non-small cell lung cancer: An immunohistochemical study with clinicopathological and prognostic implications. *Virchows Arch.*, 2019; 474(2): 159-168.
 20. Meniawy TM, Lake RA, McDonnell AM, Millward MJ, Nowak AK, PD-L1 on peripheral blood T lymphocytes is prognostic in patients with non-small cell lung cancer (NSCLC) treated with EGFR inhibitors. *Lung Cancer*, 2016; 93: 9-16.
 21. Nadal E, Massuti B, Domine M, Garcia-Campelo R, Cobo M, Felip E, Immunotherapy with checkpoint inhibitors in non-small cell lung cancer: insights from long-term survivors. *Cancer Immunol Immunother.*, 2019; 68(3): 341-352.
 22. Ng Kee Kwong F, Laggner U, McKinney O, Croud J, Rice A, Nicholson AG, Expression of PD-L1 correlates with pleomorphic morphology and histological patterns of non-small cell lung carcinomas. *Histopathology*, 2018; 72(6): 1024-1032.
 23. Omori S, Kenmotsu H, Abe M, Watanabe R, Sugino T, Kobayashi H, Nakashima K, Wakuda K, Ono A, Taira T, Naito T, Murakami H, Ohde Y, Endo M, Akiyama Y, Nakajima T, Takahashi T, Changes in programmed death ligand 1 expression in non-small cell lung cancer patients who received anticancer treatments. *Int J Clin Oncol.*, 2018; 23(6): 1052-1059.
 24. Passiglia F, Bronte G, Bazan V, Natoli C, Rizzo S, Galvano A, Listì A, Cicero G, Rolfo C, Santini D, Russo A, PD-L1 expression as a predictive biomarker in patients with NSCLC: a pooled analysis. *Oncotarget*, 2016; 7(15): 19738-19747.
 25. Ratcliffe MJ, Sharpe A, Midha A, Barker C, Scott M, Scorer P, Al-Masri H, Rebelatto MC, Walker J, Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cutoffs in non-small cell lung cancer. *Clin Cancer Res.*, 2017; 23(14): 3585-3591.
 26. Savic Prince S, Bubendorf L, Predictive potential and need for standardization of PD-L1 immunohistochemistry. *Virchows Arch.*, 2019; 474(4): 475-484.
 27. Schmidt LH, Kümmel A, Görlich D, Mohr M, Bröckling S, Mikesch JH, Grünewald I, Marra A, Schultheis AM, Wardelmann E, Müller-Tidow C, Spieker T, Schliemann C, Berdel WE, Wiewrodt R, Hartmann W, PD-1 and PD-L1 Expression in NSCLC indicate a favorable prognosis in defined subgroups. *PLoS One*, 2015; 10(8): 1-15.
 28. Sepesi B, Cuentas EP, Canales JR, Behrens C, Correa AM, Vaporciyan A, Weissferdt A, Kalhor N, Moran C, Swisher S, Wistuba I, Programmed death cell ligand 1 (PD-L1) is associated with survival in stage I non-small cell lung cancer. *Semin Thorac Cardiovasc Surg.*, 2017; 29(3): 408-415.
 29. Sheffield BS, Fulton R, Kalloger SE, Milne K, Geller G, Jones M, Jacquemont C, Zachara S, Zhao E, Pleasance E, Laskin J, Jones SJ, Marra MA, Yip S, Nelson BH, Gown AM, Ho C, Ionescu DN, Investigation of PD-L1 biomarker testing methods for PD-1 axis inhibition in non-squamous non-small cell lung cancer. *J Histochem Cytochem.*, 2016; 64(10): 587-600.
 30. Soo RA, Lim SM, Syn NL, Teng R, Song R, Mok TSK, Cho BC, Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: Current controversies and future directions. *Lung Cancer*, 2018; 115: 12-20.
 31. Tsai EB, Pomykala K, Ruchalski K, Genshaft S, Abtin F, Gutierrez A, Kim HJ, Li A, Adame C, Jalalian A, Wolf B, Garon EB, Goldman JW, Suh R, Feasibility and safety of intrathoracic biopsy and repeat biopsy for evaluation of programmed cell death ligand-1 expression for immunotherapy in non-small cell lung cancer. *Radiology*, 2018; 287(1): 326-332.
 32. Tsao MS, Le Teuff G, Shepherd FA, Landais C, Hainaut P, Filipits M, Pirker R, Le Chevalier T, Graziano S, Kratze R, Soria JC, Pignon JP, Seymour L, Brambilla E, PD-L1 Protein expression assessed by immunohistochemistry is neither prognostic nor predictive of benefit from adjuvant chemotherapy in resected non-small cell lung cancer. *Ann Oncol.*, 2017; 28(4): 882-889.
 33. Yoneda K, Imanishi N, Ichiki Y, Tanaka F, Immune checkpoint inhibitors (ICIs) in non-small cell lung cancer (NSCLC). *J UOEH*, 2018; 40(2): 173-189.