Association of histologic subtypes with genetic alteration and PD-L1 expression in pulmonary adenocarcinoma

BANTITA PHRUTTINARAKORN¹, THANYANAN REUNGWETWATTANA² and PIMPIN INCHAROEN¹

¹Department of Pathology; ²Division of Medical Oncology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Received January 29, 2020; Accepted June 11, 2020

DOI: 10.3892/mco.2020.2082

Abstract. Genetic alteration and programmed death-ligand 1 (PD-L1) expression have been revealed to be associated with various subtypes of pulmonary adenocarcinoma (ADC). The present study aimed to explore the association between histological subtypes and genetic alterations and PD-L1 expression. A total of 375 cases of pulmonary ADC were included. Genetic alterations were determined using next generation sequencing (NGS) in 136 cases. PD-L1 expression was detected by immunohistochemistry (based on clone 22C3) in the remaining 239 cases. Mutations in the epidermal growth factor receptor gene (EGFR) were detected in 76 (55.8%) cases associated with the papillary subtype (P=0.038). Mutations in the Kirsten rat sarcoma viral oncogene homolog gene (KRAS) were present in 46 (33.8%) cases associated with the lepidic subtype (P<0.001) and mucinous ADC (P=0.037). PD-L1 expression was identified in 63 (26.4%) cases associated with the solid subtype (P<0.001). In conclusion, the present study demonstrated that EGFR and KRAS mutations, alongside PD-L1 protein expression are significantly associated with specific subtypes of pulmonary ADC. These results should aid our ability to accurately select appropriate areas of the heterogeneous tumor for molecular testing methods and to predict patient outcomes and prognosis.

Introduction

Lung cancer is the common carcinoma worldwide in both men and women, and is the leading cause of carcinoma-related death (1). Lung cancer is classified into two main subtypes: Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which are identified in 85 and 15% of diagnosed patients, respectively. NSCLC may be classified further into three subtypes: Adenocarcinoma (ADC) is the most common type (60%), followed by squamous cell carcinoma (SCC) and large cell carcinoma (LCC). The incidence of ADC has increased greatly over the course of the last few decades (2). Several previous studies have identified genetically driven mutations associated with the tyrosine kinase receptor pathway in patients with NSCLC, especially in the case of ADC, including mutations of the genes for epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS) and v-raf murine sarcoma viral oncogene homolog B1 (BRAF), in addition to translocation of the anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1) and rearranged during transfection (RET) genes (3–8), which have led to novel treatment outcomes involving specific targeted tyrosine kinase inhibitors (TKIs). In terms of the histological evaluation of ADC, the recently formed International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classified invasive ADC according to its predominant growth pattern, including lepidic, acinar, papillary, micropapillary and solid subtypes, as well as ADC variants, including mucinous, colloid, enteric and fetal ADC (9). Recently published studies have demonstrated that the histological subtypes of pulmonary ADC are associated with 5-year survival, overall survival and disease-free survival rates across all tumor stages during treatment with adjuvant therapy (4,10–11). Histological subtypes and molecular factor correlations have also been reported on. Previous studies revealed that the EGFR mutation is associated with the lepidic and papillary subtypes (4,12), whereas solid subtypes are predominantly associated with the ALK mutation (13). Another study suggested that the predominance of papillary features in ADC is predictive of the response to gefitinib, a finding that implied that the EGFR mutation could be more common among this subtype (14).

In advanced stage lung cancer, a large body of evidence derived from clinical trials has led to an understanding that immunotherapy is more effective than systemic chemotherapy in terms of treating unresectable or advanced NSCLC (15–19). In the field of cancer immunotherapy, an understanding of the complex interplay between the tumor and the immune systems has been proposed. Programmed death 1 (PD-1) protein, a T-cell co-inhibitory receptor that binds to its ligand, programmed death-ligand 1 (PD-L1), serves a pivotal role.
in regulating T-cell activation and proliferation, functioning as an immune checkpoint. The mechanism of PD-1/PD-L1 interaction provides one of the major pathways used by certain tumors to escape immune surveillance (20,21). Studies using various PD-L1 detection antibodies and immunohistochemistry (IHC) assays have identified that a high level of PD-L1 expression in tumor cells is associated with poor prognosis in NSCLC (22,23), whereas in other studies, PD-L1 expression was associated with longer survival rates (24,25). The association between PD-L1 expression and clinicopathological characteristics, including histopathological parameters, revealed no significant correlations in certain studies (23,26), whereas another study identified that PD-L1 expression was associated with more advanced tumor status, node involvement status and the pathological stage. A further study suggested that PD-L1 expression was likely to be predominantly associated with the solid subtype (27).

This aim of the present study was to identify the association between histological subtypes of pulmonary ADC in the Thai population and genetic alterations identified by next-generation sequencing (NGS), as well as PD-L1 expression identified by PD-L1 (clone 22C3) IHC. The association among clinicopathological parameters and PDL1 expression was also explored.

**Patients and methods**

*Patients.* The study composed of 375 cases of pulmonary ADC diagnosed at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (Bangkok, Thailand) during 2013–2017 divided into two separated groups. A total of 136 cases in the first group had known genetic alteration detected by NGS and 239 cases in the second group had PD-L1 expression testing by IHC. Clinical features and patient characteristics including age, sex, smoking history, specimen size, specimen site, and staging were obtained from the patient's medical record.

**Histologic evaluation.** The pathologic report was retrospectively reviewed in all cases. The glass slides from transbronchial biopsy (TTNB), Video-assisted thoracoscopic surgery (VATS) for wedge resection, lobectomy, pleural biopsy, and tumor removal specimen were retrieved and reviewed. The histologic subtype from all cases was recorded as predominant lepidic, acinar, papillary, micropapillary or solid subtype and mucinous ADC. The histologic subtypes are examined by the several first senior pathologists who assigned the final pathological reports. The pathology trainee and the second senior pathologists who assigned the final pathological reports were using Fisher's exact/ Chi-square test. Odds ratio (OR) was calculated in predominant histologic subtypes correlate with EGFR and KRAS mutation. The association between PD-L1 expression and clinicopathologic variables were using Fisher's exact/ Chi-square test. OR was calculated in predominant histologic subtypes associate with PD-L1 expression.

**Genetic mutation analysis.** All patients in genetic mutation group are performed by next generation sequencing (NGS). DNA extracted from NSCLC FFPE tumor using QIAsymphony DSP DNA Mini kit in the QIAsymphony SP system (Qiagen Inc.) with the protocol provided by the manufacturer. A total of 20 ng of extracted DNA has been measured by Qubit fluorometer using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Inc.) before proceeded to the DNA library step. Genomic DNA was fragmented then selected target regions of 45 genes associated with lung cancer, using Human Lung Cancer Panel (NGHS-005X) from GeneRead DNAseq Targeted Panels v2 (Qiagen Inc.) according to the manufacturer's instructions. The target genes of the Human Lung Cancer Panel (NGHS-005X) are as follow: AKT1, ALK, APC, ATM, BA13, BAP1, BRAF, CDKN2A, EGFR, EPHA5, ERBB2, ERBB4, FXBX7, FGFR1, FGFR2, GRMS, KDR, KEAPI, KIT, KMT2D, KRAS, LRP1B, MDM2, MET, MLH1, MUC16, MYC, NF1, NFE2L2, NOTCH1, PDGFRB, PIK3CA, PIK3CG, PKHD1, PTEN, RARB, RBI, RET, ROS1, RUNX1, SMAD4, SMARCA4, SOX2, STK11 and TP53. Variant discovery analysis was performed with VarSeq v2.1.1 (Golden Helix, Inc.). Only variants that have read depths (DP)≥10 x coverage, genotype quality (GQ)≥20 and presented more than 3% were selected.

**PD-L1 IHC.** In this study used Dako PD-L1 IHC 22c3 pharmDx SK006 IHC assay by using monoclonal mouse anti-PD-L1, clone 22c3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue and using Envision FLEX visualization system on Autostainer link 48 platform. The tonsil tissues were used as the internal control.

A minimum of 100 viable tumor cells are considered adequate for PD-L1 evaluation. Any perceptible membrane staining of tumor cells are included in the scoring. PD-L1 protein expression is determined by using tumor proportion score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The immune cells and normal cells are not included in the scoring. The TPS are recorded in negative/no PD-L1 expression (TPS<1%), weak positive PD-L1 expression (TPS1–49%), and strong positive/high PD-L1 expression (TPS≥50%) (28).

**Statistical analysis.** The relationship between genetic mutations and histologic subtypes was evaluated using Fisher exact/χ² test. Odds ratio (OR) was calculated in predominant histologic subtypes correlate with EGFR and KRAS mutation. The association between PD-L1 expression and clinicopathologic variables were using Fisher's exact/ Chi-square test. OR was calculated in predominant histologic subtypes associate with PD-L1 expression.

**Results**

**Genetic alterations determined from the NGS study.**

**Patient characteristics.** From a total of 136 patients with known genetic alterations identified via the NGS analysis, 82 (60.2%) cases were women and 54 (39.7%) cases were men. The range of ages, and median age were found to be 28-65 years, respectively. Specimens were collected from the primary site in 109 (80.1%) of the cases, whereas they were collected from the metastatic site, including brain, bone, lymph node, liver, pleura and pericardium, in the other 27 (19.9%) cases. The size of tissue sample taken in 101 (74.3%) cases was ≥0.5 cm in its greatest dimension based on lobectomy, wedge resection, excision, and biopsy, whereas 35 (25.7%) cases were of biopsy specimen size <0.5 cm in diameter. A total of
78 (57.3%) cases were non-smokers, whereas 58 (42.7%) cases were ex- and current smokers. The majority of the patients [103 (75.7%) cases] were at stage IV of the disease. The patient characteristics are highlighted in Table I.

Association between histological features and genetic mutation. Based on the 136 samples, the tumors were classified into acinar, papillary, lepidic, micropapillary and solid predominant subtypes in 57 (41.2%), 24 (17.6%), 20 (14.7%), 6 (4.4%) and 3 (2.2%) cases, respectively. The mucinous ADC variant was identified in 26 (19.1%) cases. The EGFR mutation was detected in 76 (55.8%) cases, followed by the identification of the KRAS mutation in 46 (33.8%) cases. BRAF, MET, AKT1, ROS1 and PTEN mutations were identified in 12 (8.8%), 11 (8.0%), 8 (5.9%), 3 (2.2%) and 1 (0.7%) cases, respectively. EGFR-KRAS, EGFR-BRAF and EGFR-KRAS-BRAF co-mutations were present in 20, 8 and 3 cases, respectively. The associations identified between the genetic mutation and the predominant histological subtype are shown in Table II. Only the EGFR and KRAS mutations were shown to have a significant level of association with the histological subtype (P=0.038 and P=0.001, respectively). Among the cases with EGFR and KRAS mutations, the papillary predominant subtype was significantly associated with the EGFR mutation (P<0.001) (Table III). Lepidic subtype and mucinous ADC variant were the parameters found to be significantly associated with the KRAS mutation (P<0.001 and P=0.037, respectively; Table III).

PD-L1 expression Patient characteristics. Out of the 239 patients, 135 (56.5%) cases were women and 104 (43.5%) cases were men. The mean age was 63 years. The range of ages, and median age were 28-89 and 63 years, respectively. Ninety-one (58.7%) patients were non-smokers, and 64 (41.3%) cases were ex- and current smokers. The majority of the patients were at stage IV of the disease (117 (85.4%) cases). The specimens were collected from the primary site in 182 (76.2%) cases, and collected from the metastatic sites, including the brain, bone, lymph node, liver, pleura and skin, in the remaining 57 (23.8%) cases. A total of 170 (71.1%) specimens taken via lobectomy, wedge resection, excision, and biopsy measured ≥0.5 cm in their greatest dimension, whereas 69 (28.9%) specimens were of biopsy size <0.5 cm in diameter (Table IV).

Association between clinicopathological parameters and PD-L1 expression. A pattern of PD-L1 expression was identified in 63 (26.4%) of the 239 patients, including 12 (5%) cases of strong positive (TPS≥50%) and 51 (21.4%) cases of weak positive (TPS≤1%) expression. A total of 176 (73.6%) cases were negative for PD-L1 expression (TPS<1%). PD-L1 expression was found to be significantly associated with specimen size ≥0.5 cm (P=0.037), whereas no significant associations were identified comparing PD-L1 expression with the parameters of age, gender, staging or site of the tumor (primary vs. metastasis; Table IV).

Association between histological features and PD-L1 expression. The most common histological subtype was the acinar subtype in 107 (44.8%) cases, followed by solid subtype in 55 (23.0%) cases, papillary subtype in 36 (15.1%) cases, lepidic subtype in 25 (10.4%) cases, micropapillary subtype in 10 (4.2%) cases, and mucinous ADC variant in 6 (2.5%) cases. The solid subtype was significantly associated with PD-L1 expression (P<0.001; OR, 3.96) in terms of positive predictive value (P=0.007; OR, 0.102; Table V).

Discussion

The present study has revealed that the most common histological subtype identified in lung ADC regarding the IASLC/ATS/ERS classification system was the acinar subtype (43.0%). The EGFR mutation was the most common mutation, which was detected in 76 out of 136 (55.8%) cases. The histological subtype with the highest level of significance in terms of its association with the EGFR mutation, based on the present study, was the papillary subtype (P=0.038; OR, 2.793). This finding concurred with those of previous studies: A significant association between EGFR and micropapillary, lepidic, acinar or papillary subtypes were identified in studies by Wang et al (29) who analyzed 153 cases, Li et al (30) who investigated 230 cases, Yoshizawa et al (31) who analyzed 440 cases, and Dong et al (32) who analyzed 330 cases. In investigating KRAS-mutated NSCLC, several
studies have demonstrated that the KRAS mutation is associated with mucinous ADC, including studies by Dong et al (32) (330 cases) and Marchetti et al (33) (58 cases). A study by Kadota et al (34), that included 864 patients with ADC revealed that mucinous ADC, as well as extracellular mucin production, were also significantly associated with the KRAS mutation. The KRAS mutation in the present study was significantly associated with mucinous ADC variant (P=0.037), a finding that was in accordance with all mentioned studies (32-34). On the other hand, in the present study, the subtype for which the predominantly significant association was identified was the lepidic (P<0.001) subtype, as opposed to the solid subtype. There was a possibility of contrast outcome, which might have been influenced by the KRAS mutation identified in our study: In 23 of 46 cases, KRAS was mainly co-mutated with BRAF and/or EGFR mutations, resulting in predominant patterns that differed with respect to other co-mutations, especially EGFR. Moreover, the acinar predominant subtype was also identified to be statistically significant as a protective prediction factor, and it was unlikely to harbour the KRAS mutation (P=0.002; OR, 0.301). The acinar pattern was predominantly found in 12 cases that harboured the BRAF mutation; however, no statistically significant association was identified in these cases (P=0.393).

PD-1/PD-L1 expression in NSCLC has been widely studied in terms of exploring significant associations with clinicopathological parameters and prognostic indicators. A meta-analysis of 11,444 cases from 47 studies by Zhang et al (35) revealed that the expression of PD-L1 was increased in males, smokers, patients with SCC, and patients with a higher histological grade, larger tumor size, nodal metastasis, and later clinical stage. Another meta-analysis of 1,157 patients from 6 studies by Wang et al (23) concluded that PD-L1 expression was significantly associated with poorly differentiated tumors, resulting in poor overall survival of the patients with NSCLC, whereas a meta-analysis of 1,550 patients from 9 studies by Pan et al (36) demonstrated that the majority of the clinicopathological features, including histological type, smoking status, tumor depth, lymph node status and tumor-lymph node-metastasis (TNM) stage, were not significantly associated with PD-L1 expression, with the exception of tumor differentiation in NSCLC. A meta-analysis of 3,128 patients from 11 studies by Yang et al (37), also failed to reveal any significant associations among clinicopathological characteristics and PD-L1 expression in patients with NSCLC. These data may be compared with the present study, in which specimen size was the only clinicopathological parameter that exerted a significant impact on PD-L1 expression. The larger tumor size (≥0.5 cm in the greatest dimension) was more significantly associated with PD-L1 expression compared with the smaller tumor size (<0.5 cm; P=0.037).
This could be accounted for by the fact that, the larger the specimen size, the more likely it is that there will be enhanced PD-L1 expression due to tumor heterogeneity. The remaining clinical parameters, including smoking status, age, sex, stage, and specimen type, were not found to be significantly associated with PD-L1 expression, and these results were in accordance with those of Pan et al (36) and Yang et al (37). The discrepancies identified in the results obtained from these meta-analyses might have been due to the differing sample sizes, differences in subtype among the patients with NSCLC,
variable levels of PD-L1 expression detected using different assays, and other interpretation criteria.

More recent studies on PD-L1 expression in pulmonary ADC have also been published. PD-L1 expression in these studies was detected according to standardized Food and Drug Administration (FDA) approved assays, including the 22C3 PharmDx assay (Agilent Technologies/Dako) (38,39), the Ventana PD-L1 SP142 assay (Ventana Medical Systems Inc.) (40), and the Ventana PD-L1 SP263 assay (Ventana Medical Systems Inc.) (41,42). Each IHC antibody clone is associated with a specific type of targeted therapy. In the present study, Dako PD-L1 IHC 22c3 pharmDx was used, which is the IHC study that makes use of pembrolizumab. Pembrolizumab has been evaluated in large-scale clinical trials; patients with TPS≥50% are eligible to receive pembrolizumab as first-line therapy, and these have been shown to have significantly improved progression-free survival and overall survival rates compared with those patients with TPS<1% or 1-49% (43). Patients with TPS≥1% subsequently treated in a trial with pembrolizumab were shown to have significantly improved overall survival rates and risk-benefit profiles that reached the level of clinical relevance compared with standard care chemotherapy (43). Among the PD-L1 expression studies in patients with pulmonary ADC using PD-L1 IHC clone 22C3, Pan et al (38) identified PD-L1 expression (TPS≥1%) in 4.1% of the total of 221 cases, including 1 (0.5%) case with TPS≥50%. Miyazawa et al (39) identified PD-L1 expression (TPS≥1%) in 21% of 78 cases, including TPS≥50% in 7 (9%) cases, percentages that were more comparable with those in our study (TPS≤1%, 21 vs. 23%; and TPS≥50%, 9 vs. 5%). They also reported that PD-L1 tends not to be expressed in early invasive ADC or well differentiated subtypes, similar to our finding that negative PD-L1 (TPS<1%) expression was found to be associated with the lepidic subtype (P=0.007). On the other hand, Driver et al (40) identified a larger number of cases of PD-L1 expression (44% of 125 cases) compared with our study, possibly due to their use of a different PD-L1 IHC clone (SP142), and the criteria for positive PD-L1 expression from their study also included positivity of infiltrating immune cells.

In histological terms, solid predominant ADC was found to be the cancer type that was consistently associated with high PD-L1 expression. The studies by Pan et al (38) and Driver et al (40) demonstrated significantly higher PD-L1 expression levels in solid vs. non-solid subtypes of ADC. Our results also revealed a significant association between the solid predominant histological subtype and PD-L1 expression, consistent with those two studies. Moreover, the large genetic and immune profiles of solid ADC published by Dong et al (42) from 194 patients with lung ADC also demonstrated that solid ADC is significantly associated with a higher smoking index, tumor mutation burden and adaptive immune resistance (highly PD-L1-expressing tumor cells and highly CD-8 positive tumor-infiltrating lymphocytes), findings which draw attention to the benefits associated with using PD-L1/PD-L1 immune checkpoint inhibitors in patients with solid predominant ADC.

It should be noted that our results did reveal certain differences compared with those of previous studies, possibly resulting from several limitations. Firstly, in pattern recognition that exist in small biopsies, which may be distortions of the true predominant pattern one would identify from the whole resection specimen. Secondly, dynamic changes in expression occurring between the primary tumour and the metastatic sites may also affect the histological pattern and/or genetic alterations or PD-L1 expression, and these possibilities should be investigated in future studies.

In conclusion, in the present study patients from a Thai population with lung ADC were collected and analyzed according to the histological subtypes in terms of their genetic mutations and PD-L1 expression levels. The EGFR and KRAS mutations were the first and second most common genetic alterations identified in the present study. The papillary histological subtype was associated with a high incidence of EGFR mutations. The lepidic subtype and mucinous ADC variant were significantly associated with the KRAS mutation. PD-L1 expression (TPS≥1%) was identified in 26% of the patients, and the solid subtype was associated with PD-L1 protein expression. Genetic mutations and the PD-L1 expression level therefore may serve as representative predictive factors for lung ADC, and may be of use in terms of targeted immunotherapeutic strategies. The histological subtype associated with the lung tumor is worthy of investigation in terms of identifying the genotype-phenotype association. These results may be helpful in selecting appropriate areas of the heterogeneous tumor feature for the patients who require personalized therapy, and predicting their prognosis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

PI conceived the study, participated in its design, coordinated the study, analyzed the data and drafted the manuscript. BP participated in study design and coordination, and drafted the manuscript. TR participated in provided clinical data and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Committee on Human Rights Related to Research Involving Human Subjects (Faculty of Medicine, Ramathibodi Hospital, Mahidol University; protocol no. 11-61-45). Consent for tissue collection and use was collected prior to experimentation.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394–422, 2018.

2. Dela Cruz CS, Tanoue LT and Matthay RA: Lung cancer: Epidemiology, etiology, and prevention. Clin Chest Med 32: 605–644, 2011.

3. Choi CM: Overview of ALK and ROS1 rearranged lung cancer. J Thorac Oncol 7: 256–237, 2013.

4. Kim J, Jang SJ, Choi CM and Ro JY: Correlation of histologic subtypes and molecular alterations in pulmonary adenocarcinoma: Therapeutic and prognostic implications. Adv Anat Pathol 23: 330–338, 2016.

5. Ou SH, Chalmers ZR, Arzada MC, Ross JS, Stephens PJ, Ali SM and Miller VA: Identification of a novel TMEM106B-ROS1 fusion variant in lung adenocarcinoma by comprehensive genomic profiling. Lung Cancer 88: 352–354, 2015.

6. Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nambu T, Sakamoto H, Tsuta K, FurUTa K, Shimada Y, et al: KIF5B-RET fusions in lung adenocarcinoma. Nat Med 18: 375–377, 2012.

7. Ding X, Zhang Z, Jiang T, Li X, Zhao C, Su B and Zhou C: Clinicopathologic characteristics and outcomes of Chinese patients with non-small-cell lung cancer and BRAF mutation. Cancer Med 6: 555–562, 2017.

8. Chen Z, Liu X, Zhao J, Yang H and Teng X: Correlation of EGFR mutation and histological subtype according to the IASLC/ATS/ERS classification of lung adenocarcinoma. Int J Clin Exp Pathol 7: 8039–8045, 2014.

9. Travis W, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, et al: International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 6: 244–285, 2011.

10. Warth A, Muley T, Meister M, Stenzinger A, Thomas M, Schirrmacher P, Schnabel PA, Budczies J, Hoffmann H and Weichert W: The novel histologic international association for the study of lung cancer/american thoracic society/european respiratory society classification system of lung adenocarcinoma is a stage-independent predictor of survival. J Clin Oncol 30: 1438–1446, 2012.

11. Russell PA, Wainer Z, Wright GM, Daniels M, Conron M and Williams RA: Does lung adenocarcinoma subtype predict patient survival?: A clinicopathologic study based on the new international association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary lung adenocarcinoma classification. J Thorac Oncol 6: 1496–1504, 2011.

12. Villa C, Cagle PT, Johnson M, Patel JD, Yeldandi AV, Raj R, DeCamp MM and Raparia K: Correlation of EGFR mutation status with predominant histologic subtype of adenocarcinoma according to the new lung adenocarcinoma classification of the international association for the study of lung cancer/american thoracic society/european respiratory society. Arch Pathol Lab Med 138: 1353–1357, 2014.

13. Pan Y, Zhang X, Li Y, Hu H, Wang L, Li H, Wang R, Ye T, Luo X, Zhang Y, et al: ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: A comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. Lung Cancer 84: 121–126, 2014.

14. Kim YH, Ishii G, Goto K, Nagai K, Tsuta K, Shinoda N, Nitadori J, Koyama T, Kosaka Y and Ochiai A: Dominant papillary subtype is a significant predictor of the response to gefitinib in adenocarcinoma of the lung. Clin Cancer Res 10: 7311–7317, 2004.

15. Reck M, Rodríguez-Areny R, Robinson AG, Hui R, Csósz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, et al: KEYNOTE-024 Investigators: Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375: 1823–1833, 2016.

16. Lin SY, Yang CY, Liao BC, Ho CC, Liao WY, Chen KY, Tsai TH, Hsu CL, Hsu WH, Su KY, et al: Tumor PD-L1 expression and clinical outcomes in advanced-stage non-small cell lung cancer patients treated with nivolumab or pembrolizumab: Real-world experience in Taiwan. J Cancer 9: 1813–1820, 2018.

17. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, et al: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 373: 1637–1659, 2015.

18. Herbst RS, Baas P, Kim D, Felip E, Pérez-Garcia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, et al: Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised, controlled trial. Lancet 387: 1540–1550, 2016.

19. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Godbole SM, Pati T, Kowalski DM, Dols MC, et al: OAK study group: Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. Lancet 389: 255–265, 2017.

20. Topalian SL, Taube JM, Anders RA and Pardoll DM: Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer 16: 275–287, 2016.

21. He J, Hu Y, Hu M and Li B: Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. Sci Rep 5: 13110, 2015.

22. Maru DM, Zhou W, Delil-Fahmi M, Georgesen JB, Wang Z, Emancipator K, Wu D, Busch-Sorensen M, Meldgaard P and Hager H: PD-L1 expression and survival among patients with advanced non-small cell lung cancer treated with chemotherapy. Transl Oncol 9: 64–69, 2016.

23. Wang A, Wang HY, Liu Y, Zhao MC, Zhang HJ, Lu ZY, Fang YC, Chen XF and Liu GF: The prognostic value of PD-L1 expression for non-small cell lung cancer patients: A meta-analysis. Eur J Surg Oncol 41: 450–456, 2015.

24. Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, Herbst RS, Gettinger SN, Chen L and Uemim DL: Prognosed death ligand-1 expression in non-small cell lung cancer. Lab Invest 94: 107–116, 2014.

25. Incorvaia L, Fanale D, Badalamenti G, Barraco N, Bono M, Corsini LR, Galvano A, Grisitina V, Listi A, Vieni S, et al: Programmed death ligand 1 (PD-L1) as a predictive biomarker for pembrolizumab therapy in patients with advanced non-small-cell lung cancer (NSCLC). Adv Ther 36: 2600–2670, 2019.

26. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, Haghhighi M, Fazlollahi L, Stoolper MB, Sonett JR, Sacher AG, et al: PD-L1 expression in non-small cell lung carcinoma: Composite of RNA, histopathology, small biopsy, and surgical resection specimens. Cancer Cytopathol 125: 896–907, 2017.

27. Zhang Y, Wang L, Li Y, Pan Y, Wang R, Hu H, Li H, Luo X, Ye T, Sun Y and Chen H: Protein expression of programmed death ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. Onco Targets Ther 7: 567–573, 2014.

28. Dako: PD-L1 IHC 22C3 pharmDx. https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013S014C.pdf.

29. Wang T, Zhang Y, Liu B, Hu M, Zhou N and Zhi X: Associations between epidermal growth factor receptor mutations and histological subtypes of lung adenocarcinoma according to the IASLC/ATS/ERS classification in Chinese patients. Thorac Cancer 8: 600–605, 2017.

30. Li H, Pan Y, Li Y, Li C, Wang R, Hu H, Zhang Y, Ye T, Wang L, Shen L, et al: Frequency of well-identified oncogenic driver mutations in lung adenocarcinoma of smokers varies with histological subtypes and graduated smoking dose. Lung Cancer 79: 8–13, 2013.

31. Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, Tsujiyama T, Travis WD, Date H, Inoue H: Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: Analysis of 440 Japanese patients. J Thorac Oncol 8: 52–61, 2013.

32. Dong YJ, Cai YR, Zhou LJ, Su D, Mu J, Chen XJ and Zhang LI: Association between the histological subtype of lung adenocarcinoma, EGFR/KRAS mutation status and the ALK rearrangement according to the novel IASLC/ATS/ERS classification. Oncol Lett 11: 2552–2558, 2016.
Marchetti A, Buttitta F, Pellegrini S, Chella A, Bertacca G, Filardo A, Tognoni V, Ferrelli F, Signorini E, Angeletti CA and Bevilacqua G: Bronchioloalveolar lung carcinomas: K-ras mutations are constant events in the mucinous subtype. J Pathol 179: 254-259, 1996.

Kadota K, Yeh YC, D'Angelo SP, Moreira AL, Kuk D, Sima CS, Riely GJ, Arcila ME, Kris MG, Rusch VW, et al: Associations between mutations and histologic patterns of mucin in lung adenocarcinoma: Invasive mucinous pattern and extracellular mucin are associated with KRAS mutation. Am J Surg Pathol 38: 1118-1127, 2014.

Zhang M, Li G, Wang Y, Wang Y, Zhao S, Haihong P, Zhao H and Wang Y: PD-L1 expression in lung cancer and its correlation with driver mutations: A meta-analysis. Sci Rep 7: 10255, 2017.

Pan ZK, Ye F, Wu X, An HX and Wu JX: Clinico-pathological and prognostic significance of programmed cell death ligand1 (PD-L1) expression in patients with non-small cell lung cancer: A meta-analysis. J Thorac Dis 7: 462-470, 2015.

Yang H, Chen H, Luo S, Li L, Zhou S, Shen R, Lin H and Xie X: The correlation between programmed death-ligand 1 expression and driver gene mutations in NSCLC. Oncotarget 8: 23517-23528, 2017.

Pan Y, Zheng D, Li Y, Cai X, Zheng Z, Jin Y, Hu H, Cheng C, Shen L, Wang J, et al: Unique distribution of programmed death ligand 1 (PD-L1) expression in East Asian non-small cell lung cancer. J Thorac Dis 9: 2579-2586, 2017.

Miyazawa T, Marushima H, Saji H, Kojima K, Hoshikawa M, Takagi M and Nakamura H: PD-L1 expression in non-small-cell lung cancer including various adenocarcinoma subtypes. Ann Thorac Cardiovasc Surg 25: 1-9, 2019.