Clinicopathological and molecular characteristics of patients with hypermutant lung cancer: A retrospective cohort study

HONGBIN ZHANG1, YUAN WANG1, QIAOXIA JI1, HONGMEI CAI1, XIANGCUN LIANG1, JIONG XIE1, HUA LI1, JUN WANG2, GUIYUN ZHU3, ERPENG TIAN4, LINGLING ZHU1, MINGMING YUAN3, RONGRONG CHEN5 and MIN ZHAO1

1Department of Oncology, Hebei Chest Hospital, Research Center of Hebei Lung Cancer Prevention and Treatment, Shijiazhuang, Hebei 050041; 2Department of Radiation Oncology, Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei 050011; 3Department of Pathology; 4Molecular Biology Laboratory, Hebei Chest Hospital, Shijiazhuang, Hebei 050041; 5Geneplus-Beijing, Beijing 102206, P.R. China

Received May 26, 2020; Accepted January 28, 2021

DOI: 10.3892/ol.2021.12590

Abstract. Tumor mutation burden (TMB) is an independent indicator used to select patients sensitive to immunotherapy. The present study aimed to investigate the clinicopathological and molecular characteristics of patients with hypermutant lung cancer to identify an economical, simple and complementary method for predicting TMB and immunotherapy responses. In total, 1,000 patients with lung cancer were randomly selected, and their samples were submitted to next-generation sequencing, with their TMB status reviewed. The threshold of hypermutation was set to 17.24 mutations (muts)/Mb. The proportion of smokers was higher in the hypermutant cohort (67.2 vs. 14.3%; P<0.0001). A similar trend was obtained for all genes tested, except for the EGFR gene. Furthermore, in the hypermutant cohort, the prevalence of microsatellite instability was extremely high (9.0%). The mutation frequency in DNA damage response (DDR) genes was notably higher in the hypermutant cohort, where several DDR-associated genes were enriched, compared with in the non-hypermutant cohort. The enrichment analysis revealed a strong association between mutations in Notch signaling and high TMB. To the best of our knowledge, the present study is the first to comprehensively investigate the clinical and genetic characteristics of patients with hypermutant lung cancer in a Chinese population. The results of the current study suggested that hypermutant lung cancer exerted distinctive clinical and genetic features, which may be used as complementary indicators for screening patients sensitive to immunotherapy.

Introduction

Lung cancer is the leading cause of cancer-associated death among males and females, accounting for an estimated 600,000 associated deaths in China in 2015 (1). Immune checkpoint blockade has emerged as a promising strategy in several malignancies, including both non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (2-6). The CheckMate-032 study investigated the efficacy of nivolumab, an anti-programmed cell death-1 (PD-1) antibody, alone or in combination with ipilimumab, an anti-cytotoxic T lymphocyte antigen-4 antibody in recurrent patients with SCLC, who progressed after one or more prior regimens (6). In the aforementioned study, the objective response rates (ORRs) were 10, 23 and 19% for patients treated with 3 mg/kg nivolumab, 1 mg/kg nivolumab + 3 mg/kg ipilimumab and 3 mg/kg nivolumab + 1 mg/kg ipilimumab, respectively (6). Among the patients with metastatic NSCLC who progressed following platinum-based chemotherapy, nivolumab was associated with significantly longer median overall survival time compared with docetaxel (OS; non-squamous NSCLC, 12.2 vs. 9.4 months; hazard ratio [HR], 0.73; 95% CI, 0.59-0.89; P=0.002; squamous NSCLC, 9.2 vs. 6.0 months; HR, 0.59; 95% CI, 0.44-0.79; P<0.001] (7,8). Other immune checkpoint inhibitors, including pembrolizumab, an anti-PD-1 antibody
[programmed death-ligand 1 (PD-L1)-positive NSCLC population, 10.4 vs. 8.5 months; HR, 0.71; 95% CI, 0.58-0.88; \( P=0.0008 \), and atezolizumab, an anti-PD-L1 antibody (13.8 vs. 9.6 months; HR, 0.73; 95% CI, 0.62-0.87; \( P=0.0003 \)), also exhibited improved OS compared with chemotherapy (9,10). However, the response rate of immune checkpoint inhibitors was relatively low in unselected patients (5). Therefore, multiple biomarkers have been investigated for selecting patients who can benefit from immunotherapy.

Tumor mutation burden (TMB) is an emerging biomarker to independently predict response to immunotherapy (11,12). For example, the retrospective analysis of the Checkmate-032 study suggested that the efficacy of nivolumab combined with ipilimumab was improved in patients with high TMB compared with those with low TMB (ORR, 46.2 vs. 22.2%) (11). Currently, the evaluation of TMB is based on expensive, large next-generation sequencing (NGS) gene panels or whole-exome sequencing. Several studies have demonstrated that single gene mutations, such as driver mutations in polymerase ε catalytic subunit A (POLE)/polymerase δ catalytic subunit gene 1 (POLD1) genes and pathogenic mutations in mismatch repair genes, are associated with high TMB, which may provide an economical, simple and complementary method for predicting TMB and response to immunotherapy (13-15). However, the aforementioned studies mainly focused on colorectal and endometrial carcinoma. The molecular characteristics that may contribute to high TMB in lung cancer have not been fully documented. Therefore, in the present study, a retrospective, cohort study was conducted to comprehensively investigate the clinicopathological and molecular features of patients with lung cancer with extremely high mutation burden (hypermutation).

In addition to TMB, other molecular biomarkers have been identified to affect patient response to immunotherapy. High levels of microsatellite instability (MSI), deficient mismatch repair and PD-L1 expression have been approved by the Food and Drug Administration (FDA) as predictive biomarkers of immunotherapy across multiple types of cancer, such as NSCLC, triple-negative breast cancer and gastric or gastroesophageal junction adenocarcinoma (16-19). Additionally, alterations in DNA damage response (DDR) genes may be associated with high TMB and improved clinical benefits from immunotherapy in patients with NSCLC (20). Tumors with co-occurring TP53/KRAS gene mutations exhibited remarkable clinical benefit from immunotherapy with PD-1 inhibitors (21). However, some driver mutations in NSCLC tend to predict a poorer response to immunotherapy, such as EGFR sensitive mutations and ALK fusions (22). Somatic mutations in PTEN, \( \beta \)-2 microglobulin (B2M), serine-threonine kinase 11 (STK11), Kelch-like ECH-associated protein 1 (KEAP1), murine double minute 2 (MDM2) and 11q13 amplification have also been negatively associated with immunotherapy response (23-27). The current study aimed to explore the prevalence of these immunotherapy-associated biomarkers in a hypermutant lung cancer cohort.

Materials and methods

Patient samples. A total of 1,000 patients with lung cancer who underwent genetic testing using the NGS technology at ZHANG et al: CLINICAL AND GENOMIC FEATURES OF HYPERMUTANT LUNG CANCER
To explore whether the DMGs that were more frequently mutated in the hypermutant cohort were enriched in certain signaling pathways, the significance of mutation enrichment was determined by applying a hyper-geometric test and was adjusted for multiple testing using the Benjamini-Hochberg false discovery rate.

**Statistical analysis.** The somatic mutation profiles in The Cancer Genome Atlas (TCGA) database from 1,031 NSCLC samples, including 562 patients with adenocarcinoma and 469 patients with squamous cell cancer, were downloaded from CBioPortal (https://www.cbioportal.org). The difference in age at diagnosis between the cohorts was evaluated using a two-tailed unpaired Mann-Whitney U test. A \( \chi^2 \) test was utilized to assess the differences in other demographic characteristics. Missing data regarding histological subtype, clinical stage and family history were not included in these statistical analyses. Spearman's rank correlation analysis was used to examine the correlation between sex and smoking history. Cox multivariate regression analysis was used to further verify the association between clinical characteristics and TMB. P≤0.05 was considered to indicate a statistically significant difference.

**Results**

**Study design and patient demographics.** The flow chart of the methodology of the present study is presented in Fig. 1A. To determine the threshold of hypermutation in patients with lung cancer, the TMB status of 1,000 selected patients with lung cancer was screened. The distribution of TMB is presented in Fig. 1B. Among these patients, the median TMB was 5 muts/Mb (range, 0-80 muts/Mb). TMB of 17.24 muts/Mb was...
defined as the threshold according to the criterion for hypermutation set in the current study. Consequently, 67 patients were considered to be hypermutant, and the remaining patients were non-hypermutant (n=933). The median TMB for the hypermutant and non-hypermutant cohorts was 22 (range, 17.28-80 muts/Mb) and 4.8 muts/Mb (range, 0-17 muts/Mb), respectively.

The clinicopathological characteristics of patients are summarized in Table I. The proportion of males (86.6 vs. 59.2%) and smokers (85.1 vs. 46.6%) was higher in the hypermutant cohort compared with in the non-hypermutant cohort (both \( P<0.0001 \)). In addition, a strong correlation between smoking and sex (male smokers vs. female smokers, 79.5 vs. 9.1%; Spearman rank correlation, 0.659; 95% CI, 0.551-0.748) was revealed in the present study (data not shown). Additionally, compared with in the non-hypermutant cohort, the proportion of patients with squamous cell carcinoma and SCLC was higher in the hypermutant cohort (22.4 vs. 13.1% and 6.0 vs. 2.6%, respectively; \( P=0.0198 \)). In addition, the age at diagnosis of hypermutant patients was significantly increased compared with that of non-hypermutant patients (\( P=0.0198 \)). Cox multivariate regression analyses further confirmed that TMB was associated with sex, smoking history and histological subtype, but not with age at diagnosis (Table SV1).

**Mutation profiles of hypermutant and non-hypermutant lung cancer.** The somatic mutation profiles of hypermutant and non-hypermutant samples are presented in Fig. 2 (top 25 genes for each group). As shown in Fig. 2, the occurrence of mutations in multiple genes was higher in the hypermutant compared with in the non-hypermutant cohort. TP53 was the most frequently mutated gene in both groups, with a mutation frequency of 89.6 and 59.9% in the hypermutant and non-hypermutant group, respectively (\( P<0.001 \)). Low-density lipoprotein receptor-related protein 1B (LRP1B) exerted the most significant difference on mutation rate between the hypermutant and non-hypermutant cohorts (67.2 vs. 14.3%; \( P<0.001 \)). In addition, 51.1% of cases with LRP1B mutations in the hypermutant cohort harbored >2 mutations, which was significantly higher compared with in the non-hypermutant cohort (21.8%; \( P=0.0002 \)) (data not shown). Only EGFR mutations were more frequently observed in the non-hypermutant cohort compared with in the hypermutant cohort (48.9 vs. 22.4%; \( P<0.001 \)).

Subsequently, the mutational profiles of common driver genes in NSCLC were compared (Fig. 3). In the hypermutant cohort (Fig. 3A), the most frequently mutated genes were EGFR and KRAS, with a mutation rate of 18 and 19%, respectively. EGFR sensitive mutations (exon 19 deletions, Leu858Arg and other missense mutations that are sensitive to first- and second-generation EGFR tyrosine kinase inhibitors) and amplification were identified in only 7.5 and 9% of hypermutant patients, respectively. BRAF non-V600E mutations were found in 4 cases in the hypermutant cohort. In the
non-hypermutant cohort (Fig. 3B), EGFR mutations were detected in 48% of cases. Furthermore, EGFR sensitive mutations were identified in 43.2% of non-hypermutant patients, and among them, 45 cases harbored EGFR secondary resistance mutations. EGFR amplification was found in 12.2% of non-hypermutant patients, with 76.3% of cases accompanied with EGFR sensitive mutations. Compared with in the hypermutant cohort, fewer patients in the non-hypermutant cohort harbored KRAS mutations (10%). ALK, ROS1 and RET rearrangements were only observed in the non-hypermutant cohort with a frequency of 6, 2.3 and 1.3%, respectively. Finally, BRAF mutations were detected in 23 cases, including 11 V600E and 12 non-V600E mutations, representing 2.5% of all patients in the non-hypermutant cohort.

Molecular features associated with immunotherapy efficacy. The genetic factors associated with immunotherapy efficacy in the hypermutant cohort were analyzed. The results revealed that MSI-high was observed in 6 cases in the hypermutant cohort (9.0%) and only 1 patient in the non-hypermutant cohort (0.1%) (data not shown). Subsequently, the prevalence of DDR variants (20,38,39) (Table SVII) between the hypermutant and non-hypermutant cohorts was compared. A total of 105 mutations were identified in DDR genes in 70.1% of patients in the hypermutant cohort, while 321 mutations in DDR genes were detected in 27.0% of patients in the non-hypermutant cohort (data not shown). Mutations in multiple DDR genes were enriched in the hypermutant cohort, including mutations in ATM, POLE/POLD1 and BRCA1/2 genes (Fig. 4A). As shown in Fig. 4B and C, 16 mutations (including one copy number variant) were detected in POLE/POLD1 genes. According to the criteria for driver mutations proposed by Campbell et al (40), no known driver mutations were detected in POLE/POLD1 genes. In addition, several mutations in POLD1 (E795Q and S816C) and POLE (A1528T and P1205L) genes were considered as non-driver mutations, according to the POLE/POLD1 variants and associated mutation burden referred by Campbell et al (40). The function of other variants remains to be characterized. TP53 and KRAS co-alterations were identified in 16.4% of patients in the hypermutant cohort, and significantly fewer in the non-hypermutant cohort (5.8%; P=0.002; data not shown). Furthermore, several genetic alterations were negatively associated with response to immunotherapy, including three PTEN loss-of-function (LOF) mutations, two B2M LOF mutations, five EGFR sensitive mutations, three STK11 LOF mutations,
one KEAP1 LOF mutation, two cases with 11q13 amplification (CCND1, FGF3, FGF4 and FGF19) and one case with MDM2 amplification (data not shown).

Identification of DMGs and enrichment analysis. To identify genes with significantly higher alteration frequency in the hypermutant group compared with in the non-hypermutant
The DMGs enriched in the hypermutant cohort are summarized in Table SVIII. Webgestalt was used for the enrichment analysis. The top 10 pathways are shown in Fig. 5A, including the Notch signaling pathway, MAPK signaling pathway and RAS signaling pathway. Mutations in the Notch signaling pathway were enriched in the hypermutant cohort, with an enrichment ratio of 3.49. This signaling pathway included NOTCH1/2/3/4, CREB-binding protein (CREBBP), E1A binding protein P300, histone deacetylase 1 (HDAC1), nicastrin (NCSTN), and mastermind-like transcriptional coactivator 2 (MAML2); these genes were completely mutated in 64.2% of cases in the hypermutant cohort (data not shown). To validate the enrichment of mutations in the Notch signaling pathway in the hypermutant cohort, the validated genes were expanded to all the genes involved in the Notch signaling pathway (41). Two-tailed unpaired Mann-Whitney U test was used to compare the number of mutations between Notch signaling mutant and wild-type cohorts. Patients with mutations in these nine genes exhibited more non-synonymous mutations in the coding regions compared with those without these mutations (median, 310 vs. 183.5; P<0.0001; Fig. 5B). Similarly, when the validated genes were expanded to all the genes involved in the Notch signaling pathway (41) (ARRDC1, CNTN6, CREBBP, EP300, HES1, HES2, HES3, HES4, HEY1, HEY2, HEYL, KAT2B, KDM5A, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NOV, NRARP, PSEN2, LFNG, ITCH, NCSTN, SPEN, JAG1, APH1A, FBXW7, FHL1, THBS2, HDAC2, MFAP2, CUL1, RFNG, NCO1, NCO2, MFAP5, HDAC1, NUMB, JAG2, MAML3, MFNG, CIR1, CNTN1, MAML1, MAML2, NUMBL, PSEN1, PSEN2, RBPJ, RBPJL, RBX1, SAP30, SP1, SWN1, CTBP1, CTBP2, ADAM10, APH1B, ADAM17, DLK1, DLI1, DLI3, DLI4, DNER, DTX1, DTX2, DTX3, DTX3L, DTX4, EGFL7), a
significant difference was also observed (median, 292 vs. 125; P<0.0001; Fig. 5B).

Discussion

It has been reported that hypermutation is frequently found in melanoma, lung and bladder cancer (42-44). The present retrospective cohort study comprehensively explored the clinicopathological and molecular features of patients with hypermutant lung cancer. The present study may provide important insights into hypermutant lung cancer among the Chinese population. To the best of our knowledge, the present study was the first to comprehensively investigate the clinical and genetic characteristics of hypermutant lung cancer in a Chinese population.

First, the clinical characteristics between cohorts were compared, and the results revealed that hypermutation was more frequently observed in certain groups of patients, including males, smokers and specific histological subtypes. A previous study investigated the association between smoking and mutational burden. The results demonstrated that the total number of point mutations in the coding regions was higher in smokers (median, 209; range, 104-1,363) compared with non-smokers (median, 18; range, 10-22) (43). Other studies also obtained similar conclusions (45,46). Additionally, the present findings were consistent with the aforementioned studies. In addition, the strong correlation between smoking and sex observed in the present study may be associated with smoking habits in the Chinese population. In China, the smoking prevalence was 47.2% (range, 46.9-47.5%) for men and 2.7% (range, 2.6-2.8%) for women in 2013 (47). Therefore, the present study hypothesized that the significantly higher rate of males in the hypermutant cohort may result from the higher number of smokers in this cohort. The current findings were consistent with a previous study demonstrating higher TMB in squamous carcinoma and SCLC compared with in adenocarcinoma (48).

Subsequently, the mutation spectra of both cohorts were analyzed. LRP1B was more frequently mutated in the hypermutant cohort. LRP1B encodes a member of the low-density lipoprotein receptor family. These receptors serve a wide variety of roles in normal cell function and development by interacting with multiple ligands (49). Several studies have demonstrated an association between LRP1B mutations and a high level of TMB in patients with NSCLC and melanoma; these studies suggested that in patients with LRP1B mutations, immune response and cell cycle regulation circuits were among the top enriched pathways (50,51). Although the mechanism underlying the association between TMB and LRP1B is not entirely clear, the current study supported the aforementioned findings. The distribution of KRAS and EGFR mutations identified in the present study was also consistent with previous studies, suggesting the positive effect of KRAS mutations and negative effect of EGFR mutations on immunotherapy response (21,52). ALK, ROS1 and RET rearrangements were only found in the non-hypermutant cohort, which may suggest a negative association with hypermutation, thereby affecting the response to immunotherapy (53).

The genetic factors affecting immunotherapy response were also analyzed. MSI-high was reported in 0.2% of patients with non-squamous NSCLC (54). In the present study, the prevalence of MSI-high was extremely high in the hypermutant cohort (9%), indicating a potential important role of MSI in hypermutation in lung cancer. The enrichment of DDR gene mutations in the hypermutant cohort suggested that mutations in DDR genes may serve as biomarkers for predicting TMB and patient response to immunotherapy. A previous study has confirmed elevated TMB and improved efficacy of immunotherapy in patients with pathogenic DDR alterations (20). Co-mutations of KRAS and TP53 were more frequently identified in the hypermutation cohort, supporting the improved clinical outcomes of patients with KRAS and TP53 co-mutations during the period of immunotherapy (21). In addition, several negative biomarkers, such as PTEN mutations and MDM2/4 amplification, in the hypermutant cohort were identified, suggesting hyper-progressive disease or disease resistant to immunotherapy, which should raise concern.

DMGs enriched in the hypermutant cohort were identified in the present study, and an enrichment analysis was performed. The results suggested that mutations in the Notch signaling pathway were associated with high TMB, which was confirmed using molecular profiles of lung cancer in TCGA database. The Notch signaling pathway activates cell proliferation and antagonizes apoptosis, as well as cross-talks with several transcriptional factors to promote epithelial-mesenchymal transition in lung cancer, thus leading to enhanced motility, invasion and metastasis of cancer cells (55). Recently, NOTCH1 has been reported to contribute to an immune-suppressive tumor microenvironment in melanoma (56). Targeting NOTCH1 may therefore affect cell proliferation and survival, and provide an immune-responsive tumor microenvironment, thus improving the efficacy of immunotherapy (56). Another study uncovered a marked association between mutations in NOTCH1/2/3 and improved outcomes in EGFR- and ALK-wild-type patients with NSCLC treated with immune checkpoint inhibitors (57). In addition, deleterious NOTCH mutations exhibited an improved effect compared with non-deleterious mutations (57). However, the underlying mechanism remains unknown. Previous studies found that tumors with Notch family gene (NOTCH1/2/3/4) mutations exerted higher TMBs in multiple types of cancer, including hepatocellular carcinoma, esophageal carcinoma, breast cancer, SCLC, head and neck cancer and cutaneous carcinoma (58,59), which may explain the predictive value of NOTCH mutations to immunotherapy response. In the present study, the strong association between NOTCH gene mutations and high TMB indicated a potential strategy for immunotherapy in patients with lung cancer with mutations in the Notch signaling pathway. However, the mutation types in genes involved in the Notch signaling pathway and the specific genes exhibiting strong predictive value to immunotherapy response should be further investigated.

The FDA has approved FoundationOne®CDx as the first companion diagnostic to identify patients with solid tumors that are TMB-high (≥20 muts/Mb) and suitable for treatment with pembrolizumab (60). A previous study conducted by Foundation Medicine described the distribution of TMB across a diverse cohort of 100,000 cases of cancer, with TMB >20 muts/Mb designated as high TMB (61). The percentages of
cases with TMB >20 muts/Mb were 17, 12.3, 11.3 and 9% for NSCLC (not otherwise specified) (n=2,636), lung adenocarcinoma (n=11,855), lung squamous cell carcinoma (n=2,102) and lung small cell undifferentiated carcinoma (n=913), respectively (61). However, in the present study, hypermutant patients (TMB ≥17.24 muts/Mb) constituted 6.7% of the whole population. TMB cut-offs may differ depending on sample type, tumor type, patient subgroup, therapy investigated and assay used. More specifically, the capture region, number of genes, sequencing depth and TMB calculation method of a panel may affect the TMB threshold. Despite the difference between TMB cut-offs and sequencing details, the findings of the present study may be valuable thanks to the scientific method for TMB cut-off determination and controlled study design.

However, there were a few limitations due to the retrospective nature of the present study. Information on whether the patients received immunotherapy after genetic testing and corresponding treatment response was not available. The efficacy of immune checkpoint inhibitors in the hypermutant cohort was of great value and could be further researched. In addition, ~78% of patients in the current study were diagnosed with adenocarcinoma. It may be interesting to explore TMB-associated molecular characteristics in adenocarcinoma only in a Chinese population.

In conclusion, the present cohort study suggested that hypermutant lung cancer exhibited distinctive genetic profiles, including high occurrence of MSI-high, high frequency of mutations in DDR genes and genes involved in the Notch signaling pathway, which may be associated with high levels of TMB. In addition, patients with hypermutant lung cancer may be more likely to have a history of tobacco use and exhibit the histological subtypes of squamous carcinoma and SCLC. These characteristics may be used as complementary indicators for screening patients sensitive to immunotherapy.

Acknowledgements
Not applicable.

Funding
The present study was supported by Government-funded clinical excellence training programme.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
HZ, YW and MZ contributed to conception and design of the study. MZ contributed to the provision of study materials or patients. HZ and MY confirm the authenticity of all the raw data. QJ, HC, RC, XL, JX and MY contributed to acquisition of data. HL, JW, GZ, ET and LZ performed data analysis and interpretation. All authors wrote the manuscript and read and approved the final manuscript.

Ethics approval and consent to participate
All procedures were in accordance with the 1964 Declaration of Helsinki and its later amendments. The study was performed under a protocol approved by the Institutional Review Board of Geneplus-Beijing. Written informed consent was provided by all participants included in the study.

Patient consent for publication
Not applicable.

Competing interests
MY and RC are employees of Geneplus-Beijing. All other authors declare that they have no competing interests.

References
1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu QX and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
2. Chung HC, Piha-Paul SA, Lopez-Martin J, Schellens JHM, Kao S, Miller WH Jr, Delord JP, Gao B, Planchard D, Gottfried M, et al: Pembrolizumab after two or more lines of prior therapy in patients with advanced small-cell lung cancer (SCLC): Results from the KEYNOTE-028 and KEYNOTE-158 studies. Cancer Res 79: CT073, 2019.
3. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, Huemer F, Losonczy G, Johnson ML, Nishino M, et al: First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 379: 2220-2229, 2018.
4. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszzi T, Fülöp A, Gottfried M, Feled N, Tafreshi A, Cuffe S, et al: Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375: 1823-1833, 2016.
5. Herbst RS, Morgensztern D and Boshoff C: The biology and management of non-small cell lung cancer. Nature 553: 446-454, 2018.
6. Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, Jäger D, Pietanza MC, Le DT, de Braud F, et al: Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): A multicentre, open-label, phase 1/2 trial. Lancet Oncol 17: 883-895, 2016.
7. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, et al: Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373: 123-135, 2015.
8. 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: 1627-1639, 2015.
9. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, et al: 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.
10. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia 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.
11. Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, Rizvi NA, Hirsch FR, Selvaggi G, Szustakowski JD, et al: Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 35: 329, 2019.
12. Hellmann MD, Cilensean TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, Minenka E, Linardou H, Burgers S, Salman P, et al: Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 378: 2093-2104, 2018.
13. Wang F, Zhao Q, Wang YM, Jin Y, He MM, Liu ZX and Xu RH: Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types. JAMA Oncol 2018; 4: 1503-1509.

14. Song Z, Cheng G, Xu C, Wang W, Shao Y and Zhang Y: Clinicopathological characteristics of POLE mutation in patients with non-small-cell lung cancer. Lung Cancer 2018; 118: 57-61.

15. Stadler ZK, Mattaglini F, Siddiqua S, Hechtman JT, Tran C, Cersek A, Yager R, Segal NH, Varghese AM, Reidy-Lagunes DL, et al.: Reliable detection of mismatch repair defect deficiency in colorectal cancers using mutational load in next-generation sequencing panels. J Clin Oncol 34: 2141-2147, 2016.

16. Marcus L, Lemery SJ, Keegan P and Pazdur R: FDA approval summary: Atezolizumab plus platinum-protein-bound for the treatment of patients with advanced or metastatic TNBC whose tumors express PD-L1. Clin Cancer Res 26: 2284-2289, 2020.

17. Fashoyin-Aje L, Donghue M, Chen H, He K, Veeraraghavan J, Goldberg KB, Keegan P, McKee AE and Pazdur R: FDA approval: Pembrolizumab for recurrent locally advanced or metastatic head and neck squamous cell carcinoma expressing PD-L1. Oncologist 24: 103-109, 2019.

18. Sul J, Blumenthal GM, Jiang X, He K, Keegan P and Pazdur R: FDA approval summary: Pembrolizumab for the treatment of patients with metastatic non-small cell lung cancer whose tumors express programmed Death-Ligand 1. Oncologist 21: 643-650, 2016.

19. Ricciuti B, Cheng ML, Recondo G, Nishino M, Uemoto R, Sholl LM and Awad MM: DNA damage response gene alterations are associated with high tumor mutational burden and clinical benefit from programmed death 1 axis inhibition in non-small cell lung cancer. J Clin Oncol 37 (Suppl 15): S9977, 2019.

20. Dong ZY, Zhong WZ, Xuan BC, Shao H, Lu ZY, Jia Y, et al.: Potentially predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Cancer Discov 6: 202-216, 2016.

21. Sade-Feldman M, Jiao YH, McNamara ER, McIvor AM, He F, et al.: PD-L1 expression is a biomarker for pembrolizumab response in NSCLC. J Thorac Oncol 13: 1349-1358, 2018.

22. Goetz MP, Gradishar WJ, Anderson BO, Abrey LE, Aft R, Allison KH, Blair SL, Burstyn-Hirsch M, Cari LA, et al.: NCCN guidelines insights: Breast cancer, version 3.2018. J Natl Compr Canc Netw 17: 118-126, 2019.

23. Li J, Luptat R, Amarasinghe KC, Thompson ER, Doyle MA, Ryland GL, Tothill RW, Halgamuge SK, Campbell IG and Gorringer KL: CONTRA: Copy number analysis for targeted resequencing. Bioinformatics 26: 1307-1312, 2010.

24. Zeh JH, Benayed R, Vassilakos P, Keshav S, Kim HR, Shin PS, Pao W, Javaherian MA, Eling SM, et al.: Mismatch repair deficiency and response to immune checkpoint blockade in urothelial cancer associated with germline mismatch repair mutations. N Engl J Med 373: 1973-1984, 2015.

25. Wang HL, Wang J, Ma L, Wang H, et al.: NCCN guidelines insights: Thyroid carcinoma, version 2.2018. J Natl Compr Canc Netw 17: 1429-1440, 2018.

26. Armstrong D, Allegra RD, Balkum-Gamez JN, Barilhowl R, Behbahaki K, Berchuck A, Berek JS, Chen LM, Cristea M, Devlin SM, et al.: Mismatch repair defects in ovarian cancer, version 1.2019. J Natl Compr Canc Netw 17: 896-909, 2019.

27. Goetz MP, Gradishar WJ, Anderson BO, Abrey LE, Aft R, Allison KH, Blair SL, Burstyn-Hirsch M, Cari LA, et al.: NCCN guidelines insights: Breast cancer, version 3.2018. J Natl Compr Canc Netw 17: 118-126, 2019.

28. Li J, Luptat R, Amarasinghe KC, Thompson ER, Doyle MA, Ryland GL, Tothill RW, Halgamuge SK, Campbell IG and Gorringer KL: CONTRA: Copy number analysis for targeted resequencing. Bioinformatics 26: 1307-1312, 2010.

29. Zeh JH, Benayed R, Vassilakos P, Keshav S, Kim HR, Shin PS, Pao W, Javaherian MA, Eling SM, et al.: Mismatch repair deficiency and response to immune checkpoint blockade in urothelial cancer associated with germline mismatch repair mutations. N Engl J Med 373: 1973-1984, 2015.
50. Chen H, Chong W, Wu Q, Yao Y, Mao M and Wang X: Association of LRP1B mutation with tumor mutation burden and outcomes in melanoma and non-small cell lung cancer patients treated with immune check-point blockades. Front Immunol 10: 1113, 2019.

51. Lan S, Li H, Liu Y, Ma L, Liu X, Liu Y, Yan S and Cheng Y: Somatic mutation of LRP1B is associated with tumor mutational burden in patients with lung cancer. Lung Cancer 132: 154-156, 2019.

52. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, Plodkowski A, Long N, Sauter JL, Rekhtman N, et al: Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol 36: 633-641, 2018.

53. Mazieres J, Drilon A, Lusque A, Mhanna L, Cortot AB, Mezquita L, Thai AA, Mascaux C, Couraud S, Veillon R, et al: Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: Results from the IMMUNOTARGET registry. Ann Oncol 30: 1321-1328, 2019.

54. Mahadevan N, Shivadasani P, Nowak J, Awad M and Sholl L: MA11.10 Identification of mismatch repair deficient lung adeno-carcinomas using targeted next-generation sequencing. J Thorac Oncol 13 (Suppl 10): S395, 2018.

55. Yuan X, Wu H, Han N, Xu H, Chu Q, Yu S, Chen Y and Wu K: Notch signaling and EMT in non-small cell lung cancer: Biological significance and therapeutic application. J Hematol Oncol 7: 87, 2014.

56. Qi H, Zmina PM, Huang AY, Askew D and Bedogni B: Inhibiting Notch1 enhances immunotherapy efficacy in melanoma by preventing Notch1 dependent immune suppressive properties. Cancer Lett 434: 144-151, 2018.

57. Zhang K, Hong X, Song Z, Xu Y, Li C, Wang G, Zhang Y, Zhao X, Zhao Z, Zhao J, et al: Identification of deleterious mutation as novel predictor to efficacious immunotherapy in NSCLC. Clin Cancer Res 26: 3649-3661, 2020.

58. Jiang D, Niu Z, Zhang J, Wang Y, Shang L, Li B, Guo J, Wang B, Zhao LQ, Wang W, et al: Notch family gene mutations associate with high tumor mutational burden in diverse cancers. J Clin Oncol 37 (Suppl 15): e14616, 2019.

59. Severson EA, Ramkissoon S, Daniel S, Vergilio JA, Gay LM, Elvin JA, Suh J, Frampton GM, Ali SM, Miller VA and Ross JS: Association of tumor mutational burden in cutaneous squamous cell carcinoma with genomic alterations in Notch family receptors. J Clin Oncol 35 (Suppl 15): e13031, 2017.

60. Subbiah V, Solit DB, Chan TA and Kurzrock R: The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) ≥10: A decision centered on empowering patients and their physicians. Ann Oncol 31: 1115-1118, 2020.

61. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, et al: Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 9: 34, 2017.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.