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Association between certain non–small cell lung cancer driver mutations and predictive markers for chemotherapy or programmed death-ligand 1 inhibition

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Abstract

This study aimed to analyze the association between driver mutations and predictive markers for some anti–tumor agents in non–small cell lung cancer (NSCLC). A cohort of 785 Chinese patients with NSCLC who underwent resection from March 2016 to November 2017 in the First Affiliated Hospital of Guangzhou Medical University was investigated. The specimens were subjected to hybridization capture and sequence of 8 important NSCLC-related driver genes. In addition, the slides were tested for PD-L1, excision repair cross-complementation group 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1), thymidylate synthase (TS) and β-tubulin III by immunohistochemical staining. A total of 498 (63.4%) patients had at least 1 driver gene alteration. Wild-type, EGFR rare mutation (mut), ALK fusion (fus), RAS mut, RET fus and MET mut had relatively higher proportions of lower ERCC1 expression. EGFR 19del, EGFR L858R, EGFR rare mut, ALK fus, HER2 mut, ROS1 fus and MET mut were more likely to have TS low expression. Wild-type, EGFR L858R, EGFR rare mut and BRAF mut were associated with lower β-tubulin III expression. In addition, wild-type, RAS mut, ROS1 fus, BRAF and MET mut had higher proportion of PD-L1 high

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expression. As a pilot validation, 21 wild-type patients with advanced NSCLC showed better depth of response and response rate to taxanes compared with pemetrexed/gemcitabine (31.2%/60.0% vs 26.6%/45.5%). Our study may aid in selecting the optimal salvage regimen after targeted therapy failure, or the chemo-regimen where targeted therapy has not been a routine option. Further validation is warranted.

KEYWORDS
chemotherapy, gene mutation, lung cancer, predictive markers, programmed death-ligand 1

1 | INTRODUCTION

According to a recently published report of cancer incidence and mortality in China, lung cancer is still the most common cancer nationwide, and is the leading cause of cancer death.[1] Over the past decade, targeted therapy has played an important role in oncology, significantly prolonging the survival time of cancer patients, as well as improving their quality of life. Driven gene mutations are important causes of lung cancer and predictors of targeted therapy, such as osimertinib for EGFR-mutated advanced non–small cell lung cancer (NSCLC) patients[2] and crizotinib for ALK-positive lung cancer patients.[3]

Non–small cell lung cancer accounts for approximately 85% of all lung cancers,[4] which are relatively insensitive to chemotherapy compared to small cell lung cancer. For NSCLC patients with gene mutations, such as EGFR or ALK mutations, targeted therapy has already become the first line treatment.[5] However, acquired resistance to targeted drugs is inevitable, and chemotherapeutic agents or immune checkpoint inhibitors will be used as alternative drugs after the failure of targeted therapy or combined drugs in the treatment.[6-8] As genotyping becomes increasingly common, it is necessary to understand the sensitivity of patients with different mutation types to different chemotherapeutic agents or immune checkpoints inhibitors.

Due to the short history of conventional genotyping, there are few studies that have reported on the relationship between different genotypes and non–targeted drugs. However, as alternatives to actual drug efficacy indicators, some known drug resistance markers or sensitive markers can predict the efficacy of certain drugs. For example, high levels of ribonucleotide reductase subunit M1 (RRM1) have been associated with resistance to gemcitabine.[9] Programmed death-ligand 1 (PD-L1) positive expression enhanced the efficacy of nivolumab in patients with advanced non–squamous NSCLC.[10]

Our study analyzes the relationship between different genotypes and the expression of some known predictive markers, thus providing information critical for individualization of immunotherapy and chemotherapy.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

A total of 785 patients diagnosed with NSCLC with pathologic stage I to IVA disease were included continuously from March 2016 to November 2017 at the First Affiliated Hospital of Guangzhou Medical University. All patients underwent radical resection for lung cancer, and formalin-fixed paraffin-embedded (FFPE) specimens were collected from the patients. Two pathologists (Dr He and Dr Gu), who were unaware of the clinical data, independently reviewed the specimens to confirm the histological subtype, staining intensity and tumor cell content. Other relevant information, including age and gender, were also collected. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University. All the patients in this study provided written informed consent.

2.2 | Next-generation sequencing-based genomic profiling

The specimens were reviewed to ensure tissue adequacy (>20% tumor nuclei) before testing. DNA was extracted from unstrained FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Duesseldorf, Germany) following the manufacturer's instructions. DNA concentration was measured using a Qubit Fluorometer (Thermo Fisher, MA, USA). A targeted next-generation sequencing method was used to identify the clinically relevant mutation profiles. Briefly, FFPE DNA was used for library construction. Hybridization capture of relevant introns and exons from *EGFR* 19del, *EGFR* L858R, *EGFR* rare, *ALK*, *HER2*, *RAS*, *RET*, *ROS1*, *BRAF* and *MET* was performed. The hybrid capture libraries were then sequenced to >5009 average unique coverage using Ion Proton Sequencers (Thermo Fisher). Sequencing data were processed using a customized bioinformatics pipeline named Otype, which was designed to simultaneously detect single nucleotide variations, short insertions and deletions, copy number variations and gene rearrangements. Finally, data interpretation was focused on genomic alterations associated with clinically available targeted treatment options according to the standards and guidelines of the NCCN, the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).

2.3 | Histological analysis

The pathologic records of the specimens and all available HE-stained tissue sections, in addition to any available sections with special stains or immunohistochemical (IHC) analysis, were reviewed.

Pathological information was collected, including maximum tumor sizes (in cm) and pathologic disease stages. Staging was based on the guidelines of the 7th edition of the TNM classification for lung cancer.[11]

2.4 | Immunohistochemical staining

Tumor sections were incubated with specific monoclonal antibodies against excision repair cross-complementation group 1 (ERCC1) epitope (clone UMAB8, Beijing Zhongshan Golden Bridge Biotechnology), RRM1 epitope (clone EP242, Beijing Zhongshan Golden Bridge Biotechnology), thymidylate synthase (TS) epitope (clone TS1, Beijing Zhongshan Golden Bridge Biotechnology) and β-tubulin-III epitope (clone TUJ1, Fuzhou Maxim Biotechnology Development).

Immunohistochemical staining showed brown-yellow granules localizing in the cytoplasm or the nucleus (different antibody with different localization). The grading of IHC positive reaction was based on the criterion of combining the staining intensity and the percentage of positive cells. Five images were randomly acquired at a magnification of ×400 for each specimen. We try to avoid the marginal zone to prevent the edge effect from affecting the evaluation. The number of all cells and positive cells were counted by using the micro-measurement grid, and the average proportion of positive cells was calculated. First, a score was given according to the staining intensity: 0 if colorless, 1 if light yellow, 2 if light brown, and 3 if dark brown. Then, the percentage of positive cells was calculated for each specimen, and a proportion score was assigned (0 if 0%, 1 if 0% to 10%, 2 if 10% to 50%, 3 if 50% to 75% and 4 if >75%). Finally, this proportion score was multiplied by the staining intensity score to obtain a final semi quantitative score, which was divided into 4 grades: −(0,1,2), +(3,4), ++(6,8) and +++(9,12). Tumors with a final score exceeding 3 were deemed IHC-positive. With regard to protein expression, − ~ + is considered low expression and ++~+++ is considered high expression.

VENTANA PD-L1 (SP142) Assay (Roche, Basel, Switzerland) was used for the IHC assessment of the PD-L1 protein in tumor cells in FFPE tumor tissue stained with the OptiView DAB IHC Detection Kit and the OptiView Amplification Kit on a VENTANA BenchMark IHC/ISH instrument. The proportion of tumor area occupied by PD-L1 expressing tumor cells (%TC, ≥1%) of any intensity was considered PD-L1 positive.

2.5 | The correlation between each driver mutation and the sensitivity markers

R studio 19.0 (R Studio) and R package ComplexHeatmap were used to generate the heatmap plot and complete linkage clustering was used to perform the hierarchical clustering of the marker expression and present the relationship between genetic features and predictive markers. We calculated the prevalence of 8 important NSCLC-related driver genes in all the samples, and mutations were grouped according to the mutation with highest abundance in the patient. According to the gene mutations, a low expression rate (ERCC1, RRM1, TS and β-tubulin III) or a positive rate (PD-L1) of the predictive markers were also calculated in each subgroup. The overall percentage was used as a reference cut-off for each sensitivity marker.

2.6 | Pilot validation

Another 21 patients with wild-type (pan-negative) advanced NSCLC were divided into 2 groups based on the treatment. Group A used anti–microtubule agents, such as docetaxel or paclitaxel, while group B used antimetabolic agents, such as gemcitabine or pemetrexed. The depth of response (DoR) and the objective response rate (ORR) were calculated as tumor remission indicators to show the drug efficacy.

2.7 | Statistical analysis

Statistical analysis was performed using R studio 19.0 and IBM SPSS Statistics 22.0 (SPSS). The *t* test, the χ^2-test and Fisher's exact test were used according to the variable type. A 2-tailed *P*-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

This study included 438 male and 347 female NSCLC patients, with a median age of 59.1 years old. There were 638 adenocarcinomas, accounting for approximately 80.0% of all the histological types, as well as 67 squamous-cell carcinomas and 14 lymphoepithelioma-like carcinomas. Approximately 60.0% of these patients were stage I. The clinicopathological characteristics of patients are summarized in Table 1. In addition, the examples representing different expression levels of each marker are demonstrated in Figure 1.

3.2 | Distribution of different mutations

A total of 498 (63.4%) patients had at least 1 driver gene alteration; 34 patients had compound mutations. Only the mutation with highest abundance is considered for patient classification (Table S1). The prevalence of each benchmark driver mutation is shown in Table 2. Wild-type patients have the highest proportion, 36.6%, followed by EGFR L858R, 23.2%, and EGFR 19del, 21.4%.

3.3 | Correlation between driver mutations and predictive markers

Correlation between driver mutations and predictive markers is summarized in Figure 2 and Table 3. For example, wild-type, EGFR rare mutation, ALK fusion, RAS mut, RET fus and MET mut had higher proportions of lower ERCC1 expression, which indicated

TABLE 1 Baseline characteristics of non–small cell lung cancer patients (N = 785)

	Frequency	Percentage(%)
Sex		
Male	438	55.80
Female	347	44.20
Age (years)	mean($\bar{X} \pm S$) 59.1 ± 10.7	
Histology		
Squamous carcinoma	67	8.54
Adenocarcinoma	638	81.27
Adenosquamous carcinoma	8	1.02
Lymphoepithelioma-like carcinoma	14	1.78
Large cell lung cancer	4	.51
Mixed small cell lung cancer	6	.76
Others/undefined	48	6.11
Clinical stage		
I	468	59.62
II	164	20.89
III	136	17.32
IVA	17	2.17

that these genotypes might be more sensitive to platinum. EGFR 19del, EGFR L858R, EGFR rare mut, ALK fus, HER2 mut, ROS1 fus and MET mut were more likely to have RRM1 low expression and EGFR 19del, EGFR L858R, EGFR rare mut, ALK fus and HER2 mut tend to have TS low expression. Wild-type, EGFR L858R, EGFR rare mut and BRAF mut were associated with lower β-tubulin III expression. In addition, wild-type, RAS mut, ROS1 fus, BRAF and MET mut had higher proportions of PD-L1 high expression, suggesting potential higher chance of response to PD-(L)1 blocking therapies.

TABLE 2 The prevalence of driver mutations

Type	Prevalence (%)
Total	785 (100)
Wild type	286 (36.56)
EGFR 19DEL	168 (21.40)
EGFR L858R	182 (23.18)
EGFR rare	19 (2.42)
RAS	77 (9.81)
ALK	25 (3.18)
RET	9 (1.15)
ROS1	8 (1.02)
BRAF	5 (0.64)
MET (mut)	3 (0.38)
HER2	2 (0.25)

Note: Concurrent mutations were grouped according to the mutation with highest abundance.

3.4 | Pilot validation of sensitivity prediction

Anti–microtubule drugs, rather than anti–metabolic agents, were consistent with recommendation in wild-type patients. Group A has 10 advanced NSCLC patients, while group B has 11 patients. There was no significant difference in gender (male 10/8, $P = 0.074$) between them, while group A was older than group B (63.3 ± 6.1/52.6 ± 8.4, $P = 0.004$). Patients in group A had higher DoR and ORR (31.2%/60.0%) than group B (26.6%/45.5%), consistent with our recommendation of anti–microtubules, such as taxanes and vinorelbine, in the treatment of NSCLC patients without active driver mutations.

4 | DISCUSSION

All previous treatment strategies for NSCLC patients were based on the whole population. It is now known that genotyping determines

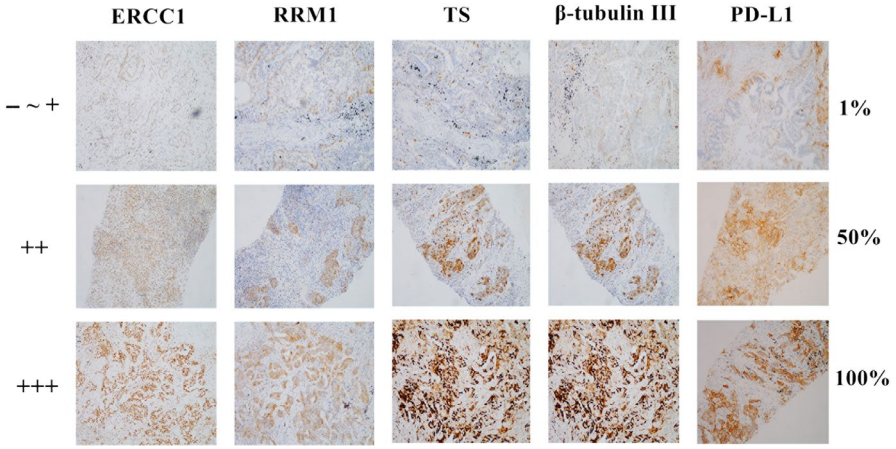

FIGURE 1 The examples representing different expression levels of ERCC1, RRM1, TS, β-tubulin III and PD-L1. –~+, ++ and +++ measure the expression of ERCC1, RRM1, TS and β-tubulin III. 1%, 50% and 100% for PD-L1

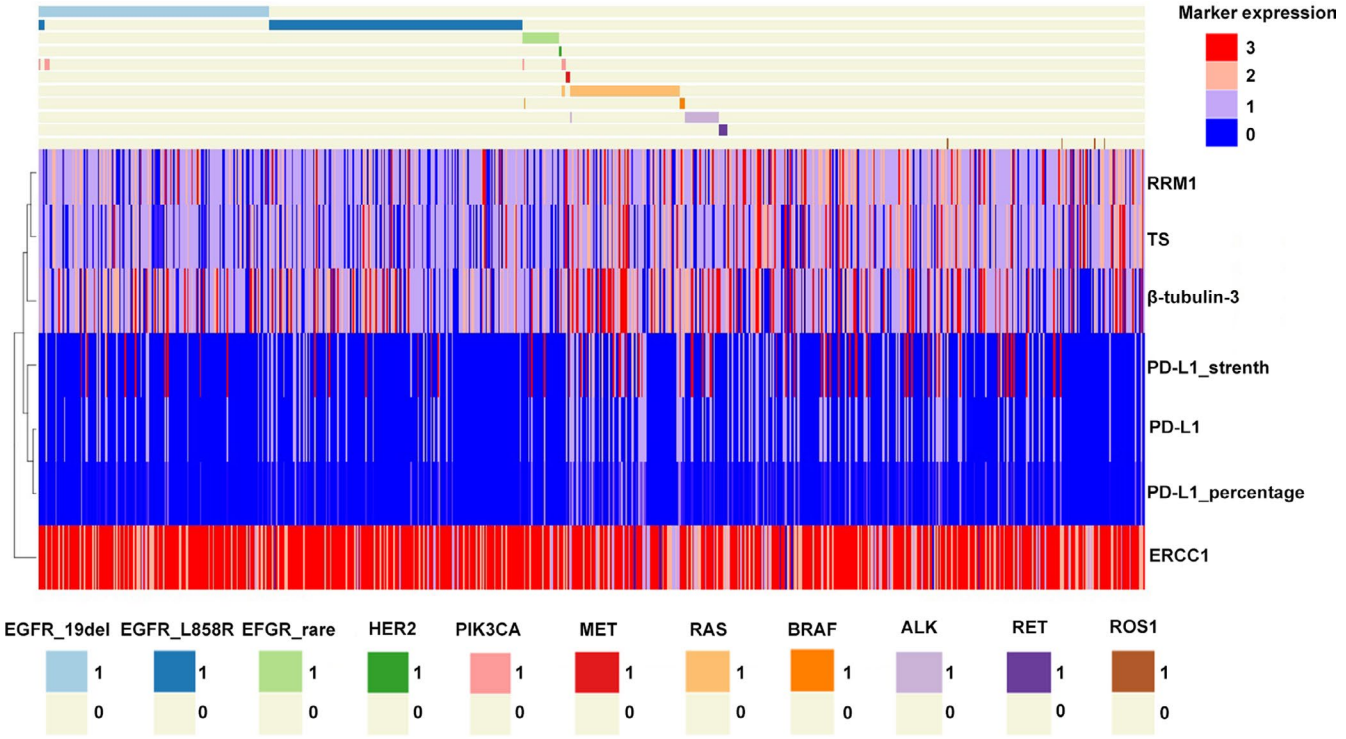

FIGURE 2 Correlation between driver mutations and predictive markers

not only the targeted therapy options but also the biological behavior of the tumor, thus different treatment strategies. Therefore, it is necessary to re-examine the conclusions of treatment strategies based on the general population in the past. One of the treatment strategies is traditional chemotherapy regimen selection. Targeted therapy has been the first choice for NSCLC patients with typical gene mutation, such as EGFR and ALK. However, the treatment of cancer is a long-term process and the acquired resistance of targeted drugs is inevitable. Furthermore, for patients harboring known gene mutations with no available targeted therapy options, chemotherapy or immunotherapy is still the standard of care.

The present study assessed the relationship between genotyping for 8 important driver genes and some outcomes of sensitivity markers of chemotherapy and immunotherapy in a large sample of NSCLC patients. EGFR mutations were still the most common gene alterations in NSCLC patients, followed by RAS and ALK. The genotyping results agreed with previous studies in the prevalence of driver mutations in Chinese NSCLC patients.[12] The low expression rate of ERCC1, RRM1, TS and β-tubulin III, and the PD-L1 positive rate, differing from other studies, might be due to, for instance, different races, proportions of histology and stages.[13-15] In particular, the issue of ERCC1 isoform and PD-L1 antibody selection for IHC is worth mentioning. Friboulet et al[16] detected and elaborated on the function of ERCC1 isoform 202 for nucleotide excision repair and cisplatin resistance. While ERCC1 monoclonal 4F9 is the specific antibody of ERCC1 isoform 202, all specimen samples in our study were tested by ERCC1 monoclonal 4F9 (clone UMAB8, Beijing Zhongshan Golden Bridge Biotechnology). Therefore, the lower expression of ERCC1 in our study means a lower expression of ERCC1 isoform 202 and the patients with lower ERCC1 isoform 202 expression are more sensitive to platinum. In addition, the study BLUEPRINT showed that the expression of PD-L1 detected by SP142 antibody is lower than other PD-L1 antibodies, but SP142 antibody, 22C3 antibody and other PD-L1 antibodies have highly consistency.[17] In addition, every PD-L1 inhibitor needs to match certain PD-L1 detecting antibody(s), and there is still no consensus on which is the optimal PD-L1 detecting antibody. In the initial stage of our PD-L1 study, the pathology department of our hospital used antibody SP142 for all NSCLC patients. The aim of the present study was to detect the expression of PD-L1 for the whole population to obtain a general level rather than for the expression of one certain patient to select drugs. Therefore, we consider SP142 to be reasonable on that basis.

Some previous studies have demonstrated the relationship between predictive markers and certain chemo agents or targeted drugs. The results revealed that tumor histology also had an impact on their correlation. Low expression of ERCC1 can predict higher objective response of platinum-based therapy in NSCLC patients with better outcome,[18,19] and squamous patients seemed to benefit more.[20] Patients with high RRM1 expression showed resistance to gemcitabine.[9] A low level of TS expression was related to clinical benefit from pemetrexed therapy.[21] Combining data from 10 studies supported that β-tubulin III could be a predictive factor for sensitivity to chemotherapy regimens containing taxanes or vinorelbine, the ORR of the chemotherapy was significantly higher in patients with low/negative expression.[22] PD-L1 expression enhanced the efficacy of nivolumab.[10]

TABLE 3 The correlation between each driver mutation and the sensitivity markers

Type	ERCC1 low %	RRM1 low %	TS low %	β-tubulin III low %	PD-L1 (+) %	Potential sensitive agents
Overall population (cut-off value)	11.7 (68/583)	66.3 (386/582)	72.0 (420/583)	57.0 (345/605)	27.6 (145/525)	-
Wild type	14.9[a] (29/194)	50.0 (97/194)	54.6 (106/194)	61.8[a] (126/204)	36.4[a] (64/176)	Platinum/Taxanes/ Vinorelbine/PD-(L)1 inhibitors
EGFR 19DEL	2.9 (4/138)	76.8[a] (106/138)	87.6[a] (120/137)	58.9 (83/141)	16.1 (20/124)	Gemcitabine/ Pemetrexed
EGFR L858R	7.2 (10/139)	81.2[a] (112/138)	82.7[a] (115/139)	60.3[a] (85/142)	18.9 (23/122)	Gemcitabine/ Pemetrexed/ Taxanes
EGFR rare	16.7[a] (2/12)	91.7[a] (11/12)	100[a] (12/12)	85.7[a] (12/14)	20.0 (3/15)	Platinum/ Gemcitabine/ Pemetrexed/ Vinorelbine/
ALK	22.7[a] (5/22)	69.6[a] (16/23)	95.7[a] (22/23)	39.1 (9/23)	25.0 (5/20)	Platinum/ Gemcitabine/ Pemetrexed
HER2	.0(0/1)	100[a] (1/1)	100[a] (1/1)	.0 (0/1)	-	Gemcitabine/ Pemetrexed
RAS	28.6[a](16/56)	58.9 (33/56)	58.9 (33/56)	39.0 (23/59)	44.9[a] (22/49)	Platinum/PD-(L)1 inhibitors
RET	14.3[a](1/7)	50.0 (3/6)	57.1 (4/7)	28.6 (2/7)	.0 (0/6)	Platinum
ROS1	.0(0/6)	66.7[a] (4/6)	66.7 (4/6)	14.3 (1/7)	60.0[a] (3/5)	Gemcitabine/PD-(L)1 inhibitors
BRAF	.0(0/5)	20.0 (1/5)	40.0 (2/5)	60[a] (3/5)	60.0[a] (3/5)	Taxanes/ Vinorelbine/PD-(L)1 inhibitors
MET (mut)	33.3[a](1/3)	66.7[a] (2/3)	33.3 (1/3)	33.3 (1/3)	66.7[a] (2/3)	Platinum/ Gemcitabine/PD-(L)1 inhibitors

Note: Low expression of ERCC1, RRM1, TS and β-tubulin III indicate better sensitivity to platinum, gemcitabine, pemetrexed and anti–microtubule agents, respectively, while positive/higher PD-L1 expression indicates better sensitivity to PD-(L)1 inhibitors. The percentage of favorable expression of each marker (low ERCC1, low RRM1, low TS, low β-tubulin III and positive PD-L1) in overall population is used as the cut-off value.
ERCC1, excision repair cross-complementation group 1; fus, fusion; mut, mutation; PD-(L)1, programmed death-ligand 1; RRM1, ribonucleotide reductase subunit M1; TS, thymidylate synthase.
[a]The rate was higher than the general level, and the drugs in the last column were considered sensitive to patients with certain genotype.
[b]Green shades mean that the rate was higher than the general level.

Based on the above results, as well as different driver gene mutations, we recommended some alternative drugs for NSCLC patients who became resistant to targeted therapy or had no optimal targeted drugs. Although there is a limitation that the data for IHC staining in the validation cohort is not applicable, the small sample test results validated our recommendation: that is to say, NSCLC patients with no EGFR mutations will have better outcomes by using anti–microtubule drugs rather than gemcitabine or pemetrexed. Few studies focus on the treatment of NSCLC patients with EGFR wild-type. A Japanese study revealed that pemetrexed-carboplatin combination was effective and well-tolerated in EGFR wild-type non–squamous NSCLC patients, resulting in an ORR of 35.8%,[22] which was lower than the ORR of group A (45.5%) in our study. The difference may be caused by small sample and NSCLC histology. Therefore, a large randomized clinical trial on the treatment efficacy comparison between anti–microtubules and gemcitabine or pemetrexed is needed.

Referring to the results, some drug sensitivity indications were consistent with previous reports in some respects. For example, NSCLC patients with ALK+ responded well to pemetrexed-based therapy, obtaining prolonged progression-free survival.[23] For advanced NSCLC patients with KRAS mutations, who were considered chemo-resistant,[24] PD-(L)1 inhibitors were available[25] and platinum-based chemotherapy were added.[26] BRAF mutation in Chinese NSCLC patients was rare, with patients not responding well to chemotherapy,[27] and limited data available. Anti PD-(L)1 was optional

and taxanes might be the most sensitive chemo agents. For EGFR-mutant patients, PD-(L)1 inhibitors were not recommended,[26] while patient with KARS, BRAF and MET mutations benefit more from immune checkpoint inhibitors than EGFR, ALK and RET patients.[25,28] With the common practice of gene mutation detection, there will be more evidence from qualified clinical studies to support these results.

Our study has provided enlightenment for clinical practice. On the one hand, detection of gene mutations can help in selecting the best available drugs for targeted therapy. On the other hand, for the patients with drug resistance after targeted cancer therapies and without specific targeted therapies, the analysis of the predictive markers of chemotherapy and immunotherapy efficacy can provide an important reference to choose the optimal chemotherapy and assess whether it is suitable for immunotherapy. In addition, the results explain why patients differ in sensitivity to various drugs from the perspective of gene mutations.

It is noteworthy that there is no clear conclusion on the relationship between predictive markers and chemotherapy efficacy; large randomized studies are needed to determine their predictive value in different settings and tumors. And for our study, there is still a need for follow-up studies to analyze the data of patients receiving these drugs and to verify the clinical significance of this study. In the future, precise treatment will be the key point in drug research and development, and more specific and selective biomarkers will be identified. Furthermore, a feasible method of continuous dynamic detection of biomarkers, especially liquid biopsy, will play an important role both in drug guidance and resistance. Thus, patients receiving individualized treatment will benefit more.

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REFERENCES

1. Chen WQ, Li H, Sun KX, et al. Report of cancer incidence and mortality in China, 2014. *Zhonghua Zhong Liu Za Zhi*. 2018;40:5-13.
2. Soria JC, Ohe Y, Vansteenkiste J, et al. Osimertinib in untreated EGFR-mutated advanced non–small-cell lung cancer. *N Engl J Med*. 2018;378:113-125.
3. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014;371:2167-2177.
4. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64:9-29.
5. NCCN. NCCN Guidelines Version 5.2018 Non-Small Cell Lung Cancer; 2018.
6. Wu SG, Shih JY. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non–small cell lung cancer. *Mol Cancer*. 2018;17:38.
7. Sharma GG, Mota I, Mologni L, Patrucco E, Gambacorti-Passerini C, Chiarle R. Tumor resistance against ALK targeted therapy-where it comes from and where it goes. *Cancers*. 2018; 10(3):62.
8. Stinchcombe TE, Borghaei H, Barker SS, Treat JA, Obasaju C. Pemetrexed with platinum combination as a backbone for targeted therapy in non–small-cell lung cancer. *Clin Lung Cancer*. 2016;17:1-9.
9. Jordheim LP, Seve P, Tredan O, Dumontet C. The ribonucleotide reductase large subunit (RRM1) as a predictive factor in patients with cancer. *Lancet Oncol*. 2011;12:693-702.
10. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. *N Engl J Med*. 2015;373:123-135.
11. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol*. 2007;2:706-714.
12. Gou LY, Wu YL. Prevalence of driver mutations in non-small-cell lung cancers in the People's Republic of China. *Lung Cancer*. 2014;5:1-9.
13. Vilmar AC, Santoni-Rugiu E, Sorensen JB. ERCC1 and histopathology in advanced NSCLC patients randomized in a large multicenter phase III trial. *Ann Oncol*. 2010;21:1817-1824.
14. Wang L, Wang R, Pan Y, Sun Y, Zhang J, Chen H. The pemetrexed-containing treatments in the non–small cell lung cancer is −/low thymidylate synthase expression better than +/high thymidylate synthase expression: a meta-analysis. *BMC Cancer*. 2014;14:205.
15. Zhang HL, Ruan L, Zheng LM, Whyte D, Tzeng CM, Zhou XW. Association between class III beta-tubulin expression and response to paclitaxel/vinorebine-based chemotherapy for non–small cell lung cancer: a meta-analysis. *Lung Cancer*. 2012;77:9-15.
16. Friboulet L, Olaussen KA, Pignon JP, et al. ERCC1 isoform expression and DNA repair in non–small-cell lung cancer. *N Engl J Med*. 2013;368:1101-1110.
17. Hirsch FR, Mcelhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol*. 2017;12:208-222.
18. Chen S, Zhang J, Wang R, Luo X, Chen H. The platinum-based treatments for advanced non–small cell lung cancer, is low/negative ERCC1 expression better than high/positive ERCC1 expression? A meta-analysis *Lung Cancer*. 2010;70:63-70.
19. Vassalou H, Stathopoulos E, Fiolitaki G, et al. Excision-repair-cross-complement-1 protein as a prognostic factor in patients with advanced non–small cell lung cancer treated with platinum-based first-line chemotherapy. *Lung Cancer*. 2013;82:324-329.
20. Lee SM, Falzon M, Blackhall F, et al. Randomized prospective biomarker trial of ERCC1 for comparing platinum and nonplatinum therapy in advanced non–small-cell lung Cancer: ERCC1 Trial (ET). *J Clin Oncol*. 2017;35:402-411.
21. Christoph DC, Asuncion BR, Hassan B, et al. Significance of folate receptor alpha and thymidylate synthase protein expression in patients with non–small-cell lung cancer treated with pemetrexed. *J Thorac Oncol*. 2013;8:19-30.
22. Kimura T, Taniguchi H, Watanabe N, et al. Phase II study of carboplatin and pemetrexed in advanced EGFR-wild-type non–squamous non–small cell lung cancer: the central Japan Lung Study Group Trial 0906. *Anticancer Res*. 2016;36:1767-1771.
23. Jo J, Kim SH, Kim YJ, et al. Efficacy of pemetrexed-based chemotherapy in comparison to non–pemetrexed-based chemotherapy in advanced, ALK+ non–small cell lung cancer. *Yonsei Med J*. 2018;59:202-210.
24. Zhang Y, Fang W, Yan Y, et al. The efficacy of first-line chemotherapy is associated with KRAS mutation status in patients with

advanced non–small cell lung cancer: a meta-analysis. *Med Oncol*. 2015;32:61.
25. Yang H, Chen H, Luo S, et al. The correlation between programmed death-ligand 1 expression and driver gene mutations in NSCLC. *Oncotarget*. 2017;8:23517-23528.
26. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. *N Engl J Med*. 2015;373:1627-1639.
27. Ding X, Zhang Z, Jiang T, et al. Clinicopathologic characteristics and outcomes of Chinese patients with non–small-cell lung cancer and BRAF mutation. *Cancer Med*. 2017;6:555-562.
28. Mhanna L, Milia J, Lusque A, Couraud S, Mascaux C, Veillon R. Efficacy of immune-checkpoint inhibitors (ICI) in non–small cell lung cancer (NSCLC) patients harboring activating molecular alterations. *J Clin Oncol*. 2018;36:172.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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