The Role of ERBB4 Mutations in the Prognosis of Advanced Non-small Cell Lung Cancer Treated With Immune Checkpoint Inhibitors

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Research

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Abstract

Introduction: Recently, immune checkpoint inhibitors (ICIs) has been reported to achieved convincing clinical benefits and significantly prolonged the overall survival (OS) of advanced non-small cell lung cancer (NSCLC) patients. Sensitivity to immunotherapy was related to several biomarkers, such as PD-L1 expression, TMB level, MSI-H and MMR. However, novel biomarkers for the prognosis to ICIs treatment need to be further investigated, and it is an urgent demand to establish a systematic hazard model to assess the efficacy of ICIs therapy for advanced NSCLC patients.

Methods: In this study, gene mutation and clinical data of NSCLC patients was obtained from the TCGA database. Then, we analyzed the detailed clinical information and mutational data of two advanced NSCLC cohorts received ICIs treatment from the cBioPortal for Cancer Genomics. The Kaplan-Meier plot method was used to perform survival analyses, selected variables were used to develop a systematic nomogram. The prognostic significance of ERBB4 in pan-cancer was analyzed by another cohort from cBioPortal for Cancer Genomics.

Results: Mutation frequencies of TP53 and ERBB4 was 54% and 8% in NSCLC, respectively. Mutual exclusive analysis in cBioPortal indicated that ERBB4 does show co-occurring mutations with TP53. Patients harbored ERBB4 mutations were confirmed to have a better prognosis for ICIs treatment, compared to ERBB4 wild type (PFS: \( p=0.0360 \); OS: \( p=0.0378 \)) and only TP53 mutations (OS: \( p=0.021 \)). The mutation status of ERBB4 and TP53 are tightly linked to DCB for ICIs treatment, PD-L1 expression, TMB value and TIICs. Finally, a novel nomogram was built to evaluate the efficacy of ICIs therapy.

Conclusion: ERBB4 mutations could serve as a predicting biomarker for prognosis of ICIs treatment. The systematic nomogram was proven to have a great potential to evaluate the efficacy of ICIs therapy for advanced NSCLC patients.

Introduction

Among various cancers, lung cancer accounts for the highest incidence and is the leading cause of cancer-related death worldwide[1]. Lung cancer can be classified into two pathological forms, including non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). NSCLC accounts for 85% of lung cancer and the 5-year survival rate of advanced NSCLC patients is approximately 5–10%[2]. For advanced NSCLC patients, immune checkpoint inhibitors (ICIs) including atezolizumab, pembrolizumab and nivolumab has achieved convincing clinical benefits and significantly prolonged the overall survival (OS) of patients[3–5]. However, the objective response rate of ICIs therapy is only nearly 17%, illustrating that the majority of advanced NSCLC fail to benefit from ICIs[6, 7]. Thus, it is a large unmet clinical need to identify the appropriate patients who may respond to ICIs therapy.

Recently, it has been found that the sensitivity to immunotherapy was related to several biomarkers, such as programmed death-ligand 1 (PD-L1) expression[3, 8, 9], tumor mutational burden (TMB)[8, 9], microsatellite instability-high (MSI-H)[10] and mismatch repair (MMR)[11]. However, only expression
status of PD-L1 is insufficient in predicting the prognosis of immunotherapy in NSCLC[12, 13]. Additionally, TMB was regarded as an appropriate predictive biomarker for immunotherapy efficacy in multiple cancers, including NSCLC[14–16]. However, the high cost of next generation sequencing (NGS) and requirement of adequate pathologic tissue make it difficult for advanced NSCLC patients to assess the level of TMB. Moreover, cancer progression and treatment will lead to the dynamic changes of TMB[17]. Tumor cell proliferation is activated though specific somatic mutation in driver genes. In contrast, NSCLC patients with mutation of EGFR or ALK might not get clinical benefits from ICIs therapy. Therefore, it is an urgent demand to establish a hazard model involving the biomarkers above and genetic alterations for the prognosis of immunotherapy.

Tyrosine kinase receptors of the ERBB family include four members: HER-1/ERBB1/EGFR, HER-2/ ERBB2, HER-3/ ERBB3, and HER-4/ ERBB4[18, 19]. The former researches indicated that the ERBB receptor family is closely associated with cell proliferation and oncogenic events[20]. Exceptionally, ERBB4 is the only member of the ERBB receptor family with growth inhibiting and differentiation stimulating ability and expression of ERBB4 has been proven to be downregulated in different types of aggressive tumors[21, 22]. Functional characterization of nine mutations of ERBB4 disclosed four types of activating mutations (K935I, D931Y and Y285C, D595V) with increased basal and ligand-induced ERBB4 phosphorylation levels[23]. Moreover, certain ERBB4 polymorphisms (SNPs rs6742399, rs6740117 and rs6747637) were firmly linked to a higher risk of lung cancer, suggesting that ERBB4 mutation may predispose to development of lung cancer[24]. The sequences of EGFR and ERBB4 kinase domains were 79% identical[25]. It is well-known that EGFR-TKIs are the first-line treatment for advanced NSCLC patients harboring EGFR mutations. Previous researches reported EGFR mutations could be regarded as biomarkers of resistance to ICIs[26, 27]. According to the latest studies, we noted that ERBB4 mutations were mainly enriched in NSCLC patients with high PD-L1 expression, compared with other ERBB family numbers[28]. Several reports suggested that patients with ERBB4 mutation may respond to ICIs treatment in esophageal cancer and cervical cancer[29, 30]. Thus, ERBB4 mutation may be a novel biomarker for lung cancer immunotherapy.

A study of two immunotherapy cohorts with clinical and mutational data[31, 32] was performed to clarify the relationship between ERBB4 mutations and the prognosis of NSCLC patients receiving ICIs treatment. In addition, we also collected The Cancer Genome Atlas (TCGA)-NSCLC cohort to explore the role of ERBB4 mutations in the tumor-infiltrating immune cells and TMB level. Finally, a systematic nomogram to predict the prognosis of NSCLC patients with ICIs therapy was established based on the clinicopathological information and mutational data.

Materials And Methods

Study design and data download

Two immunotherapy cohorts with clinical and mutational data were collected from the cBioPortal. One of the cohorts [MSKCC, J Clin Oncol 2018] was 240 advanced NSCLC patients with mutational data received
ICI therapy (pembrolizumab/ pembrolizumab+ipilimumab) and 86 of them accepted PD-L1 expression assessment[31]. Efficacy of the immunotherapy was assessed according to RECIST guidelines and durable clinical benefit (DCB) was defined as the time that patients remained sensitive to ICI treatment (complete response [CR]/partial response [PR] or stable disease [SD] that lasted > 6 months). PFS (Progress free survival) was assessed as the time from the date of ICIs treatment to the date of progression or death in this cohort. The other of the cohorts [TMB and Immunotherapy (MSKCC, Nat Genet 2019)] included 350 advanced NSCLC patients who received FDA-approved ICIs therapy[32]. OS was assessed as the date of death or last follow-up in this cohort. The assessment of TMB was reported in the both clinical trials. In addition, we also analyzed somatic mutation and survival data from the Cancer Genome Atlas (TCGA)-LUAD and -LUSC cohort.

**Development of nomogram**

The prognosis univariates were analyzed using the Kaplan-Meier method, and the log rank tests were used to detect significant differences. A nomogram was formulated based on the results of the univariate analyses and by using the “rms” package of R software version 3.1.2. Accuracy and discriminative value of the nomogram was estimated by concordance index (C-index) and Calibration plot.

**Bioinformatic analysis**

The gene mutation expression and clinical data was downloaded from TCGA portal. LUAD and LUSC patients were designed to divided into two groups according to mutation status of ERBB4, respectively. Gene set enrichment analysis (GSEA) was performed using GSEA software 4.1.0. A normal p-value <0.05 was considered significantly enriched. CIBERSORT[33] was used to evaluate the proportions of 22 tumor-infiltrating lymphocyte subsets in tumor samples. Then, relative abundance of immune cell infiltration was estimated in patients with different ERBB4 status. The number of permutations was set to 1000, and the criterion for successful computation of a sample was threshold p-value 0.05.

**Statistical analysis**

All statistical analyses were conducted using the GraphPad Prism software 7.0 and R software 3.6.3. Student’s t tests and one-way ANOVA test were used to determine statistical significance. Survival curves were depicted using the Kaplan-Meier method and compared using the log-rank test. For all comparisons, a two-tailed p-value <0.05 was considered statistically significant.

**Results**

**The role of ERBB4 and TP53 mutation in the prognosis of NSCLC patients analyzed by cBioPortal**

Mutation frequencies of TP53 and ERBB4 was 54% and 8% in NSCLC, respectively (Fig. 1A). In the both cohorts, mutual exclusive analysis in cBioPortal indicated that ERBB4 does show co-occurencing mutations with TP53 (p < 0.01). Moreover, TP53 mutation was found in all ERBB4-mutated patients of the both cohorts. Then, relationship between the survival of NSCLC patients and ERBB4 mutation statues
was further analyzed. According to the ERBB4 gene mutation status, advanced NSCLC patients treated with ICIs were divided into ERBB4-MT and ERBB4-WT groups, and then KM analysis was performed. We found that the PFS and OS time of the ERBB4-MT NSCLC patients treated with ICIs was longer than that of the ERBB4-WT patients (the median PFS: 9.2 VS 3.17 months, \( p = 0.0360 \); the median OS: 21 VS 11 months, \( p = 0.0378 \), Fig. 1B and C). In addition, both TCGA-LUAD and -LUSC somatic mutation and survival data was downloaded from the Genomic Data Commons. We found that ERBB4 gene mutation only predict prognosis in the TCGA-LUSC patients who did not receive ICI treatment (LUSC: \( p = 0.014 \); LUAD: \( p = 0.785 \), Fig. 1D and E). Therefore, ERBB4-mutated patients exhibited a better prognosis than those with ERBB4 wild type treated with ICIs.

**The role of ERBB4 and TP53 mutation in outcome for ICIs treatment analyzed by cBioPortal**

Firstly, due to TP53 mutation found in all ERBB4-mutated patients of the both cohorts, we divided patients harboring TP53 mutation into ERBB4-MT group (ERBB4 and TP53 comutation), TP53-MT group (TP53 mutation with ERBB4-wildtype) and WT group (without TP53 or ERBB4 mutation). Analysis of the datasets from the cBioPortal showed that ERBB4 and TP53 comutation was associated with an improved sensitivity to immunotherapy. Although the difference of PFS between ERBB4-MT and TP53-MT was not significant (\( p = 0.135 \), Fig. 2A), the median PFS in ERBB4-MT group was 9.2 months compared to 4.33 months in TP53-MT group. In addition, patients harboring ERBB4 mutation benefited more from ICIs treatment and achieved a longer OS time than those in the TP53-MT group (21 months VS 8 months, \( p = 0.021 \), Fig. 2B). As depicted in Fig. 2C, the percentage of DCB were high in the ERBB4-MT group than that in the TP53-MT group and WT group (60% VS 31.9% VS 24%, ERBB4-MT VS TP53-MT group: \( p = 0.035 \); ERBB4-MT VS WT group: \( p = 0.021 \)).

**The role of ERBB4 and TP53 mutation in the TMB level and PD-L1 expression analyzed by cBioPortal and TCGA**

In addition, the mutation status of ERBB4 and TP53 is closely associated with the TMB level and PD-L1 expression (Fig. 2D, E, F, G and H). ERBB4 and TP53 comutation is related to a higher PD-L1 score (ERBB4-WT VS MT group: \( p = 0.022 \), Fig. 2D) in advanced NSCLC patients. However, there is no significant difference of PD-L1 expression between ERBB4-WT group and TP53-WT group (\( p = 0.609 \)). In the both cohorts, a higher TMB value were confirmed in the ERBB4-MT group compared to TP53-MT and WT group (Rizvi’s research: TP53-MT group: \( p = 0.002 \), WT group: \( p < 0.001 \); Samstein’s research: TP53-MT group: \( p < 0.001 \), WT group: \( p = 0.001 \), Fig. 2E and 2F). Moreover, ERBB4 and TP53 mutation were associated with a higher TMB value in both TCGA-LUAD and -LUSC cohorts, respectively (\( p < 0.001 \), Fig. 2G and 2H).

**Construction of nomogram for the prognosis of immunotherapy**

Nomogram to predict the PFS of eighty-three NSCLC patients of the selected cohort was based on integrated information of clinicopathologic features, targeted sequencing and PD-L1 expression. Firstly, we identified variables for nomogram construction by univariate analyses. Multiple variables were proved
to be significantly linked to the PFS of NSCLC patients with ICI treatment, including ERBB4 mutation ($p = 0.0079$), EGFR mutation ($p = 0.0152$), smoking ($p = 0.0040$), treatment lines ($p = 0.0097$), TMB ($p = 0.0059$) and PD-L1 expression ($p = 0.0113$) (Fig. 3A–F). Moreover, the univariate analyses indicated that the advanced NSCLC patients with ERBB4 mutation, ever smoking, first-line ICIs administration, elevated expression of PD-L1 ($\geq 50\%$ percentage), or a high TMB score ($\geq 75\%$th percentage) could derive survival benefits from immunotherapy. However, EGFR mutation might predict the poor prognosis of NSCLC treated by ICIs. A systematic nomogram was formulated based on these variables (Fig. 3G) This novel nomogram could help clinical physicians to easily obtain a point of each variable, and then the total point was evaluated as the sum of all variable points. Therefore, we could assess the efficacy of ICIs therapy for advanced NSCLC patients in advance. The nomogram demonstrated good accuracy in estimating the PFS of advanced NSCLC patients with ICIs therapy, with a bootstrap-corrected C index of 0.75 (95% CI, 0.72 to 0.78). In addition, calibration plots graphically showed great predictive performance in Fig. 3H (the dashed lines in the calibration plots correspond to a 10% margin of error). Therefore, ERBB4 mutation could be regarded as a novel prognostic biomarker for ICIs treatment, and could have a connection with factors that closely associated with efficacy of immunotherapy.

The role of ERBB4 mutation in Tumor-infiltrating immune cell modulation and enrichment pathway analysis of ERBB4 mutation

The relationship between ERBB4 mutation and tumor-infiltrating immune cells was analyzed using CIBERSORT algorithm. As shown in Fig. 4A, we discovered that CD8 T cells, activated memory CD4 T cells, follicular helper T cells and M1 macrophages were more enriched in ERBB4 mutant LUAD group, nevertheless, memory resting CD4 T cells, dendritic cells and Mast cells were enriched in wild-type LUAD group. As for LUSC group, activated memory CD4 T cells, follicular helper T cells and M1 macrophages were more enriched in ERBB4 mutant group (Fig. 4B). Therefore, ERBB4 deficiency might activate the antigen presentation process and cellular immunity and led to the change in the sensitivity to immunotherapy in advanced NSCLC patients.

GSEA analysis performed with TCGA-LUAD revealed that Cell Cycle, Oocyte Meiosis, Spliceosome, RNA Degradation, Nucleotide Excision, Repair DNA Replication, Notch Signaling Pathway, Progesterone Mediated Oocyte Maturation, Pyrimidine Metabolism, Ubiquitin Mediated Proteolysis pathway significantly enriched in samples with ERBB4 mutation (Fig. 5A). As for TCGA-LUSC samples, Porphyrin and Chlorophyll Metabolism, Galactose Metabolism, Sphingolipid Metabolism, Pentose and Glucuronate Interconversions, Glutathione Metabolism Pathway significantly enriched in samples with ERBB4 mutation (Fig. 5B). However, Glioma pathway were enriched in samples with wild type.

Prognostic value of ERBB4 in pan-cancer

Prognostic value of ERBB4 was analyzed as an independent external validation in the cohort [TMB and Immunotherapy (MSKCC, Nat Genet 2019)] [32] in cBioPortal for Cancer Genomics. The mutation of ERBB4 led to a higher TMB level in various cancers ($p < 0.0001$) (Fig. 5C). The Kaplan–Meier survival
analysis indicated that the mutation status of ERBB4 was associated to the prognosis of cancer patients ($p = 0.0130$) (Fig. 5D).

**Discussion**

It is worth noting that the PD-L1 expression or TMB value did not show satisfied efficiency in selecting patients who might benefit from immunotherapy. Recently, some specific gene mutations were disclosed to have an intimate relationship with the efficacy of ICI treatment. Not all mutations of TP53, which was found the most prevalent altered in NSCLC, were shown equal in the outcome among patients received ICIs treatment. For the patients receiving atezolizumab or docetaxel, the better survival benefit of ICIs treatment could be found in PD-L1 positive patients with the TP53 mutation\[28\]. Moreover, TP53 co-mutation with EGFR/STK11/KRAS/ATM have been proved to have predictive value for outcome of ICIs in NSCLC\[34–37\]. Therefore, these gene mutations might be beneficial to predicting the prognosis of ICIs treatment for advanced patients with mutated-TP53. In addition, Wang et al\[38\] indicated that the mutation of FGFR4 might serve as a novel biomarker in modulating the TIME and correlated with the prognosis of NSCLC patients. Data from the Cancer Genome Atlas showed that loss of ERBB4 gene copy numbers was found in different cancer types, including esophageal, lung, bladder and cervical carcinoma\[39\]. A similar pattern can be proven from mRNA expression analyses of a large fraction of tumor cell lines. RNA sequencing data from the Cancer Cell Line Encyclopedia (CCLE), indicate that mRNA expression of ERBB4 is down-regulated in different tumor-derived cell lines\[39\]. Therefore, functional deficiencies of ERBB4 might promote tumor growth in various types of cancers and associate with the poor prognosis of cancer patients\[40, 41\]. The main signaling pathways associated to ERBB4 are the Ras-MAPK-ERK and PI3K-Akt pathways, which moderated cell cycle cessation and differentiation\[42, 43\]. Naresh et al indicates that somatic mutations of ERBB4 in cancer suppressed both pathways and led to cell proliferation rather than differentiation\[22, 40, 41\]. Thus, tumor suppressor-like function of ERBB4 is strongly supported. Studies on the role of ERBB4 in the immune system is relatively new. It was reported that activation of ERBB4 receptors might lead to macrophage apoptosis in a mouse model of colitis\[44\]. Moreover, in another mouse model of cardiac and skin fibrosis, activation of ERBB4 receptors on macrophages contributed to the attenuation of inflammation and fibrosis\[45, 46\]. Based on these former studies, we conducted research to further investigate the predictive value of ERBB4 mutations in advanced NSCLC with ICIs treatment.

In this study, we found that ERBB4 does show co-occurring mutations with TP53. Moreover, ERBB4 and TP53 comutation was associated with a clinical benefit and survival improvement, compared with the only mutation of TP53. Though their PFS time did not show significant difference between ERBB4-TP53 comutation and only TP53 mutation, the OS of patients with ERBB4 and TP53 comutation was proven to be prolonged through ICIs therapy. Additionally, the comutation of TP53 and ERBB4 was closely related to other predictive biomarker of ICIs therapy, such as the expression of PD-L1, TMB value and TILCs. NSCLC patients harboring ERBB4 and TP53 comutations might boost TMB and PD-L1 expression. Moreover, ERBB4 and TP53 deficiencies could moderate the infiltrating immune cells and augment tumor immunogenicity by activating the process of antigen presentation and anticancer cellular immunity in
patients with NSCLC. Thus, ERBB4 mutation might be closely associated with an additional clinical benefit for patients with mutated TP53. SGEA enrichment analysis showed that these ERBB4-MT groups were mainly associated with Cell Cycle, Oocyte Meiosis, Spliceosome and RNA Degradation pathways in LUAD and Porphyrin and Chlorophyll Metabolism, Galactose Metabolism, Sphingolipid Metabolism Pentose pathways in LUSC, respectively. According to the results of Cox regression, we formulated a novel nomogram based on the mutation statues of EGFR and ERBB4, PD-L1 expression, TMB level and other clinicopathological features for advanced NSCLC patients with ICIs therapy. The novel nomogram might help patients and physicians to estimate the clinical benefit of ICIs therapy and determine the appropriate therapeutic plan and follow-up before treatment for patients with NSCLC.

The existing reports indicated that the mechanism of ERBB4 in advanced NSCLC patients with ICIs treatment is still needed to further explore. Our research is the first to investigate ERBB4 mutation might serve as a novel biomarker in modulating the TIME and correlate with the prognosis of NSCLC patients with ICIs therapy. In addition, tendency between ERBB4 and TP53 mutation and their role in PFS and OS of NSCLC patients with ICIs therapy were clearly clarified. Functional deficiencies of ERBB4 contributed to the poor prognosis of NSCLC patients. The mutation of ERBB4 was closely associated with the change of cancer phenotype and showed predictive value in mutated-TP53 patients with ICIs therapy. However, we still needed to demonstrate several limitations in this study. Firstly, because of small clinical sample size in this research, further analysis of ERBB4 function in NSCLC with ICIs therapy is needed. In addition, the molecular mechanism underlying the association of ERBB4 mutation with modulation of the TIME and a higher TMB and PD-L1 expression in NSCLC are still unclear. The full implications of ERBB4 mutation remain elusive and require further study.

**Conclusion**

ERBB4 could serve as a novel biomarker for advanced NSCLC treated by ICIs. The mutation status of ERBB4 and TP53 are tightly linked to prognosis for ICIs treatment, PD-L1 expression TMB value and TIIICs. The systematic nomogram was formulated based on these biomarkers to assess the efficacy of ICIs therapy for advanced NSCLC patients.

**Abbreviations**

ERBB4: erb-b2 receptor tyrosine kinase 4; NSCLC: Non-small cell lung cancer; MMR: Mismatch repair; MSI-H: Microsatellite instability-high; PD-L1: Programmed death ligand 1; TMB: Tumor mutational burden; ICIs: Immune checkpoint inhibitors; TIIICs: Tumor-infiltrating immune cells; TCGA: The Cancer Genome Atlas; PFS: Progress free survival; OS: Overall survival; GSEA: Gene set enrichment analysis; NGS: Next-generation sequencing.

**Declarations**

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Not applicable.

**Authors’ contributions**

XLH has made substantial contributions to the design of the work, the analysis and interpretation of data. RRW and HLX ensured that questions related to the accuracy or integrity of any part of the work. WJJ and KHT have approved the submitted version. All authors have read and approved the manuscript.

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**Availability of data and materials**

All data generated during this study are included in this published article. The datasets generated in the current study are available in the cBioportal for Cancer Genomics[47, 48] (http://www.cbioportal.org/).

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no potential conflicts of interest.

**References**

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: a cancer journal for clinicians. 2020;70(1):7-30.

3. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393(10183):1819-30.

4. Horn L, Spigel DR, Vokes EE, Holgado E, Ready N, Steins M, et al. Nivolumab Versus Docetaxel in Previously Treated Patients With Advanced Non-Small-Cell Lung Cancer: Two-Year Outcomes From Two Randomized, Open-Label, Phase III Trials (CheckMate 017 and CheckMate 057). Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2017;35(35):3924-33.
5. Leighl NB, Hellmann MD, Hui R, Carcereny E, Felip E, Ahn MJ, et al. Pembrolizumab in patients with advanced non-small-cell lung cancer (KEYNOTE-001): 3-year results from an open-label, phase 1 study. The Lancet Respiratory medicine. 2019;7(4):347-57.

6. Park YJ, Kuen DS, Chung Y. Future prospects of immune checkpoint blockade in cancer: from response prediction to overcoming resistance. Experimental & molecular medicine. 2018;50(8):109.

7. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine. 2012;366(26):2455-65.

8. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, Gainor JF, et al. First-Line Nivolumab Plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2019;37(12):992-1000.

9. Ott PA, Bang YJ, Piha-Paul SA, Razak ARA, Bennouna J, Soria JC, et al. T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2019;37(4):318-27.

10. Li Q, Zhang B, Niu FN, Ye Q, Chen J, Fan XS. [Clinicopathological characteristics, MSI and K-ras gene mutations of double primary malignancies associated with colorectal cancer]. Zhonghua yi xue za zhi. 2020;100(4):301-6.

11. Mandal R, Samstein RM, Lee KW, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. Science. 2019;364(6439):485-91.

12. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. The New England journal of medicine. 2016;375(19):1823-33.

13. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(19):5064-74.

14. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. Molecular cancer therapeutics. 2017;16(11):2598-608.

15. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD. Tumor neoantigens: building a framework for personalized cancer immunotherapy. The Journal of clinical investigation. 2015;125(9):3413-21.

16. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351(6280):1463-9.
17. Cyriac G, Gandhi L. Emerging biomarkers for immune checkpoint inhibition in lung cancer. Seminars in cancer biology. 2018;52(Pt 2):269-77.

18. Bouchez C, Pluvy J, Soussi G, Nguyen M, Brosseau S, Tourne M, et al. Epidermal growth factor receptor-mutant non-small cell lung cancer and choroidal metastases: long-term outcome and response to epidermal growth factor receptor tyrosine kinase inhibitors. BMC cancer. 2020;20(1):1186.

19. Li MJ, He Q, Li M, Luo F, Guan YS. Role of gefitinib in the targeted treatment of non-small-cell lung cancer in Chinese patients. Oncotargets and therapy. 2016;9:1291-302.

20. Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature. 2018;554(7691):189-94.

21. Muraoka-Cook RS, Feng SM, Strunk KE, Earp HS, 3rd. ErbB4/HER4: role in mammary gland development, differentiation and growth inhibition. Journal of mammary gland biology and neoplasia. 2008;13(2):235-46.

22. Naresh A, Long W, Vidal GA, Wimley WC, Marrero L, Sartor CI, et al. The ERBB4/HER4 intracellular domain 4ICD is a BH3-only protein promoting apoptosis of breast cancer cells. Cancer research. 2006;66(12):6412-20.

23. Kurppa KJ, Denessiouk K, Johnson MS, Elenius K. Activating ERBB4 mutations in non-small cell lung cancer. Oncogene. 2016;35(10):1283-91.

24. Zhang Y, Zhang L, Li R, Chang DW, Ye Y, Minna JD, et al. Genetic variations in cancer-related significantly mutated genes and lung cancer susceptibility. Annals of oncology : official journal of the European Society for Medical Oncology. 2017;28(7):1625-30.

25. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. The EMBO journal. 2000;19(13):3159-67.

26. Garassino MC, Gelibter AJ, Grossi F, Chiari R, Soto Parra H, Cascinu S, et al. Italian Nivolumab Expanded Access Program in Nonsquamous Non-Small Cell Lung Cancer Patients: Results in Never-Smokers and EGFR-Mutant Patients. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2018;13(8):1146-55.

27. Lee CK, Man J, Lord S, Links M, Gebski V, Mok T, et al. Checkpoint Inhibitors in Metastatic EGFR-Mutated Non-Small Cell Lung Cancer-A Meta-Analysis. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2017;12(2):403-7.

28. Wang H, Shan Q, Guo J, Han X, Zhao C, Li H, et al. PDL1 high expression without TP53, KEAP1 and EPHA5 mutations could better predict survival for patients with NSCLC receiving atezolizumab. Lung cancer. 2021;151:76-83.

29. Ngoi NYL, Heong V, Lee XW, Huang YQ, Thian YL, Choo BA, et al. Tumor molecular profiling of responders and non-responders following pembrolizumab monotherapy in chemotherapy resistant advanced cervical cancer. Gynecologic oncology reports. 2018;24:1-5.

30. Yan T, Cui H, Zhou Y, Yang B, Kong P, Zhang Y, et al. Multi-region sequencing unveils novel actionable targets and spatial heterogeneity in esophageal squamous cell carcinoma. Nature communications.
2019;10(1):1670.

31. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, 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. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(7):633-41.

32. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nature genetics. 2019;51(2):202-6.

33. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nature methods. 2015;12(5):453-7.

34. Chen Y, Chen G, Li J, Huang YY, Li Y, Lin J, et al. Association of Tumor Protein p53 and Ataxia-Telangiectasia Mutated Comutation With Response to Immune Checkpoint Inhibitors and Mortality in Patients With Non-Small Cell Lung Cancer. JAMA network open. 2019;2(9):e1911895.

35. Dong ZY, Zhong WZ, Zhang XC, Su J, Xie Z, Liu SY, et al. Potential Predictive Value of TP53 and KRAS Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23(12):3012-24.

36. Biton J, Mansuet-Lupo A, Pecuchet N, Alifano M, Ouakrim H, Arrondeau J, et al. TP53, STK11, and EGFR Mutations Predict Tumor Immune Profile and the Response to Anti-PD-1 in Lung Adenocarcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2018;24(22):5710-23.

37. Skoulidis F, Byers LA, Diao L, Papadimitrakopoulou VA, Tong P, Izzo J, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. Cancer discovery. 2015;5(8):860-77.

38. Wang L, Ren Z, Yu B, Tang J. Development of nomogram based on immune-related gene FGFR4 for advanced non-small cell lung cancer patients with sensitivity to immune checkpoint inhibitors. Journal of translational medicine. 2021;19(1):22.

39. Segers VFM, Dugaucquier L, Feyen E, Shakeri H, De Keulenaer GW. The role of ErbB4 in cancer. Cellular oncology. 2020;43(3):335-52.

40. Long W, Wagner KU, Lloyd KC, Binart N, Shillingford JM, Hennighausen L, et al. Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. Development. 2003;130(21):5257-68.

41. Jones FE, Welte T, Fu XY, Stern DF. ErbB4 signaling in the mammary gland is required for lobuloalveolar development and Stat5 activation during lactation. The Journal of cell biology. 1999;147(1):77-88.

42. Iwakura Y, Nawa H. ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease. Frontiers in
cellular neuroscience. 2013;7:4.

43. Telesco SE, Vadigepalli R, Radhakrishnan R. Molecular modeling of ErbB4/HER4 kinase in the context of the HER4 signaling network helps rationalize the effects of clinically identified HER4 somatic mutations on the cell phenotype. Biotechnology journal. 2013;8(12):1452-64.

44. Schumacher MA, Hedl M, Abraham C, Bernard JK, Lozano PR, Hsieh JJ, et al. ErbB4 signaling stimulates pro-inflammatory macrophage apoptosis and limits colonic inflammation. Cell death & disease. 2017;8(2):e2622.

45. Vermeulen Z, Hervent AS, Dugaucquier L, Vandekerckhove L, Rombouts M, Beyens M, et al. Inhibitory actions of the NRG-1/ErbB4 pathway in macrophages during tissue fibrosis in the heart, skin, and lung. American journal of physiology Heart and circulatory physiology. 2017;313(5):H934-H45.

46. De Keulenaer GW, Feyen E, Dugaucquier L, Shakeri H, Shchendrygina A, Belenkov YN, et al. Mechanisms of the Multitasking Endothelial Protein NRG-1 as a Compensatory Factor During Chronic Heart Failure. Circulation Heart failure. 2019;12(10):e006288.

47. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer discovery. 2012;2(5):401-4.

48. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Science signaling. 2013;6(269):pl1.

Figures
Figure 4

ERBB4 mutation is correlated with tumor-infiltrating immune cells. A. Violin plot displays the differentially infiltrated immune cells between ERBB4-mutant groups and ERBB4-wild group in LUAD-TCGA cohort. B. Violin plot displays the differentially infiltrated immune cells between ERBB4-mutant groups and ERBB4-wild group in LUSC-TCGA cohort.