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Research Article

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USH2A mutation and specific driver mutation subtypes are associated with clinical efficacy of immune checkpoint inhibitors in lung cancer

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Abstract: This study aimed to identify subtypes of genomic variants associated with the efficacy of immune checkpoint inhibitors (ICIs) by conducting systematic literature search in electronic databases up to May 31, 2021. The main outcomes including overall survival (OS), progression-free survival (PFS), objective response rate (ORR), and durable clinical benefit (DCB) were correlated with tumor genomic features. A total of 1546 lung cancer patients with available genomic variation data were included from 14 studies. The Kirsten rat sarcoma viral oncogene homolog *G12C* ($KRAS^{G12C}$) mutation combined with tumor protein P53 (*TP53*) mutation revealed the promising efficacy of ICI therapy in these patients. Furthermore, patients with epidermal growth factor receptor (*EGFR*) classical activating mutations (including $EGFR^{L858R}$ and $EGFR^{\Delta 19}$) exhibited worse outcomes to ICIs in OS (adjusted hazard ratio (HR), 1.40; 95% confidence interval (CI), 1.01–1.95; P=0.0411) and PFS (adjusted HR, 1.98; 95% CI, 1.49–2.63; P<0.0001), while classical activating mutations with $EGFR^{T790M}$ showed no difference compared to classical activating mutations without $EGFR^{T790M}$ in OS (adjusted HR, 0.96; 95% CI, 0.48–1.94; P=0.9157) or PFS (adjusted HR, 0.72; 95% CI, 0.39–1.35; P=0.3050). Of note, for patients harboring the Usher syndrome type-2A (*USH2A*) missense mutation, correspondingly better outcomes were observed in OS (adjusted HR, 0.52; 95% CI, 0.32–0.82; P=0.0077), PFS (adjusted HR, 0.51; 95% CI, 0.38–0.69; P<0.0001), DCB (adjusted odds ratio (OR), 4.74; 95% CI, 2.75–8.17; P<0.0001), and ORR (adjusted OR, 3.45; 95% CI, 1.88–6.33; P<0.0001). Our findings indicated that, *USH2A* missense mutations and the $KRAS^{G12C}$ mutation combined with *TP53* mutation were associated with better efficacy and survival outcomes, but *EGFR* classical mutations irrespective of combination with $EGFR^{T790M}$ showed the opposite role in the ICI therapy among lung cancer patients. Our findings might guide the selection of precise targets for effective immunotherapy in the clinic.

Key words: Immune checkpoint inhibitor (ICI); Lung cancer; Usher syndrome type-2A (*USH2A*) missense mutation; Kirsten rat sarcoma viral oncogene homolog *G12C* ($KRAS^{G12C}$) mutation combined with tumor protein P53 (*TP53*) mutation; Epidermal growth factor receptor (*EGFR*) mutation

1 Introduction

Recent studies have indicated that immune checkpoint inhibitors (ICIs), including antibodies targeting programmed death-(ligand) 1 (PD-(L)1) or cytotoxic T-lymphocyte antigen-4 (CTLA-4), have prolonged the survival of lung cancer patients to some extent (Brahmer, 2013; Steven et al., 2016; Gao et al., 2022).

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However, objective response and durable clinical benefit (DCB) only occur in a minority of patients (Yarchoan et al., 2017), and effective genomic biomarkers associated with the potential response of ICIs still remain poorly characterized in the present guidelines. To identify novel genomic predictors of response to ICIs, a string of recent research has performed high-throughput genomic profiling of tumor samples from patients treated with ICIs, by either whole-exome sequencing (WES) or targeted next-generation sequencing (NGS). However, a considerable number of studies were restricted by the inclusion of small patient cohorts, which resulted in contradictory conclusions (Jeanson et al., 2019; Mazieres et al., 2019; Gao et al., 2020; Arbour et al., 2021) and limited their power to detect novel genomic biomarkers. Therefore, a comprehensive analysis was indispensable to assess the potential value of established genomic biomarkers and to enable the discovery of biomarkers with sufficient sensitivity.

In this present study, we performed individual patient-level analyses from published clinical research data to examine the genomic features correlated with clinical and survival outcomes, including the DCB, objective response rate (ORR), overall survival (OS), as well as progression-free survival (PFS) data of lung cancer patients treated with ICIs. In particular, our study demonstrated the predictive value of the mutation subtypes of canonical driver genes, including Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and epidermal growth factor receptor (*EGFR*), in immunotherapy. More importantly, we identified novel target genes such as Usher syndrome type-2A (*USH2A*), which could be robust biomarker and serve to predict the clinical efficacy of immunotherapy.

2 Methods

2.1 Study eligibility and data acquisition

We conducted systematic literature search in the databases of Embase, PubMed, and Cochrane Library to identify relevant studies published up to May 31, 2021. The prospective pooled analysis protocol was registered on the PROSPERO database on September 6, 2021, and was regularly updated (ID: CRD42021271903). Two investigators (YANG and FENG) independently performed search on the databases. The search terms were as follows: ("lung cancer" or "lung carcinoma" or "cancer of lung" or "lung neoplasm") AND ("mutation" OR "mutations" OR "genomic alteration" OR "genomic variant" OR "genomic alterations" OR "genomic variants") AND ("immune checkpoint inhibitor" OR "immune checkpoint inhibitors" OR "ICI" OR "ICIs" OR "immune checkpoint blockers" OR "immune checkpoint blockade" OR "ICB" OR "ICBs" OR "PD-1" OR "PD-L1" OR "CTLA-4" OR "Ipilimumab" OR "Tremelimumab" OR "Nivolumab" OR "Pembrolizumab" OR "Lambrolizumab" OR "Atezolizumab" OR "Avelumab" OR "Durvalumab"). When duplicate reports were identified, the one with larger sample size and more detailed clinical profiling was included. In addition, to discern potentially missing articles, the references in the included articles were also reviewed.

After the articles were obtained, studies were accessed according to the following inclusion criteria: (1) patients were diagnosed with lung cancer and treated with ICIs; (2) individual patient-level clinical profile of objective response, when DCB, OS, or PFS was given in the article; (3) variant-level genomic profiling of patient cohorts was available; (4) the size of patients attainable for analysis was at least five; (5) studies published in English.

Two investigators (YANG and FENG) inspected the retrieved articles to screen potentially applicable studies. Disagreements over particular articles were discussed and the consensus of all investigators was eventually reached.

2.2 Data extraction

Two investigators (YANG and FENG) independently extracted data from each screened study. Disagreements or inconsistencies were discussed and determined with the corresponding author, and consensus was reached with all investigators. The information extracted from each study included title, first author, year of publication, sample size, and sequencing method.

First, we extracted the individual patient-level information from each study: patient ID, sample source, histological type, gender, age, smoking status, treatment, disease outcomes (objective response, DCB, OS, and PFS), and sequencing method. When duplicate patients were identified, the one with detailed clinical information was included. "Responder" (R) was defined as response evaluation criteria in solid tumors (RECIST) version 1.1 criteria-based response

(Eisenhauer et al., 2009) with complete response (CR) or partial response (PR); "Non-responder" (NR) was defined as stable disease (SD) or progressive disease (PD); DCB was defined as CR, PR, or SD lasting longer than six months; all other patients were considered to have no durable benefit (NDB) (Hellmann et al., 2018).

The following variant-level genomic data were extracted from each study: patient ID, chromosome, start position, reference allele, and variant allele, which were based on the hg19 human genome. The analysis and interpretation of genomic variants were performed by SNPnexus (https://www.snp-nexus.org) (Oscanoa et al., 2020). The top 20 most frequently mutated genes were included in our study.

2.3 Statistical analysis

The hazard ratios (HRs) of OS and PFS were calculated by applying both univariate and multivariate Cox's proportional hazard regression analyses. The odds ratios (ORs) of ORR and DCB were estimated by applying both univariate and multivariate logistic regression analyses. To adjust for potential confounding factors, including age and gender, multivariate models were employed. For OR equal to 0, Fisher's exact test was performed to estimate its 95% confidence interval (CI). For survival analyses, the log-rank test was performed to compare the Kaplan-Meier survival curves. All statistical analyses were performed using R 4.1.0 and GraphPad Prism 7. Data visualization was conducted by the R packages "ComplexHeatmap" (Gu et al., 2016), ggplot2, forestplot, and survminer.

3 Results

3.1 Genomic biomarker landscape of immunotherapy efficacy in lung cancer

We performed systematic literature research of the PubMed, Embase, and Cochrane Library databases to identify studies reporting the association between genomic variation and the efficacy of ICIs, which were published up to May 31, 2021. The pooled analysis (Fig. S1) included a total of 1546 patients across 14 studies (Table 1) (Rizvi NA et al., 2015; Gandara et al., 2018; Hellmann et al., 2018; Miao et al., 2018; Rizvi H et al., 2018; Samstein et al., 2019; Abou Alaiwi et al., 2020; Anagnostou et al., 2020; Jia et al., 2020;

Table 1 Basic characteristics of included studies

Study	Sequencing platform	Sample size after screening	Percentage of all lung cancer patients (%)					
			Age ≥65 years	Male gender	Ever smoker	*EGFR* mutation	*KRAS* mutation	*USH2A* mutation
Miao et al., 2018	WES	30	20.00	36.67	73.33	43.33	26.67	33.33
Pender et al., 2021	WGS	26	19.23	38.46		7.69	30.77	30.77
Abou Alaiwi et al., 2020	Oncopanel	331		47.43		10.88	40.48	
Samstein et al., 2019	MSK-IMPACT	139	62.59	46.76		15.11	37.41	
Frigola et al., 2021	WES	44	36.36	63.64	81.82	9.09	25.00	43.18
Hellmann et al., 2018	WES	75	52.00	49.33	80.00	17.33	32.00	40.00
Rizvi et al., 2015	WES	34	32.35	44.12	79.41	5.88	23.53	5.88
Rizvi et al., 2018	MSK-IMPACT	239	53.97	49.37	80.75	10.88	35.98	
Gandara et al., 2018	Targeted gene panel	429	43.36	26.81	82.05	10.02	9.09	
Fang et al., 2021	WES	69			49.28	15.94	5.80	24.64
Anagnostou et al., 2020	WES	89	49.44	51.69	39.33	6.74	32.58	24.72
Jia et al., 2020	ctDNA-WES	10	70.00	90.00	80.00	20.00	0	30.00
Moding et al., 2020	CAPP-Seq	23			78.26	17.39	8.70	4.35
Reuss et al., 2020	WES-IMPACT	8	37.50	75.00	100.00	0	50.00	25.00

WES: whole-exome sequencing; WGS: whole-genome sequencing; MSK-IMPACT: Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; ctDNA: circulating tumor DNA; CAPP-Seq: cancer personalized profiling by deep sequencing; *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homolog; *USH2A*: Usher syndrome type-2A.

Moding et al., 2020; Reuss et al., 2020; Fang et al., 2021; Frigola et al., 2021; Pender et al., 2021).

The genomic variations covering missense mutations, nonsense mutations, frameshifts, in-frame alterations (deletions and insertions), splice site alterations, and other alterations (silent, intron alterations, untranslated region alterations, etc.) include the mutant forms with important structural and functional differences (Sabapathy and Lane, 2018). Therefore, we estimated the effect size of each gene categorized by its mutation types (subtypes less than 15 cases were not included). Then, both univariable logistic regression analyses and univariable Cox's proportional hazard regression analyses were performed to evaluate the effect size for clinical and survival outcomes, including DCB, ORR, OS, and PFS. Multivariable models were also applied to reweigh the potential confounding effects (age and gender) (Tables S1–S8).

In order to reveal gene subtypes with significantly higher ORs of DCB and response after adjustment, we visualized the results via heatmaps (Fig. 1, multivariable analyses). Consistent results were exhibited for ORR and DCB in univariable analyses (Figs. S2c and S2d) and survival outcomes (Figs. S2a and S2b, multivariable analyses; Figs. S2e and S2f, univariable analyses). The findings were in line with reported research—that is, patients typically with missense mutations of tumor protein P53 (*TP53*) (Sun et al., 2020), titin (*TTN*) (Jia et al., 2019), mucin 16 (*MUC16*) (Yu et al., 2020; Zhang et al., 2020), FAT atypical cadherin 1 (*FAT1*) (Fang et al., 2019; Zhu et al., 2021), type 2 ryanodine receptor (*RYR2*), or zinc finger homeobox 4 (*ZFHX4*) (Cai et al., 2018) showed favorable clinical outcomes compared to wild type, while patients with *EGFR* missense mutations gained significantly less benefit (Lee et al., 2017; Fang et al., 2019). Employing this method, we also discovered several novel potential biomarkers (such as *USH2A* missense mutation, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4 (*SMARCA4*) missense mutation, and protein tyrosine phosphatase receptor type D (*PTPRD*) mutation) that could be used for lung cancer patients to significantly benefit from ICI therapy. Among the signatures listed above, the *USH2A* missense mutation was found to be associated with the highest OR with significance in most subgroups.

3.2 Association between favorable outcomes in response to ICIs and *KRAS^{G12C}-TP53* co-mutation

In order to investigate the treatment efficacy of ICIs in *KRAS* mutant patients, patients were stratified by three major subtypes (Fig. 2a). Among the 291 *KRAS* mutant patients in total, 285 (97.9%) carried missense variants, 161 (55.3%) harbored *G12C*, and 55 (18.9%) and 69 (23.7%) harbored *G12D* and other missense mutations (excluding *G12C* and *G12D*), respectively.

The OS and PFS of *KRAS* mutant patients that harbored *G12C* missense mutation were proved to be prolonged across most examined subgroups compared with *KRAS* wild-type lung cancer patients. Notably, significantly superior OS (adjusted HR 0.70, 95% CI 0.49–0.99, P=0.05; HR 0.68, 95% CI 0.29–0.96, P=0.03) and PFS (adjusted HR 0.48, 95% CI 0.29–0.79, P=0.004; HR 0.48, 95% CI 0.29–0.79, P=0.004) were observed for *KRAS^{G12C}-TP53* co-mutation patients compared to other mutation subtypes (Figs. 2b and S3a). For all *KRAS* subtypes other than *G12C*, few significant differences were observed. The likelihood of response to ICIs was stratified by *KRAS* mutational status, and a strong association was found between the existence of *KRAS^{G12C}* mutation and higher ORR (P<0.05, irrespective of most subgroups but not in *TP53* wild-type or PD-(L)1+CTLA-4) and DCB (adjusted OR, 2.33; 95% CI, 1.05–5.18; P=0.04, in *TP53* mutant subgroup) (Fig. S4), which were in line with the results for HR.

We further examined the correlation of *KRAS^{G12C}* with clinical outcomes in ICI-treated lung cancer and non-small-cell lung cancer (NSCLC), separately (Figs. S3b–S3i), in *TP53* mutant patients. Compared with the *KRAS* wild-type, *KRAS^{G12C}* mutations accumulated in responders (adjusted OR, 3.89; 95% CI, 1.89–7.98; P<0.001, all lung cancer) and DCB (adjusted OR, 2.23; 95% CI, 1.05–5.18; P=0.04, all lung cancer) (Figs. S3b, S3c, S3f, and S3g), and these were also correlated with prolonged survival in both OS (adjusted HR, 0.70; 95% CI, 0.49–0.99; P=0.05, all lung cancer) and PFS (adjusted HR, 0.48; 95% CI, 0.29–0.79; P=0.004, all lung cancer) (Figs. S3d, S3e, S3h, and S3i), where NSCLC patients showed consistent outcomes, as mutually proven by reported research (Assoun et al., 2019; Moding et al., 2020). This indicated that *KRAS^{G12C}-TP53* co-mutation was associated with favorable outcomes in ICI therapy.

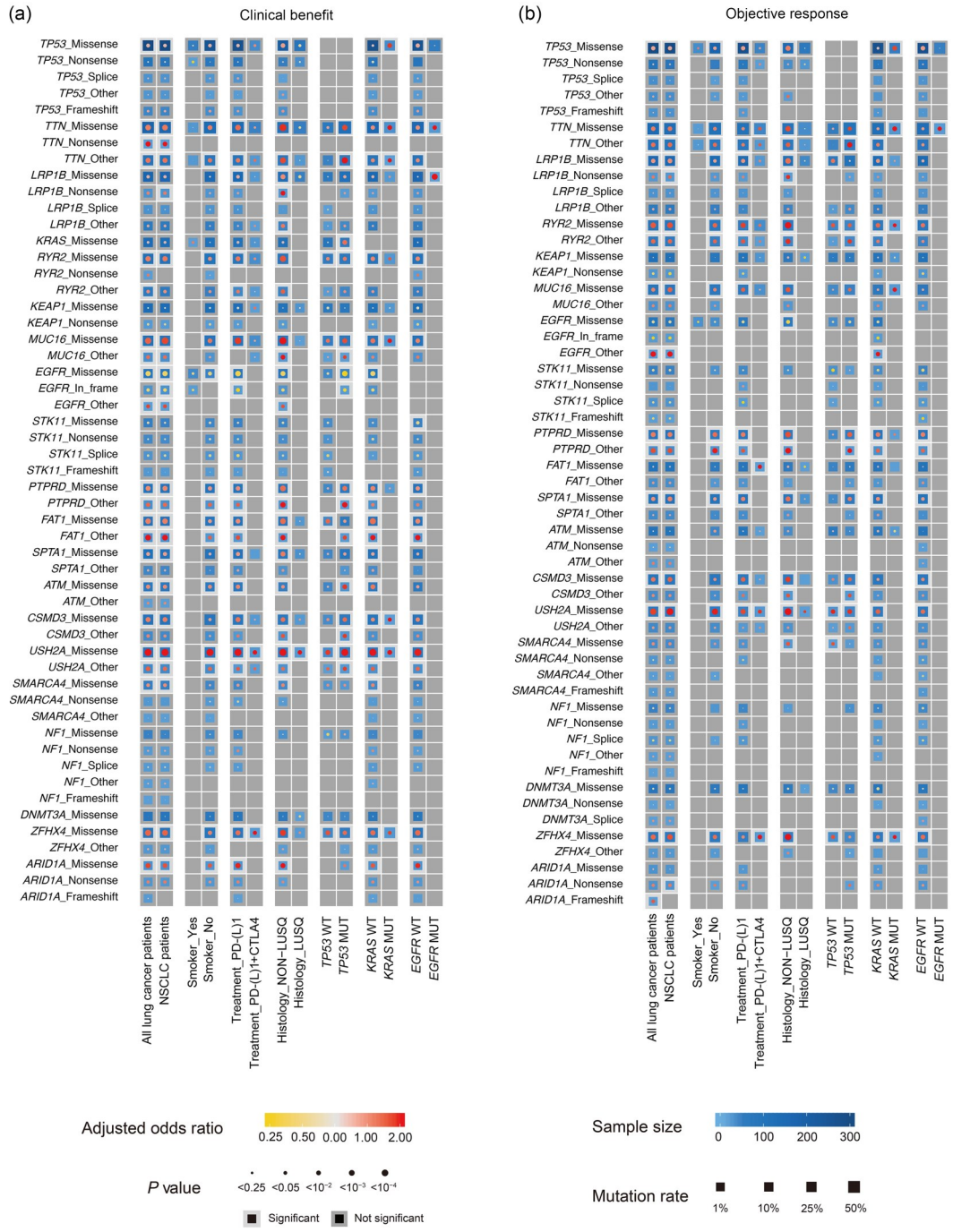

Fig. 1 Summary of gene subtypes with clinical and genomic features associated with clinical benefit (a) and objective response (b) in lung cancer patients treated with immune checkpoint inhibitors after adjustment. The following parameters were determined for all lung cancer and NSCLC patients: smoker (Yes or No), treatment (PD-(L)1 or PD-(L)1+ CTLA-4), histology (NON-LUSQ or LUSQ), *TP53* mutation status (WT or MUT), *KRAS* mutation status (WT or MUT), and *EGFR* mutation status (WT or MUT). The adjusted odds ratios of clinical benefit were described from 0.25 to 2.00 in yellow to red circled at the center of each cell, of which the diameter represents the *P*-value. The sample size was described from 0 to 300 in light to dark blue in the square at each cell, of which the side length represents mutation rate. The statistical significance was shown in the background of each cell, where dark grey represents no significance and light grey represents significance. NSCLC: non-small-cell lung cancer; PD-(L)1: programmed death-(ligand) 1; CTLA-4: cytotoxic T-lymphocyte antigen-4; NON-LUSQ: non-lung squamous cancer; LUSQ: lung squamous cancer; *TP53*: tumor protein P53; WT: wild-type; MUT: mutation; *KRAS*: Kirsten rat sarcoma viral oncogene homolog; *EGFR*: epidermal growth factor receptor.

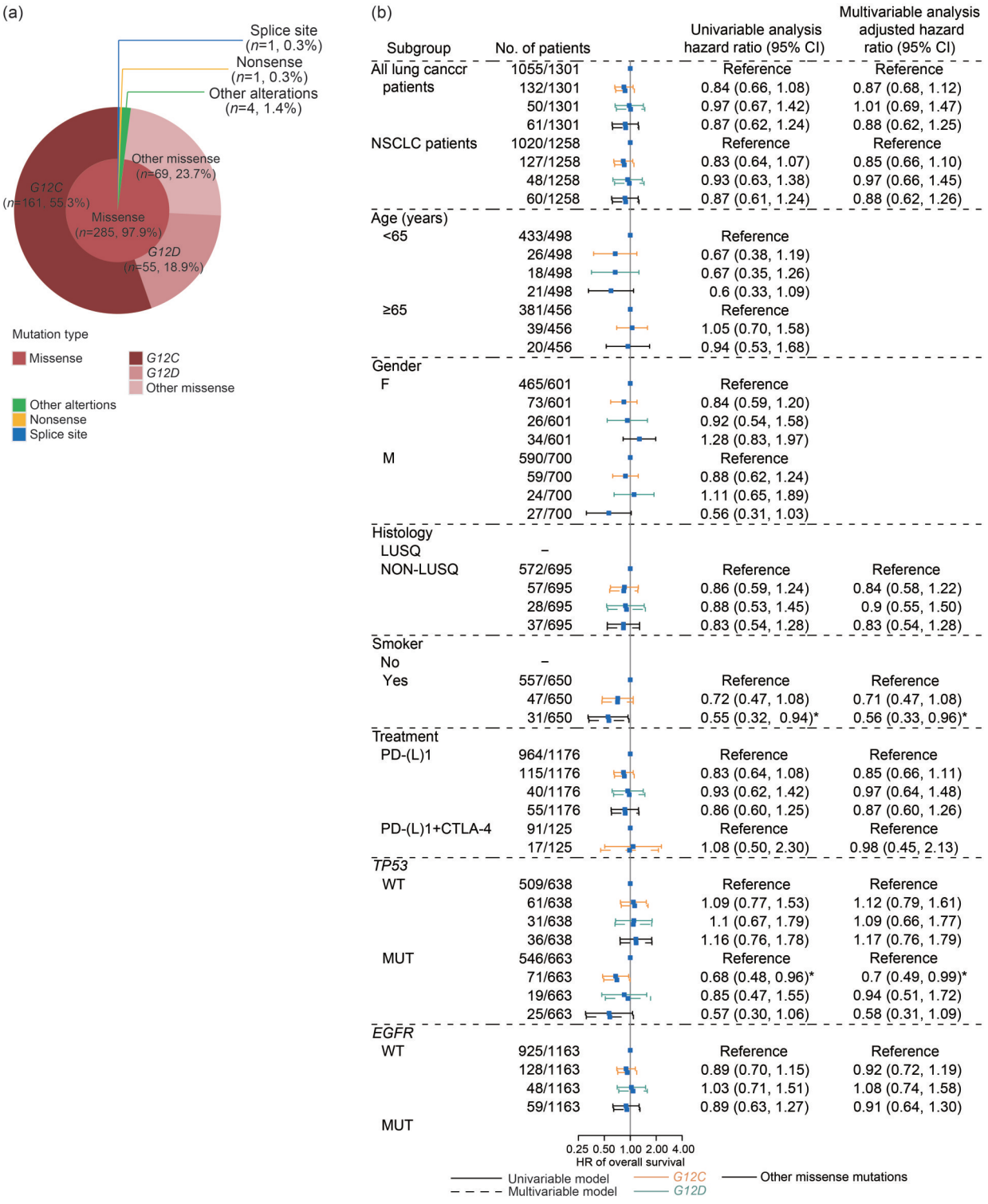

Fig. 2 Pie charts and forest plot of patients with different *KRAS* mutation subtypes. (a) Pie charts of all lung cancer patients with *KRAS* mutations showing the proportions of different *KRAS* mutation subtypes in our cohort based on 14 studies. (b) Forest plot describing the HR of OS in patients classified by *KRAS* mutation subtypes (orange: *G12C*; green: *G12D*; black: other missense), categorized by subgroups. The dotted lines represent 95% CI after adjustment and the solid lines represent unadjusted 95% CI. *KRAS*: Kirsten rat sarcoma viral oncogene homolog; HR: hazard ratio; OS: overall survival; F: female; M: male; LUSQ: lung squamous cancer; NON-LUSQ: non-lung squamous cancer; PD-(L)1: programmed death-(ligand) 1; CTLA-4: cytotoxic T-lymphocyte antigen-4; WT: wild-type; MUT: mutation; CI: confidence interval; NSCLC: non-small-cell lung cancer; *TP53*: tumor protein P53. * P<0.05, compared with reference.

3.3 Negative effects of *EGFR* classical activating mutations on prognosis and response to ICIs

To our knowledge, lung cancer patients with classical *EGFR* mutations ($EGFR^{L858R}$ missense mutation, $EGFR^{L858R}$, and in-frame deletions in exon 19 of *EGFR* ($EGFR^{ex19}$)) showed worse outcomes for PD-(L)1 blockade treatment (Hastings et al., 2019). Thus, we estimated the effect size of *EGFR* classical activating mutations in our cohort to verify the effectiveness of our method and to investigate the potential predictive value of *EGFR* mutation subtypes in immunotherapy.

We first conducted the investigation on individuals harboring *EGFR* classical activating mutations, and found that lung cancer patients with these mutations showed worse clinical and survival outcomes (Figs. 3a–3h) in terms of DCB (multivariable analysis adjusted OR 0.17, 95% CI 0.07–0.44, $P<0.001$; univariable analysis OR 0.18, 95% CI 0.07–0.46, $P<0.001$), ORR ($n=36/38$; multivariable analysis adjusted OR 0.18, 95% CI 0.04–0.76, $P=0.02$; univariable analysis OR 0.18, 95% CI 0.04–0.74, $P=0.02$), OS (multivariable analysis adjusted HR 1.40, 95% CI 1.01–1.95, $P=0.04$; univariable analysis HR 1.32, 95% CI 0.96–1.82, $P=0.09$), and PFS (multivariable analysis adjusted HR 2.08, 95% CI 1.56–2.76, $P<0.001$; univariable analysis HR 1.98, 95% CI 1.49–2.63, $P<0.001$), as supported by a previous report (Cai et al., 2018). The outcomes of NSCLC patients were concordant with those of all lung cancer patients. The forest plots containing detailed data in all stratified groups were shown in Fig. S5.

Next, patients with classical activating mutations were divided by whether they further developed *T790M* missense mutation to evaluate clinical efficacy of ICIs in tyrosine kinase inhibitors (TKIs)-resistant patients (Figs. 3i–3l). T790M is the most commonly acquired resistant mutation, which mainly occurs due to treatment with first-generation or second-generation TKIs for years (Sequist et al., 2011; Yu et al., 2013; Wu and Shih, 2018). From the total of 58 *EGFR* classical activating mutation patients, 17 (29.31%) were combined with *T790M* missense mutation.

The DCB for activating mutation with *T790M* ($n=14/16$; multivariable analysis adjusted OR 1.53, 95% CI 0.21–11.26, $P=0.68$; univariable analysis OR 1.53, 95% CI 0.21–11.26, $P=0.68$) exhibited no difference compared with that for activating mutation without *T790M*. Among all lung cancer patients, non-responders were not more frequently found when activating mutation with *T790M* was present ($n=15/15$; OR 0.00, 95% CI 0.00–3.30, $P=0.51$; Fisher's exact test) compared with activating mutation without *T790M*. For survival outcome, the results showed no significant difference between activating mutation with and without *T790M* in OS (adjusted HR 0.96, 95% CI 0.48–1.94, $P=0.31$, all lung cancer) or PFS (adjusted HR 0.72, 95% CI 0.39–1.35, $P=0.92$, all lung cancer). Taken together, we observed no significant difference in clinical or survival outcomes between *EGFR* activating mutant patients with and without *T790M*, which indicated that patients harboring the *EGFR* classical activating mutation presented similar clinical efficacy of ICIs regardless of acquiring drug resistance to TKIs.

In addition, we examined the categorized effect size of missense mutation inside or outside the tyrosine kinase domain (codon 713–965) in *EGFR*, and all subtypes listed above were integrated into forest plots (Figs. S6 and S7). For most applicable data, patients carrying activating mutations failed to derive significant clinical benefit from ICIs, while no significance was observed among other missense mutations inside or outside the tyrosine kinase domain.

3.4 Association between *USH2A* missense mutation and favorable clinical outcome in ICI-treated lung cancer patients

After systematically estimating genes proposed as classical mutations related to benefits from ICIs, we sought to identify novel biomarkers via pooled analyses. The *USH2A* missense mutation was one of the top 20 most frequently mutated genes among lung cancer patients (114/404, 28.22%). Interestingly, the stratified analysis illustrated that *USH2A* missense mutation strongly correlated with better outcomes (Figs. 1a, 1b, and S2), though there had been no previous report showing the association between *USH2A* and the efficacy of ICIs. Therefore, we questioned whether *USH2A* could serve as a robust biomarker in predicting the clinical efficacy of ICIs.

By applying our method described above, patients harboring *USH2A* missense mutation were included for further analyses (Fig. S8). As shown in Fig. 4, *USH2A* missense mutated patients exhibited significantly higher ORR and rate of DCB and a promising survival outcome among both lung cancer and NSCLC patients. For lung cancer patients with *USH2A* missense

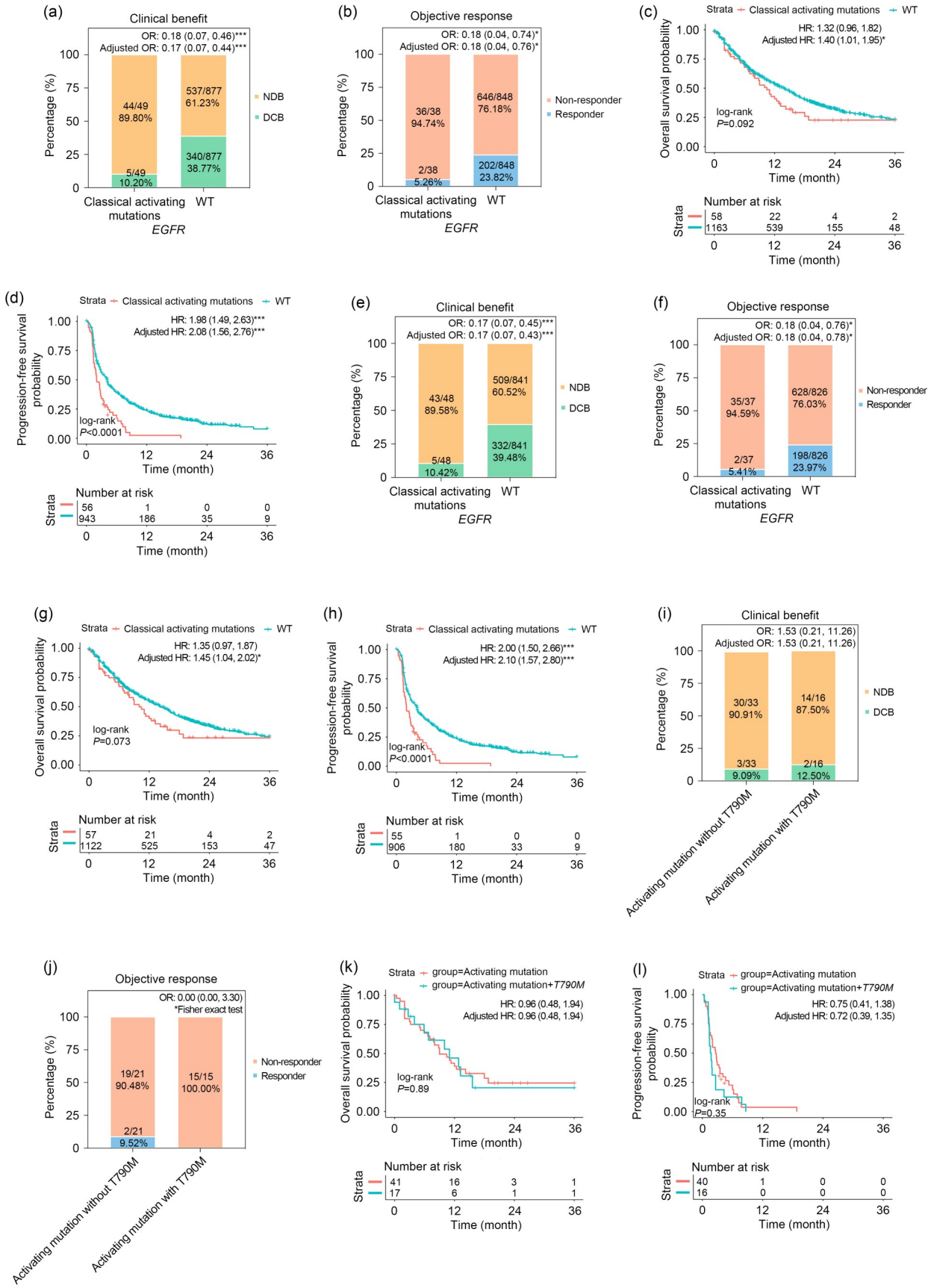
(a)
Clinical benefit
OR: 0.18 (0.07, 0.46)***
Adjusted OR: 0.17 (0.07, 0.44)***
Percentage (%)
44/49
89.80%
5/49
10.20%
537/877
61.23%
340/877
38.77%
NDB
DCB
Classical activating mutations
WT
EGFR
(b)
Objective response
OR: 0.18 (0.04, 0.74)*
Adjusted OR: 0.18 (0.04, 0.76)*
36/38
94.74%
2/38
5.26%
646/848
76.18%
202/848
23.82%
Non-responder
Responder
(c)
Strata Classical activating mutations WT
HR: 1.32 (0.96, 1.82)
Adjusted HR: 1.40 (1.01, 1.95)*
Overall survival probability
log-rank
P=0.092
Time (month)
Number at risk
58 22 4 2
1163 539 155 48
(d)
Strata Classical activating mutations WT
HR: 1.98 (1.49, 2.63)***
Adjusted HR: 2.08 (1.56, 2.76)***
Progression-free survival probability
log-rank
P<0.0001
Number at risk
56 1 0 0
943 186 35 9
(e)
Clinical benefit
OR: 0.17 (0.07, 0.45)***
Adjusted OR: 0.17 (0.07, 0.43)***
43/48
89.58%
5/48
10.42%
509/841
60.52%
332/841
39.48%
(f)
Objective response
OR: 0.18 (0.04, 0.76)*
Adjusted OR: 0.18 (0.04, 0.78)*
35/37
94.59%
2/37
5.41%
628/826
76.03%
198/826
23.97%
(g)
Strata Classical activating mutations WT
HR: 1.35 (0.97, 1.87)
Adjusted HR: 1.45 (1.04, 2.02)*
Overall survival probability
log-rank
P=0.073
Number at risk
57 21 4 2
1122 525 153 47
(h)
Strata Classical activating mutations WT
HR: 2.00 (1.50, 2.66)***
Adjusted HR: 2.10 (1.57, 2.80)***
Progression-free survival probability
log-rank
P<0.0001
Number at risk
55 1 0 0
906 180 33 9
(i)
Clinical benefit
OR: 1.53 (0.21, 11.26)
Adjusted OR: 1.53 (0.21, 11.26)
30/33
90.91%
3/33
9.09%
14/16
87.50%
2/16
12.50%
Activating mutation without T790M
Activating mutation with T790M
(j)
Objective response
OR: 0.00 (0.00, 3.30)
*Fisher exact test
19/21
90.48%
2/21
9.52%
15/15
100.00%
(k)
Strata group=Activating mutation
group=Activating mutation+T790M
HR: 0.96 (0.48, 1.94)
Adjusted HR: 0.96 (0.48, 1.94)
Overall survival probability
log-rank
P=0.89
Number at risk
41 16 3 1
17 6 1 1
(l)
Strata group=Activating mutation
group=Activating mutation+T790M
HR: 0.75 (0.41, 1.38)
Adjusted HR: 0.72 (0.39, 1.35)
Progression-free survival probability
log-rank
P=0.35
Number at risk
40 1 0 0
16 0 0 0

Fig. 3 Clinical benefit, ORR, OS, and PFS for *EGFR* classical activating mutations compared with *EGFR* wild-type in all lung cancer or NSCLC patients treated with ICIs. (a) Clinical benefit rate in all lung cancer patients with *EGFR* classical activating mutations and *EGFR* wild-type patients. The overall rate and proportion were indicated on each bar in black. The statistics were calculated applying univariate and multivariate logistic regression analyses. (b) ORR in all lung cancer patients with *EGFR* classical activating mutation and *EGFR* wild-type patients. (c) OS in all lung cancer patients with *EGFR* classical activating mutation (*n*=58) compared with *EGFR* wild-type patients (*n*=1163). The statistics were calculated applying univariate and multivariate Cox's proportional hazard regression analyses. (d) PFS in all lung cancer patients with *EGFR* classical activating mutations (*n*=56) compared with *EGFR* wild-type patients (*n*=943). (e) Clinical benefit rate in NSCLC patients with *EGFR* classical activating mutations and *EGFR* wild-type patients. (f) ORR in NSCLC patients with *EGFR* classical activating mutation and *EGFR* wild-type patients. (g) OS in NSCLC patients with *EGFR* classical activating mutation (*n*=57) compared with wild-type patients (*n*=1122). (h) PFS in NSCLC patients with *EGFR* classical activating mutation (*n*=55) compared with *EGFR* wild-type patients (*n*=906). (i) Clinical benefit rate in all lung cancer patients with *EGFR* classical activating mutation without *T790M* and *EGFR* classical activating mutation with *T790M*. The overall rate and proportion were indicated on each bar in black. (j) ORR in all lung cancer patients with *EGFR* classical activating mutation without *T790M* and *EGFR* classical activating mutation with *T790M* patients, as shown by Fisher's exact test. (k) OS in all lung cancer patients with *EGFR* classical activating mutation with *T790M* (*n*=17) compared with patients harboring *EGFR* classical activating mutation without *T790M* (*n*=41). (l) PFS in all lung cancer patients with *EGFR* classical activating mutation with *T790M* (*n*=16) compared with patients harboring *EGFR* classical activating mutation without *T790M* (*n*=40). ORR: objective response rate; OS: overall survival; PFS: progression-free survival; *EGFR*: epidermal growth factor receptor; NSCLC: non-small-cell lung cancer; ICIs: immune checkpoint inhibitors; DCB: durable clinical benefit; NDB: no durable benefit; WT: wild-type; OR: odds ratio; HR: hazard ratio. * *P*<0.05; *** *P*<0.001.

mutation (Figs. 4a–4d), the rate of DCB reached 70% (71.76% in all lung cancer patients; adjusted OR 4.74, 95% CI 2.75–8.17, $P<0.001$) and the ORR exceeded 50% (51.56% in all lung cancer patients; adjusted OR 3.45, 95% CI 1.88–6.33, $P<0.001$). Following clinical benefit and objective response, patients harboring the *USH2A* missense mutation had significantly prolonged OS (adjusted HR, 0.52; 95% CI, 0.32–0.82; $P=0.006$, all lung cancer) and PFS (adjusted HR, 0.51; 95% CI, 0.38–0.69; $P<0.001$, all lung cancer) compared with *USH2A* wild-type patients. The results were in parallel with NSCLC patients (Figs. 4e–4h). These findings supported the predictive utility of *USH2A* in ICI-treated lung cancer patients.

4 Discussion

The development of ICIs has revolutionized the therapeutic landscape in many solid malignancies, including lung cancer. Nonetheless, those who can acquire benefits constitute only a small proportion of patients (Yarchoan et al., 2017), emphasizing the urgent need for biomarkers to predict the sensitivity and resistance of ICIs. Driven by the advances in high-throughput sequencing methods, clinical scientists began to perform genomic sequencing to elucidate the genomic features associated with ICI efficacy. Nevertheless, the inclusion of small patient cohorts restricted the power of this technology to identify novel biomarkers and even led to debatable conclusions (Fang et al., 2019; Jeanson et al., 2019; Mazieres et al., 2019; Gao et al., 2020). This study is the first to perform individual-level pooled analyses to comprehensively characterize the predictive values of commonly mutated genes in ICI-treated lung cancer patients.

Firstly, we focused on the roles of two well-established driver mutations, *KRAS* and *EGFR*, in ICI-treated lung cancer patients. The roles of different *KRAS* mutation subtypes in immunotherapy are still under debate (Wu and Shih, 2018; Gao et al., 2020; Liu et al., 2020). Therefore, we performed stratified analyses and found that patients harboring $KRAS^{G12C}$-*TP53* showed favorable clinical and survival outcomes. Previous studies based on published datasets suggested that *TP53*-*KRAS* co-mutated patients exhibited inflamed tumor microenvironment (TME) characteristics, including elevated expression of PD-L1 and accumulation of cluster of differentiation 8-positive ($CD8^{+}$) T cells (Dong et al., 2017). Further in silico analysis showed that the alterations of *TP53* and *KRAS* were associated with deficient mismatch repair or DNA damage repair, leading to increased tumor mutational burden (TMB) and immunogenicity (Dong et al., 2017; Canon et al., 2019). Therefore, combined treatment with ICIs and $KRAS^{G12C}$ inhibitor might provide new opportunities for $KRAS^{G12C}$-*TP53* co-mutated patients. Patients with *EGFR* classical activating mutations, including $EGFR^{A19}$ and $EGFR^{L858R}$, have been consistently reported to rarely derive benefits from ICIs (Gainor et al., 2016; Cai et al., 2018;

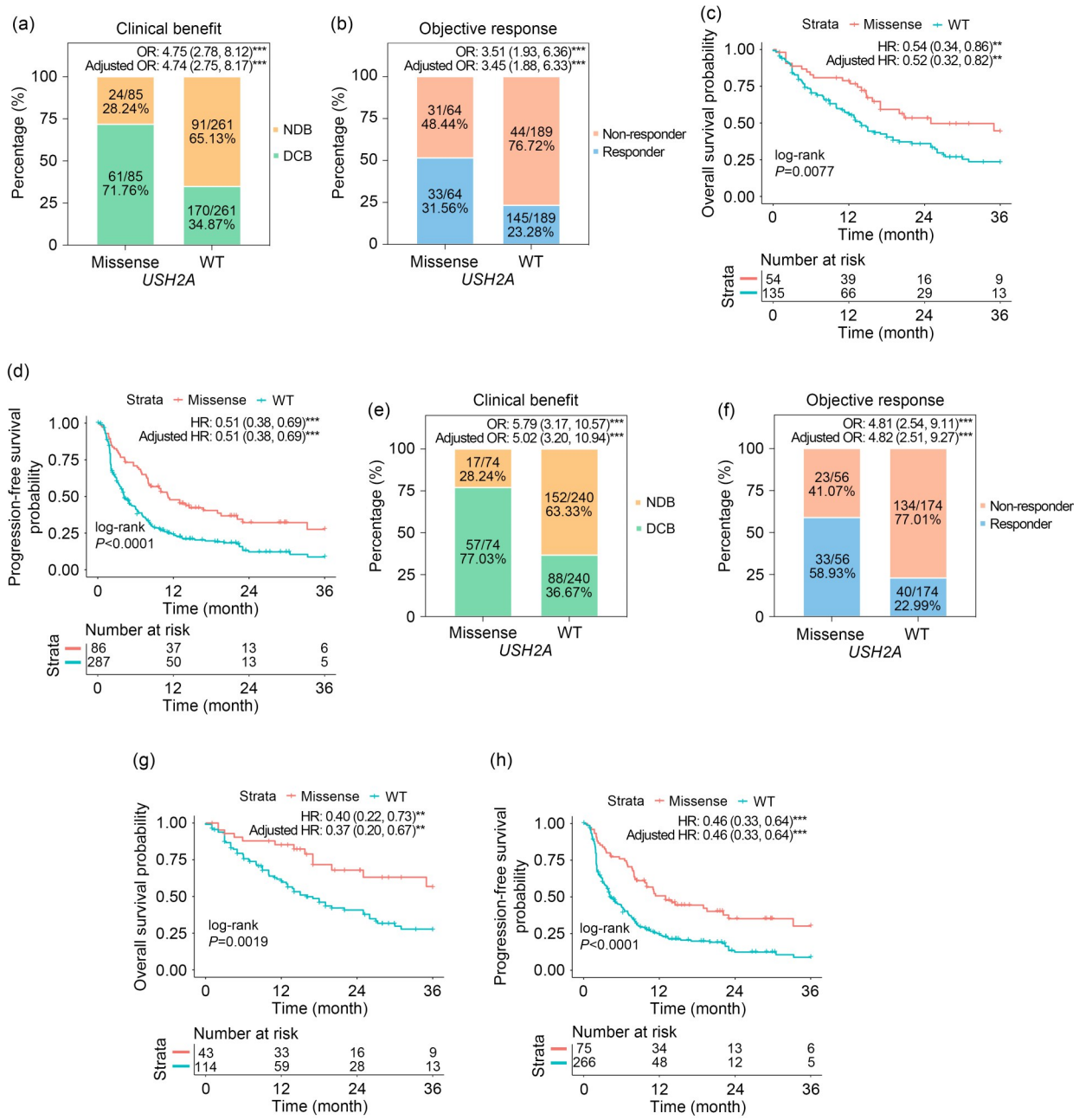

Fig. 4 Clinical benefit, ORR, OS, and PFS of all lung cancer and NSCLC patients with *USH2A* missense mutation and *USH2A* wild-type to ICIs. (a) Clinical benefit rate in all lung cancer patients with *USH2A* missense mutation and wild-type patients. The overall rate and proportion were indicated on each bar in black. The statistics were calculated applying univariate and multivariate logistic regression analyses. (b) ORR in all lung cancer patients with *USH2A* missense mutation and wild-type patients. (c) OS in all lung cancer patients with *USH2A* missense mutation (*n*=54) compared with wild-type patients (*n*=135). The statistics were calculated by univariate and multivariate Cox's proportional hazard regression analyses. (d) PFS in all lung cancer patients with *USH2A* missense mutation (*n*=86) compared with wild-type patients (*n*=287). (e) Clinical benefit rate among NSCLC patients with *USH2A* missense mutation and wild-type patients. (f) ORR among NSCLC patients with *USH2A* missense mutation and *USH2A* wild-type patients. (g) OS in NSCLC with *USH2A* missense mutation (*n*=43) compared with wild-type patients (*n*=114). (h) PFS in NSCLC with *USH2A* missense mutation (*n*=75) compared with wild-type patients (*n*=266). ORR: objective response rate; OS: overall survival; PFS: progression-free survival; *USH2A*: Usher syndrome type-2A; NSCLC: non-small-cell lung cancer; ICIs: immune checkpoint inhibitors; DCB: durable clinical benefit; NDB: no durable benefit; WT: wild-type; OR: odds ratio; HR: hazard ratio. ** *P*<0.01; * *P*<0.001.**

Kumagai et al., 2021), which is consistent with our results. Tumor cells with *EGFR* activating mutations commonly induced immunosuppressive TME (Peng et al., 2015; Sugiyama et al., 2020; Cho et al., 2021). The non-inflamed TME established by aberrant EGFR signaling resulted in resistance to ICIs, which was also observed in $EGFR^{T790M}$ mutant tumors (Hata et al., 2017). Thus, to investigate the treatment potential of ICIs in *EGFR*-mutated patients, it is necessary to target the immunosuppressive pathway caused by dysregulated EGFR signaling.

Importantly, we firstly identified the predictive value of *USH2A* missense mutations in immunotherapy. Usherin protein, encoded by *USH2A*, is a transmembrane protein, whose extracellular domain occupies about 97% of the whole protein (Weston et al., 2000; Toualbi et al., 2020). Previous studies have revealed that the dysfunction of Usherin caused by alterations of *USH2A* was associated with retinitis pigmentosa (Rivolta et al., 2002) and *USH2A* (Eudy et al., 1998). In lung adenocarcinoma, the *USH2A* mutation is also among the most frequently mutated genes to predict neoantigens (Cai et al., 2018). Sun et al. (2021) illustrated that *USH2A* mutant tumors exhibited higher TMB and immune cell infiltration into tumor tissues with stronger immunogenicity, as well as the excessive expression of immune checkpoint genes, including *PD-1*, *PD-L1*, and *CTLA-4* in colon adenocarcinoma. Moreover, *USH2A* has been studied as a top mutated gene with predicted human leukocyte antigen-DR β1 (HLA-DRB1)-binding neo-peptides at the RNA level in lung adenocarcinoma patients, suggesting that *USH2A* may play a role in identifying $CD4^+$ T cell epitopes for immune surveillance. Here, it was speculated that the mutated *USH2A* could serve to derive a neoantigen peptide that recruits immune cells and elicits a subsequent anti-tumor immune response. Detecting mutations of *USH2A* and other critical genes with next-generation sequencing is simple enough, and their relevance for predicting clinical outcomes in response to ICIs is known. Compared to relatively discordant results from different methods and platforms to reveal the expression of PD-L1 and TMB (Tsao et al., 2018; Addeo et al., 2019), the combination of *USH2A* and genes listed above may be a better choice as biomarkers for clinical decisions on ICI therapy.

Taken together, to our current knowledge, this study is the largest and most comprehensive pooled individual-level analyses in lung cancer so far, providing evidence for the potential predictive value of two common driver mutations in lung cancer. More importantly, we found that patients harboring *USH2A* missense mutations could derive promising clinical and survival benefits from ICIs. Thus, our work might provide valuable clues for precision cancer immunotherapy.

However, this study has several limitations. Firstly, more than 70% of the included samples were sequenced by targeted gene panels, which only covered 300–500 genes. Compared with WES, the restricted detection of genomic variations lowered the statistical power to screen novel biomarkers. Secondly, the clinical and survival outcomes of some included patients are not available, which might result in some potential bias. Thirdly, most of the available genomic profiling is in mutation annotation format (MAF), which can only provide relatively limited information on genomic variations. Ideally, it would be promising to obtain the raw genomic data of these included cohorts in FASTQ or BAM format, from which we could possibly infer copy number variations (Talevich et al., 2016), HLA class I haplotypes (Szolek et al., 2014), or tumor-filtrating T cell fractions (Bentham et al., 2021) for each sample. Integrating these features into the current study might provide new insights into precision cancer immunology.

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Author contributions

Yihua WU and Dajing XIA contributed to the conception and design of the study. Yihua WU, Dexin YANG, and Yuqin FENG developed the workflow and methodology. Dexin YANG, Yuqin FENG, Haohua LU, Kelie CHEN, Jinming XU, Peiwei LI, and Tianru WANG contributed to data collection, data analysis, and interpretation. Yuqin FENG, Dexin YANG, Yihua WU, and Dajing XIA contributed to writing and review of the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Dexin YANG, Yuqin FENG, Haohua LU, Kelie CHEN, Jinming XU, Peiwei LI, Tianru WANG, Dajing XIA, and Yihua WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Figs. S1–S8; Tables S1–S8