# Association of EPHA3 mutations with tumor mutational burden and efficacy of immune checkpoint inhibitors in cancer patients

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# Abstract

Background: The erythropoietin-producing human hepatocellular receptor A3 *(EPHA3*), which was identified as a tumor antigen targeted by a lytic T-cell response in melanoma patients, is relatively tumor specific. Moreover, *EPHA3* is frequently mutated in human cancers, and the association of *EPHA3* mutations with immunotherapy efficacy in cancer patients has not been reported to date.

Methods: We conducted a retrospective analysis of 1756 patients with 11 cancer types after immune checkpoint inhibitor (ICI) monotherapy identified from The Cancer Genome Atlas (TCGA) data portal in April 2021. The associations between *EPHA3* mutations and tumor mutational burden (TMB), durable clinical benefit (DCB), and overall survival (OS) was assessed.

Results: Among all 1756 patients, the mutational frequency of *EPHA3* was 6.7%. The TMB of patients with *EPHA3* mutations was substantially higher than that in patients without the mutations (odds ratio [OR], 5.35, 95% confidence interval [CI], 3.58–8.04; $P < .001$). *EPHA3* mutations were significantly associated with better DCB (median: 52.3% vs 32.8%; OR, 2.24; 95%CI, 1.15–4.41; $P = .011$) and OS (median: 40.0 vs 16.0 months; hazard ratio, 0.57; 95% CI, 0.42–0.77; $P < .001$) compared with *EPHA3* wild-type. Multivariable and subgroup analyses showed that these associations were independent of all known covariates, including TMB.

Conclusions: These findings indicate that *EPHA3* mutations may be associated with higher TMB, better immune response, and better survival than *EPHA3* wild-type. Despite the need for validation, cancer patients with *EPHA3* mutations might be appropriate candidates for ICI monotherapy.


# Background

Immune checkpoint inhibitors (ICIs) are changing the standard of care for many cancer types. Positive predictive markers for ICI treatment include microsatellite instability, elevated tumor mutational burden (TMB), and programmed cell death ligand 1 (PD-L1) overexpression [1]. Recent studies have identified a number of gene mutations as possible ICI predictive markers, including *KRAS* [2], *LRP1B* [3]*, POLE,* and *POLD1* [4]

The erythropoietin-producing human hepatocellular (EPH) receptor tyrosine kinases control cell to cell adhesion, and contribute to cell migration and axon guidance during development and homeostasis [5, 6]. *EPHA3,* which encodes EPH receptor A3, is highly expressed in some types of cancer cells, but is almost absent in normal adult tissues, making it relatively tumor specific [7, 8]. Although its role in cancers is largely unknown, *EPHA3* was identified as a tumor antigen targeted by a lytic T-cell response in melanoma patients [9], suggesting a potential role in immunotherapy. *EPHA3* is frequently mutated in human cancers, including lung adenocarcinoma, melanoma, and colorectal carcinoma [10-13]. To our knowledge, the relationship between *EPHA3* mutations and immunotherapy outcomes has not yet been reported. Here, we investigated whether *EPHA3* mutations are associated with TMB and clinical outcomes in cancer patients after ICI monotherapy.

## Methods

# Patient selection

The somatic mutational and clinical data from The Cancer Genome Atlas (TCGA) datasets were downloaded using cBioPortal [6]. Patients treated with ICI monotherapy (atezolizumab, avelumab, durvalumab, ipilimumab, nivolumab, pembrolizumab, or tremelimumab) and tumor tissue sequenced by whole genome, whole exome, or a target gene panel including the *EPHA3* gene were included. Patients were excluded if they were treated with ICI combination therapy (single ICI combined with chemotherapy, targeted therapy, or another ICI) or if treatment data was missing. This study was deemed exempt from institutional board approval and patient informed consent was waived due to the retrospective nature of the study and use of anonymized data.

# TMB classification

TMB was converted into binary variables to minimize the influence of cancer types, sequencing scope (target gene panel, whole-exome, or whole-genome), and study conditions on the analyses regarding TMB. Briefly, subsets were defined as groups of samples of the same cancer type with the same dataset sequencing scope. In each subset, the TMB score was directly used, if provided; otherwise, it was defined as the total number of nonsynonymous alterations [8, 9]. Subsets were excluded if they comprised fewer than 10 samples. TMB in the subsets was classified by percentile: high (top 20%) and non-high TMB (bottom 80%) [1].

# Statistical analyses

The primary endpoints were TMB, durable clinical benefit (DCB; complete, partial, or stable response for > 6 months), and overall survival (OS). Between-group comparisons were conducted using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. The association of *EPHA3* mutations with TMB and DCB were assessed using logistic regression models; the results were presented as odds ratios (ORs) with 95% confidence intervals (CIs), using *EPHA3* wild-type as the reference. The OS rates were compared using log-rank tests and plotted using the Kaplan–Meier method. The association between *EPHA3* mutations and OS was assessed using Cox proportional hazards regression models; the results were presented as hazard ratios (HRs) with 95% CIs, using *EPHA3* wild-type as the reference. The multivariate analyses of the associations between *EPHA3* mutations and TMB incorporated age, sex, cancer type, line of treatment, sample type, sequencing scope, and tumor purity. TMB was incorporated in the multivariate analyses of associations between *EPHA3* mutations and DCB and OS. Subgroup analyses were conducted to evaluate whether the associations between *EPHA3* mutations and TMB, DCB, and OS were dependent on these covariates. To conduct a subgroup analysis, at least 10 patients with *EPHA3* mutations were required. Two-sided $P <$

0.05 was considered statistically significant. Analyses were performed using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

# Results

Eleven datasets (Additional file 1) including 1756 ICI monotherapy patients involving 11 cancer types were identified. The selection flowchart is shown in Additional file 2. The clinical characteristics of the patients according to *EPHA3* status are summarized in Table 1. The main cancer types included melanoma (24.0%, n = 421) and non-small cell lung cancer (NSCLC, 22.7%, n = 398). All patients had locally advanced stage, recurrent or metastatic disease, and received palliative therapy. Among 1756 patients, the mutational frequency of *EPHA3* was 6.7%, and the most *EPHA3* mutations were found in patients with melanoma (10.0%) and NSCLC (10.3%).

TMB was substantially higher in patients with compared to patients without *EPHA3* mutations (Fig. 1A, Table 2; OR, 5.35; 95%CI, 3.58–8.04; $P < .001$). The *EPHA3* mutations were significantly associated with better DCB (Fig. 1B, Table 2; median: 52.3% vs 32.8%; OR, 2.24; 95%CI, 1.15–4.41; $P = .011$) and OS (Fig. 1C, Table 2; median: 40.0 [95%CI, 26.7–not reached] vs 16.0 [95%CI, 14.4–18.0] months; HR, 0.57; 95%CI, 0.42–0.77; $P < .001$) compared to *EPHA3* wild-type. The associations of *EPHA3* mutations and DCB and OS remained statistically significant in the multivariable analyses after controlling for all known covariates, including TMB (Table 2). In the subgroup analysis, all ORs showed that patients with *EPHA3* mutations had a higher TMB, and all ORs for DCB and HRs for OS favored *EPHA3* mutations except for the urinary tract OS subgroup (Fig. 2).

We investigated whether the association between *EPHA3* mutations and clinical outcomes might be attributable to general prognostic benefits of *EPHA3* mutations, unrelated to ICI monotherapy. In all 11 datasets included in the analysis, we identified the DCB of 166 patients who received ICI combination therapy and of 273 patients not treated with ICIs, there was no association between *EPHA3* mutations and DCB, respectively (Fig. 3). We identified the OS of 306 patients treated with ICI combination therapy, and found no association between *EPHA3* mutations and OS (Fig. 3).

# Discussion

To our knowledge, this is the first study to investigate the association of *EPHA3* mutations and immunotherapy outcomes in cancer patients. *EPHA3* mutations were found to be associated with higher TMB and better DCB and OS compared with *EPHA3* wild-type. This association was independent of age, sex, cancer type, line of treatment, sample type, sequencing scope, and tumor purity.

It is well known that higher TMB is associated with better responses to ICI treatment in cancer patients [1]. To determine if these responses were primarily attributable to differences in TMB, we conducted multivariable and subgroup analyses according to TMB. The magnitude of the OS benefit associated with *EPHA3* mutations decreased but was still significant after adjustments by TMB. Moreover, subgroup

analyses showed OS benefits regardless of TMB. Similar results were indicated regarding DCB. These findings suggested that *EPHA3* mutations were likely to be clinically meaningful, and underscored the robustness of *EPHA3* mutations as a predictive biomarker of ICIs.

This study had several limitations. First, it was a retrospective analysis with potential selection bias. Second, different *EPHA3* mutation subtypes were not compared due to the small number of patients per subgroup. Third, PD-L1 influence on the associations between *EPHA3* mutations and TMB, DCB, and OS were not assessed because PD-L1 expression was only known in 108 of 1756 (6.2%, Table 1) patients included in the analysis, and the statistical power was insufficient for reasonable analysis. Finally, we did not assess the regulation of immune system signaling pathways, antigen processing and presentation, and cell cycle checkpoints, which may be related to immunotherapy responses.

## Conclusion

This study showed that *EPHA3* mutations were associated with higher TMB and better clinical efficacy in terms of DCB and OS in patients treated with ICI monotherapy compared to patients with *EPHA3* wild-type. Mechanistic studies into the function of *EPHA3* mutations are needed, as well as prospective validation of its predictive role in ICI responses.

## Abbreviations

CI, confidence interval; DCB, durable clinical benefit; EPH, erythropoietin-producing human hepatocellular; HR, hazard ratio; ICI, immune checkpoint inhibitor; OR, odds ratio; OS, overall survival; PD-L1, programmed cell death ligand 1; TCGA, The Cancer Genome Atlas; TMB, tumor mutational burden.

## Declarations

# Ethics approval and consent to participate

Not applicable.

# Consent for publication

Not applicable.

# Availability of data and materials

The datasets used during the current study are available from cBioPortal at https://www.cbioportal.org/datasets (Additional file 1).

# Competing interests

The authors declare that they have no competing interests.

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# Authors' contributions

Siqi Zhang, Mengge Zheng, Deheng Nie, and Fujun Han wrote the manuscript; Huimin Tian and Wenjia Liu collected related literature and performed the data analysis; Fengli Pei, Wenhui Liu, helped in drafting figures; Fujun Han provided guidance and revised the manuscript. All authors read and approved the final manuscript.

# Acknowledgements

Not applicable.

# Authors' information

Not applicable.

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# Tables

Table 1

Patient characteristics by *EPHA3* status.

| Characteristic | Total patients (n=1756) | | *EPHA3*wt (n=1639) | | *EPHA3*m (n=117) | | *P* value |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Age (years) | | | | | | | .061 [a] |
| < 65 | 896 | 50.0 | 848 | 51.7 | 48 | 41.0 | |
| ≥ 65 | 748 | 42.6 | 689 | 42.0 | 59 | 50.4 | |
| Unknown | 112 | 6.4 | 102 | 6.2 | 10 | 8.5 | |
| Median (range) | 63 (15-92) | | 63 (15-92) | | 67 (25-87) | | .012 [a] |
| Sex | | | | | | | .115 |
| Male | 1087 | 61.9 | 1023 | 62.4 | 64 | 54.7 | |
| Female | 669 | 38.1 | 616 | 37.6 | 53 | 45.3 | |
| Cancer type | | | | | | | < .001 |
| Melanoma | 421 | 24.0 | 379 | 23.1 | 42 | 35.9 | |
| NSCLC | 398 | 22.7 | 357 | 21.8 | 41 | 35.0 | |
| Urinary tract | 214 | 12.2 | 202 | 12.3 | 12 | 10.3 | |
| Renal cell carcinoma | 152 | 8.7 | 152 | 9.3 | 0 | 0 | |
| Head and neck | 126 | 7.2 | 123 | 7.5 | 3 | 2.6 | |
| Brain glioma | 116 | 6.6 | 113 | 6.9 | 3 | 2.6 | |
| Colorectal | 97 | 5.5 | 93 | 5.7 | 4 | 3.4 | |
| Esophagogastric | 90 | 5.1 | 87 | 5.3 | 3 | 2.6 | |
| Breast | 37 | 2.1 | 34 | 2.1 | 3 | 2.6 | |
| Hepatocellular carcinoma | 27 | 1.5 | 26 | 1.6 | 1 | 0.9 | |
| Unknown primary | 78 | 4.4 | 73 | 4.5 | 5 | 4.3 | |
| Line of treatment | | | | | | | .021 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 343 | 19.5 | 312 | 19.0 | 31 | 26.5 | |
| ≥ 2 | 253 | 14.4 | 231 | 14.1 | 22 | 18.8 | |
| Unknown | 1160 | 66.1 | 1096 | 66.9 | 64 | 54.7 | |
| Sample type | | | | | | | .001 |
| Primary | 561 | 31.9 | 538 | 32.8 | 23 | 19.7 | |
| Metastatic | 633 | 36.0 | 592 | 36.1 | 41 | 35.0 | |
| Unknown | 562 | 32.0 | 509 | 31.1 | 53 | 45.3 | |
| Sequencing | | | | | | | .152 |
| Whole-exome or genome | 355 | 20.2 | 325 | 19.8 | 30 | 25.6 | |
| Target gene panel | 1401 | 79.8 | 1314 | 80.2 | 87 | 74.4 | |
| Tumor purity | | | | | | | .204 |
| <50% | 592 | 33.7 | 561 | 34.2 | 31 | 26.5 | |
| ≥50% | 458 | 26.1 | 425 | 25.9 | 33 | 28.2 | |
| Unknown | 706 | 40.2 | 653 | 39.8 | 53 | 45.3 | |
| TMB | | | | | | | <.001 |
| Bottom 80% | 1361 | 77.5 | 1311 | 80.0 | 50 | 42.7 | |
| Top 20% | 395 | 22.5 | 328 | 20.0 | 67 | 57.3 | |
| PD-L1 (%) | | | | | | | .617 [a] |
| < 1 | 60 | 3.4 | 55 | 3.4 | 5 | 4.3 | |
| ≥ 1 | 48 | 2.7 | 44 | 2.7 | 4 | 3.4 | |
| Unknown | 1648 | 93.8 | 1540 | 94.0 | 108 | 92.3 | |

[a]: Patients with unknown characteristics was not included in the comparison.

Abbreviation: m, mutations; wt, wild-type.

Table 2

Multivariable regression models for associations between *EPHA3* status and TMB and immunotherapy outcomes

| | TMB | | OS | | DCB | |
|---|---|---|---|---|---|---|
| | OR(95% CI) | *P* value | HR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Unadjusted | 5.35 (3.58-8.04) | <.001 | 0.57 (0.42-0.77) | <.001 | 2.24 (1.15-4.41) | .011 |
| Adjusted by other covariables [a] | 5.86 (4.19-8.19) | <.001 | 0.57 (0.42-0.77) | <.001 | 2.70 (1.56-4.67) | .003 |
| Adjusted by TMB | N.A. | N.A. | 0.68 (0.50-0.92) | .013 | 1.60 (0.92-2.79) | .164 |
| Adjusted by TMB and other covariables [a] | N.A. | N.A. | 0.67 (0.49-0.92) | .012 | 1.99 (1.11-3.57) | .052 |

The top 20th percentile was used as the cutoff to dichotomize tumor mutational burden (TMB) into high TMB and non-high TMB. The association between *EPHA3* status and TMB and durable clinical benefit (DCB) was assessed using logistic regression models. The results were presented as odds ratios (ORs) with 95% confidence intervals (CIs) using *EPHA3* wild-type as the reference. The association between *EPHA3* status and overall survival (OS) was assessed using Cox proportional hazards regression models. The results were presented as hazard ratios (HRs) with 95% CIs using *EPHA3* wild-type as the reference.

[a]: age, sex, cancer type, line of treatment, sample type, sequencing scope, and tumor purity.

Abbreviations: N.A., not applicable.

# Figures

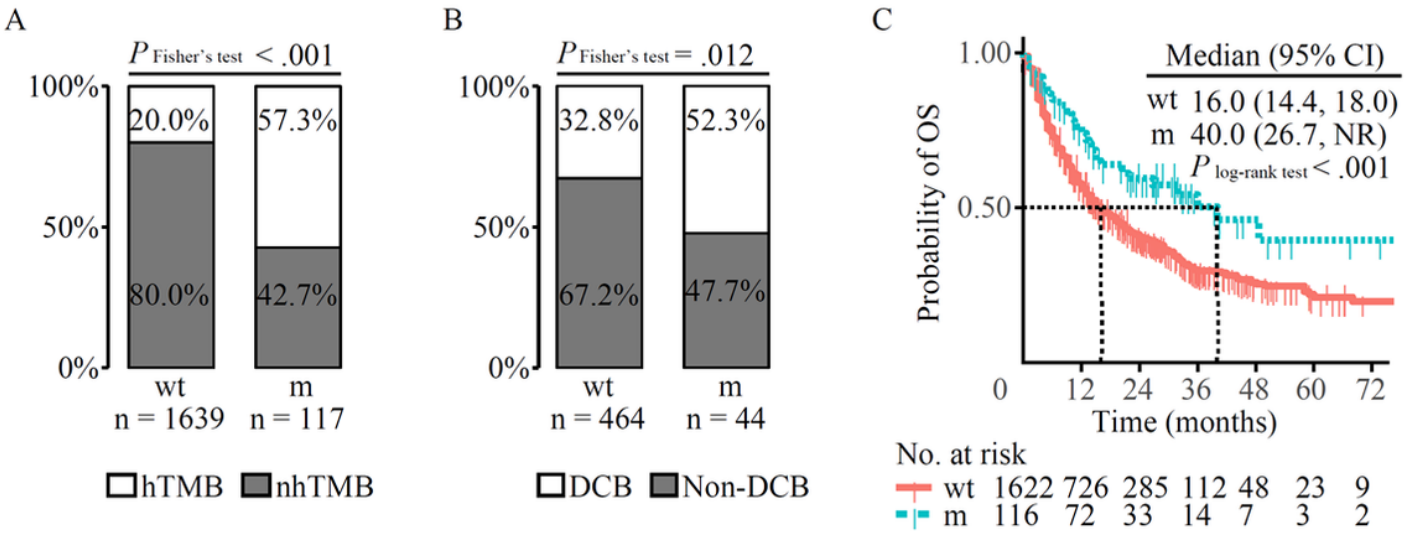
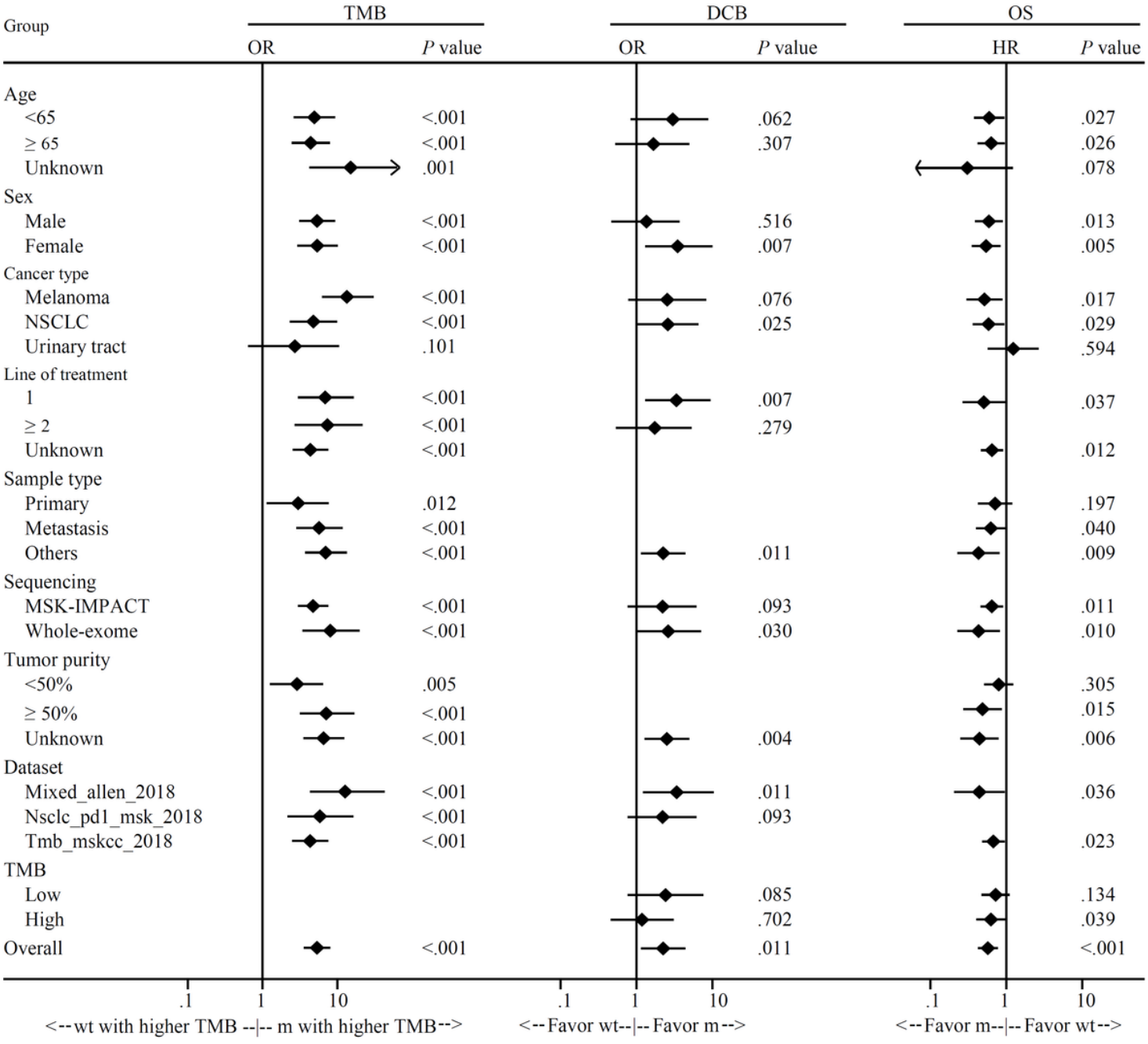



**Figure 1**

Association of *EPHA3* mutation with TMB and outcomes in patients after ICI monotherapy.

Tumor mutational burden (TMB) was classified by percentile into high TMB (top 20%) and non-high TMB (bottom 80%).

Abbreviations: NR, not reached; m, mutations; wt, wild-type.

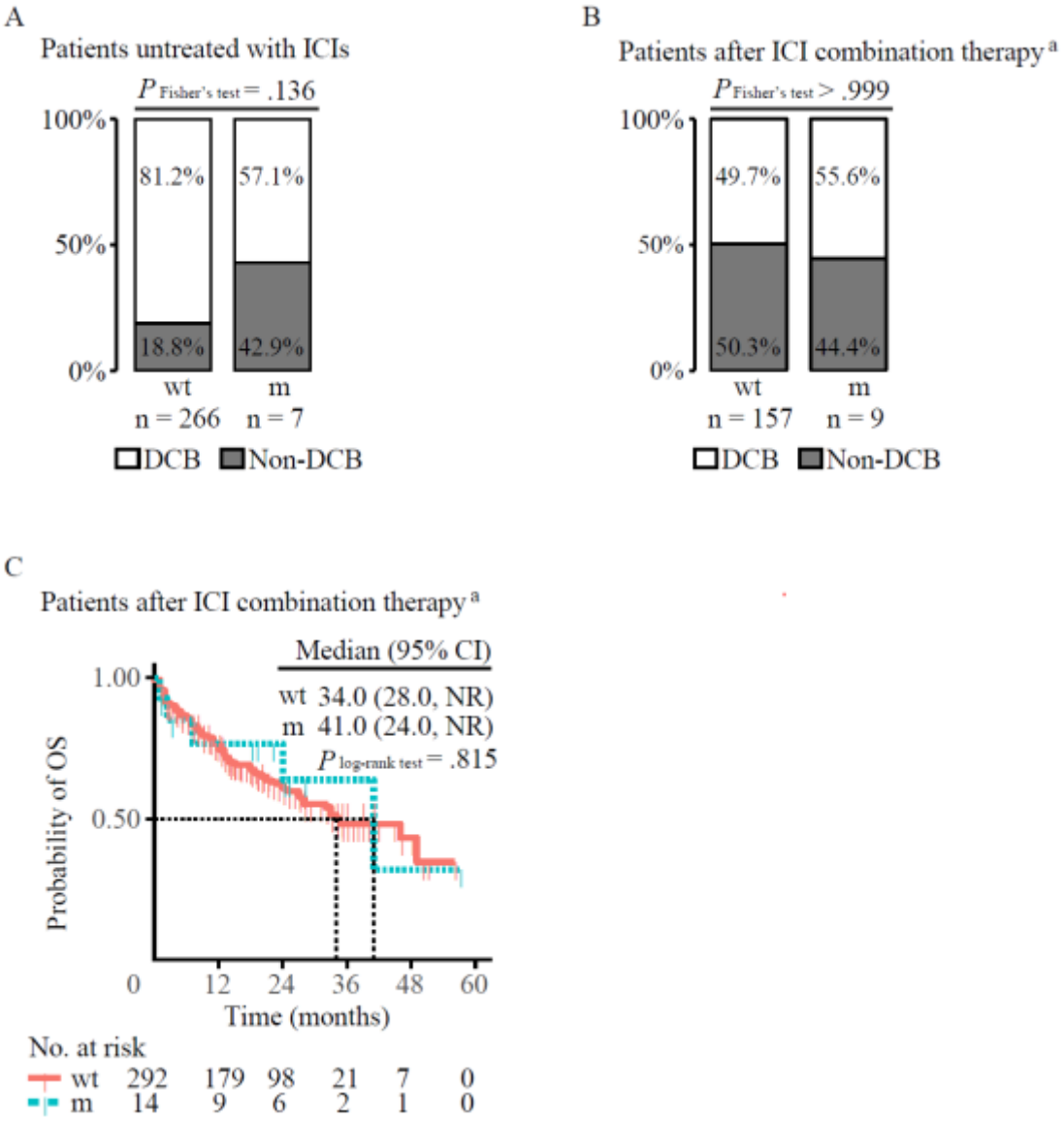



## Figure 2

Subgroup analyses of associations of *EPHA3* mutation with TMB and patient outcomes after ICI monotherapy.

Tumor mutational burden (TMB) was classified by percentile into high TMB (top 20%) and non-high TMB (bottom 80%). Forest plots show odds ratios (OR) or hazard ratios (HR) of *EPHA3* mutations versus *EPHA3* wild-type for TMB, DCB, and OS according to age, sex, cancer type, line of treatment, sample type, sequencing scope, tumor purity, and TMB. At least 10 patients with *EPHA3* mutations were required for a subgroup analysis.

Abbreviations: m, mutations; wt, wild-type.




## Figure 3

Outcomes of patients not treated with ICI monotherapy by *EPHA3* status

[a]: Immune checkpoint inhibitor (ICI) combination therapy is single ICI combined with chemotherapy, targeted therapy, or another ICI.

Abbreviations: NR, not reached; m, mutations; wt, wild-type

# Supplementary Files

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- Additionalfile1.doc
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