BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

# Online Supplementary Material

H. Bai, J. Duan, C. Li et al. EPHA mutation as a predictor of immunotherapeutic efficacy in lung adenocarcinoma

**Contents:**

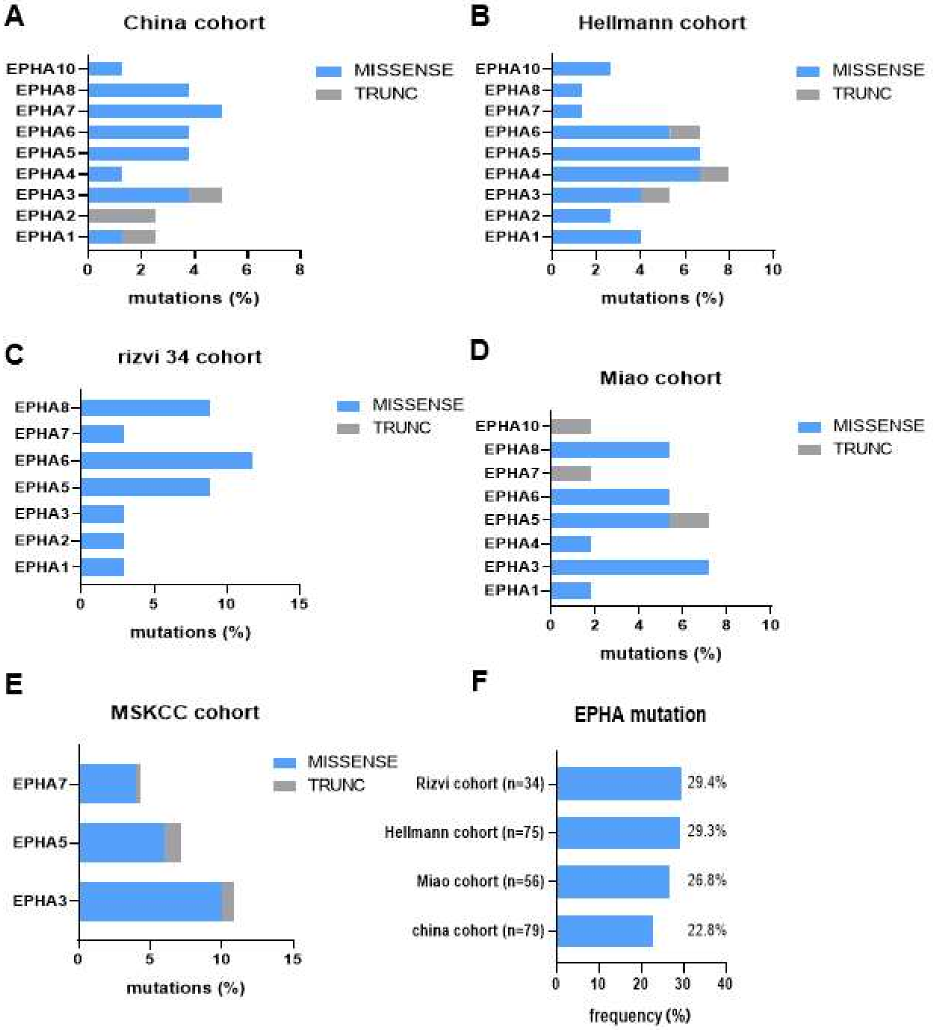
**Supplemental Tables: 1-13**

**Supplemental Figures: 1-6**

Bai H, *et al. J Immunother Cancer* 2020; 8:e001315. doi: 10.1136/jitc-2020-001315

**Supplemental Figure 1. The spectrum of EPHA mutation in different cohorts of NSCLC patients**

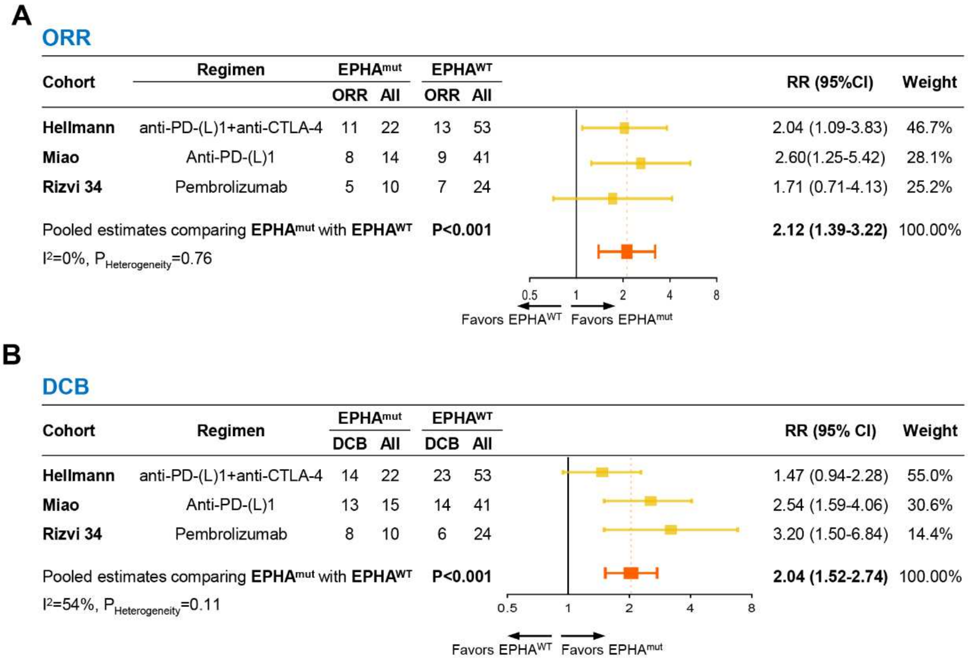
The frequency of different EPHA subtypes with MISSENSE and TRUNC variation results in discovery and validation cohorts**.**



BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Figure 2. Association between EPHA mutation and ORR and DCB in the validation cohorts**

**A.** Pooled estimates of ORR, squares in light blue represent study-specific HRs. The rhombus in orange indicate the pooled HRs. Horizontal lines indicate the 95% CIs. The P values for heterogeneity and the values of $I^2$ are from the meta-analyses of study-specific HRs. **B.** Pooled estimates of DCB. The squares in yellow represent study-specific risk rates (RRs). The square in orange indicate the pooled HRs. Horizontal lines indicate the 95% CIs. The P values for heterogeneity and the values of $I^2$ are from the meta-analyses of study-specific HRs.



| Cohort | Regimen | $EPHA^{mut}$ ORR | $EPHA^{mut}$ All | $EPHA^{WT}$ ORR | $EPHA^{WT}$ All | RR (95%CI) | Weight |
|---|---|---|---|---|---|---|---|
| **Hellmann** | anti-PD-(L)1+anti-CTLA-4 | 11 | 22 | 13 | 53 | 2.04 (1.09-3.83) | 46.7% |
| **Miao** | Anti-PD-(L)1 | 8 | 14 | 9 | 41 | 2.60(1.25-5.42) | 28.1% |
| **Rizvi 34** | Pembrolizumab | 5 | 10 | 7 | 24 | 1.71 (0.71-4.13) | 25.2% |
| Pooled estimates comparing **$EPHA^{mut}$** with **$EPHA^{WT}$** | | | | **P<0.001** | | **2.12 (1.39-3.22)** | 100.00% |

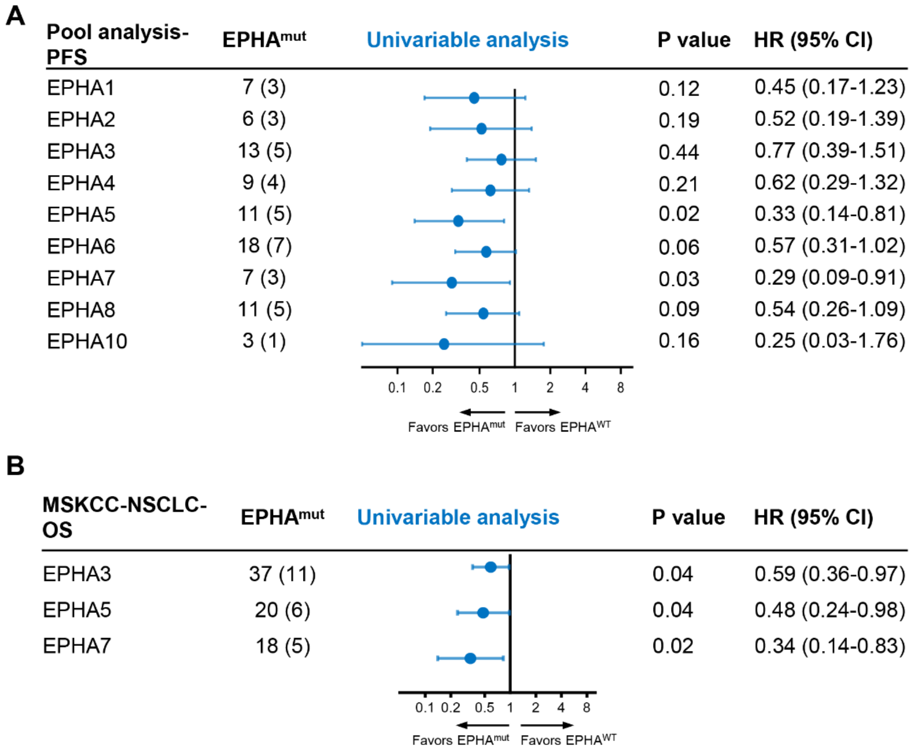
$I^2$=0%, $P_{Heterogeneity}$=0.76

| Cohort | Regimen | $EPHA^{mut}$ DCB | $EPHA^{mut}$ All | $EPHA^{WT}$ DCB | $EPHA^{WT}$ All | RR (95% CI) | Weight |
|---|---|---|---|---|---|---|---|
| **Hellmann** | anti-PD-(L)1+anti-CTLA-4 | 14 | 22 | 23 | 53 | 1.47 (0.94-2.28) | 55.0% |
| **Miao** | Anti-PD-(L)1 | 13 | 15 | 14 | 41 | 2.54 (1.59-4.06) | 30.6% |
| **Rizvi 34** | Pembrolizumab | 8 | 10 | 6 | 24 | 3.20 (1.50-6.84) | 14.4% |
| Pooled estimates comparing **$EPHA^{mut}$** with **$EPHA^{WT}$** | | | | **P<0.001** | | **2.04 (1.52-2.74)** | 100.00% |

$I^2$=54%, $P_{Heterogeneity}$=0.11

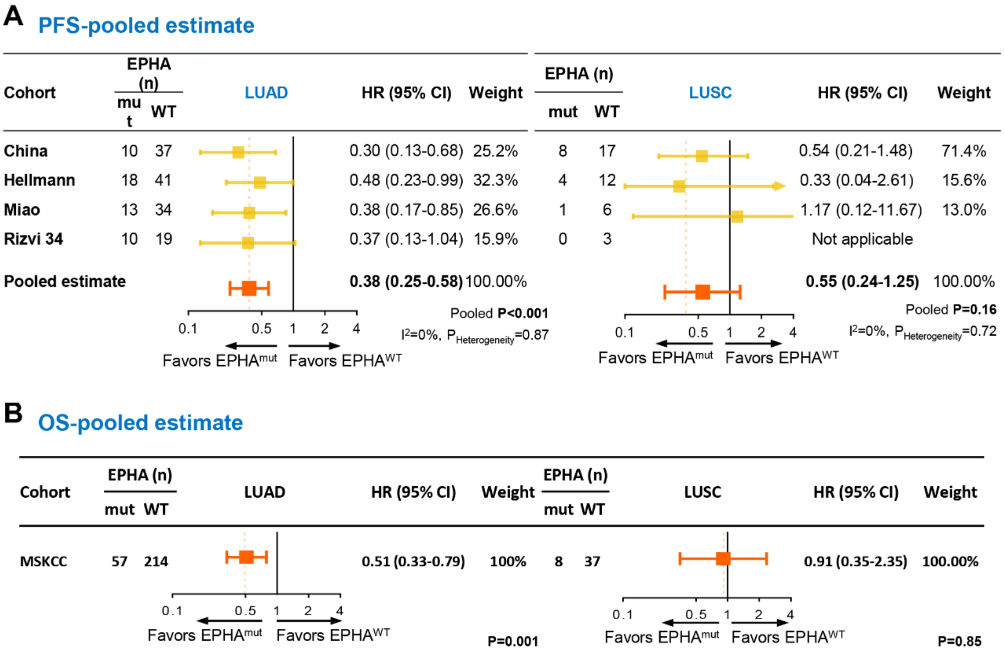
**Supplemental Figure 3. The association between different EPHA subtypes and immunotherapeutic effect in pooled cohorts analysis**

**A.** The association between different EPHA subtypes and PFS in pooled cohorts (training and validation) analysis. **B.** The association between different EPHA subtypes and OS in MSKCC cohort.



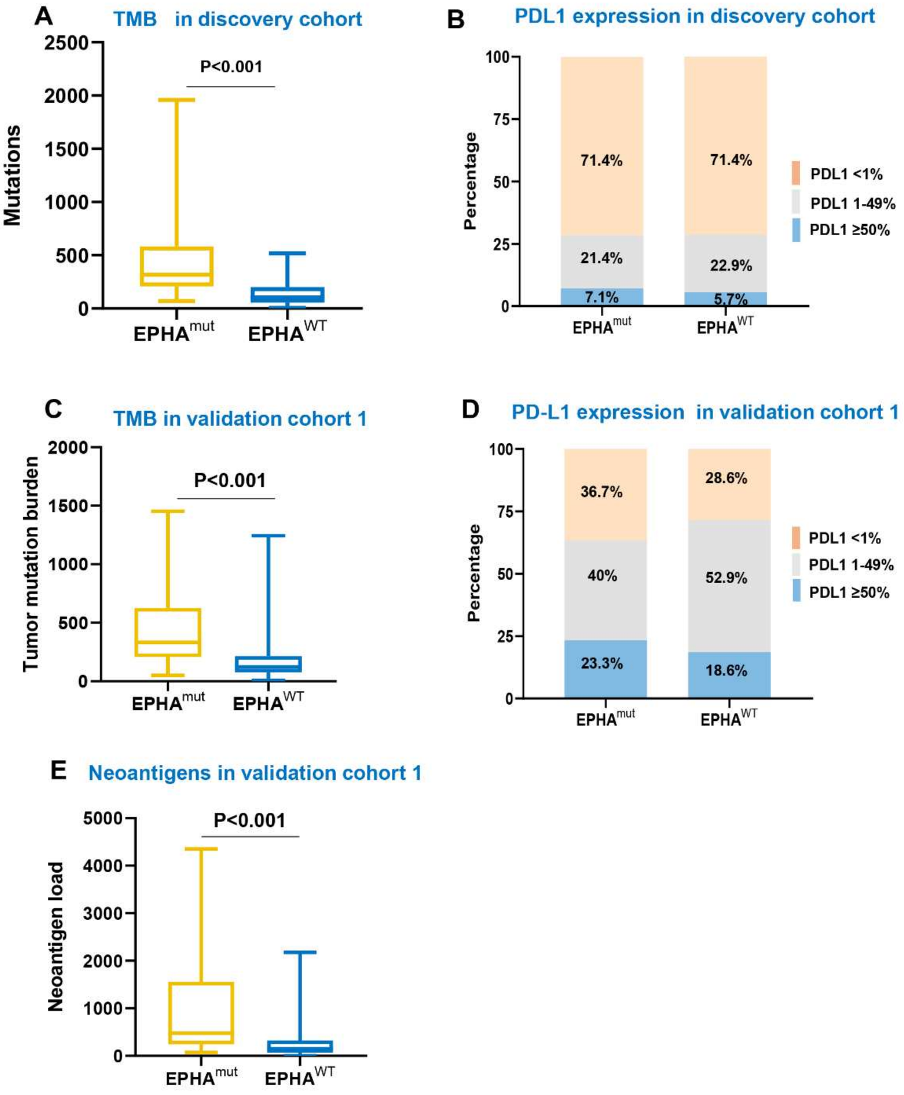
**Supplemental Figure 4. Association between EPHA mutations and clinical benefit to anti-PD-1/L1 therapy in LUAD and LUSC.**

**A-B.** Pooled estimates of HRs of PFS and OS in LUAD and LUAC in discovery cohort and validation cohorts. The squares in yellow represent study-specific HRs. The square in orange indicate the pooled HRs. Horizontal lines indicate the 95% CIs.



**Supplemental Figure 5. Association between EPHA mutation and TMB or neoantigen load in discovery and validation cohort 1**

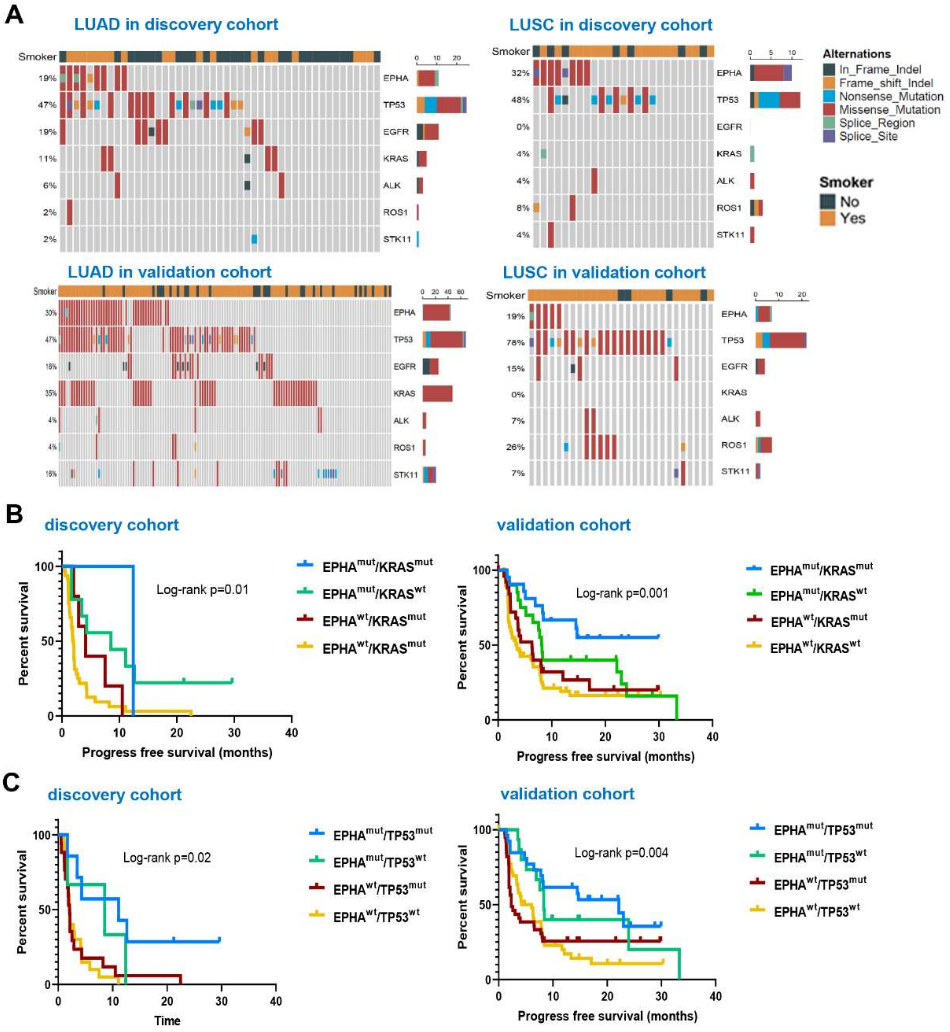
**A.** The comparation of tumor mutation burden (TMB) and predicted neoantigens between EPHA mutation and EPHA wide-type group in NSCLC in discovery cohort. **B.** The association of PDL1 expression between EPHA mutation and EPHA wide-type group in discovery cohort. **C.** The comparation of tumor mutation burden (TMB) between EPHA mutation and EPHA wide-type group in NSCLC in validation cohort 1. **-D.** The association of PDL1 expression between EPHA mutation and EPHA wide-type group in validation cohort 1.**E.** The comparation of predicted neoantigens between EPHA mutation and EPHA wide-type group in NSCLC in validation cohort 1.



BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Figure 6. The overlap between EPHA mutation and Oncogenic genes in LUAD and LUSC in discovery and validation cohort 1**

**A.**The co-occurrence of oncogenic genes and smoking status in LUAD and LUSC in the discovery cohort and in the validation cohort.**B.** The effect of co-mutation in predicting survival of immunotherapy in LUAD.



BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 1. Data source.**

| Data source | Tumor | N | Sequencing | RNAseq | Clinical outcome |
|---|---|---|---|---|---|
| China cohort | Non-small-cell lung carcinoma | 79 | WES | — | ORR; DCB; PFS |
| Rizvi 34cohort | Non-small-cell lung carcinoma | 34 | WES | — | ORR; DCB; PFS |
| Hellmann cohort | Non-small-cell lung carcinoma | 75 | WES | — | ORR; DCB; PFS |
| Miao cohort | Non-small-cell lung carcinoma | 56 | WES | — | ORR; DCB; PFS |
| MSKCC cohort | Pan-cancer | 1662 | NGS panel | — | OS |
| TCGA | Lung Adenocarcinoma | 566 | WES | 510 | PFS, OS |
| TCGA | Lung Squamous Cell Carcinoma | 484 | WES | 484 | PFS, OS |
| TCGA | Head and neck carcinoma | 523 | WES | 515 | — |
| TCGA | Bladder carcinoma | 413 | WES | 408 | — |
| TCGA | Esophageal carcinoma | 182 | WES | 181 | — |
| TCGA | Skin Cutaneous Melanoma | 448 | WES | 443 | — |

TCGA, The Cancer Genome Altlas; WES, whole-exome sequencing; ORR, objective response rate; DCR, disease control rate; DCB, durable clinical benefit; PFS, progression-free survival; OS, overall survival.

**Supplemental Table 2. Gene list of immune gene signature.**

| Classification | Genes |
|---|---|
| Immune checkpoint | PD-1, PD-L1, PD-L2, LAG3, CTLA4, TIM3, VTCN1 |
| T-effector and INFγ pathway | GBP1, IFI16, IFI30, IFNG, IRF1, STAT1, TAP1, TAP2, FAS, PSMB9, IL15RA, GZMA, GZMB, EOMES, CXCL10, CXCL9, CXCL11, TBX21, PRF1 |
| T cell receptor | CD27, GRAP2, LCK, PTPRCAP, CCL5, IL2RB, IKZF3, CD3G, CD74, CD3D, CD8A, CD4, TIGIT |
| Tumor microenvironment | IDO1, PTGS2, IL1B, IL18, IL6, IL12A, TNF, CD73 |

**Supplemental Table 3. Gene list of TGFβ signaling.**

| Classification | Genes |
|---|---|
| **TGF-β signaling** | TGFB, TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, TGFBRAP1, ACVR1, ACVR1B, ACVR1C, ACVR2A, ACVR2B, ACVRL1, BMP10, BMP15, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMPR1A, BMPR1B, BMPR2, GDF11, GDF2, INHA, INHBA, INHBB, INHBC, INHBE, NODAL, SMAD1, SMAD2, SMAD3, SMAD4, SMAD5, SMAD6, SMAD7, SMAD9, SPTBN1, ZFYVE9 |

**Supplemental Table 4. Patient characteristics in China cohort**

| Characteristics | No. (%) |
|---|---|
| **No. of patients** | 79 (100) |
| **Median age, years (IQR)** | 55 (47-61) |
| **Sex (male)** | 54 (68.4) |
| **Histology (squamous carcinoma)** | 25 (31.6) |
| **Smoking status (ever)** | 39 (49.4) |
| **Metastasis** | |
| Metastatic sites≥3 | 28 (35.4) |
| Liver metastasis | 18 (22.8) |
| Brain metastasis | 14 (17.7) |
| **ECOG** | |
| 0 | 19(24.1) |
| 1 | 60(75.9) |
| **LDH** | |
| ≥250 U/L | 36 (45.6) |
| <250 U/L | 43 (54.4) |
| **Treatment** | |
| Anti-PD-1 | 74 (93.7) |
| Anti-PD-L1 | 5 (6.3) |
| **Lines of therapy** | |
| Second | 38 (48.1) |
| Third or subsequent | 41 (51.9) |
| **PDL1 expression** | |
| <1% | 35(44.3) |
| 1-49% | 11(13.9) |
| >50% | 3(3.8) |
| Unknown | 30(38) |
| **Best overall response** | |
| CR | 1(1. 3) |
| PR | 14 (17.7) |
| SD | 20 (25.3) |
| PD | 44 (55.7) |
| **ORR (%)** | 19.0 |
| **PFS, months (median)** | 2.1 |
| **Mutation** | |
| EGFR | 9 (11.4) |
| STK11 | 3 (3.8) |
| TP53 | 39 (49.4) |
| KRAS | 8 (10.1) |
| EPHA | 16 (20.3) |

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 5. Univariable analysis and Multivariable cox regression analyses of PFS in China cohort**

| **Progress free survival** | | | | |
|---|---|---|---|---|
| **Parameter** | **Univariable analysis** | | **Multivariable analysis** | |
| | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** |
| **Age (≥65 vs. <65)** | 0.92 (0.56-1.50) | 0.73 | 1.48 (0.83-2.62) | 0.19 |
| **Sex (male vs. female)** | 0.48 (0.29-0.79) | 0.004 | 0.56 (0.27-1.15) | 0.12 |
| **Histology (non-LUSC vs. LUSC)** | 1.32 (0.80-2.18) | 0.28 | 1.04 (0.60-1.82) | 0.89 |
| **ECOG (1 vs. 0)** | 1.03 (0.65-1.63) | 0.90 | | |
| **Smoke (ever vs. never)** | 0.48 (0.30-0.76) | 0.002 | 0.64 (0.32-1.25) | 0.19 |
| **Line of treatments (later vs. 2)** | 1.17 (0.74-1.85) | 0.52 | | |
| **Metastatic sites (≥3 vs. <3)** | 1.34 (0.83-2.18) | 0.23 | | |
| **LDH (≥250 U/L vs <250 U/L)** | 1.03 (0.65-1.63) | 0.91 | | |
| **TMB (≥median vs. <median)** | 0.46 (0.28-0.73) | 0.003 | 0.67 (0.40-1.11) | 0.12 |
| **TMB (top 20% vs. others)** | 0.69 (0.38-1.24) | 0.21 | | |
| **PD-L1 expression** | | | | |
| **<1% (reference)** | | 0.60 | | 0.11 |
| **1-49%** | 0.72 (0.35-1.47) | 0.37 | 0.66 (0.30-1.42) | 0.28 |
| **>50%** | 0.48 (0.11-2.00) | 0.31 | 0.55 (0.13-2.46) | 0.44 |
| **unknown** | 0.80 (0.48-1.33) | 0.39 | 0.49 (0.28-0.87) | 0.02 |
| **EGFR (mut vs. WT)** | 1.70 (0.83-3.48) | 0.15 | | |
| **KRAS (mut vs. WT)** | 0.75 (0.36-1.56) | 0.46 | | |
| **STK11(mut vs. WT)** | 1.05(0.33-3.36) | 0.94 | | |
| **TP53 (mut vs. WT)** | 0.91 (0.57-1.44) | 0.68 | | |
| **EPHA (mut vs. WT)** | 0.38 (0.21-0.68) | 0.001 | 0.36 (0.19-0.71) | 0.003 |

**Supplemental Table 6. Patient characteristics in validation cohort 1**

| **Characteristics** | **No. (%)** |
|---|---|
| **No. of patients** | 165 (100) |
| **Median age, years (IQR)** | 63 (57-70) |
| **unknown** | 15(9.1) |
| **Sex (male)** | 77 (46.7) |
| **Histology (squamous carcinoma)** | 27 (16.4) |
| **Smoking status (ever)** | 131 (79.4) |
| **ECOG** | |
| 0 | 30(18.2) |
| 1 | 45(27.3) |
| unknown | 90(54.5) |
| **Treatment** | |
| Anti-PD-(L)1 | 90(54.5) |
| Anti-PD-(L)1+CTLA4 | 75 (45.5) |
| **Lines of therapy** | |
| One/Second | 89 (53.9) |
| Third or subsequent | 20 (12.1) |
| unknown | 56(33.9) |
| **PDL1 expression** | |
| <1% | 31(18.8) |
| 1-49% | 49(29.7) |
| >50% | 20(12.1) |
| Unknown | 65(39.4) |
| **Best overall response** | |
| CR | 5(3) |
| PR | 48 (29.1) |
| SD | 58(35.2) |
| PD | 47 (28.5) |
| NE | 7(4.2) |
| **ORR (%)** | 32.1 |
| **PFS, months (median)** | 6.5 |
| **Mutation** | |
| EGFR | 17 (10.3) |
| STK11 | 23 (13.9) |
| TP53 | 89(53.9) |
| KRAS | 47 (28.5) |
| EPHA | 47 (28.5) |

**Supplemental Table 7. Univariable analysis and Multivariable analysis COX regression analyses of PFS in the validation cohort 1**

| **Progress free survival** | | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Univariable analysis** | | **Multivariable analysis** | | **Multivariable analysis** | |
| | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** |
| **Sex (male vs. female)** | 1.26 (0.87-1.81) | 0.23 | 1.45 (0.97-2.17) | 0.07 | 1.47 (0.99-2.17) | 0.06 |
| **Age** | | | | | | |
| <65 (reference) | | 0.18 | | 0.03 | | 0.02 |
| ≥65 | 0.91 (0.61-1.36) | 0.64 | 0.60 (0.40-0.94) | 0.02 | 0.67 (0.44-1.02) | 0.06 |
| unknown | 1.59 (0.**89**-2.82) | 0.11 | 1.99 (1.03-3.86) | 0.05 | 2.40 (1.22-4.71) | 0.01 |
| **Smoking (ever vs. never)** | 0.61 (0.39-0.94) | 0.03 | 0.72 (0.45-1.13) | 0.15 | 0.80 (0.50-1.27) | 0.35 |
| **Histology (no-LUSC vs. LUSC)** | 0.94 (0.56-1.57) | 0.80 | 0.50 (0.28-0.88) | 0.02 | 0.82 (0.46-1.47) | 0.51 |
| **PD-L1 expression** | | | | | | |
| <1% (reference) | | 0.09 | | 0.11 | | 0.09 |
| 1-49% | 0.91 (0.53-1.56) | 0.73 | 1.36 (0.77-2.40) | 0.29 | 1.57 (0.89-2.79) | 0.12 |
| ≥50% | 0.38 (0.17-0.86) | 0.02 | 0.53 (0.23-1.21) | 0.13 | 0.62 (0.27-1.44) | 0.27 |
| unknown | 0.98 (0.59-1.63) | 0.95 | 1.02 (0.59-1.77) | 0.93 | 1.10 (0.63-1.90) | 0.77 |
| **Treatment (PD-(L)1 vs. CTLA-4)** | 0.92 (0.76-1.10) | 0.35 | | | | |
| **Datasets** | | | | | | |
| Rizvi 34 cohort (reference) | | 0.51 | | | | |
| Miao cohort | 0.91 (0.61-1.36) | 0.50 | | | | |
| Hellmann cohort | 1.59 (0.89-2.82) | 0.81 | | | | |
| **TMB (≥median vs. <median)** | 0.35 (0.24-0.51) | <0.001 | | | 0.33 (0.21-0.52) | <0.001 |
| **TMB (top 20% vs. others)** | 0.31 (0.17-0.56) | <0.001 | 0.31 (0.17-0.59) | <0.001 | | |
| **KRAS (mut vs. WT)** | 0.62 (0.40-0.95) | 0.03 | 0.64 (0.40-1.02) | 0.06 | 0.76 (0.48-1.20) | 0.23 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **TP53 (mut vs. WT)** | 0.77 (0.53-1.12) | 0.17 | | | | |
| **EGFR (mut vs. WT)** | 1.60 (0.90-2.81) | 0.11 | | | | |
| **STK11 (mut vs. WT)** | 1.47 (0.88-2.47) | 0.15 | | | | |
| **EPHA (mut vs. WT)** | 0.48 (0.31-0.74) | <0.001 | 0.59 (0.37-0.96) | 0.03 | 0.63(0.38-1.04) | 0.07 |

65 **Supplemental Table 8. Univariable analysis and Multivariable analysis COX regression**

66 **analyses of OS in NSCLC in validation cohort 2**

| **Overall survival** | | | | |
|---|---|---|---|---|
| **Parameter** | **Univariable analysis** | | **Multivariable analysis** | |
| | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** |
| **Histology (no-LUSC vs. LUSC)** | 1.30 (0.91-1.87) | 0.15 | 1.13 (0.78-1.65) | 0.51 |
| **Age (≥65 vs. <65)** | 1.08 (0.82-1.41) | 0.59 | 0.99 (0.75-1.29) | 0.89 |
| **Sex (male vs. female)** | 1.17 (0.90-1.53) | 0.25 | 1.14 (0.87-1.50) | 0.34 |
| **TMB (≥median vs <median)** | 0.99 (0.77-1.31) | 1 | | |
| **TMB (top 20% vs. others)** | 0.49 (0.33-0.72) | <0.001 | 0.60 (0.39-0.93) | 0.02 |
| **Treatment (anti-PD-(L)1 vs. anti-PD-1+CTLA4)** | 0.41(0.21-0.81) | 0.01 | 0.41 (0.21-0.82) | 0.01 |
| **EPHA (mut vs. WT)** | 0.48 (0.33-0.71) | <0.001 | 0.63 (0.41-0.98) | 0.04 |

67

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 9. Univariable analysis and Multivariable cox regression analyses of PFS in LUAD and LUSC of China cohort**

| | **Lung adenocarcinoma** | | | | **Lung squamous cell carcinoma** | | | |
|---|---|---|---|---|---|---|---|---|
| **Progress free survival** | **Univariable analysis** | | **Multivariable analysis** | | **Univariable analysis** | | **Multivariable analysis** | |
| **Parameter** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** |
| **Age (≥65 vs.<65)** | 0.84(0.43-1.64) | 0.61 | 1.34(0.6-3) | 0.47 | 1.39(0.59-3.29) | 0.46 | 1.94(0.72-5.2) | 0.19 |
| **Sex (male vs. female)** | 0.42(0.22-0.8) | **0.01** | 0.50(0.2-1.22) | 0.13 | 1.13(0.26-4.93) | 0.87 | 0.78(0.11-5.34) | 0.80 |
| **ECOG (1 vs. 0)** | 1.07(0.57-2.01) | 0.83 | | | 0.89(0.40-2.02) | 0.79 | | |
| **Smoke (ever vs. never)** | 0.37(0.19-0.74) | **0.004** | 0.76(0.31-1.88) | 0.55 | 0.72(0.28-1.86) | 0.50 | 0.32(0.09-1.13) | 0.08 |
| **Line of treatments (later vs. 2)** | 1.19(0.66-2.15) | 0.55 | | | 1.22(0.51-2.93) | 0.66 | | 0.28 |
| **Metastatic sites (≥3 vs. <3)** | 1.27(0.7-2.31) | 0.43 | | | 1.49(0.50-4.48) | 0.47 | 1.6(0.78-3.27) | 0.20 |
| **LDH（≥250 U/L vs <250 U/L）** | 0.83(0.45-1.53) | 0.55 | | | 1.23(0.52-2.90) | 0.64 | 1.05(0.51-2.14) | 0.90 |
| **TMB (≥median vs. <median)** | 0.33(0.17-0.64) | **0.003** | 0.41(0.2-0.85) | **0.02** | 0.62(0.26-1.47) | 0.28 | 0.93(0.29-3.00) | 0.90 |
| **PDL1 expression** | | | | | | | | |
| (<1% reference) | | 0.46 | | 0.15 | | 0.88 | | 0.62 |
| ≥1% | 1.29(0.84-1.97) | 0.24 | 1.41(0.89-2.25) | 0.14 | 1.08(0.6-1.96) | 0.79 | 1.27(0.68-2.37) | 0.46 |
| unknown | 0.73(0.41-1.29) | 0.28 | 1.02(0.55-1.89) | 0.96 | 1.08(0.55-2.14) | 0.82 | 1.06(0.53-2.11) | 0.87 |
| **EGFR (mut vs. WT)** | 1.73(0.75-3.99) | 0.20 | | | | | | |
| **KRAS (mut vs. WT)** | 0.69(0.29-1.65) | 0.41 | | | 0.5(0.07-3.81) | 0.50 | | |
| **STK11(mut vs. WT)** | 1.01(0.24-4.22) | 0.99 | | | 1.67(0.21-12.96) | 0.62 | | |
| **TP53 (mut vs. WT)** | 0.66(0.36-1.21) | 0.18 | | | 2.22(0.85-5.8) | 0.10 | | |
| **EPHA (mut vs. WT)** | 0.30(0.13-0.68) | **0.004** | 0.34(0.12-0.95) | **0.04** | 0.54(0.21-1.43) | 0.21 | 0.48(0.12-1.92) | 0.30 |

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 10. Univariable analysis and Multivariable cox regression analyses of PFS in LUAD and LUSC of validation cohort 1**

| | **Lung adenocarcinoma** | | | | **Lung squamous cell carcinoma** | | | |
|---|---|---|---|---|---|---|---|---|
| **Progress free survival** | **Univariable analysis** | | **Multivariable analysis** | | **Univariable analysis** | | **Multivariable analysis** | |
| **Parameter** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** | **HR (95%CI)** | **P value** |
| **Age** | | | | | | | | |
| <65(reference) | | 0.28 | | **0.00** | | 0.44 | | 0.67 |
| ≥65 | 0.95(0.62-1.47) | 0.82 | 0.62(0.39-0.98) | **0.04** | 0.51(0.18-1.44) | 0.20 | 0.63(0.17-2.32) | 0.48 |
| unknown | 1.64(0.85-3.16) | 0.14 | 2.37(1.12-5.02) | 0.02 | 0.75(0.16-3.60) | 0.72 | 0.43(0.05-4.06) | 0.46 |
| **Sex (male vs. female)** | 1.41(0.94-2.11) | 0.10 | 1.62(1.07-2.44) | **0.02** | 0.59(0.21-1.68) | 0.32 | 0.61(0.14-2.65) | 0.51 |
| **smoking (previous/current vs. never)** | 0.50(0.32-0.79) | **0.003** | 0.72(0.44-1.19) | 0.20 | 3.36(0.44-25.49) | 0.24 | 3.54(0.31-40.42) | 0.31 |
| **PDL1** | | | | | | | | |
| <1% | | **0.07** | | 0.16 | | 0.96 | | 0.92 |
| 1-49% | 0.91(0.51-1.62) | 0.74 | 1.53(0.8-2.93) | 0.20 | 1.23(0.31-4.96) | 0.77 | 1.52(0.32-7.09) | 0.60 |
| ≥50% | 0.32(0.13-0.8) | 0.01 | 0.59(0.22-1.58) | 0.30 | 0.86(0.14-5.19) | 0.87 | 1.03(0.11-9.57) | 0.98 |
| unknown | 1(0.58-1.72) | 0.99 | 1.08(0.58-2.02) | 0.80 | 0.96(0.24-3.86) | 0.95 | 1.51(0.26-8.70) | 0.64 |
| **Treatment (PD-1/PDL1 vs. CTLA4)** | 0.88(0.71-1.08) | 0.22 | | | 1.06(0.66-1.73) | 0.80 | | |
| **KRAS (mut vs. WT)** | 0.57(0.37-0.89) | **0.01** | 0.81(0.50-1.30) | 0.38 | | | | |
| **TP53 (mut vs. WT)** | 0.79(0.52-1.19) | 0.25 | | | 0.88(0.29-2.71) | 0.82 | | |
| **EGFR (mut vs. WT)** | 1.92(1.05-3.54) | **0.04** | 1.03(0.51-2.08) | 0.93 | 0.92(0.21-4.04) | 0.91 | | |
| **STK11 (mut vs. WT)** | 1.49(0.85-2.59) | 0.16 | | | 1.10(0.25-4.83) | 0.90 | | |
| **EPHA (mut vs. WT)** | 0.43(0.27-0.69) | **<0.001** | 0.53(0.31-0.90) | **0.02** | 0.50(0.11-2.20) | 0.36 | 0.4(0.06-2.75) | 0.35 |
| **TMB (≥median vs.<median)** | 0.32(0.21-0.49) | **<0.001** | 0.39(0.23-0.66) | **0.004** | 0.49(0.19-1.28) | 0.15 | 0.8(0.19-3.32) | 0.76 |

**Supplemental Table 11. Univariable analysis and Multivariable cox regression analyses of OS in LUAD and LUSC of validation cohort 2**

| **Overall survival** | **Lung adenocarcinoma** | | | | **Lung squamous cell carcinoma** | |
|---|---|---|---|---|---|---|
| **Parameter** | **Univariable analysis** | | **Multivariable analysis** | | **Univariable analysis** | |
| | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** | **HR (95% CI)** | **P** |
| **Age (≥65 vs. <65)** | 1.13(0.83-1.55) | 0.43 | | | 0.75(0.38-1.49) | 0.41 |
| **Sex (male vs. female)** | 1.15(0.85-1.56) | 0.38 | | | 0.94(0.44-2.02) | 0.88 |
| **TMB (≥median vs <median)** | 1.00(0.74-1.36) | 0.99 | | | 1.62(0.81-3.23) | 0.17 |
| **TMB (top 20% vs. others)** | 0.60(0.39-0.91) | **0.02** | 0.73(0.45-1.17) | **0.19** | 1.07(0.46-2.49) | 0.87 |
| **Treatment (anti-PD-(L)1 vs. anti-PD-1+CTLA4)** | 0.39(0.17-0.88) | **0.02** | 0.38(0.17-0.88) | **0.02** | 0.04(0-34.61) | 0.35 |
| **EPHA (mut vs. WT)** | 0.51(0.33-0.79) | **0.002** | 0.61(0.38-1.00) | **0.05** | 0.91(0.35-2.35) | 0.84 |

Abbreviations: TMB, tumor mutational burden; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; HR, hazard ratio; LUSC, lung squamous carcinoma; LUAD, lung adenocarcinomas; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; ORR, Objective response rate; EGFR, epidermal growth factor receptor; KRAS, kirsten rat sarcoma viral oncogene; STK11, serine/threonine kinase 11; TP53, tumor protein p53; EPHA, Ephrin type-A receptors; WT, wildtype.

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 12. P values adjusted by FDR with immune gene signature between $\mathit{EPHA}^{mut}$ and $\mathit{EPHA}^{wt}$ in multi-type tumors.**

| symbol | HNSC | BLCA | ESCA | SKCM | LUAD | LUSC |
|---|---|---|---|---|---|---|
| CCL5 | 0.958 | 0.994 | 0.998 | 0.722 | 0.061 | 0.841 |
| CD274 | 0.879 | 0.994 | 0.998 | 0.658 | 0.308 | 0.841 |
| CD27 | 0.879 | 0.994 | 0.998 | 0.721 | 0.530 | 0.797 |
| CD3D | 0.934 | 0.994 | 0.998 | 0.721 | 0.238 | 0.797 |
| CD3G | 0.879 | 0.994 | 0.998 | 0.674 | 0.183 | 0.797 |
| CD4 | 0.879 | 0.994 | 0.998 | 0.674 | 0.473 | 0.841 |
| CD74 | 0.958 | 0.994 | 0.998 | 0.674 | 0.007 | 0.841 |
| CD8A | 0.879 | 0.994 | 0.998 | 0.722 | 0.004 | 0.797 |
| CTLA4 | 0.879 | 0.994 | 0.998 | 0.658 | 0.036 | 0.797 |
| CXCL10 | 0.958 | 0.994 | 0.998 | 0.658 | 0.002 | 0.797 |
| CXCL11 | 0.958 | 0.994 | 0.998 | 0.658 | 0.024 | 0.797 |
| CXCL9 | 0.958 | 0.994 | 0.998 | 0.658 | 0.002 | 0.797 |
| EOMES | 0.831 | 0.994 | 0.998 | 0.848 | 0.061 | 0.797 |
| FAS | 0.879 | 0.994 | 0.998 | 0.658 | 0.183 | 0.841 |
| GBP1 | 0.879 | 0.994 | 0.998 | 0.658 | 0.052 | 0.841 |
| GRAP2 | 0.879 | 0.994 | 0.998 | 0.674 | 0.473 | 0.797 |
| GZMA | 0.958 | 0.994 | 0.998 | 0.716 | 0.031 | 0.797 |
| GZMB | 0.958 | 0.994 | 0.998 | 0.674 | 0.002 | 0.797 |
| HAVCR2 | 0.879 | 0.994 | 0.998 | 0.837 | 0.631 | 0.875 |
| IDO1 | 0.958 | 0.994 | 0.998 | 0.658 | 0.863 | 0.797 |
| IFI16 | 0.879 | 0.994 | 0.998 | 0.674 | 0.363 | 0.797 |
| IFI30 | 0.958 | 0.994 | 0.998 | 0.674 | 0.957 | 0.797 |
| IFNG | 0.958 | 0.994 | 0.998 | 0.674 | 0.015 | 0.797 |
| IKZF3 | 0.958 | 0.994 | 0.998 | 0.674 | 0.117 | 0.797 |
| IL12A | 0.831 | 0.994 | 0.998 | 0.674 | 0.473 | 0.797 |
| IL15RA | 0.958 | 0.994 | 0.998 | 0.837 | 0.919 | 0.974 |
| IL18 | 0.879 | 0.994 | 0.998 | 0.848 | 0.153 | 0.797 |
| IL1B | 0.879 | 0.994 | 0.998 | 0.941 | 0.691 | 0.797 |
| IL2RB | 0.879 | 0.994 | 0.998 | 0.674 | 0.153 | 0.916 |
| IL6 | 0.879 | 0.994 | 0.998 | 0.674 | 0.473 | 0.974 |
| IRF1 | 0.958 | 0.994 | 0.998 | 0.674 | 0.547 | 0.841 |
| LAG3 | 0.958 | 0.994 | 0.998 | 0.674 | 0.034 | 0.797 |
| LCK | 0.879 | 0.994 | 0.998 | 0.674 | 0.429 | 0.797 |
| NT5E | 0.958 | 0.994 | 0.511 | 0.674 | 0.258 | 0.797 |
| PDCD1 | 0.879 | 0.994 | 0.998 | 0.674 | 0.072 | 0.797 |
| PDCD1LG2 | 0.958 | 0.994 | 0.998 | 0.674 | 0.221 | 0.841 |
| PRF1 | 0.958 | 0.994 | 0.998 | 0.674 | 0.473 | 0.797 |
| PSMB9 | 0.958 | 0.994 | 0.998 | 0.658 | 0.221 | 0.797 |
| PTGS2 | 0.831 | 0.994 | 0.998 | 0.837 | 0.289 | 0.797 |
| PTPRCAP | 0.879 | 0.994 | 0.998 | 0.674 | 0.672 | 0.797 |

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

| | | | | | | |
|---|---|---|---|---|---|---|
| STAT1 | 0.958 | 0.994 | 0.998 | 0.658 | 0.002 | 0.797 |
| TAP1 | 0.879 | 0.994 | 0.998 | 0.674 | 0.085 | 0.841 |
| TAP2 | 0.879 | 0.994 | 0.998 | 0.658 | 0.473 | 0.918 |
| TBX21 | 0.879 | 0.994 | 0.998 | 0.674 | 0.238 | 0.875 |
| TIGIT | 0.958 | 0.994 | 0.998 | 0.674 | 0.061 | 0.817 |
| TNF | 0.958 | 0.994 | 0.998 | 0.674 | 0.334 | 0.817 |
| VTCN1 | 0.879 | 0.994 | 0.998 | 0.674 | 0.957 | 0.797 |

Abbreviations: HNSC, head and neck squamous cell carcinoma; BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; ESCA, esophageal carcinoma; BRCA, breast invasive carcinoma;

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

**Supplemental Table 13. P values adjusted by FDR with *TGF-β* signaling between *EPHA*$^{mut}$ and *EPHA*$^{wt}$ in multi-type tumors.**

| symbol | HNSC | BLCA | ESCA | SKCM | LUAD | LUSC |
|---|---|---|---|---|---|---|
| ACVR1 | 0.567 | 0.177 | 0.395 | 0.300 | 0.236 | 0.078 |
| ACVR1B | 0.991 | 0.577 | 0.959 | 0.379 | 0.863 | 0.650 |
| ACVR1C | 0.297 | 0.596 | 0.917 | 0.776 | 0.410 | 0.457 |
| ACVR2A | 0.133 | 0.596 | 0.395 | 0.932 | 0.272 | 0.457 |
| ACVR2B | 0.892 | 0.975 | 0.717 | 0.916 | 0.119 | 0.044 |
| ACVRL1 | 0.409 | 0.950 | 0.858 | 0.396 | 0.727 | 0.720 |
| BMP10 | 0.861 | 0.177 | NA | 0.626 | 0.929 | 0.992 |
| BMP15 | 0.962 | 0.950 | NA | 0.661 | 0.028 | 0.989 |
| BMP2 | 0.567 | 0.577 | 0.320 | 0.626 | 0.221 | 0.414 |
| BMP3 | 0.613 | 0.660 | 0.059 | 0.916 | 0.247 | 0.461 |
| BMP4 | 0.214 | 0.596 | 0.937 | 0.776 | 0.035 | 0.989 |
| BMP5 | 0.193 | 0.950 | 0.815 | 0.661 | 0.575 | 0.989 |
| BMP6 | 0.133 | 0.381 | 0.320 | 0.215 | 0.929 | 0.989 |
| BMP7 | 0.802 | 0.219 | 0.075 | 0.272 | 0.015 | 0.457 |
| BMPR1A | 0.613 | 0.166 | 0.226 | 0.615 | 0.708 | 0.461 |
| BMPR1B | 0.802 | 0.975 | 0.719 | 0.615 | 0.708 | 0.989 |
| BMPR2 | 0.535 | 0.177 | 0.579 | 0.338 | 0.467 | 0.935 |
| GDF11 | 0.133 | 0.975 | 0.172 | 0.008 | 0.942 | 0.797 |
| GDF2 | 0.802 | 0.330 | NA | 0.916 | 0.608 | 0.989 |
| INHA | 0.214 | 0.381 | 0.299 | 0.071 | 0.249 | 0.650 |
| INHBA | 0.895 | 0.177 | 0.821 | 0.472 | 0.606 | 0.797 |
| INHBB | 0.613 | 0.177 | 0.080 | 0.062 | 0.929 | 0.989 |
| INHBC | 0.503 | 0.950 | 0.143 | 0.062 | 0.608 | 0.414 |
| INHBE | 0.613 | 0.577 | 0.533 | 0.472 | 0.929 | 0.650 |
| CERS1 | 0.297 | 0.950 | 0.342 | 0.300 | 0.727 | 0.078 |
| NODAL | 0.685 | 0.372 | 0.395 | 0.916 | 0.112 | 0.797 |
| SMAD1 | 0.626 | 0.219 | 0.320 | 0.690 | 0.058 | 0.874 |
| SMAD2 | 0.802 | 0.177 | 0.320 | 0.916 | 0.800 | 0.989 |
| SMAD3 | 0.294 | 0.403 | 0.320 | 0.215 | 0.068 | 0.129 |
| SMAD4 | 0.409 | 0.177 | 0.560 | 0.916 | 0.929 | 0.797 |
| SMAD5 | 0.739 | 0.177 | 0.080 | 0.932 | 0.221 | 0.989 |
| SMAD6 | 0.133 | 0.596 | 0.172 | 0.385 | 0.005 | 0.720 |
| SMAD7 | 0.319 | 0.252 | 0.172 | 0.307 | 0.550 | 0.989 |
| SMAD9 | 0.262 | 0.177 | 0.065 | 0.472 | 0.173 | 0.720 |
| SPTBN1 | 0.626 | 0.317 | 0.255 | 0.916 | 0.608 | 0.989 |
| TGFB1 | 0.802 | 0.350 | 0.320 | 0.916 | 0.708 | 0.414 |
| TGFB2 | 0.199 | 0.596 | 0.549 | 0.215 | 0.231 | 0.989 |
| TGFB3 | 0.199 | 0.177 | 0.434 | 0.626 | 0.929 | 0.797 |
| TGFBR1 | 0.904 | 0.177 | 0.830 | 0.661 | 0.708 | 0.797 |
| TGFBR2 | 0.669 | 0.219 | 0.229 | 0.916 | 0.205 | 0.797 |

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

| | | | | | | |
|---|---|---|---|---|---|---|
| TGFBR3 | 0.925 | 0.950 | 0.172 | 0.494 | 0.015 | 0.989 |
| TGFBRAP1 | 0.802 | 0.166 | 0.402 | 0.071 | 0.929 | 0.958 |
| ZFYVE9 | 0.193 | 0.672 | 0.172 | 0.916 | 0.306 | 0.935 |

Abbreviations: HNSC, head and neck squamous cell carcinoma; BLCA, bladder urothelial carcinoma; SKCM, skin cutaneous melanoma; ESCA, esophageal carcinoma; BRCA, breast invasive carcinoma;