Journal for ImmunoTherapy of Cancer

Mutation-guided chemotherapy-free strategy in first-line immunotherapy for low PD-L1-expressing non-squamous NSCLC

Hui Li,[1] Jingjing Liu,[2] Liang Zhang,[3] Yu Xu,[4] Xinyue Wang ,[5] Shaowei Lan,[1] Peng Cui,[4] Guoqiang Wang,[4] Shangli Cai,[4] Ying Cheng [2]

To cite: Li H, Liu J, Zhang L, *et al*. Mutation-guided chemotherapy-free strategy in first-line immunotherapy for low PD-L1-expressing non-squamous NSCLC. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e009693. doi:10.1136/jitc-2024-009693

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jitc-2024-009693).

HL, JL, LZ and YX contributed equally.

Accepted 05 November 2024

For numbered affiliations see end of article.

Correspondence to
Prof Shangli Cai;
shangli.cai@brbiotech.com

Professor Ying Cheng;
jl.cheng@163.com

ABSTRACT

Background The necessity of platinum-doublet chemotherapy in first-line immunotherapy for non-squamous non-small cell lung cancer (nsqNSCLC) with programmed death-ligand 1 (PD-L1) expression on less than 50% of tumor cells remains poorly investigated. Biomarkers predicting this necessity can guide chemotherapy-free treatment to minimize unnecessary toxicity.

Methods Treated with immune checkpoint inhibitor monotherapy (ICI-mono), chemotherapy, or combination (ICI-chemo), 790 low PD-L1-expressing nsqNSCLCs (in-house: n=83; public: n=707) were analyzed for development and validation of the interaction score for additional chemotherapy (ISAC). Transcriptomic (public, n=11) and multiplex immunofluorescence data (in-house, n=100) were analyzed to evaluate the immune microenvironment.

Results ICI-chemo, compared with ICI-mono, tended to prolong progression-free survival (PFS; HR=0.72, p=0.004) and overall survival (OS; HR=0.77, p=0.071) as first-line therapy in low PD-L1-expressing nsqNSCLCs. The added value of chemotherapy was observed in the ISAC-low subgroup (PFS: HR=0.48, p<0.001; OS: HR=0.53, p=0.001) rather than the ISAC-high subgroup (PFS: HR=1.08, p=0.65; OS: HR=1.14, p=0.56). This predictive utility was independent of tumor mutational burden and PD-L1 expression, indicated by subgroup and multivariable analyses. A high ISAC was associated with adaptive immune resistance reflected by more proinflammatory (eg, $CD8^+$ T cells and M1 macrophages) rather than anti-inflammatory tumor-infiltrating immune cells (eg, M2 macrophages) and high expression of immune checkpoints except for PD-L1 (eg, programmed cell death protein-1).

Conclusion A high ISAC was identified as a significant predictor for virtually no added value of platinum-doublet chemotherapy for first-line ICI treatment in low PD-L1-expressing nsqNSCLC. Our findings may help refine personalized therapeutic strategies for nsqNSCLC, thereby improving efficacy and reducing undue toxicity.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In the first-line setting of non-squamous non-small cell lung cancers (nsqNSCLCs) with programmed death-ligand 1 (PD-L1)<50%, randomized controlled trials typically compared immune checkpoint inhibitor (ICI) plus chemotherapy with chemotherapy alone rather than head-to-head with ICI monotherapy (ICI-mono). Whether synergistic effects exist between ICI and chemotherapy remains hotly debated, leaving the question open as to whether chemotherapy is unavoidably required in combination with ICI in the first-line treatment.

WHAT THIS STUDY ADDS

⇒ The interaction score for additional chemotherapy (ISAC) was developed and validated using 790 low PD-L1-expressing nsqNSCLCs. A high ISAC was identified as a significant predictor for virtually no added value of chemotherapy to ICI and adaptive immune resistance reflected by more proinflammatory rather than anti-inflammatory tumor-infiltrating immune cells and high expression of immune checkpoints except PD-L1 (eg, programmed cell death protein-1).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ In addition to the PD-L1≥50% nsqNSCLC where ICI-mono was approved and recommended as first-line therapy, the ISAC can further distinguish nearly half of PD-L1<50% nsqNSCLCs to be exempted from chemotherapy in the first-line setting, thereby reducing undue toxicity and improving quality of life.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) targeting programmed death-(ligand) 1 (PD-(L)1) have revolutionized the treatment landscape of non-small cell lung cancer (NSCLC), offering long-term disease control.[1] As durable responses to ICI monotherapy (ICI-mono) occur in only a tiny minority, research efforts have concentrated on using other treatments as adjuvants to maximize the long-term benefits of ICI.[2] Platinum-doublet chemotherapy, the once-standard first-line treatment for non-squamous NSCLC (nsqNSCLC),[3] has been tested in multiple trials as a

combination regimen with ICI in the first-line setting.[4–8] However, these trials typically compared ICI plus chemotherapy (ICI-chemo) with chemotherapy alone rather than head-to-head with ICI-mono.[4–8] Despite that ICI-chemo has been demonstrated to provide survival benefits over chemotherapy alone,[4–8] this fails to answer the central question as to which patients need chemotherapy to increase ICI efficacy.

In the nsqNSCLC with PD-L1 expression on greater than 50% of tumor cells (tumor proportion score (TPS)≥50%), the survival benefit of ICI-mono versus chemotherapy was comparable to that of ICI-chemo versus chemotherapy in randomized controlled trials (RCTs),[5–7 9–11] leading healthcare providers to depend on PD-L1 TPS≥50% as a threshold for ICI-mono. Subsequent retrospective studies have also confirmed similar survival on ICI-mono and ICI-chemo in the TPS≥50% population.[12 13] On the other hand, in the low PD-L1-expressing nsqNSCLC, whether a synergistic effect exists between ICI and chemotherapy remains hotly debated,[14 15] leaving the question open as to whether chemotherapy is unavoidably required in combination with ICI in the first-line treatment.

Hong *et al* previously identified *CDKN2A* alterations as biomarkers indicating no benefits from ICI-chemo over ICI-mono in NSCLC.[15] However, *CDKN2A* alterations occurred primarily in squamous NSCLC rather than in nsqNSCLC, suggesting that the predictive significance of *CDKN2A* in NSCLC may be largely attributed to the squamous subtype. Here, we focused on low PD-L1-expressing nsqNSCLC and sought to develop the interaction score for additional chemotherapy (ISAC) to predict the benefit from ICI-chemo over ICI-mono as first-line therapy by analyzing an in-house database and four public cohorts. In addition, we aimed to further explore its correlation with the immune microenvironment via transcriptomic analysis on a public data set and in-house multiplex immunofluorescence experiments.

MATERIALS AND METHODS

Patients for ISAC development and validation

The features of immunotherapy cohorts are displayed in online supplemental table S1 (eg, regimen, setting, sample size, treatment lines, outcome, testing method of PD-L1 and mutations). First, the in-house cohort includes 150 patients with advanced NSCLC treated with programmed cell death protein-1 (PD-1)/PD-L1 monotherapy at Jilin Cancer Hospital, who had mutational data from sequencing either pretreatment circulating tumor DNA (ctDNA, 150-gene panel, 3D Medicines) or cancer tissue (whole-exome sequencing (WES) or 520-gene panel, Burning Rock Biotech). The baseline characteristics of included in-house samples are shown in online supplemental table S2. In addition, other four publicly available cohorts from different sources (Memorial Sloan-Kettering Cancer Center (MSKCC), MD Anderson Cancer Center (MDACC), Broad Institute of Massachusetts Institute of Technology, and the "immunotherapeutic predictive and cancer prognostic biomarkers" (IMPACT) platform) were acquired from manuscripts' appendices or online databases.[15–20] The adenosquamous and not otherwise specified NSCLCs were not included in our analysis.

This report follows the Strengthening the Reporting of Observational Studies in Epidemiology reporting guideline.

Mutational analysis

The detailed sequencing methods of the 150-gene panel, the 520-gene panel, and WES were described in previous studies.[8 21 22] Mutated genes were restricted to non-silent mutations consisting of non-sense, missense, frameshift, inframe, splice site, translation start site, and non-stop mutations. Truncating mutations of oncogenes were excluded because most of these are passenger mutations with limited cancer-promoting function.

Since the MDACC cohort only contains mutational data of 70 genes, tumor mutational burden (TMB) was not calculated in this cohort and the genomic analysis in our study was limited to the key genes shared by all cohorts. The list of key genes and signaling pathways is shown in online supplemental table S3. This definition of pathways was derived from previous studies.[23–26]

ISAC development and validation

In the first-line training set, the interaction effect on progression-free survival (PFS) between treatment (ICI-chemo vs ICI-mono) and the mutational status of each gene and pathway with a mutational rate over 5.0% (mutation vs wild-type) was calculated. The interaction terms with a potential value for prediction (HR<0.67 or >1.50, p<0.30) were included in the following multivariable analysis, incorporating the treatment variable, all the mutational events that meet the aforementioned criteria, and their interaction terms with the treatment variable. The coefficient of each mutational event was determined by the natural logarithm of the HR of its interaction term. The formula for the ISAC is as follows:

$$ISAC = \sum \left[(Mutational\ event \times \ln(interaction\ HR\ in\ multivariable\ model) \right]$$

Prognostic analysis

To explore the prognostic effect of the ISAC, we downloaded data from the cBioPortal database for all patients with stage IV nsqNSCLC.[27 28] After deduplication, a total of 1,012 patients with overall survival (OS) and mutational data were included for prognostic analysis (baseline characteristic: online supplemental table S4).[23 29–33]

Transcriptomic analysis

From the data published by Ravi *et al*,[20] we found 11 low PD-L1-expressing nsqNSCLC with both mutation and messenger RNA (mRNA) data and 24 high PD-L1-expressing samples with mRNA data to explore the transcriptomic correlates of the ISAC (baseline characteristic: online supplemental table S5).

For Gene Set Enrichment Analysis (GSEA), the javaGSEA desktop application (GSEA V.4.0.1) was used

to investigate the gene signatures significantly different between the high-ISAC and low-ISAC samples.[34] The normalized enrichment score is the primary statistic for assessing the enrichment of gene sets.

Cell-type identification by estimating relative subsets of RNA transcripts (CIBERSORT), an online method (https://cibersort.stanford.edu/index.php) for characterizing cell composition of complex tissues from their gene expression profiles,[35] was applied to the enumeration of hematopoietic subsets in mRNA mixtures. CIBERSORT outperformed other methods concerning noise, unknown mixture content, and closely related cell types.[35]

Multiplex immunofluorescence staining

The paraffin blocks of advanced nsqNSCLC with low PD-L1 expression (n=100) were prepared using routine methods and cut into 5 µm slides for staining. Multiplex immunofluorescence staining was performed using the PANO 7-plex IHC kit (Panovue, Beijing, China) to visualize the expression of CD3, CD8, CD56, CD68, PD-1, PD-L1, and CD163. Whole-slide scanning fluorescent images were obtained by the Olympus VS200 (Germany) and analyzed using the QuPath software. Detailed methods were described in previous published articles.[36]

Statistical analysis

To assess the between-group difference, we performed (1) the Fisher exact test for categorical variables, (2) the Mann-Whitney or unpaired t-test for continuous variables, and (3) the Kaplan-Meier method, the log-rank method, and the Cox regression (HR and 95% CI) for survival variables. To evaluate correlation, we implemented the Spearman analysis. All statistical analyses mentioned above were performed using IBM SPSS Statistics V.22 or R V.4.1.2. The nominal level of significance was set as 5%, and all 95% CIs were two-sided.

RESULTS

Clinical features of the low PD-L1-expressing nsqNSCLCs for ISAC development and validation

The patient flow is illustrated in figure 1. Of the total 2,016 nsqNSCLCs obtained from the in-house database, three immunotherapy cohorts (MDACC, MSKCC, and Broad), and the IMPACT database,[15–20] patients without PD-L1 results (n=769) or with high tumorous PD-L1 expression (TPS≥50. n=347), and those who had undergone three or more lines of prior therapy (n=34) or treated with anti-cytotoxic T-cell lymphocyte-4 (anti-CTLA-4, n=76) were excluded. The remaining 790 patients were classified into three sets. The "first-line total set" comprised low PD-L1-expressing nsqNSCLCs receiving first-line ICI-chemo (n=287) or ICI-mono (n=147). Additionally, collected from the IMPACT database, 70 nsqNSCLCs were treated with first-line platinum-doublet chemotherapy originally in an RCT where crossover to ICI was available. These 70 patients were used for the comparison of three first-line treatment options, that is, ICI-chemo, ICI-mono, and chemotherapy alone. Moreover, patients who received second/third-line ICI-chemo (n=15) or ICI-mono (n=271) were included in the "second/third-line test set". The baseline characteristics of the analyzed patients are shown in online supplemental table S6.

ISAC development and validation

The first-line total set was used for ISAC training and validation. Of these, no significant disparity in survival was observed among subjects from different sources (online supplemental figure S1A), suggesting a high level of consistency. The median PFS (mPFS) was 8.3 months in the ICI-chemo group, 5.3 months in the ICI-mono group, and 5.8 months in the chemotherapy group. The ICI-chemo combination delayed progression or death compared with ICI-mono (HR=0.72, 95% CI 0.58 to 0.90, p=0.004) or chemotherapy alone (HR=0.63, 95% CI 0.47 to 0.84, p=0.002, online supplemental figure S1B). These findings are consistent with previously published data on the low PD-L1-expressing subgroup.[5 9 37]

These individuals were randomized into a training set and a validation set (ratio, 2:1; n=290 and 144, respectively) in a treatment and PD-L1 expression-matched manner. In the training set, the interaction effect on PFS between treatment (ICI-chemo vs ICI-mono) and the mutational status of each gene and pathway with a mutational rate over 5.0% (mutation vs wildtype) was calculated (online supplemental table S7). Interaction effects are often difficult to reach statistical significance due to the requirement for large sample sizes. Here, in filtering for mutational events to develop the ISAC, we relaxed the restriction on p values (<0.30) but additionally added the requirement for HR (<0.67 or >1.50). Thereby, it is possible to avoid missing mutational events with predictive values.

In total, 10 mutational events were qualified, including *EGFR*, *TP53*, *STK11*, *PI3KCA*, *NF1*, *APC*, *ATM*, and pathways of WNT, receptor tyrosine kinase, and homologous recombination repair (HRR, figure 2A). *APC* and *ATM* belong to the WNT and HRR pathways, respectively. To avoid duplication, we did not include *APC* and *ATM* in our modeling, but rather the WNT and HRR pathways. In addition, the previously reported *CDKN2A* mutation, which was associated with being unsuitable for ICI-chemo treatment,[15] showed a similar trend in the training set (HR=1.80, p=0.43; online supplemental table S7). However, due to its low mutational rate (3.1%) and its failure to meet the p value requirement (<0.30), it was not considered for inclusion in the ISAC.

The coefficient of each mutational event was determined by a multivariable model (online supplemental methods, figure 2B and online supplemental table S8). As illustrated by calibration curves, the actual and ISAC-predicted 6/12 month PFS rates were highly consistent (figure 2C). The ISAC was irrelevant to age, sex, PD-L1 expression, and TMB (online supplemental table S9), but a high ISAC was linked with poor prognosis in the stage I–III and stage IV nsqNSCLCs obtained from the cBioPortal

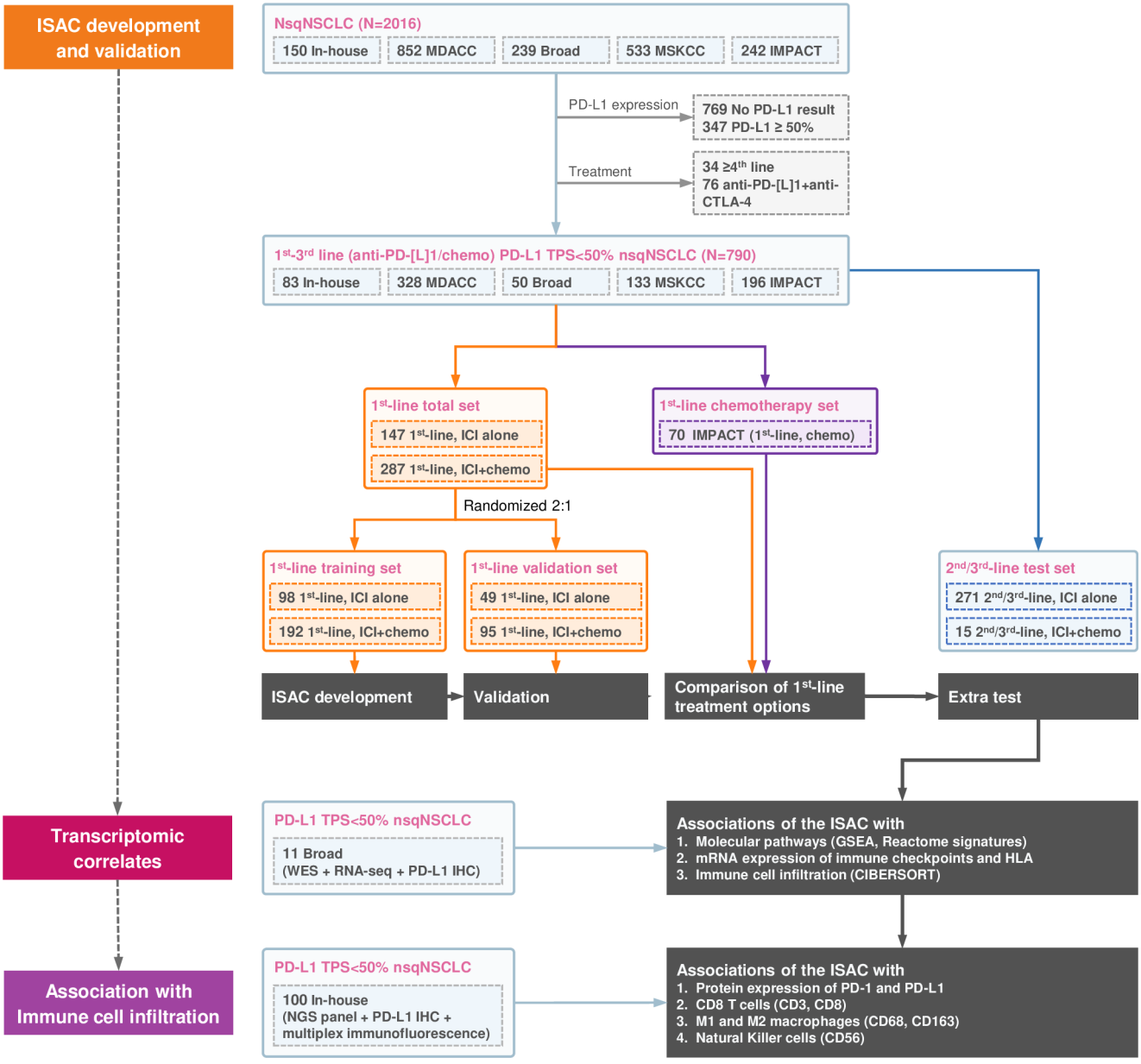

Figure 1 Schematic diagram. anti-CTLA-4, anti-cytotoxic T-cell lymphocyte-4; CIBERSORT, cell-type identification by estimating relative subsets of RNA transcripts; GSEA, gene set enrichment analysis; HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; IHC, immunohistochemical staining; IMPACT, immunotherapeutic predictive and cancer prognostic biomarkers; ISAC, interaction score for additional chemotherapy; MDACC, MD Anderson Cancer Center; mRNA, messenger RNA; MSKCC, Memorial Sloan-Kettering Cancer Center; NGS, next-generation sequencing; nsqNSCLC, non-squamous non-small cell lung cancer; PD-1, programmed cell death protein-1; PD-(L)1, programmed death-(ligand) 1; TPS, tumor proportion score; WES, whole-exome sequencing.

database (online supplemental figure S2), suggesting the aggressiveness of high-ISAC nsqNSCLCs.

In the training set, for each cut-off value ranging from 10th to 90th percentiles, we calculated the treatment effect (ICI-chemo vs ICI-mono) in the below cut-off and the above cut-off subgroups (figure 2D). To define the largest group of patients who could be spared chemotherapy, the cut-off was chosen at 0.000, where the benefit from ICI-chemo over ICI-mono was considerable in the ISAC-low subgroup (proportion: 53.8%, HR=0.44, 95% CI 0.31 to 0.64, p<0.001) while negligible in the ISCA-high subgroup (proportion: 46.2%, HR=1.08, 95% CI 0.70 to 1.65, p=0.67, figure 2E). This cut-off was applicable across different subgroups defined by age, sex, TMB, and PD-L1 expression (online supplemental figure S3). In the validation set, similar predictive power was observed (low-ISAC: HR=0.61, 95% CI 0.40 to 0.92, p=0.016; high-ISAC: HR=1.01, 95% CI 0.47 to 2.15, p=0.98, figure 2F).

Compared with ICI-chemo, patients experiencing progression on ICI-mono still have the chance to receive platinum-doublet chemotherapy as subsequent treatment. Given this, merely estimating PFS benefits is not sufficient

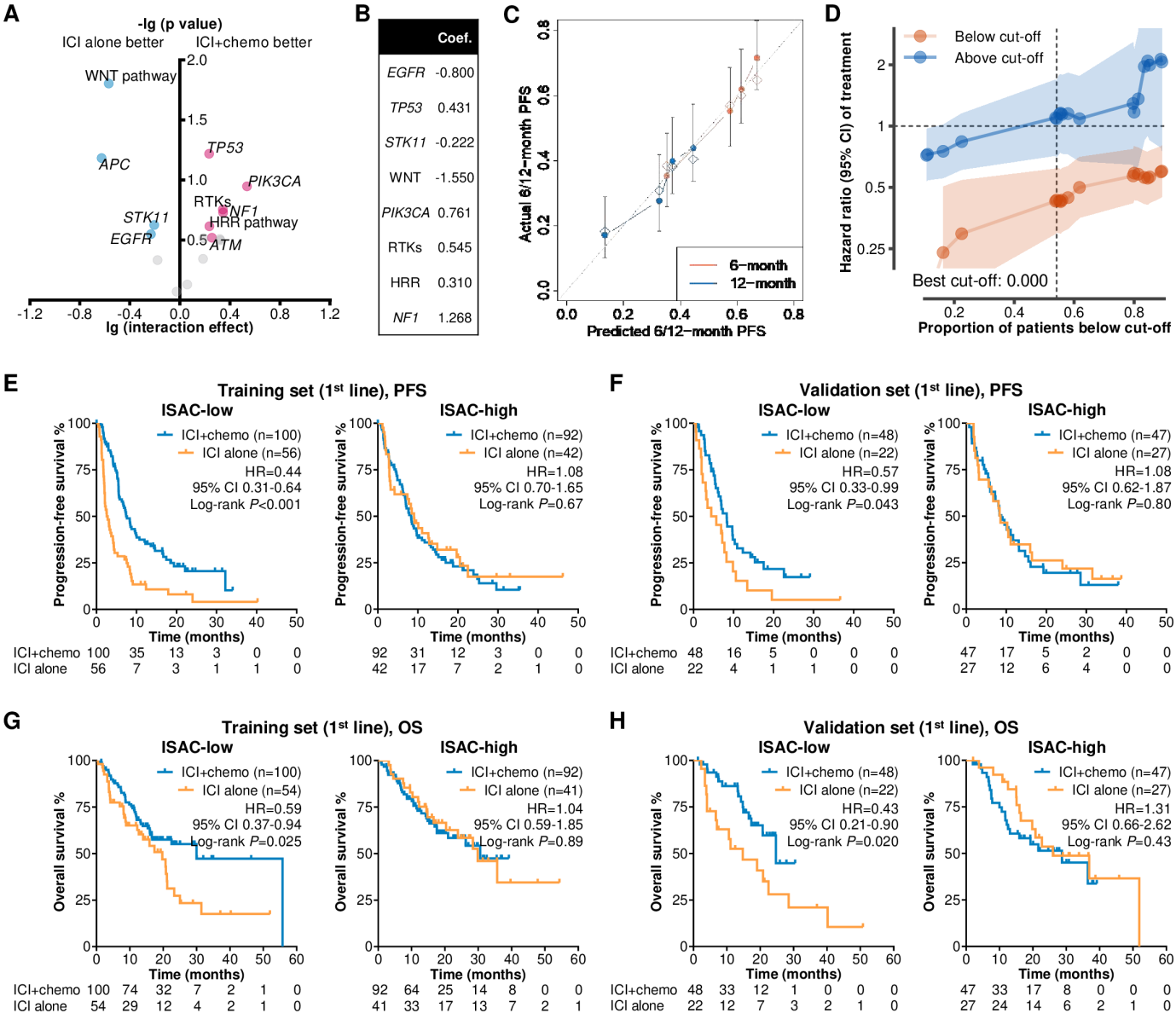

Figure 2 Development and validation of the predictive power of the ISAC. (A) Interaction effect between mutational events (mutated vs wild-type) and treatments (ICI-chemo vs ICI-mono) on PFS. (B) Coefficients of the mutational events for developing the ISAC in the multivariable model. (C) Calibration curves illustrating the association between the actual 6/12 month PFS rate and ISAC-predicted 6/12 month PFS rate in the training set. Solid circle: ideal results. Hollow rhombus and 95% CI: results after bootstrap resampling for 1,000 times. (D) Treatment effect (ICI-chemo vs ICI-mono) in the below cut-off and the above cut-off subgroups. (E–F) PFS benefits from ICI-chemo over ICI-mono in the ISAC-low and the ISAC-high subgroups of the training set (E) and the validation set (F). (G–H) OS benefits from ICI-chemo over ICI-mono in the ISAC-low and the ISAC-high subgroups of the training set (G) and the validation set (H). ICI, immune checkpoint inhibitor; ISAC, interaction score for additional chemotherapy; OS, overall survival; PFS, progression-free survival.

for comparing ICI-chemo and ICI-mono. OS data were available in most patients (287/290) and exhibited an identical trend as PFS (figure 2G,H), further corroborating the predictive value of the ISAC. Moreover, in the second/third-line extra set where patients were likely to have received platinum-doublet chemotherapy, similar predictive utility was observed (online supplemental figure S4). In summary, the benefit from ICI-chemo over ICI-mono was revealed in the low PD-L1-expressing nsqNSCLCs with a low, rather than a high ISAC.

ISAC and survival on first-line treatment options

Among all the patients receiving first-line treatments in our study, we compared survival outcomes on different regimens separately in the ISAC-low and the ISAC-high subgroups. First, the low-ISAC nsqNSCLCs responded poorly to ICI-mono but well to chemotherapy (mPFS: 3.2 vs 7.6 months, HR=1.69, 95% CI 1.12 to 2.54, p=0.008, figure 3A). On top of chemotherapy, additional ICI improved long-term rather than short-term PFS (1-year PFS rate: 35.2% vs 36.3%, p=0.40; 2-year PFS rate: 19.3%

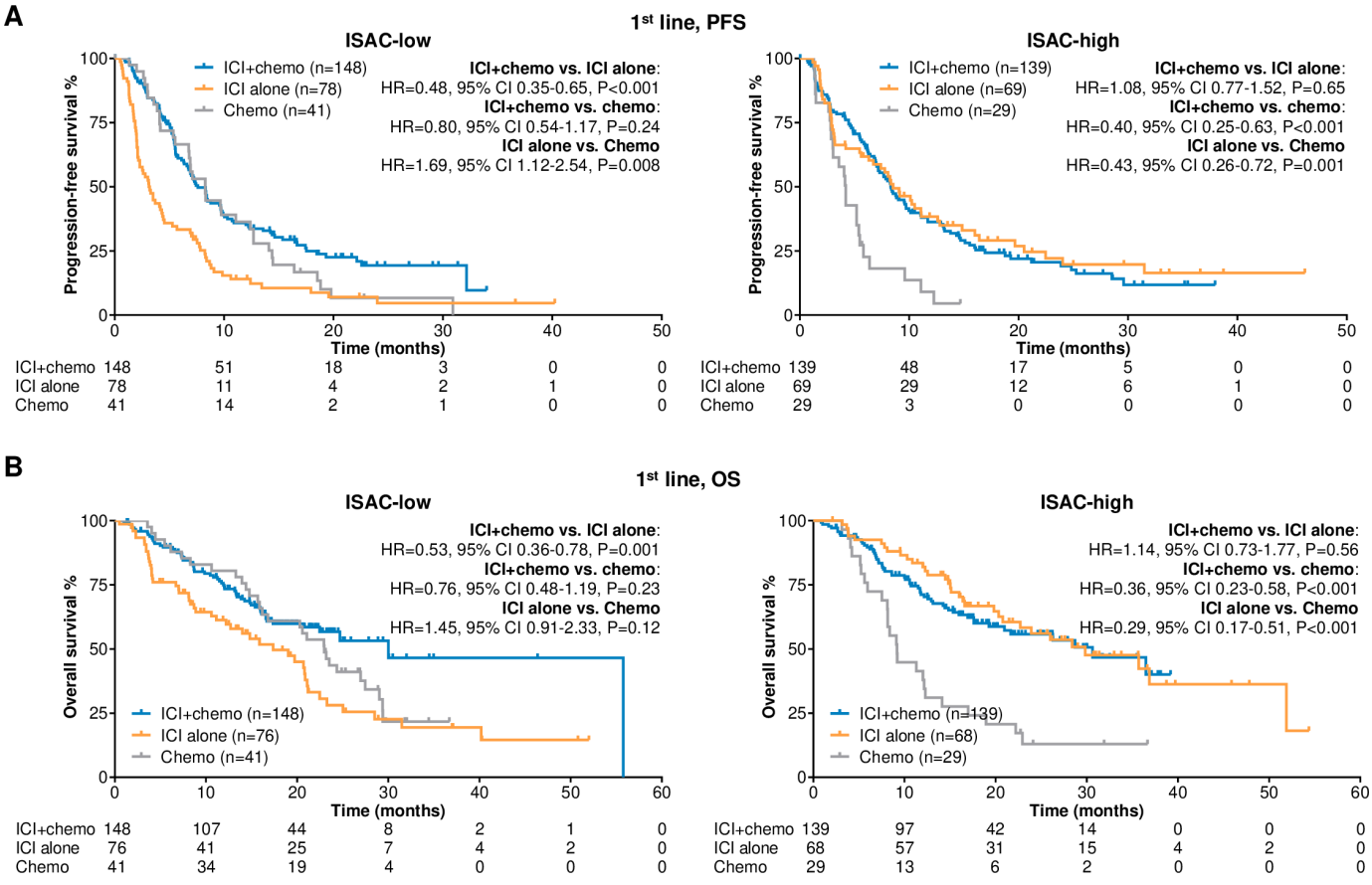

Figure 3 Association between the ISAC and survival on first-line treatment options. (A–B) PFS (A) and OS (B) on first-line ICI-chemo, ICI-mono, and chemotherapy alone in the ISAC-low and ISAC-high subgroups. ICI, immune checkpoint inhibitor; ISAC, interaction score for additional chemotherapy; OS, overall survival; PFS, progression-free survival.

vs 4.7%, p=0.005; figure 3A), which successfully translated into a long-term OS benefit (3-year OS rate: 46.6% vs 21.7%, p=0.031, figure 3B). This pattern of long-term instead of short-term benefits is aligned with prior ICI performance in nsqNSCLCs.[9 10 38 39]

In contrast, the high-ISAC nsqNSCLCs responded well to ICI-mono but poorly to chemotherapy (mPFS: 8.5 vs 4.2 months, HR=0.43, 95% CI 0.26 to 0.72, p=0.001, figure 3A). Based on ICI, the combination with chemotherapy only slightly increased the short-term PFS (4 month-PFS rate: 79.1% vs 66.3%, p=0.066; 6 month-PFS rate: 65.9% vs 61.8%, p=0.34, figure 3A) which failed to elicit both short- and long-term OS benefits (figure 3B). ICI-mono benefited the PD-L1-low/ISAC-high patients to the level comparable with the PD-L1-high patients in cohorts from in-house and public sources (PFS: p=0.97; OS: p=0.67; online supplemental figure S5).

PD-L1 expression and TMB were not significantly associated with the ISAC (online supplemental table S9) whose predictive value was also robust in the subgroups defined by PD-L1 expression (online supplemental figure S6) and TMB (online supplemental figure S7). Moreover, regardless of whether the ISAC was treated as a categorical (high vs low) or a continuous variable, its predictive function was independent of PD-L1 expression and TMB in the multivariable models (online supplemental table S10). Taken together, the ISAC may serve as an independent predictor guiding exemption from chemotherapy in the first-line treatment of low PD-L1-expressing nsqNSCLCs.

Immune-related associations of the ISAC

The Broad cohort includes 11 low PD-L1-expressing advanced nsqNSCLCs with both WES and tissue-bulk RNA-seq data.[20] GSEA between the ISAC-high and the ISAC-low subgroups revealed 144 and 33 pathways respectively enriched in these two subgroups (online supplemental table S11). Of these, 12 immunity-related pathways were illustrated in figure 4A, concerning integrin, interactions between lymphoid and non-lymphoid cells, antigen presentation, B/T cell receptor (B/TCR), interleukins, interferons, and PD-1. We also compared the ISAC-high group with other 24 high PD-L1-expressing samples (TPS≥50) and found that the results of all immunity-related pathways were non-significant (p>0.05) except the gene set namely "antigen activates BCR leading to generation of second messengers" (enriched in high PD-L1-expressing samples, p=0.027; online supplemental table S11). These results suggested adaptive immune resistance in the tumor immune microenvironment (TIME) of the PD-L1-low/ISAC-high nsqNSCLCs, and the degree of this

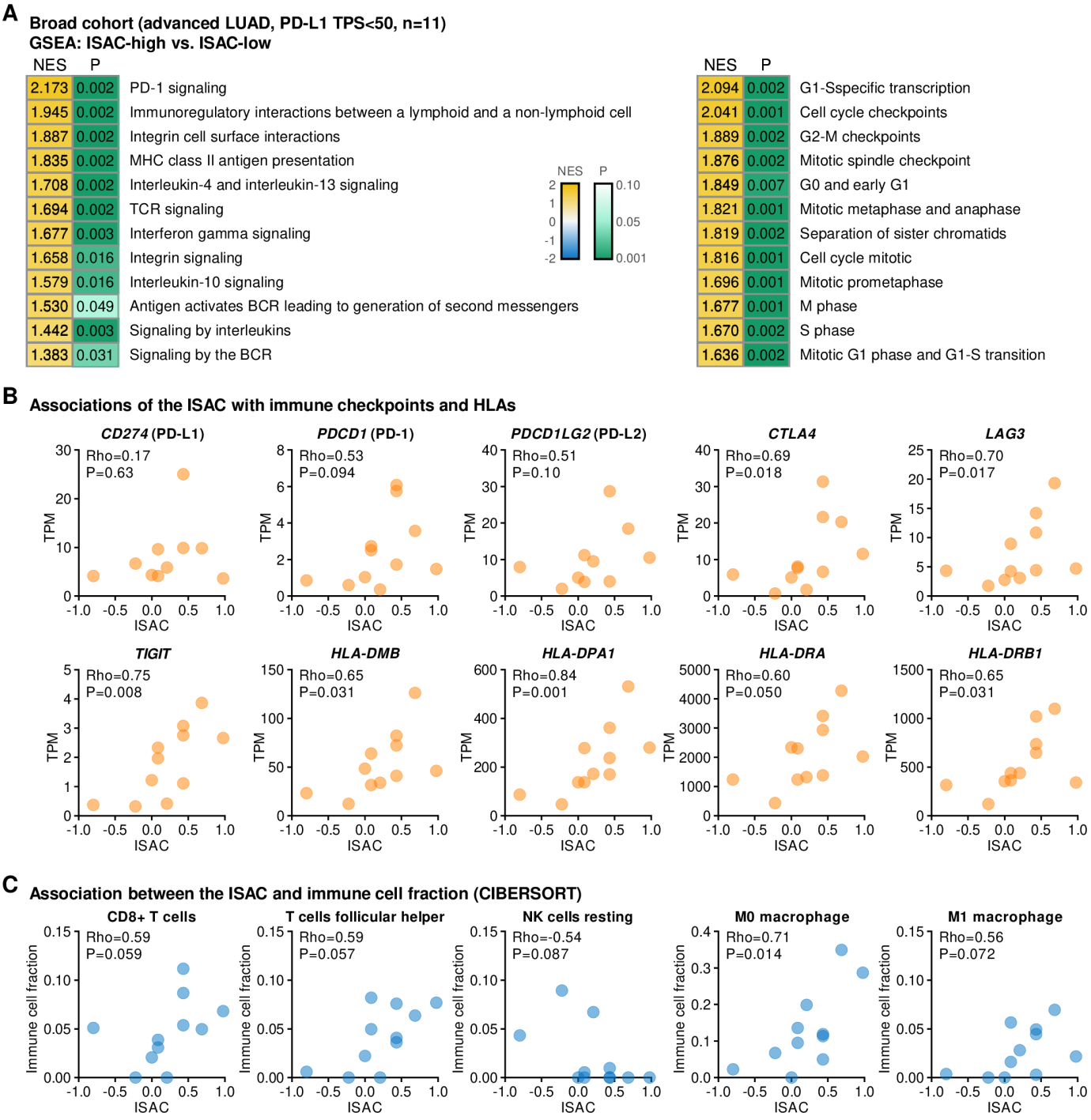

Figure 4 Associations of the ISAC with GSEA signatures, expression of immune checkpoints, and immune cell infiltration in the Broad cohort. (A) .GSEA between the ISAC-low and the ISAC-high subgroups. (B–C) . Associations of the ISAC with immune checkpoints, HLAs (B), and immune cell fraction deconvoluted by the CIBERSORT (C). BCR, B cell receptor; CIBERSORT, cell type identification by estimating relative subsets of RNA transcripts; CTLA-4, cytotoxic T-cell lymphocyte-4; GSEA, gene set enrichment analysis; HLA, human leukocyte antigen; ISAC, interaction score for additional chemotherapy; LUAD, lung adenocarcinoma; MHC, major histocompatibility complex; NES, normalized enrichment score; NK, natural killer; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; TCR, T cell receptor; TPS, tumor proportional score.

resistance was similar to that in the high PD-L1-expressing nsqNSCLCs.

Additionally, in the comparison between the ISAC-high and the ISAC-low samples, 6 out of the 10 most significantly enriched gene sets are related to cell cycle (online supplemental table S11), suggesting the connection between a high ISAC and activation of cell cycle pathways that have been previously suggested to be associated with favorable ICI efficacy.[40] In figure 4A, all the cell cycle-related gene sets with a significant result were presented.

In The Cancer Genome Atlas study, expression of human leukocyte antigens (HLAs) and immune checkpoints largely segregated tumors by immune subtypes, perhaps indicative of their role in shaping the TIME.[41] Here, we observed that the ISAC was not significantly associated with *CD274* (PD-L1) expression (p=0.63, figure 4B), consistent with previous results in the first-line total set (online supplemental table S9). Despite this, positive correlations were revealed between the ISAC and other immune checkpoints (eg, *PDCD1*, *PDCD1LG2*, *CTLA4*, *LAG3*, and *TIGIT*) and several HLAs (figure 4B). In addition, the ISAC trended to be linked with more $CD8^+$ T cells, follicular helper T cells, and M0/1 macrophages and fewer resting NK cells (figure 4C; online supplemental table S12), which were deconvoluted from RNA-seq data by the CIBERSORT algorithm.

Technically, deconvolution is unable to recognize the localization of tumor-infiltrating immune cells, for example, tumorous or stromal area. To address this issue, we implemented multiplex immunofluorescence staining in 100 low PD-L1-expressing advanced nsqNSCLCs. A higher ISAC was associated with more cells with PD-1 rather than PD-L1 positivity in both tumorous and stromal areas (figure 5A), consistent with previous results (figure 4B and online supplemental table S9). Among all results, the correlations of the ISAC with $CD8^+$ cells and the proportion of inflammatory M1 macrophages among all macrophages (M1: $CD68^+/CD163^-$; M2: $CD68^+/CD163^-$) were the most significant, and these correlations were consistent in both tumor and stromal regions (figure 5A). Representative images are displayed in figure 5B with arrowheads highlighting the staining of CD8, CD68 and CD163. These observations in terms of immune pathways, immune checkpoints, HLAs, and immune cells indicate a high level of adaptive immune resistance in the ISAC-high subgroup of low PD-L1-expressing nsqNSCLCs, which may explain why ICI-mono can achieve good efficacy in this population, thereby making the addition of chemotherapy unnecessary.

DISCUSSION

In this study, we developed and validated the ISAC as a predictive biomarker for the added value of platinum-doublet chemotherapy for first-line ICI monotherapy in low PD-L1-expressing nsqNSCLCs. As summarized in figure 6, equivalent survival outcomes on ICI-chemo and ICI-mono were observed in the ISAC-high subgroup, indicating the potential exemption from chemotherapy.

In the ISAC-high nsqNSCLCs with low PD-L1 expression, the favorable response to ICI-mono may be attributed to adaptive resistance induced by overregulation of activated tumor-infiltrating leukocytes (TILs), as reflected by (1) the enrichment of immune-related pathways in GSEA, (2) a higher proportion of proinflammatory versus anti-inflammatory TILs, and (3) elevated expression of immune checkpoints and HLAs. Chemotherapeutic agents are widely considered to facilitate ICIs by leading to immunogenic apoptosis of tumor cells and depleting suppressive tumor-infiltrating immune cells.[42] Here, we found that a high ISAC was associated with high expression of genes related to the pathways concerning apoptotic factor-mediated response and less infiltration of anti-inflammatory M2 macrophage in the tumorous area. Therefore, there is little room for chemotherapy to enhance outcomes in the ISAC-high subgroup, likely resulting in negligible synergistic effects of chemotherapy on ICI-mono Conversely, in the ISAC-low subgroup, ICI-chemo approximately doubled the 3-year OS rate compared with ICI-mono and chemo, suggesting a strong synergy.

Recently, Hong *et al* reported no synergy between ICI and chemotherapy in the first-line treatment for NSCLC, as evidenced by the equivalent OS in patients treated with ICI-mono with subsequent chemotherapy (sequentially) and those treated concurrently.[15] However, in their MDACC cohort, the ICI-chemo subgroup had a smaller proportion of squamous histology, lower PD-L1 expression, and higher disease burden (stage IVb and brain/liver metastasis), compared with those treated sequentially.[15] These biases may lead to an underestimation of the benefits of combination therapy. Here, besides the MDACC cohort, we also included in-house patients and those from other public sources and restricted the population for analysis to low PD-L1-expressing nsqNSCLC, where ICI-chemo delayed progression and death compared with ICI-mono, consistent with a published network meta-analysis.[43]

The treatment effect in the total population cannot simply be generalized to every individual due to the severe heterogeneity in nsqNSCLC, warranting biomarker analyses. Hong *et al* discovered *CDKN2A* alterations as predictors indicating no benefits from ICI-chemo over ICI-mono in NSCLC,[15] which was not validated in our study focusing on low PD-L1-expressing nsqNSCLC. *CDKN2A* is predominantly mutated in squamous NSCLC, not nsqNSCLC,[44 45] leading to the possibility that the predictive value of *CDKN2A* alterations in NSCLC may primarily be attributed to the squamous subtype. Although the ICI-containing regimens brought similar benefits in non-squamous and squamous NSCLCs,[1 46 47] this does not imply consistent potencies of predictive biomarkers for immunotherapy in both subtypes,[48 49] presumably owing to distinct pathophysiology,[45] mutational landscape,[50] and immune microenvironment.[51]

Usually, researchers identified "qualitative" predictive biomarkers that were significantly linked with survival in the intervention arm but not in the control arm.[15 52–54] However, the difference in the association of a biomarker with survival across treatment arms (estimated by the interaction effect between biomarker and treatment choices) is an essential proof of its predictive utility for guiding treatment choices.[55 56] Here, we calculated the interaction effect between each mutational event and treatment option in the training set and developed the ISAC accordingly. The ISAC performed well in the validation set and

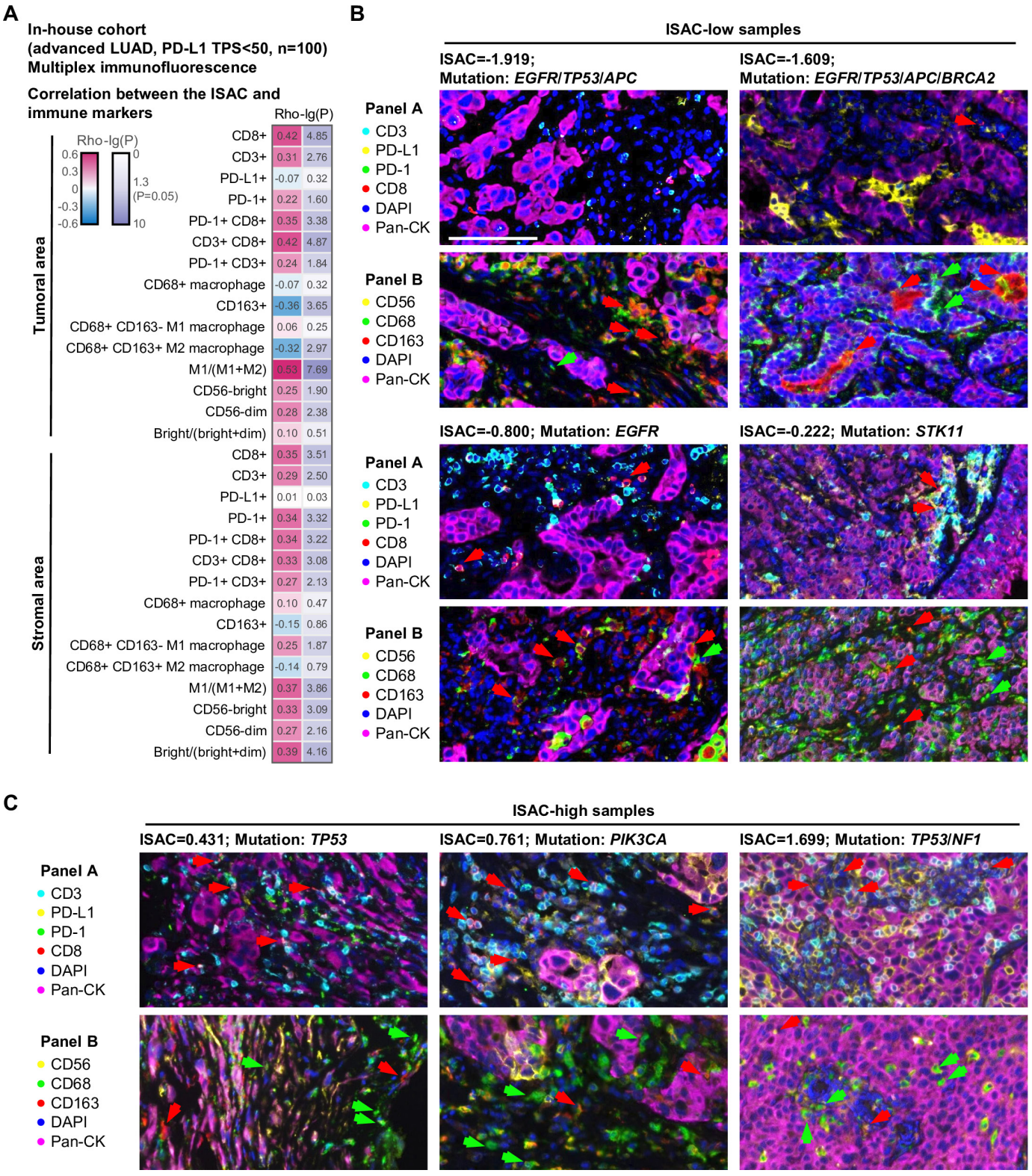

Figure 5 Associations between the ISAC and immune cell infiltration estimated by multiple immunofluorescences in the in-house cohort. (A) Correlation between the ISAC and the intensities of immune cell markers. (B–C) Representative images of the ISAC-low (B) and the ISAC-high samples (C). The ISAC for each sample and its associated mutations are at the top of the graph. Each sample has two representative images, linked with two different panels (panel A: CD3, CD8, PD-1, PD-L1, DAPI, and Pan-CK; panel B: CD56, CD68, CD163, DAPI, and Pan-CK). In panel A, red arrows indicate CD8. In panel B, green and red arrows indicate CD68 and CD163, respectively. Scale bar=100 µm. DAPI, 4',6-diamidino-2-phenylindole; ISAC, interaction score for additional chemotherapy; LUAD, lung adenocarcinoma; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; TPS, tumor proportional score.

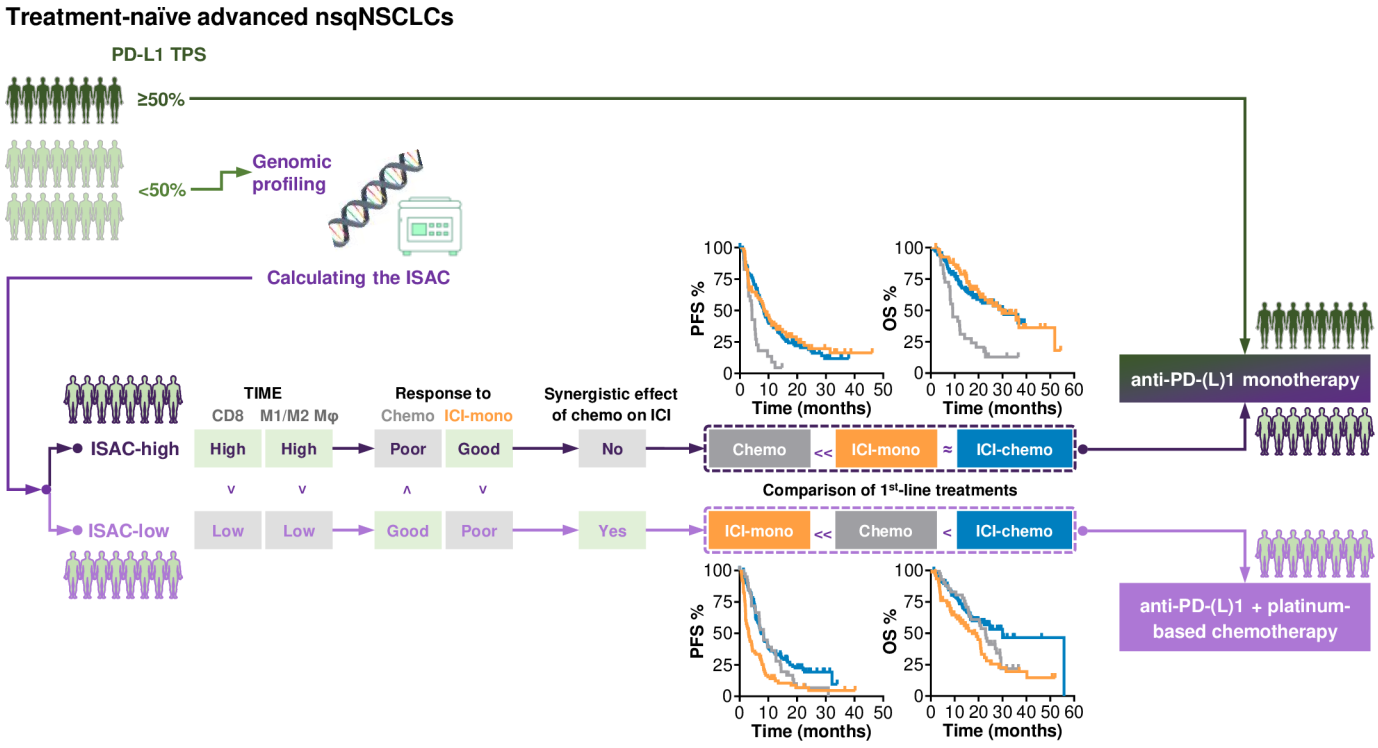

Figure 6 Summary. ICI, immune checkpoint inhibitor; ISAC, interaction score for additional chemotherapy; nsqNSCLC, non-squamous non-small cell lung cancer; OS, overall survival; PD-(L)1, programmed death-(ligand) 1; PFS, progression-free survival; TIME, tumor immune microenvironment; TPS, tumor proportional score.

its predictive utility was independent of PD-L1 and TMB, holding great promise by its broad applicability.

As for limitations, the retrospective nature of this study might confound the outcomes. The ISAC needs validation in prospective cohorts before adoption in clinical practice. The patients treated with ICI-chemo or ICI-mono were obtained from multiple sources, and this multicohort design could balance experimental features and reduce their confounding impacts to some extent. However, the heterogeneity in methods for assessing TMB and PD-L1 across cohorts may introduce biases. The TMB data used in this study were all derived from WES or panels containing more than 150 genes, and the TMB estimated by these large panels has been reported to be highly correlated with those acquired from WES.[18 21 57] The PD-L1 antibodies used in different cohorts are varied (SP263, 22C3, 28–8, E1L3N, and JS311). Of these, the two antibodies for laboratory-developed tests (JS311 and E1L3N) showed great consistency with the other three for PD-L1 standardized assays (22C3, 28–8, and SP263) in previous cross-correlation studies.[58–60] Taken together, the varied methods of assessing TMB and PD-L1 may not impact greatly the outcomes. Moreover, patients undergoing cisplatin/carboplatin plus pemetrexed were all obtained from an RCT recruiting patients solely from China. First, ethnicity was not associated with the efficacy of platinum-doublet chemotherapy in previous trials.[61 62] Second, TMB and PD-L1 expression at baseline were comparable between patients treated with chemotherapy alone and those undergoing ICI-chemo or ICI-mono. Given these, it can be speculated that the conclusion of our study may not be influenced by the single source of the first-line chemotherapy set. Furthermore, copy number variations (CNVs) were not considered in this study, since they are challenging to accurately detect in ctDNA and the cancer samples with a low purity. The potential value of CNVs could be further explored using laser-capture microdissection techniques.

CONCLUSIONS

Through a large retrospective analysis, a high ISAC was identified as a significant predictor for adaptive immune resistance induced by over-regulation of activated TILs and virtually no synergistic effect of platinum-doublet chemotherapy on ICI in low PD-L1-expressing nsqNSCLC. The ISAC-high nsqNSCLCs exhibited short survival on chemotherapy while equivalently long survival on ICI-mono and ICI-chemo, suggesting the feasibility of chemotherapy-free treatment to reduce toxicity. Conversely, favorable responses to chemotherapy while poor responses to ICI were revealed in the ISAC-low subgroup, where a synergistic effect of chemotherapy on ICI was observed, indicating the necessity of chemotherapy as an adjuvant for ICI in this subpopulation. In addition to the PD-L1≥50% subgroup where ICI-mono was approved and recommended as first-line therapy,[3] the ISAC can further distinguish nearly half of PD-L1<50% nsqNSCLCs to be exempted from chemotherapy in the first-line setting. Our findings may help refine personalized therapeutic

strategies for nsqNSCLC, thereby improving efficacy and reducing undue toxicity.

Author affiliations
[1]Translational Oncology Research Lab, Jilin Provincial Key Laboratory of Molecular Diagnostics for Lung Cancer, Jilin Cancer Hospital, Changchun, Jilin, China
[2]Department of Thoracic Oncology, Jilin Cancer Hospital, Changchun, Jilin, China
[3]Oncology Department, Jilin Cancer Hospital, Changchun, Jilin, China
[4]Burning Rock Biotech, Guangzhou, Guangdong, China
[5]Postdoctoral Research Workstation, Jilin Cancer Hospital, Changchun, Jilin, China

Acknowledgements We thank Dizai Shi (Stitch) for his emotional support and the researchers and patients involved in the public data sets analyzed in the present study.

Contributors Conceptualization: YX, and SC. Data curation: HL, JL, LZ, YX, XW, SL, and YC. Formal Analysis: YX and PC. Funding acquisition: YC. Investigation: YX and PC. Methodology: YX. Project administration: HL, GW, SC, and YC. Resources: All authors. Software: YX and PC. Supervision: SC and YC. Validation: GW. Visualization: YX. Writing—original draft: YX. Writing—review and editing: All authors. Guarantors: SC and YC.

Funding This work was funded by the Jilin Provincial Department of Science and Technology (YDZJ202202CXJD009, 20210303002SF, YDZJ202302CXJD059, and 20210204031YY to YC).

Competing interests The authors declare no potential conflicts of interest, except the employment of YX, GW, and SC in Burning Rock Biotech.

Patient consent for publication Not applicable.

Ethics approval All human sample collection and usage followed the principles of the Declaration of Helsinki and were approved by the Institution Review Board of the Jilin Cancer Hospital (202012-01). Written consent was received from all the included patients.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The authors declare that relevant data supporting the findings of this study are available within the paper and its Supplementary files. The references of all the included data sets are shown in online supplementary table S1. Due to ethical and privacy concerns, we are unable to publish their full data in our study. Readers could contact the corresponding authors for access to individual patient-level data for non-commercial purposes.

ORCID iDs
Xinyue Wang http://orcid.org/0000-0002-8634-9590
Ying Cheng http://orcid.org/0000-0001-9908-597X

REFERENCES

1 Ettinger DS, Wood DE, Aisner DL, *et al*. Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022;20:497–530.
2 Camidge DR, Doebele RC, Kerr KM. Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. *Nat Rev Clin Oncol* 2019;16:341–55.
3 National Comprehensive Cancer Network. Non-small cell lung cancer (Version 4.2023). n.d. Available: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
4 Brahmer JR, Lee J-S, Ciuleanu T-E, *et al*. Five-Year Survival Outcomes With Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Metastatic Non-Small-Cell Lung Cancer in CheckMate 227. *J Clin Oncol* 2023;41:1200–12.
5 Gandhi L, Rodríguez-Abreu D, Gadgeel S, *et al*. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* 2018;378:2078–92.
6 West H, McCleod M, Hussein M, *et al*. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 2019;20:924–37.
7 Gogishvili M, Melkadze T, Makharadze T, *et al*. Cemiplimab plus chemotherapy versus chemotherapy alone in non-small cell lung cancer: a randomized, controlled, double-blind phase 3 trial. *Nat Med* 2022;28:2374–80.
8 Wang Z, Wu L, Li B, *et al*. Toripalimab Plus Chemotherapy for Patients With Treatment-Naive Advanced Non–Small-Cell Lung Cancer: A Multicenter Randomized Phase III Trial (CHOICE-01). *JCO* 2023;41:651–63.
9 Herbst RS, Giaccone G, de Marinis F, *et al*. Atezolizumab for First-Line Treatment of PD-L1–Selected Patients with NSCLC. *N Engl J Med* 2020;383:1328–39.
10 Reck M, Rodríguez-Abreu D, Robinson AG, *et al*. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 2016;375:1823–33.
11 Sezer A, Kilickap S, Gümüş M, *et al*. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. *Lancet* 2021;397:592–604.
12 Pérol M, Felip E, Dafni U, *et al*. Effectiveness of PD-(L)1 inhibitors alone or in combination with platinum-doublet chemotherapy in first-line (1L) non-squamous non-small-cell lung cancer (Nsq-NSCLC) with PD-L1-high expression using real-world data. *Ann Oncol* 2022;33:511–21.
13 Pons-Tostivint E, Hulo P, Guardiolle V, *et al*. Real-world multicentre cohort of first-line pembrolizumab alone or in combination with platinum-based chemotherapy in non-small cell lung cancer PD-L1 ≥ 50. *Cancer Immunol Immunother* 2023;72:1881–90.
14 Tanimura K, Takeda T, Kataoka N, *et al*. First-Line Chemoimmunotherapy versus Sequential Platinum-Based Chemotherapy Followed by Immunotherapy in Patients with Non-Small Cell Lung Cancer with ≤49% Programmed Death-Ligand 1 Expression: A Real-World Multicenter Retrospective Study. *Cancers (Basel)* 2023;15:4988.
15 Hong L, Aminu M, Li S, *et al*. Efficacy and clinicogenomic correlates of response to immune checkpoint inhibitors alone or with chemotherapy in non-small cell lung cancer. *Nat Commun* 2023;14:695.
16 Liu Y, Zhang Y, Xie W, *et al*. IMPACT: A web server for exploring immunotherapeutic predictive and cancer prognostic biomarkers. *Clin Transl Med* 2023;13:e1354.
17 Rizvi NA, Hellmann MD, Snyder A, *et al*. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8.
18 Rizvi H, Sanchez-Vega F, La K, *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. *JCO* 2018;36:633–41.
19 Samstein RM, Lee C-H, Shoushtari AN, *et al*. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019;51:202–6.
20 Ravi A, Hellmann MD, Arniella MB, *et al*. Genomic and transcriptomic analysis of checkpoint blockade response in advanced non-small cell lung cancer. *Nat Genet* 2023;55:807–19.
21 Wang Z, Duan J, Cai S, *et al*. Assessment of Blood Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Patients With Non-Small Cell Lung Cancer With Use of a Next-Generation Sequencing Cancer Gene Panel. *JAMA Oncol* 2019;5:696–702.
22 Chen X, Fang L, Zhu Y, *et al*. Blood tumor mutation burden can predict the clinical response to immune checkpoint inhibitors in advanced non-small cell lung cancer patients. *Cancer Immunol Immunother* 2021;70:3513–24.
23 Chen J, Yang H, Teo ASM, *et al*. Genomic landscape of lung adenocarcinoma in East Asians. *Nat Genet* 2020;52:177–86.

24 Xu J-Y, Zhang C, Wang X, *et al*. Integrative Proteomic Characterization of Human Lung Adenocarcinoma. *Cell* 2020;182:245–61.
25 Gillette MA, Satpathy S, Cao S, *et al*. Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma. *Cell* 2020;182:200–25.
26 Schoenfeld AJ, Rizvi H, Bandlamudi C, *et al*. Clinical and molecular correlates of PD-L1 expression in patients with lung adenocarcinomas. *Ann Oncol* 2020;31:599–608.
27 Cerami E, Gao J, Dogrusoz U, *et al*. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2:401–4.
28 Gao J, Aksoy BA, Dogrusoz U, *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6:l1pl1.
29 Lengel HB, Mastrogiacomo B, Connolly JG, *et al*. Genomic mapping of metastatic organotropism in lung adenocarcinoma. *Cancer Cell* 2023;41:970–85.
30 Caeser R, Egger JV, Chavan S, *et al*. Genomic and transcriptomic analysis of a library of small cell lung cancer patient-derived xenografts. *Nat Commun* 2022;13:2144.
31 Campbell JD, Alexandrov A, Kim J, *et al*. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet* 2016;48:607–16.
32 Jee J, Lebow ES, Yeh R, *et al*. Overall survival with circulating tumor DNA-guided therapy in advanced non-small-cell lung cancer. *Nat Med* 2022;28:2353–63.
33 Ellrott K, Bailey MH, Saksena G, *et al*. Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. *Cell Syst* 2018;6:271–81.
34 Subramanian A, Tamayo P, Mootha VK, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–50.
35 Newman AM, Liu CL, Green MR, *et al*. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015;12:453–7.
36 Chen Y, Jia K, Sun Y, *et al*. Predicting response to immunotherapy in gastric cancer via multi-dimensional analyses of the tumour immune microenvironment. *Nat Commun* 2022;13:4851.
37 Mok TSK, Wu Y-L, Kudaba I, *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;393:1819–30.
38 Hellmann MD, Paz-Ares L, Bernabe Caro R, *et al*. Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer. *N Engl J Med* 2019;381:2020–31.
39 Rizvi NA, Cho BC, Reinmuth N, *et al*. Durvalumab With or Without Tremelimumab vs Standard Chemotherapy in First-line Treatment of Metastatic Non-Small Cell Lung Cancer: The MYSTIC Phase 3 Randomized Clinical Trial. *JAMA Oncol* 2020;6:661–74.
40 Dong Z-Y, Zhong W-Z, Zhang X-C, *et al*. Potential Predictive Value of *TP53* and *KRAS* Mutation Status for Response to PD-1 Blockade Immunotherapy in Lung Adenocarcinoma. *Clin Cancer Res* 2017;23:3012–24.
41 Thorsson V, Gibbs DL, Brown SD, *et al*. The Immune Landscape of Cancer. *Immunity* 2018;48:812–30.
42 Salas-Benito D, Pérez-Gracia JL, Ponz-Sarvisé M, *et al*. Paradigms on Immunotherapy Combinations with Chemotherapy. *Cancer Discov* 2021;11:1353–67.
43 Liu J, Li C, Seery S, *et al*. Identifying optimal first-line interventions for advanced non-small cell lung carcinoma according to PD-L1 expression: a systematic review and network meta-analysis. *Oncoimmunology* 2020;9:1746112.
44 Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature New Biol* 2018;553:446–54.
45 Gridelli C, Rossi A, Carbone DP, *et al*. Non-small-cell lung cancer. *Nat Rev Dis Primers* 2015;1:15009.
46 Huo G, Liu W, Chen P. Inhibitors of PD-1 in Non-Small Cell Lung Cancer: A Meta-Analysis of Clinical and Molecular Features. *Front Immunol* 2022;13:875093.
47 Liu L, Bai H, Wang C, *et al*. Efficacy and Safety of First-Line Immunotherapy Combinations for Advanced NSCLC: A Systematic Review and Network Meta-Analysis. *J Thorac Oncol* 2021;16:1099–117.
48 Zhang F, Wang J, Xu Y, *et al*. Co-occurring genomic alterations and immunotherapy efficacy in NSCLC. *NPJ Precis Oncol* 2022;6:4.
49 Bai H, Duan J, Li C, *et al*. EPHA mutation as a predictor of immunotherapeutic efficacy in lung adenocarcinoma. *J Immunother Cancer* 2020;8:e001315.
50 Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. *Nat Rev Cancer* 2019;19:495–509.
51 Yan T, Ma G, Wang K, *et al*. The Immune Heterogeneity Between Pulmonary Adenocarcinoma and Squamous Cell Carcinoma: A Comprehensive Analysis Based on lncRNA Model. *Front Immunol* 2021;12:547333.
52 Motzer RJ, Robbins PB, Powles T, *et al*. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. *N Med* 2020;26:1733–41.
53 Braun DA, Hou Y, Bakouny Z, *et al*. Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *N Med* 2020;26:909–18.
54 Motzer RJ, Choueiri TK, McDermott DF, *et al*. Biomarker analysis from CheckMate 214: nivolumab plus ipilimumab versus sunitinib in renal cell carcinoma. *J Immunother Cancer* 2022;10:e004316.
55 Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *BMJ* 2003;326:219.
56 Ballman KV. Biomarker: Predictive or Prognostic? *J Clin Oncol* 2015;33:3968–71.
57 Tang Y, Li Y, Wang W, *et al*. Tumor mutation burden derived from small next generation sequencing targeted gene panel as an initial screening method. *Transl Lung Cancer Res* 2020;9:71–81.
58 Yang J, Dong L, Yang S, *et al*. Safety and clinical efficacy of toripalimab, a PD-1 mAb, in patients with advanced or recurrent malignancies in a phase I study. *Eur J Cancer* 2020;130:182–92.
59 Adam J, Le Stang N, Rouquette I, *et al*. Multicenter harmonization study for PD-L1 IHC testing in non-small-cell lung cancer. *Ann Oncol* 2018;29:953–8.
60 Rimm DL, Han G, Taube JM, *et al*. A Prospective, Multi-institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. *JAMA Oncol* 2017;3:1051–8.
61 Zinner RG, Obasaju CK, Spigel DR, *et al*. PRONOUNCE: randomized, open-label, phase III study of first-line pemetrexed + carboplatin followed by maintenance pemetrexed versus paclitaxel + carboplatin + bevacizumab followed by maintenance bevacizumab in patients ith advanced nonsquamous non-small-cell lung cancer. *J Thorac Oncol* 2015;10:134–42.
62 Scagliotti GV, Parikh P, von Pawel J, *et al*. Phase III Study Comparing Cisplatin Plus Gemcitabine With Cisplatin Plus Pemetrexed in Chemotherapy-Naive Patients With Advanced-Stage Non–Small-Cell Lung Cancer. *JCO* 2008;26:3543–51.