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JAK2 Loss Arising From Tumor-Spread-Through-Air-Spaces (STAS) Promotes Tumor Progression by Suppressing CD8+ T Cells in Lung Adenocarcinoma: A Machine Learning Approach

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ABSTRACT

Background: Tumor spread through air spaces (STAS) is a recently discovered risk factor for lung adenocarcinoma (LUAD). The aim of this study was to investigate specific genetic alterations and anticancer immune responses related to STAS. By using a machine learning algorithm and drug screening in lung cancer cell lines, we analyzed the effect of Janus kinase 2 (JAK2) on the survival of patients with LUAD and possible drug candidates.

Methods: This study included 566 patients with LUAD corresponding to clinicopathological and genetic data. For analyses of LUAD, we applied gene set enrichment analysis (GSEA), in silico cytometry, pathway network analysis, in vitro drug screening, and gradient boosting machine (GBM) analysis.

Results: The patients with STAS had a shorter survival time than those without STAS ($P < 0.001$). We detected gene set-related downregulation of JAK2 associated with STAS using GSEA. Low JAK2 expression was related to poor prognosis and a low CD8+ T-cell fraction. In GBM, JAK2 showed improved survival prediction performance when it was added to other parameters (T stage, N stage, lymphovascular invasion, pleural invasion, tumor size). In drug

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Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Min KW. Methodology: Choi S, Kim HS, Noh YK, Min KW. Data curation and formal analysis: Choi S, Kim HS, Min KW, Lee JY, Moon JY, Jung US, Kwon MJ, Kim DH, Son BK, Pyo JS, Ro SK. Funding acquisition: Noh YK. Validation: Min KW, Noh YK. Writing - original draft: Choi S, Min KW. Writing - review & editing: Choi S, Kim HS, Min KW, Noh YK, Lee JY, Moon JY, Jung US, Kwon MJ, Kim DH, Son BK, Pyo JS, Ro SK.

screening, mirin, CCT007093, dihydroreteneone, and ABT737 suppressed the growth of lung cancer cell lines with low JAK2 expression.

Conclusion: In LUAD, low JAK2 expression linked to the presence of STAS might serve as an unfavorable prognostic factor. A relationship between JAK2 and CD8+ T cells suggests that STAS is indirectly related to the anticancer immune response. These results may contribute to the design of future experimental research and drug development programs for LUAD with STAS.

Keywords: JAK2; Lung Adenocarcinoma; Tumor Spread Through Air Spaces (STAS); CD8+ T Cells; Machine Learning

INTRODUCTION

Lung cancer is the most dangerous and complex disease. In general, an early American Joint Committee on Cancer (AJCC) stage of lung cancer requires simple curative intent and local therapy, with low recurrence or cancer-related death.^{1,3} Some patients with early-stage disease have a risk of local recurrence and metastatic potential even though curative resection is operable.^{4,5} In patients with advanced or disseminated disease, a more comprehensive, multidisciplinary approach and systemic therapy are necessary.⁶⁻⁹ Recent advances in targeted therapy and immunotherapy for advanced stages have improved the lung cancer survival rate, leading to better clinical outcomes. Some patients in advanced lung cancer stages with long-term survival have also been reported.¹⁰⁻¹³

Several studies have aimed to determine the reason or risk factors for early but dismal lung cancer and investigated molecular markers associated with its detection and prognosis.¹⁴⁻¹⁶ Tumor spread through air spaces (STAS) is a recently discovered risk factor in non-small cell lung cancer (NSCLC). The clinical significance of STAS was actively investigated after its first mention in the World Health Organization (WHO) classification of lung adenocarcinoma (LUAD).¹⁷⁻¹⁹ Recently, the presence of STAS in stage I LUAD and squamous cell carcinoma was associated with a low survival rate compared to the absence of STAS. Additionally, according to the STAS grading system, tumors are divided into two grades, Grade 1 and Grade 2, based on their distance from the main tumor mass (with a cutoff of 2,500 micrometers). Grade 2 is associated with a poorer prognosis than grade 1. On the basis of previous results, the clinicopathological meaning of STAS should be further investigated.^{20,21}

In locally advanced or metastatic NSCLC, different biomarkers have been assessed to determine molecular target therapy with conventional chemotherapy and radiotherapy. In clinical practice, targeted molecular drugs against epidermal growth factor receptor and anaplastic lymphoma kinase and immunotherapies targeting the immune checkpoints programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) contribute to favorable survival for patients with NSCLC.²² Despite the best efforts of clinicians, patients with NSCLC may experience relapse or disease progression, and this situation mandates further emerging targets and novel therapies.

The present study aimed to evaluate clinicopathological factors and survival rates according to the presence of STAS in patients with LUAD. By using bioinformatic analyses, we investigated specific genetic alterations and anticancer immune responses related to STAS. We identified Janus kinase 2 (JAK2), a novel target gene associated with STAS, and then performed pathway network analyses. We investigated drug screening in NSCLC cell lines

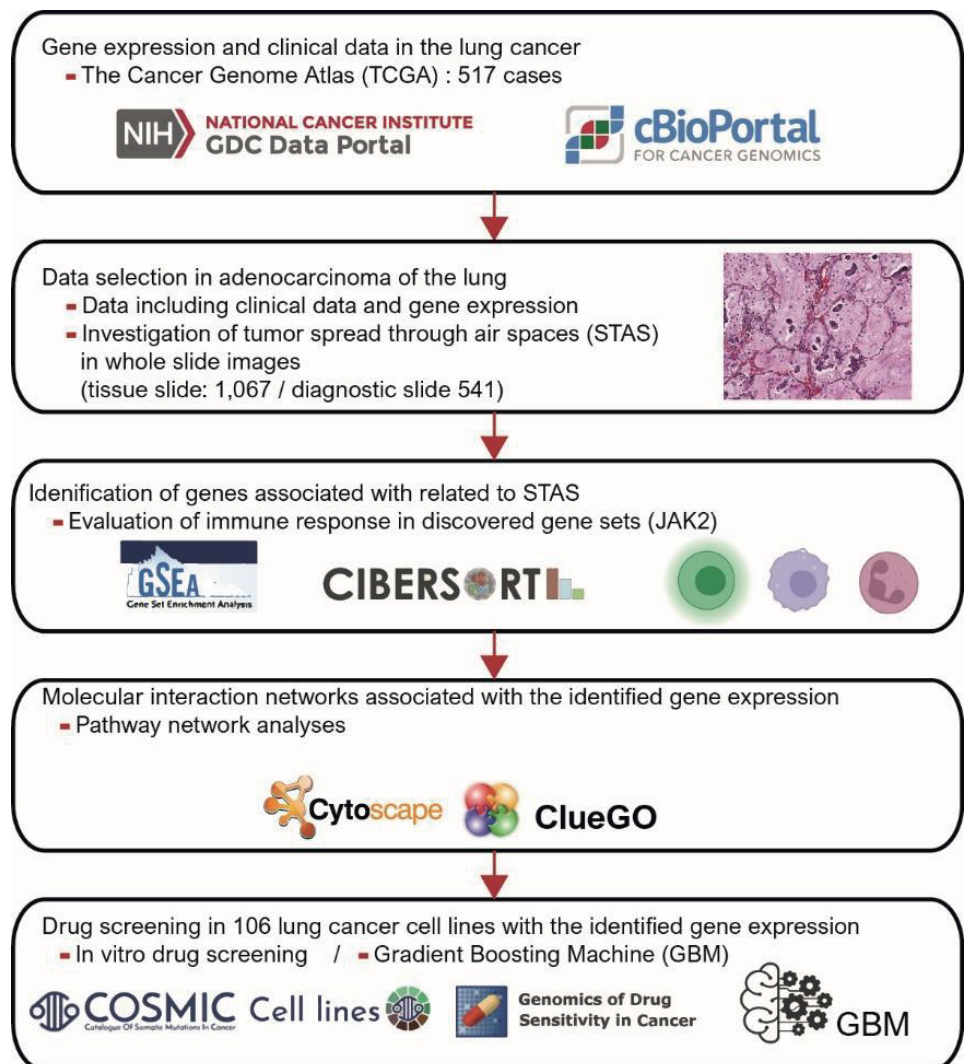


Fig. 1. Schematic diagram depicting the plan of the study.

with JAK2 expression. Using a machine learning (ML) algorithm, we analyzed the effect of JAK2 on the survival of patients with LUAD (Fig. 1).

METHODS

Patient selection and histopathological review

This study included 566 patients with LUAD from National Cancer Institute, Genomic Data Commons (GDC) Data Portal (<https://gdc.cancer.gov/about-data/publications/pancanatlas>) and cBioPortal (<https://www.cbioportal.org/>). The presence of STAS was evaluated through whole-slide images (tissue slides 1067/diagnostic slides 541) for LUAD. STAS was defined as described by Kadota et al.¹⁸, namely, tumor cells within air spaces in the lung parenchyma beyond the edge of the main tumor composed of three morphological patterns: 1) micropapillary structures consisting of papillary structures without central fibrovascular cores, which occasionally form ring-like structures within air spaces; 2) solid nests or tumor

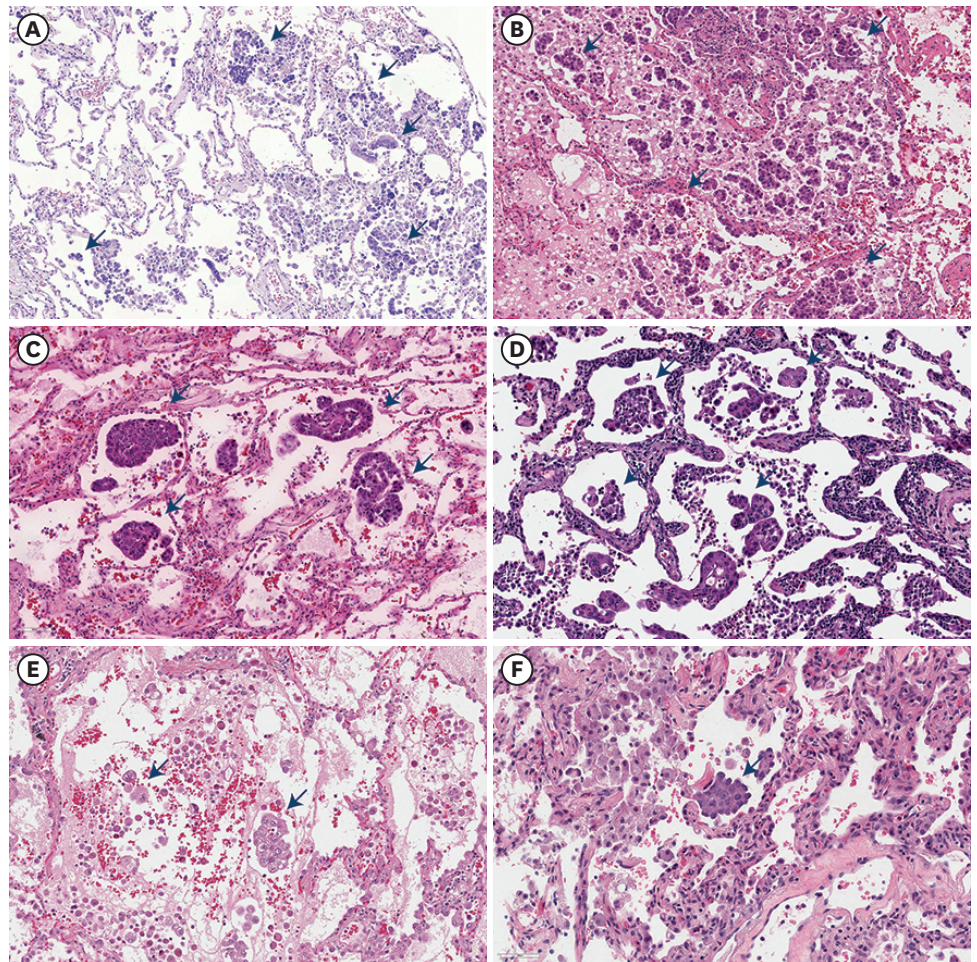


Fig. 2. Representative microphotographs revealing tumor spread through air spaces: (A, B) scattered tumor clusters (black arrow) within the air space (original magnification $\times 100$) (C, D) multiple solid clusters (black arrow) (original magnification $\times 200$) (E, F) single cluster (black arrow) (original magnification $\times 200$).

islands consisting of solid collections of tumor cells filling air spaces; and 3) single cells consisting of scattered discohesive single cells (Fig. 2).

Gene sets, in silico cytometry, and network analysis based on the TCGA database

We selected 517 patients with corresponding RNA-Seq data among 566 LUAD patients. We analyzed significant gene sets using gene set enrichment analysis (GSEA, version 4.3.2).²³ Curated gene sets (C2, 6,495 sets), oncogenic gene sets (C6, 189 gene sets) and immunologic gene sets (C7, 5,219 sets) were used to identify gene sets associated with the presence of STAS. For this analysis, 1,000 permutations were used to calculate *P* values, and the permutation type was set to phenotype; the following cutoffs were used: $P < 0.05$, false discovery rate of < 0.05 and a family wise-error rate of < 0.1 . Through GSEA, expression of JAK2, a gene associated with the presence of STAS, was determined to be low (scores < 361.72) or high (scores > 361.72).

We applied in silico cytometry to investigate proportions of immune cells (<https://cibersortx.stanford.edu/>). For grouping of networks based on functionally enriched Gene Ontology (GO) terms and pathways, pathway network analyses were visualized using Cytoscape

software (version 3.9.1) and ClueGo (version 2.5.8), an application for gene ontology analysis.^{24,25} We used the TIDE tool to identify two biomarkers: TIDE (Tumor Immune Dysfunction and Exclusion) and interferon-gamma (IFN- γ).²⁶

ML algorithm

We integrated JAK2 with clinicopathological parameters (T stage, N stage, tumor size, pleural invasion, lymphovascular invasion, histological subtypes) to construct prognostic models for survival prediction by applying ML algorithms for 517 cases (randomization: training set, 70%; validation set, 30%).^{27,28} A learning algorithm was independently applied to select and combine multiple covariates from gradient boosting machines (GBM) based on multivariate Bernoulli models. To explore the performance of the GBM method, a receiver operator characteristic (ROC) curve was used.

Drug screening

We analyzed the relationship between anticancer drug sensitivity and JAK2 expression based on the Genomics of Drug Sensitivity in Cancer (GDSC) dataset.²⁹ One hundred six NSCLC cell lines were divided into high and low groups based on zero JAK2 expression (**Supplementary Table 1**). In NSCLC cell lines with low (< 0 based on the z score) or high (> 0) JAK2 expression, drug response was defined as the natural log of the half-maximal inhibitory concentration (LN IC50). A drug was identified as effective when the calculated LN IC50 value was decreased in cell lines with low JAK2 expression and increased in those with high JAK2 expression, i.e., when an inverse correlation was observed.

Statistical analysis

Correlations between clinicopathological parameters and JAK2 were analyzed using the χ^2 test. Student's *t*-test and/or Spearman's correlation analysis were applied to examine differences among continuous variables. Survival curves were generated by the Kaplan–Meier method and compared using the log-rank test. A two-tailed *P* value of < 0.05 was considered statistically significant. All data were analyzed using R software packages and SPSS statistics (version 25.0, IBM Corp., Armonk, NY, USA).

Ethics statement

The TCGA program has established the following policy to clarify freedom of TCGA and non-TCGA users to publish or present on findings using TCGA data. There are no limitations on reports containing analyses using any TCGA data set if the data set meets the following freedom-to-publish criteria: A global analysis publication has been published on that tumor type.

RESULTS

Effect of tumor STAS

The patients with STAS had a shorter survival time than those without STAS (disease-specific survival [DSS], $P < 0.001$; overall survival [OS], $P < 0.001$) (**Fig. 3A and B**). The presence of STAS was associated with advanced N stage ($P = 0.02$), poorly differentiated histologic grade ($P = 0.019$), pleural invasion ($P = 0.031$), and lymphovascular invasion ($P = 0.002$) (**Table 1**).

Using GSEA, we identified a JAK2 regulation-related gene set associated with the presence of STAS (**Fig. 3C**). In particular, JAK2 expression was lower in primary LUAD tissues than in normal lung tissues ($P < 0.001$) (**Fig. 3D**). In survival analyses of JAK2 expression, unfavorable

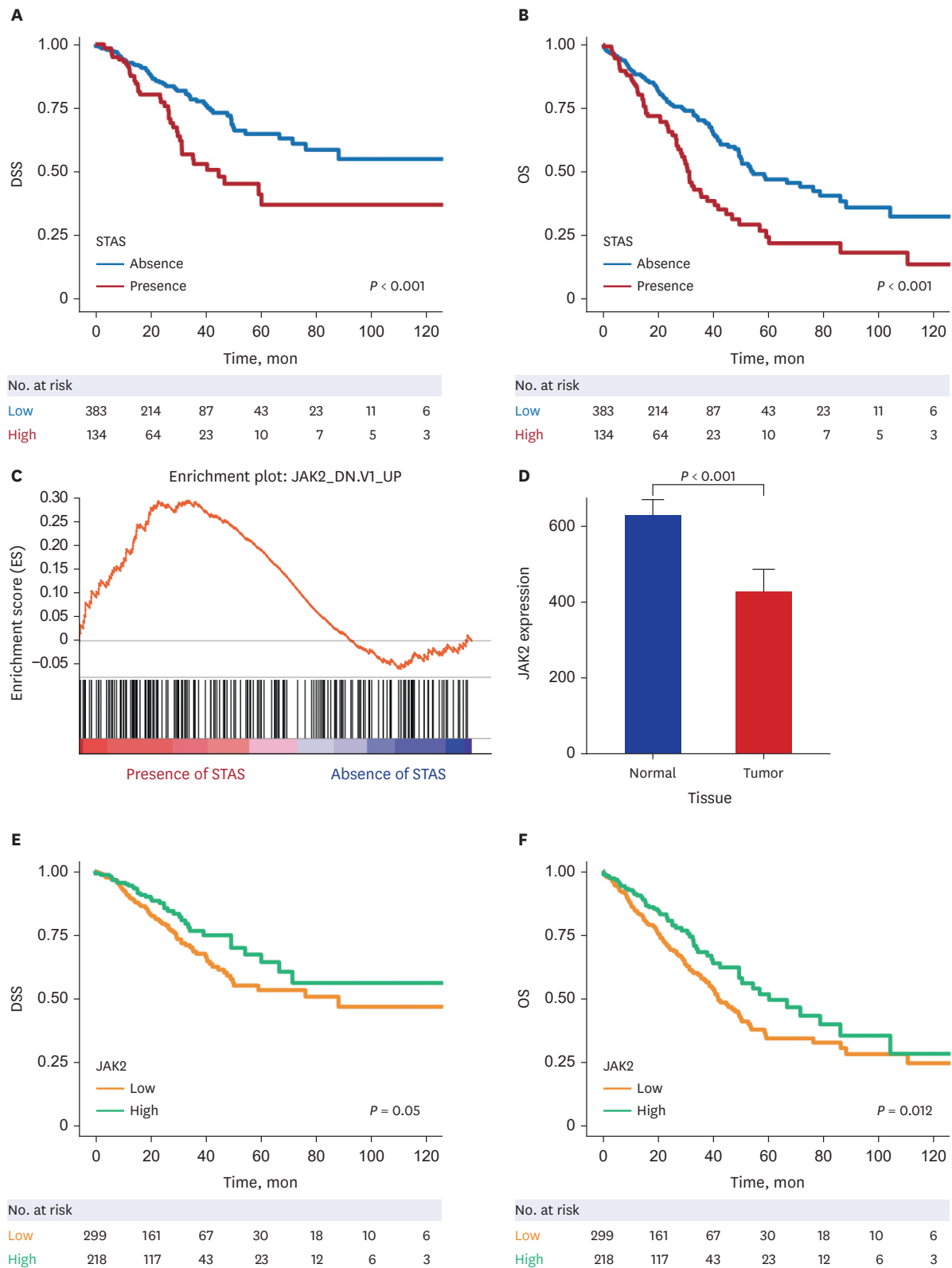


Fig. 3. Survival analyses, gene set enrichment analysis and JAK2 expression. **(A, B)** Tumor STAS was associated with a low survival rate in patients with lung adenocarcinoma. **(C)** Gene set enrichment analysis of JAK2 regulation. **(D)** Bar plots: JAK2 expression was lower in lung adenocarcinoma (DSS, $P < 0.001$; OS, $P < 0.001$). **(E, F)** Low JAK2 expression was related to short survival time in patients with lung adenocarcinoma (DSS, $P < 0.050$; OS, $P = 0.012$). STAS = spread through air spaces, JAK2 = Janus kinase 2, DSS = disease-specific survival, OS = overall survival.

Table 1. Correlation between clinicopathological parameters and tumor STAS in patients with lung adenocarcinoma

Parameters	STAS		P value (χ^2)
	Absence (n = 383)	Presence (n = 134)	
Age, yr	65.6 ± 9.8	64.8 ± 10.2	0.467 ^a
Tumor size, cm	3.9 ± 2.4	3.8 ± 2.3	0.695 ^a
Sex			0.507
Male	174 (45.4)	66 (49.3)	
Female	209 (54.6)	68 (50.7)	
Smoking status			0.201
Non-smoker	72 (18.8)	18 (13.4)	
Former or current	311 (81.2)	116 (86.6)	
T stage			0.881 ^b
1	132 (34.5)	43 (32.1)	
2	151 (39.4)	58 (43.3)	
3	62 (16.2)	19 (14.2)	
4	38 (9.9)	14 (10.4)	
N stage			0.020^c
0	267 (69.7)	78 (58.2)	
1	64 (16.7)	32 (23.9)	
2	51 (13.3)	23 (17.2)	
3	1 (0.3)	1 (0.7)	
Tumor mass			0.999
Single	369 (96.3)	129 (96.3)	
Multiple	14 (3.7)	5 (3.7)	
Histological subtype			0.019^b
Lepidic	6 (1.6)	1 (0.7)	
Acinar	90 (23.5)	23 (17.2)	
Papillary	79 (20.6)	30 (22.4)	
Micropapillary	35 (9.1)	30 (22.4)	
Solid	173 (45.2)	50 (37.3)	
Pleural invasion			0.031
Negative	271 (70.8)	80 (60.2)	
Positive	112 (29.2)	53 (39.8)	
Lymphovascular invasion			0.002
Negative	258 (67.4)	70 (52.2)	
Positive	125 (32.6)	64 (47.8)	
Margin status			0.999
Negative	368 (96.1)	129 (96.3)	
Positive	15 (3.9)	5 (3.7)	

Values are presented as mean ± standard deviation or number (%).

T or N stage, AJCC 8th edition.

STAS = spread through air spaces, T = tumor (American joint committee on cancer [AJCC] staging system, 8th edition), N = lymph nodes (AJCC staging system, 8th edition).

^aStudent's *t*-test.

^bLinear by linear association test.

^cNO vs. N1, 2 or 3.

P < 0.05 is shown in bold.

survival was found for patients with low JAK2 expression (DSS, *P* = 0.050; OS, *P* = 0.012) compared with those with high JAK2 expression (**Fig. 3E and F**). The statistical significance of OS remained after adjustment for confounding factors, including T stage, N stage, pleural invasion, and lymphovascular invasion (*P* = 0.020). However, the statistical significance for DSS disappeared after adjustment (*P* = 0.073) (**Table 2**). In 497 patients, excluding those with positive surgical margins, low JAK2 expression was associated with worse OS (*P* = 0.018) and DSS (*P* = 0.063), with a statistically significant association observed only for OS.

Low JAK2 expression was related to large tumor size (*P* = 0.043), young age (*P* = 0.014), advanced N stage (*P* = 0.029), pleural invasion (*P* = 0.041) and lymphovascular invasion (*P* = 0.011) (**Table 3**).

Table 2. Disease-free and overall survival analyses according to JAK2 expression

Variables	Univariate ^a	Multivariate ^b	HR	95% CI	
Disease-specific survival					
JAK2 (high vs. low)	0.050	0.073	1.432	0.967	2.122
T stage (1 vs. 2, 3, 4)	0.016	0.989	0.997	0.608	1.634
N stage (0 vs. 1, 2, 3)	< 0.001	0.008	1.838	1.174	2.877
Lymphovascular invasion (absence vs. presence)	< 0.001	0.048	1.575	1.004	2.472
Pleural invasion (absence vs. presence)	< 0.001	0.003	1.931	1.257	2.966
Overall survival					
JAK2 (high vs. low)	0.012	0.020	1.443	1.060	1.964
T stage (1 vs. 2, 3, 4)	0.003	0.912	1.022	0.697	1.498
N stage (0 vs. 1, 2, 3)	< 0.001	0.000	1.890	1.327	2.691
Lymphovascular invasion (absence vs. presence)	< 0.001	0.035	1.463	1.027	2.085
Pleural invasion (absence vs. presence)	< 0.001	0.000	1.917	1.370	2.681

JAK2 = Janus kinase 2, HR = hazard ratio, CI = confidence interval, T = tumor (American joint committee on cancer [AJCC] staging system, 8th edition), N = lymph nodes (AJCC staging system, 8th edition).

^aLog rank test.

^bCox proportional hazard model.

P < 0.05 is shown in bold.

Survival prediction of JAK2 using machine learning analyses

We compared the performance of two survival prediction models for predicting survival rates (Model 1; T and N stage, lymphovascular invasion, tumor size, pleural invasion, solid predominant histological subtype and micropapillary predominant histological subtype and JAK2 versus Model 2; T and N stage, lymphovascular invasion, tumor size, pleural invasion, solid predominant histological subtype, micropapillary predominant histological subtype). ROC curves were generated (area under the curve: Model 1, 0.774; Model 2, 0.746). We found that the prediction model showed improved prognostic performance when JAK2 was added (Fig. 4A and B).

Immune cell profiles and pathway network analysis according to JAK2

We analyzed the relationship between JAK2 expression and the immune cell fraction. Decreased CD8⁺ T cells (*P* = 0.014), M1 macrophages (*P* < 0.001) and CD4⁺ memory T cells (*P* < 0.001) were detected in patients with low JAK2 expression. High JAK2 expression was associated with decreased B cells (*P* = 0.010). CD274 expression (encoding PD-L1) was reduced in patients with low JAK2 expression (*P* < 0.001). Low JAK2 expression was associated with high TIDE signature score (*P* < 0.001) and low IFN- γ (*P* < 0.001). JAK2 expression was decreased in patients with anti-PD1 therapy response, but it was not statistically significant (*P* = 0.859). The changes in immune cell profiles related to the presence or absence of STAS did not show statistical significance (Supplementary Fig. 1).

Pathway network analyses demonstrated that JAK2 was directly or indirectly linked to pathways including SLC2A4 vesicle translocation, insulin secretion, immune response and interleukin-6 (IL-6) signaling (Fig. 5).

Drug screening

We analyzed drug sensitivity in NSCLC cell lines. Spearman's correlation analysis showed a high positive correlation between JAK2 and the LN IC50 value for four anticancer drugs: mirin, CCT007093, dihydrorotenone and ABT737. As effective drugs targeting JAK2, these 4 drugs effectively inhibited the growth of NSCLC with low JAK2 expression (Fig. 6).

Table 3. Correlation between clinicopathological parameters and JAK2 in patients with lung adenocarcinoma

Parameters	JAK2		P value (χ^2)
	Low (n = 299)	High (n = 218)	
Age, yr	64.5 ± 10.1	66.7 ± 9.6	0.014^a
Tumor size, cm	4.1 ± 2.3	3.7 ± 2.3	0.043^a
Sex			0.143
Male	147 (49.2)	93 (42.7)	
Female	152 (50.8)	125 (57.3)	
Smoking status			0.630
Non-smoker	50 (16.7)	40 (18.3)	
Former or current	249 (83.3)	178 (81.7)	
T stage			0.292 ^b
1	96 (32.1)	79 (36.2)	
2	121 (40.5)	88 (40.4)	
3	51 (17.1)	30 (13.8)	
4	31 (10.4)	9.6 (21.0)	
N stage			0.029^c
0	188 (62.9)	157 (72)	
1	62 (20.7)	34 (15.6)	
2	48 (16.1)	26 (11.9)	
3	1 (0.3)	1 (0.5)	
Tumor mass			0.341
Single	286 (95.7)	212 (97.2)	
Multiple	13 (4.3)	6 (2.8)	
Histological subtype			0.077 ^b
Lepidic	2 (0.7)	5 (2.3)	
Acinar	62 (20.7)	51 (23.4)	
Papillary	70 (23.4)	39 (17.9)	
Micropapillary	44 (14.7)	21 (9.6)	
Solid	121 (40.5)	102 (46.8)	
Pleural invasion			0.041
Negative	192 (64.4)	159 (72.9)	
Positive	106 (35.6)	59 (27.1)	
Lymphovascular invasion			0.011
Negative	176 (58.9)	152 (69.7)	
Positive	123 (41.1)	66 (30.3)	
Margin status			0.841
Negative	287 (96.0)	210 (96.3)	
Positive	12 (4.0)	8 (3.7)	

Values are presented as mean ± standard deviation or number (%).

T or N stage, AJCC 8th edition.

JAK2 = Janus kinase 2, T = tumor (American joint committee on cancer [AJCC] staging system, 8th edition), N = lymph nodes (AJCC staging system, 8th edition).

^aStudent's t-test.

^bLinear by linear association test.

^cNO vs. N1, 2 or 3.

P < 0.05 is shown in bold.

DISCUSSION

STAS has been actively investigated for its clinical significance and regarded as associated with worse clinical outcomes in NSCLC, though some controversies exist.^{30,31} In the present study, patients with STAS had worse DFS and OS. Subsequently, we investigated genetic alterations to explain the poor clinical outcome according to STAS. GSEA suggested that JAK2 expression is associated with STAS. Thus, we focused on JAK2 expression in patients with LUAD.

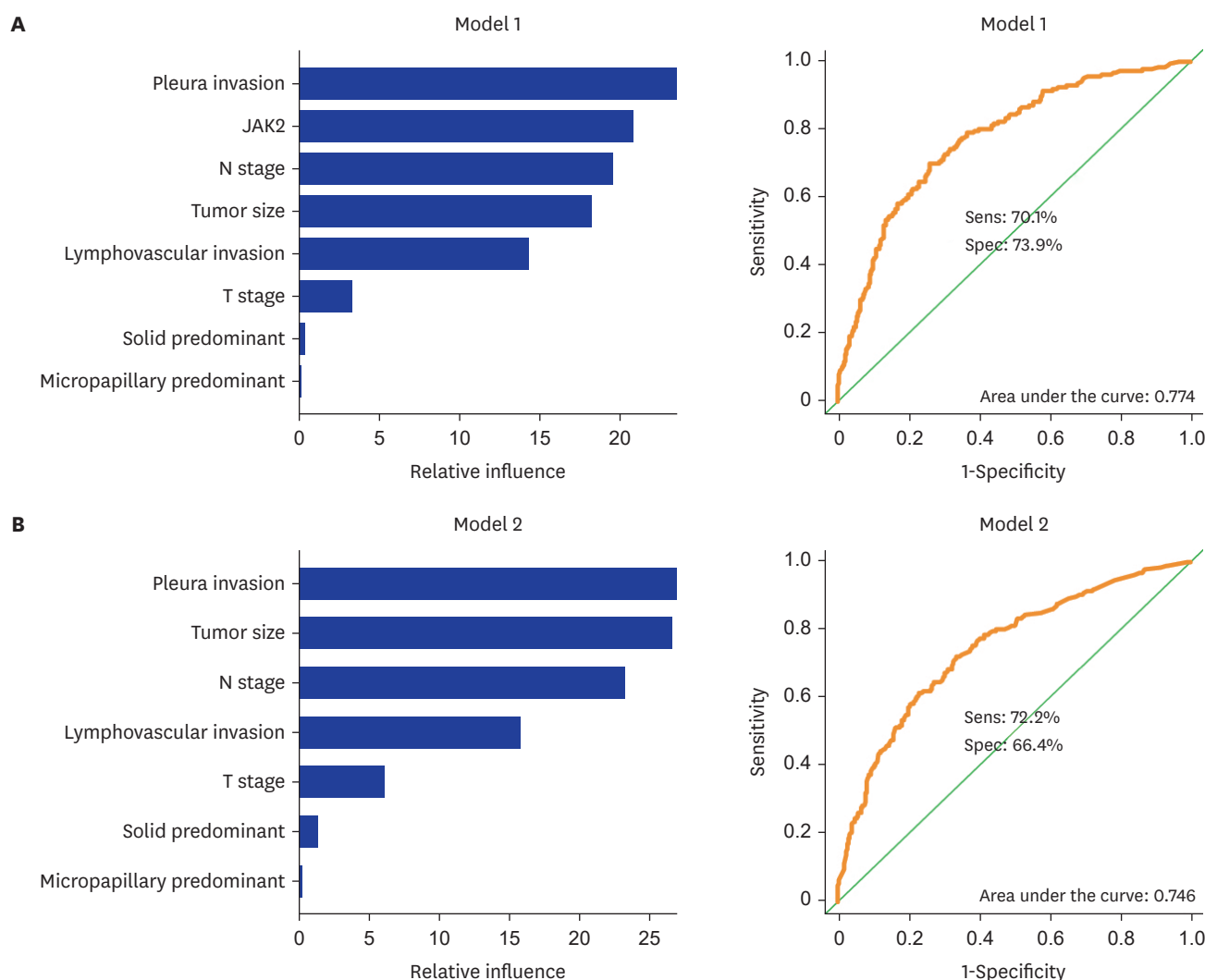


Fig. 4. We applied supervised machine learning models for prognostic prediction using a GBM. The covariates included confounding factors: **(A)** Model 1; JAK2, T stage, N stage, tumor size, lymphovascular invasion, pleural invasion, solid predominant histological subtype and micropapillary predominant histological subtype versus **(B)** Model 2; T stage, N stage, tumor size, lymphovascular invasion, pleural invasion, solid predominant histological subtype and micropapillary predominant histological subtype. And their relative importance based on survival. Receiver operating characteristic curves for the GBM models were generated based on a multivariate Bernoulli model. JAK2 = Janus kinase 2, T = tumor (American joint committee on cancer [AJCC] staging system, 8th edition), N = lymph nodes (AJCC staging system, 8th edition), GBM = gradient boosting machine.

JAK2 has been regarded as a biomarker for the prognosis of acute myelogenous leukemia (AML) and targeted for novel treatment in the modern AML treatment era of relapse after hematopoietic stem cell transplantation and treatment-related toxicities.³² Hepatocellular carcinoma (HCC) is a complex and problematic solid cancer. Sonohara et al.³³ reported the clinical significance of JAK2 in HCC treatment, with somewhat contrary results compared to previous studies. High expression of JAK2 in background normal liver tissue of resected HCC might be a good prognostic biomarker. In addition, the clinical significance of JAK2 in cancer treatment and prognosis has been reported in pancreatic ductal adenocarcinoma and metastatic breast cancer.^{34,35} In NSCLC, JAK2 and its related signaling pathway STAT were found to be associated with PD-L1 upregulation accompanied by simultaneous amplification.³⁶ In our study, JAK2 expression was lower in LUAD. Following the survival curve comparison by STAS frequency, low JAK2 expression was negatively associated with

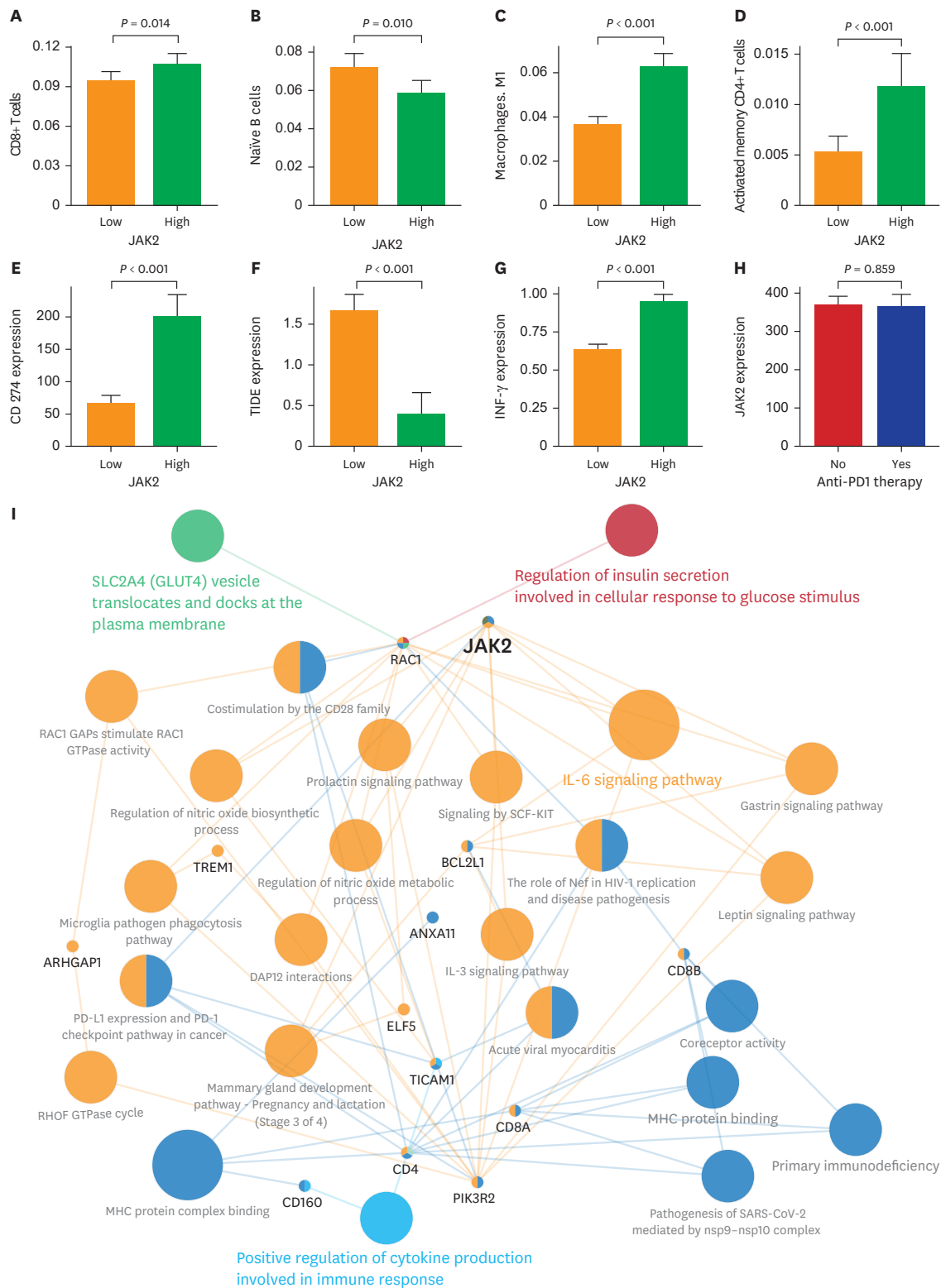


Fig. 5. Bar plots of JAK2 expression according to immune cells. Bar plot of (A) CD8+ T cells, (B) naïve B cells, and (C) macrophages. M1, (D) activated memory CD4+ T cells, (E) CD 274 expression, (F) TIDE signature score, (G) IFN- γ expression between samples with low (orange) and high (green) JAK2 expression ($P = 0.014$, $P = 0.010$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively), (H) JAK2 expression between no-responder (red) and responder (blue) patients ($P = 0.859$) (error bars: standard errors of the mean). (I) Grouping of networks based on functionally enriched Gene Ontology (GO) terms and pathways: JAK2 was directly or indirectly linked to SLC2A4 vesicle translocation, insulin secretion, immune response and IL-6 signaling pathway. JAK2 = Janus kinase 2, PD-1 = programmed death 1, TIDE = Tumor Immune Dysfunction and Exclusion, IFN- γ = interferon-gamma, IL-6 = interleukin-6.

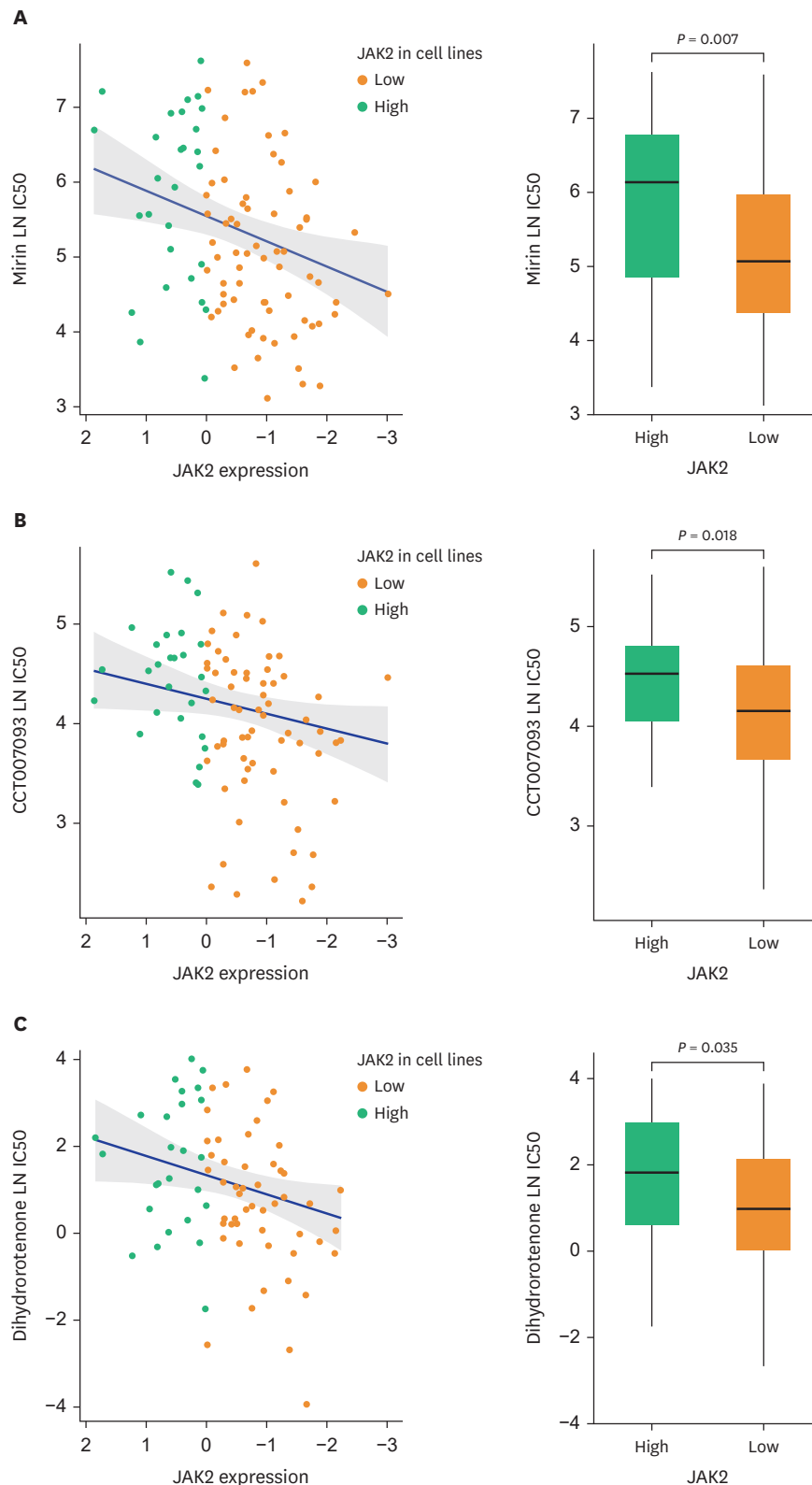


Fig. 6. Genomics of Drug Sensitivity in Cancer (GDSC) database analysis. Spearman's correlations showing the natural log of the half-maximal inhibitory concentration (LN IC50) values of (A, left) Mirin ($r = 0.275$, $P = 0.005$), (B, left) CCT007093 ($r = 0.195$, $P = 0.047$), (C, left) dihydrorotenone ($r = 0.250$, $P = 0.023$) and (D, left) ABT737 ($r = 0.222$, $P = 0.042$) [green, high JAK2 expression; orange, low JAK2 expression]. Bar plot showing the LN IC50 values of (A, right) mirin, (B, right) CCT007093, (C, right) dihydrorotenone and (D, right) ABT737 between non-small cell lung cancer cell lines with high (green) and low (orange) JAK2 expression. JAK2 = Janus kinase 2.

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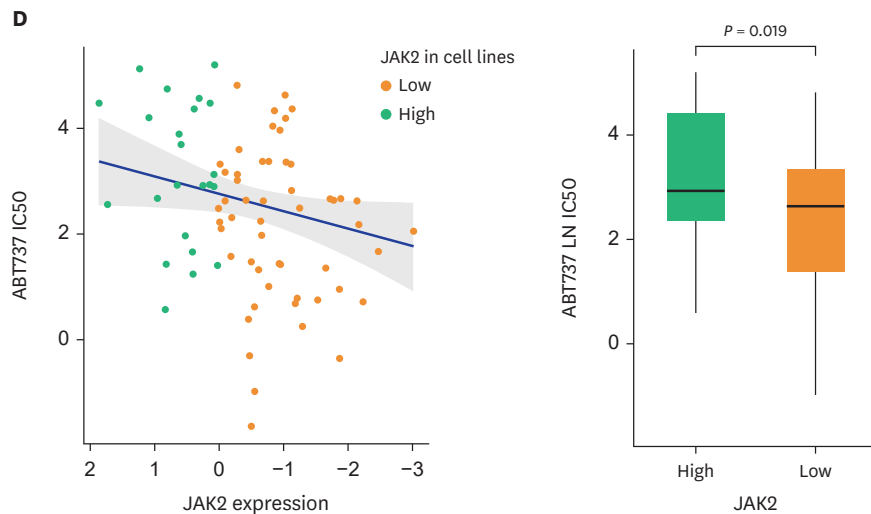


Fig. 6. (Continued) Genomics of Drug Sensitivity in Cancer (GDSC) database analysis. Spearman's correlations showing the natural log of the half-maximal inhibitory concentration (LN IC₅₀) values of (A, left) Mirin ($r = 0.275$, $P = 0.005$), (B, left) CCT007093 ($r = 0.195$, $P = 0.047$), (C, left) dihydrorotenone ($r = 0.250$, $P = 0.023$) and (D, left) ABT737 ($r = 0.222$, $P = 0.042$) [green, high JAK2 expression; orange, low JAK2 expression]. Bar plot showing the LN IC₅₀ values of (A, right) mirin, (B, right) CCT007093, (C, right) dihydrorotenone and (D, right) ABT737 between non-small cell lung cancer cell lines with high (green) and low (orange) JAK2 expression.
JAK2 = Janus kinase 2.

LUAD survival. In clinicopathological analysis and comparison, well-known risk factors for recurrence and poor prognosis, pleural invasion and lymphovascular invasion were significantly higher in the low JAK2 expression group.² From these findings, JAK2, which was previously regarded as an important molecular marker in solid and blood malignancies, might also have a significant role in NSCLC.

In our study, low JAK2 expression was associated with decreased CD8⁺ T cells and macrophages. M1, memory CD4⁺ T cells, and CD 274. The PD-L1-PD-1 axis is an inhibitory system that suppresses the effector functions of activated T cells and functions to inhibit the autoimmune response.³⁶ From these findings, we assume that low JAK2 expression could be related to suppressed immune cell function via increased PD-L1. Therefore, JAK2 might be used as a target or related molecule in developing anticancer drugs and anti-PD-L1 drugs. Next, in pathway network analysis, JAK2 was directly or indirectly linked to the immune response and IL-6 signaling pathway. From these findings, we propose that JAK2 is a potent biomarker for cancer treatment and prediction of prognosis, directly or indirectly.

In vitro drug screening showed that mirin (MRE11 [DNA repair-related protein] - targeted inhibitor), CCT007093 (PPM1D [cell cycle-related protein - targeted inhibitor]), dihydrorotenone (a potent mitochondrial inhibitor), and ABT737 (BCL family [apoptosis regulation] - targeted inhibitor) effectively killed NSCLC cells with low JAK2 expression. With further investigation and experiments, these potent drugs might become alternatives for lung cancer treatment in patients with low JAK2 expression.

In machine learning analyses with well-known prognostic parameters (T stage, N stage, lymphovascular invasion, pleural invasion, tumor size), JAK2 showed improved survival prediction performance when it was added to the above parameters. Again, our study shows the valuable function of JAK2 in lung cancer treatment outcome prediction and prognosis estimation.

This study has some limitations. First, this retrospective study did not consider complex genetic alterations during cancer progression. Second, our results based on in silico analyses need to be experimentally validated. Third, using machine learning, more clinicopathological parameters should be considered for optimization of the survival prediction model.

In summary, we found a significant relationship between JAK2 and STAS in LUAD patients. Low JAK2 expression was related to a shorter survival time with low CD8+ T cells, which was verified by in silico cytometry. In drug screening, mirin, CCT007093, dihydroretene, and ABT737 suppressed the growth of NSCLC with low JAK2 expression. Our results should prompt future studies based on experiments and a larger number of LUAD patients.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

JAK2 expression in lung cancer cell lines in the genomics of drug sensitivity in cancer

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Supplementary Fig. 1

Bar plots of Tumor spread through air spaces (STAS) according to immune cells. Bar plot of (A) CD8+ T cells, (B) naïve B cells, and (C) macrophages. M1, (D) activated memory CD4+ T cells, (E) CD 274 expression, (F) TIDE (Tumor Immune Dysfunction and Exclusion) signature score, (G) interferon- γ expression between samples with absence (blue) and presence (red) of STAS ($P = 0.371$, $P = 0.916$, $P = 0.916$, $P = 0.659$, $P = 0.974$, $P = 0.314$ and $P = 0.102$, respectively), (H) Anti-PD1 therapy response according to STAS [no-responder (gray) and responder (green) patients] ($P = 0.859$) (error bars: standard errors of the mean).

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REFERENCES

1. Detterbeck FC, Boffa DJ, Kim AW, Tanoue LT. The eighth edition lung cancer stage classification. *Chest* 2017;151(1):193-203.
[PUBMED](#) | [CROSSREF](#)
2. Wang C, Wu Y, Shao J, Liu D, Li W. Clinicopathological variables influencing overall survival, recurrence and post-recurrence survival in resected stage I non-small-cell lung cancer. *BMC Cancer* 2020;20(1):150.
[PUBMED](#) | [CROSSREF](#)
3. Yun JK, Lee HP, Lee GD, Kim HR, Kim YH, Kim DK, et al. Recent trends in demographics, surgery, and prognosis of patients with surgically resected lung cancer in a single institution from Korea. *J Korean Med Sci* 2019;34(45):e291.
[PUBMED](#) | [CROSSREF](#)

4. Ekeke CN, Mitchell C, Schuchert M, Dhupar R, Luketich JD, Okusanya OT. Early distant recurrence in patients with resected stage I lung cancer: a case series of "Blast Metastasis". *Clin Lung Cancer* 2021;22(1):e132-5.
[PUBMED](#) | [CROSSREF](#)
5. Masago K, Seto K, Fujita S, Sasaki E, Hosoda W, Kuroda H. Long-term recurrence of completely resected NSCLC. *JTO Clin Res Rep* 2020;1(3):100076.
[PUBMED](#) | [CROSSREF](#)
6. Seung SJ, Hurry M, Walton RN, Evans WK. Real-world treatment patterns and survival in stage IV non-small-cell lung cancer in Canada. *Curr Oncol* 2020;27(4):e361-7.
[PUBMED](#) | [CROSSREF](#)
7. Miller M, Hanna N. Advances in systemic therapy for non-small cell lung cancer. *BMJ* 2021;375(2363):n2363.
[PUBMED](#) | [CROSSREF](#)
8. Park S. Dancing with the surgeon: neoadjuvant and adjuvant immunotherapies from the medical oncologist's perspective. *J Chest Surg* 2023;56(2):67-74.
[PUBMED](#) | [CROSSREF](#)
9. Jung JM, Hyun SJ, Kim KJ. Surgical impacts of metastatic non-small cell lung cancer to the thoracic and lumbar spine. *J Korean Med Sci* 2021;36(7):e52.
[PUBMED](#) | [CROSSREF](#)
10. Reck M, Remon J, Hellmann MD. First-line immunotherapy for non-small-cell lung cancer. *J Clin Oncol* 2022;40(6):586-97.
[PUBMED](#) | [CROSSREF](#)
11. Brahmer JR, Lee JS, Ciuleanu TE, Bernabe Caro R, Nishio M, Urban L, 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(6):1200-12.
[PUBMED](#) | [CROSSREF](#)
12. Isbell JM, Li BT, Gomez DR. The emerging role of local therapy in oligometastatic non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2022;163(3):819-25.
[PUBMED](#) | [CROSSREF](#)
13. Yoo S, Cho WC, Lee GD, Choi S, Kim HR, Kim YH, et al. Long-term surgical outcomes in oligometastatic non-small cell lung cancer: a single-center study. *J Chest Surg* 2023;56(1):25-32.
[PUBMED](#) | [CROSSREF](#)
14. Jung YJ, Oh JJ, Kim Y, Jung JH, Seok M, Lee W, et al. Clinical validation of a protein biomarker panel for non-small cell lung cancer. *J Korean Med Sci* 2018;33(53):e342.
[PUBMED](#) | [CROSSREF](#)
15. Kim DJ, Kim WJ, Lim M, Hong Y, Lee SJ, Hong SH, et al. Plasma CRABP2 as a novel biomarker in patients with non-small cell lung cancer. *J Korean Med Sci* 2018;33(26):e178.
[PUBMED](#) | [CROSSREF](#)
16. Lee S, Kang HG, Choi JE, Lee JH, Kang HJ, Baek SA, et al. The different effect of VEGF polymorphisms on the prognosis of non-small cell lung cancer according to tumor histology. *J Korean Med Sci* 2016;31(11):1735-41.
[PUBMED](#) | [CROSSREF](#)
17. Warth A, Muley T, Kossakowski CA, Goeppert B, Schirmacher P, Dienemann H, et al. Prognostic impact of intra-alveolar tumor spread in pulmonary adenocarcinoma. *Am J Surg Pathol* 2015;39(6):793-801.
[PUBMED](#) | [CROSSREF](#)
18. Kadota K, Nitadori JI, Sima CS, Ujiie H, Rizk NP, Jones DR, et al. Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas. *J Thorac Oncol* 2015;10(5):806-14.
[PUBMED](#) | [CROSSREF](#)
19. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon, France: IARC Press; 2015.
20. Yanagawa N, Shiono S, Endo M, Ogata SY. Tumor spread through air spaces is a useful predictor of recurrence and prognosis in stage I lung squamous cell carcinoma, but not in stage II and III. *Lung Cancer* 2018;120:14-21.
[PUBMED](#) | [CROSSREF](#)
21. Han YB, Kim H, Mino-Kenudson M, Cho S, Kwon HJ, Lee KR, et al. Tumor spread through air spaces (STAS): prognostic significance of grading in non-small cell lung cancer. *Mod Pathol* 2021;34(3):549-61.
[PUBMED](#) | [CROSSREF](#)

22. Sim J, Kim H, Hyeon J, Choi Y, Han J. Anaplastic lymphoma kinase (ALK)-expressing lung adenocarcinoma with combined neuroendocrine component or neuroendocrine transformation: implications for neuroendocrine transformation and response to ALK-tyrosine kinase inhibitors. *J Korean Med Sci* 2018;33(15):e123.
[PUBMED](#) | [CROSSREF](#)
23. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, 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(43):15545-50.
[PUBMED](#) | [CROSSREF](#)
24. Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. *Bioinformatics* 2013;29(5):661-3.
[PUBMED](#) | [CROSSREF](#)
25. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009;25(8):1091-3.
[PUBMED](#) | [CROSSREF](#)
26. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat Med* 2018;24(10):1550-8.
[PUBMED](#) | [CROSSREF](#)
27. Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. *Mod Pathol* 2011;24(5):653-64.
[PUBMED](#) | [CROSSREF](#)
28. Jung WY, Min KW, Oh YH. Increased VEGF-A in solid type of lung adenocarcinoma reduces the patients' survival. *Sci Rep* 2021;11(1):1321.
[PUBMED](#) | [CROSSREF](#)
29. Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, et al. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res* 2013;41:D955-61.
[PUBMED](#)
30. Blaauwgeers H, Flieder D, Warth A, Harms A, Monkhurst K, Witte B, et al. A prospective study of loose tissue fragments in non-small cell lung cancer resection specimens: an alternative view to "spread through air spaces". *Am J Surg Pathol* 2017;41(9):1226-30.
[PUBMED](#) | [CROSSREF](#)
31. Thunnissen E, Blaauwgeers HJ, de Cuba EM, Yick CY, Flieder DB. Ex vivo artifacts and histopathologic pitfalls in the lung. *Arch Pathol Lab Med* 2016;140(3):212-20.
[PUBMED](#) | [CROSSREF](#)
32. Ikezoe T, Kojima S, Furihata M, Yang J, Nishioka C, Takeuchi A, et al. Expression of p-JAK2 predicts clinical outcome and is a potential molecular target of acute myelogenous leukemia. *Int J Cancer* 2011;129(10):2512-21.
[PUBMED](#) | [CROSSREF](#)
33. Sonohara F, Nomoto S, Inokawa Y, Hishida M, Takano N, Kanda M, et al. High expression of Janus kinase 2 in background normal liver tissue of resected hepatocellular carcinoma is associated with worse prognosis. *Oncol Rep* 2015;33(2):767-73.
[PUBMED](#) | [CROSSREF](#)
34. Song Y, Tang MY, Chen W, Wang Z, Wang SL. High JAK2 protein expression predicts a poor prognosis in patients with resectable pancreatic ductal adenocarcinoma. *Dis Markers* 2020;2020:7656031.
[PUBMED](#) | [CROSSREF](#)
35. Economopoulou P, Kotoula V, Koliou GA, Papadopoulou K, Christodoulou C, Pentheroudakis G, et al. Prognostic impact of Src, CDKN1B, and JAK2 expression in metastatic breast cancer patients treated with trastuzumab. *Transl Oncol* 2019;12(5):739-48.
[PUBMED](#) | [CROSSREF](#)
36. Ikeda S, Okamoto T, Okano S, Umemoto Y, Tagawa T, Morodomi Y, et al. PD-L1 is upregulated by simultaneous amplification of the PD-L1 and JAK2 genes in non-small cell lung cancer. *J Thorac Oncol* 2016;11(1):62-71.
[PUBMED](#) | [CROSSREF](#)