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Original Article

Integrated pathway analysis of small cell lung cancer: NOTCH alterations as an independent favorable prognostic factor and DNA damage repair alterations as correlates of tumor mutational burden

Elif Sertesen Camoz, Cengiz Karacin

Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Department of Medical Oncology, University of Health Sciences, Ankara, Türkiye

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Corresponding Author: Elif Sertesen Camoz, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Department of Medical Oncology, University of Health Sciences, Ankara, Türkiye
Email: sertesen@gmail.com

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Abstract

Aim: Small cell lung cancer (SCLC) is characterized by near-universal TP53/RB1 inactivation and high mutation burden, yet prognostic biomarkers beyond clinical stage remain limited. This study aimed to characterize pathway-level genomic alterations—including DNA damage repair (DDR), NOTCH, mismatch repair (MMR), and MYC family—and evaluate their associations with tumor mutational burden (TMB) and overall survival in a whole-genome sequenced SCLC cohort.
Materials and Methods: We analyzed genomic and clinical data from 120 primary SCLC tumors in the University of Cologne cohort, accessed via cBioPortal. A 34-gene panel spanning homologous recombination, DNA damage signaling, Fanconi anemia, MMR, NOTCH (NOTCH1/2), cell cycle regulators (TP53, RB1), and MYC family genes was interrogated. Pathway co-occurrence was assessed using Fisher's exact tests. TMB comparisons used Mann-Whitney U tests. Overall survival was analyzed via Kaplan-Meier estimates, log-rank tests, and multivariable Cox proportional hazards regression adjusted for age, sex, tumor stage, and chemotherapy.
Results: TP53 (85.8%) and RB1 (72.5%) alterations co-occurred strongly (odds ratio [OR] = 20.6, $p < .001$). DDR pathway alterations were present in 26.7% of tumors, with homologous recombination-altered cases showing markedly elevated TMB (median 12.6 vs 7.0, $p = .001$). NOTCH pathway alterations occurred in 16.7% of tumors. In multivariable Cox regression, NOTCH alterations were an independent favorable prognostic factor (hazard ratio [HR] 0.48, 95% confidence interval [CI] 0.24 – 0.94, $p = .03$) after adjustment for age, sex, advanced stage, and chemotherapy. DDR alterations did not independently predict survival (HR 1.14, $p = .64$). Advanced stage independently predicted worse survival (HR 2.38, $p < .001$), while chemotherapy was associated with better survival (HR 0.44, $p = .001$).
Conclusion: In this SCLC cohort, NOTCH and DDR pathways demonstrate distinct biological roles: NOTCH alterations serve as an independent favorable prognostic marker, while DDR alterations associate with elevated TMB without independent prognostic impact. These findings validate NOTCH as a prognostic biomarker in SCLC and support pathway-level genomic assessment in clinical risk stratification.

Keywords: Small cell lung carcinoma, receptors, notch, deoxyribonucleic acid damage, tumor burden, survival analysis

INTRODUCTION

Small cell lung cancer (SCLC) is an aggressive neuroendocrine malignancy accounting for approximately 13 – 15% of all lung cancer cases and is characterized by rapid proliferation, early metastatic dissemination, and poor overall survival, with 5-year survival rates below 7% for extensive-stage disease (1). Despite being recognized as a highly lethal tumor type, therapeutic advances have historically lagged behind those achieved in non-small cell lung cancer (NSCLC). The recent addition of immune checkpoint inhibitors (atezolizumab, durvalumab, and

adebrelimab) to platinum-etoposide chemotherapy represented the first meaningful change in first-line therapy in three decades (2-4), yet median overall survival improvement remains modest (approximately 2 – 3 months).

At the genomic level, SCLC is defined by near-universal biallelic inactivation of TP53 and RB1 tumor suppressor genes, occurring in more than 90% of cases (5,6). Additional recurrent alterations involve genes in the NOTCH signaling pathway, chromatin modifiers such as CREBBP and EP300, and amplification of MYC family members (MYC, MYCL, MYCN) (5-7). Comprehensive molecular subtyping has further refined SCLC classification, identifying distinct transcriptional subtypes with potentially divergent therapeutic vulnerabilities (8-10). Despite extensive genomic characterization, translation of these findings into clinically actionable prognostic or predictive biomarkers has been limited.

DNA damage repair (DDR) pathway alterations have gained attention in SCLC due to their biological plausibility as predictors of response to platinum-based chemotherapy, poly (ADP-ribose) polymerase (PARP) inhibitors, and immunotherapy (11,12). Homologous recombination deficiency, mismatch repair deficiency, and defects in DNA damage signaling genes such as ATM and ATR have been associated with elevated tumor mutational burden (TMB) across cancer types, potentially enhancing tumor immunogenicity (13,14). However, the prognostic significance of DDR alterations in SCLC remains inconsistent across studies (11,12), and comprehensive pathway-level analyses combining multiple DDR components remain limited.

The NOTCH signaling pathway represents another frequently altered axis in SCLC, with inactivating mutations reported in up to 25% of cases (5,15). NOTCH functions as a tumor suppressor in the neuroendocrine context by inhibiting the neuroendocrine differentiation program; its loss of function has been proposed to contribute to SCLC pathogenesis (15,16). Preclinical studies have demonstrated that NOTCH activation suppresses SCLC proliferation and induces lineage plasticity toward a non-neuroendocrine phenotype (15). Nevertheless, the independent prognostic impact of NOTCH pathway alterations in SCLC, adjusted for established clinical risk factors such as tumor stage and chemotherapy receipt, requires further validation in well-characterized cohorts with comprehensive clinical annotation (17,18).

Prior SCLC genomic studies have typically analyzed individual gene alterations or focused on single pathways in isolation. Integrated pathway-level analyses that simultaneously evaluate DDR, NOTCH, mismatch repair, and cell cycle pathways in relation to both TMB and overall survival remain scarce. Moreover, multivariable survival analyses adjusting for established clinical covariates are infrequently reported for pathway-level alterations in SCLC.

In this study, we performed an integrated pathway-level genomic analysis of a whole-genome sequenced SCLC cohort (n = 120) (5) with comprehensive clinical annotation, accessed through cBioPortal (19,20). We specifically aimed to: (i) characterize the landscape of pathway alterations spanning DDR subgroups (homologous recombination, DNA damage signaling, Fanconi anemia), mismatch repair, NOTCH, and MYC family genes; (ii) assess co-occurrence and mutual exclusivity patterns between pathways; (iii) compare TMB levels across altered versus wild-type pathway groups; and (iv) evaluate the independent prognostic significance of pathway alterations using multivariable Cox proportional hazards regression adjusted for age, sex, tumor stage, and chemotherapy.

MATERIALS AND METHODS

Ethics Statement

No institutional ethics committee approval was required for this study because the analysis used exclusively publicly available, de-identified genomic and clinical data and did not involve any direct interaction with human subjects, access to identifiable patient information, or use of biological specimens. This study was conducted in accordance with the Declaration of Helsinki and applicable Turkish national regulations governing research with publicly available de-identified data. The analysis used publicly available de-identified genomic and clinical data from the cBioPortal for Cancer Genomics (https://www.cbioportal.org; study ID: sclc_ucologne_2015), originally generated under institutional ethics committee approval at the University of Cologne as previously reported (5). Per the policy of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital and consistent with international standards (ICMJE recommendations), secondary analyses of publicly available de-identified data do not constitute research on identifiable human subjects and do not require additional institutional ethics committee approval or patient informed consent. Original informed consent was obtained by the primary investigators of the source cohort as detailed in the source publication.

Cohort Selection and Data Source

Genomic and clinical data were obtained from the publicly available Small Cell Lung Cancer cohort from the University of Cologne (5; hereafter referred to as the UCologne cohort), accessed through the cBioPortal for Cancer Genomics (https://www.cbioportal.org; study ID: sclc_ucologne_2015) (19,20). This cohort was selected based on several methodological considerations: (i) it represents the largest publicly available whole-genome sequenced SCLC cohort with matched clinical annotation; (ii) all tumors were profiled using a standardized whole-genome sequencing pipeline, avoiding technical heterogeneity inherent to combining targeted panel and exome-based datasets; (iii) the cohort provides comprehensive clinical variables including overall survival, Union for International Cancer Control (UICC) tumor stage, chemotherapy and radiation therapy status, age, sex, and smoking history, enabling multivariable survival modeling; and (iv) the cohort has served as a reference dataset in SCLC genomics, facilitating comparability with prior work.

Gene Panel and Pathway Definitions

A 34-gene panel was constructed to capture alterations in five functionally defined pathways and key co-driver genes. Pathway memberships were assigned as follows: homologous recombination (HR): BRCA1, BRCA2, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L; DNA damage signaling (DDS): ATM, ATR, CHEK1, CHEK2, TP53BP1, MDC1; Fanconi anemia (FA): FANCA, FANCD2, FANCE, FANCF, FANCI, FANCL, BRIP1; mismatch repair (MMR): MLH1, MSH2, MSH6, PMS2, EPCAM; NOTCH pathway: NOTCH1, NOTCH2; tumor suppressors/co-drivers: TP53, RB1; MYC family: MYC, MYCL, MYCN. The composite DDR pathway comprised HR, DDS, and FA genes (22 genes total). Somatic mutations were queried through the cBioPortal interface using standard variant annotation (non-synonymous, splice-site, nonsense, frameshift, and missense mutations classified as pathogenic or likely pathogenic). For missense variants, pathogenicity was inferred using cBioPortal's default annotations, which incorporate OncoKB and MSK-based functional classifications flagging variants as putative drivers, likely oncogenic, or oncogenic based on curated pathway knowledge and published functional evidence. Formal re-adjudication of individual missense variants of uncertain significance (VUS) using additional in silico predictors was not performed, and VUS were not systematically excluded; this inclusive strategy was chosen to preserve statistical sensitivity for pathway-level signal detection in a modest cohort but may include some passenger events, which we address in the Discussion.

Clinical Variables

The following clinical variables were extracted from the cohort metadata: age at diagnosis (continuous), sex (male/female), UICC tumor stage (I – IV), chemotherapy receipt (yes/no), radiation therapy receipt (yes/no), smoking pack-years (continuous, when available), overall survival (months), and overall survival status (deceased/alive). TMB (nonsynonymous) values were obtained from the original cohort annotations. For multivariable analyses, UICC stage was dichotomized into early (stages I – II) and advanced (stages III – IV) disease.

Statistical Analysis

Pathway alteration frequencies were computed as the proportion of samples harboring at least one alteration in any gene within the pathway. Co-occurrence and mutual exclusivity patterns between pathway pairs were assessed using Fisher's exact tests, with odds ratios (OR) and P values reported. TMB comparisons between pathway-altered and wild-type groups used the Mann-Whitney U test, with medians and interquartile ranges (IQR) reported. Overall survival was estimated using the Kaplan-Meier method with Greenwood confidence intervals. Between-group survival comparisons used the log-rank test. Multivariable Cox proportional hazards regression models were fitted to estimate hazard ratios (HR) and 95% confidence intervals (CI), adjusting for age (continuous), sex (male vs female), advanced stage (III – IV vs I – II), and chemotherapy receipt (yes vs no). Both univariate and multivariable models were constructed for NOTCH and DDR pathway alterations, as well as a composite pathway burden score (sum of DDR, MMR, and NOTCH alterations, range 0 – 3). Models were assessed using likelihood ratio tests. All statistical analyses were performed using Python 3.11 (pandas, numpy, scipy), with Cox regression implemented via Newton-Raphson estimation of the partial likelihood. p values $< .05$ were considered statistically significant. Given the exploratory pathway-focused study design with four pre-specified biologically motivated pathways, we did not apply formal multiple-testing correction (e.g., Bonferroni or Benjamini-Hochberg) to the primary analyses. Secondary and subgroup comparisons (e.g., stage-stratified analyses and pathway-burden associations) were interpreted with additional caution given the increased Type I error risk from multiple comparisons. Findings are consistently framed in terms of effect size, confidence intervals, and biological consistency rather than isolated P-value thresholds. p values are reported to two decimal places (e.g., $p = .03$), with three decimal places for values $< .01$ (e.g., $p = .002$) and as $p < .001$ for very small values, in accordance with AMA Manual of Style 11th edition.

Patient-Derived Xenograft Duplicates

To prevent artificial inflation of sample size and biased survival estimates, patient-derived xenograft (PDX) relapsed sample duplicates present in the broader SCLC dataset were identified and excluded from analyses. The UCologne primary cohort (n = 120) was used for all primary analyses, representing unique primary tumor specimens from individual patients.

RESULTS

Cohort Characteristics

The UCologne cohort comprised 120 primary SCLC tumors with available genomic and clinical data. The median age at diagnosis was 65 years (IQR: 59 – 71), with 63.3% male patients (n = 76) and 36.7% female patients (n = 44). UICC tumor stages were distributed as follows: stage I (n = 42, 35.0%), stage II (n = 22, 18.3%), stage III (n = 32, 26.7%), and stage IV (n = 22, 18.3%), with two cases missing stage information. Overall survival data were available for 110 patients (91.7%), with 74 deaths recorded during follow-up (67.3% event rate). All survival analyses (Kaplan-Meier estimates and Cox regression) were performed on this survival-available subset (n = 110); This survival-available subset (n = 110) was used for all Kaplan-Meier and Cox analyses; corresponding curves are shown in the Results section. Genomic frequency analyses and pathway co-occurrence assessments used the full genomic cohort (n = 120), which accounts for the different denominators between figures. Chemotherapy was administered in 53.3% of patients (64/120) and radiation therapy in 38.3% (46/120). Detailed cohort characteristics are summarized in Table 1.

Table 1. Baseline cohort characteristics (n = 120)

Characteristic	Value
Age, years (median, IQR)	65 (59 – 71)
Sex, n (%)	
Male	76 (63.3)
Female	44 (36.7)
UICC Stage, n (%)	
I	42 (35.0)
II	22 (18.3)
III	32 (26.7)
IV	22 (18.3)
Missing	2 (1.7)
Chemotherapy, n (%)	64 (53.3)
Radiation therapy, n (%)	46 (38.3)
OS data available, n (%)	110 (91.7)
Deaths during follow-up, n (%)	74 (67.3 of available)

IQR: interquartile range, OS: overall survival, UICC: Union for International Cancer Control

Pathway Alteration Landscape

Consistent with established SCLC biology, alterations in TP53 (85.8%, 103/120) and RB1 (72.5%, 87/120) were the most frequent events and demonstrated strong co-occurrence (OR = 20.6, p < .001), with 70.0% (84/120) of tumors harboring dual inactivation. The composite DDR pathway was altered in 26.7% (32/120) of tumors, comprising HR alterations in 10.0% (12/120), DDS alterations in 14.2% (17/120), and FA pathway alterations in 5.8% (7/120). MMR alterations were observed in 5.0% (6/120). NOTCH pathway alterations affected 16.7% (20/120) of tumors, with NOTCH1 alterations in 13.3% (16/120) being more common than NOTCH2 alterations. MYC family alterations were infrequent (2.5%). Individual gene frequencies and pathway-level alteration rates are shown in Figure 1.

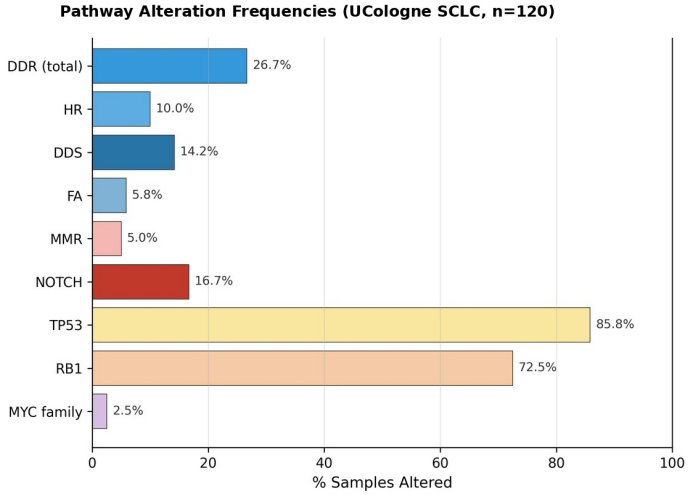

Figure 1. Pathway alteration frequencies in the UCologne SCLC cohort (n = 120); Bar chart showing alteration frequencies of pathway-level and individual gene events across DDR (total, homologous recombination, DNA damage signaling, Fanconi anemia, mismatch repair), NOTCH, TP53, RB1, and MYC family categories

Among DDR genes, the most frequently altered were ATR (4.2%), followed by ATM, CHEK1, and BRIP1 (3.3% each). Detailed oncoprint visualization (Figure 2) illustrates the distribution of alterations across the cohort, sorted by pathway status.

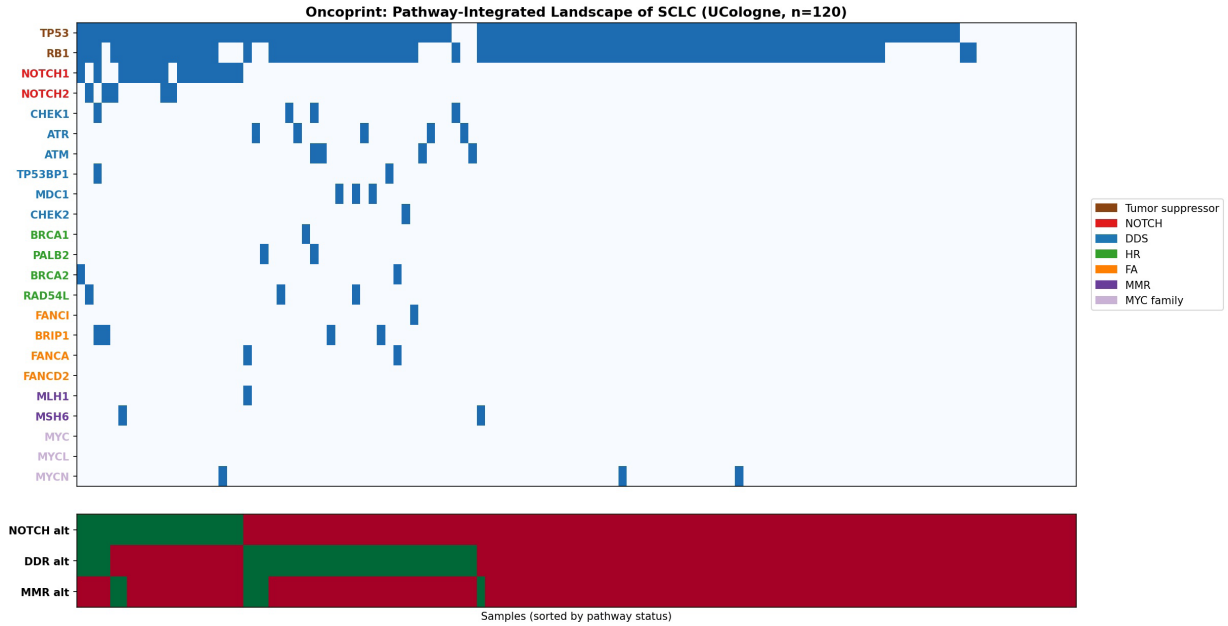

Figure 2. Oncoprint visualization of pathway-level alterations in 120 SCLC tumors. Each column represents an individual tumor; rows represent selected genes, with gene labels colored by pathway membership (legend at right); The lower tracks indicate NOTCH, DDR, and MMR pathway alteration status for each tumor (green, altered; red, wild-type); Tumors are sorted by pathway status

Pathway Co-occurrence and Mutual Exclusivity

Pairwise pathway co-occurrence analyses revealed that, beyond the well-established TP53/RB1 co-occurrence (OR = 20.6, p < .001), no pathway pairs reached statistical significance (Figure 3). However, notable non-significant trends included: (i) DDR and NOTCH alterations showed a trend toward mutual exclusivity (OR = 0.64, p = .59), with only 4 cases harboring both; (ii) NOTCH and TP53 alterations co-occurred in all 20 NOTCH-altered cases, suggesting NOTCH alterations arise on a TP53-altered background (Fisher OR = infinity, p = .07). These patterns are biologically consistent with the sequential model of SCLC pathogenesis in which TP53/RB1 loss precedes secondary events.

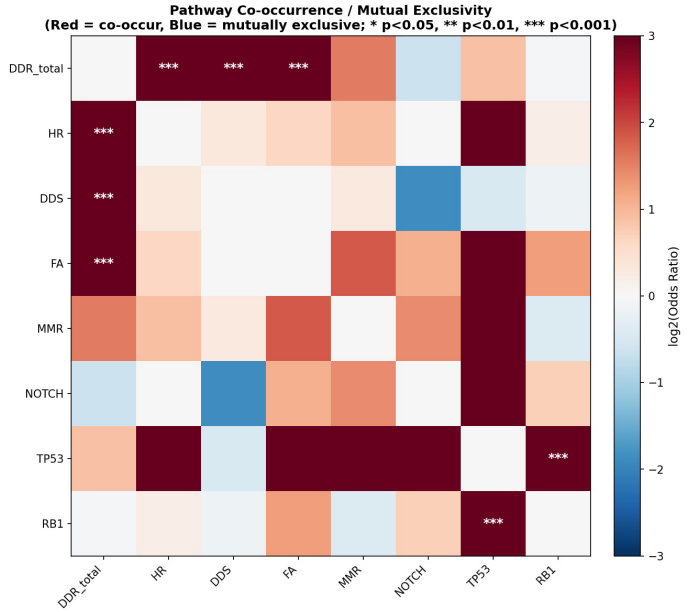

Figure 3. Pathway co-occurrence and mutual exclusivity heatmap; Pairwise Fisher's exact test odds ratios (color scale) and P values are shown for major pathway pairs; The strong co-occurrence between TP53 and RB1 alterations (OR = 20.6, p < .001) is the most prominent feature

DDR and NOTCH Alterations Are Associated with Elevated Tumor Mutational Burden

TMB distributions were compared between pathway-altered and wild-type groups (Figure 4). DDR-altered tumors exhibited significantly higher median TMB compared with DDR-wild type tumors (9.95 vs 6.82, p < .001). Among DDR subgroups, HR-altered tumors showed the most pronounced TMB elevation (median 12.6, IQR 10.9 – 16.5, vs 7.0, IQR 4.6 – 10.1 in HR-wild type; p = .001). DDS-altered tumors also showed elevated TMB (median 10.0 vs 7.0, p = .03). FA-altered tumors and MMR-altered tumors did not show statistically significant TMB differences, likely reflecting limited sample sizes (n = 7 and n = 6, respectively). NOTCH-altered tumors demonstrated modestly elevated TMB (median 8.5 vs 7.0, p = .03). These findings are consistent with the mechanistic link between impaired DDR function, accumulated mutations, and elevated overall mutational burden.

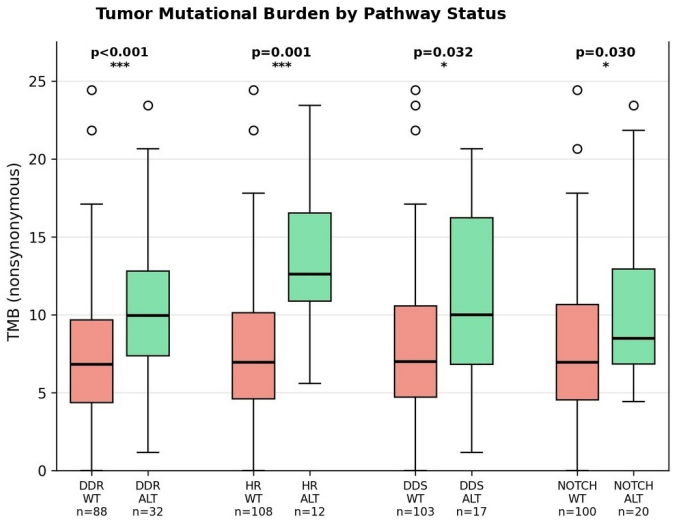

Figure 4. Tumor mutational burden (TMB) by pathway status; Boxplots comparing tumor mutational burden (nonsynonymous mutations) between pathway-altered and wild-type groups (DDR, HR, DDS, NOTCH); Median values and interquartile ranges are shown; p-values from Mann-Whitney U test

NOTCH Alterations Are an Independent Favorable Prognostic Factor

In univariate Kaplan-Meier analysis, NOTCH-altered SCLC demonstrated a marked trend toward prolonged overall survival compared with NOTCH-wild type cases (median OS 72 vs 22 months, log-rank p = .07; Figure 5). While this univariate association did not reach conventional statistical significance, it prompted multivariable analysis to determine whether the effect persisted after adjustment for established prognostic covariates.

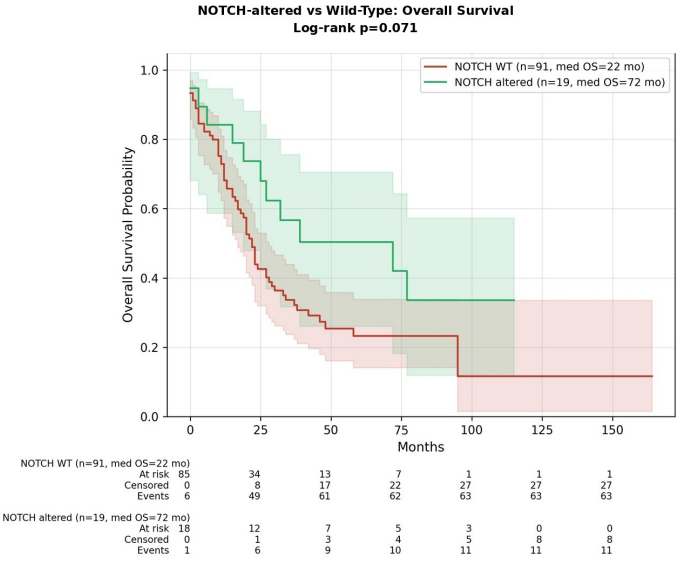

Figure 5. Overall survival (OS) by NOTCH pathway status; Kaplan-Meier overall survival curves comparing NOTCH-altered (n = 19) and NOTCH-wild type (n = 91) tumors in the survival-available subset (n = 110); Median OS: 72 vs 22 months; log-rank p = 0.17; Number-at-risk table shown below

In multivariable Cox regression adjusting for age, sex, advanced stage (III – IV), and chemotherapy receipt, NOTCH alterations emerged as an independent favorable prognostic factor (HR 0.48,

95% CI 0.24 – 0.94, p = .03; Figure 6 and Table 2). Advanced stage was independently associated with increased mortality (HR 2.38, 95% CI 1.46 – 3.89, p < .001), while chemotherapy receipt was independently associated with improved survival (HR 0.44, 95% CI 0.27 – 0.72, p = .001). Age and sex did not independently predict survival. In a full multivariable model simultaneously including NOTCH and DDR alterations, NOTCH retained its independent favorable prognostic significance (HR 0.48, p = .03), while DDR alterations showed no independent survival effect (HR 1.14, 95% CI 0.66 – 1.99, p = .64). These findings indicate that NOTCH and DDR pathways have distinct roles in SCLC: NOTCH alterations carry independent prognostic weight, whereas DDR alterations relate primarily to mutational burden without an independent survival impact.

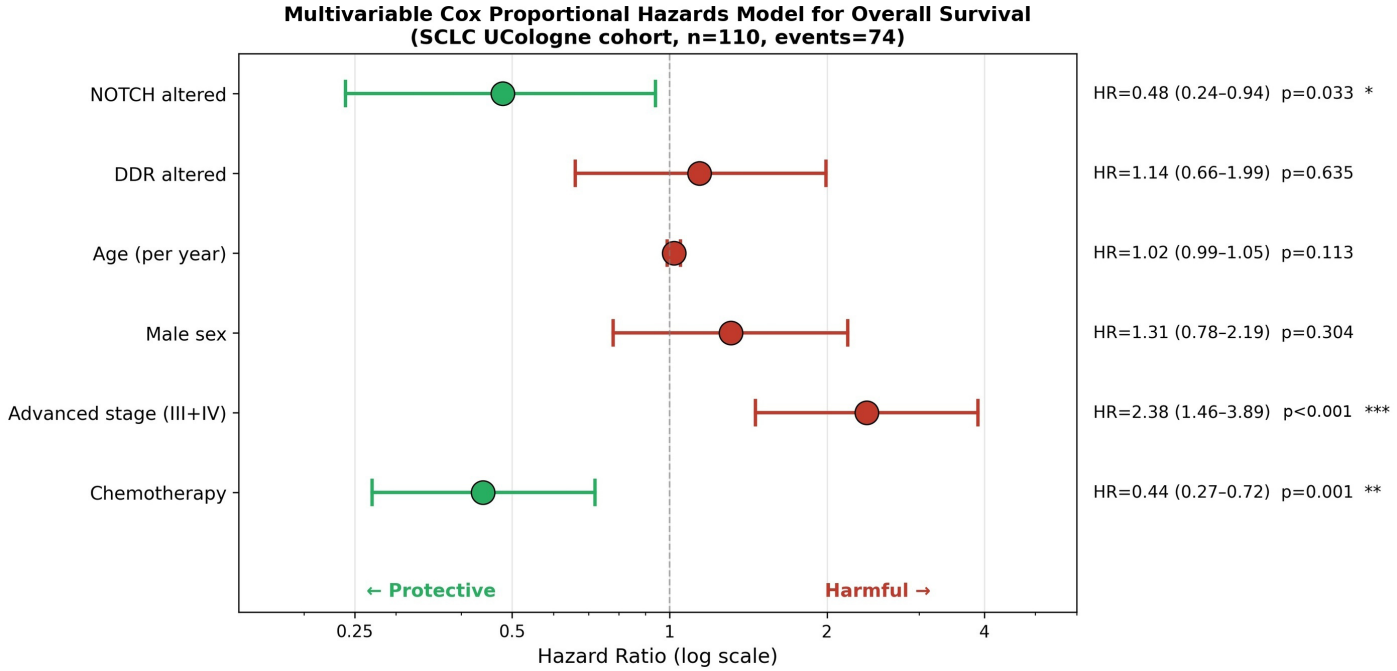

Figure 6. Forest plot of multivariable Cox proportional hazards model for overall survival. Hazard ratios with 95% confidence intervals are shown for NOTCH alteration, DDR alteration, age, sex, advanced stage, and chemotherapy receipt; NOTCH alterations confer an independent favorable prognostic effect (HR 0.48, p = .03), while advanced stage (HR 2.38, p < .001) and absence of chemotherapy (chemotherapy HR 0.44, p = .001) carry the strongest adverse and protective effects, respectively

Table 2. Multivariable Cox proportional hazards model for overall survival

Variable	HR	95% CI	p value
NOTCH altered	0.48	0.24 – 0.94	.03
DDR altered	1.14	0.66 – 1.99	.64
Age (per year)	1.02	0.99 – 1.05	.11
Male sex	1.31	0.78 – 2.19	.30
Advanced stage (III+IV)	2.38	1.46 – 3.89	< .001
Chemotherapy	0.44	0.27 – 0.72	.001

Model fit: n = 110, events = 74; Likelihood ratio test p < .001; CI: confidence interval, DDR: DNA damage repair, HR: hazard ratio

Stage-Stratified Analysis Supports Consistent NOTCH Prognostic Effect

Stratified Kaplan-Meier analyses suggested that the favorable survival association of NOTCH alterations was consistent across both early-stage (I – II) and advanced-stage (III – IV) SCLC (Figure 7). In early-stage disease, NOTCH-altered patients (n = 13) had a median overall survival of 77 months compared with 28 months in NOTCH-wild type patients (n = 48). In advanced-stage disease, median overall survival was 32 months in NOTCH-altered patients (n = 6) versus 20 months in NOTCH-wild type patients (n = 43), though the small NOTCH-altered subgroup in advanced stage limits statistical interpretation. The consistent direction of effect across stages strengthens confidence in the multivariable finding.

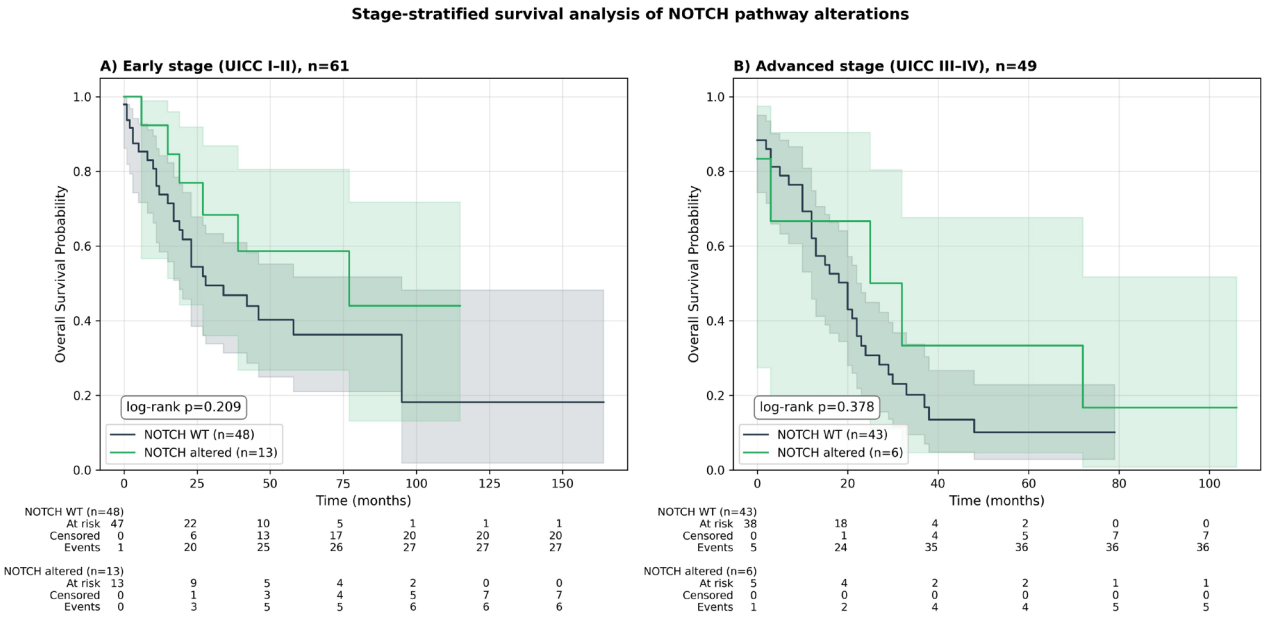

Figure 7. Stage-stratified Kaplan-Meier survival analysis for NOTCH alterations; (**A**) Early-stage disease (UICC I – II): NOTCH-altered (n = 13) median OS 77 months vs NOTCH-wild type (n = 48) 28 months; (**B**) Advanced-stage disease (UICC III – IV): NOTCH-altered (n = 6) median OS 32 months vs NOTCH-wild type (n = 43) 20 months; Consistent direction of effect across stages supports the multivariable finding

Pathway Burden and Survival

Patients were categorized by the number of altered pathways (DDR, MMR, and NOTCH combined; TP53 and RB1 excluded due to their near-universal alteration). Of the 120 patients, 71 (59.2%) had no pathway alterations, 40 (33.3%) had one altered pathway, and 9 (7.5%) had two altered pathways. Median overall survival values by pathway burden were 21, 24, and 72 months for 0, 1, and 2 altered pathways, respectively (Figure 8). In multivariable Cox regression, pathway burden as a continuous variable showed a borderline association with improved survival (HR 0.72 per additional altered pathway, 95% CI 0.51 – 1.02, p = .07). These findings should be interpreted cautiously given the small sample size in the 2-pathway group.

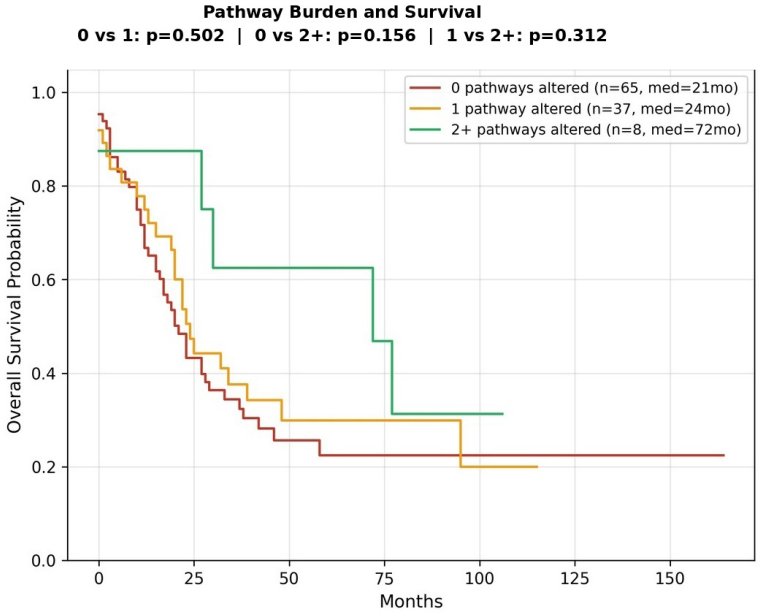

Figure 8. Pathway burden and overall survival; Kaplan-Meier curves stratified by number of altered pathways (0, 1, or ≥ 2 combined DDR, MMR, NOTCH alterations; TP53 and RB1 excluded due to near-universal alteration); Median OS values: 21, 24, and 72 months for 0, 1, and 2 altered pathways, respectively; Pairwise log-rank p-values: 0 vs 1: p = 0.052; 0 vs ≥ 2: p = 0.056; 1 vs ≥ 2: p = 0.012

DISCUSSION

In this integrated pathway-level analysis of 120 whole-genome sequenced SCLC tumors, we demonstrate that NOTCH pathway alterations are an independent favorable prognostic factor (adjusted HR 0.48, p = .03), while DDR pathway alterations—particularly homologous recombination defects—are strongly associated with elevated TMB (p = .001) but do not independently predict overall survival. These findings delineate distinct biological and clinical roles for these two pathways in SCLC and support the utility of pathway-level genomic assessment beyond single-gene analysis.

Our findings regarding NOTCH are consistent with, and extend, a growing body of evidence supporting NOTCH as a tumor suppressor in SCLC (15,16). The seminal study by George and colleagues originally reported inactivating NOTCH family mutations in approximately 25% of SCLC tumors and demonstrated that NOTCH activation in preclinical models reduced tumor burden and prolonged survival (5,15). Subsequent work by Ardeshir-Larijani and colleagues in an extensive-stage SCLC cohort (n = 67) also found NOTCH mutations to be associated with improved survival in multivariable analysis (17). Our study contributes further validation using a whole-genome sequenced cohort with broader stage distribution, distinct covariate adjustment, and confirms the approximate effect size (HR 0.48) observed in prior work (HR 0.37) (17). A methodological consideration deserves comment: while NOTCH alterations were not associated with a statistically significant survival difference in unadjusted Kaplan-Meier analysis (Figure 5), they emerged as an independent favorable prognostic factor in the multivariable Cox model (HR 0.48, p = .03). This pattern - non-significant univariate association becoming significant after covariate adjustment - typically reflects unbalanced distribution of a confounder that masks the effect in unadjusted analysis. In our cohort, tumor stage was the strongest independent prognostic factor (HR 2.38, p < .001), and its inclusion in the multivariable model appears to have unmasked the NOTCH-associated survival benefit. Additionally, the surgical-resection composition of the source cohort (with 54% early-stage disease, higher than typical population-based SCLC series where extensive-stage disease predominates) affects univariate estimates. These considerations warrant cautious interpretation and reinforce the need for validation. The consistency of the effect direction across independent cohorts strengthens NOTCH as a candidate prognostic biomarker (18). Nevertheless, our data - derived from a pre-chemoimmunotherapy cohort - do not distinguish whether NOTCH alterations represent a purely prognostic marker (indicating better outcome regardless of therapy) or a predictive marker for benefit from specific treatments; the favorable association observed here should therefore be interpreted as prognostic rather than predictive. External validation in contemporary chemoimmunotherapy cohorts (e.g., IMpower133-derived or CASPIAN-derived correlative studies) will be essential to clarify whether the NOTCH prognostic effect persists under current standard-of-care regimens and whether NOTCH status might interact with immunotherapy response.

The mechanistic basis for NOTCH-associated favorable prognosis likely relates to NOTCH's inhibitory effect on neuroendocrine differentiation and its role in driving lineage plasticity toward less proliferative, more immunogenic phenotypes. Recent molecular subtyping schemes (SCLC-A, SCLC-N, SCLC-P, SCLC-Y) have identified the inflamed/non-neuroendocrine SCLC-I subtype as associated with immune checkpoint inhibitor benefit (8,9), and NOTCH activation has been mechanistically linked to this phenotype (15). Our clinical findings are therefore biologically consistent with emerging molecular subtyping frameworks.

Regarding DDR alterations, our observation of significantly elevated TMB in DDR-altered tumors (particularly the HR subgroup) aligns with established literature in both SCLC and broader lung cancer contexts. Park and colleagues previously reported similar TMB elevation in DDR-altered SCLC (11), and the biological mechanism—defective DNA repair leading to mutation accumulation—is well-characterized across cancer types. The clinical relevance of this association lies in the potential utility of DDR alterations as surrogate biomarkers for high TMB in settings where direct TMB measurement is unavailable or as predictors of response to immune checkpoint inhibitors, which are now standard in first-line SCLC therapy.

Notably, we observed no independent prognostic impact of DDR alterations on overall survival after adjustment for clinical factors. This finding contrasts with some reports suggesting unfavorable prognosis in DDR-altered advanced lung cancers (12), but is consistent with studies in SCLC specifically, where the prognostic signal of DDR alterations has been inconsistent (11). The null prognostic association combined with the positive TMB association suggests that DDR alterations may function more as predictive biomarkers (for chemotherapy or immunotherapy response) than as prognostic biomarkers per se. Future studies with detailed treatment response data, particularly in the context of chemoimmunotherapy, are needed to clarify this distinction.

An intriguing secondary observation was the trend toward better survival with increasing pathway alteration burden (0 vs 1 vs 2 altered pathways; HR 0.72 per additional pathway, p = .07). Though not reaching statistical significance and limited by small sample sizes, this counterintuitive pattern is consistent with the hypothesis that mutation-rich tumors may generate greater neoantigen load and enhanced immunogenicity, potentially translating into improved survival in the current chemoimmunotherapy era. This hypothesis warrants validation in larger cohorts with treatment-era stratification.

Several limitations warrant consideration. First, this analysis is based on a single cohort; external validation in an independent SCLC dataset is required to confirm generalizability. Second, the sample size—while substantial for whole-genome sequenced

SCLC—limits statistical power for subgroup analyses, particularly for rare pathway combinations and advanced-stage NOTCH-altered cases (n = 6). Third, the cohort predominantly comprises patients diagnosed in the pre-immunotherapy era, and thus our findings may not fully translate to contemporary first-line chemoimmunotherapy practice. Specifically, the UCologne cohort was accrued prior to the routine incorporation of immune checkpoint inhibitors into first-line SCLC therapy; treatment data indicate that patients received chemotherapy only, with no immunotherapy administered to any patient in this cohort. Whether the NOTCH prognostic association we identify remains valid - or whether it shifts to become predictive of chemoimmunotherapy benefit - in the current era of standard-of-care regimens (e.g., platinum-etoposide plus atezolizumab as in IMpower133 or plus durvalumab as in CASPIAN) is an important open question that our study cannot address. Prospective correlative analyses embedded in contemporary chemoimmunotherapy trials will be essential to answer this question. Fourth, TMB was calculated from whole-genome sequencing-derived nonsynonymous mutation counts; harmonization with clinically standardized TMB assays (e.g., mutations per megabase by targeted panels) is imperfect. Fifth, functional consequences of individual mutations (e.g., loss-of-function versus VUS) were not differentiated, potentially diluting biological signals. Sixth, as a retrospective analysis of publicly available data, we could not independently verify clinical annotations or access additional treatment-specific outcomes. Seventh, given the exploratory nature of pathway-level analyses and the multiplicity of comparisons performed, findings should be interpreted as hypothesis-generating for non-primary analyses.

Despite these limitations, our integrated pathway-level approach provides a framework for understanding the distinct biological and clinical roles of NOTCH, DDR, and other key pathways in SCLC. The validated favorable prognostic association of NOTCH alterations, if confirmed in additional cohorts, could inform patient counseling and potentially stratification for clinical trials. The association of DDR alterations with elevated TMB supports their investigation as predictive biomarkers for immunotherapy response in ongoing SCLC trials.

CONCLUSION

In a whole-genome sequenced SCLC cohort, NOTCH pathway alterations independently predicted favorable overall survival (adjusted HR 0.48), while DDR pathway alterations—especially homologous recombination defects—were strongly associated with elevated TMB without independent prognostic impact. These findings validate and extend prior evidence for NOTCH as a prognostic biomarker in SCLC and support pathway-level genomic characterization as a clinically informative framework. An additional limitation concerns the stage-stratified analyses. Of the 20 NOTCH-altered tumors, only 6 were classified as advanced-stage (UICC III-IV) - reflecting the surgical resection bias of the source cohort and the resulting underrepresentation of extensive-stage SCLC, which comprises the majority of SCLC in real-world clinical practice. Consequently, our observation that the favorable prognostic effect of NOTCH alterations appears consistent across stage subgroups (Figure 7) should be interpreted with substantial caution, as it is derived from a small advanced-stage NOTCH-altered subgroup (n = 6). This finding should be regarded as hypothesis-generating and requires confirmation in extensive-stage-enriched cohorts. Prospective studies incorporating treatment response and external validation in independent cohorts are warranted to translate these observations into clinical practice.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This study did not require institutional ethics committee approval as it analyzed exclusively publicly available, de-identified genomic and clinical data from the cBioPortal for Cancer Genomics platform (study ID: sclc_ucologne_2015). Per the policy of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital and consistent with international standards (ICMJE recommendations and the Declaration of Helsinki), secondary analyses of publicly available de-identified data do not constitute research on identifiable human subjects and do not require additional institutional review board approval or patient informed consent. The original primary data collection was performed under ethics committee approval at the University of Cologne, as detailed in the source publication, and original informed consent was obtained by the primary investigators.

Informed Consent
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REFERENCES

1. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. Nat Rev Dis Primers. 2021;7:3.
2. Horn L, Mansfield AS, Szczęsna A, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med. 2018;379:2220-9.
3. Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. Lancet. 2019;394:1929-39.
4. Wang J, Zhou C, Yao W, et al. Adebrelimab or placebo plus carboplatin and etoposide as first-line treatment for extensive-stage small-cell lung cancer (CAPSTONE-1): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2022;23:739-47.
5. George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. Nature. 2015;524:47-53.

6. Peifer M, Fernández-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet. 2012;44:1104-10.
7. Mollaoglu G, Guthrie MR, Böhm S, et al. MYC drives progression of small cell lung cancer to a variant neuroendocrine subtype with vulnerability to aurora kinase inhibition. Cancer Cell. 2017;31:270-85.
8. Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. Nat Rev Cancer. 2019;19:289-97. Erratum in: Nat Rev Cancer. 2019;19:415.
9. Gay CM, Stewart CA, Park EM, et al. Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities. Cancer Cell. 2021;39:346-60.e7.
10. Sivakumar S, Moore JA, Montesion M, et al. Integrative analysis of a large real-world cohort of small cell lung cancer identifies distinct genetic subtypes and insights into histologic transformation. Cancer Discov. 2023;13:1572-91.
11. Park S, Lee H, Lee B, et al. DNA damage response and repair pathway alteration and its association with tumor mutation burden and platinum-based chemotherapy in SCLC. J Thorac Oncol. 2019;14:1640-50.
12. Dai J, Jiang M, He K, et al. DNA damage response and repair gene alterations increase tumor mutational burden and promote poor prognosis of advanced lung cancer. Front Oncol. 2021;11:708294.
13. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017;9:34.
14. Ricciuti B, Wang X, Alessi JV, et al. Association of high tumor mutation burden in non-small cell lung cancers with increased immune infiltration and improved clinical outcomes of PD-L1 blockade across PD-L1 expression levels. JAMA Oncol. 2022;8:1160-8. Erratum in: JAMA Oncol. 2022;8:1702.
15. Lim JS, Ibaseta A, Fischer MM, et al. Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. Nature. 2017;545:360-4.
16. Kikuchi H, Sakakibara-Konishi J, Furuta M, et al. Expression of Notch1 and Numb in small cell lung cancer. Oncotarget. 2017;8:10348-58.
17. Ardeshir-Larijani F, Wildey G, Fu P, et al. Frequency of NOTCH pathway mutation in primary tumor of SCLC compared to metastatic biopsies and association with better survival. J Clin Oncol. 2018;36:e20574.
18. Roper N, Velez MJ, Chiappori A, et al. Notch signaling and efficacy of PD-1/PD-L1 blockade in relapsed small cell lung cancer. Nat Commun. 2021;12:3880.
19. 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. Erratum in: Cancer Discov. 2012;2:960.
20. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.