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# Clinical Outcomes and Neuroendocrine Features of Transformed versus Primary Small-Cell Lung Cancer

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## Abbreviations

**AJCC:** American Joint Committee on Cancer

**ABCP:** atezolizumab plus bevacizumab plus carboplatin plus paclitaxel

**CI:** confidence interval

**CgA:** chromogranin A

**CEA:** carcinoembryonic antigen

**DOR:** duration of response

**ES-SCLC:** extensive-stage SCLC

**EGFR-TKI:** epidermal growth factor receptor tyrosine kinase inhibitor

**ECOG PS:** Eastern Cooperative Oncology Group performance status

**ECT/EPT:** etoposide plus carboplatin/cisplatin plus atezolizumab

**FFPE:** formalin-fixed paraffin-embedded

**H&E:** hematoxylin and eosin

**HR:** hazard ratio

**ICIs:** immune checkpoint inhibitors

**IHC:** immunohistochemistry

**LUAD:** lung adenocarcinoma

**mPFS:** median PFS

**mOS:** median OS

**NSCLC:** non-small cell lung cancer

**NSE:** Neuron-specific enolase

**NGS:** next-generation sequencing

**NE:** neuroendocrine

**ORR:** objective response rate

**OS:** overall survival

**P-SCLC:** primary SCLC

**PD-L1:** programmed cell death-ligand 1

**proGRP:** pro-gastrin-releasing peptide

**PFS:** progression-free survival

**PD:** progressive disease

**SCLC:** Small-cell lung cancer

**Syn:** synaptophysin

**T-SCLC:** transformed SCLC

**TPS:** tumor proportion score

## Abstract

**Introduction**: The clinical outcomes of transformed small-cell lung cancer (T-SCLC) was previously considered comparable with primary SCLC (P-SCLC). However, whether T-SCLCs and P-SCLCs differ in the era of immunotherapy remains unclear.

**Methods**: Clinical outcomes were retrospectively analyzed. Overall survival (OS) was estimated using the Kaplan–Meier method and Cox regression. Linear correlation and regression analyses were used to assess prognostic value of baseline neuron-specific enolase (NSE). Hierarchical clustering was used to group neuroendocrine (NE) markers of T-SCLC by immunohistochemical results.

**Results:** Between March 2018 and March 2023, 206 patients with T-SCLC (n = 42) and P-SCLC (n = 164) were enrolled in the study. The median OS (mOS) of T-SCLC cohort was significantly shorter than that of the P-SCLC cohort (11.7 vs. 12.9 months, $P$ = 0.033). In the T-SCLC cohort, the mOS of chemoimmunotherapy significantly outlasted that of chemotherapy (15.4 vs. 8.5 months, $P$ = 0.001). The optimal baseline NSE cutoff values differed between T-SCLC (19.7 ng/ml) and P-SCLC (74.8 ng/ml), and a high NSE level was associated with poorer mOS in both T-SCLC (10.0 vs. 16.5 months, $P$ = 0.003) and P-SCLC (10.8 vs. 16.5 months, $P$ < 0.001). The cluster with stronger expression of NE markers in T-SCLC exhibited longer mOS (14.3 vs. 10.3 months, $P$ = 0.030).

**Conclusion:** T-SCLC had a statistically poorer prognosis than P-SCLC, but the difference was modest. Chemoimmunotherapy might improve the outcomes of T-SCLC. Patients with T-SCLC who show stronger neuroendocrine features may have a poorer prognosis.


**Keywords:** Small cell lung cancer; Transformation; Treatment outcomes; Immunotherapy; Biomarkers.

## 1. Introduction

Small-cell lung cancer (SCLC) comprises 13–15% of all lung cancer cases.[1,2] Due to its aggressive nature and early metastasis, two-thirds of the patients are initially diagnosed with extensive-stage SCLC (ES-SCLC), with a 5-year survival rate of < 7%.[1-4] For decades, platinum-doublet chemotherapy dominated SCLC treatment, until the advent of immune checkpoint inhibitors (ICIs) significantly changed the therapeutic paradigm. [3,5-9] Recent studies have explored regimens combining anti-angiogenesis drugs (anlotinib and bevacizumab) with ICIs and standard chemotherapy, providing new treatment options for SCLC.[10,11]

Transformed SCLC (T-SCLC) is considered a special type of SCLC; 3–14% of non-small cell lung cancer (NSCLC) cases may histologically transform into SCLC following treatment with epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). The pathological transformation was considered one of the mechanisms of resistance to EGFR-TKI.[12-16]

Previous studies have reported that once histological transformation occurs, the clinical outcomes of T-SCLC treated with chemotherapy are comparable with those of primary SCLC (P-SCLC).[16-18] Therefore, platinum-based chemotherapy is currently recommended for T-SCLC. However, the efficacy of chemotherapy plus programmed cell death-ligand 1 (PD-L1) inhibitors or TKI in this setting remains uncertain, and research on prognostic biomarkers for T-SCLC is still limited.

Neuron-specific enolase (NSE) and pro-gastrin-releasing peptide (proGRP) are classic serum tumor markers used for the diagnosis, treatment response evaluation, and prognosis of SCLC.[19-24] NSE is a superior biomarker for predicting outcomes in patients with SCLC compared with ProGRP.[25] Jorgensen constructed a survival prognosis model based on the analysis of NSE and other factors (stage, sex, and lactate dehydrogenase, among others). High levels of serum NSE are the most important negative indicator of prognosis.[26] However, few studies have explored the relationship between NSE and the prognosis of T-SCLC.

Here, we retrospectively compared the clinical outcomes of patients with T-SCLC and those with P-SCLC in the era of immunotherapy, and we analyzed serum tumor markers and tissue immunohistochemical profiles to identify effective prognostic factors for T-SCLC.

## 2. Materials and methods

### 2.1. Patient Selection and Study Design

This single-center retrospective study enrolled patients diagnosed with P-SCLC and T-SCLC at Guangdong Provincial People's Hospital between March 2018 and March 2023. The study was approved by the Research Ethics Committee of Guangdong Provincial People's Hospital, Guangzhou, China (KY2025-053-01). Written informed consent was obtained from all enrolled patients.

Patients were included in the study if they met the following criteria: **P-SCLC cohort:** histologically confirmed stage IVA or IVB (American Joint Committee on Cancer (AJCC) eighth edition), pure SCLC at initial diagnosis; **T-SCLC cohort:** histologically confirmed stage IVA or IVB (AJCC eighth edition), either pure or combined SCLC that transformed from NSCLC with *EGFR* mutation following EGFR-TKI therapy.

Patients with limited-stage disease (n = 271), those who received best supportive care (n = 138), and those lost to follow-up after initial therapy (n = 99) were excluded. Additionally, two patients with T-SCLC and one patient with P-SCLC who initiated ICIs after completing second-line (2L) treatment were excluded. One patient with T-SCLC harboring ALK-EML4 fusion and EGFR wild-type was also excluded (Fig. 1).

### 2.2. Data collection

The following clinical characteristics, treatment details and laboratory results were collected: age, sex, smoking status, Eastern Cooperative Oncology Group performance status (ECOG PS), TNM stage (AJCC eighth edition), brain metastasis status, pathological and immunohistochemistry (IHC) information, serum tumor markers, next-generation sequencing (NGS) data, treatment regimens, and survival outcomes.

### 2.3. Study outcomes

**Definition of baseline:** In this study, we established the time of pathological transformation as the baseline for T-SCLC, and treatment initiated after transformation was defined as first-line (1L) treatment. The baseline for P-SCLC was the date of initial pathological diagnosis of SCLC.

Treatment response was evaluated by the investigators based on the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). The Clopper–Pearson method was used to calculated the 95% confidence interval (CI) for the objective response rate (ORR).

For each line of therapy, progression-free survival (PFS) was defined as the

interval from treatment initiation to the first occurrence of radiographically confirmed progressive disease (PD) or death from any cause, whichever occurred first. Patients who did not experience PD or death during that line of therapy were censored at the date of their last disease assessment for that line.

Overall survival (OS) was defined as the interval from the initiation of 1L treatment to death from any cause. Patients without a recorded death at the time of last follow-up were censored. Notably, because of differing baseline definitions, OS for T-SCLC was measured from the time of histological transformation, while OS for P-SCLC was measured from the time of initial diagnosis. Therefore, comparisons between the two cohorts should be interpreted with caution. The data cut-off date was June 22, 2024.

### 2.4. Sample Collection

Tumor tissues were collected via tissue biopsy and prepared as formalin-fixed paraffin-embedded (FFPE) samples. Blood samples were collected with an anticoagulant. Genomic DNA was extracted using a TIANamp Genomic DNA kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions.

### 2.5. IHC Staining and NGS

Formalin-fixed paraffin-embedded samples of lung cancer tissues were subjected to hematoxylin and eosin (H&E) and immunohistochemical staining. Immunohistochemical staining of Ki67, chromogranin A (CgA), synaptophysin (Syn), and CD56 (NCAM) were conducted. Positive cells were scored as expressing <10% (negative), 10% to 25% (1+), >25% to 75% (2+), and > 75% (3+).

The PD-L1 tumor proportion score (TPS) was assessed using the DAKO 22C3 antibody (Dako, Carpinteria, CA, USA).

Tissue and liquid biopsy samples from patients were submitted to two commercial laboratories, Burning Rock Biotech and Nanjing Geneseeq Technology Inc. (Nanjing, China), for genetic analysis. Targeted capture sequencing was performed using an Illumina sequencing platform (Illumina, CA, USA) with commercial panels to detect 168, 196, and 520 lung cancer-associated gene alterations. NGS data were reanalyzed based on genes commonly detected across all panels.

### 2.6. Statistical Analysis

Descriptive statistics were used to summarize the patient characteristics. Continuous variables that followed a normal distribution were compared using Student's t-test, while non-normally distributed data were compared using the Mann-Whitney U test. Categorical variables were compared using Pearson’s

chi-square test or Fisher's exact test. Median PFS (mPFS) and median OS (mOS) were estimated using the Kaplan–Meier method, with 95% CI calculated by log–log transformation. Survival differences between groups were compared using hazard ratio (HR) derived from Cox regression. The optimal cutoff value for baseline NSE was determined using the R package *Survminer* version 0.4.9, which minimizes the p-values of the maximally selected rank statistics in the survival analysis of OS. When conducting linear correlation and regression analyses, baseline NSE, carcinoembryonic antigen (CEA), and OS data were transformed, if necessary, to approximate a normal distribution. Clustering heatmaps were generated using the R package *pheatmap* version 1.0.12, employing hierarchical clustering based on Gower distance.

All statistics and figures were generated using GraphPad Prism version 10.1 (GraphPad Software, San Diego, CA, USA), IBM SPSS Statistics version 24 (IBM, Armonk, NY, USA), and R version 4.3.2. Two-sided *P* values < 0.05 were considered statistically significant.

## 3. Results

### 3.1. Patient characteristics

A total of 718 patients were screened, of whom 206 who met the eligibility criteria were enrolled in the P-SCLC (n = 164) and T-SCLC (n = 42) cohorts (Fig. 1). The median follow-up time was 32.4 months (95% CI: 28.4–34.6) for the P-SCLC cohort and 31.7 months for the T-SCLC cohort, with 95% CI not reached. The patients' baseline characteristics for the two cohorts are summarized in Table 1.

Within the P-SCLC cohort, the median age at initial diagnosis was 63 years (interquartile range [IQR]: 57–68). The majority of patients were male (n = 156, 95.1%) and smokers (n = 135, 82.8%). A total of 104 patients (63.4%) who received immunotherapy combined with chemotherapy as 1L or 2L treatment were enrolled in the P-SCLC$_{chemo+I/O}$ group, whereas the remaining 60 patients (36.6%) who received chemotherapy alone were enrolled in the P-SCLC$_{chemo}$ group.

Within the T-SCLC cohort, the median age at first diagnosis of SCLC transformation was 52.5 years (interquartile range [IQR]: 44.8–58.8). most patients were male (n = 24, 57.1%) and nonsmokers (n = 30, 71.4%). All patients were initially diagnosed with lung adenocarcinoma (LUAD) with *EGFR* mutations and received targeted therapy with EGFR-TKI until the LUAD pathologically transformed to SCLC. A total of 20 patients (47.6%) who received immunotherapy combined with chemotherapy ± anti-angiogenic therapy after SCLC transformation were enrolled in the T-SCLC$_{chemo+I/O}$ group, while the other 22 (52.4%) who did not receive immunotherapy were enrolled in the T-

237 $SCLC_{chemo}$ group.

238 We compared the mutated genes in the P-SCLC (n = 20) and T-SCLC (n = 27)
239 cohorts with the available baseline (as defined in Methods) NGS data. *TP53*
240 (95%), *RB1* (70%), *KMT2D* (25%), *POLE* (20%) and *TERT* (15%) were the
241 most frequently mutated genes in the P-SCLC cohort, while *EGFR* (100%),
242 *TP53* (96%), *RB1* (63%), *PIK3CA* (41%), *PTEN* (19%) and *TERT* (19%) were
243 most common in the T-SCLC cohort (Supplementary Fig. 1*A*). *TP53*, *RB1*,
244 *PIK3CA*, *TERT*, *CREBBP, NTRK1, SOX2* and *ERBB4* were frequently mutated
245 in both cohorts (Supplementary Fig. 1*B*).

246 **3.2. Clinical outcomes of T-SCLC versus P-SCLC**

247 A total of 42 patients with T-SCLC and 164 patients with P-SCLC were enrolled
248 in this study. The mOS of T-SCLC was 11.7 months (95% CI: 9.8–14.8),
249 significantly shorter than that of the P-SCLC cohort, which had a mOS of 12.9
250 months (95% CI: 10.9–15.2, $P = 0.033$; Fig. 2*A*).

251 Further analyses were conducted based on treatments. Among patients from
252 the T-SCLC cohort treated with chemoimmunotherapy (regardless of
253 bevacizumab) and chemotherapy (regardless of TKI), the mOS was 6.9 months
254 longer in the $T\text{-}SCLC_{chemo+I/O}$ group (n = 20) than the $T\text{-}SCLC_{chemo}$ group (n =
255 22) ($T\text{-}SCLC_{chemo+I/O}$ versus $T\text{-}SCLC_{chemo}$: 15.4 months [95% CI: 11.0–17.3]
256 versus 8.5 months [95% CI: 6.7–11.7], $P < 0.001$; Fig. 2*B*). Similarly, within the
257 P-SCLC cohort, the mOS was significantly longer in the $P\text{-}SCLC_{chemo+I/O}$ group
258 (n = 104) than the $P\text{-}SCLC_{chemo}$ group (n = 60) ($P\text{-}SCLC_{chemo+I/O}$ versus P-
259 $SCLC_{chemo}$: 13.9 months [95% CI: 11.3–18.4] versus 11.0 months [95% CI: 9.0–
260 14.6], $P = 0.014$; Fig. 2*B*). Compared to chemotherapy alone,
261 chemoimmunotherapy was a protective factor for OS in both the T-SCLC cohort
262 (HR = 0.34, 95% CI: 0.17–0.65, $P = 0.001$) and the P-SCLC cohort (HR = 0.63,
263 95% CI: 0.44–0.91, $P = 0.014$; Fig. 2*B*). We further compared the ORR to
264 chemoimmunotherapy between the two groups. Among 20 evaluable cases in
265 the $T\text{-}SCLC_{chemo+I/O}$ group, the ORR was 45% (95%CI: 23.1%-68.5%), whereas
266 among 100 evaluable cases in the $P\text{-}SCLC_{chemo+I/O}$ group, the ORR was 52%
267 (95%CI: 41.8%-62.1%). Similarly, for chemotherapy, 19 evaluable cases in the
268 $T\text{-}SCLC_{chemo}$ group had an ORR of 26.3% (95%CI: 9.1%-51.2%), compared to
269 44.1% (95%CI: 31.1%-57.6%) for 59 evaluable cases in $P\text{-}SCLC_{chemo}$ group.

270 **3.3. The clinical outcomes based on different treatment strategies in T-**
271 **SCLC**

272 We used a swimming plot to display treatment regimens and PFS for each line
273 of therapy, aiming to assess their prognostic impact in patients with T-SCLC
274 (Fig 3*A*). The most common regimens in the $T\text{-}SCLC_{chemo+I/O}$ group were
275 atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP) (60.0%,

12/20) and etoposide plus carboplatin/cisplatin plus atezolizumab (ECT/EPT) (25.0%, 5/20). The ABCP regimen (n = 12) achieved an ORR of 66.6% (95%CI: 34.9%-90.1%), including 8 cases of PR, 1 of SD, and 3 of PD. By contrast, no patients treated with the ECT/EPT (n = 5) regimen achieved PR, with 2 showing SD and 3 showing PD. Furthermore, mPFS was 6.4 months (95% CI: 3.7–7.7) for the ABCP regimen versus 3.6 months (95% CI: 1.9–NA) for the ECT/EPT regimen, corresponding to a significantly lower hazard ratio of 0.26 (95% CI: 0.07–0.92, *P* = 0.037; Supplementary Fig. 1*C*). Although not statistically significant, the ABCP regimen showed a numerically longer mOS of 15.1 months (95% CI: 10.5–19.8) compared to 11.0 months (95% CI: 8.7–NA) with the ECT/EPT regimen, and a lower HR of 0.49 (95% CI: 0.16–1.49, *P* = 0.210; Supplementary Fig. 1*D*). The remaining 3 out of 20 patients were patients T5, T8 and T10. Patient T5 received 2L treatment with atezolizumab, bevacizumab, paclitaxel and osimertinib, resulting in a PFS of 5.5 months and a best response of SD. Patient T8 received 2L treatment with atezolizumab, bevacizumab and paclitaxel, resulting in a PFS of 2.5 months and a best response of PD. Patient T10 received 1L treatment with atezolizumab, carboplatin and paclitaxel, resulting in a PFS of 8.6 months and a best response of PR (Fig 3*A*).

We compared the 1L treatments to further evaluate the efficacy of immunotherapy and TKI for patients with T-SCLC. The mPFS for 1L chemotherapy alone (*1L chemo*; n = 9 ), chemotherapy plus TKI (*1L chemo+TKI*; n = 10), and chemotherapy plus *I/O* (*1L chemo+I/O*; n = 7; including 4 cases with bevacizumab and 3 without) was 4.4 months (95% CI: 1.1–6.7), 4.4 months (95% CI: 1.4–5.4), and 6.5 months (95% CI: 3.6–8.6), respectively. Compared with *1L chemo*, the HR was 1.06 (95% CI: 0.42–2.71, *P* = 0.903) for *1L chemo+TKI* and 0.40 (95% CI: 0.13–1.23, *P* = 0.110) for *1L chemo+I/O* (Fig 3*B*). The mOS was comparable across all three regimens: 8.5 months (95% CI: 1.1–15.9) with *1L chemo*, 8.7 months (95% CI: 4.5–16.2) with *1L chemo+TKI*, and 10.5 months (95% CI: 8.7–NA) with *1L chemo+I/O*, respectively. Compared with *1L chemo*, the HR was 0.68 (95% CI: 0.25–1.84, *P* = 0.450) for *1L chemo+TKI* and 0.45 (95% CI: 0.15–1.35, *P* = 0.153) for *1L chemo+I/O* (Fig 3*C*).

Of the 26 patients across the three treatment groups, 22 were evaluable for ORR assessment. The ORR was 25.0% (95%CI: 3.2%-65.1%) in the chemotherapy group (n = 8), 28.5% (95%CI: 3.7%-71.0%) in the chemotherapy plus TKI group (n = 7), and 57.1% (95%CI: 18.4%-90.1%) in the chemotherapy plus I/O group (n = 7).

We investigated the relationship between the duration of response (DOR) to TKI therapy before transformation and OS after transformation in T-SCLC patients. A total of 8 and 11 patients with available data were included in the analysis for the $T\text{-}SCLC_{chemo}$ and $T\text{-}SCLC_{chemo+I/O}$ groups, respectively. In the

T-SCLC$_{chemo+I/O}$ group, DOR was significantly correlated with OS (r = 0.61, P = 0.044; Supplementary Fig. 2*A*), whereas no such correlation was observed in the T-SCLC$_{chemo}$ group (r = 0.16, P = 0.709; Supplementary Fig. 2*B*).

Moreover, in the T-SCLC$_{chemo+I/O}$ group, there was no statistically significant difference in mOS between patients with EGFR L858R (n = 6) and those with EGFR 19del (n = 11) (L858R versus 19del: 15.5 months [95% CI: 10.5–NA] versus 16.5 months [95% CI: 12.8–19.8], *P* = 0.780; Supplementary Fig. 2*C*). Similarly, no significant difference was observed between EGFR L858R (n = 6) and EGFR 19del (n = 15) in the T-SCLC$_{chemo}$ group(L858R versus 19del: 9.7 months [95% CI: 4.5–NA] versus 8.5 months [95% CI: 4.5–10.0], *P* = 0.224; Supplementary Fig. 2*D*).

### 3.4. NSE as a Useful Prognostic Indicator for both T-SCLC and P-SCLC

A total of 139 patients with P-SCLC and 38 patients with T-SCLC who have available baseline NSE and CEA data were enrolled in this analysis. The optimal cutoff value of baseline NSE levels in predicting prognosis differed between P-SCLC (74.8 ng/ml) (Supplementary Fig. 3*A*) and T-SCLC (19.7 ng/ml) (Supplementary Fig. 3*B*). A high baseline NSE level was identified as a risk factor in both the P-SCLC cohort (HR = 1.94, 95% CI: 1.31–2.88, *P* < 0.001) and the T-SCLC cohort (HR = 3.28, 95% CI: 1.50–7.20, *P* = 0.003), and was associated with shorter mOS in both cohorts: 10.8 months (95% CI: 9.4–12.0) vs. 16.5 months (95% CI: 13.4–20.8) for P-SCLC$_{NSE-H}$ vs. P-SCLC$_{NSE-L}$ (Fig. 4*A*), and 10.0 months (95% CI: 7.5–12.6) vs. 16.5 months (95% CI: 10.0–19.8) for T-SCLC$_{NSE-H}$ vs. T-SCLC$_{NSE-L}$ (Fig. 4*B*).

A three-dimensional scatter plot was constructed to explore the predictive value of serum tumor markers in patients with T-SCLC (Fig. 4*C*). Baseline NSE, CEA, and OS data were transformed to approximate a normal distribution. According to the results of the linear correlation analysis, baseline CEA (transformed to “SQRT(LN(CEA*10))”) (SQRT stands for square root, LN stands for natural logarithm) and OS did not show a significant linear correlation (r = -0.20, *P* = 0.272). However, there was a linear correlation between baseline NSE (transformed to “10-100/NSE”, as variable *X*)) and OS (as variable *Y*), and further simple linear regression analysis was performed to calculate the regression equation: $Y = -1.483*X + 21.05$ (r = -0.68, *P* < 0.001). In patients with P-SCLC, no significant linear correlation was found between baseline NSE (transformed to “LN(NSE)”) and OS (transformed to “SQRT(OS)”) (r = -0.13, *P* = 0.184) (Supplementary Fig. 3*C*).

### 3.5. Stronger expression of neuroendocrine (NE) markers in IHC indicated poorer outcomes for T-SCLC

We analyzed the expression of three common NE markers—CgA, Syn, and

CD56 (NCAM)—in P-SCLC (n = 136) and T-SCLC (n = 36), both of which had available IHC data. Marker expression was assessed using a four-level intensity scale: *negative*, *1+*, *2+*, and *3+*. Except for CgA (*P* = 0.021), no significant differences were observed in the expression of Syn (*P* = 0.175) and CD56 (*P* = 0.402) between the two groups (Fig. 5*A*).

However, when the data were re-analyzed (Supplementary Fig. 3*D*) using a binary classification (*3+* vs. all other levels), T-SCLC showed a significantly higher proportion of *3+* expression for CgA (11.1% vs. 0.7%, *P* = 0.007) and Syn (44.4% vs. 25.7%, *P* = 0.047) compared to P-SCLC. No significant difference was observed for CD56 (*3+* expression: 66.7% in T-SCLC vs. 72.8% in P-SCLC; *P* = 0.605). Patients with T-SCLC were clustered into two groups according to the three NE markers (Fig. 5*B*). Most patients in the NE-high (NE-H) group (16/18) exhibited strong (3+) expression of at least two NE markers, whereas all patients in the NE-low (NE-L) group (n = 18) showed strong expression (3+) for fewer than two NE markers. The mOS of patients in the NE-H group was 10.3 months (95% CI: 7.6–12.8), which was significantly shorter than the 14.3 months (95% CI: 8.7–19.0) observed in the NE-L group, with a HR of 2.35 (95% CI: 1.09-5.08, *P* = 0.030; Fig. 5*C*).

Patients with P-SCLC were clustered into two groups using the same method (Supplementary Fig. 3*E*). However, subsequent survival analysis showed no significant difference in mOS between the two groups (NE-H vs. NE-L: 12.6 months [95% CI: 10.4–14.6] vs. 15.3 months [95% CI: 9.8–42.3]), with a HR of 1.74 (95% CI: 1.00–3.03, *P* = 0.050) for the NE-H group (Supplementary Fig. 3*F*).

## 4. Discussion

By restricting the enrollment period to March 2018 to March 2023, this retrospective study is the first to compare therapeutic strategies and clinical outcomes between patients with T-SCLC and P-SCLC during the era of immunotherapy. Although patients with T-SCLC showed a statistically significant poorer prognosis (mOS: 11.7 vs. 12.9 months), the difference was relatively small.

The mOS of 1L chemoimmunotherapy (including 4 cases with bevacizumab and 3 without) was only numerically longer than that of 1L chemotherapy alone, without reaching statistical significance. These findings are consistent with the results reported by Felix *et al*, although it should be noted that their chemoimmunotherapy regimen did not include bevacizumab.[27]

However, when patients who received 1L or 2L chemoimmunotherapy (regardless of bevacizumab) were included in the $T\text{-}SCLC_{chemo+I/O}$ group, we found that chemoimmunotherapy significantly improved the mOS for T-SCLC,

extending the mOS by approximately 6.9 months compared with chemotherapy alone. This finding is consistent with a previous study involving 47 patients with T-SCLC, of whom only 11 received chemoimmunotherapy.[28] In our study, we included a balanced sample size between the T-SCLC$_{chemo+I/O}$ group (n = 20) and the T-SCLC$_{chemo}$ group (n = 22), and limited the use of PD-L1 inhibitors to 1L and 2L settings. This design provides more complete data (only one case censored) and further supports PD-L1 inhibitor–based combinations in T-SCLC, although the influence of bevacizumab use and treatment lines in the T-SCLC$_{chemo+I/O}$ group could not be controlled due to the small sample size.

Further studies are needed to evaluate the efficacy of the ABCP regimen compared to ECT/EPT in T-SCLC. Marcoux et al. reported that paclitaxel might yield a relatively higher clinical response rate in T-SCLC than in P-SCLC. [18] Based on the findings from previous studies, such as IMpower150[29] and ATTLAS[30], we consider that the ABCP regimen may be effective in treating both SCLC and potential residual NSCLC components in T-SCLC. However, in our study, ABCP showed only a trend of mOS improvement compared to ECT/EPT, without reaching statistical significance. Nevertheless, notable improvements in PFS and ORR were observed with the ABCP regimen. These results should be interpreted with caution due to the small sample size and variation in treatment lines, which may have introduced bias.

Our study found no mPFS or mOS benefit from adding 1L TKI to chemotherapy in patients with T-SCLC. Similarly, a multicenter study showed only a numerically longer mPFS and comparable mOS with the combination treatment.[27] In contrast, a single-center study from China reported a significantly longer mPFS with TKI plus chemotherapy, but no significant difference in mOS was observed.[31] Our findings, along with evidence from multiple studies, suggest that the use of TKI combined with chemotherapy in T-SCLC should be carefully considered.

Our analysis of serum NSE levels and tumor IHC profiles suggests that a stronger NE phenotype may be associated with poorer prognosis in T-SCLC. Higher baseline NSE levels were associated with shorter mOS in both P-SCLC and T-SCLC. The difference in average baseline NSE levels may have contributed to the variation in optimal cutoff values, indicating underlying biological differences between the two subtypes. Therefore, when estimating prognosis based on baseline NSE levels, it is important to consider the differences between P-SCLC and T-SCLC. Further analysis indicated that although T-SCLCs may retain LUAD components, OS was not associated with baseline CEA levels. In addition, based on the IHC results, T-SCLC were classified into two clusters. Cluster NE-H had a higher expression of three NE markers (CgA, Syn, and CD56) and a significantly worse prognosis than cluster NE-L. The stronger NE marker expression in cluster NE-H may indicate a more

complete pathological transformation, making these tumors more similar to P-SCLC. Previous studies have suggested that higher levels of NE differentiation may be associated with increased tumor aggressiveness[32,33], which may contribute to the poorer prognosis observed in the NE-H group.

Our study is subject to the inherent biases of a single-center, retrospective design. Additionally, the small sample size in the T-SCLC cohort limited our analysis, so we had to include patients who received 2L chemoimmunotherapy and were unable to fully account for the potential confounding effect of bevacizumab. Future prospective studies are warranted to clarify the role of bevacizumab in T-SCLC. When analyzing treatment regimens, there is also a potential selection bias: patients with a better performance status and fewer side effects are more likely to receive multi-drug combination treatment, such as the ABCP regimen. Besides, due to substantial missing data, we were unable to perform an analysis of ProGRP, which warrants further investigation into its potential prognostic value in T-SCLC. Finally, the insufficient tissue samples limited further exploration of how NE-related pathways influence the prognosis of T-SCLC.

## 5. Conclusion

The prognosis of T-SCLC was statistically poorer than that of P-SCLC, but the difference was modest. Our findings suggest a potential benefit of chemoimmunotherapy in T-SCLC, providing valuable insights for treatment selection, though further validation in prospective studies is needed. Stronger NE features, reflected by baseline NSE levels or IHC NE markers, may indicate a poorer prognosis in T-SCLC.

## Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Acknowledgements

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## Figure Legends

**Figure 1**. Flowchart of enrolled patients and analyses. P-SCLC, primary SCLC; T-SCLC, transformed SCLC; I/O, immunotherapy; chemo, chemotherapy; 1L, first-line; 2L, second-line; OS, overall survival; PFS, progress free survival; ORR, objective response rate; NE, neuroendocrine.

**Figure 2.** Kaplan-Meier analysis of the P-SCLC and T-SCLC cohorts and additional analysis based on treatments. **A.** OS of patients with P-SCLC and T-SCLC. **B.** OS of patients with P-SCLC and T-SCLC received chemotherapy (chemo) or chemoimmunotherapy (chemo+I/O). Cox regression was used to calculate the HR and p-value for OS. P-SCLC, primary SCLC; T-SCLC, transformed SCLC; I/O, immunotherapy; chemo, chemotherapy; mOS, median overall survival; HR, hazard ratio; CI, confidence interval.

**Figure 3.** Clinical outcomes of patients with T-SCLC based on different treatment strategies. **A.** Swimming plot of PFS and OS across each line of treatment. PFS **(B)** and OS **(C)** of patients treated with first-line (1L) chemotherapy alone (chemo), chemotherapy plus TKI (chemo+TKI) and chemoimmunotherapy (regardless of bevacizumab; chemo+I/O). Cox regression was used to calculate the HR and p-value for PFS and OS.

1L–4L, first-line to fourth-line; TKI, tyrosine kinase inhibitor; PD-L1, programmed cell death-ligand 1; T-SCLC, transformed SCLC; PFS, progress free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

**Figure 4.** Prognostic value of serum biomarkers. OS of P-SCLC **(A)** and T-SCLC **(B)** with high and low baseline NSE. **C.** A three-dimensional scatter plot, linear correlation and simple linear regression of baseline NSE, baseline CEA and OS for T-SCLC. Cox regression was used to calculate the HR and p-value for PFS and OS. Abbreviations: P-SCLC, primary SCLC; T-SCLC, transformed SCLC; OS, overall survival; HR, hazard ratio; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; SQRT, square root; LN, natural logarithm; CI, confidence interval.

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## List of supplementary material

Supplementary Figures 1-3: Supplementary Figures.pdf

Supplementary Table 1: Supplementary Table 1.xlsx

622 Table 1. Baseline demographics and clinical characteristics.

| | P-SCLC | T-SCLC | P value |
|---|---|---|---|
| | ***N* = 164** | ***N* = 42** | |
| Age, median [IQR] | 63.0 [57.0;68.0] | 52.5 [44.8;58.8] | <0.001 |
| Sex: | | | <0.001 |
| Female | 8 (4.9%) | 18 (42.9%) | |
| Male | 156 (95.1%) | 24 (57.1%) | |
| Smoking: | | | <0.001 |
| Former/current | 135 (82.8%) | 12 (28.6%) | |
| Never | 28 (17.2%) | 30 (71.4%) | |
| Not reported | 1 | 0 | |
| NSE, median [IQR] | 58.8 [34.4;102] | 29.9 [17.8;87.2] | 0.003 |
| Missing NSE data | 25 | 4 | |
| PD-L1 TPS: | | | 0.522 |
| <1% | 37 (75.5%) | 19 (90.5%) | |
| 1–49% | 11 (22.4%) | 2 (9.5%) | |
| ≥50% | 1 (2.0%) | 0 (0.0%) | |

| | P-SCLC | T-SCLC | P value |
|---|---|---|---|
| | ***N* = 164** | ***N* = 42** | |
| Not reported | 115 | 21 | |
| Stage: | | | 0.236 |
| IVA | 53 (32.3%) | 9 (21.4%) | |
| IVB | 111 (67.7%) | 33 (78.6%) | |
| Brain metastasis: | | | 0.001 |
| No | 68 (61.8%) | 9 (27.3%) | |
| Yes | 42 (38.2%) | 24 (72.7%) | |
| Not reported | 54 | 9 | |
| ECOG PS: | | | 0.428 |
| 0 | 2 (1.5%) | 2 (4.9%) | |
| 1 | 123 (92.5%) | 37 (90.2%) | |
| 2 | 8 (6.0%) | 2 (4.9%) | |
| Not reported | 31 | 1 | |
| Histology: | | | <0.001 |
| SCLC | 164 (100%) | 28 (66.7%) | |

| | P-SCLC | T-SCLC | P value |
|---|---|---|---|
| | ***N* = 164** | ***N* = 42** | |
| SCLC + adenocarcinoma | 0 (0.0%) | 14 (33.3%) | |
| Ki67(%), median [IQR] | 90.0 [80.0;90.0] | 90.0 [80.0;90.0] | 0.745 |
| Missing Ki67(%) data | 9 | 9 | |

Data was presented as n (%) or median [IQR]

Abbreviations: NSE, neuron-specific enolase; PD-L1, programmed cell death-ligand 1; TPS, tumor proportion score; ECOG PS, Eastern Cooperative Oncology Group performance status; SCLC, small-cell lung cancer.

1. T-SCLC had a modestly poorer prognosis compared to P-SCLC.
2. Chemoimmunotherapy, with or without bevacizumab, may provide benefit in T-SCLC.
3. Baseline serum NSE could be a useful prognostic indicator in T-SCLC.
4. High expression of CgA, Syn, and CD56 indicated poor prognosis in T-SCLC.

## Figure Legends

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**Supplementary Figure 1.** Genomic and clinicopathologic characteristics of patients with T-SCLC and P-SCLC. **A.** Comparison of the top mutated genes and clinicopathologic characteristics between patients with T-SCLC and P-SCLC. **B.** Venn diagram of the top mutated genes in patients with T-SCLC and P-SCLC. **C.** PFS of ABCP regimen vs. ECT/EPT regimen in patients with T-SCLC. **D.** OS of ABCP regimen vs. ECT/EPT regimen in patients with T-SCLC. Abbreviations: SCLC, small-cell lung cancer; P-SCLC, primary SCLC; T-SCLC, transformed SCLC; cn_amp, copy number amplification; cn_del, copy number deletion; ABCP, atezolizumab plus bevacizumab plus carboplatin plus paclitaxel; ECT/EPT, etoposide plus carboplatin/cisplatin plus atezolizumab; OS, overall survival; PFS, progress free survival.

**Supplementary Figure 2.** The impact of DOR and *EGFR* mutation status on OS for T-SCLC. **A.** Correlation analysis between DOR and OS in the T-$SCLC_{chemo+I/O}$ group. **B.** Correlation analysis between DOR and OS in the T-$SCLC_{chemo}$ group. **C.** Survival analysis between L858R and 19del in the T-$SCLC_{chemo+I/O}$ group. **D.** Survival analysis between L858R and 19del in the T-$SCLC_{chemo}$ group. Abbreviations: DOR, duration of response; *EGFR*, epidermal growth factor receptor; OS, overall survival.

**Supplementary Figure 3.** Exploration of NE markers and OS. **A, B.** The optimal cutoff value of baseline NSE levels in predicting prognosis for P-SCLC(A) and T-SCLC(B). **C.** The scatter plot of NSE and OS for P-SCLC. **D.** Comparison of NE marker expression between T-SCLC and P-SCLC (3+ vs. all other levels) **E.** A heatmap showing patients with P-SCLC categorized into NE-H group and NE-L group based on expression levels of three NE markers (CgA, Syn, and CD56). **F.** OS of NE-H group and NE-L group in patients with P-SCLC. Abbreviations: SCLC, small-cell lung cancer; P-SCLC, primary SCLC; T-SCLC, transformed SCLC; OS, overall survival; NSE, neuron-specific enolase; SQRT, square root; LN, natural logarithm; NE, neuroendocrine; NE-H, NE-high; NE-L, NE-low.