Original paper

Clinicopathological and molecular features of non-small cell lung cancer that transform to small-cell lung cancer: Case reports and literature review

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The purpose of this study is to explore the clinical and pathological characteristics as well as the molecular pathogenesis of patients with non-small cell lung cancer (NSCLC) transforming into small cell lung cancer (SCLC).

We investigated 14 patients with advanced NSCLC that transformed into SCLC. Whole genome sequencing (WES) was applied to analyse 14 tumour specimens (including NSCLC and SCLC specimens from each patient) from 7 patients to detect genetic predictive factors for small-cell transformation. The clinicopathological characteristics of these 14 patients were collected and analysed. In addition, a detailed literature review was conducted to identify similar cases of transformation from NSCLC to SCLC.

Fourteen cases were included. The basic condition of patients who had undergone the transformation was found to be similar to those individuals without any transformation. After SCLC transformation, the mutation spectrum changed: C>T decreased and C>A increased. In comparison to the initial NSCLC, the copy number variants (CNV) burden in the transformed SCLC increased considerably in a subset of patients. Clonal evolution analysis revealed intriguing connections and notable differences between the genetic clones of the initial NSCLC and the transformed SCLC. It was found that the process of transformation took a longer time in females compared to males. Furthermore, it was observed that the transformation time for LADC was longer compared to squamous cell carcinoma (SCC). Additionally, the analysis revealed that after completion of the transformation, the OS time for males was found to be longer than that for females.

Secondary biopsy is a crucial step in assessing the genetic and histological alterations that occur after a patient develops resistance to their initial treatment. This procedure is vital not only for individuals who have been treated with tyrosine kinase inhibitors but also for those who have undergone chemotherapy or immunotherapy. One interesting finding is that the mutation rate of p53 and RB1 in transformed SCLC is lower compared to *de novo* SCLC. Specifically, there is a decrease in the C > T mutation and an increase in the C > A mutation following transformation. Moreover, the transformed SCLC appears to originate from the major clones of the initial NSCLC.

Key words: NSCLC, SCLC, transformation, whole exome sequencing (WES), epidermal growth factor receptor (EGFR).

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. The World Health Organisation (2021) classifies lung cancer into two main histological types: non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Adenocarcinoma and squamous cell carcinoma are the main types of NSCLC. In recent years, the development of lung cancer treatment strategies has made great progress. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are the first-line treatment for a subset of lung cancers with EGFR-activated mutations [2–4]. However, drug resistance usually develops in most patients after approximately one year [5, 6]. Histological transformation into SCLC contributes to approximately 3–14% of TKI resistance in EGFR mutant NSCLC, as observed in repeat biopsy samples from patients [7–9]. The development of immunotherapy has led to the widespread use of immune checkpoint inhibitors that block programmed death 1 / programmed death ligand 1 (PD-L1) in the treatment of advanced lung cancers without sensitive mutations [10–13]. Furthermore, the transformation of NSCLC into SCLC is considered a resistance mechanism of immune checkpoint inhibitors [14]. The transformation of NSCLC into SCLC is also observed in patients undergoing chemotherapy and other targeted therapies [15, 16]. However, unlike TKIs, repeated biopsy is generally not recommended for NSCLC progression treated with chemotherapy and immune checkpoint inhibitors [17]. The aim of this systematic review is to determine the clinicopathological features and molecular pathogenesis of patients with transformation from NSCLC to SCLC.

Material and methods

Study design

The Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University approved this study (approval number: (2021) IIT(716)). All patients provided informed consent. Tissues were collected after obtaining written informed consent once the study was approved. To identify patients with advanced NSCLC that had transformed into SCLC, we conducted an electronic search of the Pathology Department database at the First Affiliated Hospital of Zhejiang University from May 2015 to May 2020 for NSCLC patients (including patients coming to our centre for consultation). Patients who had a previous history of SCLC or pulmonary neuroendocrine tumour were not included in the study. Additionally, patients who were initially diagnosed with SCLC based on their initial pathological specimens were also excluded from the research. In this study, we conducted a screening of a specific group of patients with NSCLC at the First Affiliated Hospital of Zhejiang University. These patients had previously undergone repetitive biopsies at the point of disease progression. Specifically, we collected a total of 28 tissue samples from 14 patients with advanced NSCLC that had transformed into SCLC. Out of these samples, 27 were obtained from the First Affiliated Hospital of Zhejiang University, while one sample was obtained from Xinchang Hospital of Traditional Chinese Medicine. However, not all of the collected tissue samples were qualified for whole exome sequencing (WES). Only samples that were obtained both before and after the transformation from 7 patients met the qualification criteria for WES analysis. Among the qualified samples, 11 cases of NSCLC were derived from the lung or bronchus, 2 cases were from the supraclavicular lymph nodes and group 4 lymph nodes, and 2 cases were from the parietal pleura. On the other hand, for SCLC, 6 cases were derived from the lung or bronchus, 3 cases were from the lymph nodes, 3 cases were from the liver, one case was from the pelvic fascia, and one case was from the bone marrow. To assess the response and progression of the disease, the investigator relied on radiologic reports and medical records. Follow-up information, including survival data, was obtained from the patients' electronic medical records and through telephone calls. The duration of survival after SCLC transformation was determined from the time of repeat biopsy until the patient's death.

Patients examination

Pathologists who specialise in lung cancer pathology carefully examined all sections of the tissue samples. The immunohistochemical (IHC) analysis was carried out at our institution using a fully automated system (Benchmark XT System, Ventana Medical Systems Inc., AZ, USA). The sections used for analysis were 4 μm thick and obtained from paraffin-fixed blocks. The IHC analysis involved the use of various antibodies, including PD-L1 (22C3, Dako, Glostrup, Denmark), CK7 (K727, Epitomics, CA, USA), P63 (GP1.4; Invitrogen, MA, USA), Napsin A (IP64; Leica, Wetzlar, Germany), TTF-1 (SPT24; Lab Vision, CA, USA), CK (pan) (AE1/AE3; Invitrogen), anaplastic lymphoma kinase (ALK; D5F3; Roche, Basel, Switzerland), Syn (EP158; Lab Vision), chromogranin (PHE5; Invitrogen), and CD56 (56C6; Invitrogen). Positive and negative controls were used to ensure the accuracy of the staining. The stained sections were then reviewed by 2 experienced pathologists.

DNA extraction

Least Genomic DNA was extracted from serial sections from tumour tissue using the GeneRead DNA FFPE Kit (Cat No.180134, QIAGEN, Hilden, Germany). The quantity and quality of the extracted DNA were determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., MA, USA) and 1% agarose electrophoresis, respectively.

EGFR mutation analysis

EGFR mutation analysis was performed using a method called the amplified refractory mutation system. The resected tumour samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. The DNA extracted from the tumour samples was used for PCR with the Mx3000PtM (Stratagene, CA, USA) using the EGFR 29 Mutations Detection Kit (Amoy Diagnostics, Xiamen, China).

Whole exome sequencing

The first biopsy specimens, operative specimens, and repeat biopsy specimens of 7 patients were analysed for WES. Matched DNA from normal lung tissue were available for 4 patients. Genomic DNA samples were captured using Agilent SureSelect Human All Exon v6 library following the manufacturer's instructions (Agilent Technologies, CA, USA). Briefly, genomic DNA was sheared to 150–220 bp small fragments using a sonicator (Covaris Inc., MA, USA). The sheared DNA was purified and treated with reagents supplied with the kit according to the manufacturer's instructions. Adapters from Agilent were ligated onto the polished ends, and the libraries were amplified by PCR. The amplified libraries were hybridised using custom probes. The DNA fragments bound to the probes were washed and eluted with the buffer provided in the kit. Next, these libraries were sequenced on the Illumina sequencing platform, and 150 bp paired-end reads were generated with an average sequencing depth of 100× for each sample. WES and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

Sequencing data quality control

Raw data were compiled in FASTQ format. To obtain high-quality reads that could be used for subsequent analysis, the raw reads were pre-processed with fastp (Version: 0.19.5). First, the adapter sequences were trimmed. Bases in sliding widows with average quality values < 20 were trimmed. Reads with ambiguous bases or those shorter than 75 bp were removed. Clean reads were aligned to the reference human genome (GRCh37) using BWA (Version 0.7.12). The mapped reads were sorted and indexed using SAMtools (Version 1.4), and Picard (Version 4.1.0.0, http://broadinstitute.github.io/picard) was used to mark and remove duplicate reads.

Variant calling

GATK (Version 4.1.0.0) was used for recalibration of the base quality score and for single nucleotide polymorphism (SNP) and insertion/deletion (INDEL) realignment to obtain analysis-ready BAM files. The final BAM files were used as input files for variant calling. Numerous annotation databases, such as Refseq, 1000 Genomes, the Catalogue of Somatic Mutations in Cancer, and OMIM were referred to during SNP&INDEL calling and annotated using ANNOVAR. Somatic single nucleotide variants (SNVs) and INDELs were then analysed using MeTect (Version 2.0) with default parameters. The tumour mutation burden (TMB) of a tumour sample was calculated as the number of non-synonymous somatic mutations (SNVs and INDELs) per megabase in coding regions.

Copy number variation analysis

Somatic copy number variants (CNVs) were identified using Control-FREEC (Version 11.3) with default parameters based on tumours and paired normal samples. We calculated the CNV burden of a tumour based on the percentage of autosomal tumour genome-bearing somatic CNVs.

Clonal evolution analysis

The clonal architecture of each tumour was determined using pyclone (Version 0.13.1) with default parameters. The VAF of each clone was used as an input for clonevol (https://github.com/hdng/clonevol), which constructed evolutionary trees and estimated the absolute percentage of each clone in each sample.

Systematic review

We systematically reviewed the literature to identify similar published reports on NSCLC transformation to SCLC. We searched PubMed on 16 April, 2023 using the words "((("carcinoma, non-small-cell lung"[MeSH Terms] OR ("carcinoma"[All Fields] AND "non-small-cell"[All Fields] AND "lung"[All Fields]) OR "non-small-cell lung carcinoma"[All Fields] OR "nsclc"[All Fields]) OR ("carcinoma, non-small-cell lung"[MeSH Terms] OR ("carcinoma"[All Fields] AND "non-small-cell"[All Fields] AND "lung"[All Fields]) OR "non-small-cell lung carcinoma"[All Fields] OR ("non"[All Fields] AND "small"[All Fields] AND "cell"[All Fields] AND "lung"[All Fields] AND "cancer"[All Fields]) OR "non small cell lung cancer"[All Fields])) AND (sclc[All Fields] OR ("small"[All Fields] AND ("cells"[MeSH Terms] OR "cells"[All Fields] OR "cell"[All Fields]) AND ("lung"[MeSH Terms] OR

"lung"[All Fields]) AND cancer[All Fields]))) AND transformation[All Fields]". We collected all the literature that reported the transformation of NSCLC into SCLC after treatment (including targeted therapy, chemotherapy, and immunotherapy). The PRISMA flow diagram showing the selection process for this systematic review is depicted in Fig 1. We reviewed the abstracts and full-text articles.

Statistical analysis

All visualisations were generated in the R statistical environment (Version 3.6.0), including Circos diagrams, histograms, and heatmaps produced by OE Biotech Co., Ltd. Transformation time and survival time were analysed using Kaplan-Meier survival analysis.

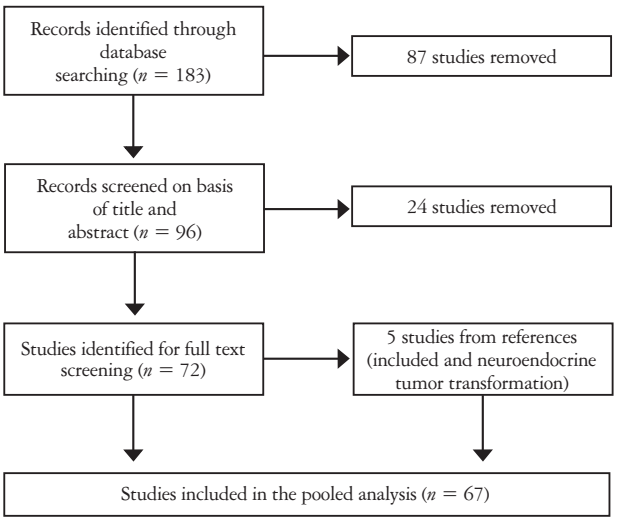

Fig. 1. PRISMA flow chart of study selection

Results

Clinicopathologic features

Baseline characteristics

During a 5-year period from 2015 to 2020, our institute observed a total of 14 cases of NSCLC transforming into SCLC in repeated biopsies. In Table I, we have summarised the clinical data of these 14 patients. The patients ranged in age between 31 and 71 years, with a median age of 57.4 years. Out of the 14 patients, 9 were men and 5 were women. Seven of the patients were smokers. Interestingly, none of the patients exhibited any specific symptoms related to the transformation from NSCLC to SCLC. It was found that 2 patients were asymptomatic, and their tumours were incidentally detected during a routine health examination. Additionally, 7 patients experienced a cough and expectoration, 2 patients had blood in their sputum, 2 patients reported chest tightness, and one patient had a fever. Three patients experienced shortness of breath, and one patient reported chest tightness. One patient had experienced haemoptysis, while another patient had shoulder and back pain.

Pre-transformation course

Eight initial diagnosis samples were obtained using needle biopsy, and 6 were surgically resected specimens. At the time of the original diagnosis, 5 cases were identified as squamous cell carcinoma (SCC) based on the expression of P40 and CK5/6, as well as the lack of expression for TTF1, CK7, and Napsin A. The other 9 cases were diagnosed as adenocarcinoma, showing positive expression for TTF1, CK7, and Napsin A, and negative expression for P40 and CK5/6. Furthermore, EGFR mutations were found in 6 of the adenocarcinoma cases, including 4 with the 19-Del mutation (one of which also had the T790M mutation), one with S768I and L858R, and one with L858R mutation. All cases were negative for CgA, Syn, and CD56 (Fig. 2).

Regarding treatment before the transformation, 4 patients (3 SCC patients and one adenocarcinoma patient) received immune checkpoint inhibitor (ICI) therapy, such as BGB-A317, nivolumab, sintilimab, and pabolizumab, alongside chemotherapy including paclitaxel, carboplatin, gemcitabine, cisplatin, and pemetrexed. Two other SCC patients received chemotherapy, and one received radiotherapy. Additionally, 6 patients with EGFR mutations in the adenocarcinoma type received EGFR-TKI treatment, such as gefitinib and icotinib, and 2 of them also underwent combination therapy with chemotherapy. Two other patients with adenocarcinoma received chemotherapy along with icotinib or erlotinib. The median time from diagnosis of advanced NSCLC to SCLC transformation was found to be 17.57 months, ranging from 3 to 47 months.

Small-cell lung cancer characteristics

All second biopsies were conducted using needle biopsy. These repeated biopsies confirmed that the patients had SCLC, a highly aggressive form of lung cancer. The confirmation of SCLC was based on the presence of at least 2 neuroendocrine markers, such as CgA, CD56, and Syn (Fig. 3, Table I), which are proteins commonly found in neuroendocrine cells. Additionally, the biopsies showed positive expression of TTF1 and CKpan, which are proteins that are typically found in SCLC cells and exhibit a distinct pattern of staining in the cytoplasm. Furthermore, Ki67 staining revealed that more than 90% of the cells had a high rate of proliferation. This characteristic of SCLC is sig-

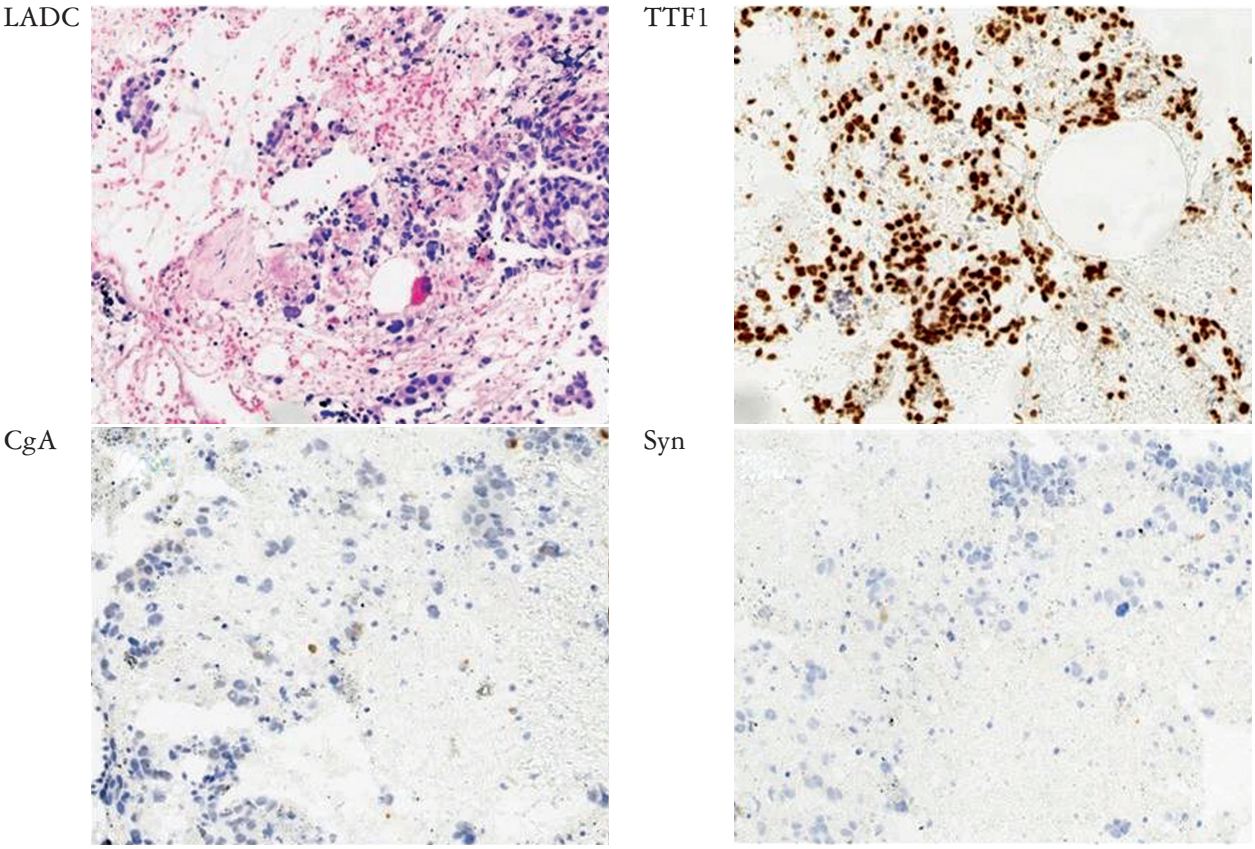

Fig. 2. Adenocarcinoma specimen. The tumour cells show obvious atypia, the cytoplasm is rich, the focal adenoid structure is seen, TTF1 is positive, and CgA and Syn are negative

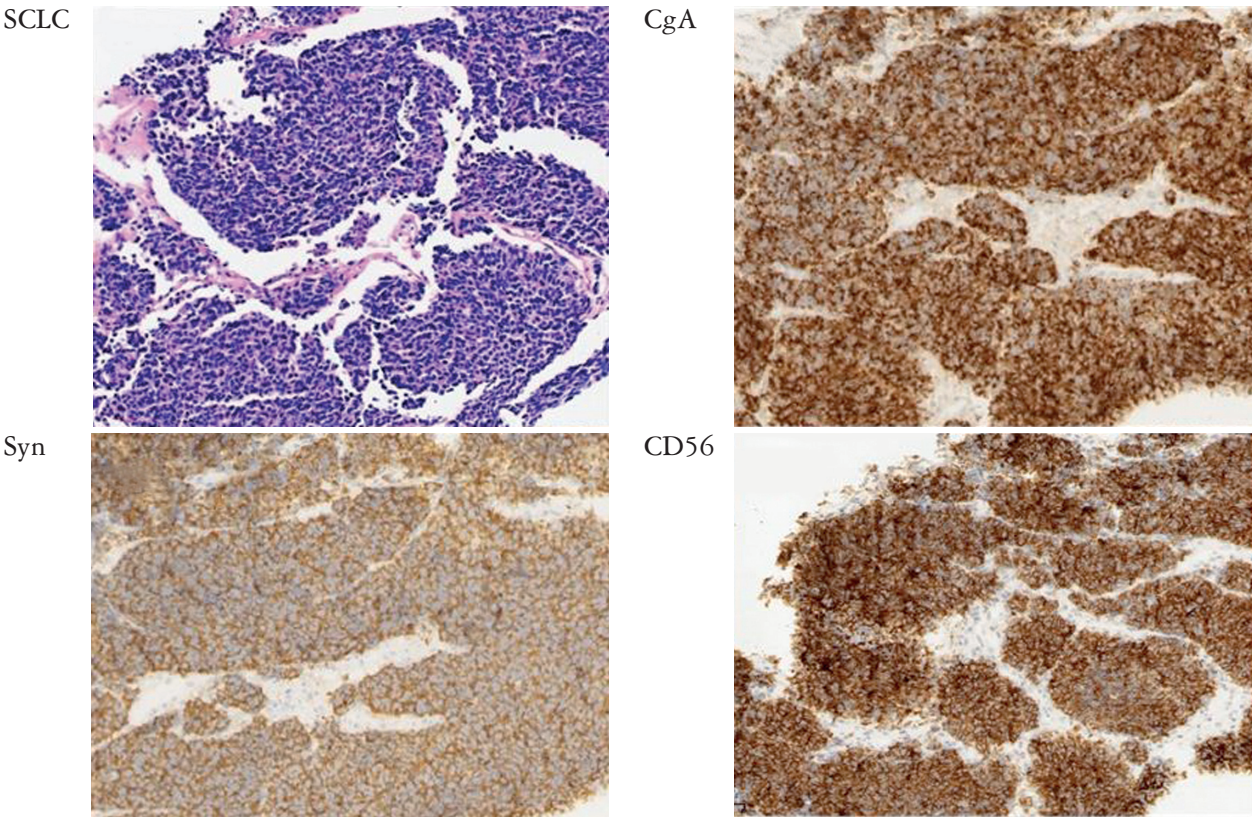

Fig. 3. Small-cell lung cancer (SCLC) specimen showing obvious cell atypia, lack of cytoplasm, fine nuclear chromatin, patchy distribution, and positive CGA, Syn and CD56

Table I. Summary of clinical and tumour genomic characteristic of patients

Source	Age	Sex	Smoking status	Histology of initial diagnosis	Genomic profile of original NSCLC	Treatment before transformation (C/S/N)	Time to transformation (months)	Histology after transformation and neuroendocrine markers	Genomic profile of SCLC	Treatment for SCLC	Patient outcome
P1	71	M	Smoker (30 cig. a day)	SCC	NOTCH1 RB1 ATM ASCL1 NRAS KRAS MET BRAF	Gemcitabine/cisplatin (1 months) > surgery > gemcitabine/ cisplatin > nivolumab > docetaxel/docetaxel >	12	SCLC +/+/+	RB1 TP53 SETD2 ERBB2 NOTCH1 ATM	Cisplatinum/etoposide	Died 6 months post SCLC dx
P2	65	M	Smoker(20 cig. a day)	SCC	TP53 PIK3CA SOX2	Radical resection > paclitaxel/cisplatin	10	SCLC +/+/+	NOTCH1 RB1 TP53 PIK3CA ASCL1 ERBB2 SETD2	No	Died 13 months post SCLC dx
P3	56	F	Never	LADC	TP53 RB1 PIK3CA	Pemetrexed/cisplatin (3 months) > pemetrexed/cisplatin/ pabolizumab/ radiotherapy > pemetrexed/ pabolizumab > pabolizumab	10	SCLC –/+/+	NOTCH1 RB1 TP53 PIK3CA ATM SOX2	Etoposide/carboplatin/ endostar > cisplatinum > etoposide/carboplatin + anlotinib > etoposide/carboplatin/ durvalumab > durvalumab > anlotinib	Survival after 12 months post SCLC dx
P4	53	M	Never	LADC	EGFR	Radical resection > icotinib (25 months) > radical resection > etoposide/ cisplatinum (4 cycles) > radiotherapy	47	SCLC –/+/+	NO	Irinotecan/cisplatinum	Died 16 months post SCLC dx
P5	57	M	Smoker (20 cig. a day)	SCC		Gemcitabine 1/ cisplatinum > radiotherapy	11	SCLC –/+/+		Not available	Loss to follow-up
P6	55	M	Smoker (20 cig. a day)	LADC	L858R	Pemetrexed/cisplatin Bevacizumab icotinib > PC	22	SCLC +/+/+			>24 months after SCLC dx (Alive)

Table I. Cont.

Source	Age	Sex	Smoking status	Histology of initial diagnosis	Genomic profile of original NSCLC	Treatment before transformation (C/S/N)	Time to transformation (months)	Histology after transformation and neuroendocrine markers	Genomic profile of SCLC	Treatment for SCLC	Patient outcome
P7	60	F	Never	LADC	19-Del	Gefitinib > osimertinib	19	SCLC –/+/+		Etoposide/ cisplatinum > icotinib > etoposide/ cisplatinum > osimertinib	Died 11 months post SCLC dx
P8	70	M	Smoker (25 cig. a day)	SCC		Paclitaxel/carboplatin/ BGB-A317 (3 cycles)	13	SCLC +/+/+		BGB-A317	Lost to follow-up
P9	54	F	Never	LADC	19-Del	Gefitinib (12 months) T790M > osimertinib (3 months)	15	SCLC –/+/+		Pemetrexed	Died 12 months post SCLC dx
P10	65	F	Never	LADC	19-Del	EGFR-TKI (details are not available)	35	SCLC +/+/+		Not available	Died 2 months post SCLC dx
P11	31	F	Never	LADC	S768I L858R	+/+/–	22	SCLC +/+/+		Pemetrexed/ cisplatin/AZD9291 > irinotecan/ carboplatin > docetaxel	Loss to follow-up after 12 months post SCLC dx
P12	44	M	Never	LADC	19Del, T790M	Mefatinib > osimertinib > pemetrexed / carboplatin	17	SCLC +/+/+		Etoposide/ cisplatinum	Survival after 6 months post SCLC dx
P13	54	M	Smoker (10 cig. a day)	LADC		Erlotinib	10	SCLC –/+/+		Etoposide/ carboplatin	Alive 3 months post SCLC dx; then lost to follow-up
P14	69	M	30-pack year	SCC		Sintilimab	3	SCLC		Symptomatic therapy	Died 2 months post SCLC dx

dx – diagnosis; EGFR – epidermal growth factor receptor; F – female; LADC – lung adenocarcinoma; M – male; NA – not available; SCLC – small-cell lung cancer; SCC – squamous cell cancer

**Age at diagnosis of initial disease*

nificant because it contributes to the rapid progression of the disease and its resistance to treatment.

After the diagnosis of SCLC, the main treatment approach for the patients was chemotherapy, as indicated in Table I. The average follow-up period for 7 patients ranged from 2 to 16 months, with a mean of 8.8 months. Unfortunately, 4 patients could not be followed up, but the remaining 3 patients were still alive at the time of analysis.

Whole exome sequencing results

Whole exome sequencing was performed on the tumour samples of all 7 patients before and after the transformation. However, only 3 patients could be analysed in detail due to various issues. Three patients did not have a normal control sample available for comparison, which is essential for identifying cancer-specific mutations. Additionally, one patient's sample did not meet the required quality standards for analysis. Hence, the analysis focused on the available samples from these 3 patients to gain insights into the genetic changes associated with the transformation of the tumour.

Mutational landscape of initial non-small-cell lung cancer and transformed small-cell lung cancer

After SCLC transformation, the mutation spectrum changed, with a decrease in the average proportion of C>T, from 44.03% to 19.53%, and an increase in the average proportion of C>A, from 16.10% to 51.9% (Fig. 4A). After SCLC transformation, TMB in patient 2 decreased; however, TMB in patients 1 and 3 increased. The TMB of NSCLC and SCLC was 12.04 and 37.94 in patient 1, and 16.46 and 91.93 in patient 3, respectively, and 42.54 and 8.05 mutations per Mb in patient 2, respectively (Fig. 4B). Thirteen known lung cancer driver genes were detected in patients with SCLC transformation, among which TP53, phosphatidylinositol-4,5-diphosphate 3 kinase catalytic subunit α (PIK3CA), and NOTCH1 had a high mutation frequency, as well as RB1 loss (Fig 4C). Compared with that of initial NSCLC, the CNV burden of transformed SCLC was decreased in all patients (patient 1, 89.73 vs. 37.09; patient 2, 69.73 vs. 37.33; patient 3, 45.33 vs. 37.14. Fig 4D). In all patients, CNVs were widespread throughout the entire ge-

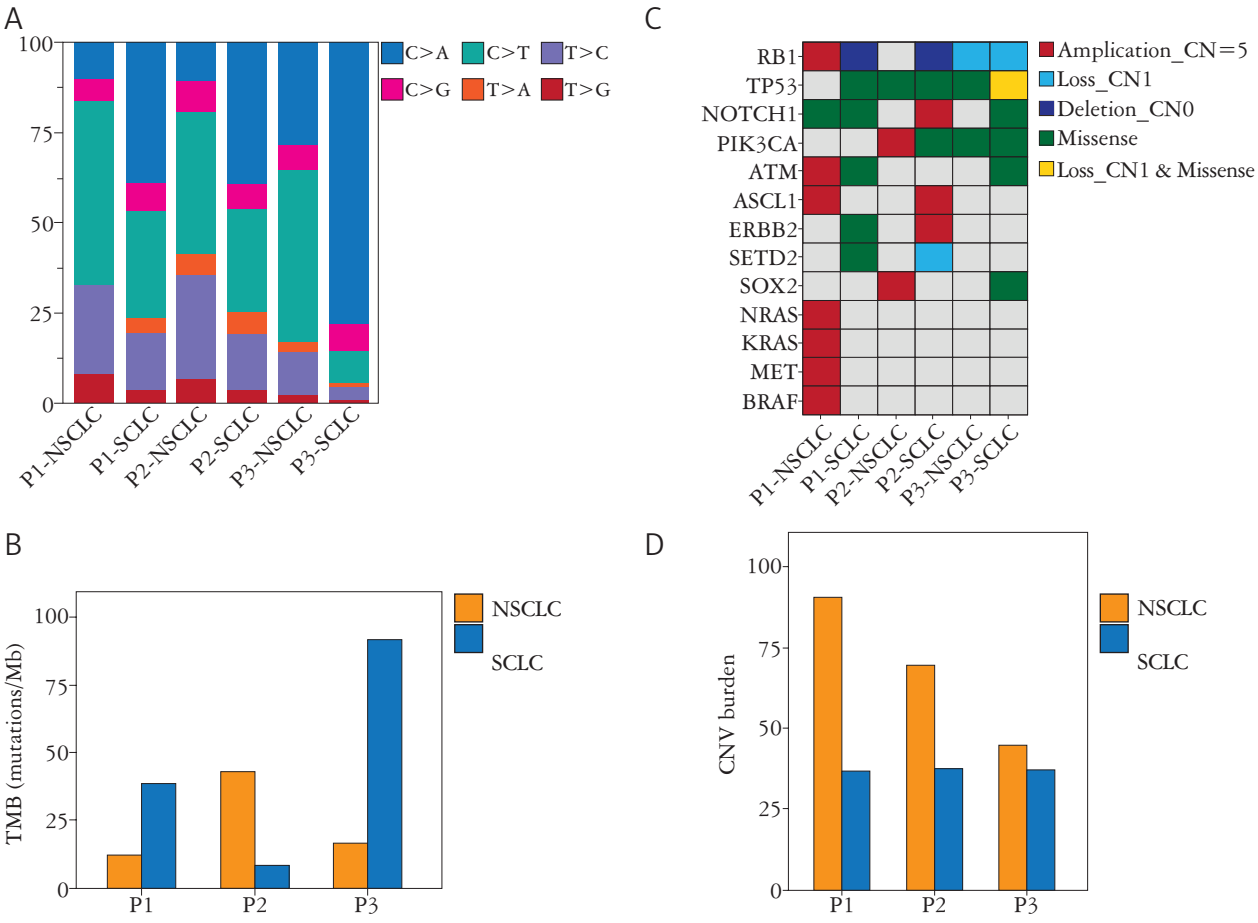

Fig. 4. A) The mutation spectrum changed after SCLC transformation. **B)** The tumour mutation burden (TMB) of the non-small cell lung cancer (NSCLC) and SCLC. **C)** Changes in lung cancer driver genes known in each sample. Each row, column, and colour represent a gene, sample, and mutation type, respectively. **D)** The copy number variants (CNV) burden of each patient before and after transformation

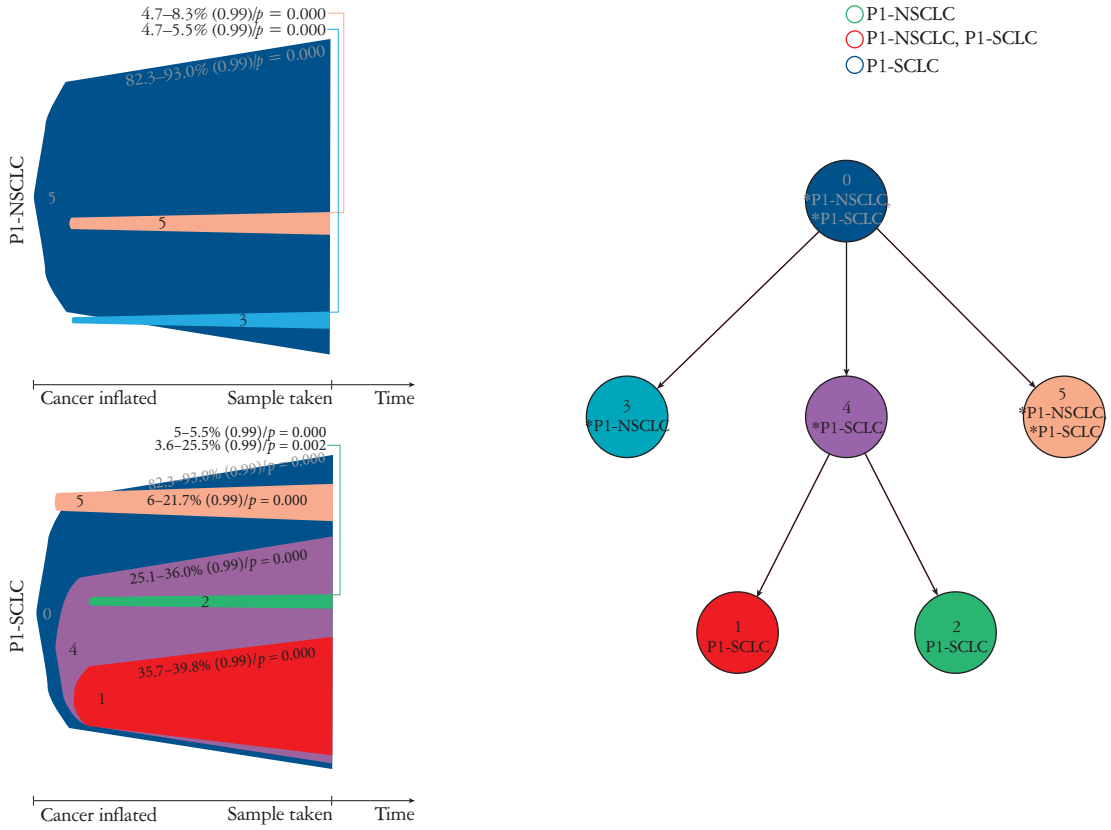

Fig. 5. The clonal evolution analysis of the clonal components of initial NSCLC and transformed SCLC of patient 1

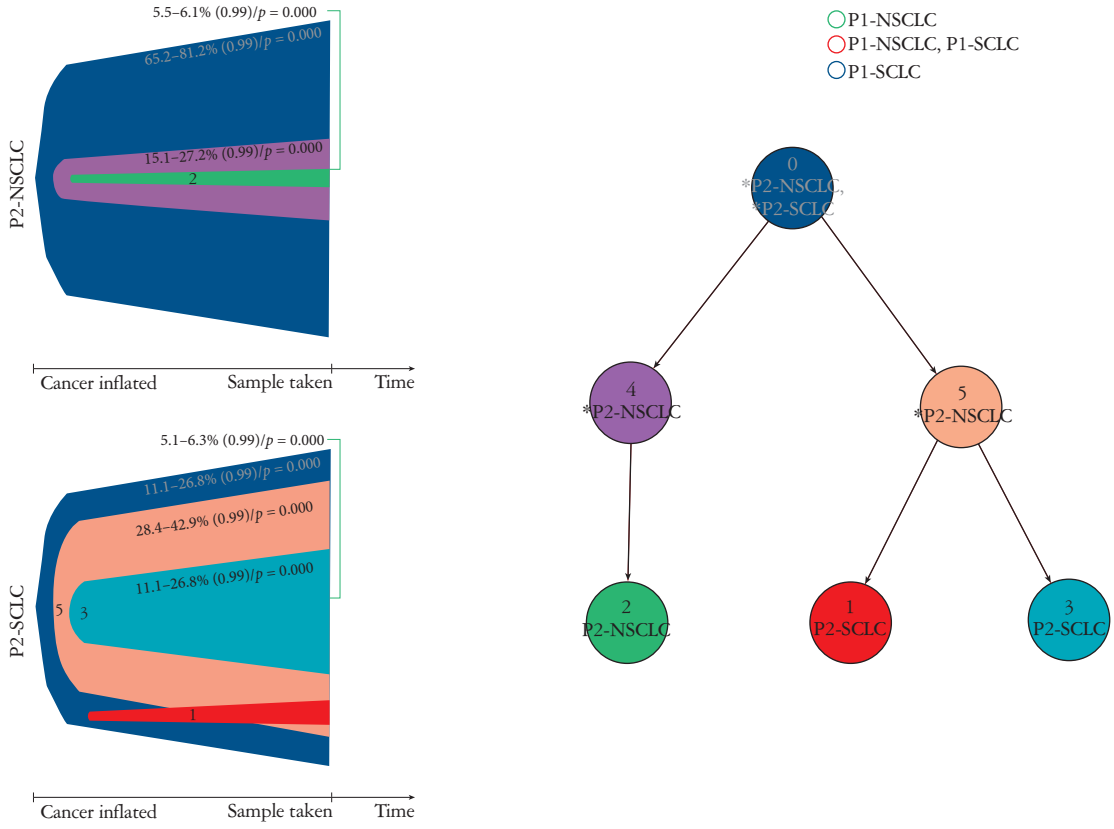

Fig. 6. The clonal evolution analysis of the clonal components of initial NSCLC and transformed SCLC of patient 2

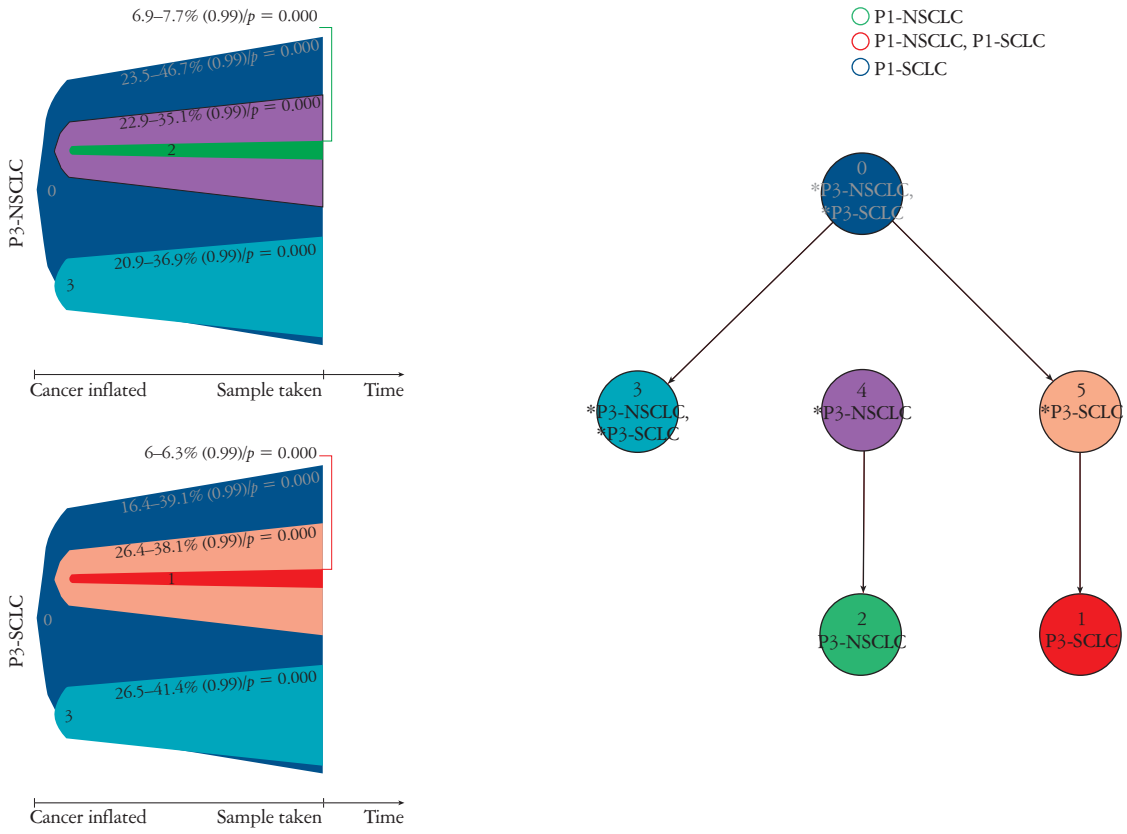

Fig. 7. The clonal evolution analysis of the clonal components of initial NSCLC and transformed SCLC of patient 3

nome of transformed SCLC compared with that of the initial NSCLC (Fig. 5).

Clonal evolution analysis

Clonal evolution analysis was performed using Clon-Evol (https://github.com/hdng/clonevol). The colours indicated the different clones. The clonal evolution analysis showed that the clonal components of the initial NSCLC and transformed SCLC were different. In patient 1, the NSCLC was mainly composed of clone 0, while the SCLC was mainly composed of clones 1 and 4 (Fig. 5). The NSCLC of patient 2 was mainly composed of clone 0, while the SCLC was mainly composed of clones 0, 3, and 5 (Fig. 6). In patient 3, the NSCLC was mainly composed of clones 0, 3, and 4, and the SCLC was mainly composed of clones 0, 3, and 5 (Fig. 7). These results suggest that the transformed SCLC clones originated directly from the major clones of the initial NSCLC.

Copy number variants burden is associated with the prognosis of patients with transformed small-cell lung cancer

For patients 1–3 in this study, the transformation time from NSCLC to SCLC was 12, 10, and 10 months, and the CNV burden of NSCLC was 89.73, 69.73, and 45.33, respectively (Fig. 8A, Table I). The overall survival (OS) of patients 1 and 2 after SCLC transformation was 6 and 13 months (patient 3 was lost to follow-up), with a CNV burden of SCLC of 37.09, 37.33, and 37.14, respectively (Fig. 8B, Table I).

The features of all cases from the systematic review and our study

We performed a systematic review of the literature to identify similar cases of NSCLC to SCLC transformation. There were a total of 140 patients with an average age of 57.9 years (26–80) in 67 studies (Suppl. Table 1, including 14 cases from our study that 2 had been published) [14–16, 18–81]. There were 66 men and 74 women. A total of 105 cases were EGFR positive before transformation, one case was uncertain, 10 cases were anaplastic lymphoma kinase (ALK) positive, one case was rearranged during transfection proto-oncogene (RET-positive, and 5 cases had neither EGFR nor ALK. Before transformation, 16 patients were treated with immunotherapy plus chemotherapy, 3 patients were not treated with immunisation and TKI (treatment unknown), and 121 patients were treated with TKI or TKI plus chemotherapy. The average transformation time was 24.5 months (1–86 months). Chemotherapy (such as etoposide, cisplatin, and carboplatin) was the main

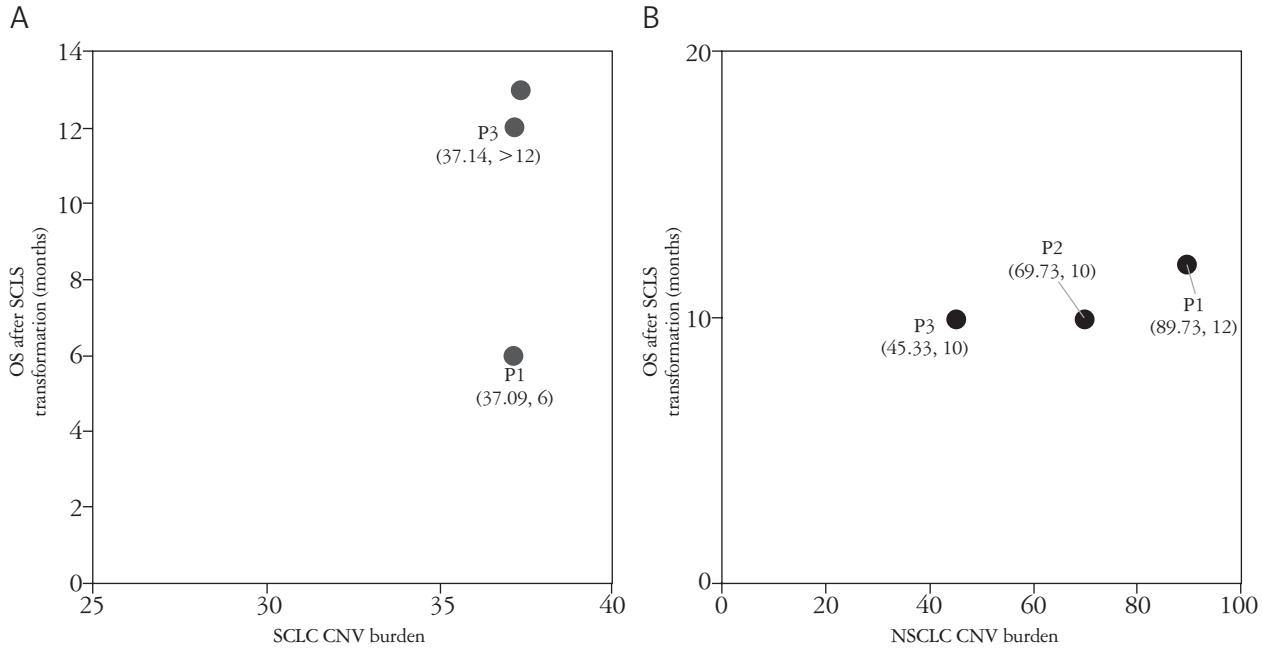

Fig. 8. A) The association between CNV burden and the time to transformation of NSCLC into SCLC. **B)** The association between CNV burden and overall survival after transformation to SCLC

treatment after transformation, supplemented by TKI or immunotherapy.

Survival time analysis

The patients from the literature (except for patients for whom we could not obtain detailed corresponding variables) and the patients from our study were included in the statistical analysis. There was no statistically significant correlation between transformation time and certain patient variables, including age, smoking status, EGFR status, ALK or RET status, *p53* mutation, and Rb1 mutations at the time of diagnosis. The transformation time was correlated with sex: the transformation time of females was long (average 28.46 months), and the transformation time of males was short (average approximately 20 months, $p = 0.014$). The transformation time was correlated with the tumour type at the initial diagnosis, the transformation time of adenocarcinoma was long (average 25.8 months), and the transformation time of squamous cell carcinoma was short (average approximately 14.6 months, $p = 0.029$). The OS after transformation was correlated with sex: the OS of females was short (average 10.1 months), and the transformation time of males was long (average approximately 14.7 months, $p = 0.014$).

Discussion

To our knowledge, this cohort represents the largest systematic review of the clinical outcomes of patients with NSCLC that has transformed into SCLC. Information from patients in the literature and patients in our centre were combined and analysed (Table II).

According to the literature, 85.8% of NSCLC cases that transformed into SCLC were treated with TKI (patients with EGFR mutation and ALK fusion), and 4.6% were treated with immunotherapy [14–16, 18–81]. Among our cases, 8 out of 14 patients were treated with TKI, accounting for a proportion of 57.1%. This indicates that TKI treatment is the main approach for patients with NSCLC that has transformed into SCLC (small-cell lung cancer), and the number of remaining cases was relatively small. The low proportion of non-TKI treatment may be attributed to the infrequent performance of re-biopsies during treatment. Therefore, it is recommended that patients who do not receive TKIs should also undergo routine re-biopsy when they develop drug resistance.

Before the transformation, none of the cases expressed neuroendocrine markers. However, after the transformation, neuroendocrine markers were expressed in all cases. The average time taken for transformation was 17.57 months, which is similar to the average transformation time mentioned in the literature (17.8 months) [82]. To perform a comprehensive analysis, the cases in the literature were combined with our cases. The average transformation time for patients treated with TKIs was 25.3 months, for patients treated with immunotherapy was 19.1 months, and for patients treated with chemotherapy was 23.7 months. This suggests

Table II. Clinical and laboratory characteristics of patients with NSCLC transforming into SCLC

Demographics	Total (N = 140)	TKI with or without chemotherapy	Immunotherapy with or without chemotherapy	Without TKI or Immunotherapy
Age, median (range)	57.9 (26–80)	56.3 (26-80)	68.5 (56–75)	63 (57–67)
Time to transformation*	24.5 (1–86)	25.3 (1–47)	19.1 (3–50)	23.7 (10–50)
Sex, No.				
Female	74	68	5	1
Male	66	52	11	3
Smoking status, No.				
Never smoker	73	72	1	0
Smoker	59	41	14	4
Not described	8	7	1	0
Histology of diagnosis				
SCC	12	0	10	2
LADC	125	119	4	2
Unknown or NOS	3	1	2	0
Mutation before transformation				
EGFR mutation	105	105	0	0
ALK or *RET* mutation	11	10	0	1
Negative for EGFR/ ALK alterations	24	5	16	3

ALK – anaplastic lymphoma kinase; EGFR – epidermal growth factor receptor; LADC – lung adenocarcinoma; SCC – squamous cell cancer

that patients with targeted gene mutations should be treated with targeted inhibitors, while patients without targeted gene mutations can be treated with immunosuppressants. In our study, out of the 14 patients, some received chemotherapy, some received immunotherapy or immunochemotherapy, and some received TKI treatment. After conducting a statistical analysis, the average overall survival time after transformation was found to be 8.6 months, which is similar to the results of a series of studies [82].

In terms of genetic mutations, the analysis revealed that C>T transitions were predominant in 3 patients, with 2 being smokers and one being a non-smoker. This finding is in contrast with a study where C>T transitions were dominant in lung cancer patients who were never-smokers and former light smokers, while C>A transitions were dominant in smokers. The occurrence of C>T transitions is caused by the deamination of cytosine to uracil. One study suggested that activation-induced high mutation of cytidine deaminase can serve as an early predictor of transformation into SCLC [83]. It was reported that C>T transitions were reduced in transformed SCLC, while C>A transversions were increased [51], which aligns with our findings.

The origin of SCLC subclones requires further investigation. Some studies have proposed that lung cancers originating from alveolar type II cells may transform into SCLC [84–86]. According to the literature, there is a trend of SCLC transformation in patients with or without an *EGFR* gene mutation [17]. In our study, among the 14 cases of SCLC transformation, there were adenocarcinoma patients with an *EGFR* mutation, as well as patients with adenocarcinoma or squamous cell carcinoma without an *EGFR* mutation. This is consistent with the notion that SCLC diverges early from NSCLC, especially in cases of EGFR TKI-resistant SCLCs, which may branch out from lung adenocarcinoma clones [37, 51].

Biallelic inactivation of TP53 and RB1 was observed in almost all of the initial *de novo* SCLCs that were analysed. Previous studies have suggested that the presence of baseline TP53 and/or RB1 mutations can be a distinguishing characteristic for patients with a higher likelihood of future transformation [37]. In our study, one patient had *p53* mutations and RB1 loss at baseline, while the other 2 patients had either a P53 mutation or RB1 loss. The dual inactivation of Rb and p53 is an important condition for the transformation of NSCLC into SCLC lineage; however, dual inactivation of Rb and p53 is not a necessary condi-

tion for the transformation from NSCLC to SCLC. Additional factors, such as *PIK3CA* and/or *Notch1* mutations, are often required [51, 82]. A retrospective study on the transformation of *EGFR* mutations into SCLC indicated that recurrent mutations in SCLC samples were TP53 (79%), RB1 (58%), and PIK3CA (27%) [82]. In our study, *TP53* and *NOTCH1* mutations were present in 100% of transformed SCLC samples, RB1 loss was observed in all samples, and *PIK3CA* mutations were found in 67% of the samples.

The transformation of SCLC can occur at any time during the disease course, with the earliest transformation observed at 3 months and the latest at 86 months after the initial lung cancer diagnosis. However, the median time for transformation was 17.57 months, which aligns with findings from a retrospective study [82]. Another study suggested that CNV burden is related to the prognosis of patients with transformed SCLC. They found that a higher CNV burden in the initial lung adenocarcinoma leads to a shorter time for transformation and a higher CNV burden in SCLC resulting in a shorter OS after transformation [51]. However, our study was unable to support this conclusion due to the limited number of cases.

In comparison to NSCLC, SCLC initially shows higher sensitivity to chemotherapy and radiotherapy. However, with the emergence of drug-resistant diseases, it rapidly relapses, leading to poor outcomes and a 5-year survival rate of less than 10%. Based on the literature and our own cases, the standard treatment for transformed SCLC involves chemotherapy, with the addition of immune and targeted therapy. However, the use of different drugs mentioned in the literature makes it difficult to perform statistical calculations, and the contribution of these rare events to SCLC development and response to therapy remains poorly understood.

Conclusions

The patients who underwent SCLC transformation had a similar basic condition compared to patients who did not experience transformation. However, after the transformation, there were significant changes in the mutation spectrum. Specifically, the occurrence of C>T mutations decreased, while the occurrence of C>A mutations increased. This alteration in the mutation pattern indicated a shift in the underlying genetic characteristics of the transformed SCLC. In addition to the genomic changes, clonal evolution analysis revealed both similarities and differences between the clones of initial NSCLC and transformed SCLC. Notably, the expression of *p53* and *RB1* genes in the transformed small cell carcinoma was found to be lower than in de novo small-cell carcinoma. This difference in gene expression levels may play a role in the transformation process and could contribute to the aggressiveness of the transformed SCLC.

To evaluate these genetic and histological changes and to select appropriate treatments after resistance, a secondary biopsy becomes crucial. It is especially important for patients who have been treated with TKIs, as well as those who have undergone chemotherapy and immunotherapy. The information obtained from the secondary biopsy enables clinicians to make informed decisions about the most suitable treatment approaches for these patients, ensuring better outcomes and increasing the chances of successful treatment.

Disclosures

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