ORIGINAL ARTICLE

Subtype of SCLC Is an Intrinsic and Persistent Feature Through Systemic Treatment

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ABSTRACT

Introduction: SCLC is an aggressive malignancy with poor outcome. Most patients have disease recurrence despite treatments with multiple modalities. Subtyping of SCLC has been proposed recently, and novel agents targeting specific subtypes are actively being investigated. In this study, we evaluated the plasticity of subtypes in paired pre- and post-treatment samples. The aim was to understand possible subtype evolution after chemotherapy resistance that could lead to alternate targeted therapy strategies.

Methods: A total of 68 samples from 32 patients with sufficient paired specimens were identified from 1998 to 2022. ASCL1, NEUROD1, and POU2F3 immunohistochemistry studies were performed on all cases, and subtyping by predominant expression was determined. Subtype comparison in each patient was performed, and expression analysis was performed on the basis of subtypes.

Results: Of 32 cases, 28 (88%) had the same subtype in pre- and first post-treatment specimens. Protein expression level of subtype-specific transcription factor remained stable after chemotherapy. Two of five (40%) NEUROD1-predominant SCLC switched to ASCL1-predominant phenotype after treatment. One case had a pitfall of scoring ASCL1 on specimen with marked crushing artifacts. One case revealed the challenge of proper subtyping for samples with borderline POU2F3 expression.

Conclusions: Subtype of SCLC generally remains the same after acquiring chemotherapy resistance. Plasticity was observed with rare cases switching from NEUROD1-predominant to ASC1-predominant SCLC. Resubtyping is unnecessary for the consideration of novel subtype-specific targeted agents, except cases with NEUROD1-predominant subtype.

Keywords: Small cell lung carcinoma; Subtype; Targeted therapy; Treatment effect

Introduction

SCLC is an aggressive malignancy with an overall 7% 5-year survival rate.[1] Although resection can be considered for patients with limited disease, most patients receive systemic therapy with or without radiation therapy as the current standard care for extensive disease.[2] Furthermore, although patients often have an initial response to etoposide in combination with platin-based chemotherapy, disease relapse and progression are typically observed. Combined chemotherapy with immunotherapy, including durvalumab and atezolizumab, has resulted in improved median overall survival

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by almost 3 months; however, longer-term survival remains elusive.[3,4]

The pathologic diagnosis of SCLC is largely based on morphologic evaluation of biopsy specimens, sometimes supported by positive expression of neuroendocrine (NE) markers, such as synaptophysin and chromogranin A, by immunohistochemical (IHC) studies.[5] Comprehensive genomic profiling studies reveal that SCLC is a relatively homogeneous entity, featuring biallelic loss of function of *RB1* and *TP53* and without tyrosine kinase oncogenic drivers.[6] Recent studies have proposed the subclassification of SCLC into four subtypes on the basis of the differential gene expression of ASCL1, NEUROD1, POU2F3, and YAP1.[7] A less understood fifth subtype with high expression of ATOH1 was also reported.[8] Immunohistochemistry has successfully been used as a surrogate to gene expression to classify SCLC into ASCL1-dominant, NEUROD1-dominant, POU2F3-dominant, and YAP1-dominant subtypes for practical clinical purposes[9–11]; however, low-level intratumoral heterogeneity has also been observed.[9,12,13] As YAP1 expression is inconsistent and unreliable by immunohistochemistry and investigation of the YAP1 subtype revealed T-cell–inflamed gene expression profile, the authors have recommended renaming this subtype as an inflamed or triple negative subtype.[14,15] Along with POU2F3, these subtypes are considered non-NE in contrast to ASCL1- and NEUROD1-dominant subtypes on the basis of molecular profiling and expression of NE markers by IHC.[6,9,16–18] Although no significant prognosis difference has been observed between the different subtypes, different targeted treatment strategies have been proposed for these individual subtypes.[19]

One important management consideration is how and when to integrate subtype-specific treatments into the current regimen. Furthermore, because the studies on subtypes of SCLC have mainly been done in untreated SCLC, the effect of treatment and resistance on the expression of these genes remains unknown. Herein, we investigated the subtypes of SCLC in paired pre- and post-treatment patient specimens.

Materials and Methods

The study was approved by the institutional review board at Mayo Clinic and performed with patient's authorization for research.

Case Selection

Natural language search in the laboratory information system of Mayo Clinic was performed for patients with a diagnosis of primary thoracic small cell carcinoma. Patients with adequate pre- and post-treatment pathology specimens were identified from years 1998 to 2022. Patients with pre- and first post-treatment specimen interval of more than 30 months were excluded, to eliminate the possibility of a second primary. Specimens from 32 patients were included in this study.

Clinicopathologic and Demographic Review

Clinical charts, including pathology reports, were abstracted to include age at diagnosis, sex, treatment, and interval time between biopsies. All histologic materials were re-reviewed by two pathologists (YCL and MCA) to confirm the diagnosis of small cell carcinoma.

Immunohistochemistry Studies

All cases were stained for ASCL1 (clone: 24B72D11.1; BD Biosciences), NEUROD1 (clone: EPR17084; Abcam), and POU2F3 (clone: polyclonal; Atlas Antibodies) for subtyping. The nuclear expression of each marker was semiquantitatively recorded for percentage of positive cells (0%–100%) and intensity of staining (0 = no stain, 1 = weak, 2 = moderate, 3 = strong). A combination H score (range 0–300) was calculated as previously described: H score = (0 × % negative) + (1 × % weakly positive) + (2 × % moderately positive) + (3 × % strongly positive cells).[20] Marker with H score less than 50 was interpreted as negative, as previously suggested.[9] Each slide was scored by two pathologists independently. Average scores were calculated and recorded for analysis. Cases with substantial discrepancy leading to subtype difference were re-reviewed by both pathologists together until consensus was reached.

Subtyping and Statistics

Each specimen was classified on the basis of the highest positively expressed H score into the following four subtypes: ASCL1 dominant, NEUROD1 dominant, POU2F3 dominant, and triple negative (defined by H scores <50 of all three markers).[9] Differences in pre- to post-treatment H scores (paired data within patient) were assessed with Wilcoxon signed rank tests. p values less than 0.05 were considered statistically significant.

Results

Clinical Data

A total of 68 specimens (from 32 patients) were included in the study, including 28 patients with one pre- and post-treatment specimen and four patients with one pre- and two post-treatment specimens. Patients' age ranged from 41 to 85 years (mean: 61.1 y). No sex predilection was identified. The clinical data are detailed in Table 1. Primary site was considered lung in 31 patients and thymus in one.

Table 1. Clinical Data

Categories	Numbers (%)
Age at diagnosis (y)	
Mean	61.1
Range	41–85
Sex	
Female	15 (47)
Male	17 (53)
Treatment received	
Chemotherapy only	9 (28)
Chemoradiation therapy	18 (56)
Resection + chemo	3 (9)
Chemo + immuno + radiation therapy	2 (6)
Duration between treatment and first post-treatment specimen (d)	
Mean	359
Range	35–753
Site of pretreatment specimen	
Lung, bronchus, and trachea	16 (50)
Lymph node	11 (34)
Pleura and pleural fluid	2 (6)
Mediastinum	2 (6)
Thymus	1 (3)
Site of first post-treatment specimen	
Lung, bronchus, and trachea	8 (25)
Lymph node	5 (16)
Bone	4 (13)
Liver	4 (13)
Pleura and pleural fluid	2 (6)
Brain	2 (6)
Soft tissue (abdomen)	2 (6)
Mediastinum	1 (3)
Chest wall	1 (3)
Adrenal gland	1 (3)
Pancreas	1 (3)
Eye	1 (3)
Site of second post-treatment specimen	
Brain	2 (50)
Lung, bronchus, and trachea	1 (25)
Liver	1 (25)

Note: All values are n (%) unless otherwise specified.
Chemo, chemotherapy; Immuno, immunotherapy.

The average duration between the start of treatment to the first post-treatment specimen was 359 days, ranging from 35 to 753 days. Treatments received included chemotherapy alone (n = 9, 28%), chemoradiation therapy (n = 18, 56%), resection followed by chemotherapy (n = 3, 9%), and combination of chemotherapy plus immunotherapy plus radiation therapy (n = 2, 6%). The treatment courses for patients are summarized in Table 1 and presented in detail in Supplementary Table 1.

IHC Subtypes

Treatment-Naive Tumor Subtypes. The sources of the pretreatment specimens were mostly from the lungs, lymph nodes, and bone (Table 1).

Treatment-naive tumors consisted of 24 (75%) cases of ASCL1 dominant, five (16%) cases of NEUROD1 dominant, one (3%) case of POU2F3 dominant, and two (6%) cases of triple negative (Table 2). In 24 ASCL1-dominant cases, seven cases were also NEUROD1 positive (H score $\geq$50). In five NEUROD1-dominant cases, two cases were also ASCL1 positive (H score $\geq$50). All ASCL1- and NEUROD1-dominant cases were POU2F3 negative (H score $<$50). The sole POU2F3-dominant case was negative for ASCL1 and NEUROD1.

Post-Treatment Tumor Subtypes

The source from the first post-treatment specimens was more often from distant metastatic sites (Table 1).

The post-treatment specimens revealed 25 (78%) cases of ASCL1-dominant, four (13%) cases of NEUROD1-dominant, two (6%) cases of POU2F3-dominant, and one (3%) case of triple negative small cell carcinoma (Table 2). In 25 ASCL1-dominant cases, five cases also co-expressed NEUROD1. Most ASCL1-dominant cases were POU2F3 negative except two cases with an H score of 50. In four NEUROD1-dominant cases, one case was also ASCL1 positive (H score = 85). All NEUROD1-dominant cases were POU2F3 negative. The two POU2F3-dominant cases were negative for ASCL1 and NEUROD1.

Comparison of Subtype Between Pre- and Post-Treatment Specimens

Of 32 cases, 28 (88%) preserved their subtype from pre- to first post-treatment specimens, including 23 (of 24) ASCL1-dominant, three (of five) NEUROD1-dominant, one (of one) POU2F3-dominant, and one (of two) triple negative cases (Fig. 1*A* and *B*). The one triple negative case with preserving subtype is the only never smoker in the study (Supplementary Table 1). Representative cases are found in Figure 2. The H score between pre- and post-treatment samples did not reveal any statistical differences indicating the expression remained stable and persistent (Fig. 3*A*–*C*). No considerable trend was observed between subtyping results and characteristics, including age, sex, local radiation, duration of treatment, and tissue types (Supplementary Table 1).

Four cases (13%) switched subtypes (Table 3): one case changed from ASCL1 dominant to NEUROD1 dominant, two cases from NEUROD1 dominant to ASCL1 dominant, and one case from triple negative to POU2F3 dominant (Fig. 1*A* and *B* and Table 3). The case (patient number [#]31) that changed from ASCL1 dominant to NEUROD1 dominant was positive for both ASCL1 (H score = 245) and NEUROD1 (H score = 205) in the treatment-naive specimen. It was positive for both ASCL1

Table 2. Subtyping and Expression of ASCL1, NEUROD1, and POU2F3 in Treatment-Naive and First Post-Treatment Samples

Subtype	Case Number (%)	ASCL1 Expression, Mean H Score (SD, Median, Range)	NEUROD1 Expression, Mean H Score (SD, Median, Range)	POU2F3 Expression, Mean H Score (SD, Median, Range)
Treatment naive				
ASCL1 dominant	24 (75)	190 (54, 188, 82-270)	36 (51, 14, 0-205)	1 (4, 0, 0-19.5)
NEUROD1 dominant	5 (16)	67 (73, 38, 0-150)	258 (38, 280, 200-285)	2 (3, 2, 0-7)
POU2F3 dominant	1 (3)	0	0	230
Triple negative	2 (6)	11 (n/a, 11, 0-22.5)	3 (n/a, 3, 0-5)	24 (n/a, 24, 0.5-47.5)
First post-treatment				
ASCL1 dominant	25 (78)	193 (60, 200, 70-298.5)	23 (40, 2, 0-150)	6 (14, 0, 0-50)
NEUROD1 dominant	4 (13)	28 (40, 13, 0-85)	258 (69, 291, 155-297.5)	0 (0, 0, 0-1)
POU2F3 dominant	2 (6)	0 (n/a, 0, 0-0)	0 (n/a, 0, 0-0)	181 (n/a, 181, 112.5-250)
Triple negative	1 (3)	0	0	0

n/a, not applicable.

(H score = 85) and NEUROD1 (H score = 155) in the post-treatment specimen as well. Nevertheless, there was a substantial difference in scoring of the ASCL1 expression (160 versus 10) of the post-treatment specimen between the two pathologists. On re-review of the ASCL1-stained slide, it was noted that the tumor cells were severely crushed and did not stain for ASCL1 in contrast to the better-preserved cells. Furthermore, the crushing artifact did not seem to affect the staining of the NEUROD1 in the same way (Fig. 4). When scoring ASCL1, one pathologist disregarded the crushed tumor cells as false negative and scored only the intact cells whereas the second pathologist took into account all cells, causing the discrepant H score. If the staining of the crushed cells is considered as a false negative owing to artifact, then the tumor would remain ASCL1 dominant with co-expression of NEUROD1.

To evaluate the interobserver agreement between pathologists, intraclass correlation coefficient between the two scoring pathologists of each marker was calculated. The intraclass correlation coefficient of ASCL1 was 0.81 (95% confidence interval [CI]: 0.70–0.88), that of NEUROD1 was 0.97 (95% CI: 0.93–0.98), and POU2F3 was 0.94 (95% CI: 0.90–0.96). Among all 68 samples, the subtype classification was concordant between the two scoring pathologists in 65 samples (96%). Two of the three discordant samples were presented in the previous paragraph. The third discordant sample was the pre-treatment sample from patient #25. That sample had similarly high expression of both ASCL1 and NEUROD1

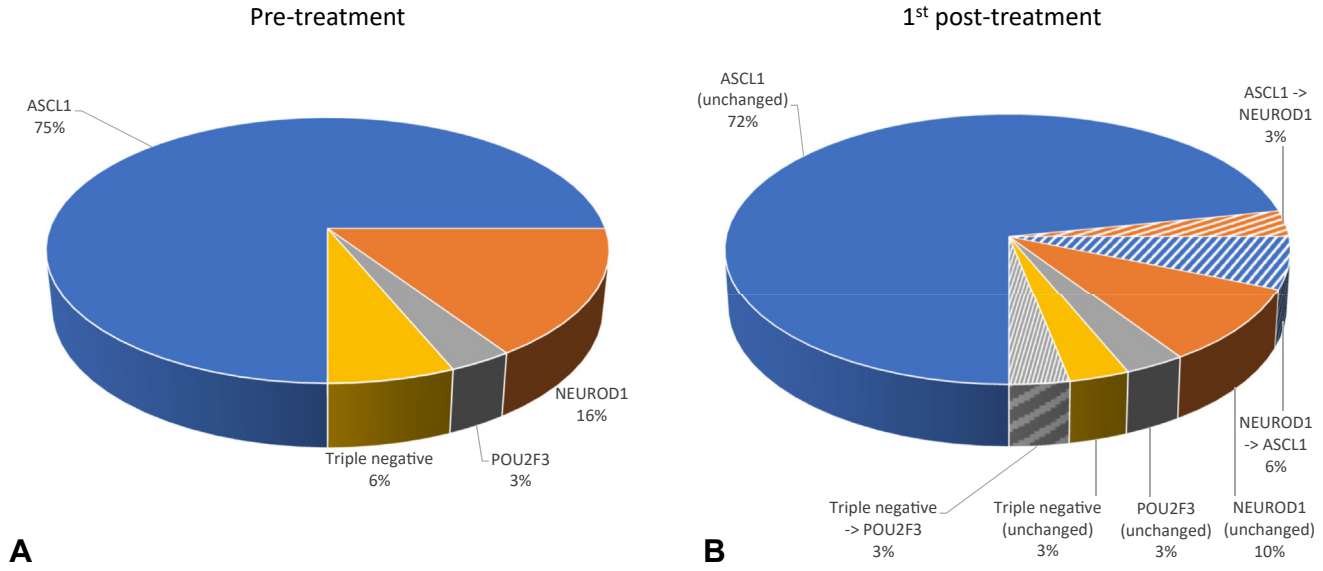

Figure 1. Pie charts of the breakdown of subtypes (*A*) pre- and (*B*) first post-treatment specimens. There were 28 (of 32) cases that maintained the pretreatment subtypes. In the four cases with change in subtype, one was from ASCL1 dominant to NEUROD1 dominant, two cases from NEUROD1 dominant to ASCL1 dominant, and one case from triple negative to POU2F3 dominant.

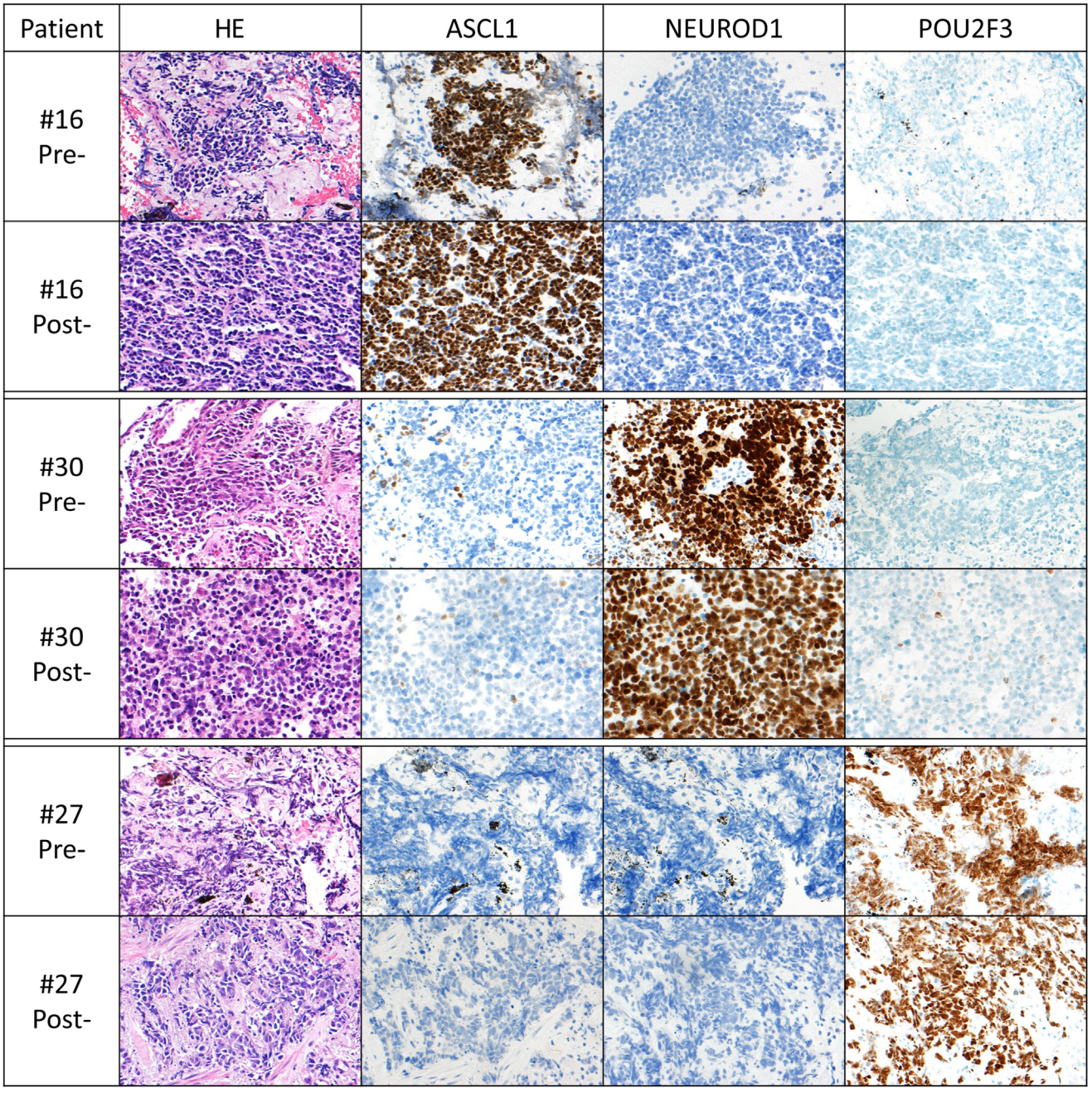

Figure 2. Representative histopathologic images illustrating maintenance of the subtypes between pre- and post-treatment tumors. Tumor cells from patient #16 remained ASCL1-dominant phenotype after disease progression, whereas tumor cells from patients #30 and #27 remained NEUROD1- and POU2F3-dominant phenotypes, respectively. Detailed H score in Supplementary Table 1. #, number; HE, hematoxylin and eosin.

and thus was scored and classified as ASCL1 dominant by one pathologist but as NEUROD1 dominant by the other.

We observed two cases with subtype change from NEUROD1 dominant to ASCL1 dominant. The first case (patient #8) was a 79-year-old man diagnosed with having SCLC by endobronchial ultrasound-guided fine-needle aspiration of mediastinal lymph nodes. The treatment-naive specimen was positive for both NEUROD1 (H score = 285) and ASCL1 (H score = 150). The patient received cisplatin plus etoposide with concurrent radiation. A post-treatment specimen obtained from an endobronchial ultrasound-guided fine-needle aspiration of mediastinal lymph nodes, 406 days after initiation of the treatment, had the tumor to be positive for ASCL1 (H score = 170) but negative for NEUROD1 (H score = 0),

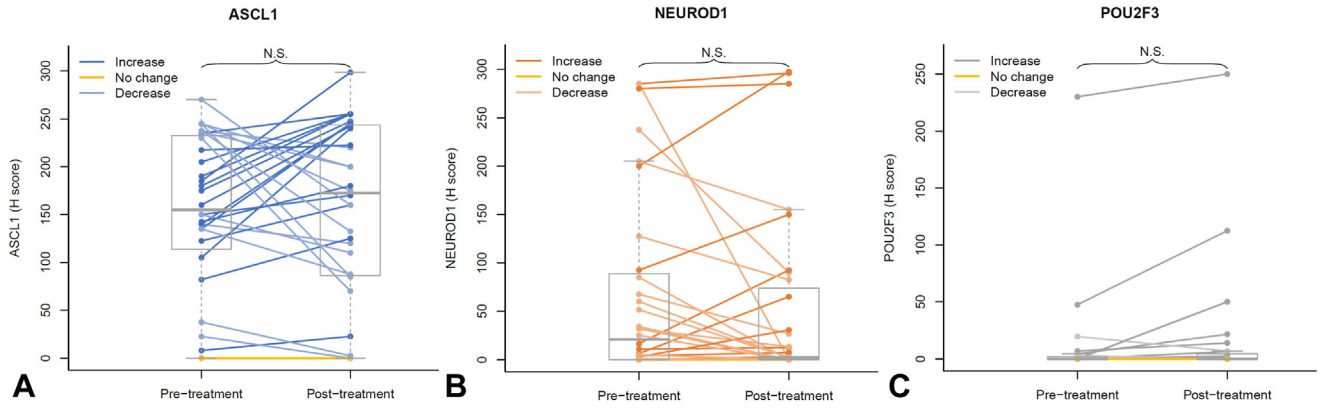

Figure 3. Protein expression of subtype-specific transcription factors in paired specimens. (*A*) ASCL1, (*B*) NEUROD1, and (*C*) POU2F3 had no significant overall change in pre- and post-treatment paired specimens. Individual increase and decrease were observed in subset of cases.

indicating a definitive change in subtype. The second case (patient #1) was a 58-year-old woman diagnosed with having SCLC by transbronchial biopsy of the left upper lobe lung. The treatment-naive specimen was positive for both NEUROD1 (H score = 237.5) and ASCL1 (H score = 140). The patient received etoposide plus cisplatin alternating with topotecan plus paclitaxel and thoracic radiation. She developed brain metastasis. A first post-treatment specimen from the resected brain metastasis, 491 days after initiation of the treatment, had a change in subtype with dominant ASCL1 with H score of 120 and NEUROD1 with an H score of 90 (Fig. 5). This patient received further Gamma knife treatment for the brain metastasis. Specimen from another craniotomy 6 months after was positive for ASCL1 (H score = 235) but negative for NEUROD1 (H score = 36.5), reaffirming the ASCL1 dominance (Fig. 5).

The last case (patient #23) that revealed a change in subtype was from a triple negative to a POU2F3-dominant subtype from a 70-year-old man treated with cisplatin plus etoposide after surgery. The treatment-naive tumor from a lobectomy specimen was negative for all three markers ASCL1 (H score = 0), NEUROD1 (H score = 0), and POU2F3 (H score = 47.5). The brain metastasis from the craniotomy resection, 507 days after initial systemic treatment, remained negative for both ASCL1 (H score = 0) and NEUROD1 (H score = 0) but positive for POU2F3 (H score = 112.5) (Fig. 6*A* and *B*). In the naive treated tumor specimen, there was a noticeable difference (15 versus 80) between the two pathologists' H score of the POU2F3 expression. When the POU2F3-stained slide was re-reviewed, the consensus was that the POU2F3 expression was truly below 50, and thus, the H score of 47.5 was upheld (Fig. 6*A* and *B*).

Four patients had more than one post-treatment specimen, allowing us to study the temporal evolution of the gene expression. Three cases had an ASCL1-dominant subtype across all biopsies and different treatments (Table 4). One patient (#1), described previously, had subtype switching from NEUROD1-dominant to ASCL1-dominant and then further evidence of ASCL1-dominant phenotype (Fig. 5). In the treatment-naive specimen, the tumor co-expressed NEUROD1 dominant (H score = 237.5) and ASCL1 (H score = 140). The subtype changed to ASCL1 dominant and maintained this phenotype in the second recurrence (Table 4). Although slight variations of ASCL1 and NEUROD1 scoring were observed, no overt trend of subtype change was appreciated.

Table 3. Paired Subtyping and Expression of ASCL1, NEUROD1, and POU2F3 in Cases With Subtype Switching

	Pretreatment (H Score)			First Post-Treatment (H Score)		
Patient	ASCL1	NEUROD1	POU2F3	ASCL1	NEUROD1	POU2F3
#31	245	205	0	85[a]	155	0
#8	150	285	3	170	0	0
#1	140	237.5	7	120	90	14
#23	0	0	47.5[a]	0	0	112.5

[a]Scoring discrepancy between pathologists.
#, number.

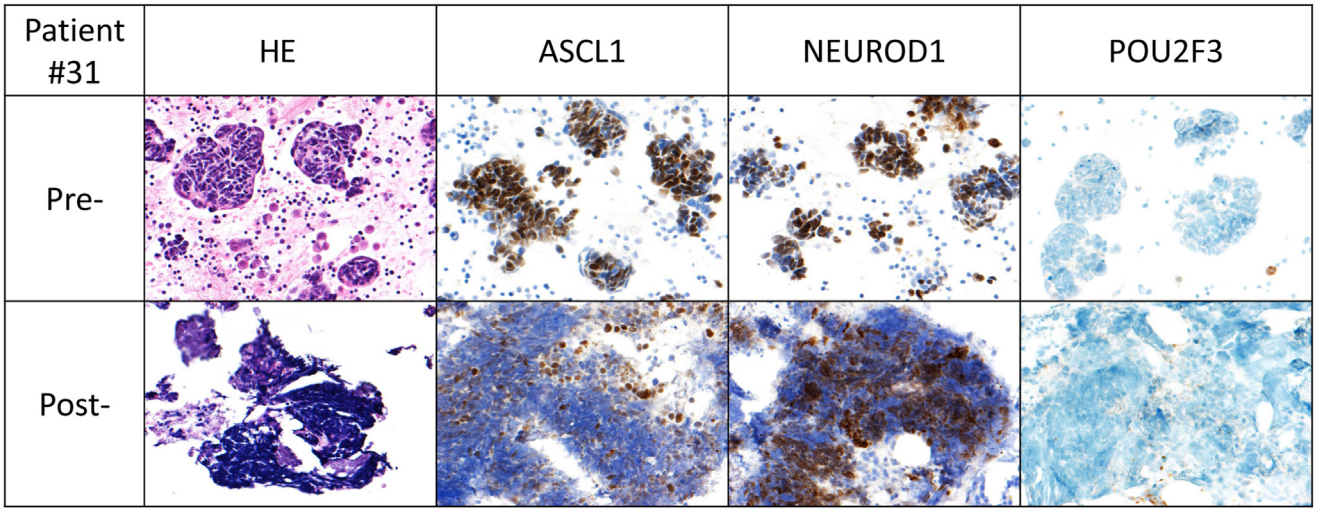

Figure 4. Histopathologic images of a case determined as switching from ASCL1- to NEUROD1-dominant subtype potentially owing to scoring pitfall. Tumor cells from pretreatment specimen had abundant expression of both ASCL1 and NEUROD1 and were classified as ASCL1 dominant because of higher H score. Tumor cells from post-treatment specimen had poor histomorphology with severe crushing artifacts. Although intact tumor cells were positive for ASCL1, crushed tumor cells were mostly negative. In contrast, considerable portion of crushed tumor cells remained positive for NEUROD1, leading to classification as NEUROD1-dominant subtype. Detailed H score in Supplementary Table 1. HE, hematoxylin and eosin.

Discussion

Owing to unsatisfactory treatment response and high progression rate with the current chemotherapy regimen for SCLC, several therapeutic strategies have been explored, including immunotherapy, DNA damage repair and cell cycle checkpoint, growth and survival signaling,

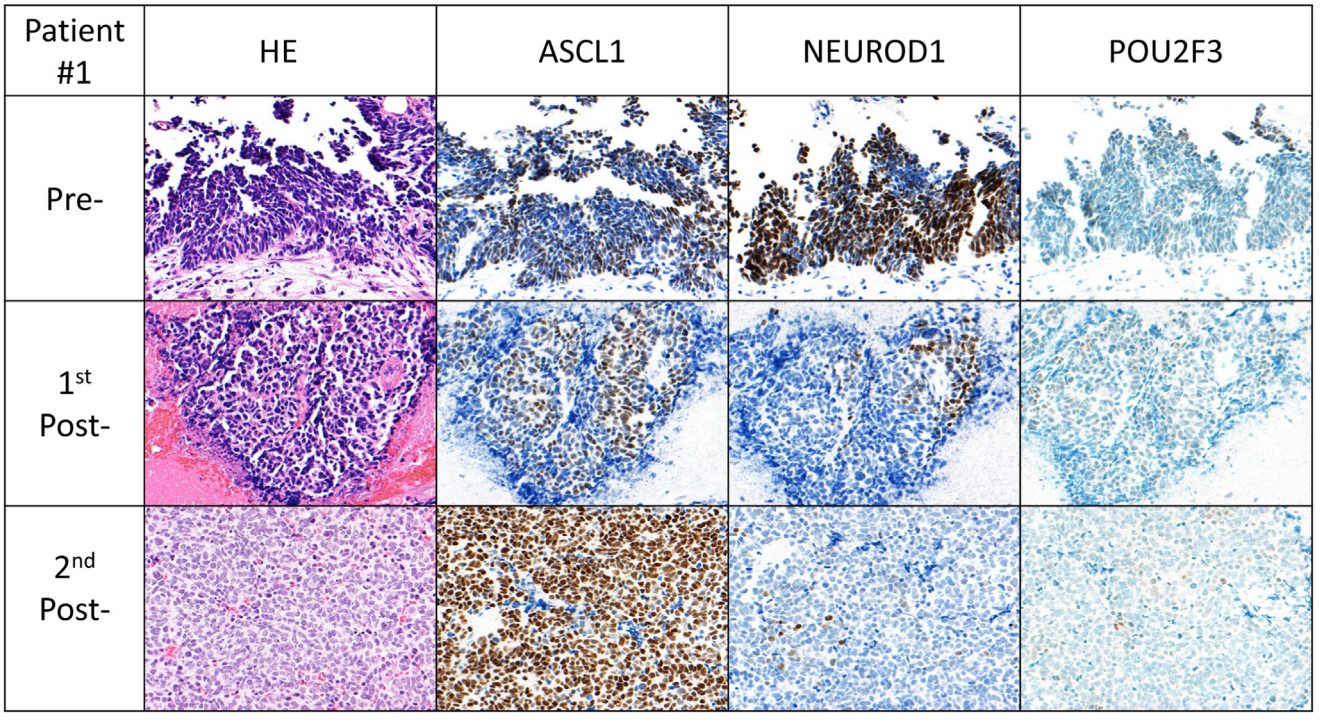

Figure 5. Representative histopathologic images revealing subtype switching from NEUROD1-dominant to ASCL1-dominant and persistence over time. Tumor cells from patient #1 had high expression of NEUROD1, low expression of ASCL1, and negative for POU2F3. First post-treatment sample revealed reversed ASCL1 and NEUROD1 expression and was subtyped as ASCL1 dominant. Second post-treatment specimen illustrated further dominant ASCL1 expression. Detailed H score in Supplementary Table 1. #, number; HE, hematoxylin and eosin.

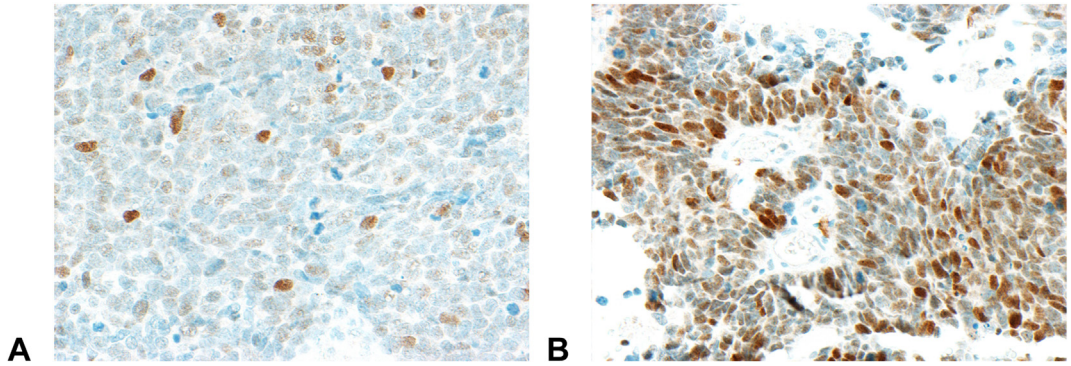

Figure 6. Immunohistochemistry-stained slide for POU2F3 revealing the challenges of POU2F3 scoring. (*A*) Pretreatment sample from patient #23 illustrated a borderline H score slightly less than 50. Weak or blush nuclear stain in abundant cells resulted in inconclusive scoring. (*B*) In comparison, more prevalent and stronger POU2F3 staining was observed in the post-treatment sample. Detailed H score in Supplementary Table 1. #, number.

and epigenetic regulators. With the recent discovery of SCLC subtypes, treatments specifically targeting these different subtypes have been proposed.[21] RNA expression studies have revealed that ASCL1-dominant SCLC possesses higher *MYCL* expression whereas *MYC* expression is highest in POU2F3-dominant SCLC.[18,22,23] It also seems that different subtypes of SCLC have distinct metabolic wiring, suggesting subtype-specific mitogenic vulnerability.[24] Therefore, preclinical data indicate that BCL2, DLL3, CREBBP, and LSD1 are amenable targets for ASCL1-dominant SCLC. Furthermore, aurora kinase, WEE-1, CHK1, and IMPDH arginine deprivation are potential targets and strategy for the other three subtypes, whereas additional individual targets have been reported, such as LSD1 for NEUROD1-dominant subtype, PARP inhibitors and IGF1R for POU2F3-dominant subtype, and immunotherapy for triple negative (or YAP1-dominant or inflamed) subtypes.[15,22,23,25,26] Other biomarkers such as SLFN11 for PARP inhibitor are also actively explored in clinical trials.[27,28] Although there is increased interest in subtypes defining therapeutic vulnerabilities, the variation in expression of these transcription factors has not currently been well-established to inform patient care.

This presented study aimed to address the question of whether the subtype of SCLC changes or remains stable during the course of treatment when disease progresses to pave the road for optimizing the timing and combination of integrating such subtype-specific novel targeted therapy into current standard care regimens. We used a unique cohort of 32 patients with paired pre- and post-treatment specimens. Owing to the rarity of patients undergoing repeated biopsies in the course of their disease and limited size of tissue obtained during sampling, assembling a cohort of paired pre- and post-treatment specimens necessary to answer our study question was extremely challenging and specimens were identified in a 24-year span. Although a small and heterogeneous data set, owing to different stages and treatment modalities, it remains, thus far, the only study addressing this important clinical question. Although previous studies have revealed conflicting evidence of how good the correlation between the RNA expression and immunoprofile, using IHC staining as a proxy for subtype delineation provides a practical surrogated approach. Nevertheless, further confirmation of these results with paired RNA expression data would be ideal although challenging owing to the small tissue size in the archived specimens. We therefore investigated the subtypes by IHC profile of each specimen and the correlation with treatment status in each individual patient.

The breakdown of subtypes in treatment-naive cases is similar to that reported in the literature.[9] The

Table 4. Serial Subtyping and Expression of ASCL1, NEUROD1, and POU2F3 in Cases With Multiple Post-Treatment Samples

	Pretreatment (H Score)			First Post-Treatment (H Score)			Second Post-Treatment (H Score)		
Patient	ASCL1	NEUROD1	POU2F3	ASCL1	NEUROD1	POU2F3	ASCL1	NEUROD1	POU2F3
#1	140	237.5	7	120	90	14	235	36.5	30
#10	135	60	0	245	2	0	227.5	12.5	6
#17	180	0	0	255	1	0	240	30	0
#28	217.5	51.5	2.5	222.5	2.5	0	167.5	77.5	0

#, number.

interobserver agreement of each of the three markers and the subtype classification concordance are high between independent scoring pathologists. Furthermore, our study reveals that the SCLC subtypes are conserved in most cases (88%) and the overall expression level generally remains similar. It has been reported from in vitro models that MYC activates Notch to dedifferentiate NE tumor cells, promoting a temporal shift in SCLC from ASCL1+ to NEUROD1+ to YAP1+ states.[29] We did not observe a shift from ASCL1+ to NEUROD1+ in the current treatment patient cohort. Some modest fluctuations of protein expression were observed in some cases, especially those having higher expression of both ASCL1 and NEUROD1. Although subtyping them by only the dominant H score could be arbitrary, the general trend remains consistent. Rare cases, for example, patients #2 and #14 (Fig. 3*A*–*C* and Supplementary Table 1), had considerable expression movement but failed to switch subtype. The overall findings indicate that the subtype of SCLC is an intrinsic characteristic of the tumor and for the most part independent from treatment effect. The subtype is likely determined during initial tumorigenesis and generally does not change in response to current treatment, over time, or after progression of the disease. This finding suggests that subtyping of SCLC can be performed on treatment-naive specimen and that repetitive sampling may not be required when a patient progresses through standard of care treatment.

Only four cases in our study had a change in subtype. Two of them (patients #31 and #23) were likely due to technical difference in the scoring methods between pathologists (Table 3). These two cases also raise the concern of scoring pitfall and issue of cutoff determination. It has been noted that ASCL1 stain, unlike NEUROD1 and POU2F3, could be considerably lost in crushed or necrotic cells owing to vulnerable antigen preservation. The caveat is that diagnostic material from biopsy specimen in SCLC typically has crushing artifact and prominent necrosis. The pretreatment specimen of patient #31 clearly illustrated the dilemma that including the crushed tumor cells in the denominator or excluding them can substantially affect the scoring and determination of subtypes (Fig. 4). A standardized scoring method would be needed to address this issue for proper classification and to avoid this pitfall. In addition, case #23 had the struggle of subtyping SCLC when an H score is near the cutoff of 50. The only one treatment-naive POU2F3-dominant case (patient #27) had diffuse and strong POU2F3 staining (Fig. 2), whereas most other cases had minimal to no POU2F3 expression. Nevertheless, the case (patient #23) that had a switch from triple negative to POU2F3 dominant had an H score just below the cutoff of 50, in contrast to the post-treatment which was 112. As the H score includes percentage of positive cells, size of specimen and sampling could account for that slight difference in score (Fig. 6*A* and *B*). Owing to the rarity of POU2F3-dominant cases found in this cohort and those reported in the literature, further studies would be desirable to revisit and establish a more definitive cutoff. Therefore, depending on the scoring scheme or interpretation, these two cases most likely conserved their phenotype for a concordance rate of 94% (30 of 32).

Two SCLC (patients #8 and #1) had a definitive change in subtype, from NEUROD1 dominant to ASCL1 dominant, comprising 40% (two of five) of treatment-naive NEUROD1-dominant SCLC cases (Fig. 5). This finding is relevant in that it was not observed in other subtypes. Furthermore, those two cases were both positive for NEUROD1 and ASCL1 but with NEUROD1 dominance in the untreated specimen, whereas the other three remaining cases of NEUROD1-dominant were NEUROD1+ and ASCL1− before and after treatment. Although more data would be needed owing to currently small sample size, this finding revealed the possibility of subtype plasticity in cases of NEUROD1-dominant subtype, which also expresses ASCL1. Interestingly, studies had revealed that the NE plasticity could be regulated by RNA-binding protein such as ZFP36L1[30] and that plasticity from NE to non-NE phonotypes can be driven by NOTCH signaling in SCLC.[31] This finding can potentially further suggest retesting of NEUROD1-dominant subtype, in particular those also positive for ASCL1, if subtype-specific targeted therapies are incorporated into treatment considerations in the future.

Given the specific therapeutic vulnerabilities of each SCLC subtype, we expect the subtypes of SCLC to emerge as biomarkers for future trials on this disease. For second-line trials, a clinical question that arose was whether the subtype remains the same as when the initial diagnosis was done. Practically, the acuity and severity of the disease when SCLC relapses do not always allow room for rebiopsy. This study reveals that in our population the subtypes remain stable in at least 88% of the patients. Given the relatively long interval between sampling in our cohort indicating stable subtype across treatments, the data suggest that rebiopsy would not be necessary except perhaps in cases of NEUROD1 that co-express ASCL1. Nevertheless, given the recent incorporation of immunotherapy in the first-line treatment of SCLC, only two patients in our cohort had immunotherapy. We expect future studies to include patients treated with immunotherapy and a need to reevaluate the evolution of subtypes after treatment with the recent Food and Drug Administration–approved combinations of immunotherapy with chemotherapy.

CRediT Authorship Contribution Statement

Ying-Chun Lo: Conceptualization, Funding acquisition, Project administration, Data curation, Writing—original draft, Writing—review and editing.

Joel Rivera-Concepcion: Data curation, Writing—review and editing.

George Vasmatzis: Conceptualization, Writing—review and editing.

Marie-Christine Aubry: Conceptualization, Data curation, Writing—review and editing.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2023.100561.

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