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Small cell lung cancer: New drugs and strategies

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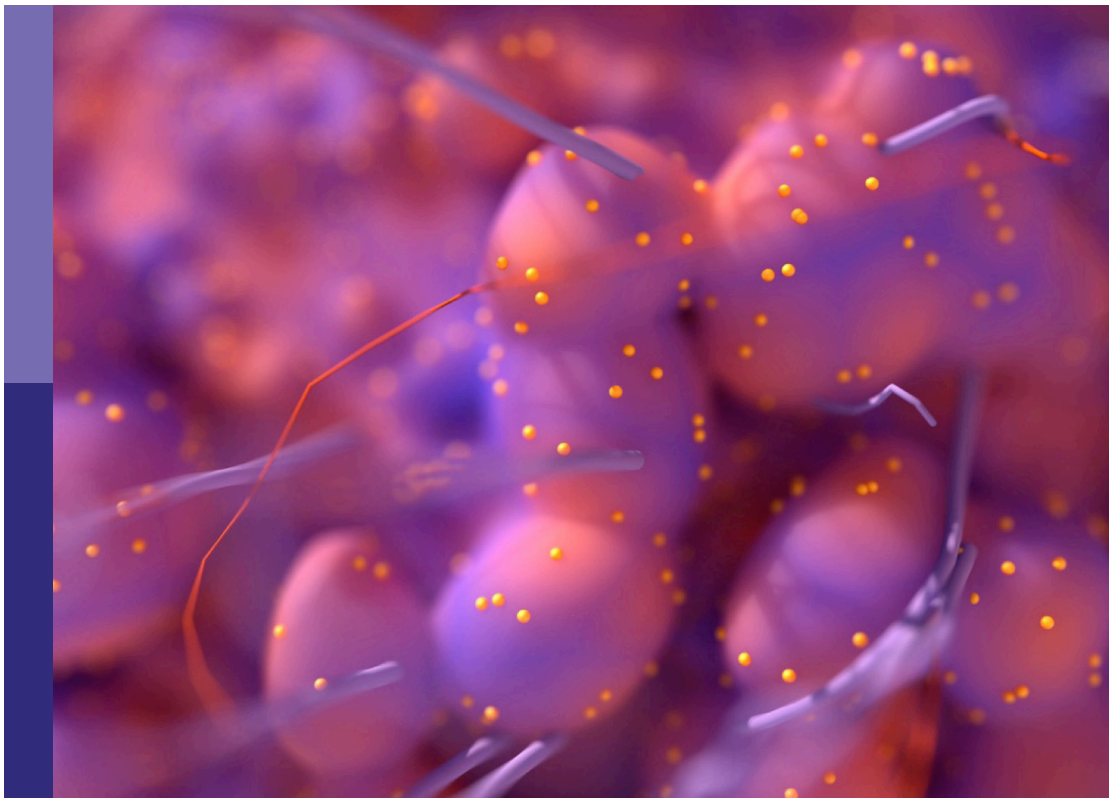

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Small cell lung cancer: New drugs and strategies

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Editorial: Small cell lung cancer: New drugs and strategies

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KEYWORDS

SCLC, immunotherapy, lurbinectedin, anti-DLL3 drugs, Aurora kinase inhibitors

Editorial on the Research Topic
Small cell lung cancer: New drugs and strategies

Small Cell Lung Cancer (SCLC) is an aggressive disease with a dismal prognosis at 5 years (1). After decades of nihilism, immune-check point inhibitors combined with chemotherapy led to a new standard first line treatment improving the overall survival rate and increasing also the number of the so-called long-survivors (2). A way that may lead to an improvement in recognizing some "Achille heels" of SCLC is to understand the biological differences in a disease considered so far like a monolith. The right direction could be the new proposal of classification that takes into account the different expressions of key transcription regulators like ASCL1-high, NEUROD1-high, POU2F3-high, and YAP1-high. This effort to categorize SCLC in different subgroups may lead to a different way to build therapeutic strategies and currently prospective trials to define the usefulness of this classification are ongoing (3). Despite the huge progress achieved in the NSCLC counterpart related to the discovery of response predictive biomarkers, these remain relatively unknown in SCLC, making personalized medicine for this malignancy still a chimera (4).

The main aim of our Research Topic is to explore new drugs and strategies in the field of SCLC, given the importance of summarizing some points related to the innovations that have emerged from the most recent clinical research (5). In particular, this issue includes fourteen articles focusing on original research (5 papers), reviewing some aspects of therapeutic strategy (7 papers), and 2 case reports to accompany the reader through all the aspects that distinguish the SCLC complex world, building a bridge between the present and future of the clinical management of this cancer.

Our Research Topic starts from the little-explored world of surgical management of early-stage SCLC, in which the risk-benefit balance of the surgical approach is still debated. In the review presented by Petrella et al., the role of surgery is reviewed in the light of literature data and the personal experience of the authors. Stage I SCLC is a really rare entity, mostly diagnosed incidentally: however, even if the rate of surgical resection remains low (1 to 6% in limited disease) lobectomy with radical lymphadenectomy is considered the gold standard surgical procedure, leading the overall survival at 5 years in nearly 50% of the patients who underwent the surgical approach. The monocentric experience reported in this paper underlines that patients with stage I pathological SCLC had a 76% of 5 years overall survival. This excellent prognosis is certainly guided by several prognostic factors including the absence of positive lymph nodes and the low diameter of the tumor. The clinical impact of the number of lymph nodes dissected (LNDs) on overall survival in N0 SCLC was assessed by Takamori et al. who queried the National Cancer Database (NCDB) exploring patients with very early SCLC (stage I-II as AJCC 7th edition) treated with a lobectomy between 2004 and 2017. They reported for the first time that SCLC patients with $\geq$3 LNDs had a significantly longer OS than those with $<$3

LNDs. The multivariate analysis confirmed that ≥3 LNDs was an independent predictor for OS. In both publications the surgical approach appears feasible and recommended particularly in stage I SCLC: however, to better define this population, an adequate lymph node sampling is of fundamental importance to consider the surgical intervention oncologically complete, while the number of lymph nodes removed remains a surrogate of the lymph node pathological situation, distinguishing the population of true N0 patients who have a decidedly excellent prognosis.

The main part of our Research Topic is dedicated to stage IV SCLC which affects more than 80% of diagnosed cases. One of the major fields of interest is related to the search for prognostic and predictive factors of response to treatments, including chemotherapy, immunotherapy or new drugs. Zhou et al. in their systematic review investigated the prognostic value of the systemic immune-inflammation index (SII) for SCLC. A set of bio humoral factors that are easy to use would be of importance to better evaluate patients to be referred to first-line treatment and to reduce costs and turnaround time for extensive, massive deep gene panel testing. SII, as reported in their paper, consists of a set of biomarkers including neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, C-reactive protein/albumin ratio, that had a prognostic role in a series of different malignant tumors. The authors concluded that also in advanced SCLC this composite biomarker tool had a relationship with prognosis and could be useful to indicate the best strategy for each patient. The role of new biomarkers for predicting the activity of immunotherapy is welcome and in the original research reported by Tang et al. C-C Motif Chemokine Ligand 5 (CCL5) expression on tumoral micro-environment has been extensively studied in a cohort of SCLC patients treated with immune-checkpoint inhibitors (ICIs). The authors found that CCL5 high expression correlated positively with overall survival and its level of expression is associated with the co-expression of other immune-checkpoint proteins like PD1/PDL1, CTLA4 among others; although its role could be further clarified in prospective trials, there are some clues about a possible role as a predictive biomarker in patients treated with ICI+DNA damage agent (PARP inhibitor). Another fascinating way to predict the efficacy of chemotherapy is to study chemosensitivity in circulating tumoral cells (CTCs). This is the main topic of the original research reported by Ju et al.. In their paper, the authors showed the results of a retrospective study conducted on SCLC patients treated with different lines of chemotherapy: they tested the susceptibility to 6 different chemotherapeutic agents monitoring CTC counts and collecting them. The reduction of CTC counts correlated positively with therapy response. Unfortunately, the administration of a newer chemotherapy line to SCLC patients based on the drug susceptibility test of CTCs failed to demonstrate a clinical activity: the weakness of a very limited sample size does not allow to draw a definitive conclusion about this experimental procedure.

Following the recent therapeutic innovations in first-line therapies and the emergence of potentially useful new drugs, the other part of our Research Topic is fully dedicated to new therapeutic strategies. Belluomini et al. extensively reviewed the available literature data about SCLC management, with a particular focus on special populations such as elderly or low-performance status patients (ECOG PS 2). This aspect has been particularly dealt with in the literature review conducted by Giunta et al. that underline the evidence and weaknesses of the first line strategy with the modern combination with CT+ICIs. The discrepancies and the difference between clinical trials results and the real-world evidence (RWE) are depicted by Rittberg et al. who described in their original research how the majority of the patients in a Canadian retrospective cohort analysis did not have the clinical characteristic to receive the triple first-line combination in the first line setting claiming the need to better understand which strategy may be really conducted in RWE.

The hopes regarding the new therapeutic strategies are entrusted also to new drugs with different mechanisms of action compared to classic chemotherapeutic agents and ICIs: in the papers of Manzo et al. and Cortinovis et al. all the findings about lurbinectedin and anti-DLL3 agents were exploited, while a focus on Aurora kinase inhibitor was extensively reviewed in the paper of Stefani et al.. SCLC is also hard to treat due to the presence of particular syndromes such as paraneoplastic syndromes that accompany its diagnosis. Ectopic Cushing's syndrome was addressed by Piasecka et al. who reviewed monocentric SCLC medical records, showing that almost 12% of the population could present with this syndrome which remains potentially underdiagnosed. Finally, some peculiar clinical aspects are presented in 2 clinical cases reported by Wang et al. and Zhang et al. about a rare phenotype switching from SCLC to NSCLC and a clinical case with a long survival due to a personalized therapeutic strategy.

In summary, new drugs and strategies will improve the prognosis of this orphan disease, but several challenges remain in the management of SCLC, including the lack the true predictive biomarkers to address the right population to newer therapeutic strategies, the lack of information regarding special populations excluded by clinical trials, the need of more insights about RWE, decreasing the gaps between clinical practice and research. We hope that this Research Topic will be of interest for the reader suggesting new ideas for future research.

Author contributions

DLC and AM: conceptualization, writing—original draft preparation, writing—review and editing, and supervision. All the authors have read and agreed to the published version of the manuscript.

Conflict of interest

AM received honoraria from Roche, AstraZeneca, Boehringer, MSD, BMS, Pfizer, Takeda, Lilly, Novartis. DLC received honoraria from Roche, AstraZeneca, BMS, MSD, Boehringer Ingelheim, Amgen, Novartis, Takeda, Lilly.

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Survival, Chemotherapy and Chemosensitivity Predicted by CTC Cultured *In Vitro* of SCLC Patients

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Purpose: The prognosis for small cell lung cancer (SCLC) patients receiving later-line treatment is very poor and there is still no standard treatments after the second-line setting. Analyzing the susceptibility of chemotherapeutic drugs with circulating tumor cells (CTCs) cultured *in vitro* may contribute to optimize the therapeutic regimen. However, so far CTCs have been barely used for studying their chemosensitivity due to the lack of technology to obtain wholly intact and viable CTCs.

Methods: Based on a retrospective study of the therapeutic response of 99 patients with unresectable SCLC, the CTC count in 14 SCLC patients was detected before and after chemotherapy to evaluate its role as a potential marker of response. Furthermore, the drug susceptibility of CTCs cultured *in vitro* obtained from ClearCell FX® System was tested and the therapy response was evaluated.

Results: All of the 99 patients received the first-line chemotherapy and the objective response rate (ORR) was 74.7%. A total of 36 patients received the second-line therapy and the average duration was 2.6 months, and only 11 cases out of them received the third-line therapy but no one responded. The change of CTC counts was identified to be correlated with therapy response. However all the five SCLC patients who were administered with the drugs according to the drug susceptibility test of CTCs for two cycles underwent progression of disease.

Conclusion: The results showed that the responses of chemotherapy are very poor in later lines and the drug susceptibility test using CTCs primary cultured *in vitro* may not benefit the improvement of therapeutic regimen of SCLC patients.

Keywords: CTC, survival, chemotherapy, chemosensitivity, small cell lung cancer

INTRODUCTION

According to the statistics, there would be 2.2 million new lung cancer cases in 2020, 15-20% of which were SCLC (1). SCLC is a highly aggressive malignancy and frequently with distant metastases at diagnosis. It is staged using the Veterans Administration Lung Study Group (VALSG) staging system, which divides SCLC patients into limited-stage (LS) diseases or extensive-stage (ES) diseases. And according to this staging system, almost two-thirds of patients have ES diseases at diagnosis (2). Even though SCLC is highly sensitive to initial chemotherapy and radiotherapy, the outcomes of newly diagnosed ES-SCLC patients are still very poor, with median progression-free survival (PFS) only about 5-6 months, median overall survival (OS) less than 10 months (2, 3). The reason for such poor survival of these patients is that the drug resistance to first-line chemotherapy emerged very quickly and the efficacy of second-line and subsequent therapies is undesirable. The standard treatment for newly diagnosed ES-SCLC at present is platinum-based doublet chemotherapy consisting of cisplatin or carboplatin plus etoposide or irinotecan alone or in combined with PD-L1 Inhibitors, and the response rates of the first-line chemotherapy are 60% to 85% (4, 5). Nevertheless, most of these patients quickly become resistant to these drugs, with a median PFS of 4 to 7 months (6–8).

Topotecan is the only Grade I recommended chemotherapeutic drug in second-line therapy for recurrent SCLC patients. There is no standard therapy for those patients eventually progressed on second-line chemotherapy. Palliative care/best supportive care (BSC) or other systemic chemotherapy can be the alternative option based on patient's performance status. Some new drugs, such as immunotherapy agents (Nivolumab and Pembrolizumab) and the multi-targeting tyrosine kinase inhibitor Anlotinib, have been approved by Chinese Food and Drug Administration (CFDA) and are already available to physicians in China, but their survival benefits are very limited and most patients cannot afford these drugs which are not included in the coverage of medical insurance reimbursement.

CTCs are tumor cells that shed from primary and metastatic sites and circulate in the peripheral blood and can be detected by many advanced technologies. Hou et al. reported that CTCs were present in 85% of SCLC patients with the abundance of 1,589 ± 5,565 cells/7.5 mL blood (9). Huang et al. found that the median number of CTCs in 24 patients measured at baseline and post-treatment was 75 (range 0-3430) and 2 (range 0-526), respectively; the median reduction of CTCs from baseline to post-treatment was 97.4% in 15 subjects (10).

As there is no standard care and the prognosis and outcomes of SCLC patients are very poor in later lines of therapy, the precision treatment holds great promise for cancer patients. With the potential to address challenges associated with drug susceptibility and the variability among the patients, analyzing the susceptibility of chemotherapeutic drugs with primary cultured CTCs *in vitro* may provide some useful information for optimizing the therapeutic regimen and prolong survival time of SCLC patients. So far CTC has been barely studied for its chemosensitivity due to the lack of technology for obtaining wholly intact and viable CTCs. In this study, the ClearCell FX System was used to get intact and viable CTCs and then the CTCs were primary cultured *in vitro* and employed for investigations of their drug sensitivity profiles.

MATERIALS AND METHODS

Patients Data

We retrospectively reviewed the medical records of patients with unresectable SCLC treated at Blinded for peer review between January 2014 and December 2019. All patients displayed measurable disease by the Response Evaluation Criteria in Solid Tumors (RECIST, Version 1.1) and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of less than or equal to 2. The information were collected including age, gender, laboratory results, diagnoses, stage, anatomic sites of involvement, sites of metastases, treatment plan, specific therapy, other medications such as supportive care agents, and performance status. The therapy response was evaluated after the two cycles of chemotherapy and every two subsequent cycles after the first evaluation until the disease progressed, and the results were recorded as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) according to RECIST 1.1 criteria.

Study Design

These studies were prospective single-institution clinical studies conducted at the Blinded for peer review. Patients aged ≥18 years with histologically or cytologically confirmed unresectable SCLC were enrolled. The therapy response was evaluated after the two cycles of chemotherapy and every two subsequent months after the first evaluation until the disease progressed, and was recorded as CR, PR,SD, and PD according to RECIST 1.1 criteria. The ORR was defined as the sum of CR plus PR. The disease control rate (DCR) was defined as the sum of CR plus PR plus SD. The treatment response was evaluated by CT scan two months after the initiation of chemotherapy and then every two months. Our study was approved by The Ethics Committee of Blinded for peer review (approval no. K19-137). All patients provided written informed consent prior to enrollment in the study.

Monitoring of CTC Counts During Chemotherapy

In this study, patients were administrated with the first-line carboplatin plus etoposide chemotherapy and some of them were screened for CTC counts test using folate receptor targeted PCR by GENO Biology in China, within one week before and after two cycles of chemotherapy.

According to the manufacturer's protocol, CTCs were enriched by lysis of erythrocytes and subsequent depletion of leukocytes. Briefly, red cell lysis buffer (v:v, 1:4) was firstly used to lyse the anticoagulant whole blood samples for 15min on ice. Then 200ml anti-CD45 coated magnetic beads were used to treat the cells for 30 min to deplete leucocytes. After that, CTCs were incubated with 10ml labeling buffer (folate-linked oligonucleotide) for 40min at

room temperature. The cells were then washed 3 times with 1ml wash buffer at 500g. Finally, the cells were treated with 120ml stripping buffer to remove the ligand-oligonucleotide conjugates. The supernatant were collected by centrifugation and neutralized by 24ml neutralization buffer for further PCR analysis. Real time quantitative polymerase chain reaction was performed using the CytoploRare® circulating lung cancer cell kit on ABI StepOne™ system (Life technologies). Two and half microliters of the prepared samples were added into a 25ml PCR reaction system following the manufacturer's instruction manual. The PCR reaction conditions were as follows: denaturation at 95°C for 2 min, annealing at 40°C for 30 s, extension at 72°C for 30 s, then cooling at 8°C for 5 min; 40 cycles of denaturation at 95°C for 10 s, annealing at 35°C for 30 s, and extension at 72°Cfor 10 s. A serial of standards containing oligonucleotides (10^{-14} to 10^{-9}M, corresponding to 2 to $2x10^5$ CTC units/3 ml blood) were used for CTC quantification. All patients' samples were tested in duplicates with 6 standards and 3 quality controls. Following the manufacturer's protocol, the mean intraassay variance (the maximum difference between duplicates) should be < 0.5 threshold cycle for the standards and quality controls, and < 1 threshold cycle for tested samples (11).

Drug Susceptibility Predicted by CTCs Primary Cultured *In Vitro*

In this study, the patients resistant to at least the first-line chemotherapy (etoposide plus cisplatin or carboplatin) were enrolled. CTCs collection and the drug susceptibility tests were done by Polaris Biology in China. About 6 chemotherapeutic agents per patient were tested based on their previous medication histories if the numbers of CTCs collected were enough. Then these patients were treated with the highly sensitive chemotherapeutic drugs according to the test results.

7.5ml of peripheral blood was collected in either EDTA or Cell-Free DNA BCT® tubes (Streck, USA) and processed within 24h, respectively. Next, red blood cell (RBC) lysis buffer was used to treat the prepared whole blood and the nucleated cell fraction was recovered. The nucleated cells were suspended in the custom ClearCell resuspension buffer, and loaded on the ClearCell FX® System(Clearbridge BioMedics, Singapore). A new CTChip was loaded on the machine and the automated protocol was run. Within an hour, the enriched CTCs were collected in a 15ml centrifuge tube in suspension format and seamlessly integrated into downstream assays. After the enrichment, the system ran a cleaning cycle to avoid cross-contamination between samples. Because of the fast metabolic rate, cancer cells can rapidly absorb glucose, which has become the basic detection principle of PET-CT. So, PET-CT was used to identify and confirmed the CTCs (11). CTCs were then transferred into a 1.5mL tube, and washed three times using 1 × PBS (with 1% penicillin and streptomycin), and then transferred into ultra-low attachment 96-well plate for short-time expansion (2-4 days). Cell viability was assessed using the eBioscience™ Indo-1 AM Calcium SensorDye (Thermo Fisher) system. Cultured CTCs were incubated with 2 umol/L calcium dye system. With this dye and drug combination, viable CTCs are shown green and dead cells are dark (**Figure 1**). Imaging was performed with the NIKONE-C1confocal microscope system.

Statistical Analyses

The statistical analysis about the correlation between changes in CTC counts and the responses to therapy was performed using IBM SPSS Statistics 23 software. The statistical significance analysis was calculated using chi-square test.

RESULTS

Patient Characteristics

In the part of retrospective study, a total of 99 patients with unresectable SCLC received platinum-based doublet chemotherapy in the first-line setting. The mean age at diagnosis was 63.99 ± 8.81 years, 92% were male, and 68.9% had ES diseases. The median PFS was 9.83 months and the ORR was 74.7%, including 3 cases with CR, 62 cases with PR, 10 cases with SD, 12 cases with PD, and 12 cases intolerant to chemotherapy or without evaluation. A total of 36 cases after progression on the first-line therapy went on to the second-line chemotherapy and 11 cases after progression on the second-line therapy went on to the third-line chemotherapy. Baseline patient characteristics are presented in **Table 1**.

Efficacy of Later-Line Chemotherapy

In the second-line setting, 36 (36.3%) patients received Irinotecan followed by platinum-based chemotherapy. For all the second-line patients, the median PFS was 2.6 months, with only 2.8% surviving for more than a year. Within the third-line setting, 11 (11.1%) patients received chemotherapy and the others received BSC. Among actively treated patients, taxane-based single-agent regimens were the most commonly used regimens followed by Irinotecan. Only 1 patient was treated with immunotherapy across all lines of therapies. The average duration of chemotherapy was 2.4 months in the third-line setting and no one responded to chemotherapy (**Table 2**). Among the patients receiving BSC, more than half of them were classified as treatment-eligible to receive active treatments as determined by their ECOG status or duration of follow-up available. So new drugs or new methods to test the susceptibility of old drugs for individual SCLC patients are urgently needed.

Correlation Between Change of CTC Counts and Treatment Response

To evaluate the change of CTC counts as a potential marker for monitoring therapy response, fourteen patients with SCLC in first-line setting were screened for CTCs counts test within one week before and after two cycles of chemotherapy, and then the patients were grouped into responsive, stable, and progressive disease based on therapeutic efficacy according to RECIST criteria 1.1. The results showed that the patients with responsive diseases had reduced CTC counts with a median decrease of 6.96 CTCs, and the patients with stable diseases had

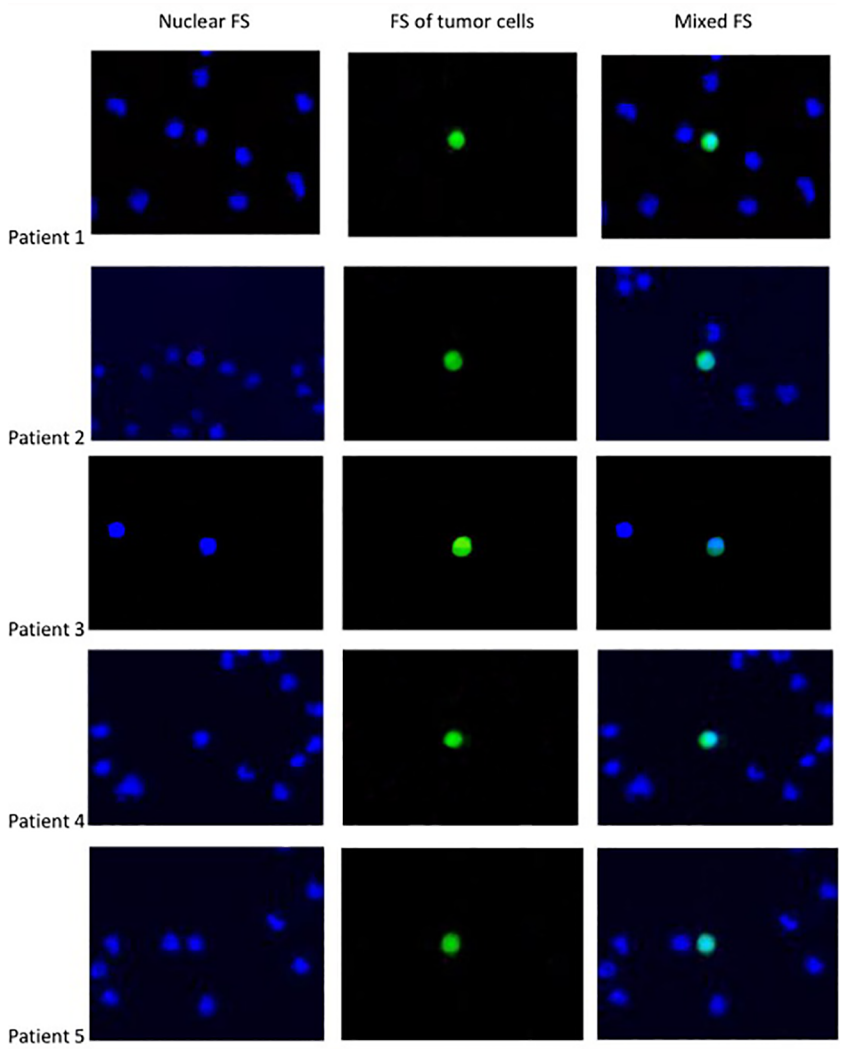

FIGURE 1 | The fluorescent staining (FS) OF CTC.

TABLE 1 | Patient characteristics.

Variables	Mean
Age (years)	63.99 ± 8.81
Sex	
Male	91
Female	8
Clinical stage	
LD	31
ED	68
Response to first-line therapy	
CR	3
PR	62
SD	10
PD	12
Intolerant or without evaluation	12

TABLE 2 | Response to chemotherapy in SCLC patients.

	Number	ORR	PFS (months)
First-line	99	74.7% (87 cases can be evaluated)	9.83
Second-line	36	11.1%	2.6
Third-line	11	0	2.4

a median decrease of 3.34 CTCs, whereas the patients with progressive diseases got a median increase of 13.05 CTCs after chemotherapy (**Table 3**), the response of therapy was significantly related with the change of CTCs counts, $p<0.001$.

Relevance Between Efficacy and Drug Susceptibility of CTCs Cultured *In Vitro*

To evaluate the clinical value of this drug susceptibility platform using CTCs primary cultured *in vitro*, ten never used chemotherapeutic agents were tested for their sensitivities in five patients with SCLC after their second-line chemotherapy. The test reports are shown in **Table 4** (not all of ten drugs were

TABLE 3 | Changes of CTC counts correlated with response of chemotherapy in SCLC patients.

Patient	Response	CTC counts		Difference
		Before therapy	After therapy	
1	PR	25.51	19.4	-6.11
2	PR	26.57	18.76	-7.81
3	SD	18.84	15.07	-3.77
4	SD	14.24	11.33	-2.91
5	PD	9	18.46	9.46
6	PD	14.98	39.21	24.23
7	PD	7.99	13.33	5.34
8	PD	9.48	15.96	6.48
9	PD	15.27	21.92	6.65
10	PD	27.75	35.58	20.83
11	PD	18.83	50.66	7.83
12	PD	6.31	27.14	31.83
13	PD	10.12	19.52	9.4
14	PD	15.07	23.52	8.45

TABLE 4 | Drug susceptibility test results.

Drug	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Docetaxel	H	H	H	R	H
Vinorelbine	R	L	H	H	
Gemcitabine	R	L	H	R	L
Paclitaxel			L		
Albumin paclitaxel				H	
Cisplatin	H				
Nedaplatin		R	H		L
Luoplatinum	M	M	M	M	
Pemetrexed		M			
Irinotecan					M

**H, Highly sensitive; M, Moderately sensitive; L, Low sensitivity; R, Resistance.*

detected in every patient because some patients had not enough CTCs). Patient 1 was highly sensitive to Docetaxel and Cisplatin, patient 2 to Docetaxel, patient 3 to Gemcitabine, Nedaplatin, Docetaxel, and Vinorelbine, patient 4 to Vinorelbine and Albumin paclitaxel, and patient 5 to Docetaxel. Four patients were treated with the highly sensitive drugs according to these results, but their disease progressed after two cycles of chemotherapy. The patient 5 didn't receive chemotherapy because of his quick disease progression and the poor ECOG status. These results showed that the drug susceptibility test of CTCs primary cultured *in vitro* may not have distinct effect on clinical efficacy of patients with SCLC.

DISCUSSION

In our study there are 99 patients with unresectable SCLC received platinum-based doublet chemotherapy in the first-line setting and the ORR was 74.7%. Due to the inaccessibility of topotecan and irinotecan was not used in first-line therapy, the patients with SCLC in our hospital received the treatment of irinotecan in second-line setting. Among them, 36 patients received Irinotecan treatment in the second-line setting, but the median PFS was only 2.6 months, and only 2.8% surviving for more than one year. In the third-line setting, 11 patients received chemotherapy, but no one responded. However, in the patients receiving BSC, more than half were classified as treatment-eligible to receive active treatments. So this study hopes to find some drugs sensitive to patients. Finally, we have shown that the culture of CTCs *in vitro* provides an opportunity to study patterns of drug susceptibility that is unique to an individual tumor although this technique is not well developed in patients with SCLC now.

CTCs circulating in the peripheral blood, with their role as a "tumor liquid biopsy", provide convenient access to all disease sites. It is conceivable that detecting and analyzing CTCs will provide insightful information in assessing the disease status and monitoring the response of anticancer drugs. However, identifying CTCs in patient blood samples is technically challenging due to the extremely low abundance of CTCs among a large number of hematologic cells. The size of circulating tumor cells (~15-20 um) is significantly different from that of red blood cells (~8 um) and white blood cells (~8-15 um). In addition, CTC has higher nuclear-cytoplasmic ratio and irregular cell morphology. These characteristics make CTCs different from other cells in fluid characteristics and flow rate (12). Researchers made great efforts to screen and separate them, because they have the potential to be used in a number of ways, for example, patient cohorts could be selected based on the drug sensitivity pre-screening, alternatively, acquired resistance to chemotherapy can be monitored throughout the progress of clinical trials.

Most PFS of patients in the CTC counts study were less than 2 months, and their OS were also very poor, and due to the limited time and fund, we can't recruit more patients and collect more samples in this study. But many studies have proved the correlation between the change of CTC counts and the response of therapy, so we didn't do the statistical evaluation. However, the technology of folate receptor targeted PCR can't get the intact and viable CTCs. There are two modes of sorting to isolate CTCs till now. One is a negative selection mode (negCTC-iChip), in which the blood sample is depleted of leukocytes by immunomagnetically targeting both the common leukocyte antigen CD45 and the granulocyte marker CD15 (13). The other is a novel platform presented in this paper for prediction of efficacy of cancer drugs based on CTCs primary cultured *in vitro*. The ClearCell® FX System, a label-free microfluidics technology that utilizes Dean Flow Fractionation principle in a spiral microfluidics system to separate the larger CTCs from smaller blood cells, driven by the CTChip® FR biochip, is one of the world's first automated cell retrieval systems that can enrich wholly intact and viable CTCs from blood in a relatively short time. The automated system performs a single-step CTCs isolation and retrieval and collects the enriched CTCs in suspension format, achieving extremely high recovery rates. CTCs with high activity and no damage can be naturally separated from other cells in the sample based on the difference of flow velocity (12). After that, the chemotherapeutic drugs susceptibility was detected in primary cultured CTCs *in vitro* and then the patients were treated according to the results obtained, but we didn't get the expected treatment response.

Although our present findings indicate that the drug susceptibility test of the CTCs cultured *in vitro* may have little effect on the clinical efficacy of SCLC, it still needs to be validated in larger studies. The other possible reason for this result may be that SCLC is a highly aggressive malignant disease and resistant to all the old drugs in our tests or that the tumor microenvironment has some influences on the drug efficacy. Krohn et al. found that many tumors acquired drug resistance and their neuroendocrine differentiation was lost during epithelial-mesenchymal transition (EMT) of SCLC cells, indicating that drug resistance is one characteristic of EMT (14). Hamilton et al. obtained a panel of SCLC CTC cell line from patients with relapsing disease, which share a primarily epithelial phenotype with high expression of EpCAM, absent phosphorylation of β-catenin and background levels of Snail (15). Maybe that's why in our study the SCLC-CTCs sensitive to some drugs but resistant *in vivo*.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of Shanghai Pulmonary Hospital (approval no. K19-137). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL, YJ, ZC and CS: substantial contributions to the conception or design, acquisition, analysis, or interpretation of data, critical revision for important intellectual content, final approval of the version to be published. DZ and LM: coordinate and to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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Prognostic Significance of the Systemic Immune-Inflammation Index (SII) in Patients With Small Cell Lung Cancer: A Meta-Analysis

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Background: Previous studies have investigated the prognostic value of the systemic immune-inflammation index (SII) in small cell lung cancer (SCLC). However, the results have been inconsistent. The study aimed to investigate the prognostic and clinicopathological significance of SII in SCLC through a meta-analysis.

Methods: The PubMed, Web of Science, Embase, Cochrane Library, and China National Knowledge Infrastructure databases were thoroughly searched. The pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to evaluate the prognostic value of the SII for survival outcomes. The combined odds ratios (ORs) and 95% CIs were used to evaluate the correlation between SII and clinicopathological features.

Results: Eight studies comprising 2,267 patients were included in the meta-analysis. Pooled analyses indicated that a high SII was significantly associated with worse overall survival (OS) (HR=1.52, 95% CI=1.15–2.00, p=0.003) but not progression-free survival (HR=1.38, 95% CI=0.81–2.35, p=0.238) in patients with SCLC. Moreover, a high SII was associated with extensive-stage SCLC (OR=2.43, 95% CI=1.86–3.17, p<0.001). However, there was a non-significant correlation between SII and age, sex, smoking history, Karnofsky Performance Status score, or initial therapeutic response.

Conclusion: Our meta-analysis demonstrated that a high SII could be an efficient prognostic indicator of OS in SCLC. We recommend adopting SII to predict OS in patients with SCLC, and SII in combination with other parameters or biomarkers may aid in addressing the clinical strategy and choosing the best treatment for an individual patient.

Keywords: systemic immune-inflammation index, meta-analysis, prognosis, small cell lung cancer, biomarker

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INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths globally, with 2,093,876 new cases diagnosed and 1,761,007 deaths annually (1). Small cell lung cancer (SCLC) is an aggressive malignancy that accounts for 15% of all lung cancer cases and causes more than 200,000 deaths per year (2). SCLC is a highly metastatic tumor strongly associated with smoking (3). SCLC is usually

classified as a limited and extensive-stage disease (LS-SCLC and ES-SCLC). Approximately 70% of patients with SCLC have ES-SCLC at diagnosis (4). The prognosis of SCLC is poor. The 1-year and 2-year overall survival (OS) rates in LS-SCLC were 58% and 21%, respectively, and they were 29.4% and 7%, respectively, for ES-SCLC (5).

Combination chemoradiotherapy followed by maintenance immunotherapy is the new standard of care for the upfront management of metastatic SCLC (6). A recent report of IMpower133 (ClinicalTrials.gov identifier: NCT02763579) showed that adding atezolizumab to carboplatin plus etoposide as the first-line treatment for ES-SCLC continued to demonstrate improved OS and a tolerable safety profile in the updated analysis, confirming the regimen as a new standard of care (7). The standard treatment for LS-SCLS is combined modality treatment, including surgery, radiation, and systemic therapy (6). Surgical resection is recommended for eligible patients with early-stage (I–IIA, T1–2N0) disease who have undergone pathologic mediastinal staging to exclude nodal involvement (6). For patients with stage IIB to IIIC (T1–T4N0–N3M0) disease, the standard of care is management with concurrent platinum-based chemotherapy and radiotherapy (6). Despite these advances in the past several decades, the survival of SCLC has not substantially improved. Therefore, it is crucial to identify reliable and novel prognostic markers for SCLC.

Recent studies have shown immunological biomarkers, such as neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, C-reactive protein/albumin ratio, and systemic immune-inflammation index (SII), have prognostic roles in a series of malignant tumors (8, 9). SII is calculated based on peripheral neutrophil, platelet, and lymphocyte counts using the following formula: platelet count × neutrophil count/lymphocyte count (10). SII has been reported as a significant prognostic biomarker for various cancers, including hepatocellular carcinoma (HCC) (11), gastric cancer (12), pancreatic cancer (13), endometrial cancer (14), non-small cell lung cancer (15–17), and bladder cancer (18). Recent studies have also investigated the prognostic value of SII in patients with SCLC; however, the results remain inconsistent (19–26). For example, some studies showed that a high SII was associated with worse survival in SCLC (21, 23), whereas others have not identified the prognostic value of SII (20, 26). Therefore, we performed a systematic and comprehensive meta-analysis to identify the prognostic and clinicopathological significance of SII in SCLC.

MATERIALS AND METHODS

Literature Search

The current meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (27). The PubMed, Web of Science, Embase, Cochrane Library, and China National Knowledge Infrastructure databases were thoroughly searched. The following search items and texts were used: ("systemic immune-inflammation index" OR "SII") AND ("small cell lung cancer" OR "SCLC"). The last search was updated on October 16, 2021. There were no limitations to the publication language. Additionally, the reference lists of pertinent articles were manually searched for potentially eligible studies.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows (1): patients pathologically diagnosed with SCLC; (2) the articles investigated the prognostic role of SII for survival outcomes, including OS or progression-free survival (PFS); (3) there was no limitation to the treatment methods, if only the treatment for patients was applied according to the standard treatment guidelines, including surgery, chemotherapy alone, immunotherapy alone, and concurrent chemoradiotherapy; (4) platelet counts, neutrophil counts, and lymphocyte counts were measured using serum-based methods before treatment; (5) a cut-off value of SII was identified; (6) hazard ratios (HRs) with 95% confidence intervals (CIs) for survival outcomes were reported in text or can be extracted from Kaplan–Meier curves; and (7) published in English or Chinese. The exclusion criteria were: (1) meeting abstracts, letters, case reports, reviews, or comments; (2) studies with insufficient data for analysis; (3) animal studies; and (4) studies that included overlapping patients. The primary endpoint was OS, defined as the period from diagnosis until death from any cause and the last follow-up period for living patients. The secondary endpoint was PFS, which was determined as the time interval from diagnosis to progression or death.

Data Extraction and Quality Assessment

Two investigators (Y.Z. and M.D.) independently reviewed all studies, and all discrepancies were resolved by discussion with a third investigator (Z.Z.) until consensus was reached. The following data were extracted from each qualified study: name of the first author, year of publication, country, study period, study design, age, Veterans Administration Lung Study Group stage, treatment, follow-up, a cut-off value of SII, determination method of cut-off value, survival outcomes, survival analysis (multivariate or univariate), HRs, and 95% CIs. If both multivariate and univariate analyses were performed, the HRs and 95% CIs of the multivariate analysis were adopted. The methodological quality of the included studies was evaluated using the Newcastle–Ottawa Scale (NOS) for cohort studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) by two independent authors (Y.Z. and M.D.). The NOS evaluates the quality of studies in three aspects: selection (0-4 points), comparability (0-2 points), and outcome (0-3 points). The NOS scores range from 0-9, and studies with NOS scores > 6 were considered high quality.

Statistical Analysis

The pooled HRs and 95% CIs were calculated to evaluate the prognostic value of the SII for survival outcomes in patients with SCLC. The heterogeneity among studies was assessed using the Cochrane Q test and I^2 statistic. A fixed-effects model was used in the absence of significant heterogeneity (I^2<50% or P for heterogeneity >0.10); otherwise, a random-effects model was utilized. The combined odds ratios (ORs) and 95% CIs

were used to evaluate the correlation between SII and clinicopathological features in SCLC. Subgroup analysis was conducted to detect the source of heterogeneity and for further investigation. Publication bias was evaluated visually using Begg's funnel plot and Egger's test. All statistical analyses were conducted using Stata 12.0 software (Stata Corp LP, Texas, USA). All statistical tests were two-sided, and statistical significance was defined as $p<0.05$.

Ethics

The requirement for ethical approval and informed consent was waived because all analyses in this study were based on previously published reports.

RESULTS

Search Results

The initial literature search identified 432 studies, and 222 records remained after excluding duplicate studies. After screening the titles and abstracts, 212 studies were removed, and 10 studies were reviewed in full text. Subsequently, two studies were eliminated for the following reasons: one study did not provide survival data and one recruited overlapping patient. Finally, eight studies comprising 2,267 patients (19-26) were included in this meta-analysis. The detailed study selection process is shown in **Figure 1**.

Baseline Characteristics of Included Studies

The characteristics of the included studies are summarized in **Table 1**. The included studies were published between 2015 and 2021. Seven studies were conducted in China (19–25) and one study was conducted in Turkey (26). The sample size ranged from 41 to 919 (median, 157). Seven studies were published in English (19, 20, 22–26), and one was published in Chinese (21). Six studies were retrospective (19, 21–24, 26), and two were prospective trials (20, 25). Six studies (19–23, 26) investigated the prognostic role of SII for OS, and five studies (21, 23–26) explored the association between SII and PFS. Seven studies (19, 21–26) recruited both LS-SCLS and ES-SCLC, and one study (20) only enrolled patients with ES-SCLC. The cut-off values of SII ranged from 479 to 1600, with a median value of 673. Six studies (20–23, 25, 26) used receiver operating characteristic curve analysis to determine the cut-off value, and two studies (19, 24) referred to the literature. HRs and 95% CIs were extracted from multivariate analysis in five studies (19, 20, 22, 23, 26) and univariate analysis in three studies (21, 24, 25). The NOS scores of the included studies ranged from 7 to 9, indicating that all included studies were of high quality. The details of the NOS scores are summarized in **Supplementary File 1**.

Impact of SII on Overall Survival in SCLC

A total of six studies with 2,167 patients (19–23, 26) reported the prognostic value of SII for OS in SCLC. A random-effects model was

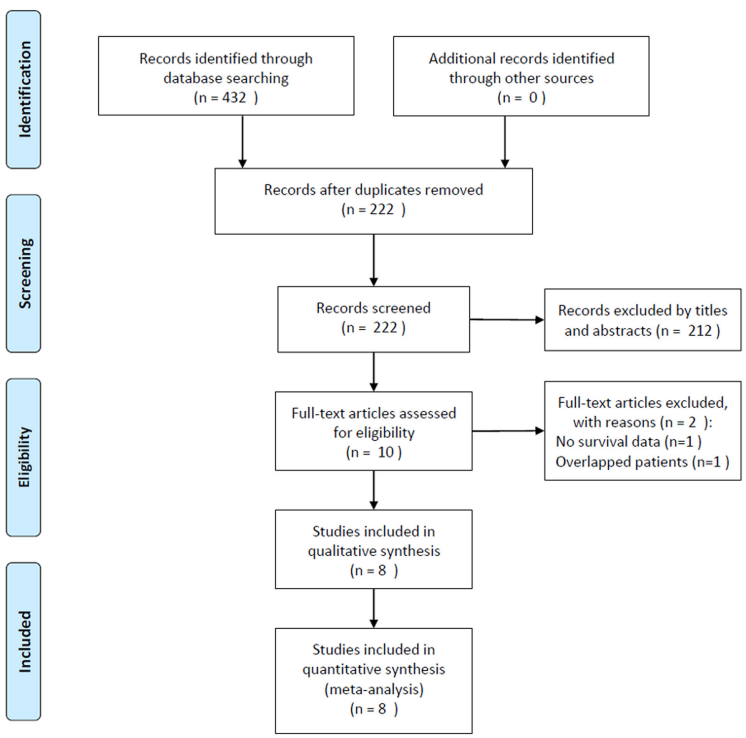

FIGURE 1 | Flow diagram of included studies for this meta-analysis.

TABLE 1 | Main characteristics of the included studies in this analysis.

Study	Year	Country	Sample size	Study period	Study design	Sex (F/M)	Age, years median(range)	VALG stage (LS/ES)	Treatment	Follow-up (month) Median(range)	Cut-off value of SII	Cut-off determination	Survival outcomes	Survival analysis	NOS score
Hong	2015	China	919	2000-2012	Retrospective	284/635	56(16-84)	552/367	CRT	To Dec 2014	1600	Literature	OS	Multivariate	7
Qi	2021	China	53	2017-2018	Prospective	19/34	65	0/53	Chemotherapy+ Targeted therapy	17.1	533	ROC analysis	OS	Multivariate	9
Teng	2021	China	98	2013-2018	Retrospective	36/62	60	50/48	CRT	To Jun 2020	571.5	ROC analysis	OS, PFS	Univariate	8
Wang	2020	China	653	2008-2009	Retrospective	231/422	56 (23-75)	384/269	CRT	NR	748.5	ROC analysis	OS	Multivariate	7
Wang	2019	China	228	2009-2015	Retrospective	69/159	58 (39-71)	114/114	CRT	46	479	ROC analysis	OS, PFS	Multivariate	8
Xiong	2021	China	41	2015-2018	Retrospective	5/36	61	7/34	Immunotherapy	To Aug 2019	730	Literature	PFS	Univariate	8
Yao	2021	China	59	2018-2020	Prospective	14/45	63 (45-78)	35/24	Chemotherapy	9.1 (1.5-24.2)	720	ROC analysis	PFS	Univariate	7
Yilmaz	2020	Turkey	216	2010-2019	Retrospective	32/184	61 (36-83)	59/157	CRT	10 (1-74)	626	ROC analysis	OS, PFS	Multivariate	8

VALG, Veterans Administration Lung Study Group; F, female; M, male; LS, limited stage; ES, extensive stage; CRT, chemoradiotherapy; OS, overall survival; PFS, progression-free survival; NOS, Newcastle-Ottawa Scale; ROC, receiver operating characteristic curve; SII, systemic immune-inflammation index.

applied because of significant heterogeneity (I^2 = 70%, Ph=0.005). As shown in **Figure 2** and **Table 2**, the pooled HR and 95% CI were HR= 1.52, 95% CI= 1.15–2.00, p=0.003, indicating that a high SII was associated with poor OS. Subgroup analysis stratified by country, sample size, study design, cut-off value, cut-off determination, survival analysis, tumor stage, and treatment were performed. The results demonstrated that a high SII remained a prognostic factor for OS in Chinese patients, with a cut-off value of ≥700, and the prognostic role was not influenced by the cut-off determination method. In addition, as shown in **Table 2**, elevated SII was associated with poor OS in patients with LS + ES but not in patients with ES.

Impact of SII on Progression-Free Survival in SCLC

Five studies consisting of 642 patients (21, 23–26) investigated the prognostic significance of the SII for PFS in SCLC. The combined HR and 95% CI were HR=1.38, 95% CI=0.81–2.35, p=0.238 (**Table 3** and **Figure 3**), which suggested that SII was not associated with PFS in patients with SCLC. The subgroup analysis suggested that elevated SII was a significant prognostic marker for poor PFS in Chinese patients with SCLC (HR=1.85, 95% CI=1.40–2.43, p<0.001; **Table 3**).

The Correlation Between SII and Clinicopathological Factors in SCLC

We investigated the association between SII and clinicopathological features, including age (≥60 vs <60 years), sex (male vs female), stage (ES vs LS), smoking history (yes vs no), Karnofsky Performance Status (KPS) score (< 80 vs ≥80), and initial therapeutic response (stable disease + progressive disease vs complete response + partial response) in SCLC. As shown in **Figure 4** and **Table 4**, a high SII was associated with ES-SCLC (OR=2.43, 95% CI=1.86–3.17, p<0.001). However, there was a non-significant correlation between SII and age, sex, smoking history, KPS score, or initial therapeutic response (**Figure 4**; **Table 4**).

Publication Bias

Begg's funnel plots and Egger's test were used to estimate the potential publication bias. The results showed that there was no significant publication bias for OS (Begg's test: p=0.851; Egger's test: p=0.223) or PFS (Begg's test: p=0.806; Egger's test: p=0.617) (**Figure 5**).

DISCUSSION

SCLC is a highly malignant carcinoma with a poor prognosis because of the elusive pathophysiology of the disease (28). Previous studies have investigated the prognostic effect of SII in SCLC; however, the conclusions are not consistent. In the present meta-analysis, data from eight studies with 2,267 patients were combined, and the results showed that an elevated SII was associated with worse OS, but not PFS. Furthermore, subgroup analysis indicated that a high SII was a significant prognostic factor for poor OS and PFS in Chinese patients with SCLC. Furthermore, a high SII was significantly correlated with ES-SCLC, suggesting that SII could indicate metastasis in SCLC. Our

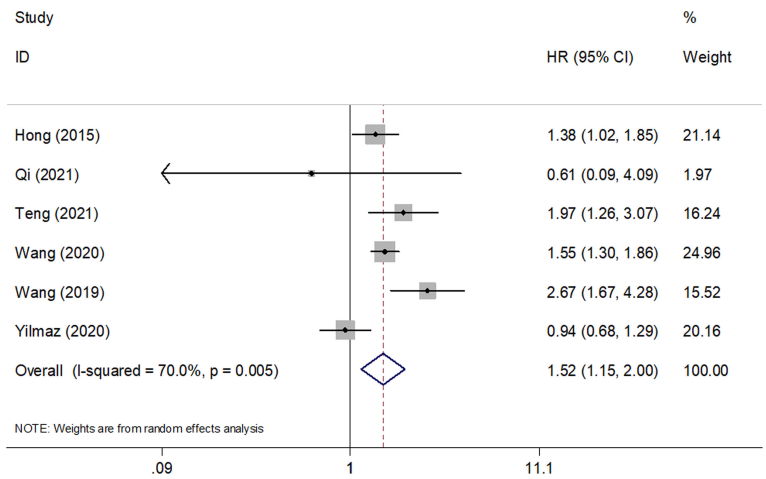

FIGURE 2 | Forest plots of pooled HRs and associated 95% CIs of the effect of high versus low SII for overall survival in patients with SCLC.

TABLE 2 | Subgroup analysis of the prognostic value of SII for overall survival in patients with SCLC.

Variables	No. of studies	No. of patients	Effects model	HR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Total	6	2,167	Random	1.52 (1.15-2.00)	0.003	70.0	0.005
Country							
China	5	1,951	Fixed	1.61 (1.41-1.85)	<0.001	46.5	0.113
Turkey	1	216	–	0.94 (0.68-1.29)	0.685	–	–
Sample size							
<200	2	151	Fixed	2.19 (1.59-3.01)	<0.001	24.2	0.267
≥200	4	2,016	Random	1.29 (0.97-1.72)	0.084	72.2	0.027
Study design							
Prospective	1	53	–	0.61 (0.09-4.11)	0.612	–	–
Retrospective	5	2,114	Random	1.55 (1.16-2.05)	0.003	74.7	0.003
Cut-off value of SII							
<700	4	595	Random	1.54 (0.83-2.85)	0.170	81.4	0.001
≥700	2	1,572	Fixed	1.50 (1.29-1.75)	<0.001	0	0.495
Cut-off determination							
ROC analysis	5	1,248	Random	1.56 (1.08-2.25)	0.018	75.5	0.003
Literature	1	919	–	1.38 (1.02-1.85)	0.034	–	–
Survival analysis							
Multivariate	5	2.069	Random	1.44 (1.05-1.98)	0.023	73.3	0.005
Univariate	1	98	–	1.97 (1.26-3.07)	0.003	–	–
Treatment							
CRT	5	1,886	Random	1.39 (1.07-1.81)	0.013	68.1	0.024
C+T/C/I	2	281	Random	1.73 (0.46-6.47)	0.418	53.9	0.141
Tumor stage							
LS+ES	5	2,114	Random	1.55 (1.16-2.05)	0.003	74.7	0.003
ES	1	53	–	0.61 (0.09-4.11)	0.612	–	–

SII, systemic immune-inflammation index; ROC, receiver operating characteristic curve; CRT, chemoradiotherapy; C+T/C/I, Chemotherapy + Targeted therapy/Chemotherapy/Immunotherapy.

meta-analysis demonstrated that SII could be applied as an effective prognostic index for poor OS in SCLC, especially in Chinese patients. To our knowledge, this is the first meta-analysis to investigate the prognostic and clinicopathological significance of SII in patients with SCLC.

SII was first identified as a useful prognostic indicator in patients with HCC in 2014 (11). SII is calculated as neutrophil × platelet/lymphocyte and is cost-effective and easily accessible. A high SII could be attributed to high neutrophil counts, high platelet counts, or low lymphocyte counts. The exact mechanisms of the prognostic value of SII in SCLC have not been fully elucidated and can be explained in the following aspects. First, neutrophils secrete cytokines and chemokines, including vascular epidermal growth factor (VEGF), to enhance tumor angiogenesis and facilitate distant

TABLE 3 | Subgroup analysis of the prognostic value of SII for progression-free survival in patients with SCLC.

Variables	No. of studies	No. of patients	Effects model	HR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Total	5	642	Random	1.38 (0.81-2.35)	0.238	81.5	<0.001
Country							
China	4	426	Fixed	1.85 (1.40-2.43)	<0.001	32.4	0.218
Turkey	1	216	–	0.77 (0.56-1.05)	0.093	–	–
Sample size							
<200	3	198	Fixed	1.64 (1.14-2.34)	0.007	41.2	0.182
≥200	2	444	Random	1.28 (0.46-3.58)	0.637	93.4	<0.001
Study design							
Prospective	1	59	–	2.13 (0.98-4.64)	0.056	–	–
Retrospective	4	583	Random	1.26 (0.69-2.30)	0.456	84.8	<0.001
Cut-off value of SII							
<700	3	542	Random	1.42 (0.71-2.82)	0.318	89.2	<0.001
≥700	2	100	Random	1.28 (0.43-3.80)	0.659	66.8	0.083
Cut-off determination							
ROC analysis	4	601	Random	1.54 (0.85-2.77)	0.151	85.2	<0.001
Literature	1	41	–	0.70 (0.26-1.89)	0.481	–	–
Survival analysis							
Multivariate	2	444	Random	1.28 (0.46-3.58)	0.637	93.4	<0.001
Univariate	3	198	Fixed	1.64 (1.14-2.34)	0.007	41.2	0.182
Treatment							
CRT	3	542	Random	1.42 (0.71-2.82)	0.318	89.2	<0.001
C+T/C/I	2	100	Random	1.28 (0.43-3.80)	0.659	66.8	0.083

SII, systemic immune-inflammation index; ROC, receiver operating characteristic curve; CRT, chemoradiotherapy; C+T/C/I, Chemotherapy + Targeted therapy/Chemotherapy/Immunotherapy.

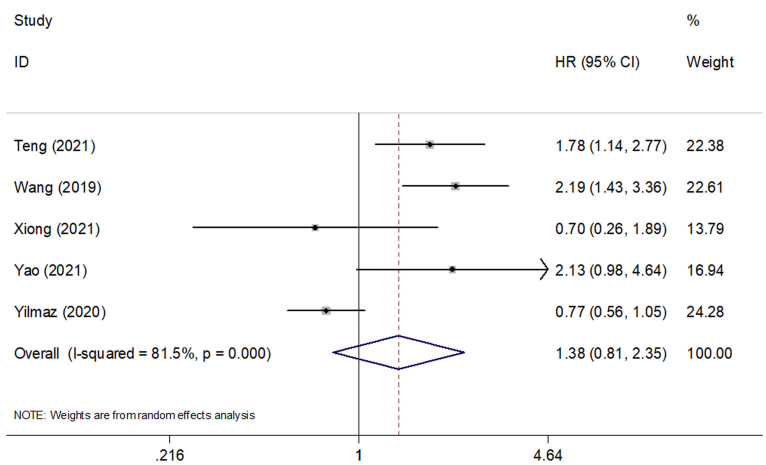

FIGURE 3 | Forest plots of pooled HRs and associated 95% CIs of the effect of high versus low SII for progression-free survival in patients with SCLC.

metastasis (29). Second, previous studies have shown that platelets play a crucial role in tumor activity. Platelets can mediate the survival and growth of tumor cells by secreting a various cytokines, such as VEGF, transforming growth factor-β, and platelet-derived growth factor (30). In addition, platelet-associated chemokines can modulate immune responses in the tumor environment and tumor angiogenesis (31). Third, lymphocytes are critically involved in cancer immune surveillance to prevent tumor development (32). Tumor-infiltrating lymphocytes are important immune cells in the tumor microenvironment and are responsible for antitumor immune responses (33). Lymphocytes play a vital role in immune defense against tumor cells, including inhibition of tumor cell proliferation and metastasis (34). Therefore, a high SII could be applied as a reliable biomarker of tumor progression and poor prognosis.

In addition, a high SII might be a consequence of a high tumor burden/metastatic/diffuse disease, which is the cause of tumor progression. For example, high SII, resulting from neutrophilia, lymphopenia, and thrombocytosis, may also be a useful prognostic indicator for postoperative survival outcomes (35) and for estimating response rates in cancer patients treated with chemotherapy (36) and immunotherapy (37). The SII is also

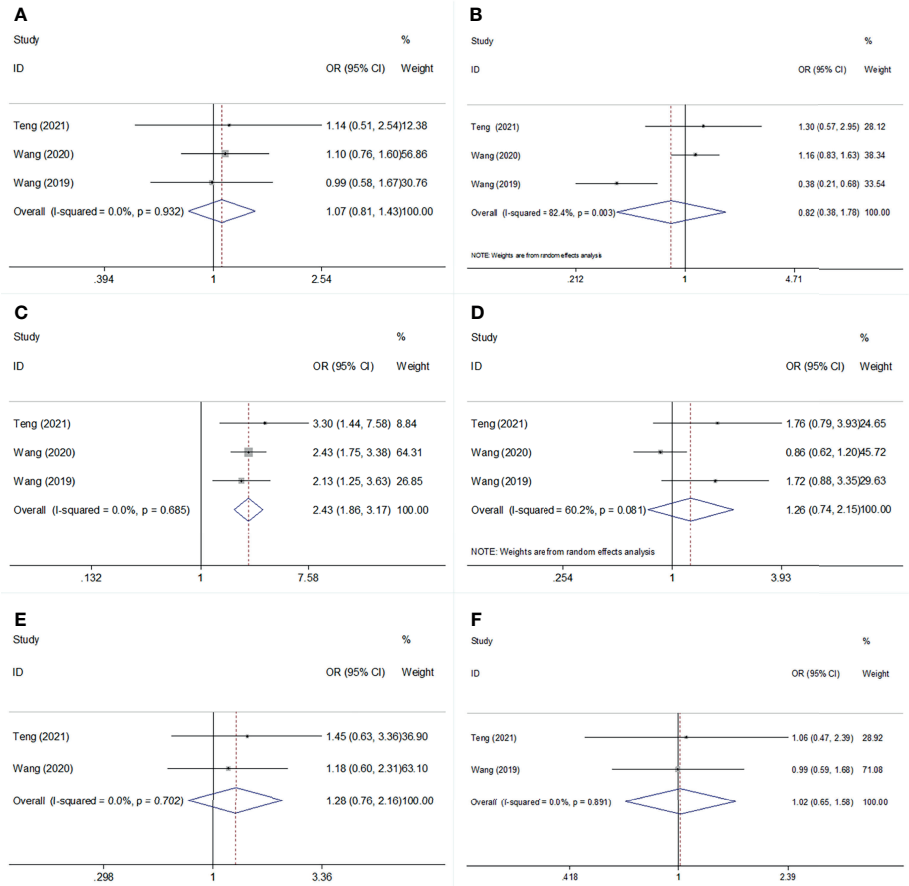

FIGURE 4 | Forest plot for the association of SII with age, sex, stage, smoking status, KPS score, and Initial therapeutic response in SCLC. **(A)** age; **(B)** sex: **(C)** stage: **(D)** smoking status; **(E)** KPS score; **(F)** Initial therapeutic response.

TABLE 4 | The correlation between SII and clinicopathological features in patients with SCLC.

Clinicopathological factors	No. of studies	No. of patients	Effects model	OR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Age (years) (≥60 vs <60)	3	979	Fixed	1.07 (0.81-1.43)	0.633	0	0.932
Sex (male vs female)	3	979	Random	0.82 (0.38-1.78)	0.619	82.4	0.003
Stage (ES vs LS)	3	979	Fixed	2.43 (1.86-3.17)	<0.001	0	0.685
Smoking history (yes vs no)	3	979	Random	1.26 (0.74-2.15)	0.397	60.2	0.081
KPS score (<80 vs ≥80)	2	751	Fixed	1.28 (0.76-2.16)	0.355	0	0.702
Initial therapeutic response (SD + PD vs CR + PR)	2	326	Fixed	1.02 (0.65-1.58)	0.947	0	0.891

SII, systemic immune-inflammation index; LS, limited stage; ES, extensive stage; KPS, Karnofsky Performance Status; SD, Stable disease; PD, Progressive disease; CR, Complete response; PR, Partial response.

a powerful tool for predicting outcomes in diffuse large B-cell lymphoma (10).

Several studies have shown the prognostic value of SII in various cancers through meta-analysis (38–40). For example, Qiu et al. showed that a high pretreatment SII predicted poor OS but not poor disease-free survival (DFS) in patients with gastric cancer, based on a meta-analysis of eight studies (38). Shui et al. reported that elevated SII was associated with poor OS, recurrence-free survival (RFS)/PFS/DFS, and cancer-specific survival in patients with pancreatic cancer in a meta-analysis including 2,365 subjects (39). In addition, Zhang et al. demonstrated that breast cancer patients with a high SII had worse OS, poorer DFS/RFS, and inferior distant metastasis-free survival than patients with a low SII (41). A recent meta-analysis of 12 studies showed that an elevated SII index was significantly

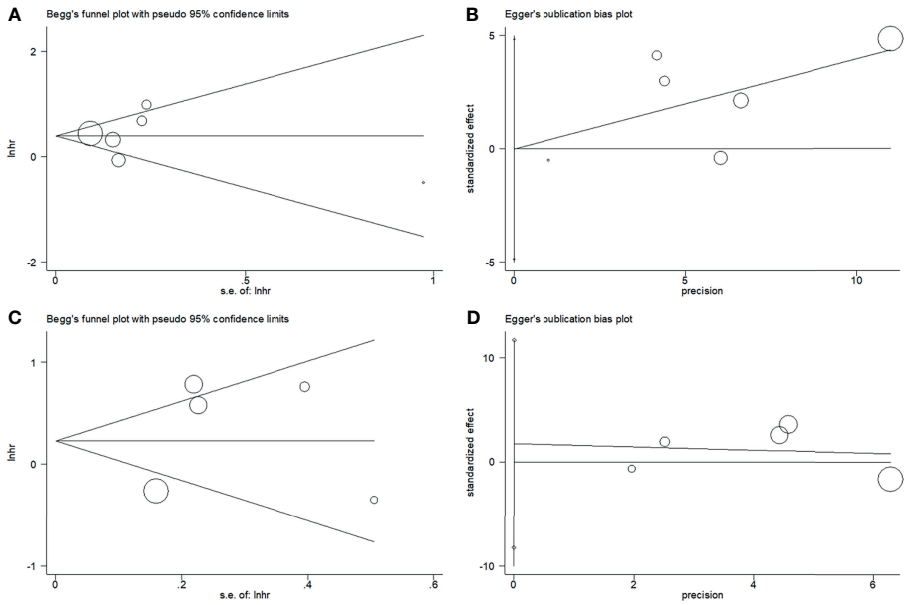

FIGURE 5 | Plots for publication bias test. **(A)** Begg's funnel plot for OS, p=0.851; **(B)** Egger's publication bias plot for OS, p=0.223; **(C)** Begg's funnel plot for PFS, p=0.806; **(D)** Egger's publication bias plot for PFS, p=0.617.

associated with poor OS, PFS, and CSS in patients with urinary system cancers (40). In the current meta-analysis, we identified a significant prognostic role of SII for OS but not for PFS. A possible reason is that the PFS is usually shorter than the OS in each study. Therefore, the difference in prognosis for PFS could not be significant in a relatively short duration.

There are some limitations to this meta-analysis need to be noted. First, the patients included in the meta-analysis were from Asia, mainly China. Therefore, our results apply to Asian patients. Second, the sample size was relatively small. Although eight studies were included, the total sample size was 2,267. Only six studies were included for OS analysis and five studies for PFS analysis. Third, most of the included studies were retrospective, and only two studies were prospective, which may have led to selection bias. Therefore, large-scale prospective trials including diverse populations are needed to validate the results of our meta-analysis.

In summary, our meta-analysis demonstrated that an elevated SII was associated with poor OS in patients with SCLC. Moreover, a high SII was predictive of ES-SCLC. We recommend adopting SII to predict OS in patients with SCLC, and SII in combination with other parameters or biomarkers may aid in addressing the clinical strategy and choosing the best treatment for each patient. Due to the limitations mentioned above, further large-scale prospective trials are needed to validate our findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YZ and MD designed and supervised the study. ZZ drafted the manuscript, carried out the literature search, and extracted the data from the eligible studies. YZ and ZZ contributed to the quality control of study inclusion and discussion. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.814727/full#supplementary-material

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CCL5 as a Prognostic Marker for Survival and an Indicator for Immune Checkpoint Therapies in Small Cell Lung Cancer

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The standard treatment for small cell lung cancer (SCLC) has not changed in decades. Recently, important advances have been made in immunotherapy. However, analysis of these trials suggests that only a small proportion of patients benefit from immune checkpoint blockade (ICB). Identifying these patients is a clinical challenge. In this study, we applied the ESTIMATE calculation to calculate immune scores in 159 cases of SCLC from two published cohorts. COX regression analysis was used to analyze the differentially expressed genes (DEGs) with high and low immune score. We found that CCL5 expression was positively correlated with survival in SCLC patients. In addition, we verified the effect of CCL5 on survival and response to treatment in another cohort that received immunotherapy. Meanwhile, Gene set enrichment analysis (GSEA) showed that genes with high expression of CCL5 were mainly enriched in immune-related activities. The result of Tumor Immune Dysfunction and Exclusion (TIDE) demonstrated that CCL5 was a potential biomarker to predict response to ICB for SCLC, which is correspondent with the result in verified cohort. These results suggest that CCL5 may be the reason for TME to maintain its immune dominance, making it a favorable factor for ICB. Therefore, CCL5 levels may help to outline the prognosis of patients with SCLC.

Keywords: CCL5, tumor microenvironment, CIBERSORT, tumor infiltrating immune cells, small cell lung cancer (SCLC)

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INTRODUCTION

Small cell lung cancer (SCLC), which accounts for 15% of all lung cancers, is a highly malignant neuroendocrine tumor (1). At present, the treatment of small cell lung cancer is limited. Surgery, platinum-containing chemotherapy and radiotherapy remain the main treatments (2, 3). SCLC responds well to chemotherapy, whereas resistance often develops rapidly after a brief remission period.

Immunotherapy refers to the use of tumor cell immunogenicity to stimulate the host to kill the tumor cells. At present, CTLA-4 and PD-1/ PD-L1 are the most popular immunotherapy targets. Immunotherapy has been approved as a second-line regimen of SCLC according to CheckMate032 and KEYNOTE-028/158 by Food and Drug Administration (FDA) (4, 5).It has also shown encouraging results in small cell lung cancer (4, 6). In IMpower-133, patients treated with etoposide/carboplatin/atezolizumab had longer clinical survival than the control group as a

first-line regime (7). However, patients with SCLC benefit much less from immunotherapy than patients with non-small cell lung cancer. SCLC tumors exhibit fewer immune cells in the tumor immune microenvironment (TIME), which may account for poor response to immune checkpoint blocking (8).Molecular markers that determine prognosis and the efficacy of immunotherapy have not been identified thus far. Meanwhile, a highly variable proportion of PD-L1 protein expression has been found in SCLC (9, 10). Unfortunately, both IMpower133, CASPIAN study and CheckMate032 study showed that there was no correlation between PD-L1 expression level and therapeutic effect of experimental groups (4, 11).

Tumor mutation burden (TMB) refers to the number of substitutions, insertions and deletions per megabyte of the exon coding region of the evaluated gene. Genomic analysis of SCLC has identified two defective tumor suppressor genes (p53 and RB1) that cause genomic instability (12).Thus,SCLC is characterized by a high mutation load, which is theoretically suitable for immunotherapy (12, 13). In Checkmate 032, tumor mutation burden was higher among patients with response to either monotherapy or combination therapy, which indicates that tumor mutation burden has prognostic value (14).But it requires more data to prove that.

In this study, aiming to discover molecular markers that play a key role in prognosis and the efficacy of immunotherapy for SCLC patients, we used ESTIMATE algorithm to calculate immune scores in SCLC samples from 159 cases of SCLC from two published cohorts and used Cox regression analysis to search for prognostic immune markers, leading to the identification of C-C Motif Chemokine Ligand 5 (CCL5). To further elucidate the potential effect of CCL5 in SCLC, we carry out the gene co-expression network analysis, CIBERSORT algorithm for estimations of the proportion of immune cell infiltrate, Tumor Immune Dysfunction and Exclusion (TIDE) algorithm for prediction of response to immune checkpoint blockade and Gene Set Enrichment Analysis. These findings may make a meaningful contribution to the development of immune therapy for SCLC patients.

MATERIALS AND METHODS

Acquisition of Data

Transcriptome RNA-seq data and clinical records of SCLC patients were obtained from the supplementary file of the studies reported by George et al. (12), Jiang et al. (15), and Roper et al. (16).

ImmuneScore Calculation

The infiltration level of immune cells was inferred from gene expression data in the studies by George and Jiang by calculating the ImmuneScore derived from the ESTIMATE algorithm using the estimate package in R (version 4.0.5). A higher ImmuneScore indicated a greater ratio of immune cell infiltrate in the TME.

Identification of DEGs Between High and Low ImmuneScore Groups

Eighty-one tumor samples in the George study and 78 tumor samples in the Jiang study were classified into the high score group or low score group based on the comparison to the median score of the ImmuneScore, respectively. The limma package in R was used to perform differential analysis of the gene expression of high score group and low score group samples. Genes with LogFC > 1.0 and false discovery rate (FDR) <0.05 were identified as DEGs. Volcano plots and heatmaps were produced by the ggplot2 and pheatmap packages in R, respectively.

Survival Analysis

The survival package in R were applied for the survival analysis. Seventy-five cases in the George study and 48 cases in the Jiang study with survival data were used for survival analysis. Seventeen cases in Roper study were used as an independent external verification cohort. The optimal cutoff point for the expression of CCL5 was determined by the "surv_cutpoint" algorithm. Kaplan–Meier (KM) analysis was performed to compare the survival outcomes of the CCL5 low and high expression groups, and log rank was used as the statistical significance test.

COX Regression Analysis

The survival package in R was used for univariate Cox regression analysis. The expression levels of the DEGs were analyzed using a univariate Cox model, and the top 15 genes ordered by p-value from small to large in univariate Cox are shown in the forest plot. CCL5 expression levels and all clinical factors in the Jiang study and Roper study were analyzed using a univariate Cox model and multivariate Cox regression model, and factors with $P < 0.05$ in the multivariate Cox regression model were identified as independent prognostic factors.

Gene Co-expression Network Analysis

Seventy-eight samples from Jiang's study were used to conduct a co-expression analysis. The correlation between the CCL5 expression level and other DEGs was calculated using R. Genes with a $P < 0.05$ were considered significant. These genes and Pearson's correlation coefficients were uploaded to Cytoscape software (http://www.cytoscape.org) (version 3.8.1) to map gene co-expression networks.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis

A total of 412 DEGs were used for GO and KEGG enrichment analyses, which were performed with the clusterProfiler and ggplot2 packages in R. Terms with both p- and q-values < 0.05 were considered significantly enriched.

Estimations of the Proportion of Immune Cell Infiltrate

The CIBERSORT algorithm in R was applied to estimate the proportion of 22 types of immune cells that had infiltrated tumor samples in the studies by George and Jiang. Samples with CIBERSORT $p < 0.05$ were selected for subsequent analysis. The overall infiltration of 22 types of immune cells in all selected samples is shown in the histogram, and the correlation between immune cells is shown in the heatmap.

Correlation Analysis of the Immune Microenvironment

The correlation between CCL5 and each type of immune cell expression level was calculated by R, and scatter plots and fitted regression lines were drawn through the ggplot2 package in R. Differential analysis for infiltrating immune cells and immune checkpoints was performed in the CCL5 low and high expression group, and the vioplot and ggpubr packages in R were used for plotting to display the outcomes of analysis.

Prediction of Response to Immune Checkpoint Blockade and Validation

Tumor Immune Dysfunction and Exclusion (TIDE, http://tide.dfci.harvard.edu/) is a computational framework developed to evaluate the potential of tumor immune escape from the gene expression profiles of cancer samples. The TIDE score computed for each tumor samples can serve as a surrogate biomarker to predict response to immune checkpoint blockade, the higher the TIDE score means the lower the possibility of response to immune checkpoint blockade. Expression data in Jiang study was uploaded for the prediction. The responding situations of durvalumab treatment in Roper cohort were used for validation. The receiver operating characteristic (ROC) curve was used to evaluate the response to ICB of CCL5. A $p < 0.05$ was considered statistically significant.

Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA) was performed using GSEA-4.1 software to elucidate the molecular mechanisms of CCL5. The gene sets used included the Hallmark gene sets, C2

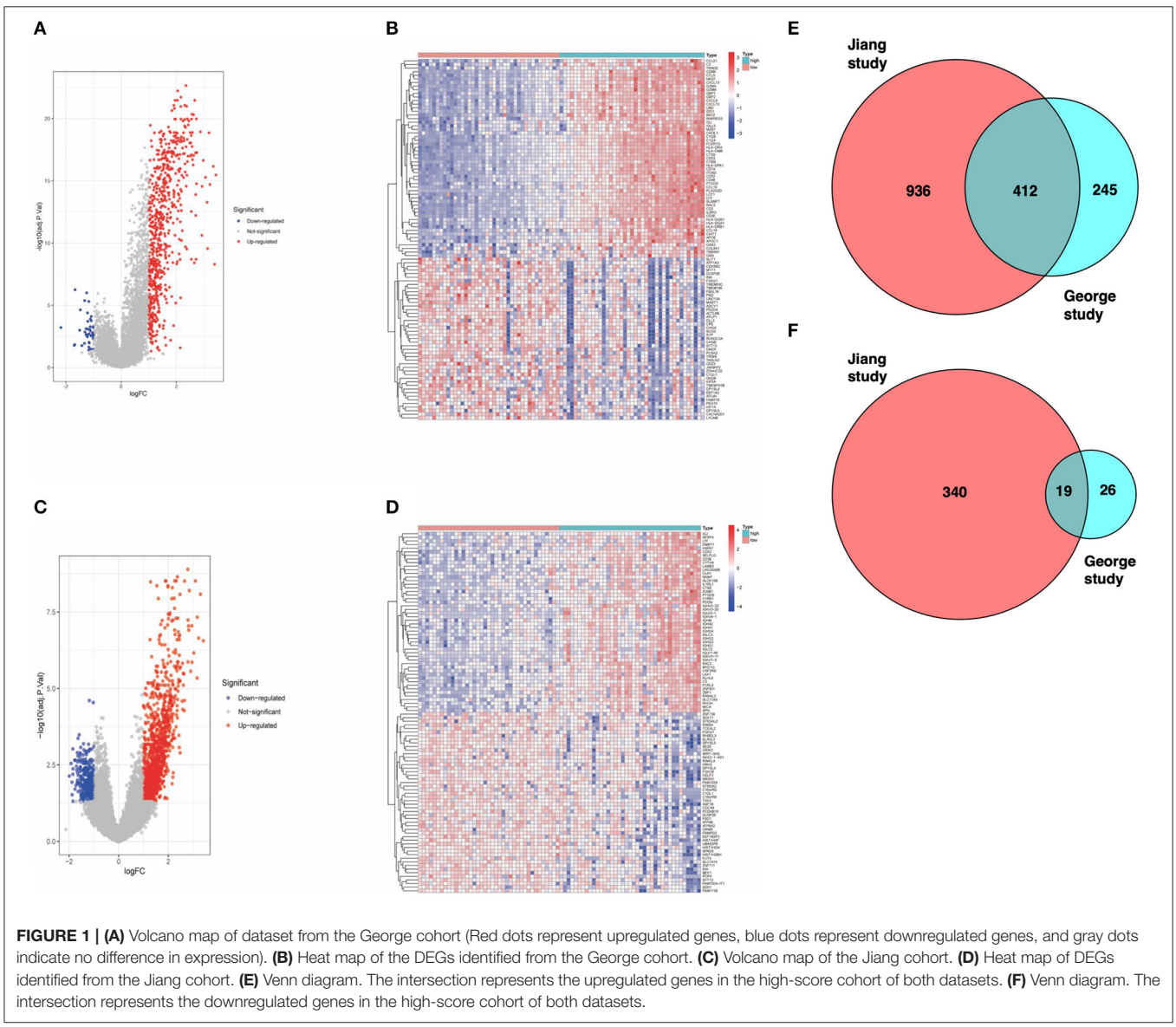

FIGURE 1 | (A) Volcano map of dataset from the George cohort (Red dots represent upregulated genes, blue dots represent downregulated genes, and gray dots indicate no difference in expression). **(B)** Heat map of the DEGs identified from the George cohort. **(C)** Volcano map of the Jiang cohort. **(D)** Heat map of DEGs identified from the Jiang cohort. **(E)** Venn diagram. The intersection represents the upregulated genes in the high-score cohort of both datasets. **(F)** Venn diagram. The intersection represents the downregulated genes in the high-score cohort of both datasets.

sets (curated gene sets), and C7 gene sets (immunologic signature gene sets). The C2 collection is divided into the following two sub-collections: Chemical and genetic perturbations (CGP) and Canonical pathways (CP) sets. Samples of 48 cases from Jiang's study divided into the CCL5 low expression group and high expression group were used for GSEA. Gene sets with $|NES| > 1$, NOM $p < 0.05$ and FDR q < 0.25 were considered significant.

RESULTS

Identification of TIME Related Genes

To assess specific changes in the immune microenvironment of SCLC, we divided patients from the study by George into high and low immunescore groups and found transcriptome differences between the two groups of samples. There were 702 DEGs in the high-score group, including 657 upregulated genes and 45 downregulated genes (**Figures 1A,B**). Similarly, we used the same algorithm to classify patients from the Jiang cohort and obtained 1707 DEGs, including 1,348 upregulated genes and 359 downregulated genes (**Figures 1C,D**). Subsequently, we found gene sets that were high or low expression in both groups with high immune scores (**Figures 1E,F**).

CCL5 Is a Protective Factor for Prognosis of SCLC

Then, we used univariate analysis to identify genes associated with prognosis in gene clusters in the Jiang cohort. Among them, the gene with the lowest *P*-value was CCL5 (**Figure 2A**). Subsequently, we divided SCLC patients from the Jiang and George cohorts into high and low CCL5 expression groups (**Supplementary Figures 1A,B**). In both cohorts, high CCL5 expression indicates better survival in patients without chromothripsis (**Figures 2B,D**).

To confirm the relationship between this gene and patients' prognosis, we performed a multivariate analysis. The result demonstrates that CCL5 is an independent protective factor with hazard ratio = 0.41 (**Figure 2C**; **Table 1**). Furthermore, we verified this result in Roper cohort, correspondence with the former cohorts, CCL5 indicates better survival in SCLC patients accepting immunotherapy (**Figure 2F**), as well as an independent protective factor (**Figure 2E**).

Co-expression Network Analysis of CCL5

Next, to explore the co-expression genes of CCL5, we calculated the Pearson correlation coefficients between DEGs and CCL5. A total of 427 genes in DEGs with $P < 0.05$ were considered as co-expression genes of CCL5 and visualized via Cytoscape (**Figure 3**; **Supplementary Table 1**). We found that LAPTM5, C3 and HLA-DPB1 were the most positively correlated with CCL5, and RUNDC3A, ATCAY and DPYSL5 were the most negatively correlated with CCL5.

KEGG/GO Biological Process Enrichment for Co-expression Genes of CCL5

KEGG and GO analyses were performed to explore the specific pathways associated with CCL5 and its co-expression genes. The KEGG pathway analysis of CCL5 interactive genes showed that cytokine-cytokine interactions and chemokine signal pathways were the most enriched pathways (**Figure 4A**) Additionally, GO analysis demonstrated that CCL5 and its co-expression genes were significantly enriched in the T cell activation pathway at biological process (BP) levels (**Figure 4B**), immune receptor activity at molecular function (MF) levels (**Figure 4C**) and collagen-containing extracellular matrix at cellular components (CC) levels (**Figure 4D**). These results suggest that CCL5 is closely related to immune-related molecules.

Potential Mechanism of CCL5 Regulating the Immune Microenvironment

To investigate the correlation between CCL5 expression and immune-related activities, the signaling pathways related to CCL5 expression were studied by GSEA. Tumor samples were divided into high and low groups according to median CCL5 expression levels. The results showed that the hallmark gene set in the CCL5-high expression group was mainly involved in apoptosis and IL2 STAT5 signaling (**Figure 5A**). In addition, the high CCL5 expression group was enriched in IL-4 signaling pathway of C7 immune gene sets, while enriched in NF-κB activation, FOXP3 target signaling pathways in other gene sets (**Figures 5B–D**). These results suggest that CCL5 may be an important factor regulating immune-related activities.

Correlation Between CCL5 and the TICs Proportion

The CIBERSORT method was used to further confirm the relationship between CCL5 expression and immune components, construct immune cell profiles and analyze the proportion of tumor infiltrating immune subtypes (**Figures 6A–C**). Eight kinds of TICs were positively correlated with CCL5 expression including CD8+ T cells, gamma delta T cells, CD4+ memory T cells, memory B cells, dendritic cells, M1 macrophages and NK cells whereas M2 macrophages was negatively correlated with CCL5 expression (**Figures 6D–K**). The above results further confirm that CCL5 expression significantly affects the immune activity in the TIME.

CCL5 Can Be an Indicator of Efficacy of ICB

To assess the response to immune checkpoint blockade (ICB) based on CCL5 expression, we firstly explored the correlation between CCL5 levels and common immune checkpoints (ICPs). CCL5 expression was associated with ICPs (programmed cell death 1 (PD1), programmed cell death ligand 1 (PD-L1), cytotoxic T lymphocyte antigen 4 (CTLA4), T cell immunoglobulin mucin 3 (TIM-3), lymphocyte activating gene 3 (LAG3), T-cell immune receptors with Ig and ITIM domains (TIGIT), etc.) in the Jiang cohort. ICPs were highly expressed in the group with high CCL5 expression (**Figure 7A**). The results showed that patients with high CCL5 expression tended to have a high level of ICPs.

Tumor Immune Dysfunction and Rejection (TIDE) is a computational framework used to simulate the two main

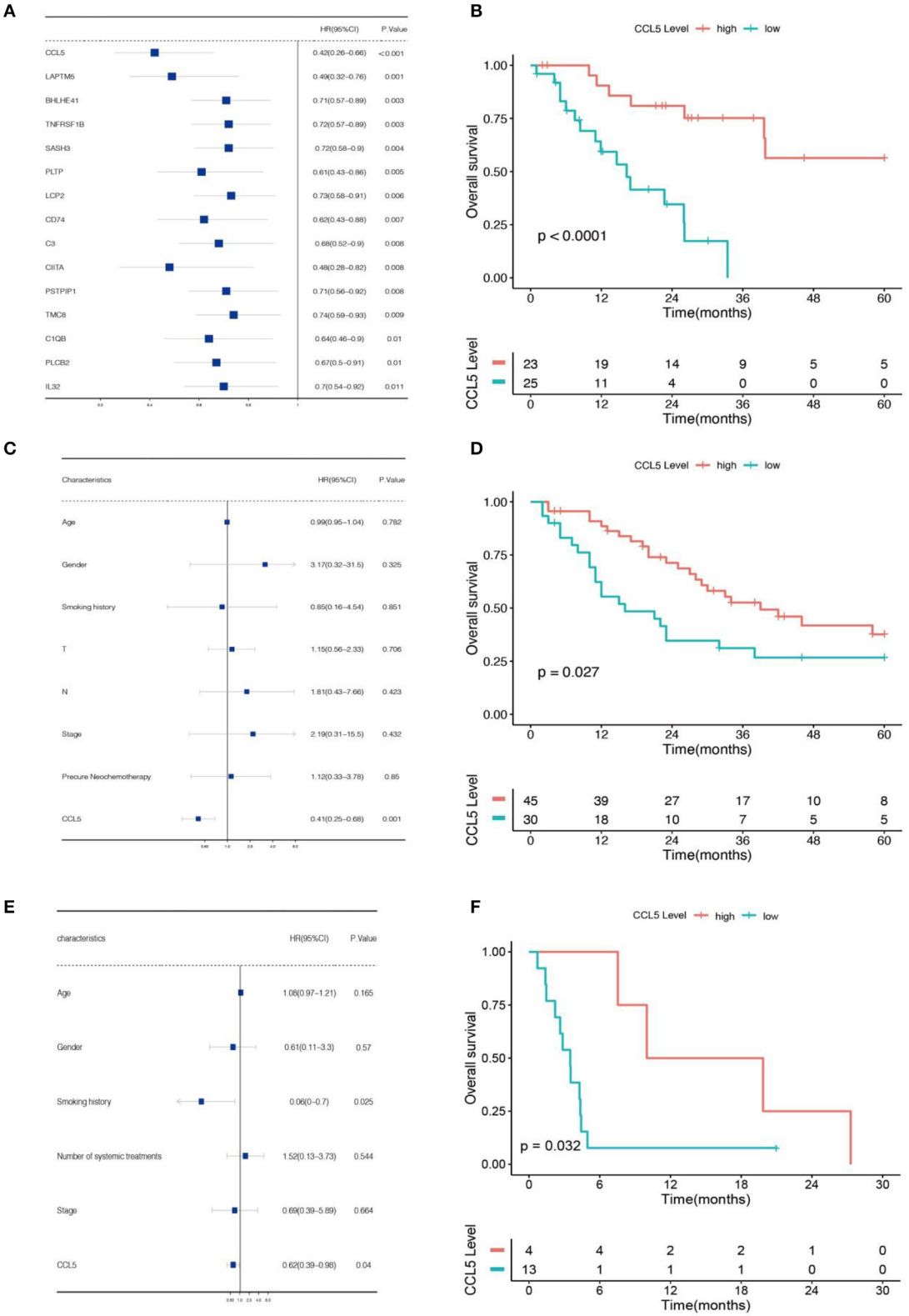

FIGURE 2 | (A) Univariate Cox regression analysis of data from the Jiang cohort. **(B)** Association between CCL5 and overall survival based on data from Jiang cohort. **(C)** Multivariate Cox regression analysis of data from the Jiang cohort. **(D)** Association between CCL5 and overall survival based on data from George cohort. **(E)** Multivariate Cox regression analysis of data from the Roper cohort. **(F)** Association between CCL5 and overall survival based on data from Roper cohort.

TABLE 1 | Univariate Cox model and Multivariate Cox regression model in the Jiang study.

Clinical characteristic	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p-Value*	HR	95%CI	*p-Value*
Age	0.99	0.94–1.03	0.545	0.99	0.95–1.04	0.782
Gender	1.02	0.3–3.48	0.974	3.17	0.32–31.5	0.325
Smoking history	0.66	0.26–1.69	0.389	0.85	0.16–4.54	0.851
T	1.61	0.99–2.62	0.056	1.15	0.56–2.33	0.706
N	2.67	1.44–4.96	**0.002**	1.81	0.43–7.66	0.423
Stage	4.21	1.71–10.37	**0.002**	2.19	0.31–15.5	0.432
Precure neochemotherapy	1.07	0.36–3.21	0.902	1.12	0.33–3.78	0.85
CCL5 expression	0.42	0.26–0.66	**0**	0.41	0.25–0.68	**0.001**

The bold values indicate the statistically significant results.

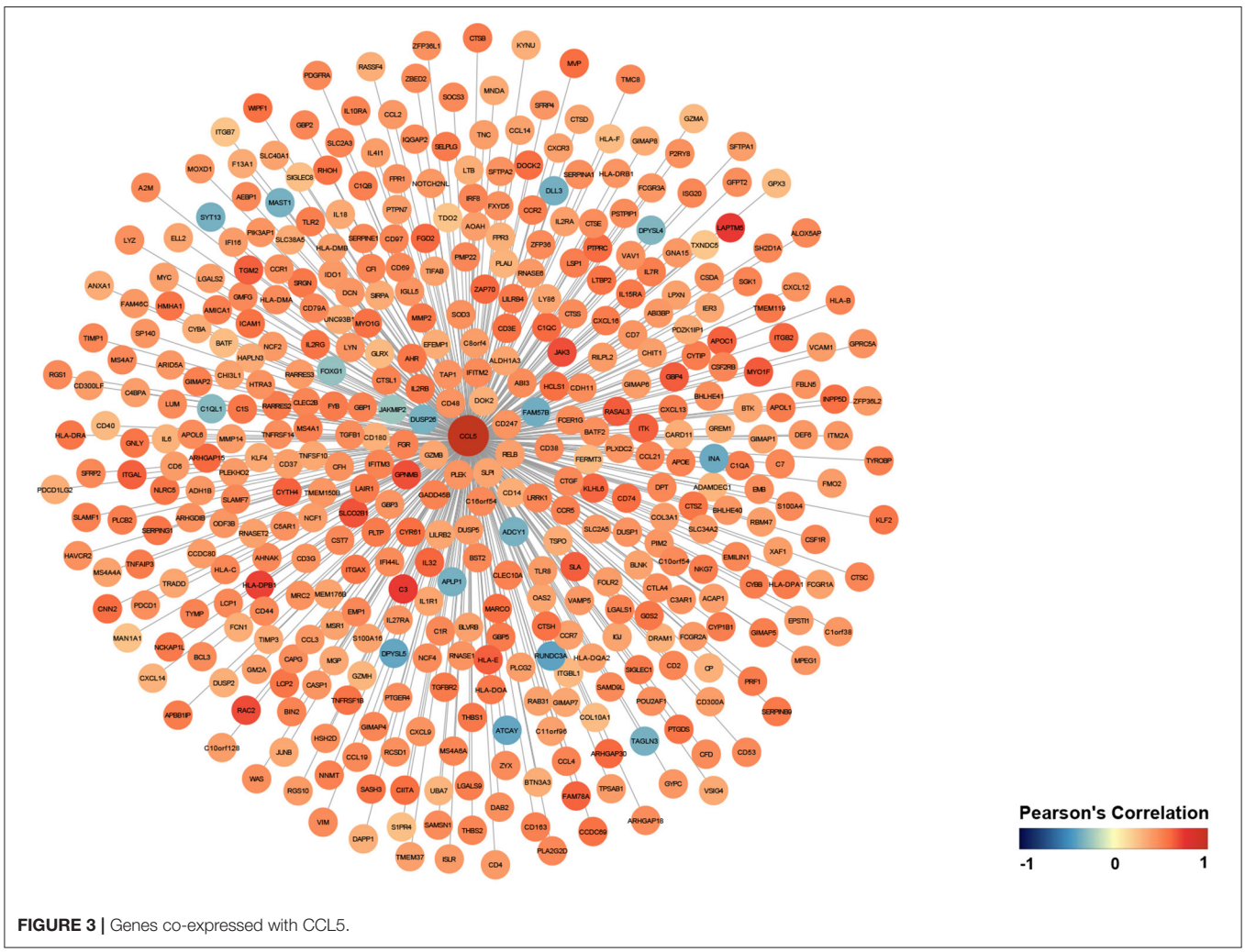

FIGURE 3 | Genes co-expressed with CCL5.

mechanisms of tumor immune evasion and can provide predictive outcomes of immune checkpoint blockade (17). Elevated TIDE scores may indicate non-response in patients with suppressive T cell infiltration. To better illustrate the predictive power of CCL5 for immunotherapy, TIDE was applied to the Jiang cohort. We were pleasantly surprised to find a negative correlation between TIDE and CCL5 (**Figure 7B**). In addition, the predicted response suggests that CCL5 may be a good predictor of immune checkpoint blockade for SCLC (**Figures 7C,D**).

To more credibly illustrate this result, we validated a positive correlation between CCL5 expression and PD-L1

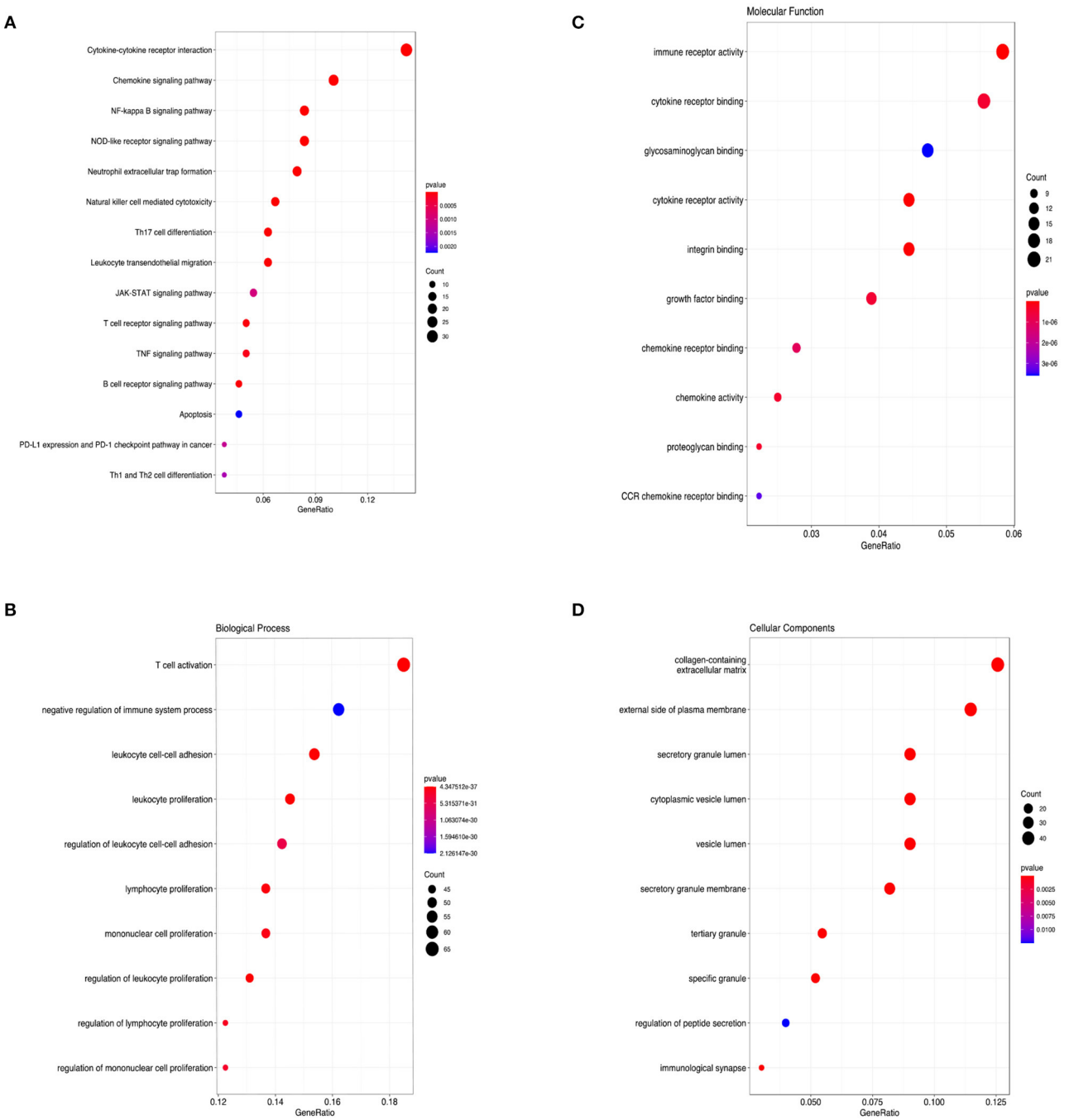

FIGURE 4 | (A) KEGG pathway enrichment analysis. **(B)** Biological process enrichment analysis. **(C)** Molecular function enrichment analysis. **(D)** Cellular component enrichment analysis.

expression in the Roper cohort, in which patients received the anti-PD-L1 antibody durvalumab and poly(adp-ribose) polymerase (PARP) inhibitor Olaparib (**Figure 7E**). The expression of CCL5 was statistically different between responders and non-responders (**Figure 7F**). CCL5 was highly expressed in the responders group. Receiver operating characteristic (ROC) curve also reveals that CCL5 is a good marker to predict the result of immunotherapy (AUC = 0.904) (**Figure 7G**).

DISCUSSION

The immune landscape of the tumor microenvironment can influence the occurrence, progression, and invasion of cancer,

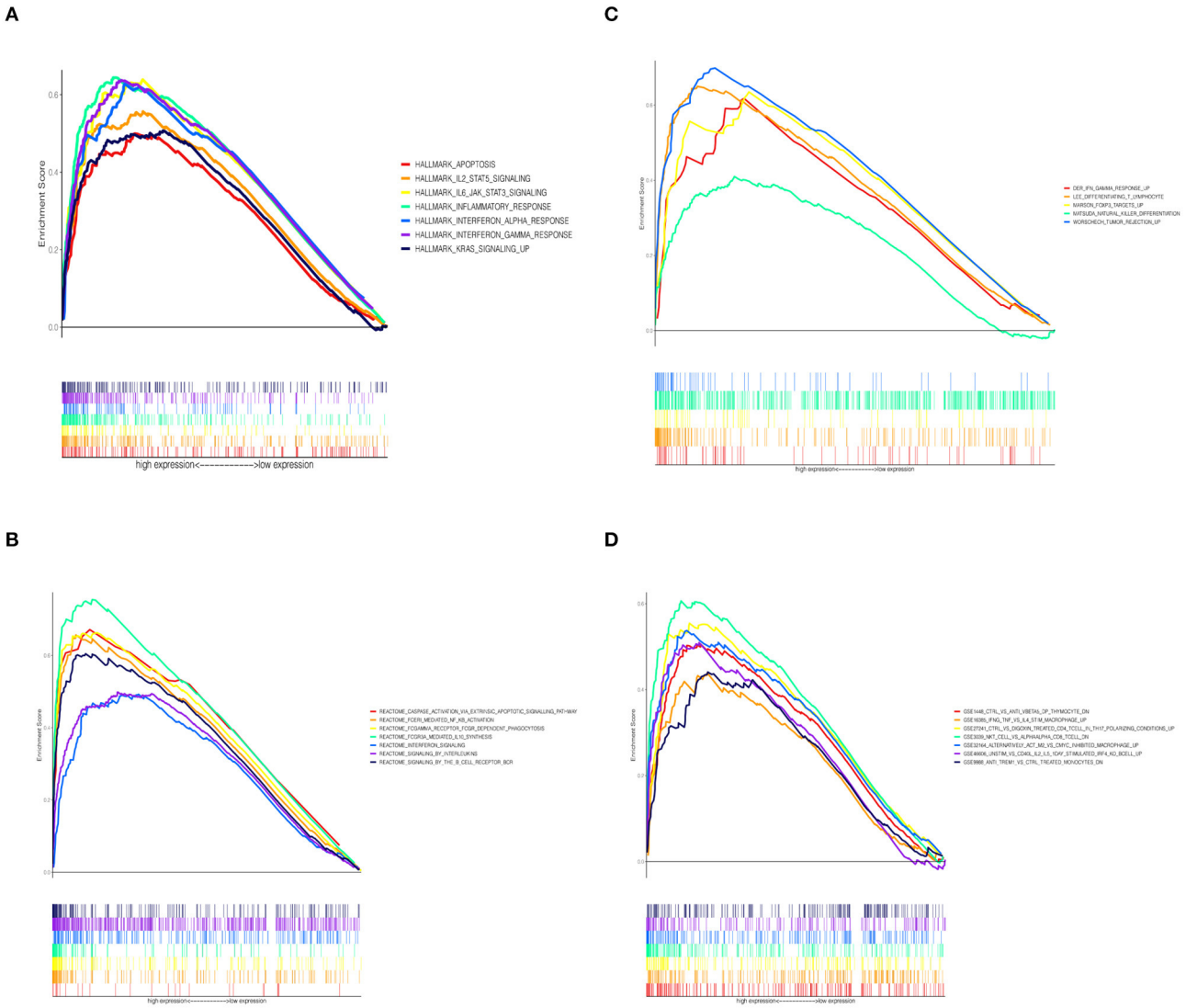

FIGURE 5 | (A) Enriched gene sets in the HALLMARK collection in samples with high CCL5 expression. **(B)** Enriched gene sets in REACTOME Pathways databases in samples with high CCL5 expression. **(C)** Enriched gene sets in Canonical Pathways gene sets in samples with high CCL5 expression. **(D)** Enriched gene sets in C7 immune gene sets in samples with high CCL5 expression.

thus influencing patient prognosis. The composition of immune cells in the microenvironment can also predict the efficacy of immunotherapy (18).

CCL5 belongs to the CC motif chemokine family and binds to its receptor CCR5 with high affinity. Different conclusions have been drawn about the role of CCL5 in tumors. Some studies accounted CCL5 for the promotion of tumor development by suppressing the immune response (19), whereas some studies regarded CCL5 as a tumor protective factor associated with high CD8+ T cell infiltration (20, 21). In this study, we first evaluated the relationship between CCL5 and survival in patients with SCLC. We found that high CCL5 expression was associated with longer survival in patients with SCLC. CCL5 is considered the target gene of NF-κB activity, leading to NF-κB activation. These effects ultimately lead to the promotion of T cell-mediated immune surveillance (22, 23). Consistent with this notion, we found that CCL5 was associated with the NF-κB pathway in KEGG enrichment and GSEA analysis. Besides CD8+ T cell, NK cells are emerging as an attractive target for immunotherapy (24, 25). SCLC metastasis is controlled by NK cells (26).NK cells are also a potential therapeutic target for small cell lung cancer (27, 28).

Similarly, the DNA damage response (DDR) inhibition activated the STING/TBK1/IRF3 innate immune pathway, leading to increased levels of chemokines such as CXCL10 and CCL5 that induced activation and function of cytotoxic T lymphocytes (29), while CCL5 recruits T cells in the tumor microenvironment via IFN (11). This is consistent with our GSEA results as well. More importantly, we found that CCL5 is associated with immune-related molecules and pathways. We

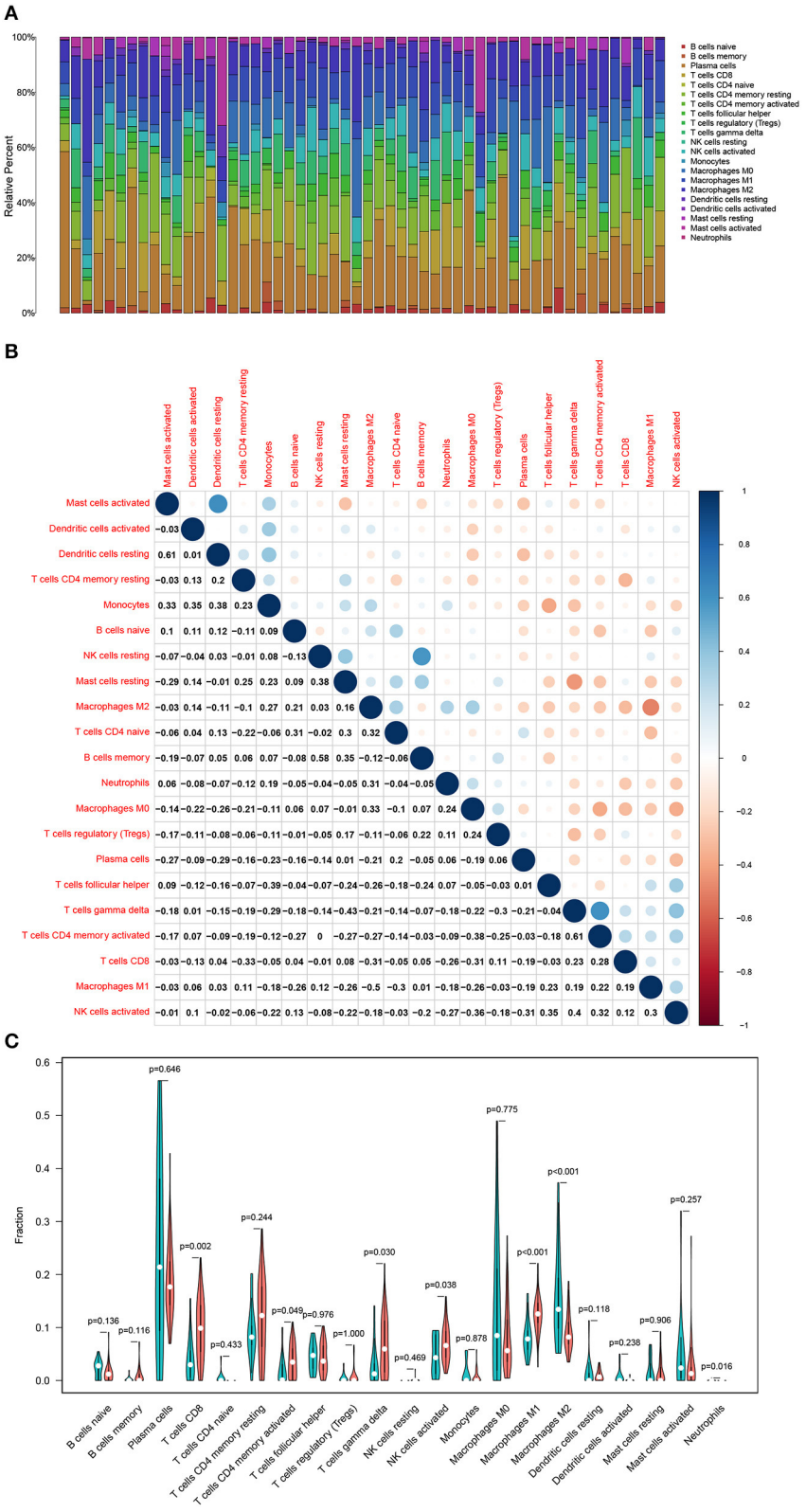

FIGURE 6 | Continued

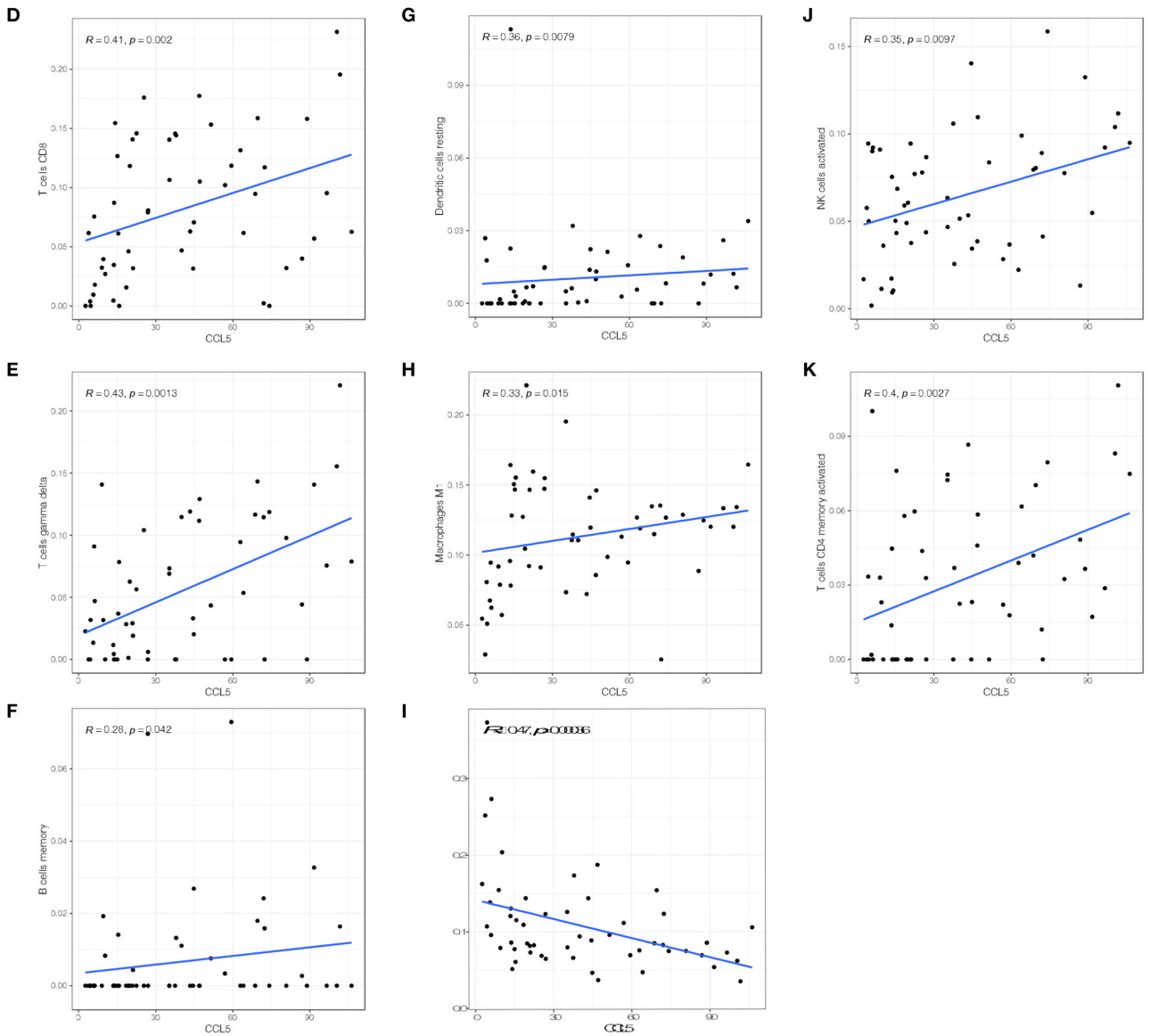

FIGURE 6 | (A) Bar plot shows the proportion of 21 types of TICs in SCLC tumor samples. **(B)** The heatmap shows the correlation between 21 TICs and the value in each small box and represents the *P*-value of the correlation between the two cells. The shadows in each tiny color box represent the corresponding correlation values between the two cells, and significance was assessed using Pearson's coefficient. **(C)** The violin plot shows the proportional differentiation of 21 immune cells relative to the median level of CCL5 expression in SCLC tumor samples with low or high CCL5 expression. **(D–K)** Scatter plot showing that the proportion of 8 TICs was correlated with CCL5 expression ($P < 0.05$). The blue line in the figure indicates the fitted line of the linear model, indicating the proportion of immune cells and CCL5 expression. Pearson's coefficient was used for the correlation test.

found that CCL5 status is associated with a variety of immune cells, including CD8 T cells (30), NK cells (31) and γδ T cells (32) that have been identified. Treg cells express the transcription factor Foxp3 and play a key role in maintaining immune homeostasis by inhibiting inflammatory responses in different biological environments (33). In most solid malignancies, high FOXP3 positive Treg infiltration in tumors is associated with poor prognosis (17); in contrast, patients with SCLC with FOXP3 positive levels have longer RFS (34). These immune-infiltrating cells are thought to promote the antitumor effects of the tumor microenvironment.

We also found statistical correlations in our data between CCL5 and immune checkpoints, including PD-1 and PD-L1 expressed in TILs. Surprisingly, patients with high CCL5 expression were predicted to respond better to immunotherapy. Thus, CCL5 may represent a potential predictor of immunotherapy. Based on the above analysis, we found a wide range of interactions between CCL5 and other immune biomarkers in SCLC.

The study has some limitations. First, the study included only three clinical cohorts. Only one cohort included immunotherapy patients. Our assumptions and results are based on a small

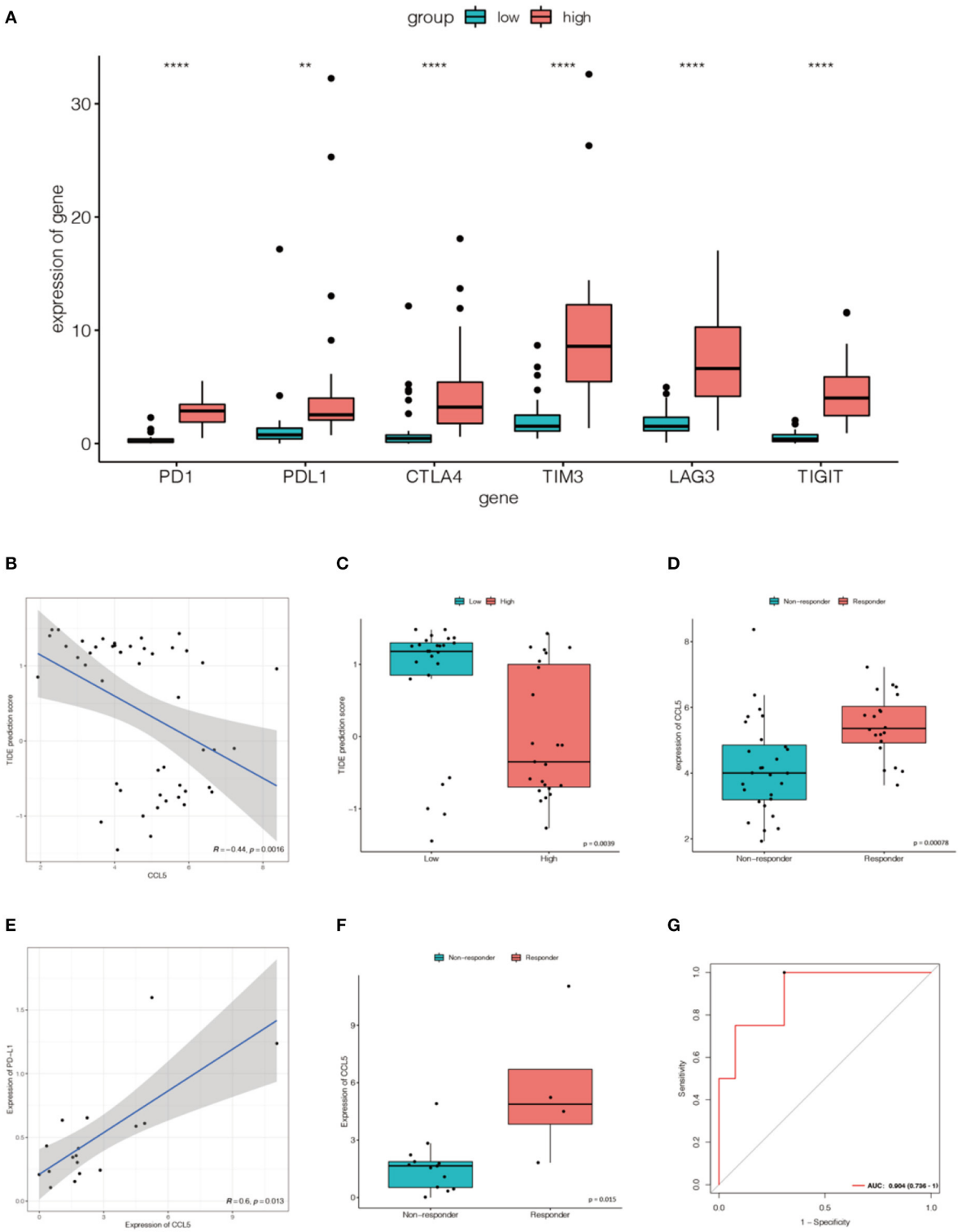

FIGURE 7 | (A) The expression of ICPs in the high CCL5 expression group was significantly greater than that in the low CCL5 expression group in the Jiang cohort (**$P < 0.001$; ****$P < 0.0001$). **(B)** Relationship between TIDE and CCL5 expression in the Jiang group. **(C)** Distribution of TIDE scores in the high- and low-expression groups. **(D)** CCL5 expression in the Jiang cohort differed between responders and non-responders. **(E)** The expression of CCL5 was positively correlated with that of PD-L1 in Roper's cohort ($p < 0.05$). **(F)** CCL5 expression in the Roper's cohort differed between responders and non-responders. **(G)** Receiver operating characteristic (ROC) curve of CCL5 in Roper's cohort (AUC = 0.904).

sample size. Prospective and multicenter studies are needed in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LG and YT: study design. YT, YH, and YN: data analysis, interpretation, and writing of the manuscript. LG, YT, and YN: revision of the manuscript. LS, YT, and YH: statistical analysis. All authors have reviewed the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.834725/full#supplementary-material

Supplementary Figure 1 | (A) The optimal cutoff point for the expression of CCL5 in the Jiang study. **(B)** The optimal cutoff point for the expression of CCL5 in the George study. **(C)** The optimal cutoff point for the expression of CCL5 in the Roper cohort.

Supplementary Table 1 | The co-expression genes of CCL5.

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The Role of Surgery in High-Grade Neuroendocrine Cancer: Indications for Clinical Practice

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Pulmonary neuroendocrine tumors (pNET) represent a particular type of malignant lung cancers and can be divided into well-differentiated low-grade NET and poorly-differentiated high-grade NET. Typical and atypical carcinoids belong to the first group while large cell neuroendocrine carcinomas (LCNEC) and small-cell lung cancers (SCLC) belong to the second one. The aim of this mini-review is to focus on the role of surgical therapy for high grade neuroendocrine tumors. SCLC has the worst prognosis among all lung cancer neoplasms: in fact, the two-year survival rate is about 5% and median survival usually ranges between 15 and 20 months. The surgical treatment of SCLC has thus infrequently been judged as a valuable aspect of the therapeutic approach, the gold standard treatment being a combination of platinum-based chemotherapy and radiotherapy. As LCNEC are rare, there is a lack of extensive literature and randomized clinical trials, therefore the curative approach is still controversial. Current treatment guidelines suggest treating LCNEC by surgical resection in non-metastatic stages and recommend adjuvant chemotherapy according to SCLC protocol. Upfront surgery is suggested in early stages (from I to IIB), a multimodality approach is recommended in locally advanced stages (III) while surgery is not recommended in stage IV LCNEC. The rate of surgical resection is quite low, particularly for SCLC, ranging from 1 to 6% in limited diseases; lobectomy with radical lymphadenectomy is considered the gold standard surgical procedure in the case of limited disease SCLC and resectable LCNEC; pneumonectomy, although reported as an effective tool, should be avoided in the light of local and distant recurrence rates.

Keywords: surgery, small cell lung cancer, large cell neuroendocrine carcinoma, lobectomy, pneumonectomy

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INTRODUCTION

Pulmonary neuroendocrine tumors (pNET) represent a particular type of malignant lung cancers and can be divided into well-differentiated low-grade NET and poorly-differentiated high-grade NET. Typical and atypical carcinoids belong to the first group while large cell neuroendocrine carcinomas (LCNEC) and small-cell lung cancers (SCLC) belong to the second one (1).

High grade neuroendocrine tumors present significantly higher mitotic rates when compared to low-grade neuroendocrine tumors; moreover, increased necrosis is commonly observed as well as their combination with other types of lung cancer like adenocarcinomas or squamous cell carcinomas (2).

The vast majority of high-grade neuroendocrine tumor patients are older and heavy smokers, with an early tendency to metastasize and a globally poor long-term prognosis with 5-year survival rates ranging from 15 to 57% (3–5).

SCLC account for 15–20% of all pulmonary tumors; among them, only 10 – 20% of cases are early-stage tumors amenable to curative local treatments; on the contrary, the vast majority of patients present huge and centrally-located lesions – very often causing superior vena cava compression and/or infiltration - and early dissemination, chemotherapy thus being the most effective fist-line treatment (6, 7). About 10% of patients suffering from SCLC present paraneoplastic syndromes such as Lambert-Eaton Syndrome, Cushing syndrome, hypercalcemia and syndrome of inappropriate antidiuretic hormone secretion (SIADH) (7).

LCNEC account for <1% of all lung cancers and 40% of affected patients are diagnosed in metastatic stage (8). Histological differential diagnosis between SCLC and LCNEC can be difficult because of the many common features shared by the two diseases, such as necrosis, neuroendocrine morphology, positive immunohistochemical staining for neuroendocrine markers and a high mitotic rate (9) (**Table 1**).

TABLE 1 | Literature review.

SCLC		
	Fox et al. (10) Lad et al. (11)	No survival benefit of neoplasm resection.
	HwihdJiang et al. (12) Lim et al. (13) Tsuchiya et al. (14)	Resected SCLC disclosed a median survival of 20 months and a 5-year survival of 11.1–52%.
	Schreiber et al. (15)	Resected T1-2 SCLC disclosed a median overall survival of 42 vs. 15 months; T3-4 22 vs. 12 months.
	Casiraghi et al. (16)	1, 5 and 10-year overall survival rates of 73.6%, 42% and 25.6%.
LCNEC		
	Iyoda et al. (17)	5-year survival rate of resected LCNEC after induction treatment is reported to be 88%, while without induction treatment it falls to 47%.
	Veronesi et al. (4)	LCNEC with mediastinal lymph node metastases show a significantly worse prognosis.
	Girelli et al. (18)	Mediastinal involvement had a significantly worse prognosis when compared to pN0 patients.
	Lo Russoet al. (19)	Radical resection should always be attempted whenever feasible and patients with nodal involvement should always receive adjuvant treatments.

THE ROLE OF SURGERY IN SMALL CELL LUNG CANCER

Small cell lung cancer (SCLC) has the worst prognosis among all lung cancer neoplasms: in fact, the two-year survival rate is about 5% and median survival usually ranges between fifteen and 20 months (7). It is characterized by early and fast diffusion, presenting a significant recurrence rate after the initial response to treatments (20). The surgical treatment of SCLC has thus infrequently been judged as a valuable aspect of the therapeutic approach, the gold standard treatment being a combination of platinum-based chemotherapy and radiotherapy (20, 21).

Two randomized controlled trials performed in the 70's and 90's evaluated the contribution of surgical resection to the therapeutic pathway of limited disease-small cell lung cancer: although some important limitations of both studies emerged, none of them was able to find any survival benefit of neoplasm resection (10, 11). In more recent times, small series of surgical resection of SCLC - focusing on different outcomes - have been reported, disclosing a median survival of 20 months and a 5-year survival of 11.1–52% (12–14). Nowadays, operated limited disease – small cell lung cancer represents only a small percentage of resected lung tumors, accounting for 0–6.1% of all resected pulmonary neoplasms (22), although several large prospective cohort studies have recently shown a potential benefit of operating early stage SCLC (15, 22–24). T1 and T2 SCLC resected diseases disclosed a median overall survival benefit of 42 vs. 15 months as well as T3 and T4 disease (22 vs. 12 months) (15). Sub-lobar resections are not suggested, as they show a significantly worse prognosis when compared to anatomical resection (24).

Locally advanced SCLC (stage IIIa) should not be considered for surgery as suggested in almost no guidelines (25–29); nonetheless, radical lymphadenectomy in N2 patients has been reported to have a valuable impact on survival in several series (15, 23, 30).

In our personal retrospective experience, we observed 65 patients suffering from SCLC and surgically treated with curative intent. Our results disclosed a median overall survival of 36 months and postoperative 1, 5 and 10-year overall survival rates of 73.6, 42, and 25.6%. In particular, patients receiving surgical radical resection and presenting a pathological stage I had a 5-year overall survival of 76.6%; on the contrary, patients undergoing induction treatments or adjuvant radiotherapy had a worse prognosis probably due to a more advanced stage with lymph node involvement. In fact, lymph node involvement together with volume and site of the tumor were significantly related to overall survival, pT4 or pN2 patients presenting 1-year overall survival rates of 50 and 57.1% respectively; none of them was alive at 5 years (16).

The role that surgery may play for treating limited-stage SCLC remains unclear due to controversial literature results and the absence of recent randomized clinical trials.

Considering how easily SCLC tends to metastasize and its high chemo-sensitivity, many recent guidelines suggest a non-surgical approach to limited disease-SCLC, recommending platinum-based chemotherapy and mediastinal radiotherapy, or chemotherapy alone with prophylactic cranial irradiation for more advanced diseases (31, 32).

Recent larger retrospective series have shown possible advantages offered by the resection of limited-stage SCLC. Encouraging 5-years overall survival rates of 48, 39, and 15% for operated patients in stage I, II and III respectively have been shown by The International Association for the Study of Lung Cancer (IASLC) Lung Cancer Staging Project in a group of 349

patients (23).Similarly, the Surveillance, Epidemiology and End Results study (SEER) disclosed a 5-year overall survival rate of 50.3% in a retrospective series of 247 resected stage I SCLC patients (33), and Yang et al. reported a 5-year survival rate of 47% in a cohort of 1,574 early stage-SCLC patients from the National Cancer Database, receiving radical resection (34).

The role of surgery in early stage SCLC might be not only limited to an improvement of overall survival but also to an appropriate histo-pathological diagnosis, thus supporting SCLC histology which could be misdiagnosed in the case of mixed forms, some NSCLC or rare tumors, thus adapting the treatment plan to the new acquired histology and helping the formulation of a different prognosis (35, 36).

To date, there is no evidence supporting surgical indication in stage II and stage IIIA SCLC. NCCN guidelines, in fact, do not recommend resecting advanced tumors as they do not benefit from surgery (35), although some recent reports seem to disclose a significant improvement in survival in stage II and stage IIIA SCLC undergoing lung resection (37). Nevertheless, whenever a surgical option is offered to SCLC patients, a careful balance between expected benefits and risks should be carried out, taking into consideration the volume extension of the planned resection, the clinical stage of the disease and the performance status of the patient; a multidisciplinary discussion is strongly recommended and every available less invasive therapeutic option should be contemplated (38).

The surgical approach to limited disease-SCLC should be standard lobectomy with lymphadenectomy which provides the best overall survival, in particular when compared to lesser resection such as wedge resection (39); on the other hand, the role of pneumonectomy in SCLC is unclear and, taking into consideration the disease's biology and the high risk postoperative course, it should be avoided even in salvage settings (40, 41).

A more effective role of surgery has been observed within a multimodality approach including chemotherapy and/or radiotherapy in patients presenting a resectable disease (42). The NCCN guidelines, in fact, recommend adjuvant chemotherapy even in the case of N0 disease at clinical staging; moreover, they suggest sequential or concurrent chemo and radiotherapy in N+ disease, reporting a more effective role of radiotherapy in pN2 disease than in isolated N1 disease (43, 44).

Worth of being reported is a combined form of SCLC and NSCLC which is relatively rare and it is defined as SCLC combined with any elements of non-small cell lung cancer (45). Incidence of combined SCLC has been reported to range from 2 to 28% and its prognosis does not significantly differ from pure SCLC after surgical resection (45).

THE ROLE OF SURGERY IN LARGE CELL NEUROENDOCRINE CARCINOMAS

It has been widely demonstrated that is quite difficult to obtain a precise diagnosis before surgery in the case of LCNEC; in the vast majority of cases, in fact, a definitive pathological confirmation is acquired by analyzing resected specimens (46–48). As LCNEC are rare, there is a lack of extensive literature and randomized clinical trials, therefore the curative approach is still controversial (49).

LCNEC shows a significantly worse prognosis when compared to other large cell non-neuroendocrine lung cancers (50, 51). A sex-related difference in terms of overall survival has occasionally been reported (52) but not further confirmed (53, 54). As for NSCLC, LCNEC with mediastinal lymph node metastases show a significantly worse prognosis (4).

Although the lack of randomized controlled trials and the retrospective nature of published studies do not allow definitive conclusions about the role of induction therapy or adjuvant treatments, it is quite well known that LCNEC is most often responsive to platinum-based neoadjuvant treatments (55). In fact, the 5-year survival rate of resected LCNEC after induction treatment is reported to be 88%, while without induction treatment it falls to 47% (17). Chemotherapy seems to play an additional beneficial role even in early stage LCNEC (4) although discordant results have been reported (56); nevertheless, taking into consideration the biological similarity of LCNEC to SCLC and the similar response rate, it seems reasonable to offer platin-based chemotherapy not only to advanced stage LCNC but also to early ones (17, 49). In our personal experience, patients with mediastinal involvement had a significantly worse prognosis when compared to pN0 patients (18); previous reports had already recommended aggressive combined approaches – as for SCLC –particularly in cases with lymph node involvement (57). Although in our experience no chemotherapy regimen conditioned overall survival, it has been widely reported that radical resection should always be attempted whenever feasible and patients with nodal involvement should always receive adjuvant treatments (19).

Current NCCN treatment guidelines suggest treating LCNEC by surgical resection in non-metastatic stages and recommend adjuvant chemotherapy according to SCLC protocol (44). Upfront surgery is suggested in early stages (from I to IIB), a multimodality approach is recommended in locally advanced stages (III) while surgery is not recommended in stage IV LCNEC.

CONCLUSION

SCLC and LCNEC are high-grade neuroendocrine neoplasms; they grow faster than other NSCLC and show a more aggressive behavior and worse prognosis. While SCLC usually present as centrally-located bulky lesions, LCNEC are more frequently diagnosed as peripheral neoplasms. They are typically detected in heavy smoker older patients in stage IV at first diagnosis in 60–80% of cases in SCLC and 40% of cases in LCNEC. The rate of surgical resection is quite low, particularly for SCLC, ranging from 1 to 6% in limited diseases; lobectomy with radical lymphadenectomy is considered the gold standard surgical procedure in the case of limited disease SCLC and resectable LCNEC; pneumonectomy, although reported as an effective tool, should be avoided in the light of local and distant recurrence rates. The surgical route should always be evaluated within a multimodality approach including chemotherapy and radiotherapy in almost every stage.

AUTHOR CONTRIBUTIONS

FP, CB, MC, and LS took part in all the aspects of the paper, idealization, writing, and revision. All authors contributed to the article and approved the submitted version.

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REVIEW
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SCLC Treatment in the Immuno-Oncology Era: Current Evidence and Unmet Needs

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Small cell lung cancer (SCLC) represents about 13%–15% of all lung cancers. It has a particularly unfavorable prognosis and in about 70% of cases occurs in the advanced stage (extended disease). Three phase III studies tested the combination of immunotherapy (atezolizumab, durvalumab with or without tremelimumab, and pembrolizumab) with double platinum chemotherapy, with practice-changing results. However, despite the high tumor mutational load and the chronic pro-inflammatory state induced by prolonged exposure to cigarette smoke, the benefit observed with immunotherapy is very modest and most patients experience disease recurrence. Unfortunately, biological, clinical, or molecular factors that can predict this risk have not yet been identified. Thanks to these clinically meaningful steps forward, SCLC is no longer considered an "orphan" disease. Innovative treatment strategies and combinations are currently under investigation to further improve the expected prognosis of patients with SCLC. Following the recent therapeutic innovations, we have reviewed the available literature data about SCLC management, with a focus on current unmet needs and potential predictive factors. In detail, the role of radiotherapy; fragile populations, such as elderly or low-performance status patients (ECOG PS 2), usually excluded from randomized studies; predictive factors of response useful to optimize and guide therapeutic choices; and new molecular targets and future combinations have been explored and revised.

Keywords: small cell lung cancer (SCLC), immune checkpoint inhibitors, immunotherapy, fragile patients, predictive factor

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1 INTRODUCTION AND THE STATE OF THE ART

Small cell lung cancer (SCLC) accounts for about 13%–15% of all new lung cancer diagnoses. About 70% of SCLC are diagnosed at an advanced stage (1). Platinum-based chemotherapy is the standard of care for both limited disease (LD) and extensive disease (ED). Although this treatment favors survival and disease control, most patients relapse, and overall survival (OS) reaches a maximum of 2 years in 21% and 7% of LD and ED, respectively (2). However, the advent of ICIs (immune checkpoint inhibitors), including PD-1 (inhibitors of programmed cell death protein 1) and PD-L1 (programmed death-ligand 1), in the therapeutic landscape of this aggressive tumor started to change the outcome of patients with ED-SCLC.

The following review reports the state of the art, as well as recent data with immunotherapy in SCLC treatment, with a focus on unmet needs and potential predictive factors.

2 THE ROLE OF ICIS IN SCLC TREATMENT

2.1 Biological Rationale

It has been hypothesized that genomic instability due to the expression of two defective tumor suppressor genes (*TP53* and *RB1*), thus perpetuating the generation of tumor-associated antigens (3) and the long-term exposure to smoke, thus inducing smoking signatures (4), makes SCLC one of the tumors with the highest tumor mutational burden (TMB) but low immunogenicity (SCLC has low MHC I expression levels, and its mutation products is difficult to be recognized by CD8 T-cell receptor). Furthermore, chemotherapy may induce immunogenic cell death that results in prompt release of pro-inflammatory cytokines and tumor antigens in the tumor microenvironment (TME), thus enhancing tumor immunogenicity (5). Although SCLC appears morphologically homogeneous, the latest data from murine models and human tumors indicate the existence of SCLC subtypes, classified on differential expression of these transcription factors: ASCL1, NEUROD1, POU2F3, or YAP1 with different therapeutic vulnerabilities (6). Among them, the SCLC-inflamed tumor, characterized by overexpression of immune genes such as those of the STING pathway, showed better survival with chemoimmunotherapy than other subtypes (7). However, although a phenotype characterized by high immune cell infiltration in TME showed a prognostic value in SCLC (8), it was not associated with other well-known candidate immune-biomarkers such as PD-L1 or TMB or with tumor response in patients treated with immunotherapy (9). Thus, by trying to understand the immune microenvironment, we get to know better the immunobiology of SCLC. Identifying the predictive biomarkers of response to immunotherapy in patients with SCLC and determining the strategies to overcome resistance to ICIs are future challenges.

2.2 Update on Treatment Options for Limited-Stage Disease

Limited-stage SCLC (LS-SCLC), meaning a tumor limited in one hemithorax and feasible radiation field, accounts for about 40% of SCLC (<5% SCLC in early stages). The role of surgery is still controversial even in early-stage SCLC, where surgery may be considered within a multimodal approach in very selected patients (10). As reported in a Cochrane systematic review published in 2017, the randomized controlled trials (RCTs) did not demonstrate a clear benefit from surgical resection in SCLC stage I–III (11). Although multiple retrospective and observational studies demonstrated the advantage of surgery for local control in the early stage I–IIA of the disease (12), the indication of surgery plus chemotherapy remains controversial. Therefore, according to the ESMO guidelines, surgery should be taken as a treatment option in patients with clinical stages I and II (cT1-2N0) and in those suspected cases with mixed SCLC histology and non-small cell lung cancer (NSCLC) (10). Otherwise, the current standard of care in SCLC of limited stage (stage I–III) consists of thoracic radiation (45 Gy in 30 fractions twice daily) plus platinum-etoposide (PE) chemotherapy (13). The advantage of this treatment is that it can be applied at full dose in patients during treatment with concomitant chemoradiotherapy (CRT) with a favorable toxicity profile. If patients are not suitable for cisplatin, carboplatin-etoposide is another treatment choice (14).

An increasing median OS was observed across CRT trials in LD-SCLC, due to technological advances and dose fractionation of radiotherapy (13). More recent trials exploring higher radiation dose schemes report even better survival outcomes, with a median OS of 37–39 months (15, 16).

However, a 70% risk of recurrence at 5 years was reported in the best-case scenarios, and maintenance or consolidation therapy strategies did not achieve a significant survival benefit (17).

Although studies are still ongoing, beneficial effects are speculated from the introduction of immunotherapy plus chemoradiation in both therapy choices, either concurrent or consolidative. The advantage of combining immunotherapy with CRT has been shown in different preclinical studies; radiotherapy used for the treatment of a primary tumor may cause the release of tumor antigens followed by a tumor-specific immune response, which is intensified by immune-stimulating elements (18). According to the abscopal effect, while radiotherapy causes a local tumor response at a targeted site, it may also cause a tumor response in non-targeted sites (metastatic disease).

We have analyzed four randomized trials, studying the concomitant therapy: immunotherapy plus CRT in LS-SCLC (**Table 1**). STIMULI (NCT02046733) is a phase II trial that studied the efficacy and tolerability of consolidation of nivolumab and ipilimumab for four cycles followed by nivolumab for 1 year versus observation after chemoradiation therapy and prophylactic cranial irradiation (PCI) in LS-SCLC (19). The statistical analysis plan considered PFS as the only primary endpoint. In total, 153 patients were randomized, and

TABLE 1 | Selected randomized clinical trial testing immunotherapy in SCLC limited disease.

Trial	Ph	Setting	Study Arm(s): E) Experimental; C) Control	*N*	Primary End-point (s)	Main Results/Status	Start Date–Estimated completion rate
STIMULI (NCT02046733)	II	Maintenance after CRT	E) nivolumab + ipilimumab C) observation	E) 78 C) 75	PFS, OS	mPFS: 10.7 vs. 14.5 [HR = 1.02 (0.66-1.58), 2-sided p = 0.93]; mOS: NR vs. 32.1 [HR = 0.95 (0.59-1.52), p = 0.82]	July 28, 2014–January 2022 (completed early in 2019)
ADRIATIC (NCT03703297)	III	Maintenance after CRT	E) durvalumab +/- tremelimumab C) placebo	724	PFS, OS	Ongoing	September 27, 2018–May 10, 2024
LU-005 (NCT03811002)	II/III	Concurrent with CRT	E) CRT + atezolizumab C) CRT	506	PFS, OS	Ongoing	May 28, 2019–December 28, 2026
ACHILES (NCT03540420)	II	Maintenance after CRT	E) atezolizumab C) observation	212	2-year survival	Ongoing	July 31 2018–December 2026
NCT04189094	II	Induction and maintenance after CRT	E) sintilimab + PE → CRT → sintilimab C) PE → CRT	140	PFS	Ongoing	January 1, 2020–July 1, 2023
NCT04308785	II	Maintenance after CRT	E) atezolizumab + tiragolumab C) atezolizumab + placebo	150	PFS	Ongoing	December 1, 2021–February 15, 2025
NCT04952597	II	Concurrent and maintenance after CRT	E) CRT + ociperlimab + tislelizumab → ociperlimab + tislelizumab E) CRT + tislelizumab → tislelizumab C) CRT	120	PFS	Ongoing	July 15, 2021–March 30, 2024

PE, platinum-etoposide; CRT, concomitant chemoradiotherapy.

after a follow-up of 22.4 months, the trial confirmed that there were no benefits in PFS or OS with the addition of nivolumab and ipilimumab (19). Furthermore, 50% of patients included in the experimental arm were unable to receive the full course of immunotherapy due to its toxicity. However, it was outlined how biobanking will be used to investigate hematological profiles and other biomarkers to define a group of patients that may benefit from the addition of immunotherapy to standard CRT. This study began in July 2014 but was terminated early in 2019 due to slow accrual. ADRIATIC (NCT03703297) is a phase III study that evaluates the efficacy of durvalumab or durvalumab plus tremelimumab compared to placebo for consolidation in patients with LS-SCLC who have not progressed after concomitant CRT (20). PFS and OS are the primary endpoints. The study started in September 2018 and will end in May 2024. LU-005 (NCT03811002) is a phase II/III trial that studies CRT compared to atezolizumab plus CRT (21). PFS is the primary endpoint of phase II and OS is the primary endpoint of phase III. Atezolizumab is administered every 3 weeks in association with radiotherapy up to 12 months in total. The stratification variables are performance status (PS 0/1 vs. 2), sex, use of chemotherapy (cisplatin vs. carboplatin), and radiation fractionation (twice daily at 45 Gy vs. once daily at 66 Gy). PCI (25 Gy in 10 fractions) is recommended in patients with a complete or almost complete response to therapy. This study opened to accrual in May 2019 and will end in December 2026. Moreover, ACHILES (NCT03540420), a phase II randomized trial comparing atezolizumab vs. observation after concurrent CRT (primary end-point is a 2-year OS rate) (22), and the NCT04189094, a phase II trial evaluating the role of adding sintilimab, an antiPD1 antibody, to chemoradiotherapy in LD-SCLC, are still ongoing. Interestingly, current ongoing trials are evaluating the combination of anti-PD1/anti-PDL1 and anti-TIGIT. In this light, the NCT04308785 represents a phase II study concentrated on the efficacy and safety of atezolizumab associated or not with tiragolumab (anti-TIGIT) as consolidation therapy in LD-SCLC patients who have not progressed during/after CRT (23), while NCT04952597 phase II trial examines the combination of ociperlimab plus tislelizumab plus concomitant CRT.

2.3 Novel Treatment Options for Extended Disease

Although current SCLC treatment remains "one size fit all", promising results were reported in the recent phase III studies including immunotherapy, which led to regulatory drug agency approval of immuno-including regimens in the first-line setting.

2.3.1 First-Line Treatment

Before the arrival of ICIs, chemotherapy with PE was considered the frontline SoC regimen for ED-SCLC for almost 30 years (24). With this regimen, ORR reached 60%–80% but responses were transient (PFS 3–6 months) and the median OS was limited (8–10 months). Recently, three phase III trials have tested the combination of ICIs (atezolizumab, durvalumab +/- tremelimumab, and pembrolizumab) with chemotherapy as first-line setting. Overall efficacy and toxicity were comparable across the studies, whereas the percentages of included patients with brain metastases or treated with PCI were different. In general, ICI introduction in the treatment landscape of SCLC represents an important and well-accepted step forward in the therapeutic strategy of ED-SCLC (**Table 2**).

The IMpower133 trial evaluated the efficacy of adding the PD-L1 inhibitor atezolizumab to the standard carboplatin-

TABLE 2 | Selected randomized clinical trial testing immunotherapy in SCLC extended disease.

Trial	Ph	Setting	Study Arm(s)	*N*	Primary End-point (s)	Main Results	Safety(AEs Grade 34)
IMpower133 (NCT02763579)	III	1-L	E) CP/ET + atezolizumab C) CP/ET + placebo	E) 201 C) 202	OS, PFS	mOS: 12.3 vs. 10.3 [HR = 0.70 (0.54-0.91), p = 0.007] mPFS: 5.2 vs. 4.3 [HR = 0.77 (0.62-0.96), p = 0.02]	Any G3/4: 57.1% vs. 56.1%; irAE: 39.9% vs. 24.5%
CASPIAN (NCT03043872)	III	1-L	E1) PE + durvalumab E2) PE + durvalumab + tremelimumab C) PE + placebo	E1) 268 E2) 268 C) 269	OS (E1 vs. C) OS (E2 vs. C)	mOS (E1 vs. C): 12.9 vs. 10.5 [HR 0.71 (0.60-0.86), p = 0.0003] mOS (E2 vs. C): 10.4 vs. 10.5	Any G/4: 60% (E1) vs. 59% (C) irAE: 20% (E1) vs. 3% (C)
Keynote-604 (NCT03066778)	III	1-L	E) PE + pembrolizumab C) PE + placebo	E) 223 C) 222	PFS, OS	mPFS: 4.5 vs. 4.3 [HR 0.75 (0.61-0.91), p = 0.0023] mOS: 10.8 vs. 9.7 [HR 0.80 (0.64-0.98), p = 0.0164]	Any G3/4: 76.7% vs. 74.9%; irAE: 24.7% vs. 10.3%
REACTION (NCT02580994)	II	1-L*	E) PE + pembrolizumab C) PE	E) 58 C) 61	PFS	mPFS: 4.7 vs. 5.4 [HR 0.84 (0.65-1.09), p = 0.194]	Any G3/4: 41.7% vs. 34.4%
NCT02359019	II single arm	Maintenance	Pembrolizumab for 2 years	45	PFS	mPFS: 1.4 12-month PFS: 13%	The only G3 ≥5% was hyponatremia
Checkmate 451 (NCT02538666)	III	Maintenance	E1) ipilimumab + nivolumab → nivolumab E2) nivolumab C) placebo	E1) 278 E2) 279 C) 273	OS (E1 vs. C)	mOS: 9.2 vs. 9.6 [HR 0.92 (0.75-1.12), p = 0.37]	Any G3/4: 52.2% vs. 8.4%
Keynote-028 (NCT02054806) Keynote-158 (NCT02628067)	Ib II	2-L and beyond	Pembrolizumab	107	ORR	ORR: 19.3% (11.4-29.4)	Any G3/4: 9.6%
Checkmate 032 (NCT01928394)	I/II	2-L and beyond	E) nivolumab + ipilimumab → nivolumab C) nivolumab	E) 96 C) 147	ORR	ORR: 21.9% vs. 11.6%	Any G3/4: 37.5% vs. 12.9%
Checkmate 331 (NCT02481830)	III	2-L	E) nivolumab C) topotecan or amrubicin	E) 284 C) 285	OS	mOS: 7.5 vs. 8.4 [HR 0.86 (0.72-1.04), p = 0.11]	Any G3/4: 13.8% vs. 73.2%
NCT01693562	I/II	2-L and beyond	Durvalumab for 12 months	21	Safety	ORR: 9.5% mPFS: 1.5 months mOS: 4.8 months	No G3/4
BALTIC (NCT02937818)	II	2-L	Durvalumab + tremelimumab	21	ORR	ORR: 9.5%	Any G3/4: 48%

CP, carboplatin; ET, etoposide; PE, platinum-etoposide; irAEs, Immune-related adverse events.
**Patients with an objective response after two cycles of induction chemotherapy with 2 cycles.*

etoposide in 403 naive patients with ED-SCLC, considering as stratifying factors sex, ECOG PS, and the presence of brain metastases (25, 26). After four cycles of treatment, PCI was included during the atezolizumab/placebo maintenance period; meanwhile, consolidation thoracic radiation was not considered. Primary endpoints were reached, with an important reduction of 30% and 23% risk of death and progression, respectively, in patients treated with atezolizumab. Median OS was 12.3 months and 10.3 months, respectively, for experimental and placebo arm (HR: 0.70; 95% CI: 0.54–0.91, p = 0.007); PFS was 5.2 months and 4.3 months, respectively, for atezolizumab and control arm (HR: 0.77; 95% CI: 0.62–0.96, p = 0.02) (26). One-year OS rate was 51.7% and 38.2% in patients undergoing chemotherapy plus atezolizumab vs. chemotherapy alone, respectively, regardless of PD-L1 expression and blood tumor molecular burden. According to these results, the combination of carboplatin, etoposide, and atezolizumab is considered as the new standard treatment for ED-SCLC in the first-line setting.

The CASPIAN trial tested the efficacy of the PD-L1 inhibitor durvalumab +/- CTLA-4 inhibitor tremelimumab, in combination with standard PE in 805 ED-SCLC naïve patients (27, 28). The control group was represented by PE alone for up to six cycles. In this trial, PCI was allowed in the control arm following chemotherapy at the investigator's discretion, but it was not allowed in the immunotherapy groups before discontinuation of all study treatments. The co-primary

endpoint was OS for durvalumab platinum-etoposide compared to chemotherapy, and for durvalumab/tremelimumab plus platinum-etoposide compared to chemotherapy. At the updated median follow-up after >3 years, combining durvalumab with platinum-etoposide significantly improved OS over only chemotherapy (12.9 vs. 10.5 months; HR: 0.71; 95% CI: 0.60–0.86; $p = 0.0003$) (27). Although the combination of durvalumab/tremelimumab plus PE numerically improved OS vs. PE, it was not statistically significant. In consideration of these results, durvalumab plus cisplatin or carboplatin etoposide has been approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) as first-line treatment in patients with ED-SCLC.

The KEYNOTE 604 trial investigated the efficacy of the PD-1 inhibitor pembrolizumab plus PE vs. chemotherapy alone in 453 ED-SCLC naive patients (29). One of its primary endpoints showed an important PFS improvement by adding pembrolizumab to chemotherapy (HR: 0.75; 95% CI: 0.61–0.91), also a prolonged OS (10.8 vs. 9.7 months); the pre-specified significance threshold was not reached (HR: 0.80; 95% CI: 0.64–0.98; $p = 0.0164$).

More recently, the REACTION trial randomized patients, with a response after two cycles of chemotherapy, to be treated with pembrolizumab in combination with chemotherapy or chemotherapy alone (30). The primary PFS endpoint was not reached (4.7 vs. 5.4 months, HR: 0.84; 80% CI: 0.65–1.09, $p = 0.194$). However, a statistically significant OS improvement (12.3 vs. 10.4 months, HR 0.73; 80% CI: 0.54–1.00) was reported.

Overall, a grade 3 or higher toxicity rate was observed during immune-chemotherapy combinations compared to chemotherapy alone, although an expected increase in immune-related AEs was reported in the experimental arms. However, data on long-term or deterioration in quality of life are limited.

2.3.2 Maintenance Therapy With ICIs

The efficacy of ICIs as a maintenance strategy in ED-SCLC is still controversial. In a phase two study, 8 weeks after the last cycle of PE chemotherapy, pembrolizumab was started as maintenance therapy for up to 2 years (31). Although both median PFS and OS were not significantly improved by pembrolizumab, the 1-year PFS and OS rate were 13% and 37%, respectively, showing that a subgroup of patients could have a clinical benefit. However, none of the biomarkers analyzed, including PD-L1, were predictive of a better response to immunotherapy.

As expected by previous studies with anti-CTLA4 ipilimumab (32) in combination with chemotherapy in ED-SCLC, no significant benefit was reported in a phase III study of immunotherapy doublet with the anti-PD-L1, nivolumab, and the anti-CTLA4, ipilimumab, as maintenance therapy for ED-SCLC (33). A total of 834 patients enrolled in the Checkmate 451 trial did not progress after receiving four cycles of platinum-based chemotherapy. These patients were randomized to receive immunotherapy with nivolumab and ipilimumab, nivolumab alone, or placebo for 2 years. The OS did not improve with nivolumab plus ipilimumab vs. placebo (HR: 0.92; 95% CI: 0.75–1.12; $p = 0.3693$) or with nivolumab vs. placebo (HR: 0.84; 95% CI: 0.69–1.02), but still there was a modest improvement in PFS with ipilimumab plus nivolumab (HR: 0.72; 95% CI: 0.60–0.87) and nivolumab (HR: 0.67; 95% CI: 0.56–0.81) compared to placebo.

Finally, the addition of an anti-PD-L1 therapy (atezolizumab or durvalumab) to the standard platinum-etoposide chemotherapy, and then keeping immunotherapy as maintenance, improved both PFS and OS (25, 28). On the other hand, the use of an anti-PD1 therapy (pembrolizumab) for the same purpose showed a similar benefit that was instead statistically significant only for PFS (29). According to previous studies in other tumors (32), although the overall benefit is only about 2 months in the extension of median survival, there are potential advantages for long-term survivors, looking at the tail of survival curves. In fact, the 2-year survival rate increased from 11% to 22%, suggesting that some patients with SCLC have a significant benefit with immunotherapy, but useful biomarkers for their *a priori* identification are still lacking.

2.3.3 Second-Line Treatment and Beyond

Unfortunately, most patients relapse within 6 months after first-line chemotherapy. The second-line treatment response rates depend on the treatment-free interval (TFI) and are approximately 20%–30% in platinum-sensitive patients (TFI ≥3 months) and 15% in platinum-resistant patients (TFI <3 months). According to clinical guidelines, the two possible second-line options for patients with ED-SCLC who progressed after platinum-based first-line chemotherapy are the topoisomerase I inhibitor topotecan and anthracycline-based regimes, including cyclophosphamide plus doxorubicin and vincristine (CAV) (10). The latest option was commonly used before a randomized trial with topotecan vs. CAV, which showed similar outcomes in both treatment arms, but intravenous topotecan showed better tolerability (34), resulting in the preferred standard of care nowadays. However, in platinum-sensitive patients, a rechallenge with PE should be also considered as a reasonable second-line option. In fact, a phase III trial recently showed that carboplatin plus etoposide had a significant improvement in PFS compared to topotecan (4.7 vs. 2.7 months, HR: 0.57; 95% CI 0.41–0.73; $p = 0.0041$), with a similar safety profile (35). In the ESMO therapeutic algorithm, lurbinectidin (selective inhibitor of RNA polymerase II) was also introduced as an alternative option for recurrent SCLC (10). This drug was recently approved by the FDA, according to the results of a phase II single-arm trial (NCT02454972), in which the single-agent lurbinectedin showed significant activity as second-line therapy. Overall, patients reported an ORR equal to 35.2% (22.2% in platinum-resistant and 45% in platinum-sensitive patients), a median duration of response up to 5.3 months (36), and a median OS of 9.3 months, with a manageable safety profile (37). Meanwhile, the combination of lurbinectedin plus doxorubicin explored in the phase III trial ATLANTIS (NCT02566993) vs. investigator's treatment choice (topotecan or CAV: cyclophosphamide, doxorubicin, vincristine) did not improve the prespecified endpoint of OS (38).

Promising preliminary antitumor activity and a good safety profile were shown with ICIs in patients progressed after standard first-line chemotherapy. The administration of pembrolizumab 200 mg every 3 weeks, as the standard dose,

was tested in a phase Ib (Keynote-028) and a phase II (Keynote-158) trial in different tumor types, including SCLC. Overall, an ORR (primary endpoint) of 19.3% was reported regardless of PD-L1 expression. On this basis, in June 2019, the FDA accelerated the approval of pembrolizumab for the treatment of metastatic SCLC patients with disease progression after receiving platinum-based chemotherapy and at least one other prior line of therapy.

The use of nivolumab in SCLC pretreated patients has been evaluated in the phase I/II Checkmate 032 (39) and in the phase III Checkmate 331 (40) clinical trials. The first one is a basket trial that studied the activity of nivolumab alone and nivolumab plus ipilimumab in different tumors including metastatic SCLC. Overall, an objective response of 10% was observed in patients treated with nivolumab alone, 23% in those treated with nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, and 19% in those treated with nivolumab 3 mg/kg plus ipilimumab 1 mg/kg. Like pembrolizumab studies, the response rate was not related to PD-L1 status. Further analysis after 18 months of follow-up showed an ORR of 11% in patients treated with nivolumab alone and of 25% in patients treated with nivolumab plus ipilimumab (41). These early results, in August 2018, led the FDA to accelerate the approval of nivolumab for pretreated SCLC patients. Recently, results of the expansion cohort of patients randomized to nivolumab vs. nivolumab plus ipilimumab were published, reporting an ORR of 11.6% in the group with nivolumab alone, and 21.9% in the combination group that experienced more frequent G3–G4 adverse events (12.9% and 37.5% in the nivolumab and nivolumab + ipilimumab group, respectively) and four deaths due to toxicity (autoimmune-related hepatitis, pneumonitis and encephalitis, and autoimmune colitis).

The second study, Checkmate 331, compared nivolumab vs. chemotherapy with topotecan or amrubicin as second-line treatment (42). Patients were grouped as platinum responders and non-responders. Although this trial did not reach its primary endpoint [median OS was 7.5 vs. 8.4 months in the nivolumab and chemotherapy arm (HR: 0.86; CI: 95%: 0.72–1.04)], the HR for OS in patients who did not respond to cisplatin was 0.71 (95% CI: 0.54–0.94). Additionally, the nivolumab group reported 55% of all grade AE vs. 90% in the chemotherapy arm (40).

Durvalumab was reported to have similar results, received every 2 weeks at a dose of 10 mg/kg, in a phase I/II study that included 21 patients with pretreated ES-SCLC disease (43). Patients were treated for up to 1 year and reported a median OS of 4.8 months, PFS of 1.5 months, and a 1-year OS rate of 27.6%. In addition, an ORR of 9.5% was recently observed with durvalumab + tremelimumab in preliminary analysis of the phase II BALTIC study (NCT02937818) (44).

The anti-PD-L1 atezolizumab as single therapy did not show significant results in pretreated patients vs. topotecan (up to six cycles) or re-induction chemotherapy in the randomized phase II IFCT-1603 study, which included 73 patients with ES-SCLC disease after failure of first-line PE-basing chemotherapy (45).

Overall, the potential use of ICIs in the second-line setting still requires further evidence. Furthermore, considering that immunotherapy is currently included in the first-line standard of care approach, it must be taken into consideration the lack of data on the role of ICIs rechallenge in patients whose disease has progressed after first-line immune-based treatment.

3 OPEN ISSUES

3.1 Radiotherapy: The Role of Consolidation Treatment and Prophylactic Cranial Irradiation

Thoracic radiotherapy (TRT) combined with chemotherapy is the standard treatment in patients with limited disease. In ED-SCLC, the importance of consolidation of TRT in patients with a good response to first-line treatment has become increasingly recognized (46). The ASTRO guidelines conditionally recommend thoracic radiotherapy to 30 Gy in 10 fractions within 6 to 8 weeks of chemotherapy completion and before maintenance immunotherapy, in patients with ED-SCLC who respond to chemotherapy and immunotherapy, and in case of residual disease in the thorax. At the ASCO 2021, SBRT was suggested to be applied more frequently in early-stage SCLC patients not eligible for resection, or who refuse surgery. Additionally, retrospective data suggest that this strategy is likely safe and effective. Therefore, ASTRO guidelines have recently incorporated SBRT as an acceptable treatment option for early-stage, node-negative, and medically inoperable SCLC.

Prophylactic cranial irradiation (PCI) is still controversial, following the publication of a Japanese randomized phase III trial that found that magnetic resonance imaging (MRI) surveillance could replace PCI for extensive-stage disease (47). In this trial, patients with ED-SCLC who responded to platinum-based doublet chemotherapy and with no brain metastases on MRI were randomly assigned (1:1) to receive PCI (25 Gy in 10 daily fractions of 2.5 Gy) or observation. The primary endpoint was OS. All patients underwent brain MRI every 3 months in a 12-month period followed by another brain MRI at 18 and 24 months after enrolment. The study showed that there was no improvement in OS with PCI therapy compared to observation (11.6 vs. 13.7 months, HR = 1.27, 95% CI 0.96–1.68, $p = 0.094$), concluding that PCI is not essential for ED-SCLC responders to initial chemotherapy, without evidence of brain metastases (48).

However, due to the incidence of brain metastases at diagnosis (about 18% of cases of ED-SCLC, which increase to 80% at 2 years), PCI is still recommended in patients who respond to treatment in both LD and ED-SCLC (49). However, active surveillance with a brain MRI every 12 weeks seems to be an acceptable option, especially to preserve patients' quality of life (48).

Although consistent data are not available for SCLC in the immunotherapy era, the safety and efficacy data obtained in NSCLC about the integration of ICIs and radiotherapy may support the feasibility of this approach. In this light, it will be crucial to better define the potential (positive and negative) synergy between local and systemic therapy in both LD and ED-SCLC, similar to what is recognized in stage III NSCLC and

in the oligometastatic/oligoprogressive setting. The patient's condition, stage, and characteristics of the disease, response to therapy, dosage and schedule of TRT/PCI, as well as the future availability of new drugs or combinations may influence the decision-making process in SCLC. In conclusion, the integration of thoracic radiotherapy in patients with ED-SCLC who after chemotherapy have persistent intrathoracic disease, as well as the role of PCI and immunotherapy in the metastatic setting, remains an important unanswered question, prioritizing the need for *ad hoc* trials.

3.2 Frail Population

Although etoposide plus carboplatin was accepted as a tolerable and equivalent regimen in terms of efficacy compared to etoposide and cisplatin, a review of the Alberta Cancer Registry showed that 32% of elderly patients (age 75+) were not treated with chemotherapy (50). Moreover, the randomized phase III trials with ICIs enrolled patients with a median age ≤70 years; they included only patients with good performance status (PS, 0-1) (23, 25, 27). In contrast, in real life, there are more and more cases of elderly patients with median age ≥70 years (51). Overall, 52% of the patients treated with chemotherapy completed all cycles and 34% of them underwent at least one dose reduction. Patients who completed all cycles with a dose reduction had a lower risk of death of 1.02 (95% CI: 0.57–1.82) compared to a risk of death of 2.72 (95% CI: 1.52–4.87) for patients who did not complete therapy. Furthermore, phase II studies aimed to point out that carboplatin and etoposide dose modifications in the elderly reported similar survival benefits *versus* standard doses (52). Therefore, elderly patients should receive standard treatment, but they may also require dose modifications.

Recently, NSCLC studies showed that immunosenescence, defined as the gradual deterioration of the immune system caused by natural advances in age, seems to be related to decreased efficacy of ICIs, regardless of the age (53). However, survival data from phase III clinical trials with ICIs are controversial in SCLC elderly population. In the KEYNOTE 604, similar magnitudes of survival benefit were reported with the use of pembrolizumab despite the patient's age (29). In contrast, in the CASPIAN trial, durvalumab was significantly effective in patients aged <65 years [HR 0.72, (95% CI: 0.56–0.92)] but not in patients aged ≥65 years [HR 0.84 (95% CI: 0.62-1.12)] (27), whereas the anti-PD-L1, atezolizumab, in the IMpower133 trial seemed to be more effective in patients aged ≥65 years [HR 0.53 (95% CI: 0.36–0.77)] than patients aged <65 years [HR 0.92 (95%: 0.64–1.32)]

In real-world data, up to 60% of elderly patients have a PS equal to 2, resulting in worse survival (54). Furthermore, they were generally also affected by at least two chronic comorbidities, followed by a higher probability of exposure to polypharmacy, which can affect the efficacy of ICIs (55). In this scenario, the REACTION trial hypothesized an interesting strategy. In this phase II trial, there were randomized patients with complete or partial response after two cycles of PE induction. In this study, 5% of the patients enrolled had an ECOG PS 2, but those patients who upgraded to PS 1 or 0 with treatment benefit were eligible for the immune-chemotherapy strategy (30). Finally, a large sample of PS 2 patients will be enrolled in the ongoing phase II SPACE trial (NCT04221529) (56) and in the phase III MAURIS trial (NCT04028050) (23), which may help to clarify whether these patients benefit or not from the addition of ICI to chemotherapy.

In terms of brain radiotherapy, the role of PCI in elderly patients is controversial. Indeed, even if PCI improved the OS in patients aged ≥70 years, it was not significantly effective among patients aged ≥80 years with SCLC (57). This finding suggests that in this group of patients, a shared decision process is necessary rather than proposing an overtreatment. Less is known in patients with ECOG-PS = 2 and those with a history of neurological conditions, such as stroke or epilepsy. Retrospective analyses have shown that PCI improves survival compared to no PCI, but its correlation with increased neurocognitive dysfunction has limited its use (57–59). In fact, a comparison of the results of cognitive tests in two RTOG trials that evaluated PCI in patients with LS-SCLC showed higher rates of cognitive decline with advanced age (60). Modern radiation techniques, hippocampal sparing, and memantine may minimize the occurrence of cognitive decline.

Geriatric Assessment Tools Geriatric oncology addresses the right approach to the care of this category of patients through the development of geriatric assessment tools to help define risks and benefits. Investigators and treating physicians are encouraged to include these tools in their clinical trials and daily practice (46). In general, clinical trials and trials that address the unique needs of the elderly are strongly recommended.

3.3 Potential Predictive Factors

SCLC is a highly aggressive tumor with still very poor prognosis marked by a very high proliferative rate and an early spread of metastasis. Moreover, despite the promising results, the magnitude of benefit with ICIs in SCLC is different from what was reported in NSCLC. Although SCLC has a high TMB, its immunosuppressive pattern in the stroma, the lack of antigen presentation, and the low expression of PD-L1 suggest a less immunogenic T-cell profile in SCLC compared to NSCLC (36, 39, 61). Similarly, a multiplexed quantitative immunofluorescence analysis in SCLC samples showed significantly lower levels of all TIL markers, MHC class II expression, and CD8+ T cells compared to NSCLC (62). However, high immune activity was reported in patients with SCLC and paraneoplastic syndromes, resulting in a better prognosis compared to patients without these syndromes (63).

Moreover, a clear therapeutic algorithm and consolidated data in special populations, like the elderly or patients with an ECOG PS ≥ 2, are still unavailable. Therefore, the identification of potential predictive factors of response to better guide the physician's choice is awaited.

3.3.1 Molecular Factors

3.3.1.1 Gene Expression Profile

The genomic profile of SCLC shows extensive chromosomal rearrangements and a high TMB. Moreover, the dual inactivation of the tumor suppressors *TP53* and *RB1* is found in most cases with SCLC (3). Sequencing analysis on both DNA

and RNA of larger cohorts of primary tumors as well as CTC-derived xenograft models confirmed this result. Furthermore, the amplification of genes from the *MYC* family (*MYC*, *MYCL*, and *MYCN*), *FGFR1* (encoding fibroblast growth factor receptor 1), and *GNAS* (encoding the α-subunit of the heterotrimeric G protein Gs) was also well described (64). Moreover, alterations in the PTEN pathway and overexpression of BCL-2 could interfere with the promotion of cell growth, proliferation, and survival in SCLC. Relapsed tumors are more frequently characterized by WNT pathway alterations, thus supposing a role for WNT signaling in chemo-resistant SCLC (65), and the heterogeneity of SCLC tumors may explain an important mechanism by which SCLC tumors evade treatment; additionally, heterogeneity itself is increased in response to treatment (66). The lineage plasticity of SCLC cells could be explained by the high levels of the stem cell transcription factor SOX2 downstream of *p53* and *RB* loss, or as a consequence of genomic amplification. Moreover, mutations in chromatin modifiers are frequent in SCLC, suggesting that alterations in epigenetic regulation may also contribute to cell fate changes. However, better understanding the TME and the molecular mechanisms underlying SCLC tumorigenesis, progression, metastasis, and response to treatment is still a challenge. Recently, some researchers have developed the first comprehensive framework to classify SCLC into four subtypes based on gene expression (6). This classification depends on the relative expression of dominant transcriptional regulators and on the substantial intra-tumoral heterogeneity that could explain the main aspects of tumor evolution, metastasis, and acquired therapeutic resistance, as well as potential targeted therapeutic strategies (64).

The first three groups are characterized by activation of the *ASCL1* (SCLC-A), *NEUROD1* (SCLC-N), and *POU2F3* (SCLC-P) genes, while the SCLC-I subtype is characterized by an inflamed gene signature with high expression of multiple immune genes, including significantly higher levels of genes indicating the presence of CD8-positive cytotoxic T cells (7). The research team first identified the four groups by applying non-negative matrix factorization to 81 SCLC patients with surgically resected tumors. The data from 276 SCLC patients enrolled in the phase III IMpower133 clinical trial were then analyzed to validate the four subtypes in the advanced stage. This study showed that SCLC-I was the most sensitive to immune checkpoint blockade, SCLC-A was the most sensitive to BCL2 inhibitors, SCLC-N was the most sensitive to Aurora kinase inhibitors (overall, more effective in those SCLC with increased MYCL expression) (67), and SCLC-P was the most sensitive to PARP inhibitors, thus suggesting different classes of drugs for different specific subtypes. This study described the subtype "*switching*" to resistance in a series of patient-derived SCLC models. Data from a mouse model also suggest that SCLC-A tends to switch to SCLC-I after being treated with chemotherapy, which could be correlated with resistance to treatment (7). Since SCLC is about 15 years behind NSCLC, in terms of developments in the field of biomarkers and personalized therapies, this emerging molecular classification represents the first step in a better understanding of the molecular pathway involved in SCLC and the choice of the best drugs for each patient, thus moving towards personalized approaches for the cure of the rare and aggressive SCLC tumor.

3.3.1.2 Liquid Biopsy

A pressing issue in the SCLC field has been the small quantity of material to be used for histological diagnosis and subsequent research. Therefore, isolating circulating tumor cells (CTCs) from the blood of SCLC patients could overcome this problem (68). However, we are still far from adequate clinical trials that concentrate on tumor material collection to identify key genetic drivers of SCLC, and while liquid biopsies may represent an important factor for exploring ICI-resistance mechanisms in SCLC, this technique itself needs more evaluation.

3.3.2 Immunological Factors

Exploratory biomarker analysis of principal phase III clinical trials showed that PD-L1 expression is not correlated to immunotherapy benefit in SCLC patients. The importance of TMB is more controversial, which seems to be predictive of nivolumab-ipilimumab benefit as the Checkmate-032 analysis suggests (69) but not predictive of atezolizumab benefit in the IMPOWER133 blood-based analysis (25). Similarly, in the CASPIAN trial, durvalumab plus chemotherapy resulted in improved OS compared to chemotherapy alone regardless of PD-L1 and TMB expression (28). Also, in the KEYNOTE 604 trial, both PFS and OS improved with the addition of pembrolizumab to chemotherapy, regardless of the combined positive PD-L1 expression score (29). Therefore, we cannot consider PD-L1 or TMB to be good predictive factors of response to immunotherapy in SCLC, at least so far.

3.3.3 Clinical Factors

According to their different microenvironments, brain metastasis and liver metastasis deserve to be mentioned as potential predictive factors of response to immune-based chemotherapy.

Indeed, the brain metastases showed an active immune microenvironment with a PD-L1 expression of 75% in SCLC samples. However, the percentage of patients with baseline brain metastases included in phase II/III clinical trials ranged from 9% to 14.2% in the immunochemotherapy arms (25, 27, 29, 30). Moreover, all trials, except the CASPIAN trial, included only asymptomatic and treated brain metastases. Thus, the limited sample size and the limited benefit in survival by adding immunotherapy to chemotherapy do not allow conclusive results. The presence of liver metastases should be considered a negative predictive factor. In particular, in the three phase III trials, anti-PD-L1 addiction to chemotherapy did not improve survival results compared to chemotherapy alone. Accordingly, in NSCLC, the occurrence of liver metastases was associated with an immune-suppressive phenotype characterized by fewer infiltrating CD8+ T-cell densities at the invasive margin in distant tumors (66) and limited immunotherapy efficacy by macrophage-mediated elimination of T cells (70). These data support the hypothesis that there is a lack of a synergistic effect of immunochemotherapy in SCLC patients affected by liver metastases.

4 NEW TARGETS AND FUTURE PERSPECTIVES

Despite the high potential immunogenicity of SCLC, the magnitude of benefit with ICIs in SCLC is not the same as that reported in NSCLC patients. Different immunophenotypes, as well as the TMEs of SCLC compared with NSCLC, may explain the different efficacy of ICIs in these two diseases (61).

Recently, other immunotherapeutic approaches, used alone or in combination with ICIs, are being explored to improve the immune response in SCLC patients. These include chimeric antigen receptor (CAR) T-cell therapy, bispecific T-cell engagers (BiTEs), antibody–drug conjugates (ADC), and immunomodulators. Multiple cell surface molecules, including CD56, CD47, and delta-like ligand 3 (DLL3), have an important expression in SCLC, thus emerging as potential therapeutic targets of CART therapy (71–73) (**Table 3**).

T cell-based therapy is an MHC-independent therapeutic option, where chimeric antigen receptors are recombinant receptors for tumor-specific antigens, engineered into T cells to allow expression, expansion, and antitumor specificity (74).

AMG 119, a DLL3-directed CART cell therapy, showed a potent antitumor response in preclinical models (75) and is being studied in an ongoing phase I trial that includes patients with advanced SCLC in progression after receiving at least one platinum-based regimen (NCT03392064). Unlike CART, BiTEs are recombinant bispecific proteins that simultaneously target a T-cell surface molecule (such as CD3) and a tumor-specific surface antigen, facilitating both T-cell adherence and antitumor response independent of MHC (76).

Preclinical studies showed that the DLL3-targeted BITEs AMG 757 demonstrated a potent and specific killing activity in SCLC cell lines as well as orthotopic and patient-derived xenograft (PDX) mouse models with DLL3 expression, by inducing T-cell activation and its redirection against tumor cells (77). AMG 757 is currently being evaluated alone or in combination with pembrolizumab in a phase I trial (NCT03319940) (78). In the updated analysis of 10 cohorts including 64 patients, AMG757 at doses up to 100 mg reported promising results in terms of response rate (43% of the disease control rate, with 13% of PR) and median response duration (6.2 months), with a relative safety profile (grade ≥3 and 4); treatment-related adverse events (AEs) occurred in 25% and 6% of cases, respectively. Cytokine release syndrome occurred in 42% of patients, mainly as mild grade toxicity (79).

Rovalpituzumab tesirine (Rova-T), a DLL3-targeted ADC, has been largely investigated in different settings of SCLC, first of all in the third-line (phase II single-arm TRINITY) (80), then in the second-line (phase III TAHOE) (81), and later as first-line maintenance therapy after platinum-based chemotherapy (phase III MERU) (82). Unfortunately, it does not show the expected activity, thus failing to improve the landscape of SCLC treatment.

Vaccines, such as fucosyl GM-1, GD3 ganglioside, polysialic acid, and dendritic cell-based p53, are also a potentially promising strategy in the management of SCLC but remaining under investigation (83).

Lefitolimod, a toll-like receptor (TLR) 9 agonist, is an immunomodulator drug studied as maintenance therapy after first-line chemotherapy in the phase II trial IMPULSE (84). Although this trial did not demonstrate an OS benefit in the intention-to-treat population, a subgroup analysis of patients with a low frequency of activated CD86+ B cells resulted in a potential OS benefit.

Promising activity in SCLC is being shown with the combination of ICIs and anti-LAG-3 (78) as well as with anti-TIM-3 agents (85), which are both correlated with the development of resistance to PD-1 blockade (80). Similarly, SKYSCRAPER-02 (NCT04256421) is a phase III randomized, double-blind, placebo-controlled trial investigating the addition of another ICI, tiragolumab (anti-TIGIT agent), to first-line atezolizumab, carboplatin, plus etoposide in patients with ES-SCLC.

Given the high expression levels of DNA damage response (DDR) proteins, such as PARP, ATR, CHK1, and WEE1 in SCLC, many DDR pathway inhibitors are under development.

Indeed, combining ICIs with small molecules, such as cyclin-dependent kinases (CDK) 4/6 inhibitors and poly(ADP-ribose) polymerase (PARP) inhibitors, is an emerging strategy. Trilaciclib, a CDK4/6 inhibitor, is being evaluated within the first-line atezolizumab, carboplatin, and etoposide in a phase II placebo-controlled trial (NCT03041311).

The PARP inhibitor, olaparib, is under investigation in phase II trials, in combination with durvalumab for relapsed SCLC. Although the first phase II study did not meet its primary endpoint (86), this combination of ICI and PARP inhibition is currently being explored (87). Furthermore, the phase III MK 7339-013/KEYLYNK-013 (NCT04624204) is currently ongoing to evaluate the combination of pembrolizumab with concurrent CRT followed by pembrolizumab with or without olaparib in LD-SCLC.

Finally, preliminary results with other targets such as Aurora A kinase inhibitor, CDK7 inhibitors, and epigenetic inhibitors showed modest further benefit in preclinical and clinical models.

The multikinase antiangiogenic anlotinib was also tested in pretreated SCLC and showed a slightly better response compared to placebo (ORR 4.9% vs. 2.6%; DCR 71.6% vs. 13.2%) (88). A higher percentage of responses was reported with the combination of the anti-VEGFR2 apatinib and camrelizumab in the phase II trial PASSION, including both chemosensitive and chemoresistant ED-SCLC (89).

5 CONCLUSIONS

SCLC is still considered the most aggressive form of lung cancer. However, the advent of immunotherapy has changed the treatment paradigm as well as the outcome of a subgroup of patients affected with extensive SCLC. In this scenario, many open issues remain. Despite the benefit from the combination of ICIs and chemotherapy reported in the recent studies, a significant percentage of patients shows disease progression within 2 years. Moreover, some categories of patients like the elderly or those with an ECOG PS of 2, largely represented in

TABLE 3 | Selected clinical studies including novel drugs/novel combinations in SCLC.

Trial	Ph	Setting	Type of approach	Study Arm(s): E) Experimental; C) Control	Primary End-point(s)	Main Results/Status	Start Date–Estimated completion rate
NCT03392064	I	Relapse/ Refractory SCLC	DLL3-directed CART cell therapy (AMG 119)	Single arm	DLTs	Suspended*	September 10, 2018–January 13, 2026
NCT03319940	I	Relapse/ Refractory SCLC	DLL3-targeted BITEs (AMG 757)	Arm A) AMG 757 Arm C) AMG 757 with Pembrolizumab Arm D) AMG 757 with additional CRS mitigation strategies Arm E-F-G) AMG 757 with different timing of administration/schedules	DLTs	Recruiting [Results from updated analysis: DCR: 43% mDOR: 6.2 mo TRAEs G3: 25% TRAEs G4: 6%]	December 26, 2017–September 12, 2024
TRINITY (NCT02674568)	II	Relapse/ Refractory SCLC [third line or later]	DLL3-targeted ADC (Rovalpituzumab Tesirine)	Single arm	ORR, OS	ORR: 12.4% (all population) 14.3% (DLL3-high population); mOS: 5.6 mo (all population) 5.7 mo (DLL3-high population); AEs G3-5: 63% [fatigue, photosensitivity reaction, pleural effusion]	January 25, 2016–October 19, 2018

TAHOE (NCT03061812)	III	Relapse/ Refractory SCLC [second line; high DLL3 expression]	DLL3-targeted ADC (Rovalpituzumab Tesirine)	E) Rovalpituzumab tesirine
				C) Topotecan
					OS	mOS: 6.3 mo vs. 8.6 mo [HR = 1.46 (1.17-1.82), $p = 0.0051$];
						mPFS: 3.0 mo vs. 4.3 mo [HR = 1.51 (1.22-1.87)];
						ORR: 15% vs. 21%;
						AEs G3-5: 56% [malignant neoplasm progression, pleural effusion, peripheral edema] vs. 57%
							April 11, 2017–February 12, 2020

MERU (NCT03033511)	III	Maintenance therapy after first-line platinum-based CT	DLL3-targeted ADC (Rovalpituzumab Tesirine)	E) Rovalpituzumab tesirine C) Placebo	OS in DLL3 high population, PFS by CRAC	mOS: 8.5 mo vs. 9.8 mo [HR = 1.07 (0.84-1.36), $p = 0.537$]; PFS evaluation by CRAC not concluded due to lack of OS benefit; AEs G3-5: 59% [pleural effusion, fatigue, peripheral edema] vs. 30%	February 7, 2017–November 20, 2019
IMPULSE (NCT02200081)	II	Maintenance therapy after first line platinum-based CT	TLR 9 agonist (Lefitolimod/MGN1703)	E) Lefitolimod/MGN1703 C) Control	OS	mOS: 279 vs. 272 days [HR = 1.14 (0.73-1.76), $p = 0.98$]**; mPFS: 90 vs. 111 days [HR not determined, $p = 0.52$]	March 2014–October 5, 2017
SKYSCRAPER-02 (NCT04256421)	III	First-line ED-SCLC	Anti-TIGIT (Tiragolumab) plus anti-PDL1 agent (Atezolizumab)	E) Tiragolumab + Atezolizumab + PE C) Placebo + Atezolizumab + PE	PFS, OS	Active, not recruiting	February 4, 2020–March 21, 2024
NCT03041311	II	First-line ED-SCLC	CDK 4/6 inhibitor (Trilaciclib/G1T28) plus anti-PDL1 agent (Atezolizumab)	E) Trilaciclib + Atezolizumab + PE C) Placebo + Atezolizumab + PE	Potential to reduce CT-induced myelosuppression	Active, not recruiting	April 7, 2017–June 2021
NCT02484404	I/II	Relapse/ Refractory SCLC	PARP inhibitor (Olaparib) plus anti-PDL1 agent (Durvalumab)	Single arm	ORR	ORR: 10.4%; mPFS: 1.8 mo; mOS: 4.1 mo	June 20, 2015–January 30, 2023
NCT04728230	I/II	First-line ED-SCLC	PARP inhibitor (Olaparib) plus anti-PDL1 agent (Durvalumab)	Single arm (+ chemotherapy and radiotherapy)	DLTs	Recruiting	January 5, 2021–July 01, 2022

(Continued)

TABLE 3 | Continued

Trial	Ph	Setting	Type of approach	Study Arm(s): E) Experimental; C) Control	Primary End-point(s)	Main Results/Status	Start Date–Estimated completion rate
MK 7339-013/ KEYLYNK-013 (NCT04624204)	III	LD-SCLC	PARP inhibitor (Olaparib) plus anti-PD1 agent (Pembrolizumab)	E) Pembrolizumab + PE (4 cycles) with CRT → pembrolizumab (9 cycles) E) Pembrolizumab + PE (4 cycles) with CRT → pembrolizumab (9 cycles) + olaparib C) PE (4 cycles) with CRT → placebo	PFS, OS	Recruiting	December 8, 2020–October 28, 2027

ALTER-1202 (NCT03059797)	II	Relapse/ Refractory SCLC [third line]	Multikinase antiangiogenetic agent (Anlotinib)	E) Anlotinib
				C) Placebo
					PFS	mPFS: 4.1 mo vs. 0.7 mo [HR = 0.19 (0.12-0.32), $p < 0.0001$];
						mOS: 7.3 mo vs. 4.9 mo [HR = 0.53 (0.34-0.81), $p = 0.0029$];
						AEs G3-4: 51.9% [hypertension, hand foot syndrome] vs. 43.6%
							March 27, 2017– May 6, 2019

PASSION (NCT03417895)	II	Relapse/ Refractory SCLC [second line]	Multikinase antiangiogenetic agent (Anlotinib) plus novel antiPD1 (Camrelizumab)	Arm A) Camrelizumab + Apatinib Arm B) Camrelizumab + Apatinib (5 days on, 2 days off) Arm C) Camrelizumab + Apatinib (7 days on, 7 days off)	ORR	ORR: 34% [ORR in chemosensitive pts 37.5%; ORR in chemoresistant pts 32.3%]; mPFS: 3.6 mo; mOS: 8.4 mo; AEs 3-4: 72.9%	February 5, 2018– March 2020

SCLC, small cell lung cancer; DLL3, delta-like ligand 3; CART, chimeric antigen receptor T cells; DLTs, dose-limiting toxicities; BITEs, bispecific T-cell engagers; CRS, cytokine release syndrome; DCR, disease control rate; mDOR, median duration of response; mo, months; ADC, antibody–drug conjugates; ORR, objective response rate; mOS, median overall survival; HR, hazard ratio; AEs, adverse events; PFS, progression-free survival; CT, chemotherapy; PE, platinum-etoposide; CRT, CRT, concomitant chemoradiotherapy; CRAC, Central Radiographic Assessment Committee; TLR, toll-like receptor; PE, platinum-etoposide; CDK 4/6, cyclin-dependent kinase 4/6; PARP, poly (ADP-ribose) polymerase; RT, radiotherapy; PTS, patients.

**Study on enrolment hold, may potentially resume. No active subjects on trial.*

***Benefit in OS was seen in patients with a low frequency of activated CD86+ B cells [HR = 0.53, (0.26–1.08)] and in patients with chronic obstructive pulmonary disease (COPD) [HR = 0.48 (0.20–1.17)].*

real-world settings, were not studied enough in clinical trials. Therefore, the identification of the predictive factors of the response could be very important in achieving better patient selection in daily clinical practice. Based on recent data from gene profiling and classification in four molecular subtypes of SCLC, as well as the correlation between these molecular subtypes and response to treatment, a strong effort is currently ongoing to personalize cancer care in SCLC tumors, moving this scenario to the new concept of *one-size-does-not-fit-all*. However, we are still far from this concept, and profound knowledge of SCLC cell biology is necessary to improve the survival of these patients.

Besides ICIs combinations, several new treatment strategies, as well as novel molecules to overcome potential mechanisms of resistance, are under investigation with promising results. Thus, is it possible to talk about an effective therapeutic algorithm in SCLC treatment in the near future? Further studies with confirmatory results and a deeper understanding of SCLC biology could be the way to answer this question and expand therapeutic opportunities in this aggressive tumor.

AUTHOR CONTRIBUTIONS

LB, JM, and SP conceived the original idea of the article, drafting, and writing the paper. LC, AI, GP, ER, EV and AV revised the scientific content of specific sections of the manuscript and participated in drafting specific section of the paper. LC, AI, GP, ER, EV, AV and JM participated in the critical revision of the paper. LB, JM, and SP participated in the critical revision and editing of the manuscript. LB, LC, AI, GP, ER, EV, AV, JM and SP conceived the original idea and provided critical revision of the manuscript as well as the final approval of the version to publish. All authors contributed to the article and approved the submitted version.

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REVIEW
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First-Line Treatment for Advanced SCLC: What Is Left Behind and Beyond Chemoimmunotherapy

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Small cell lung cancer (SCLC) is still a lethal disease. Three phase III randomized clinical trials (IMpower133, CASPIAN, and KEYNOTE-604) have highlighted the survival gain of adding immune checkpoint inhibitors to first-line standard chemotherapy in advanced SCLC patients. In this review, we discuss the data from the three trials above. Furtherly, we analyze issues that still need to be elucidated, like the role of biomarkers, poor performance status at baseline, the presence of brain metastases, and the platinum compound's choice. Moreover, we depict the future of SCLC first-line therapy management, focusing on new therapeutic strategies currently under investigation.

Keywords: small cell lung cancer (SCLC), immunotherapy, chemotherapy, biomarkers, first-line therapy

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INTRODUCTION

Small cell lung cancer (SCLC), representing <20% of all cases of lung cancer worldwide, is still a lethal disease, with an estimated 5-year overall survival (OS) of 7% (1). The extensive stage (ES), which means the tumor is not amenable to radical radiotherapy due to its extent, is characterized by the poorest prognosis. Systemic treatments for ES disease have been implemented over the years, starting with single-agent chemotherapy (CT) in the 1970s (2). A platinum-based doublet with either etoposide or irinotecan became first-line standard CT, with a similar efficacy (i.e., median OS of ∼10 months) but a different safety profile (3).

At the end of 2010s, results from three phase III randomized clinical trials, the IMpower133 (4), CASPIAN (5), and KEYNOTE-604 (6), were published. These studies have demonstrated a significant improvement in OS by adding immune checkpoint inhibitors (ICIs) to CT, thus, opening a new era in treating advanced SCLC patients.

This review will analyze some relevant aspects of the three trials above. Furtherly, we will focus on some related still open issues like potential biomarkers, poor performance status (PS), brain metastases, and the platinum compound's choice. We will then discuss the new lines of research about the first-line treatment of advanced SCLC, depicting the future in this therapeutic scenario.

EVIDENCE ON FIRST-LINE CHEMOIMMUNOTHERAPY

IMpower133 is a double-blind, placebo-controlled, phase 3 trial where treatment naïve patients with ES-SCLC were randomly assigned (1:1 ratio) to receive carboplatin and etoposide with or without atezolizumab, an anti-PD-L1 antibody (4). After an induction phase consisting of four 21-day cycles, a maintenance phase with atezolizumab or placebo was offered

until disease progression or unacceptable toxicity. Main patients' characteristics are resumed in **Table 1**. Co-primary endpoints were progression-free survival (PFS) and OS. Median PFS was 5.2 months [95% confidence interval (CI): 4.4–5.6] and 4.3 months (95% CI: 4.2–4.5) in the experimental and control arm, respectively ($p = 0.02$), while median OS was 12.3 months (95% CI: 10.8–15.9) and 10.3 months (95% CI: 9.3–11.3) in the experimental and control arm, respectively ($p = 0.007$). The objective response rate (ORR) among the two treatment groups was similar (60.2 vs. 64.4% in the experimental and control arm, respectively), as also the safety profile (4) (**Table 1**). The updated results with 22.9 months of median follow-up have confirmed a median OS of 12.3 and 10.3 months in the experimental and control arm, respectively (HR: 0.76, 95% CI: 0.60–0.95, $p = 0.0154$), with 34 and 21% of patients alive at 18 months in the two arms (7).

CASPIAN is an open-label phase 3 trial in which untreated patients with ES-SCLC were randomly assigned (1:1:1 ratio) to receive durvalumab (anti-PD-L1 drug) plus platinum-etoposide or tremelimumab (anti-CTLA-4 antibody) and platinum-etoposide, or platinum-etoposide alone (5). Patients in the CT control arm received up to six cycles of platinum-etoposide. The immunotherapy was administered as maintenance in the experimental arms after four cycles of concomitant chemoimmunotherapy until disease progression or unacceptable toxicity. In **Table 1**, the main patients' characteristics are reported for the control arm and durvalumab plus platinum-etoposide arm. Median OS, the primary study endpoint, was 13.0 months (95% CI: 11.5–14.8) with durvalumab plus platinum-etoposide vs. 10.3 months (9.3–11.2) with platinum-etoposide ($p = 0.0047$). Median PFS was similar between the same two arms (5.1 vs. 5.4 months, respectively), whilst investigator-assessed ORR was higher in durvalumab than control arm (79 vs. 70%, respectively). No relevant difference in adverse events was highlighted between the two arms except for a slightly higher incidence of neutropenia and anemia in the control arm (5) (**Table 1**). The updated results published in 2021 substantially confirmed the OS improvement after a median follow-up time of 25.1 months, being 12.9 and 10.5 months in the experimental and control arm, respectively (HR: 0.75, 95% CI 0.62–0.91, $p = 0.0032$) (8). Notably, the addition of tremelimumab to durvalumab and platinum-based chemotherapy did not show a significant improvement in OS vs. platinum–etoposide, with a median OS of 10.4 months (95% CI: 9.6–12.0) vs. 10.5 months (9.3–11.2), respectively, but increased serious adverse events and treatment-related deaths (PMID: 33285097).

KEYNOTE-604 is a double-blind, placebo-controlled, phase 3 trial where untreated patients with ES-SCLC were randomly assigned (1:1 ratio) to receive platinum-etoposide with or without pembrolizumab, an anti-PD-1 antibody (6). The main patients' characteristics are resumed in **Table 1**. PFS and OS were the two primary endpoints of this study. The median PFS was 4.5 months (95% CI: 4.3–5.4) and 4.3 months (95% CI: 4.2-−4.4) in the experimental and control arm, respectively ($p = 0.0023$), while the median OS was 10.8 months (CI 95%: 9.2–12.9) and 9.7 months (95% CI: 8.6–10.7), in the experimental and control arm, respectively ($p = 0.0164$). A higher ORR was recorded in the experimental arm (70.6%) compared to the control arm (61.8%). The safety profile was similar between the two arms (**Table 1**).

POTENTIAL BIOMARKERS

Among those biomarkers that have been explored to predict the efficacy of anti-PD-(L)1 antibodies as cancer therapy, PD-L1 is undoubtedly the most studied (9). Patients with PD-L1 positive SCLC, defined by immunohistochemical staining in over 5% of tumor cells, showed better survival in a retrospective series (10). However, another work pointed out that tumoral cells from SCLC specimens were negative for PD-L1 expression, whilst it was expressed in macrophages and correlated with tumor-infiltrating lymphocytes (TILs) (11). The different assays used to detect PD-L1 expression have made the scenario more complex (12). In the IMpower133 trial, PD-L1 testing was not performed during screening for two main reasons: an expected high rate of inadequate samples and the previous results from the phase I trial that had not shown an association between SCLC response and PD-L1 expression (4, 13). Likewise, in the CASPIAN trial, PD-L1 testing was not required for enrollment (8); it was optionally tested in archival tissue as a part of an ancillary analysis (14), confirming the low rate of PD-L1 positive tumoral cells and the lack of prognostic value when investigated as a continuous variable. In the KEYNOTE-604 trial, PD-L1 was retrospectively assessed using the combined positive score (CPS), defined as the number of PD-L1-staining cells divided by the total number of viable tumor cells times 100 (6). This estimate was based on the previous phase II KEYNOTE-158 trial (15). Patients with CPS $\geq$ 1%, CPS $<$ 1% and unknown were about 40, 40, and 20%, respectively. The subgroup analyses did not observe differences between CPS $\geq$ 1% and CPS $<$ 1% groups in PFS and OS. An exploratory analysis from the IMpower133 trial has not shown a predicted OS and PFS difference by each PD-L1 IHC subgroup (7).

The tumor mutational burden (TMB), an indirect measure of the tumor's neoantigen load, has been deeply investigated as a potential biomarker for immunotherapy in human cancer (16). Concerning the SCLC, data from the Checkmate 032 trial, with nivolumab vs. nivolumab plus ipilimumab in pretreated patients, suggested a role for the TMB as a potential predictive biomarker, given the high tumor responses achieved by the combination therapy in patients with high TMB compared to nivolumab (17). Similarly, the TMB did not predict either OS or PFS by an exploratory analysis of the IMpower133 trial (7). The recent FDA's approval of pembrolizumab for patients with any cancer type characterized by $\geq$10 mutations/megabase (mut/Mb) who had progressed to one previous treatment line without a valid alternative option has raised several criticisms. Particularly for the SCLC, it seems unlikely that clinicians will offer pembrolizumab to their patients exclusively based on a high TMB (18–20).

In conclusion, to date, neither PD-L1 nor TMB can be used in clinical practice as predictive biomarkers for ES-SCLC (**Figure 1**).

TABLE 1 | Main characteristics of enrolled patients in the phase III clinical trials Impower133, CASPIAN, and KEYNOTE-604.

Trial	IMpower133 (4)		CASPIAN (5)			KEYNOTE-604 (6)	
	Experimental arm	Control arm	Experimental arm1*	Experimental arm2*	Control arm	Experimental arm	Control arm
Therapy	CbE + atezolizumab	CbE + placebo	PE + durvalumab	PE + Durvalumab + Tremelimumab	PE	PE + pembrolizumab	PE + placebo
No of patients	201	202	268	268	269	228	225
PS				NR			
- 0	36.3%	33.2%	37%		33%	26.3%	24.9%
- 1	63.7%	66.8%	63%		67%	73.7%	75.1%
Brain metastases at baseline	8.5%	8.9%	10%	NR	10%	14.5%	9.8%
Platinum compound				NR			
- Cisplatin	0%	0%	78%		78%		27.9% (both arms)
- Carboplatin	100%	100%	25%		25%		68.5% (both arms)
PFS, median (range), mo.	5.2 (4.4–5.6)	4.3 (4.2–4.5)	5.1 (4.7–6.2)	NR	5.4 (4.8–6.2)	4.5 (4.3–5.4)	4.3 (4.2–4.4)
OS, median (range), mo.	12.3 (10.8–15.9)	10.3 (9.3–11.3)	13 (11.5–14.8)	NR	10.3 (9.3–11.2)	10.8 (9.2–12.9)	9.7 (8.6–10.7)
Grade ≥ 3 AEs	58.1%	57.6%	62%	NR	62%	76.7%	74.9%

AEs, adverse events; CbE, carboplatin + etoposide; mo, months; PE, cisplatin + etoposide; PFS, progression-free survival; NR, not reported; OS, overall survival; PS, performance status.

**Patients were allowed to switch between carboplatin and cisplatin at the investigator's discretion.*

FIGURE 1 | Clinical practical questions and current answers about first-line chemoimmunotherapy for extensive-stage small-cell-lung cancer. chemo-IO, chemoimmunotherapy; ECOG PS, Eastern Cooperative Oncology Group Performance Statis; mets, metastases; PCI, prophylactic cranial irradiation; PD-L1, programmed cell death ligand-1; TMB, tumor mutational burden.

POOR PERFORMANCE STATUS AT BASELINE

One of the challenging issues in treating advanced SCLC patients is their deterioration of PS before starting first-line therapy. The NCCN guidelines suggest the exclusive use of supportive care when poor PS (≥2) is not due to SCLC. In contrast, the use of systemic therapy is not discouraged when poor PS is a consequence of SCLC (21); given the high chemosensitivity of SCLC, rapid response and symptomatic improvement with CT is expected, even if at the cost of higher toxicity than patients with good PS (22, 23). However, some specific situations may require a delay in systemic treatment start, like the presence of symptomatic brain metastases or epidural/cord compression. In these cases, a priority to radiotherapy (RT) is given (21).

Chemoimmunotherapy should not be offered to ES-SCLC patients with PS ≥2 as they were not enrolled in the three mentioned phase 3 trials (4–6). A single-arm phase 2 trial is currently recruiting PS 2 patients with ES-SCLC to investigate the impact on OS of adding atezolizumab to carboplatin-etoposide, adopting the schedule of the IMpower133 trial (NCT04221529). On the other hand, there are several reports about CT alone in patients with poor PS. A single-arm phase 2 clinical trial enrolled advanced SCLC patients with PS 2 or age ≥ 70 years, showing that the combination of weekly paclitaxel (80 mg/m^2) and carboplatin [area under the curve (AUC) 2], given on days 1, 8, 15 every 4-week cycle for up to six cycles, was feasible with few toxicities and led to a median OS of 7.2 months (24). A Japanese phase 3 randomized trial compared carboplatin plus etoposide with split doses of cisplatin plus etoposide in elderly or poor-risk SCLC patients (25). Eighteen and eight percent of enrolled patients were PS 2 and 3, respectively. Notably, PS 2-3 patients had a median OS of 8 months and PS 3

patients aged <70 years of 7 months, regardless of treatment allocation (25).

Similarly, in PS ≥ 2 non-small cell lung cancer (NSCLC) patients, the benefit of ICIs is still controversial. However, adopting frailty-assessing scales (26) or prognostic models, including the inflammatory indexes (27, 28), could assist clinical decisions. Likewise, those could be explored as helpful tools for PS2 SCLC patients (**Figure 1**).

BRAIN METASTASES IN THE CHEMOIMMUNOTHERAPY ERA

Another critical aspect in the clinical management of SCLC patients is relative to their high risk of developing synchronous or metachronous brain metastases (29). Brain metastases could be symptomatic or incidental lesions at the imaging, particularly at the contrast-enhanced magnetic resonance imaging (MRI), which is more sensitive than the computed tomography scan (CT scan) (30).

Prophylactic cranial irradiation (PCI) has been offered since the 1970s to reduce the intracranial failure rate following CT in SCLC patients (31). Two randomized clinical trials demonstrated that PCI minimizes the risk of developing symptomatic brain metastases after CT, although this did not translate into a statistically significant OS benefit (32, 33). The percentage of enrolled patients who received PCI in the IMpower133 and KEYNOTE-604 was 11 and 13%, respectively, whilst in the CASPIAN trial, PCI was allowed only in the control arm after completion of CT, and 8% of patients in this arm received it (4–6). Noteworthy, in the IMpower133 trial, time to intracranial progression was longer in patients receiving CT + atezolizumab vs. CT only (20.2 vs. 10.5 months, respectively), even though they did not receive PCI (16.7 vs. 9.8 months, respectively) (34). This evidence further questioned the role of PCI in the era of chemoimmunotherapy. Furthermore, the optimal timing of PCI (before or after the CT induction phase) and the subsequent follow-up schedule remain controversial.

Therefore, in the absence of robust data supporting PCI use in patients eligible for chemoimmunotherapy, an individualized approach should be pursued considering brain magnetic resonance imaging (MRI) follow-up as a valid alternative option (35).

Moreover, brain metastases at baseline were not an exclusion criterion for the three randomized trials (4–6), provided they were asymptomatic or treated and stable off steroids and anticonvulsants. It means we do not currently have data about chemoimmunotherapy in SCLC patients with active symptomatic brain metastases, which represents a considerable proportion of diagnosed patients and remains an unmet clinical need (**Figure 1**).

CHEMOTHERAPY BACKBONE: CISPLATIN OR CARBOPLATIN

Platinum compounds are the mainstay of chemotherapeutic regimens in SCLC patients. The COCIS meta-analysis halted the long debate about the best platinum compound for ES-SCLC, showing substantial equivalence in efficacy between carboplatin and cisplatin, albeit with different safety profiles (3). Nevertheless, in the chemoimmunotherapy era, the question reappeared. In the Impower133 trial, only carboplatin was allowed (4). In the other two trials, about one-quarter of enrolled patients received cisplatin (5, 6), reflecting the clinical practice of broader adoption of carboplatin. Subgroups analyses from the two trials showed a substantial similarity between the two drugs (5, 6). Therefore, carboplatin might be favored in this setting, considering the heavier side effects of cisplatin and the need for corticosteroids as antiemetic prophylaxis (**Figure 1**).

THE FUTURE OF FIRST-LINE THERAPY IN SCLC

Several ongoing trials are evaluating the addition of an anti-PD(L)1 agent to CT in the first-line setting (**Table 2**). However, what is new in this setting is the investigation of other molecules in addition to chemoimmunotherapy.

The role of neoangiogenesis in SCLC is well-established, with the vascular endothelial growth factor (VEGF) and its receptor (VEGFR) as the central molecular axis involved (36–38); a higher serum concentration of VEGF correlates with poor survival (39). Bevacizumab, a humanized anti-VEGF monoclonal antibody, did not prolong the survival of advanced SCLC patients when added to CT compared to CT alone (40, 41). Antiangiogenic tyrosine kinase inhibitors (TKIs), like sorafenib and vandetanib, failed to improve the survival of chemorefractory patients (42), although they are currently under evaluation in association with CT in the first-line setting (**Table 2**). In the latest years, combining immunotherapy and antiangiogenic agents has been explored as a therapeutic strategy in several cancer types based on the potential synergy between these two drug classes (43); the antiangiogenic drugs could promote T-cell infiltration in tumors and reduce immunosuppression, thus enhancing the effect of immunotherapy. To date, several clinical trials have been investigating the association of chemoimmunotherapy with antiangiogenic drugs in the first-line setting and the association of ICIs and antiangiogenic agents as maintenance therapy (**Table 2**). Notably, the AK112, a bispecific antibody against PD-1 and VEGF, is currently being investigated with carboplatin and etoposide in a phase I trial (NCT05116007).

Other novel drugs are currently being tested with chemoimmunotherapy in the first-line setting (23). New immunomodulatory agents under investigation could potentiate the effect of anti-PD-(L)1 antibodies though their effect on specific immune targets like: the LAG3, expressed on activated T and NK cells (44); TIGIT, upregulated by activated T cells and regulatory cells (45); ILT4, expressed in myeloid cells (46); CD27, involved in T cell proliferation and differentiation to memory and effector cells (47) (**Table 2**). Poly ADP-ribose polymerase inhibitors (PARPi) have been approved in ovarian cancer, prostate cancer and breast cancer

TABLE 2 | Ongoing clinical trials evaluating new combination strategies as first-line or maintenance therapy.

Setting	Chemotherapy	Investigational drug(s)	National clinical trial number
CT + anti-PD-(L)1			
First-line therapy	CbE	HLX10 (anti-PD-1)	NCT04063163
First-line therapy	PE	Toripalimab (anti-PD-1)	NCT04012606
First-line therapy	Paclitaxel-albumin + Carboplatin	Shr-1210 (anti-PD-1)	NCT04790539
First-line therapy	CbE	ZKAB001 (anti-PD-L1)	NCT04878016
First-line therapy	CbE	SHR-1316 (anti-PD-L1)	NCT03711305
First-line therapy	CbE	LP002 (anti-PD-L1)	NCT04740021
CT + anti-VEGF			
First-line therapy	PE	Anlotinib	NCT04675697
First-line therapy	PE	AL3810	NCT04254471
CT + anti-PD-(L)1 + anti-VEGF			
First-line therapy	PE	AK112 (Anti-PD-1 and VEGF Bispecific Antibody)	NCT05116007
First-line therapy	PE	Durvalumab + Anlotinib	NCT04660097
First-line therapy	PE	Toripalimab + Anlotinib	NCT04731909
First-line therapy	PE	Camrelizumab + Apatinib	NCT05001412
Maintenance therapy	No	Vorolanib + Atezolizumab	NCT04373369
Maintenance therapy	No	Camrelizumab + Apatinib	NCT04901754
Maintenance therapy	No	Tislelizumab + Anlotinib	NCT04620837
CT + Anti-PD-1 + other drugs			
First-line therapy	PE	Pembrolizumab + MK-4830 (anti-ILT4)	NCT04924101 (KEYNOTE-B99)
First-line therapy	PE	Pembrolizumab + MK-5890 (anti-CD27)	NCT04924101 (KEYNOTE-B99)
First-line therapy	PE	Sintilimab + IBI110 (anti-LAG3)	NCT05026593
First-line therapy	PE	Atezolizumab + Tiragolumab (anti-TIGIT)	NCT04256421 (SKYSCRAPER-02)
First-line therapy	PE	Durvalumab + Olaparib (PARPi)	NCT04728230
First-line therapy	PE	Tislelizumab + 177Lu-DOTATATE	NCT05142696
First-line therapy	PE	Nivolumab + BMS-986012 (fucosyl-GM1)	NCT04702880
First-line therapy	PE	Atezolizumab + LB-100 (PP2Ai)	NCT04560972
Maintenance therapy	No	Durvalumab + Ceralasertib (ATRi)	NCT04699838
Maintenance therapy	No	Atezolizumab + Lurbinectedin	NCT05091567
Maintenance therapy	No	Atezolizumab + Niraparib + Temozolomide	NCT03830918
Maintenance therapy	No	Camrelizumab + Fluzoparib (PARPi)	NCT04782089
Maintenance therapy	No	Atezolizumab + Talazoparib (PARPi)	NCT04334941
Maintenance therapy	No	Durvalumab + AZD2811 (AurKBi)	NCT04745689

ATRi, ATR inhibitor; AurKBi, Aurora Kinase B inhibitor; CbE, carboplatin + etoposide; CT, chemotherapy; PARPi, PARP inhibitor; PE, cisplatin + etoposide; PP2Ai, Protein phosphatase 2 A inhibitor.

and are currently under investigation in SCLC, given their potential of enhancing cytotoxic response to chemotherapy, radiotherapy, and immunotherapy (48). A clinical trial with the PARPi olaparib added to chemoimmunotherapy as first-line therapy in ES-SCLC patients (NCT04728230) is ongoing. However, PARPi have currently shown limited activity in SCLC patients, suggesting that a better selection of patients is needed (49). Other drugs investigated in combination with chemoimmunotherapy are the 177Lu-DOTATATE, a somatostatin receptor-targeted radionuclide therapy; BMS-986012, an anti-fucosyl-GM1 monoclonal antibody; and LB-100, a protein phosphatase 2A (PP2A) inhibitor (**Table 2**).

In parallel, translational research focused on identifying specific subgroups of patients who do benefit—or do not—from immunotherapy. In the latest years, immune signatures have been developed and studied in several cancer types (50). Specifically for SCLC, two recently published works shed light on this topic. Xie et al. have built up a prognostic 10-gene immune-related signature (ARAF, HDGF, INHBE, LRSAM1, NR1D2, NR3C1, PLXNA1, PML, SP1, and TANK), able to predict SCLC patients' survival; however, this model needs validation as a predictive tool for immunotherapy (51). Gay et al. have identified four SCLC subtypes based on the expression of three transcription factors (i.e., ASCL1, NEUROD1, and POU2F3); if those are all not expressed, an inflamed gene signature showed a similar correlation between SCLC subtypes and their vulnerability to specific drugs (52). Also for this molecular classification, validation is needed mandatory.

CONCLUSIONS

The addition of ICIs to standard chemotherapy represents a milestone in the first-line therapeutic scenario of ES-SCLC. Results from the three phase III randomized clinical trials are consistent, with OS gain across all patients' subgroups. However, primary resistance to chemoimmunotherapy is still challenging for ES-SCLC patients. More research efforts are needed to answer specific questions, like identifying responding patient according to their clinical and molecular characteristics, adding novel anticancer drugs to chemoimmunotherapy, and optimizing the therapeutic strategy for patients with symptomatic brain metastases.

AUTHOR CONTRIBUTIONS

AA and GLB: conceptualization and supervision. EG: writing—original draft and methodology. AR and GLB: validation and review and editing. All authors contributed to the article and approved the submitted version.

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Personalized treatment of extensive stage small cell lung cancer: A case report and literature review

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A 50-year-old female patient presented with post-exercise dyspnea in September 2016, and was subsequently diagnosed with SCLC with multiple brain and spinal metastases. The first-line treatment was etoposide combined with cisplatin and synchronously performed radiotherapy for the brain and spinal cord metastases. She was treated with anlotinib after disease progression in December 2018 and continued to have clinical benefit for nearly 25 months. Unexpectedly, the patient can still benefit from further combination treatment with durvalumab after another disease progression in February 2021. Thus, it may be a potential option to use anlotinib along with immunotherapy after the anlotinib resistance in SCLC, but more clinical data are still needed to confirm it. Moreover, ctDNA dynamic monitoring was performed and reflected the outcome of the process of treatment.

KEYWORDS

extensive-stage small cell lung cancer, anlotinib, durvalumab, long survival, NGS, bTMB

Introduction

Small cell lung cancer (SCLC) is a malignant tumor with aggressive, rapid progression, and metastatic potential, accounting for about 10%–15% of lung cancer cases. At present, chemotherapy is still the main treatment for SCLC, and only a small number of patients can receive second-line treatment with limited benefit. Immune checkpoint inhibitors have shown good clinical effects in the first-line and backward treatment of SCLC, but their absolute benefit for SCLC is still limited (1, 2). Besides, there have been attempts to research antiangiogenic agents for SCLC, but previous studies have demonstrated that most antiangiogenic agents and the combination drug regimens for

treating first-line or posterior SCLC have failed (3, 4). Thus, it is still necessary to explore more effective and safe new drugs and therapeutic schedules for SCLC. As a novel multi-target tyrosine kinase inhibitor (TKI), anlotinib can inhibit vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and c-Kit at the same time, which can inhibit both angiogenesis and tumor growth. The study of ALTER1202 demonstrated that, compared with placebo, the progression-free survival (PFS) and overall survival (OS) were significantly improved in the third-line and above treatment of SCLC. Here, we present one case of an advanced SCLC patient who had received concurrent chemoradiotherapy (cCRT) and long-term benefit from anlotinib monotherapy after multiple lines of chemotherapy. Moreover, the combination of anlotinib and durvalumab still resulted in durable PFS and the tolerance was good enough after the disease progression.

Case presentation

A 50-year-old female was admitted to our hospital on 18 September 2016 due to post-exercise dyspnea and lower extremity parethesia. She had no cigarette history, no family history, but was allergic to sulfa. Enhanced chest CT indicated central lung cancer in the middle and lower hilum of the right lung with pulmonary atelectasis, invasion of the right hilar vessels, and mediastinal lymph node metastasis. Magnetic resonance imaging (MRI) of her brain and spinal cord revealed multiple brain and spinal metastases (Figures 1Ai,ii). Bronchoscopic biopsy pathology examination showed evidence of small cell carcinoma (Figure 2). An extensive stage of SCLC was diagnosed. The patient initially received inductive chemotherapy with "etoposide 100 mg/m^2 (d1–d5) + cisplatin 120 mg/m^2 (d1)" for two cycles on 23 September 2016 and 13 October 2016. Evaluation by CT scan showed a partial response (PR) based on the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Concurrent chemoradiotherapy (cCRT) was sequentially delivered, followed by two more cycles of adjuvant chemotherapy. The radiotherapy regimen including GTV/CTV 60/50 Gy/20 times for right lung and right hilar lymph node lesions, CTV 40 Gy/20 times for spinal cord metastasis at T2–3, CTV 30 Gy for intracranial metastasis of the whole brain (right frontal lobe, right paracentral lobule), and 50 Gy/15 times for GTV intracranial metastasis. During cCRT, the patient developed grade 1–2 gastrointestinal adverse reactions and grade 4 granulocytopenia and thrombocytopenia, so the adjuvant chemotherapy was suspended. The response was categorized as PR (Figures 1Bi,ii).

Unfortunately, the patient suffered multifocal metastases on October 9, 2018. PET/CT found a new lesion in the right lower lobe with multiple new hypermetabolic mediastinal lymph nodes. Meanwhile, a plasma ctDNA test was performed and six gene missense mutations were found, including CREBBP, KIT, MUTYH, MYC, PREX2, and SMO. Blood tumor mutational burden (bTMB), defined as the number of somatic,

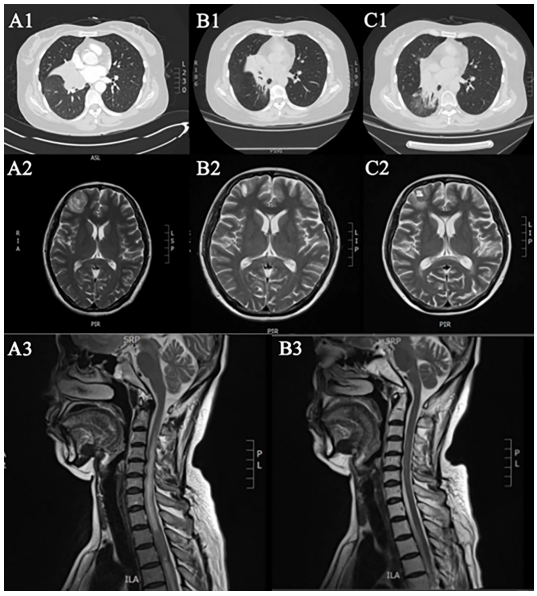

FIGURE 1
Imaging during chemotherapy. **Ai-iii** First diagnosis. **Bi-iii** After radiotherapy and chemotherapy. **(Ci, ii)** Progress after the second chemotherapy.

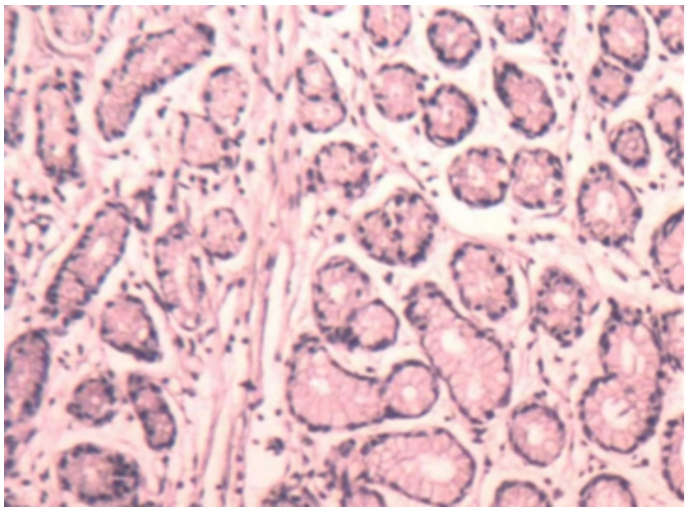

FIGURE 2
Bronchoscopic pathology showed small cell lung cancer.

coding, base substitution, and indel mutations per megabase (Mb) of genome examined, was calculated from the GENETRON OncoPanscan 825 Panel NGS platforms. "TMB high" was defined as cases with a TMB of ≥10 muts/Mb, and the bTMB of this patient was 23.33 muts/Mb. Since there were no approved immunotherapy drugs for SCLC, the patient just received another two cycles of "etoposide + lobaplatin" chemotherapy on 18 October 2018 and 12 November 2018. But the response was categorized as progressive disease (PD) (Figures 1Ci,ii).

The patient then started taking anlotinib (12 mg d1–d14/q3w) from December 2018 until the scale of the pulmonary lesion shrank and cavitated (Figure 3A). Later, the dose of anlotinib was reduced to 10 mg because of paronychia. The consolidation of lung lesions was reviewed on 14 May 2020 (Figure 3B), and the dose of anlotinib was increased to 12 mg

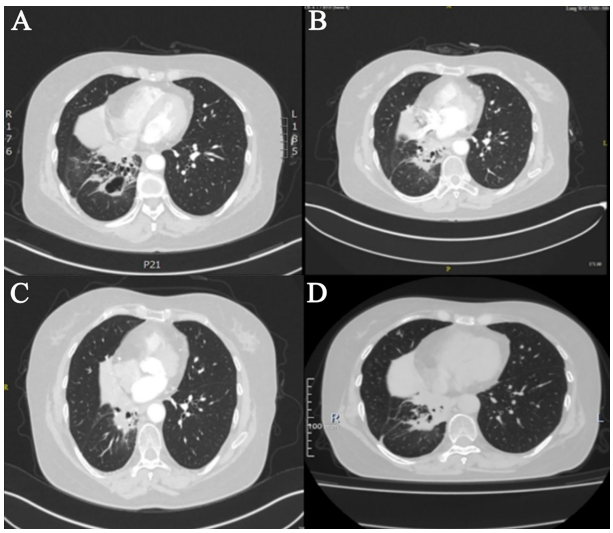

FIGURE 3
Imaging during Anlotinib treatment. **(A)** In December 2019, after anlotinib treatment, cavities formed. **(B)** In May 2020, void consolidation. **(C)** In November 2020, after the dose was increased, the lesions shrank again. **(D)** In October 2021, anlotinib combined with durvalumab shrink the lesion.

considering the risk of disease progression. During this period, the best efficacy was PR (Figure 3C), and no adverse reactions were reported.

In January 2021, she was referred for combination therapy of anlotinib along with durvalumab. As she had side effects during the previous anlotinib treatment, when adding durvalumab on this basis, to avoid the aggravation of side effects, the dose of durvalumab was adjusted and reduced to 1,000 mg q4w. The current clinical effectiveness was PR (Figure 3D). The last follow-up time was 28 March 2022. A ctDNA test was performed to monitor the effect of the treatment with bTMB reduced to 0 muts/Mb, which indicated the continuous benefit of anlotinib plus durvalumab.

In summary, the complete treatment pathway for patients is demonstrated in Figure 4.

Discussion

Extensive-stage SCLC accounts for about 60%–65% of SCLC (5). Previous studies have shown that the prognosis of extensive-stage SCLC is bleak, which has a median survival time of approximately 10 months and a less than 5% five-year survival rate with first-line chemotherapy regimens (6). Here, we report a case of a patient who achieved a response of nearly 25 months with the third-line treatment of anlotinib after the failure of second-line chemotherapy, and continued to achieve durable PFS and whose tolerance was satisfactory with subsequent anlotinib along with a durvalumab regimen after its progression.

Angiogenesis plays an important role in tumor growth, proliferation, and metastasis in SCLC (7). Thus, the anti-angiogenic drugs may play an important role in the treatment of SCLC as well. A study of 24 SCLC patients with the anti-angiogenic drug sunitinib, who had received at least one line of chemotherapy or concurrent chemoradiotherapy, showed that the ORR was 19%, and the median PFS and OS were 1.4 months and 5.6 months, respectively (8). Pazopanib is an inhibitor of the tyrosine kinase VEGFR2, PDGFR, and c-kit. In the study by Koinis et al. (9), patients after first-line platinum-based chemotherapy were included and divided into platinum-sensitive groups and platinum-resistant groups. In the overall 58 patients, the ORR was 13.8%, and the median PFS and OS were 2.5 months and 6.0 months, respectively. It seems that anti-angiogenic drug therapy plays a role in the second/third and above-line treatment of SCLC. But the number of study cases is small among those studies, which needs to be verified by a larger sample size. Anlotinib, as a novel TKI, can inhibit VEGFR, FGFR, PDGFR, and c-Kit at the same time, as well as inhibit angiogenesis and tumor growth. The ALTER1202 study, a randomized, double-blind, placebo-controlled multicenter phase II study of anlotinib in third-line and above treatment of SCLC, has been conducted in 2018. The results of ALTER1202 showed (10, 11) that patients with progressive or recurrent SCLC who were treated with anlotinib after second-line treatment had significant clinical benefits compared with placebo. Patients had a favorable clinical benefit of 3.4-month improvement in PFS (HR = 0.19, p <0.0001) and 2.4-month improvement in OS (HR = 0.53, p = 0.0029) in the anlotinib group. In our case, anlotinib was prescribed for the patient after disease progression from chemotherapy. In August 2019, anlotinib was approved for the treatment of SCLC that has

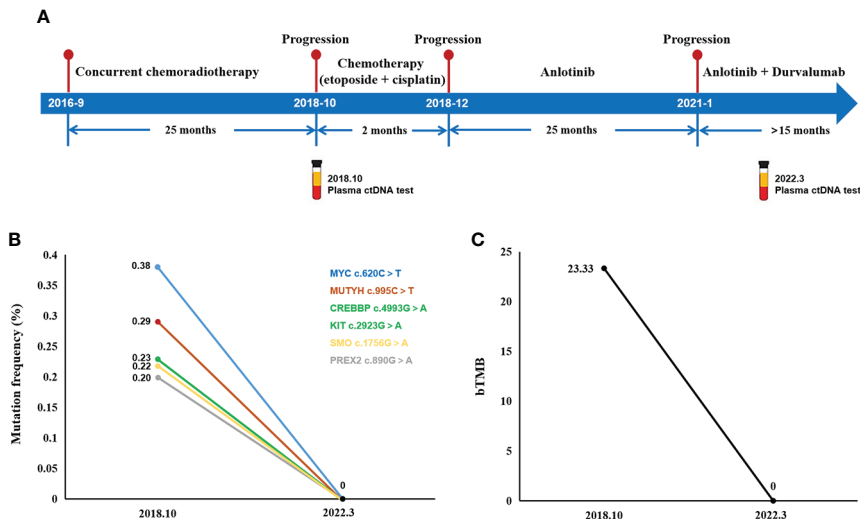

FIGURE 4
Treatment course and ctDNA NGS test results. **(A)** The complete treatment path of the patient. **(B)** Changes in gene mutation frequency and bTMB **(C)** between 2018 and 2022.

progressed or relapsed after at least two prior chemotherapy regimens in China, and it is the only approved anti-angiogenic drug for the treatment of SCLC.

Immunotherapy has changed the treatment outcomes of advanced lung cancer. In the first-line treatment, atezolizumab combined with etoposide and carboplatin-improved OS from 10.3 months to 12.3 months compared with chemotherapy (1). The OS was also significantly better in the durvalumab + etoposide +/− carboplatin group than in the chemotherapy group (13.0 months *vs* 10.3 months) (12). Therefore, the above regimens have been approved for first-line treatment of extensive-stage SCLC in many countries. There are also case reports indicating that the PD-L1 antibody durvalumab can achieve a response of 7 months in third-line treatment of extensive-stage SCLC (13).

However, the clinical effectiveness of the anti-PD-1 inhibitors nivolumab and pembrolizumab in the third-line treatment of SCLC is still controversial. In the Checkmate032 study, the ORR was 10% in patients with relapsed SCLC treated with nivolumab 3 mg/kg, 23% in nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg), and 19% in nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) (2). In this study, the duration of response was 17.9 months, the PFS was 1.4 months, and the OS was 5.6 months in the analysis of the third-line treatment with nivolumab alone (14). Similar results were shown in the KEYNOTE-028/158 study, which showed that the median PFS was 2.0 months and the median OS reached 7.7 months in the third-line and above treatment for SCLC (15). Based on the poor outcomes, Bristol-Myers Squibb (BMS) announced the withdrawal of nivolumab for the SCLC indication in the United States, and pembrolizumab also voluntarily withdrew its application for the SCLC indication in consultation with the FDA in 2021.

In recent years, the synergistic anti-tumor effect of anti-angiogenic drugs combined with immune checkpoint inhibitors has been supported by several studies. In preclinical (16) studies, anti-angiogenic drugs can promote the normalization of tumor vessels and regulate the immune microenvironment in many ways, which in turn activates the immune system. The mechanisms include promoting the maturation of dendritic cells, restoring the mobilization and infiltration of T cells, influencing the adhesion of lymphocytes, and reducing the induction and proliferation of inhibitory immune cells. At the same time, various innate and acquired immune cells are involved in the formation of blood vessels in tumors, and immune checkpoint inhibitors can promote tumor vascular normalization (17). This combination regimen has shown some efficacy for treating advanced SCLC. In the PASSION study of second-line treatment of SCLC (18), the efficacy results showed that the ORR was 33.9% and the PFS was 2.8 months in the overall population of apatinib combined with camrelizumab. The analysis showed that the ORR in the chemoresistant population and chemosensitive population was similar to that in the overall population.

However, although anti-angiogenic or anti-angiogenic drugs combined with immune checkpoint inhibitors for treating SCLC have shown preliminary efficacy, there are still many issues to be discussed, such as the suitable treatment population and the dose of treatment. In the combination therapy, the appropriate dose of anti-angiogenic drugs and the medication regimen are still worth exploring. Lin et al. (19) found that low-dose anti-angiogenic drug therapy may play an immune-promoting role by enhancing M1 polarization of macrophages and enhancing CD8+ T-cell function, while high-dose anti-angiogenic drug therapy may lead to immunosuppression of the microenvironment.

Moreover, liquid biopsy refers to the analysis of tumor-derived components in body fluids, among which circulating tumor DNA (ctDNA) has been used for dynamic monitoring of tumor changes, therapeutic effects, and patient prognosis in many cancers, including NSCLC (20), melanoma (21), and colorectal cancer (22). Compared with traditional tissue biopsy, ctDNA was noninvasive and could solve the problem of tumor heterogeneity. In SCLC, a few studies have shown that high pre-treatment ctDNA levels were associated with a poor prognosis in PFS and OS (23, 24), and plasma ctDNA could monitor dynamically the effect of treatment (25). The detection of ctDNA in LS-SCLC patients after curative treatment predicts disease recurrence and death (26). Additionally, ctDNA is a prognostic determinant in patients with SCLC treated with atezolizumab, and ctDNA is strongly associated with prognosis in SCLC patients treated with second-line immunotherapy (27). Although patients benefited from immunotherapy regardless of bTMB status in the IM133 clinical trial, it was found that patients with bTMB ≥16 were more likely to benefit from immunotherapy (28). In conclusion, liquid biopsy methods provide effective baseline analysis and longitudinal surveillance of LS and ES disease and have now been included in expanded SCLC studies and trials. We look forward to the results of these studies, particularly prospective studies of the role of ctDNA in predicting the efficacy of immunotherapy in SCLC.

Here, we found that patients with extensive SCLC can benefit from anti-angiogenic therapy plus immunotherapy depending on the situation. The efficacy may be assessed by ctDNA or bTMB, but further research is also needed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation

and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Authors HYW, XNW, SXJ, and JNZ collected the clinical information, diagnostic information, therapeutic information, and images of the patients. HYW wrote the manuscript. YH identified the case and submitted the manuscript. JNZ and CHZ revised the manuscript. YJZ and JL proofread the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Is ectopic Cushing's syndrome underdiagnosed in patients with small cell lung cancer?

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Introduction: Ectopic Cushing's syndrome (ECS) is an uncommon disorder. Recently, however, a larger proportion of patients with endogenous Cushing's syndrome (CS) had ECS than has previously been reported.

Objective: The aim of this study was to determine whether ECS is an underdiagnosed disorder in patients with small-cell lung cancer (SCLC).

Materials and methods: Medical records from consecutive patients diagnosed with SCLC at our hospital between 2013 and 2019 were reviewed (N = 213; mean age 69.5 ± 9 years; range, 36–89 years). The probability of having ECS was evaluated by review of biochemical and clinical features, including presence of recent onset diabetes mellitus, therapy resistant hypertension and/or spontaneous hypokalaemia.

Results: Of 213 identified patients with SCLC, one (0.5%) patient had confirmed ECS, two (1%) patients had probable ECS, and twenty-three (11%) patients had possibly ECS. Patients with SCLC and possibly or probable ECS exhibited a significantly shorter survival than patients only with SCLC (8 vs. 14 months, respectively).

Conclusions: Our findings indicate that ECS is underdiagnosed in patients with SCLC. Given the serious consequences of untreated ECS, the low detection rate highlights the need to improve endocrine work-up of patients with SCLC who present with biochemical and clinical features associated with ECS. Prospective studies are needed to establish a reliable assessment of the incidence of ECS and to optimise early detection strategies.

KEYWORDS

ectopic ACTH-production, hypercortisolism, small-cell lung cancer (SCLC), paraneoplastic syndrome, ectopic Cushing's syndrome

Introduction

Ectopic Cushing's syndrome (ECS) is an uncommon endocrine disorder caused by autonomous and excessive adrenocorticotropic hormone (ACTH) secretion from a tumour not originating in the pituitary gland (1, 2). The increased ACTH production subsequently causes hypercortisolism, that is often severe and characterised by treatment resistant hypertension, pronounced insulin resistance and hyperglycaemia, severe hypokalaemia and muscle weakness. Furthermore, due to the greatly increased ACTH production, hyperpigmentation of the skin and oral mucosa is also common in patients with ECS. In contrast to patients with Cushing's syndrome (CS) of pituitary or adrenal origin, hypercortisolism in patients with ECS usually develops rapidly (weeks) and typical features such as central obesity with abdominal striae may be absent (3).

Ectopic Cushing's syndrome is considered to account for approximately 5–15% of all patients with endogenous CS. Half of these are caused by lung tumours, either bronchial carcinoids or small cell lung cancer (SCLC) (1, 2, 4, 5). Recently, however, we found a higher proportion of ECS among patients with CS than has previously been reported (6). Of 80 patients diagnosed with CS in the Västra Götaland Region between 2002 and 2018, 21 (26%) had ectopic CS, of whom 8 had lung cancer.

In previous reports, between 1 and 6% of patients with SCLC had concomitant ECS (7–10). However, due to atypical presentation, rapid progression and diagnostic difficulties, the prevalence of ECS in patients with SCLC may still be underestimated.

The aim of this study was to evaluate the prevalence of clinical characteristics in patients with SCLC that may be related to ECS. Our main hypothesis is that ECS is an underdiagnosed comorbidity in patients with SCLC.

Materials and methods

Design

This was a retrospective study including patients who were diagnosed with SCLC at the Sahlgrenska University Hospital in Sweden between January 1st 2013 and December 31st 2019. Approximately half of all patients diagnosed with lung cancer in the Västra Götaland County, with 1.8 million inhabitants, and all patients with lung cancer diagnosed in north part of the Halland County (around 100 000 inhabitants) are referred to the Department of Respiratory Medicine at the Sahlgrenska University Hospital for evaluation and treatment. In Sweden, diagnostic codes are provided during all hospital visits and registered according to the diagnosis-related group (DRG) registry. To identify patients with SCLC, a search in the DRG-registry at the hospital was performed by using the specific ICD-10 code for SCLC (C34.9B).

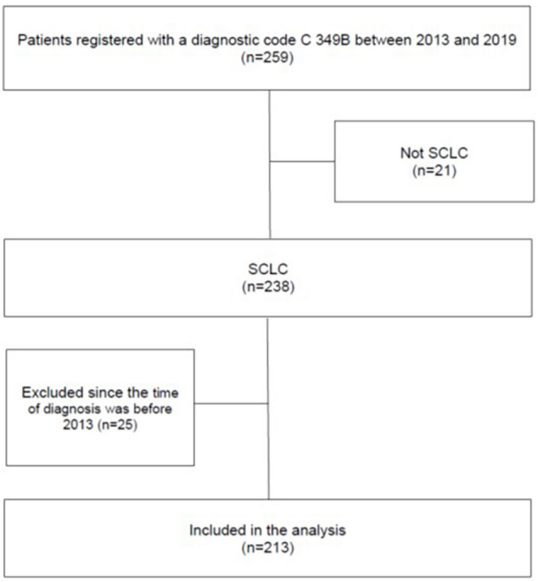

FIGURE 1
Flow chart showing the selection of patients in the study. The patients were identified by a search in the DRG-registry at the Sahlgrenska University Hospital by using the ICD-10 code for SCLC.

Patients

In total, 259 patients who had received a diagnostic code for SCLC during the study period were identified. Of these, 21 patients with histopathologically confirmed lung cancer of other aetiologies than SCLC were excluded. Additionally, 25 patients were excluded since the time of diagnosis was before 2013. Thus, the final cohort consisted of 213 patients with histopathologically confirmed SCLC (**Figure 1**). None of the patients had previously received a diagnosis of ECS.

Data collection and identification of patients with ectopic Cushing's syndrome

The medical records of all patients were reviewed and data on clinical features, biochemical data, imaging and histopathological diagnosis were collected, including data on: (a) new onset therapy-resistant hypertension; (b) new onset diabetes mellitus; (c) clinically significant hypokalaemia ($\leq$3.0 mmol/L); (d) documented hyperpigmentation; and (e) documented presence of Cushingoid features. Therapy-resistant

hypertension was defined as high blood pressure despite concurrent use of three different antihypertensive agents (11).

To validate the diagnosis of ECS, the medical records of all patients identified by the DRG registry were reviewed by an experienced endocrinologist. The diagnosis of ECS was considered to be "confirmed," "probable," or "possible," based on the criteria provided in Table 1.

Ethics

The study was approved by the Regional Research Ethics Committee in Gothenburg, Sweden (reference number 814–18; approved 26 November 2018) and conducted according to the Declaration of Helsinki.

Statistics

All data were analysed in IBM SPSS version 25.0.0.0. Categorical data are presented as number of subject (%). Normally distributed variables are presented as mean ± standard deviation (SD) and non-normally distributed variables as median (interquartile range; IQR or range). Kaplan–Meier curves were used to illustrate survival and the Log rank test to estimate the difference between patients with and without ECS.

Results

In total, 213 patients (128 women, 60%) were diagnosed with SCLC between 2013 and 2019. The mean age at diagnosis was 69.5 ± 9 (range, 36–89) years. Of 213 patients, 14 (7%) had severe hypokalaemia (serum potassium ≤3 mmol/l), 12 (6%) had therapy resistant hypertension and 4 patients (2%) had new-onset diabetes mellitus (Table 2). Cushingoid features were documented in 6 (3%) patients and hyperpigmentation in one. S-Cortisol had been measured in 35 patients, of whom 9 had concentrations at 8 AM >900 nmol/L.

Based on the criteria presented in Table 1, including presence of therapy-resistant hypertension, new-onset diabetes mellitus, and/or clinically significant hypokalaemia (≤3 mmol/L K), one (0.5%) patient was considered to have confirmed ECS, two (1%) patients had probable ECS, and 23 (11%) patients had possible ECS.

TABLE 2 Characteristics of patients without ectopic Cushing's syndrome (ECS) and with possible ECS.

	All (*N* = 213)	Patients without ECS (*N* = 187)	Possible ECS (1) (*N* = 23)
Age (years), mean ± SD	69.5 ± 9	69 ± 9	70.6 ± 8
Female gender, *N* (%)	128 (60)	110 (58)	17 (71)
Hypertension, *N* (%)	105 (49)	85 (45)	17 (71)
Therapy-resistant hypertension, *N* (%)	12 (5.6)	0	9 (39)
New-onset diabetes mellitus, *n* (%)	4 (1,9)	0	2 (9)
Hypokalaemia (<3 mmol/L K), *N* (%)	14 (7)	0	12 (52)

Confirmed ectopic Cushing's syndrome

Case 1

An active 81-year-old man with a history of smoking and prostate cancer presented with a few months history of muscle weakness, dyspnoea and peripheral oedema. Hyperpigmentation was noticed and documented, but not cushingoid features. At presentation the patient had therapy-resistant hypertension and hypokalaemia (s-potassium 2.9 mmol/L) that required treatment with 6 g potassium chloride daily and spironolactone. He did not have diabetes mellitus. Imaging studies reviled multiple lesions in the lungs and liver metastases. Bronchoscopy was performed and biopsy confirmed SCLC. ECS was suspected and endocrine work-up revealed S-cortisol at 8 AM of 1,850 nmol/L (normal 102–535 nmol/L), urinary free cortisol 3,660 nmol/L (normal 55–215 nmol/L) and p-ACTH 129 pmol/L (normal 2–11 pmol/L). The patient's condition deteriorated rapidly and 7 days after SCLC was diagnosed he died before any specific treatment was started.

Probable ectopic Cushing's syndrome

Case 2

A 67-year-old woman with a history of 100 pack-years of smoking, hypertension, primary hyperparathyroidism, osteoporosis and alcohol overconsumption, presented with a pathological radius fracture. Laboratory examination revealed severe hypokalaemia (1.9 mmol/L) and treatment with potassium supplements was started (6 g per day).

TABLE 1 Definitions of confirmed, probable and possible ectopic Cushing's syndrome used in this study.

Confirmed	Biochemical analyses confirming ectopic CS, including serum cortisol, plasma ACTH, and urinary free cortisol
Probable	Presence of two of the following: (i) new onset therapy resistant hypertension, (ii) new onset diabetes mellitus, (iii) clinically significant hypokalaemia (≤3.0 mmol/L)
Possible	Presence of one of the following: (i) new onset therapy resistant hypertension, (ii) new onset diabetes mellitus, or (iii) clinically significant hypokalaemia (≤3.0 mmol/L)

A few days later, the patient was readmitted due to bowel perforation. Serum potassium was 2.9 mmol/L, despite potassium supplementation. During emergency laparotomy, metastases in the liver and gall bladder were discovered. Histopathological examination revealed SCLC. Chest CT revealed a tumour in the left lung hilum, mediastinal lymph node metastases and pleural effusion.

Due to therapy resistant hypertension and hypokalaemia, screening for secondary hypertension was performed and displayed high s-cortisol (3,370 nmol/L) and normal aldosterone/renin ratio [27 mIU/L (normal 4–65 mIU/L)]. Due to hyperglycaemia treatment with insulin was initiated. No further endocrinological work-up was performed. Fifteen days after SCLC was confirmed, palliative chemotherapy was started. Unfortunately, the patient's condition deteriorated thereafter, and 3 weeks later she died due to pneumonia with neutropenic fever.

Case 3

A 67-year-old man with a history of cardiovascular disease and a recently diagnosed diabetes mellitus presented with dyspnoea on exertion. Clinical examination was consistent with heart failure that was treated with diuretics. On imaging, tumours were identified in lungs, liver, spleen, kidneys, pancreas, adrenal glands and mediastinal lymph nodes. Lymph node biopsy revealed SCLC. Despite administration of three antihypertensive drugs, the patient's blood pressure remained above 140/90 mmHg, potassium supplementation was started due to hypokalaemia and insulin due to hyperglycaemia. Palliative cytostatic treatment was started. Three days later the patient died with neutropenic sepsis despite treatment with broad spectrum antibiotics.

Possible ectopic Cushing's syndrome

Twenty-three patients (17 women) were considered to possibly have ECS; 12 patients with severe hypokalaemia, 9 patients with therapy resistant hypertension and two patients with new onset diabetes mellitus. The mean age at diagnosis in patients with possible ECS was 71 ± 8 years. Neither hyperpigmentation nor cushingoid features were documented in any of these patients. S-cortisol was measured in two, both having high concentrations at 8 AM (1,000 and 1,120 nmol/L, respectively).

Survival

At the end of the study, 197 of 213 patients with SCLC had deceased. The median survival time was 13 months (range 1 day to 7.9 years).

The patient with confirmed ECS died 7 days after diagnosis, and the patients with probable ECS died after 19 and 89 days, respectively. The median survival time in patients with possible ECS was 8 months (range 1 day to 2.6 years), compared to

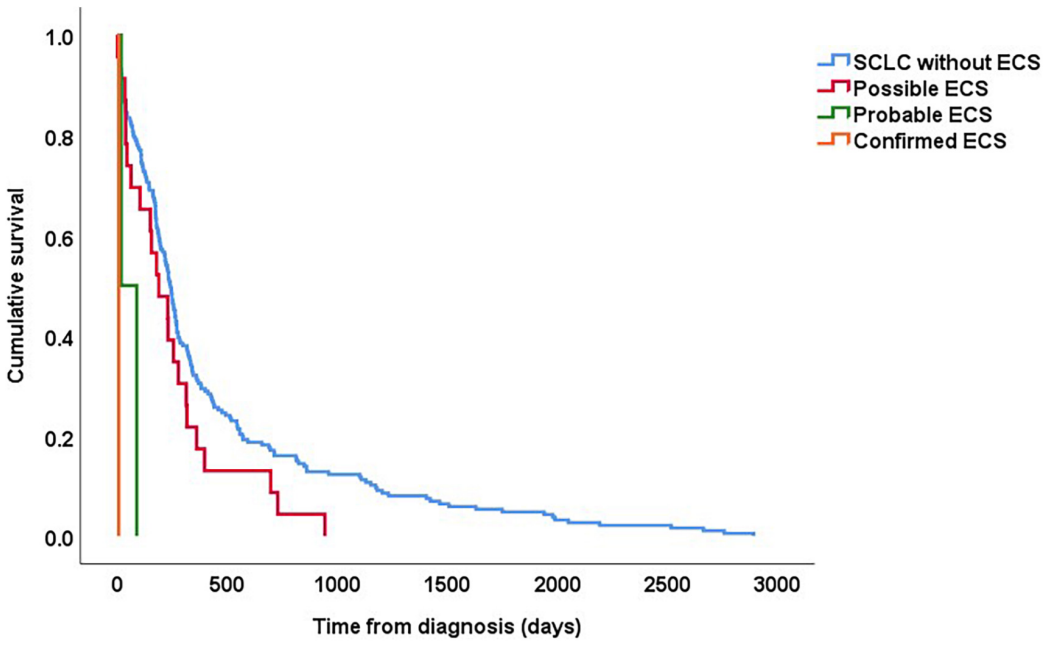

FIGURE 2
Kaplan–Meier curves showing survival in patients with SCLC based on whether they had "confirmed," "probable," and "possible" ECS. For all patients, follow-up started on the date of histological diagnosis.

14 months (range 2 days to 7.9 years) in patients without suspicion of ECS (Figure 2).

Discussion

In this retrospective study on an unselected cohort of 213 consecutive patients with SCLC we found a low prevalence of ECS; one patient was considered to have confirmed ECS, and two patients probably had the disorder. However, we also found that approximately 10% of the patients had clinical characteristics that are associated with ECS, i.e., either severe hypokalaemia, therapy resistant hypertension or new onset diabetes mellitus. We find it therefore likely that a substantial number of patients with SCLC may have had an undiagnosed ECS.

The prevalence of ECS among patients with SCLC has previously been studied in three relatively large studies (Table 3). In two of these studies, conducted at two different hospitals in Toronto Canada during the 1990's, 23 of 545 (4.5%) and 14 of 840 (1.6%) patients with SCLC had ECS. More recently, 23 of 383 (6%) patients with SCLC from France were considered to have had ECS. Thus, between 1.6 and 6% of patients with SCLC develop ECS, where different definitions of ECS, as well as different thresholds for investigating the patients at individual centres, may explain some of the varying prevalence. However, given that all of these studies are retrospective, it is also likely that several cases of ECS remained undiagnosed. In our cohort only three of 213 (1.4%) patients with SCLC had either confirmed or probable ECS, also indicating an underdiagnosis of the disorder.

As far as we know, this is the first study aimed at estimating how often ECS may be undiagnosed in patients with SCLC. By collecting data on signs, symptoms and biochemical parameters that are characteristic for ECS, we identified 23 patients that possibly had undiagnosed ECS. Obviously, we cannot claim that

TABLE 3 Summary of previous studies describing the prevalence of ECS among patients with SCLC.

Author country (references)	Period	No of patients with SCLC	No (%) of patients with ECS	Diagnostic criteria for ECS	Median survival from diagnosis	Limitations
Nagy-Mignotte et al. (10) France	1998–2012	383	23 (6)	Two or more of the following: • P-cortisol >550 nmol/L • S-potassium <3.2 mmol/L • P-glucose >5.8 mmol/L (without prior history of diabetes) • P-ACTH >15 pmol/L • 24-h urinary free cortisol >300 nmol/day	6.6 months	Retrospective study
Delisle et al. (9) Canada	1971–1991	840	14 (1.6)	Two or more of the following: • P-cortisol >600 nmol/L, and loss of diurnal variation and/or lack of suppressibility by dexamethasone • S-potassium ≤3,2 mmol/L • P-ACTH >22 pmol/L • 24-h urinary free cortisol >400 nmol/day	5.5 months	Retrospective study
Shepherd et al. (8) Canada	1980–1990	545	23 (4.5)	Signs and symptoms of hypercortisolism and two or more of the following: • P-cortisol >660 nmol/L, and loss of diurnal variation and lack of suppressibility by dexamethasone • S-potassium <3 mmol/L • P-ACTH >22 pmol/L • 22-h urinary free cortisol >400 nmol/day	6.2 months	Retrospective study
Dimopoulos et al. (7) United States	1979–1989	90	11 (12)	Clinical and laboratory findings associated with hypercortisolism i.e., • Elevated corticosteroids • Hypokalaemia • Metabolic alkalosis • Muscle weakness • Diabetes • Hypertension	12 days from initiation of CHT	Study population limited to patients with SCLC who died within 90 days after chemotherapy was started; Definition of ECS was not formulated clearly

all these patients had undiagnosed ECS. However, we would like to suggest that patients with SCLC, as well as patients with other neoplastic diseases associated with ECS, who present with severe hypokalaemia, therapy-resistant hypertension and/or new onset diabetes mellitus, should undergo biochemical testing to either confirm or exclude endogenous hypercortisolism. The hypercortisolism in patients with ECS is often severe where all the characteristic clinical features are present. This is, however, not the case in all patients, who instead present only with some of the features (7–10). In our opinion, the biochemical testing should therefore not only be performed in patients with pronounced hypercortisolism, but in all patients who present with either severe hypokalaemia, therapy resistant hypertension or new onset diabetes mellitus.

Small-cell lung cancer is a highly malignant disease with a poor prognosis and low 5-years survival rate (12). Among factors that have a negative influence on survival is the presence of ECS (7, 9, 10). In a study on survival in patients with SCLC, patients with ECS (n = 23) had a median survival of 6.6 months, which was significantly shorter compared with patients without any paraneoplastic syndrome (13 months) as well as patients with syndrome of inappropriate antidiuretic hormone secretion (8.5 months) (9). Also, in a retrospective study by Osswald, patients with ECS due to SCLC had a median survival rate of 5 months, which was significantly shorter than in patients with other forms of ECS (53–119 months) (13). In our cohort, the three patients with confirmed or probable ECS died within 3 months. In addition, the median survival time in patient with "possible" ECS was significantly shorter than in patients without a suspicion of ECS (8 v .14 months). We therefore consider it likely that some of these patients may have had undiagnosed ECS, although some of these patients may also have had a more advanced SCLC where e.g., hypokalaemia is more common than in patients in better general condition.

It is well known that patients with CS, irrespective of aetiology, have increased mortality (14–17). In many cases the cause of death is a direct consequence of the hypercortisolism *per se*, i.e., pulmonary embolism or sepsis, and not the underlying tumour (14). Thus, prompt and adequate treatment with cortisol-lowering drugs, thromboprophylaxis, prophylactic broad-spectrum antibiotics as well as prophylactic treatment against opportunistic microorganisms may improve the prognosis in many patients with ECS, and in some cases be life-saving. In fact, any diagnostic delay can be fatal. Furthermore, quality of life is severely impaired in patients with CS, and improves substantially following correction of the hypercortisolism (18). Therefore, it is essential to detect EAS as early as possible, initiate appropriate treatment, even in patient with incurable disease.

The major limitation of this study, as in all previous studies investigating the prevalence of ECS in patients with SCLC, is the retrospective design. Indeed, we cannot confirm that ECS is an underdiagnosed disorder, this is only an assumption. To solve the question a prospective trial, where consecutive patients with SCLC are screened for endogenous hypercortisolism, is needed.

Conclusion

Our findings indicate that ECS is an underdiagnosed disorder in patients with SCLC. Given the serious consequences and poor prognosis of untreated ECS, the low detection rate highlights the need to improve knowledge of ECS among healthcare providers and to optimise early detection strategies for ECS.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MP: data curation, formal analysis, investigation, methodology, project administration, software, visualisation, and writing – original draft preparation. ML, EP, and LO: writing – reviewing and editing. OR: conceptualisation, formal analysis, funding acquisition, methodology, resources, software, supervision, validation, visualisation, and writing – reviewing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Lurbinectedin in small cell lung cancer

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Few treatment options are available for patients with small cell lung cancer (SCLC) in progression after a first-line therapy. A novel therapeutic approach is represented by lurbinectedin, a synthetic derivative of trabectedin that works by inhibiting oncogenic transcription and promoting apoptosis in tumor cells. A phase II basket trial demonstrated the activity of lurbinectedin at the dose of 3.2 mg/m^2 in patients with SCLC who had failed a previous chemotherapy, with a response rate of 35.2%, a median progression-free survival (mPFS) of 3.5 months, and a median overall survival (mOS) of 9.3 months. Common severe adverse events (grades 3–4) were hematological disorders, including anemia (9%), leukopenia (29%), neutropenia (46%), and thrombocytopenia (7%). On the basis of the positive results of this phase II study, on June 2020, lurbinectedin was approved by the Food and Drug Administration as second line for SCLC patients in progression on or after platinum-based therapy. The subsequent phase III trial comparing the combination of lurbinectedin plus doxorubicin vs. CAV (cyclophosphamide, Adriamycin, and vincristine) or topotecan did not demonstrate an improvement in overall survival, although the experimental arm showed a superior safety profile. Combinations of lurbinectedin with other drugs, cytotoxic agents and immune checkpoint inhibitors, are currently under investigation. The results of these studies should better define the optimal clinical application of lurbinectedin.

KEYWORDS

SCLC, lurbinectedin, topotecan, chemotherapy, second line

Introduction

One of the most aggressive lung cancers is represented by small cell lung cancer (SCLC) (1), with an overall survival (OS) at 5 years of <10% and a median overall survival (mOS) of 9–11 months for patients in metastatic setting (2, 3). The most significant risk factor for developing SCLC is a history of tobacco exposure. Despite an extensive genetic characterization of SCLCs in recent years (4–7), no clear targetable alteration has emerged (6). For roughly 30 years, outcomes for patients with extensive-stage ES-SCLC have remained substantially unchanged (8–12), and only recently the combination of immune checkpoint inhibitors and standard platinum-based chemotherapy has changed the therapeutic paradigm in the first-line setting—thanks to the positive results observed in the IMpower133 and CASPIAN trials (13, 14).

In the IMpower133 trial, patients with metastatic SCLC naive for treatment were treated with atezolizumab or placebo plus carboplatin and etoposide every 3 weeks for four cycles followed by maintenance treatment with atezolizumab or placebo. This trial showed a median OS of 12.3 months (95% CI: 10.8–15.9 months) in the experimental arm vs. 10.3 months (95% CI: 9.3–11.3 months) for placebo [hazard ratio (HR) 0.70; 95% CI: 0.54–0.91; p = 0.0069) and a median progression-free survival (mPFS) of 5.2 months (95% CI: 4.4–5.6 months) for atezolizumab vs. 4.3 months (95% CI: 4.2–4.5 months) for placebo (HR 0.77; 95% CI: 0.62–0.96; p = 0.017). Benefits were consistent across patient subgroups (13). In the CASPIAN trial, patients with extensive-stage SCLC, naive for treatment, were randomized 1:1:1 to receive platinum-based chemotherapy (either carboplatin or cisplatin and etoposide) plus durvalumab, with or without tremelimumab every 3 weeks for 4 cycles followed by maintenance with durvalumab on day 1 every 4 weeks, or up to six cycles of platinum-based chemotherapy (standard arm). The combination of durvalumab and chemotherapy leads to a statistically significant improvement in OS [mOS of 12.9 months 95% CI: 11.3–14.7 months) for durvalumab plus chemotherapy vs. 10.5 months (95% CI: 9.3–11.2 months) for standard arm; HR 0.75; 95% CI: 0.62–0.91; p = 0.0032] (14). Based on the results of these two randomized trials, the first-line treatment for extensive-stage SCLC is currently platinum (carboplatin or cisplatin) plus etoposide and atezolizumab or durvalumab (15).

Unfortunately, almost all patients with metastatic disease relapse, notwithstanding high response rates (RRs) to first-line therapy. Patients with relapsed SCLCs are usually classified into platinum-sensitive, platinum-resistant, and platinum-refractory according to the treatment-free interval (TFI) (16). RRs to second-line chemotherapy are generally 20%–30% in platinum-sensitive patients (i.e., TFI >3 months) and 15% in platinum-resistant patients (i.e., TFI <3 months). Patients not responding or progressing during chemotherapy (platinum-refractory) have very poor outcomes, and further systemic therapy may not be helpful. Until 2020, topotecan, a topoisomerase 1 inhibitor, was the only second-line treatment approved for SCLC patients, with modest activity in sensitive disease. The efficacy of topotecan was evaluated in a randomized phase III clinical trial vs. the CAV (cyclophosphamide, Adriamycin, and vincristine) regimen; topotecan showed similar objective response rates (ORRs: 24.3% vs. 18.3%, p = 0.29), time to progression (13.3 vs. 12.3 weeks), and OS (25 vs. 24.7 weeks) but a better tolerability than CAV (17). The efficacy of topotecan was then evaluated in another phase III trial, in which topotecan was compared with best supportive care, showing a statistically significant prolongation of OS (25.9 vs. 13.9 weeks, p = 0.0104), better symptom control and a slower worsening of quality of life in patients with relapsed SCLC, of whom half were platinum-resistant. Adverse events (AEs) (particularly hematological) were however considerable, with 6% toxic deaths (18). Different toxicity profiles between oral and intravenous (i.v.) topotecan emerged from a subsequent phase III trial, which also demonstrated similar efficacy (19). Therefore, either oral or i.v. topotecan is recommended as second line in platinum-resistant or -sensitive relapsed SCLC, with CAV as an alternative option. Indeed, in a meta-analysis of 1,347 patients treated with topotecan, an RR of 17% was reported in patients with refractory-relapsed disease vs. 27% in those with sensitive disease (20). For sensitive relapsed SCLC, a recent randomized phase III study of second-line treatment compared the rechallenge with platinum-based chemotherapy and topotecan: roughly one-third of enrolled patients had limited disease at diagnosis (21). The rechallenge chemotherapy resulted in a longer mPFS than topotecan (4.7 vs. 2.7 months; HR 0.57; 90% CI 0.41–0.73; p = 0.0041). Therefore, rechallenge chemotherapy can be considered a reasonable alternative as second line for patients with sensitive relapsed SCLC. Overall, limited treatment options are currently available for patients with relapsed SCLC.

Lurbinectedin (PM01183) is a tetrahydropyrroloquinoline with better antitumor activity than trabectedin through the addition of a portion of tetrahydro β-carboline (22). This drug induces a specific degradation of transcribing RNA Pol II with the accumulation of DNA breaks, leading to apoptosis in tumor cells. The drug, covalently binding to CG-rich regions located within the affected gene, blocks the DNA repair mechanism, causing RNA polymerase II elongation arrest and therefore degradation (23). Furthermore, in transcriptionally dependent tumor cells such as SCLC cells, lurbinectedin could cause a separation of transcription factors from their target promoters, with the block of its transactivating activity. It may also influence the tumor microenvironment *via* suppression of tumor proliferation, matrix remodeling, angiogenesis, and immune suppression (24–26). Moreover, in mice with xenografted tumors, the combination of lurbinectedin and doxorubicin, which has a different mechanism of action and a different toxicity profile, showed a synergistic antitumor activity,

supporting the rationale of the combination of these two drugs (27).

Phase I studies

Elez et al. (28) evaluated in a phase I trial the safety and activity of lurbinectedin (PM01183) in 31 patients with advanced solid tumors (Table 1). The drug clearance was independent of body surface area (BSA), and a flat dose of 7 mg intravenously as a 1-h infusion every 3 weeks was recommended. The most frequent severe adverse effect was myelosuppression, occurring in 40% of patients, usually transient and manageable, never febrile. Fatigue, nausea, and vomiting were mild. A partial response was observed in a patient with pancreatic adenocarcinoma. A dose escalation study of lurbinectedin combined with doxorubicin 50 mg/m^2 every 3 weeks was conducted by Calvo et al. (29). The starting dose of lurbinectedin was 3.5 mg [i.e., 50% of that suggested by Elez et al. (28)]. The dose escalation phase enrolled 74 patients, in whom the most common tumor type was SCLC (n = 28, 38%). Four dose levels were evaluated (3.5–5 mg). Most dose-limiting toxicities were hematological. The recommended flat dose of lurbinectedin was 4 mg in the combined regimen. Twenty-seven patients with relapsed SCLC were treated with the above therapy. Twelve patients (44%) had platinum-sensitive disease (relapse after at least 90 days) and received the protocol therapy as second line. The other 15 patients (56%) had platinum-resistant disease (time to relapse shorter than 90 days) and received the therapy as second, third, or fourth line of treatment. Median age was 62 years (range 48–73). Eight patients (29.6%) received 45 cycles of lurbinectedin alone after doxorubicin discontinuation. Grade 3 of higher toxicities comprised febrile neutropenia, fatigue, mucositis, and pneumonia. However, myelosuppression was transient and reversible for patients treated with the recommended dose of lurbinectedin. The most common adverse effects related to single-agent lurbinectedin were fatigue (n = 8, 100%), decreased appetite (50%), and alopecia (38%). ORR was 57.7%, and disease control rate (DCR) was 69.2%. As second line, ORR was 66.7% (14 of 21 patients). Moreover, ORR was 91.7% for 12 patients with platinum-sensitive disease [two complete (16.7%) and nine partial (75%) responses] and 33.3% for nine patients with platinum-resistant disease. DCR was 100% in sensitive and 55.6% in resistant disease. mPFS was 4.1 months. As second line, PFS was 4.7 months (5.8 months for sensitive and 3.5 months for resistant disease). Seven patients achieved a PFS lasting over 6 months. An expansion cohort of the above study, including SCLC patients relapsed after no more than one prior therapy, was successively treated with a lower dose of doxorubicin (40 mg/m^2) to reduce the incidence of severe myelosuppression (30). Moreover, lurbinectedin dose has been modified at 2 mg/m^2 of BSA based on the finding that patients with the lowest BSA had an increased risk of severe thrombocytopenia with the flat dose of lurbinectedin. On the other hand, the maximum lurbinectedin dose was capped at 4 mg for patients with BSA higher than 2 m^2 to prevent unexpected toxicities. Twenty-eight patients were recruited in the expansion cohort: 18 (64%) had platinum-sensitive and 10 (36%) had platinum-resistant disease, including six patients with refractory tumor progressing within 30 days from platinum-based chemotherapy. Responding patients could continue to receive single-agent lurbinectedin at 4 mg/m^2 every 3 weeks after 10 courses of combined regimen. ORR was 36% [one complete (4%) and nine partial (32%) responses], PFS was 3.3 months, and OS was 7.9 months. DCR was 72%. In the subgroup analysis, ORR 50%, PFS 5.7 months, and OS 11.5 months were recorded for patients with platinum-sensitive disease, while ORR 10%, PFS 1.3 months, and OS 4.6 months were recorded for patients with platinum-resistant disease. The main toxicity was confirmed as transient

TABLE 1 Clinical studies with Lurbinectedin in solid tumors and SCLC.

Study	Author	Setting	Pts	Treatment	Response rate (%)	Disease control rate (%)	Progression-free survival (months)	Overall survival (months)	Toxicity
Phase I	Elez ME, 2014	Advanced solid tumors	31	Lurbinectedin	3.6	32.6	–	–	Myelosuppression, nausea,vomiting fatigue
Phase I	Calvo E, 2017	SCLC, pretreated	27	Lurbinectedin + doxorubicin	57.7	69.2	4.1	–	Febrile neutropenia fatigue, mucositis, pneumonia
Phase I	Ponce S, 2019	SCLC, pretreated	7 12	Lurbinectedin + paclitaxel Lurbinectedin + irinotecan	71 25	71 67	4.8 5.6	- -	Grade 4 neutropenia, febrile neutropenia Grade 4 neutropenia, fatigue, nausea
Phase II	Trigo J, 2020	SCLC second-line	105	Lurbinectedin	35	68	3.5	9.3	Myelosuppression, febrile neutropenia
Phase III	Paz-Ares L, 2021	SCLC first line	613	Lurbinectedin + doxorubicin vs Topotecan or CAV	31 vs 29		4.0 vs 4.0, HR: 0.831, p=0.043	8.6 vs 7.6, HR: 0.967, p=0.703	Myelosuppression, liver toxicity

CAV: cyclophoshamide, doxorubicin, vincristine.

myelosuppression. Non-hematological events were mild or moderate and included fatigue, nausea, decreased appetite, vomiting, and alopecia.

Phase II studies

The evidence of lurbinectedin activity in SCLC came from a cohort of a single-arm, open-label, phase II basket trial conducted by Trigo et al. (31). The authors recruited 105 patients with advanced SCLC pretreated with only one previous line of treatment (immunotherapy was allowed, alone or in combination with chemotherapy) and an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or lower. Unfortunately, the trial did not include patients with known central nervous system (CNS) involvement, missing a crucial information about the activity of lurbinectedin this setting. All patients were treated with 3.2 mg/m^2 lurbinectedin administered as a 1-h i.v. infusion once every 3 weeks until disease progression or unacceptable toxicity. According to the investigator assessment, after a median follow-up of 17.1 months, the study met its primary endpoint with an RR of 35.2% (95% CI: 26.2–45.2) in the entire cohort. In a preplanned analysis of overall response by treatment-free interval (TFI), the RR in the subgroup of 60 patients who had a sensitive disease (TFI of 90 days or longer) was 45.0% (95% CI: 32.1–58.4) with a median duration of response of 6.2 months (95% CI: 3.5–7.3). On the contrary, the subgroup of 45 patients with poor prognosis (TFI of less than 90 days) achieved an overall response of 22.2% (95% CI: 11.2–37.1) and median duration of response of 4.7 months (95% CI: 2.6–5.6). The mPFS was 3.5 months (95% CI: 2.6–4.3) in the study population: 4.6 months (95% CI: 2.8–6.5) in patients with a sensitive disease and 2.6 months (95% CI: 1.3–3.9) in patients with resistant disease, while the mOS was 9.3 months (95% CI: 6.3–11.8) in the overall population, 11.9 months (95% CI: 9.7–16.2) in patients with a sensitive disease, and 5.0 months (95% CI: 4.1–6.3) in patients with resistant disease. In a *post-hoc* exploratory analysis, lurbinectedin activity was observed in a small group of eight patients (8%) who had received previous immunotherapy, where five of them had durable responses according to investigator assessment. The most common grade 3–4 AEs were hematological disorders, including anemia (9%), leukopenia (29%), neutropenia (46%), and thrombocytopenia (7%). Serious treatment-related AEs were recorded in 10% of patients, principally due to neutropenia and febrile neutropenia [five (5%) patients for each]. However, no treatment-related deaths occurred. Other mild or moderate toxicities were fatigue (58%), nausea (32%), decreased appetite (21%), vomiting (18%), diarrhea (15%), and pneumonia (2%). The most common biochemical abnormalities were creatinine (83%) and transaminase (alanine aminotransferase: 72%; aspartate aminotransferase: 45%) increases. It is worth to note that 47 (45%) patients were still able to receive further antitumor treatments after lurbinectedin such as paclitaxel, carboplatin, etoposide, and topotecan. The results for the subset of patient candidates in this phase II study for a platinum rechallenge according to the National Comprehensive Cancer Network (NCCN) guidelines (TFI from the first line ≥180 days) were presented by Subbiah et al. (32). The authors reported an ORR of 60.0% (95% CI: 36.1–86.9) in the 20 patients treated with lurbinectedin with TFI ≥180 days, an median duration of response (mDoR) of 5.5 months (95% CI: 2.9–11.2), and a (DCR) of 95.0% (95% CI: 75.1–99.9). Median OS was 16.2 months (95% CI: 9.6–upper level not reached) and PFS was 4.6 months (95% CI: 2.6–7.3) after a median follow-up of 15.6 months. Of note, 60.9% and 27.1% of patients were still alive after 1 and 2 years, respectively. Taken together, these data were particularly encouraging in terms of response and survival, in comparison with historical controls, in both groups of patients with resistant and sensitive disease. Furthermore, lurbinectedin showed an acceptable safety profile, with manageable reversible myelosuppression as main toxicity. However, the absence of a control group and of patients with brain involvement represents a caveat.

Based on these positive results of a phase II study, on 15 June 2020, lurbinectedin has been approved by the Food and Drug Administration (FDA) for patients with SCLC in progression on or after platinum-based chemotherapy.

Phase III studies

The ATLANTIS study is an open-label, randomized, multicenter phase III trial evaluating in second line the efficacy of the combination of lurbinectedin and doxorubicin compared to the investigator's choice of chemotherapy with CAV (cyclophosphamide/doxorubicin/vincristine) or topotecan. The study enrolled pretreated patients with histologically or cytologically confirmed diagnosis of limited- or extensive-stage SCLC whose disease progressed after one prior platinum-containing line (33). Patients should have chemotherapy-free interval (CTFI; time from the last dose of first-line chemotherapy to the occurrence of progressive disease) ≥30 days and could have received prior immune checkpoint inhibitor therapy. Other inclusion criteria were adequate hematological, renal, metabolic, and hepatic function and a washout of at least 3 weeks since last prior anticancer treatment. Patients may have received whole-brain radiotherapy and prophylactic cranial irradiation or palliative radiation and concluded at least 4 and 2 weeks ago, respectively. In the trial, 613 patients were randomized to receive doxorubicin 40 mg/m^2 on day 1, followed by lurbinectedin 2 mg/m^2 on day 1 every 21 days or physician's choice of CAV (cyclophosphamide 1,000 mg/m^2 on day 1, doxorubicin 45 mg/m^2 on day 1, and vincristine 2.0 mg total on day 1 of each

21-day cycle) or topotecan 1.5 mg/m^2 on days 1–5 every 21 days until progression of disease, investigator decision, unacceptable toxicity, or withdrawal of consent (34).. Primary granulocyte colony-stimulating factor (G-CSF) prophylaxis has been received by all patients in all treatment arms. Stratification factors of the study were ECOG PS, CTFI, baseline brain metastasis, prior immunotherapy, and investigator's choice between CAV and topotecan. The OS was the primary endpoint. The secondary endpoints were the difference in OS between the experimental arm and CAV, OS and PFS in patients with or without CNS involvement, PFS by independent review committee (IRC), ORR per IRC, and duration of response (DoR) per IRC. Unfortunately, the trial did not meet the primary endpoint: the difference in OS between two arms was not statistically significant, and it translated into a small improvement in OS, from 7.6 months for the control arm to 8.6 months for the experimental arm. No factors were associated with a benefit in OS based on stratification analysis. IRC mPFS was 4.0 for both arms, with an HR in favor of the combination of lurbinectedin/doxorubicin of 0.831 and a p-value of 0.043, which translated to an improvement of PFS at 6 months (31% vs. 24%) and at 12 months (10% vs. 4%). A benefit from the experimental arm was observed for patients with a longer CTFI (>180 days) and treated with immunotherapy plus chemotherapy in the first line (35). Similar RRs were reported in the two arms: 31% in the experimental arm vs. 29% in the standard arm, with a greater benefit from the experimental treatment for patients with a longer CTFI (49% vs. 29%). Moreover, the mDoR was longer in the lurbinectedin combination arm: 5.7 months vs. 3.8 months ($p = 0.0012$). Principal grade 3–4 AEs and laboratory abnormalities were hematological disorders, including anemia, neutropenia, febrile neutropenia, and thrombocytopenia, and they were more common in the control arm (AE grade ≥3: 75% control arm vs. 47% experimental arm), with a greater delay of the treatment in this arm (34% vs. 26%). Although this phase III trial did not meet its primary endpoint and showed comparable efficacy results in the two arms, lurbinectedin plus doxorubicin showed a superior safety and tolerability profile compared to that of the control arm with a significantly lower incidence of hematological toxicities. Moreover, the ATLANTIS trial confirmed CTFI as the most important prognostic factor for second-line SCLC treatment.

Discussion

The positive results of the pivotal phase II study of Trigo et al. (31) led to the accelerated approval by the FDA of lurbinectedin at the dose of 3.2 mg/m^2 for metastatic SCLC in progression after first-line chemotherapy. Lurbinectedin compared favorably to other second-line regimens in terms of activity, such as topotecan and CAV and demonstrated a better safety profile, representing a new treatment option for the second-line therapy of SCLC. However, several issues remain to be addressed: Why did lurbinectedin fail to improve OS in the ATLANTIS study? What is the activity of lurbinectedin in SCLC patients with brain metastases? What is the role of new combinations of lurbinectedin with other cytotoxic agents and immune checkpoint inhibitors? What will be the role of predictive factors?

The phase III trial comparing the efficacy of lurbinectedin plus doxorubicin vs. CAV or topotecan failed to demonstrate a better OS, although a superior safety and tolerability profile was shown by the experimental combination. A possible explanation of the negative results of the phase III trial could be the lower dose of lurbinectedin in combination with doxorubicin compared with the higher dose used in the phase II trial (2.0 mg/m^2 vs. 3.2 mg/m^2) that provided the maximum benefit of the drug. Unfortunately, the ATLANTIS trial did not include an experimental treatment arm of lurbinectedin as a single agent. That would have been important to confirm in a phase III trial the superiority of single-agent lurbinectedin over topotecan.

For the second question, it is unknown to date whether lurbinectedin has CNS penetration, and most of the trials with lurbinectedin have excluded patients with brain metastases. In the ATLANTIS study, patients with a history of CNS metastases were allowed and roughly 15% of patients had a baseline CNS involvement. Median OS was 4.6 and 6.6 months for patients randomized to lurbinectedin + doxorubicin and control group, respectively. Therefore, further evaluation of lurbinectedin activity in patients with CNS metastases is needed.

For the third question, new combinations of lurbinectedin with other cytotoxic agents and immune checkpoint inhibitors are currently being explored (Table 2). Two phase I trials evaluated the feasibility of the combination of lurbinectedin with paclitaxel or irinotecan in SCLC patients pretreated with at least one platinum-based chemotherapy (36, 37). The recommended dose of lurbinectedin was 2.2 mg/m^2 on day 1 in combination with paclitaxel 80 mg/m^2 on days 1 and 8 every 3 weeks. The RR in SCLC patients was 71% (67% in patients with a CTFI >90 days), with a median duration of response of 2.3 months (36). This combination was well tolerated. The most frequent toxicities were fatigue (57.1%), peripheral sensory neuropathy (57%), nausea (42.9%), and diarrhea (42.9%). The recommended dose of lurbinectedin was 2.0 mg/m^2 on day 1 in combination with irinotecan 75 mg/m^2 on days 1 and 8 plus G-CSF every 3 weeks (37). The RR in 12 SCLC patients was 25% (38% in patients with a CTFI >90 days), and the median duration of response was 4.6 months. Main toxicities were fatigue, gastrointestinal events, and hematological. The LUPER Trial (38) is a phase I/II trial involving SCLC patients who have progressed from a first-line chemotherapy-based treatment, with the aim to explore the feasibility and activity of the combination of lurbinectedin with pembrolizumab. In the phase I stage of the trial, patients will receive pembrolizumab plus lurbenectedin at a starting dose of 2.4 mg/m^2, then this dose will be escalated. In the phase II stage, patients will receive

TABLE 2 Ongoing studies with Lurbinectedin.

Trial	Phase	Diagnosis	Line of treatment	Pts	Treatment	Endpoints
LUPER NCT04358237	I/II	SCLC	Second-line	42	Lurbinectedin + pembrolizumab	ORR
NCT04610658	I/II	SCLC	Second-line	57	Ipilimumab + nivolumab + lurbenectedin	DCR
NCT04802174	I/II	SCLC and High Grade Neuroendocrine tumors	Advanced	75	Lurbinectedin and berzosertib	ORR
IMFORTE NCT05091567	III	SCLC not progressed after carboplatin, etoposide and atezolizumab	Maintenance	690	Lurbinectedin + atezolizumab vs atezolizumab	PFS, OS
Emerge 402 NCT04894591	IV	SCLC	Second-line	300	Lurbinectedin	ORR

Pts, patients; ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival.

pembrolizumab plus lurbinectedin at the dose found in the first phase. Primary outcome measure for phase II is ORR; estimated enrollment is for 42 patients. Results are awaited in September 2023. A similar phase I/II trial (39) is also involving SCLC patients who have progressed from a first-line chemotherapy-based treatment. In the phase I stage of the trial, patients will receive ipilimumab and nivolumab plus lurbenectedin at three different doses (1.5, 2.6, and 3.2 mg/m^2). In the phase II stage, patients will receive ipilimumab and nivolumab plus lurbinectedin at the recommended dose found in the first phase. The primary outcome measure for phase II is DCR; estimated enrollment is for 57 participants. Results are awaited in October 2025. Another phase I/II trial (40) is enrolling patients with SCLC and high-grade neuroendocrine tumors who have failed to respond to previous standard treatments. Patients will receive a combination of lurbinectedin and berzosertib, an ataxia telangiectasia and Rad3-related (ATR) protein kinase inhibitor. The inhibition of ATR is cytotoxic in SCLC, and berzosertib has been found to be active in combination with topotecan in this clinical setting (41). Primary outcome measure is clinical RR, calculated as the fraction of patients who will experience a partial response (PR) or a complete response (CR); estimated enrollment is for 75 participants; study completion date is awaited in December 2026. The IMforte trial (42) is a phase III trial designed for patients with ES-SCLC who have already received a first-line induction therapy with carboplatin, etoposide, and atezolizumab and are found to have at least a stable disease or ongoing response. In arm A, patients will receive the combination of atezolizumab and lurbinectedin, while in arm B, patients will receive standard maintenance therapy with atezolizumab. PFS and OS are the primary outcome measures. Estimated enrollment is for 690 participants. Results are awaited in March 2025. The EMERGE 402 trial (43) is a phase IV trial that aims to report the efficacy and the AEs tied to lurbinectedin in the second-line ES-SCLC setting. ORR is the primary outcome measure. Estimated enrollment is for 300 participants. Results are awaited in June 2024.

For the fourth question, we currently do not have biomarkers to identify SCLC patients responding to lurbinectedin or to other agents. However, Schlafen-11 (SLFN11), a predictive biomarker of response to cisplatin and to other DNA-damaging agents such as poly ADP ribose polymerase (PARP) inhibitors in multiple cancer types including SCLC, has been recently identified as a promising predictive biomarker of response also to lurbinectedin. An *in vitro* and *in vivo* study showed that cell lines with a high expression of SLFN11 protein were more sensitive to single-agent lurbinectedin (44). Moreover, in SLFN11-low SCLC cell lines that are resistant to lurbinectedin, the addition of ceralasertib, an ATR inhibitor, resensitized resistant cells, providing a rationale for combining lurbinectedin with ATR inhibitors to overcome resistance in SCLC with low SLFN11 expression. Therefore, SLFN11 immunohistochemistry (IHC) could be translated into the clinical setting and be used in clinical studies with lurbinectedin in SCLC. Moreover, the recent identification by Rudin et al. (45) of four different molecular subtypes of SCLC defined by differential expression of four key transcription regulators highlights the heterogeneity of SCLC and could allow a better customization of treatments.

In conclusion, lurbinectedin has demonstrated significant activity as a single agent in second-line therapy of SCLC, especially in platinum-sensitive patients, but failed to demonstrate an improvement in OS when combined with doxorubicin compared with CAV or topotecan. New combinations of lurbinectedin with other cytotoxic drugs and with immune checkpoint inhibitors are currently under investigation. The results of these studies should better define the optimal clinical application of lurbinectedin in SCLC.

Author contributions

Conceptualization, AnM and AlM; writing—original draft preparation, AnM, VS, GC, GP, and AlM; writing—review and editing, AnM, VS, GC, GiuP, AgM, RC, GE, FL, EM, CM, PM, GT, RDC, CP, MP, GiuP, NN, and AlM; supervision, AlM; All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

AlM declares the following conflicts of interest: Speaker's fee or advisory board: MSD, BMS, Boehringer, Pfizer, Roche, AstraZeneca, Novartis, Takeda, Eli-Lilly. NN declares the following personal financial interests (speaker's fee and/or advisory boards): MSD, Qiagen, Bayer, Biocartis, Incyte, Roche, BMS, MERCK, Thermofisher, Boehringer Ingelheim, Astrazeneca, Sanofi, Eli Lilly; Institutional financial interests (financial support to research projets):MERCK, Sysmex, Thermofisher, QIAGEN, Roche, Astrazeneca, Biocartis. Non-financial interests:President, International Quality Network for Pathology (IQN Path); President, Italian Cancer Society (SIC).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Real-world eligibility for platinum doublet plus immune checkpoint inhibitors in extensive-stage small-cell lung cancer

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Introduction: Small cell lung cancer (SCLC) is a rapidly progressing aggressive malignancy. Durvalumab in CASPIAN and atezolizumab in IMPower133 were found to improve overall survival (OS) for extensive-stage SCLC. Here we evaluate the proportion of real-world ES SCLC patients who may be eligible for first-line immune checkpoint inhibitor (ICI) with platinum doublet.

Methods: A retrospective cohort analysis was conducted of referred ES SCLC between 2015 and 2017 in British Columbia, Canada. Patient demographics, staging, treatment, and survival data were collected through the Cancer Registry. Retrospective chart review was completed to extract past medical history and missing variables. CASPIAN/IMPower133 excluded patients with autoimmune diseases, active infection, and performance status (PS) ≥2.

Results: Between 2015 and 2017, 349 patients were diagnosed with ES SCLC. In patients who received platinum-doublet chemotherapy (n=227), 15 had medical contraindication to ICI: inflammatory bowel disease (n=4), rheumatoid arthritis (n=4), idiopathic pulmonary fibrosis (n=3), lupus (n=1), Sjogren's (n=1), Takayasu arteritis (n=1), and active tuberculosis (n=1). ECOG PS was 0–1 in 96 (45%), PS was 2 in 61 (29%), and ≥3 in 51 (10%). Prior to cycle 1, 82 (36%) patients were eligible for ICI in addition to platinum doublet, 23% of the entire ES population. After cycles 1 and 2, additional 15 (7%) and 8 (4%) patients became PS 0–1, respectively. mOS for ES SCLC who received first-line platinum doublet, non-platinum chemotherapy, and best supportive care was 8.4 1.9 and 1.5 months (p<0.001).

Discussion: By CASPIAN/IMpower133 trial eligibility, only 36% of our real-world platinum-treated patients would have been eligible for the addition of ICI, which is 23% of the entire ES population in one Canadian province. After one or two cycles of chemotherapy, an additional 11% of patients showed PS improvement to 0–1. While the results of CASPIAN/IMpower133 are

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practice-changing, the majority of the patients will not meet clinical trial eligibility and clinical trials including patients with poor PS are necessary.

KEYWORDS

small-cell lung cancer (SCLC), real world, durvalumab, atezolizumab, platinum doublet, immunotherapy

Introduction

Lung cancer continues to be the global leading cause of cancer-related death (1). Although non-small-cell lung cancer (NSCLC) accounts for most lung cancer diagnoses, there have been significant treatment advancements over the last 10 years including the maintenance pemetrexed, identification and targeting of driver mutations, and use of immune checkpoint inhibitors (ICIs) each resulting in improved survival in treated patients (2–5). Unfortunately, similar advances in small-cell lung cancer (SCLC) have not been observed with first-line treatment remaining platinum etoposide for over two decades.

SCLC accounts for just 13% of new lung cancer diagnosis and is characterized as a rapidly progressive neuroendocrine tumor with two-thirds of patients diagnosed with extensive-stage (ES) disease (6). Smoking continues to be the primary risk factor for SCLC with >98% of new SCLC cases having a smoking history (7). ICIs were first evaluated in SCLC post platinum-based chemotherapy. Pembrolizumab was approved as ≥2 lines of therapy based on an overall response rate of 19.3% by the Food and Drug Administration (FDA) (8). Results from second-line nivolumab were originally encouraging and received FDA-accelerated approval; however, confirmatory trials did not find an improved overall survival (OS) and thus the indication was withdrawn (9, 10).

First-line treatment has been unchanged for multiple decades given the high responsiveness to first-line platinum-based chemotherapy. Current SCLC treatment is not guided by molecular profiling due to the lack of targetable mutations (11). This has led to the evaluation of ICI in the first-line setting in conjunction with platinum-based chemotherapy in multiple trials. CASPIAN evaluated platinum-based chemotherapy alone and platinum-based chemotherapy in addition to durvalumab with and without tremelimumab. Platinum-based chemotherapy plus durvalumab improved OS compared with platinum-based chemotherapy alone from 10.3 to 13.0 months; however, the addition of durvalumab and tremelimumab did not improve OS (12, 13). IMpower133 similarly considered atezolizumab in addition to platinum-based chemotherapy with an improved OS from 10.3 to 12.3 months (14). KEYNOTE-604 evaluated platinum-based chemotherapy with or without pembrolizumab, which found a non-significant trend toward improved OS (15). However, in the management of real-world SCLC patients, contraindications limit the use of ICIs, most notably history of autoimmune diseases, active infection, and poor performance status (PS).

SCLC patients continued to have poor outcomes and unmet systemic therapy needs. ICIs, in addition to platinum-doublet chemotherapy, are the new first-line standard of care in ES or relapsed SCLC; however, it is not known what proportion of ES SCLC patients will be eligible to benefit from combination therapy. Here we retrospectively evaluate the eligibility of first-line ICIs in a pre-ICI population to forecast the expected use of ICIs in a Canadian landscape, which currently does not publicly fund ICIs in addition to platinum doublet.

Methods

Population

British Columbia has a population of 5.1 million people with centralized cancer-care delivery through six cancer centers and over 40 satellite community oncology network sites. The Outcomes and Surveillance Integration System contains diagnosis information, baseline characteristics, and patient outcomes for all referred lung cancer patients in British Columbia. Currently, in Canada, ICIs in addition to platinum doublet are not reimbursed through the public medical system, and durvalumab is only available with the addition of chemotherapy through a patient access program.

A retrospective cohort study was conducted at BC Cancer of patients diagnosed with SCLC between 1 January 2015 and 31 December 2017. Baseline patient demographics, Eastern Cooperative Oncology Group (ECOG) PS, cancer staging, treatment (chemotherapy and radiotherapy), and survival were collected using the Outcomes and Surveillance Integration System, electronic medical records, and billing administration database for chemotherapy. Past medical history and missing data were obtained through a manual chart review.

Statistical analysis

Statistical Package for the Social Sciences software version 28 was used to produce descriptive statistics using chi-square and Mann–Whitney U tests. OS was calculated from date of diagnosis using the Kaplan–Meier curves and compared using the log rank test. Patients were censored at last known follow-up. Statistically significant p-value were set at <0.05.

Ethics statement

This study was conducted with the University of British Columbia/BC Cancer Research Ethics Board approval (H19-02381). A waiver of consent was granted to extract and analyze data for this retrospective review.

Results

Between 2015 and 2017, 519 patients were diagnosed with SCLC of which 349 (67%) were diagnosed with ES SCLC. The baseline characteristics are found in Table 1. Within the population, 2% of the patients were lifelong non-smokers with 62% actively smoking at the time of cancer diagnosis. Baseline PS was 0–1 for 114 (33%) patients, PS 2 for 90 (26%) patients, and ≥3 for 139 (40%) patients of the population.

Systemic therapy was administered to 253 (72%) patients with 227 (90%) patients receiving first-line platinum-doublet chemotherapy (Table 1 and Figure 1). Of the ES patients who received first-line platinum doublet, 34% received cisplatin, 55% carboplatin, and 11% switched between cisplatin and carboplatin. Only one cycle of platinum doublet was received by 37 patients, and 10 patients received only two cycles. First-line single-agent etoposide was administered to 24 (9%) patients, and the remaining 96 (28%) received best supportive care alone. Two lines of therapy were received by 155 (44%) patients and ≥3 lines by 4%.

Medical contraindications to ICIs were found in 15 patients who received platinum-doublet chemotherapy. These included inflammatory bowel disease (n=4), rheumatoid arthritis (n=4), idiopathic pulmonary fibrosis (n=3), lupus (n=1), Sjogren's (n=1), Takayasu arteritis (n=1), and active tuberculosis (n=1). There were additional five patients who had autoimmune diseases, namely mild psoriasis (n=3) and stable hyperthyroidism (n=2) that were considered ICI eligible.

In patients who received platinum-doublet chemotherapy, the baseline ECOG PS was 0–1 in 96 (45%), 2 in 61 (29%), and ≥3 in 51 (10%) (Figure 2). Prior to cycle 2, 15 (7%) patients with ECOG PS ≥2 improved to PS 1. Prior to cycle 3, eight (4%) patients that were PS ≥2 prior to cycle 2 improved to PS 1.

TABLE 1 Baseline characteristics and treatment history of extensive stage small cell lung cancer patients.

N (%)	Extensive stage (n = 349)
Age (median), years	68
Sex	
Male	173 (49%)
Female	176 (51%)
Smoking Status	
Never	6 (2%)
Former	124 (35%)
Active	215 (62%)
Unknown	4 (1%)
Smoking years (median)	50
ECOG PS	
0-1	114 (33%)
2	90 (26%)
3-4	139 (40%)
Unknown	6 (2%)
CNS Metastases	118 (34%)
First line chemotherapy	
Platinum doublet	227 (65%)
Cisplatin doublet	76 (34%)
Carboplatin doublet	125 (55%)
Switch platinum doublet	26 (11%)
Single agent etoposide	24 (7%)
Other	2 (1%)
Second line chemotherapy	66 (19%)
Platinum doublet	28 (8%)
Single agent etoposide	4 (1%)
Topotecan	13 (4%)
Irinotecan	11 (3%)
CAV	10 (3%)
Third line chemotherapy	15 (4%)
Platinum doublet	3 (1%)
Single agent etoposide	0
Topotecan	5 (1%)
Irinotecan	4 (1%)
CAV	1 (<1%)
Thoracic Radiation	130 (37%)
PCI	13 (4%)
WBRT	117 (34%)

N, number; ECOG PS, Eastern Cooperative Oncology Group Performance Status, CNS, central nervous system; PCI, prophylactic cranial radiation; WBRT, whole brain radiation.

Prior to cycle 1, 96 (45%) patients were eligible for ICIs in addition to platinum doublet if inclusion criteria included ECOG PS 0–1, 28% of the entire ES population. Baseline characteristics were similar between eligible and ineligible patients (Table 2). If eligibility was extended to include ECOG PS 2, an additional 61 (29%) patients would have become eligible. With the inclusion of PS 0–1, after cycle 1 was administered, another 15 (7%) patients would have become eligible, and after cycle 2, an additional 8 (4%) patients would have been eligible.

The median OS for ES SCLC who received first-line platinum-doublet chemotherapy, non-platinum-doublet chemotherapy, and best supportive care was 8.4 (95% CI 7.6–9.3), 1.9 (95% CI 0.9–2.9),

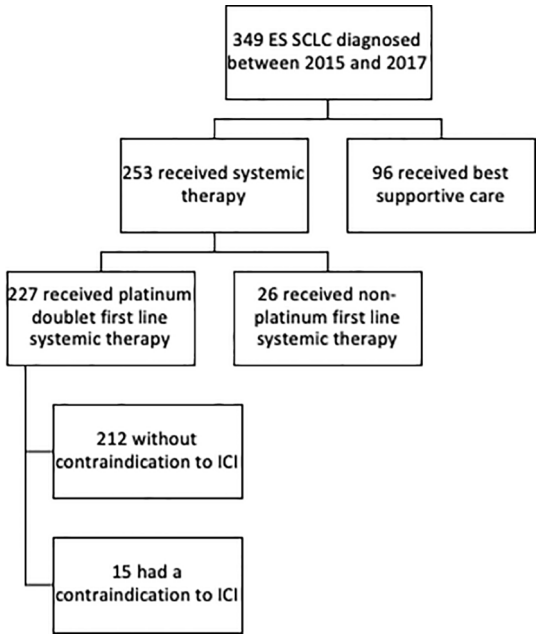

FIGURE 1
Consort diagram of extensive stage small cell lung cancer dataset assessing eligibility for ICI.

and 1.5 (95% CI 1.2–1.9) months (p<0.001), respectively. The median OS for PS 0–1, 2, and ≥3 for patients who received platinum doublet was 10.6 (95% CI 8.5–12.8), 6.0 (95% CI 4.3–7.6), and 7.0 (95% CI 4.4–9.5) months (p<0.001), respectively (Figure 3).

Discussion

Our real-world SCLC population demonstrates that a minority of patients meet the clinical trial eligibility criteria for platinum doublet plus ICIs once this is publicly reimbursed in Canada. While a small subset of patients was excluded due to immunotherapy contraindications, the majority of the patients were not eligible on the basis of poor PS. Consequently, the evidence from the large phase III clinical trials, CASPIAN, and IMpower133, needs to be interpreted cautiously due to the extrapolation of the benefits to the real-world symptomatic ES SCLC patient.

Over half of our real-world ES PS population had an ECOG PS of ≥2 at diagnosis. While chemotherapy and ICI treatments have been widely used in good PS patients with lung cancer, due to the evidence in NSCLC, the feasibility is much less clear in PS 2 patients and is not currently informed by randomized clinical trials (16). A prospective randomized phase II clinical trial of ICIs with or without carboplatin and paclitaxel in advanced NSCLC with PS 2 is yet to be reported (17). In NSCLC consensus guidelines, expert opinion suggests that there are concerns regarding the safety and tolerability of combined chemotherapy plus single-agent immunotherapy with poor PS (18). Given the concerns regarding the toxicity of combination therapy with NSCLC, ES SCLC patients with PS ≥2 should be treated cautiously.

NSCLC and SCLC differ in the timing and rate of response to chemotherapy with the latter being more responsive in a shorter time frame (5). With this disease behavior, one may use combination therapy despite poor PS with the expectation that the disease would respond rapidly to chemotherapy. In our study, we examined the improvement in PS after one cycle of chemotherapy in patients with pretreatment PS ≥2 and noted that 17% improved to PS 0–1. As clinical improvement may take time, ECOG PS after two cycles was also collected and improved to PS 0–1 from PS ≥2 occurred in 18% of the patients who received at least three cycles. The significant attrition from cycle to cycle is notable; 17% of all platinum-treated patients only received one cycle, a reflection of the disease process and the tolerability of platinum doublet alone in this symptomatic

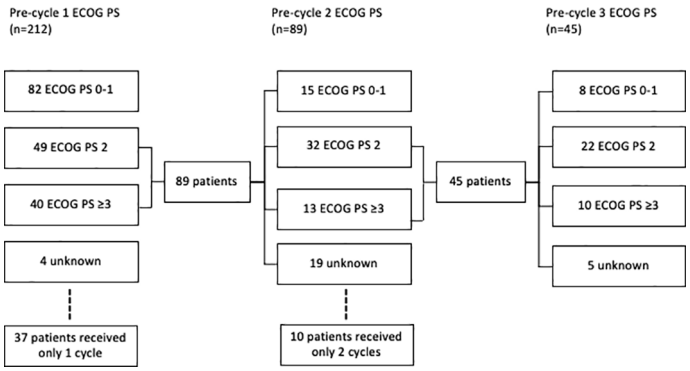

FIGURE 2
Consort diagram of ECOG of small cell lung cancer patients treated with platinum doublet prior to cycle 1, 2, and 3.

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TABLE 2 Extensive stage small cell lung cancer patients who received first line platinum doublet by eligibility for first line chemotherapy with immune checkpoint inhibitor.

N = 227 (%)	Ineligible for platinum with ICI (n = 131)	Eligible for platinum with ICI (n = 96)	p-value
Age (median), years	66	66	0.263
Sex			
Male	58 (44%)	54 (56%)	0.075
Female	73 (56%)	42 (44%)	
Smoking Status			
Never	1 (1%)	3 (3%)	0.302
Former	41 (31%)	34 (35%)	
Active	89 (68%)	59 (62%)	
Smoking years (median)	50	40	0.089
ECOG PS			
0-1	6 (4%)	96 (100%)	<0.001
2	65 (50%)		
3-4	56 (43%)		
Unknown	4 (3%)		
CNS Metastases	57 (44%)	45 (47%)	0.615

N, number; ICI, immune checkpoint inhibitor; ECOG PS, Eastern Cooperative Oncology Group Performance Status, CNS, central nervous system

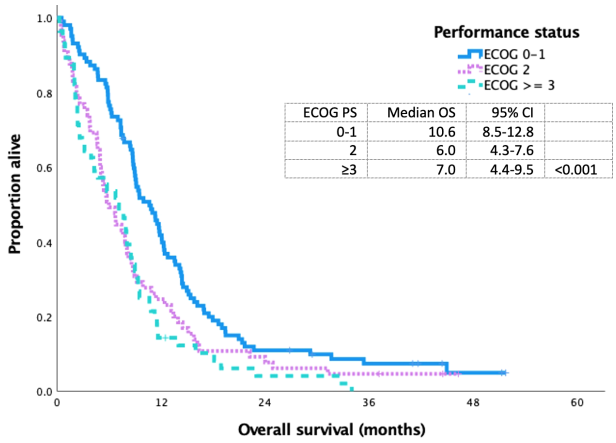

FIGURE 3
Kaplan Meier curve of extensive stage small cell lung cancer patients treated with platinum doublet chemotherapy based on ECOG 0-1 VS 2 VS ≥ 3.

population. A careful extension of the CASPIAN and IMpower133 data to selected PS ≥2 patients may be appropriate as treatment may result in a brisk improvement in the functional status (19).

An alternative strategy for poor PS patients may be a phased-in approach with platinum chemotherapy alone for the first cycle, followed by the addition of immunotherapy in the second cycle. It is unclear if similar survival benefits to the large phase III studies will be realized with this pragmatic approach. Similar to prior studies, in our cohort, poor PS remained a significant negative prognostic determinant, so it would be difficult to determine whether the difference in outcomes was a consequence of the PS or the alternate treatment scheme (7, 11). A stepped approach to treatment may mitigate toxicity risks and enable appropriate patients to receive ICIs with their platinum backbone.

Importantly, it must also be recognized that patients with poor PS (≥2) are not represented in CASPIAN or IMpower133; in addition, there is an important underrepresentation of older adults. This results in uncertainty of the clinical benefit in these patients (11). Additionally, the inclusion of patients with brain metastases in ES SCLC trials is important given their high prevalence (20). CASPIAN allowed the enrollment of patients with brain metastases; however, it required patients to be either

asymptomatic or treated off steroids and anticonvulsants (12). IMpower133 required asymptomatic brain metastases to be treated prior to enrollment (14). This real-world study demonstrates that clinical trial eligibility criteria restrict enrollment and do not reflect the average patient with ES SCLC, compromising the external validity. This forces the clinician to practice with an evidence gap for patients who have a very narrow therapeutic window. While real-world evidence can act to supplement our knowledge, more pragmatic clinical trial design is needed for this symptomatic subset of lung cancer patients (18).

SCLC is a heterogenous malignancy with four subtypes defined by varied expression of transcription factors (21, 22). Conclusive biologic, molecular, and clinical markers have not been identified to help identify which ES SCLC will most benefit from ICIs; however, preliminary findings suggest that patients with an inflamed gene signature, based on transcription factors, may obtain the most benefit (21). One ongoing challenge with SCLC is the lack of targetable mutations due to the prevalence of tumor suppressor gene deletions and loss of function mutations as opposed to activating mutations (22). Currently, molecular profiling does not impact treatment selection, however it may in the future.

Our study is limited by the retrospective nature of this analysis. Past medical history and ECOG PS were collected through a manual chart review, which was limited by the accuracy of physician documentation. In addition, the other known prognostic factors for SCLC such as weight loss and laboratory values were not routinely collected. Our strengths include the real-world cohort representing a wide variety of baseline health states from a diverse geographic and socioeconomic population.

Conclusion

Our real-world SCLC population demonstrates that by CASPIAN and IMpower 133 trial eligibility, up to 36% of patients who received platinum doublet would have been eligible for the addition of ICIs, 23% of the entire ES population. After one or two cycles of chemotherapy, an additional 11% of patients showed PS improvement to 0–1. While the results of the phase III studies are practice-changing, a significant proportion of ES patients do not meet the eligibility criteria. Clinical trials that are inclusive of poor PS patients will help address the evidence gap and will be practice-informing.

Data availability statement

The datasets presented in this article are not readily available because of ethics approval. Requests to access the datasets should be directed to cho@bccancer.bc.ca.

Ethics statement

This study was conducted with University of British Columbia/BC Cancer Research Ethics Board approval (H19-02381). A waiver of consent was granted to extract and analyze data for this retrospective review.

Author contributions

RR was involved with data acquisition and data interpretation, drafted the original manuscript, and provided final approval of the manuscript. BL was involved with data acquisition and input into the original manuscript, and provided final approval of the manuscript. ZA-H was involved with data acquisition and input into the original manuscript, and provided final approval of the manuscript. CH was involved with research conception and design, data acquisition, and data interpretation, aided to the drafted original manuscript, and provided final approval of the manuscript version.

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Conflict of interest

RR declared research grants from AstraZeneca. CH declared research grants from AstraZeneca, EMD Serono, and Roche. CH declared honoraria for advisory boards from AbbVie, Amgen, AstraZeneca, Bayer, BMS, Eisai, EMD Serono, Merck, Novartis, Pfizer, Roche, and Takeda.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Lung adenocarcinoma relapse with emerging EGFR mutation following complete response of small cell lung cancer warrants routine re-biopsy: A case report

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Transformation of small cell lung cancer (SCLC) to lung adenocarcinoma (LUAD) is rarely reported. Here, we report a case initially presented with SCLC and was diagnosed as LUAD when the lesion relapsed at the same site. A 56-year-old patient with SCLC who received etoposide and cisplatin chemotherapy combined with radiotherapy achieved a complete radiological response. After 28 months of stable disease, a computed tomography scan revealed a new lesion at the same site as the primary tumor. Pathological examination suggested a LUAD with an emerging *EGFR* exon 19 deletion. The patient was then treated with icotinib and achieved a near-complete radiological response. Nineteen months later, the patient developed resistance caused by *EGFR* T790M mutation and received treatment with osimertinib. At the last follow-up in January 2022, the patient was symptom-free. This case warrants re-biopsy and genetic testing as a routine operation when SCLC relapses at the same site as the primary tumor for an extended period, and prospective investigation is required.

KEYWORDS

non-small cell lung cancer, small cell lung cancer, lung adenocarcinoma, histological transformation, EGFR

Introduction

Non-small cell lung cancer (NSCLC) transformation to small cell lung cancer (SCLC) following treatment with tyrosine kinase inhibitors (TKIs) or immunotherapy has frequently been reported to be a mechanism of acquired resistance (1, 2). The post-treatment occurrence of histological transformation from NSCLC to SCLC is up to 14%

(3), but cases of SCLC transformation to lung adenocarcinoma (LUAD) are rare (4, 5), and the clinical significance and best treatment option underlying such cases are still unknown.

Herein, we report a case of SCLC-LUAD histological change. To our knowledge, this is the first report of *EGFR* mutant LUAD transformed from *EGFR* wild-type SCLC after chemoradiotherapy. We present the following case following the CARE reporting checklist.

Case report

A 56-year-old man with 30-pack-year smoking history experienced a dry cough and shortness of breath during exercise for one month. He had well-controlled diabetes for eight years by metformin and no significant family history of cancer. Chest computed tomography (CT) revealed a left hilar mass with obstructive pneumonia of the left upper lobe (Figure 1A). Hematoxylin-eosin (H&E) staining of the bronchoscopic specimens showed small round and poorly differentiated cells without non-small cells (Figure 2A). Immunohistochemistry (IHC) staining showed that the biopsy was negative for Napsin-A, a marker for LUAD (Figure 2B). Taken together, this patient was diagnosed with left central SCLC (T3N0M0, limit-stage, stage IIB, according to the eighth edition American Joint Commission on Cancer classification criteria) (Figure 3). A targeted comprehensive genomic profiling (CGP), which contains 654 cancer-related genes, was performed on the tumor tissue by next-generation sequencing (NGS). The CGP assay detected 18 mutations, such as *TP53* (c.159G>A, p.W53*, 75.8%) and *RB1* (c.2239dupG, p.E747Gfs*4, 70.4%) (Table 1). Concomitant inactivation of p53 and RB, which is nearly universally in SCLC, is deemed as an essential initiating molecular event (6, 7). The patient, an employee with national medical insurance, was not religious and actively cooperated during the diagnosis.

Six cycles of etoposide and cisplatin (EP) concurrent with thoracic radiotherapy followed by prophylactic cranial irradiation were given to the patient (etoposide: 120 mg/m^2 for the first 2 cycles, 100 mg/m^2 for the rest of 4 cycles, on days 1-3; cisplatin: 75 mg/m^2 days 1-3; chest radiation therapy: 55Gy/25F, concurrently with the 3-4 cycles of EP treatment; brain radiation therapy: 25Gy/10F, between the fourth and fifth cycles of EP treatment), and resulted in a complete radiological response (CR), according to the Response Evaluation Criteria in Solid Tumors version 1.1 (Figure 1B, 3). The patient showed good tolerance to chemoradiotherapy with a transient gastrointestinal response.

Twenty-eight months after CR, the patient was readmitted with a dry cough. A CT scan revealed a new lesion at the same site of the primary tumor (Figure 1C). H&E staining of the bronchoscopic re-biopsy displayed adenoid structure (Figure 2C). Napsin-A was positive in the IHC analysis (Figure 2D). The results of CT, H&E staining, and IHC suggested the diagnosis with left central LUAD (T4N0M0, stage IIIA). The CGP assay on bronchoscopic biopsy revealed

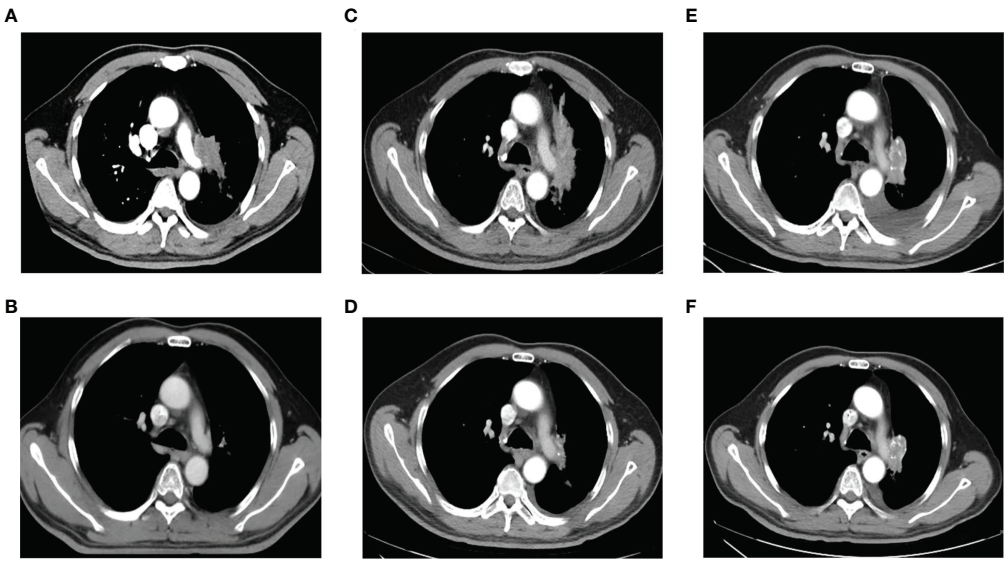

FIGURE 1
Chest computed tomography at initial diagnosis, during and after treatment. **(A)**. At initial diagnosis. **(B)**. After treatment of EP combined with radiotherapy. **(C)**. At the first relapse. **(D)**. After treatment with chemotherapy plus icotinib. **(E)**. After icotinib resistance. **(F)**. After treatment of osimertinib.

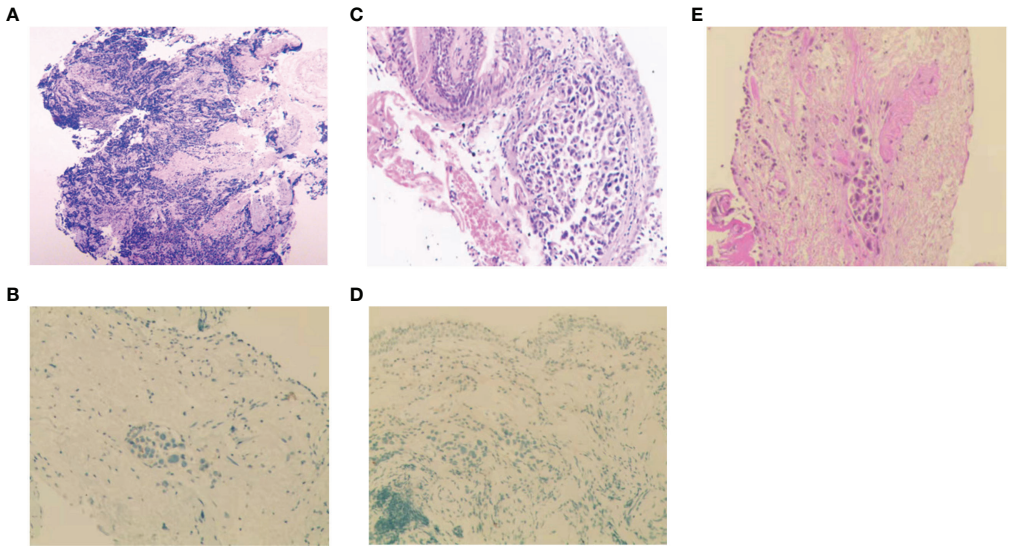

FIGURE 2
Histopathologic analysis at initial diagnosis, during and after treatment. **(A-D)** H&E staining and IHC analysis of Napsin-A at initial diagnosis **(A, B)**, at the first relapse **(C, D)**. **(E)** H&E staining after icotinib resistance.

two mutations, *EGFR* (c.2240_2257del, p.L747_P753delinsS, 2.22%) and *TP53* (c.1010G>C, p.R337P, 4.26%) (Table 1). Based on the diagnosis, he was given the first-generation EGFR-TKI icotinib plus chemotherapy (pemetrexed: 500 mg/m^2, days 1; cisplatin: 75 mg/m^2, days 1-3; icotinib: 125 mg, t.i.d., p.o.) (Figure 3). After six cycles of chemotherapy plus icotinib followed by icotinib maintenance, the patient achieved a near-complete radiological response (Figure 1D, 3).

After 19 months of stable disease, a chest CT disclosed a mass occupying the left upper hilar portion with obstructive pneumonia (Figure 1E). Histopathological analysis of the bronchoscopic re-biopsy reported LUAD structure (Figure 2E). Taken together, the diagnosis of left central LUAD was confirmed (T4NXM1, stage IVA) (Figure 3). *EGFR* T790M was detected in blood plasma by the NGS CGP assay. The patient began to take osimertinib (80mg, q.d., p.o.) and achieved a partial response (Figures 1F, 3).

During the 6-year treatment period, the patient adopted the best self-care and went to the outpatient clinic monthly for further consultation. Any changes in medication and symptoms were recorded, and there were no uncontrolled adverse events.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional review board of Hangzhou Cancer Hospital and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

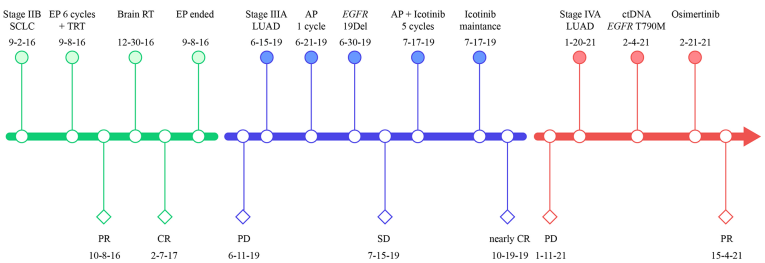

FIGURE 3
Treatment timeline of our case. SCLC, small-cell lung cancer; LUAD, lung adenocarcinoma; EP, etoposide plus cisplatin; TRT, thoracic radiation therapy; RT, radiation therapy; AP, cisplatin plus pemetrexed; PR, partial response; CR, complete response, PD, progressive disease; EGFR, epidermal growth factor receptor; 19Del, exon 19 deletion.

TABLE 1 Genetic variants in the case revealed by the CGP assay.

	Gene	Variant type	Exon	HGVSc	HGVSp	VAF/CNV
SCLC	*BARD1*	SNV	4	c.1178G>T	p.G393V	51.95%
	BCR	SNV	16	c.2954A>C	p.K985T	31.43%
	CCND3	SNV	5	c.732G>T	p.Q244H	76.15%
	FANCA	SNV	23	c.2149A>T	p.M717L	64.29%
	MERTK	SNV	15	c.2021T>C	p.M674T	19.29%
	NF1	SNV	33	c.4420G>A	p.A1474T	45.92%
	POT1	SNV	7	c.220A>G	p.K74E	18.74%
	RB1	Insertion	22	c.2239dupG	p.E747Gfs*4	70.44%
	RPTOR	Deletion	30	c.3574_3582 delGTCTACGAC	p.V1192_D1194 delVYD	12.88%
	TP53	SNV	4	c.159G>A	p.W53*	75.84%
	CALR	CNV	NA	NA	NA	4.62
	CDK4	CNV	NA	NA	NA	4.95
	GNA13	CNV	NA	NA	NA	4.78
	IRS2	CNV	NA	NA	NA	72.98
	KLF5	CNV	NA	NA	NA	63.79
	MCL1	CNV	NA	NA	NA	4.62
	PPM1D	CNV	NA	NA	NA	4.77
	RPTOR	CNV	NA	NA	NA	4.59
First Relapse	*EGFR*	Deletion	19	c.2240_2257 del	p.L747_P753 delinsS	2.22%
	TP53	SNV	10	c.1010G>C	p.R337P	4.26%
Second Relapse	*EGFR*	Deletion	19	c.2240_2257 del	p.L747_P753 delinsS	0.57%
	EGFR	SNV	20	c.2369C>T	p.T790M	0.62%

CGP, comprehensive genomic profiling; HGVS, Human Genome Variation Society; VAF, variant allel frequency; CNV, copy number variation; NA, not available.

Discussion

Transformation to LUAD from SCLC is rarely reported (4, 5, 8–10) Abeloff et al. documented the emergency of non-small cell components by H&E staining in autopsies of SCLC patients (4). Wang et al. reported a case of SCLC-LUAD transformation after the initial tumor vanished (5). Morinaga et al. described a case of LUAD with *EGFR* 19Del. The patient underwent LUAD-SCLC-LUAD pathological change with the same EGFR mutation (10). Sequist et al. presented a LUAD patient with *EGFR* L858R, who also underwent a pathological change of LUAD-SCLC-LUAD with *EGFR* L858R maintained (9). Takagi et al. recorded a case who underwent sequential LUAD, SCLC and LUAD pathological change with *EGFR* L861Q retained (8) To the best of our knowledge, our case firstly reported SCLC-LUAD transformation with emerging *EGFR* 19Del.

Ouadah et al. suggested that neuroendocrine cells are stem cells that can give rise to alveolar type 2 cells (11). Oser et al. suggested that activation of EGFR signaling could be essential for the fully differentiated alveolar-cell phenotype, and SCLC could resume adenocarcinoma histology when the EGFR signaling was restored (3). And in our case, in addition to the IHC biomarker, *EGFR* E19Del was detected when the transformation to LUAD occurred after CR following EP treatment, which may explain the SCLC-LUAD change.

It should be noted that the diagnosis was based on biopsy, H&E staining, and IHC. However, a biopsy is subjected to spatial selection bias due to intratumor heterogeneity. In this case, there was no shared mutations between the initial and relapsed tumor tissues in the targeted CGP assay by NGS (Table 1). Although the treatment with EP combined with radiotherapy was very effective, and the patient achieved a complete radiological response, we could not rule out the probability of a mixed histologic type in the initial tumor, in which case there might be a mixture of SCLC and LUAD in the initial diagnosis, but SCLC was predominant. After chemoradiotherapy, adenocarcinoma gradually became prominent, with SCLC dwindling.

It is also possible that the LUAD might be a metachronous primary tumor since the genetic variations detected in the SCLC and LUAD biopsy tissues of this patient were utterly different (Table 1). However, LUAD is more commonly localized peripherally than in a central location. The emerging LUAD appeared at the same site of the original lesion (both by imaging and bronchoscopy) significantly lowers the possibilities of metachronous primary tumor.

Patients with extensive-stage SCLC usually respond well to first-line chemoradiotherapy, but the resistance often develops within 6 months (12). However, few patients have extended progression-free survival over two years (13). Chen et al. reported that patients with *TP53* mutation or more than five mutations have more prolonged progression-free survival to first-line chemotherapy (14). In this case, the patient with SCLC had 18 mutated genes, including *TP53* and *RB1* which are nearly universally inactivated in SCLC (6).

Although icotinib, approved by the National Medical Products Administration, is a standard of care in China for EGFR mutant NSCLC (15, 16), icotinib plus chemotherapy is not yet. However, the co-mutation of TP53 (17, 18) and low frequency of EGFR mutation (4.26%) (19)of the patient might dampen the benefit of EGFR TKI alone. Meanwhile, the result of phase 3 study NEJ009 suggested EGFR TKI plus chemotherapy might benefit patients with EGFR patient (20). In addition, our case had shown good response and tolerance to chemoradiotherapy. Taken together, the patient was given the first-generation EGFR-TKI icotinib plus chemotherapy.

Conclusion

In conclusion, we reported a rare case with histological evolution from *EGFR* wild-type SCLC to *EGFR* mutant LUAD. Re-biopsy and genetic testing provided a more accurate diagnosis, which guided the choice of subsequent precise treatments, therefore, it should be recommended as a routine operation when SCLC relapses at the same site of the primary tumor after a long remission period.

Data availability statement

The datasets presented in this article are not readily available because of ethical/privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

BX contributed to the conception and design and provided administrative support. MZ contributed to treat the patient, analysis and wrote the manuscript. YT and JW analyzed the data. All authors contributed to the article and approved the submitted version.

Conflict of interest

Author JW is, and QL was employed by Berry Oncology Corporation Beijing, China.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Unweaving the mitotic spindle: A focus on Aurora kinase inhibitors in lung cancer

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Lung cancer is one of the most aggressive malignancies, classified into two major histological subtypes: non-small cell lung cancer (NSCLC), that accounts for about 85% of new diagnosis, and small cell lung cancer (SCLC), the other 15%. In the case of NSCLC, comprehensive genome sequencing has allowed the identification of an increasing number of actionable targets, which have become the cornerstone of treatment in the advanced setting. On the other hand, the concept of oncogene-addiction is lacking in SCLC, and the only innovation of the last 30 years has been the introduction of immune checkpoint inhibitors in extensive stage disease. Dysregulation of cell cycle is a fundamental step in carcinogenesis, and Aurora kinases (AURKs) are a family of serine/threonine kinases that play a crucial role in the correct advance through the steps of the cycle. Hyperexpression of Aurora kinases is a common protumorigenic pathway in many cancer types, including NSCLC and SCLC; in addition, different mechanisms of resistance to anticancer drugs rely on AURK expression. Hence, small molecule inhibitors of AURKs have been developed in recent years and tested in several malignancies, with different results. The aim of this review is to analyze the current evidences of AURK inhibition in lung cancer, starting from preclinical rationale to finish with clinical trials available up to now.

KEYWORDS

lung, cancer, oncology, Aurora kinase (AURK), mitosis, AURK inhibitors

Introduction

Despite the continuous progress in understanding its biology and discovering new potential targets, lung cancer is responsible for the highest number of cancer-related deaths in Italy (1). Non-small cell lung cancer (NSCLC) represents about 85% of lung cancer new diagnoses and it is a heterogeneous disease, often characterized by the presence of a driver mutation (oncogene-addicted disease) for which a targeted drug is available. The introduction of immune checkpoint inhibitors (ICIs) has changed the history of non-oncogene addicted disease: immunotherapy, alone or in combination with chemotherapy, represents the standard first-line treatment, reaching the biggest benefit in patients with strong expression of PD-L1 (5 years OS: 31.9% vs 16.3% with platinum-based chemotherapy) (2).

Small-cell lung cancer (SCLC) represents the other 15% of lung cancer diagnoses; it is an aggressive disease, with a high proliferation rate and a high dissemination potential, in fact most cases are diagnosed at an advanced stage. Genomic profiling of SCLC identified p53 and pRB as the most frequently altered genes (3), but no targeted therapies are available up to now. Therefore, SCLC is treated as a single entity and platinum-based chemotherapy has been considered the standard of care for the last thirty years. Since the results of IMpower133 and CASPIAN trials, immunotherapy in combination with platinum-etoposide has become the new recommended first-line treatment; although the global benefit of ICIs is small (ΔmOS=2 months), about 15-18% of patients experience a long-term benefit, being alive at 18 months after treatment start (4, 5).

Due to the limited options available after the failure of first-line regimens, particularly in SCLC, research efforts must focus on expanding the therapeutic strategies for lung cancer. An increasing attention has been focused on cell cycle regulators targeting drugs. One of the main actors in cell cycle are Aurora kinases (6, 7). Their importance was initially highlighted by genetic studies on mutants demonstrating their role in the abnormal mitotic spindle formation (from which the name "aurora", resembling aurora borealis) and cytokinesis failure. In this review, we will focus on the rationale of targeting Aurora kinases in lung cancer, disclosing the results of the available clinical trials.

Biology of Aurora kinases

Aurora kinases (AURKs) are a family of serine/threonine kinases that plays fundamental roles in cell cycle, particularly in mitotic spindle formation and in chromosome segregation. In mammals, there are three known members of this family: Aurora kinase A (AURKA), Aurora kinase B (AURKB) and Aurora kinase C (AURKC). AURKs are composed of three domains: a N-terminal domain the kinase domain and a C-terminal domain. The catalytic domain shares >70% of homology among the three isoforms (8) and is composed of a β-stranded lobe and an α-helical lobe, linked by a hinge region; the two lobes create a deep cleft where the ATP-binding pocket lies (9). The non-catalytic domains are likewise essential for the correct functions of AURKs: the N-terminal domain mediates the intracellular localization, while the C-terminal domain binds to specific co-factors that shape their conformation (10). The kinase action is only activated after auto-phosphorylation of a specific threonine residue in the catalytic domain.

The specific roles of Aurora kinases depend on the different intracellular localization and the meticulous temporal expression during the cellular cycle. Transcription of AURKs is regulated by cell cycle-dependent factors that bind to cell cycle-dependent elements (CDE) in their promoters (11). AURKC seems to be significantly expressed only in cells undergoing meiosis (i.e., spermatocytes and oocytes) and its biological functions are not well understood. Although it is overexpressed in many cancer types (12), its oncogenic role is unclear; however, it may be responsible for centrosome amplification and multinucleation of cancer cells, conferring survival advantage (13). AURKA and AURKB are, on the contrary, expressed in every cell undergoing mitosis.

AURKA levels rise from G2 phase to early mitotic phases (14–16); at first, AURKA can be found in the pericentriolar matrix and, after activation by co-factor Ajuba, it contributes to centrosome maturation: AURKA recruits several proteins essential to microtubule nucleation, stabilization and spindle assembly, like centrosomin, γ-tubulin ring complex (γ-TuRC) and D-TACC/maskin (17, 18). During late prophase, AURKA phosphorylates cyclin B1-Cyclin-Dependent Kinase 1 (CDK1), which, in turn, provokes the nuclear envelope breakdown (NEBD) by activating the Ran GTPase pathway. After NEBD, AURKA is responsible for centrosome separation by phosphorylating kinesin Eg5, which generates a sliding movement on anti-parallel microtubules pushing the centrosomes away (19). Cyclin B1-CDK1 complex also activates the spindle assembly factor TPX2, which binds to AURKA and, together, they create the bipolar mitotic spindle (20–22).

During early mitosis, AURKB phosphorylates histone H3 in order to release heterochromatin protein 1 (HP-1) from heterochromatin; this event might facilitate chromosome condensation, but evidence is unclear in mammalian cells (23, 24). Then, during prophase, AURKB regulates the attachment of microtubules of mitotic spindle to kinetochores. Kinetochores are protein complexes that bind to chromatin domains which act as a platform called centromeres. AURKB is a member of the error correction (ER) machinery, a control system that detects tension between centromere and

kinetochore and stabilizes correct chromosome biorientation (amphitelic), whereas it inhibits incorrect "tensionless" attachments (such as synthelic, monothelic and merotelic) (25). Furthermore, in case of incorrect attachments, AURKB activates the spindle assembly checkpoint (SAC) that prevents sister chromatids separation and mitotic exit (26, 27). During metaphase, AURKB takes part of the chromosome passenger complex (CPC), together with INCENP (inner centromeric protein), Survivin and Borealin, and relocates to the midzone (28). It has been shown in yeasts that AURKB promotes sister chromatid separation by recruiting Shugoshin 1 (SGO1), that removes Cohesin from centromeres (29). Lastly, AURKB plays an essential role in cytokinesis: the activation of RhoA GTPase determines actine polymerization and the formation of the contractile ring; phosphorylation of vimentin, desmin and GFAP creates the cleavage furrow (30).

Given the crucial roles in cell cycle, activity of Aurora kinases must be finely regulated, particularly in case of DNA damages. When G2 checkpoint is activated by double strand breaks, ATM and ATR phosphorylate checkpoint kinase Chk1/Chk2, that not only inhibits cyclin B1, but also AURKA and AURKB; AURKB is also blocked by PARP1 (31, 32).

Tumorigenic potential of Aurora kinases

Dysregulation of Aurora kinases can lead to proliferative and survival advantages in many tumors. Although there are no validated methods to assess AURK overexpression, different techniques could be used including immunohistochemistry, FISH and comparative multiplex RT-PCR, that can detect differential AURK-mRNA expression in normal and tumor tissues. Overexpression of AURKA is found in different cancers, including lung carcinomas, and is an established poor prognostic factor in lung, breast and colorectal cancers (33–35). The induction of AURKA overexpression *in vitro* did not demonstrate the capacity of transforming cell lines or generating malignant tumors in murine models, so Aurora A might rather be a promoting factor than an oncogene (36). In fact, AURKA overexpressing cells are characterized by multipolar spindle formation and unequal chromosome segregation, leading to aneuploidy and a potentially precancerous state. Moreover, abnormal AURKA activity hyperactivates oncogenic pathways like NFκβ, BCR/ABL and Pi3K/Akt, resulting in increased cell proliferation, survival and transformation. AURKA is also able to upregulate telomerase activity *via* hyperactivation of Myc, leading to increased survival (37). Lastly, AURKA is linked to epithelial-to-mesenchymal transition (EMT) and metastatic potential in several cancer (38, 39). Yoo and colleagues recently showed that AURKA and AURKB confer an "invasiveness signature" in lung adenocarcinoma, indeed their simultaneous inhibition *in vitro* and in a murine model of lung adenocarcinoma reduced tumor invasion (40).

AURKB is found overexpressed in many cancer types (41, 42) and is a negative prognostic factor in NSCLC and hepatocellular carcinoma amongst other tumors (43, 44). Abnormal expression of AURKB is linked to aneuploidy and micronuclei formation, in fact its overexpression alters chromosome segregation and SAC activation (45); in p53-deficient cells, these effects are even augmented (46, 47).

AURKs dysregulation is also responsible for resistance to several antineoplastic drugs. In a recent study by Tagal and colleagues, it was shown that AURKs could determine a switch from the proliferative cell cycle to polyploid growth and multinucleation in lung cancer cell lines, resulting in the formation of polyploid giant cancer cells (PGCC) (48). These cells seem to be associated with resistance to many antimitotic drugs, tumor relapse, immunosuppression, cancer stem cell production, and modulation of the tumor microenvironment (49). Expression of aurora A kinase is correlated with cisplatin resistance in NSCLC: *in vitro* data of 102 NSCLC patients treated with surgery and adjuvant cisplatin-based chemotherapy showed that AURKA expression was elevated in cisplatin-resistant lung cancer cells. Furthermore, its inhibition reversed the migration ability of cisplatin-resistant cells (50). High levels of AURKA are also associated with cisplatin resistance in JAK2-mutated myeloma cells (51).

AURKB's expression modulates the activity of taxanes in NSCLC cells and the assessment of its levels in histological samples could be developed as a predictive biomarker. It has been shown that mRNA expression of AURKB in NSCLC cell lines inversely correlated with resistance to both docetaxel ($p = 0.004$) and paclitaxel ($p = 0.007$). Furthermore, inhibition of AURKB activity with barasertib also demonstrated a strong dose-dependent efficiency in triggering paclitaxel resistance. The results of the study bring to a paradox: overexpression of AURKB reduces survival in chemotherapy-naive patients but, on the other hand, it appears to have a beneficial effect in patients treated with taxane regimens (52).

Aurora kinases in NSCLC

In a large cohort of NSCLC patients ($n = 362$) AURKA was highly overexpressed in the tumor tissues compared to corresponding normal lung tissue. In univariate analyses it resulted a significantly increased hazard ratio and poor disease-free survival in patients with a high gene expression of both AURKA ($HR = 2.813$, $p \leq 0.001$) and its co-factor TPX2 ($HR = 1.826$, $p = 0.007$). Similarly, AURKA expression confirmed to be a statistically significant prognostic marker using multivariate analyses ($p = 0.006$) (35).

A study including 11 NSCLC cell lines investigated the preclinical efficacy of MK-5108, a strong inhibitor of AURKA that had shown a potent preclinical activity in malignancies of breast, cervical, colon, ovarian, and pancreatic origin (53). MK-5108 was tested as a single agent and in combination with cisplatin and docetaxel. Concurrent treatment of MK-5108 with cisplatin or docetaxel synergistically inhibited cell growth, with the docetaxel combination performing better. In sequential administration, treatment with docetaxel followed by MK-5108 registered greater growth inhibition than the inverse, even if concurrent treatment remained superior (54).

Different preclinical studies focused on the role of AURKs in oncogene-addicted NSCLC and in particular on their role in the induction of resistance to targeted therapies. Activating mutations in the Epidermal Growth Factor Receptor (EGFR) gene are the most frequent mutations and they can be found in 14–17% of advanced NSCLC in European populations (55). Tumors with common mutations are sensitive to EGFR tyrosine kinase inhibitors (EGFR TKIs), but eventually these patients will develop resistance which will lead to disease progression. Treatment-induced activation of AURKA seems to be associated with *in vitro* and *in vivo* resistance to EGFR inhibitors. In response to chronic EGFR inhibition, AURKA can be activated by the overexpression of TPX2, which facilitate its auto-phosphorylation; TPX2 is normally degraded by a ubiquitin E3 ligase, which is intra-nuclear in both parental and resistant cells (56). In contrast, in resistant cells TPX2 delocalize in the cytosol, separate from the complex responsible for its degradation, leading to its accumulation. Aurora kinase inhibitors suppress this adaptive survival program, increasing the magnitude and duration of EGFR inhibitor response in preclinical models. The suppression of AURKA-driven residual disease could become an important weapon against the acquired resistance in these diseases. The combination of an aurora kinase inhibitor with a third-generation anti-EGFR agent resulted in a synergistic reduction in cell growth in all models (57). In addition, AURKA overexpression is linked to acquired resistance to EGFR-TKI *via* epithelial-mesenchymal transition (EMT), and AURKA inhibitor alisertib has shown to restore NSCLC cells sensitivity to EGFR-TKI and to partially reverse EMT (58). AURKA inhibition with shRNA also demonstrated to partially reverse fibroblast-mediated resistance to gefitinib in EGFR-mutated NSCLC cells co-cultured with stromal cells (59).

Another study showed that resistant EGFR-mutated NSCLC cells without the p.T790M or other acquired mutations are sensitive to AURKB inhibitors barasertib and S49076. In most acquired resistant cells in fact the phospho-histone H3 (pH3), a major product of AURKB, resulted increased and its levels reduced after treatment with AURKB inhibitors, triggering G1/S arrest, polyploidy and, eventually, cell cycle arrest and cell death. The results support the role of AURKB activation in acquired resistance to EGFR TKIs, making AURKB a potential target in NSCLC progressed to anti-EGFR therapy and not carrying resistance mutations (60). AURKB inhibitors are potent enhancers of osimertinib-induced apoptosis and can play an important role in overwhelming acquired resistance to third generation TKIs. Osimertinib resistance caused by EMT activates the ATR-CHK1-Aurora B signaling cascade and generates hypersensitivity to AURKB inhibitors by activating BIM-mediated mitotic catastrophe. AURKB inhibition stabilizes BIM through reduced Ser87 phosphorylation, and transactivates PUMA through FOXO1/3. In this way a combined inhibition of EGFR and AURKB not only efficiently eliminates cancer cells but also overcomes resistance beyond EMT (61).

AURKA and B have also shown to phosphorylate KRAS downstream effectors, playing a synergic oncogenic role with KRAS mutations. Dos Santos et al. demonstrated that KRAS positively modulated AURKA and AURKB expression by regulating their transcription or mRNA stability. They also assessed that simultaneous pharmacological inhibition of AURKA and AURKB activity *in vitro*, as well as their targeting by RNA interference, reduced cell growth and proliferation and promoted apoptosis in a KRAS-dependent manner. Unfortunately, these results were not confirmed in *in vivo* xenografts model; however, this study suggests that aurora kinases could be targeted in KRAS-mutated NSCLC (62). According to results presented at the IASLC 2022 World Conference on Lung Cancer, Lee et al. demonstrated that the addition of AURKA inhibitor VIC-1911 to KRAS inhibitor sotorasib led to increased cell death in resistant cancer cells compared to sensitive ones, suggesting that AURKA inhibition may overcome sotorasib resistance. In addition, the combined inhibition of AURKA and WEE1 led to a synergistic increase in the death of KRAS-mutated lung cancer cells with acquired resistance to sotorasib, even greater than sotorasib plus VIC-1911 (63).

AURK inhibitors were investigated as radiosensitizing agents by Liu et al. in NSCLC cell lines. MLN8237 (alisertib) was assessed together with the effect of radiation and, after treatment, p53-proficient HCC2429 and H460 cell lines increased their sensitivity to the lethal effect of radiation, with a dose enhancement ratio (DER) of 1.33 ($p < 0.05$) and 1.35 ($p < 0.05$), respectively; on the other hand, there was no significant enhanced effect in the naturally p53-deficient and radiation-resistant H1299 cells with a DER of 1.02 ($p > 0.05$). These data suggest that lower doses of radiation could achieve an equivalent antitumor effect when administered in combination with MLN8237 compared to radiation alone *in vitro*, especially in p53-competent cells (64).

Taking into account these early signs of preclinical activity, the role of AURK inhibitors in NSCLC has also been investigated in clinical trials (synthetized in Table 1).

A multicenter, 5-arm, phase II trial investigated the safety and activity of single-agent alisertib in various advanced and pretreated solid tumors (n = 249). Alisertib was administered orally in 21-day cycles at the recommended dose of 50 mg twice

TABLE 1 Trials evaluating AURK-I in NSCLC.

FIRST AUTHOR	Type of study	N° of patients (TOT/NSLC)	Drug	Outcome
Melichar B	Phase II	249/26	Alisertib	ORR 4% (SD 74%)
Godwin JL	Phase I/II	18/18	Alisertib + Erlotinib	ORR 6% (SD 28%)
Arkenau HT	Phase I	49/7	AT9283	ORR 0% (SD 29%)
Semrad TJ	Phase I	17/5	Alisertib	ORR 0%
Blakely CM	Phase I	10/10	Alisertib + Osimertinib	ORR 10% (SD 60%) mPFS 9.4 months
Mross K	Phase I	121/4	BI 811283	ORR 0%
Schoffski P	Phase II	223/56	Danusertib	ORR 2% PFR at 4 months 10.4% mPFS 9.2 weeks mOS 7.6 months
Boss DS	Phase I	59/3	Barasertib	ORR 0%

daily for 7 days followed by a break of 14 days. The study included 26 patients with NSCLC and an objective response (OR) was registered in just 1 (4%, 0-22) of 23 evaluable patients, while 17 (74%, 52-90) achieved a stable disease (SD). In the NSCLC cohort, 25 patients (96%) experienced an adverse event (AE) of any grade and the most frequent drug-related grade 3-4 adverse events included neutropenia (62%), leukopenia (27%), fatigue and anemia (both 19%). Despite the manageable toxicity profile, the activity data of alisertib were not particularly promising in patients with NSCLC and did not support further clinical assessment in this disease, in contrast to breast cancer and SCLC (65).

Godwin and colleagues assessed whether the combination of erlotinib and alisertib exerted a synergistic action in EGFR wild-type NSCLC in a phase I/II clinical trial. 18 patients with recurrent or metastatic EGFR wild-type NSCLC were treated and the combination of alisertib and erlotinib proved to be tolerable. Common drug-related adverse events of any grade were fatigue (89%), anemia (83%), leukopenia (78%), dyspnea (78%), diarrhea and anorexia (61%), while drug-related grade 3/4 adverse events included neutropenia and leukopenia (33%), febrile neutropenia, lymphopenia, and anemia (11%). The maximum tolerated dose (MTD) was 150 mg daily for erlotinib with 40 mg BID for alisertib. Disease responses were also noted, including one patient with a partial response who completed 10 cycles, and 5 patients who achieved SD (66).

A single-center phase I study including 17 patients with refractory advanced solid tumors investigated the safety and tolerability of alisertib combined with weekly irinotecan (100 mg/m^2 on day 1 and 8 of a 21-day cycle). Alisertib was administered orally twice per day on days 1-3 and 8-10 with an escalating dose of 20-60 mg. The MTD was 20 mg twice per day and the dose-limiting toxicities were diarrhea, dehydration, and neutropenia. Furthermore, it was registered one fatal cardiac arrest at the highest dose level tested which was possibly related to drug. No objective responses were observed in patients with NSCLC. Due to the weak activity and most of all to the poor tolerance, the use of alisertib in combination with irinotecan did not show appealing results (67).

Blackely et al. presented at the 2021 ASCO Annual Meeting the promising preliminary results of intermittent dosing of alisertib (30 mg BID on days 1-3, 8-11, and 15-17 of a 28-day cycle) in combination with osimertinib (80 mg daily) in patients with EGFR-mutated lung adenocarcinoma resistant to osimertinib monotherapy. In this phase Ia clinical trial (NCT04085315) 6 patients were treated with 30 mg BID and 4 patients with 40 mg BID intermittent dosing schedule of alisertib. The most commonly reported adverse events were diarrhea (70%), fatigue (60%), alopecia (50%) and neutropenia (50%), all of them of grade 1 or 2; two patients (20%) experienced grade 3 or grade 4 neutropenia, both patients were treated at the 40 mg BID intermittent dose of alisertib. Intermittent alisertib 30 mg BID was identified as the MTD and recommended phase 2 dose in combination with osimertinib 80 mg daily. The ORR was 10% (1/10) and DCR 70% (7/10). The median PFS was 9.4 months (2.0 months - N.R.) (68).

AT9283, an inhibitor of AURKA and AURKB, has been assessed in a phase I dose-escalation study in 49 patients with advanced solid tumors including NSCLC (n = 7). This drug was generally well tolerated with reversible dose-related toxic effects such as myelosuppression, gastrointestinal disturbance, fatigue, and alopecia. No objective responses were observed; however, four patients with esophageal cancer (n = 1), colorectal cancer (n = 1), and NSCLC (n = 2) demonstrated prolonged SD of more than 6 months (69).

The role of another AURKB inhibitor (BI 811283) was investigated in a phase I, dose-escalation study involving 121 patients with advanced solid tumors. The drug was administrated *via* 24-hours infusion on Days 1 and 15 of a 4-week cycle (schedule A) or Day 1 of a 3-week cycle (schedule B) and the MTDs obtained were 125 mg and 230 mg respectively. 4 patients with NSCLC were included in this study: 3 were treated with schedule A and 1 with schedule B. All patients in both treatment schedules experienced at least one adverse event. The most

common dose-limiting toxicities were hematological events, particularly neutropenia. Pharmacodynamic assessments showed a decrease in phosphorylated histone H3 (pHH3) which indicated Aurora B kinase inhibition. No patient achieved an OR, even if 30% in schedule A and 33% in schedule B reported a clinical benefit and a stabilization of the disease. Despite a good safety profile, the anti-tumor activity observed does not support the development of the drug in solid tumors (70).

In a prospective, phase II, open-label, multi-institutional study, Danusertib (PHA-739358, a pan-AURK inhibitor) was adopted as single agent for treating patients with different advanced cancers including NSCLC as second line treatment. Patients were treated with danusertib 500 mg/m^2 given as 24-h i.v. infusion every 14 days until progression or unacceptable toxicity. Danusertib showed marginal antitumor activity with a manageable safety profile. In the 56 patients with metastatic NSCLC the progression-free rate (PFR, the primary outcome) at 4 months was 10.4% (16.1% in squamous subgroup, where the only objective RECIST response was obtained). The mPFS was 9.2 weeks and the mOS 7.6 months. AEs were reported in 83.3% of patients. The most frequent drug-related AEs were fatigue (67.9%), nausea (39.3%), diarrhea (28.6%), anorexia (28.6%), vomiting (16.1%), alopecia (23.2%), constipation (10.7%), anemia and neutropenia (74.5% of events CTC grade 3 or 4) (71).

Barasertib (AZD1152), another Aurora kinases inhibitor, was tested in two phase I studies. Patients with different advanced solid malignancies were treated with escalating doses (100-650 mg) administered as a 2-h infusion every 7 days or 14 days. The MTD was respectively 200 mg and 450 mg, and neutropenia was the most frequent adverse event and dose-limiting toxicity. Grade 3-4 neutropenia occurred in 58% and 43% of patients. No OR were observed at any dose or schedule, although 15 patients (25%) achieved a SD. However, only 3 patients had NSCLC and that is why the role of barasertib is far from being defined in this type of tumor (72, 73).

Aurora kinases in SCLC

Even after the introduction of immunotherapy in the first-line setting, the majority of patients with SCLC experiences an inexorable disease progression in less than 12 months (4, 5). Unfortunately, effective treatments are not available after disease progression to first-line therapy: topotecan is currently the standard of care, with limited results (74). These poor outcomes highlight the need for a better molecular knowledge of the disease to develop new therapeutic strategies. The most common genetic mutations of SCLC are related to p53 and RB1, but none of these represent a druggable therapeutic target. Amplification of MYC family genes was also found in about 20% of SCLCs (75) and in 30-50% of SCLC cell lines (76) and is associated with treatment resistance, tumor progression and poor outcomes (77, 78). Recent studies have shown that the SCLCs family can be divided into four distinct subtypes based on the differential expression of four transcription factors (79); two of these subgroups, characterized by a high expression of ASCL-1 (SCLC-A) or NEUROD1 (SCLC-N), share a neuroendocrine phenotype; the other two subgroups can be divided on the basis of the expression of POU2F3 (SCLC-P) or of the lack of expression of the three transcription factors (SCLC-I). This last subgroup is instead characterized by the expression of an immunogenic signature, including immune checkpoints and human leukocyte antigens (HLAs), therefore the denomination "inflamed" (80). In a study conducted on murine models, SCLC-N appeared to be associated with MYC amplifications (81, 82). In fact, data suggest that MYC promotes a variant subset of SCLC with lower expression of neuroendocrine markers and with more aggressive features, that could originate from ASCL1+ progenitor cells which, over time, transition to an ASCL1-low/NEUROD1-high state due to the indirect effect of MYC on NEUROD1 signaling (83). Despite these findings, it is still difficult to exploit MYC in a therapeutic way. Nevertheless, from synthetic lethality screenings, AURK inhibitors appeared promising candidate targets. Mollaoglu et al. demonstrated that MYC-driven SCLC cell lines were sensible to AURKA inhibitor Alisertib and AURKB inhibitor Barasertib. To assess AURK inhibition *in vivo*, murine models bearing MYC-amplified SCLC received Alisertib alone, chemotherapy alone or chemotherapy + Alisertib. While single agent alisertib or chemotherapy didn't show durable results, mice who received the combination had the highest 30-day survival rate (47% vs 5% for chemo-treated vs 8% for Alisertib-treated) (83).

Antitumor activity *in vivo* of these molecules was tested in few clinical trials (synthetized in Table 2). A phase I dose-escalation trial tested Danusertib as a 24-hour infusion with and without G-CSF in patients with advanced pretreated solid tumors. Among the 56 patients enrolled in the study, 2 had

TABLE 2 Trials evaluating AURK-I in monotherapy.

FIRST AUTHOR	Type of study	N° of patients (TOT/SCLC)	Drug	Outcome
Schoffski P	Phase II	219/18	Danusertib	ORR 0%, mPFS 8.1 weeks, mOS 11.4 months
Melichar B	Phase I-II	249/60	Alisertib	ORR 21%
Cohen RB	Phase I	56/2	Danusertib (24h infusion)	ORR 50%
Carducci M	Phase I	105/3	AMG 900	ORR 0%

SCLC. One of these patients experienced an objective tumor response that lasted for 23 weeks receiving 1,000 mg/m^2 Danusertib + G-CSF, subsequently reduced to 750 mg/m^2 for hypercreatininemia G2. Drug related SAEs occurred in 21% of all patients (12/56), 9 (22%) in the group treated with Danusertib alone and 3 (19%) in the group treated with Danusertib + G-CSF (84).

A subsequent multi-cohort phase II study included 18 patients with SCLC who had failed at least two prior lines of therapy that were treated with Danusertib (multi AURK-inhibitor). Unfortunately, none of these patients was progression-free at the four-month treatment assessment. Final results have shown a mPFS of 8.11 weeks and a mOS of 11.4 months. Regarding its safety profile, Danusertib confirmed what had already emerged from previous studies: the most frequent treatment-related non-hematological AEs were asthenia/fatigue (61%, 11/18) and nausea (38.9%, 7/18); neutropenia was the most common hematological toxicity (100%) as well as the most frequent grade 3–4 event (88.9%, 16/18) (71).

A five-arm phase II study investigated the activity of Alisertib in 60 patients with pretreated SCLC. Results have shown that, among response-assessable patients, an OR was obtained in 21% (10/48). The most frequent drug-related grade 3–4 adverse events included neutropenia, leukopenia, and anemia (65).

Lastly, a phase I trial studied AMG 900, an orally administered pan-Aurora Kinase inhibitor in patients with advanced solid tumor. Among the 105 patients treated in this trial, 3 patients of the escalation cohort had SCLC. Unfortunately, none of them obtained an OR with the treatment. Regarding the safety profile, treatment-related AE with grade ≥ 3 occurred in 61 patients (58%); the most common one was neutropenia (n=44, 42%). The most common non hematological AEs were fatigue and diarrhea (85).

The activity and safety of the association of chemotherapy with aurora kinase inhibitors was evaluated in a few clinical trials (synthetized in Table 3). In the previously reported phase I study investigating the combination of alisertib and irinotecan in solid tumors, 3 of 17 patients had a diagnosis of SCLC. Although one PR occurred in a patient with SCLC among the 11 evaluable patients (9%), the toxicity profile showed significant rates of toxicities hematological and gastrointestinal toxicities, leading the authors to conclude that the combination of Alisertib and Irinotecan was not well tolerated in adult patients and to stop the planned expansion cohort (67).

Another phase I trial in patients with advanced solid tumors tested the combination of Alisertib and nab-paclitaxel, with the rationale of combining their antimitotic action. Among the 31 patients treated in the dose-escalation phase, 5 had a diagnosis of SCLC. Results have shown that one patient with refractory SCLC achieved a partial response that lasted for more than two years, until treatment was discontinued due to neurological toxicities. Two other patients with SCLC achieved a SD that lasted more than four months. These data led to an OR of 6.3% (1/16) and a DCR of 31.3% (5/16) among the 16 evaluable patients. Regarding the safety profile, the most common treatment-related AEs included alopecia (64.5%), diarrhea (41.9%), oral mucositis (41.9%), anorexia (38.7%), fatigue (38.7%), and nausea (35.5%). The most common laboratory abnormalities were leukopenia (80.6%), neutropenia (77.4%) and anemia (77.4%) (86).

A randomized double-blind phase II study assessed paclitaxel + alisertib/placebo as a second line treatment after platinum-based chemotherapy in 178 patients with SCLC, stratified by relapse type (sensitive vs refractory/resistant); mPFS was 3.32 months in the Alisertib + Paclitaxel arm versus 2.17 months in the Placebo + Paclitaxel arm (p=0.113), while mOS was 6.86 months versus 5.58 months (p=0.714). The DCR was 58% in the experimental arm versus 46% in the control arm, and ORR was 22% and 18% respectively. Slightly better results were shown in the subgroup of resistant/refractory patients. In addition, C-Myc-positive patients and those with mutations in genes involved in cell cycle regulation (CDK6, RBL1, RBL2, RB1) also showed better outcomes with Alisertib than with Placebo. The incidence of grade 3 or higher drug-related AEs was 67% with Alisertib + Paclitaxel versus 25% with Placebo + Paclitaxel; the most common AEs were neutropenia, febrile neutropenia, leukopenia, anemia, diarrhea and stomatitis (87).

The combination of Alisertib + Docetaxel was evaluated in a phase I clinical trial in the context of solid tumors eligible for Docetaxel therapy as determined by the investigator. Among the 41 patients that participated, only one patient had a diagnosis of SCLC and did not achieve an objective response. Treatment-related grade 3 or higher AEs involved 39 patients (95%), and the most common one was neutropenia (n=34, 83%) (88).

Lastly, it is worth reporting the case of a nonsmoker patient with SCLC harboring a novel JAZF1-MYCL1 gene fusion and

TABLE 3 Trials evaluating AURK-I in combination with chemotherapy.

FIRST AUTHOR	Type of study	N° of patients (TOT/SCLC)	Drugs	Outcome
Semrad TJ	Phase I	17/3	Irinotecan + Alisertib	ORR 33%
Graff JN	Phase 1	41/1	Docetaxel + Alisertib	ORR 0%
Lim KH	Phase I	31/5	Nab-paclitaxel + Alisertib	ORR 60%
Owonikoko TK	Phase II	178/178	Alisertib/Placebo + Paclitaxel	ORR 22% vs 18%

lacking alterations in TP53 and RB1. The patient had previously been treated with chemo-radiotherapy in the setting of limited stage disease; subsequently, after disease recurrence, the patient was enrolled in a clinical trial with Alisertib as his fourth-line regimen and achieved an almost complete response after ten cycles; the patient discontinued treatment after approximately 18 months of therapy (23 cycles) due to disease progression, and after the failure of subsequent chemotherapy lines, obtained an excellent disease control with Nivolumab (89).

Conclusions

The role of Aurora kinases in regulating cell cycle and safeguarding the correct transmission of genome to daughter cells is well established. Dysregulation of AURKs showed to promote tumorigenesis with different mechanisms, particularly causing aneuploidy and favoring genome instability. In addition, overexpression of AURKs is related to antineoplastic drug resistance, particularly platinum compounds and EGFR-TKIs in the case of lung cancer. Despite the strong rationale in the use of AURK inhibitors against cancer, significant clinical activity was demonstrated in hematological malignancies (90–93) but not in many solid tumors; this different outcome might be explained by the higher proliferation rate and clonality of the formers. In NSCLC, AURK inhibitors showed weak antitumor activity; nevertheless, preclinical studies and early data from clinical studies support their investigation in combination with EGFR-inhibitors. An ongoing clinical trial is evaluating safety and activity of the combination of osimertinib + alisertib or sapanisertib (an oral inhibitor of TOR complex 1 and 2) in osimertinib-resistant EGFR-mutated lung cancer (NCT04479306). Similarly, another clinical trial will study AURKA inhibitor LY3295668 in combination with osimertinib in patients with advanced EGFR-mutant NSCLC who have received a third generation EGFR-TKI (NCT05017025). In SCLC, early-phase clinical trials showed appreciable signals of activity of AURK inhibitors, particularly in combination with taxanes, but these results need to be validated in phase III randomized trials. In addition, considering the better outcomes obtained in cMyc-positive tumors, efforts should be made to apply the concept of precision medicine even in SCLC; the four subgroups based on differential expression of transcription factors ASCL1, NEUROD1, POU2F3 and YAP1 could provide a reproducible method of classifying SCLC for this scope, considering that cMyc tends to be overexpressed in SCLC-N subtype.

Author contributions

ASte and GP did write the review, FS, AStr, EV, SF, DB, FM, IS GV, and MF did review all the specific literature and pooled all available data from peer-reviewed journal and featured oncology meetings, ED'A, MDS, and CC did critically review all the drafts and GT and EB did coordinate the whole work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

EB received advisory and speakers' fee from MSD, Astra-Zeneca, Celgene, Pfizer, Helsinn, Eli-Lilly, BMS, Novartis, and Roche.

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Publisher's note

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Clinical impact of number of lymph nodes dissected on postoperative survival in node-negative small cell lung cancer

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Objectives: Small cell lung cancer (SCLC) is a lethal histologic subtype of lung cancer. Although the Commission on Cancer recommends pathological examination of at least 10 lymph nodes dissected (LNDs) for resected early-stage non-small cell lung cancer, its survival benefit of LNDs in patients with early-stage SCLC is unknown.

Methods: The National Cancer Database was queried for SCLC patients with clinical stage I-II and clinical N0, NX disease per AJCC 7th edition who had undergone lobectomy between 2004 and 2017. Overall survival of SCLC patients by the number of LNDs was compared using Log-rank tests. Univariate and multivariable Cox proportional hazards analyses were performed.

Results: In total, 688 (42%), 311 (20%), 247 (16%), 196 (12%), 126 (8%), and 36 (2%) of 1,584 patients with early-stage SCLC had ≥10, 7-9, 5-6, 3-4, 1-2, and 0 LNDs, respectively. The sequential improvement in the HRs was no longer evident if the number of LNDs exceeds 4. Patients with ≥3 LNDs (n = 1,422) had a significantly longer overall survival than those with <3 LNDs (n = 162) (hazard ratio for death: 0.76, 95% confidence interval: 0.62–0.94, P = 0.0087). Multivariate analysis revealed that ≥3 LNDs was an independent factor for predicting overall survival (hazard ratio for death: 0.76, 95% confidence interval: 0.61–0.93, P = 0.0083).

Conclusions: Although we are reluctant to recommend a definitive "optimal number" of LNDs, our findings suggest the prognostic and therapeutic roles for performing ≥3 LNDs in patients with early-stage SCLC who undergo lobectomy.

KEYWORDS

cancer, prognosis, lung small cell lung cancer, lymph node dissection, surgery, survival

Introduction

Lung cancer is one of the most fatal malignancies worldwide (1). The standard therapy for resectable lung cancer is lobectomy and thoracic lymphadenectomy (2, 3). The majority of lung cancers are classified as non-small cell lung cancer (NSCLC), and therefore the majority of studies have centered around this histologic subtype. With regard to patients with early-stage NSCLC, the required extent of thoracic lymphadenectomy has been debated (4–8). Several previous studies reported that systemic lymph node (LN) dissection provided a longer disease-free survival and overall survival (OS) than mediastinal LN sampling in patients with early-stage NSCLC (5, 6). National Comprehensive Cancer Network (NCCN) guidelines advocate sampling of at least three N2 stations or a complete mediastinal dissection (9). The Commission on Cancer (CoC) recommends that at least 10 LNs should be pathologically examined for resected early-stage NSCLC (10, 11).

Small cell lung cancer (SCLC) is the most lethal histologic subtype of lung cancer for which there have been small advances in treatment over the past decade (12). For early-stage SCLC, the use of surgery is recommended based on retrospective or single arm studies (13, 14). The International Association for the Study of Lung Cancer database for the 7th editions of the International Staging System showed that SCLC patients with clinical T1a disease who underwent surgery had 93% survival at 12 months and 73% at 24 months. SCLC patients with clinical T1b disease who received surgery had 89% survival at 12 months and 76% at 24 months (15). In general, SCLC patients who are candidates for surgery are rare, since most patients with SCLC present with locally advanced or distant metastases (12). Due to the rarity of SCLC patients who are candidates for surgery, the required extent of thoracic lymphadenectomy for early-stage SCLC has not been comprehensively investigated. The aim of the current study is to examine the prognostic significance of the number of LNs dissected (LNDs) in patients with early-stage SCLC who receive curative lung resection.

Materials and methods

National cancer database (NCDB)

The NCDB is a joint project between the CoC of the American College of Surgeons and the American Cancer Society. The CoC's NCDB and the hospitals participating in the CoC NCDB are the source of the de-identified data used herein; they have not verified and are not responsible for the statistical validity of the data analysis or the conclusions derived by the authors. The data is considered as hospital-based rather than population-based (16). The access to the NCDB participant use file was granted to T.K. Based on the use of only de-identified data, the study was exempted by the Parkview institutional review board.

Patients with SCLC diagnosed and captured in the NCDB between 2004 and 2017 were selected (n = 283,347). Of these, patients with clinical stage I-II disease were included (n = 23,653). Patients with clinical N0 and NX disease were selected (n = 17,023). Patients who underwent surgery (lobectomy) were then selected (n = 2,057). Of these, patients with information about number of lymph nodes dissected were included (n = 1,882). Patients whose survival data were available and who survived at least 1 month past the date of diagnosis were then selected (n = 1,827). Patients with neoadjuvant chemotherapy or radiation therapy were excluded (n = 1,768). Of these, patients with pTX or blank were excluded (n = 1,584). The study flow diagram of case eligibility is shown in Figure 1.

Clinical demographics including age (<70 vs. 70+), sex (male vs. female), race (whites vs. others), insurance (insured vs. uninsured), institutions (academic vs. others), Charlson-Deyo comorbidity score (0–1 vs. ≥2), years of diagnosis (2004-2010 vs. 2011-2017), histology (SCLC, not otherwise specified [NOS] vs. others), pathologic T stage (T0-1 vs. T2-4), pathologic N stage (N0 vs. N1-2), tumor size (<30 mm vs. ≥30 mm), resected margin status (other vs. negative), adjuvant chemotherapy (yes vs. no/unknown), and adjuvant chest radiation (yes vs. no/unknown) were collected. We chose the median year (2010) as a cut-off for breaking up the years of diagnosis based on the previous report, indicating that the mortality from SCLC has been declining in a linear fashion (17).

Statistical analysis

Kaplan-Meier curves by the number of LNDs were compared using the log-rank test. The associations between the number of LNDs and clinical demographics were assessed by chi-squared test and Fisher's two-sided exact test where appropriate. Univariate and multivariable Cox proportional hazards analyses were performed using JMP® 14.0 (SAS Institute Inc., Cary, NC, USA). A two tailed, $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

The study flow diagram of case eligibility is shown in Figure 1. Of note, this study analyzed only SCLC patients who received lobectomy. Patient characteristics (n = 1,584) are summarized in Table 1. In total, 688 (42%), 85 (5%), 112 (7%), 114 (7%), 110 (7%), 137 (9%), 105 (7%), 91 (6%), 87

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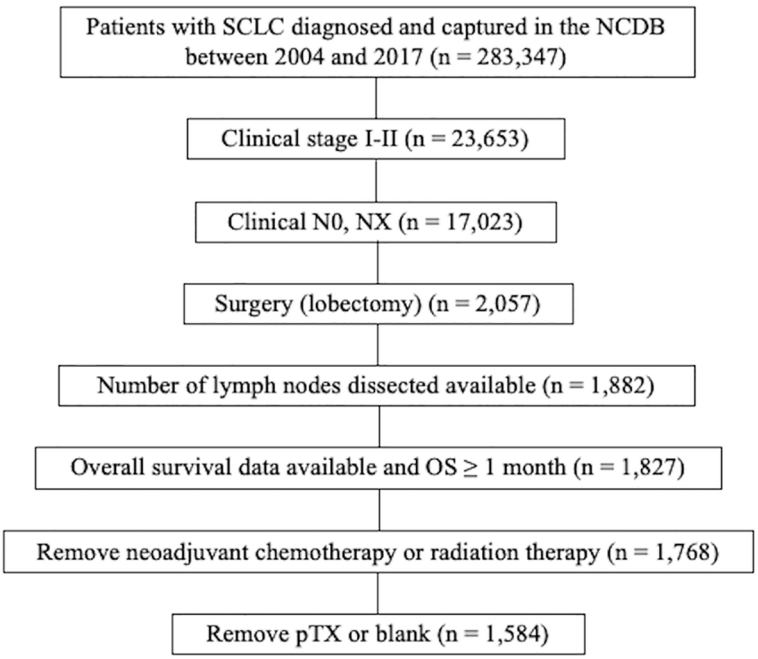


FIGURE 1
Study flow diagram of case eligibility. SCLC, small cell lung cancer; NCDB, National Cancer Database; AJCC, American Joint commission on cancer; OS, overall survival.

TABLE 1 Patient characteristics of resected clinical stage I-II (AJCCv7) small cell lung cancer (n = 1,584).

Factors		Value or no. of patients
Age	<70	947 (60%)
	≥70	637 (40%)
Sex	male	713 (45%)
	female	871 (55%)
Race	whites	1,449 (91%)
	others	135 (9%)
Insurance status	uninsured	22 (1%)
	insured	1,562 (99%)
Institution	academic	601 (40%)
	others	983 (60%)
Charlson-Deyo score	0-1	1,336 (84%)
	≥2	248 (16%)
Year of diagnosis	2004-2010	404 (26%)
	2011-2017	1,180 (74%)
Histology	SCLC NOS	1,191 (75%)
	others	393 (25%)
Pathologic T stage	T0-1	963 (61%)
	T2-4	621 (39%)
Pathologic N stage	N0	1,246 (79%)
	N1-2	311 (19%)

(Continued)

TABLE 1 Continued

Factors		Value or no. of patients
	NX	27 (2%)
Tumor size	<30mm	493 (31%)
	≥30mm	1,091 (69%)
Resected margin status	other	76 (5%)
	negative	1,508 (95%)
Adjuvant chemotherapy	yes	1,052 (66%)
	multiagent chemotherapy	976 (61%)
	single agent chemotherapy	29 (2%)
	unknown	47 (3%)
	no	477 (30%)
	unknown	55 (4%)
Adjuvant chest radiation	yes	423 (27%)
	no/unknown	1,161 (73%)
Number of lymph nodes dissected	0	36 (2%)
	1	39 (2%)
	2	87 (6%)
	3	91 (6%)
	4	105 (7%)
	5	137 (9%)
	6	110 (7%)
	7	114 (7%)
	8	112 (7%)
	9	85 (5%)
	≥10	668 (42%)

AJCC, American Joint Commission on Cancer; SCLC, small cell lung cancer; NOS, not otherwise specified.

(6%), 39 (2%), and 36 (2%) of 1,584 patients with early-stage SCLC had ≥10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 LNDs, respectively. As shown in Table 2, patients with ≥3 LNDs were significantly associated with insured (P = 0.0194) and years of diagnosis (P = 0.0263) per univariate analysis. In patients with cN0/pN1-2 disease (n = 311), 295 (95%) had LNDs≥3, and 16 (5%) had LNDs<3. In patients with cN0/pN0 disease (n = 1,246), 1,122 (90%) had LNDs≥3, and 124 (10%) had LNDs<3. Significantly more patients in the cN0/pN1-2 group received LNDs≥3 than in the cN0/pN0 group (P = 0.0075). With the aim of analyzing the effect of LNDs on nodal upstaging, pN+ rates ([pN+ cases] divided by [pN+ cases + pN0 cases]) according to the number of LNDs were calculated (Supplementary Figure 1). The sequential increase in the nodal upstaging was suggested if the number of LNDs increased.

Univariate survival analyses in patients with early-stage SCLC according to the number of LNs dissected

The Kaplan-Meier curve comparing OS according to the number of LNDs in patients with early-stage SCLC is shown in Figure 2. The OS was not significantly influenced by the number of LNDs (P = 0.1194). Multivariable COX regression analysis in subgroup-by-subgroup comparisons according to LNDs was performed to find the appropriate cut point. Figure 3 shows the hazard ratios [HR] for death by the number of LNDs. The sequential improvement in the HRs was no longer evident if the number of LNDs exceeds 4. A minimum of 3 LNs evaluation was needed to improve the mortality risk over compared with that without LNDs. As shown in Figure 4, patients with ≥3 LNDs had a significantly longer OS than those with <3 LNDs (median OS: 65.6 vs. 41.0 months, HR for death: 0.76, 95% confidence interval [CI]: 0.62–0.94, P = 0.0087). Since the NCCN guidelines recommend surgery for selected patients with T1-2/N0 SCLC and consider it for some patients with T3/N0 SCLC while surgery is not recommended for T4/N0 disease if invasive mediastinal lymph node staging is negative, a prognosis subgroups analysis stratified by T stage was conducted. In subgroup analysis of patients with T0-2 disease, patients with ≥3 LNDs had a significantly longer OS than those with <3 LNDs (P = 0.0006; Supplementary Figure 2A). However, in subgroup analysis of patients with T3-4 disease, OS of patients with ≥3 LNDs was similar to that of patients with <3 LNDs (P = 0.9968; Supplementary Figure 2B). From Figure 2, it can be seen that only the LND = 0 group had the worst OS and

TABLE 2 Patient characteristics of resected clinical stage I-II (AJCCv7) small cell lung cancer according to number of lymph nodes dissected (n = 1,584).

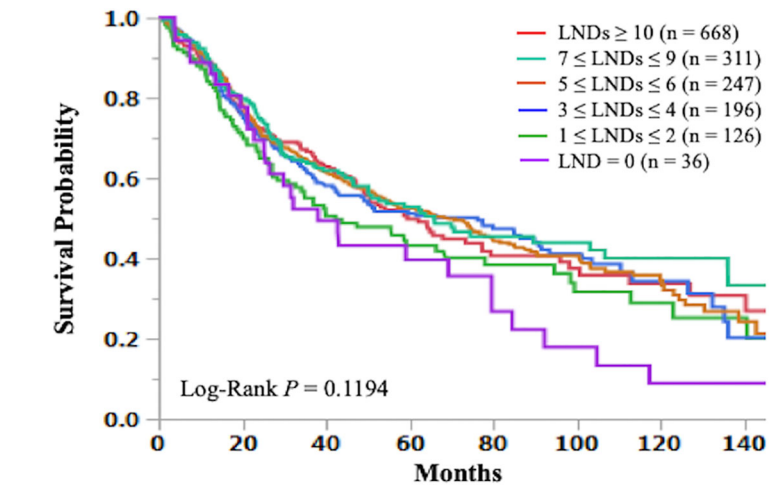
Factors		Number of lymph nodes dissected		*P* value
		≥3 (n = 1,422)	<3 (n = 162)	
Age	<70	847 (60%)	100 (62%)	0.5945[a]
	≥70	575 (40%)	62 (38%)	
Sex	male	643 (45%)	70 (43%)	0.6264[a]
	female	779 (55%)	92 (57%)	
Race	whites	1,301 (91%)	148 (91%)	0.9542[a]
	others	121 (9%)	14 (9%)	
Insurance status	uninsured	X	X	0.0194[b]
	insured	Y	Y	
Institution	academic	547 (38%)	54 (33%)	0.2020[a]
	others	875 (62%)	108 (67%)	
Charlson-Deyo score	0-1	1,200 (84%)	136 (84%)	0.8845[a]
	≥2	222 (16%)	26 (16%)	
Year of diagnosis	2004-2010	351 (25%)	53 (33%)	0.0263[a]
	2011-2017	1,071 (75%)	109 (67%)	
Histology	SCLC NOS	1,060 (75%)	131 (81%)	0.0776[a]
	others	362 (25%)	31 (19%)	
Pathologic T stage	T0-1	863 (61%)	100 (62%)	0.7974[a]
	T2-4	559 (39%)	62 (38%)	
Pathologic N stage*	N0	1,122 (79%)	124 (89%)	0.0075[b]
	N1-2	295 (21%)	16 (11%)	
Tumor size	<30mm	441 (31%)	52 (32%)	0.7773[a]
	≥30mm	981 (69%)	110 (68%)	
Resected margin status	other	65 (5%)	11 (7%)	0.2105[a]
	negative	1,357 (95%)	151 (93%)	
Adjuvant chemotherapy	yes	908 (64%)	97 (60%)	0.3193[a]
	no/unknown	514 (36%)	65 (40%)	
Adjuvant chest radiation	yes	380 (27%)	43 (27%)	0.9609[b]
	no/unknown	1,042 (73%)	119 (73%)	

AJCC, American Joint Commission on Cancer; SCLC, small cell lung cancer; NOS, not otherwise specified, X and Y number less than 10 cannot be reported according to NCDB agreement.
[a]χ^2 test.
[b]Fisher's 2-sided exact test.
*cases with pathologic NX (n = 37) were excluded.

separated from the others. Therefore, we subsequently excluded the LND = 0 group, and analyzed the OS data. Patients with ≥3 LNDs tended to have a longer OS than those with LNDs = 1, 2 (P = 0.0683; Supplementary Figure 3). In addition, patients with ≥10 LNDs did not have a significantly longer OS than those with <10 LNDs (HR for death: 0.95, 95% CI: 0.82–1.09, P = 0.6296, Supplementary Figure 4). The subgroup analysis of cN0/pN0 patients showed that patients with ≥3 LNDs had a significantly longer OS than those with <3 LNDs (P = 0.0041; Supplementary Figure 5). The survival curve comparing OS according to the number of LNDs is shown in Supplementary Figure 6. The OS was significantly influenced by the number of LNDs (P = 0.0178).

Univariate and multivariable analyses of OS in early-stage SCLC patients who underwent curative lobectomy

The results of univariate and multivariable analyses for OS in early-stage SCLC patients are shown in Table 3. Univariate analysis showed that age ($P < 0.0001$), sex ($P = 0.0015$), Charlson-Deyo score ($P = 0.0018$), pathologic T stage ($P < 0.0001$), pathologic N stage ($P < 0.0001$), tumor size ($P = 0.0055$), resected margin status ($P = 0.0114$), adjuvant chemotherapy ($P = 0.0004$), and ≥3 LNDs (HR for death: 0.76, 95% CI: 0.62–0.94, $P = 0.0115$) were significantly associated with OS. In multivariable analysis, age ($P <$



At risk (year)	0	1	2	3	4	5	6	7	8	9	10
LNDs ≥ 10	668	590	467	382	280	216	151	102	73	48	29
7 ≤ LNDs ≤ 9	311	271	206	170	130	103	84	62	39	23	13
5 ≤ LNDs ≤ 6	247	217	169	145	101	67	48	34	26	20	13
3 ≤ LNDs ≤ 4	196	175	142	113	88	69	44	32	29	19	14
1 ≤ LNDs ≤ 2	126	107	85	64	51	33	24	20	18	14	10
LND = 0	36	33	26	19	15	12	9	8	5	4	2

FIGURE 2
The Kaplan-Meier curves of overall survival in early-stage small cell lung cancer patients who underwent curative lobectomy according to the number of lymph nodes dissected (≥ 10 vs. 7-9 vs. 5-6 vs. 3-4 vs. 1-2 vs. 0) are shown. LND; lymph node dissected.

0.0001), sex (P = 0.0039), Charlson-Deyo score (P = 0.0063), pathologic T stage (P < 0.0001), pathologic N stage (P < 0.0001), resected margin status (P = 0.0341), adjuvant chemotherapy (P < 0.0001), and ≥3 LNDs (HR for death: 0.76, 95% CI: 0.61–0.93, P = 0.00083) were independent factors for predicting OS. In the subgroup multivariate analysis of OS in the cN0/pN1-2 group (n = 311) showed that LNDs≥3 was an independent prognostic factor (HR for death: 0.52, 95% CI: 0.30–0.96, P = 0.0372; Supplementary Table 1).

Discussion

In the current study, we reported for the first time that SCLC patients with ≥3 LNDs had a significantly longer OS than those

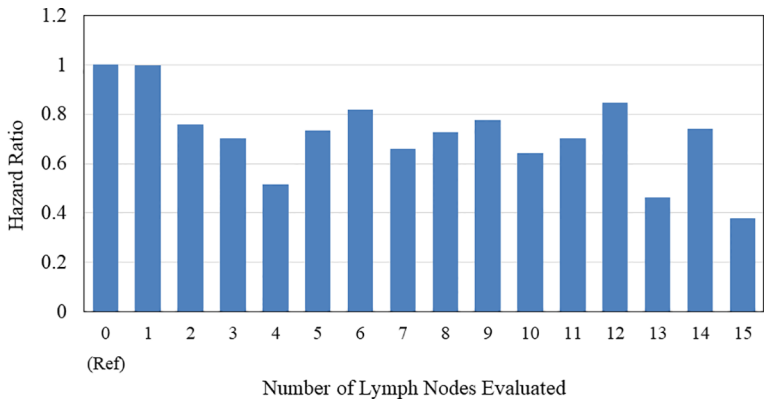


FIGURE 3
Hazard ratios for death by the number of lymph nodes evaluated are shown.

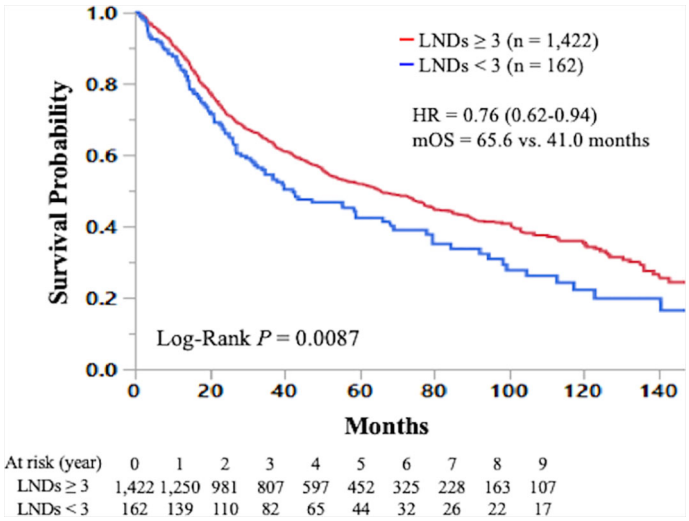


FIGURE 4
Kaplan-Meier curve of overall survival in early-stage small cell lung cancer patients who underwent curative lobectomy according to the number of lymph nodes dissected (≥3 vs. <3) is shown. LND; lymph node dissected; HR, hazard ratio; mOS, median overall survival.

with <3 LNDs. The multivariate analysis showed that ≥3 LNDs was an independent predictor for OS. Of note, the HR for death was 0.75 in patients with ≥3 LNDs compared with those with <3 LNDs, which suggests its significant prognostic and therapeutic impact. Further analyses showed that the difference in OS was not significant for cut-off of 10 LNDs. Given that the CoC recommends pathological examination of at least 10 LNs for resected early-stage NSCLC (10, 11), the appropriate cut-off for the minimal number of LNDs in early-stage SCLC may be less than that in NSCLC. Although we are reluctant to recommend a definitive "optimal number" of LNs evaluated, our findings suggested the prognostic and therapeutic roles for performing ≥3 LNDs in patients with resectable SCLC.

The recommended number of surgical LNDs for early-stage SCLC has never been investigated in the past clinical trials. This is due to the rarity of early-stage SCLC patients who are candidates for surgery. According to the previous report, stage I disease accounts for less than 5% of patients with SCLC, and patients with disease in excess of T1-2, N0 did not benefit from surgery (18). Given that highly selected SCLC patients are candidate for surgery, future randomized trials investigating the required extent of thoracic lymphadenectomy for early-stage SCLC may not be possible. Although our study was a retrospective study, the largest cancer database enrolled a total of 1,584 patients with resected SCLC, and suggested that at least 3 LNDs is recommended for early-stage SCLC.

We consider that survival gain resulting from LNDs is due to both diagnostic and therapeutic roles of LNDs. Regarding diagnostic role, the high-quality LNDs allow for accurate stage migration, subsequently optimal postoperative treatment, and improves patients' prognosis (16). Pathological nodal upstaging cases are identified in 10-20% of patients with clinical node-negative NSCLC (19). We presumed that the benefit from more LNDs was more accurate detection of nodal involvement in cN0 patients. Therefore, we conducted additional analysis regarding pN+ rates ([pN+ cases] divided by [pN+ cases + pN0 cases]) as shown in Supplementary Figure 1. As the number of LNDs increased, the upstaging rate of nodal status sequentially increased, which suggests that the high-quality LNDs enables accurate nodal staging. According to the NCCN guideline, postoperative chest radiation therapy is indicated for the patients with node-positive SCLC (9). With regard to therapeutic role, adequate LNDs can remove any remaining metastatic LNs and increase the cure rate. According to the previous report, the number of LNDs less than or equal to 15 was an independent predictor of higher probability of local recurrence in patients with completely resected pathological stage I NSCLC (20).

There are several limitations in association with our study. First, NCDB databases lack the data regarding the LN stations of the LNs investigated, which has been reported to be associated with OS in patients with NSCLC (21–24). The anatomical location of the positive LN stations has a significant effect on the prognostic value of the proportion of positive LNs (25). Second, the number of LNs removed is influenced by surgeon and pathologist procedures. Regarding surgeon procedure, if some LNs are removed in fragments, as often occurs during lung cancer resections, the pathologist can end up identifying a greater number of total LNs. From the standpoint of the pathologist, failure to remove and examine pathologically the peribronchial

TABLE 3 Multivariable analyses of overall survival in patients with resected clinical stage I-II (AJCCv7) small cell lung cancer.

Factors		Univariate HR (95% CI) *P* value	Multivariable HR (95% CI) *P* value
Age	<70	0.67 (0.59-0.77)	0.70 (0.60-0.80)
	≥70 (Ref)	< 0.0001	< 0.0001
Sex	female	0.80 (0.70-0.92)	0.81 (0.71-0.94)
	male (Ref)	0.0015	0.0039
Race	whites	1.01 (0.80-1.30)	0.94 (0.74-1.22)
	others (Ref)	0.9398	0.6421
Insurance status	uninsured	0.68 (0.32-1.23)	0.67 (0.32-1.22)
	others (Ref)	0.2166	0.2085
Institution	academic	1.05 (0.91-1.21)	0.96 (0.83-1.11)
	others (Ref)	0.4994	0.6215
Charlson-Deyo score	0-1	0.75 (0.63-0.89)	0.77 (0.65-0.93)
	≥2 (Ref)	0.0018	0.0063
Year of diagnosis	2004-2010	0.87 (0.74-1.02)	0.86 (0.73-1.00)
	2011-2017 (Ref)	0.0840	0.0602
Histology	others	1.05 (0.89-1.22)	1.01 (0.86-1.19)
	SCLC NOS (Ref)	0.5787	0.8631
Pathologic T stage	T0-1	0.67 (0.58-0.76)	0.69 (0.58-0.83)
	T2-4 (Ref)	<0.0001	< 0.0001
Pathologic N stage	N0	0.45 (0.39-0.52)	0.44 (0.38-0.51)
	N1-2/NX (Ref)	< 0.0001	< 0.0001
Tumor size	<30mm	0.81 (0.70-0.94)	1.09 (0.90-1.32)
	≥30mm (Ref)	0.0055	0.3864
Resected margin status	negative	0.68 (0.52-0.91)	0.73 (0.55-0.98)
	other (Ref)	0.0114	0.0341
Adjuvant chemotherapy	yes	0.77 (0.67-0.89)	0.73 (0.62-0.85)
	no (Ref)	0.0004	< 0.0001
Adjuvant chest radiation	no/unknown	1.02 (0.88-1.19)	0.97 (0.81-1.15)
	yes (Ref)	0.8022	0.6959
Number of lymph nodes dissected	3≥	0.76 (0.62-0.94)	0.76 (0.61-0.93)
	<3 (Ref)	0.0115	0.0083

AJCC, American Joint Commission on Cancer; SCLC, small cell lung cancer; NOS, not otherwise specified; Ref, reference.

LNs that are removed but not separately labeled by the surgeon during a lobectomy can lead to failure of identifying N1 LN involvement (26–29) Third, this is a retrospective study in association with a bias from surgeon's decisions. Surgeons may take more LNs in the middle of surgery if the LNs look suspicious of metastases. However, our study showed that patients with ≥3 LNDs had a significantly longer OS than those with fewer LNDs. Thus, the bias arising from surgeons' choice may not significantly contribute to longer OS in patients with fewer LNDs in the current study. Fourth, NCDB is lacking in the information about how surveillance was conducted, how patients were staged preoperatively, adjuvant treatment, operative time, and center-level effects. Further advanced study is needed to reach the definitive conclusions.

In conclusion, our retrospective analysis using the largest cancer database showed for the first time that patients with ≥3 LNDs had a significantly longer OS than those who had undergone fewer LNDs, suggesting prognostic and therapeutic roles for performing ≥3 LNDs. Further research is warranted to validate these findings.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Ethics statement

Based on the use of only de-identified data, the study was exempted by the Parkview institutional review board. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

ST contributed to the interpretation of data, and wrote the manuscript. TK contributed to all of the ideas of the current study and methods of analyzing the data. EP supervised the writing of the manuscript. All authors significantly contributed to this study. All authors read and approved the final manuscript.

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Conflict of interest

TK received travel fee from Merck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.962282/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Pathological nodal positive rates ([pN+ cases] divided by [pN+ cases + pN0 cases]) according to the number of lymph nodes dissected (≥ 10 vs. 7-9 vs. 4-6 vs. 1-3) are shown.

SUPPLEMENTARY FIGURE 2
Kaplan-Meier curve of overall survival in **(A)** T0-2 and **(B)** T3-4 small cell lung cancer patients who underwent curative lobectomy according to the number of lymph nodes dissected (≥3 vs. <3) is shown. LND; lymph node dissected.

SUPPLEMENTARY FIGURE 3
The Kaplan-Meier curves of overall survival in early-stage small cell lung cancer patients with pN0 who underwent curative lobectomy according to the number of lymph nodes dissected (≥ 10 vs. 7-9 vs. 5-6 vs. 3-4 vs. 1-2 vs. 0) are shown. LND; lymph node dissected.

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Harnessing DLL3 inhibition: From old promises to new therapeutic horizons

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Small-cell lung cancer (SCLC) is an aggressive neuroendocrine tumor with a high relapse rate, limited therapeutic options, and poor prognosis. The combination of chemotherapy and immune-checkpoint inhibitors brings a new therapeutic era, although the lack of predictive biomarkers of response reduces the efficacy of applying the treatment to the entire population of patients with SCLC. The lack of treatments able to bind to a specific target has always been a substantial difference to the non-small cell lung cancer (NSCLC) counterpart. Delta-like canonical Notch ligand 3 is a protein frequently overexpressed in SCLC and is therefore being explored as a potentially promising therapeutic target in high-grade neuroendocrine lung cancer. In this article, we critically review the activity and efficacy of old DLL3 inhibitors antibody-drug conjugate (ADC) and their failures through new compounds and their possible applications in clinical practice, with a focus on new molecular classification of SCLC.

KEYWORDS

DLL3, small-cell lung cancer, rovalpituzumab tesirine, tarlatamab, molecular classification

Introduction: The role of targeted therapies in SCLC

Small-cell lung cancer (SCLC) represents the most aggressive phenotype within the spectrum of all lung neuroendocrine tumors with rapid proliferation and chemoresistance to conventional antiblastic treatments. This results in poor prognosis in case of advanced stage at diagnosis (1, 2).

After more than 30 years, the first-line therapeutic paradigm for advanced stage has been changed by introducing new agents in combination with chemotherapy such as immune check-point inhibitors. This had led to an improvement of the median survival of these patients beyond 12 months (3, 4).

A major advance in modern oncological therapy in the field of lung neoplasms has been the identification of genetic factors, mostly linked to point mutations, deletions, insertions, translocations leading to the identification of tumor subtypes sensitive to molecularly targeted therapies. In addition to this, the evidence of some predictive

markers of response to immunotherapy, although inaccurate, have determined the greatest impact on survival in NSCLC, changing the natural history of this disease (5, 6).

The lack of predictors of response to the most modern treatments leads to the failure of so-called precision medicine in SCLC.

For example, analysis of biomolecular factors of patients considered to be strongly benefiting from chemo-immunotherapy treatment (i.e., longer than 18 months) with atezolizumab did not lead to evidence of benefit in any subgroup, regardless of the status of the biomarkers analyzed (7).

The exploratory analysis conducted in the CASPIAN study points in the same direction. The association between the antigen presentation factors (HLA class 1/2 alleles) and the overall survival (OS) showed that the presence of the HLA-DQB1 * 03: 01 allele was associated with a longer OS in the durvalumab + tremelimumab and chemotherapy arm, but not in the other arms, providing a proof of concept for further studies in the future (8).

Although SCLC is characterized by numerous genomic alterations typically caused by a specific pathogenic noxa (cigarette smoke), the study of these alterations has not led to the determination of specific drugs. Within some seminal works, whole-exome sequencing of SCLC tumor surgical samples in a treatment-naïve population confirmed the already known genetic features of this disease, characterized by a high mutational burden (8.6 mut/Mb), universal loss-of-function mutations in TP53 and RB1 and rare actionable targetable mutations in KIT, PIK3CA, BRAF and amplification of FGFR1, SOX2 and MYC (9).

Recently, new avenues have opened up with the evaluation of different SCLC subtypes defined by the differential expression of four key transcription regulators: ASCL1, NeuroD1, YAP1 and POU2F3 (10). The reveal of potential therapeutic vulnerabilities of these subtypes may constitute a step forward in personalized SCLC medicine (11).

In oncology, an ideal target is generally a molecular alteration that is more highly expressed in tumor tissue than in healthy cells and represents a factor that substantially promotes and supports cell proliferation and that can be blocked by a specific therapy leading to cell apoptosis.

Delta-like canonical Notch ligand 3 (DLL3) is an inhibitory ligand of the Notch pathway that is highly conserved in developing lung neuroendocrine cells. Therefore, the resulting downregulation involves the growth of neuroendocrine tumor cells (12).

DLL3 is overexpressed on the cell surface of neuroendocrine tumor cells in about 80% of SCLCs, whereas it is normally expressed in the cytoplasmic area in healthy cells (13).

DLL3 expression is also regulated by the transcription factor achaete-scute homolog 1 (ASCL1) which, in recent works, has been recognized as an oncogenic driver whose alteration is present in about 60% of all SCLCs.

The differential expression profile of DLL3 in normal vs. oncogenic tissue makes this target particularly interesting from a therapeutic point of view (14).

In recent years, the establishment of DLL3 as a unique target in SCLC has accelerated the development of novel and promising therapeutic agents.

The history of drug development involving the manipulation of this target has led to mixed results starting with older antibody-drug conjugate (ADC) such as rovalpituzumab tesirine (ROVA-T) through renewed interest in immuno-oncological agents such as bispecific T-cell engager (BiTE) and chimeric antigen receptor T cell (CAR-T), with their attendant failures, to new compounds and their possible applications in clinical practice, with a focus on a new molecular classification of SCLC.

Further development of these drugs could lead to the beginning of a new era of specific and highly active therapies in the therapeutic strategy of SCLC and other neuroendocrine neoplasms (15).

In this review, we will critically focus on the development of treatments against DLL3 and their perspective in clinical practice.

DLL3 expression and its role in high grade neuroendocrine lung cancer

DLL3 is a member of the Delta/Serrate/Lag2 (DSL) Notch receptor ligands, together with DLL1, DLL4, JAG1, and JAG2. Notch signaling is a highly conserved pathway involved in cell proliferation, differentiation, and apoptosis, which plays a pivotal role in the development of pulmonary neuroendocrine cells and is thus directly involved in the pathogenesis of certain tumors such as SCLC. DLL3 is an inhibitory ligand for the Notch receptor, normally located in the Golgi apparatus

Abbreviations: ADC, Antibody-drug conjugate; AE, Adverse event; ASCL1, achaete-scute homolog 1; BiTE, Bispecific T-cell engager; CAR-T, Chimeric antigen receptor T cell; CE, Cisplatin/carboplatin and etoposide; CRS, Cytokine release syndrome; DCR, Disease control rate; DLL3, Delta-like canonical Notch ligand 3; DLT, Dose limiting toxicities; DOR, Duration of response; DSL, Delta/Serrate/Lag2; ICI, Immune checkpoint inhibitor; IHC, Immunohistochemistry; LCNEC, Large-cell neuroendocrine carcinoma; mOS, Median overall survival; mPFS, Median progression-free survival; NE, neuroendocrine; NeuroD1, Neurogenic differentiation factor 1; NSCLC, Non-small-cell lung cancer; NGS, Next generation sequencing; ORR, Objective response rate; OS, Overall survival; PFS, Progression-free survival; POU2F3, POU domain class 2 homeobox 3; PR, Partial response; ROVA-T, Rovalpituzumab tesirine; SAE, Serious adverse event; SCLC, Small-cell lung cancer; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; TEAE, Treatment-emergent adverse event; YAP1, Yes-associated protein 1.

in healthy cells. DLL3/Notch binding prevents dislocation of the receptor on the cell surface and emerges on the cell membrane when it is pathologically overexpressed, resulting in aberrant growth of neuroendocrine tumor cells, including SCLC and large-cell neuroendocrine carcinoma (LCNEC) (13, 16). In a recent study, 63 patients with SCLC underwent immunohistochemistry (IHC) for DLL3: 52 patients (83%) were positive for DLL3 expression, with 20 patients (32%) showing high expression of DLL3 (positive in at least 50% of cancer cells) (17). DLL3 is not only involved in SCLC, but is also expressed in other tumor types of neuroendocrine origin, including melanoma, glioblastoma multiforme, small cell bladder cancer and castration-resistant prostate cancer (18). DLL3 expression is regulated by ASCL1, a transcription factor required for the proper development of pulmonary neuroendocrine cells, which is recognized as an oncogenic driver in ∼60% of all SCLCs (14, 19). ASCL1 is one of four key transcription factors whose expression underlies the emerging molecular classification of SCLC. In contrast to the increasingly targeted drugs for patients with lung adenocarcinoma involving EGFR, ALK, ROS1, RET, BRAF, MET and NTRK, SCLC is still perceived and treated as a single disease, a "homogenous" entity without clinically relevant molecular subtypes. However, based on the expression of several neuroendocrine (NE) markers, such as chromogranin A (CHGA), synaptophysin (SYP), neural cell adhesion molecule 1 (NCAM1/CD56) and gastrin-releasing peptide (GRP) SCLC can be classified into neuroendocrine-high (NE-high) or neuroendocrine-low (NE-low) tumor. The NE-high and NE-low subtypes show distinct genetic alterations and a different susceptibility to immune checkpoint inhibitors (ICIs), suggesting that some sort of biological heterogeneity also exists for SCLC (20). The biological heterogeneity of SCLC has started to emerge through studies based mainly on preclinical models such as genetically engineered mouse models (GEMMs) and patient-derived xenografts (PDXs) (21). Recent genomic profiling studies have defined SCLC molecular subtypes based on the relative expression of key transcription regulators, including ASCL1, NeuroD1 (neurogenic differentiation factor 1), YAP1 (yes-associated protein 1), and POU2F3 (POU domain class 2 homeobox 3). Multiple independent researchers have proposed a consistent nomenclature for these SCLC subtypes: SCLC-A (A=ASCL1), SCLC-N (N=NeuroD1), SCLC-Y (Y=YAP1) and SCLC-P (P=POU2F3). SCLC-A and SCLC-N show high expression of NE markers. In contrast, SCLC-Y and SCLC-P are considered non-NE tumor subtypes. Whole genome sequencing (WGS) revealed an enormous mutational burden and a high number of genetic alterations that characterize each SCLC subtype more or less specifically: ∼90% biallelic loss of TP53 and RB1, overexpression/amplification of cyclin D1 (CCD1), inactivation of cyclin dependent kinase inhibitor 2A (CDKN2A) and alteration in several genes involved in cell cycle regulation (CDK4/6), receptor kinase signaling (KIT, FGFR1), transcriptional regulation (CREBBP, MYC), apoptosis (SOX2, BCL2) and neuroendocrine differentiation/Notch signaling (22). Therefore, DLL-3 inhibitors now represent a potential therapeutic target approach in NE-high SCLC-A, underlining the importance of the emerging concept that heterogeneity in SCLC is primarily based on neuroendocrine differentiation, molecular subtype, and gene expression profile.

The old journey: First generation DLL3 inhibitors

Rovalpituzumab tesirine is a first-in-class DLL3-targeted antibody-drug conjugate consisting of the humanized DLL3-specific IgG1 monoclonal antibody SC16, the DNA cross-linking agent SC-DR002 and a protease-cleavable linker that covalently binds SC-DR002 to SC16. Rudin et al. evaluated single-agent ROVA-T in SCLC or LCNEC with measurable progressive disease previously treated with one or two chemotherapeutic regimens, including a platinum-based regimen, in a first-in-human, open-label, phase I study (16). The primary objective of the study was to assess the safety of ROVA-T; secondary objectives were to characterize the pharmacokinetics and immunogenicity of ROVA-T, estimate its antitumor activity, and establish the recommended phase II dose and schedule. Eighty-two (82) patients were enrolled, 74 SCLC and 8 LCNEC (excluded from main endpoint analyses). All patients received at least one dose of ROVA-T. The maximum tolerated dose was reported to be 0.4 mg/kg every 3 weeks, but this dose was associated with an unacceptable level of delayed toxic effects. Consequently, the recommended dose and schedule of ROVA-T was defined as two cycles of 0.3 mg/kg every 6 weeks. Sixty-six (88%) patients reported treatment-related adverse events (ADs) of any grade, 28 (38%) patients of grade 3 or worse. Thrombocytopenia, serous effusions, and skin reactions were the most frequent treatment-related ADs of grade 3 or worse. Eighteen (22%) patients discontinued treatment due to ADs. Of the 65 patients assessable for activity analyses, 11 (17%) achieved a confirmed objective response and 35 (54%) stable disease. The median duration of response (DOR) was 5.6 months, and the median progression-free survival (mPFS) was 3.1 months. Median OS (mOS) was 4.6 months in the 68 patients treated with the active dose levels of ROVA-T. Considering the 29 DLL3-high patients, ten (35%) had a confirmed objective response, mPFS was 4.5 months, and mOS was 5.8 months. Udagawa and colleagues conducted a similar phase I, open-label, dose-escalation study among the Japanese population (23). SCLC patients, pre-treated with at least two systemic regimes, including one platinum-based regimen, received 2 doses of ROVA-T (0.2 or 0.3 mg/kg) every 6 weeks. Retreatment was allowed for patients who tolerated initial doses and achieved a disease control for at least 12 weeks after the last dose, but only one patient was retreated with ROVA-T. The primary objective was to assess safety and tolerability; secondary objectives were

pharmacokinetics and preliminary efficacy. DLL3 expression was classified as high (≥75%), positive (≥25%), or negative (< 25%) as IHC-based score. A total of twenty-nine (29) patients were enrolled, 6 in the 0.2 mg/kg cohort and 23 in the 0.3 mg/kg cohort. As expected, most patients (64%) expressed high-DLL3. All patients experienced at least one treatment-emergent adverse event (TEAE) of any grade; 15 patients reported AD of grade ≥3. These safety findings were accompanied by low activity: 3 (10%) patients achieved a confirmed partial response (PR). The median DOR was 3.0 months, mPFS was 2.2 months, and mOS was 5.8 months. All responders received 0.3 mg/kg ROVA-T and had tumors with high DLL3 expression. In patients with high DLL3 expression, mOS was 7.4 months compared to 5.1 months in non- high DLL3 patients (23). Despite the premise, ROVA-T was evaluated by Hann and colleagues in a phase I, multicenter, open-label study in a chemo-naïve population. After an initial chemotherapy cycle, ROVA-T was administered as monotherapy sequentially or in combination with cisplatin/carboplatin and etoposide (CE). The primary endpoint was safety, while secondary endpoints included efficacy and pharmacokinetic assessment of ROVA-T combined with CE. Based on preliminary safety and efficacy data, patients who received lower doses of ROVA-T (0.1 or 0.2 mg/kg) in combination with CE were selected for further evaluation. Drug-related TEAEs of any grade occurred in 14 (100%) patients, while serious adverse events (SAEs) were observed in 13 (93%) patients. Seven patients (50%) achieved a confirmed objective response rate (ORR), mPFS was 5.2 months and mOS was 10.3 months. These results do not suggest any efficacy benefit of frontline combination treatment with ROVA-T (24). Despite these discouraging results, the phase II TRINITY study was conducted in a population of relapsed or refractory SCLC. All patients received ROVA-T 0.3 mg/kg every 6 weeks for two cycles as initial treatment and a retreatment was allowed in patients who had benefited from the first cycle. The co-primary endpoints were ORR and OS. Secondary endpoints were DOR, disease control rate (DCR), and PFS. Three hundred thirty-nine patients were enrolled and received at least one dose of ROVA-T, most of them (70%) with DLL3-high. The ORR for the entire population was 12.4%, mPFS was 3.5 months, and mOS was 5.6 months with no significant difference in the DLL3-high subgroup. Almost all patients reported at least one TEAE, grade 3 or 4 TEAEs were observed in 179 (54%) patients. The most frequent severe ADs were in order of incidence: cutaneous reaction, edema, and pleural effusion (54 vs. 38 vs. 32%, respectively) (25). Lastly, the activity of ROVA-T was evaluated in two phase III studies. The TAHOE study was an open-label, two-to-one randomized, phase III study that compared the efficacy and safety of ROVA-T vs. topotecan in patients with DLL3-high SCLC progressed during or after first-line platinum-based chemotherapy. The primary endpoint was OS. The ROVA-T schedule used was the same as previously described in phase II studies. After enrolment of 444 patients, the study was stopped because OS with ROVA-T was shorter than with topotecan, and statistical tests for efficacy endpoints were not performed as originally planned. mOS was 6.3 months in the ROVA-T arm and 8.6 months in the topotecan arm; mPFS was 3.0 and 4.3 months, respectively. ORR was 15% in the ROVA-T arm vs. 21% in the topotecan arm, with grade 3 or higher ADs reported in the ROVA-T arm in 64% of patients (26). The second phase III study explored ROVA-T in the maintenance phase. MERU was a phase III randomized in a 1:1 ratio, double-blind, placebo-controlled study that enrolled patients with SCLC who had achieved disease control after four cycles of first-line platinum-based chemotherapy, measured as stable disease, partial response, or complete response according to RECIST v.1.1. The primary endpoints were PFS and OS in the population with DLL3-high tumors. After enrolment of 748 patients, the study was stopped early due to the OS-based futility analysis. In the high DLL3 population (61%), mOS was 8.5 months in the ROVA-T arm and 9.8 months in the placebo arm and ORR was 10 and 5%, respectively. The mOS for all randomized patients (secondary endpoint) was 8.8 months in the ROVA-T arm and 9.9 months in the placebo group. No significance was observed in the mOS of the population with DLL3-high tumors vs. DLL3-low. However, with regard to PFS, a favorable trend was observed for DLL3-high tumors. Overall, 343 (93%) patients in the ROVA-T arm and 304 (82%) in the placebo arm experienced at least one TEAE; TEAEs of grade greater than or equal to 3 occurred in 217 patients (59%) in the ROVA-T arm and 111 (30%) in the placebo arm (27). ROVA-T was also evaluated in combination with ICIs in a phase I–II study. The primary endpoint was to assess the safety and tolerability of administering ROVA-T in combination with nivolumab or nivolumab plus ipilimumab; the secondary endpoint was antitumor activity. Fourty two patients were enrolled, 30 in cohort 1 (nivolumab) and 12 in cohort 2 (nivolumab plus ipilimumab). Overall, 23 (55%) patients were DLL3 high. Four patients experienced dose-limiting toxicities (DLTs), of which one belonged to cohort 1 and three to cohort 2. All 42 patients reported one or more TEAEs, with 38 (91%) patients reporting grades ≥3. In cohort 1, the confirmed ORR was 27.6%, mPFS was 4.8 months, and mOS was 7.4 months; in cohort 2, the confirmed ORR was 36.4%, mPFS was 4.1 months, and mOS was 11.0 months. For the entire sample ORR was 30%, mOS 7.4 months, and PFS 4.2 months (28) (see Table 1). In all these trials, ROVA-T showed a unique toxicity profile, with pleural and pericardial effusion, peripheral oedema, cutaneous reaction, and thrombocytopenia among the most common ADs. The mechanism of these toxic effects is unclear, but the most likely explanation is premature linker lysis, which causes systemic release of the DNA cross-linking agent SC-DR002. Although ROVA-T was the first target therapy studied for SCLC, the lack of predictive biomarkers, the unique toxicity profile shown in all clinical studies, and the modest clinical activity led to the discontinuation of development of this drug (29).

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TABLE 1 ROVA-T trials: Characteristics and results.

	Phase	Treatment	Line	Patients (*n*)	DLL3 high (%)	ORR (%)	PFS (m)	OS (m)	any grade AE (%)	≥3 AE (%)
Rudin (16)	I	ROVA-T	Pretreated	74	45	17	3.1	4.6	88	38
Udagawa (23)	I	ROVA-T	Pretreated	29	64	10	2.2	5.8	100	52
Hann (24)	I	CE plus ROVA-T (cohort 3)	First	14	100	50	5.2	10.3	100	93
Morgensztern (25)	II	ROVA-T	Pretreated	339	70	12.4	3.5	5.6	99	54
Blackhall (26)	III	ROVA-T vs. topotecan	Second	444	100	15	3	6.3	95	64
Johnson (27)	III	ROVA-T vs. placebo	Maintenance	748	61	10	/	8.5	93	59
Malhotra (28)	I-II	ROVA-T plus nivolumab/ROVA-T plus nivolumab and ipilimumab	Pretreated	42	55	30	7.4	4.2	100	91

CE, cisplatin/carboplatin and etoposide; DLL3, Delta-like canonical Notch ligand 3; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; AE, adverse event; ROVA-T, rovalpituzumab tesirine.

The new journey: Second generation DLL3 inhibitors

AMG 757 is a first-in-class bispecific T-cell engager antibody consisting of two domains. One domain binds the DLL3 on tumor cells and the other binds the CD3 part of the T-cell receptor. In this way, AMG757 connects DLL3-positive tumor cells and T-cells, producing both tumor cells lysis and T-cells activation. In addition, this binding causes the production of cytokines that overwhelm the immunosuppressive environment of the tumor (18, 30–32). The structure of the antibody allows an extended half-life of 9.8 days. *In vitro*, low doses of AMG 757 are sufficient to induce the killing of DLL-3 positive tumor cells by T-cells without effects on DLL3-negative cells, including normal cells. These pharmacokinetic properties allow delayed administrations in humans (30–32).

Giffin and colleagues evaluated AMG 757 efficacy in cell lines and xenograft mouse models derived from SCLC patients. They demonstrated that once-weekly administration of AMG 757 induces T-cell activation and expansion in xenograft and orthotopic mouse models derived from patients with SCLC tumors in. *In vitro*, AMG 757 leads to T-cell activation, the production of proinflammatory cytokine and the release of cytotoxic granules. Engaged T-cells kill SCLC cell lines, including those with low levels of DLL3 expression. *In vivo*, the authors evaluated the activity of AMG 757 in mouse models of patient-derived SCLC xenografts. Treatment with AMG 757 induced overall significant reduction in tumor volume. The activity was also evaluated in orthotopic SCLC models with weekly intravenous infusion. Similarly, AMG 757 treatment led to a significant reduction in tumor growth in these models. A single administration induced a significant increase in the number of human CD4+ and CD8+ T cells. In non-clinical toxicological studies, AMG 757 was well tolerated at the maximum dose of 4.5 mg/kg, confirming low DLL3 expression on normal cells (31).

Clinical experience in humans is also reassuring. An ongoing phase I study evaluated AMG 757 monotherapy in combination with anti-PD1 therapy and additional cytokine release syndrome (CRS) mitigation strategies in adult SCLC patients who had progressed or recurred after at least 1 platinum-based chemotherapy (NCT03319940). AMG 757 was administered intravenously once every 2 weeks at escalating doses up to 10 mg (0.003e10.0 mg). As of 7 august 2020, the study enrolled 40 patients with a median age 64 (44–80). Preliminary results from the monotherapy arm showed a median treatment duration of 6.1 weeks (0.1–59.4). AEs were reported in 39 (97.5%) patients and 4 (10%) discontinued treatment due to such effects. 32 (80%) were treatment-related, including 7 (17.5%) grade ≥3 and 1 (2.5%) grade 5 pneumonitis. Cytokine release syndrome (CRS) occurred in 18 (45%) patients, grade 1 or 2, none grade 3. The symptoms of CRS were fever and

hypotension, occurred within 24 h of the first two doses (or during the first 24 h) and were reversible. There were no reports of interruption or discontinuation of treatment due to CRS. A confirmed partial response (PR) was observed in 6 (15.8%) patients and stable disease in 11 (28.9%) patients. Patients with confirmed PR were mostly heavily pre-treated with a median of 2 (1–4) prior lines of therapy. They had a DOR between 1.9 and 9.4 months. Tumor shrinkage occurred irrespective of DLL3 expression (range 55–300) (33). The trial is still active and recruiting.

Another novel therapy targeting DLL3 is AMG 119, a chimeric antigen receptor T cell (CAR-T). T cells are taken from the patient and genetically modified *ex vivo* to express a chimeric antigen receptor that targets DLL3. Subsequently, cytotoxic T-cells are re-administered to the patient to recognize and kill DLL3-positive cells. Unlike AMG 757, AMG 119 can induce long-lasting antitumor activity with a single administration (34).

Preclinical data have shown that AMG 119 has high potency and specificity for DLL3-positive tumor cells. *In vitro*, AMG 119 is shown to enhance T-cell cytotoxic activity and pro-inflammatory cytokine production. *In vivo*, AMG 119 induces tumor shrinkage in xenograft models (34).

Clinical data are immature. NCT03392064 is an open-label, phase I study evaluating the safety and tolerability of AMG 119. Secondary endpoints include ORR, PFS and OS. Eligible patients are adult patients with SCLC that has progressed after at least one platinum-based chemotherapy. AMG 119 is administered intravenous once. The trial is currently suspended.

Discussion and future perspectives

Precise and effective therapy for SCLC represents an unmet medical need. Some progress has been made using modern technologies and next generation sequencing (NGS), but a thorough understand of the biology of SCLC is crucial.

DLL3 is an atypical ligand of the Notch receptor family that is found on the surface of tumor cells and in over 80% of SCLC. It should be noted that expression in normal lung tissue is low or null. The Notch pathway is associated with cancer proliferation and DLL3 participates in neuroendocrine tumorigenesis. Moreover, DLL3 is associated with a poor prognosis, particularly in some rare neuroendocrine subtypes (35).

Based on the high DLL3 expression in SCLC and LCNEC, DLL3 represents an interesting and novel targeted therapy.

In recent clinical trials, ROVA-T, a DLL3-targeting Ab-drug conjugate, has been tested as a novel antitumor drug. However, the phase III trials TAHOE and MERU (26, 27) demonstrated a shorter OS than standard therapy. Consequently, its development was permanently discontinued in August 2019. The absence of predictive biomarkers was a reason for the failure of Rova-T development. DLL3 expression was evaluated as biomarker, but while an enrichment of responses was observed in early studies in DLL3-high tumors, these results were not confirmed in phase 3 trials, although TAHOE trial enrolled only DLL3 high patients, thus the predictive role of DLL3 as biomarker was not tested in the same way as earlier trials (26). The combination of Rova-T with nivolumab + ipilimumab or nivolumab in the case of progressive disease (NCT03026166) was also discontinued after the DLT evaluation phase of the cohort (28).

Translational research is also investigating possible mechanisms of resistance to Rova-T, but there are currently no clinical implications (36).

Antibody drug conjugates are among the fastest growing drug classes in oncology; for example, the recent evolution in ADCs is evident in breast cancer; DESTINY-breast03 trial compared the efficacy and safety of trastuzumab deruxtecan (T-DXd), an ADC that combines the humanized anti-HER2 mAb trastuzumab with the topoisomerase inhibitor deruxtecan *via* a protease-cleavable peptide linker, with those of trastuzumab emtansine (T-DM1), an ADC composed of the anti-HER2 mAb trastuzumab connected to the microtubule inhibitor emtansine *via* a noncleavable linker, in patients with advanced HER2 positive breast cancer previously treated with trastuzumab and a taxane. In these patients the risk of disease progression or death was lower among who received trastuzumab deruxtecan than among who received trastuzumab emtansine (37). In this way, future development of rovalpituzumab, as mAb targeting DLL3 in the structure of an ADC, could include a different and more consistent linker, an increasing payload loading, novel and more powerful payloads or more innovative payloads that could overcome resistance to previous therapies (38).

Despite the discontinuation of ROVA-T development, new molecules targeting DLL3, such as near-infrared photoimmunotherapy, AMG 757, and AMG 119, have been explored with some promising data.

AMG 757 is a bispecific T-cell engager (BiTE). As bispecific recombinant proteins that target a T-cell surface molecule and a tumor-specific surface antigen, they promote T-cell adherence and anti-tumor response through an MHC-independent strategy (39). AMG 757 alone and in combination with pembrolizumab is being evaluated in a phase I study (NCT03319940) and is also being evaluated in combination with AMG 404 in a phase I/II study (NCT04885998). In addition, a phase II trial (NCT05060016) is ongoing in subjects with pre-treated, relapsed/refractory SCLC, in which tarlatamab, a half-life extended bispecific T-cell engager (HLE BiTE immune therapy) targeting DLL3, is being evaluated.

HPN328 is a tri-specific, half-life extended, T-cell engager targeting DLL3 and designed to minimize off-target toxicities. Interim results from an ongoing phase 1/2a study (NCT04471727) in patients with small cell lung cancer and other neuroendocrine cancers have shown promising results with regard to toxicity, and dose escalation is ongoing (40).

AMG 119 is a therapy based on CAR-T cells targeting DLL3. A phase I study (NCT03392064) was conducted in relapsed/refractory SCLC (currently suspended).

Specifically, Chen et al. (41) investigated the efficacy of DLL3-targeted bispecific antibody and CAR-T cells alone or in combination with immunotherapy.

The bispecific antibody and CAR-T showed activity in blocking the tumor growth *in vivo*. The association with the PD-1 inhibitor increased the activity of the DLL3 bispecific antibody, but not that of CAR-T cells. Although the results are rather encouraging, further studies are needed to verify this possible approach.

A new type of therapy, the near-infrared photoimmunotherapy has been providing intriguing results. Near-infrared photoimmunotherapy is an anticancer treatment technology that uses an Ab-photosensitizer conjugate followed by exposure to near-infrared light to damage cancer cells (42).

Incubating cells with ROVA-IR700 (ROVA-T conjugated with an IR700 photosensitizer) resulted in significant cell lysis upon exposure to near-infrared light. ROVA-IR700 has also been shown to shrink xenografts in mice (42).

Recently, another interesting therapeutic approach is radioimmunotherapy for SCLC. It consists of radiolabeling the anti-DLL3 antibody SC16 with the therapeutic radioisotope Lu-177 that emits beta particle. [^{177}Lu] Lu-DTPA-CHX-A″-SC16 binds to DLL3 on SCLC cells and delivers targeted radiotherapy into the cancer cells, preserving healthy tissue.

A systemic radioimmunotherapy strategy employing a monoclonal antibody with high specificity for DLL3 is the basis of a proof-of-principle study conducted by Tully and colleagues in tumor-bearing mice (43).

The study investigated the preclinical efficacy and toxicity of 177Lu-labeled SC16 for the treatment of human SCLC in tumor-bearing mice. The results show impressive efficacy in mouse models of subcutaneous xenograft of SCLC, with moderate and transient hematologic toxicity and no significant hepatotoxicity.

These findings support [^{177}Lu] Lu-DTPA-CHX-A″-SC16 as a potential development for clinical translation. Moreover, the possibility of using 89Zr-immunoPET to identify who would benefit more from targeted radioimmunotherapy with [^{177}Lu]Lu-DTPA-CHX-A″-SC16 could represent a clinically meaningful opportunity (44).

Although the results are preliminary and need to be confirmed in further studies, they are appealing.

More recently, immunotoxin therapy is becoming a promising way to treat cancer. Ataee el al. (45) have designed two recombinant immunotoxins against DLL3 containing single-chain variable fragment rovalpituzumab antibody, which will require further experimental analysis.

All the above-mentioned findings are relevant to provide progress in the treatment of SCLC. Preclinical and clinical data show some encouraging outcomes.

The development of novel targeted therapies in SCLC is crucial and extremely challenging. The role of these drugs, alone or in combination with immunotherapy, radiotherapy, or other molecules, is being studied and it is hoped that they will change the scenario of SCLC treatment.

As SCLC is still a deadly disease, more attention should be paid to improving its therapeutic strategy. Strategies include advances in genomic profiling and biological pathways to identify potential tailored therapies and novel molecular targeted therapies. With regard to surface molecules, the identification of an affective antibody drug conjugate could be an attractive therapeutic target in the future, as well as radioimmunotherapy.

The outcomes of ongoing clinical trials and future research could contribute to breakthroughs in the treatment of SCLC.

Author contributions

DC and SC designed the work. FC, MA, LS, MM, NC, ES, and SC contributed to data analysis and interpretation. MM drafted the first draft. All authors critically revised the work for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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