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REVIEW

Liquid biopsy in diagnosis and monitoring of treatment efficacy in patients with small cell lung cancer

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Abstract

Small-cell lung cancer (SCLC) remains one of the deadliest cancers worldwide. Patients' survival remains poor due to its rapid growth, high metastatic rate and limited possibilities of treatment. For many years, SCLC management has been based mostly on chemo and radiotherapy. However, new therapeutic approaches have been proposed in the past few years, including immunotherapy, which is currently implemented in clinical practice. Unfortunately, in many cases, response to therapy, especially chemotherapy, remains poor, or the patient becomes resistant to initially effective treatment. One of the crucial problems during SCLC patient care is a lack of appropriate predictive biomarkers for various therapeutic approaches. Another critical issue is the scarcity of collected tissue during biopsy, which may be insufficient or of too poor quality for analysis. A liquid biopsy might be the key to solving both of those problems as it is collected in a non-invasive way and enables the measurement of various biomarkers, including circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs). In this review, we discuss various approaches to potentially incorporating liquid biopsy into clinical application - as a companion to imaging during SCLC diagnostics, a new approach to molecular subtyping, and a material enabling predictive or prognostic biomarkers assessment. We also summarize ongoing clinical trials encompassing SCLC patients in which liquid biopsy is collected and examined.

Keywords Small-cell lung cancer · SCLC · Liquid biopsy · Next generation sequencing · CfDNA · CfRNA

Introduction

Small-cell lung cancer (SCLC) represents approximately 15% of lung cancer incidents worldwide [1]. Due to its rapid growth, high vascularity and wide ability to form metastases, it is often diagnosed at an extensive stage (ES-SCLC) [2, 3]. Therefore, SCLC is one of the deadliest cancers in the world, and the five-year survival rate is observed in only 7% of patients [1, 4]. SCLC is known as highly associated with tobacco exposure [5, 6], and still occurs more often in males than in females. However, this distinction is blurring [3].

As proposed therapies remain ineffective in a large subset of patients, a better understanding of sensitivity to treatment in the SCLC cohort is still needed [7–9]. The majority of SCLC cases are associated with *TP53 (Tumor Protein 53)* and *RB1 (Retinoblastoma)* mutations [2, 3, 10]. However, it is known that its molecular landscape is more complex. In recent years, further classification of SCLC has been proposed, including its division into four molecular subtypes: SCLC-A, SCLC-N, SCLC-P and SCLC-Y. Those subtypes are associated with overexpression of transcriptional factors: Achaete-Scute Family BHLH Transcription Factor 1 (ASCL1), Neuronal Differentiation 1 (NEUROD1), POU Class 2 Homeobox 3 (POU2F3) and Yes1 Associated Transcriptional Regulator (YAP1) [7, 11, 12]. To improve the effectiveness of implemented therapies, clinical trials analysing different therapeutical approaches, including divergent strategies in specific molecular subtypes, are currently underway [9, 11].

However, immunotherapy with anti-PD-L1 antibodies (atezolizumab, durvalumab) in combination with chemotherapy is the most effective in patients with tumor characterized by high inflammatory reaction. In particular proposed categorization, this type of tumor is characterised by high

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expression of *YAP1* gene or without expression of any of the genes mentioned above [7, 12, 13]. Recent genomic and transcriptomic analyses shed light on SCLC as a tumor that should be treated with a personalized approach. However, some difficulties can be found in this strategy.

One of the problems with the analysis of the SCLC genomic landscape is the small size and poor quality of tissue samples, which are collected during fine-needle aspiration biopsy and, subsequently, used for molecular examination [7, 12, 13]. Moreover, tissue collection is not being repeated in most cases, and it is impossible to observe changes in tumor molecular features associated with acquired resistance to therapy [14]. Implementing liquid biopsy into the clinical routine could solve the abovementioned problems, as its collection is non-invasive and may be performed multiple times with minimal risk [14]. Moreover, a one-site biopsy may be associated with bias due to the heterogeneity of the tumor. Whereas, liquid biopsy is representative of whole cancerous tissue, and this limitation does not occur in case of its analysis [15].

Considering the increasing interest in non-invasive tests in the oncological patients, in this review, we will summarize current knowledge about the possible usage of liquid biopsy in SCLC patients. Particularly we indicate its role as an alternative for challenging tumor tissue assessment, for instance, in cases where tissue is unavailable for analysis. In addition, we summarize ongoing clinical trials in which liquid biopsy has been implemented.

Liquid biopsy

The term *liquid biopsy* encompasses a collection of liquid materials, most commonly peripheral blood, which enables assessment of tumor-derived products [16, 17], including circulating free nucleic acids - DNA (cfDNA) [18] and RNA (cfRNA) [19], circulating tumor cells (CTCs) [18], tumor-educated platelets (TEPs) [20] and extracellular vesicles (EVs) [21]. Except for blood, other materials such as urine, cerebrospinal fluid (CSF), pleural effusion or ascites can be examined as a liquid biopsy (Fig. 1) [16, 17].

The main advantage of liquid biopsy over tissue usage is its minimal invasive collection. Therefore, its analysis may be performed repeatedly, enabling patients' regular monitoring without requiring re-biopsies [22]. Liquid biopsy is a source of cfRNA, such as microRNAs (miRNAs), which, due to their role as epigenetic regulators of genes expression, are known to take part in various processes, including tumorigenesis [23]. Specific miRNAs are under consideration as prospective prognostic and predictive factors in oncology patients [23–26]. Liquid biopsy may therefore represent a non-invasive, yet valuable source of numerous factors of potential clinical significance. Moreover, while results of single-site biopsy may not correspond to the whole tumor tissue [27, 28], this problem does not exist in the case of liquid biopsy, as tumor-derived components are shed to body fluids by all apoptotic and necrotic cells [15, 28, 29].

One of the main limitations of liquid biopsy usage seems to be the fragility and low concentration of its biomarkers [30]. Accordingly, highly sensitive methods may be required for extraction and detection of these factors [31, 32]. Moreover, a problem during cfDNA analysis may be the differentiation of nucleic acid originating from healthy tissue from this released from the tumor (circulating tumor DNA, ctDNA) [31].

Despite numerous advantages, currently, liquid biopsy is frequently used in research studies or during clinical trials [33–35]. Its routine use is minimal, and only a small set of tests have been approved in clinical practice such as *Epidermal Growth Factor Receptor* (*EGFR*) gene mutations testing in non-small cell lung cancer [35]. Wider liquid biopsy implementation into everyday oncological practice requires broader validation and standardization of procedures to make results more comparable [36, 37].

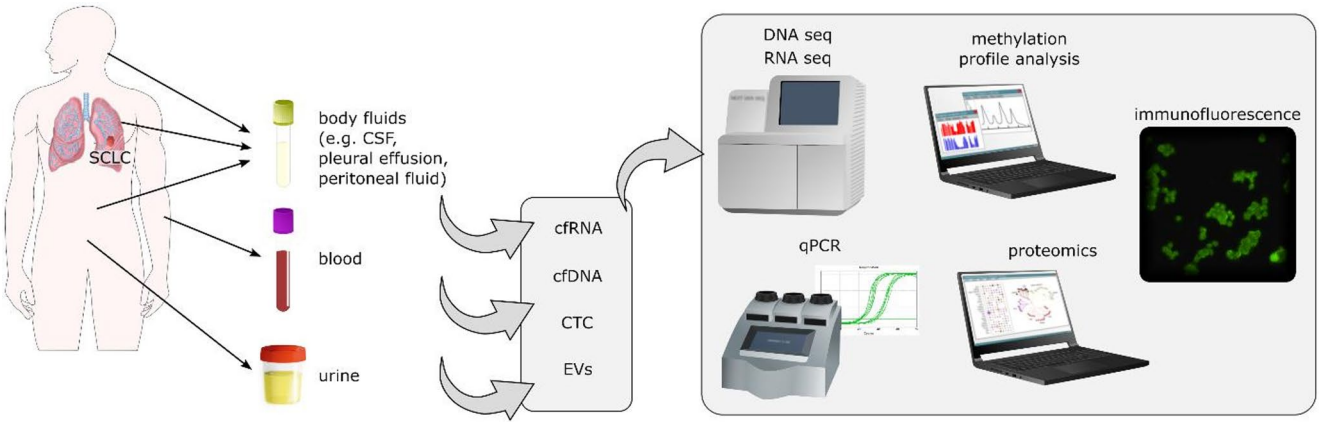

Fig. 1 Materials used in liquid biopsy and their further analysis options cfDNA– circulating free DNA; cfRNA– circulating free RNA; CTCs– circulating tumor cells; EVs– extracellular vesicles (this figure was drawn using Inkscape 1.3)

Liquid biopsy in SCLC patients

Liquid biopsy is considered as a complement [38, 39] or alternative to tissue examination [40, 41] in various oncological cohorts. Its usage seems especially beneficial in cancers such as SCLC, where tissue sample collection may be problematic [42]. Moreover, plasticity and the occurrence of dynamic molecular changes in SCLC are suggested [43, 44], and, therefore, the possibility of tumor molecular landscape re-testing seems to be especially important [45]. Numerous possible clinical applications of liquid biopsy analysis are proposed in the SCLC patients, including early diagnostics, subtyping, prognosis assessment, and supporting therapeutic decision-making. Currently, subsets of clinical trials, involving patients with small cell lung cancer, implement analysis of liquid biopsy-based markers. However, most of those trials include a small number of patients and are not specifically concerned to liquid biopsy evaluation but treat it as an additional possibility of outcome measurement (Table 1.).

SCLC detection and differentiation with NSCLC

Several new approaches to SCLC detection using liquid biopsy were suggested. Studies mostly focused on different methods of circulating free nucleic acid sequencing. In the study performed by Nunes et al., methylation assessment was proposed as a tool for either the differentiation of lung cancer subtypes or the early detection of SCLC [46]. Higher methylation levels of *HOXA9* (*Homeobox A9*) and *RASSF1A* (*Ras Association Domain Family Member 1*) genes were pointed out as characteristic of SCLC in comparison to non-small-cell lung cancer (NSCLC). Analysis of those two genes enabled the detection of SCLC with 64% sensitivity and approximately 96% specificity. However, a small subset of liquid biopsies collected from SCLC patients (only 19 SCLC versus 110 NSCLC) is a limitation of this study [46].

The potential of methylation analysis in the SCLC patients was proposed by Chemi et al. [47]. This methylome study was based on NGS combined with a methylation-based machine learning classifier. This combination enabled the appropriate classification of 93% of patients with limited stage SCLC (LS-SCLC) with area under the receiver operating characteristic curve (AUROC) amounted to 0.986 and 100% of patients with extensive stage of SCLC with AUROC equal to 1.0. Moreover, it was pointed out that the calculated methylation score correlates positively with the disease stage and that methylation of genes may have prognostic value. Patients with low methylation scores had significantly longer overall survival (OS) than those with high ones. As the authors pointed out, using liquid biopsy solves the problem of material scarcity, which is common in the case of tissue testing [47, 48].

Xue et al. constructed and validated two custom tools–ESim-seq (Early Screening tech with Integrated Model) and LCSC model (Lung Cancer Subtype multiple Classification) for non-invasive lung cancer detection and differentiation of SCLC from NSCLC. ESim-seq is a tool that assesses four fragmentomics (fragmentation patterns and end motifs in cfDNA) and four methylation features. After its validation, it reached 89.6% sensitivity and 97.4% specificity overall for diagnosis of SCLC, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). The LCSC model was based on fragmentomics and methylation analysis. It was indicated that it allows the distinguish SCLC and NSCLC patients with the area under the curve (AUC) of 0.961. However, one of the main limitations of the study by Xue et al. is the inclusion of SCLC patients only with extensive stage and metastatic disease. Therefore, it is unclear if the abovementioned tools will be useful in patients with LS-SCLC [49].

Despite proposed ctDNA usage, the implementation of CTCs in lung cancer diagnosis and differentiation is also being suggested. Seo et al. proposed SCLC detection with morphometric analysis of rare CTCs based on High Definition Single Cell Assay (HDSCA). The authors indicated that populations of CTCs in blood collected from SCLC patients are highly heterogenous. However, the number of rare CTCs is significantly higher in SCLC patients compared to healthy subjects. Finally, this approach enabled the appropriate classification of all 24 samples (including 14 samples collected from SCLC patients and 10 from healthy donors) [44].

Monitoring of lung cancer transformation

Currently, according to ESMO (European Society for Medical Oncology) guidelines, in NSCLC patients, p.ThrT790Met mutation in *EGFR* gene may be initially analysed with the usage of liquid biopsy instead of tissue testing [50]. Recently, serial liquid biopsies have been proposed to monitor and predict NSCLC to SCLC transformation. Histological transformation of NSCLC to SCLC may occur as a resistance mechanism to treatment with EGFR tyrosine kinase inhibitors. However, even though the studies of Vendrell et al. and Mooradian et al. pointed out the advantages of liquid biopsy, it was suggested that such analysis is insufficient for this purpose [45, 51]. Mooradian et al. described the case of 63-year old man with SCLC transformed from adenocarcinoma. They suggested that molecular monitoring of ctDNA may provide insight into tumor heterogeneity, which is unavailable during tissue assessment. However, simultaneously, the authors pointed out that liquid biopsy genotyping may be associated with a high level of false

Table 1 Summary of liquid biopsy-based clinical trials that are ongoing or recruiting patients with small cell lung cancer. Data were collected from the ClinalTrials.gov database (http://clinicaltrials.gov/ (accessed on 13 November 2024). cfDNA– circulating free DNA; ctDNA– circulating tumor DNA; ES-SCLC– extensive stage small cell lung cancer; VAF– variant allele frequency; WES– whole exome sequencing

Clinical Trials ID (Duration)	Title	Study Status	Location (Sponsor)	Number of Participants (Trial Type)	Utilization of Liquid Biopsy-Based Biomarkers in the Trial
NCT03382561 (05.2018–12.2024)	Cisplatin/Carboplatin and Etoposide With or Without Nivolumab in Treating Patients With Extensive Stage Small Cell Lung Cancer	Active, not recruting	United States (National Cancer Institute)	160 (Interventional)	Serial evaluation of ctDNA and exploration whether clinical outcome is associated with fluctuations in DNA levels following the administration of therapy
NCT04562337 (10.2020–10.2024)	SHR-1316 Combined With Chemotherapy and Chest Radiotherapy in ES-SCLC	Active, not recruting	China (Shandong Cancer Hospital and Institute)	67 (Interventional)	Change of ctDNA status in blood before and after receving treatment
NCT03670056 (12.2018–12.2024)	Ipilimumab and Nivolumab in Recurrent Extensive Stage Small Cell Lung Cancer After Receiving Platinum-based Chemotherapy	Active, not recruting	United States (Yale University)	25 (Interventional)	Evaluation of ctDNA as a marker for response to therapy (secondary objective of the study) Assessment if the change VAF from baseline sample to the 4 week sample predicts response to therapy and correlate to objective response rate
NCT04487756 (03.2021–10.2024)	Combination of Atezolizumab With Dendritic Cell Vaccine in Patients With Lung Cancer (VENEZO-LUNG)	Active, not recruting	Spain (Instituto Oncológico Dr Rosell)	20 (Interventional)	Tumoral cfDNA analysis by WES (part of the translational substudy)
NCT03811002 (07.2019–08.2025)	Testing the Addition of a New Immunotherapy Drug, Atezolizumab (MPDL3280A), to the Usual Chemoradiation (CRT) Therapy Treatment for Limited Stage Small Cell Lung Cancer (LS-SCLC)	Active, not recruting	United States, Japan (National Cancer Institute)	545 (Interventional)	Characterization of the concordance between molecular subtypes obtained via ribonucleic acid sequencing (RNAseq) on tumor tissue and methylation analysis on cfDNA
NCT05257551 (07.2022–03.2026)	Tempus Sculptor Study: Small Cell Lung Cancer (SCLC) Observational Study	Recruting	United States (Tempus AI)	50 (Observational)	Evaluation of the potential for liquid biopsy in SCLC. Usage of blood based assays to assess the complementarity of circulating biomarkers to tissue based methods
NCT05623319 (03.2023–08.2026)	Pembrolizumab and Olaparib Treatment of Extensive Small Cell Lung Cancer (ES-SCLC) (THOR)	Recruting	Italy (Istituto Scientifico Romagnolo per lo Studio e la cura dei Tumori)	60 (Interventional)	Evaluation of Programmed Cell Death 1 Ligand (PD-L1) expression, DNA damage repair (DDR) alterations, Tumor Mutational Burden (TMB) and MicroSatellite Instability (MSI) on biopsy and blood samples collected before, during and after the treatment
NCT06529081 (04.2024– 12.2029)	Radiotherapy Strategies for Use in Combined Treatment of Small-cell Lung Cancer	Recruting	Poland (Copernicus Memorial Hospital)	165 (Interventional)	Utilization of ctDNA analysis as a potential marker to determine the time to progression and assess the benefits derived from the administered radiotherapy

negative results [51]. A graphical comparison of tissue and liquid biopsy in SCLC is present in Fig. 2.

Vendrell et al. performed genotyping of either tumor tissue or liquid biopsy collected at different time points, including collection before histological transformation, from three patients with transformed SCLC. Even though the cohort was small, analysis of results suggested that analysis of liquid biopsy may provide information which might be omitted in case of one-site biopsy genotyping. Finally, the authors suggested that patients' liquid biopsy monitoring seems beneficial compared to testing only one of this materials [45].

Molecular subtyping

Suggestions of benefits arising from SCLC division into molecular subtypes and propositions for implementing divergent therapeutical approaches based on them caused an urgent need to find tools enabling effective subtyping. Currently, the most frequently proposed methods for SCLC subtyping are transcriptomic analysis of tumor tissue [7, 52] and immunohistochemistry [48, 53]. However, the potential utility of liquid biopsy in molecular subtyping is also being suggested.

Chemi et al. [47] and Heeke et al. [54] proposed an analysis of cfDNA methylation to enable SCLC subtyping. Chemi et al. identified subtypes of SCLC (according to A, N, P, Y molecular subtyping) by cfDNA methylation analysis. They found that 73% and 13% of methylation profiles of the cfDNA samples were characteristic for ASCL1 and NEUROD1 subtypes, respectively. 14% of methylation profiles were classified as double negative subtype (without ASCL1 and NEUROD1 expression). The spread of the subtypes was close to immunohistochemistry (IHC) analysis from SCLC tissue samples. However, this study was a less comprehensive approach to subtyping, i.e. instead of four subtypes, researchers proposed only three: SCLC-A, SCLC-N and double-negative [47].

Heeke et al. found that methylation status was in concordance between cfDNA and DNA extracted from tissue samples. Authors stated that DNA methylation-based classifier, enabling differentiation of four molecular subtypes, was suggested as convenient for use in tissue samples or plasma. Moreover, it was highlighted that analysis of cfDNA extracted from plasma samples collected at the progression

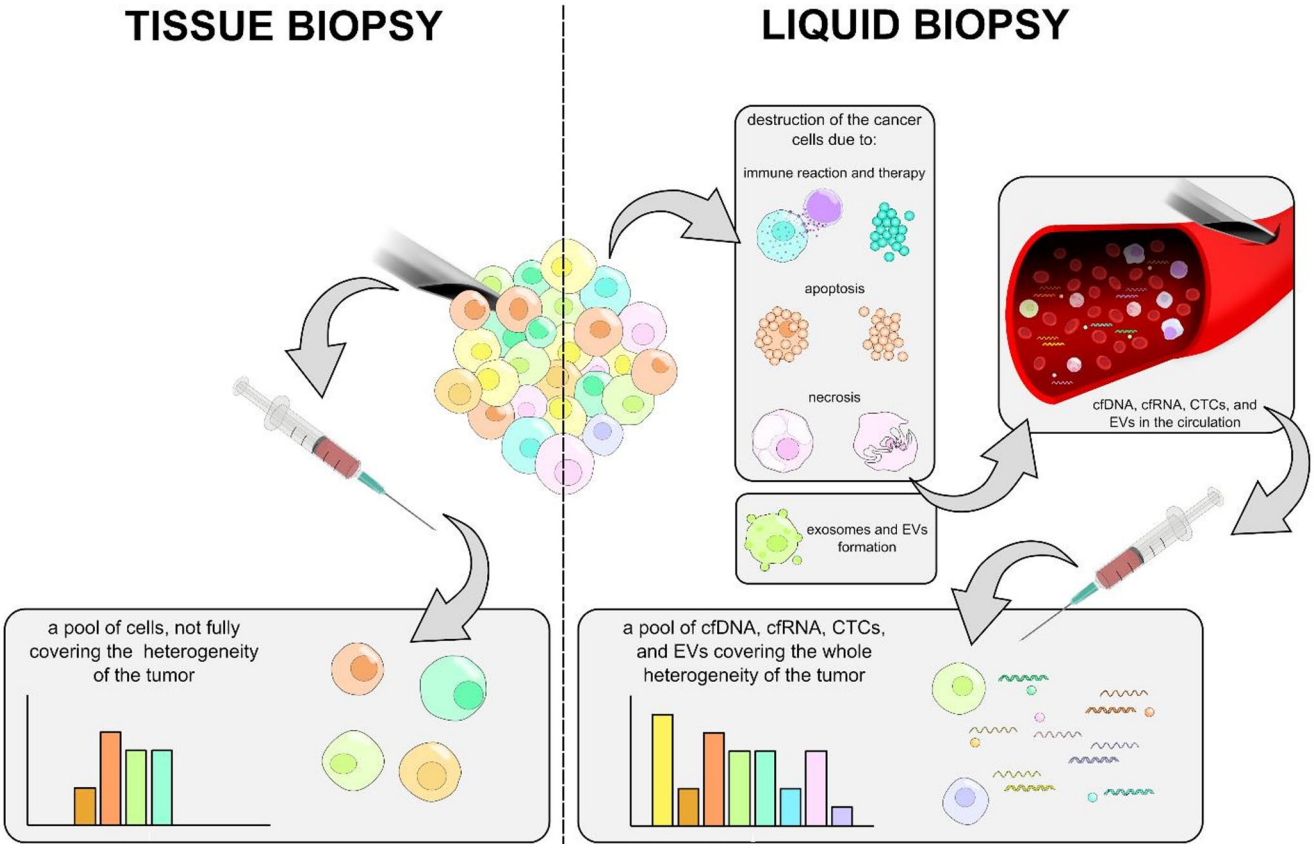

Fig. 2 Graphical comparison of tissue biopsy and liquid biopsy in SCLC
cfDNA– circulating free DNA; cfRNA– circulating free RNA; CTCs– circulating tumor cells; EVs– extracellular vesicles (this figure was drawn using Inkscape 1.3)

might be feasible for monitoring molecular changes occurring during therapy, including subtype switching [54].

Another approach to SCLC subtyping with liquid biopsy was proposed by Hiatt et al. In this study, the authors presented a custom panel called SCLCpheno-seq. SCLCpheno-seq enables targeted sequencing of specific cfDNA sites. Obtained results were subsequently translated to information about transcription factors (TFs) activity, which allows classification of the samples into specific molecular subtypes. Even though the results of this study seem promising, the lack of SCLC-P specimens among tested samples indicates that this method requires broader validation and assessment which allows distinguishing all SCLC subtypes. Despite SCLC subtyping, SCLCpheno-seq was considered feasible for NSCLC and SCLC differentiation. Moreover, the authors suggested that it might be helpful in monitoring of lung cancer histological transformation [55].

Monitoring of metastatic spread

Various parameters present in body fluid have been already proposed as related to metastatic spread in SCLC patients. It was pointed that higher levels of annexin A1 [56] and S100B protein [57] in patients' sera or downregulation of lncRNA XR_429159.1 in peripheral blood mononuclear cells [58] are associated with greater risk of formation brain metastases (Fig. 3). However, even though studies on significance of liquid biopsy in patients with metastatic tumors have already shown potential benefits arising from ctDNA and CTCs analysis [59–62], information about their role in metastasis monitoring or detection in SCLC patients is currently limited. Moreover, most studies including SCLC patients are not restricted to them but focus on several tumor types.

Shen et al. analysed potential application of CTCs in various oncological cohorts, among others in SCLC patients. Detection of CTCs was performed with single-cell metabolic assay and sequencing (scMet-Seq) i.e. method based on immunostaining of circulating tumor cells and, subsequently, their whole genome sequencing to analyse copy number alternations (CNAs). Authors assessed weather it is possible to implement CTCs analysis into diagnosis of SCLC metastases. Firstly, it was shown that count of CTCs differ between patients with metastatic SCLC and those

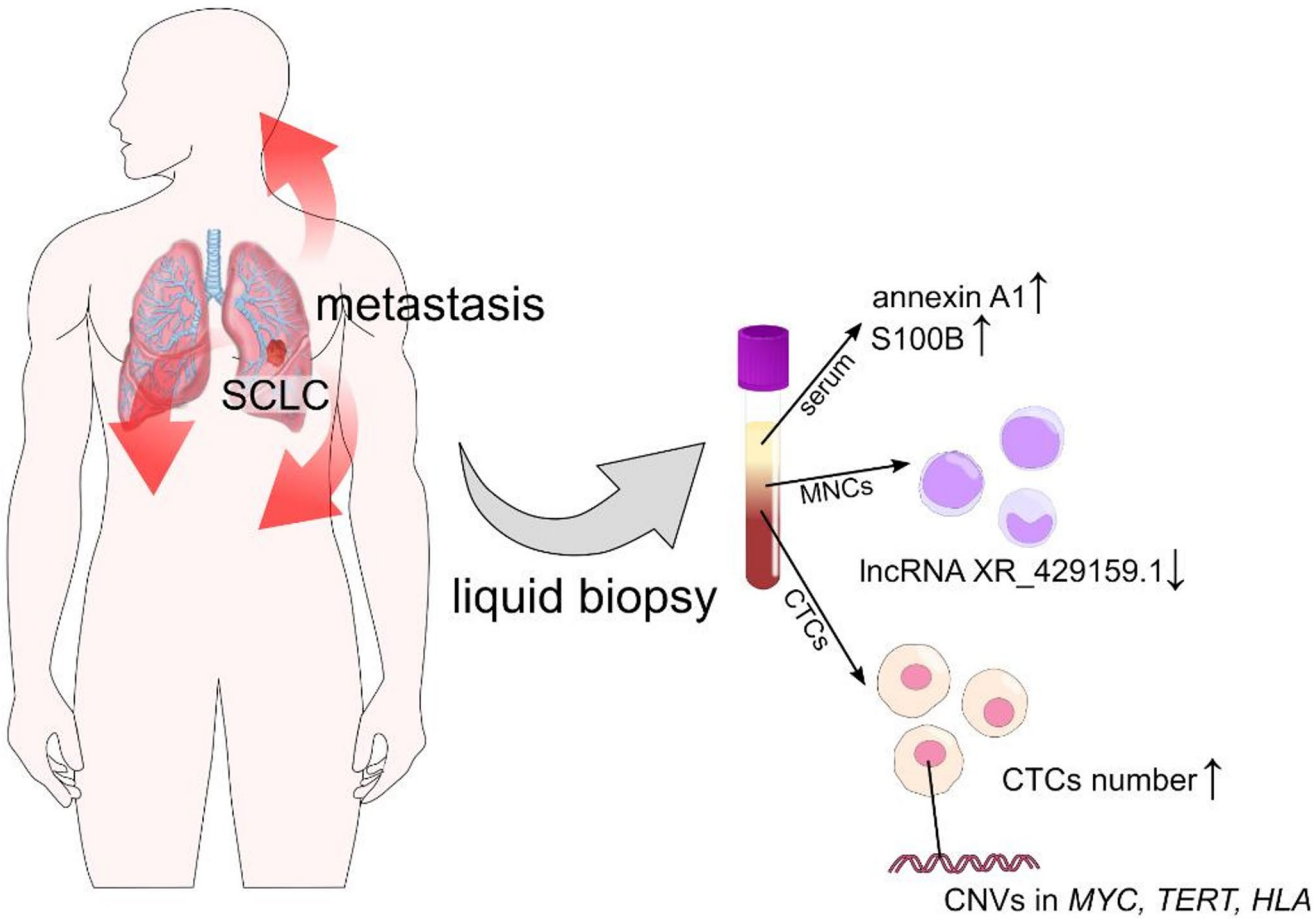

Fig. 3 Monitoring of metastatic potential of SCLC using liquid biopsy (description in the text; this figure was drawn using Inkscape 1.3)

with SCLC but without metastases or in high-risk controls (≥3.0/ml in 88% of metastatic SCLC patients vs. <3.0/ml in all patients without metastases and high-risk controls). Secondly, it was indicated that it was possible to detect metastatic SCLC with 90% sensitivity and 100% specificity with usage of scMet-Seq (Fig. 3) [63].

Ni et al. performed study which encompassed 16 lung cancer patients, including 4 SCLC cases and 1 patients with mixed LUAD and SCLC histology. Authors performed CTCs whole genome sequencing. Results of this study suggested that presence of copy number variations (CNVs) combination in specific loci (including *MYC*, *TERT* and *HLA* genes) may be crucial for the formation of metastases in lung cancer cohort. Moreover, it was tentatively suggested that analysis of CTCs CNV patterns may enable either tumor detection or classification due to its differences between LUAD and SCLC types (Fig. 3) [64].

Another opportunity to expand knowledge about CNS metastases in patients with solid tumors, including SCLC, is BrainStorm. This clinical trial is planned to include 600 oncological patients and to implement various outcome measures. Among others, study will be based on liquid biopsy analysis i.e. on deep targeted sequencing of ctDNA extracted from cerebrospinal fluid (CSF) and plasma collected from patients. Although, this trial is still ongoing and is scheduled to be completed in 2028, therefore the first published results are based on an analysis of small subset of data [65, 66].

Patient stratification

Even though various new approaches to SCLC treatment, including immunotherapy administration, are being proposed, appropriate predictive biomarkers for specific drugs have not yet been established [67–69]. The ability to comprehensively stratify SCLC patients' outcomes is limited [70–72], as well. Due to its simple collection and broad spectrum of present biomarkers [30], liquid biopsy seems to be a promising tool for either disease prognosis, its monitoring alongside radiological assessment or predicting the effectiveness of therapy. Moreover, liquid biopsy implementation into clinical trials and scientific studies seems beneficial because it enables minimally invasive testing.

Hitherto, most of the studies have focused on ctDNA analysis, although CTCs implementation is suggested as potentially beneficial, as well. The prospective role of those markers and proposed methods of their evaluation during SCLC management were discussed in details in specific subsections.

Prognosis

Circulating DNA methylation analysis seems to be feasible for SCLC and NSCLC distinguishing or differentiation of SCLC molecular subtypes, but it may also be useful in patients' prognosis assessment. Ul Haq et al. used machine learning to distinguish SCLC patients into two clusters based on cfDNA methylome patterns. The study included 74 SCLC patients (32 with LS-SCLC and 42 with ES-SCLC). Patients classified in cluster A had better OS, and those in cluster B– had worse OS with the median OS of 21 and 13 months, respectively. Methylation loss in long noncoding RNA was suggested as potentially associated with a more aggressive course of SCLC. Hypermethylation of repeats in short and long-interspersed nuclear elements was pointed as another feature with potential prognostic value. Moreover, the authors indicated that the methylome pattern assessed with cfDNA is representative of tumor tissue as well [73].

Zhang et al. pointed out the potential prognostic value of ctDNA level monitoring and its whole exome sequencing (WES). The cohort included 69 ES-SCLC patients with serial blood collections, among others, at the time of diagnosis and the moment of progression. It was shown that ctDNA level changed with the course of the disease, specifically decreased after two cycles of chemotherapy and increased in the case of progression. Moreover, it was pointed out that the ctDNA level was consistent with the radiographic assessment of tumor burden. High baseline ctDNA was suggested as an independent prognostic factor of poor progression-free survival. The authors suggested that PTEN (Phosphatase And Tensin Homolog) expression may be associated with resistance to chemotherapy as *PTEN* deletion was detected only in the samples collected at the progression. Finally, combined analysis of ctDNA and tissue was suggested as more beneficial than only tissue testing as it improved the actionable mutation detection rate [74].

Analysis of ctDNA variant allele frequency (VAF) was also proposed as a tool for predicting patients' outcomes. Nong et al. study performed with liquid biopsy showed that analysis of subclonal architecture with average VAF measurement may have prognostic value alongside different factors, such as tumor mutation burden or stage of the disease. Higher than median average VAF levels were shown to be associated with either lower PFS (5.3 versus 10.0 months) or OS (9.3 months versus 25.0 months). Moreover, it was described that ctDNA level correlates with outcomes assessed with imaging. Authors concluded that liquid biopsy may be treated as an alternative for tissue in case of its unavailability for testing [75].

Contrarily to the abovementioned study, Iams et al. did not observe a correlation between ctDNA VAF and PFS

or OS in the SCLC cohort [76]. However, the number of patients in this study was small (14 SCLC patients compared to 22 patients in Nong et al. study) and encompassed only patients with limited stage of disease. At the same time, Nong et al. included patients with limited or extensive SCLC [75, 76]. Nevertheless, Iams et al. proposed different predictor of patient outcomes, i.e. time necessary for ctDNA clearance [76].

Feng et al. suggested that in the SCLC patients, it was possible to assess prognosis based on the analysis of a single gene. For that purpose, the authors distinguished patients into three groups depending on changes in *RB1* gene in the baseline sample and after two cycles of chemotherapy. It was indicated that patients in which *RB1* mutations was detected in both time points had the worst prognosis with the lowest median OS and PFS compared to the other two groups of patients (i.e. patients in whom *RB1* mutation was not detected in either of two samples or was detected only in the baseline sample). Analogous analysis was performed for *TP53* mutations. However, its prognostic ability was poorer. The authors proposed molecular tumor burden index (mTBI) as a second potentially beneficial marker for prognosis in SCLC patients, as its elevation observed after six cycles of chemotherapy preceded radiographic progression in 64.7% (11 of 17) of patients. Moreover, it was suggested that mTBI monitoring may help identify metastatic spread during therapy [77].

The role of CTCs in the prognosis of SCLC patients has also been described. Results of the study performed by Hou et al. demonstrated that the number of CTCs in peripheral blood may be treated as an independent prognostic factor either before the start of the chemotherapy or after the administration of its first cycle. Researchers proposed the presence of 50 CTCs in 7.5 mL of blood as a cut-off value for patients' outcome stratification. In both time points, the number of CTCs$\geq$50 was defined as unfavorable and $<$50 CTCs– as favorable for PFS and OS. Moreover, it was pointed out that presence of apoptotic CTCs in the baseline sample is also associated with worse PFS and OS [78].

Predictive value

Targeted sequencing of cfDNA collected from SCLC patients was implemented in various studies and enabled the detection of numerous alterations with potential clinical utility in therapy guidance. The study of Devarakonda et al. encompassed 564 SCLC patients. The blood samples were collected either at the diagnosis or at the relapse. It was indicated that the molecular landscape was different between those samples. Among others, alterations of *AR* (*Androgen Receptor*) and *APC* (*APC Regulator Of WNT Signaling Pathway*) were more prevalent in relapse samples. It was suggested that mutations in genes such as *BRCA1* (*BRCA1 DNA Repair Associated*), *FBXW7* (*F-Box And WD Repeat Domain Containing 7*), *CCNE1* (*Cyclin E1*) and *ARID1A* (*AT-Rich Interaction Domain 1 A*) may have predictive value in patients treated with targeted therapies. Finally, the authors suggested the potential role of liquid biopsy in detecting alterations crucial during patients' progression and as a tool for expanding knowledge necessary in developing new therapeutic targets [79].

Sivapalan et al. tested the potential utility of ctDNA analysis in early prediction of response to treatment. The research included SCLC patients treated with different chemotherapeutics, immunotherapeutics, or combinations. Based on the results of sequence alterations and plasma aneuploidies obtained during targeted sequencing, cell-free tumor load (cfTL) was assessed. It was indicated that, independently from the implemented therapeutic approach, monitoring of ctDNA kinetics showed predictive value and was in concordance with radiological response. Moreover, analysis of molecular response enabled the prediction of patients' outcomes (both OS and PFS) with similar or even better accuracy compared to imaging [80].

Therapy monitoring

Numerous potential utilities of ctDNA examination were presented by Almodovar et al. In this study, targeted sequencing was performed using 140 plasma samples collected from 27 SCLC patients at various time points. Among others, it was pointed out that ctDNA analysis seems to be feasible as an early predictor of disease progression, as in 36% of patients (9 of 25 patients who had sufficient blood collections), changes in mutational landscape preceded radiographic progression. It was also indicated that cfDNA sequencing might be beneficial during guiding therapy decision-making. It enabled early detection of therapy inefficacy, which may hasten the decision to change the intensity or the treatment path. Based on five patients' cases, Almodovar et al. suggested that cfDNA sequencing may be beneficial in monitoring SCLC recurrence alongside imaging. Even though the results of this study suggested various potentially beneficial options for liquid biopsy implementation, it should be remembered that the studies were conducted in a very small group of patients. Moreover, the time of blood collection varied between patients, so it was more challenging to draw clear conclusions [13].

The study of Mohan et al. encompassed 69 patients with SCLC, including 39 with limited stage and 30 with extensive stage of disease. Despite targeted sequencing, authors applied whole genome sequencing of cfDNA. CNA analysis was pointed out as being eligible for patient monitoring. It was indicated that, alongside *TP53* targeted sequencing,

it may be helpful to distinguish samples that require more extensive analysis. Simultaneously, the authors suggested that an approach based on targeted sequencing could benefit patient stratification in clinical trials [14].

Zhang et al. evaluated SLFN11 (Schlafen Family Member 11) expression in tumor samples and in circulating tumor cells longitudinally to determine its potential role as a biomarker of response to treatment. In 196 SCLC tissues, 51% expressed SLFN11 evaluated by immunohistochemistry (IHC). In addition, 69% (20/29) extra-thoracic high-grade neuroendocrine tumors expressed SLFN11. SLFN11-positive CTCs were detected in 55% of available blood samples. The authors observed that patients receiving platinum-based therapy had the lowest number of CTCs and a lowest percentage of SLFN11-positive CTCs and it was correlated with clinical response in long-term follow-up [81]. Therefore, the authors proposed to monitor the clinical response to treatment of SCLC patients using CTCs and SLFN11 expression. This is meaningful in view of the new potential treatment option. In randomized phase II study, efficacy of maintenance atezolizumab therapy was compared to effectiveness of atezolizumab in combination with talazoparib (inhibitor of poly ADP ribose polymerase, PARP) in patients with SLFN11 (tissue expression detected by IHC) positive ES-SCLC. Authors showed that the combination of anti-PD-L1 immunotherapy and PARP inhibitor improved PFS in SLFN11-selected patients [82]. In this perspective, SLFN11 analysis in liquid biopsy appears to be an excellent biomarker of treatment efficacy and a possible qualifying predictor for personalized therapies.

Messaritakis et al. proposed an evaluation of the DLL3 (Delta Like Canonical Notch Ligand 3), cytokeratins (CK), CD45 and vimentin (Vim) for the detection and characterization of CTCs. Expression of these markers was analyzed by immunofluorescence staining [83]. Authors obtained peripheral blood samples from LS-SCLC ($n=37$) and ES-SCLC ($n=71$). Samples were collected from treatment-naïve patients, after one chemotherapy cycle and in the moment of disease progression. In 74% of treatment-naïve patients DLL3+/CD45– CTCs were detected. In patients who received one cycle of chemotherapy, decrease in both the detection rate and the absolute number of DLL3+/CD45– CTCs was observed. DLL3 expression could be detected in immunofluorescence assay on Vim+, Vim–, CK + and CK– CTCs. In the moment of disease progression, both the detection rate and the absolute number of DLL3+/CD45– CTCs were increased compared to the values of these parameters after first cycle of chemotherapy [83]. DLL3+/CD45– CTCs at baseline was significantly associated with decreased progression-free survival whereas their detection on disease progression was associated with decreased overall survival. The authors suggest that DLL3 expression on SCLC CTCs changes dynamically during the treatment and disease course. Therefore, authors proposed that analysis of DLL3 presence on CTCs and the number of DDL+/CD45– CTCs may be a valuable biomarker related to treatment efficacy.

This is of outstanding importance, as the drug tarlatamab has been registered, which is a bispecific antibody targeting DLL3 and CD3. The registration study involved patients with advanced SCLC after two or more prior lines of treatment, including at least one platinum-based regimen [84]. Moreover, ongoing clinical trial [85] may offer the possibility of using this drug in patients with LS-SCLC, so liquid biopsy tests targeting DLL3 seem to be a valid approach to develop prognostic and predictive factors in treatment personalization for SCLC patients. The Notch1/DLL3 pathway and tarlatamab mechanism of action are presented in Fig. 4.

Future perspectives

In the era of massive changes towards SCLC management, including the broad introduction of anti-PD-L1 immunotherapy into clinical practice, assessment of optimal patient stratification tools seems crucial. Currently, many approaches to liquid biopsy testing are being suggested, with the most significant emphasis on ctDNA analysis. However, further validation of proposed approaches is still necessary, and different biomarkers, such as CTCs, are also being investigated.

Currently, the most widely studies on SCLC patients were focused on the proposition of its subtyping based on molecular features [86]. It seems especially beneficial as great hopes are placed on therapy selection depending on the molecular subtype. However, the possibility of predicting response to specific regimens is still insufficient [70]. A liquid biopsy might be essential for SCLC subtyping and monitoring tumor plasticity associated with its resistance to initially effective therapy [44, 87, 88].

Recently, the attention of the scientists and clinicians has been focused on use of patient-derived cells in clinical and preclinical applications. They include formation of patient-derived xenografts (PDX) and organoids (PDO). Such formations may mirror tumor heterogeneity, genetic and somatic alterations and drug response [89–91]. However, the use of the models is limited, as the collection of cells from biopsy or tumor resection is invasive, and, in case of SCLC, the surgical procedures are rarely performed [89, 92]. Therefore, CTCs are a better choice due to ease of collection (liquid biopsy) [90, 91, 93, 94]. Both CTC-derived xenografts (CDX) and organoids (CTCDO) are studied more and more frequently and their preclinical and clinical use has been recently proposed. Their implementations

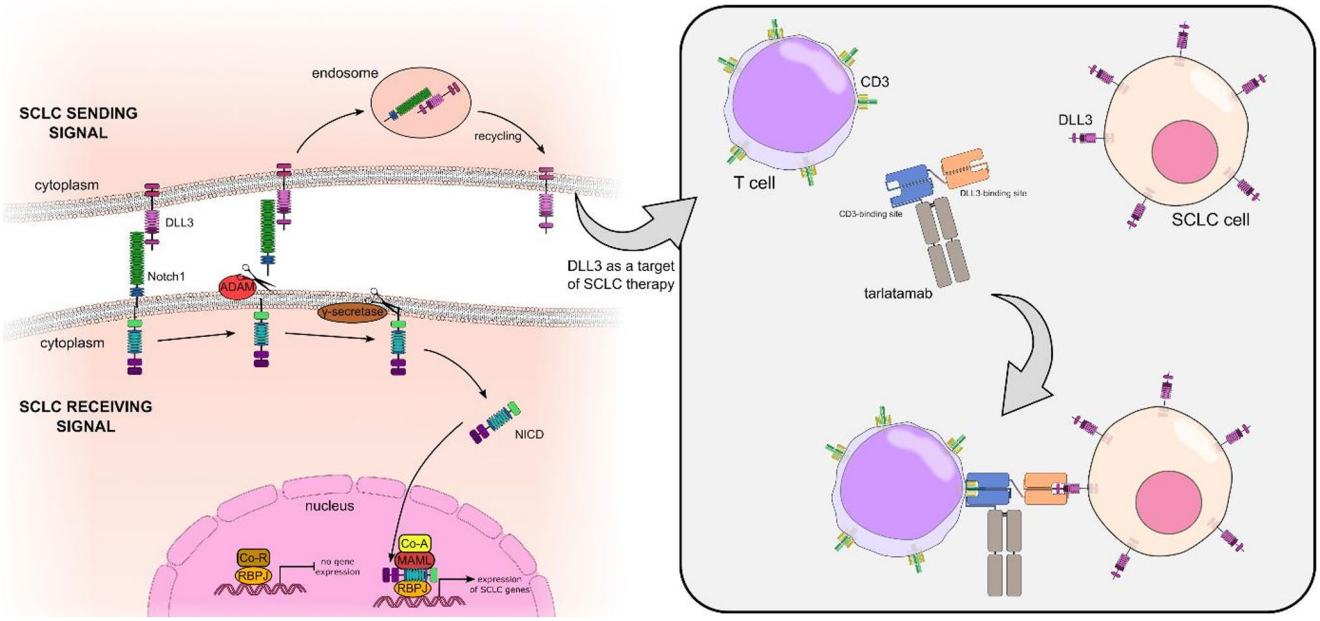

Fig. 4 Notch1 pathway dependent on DLL3, and tarlatamab mechanism of action (this figure was drawn using Inkscape 1.3)
ADAM– disintegrin and metalloproteinase domain-containing protein; Co-A– coactivator; Co-R– corepressor; DLL3– delta-like canonical Notch ligand 3; MAML– mastermind-like protein; NICD– Notch intracellular domain; RBPJ– recombination signal binding protein for immunoglobulin kappa J region

include study of the cancer biology (in in vitro, in vivo, and ex vivo models) to a great extent, and possible contribution to the better prognosis and treatment monitoring has been suggested [89–91, 93]. CTC-derived models may possess features indicating their pro-metastatic potential before the metastases occur in patient [89, 93], they reflect the patient's response to treatment, what could facilitate therapy decision-making and implementing personalized treatment [89–93], and are an advanced model to study cancer cells interactions with extracellular matrix (ECM) and immunological cells, what may have both clinical and preclinical applications [91, 92, 95, 96]. As both models have their advantages and limitations, organoids seem to be a better option due to higher reproducibility, higher similarity to patient's tissues and being more cost- and time-efficient [92, 96]. On the other hand, in vitro models are limited in reproducing the full spectrum of factors related to cancer, e.g. presence of vasculature or complexity of cancer microenvironment, therefore organoid models need further development and optimization [91, 92, 94, 96].

Many studies are focused on the benefits of implementing machine learning (ML) and artificial intelligence (AI) in the medical field. It is suggested that the use of new technologies may be further valuable in lung cancer patient management, e.g. during clinical [97] and radiological evaluation [98] or pathomorphological analysis of tissue [72, 99]. Studies concerned with combining ML or AI with non-invasive material testing in SCLC patients are currently scarce [100]. However, it is possible that a more innovative approach would make liquid biopsy results even more applicable in the future.

With ongoing clinical trials bringing us closer to introducing new therapeutic options for SCLC patients, the necessity to define tools for effective and high-throughput stratification of patients becomes increasingly urgent. Liquid biopsy is one of the materials with promising clinical applications. However, before its implementation into clinical practice will be possible, limitations associated with its usage must be overcome. Currently, the most critical issue seems to be the standardization and validation of proposed methods for liquid biopsy analysis in a large group of SCLC patients.

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Declarations

Competing interests The authors declare no competing interests.

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