Are there any theranostic biomarkers in small cell lung carcinoma?

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Abstract: Small cell lung cancer (SCLC), an aggressive lung tumour with a poor prognosis, has a high load of somatic mutations, mainly induced by tobacco carcinogens given the strong association with smoking. Advances in genomic, epigenetic and proteomic profiling have significantly improved our understanding of the molecular and cellular biology of SCLC. Given the high mutational burden of SCLC the immune microenvironment is another exciting area under investigation even if it seems to be quite distinct from that of other solid tumours. In this review we will outline the current progress in molecular etiology of SCLC mentioning some key markers considered promising theranostic biomarkers.

Keywords: Small cell lung cancer (SCLC); biomarkers; theranostics

Introduction

Small cell lung cancer (SCLC) is an aggressive neuroendocrine neoplasia, strongly related to smoking attitude, characterized by rapid growth and very poor overall survival (1). It accounts for 13–15% of all lung cancers and represents the sixth common cause of death for malignant tumours (2).

Histological evaluation is mandatory. According to the World Health Organization (WHO), morphology and immunohistochemistry should be performed for the final diagnosis (3). Prognosis and management of patients are mainly related to tumour staging (4).

Most patients are diagnosed with advanced disease often with a metastatic dissemination [extensive stage (ES)]. Despite several research efforts, the survival remains poor and only slight improvements have been made for appropriate management and effective therapies. Based on the most recent ESMO guidelines, the management of the disease consists of chemotherapy and radiotherapy.

A surgical approach is limited to a small percentage of localized cancers (T1-2, N0-1, M0) (1). The standard chemotherapy has not significantly changed in the last decades. Despite a good response after the first treatments, due to the elevated proliferative index and the sensitivity to DNA damaging drugs, the neoplasia acquires a chemo-resistance whose mechanisms remain unclear. Unlike NSCLC, a targeted therapy for SCLC has not yet been defined. The biology of SCLC is indeed not completely understood. Several studies have focused on the research of the molecular mechanisms responsible for the development, clinical behaviour and tumour landscape but the results are not conclusive (5). In the last five years some advances at the molecular level have resulted in the development of prognostic biomarkers and novel target agents (*Figure 1*).

In this review we summarized some of the most important reports on biomarkers in the sections entitled: (I) cancer biomarkers (from genetic to post-transcriptional alterations); (II) immune microenvironment biomarkers; (III) circulating biomarkers.
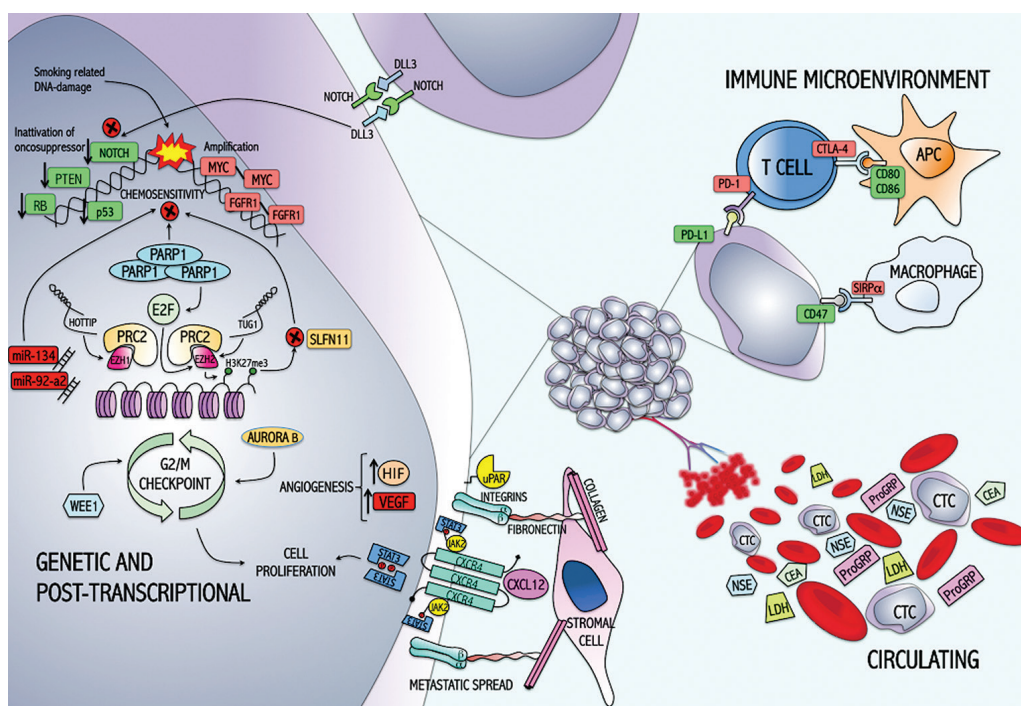
Figure 1 Biomarkers in SCLC reported in the text. On the left the key biomarkers involved in the genetic, epigenetic and post-transcriptional landscape. On the right the cancer interaction with the immune microenvironment and the circulating biomarkers. SCLC, small cell lung cancer.

Cancer biomarkers (from genetic to post-transcriptional alterations)

The molecular events which cause SCLC have not been clearly defined and a whole genetic characterization remains challenging. Only a few cases are treated by surgery and therefore tumour tissue available for molecular analysis is lacking. Recently, some efforts have been done to obtain more in depth information about the molecular signature of the neoplasia.

TP53 and *Rb* have been the most important molecular pathways investigated in SCLC. The inactivation of the two genes in a mouse model caused a high incidence of murine SCLC similar to the humans. This finding, together with the high penetrance of murine SCLC, was considered an important first step for testing new treatment modalities for SCLC (6).

More systematic genomic analyses were carried out several years later. In 2012 Peifer *et al.* conducted a wide analysis by using SNP array, exome sequencing, transcriptome and genome sequencing on a broad number of SCLC specimens. The results confirmed the inactivation of *Rb* and *TP53*, revealed mutations in *PTEN*, in *SLIT2*, and *EPHA7*, and focal amplifications of the *MYC* family and *FGFR1*. Furthermore, recurrent mutations in histone-modifying genes, *CREBBP*, *EP300*, and *MLL* were detected. These results increased the knowledge of the main biological events for the development of the neoplasia with the identification of new potential targetable genome alterations (7).

In the same year, Sos *et al.* performed a genomic and chemical vulnerability analysis on SCLC cell lines in order to identify therapeutically relevant genome alterations. The authors found that a subset of SCLC was susceptible to the action of Aurora B kinase inhibitors. This was another step to further investigation of the molecular basis in SCLC and consequently to a rational therapeutic approach (8).

A more in depth analysis on human tissue, SCLC and lymphoblastoid cell lines identified new recurrent somatic mutations. Among them, mutations in kinases, G-protein-coupled receptors, chromatin-modifying protein and *SOX* gene family members were detected. In addition, analysis of RNA sequencing data showed a recurrent *RLF-MYCL1* fusion whose silencing obtained in cell lines decreased the

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proliferative activity (9).

Genome-wide copy number analysis found that amplifications of loci of *MYC* family genes were frequent and mutually exclusive. *KIAA1432*, was also identified as amplified in SCLC with frequent fusion transcripts in several amplified regions. This suggested a simultaneous occurrence of amplification and fusion of genes in a related way (10).

In 2015 the same group further explored the genes frequently mutated and expressed in SCLC potentially targetable in therapy. In addition to the most frequent *TP53*, *Rb1* and *PTEN* alterations other genes, in particular *TMEM132D*, *SPTA1* and *VPS13B*, were found, representing other therapeutic targets for the neoplasm (11).

In a relevant number of SCLC cases together with the main inactivation of *TP53* and *Rb*, an inactivated mutation of *NOTCH* family genes was detected. This group of genes appeared involved in neuroendocrine differentiation in SCLC, as confirmed in a pre-clinical mouse model (12).

More recently the protein DLL-3 has been studied as a potential targetable biomarker in SCLC. This protein is normally expressed in the fetal brain where it acts on the somitogenesis and is an inhibitory ligand for the Notch pathway, suppressing oncogenesis and tumour growth.

In 2015 Saunders *et al*. discovered the association between DLL3 expression and the neuroendocrine phenotype. The study, carried out on xenograft models and human neoplastic tissue, demonstrated the up-regulation and the aberrant expression of DLL3 in SCLC. In this tumour, the employment of an anti-DLL3 treatment resulted effective in eradicating tumour initiating cells (13).

Based on these results several subsequent studies and clinical trials further investigated the role of DLL-3 and the efficacy of the administration of conjugated drugs for a target therapy in SCLC (14,15).

Some studies focused on the role of chromatin remodeling by chromatin modifying complex Polycomb Repressive Complex 2 (PRC2). This macromolecule is composed of subunits among which EZH1 or EZH2 (enhancer of zeste homolog), two histone H3K27 methyltransferases.

Already in 2013 the PRC2 overexpression and PRC2-target gene repression, such as cellular adhesion-related genes, was demonstrated to be associated with a poor prognosis in SCLC (16).

In the same year, Coe *et al*. studied the function of EZH2 as an oncogene. The high and aberrant expression of the histone methyltransferase was considered responsible

for the hypermethylation of PRC2-target genes with consequent pro-tumourigenic functions *in vitro* (17).

The protein was discovered to work also on pro-apoptotic genes (*Puma* and *Bad*) whose inhibition promoted the proliferative activity and interfered with p21 levels (18). More recently findings from multiple patient-derived xenografts have linked the up-regulation of EZH2 with H3K27me3 associated *SLFN11* gene silencing as a frequent mechanism of acquired chemoresistance in SCLC (19). Several EZH2 inhibitors are now under investigation for the treatment of different malignancies but not yet for SCLC.

An important role in SCLC biology has been demonstrated by non-coding RNA (ncRNA).

NcRNA are transcripts not translated into protein that are involved in key molecular processes (gene expression, genetic imprinting, histone modification, chromatin dynamics, etc.) through the interaction with all kinds of molecules. Several types of non-coding RNA are known. Among them, small non-coding RNA (sncRNA) and long non-coding RNA (lncRNA) are functionally important in SCLC. The distinction between the two groups is based on the number of nucleotides (less than and more than 200, respectively).

A lncRNA, CCAT2 (colon cancer-associated transcript 2), was detected in SCLC tissue and cell lines and its over-expression associated with advanced stage and poor prognosis (20). Recently the same group has identified a novel lncRNA, BLACAT1 (bladder cancer-associated transcript 1) involved in the biology of SCLC. BLACAT1 expression was higher in SCLC and was related to a suppressive activity in proliferation, migration and invasion, suggesting an oncogenic function. The authors concluded that the BLACAT1 acted as an oncogene correlated with a worse clinical status and outcome in SCLC patients (21).

Niu *et al*. have found that the expression of a splice variant of LIM-kinase-2 (LIMK2b) and its binding with EZH2 was regulated by the lncRNA TUG1 (taurine up-regulated gene 1). TUG1 was over-expressed in SCLC tissues promoting cell growth and chemo-resistance (22).

Similarly, the pathway of EZH1 protein was regulated by another lncRNA, HOTTIP (HOXA transcript at the distal tip) whose amplification in SCLC was associated with a more aggressive biological behavior (23).

In the group of ncRNA, miRNAs (microRNA) were also studied in SCLC.

Several miRNAs, such as miR-134 and miR-92a-2, were suggested to be involved in the mechanism of chemo-resistance by interfering in different processes. Some of these

are the HOXA1 function, ZEB2 expression, regulation of apoptosis and autophagy, Kras/mapk pathway, PARP1 activity and epithelial mesenchymal transition (24-32).

A mechanism often damaged in the complex biology of SCLC is DNA repair. In this context, PARP1 [poly (ADP-ribose) polymerase 1] plays a fundamental role. The protein is involved in the repair of single-stranded DNA (ssDNA) and is found over-expressed in many gynecological cancers. Given the high level of DNA damage PARP protein levels are upregulated in SCLC more than other cancers, as demonstrated by a study of proteomic and gene expression profile (33).

Based on these data, the authors speculated on the usefulness of PARP1-inhibitors, evaluating the combination with other treatments. This inhibition acts on the E2F1 pathways, strengthening the therapeutic efficacy of inducing double-strand DNA break agents (33).

The efficacy of a PARP inhibitor BMN 673 was tested *in vitro* and in xenograft models by Cardnell *et al.* (34). Drug sensitivity was associated with high expression of DNA repair proteins while resistance was related to baseline activation of PI3K/mTOR pathway.

The association between PARP inhibitors and alkylating agents in SCLC has been reported recently. Interestingly, the expression of *SLFN11* (schlafen family member 11), a guardian of the genome that promotes cell death in response to DNA damage, emerged as a strong predictor of the SCLC sensibility to the combination therapy of PARP inhibitors with temozolomide. Several clinical trials of a number of PARP inhibitors are ongoing in patients with SCLC (5,35).

Gardner *et al.* confirmed the link between the high levels of *SLFN11* and the chemo-sensitivity of SCLC and calls into question the remodeling of chromatin EZH2-mediated as being responsible for silencing of *SLFN11*, re-establishing *in vitro* the levels of protein after EZH2-inhibition (19).

Another factor which emerged as a promising target for therapy in SCLC is WEE1, a kinase regulating cell cycle progression. The efficacy of WEE1 inhibitor (AZD1775) is limited by a common resistance. Sen *et al.* focused on the resistance mechanisms in order to enhance the treatment response. The authors found that the expression of AXL, a receptor tyrosine kinase, promoted the resistance by silencing mTOR cascade and activating an alternative DNA damage repair pathway (36).

Chemoresistance and early metastatic spread are the main factors for SCLC aggressive behavior. Here below

are reported some of the most important key post-transcriptional molecular factors involved in metastatic spread.

The chemokine receptor CXCR4 was reported to be involved in SCLC progression in 2002 by Kijima *et al.* The authors found that all SCLC cell lines expressed CXCR4 receptors as well as c-kit even if in a lower percentage, suggesting their role in the pathogenesis of the neoplasm. The administration of small-molecule inhibitors demonstrated that the two pathways were cooperative in biological and biochemical functions and therefore the inhibition of both signals could be a targetable marker (37).

The high expression of CXCR4 was also confirmed in 2003 both on cell lines and primary tumour samples. Insights on its ligand CXCL12 and interaction with integrin signaling suggested a key role in cell motility and migration. This link gives resistance to chemotherapy thus its inhibition was reported as new therapeutic strategies for SCLC patients (38,39).

Further investigations demonstrated that this pathway was mainly mediated by a2, a4, a5, and b1 integrins whose activation resulted in an increased adhesion of SCLC cells to fibronectin and collagen. Stromal cells offer protection against chemotherapy-induced apoptosis thus the inhibition of this mechanism such as the T14 molecule and its derivatives and Plerixafor (AMD3100) could be helpful in overcoming the resistance (40,41).

Other works demonstrated alternative roles for CXCR4. In 2009 Pfeiffer *et al.* focused on the activation of the JAK2/STAT3 pathway by the link of CXCR4/CXCL12. The authors observed high levels of phosphorylated STAT3 in SCLC tissues, suggesting an important role in tumour biology. In cell lines STAT3 phosphorylation was demonstrated to be increased after CXCL12 stimulation through a cascade involving JAK2. These results supported an activity of CXCR4 both in VCAM1-mediated cell adhesion, spreading and cell growth through JAK2/STAT3 as mediators (42).

The inhibitor effect of the blockade of CXCR4 was investigated in an orthotopic xenograft mouse model. In mice with chemoresistant tumours, the administration of the molecule AMD3100 reduced tumour size and metastases formation, suggesting that the combination of CXCR4 inhibitors and standard chemotherapy could improve the neoplastic response and survival (43).

CXCR4 was confirmed to be expressed at high levels in SCLC and to correlate with bone and brain metastases, acting as an independent prognostic factor for survival (44).

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The combination of the CXCR4 antagonist LY2510924 to chemotherapy was the object of a multicenter, open-label, randomized phase II study but the addition of this drug in extensive-disease SCLC did not seem to improve therapeutic efficacy (45).

High levels of CXCR4 and uPAR (urokinase receptor, a key factor expressed in renal tumours) were associated with increased lymph-nodal metastases and worse prognosis. In cell lines, their co-expression showed a higher invasive and migrating capacity. When injected in mice these were responsible for an increase in tumour size and the induction of lung metastases (46).

Overexpression of uPAR and HIF-1α/HIF-2α (transcription factors, involved in the angiogenetic process) were detected in tissues of SCLC patients with shorter overall survival (47). HIF-1α overexpression was more recently confirmed as an independent poor prognostic factor in SCLC patients (48).

Overexpression of VEGF, another important pathway involved in angiogenesis and metastatic processes, was found strictly related to high vascularization and nodal metastasis in ES-SCLC patients.

Nevertheless, at multivariate analysis, VEGF expression was the most important factor influencing overall survival. Higher expression of VEGF and survivin (inhibitor of apoptosis) was also detected in neoplastic tissue of SCLC patients compared to the control group and their expression was significantly associated with lymph node metastasis and shorter overall survival. Both factors resulted as independent poor prognostic factors (49).

Immune microenvironment biomarkers

Preclinical and clinical studies in the oncological field have demonstrated that the immune system has a crucial role in recognizing and eradicating tumour cells, thus providing a rationale for immunotherapy in different tumours (50).

In addition to its anticancer abilities, the immune system can also promote the growth and proliferation of tumour cells, since the microenvironment often contains immunosuppressive cell types such as Tregs, myeloid-derived suppressor cells and tumour-associated macrophages (51). Thus, the imbalance between immune-inhibitory and -stimulating mechanisms in the tumour microenvironment is crucial in determining tumour immune response and the rate of tumour progression (51).

The immune system has also been demonstrated to be involved in the pathophysiology of SCLC, particularly in relation to the frequent occurrence of paraneoplastic

disorders (e.g., Lambert-Eaton myasthenic syndrome) and the positive predictive impact of immune activity on patient prognosis (50).

Immune checkpoint pathways involving the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein-1 (PD-1) receptors and their ligands dampen the T-cell immune response (52) and represent important biomarkers in different tumour types both for prognostic and therapeutic purposes. Nevertheless at present limited data have been reported on immune checkpoint blockade in patients with SCLC and only a few studies were investigated the prognostic and predictive role of this marker.

CTLA-4 is a transmembrane protein receptor expressed on the surface of T cells which regulate responses in the early stages of T-cell activation (53). CTLA-4 outcompetes the CD28 receptor for binding to its ligands, CD80/CD86, expressed by antigene-presenting cells (APCs), providing an inhibitory rather than stimulatory signal to the T cell. Thus, CTLA-4 is involved in the limitation of inflammatory responses and the tolerance to self-antigens, preventing autoimmunity. However, CTLA-4 may also play a detrimental role by inhibiting antitumor immunity and its blockade, therefore, may remove the inhibitory signal and stimulate antitumor immunity. It was the first immune checkpoint receptor to be targeted by a therapeutic agent (Ipilimumab) and it was tested in some trials, with positive results for progression-free survival but not for overall survival (5).

PD-1 is another important immune checkpoint receptor largely studied in oncology (54). It belongs to the CD28 family protein and it is expressed on the surface of T cells, regulating their activation and proliferation. It has been widely demonstrated that binding of PD-1 (expressed on activated tumor-infiltrating T cells) to PD-L1 (expressed on tumor cells and on tumor immune microenvironment) can inhibit antitumor immune responses in different malignancies, such as NSCLC, melanoma and renal cell cancer (55,56). In 2016, a multicentre, open-label, phase I/II trial of nivolumab (monoclonal antibody against PD-1) with or without ipilimumab in patients with recurrent ES-SCLC showed a better 1-year survival (57) leading to the incorporation of the nivolumab and ipilimumab combination as a National Comprehensive Cancer Network (NCCN) guideline recommendation for the second-line treatment of ES-SCLC. However, the efficacy of checkpoint inhibitors in patients with SCLC needs to be confirmed in randomized trials, and these treatments have not been formally approved for the treatment of SCLC (5). Only

a few studies have investigated the distribution of PD-L1 expression in SCLC tissue samples, reporting contradictory data concerning immunostaining and correlation with clinical outcomes.

A paper exploring the PD-1/PD-L1 expression pattern in SCLC was published in 2015 by Schultheis *et al.*, and none of the patients showed positive PD-L1 immunostaining in the tumour cell component, while 18.5% of them showed positivity in tumour-infiltrating macrophages and 48% in T-lymphocytes (58).

Other interesting studies showed a correlation between positive PD-L1 immunostaining and a longer overall survival (59) or disease-free survival (60). A comparative study using the three different antibodies was done by Takada *et al.* and reproducible data were found only when a 5% cut-off of PD-L1 positive immunostaining was chosen (61).

The predictive role of PD-L1 expression on tumour and different immune cell components was investigated by two studies that focused on brain metastases of SCLC and a positive prognostic role was found for PD-L1 expression, in particular in T-lymphocytes, supporting the hypothesis that an active immune microenvironment may be targettable in metastatic SCLC (62,63).

In 2016, for the first time, copy number alteration of CD274, the gene encoding PD-L1, was examined to determine in detail genomic alterations that may affect PD-L1 expression levels in SCLC. The authors reported that a subset (2%) of SCLC showed amplification of CD272, resulting in high immunohistochemical expression of PD-L1, concluding that the amplification of this region could be particularly sensitive to therapeutic PD-1/PD-L1 (64). In 2017 the first and only article about a negative prognostic impact of PD-L1 expression in SCLC was published by Chang *et al.* A direct correlation was found between tumour cells an T-lymphocyte PD-L1 expression, as well as with advanced disease stage (IV); multivariate analysis revealed that high tumour PD-L1 expression and stage IV disease represented two independent risk factors for poor overall survival (65).

Interestingly, the role of immune microenvironment in SCLC focusing on PD-L1 expression and FOXP3-positive tumour infiltrating T-lymphocytes was investigated in a large number of non-metastatic and metastatic SCLC tests. PD-L1 was more frequently detected in non-metastatic SCLC cases, leading to the hypothesis that downregulation of PD-L1 could be related to a high invasion potential. Another intriguing result was the potential prognostic impact of FOXP3+T-lymphocytes in the different stages

of SCLC (66). More studies are needed to clarify the role of PD-L1 in SCLC, in terms of prevalence of PD-L1 expression and in evaluating its role in predicting response to the treatment and outcome.

Overexpression of CD47, a cell-surface molecule that inhibits activation and phagocytic activity of macrophages, was found on the surface of human SCLC cells and has been implicated in immune escape by tumours (67). To date, only experimental studies have been performed, and clinical exploration of CD47/SIRPα inhibition as a therapeutic strategy for SCLC is expected to begin.

Circulating biomarkers

Several efforts have been made for years to find cost-effective and non-invasive serum markers able to indicate the biology and the behavior of SCLC. Several serum biomarkers have been identified and reported in publications. However despite extensive studies, cancer detection and follow-up via a single marker remains difficult due to the low sensitivity, specificity and reproducibility of serum biomarkers. Multiple biomarkers rather than a single serum marker testing could be the most sensitive approach in the future.

The significance of neuron specific enolase (NSE) in SCLC has been investigated and reported to have a role in the diagnosis, treatment and follow-up since the mid-eighties. The levels of NSE were found to have a stronger correlation to disease extent and response to treatment in patients in comparison to other markers [such as carcinoembryonic antigen (CEA); calcitonin, ferritin, lactate dehydrogenase (LDH)] (68,69). The sensitivity and specificity of NSE serum marker has been addressed by several authors often with contradictory results. NSE levels in SCLC patients were considered to have a good specificity, whereas the sensitivity appeared depending on the stage of disease. Indeed, the levels were elevated in a majority of ES-SCLC and in a less percentage of limited one (70).

NSE measurements before therapy also resulted useful in evaluating response duration, as emerged from a multivariate analysis (71).

More recently, a meta-analysis about the association of serum NSE levels and the prognosis of SCLC confirmed that patients with high levels of NSE showed a shorter overall survival than patients with low ones, attributing a prognostic value to the marker (72).

In patients treated with first line platinum-based chemotherapy high levels of serum NSE as well as lactate

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dehydrogenase (LDH) had a worse progression free survival and overall survival, confirming a suggested prognostic and predictive function (73).

A high level of serum LDH, a metabolic enzyme detected in patients with several types of malignancies, was identified as a prognostic factor to better stratify patients with SCLC (74). Several studies found a significant relation between serum LDH levels and outcome either in limited-stage and extensive disease SCLC (75,76).

A potential validation for the use of LDH in monitoring treatment response and for discriminating different forms of diseases has also recently been proposed by Chen *et al.* (77).

Serum pro-gastrin-releasing peptide (ProGRP), a neuropeptide involved in several physiological mechanisms, was reported as a useful marker for treatment monitoring and survival in SCLC (78).

However the prognostic and predictive value of ProGRP remained doubtful as emerged from the results of multivariate analyses especially if compared to other markers such as NSE (79,80).

Higher levels of carcinoembryonic antigen (CEA), an oncofetal protein, in patients with ES-SCLC and a strong correlation with response and survival was firstly reported by Sculier *et al.* in 1985 (81).

The relevance of CEA serum concentrations in SCLC patients in predicting the outcome was suggested by Zhu *et al.* In extensive-stage disease, normal serum CEA levels together with thoracic irradiation therapy and more than 4 cycles of chemotherapy were independent prognostic factors for overall survival in SCLC (82).

CEA has also been recently investigated as predictor of brain metastasis and survival in prophylactic cranial irradiated SCLC patients (83).

CTCs (circulating tumour cells) are cancer cells that are shed into vessels from tumors and have the potential to develop metastatic lesions. SCLC is the cancer with the higher level of CTCs. Given the metastatic potential of these cells, many studies have been carried out to understand their diagnostic, prognostic and predictive value in neoplastic diseases and their utility as biomarker or as "liquid biopsy". The prognostic value of CTCs in SCLC was controversial until the early 2000s mainly due not yet well developed technologies for detection (84).

In 2012, Hou *et al.* found that the CTCs, apoptotic CTCs and CTM (circulating tumor microemboli) number after one cycle of chemotherapy were independent prognostic factors for SCLC (85).

Several other groups confirmed more recently the link between CTCs and poor prognosis in SCLC patients (86-88). However the role of CTCs as predictors to therapeutic response remains quite debated. Wang *et al.* found relation between the CTCs levels and serum NSE but not with the therapy responsiveness (89).

The decrease in the number of CTCs in response to a second-line chemotherapy was proposed to be related to the effect on the metastases or resident SCLC lesions rather than an effective tumor progression blockage (90).

Several studies deepened the molecular biology of CTCs and highlighted different genetic profiles responsible of chemosensitive or chemorefractory SCLC (91,92). Several works have recently focused on the expression of different CTC biomarkers, such as receptor of tyrosine kinase, CXCR4, VEGFR2, Bcl-2, etc. and their correlation with prognosis and chemo-resistance mechanisms (93-95).

The CTCs are the responsible of the metastatic dissemination of SCLC. This is proven by the expression of epithelial-mesenchymal transition (EMT) phenotype in SCLC cell lines derived from primary tumors and metastases. The presence of a CTC with a mesenchymal-like phenotype, based on the low expression of E-cadherine and the high expression of c-MET, was associated with longer survival (96).

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Footnote

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