Perspectives in small cell lung cancer

George Fotopoulos¹
Elias Koteas¹
Konstantinos Syrigos¹, ²

¹ Oncology Unit, Third Department of Medicine, University of Athens, Athens, Greece
² Yale School of Medicine, New Haven, CT, USA

Address for correspondence:
George Fotopoulos
Oncology Unit, Third Department of Medicine, University of Athens, Sotiria Hospital
Mesogeion Avenue 152
11527 Athens, Greece
Tel.: +302107700220
E-mail: fotopoulos.george1@gmail.com

Abstract

Small cell lung cancer remains one of the most lethal malignancies worldwide with a 5 year survival rate at 7%. An estimated 1.6 million new lung cancers are diagnosed worldwide each year. The development of lung cancer has multiple steps, involving proliferation, mutagenesis and carcinogenesis occurring in a span of years and resulting from exposure to tobacco and other carcinogens. In the last 15 years the landscape of molecular genetics of small cell lung cancer has been clarified. As we are entering the era of genomic profiling, there have been some notable discoveries in terms of profiling and researching targeted therapies. A framework for translating the most promising discoveries into clinical trials and, ultimately clinical practice must be our goal.

KEY WORDS: SCLC, targeted therapies, genomics, molecular profiling, translational research, immunotherapy and SCLC.

Introduction

Small cell lung cancer (SCLC) remains one of the most lethal malignancies worldwide with a 5 year survival rate less than 7%. An estimated 1.6 million new lung cancers are diagnosed worldwide each year. The highest incidence rates in males are observed in Central/Eastern and Southern Europe (57 and 49 per 100,000, respectively), whereas in women the highest rates are found in Northern Europe (36 per 100,000) (1). On the other hand the proportion of lung cancer in the United States that is classified as SCLC has steadily decreased. This was illustrated by an analysis of the Surveillance, Epidemiology, and End Results (SEER) database, in which the proportion of SCLC declined from 17% in 1986 to 13% in 2002 (2). This is due to the fact that this malignancy occurs almost exclusively in smokers, and the policy regarding smoking in the United States is very strict.

SCLC differs from other lung malignancies for its doubling time, high growth fraction and metastatic dynamic. Although initially chemosensitive and radiosensitive (3, 4), it usually relapses and at that point it is almost always non-curable and lethal with limited treatment options with almost every patient. Especially for those who relapse while on treatment or within the first ninety days, there is a vast need for more efficient treatment options.

The scope of this review is to explore where research stands nowadays in terms genomics and new treatment modalities.

Material and methods

An independent review of PubMed and Science Direct database was performed up to January 2016 using combinations of terms such small cell lung cancer, chemotherapy, radiotherapy, genomics in small cell lung cancer, molecular pathogenesis and targeted therapy in small cell lung cancer. We set no geographical restrictions. All case reports and non English articles were excluded. The abstracts were screened to identify those studies and review articles we judged relevant to our objectives. Once duplicates were identified and removed, the retrieved articles were then reviewed by two separate Authors for inclusion or exclusion. Once all articles to be included were identified, the references of all included articles were reviewed to identify any additional applicable publications that may have been missed by the digital search. References from these articles were also obtained, and review articles are cited to provide readers with more details than this review has room for.

Epidemiology and pathology

As we have mentioned earlier almost two million new cases will be diagnosed with SCLC worldwide this year (1). Approximately 40% will be diagnosed with
limited disease (involving a single radiotherapy port) and 60% with extensive disease (5). SCLC accounts for approximately 15% of all bronchogenic carcinoma, shows a strong correlation with cigarette smoking and is extremely rare in persons who have never smoked. It is of neuroendocrine origin and along large cell neuroendocrine carcinoma are high grade tumors (6). SCLC is composed of a pleomorphic population of small cells (with a cell size no larger than the size of three resting lymphocyte nuclei). These may be round, oval, angulated, and with variable amounts of cytoplasm; the classic “oat cell” has virtually no cytoplasm, while the “intermediate” subtype may have a small amount of palely eosinophilic cytoplasm. The nuclei are typically hyperchromatic and either have a dispersed “salt and pepper” chromatin or a homogeneous dispersed chromatin. The cells are fragile and the tumors are generally extensively necrotic, both of which may contribute to the difficulty in establishing a histologic diagnosis. About 5% of cases may also be combined with non-small cell elements, such as squamous carcinoma or adenocarcinoma and still responds at least initially to therapy directed to small cell carcinoma. According to the WHO, such tumors should be subsumed under the “combined” rubric, listing both components present. Tumors should be comprised of at least 10% of a second component for classification as a combined carcinoma. These findings have led to the hypothesis that pulmonary carcinogenesis occurs in a pluripotent stem cell capable of differentiation along several pathways. Further supporting this is the observation that approximately 14% of NSCLC tumors with EGFR mutations acquire SCLC morphology and expression of neuroendocrine markers as EGFR-inhibitor resistance evolves, and that these tumors are sensitive to standard SCLC regimens (7).

**Genomics (Tab. 1)**

The development of lung cancer has multiple steps, involving proliferation, mutagenesis and carcinogenesis occurring in a span of years and resulting from exposure to tobacco and other carcinogens. Three seminal papers in the last 15 years have clarified the landscape of molecular genetics of small cell lung cancer (8-10) and the key points will be presented here. P53 mutations are detected in up to 90% of SCLC while loss of the retinoblastoma gene (RB1) function at 13q14 is nearly ubiquitous in SCLC. Haploinsufficiency due to loss of material on chromosome 3p at multiple break sites leads to absent or lower expression of many putative tumor-suppressor genes in the majority of SCLCs (11). The FHIT gene located to 3p14.2, the RASSF1A located at 3p21.3 and TGFBR2 at 3p21.3.22, all tumor suppressor genes are absent in almost all cases of SCLC (12, 13). On the other hand loss of PTEN is observed in only 2 to 4% of SCLC but rates of PTEN/PI3K pathway alterations overall may be significantly higher and appear to play a role in promoting SCLC tumorigenesis (14). A study sequencing 53 SCLC tumors and cell lines identified a large number of DNA alterations in each sample. Guanine (G) to thymidine (T) transversions (interchanges of purines and pyrimidines) were especially common, consistent with the known association between these types of mutations and smoking. Among the observed mutations, 22 genes were identified as being commonly altered in SCLC. In addition to those previously established in SCLC (TP53, RB1), novel observations from this study included SOX2 amplifications and RLF-MYCL1 fusions which represent potential therapeutic targets (15).

Regarding growth factor loops, upregulation of wild type c-Kit and its ligand stem cell factor is detected in

**Table 1 - Genes involved in the pathogenesis of small cell lung cancer.**

<table>
<thead>
<tr>
<th>Targeted Gene</th>
<th>Alteration Prevalence</th>
<th>Altered Gene Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53</td>
<td>90%</td>
<td>Deletion</td>
</tr>
<tr>
<td>RB1</td>
<td>100%</td>
<td>Deletion</td>
</tr>
<tr>
<td>FHIT1</td>
<td>100%</td>
<td>Deletion</td>
</tr>
<tr>
<td>RASSF1A</td>
<td>100%</td>
<td>Deletion</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>100%</td>
<td>Deletion</td>
</tr>
<tr>
<td>PTEN</td>
<td>4%</td>
<td>Deletion</td>
</tr>
<tr>
<td>c-KIT</td>
<td>90%</td>
<td>Uptregulation</td>
</tr>
<tr>
<td>MYC 1,2,3</td>
<td>&lt;5%</td>
<td>Uptregulation</td>
</tr>
<tr>
<td>G1B</td>
<td>&gt;5%</td>
<td>Uptregulation</td>
</tr>
<tr>
<td>PARP</td>
<td>&lt;5%</td>
<td>Uptregulation</td>
</tr>
<tr>
<td>EZH2</td>
<td>&lt;5%</td>
<td>Uptregulation</td>
</tr>
</tbody>
</table>
up to 90% of SCLC (16) and MYC family members (MYC, MYCL1, and MYCN) are amplified in approximately 20% of SCLC (8). Gene expression arrays and CGH analyses have shown upregulation of the islet-1 and the forkhead box protein G1B transcription factors (17).

Telomerase is a ribonucleoprotein enzyme that compensates for telomere shortening during cell division by synthesizing telomeric DNA, thereby maintaining telomere length. In normal somatic cells, telomerase activity is usually undetectable, with the exception of some cell types that possess the ability to divide indefinitely (e.g., hematopoietic cells, hair follicles, intestinal crypt cells, and basal cells of the epidermis). Activation of telomerase is detected in approximately 90% of SCLCs (18). In cancer cells, telomerase activity correlates with the stabilization of telomere length and cellular immortalization.

Lastly in contrast to other types of lung cancer, mutations in the EGFR and KRAS oncogenes are rare, while there is a higher expression of PARP1 and EZH2 (8-10).

Current treatment standards for small cell lung cancer

Response of SCLC in frontline chemotherapy is impressive, ranging up to 60% (19) only to be outshone by the subsequent resistance to further line of therapies after disease progression. The current standard of care for limited SCLC (one that can be encompassed in a single radiation field) is concurrent chemoradiation (20) and for extensive disease is chemotherapy (21), with the backbone being cisplatin –, etoposide doublet. Furthermore it is well validated nowdays that if a good response is achieved, there is a further benefit in both settings with the addition of prophylactic cranial irradiation (22). Regarding chemoradiation, clinical trials have established the importance of beginning radiotherapy as early as possible (23) and the superiority of the hyperfractionated scheme (24).

Upon progression, topotecan is the only second line drug with and approval by the Food and Drug Administration (FDA) (21) alas with disappointing response rates. Response rates in second line treatments depend on the duration of disease control from the frontline treatment. Patients who relapse after three months have a response rate of 25% and also a rechallenge with the same platinum doublet can be offered (25), but that rate drops to 6% if the relapse occurs before this time point (26). Temozolomide demonstrated in a phase two study (27) a response rate of 38% in patients with brain metastasis, while other options in the second line setting are taxanes, gemcitabine, vinorelbine and irinotecan (21). The Japanese Cooperative Oncology Group demonstrated the superiority of platinum-etoposide versus platinum-etoposide (28) but these results could not be replicated in further studies (29-31). In the third line setting no consensus exists on treatment and responses are low (26).

Despite vigorous efforts by clinical investigators and researchers the list of unsuccessful drugs for SCLC is long. More than forty failed phase 3 studies since 1970 have taken place, and as result therapeutic options have not changed for the last thirty years (32) (Tab. 2). As a result for this lack of major breakthroughs the 5 year survival rate is less than 7% for all stages in SCLC (33).

Recently there has been a genomic breakthrough in oncology and translational research has advanced in SCLC. As we have already forementioned, now more than ever, we have a better understanding of its biology and molecular hallmarks and we have identified a number of potential targets at the molecular level. Since the need for effective treatment for patients diagnosed with SCLC has become more critical than ever we should further explore these potential approaches.

Molecularly targeted therapies

The insulin-like growth factor 1 receptor (IGF-1R) pathway is a potent autocrine growth factor pathway that appears to be involved in the pathogenesis of SCLC (34). Binding of IGF-1 and IGF-2 to a lesser extent, to IGF-1R results in a conformational change in the receptor that leads to its activation. The activated receptor then recruits insulin receptor substrate that serves as a docking protein to link the receptor to the PI3 kinase and the MAP kinase pathways, resulting in cellular proliferation and inhibition of programmed cell death.

Cixutumumab, a monoclonal antibody that binds to the IGF-1R, blocks the binding of IGF-1 and prevents these pathways from being activated. A three arm randomized phase II study of cisplatin and etoposide ver-

Table 2 - Demographics of the 52 randomized trials.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of trials</td>
<td>52</td>
</tr>
<tr>
<td>Treatment arms</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Median year of trial initiation</td>
<td>1990 (1980-2006)</td>
</tr>
<tr>
<td>Randomized Patients in all Trials</td>
<td>10262</td>
</tr>
<tr>
<td>Male patients (%)</td>
<td>75%</td>
</tr>
<tr>
<td>Female patients (%)</td>
<td>25%</td>
</tr>
<tr>
<td>Trials assigning PCI when CR/PR (%)</td>
<td>37%</td>
</tr>
<tr>
<td>Trials with statistical significance in OS (%)</td>
<td>25%</td>
</tr>
</tbody>
</table>
sus with either vismodegib or cixutumumab for patients with extensive stage-small cell lung cancer was presented in 2013 at the American Society of Clinical Oncology meeting (35). There was no significant improvement in progression-free survival, overall survival, or response rate in those receiving cixutumumab in combination with cisplatin plus etoposide.

We stated earlier that SCLC has a high expression of PARP1 (8). PARP1 is a protein that is important for repairing single-strand breaks in the DNA. If such nicks persist unrepaired until DNA is replicated (which must precede cell division), then the replication itself can cause double strand breaks to form (36). Drugs that inhibit PARP1 cause multiple double strand breaks to form in this way, and in tumors with BRCA1, BRCA2 or PALB2 mutations these double strand breaks cannot be efficiently repaired, leading to the death of the cells. Normal cells that don’t replicate their DNA as often as cancer cells, and that lack any mutated BRCA1 or BRCA2 still have homologous repair operating, which allows them to survive the inhibition of PARP (37). Talazoparib, after trials for advanced hematological malignancies and for advanced or recurrent solid tumors (38) has shown a high level of activity in preclinical studies with SCLC cells. A cohort of patients with SCLC is now being treated with BMN 673 in a phase I study (38).

Using a mouse model in which deletion of Rb1 and Trp53 in the lung epithelium of adult mice induces SCLC, researchers (39) found that the Hedgehog signaling pathway is activated in SCLC cells independently of the lung microenvironment. Constitutive activation of the Hedgehog signaling molecule Smoothened (Smo) promoted the clonogenicity of human SCLC in vitro and the initiation and progression of mouse SCLC in vivo. Reciprocally, deletion of Smo in Rb1 and Trp53-mutant lung epithelial cells strongly suppressed SCLC initiation and progression in mice. Furthermore, pharmacological blockade of Hedgehog signaling inhibited the growth of mouse and human SCLC, most notably following chemotherapy. These findings showed a crucial cell-intrinsic role for Hedgehog signaling in the development and maintenance of SCLC and identified that the Hedgehog pathway could be targeted to slow the progression of disease and delay cancer recurrence in individuals with SCLC.

Although a phase II study evaluated the hedgehog inhibitor vismodegib in patients with previously untreated SCLC (35) and there was no significant improvement in progression-free survival, overall survival, or response rate in those receiving vismodegib, this is a promising pathway to be further explored.

Although Myc (8) has been recognized as a potentially important target for many years, designing a drug to directly inhibit Myc activity has been challenging. Myc are amplified in 20% of cases of SCLC (8), and in these tumors may be more sensitive in certain target ed drugs like bromodomain inhibitors (40). Recently the results of a phase two clinical trial of a single agent, alisertib, were published (41). Response rate of 21% was noted in 47 patients with recurrent or progressive SCLC, with the highest response rate observed in the platinum refractory subs, et. If the activity of these inhibitors is validated in subsequent clinical trials, then Myc amplified SCLC could be the first genomically defined subgroup with a specific targeted agent.

Several different tyrosine kinase inhibitors targeting c-kit, EGFR, and Src have been studied in patients with SCLC. However, none of these have had significant clinical activity (42-44).

Another frequently abnormal modulator in SCLC is bcl-2. However, targeted therapy directed at this and other proapoptotic proteins have not demonstrated significant clinical activity (45-48).

Immunotherapy and other targets

The landscape in oncology has changed drastically in the last few years with the progress of immunology. Melanoma, non small cell lung cancer, colon cancer are but a few examples now treated with the addition of immunological agents in the known therapies. Now- days several immunotherapy approaches are under investigation in SCLC (49). Immune checkpoint blockade alone or in combination with chemotherapy is a promising approach.

Nivolumab was tested in a phase I/II study where 128 patients were treated either with nivolumab alone or with a combination of nivolumab plus ipilimumab (50). Patients all had received prior platinum-based chemotherapy, and patients were not preselected based upon PD-1 expression. In the 40 patients treated with nivolumab alone, there were seven objective responses. In the 40 patients treated with a combination of nivolumab plus ipilimumab, there were eight objective responses and an additional 7 patients with stable disease ultimately went on to have a partial response following the data cutoff. These are but preliminary results but promising.

Pembrolizumab, another anti-PD1 antibody, was evaluated in a phase IB study. Twenty patients with SCLC expressing PD-L1 by immunohistochemistry were treated with pembrolizumab (51). All patients had received prior platinum-based combination chemotherapy. In preliminary results presented at the 2015 ASCO meeting, objective responses were observed in seven cases.

Ipilimumab is a monoclonal antibody to CTLA-4 (cyto- toxic T lymphocyte- associated antigen 4) that blocks ligand binding to CTLA-4 and T cell activation occurs. It has been evaluated in combination with paclitaxel and carboplatin in a randomized phase II study that included 130 patients with chemotherapy naive SCLC (52). The study had three arms. One arm was to receive ipilimumab concurrently with chemotherapy. The second arm was sequential phased with chemotherapy initially followed by chemotherapy plus ipilimumab and the third arm with standard chemotherapy without ipilimumab. In the SCLC patient cohort, the phased administration of ipilimumab with chemotherapy improved progression-free survival based upon immune-related response criteria (HR 0.64 95% CI 0.40-1.02;
There was a trend toward prolonged overall survival in the phased treatment group that received chemotherapy followed by chemotherapy plus ipilimumab (median overall survival twelve versus nine months for chemotherapy alone, p=0.13). There was no significant difference between standard chemotherapy and concurrent administration of ipilimumab. Several trials of immunotherapy in the first-line and relapsed settings are now ongoing, such as the investigation of a programmed cell death protein 1 (PD-1) inhibitor (nivolumab) combined with ipilimumab (NCT01928394). Other immunotherapy approaches investigated are immunologically targeted toxins such as BB 10901, a humanized murine monoclonal antibody (huN-901) that binds to CD56 conjugated to the tubulin toxin maytansinoid cytotoxin DM1 and dendritic cells transduced with full-length wild-type p53 gene, which are delivered via an adenovirus (53, 54).

Another drug target with potential activity in SCLC is FGFR. Alterations (e.g., amplification or mutation) in FGFR family members have been described in a small subs, of SCLC tumors; and some, but not all, SCLC models with FGFR1 amplification have demonstrated sensitivity to FGFR inhibitors (55). Drugs targeting FGFR family members that are currently in clinical investigation for SCLC include JNJ-42756493 (a pan-FGFR inhibitor) and BIBF1120 [a multitargeted drug that inhibits FGFR, vascular endothelial growth factor receptor (VEGFR), and platelet, et-derived growth factor receptor (PDGFR). NCT01703481 and NCT014 41297]. However, the extent to which SCLC with mutations and/or amplifications in FGFR family member genes are dependent on the FGFR pathway is not yet known.

SCLC expresses numerous gangliosides such as fucosyl GM1, polysialic acid, GM2, GD2 and GD3 that are not expressed on most normal tissue. These antigens have been studied as potential targets for a vaccine approach (56). The most extensively studied is the anti-idiotypic antibody (BEC-2) which mimics GD3; BEC2 was evaluated in an international phase III trial (57). Following definitive therapy, 515 LS-SCLC responding patients were randomly assigned to BEC2 or observation. No improvement in survival was seen with the vaccine compared to observation. Additional vaccines in clinical testing target the fucosyl GM1 ganglioside (Fuc-GM1) and polysialic acid (polySA), a component of the neural cell adhesion molecule (NCAM).

Lastly, bendamustine, an alkylating agent approved for the treatment of chronic lymphocytic leukemia was evaluated (120 mg/m² IV day 1 and 2 every 3 weeks) in relapsed SCLC after second or third line therapy. Of 33 evaluable patients 10 showed an objective response (58).

Translational research in SCLC

Translational research in SCLC is difficult and underdeveloped. The main scientific reason for this is tissue limitations. Few cells are needed for diagnosis, surgical resection is not common, re-biopsy at progression is not done and there is no next generation sequencing in the clinical setting. Other problems are the molecular complexity of the disease, the rapid pace and the poor understating of the mechanisms of resistance in recurrent disease. If we take into account the poor investment for SCLC research we have a clear picture why there is stagnation in the field. For example in 2012, the National Cancer Institute (NCI) research portfolio contained 745 projects that included lung cancer research, but only 17 of those had a focus on SCLC (59) and since 2007 only 100 interventional trials in SCLC have been registered, and inadequate number to make a meaningful impact in patients outcome.

Perspective

SCLC may be a minor percentage of lung cancer patients in the US, but poses a real issue in other parts of the world where smoking is widespread. An upgrade in translational research is much needed and translating these findings into clinical trials and new standard of cares. A much needed drug development program that incorporates discovery and prioritization of candidate drugs in preclinical models to active treatments. Toward this goal, a major initiative to characterize drug sensitivity is ongoing at the NCI through the Developmental Therapeutics Program, which is investigating more than 400 targeted drugs and 100 FDA-approved oncology therapies in a panel of >60 SCLC cell lines. Results from this drug screen and other ongoing preclinical efforts-combined with an integrated analysis of the molecular profiles of these SCLC cell lines (e., mutations, amplifications, deletions, or alterations at the protein or pathway levels that correspond to drug sensitivity) - will provide important leads that can be validated in the laboratory and taken forward into clinical trials (60).

Another problem is the paucity of data regarding whole-exome DNA sequencing in SCLC. In contrast to NSCLC for which data for more than 1000 tumors have been published (61) in SCLC this is the case in only 82 cases (62). This is very unsettling because this disease carries a large number of mutations per tumor (62) almost four times more than breast and ten times more than prostate cancer, with the majority of them being passengers mutations. This makes it more difficult to definitively identify those mutations that are drivers of cancer growth and invasion. To date genomic studies of SCLC have been insufficiently powered to identify recurrent mutations.

To improve outcomes for patients with SCLC, therapies are needed that improve the durability of responses to front-line therapy (including an increased number of patients with LS-SCLC who have long-term survival) and have activity after disease relapse. To achieve this, we need to apply an approach similar to what has been done in NSCLC and other cancers, including in-depth characterization of potential drug targets and activated pathways present in SCLC, fol-
lowed by translation of the most promising drug targets into the clinic. Furthermore, beyond profiling of treatment-naïve tumors, there is an urgent need to better understand what drives therapeutic resistance in recurrent SCLC, because chemotherapy resistance to second-line and later treatments remains a key factor in poor patient outcomes.

As we stated earlier the major issue that needs to be addressed is adequate tissue for molecular profiling. Regardless of whether it is a surgical disease or not we must implement this in our daily clinical practice for research purposes not only at the beginning of the disease but also at the time of progression. Other investigators have demonstrated the potential of liquid biopsy, meaning obtaining cells from a patient circulation, propagating them in mouse models for molecular profiling and drug sensitivity (63, 64).

We must not forget that apart from the lung, small cell carcinoma can arise also in extrapulmonary sites, such as the urinary bladder (65) which are managed accordingly. Biopsies could be obtained from these sites and joint efforts could advance our understanding in small cell carcinoma biology.

Summary

SCLC is an aggressive disease affecting millions of people worldwide especially smokers. It is a high lethal disease and no significant progress has been made in terms of treatment in the past thirty years. This is so due to several factors with the two major ones being lack of tissue samples for translational research and lack of interest from researchers in comparison with other malignancies. Despite these challenges, as we are entering the era of genomic profiling, there have been some notable discoveries in terms of profiling and researching targeted therapies. Important steps toward this goal will include a coordinated effort to collect adequate SCLC tumor tissue for research from treatment-naïve and refractory patients, funding and support to a scientific community of SCLC investigators, and a framework for translating the most promising discoveries into clinical trials and, ultimately clinical practice.

Conflict of interest

The Authors declare no conflict of interest.

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