REVIEW ARTICLE

Development of molecularly targeted agents and immunotherapies in Small Cell Lung Cancer

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Abstract

Small cell lung cancer (SCLC) is a smoking-induced malignancy with multiple toxin-associated mutations, which accounts for 15% of all lung cancers. It remains a clinical challenge with a rapid doubling time, early dissemination and poor prognosis. Despite multiple clinical trials in SCLC, platinum-based chemotherapy remains the mainstay of treatment in the advanced first line setting. Good initial responses are nevertheless inevitably followed by disease relapse and survival remains poor. There are currently no molecularly targeted agents licensed for use in SCLC. Advances in sequencing the cancer genome and other high-throughput profiling technologies have identified aberrant pathways and mechanisms implicated in SCLC development and progression. Novel anti-tumour therapeutics that impact these putative targets are now being developed and investigated in SCLC. In this review, we discuss novel anti-tumour agents assessed in SCLC with reference to the complex molecular mechanisms implicated in SCLC development and progression. We focus on novel DNA damage response inhibitors, immune checkpoint modulators and antibody-drug conjugates (ADCs) that have shown promise in SCLC, and which may potentially transform treatment strategies in this disease. Finally, we envision the future management of SCLC and propose a biomarker-driven translational treatment paradigm for SCLC that incorporates next generation sequencing studies with patient tumours, circulating plasma DNA and functional imaging. Such modern strategies have the potential to transform the management and improve patient outcomes in this disease.

Introduction

Lung cancer is the leading cause of cancer-related deaths globally [1, 2]. Small cell lung cancer (SCLC) is an aggressive neuroendocrine subtype of lung cancer with a propensity to present with metastatic disease (extensive stage disease; ED) at an early stage [3, 4]. Our understanding of the complex molecular mechanisms and pathways underpinning the development and progression of SCLC has improved with recent advancements in cancer genome sequencing and other high throughput profiling technologies [5-12]. However, despite these advances, there have been no significant improvements in the development of anti-tumour strategies for this disease. A plethora of molecular targeted agents have been investigated in SCLC and most have failed to demonstrate clinical benefit [13-45]. This is likely due to a combination of the molecular targets selected, their functional relevance in the pathogenesis of this aggressive disease and the lack of predictive biomarkers for their clinical efficacy in SCLC, making appropriate patient selection challenging.

Given the richness of the leads that have been produced with high throughput profiling technologies in other solid malignancies as well as liquid tumours, there is now a fresh impetus to exploit these technologies in SCLC. Here we detail novel therapies that have been investigated in patients with SCLC in the context of our expanding knowledge of the molecular biology underpinning the disease. We also propose treatment strategies that we believe will provide SCLC patients with the greatest chance of clinical benefit.

Development of novel agents in SCLC: the challenges

A number of molecularly targeted therapies have been evaluated in SCLC either as monotherapy or in combination with other anti-tumour agents. These include clinical trials in the first-line setting, as maintenance therapy and in relapsed SCLC **(Table 1-2)** [13-45]. The majority of these trials have not found a benefit probably due to both disease and trial-related issues. For example, undertaking clinical trials in SCLC is particularly challenging as patients commonly present with disease-related symptoms requiring urgent systemic chemotherapy. Therefore, the time required in performing comprehensive genomic analysis and screening for clinical trial entry is

often to the detriment of the patients' performance status and symptoms. This is highlighted by the relatively small number of patients enrolled into clinical trials of targeted therapies in SCLC **(Table 1-2)** [13-45]. As SCLC is a chemosensitive disease, with platinum-based chemotherapy inducing high response rates of 75-95% and improving patient survival, it is also difficult to demonstrate an improved response rate from targeted therapies used as adjuncts to chemotherapy in the first-line treatment of SCLC without very large numbers of patients [46]. However, despite these initial high response rates observed with chemotherapy, patients with SCLC inevitably relapse. An attractive approach is thus to explore the use of molecularly targeted therapies in the maintenance setting to prolong treatment responses. This approach is especially relevant in SCLC, as patients on relapse often undergo rapid clinical deterioration, making treatment within or outwith a clinical trial challenging.

Drug development in SCLC: pitfalls and promises

Targeting angiogenesis, cell signalling and apoptosis.

Overall, clinical trials evaluating molecularly targeted therapies that inhibit angiogenesis, cell signalling and apoptosis in SCLC have been disappointing **(Tables 1-2; Figure 1)**. Angiogenesis plays an important role in tumour growth, invasion and metastasis [47]. Vascular endothelial growth factors (VEGFs) stimulate cancer cell migration, invasion, vascular permeability and vessel formation, and have been shown to increase tumour growth and angiogenesis in pre-clinical models [48]. Increased levels of VEGF-A and high vascular counts are associated with a poor prognosis in SCLC [48-50]. Moreover, aberrations within the VEGF pathway have been identified in SCLC, the majority of which occur downstream of the VEGF:VEGFR interaction **(Table 3; Figure 1)** [5-7]. These pre-clinical studies suggest that targeting angiogenesis is an attractive strategy for the treatment of SCLC. Disappointingly, nearly all trials of targeted agents inhibiting angiogenesis have demonstrated little or no clinical benefit in SCLC **(Tables 1-2; Figure 1)** [13, 19-22, 25-28, 30, 31, 33-35, 37, 41, 45].

An area of potential promise is sunitinib, a small molecule inhibitor of multiple tyrosine kinase receptors, that has shown anti-tumour activity in clinical trials **(Figure**

1; Table 1-2) [51]. Ready and colleagues investigated maintenance sunitinib after platinum and etoposide chemotherapy in untreated ED SCLC. Progression free survival (PFS) and overall survival (OS) of patients receiving maintenance sunitinib were 3.7 and 9.0 months compared to 2.1 and 6.9 months, respectively, in those patients receiving placebo. The improvement in PFS was significant (P=0.02). The most common grade 3-4 toxicities were fatigue (19%) and neutropenia (14%) [22]. Spigel and colleagues demonstrated the anti-tumour potential (PFS 7.6 months; OS at 1 year was 54%) of sunitinib as maintenance therapy in patients with ED SCLC with no evidence of progressive disease after first line chemotherapy with irinotecan and carboplatin [26].

Sunitinib as monotherapy in patients with relapsed SCLC has not been well studied. Sunitinib was poorly tolerated (63% grade 3-4 thrombocytopenia; 25% grade 3-4 neutropenia) in a phase II study, with little activity in patients who relapsed/progressed after chemotherapy [35]. Recently, Abdelraouf and colleagues reported anecdotal responses, comprising a RECIST partial response lasting 10 months and a second patient with stable disease that lasted 20 months, out of a small cohort of nine patients (7 chemosensitive and 2 chemotherapy-naïve) treated with sunitinib monotherapy [52]. Grade 3-4 thrombocytopenia and neutropenia were observed in 33% of patients treated with sunitinib. The activity of sunitinib in SCLC may thus warrant further investigation. Future studies should incorporate the exploration of predictive biomarkers of response to identify patients with SCLC who are most likely to benefit from treatment with sunitinib.

Aberrations in EGFR, c-KIT, PI3K/AKT/mTOR, IGFR1 and hedgehog signalling pathways have been identified in SCLC **(Table 3; Figure 1)** [5-12, 53]. Preclinical studies have reported overexpression of c-kit and IGFR1, and activation of Hedgehog signalling in SCLC [38, 53-55]. Ross and colleagues performed targeted exome sequencing of 236 cancer-related genes in ED SCLC and identified molecular aberrations in a number of cell-signaling networks including rapamycin-insensitive companion of mTOR (RICTOR; 10%), stem cell factor receptor tyrosine kinase (c-KIT;

7%), PI3K catalytic subunit alpha (PIK3CA; 6%), epidermal growth factor receptor (EGFR; 5%), phosphatase and tensin homolog (PTEN; 5%) and KRAS (5%) **(Table 3)** [6]. Similarly, Umemura and colleagues identified high prevalence of genetic mutations including PIK3CA (6% of cases), PTEN (4%), RICTOR (9%) and mTOR (4%) **(Table 3)** [11]. Therapies targeting EGFR, c-KIT, PI3K/AKT/mTOR, IGFR1 and hedgehog pathway inhibitors have disappointingly demonstrated minimal efficacy in first-line, maintenance and relapsed SCLC **(Tables 1-2; Figure 1)** [14, 15, 18, 24, 29, 38, 40, 43].

Therapeutic strategies that induce apoptosis in SCLC have also been explored in preclinical and clinical studies. The anti-apoptotic protein BCL-2 is expressed in SCLC and correlates with poor prognosis [56]. In addition, inhibition of the proteasome by bortezomib is associated with a reduction in BCL-2 levels and induction of apoptosis in SCLC cell lines [57]. Similarly, HDAC inhibition reduces the growth of SCLC cell lines and xenografts [58]. Targeted therapies that inhibit BCL-2 family proteins, HDACs and the proteasome have demonstrated no benefit in patients with SCLC **(Tables 1-2; Figure 1)** [16, 23, 32, 36, 39, 42].

Overall, despite both widespread comprehensive genome analysis and pre-clinical studies implicating angiogenesis, cell signalling and apoptosis in the pathogenesis of SCLC, therapies that target these pathways have demonstrated little or no efficacy in clinical trials **(Tables 1-3; Figure 1)** [5-7, 11, 13-16, 18-43, 45, 47-50, 53-55, 57, 58]. These results are likely multifactorial. The aberrations identified in recent genome analyses may be present, but are probably not critical for the pathogenesis of SCLC. Therefore, translational studies to understand the consequence of these aberrations are urgently required. In addition, if such abnormalities are critical for the progression and development of SCLC, then only those patients harboring such aberrations are likely to benefit from treatment and therefore, clinical trials of targeted therapies in small-unselected populations are unlikely to show clinical benefit. Finally, most of those agents discussed have been developed for other tumour types rather than SCLC. In the future, it will be important to discover new

targets and develop targeted therapies based on the underlying biological drivers of SCLC.

Targeting DNA repair pathways

Transcriptional regulation and DNA repair pathways have been implicated in SCLC development. Sequencing of SCLC surgical samples has identified a high prevalence of inactivating mutations in *TP53* and *RB1*, amplification of *MYC* family members and mutations of histone modifiers (CREBBP and EP300) **(Table 3)** [6, 9-11]. Similarly, SCLC has a very high level of poly(ADP-ribose) polymerase 1 (PARP1) expression compared to other solid tumours implicating a potential role in SCLC [59]. PARP1 is a highly abundant nuclear protein that is activated in response to DNA damage [60]. The PARP1 enzyme has a critical function in DNA damage recognition and subsequent repair through base excision repair and has a role in homologous recombinant repair [17, 60]. Loss of PARP1 activity in pre-clinical models leads to the accumulation of DNA strand breaks along with sensitization to radiotherapy and chemotherapy [17, 60-62]. Sensitization seems to be a result of increased DNA damage and delayed DNA damage repair [17]. Platinum-based chemotherapy induces high response rates in SCLC, and the use of PARP inhibitors to prevent the ability of cancer cells to repair DNA damage induced by cytotoxic agents is thus an attractive therapeutic strategy for the treatment of SCLC **(Figure 1).**

The safety of the PARP inhibitor veliparib in combination with cisplatin and etoposide in first-line treatment of ED SCLC has been evaluated in the clinic **(Table 1)** [63]. The addition of veliparib to cisplatin and etoposide did not improve the response rate beyond that expected for chemotherapy alone [63]. PARP inhibitors may demonstrate clinical benefit as maintenance treatment in patients with platinum sensitive disease through the prolonged inhibition of DNA repair pathways. Olaparib is currently being evaluated in a randomised placebo-controlled phase II study as maintenance therapy in SCLC after RECIST response to first-line chemotherapy (ISRCTN 73164486) **(Table 1)**. Wainberg and colleagues evaluated the PARP inhibitor BMN-673 in ED SCLC patients that progressed after platinum-based chemotherapy **(Table 2)** [44]. Single agent treatment was well tolerated (4.3% grade

3-4 toxicities) and two of 11 evaluable patients (18%) with SCLC demonstrated RECIST partial responses [44].

Multiple putative predictive biomarkers of platinum sensitivity and clinical outcomes in SCLC have been investigated [3, 64]. Karachaliou and colleagues identified a gene expression signature from 184 patients with SCLC treated with cisplatin and etoposide [3]. This favorable expression signature (low levels of excision repair cross complementation group 1, pyruvate kinase isoform M2, DNA topoisomerase I and DNA topoisomerase II mRNA) correlated with improved PFS and OS in patients with both limited disease (LD) ($P<0.001$ and $P=0.007$, respectively) and ED ($P=0.007$ and $P=0.011$, respectively) SCLC [3]. BMN-673 demonstrates anti-tumour activity in SCLC cell lines and xenografts [65]. The elevated expression of 17 DNA repair proteins as well as higher levels of expression of a novel "DNA repair protein score" predicted anti-tumour responses to BMN-673. In addition, PI3K activation was associated with resistance to BMN-673 [65]. These findings need to be validated in patients with SCLC as they may identify biomarkers that may predict for response and resistance to BMN-673. Germline *BRCA1* and *BRCA2* mutations in SCLC are low. However, the numerous somatic aberrations seen within DNA repair proteins mean SCLCs possess a BRCAness phenotype with potential associated sensitivity to platinum therapy and novel targeted agents such as PARP and ataxia telangiectasia and Rad3-related protein (ATR) inhibitors [66]. Therapies that target DNA repair defects in SCLC in an appropriately selected population have the potential to transform the treatment of this aggressive disease. The proposed BRCAness phenotype of SCLC, molecular alterations in transcriptional regulation, and observed early anti-tumour responses to BMN-673 in relapsed SCLC warrant further investigation with the exploration of biomarker-defined patient subgroups.

Targeting the immune system

Immune modulation provides an attractive method to harness a patients immune system to achieve tumour control, stabilisation and potential eradication of disease [67]. T-cells play a central role in cell-mediated immunity **(Figure 2)**. Briefly, T-cell activation occurs when exposure to tumour antigen is followed by antigen

independent co-regulatory signals. This leads to the activation and mobilisation of T-cells **(Figure 2)** [68, 69]. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is expressed on activated T-cells and down regulates the T-cell response [70]. Activated T-cells mobilise to the tumour microenvironment, where if they recognise antigens expressed on the tumour cell, they proliferate, release cytolytic enzymes and secrete cytokines that attract other members of the immune system and eliminate tumour cells **(Figure 2)**. In comparison, programmed cell death protein-1 (PD-1) is expressed on activated T-cells, which binds to its ligand PD-L1, causing T-cell inhibition. Tumour cells hijack this pathway by expressing PD-L1 on their cell surface and directly suppressing anti-tumour cytotoxic T-cell activity and thus evading the immune system **(Figure 2).** Immune therapies that overcome inhibition of the immune system by targeting CTLA-4, PD-1 and PD-L1 have shown great promise as novel anti-cancer therapies in multiple malignancies [67].

Ipilimumab (CTLA-4 monoclonal antibody) has been evaluated in combination with carboplatin and paclitaxel chemotherapy in first line treatment of patients with ED SCLC **(Table 1)** [71]. In a pivotal 3-arm phase II trial, patients received either 6 cycles of carboplatin and paclitaxel with or without ipilimumab or with ipilimumab in a phased schedule [71]. Phased ipilimumab was associated with improved immune related PFS when compared to the control arm (P=0.03) [71]. There was a trend towards improved OS in the phased ipilimumab arm (P=0.13). The incidence of grade 3-4 toxicities was greater with ipilimumab treatment (phased 50%, concurrent 43%) than without (30%). This study did not identify any potential biomarkers that may predict for response to ipilimumab treatment in patients with SCLC. Further clinical trials are now ongoing to evaluate ipilimumab with and without chemotherapy in LD and ED SCLC (NCT01331525, NCT02046733 and NCT01450761).

Nivolumab and pembrolizumab are novel high-affinity, humanized monoclonal antibodies that block PD-1. Nivolumab is currently being investigated as monotherapy or in combination with ipilimumab in advanced tumours, including SCLC (NCT01928394). Monoclonal antibodies that block PD-L1 (BMS 936559, MEDI4736 and MPDL3280A) are also being evaluated in early phase clinical trials in

advanced cancers. Pembrolizumab demonstrated promising antitumor activity in the KEYNOTE-028 trial involving patients with heavily pretreated, PD-L1–positive, extensive-stage SCLC **(Table 2)** [72]. Patients received 10 mg/kg of intravenous pembrolizumab once every 2 weeks. Response was assessed every 8 weeks for the first 6 months, then every 12 weeks thereafter. Overall, the safety and toxicity profile was consistent with previous studies with pembrolizumab in other tumor types. Treatment-related adverse events affecting two or more patients occurred in 14 patients; two patients had grade 3-5 adverse events, including one case of colitis that resulted in the patient's death. One case of grade 2 autoimmune thyroiditis resulted in treatment interruption [72]. The overall response rate to pembrolizumab in antitumor activity was 35% (95% CI [15%, 59%]), and the responses appeared durable, with six of seven ongoing at data cutoff. The median time to response was 8.6 weeks (range: 7.7-16.1 weeks), while the median duration of response was 29.1 weeks (range: 0.1-29.1 weeks). Five of seven patients who had a disease response had reduction in tumor size from baseline of 50% or greater [72]. Interestingly, six of seven responses occurred after 8 weeks of initiating treatment, and one patient who had stable disease at 8 weeks then had a partial response at 16 weeks.

The distinct roles of CTLA-4 and PD-1/PD-L1 in regulating T-cell activation suggests there is merit in exploring dual blockade **(Figure 2)**. Combined blockade with CTLA-4, PD-1 and PD-L1 antibodies inhibit growth in pre-clinical melanoma models [73]. CTLA-4 inhibition has been shown to upregulate PD-1 on tumour infiltrating cells while PD-1 inhibition has been shown to upregulate CTLA-4 on tumour infiltrating cells [73]. This potentially provides a mechanism of resistance to immune monotherapy and therapeutic strategies that block CTLA-4 and PD-1 may demonstrate synergistic benefit in patients. The phase I/II CheckMate 032 study of nivolumab with or without ipilimumab for patients with recurrent SCLC demonstrated that both nivolumab monotherapy and the nivolumab/ipilimumab combination therapy showed activity and durable responses in patients with SCLC **(Table 2)** [74]. The study comprised four arms, and included 128 patients with progressive disease after one or more lines of therapy, including a first-line platinum-based regimen. One arm received 3 mg/kg of intravenous nivolumab every

2 weeks (40 patients), and two arms received 1 mg/kg of intravenous nivolumab in combination with either 1 mg/kg or 3 mg/kg of intravenous ipilimumab every 3 weeks for four cycles (3 patients and 47 patients, respectively). Data have not been reported from the fourth arm, which included 3 mg/kg of intravenous nivolumab plus 1 mg/kg of intravenous ipilimumab every 3 weeks for four cycles [74].

Fifteen percent of patients in the arm treated with nivolumab monotherapy had grade 3-4 adverse events; 34% had grade 3-4 adverse events in the arm treated with nivolumab plus the higher dose (3 mg/kg) of ipilimumab. One patient died from treatment-related causes (myasthenia gravis) in the combination arm receiving the higher dose of ipilimumab. The ORR was 18% with nivolumab monotherapy and 17% with nivolumab/ipilimumab. The disease control rate was 38% with monotherapy and 54% with combination therapy. The median OS was 4.4 months with monotherapy (95% CI [2.9, 9.4]) and 8.2 months with combination therapy (95% CI [3.7, not reached]). Overall, the response rate and frequency of tumor reduction suggested increased effects with the combination, activity was observed in both platinum-sensitive and platinum-resistant/refractory disease. Interestingly, responses occurred regardless of PD-L1 expression.

Only a subset of patients treated with immune therapies experience durable and long-term disease control [67, 72, 74]. It will therefore be important to identify predictive biomarkers of response to identify those patients that will benefit most from CTLA-4 and/or PD-1/PD-L1 targeting. For example, PD-L1 expression is currently the closest we have to a predictive biomarker at this point of time for PD-1/PD-L1 inhibitors, but is clearly imperfect [67]. Variations in laboratory assays, tumour type and previous treatments may contribute to observed heterogeneity in PD-L1 expression [67]. Making treatment decisions on such a biomarker requires careful consideration since many who are biomarker negative may still benefit. It is likely that the selection of biomarkers for PD-L1/PD-1 inhibitors will involve additional factors, such as non-synonymous mutational load. Candidate neo-antigens have been identified that bind with high affinity to MHC class I receptors, which may predict for anti-tumour responses to both CTLA-4 and PD-1 treatments

[75]. Patients with NSCLC harbouring tumors with high candidate neoantigen burden had improved outcomes compared to controls when treated with pembrolizumab (median PFS 14.5 months versus 3.5 months p=0.002) [76]. The application of neoantigen burden as a predictive biomarker of response to different immunotherapies is promising and should be explored in future clinical trials.

Antibody-drug conjugates (ADCs)

A number of antibodies that target tumor surface antigens have provided effective anti-cancer therapies [77]. However, many unmodified antibodies lack therapeutic activity and are instead being exploited to deliver potent cytotoxic drugs in the form of ADCs. Neural cell adhesion molecule (also called CD56) is expressed on the cell surface of SCLC (approximately 74%) [78, 79]. Lorvotuzumab mertansine (LM) is an antibody drug conjugate (ADC) containing a CD56 binding antibody (N901) bound to the microtubulin disruptive agent DM-1. LM has demonstrated activity in pre-clinical models of SCLC both as monotherapy and in combination with chemotherapy [79, 80]. In two Phase I studies involving CD56-positive solid tumors, the CBR (partial responses plus stable disease for ≥ 75 days) was 25% (17/68 patients) in patients with small cell lung cancer (SCLC) from among 113 patients treated with LM [81]. Despite initial promise, a phase II study of LM in combination with carboplatin and etoposide was stopped early by the independent data monitoring committee due to concerns with efficacy of the combination and LM has not been developed further in SCLC.

Rovalpituzumab tesirine, an ADC comprised of a humanized anti-delta-like 3 (DLL3) monoclonal antibody conjugated to the DNA-damaging pyrrolobenzodiazepine (PDB) dimer toxin, has shown promising activity in SCLC [82]. DLL3 expression by immunohistochemistry is elevated in SCLC (72 to 85%), compared to adenocarcinoma (3.7%), squamous cell carcinoma (0%) and normal lung tissue (0%) [82]. DDL3 is overexpressed in primary SCLC and patient derived xenografts compared to normal tissue [82]. Treatment with rovalpituzumab tesirine induced durable responses in SCLC patient derived xenograft tumour models and eliminated tumour initiating cells which are not effected by standard therapies and are thought

to be responsible for the rapid disease relapse seen clinically in SCLC [82]. The efficacy of rovalpituzumab tesirine is a consequence of targeted delivery of PDB dimer toxin either as naked anti-DLL3 antibody or free PDB dimer toxin had anti-tumour activity [82]. These encouraging pre-clinical data are supported by a recent first-in-human phase I trial of rovalpituzumab tesirine in patients with recurrent SCLC. In all evaluable patients (n=32), the overall response rate was 22% (n=7 partial response), together with disease stabilization in 53% (n=17) of patients [83]. Sixteen patients had confirmed DLL3 positivity (H-Score ≥ 120) and of these, 7 patients (44%) had partial response and 8 patients (50%) achieved stable disease [83]. Grade 3-4 toxicities were capillary leak syndrome (14%) and thrombocytopenia (6%) [83]. Rovalpituzumab tesirine in DLL3 positive patients is a promising treatment strategy, and the first in this class of drugs to show activity in SCLC. If these results can be reproduced in the planned follow-on trials, then rovalpituzumab tesirine has the potential to transform the treatment of relapsed SCLC.

Developing targeted therapies in SCLC: how can we improve?

Over the past 10 years, molecularly targeted agents have altered the management of different malignancies, with significant patient benefit observed in biomarker selected groups of patients. Despite the successes observed in multiple solid tumours, there are still no targeted therapies approved for use in SCLC and there are a number of reasons for these short fallings. Firstly, most agents explored in SCLC have showed promise in other tumour types and have not been discovered or developed specifically against the underlying biology of SCLC. Secondly, despite our increase in the understanding of the molecular characteristics of SCLC, the development of SCLC targeted treatments has continued in small underpowered phase II studies involving unselected patients. We have learnt little from such studies, and they have failed to demonstrate clinical benefit in selected groups of patients with SCLC, resulting in a paucity of phase III studies involving targeted agents in this disease.

Translational research that identifies molecular aberrations from extensive whole genome analysis that are critical for SCLC development is urgently required.

Correlation of these findings with clinical endpoints is of paramount importance and these studies have the potential to identify predictive biomarkers that determine patient cohorts most likely to respond to molecularly targeted therapies and immunotherapies. One constraint to such translational studies is the paucity of tissue for molecular studies since SCLC is rarely resected and serial biopsies are a major challenge. Therefore, alternative sources of tissue including circulating tumour cells (CTCs) and circulating plasma DNA (cpDNA) needs to be identified for such translational studies. Moreover, translational studies that can identify molecular mechanisms of resistance in patients progressing on treatment with targeted therapies and immunotherapies will be key to developing combination approaches for the treatment of SCLC. These strategies have the potential to overcome and reverse resistance to treatments, so as to provide further clinical benefit for patients with SCLC.

Taking all this together, we therefore propose a novel paradigm for SCLC that will incorporate the upfront and sequential molecular analyses of patient tissue and functional imaging studies to optimally select and monitor patients who may benefit from such treatments **(Figure 3).** With this treatment pathway, patients with SCLC should undergo a tumour biopsy at diagnosis to obtain suitable tissue for molecular analysis alongside the collection of blood specimens for the isolation of CTCs, cpDNA and microRNA (miRNA) **(Figure 3)**. Such translational studies may identify genetic aberrations and molecular signatures that predict treatment response and resistance to therapies. In addition, co-clinical studies with patient tumour and/or CTC derived xenografts (PDX) may potentially be used to guide therapeutic strategies for individual patients. These models have already shown promise with CTC derived xenografts from patients with SCLC [84]. One limitation currently is the time taken to grow PDXs as clinically SCLC progresses rapidly and treatment decisions may be required prior to knowledge being obtained from such model systems. However, as technologies develop, these PDXs may have a critical role in guiding therapeutic strategies to overcome drug resistance and therefore play an important role in delivering precision medicine in SCLC. Overall, these hypothesis-testing, biomarker-driven translational studies have the potential to identify patients most likely to

respond to targeted therapies, immunotherapies and ADCs, and to provide greater and more durable clinical benefit to patients with SCLC. Furthermore, serial collection of tissue, CTCs, cpDNA and miRNA, with the development of PDX models, will enable 'real-time' monitoring with sequential molecular analyses of tumour biology while on targeted treatment and at progression.

Conclusions

SCLC has lagged behind other tumour types in terms of a canvas for novel anti-cancer drug discovery and biomarker development. In the future, it is likely that with our increased understanding of the underlying biology of SCLC and the identification and validation of molecular signatures associated with this disease, we may finally begin to see a new era of effective targeted agents, immunotherapeutics and ADCs developed for the treatment of SCLC.

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FIGURES

Figure 1: Molecular targets in small cell lung cancer

The figure simplifies the complex network of plasma membrane (PM) receptors, intracellular signaling pathways and cellular functions that have been implicated in small cell lung cancer (SCLC) development and progression. Targeted therapies (blue) that impact these pathways have been investigated in SCLC. Genetic aberrations identified in SCLC that are actionable (green) and non-actionable (red) are shown. Targets include the epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptors (VEGFR), vascular endothelial growth factor (VEGF), stem cell factor receptor (C-Kit), insulin-like growth factor receptor (IGFR), rat sarcoma (RAS), B-cell lymphoma (BCL-2), mammalian target of rapamycin (mTOR), Src and Poly-ADP ribose polymerase (PARP). Therapies highlighted have activity against multiple targets (*).

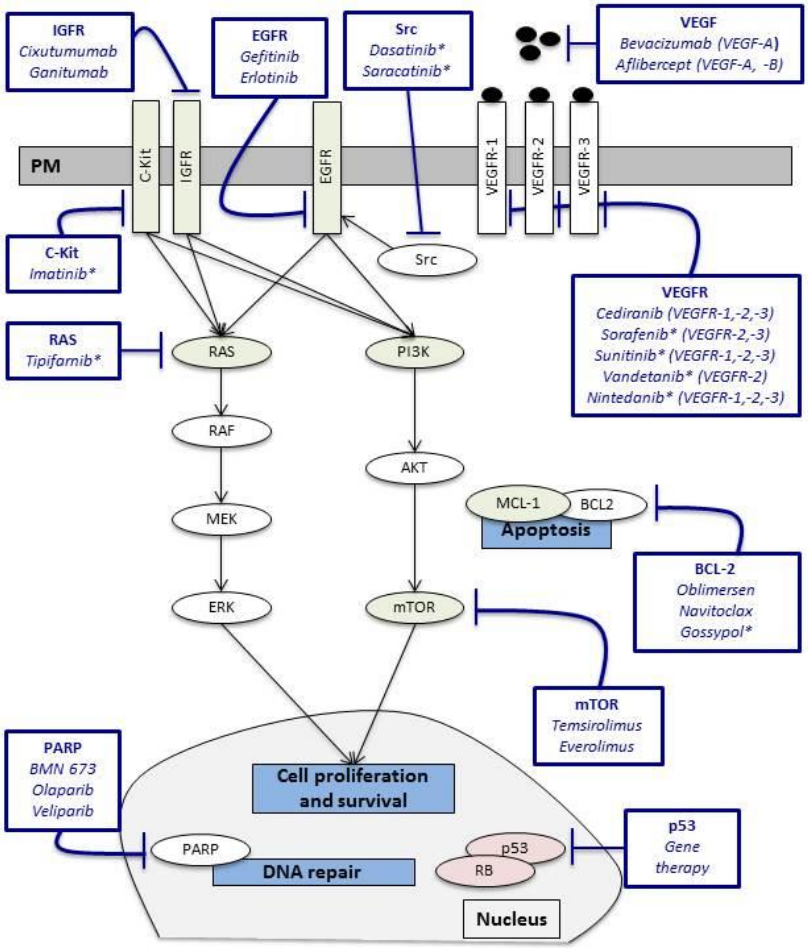


Figure 2: Targeting the immune system in small cell lung cancer

Initial T-cell activation occurs within lymph nodes (priming phase). Tumour antigen (Ag) is presented by the major histocompatibility complex (MHC) of the antigen presenting cells (APC) to the T-cell receptor (TCR) of the T-cell. This is followed by antigen independent coregulatory signals in which CD28 on the T-cell binds B7 on the APC leading to T-cell activation and mobilisation. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that is expressed on the activated T-cell competes with CD28 for binding to B7 and down regulates the T-cell response. Activated T-cells mobilise to the tumour microenvironment and if they recognise antigens expressed on the MHC of the tumour cell they proliferate, release cytolytic enzymes and secrete cytokines that attract other members of the immune system and eliminate the tumour (effector phase). Programmed cell death protein-1 (PD-1) is found on the surface of activated T-cells where it binds to its ligands PD-L1, causing T-cell inhibition and down regulation of the acquired T-cell response. Tumour cells may hijack this pathway by expressing PD-L1 on their cell surface and directly suppressing anti-tumour cytotoxic T-cell activity and evading the immune system. Immunotherapies (blue) inhibit down regulation of the immune response.

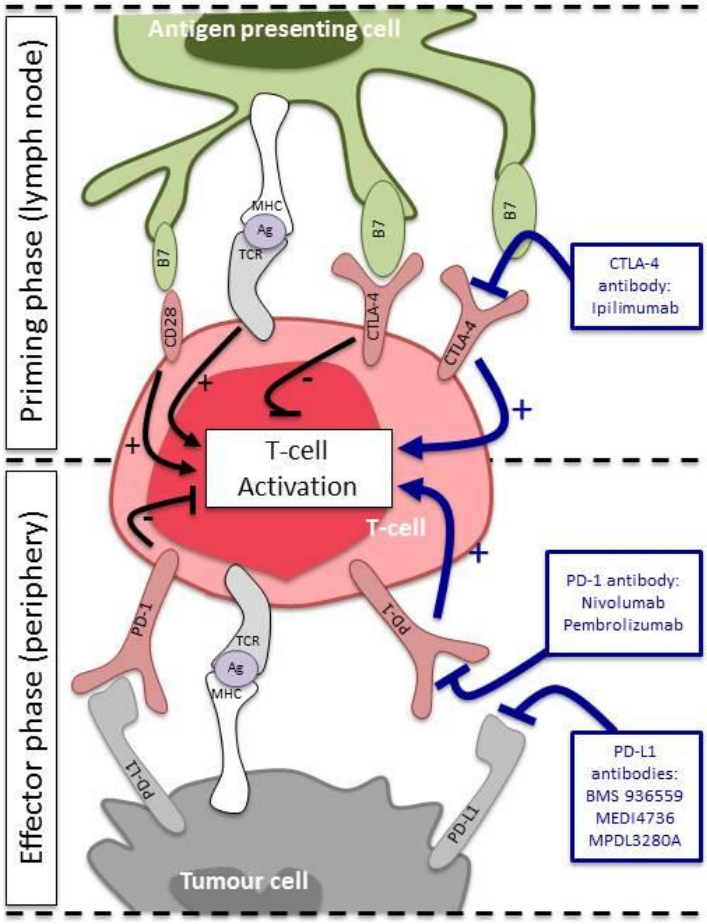
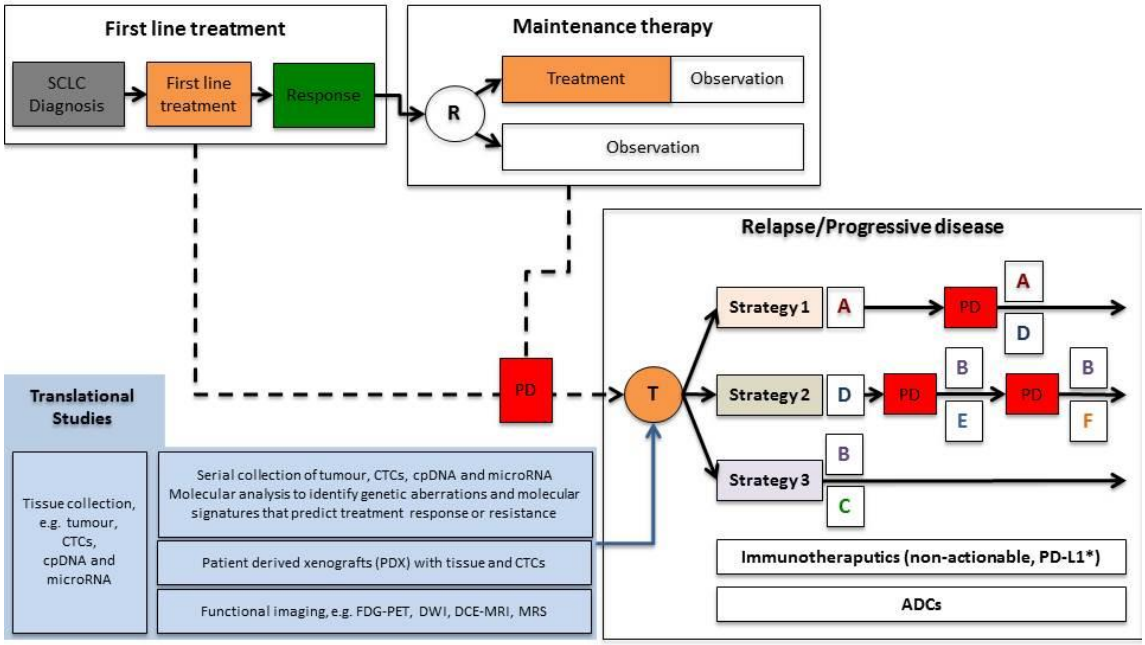


Figure 3: Future treatment paradigm for small cell lung cancer

In the future, patients diagnosed with SCLC should routinely undergo tissue biopsy and serial whole blood sampling to isolate circulating tumour cells (CTCs), circulating plasma DNA (cpDNA) and/or microRNA (miRNA) for translational studies (pale blue boxes). These studies will provide molecular analysis of individual patient tumours and development of patient derived xenografts to determine therapies (orange shading) for patients who undergo observation, maintenance therapy or eventual treatment of relapsed disease. Functional imaging will be assessed alongside molecular studies to identify patients with progressive disease at an early stage. Patients that receive targeted therapy (T) will have ongoing translational studies from serial collections (blue shading) to determine molecular changes that predict resistance to current therapies (strategy 1, strategy 2 and strategy 3). At progressive disease (PD; red shading) molecular analysis will allow adaption/addition of therapy (A, B, C, D, E and F) to overcome resistance and provide further clinical benefit for patients. Immunotherapy should be considered in patients without actionable molecular changes or patients with PDL-1 positive tumours. *PD-L1 testing currently is very heterogeneous and extreme caution should be used when applying it as a biomarker of response. It is likely that selection biomarkers for PD-L1/PD-1 inhibitors will involve additional factors, such as non-synonymous mutational load. Patients expressing antigens for antibody-drug conjugate (ADCs) should be considered for such therapies.



TABLES

Table 1: Targeted therapies in first line treatment of small cell lung cancer

Putative Target	Agent	Author	Phase	Therapy	Setting	Outcome
Targeting angiogenesis						
VEGF-A	**Bevacizumab**	Pujol[2015] Patton[2006] Spigel[2008] Ready[2011] Spigel[2009] Spigel[2011]	II/III II II II II II	Combination Monotherapy Combination Combination Combination Combination	F (M) R M F (M) F F (M) F (M) R	Negative 15m OS Stopped 7m PFS, 11.6m OS 9.1m TTP, 12.1m Negative
RAF-1, VEGFR-2, VEGFR-3 and PDGFRβ	**Sorafenib**	Sharma[2014]	II	Combination	F (M)	7.4m OS
VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, c-KIT, FLT-3 and RET	**Sunitinib**	Spigel[2012] Ready[2015]	II II	Monotherapy Monotherapy	M M R	7.6m PFS Improved PFS not OS
VEGFR-2, EGFR and RET	**Vandetanib**	Arnold[2007]	II	Monotherapy	M R	Negative
Targeting cell signaling						
BCR-Abl, c-KIT and PDGFR	**Imatinib**	Johnson[2003] Spigel[2007] Schneider[2010]	II II II	Monotherapy Monotherapy Monotherapy	F M F (M)	0.8m TTP 5.4m PFS, 8.4m OS 4.3m PFS, 7.8m OS
mTOR	**Temsirolimus**	Pandya[2007]	II	Monotherapy	M	2.5m PFS
IGF1R	**Cixutumumab**	Belani[2013]	II	Combination	F R	Negative
Smoothened	**Vismodegib**	Belani[2013]	II	Combination	F R	Negative
Targeting apoptosis						
BCL-2	**Oblimersen**	Rudin[2008]	II	Combination	F	Negative
BCL-2, MCL-1, BCL-W, BCL-XL	**Obatoclax**	Langer[2014]	II	Combination	F	Negative
Targeting DNA repair defects						
PARP	**Veliparib** **Olaparib**	Owonikoko[2014] Ongoing	I II	Combination Monotherapy	F M R	Negative ISRCTN73164486
Targeting the immune system						
CTLA-4	**Ipilimumab**	Reck[2013] Ongoing Ongoing Ongoing	II II II III	Combination Combination Combination Combination	F F F (M) F	Improved iRPFS NCT01331525 NCT02046733 NCT01450761

VEGF – Vascular endothelial growth factor; VEGFR – Vascular endothelial growth factor receptor; PDGFR – Platelet derived growth factor receptor; c-KIT – Stem cell factor receptor; FLT3 – FMS-like tyrosine kinase 3; RET – Rearranged during transfection tyrosine kinase; mTOR - mammalian target of rapamycin; IGFR – Insulin like growth factor receptor; BCL-2 – B-cell lymphoma; MCL-1 – Myeloid cell leukemia 1; HDAC – Histone deacetylase; F – First-line; F (M) – First-line followed by maintenance; S – Second-line; S (M) – Second-line followed by maintenance; R – Randomised; PFS – Progression free survival; TTP – Time to progression; OS – Overall survival; m – Months; CS – Case study

Table 2: Targeted therapies in treatment of relapsed small cell lung cancer

Putative Target	Agent	Ref	Phase	Therapy	Setting	Outcome
Targeting angiogenesis						
VEGF-A	**Bevacizumab**	Jalal[2010] Waterhouse[2010] Mountzios[2012]	II II II	Combination Combination Combination	R (M) R R	14.7w PFS, 30w OS 17.4w PFS, 31.6w OS 2.7m PFS, 6.3m OS
RAF-1, VEGFR-2, VEGFR-3 and PDGFRβ	**Sorafenib**	Gitlitz[2010]	II	Monotherapy	R	6.7m OS^{PS}, 5.3m OS^{PR}
VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, c-KIT, FLT-3 and RET	**Sunitinib**	Han[2013]	II	Monotherapy	R	1.4m PFS, 5.6m OS
VEGFR-1 and VEGFR-2	**Aflibercept**	Allen[2014]	II	Combination	R Ra	Negative
VEGFR-1, VEGFR-2 and VEGFR-3, PDGF and c-Kit	**Pazopanib**	Gandhi[2012]	II	Monotherapy	R	14.1w PFS
Targeting cell signaling						
EGFR	**Gefitinib**	Moore[2006]	II	Monotherapy	R	50d TTP
BCR-Abl, c-KIT and PDGFR	**Imatinib**	Johnson[2003] Krug[2005]	II II	Monotherapy Monotherapy	R R	1.2m TTP All PD by 4w (n=12)
mTOR	**Everolimus**	Tarhini[2010]	II	Monotherapy	R	1.3m PFS, 6.7m OS
Targeting apoptosis						
BCL-2, BCL-W, BCL-XL	**Navitoclax**	Rudin[2012]	II	Monotherapy	R	1.5m PFS, 3.2m OS
BCL-2, BCL-XL, BCL-W, MCL-1	**Gossypol**	Heist[2010]	I/II	Combination	R	17.4w PFS^{PS}, 11.7w ^{PR}
Proteasome	**Bortezomib**	Lara[2006]	II	Monotherapy	R	1m PFS, 3m OS
HDAC	**Panobinostat**	De Marinis[2013]	II	Monotherapy	R	Negative
Targeting DNA repair defects						
PARP	**BMN673**	Wainberg[2014]	I	Monotherapy	R	18% RR
Targeting the immune system						
PD-1/CTLA-4	**Nivolumab Ipilimumab**	Antonia[2015]	I/II	Combination	R	Nivolumab 18% ORR and 4.4m OS, Combination 17% ORR and 8.2m OS
Antibody-drug conjugates						
CD56	**Lorvotuzumab mertansine**	Beck[2012]	I	Monotherapy	R	25% PR/SD
DLL3	**Rovalpituzumab tesirine**	Rudin[2015]	I	Monotherapy	R	

VEGF – Vascular endothelial growth factor; VEGFR – Vascular endothelial growth factor receptor; PDGFR – Platelet derived growth factor receptor; c-KIT – Stem cell factor receptor; FLT3 – FMS-like tyrosine kinase 3; RET – Rearranged during transfection tyrosine kinase; mTOR - mammalian target of rapamycin; BCL-2 – B-cell lymphoma; MCL-1 – Myeloid cell leukemia 1; HDAC – Histone deacetylase; CD56 – Neural cell adhesion molecule; DLL3 – delta-like 3; F – First-line; F (M) – First-line followed by maintenance; R – Relapsed; R (M) – Relapsed followed by maintenance; Ra – Randomised; PFS – Progression free survival; TTP – Time to progression; OS – Overall survival; m – Months; ^{PR} – Platinum resistant; ^{PS} –Platinum sensitive; d – Days; PR – Partial response; SD – Stable disease

Table 3: Molecular basis of small cell lung cancer

Ref	Bordi et al 2014	Ross et al 2014*	Wakuda et al 2014	Umemura et al 2014*	Rudin et al 2012*	Peifer et al 2012*
Patients	113	98	60	47	36	99
Technique	DNA from FFPE	DNA from FFPE	DNA from FFPE (50), FF (8) and pleural effusions (7)	DNA from FFPE (43) and MFPE (12).	DNA from FF	DNA from FF
Coverage	6 gene panel	236 cancer genes	9 gene panel	Whole exome and copy number analysis	Whole exome, transcriptome and copy number analysis	Whole exome sequencing and transcriptome analysis
Aberrations	Mutation	Mut and amp	Mut and amp	Mut and amp	Mut and amp	Mut and amp
EGFR	1.8%	5%	1.7%	NR	**Mutation hotspots**	
BRAF	0%	NR	0%	2.1%	TP53	
c-KIT	0%	7%		NR	RB1	
c-MET	4.4%	NR	1.7%	NR	PIK3CA	
KRAS	0%	5%	1.7%	2.1%	CDKN2A	**Mutations**
NRAS		NR	0%	NR	PTEN	TP53
PDGFR	0%	NR		NR	EP300	RB1
TP53		86%		78.7%	MLL2	CREBBP
RB1		54%		44.7%		EP300
MLL2		17%		NR	**Mutation clustering**	MLL
RICTOR		10%		8.5%	PIK3CA	PTEN
MYCL1		8%		8.5%	AKT1-3	SLIT2
FGF10		8%		NR	MTOR	EPHA7
LRP1B		7%		NR	NOTCH1-3	**Amplification**
PIK3CA		6%	15%	6.4%	SMO	MYC family
PTEN		5%		4.3%	SOX 3-6,9,11,14,17	FGFR1
MYST3		5%		NR		
MEK1		NR	0%	4.3%	**Amplification**	
AKT		NR	1.7%	12.8%	SOX2	
HER2		NR	0%	NR	MYC	
CREBBP		3%		4.3%	KIT	

DNA – deoxyribose nucleic acid; FFPE – formalin fixed paraffin embedded; FF – fresh frozen; Mut – mutation; Amp – amplification; NR – not recorded; Not assessed (grey shading); *please see original references for exhaustive list of aberrations.