Published in final edited form as:

Curr Probl Cancer. 2012 May ; 36(3): 156–173. doi:10.1016/j.crrproblcancer.2012.03.005.

Novel Therapeutic Approaches for Small Cell Lung Cancer: The Future has Arrived

M. Catherine Pietanza, M.D.¹ and Charles M. Rudin, M.D., Ph.D.²

¹Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY

²The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD

Keywords

SCLC; tyrosine receptor kinases; developmental pathways; immunology; oncolytic replication-selective viruses

Introduction

Thirty thousand patients are diagnosed with small cell lung cancer (SCLC) in the United States each year [1]. Tobacco exposure is strongly associated with the development of this disease. SCLC has a unique natural history with a much shorter doubling time, higher growth fraction and earlier development of widespread metastases than any of the non-small cell histologies. Although SCLC is characterized by a superior initial response rate to chemotherapy and radiation, even responsive disease is typically associated with relapse within months after treatment, with the rapid development of chemo-resistance, leading to a dismal five year survival rate of 5% [2].

Few improvements have been made in the fundamentals of SCLC treatment. Notably, the standard chemotherapy regimen used for initial disease has not changed over the last three decades. First-line treatment for SCLC involves combination chemotherapy with cisplatin or carboplatin plus etoposide (with the addition of radiation therapy in limited stage), which results in a 60-80% overall response rate. However, all patients with extensive stage SCLC, and the majority of patients with limited SCLC, suffer relapse within months of completing initial therapy. Topotecan (Hycamtin; GlaxoSmithKline) is the only approved agent for recurrent or progressive SCLC, based on the results of three phase III trials [3-5]. There are no accepted regimens for patients whose disease has progressed after first- and second-line treatments for SCLC. As the prognosis for patients with recurrent SCLC is poor, there is a clear need for the development of new therapeutic strategies. Focusing on common tumor-specific molecular and cellular abnormalities may identify novel therapeutic targets in this challenging disease.

© 2012 Mosby, Inc. All rights reserved.

Reprint requests: Dr. M. Catherine Pietanza Memorial Sloan-Kettering Cancer Center 1275 York Avenue New York, NY 10021 TEL: 212-639-6373 FAX: 212-794-4357 pietanzm@mskcc.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DNA Repair

Temozolomide

SCLC is characterized by frequent aberrant methylation and epigenetic silencing of the *MGMT* gene, located on chromosome 10q26, which encodes the DNA-repair protein O⁶ alkyl-guanine (O⁶-AG) DNA alkyltransferase (MGMT) [6-8]. This protein removes alkyl groups from the O⁶ position of guanine, a common site of DNA modification. High levels of MGMT activity in cancer cells blunt the therapeutic effects of alkylating agents, and thus can be an important determinant of treatment failure [6, 8]. Epigenetic silencing of *MGMT* via hypermethylation of specific CpG islands of its promoter leads to loss of MGMT activity and improved sensitivity to alkylating agents [6, 8]. Left unrepaired, chemotherapy-induced lesions trigger cytotoxicity and apoptosis. Temozolomide is an oral alkylating agent, currently approved for use in patients with glioblastoma multiforme and in refractory astrocytoma. It also has activity in metastatic melanoma. In the phase III study of temozolomide in glioma, *MGMT* promoter methylation status was analyzed retrospectively and found to be an independent favorable prognostic factor [9]. A recent, large Radiation Thoracic Oncology Group study has confirmed these findings and has shown that *MGMT* promoter methylation is associated with response to temozolomide in glioma [10].

Given the strong rationale to study temozolomide in patients with SCLC, including the known single agent efficacy of alkylating agents in this malignancy [11] and the ability for the agent to cross the blood-brain barrier, a phase II clinical trial of temozolomide for relapsed sensitive or refractory SCLC has been performed [12]. Sixty-four patients who had received one or two lines of prior therapy were enrolled. The primary endpoint was overall response rate (ORR) according to RECIST[13], assessed separately for sensitive and refractory disease [14]. Temozolomide therapy was well tolerated, and associated with a 20% ORR [95% CI: 11% to 32%] in this patient population. In the sensitive group (n = 48), one complete response and 10 partial responses were noted for a 23% ORR [95% CI: 12% to 37%], while two responses were seen in the refractory cohort (n = 16) for a 13% ORR [95% CI: 2% to 38%]. Responses also were noted in patients receiving temozolomide as third-line treatment and in those with brain metastases [12]. Median time to progression (TTP) and overall survival (OS) for all treated patients were 1.6 months [95% CI: 0.9 to 3.0 months] and 5.8 months [95% CI: 4.2 to 7.0 months], respectively. For the sensitive and refractory patients, median TTP were 1.6 months [95% CI: 0.9 to 3.5 months] and 1 month [95% CI: 0.8 to 3.4 months], respectively, and OS were 6.0 months [95% CI: 4.2 to 7.2 months] and 5.6 months [95% CI: 2.5 to 7.7 months], respectively [12]. Activity in refractory and relapsed disease is of particular interest, as topotecan is minimally active in this context with reported response rates of only 2 – 6%. Importantly, as part of this study, tumor samples were obtained for *MGMT* promoter methylation analysis [12]. In patients for whom *MGMT* methylation status could be determined (N = 27), the overall promoter methylation rate was 48%. Patients with tumors demonstrating *MGMT* promoter methylation had a better response to treatment compared to those with unmethylated *MGMT*, although the comparison did not reach statistical significance [12]. A larger number of cases will need to be studied to further validate the association between MGMT promoter methylation and efficacy of temozolomide. In SCLC clinical trials, such testing for potential predictive markers of benefit from treatment is imperative.

ABT-888 (Veliparib)

To potentiate the activity of temozolomide and possibly to overcome resistance, the addition of a second agent aimed at DNA repair may be necessary. High Poly (ADP-Ribose) Polymerase (PARP)-1 expression has been demonstrated in SCLC cell lines by proteomic profiling and in archival SCLC tumors by immunohistochemistry [15]. Increased PARP

activity is one of the mechanisms by which tumor cells avoid apoptosis caused by DNA damaging agents such as temozolomide. PARP-1 initiates the base excision repair (BER) pathway upon recognition of single-stranded DNA breaks [16-18]. Inhibition of PARP-1 sensitizes tumor cells to cytotoxic agents by blocking initial recognition of DNA damage. Persistence of multiple single strand lesions in the genome, which would normally be repaired through the BER system, ultimately leads to accumulation of double-strand breaks resulting in apoptosis or mitotic catastrophe during DNA replication [19, 20]. Thus, the addition of ABT-888, an orally bioavailable small molecule inhibitor of PARP, to temozolomide may lead to improved response rates and outcomes in patients with relapsed SCLC, and merits clinical testing. A multi-center phase II study comparing temozolomide and ABT-888 versus temozolomide and placebo in patients with relapsed sensitive and refractory SCLC is due to open in the early part of 2012.

Histone Deacetylation

Vorinostat

In addition to hypermethylation of DNA, histone regulation is an important epigenetic modification that has emerged as a mechanism leading to tumorigenesis [21]. Histones are a family of basic proteins that serve as structural and regulatory components of chromatin, which serves to pack linear DNA efficiently within the cell. The fundamental subunit of chromatin is the nucleosome [22]. Transcriptional activity occurs only when nucleosomes are decondensed as euchromatin, in a process that requires methylation, acetylation, and phosphorylation of histones [22]. Clinically important in cancer treatment is histone acetylation, which is controlled by a family of acetyltransferases and deacetylases (HDACs). Acetylation of histones leads to increased accessibility of promoter regions and increased transcription of genes [23]. This process is opposed by HDACs, which remove lysine residues from histone tails and nonhistone proteins, thereby preventing chromatin relaxation and gene transcription [23]. HDAC inhibition exhibits antitumor effects by upregulating apoptosis pathways, blocking tumor angiogenesis, inhibiting intracellular stress response pathways, increasing generation of intracellular reactive oxygen species, and impairing handling of misfolded proteins [24]. Given that HDAC inhibitors enhance pro-apoptotic pathways, they have been tested with multiple conventional chemotherapeutic agents, including platinum, taxanes, gemcitabine, fluorouracil and epirubicin [24].

A well studied HDAC inhibitor is vorinostat, which is approved for the treatment of refractory cutaneous T-Cell lymphoma. Synergistic activity of vorinostat and topotecan has been observed in chemo-sensitive and chemo-refractory SCLC cell lines [25]. A phase I/II study evaluating vorinostat and topotecan in patients with chemo-sensitive recurrent SCLC was closed early due to slow accrual (clinicaltrials.gov identifier NCT00697476). An ongoing phase I/II clinical trial investigates the combination of vorinostat with carboplatin and etoposide in the first-line treatment of patients with extensive stage SCLC (clinicaltrials.gov identifier NCT00702962).

Nonreceptor Oncogenes

Bcl-2 Genes

Bcl-2 is one of a family of proteins that regulate cell death and mechanisms such as apoptosis, necrosis and autophagy [26-28]. The Bcl-2 family is comprised of anti-apoptotic proteins (bcl-2, bcl-x, and mcl-x) and pro-apoptotic proteins (bax, bak and bad), the balance between which controls cell death [29]. Bcl-2, in particular, promotes cell survival by inhibiting the pro-apoptotic actions of bax and bak. Overexpression of Bcl-2 is associated with both malignant transformation and chemotherapeutic resistance. Bcl-2 upregulation is present in 75-95% of SCLC, associated with gene amplification of the *BCL2* locus on

chromosome 18q21 [30-34]. Inhibition of bcl-2 shows anti-tumor activity against SCLC cell lines and in xenograft models [35-37].

Oblimersen, an antisense oligonucleotide targeting bcl-2, was one of the first bcl-2 inhibitors in clinical development. The Cancer and Leukemia Group B conducted a randomized phase II trial of carboplatin and etoposide with or without oblimersen in patients with extensive stage SCLC. The response rates were similar in both arms, 61% (95% CI, 45 – 76%) for the arm with oblimersen and 60% (95% CI, 32 – 84%) for the arm without oblimersen, $p = 1.0$. Oblimersen failed to show an improvement in survival when added to etoposide and carboplatin in patients, which was the primary endpoint of the trial [38]. In fact, one year survival for patients receiving oblimersen, carboplatin and etoposide was 24% (95% CI, 12 – 40%) compared to 47% (95% CI, 21 – 73%) for patients in the carboplatin and etoposide alone arm. The hazard ratio for overall survival was 2.0 ($p = .02$), suggesting worse outcome for patients receiving oblimersen. The toxicities were similar in both arms. The authors suggested that oblimersen was not sufficiently suppressing intracellular bcl-2 in SCLC patients, thus accounting for the poorer outcome in the arm that included the agent [38].

Navitoclax, a selective high-affinity small molecule inhibitor of bcl-2 and bcl-x_L [39, 40], binds to bcl-2 in a protein domain similar to that of naturally occurring Bcl-2 inhibitors, such as bax or bak. Although preclinical studies of single agent navitoclax, or the closely related parent molecule ABT-737, resulted in dramatic tumor responses in SCLC cell line xenograft tumors [36, 37, 40, 41], a recent phase II study of the agent demonstrated limited activity in patients with recurrent and progressive SCLC after at least one prior therapy. However, as multiple preclinical models support that ABT-737 and navitoclax, through lowering of the tumor apoptotic threshold, enhance the efficacy of standard cytotoxic agents against SCLC and other solid tumors [42-48], a phase I trial of navitoclax with first-line combination chemotherapy for SCLC has been completed, the results of which are not yet available (clinicaltrials.gov identifier NCT00878449).

A third agent, obatoclax mesylate, which is a small-molecule BH3-mimetic that exhibits binding affinity for a range of bcl-2 family members, including bcl-2, bcl-XL, and mcl-1, has been evaluated in various clinical scenarios. A phase II trial of obatoclax mesylate added to topotecan did not exceed the historic response rate seen in topotecan alone in patients with relapsed SCLC [49]. The efficacy of obatoclax mesylate in combination with carboplatin and etoposide was investigated in patients with chemotherapy-naïve extensive-stage SCLC [50]. Patients were randomized to obatoclax mesylate with carboplatin and etoposide followed by maintenance obatoclax or carboplatin and etoposide alone. Although obatoclax in combination with carboplatin and etoposide failed to meet the primary endpoint of improved response rate, the combination demonstrated a trend for improved ORR (65% for the obatoclax arm versus 54% for the control arm ($p=0.107$)), PFS (6 months versus 5.4 months ($p=0.084$)) and OS (10.6 months versus 9.9 months ($p=0.052$)). Obatoclax mesylate markedly decreased the rate of refractoriness to the initial 6 cycles of chemotherapy by 40%, consistent with its *in vitro* synergy [50]. Based on these results, a phase III testing of this agent is being planned.

Receptor Tyrosine Kinases and Growth Factors

Insulin-Like Growth Factor-1 Receptor

The insulin-like growth factor receptor (IGF-1R), a member of the insulin receptor subclass of receptor tyrosine kinase, is activated by the ligands IGF-1 and IGF-2 and triggers mitogenic and anti-apoptotic signaling pathways contributing to cellular transformation and malignant growth [51]. IGF-1R is autophosphorylated upon ligand binding and activates the

PI3K-AKT pathway, strongly implicated in the development and growth of cancer cells, as well as resistance to chemotherapy [51, 52]. Signaling through PI3K also can activate the Raf/MEK/Erk pathway to promote metastasis [52]. Current data suggest that IGF-1 and IGF-2 drive tumor cell proliferation and survival through multiple oncogenic pathways [53].

High plasma levels of IGF-1 have been associated with an increased risk for lung cancer [54]. IGF-1R and its ligand, IGF-1, are expressed at high levels in SCLC cell lines [51, 55, 56]. Resected primary SCLC demonstrate both over-expression of IGF-1R protein and *IGF-1R* gene amplification [57]. Taken together, these observations suggest a link between tumor IGF-1R signaling and SCLC proliferation, survival, and chemotherapeutic resistance. Therefore, targeted inhibition of IGF-1R represents an attractive approach to enhancing chemotherapeutic efficacy and inhibiting tumor recurrence in patients with SCLC. Two monoclonal antibodies (IMC-A12 and MK-0646) have been evaluated in patients with extensive stage disease in phase II studies and results are pending. The ECOG 1508 study has evaluated IMC-A12 together with cisplatin and etoposide in a randomized trial to determine if IGF-1R inhibition leads to improved progression-free survival (clinicaltrials.gov identifier NCT00887159). The efficacy and safety of MK-0646 has been studied in a single arm trial (clinicaltrials.gov identifier NCT00869752); analysis of blood samples for biomarker and pharmacogenetic correlative studies (for example, changes in expression of IGF biomarkers, haplotype tagging analysis of the IGF-1R) is in progress.

Developmental Pathways

Hedgehog Pathway

The Hedgehog (Hh) signaling pathway is a critical regulator of proliferation and differentiation during embryonic development. This pathway has been shown to be essential in early lung formation and development through epithelial-mesenchymal interactions [58, 59]. There are three known ligands of this pathway in humans: Sonic Hedgehog (SHh), Indian Hedgehog (IHh), and Desert Hedgehog (DHh). The signaling cascade is initiated by Hh binding to the Patched-1 receptor (Ptch-1), a twelve transmembrane protein. In the absence of Hh ligand, Ptch-1 constitutively inhibits the seven-transmembrane protein Smoothened (Smo), and renders the pathway inactive. However, binding of Hh ligand to Ptch-1 relieves the inhibition of Smo, which then activates a protein complex and downstream transcription of Hh targets in the nucleus, including Gli-1 and Ptch-1, leading to upregulation of target genes important for cellular proliferation.

The Hedgehog pathway is an oncogenic driver of tumors by two predominant mechanisms. The first is by direct mutations affecting key regulators of the pathway, as is commonly seen in basal cell carcinoma and medulloblastoma, which are remarkably sensitive to pharmacologic inhibition [60-64]. The second mechanism is by ligand-dependent activation of the Hedgehog pathway, which can be either autocrine or paracrine, and has been reported in multiple tumor types, including SCLC, NSCLC, prostate, colorectal, and pancreas [59, 65-69]. Importantly, recent *in vivo* data show that Hedgehog signaling in SCLC preclinical models occurs in a cell-autonomous manner, independent of the lung microenvironment [65].

Hedgehog signaling may play a significant role in the development and proliferation of SCLC. In normal lung development, Hh signaling is essential in early stromal development and branching morphogenesis of the embryonic airways [70]. Some lines of evidence suggest that SCLC is a relatively undifferentiated airway epithelial tumor that may recapitulate aspects of early lung development [71]. As in early lung formation, Hedgehog signaling has been implicated in SCLC initiation [65]. Both SHh and Gli-1 are expressed, and upregulated relative to normal airway epithelium, in SCLC [59]. Constitutive activation

of the pathway promotes tumor progression, and deletion of Smo suppresses initiation and progression in murine models of SCLC [59, 65]. The growth of both mouse and human SCLC can be inhibited by pharmacologic blockade of Smo [59, 65]. Following chemotherapy, Hedgehog pathway inhibition may delay or prevent recurrence of residual disease [65, 72].

Additionally, Hedgehog signaling has a role in regulating stem cell maintenance and differentiation, which may be related to its function in tumorigenesis in some models. Abnormal activation of embryonic developmental programs, including Hedgehog, has been implicated in lung tumorigenesis [58, 73]. Inhibition of Hh signaling in both *in vitro* and *in vivo* models has been associated with a loss of tumorigenic potential and improved survival [74-78]. In SCLC cell lines, Hh inhibition was reported to decrease cell growth primarily via a progenitor population [79]. These data support a model in which clonogenic recurrence of SCLC is dependent on a subset of progenitor cells that are relatively chemotherapy-resistant and which may depend on the Hh developmental pathway.

These several lines of evidence suggest that the addition of a Hh inhibitor to conventional chemotherapy may promote a more sustained treatment response and ultimately improve clinical outcomes in SCLC. As such, the ECOG 1508 phase II randomized trial in patients with extensive stage disease includes an arm evaluating whether the addition to cisplatin and etoposide of GDC-0449 (Genentech), an inhibitor of Smoothened, improves progression free survival (clinicaltrials.gov identifier NCT00887159). The trial has completed accrual and final results are pending. Similar clinical trials in extensive stage SCLC are being planned using either IPI-926 (Infinity Pharmaceuticals, Inc.) or LDE225 (Novartis Oncology), both of which are competitive inhibitors of Smoothened.

Immunotherapy

Antigen-Specific Vaccines: Gangliosides

Gangliosides are a subgroup of glycolipids that are components of the cell membrane. Gangliosides are found on all eukaryotic cells, and in particularly high concentration in cells of the central nervous system [80]. They are thought to contribute to numerous biologic functions, including cell-cell adhesion, cell signaling and motility [80]. Increased expression of gangliosides has been demonstrated in SCLC as well as other malignancies [80]. As such, gangliosides are thought to be potential targets for cancer immunotherapy. In a small pilot study, 15 patients with SCLC were treated with the anti-idiotype BEC2 vaccine, for which the ganglioside antigen GD3 is the target [81]. As prolonged survival was observed in these patients, a phase III trial evaluating the BEC2 vaccine in limited stage SCLC was led by the European Organization for Research and Treatment of Cancer [82]. While the overall results showed no improvement in progression-free or overall survival for the vaccine, a trend toward improved survival ($p=0.085$) was noted in the 33% of patients who mounted an immune response to GD3 [82].

A variety of other glycolipid antigens are expressed on SCLC cells and represent potential targets for immunotherapy. Fucosyl GM-1 is a ganglioside expressed in 75% of SCLC specimens, but only rarely in normal tissue or NSCLC and other tumors [83]. N-propionylated polysialic acid, a component of embryonic neural cell adhesion molecule, is a polymer of more than twenty negatively-charged alpha 2-8 linked sialic acid residues, which is involved in cell motility and development. It is expressed abundantly in SCLC and not in normal tissues [84, 85]. Several other gangliosides, such as GD2, GD3 and Globo-H, are expressed on multiple tumors. Each of these gangliosides has been conjugated to keyhole limpet hemocyanin (KLH), an immunogenic carrier protein and administered with an immunologic adjuvant, such as QS-21 (Antigenics, Inc), OPT-821 (Optimer

Pharmaceuticals), and Bacillus Calmette-Guerin (BCG). These individual vaccines have been evaluated in patients with SCLC, as well as other malignancies (i.e. those expressing GD2, GD3 and Globo-H), and have been shown to be safe and immunogenic [81, 86] [87-92].

A pentavalent vaccine against SCLC targeting GD2, GD3, fucosyl GM1, Globo H and N-propionylated polysialic acid has been developed, as these are among the most widely expressed target antigens on SCLC, and preclinical studies suggested that a pool of antibodies against these five antigens is superior to any single antibody moiety [93, 94]. There is an ongoing pilot trial evaluating the safety and immunogenicity of this pentavalent vaccine, conjugated to KLH and administered with OPT-821 adjuvant (Optimer Pharmaceuticals), for SCLC patients who have completed planned first-line therapy and have maintained a partial or complete response (clinicaltrials.gov identifier NCT01349647). The primary endpoints are toxicity and immunogenicity, as determined by serologic assays.

Antigen-Independent Immunotherapy: Ipilimumab

Ipilimumab is a humanized IgG1 monoclonal antibody against cytotoxic T-lymphocyte antigen-4 (CTLA-4). By blocking the inhibitory signal provided by CTLA-4, this class of antibodies can prolong the activation and proliferation of tumor-directed cytotoxic T cells, thus promoting an anti-tumor immune response [95]. The US Food and Drug Administration recently approved ipilimumab for the treatment of patients with metastatic melanoma, as it showed a survival benefit in a large phase III trial [96].

Ipilimumab is undergoing evaluation in lung cancer as well. A randomized, double-blind, three arm phase II trial in patients with untreated stage IIIB/IV NSCLC or extensive stage SCLC was performed to evaluate the efficacy and safety of paclitaxel and carboplatin with or without ipilimumab given on two dosing schedules [97]. Among the 204 patients with NSCLC, ipilimumab in combination with paclitaxel and carboplatin met its primary endpoint by demonstrating superiority in progression free survival compared with paclitaxel/carboplatin alone. Ipilimumab did not potentiate the toxicities of the chemotherapy, but was associated with moderate immune-related adverse events. A phased dosing schedule, in which ipilimumab was started in cycle three, appeared to demonstrate better efficacy than a concurrent regimen.[97] Among the 130 patients with SCLC, the phased dosing schedule of ipilimumab given with paclitaxel and carboplatin appeared to improve immune-related PFS (median 6.4 months for the phased ipilimumab arm versus 5.3 months for the control arm ($p = 0.03$)), immune-related best overall response rate (71% (95% CI, 55 – 84) versus 53% (95% CI, 38 – 68)) and OS (median 12.9 months versus 9.9 months ($p = 0.13$)), compared to paclitaxel and carboplatin, while the concurrent regimen did not lead to improved outcomes. Similar to the NSCLC cohort, immune-related adverse events were observed in the ipilimumab arms [98]. Given these favorable results, a randomized, multicenter, double blind phase III trial comparing the efficacy of platinum/etoposide with or without ipilimumab in patients with newly diagnosed extensive stage disease SCLC, with overall survival as the primary endpoint, is currently being launched (clinicaltrials.gov identifier NCT01450761).

Anti-Cancer Viruses

Seneca Valley Virus

Oncolytic replication-selective viruses represent a novel class of anti-cancer agents. In cancer cells permissive for viral replication, the initial viral inoculum can be amplified through selective replication, causing elimination of the infected cells by virus-mediated cytolysis. As viral progeny are released, neighboring cancer cells may be infected, resulting in an amplifying series of infection, replication, and oncolysis [99]. Seneca Valley Virus

(SVV-001) is a replication-competent picornavirus with potent and selective tropism for neuroendocrine cancer cell types, including SCLC [100]. In SCLC xenografts, SVV-001 doses 10^8 vp/kg led to complete and durable eradication of tumors in all mice [100].

A first-in-human, first-in-class phase I clinical trial of SVV-001 was conducted in 30 patients with cancers with neuroendocrine features, including SCLC.[101] SVV-001 was well tolerated without dose limiting toxicities at each of the five dose cohorts studied, ranging from 10^7 to 10^{11} vp/kg. All patients, including those that had been extensively pretreated, were able to mount an effective humoral immune response, clearing detectable infectious SVV-001 within one month of virus administration [101]. Among patients with SCLC, *in vivo* intratumoral viral replication was observed, with blood levels of circulating free virus peaking several days after administration several fold higher than the dose of SVV-001 given. Higher neutralizing antibody titers were observed in those with SCLC compared to other patients treated at the same dose. In one patient with SCLC, persistent SVV-001 production was specifically evident in malignant tumor. One patient with previously progressive chemorefractory SCLC remained progression-free for 10 months after SVV-001 administration, and lived for more than three years after treatment [101].

Based on these initial findings, a phase II study of SVV-001 has been initiated in the North Central Treatment Group (clinicaltrials.gov identifier NCT01017601). This randomized study evaluates post-chemotherapy administration of a single dose of SVV-001 intravenously versus placebo in 90 patients with extensive stage SCLC.

Moving Forward in SCLC

Recently, there have been improvements in the understanding of the malignant transformation, clonogenic potential, tumor growth, and metastatic spread in lung cancer. High throughput technologies such as genomics, epigenomics, transcriptomics (i.e. gene expression and miRNA expression arrays) and proteomics have been instrumental in this [102]. However, these global approaches have been most extensively applied in the context of NSCLC. Limited analyses to date of SCLC have revealed distinct abnormalities that could serve as drug targets [103-106]. The first full sequencing of a SCLC cell line (NCI-H209) genome recently was completed specifically to determine the mutational burden associated with cigarette smoke [107]. A total of 22,910 somatic substitutions, 65 insertions and deletions, 334 copy number segments and 58 structural variants were identified [107]. There are ongoing studies to sequence the genome of SCLC tumors, and to conduct additional “omic” analyses, which are likely to provide further insights into the biology of the disease.

A primary xenograft model, which depends on the immediate transfer of human SCLC from chemo-naïve patients to recipient mice without intervening tissue culture or cell line derivation *ex vivo*, has been developed [108]. Based on gene expression profiling, these primary xenograft models appear to retain features of the primary tumor that are lacking in xenograft models developed from cell lines [108]. Such primary tumor models may represent an attractive platform for preclinical therapeutic testing in SCLC.

Further work in SCLC to develop molecularly targeted agents must depend on strong pre-clinical models, ongoing use of high throughput methodologies, and extensive evaluation of predictive markers of benefit in clinical trials. Combining these approaches, we may begin to change prognosis for patients with this disease.

Conclusion

Distinct pathways contribute to the pathogenesis of SCLC, leading to its unique biology and clinical features. Emerging definition of these basic molecular and cellular changes is fostering the development of novel therapeutic strategies, some of which are outlined here. Ultimately, continued rational target-based approaches should lead to improved survival of patients with SCLC.

References

1. Siegel R, et al. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin.* 2011; 61:212–236. [PubMed: 21685461]
2. Merrill RM, et al. Conditional survival among patients with carcinoma of the lung. *Chest.* 1999; 116:697–703. [PubMed: 10492274]
3. von Pawel J, et al. Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. *J Clin Oncol.* 1999; 17:658–667. [PubMed: 10080612]
4. O'Brien ME, et al. Phase III trial comparing supportive care alone with supportive care with oral topotecan in patients with relapsed small-cell lung cancer. *J Clin Oncol.* 2006; 24:5441–5447. [PubMed: 17135646]
5. Eckardt JR, et al. Phase III study of oral compared with intravenous topotecan as second-line therapy in small-cell lung cancer. *J Clin Oncol.* 2007; 25:2086–2092. [PubMed: 17513814]
6. Esteller M, Herman JG. Generating mutations but providing chemosensitivity: the role of O6-methylguanine DNA methyltransferase in human cancer. *Oncogene.* 2004; 23:1–8. [PubMed: 14712205]
7. Toyooka S, et al. DNA methylation profiles of lung tumors. *Mol Cancer Ther.* 2001; 1:61–67. [PubMed: 12467239]
8. Gerson SL. Clinical relevance of MGMT in the treatment of cancer. *J Clin Oncol.* 2002; 20:2388–2399. [PubMed: 11981013]
9. Hegi ME, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005; 352:997–1003. [PubMed: 15758010]
10. Gilbert MR, et al. RTOG 0525: A randomized phase III trial comparing standard adjuvant temozolomide with a dose-dense schedule in newly diagnosed glioblastoma. *J. Clin. Oncol.* 2011; 29(Suppl) abstract 2006.
11. Green RA, et al. Alkylating agents in bronchogenic carcinoma. *The American journal of medicine.* 1969; 46:516–525. [PubMed: 5791000]
12. Pietanza MC, et al. Phase II Study of Temozolomide for Relapsed Sensitive or Refractory Small Cell Lung Cancer. *Journal of Thoracic Oncology.* 2010; 5:S507.
13. Therasse P, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000; 92:205–216. [PubMed: 10655437]
14. von Pawel J, et al. Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. *J Clin Oncol.* 1999; 17:658–667. [PubMed: 10080612]
15. Byers LA, et al. Identification of signaling pathways active in small cell lung cancer compared to non-small cell lung cancer by proteomic profiling. *AACR Annual Meeting.* 2010 Abstract 5556.
16. Schreiber V, et al. Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. *J. Biol. Chem.* 2002; 277:23028–23036. [PubMed: 11948190]
17. Memisoglu A, Samson L. Base excision repair in yeast and mammals. *Mutat. Res.* 2000; 451:39–51. [PubMed: 10915864]
18. Tentori L, Graziani G. Chemopotential by PARP inhibitors in cancer therapy. *Pharmacol. Res.* 2005; 52:25–33. [PubMed: 15911331]

19. Liu X, et al. Potentiation of temozolomide cytotoxicity by poly(ADP)ribose polymerase inhibitor ABT-888 requires a conversion of single-stranded DNA damages to double-stranded DNA breaks. *Mol Cancer Res*. 2008; 6:1621–1629. [PubMed: 18922977]
20. Plummer ER. Inhibition of poly(ADP-ribose) polymerase in cancer. *Curr Opin Pharmacol*. 2006; 6:364–368. [PubMed: 16753340]
21. Jones PA, Baylin SB. The epigenomics of cancer. *Cell*. 2007; 128:683–692. [PubMed: 17320506]
22. Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293:1074–1080. [PubMed: 11498575]
23. Gregory PD, et al. Histone acetylation and chromatin remodeling. *Experimental cell research*. 2001; 265:195–202. [PubMed: 11302684]
24. Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. *J Clin Oncol*. 2009; 27:5459–5468. [PubMed: 19826124]
25. Bruzzese F, et al. Synergistic antitumor effect between vorinostat and topotecan in small cell lung cancer cells is mediated by generation of reactive oxygen species and DNA damage-induced apoptosis. *Mol Cancer Ther*. 2009; 8:3075–3087. [PubMed: 19887547]
26. Cory S, et al. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene*. 2003; 22:8590–8607. [PubMed: 14634621]
27. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008; 132:27–42. [PubMed: 18191218]
28. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. *Blood*. 2008; 111:3322–3330. [PubMed: 18362212]
29. Haura EB, et al. Antiapoptotic signaling pathways in non-small-cell lung cancer: biology and therapeutic strategies. *Clin Lung Cancer*. 2004; 6:113–122. [PubMed: 15476597]
30. Kaiser U, et al. Expression of bcl-2--protein in small cell lung cancer. *Lung Cancer*. 1996; 15:31–40. [PubMed: 8865121]
31. Ikegaki N, et al. Expression of bcl-2 in small cell lung carcinoma cells. *Cancer Res*. 1994; 54:6–8. [PubMed: 8261463]
32. Ikegaki N, et al. Relationship between bcl-2 and myc gene expression in human neuroblastoma. *Cancer Lett*. 1995; 91:161–168. [PubMed: 7767905]
33. Jiang SX, et al. Expression of bcl-2 oncogene protein is prevalent in small cell lung carcinomas. *The Journal of pathology*. 1995; 177:135–138. [PubMed: 7490679]
34. Stefanaki K, et al. Immunohistochemical detection of bcl2, p53, mdm2 and p21/waf1 proteins in small-cell lung carcinomas. *Anticancer Res*. 1998; 18:1167–1173. [PubMed: 9615783]
35. Hann CL, et al. Therapeutic efficacy of ABT-737, a selective inhibitor of BCL-2, in small cell lung cancer. *Cancer Res*. 2008; 68:2321–2328. [PubMed: 18381439]
36. Oltersdorf T, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*. 2005; 435:677–681. [PubMed: 15902208]
37. Tahir SK, et al. Influence of Bcl-2 family members on the cellular response of small-cell lung cancer cell lines to ABT-737. *Cancer Res*. 2007; 67:1176–1183. [PubMed: 17283153]
38. Rudin CM, et al. Randomized phase II Study of carboplatin and etoposide with or without the bcl-2 antisense oligonucleotide oblimersen for extensive-stage small-cell lung cancer: CALGB 30103. *J Clin Oncol*. 2008; 26:870–876. [PubMed: 18281659]
39. Park CM, et al. Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J Med Chem*. 2008; 51:6902–6915. [PubMed: 18841882]
40. Tse C, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res*. 2008; 68:3421–3428. [PubMed: 18451170]
41. Shoemaker AR, et al. Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. *Clin Cancer Res*. 2008; 14:3268–3277. [PubMed: 18519752]
42. Shoemaker AR, et al. A small-molecule inhibitor of Bcl-XL potentiates the activity of cytotoxic drugs in vitro and in vivo. *Cancer Res*. 2006; 66:8731–8739. [PubMed: 16951189]
43. Li R, et al. ABT-737 synergizes with chemotherapy to kill head and neck squamous cell carcinoma cells via a Noxa-mediated pathway. *Molecular pharmacology*. 2009; 75:1231–1239. [PubMed: 19246337]

44. Kim KW, et al. Combined Bcl-2/mammalian target of rapamycin inhibition leads to enhanced radiosensitization via induction of apoptosis and autophagy in non-small cell lung tumor xenograft model. *Clin Cancer Res.* 2009; 15:6096–6105. [PubMed: 19773376]
45. Hikita H, et al. The Bcl-xL inhibitor, ABT-737, efficiently induces apoptosis and suppresses growth of hepatoma cells in combination with sorafenib. *Hepatology.* 2010; 52:1310–1321. [PubMed: 20799354]
46. Jain HV, Meyer-Hermann M. The molecular basis of synergism between carboplatin and ABT-737 therapy targeting ovarian carcinomas. *Cancer Res.* 2011; 71:705–715. [PubMed: 21169413]
47. Zall H, et al. Chemotherapeutic drugs sensitize human renal cell carcinoma cells to ABT-737 by a mechanism involving the Noxa-dependent inactivation of Mcl-1 or A1. *Molecular cancer.* 2010; 9:164. [PubMed: 20576107]
48. Tan N, et al. Navitoclax enhances the efficacy of taxanes in non-small cell lung cancer models. *Clin Cancer Res.* 2011; 17:1394–1404. [PubMed: 21220478]
49. Paik PK, et al. A phase II study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in relapsed small cell lung cancer. *Lung Cancer.* 2011; 74:481–485. [PubMed: 21620511]
50. Langer CJ, et al. A randomized phase II study of carboplatin and etoposide with or without pan-BCL-2 antagonist obatoclax in extensive-stage small cell lung cancer. *Journal of Clinical Oncology.* 2011; 29(suppl) abstr 7001.
51. Fischer B, et al. Targeting receptor tyrosine kinase signalling in small cell lung cancer (SCLC): what have we learned so far? *Cancer Treat Rev.* 2007; 33:391–406. [PubMed: 17368733]
52. Samani AA, et al. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocr. Rev.* 2007; 28:20–47. [PubMed: 16931767]
53. Bruchim I, et al. Targeting the IGF1 axis in cancer proliferation. *Expert Opin Ther Targets.* 2009; 13:1179–1192. [PubMed: 19663648]
54. Yu H, et al. Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis. *J. Natl. Cancer Inst.* 1999; 91:151–156. [PubMed: 9923856]
55. Sattler M, Salgia R. Molecular and cellular biology of small cell lung cancer. *Semin Oncol.* 2003; 30:57–71. [PubMed: 12635090]
56. Izzycki T, et al. Serum levels of IGF-I and IGFBP-3 in patients with lung cancer during chemotherapy. *Oncol Res.* 2006; 16:49–54. [PubMed: 16783968]
57. Badzio A, et al. Increased insulin-like growth factor 1 receptor protein expression and gene copy number in small cell lung cancer. *J Thorac Oncol.* 2010; 5:1905–1911. [PubMed: 21124078]
58. Peacock CD, Watkins DN. Cancer stem cells and the ontogeny of lung cancer. *J Clin Oncol.* 2008; 26:2883–2889. [PubMed: 18539968]
59. Watkins DN, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature.* 2003; 422:313–317. [PubMed: 12629553]
60. Daya-Grosjean L, Couve-Privat S. Sonic hedgehog signaling in basal cell carcinomas. *Cancer Lett.* 2005; 225:181–192. [PubMed: 15978322]
61. Xie J, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature.* 1998; 391:90–92. [PubMed: 9422511]
62. Lee Y, et al. A molecular fingerprint for medulloblastoma. *Cancer Res.* 2003; 63:5428–5437. [PubMed: 14500378]
63. Von Hoff DD, et al. Inhibition of the Hedgehog Pathway in Advanced Basal-Cell Carcinoma New England. *Journal of Medicine.* 2009; 361:1–9.
64. Rudin CM, et al. Treatment of Medulloblastoma with Hedgehog Pathway Inhibitor GDC-0449. *New England Journal of Medicine.* 2009; 361:1–6. [PubMed: 19528190]
65. Park KS, et al. A crucial requirement for Hedgehog signaling in small cell lung cancer. *Nat. Med.* 2011
66. Yuan Z, et al. Frequent requirement of hedgehog signaling in non-small cell lung carcinoma. *Oncogene.* 2007; 26:1046–1055. [PubMed: 16909105]
67. Sanchez P, et al. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. *Proc Natl Acad Sci U S A.* 2004; 101:12561–12566. [PubMed: 15314219]

68. Varnat F, et al. Human colon cancer epithelial cells harbour active HEDGEHOG-GLI signalling that is essential for tumour growth, recurrence, metastasis and stem cell survival and expansion. *EMBO Mol Med.* 2009; 1:338–351. [PubMed: 20049737]
69. Thayer SP, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature.* 2003; 425:851–856. [PubMed: 14520413]
70. Pepicelli CV, et al. Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr Biol.* 1998; 8:1083–1086. [PubMed: 9768363]
71. Liu H, et al. Predicting survival within the lung cancer histopathological hierarchy using a multi-scale genomic model of development. *PLoS Med.* 2006; 3:e232. [PubMed: 16800721]
72. Travaglione, V., et al. A novel HH pathway inhibitor, IPI-926, delays recurrence post-chemotherapy in a primary human SCLC xenograft model. 99th AACR Annual Meeting; 2008. p. 4611
73. Watkins, DN.; Peacock, CD. American Society of Clinical Oncology Educational Book. ASCO; 2007. Small Cell Lung Cancer: Carcinoma or Primitive Neural Tumor; p. 428-431.2007
74. Watkins DN, et al. Hedgehog signaling: progenitor phenotype in small-cell lung cancer. *Cell Cycle.* 2003; 2:196–198. [PubMed: 12734424]
75. Merchant AA, Matsui W. Targeting Hedgehog--a cancer stem cell pathway. *Clin Cancer Res.* 2010; 16:3130–3140. [PubMed: 20530699]
76. Dierks C, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell.* 2008; 14:238–249. [PubMed: 18772113]
77. Zhao C, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature.* 2009; 458:776–779. [PubMed: 19169242]
78. Liu S, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res.* 2006; 66:6063–6071. [PubMed: 16778178]
79. Tian F, et al. Effects of the Hedgehog pathway inhibitor GDC-0449 on lung cancer cell lines are mediated by side populations. *Clinical and experimental medicine.* 2011
80. Brezicka T, et al. Reactivity of monoclonal antibodies with ganglioside antigens in human small cell lung cancer tissues. *Lung Cancer.* 2000; 28:29–36. [PubMed: 10704706]
81. Grant SC, et al. Long survival of patients with small cell lung cancer after adjuvant treatment with the anti-idiotypic antibody BEC2 plus Bacillus Calmette-Guerin. *Clin Cancer Res.* 1999; 5:1319–1323. [PubMed: 10389914]
82. Giaccone G, et al. Phase III study of adjuvant vaccination with Bec2/bacille Calmette-Guerin in responding patients with limited-disease small-cell lung cancer (European Organisation for Research and Treatment of Cancer 08971-08971B; Silva Study). *J Clin Oncol.* 2005; 23:6854–6864. [PubMed: 16192577]
83. Brezicka FT, et al. Coexpression of ganglioside antigen Fuc-GM1, neural-cell adhesion molecule, carcinoembryonic antigen, and carbohydrate tumor-associated antigen CA 50 in lung cancer. *Tumour Biol.* 1992; 13:308–315. [PubMed: 1337798]
84. Komminoth P, et al. Polysialic acid of the neural cell adhesion molecule distinguishes small cell lung carcinoma from carcinoids. *Am J Pathol.* 1991; 139:297–304. [PubMed: 1651057]
85. Lantuejoul S, et al. Neural cell adhesion molecules (NCAM) and NCAM-PSA expression in neuroendocrine lung tumors. *Am J Surg Pathol.* 1998; 22:1267–1276. [PubMed: 9777989]
86. Dickler MN, et al. Immunogenicity of a fucosyl-GM1-keyhole limpet hemocyanin conjugate vaccine in patients with small cell lung cancer. *Clin Cancer Res.* 1999; 5:2773–2779. [PubMed: 10537341]
87. Krug LM, et al. Vaccination of patients with small-cell lung cancer with synthetic fucosyl GM-1 conjugated to keyhole limpet hemocyanin. *Clin Cancer Res.* 2004; 10:6094–6100. [PubMed: 15447995]
88. Krug LM, et al. Vaccination of small cell lung cancer patients with polysialic acid or N-propionylated polysialic acid conjugated to keyhole limpet hemocyanin. *Clin Cancer Res.* 2004; 10:916–923. [PubMed: 14871967]
89. Krug LM, et al. Immunization with N-propionyl polysialic acid-KLH conjugate in patients with small cell lung cancer is safe and induces IgM antibodies reactive with SCLC cells and bactericidal against group B meningococci. *Cancer Immunol Immunother.* 2011

90. Chapman PB, et al. Vaccination with a bivalent G(M2) and G(D2) ganglioside conjugate vaccine: a trial comparing doses of G(D2)-keyhole limpet hemocyanin. *Clin Cancer Res.* 2000; 6:4658–4662. [PubMed: 11156217]
91. Slovin SF, et al. Carbohydrate vaccines in cancer: immunogenicity of a fully synthetic globo H hexasaccharide conjugate in man. *Proc. Natl. Acad. Sci. USA.* 1999; 96:5710–5715. [PubMed: 10318949]
92. Gilewski T, et al. Immunization of metastatic breast cancer patients with a fully synthetic globo H conjugate: A phase I trial. *Proc. Natl. Acad. Sci. USA.* 2001; 98:3270–3275. [PubMed: 11248068]
93. Zhang S, et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. *Int J Cancer.* 1997; 73:42–49. [PubMed: 9334808]
94. Livingston PO, et al. Antigen expression on SCLC cell lines confirms selection of a quadrivalent vaccine against SCLC containing GM2, fucosyl GM1, Globo H, and polysialic acid. *Proc Amer Assoc Cancer Res.* 2003
95. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. *J Clin Oncol.* 2008; 26:5275–5283. [PubMed: 18838703]
96. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010; 363:711–723. [PubMed: 20525992]
97. Lynch TJ, et al. Overall survival and progression free survival results for a randomized phase II trial of ipilimumab and paclitaxel/carboplatin in first-line Stage IIIB/IV Non-small cell lung cancer. *Ann. Oncol.* 2010;21.
98. Reck M, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in extensive disease-small cell lung cancer: Results from a phase 2 trial. *Journal of Thoracic Oncology.* 2011; 9:S310.
99. Yamamoto M, Curiel DT. Current issues and future directions of oncolytic adenoviruses. *Molecular therapy : the journal of the American Society of Gene Therapy.* 2010; 18:243–250. [PubMed: 19935777]
100. Reddy PS, et al. Seneca Valley virus, a systemically deliverable oncolytic picornavirus, and the treatment of neuroendocrine cancers. *J. Natl. Cancer Inst.* 2007; 99:1623–1633. [PubMed: 17971529]
101. Rudin CM, et al. Phase I clinical study of Seneca Valley Virus (SVV-001), a replication-competent picornavirus, in advanced solid tumors with neuroendocrine features. *Clin Cancer Res.* 2011; 17:888–895. [PubMed: 21304001]
102. Ocak S, et al. High-throughput molecular analysis in lung cancer: insights into biology and potential clinical applications. *Eur. Respir. J.* 2009; 34:489–506. [PubMed: 19648524]
103. Olejniczak ET, et al. Integrative genomic analysis of small-cell lung carcinoma reveals correlates of sensitivity to bcl-2 antagonists and uncovers novel chromosomal gains. *Mol Cancer Res.* 2007; 5:331–339. [PubMed: 17426248]
104. Voortman J, et al. Array comparative genomic hybridization-based characterization of genetic alterations in pulmonary neuroendocrine tumors. *Proc Natl Acad Sci U S A.* 2010; 107:13040–13045. [PubMed: 20615970]
105. Bangur CS, et al. Identification of genes over-expressed in small cell lung carcinoma using suppression subtractive hybridization and cDNA microarray expression analysis. *Oncogene.* 2002; 21:3814–3825. [PubMed: 12032850]
106. Cho NH, et al. Comparative proteomics of pulmonary tumors with neuroendocrine differentiation. *Journal of proteome research.* 2006; 5:643–650. [PubMed: 16512680]
107. Pleasance ED, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature.* 2010; 463:184–190. [PubMed: 20016488]
108. Daniel VC, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. *Cancer Res.* 2009; 69:3364–3373. [PubMed: 19351829]