Small Cell Lung Cancer: Can Recent Advances in Biology and Molecular Biology Be Translated into Improved Outcomes?

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Introduction

Small cell lung cancer (SCLC) is one of the four major histologic types of lung cancer. The incidence of SCLC in developed countries has declined in recent years, presumably due to changes in cigarette composition. In the United States (US) SCLC is estimated to represent about 16% of new lung cancer diagnoses, which equates to about 35,000 new cases annually. In underdeveloped countries the percentage of SCLC cases may be higher. SCLC presents with a very large number of genetic alterations, including tumor suppressor genes, copy number gains and other somatic mutation in transcription factors, enzymes involved in chromatin modification, receptor tyrosine kinases and their downstream signaling components [1]. SCLC has a high propensity for early spread and a high initial responsiveness to cytotoxic chemotherapy usually followed by rapid development of resistance. Thus, essentially all patients of any stage receive a doublet combination of etoposide with cisplatin or carboplatin. For the rare patient without nodal involvement, the chemotherapy may follow surgery and for the patient with nodal disease without distant metastases, a combination of chemotherapy with chest radiotherapy is usually given concurrently. Unfortunately, these therapies are short in duration and not curative in most instances with 5-year survival rates below 7%. No major treatment advances have occurred over the past 30 years[2]. Since the approval of topotecan in 1996, the US Food and Drug Administration (FDA) has not approved any new drugs for the treatment of SCLC patients [3]. For these reasons SCLC was declared a "recalcitrant" cancer in the US. However, considerable therapeutic opportunities, including targeted therapies, exist because of recent developments in understanding the biology and molecular biology of SCLC in part due to the new model systems.

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Etiology and cell of origin of SCLC and related neuroendocrine tumors

A. SCLC

SCLC is almost always smoking-induced, and exhibits a high frequency of aberrations involving both oncogenes and tumor suppressors [4, 5].

It is important to understand the specific cell type(s) present in the lung that are capable of transformation to SCLC, with special emphasis on their proliferation, differentiation, and migration control programs, so that the malignancy can be viewed within a framework of normal biological processes [6]. Unlike the cells of origin for two other major lung cancer subtypes, adenocarcinoma (alveolar type 2 cells [7]) and squamous cell carcinoma (presumed to be tracheo-bronchiolar basal cells [8]), a rare sensory cell type termed pulmonary neuroendocrine cells are the predominant cell of origin for SCLC [9]. Dr. Krasnow's laboratory has demonstrated that the transformation processes for each of these cell types are similar in that characteristic oncogenes such as *EGFR, KRAS*, etc. (for lung adenocarcinoma [10]) and tumor suppressors such as *RB1, TP53*, etc. (for SCLC [4]) promote self-renewal in the corresponding normal cell types of origin [11] (MAK unpublished findings). These findings help explain why mutated versions of those genes are such powerful transformation inducers and why the mature cancer cells often phenotypically resemble their normal counterparts. Moreover, by assessing copy number neutral loss-of-heterozygosity mutations in human SCLC biopsies, Dr. Peifer's laboratory has discovered that *TP53* mutations likely occur earlier in SCLC tumorigenesis than mutations to *RB1* (MP unpublished findings). This suggests that the fidelity of RB1's programmed role(s) in pulmonary neuroendocrine cells is somewhat sensitive to the status of TP53.

An important distinguishing feature between SCLC and adenocarcinoma, on the other hand, appears to be the acquisition of additional mutations once transformation is achieved. Based on computational assessment of subclonal architecture in biopsied human SCLC versus adenocarcinoma samples by high-throughput sequencing, it appears that SCLC typically has significantly reduced genetic heterogeneity compared to adenocarcinoma [4]. The basic biological and clinical implications of this finding are so far unclear.

While pulmonary neuroendocrine cells are agreed to be the predominant SCLC cell of origin[9] (at least in mice), a question remains whether they are the only possible cell of origin, especially in light of additional oncogenes and tumor suppressors discovered recurrently mutated in human SCLC patients [4]. In order to take an unbiased approach as to which genes might potentially induce SCLC transformation in different lung epithelial cell types, Dr. Berns' laboratory has combined the canonical SCLC-relevant genetically engineered mouse model (GEMM), wherein both *Rb1* and *Trp53* are deleted from any cell type of interest [12], with transposon-mediated insertional mutagenesis to generate large numbers of epithelial clones with distinct mutations in individual animals [13]. This system allows the experimenter to assess whether, in a background of compound *Rb1/Tp53* deletions, randon additional mutations can transform previously refractory cell types in the lung to SCLC. These experiments verified the unrivaled vulnerability of pulmonary neuroendocrine cells to SCLC transformation, but also pointed to an, albeit less efficient, vulnerability of alveolar type 2 cells (AB unpublished findings). The relevant gene(s) that,

when mutated in combination with *Rb1* and *Trp53* can induce alveolar type 2 cells to adopt a SCLC phenotype, remain unclear, but will be important targets for both basic biological and clinical investigations. Finally, because many orders of magnitude more alveolar type 2 cells are present in adult mouse and human lungs than pulmonary neuroendocrine cells [14, 15], these findings suggest that alveolar type 2 cells may in fact be a relevant target cell type for SCLC transformation in human patients.

In addition, the Berns laboratory has generated GEMMs designed to constitutively activate expression of candidate oncogenes, notably *Mycl* and *Nfib*, together with *Rb1/Trp53* compound deletion in the mouse lung epithelium. Activated together, Mycl and Nfib accelerated SCLC development, including increasing both the growth rate of primary tumors and the appearance of metastases in the liver, bone, and kidney (AB unpublished findings). Interestingly, when studied in isolation, the two phenotypes were genetically separable: Mycl activation explained the primary tumor growth phenotype, and Nfib the metastatic progression. These results provide a rational framework to clinically intervene in two critical steps of SCLC tumorigenesis.

The growing understanding of the biologic and molecular characteristics of the SCLC cell of origin has therapeutic implications. Recently, the potential SCLC stem cell niche has emerged as a potential therapeutic target. Treatment strategies under investigation that may result in preferential targeting of SCLC stem cells include inhibition of developmental pathways such as WNT and Hedgehog, the transcription factors ASCL1 and NEUROD1, and the focal adhesion kinase (FAK) and PI3K/mTOR signaling pathways.

Aside from proliferation and transformation, the differentiation control program of pulmonary neuroendocrine and malignant SCLC cells, most notably the remarkable plasticity evident in response to commonly administered cancer therapies [16-18], requires increased understanding. By analyzing global gene expression patterns in a large panel of SCLC cell lines, as well as primary human SCLC samples, the laboratories of Drs. Massion and Quaranta have demonstrated that the samples can be subdivided into two general classes: those exhibiting a 'neuroendocrine' expression pattern versus those exhibiting a 'mesenchymal-like' pattern (PPD and VQ unpublished findings). Key genes underpinning the two states include transcription factors and cell adhesion proteins. Interestingly, both classes can coexist in a primary SCLC tumor biopsied from the same individual patient, and chemotherapy (e.g., topoisomerase inhibitor) can modulate representation of the two classes within the overall tumor. Previous studies have implicated the Ras/MAPK [19] and Notch [4] signaling pathways in controlling differentiation of pulmonary neuroendocrine and/or SCLC cells to alternative fates, and comprehensive integration of these results with those previous should shed new light on cellular heterogeneity in SCLC.

In addition to heterogeneity within and between individual SCLCs, phenotypic conversion to SCLC is a newly identified mechanism of drug resistance observed in lung adenocarcinomas progressing on EGFR tyrosine kinase inhibitor (TKI) treatment, occurring in roughly 15% of patients [16, 18]. In most cases these adenocarcinoma-to-SCLC transformed tumors retain the original EGFR mutation, arguing against them being newly generated primary tumors posttherapy, but can continue to proliferate malignantly despite downregulation of EGFR

protein. Moreover, in a recent study, 10/10 patients with adenocarcinoma-to-SCLC transformed tumors exhibited homozygous deletion of the *RB1* gene, suggesting that *RB1* deletion is required for phenotypic conversion [16]. However, *RB1* was also found deleted in 1/9 adenocarcinoma cases where TKI resistance was achieved independently of SCLC conversion [16], suggesting that *RB1* deletion alone is insufficient explain the phenotypic switch.

Finally, the migration control program of normal pulmonary neuroendocrine cells and malignant SCLC cells is extremely relevant to both understanding and treating SCLC metastasis. Dr. Krasnow's laboratory recently demonstrated a novel form of epithelial cell migration exhibited by normal pulmonary neuroendocrine cells during lung epithelial development, termed 'slithering,' which is used to organize neuroendocrine cells into stereotyped clusters [20]. The normal slithering program involves transient activation of an epithelial-to-mesenchymal transition (EMT) wherein recently specified pulmonary neuroendocrine cells migrate over and around other epithelial cells to find one another, without ever invading basally into the lung mesenchyme. Further investigation of the slithering program should hopefully reveal molecular dependencies that can be targeted in SCLC to attenuate or perhaps even prevent metastasis to extrapulmonary organs, which is the major cause of patient death [17].

B. Pulmonary Carcinoids

Pulmonary carcinoids are low and intermediate grade neuroendocrine malignancies characterized by the expression of neuroendocrine differentiation markers and a low proliferation index. Pulmonary carcinoids (like carcinoids arising in other sites) are divided in typical (TC) and atypical carcinoids (AC) on the basis of histopathological objective criteria which are <2 mitosis/2mm^2 and lack of necrosis for TC and 2-10/2mm^2 mitoses and/or focal necrosis for the AC[21]. While the etiology is still not completely understood, carcinoids appear to arise from a different stem/progenitor cell than the high grade neuroendocrine lung tumors; SCLC and large cell neuroendocrine carcinoma (LCNEC). The first clue to them being different is a possible preneoplastic lesion "Diffuse Idioplastic Neuroendocrine Cell Hyperplasia" (DIPNECH) unique for pulmonary carcinoids and their occurrence in the setting of MEN1 disease (5% of carcinoids arise in MEN1 disease and less than 5% on the background of DIPNECH). However, 20 to 40% of carcinoids display somatic double allelic inactivation of *MEN1* with mutation of one allele and allele loss (LOH) at the MEN1 gene (11q.13) location[22]. A more definitive clue was provided by the results of integrated genome analysis on 61 carcinoids (29 genomes, 5 exosomes, 69 RNA-Seq)[23], which revealed a distinct mutational profile with 52% of the mutations affecting chromatin remodelling genes (*MEN1, PSIP1, ARID1A*), 34% belonging to the methylation complex and 25% to the SWI/SNF complex, in a mutually exclusive fashion. Overall, 73% of candidate drivers are found in pulmonary carcinoids. In contrast to high grade NE tumors, pulmonary carcinoids have a low rate of mutation (0.4 Muts/Mb), no significant focal copy number alteration, and *TP53* or *RB1* mutations/loss are very rare and never found together. Gene expressions profiling revealed wide differences. The pulmonary carcinoids appear to be etiologically independent of the high grade neuroendocrine tumors (SCLC / LCNEC)

with no transitions or combinations occurring in patients. The molecular pathology and biology of carcinoids from other sites may be different.

C. LCNEC

LCNEC is a highly aggressive malignancy whose prognosis approaches that of SCLC[24, 25]. Based on similarity in expression profiling studies, a close biologic relationship between LCNEC and SCLC has been suggested [26]. In the new WHO classification, LCNEC is categorized into the same category with SCLC under the "neuroendocrine carcinoma", as LCNEC has many genetic features shared with SCLC despite some exceptions. Whether LCNEC should be therapeutically treated as SCLC or NSCLC remains controversial[27, 28]. Currently, there are only limited comprehensive molecular data on genomic alterations in LCNEC[29]. Such studies can be anticipated to yield insight into the biologic relationship between LCNEC and SCLC, which may inform clinical management of patients with these tumors. An analysis of a series of LCNEC utilizing custom targeted next-generation sequencing of 300 key cancer genes using the MSKIMPACT™ test, suggested that LCNEC comprises distinct molecular subsets: SCLC-like (characterized by *RB1 + TP53* co-mutation and absent/rare NSCLC-type mutations) and NSCLC-like (characterized by the presence of NSCLC-type mutations, including *KRAS, STK11, KEAP1, MAP2K1*). The clinical relevance of these findings is under investigation.

Genome, epigenome, and proteome studies

SCLC sequencing efforts [30, 31] on 110 whole genomes found evidence for a nearly universal and bi-allelic loss of *TP53* and *RB1*[4] (Figure 1). Rare tumors lacking *RB1* mutations showed alternative mechanisms of RB1 activation by overexpression of Cyclin D1 due to chromothripsis events affecting chromosome 3 and 11. Alterations (point mutations, small deletions) of the *PTEN* gene, located at 10q23.3, are observed in 10-18% of SCLC tumors [32] and deregulation of MYC function has also been noted to be important in SCLC[33]. In addition, the analysis of somatic rearrangements showed that translocation within *TP73* lead to the generation of an oncogenic version of the gene, *TP73 ex2 or TP73 ex2-3*[4]. About 25% of human SCLC tumors showed inactivating mutations of *NOTCH* family genes suggesting that these genes are tumor suppressors in SCLC. This notion was further supported by the observation that activation of Notch signaling leads to significantly fewer tumors and a prolonged survival in SCLC transgenic mouse models [4].

In a large transcriptome analysis of 19 fresh frozen tumors and 23 cell lines, 60 fusion events were detected but none of them involved any targetable kinases. Only two genes were involved in recurrent fusion events: *RLF* and *PTV1* - in accordance with the previously described low abundance of recurrent fusion events in SCLC [31]. Combining these results with copy number analyses of these tumors showed that *RLF-* and *PTV1*-fusions most likely evolved as a byproduct of *MYCL1* and *MYC* amplifications, respectively[34].

The clinical characteristics and multigene mutation profiling of SCLC in never-smokers [35, 36] has been evaluated. The never-smokers with SCLC (50/391) had a better prognosis than smokers with SCLC in the Korean cohort. Although they found that the *EGFR* mutation rate was high in never-smokers, it is still unclear how effective EGFR TKIs

are for *EGFR*-mutated SCLC. Other mutations found were *TP53, RB1, PTEN, MET* and *SMAD4*. Therefore, further global investigation of SCLC to determine differences in genetic or clinical characteristics between never-smokers and smokers is warranted.

Epigenetic Changes

A global CpG-site methylation analysis on 47 SCLC tumors (34 fresh frozen specimens, 6 patient derived xenografts (PDX), 7 cell lines) found that PDX samples better represent the methylome of primary tumors than cell lines. Furthermore, by using methylome and transcriptome analysis, distinct subtypes of SCLC can be defined that are indistinguishable by standard histological approaches [37]. EZH2 showed increased overexpression in comparison to normal lung tissue and this increase was correlated with a higher methylation of the *EZH2* promoter [38]. Recent developments in drug discovery [39, 40] and pre-clinical data suggest that EZH2 is amenable to targeted therapy.

Proteomic Changes

Proteomic analyses of SCLC cell lines and tumors led to the discovery that PARP1 is overexpressed and that PARP inhibition has activity in pre-clinical models and in a subset of SCLC patients [41, 42], with proteomic markers of DNA repair and PI3K pathway activation predictive for response of PARP inhibition in SCLC [43]. Beyond PARP, proteomic analysis revealed other potential targets, such as EZH2[41] and Chk1[44].

Tumor evolution and targeting in preclinical models

Progress has been made in 1) the development and use of sophisticated SCLC GEMMs and PDXs, 2) the identification and propagation of circulating tumor cells (CTCs) from SCLC patients and 3) renewing interest in available SCLC cell lines [45].

Mouse models of cancer are initiated by oncogene and tumor suppressor mutations engineered into the mouse genome. These initiating mutations cause cancer in the mouse or, at minimum, predispose to the development of tumors[46] (Figure 2). Additional acquired somatic genetic alterations have been described in GEMMs, suggesting that additional mutations might contribute to tumor formation or progression[47, 48].

Comprehensive exome sequencing of primary and metastatic SCLC tumors from the *Trp53; Rb1*-mutant GEMM [12, 49] showed GEMM SCLC tumors harbor very few point mutations, most likely because the mice develop SCLC without exposure to cigarette smoke. The most frequently observed alterations in mouse SCLC were *Mycl* (encoding the L-myc oncogene) amplification, inactivating point mutations targeting the tumor suppressor Pten and DNA copy number loss of Chr19, which encodes the mouse *Pten* gene. Consistent with *Pten* loss acting as an important driver in SCLC, deletion of *Pten* in the *Trp53, Rb1* model accelerated tumor progression[50, 51]. Genomic analysis of the triple mutant *Trp53/Rb1/Pten* tumors revealed persistence of the *Mycl1* DNA amplification. This implies that MYCL1 is a key driver in SCLC. Analysis of the clonal evolution of tumors in the SCLC GEMM identified spread of multiple primary tumor subclones to regional thoracic lymph nodes. Considering the fecundity with which SCLC establishes multiple genetically-distinct metastatic

subclones in regional lymph nodes, it is intriguing to wonder if these nodes might serve as a reservoir of disease after treatment, as in other cancer models [52].

Dr. MacPherson's lab performed genomic characterization of human SCLC cell lines and tumor tissues to identify recurrent mutated potential driver genes. They showed the histone methylase *MLL2/KMT2D* as a putative tumor suppressor with frequent truncating mutations. Use of a conditional *Mll2* knockout mouse suggested that *Mll2* loss can cooperate with loss of *Trp53* to promote cellular proliferation in mouse embryo fibroblasts, promote lung cancer and expand the tumor spectrum in the *Trp53/Rb1* mouse model. The accelerated *Trp53/Rb1/Pten* triple mutant SCLC model[49, 50] was used to investigate the role of Mycl as a driver of SCLC tumorigenesis. Conditional deletion of *Mycl1* potently suppressed SCLC tumorigenesis, complementary to the Berns' lab finding that overexpression of Mycl promoted SCLC[53]. These results confirm the role of *MYCL1* as critical driver in SCLC, and implicate *MLL2* loss and more broadly epigenetic dysregulation, as important events in a subset of human SCLC.

Human SCLC often exhibits a dramatic response to platinum-based chemotherapy, only to subsequently recur and become resistant to subsequent treatments [45]. Preclinical models have largely failed to recapitulate this pattern of response to therapy. CTCs and CTC-derived xenografts (CDXs) may be a useful model for genetic characterization and preclinical studies assessing the development of resistance. Patients with SCLC exhibit a high burden of CTCs as previously described [54]. Dr. Dive's group demonstrated that CTCs from SCLC patients efficiently form tumors when implanted into immunocompromised mice. Importantly, the CDX tumors recapitulated the histological features of human SCLC, and mirrored the response to chemotherapy of the patient from which the cells were obtained. The genomic profile of expanded CDX tumors was also similar to the parental CTCs. It is enticing to envision using these models to uncover the genetic evolution of SCLC between initial therapy and the emergence of resistance [55]. Additional samples are needed to validate this approach, but coupling preclinical therapeutic studies using CDXs with analysis of genetic progression is a potentially powerful approach to the identification of the mediators of chemotherapy resistance and to targeting these pathways with rationally designed drugs

Developmental signaling pathways

The WNT[56] and Hedgehog (Hh)[57] signaling pathways are frequently disrupted in SCLC. SmoM2 is a mutant form of the smoothened receptor derived from human basal cell skin cancer that is constitutively active independent of the Hh ligand. Cells expressing this allele ($Smo^{M2/+}$) display constitutive activation of the Hh signaling pathway. The Sage lab compared $Rb1^{lox/lox}/Trp53^{lox/lox}/Smo^{M2/+}$ mice to $Rb1^{lox/lox}/Trp53^{lox/lox}/Smo^{lox/lox}$ mice [57] and observed that the wild type *Smo* ($Smo^{M2/+}$) had more, larger and earlier tumors. Inhibition of Smo through silencing or with LDE225 therapy, a smoothened inhibitor, inhibited colony growth and inhibition of chemo-resistant SCLC tumors was greater than inhibition of chemonaive SCLC tumors, indicating that Smo inhibitors may preferentially inhibit SCLC stem cells. The same group conducted studies indicating that the combination of E/P plus LDE225 produced superior *in vivo* growth inhibition compared to E/P alone or LDE225 alone. Thus, there is a definite role for Hedgehog signaling in SCLC. Based on

these and other data, a randomized phase II trial in first-line SCLC therapy comparing etoposide/cisplatin (E/P) to etoposide/cisplatin plus vismodegib with vismodegib maintenance was designed. Unfortunately, there were no differences in efficacy with median PFS of 4.4-4.6 months in the two arms [58].

The WNT pathway may be involved in SLC as well as NSCLC pathogenesis. This pathway deserves further investigation in SCLC as a number of druggable targets exist in this signal pathway [56].

ASCL1, a transcription factor, regulates tumor initiating capacity in SCLC and is required during development of neural and neuroendocrine lineages[9]. Interestingly, the expression of ASCL1 and NEUROD1, another transcription factor, are mutually exclusive in SCLC cell lines. ASCL1 activates NOTCH signaling by direct regulation of DLL1 expression. NOTCH signaling represses ASCL1 expression via the transcriptional repressor HES1. ASCL1 but not NEUROD1 is required for neuroendocrine tumor formation in the Tr*p53/Rb1/p130* mouse model. ASCL1 directly regulates genes in SCLC tumor growth including *MYC, NFIB, RET* and genes in the NOTCH pathway [59] (and unpublished data JE Johnson).

NEUROD1 appears to play an important in some SCLC cell lines [60, 61]. Studies have indicated that ERK 1/2 signaling is low in all SCLC cell lines and that blocking ERK activity has no effect on cell growth while activating ERK inhibits SCLC growth. NEUROD1 can inhibit ERK and stimulate metastases. SCLC cell lines such as NCI-H2171, NCI-H82, and NCI-H1962 that express NeuroD1 produce fast growing tumors in mouse xenografts. In cell lines with high NEUROD1 expression, knockdown of NEUROD1 inhibits formation of soft agar colonies and cell migration *in vitro* and metastases *in vivo*. NEUROD1 binds the *TrkB* promoter and knockdown of TrkB suppressed the growth and inhibition of TrkB by chemical means blocked SCLC proliferation *in vitro* and *in vivo*.

New SCLC targets and drugs

Apoptotic agents

A key therapeutic goal is the induction of tumor cell death to achieve regression. The anti-apoptotic protein Bcl-2 is overexpressed in many cancers, including 40-60% of SCLCs [62-64]. Treatment of SCLC PDX models with ABT-737, a Bcl-2/Bcl-xL inhibitor, resulted in reduced tumor growth, but the response was limited [65]. Characterization of ABT-737 resistant tumor lead to the identification of PI3K/mTOR inhibitors as possible agents that could augment ABT-737 responses. The mTOR inhibitor rapamycin dramatically enhanced ABT-737 activity in the PDX models, possibly by inducing the pro-apoptotic proteins BAX and BAK1.

The Bcl-2/Bcl-xL/Bcl-w inhibitor ABT-263 also promotes apoptosis in SCLC cell lines, and a highthroughput drug screen showed that high expression of the pro-apoptotic regulator BIM and low expression of the anti-apoptotic MCL1 gene correlated strongly with sensitivity[66, 67]. However, a phase II trials of ABT-263 monotherapy revealed minimal clinical activity[68]. In SCLC cell lines, cells with high MCL-1 expression exhibited relative resistance to ABT-263 but inhibition of mTOR activity using AZD8055 suppressed MCL-1

protein levels and sensitized cells to ABT-263. This combination also suppressed growth of autochthonous Tr*p53/Rb1* double mutant GEMM SCLC tumors [67]. Taken together, these studies[67, 69] suggest that dual inhibition of Bcl-2/Bcl-xl and mTOR might be a rational therapeutic approach in SCLC.

eIF4E inhibitors

eIF4A is sufficient and required for MYC driven T-ALL and lymphoma models [70]. Silvestrol is a natural compound that blocks eIF4A RNA helicase and effectively inhibits T-ALL cells and many, but not all, SCLC cell lines. Silvestrol sensitive transcripts include MYC, NOTCH, MyB and others[71].

Aurora kinase inhibitors

High expression of Aurora kinase A or B imparts a poor prognosis in lung cancers [72, 73]. This is in keeping with several trials showing that cell cycle gene signatures are also associated with prognosis. Aurora kinase tyrosine inhibitors may be directed at Aurora A, Aurora B or may inhibit all Aurora kinases at concentrations that can be achieved in humans. Aurora kinases inhibitors of all classes have been shown to inhibit the growth of several NSCLC cell lines *in vitro* and in preclinical mouse models. It is not clear whether any class of Aurora kinase inhibitor is preferred.

In SCLC cell lines, Sos et al showed that the specific Aurora A inhibitors MLN8237 and PHA680632 inhibited many, but not all, SCLC cell lines and that there was a significant association between *MYC* amplification and sensitivity to the TKI [74]. The specific Aurora Kinase B inhibitor AZD1152 was studied on a panel of SCLC cell lines and there was a significant relationship between *MYC* and *MYCL1* amplification and expression and drug sensitivity. There was also a significant relationship between a reported MYC gene signature and sensitivity[75].

AZD1152 was studied in patients with hematologic malignancies and while there was some evidence of activity, hematologic toxicity was considerable. MLN8237 (alisertib) has been studied specifically in patients with SCLC, breast and ovarian cancers. In the phase II study in SCLC there was an objective response rate of 21% that included an OR of 19% in those with a sensitive relapse and 27% in those with a resistant relapse [76]. The overall PFS was 2.6 months in the sensitive and 1.4 months in the resistant relapse. Millenium conducted trials of the combination of MLN8237 plus paclitaxel in patients with breast and ovarian cancers and found that the agents could be combined safely. Thus, they have instituted an ongoing randomized phase II trial comparing paclitaxel alone to the combination of paclitaxel plus MLN8237 in patients with SCLC who progress after initial etoposide/platinum therapy (NCT02038647).

Notch inhibitors

MEDI0639 is a human monoclonal IgG1 antibody directed against Delta-like ligand 4 (DLL4) in the NOTCH developmental signaling pathway and believed to block angiogenesis by promoting formation of non-functional vasculature and inhibiting tumor initiating (stem) cells (TICs) [77]. A phase Ib/II study of OMP-59R5, a fully human monoclonal IgG2 antibody

targeting the Notch 2/3 receptors, in combination with etoposide and platinum in untreated extensive-stage SCLC showed promise with 13/16 (81.3%) attaining a partial response and 3 achieving stable disease [78]. Demcizumab is another monoclonal antibody directed against DLL4 [79] with ongoing trials in NSCLC (NCT02259582, NCT01189968).

FGFR inhibitors

The fibroblast growth factor receptor (FGFR) family represents promising targets for the development of targeted therapies in SCLC. Several studies have reported that the *FGFR1* gene is amplified in 5-6% of SCLC patients [80]. A study with 83 SCLC patients explored the correlations between FGFR1 and its ligands and the results from Dr. Hirsch's group showed that a subset of SCLCs were potentially characterized by activated FGF/FGFR1 pathway, as is evidenced by positive FGFR1, FGF2, FGF9 protein and/or mRNA expression or gene copy number [81]. Combined analysis of FGFR1 and ligand expression may allow selection of SCLC patients for FGFR1 inhibitor therapy. Dr. Hirsch's group is studying the FGFR inhibitor ponatinib in a biomarker driven trial (NCT01935336). Another phase II trial to assess the efficacy and safety of lucitanib [82], an inhibitor of FGFR1-3, VEGFR1-3, and PDGFRα/β, in patients with advanced lung cancer is currently recruiting participants (NCT02109016).

PIC3CA

Whole exon sequencing (n = 51) and copy number analysis (n =47) on Japanese SCLC patients [83] detected genetic alterations in the PI3K/AKT/mTOR pathway in 36% of the tumors. Importantly, the SCLC cells harboring active PIK3CA mutations were potentially targetable with currently available PI3K inhibitors. Therefore, a sequencing-based comprehensive analysis could stratify SCLC patients for potential therapeutic targets.

A possible approach to targeting cancer stem cells is through dual inhibition of PI3 kinase and mTOR using VS5584. In models of SCLC, breast, and ovarian cancer, VS5584 demonstrates preferential targeting of the stem niche, as defined by aldefluor, tumorsphere, and limiting dilution assays. Both PI3K and mTOR inhibition appear requisite for these effects. In the SCLC model, VS5584 also demonstrated marked reduction in tumor initiating capacity in contrast to platinum/etoposide, which had essentially no activity [84]. VS5584 is currently in early phase clinical trials.

RET inhibitors

An activating M918T *RET* somatic mutation in a metastatic SCLC tumor specimen has been described [85]. SCLC cell lines, which have the stable overexpression of both mutant M918T and wild-type *RET*, became sensitized to the RET TKIs, vandetanib and ponatinib. These results indicate that a subpopulation of SCLC patients may derive benefit from TKIs targeting RET.

Transcription-targeting drugs

A high-throughput cellular screen of a diverse chemical library discovered that SCLC is sensitive to THZ1, a covalent inhibitor of CDK7. Moreover, expression of super-enhancer-associated transcription factor genes, including MYC family proto-oncogenes and

neuroendocrine lineage-specific factors, is highly vulnerability to THZ1 treatment [83, 86]. Hence, the downregulation of these transcription factors may contribute to SCLC sensitivity to transcriptional inhibitors and THZ1 may represent a prototype drug for tailored SCLC therapy.

Cytokines and growth factor binding to their cognate receptors leads to activation of the Janus Kinase- Signal Transducer and Activator of Transcription (JAK-STAT) pathway. STATs result in cell proliferation and activation of both tumor and inflammatory cells [87, 88]. STAT 3 is phosphorylated in NSCLC and pancreatic cancer and predicts for poor survival. There are a number of JAK inhibitors in development. Ruxolitinib (INCB1824), an inhibitor of JAK1/2, is FDA approved for myelofibrosis and tofacitinib [89], an inhibitor of JAK1/3, is approved in rheumatoid arthritis. Ruxolitinib has been evaluated *in vitro* in NSCLC cell lines and will inhibit STAT 3 activation [90]. Randomized trials of ruxolitinib in addition to cisplatin/pemetrexed, docetaxel and erlotinib are in progress in NSCLC. In SCLC, evaluation of on line databases as well as tumors and cell lines demonstrated that 30-40% have copy number gain for *JAK1/2* gene. *In vitro* and animal data indicates that targeting JAK2 with siRNA will inhibit the growth of SCLC. AZD1480 (which targets JAK1/2/3, FLT3 and Aurora kinase) has single agent activity as well as synergy with existing chemotherapy [91].

Novel cytotoxic chemotherapeutic agents

Phase II trials of amrubicin conducted in the US [92] and Asia [93] showed that amrubicin has activity in SCLC patients with had progressed after first line etoposide/platinum therapy. Activity was observed both in "sensitive" and "resistant" relapse. These studies were followed by a randomized phase III trial comparing amrubicin to topoteacan chemotherapy in patients who progressed on etop/platinum [94]. Unfortunately, amrubicin, while suggestive of some clinical benefit, was not superior to topoteacan in this trial and thus has not been approved for use in SCLC except in Asia.

Since palifosfamide plus doxorubicin showed supportive results in soft tissue sarcoma (NCT00718484), a phase I trial with palifosfamide plus carboplatin and etoposide is in process (NCT01555710). Another global phase II trial with aldoxorubicin [95] in patients with relapsed and refractory SCLC is in process (NCT02200757). The Southwest Oncology Group pooled data from trials in second- and/or third-line ES-SCLC. Univariate and multivariate Cox regression models were fit to assess the relationship between baseline characteristics and PFS and OS. Of 329 patients, 151 were platinum sensitive and 178 refractory. In this analysis platinum sensitivity status was not associated with OS, however prognostic groups with differential OS outcomes (high, intermediate and poor risk) were identified. Elevated lactate dehydrogenase (LDH), weight loss, performance status and male sex were all associated with worse OS. The authors concluded that, platinum sensitivity status was no longer independently associated with OS and that validation of this model in an independent SCLC dataset is warranted [96].

FAK inhibitors

Focal adhesion kinases (FAK) play a critical role in cancer initiation and proliferation as well as resistance to chemotherapy and radiation. Importantly, inhibition of FAK with the novel TKI VS6063 has demonstrated reduction in tumor initiating capacity (i.e. deplete cancer stem cells) in contrast to paclitaxel [97]. VS6063 is currently in early phase clinical trials for SCLC, however, negative results were recently announced in a maintenance setting trial conducted in malignant pleural mesothelioma [98].

CXCR4 inhibitors

Chemokine receptor 4 (CXCR4) is a G protein-coupled chemokine receptor that is functionally expressed or overexpressed in a number of cancers [99, 100]. CXCR4 plays a role in invasion, survival, angiogenesis and metastasis [101]. Elevated CXCR4 expression is associated with inferior outcome in NSCLC [102]. LY2510924 is a peptide antagonist of CXCR4 blocking the signal cascade of SDF-1, which has *in vitro* and *in vivo* activity in a number of cell lines and tumor models. A randomized phase 2 trial of this agent combined with carboplatin/etoposide vs. carboplatin/etoposide alone was conducted, however, it increased toxicity and failed to demonstrate any evidence of benefit in terms of either PFS or OS [103].

PARP inhibitors

Poly ADP ribose polymerase (PARP) is critical for DNA damage repair [104]. Given that all current treatment for SCLC relies on DNA damaging agents (e.g. chemotherapy and radiotherapy) and that resistance to these modalities is at least in part due to repair of DNA damage, inhibition of DNA damage repair is an extremely logical approach. Single agent activity of the PARP inhibitors olaparib, rucaparib, talazoparib and ABT-888 (veliparib) and synergy with platinum and etoposide was demonstrated in cell lines and animal models[41, 43, 105]. A randomized, Phase 2, double-blind Small cell lung cancer Trial of Olaparib as Maintenance Program (or "STOMP") which compares PARP inhibition to placebo following first-line chemotherapy (Cancer Research UK, trial number CRUK/10/037) has recently closed to recruitment and results are awaited [106]. A phase I trial combining veliparib with cisplatin and etoposide (ECOG-ACRIN E2518) [107] successfully completed and is now active as a randomized phase II trial. The newer PARP inhibitor talazoparib kills SCLC cells more efficiently than the older PARP inhibitor olaparib[43] and had single agent activity in SCLC in a recently completed Phase 1 trial [42]. High levels of PARP and other proteins involved in DNA damage repair, like FANCD2 and pCHK2, are strongly associated with the sensitivity of SCLC cells to talazoparib. High expression of a "DNA repair protein score" is also associated with greater response to talazoparib, while higher expression of a "PI3K score" is more resistant to the drug [108].

Antibody drug and radiotherapeutic conjugates

Antibody drug conjugates (ADCs) have been some of the most active agents developed in oncology, combining the specificity of antibodies with the cytotoxicity of traditional chemotherapeutic agents [109]. An ADC, lorvotuzamab mertansine, targeting the neural cell adhesion molecule CD56 found on over 90% of SCLC had excellent activity *in vitro*, in

preclinical models, as well as single agent activity [110]. However, further development was abandoned due to unacceptable toxicity when combined with carboplatin/etoposide [111].

SC16LD6.5 (rovalpituzumab tesirine) is an ADC that targets SCLC and LCNEC tumors by way of binding the atypical Notch ligand delta-like ligand 3 (DLL3) on the cell surface and then delivering the DNA damaging agent D6.5, pyrrolobenzodiazepine dimer toxin. Data from a tissue microarray shows that the majority of SCLCs express DLL3 by IHC analysis and, using an H score, high expression (H-score >120) was observed in 60%; moderate expression (H-score 60-120) in 22% and low expression (H-score <60) in 18%. Similar rates (61%, 14% and 25%) were found in patients on the phase 1 trial (NCT01901653). The ADC inhibits the growth of SCLC PDX models in relation to the level of expression of DLL3 and the growth inhibition was far superior to the combination of etoposide/cisplatin[112]. Overall response rate in the Phase 1 was 20%, with 70% receiving clinical benefit. For those with an H-score of 180 or above, the ORR was 39% and clinical benefit was 75% [113].

Targeting moieties (including antibodies and small peptides) can be conjugated to radioisotopes. Radioisotopes that have the potential for use as cancer therapeutics include alpha emitting agents, which have high energy but short path lengths, and beta emitters, with lower energy, but longer path lengths. The latter agents uniquely possess the possibility of overcoming tumor heterogeneity by "crossfire effect", i.e. the particle travels a distance of > 1 cell and therefore has the ability to damage cells that may not express the target. In addition, gamma emitting radioconjugates (which can be a separate agent or the same drug, depending upon the radioisotope) can be used for imaging and therefore allow for real time assessment of the presence of the target within a tumor site. Somatostatin receptors, specifically SSTR2, are frequently expressed in neuroendocrine cancers, including SCLC. A preliminary trial demonstrated the feasibility of targeting SSTR2 with a rhenium 188 labeled SSTR2 peptide fragment [114].

EZH2 inhibitors

Based on the sequencing the entire coding region and the intron–exon boundaries of MAX in lung cancer cell lines, EZH2 and BRG1 can be biomarkers to predict sensitivity to an EZH2 specific inhibitor, GSK126, plus etoposide [115, 116].

Antiangiogenic agents

Targeted therapy trials in SCLC that did not statistically reach their endpoints include antiangiogenic agents like thalidomide, bevacizumab and sorafenib [117-121]. A phase II study of cediranib (single agent) failed to demonstrate any objective response in recurrent or refractory SCLC [122]. Similarly vandetanib in maintenance setting [123] and aflibercept (VEGF-trap) in a phase II clinical trial testing aflibercept with or without topotecan did not show any significant clinical benefit [124]. Sunitinib as a single agent was tested in a single arm Phase II trial (EORTC-08061) in patients with chemo-naive extensive small cell lung cancer or who had a "chemosensitive" relapse. The trial was stopped early due to poor accrual [125]. However, sunitinib maintenance met its primary endpoint of prolonged PFS in a randomized Phase II CALGB trial[126].

Other pathway inhibitors

Clinical trials of signaling pathway inhibitors for c-kit and PDGFR (imatinib in all comers or in KIT positive patients), MET/HGF or IGF1R (AMG 102 or AMG 479, respectively, in combination with platinum-based chemotherapy) and AKT/mTOR (everolimus, temsirolimus etc.) have had disappointing results despite pre-clinical evidence of activity [127-133].

Wee1 inhibitors

AZD1775 is a small molecule wee-1 kinase inhibitor believed to abrogate the G2/M checkpoint, leading to mitotic catastrophe and cell death in the presence of chemotherapy induced DNA damage in *TP53* mutant SCLC tumors [134].

Radiation therapy

Prophylactic cranial irradiation (PCI) is known to reduce the risk of brain metastases and improves survival, however higher dose PCI was not found to be better. Neurocognitive changes mainly occur after high doses and in elderly patients, but are also observed without PCI. In a phase 3 randomized controlled trial 498 patients were assigned (1:1) to receive either thoracic radiotherapy (30 Gy in ten fractions) or no thoracic radiotherapy. All underwent PCI. Overall survival at 1 year was not significantly different between the groups, 33% (95% CI 27–39) for the thoracic radiotherapy group versus 28% (95% CI 22–34) for the control group (hazard ratio [HR] 0.84, 95% CI 0.69–1.01; p=0.066). However, in a secondary analysis, 2-year overall survival was 13% (95% CI 9–19) versus 3% (95% CI 2–8; p=0.004). Progression was less likely in the thoracic radiotherapy group than in the control group (HR 0.73, 95% CI 0.61–0.87; p=0.001). At 6 months, PFS was 24% (95% CI 19–30) versus 7% (95% CI 4–11; p=0.001). The authors concluded that thoracic radiotherapy in addition to PCI should be considered for all patients with ES-SCLC who respond to chemotherapy [135].

SCLC immunotherapy

The development of immunotherapies for SCLC is not a novel endeavor [136-140], but recent successes in this field for other cancer types suggest that these approaches may provide a degree of prolonged clinical benefit that has not been observed in SCLC patients with traditional treatments [141] (Figure 4).

SCLC patients with paraneoplastic syndromes develop T-cell responses [142, 143], appear to live longer that than SCLC patients without such syndromes[144] and long-term survivors of SCLC maintained a high ratio of effector T-cells to regulatory T-cells [145]. One would expect SCLC tumors to be sensitive to the activation of T-cell checkpoints due to the high mutation burden in these tumors [30, 31], even if immunosuppression mechanisms may limit the efficacy of T-cell clones [146, 147]. A significant increase in survival in patients with a high number of lymphocytes in their blood has been reported[148]. In addition, neural-specific antibodies can be used as diagnostic and prognostic biomarkers for SCLC patients in large cohorts [149]. Importantly, there is a strong correlation between auto-immune phenotypes or paraneoplastic

syndromes and exceptional longevity in SCLC patients, suggesting that activation of the immune system is beneficial to these patients in the long term.

Manipulation of T-cell immune checkpoints, particularly CTLA-4, PD-1, and PD-L1, [150] in SCLC to enhance the anti-cancer effects of T-cells has been or is being evaluated. In a phase II clinical trial in ES-SCLC patients ipilimumab, a fully human IgG1 cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) monoclonal antibody, in combination with standard chemotherapy, improved the immune-related progression-free survival [151] and a phase III trial is in progress (NCT01450761). In the phase I/II CheckMate-032 study, SCLC patients with progressive disease after >1 prior line of therapy received nivolumab (a fully human IgG4 programmed death 1 (PD-1) inhibitor antibody) + ipilimumab or nivolumab monotherapy. The combination or monotherapy showed activity and durable responses with tolerable toxicity. Overall response was reported in 7/40 pts (18%) with nivolumab alone and in 8/46 pts (17%) in the combination therapy [152]. In the phase IB KEYNOTE-028 study SCLC patients with PD-L1 positive tumor who failed or were ineligible for standard therapy were treated with pembrolizumab (humanized monoclonal IgG4 PD-1 inhibitor antibody); the overall response was reported to be 35% and safety profile consistent with other PDL-1 studies [153]. Another two studies are exploring the efficacy of combination of pembrolizumab with chemotherapy/radiotherapy for extensive SCLC (NCT02359019, NCT02402920). Additionally, blocking PD-L1 signaling by infiltrating macrophages may be sufficient to generate a positive response from T-cells and improve the survival of SCLC patients [154]. A number of clinical trials including or focusing on SCLC patients with PD-1, PD-L1 or CTLA-4 inhibitors are ongoing (e.g., NCT01693562, -02261220, -02046733, 02538666).

Gangliosides are cell surface oligoglycosylceramides that contain a sialic acid linked onto the sugar chain and are predominantly found in the nervous system and one such antigen, Fucosyl-GM1 (Fuc-GM1), is selectively expressed at high levels on the majority of SCLC tumors[155]. In the last 15 years, vaccines have been developed against Fuc-GM1[156, 157], and these have demonstrated safety and immunogenicity in pilot trials enrolling patients with SCLC who have completed first-line chemotherapy[158]. A phase I/II clinical trial using a high-affinity antibody against this antigen is ongoing (BMS-986012, NCT02247349).

Chimeric antigen receptor (CAR) T-cells [159, 160] have a possible use in SCLC and potential targets of CAR T-cells in SCLC can be identified from auto-antibodies found in patients. In particular, NCAM/CD56 is an attractive target with high expression on the surface of SCLC cells. Pre-clinical data indicate potent anti-tumor effects of CD56 CAR T-cells in SCLC. The expression of CD56 on other cells, including neural tissue and natural killer (NK) cells, might limit the use of this particular antigen, but this strategy may be developed with other SCLC-specific cell surface markers and in combination with other immunotherapies.

CD47 is a cell-surface molecule that acts as a "marker of self" and prevents cells of the innate immune system from attacking hematologic malignancies and certain types of solid tumors [161-163]. CD47 normally promotes immune evasion by signaling through SIRPα, an inhibitory receptor on macrophages and other myeloid cells [164]. CD47 levels are high on the surface of SCLC cells and pre-clinical data from human cell lines and xenografts suggest that blocking CD47 strongly promotes the phagocytosis of SCLC cells by macrophages and

inhibits tumor growth by T cellmediated processes[165]. It is possible that the anti-cancer effects of anti-CD47 strategies could be enhanced with the concomitant activation of T-cell checkpoints.

SCLC resources

The Recalcitrant Cancer Research Act of 2012 (H.R. 733) that was signed into a bill in early 2013 stipulates the National Cancer Institute (NCI) to "develop scientific frameworks" in SCLC research as this cancer fulfils the criteria of a recalcitrant disease.

The available resources to conduct broad scale SCLC research are suboptimal. New initiatives are needed that would include changes in standard of care and standardization of tissue collection protocols to gain access to specimens that reflect the dynamic biology of the disease. The National Cancer Institute has conducted a preliminary high throuput drug screen on a panel of 63 SCLC cell lines using 103 approved oncology drugs and 420 investigational agents. mRNA and miRNA gene expression profiles were determined for all cell lines and correlations between mRNA/miRNA expression patterns and drug sensitivity were robust and provide another approach to begin to understand the molecular determinants of therapy response and resistance in SCLC. Additionally this approach may potentially identify new drugs and drug combinations with improved efficacy in this recalcitrant disease.

Summary

Despite the paucity of therapeutic advances in SCLC, considerable progress in understanding the biology, molecular biology, model systems and potential therapeutic targets has been made. Studies of early lung and neuroendrocrine cell development models have provided insights into the cell of origin for SCLC. New GEMMs have illustrated the universal importance of *TP53* and *RB1* gene mutations in the pathogenesis and the potential role of additional genetic changes as well as changes in transcription factor expression. PDXs and CDXs provide new means for preclinical testing of new therapies. Molecular studies have identified the high mutation burden found in SCLC and have identified differences between SCLC, carcinoids and large cell neuroendocrine tumors. Potential therapeutic targets including EZH2, PARP, CDK1, MCL1, BCL2, BIM, SHH (Sonic Hedgehog), WNT, NOTCH1, Aurora Kinase, FGFR, PIK3CA, RET, THZ1, JAK-STAT, FAK, CXCR4, PD-L1, Fuc-GM1, CD56 and CD47. Ongoing and future clinical trials have to show which of these candidates can be translated into an effective targeted therapy. Thus, the future of improving outcomes for SCLC patients appears promising but there are still a number of unanswered questions which need to be addressed in the future and these are outlined below.

Small Cell Lung Cancer Major Questions of Translational Relevance

1. **What are the mechanisms underlying the universal development of chemoresistance?** SCLC is in nearly every case very sensitive to platinum-etoposide chemotherapy with dramatic clinically beneficial responses. However, in nearly every case the tumors become resistant to this chemotherapy. What is the

mechanism of this resistance, are there ways to avoid it, and what are additional therapies that could kill such resistant tumor cells?

2. **What are the mechanisms of highly metastatic behavior in SCLC?** SCLC is in nearly every case highly metastatic from the time of clinical diagnosis. What are the most important mechanisms responsible for the metastatic behavior and can these be therapeutically targeted? A subset of this question, is that SCLC appear to be much enriched in cancer stem cells ("tumor initiating cells", TICs) and does targeting stem cell signaling pathways provide effective therapy for primary and metastatic sites of SCLC?

3. **Are there therapeutic targets of "acquired vulnerability" associated with RB1 or TP53 mutations in SCLC?** Tumor suppressor genes RB1 and TP53 abnormalities are essentially universal in SCLCs. Are there acquired vulnerabilities associated with changes in these two genes that can be therapeutically targeted?

4. **Do the many other mutations occurring in human SCLC (but not in GEMMs) provide therapeutic targets – "acquired vulnerabilities for human SCLC?"** Current evidence indicates that human SCLCs have many other genetic (mutations) and epigenetic changes besides those involving the key oncogenic drivers (such as TP53, RB1, LMYC, NFIB). By contrast, mouse GEMM SCLC have very few additional changes. Presumable the differences in mutation rates result from exposure to cigarette carcinogens in humans but not in mice. Do the other mutations in human SCLC provide acquired vulnerabilities of therapeutic vulnerabilities, do any these represent "synthetic lethalities" with the main driver oncogene changes, and are there several vulnerabilities common across multiple SCLCs or are these vulnerabilities "private" and found in only individual tumors?

5. **Can we develop therapeutic strategies targeting important SCLC driver transcription factors?** Several transcription factors appear to be very important in the growth and survival of SCLC including ASCL1, NeuroD1, myc family members, and SOX2). Are there therapeutic strategies that can be directed against these key transcription factors and do they provide quantitatively multiple logs of tumor cell kill to allow development of curative strategies?

6. **What are the important differences in human preclinical models of SCLC that influence discovery and validation of new therapies?** Currently there are several types of human preclinical models of SCLC including SCLC lines, SCLC cell line xenografts (CDXs), patient derived SCLC xenografts (PDXs), and circulating SCLC tumor cells isolated from peripheral blood of SCLC patients. While these all share common genetic abnormalities found in SCLC (such as TP53, RB1, myc family members), we need to know what are the molecular differences between these different models and SCLC tumor samples and whether preclinical therapeutic responses are similar or different between these models. Can we continue to use all of the models for development of new therapies or do we need to only use one type of preclinical model?

7. **Are the differences between human SCLC and GEMM of SCLC that influence the discovery and validation of new therapies?** Genetically engineered mouse models of lung cancer (GEMMs) appear to be very similar to human SCLC. However, it appears that mouse GEMM SCLC do not appear to be sensitive to platin-etoposide chemotherapy. What are the differences between human and the GEMM models of SCLC that explain this discrepancy? Are there important differences between human and GEMM preclinical models that could influence the discovery and validation of new SCLC therapies?

8. **What are the biologic reasons for SCLCs expressing ASCL1 vs NeuroD1 as a lineage oncogene?** The majority of human SCLCs have ASCL1 as the major neuroendocrine lineage driver gene. However, a subset of human SCLCs express high levels of NeuroD1 and not ASCL1. Currently, there is no evidence that mouse lung neuroendocrine cells can express Neuro D1. This raises the question of whether small cell cancers predominantly expressing NeuroD1 arise in the human lung or in some other primary site.

9. **Do genetic abnormalities in histologically normal epithelium of SCLC patients provide diagnostic and chemopreventative targets?** There appear to be a much greater frequency of genetic abnormalities in the histologically normal epithelium of patients with SCLC compared to those with NSCLC. Does this information provide ways for early diagnosis or implementation of chemoprevention strategies for SCLC using these genetic alterations as molecular biomarkers?

Most recently the US National Cancer Institute released a Request for Application (RFA) (PAR-16-049, PAR-16-050, PAR-16-051) for grants specifically focusing on SCLC, and hopefully through these grants many of the above questions will be addressed.

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Appendix

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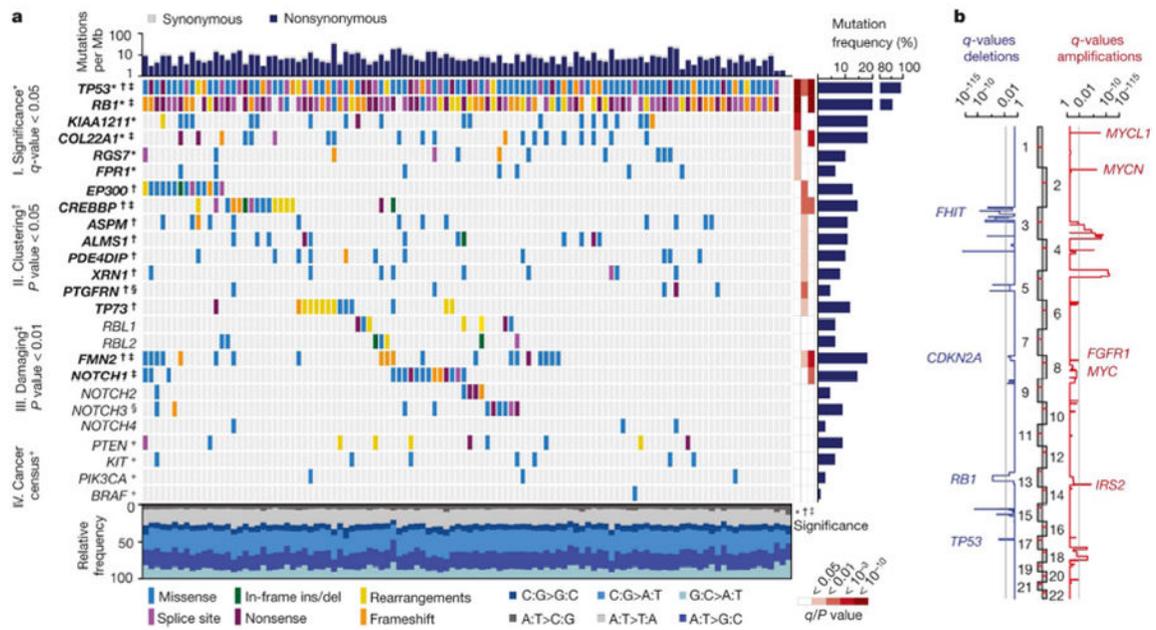
Figure 1. Genomic alterations in SCLC

A, Tumor samples are arranged from left to right. Alterations of SCLC candidate genes are annotated for each sample according to the colour panel below the image. The somatic mutation frequencies for each candidate gene are plotted on the right panel. Mutation rates and type of base-pair substitution are displayed in the top and bottom panel, respectively. Significant candidate genes are highlighted in bold (*corrected q-values,0.05, {P,0.05, {P, 0.01). The respective level of significance is displayed as a heatmap on the right panel. Genes that are also mutated in murine SCLC tumors are denoted with a 1 symbol. Mutated cancer census genes of therapeutic relevance are denoted with a 1 symbol. **B,** Somatic copy number alterations determined for 142 human SCLC tumors by single nucleotide polymorphism (SNP) arrays. Significant amplifications (red) and deletions (blue) were determined for the chromosomal regions and are plotted as q-values (significance,0.05). George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524:47-53
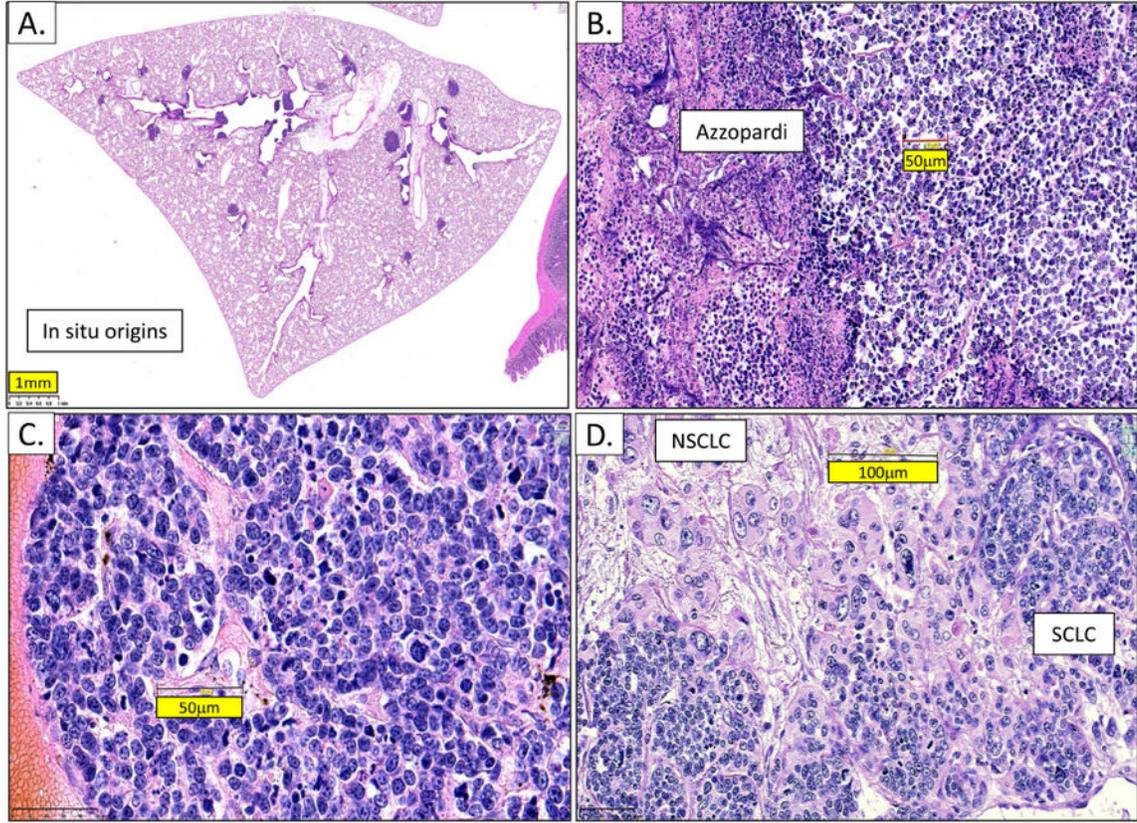
Figure 2. GEMMs for SCLC and its origins

A and **C**, Berns laboratory, (p53/Rb1 double CKO); **B** and **D**, Sage laboratory, (p53/Rb/p130 triple CKO). A, Whole lung section demonstrating multiple in situ lesions arising in large airways and a few small invasive carcinomas. B, SCLC with area of necrosis and Azzopardi effect adjacent to a focus of LCNEC. C, High power view of SCLC morphology. D, Combined SCLC carcinoma, with focal areas of poorly differentiated NSCLC. NSCLC, non–small-cell lung carcinoma; SCLC, small-cell lung carcinoma.

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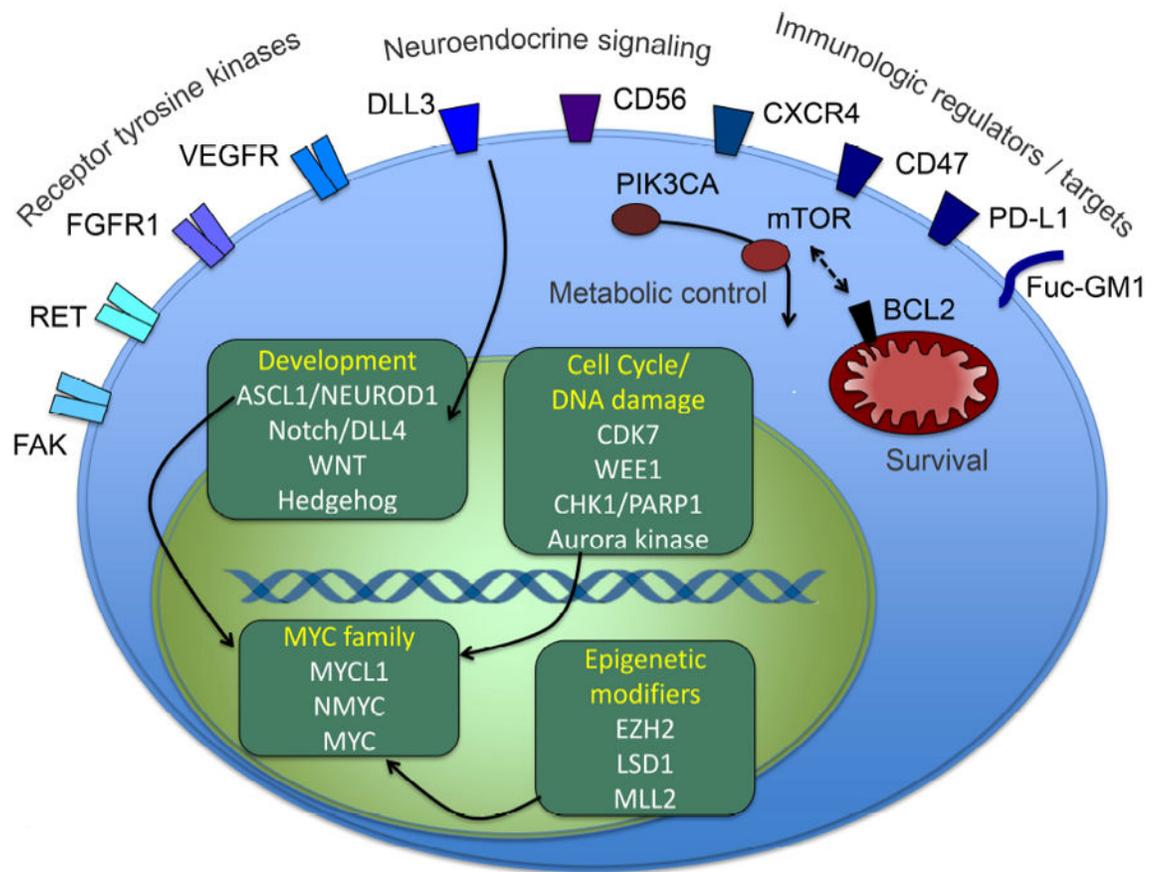
Figure 3. Some of the many areas of current therapeutic interest in small cell lung cancer
Cell surface targets include a number of receptor tyrosine kinases implicated in proliferative signaling, invasion, and angiogenesis; factors regulating neuroendocrine differentiation that are being explored as targets for antibody drug conjugates; immunologic regulators; and targets for tumor-specific vaccine strategies. Intracellular pathways of particular interest include metabolic and apoptotic regulators, cell cycle checkpoint controls, developmental signaling pathways, the MYC family of transcriptional regulators, and epigenetic modifiers of histones that affect chromosomal accessibility and gene expression.
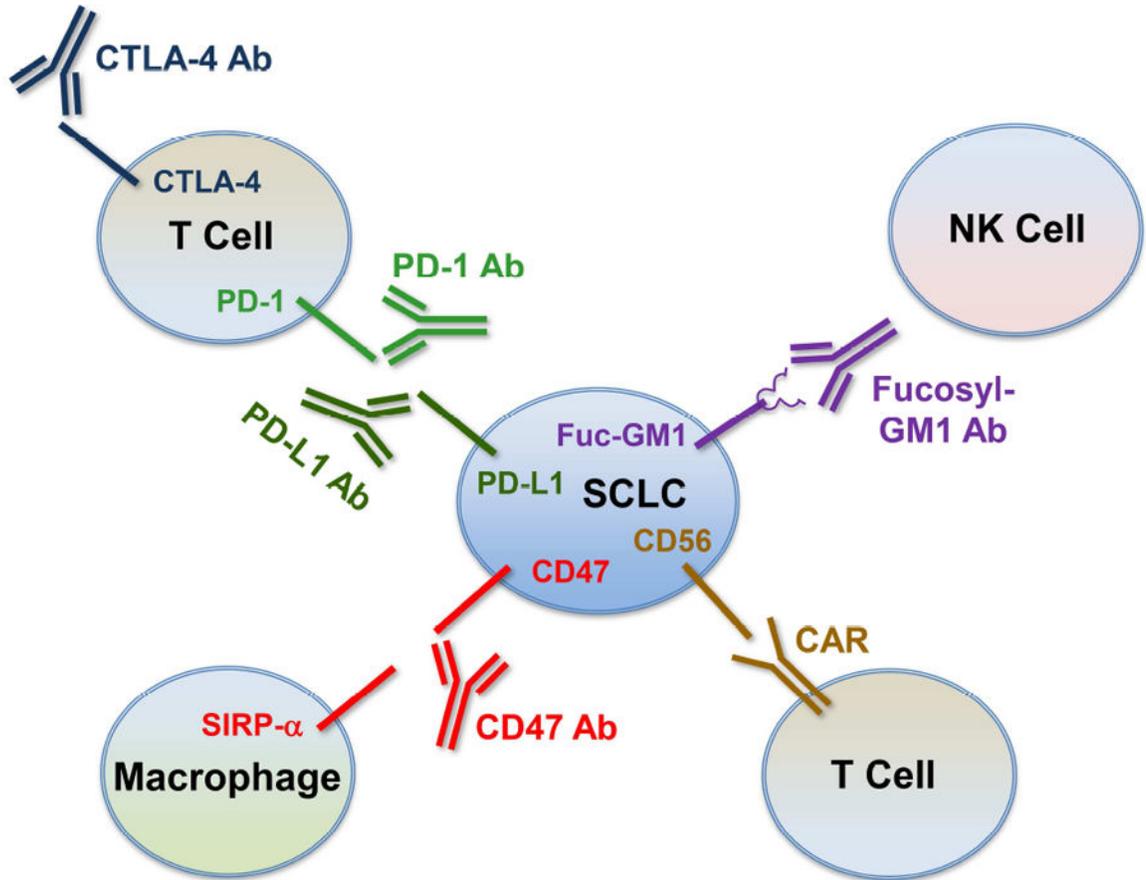
Figure 4. Immunotherapies against SCLC

Similar to other cancers, blockade of immune checkpoints is thought to be a promising strategy in SCLC. For instance, blockade of CTLA-4 or the interactions between PD-1 (at the surface of T cells) and PD-L1 (expressed on tumor cells or in the tumor microenvironment) with specific antibodies (Ab) may enhance the anti-cancer effects of T cells (T). Similarly, blockade of myeloid checkpoints such the CD47 receptor could enhance the activity of macrophages (M) against SCLC cells. Finally, a number of epitopes may be specific to neuroendocrine SCLC cells (e.g. CD56/NCAM or the ganglioside antigen Fuc-GM1) and could be targeted with chimeric antigen receptor (CAR) T cells or monoclonal antibodies (which could lead to the activation of antibody-dependent cell-mediated toxicity via NK cells). Note that these strategies may be used as single agents or in combination with each other or with chemotherapy.

Table 1

Agent	Target	Phase of trials	References
Erismodegib, Sonidegib (LDE225)	Smoothened (Hedgehog antagonist)	Preclinical, I	57
Vismodegib (GDC-0449)	Smoothened (Hedgehog antagonist)	Preclinical, I, II	58
ABT-737	Bcl-2, Bcl-xL	Preclinical	65, 69
Navitoclax (ABT-263)	Bcl-2, Bcl-xL, Bcl-w	Preclinical, I, II [*]	66, 67, 68
Silvestrol	EIF4E	Preclinical	70, 71
PHA-680632	Aurora Kinases	Preclinical	74
Alisertib (MLN8237)	Aurora A Kinase	Preclinical, I, II	74, 76
Barasertib (AZD1152)	Aurora B Kinase	Preclinical, I [*]	75
MEDI0639	DLL4	Preclinical, I	77
Tarextumab (OMP-59R5)	Notch 2/3	Preclinical, I, II	78
Demcizumab (OMP-21M18)	DLL4	Preclinical, I, II (NSCLC)	79
Ponatinib (AP24534)	FGFR1, PDGFRa, VEGFR2	Preclinical, I, II	80, 81
Lucitanib (E-3810)	FGFR1-3, PDGFRα/β, VEGFR1-3	Preclinical, I, II	82
VS-5584	mTOR/PI3K	Preclinical, I	84
AZD8055	mTOR	Preclinical, I [*]	67
Ponatinib, Vandetinib, Alectinib, Cabozaninib	RET	Preclinical, I, II	85
THZ1	CDK7	Preclinical	86
Ruxolitinib (INCB1824)	JAK1/2	Preclinical, I, II (NSCLC)	90
Tofacitinib (CP-690550)	JAK3	Preclinical	89
AZD1480	JAK2	Preclinical, I [*]	91
Amrubicin	Topoisomerase II	Preclinical, I, II, III, Marketed	92, 93, 94
Palifosfamide	Alkylation	Preclinical, I, II, III	
Aldoxorubicin	Anthracycline	Preclinical, I, II	95
Defactinib (VS 6063)	FAK	Preclinical, I, II (NSCLC, Meso)	97, 98
LY2510924	CXCR4	Preclinical, I, II	103
Olaparib (AZD2281)	PARP1/2	Preclinical, I, II	41, 106
Rucaparib (AG-014699, PF-01367338)	PARP1	Preclinical, I, II	41
Talazoparib (BMN-673)	PARP1/2	Preclinical, I, II	42, 43,
Veliparib (ABT-888)	PARP1/2	Preclinical, I, II	105, 107
Lorvotuzumab Mertansine	CD56	Preclinical, I, II [*]	110, 111
Rovalpituzumab Tesirine (SC16LD6.5)	DLL3	Preclinical, I, II	112, 113
GSK126	EZH2	Preclinical	115, 116
Sorafenib	RAF1, BRAF, PDGFRβ, VEGFR2	Preclinical, I, II [*]	117
Bevacizumab	VEGF	Preclinical, I, II, III [*]	118, 121
Thalidomide	Angiogenesis	Preclinical, I, II, III	119, 120
Cediranib (AZD2171)	VEGFR1-3, FLT1/4, cKit, PDGFRβ, FGFR1	Preclinical, I, II [*]	122

Agent	Target	Phase of trials	References
Vandetanib	VEGFR2	Preclinical, I, II	123
Aflibercept	VEGF Trap	Preclinical, I, II	124
Sunitinib	VEGFR1-3, PDGFRβ, c-KIT, FLT3, RET	Preclinical, I, II	125, 126
Imatinib	PDGFR, c-Kit	Preclinical, I, II[*]	127, 128
Rilotumumab (AMG 102)	HGF	Preclinical, I, II[*]	129
Ganitumab (AMG 479)	IGF-1R	Preclinical, I, II	130
Everolimus	mTOR	Preclinical, I, II	131, 132
Temsirolimus	mTOR	Preclinical, I, II	133
AZD1775 (MK-1775)	WEE1	Preclinical, I, II	134
Ipilimumab	CTLA-4	Preclinical, I, II, III	151, 152
Nivolumab	PD-1	Preclinical, I, II, III	152
Pembrolizumab	PD-1	Preclinical, I, II	153
BMS-986012	Fucosyl-GM1	Preclinical, I, II	158
CAR T Cells	CD56	Preclinical	159, 160
Anti CD 47 antibodies	CD47	Preclinical, I	165

[*]Clinical development halted at the time.