REVIEW

New Approaches to SCLC Therapy: From the Laboratory to the Clinic

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ABSTRACT

The outcomes of patients with SCLC have not yet been substantially impacted by the revolution in precision oncology, primarily owing to a paucity of genetic alterations in actionable driver oncogenes. Nevertheless, systemic therapies that include immunotherapy are beginning to show promise in the clinic. Although, these results are encouraging, many patients do not respond to, or rapidly recur after, current regimens, necessitating alternative or complementary therapeutic strategies. In this review, we discuss ongoing investigations into the pathobiology of this recalcitrant cancer and the therapeutic vulnerabilities that are exposed by the disease state. Included within this discussion, is a snapshot of the current biomarker and clinical trial landscapes for SCLC. Finally, we identify key knowledge gaps that should be addressed to advance the field in pursuit of reduced SCLC mortality. This review largely summarizes work presented at the Third Biennial International Association for the Study of Lung Cancer SCLC Meeting.

Keywords: SCLC; Gene mutations; Therapy; Neuroendocrine; ASCL1

Introduction

SCLC accounts for approximately 13% of all new lung cancer diagnoses.[1] SCLC exhibits many of Hanahan and Weinberg's[2,3] hallmarks of cancer to an exaggerated degree, including propensity for early metastasis, rapid cell division, high levels of replication stress, the ability to cope with certain oxidative and metabolic stresses, and evasion of apoptosis and the effector cells of the immune system. Together, these factors contribute to an exceedingly poor prognosis with patient survival measured in months, not years, that has led to a recalcitrant cancer designation for SCLC by the National Cancer Institute (NCI).

A meeting summary of the 2017 International Association for the Study of Lung Cancer SCLC Workshop posed the question: "Can recent advances in our understanding of tumor biology be translated into improved outcomes?"[4] This question has been answered affirmatively by the recent addition of immune checkpoint blockade to first-line chemotherapy in extensive stage SCLC, which constituted the first significant improvement in systemic therapy after several decades.[5] However, the magnitude of the treatment effect, although encouraging, was modest, and highlighted a clear need to improve the effectiveness of immunotherapy in SCLC.

Although the addition of immunotherapy in the treatment of SCLC is rapidly reaching a relatively mature stage, the exploration of underlying disease mechanisms and the development of candidate predictive biomarkers remains in its comparative infancy. It is increasingly appreciated that there are discrete molecular subtypes of SCLC that can differ in their response to different therapies in preclinical models of the disease, providing a rich and untapped vein to mine for new therapeutic liabilities (reviewed in Rudin and Poirier et al.).[6] In the light of limited durability of benefit from current therapies, it is of critical need to continue exploring new biomarker-directed therapeutic strategies and treatment combinations in the laboratory and in the clinic (Fig. 1). Further exploration of the processes that drive different molecular subtypes of SCLC and the therapeutic liabilities induced by these states is warranted.

In this review, we highlight recent advances in SCLC research from literature and unpublished data from a variety of researchers in the field, as presented in the 2019 International Association for the Study of Lung Cancer SCLC Workshop.

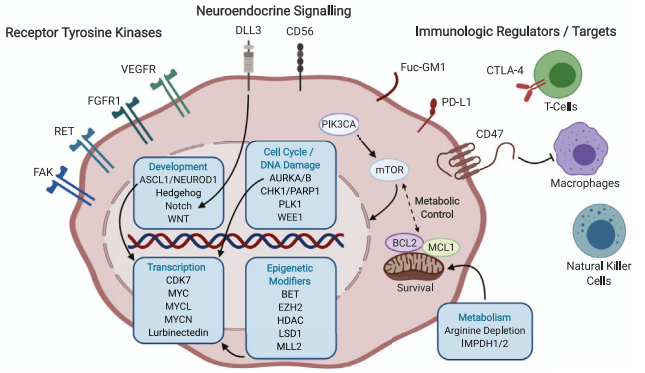


Figure 1. Some of the many areas of current therapeutic interest in SCLC. Cell surface targets include a number of the following: (1) receptor tyrosine kinases implicated in proliferative signaling, invasion, and angiogenesis; (2) factors regulating neuroendocrine differentiation that are being explored as targets for antibody drug conjugates; and (3) immunologic regulators and targets for tumor-specific vaccine strategies. Intracellular pathways of particular interest include: (1) metabolic and apoptotic regulators; (2) cell cycle and DNA damage checkpoint controls; (3) developmental signaling pathways; (4) transcriptional regulators, including the MYC family of transcription factors; and (5) epigenetic modifiers of histones that affect chromosomal accessibility and gene expression. FAK, focal adhesion kinase; RET, ret proto-oncogene; FGFR1, fibroblast growth factor receptor 1; VEGFR, vascular endothelial growth factor receptor; DLL3, delta-like 3 (Drosophila); CD56, neural cell adhesion molecule 1; Fuc-GM1, fucosyl-monosialotetrahexosylganglioside; PD-L1, programmed death ligand-1; CTLA4, cytotoxic T-lymphocyte associated protein 4; MHC 1, Major Histocompatibility Complex 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; mTOR, mammalian target of rapamycin; BCL2, B-cell lymphoma 2; MCL1, MCL1 apoptosis regulator, BCL2 family member; ASCL1, achaete-scute family bHLH transcription factor 1; NEUROD1, neuronal differentiation 1; WNT, wingless-type MMTV integration site family member; AURKA/B, Aurora kinase A/B; PLK1, Polo-like Kinase 1; WEE1, WEE1 G2 checkpoint kinase; CHK1, checkpoint kinase 1; PARP1, poly-ADP ribose polymerase 1; CDK7, Cyclin Dependent Kinase 7; MYCL, MYCL proto-oncogene, BHLH transcription factor; MYCN, MYCN proto-oncogene, BHLH transcription factor; MYC, MYC proto-oncogene, BHLH transcription factor; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; LSD1, lysine (K)-specific demethylase 1A; MLL2, myeloid or lymphoid or mixed-lineage leukemia 2; HDAC, Histone Deacetylase; BET, bromodomain and extra-terminal domain; IMPDH1/2, inosine monophosphate dehydrogenase 1/2.

Diagnostic and Molecular Pathology

The WHO classification schema for pulmonary neuroendocrine tumors was updated in 2015 and includes three primary histologic classes: SCLC, large cell neuroendocrine carcinoma (LCNEC), and pulmonary carcinoid tumors.[7] Histologically, SCLC is seen as a "small round blue cell" tumor under hematoxylin and eosin staining (Fig. 2*A*) and is characterized by high proliferative index as assessed by Ki67 immunohistochemistry (Fig. 2*B*). Carcinoid tumors have a much lower proliferative index. In addition, histologic subcategories within SCLC and LCNEC include combined SCLC and combined LCNEC, which refer to mixed histology tumors containing other non–small cell components

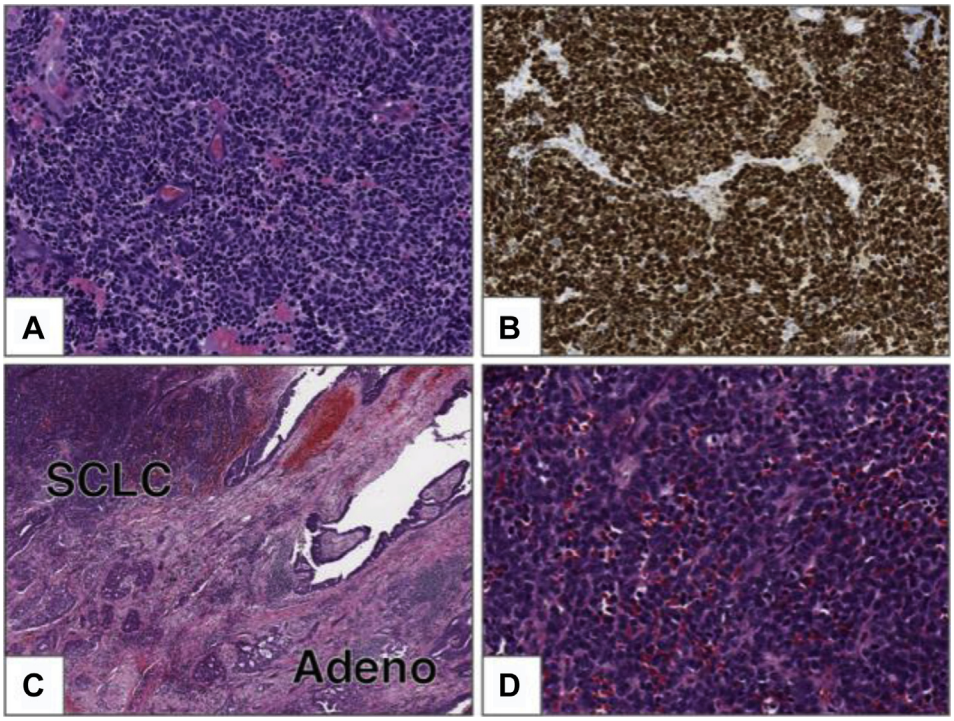


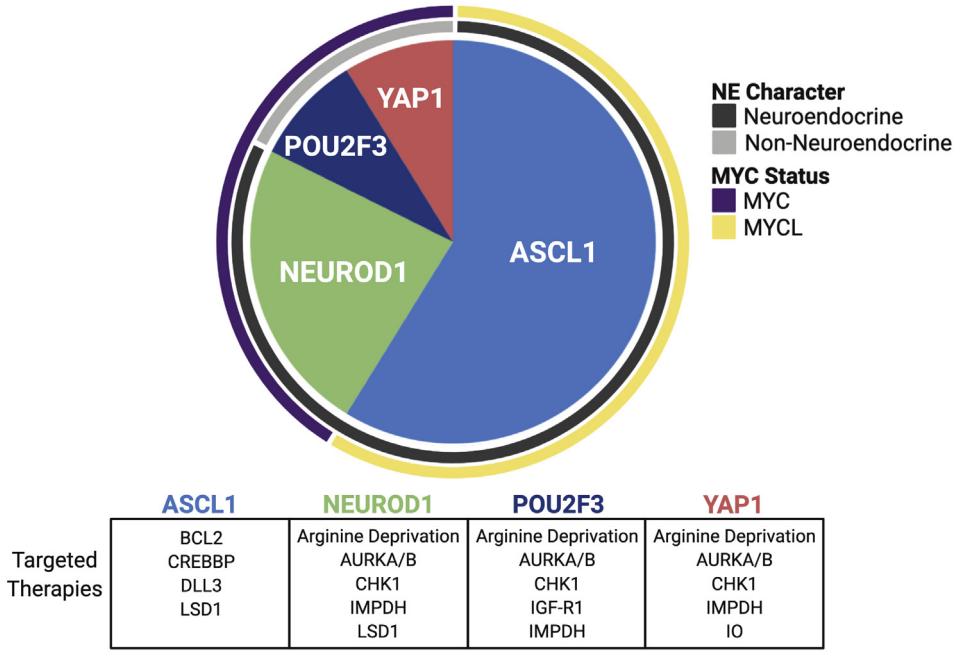
Figure 2. Small cell carcinoma. *(A)* This tumor is composed of small cells with scant cytoplasm, finely granular chromatin and frequent mitoses. Nucleoli are absent. *(B)* Ki-67 shows strong nuclear staining in 100% of the tumor cells. Combined small cell carcinoma and adenocarcinoma. *(C)* Low power image of tumor composed of two components: small cell carcinoma (upper left) and adenocarcinoma with acinar pattern (lower right). *(D)* High power image of the SCLC component from *C*.

(Fig. 2*C* and *D*). Within SCLC, combined SCLC represents a substantial fraction of cases; in one series, 16% of SCLC tumors had combined large cell carcinoma components, 9% had combined adenocarcinoma, and 3% had combined squamous cell carcinoma.[8]

The histologic appearance of SCLC can resemble other tumor types, notably carcinoid with crush artifact, Ewing sarcoma, desmoplastic round cell tumor, Merkel cell carcinoma, *SMARCA4*- or *SMARCB1*-deficient cancers, or basaloid squamous cell carcinoma. Differentiation from LCNEC is based primarily on tumor cell size and nuclear or cytoplasmic characteristics, SCLC being comparatively smaller in diameter and with a greater nuclear-to-cytoplasmic ratio. Other distinguishing features of SCLC include: (1) finely granular and uniform nuclear chromatin; (2) absent, or inconspicuous nucleoli; (3) nuclear molding; and (4) a fusiform shape. High mitotic counts are an important differentiator of carcinoids and SCLC; however, in the setting of metastatic pulmonary carcinoids, recent data reveal that recurrent and metastatic carcinoids can shift toward more aggressive growth, with a higher proliferation rate.[9]

SCLC is known for its highly metastatic nature. One newly investigated model of invasive spread that has been proposed as a putative prognostic indicator in SCLC, is "spread through air spaces" (STAS). STAS has been found to be associated with poor outcome in NSCLCs.[10,11] Recent data indicate that STAS is also prognostic in lung neuroendocrine tumors including SCLC, LCNEC, and atypical carcinoid.[12]

Common genetic lesions in SCLC are simultaneous pathognomonic inactivation of the tumor suppressor genes *TP53* and *RB1*, *MYC* family copy number gain, and inactivating mutations in epigenetic readers and writers and *NOTCH* family members.[13-15] Although current clinical pathology guidelines consider all pure SCLC as a single disease entity, recent gene expression profiling of both human patient SCLC tumors and cell lines, and representative murine models, suggests that biologically discrete subtypes of the disease exist, that can be distinguished on the basis of relative expression of three or four key transcriptional regulators: *ASCL1*, *NEUROD1*, *POU2F3*, and *YAP1* (Fig. 3). ASCL1 and NEUROD1 are critical factors in normal neuroendocrine development.[16,17] Concomitant disruption of *Ascl1*, *Trp53*, *Rb1,* and Rb1 family member *Rbl2* in a genetically engineered mouse model (GEMM) of SCLC, suggests that *Ascl1* is required for SCLC tumorigenesis in this model, whereas *NEUROD1* is dispensable.[18,19] In human cancers, *ASCL1* and *NEUROD1* bind to distinct super enhancer loci in *ASCL1*-high (SCLC-A) and *NEUROD1*-high (SCLC-N) tumors,[18-20] respectively, and seem to drive differential gene expression. Although *ASCL1* and *NEUROD1* define distinct molecular subtypes of disease, some tumors



	ASCL1	NEUROD1	POU2F3	YAP1
Targeted Therapies	BCL2 CREBBP DLL3 LSD1	Arginine Deprivation AURKA/B CHK1 IMPDH LSD1	Arginine Deprivation AURKA/B CHK1 IGF-R1 IMPDH	Arginine Deprivation AURKA/B CHK1 IMPDH IO

Figure 3. Diagram of the relative abundance, MYC status, and NE character of the four molecular subtypes of SCLC, each identified by their key transcriptional regulator. These subtypes may exhibit distinct targetable vulnerabilities, which are represented in the table beneath the pie chart. Proportions of each subtype are as follows: ASCL1 (0.70, 95% CI: 0.60-0.79), NEUROD1 (0.11, 95% CI: 0.06-0.20), YAP1 (0.02, 95% CI: 0.01-0.09), POU2F3 (0.16, 95% CI: 0.10-0.26). ASCL1, achaete-scute homolog 1; AURKA/B, Aurora kinase A/B; BCL2, B-cell lymphoma 2; CREBBP, CREB-binding protein; CHK1, checkpoint kinase 1; DLL3, delta-like ligand 3; IMPDH, inosine-5' monophosphate dehydrogenase; IGF-R1, insulin-like growth factor 1 receptor; IO, immuno-oncology; LSD1, lysine-specific histone demethylase 1; NE, neuroendocrine; NEUROD1, neurogenic differentiation factor 1; POU2F3, POU class 2 homeobox 3; YAP1, yes-associated protein 1.

express both factors to different extents, and the lineage relationship among these subsets has not been fully defined. In contrast, *POU2F3*-positive SCLC (SCLC-P) seems to have a distinct transcriptional signature from other subsets.[21] *POU2F3* biology and its role in this subtype of SCLC is discussed in detail in another section of this review. The *YAP1*-high subtype (SCLC-Y) has been the least extensively characterized in terms of determinants of differential gene expression; however, this subtype is seen to be enriched in human cell lines with detectable RB1 protein by Western blot. It is unclear if *YAP1* itself is a driver of this phenotype,[22] or a marker thereof. The pathologic distinctions among these transcriptionally defined subtypes, in terms of natural history of disease and therapeutic outcome, have not been fully investigated and represent substantial unmet needs.

Cell of Origin, Tumor Initiation, and Lineage-Related Pathways

Mouse model studies have suggested that a predominant cell of origin for SCLC is the neuroendocrine (NE) cell,[23,24] which, according to our current understanding, is also the proposed cell of origin of pulmonary carcinoids and LCNECs. However, interpretation of these studies is complicated by technical issues related to inhalation of viruses using cell-type–specific promoters. To further characterize the function of NE cells in the normal airway epithelium, lineage tracing approaches have been employed in animal models, to track the fate of NE cells after airway injury.[25] This work reported that only a subset of the NE cell population proliferates after lung injury. Interestingly, this subset of cells seem to be the same population that proliferates after sequential injuries, suggesting that these cells possess a unique capacity for self-renewal. This rare population of NE cells has been termed NE^{stem} and is characterized by *Notch2* expression. Consistent with the loss of *RB1* and *TP53* in human tumors, loss of both tumor suppressors in the NE population in mice led to constitutive activation of the self-renewing NE cell population. In accordance with frequent loss of function alterations in the NOTCH pathway in human SCLC, pharmacologic Notch inhibition in the mouse blocked NE cell reprogramming and clonal expansion.[25] Loss of function of *RB1*, *TP53,* and NOTCH presumably lock NE progenitors into a self-renewal program and thereby contribute to transformation.

Although NE cells have been implicated as a major cell of origin, recent studies from animal models suggest that they may not be the only cells eligible for transformation to SCLC. A recent study on the molecular

profiles of nuclear factor I/B (*Nfib)*-amplified mouse tumors proposed the existence of alternative (yet to be identified) cell(s) of origin that impact metastatic trajectories.[26] Another recent study on human SCLC cell lines discovered a subset of SCLCs lacking expression of neuroendocrine markers and harboring a tuft cell signature.[21] Tuft cells, also known as brush cells, are marked by expression of *POU2F3*, *TRMP5,* and *ASCL2*. These cells have been identified in the lung by single cell transcriptome sequencing.[27,28] Using a transcription factor-focused clustered regularly interspaced short palindromic repeats (CRISPR) knockout screen, it was reported that SCLC cell lines expressing tuft cell markers, are exquisitely dependent on the transcription factor POU domain, class 2, transcription factor 3 (POU2F3), a lineage specific marker and master regulator of tuft cell fate. Chromatin profiling of human POU2F3-positive SCLC cell lines reveals that *POU2F3* is responsible for maintaining a tuft cell enhancer landscape. This indicates that the tuft cell may be a potential cell of origin for SCLC; or alternatively, a different cell of origin may have the capacity to transdifferentiate to a state resembling the tuft cell. Established SCLC cell lines with a tuft cell program, are particularly vulnerable to IGF1R inhibitors,[21] highlighting the possibility of subtype-specific therapies for SCLC. A randomized phase 2 study of cisplatin and etoposide alone or in combination with either vismodigib, a Hedgehog inhibitor, or cixutumumab, an IGF1R-directed monoclonal antibody, was negative.[29] However, this trial was conducted without biomarker selection and the number of patients with POU2F3-positive tumors in each arm, would likely be few.

As molecular subtypes of SCLC are being increasingly appreciated, there is a need to define the underlying epigenetic states of each subtype. Chromatin immunoprecipitation for acetylated H3K27 to evaluate the SCLC subtype-specific super-enhancer landscape revealed distinct super-enhancer profiles among human SCLC-A, SCLC-N, and SCLC-P (J.E. Johnson et al., and C.L. Christensen, et al., unpublished data, 2019).[18,20] Further examination of the SCLC-A subtype by immunoprecipitation-mass spectrometry approaches identified three putative Achaete-scute homolog 1 (ASCL1)-interacting partners, including two transcription factors, NK2 homeobox 1 (NKX2-1) and Prospero homeobox protein 1 (PROX1), and a nuclear import protein, Karyopherin Subunit Beta 1 (KPNB1). Strong overlap was found among the genomic targets of ASCL1, NKX2-1, and PROX1, delineating a putative transcription factor network. Further studies of KPNB1 in human SCLC cells reported that inhibition of KPNB1 led to decreased viability and colony formation of ASCL1-positive cells (J.E. Johnson et al., unpublished data, 2019). These findings suggest that there is a potential for developing subtype-specific therapies for SCLC. Other unbiased approaches are being used to discriminate SCLC subtypes. A recent study investigated SCLC subtypes from a gene regulatory network perspective, seeking to account for genetic, epigenetic, and intrinsic stochastic noise within gene expression data.[30] Theoretical modeling with Boolean logic predicted four subtypes among SCLC (named NE, NE-v1, NE-v2, and Non-NE) with a high degree of overlap with other proposed subtypes.[6] For example, NE resembles the SCLC-A, NE-v1 resembles the SCLC-N, and the Non-NE resembles the SCLC-Y subtypes. Interestingly, the NE-v2 subtype seemed distinct from the NE cluster, although both express characteristic markers of SCLC-A, suggesting further heterogeneity among the SCLC-A cells, with predicted differences in cell morphology, gene expression, and drug response. The NE-v2 state was present in classic (*Rb1;Trp53;Rbl2*, RPR2) GEMMs, but largely absent from variant (*Rb1;Trp53;Myc*, RPM) GEMMs, consistent with higher expression of *ASCL1* and other canonical neuroendocrine genes in the RPR2 model. Variant MYC-driven GEMMs, in contrast, were enriched for NE-v1 and Non-NE phenotypes. Importantly, these subtypes are postulated to be dynamic states of transition, likely with different thresholds for change.[30] Indeed, silico predictions of master regulators and destabilizers of these states, highlight potential mechanisms of tumor plasticity, that will require functional studies to validate.

Tumor Progression, Intratumoral Heterogeneity, and Metastasis

New advances in CRISPR-Cas9 genome engineering technology and mouse genomics have provided a platform with which to functionally interrogate driver genes and provide insight into the mechanisms of SCLC tumor progression. SCLC frequently harbors genomic alterations in genes encoding the histone acetyl transferases *CREBBP* and *EP300* with a significant enrichment of hotspot mutations in the histone acetyltransferase domain. Early functional studies in cell lines pointed to a tumor-suppressive function of CREB-binding protein (CREBBP) and E1A Binding Protein P300 (EP300) in SCLC.[14] Recent CRISPR-based approaches to alter genes in early RPR2 mouse tumor cells further revealed a tumor-suppressive role for *Crebbp* in SCLC.[31] In an autochthonous mouse model for SCLC, inactivation of *Crebbp* (*Rb1*$^{fl/fl}$;*Trp53*$^{fl/fl}$;*Crebbp*$^{fl/fl}$) accelerated tumor formation. Loss of *Crebbp* led to reduced levels of histone acetylation, which impacted the transcription of cellular adhesion genes.[31] Using a similar approach, the authors tested the oncogenic potential of other mutations. *TP73* is altered by mutation or genomic rearrangement in

approximately 13% of human SCLC,[13] and this is predicted to lead to loss of tumor protein P73 (TP73) function or expression of dominant negative TP73 isoforms. Targeting *Trp73* with sgRNAs directed at its transactivation domain accelerated tumor growth consistent with a tumor-suppressive role for TP73.[32] Ectopic expression of *Fgfr1* promoted tumor growth; whereas, conversely, *Fgfr1* loss inhibited tumor growth, suggesting an oncogenic role for Fibroblast growth factor receptor 1 (FGFR1) consistent with its amplification in approximately 6% of SCLC.[13,32,33] Interestingly, this approach validated unpublished findings that *Fgfr1* alterations impact anatomical tumor location in GEMMs (A. Berns et al., unpublished data, 2019), suggesting a potential cell-type–specific impact of FGFR1 in SCLC.

MYC family transcription factors (*MYC*, *MYCL,* and *MYCN*) are frequently amplified in SCLC tumors. Intriguingly, genomic alterations of these *MYC* paralogs occur in a mutually exclusive manner in SCLC.[34] Using a MYC-driven GEMM and a pharmacologic vulnerability screen in SCLC cell lines, oncogenic activation of *MYC* was found to pose a specific susceptibility to Aurora kinase inhibition.[35,36] A recent study employed CRISPR-activation approaches in mouse cell lines with *Rb1;Trp53* loss to study the effects of *MYC* paralogs (C, L, and N-MYC).[37] *Myc*-activated cells recapitulated previously discovered *MYC*-dependent drug sensitivities including to Aurora A inhibitors.[35,36,38] Mechanistically, MYC was found to epigenetically repress *BCL2* transcription through its interactions with DNA (cytosine-5)-methyltransferase 3A (DNMT3a) and MIZ1. This interaction results in the methylation of the *BCL2* promoter with a subsequent decrease in B-cell lymphoma 2 (BCL2) protein expression, consistent with observations that the *BCL2* promoter is silenced by DNA methylation in the variant SCLC subtype.[39] MYC activation also increases DNA damage signaling, apoptosis, and is associated with increased BH3-apoptotic priming and MCL1 dependency, providing a rationale for MYC-driven SCLC sensitivity to DNA damage checkpoints like Checkpoint kinase 1 (CHK1) inhibition.[37] Indeed, combining MYC-dependencies (CHK1 plus Aurora Kinase A [AURKA] inhibition) suppressed tumor growth and enhanced survival of MYC-driven GEMMs compared with the standard of care chemotherapy.[37] These studies suggest the ability to exploit MYC-specific therapeutic vulnerabilities in SCLC.

On the basis that *RB1* is almost universally lost in SCLC, two recent studies discovered a dependency of *RB1*-deficient SCLC cells on Aurora kinases.[40,41] Specifically, a CRISPR-based synthetic lethal screen determined that *RB1* loss is synthetic lethal with Aurora B kinase inhibition in SCLC cell lines.[40,41] *RB1*-mutant SCLC cells are sensitive to AZD2811, an Aurora B kinase (AURKB) inhibitor, similar to what has been found in *MYC*-overexpressing tumors.[35,36,38,42] Together, this suggests that both *RB1* loss and *MYC* overexpression may be required for the sensitivity of SCLC to Aurora kinase inhibitors. A phase 1 clinical trial is ongoing for AZD2811 as a monotherapy in patients with relapsed or refractory SCLC. It will be important to determine whether responses in the AZD2811 trial correlate with MYC status as was observed in earlier clinical trials treating patients with SCLC with combination alisertib and paclitaxel.[43] Together, these studies highlight the importance of collecting biomarker information during clinical trials to identify relevant patient populations for larger definitive studies.

In addition to MYC, NOTCH signaling has been implicated as a major pathway contributing to transcriptional heterogeneity in SCLC.[44] Although Notch can be tumor-suppressive and exhibits frequent loss of function alterations in SCLC,[13] it can also be oncogenic. Indeed, NOTCH activation is found in a subset of mouse and human tumors. NOTCH-high tumors express RE1-Silencing Transcription factor (REST), a transcriptional repressor that can suppress neuroendocrine gene expression, thereby contributing to a non-neuroendocrine cell fate. MYC-high SCLC is also associated with a non-neuroendocrine fate, and MYC can drive a non-neuroendocrine phenotype in GEMM.[13,35,45] Consistently, in a large panel of human SCLC cell lines, *MYC*, *NOTCH, REST,* Hippo, and Transforming Growth Factor Beta pathways are highly associated with non-neuroendocrine SCLC fate.[45] In contrast, neuroendocrine-high cell lines express lower levels of those genes and related pathways (i.e., *MYC*, *NOTCH*, *REST*) and higher levels of *ASCL1* and *NKX2-1*. Understanding the functional relevance of these pathways and their relationships to one another constitutes a major unmet need in SCLC, because these pathways may be therapeutically relevant.

SCLC is a highly metastatic tumor type, which is a major cause of morbidity. Recent work has shed light on mechanisms of metastasis using GEMMs. Data from multiple groups reported that the transcription factor NFIB promotes metastases in SCLC.[46-48] NFIB is important for lung and brain development and *Nfib* is genomically amplified in classic SCLC tumors from GEMMs,[49] in which it has been implicated as an ASCL1 target gene.[18] NFIB is also highly expressed in ASCL1-low MYC-driven variant tumors from GEM models, in which it is not amplified but seems to be a MYC target gene.[36] Overexpression of *NFIB* in classic GEMMs accelerates tumor formation and metastases, and this is caused by NFIB's ability to open chromatin and promote a pro-metastatic neuronal gene expression program.[46-48] A recent study suggests that metastatic tumor cells are

more neuronal than neuroendocrine and exhibit protrusions that resemble axons; knockdown of genes implicated in protrusion formation inhibited metastatic capacity, suggesting a potentially new avenue for blocking metastases.[50] Neurogenic differentiation 1 (NEUROD1) has also been implicated in SCLC migration and is associated with neuronal gene expression programs,[18,51,52] but the relationship among NFIB, NEUROD1, and neuronal behavior, if there is one, is currently not well understood. More recently, it was reported that the cell of origin impacts the metastatic trajectory of tumors in GEMMs and therefore, different mechanisms of metastases may occur in SCLC.[26] Further studies are needed to determine whether these metastatic programs could be pharmacologically inhibited for patient benefit.

To further delineate heterogeneous phenotypes in SCLC, new technologies are being explored including single cell transcriptome sequencing and mass cytometry (CyTOF). CyTOF is a mass cytometry approach coupling antibodies to rare earth metals, similar to the fluorophores of traditional flow cytometry. CyTOF allows for increased multiplexing capacity and sensitivity per cell. CyTOF is being employed to investigate intra- and intertumoral heterogeneity among patient-derived xenograft (PDX) samples to identify novel populations of SCLC, and to probe mechanisms of chemotherapy resistance (J. Lehman et al., unpublished data, 2019). These unbiased approaches to investigate tumor cell populations, will undoubtedly increase our understanding of tumor heterogeneity, plasticity, and treatment resistance mechanisms.

Large-Scale Molecular Profiling

Large-scale profiling studies have provided valuable insight into the epi-genomic, genomic, proteomic, and metabolomic landscape of SCLC tumors.[14,15,18,20,39,53-55] Quantification of intratumoral heterogeneity using genome sequencing data of SCLC tumors reported that subclonal diversity was threefold lower in SCLC, than in lung adenocarcinomas.[13] This disparity may point to pronounced differences in the evolution and progression of SCLC and lung adenocarcinomas. This view is further challenged by the evolutionary dynamics of lung adenocarcinomas with mutations in the *EGFR* gene that undergo histologic transdifferentiation to SCLC through a poorly understood mechanism of lineage plasticity. SCLC tumors may originate from multiple cell types, whereas lung adenocarcinomas have been reported to mainly develop from type II alveolar cells.[56,57] Despite their reportedly distinct cells of origin, *EGFR*-mutant lung adenocarcinomas have been reported to transform to SCLC after treatment with tyrosine kinase inhibitors.[58-60] The molecular mechanisms of this phenomenon have been strongly associated with the loss of *RB1*.[61] A recent study on serial sampling and longitudinal sequencing of four transdifferentiated *EGFR*-mutant lung adenocarcinomas provided further insight into the genomic and evolutionary dynamics of transformed SCLC tumors.[62] The genomic profiles of these cases revealed a branched evolution, in which all SCLC-transformed cases harbored early clonal *EGFR* mutations and early clonal dual inactivation of *TP53* and *RB1*. As part of the evolutionary early branching, acquired *EGFR* resistance mutations and other driver gene alterations (e.g., *MYC*, *PIK3CA*, *PTEN*, *ARID1A*) were found to be late events and branch specific. Private tumor-specific late-branching events were enriched for apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)-mutational signatures.[62] This observation is in line with previous studies on the evolution of lung adenocarcinomas that revealed reduced rates of smoking-related (C> A transversions in the branches and increased APOBEC-related subclonal mutations.[57,63-65] In the light of evolutionary dynamics of lung adenocarcinomas, further studies on serial SCLC tumor samples are required to obtain more insight on genomic changes throughout tumor progression and therapy resistance.

In a recent study, whole exome sequencing of 30 chemotherapy-resistant SCLC tumors was performed; and for 12 of these cases, the comparative analysis of matched treatment-naive tumors provided information on potential mechanisms of resistance to chemotherapy.[66] The genomic profile of chemotherapy-resistant tumors mirrored the alterations identified in treatment-naive SCLC. Tumors from chemo-refractory patients with SCLC were reported with recurrent somatic alterations and with transcriptional up-regulation of wingless-related integration (WNT) pathway genes. WNT activation was enriched in patient tumors that specifically harbored low levels of *ASCL1* expression. In addition, some patient cases were found with alterations in mismatch repair genes and amplification of *ABCC1*, which in earlier studies was identified in chemotherapy-resistant SCLC cell lines.[66] These analyses warrant further studies on WNT pathway genes and their role in distinct genomic and transcriptional subgroups of SCLC.

SCLC tumors with low expression of *ASCL1* generally harbor high expression levels of the *MYC* transcription factor. In addition to their distinct expression patterns, *MYC*-dependent SCLC tumors report therapeutic vulnerabilities to Aurora kinase inhibitors.[35,36] Metabolomics of human SCLC cell lines and mouse-derived SCLC tumors revealed distinct metabolic profiles of *MYC*-driven tumors. Metabolite set enrichment analyses pointed to an inosine monophosphate dehydrogenase (IMPDH) dependency in *ASCL1*^{low} cells.[54] The enzymatic

activities of IMPDH1/2 allow for de novo guanosine nucleotide synthesis, and *ASCL1*^{low} SCLC cells depend on this mechanism for cell survival. IMPDH1/2 inhibitors suppressed cell growth in xenograft models of *ASCL1*^{low} SCLC cell lines, and GEMMs of *ASCL1*^{low}/*MYC*^{high} tumors were sensitive to the combined inhibition of IMPDH1/2 with chemotherapy.[54] In addition to guanosine metabolism, recent studies also revealed subtype-specific dependencies of SCLC on arginine.[55] Arginine has pleiotropic cellular functions in nitric oxide signaling, polyamine biosynthesis, and mammalian target of rapamycin (mTOR) activation. Pharmacologic interventions revealed that *MYC*-driven SCLC tumor cells are dependent on arginine for mTOR pathway activity and polyamine biosynthesis. Pharmacologic depletion of arginine was found to promote survival of mice bearing *MYC*-driven SCLC tumors in GEMMs, human cell line xenografts, and PDX models.[55] Arginine deprivation agents such as pegylated arginine deiminase (ADI-PEG20) and pegylated human arginase have been in clinical trials for SCLC, and these data suggest that biomarker analysis may be critical to interpretation of these findings.[67]

Platforms for Discovery

In contrast to the large-scale genome sequencing efforts of primary lung adenocarcinoma that proved to be a fertile ground for drug target discovery, genomic surveys of SCLC tumors have produced few directly targetable alterations.[13-15] A growing theme among recent discoveries has been the importance of neuroendocrine transcriptional networks, epigenetic modifiers, metabolism, and global transcriptional addiction in the maintenance of SCLC tumors. This reality has forced a strategic shift in therapeutic discovery efforts away from target nomination from the set of recurrent genetic alterations in spontaneous human tumors and toward pharmacologic targeting of candidate pathways, targeted gene disruption, synthetic lethal screens, and chemistry-first screening as platforms for target identification. The field is increasingly gravitating toward approaches that combine multiomic characterization, diverse chemical libraries that include natural products, and focused gene disruption of defined target classes, such as epigenetic modifiers and kinases using CRISPR-Cas9 genome engineering technology in cell lines and mouse models, as a means to discover new SCLC biology.

Landmark studies to identify the key drivers of drug response in cancer cell lines uncovered several important chemical-genetic or chemical-epigenetic interactions that have subsequently been validated in patients.[68] These initial studies focused on over 1000 unique cell lines across cancer types and a relatively small number of drugs with known antineoplastic activity to power the discovery of molecular predictors of response. A study focusing on SCLC cell lines screened 526 compounds in 63 SCLC cell lines and included gene and miRNA expression measurement; this study constitutes one of the largest public chemical screens in SCLC cell lines.[69] Recent efforts in lung cancer have focused on screening larger and more diverse sets of compounds to discover "therapeutic triads" of novel targets, compounds, and response biomarkers. In NSCLC, greater than 100 cell lines were recently screened with a 200,000 compound diversity-oriented chemical library as part of the precision oncology probe set lung project.[70] This effort has been expanded to include 30 SCLC cell lines to identify compounds with selectivity for SCLC over NSCLC, increasing the total chemical space explored by two orders of magnitude over the previous benchmark study (J. Minna et al., unpublished data, 2019). Natural product screening for new classes of biologically active compounds and identification of their relevant targets through forward genetic approaches may further augment screens of synthetic compound libraries.

CRISPR-Cas9 gene-disruption screens have revolutionized forward genetics in human cell lines. This powerful technology has been recently employed to uncover transcription factor dependencies in SCLC cell lines, as discussed above.[21] Dropout screens targeting developmental pathways or genes required for stem cell maintenance, kinases, or other classes of proteins may uncover previously unappreciated subtype-specific gene dependencies. Screens that target genes in tandem, or that combine chemical perturbation with gene disruption, constitute a powerful technology for interrogating genetic or chemical-genetic interactions. One recent study identified a role for microtubule associated serine/threonine kinase 1 (MAST1) in driving cisplatin resistance through impingement on mitogen-activated protein kinase signaling through a kinome-wide RNAi screen in combination with cisplatin.[71] Screens of this nature constitute a particularly powerful discovery engine.

Discovery platforms by their nature generate large-scale datasets that require specialized expertise to curate and mine for biological signal. Pharmacogenomic data portals are a useful resource to make these datasets readily accessible to a broad audience. Recently, CellMinerCDB has been established as a web-based data portal (https://discover.nci.nih.gov/cellminercdb/) that combines drug response and multiomics data from multiple cell line datasets (the NCI-60, NCI-SCLC, Sanger/MGH Genomics of Drug Sensitivity in Cancer [GDSC], and Broad Cancer Cell Line Encyclopedia/Cancer Therapeutics Response Portal [CCLE/CTRP]). In addition, it also provides integrative and exploratory tools to mine these data for potential molecular predictors of response.[72] Beyond

studying a large panel of cell lines derived from various cancer types, a SCLC-specific data portal will be launched (SCLC-CellMinerCDB) providing analysis tools for drug screening data from 118 SCLC cell lines from four drug screen portals (NCI- Development Therapeutics Program, CCLE, GDSC, CTRP) and the University of Texas Southwestern data. SCLC-CellMinerCDB will allow cross database analysis for whole genome, somatic alterations (mutations and copy number alterations), DNA methylome, gene expression, and proteomic data. This integrative approach will allow for cross-comparisons of SCLC and other cancer cell lines, and for pharmacogenomic data exploration to link drug treatment response to genomic and molecular characteristics.

These discovery efforts ultimately demand clean genetic models for mechanistic validation. The *Rb1;Trp53* GEMM developed in the laboratory of Anton Berns, which gives rise to lung tumors that closely resemble human SCLC-A, continues to be a widely used model for mechanistic validation of potentially cooperating genetic events; although, it has a long latency of 10 to 12 months.[73] This model has proven to be useful in ranking therapeutic liabilities that may arise from the underlying biology of a given genetic lesion. An ever-expanding number of triple- and quadruple-transgenic GEMMs on the basis of the original double-transgenic model have expedited tumor latency and are helping to unravel the early steps of SCLC carcinogenesis and validate new therapeutic approaches.

GEMMs have many advantages; however, in some instances, certain hypotheses cannot be tested in mice owing to a lack of direct homology between mouse and human. Pulmonary neuroendocrine cells (PNECs) are considered by many to be a likely cell of origin for SCLC. Because these relatively rare cells constitute less than 1% of an adult human lung and there are no effective in vitro culture systems, primary PNECs have not been studied directly. The generation of PNECs and SCLC-like tumors from human embryonic stem cells through chemical and genetic recapitulation of the key events in SCLC carcinogenesis holds promise as a clean in vitro genetic model for the initiation of human SCLC.[74] In this system, NOTCH inhibition and RNAi knockdown of *TP53* and *RB1* in lung progenitor cells results in a massive expansion of PNECs to constitute 10% to 30% of the culture population.

Together, complementary mouse and in vitro human model systems enable the discovery and preclinical validation of disease-relevant molecular alterations identified in spontaneous human cancers and their relation to therapeutic responses. A deeper understanding of the mechanistic basis of drug sensitivity is likely to be a source of candidate predictive biomarkers of response.

Biomarkers

Liquid Biopsies for Molecular Profiling in Patients With SCLC

Serial analysis of tumor biopsies beyond the initial diagnostic specimens are not routinely performed in patients with SCLC, which limits molecular studies and biomarker assessments of treatment-induced changes in this cancer type. The analysis of blood-based tumor components such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and tumor-derived extracellular vesicles (EVs) could provide alternative opportunities to monitor the molecular phenotype of a patient's tumor throughout the disease, and to assess biomarkers of treatment response and tumor progression.

Compared with other lung cancers, patients with SCLC have the greatest number of CTCs.[75] The number of CTCs has been found to serve as a prognostic marker for clinical response to therapy.[76] Furthermore, isolated CTCs from patients with SCLC can be used to generate CTC-derived xenograft models (CDX), which allow for the enrichment and subsequent analysis of the tumor material.[77] The generation of serial CDX proved feasible at multiple time points during treatment. The established tumor models were reported to faithfully reflect the matched patient tumor, and to recapitulate the mutational landscape and transcriptional diversity found in SCLC tumors.[77,78] Furthermore, pharmacologic drug screening using CDXs mirrors the therapeutic responses of the donor patient.[77,78] The establishment of CDX requires considerable resources and time, which limits the chances for a donor patient to benefit from preclinical interrogations of their own tumor. New approaches and technologies are focusing on the direct study of isolated CTCs, which provide prospects for *ex vivo* expansion and short-term culture for gene manipulation and preclinical drug screening.[79] Thus, CTCs and CDXs hold promise as additional tools to gain mechanistic insights into treatment sensitivity and resistance.

CTC-derived nucleic acids or ctDNA similarly allow for longitudinal disease monitoring of patients with SCLC.[80,81] Customized targeted sequencing panels aid in assessing and tracking tumor-related mutations and copy number changes. SCLC-associated genomic alterations were successfully identified in up to 85% of plasma samples, as recently reported by genomic profiling studies using ctDNA.[80] Tracking such genomic alterations in the ctDNA of patients with SCLC also served as an indicator of disease relapse.[80] Furthermore, comparative analyses of CTC-derived nucleic acids from chemo-sensitive and chemo-refractory patients with SCLC revealed distinct copy number profiles, which may serve as biomarkers for patient stratification.[81]

Table 1. Recent and Ongoing Clinical Trials

Agent	Target	Trial Phase	Clinical Trial ID(s)
Navitoclax (ABT-263)	BCL-2, BCL-xL, BCL-W	I, II	NCT03366103
APG-1252	BCL-2, BCL-xL, BCL-W	I	NCT03080311, NCT03387332
ABBV-075	BET	I	NCT02391480
Lurbinectedin (PM01183)	CG-rich promoter sequences	III	NCT02566993
Prexasertib (SRA737)	CHK1	II	NCT02735980
Rova-T	DLL3	III	NCT03543358
AMG 757	DLL3	I	NCT03319940
AMG 119	DLL3	I	NCT03392064
BMS-986012	FucGM1	I, II	NCT02247349, NCT02815592
Vistusertib	mTORC1/2	I, II	NCT03366103
Olaparib	PARP	I, II	NCT02446704, NCT03009682, NCT02769962, NCT03532880, NCT03923270, NCT02511795
Talazoparib	PARP	II	NCT03672773
Veliparib	PARP	II	NCT03227016
Rucaparib	PARP	II	NCT03958045
Niraparib	PARP	II II	NCT03830918 NCT03516084

BCL-2, B-cell lymphoma 2; BCL-xL, B-cell lymphoma-extra large; BET, Bromodomain and extra-terminal domain; CHK1, checkpoint kinase 1; DLL3, delta-like ligand 3; FucGM1, Ganglioside fucosyl-GM1; mTORC1/2, mTOR Complex 1/2; PARP, Poly (ADP-ribose) polymerase.

The isolation and characterization of EVs such as exosomes may constitute an alternative source for multiple analytes in liquid biopsies. EVs contain cytosolic proteins, lipids, and nucleic acids, and are released from all cells including cancer cells.[82] Tumor-related EVs can be found in patients with cancer and it is critical to distinguish those from EVs of normal cells by tracking tumor-specific markers. To this end, a recent study reported that Glypican-1 positive exosomes can serve as a diagnostic marker for pancreatic cancer.[83] The signal of EV–associated *Glypican-1* RNA was found to be further amplified with the use of molecular beacons and lipoplex nanoparticles.[84] SCLC tumors harbor several tumor-specific expression markers,[6] including Delta-like ligand 3 (*DLL3)*.[85] The technological advances in EV capture and analyses could be resourceful for studying SCLC tumor markers and could aid in diagnosis and in monitoring disease progression.

Targeted Therapies

DNA Damage Response Inhibition

Compared with lung adenocarcinoma, SCLC tumors exhibit high expression levels of DNA damage response (DDR) proteins.[53] Many DDR proteins, such as Poly (ADP-ribose) polymerase (PARP), ataxia telangiectasia and Rad3-related protein (ATR), CHK1, and WEE1, have small molecule inhibitors in various stages of development (Table 1). Among the DDR proteins, PARP is perhaps the most actively studied drug target as a single agent and in combination with other therapies.

The mechanism of action of PARP inhibitors is twofold: to inhibit the formation of poly-ADP ribose at single-strand breaks and to prevent the release of PARP complexes from DNA single-strand breaks. Replication forks stall when they encounter PARP trapped on DNA, which can ultimately result in double-strand breaks. It is now recognized that PARP inhibitors differ in their PARP-trapping potency, which is a crucial factor for cytotoxicity of these agents.[86] In addition to their action as single agents, PARP inhibitors can synergize with other agents that induce single-stranded breaks such as the alkylating agent temozolomide (TMZ).[86-88] Schlafen family member 11 (SLFN11) has been tested as a biomarker of PARP inhibitor sensitivity in SCLC in preclinical models and as an exploratory clinical correlate.[87,89]

The efficacy of PARP inhibitors has been tested in various settings in patients with SCLC.[43,89-91] Although there is reproducible evidence of efficacy, the magnitude of benefit seems modest in the lack of predictive biomarkers to assist in patient selection. Emerging data from the preclinical and clinical arena are coalescing to inform the best strategies to successfully incorporate this class of agents into SCLC treatment. A recent clinical study of the PARP inhibitor (PARPi), olaparib, in combination with TMZ in patients with SCLC included a co-clinical research program to establish PDX models and further interrogate patient-specific treatment responses.[91] Longitudinal SCLC xenograft models established from tumor material from this trial recapitulated the clinical responses of the patients to chemotherapy and to the combination treatment of TMZ with PARPi.[78] This co-clinical framework provides a valuable platform for in-depth functional studies of treatment responses and biomarker discovery.

Synergy between DDR inhibitors and ionizing radiation (IR) therapy has been found in various tumor types.[92-95] The addition of the PARPi talazoparib to escalating doses of IR reflected potentiation of the IR effect in a panel of SCLC cell lines, using both short-term cytotoxicity and long-term clonogenic assays.[96] Moreover, IR potentiation correlates with PARP-trapping ability of PARP inhibitors with a stronger effect recorded with talazoparib compared with veliparib, which has been found to have the weakest PARP-trapping effect. Similar potentiation effects were noted in in vivo models using SCLC PDX representative of chemo-naive and chemo-resistant disease. An investigator-initiated clinical trial is now testing the combination of olaparib and low-dose fractionated thoracic radiation (10 × 3 Gy over 2 w) (NCT03532880).

G2/M Mitotic Checkpoint Inhibition

The DNA damage checkpoint represents an attractive target in SCLC, because of the aberrant expression of various DNA damage response genes and intrinsic cellular vulnerabilities.[37,53,97] In addition to Aurora kinases and WEE1, PLK1 is a node in the G2/M mitotic checkpoint that plays a major role in driving centrosome disjunction and separation. It is frequently overexpressed in human cancers including lung cancer and its inhibition leads to a characteristic polo arrest phenotype, leading to cell cycle arrest in mitosis owing to monopolar spindles and apoptosis.[98,99] Inhibitors of PLK1 previously evaluated in preclinical and clinical settings include SBE, onvansertib (NMS-P937), Ro3280, MLN0905, HMN-214, GSK461364, Rigosertib (ON-01910), Volasertib (BI6727), and BI2536.[100] Agnostic low-throughput screening revealed exquisite sensitivity of SCLC cell lines and in vivo models to different PLK1 inhibitors; in particular, onvansertib and volasertib (T. Owonikoko, et al., unpublished, 2019). In addition, *TP53* gene mutation and MYC expression seemed to predict for sensitivity to this class of agents,[37,42] consistent with observations in other tumor types. The combination of PARPi and other targeted agents that act through G2 checkpoint blockade such as ATR, CHK1, and WEE1 inhibitors has been the focus of preclinical testing.[97,101-103]

Genomic instability characterized by high tumor mutational burden (TMB) and the presence of microsatellite instability or BRCA mutations have been associated with sensitivity to immunotherapeutic agents targeting the PD-1 signaling axis. PARP inhibition was reported to induce an immune response and increase the efficacy of PD-1 targeted immunotherapy agents through a mechanism that implicates T-cell activation through the stimulator of interferon genes (STING) pathway.[104] Furthermore, the combination of PARP inhibitor olaparib and anti-PD1 therapy was synergistic in a syngeneic transplantable GEMM (RPR2) of SCLC with increased cytotoxic T-cell infiltration in SCLC tumors treated with the combination compared with either agent alone.[105] A similar observation was made with the combination of a CHK1 inhibitor (prexasertib or SRA737) and anti-PD1 antibodies in a classic SCLC GEMM.[106] Both PARP and CHK1 inhibitors were found to induce increased expression of PD-L1, IFNβ, C-X-C motif chemokine 10 (CXCL10), and C-C Motif Chemokine ligand 5 (CCL5). In addition, the synergistic interaction of PARP and CHK1 inhibitors with PD-1 targeted agents was found to be strongly dependent on the activation of the cyclic GMP-AMP Synthase-STING pathway.[105,106] These preclinical data support the clinical evaluation of CHK1 and PARPi as rational combination partners for immunotherapy in SCLC.[107]

Apoptosis

BCL2, a key regulator of the intrinsic apoptotic pathway, is overexpressed in a subset of SCLC. Inhibitors of this pathway have been evaluated in patients with recurrent SCLC but the promise for clinical use has been limited because of modest efficacy along with high rates of hematologic toxicity.[108,109] ABT-263 (navitoclax) is a subnanomolar inhibitor of BCL2, BCL2L1 (BCL-XL), and BCL2L2 (BCL-W). Navitoclax has reported efficacy in preclinical models of SCLC.[110] Preclinical data using cell line and xenograft models exhibited synergy with concurrent inhibition of the PI3K/mTOR and BCL2 pathways.[111-113] A phase I and II study is currently evaluating the safety of this combination using navitoclax and vistusertib, a dual TORC1/2 kinase inhibitor, in patients with relapsed SCLC (NCT03366103). In addition, APG-1252, which also inhibits BCL2, BCL2L1, and BCL2L2, is also being tested as a single agent in relapsed SCLC (NCT03387332, NCT03080311). Preclinical studies suggest that BCL2 inhibitors may be most relevant to classic SCLC-A subsets,[37] suggesting biomarker information will be important in these clinical trials. As mentioned earlier, MYC can repress *BCL2* expression, consistent with functional studies showing that MYC-driven SCLC is more reliant on MCL1 than BCL2.[37] These studies also suggested that high BCL2 expression in the SCLC-A subset may be responsible for the relative resistance of these cells to Aurora kinase inhibition. Future studies are warranted to determine the utility of BCL2 family targets and to identify novel combination strategies that can sensitize SCLC to apoptosis.

Lurbinectedin (PM01183)

Lurbinectedin (PM01183) is a synthetic analog of trabectedin (Yondelis, ET-743), and belongs to the natural marine-based tetrahydroisoquinoline family of antitumor agents. It is a selective inhibitor of active transcription and binds to CG-rich sequences within the promoter region of select genes, leading to irreversible stalling and degradation of elongating RNA polymerase II on the DNA template, generation of single- and double-strand DNA breaks, and subsequent cell death.[114,115] In preclinical models, lurbinectedin can also reduce type 2 tumor-associated macrophages and modulate the inflammatory tumor microenvironment.[116,117] A phase I trial of doxorubicin (50 mg/m^2) and lurbinectedin (4 mg) as second-line therapy in 48 patients with relapsed SCLC reported a response rate of 37% to 67% with myelosuppression as the main toxicity.[118] As a single agent, lurbinectedin also revealed impressive efficacy in relapsed SCLC, particularly in patients with platinum-sensitive relapse with a response rate of 44% and median overall survival of 15.8 months.[119] The ATLANTIS study is a phase III trial of randomized patients with relapsed SCLC to receive doxorubicin plus lurbinectedin on the experimental arm and topotecan or a three-drug regimen (cyclophosphamide, doxorubicin, vincristine) on the control arm (NCT02566993). The study has completed accrual and final results are awaited to provide basis for potential regulatory approval.

Delta-Like Ligand 3

Delta-like canonical Notch ligand 3 (DLL3) is a Notch ligand with restricted expression in SCLC and other neuroendocrine tumors and a validated target for therapy. In a phase 1 clinical trial of rovalpituzumab teserine (Rova-T; SC16LD6.5), a DLL3-targeted antibody drug conjugate, objective responses were observed in 16% of 56 patients with recurrent SCLC. This was associated with a median overall survival (OS) of 5.8 months.[120] The follow-up TRINITY study enrolled 339 eligible patients with recurrent DLL3-positive SCLC to receive Rova-T (0.3 mg/kg IV every 6 weeks for two doses) as third-line treatment or beyond. Approximately 70% of these patients had tumors with high DLL3 expression (i.e., $\geq$75% cells DLL3-positive) using a standard companion immunohistochemistry assay. The confirmed response rate was 18% in all patients and 19.7% in patients with high DLL3. Median OS was identical in both populations at 5.6 months and 5.7 months, respectively. Treatment emergent adverse events attributable to Rova-T occurred in 91% of patients with serious adverse events in 30% of patients. Fatal adverse events were recorded in 10 patients.[121] TAHOE study was a randomized phase III trial of Rova-T versus topotecan as second-line treatment in recurrent SCLC (NCT03061812). This study was discontinued owing to a shorter survival noted in patients on the experimental arm versus the control arm. MERU study was a phase III trial to evaluate Rova-T as a maintenance therapy after frontline platinum-based chemotherapy in patients with extensive stage SCLC (NCT03033511). This study was terminated owing to a lack of survival benefit at the interim analysis. On the basis of these two negative studies, research and development of Rova-T has been discontinued. Rova-T dosing was limited by a toxicity profile that has been attributed to its pyrrolobenzodiazepine warhead, including persistent pleural and pericardial effusions, peripheral edema, and in some cases, anasarca. These toxicities are believed to be an off-target liability of the antibody-drug conjugate, and therefore DLL3 remains an intriguing target for multiple alternative therapeutic strategies including bispecific T-cell engagers (AMG 757) and a chimeric antigen receptor T-cell therapy (AMG 119).[121]

AMG 757 is a bispecific T-cell engager antibody construct that binds to DLL3 on the cancer cell surface and the CD3 on cytotoxic lymphocytes as a method to directly recruit immune effector cells into the tumor microenvironment. Binding brings the T-cell and cancer cell into close proximity, leading to T-cell receptor independent activation.[122,123] In preclinical models, AMG 757 exhibited strong potency against SCLC cell lines with varying levels of DLL3 receptor density. It also achieved effective antitumor effects in orthotopic models of SCLC. Finally, AMG 757 revealed good tolerability and dose-proportional exposure with an extended half-life in toxicology studies in cynomolgus monkeys.[124] The agent is currently in human clinical testing in a first-in-human phase I clinical trial designed to evaluate its safety, tolerability, and pharmacokinetics (NCT03319940). Two populations of patients with SCLC will be enrolled including patients with relapsed or refractory SCLC (part A), and as maintenance in patients with ongoing clinical benefit after frontline platinum-based chemotherapy (part B).

From a diagnostic perspective, the use of ^{89}Zr-SC16, a positron emission tomography radiotracer, is under development for in vivo imaging and as a companion diagnostic to optimize the selection of patients for treatment with DLL3-directed pharmacophores. ^{89}Zr-labeled SC16 antibody successfully delineated normal tissue from subcutaneous and orthotopic SCLC PDX models.[125] Radiotracer accumulation in tumors was directly correlated with the degree of DLL3 expression, and also correlated with response to SC16LD6.5 (Rova-

T) therapy in SCLC PDX models.[125] Ongoing attempts to further improve the imaging characteristics of the ^{89}Zr-labeled-SC16 radiotracer include evaluation of different site-selective linker chemistries with improved in vivo stability.[126]

Epigenetic inhibitors

Bromodomain and extra-terminal motif protein (BET) proteins recognize acetylated histones and recruit proteins to promoters and enhancers, especially super enhancers.[127-129] A number of small molecule BET inhibitors are currently in clinical development.[130] This class of agents inhibits the expression of cancer-related target genes including *MYC*, *MYCN*, *IL7R*, *FOSL1*, *AR*, *ER*, *BCL2*, *BCL6*, *PAX5*, *CDK4*, and *CDK6*. Limited efficacy of BET inhibitors was observed as a single agent in preclinical models of SCLC; however, activity was more promising when combined with BCL2 inhibitors or cytotoxic chemotherapy.[131-133] A study of the BET inhibitor ABBV-075 in combination with the BCL2 inhibitor venetoclax in patients with cancer, including SCLC, recently completed accrual (NCT02391480).

The polycomb repressive complex 2 (PRC2) maintains epigenetic gene silencing during normal development and tissue differentiation by methylating histone H3 at lysine 27 (K27), an inhibitory chromatin mark.[134] The methyltransferase subunit of PRC2 can be enhancer of zeste homolog 1 or 2 (EZH1/2), which use the cofactor S-adenosyl methionine as a methyl donor for H3K27 mono-, di- and tri-methylation reactions. EZH2 is known to be overexpressed or mutated in multiple cancers.[135] Although EZH2 is not frequently mutated in SCLC, it can be overexpressed at the mRNA and protein levels relative to other cancer types.[39,53] Small molecule S-adenosyl methionine mimetics that selectively inhibit EZH1/2 to different degrees are in active development; however, they have thus far exhibited limited single agent activity in preclinical models of SCLC. One study found that EZH1/2 inhibitors could reverse an epigenetic mechanism of acquired chemoresistance caused by epigenetic silencing of SLFN11.[136] In this context, inhibition of EZH1/2 could rescue SLFN11 expression and synergize with a variety of DNA damaging agents in vitro and in vivo. These results supported the initiation of a phase I and II trial evaluating the safety and tolerability of valemetostat (DS-3201b), a potent dual EZH1/2 inhibitor, in combination with irinotecan in patients with recurrent SCLC (NCT03879798).

Immunotherapy

After decades without a change in the standard of care, 2018 produced a new landmark in the treatment of SCLC, with the US Food and Drug Administration approval of atezolizumab (anti–PD-L1) in combination with first-line platinum doublet chemotherapy for extensive stage disease.[5] This approval established immune checkpoint blockade as a new treatment for SCLC, as it has been for several other lung cancer subtypes. IMPOWER 133 was a randomized phase III study of carboplatin and etoposide with or without atezolizumab. The addition of atezolizumab resulted in a statistically significant improvement in progression-free survival (PFS) (HR = 0.77, 95% CI: 0.62–0.96, $p = 0.02$) and OS (HR = 0.70, 95% CI: 0.54-0.91, $p = 0.007$). No significant difference in objective response rate was observed. In an exploratory analysis, blood-based TMB was not predictive of benefit from the addition of atezolizumab to chemotherapy. Despite the milestone significance of this advance, the benefits of atezolizumab in this context were limited—the improvements in median PFS and OS were approximately 1 and 2 months, respectively, with just over half of the patients treated with the triplet regimen alive at the 1-year mark.[5]

Initial results from the phase III CASPIAN study, similarly involving first-line carboplatin-etoposide or cisplatin-etoposide with or without durvalumab, were recently presented.[119] This study reported very similar improvements in both PFS (HR = 0.78, 95% CI: 0.65–0.94) and OS (HR = 0.73, 95% CI: 0.59–0.91) to those seen in IMPOWER 133, using a different PD-L1 inhibitor. Objective response rate was modestly higher with durvalumab (79% versus 70%; OR 1.64, 95% CI: 1.11–2.44). Results of the third arm of the CASPIAN study, adding the CTLA-4 inhibitor tremelimumab to platinum, etoposide, and durvalumab, have not yet been reported.

Excitement about the application of immune checkpoint blockade in unselected patients with SCLC was further tempered by negative results in other studies, in which the activity of immune checkpoint inhibitors alone was tested. Of note, the CheckMate 331 study, a randomized phase III study of nivolumab (anti-PD1) versus standard of care topotecan in the second-line (recurrent metastatic) setting did not reveal statistically significant improvement in both PFS and OS.[137] Posthoc subset analysis suggested a benefit from nivolumab in patients defined as chemo-resistant on the basis of duration of response to first-line platinum-based chemotherapy (HR = 0.71; 95% CI: 0.54–0.94), a population in particular need of better therapeutic options; but this result would require confirmation in a dedicated prospective study.

Putting the results of IMPOWER 133 and CASPIAN into context with other studies of immune checkpoint blockade in SCLC, it is important to emphasize that the modest improvements in median PFS and OS do not tell the full story. Notably, across several studies, a small subset of patients with SCLC seem to benefit

substantially from treatment with immune checkpoint blockade, with durable responses observed in patients treated with either single agent PD-(L)1 therapy or combined PD-(L)1 plus CTLA4 blockade.[138-141] The CheckMate 032 study was a randomized study of nivolumab and nivolumab plus ipilimumab (anti–CTLA-4). In late 2018, nivolumab was granted accelerated US Food and Drug Administration approval for third-line treatment of metastatic SCLC, based primarily on response data from a subset of patients treated on the nivolumab arm of this study. Treatment with nivolumab in the third-line setting was associated with a response rate of only 12%; but notably, these responses were durable for at least 6 months in 77% and at least 12 months in 62% of cases.

Together, these observations suggest two directions of active investigation in SCLC immunotherapy. First, to further define predictive biomarkers identifying patients with SCLC for whom immune checkpoint blockade might offer a durable benefit; and second, to build on the initial success of chemoimmunotherapy through new combinations that might impact a broader fraction of patients. Both strategies are being actively pursued.

Biomarkers of Immune Response

High TMB is increasingly recognized as an important determinant of the likelihood of response to immune checkpoint blockade across disease types.[142] Given that SCLC is predominantly a disease associated with the chemical mutagenic effects of substantial tobacco exposure, it was not clear whether any predictive TMB threshold could be established in a patient population typified by consistently high clonal mutational load. However, recent exploratory analyses from the CheckMate 032 study found that TMB could serve as a predictive biomarker in SCLC.[140] Patients with tumors in the highest tertile of TMB seemed more likely to benefit from either nivolumab or the combination of ipilimumab and nivolumab, as measured by PFS and OS. These differences were most striking in the combination arm, with 1-year survival of 62.4% in the highest TMB tertile, versus 19.6% and 23.4% survival in the middle and lowest tertile, respectively.

PD-L1 expression is also a predictive biomarker for immunotherapy across several solid tumors. However, the frequency and intensity of PD-L1 staining in SCLC tumor cells is quite low compared with NSCLCs and other solid tumors.[143] Initial data from the CheckMate 032 study suggested no correlation between PD-L1 expression and clinical benefit.[138] However, in the Keynote-028 and Keynote-158 studies of pembrolizumab (anti-PD1), PD-L1 expression, especially combined expression on tumor and immune stromal cells, was associated with improved response to pembrolizumab (35.7% versus 6%).[139,141]

Novel Immunotherapy Combinations and Targets

Beyond PD-(L)1 and CTLA-4, a number of other combinatorial approaches to augment the efficacy of immune checkpoint blockade are under active exploration. One approach showing remarkable preclinical promise are combinations of agents targeting DNA damage repair pathways and cell cycle regulators, including PARP1 and CHK1/2.[105] CDK7 is a central regulator of cell cycle progression, controlling the activity of multiple other CDK complexes involved in both G1-S and G2-M transitions. The recognition of CDK7 as a vulnerability that could be selectively targeted with an initial inhibitor, THZ1, was reported in 2015.[20] A novel and more selective inhibitor of CDK7, YKL-5-124, has recently been characterized.[144] YKL-5-124 is being extensively evaluated in preclinical models of SCLC, both as a single agent and in combination with PD-1 blockade (H. Zhang; C.L. Christensen et al., unpublished data, 2019).

Ganglioside fucosyl-GM1 (FucGM1) is a tumor-associated antigen with restricted expression in SCLC that is absent in most normal tissues.[145] BMS-986012 is a nonfucosylated, fully human IgG1 antibody that binds specifically to FucGM1. It revealed strong antitumor efficacy against SCLC cell lines and xenograft models when used as a single agent and in combination with chemotherapy and immunomodulatory agents.[145] The safety of BMS-986012 in combination with a platinum doublet was tested in patients with SCLC (NCT02949895, NCT02815592). In addition, BMS-986012 was combined with nivolumab (NCT02247349) in relapsed patients with SCLC and was safe and tolerable.[146] A promising response rate greater than 20% was recorded in this study and future development of the regimen is awaited.

A final immunologic strategy under rigorous interrogation by multiple SCLC investigators involves the harnessing of key components of the innate immune system including macrophages and natural killer cells. Macrophages are found in SCLC stroma, and many SCLCs express CD47, the “don’t eat me” signal that inhibits macrophage phagocytosis. In preclinical GEMM and PDX SCLC models, CD47 blockade can induce tumor responses in vivo.[147] SCLC can lose expression of Major Histocompatibility Complex (MHC) class I,[148] which may, in part, explain why this tumor type seems less responsive to PD-1 blockade, directed primarily at activating cytolytic T-cell responses. Loss of MHC class I on the cell surface may make SCLC susceptible to natural killer cell–activating therapies, an area of active investigation. A recent study reported that loss of MHC class I

may be driven by PRC2, and suggests the tantalizing possibility that resistance to immunotherapy may arise in SCLC through an epigenetic mechanism with the potential for pharmacologic intervention.[149]

In summary, despite recent advances in SCLC research, many key knowledge gaps remain in our understanding of the underlying pathobiology that drives this disease, and how the therapeutic liabilities that it causes can be exploited in the clinic. We have identified eight thought-provoking questions that are of significant interest to the field:

1. Which therapeutic liabilities and treatment outcomes are associated with different molecular subtypes of SCLC (SCLC-A, SCLC-N, SCLC-Y, SCLC-P)?
2. To what extent will molecular subtypes of SCLC be used in the diagnosis of SCLC? What are the best biomarkers for molecular subtype discrimination?
3. What are the potential cells of origin of SCLC and what is the impact on the natural history of the disease? Are different SCLC characteristics driven by genetics, cell of origin, lineage plasticity, or some combination of factors?
4. What roles do NOTCH and WNT signaling play in intratumoral heterogeneity and the development of acquired resistance? What are the functional roles of different cell populations to the extent that they can be defined?
5. What are the underlying mechanisms of lineage plasticity? Can this be understood and targeted?
6. How can metabolic liabilities and nutrient-sensing pathways be exploited?
7. What are the most effective ways to measure different circulating analytes with respect to early detection, treatment response, relapse, prognosis, etc.?
8. How can we improve immunotherapy outcomes? Can we identify predictive biomarkers?

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