REVIEW

Killing SCLC: insights into how to target a shapeshifting tumor

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Small cell lung cancer (SCLC) is a rapidly growing, highly metastatic, and relatively immune-cold lung cancer subtype. Historically viewed in the laboratory and clinic as a single disease, new discoveries suggest that SCLC comprises multiple molecular subsets. Expression of MYC family members and lineage-related transcription factors ASCL1, NEUROD1, and POU2F3 (and, in some studies, YAP1) define unique molecular states that have been associated with distinct responses to a variety of therapies. However, SCLC tumors exhibit a high degree of intratumoral heterogeneity, with recent studies suggesting the existence of tumor cell plasticity and phenotypic switching between subtype states. While SCLC plasticity is correlated with, and likely drives, therapeutic resistance, the mechanisms underlying this plasticity are still largely unknown. Subtype states are also associated with immune-related gene expression, which likely impacts response to immune checkpoint blockade and may reveal novel targets for alternative immunotherapeutic approaches. In this review, we synthesize recent discoveries on the mechanisms of SCLC plasticity and how these processes may impinge on antitumor immunity.

Small cell lung cancer (SCLC) is known as a neuroendocrine lung cancer that is highly aggressive and metastatic with dismal patient outcomes (Fig. 1; Rudin et al. 2019; Poirier et al. 2020). In recent years, our understanding of SCLC has undergone a series of shapeshifting transformations. For decades, SCLC was treated in the clinic and in the laboratory as a single disease, with a slow-growing appreciation for heterogeneity in tumor cell morphology, gene expression, viral tropism, and other characteristics. More recently, SCLC has been stratified based on expression of MYC family members and lineage-defining transcription factors ASCL1, NEUROD1, and POU2F3 (and initially YAP1, which has recently been called into question) (Rudin et al. 2019; Poirier et al. 2020). These molecular stratifications are important because they correlate with therapeutic responsiveness in preclinical and clinical studies. However, recent findings suggest that SCLC exhibits remarkable subtype plasticity, bringing our notion of SCLC full circle—from one disease, to a disease of multiple subtypes, and (potentially) back to one highly plastic disease. In this review, we (1) provide a brief historical overview of our changing perspective of SCLC, (2) discuss our current understanding of the therapeutic relevance of SCLC molecular subsets, (3) synthesize recent findings on mechanisms and factors regulating SCLC plasticity and how these mechanisms may impact response to immunotherapy, and (4) speculate on future directions in SCLC research.

While many timely SCLC reviews have been written by our colleagues (Rudin et al. 2019, 2021; Poirier et al. 2020; Ko et al. 2021; Schwendenwein et al. 2021), here we focus specifically on the molecular subtypes of SCLC and the emerging evidence supporting their relationships to drug sensitivities and immune response, and their highly plastic nature.

Early studies documenting phenotypic heterogeneity in SCLC

Following years of optimizing the growth of human SCLC cell lines in culture in the 1970s, Carney et al. (1985) and Gazdar et al. (1985) published back-to-back studies characterizing 50 newly created SCLC cell lines. This detailed characterization found that 70% of cell lines exhibited "classic" morphology with tightly packed, neurosphere-like aggregates and high expression of proteins associated with neuroendocrine fate. The remaining 30% of cell lines were termed "variant," a fraction of which grew in the classic morphology but harbored reduced neuroendocrine (NE) marker expression, and the remaining of which exhibited a more loosely attached morphology, resembling

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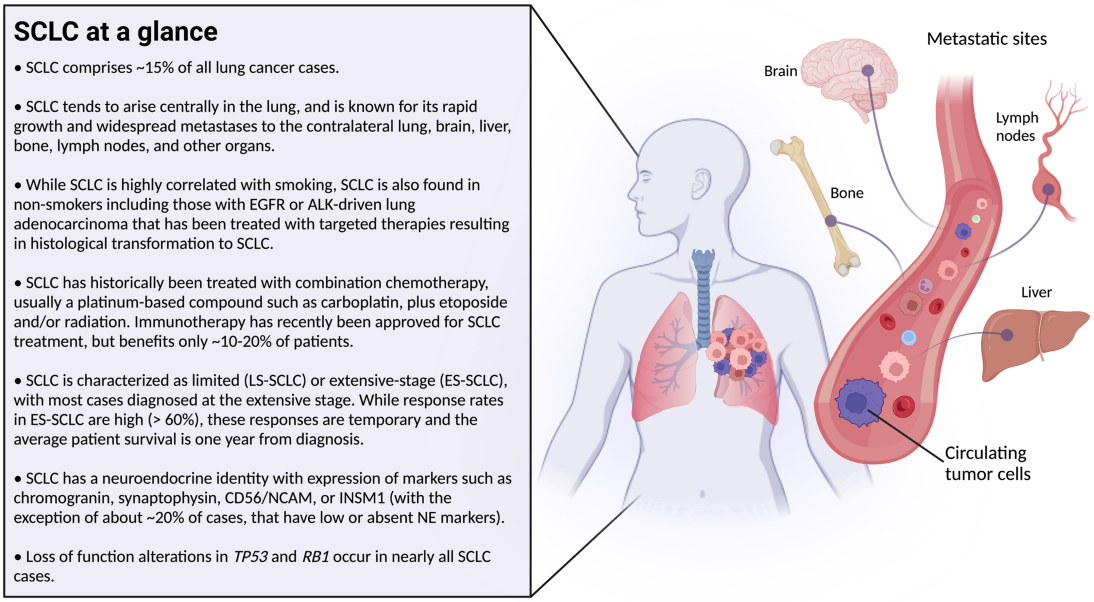

Figure 1. Basic clinical background on small cell lung cancer (SCLC) (in the text box; *left*) with illustrated sites of metastatic spread (*right*).

undifferentiated large cell carcinomas. The latter "variant" lines with reduced NE markers, the investigators presciently observed, tended to harbor *MYC* amplifications and numerous hallmarks of aggressive behavior: The MYC-high cell lines were more often derived from patients after treatment, grew faster, exhibited radio resistance, and were associated with poor response to therapy and shorter survival times (Radice et al. 1982; Little et al. 1983; Johnson et al. 1987). *MYCN* and *MYCL* amplifications and overexpression (and, in the case of *MYCL*, genomic rearrangements) were also observed in cell lines in a mutually exclusive manner with *MYC* (Nau et al. 1985, 1986; Wistuba et al. 2001; Kim et al. 2006). These studies represented some of the first examples of SCLC heterogeneity. Through comprehensive gene expression analyses of human SCLC tumors and cell lines, we now know that the non-NE SCLCs are enriched for *MYC* and markers that signal activation of Notch/Rest, Hippo/Yap1, TGF-β, and epithelial–mesenchymal transition (EMT) pathways (Zhang et al. 2018). The biological and clinical significance of these findings is becoming increasingly appreciated over time.

With the emergence of whole-genome sequencing, gene expression, and methylation profiling, we learned that loss of TP53 and RB1 function are near-universal events in SCLC (Peifer et al. 2012; Rudin et al. 2012; George et al. 2015; Poirier et al. 2015). Notch receptor loss-of-function (LOF) alterations are found in ~25% of SCLC and are mutually exclusive with alterations in chromatin remodeling genes *CREBBP* and *EP300*. In contrast to the personalized treatment approaches achieved with kinase alterations in lung adenocarcinoma, these common genomic alterations in SCLC did not immediately implicate targeted therapy approaches for specific genetic subsets. Instead, SCLC began to be stratified by expression of ASCL1 (a lineage-specific developmental transcription factor driving NE fate) and NEUROD1 (another basic helix–loop–helix transcription factor important for brain development) (Borges et al. 1997; Cau et al. 1997; Ito et al. 2000; Neptune et al. 2008; Osborne et al. 2013; Borromeo et al. 2016). Poirier et al. (2013) discovered that a relatively high NEUROD1/ASCL1 ratio correlated with sensitivity to infection with Seneca Valley virus (SVV), which preferentially inhibited growth of this subset of tumors. The receptor for SVV infection was later determined to be anthrax toxin receptor 1 (ANTXR1) (Miles et al. 2017). The SVV study represents one of the first examples of molecular subtype stratification of SCLC for therapeutic purposes in a preclinical setting.

Heterogeneity in therapeutic responses: ASCL1 vs. MYC

In the last decade, examples abound elucidating SCLC subtype-specific therapeutic responses. Multiple groups found that MYC-high subtypes of SCLC correspond with ASCL1-low samples, and this subset of SCLC responds to Aurora A/B kinase inhibition in cell lines and animal models (Hook et al. 2012; Sos et al. 2012; Helfrich et al. 2016; Cardnell et al. 2017; Mollaoglu et al. 2017; Dammert et al. 2019). More recently, CRISPR-mediated activation of specific Myc family members elegantly demonstrated that *Myc*, as opposed to *Mycn* or *Mycl*, promotes sensitivity to AURKA inhibition (Brägelmann et al. 2017; Dammert et al. 2019). These findings parallel results in clinical trials of chemotherapy-relapsed SCLC patients who received paclitaxel with or without the AURKA inhibitor alisertib, where patients with MYC-high tumors

had significantly improved progression-free survival when receiving alisertib (Owonikoko et al. 2020).

In contrast to the MYC-associated vulnerabilities, Delta-like-ligand 3 (DLL3) tends to be expressed most highly in ASCL1-high SCLC, consistent with its identification as an ASCL1 target gene (Saunders et al. 2015; Borromeo et al. 2016; Zhang et al. 2018). DLL3 is a Notch pathway inhibitory ligand, and its high surface expression in the ASCL1 subtype is consistent with the mutual antagonism observed between ASCL1 and the Notch pathway (Chen et al. 1997; Morrison et al. 2000; Ball 2004; Somasundaram et al. 2005; Lim et al. 2017), discussed in more detail later. The DLL3 targeted antibody drug conjugate rovalpituzumab tesirine (Rova-T) has been evaluated in clinical trials and was more effective against DLL3-expressing tumors, albeit limited by toxicities (Saunders et al. 2015; Rudin et al. 2017; Morgensztern et al. 2019). Additional DLL3 targeting approaches are currently under development (Owen et al. 2019).

In addition to the two clinical examples above, preclinical studies have stratified SCLC molecular subsets based on unique cell death phenotypes. BH3 profiling, a functional assay that measures mitochondrial depolarization in response to stimulation with various BH3 peptides, demonstrated that MYC-high SCLC is more apoptotically primed than MYC-low cell lines (Dammert et al. 2019). This observation is consistent with higher basal levels of apoptosis in *Myc*-high compared with *Myc*-low tumors in genetically engineered mouse models (GEMMs) (Mollaoglu et al. 2017). BH3 profiling also identified MCL1 as an apoptotic vulnerability in MYC-high cell lines, but this remains to be evaluated in preclinical GEMMs, hampered by the reduced affinity of the MCL1-specific inhibitor S63845 to murine MCL1 (Kotschy et al. 2016; Brennan et al. 2018). In contrast, multiple groups have noted that the prosurvival protein BCL2 is enriched in the classic ASCL1-high (*MYC*-low) subset of SCLC (Augustyn et al. 2014; Poirier et al. 2015; Borromeo et al. 2016; Cardnell et al. 2017; Lochmann et al. 2018; Dammert et al. 2019). Indeed, *BCL2* has been shown to be an ASCL1 target gene (Augustyn et al. 2014; Borromeo et al. 2016) that can be repressed by MYC expression (Dammert et al. 2019), providing rationale to revisit the efficacy of BCL2 inhibitors in the ASCL1-high setting. A more recent study suggests that the machinery required for extrinsic apoptosis and necroptosis is lowly expressed in SCLC, but a subset of SCLC is enriched for ferroptosis pathway genes (Bebber et al. 2021). SCLC cells that are ASCL1-low (often expressing multiple EMT markers) tend to be more sensitive to ferroptosis induction (Bebber et al. 2021), reminiscent of studies suggesting mesenchymal fate is predictive of ferroptosis sensitivity in multiple cancer types (Hangauer et al. 2017; Viswanathan et al. 2017; Jiang et al. 2021). Recent studies also suggest SCLC can undergo pyroptosis (Wu et al. 2021). How SCLC molecular subsets are wired for sensitivity to diverse forms of cell death and how cell death processes impact immune response should be important areas of further study.

In addition to differences in cell death regulation, MYC-high/ASCL1-low SCLC tends to be more proliferative and glycolytic with unique metabolic vulnerabilities (Mollaoglu et al. 2017; Huang et al. 2018a; Cargill et al. 2021). Unbiased metabolite profiling experiments on cell lines and murine tumors have identified key metabolic differences in ASCL1-high versus ASCL1-low SCLC subtypes. For example, Huang et al. (2018a, 2021) found that purine pathway metabolites, particularly guanosine nucleotides, are highly enriched in ASCL1-low cell lines. ASCL1-low cell lines and tumors have higher expression of *IMPDH1* and *IMPDH2*, known MYC target genes that are key for guanine nucleotide biosynthesis (Mannava et al. 2008). Increased IMPDH expression is associated with dependency on these enzymes, conferring sensitivity to mycophenolic acid (MPA) in vitro and mizoribine in vivo, both of which are used in clinical settings as immunosuppressants (Dayton et al. 1992). Metabolite profiling studies were also key to the discovery that *Myc*-high/*Ascl1*-low tumors are exquisitely sensitive to depletion of exogenous arginine with pegylated arginine deiminase (ADI-PEG20) in GEMMs and human xenografts (Chalishazar et al. 2019). Taken together, MYC-high/ASCL1-low SCLC appears to be highly proliferative and glycolytic, but primed for cell death and/or ferroptosis specifically, suggesting this aggressive level of tumor growth confers unique therapeutic vulnerabilities.

Inactivating mutations in *MAX*, the protein product of which is a heterodimer of MYC family members, are found in ~6% of SCLC (Romero et al. 2014). MAX was found to be a tumor suppressor whose loss leads to derepression of serine and one-carbon pathway genes (Augert et al. 2020). Recent studies suggest that MAX-deficient SCLC is predominantly of the ASCL1 subtype and does not depend on any MYC family member (Llabata et al. 2021). These studies beg the question of whether a non-MYC family member state of SCLC is phenotypically and/or metabolically distinct from other molecular subsets.

Despite the generally higher rate of proliferation and cell cycle vulnerabilities associated with MYC-high/ASCL1-low SCLC, a recent study showed that it is the NE-high ASCL1$^+$ subgroup of SCLC that has high replication stress, which responds preferentially well to ATR inhibition in clinical trials (Thomas et al. 2021). Other preclinical studies have suggested that SCLC is vulnerable to ATR and CHK1 kinase inhibition (Cardnell et al. 2017; Doerr et al. 2017; Sen et al. 2017; Dammert et al. 2019), but some studies suggest that MYC-high SCLC is the most sensitive. Thus, there remain outstanding questions as to how SCLC subsets are metabolically wired to drive proliferation, and how this wiring creates therapeutic vulnerabilities. Given that metabolic and proliferative characteristics of in vivo tumors are not easily mimicked in a dish (Muir et al. 2018), it will be crucial to elucidate these vulnerabilities further in physiologically relevant systems.

A revised and evolving classification scheme based on lineage-related transcription factors

The field has recently converged on finer resolution of SCLC molecular subsets by gene expression analysis,

defined by ASCL1 (SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P), or (initially) YAP1 (SCLC-Y). The majority of SCLCs express ASCL1, estimated at ~70% of cases by gene expression (Rudin et al. 2019) and protein analyses (Baine et al. 2020). NEUROD1 is expressed in ~11% of SCLC cases by gene expression (Rudin et al. 2019) but is detected in ~45% of cases at the protein level (Baine et al. 2020), likely because bulk gene expression underestimates the frequency of samples with sparsely positive cells. When analyzed at the protein level, the majority of NEUROD1$^+$ tumor samples also have ASCL1$^+$ cells, while ~17% of SCLC cases are NEUROD1-predominant (Baine et al. 2020). Both SCLC-A and SCLC-N subtypes tend to be NE-high and express *MYCL*, with SCLC-N having distinct neuronal signatures compared with SCLC-A (Borromeo et al. 2016; Zhang et al. 2018; Chan et al. 2021; Patel et al. 2021). While most studies have not carefully sought to determine whether ASCL1 and NEUROD1 are coexpressed at the protein level in the same tumor cells, single-cell transcriptional approaches have suggested that a minority subset of tumor cells (7%–9%) expresses both markers simultaneously in mouse and patient tumors (Ireland et al. 2020; Chan et al. 2021). The co-occurrence of ASCL1 and NEUROD1 in individual tumor cells could portend subtype plasticity and the presence of transitional states and/or could reflect the inadequacy of individual markers to define a single subtype, discussed in more detail later.

POU2F3 is a master driver of the tuft or brush cell lineage, a rare cell type in the lung and other tissues that exhibits chemosensory functions (Montoro et al. 2018; Plasschaert et al. 2018; Huang et al. 2018b). POU2F3 expression is observed in ~16% of SCLC cases by gene expression and in ~7% of cases at the protein level, which tend to be mutually exclusive with ASCL1 and NEUROD1. SCLC-P tumors have a low neuroendocrine gene expression program, consistent with their low expression of ASCL1 and NEUROD1. SCLC-P tumor cell lines depend on POU2F3 for viability, and this tumor subset has been shown to preferentially depend on IGF-1R and PARP signaling (Huang et al. 2018b; Gay et al. 2021) and may be enriched for MCL1 expression (Yasuda et al. 2020), reiterating the notion that distinct SCLC subtypes harbor unique therapeutic vulnerabilities. Further studies will be needed to determine whether POU2F3 co-occurs with other subtype markers in the same tumor cells and whether it can arise during subtype evolution.

Importantly, GEMM tumors that express high levels of ASCL1, such as those from the *Rb1*$^{fl/fl}$;*p53*$^{fl/fl}$ (RP), *Rb1*$^{fl/fl}$;*p53*$^{fl/fl}$;*Pten*$^{fl/fl}$ (RPP), *Rb1*$^{fl/fl}$;*p53*$^{fl/fl}$;*Rbl2*$^{fl/fl}$ (RPR2), and *Rb1*$^{fl/fl}$;*p53*$^{fl/fl}$;*Rlf-Mycl* fusion mice, are highly neuroendocrine and express or amplify *Mycl* (Dooley et al. 2011; Huijbers et al. 2014; McFadden et al. 2014; Semenova et al. 2016; Mollaoglu et al. 2017; Ciampricotti et al. 2021), similar to human tumors. In contrast, tumors driven by *Myc* (*Rb1*$^{fl/fl}$;*p53*$^{fl/fl}$;*LSL-Myc*$^{T58A/T58A}$ [RPM] mice) often exhibit reduced ASCL1 and *Mycl* and can express NEUROD1, POU2F3, and/or YAP1 in a context-specific manner (Mollaoglu et al. 2017; Ireland et al. 2020). Thus, these molecular subtype states appear to be conserved between mice and humans, although the mechanisms that lead to these states are not yet fully understood. Interestingly, these studies demonstrate that NE cells have the capacity to give rise to ASCL1$^+$, NEUROD1$^+$, and YAP1$^+$ subtypes, given specific genetic conditions. In contrast, POU2F3$^+$ tumors arose in the presence of stabilized mutant MYC from an unknown cell of origin using CMV-Cre (Ireland et al. 2020), potentially a tuft cell, but this requires further study to definitively conclude. It is important to note that this finding does not exclude the possibility that SCLC-P tumors could arise from a NE cell of origin, given the proper (epi)genetic conditions (Ireland et al. 2020; Olsen et al. 2021; Chen et al. 2022). Although NE cells are recognized as a major cell of origin for SCLC (Park et al. 2011; Sutherland et al. 2011), it has been demonstrated that other lung cells, including club and alveolar type II (AT2) cells, can give rise to NE-high SCLC in the context of high MYC and loss of *Rb1* and *p53* (Olsen et al. 2021; Chen et al. 2022). These findings emphasize that SCLC may arise from multiple cell lineages in a context-specific manner and that adult differentiated cells can exhibit a high degree of cellular plasticity under potent genetic conditions.

Recent studies have called into question the existence or relevance of YAP1 expression in SCLC (Baine et al. 2020; Gay et al. 2021). YAP1 was originally determined to represent ~2% of SCLC cases by gene expression analysis (Rudin et al. 2019). However, when SCLC cell lines were taken into consideration, the frequency of YAP1$^+$ SCLC has been estimated to be as high as ~10% (Ireland et al. 2020), and a recent study of human tissue also reported YAP1 protein in ~10% of SCLC (Owonikoko et al. 2021). SCLC-Y was noted to be non-NE and express multiple markers associated with EMT, including vimentin (VIM), SNAI2, and CD44 (Zhang et al. 2018; Rudin et al. 2019; Ireland et al. 2020). Importantly, recent analyses suggest that *YAP1* mRNA expression fails to distinguish a clear subset of tumors that is distinct from the ASCL1, NEUROD1, and POU2F3 subsets (Gay et al. 2021). Consistent with that notion, protein analyses of 174 tumor samples found low expression of YAP1 that did not appear to be exclusive of other subtypes (Baine et al. 2020). As YAP1 appears to be more commonly expressed in SCLC cell lines than tumors, this calls into question whether microenvironmental or mechano–transduction signals may induce or select for YAP1 (Sun and Irvine 2016). YAP1 can also be present in stromal cells, which makes it difficult to distinguish from tumor cells with bulk analysis approaches. However, YAP1 can be expressed in late stage tumors in multiple mouse models of SCLC (Ireland et al. 2020; Wu et al. 2021) and has been detected in human SCLC circulating tumor cell-derived xenografts (CDX) at variable proportions (Pearsall et al. 2020). These data suggest that YAP1 could be the result of stromal contamination and/or could represent a late stage of SCLC progression. Toward the latter possibility, recent functional studies show that ectopic expression of YAP1 or TAZ (WWTR1) can promote a non-NE fate (Horie et al. 2016; Wu et al. 2021; Jin et al. 2022). Conversely, recent studies suggest that YAP1 may serve as a prognostic

biomarker for limited-stage disease and tumors exhibiting a T-cell inflamed phenotype (Tlemsani et al. 2020; Owonikoko et al. 2021). Finally, to further complicate the issue, YAP1 has been associated with *RB1* proficiency in cell line studies (McColl et al. 2017; Tlemsani et al. 2020), questioning whether these cells are large cell neuroendocrine, which share morphological similarities with variant SCLC (Sonkin et al. 2019). Hence, the controversy surrounding the clinical significance of YAP1 will be an active area of investigation to resolve these issues and to evaluate its potential implications for immunotherapy response, discussed in more detail below.

In the midst of the development and refinement of the SCLC molecular classification scheme (Rudin et al. 2019), numerous studies began to suggest that tumor heterogeneity can change, particularly during the course of chemotherapy treatment. CDX and patient-derived xenograft (PDX) models provide a unique means to interrogate changes in tumor composition of individual human tumors before, during, and after a given therapy. Specifically, CDX/PDX models have been used to identify molecular and phenotypic changes associated with chemoresistance in SCLC. With these and other models, multiple studies demonstrated that MYC expression and the non-NE SCLC phenotype increase following chemotherapy treatment, while NE identity decreases (Drapkin et al. 2018; Wagner et al. 2018; Chalishazar et al. 2019; Simpson et al. 2020; Stewart et al. 2020; Gay et al. 2021; Huang et al. 2021). For example, in a cohort of 19 SCLC PDX models, a MYC transcriptional signature correlated with resistance to etoposide and platinum (Drapkin et al. 2018). In a matched pair of CDX, the percentage of MYC^+ cells increased with tumor progression, concordant with increased expression of Notch signaling and EMT markers (Simpson et al. 2020). These findings are consistent in GEMMs, where high expression of MYC or MYCN and low expression of NE markers correspond with poor response to chemotherapy (Mollaoglu et al. 2017; Grunblatt et al. 2020). In human cell lines, MYC protein levels also increased (while MYCL decreased) following selection for chemoresistance (Wagner et al. 2018; Chalishazar et al. 2019; Huang et al. 2021). ASCL1 was also found to be significantly reduced in chemotherapy-resistant cell lines and in a cohort of human tissue samples following chemotherapy relapse (Wagner et al. 2018). Taken together, these findings suggest that $ASCL1^+$ tumor cells are more vulnerable to chemotherapy and potentially outcompeted by non-NE or MYC-high cells during treatment, and/or that $ASCL1^+$ cells can dedifferentiate to a non-NE phenotype as a mechanism of acquired resistance. Indeed, MYC and ASCL1 have been identified in distinct superenhancers (Christensen et al. 2014; Borromeo et al. 2016), suggesting that epigenetic and transcriptional states may evolve during chemotherapy resistance. Moreover, in a panel of eight CDX/PDX models, intratumoral heterogeneity (as defined by the average distance between the normalized expression profiles of each cell and all other cells in a sample) increased in chemotherapy-relapsed samples (Stewart et al. 2020). This study further supports the provocative notion that pre-existing heterogeneity allows selection to occur during therapy and/or that cells have the capacity for plasticity during resistance (Fig. 2). Importantly, the underlying mechanisms that promote transcriptional heterogeneity and/or plasticity are still relatively undefined and a major focus of current investigations.

Mechanisms of SCLC plasticity

Plasticity is a crucial mechanism that endows tumor cells with the capacity to convert to a distinct cell identity in response to processes, including external cues and/or stress, and has been implicated in drug resistance (Boumahdi and de Sauvage 2020). One of the first demonstrations of SCLC plasticity originated from studies of the RP GEMM. Calbo et al. (2011) described the coexistence of NE and non-NE tumor cell populations in murine SCLC. Akin to the human SCLC cell lines generated by Gazdar et al. (1985), NE cells exhibited a nonadherent growth pattern when cultured in vitro, characterized by high expression of *Mycl* and *Ascl1*. Conversely, non-NE cells derived from the RP model displayed a propensity for adherent growth and exhibited phospho-ERK signaling and mesenchymal-like characteristics, including expression of CD44 and VIM (Calbo et al. 2011; Kwon et al. 2015; Zhang et al. 2018). Importantly, genomic studies of NE and non-NE clonal cell lines derived from a single tumor shared genomic aberrations, suggesting that SCLC tumor cells have the capacity to undergo phenotypic switching. Interestingly, enforced expression of oncogenic KrasV12 drove the dedifferentiation of NE cells to a more non-NE state (Table 1; Calbo et al. 2011). Ras is a master driver of mitogen-activated protein kinase (MAPK) signaling, and early studies suggested that MAPK activation can promote cell cycle arrest and a reduction in NE markers in a small number of SCLC cell lines (Ravi et al. 1998, 1999). Recent studies have confirmed that hyperactivation of RAS, MEK, or ERK can suppress NE differentiation in SCLC and inhibit the growth of NE SCLC cells (Caeser et al. 2021; Inoue et al. 2021). Ras/Raf/Mek/Erk pathway mutations are conspicuously low in SCLC compared with lung adenocarcinoma, consistent with an incompatibility of Ras activation with neuroendocrine fate. While MAPK activation clearly facilitates lung tumor growth of the adenocarcinoma lineage, NE-high SCLC appears to suppress MAPK signaling through mechanisms that are still not fully understood.

Notch pathway activity is a recurrent mechanism implicated in lineage switching in SCLC (Lim et al. 2017; Ireland et al. 2020; Patel et al. 2021; Wu et al. 2021). ASCL1 and the Notch pathway have a mutually antagonistic relationship in lung development and cancer. For example, HES1, a highly conserved target of Notch signaling, can act as a transcriptional repressor of *ASCL1* expression (Chen et al. 1997). Consistently, during lung development, *Hes1* or *Notch1/2/3* knockout expands NE progenitor cells (Ito et al. 2000), while *Ascl1* loss ablates NE cells (Borges et al. 1997; Kiyokawa and Morimoto 2020). Notch signaling can also promote the proteasomal degradation of

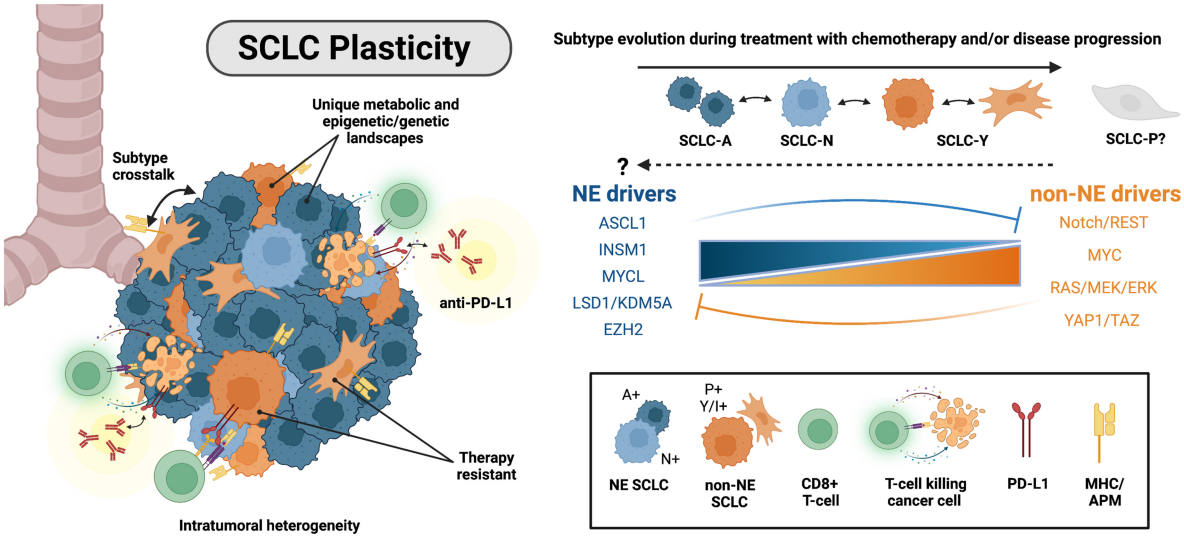

Figure 2. Intratumoral heterogeneity and mechanisms of plasticity in SCLC. (*Left*) SCLC cells within individual human tumors are classified as NE-high (SCLC-A and SCLC-N subtypes) or non-NE SCLC (SCLC-Y and SCLC-P subtypes). Non-NE tumor cells are immunomodulatory and have an increased response to immune checkpoint blockade (ICB). (*Top right*) SCLC cells demonstrate subtype plasticity. SCLC-A cells can evolve to SCLC-N and SCLC-Y. It remains unknown whether (1) SCLC-P can evolve to or from the other subtypes or (2) non-NE cells can convert back to a NE-high phenotype. (*Middle right*) Molecular mechanisms implicated in driving NE to non-NE SCLC cell fate transition.

ASCL1 in NE cells (Sriuranpong et al. 2002). Together, these studies demonstrate a critical role for ASCL1/Notch mutual antagonism during lung development. ASCL1 transcriptionally induces expression of Notch-activating ligand *Dll1*; DLL1 then acts on neighboring cells to promote Notch signaling, which represses ASCL1 in those neighboring cells (Nelson et al. 2009; Shimojo et al. 2016; Kiyokawa and Morimoto 2020)—a pattern-forming process termed "lateral inhibition." Lateral inhibition leads to a "salt and pepper"-like pattern of cell identities, which are observed during lung development (Morimoto et al. 2010) and in tumors from mouse models of SCLC (Lim et al. 2017). In SCLC, Notch directly activates expression of *HES1* and the neuronal transcriptional repressor *REST*. REST silences NE target genes and promotes a non-NE switch in tumor cells (Table 1; Lim et al. 2017). Thus, Notch signaling is highly associated with the non-NE, ASCL1-low SCLC state. Consistently, SCLC tumors with *NOTCH* LOF tend to express ASCL1 and have a NE-high phenotype (Lim et al. 2017; Ireland et al. 2020). SCLC tumors with active Notch signaling also express EMT markers, like VIM and ZEB1/2, YAP1/TAZ, and MYC—potential drivers of SCLC plasticity, discussed below.

Recent studies demonstrate that MYC is sufficient to promote the conversion of classic SCLC to a variant morphology and to drive SCLC sequentially from an ASCL1$^+$ to a NEUROD1$^+$ to a YAP1$^+$ state from a NE cell of origin (Mollaoglu et al. 2017; Ireland et al. 2020). Single-cell transcriptional profiling of RPM tumors in vitro and in vivo suggested that MYC promotes subtype evolution (Ireland et al. 2020), with similar results in human cell lines (Table 1; Patel et al. 2021). Mechanistically, MYC directly activates pro-Notch factors, induces REST expression, and converts cells from an NE-high to an NE-low state (Ireland et al. 2020). Consistently, human NE-low SCLC is more likely to be *NOTCH* wild type and express *MYC* (Ireland et al. 2020). Functional studies suggest that MYC depends on Notch (Ireland et al. 2020) or REST in a Notch-independent manner (Patel et al. 2021) for SCLC subtype plasticity; discrepancies in these two studies may imply that genetic or environmental context may determine how MYC promotes plasticity.

Mechanisms driving SCLC plasticity may co-opt normal processes that occur during lung development and injury repair. In normal lungs, a specific subpopulation of *Notch2*$^+$ NE cells (termed "NE stem cells") responds to injury with proliferation, outward migration, and subsequent differentiation into other lung cell fates—a process that is restrained by Rb1 and p53 (Ouadah et al. 2019). Loss of Rb1/p53 can promote self-renewal of *Notch2*$^+$ NE stem cells, even in the absence of injury. Following injury, Notch signaling is necessary and sufficient to initiate deprogramming, which is critical for differentiation into other cell fates. Constitutive Notch is not sufficient to induce transit amplification or differentiation of deprogrammed cells into alternate lung cell fates, like club or AT2 cells, suggesting a secondary signal is required. Given the ability of MYC to drive cell cycle entry and non-NE fate in SCLC, the secondary signal may impinge on MYC during lung injury response, although this role for MYC remains unexplored.

Notch pathway ligands and receptors are transcriptional targets of the paralogs YAP1 and TAZ (WWTR1) (Totaro et al. 2018), transcriptional coactivators and downstream effectors of the Hippo signaling pathway. YAP1/TAZ are

Table 1. *Mechanisms driving SCLC plasticity and their effect on tumor cell immunogenicity*

Gene	Tumor cell phenotype	Immunogenicity	Reference
CREBBP/EP300	Loss of *Crebbp/Ep300* decreased levels of H3K27Ac and epithelial marker expression and increased mesenchymal markers associated with non-NE SCLC. In another study, low H3K27Ac was observed in NE-high SCLC. Thus, the impact of CREBBP/EP300 activity on the NE phenotype of SCLC is still ambiguous.	Not examined	Jia et al. 2018; Inoue et al. 2021
EZH2	Genetic and/or pharmacological inactivation of EZH2 in human and murine NE cells promoted their conversion to a non-NE phenotype.	Reactivation of MHC-I expression resulted in increased T-cell-mediated tumor cell killing. EZH2i synergizes with ICB and STING agonism to enhance antitumor immunity.	Burr et al. 2019; Mahadevan et al. 2021
KDM5A	Genetic and/or pharmacological inactivation of KDM5A in ASCL1$^+$ human and murine SCLC tumor cells decreased NE marker expression, including ASCL1 and induced NOTCH signaling, via activation of NOTCH2.	Not examined	Oser et al. 2019
RAS/MEK/ERK	Enforced expression of oncogenic RasV12 in NE cells derived from RP mice or RasV12 or EGFR R858 in NE human cell lines drove a transition to an adherent non-NE-like phenotype via hyperactivation of ERK.	Not examined	Calbo et al. 2011; Inoue et al. 2021
LSD1	Pharmacological inhibition of LSD1 resulted in the activation of NOTCH signaling and reduced expression of ASCL1.	Ectopic expression of ZFP36L1, an LSD1-regulated gene, triggered MHC-I expression and the induction of immune signatures, including an IFN-γ gene signature.	Takagi et al. 2017; Augert et al. 2019; Chen et al. 2021a
MYC	Enforced expression of MYC drives SCLC subtype switching from SCLC-A to SCLC-N to SCLC-Y. MYC induces activation of the NOTCH/REST pathway and EMT transcriptional programs. MYC expression in the context of *Rb1/p53* loss allows SCLC tumorigenesis in multiple cells of origin.	*MYC* expression is significantly associated with clinical benefit to ICB vs. *MYCL* or *MYCN*.	Mollaoglu et al. 2017; Ireland et al. 2020; Patel et al. 2021; Olsen et al. 2021; Roper et al. 2021; Chen et al. 2022
NOTCH/REST	Induces REST expression and promotes non-NE phenotype	High Notch correlates with clinical benefit to ICB. Ectopic N1ICD enhances expression of antigen-presenting machinery in human SCLC cell lines.	Lim et al. 2017; Roper et al. 2021
YAP1/TAZ	YAP1 overexpression promotes non-NE phenotype switching in mouse models of SCLC. TAZ manipulation alters NE/non-NE cell fate markers.	SCLC-Y may be related to SCLC-I, the subtype most sensitive to ICB. High YAP1 expression correlates with increased HLA and MHC-I expression in human SCLC tumors and cell lines.	Tlemsani et al. 2020; Owonikoko et al. 2021; Wu et al. 2021; Jin et al. 2022

negatively regulated by Hippo kinases and have been implicated in SCLC plasticity and tumor progression (Zhang et al. 2018; Ireland et al. 2020; Wu et al. 2021). *YAP1* positively correlates with expression of *TAZ*, *NOTCH1/2/3*, and *REST* in human SCLC cell lines (Tlemsani et al. 2020). In SCLC tumors of RPP mice with constitutively activated forms of YAP1 (i.e., YAP-S127A or YAP-5SA), NE differentiation was lost and non-NE markers, including *Notch2*, *Vim*, and Notch targets HES1 and REST, were expressed (Table 1; Wu et al. 2021), suggesting that YAP1/TAZ may act through Notch to promote NE dedifferentiation. Consistently, SCLC tumors of RPP GEMMs with genetic deletion of *Yap1* and *Taz* lacked HES1 expression. YAP1/TAZ may also have a Notch-independent role in driving non-NE fate of SCLC, as YAP1 activation in RPP GEMM tumors prevented NE differentiation, even

when Notch activity was blocked via γ-secretase inhibition or *Rbpj* knockout (Wu et al. 2021). Whether YAP1/TAZ act through MYC or Ras to induce non-NE SCLC fate is yet to be reported.

Increasing evidence supports a key role for epigenetic regulators in SCLC plasticity. Initially identified as a mechanism driving chemoresistance (Gardner et al. 2017), the histone methyltransferase (HMT) EZH2 has been implicated in controlling NE cell fate. EZH2 is the enzymatic subunit of the Polycomb-repressive complex 2 (PRC2), involved in gene repression through catalyzing trimethylation of histone H3K27. Consistent with an emerging role for EZH2 in the maintenance of NE cell fate, pharmacological inhibition of EZH2 in NE-high SCLC cell lines triggered cellular adherence and the transition to a non-NE phenotype (Table 1; Mahadevan et al. 2021). Interestingly, the cell fate change induced by EZH2 was concomitant with derepression of MHC-I expression (Burr et al. 2019; Mahadevan et al. 2021), implicating EZH2 in immune cell evasion, as discussed in greater detail below. Truncating mutations in the H3K4 HMT gene *KMT2D* (also known as *MLL2*) have been identified in primary SCLC tumors and cell lines (Augert et al. 2017). Human SCLC cell lines harboring either homozygous or heterozygous mutations in *KMT2D* displayed a reduction in histone H3K4 monomethylation, a histone mark that has been associated with active enhancers (Heintzman et al. 2007). The functional role of KMT2D in SCLC tumorigenesis and tumor cell plasticity remains unexplored.

The histone acetyltransferases (HATs) CREBBP (also known as CBP) and its paralog, EP300 (also known as p300), are commonly mutated in SCLC (George et al. 2015; Augert et al. 2017). CREBBP/EP300 have been implicated in cell cycle control and transcriptional control of enhancers (Jin et al. 2011; Attar and Kurdistani 2017), but whether they promote NE versus non-NE SCLC fate remains uncertain. In one study, conditional loss of *Crebbp/Ep300* in RP GEMMs significantly reduced H3K27Ac levels and increased sensitivity to HDAC inhibition (Jia et al. 2018). In this study, *Crebbp/Ep300* loss led to a decrease in epithelial markers such as CDH1 (encoding E-cadherin), associated with NE-high SCLC, and a gain in mesenchymal markers that are associated with non-NE SCLC, including ZEB1 and VIM (Jia et al. 2018). In contrast, another study showed that H3K27Ac levels are reduced in NE-high SCLC and that ERK-mediated activation of CREBBP/EP300 is important for non-NE lineage transformation (Table 1; Inoue et al. 2021). Interestingly, *CREBBP/EP300* genomic alterations are mutually exclusive with Notch pathway mutations, such that it is tempting to speculate that Notch could signal through CREBBP/EP300 to promote non-NE fates. Further studies are warranted to reconcile these disparate findings and conclusively delineate the contribution of CREBBP/EP300 to SCLC cell fate.

Notably, multiple epigenetic factors implicated in SCLC cell fate alter Notch activity. Inactivation of the histone demethylases LSD1/KDM1A and KDM5A, both of which demethylate H3K4, induces NE dedifferentiation via down-regulation of ASCL1 (Mohammad et al. 2015; Takagi et al. 2017; Augert et al. 2019; Oser et al. 2019). LSD1/KDM1A and KDM5A have been shown to act synergistically to repress Notch and promote ASCL1 expression (Oser et al. 2019), suggesting independent or parallel mechanisms of action (Table 1). Additionally, histone deacetylase inhibitors like Trichostatin A can induce NE dedifferentiation in SCLC by activating Notch and REST (Augert et al. 2019; Wu et al. 2021). Finally, while genes encoding chromatin remodelers CHD7 and PBRM1 (whose protein product is part of the SWI/SNF remodeling complex) are also found altered in SCLC (George et al. 2015; Augert et al. 2017), their impact on SCLC cell fate and plasticity is relatively unexplored. Together, these studies emphasize that changes in chromatin structure are undoubtedly critical for SCLC plasticity. However, it is still unclear how the epigenetic regulators discussed above impact specific SCLC-A, SCLC-N, SCLC-P, and SCLC-Y states. Whether and how plasticity drivers, including Ras, Notch, Myc, and Yap1/Taz, impinge on these epigenetic regulators in SCLC are also largely unknown.

It is likely that additional molecular mechanisms underlie lineage plasticity. NFIB, a transcription factor with an oncogenic role in SCLC (Semenova et al. 2016; Wu et al. 2016), drives a global increase in chromatin accessibility that augments the metastatic capacity of SCLC tumor cells (Denny et al. 2016). Also, in the developing lung, INSM1 is a key regulator of pulmonary NE cell differentiation (Jia et al. 2015), while in SCLC cell lines, a reciprocal relationship between INSM1 and YAP1 has been demonstrated (McColl et al. 2017; Tlemsani et al. 2020). INSM1 can inhibit Notch activity by repressing *Hes1* (Jia et al. 2015), and INSM1 may also be inhibited by Notch in a mutually inhibitory relationship (Fujino et al. 2015). While ASCL1 knockout prevents tumorigenesis in classic SCLC models (Borromeo et al. 2016), ASCL1 knockout in the context of MYC-driven tumors leads to a neural crest and/or mesenchymal stem-like state with activation of many of the pathways associated with non-NE tumors, including Notch/Rest, TGF-β, and Hippo/Yap1 (Olsen et al. 2021). In addition, ASCL1 loss leads to induction of SOX9 (Olsen et al. 2021), which is associated with non-NE SCLC fate and can act downstream from MAPK signaling (Inoue et al. 2021). SOX9 mediates lineage plasticity in other tissues (Christin et al. 2020; Nouri et al. 2020) but remains relatively unexplored in SCLC. Computational methods have been used to predict candidate regulators of lineage plasticity and cell fate changes in SCLC (Wooten et al. 2019; Chan et al. 2021; Chauhan et al. 2021). These studies support a role for Ras/Mapk, Notch, Myc, Yap1/Taz, and epigenetic factors as determinants of SCLC plasticity, and more factors remain to be explored. Moving forward, unbiased -omic methodologies may represent powerful approaches to identify key drivers and mechanisms of subtype switching.

Although multiple mechanisms that drive NE dedifferentiation of SCLC toward a non-NE phenotype have been identified, the full extent of SCLC plasticity is still not well understood. For example, while Notch activation has been shown to promote an NE-to-non-NE transition in SCLC, studies suggest that Notch blockade cannot

reverse cells back to an NE-high state (Lim et al. 2017; Ireland et al. 2020; Wu et al. 2021). In fact, little to no evidence exists to date to support the occurrence of a non-NE-to-NE transition in SCLC. However, other non-NE, epithelial cancers can undergo NE transformation in the context of resistance to targeted therapies. Specifically, EGFR-driven lung adenocarcinoma is known to convert to SCLC during resistance to tyrosine kinase inhibition (Niederst et al. 2015; Oser et al. 2015; Quintanal-Villalonga et al. 2021). Analysis of these samples suggest that adeno-to-SCLC conversion has the capacity to give rise to all four subtypes, albeit YAP1 expression was higher in the lung adenocarcinoma component (Quintanal-Villalonga et al. 2021). Prostate adenocarcinoma treated with androgen deprivation therapy can also convert to neuroendocrine prostate carcinoma (NEPC) that shares many similarities to SCLC, including expression of ASCL1 and NEUROD1 (Cejas et al. 2021; Kaarijärvi et al. 2021). Whether non-NE subtypes of SCLC can use similar mechanisms of plasticity to transition to an NE-high state remains an open question. Moreover, whether the SCLC cell of origin dictates or constrains the directionality of SCLC plasticity is yet to be investigated. Advanced lineage tracing approaches could be used to gain a deeper understanding of tumor evolution during disease progression and in response to chemotherapy. Specifically, CRISPR- or barcode-based lineage tracing paired with single-cell technologies, such as those recently used in the context of hematopoietic malignancies (Fennell et al. 2022), could be used to identify nongenetic mechanisms that govern SCLC plasticity and drug resistance. Combining epigenetic drugs with additional therapies may hold promise for treating SCLC and will undoubtedly be an area of increased focus in the coming years.

Tumor heterogeneity and the immune microenvironment

SCLC has a high tumor mutational burden (TMB) (Alexandrov et al. 2013) and harbors a prevalence of C > A transversions—a result of DNA adducts forming between tobacco carcinogens and guanine nucleotides (Pleasance et al. 2010; Alexandrov et al. 2013, 2016; George et al. 2015). While high TMB may correlate with increased neoantigen production (Schumacher and Schreiber 2015) and favorable response to immune checkpoint inhibitors anti-PD-L1/PD1 in NSCLC (Ready et al. 2019), this does not appear to be the case for SCLC (Hellmann et al. 2018; Horn et al. 2018; Paz-Ares et al. 2019). Indeed, the recent approval of immunotherapy (anti-PD-L1 and atezolizumab [Atezo]) in combination with chemotherapy for first-line treatment of SCLC provides only marginal benefit for ~10%–20% of patients (Horn et al. 2018). Multiple studies have therefore sought to uncover the mechanisms restricting immune cell infiltration in SCLC and whether it can be attributed to an absence of tumor antigens or defects in antigen presentation machinery. Indeed, it was noted in the 1980s that compared with other tumor types, SCLC exhibits remarkably low expression of class 1 MHC antigens, including human leukocyte antigens (HLAs) and β-2-microglobulin (B2M) (Doyle et al. 1985). The identification of additional mechanisms that underpin SCLC's lack of immunogenicity is an area of active investigation. Additionally, the search for therapeutic approaches to warm up the immune landscape of SCLC is a high priority in the field.

The classification of SCLC as an "immune-cold" disease lacking infiltration of cytotoxic immune cells (Busch et al. 2016) has recently been revisited, sparked by the stratification of SCLC into four transcriptional subtypes (Rudin et al. 2019). Using bulk transcriptional profiles from SCLC patient samples, several groups have recently discovered that the immune landscape differs among SCLC subtypes (Best et al. 2020; Dora et al. 2020; Cai et al. 2021; Gay et al. 2021; Roper et al. 2021). Expression of immune-associated genes such as PD-L1 and MHC-I were repressed in SCLC-A tumors and in NE tumors of other tissue origins, consistent with the expression profile of pulmonary NE cells (Sutherland et al. 2011; Cai et al. 2021). However, a subset of ASCL1$^+$ tumors exhibited high HLA expression and high T-cell and NK cell scores (transcriptional signatures used to predict T-cell and NK cell infiltrate in solid tumors), suggesting that additional biomarkers may be required to stratify response to immune checkpoint blockade (ICB) (Best et al. 2020). SCLC-N tumors appear to be the most immune-cold subset of SCLC, lacking expression of HLA- and antigen-presenting genes with the lowest T-cell and NK cell scores of the four subtypes (Best et al. 2020; Chan et al. 2021; Gay et al. 2021). Consistent with NEUROD1's association with low immune infiltration, permissivity for SVV infection (which is enriched in NEUROD1$^+$ cells) was shown to be associated with lower type I interferon (IFN) innate immune gene expression (Fig. 3; Miles et al. 2017).

Interestingly, Gay et al. (2021) recently identified a novel inflamed (I) subtype, denoted SCLC-I, identified based on its low expression of ASCL1, NEUROD1, and POU2F3 and their associated transcriptional signatures. Critically, it was coined "inflamed" due to elevated expression of immune checkpoint molecules and HLAs, as well as high immune cell infiltration (Gay et al. 2021).

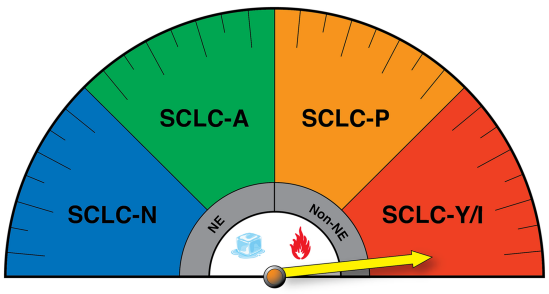

Figure 3. SCLC transcriptional subtypes display distinct immunogenic profiles that may impact response to immune checkpoint blockade (ICB). (NE) Neuroendocrine, (non-NE) nonneuroendocrine. The yellow arrow reflects potential enhanced responsiveness of the SCLC-Y/I subtypes to ICB, while SCLC-N has been suggested to be the most immune-cold.

Up-regulation of IFN-γ response genes and high expression of HLA were similarly reported in an independent SCLC-Y patient cohort (Owonikoko et al. 2021) and in SCLC-Y cell line samples (Tlemsani et al. 2020), adding fuel to the debate over the existence of SCLC-Y and its relationship to the newly annotated SCLC-I subtype. This highlights the need to re-evaluate the nomenclature used to classify SCLC subtypes. For example, while *YAP1* mRNA expression alone might be insufficient to define a distinct subtype, use of well-validated transcriptional signatures of the HIPPO pathway may serve as more powerful classification tools and help address discrepancies in the field (McColl et al. 2017; Wang et al. 2018; Pearsall et al. 2020; Zhang et al. 2020b). Nevertheless, translating such a classification scheme to a clinical diagnostic tool remains a significant challenge for the field. Likely, it will be important to use a panel of markers, including NE markers and histopathology, to better define SCLC-A, SCLC-N, and SCLC-P states, rather than the use of a single transcriptional marker (Rekhtman 2022). This may be particularly important for identifying non-NE subtypes; for example, POU2F3 immunostaining in combination with morphological phenotyping may serve as the most robust diagnostic approach for SCLC-P tumors.

The emergence of distinct immune cell profiles per SCLC subtype has raised new questions related to the efficacy of ICB in SCLC patients. Indeed, it is tantalizing to hypothesize that patients harboring SCLC-P and/or SCLC-Y/I subtypes may exhibit more durable responses to ICB due to relatively higher expression of antigen presentation machinery (APM) genes and their low-NE phenotype (Fig. 3). Recent studies by Gay et al. (2021) were the first to directly address this hypothesis in a retrospective analysis of SCLC patients enrolled in IMpower133, the first randomized trial to demonstrate improvements in progression-free and overall survival (OS) when Atezo was combined with platinum chemotherapy in treatment-naive SCLC patients (Horn et al. 2018; Gay et al. 2021). While this study was not powered for subtype stratification, a significant median OS benefit was observed for SCLC-I in the combination treatment arm (EP + Atezo 18.2 mo vs. EP + placebo 10.4 mo). Moreover, SCLC-P tumors showed a trend for OS benefit in the combination ICB arm (EP + Atezo 9.6 mo vs. EP + placebo 6.0 mo), consistent with high expression of HLA and APM genes in this subset. It should be noted, however, that SCLC-P tumors exhibited the poorest outcomes overall regardless of the treatment arm (Gay et al. 2021). This finding urges analysis on a larger number of samples to determine whether SCLC-P truly exhibits increased resistance to therapy compared with other non-NE SCLC tumors. Using gene expression from tumors in an independent cohort of 20 SCLC patients, a direct correlation between Notch pathway activation and improved response to ICB was found (Roper et al. 2021). By some metrics, *MYC* expression also correlated with clinical benefit to ICB therapy, while *MYCL* and *MYCN* displayed no enrichment (Roper et al. 2021). Together, these studies reveal an association between a low-NE differentiation state and enhanced antitumor immunity. To date, however, no clinical trials have stratified patients based on SCLC subtype or degree of NE differentiation. Indeed, stratification could be hampered by the high level of intratumoral heterogeneity observed in patient samples (Baine et al. 2020; Ireland et al. 2020; Stewart et al. 2020), the source of material analyzed, and/or the highly plastic nature of SCLC tumor cells (Ireland et al. 2020; Patel et al. 2021). Given that Notch receptor genes and many epigenetic regulators are genomically altered in SCLC, it would be valuable to determine how these genomic alterations impact plasticity and whether they are predictive of therapeutic responses. Moreover, recent studies that have reconstructed the phylogenetics of SCLC revealed that the majority of somatic alterations (~80%) represent "trunk" mutations (Chen et al. 2021b; Zhou et al. 2021). It remains unknown whether these alterations could serve as useful neoantigens for immunotherapeutic purposes (Levine et al. 2019).

Given the lack of durable response to ICB in the majority of SCLC patients, several studies have sought to evaluate combination therapies to enhance the efficacy of ICB. Targeting DNA damage response (DDR) pathways with either PARP or CHK1 inhibition led to increased PD-L1 expression on the surface of SCLC tumor cells (Sen et al. 2019). Interestingly, CHK1 inhibition alone was sufficient to induce elevated T-cell infiltration in vivo; however, tumor eradication was only observed when CHK1 inhibition was combined with anti-PD-L1 treatment. $CD8^+$ T-cell recruitment to the tumor microenvironment following DDR inhibition was mediated by activation of a STING–TBK1–IRF3 pathway (Sen et al. 2019). Selective inhibition of CDK7 by YKL-5-124 also caused replication stress and displayed synergy when combined with chemotherapy and ICB (Zhang et al. 2020a). However, in contrast to targeting the DDR, CDK7 inhibition appears to elicit its effects in a STING-independent manner (Zhang et al. 2020a).

The innate immune system has also been implicated in the control of SCLC tumorigenesis. Using a syngeneic RP cell line model, Best et al. (2020) implicated NK cells, but not $CD8^+$ T cells, as key regulators of SCLC metastasis. Indeed, mice genetically engineered to lack NK cells were unable to control the dissemination of RP cells following intravenous injection. Critically, and in line with the emergence of NK cell immunotherapeutic approaches (Huntington et al. 2020), chemical or genetic activation of NK cells ameliorated SCLC metastasis, an effect that synergized with T-cell activation. NK cell-mediated killing, however, does not appear to correlate with low MHC-I expression (Stam et al. 1989), and instead was recently shown to be reliant on expression of the NK cell-activating receptor NKG2D/MICA (Zhu et al. 2021). Interestingly, HDAC inhibition enhances NK cell-mediated killing through the depression of NKG2D expression, highlighting an important role of epigenetic regulation in antitumor immunity (Liu et al. 2018; Zhu et al. 2021). SCLC-P and the inflamed SCLC-I subtype exhibit the highest expression of MICA and harbor a high NK cell score (Cursons et al. 2019; Best et al. 2020; Gay et al. 2021), suggesting that NK cell immunotherapies may be most effective in NE-low SCLC subtypes—an observation that requires further validation in the preclinical and clinical settings.

Recent single-cell transcriptomic analysis has provided unprecedented insights into the phenotype of infiltrating immune cells in 21 SCLC patient samples (Chan et al. 2021). A profibrotic monocytic/macrophage population was shown to be uniquely abundant in SCLC compared with levels seen in lung adenocarcinoma and normal lung tissue. While the functional significance of this macrophage population remains unknown, an association was observed with a rare, prometastatic *PLCG2*$^{+}$ tumor cell population, suggesting a potential protumorigenic role of the macrophages. Other studies have explored therapies that harness the anticancer activity of myeloid cells. Blocking antibodies against CD47 display efficacy in preclinical models by inducing macrophage phagocytosis of SCLC tumor cells (Weiskopf et al. 2016). Interestingly, lurbinectedin, recently provisionally approved for the treatment of metastatic platinum-resistant SCLC (Trigo et al. 2020; Singh et al. 2021), demonstrates efficacy in reducing tumor-associated macrophages in preclinical studies (Belgiovine et al. 2017). It is therefore of interest to explore whether the antitumor effects of lurbinectedin are due to effects on the tumor or macrophage compartments or both.

Increasing SCLC immunogenicity through the induction of phenotype switching

The long-standing observation that SCLC exhibits low MHC-I expression (Doyle et al. 1985), despite the lack of genetic alterations in APM components, implies epigenetic and transcriptional mechanisms of APM control. Indeed, recent studies have uncovered a crucial role for the PRC2 complex, comprising core subunits EZH2, EED, and SUZ12, in the transcriptional repression of MHC-I in SCLC (Burr et al. 2019; Mahadevan et al. 2021). In a genome-wide CRISPR–Cas9 screen, Burr et al. (2019) identified sgRNAs targeting *EED* and *SUZ12* as key regulators of MHC-I expression. This appears to be a conserved mechanism in neuroendocrine tumors, as genetic or pharmacological inhibition of EZH2 restored MHC-I cell surface expression in neuroblastoma, Merkel cell carcinoma, and SCLC cell lines (Burr et al. 2019), a finding that was independently validated by Mahadevan et al. (2021). Similar to the SCLC-I subtype, high MHC-I cell surface expression was associated with a low-NE cell phenotype and derepression of the cGAS–STING pathway (Mahadevan et al. 2021). Critically, EZH2 inhibition resulted in increased T-cell-mediated tumor cell killing (Burr et al. 2019) and was shown to synergize with STING agonists to enhance tumor cell clearance in vivo (Mahadevan et al. 2021). EZH2 inhibitors are currently being evaluated in patients with recurrent SCLC (NCT038979798), based on earlier studies implicating EZH2 in chemoresistance (Sato et al. 2013; Gardner et al. 2017).

Alternative mechanisms of MHC-I regulation associated with subtype switching in SCLC are now emerging. Recently, the RNA-binding protein ZFP36L1 was identified in a CRISPR/Cas9 genomic screen designed to identify the molecular mechanisms underlying LSD1 inhibitor (ORY-1001) sensitivity in SCLC tumor cells (Chen et al. 2021a). Interestingly, high expression of HLA-B and HLA-C is observed in ZFP36L1-activated SCLC tumor cells. Moreover, the phenotype of ZFP36L1-activated cells resembled that of the SCLC-I subtype (Gay et al. 2021), characterized by enrichment of mesenchymal and IFN-γ gene signatures and a loss of NE markers and ASCL1 target genes. Together, these emerging studies offer great promise for the use of therapeutic agents to prime SCLC tumors for immune rejection through inducing NE cell dedifferentiation. It is notable, however, that multiple factors associated with non-NE fate (i.e., Myc, Notch, Yap1, and Ezh2) have been associated with chemotherapy resistance and/or tumor aggressiveness (Gardner et al. 2017; Lim et al. 2017; Mollaoglu et al. 2017; Wagner et al. 2018; Ireland et al. 2020; Wu et al. 2021), warranting caution for implementing therapeutic approaches that promote non-NE fates.

Future directions

Recent advances have transformed our view of SCLC and shed light on approaches that could improve outcomes for patients with specific molecular subsets of SCLC. However, the remarkable transcriptional and epigenetic plasticity in SCLC suggests that defining molecular states is a challenging notion, especially for clinical purposes. Moreover, the capacity for plasticity implies that drug resistance will be a continual obstacle to successful treatment. It is critically important that we improve our understanding of the mechanisms of SCLC plasticity and determine how to harness this knowledge to improve the efficacy of combination therapies and immunotherapy. In addition to the gaps in knowledge discussed previously in this review, we propose additional key areas of study:

- An outstanding question is whether early studies identifying MYC-high therapeutic vulnerabilities pertain to all ASCL1-negative subgroups or specific ones; i.e., which therapeutic sensitivities are NEUROD1-specific versus POU2F3-specific or triple-negative ASCL1/NEUROD1/POU2F3-specific? How does C-MYC function differently from other MYC family members like MYCL? Is dosage important? How do MYC family members differ in function? Mechanistic studies in cell lines and animal models will more definitively address these questions.
- While modulation of key factors such as Ras, Notch/Rest, Myc, Yap1/Taz, and epigenetic regulators has been shown to impact NE and non-NE fates, how these signals are coordinated and interrelated is not well understood. Deciphering the basic mechanisms of how extracellular signals impinge on these factors and epigenetic regulators such as the PRC2 complex, Crebbp/p300, Lsd1, Kdm5a, Zfp36l1, Ets family members, and others will be important for manipulating cell fate. How these factors control each other from a network perspective and whether they function in the

same or parallel and distinct pathways remain unknown.

- Since the refinement of the four SCLC molecular subtypes by Rudin et al. (2019), a novel transcriptional subtype based on the expression of ATOH1 has emerged from PDX studies (Westerman et al. 2007; Simpson et al. 2020). ATOH1 is another bHLH proneural transcription factor that is important for inner ear and cerebellar development. ATOH1$^+$ SCLC CDX models appear to exhibit a distinct transcriptional signature when compared with SCLC-A, SCLC-N, and SCLC-P subtypes and correlate with expression of the mesenchymal marker VIM. Whether ATOH1 is expressed in primary human SCLC tumors has been questioned, highlighting the need to better understand this observation.
- Vascular endothelial (VE)-cadherin-positive SCLC tumor cells have been frequently observed in SCLC CDX models (Williamson et al. 2016), and the signals and mechanisms that promote the process of vascular mimicry (VM) and how they relate to SCLC molecular subtypes are unknown. VM may be an important process for therapeutic intervention given that VM correlates with poor outcomes and chemotherapy resistance in CDX studies (Williamson et al. 2016).
- Given that tumors often harbor cells in multiple subtype states (Baine et al. 2020; Ireland et al. 2020), there is likely extensive cross-talk among populations of cells (Fig. 2) that provide survival advantages in the face of environmental stresses. Cell–cell interactions have been implicated in metastases, but how subtype states interact with each other during hypoxia, nutrient deprivation, and chemotherapy-induced stresses are largely unknown.
- Exciting new studies suggest that cell fate or lineage can impact expression of antigen presentation and immune response machinery, providing renewed hope that these mechanisms can be exploited to improve immunotherapy responses. A key area of future investigation concerns how to increase immune cell recognition and response to ICB.
- SCLC is thought of as a relatively immune-cold tumor, but single-cell approaches have been crucial in illuminating novel immune populations like the profibrotic monocytic/macrophage population associated with the prometastatic *PLCG2*$^+$ population (Chan et al. 2021). Immune populations such as these may be important in the pathogenesis of SCLC and/or may be targeted to enhance therapeutic responses.

Competing interest statement

T.G.O. is named on a patent related to SCLC subtype biomarkers (US11124841B2). K.D.S. and A.S.I. declare no competing interests..

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Killing SCLC: insights into how to target a shapeshifting tumor

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