Editorial Commentary

Role of CDK4/6 inhibitors in targeting Rb proficient small cell lung cancer

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Small cell lung cancer (SCLC) is a high-grade neuroendocrine carcinoma, and remains one of the deadliest cancers with limited therapeutic options, resulting in a dismal 5-year overall survival of 5% (1). This continued lack of treatment progress in SCLC contrasts with non-small cell lung cancer (NSCLC), where remarkable advances have been made, especially with targeted drugs for molecularly defined patient subsets (e.g., *EGFR* mutated cancers; *ALK* and *ROS1* fusions) (2). Although recent clinical trials have shown that immunotherapy with PD-L1 blockade can improve survival in patients with extensive stage SCLC (3,4), the benefits remain restricted to a minority of patients and are less impressive than the effect of immunotherapy in other cancer types.

SCLC is characterized by bi-allelic inactivation of two major tumor suppressor genes, *TP53* and *RB1*, and lack of any actionable driver mutations. One third of patients can exhibit high tumor mutation burden. Recent investigations have mainly focused on characterization of biological underpinnings of SCLC. Major advances include the identification of the role of tumor heterogeneity and lineage plasticity (5), the discovery of molecular subgroups defined by differential expression of transcription factors (6-8), and potential subtype-specific therapeutic vulnerabilities. SCLC is now divided into four major subgroups—SCLC-A—defined by upregulated ASCL1, SCLC-N with upregulated NEUROD1, and SCLC-P with POU2F3 and SCLC-Y associated with YAP1 (7,9). Each subgroup has distinct biological implications, metastatic proclivity and classification of a patient's tumor as one of these subgroups may serve as a biomarker defining vulnerability to novel therapies.

More recently, Gay *et al.* identified a new SCLC-I or "inflamed" subgroup, with elevated expression of immune markers. In a retrospective correlative analysis, the SCLC-I subgroup also showed potential benefit from chemotherapy combined with PD-L1 blockade (6). The study also showed that SCLC-A cell lines have enhanced sensitivity to BCL2 inhibitors, SCLC-P cell lines show enhanced sensitivity to PARP inhibitors and antimetabolite, SCLC-N cells may be sensitive to Aurora kinase inhibitors, and SCLC-I may be sensitive to BTK inhibitors (6). However, further validation in larger clinical cohorts and prospective trials are needed to confirm the clinical utility of these subtypes. Recent evidence also suggests that SCLC-N may also be particularly sensitive to the neuroendocrine specific oncolytic virus SVV-001, given upregulation of the receptor for viral entry TEM8 (10).

The study by Febres-Aldana *et al.* (11) highlights an important novel subgroup of SCLC, defined by proficiency of the tumor suppressor RB, with increased sensitivity to CDK4/6 inhibitors. This paper is important for several

reasons (1) defining a distinct subgroup of SCLC and a potential treatment option (2) characterizing RB proficient SCLC and its defining features including aggressive clinical course and association with NSCLC (3) improving the technical methodology for identification of an RB proficient SCLC subgroup, through highlighting the limitations of next generation sequencing (NGS) and the potential utility of immunohistochemical (IHC) testing in this context.

SCLC tumorigenesis has long been thought to be driven by dual inactivating mutations in the tumor suppressors *TP53* and *RB1* (12). In mouse models of SCLC inactivation of both *TP53* and *RB1* was necessary for development (13,14) of SCLC. However, contrary to this established model, Febres-Aldana *et al.* sought to identify a subgroup of SCLC with proficient RB that had not been previously well characterized. Febres-Aldana *et al.* examined RB expression in 208 samples from patient with *de novo* SCLC. They specifically excluded samples from tumors with known histological neuroendocrine transformation of NSCLC in association with the development of resistance to EGFR inhibitors (14-18). Fourteen samples of SCLC were identified as RB proficient. These tumors were concentrated in the samples of patients with limited stage disease and were associated with combined SCLC with other histologies. These samples demonstrated lower expression of traditional markers of neuroendocrine differentiation including synaptophysin, chromogranin A, INSM1 and CD56 and were also associated with the "neuroendocrine low" transcriptomic features with low expression of transcriptomic factors ASCL1 and NEUROD1 which define the neuroendocrine enriched subgroups SCLC-A and SCLC-N. Interestingly, mutational profiling in this subgroup revealed increased *CCND1* and *CDKN2A* as well as mutations more commonly associated with NSCLC including in *FGFR1* and *EGFR* amplifications, *KEAP1* mutations, and *STK* mutations or loss. The lack of neuroendocrine differentiation and association with histologically combined tumors and mutations more commonly seen in NSCLC suggests that the SCLC tumors within this group may have started as NSCLC and through plasticity transformed to SCLC. Given this possible association with NSCLC one might expect that the patients with the RB proficient SCLC to have improved survival compared to more typical RB deficient SCLC. However, it was noted that the RB proficient subgroup in this study had a significantly more aggressive clinical course and poor prognosis and were often primarily refractory to standard treatments with carboplatin and etoposide, a regimen that is considered highly effective—at least initially—with SCLC. The authors also highlight that despite the similarities to NSCLC on genomic profiling, the reviewed tissue samples from the RB proficient SCLC tumors were identical to classic SCLC with similar histomorphology and elevated Ki-67%.

In order to confirm RB proficiency, the authors of this paper used both NGS and IHC to detect mutations in RB and functional activity. They used the MSK-IMPACT platform for detection of single nucleotide variants, indels, copy number alterations, and structural variants. In addition to the standard analysis, for all samples lacking *RB1* alterations they also performed manual review of sequencing. For the IHC analysis they performed IHC for RB and IHC for p16 and CyclinD1 to characterize RB functional status. The rationale was that p16 elevation is a marker of RB inactivation, while CyclinD1 is dependent upon RB. Thus, low p16 and elevated CycinD1 were considered markers of RB protein expression and function. In their analysis they noted that of 208 *de novo* SCLC cases 184 demonstrated loss of RB expression, and 24 retained RB expression. By NGS, 138 were noted to have *RB1* alterations with an additional 27 cases only identified after manual review for a total of 165 with RB loss. They used a combination of NGS and IHC to group the samples into 4 groups: RB lost/*RB1* mutated (n=155), RB lost/*RB1* wild type (n=29), RB proficient/*RB1* mutated (n=10) and RB proficient/*RB1* wild type (n=14). Functional activity of RB was examined with IHC for p16 and CyclinD1. They noted that in the tumors which were RB proficient /*RB1* wildtype, P16 was low and CyclinD1 was elevated establishing that RB was functional. However, the presence of an RB proficient/*RB1* mutated group presents problems for those who might rely on NGS alone for RB testing. They suggest that this study provides evidence that NGS testing may miss cases of RB proficient tumors and that IHC testing of RB in combination with p16 and CyclinD1 represents a more effective way to identify RB proficient subgroups.

A previous study in SCLC cell lines reported similar findings. Sonkin *et al.* examined 48 SCLC cell lines from the Cancer Cell Line Encyclopedia database. Eight out of these 48 cell lines were found to be *RB1* WT. *RB1* WT SCLC lines were found to be enriched for loss of neuroendocrine lineage markers with *CDKN2A* inactivation, or *CCND1* amplification. Interestingly, 2 *RB1* WT SCLC cell lines that were included in a drug screen were found to be sensitive to

CDK4/6 inhibition (19).

The importance of RB proficient SCLC was also recently evaluated in another paper by Wildey *et al.* (20) In this study the authors analyzed patient samples of SCLC tumors as well as cell lines and xenograft tumors for RB1 protein expression. They also report on the first two patients treated on a clinical trial of CDK4/6 inhibitors in patients with retained RB1 expression with SCLC. The authors of this study performed a series of experiments including examining a cohort of tissues samples from 62 patients with SCLC. They noted these samples came from a variety of body sites and included primary tumors and metastatic lesions. In this study, RB proficient tumors detected by IHC did have some association with neuroendocrine markers and were also associated with YAP1 expression. They also noted on concurrent studies that *RB1* mutations on NGS did not seem to correlate with RB1 protein expression and function and that transcriptomic characterization of a RB1 loss of function phenotype may be another way to accurately detect RB1 proficient tumors. The authors also tested the sensitivity of SCLC cell lines with RB1 proficiency to CDK4/6 inhibitors and found that RB1 proficiency was required for sensitivity to CDK4/6 inhibition. Perhaps the most interesting data from this paper is the report of a clinical trial of CDK4/6 inhibitor abemaciclib in patients with RB1 proficient SCLC. They noted that 2 patients had been enrolled and although the first patient did not respond, the 2nd patient had an excellent response to CDK4/6 inhibition. The trial used NGS for screening and the first patient enrolled had RB1 proficiency on NGS but on IHC RB1 was lost suggesting that this was not a truly RB proficient tumor. This further underscores the importance of IHC staining of both RB and potentially p16 and CyclinD1 to confirm RB expression and function.

The study by Febres-Aldana *et al.* is important as it supports the evidence that SCLC is a deeply heterogenous tumor type and is closely associated with plasticity both within SCLC subtypes (including SCLC-A to SCLC-N) (5) and with NSCLC transforming over time to SCLC. The histological transformation of NSCLC to SCLC as a mechanism of therapeutic resistance in tyrosine kinase inhibitors, mainly targeting EGFR, is well documented. However, *de novo* SCLC with genomic features of NSCLC suggest true heterogeneity within SCLC even prior to therapy. Emerging evidence suggests that heterogeneity within SCLC and neuroendocrine transformation may be driven by epigenetic changes, including chromatin remodeling, which may play role in neuroendocrine differentiation of NSCLC (21). Although the role of MYC is well documented in driving transitions between SCLC subtypes including A to N, there is also evidence that epigenetic regulators may influence SCLC-A to SCLC-N lineage plasticity (22). Although NGS testing is increasingly done across cancer types, the importance of other factors driving potentially important therapeutic subtypes including those determined by master transcriptomic regulators and epigenetic changes require further investigation. In addition, the role of IHC in defining clinically meaningful subgroups as a biomarker of potential treatment response is another important area to target in future clinical trials.

SCLC is an aggressive disease with a dismal prognosis. In addition, many patients who present with advanced SCLC have a poor performance status, finding alternative therapies that may be better tolerated than systemic chemotherapy is critical. Molecular subtyping of SCLC and evidence of plasticity has established the heterogeneous and dynamic nature of SCLC, which poses a major challenge of resistance to therapeutic interventions. However, the improved understanding of the biology of this disease and particularly the vulnerabilities of SCLC subgroups to specific therapies is necessary and will lead to higher efficacy of clinical trials. Given the findings of recent studies, moving forward one could see a future where all patients with SCLC undergo IHC-based subtyping of their cancer to help direct treatment and provide important prognostic information about the disease course. Although these studies represent significant advances forward—the number of patients that fall into these subgroups with more established therapies is small. Further work is urgently needed to develop durable treatment options and to identify combinatorial regimens to enhance the efficacy of immunotherapy for patients with SCLC. Moreover, given the plastic nature of SCLC, disease monitoring throughout treatment and therapy switching may be essential to achieve long-term survival. One important area of investigation that the study by Febres-Aldana *et al.* encourages is whether extra pulmonary high grade neuroendocrine cancers or other cancers that undergo neuroendocrine differentiation including prostate cancer and pancreatic cancer (22) also share a subgroup with RB proficiency that may be targeted with CDK4/6 inhibition. Finally, the studies described here focus on the tumor intrinsic factors, and the role of tumor extrinsic factors-such as the unique interactions between tumor cells and the tumor immune microenvironment and stroma represent an important area of investigation.

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