Biomarkers of Small Cell Lung Cancer

Subjects: Oncology

Contributor: Anna Keogh, Stephen Finn, Teodora Radonic

Small cell lung cancer (SCLC) is a high-grade neuroendocrine malignancy with an aggressive behavior and dismal prognosis. 5-year overall survival remains a disappointing 7%. Genomically, SCLCs are homogeneous compared to non-small cell lung cancers and are characterized almost always by functional inactivation of RB1 and TP53 with no actionable mutations. Additionally, SCLCs histologically appear uniform. Thus, SCLCs are managed as a single disease with platinum-based chemotherapy remaining the cornerstone of treatment. Recent studies have identified expression of dominant transcriptional signatures which may permit classification of SCLCs into four biologically distinct subtypes, namely, SCLC-A, SCLC-N, SCLC-P, and SCLC-I. These groups are readily detectable by immunohistochemistry and also have potential predictive utility for emerging therapies, including PARPi, immune checkpoint inhibitors, and DLL3 targeted therapies. In contrast with their histology, studies have identified that SCLCs display both inter- and intra-tumoral heterogeneity. Identification of subpopulations of cells with high expression of PLCG2 has been linked with risk of metastasis. SCLCs also display subtype switching under therapy pressure which may contribute furthermore to metastatic ability and chemoresistance.

Keywords: small cell lung cancer ; biomaker ; Pathogenesis

1. Introduction

Small-cell lung cancer (SCLC) is a high-grade neuroendocrine carcinoma accounting for 15% of all lung cancers. They arise predominantly in current or former heavy smokers. Prognosis is exceptionally poor, with 5 year overall survival (OS) of 7% [1]. They are predominantly centrally located in the major airways and almost always involve the mediastinal lymph nodes. Approximately 5% of cases, however, arise peripherally in the lungs [2]. Metastasis occurs early, with up to two thirds of patients having widespread disease at initial presentation. The most common sites of metastasis include liver, bone, brain, ipsilateral and contralateral lung, and adrenal glands.

Histologically, SCLCs are homogenous and characterized by small or intermediate size cells with granular chromatin, inconspicuous nucleoli, and scant cytoplasm. High mitotic activity and apoptosis are prominent. According to the 5th edition of the WHO Thoracic Malignancies classification, most SCLCs express at least one positive neuroendocrine (NE) marker (Chromogranin A, Synaptophysin, CD56, and INSM1) on immunohistochemistry (IHC) [3]. Genetically, SCLCs are homogenous also, with biallelic loss of function of TP53 and RB seen in virtually all SCLCs.

Currently, SCLC is treated as a single disease, with platinum-based chemotherapy remaining the cornerstone of treatment. Early-stage disease is treated with surgery and adjuvant platinum chemotherapy, locally advanced disease with concurrent radiation and platinum-based chemotherapy, and metastatic disease with systemic chemotherapy with or without immunotherapy [4]. SCLC is extremely sensitive to chemotherapy initially, with objective response rate (ORR) to first line chemotherapy of over 60% even in patients with metastatic disease [5]. Unfortunately, for the majority, this response is transient with relapse occurring early. Prognosis is poor with overall survival (OS) of less than 2 years in patients with early stage disease and approximately one year for patients with metastatic disease [1].

Over the past 20 years, there has been no improvement in the survival or response rate to chemotherapy in SCLC patients [5][6]. Therefore, there is a desperate need for a new approach in this setting. Currently, there are no biomarkers clinically available to guide treatment for targeted therapies in SCLC.

Since 2018, the PD-L1 blockade with atezolizumab and durvalumab has been incorporated as part of a frontline regimen after two large phase III trials, Impower133 [7] and CASPIAN, showed benefit [8]. However, the overall improvement in survival was modest compared to other solid tumors and only a small subset of patients seemed to derive benefit. PD-L1 IHC, a predictive biomarker for PD-L1 inhibitors in many solid tumors, failed to predict a response in SCLC and the recent approval of immunotherapy did not require PD-L1 IHC. Thus, there is a need for the development of a predictive biomarker to identify this subset of patients who can derive benefit from Immunotherapy.

Multiple recent studies have classified SCLC into four biologically distinct subtype based on differential expression of transcriptional factors: ASCL1, NEUROD1, and POU2F3 or SCLC-A, SCLC-N, and SCLC-P respectively [9][10]. A fourth subtype is negative for all three transcription factors and has a high infiltration of inflammatory cells, classified as an immune phenotype or SCLC-I [10].

There is emerging evidence that the newly defined subtypes of SCLC may have specific therapeutic vulnerabilities and therefore, may solve the current unmet need for predictive biomarkers to guide therapy selection in SCLC.

SCLC also demonstrates plasticity as well as intra- and inter-tumoral heterogeneity with implications on chemoresistance.

2. Pathogenesis

TP53 and RB1 (RB Transcriptional Corepressor 1) biallelic loss of function is obligatory in SCLC [11][12][13]. This inactivation of tumor-suppressor genes (TSGs) is the initiating step in oncogenesis of SCLC. This differs from most solid tumors, including non-small cell lung cancer (NSCLC), where activation of oncogenic drivers is required [14].

The vast majority of SCLCs arise in patients with a history of heavy smoking. Only rarely can they occur de novo in non-smokers [15]. This smoking etiology is reflected by the characteristic tobacco carcinogen-associated molecular signature of G > T and C > A transversions seen at high frequency (28%) and a high tumor mutational burden (TMB) [13].

Most patients are diagnosed with SCLC at an advanced stage and rarely undergo surgical resection. As a result, small tissue biopsies or cytology samples are the only biological material available. Research into the tumorigenesis of SCLC has been largely hampered by the low number of samples available. However, new research techniques such as genetically engineered mouse models (GEMMs), development of SCLC cell lines, patient-derived in vivo models, as well as analysis of mechanisms of acquired therapeutic resistance through transcriptomic and proteomic approaches, have enabled a deeper understanding of SCLC.

3. Potential Clinical Utility of Emerging Subtypes

The detection of biological heterogeneity based on master transcriptional activators has been a huge advancement in the understanding of SCLC and has potential to translate into clinical practice in the future. Each of these subtypes have distinct biological activity and therapeutic vulnerabilities which will hopefully lead to the development of novel diagnostic, prognostic, and predictive biomarkers to aid in the pathologic assessment and guide clinical decisions for these aggressive tumors.

3.1. Subtypes as Emerging Diagnostic Biomarkers

Diagnosis of SCLC is made by histopathological examination. Because of the central location of the tumors, biopsies are most often obtained by bronchoscopy with or without endobronchial ultrasound. As a result, diagnosis is made on scant biopsy specimens or cytology. Due to the aggressive nature of SCLC, workup including diagnosis and staging should be performed as quickly as possible after presentation.

Morphologically, SCLCs are composed of small cells (<3 resting lymphocytes) with scant cytoplasm, finely granular nuclei, and inconspicuous nucleoli, indistinct cell boarders, high mitotic count, apoptosis, and frequent necrosis. Tumors are usually densely packed, forming a sheet-like pattern [3]. Morphologically, SCLC are homogenous compared to NSCLC, although rare variations exist [16].

Historically, SCLC is a morphological diagnosis made on the haematoxylin and eosin (H&E) stain. However, IHC is widely used in pathology practice. Histologically, the differential diagnosis includes other neuroendocrine tumors, especially large cell neuroendocrine carcinoma (LCNEC), basaloid squamous cell carcinoma, undifferentiated SMARCA4 tumor, small round blue cell tumors, and lymphoma. Both clinical course and treatments vary widely across these malignancies, and therefore, accurate diagnosis is essential.

NE markers are positive in 90 to 95% of SCLC cases. However, 5–10% can be positive only with CD56, sometimes only weakly, or even negative, creating a major diagnostic challenge especially in those where morphology is not well preserved.

As a result, large IHC panels are needed to exclude other differentials, often delaying diagnosis. In such cases, or whenever the differential diagnosis with low-grade NE tumors, NSCLC or LCNEC, arises, the loss of RB IHC and a

mutant staining for TP53 (either loss of TP53 or a strong diffuse staining) is helpful. This pattern is seen in SCLC representing its genetic hallmark [13]. This approach is not yet validated but might be of great value in small or crushed biopsies. In difficult cases, the addition of p16 IHC might be of additional assistance; strong and diffuse p16 reflects loss of Rb through its negative regulatory loop [17].

POU2F3, the novel transcriptional factor expressed in 7% of SCLC, is showing great promise as a diagnostic biomarker in $NE^{low/-}$SCLCs. Baine et al. analyzed POU2F3 IHC expression in SCLC (n = 123) and other major lung cancer types (n = 433) [18]. Expression was diffusely positive in 70% of SCLCs that were fully NE negative. Expression was negative in NE SCLCs and therefore, is best suited as a second step marker in suspected $NE^{low/-}$SCLC. Although not fully restricted to SCLC (positive staining was seen in basaloid SCC and LCNEC in 22% and 12% of cases, respectively), it is highly selective, and therefore is useful as a multipurpose IHC, similar to TTF-1. The addition of POU2F3 IHC, therefore, would aid in a more efficient diagnostic pathway for $NE^{low/-}$SCLCs, eliminating the use of larger IHC panels and conserving tissue for a further workup, such as detection of predictive biomarkers, in the future.

3.2. SCLC Subtypes as Prognostic Biomarkers

None of the four distinct subtypes give clear prognostic information. SCLC-A and SCLC-N are associated with immune cold tumors [19] and low NE subtypes are associated with chemoresistance. However, few studies have evaluated the prognosis of these subtypes. SCLC-P has been identified as a poor prognostic factor in one study [20] and conflicting results exist as to whether high YAP1 expression is associated with poor prognosis [19][21]. For now, SCLC subtype behavior in a clinical context is still not clarified.

3.3. Predictive Biomarkers

In stark contrast to NSCLC, SCLC has been treated as a single disease, with a one-size-fits-all model, and with platinum-based chemotherapy remaining the cornerstone of treatment. Over 20 years, there has been no improvement in the survival or response rate to chemotherapy of SCLC patients and therefore, there is a desperate need for new approaches in this setting [5].

Currently, there are a number of promising biomarkers in the pipeline which may be used to guide clinical decisions for these patients in the future.

3.1. SCLC Subtypes as Predictive Biomarkers

The four described SCLC subtypes, SCLC-A, SCLC-N, SCLC-P, and SCLC-I, described by distinct translational activators, have been shown to have distinct therapeutic vulnerabilities. Using in vitro cytotoxic assays, Gay et al. identified that the SCLC-A subtype have increased sensitivity to BCL2 inhibitors, the SCLC-N subtype, to aurora kinase inhibitors, and the SCLC-P subtype, to PARPi and antimetabolites [10]. The SCLC-P subtype had increased sensitivity to PARPi independent of SLFN11 (an emerging predictive biomarker for PARPi in SCLC, see below), suggesting there is an alternate pathway of efficacy which potentially widens the cohort of patients who will derive benefit from this promising new target in SCLC. Within SCLC-A, there is a bimodal expression of SLFN11, and if separated by high and low expression, there is a stark difference in cisplatin sensitivity and to PARPi with olaparib [10].

To identify therapeutic vulnerabilities, Gay et al. also identified unique protein expression patterns between groups which may serve as predictive biomarkers. SCLC-A and SCLC-N subtypes have a high expression of DLL-3, which is absent in SCLC-P and SCLC-I groups. A subset of SCLC-A also has high expression of SLFN11, which predicts a response to PARPi (see below). The SCLC-N group has a high expression of somatostatin receptor 2 (SSTR2), which can be targeted by somatostatin analogues such as octreotide.

The response of subtypes to these targeted therapies requires validation. However, the identification of distinct therapeutic vulnerabilities within these four subtypes highlights that a one-size-fits-all-model may not be appropriate for managing this disease. This will certainly pave the way for biomarker-driven clinical trials in the future introducing SCLC into the world of precision oncology.

3.2. SCLC-I Predicts Response to Immunotherapy

SCLCs have a high TMB and, therefore, are predicted to induce strong T-cell responses. NSCLC are also associated with a similarly high TMB, and as such, have shown responsiveness to immunotherapy. Immunotherapy by means of targeting PD1/PD-L1, either as a monotherapy or in combination with chemotherapy, has become the first-line treatment for patients with stage IV NSCLC lacking a driver mutation [22]. PD-L1 expression on tumor cells (tumor proportion score) by

IHC is a strong predictor of response, with cut offs of ≥1% and ≥50% used as selection criteria for combination therapy and monotherapy, respectively [23][24][25]. Additionally, TMB status is an important predictive biomarker with high TMB predicting improved objective response to durable benefit, and progression-free survival independent of PD-L1 expression [26]. High TMB in NSCLC is associated with immune cell infiltration and an inflammatory T-cell mediated response which may explain the increased sensitivity to immunotherapy [27].

Although NSCLC and SCLC have a similar TMB profile, they exhibit a differential response to immunotherapy. Clinical trials involving immunotherapy in SCLC have yielded disappointing results, with only a subset of patients deriving a benefit [28]. PD-L1 IHC is rarely expressed in SCLC and does not predict a response to immunotherapy [29]. The differential responsiveness between SCLC and NSCLC may lie within the interaction of SCLC and its surrounding environment, including immune surveillance [30]. SCLC exhibits an extremely cold T-cell receptor (TCR) repertoire and lower immune infiltrate compared to NSCLCs despite having similar TMBs [31]. SCLCs also have low infiltration by immune cells, specifically, cytotoxic T cells [32].

Currently, there are no biomarkers that predict a response to immunotherapy in SCLC.

The novel, low-NE subtype identified by Gay et al., SCLC-I, demonstrates an ‘inflamed’ phenotype and has a high expression of both CD8A and CD8B, suggesting greater cytotoxic T-cell infiltration [10].

The Impower133 trial was the first randomized trial demonstrating PFS and OS improvements with Immunotherapy in SCLC [29]. Using data from this trial, Gay et al. retrospectively stratified survival data by SCLC subtype and the identified OS benefit was seen in the SCLC-I group compared to all others in the platinum-etoposide (EP) plus atezolizumab arm, but not in the EP plus placebo arm (HR, 0.566; 95% CI, 0.321–0.998) [10]. This represents clinical data that the SCLC-I subtype is a candidate biomarker for predicting a response from the immune checkpoint blockade in SCLC.

Additionally, the SCLC-N subtype has been shown to have the most immunosuppressive TME and therefore, may derive no benefit from IO [33]. These studies highlight the need to match tumor subtype to therapy to maximizes the response in patients. Future prospective trials are needed to elucidate the clinical relevance of the novel transcriptional subsets in SCLC.

3.3. SLFN11 as a Predictive Biomarker

Resistance to cell death and genomic instability are important hallmarks of cancer [34]. Small molecule inhibitors that directly damage DNA response (DDR) have gained increasing interest over the years. Poly (ADP-ribose) polymerase (PARP) are a family of proteins who play a critical role in DNA repair. When induced by a DNA lesion, PARP catalyzes the addition of poly (ADP-ribose) chains to target proteins, which mediates the recruitment of additional DNA repair factors to the damaged DNA [35]. PARP inhibitors (PARPi) have been studied most in cancers with deficient homologous recombination repair (HRR) pathways which depend on PARP-mediated base excision for repair.

Schlafen 11 (SLFN11) is a DNA/RNA helicase that induces an irreversible replication block and it has been identified as a predictive biomarker of DNA-damaging agents and PARPi in preclinical settings in SCLC [36], as well as many other solid tumors, including breast [37], ovarian [38], colorectal [39], and gastric cancer [40], and mesothelioma [41].

SLFN11 also predicts the response to other DNA-damaging agents, including platinum salts, topoisomerase I inhibitors, topoisomerase II inhibitors, alkylating agents, and antimetabolites. Low expression also predicts resistance to these agents [42].

Clinical detection of SLFN11 can be made using IHC. Multiple studies have demonstrated that detection of SLFN11 is made by the presence of any nuclear staining [42]. Conversely, negative SLFN11 tumors have a complete absence of staining. Therefore, the distinction between SLFN11-positive and -negative tumors can be easily made in clinical practice by IHC.

Unlike other biomarkers, SLFN11 induces its cellular response to PARPi independently of HRR. Instead, ‘PARP trapping’ occurs, whereby PARPi traps PARP1/2 at the site of DNA damage preventing repair [43]. SLFN11 induces cell cycle arrest in the S-phase, which leads to a stalling of replication forks resulting in fork breakage and replisome disassembly [44]. SLFN11 and PARPi are believed to have synergistic effects through this ‘trapping’ mechanism [45]. PARP trapping varies among different PARPi, with talazoparib being the strongest and veliparib being the weakest [42][43][45][46][47]. The predictive strength of SLFN11, therefore, may be PARPi class dependent.

A phase II trial of relapsed SCLC treated with temolizomide plus veliparib (PARPi) versus temolizomide plus placebo found no significant difference in 4-month PFS or median OS [48]. Retrospective analysis, identifying SLFN11-positive patients using IHC on archival tissue, identified that SLFN11-positive patients had a significantly longer PFS and OS compared to SLFN11-negative patients in the temozolomide plus veliparib group [48]. This represented the first clinical data that SLFN11 is a candidate biomarker for PARPi in SCLC.

A phase II randomized clinical trial of atezolizumab in combination with talazoparib versus atezolizumab alone in SCLC is currently ongoing (NCT04334941) for patients with SLFN11-positive extensive-stage SCLC. As mentioned above, talazoparib has the strongest PARP-trapping ability and may confer additional benefit compared to other PARPi in SLFN11-positive patients. This is the first clinical trial selecting patients based on SLFN11 status.

Lurbinectedin, an alkylating agent, recently received FDA-accelerated approval as a second line treatment option for metastatic SCLC [49]. SLFN11 has been shown as a candidate biomarker for lurbinectedin sensitivity. SLFN11-low SCLC cell lines are resistant to lurbinectedin [50]. However, the addition of an ATR (Ataxia telangiectasia mutated and Rad3-related) inhibitor re-sensitizes these cells [50]. This confirms SLFN11's role as a master regulator of DNA damage response, independent of ATR, with the absence of SLFN11 leading to synthetic lethality when ATR is inhibited. Therefore, the addition of an ATR inhibitor to lurbinectedin in SLFN11-low tumors may overcome resistance.

SLFN11 has also been shown to be detectable in CTCs in SCLC and may represent a candidate biomarker to monitor patients via non-invasive liquid biopsy [51] (discussed below).

3.4. DLL3 as a Predictive Biomarker

Delta-like ligand 3 (DLL3) is a ligand that inhibits the Notch pathway and is upregulated and aberrantly expressed on the cell surface in up to 80% of SCLCs, specifically, NE subtypes (SCLC-A and SCLC-N) [52][53]. In contrast, DLL3 is only expressed at very low levels in a few normal cells and is exclusively cytoplasmic [54]. This selective expression has gained a lot of attention as an attractive biomarker in SCLC.

Rovalpituzumab tesirine (Rova-T) is an antibody–drug conjugate composed of a DLL3-targeting immunoglobulin G1 monoclonal antibody tethered to pyrrolobenzodiazepine (PBD), a toxic DNA crosslinking agent, by means of a protease-cleavable linker [52]. Rova-T was designed to deliver cytotoxic treatment to DLL3, expressing SCLC cells while sparing healthy cells, reducing side effects. Phase II and III clinical trials using RovaT as a third line and beyond treatment for participants with relapsed or refractory DLL3 expressing SCLC exhibited an inferior OS compared to patients on current standard second-line chemotherapy, topotecan, and therefore, it was discontinued [55].

Despite this, DLL3 remains an attractive target due to its specificity and high expression in SCLC. Novel therapeutic targets, such as anti-DLL3 bispecific T-cell engager, are in development [56]. Additionally, a recent study used a radiolabeled anti-DLL3 mAb SC16 with the therapeutic radioisotope Lutetium-177 in GEMMs. This radioisotope specifically attaches to SCLC cells to deliver targeted radiotherapy, minimizing radiation to healthy cells. Results showed antitumor efficacy and a low toxicity profile, making it a strong potential for clinical translation [57].

References

1. Rudin, C.M.; Brambilla, E.; Faivre-Finn, C.; Sage, J. Small-cell lung cancer. Nat. Rev. Dis. Primers 2021, 7, 3.
2. Travis, W.D. Pathology and diagnosis of neuroendocrine tumors: Lung neuroendocrine. Thorac. Surg. Clin. 2014, 24, 257–266.
3. Organisation WHO. Thoracic Tumours, 5th ed.; WHO Classification of Tumours Editorial Board: Lyon, France, 2021.
4. Kalemkerian, G.P.; Loo, B.W.; Akerley, W.; Attia, A.; Bassetti, M.; Boumber, Y.; Decker, R.; Dobelbower, M.C.; Dowlati, A.; Downey, R.J.; et al. NCCN Guidelines Insights: Small Cell Lung Cancer, Version 2.2018. J. Natl. Compr. Cancer Netw. 2018, 16, 1171–1182.
5. Lattuca-Truc, M.; Timsit, J.-F.; Levra, M.G.; Ruckly, S.; Villa, J.; Dumas, I.; Pinsolle, J.; Ferrer, L.; Guillem, P.; Moro-Sibilot, D.; et al. Trends in response rate and survival in small-cell lung cancer patients between 1997 and 2017. Lung Cancer 2019, 131, 122–127.
6. Howlader, N.; Forjaz, G.; Mooradian, M.J.; Meza, R.; Kong, C.Y.; Cronin, K.A.; Mariotto, A.B.; Lowy, D.R.; Feuer, E.J. The Effect of Advances in Lung-Cancer Treatment on Population Mortality. N. Engl. J. Med. 2020, 383, 640–649.

7. Horn, L.; Mansfield, A.S.; Szczęsna, A.; Havel, L.; Krzakowski, M.; Hochmair, M.J.; Huemer, F.; Losonczy, G.; Johnson, M.L.; Nishio, M.; et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. N. Engl. J. Med. 2018, 379, 2220–2229.
8. Paz-Ares, L.; Dvorkin, M.; Chen, Y.; Reinmuth, N.; Hotta, K.; Trukhin, D.; Statsenko, G.; Hochmair, M.G.; Özgüroğlu, P.M.; Ji, J.H.; et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): A randomised, controlled, open-label, phase 3 trial. Lancet 2019, 394, 1929–1939.
9. Rudin, C.M.; Poirier, J.T.; Byers, L.A.; Dive, C.; Dowlati, A.; George, J.; Heymach, J.V.; Johnson, J.E.; Lehman, J.M.; MacPherson, D.; et al. Molecular subtypes of small cell lung cancer: A synthesis of human and mouse model data. Nat. Rev. Cancer. 2019, 19, 289–297.
10. Gay, C.M.; Stewart, C.A.; Park, E.M.; Diao, L.; Groves, S.M.; Heeke, S.; Nabet, B.Y.; Fujimoto, J.; Solis, L.M.; Lu, W.; et al. Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities. Cancer Cell 2021, 39, 346–360.e7.
11. Cui, M.; Augert, A.; Rongione, M.; Conkrite, K.; Parazzoli, S.; Nikitin, A.Y.; Ingolia, N.; MacPherson, D. PTEN Is a Potent Suppressor of Small Cell Lung Cancer. Mol. Cancer Res. 2014, 12, 654–659.
12. Rudin, C.M.; Durinck, S.; Stawiski, E.W.; Poirier, J.; Modrusan, Z.; Shames, D.S.; Bergbower, E.; Guan, Y.; Shin, J.; Guillory, J.; et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat. Genet. 2012, 44, 1111–1116.
13. George, J.; Lim, J.S.; Jang, S.J.; Cun, Y.; Ozretić, L.; Kong, G.; Leenders, F.; Lu, X.; Fernández-Cuesta, L.; Bosco, G.; et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015, 524, 47–53.
14. Brambilla, E.; Gazdar, A. Pathogenesis of lung cancer signalling pathways: Roadmap for therapies. Eur. Respir. J. 2009, 33, 1485–1497.
15. Varghese, A.M.; Zakowski, M.F.; Yu, H.A.; Won, H.H.; Riely, G.J.; Krug, L.M.; Kris, M.G.; Rekhtman, N.; Ladanyi, M.; Wang, L.; et al. Small-Cell Lung Cancers in Patients Who Never Smoked Cigarettes. J. Thorac. Oncol. 2014, 9, 892–896.
16. Rekhtman, N. Lung neuroendocrine neoplasms: Recent progress and persistent challenges. Mod. Pathol. 2021, 35, 36–50.
17. Yuan, J.; Knorr, J.; Altmannsberger, M.; Goeckenjan, G.; Ahr, A.; Scharl, A.; Strebhardt, K. Expression of p16 and lack of pRB in primary small cell lung cancer. J Pathol. 1999, 189, 358–362.
18. Baine, M.K.; Febres-Aldana, C.A.; Chang, J.C.; Jungbluth, A.A.; Sethi, S.; Antonescu, C.R.; Travis, W.D.; Hsieh, M.-S.; Roh, M.S.; Homer, R.J.; et al. POU2F3 in SCLC: Clinicopathologic and genomic analysis with a focus on its diagnostic utility in neuroendocrine-low SCLC. J. Thorac. Oncol. 2022.
19. Owonikoko, T.K.; Dwivedi, B.; Chen, Z.; Zhang, C.; Barwick, B.; Ernani, V.; Zhang, G.; Gilbert-Ross, M.; Carlisle, J.; Khuri, F.R.; et al. YAP1 Expression in SCLC Defines a Distinct Subtype With T-cell–Inflamed Phenotype. J. Thorac. Oncol. 2020, 16, 464–476.
20. Ding, X.-L.; Su, Y.-G.; Yu, L.; Bai, Z.-L.; Bai, X.-H.; Chen, X.-Z.; Yang, X.; Zhao, R.; He, J.-X.; Wang, Y.-Y. Clinical characteristics and patient outcomes of molecular subtypes of small cell lung cancer (SCLC). World J. Surg. Oncol. 2022, 20, 1–8.
21. McColl, K.; Wildey, G.; Sakre, N.; Lipka, M.B.; Behtaj, M.; Kresak, A.; Chen, Y.; Yang, M.; Velcheti, V.; Fu, P.; et al. Reciprocal expression of INSM1 and YAP1 defines subgroups in small cell lung cancer. Oncotarget 2017, 8, 73745–73756.
22. Hanna, N.H.; Temin, S.; Masters, G. Therapy for Stage IV Non–Small-Cell Lung Cancer Without Driver Alterations: ASCO and OH (CCO) Joint Guideline Update Summary. JCO Oncol. Pract. 2020, 16, e844–e848.
23. Pérol, M.; Felip, E.; Dafni, U.; Polito, L.; Pal, N.; Tsourti, Z.; Ton, T.; Merritt, D.; Morris, S.; Stahel, R.; et al. Effectiveness of PD-(L)1 inhibitors alone or in combination with platinum-doublet chemotherapy in first-line (1L) non-squamous non-small-cell lung cancer (Nsq-NSCLC) with PD-L1-high expression using real-world data. Ann. Oncol. 2022, 33, 511–521.
24. Gandhi, L.; Rodríguez-Abreu, D.; Gadgeel, S.; Esteban, E.; Felip, E.; De Angelis, F.; Domine, M.; Clingan, P.; Hochmair, M.J.; Powell, S.F.; et al. Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. N. Engl. J. Med. 2018, 378, 2078–2092.
25. Herbst, R.S.; Baas, P.; Kim, D.-W.; Felip, E.; Pérez-Gracia, J.L.; Han, J.-Y.; Molina, J.; Kim, J.-H.; Arvis, C.D.; Ahn, M.-J.; et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 2015, 387, 1540–1550.

26. Hellmann, M.D.; Nathanson, T.; Rizvi, H.; Creelan, B.C.; Sanchez-Vega, F.; Ahuja, A.; Ni, A.; Novik, J.B.; Mangarin, L.M.; Abu-Akeel, M.; et al. Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer. Cancer Cell 2018, 33, 843–852.e4.

27. Ricciuti, B.; Wang, X.; Alessi, J.V.; Rizvi, H.; Mahadevan, N.R.; Li, Y.Y.; Polio, A.; Lindsay, J.; Umeton, R.; Sinha, R.; et al. Association of High Tumor Mutation Burden in Non–Small Cell Lung Cancers with Increased Immune Infiltration and Improved Clinical Outcomes of PD-L1 Blockade Across PD-L1 Expression Levels. JAMA Oncol. 2022.

28. Chung, H.C.; Piha-Paul, S.A.; Lopez-Martin, J.; Schellens, J.H.M.; Kao, S.; Miller, W.H., Jr.; Delord, J.-P.; Gao, B.; Planchard, D.; Gottfried, M.; et al. Pembrolizumab After Two or More Lines of Previous Therapy in Patients with Recurrent or Metastatic SCLC: Results From the KEYNOTE-028 and KEYNOTE-158 Studies. J. Thorac. Oncol. 2020, 15, 618–627.

29. Liu, S.V.; Reck, M.; Mansfield, A.S.; Mok, T.; Scherpereel, A.; Reinmuth, N.; Garassino, M.C.; De Castro Carpeno, J.; Califano, R.; Nishio, M.; et al. Updated Overall Survival and PD-L1 Subgroup Analysis of Patients with Extensive-Stage Small-Cell Lung Cancer Treated with Atezolizumab, Carboplatin, and Etoposide (IMpower133). J. Clin. Oncol. 2021, 39, 619–630.

30. Kim, R.; Emi, M.; Tanabe, K. Cancer immunoediting from immune surveillance to immune escape. Immunology 2007, 121, 1–14.

31. Chen, M.; Chen, R.; Jin, Y.; Li, J.; Hu, X.; Zhang, J.; Fujimoto, J.; Hubert, S.M.; Gay, C.M.; Zhu, B.; et al. Cold and heterogeneous T cell repertoire is associated with copy number aberrations and loss of immune genes in small-cell lung cancer. Nat. Commun. 2021, 12, 1–13.

32. Hamilton, G.; Rath, B. Immunotherapy for small cell lung cancer: Mechanisms of resistance. Expert Opin. Biol. Ther. 2019, 19, 423–432.

33. Chan, J.M.; Quintanal-Villalonga, A.; Gao, V.R.; Xie, Y.; Allaj, V.; Chaudhary, O.; Masilionis, I.; Egger, J.; Chow, A.; Walle, T.; et al. Signatures of plasticity, metastasis, and immunosuppression in an atlas of human small cell lung cancer. Cancer Cell 2021, 39, 1479–1496.e18.

34. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. Cell 2011, 144, 646–674.

35. Keung, M.; Wu, Y.; Vadgama, J. PARP Inhibitors as a Therapeutic Agent for Homologous Recombination Deficiency in Breast Cancers. J. Clin. Med. 2019, 8, 435.

36. Allison Stewart, C.; Tong, P.; Cardnell, R.J.; Sen, T.; Li, L.; Gay, C.M.; Masrorpour, F.; Fan, Y.; Bara, R.O.; Feng, Y.; et al. Dynamic variations in epithelial-to-mesenchymal transition (EMT), ATM, and SLFN11 govern response to PARP inhibitors and cisplatin in small cell lung cancer. Oncotarget 2017, 8, 28575–28587.

37. Coussy, F.; El-Botty, R.; Château-Joubert, S.; Dahmani, A.; Montaudon, E.; Leboucher, S.; Morisset, L.; Painsec, P.; Sourd, L.; Huguet, L.; et al. BRCAness, SLFN11, and RB1 loss predict response to topoisomerase I inhibitors in triple-negative breast cancers. Sci. Transl. Med. 2020, 12, eaax2625.

38. Zoppoli, G.; Regairaz, M.; Leo, E.; Reinhold, W.C.; Varma, S.; Ballestrero, A.; Doroshow, J.H.; Pommier, Y. Putative DNA/RNA helicase Schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents. Proc. Natl. Acad. Sci. USA 2012, 109, 15030–15035.

39. Tian, L.; Song, S.; Liu, X.; Wang, Y.; Xu, X.; Hu, Y.; Xu, J. Schlafen-11 sensitizes colorectal carcinoma cells to irinotecan. Anti-Cancer Drugs 2014, 25, 1175–1181.

40. Takashima, T.; Taniyama, D.; Sakamoto, N.; Yasumoto, M.; Asai, R.; Hattori, T.; Honma, R.; Thang, P.Q.; Ukai, S.; Maruyama, R.; et al. Schlafen 11 predicts response to platinum-based chemotherapy in gastric cancers. Br. J. Cancer 2021, 125, 65–77.

41. Rathkey, D.; Khanal, M.; Murai, J.; Zhang, J.; Sengupta, M.; Jiang, Q.; Morrow, B.; Evans, C.N.; Chari, R.; Fetsch, P.; et al. Sensitivity of Mesothelioma Cells to PARP Inhibitors Is Not Dependent on BAP1 but Is Enhanced by Temozolomide in Cells with High-Schlafen 11 and Low-O6-methylguanine-DNA Methyltransferase Expression. J. Thorac. Oncol. 2020, 15, 843–859.

42. Zhang, B.; Ramkumar, K.; Cardnell, R.J.; Gay, C.M.; Stewart, C.A.; Wang, W.-L.; Fujimoto, J.; Wistuba, I.I.; Byers, L.A. A wake-up call for cancer DNA damage: The role of Schlafen 11 (SLFN11) across multiple cancers. Br. J. Cancer 2021, 125, 1333–1340.

43. Murai, J.; Huang, S.-Y.N.; Renaud, A.; Zhang, Y.; Ji, J.; Takeda, S.; Morris, J.; Teicher, B.; Doroshow, J.H.; Pommier, Y. Stereospecific PARP Trapping by BMN 673 and Comparison with Olaparib and Rucaparib. Mol. Cancer Ther. 2014, 13, 433–443.

44. Branzei, D.; Foiani, M. Maintaining genome stability at the replication fork. Nat. Rev. Mol. Cell Biol. 2010, 11, 208–219.

45. Murai, J.; Feng, Y.; Yu, G.K.; Ru, Y.; Tang, S.-W.; Shen, Y.; Pommier, Y. Resistance to PARP inhibitors by SLFN11 inactivation can be overcome by ATR inhibition. Oncotarget 2016, 7, 76534–76550.
46. Murai, J.; Huang, S.-Y.N.; Das, B.B.; Renaud, A.; Zhang, Y.; Doroshow, J.H.; Ji, J.; Takeda, S.; Pommier, Y. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer Res. 2012, 72, 5588–5599.
47. Pommier, Y.; O'Connor, M.J.; de Bono, J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Sci. Transl. Med. 2016, 8, 362ps17.
48. Pietanza, M.C.; Waqar, S.N.; Krug, L.M.; Dowlati, A.; Hann, C.L.; Chiappori, A.; Owonikoko, T.K.; Woo, K.M.; Cardnell, R.J.; Fujimoto, J.; et al. Randomized, Double-Blind, Phase II Study of Temozolomide in Combination with Either Veliparib or Placebo in Patients with Relapsed-Sensitive or Refractory Small-Cell Lung Cancer. J. Clin. Oncol. 2018, 36, 2386–2394.
49. Trigo, J.; Subbiah, V.; Besse, B.; Moreno, V.; López, R.; Sala, M.A.; Peters, S.; Ponce, S.; Fernández, C.; Alfaro, V.; et al. Lurbinectedin as second-line treatment for patients with small-cell lung cancer: A single-arm, open-label, phase 2 basket trial. Lancet Oncol. 2020, 21, 645–654.
50. Kundu, K.; Cardnell, R.J.; Zhang, B.; Shen, L.; Stewart, C.A.; Ramkumar, K.; Cargill, K.R.; Wang, J.; Gay, C.M.; Byers, L.A. SLFN11 biomarker status predicts response to lurbinectedin as a single agent and in combination with ATR inhibition in small cell lung cancer. Transl. Lung Cancer Res. 2021, 10, 4095–4105.
51. Zhang, B.; Stewart, C.A.; Wang, Q.; Cardnell, R.J.; Rocha, P.; Fujimoto, J.; Soto, L.M.S.; Wang, R.; Novegil, V.; Ansell, P.; et al. Dynamic expression of Schlafen 11 (SLFN11) in circulating tumour cells as a liquid biomarker in small cell lung cancer. Br. J. Cancer 2022, 127, 569–576.
52. Saunders, L.R.; Bankovich, A.J.; Anderson, W.C.; Aujay, M.A.; Bheddah, S.; Black, K.; Desai, R.; Escarpe, P.A.; Hampl, J.; Laysang, A.; et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci. Transl. Med. 2015, 7, 302ra136.
53. Tanaka, K.; Isse, K.; Fujihira, T.; Takenoyama, M.; Saunders, L.; Bheddah, S.; Nakanishi, Y.; Okamoto, I. Prevalence of Delta-like protein 3 expression in patients with small cell lung cancer. Lung Cancer 2017, 115, 116–120.
54. Geffers, I.; Serth, K.; Chapman, G.; Jaekel, R.; Schuster-Gossler, K.; Cordes, R.; Sparrow, D.; Kremmer, E.; Dunwoodie, S.; Klein, T.; et al. Divergent functions and distinct localization of the Notch ligands DLL1 and DLL3 in vivo. J. Cell Biol. 2007, 178, 465–476.
55. Matsuo, K.; Taniguchi, K.; Hamamoto, H.; Inomata, Y.; Komura, K.; Tanaka, T.; Lee, S.; Uchiyama, K. Delta-like canonical Notch ligand 3 as a potential therapeutic target in malignancies: A brief overview. Cancer Sci. 2021, 112, 2984–2992.
56. Hipp, S.; Voynov, V.; Drobits-Handl, B.; Giragossian, C.; Trapani, F.; Nixon, A.E.; Scheer, J.M.; Adam, P.J. A Bispecific DLL3/CD3 IgG-Like T-Cell Engaging Antibody Induces Antitumor Responses in Small Cell Lung Cancer. Clin. Cancer Res. 2020, 26, 5258–5268.
57. Tully, K.M.; Tendler, S.; Carter, L.M.; Sharma, S.K.; Samuels, Z.V.; Mandleywala, K.; Korsen, J.A.; Reyes, A.M.D.; Piersigilli, A.; Travis, W.D.; et al. Radioimmunotherapy Targeting Delta-like Ligand 3 in Small Cell Lung Cancer Exhibits Antitumor Efficacy with Low Toxicity. Clin. Cancer Res. 2022, 28, 1391–1401.