

Targeting DNA damage repair in small cell lung cancer and the biomarker landscape

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Abstract: Small cell lung cancer (SCLC) is an aggressive malignancy that accounts for 14% of all lung cancer diagnoses. Despite decades of active research, treatment options for SCLC are limited and resistance to the few Food and Drug Administration (FDA) approved therapies develops rapidly. With no approved targeted agents to date, new therapeutic strategies are desperately needed. SCLC is characterized by high mutation burden, ubiquitous loss of TP53 and RB1, mutually exclusive amplification of MYC family members, thereby, high genomic instability. Studies in the past few years have demonstrated the potential of targeting the DNA damage response (DDR) pathway as a promising therapeutic strategy for SCLC. Inhibitors targeting DDR proteins have shown promise in preclinical models, and are under clinical investigation as single agents and in combination with cytotoxic therapies. Recent efforts to expand the therapeutic arsenal toward SCLC have focused in part on immune checkpoint inhibitors, such as agents targeting the receptor-ligand pair programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1). Clinical trials have confirmed activity of these agents in extensive stage (ES)-SCLC. However, while several patients had dramatic responses, overall response rates to immune checkpoint blockade (ICB) remain poor. As a result, there is an urgent need to develop rational combination therapies to enhance response rates to immunotherapy in SCLC. Identification of predictive biomarkers for patient stratification, identifying effective combinations to overcome adaptive resistance to DDR-targeted therapies and identifying strategies to enhance response to immunotherapy are areas of active investigation in SCLC.

Keywords: DNA damage response (DDR); biomarkers; immunotherapy; small cell lung cancer (SCLC)

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The “recalcitrant cancer”

With an estimated 250,000 cases worldwide annually, small cell lung cancer (SCLC) is the sixth major cause of cancer-related mortality (1-7). SCLC is the most aggressive form of lung cancer, characterized by rapid tumor growth and early metastatic spread (8,9). Currently, chemotherapy remains the cornerstone of treatment for both limited stage (LS) and extensive stage (ES)-SCLC, with a majority of patients obtaining an objective response with first-line

treatment, as described by Farago and Keane (current standards for clinical management of SCLC) also in this issue. However, despite high rates of response initially, the duration of clinical response is extremely limited in most cases and almost all patients relapse—often within a few months (5,6,8,9). Recently, clinical trials have demonstrated that some SCLC patients can obtain durable responses to immunotherapy and this has led to the addition of nivolumab with or without ipilimumab to the NCCN treatment guidelines as the first non-chemotherapy

option for relapsed SCLC (10,11). However, beyond this there are currently no targeted agents for the treatment of SCLC approved by the United States Food and Drug Administration (FDA).

In 2012, SCLC was recognized by US Congress as one of two “recalcitrant cancers” (the second being pancreatic cancer) based on its 5-year survival rate of <7%. The Recalcitrant Cancer Research Act led to new funding mechanisms and a renewed focus on translational research in SCLC intended to speed the pace of translational research and discovery for this disease (12). In the past several years, investigators have identified many new, highly promising drug targets through translational investigations of preclinical models and patient tissues (5,6). Among these are several drugs targeting the proteins involved in DNA damage response (DDR) such as poly-ADP-ribose polymerase (PARP), checkpoint kinase 1 (CHK1), ataxia telangiectasia and Rad3-related protein (ATR), and WEE1 (13). The finding that DDR targets are more highly expressed in SCLC (14)—together with data demonstrating preclinical activity of DDR inhibitors in SCLC models (14-19)—has had immediate translational implications, as several DDR inhibitors have been developed and are either approved for other cancer types (e.g., PARP inhibitors) or are in clinical trials (20). Only a small number of DDR inhibitor trials have included SCLC patients to date (*Table 1*). However, the available data suggest promising activity in a subset of SCLC patients. Moreover, candidate biomarkers are emerging that may aid in identifying subsets of SCLC with greatest vulnerability to specific DDR inhibitors.

In this review, we will discuss aspects of SCLC biology that may make it especially susceptible to DDR targeting approaches, preclinical and clinical data for several DDR inhibitors, and candidate biomarkers that may help identify patients who would benefit from DDR targeting. In recent years cancer treatment has been revolutionized with the clinical development of antibodies targeting immune checkpoints (21,22). In addition, there is active research to understand what drives immunogenicity in a cancer and the role of DDR pathways in this context has recently been in focus. Here, we will also discuss the mechanisms by which targeting the DDR pathways could regulate the antitumor immune response to immune checkpoint blockade (ICB) and describe clinical efforts to combine DDR targeted therapies and ICB in SCLC.

Genomic instability—a hallmark of SCLC

Cigarette smoking contributes significantly to the

accumulation of DNA damage and is a major cause of SCLC (>95% of patients): in particular, the risk of developing SCLC increases with the number of cigarettes smoked each day and with the duration of smoking (23,24). A previous study exploring the mutational burden associated with tobacco smoking in SCLC performed deep sequencing on SCLC cell line, NCI-H209 (25) and identified a total of 22,910 somatic substitutions, including 134 in coding exons. Interestingly, even in this single cell line profiling study, they identified several distinctive point mutation patterns that reflected the effect of carcinogens present in cigarette smoke (25). They also observed signatures of the partially successful attempts of the cells’ surveillance machinery to repair DNA damage (25). Unsurprisingly, SCLC carries a high mutation burden and genomic instability (26).

On top of the mutational burden induced by tobacco exposure, almost all SCLC tumors have a loss of *TP53* and *RBI* and amplification of the *MYC* family of oncogenes (27,28). Defective *TP53* and *RBI*, along with aberrant activation of the oncogene *MYC*, results in rapid proliferation and, consequently, replication stress in SCLC. In this background, SCLC cells become dependent on intact DDR pathways for survival. Any further perturbation to the DDR or cell cycle checkpoint regulation can potentially lead to mitotic catastrophe and cell death.

Earlier studies have reported the vulnerability of *TP53* defective cancer cells to DDR inhibitors (29,30). In the last few years data from proteomic and genomic studies of SCLC have also highlighted changes in the DDR pathways in SCLC. Proteomic analysis of 34 SCLC and 74 non-small cell lung cancer (NSCLC) cell lines demonstrated that SCLC had significantly higher levels of E2F1-regulated factors including DNA repair proteins such as PARP, ATR, CHK1/2, DNA-dependent protein kinase (DNA-PK), and ataxia telangiectasia-mutated (ATM) (14). Subsequent studies confirmed the overexpression of *ATR*, *CHEK1* and *WEE1* in SCLC patient tumors as compared to normal lung (17-19). A recent study by Doerr *et al.* further demonstrated by transcriptomic analysis of different subtypes of lung cancer that SCLC has a significantly higher expression of genes involved in the DDR (such as *CHEK1*, *CDC25A*, *B* and *C*) than other lung cancer subtypes (31). These new insights into the biology of SCLC represent an intriguing possibility of targeting DDR proteins (13,32) (*Figure 1*).

Targeting DDR—an “Achilles’ heel” of SCLC?

Recent studies have demonstrated the therapeutic

Table 1 DDR-targeted therapy clinical trials in SCLC (list includes ongoing trials as of 12/2017)

Trial	Treatment	Indication
PARP inhibitor trials in SCLC		
NCT03227016	Phase 1: veliparib alone; phase 2: veliparib + topotecan	Relapsed/refractory ES-SCLC
NCT02734004	Phase 1/2: MEDI4736 (anti-PD-L1) in combination with olaparib	Advanced solid tumors including ES-SCLC cohort
NCT02289690	Phase 2: carboplatin/etoposide +/- veliparib	Treatment-naïve ES-SCLC
NCT02769962	Phase 1/2: CRLX101 (camptothecin nanoparticle) + olaparib	Relapsed/refractory ES-SCLC
NCT01642251	Phase 1/2: cisplatin/etoposide +/- veliparib	Treatment-naïve ES-SCLC
NCT02498613	Phase 2: olaparib + cediranib (anti-VEGFR TKI)	Advanced solid tumors including ES-SCLC cohort
NCT02446704	Phase 1/2: olaparib + TMZ	Relapsed/refractory ES-SCLC
NCT03009682	Phase 2: olaparib monotherapy	Relapsed/refractory ES-SCLC harboring HR mutations
NCT02511795	Phase 1b: olaparib + AZD1775 (WEE1 inhibitor)	Advanced solid tumors including ES-SCLC cohort
ATR inhibitor trials including SCLC		
NCT02487095	Phase 1/2: topotecan + VX970	Advanced small cell cancers
NCT02589522	Phase 1: VX-970 + WBRT	Brain metastases from tumors
NCT02223923	Phase 1: AZD6738 +/- RT	Advanced solid tumors
NCT02723864	Phase 1: veliparib + VX-970 + cisplatin	Advanced solid tumors
NCT02595931	Phase 1: VX-970 + irinotecan	Advanced solid tumors
NCT02157792	Phase 1: VX-970 + chemotherapy	Advanced solid tumors
NCT03188965	Phase 1: BAY1895344 monotherapy	Advanced solid tumors
CHK inhibitor trials including SCLC		
NCT02735980	Phase 2: prexasertib monotherapy	Relapsed/refractory ES-SCLC
NCT02797964	Phase 1: SRA737 monotherapy	Advanced solid tumors
NCT02797977	Phase 1: SRA737 + gemcitabine +/- cisplatin	Advanced solid tumors
NCT02873975	Phase 2: prexasertib monotherapy	Advanced solid tumors with HR deficiency or replicative stress
NCT03057145	Phase 1: prexasertib + olaparib	Advanced solid tumors
Wee1 inhibitor trials including SCLC		
NCT02482311	Phase 1: AZD1775 monotherapy	Advanced solid tumors including ES-SCLC cohort
NCT02511795	Phase 1b: AZD1775 + olaparib	Advanced solid tumors including ES-SCLC cohort
NCT02593019	Phase 2: AZD1775 monotherapy	Relapsed/refractory ES-SCLC
NCT02688907	Phase 2: AZD1775 monotherapy	Relapsed/refractory ES-SCLC with MYC amplifications or CDKN2A + TP53 mutations
NCT02937818	Phase 2: AZD1775 + carboplatin (arm)	Relapsed/refractory ES-SCLC
NCT01748825	Phase 1: AZD1775 monotherapy	Advanced solid tumors
AURKA or AURKB inhibitor trials including SCLC		
NCT03216343	Phase 1: chiauranib	Relapsed/refractory ES-SCLC
NCT03092934	Phase 1/2: AK-01	Advanced solid tumors including ES-SCLC cohort
NCT02719691	Phase 1b: MLN0128 (TORC1/2 inhibitor) + alisertib	Advanced solid tumors
NCT02134067	Phase 1: TAS-119 + paclitaxel	Advanced solid tumors
NCT01118611	Phase 1: GSK1070916A	Advanced solid tumors

SCLC, small cell lung cancer; ES-SCLC, extensive stage-SCLC; DDR, DNA damage response; PARP, poly-ADP-ribose polymerase; PD-L1, programmed death-ligand 1; CEGFR, vascular endothelial growth factor; TKI, tyrosine kinase inhibitor; TMZ, temozolomide; WBRT, whole brain radiation therapy; RT, radiation therapy; AURKA/B, Aurora A/B.

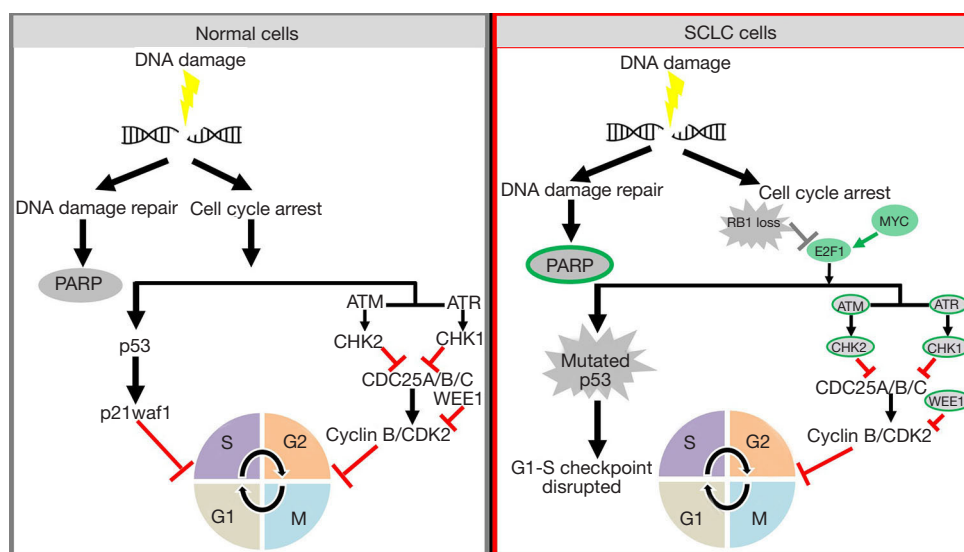


Figure 1 Targeting DDR vulnerabilities of SCLC. Ubiquitous loss of TP53 and RB1 disrupts the G1-S cell cycle checkpoint SCLC making the cells dependent on G2-M cell cycle checkpoint for arrest upon DNA damage. MYC family amplifications and increased gene and protein expression of multiple DDR pathway molecules (indicated in green) suggest they may be effective targets in SCLC. DDR, DNA damage response; SCLC, small cell lung cancer; PARP, poly-ADP-ribose polymerase; ATM, ataxia telangiectasia-mutated; ATR, ataxia telangiectasia and Rad3-related protein.

opportunities of targeting DDR pathways in highly aggressive cancers, including SCLC (14,15,17,18,33-37). The DDR network is a complex and dynamic system with almost 450 integral proteins. Five major DNA repair pathways are known: base excision repair (BER) to repair single-strand breaks (SSBs); homologous recombination repair (HRR) and non-homologous end-joining (NHEJ) to repair double-strand breaks (DSBs); mismatch repair (MMR) to repair replication errors, and nucleotide excision repair (NER) to repair bulky adducts (13,34-37). The different DDR pathways have built in redundancy to a certain extent that allows many of them to serve as a compensatory mechanism when other DDR pathways are compromised.

The transformation of a normal cell to a cancerous one requires, amongst other features, the acquisition of the ability of uncontrolled proliferation and failure of efficient and accurate DNA repair (38,39). The loss of robust DNA damage repair has important implications in tumor growth, cancer progression and therapeutic responses. Some common features of highly aggressive human malignancies include high replication stress, aberrant cell cycle control, and functional defects in one or more of the DNA repair pathways. The cellular stress is further

enhanced with frequent overexpression and activation of oncogenes inducing proliferation such as *MYC* or *CCNE1* or loss of tumor suppressor genes such as *TP53* that further disrupt the balance of replication, transcription and cell cycle checkpoint control (40). As mentioned above, the mutational landscape of SCLC is complex and varied, but includes the ubiquitous functional inactivation of both *TP53* and *RB1* (26-28). Other notable abnormalities of SCLC are the frequent amplifications of the oncogenic transcription factors *MYC* and *SOX2* in 27% of cases (26-28). Several studies have demonstrated the role of *MYC* as a vital regulator of cell growth and proliferation by enhancing the expression of genes controlling cell cycle (e.g., cyclins, CDKs, dNTP biosynthetic enzymes and replication factors), and repressing anti-proliferative genes (e.g., CDK inhibitors) (41,42). Also, *MYC* physically interacts with the pre-replicative complex and co-localizes with replication foci in early S phase regardless of transcription and hence drives cell cycle progression by directly controlling replication initiation (40,43). Hence, *MYC* overexpression and/or amplification affects replication initiation and potentially leads to premature origin firing and subsequent replication stress.

DNA-damaging chemotherapy in SCLC

By removing many of the mechanisms through which cells would normally halt the cell cycle to respond to errors in DNA replication or extrinsic DNA damage, cancer cells can proliferate at much higher rates. However, this characteristic also makes highly proliferative cancers more susceptible to the DNA damage induced by chemotherapy or radiation. This explains the initial high response rates seen in SCLC with chemotherapies that broadly target the process of cell division or DNA replication, including platinum agents (e.g., cisplatin, carboplatin) and topoisomerase I/II inhibitors (e.g., etoposide, irinotecan) (44,45).

Platinum-chemotherapy

Cisplatin and the cisplatin analog carboplatin have defined the backbone of SCLC chemotherapy for several decades (44,45). Platinum compounds bind to reactive centers on purine residues within DNA and generate cisplatin-purine cross-links to induce DNA damage, block cell division and, ultimately, promote apoptosis (46). Numerous studies have identified deficient DDR as a potential marker of platinum sensitivity and the upregulation of DDR machinery as a potential mechanism of platinum resistance (46).

Type II topoisomerase (Topo II) inhibitors

Etoposide is widely used in combination with platinum-chemotherapy in first-line treatment for SCLC (44,45). Etoposide targets Topo II, an enzyme class that plays a critical role during DNA replication (47). Specifically, Topo II cleaves double-stranded DNA to permit passage of intact helical DNA before ligating the cleavage site. Etoposide prevents this ligation event by stabilizing the complex formed by Topo II and the 5' cleaved ends of the DNA, resulting in stable, protein-linked double strand breaks in DNA and subsequent apoptosis (47).

Type I topoisomerase (Topo I) inhibitors

The Topo I inhibitors topotecan and irinotecan are commonly used in relapsed/refractory SCLC following resistance to platinum and Topo II targeting therapies (44,45). However, irinotecan is often also used (outside the United States) in the frontline setting in combination with platinum therapy. Topotecan is currently the only FDA-approved therapy for relapsed/refractory SCLC. The Topo I

enzyme class is responsible for creating single strand breaks in DNA to relieve torsional strain created by twisting and supercoiling. Binding of topotecan or irinotecan to the Topo I-DNA complex prevents repair of these single strand nicks and, ultimately, to unrepaired double strand breaks and apoptosis (48).

DDR-targeted therapies in SCLC

In the last few years, targeted agents for DDR pathways have opened exciting new avenues for research and treatment in several cancers including SCLC. As mentioned above, DDR proteins were seen to be overexpressed in SCLC *in vitro*. Follow-on studies confirmed the higher gene expression of DDR pathway mediators such as *PARP*, *CHEK1*, *ATM* and *ATR* in SCLC patient tumors as compared to normal lung and/or NSCLC tumors (14,17-19,31,49). In addition, a high-throughput small molecule drug screen also identified DDR proteins, including *CHK1* as a candidate therapeutic target in SCLC (50). Since then, several DNA repair inhibitors have been evaluated in SCLC preclinical models and clinical trials. Although DDR inhibitors have shown activity as monotherapy in SCLC models and in some patients (e.g., the PARP inhibitor talazoparib) (20), combinations with cytotoxic chemotherapies, other targeted agents (e.g., DDR inhibitor combinations), or possibly with immunotherapy are predicted to provide greater clinical response by increasing the percentage of patients likely to respond and potentially the duration of response.

Targeting PARP in SCLC treatment

PARPs are a family of nuclear protein enzymes involved in the DDR. Previous reports have extensively characterized the key role of PARP in DDR and repair of SSB through BER (51-54). More recently PARP's role in homologous recombination (HR) and NHEJ was identified. PARP1 regulates the restart of replication forks after DNA damage repair by the attachment of ADP-ribose units to multiple proteins.

PARP inhibitors exert their effect by two main mechanisms first by trapping the enzyme to the SSBs by preventing the utilization of NAD⁺ (55); second, PARP1 inhibitors inhibit PARylation and therefore binding of PARP to DNA (55). The resulting PARP-DNA complexes lead to collapsing and stalling of replication forks and ultimately to the conversion of SSBs to DSBs leading to

apoptosis (51-55). In cells with intact HR pathway, loss of PARP is not lethal. However, PARP targeting is highly effective in the context of synthetic lethality in models with existing deficiency in DNA repair, such as deleterious mutations in *BRCA1/2*. BRCA1 and 2 deficient cells are rendered defective in the ability to repair through HR and depend on error-prone NHEJ for DNA repair (56-60).

To date, three PARP inhibitors olaparib (AZD-2281/KU-0059436), niraparib (MK-4827) and rucaparib (AG-014699/PF-01367338) have been approved by the US FDA for the treatment of biomarker defined subsets of ovarian cancer (61). In SCLC, however, PARP inhibition is independent of BRCA1 status and likely depends more on intrinsically high levels of replication stress and genomic instability (14). In addition to the FDA-approved agents, PARP inhibitors that are currently undergoing active clinical investigation in SCLC also include CEP-9722, E7016/GPI-21016, talazoparib (BMN-673), and veliparib (ABT-888).

Although most PARP inhibitors are comparable in their enzymatic inhibition of PARP activity, they vary by the degree to which they induce direct cytotoxicity through PARP trapping. Among PARP inhibitors that are approved or in advanced stages of clinical trial testing, the lowest amount of trapping is seen with veliparib (making it more tolerable in combination with chemotherapy); moderate trapping with drugs such as olaparib, rucaparib, and niraparib; and the highest degree of trapping with talazoparib (formerly BMN-673) (55). Based on preclinical results described below, several clinical trials have been initiated to test the activity of these drugs in SCLC patients. Three main therapeutic approaches have been tested in SCLC patients to date: (I) single agent talazoparib in platinum-sensitive relapsed SCLC; (II) the combination of temozolomide (TMZ) with veliparib or olaparib in relapsed SCLC (NCT01638546, NCT02446704); and (III) the addition of veliparib to front-line platinum-etoposide therapy. None of these trials required biomarker testing as part of the eligibility criteria, although some exploratory biomarker analyses have been performed retrospectively. Available results from these clinical studies are outlined below.

Single agent activity of PARP inhibitors in SCLC preclinical models

In previous studies, we demonstrated single agent activity of PARP inhibitors, olaparib and rucaparib, in SCLC preclinical models (14). A subsequent investigation further demonstrated the single agent potential of another PARP

inhibitor, talazoparib, in SCLC preclinical models (15). In the first PARP inhibitor trial to include SCLC patients, single agent talazoparib was tested in an expansion cohort of platinum-sensitive patients following relapse as part of the first-in-man phase 1 trial (20). Talazoparib is a highly potent PARP inhibitor that works through two mechanisms— inhibition of PARP enzymatic activity and direct cytotoxicity induced by trapping PARP-DNA complexes at the sites of single-strand DNA breaks (so-called “PARP trapping”). High response rates were observed in BRCA-mutated breast and ovarian cancer patients treated on this phase 1 study and clinical benefit from this drug in BRCA-mutated breast cancer patients was recently confirmed in a phase 3 trial (62). Patients in the SCLC cohort (n=23) were treated with 1.0 mg daily by mouth [the established maximum tolerated dose (MTD)]. Two patients (9%) had partial responses (PRs) (duration 12.0 and 15.3 weeks, respectively) and four patients (18%) had stable disease (SD) (lasting >16 weeks). Median progression free survival (PFS) for the overall SCLC cohort was 11.1 weeks (20). This is the only clinical trial to date of single agent PARPi (or of talazoparib) for relapsed SCLC patients.

Strategies of combining PARP inhibitors with chemotherapy in SCLC preclinical models

Several preclinical studies have also demonstrated the effect of PARP inhibitors alone or in combination regimens with standard chemotherapy in SCLC models. For example, we found that olaparib in combination with cisplatin and etoposide in multiple SCLC cell lines caused approximately 80% more cell killing than in control cells and the cell count was significantly lower than the cell count after treatment with olaparib alone ($P<0.05$ for both) (14). Furthermore, treatment of H69 cells with olaparib in combination with irinotecan, another chemotherapeutic commonly used in the treatment of SCLCs, also resulted in a greater decrease in tumor cell viability than either agent alone ($P=0.03$ olaparib/irinotecan *vs.* control; $P=0.007$ olaparib/irinotecan *vs.* irinotecan alone) (14). In a study investigating the efficacy of PARP inhibitor, veliparib, as a single agent and in combination with cisplatin, carboplatin, and etoposide, Owonikoko *et al.*, demonstrated that veliparib potentiates standard cytotoxic agents and the effect is correlated with platinum sensitivity, DNA-PKcs expression and a 5-gene (*GLS*, *UBEC2*, *HACL1*, *MSI2* and *LOC100129585*) expression profile in SCLC cell lines (63). In another study, Lok *et al.*, demonstrated that PARP inhibition by talazoparib is synergistic in combination

with DNA alkylator, TMZ in SCLC cell lines and patient-derived xenografts (PDX) models (64).

Based on phase 2 data showing activity of TMZ in relapsed SCLC (65) and preclinical data demonstrating activity of TMZ in combination with PARP inhibitors (64), two clinical trials have tested this combination in SCLC. In the first study, 100 patients with 1–2 prior lines of therapy were treated with TMZ with either veliparib or placebo (TMZ 150–200 mg/m²/day on days 1–5 and veliparib/placebo 40 mg po twice daily on days 1–7) (66). The patient population included 59% platinum-refractory patients and 33% 3rd line patients, which is notable since these are features known to be associated with lower response rates and shorter survival. Although the primary endpoint of improved 4-month PFS was not met in this trial, patients receiving the combination of TMZ/veliparib had an almost 3-fold higher response rate as compared to the TMZ/placebo arm (39% vs. 19%). Median overall survival (OS) was 8.2 months in TMZ/veliparib arm and 7.0 months in the TMZ/placebo arm (P=0.50). However, a significantly longer PFS and OS were observed in patients receiving TMZ/veliparib combination who had detectable schlafen 11 (SLFN11) by immunohistochemistry. The putative DNA/RNA helicase SLFN11 has been shown by others and us to be a candidate predictive marker of chemotherapy and PARP inhibitor sensitivity (49,64,67,68). A second TMZ-PARP inhibitor trial using olaparib is now ongoing. Data presented from the first 13 patients, though early, showed promising activity with a response rate of 48% and median PFS of 5.6 months (69).

In the frontline setting, the PARP inhibitor veliparib has been explored in combination with cisplatin-etoposide (ECOG-ACRIN 2511; NCT01642251) and with carboplatin-etoposide (NCT02289690). In the first trial, 128 eligible patients with ES-SCLC were randomized to four cycles of cisplatin plus etoposide (75 and 100 mg/m², respectively) with either veliparib (100 mg po twice daily on days 1–7) or placebo. No maintenance veliparib was given in this trial. As of December 2016 (median follow up 18.5 months), the primary endpoint of PFS was 6.1 months in the cisplatin-etoposide plus veliparib arm versus 5.5 months in the cisplatin-etoposide-placebo arm (P=0.06), with an adjusted one-sided PFS HR of 0.63 (P=0.01). A trend toward improved OS was also observed in patients receiving chemotherapy in combination with veliparib, although this did not reach statistical significance (OS 10.3 vs. 8.9 months, P=0.17). Similarly, response rates were numerically higher in the veliparib arm (72% vs. 64%), but not significantly

different (P=0.57). The most frequent adverse events in both groups were neutropenia (grade 3/4: 49% in veliparib arm; 32% placebo arm); leukopenia (grade 3/4: 19% in veliparib arm; 14% placebo arm); and anemia (grade 3/4: 19% in veliparib arm; 12% placebo arm). A randomized phase 1/2 study of carboplatin-etoposide with or without veliparib which incorporates a higher dose of veliparib with chemotherapy (recommended phase 2 dose 240 mg po BID days –2 to 12) as well as veliparib in the maintenance setting is ongoing (NCT2289690) (70). The finding from the cisplatin-etoposide +/- veliparib trial suggest that a biomarker selection strategy (such as SLFN11 or other candidate markers) will be needed to identify those patients likely to receive the greatest benefit from the addition of PARP inhibition to standard chemotherapy. In addition to these studies, there are now nine trials of PARP inhibitors at various stages for SCLC, including trials testing for the first time the combination of the PARP inhibitor olaparib with immunotherapy (MEDIOLA trial, NCT02734004) (Table 1).

Combining inhibition of PI3K and PARP in SCLC

Several studies have shown that combined inhibition of PARP and PI3K is a potentially effective treatment regimen for breast cancers with elevated activity of the PI3K pathway (71,72). Activation of the PI3K pathway is a common feature of many tumor types. PI3K pathway activation leads to the stimulation of cell growth, motility, survival, and metabolism, and also sensing of DDR (73,74). In a subsequent study, Cardnell *et al.*, further showed that the proteins most significantly upregulated following treatment with the PARP inhibitors olaparib and rucaparib belonged to the PI3K/mTOR pathway (p-mTOR, p-AKT, and pS6) (P=0.02), and amongst the most significantly down-regulated proteins were LKB1 and its targets AMPK and TSC, which negatively regulate the PI3K pathway (P=0.042) (16). The study concluded that the observed increase in PI3K/mTOR pathway activation following PARP inhibition results from decreased ATP usage and a subsequent decrease in stress response signaling via LKB1. The data further suggested that combining PARP and PI3K inhibitors enhances the effect of either agent alone in *in vitro* and *in vivo* SCLC models (16). Although there is a strong preclinical rationale for combining inhibitors of PARP and PI3K, the therapeutic possibilities for this combination are less clear. The main concerns would be that of toxicity, patient selection and finally the resistance to targeted therapies as Juvekar *et al.*'s identification of a drug resistant “pushing margin” that continues to grow

during treatment with a PI3K inhibitor (72). However, this combination certainly warrants consideration for clinical trial and may pave the way for wider use of PARP inhibitors in SCLC patients.

Targeting ATR in SCLC

Another DDR protein implicated in SCLC is ATR. The ATR-CHK1 axis is part of a complex signaling network that is activated upon genotoxic stress and DNA damage. In the presence of extended stretches of ssDNA that are coated with replication protein A (RPA), ATR gets recruited by interactions with ATRIP. Once activated, ATR phosphorylates and activates multiple targets including CHK1. Upon stimulation, ATR-CHK1 axis enforces halting of cell cycle progression at the G2-M phase until damage is repaired (75-78). Preclinical observations suggest that ATR inhibitors may be especially active in *TP53* and/or *ATM* deficient models from other tumor types (79). Beyond its role in regulating p53-mediated apoptosis, ATM is also reported to be involved in DNA DSB repair, particularly through the HR pathway, with a less well-defined role in the NHEJ pathway. Hence, in ATM deficient tumors (up to ~8% of SCLC), DSB repair is dependent primarily on the ATR/CHK1 axis (19,78). Recent reports find activity of ATR inhibitors in *in vitro* and *in vivo* models of SCLC (31). Clinically, results from a phase 1, first-in-class trial with the ATR inhibitor VX-970 included a complete response (CR) in a colorectal cancer patient with ATM loss and a durable PR in a *BRCA1* mutant ovarian cancer, further supporting a role for ATR inhibitors in DDR-deficient tumors. Several ATR inhibitors are now in clinical trial alone or in combination with chemotherapy, radiation and PARP inhibitors, including VX-970 and VX-803 (EMD Serono), AZD6738 (AstraZeneca) and BAY-1895344 (Bayer). Notably, several of these trials include SCLC patients (NCT02487095; NCT02589522), while others enroll across solid tumors. The clinical outcomes and biomarker analyses of these studies will guide future investigations into ATR inhibitors for SCLC.

Targeting CHK1 in SCLC

The combined loss of *TP53* and *RB1* in SCLC leads to defective cell cycle arrest at the G1/S phase and hence an enhanced dependency on the G2/M phase for repair upon DNA damage. We previously showed that SCLC cell lines have a median of 4.5-fold higher (range,

2–7 folds) CHK1 protein expression than NSCLC cell lines ($P=0.001$) (18). This was further validated in SCLC tumors which were shown to have 4.2-fold higher CHK1 expression at the mRNA levels ($P<0.0001$) *vs.* normal lung. Based on this, we tested the activity of a second generation CHK1 inhibitor, prexasertib (80), as a single agent or combined with chemotherapy in both platinum sensitive and resistant SCLC models. In a comprehensive panel of 39 human and murine SCLC cell lines, prexasertib showed remarkable single agent activity with half-maximal inhibitory concentration (IC50) of prexasertib <100 nM in 50% human SCLC cell lines tested, as well as activity in SCLC syngeneic, genetically-engineered mouse (GEM) and chemoresistant models. In addition, prexasertib potentiated the effect of cisplatin in chemoresistant and chemosensitive models, a finding that has potential clinical relevance. CHK1 inhibition also reversed PARP inhibitor resistance in preclinical models (18). Similar findings were reported recently in an independent study by Doerr *et al.*, where transcriptome analysis showed a significant overexpression of genes involved in the DDR, specifically *CHEK1*, in SCLC compared to lung adenocarcinoma. They further showed that the mRNAs encoding the phosphatases *CDC25A*, *B* and *C* (substrates of checkpoint kinases) are expressed at significantly higher levels in SCLC, compared to lung adenocarcinomas (ADC) and squamous cell carcinomas (31). Following these observations, they demonstrated that CHK1 inhibitor, PF-477736, induced genotoxic damage and apoptosis in human and murine SCLC cell lines, but not in lung adenocarcinoma *in vitro* and *in vivo* models (31).

Together, these findings provide a strong rationale for targeting CHK1 and other G2/M checkpoint proteins such as WEE1. Previous phase I clinical trial data with the CHK1 inhibitor MK-8776 (Merck) showed PRs in only 2/30 evaluable patients, but SD in an additional 13/30. However, this was a solid tumor population that did not select for DDR deficiencies and included no SCLC patients. Additional data from a phase I trial of the CHK1 inhibitor prexasertib (Eli Lilly) in patients with advanced refractory squamous NSCLC, head and neck cancers and anal cancers reported similar response rates [PR in 4.4% (2/45); SD in 33% (15/45)] (81). Notably, these cancers also have a significantly high rate of *TP53* mutation. Building on the preclinical and phase 1 results, prexasertib has now entered a phase II trial for SCLC patients with relapsed disease (NCT02735980). This trial is ongoing and will assess single agent activity in 116 patients with platinum

sensitive or refractory SCLC. Additional CHK1 inhibitors in clinical investigation include GDC-0575 (Genentech; NCT01564251) and SRA737 (Sierra Oncology; NCT02797964 and NCT02797997). Studies in triple-negative breast cancers and head and neck cancers (in which *TP53* mutations often occur) have demonstrated that CHK1 inhibitors can augment the effects of DNA-damaging treatments, findings that have led to trials investigating combinations with CHK1 inhibitors with platinum chemotherapy or PARP inhibitors (NCT02797977; NCT03057145).

Targeting *WEE1* in SCLC

WEE1 tyrosine kinase is a crucial component of the G2-M cell cycle checkpoint that halts the entry of the cell into mitosis upon DNA damage. Upon successful completion of the G2/M phase, the cell's entry into mitosis is governed by the phosphorylation status of CDK1, also known as CDC2, and its association with cyclin B. *WEE1* acts as a negative regulator of entry into mitosis at the G2-M transition by preventing the entry of cyclin B complexed with CDK1 activated in the cytoplasm (82-84).

Analysis of *WEE1* mRNA expression levels in 68 SCLC and 26 normal lung tissue samples showed that compared with normal lung tissue samples, SCLC samples had >4-fold higher *WEE1* expression ($P < 0.0001$) (17). Furthermore, profiling of 63 SCLC and 114 NSCLC cell lines showed significantly higher *WEE1* protein expression in SCLC cell line samples [$P < 0.0001$; fold change (FC) = 1.66] (17). The study further showed that a significant subset (~70%) of SCLC cell lines were sensitive to the *WEE1* inhibitor, AZD1775 (AstraZeneca). Using high-throughput proteomic profiling, we found that SCLC models with primary and acquired resistance to AZD1775 expressed high levels of receptor tyrosine kinase, AXL and phosphorylated S6 and resistance could be overcome with *WEE1*/AXL or *WEE1*/mTOR inhibitor combinations in cell lines and mouse models (17). This study further demonstrates that AXL-mediated activation of CHK1 could lead to redundancy and consequent resistance to *WEE1* inhibition in SCLC (17).

Currently, AZD1775 is the only *WEE1* inhibitor in clinical development (85-87). Early data from the trial showed that two of four patients with SCLC had a response to single-agent AZD1775 (88), although the final study results have not yet been published. Single agent (NCT02482311; NCT02593019) and combination studies with platinum chemotherapy (NCT02937818) and PARP

inhibitors (NCT02511795) are being investigated for SCLC and other cancer types. These include a study specifically targeting SCLC with *MYC* amplifications (NCT02688907).

Targeting *Aurora kinase (AURK)* in SCLC

The AURKs belong to a family of highly conserved serine/threonine kinases that are important for faithful transition through mitosis. The function and expression of Aurora A is regulated by the cell cycle and the protein controls important mitotic events including centrosome maturation, chromosome alignment, chromosome segregation, and cytokinesis (89). Indeed, genetic inhibition of Aurora A (*AURKA*) has been shown to inhibit the proliferation of SCLC cells (90). A small molecule Aurora B (*AURKB*) inhibitor, barasertib (AZD1152) has also been shown to inhibit the growth of SCLC *in vitro* and *in vivo* (91). Recent reports have shown activity of *AURK A* targeting in SCLC with high *MYC* and *NEUROD1* expression (92). Alisertib (MLN8237; Takeda) is a selective inhibitor of *AURKA*, with an IC₅₀ of 1.2 nM against *AURKA* and 396.5 nM against *AURKB* (93). Multiple studies have demonstrated the preclinical activity of alisertib in a wide range of human tumor models including lung, prostate, ovarian, and lymphoma cells (94-96). A multicenter phase I/II study of alisertib in patients with various solid tumors (including SCLC), defined the recommended phase II dose and schedule and established the dose-limiting toxicities (97,98). The phase II expansion cohort of recurrent SCLC patients showed 21% objective response rate (ORR) with single agent alisertib treatment. In a subsequent randomized phase II study, alisertib (or placebo) was then combined with paclitaxel as second-line therapy for SCLC patients. In that study, a significant improvement in PFS was observed (101 *vs.* 66 days, $P = 0.038$) with alisertib in the overall population. A trend toward improvement in ORR and OS was also observed, although this was not statistically significant. An exploratory analysis was then performed to determine if there was enhanced activity in patients whose cancer expressed cMYC protein. In 46 evaluable patients, those with cMYC positive tumors (defined as those with IHC intensity of 1-3+) who received the alisertib/paclitaxel combination had significantly improved PFS as compared to those with cMYC negative tumors (IHC intensity of 0). Specifically, the hazard ratio (HR) comparing alisertib/paclitaxel *vs.* paclitaxel alone in cMYC positive was 0.29 (0.12-0.72) *vs.* a HR in the cMyc-negative population

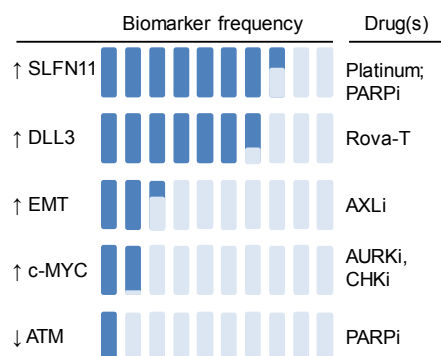


Figure 2 Landscape of predictive biomarkers in SCLC. Recent preclinical studies identified predictive biomarkers of response to DDR-targeted therapies in SCLC. SLFN11 predicts sensitivity to PARP inhibitors and chemotherapy; AXL and/or EMT predict resistance to PARP and WEE1 targeting; high ATM expression predicts resistance to PARP and ATR-targeted therapies; high MYC expression and amplification predicts sensitivity to AURK and CHK1 inhibitors; high expression of the inhibitory Notch ligand Delta-like protein 3 (DLL3) predicts response to Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate. Approximate proportions for each biomarker are estimated based on a combination of reported patient tumor and/or patient-derived xenograft expression and (where available) response rates in specific biomarker-selected populations [DLL3 (PMID: 27932068), MYC (PMID: 28490518), SLFN11/ATM/EMT (PMID: 28212573)]. For example, while >50% DLL3 expression was noted in over two-thirds of evaluated patient samples, an objective response was observed in only 18% of evaluable patients, suggesting proportion of SCLC sensitive to DLL3 is less than that which expresses high DLL3 (PMID: 27932068). SCLC, small cell lung cancer; SLFN11, schlafen 11; PARP, poly-ADP-ribose polymerase; DDR, DNA damage response; EMT, epithelial-mesenchymal transition; ATM, ataxia telangiectasia-mutated; ATR, ataxia telangiectasia and Rad3-related protein; AURK, Aurora kinase; CHK1, checkpoint kinase 1; PDX, patient-derived xenograft.

of 11.8 (range, 1.52–91.2) (binary $P=0.0006$). Given the relatively small number of patients and retrospective nature of the analysis, a prospective study is needed to further validate the predictive value of cMYC in the clinic (99,100). Together, these clinical data suggest AURKA inhibition is a promising treatment strategy in SCLC (99). Additional inhibitors of AURKA and AURKB are currently in clinical development including chiauranib (Chipscreen Biosciences), AK-01 (AurKa Pharma), TAS-119 (Taiho Oncology) and GSK1070916A

(GlaxoSmithKline) with several trials specifically focusing on SCLC (NCT03216343; NCT03092934).

The biomarker landscape in SCLC

Key to the success of any targeted agent is an appropriate patient selection strategy. By leveraging a large number of preclinical models, candidate biomarkers have recently been identified for several DDRi in SCLC. These include SLFN11 and epithelial-mesenchymal transition (EMT) as predictive markers of chemotherapy and PARP inhibitor sensitivity (49,64,67,68,101); MYC (and TTF1) as markers of CHK1 and AURK inhibitor sensitivity (18,92,102,103); and AXL/mTOR axis as the predictive marker of WEE1 inhibitor resistance (17). These findings suggest that biomarker profiling of patient samples could ultimately help guide treatment decisions in the clinic, moving us towards the goal of personalized SCLC treatment (Figure 2).

The rise of SLFN11

The Schlafen (SLFN) family (from the word schlafen, which in German means sleeping) includes several mouse and human genes (104–108). Multiple reports in the past year have highlighted the role of SLFN11 as a critical determinant of response to chemotherapy and PARP inhibitors in SCLC *in vitro* and *in vivo* models. Polley *et al.*, screened 63 human SCLC lines and three NSCLC lines for response to 103 FDA-approved oncology agents and 423 investigational agents (68). They found that high *SLFN11* expression was associated with sensitivity to the PARP-1 inhibitors ($R = -0.42$) (68). This finding was further substantiated in two subsequent papers. Lok *et al.*, demonstrated that SLFN11 expression correlates with response to multiple PARP inhibitors and loss of *SLFN11* conferred resistance to PARP inhibition in SCLC cell line and animal models (64). Independently, using a high-throughput, integrated proteomic, transcriptomic, and genomic analysis of SCLC PDXs and profiled cell lines, Allison Stewart *et al.*, demonstrated that high levels of SLFN11 (as well as high E-cadherin and low ATM) predicted sensitivity to PARP inhibition and chemotherapy (49). They further showed that SLFN11 expression was downregulated after treatment with cisplatin or PARP inhibitors, and knockdown of *SLFN11* reduced *in vitro* sensitivity and drug-induced DNA damage (49). SLFN11 expression is regulated by multiple mechanisms (105) including methylation, which is often regulated by the

catalytic subunit of the Polycomb repressive complex 2, enhancer of zeste homologue 2 (EZH2), discussed below.

EZH2 has been previously shown to be overexpressed in many tumors, including SCLC (14,109). Of its more important cellular functions, EZH2 plays a crucial role in methylation via chromatin modification and activation of DNA methyltransferases (110-112). Its other functions include suppression of apoptosis, increased cell proliferation and activation of ASCL1 expression (via suppression of TGF- β signaling) (113). Expression of the *EZH2* gene is under the direct control of the E2F family of transcription factors, including E2F1 (110-112). Interestingly, a study published in 2017 demonstrated that overexpression of EZH2 downregulates *SLFN11* via histone modification and methylation thereby making the SCLC resistant to chemotherapy (101). Furthermore, cisplatin and EZH2 inhibitor combination was successful in overcoming drug resistance in both chemo-sensitive and resistant SCLC models. Furthermore, EZH2 inhibition was found to prevent *SLFN11* loss and subsequent resistance to chemotherapy and PARP targeting (101), suggesting an intriguing possibility of combining EZH2 inhibitors with standard chemotherapy in the clinic for SCLC patients.

Role of MYC in DDR inhibitor response

After *TP53* and *RB1*, *MYC* family amplifications are the next most common form of genetic alterations. Amplifications and/or overexpression of *MYC* family genes (*MYC*, *MYCL* and *MYCN*) are mutually exclusive and are observed in about 20% of tumors and cell lines (27,28,114-117). *MYC* amplification is a known driver of genomic instability and replication stress. In SCLC, higher *MYC* expression in the mouse lung promotes an aggressive phenotype, highly metastatic tumors and rapid development of chemotherapy resistance similar to human SCLC (92,118). However, inhibitors of *MYC* have yet to be translated into clinically viable therapies.

Studies from our group and others have demonstrated the potential of targeting *MYC* indirectly as a synthetic lethal strategy in SCLC. A recent study from our group demonstrated that higher expression of and/or *MYC* amplification predicted sensitivity to *CHK1* targeting in SCLC models (18). We further showed that knockdown of *MYC* made SCLC cells more resistant to *CHK1* inhibitors, indicating the functional role of *MYC* in DDR inhibitor response (18). Using model-based clustering, Cardnell *et al.*, found two major proteomic subtypes of SCLC characterized

by either high thyroid transcription factor-1 (TTF1)/low cMYC protein expression or high cMYC/low TTF1 (102). The study further demonstrated that protein levels of TTF1 and cMYC predict response to targeted therapies including AURK, BCL2, and HSP90 inhibitors (102). Combined genomic and chemical vulnerability analysis in a panel of 60 SCLC cell lines demonstrated that sensitivity to structurally diverse AURK inhibitors VX680, MLN8237, PHA680632, and ZM447439 was significantly enriched in *MYC* amplified cells (P=0.004 MLN8237; P=0.003 PHA680632; P=0.01 VX680; P=0.01 ZM447439) (103). In an elegant study using SCLC GEM models, Mollaoglu *et al.*, showed that SCLC with high *MYC* expression is vulnerable to AURK inhibition and chemotherapy and the combination causes tumor suppression and significant survival benefit (92). Mollaoglu *et al.*, (92) and our data (18) demonstrate that *MYC* expression is observed in a larger cohort of SCLC patients than *MYC* amplification alone and some patients have high *MYC* expression even without amplification. The data thus suggests that *MYC* expression rather than *MYC* amplification alone may identify additional *MYC* “positive” patients and therefore may be a more sensitive biomarker to identify patients who may respond to certain DDR inhibitors. SCLC patients with *MYC* amplifications are being enrolled on an active clinical trial with the WEE1 inhibitor AZD1775 (NCT02688907). Trial designs such as this along with detailed mechanistic studies are warranted to establish the role of *MYC* in different SCLC subtypes.

Role of EMT and AXL in SCLC

EMT has emerged as a key regulator of metastasis, resistance to targeted therapy, chemotherapy, and radiation; as well as an emerging role in immune escape (119,120). Allison Stewart *et al.*, recently demonstrated that in SCLC preclinical models, resistance to PARP inhibitors is frequently associated with EMT and low expression of *CDH1* (*E-cadherin*). High levels of *SLFN11* and E-cadherin and low ATM were significantly associated with *in vitro* sensitivity to cisplatin and topoisomerase1/2 inhibitors in SCLC. Interestingly, SCLC with loss of E-cadherin and high EMT signature scores displayed alterations in expression of the miR200 family and key neuroendocrine and SCLC genes (e.g., *NEUROD1*, *ASCL1*, *ALDH1A1*, *MYCL*) (49). In a 76-gene EMT signature in NSCLC, the mesenchymal phenotype was strongly correlated with the expression of the receptor tyrosine kinase, AXL (121).

AXL, activated by a complex interaction between its ligand growth arrest-specific protein 6 (Gas6) and phosphatidylserine, regulates various vital cellular processes, including proliferation, survival, motility, and immunologic response (122-124). AXL has been suggested to promote both intrinsic and acquired resistance to chemotherapeutic, immunotherapeutic and molecularly targeted agents in both solid and hematologic malignancies, including acute myeloid leukemia, NSCLC, gastric and colorectal ADC, and breast and prostate cancers (121,125,126). Thus, several AXL inhibitors are currently in preclinical and clinical development, but thus far, AXL's role in SCLC had not been well studied. A recent study from our group demonstrated that higher basal expression of AXL is associated with primary resistance to WEE1 targeting (17). Moreover, AXL expression is upregulated upon acquired resistance to WEE1 inhibitor, AZD1775 and co-inhibition of AXL overcomes resistance in SCLC models (17). Further exploration is needed to determine if AXL contributes functionally to additional DDR inhibitor resistance as it is frequently overexpressed in tumors that have undergone EMT.

Future perspective—DDR-immune system crosstalk and its therapeutic implications in SCLC

Earlier studies showed that defects in the DDR network and function could lead to changes in the immune microenvironment of tumors (127). Therapeutically, IL-2 and intravesicular Bacillus Calmette-Guerin (BCG) have long track-records of being used to activate the host immune system for multiple cancer types including melanoma, renal cell carcinoma and bladder cancer (128-130). In the past several years, many cancer immunotherapy regimens have been developed that use therapeutic antibodies against inhibitory signaling molecules on tumor and immune cells (131,132). Common targets of these monoclonal antibodies include the immune checkpoints PD-1, PD-L1, and cytotoxic T-lymphocyte associated protein 4 (CTLA4) (132-137). Since the first FDA approval of ipilimumab (anti-CTLA4) for melanoma, several such ICB agents have been approved for patients with advanced cancers, including NSCLC (138-140).

Though very promising, ICBs vary in their activity across different cancer types and hence there is an active effort to develop therapeutic strategies including novel drug combinations that may enhance the anti-tumor efficacy of ICBs in cancers with relatively low response rates to

immunotherapy. Several studies have now reported high mutational burden as a potential predictor of effective immunotherapy response (141) however, despite having one of the highest mutational burdens, SCLCs (26), the clinical efficacy of checkpoint inhibitors in this disease seems to be far less than as expected. Antibodies targeting PD-1 or PD-L1 are being actively evaluated in clinical trials for SCLC alone and in combination with other therapeutic agents or radiation (142). Recently, nivolumab with or without ipilimumab was added to the National Comprehensive Cancer Network guidelines as a treatment option for relapsed SCLC (11). However, only a minority of SCLC patients respond to anti-PD-1 monotherapy (~10%) or the anti-PD1/anti-CTLA4 combination (~23%) (10,143). Notably, SCLC has relatively immunosuppressed phenotypes with low levels of infiltrating T cells and reduced antigen presentation (144-146). Furthermore, PD-L1 levels appear to be lower in SCLC than in NSCLC (147), which is paradoxical given the high overall mutation burden in SCLC (25) and might at least partially explain the poor outcomes from anti-PD-1/PD-L1 trials (*Table 2*).

In other cancer types, defects in the DDR pathway have been associated with enhanced response to ICB (153,154). For example, somatic alterations in DDR genes such as POLE, POLD1 and MSH2 have been linked with durable benefit to ICB response in patients, suggesting that defects in DDR network and changes in the genomic landscape (such as higher mutation burden and increased levels of predicted neo-antigens) may contribute to enhanced ICB response (141). Recently, our group and others found that co-targeting DDR proteins such as PARP and CHK1 can increase expression of PD-L1 and anti-tumor immune response of anti-PD-L1 in SCLC and breast cancer models (155,156). These findings suggest that DDR targeting through pharmacologic inhibition could potentially enhance the anti-tumor immune response to ICB in SCLC and other malignancies (157-159).

Conclusions

Proteomic, genomic and transcriptomic profiling have helped identify novel, promising DDR targets for SCLC like PARP, CHK1, WEE1 and ATR. Drugs targeting these proteins are currently in various phases of clinical development. Identification of predictive biomarkers for DDR-targeted therapies and identifying optimal combination regimens to overcome adaptive resistance to DDR-targeted therapies are areas of active investigation in SCLC. The importance of liquid biopsies and blood-based

Table 2 Reported immunotherapy trials in SCLC

Reference	Trial details
Antonia SJ <i>et al.</i> (CheckMate 032) (10)	<p>Phase 1/2: LS or ES-SCLC patients with progression after prior platinum therapy</p> <p>Patients received nivolumab (nivo) 3 mg/kg q2w (every 2 weeks) until PD or toxicity or nivo/ipilimumab (ipi) (1+1 mg/kg, or 1+3 mg/kg, or 3+1 mg/kg) q3w (every 3 weeks) ×4 cycles, followed by nivo 3 mg/kg q2w until PD or toxicity</p> <p>ORR was 10% (10/98) in nivo alone, 33% (1/3) for nivo 1 + ipi 1, 23% (14/61) for nivo 1 + ipi 3 and 19% (10/54) for nivo 3 + ipi 1 (21% or 25/118 total for nivo + ipi)</p> <p>PD-L1 expression $\geq 1\%$ in 17% and $\geq 5\%$ in 5% of patients analyzed using 28-8 (Dako) antibody</p> <p>Responses were irrespective of PD-L1 expression</p>
Antonia SJ <i>et al.</i> (CheckMate 032 exploratory analysis) (148)	<p>Evaluable patients from CheckMate 032 were divided into low/medium/high TMB (<143, 143–247, and >247 missense mutations, respectively)</p> <p>Breakdown into three tertiles compared favorably to Carbone DP <i>et al.</i> (149) CheckMate 026 data for NSCLC</p> <p>Improved ORR, 1-year PFS and 1-year OS for both nivo alone and nivo/ipi with high TMB</p> <p>Low/medium/high ORRs: 4.8%, 6.8%, 21.3% (nivo alone) and 22.2%, 16.0%, 46.2% (nivo + ipi); 1-year PFS: not-calculable, 3.1%, 21.2% (nivo alone) and 6.2%, 8%, 30% (nivo + ipi); 1-year OS: 22.1%, 26%, 35.2% (nivo alone) and 23.4%, 19.6%, 62.4% (nivo + ipi)</p>
Ott PA <i>et al.</i> (KEYNOTE-028) (150)	<p>Pre-treated ES-SCLC with PD-L1 expression $\geq 1\%$ were treated with pembrolizumab (pembro) 10 mg/kg q2w for 24 months or until PD or toxicity</p> <p>Forty-six of 145 (31.7%) evaluated patients had PD-L1 expression $\geq 1\%$ using 22C3 (Merck) antibody</p> <p>Twenty-four patients total treated, 1 CR, 7 PRs (ORR of 33% or 8/24)</p>
Gadgeel SM <i>et al.</i> (151)	<p>Phase II study of pembro 200 mg flat-dose q3w for maximum of 2 years following 4–6 cycles of platinum/etoposide with PR or SD (pembro maintenance)</p> <p>PD-L1 staining only positive in 1/35 patients; maintenance pembro failed to improve PFS compared to historical controls</p>
Reck M <i>et al.</i> (152)	<p>Phase II study featuring 1:1:1 randomization between carbo/taxol + placebo + ipi (concurrent) or + placebo followed by + ipi (phased); no improvement in PFS or OS</p>

PD, progressive disease; ORR, objective response rate; TMB, tumor mutational burden; PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; SCLC, small cell lung cancer; LS-SCLC, limited stage SCLC; ES-SCLC, extensive stage SCLC; PD-L1, programmed death-ligand 1; NSCLC, non-small cell lung cancer; CR, complete response.

biomarker identification strategies are additional areas of interest in this disease. Immuno-oncology and the use of ICB in SCLC treatment is an area of intensive ongoing investigation. Optimizing strategies to activate the immune system would be one of most interesting future areas of

research in this disease. The results from the ongoing DDR-targeted clinical trials would indicate whether we can finally expand and improve the therapeutic options for this recalcitrant disease for which the treatment options have remained stagnant for over three decades.

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Footnote

Conflicts of Interest: LA Byers has served as an advisory board member and/or consultant to AstraZeneca, Medivation, and AbbVie.

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