European Journal of Cancer 119 (2019) 132–150

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Review

Challenging the recalcitrant disease—developing molecularly driven treatments for small cell lung cancer

Daisy W.S. Mak [a], Su Li [a], Anna Minchom [b,*]

[a] *Lung Unit, Royal Marsden Hospital, Downs Road, Sutton, Surrey, SM2 5PT, UK*
[b] *Drug Development Unit, Royal Marsden/Institute of Cancer Research, Downs Road, Sutton, Surrey, SM2 5PT, UK*

Received 20 February 2019; received in revised form 11 April 2019; accepted 26 April 2019
Available online 21 August 2019

KEYWORDS
Small cell lung cancer;
Molecular analysis;
Cell signalling;
Immunotherapy;
DNA repair;
Tumour suppressor gene;
Cancer epigenetic

Abstract Small cell lung cancer (SCLC) has been described as a 'recalcitrant' disease characterised by poor survival and with little progress made in developing novel treatments in the last decades. However, recent drug developments have opened some potential therapeutic avenues. In this review, the genomic landscape of SCLC is explored, in particular the Notch pathway and attempts to target the key node DLL3. The likely primary importance of MYC to SCLC subtype transformation and recent attempts to drug MYC are discussed. Bcl-2 is a druggable protein, highly expressed in SCLC, and relevant Bcl-2 targeting drugs are reviewed. None of these drug targets are, however, as advanced their development as the field of immunotherapy for SCLC. The key developments in single agent PD-L1 and PD-1 inhibitors and in combination with chemotherapy have led to the only recent licencing approvals for SCLC in recent years and will likely pave the way for future rational drug combinations. Drug development in SCLC poses its own challenges with rapid clinical deterioration often precluding trial entry. Effective drug development in a biomarker-driven approach depends on early patient screening and use of circulating biomarkers. Given recent developments, we may hope to be at the start of an era of greater progress in the treatment of SCLC.

* *Corresponding author*: Drug Development Unit, Royal Marsden Hospital/ Institute of Cancer Research, Downs Road, Sutton, Surrey, SM2 5PT, United Kingdom.
E-mail address: Anna.Minchom@icr.ac.uk (A. Minchom).

https://doi.org/10.1016/j.ejca.2019.04.037
0959-8049/

1. Small cell lung cancer and standard-of-care treatment

Over the last two decades, developments in the treatment of lung cancer have resulted in decreasing incidence and mortality rates in a number of countries [1]. However, improvement in thoracic surgery, identification of genomic and molecular changes, and advancements in biomarker-driven therapies benefit mainly patients with non-small cell cancer (NSCLC). Small cell lung cancer (SCLC) represents 15% of all new lung cancer cases [2]. Europe has one of the highest age-standardised rates (ASRs) of lung cancer in the world (ASR 3.54–4.25 per 100,000 depending on region and around 470,000 new cases diagnosed in one year) [3]. In the United States, the age-adjusted incidence rate of SCLC was 5.89 per 100,000 in 2018, translating to around 35,000 patients with newly diagnosed SCLC in last year [2]. Mirroring the trend of tobacco cessation, on average, there is a decrease of incidence rate of 3.1% per year from 1988 to 2015 [2]. The prognosis of SCLC, however, remains grave with 5-year survival of less than 10% in some European countries [4–6].

Patients presenting with limited stage disease (T1-2 N0 as per TNM staging) are best managed by surgery followed by adjuvant chemotherapy; this constitutes less than 5 percent of patients [7]. The standard of care for limited stage (inoperable) SCLC is combination of chemoradiation, whereas that for extensive stage SCLC (ES-SCLC) is platinum-based chemotherapy followed by thoracic and cranial irradiation in sensitive disease [8]. Known as the 'recalcitrant tumour', although it is sensitive to platinum-based chemotherapy and radiotherapy, the disease follows a relapse-refractory course [9]. The response rates (RRs) to the standard first-line etoposide and cisplatin or carboplatin is close to 70% [10]. However, outcomes remain poor with progression-free survival (PFS) of only 5.5 months [10]. Topotecan is approved by the US Food and Drug Administration (FDA) in the second-line setting based on a phase III study showing superior overall survival (OS) compared with best supportive care [11].

In the past decades, the landscape of the management of advanced SCLC has changed little. In recent few years, however, in line with expanding molecular and genomic techniques and the rapidly evolving field of drug discovery, there has been a resurgence of interest in SCLC. This review focuses on the molecular features of this disease and potential therapeutic applications (see Fig. 1).

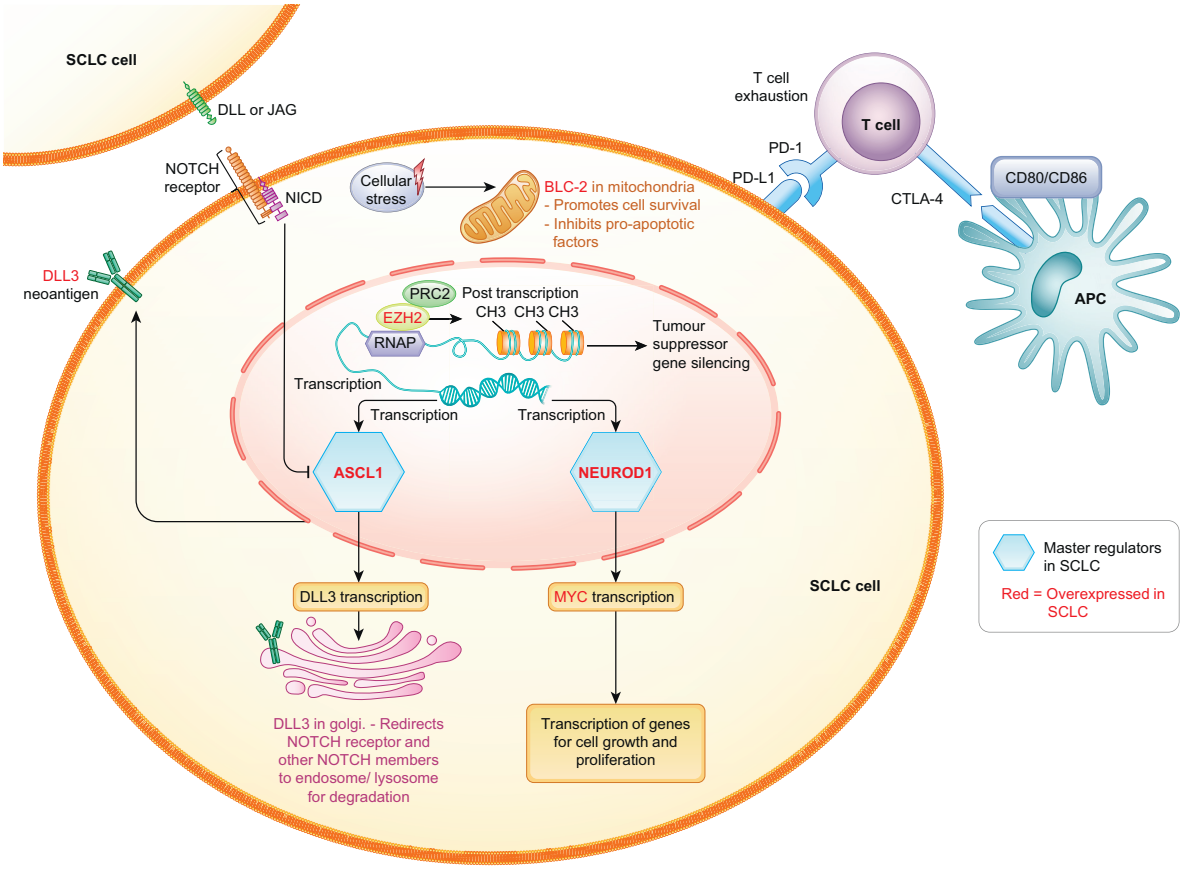

Fig. 1. Targets in small cell lung cancer: Notch pathway, MYC, BCL-2, epigenome and immune microenvironment. APC, antigen presenting cell; ASCL1, achaete-scute homologue 1; BCL-2, B-cell lymphoma 2; DLL, delta-like ligand; JAG, jagged ligand; EZH2, enhancer of zeste homologue 2; NICD, Notch intracellular domain; NEUROD1, neurogenic differentiation 1 (NeuroD1); PRC2, polycomb repressive complex 2: RNAP, RNA polymerase.

2. ASCL1 and the Notch pathway

SCLC, together with large cell neuroendocrine carcinoma (LCNEC), is classified as high-grade neuroendocrine (NE) tumour of lung under the 2015 World Health Organisation classification system [12]. Pulmonary neuroendocrine cells (PNECs) are precursors of SCLC. In mouse models, by replicating oncogenic mutations and losses of tumour suppressor genes, a subset of these cells are transformed into SCLC [13,14]. Critical to the development of SCLC is the transcription factor achaete-scute homologue-1 (ASCL1). ASCL1 commits epithelial cells into neuroendocrine fate and is highly expressed in classic SCLC and LCNEC [15]. ASCL1 is expressed in 77% of SCLC [16]. ASCL1 has been shown to be important to maintain stemness, cell cycle progression and mitosis via specific pathways such as MAPK, tubulin binding, unfolded protein response and stem cell differentiation [17]. ASCL1 binds near *MYCL1*, nuclear factor I B *(NFIB)* and SRY-box 2 *(SOX2)*, suggesting direct regulation of these oncogenes [18–20].

One of the important pathways to regulate ASCL1 is the Notch signalling pathway. The Notch receptor has four isoforms: Notch1, 2, 3 and 4 [21], which serve as a membrane-tethered transcription factor. There are five Notch ligands: delta-like ligand (DLL)1, DLL3, DLL4, jagged ligand (JAG)1 and JAG2 [22]. While the Notch signalling pathway is oncogenic in a number of tumour types such as T-cell acute lymphoblastic leukaemia and NSCLC [23], its role in neuroendocrine tumour is suppressive [24]. In SCLC, there are inactivating mutations in the *Notch* family genes in 25% of patients [16]. By reactivating Notch signalling pathways, the neuroendocrine markers and metastatic capability of SCLC is reduced with the number of tumours and survival of transgenic mouse models prolonged [25].

2.1. DLL3 and rovalpituzumab tesirine

The Notch ligands DLL1, DLL4, JAG1 and JAG2 activate the Notch receptors [26]. DLL3 is downstream of ASCL1 differentiation pathway and mainly situated in the Golgi apparatus and thus is unable to activate Notch signalling [27]. Structurally, it has reduced the number of epidermal growth factor repeats and spacing of cysteine residues within the delta/serrate/LAG-2 (DSL) domain, which are important for Notch binding, compared with DLL1 and 4 [28]. In addition, it inhibits Notch pathway by redirecting or retaining Notch receptor and DLL1 to late endosome/lysosomal compartments or the Golgi apparatus, respectively, for destruction, and thus preventing them from reaching cell surface [29]. Thus, by inhibiting Notch signalling pathway, which negatively affects ASCL1, it is postulated that DLL3 may be associated with neuroendocrine phenotype and plays a functional role in carcinogenesis in neuroendocrine tumours. Some DLL3 is located on the cell surface where it acts as a 'Trojan horse' tumour-specific antigen [30]. Whole-transcriptome sequencing has shown that compared with normal tissue, DLL3 is significantly overexpressed in SCLC patient-derived xenografts [31]. About 70% of SCLC patients have DLL3 detected on the cell surface [32]. The antibody–drug conjugate (ADC) rovalpituzumab tesirine (Rova-T^{TM}) is a DLL3 IgG1 monoclonal antibody conjugated to cell-cycle independent pyrrolobenzodiazepine (PBD) dimer toxin: a DNA toxic cross-linking agent [33]. A promising phase I study demonstrated a 38% objective RR in the 26 patients who had high DLL3 expression as defined by expression in 50% of more of tumour cells [34]. Grade 3/4 adverse events include thrombocytopaenia, serosal effusions and increased lipases [34]. In the phase 2 TRINITY study, in which 67% of 199 patients received the drug in a third-line setting, the overall response rate (ORR) was 16%, whereas the median overall survival (OS) was 5.6 months. In patients with high DLL3 expression (DLL3 expressed in $\geq$75% tumour cells by immunohistochemistry), the PFS was 4.1 months, whereas the median OS was 6.7 months [35]. In the phase III MERU study, Rova-T was given as maintenance therapy in patients with ES-SCLC who had derived benefit from first-line platinum-containing regimen in maintenance setting [36], whereas in the phase III TAHOE study, Rova-T was compared with the current standard of care (topotecan) in the first relapse setting, in patients with advanced or metastatic SCLC having high DLL3 expression [37]. Unfortunately, recruitment to TAHOE has been halted in December 2018 as preliminary data show shorter OS in the Rova-T arm than in the topotecan arm [38]. It is unclear what to attribute this disappointing outcome to. The drug has a unique toxicity profile which is probably more related to PBD rather than DLL3 inhibition, as the ligand is not expressed in serosal surface, vasculature, platelet or megakaryocytes [34], so it maybe that the DLL3 target is worthy of further investigation with a less toxic ADC payload. There are currently trials of combination with platinum/etoposide and ipilimumab/nivolumab recruiting [39,40].

3. NEUROD-1 and MYC

Another key player in the oncogenesis of SCLC is the transcription factor neurogenic differentiation 1 (NeuroD1), a member of the basic helix–loop–helix transcription factors. It is important in promoting neuronal differentiation [41,42]. NeuroD1 is expressed in 15% of SCLC [41]. Targets include the oncogene *MYC*, *TrkB* (tyrosine kinase tropomyosinrelated kinase B) and neural cell adhesion molecule (*NCAM*) [42].

NeuroD1 does not appear to be essential in the development of SCLC in mouse models, unlike ASCL1 [43]. In contrast to ASCL1, in mice models with knock-out of *NEUROD1*, PNECs are still present [44]. Stratification of

SCLC by gene expression patterns found three subtypes: ASCL^{High}(70%), NEUROD1^{High}(15%) and ASCL^{Low}/NEUROD1^{Low}(15%) [43]. ASCL^{High} and NEUROD1^{High} subtypes show clear heterogeneity including distinct genetic alterations and epigenetic profiles, morphologies and different expression of NE markers [45,46]. The more commonly occurring SCLC developed from *Rb/p53* mutant mouse model resembles the ASCL1^{High} subtype [43] and is termed the 'classic' SCLC, while the 'variant' SCLC exhibits faster doubling time, frequent *MYC* amplification, reduced NE marker and loose aggregated morphology [45,47,48] and was found to correlate with NeuroD1 expression [49]. It is postulated that early tumour cells initially exhibit 'classic' SCLC phenotype; with time, it acquires the NEUROD1^{High} state coinciding with the variant morphology and NE-low phenotype, and this process is fuelled by MYC activation [50].

Given the key role, the transcription factor MYC appears to play in SCLC subtype transformation, and in its relation to NeuroD1 efforts have been made to determine the prognostic implications of MYC and the therapeutic potential of drugging MYC. *MYC* family members (*MYC, MYCL* and *MYCN*) are amplified in approximately 20% of SCLCs and are associated with a worse prognosis [51]. A retrospective study found that SCLC harbouring *MYC* amplification had a significantly shorter OS (4.7 weeks) *versus* patients without *MYC* amplification (26.2 weeks) (p = 0.02, 95% confidence interval [CI] 1.36–10.26 weeks) [52]. Deletion of *MYC* in SCLC models result in suppression of tumour growth [53]. There are inherent difficulties in targeting MYC as MYC lacks a specific active site and is located in the nucleus [54]. Various strategies have been used to target MYC indirectly (see Fig. 2).

3.1. MYC and Aurora kinase inhibitors

Screening of genetic libraries to identify oncogene dependencies has found that *MYC*-amplified tumours demonstrated dependency on Aurora B kinase activity [55]. Aurora kinases are key regulators in mitosis [56]. Aurora-A stabilises MYCN through direct physical contact and sequesters it from ubiquitin-mediated degradation [57]. Aurora kinase inhibitors inhibit growth in *MYC*-amplified cell lines [58]. Trial of Aurora kinase inhibitors in the non-*MYC*–selected SCLC has reported. Alisertib, an Aurora A kinase inhibitor, has been trialled in phase II study in combination with paclitaxel against paclitaxel alone in relapsed SCLC and showed a non-significant trend towards improved OS against paclitaxel alone (OS alisertib with paclitaxel 186 days *versus* paclitaxel with placebo 165 days; hazard ratio [HR] 0.79; p = 0.209). Grade 3 toxicity was higher with the combination (drug-related grade 3 AE 67% *versus* 25%) [59]. A phase I study on AZD2811, an Aurora kinase B inhibitor, in patients with advanced solid tumours is recruiting (NCT02579226), and a phase II trial investigating single agent AZD2811 in relapsed SCLC has completed recruitment with results awaited (NCT03366675).

3.2. MYC and CDK inhibitors

Cyclin-dependent kinases (CDKs) are regulators of MYC function [60,61]. CDK2 engages in extensive cross-regulation with MYC [62]. CDK7 and CDK9 are required for MYC-dependent transcription and growth by phosphorylating a subunit of the carboxy-terminal domain of RNA polymerase II [63–65]. CDK inhibitors in clinical development thus far tend to be non-specific [66]. Clinical trials of CDK9 inhibitors, including flavopiridol and dinaciclib, reveal suboptimal response and severe adverse effects [66–70]. Seliciclib, another CDK inhibitor with CDK9 activity tested in phase I studies, also displayed unacceptable toxicities and unsatisfactory response [71,72]. With tools to enhance their selectivity, it may be anticipated that the treatment efficacy and profound adverse effects of CDK9 inhibitors could be improved.

3.3. MYC and BET inhibitors

Another strategy to target *MYC* transcription is inhibiting BRD4 (bromodomain-containing 4). BRD4 is a member of the bromodomain and extra terminal domain (BET) family [73]. BET proteins are important for recruitment of

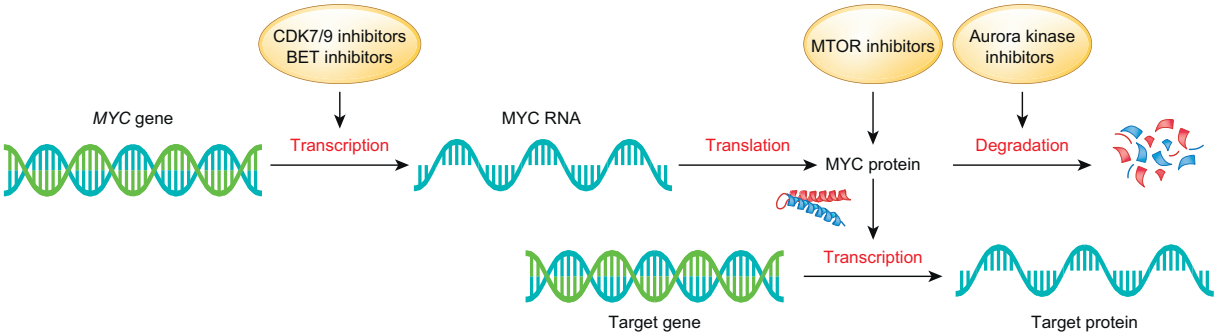

Fig. 2. MYC regulation and mechanisms of action of MYC-targeting drugs. BET, bromodomain and extraterminal motif; CDK, cyclin-dependent kinase; mTOR, mammalian target of rapamycin; RNA, ribonucleic acid.

P-TEFb to acetylated histones, with *MYC* transcription being heavily dependent on the process [74]. JQ1 is a potent BET4 inhibitor and an archetypal molecular of its class. It displaces BRD4 from the super-enhancers within the *MYC* oncogene [75] and induces differentiation, cycle arrest and apoptosis in *MYCN* amplified neuroblastoma models [76]. *In vitro* and *in vivo* antitumour effect is also observed in haematological malignancies and pancreatic cancer with *MYC* overexpression [77–79]. However, as the effects of single-agent BET inhibitors are limited, research on combination therapy with small-molecule inhibitors, immune checkpoint inhibitors and other epigenetic therapies is ongoing [80].

3.4. MYC and PI3K/AKT/MTOR pathway inhibitors

The PI3K/AKT/MTOR pathway is frequently upregulated in cancers [81]. Mammalian target of rapamycin (mTOR) is a serine/threonine-specific protein kinase that regulates phosphorylation of p70S6 kinase (p70) and 4EBP1: proteins responsible for translation and expression of D-type cyclins and MYC [82]. Targeting messenger RNA translation by inhibiting the PI3K/AKT/TOR pathway is, therefore, a means of blocking *MYC* gene products. Blocking this pathway decreased MYC level and was shown to have therapeutic efficacy in several tumour models [81,83–86]. Studies have indicated that PI3K/AKT/TOR pathway is widely expressed and activated in SCLC [87,88]. Sixty-two percent of SCLC demonstrates high levels of phosphorylated Akt [89], whereas whole exome sequencing and copy number analysis showed genetic alterations in near 40% in the PI3K/AKT/mTOR pathway [90]. Temsirolimus has been tested in maintenance setting. The median OS and 1-year PFS were 2.2 months (95% CI 1.8–2.9 months) and 4.7% (95% CI 0.2–9.2%), respectively [91]. Everolimus has been tested in the relapsed setting in 40 patients but yielded only at most partial response (PR) rate of 3% and stable disease (SD) rate of 23% at 6 weeks, with median OS at 6.7 months (95% CI 4.0–8.6 months) and time to progression at 1.3 months (95% CI 1.4–1.4 months) [92]. Although survival was not greatly impacted in these trials, the investigation of drugs targeting the PI3K pathway in the *MYC*-amplified SCLC setting would be interesting.

4. Targeting Rb

SCLC is deemed a highly mutated cancer through prolonged exposure to tobacco smoke and other carcinogens [93,94]. *p53* and *Rb1* are tumour suppressor genes that are almost universally inactivated in SCLC [95]. p53 is activated in response to cellular stress resulting in cell cycle arrest, senescence and apoptosis [95]. Rb1 arrests cell cycle in G1 to S phase via E2F inhibition [96]. Loss of function mutations in *p53* and *Rb1* are difficult to therapeutically target. Preclinical data utilising genetic and drug screening methods in SCLC cell lines with *Rb1* loss identified high dependency on proteins related to chromosome segregation in mitosis including Aurora A and B kinase, and cell lines were sensitive to Aurora kinase inhibition [97,98]. Aurora kinase inhibition has shown activity in *MYC*-amplified cell lines as discussed previously; these studies demonstrate evidence that the mechanism may be independent of *MYC* amplification. Instead, sensitivity to Aurora kinase inhibition may be due to the role Rb has in mitotic fidelity rendering cells with *Rb1* loss vulnerable to inhibition of factors such as Aurora kinase that are involved in faithful transition through mitosis.

5. Targeting BCL-2

BCL-2 is an anti-apoptotic protein found on the inner mitochondrial membrane [99]. BCL-2 is highly expressed in approximately 65% of SCLC [100,101]. Navitoclax (ABT-263) is a potent inhibitor of BCL-2, BCL-XL and BCL-w. In a phase II trial in recurrent SCLC, the observed RR was low (1/39 patients) and significant thrombocytopaenia occurred [102]. To mitigate this toxicity, navitoxlax was re-engineered and venetoclax (ABT-199) developed. Venetoclax specifically binds to BCL-2 but not to BCL-XL or BCL-w and induces apoptosis in BCL-2–dependent tumours without causing thrombocytopaenia [103]. Single-agent venetoclax is currently approved for use in patients with chronic lymphocytic leukaemia with 17p deletion. Using a high-throughput drug screen, venetoclax was tested across approximately 800 various solid tumour cancer cell lines and a large subset of SCLC was found to be hypersensitive to BCL-2 inhibition and response correlated to BCL-2 expression [104]. It is noted that the clinical data from navitolax has been disappointing, despite promising preclinical data [105,106]; however, the leading hypothesis to explain this failure is due to BCL-2 not being sufficiently inhibited because of dose-limiting thrombocytopaenia. This would suggest a rationale for biomarker-directed clinical trials of venetoclax in SCLC with high BCL-2 expression levels, and a phase I trial on venetoclax with a BET inhibitor ABBV-075 is currently recruiting (NCT02391480). A novel Bcl-2/Bcl-xL inhibitor APG-1252 has been tested in a phase I study including 6 relapse SCLC patients [107]. No haematological toxicity has been reported, and dose escalation is ongoing.

6. Targeting the DNA damage response

DNA damage response (DDR) pathways are highly complex systems that repair DNA damage within cells. Small cell lung cancer often exhibits high gene expression of DDR pathway nodes such as checkpoint kinase 1 (CHK1), ataxia-telangiectasia mutated protein kinase

(ATM), ataxia telangiectasia and Rad3 related protein (ATR) and poly (ADP-ribose) polymerase (PARP) [108–113] (see Fig. 3). It could be postulated that DDR drugs may have single-agent activity in small cell lung cancer and potential synergistic activity with DNA damaging chemotherapy such as platinum agents (which form the backbone of standard SCLC treatment).

6.1. Lurbinectedin

Lurbinectedin is a novel anticancer drug that triggers the degradation of RNA polymerase II. There follows stalling of the replication fork and accumulation of DNA breaks [114]. There is also evidence of modulation of the tumour microenvironment [115]. Lurbinectedin was tested in phase II as a single agent in relapsed SCLC [116]. In the 61 patients, ORR was 39.3% (95% CI 27.1–52.7%), median PFS was 4.1 months (95% CI 2.6–5.7 months) and OS was 11.8 months (95% CI 9.6–15.9 months). Myelosuppression was common, with 44% of patients experiencing grade 3/4 neutropenia [117]. A phase Ib trial of lurbinectedin in relapsed SCLC in combination with doxorubicin and in combination with paclitaxel has been presented [118]. The doxorubicin dose of 50 mg/m^2 was subsequently expanded at a 20% dose reduction because of myelosuppression. ORR was 67% (14 patients) in the doxorubicin 50 mg/m^2 combination arm, 37% (10 patients) in the doxorubicin 50 mg/m^2 combination arm, 71% (5 patients) in the paclitaxel combination arm. The authors report PFS of 3.2–5.8 months in those with platinum-sensitive disease; the rate in non-platinum sensitive disease was not reported. Interestingly, the ORR was only 25% in the lurbinectedin arm, supporting ongoing development of combinations with chemotherapy, although this is challenging with the high rates of myelosuppression seen. The authors report that dose reductions and granulocyte-colony stimulating factor (G-CSF) made the myelosuppression manageable.

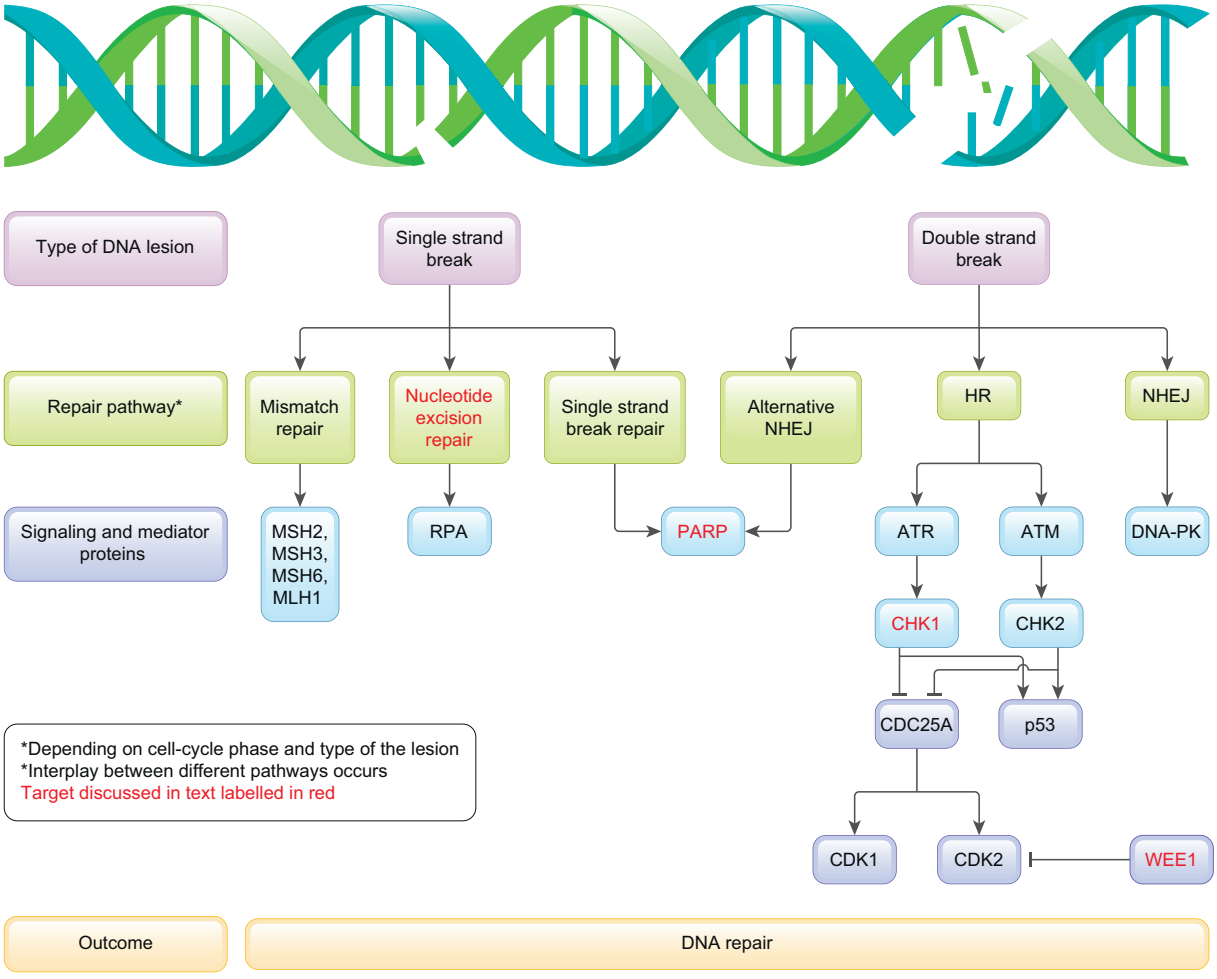

Fig. 3. Selected DNA damage response pathways demonstrating signalling proteins and potential drug targets in SCLC. ATM, ataxia-telangiectasia mutated protein kinase; ATR, ataxia telangiectasia and Rad3-related protein; CDC25A, cell division cycle 25 homologue A; CDK, cyclin-dependent kinase; CHK, checkpoint kinase; DNA-PK, DNA-dependent protein kinase; HR, homologous recombination; MLH1, MutL homologue 1; MSH, MutS protein homologue; NHEJ, non-homologous end joining; PARP, poly ADP ribose polymerase; RPA, replication repair protein A; WEE1, Wee-like protein kinase 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Lurbinectedin has received orphan drug designation from the European Medicines Agency (EMA) [119] and FDA [120], and a phase III trial of lurbinectedin in combination with doxorubicin against physicians' choice in relapsed SCLC is underway [121].

6.2. PARP inhibition

PARP is a family of nuclear protein enzymes which play an important role in single-strand DNA break repair [122,123]. PARP is highly expressed in SCLC. In a study on SCLC cell lines, PARP protein expression was scored based on a composite score of percentage of cells staining positive and staining intensity. The mean score was 262 for SCLC (n = 12), compared with 237 for LCNEC (n = 20), 230 for atypical carcinoid (n = 9), 197 for typical carcinoid (n = 55), 120 for squamous NSCLC (n = 24) and 104 for adenocarcinoma NSCLC (n = 24) [110]. At the mRNA level, PARP1 had the greatest differential level between SCLC and NSCLC ($p < 0.0001$) [110]. As a single agent in maintenance setting in a randomised, placebo-controlled phase II study, the PARP inhibitor olaparib failed to prolong PFS and OS when compared with the placebo, with a high level of drug discontinuation in the treatment arm at 35% [124]. Talazoparib, a highly potent PARP inhibitor, was tested in a first-in-man phase I trial. In an expanded cohort including 23 SCLC who had platinum-sensitive relapse, talazoparib was able to induce PR and SD in 27% of patients, with PFS reported to be 11.1 weeks [125]. Velaparib has been tested in combination with cisplatin and etoposide against placebo in phase II which showed a modest signal of efficacy (PFS 6.1 *versus* 5.5 months; p = 0.01; OS 10.3 *versus* 8.9 months; p = 0.17) [126]. Velaparib has been shown to be active in combination with temozolomide in preclinical study [127]. This observation was put forward to a phase II study in a relapse-refractory population [128]. Despite temozolomide/velaparib combination demonstrating an improved RR (39 *versus* 19%, p = 0.016), it did not translate into better PFS and OS, and severe haematological toxicities were more frequent in the combination arm. Despite this disappointing result, the group with detectable SCFN11 (a putative DNA/RNA helicase) by immunohistochemistry at diagnosis demonstrated a trend towards better OS in the temozolomide/velaparib group [128]. A phase I/II study of olaparib and temozolomide is ongoing in relapse/refractory setting [129]. Combining PARP inhibitor with other molecularly targeted therapy is also mechanistically feasible; a study has demonstrated proteins most elevated after PARP inhibition *in vitro* belong to the PI3K/mTOR pathway [130].

6.3. CHK inhibition

CHK1 forms a complex signalling network with ATR, and this CHK1-ATR axis facilitates S and G2 checkpoint arrest [131,132]. In ATM-deficient deficient tumours, which makes up of 8% of SCLC, double-strand repair relies on ATR/CHK axis [108]. VX-970 is a first-in-class ATR inhibitor. In a phase I study, VX-970 was well tolerated with no dose-limiting toxicities. A colorectal cancer patient with ATM loss was on single-agent VX-970 for 20 months, so demonstrating the potent synthetic lethality possible [133]. VX-970 is able to sensitise cells to chemotherapeutic agents, causing replication fork stalling in preclinical studies [134,135]. This forms the basis of combining future generations of ATR inhibitor with these chemotherapy as well as other DDR inhibitors such as PARP. AZD6738, another ATR inhibitor, is also in development [136].

6.4. WEE1 inhibition

Wee-like protein kinase 1 (WEE1) is a crucial tyrosine kinase to halt cell from progressing from G2 to M phase of a cell cycle upon DNA damage [137]. WEE1 mRNA and WEE1 protein are significantly overexpressed in SCLC [112]. The WEE1 inhibitor AZD1775 is being studied as a single agent [138] (NCT02593019) and in combination with chemotherapy (NCT02937818) and olaparib (NCT02511795) [138].

7. Targeting the epigenome

The concept of epigenetics has been refined and is generally accepted as 'the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence'. [139] Epigenetic changes include posttranslational modifications involving amino acids of histones, histones variants and methylation and acetylation modifications of DNA bases [140].

EZH2 (zeste homologue 2) is the functional enzymatic component of a histone methyltransferase called polycomb repressive complex 2 (PRC2), which is the major enzyme that methylates lysine-27 of H3-K27 (histone H3) [141]. In many cancers, including SCLC, EZH2 is upregulated and overexpressed, with high EZH2 levels correlating with advanced stage of disease and poor prognosis [110,141]. EZH2 is likely a transcriptional repressor silencing tumour suppressor genes [141]. With recent progress in deciphering the high-resolution structure of EZH2 and its interaction with PRC2 [142], small molecule inhibitors targeting this histone methyltransferase are expected.

The histone modifying enzyme, lysine demethylase 1 (LSD1) is overexpressed in many human cancers including lung, breast, prostate and haematological malignancies [143]. LSD1 contributes to maintenance of genome-wide DNA methylation by demethylating and inhibiting turnover of the DNA methyltransferase [144]. It was found that a subset of SCLC lines and primary

Table 1
Selected non-immunotherapy trials in small-cell lung cancer.

Target class	Target	Drug	Phase	Results/recruitment status	Reference
Notch pathway	DLL3	Rovalpituzumab tesirine	II	In DLL3-high patients, mPFS 4.1 months, mOS 6.7 months	[35]
		Rovalpituzumab tesirine (maintenance after first-line chemotherapy in DLL3-high patients)	III	Recruiting	NCT03033511 https://clinicaltrials.gov/ct2/show/NCT03033511
		Rovalpituzumab tesirine (in relapsed disease *versus* topotecan in DLL3-high patients)	III	Active, not recruiting	NCT03061812 https://clinicaltrials.gov/ct2/show/NCT03061812
		Rovalpituzumab tesirine in combination with nivolumab or nivolumab + ipilimumab	I/II	Completed recruitment	[182] NCT03026166 https://clinicaltrials.gov/ct2/show/NCT03026166
		Rovalpituzumab tesirine in combination with first-line chemotherapy in DLL3-high patients	I	Terminated (strategic considerations)	[39] NCT02819999 https://clinicaltrials.gov/ct2/show/NCT02819999
Auroras	Aurora A	Alisertib in combination with paclitaxel (*versus* paclitaxel alone)	II (randomised)	mPFS 101 days combination *versus* 66 days alisertib alone (HR 0.77; 95% CI 0.557−1.067; p = 0.113) mOS 186 days combination *versus* 165 days alisertib alone (HR 0.93; 95% CI 0.652−1.341; p = 0.714)	[59]
	Aurora B	AZD2811	I (in multiple solid tumours)	Recruiting	[183] NCT02579226 https://clinicaltrials.gov/ct2/show/NCT02579226
	Aurora B	AZD2811	II	Terminated (early detection of the purpose of the study)	NCT03366675 https://clinicaltrials.gov/ct2/show/NCT03366675
DNA damage response (DDR)	PARP	Olaparib maintenance (*versus* placebo)	III	mOS 8.9 (90% CI 7.0−11.9), 9.9 (90% CI 7.6−12.9) and 9.0 (90% CI 6.6−11.8) months in the placebo, olaparib bd and tds arms, respectively. There was no significant difference in OS between olaparib and placebo for either the bd (Cox adjusted HR 0.97; 90% CI 0.69−1.37; stratified logrank p=0.73) or the tds arm (1.05; 90% CI 0.76−1.46; p=0.73).	[124]
	PARP	Olaparib in combination temozolomide	I/II	ORR 41.4% (95% CI 23.5−59.3%), mPFS 87 days (95% CI 48−159 days) mOS 220 days (95% CI 140−308) mDOR 103 days	[129]
	PARP	Veliparib in combination with cisplatin and etoposide [CE] (*versus* CE with placebo)	II (randomised)	mPFS CE/velaparib 6.1 and 5.5 months CE/placebo (unstratified HR 0.75 [one-sided p = 0.06]) mOS CE/velaparib 10.3 and 8.9 months CE/placebo (unstratified HR 0.83 [one-sided	[126]

(*continued on next page*)

Table 1 (*continued*)

Target class	Target	Drug	Phase	Results/recruitment status	Reference
				p = 0.17])	
	PARP	Veliparib in combination with temozolomide (*versus* temozolomide with placebo)	II (randomised)	4-month PFS rate 36% temozolomide/veliparib and 27% temozolomide/placebo (p = 0.19). mOS 8.2 months temozolomide/veliparib (95% CI 6.4–12.2) and 7.0 months temozolamide/placebo (95% CI 5.3–9.5) (p = 0.50)	[128]
	ATR and PARP	AZD6738 in combination with olaparib	II	Recruiting	NCT02937818 https://clinicaltrials.gov/ct2/show/NCT02937818
	ATR	VX-970 in combination with topotecan	I/II	Recruiting	NCT02487095 https://clinicaltrials.gov/ct2/show/NCT02487095
	CHK1	SRA-737 in combination with gemcitabine plus cisplatin (*versus* in combination with low-dose gemcitabine)	I/II (in multiple solid tumours)	Active, not recruiting	NCT02797977 https://clinicaltrials.gov/ct2/show/NCT02797977
	WEE1	AZD1775	II	Active, not recruiting	NCT02593019 https://clinicaltrials.gov/ct2/show/NCT02593019
	WEE1	AZD1775 in combination with carboplatin	II	Recruiting	NCT02937818 https://clinicaltrials.gov/ct2/show/NCT02937818
	WEE1	AZD1775 in combination with olaparib	Ib (in multiple solid tumours)	Active, not recruiting	NCT02511795 https://clinicaltrials.gov/ct2/show/NCT02511795
	RNA polymerase II	Lurbinectedin	II	19 patients (38%) had PR. 20 patients (40%) had disease stabilisation, 6 of them for > 4 months. mDOR 5.3 months (CI 95% 2.8–8.8) and mPFS 4.2 months (CI 95% 2.8–6.3)	[116]
		Lurbinectedin in combination with doxorubicin	I	ORR 67% (95% CI 43–85%) mPFS 4.7 months (95% CI 3.5 months-not reached)	[184]
		Lurbinectedin in combination with doxorubicin (expanded cohort from the above trial, with dose reduced)	Ib	ORR 37% mPFS 3.4 months (95% CI 1.5–6.2 months) mOS 7.9 months (95% CI 4.9–11.5 months)	[185]
		Lurbinectedin in combination with paclitaxel	I	ORR 71% mPFS 4.8 months	[118]
		Lurbinectedin in combination with doxorubicin *versus* cyclophosphamide plus doxorubicin plus vincristine(CAV) or topotecan	III	Active, not recruiting	[186]
Bcl-2	Bcl-2	APG-1252	I	Well tolerated and one partial response seen	[107]
	Bcl-2	Navitoclax (ABT-263)	II	PR in one (2.6%) patient and stable disease in 9 (23%) patients. mPFS 1.5 months mOS 3.2 months. Grade 3/4 thrombocytopaenia in 41%	[187]

	Bcl-2 (and mTOR)	Navitoclax and vistusertib	I/II	Recruiting	NCT03366103 https://clinicaltrials.gov/ct2/show/NCT03366103
	Bcl-2 (and BET inhibitor)	Venetoclax and ABBV-075	I	Completed recruitment	NCT02391480 https://clinicaltrials.gov/ct2/show/NCT02391480
	Bcl-2/Bcl-xL	APG-1252	I	Recruiting	[107] NCT03080311 https://clinicaltrials.gov/ct2/show/NCT03080311
Epigenome	LSD1	GSK2879552	I	Recruitment terminated as the risk benefit does not favour continuation of the study	NCT02034123 https://clinicaltrials.gov/ct2/show/NCT02034123

PR, partial response; mPFS, median progression-free survival; mOS, median overall survival; DLL, delta-like ligand; ATR, ataxia telangiectasia and Rad3-related protein; PARP, poly ADP ribose polymerase; HR, hazard ratio; CI, confidence interval; BET, bromodomain and extraterminal motif; WEE1, Wee-like protein kinase 1; LSD1, lysine demethylase 1; mTOR, mammalian target of rapamycin.; mDOR, median duration of response

samples showing growth inhibition by an LSD inhibitor, GSK2879552, exhibited DNA hypomethylation in a signature probe set, thus providing a useful predictive biomarker for use in SCLC [145]. A phase I trial of this LDS1 inhibitor in relapsed/refractory SCLC was initiated but was recently terminated as the risk benefit in relapsed refractory SCLC does not favour continuation of the study (NCT02034123).

A summary of selected non-immunotherapy trials on SCLC is shown in Table 1.

8. Targeting the immune system

Immunotherapy has revolutionised the treatment of many advanced cancers. Several studies in SCLC have failed to meet their primary end-point. Maintenance pembrolizumab, a PD-1 inhibitor, after first-line chemotherapy was investigated in a phase II trial with a discouraging PFS of 1.4 months and OS of 9.6 months [146]. Maintenance ipilimumab and nivolumab after first-line chemotherapy was investigated in a phase III study [147]; however, it has recently been announced that it has not met its primary end-point of OS [148]. There has been a recent announcement [149] that CheckMate 331, a phase III study comparing nivolumab *versus* topotecan in relapsed disease failed to meet its primary OS end-point [150]. This is disappointing especially as it might have been predicted that SCLC would respond well to immunotherapy. Cancers with a high number of mutations may be susceptible to immune targeting as mutations generate neoantigens that are potential immune targets [151]. SCLC has a relatively high mutational burden [152]. This is in keeping with the genomic landscape of heavy smoking exposure and high levels of DNA repair deficits. Tumour mutational burden (TMB) has been investigated as an emerging predictive biomarker to response to immunotherapy in NSCLC [153] where it has been shown that nivolumab, a PD-1 inhibitor, combined with ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, was more effective than chemotherapy in patients with TMB >10 mutations/megabase (MB) (PFS 7.2 *versus* 5.5 months; HR = 0.58; p = 0.001), whereas in patients with TMB <10 mutations/MB, there was no significant difference between the groups (PFS 3.2 *versus* 5.5 months; HR = 1.07; CI 0.84–1.35). Indeed, stratifying SCLC by TMB results has proved a more fruitful approach than unselected cohorts. In a phase II study (CheckMate 032) in relapsed SCLC, patients receiving either nivolumab or nivolumab with ipilimumab were grouped into 'low', 'medium' or 'high' TMB groups. TMB low was defined as <143 mutations/MB, TMB medium as 143–247 mutations/MB and TMB high as ≥ 248 mutations/MB. ORR to nivolumab was 4.8% in TMB low, 6.3% in TMB medium and 21.3% in TMB high. The

combination ipilimumab/nivolumab ORR was 22.2% in TMB low, 16.0% in TMB medium and 46.2% in TMB [154]. In the same study, PD-L1 expression was investigated as a predictive biomarker of response. PD-L1 expression did not predict response and was not associated with tumour mutational burden [155]. Based on the results of CheckMate 032, nivolumab has been granted accelerated approval by the FDA as third-line treatment [156].

There is rationale for combining immunotherapy and chemotherapy based on the concept of chemotherapy-induced tumour damage releasing tumour-specific antigens, thus increasing activation of T-cells [157,158]. A phase III trial, IMpower 133 [159] assessed atezolizumab, a PD-L1 inhibitor, in combination with first-line chemotherapy plus maintainence atezolizumab *versus* standard chemotherapy. This demonstrated a significant benefit in PFS and OS (PFS 5.3 *versus* 4.3 months; HR 0.77; p = 0.02, OS 12.3 *versus* 10.3 months; HR 0.7; p = 0.007). Although the OS difference is modest at 2 months, this represents a significant proportion of the expected survival, given that 38.2% of the patients in the standard arm lived until 1 year compared with 51.7% in the atezolizumab group. An exploratory analysis of TMB concluded TMB was not predictive of benefit (HR for progression/death in <10 mutations/MB 0.70, 95% CI 0.45–1.07; >10 mutations/MB 0.68, 95% CI 0.47–0.97; >16 mutations/MB 0.63, 95% CI 0.35–1.15). Atezolizumab has been granted FDA approval in the first-line setting in combination with chemotherapy. In contrast, a first-line phase III trial [160] of ipilimumab in combination with standard chemotherapy and continued as a maintenance therapy *versus* standard chemotherapy alone failed to show a benefit in adding immunotherapy (PFS 4.6 *versus* 4.4 months; OS 11 *versus* 10.9 months). It has been speculated that the difference between the trials could be due to the differential action of CTLA-4 *versus* PD-1 blockade. CTLA-4 regulates peripheral T cell activation, whereas PD-1 suppresses T cell activation in the tumour microenvironment, and potentially this tumour microenvironment effect is necessary to antitumour activity. It is also possible that chemotherapy reduces the early T cell activation associated with CTLA-4 blockade but preserves the intratumoural response related to PD-L1 inhibition. A phase I study combining the anti–PD-L1 antibody durvalumab with the anti–CTLA-4 antibody tremelimumab in relapse SCLC is ongoing [161]. Preliminary data have shown that the combination is tolerable with favourable activity seen (ORR 13.3% with 2 complete response, 2 PR; 95% CI 3.8–30.7%; PFS 8 months, 95% CI 1.0–1.9 months; OS 7.9 months, 95% CI 3.2–15.8 months).

There are a vast number of early phase trials investigating the combination of immunotherapy and targeted drugs. There are some potential combinations that hold potential for investigation in SCLC. For example, MYC plays a global role in immune modulation. Multiple groups have demonstrated that MYC inactivation downregulates immune checkpoint CD47 and PD-L1 gene expression, enhancing anti-tumour response [162–166]. Others have shown widespread cytokine changes and T-cell migration into the tumour microenvironment with associated tumour responses with MYC targeting [167–169]. Others have shown MYC regulates via MHC [170,171]. A summary of immunotherapy trials is shown in Table 2.

9. Drug development challenges

The challenges encountered in the research of SCLC are manifold. First, the rapidity in disease presentation and the declining performance status of the patient often precludes trial entry. Patients are, almost universally, ex-smokers or current smokers with high levels of comorbidity.

Second, molecular characterisation is challenging. This is partially due to the deteriorating condition of patients that precludes pretrial screening, and also, as the vast majority of patients are treated with chemotherapy and radiation, there may be a lack of resected specimens. Analysis of circulating biomarkers promises a non-invasive, rapid turnaround molecular prescreening tool. Circulating tumour cells (CTCs) are present in 85% of SCLC patients [171]. CTC pretreatment level and level change after one cycle of chemotherapy are independent prognostic factors [172] and are tumourigenic when injected into immunocompromised mice, forming CTC-derived explants [173]. Analysis of a wide panel of genes frequently altered in SCLC showed similar changes in CTCs [174]. Enumeration of CTCs has been applied to clinical studies as a biomarker to predict response or resistance [175,176]. Further studies to appreciate the heterogeneity of CTCs by exploring the genomic, transcriptome, and epigenetic variation, at diagnosis, during treatment resistance, and in metastatic setting are required. Cell-free DNA (cfDNA) is tumour-derived fragmented DNA in the blood [177]. In a study, 140 plasma samples of cfDNA from 27 patients with SCLC were available for analysis, and disease-associated mutations were detected in 85% of samples [178]. This detection rate is similar to that of CTCs [171,179]. The same study showed that allelic frequencies and copy number alterations in the cfDNA tracked closely to treatment response and identified disease recurrence before radiological progression. A study aimed to use a CTC gene panel to predict chemotherapy sensitivity using a panel of frequently amplified or deleted genes in SCLC (e.g. *TP53*, *Rb1*, *PTEN*, *SOX2*) [174]. The authors failed to build a model to predict chemosensitivity; however, the analysis included extended whole genome-wide copy number aberration profiles which did predict chemosensitivity. These unique biomarkers set the stage for

future studies in SCLC as prognostic and predictive markers, to monitor treatment response and to decipher mechanism of resistance. However, caution should be used as the CTCs may be phenotypically different from the cells taken from solid tumour biopsy as demonstrated in a study that showed significantly high sensitivity to chemotherapy in CTC compared with cell lines from the same patients derived from tumour biopsy [180].

Table 2
Selected immunotherapy trials in small-cell lung cancer.

Drug	Line of treatment	Phase	Results/recruiting	Reference
Maintenance pembrolizumab	After first-line chemotherapy	II	PFS 1.4 months (95% CI 1.3–2.8) OS 9.6 months (95% CI 7.0–12) 1-year OS 37%	[146]
Pembrolizumab	Relapsed	II	PFS 2 months OS 9.1 months ORR 18.7% (95% CI 11.8–27.4) overall; ORR 35.7% (15/42; 95% CI 21.6–52.0) in PD-L1 positive ORR 6.0% (3/50; 95% CI 1.3–16.5) in PD-L1 negative	[187]
Pembrolizumab in combination with paclitaxel	Relapsed	II	ORR 23.1% PFS 5.0 months (95% CI 2.7–6.7) OS 9.2 m (95% CI 6.6–15.1)	[188]
Maintenance ipilimumab + nivolumab or nivolumab (*versus* surveillance)	After first-line chemotherapy	III	No data published but failed to meet end-point for OS announced November 2018	[147] [148]
Nivolumab (*versus* topotecan)	Relapsed	III	No data published but failed to meet end-point for OS announced Oct 18.	[150] [149]
Nivolumab alone *versus* nivolumab plus ipilimumab	Relapsed	I/II open label	ORR nivolumab 10%, 6-month PFS rate 17.2%, 12-month OS rate 28.3%, 18-month OS rate 20%; ORR nivolumab 1 mg/kg + ipilimumab 3 mg/kg 23% nivolumab 3 mg/kg + ipilimumab 1 mg/kg 19%	[189,190]
Atezolizumab + chemotherapy + atezolizumab maintenance (*versus* etoposide + platinum)	First line	I/III	Immunotherapy *versus* control PFS 5.2 months *versus* 4.3 months (HR 0.77; 95% CI 0.62–0.96; p = 0.02) OS 12.3 months *versus* 10.3 months (HR 0.70; 95% CI 0.54–0.91; p = 0.007) 1-year survival 51.7% *versus* 38.2%	[159]
Ipilimumab + etoposide + platinum + maintenance ipilimumab (*versus* etoposide + platinum)	First line	III	Immunotherapy *versus* chemotherapy alone PFS 4.6 months *versus* 4.4 months (HR 0.85; 95% CI 0.75–0.97) OS 11.0 months *versus* 10.9 months (HR 0.94; 95% CI 0.81 to 1.09; p = 0.38)	[160]
Durvalumab + tremelimumab	Relapsed	I	PFS 1.8 months OS 7.9 months ORR 13.3% with DOR 18.9 months	[161]

CI, confidence interval; HR, hazard ratio; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; DOR, duration of response

Third, human SCLC often responds to platinum-based chemotherapy dramatically, only to relapse later and become resistant to treatment. Preclinical models have largely failed to reciprocate this pattern of response [181]. Focus to improve these models including use of CTC-derived explants could potentially identify mediators of chemotherapy resistance, target these pathways by rationally designed drugs and guide therapeutic decision.

10. Conclusion

In the last decades, SCLC has remained a lethal disease. A recurring issue is disappointment in clinical trials despite identification of a putative target and promising results in preclinical models. This is discernible in the recent development of rovalpituzumab. However, different from previous failure to identify a crucial target, the shorter survival of rovalpituzumab might be related to its adverse effects associated with the toxin included in the ADC. Targeting DLL3 may yet still have a future with alternative drugs or ADC payloads.

Immunotherapy has given hope in the treatment of SCLC. However, while atezolizumab (in combination with chemotherapy) and nivolumab are recently approved in first- and third-line settings, respectively, maintenance treatment with pembrolizumab produced less than expected results. Incorporating TMB is a strategy that has proved to be successful. This underscores the importance of positioning an immunotherapy in the treatment algorithm, as well as selection of predictive biomarkers. There are some challenges in introducing TMB into standard clinical practice, due to the need to obtain biopsy samples with high DNA quantity, standardisation of sequencing panels and establishing the level of what constitutes 'high TMB'. Results from recent trials demonstrates that combining immunotherapy with chemotherapy not only has a solid rationale but also can yield effective results.

Other therapeutic combination strategies include DDR drugs in combination with chemotherapy and immunotherapy. Insights from synthetic lethality in genetic mutation and cross-talks between molecular pathways also make combining different molecular targeted therapies, such as PARP and inhibitors of the PI3k/AKT, of interest.

Promising biomarkers candidates in SCLC are manifold but complex. MYC is an intriguing target which requires further work. For example, although MYC sensitises SCLC to Aurora kinase inhibition, sensitivity to drug may also be mediated by Rb loss.

The optimal development of targeted therapy in SCLC requires early identification of biomarkers. Biomarker screening at disease presentation in preparation for entry to trials in the post first-line maintenance or second-line setting should be considered. Repeated tumour or liquid biopsy at the time of disease progression may help to better understand resistance mechanisms. Finally, enrolling patients with SCLC into molecularly driven 'basket trials' may accelerate drug development. With concerted effort and accelerating pace in research, we can hope that new, personalised interventions can be delivered to patients with SCLC and witness meaningful changes in the outcome in SCLC patients in the future.

Conflict of interest statement

None declared.

References

[1] Wong M, Lao X, Ho K, Goggins W, Tse S. Incidence and mortality of lung cancer: global trends and association with socioeconomic status. Sci Rep 2017;7(1):14300. https://doi.org/10.1038/s41598-017-14513-7.

[2] Noone A, Howlader N, Krapcho M, Miller D, Brest A, Yu M, et al. SEER cancer statistics review. Bethesda, MD: National Cancer Institute; 1975-2015. based on November 2017 SEER data submission, posted to the SEER web site, https://seer.cancer.gov/csr/1975_2015/. [Accessed April 2018].

[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin 2018;68(6):394–424.

[4] Janssen-Heijnen ML, Karim-Kos HE, van der Drift MA, Groen HJ, Ho VK, Koning C, et al. Modest improvements of survival for patients with small cell lung cancer aged 45 to 59 years only, diagnosed in The Netherlands, 1989 to 2008. J Thorac Oncol 2012;7(1):227–32.

[5] Lassen U, Osterlind K, Hansen M, Dombernowsky P, Bergman B, Hansen HH. Long-term survival in small-cell lung cancer: posttreatment characteristics in patients surviving 5 to 18+ years–an analysis of 1,714 consecutive patients. J Clin Oncol 1995;13(5):1215–20.

[6] Dayen C, Debieuvre D, Molinier O, Raffy O, Paganin F, Virally J, et al. New insights into stage and prognosis in small cell lung cancer: an analysis of 968 cases. J Thorac Dis 2017;9(12):5101–11.

[7] Yu JB, Decker RH, Detterbeck FC, Wilson LD. Surveillance epidemiology and end results evaluation of the role of surgery for stage I small cell lung cancer. J Thorac Oncol 2010;5(2):215–9.

[8] Früh M, De Ruysscher D, Popat S, Crinò L, Peters S, Felip E. Small-cell lung cancer (SCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2013;24(suppl_6):vi99–105.

[9] Mascaux C, Paesmans M, Berghmans T, Branle F, Lafitte JJ, Lemaitre F, et al. A systematic review of the role of etoposide and cisplatin in the chemotherapy of small cell lung cancer with methodology assessment and meta-analysis. Lung Cancer 2000;30(1):23–36.

[10] Foster NR, Qi Y, Shi Q, Krook JE, Kugler JW, Jett JR, et al. Tumor response and progression-free survival as potential surrogate endpoints for overall survival in extensive stage small-cell lung cancer: findings on the basis of North Central Cancer Treatment Group trials. Cancer 2011;117(6):1262–71.

[11] O'Brien ME, Ciuleanu TE, Tsekov H, Shparyk Y, Cuceviá B, Juhasz G, et al. Phase III trial comparing supportive care alone

with supportive care with oral topotecan in patients with relapsed small-cell lung cancer. J Clin Oncol 2006;24(34):5441–7.
[12] Travis W, Brambilla E, Burke A, Marx A, Nicholson A. WHO classification of tumours of the lung, pleura, thymus and heart. In: International Agency for Research on Cancer. Lyon. 4th edition, vol. 7; 2015.
[13] Sutherland K, Proost N, Brouns I, Adriaensen D, Song J, Berns A. Cell of origin of small cell lung cancer: inactivation of Trp53 and Rb1 in distinct cell types of adult mouse lung. Cancer Cell 2011;19(6):754–64.
[14] Park K, Liang M, Raiser D, Zamponi R, Roach R, Curtis S, et al. Characterization of the cell of origin for small cell lung cancer. Cell Cycle 2011;10(16):2806–15.
[15] Borges M, Linnoila R, van de Velde H, Chen H, Nelkin B, Mabry M, et al. An achaete-scute homologue essential for neuroendocrine differentiation in the lung. Nature 1997;386(6627):852–5.
[16] George J, Lim JS, Jang SJ, Cun Y, Ozretic L, Kong G, et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524(7563):47–53.
[17] Donakonda S, Sinha S, Dighe SN, Rao MRS. System analysis identifies distinct and common functional networks governed by transcription factor ASCL1, in glioma and small cell lung cancer. Mol Biosyst 2017;13(8):1481–94.
[18] Dooley AL, Winslow MM, Chiang DY, Banerji S, Stransky N, Dayton TL, et al. Nuclear factor I/B is an oncogene in small cell lung cancer. Genes Dev 2011;25(14):1470–5.
[19] McFadden DG, Papagiannakopoulos T, Taylor-Weiner A, Stewart C, Carter SL, Cibulskis K, et al. Genetic and clonal dissection of murine small cell lung carcinoma progression by genome sequencing. Cell 2014;156(6):1298–311.
[20] Rudin CM, Durinck S, Stawiski EW, Poirier JT, Modrusan Z, Shames DS, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44(10):1111–6.
[21] Kumar R, Juillerat-Jeanneret L, Golshayan D. Notch antagonists: potential modulators of cancer and inflammatory diseases. J Med Chem 2016;59(17):7719–37.
[22] Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Canc 2003;10(3):756–67.
[23] Espinoza I, Miele L. Notch inhibitors for cancer treatment. Pharmacol Ther 2013;139(2):95–110.
[24] Kunnimalaiyaan M, Chen H. Tumor suppressor role of Notch-1 signaling in neuroendocrine tumors. Oncol 2007;12(5):535–42.
[25] George J, Lim J, Jang S. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524(7563):47–53.
[26] Ntziachristos P, Lim J, Sage J, Aifantis I. From fly wings to targeted cancer therapies: a centennial for notch signaling. Cancer Cell 2014;25(3):318–34.
[27] Chapman G, Sparrow D, Kremmer E, Dunwoodie S. Notch inhibition by the ligand DELTA-LIKE 3 defines the mechanism of abnormal vertebral segmentation in spondylocostal dysostosis. Hum Mol Genet 2011;20(5):905–16.
[28] Fehon R, Kooh P, Rebay I, Regan C, Xu T, Muskavitch M, et al. Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila. Cell 1990;61(3):523–34.
[29] Loomes K, Stevens S, O'Brien M, Gonzalez D, Ryan M, Segalov M, et al. Dll3 and Notch1 genetic interactions model axial segmental and craniofacial malformations of human birth defects. Dev Dynam 2007;236(10):1943–51.
[30] Gazdar A, Bunn P, Minna J. Small-cell lung cancer: what we know, what we need to know and the path forward. Nat Rev Canc 2017;17(12):725–37.
[31] Dylla S. Toppling high-grade pulmonary neuroendocrine tumors with a DLL3-targeted trojan horse. Mol Cell Oncol 2016;3(2):e1101515.
[32] Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 2015;7(302):302ra136.
[33] Hartley J. The development of pyrrolobenzodiazepines as antitumour agents. Expert Opin Investig Drugs 2011;20(6):733–44.
[34] Rudin CM, Pietanza MC, Bauer TM, Ready N, Morgensztern D, Glisson BS, et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. Lancet Oncol 2017;18(1):42–51.
[35] Carbone DP, Morgensztern D, Le Moulec S, Santana-Davila R, Ready N, Hann CL, et al. Efficacy and safety of rovalpituzumab tesirine in patients with DLL3-expressing, ≥ 3rd line small cell lung cancer: results from the phase 2 TRINITY study. J Clin Oncol 2018;36(15_suppl).
[36] AbbVie A. Study of rovalpituzumab tesirine as maintenance therapy following first- line platinum-based chemotherapy in participants with extensive stage small cell lung cancer (MERU). https://ClinicalTrials.gov/show/NCT03033511; 2017.
[37] AbbVie Study. Comparing rovalpituzumab tesirine versus topotecan in subjects with advanced or metastatic small cell lung cancer with high levels of delta-like protein 3 (DLL3) and who have first disease progression during or following front-line platinum-based chemotherapy (TAHOE). 2017. https://ClinicalTrials.gov/show/NCT03061812.
[38] AbbVie. Phase III rova-T trial halted in small cell lung cancer. 2018. https://wwwonclivecom/web-exclusives/phase-iii-rovat-trial-halted-in-small-cell-lung-cancer.
[39] Hann CL, Morgensztern D, Dowlati A, Burns TF, Jotte RM, Pennell NA, et al. A study of rovalpituzumab tesirine in frontline treatment of patients with DLL3 expressing extensive small cell lung cancer. J Clin Oncol 2017;35(15_suppl):TPS2598 [TPS].
[40] AbbVie. 1538Tip - a phase 1/2 study on safety of rovalpituzumab tesirine in combination with nivolumab or nivolumab + ipilimumab in small cell lung cancer. Ann Oncol 2017;28(suppl_5):v539–42. https://doi.org/10.1093/annonc/mdx386.
[41] Westerman BA, Breuer RH, Poutsma A, Chhatta A, Noorduyn LA, Koolen MG, et al. Basic helix-loop-helix transcription factor profiling of lung tumors shows aberrant expression of the proneural gene atonal homolog 1 (ATOH1, HATH1, MATH1) in neuroendocrine tumors. Int J Biol Mark 2007;22(2):114–23.
[42] Osborne JK, Larsen JE, Shields MD, Gonzales JX, Shames DS, Sato M, et al. NeuroD1 regulates survival and migration of neuroendocrine lung carcinomas via signaling molecules TrkB and NCAM. Proc Natl Acad Sci U S A 2013;110(16):6524–9.
[43] Borromeo MD, Savage TK, Kollipara RK, He M, Augustyn A, Osborne JK, et al. ASCL1 and NEUROD1 reveal heterogeneity in pulmonary neuroendocrine tumors and regulate distinct genetic programs. Cell Rep 2016;16(5):1259–72.
[44] Neptune ER, Podowski M, Calvi C, Cho JH, Garcia JG, Tuder R, et al. Targeted disruption of NeuroD, a proneural basic helix-loop-helix factor, impairs distal lung formation and neuroendocrine morphology in the neonatal lung. J Biol Chem 2008;283(30):21160–9.
[45] Gazdar AF, Carney DN, Nau MM, Minna JD. Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological, and growth properties. Cancer Res 1985;45(6):2924–30.
[46] Poirier JT, Gardner EE, Connis N, Moreira AL, de Stanchina E, Hann CL, et al. DNA methylation in small cell lung cancer defines distinct disease subtypes and correlates with high expression of EZH2. Oncogene 2015;34(48):5869–78.
[47] Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, et al. Establishment and identification of small cell

lung cancer cell lines having classic and variant features. Cancer Res 1985;45(6):2913–23.
[48] Johnson BE, Russell E, Simmons AM, Phelps R, Steinberg SM, Ihde DC, et al. MYC family DNA amplification in 126 tumor cell lines from patients with small cell lung cancer. J Cell Biochem Suppl 1996;24:210–7.
[49] Poirier J, Dobromilskaya I, Moriarty W, Peacock C, Hann C, CM R. Selective tropism of Seneca Valley virus for variant subtype small cell lung cancer. J Natl Cancer Inst 2013;105(14):1059–65.
[50] Mollaoglu G, Guthrie MR, Bohm S, Bragelmann J, Can I, Ballieu PM, et al. MYC drives progression of small cell lung cancer to a variant neuroendocrine subtype with vulnerability to Aurora kinase inhibition. Cancer Cell 2017;31(2):270–85.
[51] Sattler M, Salgia R. Molecular and cellular biology of small cell lung cancer. Semin Oncol 2003;30(1):57–71.
[52] Alves Rde C, Meurer RT, Roehe AV. MYC amplification is associated with poor survival in small cell lung cancer: a chromogenic in situ hybridization study. J Cancer Res Clin Oncol 2014;140(12):2021–5.
[53] Kim D-W, Wu N, Kim Y-C, Cheng PF, Basom R, Kim D, et al. Genetic requirement for Mycl and efficacy of RNA Pol I inhibition in mouse models of small cell lung cancer. Genes Dev 2016;30(11):1289–99.
[54] Chen H, Liu H, Qing G. Targeting oncogenic Myc as a strategy for cancer treatment. Signal Transduct Target Ther 2018;3(5): 2018. https://doi.org/10.1038/s41392-018-0008-7. Feb 23.
[55] Sos ML, Dietlein F, Peifer M, Schöttle J, Balke-Want H, Müller C, et al. A framework for identification of actionable cancer genome dependencies in small cell lung cancer. Proc Natl Acad Sci Unit States Am 2012;109(42):17034–9.
[56] Bischoff JR, Plowman GD. The Aurora/Ipl1p kinase family: regulators of chromosome segregation and cytokinesis. Trends Cell Biol 1999;9(11):454–9.
[57] Otto T, Horn S, Brockmann M, Eilers U, Schuttrumpf L, Popov N, et al. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer Cell 2009;15(1):67–78.
[58] Helfrich BA, Kim J, Gao D, Chan DC, Zhang Z, Tan A-C, et al. Barasertib (AZD1152), a small molecule Aurora B inhibitor, inhibits the growth of SCLC cell lines in vitro and in vivo. Mol Cancer Ther 2016;15(10):2314–22.
[59] Owonikoko T, Nackaerts K, Csoszi T, Ostoros G, Baik C, Ullmann CD, et al. OA05.05 randomized phase 2 study: Alisertib (MLN8237) or placebo + paclitaxel as second-line therapy for small-cell lung cancer (SCLC). J Thorac Oncol 2017;12(1):S261–2.
[60] Molenaar JJ, Ebus ME, Geerts D, Koster J, Lamers F, Valentijn LJ, et al. Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. Proc Natl Acad Sci U S A 2009;106(31):12968–73.
[61] Chipumuro E, Marco E, Christensen CL, Kwiatkowski N, Zhang T, Hatheway CM, et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. Cell 2014;159(5):1126–39.
[62] Hydbring P, Castell A, Larsson LG. MYC Modulation around the CDK2/p27/SKP2 Axis. Genes 2017;8(7).
[63] Gargano B, Amente S, Majello B, Lania L. P-TEFb is a crucial co-factor for Myc transactivation. Cell Cycle 2007;6(16):2031–7.
[64] Huang CH, Lujambio A, Zuber J, Tschaharganeh DF, Doran MG, Evans MJ, et al. CDK9-mediated transcription elongation is required for MYC addiction in hepatocellular carcinoma. Genes Dev 2014;28(16):1800–14.
[65] Larochelle S, Amat R, Glover-Cutter K, Sanso M, Zhang C, Allen JJ, et al. Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. Nat Struct Mol Biol 2012;19(11):1108–15.
[66] Morales F, Giordano A. Overview of CDK9 as a target in cancer research. Cell Cycle 2016;15(4):519–27.
[67] Karp JE, Garrett-Mayer E, Estey EH, Rudek MA, Smith BD, Greer JM, et al. Randomized phase II study of two schedules of flavopiridol given as timed sequential therapy with cytosine arabinoside and mitoxantrone for adults with newly diagnosed, poor-risk acute myelogenous leukemia. Haematologica 2012;97(11):1736–42.
[68] Lanasa MC, Andritsos L, Brown JR, Gabrilove J, Caligaris-Cappio F, Ghia P, et al. Final results of EFC6663: a multicenter, international, phase 2 study of alvocidib for patients with fludarabine-refractory chronic lymphocytic leukemia. Leuk Res 2015;39(5):495–500.
[69] Stephenson JJ, Nemunaitis J, Joy AA, Martin JC, Jou YM, Zhang D, et al. Randomized phase 2 study of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus erlotinib in patients with non-small cell lung cancer. Lung Cancer 2014;83(2):219–23.
[70] Mita MM, Joy AA, Mita A, Sankhala K, Jou YM, Zhang D, et al. Randomized phase II trial of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus capecitabine in patients with advanced breast cancer. Clin Breast Canc 2014;14(3):169–76.
[71] Le Tourneau C, Faivre S, Laurence V, Delbaldo C, Vera K, Girre V, et al. Phase I evaluation of seliciclib (R-roscovitine), a novel oral cyclin-dependent kinase inhibitor, in patients with advanced malignancies. Eur J Cancer 2010;46(18):3243–50.
[72] Benson C, White J, De Bono J, O'Donnell A, Raynaud F, Cruickshank C, et al. A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. Br J Canc 2007;96(1):29–37.
[73] Zeng L, Zhou MM. Bromodomain: an acetyl-lysine binding domain. FEBS Lett 2002;513(1):124–8.
[74] Itzen F, Greifenberg AK, Bösken CA, Geyer M. Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation. Nucleic Acids Res 2014;42(12):7577–90.
[75] Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011;146(6):904–17.
[76] Puissant A, Frumm SM, Alexe G, Bassil CF, Qi J, Chanthery YH, et al. Targeting MYCN in neuroblastoma by BET bromodomain inhibition. Cancer Discov 2013;3(3):308–23.
[77] Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci U S A 2011;108(40):16669–74.
[78] Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011;478(7370):529–33.
[79] Mazur PK, Herner A, Mello SS, Wirth M, Hausmann S, Sanchez-Rivera FJ, et al. Combined inhibition of BET family proteins and histone deacetylases as a potential epigenetics-based therapy for pancreatic ductal adenocarcinoma. Nat Med 2015;21(10):1163–71.
[80] Doroshow DB, Eder JP, LoRusso PM. BET inhibitors: a novel epigenetic approach. Ann Oncol 2017;28(8):1776–87.
[81] Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Canc 2004;4(5):335–48.
[82] Hosoi H, Dilling MB, Liu LN, Danks MK, Shikata T, Sekulic A, et al. Studies on the mechanism of resistance to rapamycin in human cancer cells. Mol Pharmacol 1998;54(5):815–24.
[83] Atkins MB, Hidalgo M, Stadler WM, Logan TF, Dutcher JP, Hudes GR, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin

kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 2004;22(5):909–18.
[84] Chapuis N, Tamburini J, Green AS, Vignon C, Bardet V, Neyret A, et al. Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. Clin Cancer Res 2010;16(22):5424–35.
[85] Frost P, Moatamed F, Hoang B, Shi Y, Gera J, Yan H, et al. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. Blood 2004; 104(13):4181–7.
[86] Yu K, Toral-Barza L, Discafani C, Zhang WG, Skotnicki J, Frost P, et al. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. Endocr Relat Cancer 2001;8(3):249–58.
[87] Fischer B, Marinov M, Arcaro A. Targeting receptor tyrosine kinase signalling in small cell lung cancer (SCLC): what have we learned so far? Cancer Treat Rev 2007;33(4):391–406.
[88] Schmid K, Bago-Horvath Z, Berger W, Haitel A, Cejka D, Werzowa J, et al. Dual inhibition of EGFR and mTOR pathways in small cell lung cancer. Br J Canc 2010;103(5):622–8.
[89] Blackhall FH, Pintilie M, Michael M, Leighl N, Feld R, Tsao MS, et al. Expression and prognostic significance of kit, protein kinase B, and mitogen-activated protein kinase in patients with small cell lung cancer. Clin Cancer Res 2003;9(6): 2241–7.
[90] Umemura S, Mimaki S, Makinoshima H, Tada S, Ishii G, Ohmatsu H, et al. Therapeutic priority of the PI3K/AKT/mTOR pathway in small cell lung cancers as revealed by a comprehensive genomic analysis. J Thorac Oncol 2014;9(9):1324–31.
[91] Pandya KJ, Dahlberg S, Hidalgo M, Cohen RB, Lee MW, Schiller JH, et al. A randomized, phase II trial of two dose levels of temsirolimus (CCI-779) in patients with extensive-stage small-cell lung cancer who have responding or stable disease after induction chemotherapy: a trial of the Eastern Cooperative Oncology Group (E1500). J Thorac Oncol 2007;2(11): 1036–41.
[92] Tarhini A, Kotsakis A, Gooding W, Shuai Y, Petro D, Friedland D, et al. Phase II study of everolimus (RAD001) in previously treated small cell lung cancer. Clin Cancer Res 2010; 16(23):5900–7.
[93] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415.
[94] Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 2009;463:184.
[95] Harris SL, Levine AJ. The p53 pathway: positive and negative feedback loops. Oncogene 2005;24:2899.
[96] Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995;81(3):323–30.
[97] Oser MG, Fonseca R, Chakraborty AA, Brough R, Spektor A, Jennings RB, et al. Cells lacking the RB1 tumor suppressor gene are hyperdependent on Aurora B kinase for survival. Cancer discovery; 2018. CD-18-0389.
[98] Gong X, Du J, Parsons SH, Merzoug FF, Webster Y, Iversen PW, et al. Aurora-A kinase inhibition is synthetic lethal with loss of the RB1 tumor suppressor gene. Cancer discovery; 2018. CD-18-0469.
[99] Hockenbery D, Nuñez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990;348(6299): 334–6.
[100] Ben-Ezra JM, Kornstein MJ, Grimes MM, Krystal G. Small cell carcinomas of the lung express the Bcl-2 protein. Am J Pathol 1994;145(5):1036–40.
[101] Jiang S-X, Sato Y, Kuwao S, Kameya T. Expression of bcl-2 oncogene protein is prevalent in small cell lung carcinomas. J Pathol 1995;177(2):135–8.
[102] Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, et al. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. Clin Cancer Res 2012; 18(11):3163.
[103] Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013;19:202.
[104] Lochmann TL, Floros KV, Naseri M, Powell KM, Cook W, March RJ, et al. Venetoclax is effective in small-cell lung cancers with high BCL-2 expression. Clin Cancer Res 2018;24(2): 360.
[105] Shoemaker AR, Mitten Mj Fau, Adickes J, Adickes J Fau, Ackler S, Ackler S Fau, Refici M, Refici M Fau, Ferguson D, Ferguson D Fau, Oleksijew A, et al. Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. 2008. 1078-0432 (Print)).
[106] Tse C, Shoemaker Ar Fau, Adickes J, Adickes J Fau, Anderson MG, Anderson Mg Fau, Chen J, Chen J Fau, Jin S, Jin S Fau, Johnson EF, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. 2008. p. 1538–7445 [Electronic)].
[107] Lakhani NJ, Rasco DW, Tolcher AW, Huang Y, Ji J, Wang H, et al. A phase I study of novel dual Bcl-2/Bcl-xL inhibitor APG-1252 in patients with advanced small cell lung cancer (SCLC) or other solid tumor. J Clin Oncol 2018;36(15_suppl):2594.
[108] Sen T, Tong P, Wang J, Byers LA. Proteomic profiling identifies ATM expression level as a predictive biomarker to ATR and PARP inhibition in small cell lung cancer (SCLC). Cancer Res 2016;76(14 Supplement):LB-132.
[109] Allison Stewart C, Tong P, Cardnell RJ, Sen T, Li L, Gay CM, 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(17): 28575–87.
[110] Byers LA, Wang J, Nilsson MB, Fujimoto J, Saintigny P, Yordy J, et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discov 2012;2(9):798–811.
[111] Doerr F, George J, Schmitt A, Beleggia F, Rehkamper T, Hermann S, et al. Targeting a non-oncogene addiction to the ATR/CHK1 axis for the treatment of small cell lung cancer. Sci Rep 2017;7(1):15511.
[112] Sen T, Tong P, Diao L, Li L, Fan Y, Hoff J, et al. Targeting AXL and mTOR pathway overcomes primary and acquired resistance to WEE1 inhibition in small-cell lung cancer. Clin Cancer Res : an official journal of the American Association for Cancer Research 2017;23(20):6239–53.
[113] Sen T, Tong P, Stewart CA, Cristea S, Valliani A, Shames DS, et al. CHK1 inhibition in small-cell lung cancer produces single-agent activity in biomarker-defined disease subsets and combination activity with cisplatin or olaparib. Cancer Res 2017; 77(14):3870–84.
[114] Santamaría Nuñez G, Robles CMG, Giraudon C, Martínez-Leal JF, Compe E, Coin F, et al. Lurbinectedin specifically triggers the degradation of phosphorylated RNA polymerase II and the formation of DNA breaks in cancer cells. Mol Cancer Ther 2016;15(10):2399.
[115] Belgiovine C, Bello E, Liguori M, Craparotta I, Mannarino L, Paracchini L, et al. Lurbinectedin reduces tumour-associated macrophages and the inflammatory tumour microenvironment in preclinical models. Br J Canc 2017;117:628.
[116] Trigo Perez JM, Leary A, Besse B, Castellano DE, Ponce Aix S, Arrondeau J, et al. Efficacy and safety of lurbinectedin (PM1183) in small cell lung cancer (SCLC): results from a phase 2 study. J Clin Oncol 2018;36(15_suppl):8570.
[117] von Pawel J, Schiller JH, Shepherd FA, Fields SZ, Kleisbauer JP, Chrysson NG, et al. Topotecan versus

cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. J Clin Oncol 1999;17(2):658.

[118] Olmedo Garcia ME, Forster M, Calvo E, Moreno V, Lopez Criado MP, Lopez-Vilarino de Ramos JA, et al. Activity of lurbinectedin as single agent and in combination in patients with advanced small cell lung cancer (SCLC). Ann Oncol 2017;28(suppl_5):mdx386.003. mdx386.003.

[119] PharmaMar PharmaMar. Receives positive opinion from EMA (COMP) for orphan drug designation of Zepsyre® (lurbinectedin) for small-cell lung cancer. 2018. https://wwwpharmamarcom/wp-content/uploads/2019/01/PR_Lurbinectedin-orphan-drug-designation-EMA_OKpdf.

[120] PharmaMar. The U.S. Food and Drug Administration (FDA) has granted Orphan Drug designation to PharmaMar's lurbinectedin. 2018. https://wwwpharmamarcom/wp-content/uploads/2018/08/PR_Lurbinectedin-orphan-drug-designation-FDApdf.

[121] Farago AF, Drapkin BJ, Lopez-Vilarino de Ramos JA, Galmarini CM, Núñez R, Kahatt C, et al. ATLANTIS: a Phase III study of lurbinectedin/doxorubicin versus topotecan or cyclophosphamide/doxorubicin/vincristine in patients with small-cell lung cancer who have failed one prior platinum-containing line. Future Oncol 2018;15(3):231–9.

[122] Ame JC, Spenlehauer C, de Murcia G. The PARP superfamily. Bioessays : news and reviews in molecular, cellular and developmental biology 2004;26(8):882–93.

[123] D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 1999;342(Pt 2):249–68.

[124] Woll P, Gaunt P, Steele N, Ahmed S, Mulatero C, Shah R, et al. P1.07-015 STOMP: a UK national cancer research network randomised, double blind, multicentre phase II trial of olaparib as maintenance therapy in SCLC: topic: drug treatment alone and in combination with radiotherapy. J Thorac Oncol 2017;12(1):S704–5.

[125] de Bono J, Ramanathan RK, Mina L, Chugh R, Glaspy J, Rafii S, et al. Phase I, dose-escalation, two-Part Trial of the PARP inhibitor Talazoparib in patients with advanced germline BRCA1/2 mutations and selected sporadic cancers. Cancer Discov 2017;7(6):620–9.

[126] Owonikoko TK, Dahlberg SE, Sica G, Wagner LI, Wade JL, Srkalovic G, et al. Randomized trial of cisplatin and etoposide in combination with veliparib or placebo for extensive stage small cell lung cancer: ECOG-ACRIN 2511 study. J Clin Oncol 2017;35(15_suppl):8505.

[127] Liu X, Shi Y, Guan R, Donawho C, Luo Y, Palma J, et al. Potentiation of temozolomide cytotoxicity by poly(ADP)ribose polymerase inhibitor ABT-888 requires a conversion of single-stranded DNA damages to double-stranded DNA breaks. Mol Cancer Res : MCR 2008;6(10):1621–9.

[128] Pietanza MC, Waqar SN, Krug LM, Dowlati A, Hann CL, Chiappori A, 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(23):2386–94.

[129] Farago AF, Drapkin BJ, Charles A, Yeap BY, Heist RS, Azzoli CG, et al. Safety and efficacy of combination olaparib (O) and temozolomide (T) in small cell lung cancer (SCLC). J Clin Oncol 2018;36(15_suppl):8571.

[130] Cardnell RJ, Feng Y, Mukherjee S, Diao L, Tong P, Stewart CA, et al. Activation of the PI3K/mTOR pathway following PARP inhibition in small cell lung cancer. PLoS One 2016;11(4):e0152584.

[131] Sorensen CS, Syljuasen RG, Falck J, Schroeder T, Ronnstrand L, Khanna KK, et al. Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. Cancer Cell 2003;3(3):247–58.

[132] Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H, et al. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 1997;277(5331):1497–501.

[133] Yap TA, Luken MJdM, Carrigan B, Roda D, Papadatos-Pastos D, Lorente D, et al. Abstract PR14: phase I trial of first-in-class ataxia telangiectasia-mutated and Rad3-related (ATR) inhibitor VX-970 as monotherapy (mono) or in combination with carboplatin (CP) in advanced cancer patients (pts) with preliminary evidence of target modulation and antitumor activity. Mol Cancer Ther 2015;14(12 Supplement 2):PR14.

[134] Fokas E, Prevo R, Hammond EM, Brunner TB, McKenna WG, Muschel RJ. Targeting ATR in DNA damage response and cancer therapeutics. Cancer Treat Rev 2014;40(1):109–17.

[135] Hall AB, Newsome D, Wang Y, Boucher DM, Eustace B, Gu Y, et al. Potentiation of tumor responses to DNA damaging therapy by the selective ATR inhibitor VX-970. Oncotarget 2014;5(14):5674–85.

[136] Yap TA, Krebs MG, Postel-Vinay S, Bang YJ, El-Khoueiry A, Abida W, et al. Phase I modular study of AZD6738, a novel oral, potent and selective ataxia telangiectasia Rad3-related (ATR) inhibitor in combination (combo) with carboplatin, olaparib or durvalumab in patients (pts) with advanced cancers. Eur J Cancer 2016;69:S2.

[137] Mir SE, De Witt Hamer PC, Krawczyk PM, Balaj L, Claes A, Niers JM, et al. In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. Cancer Cell 2010;18(3):244–57.

[138] Bauer TM, Jones SF, Greenlees C, Cook C, Mugundu GM, Jewsbury PJ, et al. Abstract CT013: a phase Ib, open-label, multicenter study to assess the safety, tolerability, pharmacokinetics, and antitumor activity of AZD1775 monotherapy in patients with advanced solid tumors: initial findings. Cancer Res 2016;76(14 Supplement):CT013.

[139] Wu C, Morris JR. Genes, genetics, and epigenetics: a correspondence. Science 2001;293(5532):1103–5.

[140] Dupont C, Armant DR, Brenner CA. Epigenetics: definition, mechanisms and clinical perspective. Semin Reprod Med 2009;27(5):351–7.

[141] Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 2008;647(1–2):21–9.

[142] Han Z, Xing X, Hu M, Zhang Y, Liu P, Chai J. Structural basis of EZH2 recognition by EED. Structure (Lond) 2007;15(10):1306–15.

[143] Lynch JT, Harris WJ, Somervaille TC. LSD1 inhibition: a therapeutic strategy in cancer? Expert Opin Ther Targets 2012;16(12):1239–49.

[144] Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, et al. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 2009;41(1):125–9.

[145] Mohammad HP, Smitheman KN, Kamat CD, Soong D, Federowicz KE, Van Aller GS, et al. A DNA hypomethylation signature predicts antitumor activity of LSD1 inhibitors in SCLC. Cancer Cell 2015;28(1):57–69.

[146] Gadgeel SM, Pennell NA, Fidler MJ, Halmos B, Bonomi P, Stevenson J, et al. Phase II study of maintenance pembrolizumab in patients with extensive-stage small cell lung cancer (SCLC). J Thorac Oncol 2018;13(9):1393–9.

[147] Ready N, Owonikoko TK, Postmus PE, Reck M, Peters S, Pieters A, et al. CheckMate 451: a randomized, double-blind, phase III trial of nivolumab (nivo), nivo plus ipilimumab (ipi), or placebo as maintenance therapy in patients (pts) with extensive-stage disease small cell lung cancer (ED-SCLC) after first-line platinum-based doublet chemotherapy (PT-DC). J Clin Oncol 2016;34(15_suppl):TPS8579 [TPS].

[148] Bristol-Myers Squibb. Bristol-myers squibb announces CheckMate -451 study did not meet primary endpoint of

overall survival with opdivo plus yervoy vs. Placebo as A maintenance therapy in patients with extensive-stage small cell lung cancer after completion of first-line platinum-based chemotherapy. 2018. updated 26th November 2018. Available from: https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-announces-checkmate-451-study-did-not-mee.

[149] Bristol-Myers Squibb. Bristol-myers squibb announces phase 3 CheckMate -331 study does not meet primary endpoint of overall survival with opdivo versus chemotherapy in patients with previously treated relapsed small cell lung cancer. 2018. updated 12th October 2018. Available from: https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-announces-phase-3-checkmate-331-study-doe.

[150] Horn L, Reck M, Gettinger SN, Spigel DR, Antonia SJ, Rupnow BA, et al. CheckMate 331: an open-label, randomized phase III trial of nivolumab versus chemotherapy in patients (pts) with relapsed small cell lung cancer (SCLC) after first-line platinum-based chemotherapy (PT-DC). J Clin Oncol 2016;34(15_suppl):TPS8578 [TPS].

[151] Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med 2017;377(25):2500–1.

[152] Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9(1):34.

[153] Hellmann MD, Ciuleanu T-E, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018;378(22):2093–104.

[154] Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 2018;33(5):853–61. e4.

[155] Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N Engl J Med 2016;375(19):1823–33.

[156] FDA. FDA. Grants nivolumab accelerated approval for third-line treatment of metastatic small cell lung cancer. 2018. https://wwwfdagov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm617370htm.

[157] Merritt RE, Mahtabifard A, Yamada RE, Crystal RG, Korst RJ. Cisplatin augments cytotoxic T-lymphocyte–mediated antitumor immunity in poorly immunogenic murine lung cancer. J Thorac Cardiovasc Surg 2003;126(5):1609–17.

[158] Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008;8:59.

[159] Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 2018;379(23):2220–9.

[160] Reck M, Luft A, Szczesna A, Havel L, Kim S-W, Akerley W, et al. Phase III randomized trial of ipilimumab plus etoposide and platinum versus placebo plus etoposide and platinum in extensive-stage small-cell lung cancer. J Clin Oncol 2016;34(31):3740–8.

[161] Cho DC, Mahipal A, Dowlati A, Chow WA, Segal NH, Chung KY, et al. Safety and clinical activity of durvalumab in combination with tremelimumab in extensive disease small-cell lung cancer (ED-SCLC). J Clin Oncol 2018;36(15_suppl):8517.

[162] Casey SC, Baylot V, Felsher DW. The MYC oncogene is a global regulator of the immune response. Blood 2018;131(18):2007.

[163] Kim EY, Kim A, Kim SK, Chang YS. MYC expression correlates with PD-L1 expression in non-small cell lung cancer. Lung Cancer 2017;110:63–7.

[164] Atsaves V, Tsesmetzis N, Chioureas D, Kis L, Leventaki V, Drakos E, et al. PD-L1 is commonly expressed and transcriptionally regulated by STAT3 and MYC in ALK-negative anaplastic large-cell lymphoma. Leukemia 2017;31:1633.

[165] Melaiu O, Mina M, Chierici M, Boldrini R, Jurman G, Romania P, et al. PD-L1 is a therapeutic target of the bromodomain inhibitor JQ1 and, combined with HLA class I, a promising prognostic biomarker in neuroblastoma. Clin Cancer Res 2017;23(15):4462.

[166] Maeda T, Hiraki M, Jin C, Rajabi H, Tagde A, Alam M, et al. MUC1-C induces PD-L1 and immune evasion in triple-negative breast cancer. Cancer Res 2018;78(1):205.

[167] Kortlever RM, Sodir NM, Wilson CH, Burkhart DL, Pellegrinet L, Brown Swigart L, et al. Myc cooperates with ras by programming inflammation and immune suppression. Cell 2017;171(6):1301–15. e14.

[168] Rakhra K, Bachireddy P, Zabuawala T, Zeiser R, Xu L, Kopelman A, et al. CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. Cancer Cell 2010;18(5):485–98.

[169] Topper MJ, Vaz M, Chiappinelli KB, DeStefano Shields CE, Niknafs N, Yen RC, et al. Epigenetic therapy ties MYC depletion to reversing immune evasion and treating lung cancer. Cell 2017;171(6):1284–300. e21.

[170] Bernards R, Dessain SK, Weinberg RA. N-myc amplification causes down-modulation of MHC class I antigen expression in neuroblastoma. Cell 1986;47(5):667–74.

[171] Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, et al. Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol : official journal of the American Society of Clinical Oncology 2012;30(5):525–32.

[172] Huang CH, Wick JA, Sittampalam GS, Nirmalanandhan VS, Ganti AK, Neupane PC, et al. A multicenter pilot study examining the role of circulating tumor cells as a blood-based tumor marker in patients with extensive small-cell lung cancer. Frontiers in oncology 2014;4:271.

[173] Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat Med 2014;20(8):897–903.

[174] Carter L, Rothwell DG, Mesquita B, Smowton C, Leong HS, Fernandez-Gutierrez F, et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. Nat Med 2016;23:114.

[175] Belani CP, Dahlberg SE, Rudin CM, Fleisher M, Chen HX, Takebe N, et al. Vismodegib or cixutumumab in combination with standard chemotherapy for patients with extensive-stage small cell lung cancer: a trial of the ECOG-ACRIN Cancer Research Group (E1508). Cancer 2016;122(15):2371–8.

[176] Pietanza MC, Litvak AM, Varghese AM, Krug LM, Fleisher M, Teitcher JB, et al. A phase I trial of the Hedgehog inhibitor, sonidegib (LDE225), in combination with etoposide and cisplatin for the initial treatment of extensive stage small cell lung cancer. Lung Cancer 2016;99:23–30.

[177] Stewart CM, Tsui DWY. Circulating cell-free DNA for non-invasive cancer management. Cancer genetics 2018;228–229:169–79.

[178] Almodovar K, Iams WT, Meador CB, Zhao Z, York S, Horn L, et al. Longitudinal cell-free DNA analysis in patients with small cell lung cancer reveals dynamic insights into treatment efficacy and disease relapse. J Thorac Oncol 2018;13(1):112–23.

[179] Hou JM, Greystoke A, Lancashire L, Cummings J, Ward T, Board R, et al. Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am J Pathol 2009;175(2):808–16.
[180] Hamilton G, Rath B, Holzer S, Hochmair M. Second-line therapy for small cell lung cancer: exploring the potential role of circulating tumor cells. Transl Lung Cancer Res 2016;5(1):71–7.
[181] Gazdar AF, Hirsch FR, Minna JD. From mice to men and back: an assessment of preclinical model systems for the study of lung cancers. J Thorac Oncol 2016;11(3):287–99.
[182] Scripture C, Selvaggi G, Lakatos I, Boynton K, Lally S, Han TH. A phase 1/2 study on safety of rovalpituzumab tesirine in combination with nivolumab or nivolumab + ipilimumab in small cell lung cancer. Ann Oncol 2017;28(suppl_5):v539–42. https://doi.org/10.1093/annonc/mdx386.
[183] Burris HA, Wang JS-Z, Johnson ML, Falchook GS, Jones SF, Strickland DK, et al. A phase I, open-label, first-time-in-patient dose escalation and expansion study to assess the safety, tolerability, and pharmacokinetics of nanoparticle encapsulated Aurora B kinase inhibitor AZD2811 in patients with advanced solid tumours. J Clin Oncol 2017;35(15_suppl):TPS2608 [TPS].
[184] Forster M, Calvo E, Olmedo Garcia ME, Lopez Criado MP, Moreno V, Soto-Matos A, et al. Lurbinectedin (PM01183) with doxorubicin (DOX), an active treatment as second-line therapy in small cell lung cancer (SCLC). J Clin Oncol 2015; 33(15_suppl):7509.
[185] Forster M, Moreno V, Calvo E, Olmedo ME, Lopez-Criado MP, Lopez-Vilariño J, et al. P1.12-20 overall survival with lurbinectedin plus doxorubicin in relapsed SCLC. Results from an expansion cohort of a phase Ib trial. J Thorac Oncol 2018;13(10):S581.
[186] Farago AF, Paz-Ares LG, Ciuleanu T-E, Fülop A, Navarro A, Bonanno L, et al. ATLANTIS: global, randomized phase III study of lurbinectedin (L) with doxorubicin (DOX) vs. CAV or topotecan (T) in small-cell lung cancer after platinum therapy. J Clin Oncol 2018;36(15_suppl):TPS8587 [TPS].
[187] Chung HC, Lopez-Martin JA, Kao SC-H, Miller WH, Ros W, Gao B, et al. Phase 2 study of pembrolizumab in advanced small-cell lung cancer (SCLC): KEYNOTE-158. J Clin Oncol 2018; 36(15_suppl):8506.
[188] Kim Y, Keam B, Ock C-Y, Song S, Kim M, Kim SH, et al. A phase II study of pembrolizumab and paclitaxel in refractory extensive disease small cell lung cancer. J Clin Oncol 2018; 36(15_suppl):8575.
[189] Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol 2016;17(7):883–95.
[190] Ready N, Farago AF, de Braud F, Atmaca A, Hellmann MD, Schneider JG, et al. Third-line nivolumab monotherapy in recurrent SCLC: CheckMate 032. J Thorac Oncol 2019;14(2): 237–44.