2020

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Recommended Citation

Jane S. Y. Sui, Petra Martin & Steven G. Gray (2020) Pre-clinical models of small cell lung cancer and the validation of therapeutic targets, *Expert Opinion on Therapeutic Targets*, 24:3, 187-204, DOI: 10.1080/14728222.2020.1732353

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To cite this article: Jane S. Y. Sui, Petra Martin & Steven G. Gray (2020) Pre-clinical models of small cell lung cancer and the validation of therapeutic targets, Expert Opinion on Therapeutic Targets, 24:3, 187-204, DOI: [10.1080/14728222.2020.1732353](https://doi.org/10.1080/14728222.2020.1732353)

To link to this article: <https://doi.org/10.1080/14728222.2020.1732353>

Published online: 26 Feb 2020.

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REVIEW

Pre-clinical models of small cell lung cancer and the validation of therapeutic targets

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ABSTRACT

Introduction: Small-cell lung cancer (SCLC) is an aggressive form of lung cancer that has a dismal prognosis. One of the factors hindering therapeutic developments for SCLC is that most SCLC is not surgically resected resulting in a paucity of material for analysis. To address this, significant efforts have been made by investigators to develop pre-clinical models of SCLC allowing for downstream target identification in this difficult to treat cancer.

Areas covered: In this review, we describe the current pre-clinical models that have been developed to interrogate SCLC, and outline the benefits and limitations associated with each. Using examples we show how each has been used to (i) improve our knowledge of this intractable cancer, and (ii) identify and validate potential therapeutic targets that (iii) are currently under development and testing within the clinic.

Expert opinion: The large numbers of preclinical models that have been developed have dramatically improved the ways in which we can examine SCLC and test therapeutic targets/interventions. The newer models are rapidly providing novel avenues for the design and testing of new therapeutics. Despite this many of these models have inherent flaws that limit the possibility of their use for individualized therapy decision-making for SCLC.

ARTICLE HISTORY

Received 30 September 2019
Accepted 17 February 2020

KEYWORDS

Small-cell lung cancer (SCLC); pre-clinical models; heterogeneity; drug resistance; liquid biopsy

1. Introduction

Small-cell lung cancer (SCLC) is an aggressive poorly differentiated neuroendocrine tumor accounts for 13% of all lung cancer cases [1]. It is one of the poorest survival rates of lung cancer, with median overall survival of approximately 10 months. At diagnosis, patients tend to present with widely metastatic disease due to rapid growth and doubling time of tumor proliferation [2–4]. SCLC occurs almost exclusively in smokers especially in heavy smokers [5], and in the Western world, the incidence of SCLC has decreased due to smoking pattern and smoking cessation programs [5].

The first staging system for SCLC was introduced by the Veterans' Administration Lung Study Group (VALSG) [6]. Staging was divided into two disease subgroups termed 'limited-stage' characterized by tumors confined to one radiation portal although local extension into ipsilateral, supraclavicular nodes could be present and 'extensive-stage' for extrathoracic metastases. In 2007, the International Association for the Study of Lung Cancer (IASLC) recommended that the seventh edition of the American Joint Committee on Cancer Staging (AJCC) and the Union Internationale Contre le Cancer (UICC) tumor, node, metastasis (TNM) staging should replace the VALSG staging system [7]. Now superseded by the 8th edition, the IASLC Lung Cancer Staging Project allows an accurate staging of SCLC with TNM, which carries important prognostic outcomes and implications for treatment in SCLC [8].

Whilst this staging system is critical to patient care, other attempts to stratify SCLC into different subgroups based on our molecular understanding of SCLC have been attempted, and were most recently discussed in depth and synthesized into a working nomenclature for SCLC based upon the expression of four key transcriptional regulators [9].

SCLC is highly responsive to cytotoxic chemotherapy in early lines of therapy [9,10]. One third of SCLC presents with early-stage disease which can be cured with surgery or concomitant cytotoxic chemotherapy with radiotherapy [10]. However, the majority of SCLC has short duration of response to therapy due to acquired therapeutic resistance with 5-year survival rates less than 7% [11].

Pre-clinical models with SCLC often show promising therapeutic possibilities. However, most often these fail to translate into clinical benefits in phase II/III clinical trials, and a salutary reminder of this in SCLC has been the discontinuation of Rova-T [12] a drug that showed exciting potential in pre-clinical models of SCLC (discussed in more detail in section 3.2). Over the last four decades, the standard treatment for SCLC has not changed with regimen consistent with platinum-based agent (cisplatin or carboplatin) in combination with etoposide [13]. Most recently, a breakthrough for treatment of SCLC involved the incorporation of the immune checkpoint inhibitor atezolizumab,

Article Highlights

- Small-cell Lung Cancer (SCLC) is an aggressive tumor with poor prognosis, characterized by the development of rapid resistance to chemotherapy.
- As surgical intervention is rare for SCLC, hypothesis testing has been hampered by a lack of available material.
- The development of pre-clinical models has allowed the identification and pre-clinical testing of novel new agents and therapeutic targets.
- Limitations exist for many of these models which preclude testing patients for individualized therapy although advances in this area suggest that this barrier may be overcome.
- Blood-based pre-clinical models may hold the key to this strategy allowing for *ex vivo* expansion of patients' tumor cells allowing for testing of samples for personalized therapy.

This box summarizes key points contained in the article.

targeting programmed cell death ligand-1 (PD-L1), into standard first-line therapy with carboplatin and etoposide for initial treatment for extensive-stage SCLC which showing a median improvement of overall survival by 2 months [14], leading to Food and Drug Administration (FDA) approval of this combination for extensive-disease-small-cell lung cancer (ED-SCLC) in March 2019 [15], but cost-benefit analysis suggests that such a treatment regimen is not cost-effective choice in the first-line setting [16]. However, a more recent analysis of the IMpower133 study with respect to adverse events (AEs) and patient-reported outcomes suggest that a positive benefit-risk profile of first-line atezolizumab plus carboplatin/etoposide in extensive-stage SCLC does exist and further support this regimen as a new standard of care is warranted [17].

Other therapies approved by the FDA for the treatment of SCLC include topotecan (a topoisomerase I poison, approved for the second-line setting with response rate of 24% [15]), while in the third-line setting, nivolumab and pembrolizumab, both programmed cell death-1 (PD-1) inhibitors have been approved [15].

Numerous attempts have been made to identify novel therapeutic targets for the treatment of SCLC.

2. What are the current pre-clinical models?

Several models to interrogate SCLC pre-clinically have been developed and are summarized in Box 1. In the following sections, we describe the current available models and the limitations associated with each.

2.1. Established cell lines

In 1971, the first established SCLC cell line was described [24]. Since then, many SCLC cell lines have been established and earlier estimates suggest that at least 300–400 lung cancer cell lines (encompassing both SCLC & NSCLC) exist [25], and the most commonly used are provided in Table 1

Cell lines established from SCLC tumors have traditionally been seen as a basic research tool, useful for hypothesis and pre-clinical drug testing (Table 2). In general, all established cell lines (including SCLC) have significant issues as regards

Box 1. Brief descriptions of the currently existing SCLC models.

- 1 Established Cell Lines. A cell line is an immortalized permanently established cell culture derived from patients that will proliferate indefinitely given appropriate fresh medium and space [18].
- 2 Primary Cell Lines. Primary cells are taken directly from the tissue and processed to establish them under optimized culture conditions. Some primary cells will spontaneously immortalize at which point they become an established cell line [19]. Others however will not and because they have not been immortalized they will eventually senesce or stop dividing.
- 3 Spheroids. Multicellular spheroids are three-dimensional spherical cellular aggregates that can be generated using either established cell lines or primary cell lines [38].
- 4 Organoids. These are three-dimensional cell clusters with near-native microanatomy that arise from self-organizing mammalian pluripotent or adult stem cells grown *in vitro* [20,21,35].
- 5 Ex Vivo Lung Models. An acellular lung model in which all cells are removed from a rat heart and lung block, and then tumor cells are reintroduced and maintained using perfusion in a bioreactor [44].
- 6 Cell Reprogramming. SCLC like tumors have been generated from either lung progenitor cells derived from human embryonic stem cells, or by forced overexpression of critical transcription factors in primary normal human bronchial epithelial cells.
- 7 Standard Xenografts. There are various models but all involve the injection of human cancer cells into immunocompromised mice, either subcutaneously, orthotopically, or systemically [67].
- 8 Distant Organ Metastasis models. These models often involve orthotopic transplantation of cell lines, which subsequently develop metastases to distant organs [22].
- 9 Syngeneic models. Such models are essentially homografts derived from immortalized mouse cancer cell lines which originated from the same inbred strain of mice. A classic example in lung cancer is the Lewis lung cancer model [23].
- 10 Genetically Engineered Mouse Models (GEMMs). In these models, manipulating the genomes of mice to have genetic alterations that characterize human tumors such as those found in SCLC, *de novo* tumors are formed *in vivo* [89].
- 11 Patient Derived Xenografts (PDXs). In these models resected human tumors are directly engrafted into immune-deficient mice, and subsequently by serial transplantation between mice [89].
- 12 Circulating Tumor cell derived explants (CDX). In these models circulating tumor cells (CTCs) isolated from a patient's blood form tumors when engrafted into immune-deficient mice and like PDXs can be maintained by serial transplantation [98].

their use in pre-clinical models. This problem with established cell lines was exemplified recently by a study demonstrating the significant issue of clonal evolution in breast cancer cell lines during long-term culture, resulting in significant cell line heterogeneity which can result in drastically different drug responses between clones [26,27]. Studies in other tumor types have shown that the epigenetic makeup of established cell lines show a distinct subset of genes which acquire *de novo* DNA methylation in cell lines which is not present in primary tumors [28], which may affect interpretation of cellular responses to new therapeutic agents tested in these cell lines.

SCLC tumor cell lines are no exception and some of the issues that have emerged over the years include the fact that (a) they exhibit genetic instability or drift during long-term passage, and (b) there is lack of interaction with other non-tumor components such as stromal, vascular, or inflammatory cells [25].

While pharmacogenomics profiling involving large collections of cancer cell lines have proven to be mostly reproducible, some discrepancies regarding drug sensitivity emerge [27], as exemplified by the study of Ben-David et al. [27],

Table 1. SCLC cell lines frequently used in pre-clinical studies. Most frequently used SCLC cell lines stratified based on the new proposed molecular subtyping [9] incorporating neuroendocrine features and differential gene expression. This table is derived from data provided in [9,129].

	Neuroendocrine		Non-Neuroendocrine	
Classification	SCLC-A 'Classic'	SCLC-N 'Variant'	SCLC-Y 'YAP1'	SCLC-P 'POU2F3'
Genomic Profile	<i>TP53^{mut}/RB1^{mut}</i>	<i>TP53^{mut}/RB1^{mut}</i>	<i>TP53^{mut}/RB1^{wt-enriched}</i>	<i>TP53^{mut}/RB1^{mut}</i>
Transcriptional Profile	<i>ASCL1^{high}</i> <i>NEUROD1^{low}</i> <i>INSM1^{high}</i> <i>L-MYC^{high}</i> <i>DLL3^{high}</i>	<i>ASCL1^{variable}</i> <i>NEUROD1^{high}</i> <i>INSM1^{high}</i> <i>C-MYC^{high}</i>	<i>ASCL1^{low}</i> <i>NEUROD1^{low}</i> <i>INSM1^{low}</i> <i>YAP1^{high}</i>	<i>ASCL1^{low}</i> <i>NEUROD1^{low}</i> <i>INSM1^{low}</i> <i>POU2F3^{high}</i>
Protein Expression (IHC)	<i>TTF-1^{high}/C-MYC^{low}</i>	<i>TTF-1^{low}/C-MYC^{high}</i>		
Cell Lines associated with each subtype	NCI-H1930 NCI-H1963 COR-L47 NCI-H1436 NCI-H2196 SHP-77 NCI-H1105 NCI-H2029 CORL-51 NCI-H510 COR-L95 NCI-H146 COR-L88 NCI-H660 NCI-H889 NCI-H1618 NCI-H2141 DMS-454 NCI-H1184 NCI-H1876 DMS-153 NCI-H69 COLO-668 NCI-H1836 NCI-H209 DMS-79 NCI-1092 NCI-H2081 DMS-53	CORL-24 NCI-H524 NCI-H1694 DMS-273 HCC-33 NCI-H446 CPC-N NCI-H82 NCI-H2171 SCLC-21 H NCI-H2227 COR-L279 NCI-H2066	NCI-H196 NCI-H2286 NCI-H1341 SW-1271 NCI-H1339 DMS-114 NCI-H841 SBC-5	NCI-H211 NCI-H526 COR-L311 NCI-H1048

which demonstrated that drastically different drug responses can occur between cell line clones. Such a situation has also been observed in SCLC in a recent *in vitro* study using $n = 63$ SCLC cell lines which found that there was no correlation between patient treatment histories and sensitivities to FDA approved SCLC treatments (Etoposide and topotecan) [29].

As such studies involving SCLC cell lines while useful, should take into account the issues raised above and potentially include some of the other pre-clinical models discussed below.

2.2. Primary cell lines

As surgical resection of SCLC is rare, the majority of early information regarding cytogenetics abnormalities in SCLC was derived using short-term culture of primary cells and cell lines [30]. This has now of course been superseded by recent genome sequencing of primary tumors isolated at surgery [31], but is important in the historical context.

One of the advantages of primary cells is that they can be used to derive patient-derived xenografts (discussed in depth in a later section) (Table 2). However, one major limitation of the use of primary cells cultured in dishes is that if you generate and propagate these SCLC primary cells in standard 2D culture (involving standard growth conditions such as serum)

a group of tumor-specific genes that are found to be expressed in both primary SCLC and xenografts is lost during this transition to tissue culture and cannot be regained when the tumors are reestablished as secondary xenografts [32]. More recently, Drapkin et al. [33] determined that short-term cultures may overcome this limitation and found that treatments of short-term cultures (STCs) of primary cells from established PDX models correlated with patient responses to experimental therapy [33,34].

2.3. Moving cell culture from two dimensions (2D) to three dimensions (3D)

One significant issue with the common culture of SCLC cell lines and primary cells is that often they are grown on a flat surface, such as the bottom of a petri dish or flask, and whilst convenient cells in the body do not naturally grow in a 2D fashion. Methods to grow cells in 3D have been developed such as spheroids and organoids (Table 2). The terminology spheroid and organoid are often used interchangeably yet there are distinct differences between them.

At their simplest spheroids can be viewed as consisting of cell aggregates generated from a single cell type or from a multicellular mixture of cells. Organoids on the other are

Table 2. Advantages and Disadvantages of the currently available SCLC pre-clinical models.

Model	Advantages	Disadvantages	Potential Applications
Established Cell Lines [89]	Widely accessible and easy to work with Ample “omic” data available	Numerous cell divisions Variations in culture conditions Homogenous (no intra-tumoral heterogeneity) Genetically unstable Specialized Media/Additives and culture conditions required Cells have limited self-renewal Can change phenotype in culture	Target Discovery Hypothesis Testing Mechanisms of Action High Throughput Screening (HTS) Target Discovery Hypothesis Testing Mechanisms of Action High Throughput Screening
Primary Cell Lines	Relatively easy to establish		
Spheroids [177]	Co-cultures are possible; no external biomaterials are required; ECM is produced by the cells; high numbers of cell–ECM interactions are established; high numbers of cell–cell interactions are established; gradients of gases, nutrients, and pH are present; spheroids can be formed without specific equipment and tools; the majority of the techniques are inexpensive; compatible with HTS.	Optimizations may be required for the formation of uniform spheroids; spheroids may be destroyed during their analysis and manipulation; few protocols and assays are standardized.	
Organoids [167, 178]	Can be developed over several weeks Suitable for HTS Maintained the genetic characteristics of the original tumors	Under-investigated and often poorly validated Often require the use of specialized basement membrane which may affect their use as a tumor model as the influence of this on organoid growth and differentiation is not currently understood. Methods for HTS not well established Low to medium efficiency Difficult to establish Difficulty in controlling nutrient delivery Limited data available	HTS drug screening and predicting patient responses to selected treatment regimens
Ex Vivo Lung Models [45]	Supports the growth of human tumor cells in a natural lung microenvironment Improved cell–cell interactions, cell–ECM interactions and presence of cell populations		Useful to study metastasis
Cell Reprogramming [47–49,166,168] Standard Xenografts	Form primary tumor, CTCs and metastatic lesions within 10–14 days Knowledge of known key alterations in SCLC can be used to force cellular reprogramming Well established Easy to work with	Difficult to achieve Limited data available Model dependent (e.g. flank versus orthotopic) Requires the use of immunodeficient hosts	Can be used on PDXs to generate stable PDX cell lines Target Discovery Hypothesis Testing Mechanisms of Action Studies of metastasis Target Discovery Hypothesis Testing
Distant Organ Metastasis models [57,58]	Well established	Intravenous tail-vein injection of SCLC cells is not a true model of metastasis per se, but merely represents a model of multiple primary tumors An alternative involves orthotopic intra-pleural injection of SCLC	
Syngeneic models	Reproducible Easily Manipulated No host breeding requirements Immunocompetence	Requires Imaging Relatively few transplantable cell lines Lack of heterogeneity Lack of native tumor microenvironment Syngeneic models often have a neo-antigen load significantly higher than found in most human cancers Challenges to breed Low immunogenicity Long latency periods, Mice develop disease at different stages and 100% penetrance is not often achievable Require continuous breeding	Widely Used Model for Immunotherapy Efficacy Studies
Genetically Engineered Mouse Models (GEMMs) [70,98,170]	Native (autochthonous growth) Provides a natural microenvironment De Novo tumorigenesis <i>in vivo</i> Tumor development is driven by the specific engineered genetic mutations/alterations Allows for tumor heterogeneity		Cancer gene validation Analysis of drug resistance mechanisms Tumor Development Studies Identification of Tumor Initiating Cells (TICs) <i>ex vivo</i> culture Studies on tumor heterogeneity Target validation Pharmacological studies
Patient Derived Xenografts (PDXs) [98,170]	Allows for tumor heterogeneity Reproduces tumor complexity	Low implantation rate Cost Requires immune-deficient host Technically challenging Generally injected subcutaneously as opposed to orthotopically	

(Continued)

Table 2. (Continued).

Model	Advantages	Disadvantages	Potential Applications
Circulating Tumor cell derived explants (CDX) [98]	Unlimited source of tumor material Closely resembles patient tumor Allows for tumor heterogeneity	Length of time to establish Lack of immune system in tumor microenvironment	Target Validation Characterization of genomic landscape Pharmacological studies Analysis of drug resistance mechanisms <i>ex vivo</i> culture
Chicken embryo chorioallantoic (CAM) assay [100, 179]	Offers a continuous circulatory system. Fast Low Cost Allows PDX engraftment	Limited time frame to study tumor growth and effects Low experimental burden	Angiogenesis Assays HTS for pre-clinical drug screening

established or derived from stem cells [35], and reflect a very accurate microanatomy to the organ that they represent. There are two critical differences between the two, namely (a) internal developmental processes drive organoid formation, spheroids develop primarily via cell-to-cell adhesion; and (b) long-term culture/expansion of cells requires an immature stem cell population to replenish dying cells. As such, organoids maintain a population of stem cells during *in vitro* culture, which guarantees their long-term viability.

The culture of SCLC as spheroids was originally used with established SCLC cell lines, as they were thought to mimic in some degree SCLC micro-metastases [36]. Since then, a subpopulation of cells with cancer-stem like properties (such as tumorsphere formation) was identified in primary cells isolated from SCLC tumors [37]. It has now been shown that circulating tumor cells (CTCs) isolated from the blood of patients with recurrent SCLC can be cultured as tumorspheres [38,39]. Compared to the same cells grown as single-cell suspensions, these tumorspheres showed enhanced resistance to topotecan and epirubicin [38]. One potential limitation of deriving tumorspheres this way is that the multicellular spheroids formed from CTCs rely on the assumption that they remain largely identical to the original tumors, but this has not been fully validated [38]. Nevertheless, given the fidelity observed for patient-derived xenografts generated using CTCs (called CDXs – please see Section 2.11) it would appear that tumorspheres derived from SCLC CTCs would indeed be similar to the primary tumor.

Tumor organoids represent a novel new technique as a pre-clinical model (Table 2), particularly when primary cells derived from the patient's tumor are used to generate what is called a patient-derived organoid or PDO [40]. PDO models have several advantages over preexisting models, including conserving the molecular and cellular composition of the original tumor [41]. Moreover, there is some evidence that PDOs can better maintain the primary and tumor cell characteristics such as gene stability, heterogeneity, and the pathological features of the primary tumors in long-term culture than established cell lines and PDXs [32,40]. Thus, organoids have advantages over the traditional model and *in vitro* tumor model. These advantages highlight the tremendous potential of tumor organoids in personalized cancer therapy, particularly preclinical drug screening, and predicting patient responses to selected treatment regimens [35,40,41].

Organoids studies of SCLC are not very common. Initial development of organoid cultures from lung cancer were first described in abstract form in 2017, when 13 lung cancer patient tissue organoids were described [42] and included some from SCLC. The organoids were compared with their human tumors histological and genetic profiles. In this regard, the SCLC organoids showed a typical neuroendocrine morphology, and maintained the genetic characteristics of the original tumors including TP53 and RB mutation. Moreover, the cancer organoids showed stronger tumor-forming capacity than direct graft of tumor tissue, and when grown as organoids had resistance to various anticancer treatments [42]. The same group recently published a more detailed protocol for growing SCLC organoids on a microfluidic-based

platform for drug sensitivity testing [43]. In this matrigel-based droplet system, SCLC cultures assemble after 72 h into 3D organoid structures ranging from thin-walled cystic structures to compact spherical masses [43]. Targeted exome sequencing between the organoids and parent tissue showed shared mutations [43]. The potential clinical utility of this system was demonstrated by using these organoids to test drug sensitivity, in this instance using the standard first-line agents' cisplatin and etoposide [43]. This would appear to be the first easy to use, cost-effective, and clinically relevant organoid-based system for direct drug sensitivity testing in SCLC [43].

2.4. *Ex vivo* lung 4D model

Basically, this is an *ex vivo* acellular lung model, created by removing all of the cells from a rat heart and lung block (Table 2). It therefore retains a natural lung matrix maintaining its three-dimensional architecture, including perfusable vascular beds and preserved airways, including the basement membranes of the alveolar septa. When tumor cells are placed into the trachea, they form nodules in the lung matrix [44]. While this technology has been shown to be suitable for studies of SCLC [45], no data currently exists on the use of this pre-clinical model to validate any potential SCLC therapeutic targets.

2.5. Cellular reprogramming/transdifferentiation models

Two recent papers have shown how SCLC can be generated by forced reprogramming of cells. In the past conditions have been established for differentiating human embryonic stem cells (hESCs) into lung progenitor cells [46] (Table 2). Using this methodology, and taking advantage of the current knowledge regarding key proteins in SCLC, Chen et al. [47] found that by inhibiting Notch, RB1, and TP53, they could generate significant numbers of Pulmonary neuroendocrine cells (PNECs), the cells suspected of being the putative precursors to SCLC cells. Furthermore, these PNECs when transplanted into immunocompromised mice formed tumors with SCLC characteristics [47].

Prior to this, Park et al. [48] had used lentivirus-based transformation of normal human bronchial epithelial cells (NHBEs) to generate cells which when xenografted into immunocompromised mice formed tumors with SCLC features. The methodology involved five targets and utilized a dominant-negative TP53 (P); expression of myristoylated AKT to inhibit PTEN (A); knockdown of RB1 (R); and overexpression of c-MYC (C) and Bcl2 (B) (called PARCB) [48,49]. One limitation of this particular methodology is that all 5 genetic manipulations were required in order to achieve SCLC development, whereas it is well established that only subsets of SCLC contain these particular mutations/alterations [48,49].

2.6. Standard xenograft models

The most commonly used tumor xenograft model in cancer research involves subcutaneous flank injection into immunocompromised mice [50], and xenografts of SCLC have been used since the 1980 s [51,52], and continue to be used extensively to the present [53] (Table 2).

2.7. Standard orthotopic models

Some of the first orthotopic models for SCLC were described in the 1990s [54,55]. More recently orthotopic models combining luciferase tags have been developed [56,57]; and in one instance several xenograft models (subcutaneous cell line vs orthotopic cell line vs subcutaneous PDX vs distant organ metastasis) have been interrogated simultaneously [58] (Table 2).

2.8. Distant organ metastasis models

The ability of SCLC cells to leave the primary tumor and establish inoperable metastases is a major cause of death and a serious impediment to successful therapy [13]. As such, distant organ metastasis models of SCLC have been developed to allow for pre-clinical interrogation of the efficacy of drugs in this situation (Table 2). The first models used tail-vein injection leading to metastases to the liver, kidneys, lymph nodes, and bone marrow [59,60]. A follow-up study of several cell SCLC lines found that although all cell lines tested (SBC-5, SBC-3, SBC-3/ADM, H69, H69/VP) formed metastatic nodules in multiple organs (liver, kidney, and lymph nodes), only SBC-5 cells reproducibly developed bone metastases [61–64].

One disadvantage of the methodology used in the previous studies is that that simple use of intravenous tail-vein injection of SCLC cells is not a true model of metastasis per se, but is merely a model of multiple primary tumors. As such, orthotopic implantation of the primary tumor followed by the subsequent development of metastases would be a more relevant pre-clinical model of SCLC. In this regard, distant metastases were observed when orthotopic intra-pleural injection of SCLC was conducted in athymic nude mice [65]. A similar approach was also used by Nomoto and colleagues, where orthotopic implantation of a GFP-tagged (green fluorescent protein) SCLC cell line (DMS273-GFP) into the lung, resulted in distant metastases to bone, brain, and lymph node (metastases common to SCLC patients) [66]. Likewise, Taromi et al. also used intrathoracic injection of H69-Luc-GFP cells to develop a metastatic model of SCLC with metastases to the liver, bone, brain, adrenal glands, and kidney [57]. In a different approach, another distant metastasis model described involving intra-cardiac injection of a luciferase (luc) tagged SCLC cell line (luc-H82) into NSG (NOD-scid gamma) mice also led to metastasis to the liver, ovaries, head, and bone [58].

2.9. Syngeneic models

Syngeneic murine models entail the injection of immunologically compatible cancer cells into immunocompetent mice (Table 2). The availability of syngeneic models to study lung cancer is very limited, and the best known in lung cancer is the Lewis lung carcinoma (LLC) model [67]. To our knowledge, there have been very few reports of syngeneic SCLC mouse models. The earliest was developed using HPV-E6/E7 transgenic mice, where two mouse SCLC cell lines PPAP-9 and PPAP 10, reform tumors when injected into syngeneic mice [68]. More recently a syngeneic model of SCLC in genetically

engineered mice (GEMM) was described [69] (and are discussed in greater detail in the next section)

Early experiments sacrificed the animals after a set period (usually 5 weeks post inoculation) and used macroscopic dissection or X-Ray radiography to detect overt metastases.

Subsequently, most studies have utilized either GFP-tagged or Luciferase-tagged SCLC cells to monitor for the presence of metastases. Such studies use either Bioluminescence imaging (BLI) or optical tomography to assess for metastases. Studies such as those by Herbst and colleagues [65] have attempted to determine if these manipulations affect the growth, metastatic spread, and testing the efficacy of chemotherapeutic agents of these SCLC metastases models. Their conclusions were that such models had close correlation between the existing clinical data suggesting that these models could be predictive of results in the clinical setting, and that the manipulations used to generate and monitor the models did not affect the results in any meaningful way [65].

2.10. Genetically Engineered Mice Models (GEMM)

GEMMs are non-patient-derived cancer models often achieved by manipulating one or more genes (Table 2). Until recently, progress in this area was limited, but more recent technological developments have led to advanced mouse models that closely recapitulate the human cancer in terms of genetic composition, interactions with the tumor microenvironment, metastasis, drug response, and drug resistance [70]. With the establishment that loss of *Rb* and *p53* are almost universally inactivated in SCLC [9], the first GEMM for SCLC was derived using a strategy to inactivate both genes and established in 2003 [71]. Two additional models were then developed incorporating triple-knockouts, the first involving *Rb/p53* and *p130* [9,72,73], the second involving knockout of *Pten* [73,74].

The *Rb/p53/p130* knockout GEMM (also known as the *TCKO* model) has been used to further investigate the roles that two other transcription factors associated with SCLC (*NEUROD1* and *ASCL1*) play in this cancer revealing discrete genomic landscapes and gene expression programs that underpin the heterogeneity seen in SCLC [75].

GEMM models have been used to show that *MYC* family members are key driver gene in SCLC. The first described was a model derived by Anton Berns group using re-derived embryonic stem cells (GEMM-ESCs) in the *Rb/p53* GEMM [76].

Having identified that *MYC-L* is an important element in SCLC, studies using the *TCKO* model revealed a population of long-term tumor-propagating cells (TPCs) with high expression of *EpCAM* and *CD24*, and elevated *MYC* activity which could be targeted using Bromo- and Extra- Terminal domain (BET) inhibitors [77]. Other studies have also used an *Rb/p53/Pten* knockout GEMM, to conduct long-time longitudinal study of CTCs in animals undergoing over 4 days of treatment with a BET inhibitor [78]. Support for the critical role-played by *MYC* family members and in particular *Mycl* has come from other GEMM derived *Mycl* models such as the *pRb^{lox/lox}, p53^{lox/lox}, p130^{lox/lox}, Mycl^{lox/lox}* derived by Kim et al. [79], and the *pRb^{fl/fl}, p53^{fl/fl}, MycT58A^{LSL/LSL}* strain [80,81].

The importance of these models is that their use has identified novel candidate targets such as Aurora Kinase [80], and that Mycl-driven tumors are susceptible to arginine depletion [53].

Another transcription factor commonly altered in SCLC is NFIB. Originally identified in an analysis of the *pRb^{fl/fl}*, *p53^{fl/fl}* model by Tyler Jacks and colleagues [82], subsequent studies in a *pRb^{fl/fl}*, *p53^{fl/fl}* *p130^{+/+}* GEMM demonstrated that NFIB promotes metastasis via changes in the chromatin landscape of these tumors [83]. GEMMs designed to overexpress NFIB have been developed confirming the oncogenic role of NFIB in SCLC [84,85].

Most recently, a GEMM involving *Crebbp* deletion in the *pRb/p53* background was shown to be a potent tumor suppressor in SCLC, and inactivation of CREBBP enhances responses to histone deacetylase inhibitors [86], a finding validated in SCLC cells, where CREBBP-mutated but not wild-type cells showed significantly lower IC50 values after treatment with a histone deacetylase inhibitor (HDACi) [87].

One of the advantages associated with GEMM models is that they can closely mimic the tumoral heterogeneity that is often observed in SCLC. In this regard, this can lead to the identification of novel therapeutic avenues such as the identification of the potential to target MYC overexpressing SCLC with either BET inhibitors [77], Aurora kinase inhibitors [80], or through arginine depletion [53]. In addition, these models have also been used to examine the differential sensitivity SCLC has to cisplatin [88].

A major limitation of GEMMs in particular for germline GEMMs is that the development of these models is 'time consuming, laborious, and expensive' [70]. Another disadvantage lies in the fact that in order to maintain these expensive models they require continuous breeding. This comes with an associated risk for genomic diversification or genomic evolution. It has been estimated that on the basis of spontaneous mutation rates, in the wild-type setting 0.96 deleterious germline mutations should arise each generation. This number will obviously be much higher in genomically unstable mice, and as colony maintenance of GEMMs relies on inbreeding, it is estimated that there is a 25% chance for a new mutation to consequently become homozygous and thus fixed in the population [89].

2.11. Patient-derived xenografts

PDX models from resected SCLC have been well established. In these models, tumors are directly engrafted into immune-deficient mice, and subsequently by serial transplantation between mice [89] (Table 2). The importance of these models is that they show both genomic and functional fidelity to the original tumors particularly with respect to sensitivity to standard therapy regimens such as etoposide and platinum (EP) [33] and to identify biomarkers predictive of chemoresistance [90]. In the study by Drapkin et al., upregulation of an MYC expression signature was associated with resistance to EP [33]. Moreover, such models have been used in a Phase II clinical trial setting in SCLC [91].

In addition to PDX models, CTC derived xenografts called CDX have been established [92]. These models have been shown to closely resemble the donor's original tumor, and mirror patient response to therapy (Table 2). Moreover, the ability to derive CDX models from blood samples opens up

the approach to allowing the generation of serial longitudinal models from the same patient throughout the course of their treatment [34,93]. Such a strategy has been successfully used by Drapkin et al., to recapitulate the evolving drug sensitivities in a patient [33], and also to identify novel potential therapeutic approaches in both chemo-naïve and chemo-refractory SCLC patients [93–95].

Given the current potential for the use of CDXs and PDXs in SCLC therapy in the clinical setting [91], how might this impact or affect our attempts to follow the 3 R initiative? Proposed 60 years ago, this initiative attempts to Replace, Reduce, and Refine the way that animals are used in research [96]. In this regard, the suggestion that for SCLC, the one mouse, one patient paradigm is worth exploring. First proposed by Malaney et al. [97], this model envisages the implantation of patient tumor samples in mice for subsequent use in drug efficacy studies. This would then allow for identification of a personalized therapeutic regimen for each patient, eliminating the cost and toxicity associated with non-targeted chemotherapeutic measures [97]. This would appear to be ideal for SCLC, yet one of the problems that may prevent this in a real-life setting is that often 'the time frame for the generation of these models often exceeds the life span of the donor patient making the "one mouse, one patient" paradigm incompatible for SCLC' [98]. In this regard, an alternative may therefore be the short-term culture of CTCs for ex vivo analyses of clinical therapeutic responses [99]. Such a strategy has recently been demonstrated by Caroline Dive and colleagues, where short-term ex vivo culture of CDX cells was utilized to develop a platform capable of screening for novel treatments [34]. Because of the limited utility of CDX models for direct patient therapy assessments, the direct culture of CTCs is being evaluated for this purpose [34]. However, the technical issue of the low numbers of CTCs in a blood draw has yet to be resolved. Moreover, such a strategy may skew toward patients with a high burden of disease.

2.12. Chicken embryo chorioallantoic (CAM) assay

The chick chorioallantoic membrane (CAM) assay has been suggested as a cost-effective versatile platform to conduct rapid PDX preclinical studies [100], with the potential to test multiple targeted therapies within 5–10 days of engraftment (Table 2). Successful engraftment of patient tumor tissue has been reported for several tumor types with take rates of up to 100% reported in some instances [100]. Very early initial studies have shown that lung cancer tissues can be successfully implanted in the CAM assay [101,102]. The tissues transplanted were described as either lung undifferentiated carcinoma [101], or squamous cell carcinoma [102]. Whilst to our knowledge no PDX studies of SCLC in the CAM assay have currently been described, there has been one SCLC study examining angiogenesis utilizing the NCI-H446 cell line [103]. This suggests that the CAM assay may therefore have utility within the SCLC setting particularly regarding rapid analysis of PDX-based studies.

3. Validation of therapeutic targets

It has been discussed in the previous sections novel potential therapies have been identified from the development of new preclinical models of SCLC. In the following sections, we will discuss how pre-clinical models have helped to identify some of these new therapeutic vistas for the treatment of SCLC.

3.1. MYC

Of the several recurrent genetic aberrations identified in SCLC, the MYC family genes (MYC, MYCL, and MYCN) have emerged as oncogenic drivers that may constitute novel therapeutically tractable targets [104]. Using the pre-clinical models discussed above various studies have identified that alterations to the MYC family may render SCLC sensitive to either Aurora Kinase inhibitors [104,105], or to BET inhibitors [106–110] (Figure 1, Table 3). Subgroup analysis of c-MYC by IHC in archival tumor biopsies from a Phase II trial (NCT02038647) of the Aurora Kinase inhibitor Alisertib ± paclitaxel in SCLC suggests that tumors with high c-MYC expression may indeed be susceptible to this compound [111], but caution is indicated as the number of samples in this subgroup analysis was restricted to $n = 33$ and further studies are therefore warranted (Table 3). In a more recent development, a Crispr-based approach in

SCLC cell lines and xenografts has identified that loss of *pRb* in SCLC renders them hyperdependent on Aurora B kinase, and as such amenable to Aurora B kinase-specific inhibitors [112]. In the same issue, Buchanan and colleagues identified a synthetic lethal interaction with RB1 and Aurora Kinase A [113]. Identifying which patients will respond to Aurora Kinase inhibitors is therefore becoming increasingly important. In this regard, a proteomic-based approach of SCLC identified two major subgroups characterized as either high TTF-1/low cMYC, or low TTF-1/high cMYC. This low TTF-1/high cMYC subgroup was confirmed as being predictive of responsiveness to Aurora Kinase inhibitors [114] (Table 3).

3.2. NOTCH/DLL3

The comprehensive profiling of tumors by George *et al.* identified the Notch pathway as being significantly affected in SCLC [31]. DLL3 is a Notch inhibitory ligand whose expression is found in ~85% of SCLCs, with minimal to absent surface expression in normal lungs and has led to the development of several DLL3 targeting approaches [115] (Figure 1, Table 3). Studies in preclinical models up to and including PDXs all suggest that DLL3 could be both an excellent biomarker and therapeutic target in SCLC [58,116–118]. However, in a recent Phase II study of one of these agents Rova-T (Rovalpituzumab

Figure 1. Targets currently under investigation in SCLC.

Table 3. Therapeutic targets under evaluation in SCLC as discussed in this review.

Target	Therapy(ies) under evaluation	Mechanism of Action	Preclinical Model data	Comments
MYC (MYC, MYCL and MYCN)	Aurora Kinase inhibitors BET inhibitors	cell cycle-regulated serine/threonine Kinases important for mitosis Inhibition of KDMs leads to decreased expression of MYC resulting in enhanced sensitivity to Aurora Kinase inhibitors	[104,105, 106–110]	Phase II trial suggests that high c-MYC is associated with sensitivity to Alisertib [111] Low TTF-1 and high cMYC expression is predictive of responsiveness to Aurora Kinase inhibitors [114] DLL3 is a Notch inhibitory ligand whose expression is found in ~85% of SCLCs [115] Failure of Rova-T in Clinical trials has led to discontinuation of development [12] IMpower133 study of Atezolizumab (an anti-PD-L1 therapy) approved by the FDA for first-line therapy [14]
DLL-3 NOTCH	ADC (Rova-T) LSD1 inhibitors	releases Taserine a compound that upon binding to DNA prevents replication Disrupts NOTCH-ASCL1 signaling	[58,116–118, 122]	
Checkpoint inhibitors	anti-PD1 anti-PDL1 anti-CTLA4	reestablishment of anti-tumor immune responses	anti-PD-L1 [180]	
SLFN11	EZH2 inhibitors PARP inhibitors	sensitizes SCLC to standard cytotoxic therapies prevents cancer cells treated with cytotoxic or DNA damaging agents from being repaired	[133 93,135]	SLFN11 may be a potential biomarker used to stratify SCLC for PARP or EZH2 inhibition [136]
CD56 (NCAM1)	ADC Lorvotuzumab Promiximab CAR-T	Releases either tubulin binding compounds (DM1 or Aurostatin E) which bind to tubulin and inhibit mitosis or Duocarmycin which binds to DNA and prevents cells replicating CD56 R-CAR+ T cells release antitumor cytokines upon co-culture with CD56(+) tumor targets	[139 141,142, 143]	Phase I/II clinical trial of Lorvotuzumab ADC in SCLC was not associated with any favorable responses [140]
TROP2	ADC Sacituzumab govitecan	Releases SN-38 (a 1000 fold more active metabolite of irinotecan) to inhibit both DNA replication and transcription		Phase II clinical trial of (n = 53) patients with metastatic SCLC, showed a safe and effective therapeutic profile, with an ORR of 14%, and median OS of 7.5 months [152]

Teserine) (Figure 1), $n = 339$ patients were assessed for DLL3 by IHC, and DLL3-high and DLL3-positive tumors were seen in 238 (70%) and 287 (85%) patients, respectively [119]. Unfortunately, the results of this trial were mostly unpromising with modest clinical activity that was associated with grade 3–5 adverse events seen in 213 (63%) patients [119]. A second Phase III trial comparing this agent with Topotecan (TAHOE) for the second-line treatment of patients with high DLL3 ($\geq 75\%$) extensive-stage SCLC was put on hold as shorter overall survival (OS) was reported for the Rovalpituzumab arm compared with the control arm of topotecan therapy. A further phase III study which was evaluating Rova-T as a first-line maintenance therapy for advanced SCLC, compared to placebo (MERU) also recently failed, as interim data from the study demonstrated no survival benefit for patients treated with Rova-T. On the basis of all these negative trial data Rova-T development has subsequently been discontinued [12].

DLL3 expression was also recently assessed in a CTC-driven biomarker study of SCLC patient responses to etoposide/platinum [120]. Remarkably it emerged that in this study of $n = 108$ treatment-naïve patients, baseline samples were taken from all patients, after one chemotherapy cycle ($n = 68$ patients; post-first cycle sample) and at the time of disease progression, before the initiation of second-line treatment ($n = 48$ patients; disease progression sample). The expression of DLL3, cytokeratins (CK), CD45, and vimentin (Vim) was characterized on the isolated CTCs from these samples. The most important finding was that prior to treatment, 74.1% of patients had detectable DLL3⁺/CD45⁺ CTCs. One-treatment cycle significantly decreased both the detection rate ($p < 0.001$) and the absolute number ($p < 0.001$) of the DLL3⁺/CD45⁺ CTCs and were associated with (a) significantly decreased progression-free survival at baseline and (b) with significantly decreased overall survival on disease progression [120]. Despite the apparent set-backs with DLL3 targeting agents, a recent study using cell lines and patient PDX models identified an LSD1-NOTCH-ASCL1 axis that was sensitive to LSD1 inhibitors [121] (Figure 1, Table 3), confirming an earlier study in SCLC cell lines and xenografts that had identified LSD1 as a therapeutic target in SCLC [122]. As such, the use of DLL3 as a biomarker may allow the stratification of patients into trials involving LSD1/NOTCH inhibitors moving forward (Table 3).

3.3. Onco-immunotherapy (checkpoint inhibitors)

At some point, tumors manage to evade our immune system often by expressing signals that inhibit the anti-tumor immune response [123]. The scientific turning point for cancer immunotherapy came with the understanding that T cell immune responses are controlled through on and off switches, so-called immune checkpoints that protect the body from possibly damaging immune responses' [124]. Blockade of these checkpoints has emerged as a new paradigm for the treatment of cancer, including SCLC [125]. The most advanced inhibitors target the following checkpoints: CTLA4 (Cytotoxic T Lymphocyte-Associated 4); PD-1 (programmed death 1 receptor) and its ligand programmed death-ligand 1 (PD-L1) (Figure 1, Table 3).

As previously mentioned in the introduction, the results of the IMpower133 study of Atezolizumab (an anti-PD-L1 therapy) have been approved by the FDA for first-line therapy in extensive-stage SCLC [14]. Similar encouraging results have also been observed in the first-line setting for the anti-PD-1 checkpoint inhibitor Durvalumab as part of the CASPIAN Phase III trial evaluating the efficacy of this agent in combination with platinum-etoposide, with or without tremelimumab (an anti-CTLA4) in treatment-naïve extensive-stage SCLC [126].

Checkpoint inhibitors are also showing promise within the second-line setting that has come from data arising from the Phase Ib KEYNOTE-028 basket trial of Pembrolizumab (anti-PD1), which demonstrated a tolerable safety profile with an OS of 9.8 months at median follow-up [127]. More recently, a pooled analysis of two trials KEYNOTE-028 and KEYNOTE-158 of patients with previously treated recurrent/metastatic small-cell lung cancer (SCLC) treated with two-or-more therapies found that the median duration of response was not reached (range, 4.1–35.8+ months), and that 61% of responders had responses lasting ≥ 18 months [128], which supports the potential use of pembrolizumab within the third-line or salvage therapy setting, and has resulted in a priority review designation by the FDA for SCLC patients following progression after ≥ 2 prior lines of therapy [129].

It must be noted that other trials of checkpoint inhibitors in SCLC have not had the same success rate as the ones described above, and the potential confounding factors have been comprehensively reviewed recently by Reguart et al. [129]. For example, compared with non-small cell lung cancer (NSCLC), levels of PD-L1 are generally low in SCLC [15]. In a recent development, DNA-damaging agents have now been shown to enhance the expression of the checkpoint inhibitor target PD-L1 and enhance anti-tumorigenic CD8 + cytotoxic T-cells, dendritic cells, and M1 macrophage populations in an SCLC model suggesting novel new therapeutic approach and regimens to treat SCLC [130,131].

3.4. SLFN11 and the DNA damage response

High expression of SLFN11 has been linked with the response to DNA-damage-inducing chemotherapies in many cancers [2,111].

An in-depth DNA methylation analysis of SCLC identified that dense clustering of high-level methylation occurred in CpG islands which was correlated with high expression of the histone methyltransferase gene EZH2. Moreover, pharmacological inhibition of EZH2 in a patient PDX was found to inhibit tumor growth [132]. Building on this observation, Poirier and colleagues then used paired SCLC chemo-naïve and chemo-resistant PDX models to identify that in the resistant tumors, EZH2 had epigenetically downregulated SLFN11 [133] (Figure 1). Combining standard cytotoxic therapies with an EZH2 inhibitor subsequently prevented both the emergence of acquired resistance and augmented chemotherapeutic efficacy in both the chemo-sensitive and chemo-resistant models of SCLC [133] (Table 3). Expression of SLFN11 has since been shown to correlate with response to poly ADP ribose polymerase (PARP) inhibitors such as Talazoparib, and IHC expression levels of SLFN11 could be used as a predictor of Talazoparib response [134]. Additional studies of PARP inhibitors using preclinical PDX and CDX models have

confirmed their potential utility in combination with either radiotherapy [135], or via WEE1 inhibitors [93] (Table 3).

In this regard, in a Phase II clinical trial of SCLC which examined the combination of the alkylating agent Temozolomide in combination with the PARP inhibitor Veliparib found that no significant difference in PFS was observed in unstratified patients, but ORR was significantly higher in patients receiving TMZ/veliparib compared with TMZ/placebo (39% v 14%; $P = .016$). Critically, however, if patients were examined for SLFN11 expression, significantly prolonged PFS (5.7 v 3.6 months; $P = .009$) and OS (12.2 v 7.5 months; $P = .014$) were observed in patients treated with TMZ/veliparib who had SLFN11-positive tumors [136]. These results suggest that SLFN11 could potentially be used to stratify SCLC into appropriate treatment arms for therapy with either PARPi or epigenetic targeting using EZH2 inhibition (Table 3).

3.5. CD56

CD56 (also known as neural cell adhesion molecule 1, NCAM1) is a membrane glycoprotein that was initially shown to be useful in the diagnosis of SCLC [137,138]. More recently, however, both antibody–drug conjugates (ADC) and chimeric antigen T cell (CAR-T) approach to target CD56 have been assessed in SCLC (Table 3, Figure 1).

The first agent developed to target CD56 was Lorvotuzumab mertansine an ADC comprising an anti-CD56 antibody linked via a cleavable disulfide linker to the tubulin-binding maytansinoid DM1 (Figure 1), which showed good pre-clinical activity in animal models of SCLC [139]. However, a Phase I/II clinical trial in combination with carboplatin/etoposide for patients with extensive-stage SCLC was associated with only modest improvements in patient tumor responses, but with significant additional toxicities most notably a higher incidence of serious infections with fatal outcomes, and it was recommended that this combination should not be considered for further development [140] (Table 3). Two additional ADCs have been developed utilizing the anti-CD56 antibody Promiximab coupled either with Duocarmycin (a DNA alkylating agent) [141], or Monomethyl auristatin E (a highly toxic antimitotic drug) [142] (Figure 1), both of which showed pre-clinical activity in animal xenograft models (Table 3). At present, there do not appear to be any clinical trials running for either of these ADCs in SCLC.

A CAR-T approach has recently been reported in which the engineered CD56 R-CAR+ T cells were found to be capable of SCLC tumor cells in *in vitro* co-cultures and inhibit tumor growth *in vivo* when tested against CD56+ human xenograft models [143] (Figure 1, Table 3).

3.6. Trop-2

Trop-2, also known as epithelial glycoprotein-1, gastrointestinal antigen 733–1, membrane component surface marker-1, and tumor-associated calcium signal transducer-2 is a transmembrane glycoprotein that is generally upregulated across all tumors (including SCLC) compared to normal cells [144], and is essential for anchorage-independent cell growth and tumorigenesis [145,146]. Subsequently,

antibodies targeting Trop-2 were been developed and subsequently Trop-2-targeted ADC (Figure 1). Of these, the most studied is sacituzumab govitecan (IMMU-132) an ADC targeting Trop-2 coupled with SN-38 (a 1000 fold more active metabolite of irinotecan) [147–149] (Table 3). It has recently achieved FDA breakthrough therapy status for the treatment of metastatic triple-negative breast cancer (mTNBC) [150,151]. Sacituzumab govitecan has also been examined in a Phase II clinical trial of ($n = 53$) patients with metastatic SCLC, showing a safe and effective therapeutic profile, and the ORR was 14%, with a median OS of 7.5 months [152] (Table 3), and it will interesting to see if future studies of this agent in SCLC patients continue to show clinical benefit.

4. Conclusion

We have come a long way in the development of pre-clinical models in SCLC. A major barrier to the lack of progress in SCLC continues to be an incomplete understanding of the heterogeneity displayed by SCLC patients' tumors, and the absence of biomarkers that could guide selection of personalized therapeutic strategies [114]. Despite this, whilst identification of novel biomarkers and candidate therapeutic targets have indeed been identified, the resulting clinical trials of these agents have not yet made any major breakthroughs in treatment options for patients with SCLC. Continued development of the novel pre-clinical models discussed in the previous sections coupled with new syntheses based on large-scale re-analysis of SCLC data will provide new vistas and avenues of patient stratification which will ultimately result in definite breakthroughs for therapy [9,99,111,153].

5. Expert opinion

Huge strides have been made in the past two decades in our understanding of SCLC based in no small part on the development of the pre-clinical models discussed in the previous sections. Despite this, the treatment options available to SCLC patients remain limited, and overall survival remains dismal.

Moving forward, how can we improve on this? Are there any alternatives that we could use? For example, do we need pre-clinical models? In a recent analysis of RNA-Seq data from small-cell neuroendocrine cancers (SCNCs) of which SCLC belongs, it was found that (a) SCNCs have a strongly convergent expression signature; and (b) As these tumors progress from adenocarcinoma to SCNCs the tumors become increasingly independent of their tissue of origin, becoming more similar to each other than to adenocarcinomas of different tissues [154]. Moreover, from a drug screening perspective, it was subsequently shown that SCNCs demonstrated common sensitivity profiles that overlap with hematological cancers, which may allow or guide treatment options beyond tissue-specific-targeted therapies [154].

Given the difficulties that researchers face regarding the development and heterogeneity seen in SCLC, can we correctly mimic SCLC? Rather than developing even more GEMMs or PDX/CDX models is there an opportunity to take the new knowledge gained from these and instead develop integrated

cancer tissue engineering models of SCLC to enable precision medicine treatments [155].

Synthetic lethality describes a situation whereby for two genes that interact, disruption of either gene alone is viable but when both genes are simultaneously disrupted the net result is a loss of cell viability [156]. Synthetic lethality screening aims to identify novel therapeutic targets and increased genetic interaction networks for targeting cancer [157]. Recently a synthetic lethal screen in SCLC identified that HDAC6 and its corresponding inhibitor ricolinostat elicited synergistic effects with BET inhibitors in SCLC [158]. In addition, it was found that Natural Killer (NK) cells are critical to this response, suggesting that these innate immune lymphoid cells play a role in SCLC tumor treatment response [158], and the suggestion that immunoepigenetic combination therapies may become a new paradigm for the treatment of cancer including SCLC [159]. Critically the first major breakthrough in the treatment of SCLC would appear to be in the oncoimmunology setting where checkpoint inhibitors have been shown to have clinical benefit and may yet be approved in the first-line setting in combination with chemotherapy [14,129]. From analyses, it would appear that Tumor Mutational Burden (TMB) may have the ability to predict patient response for checkpoint inhibitors [160,161]. Intriguingly, neoantigen-directed immune escape in NSCLC was recently shown to involve an epigenetic mechanism (hypermethylation of genes that contain neoantigenic mutations) [162], suggesting that immunoepigenetic strategies may become important moving forward in both the selection and treatment of patients with SCLC.

Novel technologies such as clustered regularly interspaced short palindromic repeats (CRISPR) based screening strategies for synthetic lethality are now beginning to identify new subsets of tumors sensitive to targeting agents such as the approach used by Oser et al. [112] to identify that SCLC with loss of Rb1 are hyperdependent on Aurora Kinase B. In a similar strategy, using a CRISPR-based activation model, Sos and colleagues demonstrated that MYC (but not MYCL or MYCN) repressed BCL2 transcription via interaction with MIZ1 and DNMT3a [163]. As a consequence of this loss of BCL2, cells were found to have elevated apoptotic priming, intrinsic genotoxic stress, and susceptibility to DNA damage checkpoint inhibitors. Moreover, a combination of combined AURK and CHK1 inhibition substantially prolonged the survival of mice bearing MYC-driven SCLC beyond that of combination chemotherapy, confirming the potential role of MYC as a candidate that may allow for genotype-based selection of targeted therapeutics in SCLC [163].

Can we identify better biomarkers to predict patients who will respond to novel therapeutics? One emerging biomarker that may be useful to assist in stratification may be to include TTF immunohistochemistry in SCLC patient workup along with actionable targets such as DLL3 and c-MYC which may aid in the correct stratification of patients for treatment [114,164]. Moreover, low-TTF expression may also indicate sensitivity to additional targets such as DNA Damage Repair agents, PLK inhibitors, etc. [114]. Cells that were TTF-high were found to be sensitive to Bcl-2 inhibitors which have been shown to have activity in pre-clinical PDX models of SCLC, when combined with rapamycin [165]. High TTF expression has also

been shown to be a potential surrogate marker for DLL3 expression, and could potentially be used to additionally stratify patients with DLL3-positive tumors [114], and this observation was recently validated in a separate cohort of SCLC [164].

One of the significant barriers to individualized therapy for SCLC remains the lack of biological material to allow for detailed analysis. In this regard, the length of time for developing PDX or CDX models is a rate-limiting step. The emergence of *ex vivo* cultures of CTCs may prove to be a critical element in the treatment paradigm of SCLC [99], and the development of new technologies such as microfluidic chip-based growth of patient organoids [43] may allow for a more nuanced analysis of patient response to therapy and/or identification of individualized personalized therapeutic treatment regimens. Another potential area where advances may occur involves the use of conditional reprogramming to generate stable PDX derived cell lines [166] suitable for organoid development and the establishment of patient-derived organoid biobanks [167,168].

What therefore would be the ideal model for SCLC therapy testing or is there one? Despite being described as small-cell, one size does not fit all in the case of this difficult to treat cancer. As discussed in previous sections the development of PDX or CDX models in mice is costly and time-consuming and does not make them easily accessible to personalized patient therapeutic testing. In this regard, it may be useful to develop organoid-based testing strategies using CTCs or alternatively, CAM assay strategies could be considered as an alternative for personalized approaches to therapy.

Given the potential breakthroughs emerging for the use of onco-immunological targeting of SCLC, the current pre-clinical models (including PDXs and CDXs) have limitations. In this instance, pre-clinical models that involve humanized mice that recapitulate the human immune system may yet prove to be an essential development to come [169–171]. In this regard, such models have been used to test onco-immunological agents such as checkpoint inhibitors in NSCLC [172–174], but we are currently unaware of any such studies in SCLC. However, whilst such a strategy looks promising it is not without limitations as the immune system generated in these models is not the same as the cancer patients (that is, whilst the PDX/CDX itself may be similar to the cancer patient, unless the humanized mouse system utilizes PBMCs from the patient, then the immune component will not truly match that of the patient). Nevertheless, we look forward to seeing studies in SCLC utilizing such approaches for pre-clinical analysis of onco-immunological therapies to treat this cancer.

Blood-based analysis of SCLC may yet prove to be critical to allow clinicians treat patients with SCLC. Recent analyses of circulating free DNA (cfDNA) longitudinally in patients with SCLC [175] raises the possibility that both strategies (cfDNA and isolation of CTCs for *ex vivo* expansion and testing) could be combined to enhance the treatment options for patients with SCLC. Moreover, the recent development of a blood-based methodology to assess TMB in NSCLC (bTMB) [176] suggests that the age of the so-called liquid biopsy is about to emerge in our treatment of SCLC.

Funding

This paper was not funded.

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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