

Fast, hungry and unstable: finding the Achilles' heel of small-cell lung cancer

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Over 95% of patients with small-cell lung cancer (SCLC) die within five years of diagnosis. The standard of care and the dismal prognosis for this disease have not changed significantly over the past 25 years. Some of the characteristics of SCLC that have defined it as a particularly virulent form of cancer – rapid proliferation, excessive metabolic and angiogenic dependence, apoptotic imbalance and genetic instability – are now being pursued as tumor-specific targets for intervention both in preclinical and early phase clinical studies. Here, we summarize areas of ongoing anti-cancer drug development, including classes of agents that target essential pathways regulating proliferation, angiogenesis, apoptotic resistance, chromosomal and protein stability, and cell–cell and cell–matrix interaction.

Introduction: new molecular targets in a refractory disease

It is estimated that lung cancer accounts for over 170 000 new diagnoses and over 160 000 deaths in 2006, making it the leading cause of cancer-related deaths in USA [1]. Small-cell lung cancer (SCLC) represents 15–20% of lung cancer cases [2]. Clinically, SCLC is highly aggressive with a rapid doubling time and early metastatic behavior; the majority of SCLC patients present with advanced disease. Newly diagnosed SCLC is typically sensitive to cytotoxic chemotherapy with reported response rates of up to 80%. Most patients relapse, however, and eventually die with chemotherapy resistant disease. With a median survival of 18 months and ten months for limited-stage (LS) and extensive-stage (ES) disease, respectively, there have only been incremental changes in the prognosis for patients with SCLC over the past 20 years.

Current recommendations for the treatment of LS SCLC, defined as a disease that is confined within the ipsilateral hemithorax that can be encompassed within a single radiation port, includes combination chemotherapy (typically etoposide and a platinum agent) and external beam radiation. A subset of LS SCLC patients with very early stage disease might also benefit from surgical resection. The recommended treatment for ES SCLC, defined as a disease that extends beyond the ipsilateral hemithorax or that cannot be encompassed within a single radiation port, is doublet chemotherapy consisting of a platinum agent and either etoposide or irinotecan, or triple therapy

with cyclophosphamide, doxorubicin and etoposide (or vincristine) [3]. For recurrent disease, recommended therapy includes topotecan monotherapy, which yields response rates of 20–30%.

Although various combinations of cytotoxic agents continue to be investigated, much clinical research on SCLC has shifted towards the identification of specific molecular changes that can be used as targets for therapy. Key features of malignant transformation include: alterations in proliferative signaling pathways, tumor-associated angiogenesis, cell-survival pathways, chromosomal stability and replication, protein folding and stability, and cell–cell and cell–matrix interactions (Figure 1). Paralleling the diversity of targets is the diversity of approaches: classes of agents currently under preclinical and clinical evaluation include antisense therapies, small-molecule inhibitors, peptide antagonists, immunotherapies and gene therapies. Here, we focus on agents under active clinical and laboratory investigation for the treatment of SCLC.

Cell proliferation: targeting growth-factor signaling pathways

Targeted inhibition of cell-surface growth-factor receptors and their downstream signaling pathways that drive cancer-cell proliferation has been a major focus of anticancer drug development over the past decade. Several receptor tyrosine kinases, including c-Kit and c-Met, are highly expressed and have been implicated in malignant transformation in SCLC. Also, a G-protein-coupled receptor – the gastrin-releasing peptide (GRP) receptor – is almost universally active in SCLC. Several of these growth-factor receptors act upstream of shared proliferative signaling pathways, including the phosphatidylinositol 3-kinase (PI 3-K)–Akt (protein kinase B)–mammalian target of rapamycin (mTOR) pathway and the RAS–RAF–MEK (MAPK–ERK) pathway. These growth-factor receptors and some of the downstream second messengers are amenable to targeted inhibition.

c-Kit

c-Kit gene encodes a transmembrane receptor tyrosine kinase of the platelet-derived growth-factor (PDGF) receptor subfamily. Upon binding of its ligand steel factor (SLF, also known as stem cell factor (SCF)), *c-Kit* homodimerizes and initiates an intracellular signaling cascade. Up to 70% of SCLCs have been reported to express *c-Kit* and SCF, although the prognostic value of this finding in unclear [4–6]. Imatinib (STI-571, GleevecTM) is a small-molecule

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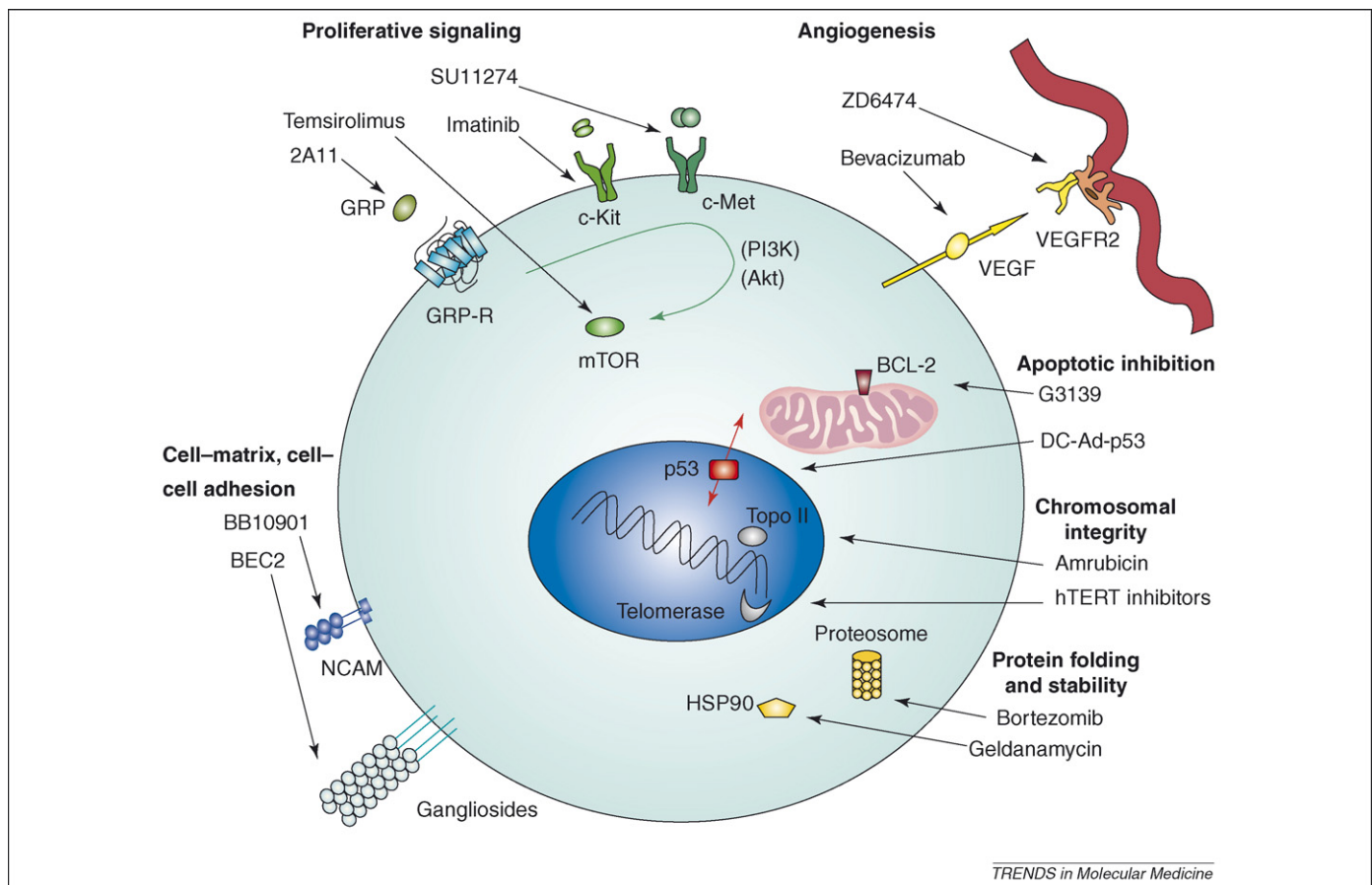


Figure 1. Diverse cellular processes that contribute to malignant transformation are being targeted through the development of new therapeutics for SCLC. Key components of proliferative signaling pathways are shown here, including tumor-associated angiogenesis, cell-survival pathways, mediators of chromosomal stability and replication, protein folding and stability, and cell-cell and cell-matrix interactions.

inhibitor of ABL tyrosine kinase, PDGF receptor and c-Kit. Imatinib inhibits SCLC cell lines and xenografts in a dose-dependent manner [7,8]. In phase II clinical trials, however, imatinib did not show activity against *de novo* or recurrent SCLC [9], as maintenance therapy after induction with etoposide and irinotecan in patients with ES SCLC [10] or in pre-screened patients with c-Kit-positive recurrent SCLC [11]. These entirely negative trials in SCLC, in contrast to the dramatic activity of this agent against gastrointestinal stromal tumors bearing c-Kit-activating mutations [12], was an early indication that, in human cancer patients, small-molecule tyrosine kinase inhibitors might be most effective when directed against mutated receptors rather than an upregulated and active receptor.

c-Met

Activation of the c-Met tyrosine kinase by its natural ligand hepatocyte growth factor (HGF, also known as scatter factor) leads to multiple cellular activities, including proliferation, angiogenesis, invasion into the extracellular matrix and wound healing [13]. c-Met is expressed in a large percentage of SCLCs [14], and tumor-specific mutations of the c-Met receptor have been described in SCLC [15]. The HGF-c-Met signaling network is targeted by various agents in preclinical and early phase clinical development, including blocking monoclonal antibodies against the ligand and the receptor, in addition to small-molecule receptor

tyrosine kinase inhibitors. Two small-molecule inhibitors of c-Met, SU011274 and PHA665752, have antitumor activity in xenografts that are derived from various human tumors [16,17], similar to humanized monoclonal antibodies against HGF [18,19]. Clinical activity of c-Met inhibitors in SCLC is currently being explored in early phase clinical trials but has not yet been reported.

Gastrin-releasing peptide

SCLC cells produce several neuropeptides, including GRP and neuromedin B [20,21]. Up to 85% of SCLCs express the related GRP receptor, neuromedin B (NMB) receptor and bombesin receptor subtype 3 (BBR-3), therefore creating an autocrine activation loop [22]. Binding of these G-protein-coupled receptors to their respective ligands results in the activation of multiple proliferative signaling cascades [23,24]. GRP also seems to have angiogenic properties, inducing endothelial-cell migration and cord formation *in vitro*, and promotion of angiogenesis *in vivo* [25].

This pathway has been targeted with small-molecule inhibitors, peptide antagonists and monoclonal antibodies. A small-molecule inhibitor of the GRP receptor, 77427, prevented angiogenesis in an *in vivo* model; this effect was specific to GRP because 77427 had no effect on VEGF-induced angiogenesis [25]. Preclinical studies have also demonstrated that treatment with bombesin-like peptide antagonists or neutralizing antibodies results in inhibition of SCLC tumor growth *in vitro* and *in vivo* [23,26,27]. In a

phase I trial of 13 relapsed SCLC patients, treatment with the anti-GRP 2A11 murine monoclonal antibody caused one complete response [28,29].

Farnesylation and receptor-associated second messengers

Several signal-transduction molecules that associate with growth-factor receptors and participate in downstream signaling are recruited to the cell membrane by linkage to lipids via farnesyl transferase. Several specific inhibitors of farnesyl transferase have been developed. Farnesyl transferase inhibitors have shown efficacy in preclinical models of SCLC, presumably through effects on the large number of farnesylated second messengers that regulate growth and survival [30]. This observation has not yet successfully translated into the clinic; in an initial phase II clinical trial of 22 patients with relapsed SCLC, the farnesyl transferase inhibitor tipifarnib (Zarnestra™, R115777) failed to show clinical activity as a single agent [31].

mTOR

The PI3K–Akt–mTOR pathway is a signaling cascade that mediates proliferation, cell growth, anchorage independence and apoptosis (reviewed in [32,33]). Activation of this pathway promotes tumorigenesis and therapeutic resistance, whereas its inhibition can lead to chemotherapy sensitization of SCLC cell lines [34,35]. Temsirolimus (CCI-779) is a mTOR inhibitor that has cytotoxic and antitumor activity in several preclinical models. In a phase II clinical trial in ES SCLC, 87 patients were randomized to receive two doses of temsirolimus after standard first-line chemotherapy. Patients treated with either dose showed no improvement in median survival compared with reported outcomes in earlier studies [36]. Additional studies to evaluate mTOR inhibitor activity in SCLC are ongoing.

Angiogenesis: targeting tumor-associated vascular growth

Angiogenesis, the stimulation of new blood vessel growth, is fundamental to normal tissue development, wound healing and reproduction. It has also been definitively associated with tumorigenesis. One of the most important promoters of angiogenesis, produced in different amounts by many human tumors, is the vascular endothelial growth factor (VEGF), which binds to specific receptors including the VEGF-receptor 2 to stimulate blood vessel growth. Clinical correlative studies have established that microvessel density and tissue VEGF expression portend a poorer prognosis in non-small cell lung cancer (NSCLC). This clinical correlation has not been definitively studied in SCLC, due in part to difficulty in obtaining SCLC biopsy samples (reviewed in [37]). Although the prognostic value of serum VEGF levels in SCLC patients is unclear [38,39], the role of angiogenesis in tumor progression and the success of anti-angiogenic agents across various tumor types has prompted investigation of angiogenic targeting in SCLC.

Vascular endothelial growth factor

Bevacizumab (Avastin™), a monoclonal antibody directed against VEGF, has demonstrated clear activity in colorectal cancer, NSCLC and breast cancer. Bevacizumab is currently

in a phase II study as maintenance therapy for LS SCLC after treatment with irinotecan and carboplatin. Early analysis suggests that bevacizumab might improve progression-free survival compared with standard therapy [40].

ZD6474 is an orally bioavailable inhibitor of VEGF-receptor 2, which also inhibits other receptor tyrosine kinases that are implicated in angiogenesis and cell proliferation. In preclinical models of SCLC, ZD6474 reduced metastatic rate and induced tumor-cell apoptosis [41]. ZD6474 is currently being evaluated in the adjuvant setting of LS and ES SCLC in a phase II cooperative group trial led by the National Cancer Institute of Canada.

Basic fibroblast growth factor

Thalidomide, an immunosuppressant and anti-inflammatory drug, also has potent anti-angiogenesis activity associated with decreased tumor production of both VEGF and a second key mediator of angiogenesis, basic fibroblast growth factor (bFGF) [42]. Thalidomide was well tolerated in combination with carboplatin and etoposide in a phase II clinical trial [43]. In a phase III study, which was stopped early due to poor accrual, 92 ES SCLC patients with chemotherapy responsive disease were randomized to receive cisplatin, etoposide, epirubicin and cyclophosphamide, with or without thalidomide. Despite the small size of the study, a statistically significant difference in survival was observed in favor of the thalidomide arm: 11.7 versus 8.7 months (hazard ratio: 0.48; 95% confidence interval: 0.24–0.93; $p = 0.03$) [44]. More patients on the thalidomide treatment arm withdrew from the study due to toxicity (33% versus 19%), notably neuropathy and constipation. Nevertheless, this result is encouraging and supports further investigation of targeted antiangiogenic therapy in SCLC. Receptor tyrosine kinase inhibitors that are active against both VEGF-receptor 2 and bFGF receptor are under development and might represent a better tolerated alternative.

Matrix metalloproteinases

An early step in angiogenesis and tumor invasion is the breakdown of the extracellular matrix mediated by matrix metalloproteinases (MMPs). Both MMP-2 and MMP-9 expression have been associated with poor prognosis in lung cancer [45]. Marimastat, an inhibitor of MMP-2 and MMP-9, was evaluated in a phase III clinical trial of ES SCLC patients who responded to first-line chemotherapy. No survival improvement was observed, and significant musculoskeletal toxicity resulted in discontinuation of therapy in 20% of patients [46]. Unfortunately, this result mirrored that in other disease types.

Apoptotic inhibition: targeting aberrant cell-survival pathways

Apoptosis is crucial for the development and homeostasis of multicellular organisms. These processes are dysregulated in tumors, promoting cancer cell survival when programmed cell death would normally be triggered. Apoptotic inhibition has key roles in both tumorigenesis and therapeutic resistance. Among the central regulators of apoptotic signaling are p53, which translates responses to DNA damage and other stresses into activation of an intrinsic

apoptotic pathway, and the B-cell CLL/lymphoma 2 (BCL-2) family of proteins that regulate an important step in apoptotic commitment – that is, permeability transition of the mitochondrial membrane. Both of these regulators are currently being targeted in SCLC in preclinical models and early phase clinical trials.

BCL-2

BCL-2 is a key regulator of the apoptotic cascade and is overexpressed in most SCLCs [47,48]. Aberrant expression of BCL-2 has been correlated with therapeutic resistance [49,50]. Antisense oligonucleotides against BCL-2 mRNA have shown antitumor activity in preclinical SCLC models [51]. Initial clinical trials, however, combining standard chemotherapy with the BCL-2 antisense oligonucleotide G3139 in patients with SCLC did not result in suppression of BCL-2 protein levels *in vivo* [52]. Consistent with inadequate target suppression, outcome in early phase clinical trials was similar to that of patients treated with chemotherapy alone [52,53]. A randomized phase II study of carboplatin and etoposide with or without G3139 suggests that G3139 does not increase response rate to standard chemotherapy [54].

A more promising alternative strategy for inhibiting BCL-2 function is the use of small-molecule inhibitors. Stabilized α -helix of BCL-2 domains (SAHB_A) is a peptide-mimetic modeled after the naturally occurring BCL-2 inhibitor BH3interacting domain death agonist (BID). SAHB_A has antitumor activity against human leukemia xenografts [55]. Nonpeptide-mimetics based on the same strategy include ABT-737, AT101 (gossypol) and GX15-070. ABT-737 exhibits remarkable cytotoxicity as a single agent or in combination with chemotherapeutic agents or γ -radiation in cell lines, and activity against human leukemia, lymphoma and SCLC xenografts [56]. Clinical trials to evaluate the effects of these agents or related derivatives are currently starting in SCLC.

p53

The *p53* gene encodes a crucial factor that regulates cell survival and damage response pathways. *p53* orchestrates a series of responses to perceived DNA damage or aberrant cell-cycle progression, triggering cell-cycle arrest, DNA damage response pathways and the apoptotic cascade [57]. *p53* is mutated in over 90% of SCLCs [58]. Preclinical studies demonstrate that adenoviral or retroviral expression of wild-type *p53* restores chemotherapy and radiation sensitivity and induces apoptosis in tumor cell lines, including SCLC [59,60].

The prolonged half-life and typically higher expression of mutant *p53* in cancer cells enable its use as an antigenic target for vaccine therapy, an approach that is supported by several preclinical studies [61,62]. A vaccine composed of dendritic cells that are transduced with an adenoviral vector expressing *p53* (DC-Ad-*p53*) has been used in combination with chemotherapy in ES SCLC patients [63]. In this study, *p53*-specific T-cell responses were observed in >50% of patients and vaccination was associated with a high rate of objective response to chemotherapy. A phase I and II dose-escalation clinical trial of the DC-Ad-*p53* vaccine in SCLC is underway.

Chromosomal stability: targeting genomic integrity and replication

An important finding in SCLC and many other solid tumors is the loss of genomic stability, ranging from individual gene mutation to gross chromosomal aberration and alterations of ploidy. Almost all SCLCs have deletions that affect multiple chromosomal sites (reviewed in [64]). Common regions of chromosome loss include 3p, 4p, 5q, 13q, 16q and 17p regions, which are loci with well-known tumor suppressor genes such as retinoblastoma (*RB*) and *p53*. Deletion of the short arm of chromosome 3 alone has been reported in up to 90% of SCLC cases. Genes that are located on 3p include retinoic acid receptor- β (RAR- β), fusion 1 (FUS1) and fragile histidine triad (FHIT), the last of which is altered in up to 80% of SCLC cases. Comparative genomic hybridization studies have reiterated findings from earlier genomic studies that showed gene amplification at multiple loci in SCLC, including 1p, 2q, 3q, 5p, 6p12, 6p15, 6p22–23, 7q, 8q, 11q, 12p, 14q and 18q. These regions encode oncogenes, including MYC, K-RAS, mixed lineage leukemia (MLL) and BCL-2.

Several agents that are currently being explored are intended to increase chromosomal instability or to interfere with cellular responses to chromosomal instability. These include a novel derivative of a traditional cytotoxic class, the topoisomerase II inhibitor amrubicin, and agents that target telomerase, the enzyme that maintains the integrity of chromosomal ends.

DNA topoisomerase II

The DNA topoisomerase II inhibitors etoposide and doxorubicin are among the most-active single agents against SCLC. Amrubicin, a synthetic anthracycline, and its 13-hydroxy metabolite amrubicinol represent a newer generation of potent topoisomerase II inhibitors [65]. A phase II study of 33 patients with previously untreated SCLC reported a response rate of 76% for amrubicin [66], and a 44-patient phase I and II study reported an 88% response rate in combination with cisplatin [67]. The latter combination was associated with a median survival time of 13.6 months.

Two recent phase II trials of amrubicin monotherapy for recurrent SCLC have also reported encouraging activity. In a phase II trial of 60 SCLC patients that were pretreated with at least one platinum-containing regimen, Seto *et al.* [68] reported response rates of 52% for chemosensitive relapse, and 50% in 16 patients with chemorefractory relapse, defined as progression within 60 days of completion of first-line therapy. Median survival was 11.7 and 10.9 months for patients with chemosensitive and chemorefractory disease, respectively [68]. These results were supported by another phase II study of SCLC patients that were treated with one or two prior therapies, in which single agent amrubicin was associated with a response rate of 53%, median survival of 8.8 months and one-year survival of 26% [69]. The results of these early phase clinical trials indicate improvements upon reported objective response rate, overall survival and one-year survival data from studies of topotecan monotherapy in recurrent SCLC, particularly for chemorefractory relapse [70]. It is unclear whether the pretreated patients in these amrubicin

trials received prior therapy with a DNA topoisomerase II inhibitor (e.g. etoposide or doxorubicin), and whether the toxicities of this agent are significant (grade 3–4 neutropenia was reported in 83–97% of patients and febrile neutropenia in 5–35%).

Further questions to be addressed include the efficacy of amrubicin in more-diverse patient populations and in randomized comparisons with standard therapy. These questions might be addressed with a phase II trial that compares amrubicin monotherapy with that of topotecan in relapsed SCLC, which is currently underway in the USA. Trials of amrubicin combination therapy that have currently started in Japan include a phase II trial of amrubicin and irinotecan in patients with relapsed disease, and a phase III trial of amrubicin and carboplatin in chemotherapy naïve elderly patients with ES SCLC.

Telomerase

Chromosome ends (telomeres) are capped by structures containing defined nucleotide repeat sequences that distinguish telomeres from DNA breaks (reviewed in [71]). Critically short or uncapped telomeres can trigger a DNA damage response pathway, leading to cell-cycle arrest and apoptosis. Telomeric repeats are synthesized by the ribonucleoprotein telomerase to preserve telomere length in replicating cells. Telomerase expression is silenced in terminally differentiated cells, but is frequently reactivated in the process of malignant transformation, facilitating unlimited proliferative capacity of cancer cells [72]. Telomerase is reactivated in most (up to 98%) SCLCs, and can serve both as a target of directed inhibition and as a tumor antigen. Immune responses and one clinical complete response have been observed in a NSCLC study of two synthetic peptide vaccines: GV1001 (human telomerase reverse transcriptase, hTERT; amino acids 611–626) and HR2882 (hTERT; amino acids 540–548) [73]. A telomerase-specific oncolytic virus, telomelysin (OBP-301), and a telomerase inhibitor, CKD601, are currently undergoing preclinical evaluation.

Retinoblastoma

RB is a crucial negative regulator of cell-cycle entry. Deletion or alteration of the *RB* gene has been reported in >90% of SCLC cases [74]. The relationship between loss of p53 and RB and development of SCLC is supported by the development of a SCLC mouse model in which p53 and RB could be conditionally lost [75]. Despite extensive characterization of RB pathway disruption in several tumor types, there have been few attempts to target this key factor in oncogenesis. An oncolytic adenovirus developed by ONYX Pharmaceuticals demonstrated preclinical evidence of specific antitumor activity against RB-deficient tumors, but has not been explored clinically.

Protein folding and stability: targeting metastable and mutant oncoproteins

Many of the key factors in malignant transformation are aberrantly expressed and/or stabilized derivatives of normal regulators of cell proliferation and cell survival. Intracellular levels of these important oncoproteins and tumor suppressors are normally tightly regulated by control both of gene transcription and translation, and of

protein degradation. Homeostatic disruption either by inhibition of protein folding or of protein degradation has been shown to have selective antitumor activity.

26S proteasome

The 26S proteasome is a multi-enzyme unit that regulates intracellular protein homeostasis via proteolytic degradation. It has several substrates that are important in cell-cycle regulation and apoptosis including cyclins, I κ B and p53 (reviewed in [76]). Proteasomal inhibition in lung cancer cell lines causes cell-cycle arrest and apoptosis associated with increased levels of p21 and decreased levels of BCL-2 [77,78]. Bortezomib (VelcadeTM), a reversible proteasome inhibitor, has received Food and Drug Administration (FDA) approval for the treatment of multiple myeloma, and can induce apoptosis in SCLC cell lines [78]. A phase I clinical trial of bortezomib in combination with topotecan for relapsed SCLC is currently being conducted at the University of California Davis.

HSP90

Heat shock protein 90 (HSP90) is a molecular chaperone, required for efficient folding of nascent 'client' proteins into fully functional tertiary structures. HSP90 inhibition is associated with disruption of multiple signaling pathways implicated in cancer, including the I- κ B kinase–nuclear factor- κ B (IKK–NF- κ B), PI 3-K–Akt and RAS–RAF–MEK pathways [79]. Treatment of SCLC lines *in vitro* with geldanamycin, an HSP90 inhibitor, inhibits growth and viability of multiple SCLC lines associated with apoptotic induction [80]. Several potent and specific inhibitors of HSP90 have been developed and are being actively evaluated in early phase clinical trials [81].

Cell–matrix and cell–cell adhesion: targeting the cancer-cell environment

Another important determinant of malignant transformation concerns tumor-cell interaction with adjacent cells and surrounding extracellular matrix. Cell–cell and cell–matrix interactions are key factors that regulate cell survival and proliferation through activation of transmembrane complexes, including cadherins and integrins, in addition to associated intracellular membrane signaling molecules such as the focal adhesion kinase. Oncogenic transformation is associated with aberrant production or structure of cell–cell and cell–matrix complexes. These tumor-specific abnormalities represent exposed cell-surface targets and have been exploited as putative antigens for antibody-based anti-cancer immunotherapies.

CD56

Neural cell adhesion molecule (NCAM) modulates neuroendocrine cell growth, migration and differentiation [82]. The *NCAM* gene encodes three distinct isoforms, one of which is CD56 [83]. NCAM is expressed in most SCLCs [84]. A humanized anti-CD56 monoclonal antibody conjugated to a cytotoxic maytansinoid drug, DM-1, (BB10901; huN901-DM1) has been developed. Early reports of a phase II clinical trial in SCLC patients with relapsed, CD56⁺ disease indicated that BB10901 was well tolerated, and clinical activity was observed in three of the first ten

patients enrolled [85]. Two clinical trials of BB10901 in SCLC are currently underway.

Gangliosides

SCLC cells produce tumor-specific gangliosides on their cell surface, including GM2, GD2, GD3 and 9-O-acetyl-CD3 [86,87]. BEC2 (mitumomab) is a mouse monoclonal antibody against CD3 combined with Bacillus Calmette-Guerin (BCG), which is used as an immune adjuvant. In a pilot study, 15 SCLC patients received a combination of anti-BEC2 and BCG in the adjuvant setting after standard chemotherapy. All patients developed anti-BEC2 antibodies and median survival in this cohort was 21 months [88]. These initially promising results did not hold up in a subsequent phase III trial of 515 patients with LS SCLC randomized to receive BEC2 and BCG or best supportive care after major response to chemotherapy and radiation; the vaccine did not improve survival in LS SCLC patients [89]. Subset analysis suggested a trend towards prolonged survival in patients who developed a humoral response to the vaccine.

Concluding remarks: challenges in SCLC therapeutic development

Current therapeutic research in SCLC represents a convergence of tumor biology, offering a detailed view of the essential components of SCLC malignant transformation and growth, and molecular pharmacology, offering a spectrum of targeted inhibitors of these important pathways. SCLC is a notoriously rapidly growing tumor with aggressive metastatic potential. Many of the key alterations that lead to constitutive activation of proliferative and metabolic pathways, loss of cell–cell and cell–matrix adhesion, disruption of apoptotic control and stimulation of angiogenic support have been defined. These same alterations that are essential to SCLC survival, growth and spread are being specifically targeted by a new generation of targeted therapeutics.

One of the lessons to be learned from the past decades of clinical research in SCLC is the need for better and more-representative preclinical models of disease. The promises of many seemingly successful strategies, showing high-level activity *in vitro* and apparent cures in immunosuppressed mice bearing xenograft tumors, have failed in clinical studies. Although cancer-cell-line-based xenograft models represent a standard testing ground for new anticancer strategies in this and other cancers, these models are an imperfect reflection of the disease. Cancer cell lines that are maintained in tissue culture are selected for properties that are optimal for *ex vivo* propagation, including growth as monolayers on plastic, in high-glucose media and in high-oxygen tension. Human cancers, by contrast, arise and are maintained in hypoxic and nutrient-poor complex environments in which selection for angiogenic drive and other survival strategies are operant. Reflective of these different selective pressures, cell-line-based SCLC xenografts tend to grow as non-invasive tumor nodules with low metastatic potential, in marked contrast to the human disease that is characterized by aggressive local invasion and early and widespread metastasis.

Multiple strategies for developing more-representative preclinical models of SCLC are being explored. One

strategy involves genetic manipulation of mice to produce murine tumors with features that are similar to human disease. A prime example of this for SCLC is based on concomitant interruption of *RB* and *p53* loci, resulting in mice that develop tumors with several features in common with human SCLC. An alternative strategy, avoiding the inherent biological differences between genetically derived murine tumors and a human cancer demonstrating complex genetic and genomic alterations, is to optimize human tumor xenograft models. One approach, which has shown great promise in multiple tumor types and is now being applied in SCLC, involves the transfer of cancer cells from the patient directly into the mouse with no intervening *ex vivo* culture. Recent data, from glioblastoma in particular, suggest that such primary heterotransplants might retain biological similarity to human disease that is lost in cancer cell lines *ex vivo* [90]. Use of these models as preclinical platforms for innovative therapeutic development is just beginning. Our own laboratory has been using such a model to evaluate the efficacy of highly selective BCL-2 inhibitors, a strategy that we think holds promise for improved treatment of SCLC.

Despite active and ongoing research into novel approaches to treat SCLC, there has been minimal progress in the clinical treatment of this disease over the past 25 years. The basis of cytotoxic intervention for SCLC in the early 1980s remains a standard of care at present. Advances in supportive care and technical advances in radiation therapy have led to the application of therapies with less toxicity and greater safety in most patients. Many strategies for molecularly targeted intervention are now being explored in both late preclinical and early phase clinical development. Referral of patients with SCLC to active clinical research centers for participation in clinical trials is of primary importance. Recent insights into the underlying biology of malignant transformation in SCLC and development of more-representative laboratory models of disease will facilitate continued focused therapeutic testing of novel anticancer strategies. The challenge and the promise of the upcoming years will be translating these insights into more-effective therapies for patients with SCLC.

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