

Novel Targets for Therapeutic Agents in Small Cell Lung Cancer

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Small cell lung cancer, novel targets, therapeutic agents, signal transduction pathways

Abstract

Small cell lung cancer (SCLC) is known to have an aggressive phenotype and often presents with distant metastasis. Despite frequent initial response to chemotherapy, it inevitably relapses within 2 years in the majority of patients. Because of the poor overall prognosis of the disease and its unique tumor biology, the opportunity for improving clinical outcome of patients with development of novel therapeutics is great. This review provides current insights into the novel molecular targets in SCLC. Cellular signal transduction pathways and their relationship to cellular functions also are discussed. Discussion of the role receptor tyrosine kinases (RTKs) have in SCLC therapeutic inhibition is emphasized. In particular, the recent development of small molecule inhibitors of RTKs such as c-Kit, c-Met, and VEGF-R and the potential for clinical trials are highlighted. (*JNCCN* 2004;2:165-172)

In 2003, an estimated 171,900 new cases of lung cancer will have been diagnosed in the United States. Approximately 16% of these are small cell lung cancer (SCLC).¹ The vast majority of SCLC cases result from long-term tobacco smoking.² Limited-disease SCLC (LD-SCLC, occurring in approximately one third of SCLC patients), with disease confined to the chest, potentially

can be cured with combinations of systemic chemotherapy and radiotherapy. Although inclusion of prophylactic cranial irradiation (PCI) has improved disease control inside the brain, long-term local control remains difficult, with the local-locoregional rate of relapse at 35% to 50% despite the most active concurrent chemoradiation protocols.³ The median survival for LD-SCLC is approximately 15 to 20 months, with 10% to 20% surviving 5 years, and the median survival with extensive-disease (ED-SCLC) is only 8 to 13 months, with merely 1% to 2% surviving 5 years. Despite the initial response to chemotherapy in most patients, more than 90% of SCLC patients eventually will be potential candidates for second-line or salvage chemotherapy.

Researchers have shown a strong desire to explore additional salvage therapeutic alternatives for SCLC patients for whom initial chemotherapy has failed. This has led to the development of novel targeted therapies for SCLC. The enthusiasm behind novel targeted therapeutics is built on the following factors: (1) because targeted therapies often are based on differential biologic parameters between malignant and nonmalignant cells, they tend to have less of the numerous systemic side effects traditional cytotoxic chemotherapies encompass; (2) mechanism and pathway of resistance would be different from cytotoxic chemotherapies; (3) potential synergistic or additive effects can be expected of the novel targeted therapeutics when combined with conventional chemotherapies.

Molecular Biology of SCLC

Many major components of cellular signaling and cell survival pathways often are altered in lung cancer cells through overexpression, mutation, or deletion. Some of these deregulated cellular circuitries have been identified and targeted as potential novel therapeutics in SCLC. Furthermore, tumor-associated antigens and angiogenesis

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in SCLC have also been explored as opportunities for immunotherapy as well as antiangiogenesis approaches in SCLC treatment.

Multiple chromosomal abnormalities have been described in SCLC, including 1p, 3p, 5p, 6q, 8q, 13q, and 17p.^{4,5} Chromosome 3p abnormalities are seen in the majority of SCLC cell lines and specimens, with the most common and consistent variant being the loss of the short arm of chromosome 3 [3p(14-25)].⁶ The deletion of 3p in SCLC cell lines was first reported by Whang-Peng et al.,⁷ with the allelic loss seen in more than 90% of cases.⁸⁻¹⁰ In addition, researchers found that the fragile histidine triad (*FHIT*) gene localizes to 3p14.2 and abnormalities of the *FHIT* gene can be seen in approximately 80% of SCLC.¹¹ *FHIT* was found to be a tumor suppressor as the transfection of the wild-type *FHIT* into lung cancer cells inhibited tumorigenicity and induced apoptosis in nude mice.¹² Loss of the chromosome 3p region can result in the deletion of other potential tumor suppressors, such as the protein-tyrosine phosphatase- γ gene, the semaphorin IV and A(V) genes,^{13,14} and finally the von Hippel Lindau tumor-suppressor gene (*VHL*).¹⁵ Located in 5q13-21 is the gene encoding the p85 α -regulatory subunit of phosphatidylinositol-3 kinase (*PI3K*) and the tumor suppressor gene *APC* seen in colorectal carcinomas. Both can be lost in SCLC.¹⁶

Receptor Tyrosine Kinases

The protein tyrosine kinases (TKs) represent the largest family of dominant oncogenes in SCLC.⁵ The two major types of TKs are receptor tyrosine kinases (RTKs) and nonreceptor TKs. Many RTKs and their ligands have been found to have an important role in the pathogenesis of human cancers, including SCLC.^{5,17,18} Examples of RTKs in SCLC include insulin-like growth factor I receptor (IGFR-I), transforming growth factor- β (TGF- β), c-Kit, c-Met, and vascular endothelial growth factor (VEGF) receptor. Although found to be important in cellular signaling in non-small cell lung cancer (NSCLC), epidermal growth factor receptor (EGFR) is not significantly overexpressed in SCLC. IGF-I is a mitogenic growth factor that is elevated in more than 95% of SCLC^{19,20} and has been linked to higher lung cancer risk in a case-control study.²¹ TGF- β serves as negative regulator of cellular proliferation in lung cancers. Important effectors of TGF- β signaling include Sma and Mad

(SMADs), which are phosphorylated by the TGF- β RTK, leading to homo- or hetero-oligomerization and nuclear translocation of the receptor complexes, and thereafter transcriptional activation of target genes for cellular growth regulation.²²

c-Kit as Therapeutic Target: Imatinib Mesylate

c-Kit (CD117) belongs to the class III RTK with five extracellular immunoglobulin-like regions, a transmembrane domain, and two tyrosine kinase domains in the cytoplasm. This RTK shares homology with other receptors, such as colony-stimulating factor-1, c-Fms, Flt3, and platelet-derived growth factor receptor (PDGFR).²³⁻²⁵ About 30% to 40% of SCLC specimens overexpress c-Kit.²⁶ The ligand for c-Kit is stem cell factor (SCF). SCF/c-Kit pathway acts both in an autocrine and paracrine fashion in activating cellular functions such as cell growth, survival, and motility for SCLC.²⁷⁻²⁹ Unlike gastrointestinal stromal tumors (GIST), we have not found any mutations of c-Kit in SCLC.³⁰

Overexpression of c-Kit has been found in SCLC, leading to initial enthusiasm of targeting c-Kit in SCLC. A recent clinical trial used the potent c-Kit targeted small molecule inhibitor imatinib mesylate (Gleevec; Novartis Pharmaceuticals, East Hanover, NJ) in SCLC. Imatinib mesylate can inhibit c-Kit, PDGF receptor, BCR/ABL, c-Abl, and c-Arg in vitro. Imatinib and similar drugs suppress the growth of SCLC cells in vitro.^{27,28,31,32} Clinical efficacy of Imatinib mesylate (600 mg daily oral dose) was evaluated in a small phase II trial in untreated or sensitive relapsed ED-SCLC patients.³³ A total of 19 patients were included (9 chemo-naive and 10 sensitive-relapsed patients). Only two grade 3 toxicities occurred. However, no objective responses were observed, with mean times to progression of 1 and 1.2 months in the untreated and sensitive-relapsed groups, respectively. Only 4 of the 19 tumor samples (21%) stained positive for c-Kit by immunohistochemistry. New clinical trials in the future using imatinib as targeted therapy would require selection of patients with c-Kit immunohistochemical expression as appropriate cellular target. An open-label, multicenter, single-arm, two-stage, pilot phase II study in patients with previously treated or untreated, c-Kit positive, ED-SCLC is currently ongoing.³³

c-Met as Therapeutic Target: SU11274 and PHA665752

c-Met is another RTK that has regulatory role in a wide number of cellular processes and signaling func-

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tions, such as cell growth and survival, apoptosis, scattering, motility, migration, angiogenesis, epithelial-mesenchymal transition (EMT), invasion, and metastasis.³⁴ In particular, *c-Met* triggers an invasive cellular program that is pivotal in EMT and tumor cells invasion and metastasis. *c-Met* is a disulfide-linked heterodimer that is activated by its natural ligand and hepatocyte growth factor (HGF; also called scatter factor, SF). The RTK is associated with multiple signal-transduction intermediates, such as Grb2, p85-PI3K, Stat-3, and Gab1.^{35,36} *c-Met* has been shown to be overexpressed and functional in SCLC.^{35,37} We recently showed that the HGF/*c-Met* pathway can be modulated in SCLC, leading to dramatic alterations in cell motility and migration as well as reactive oxygen species (ROS) production.³⁵ In addition, HGF/*c-Met* signaling leads to activation of focal adhesion proteins, such as p125FAK, PYK2, and paxillin.³⁵ Activating mutations of *c-Met* have been identified in numerous solid tumors as well.³⁴ We recently identified novel juxtamembrane and semaphorin (sema) domain mutations of *c-Met* in SCLC and have shown that the juxtamembrane mutations regulate cytoskeletal functions and induce tyrosine phosphorylation of cellular proteins.³⁰ HGF has been found to be highly elevated in SCLC patients when compared with normal control subjects or with those treated patients with no evidence of disease.³⁸ HGF potentially can serve as a serum tumor biomarker in SCLC.³⁸

Various attempts of inhibiting *c-Met* in vitro have been reported in the literature.³⁴ Examples include peptide inhibition, nonselective kinase inhibitor such as the natural alkaloid K252a, and *c-Met* inhibition through ribozyme-mediated down-regulation. Small molecule inhibitors targeting specifically against *c-Met* represent an attractive novel targeted therapeutic approach.

We have recently reported a novel small molecule inhibitor of *c-Met*, SU11274 ([[(3Z)-N-(3-chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-N-methyl-2-oxo-2,3-dihydro-1H-indole-5-sulfonamide]).³⁹ SU11274 has specific inhibition of *c-Met* and its oncogenic variant Tpr/Met (a fusion oncoprotein generated by chromosomal rearrangement putting the transcription promoter region, Tpr, upstream of the truncated *c-Met* gene). Our laboratory showed that inhibition of the Met kinase activity by SU11274 led to time- and dose-dependent reduced cell growth of

Tpr/Met transformed BaF3 cells, and the SCLC H69 and H345 cells.³⁹ H69 cells overexpress the juxtamembrane mutation of *c-Met* R988C, and H345 cells overexpress the wild type *c-Met*.³⁰ We found that the inhibitor blocked phosphorylation of Akt, GSK-3 β , and the pro-apoptotic transcription factor FKHR. The characterization of SU11274 as an effective inhibitor of *c-Met* tyrosine kinase activity illustrates the potential of targeting *c-Met* therapeutically in cancers associated with activated forms of this kinase, such as SCLC.

Another selective small molecule inhibitor of *c-Met*, PHA665752, has been developed. This agent inhibits *c-Met*-dependent phenotypes in vitro and exhibits cytoreductive anti-tumor activity in vivo.⁴⁰ PHA665752 potently inhibited HGF-induced and constitutive *c-Met* phosphorylation, as well as HGF and *c-Met*-driven phenotypes such as cell morphology, proliferation, and survival, cell motility, and invasion of a variety of tumor cells. It also inhibited the phosphorylation of various downstream signaling mediators such as Gab-1, ERK, Akt, Stat-3, PLC- γ , and FAK in various tumor cell lines. PHA665752 inhibited *c-Met* phosphorylation in tumor xenografts for up to 12 hours after a single dose, and the inhibition was associated with dose-dependent tumor growth inhibition or delay over a repeated administration schedule.⁴⁰

Taken together, these studies show the feasibility of selectively targeting *c-Met* with selective small molecule ATP-competitive inhibitors of the RTK as a novel therapeutic approach in SCLC. It can be anticipated that novel compounds such as these would enter into clinical trials in the near future.

PI3K/Akt/mTOR Pathway

Phosphatidylinositol 3'-kinase (PI3K) is a key signaling molecule involved in diverse cellular regulation such as adhesion, motility, migration, proliferation, and viability.^{41,42} Constitutive activation of PI3K signaling pathway has been reported in SCLC, mediating anchorage-independent proliferation via Akt (protein kinase B) and p70-S6K-dependent pathway.⁴³ On activation, *c-Met* can recruit and associate with PI3K, which eventually leads to downstream pathway activation of Akt.⁴⁴ Akt in turn inhibits caspase-9, Bad, and the forkhead (FKHR) transcription factors.^{45,46} The mammalian target of

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rapamycin (mTOR) is a protein kinase that normally acts as a checkpoint for nutritional status,^{47,48} and is an important signaling intermediate molecule downstream of the PI3K/Akt pathway that inhibits apoptosis.^{49,50} mTOR is a large (M_r ~289,000) multidomain serine/threonine kinase, and is a member of the PI3K family of protein kinases based on homology within the catalytic domain. Although signals that activate mTOR have not been well understood, phosphorylation of Akt and protein interactions via the mTOR NH₂-terminal multiple repeat HEAT motifs are among the possible mechanisms. The p70-S6 kinase (p70-S6K) and the translation inhibitor 4E-BP-1 are the two best-characterized mTOR substrates. Growth factor activation of the PI3K pathway results in phosphorylation and activation of p70-S6K by mTOR or PDK-1. It is currently not known if c-Met pathway can modulate the mTOR signaling cascade in lung cancer.

PI3K/Akt/mTOR Pathway as Therapeutic

Target: CCI-779

Rapamycin has been shown to inhibit growth of SCLC cell lines correlating with inhibition of the p70-S6K phosphorylation, which is constitutively activated in SCLC.⁵¹ Thus, the rapamycin-sensitive p70-S6K pathway may provide a novel therapeutic target in SCLC therapy. Several mTOR inhibitors have been used in clinical trials. CCI-779 is a novel ester analogue of the immunosuppressive agent rapamycin (sirolimus) that exerts cytostatic effects by the inhibition of the translation of cell-cycle regulatory proteins. Objective response was seen with CCI-779 in a lung cancer patient in phase I studies.⁵² The Eastern Cooperative Oncology Group (ECOG) has proposed the use of CCI-779 in clinical trial for patients with ED-SCLC post-induction chemotherapy.

Bcl-2 and Apoptosis

When bound to Bcl-2 or Bcl-X, Bad promotes cell death. The Bcl-2 oncogene is an inhibitor of apoptotic cell death that is expressed in up to 93% of SCLC.⁵³ Overexpression of Bcl-2 was shown to confer chemoresistance.⁵⁴

Bcl-2 as Therapeutic Target: Oblimersen (G3139)

Immunohistochemical detection of Bcl-2 in SCLC showed its expression up to 93% of cases.⁵³ Antisense oligonucleotide approach has been attempted to tar-

get Bcl-2 therapeutically. Oblimersen (G3139) is an 18-base oligonucleotide, complimentary to the Bcl-2 mRNA, hypothesized to inhibit Bcl-2 protein production. In a phase I study, 12 chemorefractory patients were treated with G3139 and paclitaxel, and researchers established that this combination was well tolerated.⁵⁵ Two patients showed stable disease at more than 30 weeks, although no objective responses occurred. Further studies determined the optimal dose of G3139 to be 7 mg/kg on days 1 to 8 by continuous infusion, etoposide at 80 mg/m² on days 6 to 8, and carboplatin at AUC5 on day 6.⁵⁶ Twelve of the 14 evaluable patients had PR (86%), and all 11 patients treated with the optimal dose were found to have PR. In light of these promising results, a larger phase II randomized trial is being performed by the Cancer and Leukemia Group B (CALGB) to assess whether adding G3139 enhances the efficacy of etoposide/carboplatin in SCLC patients.

Tumor-Associated Antigens and Immunotherapy

A variety of tumor-associated cell-surface antigens have been identified as potential immunotherapeutic targets in SCLC. Examples include the gangliosides GM2, GD2, GD3, and fucosyl GM1.⁵⁷ SCLC cells also express unique cell adhesion molecules such as neural cell adhesion molecule (NCAM, CD56) that can potentially be targeted therapeutically.

NCAM (CD56) as Therapeutic Target

Specific anti-NCAM antibodies have been designed to target SCLC using the strategy of conjugating toxins such as diphtheria toxin or ricin to the antibodies. Similar immunotherapy with toxin-conjugated antibody has been developed in cutaneous T-cell lymphoma (CTCL) targeted therapy using IL-2 receptor antibody, DAB(389)IL-2 (denileukin diftitox; ONTAK).⁵⁸ One such example in SCLC is the anti-NCAM antibody N901 that is conjugated with blocked ricin (N901-bR). Used in a phase I trial in extensive stage SCLC, N901-bR showed one PR in 21 enrolled patients.^{59,60}

Gastrin-Releasing Peptide (GRP) as Therapeutic Target

A mouse monoclonal antibody against GRP, 2A11, was designed to block and inhibit its autocrine growth stimulatory effects in SCLC cell lines and mouse mod-

els.⁶¹ One of 12 evaluable SCLC patients treated with 2A11 was reported to have CR lasting for 4 months, and three others showed stable disease in a phase II trial.⁶²

Cell Surface Glycosphingolipid Antigen GD3 as Therapeutic Target

Active immunization has been explored to add to chemotherapy to eliminate residual SCLC disease. An example is the BEC2/BCG (Bacillus Calmette-Gur in) immunization, which takes advantage of the potent immune adjuvant BCG to magnify the immune response generated against the anti-idiotypic mouse IgG_{2b} monoclonal antibody BEC2.⁵⁷ BEC2 structurally mimics GD3 ganglioside and has been used previously in immunization of melanoma patients.^{63,64} The median overall survival and progression-free survival were longer than expected in patients who had received the BEC2/BCG immunization, when compared with a prior control group of 31 patients representative of SCLC patients in the literature.⁵⁷

Vascular Endothelial Growth Factor (VEGF) and Angiogenesis

Solid tumor progression is critically dependent on angiogenesis, a process regulated at least partially by tumor-derived growth factors such as VEGF, which activates signaling responses in the host endothelial cells, thereby resulting in new tumor blood vessel formation from the existing host vasculature. VEGF is the most potent known mediator of angiogenesis, essential for the growth and metastasis of tumors. In a study of 68 SCLC patients, high pretreatment serum levels of VEGF were associated with poor survival.⁶⁵ Two high-affinity receptors for VEGF with associated tyrosine kinase activity have been identified on the endothelium of human vasculature: fms-like tyrosine kinase (Flt-1) and kinase insert domain-containing receptor (KDR). KDR signaling is thought to bear a more important role in mediating tumor progression.^{66,67}

VEGF and VEGF-R as Therapeutic Targets: Bevacizumab and ZD6474

Anti-angiogenesis approach against the important molecule in angiogenesis VEGF has entered clinical trials using the anti-VEGF antibody (Bevacizumab) and small molecule tyrosine kinase inhibitor (ZD6474) against its receptor. The murine monoclonal antibody

VEGF A.4.6.1 recognizes all VEGF-A isoforms with high-affinity and inhibits many human tumor cell lines in nude mice. VEGF A.4.6.1 was thereafter "humanized" to bevacizumab in 1997.⁶⁸ Bevacizumab specifically blocks binding of VEGF to its high-affinity receptors KDR and Flt-1, thereby inhibiting the angiogenic effects of VEGF.⁶⁸ Bevacizumab has already come to clinical fruition with the dose and tolerability established in phase I studies in advanced cancers.^{69,70} The ECOG has proposed studying the treatment with bevacizumab in combination with etoposide/cisplatin (EP) in untreated ED-SCLC patients.

ZD6474 is a potent small molecule tyrosine kinase inhibitor of the VEGF-receptor, KDR.⁷¹ It also has some activity against EGFR in addition to its anti-angiogenesis property, making it a dual TKI. A randomized trial using ZD6474 as maintenance therapy versus placebo for SCLC patients (LD and ED) who have experienced complete or partial remission is ongoing under the National Cancer Institute of Canada Clinical Trial Group (NCIC-CTG).

Reactive Oxygen Species

Reactive oxygen species (ROS) such as O₂^{•-}, OH[•], NO[•], and H₂O₂, are now known to play key role in regulation of various cellular processes, including gene expression, proliferation, and motility, with the effects dependent on the relative levels of the ROS.^{72,73} ROS are normal metabolic by-products continuously generated from the mitochondria of most cells. ROS not only is essential for cell defense mechanism, they also play a pathogenetic role in oxidative damages in DNA, proteins, and lipids. Cigarette smoking can generate more than 10¹⁴ free radicals per puff. ROS generated from tobacco smoking can induce toxicity, contributing eventually to the pathogenesis of SCLC.⁷⁴ Superoxide dismutase has been studied as a target for the selective apoptosis of transformed cells.⁷⁵ We recently have shown the generation of ROS via HGF stimulation of the RTK c-Met.³⁵ Researchers have suggested that ROS generated may act as second messengers to regulate the activities of redox-sensitive enzymes, including protein kinases and protein phosphatases. Changes in the oxidative state inside the cell can lead to an imbalance of the fine homeostatic balance between the protein kinases and phosphatases, thereby perturbing various cellular processes. Although

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using the ROS signaling pathway modulation in the clinical setting is premature, it is a promising area of translational research with the goal of employing antioxidants in modulating the ROS pathways in chemoprevention or chemotherapy.

Conclusions

SCLC is a difficult disease to treat, with a very poor clinical outcome despite current standard therapies. With the information obtained from the molecular and cellular biology of SCLC, multiple novel therapeutic approaches have been undertaken to combat the disease. Researchers hope that combinations of traditional cytotoxic chemotherapy with novel therapeutics will eventually lead to better prognosis for this devastating disease.

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