Characterization of c-kit Expression in Small Cell Lung Cancer: Prognostic and Therapeutic Implications

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ABSTRACT

Purpose: **The tyrosine-kinase receptor c-kit and its ligand stem cell factor are coexpressed in many small cell lung cancer (SCLC) cell lines, leading to the hypothesis that this coexpression constitutes an autocrine growth loop. To further evaluate the frequency and pathogenic relevance of c-kit expression, tumor tissue together with the corresponding clinical data of SCLC patients was analyzed.**

Experimental Design: **Tumor tissue of 102 consecutive SCLC cancer patients was analyzed immunohistochemically using an affinity-purified polyclonal c-kit antibody. Immunostaining data were correlated with survival and other relevant clinical parameters.**

Results: **A positive c-kit expression was observed in 37% of patients. c-kit expression was associated with decreased survival in the likelihood-ratio-forward selection model of the Cox regression including clinically relevant risk factors (c-kit expression, age, gender, stage, tumor stage, node stage, metastasis stage, weight loss, performance status, response to chemotherapy, lactate dehydrogenase, neuron-specific enolase, hemoglobin). Only c-kit expression [hazard ratio, 2.00; confidence interval (CI), 1.17–3.41; $P = 0.012$], response to chemotherapy (hazard ratio, 4.49; CI, 2.36–8.55; $P < 0.001$), and tumor stage (hazard ratio, 2.11; CI, 1.18–3.74; $P = 0.008$) were explanatory prognostic factors. These factors and all possible interactions between them were further analyzed in a second Cox regression model. As expected, response to chemotherapy had the highest impact on survival (hazard ratio, 3.06; CI, 1.69–5.54; $P < 0.001$). In patients with extensive disease, minor response to chemotherapy, and positive c-kit expression, the risk to die increased to 8.4 (hazard ratio, 2.74; CI, 1.52–4.91; $P = 0.002$). In a Kaplan-Meier analysis median survival of patients with minor response to chemotherapy and extensive stage was 288 days (CI, 255–321 days) when c-kit expression was negative compared with only 71 days (CI, 0–237 days) for c-kit-positive patients (log rank test: $P = 0.003$).**

Conclusions: **c-kit represents a new prognostic factor in SCLC. c-kit expression is of particular clinical relevance in patients with advanced disease and poor response to chemotherapy. Given the very limited therapeutic options and unfavorable prognosis of these patients, clinical studies aimed at targeting c-kit (*e.g.*, STI571) are clearly warranted.**

INTRODUCTION

Lung cancer is the leading cause of cancer-related death in Western industrial countries. SCLC[2] represents 15–25% of all lung cancer types and is distinguished from non-small cell lung cancer by its rapid tumor doubling time, high growth fraction, and early development of widespread metastases (1). Without therapy the patients' life expectancy is less than 4 months (2). Even with the introduction of chemotherapy, 5-year survival rates range only from 3–7% (3, 4), and within the last 20 years survival improved only modestly (5). Clearly, a better understanding of the molecular biology of SCLC is necessary to deduce new therapeutic strategies that will benefit patients suffering from this disease.

A variety of molecular markers has been implicated both in pathogenesis and prognosis of SCLC (6, 7). Paracrine or autocrine signal transduction pathways have been a popular hypothesis used to explain deregulated SCLC growth (7). An attractive candidate for such an oncogenic mechanism is the c-kit/SCF system (8, 9). The c-kit proto-oncogene is the cellular counterpart of the transforming gene of the Hardy-Zuckerman feline sarcoma virus (10) and encodes a transmembrane tyrosine kinase growth factor receptor belonging to the platelet-derived growth factor receptor family (11, 12). Its ligand SCF (kit-ligand or steel factor) is a hemopoietic growth factor that supports the proliferation of multiple hemopoietic cell lines (13, 14). Recent evidence indicates that SCLC cell lines and tumors express the mRNA for the c-kit receptor and for SCF, suggesting that these gene products constitute an autocrine loop mediating tumor cell survival and growth (15, 16). However, most of

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[2] The abbreviations used are: SCLC, small cell lung cancer; CT, computed tomography; LD, limited disease; ED, extensive disease; CI, confidence interval; TBS, Tris-buffered saline; LDH, lactate dehydrogenase; NSE, neuron-specific enolase; TNM, tumor, node, metastasis; SCF, stem cell factor; VALG, Veterans Administration Lung Study Group.

this knowledge is derived from cell culture experiments or animal models and is based on RNA or DNA analysis only. The clinical impact of c-kit protein expression in SCLC patients is unknown. To further substantiate this hypothesis, we examined whether c-kit expression influences survival in SCLC.

PATIENTS AND METHODS

Study Population. The study population consisted of consecutive patients with newly diagnosed SCLC investigated at the Pulmonary Division of Mainz University Hospital (Mainz, Germany) between 1989 and 1999. Tissue samples were obtained before therapy at the time of bronchoscopy (96%), mediastinoscopy (1%), or surgery (3%). Only one tumor sample was obtained from a cervical lymph node metastasis; all other samples were derived from the primary tumor side. Clinical data were collected from chart review. The staging procedure for the majority of patients was standardized including a fiberoptic bronchoscopy (100% of patients), routine laboratory parameters (100%), chest CT (100%), abdomen CT (96%), bone scan (94%), and brain scan (67%). One hundred two patients [age, 61.7 ± 9.1 years (mean ± SD); 82% male] were included in the study. Thirty patients (29%) had LD and 70 patients (69%) had ED according to the criteria of the VALG (2). In two patients the clinical documentation did not allow a definite classification (Table 1). In 88% of patients chemotherapy was performed as first-line treatment, mostly based on a cyclophosphamide and adriamycin combination: 33% of patients with adriamycin (50 mg/m^2, day 1), cyclophosphamide (1000 mg/m^2, day 1), and vincristine (2 mg/m^2, day 1); and 53% of patients with adriamycin (50 mg/m^2, day 1), cyclophosphamide (1000 mg/m^2, day 1), etoposide (100 mg/m^2, days 1, 2, and 3). Five percent of patients were treated with cisplatin (50 mg/m^2, days 1 and 7) and etoposide (100 mg/m^2, days 3, 4, and 5), whereas other combinations were applied in 10% of patients. The median number of cycles was four (mean, 4.1 ± 2.0 cycles); 17 patients (18.9%) received one cycle, 7 patients (7.8%) received two cycles, 9 patients (10%) received three cycles, 13 patients (14.4%) received four cycles, 7 patients (7.8%) received five cycles, 34 patients (37.8%) received six cycles, and 3 patients (3.3%) received seven cycles. Response was evaluated after two cycles of chemotherapy or if new clinical symptoms occurred. Response to chemotherapy was classified according to WHO criteria in complete response, partial response, stable disease, or progressive disease. Complete response was achieved in 23% of patients, partial response in 27%, and stable disease in 7% of patients. Forty-three percent of patients progressed during therapy. Fifteen of 30 LD patients (50%) and 17 of 70 ED patients (24%) received radiotherapy of the primary tumor (45–60 Gy). The two patients with no definite clinical stage did not receive radiotherapy. Four patients with complete response underwent surgical resection of the primary tumor site. The majority of patients were followed regularly in a time frame of 2–4 months. The survival time was calculated from the date of histological diagnosis.

Table 1 Baseline characteristics of the study population

	Number evaluated (n = 102)	%	Not evaluable
Age	61.7 ± 9.9[a]		
Sex	84 males	82	
Smoker or former smoker	91	91	2
Performance status			1
WHO 0	3	3	
WHO I	49	49	
WHO II	36	36	
WHO III	13	13	
Extent of the disease			2
Limited	30	30	
Extensive	70	70	
Stage			2
I	3	3	
II	6	6	
III	41	41	
IV	50	50	
T stage			4
T1	5	5	
T2	16	16	
T3	16	16	
T4	61	62	
N stage			3
N0	9	9	
N1	14	14	
N2	49	50	
N3	27	27	
M stage			1
M0	51	50	
M1	50	50	
LDH (units/liter)	361 ± 262[a]		4
NSE (ng/ml)	50 ± 71[a]		2
Hemoglobin	13.6 ± 1.7[a]		2

[a] Mean ± SD.

Immunohistochemistry. After bronchoscopy, surgery, or mediastinoscopy, fresh tumor tissue specimens were immediately formalin fixed. The probes were paraffin-embedded, cut in 3-μm sections, mounted on silane-coated slides, and baked overnight at 60°C. The paraffin was removed in xylene (2 × 5 min), rehydrated through graded alcohol (100%, 96%, and 70% for 5 min, respectively), and finally washed for 5 min in distilled water. The slides were cooked in citrate buffer (pH 6.0), using a microwave, three times for 5 min. After rinsing the slides with TBS (pH 7.4) for 5 min, the specimens were treated with 3% hydrogen peroxide for 15 min at 37°C and washed again in Tris (pH 2 × 5 min). Unspecific binding of biotin and avidin was blocked by incubating the slides in blocking solution for 15 min each (Vector Laboratories, Inc., Burlingame, CA). Again, the slides were washed with TBS (3 × 5 min). Subsequently, nonspecific binding sides were blocked with 2% of BSA (Sigma Chemical Co., St. Louis, MO) in TBS supplemented with goat serum (1:60; DAKO, Hamburg, Germany) for 60 min at room temperature. The primary antibody against c-kit was raised in rabbits by immunization with a peptide of the COOH-terminal domain of the intracellular part of the receptor (sequence, SVGSTASSSQPLLVHDDV). Using corresponding peptide as affinity matrix, the polyclonal antibody was purified to a final concentration of 0.4 mg/ml. The purified antibody was diluted 1:200 in TBS with 1% BSA and incubated overnight at 4°C. Thereafter, the slides were rinsed with TBS (3 × 5 min). For the detection of the primary antibody, a commercially available detection kit (ABC kit, XHC01, Unitect; Dianova, Hamburg, Germany) was used. The sections were incubated with biotiny-

lated antirabbit antibodies from goat (1:100) for 15 min at 37°C (Dianova). After rinsing with three changes of TBS, the ABC reagent (avidin and biotinylated horseradish peroxidase) was added for 30 min at 37°C. Again, the sections were washed in TBS three times (5 min). The 3,3′-diaminobenzidine solution [0.1% 3,3′-diaminobenzidine and 0.3% H_2O_2 in 0.05 Tris (pH 7.5)] was added for 5–10 min. Afterward, the slides were rinsed for 10 min under tap water. Counterstaining was performed in hematoxylin solution (Mayer, 1:10; Merck, Darmstadt, Germany) for 3 min. After washing with water the dehydration of the tissue was performed using increasing alcohol concentrations (70%, 96%, and 100% ethanol for 3 min). After incubation in 100% xylol for 5 min, the sections were covered with Entellan Neu (Merck). Twelve to 16 slides were processed in one session. One slide with c-kit overexpressing gastrointestinal stroma tumor tissue was included in each staining run. As a negative control, the same procedure was followed, but with the substitution of an unrelated rabbit antibody instead of the c-kit antibody. The slides were classified independently by two investigators (M.B. and F.B.) as positive or negative. Positive slides showed c-kit-positive cells including membrane staining in at least 10% of all tumor cells. Slides showing a weak cytoplasmatic signal without membrane staining were defined as negative.

Statistical Analysis. Standard descriptive statistics such as mean, SD, and frequencies were calculated to describe the study population, the potential risk factors, and the labor chemical parameters (LDH serum levels, NSE serum levels, hemoglobin levels). One of the principal aims of this study was the examination of the prognostic value of risk factors on the survival of patients with SCLC, taking into particular consideration the influence of c-kit expression. Features considered as potential explanatory factors were: c-kit expression (positive *versus* negative), stage (LD *versus* ED), performance status (classified according to WHO 0–1 *versus* 2–4), gender, age as a continuous variable and dichotomized (cutpoint, 70 years), T stage (T1–2 *versus* T3–4; Ref. 18), N stage (N0–1 *versus* N2–3; Ref. 18), M stage (18), LDH serum levels (>240 units/liter *versus* ≤240 units/liter), NSE serum levels (>20 ng/liter *versus* ≤20 ng/liter), hemoglobin levels (>12 g/dl *versus* ≤12 g/dl), weight loss (>10% *versus* ≤10%), response to chemotherapy (only patients with chemotherapy as first-line treatment: complete response *versus* partial response, stable disease and progressive disease; WHO criteria). Survival time was calculated from diagnosis to death or from diagnosis to last examination, if the patient survived. In the latter case the survival time was regarded as censored. Cox regression was applied to analyze the prognostic value of these risk factors with respect to survival using a forward stepwise selection with the likelihood ratio criterion (inclusion/exclusion criteria: $P \leq 0.05/P > 0.10$, respectively). Because 8% of patients with at least one missing value regarding the risk factors had to be omitted from this analysis, a second Cox regression with all risk factors selected by the first Cox regression was performed to analyze more patients. In four patients the chart reports of the CT scans were not definite, and the original CT scans were not available. Therefore, we could not define the exact T stage in four patients, N stage in three patients, and M stage in one patient. In addition, blood LDH levels were missing in four patients, and NSE and hemoglobin levels in two patients. One patient chart was without any specification of the patient's performance status. Similarly, a Cox regression with a forward and a Cox regression with a backward stepwise selection with the likelihood ratio criterion was used while performing the second analysis (inclusion/exclusion criteria: $P \leq 0.05/P > 0.10$, respectively). Interactions between all risk factors, including triple interactions, were considered as well. To illustrate the effect of the explanatory risk factors, Kaplan-Meier survival curves are shown for relevant parameter constellations. Because c-kit expression was the main objective of this study, the dependency of this parameter on other risk factors was explored using the Fisher's exact test for 2×2 contingency tables and the χ^2 test for larger tables. All analyses are regarded as explorative. Statistical analysis was performed using SPSS software 9.0.1.

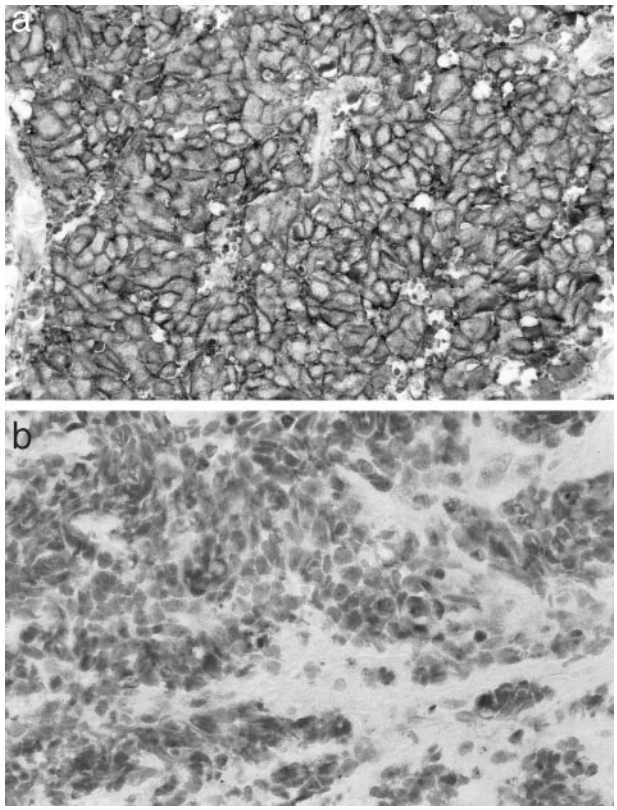

Fig. 1 Immunohistochemical staining of SCLC using a affinity-purified polyclonal antibody against c-kit. *a*, positive staining, including membrane staining, in at least 10% of tumor cells as observed in 38 of 102 SCLC tissues. *b*, negative result as observed in 64 (61%) of all SCLC tissues.

RESULTS

c-kit Immunostaining. Positive staining for c-kit was observed in 38 of 102 patients (37%). Positive slides showed clear cytoplasmatic staining, including membrane staining in at least 10% of all tumor cells (Fig. 1*a*). A typical negative result is shown in Fig. 1*b*. Because the analyzed paraffin blocks were collected over a time period of 10 years, we compared the blocks older than the median (5.83 years) with blocks younger

Table 2 Explanatory prognostic factors accepted in the likelihood forward selection model of the Cox regression

	Hazard ratio	95% CI	P^a
c-kit expression[b]	2.00	1.17–3.41	0.012
Stage[c]	2.11	1.18–3.74	0.008
Response to chemotherapy[d]	4.49	2.36–8.55	<0.001

[a] According to the likelihood ratio test. p-value according to the score test.
[b] c-kit expression in at least 10% of tumor cells was defined as positive.
[c] LD *versus* ED according to VALG (Ref. 2).
[d] Complete response *versus* partial response, stable disease, and progressive disease (WHO criteria).

Table 3 All other clinical parameters analyzed and not regarded as explanatory in the likelihood forward selection model of the Cox regression

	P^a
Performance status[b]	0.406
Gender	0.987
Age[c]	0.571
T stage[d]	0.503
N stage[e]	0.112
M stage[f]	0.712
LDH (>240 units/liter)[g]	0.076
NSE (>20 ng/liter)[h]	0.840
Hemoglobin (<12 mg/dl)[i]	0.717
Weight loss (>10%)	0.585

[a] According to the score test.
[b] Performance status classified according to WHO 0–I *versus* II–IV.
[c] Age <70 years *versus* >70 years.
[d] T1–2 *versus* T3–4 (Ref. 17).
[e] N0–1 *versus* N2–3 (Ref. 17).
[f] Distant metastasis.
[g] LDH serum levels >240 U/liter *versus* <240 U/liter.
[h] NSE serum levels >20 ng/liter versus <20 ng/liter.
[i] Hemoglobin levels >12 g/dl *versus* <12 g/dl.

than the median to detect a potential loss or masking of the antigen over time. No decrease of c-kit expression due to long storage was found (old blocks, 47% c-kit positivity; newer blocks, 28%; Fisher's exact test, $P = 0.065$).

Prognostic Significance of c-kit Expression. Among the clinical parameters analyzed, only c-kit expression, tumor stage, and response to chemotherapy were of prognostic relevance regarding survival (Table 2). LDH serum levels and lymph node involvement were of borderline relevance. All other parameters were not explanatory (Table 3). This was also true when the patients with at least one missing value regarding the nonrelevant risk factors were included in the second analysis. The forward and the backward selection process of the second Cox regression lead to the same result. Again, response to chemotherapy as well as the interaction between response to chemotherapy, stage, and c-kit expression were explanatory risk factors (Table 4). The risk to die from SCLC for patients with minor response to chemotherapy was three times higher compared with patients with complete response to chemotherapy. If minor response to chemotherapy is combined with detectable c-kit expression and ED, this risk further increased by a factor of 2.74 compared with patients with minor response to chemotherapy but LD or negative c-kit expression. Thus, patients characterized by positive c-kit expression, minor response to chemotherapy, and ED had an 8.38-fold increased risk to die compared with patients with negative c-kit expression, complete response to chemotherapy, and LD. In patients who responded to chemotherapy no influence of c-kit on survival could be found. In addition, Kaplan-Meier analysis demonstrated that patients with positive c-kit expression, ED, and minor response to chemotherapy survived shorter (median survival time, 71 days; CI, 0–237 days) compared with the same patient group with c-kit-negative tumors (median survival time, 288 days; CI, 255–321 days; log rank test, $P = 0.003$; Fig. 2). In patients with complete response to chemotherapy the survival curves of patients with c-kit-positive *versus* negative tumors and LD *versus* ED are similar (data not shown). A log rank test was performed to evaluate the impact of tumor stage (LD *versus* ED) in c-kit-positive patients. The comparison between c-kit-positive LD and ED patients ($n = 38$) revealed only a borderline prognostic difference (251 days *versus* 187 days; $P = 0.074$, log rank test). This was in contrast to the more pronounced difference between the two stages in c-kit-negative patients (388 days *versus* 274 days; $P = 0.010$, log rank test).

Table 4 Explanatory factors accepted by the second Cox regression with a forward and a backward stepwise selection. c-kit expression, stage, response to chemotherapy, and all possible interactions were included in the model

	Hazard ratio	95% CI	P^a
Response to chemotherapy[b]	3.06	1.69–5.54	<0.001
Response to chemotherapy[b]/c-kit expression[c]/stage[d]	2.74	1.52–4.91	0.002

[a] According to the likelihood ratio test.
[b] Complete response *versus* partial response, stable disease, and progressive disease (WHO criteria).
[c] c-kit expression in at least 10% of tumor cells was defined as positive.
[d] Limited *versus* extensive disease according to VALG (2).

Correlation of c-kit with Clinical Parameters. c-kit expression was associated with lower tumor stage (LD *versus* ED; $P = 0.046$). A borderline association was observed between c-kit and lower T stage ($P = 0.068$; Table 5) *i.e.*, c-kit is more often expressed lower tumor stages with better prognosis. c-kit expression and all other clinical parameters were not associated with each other.

DISCUSSION

In this study, a clear-cut positive expression of c-kit was observed in 37% of patients. In previous analyses a higher fraction of c-kit-positive tumors was observed. These studies were based mainly on cell line analyses (15, 16, 18–20). To our knowledge, there are only two studies in which c-kit protein expression was analyzed directly in tumor tissue of SCLC patients. Natali *et al.* (21) found c-kit protein expression in seven of seven fresh frozen tumor samples. Matsuda *et al.* (22) analyzed fresh frozen SCLC tissue specimens immunohistochemically. Only 5 samples showed strong receptor expression, 9 samples showed moderate expression, and 11 samples no expression. Potential explanations for this discrepancy are the

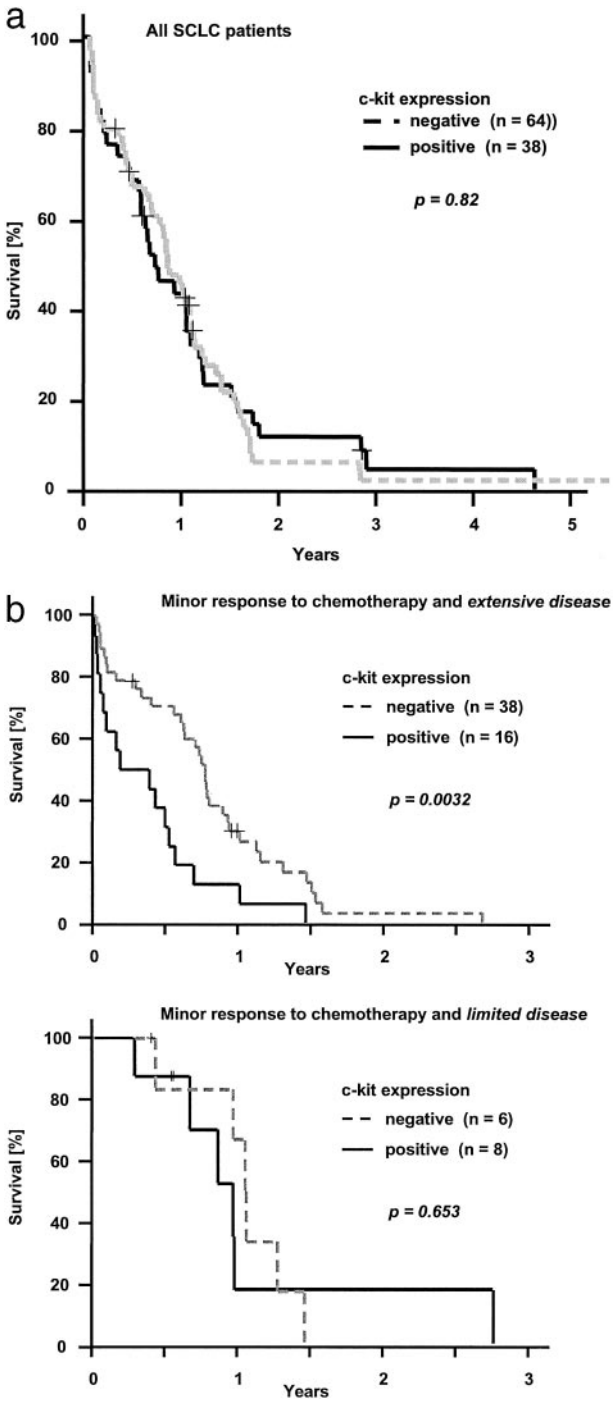

Fig. 2 Association of c-kit expression in patients with SCLC ($n = 102$) with survival time. *a*, survival curves of all patients according to negative and positive c-kit expression are shown. *b*, patients with minor response to chemotherapy were stratified to either ED or LD. Survival curves of negative and positive c-kit expression are shown.

Table 5 Association between c-kit expression and stage, performance status, gender, age, TNM classification, LDH, NSE, hemoglobin, and response to chemotherapy. The dependency was explored using Fisher's exact test for 2 × 2 contingency tables or χ^2 test

	c-kit expression[a]		
	Negative *n* (%)	Positive *n* (%)	*P*[b]
Stage[c]			
LD	14 (47)	16 (53)	0.046
ED	48 (69)	22 (31)	
Performance status[d]			
0–1	30 (58)	22 (42)	0.411
2–4	33 (67)	16 (33)	
Gender			
Male	52 (62)	32 (38)	0.793
Female	12 (67)	6 (33)	
Age[e]			
<70 yr	54 (66)	28 (34)	0.206
>70 yr	10 (50)	10 (50)	
T stage[f]			
1	2 (40)	3 (60)	0.068
2	6 (38)	10 (62)	
3	9 (56)	7 (44)	
4	43 (70)	18 (30)	
N stage[g]			
0	5 (56)	4 (44)	0.403
1	6 (43)	8 (57)	
2	33 (67)	16 (33)	
3	17 (63)	10 (37)	
M stage[h]			
0	30 (59)	21 (41)	0.539
1	33 (66)	17 (34)	
LDH[i]			
<240 units/liter	18 (53)	16 (47)	0.193
>240 units/liter	43 (67)	21 (33)	
NSE[j]			
<20 ng/liter	25 (61)	16 (39)	1.000
>20 ng/liter	37 (63)	22 (37)	
Hemoglobin[k]			0.400
>12 g/dl	54 (64)	30 (36)	
<12 g/dl	8 (50)	8 (50)	
Weight loss			1.000
No weight loss	29 (62)	18 (38)	
Weight loss >10% of body weight	28 (61)	18 (39)	
Response to chemotherapy[l]			0.319
Complete response	11 (52)	10 (48)	
Minor response	44 (65)	24 (35)	

[a] c-kit expression in at least 10% of tumor cells was defined as positive.
[b] According to Fisher's exact test or χ^2 test.
[c] Limited *versus* extensive disease according to VALG (2).
[d] Performance status is classified according to WHO 0–1 *versus* II–IV.
[e] Age <70 years *versus* >70 years.
[f] Ref. 17.
[g] Ref. 17.
[h] Distant metastasis.
[i] LDH serum levels >240 units/liter *versus* <240 units/liter.
[j] NSE serum levels >20 ng/liter *versus* <20 ng/liter.
[k] Hemoglobin levels >12 g/dl *versus* <12 g/dl.
[l] Complete response *versus* partial response, stable disease, and progressive disease (WHO criteria).

use of fresh frozen tissues compared with paraffin-embedded tissue in our study. Furthermore, our definition of positive immunostaining was relatively strict: only tumors with clear-cut membrane staining were accepted as positive. Another factor may be the comparatively large number of patients evaluated. This is the first study evaluating c-kit receptor status in a patient population with more than 100 samples. However, the results should be interpreted with caution, because other methods with

higher sensitivity (*e.g., in situ* hybridization) could result in a higher distribution of c-kit expression. Although the study was performed in one unique center, a more homogeneous patient population regarding treatment would be preferable. Therefore, confirmation of our results in future prospective studies is clearly warranted.

In the statistical models only response to chemotherapy (hazard ratio, 3.06) as well as response to chemotherapy, stage, and c-kit expression together (hazard ratio, 2.74) were relevant risk factors regarding survival in patients with SCLC (*i.e.,* patients having ED and minor response to chemotherapy with positive c-kit expression had a 174% increased risk to die compared with c-kit-negative patients). In patients with ED, minor response to chemotherapy, and c-kit expression, this risk was increased by 738% compared with patients having LD, complete response to chemotherapy, and no detectable c-kit expression. c-kit expression was even associated with shorter survival in patients with poor prognosis (ED and minor response to chemotherapy). In this group, survival of c-kit-positive patients was 71 days compared with 288 days for c-kit-negative patients. Thus, the results of this study support the hypothesis that c-kit plays a critical role in the pathogenesis of SCLC. It remains unclear whether this impact is due to an autocrine or paracrine growth stimulation as suggested by *in vitro* studies (15, 16, 20). This question remains to be answered, in particular because staining of SCF in paraffin-embedded tissue is not well established yet.

The phenomenon that oncogenes influence survival only in relatively advanced carcinomas has already been observed in previous studies. For instance, the p53 antagonist mdm-2 influenced survival only in patients with advanced ovarian cancer (23). Similarly, the expression of c-erb-B2, another membrane receptor with tyrosine kinase activity, was a prognostic factor only in advanced stages of SCLC (24) and ovarian cancer (25).

The biological mechanism responsible for the influence of c-kit on prognosis will be subject of future studies. In contrast to other types of tumors in which c-kit mutations lead to a constitutive activation, SCLC is not thoroughly analyzed for potential mutations. A single c-kit mutation in SCLC has been identified. The amino acid substitution at the transmembrane domain (methionine to leucine substitution due to an A to C transversion at codon 541) of as yet unclear functional consequence seems to be relatively rare because it was observed in only 1 of 15 SCLC cell lines and in only 1 of 13 primary tumor samples (9).

Several lines of evidence support the concept that c-kit expression is of clinical relevance in SCLC. First, c-kit expression clearly identified SCLC patients with a particular poor prognosis. Statistical analysis showed that c-kit is an explanatory factor. This observation is even more relevant because c-kit is not only a biochemical marker, but the involvement of c-kit in an autocrine, paracrine, or endocrine growth loop may represent a molecular mechanism behind aggressive tumor growth (26, 15, 16). Thus, this observation provides a rationale for therapeutic intervention aimed at c-kit expression, particularly in c-kit-positive SCLC patients with ED, a patient population to whom clinicians currently have hardly anything to offer, and in whom new therapeutic strategies are urgently warranted. Therapeutic concepts could include inhibition of c-kit by humanized monoclonal antibodies. This approach has already been successfully applied in breast cancer patients using a humanized monoclonal antibody (herceptin, trastazumab) that blocks the extracellular domain of the HER/*neu* receptor (27). A chimeric monoclonal antibody against the epidermal growth factor receptor, given alone or in combination with radiation or chemotherapy, showed impressive antitumor activity in different cancer types (28, 29). Another attractive target within c-kit is the tyrosine kinase domain. A tyrosine kinase inhibitor that recently attracted much interest is STI571 (30, 31). It has been shown that STI571, which is effective in the treatment of chronic myeloic leukemia (30, 32, 33) does not only inhibit cellular Abl tyrosine kinase activity but also the platelet-derived growth factor and c-kit receptor tyrosine kinases at similar concentrations (34). STI571 also inhibits proliferation of c-kit-positive SCLC cell lines (35, 36). These *in vitro* experiments and the observations summarized in this manuscript support treatment with tyrosine kinase inhibitors such as STI571 as a promising strategy in c-kit-positive SCLC patients. Trials using the c-kit inhibitor STI571 have already been initiated in other cancer types, some with impressive results (37, 38). Other strategies to interrupt the c-kit autocrine loop include antisense RNA constructs targeting c-kit and SCF (39, 40). Finally, these observations are also relevant in the context of the design of future clinical studies aimed at c-kit inhibition. Because only 37% of all SCLC patients were c-kit positive, patients included in such trials have to be stratified according to c-kit expression.

In conclusion, c-kit represents a new prognostic factor in SCLC. c-kit expression is of particular clinical relevance in patients with advanced disease and poor response to chemotherapy. Given the very limited therapeutic options and unfavorable prognosis of these patients clinical studies aimed at targeting c-kit are clearly warranted.

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