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Lack of *c-kit* exon 11 activating mutations in c-KIT/CD117-positive SCLC tumour specimens

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Abstract

Previous studies have shown that STI571, a selective tyrosine kinase inhibitor of c-KIT, is highly effective in c-KIT/CD117-positive gastrointestinal stromal tumours (GIST), especially those that have activating mutations in the *c-kit* exon 11 that encodes the juxtamembrane (JM) domain of the c-KIT oncoprotein. We examined the prevalence of activating exon 11 *c-kit* mutations in 26 small-cell lung cancer (SCLC) cases in order to explore whether this disease is also a potential target for treatment with STI571. Expression of c-KIT, estimated by immunohistochemistry, was demonstrated in 14 out of 22 SCLC samples (64%); nine samples showed moderate to strong staining (41%), five samples were weakly positive (23%), whereas eight samples (36%) were negative for CD117. Next, we examined the mutational status of exon 11 of the *c-kit* gene, by single-stranded conformational polymorphism (SSCP) and sequencing in all of the cKIT/CD117-positive tumours. However, no activating mutations in the *c-kit* exon 11 were found by either technique. Apparently, c-KIT oncoprotein expression in SCLC was not correlated with activating mutations in *c-kit* exon 11. In analogy to GISTs, our results could imply that SCLC patients would not benefit from treatment with STI571.

Keywords: Small-cell lung cancer; c-KIT/CD117; Tyrosine kinase receptor/inhibitor; Exon 11 activating mutations; Juxtamembrane domain; SSCP; STI571

1. Introduction

The vast majority of small-cell lung cancers (SCLC) express the c-KIT oncoprotein, a 145-kDa transmembrane glycoprotein [1]. This c-KIT protein, also known as CD117, is a member of the type III receptor tyrosine kinase family [2]. Steel factor, which is also designated stem cell factor (SCF), is the cognate ligand of this receptor. Activation of tyrosine kinase activity through dimerisation of the c-KIT receptor occurs upon binding of the cognate ligand and results in the phosphorylation of a variety of substrates involved in intracellular signal transduction [3]. The kinase activity of c-KIT has been implicated in the pathophysiology of a variety of human malignancies including SCLC, gastrointestinal stromal tumour (GIST), melanoma, germ cell tumours and neuroblastoma [4]. Autocrine or paracrine activation of c-KIT kinase by SCF has been postulated for a number of these malignancies. Apart from SCF-mediated c-KIT receptor activation, ligand-independent constitutive activation of this tyrosine kinase receptor through specific mutations in the *c-kit* gene has also been documented [4–6]. These activating mutations result in ligand-independent dimerisation and autophosphorylation of the c-KIT receptor, and activation of its tyrosine kinase activity may lead to uncontrolled cell proliferation and stimulation of downstream signalling pathways. Activating mutations of the *c-kit* gene are primarily located in the region between the transmembrane and tyrosine kinase domains, known as the juxtamembrane (JM) domain encoded by *c-kit* exon11 [5].

GISTs, the most common mesenchymal neoplasm in the human gastrointestinal tract, are almost exclusively positive for c-KIT, ranging from 89 to 100% [7,8], and commonly have activating mutations (approximately 70%) in the *c-kit* gene [6,7,9–11]. The vast majority of

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these mutations in GIST were confined to amino acid modifications in the JM domain of c-KIT. These activating mutations, which represent small in-frame deletions, point mutations or both, were most frequently found in the proximal part of exon 11 at codons 550–562 [5–7,9,10]. In the more distal part of exon 11, a few insertions located outside of the JM domain (duplicated 3-prime exon 11 codons) and a sporadic deletion of codon 579 have also been documented [7].

STI571 (Glivec®, Gleevec®, Imatinib) is a small synthetic molecule that shows selective inhibition of tyrosine kinase activity and functions through competitive inhibition at the adenosine 5′-triphosphate (ATP)-binding site of the enzyme [12]. STI571 was originally developed as an inhibitor of BCR-ABL kinase activity, but also showed selective inhibition of the tyrosine kinase activity of c-KIT oncoprotein and platelet-derived growth factor (PDGF) receptors α and β [13]. In chronic myeloid leukaemias (CML) and acute lymphoblastic leukaemias (ALL), this tyrosine kinase inhibition is directed against BCR-ABL, a constitutively activated tyrosine kinase which is the product of the Philadelphia (Ph) chromosome [13]. STI571 induces complete haematological remissions in almost all CML patients and it has substantial inhibitory activity on tumour cell growth of Ph-positive ALL patients [13,14]. The results of a phase I study on the safety and efficacy of STI571 in metastatic GISTs provided evidence for a role of c-KIT in GISTs and demonstrated the potential of this drug in the management of this chemo-resistant disease [15,16]. Furthermore, preliminary results from a phase II trial of STI571 in the treatment of patients with unresectable or metastatic GIST (initiated in July 2000; CSTI571 B2222) showed that STI571 induces remarkable response rates, in total contrast to the lack of activity of conventional cytotoxic drug treatments (reviewed in [4,16]). A major result of that multicentre study was that GIST patients with different *c-kit* gene mutations showed different responses to treatment with STI571. Patients with mutations in exon 11 responded extremely well compared with patients with mutations in exon 9 or 7 or wild-type (wt) *c-kit* sequences. Whether STI571 can be successfully used in other c-KIT-positive tumours and whether the mutational status of *c-kit* exon 11 influences the treatment outcome of other potential target tumours is presently not known.

SCLC, a distinct clinicopathological entity among lung cancers, has a highly aggressive clinical course and results in significant morbidity and mortality [17]. Despite years of clinical research, the prognosis for SCLC patients treated with combination chemotherapy has only minimally improved. Since over 70% of SCLC express the c-KIT oncoprotein [18], STI571 may prove to be useful in the chemotherapeutic treatment of this disease. Evidently, the mutational status of the *c-kit* gene plays an important role in the treatment of GIST with STI571. As yet, no information on the mutational status of the *c-kit* gene in SCLC has been reported. Therefore, we examined the prevalence of activating *c-kit* exon 11 mutations in 26 SCLC samples.

2. Patients and methods

2.1. Patients and tumours

A total of 26 SCLC samples were included in this study. Paraffin-embedded tumour specimens were retrieved from the archives of the department of pathology, Erasmus MC, that were collected between 1980 and 2002. The SCLC specimens consisted of 14 primary tumour biopsies and 12 samples from metastases from confirmed primary pulmonary small cell carcinoma patients. Furthermore, four paraffin-embedded GIST samples served as a positive control for *c-kit* exon 11 DNA mutations. Histological sections (5 μm) were cut from the paraffin blocks for routine haematoxylin/eosin (H&E) and immunohistochemical staining (CD117). Two additional sections (20 μm) were cut for molecular analysis. For all tumour samples included in the analyses, the number of tumour cells represented at least 70% of total nucleated cells (range 70–95%, median 85%), as judged by H&E staining.

2.2. Immunohistochemistry

Paraffin sections were immunostained with a polyclonal anti-CD117 antibody (anti-human c-KIT, code No. A4502, DAKO, Glostrup, Denmark) using a standard streptavidin-biotin procedure in an automated immunostainer (Mark V, DPC, Los Angeles, CA, USA). Diaminobenzidine was used as a chromogen and no heat-mediated or protease-assisted antigen retrieval methods were applied for the detection of CD117. Appropriate positive and negative controls were incorporated in each run of the immunostainer. The immunohistochemical CD117 staining intensity was scored visually using a three-tiered scale, samples with $\leqslant 5\%$ of weakly stained cells were considered to be CD117-negative (−), samples with $>5 \leqslant 20\%$ positive cells with weak to moderate staining intensity were scored as weakly positive (±) and samples showing $>20 \leqslant 85\%$ of positive cells with moderate to strong intensity were scored as positive (+). Notably, GISTs samples were scored as positive (+) when $\geqslant 50\%$ of the tumour cells showed strong staining intensity.

2.3. DNA isolation and c-kit exon 11 amplification

DNA was isolated from paraffin-embedded tissue blocks using the QIAGEN DNeasyTM Tissue Kit (Westburg, Leusden, The Netherlands) and the final

eluate was diluted in ultra-pure water to 100 μg/ml before amplification and single-stranded conformational polymorphism (SSCP) analysis. Primers used for the amplification of the juxtamembrane domain of exon 11 of the *c-kit* gene were cKITex11Fw1: 5′-CCA-GAG-TGC-TCT-AAT-GAC-TGA-GAC-3′ and cKITex11Rv3: 5′- ACT -CAG-CCT-GTT-TCT- GGG - AAA - CTC - 3′. These primers were designed by Oligo 6.0 primer analysis software (Medprobe, Oslo, Norway), purchased from Sigma-Genosys (Zwijndrecht, The Netherlands) and used for polymerase chain reaction (PCR), SSCP and cycle-sequencing. In order to check for duplicated codons at the 3′ termini of this exon, two additional primers were designed that spanned both exon/intron junctions of this particular exon. These primers; KIT11A: 5′-AGG-TGA-TCT-ATT-TTT-CCC-TTT-C-3′ and KIT11B: 5′-GTG-ACA-TGG-AAA-GCC-CCT-G-3′ were purchased from Invitrogen-Life Technologies (Merelbeke, Belgium). Intronic sequences were according to *c-kit* genomic sequence Genbank number U63834 and mRNA sequences were according to Genbank X06182. DNA amplification and the PCR-based SSCP were carried out in a Peltier Thermal Cycler DNA Engine (PTC-200) from MJ Research, Inc. (Watertown, MA, USA). A total of 100 ng of tumour sample DNA was used as a template for both normal amplification and PCR-based SSCP. Normal amplification was performed in 50 μl 1×PCR buffer (Roche Applied Science, Almere, The Netherlands) containing 1.5 mM $MgCl_2$ and in the presence of 300 nM forward and reverse primers, 250 μM deoxyribonucleoside 5′-triphosphates (dNTPs) (Promega, Leiden, The Netherlands) and 2.5 units of an unique enzyme mix of *Taq* and *Tgo* DNA polymerases with proof-reading activity (Roche). Cycle conditions were: 95 °C denaturation for 4 min followed by 40 cycles of 95 °C denaturation for 30 s, 55 °C annealing for 45 s and 68C extension for 45 s with an extra extension step for another 6 min at 68 °C after the last cycle. Resulting PCR products were analysed on high-resolution 2% (w/v) MetaPhor® agarose (Bio-Whittaker Molecular Applications, Rockland, ME, USA) on which small in-frame deletions/insertions (⩾3 bp) were readily detected.

2.4. PCR-based SSCP and sequence analysis of c-kit exon 11

All PCR-based SSCP reactions were performed in 25 μl 1×PCR buffer containing 1.5 mM $MgCl_2$, 300 nM forward and reverse primers, 'low-A' dNTPs, i.e. 400 μM deoxyguanosine 5′-triphosphate (dGTP), 400 μM deoxythymidine 5′-triphosphate (dTTP), 400 μM deoxycytidine 5′-triphosphate (dCTP) and 80 μM dATP (all dNTPs were from Promega, Leiden, The Netherlands), 0.25 μl [α-32P]-dATP (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) and 1 unit of *Taq* polymerase (Promega). Cycle conditions for PCR-based SSCP were: 94 °C denaturation for 4 min followed by 28 cycles of 94 °C denaturation for 45 s, 55 °C annealing for 45 s and 72 °C extension for 1 min with an extra extension step for another 6 min at 72 °C after the last cycle. Samples were fully denatured by the addition of an equal volume of loading buffer (95% (v/v) formamide, 10 mM NaOH, 0.03% (w/v) bromophenol blue and 0.03% (w/v) xylene cyanol) and subsequently incubated at 95 °C for 6 min. After denaturation,

Table 1
Immunohistochemical staining of CD117 and mutational analysis of *c-kit* exon 11 in tumour samples of SCLC and GIST patients

		Mutational analysis of *c-kit* exon 11		
Tumours	(P/M[a])	CD117/ c-KIT[b]	Del/insert/ pm[c]	DNA sequence[d]
SCLC1	(M)	+	No	wt
SCLC2	(P)	+	No	wt
SCLC3	(P)	+	No	wt
SCLC4	(M)	+	No	wt
SCLC5	(P)	+	No	wt
SCLC6	(M)	+	No	wt
SCLC7	(P)	+	No	wt
SCLC8	(P)	+	No	wt
SCLC9	(P)	+	No	wt
SCLC10	(M)	±	No	wt
SCLC11	(M)	±	No	wt
SCLC12	(P)	±	No	wt
SCLC13	(M)	±	No	wt
SCLC14	(M)	±	No	wt
SCLC15	(M)	−	No	wt
SCLC16	(M)	−	No	ND
SCLC17	(M)	−	No	ND
SCLC18	(P)	−	No	ND
SCLC19	(P)	−	No	ND
SCLC20	(M)	−	No	ND
SCLC21	(P)	−	No	ND
SCLC22	(P)	−	No	ND
SCLC23	(M)	ND	No	ND
SCLC24	(P)	ND	No	ND
SCLC25	(P)	ND	No	ND
SCLC26	(P)	ND	No	ND
GIST1	(P)	+	pm: 558; del: 559–560	mt
GIST2	(P)	+	No	wt
GIST3	(P)	+	del. 557 + 558	mt
GIST4	(P)	+	del. 557 + 558	mt

[a] Primary tumours; M, metastasis.

[b] CD117 immunostaining; (−): samples with ⩽5% of positive cells with a weak staining intensity, (±): samples with >5⩽20% positive cells with weak to moderate staining intensity, (+): samples with >20⩽85% of positive cells with moderate to strong staining intensity. ND; not determined.

[c] Exon 11 DNA aberrations (del, deletion; insert, insertion; pm, point mutation) were detected by polymerase chain reaction (PCR) analysis and the single-stranded conformation polymorphism (SSCP) method.

[d] The complete nucleotide sequence of the juxtamembrane (JM) domain of the *c-kit* gene (exon 11, codons 550–581) was sequenced in two directions. wt, Wild-type; mt, mutant; ND, not determined; SCLC, small-cell lung cancers; GIST, gastrointestinal stromal tumours.

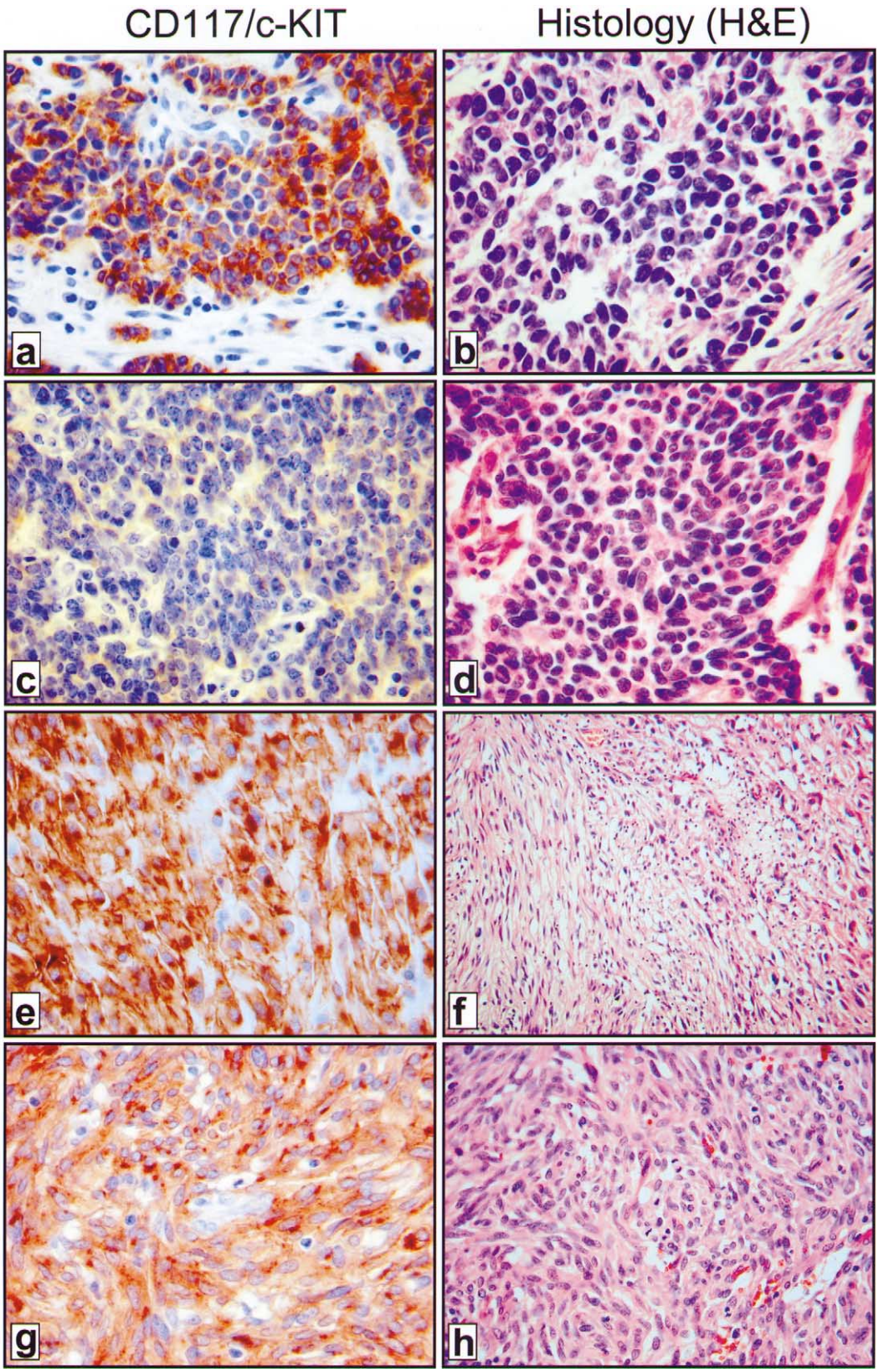

Fig. 1. Representative photomicrographs of small-cell lung cancers (SCLC) (a–d) and gastrointestinal stromal tumours (GIST) (e–h). CD117/c-KIT immunostaining (a, c, e and g) and conventional histology (H&E staining; b, d, f and h) are indicated. Representative examples of CD117-positive, (a and b) and-negative (c and d) SCLC samples are shown. An activating *c-kit* exon 11 mutation was not found in Neither SCLC sample. Representative CD117-positive GIST samples with (e and f) and without (g and h) *c-kit* exon 11 activating mutations are shown for comparison. Original magnification of the photomicrographs 400× (a–e and g) or 200× (f and g).

samples were immediately placed on ice and then 4 μl aliquots were loaded onto a 10% (w/v) non-denaturing polyacrylamide gel containing 10% (v/v) glycerol. SSCP analysis was carried out at a constant 12 W for 16-20 h at room temperature. PCR products showing an aberrant single-stranded conformation pattern were used for confirmational sequencing analysis.

Before sequencing, the PCR products were purified using QIAquick technology according to the manufacturer's recommendations. The purified PCR products of *c-kit* exon 11 were sequenced in both directions using the ABI Prism BigDye® Terminator Cycle Sequencing Kit on an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

3. Results

3.1. Immunohistochemical analysis of CD117 (c-KIT)

The immunohistochemical data of CD117 are summarised in Table 1. Eight of the 22 analysed SCLC samples (36%) were CD117-negative (−), 5 samples (23%) showed weak staining (±) and nine samples (41%) had moderate to strong CD117 immunostaining (+). Thus, 64% of the SCLC samples showed expression of c-KIT/CD117. The immunostaining in SCLC was exclusively cytoplasmic and the staining intensity was variable within each sample. Furthermore, the SCLC samples showed no accentuation of the perinuclear Golgi apparatus as was seen in GISTs. Representative examples of CD117-positive and-negative SCLC samples are shown in Fig. 1a and c, respectively. H&E staining demonstrated that nearly all of the nucleated cells in these SCLC samples consisted of tumour cells (Fig. 1b and d). In order to compare the c-KIT-positive SCLC with a widely accepted c-KIT-positive tumour type, four GIST samples were included and representative CD117-positive GIST samples are shown in Fig. 1e–h. The tumour cells of these GIST samples showed strong cytoplasmic staining with occasional perinuclear accentuation (Fig. 1e and g). Furthermore, the normal histology of these GIST specimens showed that the majority of the tumour cells had a spindle-like morphology and were in a fasicular arrangement (Fig. 1f and h).

3.2. Mutational analysis of c-kit exon 11

To investigate whether activating mutations in exon 11 of the *c-kit* gene were underlying the c-KIT/CD117 oncoprotein expression, we determined the mutational status of *c-kit* exon 11. Initially, we screened 26 different SCLC samples for the presence of small deletions and/or insertions using specific primers spanning the complete exon 11 coding sequences for the JM domain of the c-KIT protein (codons 550–590). PCR products were analysed on a high-resolution agarose gel. Using this sensitive technique, we could readily detect *c-kit* exon 11 variant PCR products in GIST samples with known deletions (data not shown). However, no aberrant PCR products were detected in either CD117-positive or -negative SCLC samples (Table 1). This suggested that activating deletions and/or insertions in the JM domain of the *c-kit* oncogene were probably not present. Next, we screened all SCLC samples by SSCP

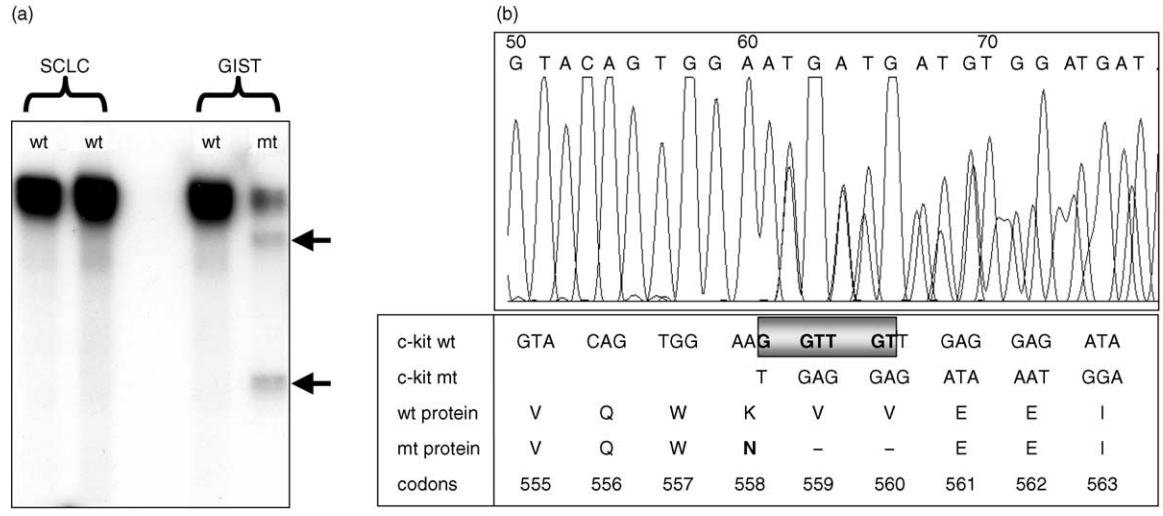

Fig. 2. Mutational analysis of exon 11 of the *c-kit* gene: (a) PCR-based single-stranded conformational polymorphism (SSCP). The two SCLC samples showed a normal, wild-type (wt) SSCP pattern. PCR-based SSCP readily detects exon 11 mutations in GIST: an exon 11 mutant (mt) sample (GIST1) showing an variant SSCP pattern versus a wt sample (GIST2). Arrows: extra SSCP bands indicating potential mutations. (b) Direct sequencing of mutant exon 11 of GIST1. Part of the chromatogram containing the mutated exon 11 sequence, the double sequence read out, the exon 11-wt and-mt sequence interpretation and its implication for the protein sequence are shown. Codon numbers and corresponding amino acids are indicated.

for the presence of more subtle *c-kit* exon 11 mutations (point mutation and or insertions/deletions of less than 3 bp). Evidently, no aberrant SSCP pattern was found in any of the SCLC samples (Table 1). Conversely, we readily detected extra bands representing variant single-stranded confirmations in three out of four analysed GIST samples (Table 1). Fig. 2a shows the SSCP analysis of two representative SCLC samples having a normal, wt SSCP pattern. *c-kit* exon 11 mutant (mt) and wt GIST samples are shown for comparison. Finally, we performed sequencing of the entire exon 11 PCR products of 15 SCLC and four GIST samples. Although most of these SCLC samples were positive for c-KIT/CD117 protein, they had wt *c-kit* exon 11 alleles (Table 1). In contrast, sequence analysis of the three GIST samples having aberrant SSCP patterns revealed a small deletion together with a point mutation in one GIST sample (Table 1 and Fig. 1b) and small deletions in two other GIST samples (Table 1). In all GISTs with exon 11 DNA aberrations, we always found *c-kit* wt sequences in addition to the mutations (Fig. 1b), so we concluded that only one of the two *c-kit* alleles was mutated in these GIST samples.

4. Discussion

Currently, there is no curative treatment for most SCLC patients and in particular those patients with extensive stage disease, who are traditionally treated with combination chemotherapy, have a very poor prognosis [19]. Recent treatment strategies in SCLC are targeted to dysregulated signalling pathways that are causally associated with tumour progression. Most SCLC cells express receptor tyrosine kinases, which may offer rational targets for molecular therapy. Receptor tyrosine kinase activity triggers signalling molecules, which have been proven to be critical in both tumour growth control and transformation. Thus, tyrosine kinase inhibitors may prove to be useful in the chemotherapeutic treatment of SCLC. Since approximately 70% of SCLC express the c-KIT tyrosine kinase oncoprotein ([18], this study), STI571 might be a potential therapeutic agent in the treatment of SCLC.

It might be expected that only cancers whose tumour cells are (partially) dependent on c-KIT activation for tumour growth and pathogenesis will respond to treatment with tyrosine kinase inhibitors. With respect to GIST, constitutive c-KIT tyrosine kinase activity, resulting from activating or gain-of-function mutations, most likely provide the driving force for tumour growth and pathogenesis [4,6,7]. The high response rate of exon 11 mutated GIST cells to STI571 strongly suggests that abrogation of this signal transduction pathway leads to cell death and/or homeostasis (reviewed in Ref. [4]). Here, we provide evidence that c-KIT oncoprotein expression in SCLC is not correlated with activating mutations in *c-kit* exon 11. To our knowledge, no other information on the mutational status of the *c-kit* gene in SCLC is currently available. Notably, similar to our SCLC study, a lack of mutations of exon 11 and exon 17 of the *c-kit* gene in c-KIT-positive angiosarcomas [20] and adenoid cystic carcinomas [21] has been reported.

Based on the GIST results, we restricted our mutational analysis in the present study to DNA aberrations in exon 11 of the *c-kit* gene. Whether mutations in other regions of the *c-kit* gene are responsible for the observed c-KIT oncoprotein expression in SCLC is presently unknown, but it seems unlikely since mutations in exon 9, encoding the extracellular (EC) domain, exon 13, encoding the 1st catalytic tyrosine kinase domain (TK1), or exon 17, encoding TK2, are rare [6,7,11]. Taken together, it seems unlikely that gene mutations contribute to *c-kit* oncogene activation and dysregulated c-KIT signalling in SCLC. So, other mechanisms are probably responsible for the observed c-KIT oncoprotein expression in SCLC. In particular, autocrine/paracrine stimulation of the c-KIT kinase activity by binding of SCF to its cognate receptor has been postulated as a potential mechanism that may contribute to the tumorigenesis of a number of malignancies including SCLC [22,23]. Notably, a number of studies reported that STI571 and some tyrphostin inhibitors of c-KIT could block the growth of c-KIT-positive SCLC cell lines [23–25]. So it would appear that SCLC cell lines are sensitive to growth inhibition by STI571 and accordingly clinical trials are underway with this drug [12,26]. However, it should be noted that while STI571 efficiently blocked SCF-mediated c-KIT activated cell growth in serum-free medium, no more than a limited effect, which appeared to be cytostatic in nature, was seen in serum-containing cell cultures. Whether this confounding factor may have an impact on the inhibitory activity of STI571 in relevant *in vivo* situations remains to be elucidated.

Recently, the results from the multicentre study phase II trial in GIST on the efficacy and safety of STI571 were reported [27] together with more detailed information on the correlation between clinical response and *c-kit* mutational status [28]. In this study, it was shown that the likelihood of a clinical response to STI571, as well as the time to treatment failure, correlated with *c-kit* mutational status. Moreover, patients with a GIST harbouring an exon 11 *c-kit* mutation had a significantly higher partial response rate (72%) than patients whose tumour had an exon 9 mutation (31.6%, $P = 0.0033$) or no detectable mutation (11.8% $P < 0.0001$). In addition, the time to treatment failure was significantly longer in patients with exon 11 mutations compared with those without exon 11 mutations ($P < 0.0001$). Interestingly, *in vitro* analysis of typical GIST-associated *c-kit* mutations confirmed that the kinase activity of these oncogenic

KIT proteins were all equally sensitive to inhibition by STI571 using clinically relevant concentrations of drug. This discrepancy between *in vivo* and *in vitro* data emphasises that the *in vitro* results have to be interpreted cautiously since they do not necessarily predict clinical response. Based on the observed association between clinical response to STI571 in GIST patients and the mutational status of the *c-kit* oncogene [28], our present results may imply that SCLC patients, including those with c-KIT oncoprotein expression, but without exon 11 mutations, will not respond to treatment with STI571.

In May 2001, STI571 entered phase II trials for SCLC [12]. Recently, the initial preliminary results of 19 SCLC patients have been reported [26]. Notably, there were no objective responses seen in these 19 SCLC patients (four of the 14 tumour blocks, which were available for immunohistochemistry were positive for CD117/c-KIT staining (29%). Although the authors stated that any conclusion from this study should take into account the small number of c-KIT-positive tumours, these first results suggest that STI571 is not very effective in SCLC patients. Presumably, therefore, the *in vivo* effectiveness of STI571 on tumour cell growth is different between GIST cells with mutation-driven constitutive activation of c-KIT signalling and SCLC cells with an autocrine stimulated cell proliferation. In light of the fascinating results of the STI571 trials in GIST, particularly the positive correlation between the presence of exon 11 mutations and the clinical response to STI571, we are eager to learn the final results of this phase II clinical trial with STI571 in SCLC. However, with respect to our present results, it may be expected that SCLC patients would not benefit from treatment with STI571.

References

1. Hibi K, Takahashi T, Sekido Y, *et al*. Coexpression of the stem cell factor and the c-kit genes in small cell lung cancer. *Oncogene* 1991, **6**, 2291–2296.
2. Reilly JT. Class III receptor tyrosine kinases: role in leukaemogenesis. *Br J Haematol* 2002, **116**, 744–757.
3. Scheijen B, Griffin JD. Tyrosine kinase oncogenes in normal hematopoiesis and hematological diseas. *Oncogene* 2002, **21**, 3314–3333.
4. Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002, **20**, 1692–1703.
5. Longley BJ, Reguera MJ, Ma Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* 2001, **7**, 571–576.
6. Rubin BP, Singer S, Tsao C, *et al*. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001, **61**, 8118–8121.
7. Miettinen M, Lasota J. Gastrointestinal stromal tumors: definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001, **438**, 1–12.
8. Dematteo RP, Heinrich MC, El-Rifai WM, Demetri G. Clinical management of gastrointestinal stromal tumors: before and after STI-571. *Hum Pathol* 2002, **33**, 466–477.
9. Taniguchi M, Nishida T, Hirota S, *et al*. Effect of c-kit mutation on prognosis of gastrointestinal stromal tumors. *Cancer Res* 1999, **59**, 4297–4300.
10. Lasota J, Jasinski M, Sarlomo-Rikala M, Miettinen M. Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. *Am J Pathol* 1999, **154**, 53–60.
11. Lasota J, Wozniak A, Sarlomo-Rikala M, *et al*. Mutations in exons 9 and 13 of KIT gene are rare events in gastrointestinal stromal tumors. A study of 200 cases. *Am J Pathol* 2000, **157**, 1091–1095.
12. Radford IR. Imatinib. Novartis. *Curr Opin Investig Drugs* 2002, **3**, 492–499.
13. Druker BJ, Sawyers CL, Kantarjian H, *et al*. Activity a of specific inhibitor of the BCR-Abl tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001, **344**, 1038–1042.
14. Goldman JM, Druker B. Chronic myeloid leukemia: current treatment options. *Blood* 2001, **98**, 2039–2042.
15. Van Oosterom AT, Judson I, Verweij J, *et al*. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001, **358**, 1421–1423.
16. Verweij J, Judson I, van Oosterom A. STI571: a magic bullet? *Eur J Cancer* 2001, **37**, 1816–1819.
17. Chute JP, Chen T, Feigal E, Simon R, Johnson BE. Twenty years of phase III trials for patients with extensive-stage small-cell lung cancer: perceptible progress. *J Clin Oncol* 1999, **17**, 1794–1801.
18. Hibi K, Takahashi T, Sekido Y, *et al*. Coexpression of the stem cell factor and the c-kit genes in small-cell lung cancer. *Oncogene* 1991, **6**, 2291–2296.
19. Giaccone G. State of the art in systemic treatment of lung cancer. *Eur J Cancer* 2001, **37**(S7), 99–101.
20. Miettinen M, Sarlomo-Rikala M, Lasota J, *et al*. KIT expression in angiosarcomas and fetal endothelial cells: lack of mutations of exon 11 and exon 17 of C-kit. *Mod Pathol* 2000, **13**, 536–541.
21. Holst VA, Marshall CE, Moskaluk CA, Frierson Jr HF. KIT protein expression and analysis of c-kit gene mutation in adenoid cystic carcinoma. *Mod Pathol* 1999, **12**, 956–960.
22. Krystal GW, Hines S, Organ C. Autocrine growth of small cell lung cancer mediated by coexpression of c-kit and stem-cell factor. *Cancer Res* 1996, **56**, 370–376.
23. Krystal GW, Honsawek S, Litz J, Buchdunger E. The selective tyrosine kinase inhibitor STI571 inhibits small cell lung cancer growth. *Clin Cancer Res* 2000, **6**, 3319–3326.
24. Krystal GW, Honsawek S, Kiewlich D, *et al*. Indolinone tyrosine kinase inhibitors block Kit activation and growth of small cell lung cancer cells. *Cancer Res* 2001, **61**, 3660–3668.
25. Wang WL, Healy ME, Sattler M, *et al*. Growth inhibition and modulation of kinase pathways of small cell lung cancer cell lines by the novel tyrosine kinase inhibitor STI 571. *Oncogene* 2000, **19**, 3521–3528.
26. Johnson BE, Fisher B, Fisher T, *et al*. Phase II study of STI571 (Gleevec) for patients with small cell lung cancer. *Proc Am Soc Clin Oncol*, 2002 (abstr **1171**).
27. Demetri GD, von Mehren M, Blanke CD, *et al*. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002, **347**, 472–480.
28. Heinrich MC, Corless CL, Blanke C, *et al*. KIT mutational status predicts clinical response to STI571 in patients with metastatic gastrointestinal stromal tumors (GISTs). *Proc Am Soc Clin Oncol*, 2002 (abstr **6**).