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REVIEW

Gastrointestinal Stromal Tumors: Morphological, Immunohistochemical and Molecular Changes Associated with Kinase Inhibitor Therapy

Mario Díaz Delgado · Alicia Hernández Amate · Sofía Pereira Gallardo · Sara Jaramillo · Juan Antonio Virizuela Echaburu · Ricardo J. González-Cámpora

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Abstract Recurrent or metastatic GISTs are currently treated with kinase inhibitors since they achieves disease control in 70–85% of patients but this response depend on *KIT* and *PDGFRA* gene mutation status. We review the morfological and molecular findings associated to kinase inhibitors administration in GISTs based on the literature on Medline and authors' own experience. The initial response to kinase inhibitors (imatinib mesylate, Gleevec, Novartis) usually is partial and depend on the mutational *KIT* or *PDGFRA* state. Amongst patients wih *KIT* mutations, the best results are achived in those harboring exon 11 (85%) and exon 9 (45%) mutations. GISTs harboring *PDGFRA* gene mutations generally respond favorably except those involving the Asp842Val mutation. In the absence of *KIT/PDGFRA* gene mutations, partial response or disease stabilization is reported in 23% and 50% of patients, respectively, and disease progression in 19%. Histological examination of tumors displaying an initial response to imatinib reveals a highly-variable reduction in the number of tumor cells, a decline in the proliferative index, myxohyaline or sclerohyaline stroma, and a varying degree of bleeding and edema, necrosis and cystification. 72% of patients with initial good response to imatinib, display metastases or new nodule growth within an existing clinically-quiescent tumor after 12–36 months of treatment. This secondary resistance is characterized by a number of well-defined morphological and molecular changes. Histologically, the new growths display increased mitotic activity, pleomorphism, an epithelioid or mixed phenotype and persistent KIT expression although more rarely, dedifferentiation and loss of KIT expression (Fig. 4), as well as trans-differentiation into a rhabdomyosarcoma or epithelial phenotype has been reported. Molecularly, 46–67% of patients present additional *KIT* mutations, generally in the kinase domain (exons 13, 14 and 17) but also in the ATP-binding domain (exons 15,16) of the same allele. Secondary *PDGFRA* mutations are very rare. Secondary mutations have not been observed in GISTs not harboring *KIT/PDGFRA* mutations, or in tumors displaying an unusual morphology or loss of CD117 expression. A number of studies highlight the presence of different resistance mutations within different new tumor nodules, as well as the simultaneous development of distinct resistant tumor subclones within a single lesion (acquired polyclonal resistance). Secondary muta-

M. Díaz Delgado
Servicio de Anatomía Patológica, Hospital de Mérida, Badajoz, Spain

A. Hernández Amate
Servicio de Anatomía Patológica, Hospital Infanta Cristina, Badajoz, Spain

J. A. Virizuela Echaburu
Servicio de Oncología Médica, Hospital Universitario V. Macarena, Seville, Spain

S. Pereira Gallardo · S. Jaramillo · R. J. González-Cámpora (✉)
Unidad de Gestión de Anatomía Patológica, Hospital Universitario Virgen Macarena, Avda. Dr. Fedriani s/n, 41009 Seville, Spain
e-mail: rcampora@us.es

tion in genes other than *KIT/PDGFRA* has only been reported in *BRAF* (Val600Glu).

Keywords Gastrointestinal stromal tumors · GIST · Imatinib mesylate, sunitinib malate · Primary resistance · Secondary resistance · Morphological changes · Molecular changes

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors of the gastrointestinal tract that differentiate towards interstitial cells of Cajal or their precursors; they are generally KIT (CD117)-positive, and display *KIT* or *PDGFRA* gene mutations [1, 2]. These tumors have recently prompted particular interest due to their good response to new targeted therapies [3].

The primary treatment for a low- or intermediate-risk gastrointestinal stromal tumor (GIST) is surgery: The lesion should be removed intact and with clear surgical margins; lymphadenectomy is not required except in pediatric GISTs [4]. Chemotherapy and radiotherapy have been assayed in patients with high-risk malignant tumors, but results to date have not been encouraging [5]. GISTs are currently treated with kinase inhibitors. The results obtained with one of these, imatinib mesylate (*Gleevec*, Novartis, USA), are particulartly promising since it achieves disease control in 70–85% of patients with advanced tumors (median progression-free survival 20–24 months, estimated median overall survival time is 36 months). These results contrast sharply with those obtained previously using doxorubicin-based chemotherapy, where the median survival was only 9 months [4].

Clinical Responses to Imatinib

Objective reponses to imatinib are neither complete nor uniform—a partial response is reported in 53% of cases, and disease stabilization in 27%—and depend on *KIT* and *PDGFRA* gene mutation status. Amongst patients wih *KIT* mutations, the best results are obtained in those harboring exon 11 (85%) and exon 9 (45%) mutations [6, 7]; while the results for GISTs involving exon 9 mutations are rather less promising, a substantial improvement is reported when the dose-rate is doubled from 400 to 800 mg/day [8]. Experience in patients with GISTs harboring exon 13 and exon 17 mutations is very limited, though a partial response or stable disease has been reported [9], except in patients with the Asn822Lys mutation, who display no response at all [6]. GISTs harboring *PDGFRA* gene mutations generally respond favorably except those involving the Asp842Val mutation [6, 8]. In the absence of *KIT/PDGFRA* gene mutations, partial response or disease stabilization is reported in 23% and 50% of patients, respectively, and disease progression in 19% [8]. Recent studies recommend adjuvant treatment with imatinib, which significantly increases recurrence-free survival times [10] (Table 1). The second kinase inhibitor used in GISTs' treatment is sunitinib malate (Sutent, Pfizer, USA), a multi-targeted tyrosine kinase inhibitor (KIT, PDGFRA, VEGFR, FLT3 and RET). Recently it has been approved as a second-line therapy for patients with imatinib-resistant or –tolerant GIST. The clinical benefits of sunitinib also depend on *KIT* and *PDGFRA* mutational status. Although short-term clinical benefits are reported in 65% of GIST patients that are refractory to imatinib, significantly better results have been obtained in patients hosting *KIT* exon 9 mutations [11]; however, only about 25% of patients switched to sunitinib therapy continue to respond favorably 1 year later [12].

The clinical responses to kinase inhibitors treatment vary considerably [4]; a partial favorable response may prompt rapid tumor attention, defined as at least a 30% decrease in the greatest tumor diameter on a CT scan (Fig. 1). In other cases, tumors may show very little radiographic change, but their growth is arrested for >6 months. A minority of patients experience continued tumor growth within the first 6 months of treatment, which is referred to as primary resistance.

Histological Finding in GISTs Treated with Imatinib Mesylate

Histological examination of tumors displaying an initial response to imatinib reveals a highly-variable reduction in the number of tumor cells, a decline in the proliferative index (mitosis, Ki67), myxohyaline or sclerohyaline stroma, and a varying degree of bleeding and edema, necrosis and cystification (Fig. 2). The number of residual CD117-positive cells reported also varies widely, and in some cases there is evidence of trans-differentiation to a smooth muscle phenotype (Fig. 3) [13, 14]. Rare instances of bone and cartilage formation have been observed, though it is not clear whether this is a result of tumor trans-differentation or stromal fibroblast metaplasia [15]. The earliest changes, observed after 5–7 days of the start of treatment, include a marked reduction in KIT phosphorylation, together with apoptosis and a decline in mitotic activity [14].

Secondary Resistance

The term "secondary resistance" is applied to those patients who, despite a good initial response to treatment,

Table 1 Mutation types and primary responses to Imatinib in vivo (*Gleevec*, Novartis, USA)

	KIT 9	KIT 11	KIT 13	KIT 17	WT	PGDFRA 12	PDGFRA 18
Mutation type	Ala502_Tyr503 dup	del del-ins sust dup	Lys642Glu Glu635Lys	Asp820Tyr Asp822His Asn822Lys		Asp561Val del del-ins dup ins	Asp842_Met845del Asp822_Met844del Ile843del_His845del Ile843del Ile843del_His845del Asp842Val Asp846Val
Type of response (%)							
Complete response	3.5	6.5	0	0	4.5	0	0
Partial response	34	57	40	25	33	100	25
Stable disease	37.5	19	20	50	28		50
Progressive disease	16	6.5	20	25 Asn822Lys	17		25 Asp846Val

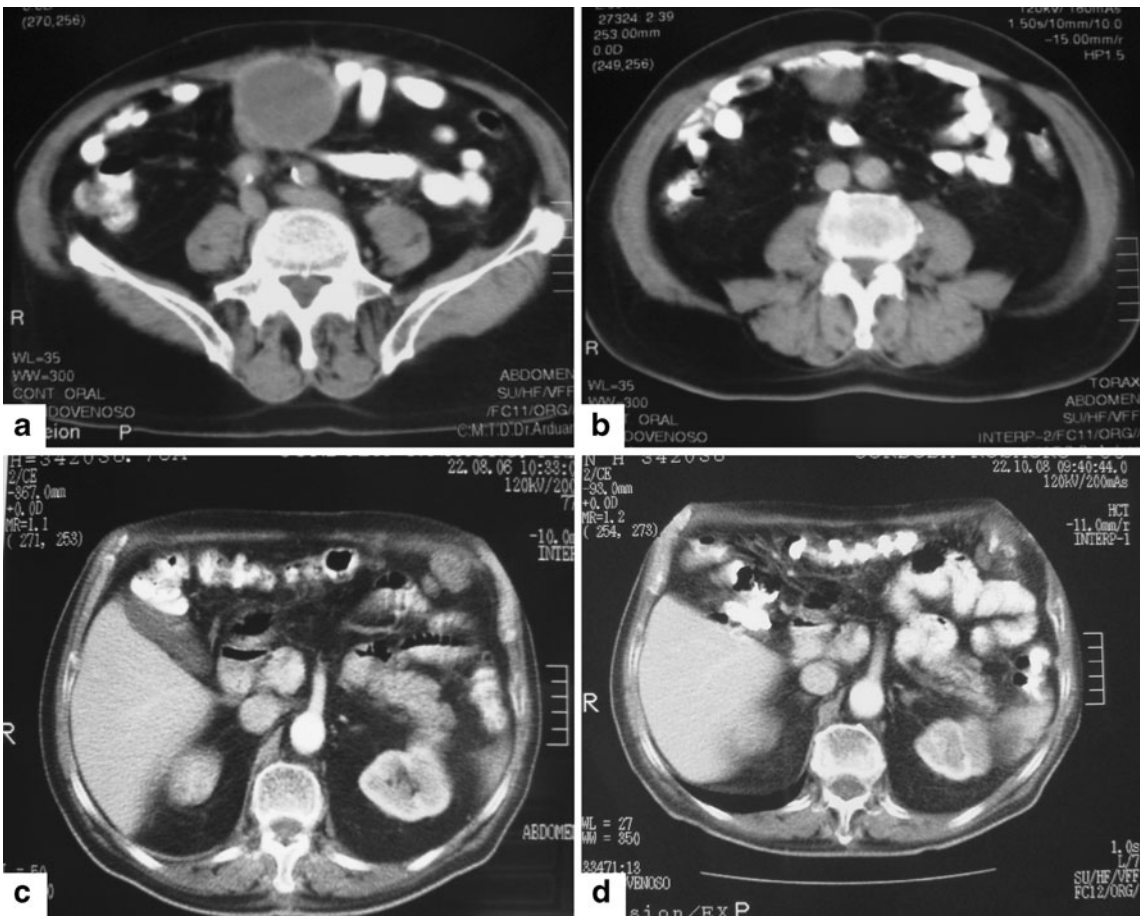

Fig. 1 **a**. Imatinib therapy (400 mg) was started in 2004, following observation of a tumor close to the anterior abdominal wall, diagnosed histologically as a GIST. **b**. In 2005, a significant partial response was observed, lasting for one year. **c**. In 2006, follow-up examination revealed new tumor growth and treatment was started with sunitinib (50 mg). **d**. By 2008, the tumor had not disappeared, but remained stable

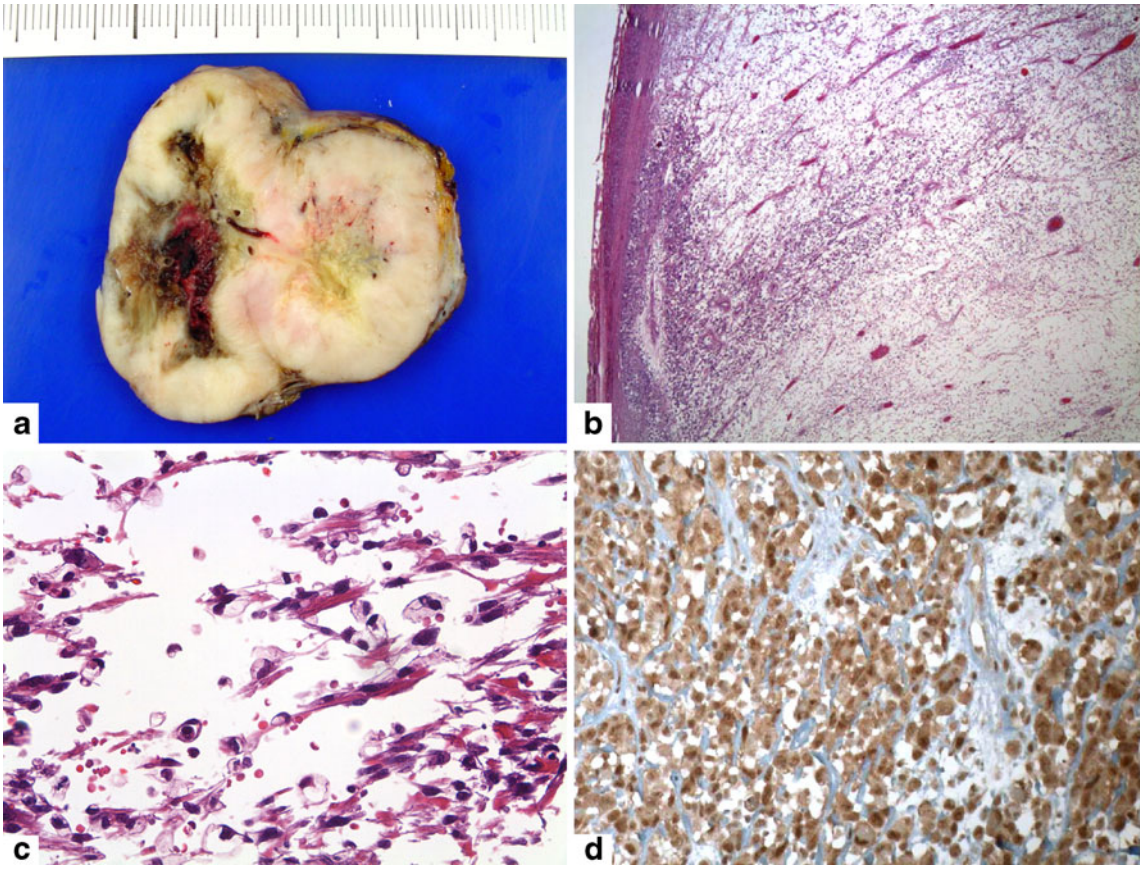

Fig. 2 **a**. Section of tumor treated with imatinib. Note intratumoral edema and bleeding, involving almost half the lesion. **b**. Panoramic image showing tumor hypocellularity and marked interstitial edema. HE X4. **c**. Detail highighting edema and tumor cells with pyknotic nuclei. HE X 40. **d**. Persistent KIT expression in viable tumor cells. CD117 X40

display metastases or new nodule growth within an existing clinically-quiescent tumor after 12–36 months of treatment. This resistance, occurring in most patients receiving imatinib (72%) [16], is characterized by a number of well-defined morphological and molecular changes. Histologically, new growths display increased mitotic activity, pleomorphism, an epithelioid or mixed phenotype and persistent KIT expression (Fig. 4); more rarely, reports have noted dedifferentiation and loss of KIT expression (Fig. 4), as well as trans-differentiation into a rhabdomyosarcoma or epithelial phenotype [17, 18].

In 46–67% of patients with secondary resistance, additional *KIT* mutations are observed, generally in the kinase domain (exons 13, 14 and 17) but also in the ATP-binding domain (exons 15,16) of the same allele [8, 16, 19] (Table 2). Secondary mutations tend to be single aminoacid substitutions, and their frequency is determined by the location of the primary KIT mutation; they are more common in GISTs harboring exon 11 than exon 9 mutations [16] (Table 2). Secondary *PDGFRA* mutations are very rare [9]. A number of studies highlight the presence of different resistance mutations within different new tumor nodules, as well as the simultaneous development of distinct resistant tumor subclones within a single lesion (acquired polyclonal resistance) [4, 9, 19](Table 3). Almost all secondary mutations, with the exception of Asn822Tyr and Tyr823-Asp, are new mutations not reported in primary lesions (9), and almost all are imatinib resistant, especially Val654Ala [20].

A number of authors have suggested that secondary mutations may be present from the outset in a small number of cells, providing a selective advantage to those cells during imatinib therapy [4]; however, there is also evidence to suggest that secondary mutations arise de novo, as a response to treatment, since these changes are not observed in cases of primary resistance and a positive correlation has been noted between the presence of

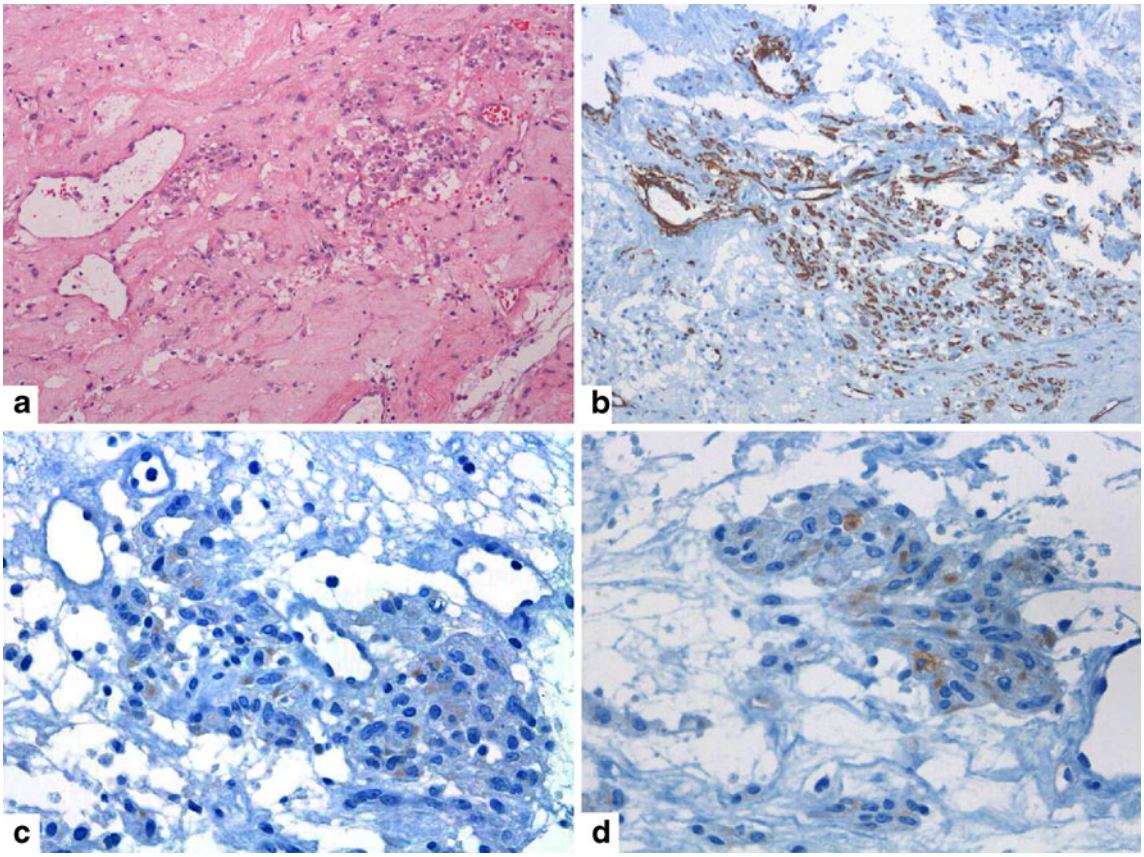

Fig. 3 Tumor treated with imatinib and excised several months after a primary partial response. Detail showing an area of tumor regression, with a marked drop in the number of cells within a hyalinized stroma. HE X 10. B. Residual cells staining positive for smooth muscle actin; the tumor also contains numerous small vessels displaying positive parietal staining. AML X10. C and D. Details showing sparse CD117 (C) and DOG 1 expression (D) in residual tumor cells. X 40

secondary mutations and the duration of imatinib therapy [16]. Secondary mutations have not been observed in GISTs not harboring *KIT/PDGFRA* mutations, or in tumors displaying an unusual morphology or loss of CD117 expression [21]. Two mechanisms have been put forward to account for the development of secondary resistance to imatinib therapy: 1) second-site mutations may stabilize the conformation of the KIT kinase which prevents imatinib binding; 2) second-site mutations may specifically interfere with imatinib binding without affecting the overall KIT kinase conformation. However, the fact that almost 50% of cases display no secondary mutations suggests that additional resistance mechanisms may be involved, such as KIT genomic amplification and the activation of an alternative receptor tyrosine-kinase protein in the absence of KIT expression [20, 22]. In this respect, Agaram et al. [23] have reported the presence of a *BRAF* gene mutation (Val600Glu) in an imatinib-resistant GIST with primary *PDGFRA* mutations, which displayed not only loss of KIT and PDGFRA protein expression but also trans-differentiation into a rhabdomyosarcoma phenotype.

Given the complex nature of secondary mutations, new kinase inhibitors are unlikely to be effective against the whole range of mutant clones. Experience to date with the new kinase inhibitors seems to indicate that, while effective in the short term, they are unlikely to achieve prolonged remission or cure. The rapamycin derivative everolimus, which specifically inhibits mTOR downstream of the AKT pathway, has recently been approved for use in conjunction with kinase inhibitors [24]. At the same time, other complementary therapeutic approaches are currently being explored, including KIT degradation via inhibition of the heat shock protein HSP-90 by IPI 504 (geldanamycin) [25], and transcriptional down-regulation of KIT and CDK by flavopiridol [26].

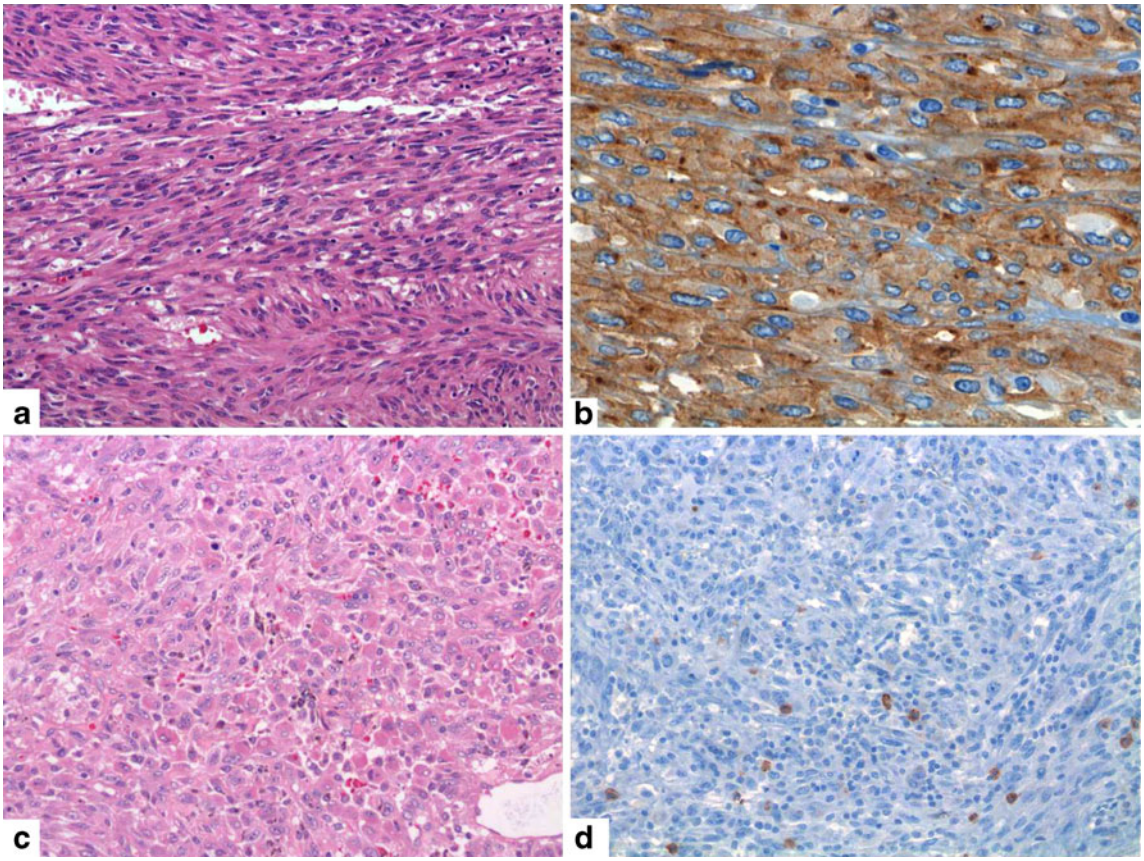

Fig. 4 A and B . Baseline GIST prior to imatinib therapy. **a** Neoplasm comprising spindle cells arranged in a fascicular pattern, containing scant eosinophilic cytoplasm. HE X20. **b** Detail showing positive staining for KIT; note cytoplasmic and Golgi pattern. CD117 X 40. C y D. New tumor growth after partial response to imatinib. **c** Cells displaying an epithelioid phenotype, with abundant eosinophilic cytoplasm. HE x40. **d** Negative staining for CD117; the only positive cells are interstitial mast cells. CD117 X 40

Table 2 KIT mutations with secondary Imatinib resistance (*Gleevec*, Novartis, USA)

KIT 13 (TK1)	KIT 14 (TK1)	KIT 15 (KI)	KIT 16 (KI)	KIT 17 (TK2)
Val654Ala[a]	Thr670Ilr[a]	Asp716Asn	Leu783Val	Cys809Gly
Val654Glu[a]	Thr670Glu[a]			Asp816His
				Asp 816Glu
				Asp816Gly
				del816
				Ls818Arg
				Asp 820Ala[a]
				Asp820Gly[a]
				Asp820Tyr[a]
				Asp 820Glu[a]
				Asp820His[a]
				Asn822Lys[a]
				Asn822Tyr[a]
				Tyr823Asp[a]

[a] most common mutations

Table 3 Multiple KIT mutations in GISTs displaying secondary resistance to Imatinib (*Gleevec*, Novartis, USA)

2 Mutations	3 Mutations	4 Mutations
Val 654 Ala	Val654Ala	Asp816Glu
Thr670Ile	Thr670Glu	Asp820Val
	Tyr823Asp	Asp820Glu
		Asp822Lys
Val654Ala	Asn818Lys	
Asn822Lys	Asn822Lys	
	Tyr823Asp	
Val 654Ala	Asp820Glu	
Asp816His (2)	Asn822Lys	
	Asn822Tyr	
Val654Ala		
Asp820G		
Asn822Tyr		
Cys809Glyn		

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