Case Report

Complete Pathologic Response to Neoadjuvant Imatinib of a Gastrointestinal Stromal Tumour in the Stomach with Concomitant Mutations in KIT

Mariem Ben Rekaya[1,4]*; Feryel Letaief Ksontini[2]*; Emna Harigua-Souiai[3]; Ryma Boujneh[2]; Ahmed Med H'mayada[1]; Mariem Kssentini[1,4]; Mouna Ayadi[2]; Mediha Trabelsi[5]; Ridha M'rad[5]; Soumaya Rammeh[1,4]

[1]Theranostic Biomarkers, UR17ES15, Faculty of Medicine of Tunis, Université Tunis El Manar, Tunisia.

[2]Medical Oncology Department, Salah Azaiez Institute, Tunisia.

[3]Laboratory of Molecular Epidemiology and Experimental Pathology – LR16IPT04, Institut Pasteur de Tunis, Université de Tunis El Manar, Tunis, Tunisia.

[4]Pathology Department, Charles Nicolle Hospital, Tunis, Tunisia.

[5]Department of Hereditary and Congenital Disorders, Charles Nicolle Hospital, Tunis, Tunisia.

Abstract

The Gastrointestinal Stromal Tumors (GISTs) are driven in 90% of cases by mutations in the KIT or PDGFRA proto-oncogene. Pathologic complete response (pCR) of GISTs to neoadjuvant Imatinib is rare and the molecular pathology is not well known. We report the case of a 41-year-old woman with a local gastric GIST. Molecular investigation showed concomitant missense mutations: Thr574Pro, Gln575Pro, Leu576Pro, Pro577Ser in exon 11. After 7 months of neoadjuvant Imatinib therapy, the tumour was downstaged to allow for partial gastrectomy. A histopathological examination revealed total pCR. These amino acid positions have never been reported to be associated with pCR. Molecular modelling and imatinib docking of the wild type versus the mutated model containing the 4 mutations in exon 11 were performed. Results showed a slight direct mutation effect and a more stable model due to the four successive prolines 573, 574, 575, 576 that incur a specific rearrangement in the protein surface to steady a conformation favourable for Imatinib binding. Patients carrying co-occurrence variants represent a heterogeneous subgroup in terms of biological and clinical behaviours. Comprehensive the role and the intrinsic molecular features of sensitive mutations can identify patients who will respond to imatinib.

Keywords: Imatinib response; pCR Co-occurrence; Mutation; KIT; DGFRA.

Introduction

The gastrointestinal stromal tumours «GISTs» are driven in 90% of cases by somatic mutations in the proto-oncogene receptor tyrosine kinase *KIT* also known as (C-KIT, CD117) or the platelet-derived growth factor receptor alpha *PDGFRA* also known as (CD140A; PDGFR2). These two genes are located in the same chromosomal region 4q2, and code for the same sub-family of proteins within the family of receptor tyrosine kinases [1]. Most primary *KIT* mutations in GISTs occur in exon 11 or exon 9, and rarely in the exons13/14 or 17. However, *PDGFRA* mutations are most often on exon 18 (mainly the p.D842V substitution) and rarely exon 12. KIT and PDGFR inhibitions are the primary therapeutic modality for unresectable and metastatic GIST [2]. Imatinib mesylate TKI(s) compete with ATP for the ATP-binding site of several receptor tyrosine kinases. It selectively blocks the activation of KIT and PDGFR receptors [3]. Complete Response (CR) is defined as the disappearance of all lesions without any new lesions [2,4]. GIST's pathological CR (pCR) after neoadjuvant imatinib therapy is rare and its molecular mechanisms are not well defined. Previous molecular findings of reported cases with pCR after neoadjuvant Imatinib showed recurrent deletion that affects specific positions amino acid in *KIT* exon 11. A recent study suggested that the mutation type and affected codon locations of *KIT* predict progression-free survival to first-line Imatinib in GISTs [5]. In this study, we report a case of pCR of a locally advanced gastric GIST treated by neoadjuvant Imatinib with concomitant mutations in exons 11 and 9 of the *KIT* gene.

Case report

Clinical findings

A 41-year-old woman, with a family history of mammary neoplasia, consulted for abdominal pain and recurrent diarrhea pit for 1 month. On clinical examination, there was nothing significant. Ultrasound and Computed Tomography (CT) scan revealed a solid mass of 9 x 10 cm very close to the small-gastric curvature filling the back cavity of the epiploons, evoking a gastric stromal tumor. Fibroscopy showed a sub-mucosal process of the small-sub-cardial curvature. Colonoscopy did not show anomalies. The biopsy showed a spindle cell tumor related to a GIST with CD117 and Dog1 strong immuno-expression. There were no mitoses in the specimen that totalised 30 fields at high magnification (x400) (Figure 1). The tumor was not surgically resectable without mutilating surgery. The decision of the multidisciplinary meeting was to start with neoadjuvant treatment. The patient had a neoadjuvant treatment with imatinib (400 mg/day). Four months after the treatment, a 40% decrease in the volume of the mass and a decrease in its density higher than 15% on the CT scan were observed, which implied a good tumor response according to the CHOI criteria [4]. Seven months after the beginning of the treatment, there was a 10% additional decrease in the tumor size without change in its density. A partial gastrectomy was performed. The Histopathological examination revealed total hyaline fibrous transformation of the GIST without viable residual tumoral cells. The surgical limits were healthy. After surgery, treatment with imatinib was continued in an adjuvant setting to make 3 years of Imatinib. After 48 months, the patient was under treatment with good tolerance and without recurrence.
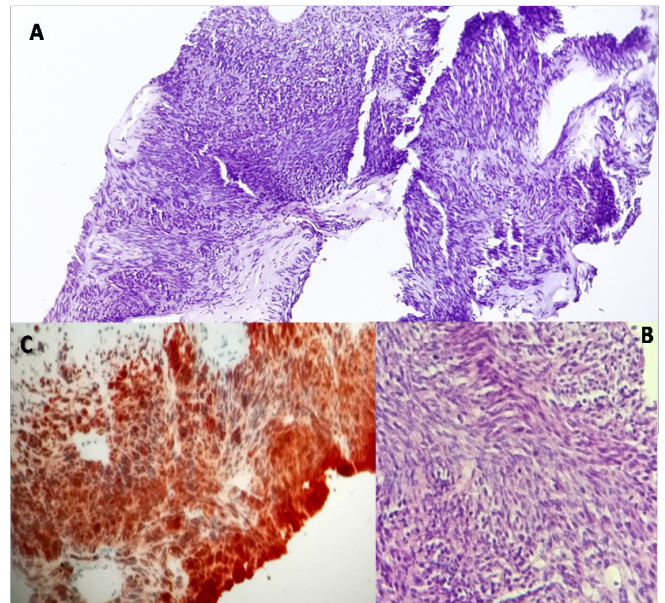

Figure 1: (A) Dense spindle cell proliferation (HEX200); **(B)** Spindle cell proliferation with little atypia and rare mitoses, (HEX400); **(C)** Spindle cells were c-Kit positives (IHCX200).

Molecular findings

Ethical approval according to the Declaration of Helsinki Principles was obtained from the medical ethics committee of the Charles Nicolle Hospital of Tunis. Four FFPE sections of 10 µm thickness from the biopsy specimen were processed for DNA extraction using QIAmp DNA Mini KIT (Qiagen) according to the manufacturer's instructions. Specific primers were designed using the Primer3 software v. 0.4.0. The details of the primer sequences, their annealing temperatures and product sizes are shown in Table 1. Five targeted sequences were amplified by Polymerase Chain Reaction (PCR) using the Qiagen hot start PCR kit. PCR conditions were as follows: 94°C for 15 min, 40 cycles of 94°C for 1 min, 55°C for 35 sec, 72°C for 45 sec and finally 30 min at 72°C. PCR products were purified using the innuPREP PCR pure Kit. PCR sequencing was performed using the Big Dye V.3.1 Terminator Kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified using the reaction Wizard™ MagneSil™ Sequencing

Table 1: List of PCR primers for amplifying and Sanger sequencing of *KIT* exons 9, 11, 17 and *PDGFRA* exons 12 and 18.

Gene	Exon	Primer	Sequence 5'→3'	Ta	Product size
KIT	Exon 9	KIT9F	TCCTAGAGTAAGCCAGGGCTT	55	284 bp
		KIT9R	TGGTAGACAGAGCCTAAACATCC		
	Exon 11	KIT11F	GATCTATTTTTCCCTTTCTCC	55	174 bp
		KIT11R	AGCCCCTGTTTCATACTGAC		
	Exon 17	KIT17F	TACAAGTTAAAATGAATTTAAATGGT	55	228 bp
		KIT17R	AAGTTGAAACTAAAAATCCTTTGC		
PDGFRA	Exon 12	PDGFRA12F	TCCAGTCACTGTGCTGCTTC	55	260 bp
		PDGFRA12R	GCAAGGGAAAAGGGAGTCTT		
	Exon 18	PDGFRA18F	ACCATGGATCAGCCAGTCTT	57	247 bp
		PDGFRA18R	GGAGGATGAGCCTGACCAG		

Ta: annealing temperature; bp: base pair

Table 2: The HGVS annotation of all somatic identified variants (pathogenic and polymorphism) and in silico-predicted function.

Genes	Target regions	Genomic descriptions	Transcript descriptions	Protein descriptions	Types	Mutation taster prediction	Rs numbers/Cosmic	Clin Var (references previously described the variant)
Kit	Exon 9	NC_000004.11:g.55592181C>A	NM_000222.2(KIT_v001):c.1505C>A	NM_000222.2(KIT_i001):p.(Ala502Asp)	Non synonyme	Disease causing	Unknown	Unknown
		NC_000004.11:g.55592224T>A	NM_000222.2:c.1540+8T>A	Protein features might be affected	Splice site changes Donor increased	Disease causing	Unknown	Unknown
		1-NC_000004.11:g.55593654A>C	NM_000222.2(KIT):c.1720A>C	NM_000222.2(KIT_i001):p.(Thr574Pro)	Non-synonymous	Disease causing	Unknown	Unknown
		2-NC_000004.11:g.55593658A>C	NM_000222.2:c.1724A>C	NM_000222.2(KIT_i001):p.(Gln575Pro)	Non-synonymous	Disease causing	Unknown	Unknown
	Exon 11	3-NC_000004.11:g.55593661T>C	NM_000222.2(KIT_v001):c.1727T>C	NM_000222.3(KIT_i001):p.(Leu576Pro)	Non-synonymous	Disease causing	rs121913513 COSM1290	Likely Pathogenic described at germinal and somatic levels [40-43].
		4-NC_000004.11:g.55593663C>T	NM_000222.2(KIT_v001):c.1729C>T	NM_000222.3(KIT_i001):p.(Pro577Ser)	Non-synonymous	Disease causing	COSM1293HGMD CI050498	Previously described in leukemia patients [26].
	C TJS EX11	5-NC_000004.11:g.55593665T>C	NM_000222.2(KIT_v001):c.1731T>C	NM_000222.2(KIT_i001):p.(=)	Synonymous	Disease causing with splice site effect	Unknown	No
	Exon 17	NC_000004.12:g.54733224C>A	NM_000222.2(KIT_v001):c.2484+32C>A		Intronic	Polymorphism	Unknown	Unknown
		NC_000004.12:g.54733236A>T			Intronic	Polymorphism	unknown	Unknown
		NC_000004.12:g.54733241C>A			Intronic	Polymorphism	rs201112416	Unknown
PDGFRA	Exon 12	NC_000004.11:g.55152143T>A	NG_009250.1(PDGFRA_v001):c.2562+13T>A		Intronic	Polymorphism	unknown	Unknown
		NC_000004.11:g.55152144T>A	NG_009250.1(PDGFRA_v001):c.2562+14T>A		Intronic	Polymorphism	unknown	Unknown
		NC_000004.11:g.55152148T>A	NG_009250.1(PDGFRA_v001):c.2562+18T>A		Intronic	Polymorphism	unknown	Unknown
		NC_000004.11:g.55152153G>C	NG_009250.1(PDGFRA_v001):c.2562+23G>C		Intronic	Polymorphism	unknown	Unknown
		NC_000004.11:g.55152154G>A	NG_009250.1(PDGFRA_v001):c.2562+24G>A		Intronic	Polymorphism	unknown	Unknown

Reaction Clean-Up System and sequencing was performed in an ABI Prism 3500 sequencer (Applied Biosystems). The five hot spot regions, including the exons 9, 11 and 17of *KIT* and 12 and 18 of *PDGFRA* and their flanking regions were simultaneously analyzed.

Sequence analysis showed the co-occurrence of 6 coding and one intronic variation in the KIT gene. Exon 9 contained two variations: the first was a missense variant, p.Ala502Asp not previously described and the second was an intronic variant predicted by mutation taster as a splice donor variant (c.1540+8T>A). For exon 11, there were 5 coding variants. Two are known, the Leu-576Pro (rs121913513 or COSM1290) and the Pro577Ser (HGM-DCI050498). Three have not been described. Two were missense variants (Gln575Pro and Thr574Pro) and one was a silencing variant (c.1731T>C; Pro577Pro). All variations identified in *KIT* exon 17 and PDGFRA exon 12 were polymorphisms with neutral effects (Table 2).

Molecular modelling

Comparative modelling of the mutated KIT protein structure using the Modeller software [6] was done. The 1T46 PDB entry of the KIT structure co-crystallized with the STI-571 inhibitor (imatinib) was considered a template. The latter structure contains multiple truncated regions. It starts at residue Gly565 and ends at residue ASN933. Only mutations on the juxta-membranous (JM) domain were included (Thr574Pro, Gln575Pro, Leu576Pro, Pro577Ser). The best scored model was further refined using the Galaxy Refine 2 tool. Then, structural alignment of the refined model and the reference structure 1T46 using PyMOL (Schrodinger, LLC. 2010) was performed. The mutated KIT model showed no conformational changes at the binding site of STI-571 (Figure 2).

Figure 2: The structure of KIT protein. Panel (A) is a representation of the protein surfaces. On the left, the 1T46 structure and on the right the mutated KIT protein model. Panel (B) is a cartoon representation of the 1T46 (on the left) and the mutated KIT model (on the right) with a zoom on the mutated residues shown in green carbon licorice representation. The STI-571 molecule is shown in pink carbon licorice on all sub-figures.

Molecular docking of imatinib into its binding site on the 1T46 structure, then on the mutated KIT model using AutoDock 4.2 was performed. Input files of the receptors and ligand were prepared using AutoDock Tools. The crystal pose was re-obtained with estimated binding energy of -14.15 kcal/mol. The best docking pose on the mutated KIT model was -12.89 kcal/mol. Since the standard deviation of this scoring function was +/-2 kcal/mol, both docking scores were considered equivalent. This confirms that the mutations on the JM domain had little to no effect on the binding mode of imatinib on the KIT protein (Figure 2).

Discussion

We report a case of a pCR to neoadjuvant imatinib of a gastric GIST. Our literature review showed that there are only 23 reported GISTs with pCR after neoadjuvant imatinib [7-24]. Mutation analyses were reported in 16 of these cases. All reported cases had deletion mutations that affected residues between 550 and 559 positions (Table 3). Only one case has co-existence of two mutations: deletion of residues 558 and 559 plus missense mutation W557C [16]. Our case displayed substitution mutations that affected codons 574, 575, 576 and 577 and a mutation in exon 9 codon 502. Both mutations in exon 11, that encode for the JM, and mutations in exon 9, that encode for the extracellular domain of KIT, allow receptor dimerisation in the absence of a ligand, thus resulting in a conformational change that relieves the suppression of the activation loop of the kinase domain [25]. The co-occurrence of sensitive mutations could increase imatinib sensitivity and explain the pCR in this study. Unfortunately, it is impossible to know if there are cis mutations (in the same allele), trans mutations (in different alleles) in distinct clones within the same tumour or even in the same tumour cell. Two mutations (Leu576Pro, rs121913513, COSM1290) and (Pro577Ser, COSM1293, HGMD CI050498) have been previously described. The Leu576Pro mutation has been found in many cancers, mainly in GISTs [26], leukemia cells [26], melanomas [27] and thymic carcinomas [28]. Pro577Ser mutation has been found in melanomas and at germinal level in leukemia patients [26].

All variations found in this study in *PDGFRA* are polymorphisms without any functional effect. It is currently admitted that mutations of *KIT* and *PDGFRA* are mutually exclusive in primary untreated GISTs [29]. Previous studies showed that the JM domain of KIT inhibits kinase activity by maintaining the receptor in an inactive conformation. Mutations in exon 11, characteristic of GISTs, lead to destabilization of the JM domain, which tends towards a more extensive conformation and can no longer exercise its regulatory activity. The kinase is then activated constitutively independently of its ligand; although a small proportion of kinase remains in self-inhibiting conformation because the mutations are in the heterozygous state [30].

Molecular modelling of the four concomitant mutations in this study suggests that the effect of the mutations of the JM domain on STI-571 efficacy is indirect. These mutations would infer a series of 4 consecutive prolines at the sequence level: a Pro573 (non mutated) followed by three Proline residues at positions 574, 575, 756 (mutated). Prolines are underrepresented in proteins, but are frequent mutations that increase protein stability [31]. Multiple (3 or more) prolines incur a specific rearrangement on the protein surface. In the case of KIT, this may lead to stabilising the JM
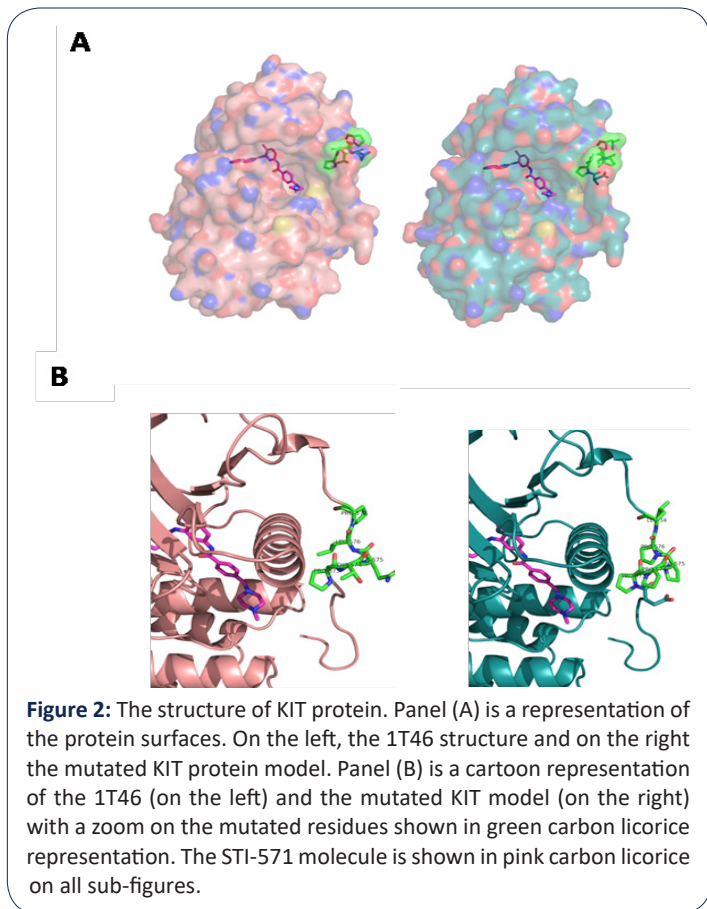
domain in a conformation favourable for STI-571 binding. Such Proline-induced molecular mechanisms have been described in other systems [32].

The therapeutic response of GISTs harbouring multiple driver or concomitant mutations in the same gene is not well known. A recent study found that a cell line of non-small-cell lung cancer with two copies of *EGFR* mutations was markedly more sensitive to EGFR-TKIs compared with parent cells with KRAS mutation alone and suggests that the presence of concomitant *EGFR* mutations affect the TKI response [33]. Studies, used high - throughput sequencing and deep sequencing, reclassified considered wild-type GISTs as *KIT* or *PDGFRA* mutated [34] and identified concomitant mutations in two downstream effectors: *BRAF* and *FGFR3* in *KIT* mutated tumours and *PIK3CA* and *KRAS* in *KIT/PDGFRA* wild-type GIST [35]. Braggio and al [36] identified complex mutations with five concomitant in-frame deletions and insertions and one in-frame deletion plus missense mutation in exon 11 of *KIT* that mainly affect codons 577 and 578 and suggested that GISTs with complex deletion and insertion *KIT* mutations have poor prognosis [36]. More recent studies showed the aggressive biology of mutation of codons 557/558 deletions/delins of exon 11

than downstream or upstream mutations of these codons in the metastatic setting and allow for prediction at the baseline, which GIST patients would develop resistance to first-line imatinib treatment earlier. Additionally, patients with deletions or delinsertion regardless of codon regions, had a significantly better complete response rate (557/558: 40%; other codons: 37.5%) compared to patients with other pathogen variants [5].

Imatinib-sensitive biomarkers of GISTs: KIT expression (CD117), *KIT* mutations in exons 9 and 11, *PDGFRA* mutations in exons 12 and 18. However, resistance biomarkers are *BRAF* mutation [37], secondary mutation in the ATP binding domain or the activation-loop domain of *KIT* (exon 13 and 14 and rarely 17) [38], or codon 842 in exon 18 of *PDGFRA*, over expression of KIT in NF1 associated GIST cells and loss of KIT expression [39] accompanied by activation of alternative pathways. A Comprehensive of all factors and mechanisms that could influence the imatinib response is crucial to better improve patients management.

Table 3: Review of the previously reported cases of locally advanced and/or or metastatic gastrointestinal stromal tumours with imatinib pathologic complete response.

Reference	Number of patients	NAD (m) *	Resected (n)	pCR** (n)	Site	Molecular findings in pCR patients	Localadvanced/Metastasis
[7]	126	10	17	2	6 colon, 4 stomach, 6 small bowel, 1 unknown	NR***	NR
[8]	90	12,2	12	1	42 stomach, 42 small bowel, 8 large bowel, 8 retroperitoneum	NR	Metastatic and or locally advanced
[9]	1	6	1	1	Stomach	W557_V559delins KIT exon 11	Liver metastasis
[10]	1	13	1	1	Small Bowel	NR	Liver metastasis
[11]	180	12	22	2	Rectum	NR	Liver metastasis
[44]	141	14	32	3	NR	NR	Metastatic and/or locally advanced
[13]	1		1	1	Stomach	NR	liver metastases
[14]	1	12	1	1	Rectum	6 pb deletion in KIT exon11	Locally advanced
[15]	46	12.9	11	1	NR	NR	Locallyadvanced primary GIST
[16]	2	2	2	1	Rectum	KIT exon 11 deletion of residues 558 and 559 plus W557C	locally advanced GIST
[17]	1	2,5	1	1	Rectum	NR	Locally advanced not metastatic
[18]	1	18	1	1	Rectum	NR	Locally advanced pelvic
[19]	9	01-Jun	6	1	Rectum	NR	Locally advanced not metastatic
[20]	1	18	1	1	Stomach	NR	Locally advanced
[21]	1	10	1	1	Extra intestinal location	NR	Locally advanced with liver metastasis
[22]	1	NR	1	1	Extra-gastrointestinal stromal tumor (rectum and bladder)	NR	Locally advanced
[23]	171	12.5/4.4-62.4	26	12	9 Stomach 11 small bowel 5 colon-rectum 1 other	KIT deletion mutation at codons 550-558 in exon 11 in all 12 patients	Locally advanced /metastatic
[24]	1	6	1	1 Near pCR	Large gastric (cardia, distal pancreas, and splenic hilum)	K558 deletion of KIT exon 11	Locally advanced
This study	1	7	1	1	Stomach	Exon 9:Ala502Asp; Exon 11:Thr574Pro, Gln575Pro, Leu576Pro, Pro577Ser	Locally advanced not metastatic

*NAD (m): Median duration of neoadjuvant Imatinib in months; ** pCR: pathologic Complete Response; ****Near pCR: viable cells <5 %, ***NR: Not reported.

Conclusion

We report a rare of pCR to neoadjuvant imatinib of a gastric GIST that harboured co-occurence of multiple *KIT* mutations in exon 11 and 9, suggesting that these amino acid modifications increase the sensitivity of GISTs to Imatinib. Molecular studies of large series with functional analyses are needed to understand GIST's complete response mechanisms.

References

1. Lasota J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. Histopathology. 2008; 53: 245-266.

2. Desai J. Response assessment in gastrointestinal stromal tumor. Int J Cancer. 2011; 128: 1251-1258.

3. Kee D, Zalcberg JR. Current and emerging strategies for the management of imatinib-refractory advanced gastrointestinal stromal tumors. Ther Adv Med Oncol. 2012; 4: 255-270.

4. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol. 2007; 25: 1753-1759.

5. Incorvaia L, Fanale D, Vincenzi B, De Luca I, Bartolotta TV, et al. Type and Gene Location of KIT Mutations Predict Progression-Free Survival to First-Line Imatinib in Gastrointestinal Stromal Tumors: A Look into the Exon. Cancers (Basel). 2021; 13.

6. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol. 1993; 234: 779-815.

7. Scaife CL, Hunt KK, Patel SR, Benjamin RS, Burgess MA, et al. Is there a role for surgery in patients with «unresectable» cKIT+ gastrointestinal stromal tumors treated with imatinib mesylate? Am J Surg. 2003; 186: 665-669.

8. Bauer S, Hartmann JT, de Wit M, Lang H, Grabellus F, et al. Resection of residual disease in patients with metastatic gastrointestinal stromal tumors responding to treatment with imatinib. Int J Cancer. 2005; 117: 316-325.

9. Melichar B, Voboril Z, Nozicka J. Pathological complete response in advanced gastrointestinal stromal tumor after imatinib therapy. Intern Med. 2005; 44: 1163-1168.

10. Chacón M, Roca E, Huertas E, Loria FS, Domenechini E. Dramatic Responses to Therapy in Rare Tumors. Journal of Clinical Oncology. 2005; 23: 1580-1582.

11. Bonvalot S, Eldweny H, Péchoux CL, Vanel D, Terrier P, et al. Impact of surgery on advanced gastrointestinal stromal tumors (GIST) in the imatinib era. Ann Surg Oncol. 2006; 13: 1596-1603.

12. Rutkowski P, Gronchi A, Hohenberger P, Bonvalot S, Schöffski P, et al. Neoadjuvant imatinib in locally advanced gastrointestinal stromal tumors (GIST): the EORTC STBSG experience. Ann Surg Oncol. 2013; 20: 2937-2943.

13. Suzuki S, Sasajima K, Miyamoto M, Watanabe H, Yokoyama T, et al. Pathologic complete response confirmed by surgical resection for liver metastases of gastrointestinal stromal tumor after treatment with imatinib mesylate. World J Gastroenterol. 2008; 14: 3763-3767.

14. Salazar M, Barata A, André S, Venâncio J, Francisco I, et al. First report of a complete pathological response of a pelvic GIST treated with imatinib as neoadjuvant therapy. Gut. 2006; 55: 585-586.

15. Andtbacka RH, Ng CS, Scaife CL, Cormier JN, Hunt KK, et al. Surgical resection of gastrointestinal stromal tumors after treatment with imatinib. Ann Surg Oncol. 2007; 14: 14-24.

16. Mandalà M, Pezzica E, Tamborini E, Guerra U, Lagonigro SM, et al. Neoadjuvant imatinib in a locally advanced gastrointestinal stromal tumour (GIST) of the rectum: a rare case of two GISTs within a family without a familial GIST syndrome. European Journal of Gastroenterology & Hepatology. 2007; 19: 711-713.

17. Hou YY, Zhou Y, Lu SH, Qi WD, Xu C, et al. Imatinib mesylate neoadjuvant treatment for rectal malignant gastrointestinal stromal tumor. World J Gastroenterol. 2009; 15: 1910-1913.

18. de Azevedo C, Paiva T, Rossi B. Pathologic complete response with neoadjuvant imatinib for locally advanced pelvic GIST. Int J Clin Oncol. 2011; 16: 279-283.

19. Machlenkin S, Pinsk I, Tulchinsky H, Ziv Y, Sayfan J, et al. The effect of neoadjuvant Imatinib therapy on outcome and survival after rectal gastrointestinal stromal tumour. Colorectal Dis. 2011; 13: 1110-1115.

20. El Mesbahi O, Brahmi SA, Akasbi Y, El Mrabet FZ, Touyar A, et al. Pathological complete response in advanced gastric stromal tumor after imatinib mesylate therapy: a case report. J Med Case Rep. 2011; 5: 197.

21. Quezada N, Acevedo F, Marambio A, León F, Galindo H, et al. Complete pathological response to Imatinib mesylate in an extraintestinal gastrointestinal stromal tumor. Int J Surg Case Rep. 2014; 5: 681-685.

22. Rediti M, Pellegrini E, Molinara E, Cerullo C, Fonte C, et al. Complete pathological response in advanced extra-gastrointestinal stromal tumor after imatinib mesylate therapy: a case report. Anticancer Res. 2014; 34: 905-907.

23. Cheng C, Tsai C, Yeh C. Clinical significance of pathological complete response in patients with metastatic gastrointestinal stromal tumors after imatinib mesylate treatment-lessons learned. Anticancer Res. 2014; 34: 6617-6625.

24. Brown JB, Pai RK, Burgess MA, Chennat J, Zureikat AH. Pathologic Complete Response in a Large Gastric GIST: Using Molecular Markers to Achieve Maximal Response to Neoadjuvant Imatinib. J Natl Compr Canc Netw. 2018; 16: 1424-1428.

25. Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011; 11: 865-878.

26. Hussain SR, Babu SG, Naqvi H, Singh P, Mahdi F. The Frequency of Mutations in Exon 11 of the c-kit Gene in Patients With Leukemia. Turk J Haematol. 2012; 29: 10-16.

27. Lasota J, Kowalik A, Felisiak-Golabek A, Zięba S, Waloszczyk P, et al. Primary malignant melanoma of esophagus: clinicopathologic characterization of 20 cases including molecular genetic profiling of 15 tumors. Modern Pathology. 2019; 32: 957-966.

28. Yoh K, Nishiwaki Y, Ishii G, Goto K, Kubota K, et al. Mutational status of EGFR and KIT in thymoma and thymic carcinoma. Lung Cancer. 2008; 62: 316-320.

29. Mei L, Smith SC, Faber AC, Trent J, Grossman SR, et al. Gastrointestinal Stromal Tumors: The GIST of Precision Medicine. Trends Cancer. 2018; 4: 74-91.

30. Chan PM, Ilangumaran S, La Rose J, Chakrabartty A, Rottapel R. Autoinhibition of the kit receptor tyrosine kinase by the cytosolic juxtamembrane region. Mol Cell Biol. 2003; 23: 3067-78.

31. Trevino SR, Schaefer S, Scholtz JM, Pace CN. Increasing protein conformational stability by optimizing beta-turn sequence. J Mol Biol. 2007; 373: 211-218.

32. Gaddie KJ, Kirley TL. Proline residues link the active site to transmembrane domain movements in human nucleoside triphosphate diphosphohydrolase 3 (NTPDase3). Purinergic Signal. 2010; 6: 327-337.

33. Tsukumo Y, Naito M, Suzuki T. Influence of EGFR-activating mutations on sensitivity to tyrosine kinase inhibitors in a KRAS mutant non-small cell lung cancer cell line. PLoS One. 2020; 15: e0229712.

34. Astolfi A, Indio V, Nannini M, Saponara M, Schipani A, De Leo A, et al. Targeted Deep Sequencing Uncovers Cryptic KIT Mutations in KIT/PDGFRA/SDH/RAS-P Wild-Type GIST. Front Oncol. 2020; 10: 504.

35. Mavroeidis L, Metaxa-Mariatou V, Papoudou-Bai A, Lampraki AM, Kostadima L, et al. Comprehensive molecular screening by next generation sequencing reveals a distinctive mutational profile of KIT/PDGFRA genes and novel genomic alterations: results from a 20-year cohort of patients with GIST from north-western Greece. ESMO Open. 2018; 3: e000335.

36. Braggio E, Braggio Dde A, Small IA, Lopes LF, Valadão M, et al. Prognostic relevance of KIT and PDGFRA mutations in gastrointestinal stromal tumors. Anticancer Res. 2010; 30: 2407-14.

37. Agaram NP, Wong GC, Guo T, Maki RG, Singer S, et al. Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. Genes Chromosomes Cancer. 2008; 47: 853-859.

38. Antonescu CR, Besmer P, Guo T, Arkun K, Hom G, et al. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res. 2005; 11: 4182-4190.

39. Mearadji A, den Bakker MA, van Geel AN, Eggermont AM, Sleijfer S, et al. Decrease of CD117 expression as possible prognostic marker for recurrence in the resected specimen after imatinib treatment in patients with initially unresectable gastrointestinal stromal tumors: a clinicopathological analysis. Anticancer Drugs. 2008; 19: 607-612.

40. Vale Rodrigues R, Santos F, Pereira da Silva J, Francisco I, Claro I, et al. A case of multiple gastrointestinal stromal tumors caused by a germline KIT gene mutation (p.Leu576Pro). Fam Cancer. 2017; 16: 267-270.

41. Neuhann TM, Mansmann V, Merkelbach-Bruse S, Klink B, Hellinger A, et al. A novel germline KIT mutation (p.L576P) in a family presenting with juvenile onset of multiple gastrointestinal stromal tumors, skin hyperpigmentations, and esophageal stenosis. The American journal of surgical pathology. 2013; 37: 898-905.

42. Park J, Yoo HM, Sul HJ, Shin S, Lee SW, Kim JG. Genetic Characterization of Molecular Targets in Korean Patients with Gastrointestinal Stromal Tumors. J Gastric Cancer. 2020; 20: 29-40.

43. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016; 374: 2209-2221.

44. Rutkowski P, Nowecki Z, Nyckowski P, Dziewirski W, Grzesiakowska U, et al. Surgical treatment of patients with initially inoperable and/or metastatic gastrointestinal stromal tumors (GIST) during therapy with imatinib mesylate. J Surg Oncol. 2006; 93: 304-311.