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Gastrointestinal stromal tumours: an actualized overview

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SUMMARY

The gastrointestinal stromal tumour (GISTs), the most common mesenchymal neoplasm in the gastrointestinal tract, has been the subject of great interest in recent years in terms of prognosis, diagnosis and treatment. Its etiology is linked to the mutation of *c-KIT* and *PDGFRA* genes, although between 5 and 15% show no signs of such mutations. It is still diagnosed using immunohistochemical staining. The first line of treatment continues to be surgery, although advances in the molecular biology of GISTs are facilitating the development of new treatment strategies. Those that act by regulating tyrosine kinase activity are of particular interest. Drugs such as imatinib and sunitinib have improved the prognosis of these patients, although the development of resistance constitutes one of the main limitations of the treatment. The aim of this review is to present an up-to-date overview of the main etiopathogenic, diagnostic and therapeutic aspects of these tumours.

Key words: Gastrointestinal stromal tumours – Mesenchymal neoplasm – *KIT* gene – Drug resistance – Patents – Nanotechnology

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INTRODUCTION

Gastrointestinal stromal tumours (GISTs) account for at least 1% of gastrointestinal neoplasms and they are the most common mesenchymal neoplasms (80%) of the digestive tract (Søreide et al., 2016). They are currently defined as mesenchymal tumours of fusiform, epithelioid or pleomorphic cells in the digestive tract, with a mutated KIT (CD 117) expression. They present a mutation in the *KIT* gene, which codifies a tyrosine kinase receptor, or in the gene that codes for the platelet-derived growth factor receptor alpha (PDGFRα) (Doyle et al., 2014). Nevertheless, there are GISTs that are KIT-negative and therefore difficult to diagnose (Demetri et al., 2004). Its incidence is variable, and there are studies in the United States with 10 to 20 cases per one million inhabitants a year (Søreide et al., 2016). The average age is between 66 and 69, regardless of gender. Its clinical presentation depends on its location, the most common symptom being abdominal pain (50%-70% of cases) (Barrios et al., 2015). Its most common location is the stomach (40-60%) and the small intestine (30-40%). Other locations such as colon, rectum, oesophagus or even pancreas are rare, predominating in these structures the presence of adenocarcinomas (Abbruzzese et al., 2004; Jiménez et al., 2016). Finally, 10-15% of GISTs manifest themselves as a metastatic illness, particularly in the peritoneum and liver (Kocakova et al., 2015).

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ETIOLOGY OF GISTS

GISTs originate in the interstitial cell of Cajal (Nilsson et al., 2005). Hirota et al. (1988) found an overexpression of KIT (94%), of CD34 (82%) or both (78%) in 58 mesenchymal tumours. KIT mutations keep the protein activated without the need for a ligand.

The KIT protein, a transmembrane glycoprotein, with the function of type III tyrosine kinase receptors, is codified by the *c-KIT* gene that contains 21 exons and is found on chromosome 4q12. It is expressed in haematopoietic stem cells, melanocytes, mastocytes, germ cells and interstitial cells of Cajal. KITs have an extracellular (EC) domain with ligand binding (SCF) and a cytoplasmatic domain with a juxtamembrane (JM) region, and two tyrosine kinase (TK1, TK2) domains separated by a kinase insertion. The juxtamembrane domain inhibits its activity in the absence of a ligand (Lasota and Miettinen, 2008). The dimerisation of the receptor activates its intrinsic tyrosine kinase activity and the autophosphorylation of its multiple tyrosine residues (568, 570, 703, 721, 730, 823, 900 and 936), which activates proliferation, apoptosis, chemotaxis and cell adhesion signaling cascades (Downs-Kelly and Rubin, 2011, Heinrich et al., 2002) such as PI3K (Phosphatidylinositol 3-kinase), Src (protein of the tyrosine kinases family), RAS-RAF-MAP kinases that include ERK, JNK and p38 MAPK, JAK/STAT (cytoplasmatic tyrosine kinases) and phospholipase C (a membrane enzyme) (Fig. 1). Platelet-derived growth factor receptor (PDGFRα) is expressed on progenitor myeloid and erythroid cells in the bone marrow, as well as on monocytes, fibroblasts, megakaryocytes, endothelial cells, glial cells and osteoblasts. The binding of its ligand (PDGFs) dimerises the receptor and activates its kinase activity, also regulating cellular proliferation (Lasota and Miettinen, 2008).

KIT or PDGFRα mutations promote constitutive activation in the absence of ligands (Heinrich et al., 2002). JM domain mutations affect the self-regulation function (gain-of-function mutation), whereas EC domain mutations affect dimerisation and promote spontaneous activation. TK domain mutations affect their enzyme activity (Heinrich et al., 2002).

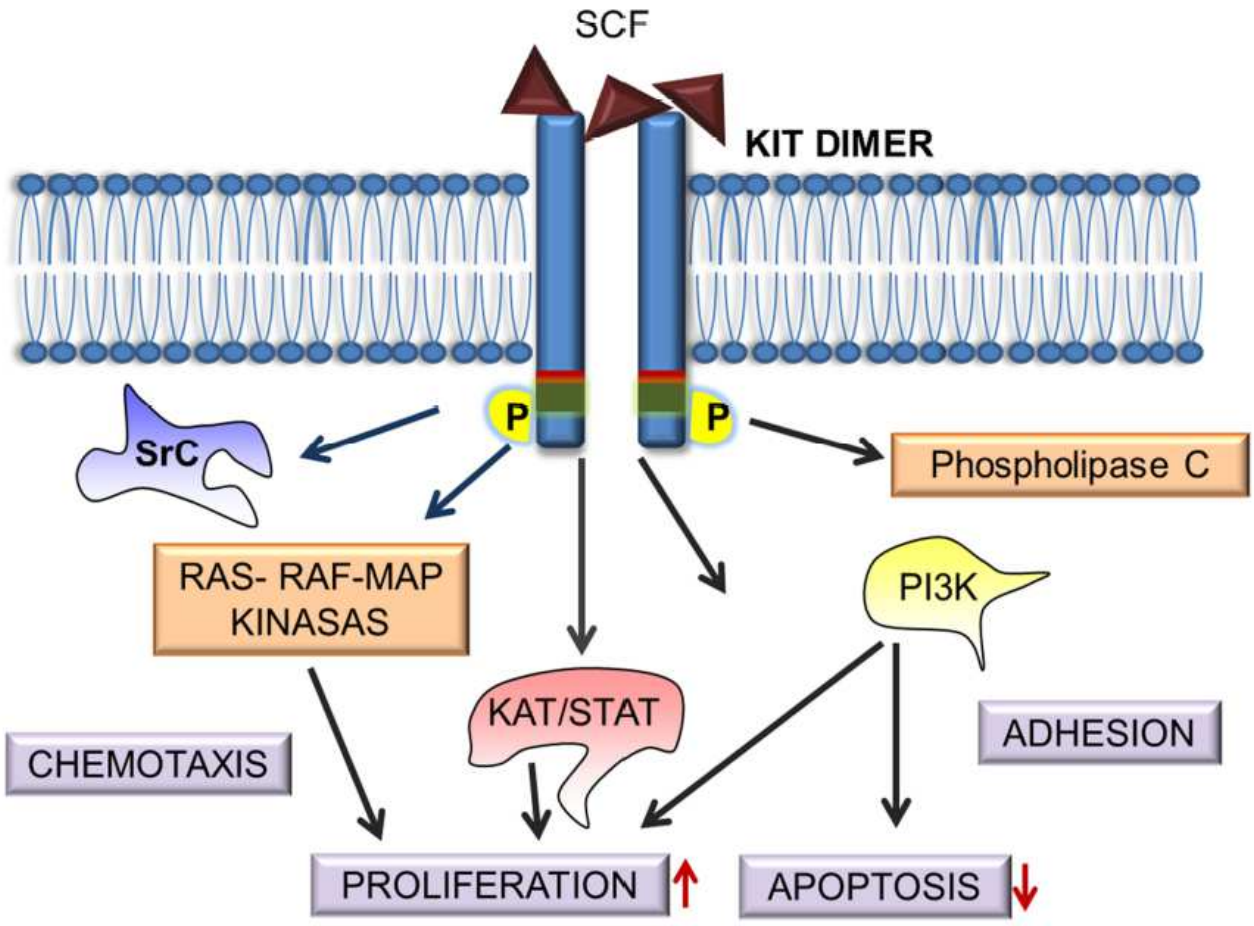

Fig 1. Physiological KIT activation is triggered by the binding of its ligand Stem Cell Factor (SCF). Dimerisation of the receptor takes place and kinase domains are activated. The phosphorilation of tyrosine residues in KIT receptors activates intracellular signaling pathways, as the PI3-K/AKT, RAS/RAF/MAPK and JAK/STAT, controlling cell proliferation, adhesion, apoptosis, survival and differentiation. Mutations keep the receptor active even without ligand binding.

The majority of GISTs are KIT-positive (95%). The most common mutations are located on exon 11 (60-70%), 9 (5-15%), 13 (≈1%) and 17 (≈1%), and occur in the JM domain (exon 11) (68%). PDGFRα (≈5%) mutations affect the TK2 domain (exon 18) (6%) (Lasota and Miettinen, 2008). A recent study detected KIT and PDGFRA mutations in 119 (53.8%) and 56 (25.3%) GIST patients, respectively (Capelli et al., 2016). However, as it was stated in these studies, variations in the methodology must be taken into account.

Other molecular alterations associated with GISTs are those that occur in the insulin growth factor (IGF), the stem cell factor (linked with imatinib resistance), the transforming growth factor β (TGF-β/Smad), SnoN, p21(WAF1)/CIP1 and p27 (KIP1)78 (Hayashi et al., 2013, Nannini et al., 2014). Wilms' tumour-1 (WT-1) has been found to be overexpressed in GISTs (Kang et al., 2010). There is a certain predisposition towards GISTS development among patients with neurofibromatosis type 1, Carney-Stratakis syndrome and Carney's triad (Bachet and Emile, 2010). In any case, the molecular bonds are complex. Thus, mutations on exon 11 have been linked with CD133 (Arne et al., 2011, Chen et al., 2012). Authors such as Mikami et al, (Mikami et al., 2013) suggest that exon 11 mutation may be a preliminary event to the development of the tumour.

DIAGNOSIS OF GISTS

Small GISTs are normally asymptomatic and are diagnosed by accident. Table 1 summarises some of the diagnostic features of GIST. There are no specific symptoms and they are linked to the tumour being located in the gastrointestinal cavity (Yacob et al., 2015). Computed tomography with contrast is the diagnostic method of choice since it allows the tumour mass to be located and the local and metastatic invasion to be detected. The tumour shows up as a solid mass with hyperdense attenuation. Positron emission tomography with 18-fludeoxyglucose (18FDG-PET) distinguishes between the scar tissue and the tumour, and allows the response to treatment to be assessed. In GISTs, 18FDG-PET is useful for stratifying the illness, assessing its spread and response to treatment (modulation of glucose metabolism). The F18-FDG-PET/CT combination has been seen as a reliable method for restaging GISTs (Bertagna et al., 2010). Endoscopic ultrasound is used for the differential diagnosing of GISTs (Pavlovic Markovic et al., 2012). In addition, laparoscopy is used in the resection of the tumour and its diagnosis (Severino et al., 2016).

Table 1. Useful differential diagnostic features of GISTs.

Criteria	GIST
Clinical criteria	Gastrointestinal tract location (> 95%) Presence of peritoneal and liver metastasis Absense of lymphatic and lung metastasis
Histopathologic and immunohistochemical criteria	Epithelioid, fusiforms, or both cells Positive immunostaining (95%) for KIT 95% Positive immunostaining (70%) for CD34 Negative immunostaining (95%) for desmin Negative immunostaining (95%) for S100
Mutations	KIT mutations (85%) PDGFRA mutations (5%)

The differential diagnosis is based on immunohistochemical tests (95% are c-KIT positive). Those that are c-KIT negative (5%) are difficult to diagnose and require the study of KIT or PDGFRA mutations. CD 117 expression is currently considered a specific GISTs marker, although 5-10% of cases are CD 117 negative, and so the use of PDGFRA, PKC0, and DOG1 is necessary (Gonzalez-Campora et al., 2011). Novelli et al. (2010) believe that testing positive for CD117 and DOG1 confirms GISTs diagnosis. The latter marker shows little immunoreactivity in other mesenchymal and non-mesenchymal tumours (Lee et al., 2010). Finally, it has been found that carbonic anhydrase isoenzyme II is overexpressed in 95% of GISTs (Parkkila et al., 2010).

ASSESSING DISEASE PROGRESSION IN GISTS

The monitoring of the response to imatinib treatment is controversial. The protocols that assess the response to treatment according to tumour size measure one (RECIST criteria) or two aspects (SWOG criteria). However, response to imatinib involves a decline in tumour density followed by a slow decrease in tumour size, which is not included in the criteria above. An alternative is the Choi criteria, which combine CAT and PET imaging in patients with metastatic GISTs, incorporating the density of the tumour (CHOI criterion) (Choi et al., 2007). The response rate using CHOI is similar to that of PET, and is more closely linked with time to progression and survival rate. Furthermore, tumours have an increased glucose consumption, therefore positron emission tomography with 18-FDG may show changes in metabolism that allow the activity and response to be assessed using a semi-quantitative method such as SUV (standardised uptake value). The European Organisation for Research and Treatment of Cancer (EORTC) has developed metabolic response criteria for assessing tumours with FDG-PET, which have prognostic value for the time to progression and overall survival. The EORTC established the response criteria through PET (Young et al., 1999), defining partial response as a ≥ 25% reduction in the maximum SUV. Among those who respond well to imatinib, the absolute SUV falls to below 2.5, which shows a significant correlation

with a higher progression-free survival rate (Van den Abbeele et al., 2002).

Analyses of 172 injuries of 40 patients with metastatic GISTs treated with imatinib (Choi et al., 2007) show that changes in tumour size and density are correlated with changes in the maximum SUV of the PET. Those whose maximum SUV declined by >70% or whose absolute SUV fell by <2.5 after two months of treatment with imatinib were defined as responding well to PET. Therefore, in the CAT scans, the decrease in the attenuation coefficient or average density measured in Hounsfield units (HU) and the decrease in tumour size are linked with a good response determined by PET (Choi et al., 2007). The Choi Criteria, which include tumour density and variations in tumour size in the CAT, are more sensitive to the imatinib response and have a higher correlation with time to progression and survival. Recently, Hensley et al. (2014) have shown the usefulness of near-infrared (NIR) imaging probes together with three-dimensional fluorescence molecular tomography (FMT) in GISTs-T1 xenografts as an alternative for assessing the response to treatment with imatinib.

PROGNOSTIC FACTORS IN GISTS

The most important prognostic factors in GISTs are tumour size, mitosis index and location (Demir et al., 2013; Kataoka et al., 2013; Cannanzi et al., 2014). GISTs may have a high risk (50% in the 10 years following diagnosis) or low risk (< 5%) of recurrence. The recurrence-free survival rate after surgery is 59.9% after 15 years. Some studies have linked poor prognosis with a location outside of the stomach, rupture of the pseudocapsule, and male gender (Joensuu et al., 2012).

Some studies suggest that the mutational status of KIT and PDGFRα predict the response to imatinib. Partial responses of 85-90% with mutation on exon 11, and of 50% when exon 9 is affected, have been found (Heinrich et al., 2003). Phase III clinical trials (Verweij et al., 2004; Blanke et al., 2008) show that patients with mutations on exon 11 have higher response to treatment, progression -free survival and overall survival rates than patients with exon 9 or no mutations (Debiec-Rychter et al., 2006). Furthermore, the relative risk of progression in exon 9 mutations is higher (171%) than that of exon 11 mutations (108%). Moreover, PDGFRα mutations have been linked with greater resistance to imatinib, particularly those on exon 18 (Corless et al., 2005,Heinrich et al., 2003). Nevertheless, although exon 11 mutations in GISTs mean a worse prognosis than wild-types or exon 9 mutations, in metastatic GISTs there is a higher imatinib response (Demetri et al., 2004; Heinrich et

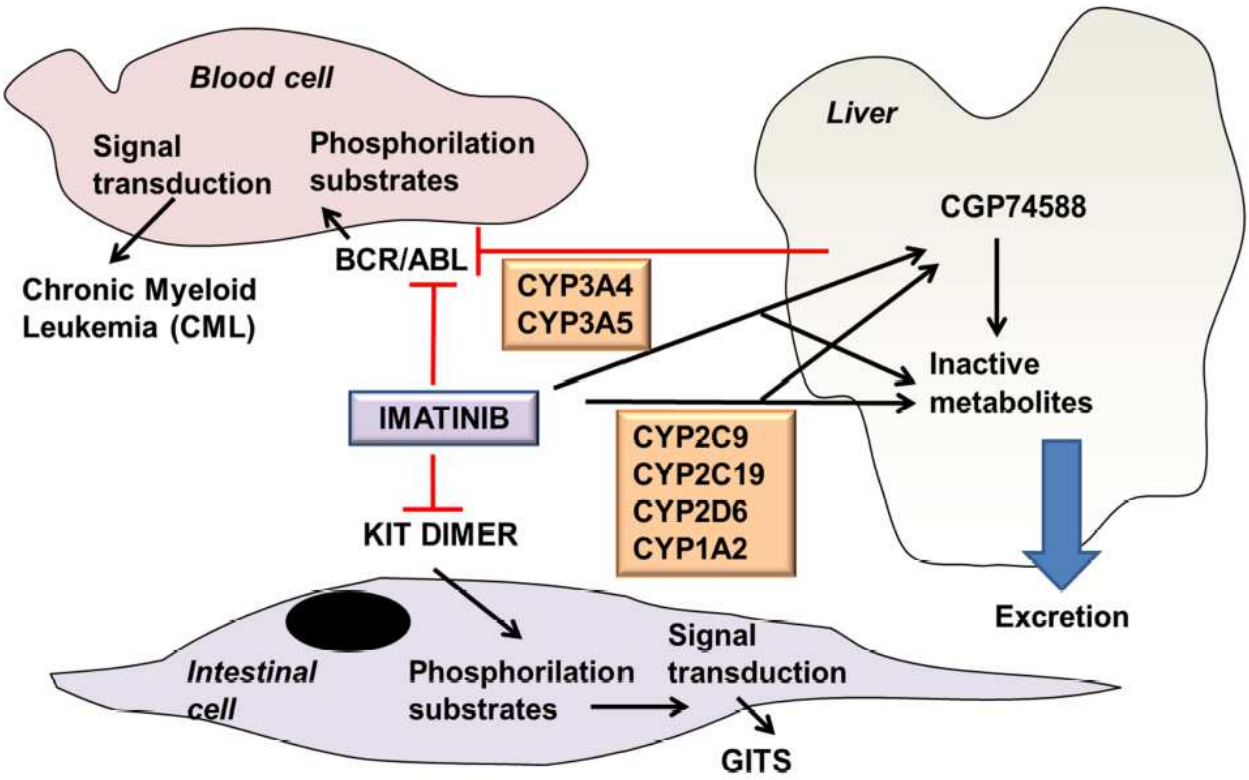

Fig 2. Imatinib Mesylate is an inhibitor of protein tyrosine kinase, interrupting KIT-mediated signal transduction, and BCR/ABL pathways. For this reason, it is used in the treatment of gastrointestinal stromal tumors (GIST), as well as different types of leukemia, being the most important Chronic Myeloid Leukemia (CML). Imatinib is metabolized in the liver to an active metabolite, so as to inactive metabolites that are excreted.

al., 2005).

The gene *GSTT1* (Lee et al., 2013) has recently been linked to a low response to treatment with imatinib in wild-type GISTs patients. The role that insulin growth factor 1 (IGF1R), the vascular endothelial growth factor (VEGF), succinate dehydrogenase (SDH) subunits (Belinsky et al., 2013; Oudijk et al., 2013) and alterations in BRAF, RAS, and the PI3K pathway (Corless et al., 2011; Daniels et al., 2011) play in resistance to treatment remains relatively unclear. On the other hand, SMA and p53 expression in GISTs has been linked with a positive prognosis (Demir et al., 2013; Zong et al., 2012). Also, the expression of the NKp30c immunosuppressive isoform, as opposed to other isoforms, suggests a lower survival rate (Delahaye et al., 2011). The prognosis value of angiogenesis, proliferation, invasiveness, and metastasis markers such as CD105 and CD31, COX-2, Ki67, MMP-9 and VEGF as indicators of higher tumour progression, has also been studied (Basilio-de-Oliveira and Nunes Pannain, 2015; Liu et al., 2015), as it has that of biomarker HER4. The latter is of special interest in gastric GISTs and could also be used as a protein target (Zhao et al., 2014). Recently, Jiang et al., (2016) have described the NIMA-interacting peptidylprolyl isomerase (PIN1) and Ki67 as two potential factors that may predict the malignancy of GIST.

TREATMENT OF GISTS

Surgery is the treatment of choice for localized GISTs. The aim is to achieve a complete resection with tumour-free margins, while keeping the pseudocapsule intact. The surgical methods for treating GISTs in their primary location have improved in recent years, including the use of endoscopic resection, or laparoscopy, as less invasive methods than conventional surgery (Huang et al., 2014). Very positive results are being obtained in the case of laparoscopy (Choi et al., 2015; Dressler et al., 2015; Severino et al., 2016). Also, the use of an endobag protects the tumour, preventing it from rupturing (Valle et al., 2014). The presence of metastasis, particularly peritoneum and liver, should be investigated. Lymphadenectomy is not necessary due to its low tendency to metastasise via lymph nodes. Oesophageal GISTs require special surgical methods, such as right-sided video-assisted thoracoscopic surgery (VAST) (Nawara et al., 2013).

GISTs treatment currently includes the use of certain drugs (Roggin and Posner, 2012) such as imatinib mesylate, a selective and competitive inhibitor of the tyrosine kinase protein that inhibits KIT, c-Abl/Arg and PDGFRA/B (Fig. 2). Imatinib acts by occupying the TK domain (ATP binding site) and preventing phosphorylation of the substrate and hence the signalling cascade for cellular proliferation. In vitro studies show that imatinib induces apoptosis, inhibiting KIT in the GIST882 and GIST780 cell lines. The tumour size decreased by 60% in rats with GIST882 (Siddiqui and Scott, 2007). The presence of KIT mutations creates resistance to imatinib via the modification of the protein domains.

Clinical trials on unresectable/metastatic diseases have shown a significant benefit with imatinib in doses of 400 mg (the FDA-approved dosage) (Demetri et al., 2002, Blanke et al., 2008). Higher doses (800 mg per day) have shown no significant difference in the response rate nor in the overall survival rate, although it has shown a difference in progression-free survival rate (50% vs 44%) (Verweij et al., 2004). Nevertheless, similar studies have found no such differences (Blanke et al., 2008), nor has toxicity been found when tested on GIST patients aged over 75 (Italiano et al., 2013). Recently, Yoo et al. (2016) have demonstrated that, despite toxicity, imatinib benefits supporting its clinical relevance for patients without treatment options. The combination of imatinib with metronomic cyclophosphamide causes a synergy in the inhibition of angiogenesis and a stabilisation in patients with advanced GISTs and imatinib and sunitinib resistance (Adenis et al., 2013). Halting treatment leads to a rapid progression of the disease, which is why it is recommended to continue until progression or intolerance (Blay et al., 2007; Le Cesne et al., 2007).

Adjunctive treatment with imatinib (400 mg/day) for 1 year significantly increased the recurrence-free and overall survival rates (Dematteo et al., 2009). Randomised SSGXVIII/AIO trials suggest that administering adjunctive imatinib for 36 months increases the recurrence-free and overall survival rates in high-risk GIST cases. In this study, patients with a high risk for GISTs recurrence after surgery (tumor greater than 10 cm in size with a mitotic rate of > 10 mitoses/50HPF or tumour greater than 5 cm in size with a mitotic rate of > 5 mitoses/50HPF or a risk of recurrence of greater than 50%) were randomized to 12 months or 36 months of post-operative Imatinib. The RFS and OS were longer in the 36 months group compared to the 12 months group (recurrence-free survival after 5 years was 66% vs 48%, and overall survival after 5 years was 92% vs 82%) (Joensuu et al., 2011).

Other studies also showed the benefits of prolonged treatment with imatinib (400mg daily over 3 years) after surgical resection to avoid recurrences, and of continued use in advanced or metastatic GISTs (Le Cesne et al., 2013). In addition, imatinib has been shown to be superior to other agents such as nilotinib (Blay et al., 2015). Two years of adjunctive therapy improves the recurrence-free survival rate of GIST patients with mutations on KIT exon 11 (Kang et al., 2013).

Neoadjuvant treatment before surgery has also

shown improvements in treatment, enabling the size of the tumour and the risk of rupture to be reduced (Eisenberg and Smith, 2011; Stiekema et al., 2013). Thus, imatinib before surgery increases the relapse-free survival rate among patients with advanced GISTs, as well as the complete tumour resection rate (Chang et al., 2015; Doyon et al., 2012).

RESISTANCE TO IMATINIB AND PROGRESSION

Resistance to imatinib, which causes a lack of response and no stabilization, and leads to progression of the disease, can be measured in three ways: by KIT or PDGFRA mutations, amplification of KIT or PDGFRA, and the alternative activation of kinases (Fig. 3). Resistance may be primary or secondary.

Primary resistance consists of a lack of a response to imatinib in the first six months of treatment (14% of patients) (Demetri et al., 2002). The most common mechanism is the initial mutational status. Patients develop new nodes or a rapid growth of a latent injury (Heinrich et al., 2006). The mutations on PDGFRA exon 18 (D842V) and on KIT exon 9 have been correlated to the response to imatinib. In fact, KIT exon 9 mutation has been related with progression of the GISTs. In these patients, imatinib at 800 mg improved response rates (67%) in comparison to imatinib at 400 mg (17%). By contrast, some mutation such as KIT exon 11 also represent a prognostic factor but reducing the risk of death.

Secondary resistance appears in patients who initially respond to treatment, but who show progression after the first six months. Heinrich et al. (2006) describe secondary resistance to imatinib with a median time until treatment failure of 20.2 months and with secondary mutations on KIT exons 13, 14, 17 and 18 (95% of cases). For unknown reasons, secondary mutations appear more frequently in patients with an initial mutation on exon 11 (60%) than in patients with an initial mutation on exon 9 (20%). Van Glabbeke et al. (2005) describe secondary resistance in 43% of the 818 patients who are progression-free after 3 months. Other studies link mutation on exon 13 with a poor progression of the disease in patients with GIST resistant to sunitinib (Kikuchi et al., 2012), and the polymorphism of transporter genes *SLC22A4* and *SLC22A5* with the development of imatinib resistance (Angelini et al., 2013). However, phase III trials show that reintroducing imatinib into the treatment of patients with resectable/metastatic GISTs with resistance, slows the progression of the disease, although it does not stop it (Kang et al., 2013).

60% of GIST patients are resistant to imatinib and must be controlled once treatment is completed. Personalized treatment of each GIST patient is considered to be ever more necessary, bearing in

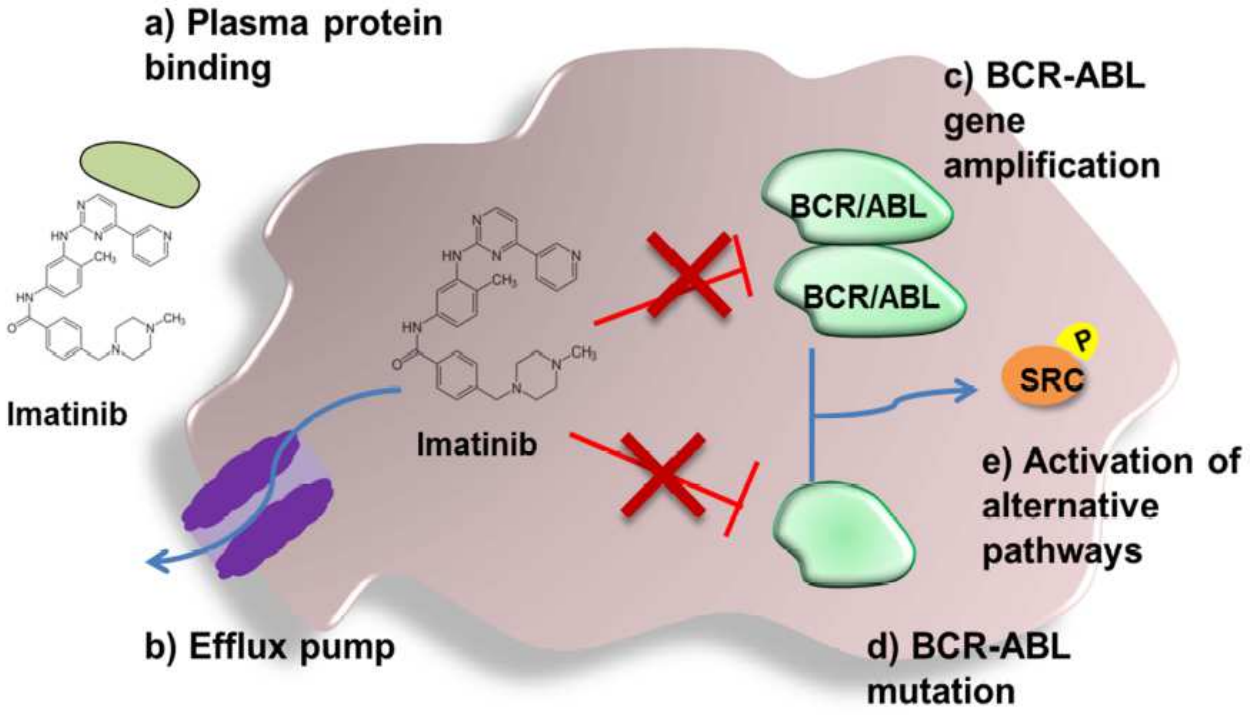

Fig 3. Mechanisms involved in the resistance to kinase inhibitors as imatinib: a) binding to plasma proteins may reduce effective drug blood concentration; b) efflux pumps release drug from cells preventing its action; c) overproduction of the target gene enhances resistance to the inhibitor; d) mutation in the oncogene allows it to escape drug action; and e) activation of alternative tirosine kinase downstream pathways.

mind that both the tumour and genetic DNA of the patient can have an impact on their response to treatment (Joensuu, 2012; Le Cesne et al., 2013; Ravegnini et al., 2015; Reichardt et al., 2012). Zalcberg et al. (2005) determined, in patients treated with imatinib (400 mg per day), that 27% presented a stabilization of the disease, 59% saw progression (median of progression-free survival of 81 days) and 18% saw no progression within the year. Similar studies with imatinib (800 mg per day) found 6% showed a partial response, 32% were stable and 48% saw progression. The median of progression-free survival and overall survival was 4 and 15 months, respectively (Rankin et al., 2004). GIST progression following treatment with imatinib can be treated with sunitinib.

Sunitinib, a tyrosine kinase inhibitor that is administered orally, has antiangiogenic properties, inhibiting vascular endothelial growth factor (VEGFR1-3) receptors, in addition to inhibiting KIT and PDGFR. A phase III study using sunitinib in cases of advanced unresectable or metastatic GISTs that are resistant or intolerant to imatinib (Demetri et al., 2006) showed an improvement in the progression, progression-free survival and overall survival time. These results have been recently supported by Goulooze et al. (2016). The sunitinib response seems to be dependent on the mutational status, with benefits in the majority of GIST molecular subtypes, the rate for which are as follows: exon 9, 42%; exon 11, 36%; PDGFRA, 25, and wild-type, 56%. Imatinib resistant patients with mutations on KIT exon 9 have a higher partial response rate with sunitinib than those with mutations on exon 11 (37% vs 5%). This has recently been corroborated (Liu et al., 2013). Progression-free survival was also higher in patients with a primary mutation on KIT exon 9 or a wild-type muta-

Table 2. Recent patents related to GIST.

Patent number	Title	Year	Inventors	Company
US 20160045501 A1	Method of optimizing the treatment of Proliferative Diseases Mediated by the Tyrosine Kinase Receptor KIT with imatinib	2016	Wang Y and Wehrle E.	Novartis Ag
WO 2016003797 A1	Therapy for gist	2016	Shah GD.	Imclone Llc
US 9402831 B2	Combination therapy of HSP90 inhibitors with BRAF inhibitors	2016	Proia D and Acquaviva J.	Synta Pharmaceutical Corp.
WO2014199244 A2	Crystalline imatinib mesylate process	2016	Pipal BR et al.	Shilpa Medicare Limited
US 20160045513 A1	Isoxazole compound for the treatment of cancer	2016	Chene P et al.	Novartis Ag
WO 2016130502 A1	Combination therapy of hsp90 inhibitors and pd-1 inhibitors for treating cancer	2016	Proia DA and Rao PE	Synta Pharmaceuticals Corp.
US 20160250218 A1	Pharmaceutical combination	2016	Stefanica MF et al.	Boehringer Ingelheim International
US 9402831 B2	Combination therapy of HSP90 inhibitors with BRAF inhibitors	2016	Proia D and Acquaviva J.	Synta Pharmaceutical Corp.
WO2014199244 A2	Crystalline imatinib mesylate process	2016	Pipal BR et al.	Shilpa Medicare Limited
WO 2016038590 A1	Process for the preparation of crystalline form i of regorafenib	2015	Gore V et al.	Mylan Laboratories
WO 2016007856 A1	Multi-drug delivery system and uses thereof	2015	Chsieh P et al.	Academia Sinica, Liang, Chi-Ming
EP 2609213 B1	Methods and compositions for diagnosing gastrointestinal stromal tumors	2015	Von Bubnoff N et al.	Albert-Ludwigs-Universität Freiburg
US 9085805 B2	Methods and compositions for diagnosing gastrointestinal stromal tumors	2015	Von Bubnoff N et al.	Albert-Ludwigs-Universität Freiburg
EP 2609213 B1	Methods and compositions for diagnosing gastrointestinal stromal tumors	2015	Von Bubnoff N et al.	Albert-Ludwigs-Universität Freiburg
WO 2016007856 A1	Multi-drug delivery system and uses thereof	2015	Chsieh P et al.	Academia Sinica, Liang, Chi-Ming
WO2015055898 A2	Compositions comprising phosphodiesterase inhibitors for use in the treatment of a solid tumor in a human patient	2014	Shito H et al.	Sartar Therapeutics Ltd
WO 2013063000 A1	Method of treating gastrointestinal stromal tumors	2013	Monahan JE and Li F.	Novartis Ag
WO 2013063003 A1	Method of treating gastrointestinal stromal tumors	2013	Monahan JE and Li F.	Novartis Ag

tion than those with a mutation on exon 11 (median of 19.4 months, 20.9 months and 5.1 months, respectively), with the same applying to overall survival (median of 26.9 months, 30.5 months and 12 months, respectively) (Heinrich et al., 2006). As regards response to sunitinib depending on secondary mutations occurring during treatment with imatinib, a greater benefit among patients with mutations on KIT exon 13 and 14 than those that had mutations on exon 17 or 18 was found (65% vs 9%) (Demetri et al., 2006). Sunitinib therefore has clinical benefits for patients with GISTs which is resistant to imatinib and which is influenced by primary and secondary mutations on the tyrosine kinase receptors. It is most effective on primary wild-type genotype or KIT mutations on exon 9.

In patients with imatinib and sunitinib resistance, activity of second-generation tyrosine kinase inhibitors such as sorafenib, nilotinib, dasatinib and regorafenib has been seen (Rammohan et al., 2013; Shirley and Keating, 2015)). The latter is considered as the third drug of choice for treating GISTs. It is a multi-kinase KIT, PDGFR and VEGFR inhibitor. Demetri et al. (2013), in a phase III clinical trial, showed the effectiveness of regorafenib in patients with resistant GISTs, obtaining a significant improvement in progression-free survival. Other studies also support the use of this drug in future GIST treatment (Waddell and Cunningham, 2013). In patients with sunitinib resistance (Gounder and Maki, 2011), the use of Nilotinib has been effective (Cauchi et al., 2012). Furthermore, SU-014813, an oral tyrosine kinase inhibitor, combined with docetaxel, is useful in sunitinib-resistant GISTs (de Jonge et al., 2011). The Bcl-2/Bcl-x(L), ABT-737 inhibitor has also been tested on imatinib-resistant GISTs, combined with imatinib, to increase apoptosis (Reynoso et al., 2011). Recently, pazopanib demonstrated an improvement in progression-free survival of patients with advanced GISTs showing resistant to imatinib and sunitinib (Mir et al., 2016)

Finally, other molecules used in clinical trials (specific KIT and PDGFRA mutation inhibitors, signal inhibitors, heat shock protein or histone deacetylase inhibitors) and immunotherapy in preclinical trials, due to the infiltration in GISTs of many tumour-infiltrating immune cells, seem to be effective (Bauer and Joensuu, 2015).

RECENT PATENT AND GITS

In recent years, a number of patents related to the treatment and diagnosis of GISTs have been developed based on various aspects (Table 2) such as inhibition of the KIT tyrosine kinase receptor with imatinib mesylate by adjusting its dose to achieve a minimal plasma concentration (US 20160045501 A1). The detection and quantifica-

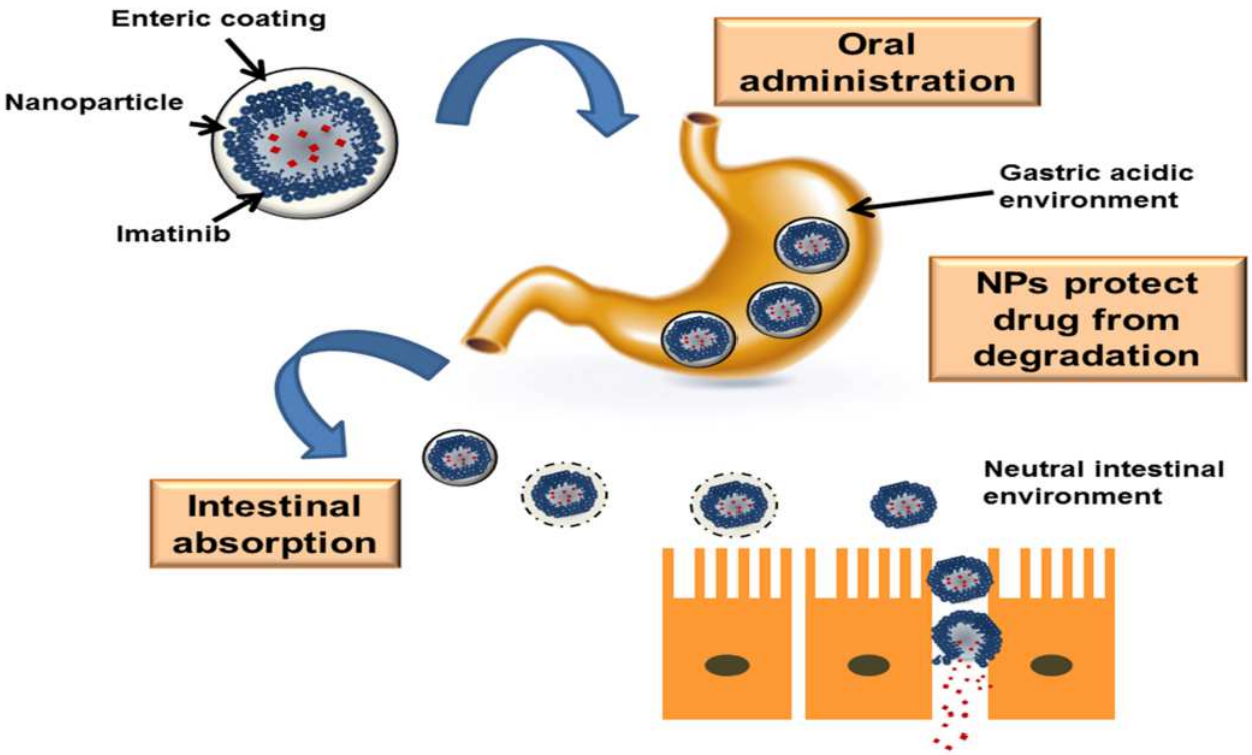

Fig 4. Incorporation of imatinib into NPs with an enteric coating avoids drug release in acidic gastric environment, so as its degradation. At intestinal neutral pH, the coating is degraded allowing intestinal absorption of NPs or free drug, reaching systemic vessels.

tion of specific alterations of genes (cKIT and PDGFRA) in patient blood samples have been also patented (EP 2609213 B1). In addition, some patents (WO 2013063000 A1, WO 2013063003 A1) developed the use of a combined therapy (dual KIT inhibitor, cKIT inhibitors, PI3K or FGFR) to overcome the limitations of imatinib or sunitinib treatments or the use of new pharmaceutical formulations such as imatinib mesylate free crystals (WO 2014199244 A2). Gore et al. (WO 2016038590 A1) disclosed a process which allows the high-throughput production of the crystalline form I of Regorafenib, an inhibitor of multiple membrane-bound and intracellular kinases. Inventions comprising PD-1 and Hsp90 inhibitors (WO 2016130502 A1) or BRAF inhibitor (US 9402831 B2) can also be found with a synergistic effect with other therapeutic agents. Phosphodiesterase inhibitors (WO 2015055898 A2), a tautomer thereof or a pharmaceutically salt thereof (Isoxazole) have also been patented (US 20160045513 A1; US 20160250218 A1). Antibodies that bind to the human platelet-derived growth factor receptor (PDGFR alpha) have been used for treatment of GISTs (WO 2016003797 A1). Recently, Von Bubnoff et al. (2015) patented a method for detecting mutations in the cKIT and PDGFRA marker genes in circulating DNA samples (EP 2609213 B1).

GISTS AND NANOTECHNOLOGY

The design of nanoparticles (NPs) in combination with drugs is leading to great advances in cancer treatment (Prados et al., 2014). In GISTs, NPs that include imatinib mesylate increases its solubility, decrease the treatment dosage and improve the pharmacokinetic profiles (Edelson et al., 2008; Jenkins and Liversidge, 2006). Liversidge et al. (2009) administer imatinib NPs orally, preventing the drug from degrading in the stomach (Fig. 4). NPs with a polyglutamic acid core and covered with chitosan (Sung and Tu, 2011), have a highly positive charge that increases the ability of the agent to permeate the intestinal mucous. Other NPs slow down the release of the drug depending on the pH of the environment (Illig et al., 2007) and redisperse NPs, creating a solution (Bhatia et al., 2008; Jenkins and Liversidge, 2006). Proposals have recently been made to encapsulate imatinib mesylate in NPs that mimic the cell membrane, so that they act as biological transporters with a long blood circulation time (Zhang et al., 2013).

By incorporating imatinib mesylate in poly(lactide -co-glycolide) NPs, a reduction in the cardiotoxicity in *in vivo* tests was achieved, in addition to an increase in its *invitro* anti-tumour effect (Marslin et al., 2015). Sorafenib has also been included into NPs to increase their activity (Carty et al., 2008). Even tyrosine kinase inhibitors such as rapamycin have been combined with NPs and albumin to treat GISTs. Binding with the SPARC protein receptor, overexpressed in this type of cell, improves its activity (Desai et al., 2010; Rossi et al., 2006).

Other agents have also been combined with NPs to treat GISTs. This is the case of albumin NPs with paclitaxel combined with perforin (Trieu et al., 2010), or cytotoxic NPs combined with ascorbate (Manganaro and Rockwell, 2012), which improves the release of the drug in a microenvironment with reactive oxygen species (tumour microenvironment). Tests with carbon nanotubes combined with UDP-glucuronosyltransferase or p53 were also carried out on GISTs, with limited results (Radominska-Pandya and Biris, 2011). Finally, some NPs bound with benzazepines that recognise the cholecystokinin B (CCK2R, CCKBR) receptor, overexpressed in GISTs, are being trialed for diagnosis, although when bound with a cytotoxin they can be used in therapy. (Hainfeld and Liu, 2010; Low and Wayua, 2013).

CONCLUSIONS

Despite GISTs is the most common mesenchymal neoplasm in the gastrointestinal tract, it is also a rare pathology. Currently, the surgical treatment is the main therapeutic strategy for localized forms, although in more advanced tumors the role of this therapy remains limited. In addition, the identification of genetic mutations such as those detected in c-KIT and PDGFRA genes, which are related with the pathogenesis of disease, has conducted to the development of effective treatments addressed to specific molecular targets. These approaches decreased tumor progression and increased patient survival in time. Drugs such as imatinib and sunitinib, which act by regulating tyrosine kinase activity, have been used with significant results, but there are also new approaches for those patients who present resistance to the previous treatments. Finally, future studies about GISTs will be necessary to develop new therapeutic strategies, so as to evaluate the appropriate treatment for every patient.

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The authors declare that they have no conflict of interest

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