Pharmacology & Therapeutics 248 (2023) 108475

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

The multifaceted landscape behind imatinib resistance in gastrointestinal stromal tumors (GISTs): A lesson from ripretinib

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ARTICLE INFO

Available online 10 June 2023

Associate Editor: S.J. Enna

Keywords:
GIST
Drug resistance
Imatinib
Tyrosine kinase inhibitors
Multi-target TKI
Ripretinib
Resistance

ABSTRACT

Gastrointestinal stromal tumors (GISTs) are rare mesenchymal sarcomas and the gold-standard treatment is represented by tyrosine kinase inhibitors (TKIs). Unfortunately, first-line treatment with the TKI imatinib usually promotes partial response or stable disease rather than a complete response, and resistance appears in most patients. Adaptive mechanisms are immediately relevant at the beginning of imatinib therapy, and they may represent the reason behind the low complete response rates observed in GISTs. Concurrently, resistant subclones can silently continue to grow or emerge *de novo*, becoming the most representative populations. Therefore, a slow evolution of the primary tumor gradually occurs during imatinib treatment, enriching heterogeneous imatinib resistant clonal subpopulations. The identification of secondary *KIT*/*PDGFRA* mutations in resistant GISTs prompted the development of novel multi-targeted TKIs, leading to the approval of sunitinib, regorafenib, and ripretinib. Although ripretinib has broad anti-KIT and -PDGFRA activity, it failed to overcome sunitinib as second-line treatment, suggesting that imatinib resistance is more multifaceted than initially thought.
The present review summarizes several biological aspects suggesting that heterogeneous adaptive and resistance mechanisms can also be driven by KIT or PDGFRA downstream mediators, alternative kinases, as well as non-coding RNAs, which are not targeted by any TKI, including ripretinib. This may explain the modest effect observed with ripretinib and all anti-GIST agents in patients.

Contents

Abbreviations: AL, Activation loop: Atrogin-1 (FOXO32); ATP-BP, ATP binding pocket; CDK, cyclin dependent kinase; CR, complete response; EORTC, European Organisation for. Research and Treatment of Cancer; ED, extracellular domain; FGF, Fibrobrast growth factor; FGFR, Fibroblast growth factor receptor; GIST, Gastrointestinal stromal tumor; JM, juxtamembrane region; lncRNA, long non-coding RNA; miR, microRNA; ncRNA, non-coding RNA; OS, overall survival; OPN, Osteopontin; PR, partial response; PFS, progression-free survival; RFS, recurrence-free survival; SD, stable disease; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; TKR, tyrosine kinase receptor; WT, wild-type.

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https://doi.org/10.1016/j.pharmthera.2023.108475
0163-7258/

1. Introduction

Gastrointestinal stromal tumors (GISTs) are mesenchymal neoplasms, and 80–90% of which are primarily driven by mutations in *KIT* or *PDGFRA* tyrosine kinase receptors (TKRs) (Corless, Barnett, & Heinrich, 2011; Hirota, 1998; Niinuma, Suzuki, & Sugai, 2018). Consequently, the development and clinical approval of the tyrosine kinase inhibitor (TKI) imatinib (Gleevec, Novartis) completely revolutionized patients' outcomes, becoming first-line therapy in 2001 (Demetri et al., 2002; Joensuu et al., 2001). Imatinib-based therapy has extended the median overall survival (OS) up to 57 months in patients with advanced, unresectable and/or metastatic GISTs, while the three-years adjuvant therapy has significantly improved recurrence-free survival (RFS) in high-risk KIT positive tumors (Reichardt, 2018). Although imatinib has improved both prognosis and survival outcomes, it is rarely curative due to the emergence of treatment-resistant cells within the tumor (Balachandran & DeMatteo, 2014). Immediate resistance can be observed in about 10% of *KIT*/*PDGFRA* wild-type (WT) GISTs and in 7–8% of tumors that harbor primary mutations in *KIT* or *PDGFRA* that are intrinsically imatinib resistant, such as *PDGFRA* D842V-mutant tumors (Corless et al., 2005; Wada, Arai, Kure, Peng, & Naito, 2016). Furthermore, the phase III EORTC 62005 trial showed that even when unresectable or metastatic GISTs have a favorable mutational pattern and responsiveness to imatinib, only 5% of patients achieve a complete response (CR) (Reichardt, 2018). The fact that most patients instead commonly achieve partial response (PR), or stable disease (SD) suggests that GIST cells could survive the treatment through the activation of adaptive processes. Imatinib withdrawal indeed leads a prompt disease progression, confirming that GIST cells are mainly stabilized in a non-proliferative state rather that inducing cell death. Hence, continuous imatinib treatment has been recommended unless unmanageable drug related side effects (Blay et al., 2007).

Regretfully, disease control in imatinib-naïve GISTs is not durable, leading to disease progression within 24 months due to acquired resistance (Serrano & Fletcher, 2019). At least twenty non-random mutations in the KIT ATP Binding Pocket (ATP-BP) or Activation Loop (AL) domains have been identified in imatinib-refractory GISTs (Oppelt, Hirbe, & Van Tine, 2017). Therefore, several drug discovery efforts have been pursuing novel TKIs that can simultaneously target imatinib-sensitive and -resistant mutants. Sunitinib (Sutent, Pfizer) was the first multi-targeted TKI approved in 2006 as second-line therapy in case of imatinib-resistant metastatic GISTs, while regorafenib (Stivarga, Bayer) was introduced in 2012 as third-line treatment of sunitinib-refractory GISTs (Demetri et al., 2006, 2013). However, both sunitinib and regorafenib have only slightly increased the median progression-free survival (PFS) of 4–6 months (Lostes-Bardaji, García-Illescas, Valverde, & Serrano, 2021). Mechanistically, sunitinib and regorafenib target secondary *KIT* mutations in the ATP-BP or AL domains, respectively, thus displaying complementarity to imatinib-resistant KIT mutations (Fig. 1A). Therefore, it has been proposed that their modest efficacy could be related to the proliferation of untargeted imatinib-resistant subclones, which gradually substitute TKI-sensitive subclones (Fig. 1B).

In 2020, ripretinib, an inhibitor with broad activity against KIT and PDGFRA (Qinlock, Deciphera Pharmaceuticals) was developed in the phase III INVISTUS trial and lately approved as the fourth-line treatment of GISTs resistant to the other approved TKIs (Blay et al., 2020). Compared to imatinib, sunitinib and regorafenib, ripretinib showed a lower nM IC_{50} range against almost the totality of KIT and PDFGRA mutants in a panel of about thirty *in vitro* cellular models (Smith et al., 2019). Specifically, while imatinib, sunitinib and regorafenib displayed weaknesses in targeting certain secondary mutations, ripretinib revealed similar efficacy toward *KIT* primary and secondary mutations. However, despite these broad *in vitro* data, the positive results from the INVICTUS trial in heavily pretreated patients, as well as promising results in prior clinical phase (Janku et al., 2020), ripretinib did not meet the primary endpoint in the recent INTRIGUE trial, which aimed to evaluate it as an alternative second-line treatment replacing sunitinib (Bauer et al., 2022). In this trial, 453 patients previously treated with imatinib and who developed therapy resistance, were randomly treated with ripretinib or sunitinib. Even if ripretinib was associated with fewer severe adverse effects (grade 3–4), no statistically significant differences in progression-free survival (PFS) between the two groups were observed.

With this premise in mind, the goal of the present review is to review preclinical findings, highlighting the emerging heterogeneity of drug-resistance mechanisms which can retrospectively explain the modest outcomes achieved with multi-targeted TKIs and the recent failure of ripretinib as second-line treatment.

2. Complexity and heterogeneity of imatinib resistance mechanisms

2.1. Mechanisms associated with KIT/PDGFRA related pathways

Activation of several intermediate factors downstream of KIT/PDGFRA plays a key role in the transduction of the oncogenic signal. As confirmed in primary mutated GIST-T1 (primary mutation in *KIT* exon 11 - Δ560–578) and GIST-882 (primary mutation in *KIT* exon 13 - K642E) cell models, the biological effects promoted by imatinib treatment can be significantly different even if KIT receptors are sensitive to the drug binding and phosphorylation of tyrosine residues is similarly inhibited (Noma et al., 2005; Tarn et al., 2006; Tuveson et al., 2001). Therefore, the efficacy of imatinib is likely not only related to tyrosine kinase receptor (TKR) inhibition, but it is rather influenced by the genomic context and the activity of other complementary and alternative signaling pathways.

Notably, the PI3K/AKT/mTOR pathway is reported to be critical in the transduction of KIT signaling in GIST. As reported by Bosbach et al., spontaneous GIST development in a mouse model harboring the oncogenic *KIT* deletion V558 can be reverted by an additional *KIT* mutation in the 719-tyrosine residue, a site that, when phosphorylated, may recruit PI3K (Bosbach et al., 2017). Given its central role in GIST biology, the association between the PI3K/AKT/mTOR signaling cascade and imatinib resistance has been of great interest. Lai and colleagues used an imatinib-resistant GIST-882IR cell line derived from the sensitive progenitor GIST-882 model and revealed that resistance cannot be only related to secondary *KIT* mutations, but also to KIT overexpression (Lai et al., 2016). The authors further showed that the underlying mechanism was the overexpression of p55PIK, a PI3K isoform, which activates the NF-κB signaling to increase KIT expression. Consequently, p55PIK inhibition resulted in decreased KIT expression and restored imatinib sensitivity in resistant cells (Lai et al., 2016). These data highlight that PI3K-mediated KIT upregulation can be an alternative to secondary *KIT* mutations as a mechanism for imatinib resistance. Further deregulation of PI3K pathways was identified in a further cellular model established from GIST-T1 after sunitinib long-time exposure. The novel cell line, GIST-T1R, which was resistant to both sunitinib and imatinib, is characterized by the downregulation of PTEN, — one of the main PI3K regulator -, leading to the activation of AKT/mTOR even under sunitinib treatment (Yang et al., 2012). Yang and co-authors reported methylation of PTEN promoter that, in turn, induces PTEN silencing and overstimulation of PI3K pathway. This suggests that PI3K deregulation can be also employed by the tumor instead of secondary KIT mutations to promote imatinib and sunitinib escape (Yang et al., 2012). Furthermore, analysis in GIST patients' samples confirmed that the PI3K/AKT/mTOR pathway can be activated in KIT negative and imatinib-resistant tumors supporting the idea of targeting this pathway as a strategy for refractory GISTs (Duan, Haybaeck, & Yang, 2020; Li et al., 2015; Patel, 2013). However, it is yet unclear whether PI3K deregulation is mostly mediated by aberrancies that act directly on the PI3K pathway, or whether it is due to stimulation *via* alternative upstream drivers able to trigger PI3K signaling.

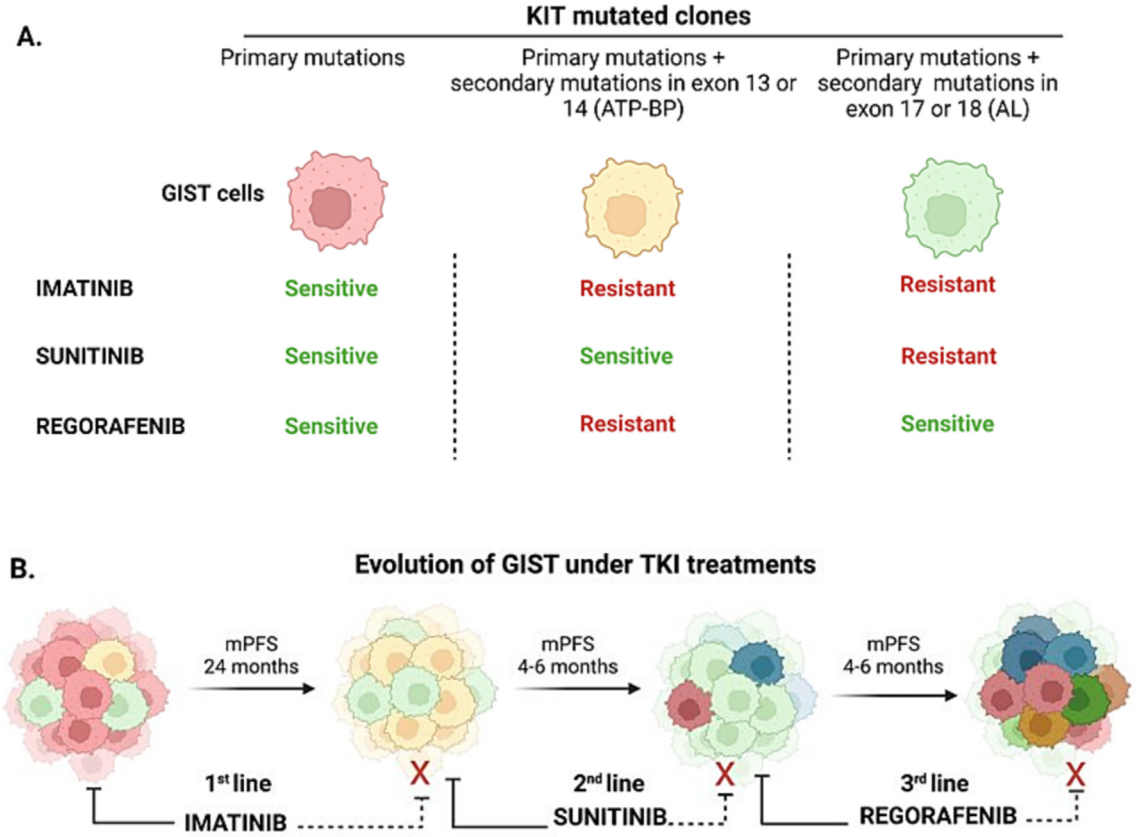

Fig. 1. A). Sensitivity of *KIT* mutants to imatinib, sunitinib and regorafenib. Imatinib, the first line treatment, almost exclusively recognizes *KIT* mutated clones that harbor primary mutations on exon 9,11,13 (around 70% of all diagnosed primary GISTs). Those exons encode for the juxtamembrane region (JM) and the extracellular domain (ED). Sunitinib and regorafenib, as multi-target TKIs, display a broader recognition capability compared with imatinib, targeting the ATP-BP and AL secondary mutations (exon 13/14 or exon 17/18), respectively. **B**). The complementarity of action depicted in panel A supports the concept that such clones could escape from sunitinib and regorafenib, as well as from imatinib. Pink indicates cell clones that are sensitive to imatinib, sunitinib and regorafenib; yellow indicates sensitivity to sunitinib but not to imatinib and regorafenib; green indicates sensitivity to regorafenib but not to imatinib and sunitinib. The same color scheme is applied to panels A and B. mPFS: median progression-free survival. Figure created with BioRender.com.

Recently, an extensive pharmacological study using the PI3K inhibitor GDC-0980 in imatinib-resistant GIST cells (GIST-430/654, GIST-T1/670, GIST-T1/816, GIST-T1/820) revealed that inhibition of the PI3K/AKT/mTOR pathway is not sufficient to remarkably affect cell proliferation and apoptosis in imatinib resistant cellular models (García-Valverde et al., 2021). In addition, substantially different IC_{50}s between cell lines were reported, suggesting that the involvement of PI3K/AKT/mTOR could vary and be cell specific. The screening data agrees with previous studies, which identified RAS/MAPK as additional KIT downstream pathway that play a key role for the stabilization of ETV1, a transcription factor highly expressed in GIST, which is needed for tumorigenesis (Chi et al., 2010; Ran et al., 2015). García-Valverde et al. also studied the effect of the inhibition of RAS/MAPK similarly to previously described for PI3K/AKT/mTOR, but they highlighted that ablation of a single pathway is not sufficient to achieve prolonged anti-tumoral effect in resistant cell models. However, when they concurrently inhibited both KIT downstream pathways, a blockade of cell proliferation and apoptosis stimulation in imatinib resistant cells were observed, independently from the secondary mutation which they harbor (García-Valverde et al., 2021). Therefore, as depicted in Fig. 2, the authors hypothesized that survival of resistant GIST cells may be sustained through pathways that remain functional (Fig. 2).

2.2. Involvement of alternative kinase drivers

In addition to KIT downstream PI3K/AKT/mTOR and RAS/MAPK signaling pathways, recent findings have highlighted a multitude of key players that can be similarly involved in TKI resistance, including alternative kinases. The involvement of alternative kinases in imatinib resistance was reported by Urbini et al. evaluating the role of the fibroblast growth factor (FGF) signaling pathway (Urbini et al., 2020). FGF2 is a ligand of FGF receptors (FGFRs) belonging to the TKR family, previously identified as an attenuator of imatinib efficacy from a panel of 220 growth factors (Li et al., 2015). Indeed, the effect of imatinib on cellular viability is counteracted in GIST-T1 and GIST-882 cell lines by simultaneous treatment with FGF2. Stimulation of the FGF pathway promotes the reactivation of the MAPK pathway and, consequently, imatinib resistance. MAPK phosphorylation was promptly prevented when co-treating cells with the FGFR inhibitor BGJ398, which restored imatinib sensitivity. No effect was observed using BGJ398 as a single treatment, suggesting that the FGF pathway is not active when KIT is functional and can be activated as an adaptive mechanism *in vitro,* although the trial was developed in imatinib-resistant patients and therefore imatinib was unlikely to exert any inhibitory activity against KIT mutations. The authors hypothesized that this could have notable clinical significance considering the ubiquitous expression of both FGFR1 and FGF2 in GIST. In accordance, imatinib treatment was recently reported to induce remarkable changes in the GIST-T1 secretome, including FGF2 release, highlighting the idea that the FGF pathway is activated as an imatinib response *via* autocrine signaling (Boichuk et al., 2020). Moreover, a further proof of a critical role for the FGF pathway in GIST was described by a second study in which FGFR2α over-expression was reported in the imatinib-resistant cell line GIST-T1R, developed from the GIST-T1 cell line through continuous treatment with imatinib and

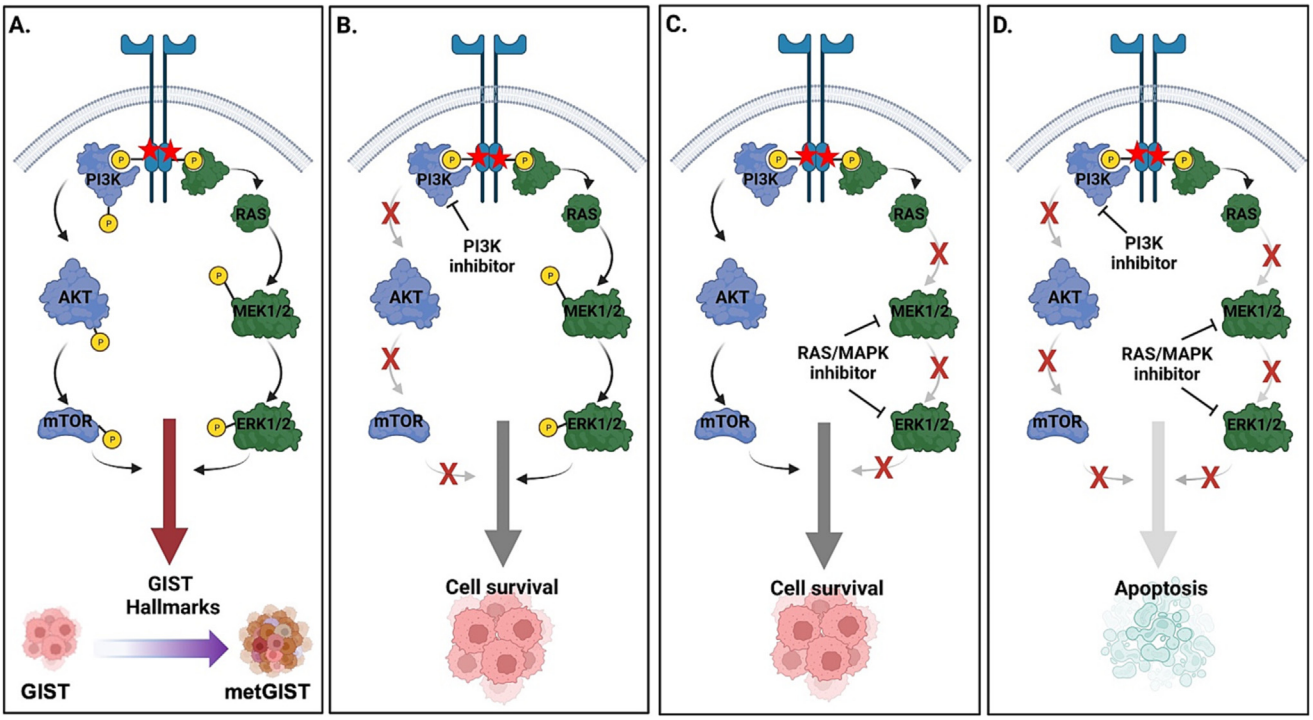

Fig. 2. PI3K/AKT/mTOR and RAS/MAPK pathways are crucial in GIST cells including those that are resistant to imatinib. **A)** In GIST resistant cells both PI3K/AKT/mTOR and RAS/MAPK pathways contribute to cell proliferation **B and C**) Single ablation of the PI3K/AKT/mTOR or RAS/MAPK pathway is not able to promote an anti-proliferative effect. **D)** Simultaneous targeting of PI3K/AKT/mTOR and RAS/MAPK pathways may promote significative reduction of cell proliferation and induce apoptosis. Red stars indicate gain of function mutations on *KIT* gene that promote constitutive and ligand-independent receptor activation. metGIST: metastatic GIST. Figure created with BioRender.com.

characterized by loss of KIT expression (Boichuk et al., 2017). The role of FGFR2α in imatinib resistance was further corroborated by analyzing the cytotoxic effect of BGJ398, shown to exclusively affect GIST-T1R cells but not the imatinib-sensitive precursor line. Overall, these data strongly indicate the contribution of FGF signaling in GIST biology in both adaptive and acquired resistance to imatinib (Astolfi, Pantaleo, Indio, Urbini, & Nannini, 2020). Subsequent studies focused on identifying additional TKRs have revealed a more multifaceted landscape with additional relevant players. Tu and colleagues reported an inverse expression between AXL and KIT using *in vitro* KIT-negative cell models (Tu et al., 2018). In these cell lines (GIST-54, GIST-62 and GIST-552), AXL knockdown impaired cell viability, while no negative consequence on cellular viability was observed in KIT positive and AXL negative cells (GIST-430), suggesting that AXL may drive GIST biology in the absence of KIT. Accordingly, AXL, as well as MET, was upregulated among 49 tyrosine kinases in cell lines derived from imatinib-resistant GISTs patients (HG129 and HG209), compared to imatinib-sensitive GIST-882 cell line (Cohen et al., 2015). Focusing on MET, imatinib-resistant HG209 cell model expresses high levels of MET and low levels KIT, whereas the opposite is observed in imatinib-sensitive cell models (GIST-T1, GIST-882). Treatment with HGF, a MET ligand, exclusively promoted a mitogenic stimulus in HG209, confirming the key role of MET in absence of KIT signaling, while no effect in KIT-driven and imatinib-sensitive cells was observed. Noteworthy, similarly to what previously described for the FGF pathway, in GIST-T1 and GIST-882 cell lines, phosphorylation of MET was increased upon imatinib treatment, suggesting the involvement of MET in adaptive mechanisms.

Besides FGF, AXL and MET pathways, no other TKRs analyzed up to date, such as the Ephrin type-A receptor 2 (EPHA2) or the epidermal growth factor receptor (EGFR), seem to independently promote GIST oncogenesis. Thus, it has been proposed that their overexpression/activation in KIT-independent cell lines is a required but not sufficient requisite to trigger imatinib resistance, and other co-activators are needed (Tu et al., 2018).

Furthermore, even if most of the research has focused on the role of TKR in GISTs, contributions mediated by non-receptor TKs have been identified. WEE1, a molecule involved in G_2/M mitosis transition, was shown to be overexpressed in GIST samples compared to surrounding non-tumor tissues and to correlate with tumor size, mitotic count and risk grade. SiRNA-mediated WEE1 inhibition promoted accelerated KIT degradation *via* autophagy in GIST-T1 and GIST-882 cells and significantly affected viability and colony formation (Liu et al., 2020). WEE1 therefore seems to act as a positive regulator of KIT expression. ABL-1, another TK widely expressed in GISTs can be related to imatinib resistance in a similar manner (Rausch et al., 2017). Imatinib is on-target against both KIT and ABL-1. Accordingly, to assess the effect of ABL-1 inhibition in GISTs, dual depletion of ABL-1 and KIT attenuates the effect observed with single KIT ablation, suggesting that ABL-1 targeting can reduce the efficiency of imatinib. Among other non-receptor kinases, aurora serine/threonine kinases A (AURKA), a key player in chromatin segregation during mitosis, was first identified as an independent and negative prognostic factor in GISTs (Lagarde et al., 2012; Yeh et al., 2014; Yen et al., 2012). Moreover, its involvement in imatinib resistance has been recently reported in the GIST-T1 cell line, thus adding more complexity to the resistance landscape driven by alternative kinases (Cheng, Wang, Lu, Fan, & Wang, 2021).

2.3. Quiescence and autophagy as integrating processes in apoptosis evasion mechanisms

In line with the clinical evidence, preclinical studies confirmed that imatinib is often ineffective at completely killing all cells in imatinib-sensitive tumors, supporting the hypothesis that some clones may survive through evasion from apoptosis (Gupta et al., 2010). Indeed, GIST-882 and GIST-T1 cell lines respond to imatinib by

transiently modifying their cellular metabolism in order to promote quiescence, a well-known adaptive mechanism widely associated with cancer drug resistance (Mellor, Ferguson, & Callaghan, 2005). For example, in GIST-882 cells, imatinib resistance was associated with the induction of specific regulators of quiescence, such as the cyclin-dependent kinase (CDK) regulator $p27^{Kip1}$ (Y. Liu et al., 2008). In addition to the APC^{CDH1}–SKP2–$p27^{Kip1}$ axis, DREAM multiprotein complex mediates quiescence activation following imatinib treatment in these cells (Boichuk et al., 2013). Inhibition of the DREAM subunit, DYRK1A kinase, in combination with imatinib, significantly increases the percentage of apoptotic cells, suggesting that inactivation of quiescence pathways may be a promising strategy to improve sensitivity to imatinib (Boichuk et al., 2013; Gupta et al., 2010).

An additional mechanistic insight into imatinib-related quiescence was recently described by García-Valverde and colleagues (García-Valverde et al., 2021), who identified the FBXO32 as the most universally upregulated gene in response to inhibition of KIT or its downstream PI3K/AKT/mTOR and RAS/MAPK pathways. The authors showed that FBXO32 is finely tuned by the KIT pathway and represents a survival factor that leads to quiescence activation and to apoptosis evasion, thus supporting its targeting in a co-treatment approach for improving drug response. Moreover, as already detailed by Ravegnini and collaborators, quiescence is not the unique adaptive mechanism and GIST cells can also promote autophagy suggesting that it could be part of a complex system aimed to counteract apoptosis(Ravegnini et al., 2017). In this context, Gupta and co-workers reported that imatinib stimulated autophagy as a survival pathway in quiescent GIST-T1 cells, whereas no markers of autophagy were observed in imatinib-resistant GIST-882 and GIST-T1R cell lines (Gupta et al., 2010).

Although evasion of apoptosis can be the result of activation of both quiescence or autophagy, direct impairment of the physiological balance between pro- and anti-apoptotic factors has also been observed. For example, the anti-apoptotic protein MCL-1, commonly upregulated in GISTs, is induced in GIST-882 cells by osteopontin (Hsu et al., 2014). All together these studies suggest an intricate network between imatinib-induced mechanisms of therapeutic adaptation involving quiescence, autophagy and apoptosis.

2.4. The emerging role of non-coding RNAs

Non-coding RNAs (ncRNAs) are untranslated single-stranded RNAs of variable length that play crucial functions both at the physiological and pathological levels, including in cancer (Amirnasr, Sleijfer, & Wiemer, 2020; Wang, Han, Sun, Chen, & Chen, 2019). Among ncRNAs, microRNAs (miRs) are well-known regulators of a large number of genes (O'Brien, Hayder, Zayed, & Peng, 2018). Although dysregulation of miRs, such as miR-221, miR-222 and miR-148b-3b, contributes to GIST biology through KIT regulation (Ihle et al., 2015; Pantaleo et al., 2016; Wang et al., 2018), several other miRs have been identified as involved in imatinib resistance. For example, miR-320 downregulation correlates with the rapid development of imatinib resistance in GIST patients (Gao et al., 2014). Similar observations have been made in *in vitro* studies, such as downregulation of miR-218 in imatinib-resistant GIST-430 cells compared to imatinib-sensitive GIST-882 cells (Fan et al., 2015). In particular, transfection of miR-218 mimic promoted imatinib sensitivity in GIST-430 cells. Interestingly, increased levels of miR-218 in GIST-430 cell line reduced AKT phosphorylation causing inhibition of the PI3K/AKT/mTOR pathway. In another study, miR expression was analyzed in tumor tissue obtained before imatinib treatment (representative of imatinib responsiveness) and in matched tissue after relapse (representative of imatinib resistance), identifying specific changes in miR levels upon GIST progression and imatinib resistance (Shi et al., 2016). Aligning with a role for PI3K dysregulation during GIST resistance, miR-518a-5p, which targets the PI3K family-member PI3KC2A, was upregulated in imatinib-resistant tumor specimens. Moreover, a recent study found that the high miR-30a levels detected in imatinib-sensitive GIST-882 and GIST-T1 cell models decreased after imatinib treatment, suggesting a potential role of this miR in drug resistance (Chen et al., 2020). Interestingly, miR-30a downregulation was related to autophagy stimulation, as confirmed by the upregulation of the mir-30a target and autophagy player Beclin-1. Notably, the use of miR-30a mimic suppresses autophagy and sensitizes GIST cells to imatinib, increasing the percentage of apoptotic cells. Based on that, the data provides evidence of autophagy initiation as a mechanism of imatinib resistance.

Many other miRNAs, ncRNAs and genes are involved in the regulation of autophagy and remain to be explored. Among these, long ncRNAs (lncRNAs) are classified as untranslated transcripts longer than 200 nucleotides that regulate gene expression at transcriptional, post-transcriptional and epigenetic levels (Marchese, Raimondi, & Huarte, 2017; Schmitt & Chang, 2016). An active role of lncRNAs in imatinib resistance was recently reported by Zhang and co-workers, who reported that overexpression of the lncRNA HOTAIR suppresses the potency of imatinib in GIST-T1 and GIST-882 cells, leading to increased expression of autophagy-related genes such as P62, Beclin1, LC3I/II and ATG2B (Zhang et al., 2021). Mechanistically, imatinib treatment was found to stimulate HOTAIR migration from the nucleus to the cytoplasm, promoting HOTAIR recognition, sponging of miR-130 and finally leading to induction of autophagy. Accordingly, with *in vitro* data, siRNA-mediated HOTAIR downregulation significantly improved imatinib sensitivity in GIST-T1-based xenograft models, while no effect was observed in mice treated with siRNA only. These results suggest that HOTAIR could represent a potential promising target in imatinib adaptive response and could increase partial response rate observed during imatinib therapy. A subsequent high-throughput RNA sequencing study found dysregulation of additional lncRNAs in GIST samples. Among them, RP11-616M22.7 was overexpressed in imatinib-resistant specimens compared to the sensitive ones (Shao et al., 2021). *In vitro,* imatinib treatment prompted a dose-dependent increase of RP11-616M22.7 in GIST-T1 and GIST 882 cell lines, suggesting that its level may be strictly regulated as an adaptive response. In line with this, co-treatment of GIST-T1 xenograft tumors with imatinib and anti-RP11-616M22.7 siRNA improved survival, supporting the idea that targeting RP11-616M22.7, and more in general lncRNAs, could be a promising strategy.

3. Concluding remarks and future perspectives

GISTs are rare mesenchymal sarcomas that are typically unresponsive to conventional chemotherapy. The identification of oncogenic mutations in *KIT* and *PDGFRA* as drivers of the majority of GISTs led to the regulatory approval of first-line imatinib, which revolutionized patients' outcomes. Unfortunately, imatinib usually promotes partial response or stable disease, rather than complete response. As shown in Fig. 3, preclinical studies and clinical results support that imatinib certainly induces apoptosis in a proportion of sensitive clones. However, a subset of cells counteracts imatinib-induced cell death through adaptive survival processes. Adaptive mechanisms are highly relevant at the initiation of imatinib therapy, and they can justify the low rate of complete responses observed in GISTs. The biological processes leading to *de novo* emergence of imatinib-resistant subclones will concurrently take place; pre-existent imatinib-resistant subclones, yet undescribed, can also "silently" continue growing until becoming the most representative population. Therefore, slow evolution of the imatinib-naïve neoplasm gradually takes place during imatinib treatment, enriching heterogenous imatinib-resistant clone subpopulations which foster the transition from stable to progressing disease within 24 months in patients with unresectable, advanced stage and metastatic GISTs.

The identification of additional and secondary *KIT*/*PDGFRA* mutations in hotspot regions of these genes prompted the development of novel multi-targeted TKIs, leading to the approval of sunitinib, regorafenib and ripretinib. Although ripretinib represented a remarkable

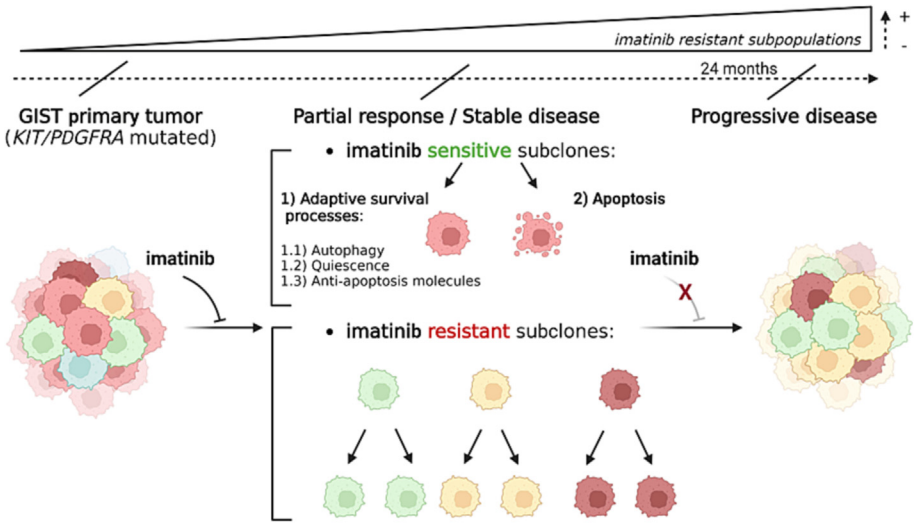

Fig. 3. Evolution of imatinib sensitive tumor mass in a resistant tumor mosaic. Primary GISTs harboring mutations in KIT or PDGFRA are commonly imatinib sensitive, but patients mostly show a partial response or stable disease rather than a complete response. As confirmed by *in vitro* and clinical evidence, a significant number of GIST cells activate adaptive survival processes, while resistant clones can be selected, leading to an imatinib-resistant and heterogeneous tumor mass and progression of disease within 24 months. The same color scheme as Fig. 1 is applied.

achievement in the effort of developing broader KIT/PDGFRA inhibitors, the phase III INTRIGUE trial failed to show the superiority of ripretinib over sunitinib as second-line treatment, thus suggesting that imatinib resistance is more multifaceted than initially though (*i.e.* secondary mutations are the key drivers of resistance). The present review highlights the heterogeneous mechanisms of therapy adaptation and resistance, which suggests the involvement of additional activated *KIT/PDGFRA* downstream mediators, alternative kinases, as well as ncRNAs. These pathways likely act in concert and therefore cannot be targeted even by multi-targeted TKIs. Indeed, drugs able to target most *KIT/PDGFRA* mutants - as in the case of ripretinib - still face the challenge of activation of multiple alternative pathways through several mechanisms. This may explain the modest effect observed for ripretinib in clinics and reveal a multifaceted, complex landscape (Fig. 4).

The present integrative analysis of ripretinib, which considers both clinical results and *in vitro* data, supports the concept that improvement of GIST management requires a paradigm shift away from the identification of the best-in-class multi-targeted TKIs. The heterogeneity of resistant mechanisms indicates that pharmacological combinations could be the answer to counteract the multitude of molecular systems involved in the failure of TKI monotherapy, albeit the value of TKI therapy is not in question, (Mokhtari et al., 2017). Regretfully, it is not fully understood which of the described pathways, if any, are the most clinically relevant. Indeed, since cell lines cannot perfectly mimic the real clinical

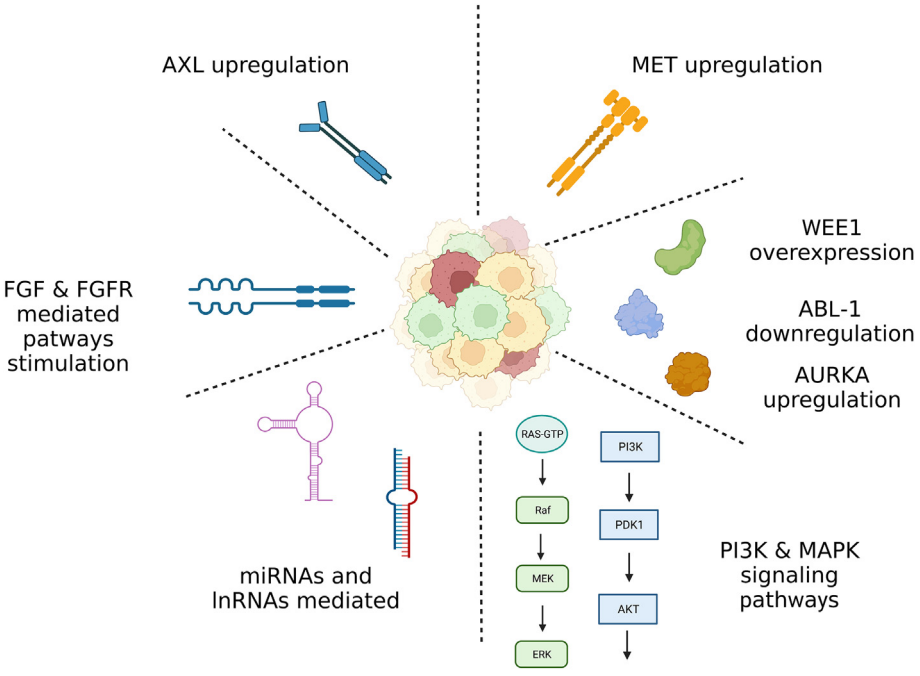

Fig. 4. GIST subclones can activate a number of pathways to overcome imatinib treatment. These mechanisms can sustain progressed disease independent of KIT/PDGFRA mutations, explaining the modest effect of multi-target TKIs, including ripretinib. The same color scheme as Fig. 1 is applied.

scenario, the mechanistic insights should be confirmed in patient-derived samples in order to define the most common and associated with imatinib resistance *in vivo*. Hence, additional studies are needed to identify additional druggable pathways, which can then be tested clinically in conjunction with established TKI therapy.

In conclusion, as learned from the ripretinib clinical failure, a deep comprehension of imatinib resistance mechanisms has been unveiled in the in the last decade, shedding light on the complex landscapes which makes overcoming imatinib resistance challenging. Most of the recognized mechanisms contributing to drug resistance seem to be intertwined and the identification of the biological pivots is crucial for clinical future success. Moreover, multidisciplinary efforts are mandatory for the identification and development of novel pharmacological options and efficient combination with readily available TKIs, getting closer to the identification of the new magic bullets for GIST management.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

C.S. has received research funding (institution) from IDRX, Blueprint, Karyopharm, Pfizer, Deciphera, and Bayer; consulting fees (advisory role) from Cogent, Immunicum AB, Deciphera and Blueprint; payment for lectures from Roche, PharmaMar, Decipehera, Bayer and Blueprint; and travel grants from Gilead, PharmaMar, Pfizer, and Bayer AG.

Acknowledgment

The research leading to these results has received funding from the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to E.C., CUP J33C22002920006. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them. TA acknowledges funding from Instituto de Salud Carlos III grant PI21/00470 co-financed by the European Regional Development Fund (ERDF).

S.M. is supported by a Fulbright fellowship (year 2022).

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