REVIEW

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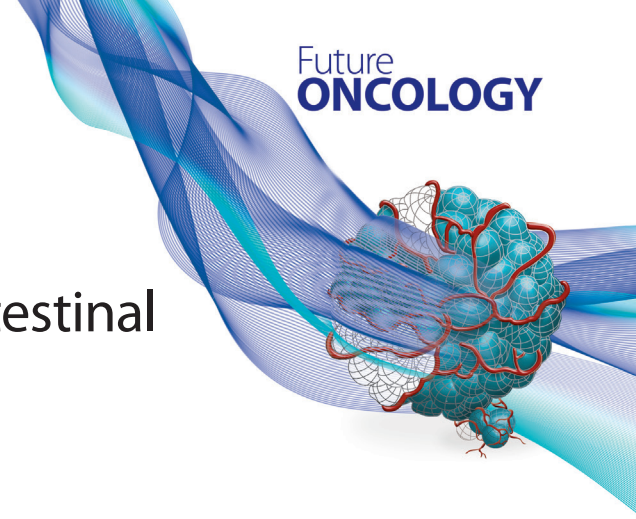

Latest advances in adult gastrointestinal stromal tumors

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Gastrointestinal stromal tumors (GISTs) are the most common GI tract mesenchymal tumors. GIST patients are optimally managed by a precision medicine approach. Herein, we discuss the latest advances in precision medicine and ongoing clinical trials relevant to GIST. Circulating tumor DNA for detection of mutational changes could replace tissue biopsies and radiographic imaging once validated. Most GISTs are *KIT/PDGFRα* mutated, and despite the good clinical response to imatinib, treatment is generally not curative, more often due to secondary mutations. New mechanisms to bypass this resistance by inhibiting KIT downstream pathways and by targeting multiple KIT or PDGFRα mutations are being investigated. Immunotherapy for GIST patients is in its infancy. These approaches may lead to more effective, less toxic therapies.

KEYWORDS

• circulating tumor DNA
• GIST • KIT • PDGFRα
• quadruple WT GIST

Gastrointestinal stromal tumors (GISTs) are the most common GI tract mesenchymal tumors, with 3.2–7.8 new cases per million diagnosed every year in the USA [1–3] and up to 10–14 cases per million in Europe [4–6]. Although less than two decades have passed since the groundbreaking discovery of their most common driver mutation [7] (*kit* proto-oncogene), GISTs have been a model of targeted therapies for solid tumors. About 80% of sporadic GISTs carry gain-of-function mutations in the *kit* proto-oncogene leading to constitutive activation of *kit* [7,8] and 5–10% have activating genomic alterations in the *PDGFRα* [9,10]. Among the GISTs lacking *KIT* and *PDGFRα* alterations (referred to as *KIT/PDGFR* WT GIST), two main distinct groups have been described. About 15% of the *KIT/PDGFR* WT GISTs harbor activating mutations in *BRAF* or more rarely in *RAS* genes [11,12], and 20–40% demonstrate loss of function of the SDH (SDH-deficient GISTs) [13,14]. Less commonly, WT GISTs can arise in the context of neurofibromatosis type I (NF1) disease, associated with loss of function of the NF1 protein [15].

The above findings led to the development of targeted tyrosine kinase inhibitors (TKIs) that block the KIT, PDGFR and BRAF signaling pathways. The initial US FDA-approved TKI for the treatment of metastatic or unresectable GIST was imatinib with activity against KIT and PDGFRα [16] pathways. Despite producing a remarkable clinical response, imatinib is rarely curative with a median time of disease progression of 18 months [17] and a median survival of 58 months [18]. Since resistance to imatinib is inevitable, other TKIs were developed to overcome this problem and are approved for patients who failed or did not tolerate imatinib treatment. Sunitinib, a potent KIT and PDGFR inhibitor was approved in second line of treatment for patients demonstrating intolerance or resistance to imatinib with a median PFS of 6 months [19]. For GIST patients refractory to both imatinib and sunitinib, regorafenib [20], a multi-TKI blocking KIT, PDGFR and BRAF

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pathways, was approved in 2013 with a median progression-free survival (PFS) of 4 months.

The success of these agents in the metastatic disease setting led to imatinib use in the perioperative setting for patients with unresectable *KIT*-mutated GIST [21,22] and in the adjuvant setting after surgical resection depending on the risk of disease recurrence [23,24].

This review serves as an update on latest advances on GIST biology and the implementation of new methods to monitor the disease with circulating tumor DNA (ctDNA), as well as on the latest clinical trials and future perspectives.

Biology of GIST: new targetable mutations

Despite the discovery of driver genetic alterations in GIST, there remain 10–15% of all adult GIST patients and many pediatric GIST patients, which are KIT/PDGFR/SDH/RAS pathway negative or as proposed in 2015, quadruple WT [15]. The RAS-pathway mutant GISTs refer to the GISTs harboring mutations in *BRAF/RAS* or *NF1*. The clinical course of quadruple WT GISTs is generally more indolent compared with the *KIT* and *PDGFR*-mutated GIST, despite their higher metastatic propensity. Overall survival in adult patients with WT GIST is more favorable compared with the mutated adult GIST patients, which could reflect a similar molecular pathway to pediatric GIST. The mainstay of treatment of quadruple WT GISTs is surgical resection, but in progressive disease the treatment is challenging because the response to the traditional TKIs is poor [25].

Over the last 3 years, whole genome analysis has led to the discovery of new genomic patterns within the quadruple WT GIST category. To this date, a distinctive but grossly heterogeneous transcriptome profile has been described.

Nannini *et al.* [26] showed that both cases of quadruple WT GISTs included in the whole genome analysis of 16 GIST patients had a genomic profile profoundly different from either *KIT/PDGFR*α-mutated or SDH-deficient GISTs. Overexpression of molecular markers (CALCRL and COL22A1) and specific oncogenes including tyrosine and cyclin-dependent kinases (*NTRK2* and *CDK6*) and one member of the ETS (E26)-transcription factor family (ERG) were found in those two cases, both of which originated from the small intestine. ETV1, another member of the ETS family is highly expressed on GISTs and plays an important role in cell proliferation in KIT mutant GISTs [27].

Interestingly, Belinsky *et al.* described a patient with quadruple WT GIST [28], who was found to have a somatic inactivation of *NF1* without the NF1 syndrome and a novel loss of function mutation in *MAX*. This case of sporadic GIST did not share any characteristics with NF1 syndrome patients with GISTs. Surprisingly, Gasparotto *et al.* [29] also found *NF1* pathogenic mutations in 13 of 22 analyzed patients with quadruple WT GIST, in the absence of NF1 syndrome. However, since in seven patients the *NF1* mutations were constitutional, the presence of an unrecognized form of sporadic NF1 syndrome was hypothesized. Somatic mutations in *NF1* in the absence of NF1 syndrome in GIST patients may have diagnostic implications as well, considering the complexities of molecular identification of mutations in the *NF1* gene (58 exons).

At least two studies have identified *ETV6-NTRK6* fusions [30,31] in quadruple WT GISTs which can be potentially targeted by crizotinib [32]. In one study, Shi *et al.* [30] used gene expression analyses and parallel sequencing in 186 GIST cases to identify 24, which were quadruple WT. Two out of the 24 WT cases in that study, harbored *FGFR1* gene fusions (*FGFR1–HOOK3, FGFR1–TACC1*) and one harbored an *ETV6–NTRK3* fusion. The patient with the *ETV6–NTRK3* fusion was treated with a TRK inhibitor in clinical development (LOXO-101) resulting in radiographic and clinical response [33]. In an independent sample set of the same study, five quadruple WT GIST cases were found, including two additional cases with *FGFR1–TACC1* and *ETV6–NTRK3* fusions. In an earlier study, Brenca *et al.* [31] discovered the *ETV6–NTRK3* fusion in one patient with rectal quadruple WT GIST. *In vitro*, the *ETV6–NTRK3* fusion unveiled a possible mechanism of activation of IGF1R downstream signaling and cells were sensitized to IGF1R inhibition. Based on evidence suggesting that the *ETV6–NTRK3* fusion product is a target of ALK inhibitors, cells from the patient harboring the *ETV6–NTRK3* fusion were sensitized to crizotinib and ceritinib treatment as well.

Lastly, another report showed involvement of the *MEN1* gene [34] which in addition to the *MAX* gene suggest that at least a subcategory of quadruple WT GISTs may have similarities with neuroendocrine tumors and in particular pheochromocytomas. Whole genome analysis was employed in nine quadruple WT GIST

patients and 18 mutations per sample were identified underscoring the molecular heterogeneity of this specific category of GISTs. In addition to the *MEN1* and *MAX* gene mutations, *TP53*, *FGFR1* and *CTNND2* mutations were observed in this study.

Further efforts are needed to better characterize and understand these genomic alterations in the quadruple WT GISTs. It is unclear whether these mutations represent secondary molecular hits implicated in disease progression or early causative events in the pathogenesis of disease. Nevertheless, the discovery of distinct genomic profiles in this heterogeneous group of GISTs might lead to new targeted therapeutic strategies that are not available for these patients otherwise.

Advances in GIST diagnosis: ctDNA

Establishing the mutational status of the tumor is pivotal for treatment-planning decisions in clinical practice. Tissue obtained from biopsies or surgical procedures is currently the gold standard source for tumor DNA analysis. Due to the invasive nature of this kind of tumor tissue sampling, repeated biopsies are not done frequently. Therefore, in reality only a static molecular picture of the disease is obtained. In addition, formalin-fixed paraffin-embedded blocks are not ideal for processing genomic analyses, where fresh DNA is more appropriate [35]. Novel technologies have emerged over the last few years, focusing on isolating ctDNA from patient-derived plasma (liquid biopsy). This approach can potentially overcome the limitations of tissue sampling. In addition, blood sampling can be obtained at any time during the disease course, providing a dynamic assessment of genetic changes over time.

The application of ctDNA specifically in GIST patients has already been published in the recent literature. In a subgroup of patients in the Phase III GRID trial, concordance of up to 84% was reported between plasma and tissue for detection of primary *kit* mutations [36]. Yoo *et al.* [37] showed 100% concordance between ctDNA and tissue sample in patients with TKI refractory GISTs treated with dovitinib. In regard to the use of ctDNA as a dynamic measurement of disease, Maier *et al.* [38] reported a correlation of the amount of mutant ctDNA and disease progression. An observational study evaluating whether the trend in the levels of ctDNA may be related to different clinical behaviors of GISTs monitored by radiological investigations is currently conducted and recruiting patients (NCT02443948). These early results are encouraging and once validated in a larger scale, ctDNA may become the standard mean of monitoring the tumor genetic profile in progressive metastatic disease and possibly of detecting recurrence or progression prior to radiographic imaging.

Advances in treatment of metastatic disease

• Novel TKIs

Three TKIs are the US FDA approved for the treatment of metastatic *KIT/PDGFR* mutant GIST – the imatinib in first line and second and third line, sunitinib and regorafenib, respectively. However, the majority of patients treated with imatinib will eventually develop primary (<180 days of therapy) or secondary (>180 days of therapy) resistance due to secondary *kit* mutations, leading to reactivation of the KIT receptor and downstream pathways. Ongoing trials are exploring the efficacy of different mechanisms to overcome this resistance to first- and second-generation-approved TKIs.

KIT downstream inhibitors

It is well established that *kit*-mutated, imatinib-resistant, GIST continues to depend on KIT signaling for cell survival and proliferation via the PI3K/mammalian target of rapamycin and the RAS/RAF/MEK/MAPK signaling pathways [39,40]. Therefore, the inhibition of KIT downstream signaling pathways is a possible mechanism to overcome resistance to TKIs, and currently various agents are being investigated.

Although the clinical trial of imatinib plus everolimus was disappointing, two Phase I trials studying PI3K inhibition in combination with imatinib in third-line treatment of GISTs are currently ongoing. The BKM120 is a pan PI3Ki (NCT01468688), whereas the BYL719 is a selective inhibitor of the PI3K catalytic p110α subunit (NCT01735968). Results of both trials are awaited; the first trial has been completed and the second is ongoing but recruitment is completed. A preceding preclinical study where the PI3K inhibitors were tested in xenograft models showed significant synergistic effect in tumor volume reduction and increase in the proapoptotic effect. The combination of imatinib with a PI3K inhibitor improved the efficacy of either agent alone with greater antitumor effect [41]. This response was found to be dependent on the KIT genotype and specific molecular characteristics.

Another possible target of the KIT downstream pathway is the transcription factor ETV1, which serves as a regulator of the interstitial cells of Cajal playing a major role in the GIST growth and survival. Dual inhibition of KIT and ETV1 by imatinib and the MEK inhibitor MEK162 demonstrated a synergistic effect on tumor growth suppression *in vitro* and *in vivo* [42]. In addition, a positive feedback circuit between the downstream activation of KIT and the ETV1 regulation of KIT expression was uncovered and may partially explain this additive effect of dual inhibition of KIT and ETV1. However, a Phase I trial of imatinib and MEK162 in heavily pretreated patients showed that the combination had only a modest activity in imatinib refractory patients with only 2/17 treated patients having durable stable disease of more than 1 year [43]. Multiple lines of prior therapy could lead to activation of alternate pathways or development of new mutations which may be responsible for this modest activity of dual KIT and MEK inhibition in this Phase I trial. For this reason, a Phase II study is exploring the effect of imatinib in combination with MEK162 in patients with untreated advanced GIST (NCT01991379).

PDGFRα TKIs

The substitution of D842V in exon 18 of *PDGFRα* is resistant to imatinib, sunitinib and regorafenib [9,44]. The D842V mutation in *PDGFRα* is homologous to the D816V mutation in *kit* which is well established as resistant to imatinib *in vitro*. This mutation results in a ligand-independent activation of PDGFRα and represents the most common mutation in patients with primarily imatinib-resistant, gastric GIST [45,46]. Two novel agents targeting this specific mutation are currently in clinical trial. Crenolanib was shown to be the first TKI with activity against the *PDGFRα* D842V mutant GIST in a Phase II trial. The medication was well tolerated and results from the 16 treated patients were encouraging (2/16 patients achieved a partial response (PR), while 5/16 patients achieved stable disease (SD) or better) [47]. A Phase III randomized placebo-controlled trial of oral crenolanib versus oral placebo in combination with best supportive care in patients with advanced or metastatic GISTs with a D842V mutation in the *PDGFRα* is currently recruiting patients at Sylvester Comprehensive Cancer Center and other sites (NCT02847429). BLU-285, a novel small-molecule kinase inhibitor, was shown to have potent activity against the *PDGFRα* D842V as well as the *KIT* exon 17 GIST mutants both *in vitro* and *in vivo* models. *In vivo*, it was well tolerated and showed a dose-dependent tumor growth inhibition [48]. This highly selective agent has the potential to cover the entirety of *KIT* primary and secondary mutants and provides maximum benefits to patients. A Phase I trial of oral BLU-285 is currently recruiting patients with advanced, refractory or D842V mutant GISTs and other refractory or relapsed solid tumors at the Sylvester Comprehensive Cancer Center and other sites (NCT02508532).

Novel multikinase & KIT inhibitors

Several multikinase inhibitors have been studied recently or are being currently studied in clinical trials with potential activity in GISTs.

Dovitinib, an inhibitor targeting VEGF receptors (VEGFRs) 1–3, FGF receptors (FGFRs) 1–3, PDGFRβ and KIT [49], was studied in two Phase II trials. In the first trial, patients with GISTs refractory to imatinib and sunitinib were included and dovitinib was found to have modest activity with a median PFS of 3.6 months for the 30 treated patients [50]. In the second trial, 38 patients who progressed on imatinib were analyzed and the median PFS was 4.6 months [51]. Subgroup analysis might identify patients who derived benefit from dovitinib.

Vatalanib, a VEGFR, KIT and PDGFR inhibitor [52] was also tested in a Phase II trial in patients who were resistant to imatinib or imatinib and sunitinib. The median time to progression was 5.8 months in second line and 3.2 months in third line [53]. Vatalanib was generally well tolerated with overall efficacy results similar to sunitinib although much better tolerated.

More recently, cabozantinib, a TKI targeting KIT, MET, AXL and VEGFR showed significant tumor regression *in vivo*, in patient-derived xenograft models of GISTs carrying different *KIT* mutations [54]. A Phase I study which included four GIST patients in third-line treatment showed long-lasting SD (6–20 months) [55]. Subsequently, a Phase II trial studying the efficacy of cabozantinib in third-line treatment of GISTs after progression on imatinib and sunitinib without further exposure to other KIT- or PDGFR-directed TKIs, is ongoing (CABOGIST – NCT02216578).

Vandetanib targets RET, VEGFR and EGFR [56] and is approved in the treatment of metastatic medullary thyroid cancer. It has been used with some success in tumors with SDH loss. A Phase II trial (NCT02015065) which completed recruitment is assessing the clinical activity of vandetanib in pediatric and adult WT SDH-deficient GIST.

Famitinib, a VEGFR2, VEGFR3, PDGFR, Flt3, Flt1 and KIT inhibitor showed partial response in a patient with treatment naive GIST who was included in a Phase I trial [57]. A Phase II is currently undergoing (recruitment is completed) and studies the efficacy of famitinib in patients with advanced or metastatic GISTs who have failed imatinib (NCT02336724).

DCC-2618 is a potent inhibitor of primary mutant *KIT* with exon 9 or exon 11 mutations paired with secondary mutations in exons 13, 14 or 17, including the D816V mutation which is refractory to all currently approved TKI [58]. A Phase I trial currently recruiting patients at Sylvester Comprehensive Cancer Center and other sites (NCT02571036) will study the pharmacokinetics and the preliminary antitumor activity in patients with advanced malignancies including GISTs who have progressed to at least imatinib.

PLX9486 is an inhibitor of mutant *KIT* – including exon 17 mutations – and also of the wild-type KIT kinase activity [59] that along with PLX3397, another potent KIT inhibitor, are combined to target complementary *KIT* secondary-mutation profiles in a Phase Ib trial patients at Sylvester Comprehensive Cancer Center and other sites (NCT02401815) for patients with advanced solid tumors including GIST (fourth line). This TKI combination has the capability to block most of the KIT mutations conferring primary and secondary resistance to TKIs.

• Immunotherapy

The advance of immunotherapy over the last few years has been unprecedented in cancer therapeutics development. As our knowledge on the tumor microenvironment and the interaction between the cancer cells and the immune system expands, more therapeutic targets are unveiled [60]. Tumor-infiltrating immune cells create independent immunosuppressive networks and play a pivotal role in tumor surveillance and progression. $CD8^+$ T cells (cytotoxic) and regulatory T cells (suppressive cells) are part of these networks and shape the relationship between the tumor and the immune system. The balance between these two opposing forces is regulated by cytokines, checkpoint proteins and other types of immune cells [61]. In addition to targeted therapies against specific immune molecules such as checkpoint inhibitors, TKIs also have the capability to manipulate the immune system. In preclinical studies on *kit* mutant GIST mouse models, imatinib was found to exert its antitumor effect not only by directly inhibiting tyrosine kinases on tumor cells but also indirectly through the immune system by activating $CD8^+$ cells and inducing regulatory T-cell apoptosis [62]. The applications of immunotherapy in GIST have been focused so far on checkpoint inhibitors, vaccine development and adoptive cell therapy.

Checkpoint inhibitors have already been incorporated in the treatment of several solid malignancies and most recently in a hematologic malignancy as well. The approved checkpoint inhibitors consist of PD1 inhibitors (nivolumab, pembrolizumab), a PD-L1 inhibitor (atezolizumab) and a CTL-4 inhibitor (ipilimumab). In the treatment of patients with a GIST or other sarcomas, checkpoint inhibitors are not yet approved, but there are a number of clinical trials investigating the efficacy of immunotherapy in GIST. A Phase Ib trial of the combination of ipilimumab and dasatinib included eight patients with GISTs in third- and fourth-line treatments, and showed a few durable Choi responses [63]. The combination of immunotherapy and a second nonimmunotherapy agent in the management of advanced GIST is the subject of several open clinical trials: imatinib plus ipilimumab (Phase I – NCT01738139), PLX3397 (TKI) plus pembrolizumab (Phase I/IIa – NCT02452424), axitinib (anti-VEGFR/KIT/PDGFR) plus pembrolizumab (Phase II – NCT02636725), metronomic cyclophosphamide plus pembrolizumab (Phase II – NCT02406781). Dual blockade of two different checkpoint proteins with PD1 and CTL-4 inhibitors is also being studied in two clinical trials: nivolumab with or without ipilimumab in metastatic or unresectable GIST (Phase II – NCT02880020) and nivolumab plus ipilimumab in unresectable sarcomas including GIST (Phase II – NCT02982486). Like the various other solid malignancies where checkpoint inhibitors are used in second and third line and gradually moved to the first line, it is likely that over the next couple of years, immunotherapy

will start gaining ground in the treatment of GIST and other sarcomas as well.

Another immunotherapy application in the treatment of patients with GIST currently being investigated is the intratumoral vaccine Intuvax (activated dendritic cells from healthy donors) that is being tested in a Phase I trial (NCT02686944) for patients with metastatic and advanced GIST patients after failure to TKIs. Due to heterogeneity of the tumor types and expression of various immunogenic antigens, sarcomas could be ideal targets for vaccines. The promising early results of Intuvax from Phase I/II trials in renal and hepatic cancers set the precedent for GIST given the rarity and complexity of this malignancy.

A newer treatment strategy with promising results in hematologic malignancies is the development of chimeric antigen receptors, which are composed of the antigen-combining regions of the heavy and light chains of antibodies with a T-cell intracellular-signaling molecule. An anti-KIT chimeric immune receptor has already been developed and showed significant reductions in tumor growth rates in GIST mouse models [64]. Clinical trials exploring the use of chimeric antigen receptor T cells in mainly other types of sarcomas are now recruiting patients.

Advances of treatment in adjuvant setting

After the success of imatinib in the advanced disease setting, its application in the perioperative setting was explored as well. In patients with primary mutant GISTs, surgical resection with negative margins remains the main therapeutic modality. However, the risk of recurrence even after complete resection can remain high depending on specific tumor characteristics – tumor location, size and mitotic rate [65]. In addition to these recurrence risk factors, tumor rupture was later validated as independent negative prognostic factor likely related to tumor spillage [66]. The most frequently used risk assessment tools for GIST recurrence are the NIH Consensus Criteria [67], the Armed Forces Institute of Pathology (AFIP) Miettinen [68] Criteria and the Modified NIH Consensus Criteria proposed in 2008 [69]. More personalized stratification models using nomograms [70] and prognostic heat maps [66] were also developed to more accurately estimate the outcomes and assist in patient counseling. While the benefit of adjuvant therapy in terms of RFS is clear in the low- and high-risk group of patients, in the intermediate group it remains challenging. For example, the 'intermediate malignant potential' group based on the AFIP-Miettinen staging system had a 69–87% RFS depending on the location of the tumor and the mitotic rate. This variability in the RFS in the intermediate group could be attributed to the broad inclusion of patients in this group, as well as the lack of standardization among the different assessment tools, emphasizing the need for integrating further prognostic markers specifically for these patients. The nomograms provide more flexibility in defining these risk groups but the intermediate group benefit remains unclear.

Incorporating additional variables such as the tumor mutational status might improve the prognostic values of these tools. However, the assessment of probability of benefit based on the predictive marker of GIST mutation has not been widely accepted as a variable in the recommendation of adjuvant imatinib therapy. At Sylvester Comprehensive Cancer Center, we perform mutation testing in all patients who are treated with adjuvant imatinib after resection of GIST. For instance, a patient with *PDGFR* D842V, *raf, NF-1, ntrk* or other imatinib-resistant mutations would not be predicted to benefit from imatinib therapy.

In contrast to the established risk factors for recurrence after resection at least for the low- and high-risk groups, the optimal duration of treatment with imatinib is less clear. Randomized trials have evaluated the role of imatinib (400 mg daily) for 1, 2 and 3 years in the adjuvant setting [23–24,71] and the imatinib arms had significantly longer PFS when compared with the placebo arms, emphasizing the need for adjuvant treatment in general. Additionally, treatment for 3 years improved RFS and OS more when compared with 1 year of adjuvant treatment. Moreover, the RFS curves of 1 versus 3 years of adjuvant imatinib overlap within 12 months of discontinuing the treatment raising the question of true recurrence reduction or just delay. However, 3 years of treatment after resection is considered standard of care currently in GIST patients with high-risk features. The value of longer than 3 years of adjuvant treatment with imatinib is unknown. Adjuvant imatinib treatment for 5 years is under a Phase II clinical trial and long-term follow-up is pending (NCT00867113).

Adjuvant treatment for high-risk *KIT* and *PDGFR*α WT GISTs is not established and there are no specific guidelines available. Although not

supported by prospective clinical trials, our practice is to treat high-risk GIST patients with an adjuvant, precision medicine approach. We recommend therapy based on the mutation results, for example, RAF inhibitor for *raf* mutant GIST, imatinib 800 mg daily for *kit* exon 9 mutant GIST, mammalian target of rapamycin inhibitor for *PI3K* mutant GIST, among others. As more targetable mutations are being uncovered in the WT GISTs, and the development of TKIs specifically for these alterations is growing, it is a matter of time before these TKIs are transferred in the adjuvant treatment setting of these GISTs.

Conclusion

Major strides have been made in the management of GIST with KIT inhibitors, making GIST one of the first solid tumors with available targeted therapies. It is time that the focus shifted toward PDGFR and downstream inhibitors as well as other type of agents beyond TKIs, such as immunotherapy or novel combinations of both. Considering that sarcomas are believed to develop from genetic alterations in mesenchymal progenitor cells, genome editing technologies have also tremendous potentials in sarcoma biology research and development of therapies offering exciting opportunities for the future.

Future perspective

• Combination treatments

Combination treatments might offer a solution for refractory and resistant GIST tumors. As mentioned in the 'Advances of treatment in metastatic disease' section, clinical trials are currently investigating combinations of TKIs targeting KIT/PDGFRα as well as KIT downstream pathways, or TKIs with checkpoint inhibitors or chemotherapeutic agents. Recently, the combination of the newer multi-TKI, pazopanib, used in advanced renal cell cancer and soft tissue sarcoma after failure to chemotherapy with trametinib, a TKI used in patients with advanced melanoma carrying the *BRAF* V600E or V600K mutations is tested in a Phase II trial (NCT02342600). Finding the right combination has become the focus of many GIST clinicians.

• Circulating tumor DNA

Moving forward from establishing GIST diagnosis, the utility of ctDNA is studied in early secondary resistance mutation detection in parallel with timely treatment adaptation in a prospective trial (NCT02331914). In addition to ctDNA, imatinib serum concentrations as well will be obtained as part of this trial at specific disease intervals, providing a detailed mutation analysis and drug concentration assessment. Researchers aim to develop a predicting model for secondary imatinib resistance based on patient phenotype and tumor genotype.

• Pharmacogenetics in GIST

As newer technologies like next-generation sequencing and ctDNA are being implemented and more targeted therapies are becoming available, the focus will eventually shift to the optimal utilization of these agents. Even in a disease as rare as GIST, there are a number of studies trying to identify specific genetic factors affecting the variability of response to TKIs [72]. More studies are needed to validate specific signatures like miRNAs and single nucleotide polymorphisms described in smaller studies. Genotyping of metabolizing and transporter genes might become the standard of care before the initiation of treatment, in order to choose the appropriate TKI dose and optimize tumor responses.

• Immunotherapy

The development of antibody-drug conjugates (ADC) is a promising novel therapeutic approach. The use of antibodies specific to the tumor cell-surface yields tumor specificity and higher potency of the conjugated cytotoxic agents. In the past decade, two such agents have been approved: ado-trastuzumab emtansine in breast cancer and brentuximab vedotin in lymphomas. In GIST, a KIT-targeting ADC with an antitubulin agent showed antiproliferating activity against GIST *kit*-mutated cell lines *in vitro*. The anti-KIT ADC developed by Abrams *et al.* [73] was highly selective in cancers with elevated *KIT* expression regardless of their mutational status, which could be effective potentially in both *kit*-mutated and WT GIST tumors.

The abscopal effect is the resolution of unradiated metastatic lesions after the addition of radiotherapy to immunotherapy and was first described anecdotally in patients with metastatic melanoma [74,75]. Radiotherapy works synergistically with immunotherapy by inducing more inflammatory tumor cell death, dendritic cell and cytotoxic T-cell activation and antigen presentation [76]. Thus, this could potentially lead to long-term remission in patients with metastatic disease. A few clinical trials are currently

available for patients with melanoma combining immunotherapy and radiotherapy to investigate the immune responses. Should this synergistic approach prove effective, it may be explored in other types of cancers such as sarcomas.

Tumor-associated macrophages (TAM) are the most common immune cells in the GIST microenvironment [77]. They are classified as classically activated M1 and alternatively activated M2, with proinflammatory and anti-inflammatory functions, respectively. Much like the balance between the cytotoxic and regulatory T cells, the polarity of the TAM subtypes is governed by the tumor cell activity and is influenced by cytokines and other immune cells in the tumor microenvironment. In GIST mouse models, M1 subtype was shown to have antitumoral activity and after treatment with imatinib TAM were polarized to the M2 subtype. Human TAM also had similar polarization – M1 at baseline and M2 after imatinib treatment [78]. Despite that fact that functional studies of the TAM are limited, therapeutic modulation of the subtypes has been demonstrated in some preclinical studies. Two early clinical trials of macrophage modulators are open: a humanized anti-CD47 monoclonal antibody (Hu5F9-G4) that inhibits an antiapoptotic signal in human macrophage is in a Phase I trial recruiting patients with advanced solid tumors (NCT02216409), and a synthetic agonist of Toll-like receptor 4, a receptor expressed by macrophages, is currently in a Phase I trial recruiting patients with metastatic or unresectable soft tissue sarcomas with the combination of radiotherapy included as a means of increasing tumor antigen release (NCT02180698).

Cancer testis antigens (CTA) are a group of proteins regulating cell differentiation and development during embryogenesis and are expressed in multiple malignancies. They are normally expressed in the primitive spermatagonium of the testis and although they are highly immunogenic they do not provoke an immunogenic response naturally. In GISTs, the expression of CTA has been associated with a more aggressive phenotype with higher risk of recurrence and poor response to imatinib [79]. In addition to their potential prognostic role in GIST, CTA are potential targets for immunotherapy.

• Adjuvant treatment

Intermediate-risk GIST tumors pose a great challenge for GIST clinicians and the role of imatinib in this subgroup of patients is controversial. The European Organization for Research and Treatment of Cancer (EORCT) 62024 [80] failed to show a significant benefit of imatinib for these patients, although according to current consensus of risk classification a significant proportion of the patients assigned in the intermediate-risk group in that prospective study would be classified now as low risk. In a recent retrospective study [81] including only intermediate-risk patients, the findings were similar and imatinib effect on outcomes were not clear. Both results suggest that intermediate-risk GISTs behave more like low-risk GIST tumors, but this is not established and this risk category is not homogenous. The addition of more prognostic tools like the mutational status might be of value although not yet largely validated. Interestingly, a Genomix index was

EXECUTIVE SUMMARY

- Once more widely clinically validated, circulating tumor DNA could substitute tissue biopsies for mutational profiling, especially in following-up patients with metastatic gastrointestinal stromal tumors (GISTs).
- Quadruple WT GISTs have a distinctive but grossly heterogeneous genomic profile. New potentially targetable genetic alterations (like *ETV6–NTRK3* fusion, *NF-1* mutations in the absence of neurofibromatosis type I disease) have been described in quadruple WT GIST.
- Novel tyrosine kinase inhibitor targeting downstream KIT pathways are in development – PI3K inhibitors (BKM120, BYL719), ETV1 inhibition by MEK inhibitors (MEK162).
- Crenolanib and BLU-285 are tyrosine kinase inhibitors with activity against the D842V *PDGFR*α mutation and currently in clinical trials.
- Multikinase inhibitors attempting to block a variety of KIT mutations are being studied.
- Applications of immunotherapy are being tested in GISTs – checkpoint inhibitors, vaccines and adoptive cell therapy.
- Optimal duration of adjuvant imatinib in high-risk mutant GISTs is not clear. Three years of adjuvant imatinib is more beneficial than 1 year of treatment. Five years of adjuvant treatment is under investigation.

used in 67 GIST samples and was able to detect high-risk patients among the intermediate-risk patients by the AFIP score by identifying genetic signatures associated with poor prognosis and metastatic propensities [82].

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