Master Thesis

MOLECULAR ALTERATIONS, PRIMARY AND SECONDARY RESISTANCE MUTATIONS IN ADVANCED GIST AND THEIR THERAPY OPTIONS: A LITERATURE REVIEW

Molekulare Alterationen, primäre und sekundäre Resistenzmutationen bei einem fortgeschrittenen GIST und deren Therapiemöglichkeiten: ein Literaturreview

submitted by

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Affidavit

I declare on my word of honour that I have written the present thesis independently and without foreign assistance from third parties, have used no other source material than those here listed and have referenced any text or contents from the used sources appropriately.

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1. List of Abbreviations

AKT3: RAC-gamma serine/threonine kinase 3

ARID1A: AT-rich interaction domain 1A

AS-PCR: allele-specific ligation PCR

BEAMing: beads, emulsion, amplification, magnetics-digital PCR

BRAF: B-raf

BSAS: bisulfite amplicon sequencing

CAPP- Seq: cancer personalized profiling by deep sequencing

CR: complete response

CSS: Carney Stratakis syndrome

CT: Carney triad

ctDNA: circulating tumour DNA

ddPCR: droplet digital PCR

DOG1: Anoctamin

EMA: European Medicines Agency

ESMO: European Society of Clinical Oncology

EURACAN: European Reference Network on Rare Adult Cancers

FGFR1: fibroblast growth factor receptor 1

GENTURIS: GENetic TUmour RIsk Syndromes

GIST: gastrointestinal stromal tumour

HGVD: Human Genome Variation Society

HIF1α: hypoxia-inducible factor 1α

HPFs: high-power fields

HSP90: heat shock protein 90

IHC: immunohistochemistry

KIT: receptor tyrosine kinase

KRAS: Kirsten rat sarcoma virus

mOS: median overall survival

mPFS: median progression free survival

NCCN: National Comprehensive Cancer Network

NF1: neurofibromatosis 1

NGS: next generation sequencing

NTRK: neurotrophic tyrosine receptor kinase

ORR: overall response rate

PDGFRA: platelet-derived growth factor receptor-α

PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

PR: partial response

PTEN: phosphatase and tensin homolog

RECIST: Response Evaluation Criteria in Solid Tumors

RNAseq: RNA sequencing

RT-PCR: reverse transcription polymerase chain reaction

SafeSEQ: Safe-sequencing system

SCF: stem cell factor

SDH: succinate dehydrogenase

SiMSen-Seq: Simple, Multiplexed, PCR-based barcoding of DNA for Sensitive mutation detection using Sequencing

TAm-Seq: Tagged-amplicon deep sequencing

TKI: tyrosine kinase inhibitor

TSC: tuberous sclerosis

TET: family of ten-eleven translocation methylcytosine dioxygenases

UMI: unique molecular identifiers

VAF: variant allele frequency

VEGF: vascular endothelial growth factor

2. Abstract

GIST is the most common sarcoma of the gastrointestinal tract. The discovery of the *KIT* and *PDGFRA* mutations as the oncogenic drivers in GIST have changed the prognosis of advanced GIST dramatically, due to targeted therapy option with TKI imatinib. However, advanced GIST patients eventually progress, while on imatinib, developing secondary resistance mutations to imatinib. Treatment with next-line TKIs results similarly with relatively rapid progression because of further evolution of resistance mutations. This represents advanced progressive GIST as genetically dynamic and heterogenous tumour, demonstrating multiple resistance mutations in different metastases and even the same metastasis. According to this, tissue biopsy cannot provide reliable information of the whole mutational burden in progressive advanced GIST. Hence, ctDNA assays are being incorporated in the recent studies to expand the detection of resistance mutations and to support targeted therapy decisions. However, the sensitivity should be improved to apply ctDNA in the routine diagnostics.

Subset of GIST patients harbour no *KIT* and *PDGFRA* mutations, describing wild-type GIST. Using larger gene panels, other oncogenic drivers in this group were detected, showing no or weak response to TKIs. The extension of wild-type gene panels and diagnostic sequence of wild-type GIST is not strong defined.

This master thesis is a literature review, including scientific data from guidelines, randomised controlled clinical trials, prospective cohort studies, retrospective cohort studies, *in vitro* studies, case series and case reports and other literature reviews. The purpose of this literature review is to answer two research questions to offer own interpretation: how and when secondary resistance mutations should be assessed and which genes should be covered in newly diagnosed and progressive advanced GIST.

3. Zusammenfassung

GIST ist das häufigste Sarkom des gastrointestinalen Traktes. Die Entdeckung von onkogenen Treibermutationen in *KIT* und *PDGFRA* verbesserte die Prognose signifikant wegen des Einsatzes von einer zielgerichteten Therapie mit einem TKI Imatinib. Jedoch zeigen sich die Patienten mit einem fortgeschrittenen GIST schließlich unter Imatinib progredient aufgrund sekundärer Resistenzmutationen gegen Imatinib. Die Therapie mit TKIs der weiteren Therapielinien führt ebenso zu einer relativ raschen Progression wegen neuer Resistenzmutationen. Dies stellt den fortgeschrittenen progredienten GIST als einen genetisch dynamischen und heterogenen Tumor mit multiplen Resistenzmutationen in verschiedenen Metastasen und sogar in einer Metastase dar. Diesbezüglich kann eine Gewebebiopsie nicht die ganze Mutationslast widerspiegeln. Flüssigbiopsien, d.h., ctDNA werden in rezenten Studien inkludiert, um den Nachweis von Resistenzmutationen zu erweitern und um die Therapieentscheidungen zu steuern. Jedoch soll die Sensitivität von ctDNA erhöht werden, um sie in der Routinediagnostik verwenden zu können.

Der kleinste Teil von allen GISTs trägt keine Mutation in *KIT* und *PDGFRA*, beschrieben als der nichtmutierte GIST. Mittels größeren Genpanels konnten andere onkogene Treibergene festgestellt werden, die kein oder niedriges Ansprechen auf TKIs zeigen. Eine optimale Erweiterung des Genpanels oder ein diagnostischer Algorithmus bei einem nichtmutierten GIST ist nicht gut definiert.

Bei dieser Masterarbeit handelt es sich um ein Literaturreview. Wissenschaftliche Daten von Leitlinien, randomisierten kontrollierten klinischen Studien, prospektiven Kohortenstudien, retrospektiven Kohortenstudien, *in vitro* Studien, Fallserien, Fallberichten und anderen Literaturreviews sind hier gesammelt und zusammengefasst. Das Ziel des Literaturreviews ist die zwei Fragestellungen zu beantworten und eine entsprechende eigene Interpretation anzubieten: wie und wann sollten die sekundären Resistenzmutationen getestet werden und welche Gene sollte ein Genpanel bei einem neu diagnostizierten und progredienten fortgeschrittenen GIST abdecken.

4. Introduction

Gastrointestinal stromal tumour (GIST) is the most common sarcoma of the gastrointestinal tract [1] and is known to be resistant to chemotherapy or radiotherapy with poor prognosis before the introduction of imatinib into treatment of GIST [4]. With the first evidence of receptor tyrosine kinase gene (*KIT*), and a few years later platelet-derived growth factor receptor-α gene (*PDGFRA*) mutations playing major role in the molecular pathology in GIST, various tyrosine kinase inhibitors (TKIs) have become the central therapy option for GIST, the first of them imatinib, showing generally good prognosis also in advanced disease [4]. Unfortunately, advanced GIST patients treated with imatinib eventually progress in average of 2 years of the treatment due to secondary resistance mutations to imatinib [4]. This describes GIST as a genetically very heterogenous disease [43]. Other TKIs, e.g., sunitinib and regorafenib are standard next-line therapy options, although showing less survival benefit because of further mutational pressure, while on these TKIs [1,35]. Novel TKIs (ripretinib, avapritinib) have obtained role in GIST treatment several years ago [1,35]. However, the extensive mutational heterogeneity in progressive advanced GIST is a major problem also for the treatment with the newest TKIs [43]. Although primary and secondary resistance mutations in *KIT* are well described and new therapy approaches including drug combinations are being investigated, patients with advanced GIST including metastatic or unresectable GIST portray a problematic group in terms of molecular diagnosis. Tissue biopsy from one or few metastases or primary tumour cannot represent the whole genetic landscape of the advanced progressive GIST because of its intralesional and interlesional heterogeneity as well as dynamic genetic change over time, harbouring multiple mutations even in one lesion [43]. Liquid biopsy, namely circulating tumour DNA (ctDNA) assays could be a new diagnostic tool in this situation, demonstrating a full spectrum of secondary resistance mutations [51,63,68]. However, ctDNA assays need to be validated for clinical praxis as well as the target group to reach the maximal sensitivity [49]. The second problem group in GIST is so-called wild-type GIST, reflecting approximately 15 % of GIST patients without *KIT* and *PDGFRA* mutations [4]. This group includes patients with alterations in other driver genes, e.g., *BRAF* (B-raf), *NF1* (neurofibromatosis 1), *KRAS* (Kirsten

rat sarcoma virus), *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α), *FGFR1* (fibroblast growth factor receptor 1), *NTRK* (neurotrophic tyrosine receptor kinase), SDH-complex (succinate dehydrogenase) encoding genes [4,32,34]. In some cases, no driver mutation can be found despite the application of large gene panels covering some hundreds of genes [27]. This means that in wild-type GIST other diagnostic steps including extended gene panel should be used. Wild-type GIST shows less or no response to TKIs [1,35] used in *KIT* and *PDGFRA* mutated GIST and therefore accurate molecular diagnostics has its therapeutic consequence.

4.1. Research Questions

1. When and how should the resistance mutations in advanced GIST be detected to maximally cover the genetic heterogeneity?
2. Which genes should be covered in advanced GIST for the first diagnosis and progressive disease and is the stepwise diagnostic appropriate?

There are no standard answers to these complex problems to be found in the guidelines for GIST (e.g., ESMO-EURACAN-GENTURIS, NCCN) [1,35], so the purpose of this master thesis is to try to answer the research questions theoretically and to find the general consensus for the molecular subclassification, based on the previous studies.

4.2. Research Tasks

1. Review of driver genes involved in GIST molecular pathogenesis.
2. Review of frequencies of primary mutations, primary resistance mutations and secondary resistance mutations in advanced GIST.

3. Brief explanation of the structure of KIT and PDGFRA proteins and their core domains.
4. Review of hereditary GISTs and GIST syndromes (GIST associated with particular clinical signs), responsible genes and their role in diagnostic algorithm.
5. Review of the tissue biopsies and ctDNA assays in GIST.
6. Review of molecular diagnosis by immunohistochemistry and molecular pathology methods.
7. Review of therapeutic options subordinated to molecular targets.
8. Review of probable future therapy options.

5. Materials and Methods

The type of this master thesis is a literature review. The section “Results” includes scientific literature of molecular pathology of sporadic and germline GIST, particularly reflecting primary and secondary resistance mutations, as well as diagnostic methods and therapeutic options. The section “Discussion” provides the author’s interpretation of diagnostic algorithm of GIST, based on the included literature.

The study group covers advanced GIST, namely metastatic, relapsed or nonresectable tumour and excludes early GIST.

The literature search started with the strongest evidence data, namely using the newest version of guidelines for GIST (ESMO-EURACAN-GENTURIS and NCCN) [1,35]. To expand the literature search, PubMed database was used, involving randomised controlled clinical trials, prospective cohort studies, retrospective cohort studies, *in vitro* studies, case series, case reports and literature reviews. The following search terms were used: GIST ‘OR’ gastrointestinal stromal tumour (‘OR’ tumor) ‘AND’ mutations / resistance mutations / wild-type / *KIT* / *PDGFRA* / *BRAF* / *SDH* / *NF1* / *PIK3CA* / *KRAS* / *NTRK* / pathology / bisulfite sequencing / methylation / epimutation / resistance / molecular heterogeneity / liquid biopsy / circulating tumour DNA / ctDNA / treatment / targeted therapy / tyrosine kinase inhibitor / TKI / imatinib / sunitinib / regorafenib / avapritinib / repritinib / nilotinib / pazopanib / sorafenib / entrectinib / larotrectinib / Carney-Stratakis / Carney triad / syndromic / hereditary / familial / germline mutations.

All the variants are displayed according to the current Human Genome Variation Society (HGVD) nomenclature (https://varnomen.hgvs.org), using protein reference sequence.

All the figures were created with BioRender.com. The author owns the licence of BioRender.

6. Results

GISTs are one of the most common sarcomas in human as well as the most common malignant mesenchymal tumours of the gastrointestinal tract. However, their incidence is rare, ranging from 0.4 to 2 cases per 100 000 per year. The origin of GIST are interstitial cells of Cajal, known as pacemaker cells in the muscular layer in the gastrointestinal tract [1]. The anatomic localizations of GIST are as follows: oesophagus (1 - 2 %), stomach (59 - 61 %), duodenum, especially the second part and involving pancreas (4 - 5 %), small intestine (jejunum and ileum) (30 %), colon (more left sided) and appendix (1 - 2 %), rectum (4 %), extragastrointestinal (1 %), i.e., omental, retroperitoneal, mesenteric, serosa of rectovaginal septum / urinary bladder / uterus, gallbladder [2]. GISTs metastasize mostly to the liver and peritoneum, with 20 % of cases showing synchronous and 30 % - metachronous metastases [3]. GIST is generally chemotherapy and radiotherapy resistant, but due to its molecular alterations, mainly in tyrosine kinase signalling pathways, targeted therapy with TKIs has become a major pillar of treatment in GIST [4]. The histological diagnosis includes positive CD117 (KIT) immunohistochemical (IHC) staining (in 95 % of cases). DOG1 (Anoctamin 1) is another IHC marker in GIST, mostly as additional IHC marker identifying CD117-negative GIST. Mitotic count per 50 high-power fields (HPFs), tumour localization, tumour size and genotype are absolutely necessary for therapeutic decisions [3]. When the treatment with TKIs is planned, the molecular pathology diagnostics is mandatory, to select the right TKI for the right patient.

6.1. Molecular Pathology of GIST

About 80 % of all GISTs harbour a *KIT* protooncogene gain-of-function mutation, leading to the ligand-independent activation of the kinase. KIT belongs to the type III receptor tyrosine kinase family; its corresponding ligand, stem cell factor (SCF), stimulates the homodimerization and activation of KIT [4]. *KIT* is localized on chromosome 4q12 and contains 21 exons [5]. KIT protein consists of extracellular immunoglobulin-like domain with ligand-binding site, transmembrane domain,

juxtamembrane domain, kinase 1 domain (with ATP-binding pocket), kinase insert domain, kinase 2 domain (with activation-loop) and C-terminal tail (Fig.1) [6].

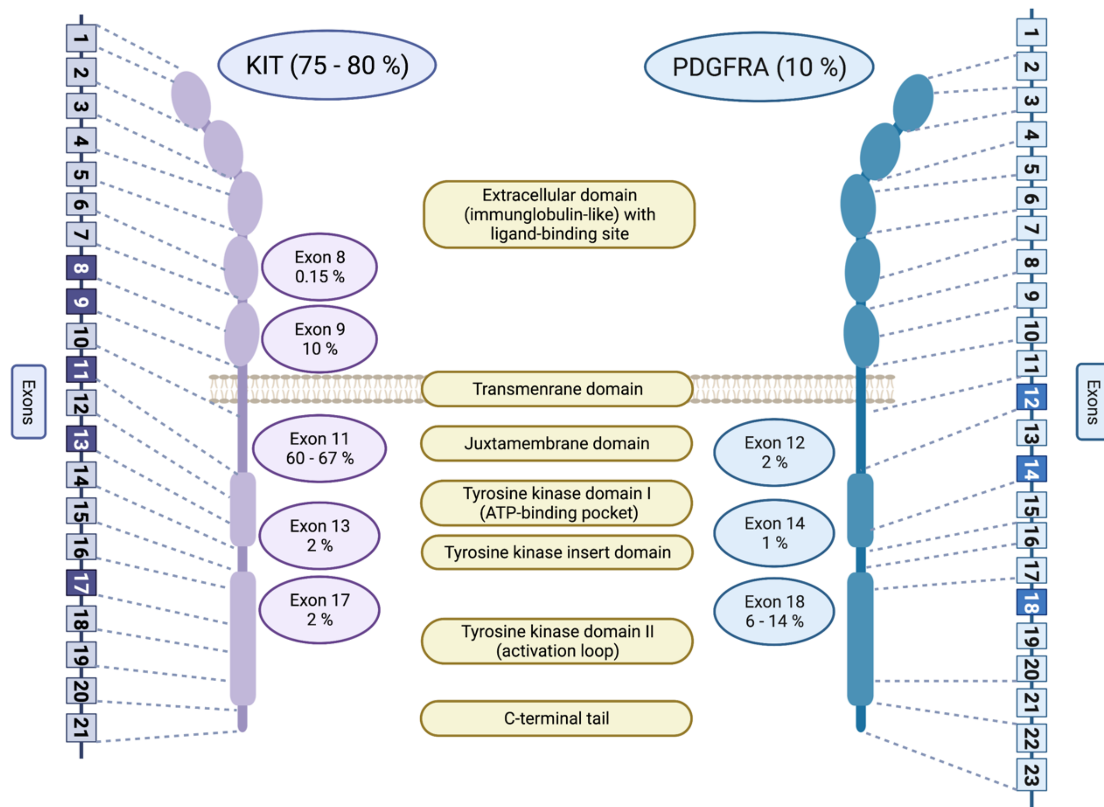

Figure 1. KIT and PDGFRA structure, domains, coding exons and the common primary mutations with frequencies. Created with BioRrender.com. Adapted from [4,6-13].

KIT (as well as PDGFRA) are dual switch kinases by having an inhibitory switch in the juxtamembrane domain and an activating switch in the activation loop. The kinase switch pocket is located near the ATP-binding pocket. By binding of the activating or inhibitory switch to the kinase switch pocket, the tyrosine kinase is being turned in the "on" or "off" state. Thus, disruption of one or both of the switches leads to ligand-independent activation of the tyrosine kinase [7]. The most common primary mutations (60 - 67%) are found in exon 11 (encodes juxtamembrane domain), including mostly in-frame deletions (60 %), rarely duplications, large deletions (more than 15 codons), insertions (2 %), substitutions (17 %, most common being p.Val559Asp and p.Leu576Pro), indels (16 %) or delins (frequent in codons 557 – 558) and splice-site mutations [4,8-12]. The majority of exon 11 mutations cluster between codons 550 and 560 [9]. Primary exon 9 (encodes extracellular domain) mutations make 10 % of *KIT* mutations and are frequent

described as tandem duplications (e.g., p.Ala502_Tyr503dup) [4,8,9,11]. Primary exon 17 (encodes activation-loop) and primary exon 13 (encodes ATP-binding pocket) mutations are rare (approximately 2 % each) and primary exon 8 mutations are very rare (0,15 %) (Fig. 1) [4,8]. According to the BFR14 trial, a multicentre randomised phase 3 study, median progression free survival (mPFS) and median overall survival (mOS) of patients with advanced GIST with exon 9 mutations were 12.6 and 55 months, respectively, and for exon 11 mutations – 39.4 and more as 73 months, respectively [12]. This reflects the poorer prognosis of patients with advanced GIST harbouring *KIT* exon 9 mutations. When dividing the *KIT* exon 11 mutations by localization in the upstream of codon 557, within codons 557 to 558 and downstream of codon 558, the mutations in codons 557 - 588 showed better complete response (CR) rate (29.3 % *vs* 41.1 % *vs* 23.1 %, p = 0.028), but worse mPFS (49.4 months *vs* 30.6 months *vs* 63.3 months, p = 0.0176) [12].

PDGFRA is as well a type III receptor tyrosine kinase and owns analogous structure to KIT. The ligand of PDGFRA is PDGFA [4]. *PDGFRA* gene is located on chromosome 4q12 and consists of 23 exons [13]. *PDGFRA* mutations are mutually exclusive, meaning that no *KIT* mutations are found in *PDGFRA* mutated GIST and *vice versa* [4]. Primary *PDGRA* mutations are found in exon 12 (encodes juxtamembrane domain) with 2 % (e.g., p.Val561Asp), exon 18 (encodes activation loop) with 6 - 14 % (the most common mutation p.Asp842Val) and exon 14 (ATP-binding pocket) (e.g., p.Asn659Lys, p.Asn659Tyr) with 0,6 % (Fig. 1) [8,11]. Autophosphorylation of KIT and PDGFRA leads to constant activation of downstream signalling pathways RAS/RAF/MEK and PI3K/AKT/mTOR, causing accelerated cell proliferation and survival (Fig. 2) [4,14].

Approximately 10 – 15 % of GIST harbour no *KIT* or *PDGFRA* mutation, former wild-type GISTs, including other rare mutations, e.g., *BRAF*, *KRAS*, *NRAS*, *HRAS*, *NF1*, *PIK3CA*, SDH complex encoding genes. SDH complex, consisting of four subunits (SDHA, SDHB, SDHC and SDHD), oxidizes succinate to fumarate in the mitochondrial Krebs cycle [4]. *SDHA* is located on chromosome 5p15 and consists of 17 exons [15], *SDHB* – on chromosome 1p36 with 8 exons [16], *SDHC* – on chromosome 1q23 with 7 exons [17], *SDHD* – on chromosome 11q23 with 6 exons [18]. Due to loss of function mutations in SDH complex encoding genes, being tumour suppressor genes, the level of the substrate succinate increase, leading to

inhibition of the enzyme family of ten-eleven translocation methylcytosine dioxygenases (TET), DNA demethylases, thus stimulating dysregulation of DNA-methylation (towards hypermethylation), stabilization of hypoxia-inducible factor 1α (HIF1α), stimulating oncogene transcription, and increasing vascular endothelial growth factor (VEGF) (Fig. 2) [4,11]. SDH-deficient GISTs have demonstrated higher genome hypermethylation comparing to SDH-proficient GISTs, assessed by 450 K Infinium Methylation array platform. Pathogenetically it can be explained by accumulation of succinate and thus toxic effects on *TET2* enzyme, that is crucial for DNA demethylation [19]. This represents a completely other pathogenesis through loss-of-function of SDH complex. SDH-deficient GIST can be sporadic or germline and part of syndrome, also being sporadic or germline (see section "GIST Syndromes"). SDHB IHC is an excellent surrogate marker for the first diagnosis of SDH-deficient GIST, demonstrating loss of expression regardless of affected SDH-subunit. There are no SDHC or SDHD antibodies for IHC available. SDHA antibodies could be used to further search for mutations in SDHA in case of loss of SDHA IHC expression [11]. SDH-deficient GIST makes to 13.9 % of GISTs, with the majority of mutations in *SDHA* (5.4 %), followed by *SDHC* promoter hypermethylation (3.9 %), *SDHB* mutations (2.5 %), *SDHC* mutations (1.9 %) and *SDHD* mutations (0.2 %) [14]. The most appropriate method for determination of *SDHC* promoter hypermethylation is not good established in the clinical practice [20]. Since only *SDHC* promoter should be covered, amplicon-based assay can be used, e.g., bisulfite amplicon sequencing (BSAS) by Illumina [21]. This method includes two CpG islands of *SDHC* promoter, namely CpG:17 and CpG:27, where the first one consists of 11 CpG sites and CpG:27 has totally 16 CpG sites. The amplicon lengths are 217 bp and 250 bp, respectively [22]. In an animal model using BSAS was demonstrated, that methylation level of 0 – 20 % is associated with high mRNA expression and methylation level of 75 – 100 % - with no mRNA expression [23].

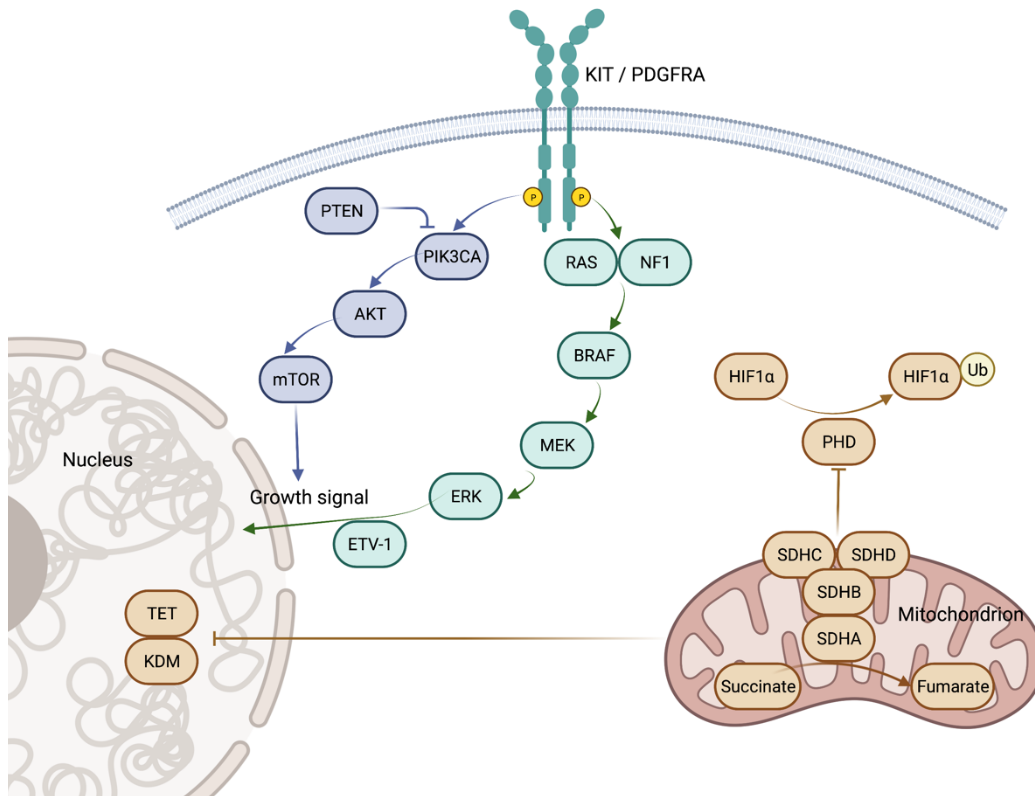

Figure 2. Oncogenic molecular pathways in GIST. Mutations in *KIT*, *PDGFRA*, *PIK3CA*, *AKT*, *NF1*, *RAS*, *BRAF*, *PTEN* leads to activation of PI3K-AKT-mTOR and RAS-RAF-MEK-ERK pathways. Accompanied with ETV1 this induces accelerated proliferation. Under normal oxygenation HIF1α is inactivated through prolyl-hydroxylase domain (PHD) through ubiquitination. Loss-of-function of SDH-complex leads to the accumulation of intracellular succinate, which inhibits PHD, thus causing increased HIF1α activity induced tumorigenesis. Additionally, succinate accumulation inhibits TET and KDM enzymes, leading to DNA and histone methylation. Created with BioRender.com. Adapted from [4,24].

BRAF, known as protooncogene, with its activating mutation p.Val600Glu in exon 15 is found mutually exclusive in the wild-type GIST, ranging from 3.5 % [9] - 3.9 % [8] – 13 % [25] of GISTs. *BRAF* mutations were found as primary (at the first diagnosis) and secondary resistance (in progressing tumours) mutations in these studies. BRAF is localized downstream of KIT or PDGFRA in the signalling pathway of RAS/RAF/MEK [4,14], reaching the same oncogenic effects as with the activation of KIT or PDGFRA.

One case with *PIK3CA* mutation p.His1047Leu in GIST was described, detected together with *KIT* exon 11 mutation [9]. In larger study group with 529 GIST patients

PIK3CA mutations were detected in 10 patients with primary tumour more than 14 cm of size and in two of them being metastatic. *PIK3CA* mutations were found in *KIT* mutated tumours and demonstrated resistance to imatinib and had short survival. The mutations were clustered in exon 1, 9 and in most of the cases exon 20 [26]. *PIK3CA* mutations are very rare in GIST and are typically detected together with *KIT* mutations. Besides, *PIK3CA* mutations are probably passenger mutations, predicting resistance of TKIs. This could be analogous explained with the PIK3CA localisation downstream of KIT or PDGFRA in the PI3K/AKT/mTOR signalling pathway [4,14], being independent of KIT and PDGFRA inhibition.

KRAS mutations are extremely rare in GIST (< 0.2 %), demonstrating few cases with p.Gly12Asp, p.Gly12Ala, p.Gly12Val, p.Gly12Arg, p.Gly13Asp mutations, in most of the cases occurring simultaneously with *KIT* or *PDGRA* mutations and only in one case as isolate mutation. *KRAS* mutations demonstrate resistance to imatinib [27]. Identically to *BRAF* mutations, KRAS stimulates the downstream signalling pathway RAS/RAF/MEK [4,14], thus illustrating the resistance to imatinib.

AT-rich interaction domain 1A gene (*ARID1A*) frameshift mutation with loss-of-function is described as potential driver mutation in wild-type GIST with no other mutations found. Since ARID1A influences accelerated phosphorylation of AKT, activation of the downstream PI3K/AKT/mTOR signalling pathway explains its role in the tumorigenesis [27].

Among *KIT* and *PDGFRA* wild-type GIST patients 1.5 % are associated with neurofibromatosis 1 (NF1), demonstrating the majority of tumours in small intestine as well as multiple GISTs in the same patient [28]. However, some authors reported cases with *NF1* and concomitant *KIT* or *PDGFRA* mutations [29,30], although in another study the *NF1* variants were benign in both of the *KIT* mutated patients [31]. In this study group germline *NF1* mutation was detected in 7 out of 22 *KIT / PDGFRA / SDH / BRAF* wild-type patients, showing previous unrecognized NF1 syndrome in adult population with the onset in the late fifth decade.

NTRK1, NTRK2, NTRK3 gene fusions are oncogenic drivers in various tumours with a frequency to 1 %. The most common fusion is known to be *ETV6-NTRK3* fusion and is the most frequent in GIST as well. The most appropriate method for the diagnostics of these fusions in GIST is NGS based RNA sequencing (RNAseq),

knowing that soft tissue spindle cell tumours show false positive results with pan-TRK IHC [11,32]. However negative pan-TRK IHC expression exclude *NTRK* fusion, so it could be used as the first diagnostic step and when positive, RNAseq should follow as confirmatory test. The detection of the fusion partner gene with reverse transcription polymerase chain reaction (RT-PCR) or FISH is not feasible. DNA based NGS sequencing is disadvantageous in case of *NTRK2* or *NTRK3* fusions due to large introns in these genes. Point mutations in NTRK are with no clinical use so far. In case of GIST the diagnostics of *NTRK* fusions should be done in *KIT*, *PDGFRA*, *SDH*, *NF1*, *BRAF* wild type genotype [32]. There are commercial NGS RNAseq gene panels available, e.g., Archer FusionPlex Sarcoma v2 for sarcoma, covering 63 genes and including all of the *NTRK1-3* genes [33].

Very rare, namely in 2 of 12 patients lacking *KIT*, *PDGFRA*, *RAS*, *SDHA-D* mutations, gene fusions involving *FGFR1* gene (*FGFR1-HOOK3* and *FGFR1-TACC1*) are described and therefore testing for *FGFR1* fusions with RNAseq in this wild-type population could be meaningful [34]. In addition, Archer FusionPlex Sarcoma v2 gene panel also targets *FGFR1-3* genes [33].

Notably, rarely no driver mutations can be found, even covering hundreds of genes. One study, analysing 8 *KIT* and *PDGFRA* wild-type GISTs with DNA based NGS gene panel of 341 cancer-related genes could not find any mutation in one patient with advanced GIST [27].

In harmony with ESMO-EURACAN-GENTURIS and NCCN guidelines the molecular pathology of GIST should include tests for *KIT*, *PDGFRA*, *BRAF*, *NF1*, *NTRK*, *FGFR* alterations, optimally with Sanger sequencing or NGS, as well as, in *KIT* / *PDGFRA* wild-type GIST – IHC for SDHB [1,35]. The precise sequence and extent of the mutation testing is not served in these guidelines and probably depends on the principles in every particular sarcoma centre.

To estimate the prognosis, if all the *KIT* and *PDGFRA* wild-type GISTs are summed up the mOS and mPFS is 54.9 months and 12.3 months, respectively [12], thus being worse compared to *KIT* and *PDGFRA* mutated GIST.

6.2. GIST syndromes

In approximately 5 % of cases GIST is associated with a syndrome and can be subdivided in SDH-proficient and SDH-deficient GIST syndrome [24].

6.2.1. SDH-proficient syndromic GIST

Familial *KIT*-mutated syndromic GIST, harbouring germline mutations in the same exons as sporadic GIST, namely exon 11, rarely exon 8, 9, 13, 17, shows autosomal-dominant inheritance pattern, affecting predominantly young patients with median age of 40 – 50 years. Clinical manifestations include multiple GISTs, hyperpigmentation, diffuse interstitial Cajal cell hyperplasia with dysphagia and urticaria pigmentosa [24].

Familial *PDGFRA*-mutated syndromic GIST is an autosomal-dominant inherited syndrome with such clinical manifestations as lipomas, inflammatory fibrous tumours, large hands and multiple GISTs, most commonly in stomach. However, diffuse hyperplasia of interstitial cells of Cajal is not typical. Predominantly females are affected, median age is 40 to 50 years. The mutations in *PDGFRA* cluster in exon 12, 14 and 18 [24].

Neurofibromatosis type 1 is an autosomal-dominant inherited disease, bearing inactivating mutations in *NF1* tumour suppressor gene on chromosome 17q11. The incidence is 1 in 2500 to 1 in 3000 people per year [36]. NF1 is characterized by variable clinical expression with mainly *café-au-lait* spots, axillary or inguinal freckles, Lisch nodules in the eye, optic glioma, bony dysplasia, neurofibromas, neuroendocrine tumours and multiple GISTs (approximately 7 % of patients), mostly in the small intestine. The median age is 49 years. These patients are resistant to imatinib, although they show mainly no aggressive clinical course [24,36,37].

6.2.2. SDH-deficient syndromic GIST

Carney-Stratakis syndrome (CSS) describes a dyad of paragangliomas and GISTs, which are usually multiple, with gastric localization and regional lymph node metastases. The median age is 30 years, most commonly affecting females. CSS is autosomal dominant inherited syndrome; loss-of-function mutation in any of the SDH-complex genes can be causal. CSS patients are resistant to imatinib. However, they demonstrate indolent course and response to sunitinib and regorafenib [24]. SDHA-deficient GIST occurs mostly due to germline mutations with the most frequent being nonsense, loss-of-function mutation p.Arg31Ter. The median age of onset is classically later as in other SDH-deficient GISTs, i.e., median 34 years [11].

Carney triad (CT) includes paragangliomas, pulmonary chondromas and multiple GISTs, mostly having gastric localization. Almost exclusively young females are affected with median age of 22 years. CT shows no inheritance and the genetic background of CT is *SDHC* promoter hypermethylation [24,38].

Germline testing and genetic counselling for the patient and relatives should be offered in the case of features for inherited GIST [24].

6.3. Primary Resistance to Imatinib in GIST

Primary resistance to imatinib means progression within 6 months of the treatment [4]. Common causes of primary resistance to imatinib are *KIT* exon 9 mutations, *PDGFRA* exon 18 p.Asp842Val mutation and wild-type GIST [39].

Exon 9 *KIT* mutations are proved to respond poorly to standard dose imatinib (400 mg), therefore dose escalation to 800 mg of imatinib daily has demonstrated risk reduction of mPFS for 61 % [10].

Avapritinib is a new type-I TKI of KIT and PDGFRA [40]. Before avapritinib patients with *PDGFRA* exon 18 p.Asp842Val mutations had poor prognosis, being resistant

to imatinib (Fig. 3) [41]. One study, including 58 advanced GIST patients with *PDGFRA* mutations, treated with imatinib, demonstrated worse prognosis in exon 18 p.Asp842Val mutation *vs* other *PDGFRA* mutations with mPFS 2.8 *vs* 28.5 months, respectively [42]. NAVIGATOR, phase 1 prospective study, included 56 patients with metastatic or unresectable GIST with primary *PDGFRA* exon 18 p.Asp842Val mutation, treated with avapritinib. Overall response rate (ORR) in this group was 91 %, mPFS was 34 months and mOS not reached. CR was seen in 9 % and partial response (PR) in 79 % of the patients [41]. Based on NAVIGATOR trial, avapritinib is now the first line therapy in the case of *PDGFRA* exon 18 p.Asp842Val mutation in advanced GIST [1,35].

KIT and *PDGFRA* wild-type GIST harbour mutations in SDH-complex encoding genes or in genes involved in RAS/RAF/MEK and PI3K/AKT/mTOR pathways as well as *NF1*, *NTRK*, *FGFR* alterations and are typically resistant to imatinib [1,14].

6.4. Secondary Resistance in GIST

Secondary resistance develops after the initial response to the treatment, mainly due to one or more additional mutations in *KIT* or *PDGFRA*, rarely because of mutations in other oncogenic driver genes [4,11]. Secondary *KIT* mutations affect imatinib binding, occurring in exon 13 and 14 (ATP-binding pocket) and exon 17 and 18 (activation-loop) [4]. The diagnostics and treatment of GIST with secondary resistance mutations are complicated due to intralesional (different mutations in one lesion) and interlesional (different mutations in different lesions) heterogeneity. In a 14-patient group (progressing on imatinib and / or sunitinib) with tissue samples from 53 metastases was nicely demonstrated, that two to five secondary mutations can be found in different metastases and two secondary mutations – in the same metastasis from different areas. Secondary resistance mutations were detected in the ATP-binding pocket (exon 13 or 14) with 42.9 - 50 % and activation-loop (exon 17 or 18) with 50 - 57.1 % (Fig. 3). The most frequent secondary resistance mutation was p.Val654Ala in *KIT* exon 13. No secondary resistance mutations were found in *KIT* or *PDGFRA* wild-type GISTs [43]. Another study confirmed the previous results,

showing that the most common secondary resistance mutation of *KIT* exon 13 is p.Val654Ala, and the most frequent mutations in exon 17 involve codons 820, 822 or 823. In a group of 109 imatinib-resistant GIST patients in 20 of them (18 %) mutations in the downstream signalling genes were detected, namely *NF1*, *PTEN*, *KRAS*, *PIK3CA*, *TSC2*, *TSC1*, *AKT3*, *BRAF*. However, apart from *BRAF* mutations all of these cases demonstrated concomitant mutations in *KIT* or *PDGFRA*. Mutations in *AKT3*, *KRAS*, *PIK3CA* (one case), *NF1* were associated with secondary resistance mutations in *KIT* or *PDGFRA*, questioning the role of secondary resistance due to downstream signalling. *PTEN*, *TSC1*, *TSC2* and *PIK3CA* (two cases) showed no secondary resistance mutations in *KIT* or *PDGFRA* [44].

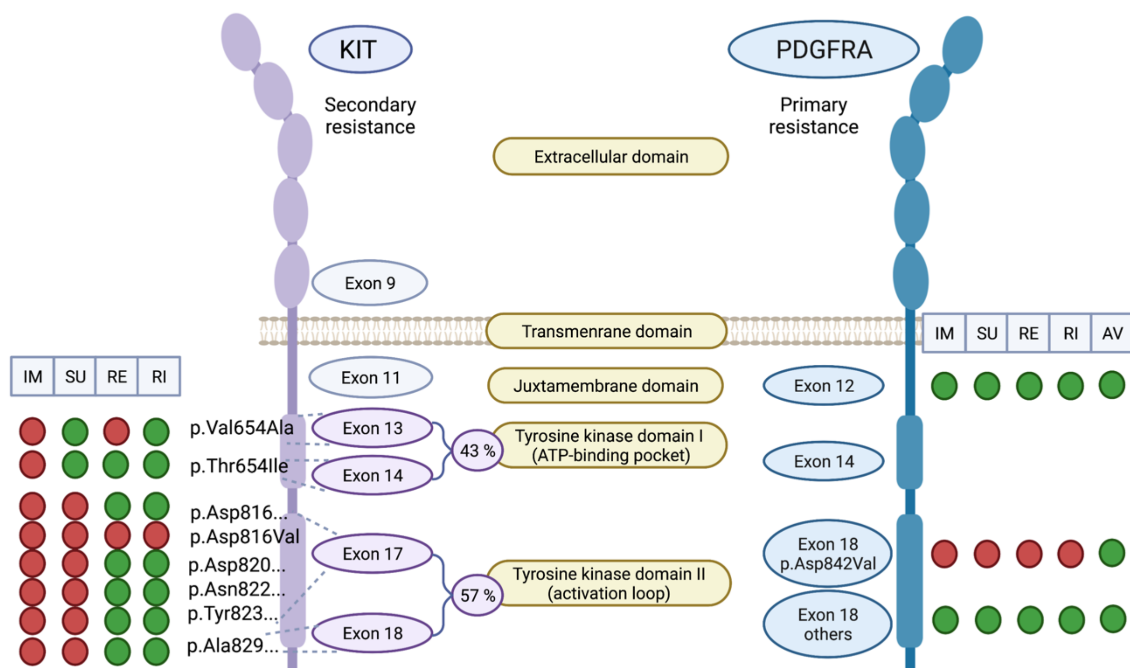

Figure 3. *KIT* secondary resistance mutations (with the most frequent mutations in exons 13, 14, 17, 18) with frequencies and *PDGFRA* primary resistance mutation (exon 18 p.Asp842Val). IM: imatinib, SU: sunitinib, RE: regorafenib, RI: ripretinib, AV: avapritinib. Red: resistant, green: sensitive. Mutations p.Asp816…, p.Asp820…, p.Asn822, p.Tyr823…, p.Ala829… describe various options of substituting amino acid in the corresponding codon. Created with BioRender.com. Adapted from [45].

Recently the secondary resistance to avapritinib in patients with *PDGFRA* exon 18 p.Asp842Val mutation treated in NAVIGATOR trial were analysed [46]. Eleven patients showed progression after initial response to avapritinib with consecutive mOS for only 5.2 months after the progression. Samples from four of eleven patients progressing on avapritinib were further analysed, discovering novel *PDGFRA* secondary resistance mutations, all of them affecting ATP-binding pocket: exon 13 with Val598Phe, exon 14 with p.Gly652Glu, p.Val658Ala, p.Pro653_His654insGlyProGln, p.Asn659Lys and exon 15 with p.Thr674Arg, p.Gly680Arg, p.Tyr676Cys (Fig. 4).

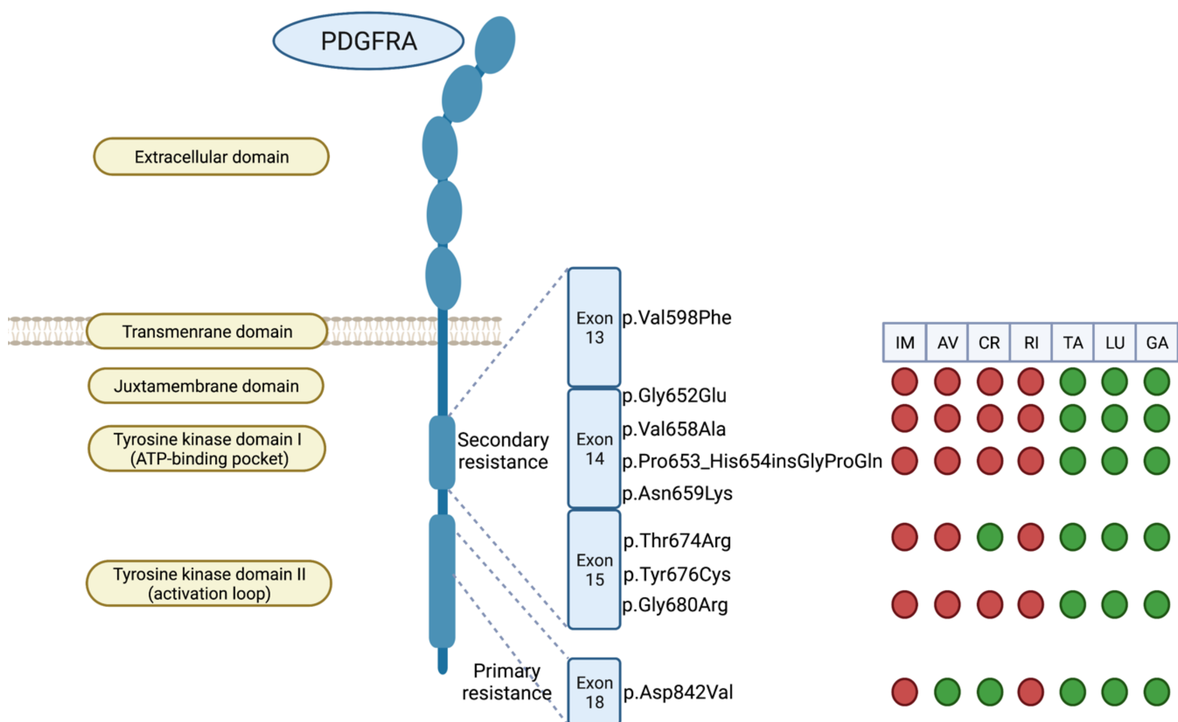

Figure 4. PDGFRA primary (exon 18 p.Asp842Val) and secondary resistance mutations (exon 13, 14, 15). The sensitivity is displayed according to *in vitro* data. Red: resistant, green: sensitive. IM: imatinib, AV: avapritinib, CR: crenolanib, RI: ripretinib, TA: tanespimycin, LU: luminespib, GA: ganetespib. Created with BioRender.com. Adapted from [46].

BRAF p.Val600Glu is also described as secondary resistance mutation to imatinib in one case report, although occurring together with another secondary resistance

mutation in *KIT* exon 13 p.Val654Ala [47]. This case illustrates some discrepancies, knowing from previous studies that *BRAF* mutations are not associated with *KIT* mutations [8,9,25,44].

6.5. Tissue Biopsies in GIST

IHC and molecular pathology testing on tumour tissue is still the standard approach in GIST. According to ESMO-EURACAN-GENTURIS guidelines, when no complete resection can be achieved (i.e., early GIST), multiple core needle biopsies should be taken from bulky tumour. The biopsies could be obtained through endoscopic ultrasonography guidance or through ultrasound / computed tomography guided percutaneous puncture [1]. However, how many biopsies should be optimally taken from primary or progressing primary tumour or metastasis, is not defined. Puncture of every metastasis in the patient with multiple metastases is distressing for the patient and is not realistic outside clinical trials. According to Choi response criteria, one of the hallmarks of progressive disease is new or increasing intratumoral nodule, described as “nodule within the mass”. Choi response criteria replace Response Evaluation Criteria in Solid Tumors (RECIST) and are the standard evaluation criteria in GIST [48]. When imaging-guided biopsy is planned, probably puncture of progressing area, e.g., “nodule within the mass”, should be taken or when possible – total surgical resection of the whole metastasis (author’s opinion).

6.6. Circulating Tumour DNA (ctDNA) in GIST

Liquid biopsy, precisely ctDNA assay, is still not clinically validated as a diagnostic method for routine use in GIST. Nevertheless, recent studies are implementing ctDNA to detect secondary resistance mutations.

Cell-free DNA is the total amount of cell-free DNA in plasma from multiple sources, including tumour; ctDNA is the fraction of cell-free DNA, that comes from tumour [49]. It is released in the circulation through tumour apoptosis, necrosis and active secretion [50]. Because of discordance between tissue and ctDNA genotyping, in case of undetected mutation the result should be confirmed by tissue examination. The major advantage of ctDNA assay is the representation of the whole tumour genomic landscape, while tissue sampling from different, multiple and difficult localized sites is often not possible. The other benefit of ctDNA assays is less invasiveness through venepuncture and therefore easier serial testing [49]. Digital PCR assays, such as droplet digital PCR (ddPCR) or beads, emulsion, amplification, magnetics-digital PCR (BEAMing) have higher sensitivity (detection rate with variant allele fraction (VAF) of 0.01 %). VAF describes the percentage of the circulating tumour DNA in plasma sample, namely the ratio of the ctDNA and the total amount of cell free DNA. For instance, VAF of 0.01 % represents one mutated copy of the tumour out of 10 000 DNA copies. Digital PCR assays are applicable for known preselected specific variants in optimally one or few genes. Targeted deep sequencing NGS-based assays are capable of detecting multiple variants in multiple preselected exons and genes, but have lower sensitivity (detection rate with VAF of 1 – 0.1 %) [49,51-53]. However, the sensitivity of NGS-based assays could be increased by improving the distinction of real variants from errors during amplification and sequencing. It can be done by using capture of ctDNA with specific probes, by PCR amplification of selected regions with short amplicon size < 150 bp for the enrichment in NGS or by tagging each fragment with unique molecular identifiers (UMI) [51,52]. Molecular barcoding with UMIs is incorporated in the library preparation by ligation of UMIs to the ends of each DNA template before the amplification. [54]. Advanced GIST, progressing on imatinib, is known to be genetically heterogenic disease, showing multiple resistance mutations not only in different metastases (interlesional heterogeneity), but also in the same metastasis (intralesional heterogeneity) [43]. Therefore, implementation of ctDNA assays in clinical praxis for this patient group could replace tissue biopsies and could even provide extended information, reflecting the whole mutational burden.

Results and conclusions from studies using different ctDNA assays in GIST are briefly summarized further. Additional information to compare the methodologies

and sensitivities was selected from few other studies with tumours of other entities. Short summary is given in table 1.

Applying allele-specific ligation PCR (AS-PCR) for plasma in GIST, the ctDNA / tissue concordance was 26.8 - 39 % in different studies [51,55,56]. Approximately 40 mutations in one gene using specific primers can be analysed with AS-PCR as demonstrated in lung carcinoma. In addition, VAF was described to range from 0.02 – 0.1 % [57].

With ddPCR 42.9 - 77 % plasma / tissue concordance was provided in GIST [51,58,59]. On the basis of water-in-oil emulsion each DNA molecule is enveloped in one of approximately 20 000 nanolitre droplets. Thus, independent PCR reaction can be performed in every droplet. Although the analytical sensitivity is very good (VAF of 0.005 – 0.01% shown in lung carcinoma), ddPCR is suitable only for hotspot mutations in one to few genes [60].

Another PCR-based method BEAMing demonstrated 64 - 100 % plasma / tissue concordance in GIST [46,5161]. Magnetic beads with primers on their surface are dispersed in water-in-oil emulsion. Each DNA molecule is connected with the magnetic bead and then amplified by PCR reaching tens of thousands of copies of the original DNA fragment. Then the allele-specific hybridization for variant detection can be performed. In patients with colorectal cancer 33 hotspot mutations with specific primers for 4 genes were analysed, showing VAF of 0.01 % [53,62].

Small amplicon NGS panel showed plasma / tissue concordance of 28.6 - 100 % in different studies in GIST [51,58,63-66]. One of the small amplicon-based methods, named tagged-amplicon deep sequencing (TAm-Seq) was used in lung carcinoma. Targeted sequencing of hotspot mutations, exons and also intronic regions from 36 genes was carried out using specific primers for the sequences of interest and performing targeted DNA enrichment by PCR with VAF of 0.05 – 1 % [67].

Capture-based NGS gene panel revealed 56 % plasma / tissue concordance in GIST [51,68,69]. An example of capture-based method is cancer personalized profiling by deep sequencing (CAPP-Seq), that was initially designed for lung carcinoma, but can be used also for other cancers. It targets 521 exons and 13 introns from 139 recurrently mutated genes. This assay demonstrated high specificity at VAF down to 0.02 % [70]. The regions of interest are enriched before

sequencing by hybridization of target sequences to antisense oligonucleotides (capture probes) [71].

Several of these studies including GIST patients could exhibit the benefit of ctDNA for detecting of secondary resistance mutations, showing the whole spectrum of tumour heterogeneity as well as helping in the therapy monitoring and for making therapy decisions, when repeating the ctDNA at every tumour progression. Besides, ctDNA provided additional information, representing new secondary resistance mutations that could not be found in tissue samples [51,63,68].

Some of the core studies, performing ctDNA with different methods in GIST were selected below, to probably find some consensus regarding target group and clinical sensitivity.

In the study with 44 GIST patients ctDNA could be detected only in patients with larger tumour size (13.4 cm *vs* 4.4 cm, p = 0.006), using UMI-based ctDNA assay targeting 28 cancer-related genes. Somatic mutations with ctDNA in *KIT* or *PDGFRA* were found in 16 of 44 samples (36 %). The VAF was 4.6 % (range 0.07 % – 48.1 %). In one case ctDNA-assay was used for disease monitoring, namely detecting secondary resistance mutations in progressing disease, where one of the mutations could not be found in tumour tissue [50].

Another NGS-based method SiMSen-Seq (Simple, Multiplexed, PCR-based barcoding of DNA for Sensitive mutation detection using Sequencing), was used as ctDNA-assay, generating specific panel for each patient, including the tumour-specific mutation in each patient as well as mutations related to imatinib and sunitinib resistance. The study group included 32 GIST patients deemed for surgery. In 9 of 32 patients ctDNA was detected at least in one sample. Median tumour size was 17 *vs* 9 cm (not statistically significant) with detectable *vs* undetectable ctDNA, respectively [72]. SiMSen-seq is a highly sensitive assay that requires low DNA input. Optimally 40 target regions can be analysed. This assay uses primers with specific loop structure, that hides and thus protects molecular barcodes during the first PCR cycles. The limit of ctDNA detection is assumed to be at VAF of < 0,1 % [73].

Capture-based assay demonstrated ctDNA detection rate of 56.3 % (18 from 32 GIST patients). The positive detection rate was significantly higher in tumours > 10 cm of size compared to ≤ 10 cm (73.9 % *vs* 11.1 %, p = 0.004) [68].

Small amplicon-based NGS for 60 genes and ddPCR for *KIT* and *PDGFRA* mutations were performed in 37 plasma samples of 18 GIST patients. The ctDNA detection rate was 28.6 % by NGS and 42.9 % by ddPCR [58].

In a prospective study with 25 GIST patients the detection rate of ctDNA detected by ddPCR for *KIT* and *PDGFRA* was 80 % (20 out of 25 patients). UMI-based NGS was used in 4 patients, providing the identification of mutation in 2 of them. Notably, NGS method showed additional secondary resistance mutations in repeated samples in progressing patients, which could not be found with ddPCR [55].

Different methods, e.g., BEAMing, detecting 64 % (14/22) and capture-based NGS, detecting 53 % (20/38), were used in one study, investigating *PDGFRA* secondary mutations. The detection rate was dependent of the median sum of all target lesions (18.2 cm *vs* 7.5 cm, meaning detected *vs* not detected) [46].

SafeSEQ (Safe-sequencing system) is another UMI-based method, allowing the detection of substitutions, insertions, deletions at VAF of 0.1 % to 0.01 %. The detection rate in GIST was 78 of 96 *KIT* mutations (81 %) [74].

Table 1. Short overview of ctDNA methods in GIST.

Method	Assay examples	VAF (% ctDNA)	Application
Digital PCR	AS-PCR	0.1 – 0.02 %	Targeted regions in one to few genes
	ddPCR	0.005 – 0.01 %	
	BEAMing	0.01 %	
NGS (targeted deep sequencing)	Short-amplicon-based: TAm-Seq	0.05 – 1 %	Targeted regions in multiple genes (gene panels)
	Capture-based: CAPP-Seq	0.02 %	
	UMI-based:		
	SiMSen-Seq	< 0.1 %	
	SafeSEQ	0.01 – 0.1 %	

6.7. Mutational Status Guided Treatment in GIST

Generally, mOS in advanced GIST is 75.9 months and mPFS is 29.7 months [75]. Imatinib, a type-II TKI of KIT, PDGFRA and ABL, is the first line therapy in advanced GIST with imatinib-sensitive primary mutations, various treatment options are established in the clinical praxis, but even more are being investigated in preclinical and clinical studies. Other TKIs (e.g., sunitinib, regorafenib, avapritinib, ripretinib) are applicable in case of imatinib resistance based on randomized clinical studies. Unfortunately, progression over time is well known, while being treated with second and further line TKIs due to emerging of further resistance mutations. To overcome the resistance to imatinib in GIST, various creative approaches are being investigated, including broad spectrum kinase inhibitors, combinations of TKIs in alternating sequence or concomitant combinations of TKIs or with downstream signalling inhibitors. TKIs bind to catalytic site in the ATP-binding pocket. Two main

groups of TKIs are distinguishing between binding to phosphorylated active kinase (type I TKIs) and unphosphorylated inactive kinase (type II TKIs) [76].

An overview of treatment options in GIST is summarized in tables 2 and 3.

Nilotinib, compared with imatinib in the interim analysis of the phase 3 randomised ENESTg1 trial, could not reach better 2-year PFS or 2-year OS in the first line in advanced GIST patients (51.6 % *vs* 59.2 %, HR 1.466, and 81.8 % *vs* 90 %, HR 1.850, respectively). Thereafter the recruitment in the trial was stopped for futility, therefore, p values are not presented [77].

Regorafenib is active in mutations in activation-loop, namely in exon 17 (e.g., p.Asp820, p.Asn822, p.Tyr823, p.Asp816 except for p.Asp816Val), exon 18 (p.Ala829Pro) and exon 14 p.Thr670Ile. Sunitinib is active in mutations in ATP-binding pocket, namely in exon 13 (including p.Val654Ala) and exon 14 p.Thr670Ile (Fig. 3) [78,79].

Sorafenib and ponatinib demonstrate similar inhibitory spectrum as regorafenib. Based on *in vitro* data masitinib, nilotinib and dasatinib have weak inhibitory potential in imatinib secondary resistance mutations, while dovitinib demonstrates similar inhibitory effect as sunitinib [76,79].

Sunitinib is a type-II KIT, PDGFRA VEGFR1-3, FLT3 and RET inhibitor. In randomized controlled phase 3 study 312 advanced GIST patients were randomised for sunitinib or placebo after progression to imatinib, resulting in mPFS with 24.1 vs 6.0 weeks, respectively, p = < 0.0001 [80]. Sunitinib is now established as second line therapy after imatinib in advanced GIST (Fig. 3) [1,35].

GRID trial, a randomised phase 3 study, investigated regorafenib vs placebo in 199 advanced GIST patients after progressing on imatinib an sunitinib. Regorafenib is a type-II multikinase inhibitor, blocking VEGFR1-3, TEK, KIT, RET, BRAF, PDGFR, FGFR. The results provided mPFS of 4.8 vs 0.9 months, p < 0.0001 [81]. The study was the basis for regorafenib as the third line therapy in advanced GIST [1,35].

In the phase 3 study VOYAGER avapritinib was compared with regorafenib in the third line in advanced GIST, no benefit regarding mPFS or mOS of avapritinib could be demonstrated [82].

Cabozantinib was evaluated in phase 2 CaboGIST trial, including advanced GIST patients progressing after imatinib and sunitinib. The mPFS was 5.5 months in this study [83].

Pazopanib, an inhibitor of KIT, VEGFR1-3, PDGFRA and PDGFRB, was investigated in phase 2 randomised trial PAZOGIST in advanced GIST patients, resistant to imatinib and sunitinib. The results provided mPFS of 3.4 vs 2.3 months, $p = 0.03$ (pazopanib vs placebo) [84].

Ripretinib is a new dual switch-control type-II tyrosine kinase inhibitor, having dual inhibitory mechanism by binding to the activation loop (activating switch) and inhibitory switch pocket in the juxtamembrane domain, that leaves the kinase in inactivated state [45,85]. INVICTUS phase 3 study compared ripretinib with placebo in advanced GIST, progressing on imatinib, sunitinib and regorafenib. The mPFS was 6.3 vs 1 month, $p < 0.0001$ and the mOS was 15.1 vs 6.6. months, respectively [85]. Ripretinib is the standard fourth line therapy option in advanced GIST [1,35].

INTRIGUE trial is a phase 3 study, comparing ripretinib with sunitinib in the second line therapy for advanced GIST. Recently the results were published, demonstrating no difference in mPFS (8.3 vs 7 months, $p = 0.36$, respectively). OS was not reached and data was immature [86].

Early results from the study PLX121-01 were recently presented. In this phase 1 / 2 study 18 patients with relapsed or refractory GIST with previous mandatory imatinib treatment (and majority already treated with sunitinib, regorafenib, ripretinib) were included, investigating novel TKI PLX9486 plus sunitinib. PLX9486 is type I TKI, blocking the active conformation of the kinase, with activity against primary *KIT* exon 9 and 11 mutations as well as activation loop mutations (exon 17 and 18). On contrary, sunitinib is known to be active in primary *KIT* mutations and secondary mutations in exon 13 and 14. Thus, the rationale of the study was finding the combination of TKIs, to inhibit broader spectrum of *KIT* mutations. The median PFS was 12 months [87].

A new approach alternating sunitinib for 3 days and regorafenib for 4 days to cover broader spectrum of secondary *KIT* mutations was implemented in small study group with 14 advanced GIST patients after progressing of all TKIs. The mPFS and

mOS was 1.9 months and 10.8 months respectively. The best response was stable disease [88].

In recent study, investigating *PDGFRA* secondary resistance in vitro, was demonstrated, that p.Val598Phe and p.Thr674Arg are sensitive to crenolanib, and further HSP90 (heat shock protein 90) inhibitors tanespimycin, luminespib, ganetespib, the last two being currently in clinical development, are inhibiting all the known secondary resistance mutations (p.Val598Phe, p.Gly652Glu, p.Val658Ala, p.Pro653_His654insGlyProGln, p.Asn659Lys, p.Thr674Arg, p.Gly680Arg, p.Tyr676Cys) (Fig. 4) [46]. HSP90 role is to stabilize labile oncogenic proteins, preventing their degradation [76]. Furthermore, p.Asp842Val mutation, known as resistant to imatinib and ripretinib, showed sensitivity to not only avapritinib but also crenolanib [46]. Phase 3 study (NCT02847429) is investigating crenolanib in *PDGFRA* p.Asp842Val mutated GIST, the results are awaited. Crenolanib is type I TKI against FLT3, PDGFRA and PDGFRB receptor tyrosine kinases [89].

Cell lines harbouring mutations in *KIT*, *PTEN*, *KRAS*, *NF1* and *TSC2* were generated and the inhibitory activity of sapanisertib, a novel ATP-competitive mTOR complex 1 and 2 inhibitor, was examined, demonstrating antiproliferative effects in all cell lines, thus leaving MEK cascade overstimulated. Combinations of sapanisertib with imatinib (in *TSC2* mutated cell lines) and especially with MEK inhibitor trametinib showed strong inhibition of AKT-phosphorylation. Notably, *PTEN* mutated cell lines displayed weak sensitivity to imatinib [44]. Phase Ib / II study, investigating MEK162 inhibitor plus imatinib in advanced GIST, is ongoing (NCT01991379).

Multiple studies, evaluating various other inhibitors of PDGFR, NTRK, FGFR, KIT, VEGF, RAF, MEK as well standard line therapies in combination with immunotherapy agents (check point inhibitors) are ongoing [90].

Table 2. Summary of EMA approved TKIs in advanced GIST.

TKI	Trial	Line	Activity / Resistance
Imatinib	BFR14 phase 3 (long-term follow-up)	1st	R: *KIT* secondary resistance mutations (exon 13, 14, 17, 18) R: *PDGFRA* exon 18 p.Asp842Val
Avapritinib	NAVIGATOR phase 1	1st	A: *PDGFRA* exon 18 p.Asp842Val
Sunitinib	NCT00075218 phase 3	2nd	A: *KIT* exon 13, 14
Regorafenib	GRID phase 3	3rd	A: *KIT* exon 14, 17, 18
Ripretinib	INVICTUS phase 3	4th	R: *KIT* exon 17 p.Asp816Val R: *PDGFRA* exon 18 p.Asp842Val

Table 3. Summary of therapies in individual cases in advanced GIST currently being investigated.

Drugs	Activity
BRAFi	*BRAF* p.Val600Glu
HSP90i	*PDGFRA* secondary resistance mutation (exon 14, 15)
Crenolanib	*PDGFRA* exon 18 p.Asp842Val (primary resistance) *PDGFRA* exon 15 p.Thr674Arg (secondary resistance)
PLX9486 plus sunitinib	*KIT* exon 13, 14, 17, 18 secondary resistance mutations
mTORi plus MEKi	*PTEN*, *KRAS*, *NF1*, *TSC2* mutations
Sunitinib	SDH-deficiency
Regorafenib	SDH-deficiency
Temozolomide	SDH-deficiency
FGFRi	SDH-deficiency
NTRKi	*NTRK* fusions

6.8. Treatment Options for KIT and PDGFRA Wild-Type GIST

Sunitinib has reached disease stabilization in small group of paediatric patients with SDH-deficient advanced GIST, showing mPFS of 15 months [91]. Regorafenib could achieve PR in 2 of 6 SDH-deficient patients with advanced GIST [92]. Temozolomide demonstrated 40 % objective response rate and 100 % disease control rate in 5 patients with SDH-deficient GIST [93]. Temozolomide is now being investigated in phase 2 trial in advanced SDH-deficient GIST patients (NCT03556384). Patients with SDH-deficient GIST are known to highly express FGFR1 and FGFR2 [94]. Rogaratinib, a novel pan-FGFR inhibitor, is currently under investigation in phase II trial with sarcoma patients with *FGFR1-4* alterations or SDH-deficient GIST (NCT04595747) [94].

NTRK inhibitors (larotrectinib, entrectinib) have demonstrated partial response in patients with GIST with *NTRK* translocations [32,95]. In a phase 2 “basket” trial, including patients with *NTRK* rearrangements and treated tumour-agnostic with larotrectinib, 3 patients with GIST participated, all of them showing PR [95]. Novel TRK inhibitors selitrectinib and repotrectinib have demonstrated activity after progression on larotrectinib or entrectinib because of resistance mutations in the NTRK kinase domain [32].

One case report demonstrated response to BRAF-inhibitor dabrafenib, followed by progression because of further *PIK3CA* gain-of-function mutation p.His1047Arg [96].

7. Discussion

Summing up the characteristics of molecular pathology in GIST, beyond routinely assessing of only *KIT* or *PDGFRA* mutations, other molecular targets should be incorporated in the standard molecular subclassification algorithm, thus providing all the information for precision therapy decisions.

For the newly diagnosed GIST, after immunohistochemical confirmation of GIST through CD117 or DOG1 IHC, the first step should include *KIT* exons 8,9,11,13,14,17,18 and *PDGFRA* exons 12, 13, 14, 15, 18 by NGS, since approximately 85 % of all GISTs demonstrate mutations in these genes.

In the case of *KIT* and *PDGFRA* wild-type situation, the second diagnostic step should incorporate the IHC of SDHB, knowing that this reflects SDH-deficiency irrespective of affected subunit of SDH-complex. SDH-deficient GIST is the largest *KIT* and *PDGFRA* wild-type group. IHC of SDHA could be used as extra step for the specific diagnosis of SDHA-deficiency to further look for mutations in this particular gene. To subordinate the causal gene (*SDHA*, *SDHB*, *SDHC*, *SDHD*), NGS gene panel with additional bisulfite amplicon NGS of *SDHC* promoter should be performed as the third diagnostic step. In the case of *KIT* and *PDGFRA* wild-type, but SDH-proficient GIST, the following rare driver genes should be covered with NGS panel: *BRAF*, *NF1*, *HRAS*, *NRAS*, *KRAS*, *PIK3CA*, *PTEN*, *TSC1*, *TSC2*, *AKT3*, *ARID1A*.

When no causal mutations are found, the fourth diagnostic step should include RNAseq, e.g., Archer FusionPlex Sarcoma v2 panel, particularly for *NTRK* and *FGFR* fusions. The pan-TRK IHC to screen for *NTRK* fusions can be excluded in author’s opinion, since in both scenarios - positive or negative staining – the RNAseq should be performed, in the first one – to confirm the IHC, and in the second one – to further search for *FGFR* fusions.

The summary of the offered four-step diagnostic algorithm is given in the figure 5.

As described in the section “Results”, extreme rarely no driver mutations in GIST can be found, representing either truly wild-type GIST or insufficient covering of genes. Therefore, probably larger NGS gene panels or even NGS whole exome or

whole genome sequencing could be justified in such cases. However, when finding mutation in a novel gene, not previous described in GIST or tumours, the causality is questionable and must be proved. In addition, the diagnostic expenditure should be balanced with therapeutic consequence. Besides, as known in the cases of *NTRK* and *FGFR* fusions or *SDHC* promoter hypermethylation, apart from DNA-level the alterations should be searched also in the level of transcriptomics and epigenetics.

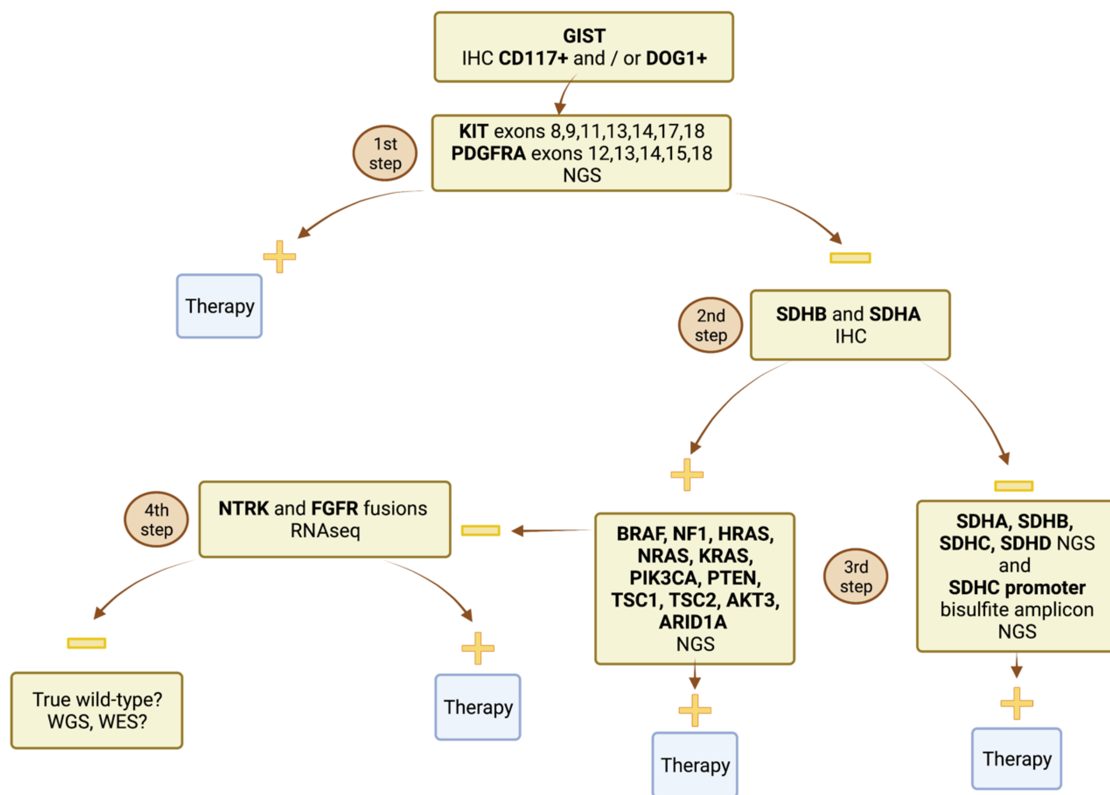

Figure 5. Diagnostic algorithm of newly diagnosed GIST in author's interpretation, based on the included literature. For details see text. Created with BioRender.com.

Advanced GIST, progressing on the first-line and further-line TKIs represents a complex group in terms of molecular diagnosis, due to its intralesional and interlesional heterogeneity as well as dynamic changes in mutational burden, while treating with different TKIs. As described in the section "Results", different metastases may harbour different mutations and, additionally, even one metastasis can harbour different mutations. Multiple tissue biopsies could be accessible from

one to few lesions, but are not feasible from every single one lesion in the clinical practice, as it could be distressful for the patient. Obtaining of tissue biopsies should be very good planned, some examples are as follows (in author's opinion): image guided biopsies of the progressing areas of the lesion (e.g., the "nodule within the mass"), image guided biopsies of the new metastasis, complete excision of the new metastasis for diagnostic purposes. Nevertheless, tissue biopsies cannot be estimated as 100 % reliable due to genetic heterogeneity in advanced progressing GIST. Hence, ctDNA assay is probably the future diagnostic method for this situation. However, to date no validation for clinical praxis is available. Still multiple studies, comparing the concordance between various ctDNA assays and tissue, have found some consensus. Firstly, the sensitivity of ctDNA assays should be improved for the implementation in the routine diagnostics. Secondly, the sensitivity of ctDNA can be increased, when used in larger tumours, i.e., > 10 cm. Thirdly, PCR based assays are more sensitive, but cover only *KIT* and *PDGFRA*; on contrary, NGS based assays are less sensitive, but incorporate other genes responsible for secondary resistance. Fourthly, ctDNA represents all the intralesional and interlesional genomic heterogeneity in GIST, providing additional information to tissue examination. Fifthly, only simple venepuncture for ctDNA is required. Sixthly, because of the simple accessibility of the blood samples, ctDNA is easy repeatable at every tumour progression, thus providing therapy monitoring and therapy guidance.

In author's opinion, due to the additional information of the ctDNA, it could be used in clinical praxis together with tissue biopsies in progressive GIST. Based on the available data from the section "Results", the secondary resistance mutations in progressing disease should be assessed in one-step NGS gene panel, covering *KIT* (especially exons 13, 14, 17, 18), *PDGFRA* (especially exons 13,14,15), *BRAF*, *PIK3CA*, *NF1*, *KRAS*, *NRAS*, *HRAS*, *PTEN*, *TSC1*, *TSC2*, *AKT3*, *ARID1A*, since they have been found accompanied by *KIT* or *PDGFRA* primary mutations. This should be repeated at every progress, as every new progress potentially predicts new resistance mutation, that should be detected, to select the right TKI. Figure 6 summarizes the diagnostic principle in progressing advanced GIST.

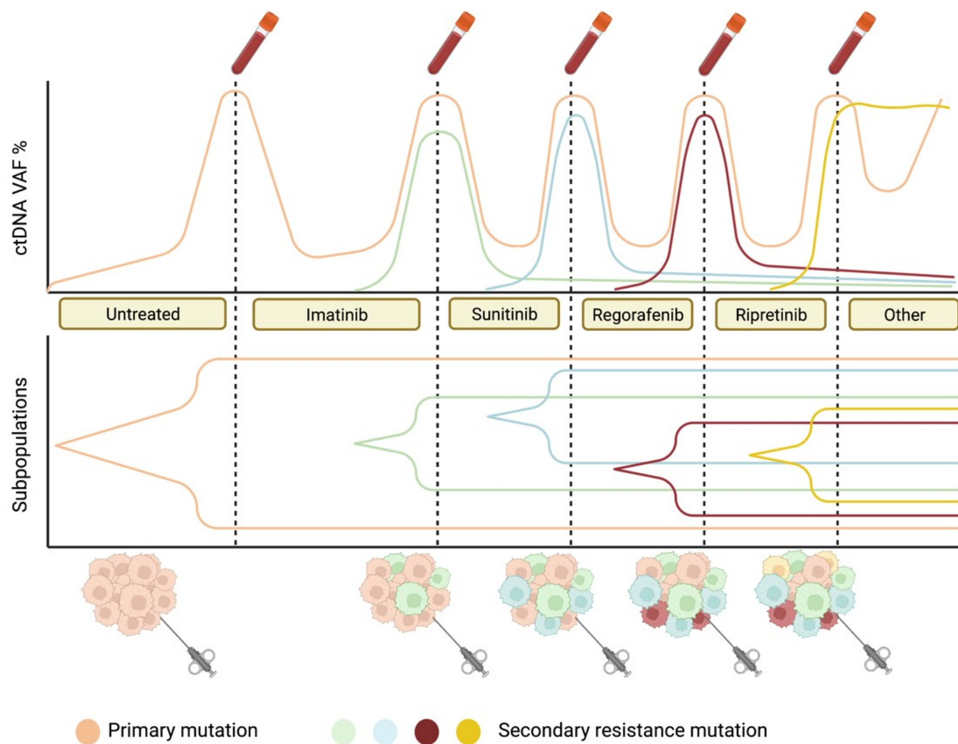

Figure 6. Principle of recording mutational spectrum in advanced GIST, progressing on TKIs over time. Tissue biopsies concomitantly with ctDNA are obtained ideally at every tumour progression to guide treatment decisions. Created with BioRender.com. Adapted from [51].

Clinical signs and young age could indicate for syndromic GIST. The first diagnostic step is to differentiate between SDH-proficient and SDH-deficient GIST by SDHB IHC as described above. In case of SDH-proficiency the NGS gene panel covering *KIT*, *PDGFRA* and *NF1* should be performed. In case of SDH-deficiency further steps as illustrated above should be carried out. In addition, genetic counselling should be offered for the patient and the family in case of the germline mutation. Figure 7 outlines diagnostic steps in syndromic GIST.

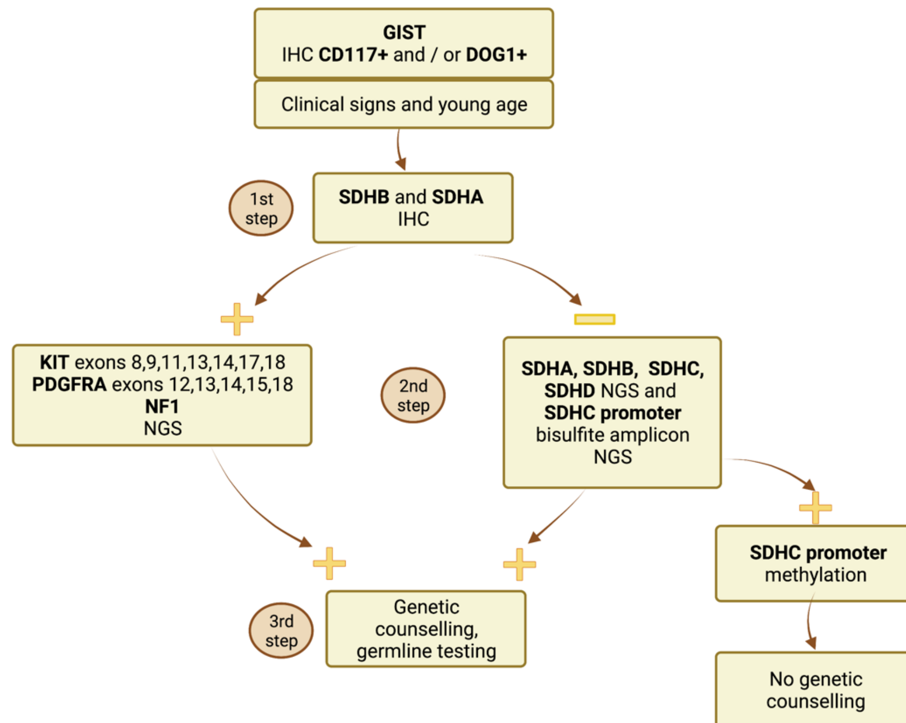

Figure 7. Diagnostic algorithm of the syndromic GIST in author's interpretation, based on the included literature. For details see text. Created with BioRender.com.

In conclusion, GIST characterises with extremely complex and dynamic genomic heterogeneity. The molecular subclassification of GIST is therefore intricate, but very important task, as these molecular targets are crucial for the selection of the right TKI. Improvement of the ctDNA technologies could offer new future diagnostic method for clinicians, particularly in progressive advanced GIST. Additionally, better understanding of the molecular mechanisms in GIST provides the implementation of novel TKIs and therapy approaches.

8. References

1. Casali PG, Blay JY, Abecassis N, Bajpai J, Bauer S, Biagini R, Bielack S, Bonvalot S, Boukovinas I, Bovee JVMG, Boye K, Brodowicz T, Buonadonna A, De Álava E, Dei Tos AP, Del Muro XG, Dufresne A, Eriksson M, Fedenko A, Ferraresi V, Ferrari A, Frezza AM, Gasperoni S, Gelderblom H, Gouin F, Grignani G, Haas R, Hassan AB, Hindi N, Hohenberger P, Joensuu H, Jones RL, Jungels C, Jutte P, Kasper B, Kawai A, Kopeckova K, Krákorová DA, Le Cesne A, Le Grange F, Legius E, Leithner A, Lopez-Pousa A, Martin-Broto J, Merimsky O, Messiou C, Miah AB, Mir O, Montemurro M, Morosi C, Palmerini E, Pantaleo MA, Piana R, Piperno-Neumann S, Reichardt P, Rutkowski P, Safwat AA, Sangalli C, Sbaraglia M, Scheipl S, Schöffski P, Sleijfer S, Strauss D, Strauss SJ, Hall KS, Trama A, Unk M, van de Sande MAJ, van der Graaf WTA, van Houdt WJ, Frebourg T, Gronchi A, Stacchiotti S; ESMO Guidelines Committee, EURACAN and GENTURIS. Gastrointestinal stromal tumours: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2022 Jan;33(1):20-33. doi: 10.1016/j.annonc.2021.09.005. Epub 2021 Sep 21.
2. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol. 2006 May;23(2):70-83. doi: 10.1053/j.semdp.2006.09.001.
3. Lugowska I, Jean-Yves B, Gelderblom H. Sarcoma and Gastrointestinal Stromal Tumours plus Cancer of Unknown Primary Site Essentials for Clinicians. ESMO Press; 2020. 91 p.
4. Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011 Nov 17;11(12):865-78. doi: 10.1038/nrc3143.
5. National Library of Medicine [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.ncbi.nlm.nih.gov/gene/3815
6. Ruan HB, Zhang N, Gao X. Identification of a novel point mutation of mouse proto-oncogene c-kit through N-ethyl-N-nitrosourea mutagenesis. Genetics. 2005 Feb;169(2):819-31. doi: 10.1534/genetics.104.027177.

7. Nemunaitis J, Bauer S, Blay JY, Choucair K, Gelderblom H, George S, Schöffski P, Mehren MV, Zalcberg J, Achour H, Ruiz-Soto R, Heinrich MC. Intrigue: Phase III study of ripretinib versus sunitinib in advanced gastrointestinal stromal tumor after imatinib. Future Oncol. 2020 Jan;16(1):4251-4264. doi: 10.2217/fon-2019-0633. Epub 2019 Nov 22.
8. Huss S, Künstlinger H, Wardelmann E, Kleine MA, Binot E, Merkelbach-Bruse S, Rüdiger T, Mittler J, Hartmann W, Büttner R, Schildhaus HU. A subset of gastrointestinal stromal tumors previously regarded as wild-type tumors carries somatic activating mutations in KIT exon 8 (p.D419del). Mod Pathol. 2013 Jul;26(7):1004-12. doi: 10.1038/modpathol.2013.47. Epub 2013 Apr 19.
9. Daniels M, Lurkin I, Pauli R, Erbstösser E, Hildebrandt U, Hellwig K, Zschille U, Lüders P, Krüger G, Knolle J, Stengel B, Prall F, Hertel K, Lobeck H, Popp B, Theissig F, Wünsch P, Zwarthoff E, Agaimy A, Schneider-Stock R. Spectrum of KIT/PDGFRA/BRAF mutations and Phosphatidylinositol-3-Kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). Cancer Lett. 2011 Dec 15;312(1):43-54. doi: 10.1016/j.canlet.2011.07.029. Epub 2011 Aug 6.
10. Debiec-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, Blay JY, Leyvraz S, Stul M, Casali PG, Zalcberg J, Verweij J, Van Glabbeke M, Hagemeijer A, Judson I; EORTC Soft Tissue and Bone Sarcoma Group; Italian Sarcoma Group; Australasian GastroIntestinal Trials Group. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. Eur J Cancer. 2006 May;42(8):1093-103. doi: 10.1016/j.ejca.2006.01.030. Epub 2006 Apr 18.
11. Brčić I, Argyropoulos A, Liegl-Atzwanger B. Update on Molecular Genetics of Gastrointestinal Stromal Tumors. Diagnostics (Basel). 2021 Jan 28;11(2):194. doi: 10.3390/diagnostics11020194.
12. Patrikidou A, Domont J, Chabaud S, Ray-Coquard I, Coindre JM, Bui-Nguyen B, Adenis A, Rios M, Bertucci F, Duffaud F, Chevreau C, Cupissol D, Pérol D, Emile JF, Blay JY, Le Cesne A; French Sarcoma Group. Long-term outcome of molecular subgroups of GIST patients treated with standard-dose imatinib in the BFR14 trial of the French Sarcoma Group. Eur J Cancer. 2016 Jan;52:173-80. doi: 10.1016/j.ejca.2015.10.069. Epub 2015 Dec 11.

13. OMIM [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.omim.org/entry/173490
14. Schaefer IM, Mariño-Enríquez A, Fletcher JA. What is New in Gastrointestinal Stromal Tumor? Adv Anat Pathol. 2017 Sep;24(5):259-267. doi: 10.1097/PAP.0000000000000158.
15. National Library of Medicine [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.ncbi.nlm.nih.gov/gene/6389
16. National Library of Medicine [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.ncbi.nlm.nih.gov/gene/6390
17. National Library of Medicine [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.ncbi.nlm.nih.gov/gene/6391
18. National Library of Medicine [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.ncbi.nlm.nih.gov/gene/6392
19. Killian JK, Kim SY, Miettinen M, Smith C, Merino M, Tsokos M, Quezado M, Smith WI Jr, Jahromi MS, Xekouki P, Szarek E, Walker RL, Lasota J, Raffeld M, Klotzle B, Wang Z, Jones L, Zhu Y, Wang Y, Waterfall JJ, O'Sullivan MJ, Bibikova M, Pacak K, Stratakis C, Janeway KA, Schiffman JD, Fan JB, Helman L, Meltzer PS. Succinate dehydrogenase mutation underlies global epigenomic divergence in gastrointestinal stromal tumor. Cancer Discov. 2013 Jun;3(6):648-57. doi: 10.1158/2159-8290.CD-13-0092. Epub 2013 Apr 2.
20. Casey RT, Ten Hoopen R, Ochoa E, Challis BG, Whitworth J, Smith PS, Martin JE, Clark GR, Rodger F, Maranian M, Allinson K, Madhu B, Roberts T, Campos L, Anstee J, Park SM, Marker A, Watts C, Bulusu VR, Giger OT, Maher ER. SDHC epi-mutation testing in gastrointestinal stromal tumours and related tumours in clinical practice. Sci Rep. 2019 Jul 15;9(1):10244. doi: 10.1038/s41598-019-46124-9.
21. Illumina [Internet]. c2022 – [cited 2022 Jul 25]. Available from: https://www.illumina.com/science/sequencing-method-explorer/kits-and-arrays/bsas.html
22. Ochoa E, Zuber V, Fernandez-Jimenez N, Bilbao JR, Clark GR, Maher ER, Bottolo L. MethylCal: Bayesian calibration of methylation levels. Nucleic Acids Res. 2019 Aug 22;47(14):e81. doi: 10.1093/nar/gkz325.

23. Masser DR, Berg AS, Freeman WM. Focused, high accuracy 5-methylcytosine quantitation with base resolution by benchtop next-generation sequencing. Epigenetics Chromatin. 2013 Oct 11;6(1):33. doi: 10.1186/1756-8935-6-33.
24. Gopie P, Mei L, Faber AC, Grossman SR, Smith SC, Boikos SA. Classification of gastrointestinal stromal tumor syndromes. Endocr Relat Cancer. 2018 Feb;25(2):R49-R58. doi: 10.1530/ERC-17-0329. Epub 2017 Nov 23.
25. Hostein I, Faur N, Primois C, Boury F, Denard J, Emile JF, Bringuier PP, Scoazec JY, Coindre JM. BRAF mutation status in gastrointestinal stromal tumors. Am J Clin Pathol. 2010 Jan;133(1):141-8. doi: 10.1309/AJCPPCKGA2QGBJ1R.
26. Lasota J, Felisiak-Golabek A, Wasag B, Kowalik A, Zięba S, Chłopek M, Wang ZF, Coates T, Kopczynski J, Gozdz S, Sarlomo-Rikala M, Miettinen M. Frequency and clinicopathologic profile of PIK3CA mutant GISTs: molecular genetic study of 529 cases. Mod Pathol. 2016 Mar;29(3):275-82. doi: 10.1038/modpathol.2015.160. Epub 2016 Jan 22.
27. Hechtman JF, Zehir A, Mitchell T, Borsu L, Singer S, Tap W, Oultache A, Ladanyi M, Nafa K. Novel oncogene and tumor suppressor mutations in KIT and PDGFRA wild type gastrointestinal stromal tumors revealed by next generation sequencing. Genes Chromosomes Cancer. 2015 Mar;54(3):177-84. doi: 10.1002/gcc.22230. Epub 2014 Nov 27.
28. Miettinen M, Fetsch JF, Sobin LH, Lasota J. Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases. Am J Surg Pathol. 2006 Jan;30(1):90-6. doi: 10.1097/01.pas.0000176433.81079.bd.
29. Yantiss RK, Rosenberg AE, Sarran L, Besmer P, Antonescu CR. Multiple gastrointestinal stromal tumors in type I neurofibromatosis: a pathologic and molecular study. Mod Pathol. 2005 Apr;18(4):475-84. doi: 10.1038/modpathol.3800334.
30. Takazawa Y, Sakurai S, Sakuma Y, Ikeda T, Yamaguchi J, Hashizume Y, Yokoyama S, Motegi A, Fukayama M. Gastrointestinal stromal tumors of neurofibromatosis type I (von Recklinghausen's disease). Am J Surg Pathol. 2005 Jun;29(6):755-63. doi: 10.1097/01.pas.0000163359.32734.f9.

31. Gasparotto D, Rossi S, Polano M, Tamborini E, Lorenzetto E, Sbaraglia M, Mondello A, Massani M, Lamon S, Bracci R, Mandolesi A, Frate E, Stanzial F, Agaj J, Mazzoleni G, Pilotti S, Gronchi A, Dei Tos AP, Maestro R. Quadruple-Negative GIST Is a Sentinel for Unrecognized Neurofibromatosis Type 1 Syndrome. Clin Cancer Res. 2017 Jan 1;23(1):273-282. doi: 10.1158/1078-0432.CCR-16-0152. Epub 2016 Jul 7.
32. Demetri GD, Antonescu CR, Bjerkehagen B, Bovée JVMG, Boye K, Chacón M, Dei Tos AP, Desai J, Fletcher JA, Gelderblom H, George S, Gronchi A, Haas RL, Hindi N, Hohenberger P, Joensuu H, Jones RL, Judson I, Kang YK, Kawai A, Lazar AJ, Le Cesne A, Maestro R, Maki RG, Martín J, Patel S, Penault-Llorca F, Premanand Raut C, Rutkowski P, Safwat A, Sbaraglia M, Schaefer IM, Shen L, Serrano C, Schöffski P, Stacchiotti S, Sundby Hall K, Tap WD, Thomas DM, Trent J, Valverde C, van der Graaf WTA, von Mehren M, Wagner A, Wardelmann E, Naito Y, Zalcberg J, Blay JY. Diagnosis and management of tropomyosin receptor kinase (TRK) fusion sarcomas: expert recommendations from the World Sarcoma Network. Ann Oncol. 2020 Nov;31(11):1506-1517. doi: 10.1016/j.annonc.2020.08.2232. Epub 2020 Sep 3.
33. ArcherDX [Internet]. c2022 – [cited 2022 Jul 26]. Available from: https://archerdx.com/research-products/solid-tumor-research/fusionplex-sarcoma/
34. Shi E, Chmielecki J, Tang CM, Wang K, Heinrich MC, Kang G, Corless CL, Hong D, Fero KE, Murphy JD, Fanta PT, Ali SM, De Siena M, Burgoyne AM, Movva S, Madlensky L, Heestand GM, Trent JC, Kurzrock R, Morosini D, Ross JS, Harismendy O, Sicklick JK. FGFR1 and NTRK3 actionable alterations in "Wild-Type" gastrointestinal stromal tumors. J Transl Med. 2016 Dec 14;14(1):339. doi: 10.1186/s12967-016-1075-6.
35. NCCN Clinical Practice Guidelines in Oncology: Gastrointestinal Stromal Tumors (GISTs). Version 1.2022.
36. OMIM [Internet]. c2022 – [cited 2022 Jun 5]. Available from: https://www.omim.org/entry/162200
37. Ricci R. Syndromic gastrointestinal stromal tumors. Hered Cancer Clin Pract. 2016 Jul 19;14:15. doi: 10.1186/s13053-016-0055-4.

38. Haller F, Moskalev EA, Faucz FR, Barthelmeß S, Wiemann S, Bieg M, Assie G, Bertherat J, Schaefer IM, Otto C, Rattenberry E, Maher ER, Ströbel P, Werner M, Carney JA, Hartmann A, Stratakis CA, Agaimy A. Aberrant DNA hypermethylation of SDHC: a novel mechanism of tumor development in Carney triad. Endocr Relat Cancer. 2014 Aug;21(4):567-77. doi: 10.1530/ERC-14-0254. Epub 2014 May 23.
39. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology. 2003 Sep;125(3):660-7. doi: 10.1016/s0016-5085(03)01046-1.
40. Bauer S, George S, von Mehren M, Heinrich MC. Early and Next-Generation KIT/PDGFRA Kinase Inhibitors and the Future of Treatment for Advanced Gastrointestinal Stromal Tumor. Front Oncol. 2021 Jul 12;11:672500. doi: 10.3389/fonc.2021.672500.
41. Jones RL, Serrano C, von Mehren M, George S, Heinrich MC, Kang YK, Schöffski P, Cassier PA, Mir O, Chawla SP, Eskens FALM, Rutkowski P, Tap WD, Zhou T, Roche M, Bauer S. Avapritinib in unresectable or metastatic PDGFRA D842V-mutant gastrointestinal stromal tumours: Long-term efficacy and safety data from the NAVIGATOR phase I trial. Eur J Cancer. 2021 Mar;145:132-142. doi: 10.1016/j.ejca.2020.12.008. Epub 2021 Jan 16.
42. Cassier PA, Fumagalli E, Rutkowski P, Schöffski P, Van Glabbeke M, Debiec-Rychter M, Emile JF, Duffaud F, Martin-Broto J, Landi B, Adenis A, Bertucci F, Bompas E, Bouché O, Leyvraz S, Judson I, Verweij J, Casali P, Blay JY, Hohenberger P; European Organisation for Research and Treatment of Cancer. Outcome of patients with platelet-derived growth factor receptor alpha-mutated gastrointestinal stromal tumors in the tyrosine kinase inhibitor era. Clin Cancer Res. 2012 Aug 15;18(16):4458-64. doi: 10.1158/1078-0432.CCR-11-3025. Epub 2012 Jun 20.
43. Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC, Fletcher CD, Corless CL, Fletcher JA. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol. 2008 Sep;216(1):64-74. doi: 10.1002/path.2382.
44. Mühlenberg T, Ketzer J, Heinrich MC, Grunewald S, Marino-Enriquez A, Trautmann M, Hartmann W, Wardelmann E, Treckmann J, Worm K, Bertram

S, Herold T, Schildhaus HU, Glimm H, Stenzinger A, Brors B, Horak P, Hohenberger P, Fröhling S, Fletcher JA, Bauer S. KIT-Dependent and KIT-Independent Genomic Heterogeneity of Resistance in Gastrointestinal Stromal Tumors - TORC1/2 Inhibition as Salvage Strategy. Mol Cancer Ther. 2019 Nov;18(11):1985-1996. doi: 10.1158/1535-7163.MCT-18-1224.

45. Lostes-Bardaji MJ, García-Illescas D, Valverde C, Serrano C. Ripretinib in gastrointestinal stromal tumor: the long-awaited step forward. Ther Adv Med Oncol. 2021 Jan 7;13:1758835920986498. doi: 10.1177/1758835920986498.
46. Grunewald S, Klug LR, Mühlenberg T, Lategahn J, Falkenhorst J, Town A, Ehrt C, Wardelmann E, Hartmann W, Schildhaus HU, Treckmann J, Fletcher JA, Jung S, Czodrowski P, Miller S, Schmidt-Kittler O, Rauh D, Heinrich MC, Bauer S. Resistance to Avapritinib in PDGFRA-Driven GIST Is Caused by Secondary Mutations in the PDGFRA Kinase Domain. Cancer Discov. 2021 Jan;11(1):108-125. doi: 10.1158/2159-8290.CD-20-0487. Epub 2020 Sep 24.
47. Zheng S, Huang KE, Pan YL, Zhou Y, Pan SD, Li X, Jia J, Zheng XL, Tao DY. KIT and BRAF heterogeneous mutations in gastrointestinal stromal tumors after secondary imatinib resistance. Gastric Cancer. 2015 Oct;18(4):796-802. doi: 10.1007/s10120-014-0414-7. Epub 2014 Aug 15.
48. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, Chen LL, Podoloff DA, Benjamin RS. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol. 2007 May 1;25(13):1753-9. doi: 10.1200/JCO.2006.07.3049.
49. Merker JD, Oxnard GR, Compton C, Diehn M, Hurley P, Lazar AJ, Lindeman N, Lockwood CM, Rai AJ, Schilsky RL, Tsimberidou AM, Vasalos P, Billman BL, Oliver TK, Bruinooge SS, Hayes DF, Turner NC. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018 Jun 1;36(16):1631-1641. doi: 10.1200/JCO.2017.76.8671. Epub 2018 Mar 5.
50. Namløs HM, Boye K, Mishkin SJ, Barøy T, Lorenz S, Bjerkehagen B, Stratford EW, Munthe E, Kudlow BA, Myklebost O, Meza-Zepeda LA.

Noninvasive Detection of ctDNA Reveals Intratumor Heterogeneity and Is Associated with Tumor Burden in Gastrointestinal Stromal Tumor. Mol Cancer Ther. 2018 Nov;17(11):2473-2480. doi: 10.1158/1535-7163.MCT-18-0174.

51. Gómez-Peregrina D, García-Valverde A, Pilco-Janeta D, Serrano C. Liquid Biopsy in Gastrointestinal Stromal Tumors: Ready for Prime Time? Curr Treat Options Oncol. 2021 Feb 27;22(4):32. doi: 10.1007/s11864-021-00832-5.
52. Moati E, Taly V, Garinet S, Didelot A, Taieb J, Laurent-Puig P, Zaanan A. Role of Circulating Tumor DNA in Gastrointestinal Cancers: Current Knowledge and Perspectives. Cancers (Basel). 2021 Sep 22;13(19):4743. doi: 10.3390/cancers13194743.
53. García-Foncillas J, Alba E, Aranda E, Díaz-Rubio E, López-López R, Tabernero J, Vivancos A. Incorporating BEAMing technology as a liquid biopsy into clinical practice for the management of colorectal cancer patients: an expert taskforce review. Ann Oncol. 2017 Dec 1;28(12):2943-2949. doi: 10.1093/annonc/mdx501.
54. De Luca G, Dono M. The Opportunities and Challenges of Molecular Tagging Next-Generation Sequencing in Liquid Biopsy. Mol Diagn Ther. 2021 Sep;25(5):537-547. doi: 10.1007/s40291-021-00542-6. Epub 2021 Jul 5.
55. Jilg S, Rassner M, Maier J, Waldeck S, Kehl V, Follo M, Philipp U, Sauter A, Specht K, Mitschke J, Lange T, Bauer S, Jost PJ, Peschel C, Duyster J, Gaiser T, Hohenberger P, von Bubnoff N. Circulating cKIT and PDGFRA DNA indicates disease activity in Gastrointestinal Stromal Tumor (GIST). Int J Cancer. 2019 Oct 15;145(8):2292-2303. doi: 10.1002/ijc.32282. Epub 2019 Apr 29.
56. Maier J, Lange T, Kerle I, Specht K, Bruegel M, Wickenhauser C, Jost P, Niederwieser D, Peschel C, Duyster J, von Bubnoff N. Detection of mutant free circulating tumor DNA in the plasma of patients with gastrointestinal stromal tumor harboring activating mutations of cKIT or PDGFRA. Clin Cancer Res. 2013 Sep 1;19(17):4854-67. doi: 10.1158/1078-0432.CCR-13-0765. Epub 2013 Jul 5.
57. Szpechcinski A, Bryl M, Wojcik P, Czyzewicz G, Wojda E, Rudzinski P, Duk K, Moes-Sosnowska J, Maszkowska-Kopij K, Langfort R, Barinow-

Wojewodzki A, Chorostowska-Wynimko J. Detection of EGFR mutations in liquid biopsy samples using allele-specific quantitative PCR: A comparative real-world evaluation of two popular diagnostic systems. Adv Med Sci. 2021 Sep;66(2):336-342. doi: 10.1016/j.advms.2021.06.003. Epub 2021 Jul 15.

58. Serrano C, Vivancos A, López-Pousa A, Matito J, Mancuso FM, Valverde C, Quiroga S, Landolfi S, Castro S, Dopazo C, Sebio A, Virgili AC, Menso MM, Martín-Broto J, Sansó M, García-Valverde A, Rosell J, Fletcher JA, George S, Carles J, Arribas J. Clinical value of next generation sequencing of plasma cell-free DNA in gastrointestinal stromal tumors. BMC Cancer. 2020 Feb 5;20(1):99. doi: 10.1186/s12885-020-6597-x.
59. Boonstra PA, Ter Elst A, Tibbesma M, Bosman LJ, Mathijssen R, Atrafi F, van Coevorden F, Steeghs N, Farag S, Gelderblom H, van der Graaf WTA, Desar IME, Maier J, Overbosch J, Suurmeijer AJH, Gietema J, Schuuring E, Reyners AKL. A single digital droplet PCR assay to detect multiple KIT exon 11 mutations in tumor and plasma from patients with gastrointestinal stromal tumors. Oncotarget. 2018 Feb 14;9(17):13870-13883. doi: 10.18632/oncotarget.24493.
60. Olmedillas-López S, García-Arranz M, García-Olmo D. Current and Emerging Applications of Droplet Digital PCR in Oncology. Mol Diagn Ther. 2017 Oct;21(5):493-510. doi: 10.1007/s40291-017-0278-8.
61. Yoo C, Ryu MH, Na YS, Ryoo BY, Park SR, Kang YK. Analysis of serum protein biomarkers, circulating tumor DNA, and dovitinib activity in patients with tyrosine kinase inhibitor-refractory gastrointestinal stromal tumors. Ann Oncol. 2014 Nov;25(11):2272-2277. doi: 10.1093/annonc/mdu386. Epub 2014 Aug 22.
62. Diehl F, Schmidt K, Durkee KH, Moore KJ, Goodman SN, Shuber AP, Kinzler KW, Vogelstein B. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. Gastroenterology. 2008 Aug;135(2):489-98. doi: 10.1053/j.gastro.2008.05.039. Epub 2008 May 15.
63. Bauer S, Herold T, Mühlenberger T, Reis AC, Falkenhorst J, Ketzer MB, Breitenbuecher F, Schuler MH, Grunewald S. Plasma Sequencing to Detect a Multitude of Secondary KIT Resistance Mutations in Metastatic Gastrointestinal Stromal Tumors. J Clin Oncol. 2015 May; 33: 10518-10518. doi: 10.1200/jco.2015.33.15_suppl.10518.

64. Kang G, Bae BN, Sohn BS, Pyo JS, Kang GH, Kim KM. Detection of KIT and PDGFRA mutations in the plasma of patients with gastrointestinal stromal tumor. Target Oncol. 2015 Dec;10(4):597-601. doi: 10.1007/s11523-015-0361-1. Epub 2015 Mar 5.
65. Wada N Kurokawa Y, Takahashi T, Hamakawa T, Hirota S, Naka T, Miyazaki Y, Makino T, Yamasaki M, Nakajima K, Takiguchi S, Mori M, Doki Y. Detecting Secondary C-KIT Mutations in the Peripheral Blood of Patients with Imatinib-Resistant Gastrointestinal Stromal Tumor. Oncology. 2016;90(2):112-7. doi: 10.1159/000442948. Epub 2016 Jan 19.
66. Kang G, Sohn BS, Pyo JS, Kim JY, Lee B, Kim KM. Detecting Primary KIT Mutations in Presurgical Plasma of Patients with Gastrointestinal Stromal Tumor. Mol Diagn Ther. 2016 Aug;20(4):347-51. doi: 10.1007/s40291-016-0203-6.
67. Guibert N, Hu Y, Feeney N, Kuang Y, Plagnol V, Jones G, Howarth K, Beeler JF, Paweletz CP, Oxnard GR. Amplicon-based next-generation sequencing of plasma cell-free DNA for detection of driver and resistance mutations in advanced non-small cell lung cancer. Ann Oncol. 2018 Apr 1;29(4):1049-1055. doi: 10.1093/annonc/mdy005.
68. Xu H, Chen L, Shao Y, Zhu D, Zhi X, Zhang Q, Li F, Xu J, Liu X, Xu Z. Clinical Application of Circulating Tumor DNA in the Genetic Analysis of Patients with Advanced GIST. Mol Cancer Ther. 2018 Jan;17(1):290-296. doi: 10.1158/1535-7163.MCT-17-0436. Epub 2017 Nov 13.
69. Arshad J, Roberts A, Ahmed J, Cotta J, Pico BA, Kwon D, Trent JC. Utility of Circulating Tumor DNA in the Management of Patients With GI Stromal Tumor: Analysis of 243 Patients. JCO Precis Oncol. 2020 Nov;4:66-73. doi: 10.1200/PO.19.00253.
70. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, Liu CL, Neal JW, Wakelee HA, Merritt RE, Shrager JB, Loo BW Jr, Alizadeh AA, Diehn M. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014 May;20(5):548-54. doi: 10.1038/nm.3519. Epub 2014 Apr 6.
71. Bratman SV, Newman AM, Alizadeh AA, Diehn M. Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq. Expert Rev

Mol Diagn. 2015 Jun;15(6):715-9. doi: 10.1586/14737159.2015.1019476. Epub 2015 Mar 16.

72. Johansson G, Berndsen M, Lindskog S, Österlund T, Fagman H, Muth A, Ståhlberg A. Monitoring Circulating Tumor DNA During Surgical Treatment in Patients with Gastrointestinal Stromal Tumors. Mol Cancer Ther. 2021 Dec;20(12):2568-2576. doi: 10.1158/1535-7163.MCT-21-0403. Epub 2021 Sep 22.
73. Ståhlberg A, Krzyzanowski PM, Egyud M, Filges S, Stein L, Godfrey TE. Simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing. Nat Protoc. 2017 Apr;12(4):664-682. doi: 10.1038/nprot.2017.006. Epub 2017 Mar 2.
74. Jeffers M, Kappeler C, Kuss I, Beckmann G, Mehnert DH, Fredebohm J, Teufel M. Broad spectrum of regorafenib activity on mutant KIT and absence of clonal selection in gastrointestinal stromal tumor (GIST): correlative analysis from the GRID trial. Gastric Cancer. 2022 May;25(3):598-608. doi: 10.1007/s10120-021-01274-6. Epub 2022 Jan 20.
75. Patrikidou A, Le Cesne A. Key messages from the BFR14 trial of the French Sarcoma Group. Future Oncol. 2017 Feb;13(3):273-284. doi: 10.2217/fon-2016-0409. Epub 2016 Sep 14.
76. Serrano C, George S, Valverde C, Olivares D, García-Valverde A, Suárez C, Morales-Barrera R, Carles J. Novel Insights into the Treatment of Imatinib-Resistant Gastrointestinal Stromal Tumors. Target Oncol. 2017 Jun;12(3):277-288. doi: 10.1007/s11523-017-0490-9.
77. Blay JY, Shen L, Kang YK, Rutkowski P, Qin S, Nosov D, Wan D, Trent J, Srimuninnimit V, Pápai Z, Le Cesne A, Novick S, Taningco L, Mo S, Green S, Reichardt P, Demetri GD. Nilotinib versus imatinib as first-line therapy for patients with unresectable or metastatic gastrointestinal stromal tumours (ENESTg1): a randomised phase 3 trial. Lancet Oncol. 2015 May;16(5):550-60. doi: 10.1016/S1470-2045(15)70105-1. Epub 2015 Apr 14.
78. Serrano C, Fletcher JA. Overcoming heterogenity in imatinib-resistant gastrointestinal stromal tumor. Oncotarget. 2019 Oct 29;10(59):6286-6287. doi: 10.18632/oncotarget.27277.
79. Serrano C, Mariño-Enríquez A, Tao DL, Ketzer J, Eilers G, Zhu M, Yu C, Mannan AM, Rubin BP, Demetri GD, Raut CP, Presnell A, McKinley A,

Heinrich MC, Czaplinski JT, Sicinska E, Bauer S, George S, Fletcher JA. Complementary activity of tyrosine kinase inhibitors against secondary kit mutations in imatinib-resistant gastrointestinal stromal tumours. Br J Cancer. 2019 Mar;120(6):612-620. doi: 10.1038/s41416-019-0389-6. Epub 2019 Feb 22.

80. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006 Oct 14;368(9544):1329-38. doi: 10.1016/S0140-6736(06)69446-4.
81. Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, Hohenberger P, Leahy M, von Mehren M, Joensuu H, Badalamenti G, Blackstein M, Le Cesne A, Schöffski P, Maki RG, Bauer S, Nguyen BB, Xu J, Nishida T, Chung J, Kappeler C, Kuss I, Laurent D, Casali PG; GRID study investigators. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013 Jan 26;381(9863):295-302. doi: 10.1016/S0140-6736(12)61857-1. Epub 2012 Nov 22.
82. Kang YK, George S, Jones RL, Rutkowski P, Shen L, Mir O, Patel S, Zhou Y, von Mehren M, Hohenberger P, Villalobos V, Brahmi M, Tap WD, Trent J, Pantaleo MA, Schöffski P, He K, Hew P, Newberry K, Roche M, Heinrich MC, Bauer S. Avapritinib Versus Regorafenib in Locally Advanced Unresectable or Metastatic GI Stromal Tumor: A Randomized, Open-Label Phase III Study. J Clin Oncol. 2021 Oct 1;39(28):3128-3139. doi: 10.1200/JCO.21.00217. Epub 2021 Aug 3.
83. Schöffski P, Mir O, Kasper B, Papai Z, Blay JY, Italiano A, Benson C, Kopeckova K, Ali N, Dileo P, LeCesne A, Menge F, Cousin S, Wardelmann E, Wozniak A, Marreaud S, Litiere S, Zaffaroni F, Nzokirantevye A, Vanden Bempt I, Gelderblom H. Activity and safety of the multi-target tyrosine kinase inhibitor cabozantinib in patients with metastatic gastrointestinal stromal tumour after treatment with imatinib and sunitinib: European Organisation for Research and Treatment of Cancer phase II trial 1317 'CaboGIST'. Eur J

Cancer. 2020 Jul;134:62-74. doi: 10.1016/j.ejca.2020.04.021. Epub 2020 May 26.

84. Mir O, Cropet C, Toulmonde M, Cesne AL, Molimard M, Bompas E, Cassier P, Ray-Coquard I, Rios M, Adenis A, Italiano A, Bouché O, Chauzit E, Duffaud F, Bertucci F, Isambert N, Gautier J, Blay JY, Pérol D; PAZOGIST study group of the French Sarcoma Groupe-Groupe d'Etude des Tumeurs Osseuses (GSF-GETO). Pazopanib plus best supportive care versus best supportive care alone in advanced gastrointestinal stromal tumours resistant to imatinib and sunitinib (PAZOGIST): a randomised, multicentre, open-label phase 2 trial. Lancet Oncol. 2016 May;17(5):632-41. doi: 10.1016/S1470-2045(16)00075-9. Epub 2016 Apr 5.
85. Blay JY, Serrano C, Heinrich MC, Zalcberg J, Bauer S, Gelderblom H, Schöffski P, Jones RL, Attia S, D'Amato G, Chi P, Reichardt P, Meade J, Shi K, Ruiz-Soto R, George S, von Mehren M. Ripretinib in patients with advanced gastrointestinal stromal tumours (INVICTUS): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2020 Jul;21(7):923-934. doi: 10.1016/S1470-2045(20)30168-6. Epub 2020 Jun 5. Erratum in: Lancet Oncol. 2020 Jul;21(7):e341.
86. Heinrich MC, Jones RL, Gelderblom H, George S, Schöffski P, von Mehren M, Zalcberg JR, Kang YK, Razak ARA, Trent JC, Attia S, Le Cesne A, Su Y, Meade JN, Wang T, Sherman ML, Ruiz-Soto R, Blay JY, Bauer S. INTRIGUE: A phase III, randomized, open-label study to evaluate the efficacy and safety of ripretinib versus sunitinib in patients with advanced gastrointestinal stromal tumor previously treated with imatinib. J Clin Oncol. 2022 April;40:36_suppl, 359881-359881. doi: 10.1200/JCO.2022.40.36_suppl.359881.
87. Wagner AJ, Severson PL, Shields AF, Patnaik A, Chugh R, Tinoco G, Wu G, Nespi M, Lin J, Zhang Y, Ewing T, Habets G, Burton EA, Matusow B, Tsai J, Tsang G, Shellooe R, Carias H, Chan K, Rezaei H, Sanftner L, Marimuthu A, Spevak W, Ibrahim PN, Inokuchi K, Alcantar O, Michelson G, Tsiatis AC, Zhang C, Bollag G, Trent JC, Tap WD. Association of Combination of Conformation-Specific KIT Inhibitors With Clinical Benefit in Patients With Refractory Gastrointestinal Stromal Tumors: A Phase 1b/2a Nonrandomized

Clinical Trial. JAMA Oncol. 2021 Sep 1;7(9):1343-1350. doi: 10.1001/jamaoncol.2021.2086.

88. Serrano C, Leal A, Kuang Y, Morgan JA, Barysauskas CM, Phallen J, Triplett O, Mariño-Enríquez A, Wagner AJ, Demetri GD, Velculescu VE, Paweletz CP, Fletcher JA, George S. Phase I Study of Rapid Alternation of Sunitinib and Regorafenib for the Treatment of Tyrosine Kinase Inhibitor Refractory Gastrointestinal Stromal Tumors. Clin Cancer Res. 2019 Dec 15;25(24):7287-7293. doi: 10.1158/1078-0432.CCR-19-2150. Epub 2019 Aug 30.
89. Fujino S, Miyoshi N, Ito A, Yasui M, Ohue M, Ogino T, Takahashi H, Uemura M, Matsuda C, Mizushima T, Doki Y, Eguchi H. Crenolanib Regulates ERK and AKT/mTOR Signaling Pathways in RAS/BRAF-Mutated Colorectal Cancer Cells and Organoids. Mol Cancer Res. 2021 May;19(5):812-822. doi: 10.1158/1541-7786.MCR-20-0600. Epub 2021 Feb 12.
90. Vallilas C, Sarantis P, Kyriazoglou A, Koustas E, Theocharis S, Papavassiliou AG, Karamouzis MV. Gastrointestinal Stromal Tumors (GISTs): Novel Therapeutic Strategies with Immunotherapy and Small Molecules. Int J Mol Sci. 2021 Jan 6;22(2):493. doi: 10.3390/ijms22020493.
91. Janeway KA, Albritton KH, Van Den Abbeele AD, D'Amato GZ, Pedrazzoli P, Siena S, Picus J, Butrynski JE, Schlemmer M, Heinrich MC, Demetri GD. Sunitinib treatment in pediatric patients with advanced GIST following failure of imatinib. Pediatr Blood Cancer. 2009 Jul;52(7):767-71. doi: 10.1002/pbc.21909.
92. Ben-Ami E, Barysauskas CM, von Mehren M, Heinrich MC, Corless CL, Butrynski JE, Morgan JA, Wagner AJ, Choy E, Yap JT, Van den Abbeele AD, Solomon SM, Fletcher JA, Demetri GD, George S. Long-term follow-up results of the multicenter phase II trial of regorafenib in patients with metastatic and/or unresectable GI stromal tumor after failure of standard tyrosine kinase inhibitor therapy. Ann Oncol. 2016 Sep;27(9):1794-9. doi: 10.1093/annonc/mdw228. Epub 2016 Jul 1.
93. Yebra M, Bhargava S, Kumar A, Burgoyne AM, Tang CM, Yoon H, Banerjee S, Aguilera J, Cordes T, Sheth V, Noh S, Ustoy R, Li S, Advani SJ, Corless CL, Heinrich MC, Kurzrock R, Lippman SM, Fanta PT, Harismendy O, Metallo C, Sicklick JK. Establishment of Patient-Derived Succinate

Dehydrogenase-Deficient Gastrointestinal Stromal Tumor Models for Predicting Therapeutic Response. Clin Cancer Res. 2022 Jan 1;28(1):187-200. doi: 10.1158/1078-0432.CCR-21-2092. Epub 2021 Aug 23.

94. Nannini M, Rizzo A, Indio V, Schipani A, Astolfi A, Pantaleo MA. Targeted therapy in SDH-deficient GIST. Ther Adv Med Oncol. 2021 Jun 28;13:17588359211023278. doi: 10.1177/17588359211023278.
95. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, Nathenson M, Doebele RC, Farago AF, Pappo AS, Turpin B, Dowlati A, Brose MS, Mascarenhas L, Federman N, Berlin J, El-Deiry WS, Baik C, Deeken J, Boni V, Nagasubramanian R, Taylor M, Rudzinski ER, Meric-Bernstam F, Sohal DPS, Ma PC, Raez LE, Hechtman JF, Benayed R, Ladanyi M, Tuch BB, Ebata K, Cruickshank S, Ku NC, Cox MC, Hawkins DS, Hong DS, Hyman DM. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. N Engl J Med. 2018 Feb 22;378(8):731-739. doi: 10.1056/NEJMoa1714448.
96. Falchook GS, Trent JC, Heinrich MC, Beadling C, Patterson J, Bastida CC, Blackman SC, Kurzrock R. BRAF mutant gastrointestinal stromal tumor: first report of regression with BRAF inhibitor dabrafenib (GSK2118436) and whole exomic sequencing for analysis of acquired resistance. Oncotarget. 2013 Feb;4(2):310-5. doi: 10.18632/oncotarget.864.