Int J Hum Genet, 20(2): 72-80 (2020)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
DOI: 10.31901/24566330.2020/20.2.749

Molecular Basis of cKit Mutation in Recurrent Gastrointestinal Stromal Tumour (GIST): A Case Report from India

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KEYWORDS Gastrointestinal Stromal Tumour. Imatinib. Kinase Inhibitors. Next-Generation Sequencing. Somatic Mutation Testing. Sunitinib. Sorafenib

ABSTRACT The development and progression of gastrointestinal stromal tumours (GISTs) are associated with mutations in the genes KIT and PDGFRA, which predict the response to tyrosine kinase inhibitors (TKI's). The confirmation of diagnosis of GIST is mainly on the basis of findings from the imaging and diagnostic tests of CD34/CD117 expression by immunohistochemistry (IHC). Approximately eighty percent of GIST with advanced disease achieves partial response or stable disease with selective TKI's. The resistance to the drug appears with prolonged usage due to emergence of acquired resistance mutations in the activation loop of cKit gene. Through this case study, the researchers aim to propose that genomic characterisation of GIST must be considered a standard practice to find aberrations that can be better managed with targeted treatment. Here, the researchers discuss a case of a 37-year-old female diagnosed with GIST based on CD34/CD117 positivity by IHC who initially responded to neo adjuvant imatinib therapy but the disease progressed after few months of treatment with each line of TKI. Somatic mutation analysis of 48 tumour-specific actionable genes by Next Generation Sequencing (NGS) was performed to identify the presence of any actionable mutations. The results revealed several primary and secondary (acquired) mutations in the KIT gene indicating resistance to TKI's imatinib, sunitinib and sorafenib but a possible response to regorafenib, a third-line TKI.

INTRODUCTION

Gastrointestinal stromal tumours (GIST) are the well-known mesenchymal neoplasms that are usually found in any part of the digestive system but common sites are the gastrointestinal tract and the stomach (Hirota et al. 1998; Nilsson et al. 2005; Tryggvason et al. 2005; Gomes et al. 2008, Miettinen et al. 2011). At a lesser frequency, GIST may arise in other organs like the appendix, gallbladder, pancreas, retroperitoneum, paravaginal and periprostatic tissues (Duensing et al. 2004). About twenty to twenty-five percent of gastric GIST, and forty to fifty percent of small intestinal GIST are clinically aggressive (West et al. 2004; Heinrich et al. 2003a), and has been estimated that about ten to twenty-five percent of patients present with metastatic disease (Judson et al. 2007).

GISTs are among the rare type of cancers that are often diagnosed incidentally and can present in different ways. Because of this, the identification of the risk factors of GIST has not been successful (Starczewska Amelio et al. 2014; Chiang et al. 2014; Fayet et al. 2014). Due to acquired mutations in KIT, changes in the response to tyrosine kinase have been observed by few studies, which were found to vary across different population, that is, seventy-five percent in a Norwegian study, seventy-nine percent in an Italian study, fifty-six percent in a Portuguese study, and seventy-four percent in a Korean study (Gomes et al. 2008; Braconi et al. 2008; Steigen et al. 2007; Kim et al. 2004).

The positivity and over-expression of CD 34 and CD 117 (KIT protein), by immunohistochemis-

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try (IHC) remains a very specific test for final diagnosis of GIST (Kerliu et al. 2014) and holds a therapeutic implication of offering as patients harbouring this marker respond more effectively to tyrosine kinase inhibitors (TKI's), imatinib mesylate (IM) or sunitinib malate. However, some patients with GIST, while initially sensitive to TKIs, gain resistance in later stages of treatment, which could be due to presence of either *de novo* (independent of treatment) or an acquired genetic alteration.

The identification of molecular basis of GIST, particularly the expression of c-Kit or tyrosine-protein kinase Kit or CD117, which is the cellular homologue of the feline sarcoma viral oncogene v-kit has greatly enhanced and changed the understanding of GIST biology (Besmer et al. 1986; Yarden et al. 1988; Qiu et al. 1988). GISTs were considered as one of the major treatment-refractory tumours where only few patients showed clinical response to conventional chemo and/or radiation therapy. Although surgery used to be the only valid therapy for GIST, the availability of molecular-targeted therapy specific for KIT/PDGFRA TKI's such as imatinib mesylate or, in the case of imatinib-resistant GIST, sunitinib malate has made the correct identification of GIST very important (Dematteo et al. 2002; Heinrich et al. 2002). It is known that about seventy-five to eighty percent of GISTs will have a mutation in cKit, which could be *de novo* or acquired, ten percent in PDGFRA and ten to fifteen percent of GISTs will not have a mutation in either of these two genes (Barnett et al. 2013).

Although IHC for KIT (CD117) is considered as a reliable diagnostic tool in the diagnosis of GIST (Steigen et al. 2009), few KIT negative cases of GIST, those showing unusual cell morphology and GISTs that progress during or after treatment with imatinib or sunitinib can be a challenge for pathologists and clinicians (Liegl et al. 2010). Though treatment with imatinib has a major significance in the management of GIST, several reports to date indicate the progression of the disease in most of the patients due to development of resistance to imatinib (Verweij et al. 2004). Hence, molecular subtyping of GIST is very important to understand the mutational characterisation, which would help for better prognosis and for planning the treatment (Heinrich et al. 2008a; Debiec-Rychter et al. 2006). A large number of GIST cases are associated with KIT mutations in exon 11 but mutations in exons 8, 9, 13, 14 and 17 have also been reported (Heinrich et al. 2003b; Zimmermann. 2017). The identification of the KIT mutations in exon 11 that encodes the juxta membrane and in exon 9, which encodes extracellular domains play a major role in understanding the mechanism of many GISTs. The mutations in exons 13 and 17 that encode for tyrosine kinase domains 1 and 2 are rare (Joensuu et al. 2012). Current learning explains that the presence and localisation of mutations in the kinase domain of cKit within the gene sequence along with the type of mutation plays an important role for planning suitable targeted treatment (Corless et al. 2011).

As most of the known actionable variants have some clinical significance, it is very essential to sequence all GIST cases to detect the presence of mutations in both KIT and PDGFRA at the time of diagnosis. The genetic mutations, associated pathways and related prognosis that are involved in a rare form of GIST called Ampulla of Vater carcinoma (AVCs) have been studied to establish biomarkers for early diagnosis of AVC and to discover molecular targets for drug therapy (Kaavya et al. 2018). A study on population screening of patients affected with AVC in Tamil Nadu in India has shown a high frequency of KRAS gene mutation, an early molecular event leading to an abnormal proliferation of the cells (Anand et al. 2016). Even though GISTs in young (under 40 years) patients are not common and not well studied, they should be considered in the diagnosis of gastrointestinal masses. In this case report, the researchers present a young patient with GIST who progressed on treatment with TKI's prescribed on the basis of IHC. The researchers investigated the therapeutic and prognostic significance of the mutational status of 48 oncogenes and tumour suppressor genes that includes KIT and PDGFRA by the use of next-generation sequencing (NGS) technology. With the present learning, the researchers realise that the KIT mutated GISTs are not homogenous with regards to prognosis and TKI affectability, based on the particular site of mutation within the KIT gene.

Objectives

To demonstrate that IHC should be followed with multigene testing by NGS to identify the *de*

novo and/or acquired resistance mutations in tumour, which have been pre-treated with TKIs and analyse the possible prognostic, therapeutic or clinical implications of the alteration detected.

To provide insights that early detection of targeted mutation can help in timely treatment thereby avoiding appearance of resistance mutations.

Case Report

A 37-year-old female was presented in September 2012 with history of progressive increase in abdominal pain for 2½ months. She underwent diagnostic laparoscopy and biopsy of peritoneal lesions based on ultrasound scans of the abdomen and pelvis. The histopathology evaluation (HPE) revealed fibrosarcomatous changes and IHC showed positivity for CD 117 and CD34 (Figs. I A, B and C), which confirmed the diagnosis of GIST. Based on HPE and IHC findings, the patient was given imatinib (400mg per day), which she responded and tolerated well. The medicine was stopped before 1 week of exploratory laparotomy-omentectomy and peritoneal debulking in March 2013 at a hospital in Bangalore. Post-operative period was uneventful and imatinib was restarted at same dose of 400mg per day for 2 weeks from postoperative day till September 2013. While still on imatinib, the patient complained of frequent abdominal pain and hence a CT scan of abdomen and pelvis was completed. The scans were suggestive of recurrent peritoneal disease due to which second-line targeted drug with sunitinib was started at a dosage of 50 mg once a day for 4 weeks and 2 weeks off. Reassessment done in July 2014, revealed a progressive disease with an increase in the number and size of the peritoneal nodules, hepatic lesion and ascites when compared with the previous study performed in April 2014. She was on follow-up with symptomatic treatment and because her family was very keen for third-line treatment, sorafenib was started from September 2014. Symptomatically, the patient did not show any improvement and ultrasound scan of the abdomen and pelvis done in October 2014 revealed mass in left lumbar region with suspicious infiltration of left kidney and masses in hepatic surface, periportal, pancreatic and lesser sac regions, thus conferring metastases. The scanning also uncovered extensive peritoneal deposits, few hypoechoic lesions in liver and moderate ascites. An ultrasound-guided biopsy of abdominal mass confirmed metastatic GIST with peritoneal metastases. Since the patient was resistant to either of imatinib, sunitinib or sorafenib, genetic testing was recommended for which a pre-genetic test counselling was carried out to understand the history of the disease.

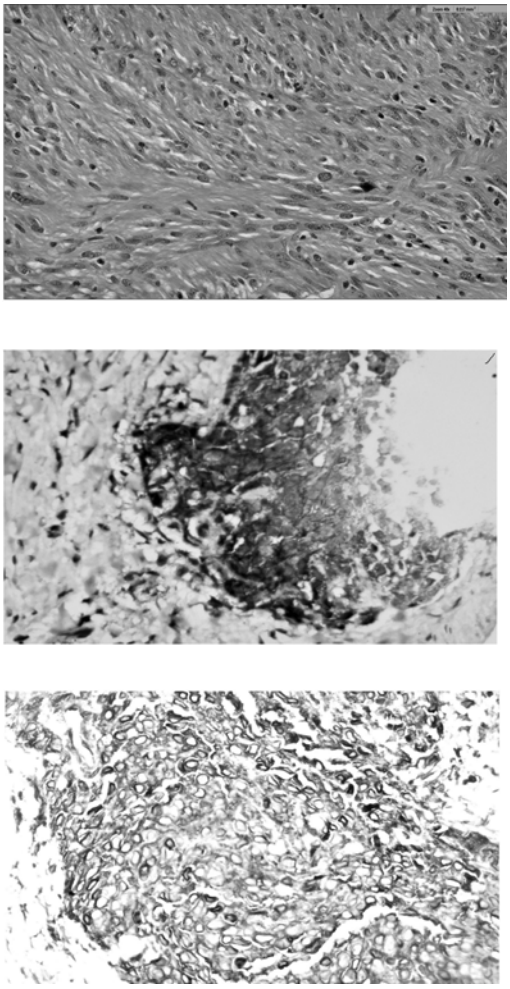

Fig. I A, B, C: A. Histological appearance of the surgical specimen. The pathological finding showed an interlacing pattern of spindle cells. H & E × 40.
B & C. The specimen was positive for CD 117 and CD 34 (KIT) immunostaining. × 200.

The approval for genetic testing of the patient's samples for this study was obtained by the Institutional Ethics Committee of Health Care Global Enterprises, Bangalore (EC Registration No: ECR/386/Inst/KA/2013/RR-19). After obtaining the informed consent, somatic mutation analysis by NGS was performed to check if any genes responsible for GIST were altered based on which further treatment could be planned. The test was performed based on Illumina Truseq amplicon cancer panel that includes 48 genes associated with solid tumours. The data generated was processed and analysed using Strand NGS and mutations that were detected were assessed for potential response to targeted therapy (actionability) and clinical relevance. She was admitted at HCG Hospital, Bangalore in November 2014 for symptomatic treatment, but gradually her general condition deteriorated and succumbed to the disease in December 2014.

METHODOLOGY

Detection of KIT by Immunohistochemistry

IHC was performed on the formalin-fixed paraffin-embedded (FFPE) biopsy sample from the peritoneal lesion. About 3 mm thick sections were cut from the paraffin block and used for hematoxylin and eosin staining (H and E staining) for HPE and IHC. In brief, the tumour sections were deparaffinised in xylene for about 15 minutes and rehydrated with increasing concentrations of ethanol. Epitope retrieval was done on epitope retrieval steamer set using epitope retrieval solution (ready-to-use). Rabbit polyclonal antibody against human KIT (A4502, DAKO) at a dilution of 1:200 and mouse monoclonal antibody against human CD 34 (Novocastra Laboratories, New Castle upon Tyne, UK) at a dilution of 1:80 were used as primary antibodies. The slides were incubated for 1 hour at room temperature with the primary antibodies followed by incubation with Envision and labelled polymer for 30 minutes, and diaminobenzidine (DAB) and substrate for 5 minutes (DAKO). The slides were then counterstained with hematoxylin for 30 seconds, washed and de-xylenated before mounting the coverslips on the tissue sections. The sections were observed under a light microscope and the cytoplasmic positivity was scored in a semi-quantitative method (0, 1+, 2+, 3+) by the pathologist based on the percentage of positive cells.

Next-Generation Sequencing for Detection of KIT

Genetic testing study was carried out after getting the approval from the Human Research Ethics Committee at Health Care Global Enterprises, India. As recommended by the committee, written consent was obtained to use the formalin-fixed, paraffin-embedded (FFPE) tumour samples for the study. The H&E slides of the FFPE samples were examined for the presence of viable tumour cells and scored for percentage of tumour nuclei by a pathologist who selected the areas of neoplastic cells. The specimen with estimated tumour nuclei $\geq$ thirty percent in indicated areas was considered for the study. About 5-micron sections of FFPE tissue was used to extract genomic DNA using a QIAamp DNA FFPE Tissue kit (Qiagen, Germany) after deparaffinisation with xylene and one hundred percent ethanol according to the manufacturer's instructions. DNA from the paired normal sample (saliva) was isolated using Oragene-DNA (OG-500) Kit (DNA Genotek, CANADA). The FFPE DNA was qualified using the Illumina Infinium assay kit (Illumina, San Diego, CA, USA). Amplicon based library was prepared from 250 ng of DNA samples using TruSeq® Amplicon-Cancer Panel (Illumina, San Diego, CA, USA). The panel provides pre-designed, optimised oligonucleotide probes to sequence hotspot mutations in > 35 kilobases (kb) of the target genome sequence. The panel consists of forty-eight genes (ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL) targeting 212 amplicons in a multiplexed reaction (Mavroeidis et al. 2018). Following the manufacturer's protocol genomic DNA was hybridised with pairs of oligonucleotide-probes that are specific to the targeted regions. The unbound probes were removed by washing.

The pair of oligonucleotide-probes were then extended and ligated to form templates. The templates were then amplified through PCR using primers that add adaptors and index tags for multiplex sequencing. Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) were used to purify the PCR products and the quality of DNA libraries were checked on Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA). The libraries were normalised, pooled and sequenced using the Illumina MiSeq system. Paired-end NGS reads obtained from the Illumina MiSeq sequencer were aligned against the hg19 reference genome using the MiSeq Reporter software from Illumina and the aligned files were imported into Avadis NGS 1.5 for QC and downstream analysis. Reads with average quality below Q20 were filtered. The Avadis NGS variant caller was used to identify single base variants (SBV) and multi-base variants (MBV) at all locations with coverage of at least 10X. Variants with Phred confidence above 50 were annotated using the dbSNP 137 database. The SNP effect analysis feature of Avadis NGS was used to identify the functional effects of the variants on Ref Seq transcripts of the genes. This test namely somatic 48 gene test detects somatic alterations in hot spot regions of 48 genes and interprets those with possible therapeutics, clinical or prognostic implications.

RESULTS

Genetic analysis by NGS detected four alterations in KIT kinase domain in exons 11 (p.Val559_Asn566del), 13 (p.Val654Ala), 14 (p.Asp677Asn) and 17 (p.Asp820His).

The variant p.Val559_Asn566del in exon 11 was a novel mutation located in the juxta membrane domain that causes an in-frame deletion that adversely affects patient outcome and are significantly associated with high-risk GIST.

The identified second mutation p.Val654Ala in exon 13 causes a missense substitution and has been reported in cancers of the large intestine and hematopoietic and lymphoid tissues (COSM12706).

The third mutation identified was p.Asp677 Asn, another novel variant that caused a missense substitution in exon 14.

The identified fourth variant p.Asp820His causes a missense substitution in exon 17 and has been reported in cancers of soft tissues and testis (COSM22379).

DISCUSSION

GISTs are the most common gastrointestinal mesenchymal tumours and are different from leiomyomas, neurogenic tumours and leiomyosarcoms (Durham et al. 2004). They differ in the presentation of symptoms, site of the tumour, biological behaviour and immunophenotype. GISTs are differentiated from these tumours through IHC based on the positive expression of proto-oncogene cKit (CD117) and CD 34 that helps in the confirmation of GIST diagnosis (Durham et al. 2004).

The management of GIST patients depend on the type and aggressiveness of the disease. Whereas, a resectable disease may need neo adjuvant/adjuvant therapy, metastatic disease may be managed by palliative tyrosine kinase inhibition (Hirota et al. 1998). A multi-targeted TKI, Imatinib mesylate was reported to be an effective therapy for GIST (Hirota et al. 1998) but several reports till date have indicated the resistance after prolonged treatment with TKI due to acquisition of primary or secondary mutations in the proto oncogene cKit (Antonescu et al. 2013). There are different targeted drugs for *de novo* mutations in cKit that have been approved for GISTs to achieve good clinical response. But, next generation agents are required to overcome the secondary or acquired resistance mutations in cKit in GIST (Liu et al. 2019).

The acquisition of secondary KIT mutation can occur on the same allele as the primary mutation and is the most common cause of drug resistance, which typically occurs after a prolonged interval of treatment (Antonescu et al. 2005; Debiec-Rychter et al. 2005). In contrast, acquired resistance in tumours lacking detectable 2nd site mutations occur in patients who have been on the drug for a shorter period of time (Antonescu et al. 2005). Development of NGS has helped in identifying more primary and drug-resistant mutations.

In this study, the researchers have discussed a case of a young woman with small intestinal GIST treated with three targeted TKI's. Treatment

was started with imatinib for which initial partial response was observed but after few months, the therapy was changed to second line with sunitinib because of recurrent peritoneal disease. Though the disease was stable for few months, reassessment revealed disease progression, and hence a third line therapy was started with sorefenib. Treatment with sorefenib did not show any symptomatic improvement, which led to metastasis to kidney, liver and pancreas. Somatic mutation analysis detected the presence of four alterations in the cKit gene in exon 11, 13, 14 and 17. Treatment with imatinib is effective in GISTs with the exon 11 mutation in cKit, but if exons 13 and 17 are also mutated, the therapeutic effect of imatinib may not be as good potentially, as it resulted in acquired resistance in almost 50 percent of cases, which was also evident in this patient (Garner et al. 2014). Clinical studies have shown that imatinib had the longest progression-free and overall survival in patients with primary exon 11 KIT mutations compared to those without KIT mutations or with other mutations (Kurokawa and Komatsu 2017).

The mutation observed in KIT gene in exon 13 was pVal654Ala that encodes tyrosine kinase 1 of KIT protein that is found to enhance cellular proliferation. The Val 654 residue is located in the imatinib binding site of KIT and this mutation disrupts drug binding, which results in resistance to the drug imatinib.

The third mutation p.Asp677Asn identified in the kinase insert domain was again a novel mutation in exon 14 resulting in constitutive activation of the cKit receptor. Clinical evidences from GIST patients have shown that secondary mutations in KIT, including p.Val654Ala and p.Asp820His, and mutations in exon 14 conferred resistance to imatinib (Chen et al. 2004; Debiec-Rychter et al. 2005; Heinrich et al. 2008a; Koyama et al. 2006).

The fourth mutation p.Asp820His detected in exon 17 (codons 810-823) is important in stabilising the activated receptor (Gounder et al. 2011). Since imatinib competes with ATP for the ATP binding site of the kinase, it prevents the downstream signalling, thus inhibiting imatinib binding to the ATP binding site resulting in constitutive and strong activation of KIT phosphorylation (Wu et al. 2014; Allgayer 2014). *In vitro* studies have suggested resistance to both imatinib and sunitinib in presence of double mutations in KIT, when the second mutation existed in the activation loop (exon 17), which in this case further indicates resistance to sunitinib due to the presence of the p.Asp820His mutation (Heinrich et al. 2008 b). Clinical trials have shown that sorafenib, another multikinase inhibitor inhibits KIT, VEGFR, PDGF and BRAF kinases and has demonstrated both pre-clinical and clinical activity against resistant GIST. It has also shown the activity in a retrospective set of refractory GIST cases previously treated with imatinib, sunitinib and nilotinib (Liegl et al. 2008). But in this patient discussed here, treatment with sorafenib for almost 2 months also did not show any remarkable response. Since new mutations are continuously evolving in GIST to maintain strong KIT signalling in the TKI setting, it has become very essential to discover drugs that act to inhibit GIST independent of the specific KIT mutations.

The results from NGS indicated the significance of another FDA approved drug regorafenib, which has been used to treat imatinib and sunitinib refractory GIST. Regorafenib has been shown to improve progression-free survival of imatinib and sunitinib resistant GISTs across different subpopulations during the analysis of clinical trials treatment. It is evident from a phase 3 randomised trial GRID, that regorafenib showed a significant improvement of 4.8 months PFS as compared to 0.9 months in the placebo arm (n=66) (Demetri et al. 2013). As this patient was resistant to all three lines of TKI's, it would have been worthwhile checking if she would have responded to regorafenib if it were administered as soon as the resistance was observed. If the molecular analysis of KIT gene was performed at the time of diagnosis itself, it would have helped to understand if the patient would have positively responded to standard imatinib/sunitinib treatment that was given as standard treatment based on the initial diagnosis of GIST.

A recent study has shown that the 3rd generation drug regorafenib regresses an imatinib-resistant recurrent GIST with a mutation in exons 11 and 17 of c-Kit in a patient-derived orthotopic xenograft (PDOX) nude mouse model (Miyake et al. 2018). Preclinical studies on the activity of vascular endothelial growth factor receptor (VEGFR) kinase inhibitor axitinib has shown an advantage in both *in vitro* and *in vivo* models of GIST with primary and secondary cKit mutations. Although

this drug has been approved for renal cell carcinoma (RCC), it has shown comparable sensitivity to TKIs in cKit mutated GISTs (Liu et al. 2019). Through a drug repositioning approach, Lu et al have shown that cabozantinib, a TKI used to treat medullary thyroid cancer and a second-line treatment for renal cell carcinoma exhibited higher potency than imatinib against primary gain-of-function mutations of cKit. The drug was able to overcome cKit gatekeeper T670I mutation and the activation loop mutations that are resistant to imatinib or sunitinib. It also showed good efficacy *in vitro* and *in vivo* in the cKit mutant-driven preclinical models of GISTs thus providing the basis for the future clinical applications of cabozantinib as an alternative anti-GISTs therapy in precision medicine (Lu et al. 2019).

CONCLUSION

Although HPE and IHC are the gold standards in the diagnosis of GISTs by using IHC panel (CD34/CD117), the researchers learn from this case that accurate molecular analysis is essential and should be considered for patients diagnosed with GIST before initiating TKI to detect the *de novo* mutations. Serial monitoring of the patients during the course of treatment is necessary to identify acquired mutations, which occur after long-term treatment with TKIs. The molecular diagnosis of GISTs guides clinicians to precision medicine and provides optimal treatment options. Molecular testing should be considered even for patients who are on treatment with imatinib to detect the emergence of acquired resistance mutation. This can be easily achieved by mutational study of the metastatic site or from the "liquid biopsy" by testing the mutations in the cell-free DNA (cfDNA). The systemic treatment of GISTs by adjuvant palliative set up should be personalised based on the genotype and other known prognostic and predictive factors.

RECOMMENDATIONS

The study emphasises that mutational analysis for known actionable genes such as KIT should be included early on as diagnostic work-up of GIST. This will not only help to confirm the diagnosis of suspect GIST that does not exhibit positive immune reactivity for CD117/DOG1 but also enable the oncologists to stratify the patients "at-risk" for relapse after curative surgery and in the case of advanced, inoperable, metastatic disease, for the selection of appropriate therapy and identify the acquired resistance in patients who are being on prolonged treatment with targeted TKI's.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Veena Ramaswamy and Dr. Tejaswini, Pathologists, Strand Life Sciences, HCG, scientific team at UAS Alumni Association Building, Veterinary College Campus, Bengaluru and at Kirloskar Business Park, Bengaluru. The authors would also like to thank the patient's family for giving consent for genetic testing and for publishing the manuscript.

ABBREVIATIONS

ATP: Adenosine triphosphate
cf DNA: Cell-free DNA
FFPE: Formalin-fixed, paraffin-embedded
GIST: Gastrointestinal stromal tumours
HPE: Histopathology evaluation
IHC: Immunohistochemistry
NGS: Next generation sequencing
TKI: Tyrosine kinase inhibitors
VEGF: Vascular endothelial growth factor

REFERENCES

Allgayer H 2014. *Recent Results in Cancer Research*. Springer.

Anand L, Padmavathi V, Venkatesh B et al. 2016. Population screening of K-*ras* gene and genetic counselling for patients affected with Ampulla of Vater in Tamil Nadu. *International Journal of Human Genetics*, 16: 3-4.

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

Antonescu CR, Romeo S, Zhang L et al. 2013. Dedifferentiation in gastrointestinal stromal tumor to an Anaplastic KIT Negative Phenotype - a diagnostic pitfall: Morphologic and molecular characterization of 8 cases occurring either de-novo or after Imatinib Therapy. *Am J Surg Pathol*. 37(3): 385–392.

Barnett CM, Corless CL, Heinrich MC 2013. Gastrointestinal stromal tumors: Molecular markers and genetic subtypes. *Hematol Oncol Clin North Am*, 27: 871-888.

Besmer P, Murphy JE, George PC et al. 1986. A new acute transforming feline retrovirus and relationship of its oncogene v-*kit* with the protein kinase gene family. *Nature* (Lond), 320*:* 415–421.

Braconi C, Bracci R, Bearzi I et al. 2008. KIT and PDGFR alpha mutations in 104 patients with gastrointestinal stromal tumors (GISTs): A population-based study. *Ann Oncol*, 19(4): 706-710.

Chen LL, Trent JC, Wu EF et al. 2004. A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. *Cancer Res*, 64: 5913–5919.

Chiang NJ, Chen LT, Tsai CR et al. 2014. Chang JS. The epidemiology of gastrointestinal stromal tumors in Taiwan, 1998–2008: A nation-wide cancer registry-based study. *BMC Cancer*, 14: 102.

Corless CL, Barnett CM, Heinrich MC 2011. Gastrointestinal stromal tumors: Origin and molecular oncology. *Nat Rev Cancer*, 11(12): 865–878.

Debiec-Rychter M, Cools J, Dumez H et al. 2005. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology*, 128 (2): 270-279.

Debiec-Rychter M, Sciot R, Le Cesne A et al. 2006. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer*, 42: 1093-1103.

Dematteo RP, Heinrich MC, El-Rifai WM et al. 2002. Clinical management of gastrointestinal stromal tumors: before and after STI-571. *Human Pathol*, 33: 466-477.

Demetri GD, Reichardt P, Kang YK et al. 2013. 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*, 381: 295-302.

Duensing A, Joseph NE, Medeiros F et al. 2004. Protein Kinase C theta (PKC theta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). *Cancer Res*, 64(15): 5127-31.

Durham MM, Gow KW, Shehata BM et al. 2004. Gastrointestinal stromal tumors arising from the stomach: A report of three children. *J Pediatr Surg*, 39(10): 1495–1499.

Fayet Y, Chasles V, Ducimetière F et al. 2014. To answer rare cancer issues: Geographical analysis of EMS sarcoma cohort in the Rhône-Alpes region. *Bull Cancer*, 101(2): 127–136.

Garner AP, Gozgit JM, Anjum R et al. 2014. Ponatinib inhibits polyclonal drug-resistant KIT oncoproteins and shows therapeutic potential in heavily pretreated gastrointestinal stromal tumor (GIST) patients. *Clin Cancer Res*, 20: 5745-55.

Gomes AL, Gouveia A, Capelinha AF et al. 2008. Molecular alterations of KIT and PDGFRA in GISTs: Evaluation of a Portuguese series. *J Clin Pathol*, 61(2): 203-208.

Gounder MM, Maki RG 2011. Molecular basis for primary and secondary tyrosine kinase inhibitor resistance in gastrointestinal stromal tumor. *Cancer Chemother Pharmacol*, 67.

Heinrich MC, Blanke CD, Druker BJ et al. 2002. Inhibition of KIT tyrosine kinase activity: A novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol*, 20: 1692-1703.

Heinrich MC, Corless CL, Demetri GD et al. 2003a. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol*, 21: 4342-4349.

Heinrich MC, Corless CL, Duensing A et al. 2003b. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science*, 299 (5607): 708-710.

Heinrich MC, Maki RG, Corless CL et al. 2008a. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol*, 26(33): 5352-5359.

Heinrich MC, Owzar K, Corless CL et al. 2008b. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol*, 26: 5360-5367.

Hirota S, Isozaki K, Moriyama Y et al. 1998. Gain-of-function mutations of KIT in human gastrointestinal stromal tumors. *Science*, 279: 577-580.

Joensuu H, Ronald P. DeMatteo RP 2012. The management of gastrointestinal stromal tumors: A model for targeted and multidisciplinary therapy of malignancy. *Annu. Rev Med*, 63: 10.1-10.12.

Judson I, Demetri G 2007. Advances in the treatment of gastrointestinal stromal tumours. *Ann Oncol*, 18(Suppl 10): x20-4.

Kaavya J, Balachandar V, Sivanandan Santhy K 2018. Ampullary carcinoma- A genetic perspective. *Mutation Research*, 776: 10-22.

Kerliu SM, Meka VS, Kerliu I et al. 2014. Small intestinal gastrointestinal stromal tumor in a young adult woman: a case report and review of the literature. *Journal of Medical Case Reports*, 8: 321.

Kim TW, Lee H, Kang YK et al. 2004. Prognostic significance of c-kit mutation in localized gastrointestinal stromal tumors. *Clin Cancer Res*, 10: 3076-3081.

Koyama T, Nimura H, Kobayashi K et al. 2006. Recurrent gastrointestinal stromal tumor (GIST) of the stomach associated with a novel c-kit mutation after imatinib treatment. *Gastric Cancer*, 9 (3): 235-239.

Kurokawa Y, Komatsu Y 2017. *Gastrointestinal Stromal Tumor.* Springer Sciences and Business Media LLC.

Liegl B, Fletcher JA, Fletcher CD 2010. Gastrointestinal stromal tumors. *Virchows Arch*, 456: 111–127.

Liegl B, Kepten I, Le C et al. 2008. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. *J Pathol*, 216: 64-74.

Liu F, Zou F, Chen C et al. 2019. Axitinib overcomes multiple imatinib resistant *cKit* mutations including the gatekeeper mutation T670I in gastrointestinal stromal tumors. *Ther Adv Med Oncol*, 11: 1–15.

Lu T, Chen C, Wang A et al. 2019. Repurposing cabozantinib to GISTs: Overcoming multiple imatinib-resistant *cKit* mutations including gatekeeper and ac-

tivation loop mutants in GISTs preclinical models. *Cancer Lett*, 447: 105-114.
Miettinen M, Lasota J 2011. Histopathology of gastrointestinal stromal tumor. *Journal of Surgical Oncology*, 104: 865–873.
Miyake K, Kawaguchi K, Kiyuna T et al. 2018. Regorafenib regresses an imatinib-resistant recurrent gastrointestinal stromal tumor (GIST) with a mutation in exons 11 and 17 of c-kit in a patient-derived orthotopic xenograft (PDOX) nude mouse model. *Cell Cycle*, 17(6): 722–727.
Nilsson B, Bumming P, Medis-Kindblom JM et al. 2005. Gastrointestinal stromal tumors: The incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era-A population-based study in western Sweden. *Cancer*, 103: 821-829.
Qiu FH, Ray P, Brown K, et al. 1988. Primary structure of c-*kit*: relationship with the CSF-1/PDGF receptor kinase family-oncogenic activation of v-*kit* involves deletion of extracellular domain and C terminus. *EMBO J*, 7: 1003–1011.
Starczewska Amelio JM, Cid Ruzafa J, Desai K et al. 2014. Prevalence of gastrointestinal stromal tumour (GIST) in the United Kingdom at different therapeutic lines: An epidemiologic model. *BMC Cancer*, 14(1): 364.
Steigen SE, Eide TJ, Wasag B et al. 2007. Mutations in gastrointestinal stromal tumors– a population-based study from Northern Norway. *APMIS*, 115: 289-298.
Steigen SE, Eide TJ 2009. Gastrointestinal stromal tumors (GISTs): A review. *APMIS*, 117: 73-86.
Tryggvason G, Gislason HG, Magnusson MK et al. 2005. Gastrointestinal stromal tumors in Iceland, 1990–2003: The Icelandic GIST study, a population-based incidence and pathologic risk stratification study. *Int J Cancer*, 117: 289-293.
Verweij J, Casali PG, Zalcberg J et al. 2004. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: Randomised trial. *Lancet*, 364: 1127–1134.
West RB, Corless CL, Chen X et al. 2004. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol*, 165(1): 107-113.
Wu L, Zhang Z, Yao H et al. 2014. Clinical efficacy of second-generation tyrosine kinase inhibitors in imatinib-resistant gastrointestinal stromal tumors: A meta-analysis of recent clinical trials. *Drug Des Devel Ther*, 8: 2061-2067.
Yarden Y, Kuang WJ, Yang-Feng T et al. 1988. Human proto-oncogene c-*kit*: A new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J*, 6: 3341–3351.
Zimmermann A 2017. *Tumors and Tumor-Like Lesions of the Hepatobiliary Tract*. Springer Science and Business Media LLC.

Paper received for publication in December, 2019
Paper accepted for publication in April, 2020