A STUDY OF OUTCOME OF METASTATIC GASTROINTESTINAL STROMAL TUMORS TREATED WITH IMATINIB AND CORRELATION WITH C-KIT MUTATION STATUS

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CERTIFICATE

This is to certify that this dissertation on "**A STUDY OF OUTCOME OF METASTATIC GASTROINTESTINAL STROMAL TUMORS TREATED WITH IMATINIB AND CORRELATION WITH C-KIT MUTATION STATUS"** is a bonafide work done by **Dr. Sanju Cyriac,** in the Department of Medical Oncology, College of Oncological sciences, Adyar, Chennai,, under my overall supervision and guidance, to my satisfaction.

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I dedicate this work to my father, **Dr Cyriac Kurian MS., DO.,** who is my constant inspiration as a doctor. I thank my wife for the mental support all through my post graduate career.

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CONTENTS

INTRODUCTION

Gastrointestinal Stromal Tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract.[1] They are very rare tumors and can arise anywhere in the gastrointestinal tract but most commonly arise from the stomach (40-65%), the small intestine (20-40%) and rectum (2-15%). Majority of the patients are asymptomatic.

The molecular hallmark of entity was discovered only 10 years back. Approximately 95% of GIST stain positive for CD117 on immunohistochemistry.[1] KIT is the cell surface transmembrane tyrosine kinase receptor of the stem cell factor. In1998, Hirota et al identified activated mutations of the KIT receptor gene in familial and sporadic cases of GIST, which were later found to occur very frequently. [2] Approximately 80 – 90% of the GIST exhibit activating KIT mutations. [3]

The most common KIT mutations affect the juxtamembrane domain encoded by exon 11 which is seen in 80% of the cases. [4,5] In addition, 12% of GISTs have a mutation in an extracellular domain encoded by exon 9. More rarely, mutations occur in the split kinase domain encoded by exon 13 or 17. Approximately 30% of GISTs that are wild type for KIT, and 5% to 8% of GISTs overall, have PDGFRA gene mutations. KIT and PDGFRA mutations are mutually exclusive in GIST. Wild-type GISTs (i.e., GISTs lacking mutations of KIT or PDGFRA) account for 12% to 15% of all adult GISTs but is more prevalent in pediatric GISTs (uptp 90%). The molecular pathogenesis and underlying biology of wild-type GISTs is a subject of ongoing research.

The prognosis of metastatic GIST was considered dismal and hardly any patient survived beyond 1 - 2 years. Traditionally, GIST is considered to be chemoresistant. The discovery of Imatinib and its use against Chronic Myeloid Leukemia led to more researches for targeted therapy. [6] Subsequently, the ability of Imatinib to inhibit KIT and PDGFR was noted. In two large phase III studies comparing imatinib dose levels (400 mg/day vs. 800 mg/day), the median progression-free survival (PFS) for either arm was approximately 20 months, and median overall survival (OS) was approximately 50 months.[7,8] So the fact that the natural history of GIST has changed was clear. Since then, there are fascinating research activities going on in the field of GIST.

Secondary resistance mechanisms eventually ensue and many patients progress after Imatinib. Several second line agents are available now to salvage such patients. These include drugs like Sunitinib, Dasatinib, Nilotinib etc. Newer pathways like mTOR inhibition are explored to add drugs to the second line list.

The clinical studies of imatinib for treatment of GISTs consistently demonstrated that genotypically defined subsets of GISTs have different outcomes during imatinib treatment. [7, 9] The presence of a KIT exon 11 mutation is associated with a significantly improved clinical outcome during imatinib therapy when compared with patients with metastatic GISTs with KIT exon 9-mutant or wild-type genotypes. Patients with KIT exon 9-mutant GIST had significantly improved PFS, but not OS, when treated with high-dose imatinib. The relative imatinib resistance of the KIT exon 9 mutant kinase when compared with KIT exon 11 mutant kinase might

explain the benefit of high-dose imatinib in KIT exon 9-mutant GIST. Based on the previous data, many GIST experts now recommend routine tumor genotyping and dose selection based on the presence or absence of a KIT exon 9 mutation.

Metastatic GIST management has taken a new turn in our country also after the introduction of Imatinib. Support programmes like GIPAP (Glivec International Patient Assistance Programme) provide free drugs to all patients with metastatic GIST. There is hardly any data about the treatment outcome of metastatic GIST in India. It would be interesting to check whether our patients behave similar to the reported series. The genotype pattern in our population also is largely unknown. This study is conducted to address both these important issues.

AIM

The aims of the study are

1. To analyse clinicopathological profile of patients with metastatic GIST
2. To determine the prognostic factors associated with treatment outcome
3. To assess the survival patterns
4. To study the genotype of tumors of patients with metastatic GIST
5. To study the relationship of genotype with treatment outcome, if any.

REVIEW OF LITERATURE

Gastrointestinal Stromal Tumors (GIST) are rare malignancies of the gastrointestinal tract. It has been labelled as the most common mesenchymal malignancy of the GIT. [1] The progress made in this relatively recently defined entity is fascinating. The better understanding about the molecular mechanisms of the disease made it possible to incorporate successfully targeted therapy into the treatment armamentarium. FDA approval of Imatinib (IM) for the management of metastatic GIST came first in 2002. Now IM is also used as an adjuvant treatment in an effort to improve the high recurrence rates. The numerous ongoing phase I/II trials aim at further improvement of the results and also using novel Tyrosine Kinase Inhibitors (TKI) for the management of GIST.

The annual incidence of GIST in United States is estimated around ~4000-5000 per year.[1] Before 2000, GIST was estimated to account for 1-3% of all GI malignancies. Prevalence must be higher as many patients with GIST live with small lesions often detected at autopsy only. There is no published data regarding the prevalence of GIST in India. The entity is described more clearly in the recent times as majority were labelled as leiomyosarcomas in the past and since immunohistochemistry is widely available nowadays.

The diagnostic criteria for GIST remained controversial and somewhat confusing before 1999. Mazur and Clark used the term GIST in 1993 as a purely descriptive term to define intra-abdominal tumors that were definitely not carcinomas

and that also failed to exhibit features of either smooth muscle or nerve cells. [10] However, pathologists recognized that there was not a completely clear differential expression of muscle or nerve antigenic markers when careful immunohistochemical analyses were performed on certain mesenchymal tumors of the gut. There onwards several names were applied to these tumors based on the different patterns of cell lineage markers described by many pathologists across the world.

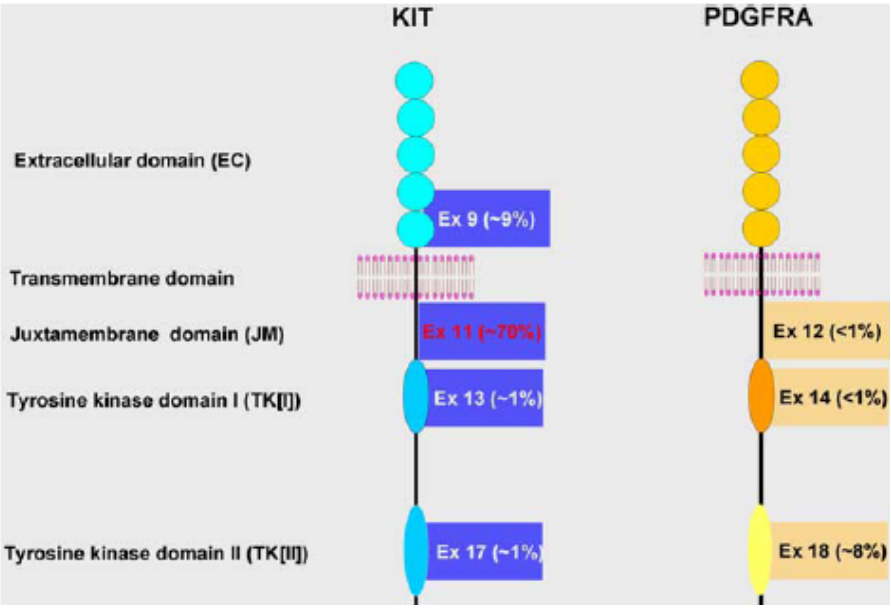
GISTs were often previously diagnosed as leiomyomas or leiomyosarcomas because of their histologic resemblance. Despite this, it had long been recognized that a subset of these tumors that arose in the bowel wall had a number of peculiar histologic features and likely represented a different entity altogether.[11] Another interesting aspect of these GI tract leiomyosarcomas were that they were exceptionally resistant to standard chemotherapy regimens compared to those arising in other anatomic sites. Modern immunohistochemical techniques developed in the 1980s helped in better differentiation of GIST from other differential diagnoses. A significant number of these tumors were noted to have absence of the characteristic muscle antigens that defined leiomyosarcomas located elsewhere in the body. Other terms were generated based on the fact that neural crest antigens such as neuron-specific enolase and S-100 could be demonstrated in GIST cells, which led to the early terminologies like plexosarcomas and gastrointestinal autonomic nerve tumors. [1]

Additional research in immunohistochemical analysis of GISTs in the early 1990s revealed that a significant proportion of these tumors expressed the CD34 antigen. [12] This antigen is well known for its presence on hematopoietic stem cells

as well as vascular and myofibroblastic cells. CD34 was initially thought to be the differentiating feature between GISTs and other spindle cell tumors of the GI tract like schwannomas or leiomyomas. However distressingly, CD34 expression was present in only approximately half of all GIST cases, and a proportion of smooth muscle and Schwann cell tumors could also express CD34. So CD34 was neither a sensitive nor a specific marker for GIST.

In 1998, Hirota et al described the expression of C-KIT protein as a feature of GISTs, further separating them from other gastrointestinal mesenchymal tumors.[2] GIST is now considered as a specific category distinct from true smooth muscle tumors and neurogenic tumors. C-KIT belongs to the class III receptor tyrosine kinases (RTKs), together with platelet-derived growth factor receptor- α (PDGFRA), colony-stimulating factor-1 receptor (CSF-1-R), vascular endothelial growth factor receptors 1 and 2 (Flt-1 and Flk-1, respectively), Flk-2, and Flt-4. The KIT and PDGFRA genes occupy a spot in chromosome 4q12.[13] The RTKs are characterized by the presence of an extracellular domain, a transmembrane domain, a juxtamembrane domain, and an intracellular domain where the 2 kinase domains are lodged [figure 1].

Figure 1



Upon binding stem cell factor, the C-KIT receptor homodimerizes and experiences conformational transformations that lead to the activation of the kinase domains. C-KIT has been attributed many physiologic functions such as cell survival, proliferation, differentiation, adhesion, and apoptosis by signaling through the MAP kinase, PI3-kinase, and JAK/STAT pathways.[13] C-KIT signaling is essential for normal erythropoiesis, lymphopoiesis, gametogenesis, and melanogenesis and for the correct development and function of mast cells. A dysfunctional activation of this RTK, therefore, has been involved in diverse neoplasias such as mastocytosis/mast cell leukemia, germ cell tumors, small cell lung carcinoma, acute myeloid leukemia, neuroblastoma, melanoma, ovarian carcinoma, and breast carcinoma, besides GISTs. [1]

The identification of a family that exhibited an autosomal dominant inheritance pattern of GIST further confirmed the oncogenic potential of mutant, uncontrollably active KIT in the pathogenesis of GIST in humans. They harbored a germline activating KIT mutation, similar to the mutations that were seen in sporadic cases of GIST on genetic analysis.[14] Often, these tumors may not present clinically until the second or third decade of life, and some even present in far advanced age. KIT mutations have also been documented in very small (less than 1 cm) GISTs that were detected incidentally and that appear morphologically benign. These findings support the hypothesis that activating mutations in the KIT protooncogene represent an early transforming mechanism in GIST oncogenesis. However, since many tumors harboring this mutation can remain small for years, there must be other key signaling steps that confer an aggressive and malignant phenotype to GIST cells. These other molecular pathways remain poorly understood. Unique elements of the downstream signaling cascades in GIST are being actively elucidated, and these appear to differ from KIT signaling in hematologic neoplasia in that the STAT5 pathway is not typically activated in GIST, whereas STAT1 and STAT3 are activated at a high level. [15]

In GISTs, KIT or PDGFRA mutations cause constitutive oncogenic signaling in the absence of their ligands. The uncontrolled RTK activity results in the activation of the PI3K-AKT and MEK-MAPK pathways accompanied by relatively low level signal transducer and activation of transcription (STAT)1 and STAT3 activation, leading to alterations in cell cycle, protein translation, metabolism, and apoptosis.[13]

There is a broad spectrum of C-KIT mutations in GISTs, ranging from 30% to 90%. The C-KIT status can constitute a prognostic factor for survival. Most of the

mutations are located in the juxtamembrane domain (exon 11), followed by the extracellular domain (exon 9), and seldom are in the kinase domains (exon 13 and 17). About 35% of GISTs lacking c- kit mutations have intragenic activation mutations in PDGFRA, the most common, located in exons 12, 14, and 18. Mutations of C-KIT and PDGFRA seem to be mutually exclusive oncogenic events in GISTs.[1]

Various types of mutation that can be found in exon 11 include missense mutations, insertions, and deletions. The distal part of KIT exon 11 can have tandem repeat mutations infrequently. These changes were mainly found in stomach and have been proposed to be associated with a quite indolent clinical course. The deletion in and around KIT exon 11 codon 557–558 is often quoted to be associated with aggressive clinical course and poor prognosis, but this is not confirmed by others. Loss of heterozygosity in the KIT locus has been associated with high proliferative activity and increased metastatic potential.[16,17]

GISTs with an exon 9 mutation are often seen with tumors of the small intestine and are frequently high-risk tumors. In the vast majority of cases, exon 9 mutations are characterized by insertion of six base pairs, a duplication of Ala and Tyr and are found in primary as well as relapsed or advanced GISTs. According to a recent study, KIT exon 13 and exon 17 mutant GISTs are slightly overrepresented among the intestinal group of GISTs, and if tumors with an exon 13 mutation occur in the stomach, they tend to be slightly larger and more aggressive than “average” gastric GISTs. The majority of KIT exon 13 and 17 mutations are substitutions and, in small intestinal GISTs, these mutations have no substantial impact on clinicopathologic features when compared to the “average” small intestinal GIST.[16]

PDGFRA mutations, identified in approximately 8% of GISTs, involve mainly (6–7%) either exon 18 (kinase activation loop) or exon 14 (ATP-binding pocket) and rarely (less than 1%) exon 12 (JM). Mutations in PDGFRA exon 14 and 18 are mostly missense mutations. The subset of GISTs with a PDGFRA mutation that is associated with a commonly benign clinical course is limited to the stomach and omentum, lack KIT expression by immunohistochemistry (IHC), and preferentially shows epithelioid morphology. GISTs with a mutation D842V in exon 18 of PDGFRA are resistant to imatinib and sunitinib.[16]

The tumor predominantly affects adults at a median age of 58 years with a slight male preponderance. GISTs can occur throughout the GI tract and are most commonly seen in the stomach (60%), jejunum and ileum (30%), duodenum (5%), colorectum (4%), and rarely the esophagus and appendix. [1] Extra gastrointestinal stromal sarcomas can occur in the omentum, mesentery, or retroperitoneum.

Clinical symptoms associated with GIST include abdominal pain, fatigue, dysphagia, satiety, and obstruction. Sometimes, patients may present with chronic GI bleeding which leads to anemia or acute GI bleeding which may be caused by erosion through the gastric or bowel mucosa or rupture into the abdominal cavity causing life-threatening intraperitoneal hemorrhage. Most of the symptomatic tumors were more than 5 cms in size. Small GISTs mainly present as incidental findings during endoscopy, surgery, or radiologic studies for other reasons, whereas patients with malignant GIST often present with disseminated disease. Vast majority of the

metastases at presentation are intraabdominal, either to the liver, omentum and peritoneal cavity. The most common site of metastases is in the liver.

Some families do have heritable mutations in KIT and PDGFRA.[14] The penetrance of these mutations is quite high, and most affected family members will develop solitary or multiple GISTs during their life span. The mean age of onset is younger than that of sporadic GISTs without gender differences. Most of these GISTs follow a benign course, and their morphology does not differ from that of their sporadic counterparts. GISTs can be part of Carney's triad (gastric GIST, paraganglioma, and pulmonary chondroma) or Carney's dyad (paraganglioma, gastric GIST), and these GISTs are often KIT/PDGFRA wild-type.[18] The genetic basis for Carney's triad is not known, although it is thought to be sporadic rather than familial. In both conditions, the presence of multiple gastric GISTs is common. The clinical features of the triad include occurrence at a young age, female predilection, tumor multifocality, slow growth, frequent metastasis, lack of response to imatinib treatment, and sometimes fatal outcome. They often affect the gastric antrum, show predominant epithelioid histology and lack the classical molecular abnormalities. So they have all the poor prognostic features of GIST.

Approximately 1-2% of GISTs occur in the pediatric age group, predominantly in the second decade. Pediatric GISTs are associated with a marked female predominance, are preferentially located in the stomach, and show mainly epithelioid morphology. Although these tumors consistently express KIT protein, the majority lack KIT or PDGFRA mutations. Unlike adult GISTs, these tumors quite often spread to lymph nodes. Interestingly, pediatric KIT wild-type GISTs lack the

typical cytogenetic deletions seen in adult KIT-mutant GISTs and progress to malignancy without acquiring large-scale chromosomal aberrations. The difference between pediatric KIT wild-type and adult GISTs of the stomach is further demonstrated by their separate clustering by gene expression profiling, and it is very likely that these tumors are a separate clinicopathologic entity.[1,19]

Time to tumor progression was significantly longer on sunitinib than on prior imatinib treatment for pediatric GIST patients, indicating that they may benefit from sunitinib as first-line treatment.[20]. In the pediatric age group, Carney's triad should be considered in any patient with GIST, especially if patients also present with lung nodules.

GISTs present most often as well-circumscribed, highly vascular tumors.[13] On gross examination, these tumors appear fleshy pink or tan-white and may show hemorrhagic foci, central cystic degenerative changes, or necrosis. There are three principial subtypes on morphological evaluation. The spindle cell subtype of GIST, accounts for about 70% of cases and is composed of cells with palely eosinophilic fibrillary cytoplasm, ovoid nuclei, and ill-defined cell borders, often with a syncytial appearance, arranged in short fascicles or whorls [Figure 2]. GIST with epithelioid cell morphology, accounting for approximately 20%, is composed of round cells with eosinophilic to clear cytoplasm arranged in sheets and nests. Finally, approximately 10% of GISTs show mixed morphology, being composed of both spindle and epithelioid cells. Variable cellularity as well as sclerotic, collagenous, or myxoid stromal changes can be seen in each subtype. Overall, GISTs are characterized as uniform and monotonous tumors.

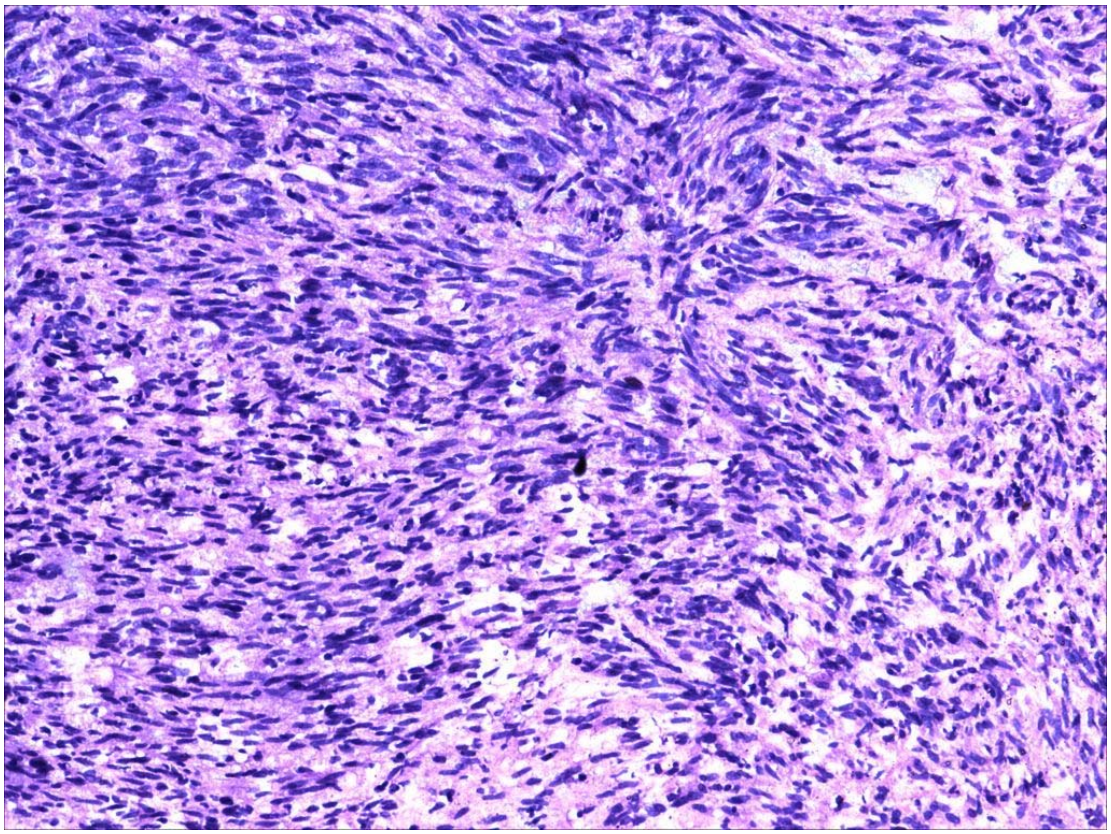


Fig.1: Sheets and fascicles of spindle cells with hyperchromatic nuclei and increased mitoses. H&E 200x

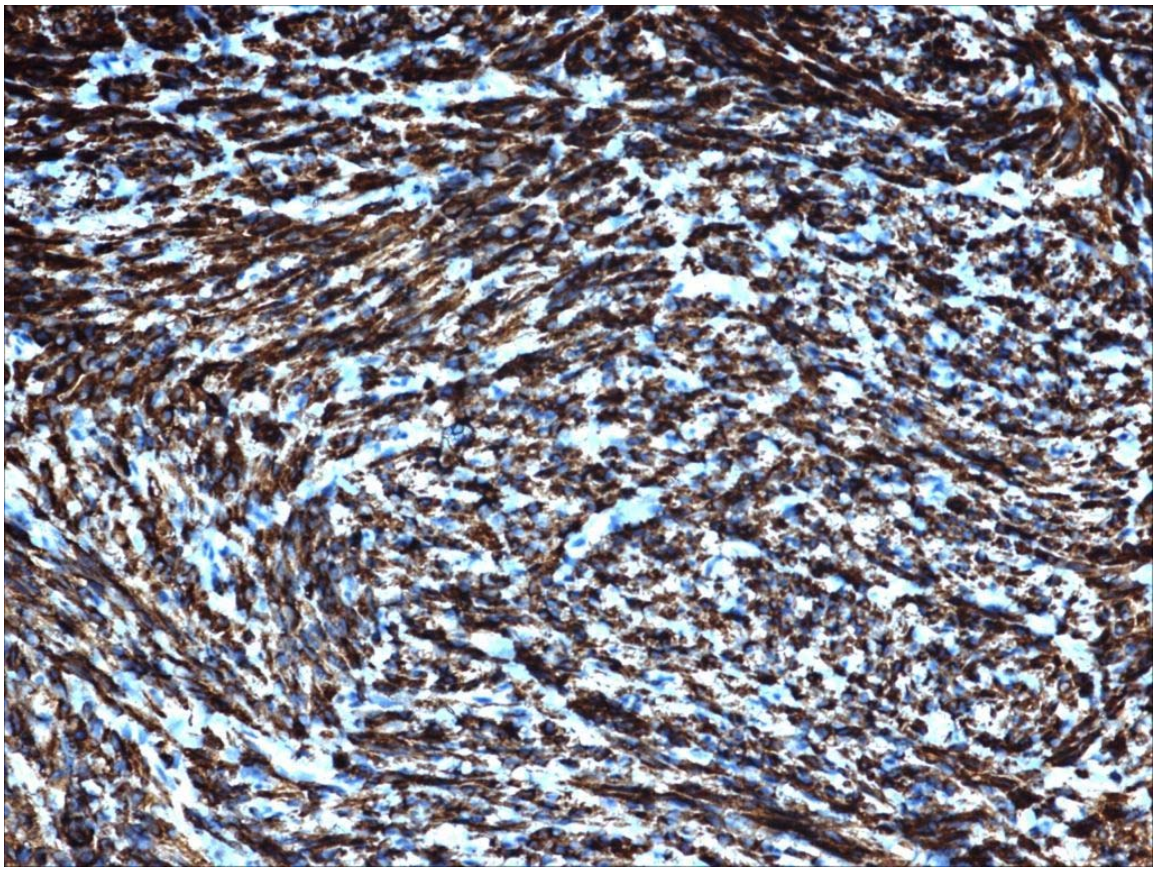


Fig.2: Tumour cells showing strong positivity for c-kit. DAB 200x

Different KIT-staining patterns can be observed in GIST.[21] Strong and diffuse cytoplasmic KIT staining often associated with dot-like staining is the most common finding [Figure 3]. A minority of cases can have exclusively dot-like or even a membranous staining pattern. The patterns of KIT staining do not correlate with the type of KIT mutation and have no impact on the likelihood of response to imatinib. However, GISTs showing weak, focal or absent KIT expression are more likely KIT wild-type. KIT-negative GISTs preferentially occur in the stomach and usually show pure epithelioid or mixed (spindle and epithelioid) cytomorphology.[22]

Other commonly expressed but less sensitive and specific markers are CD34, h-caldesmon, and SMA.[13] CD34 is expressed in approximately 80% of gastric tumors, 50% of those in the small intestine, and 95% of GISTs in the esophagus and rectum. H-caldesmon is expressed in more than two-thirds of GISTs and SMA in 30%. S-100 and cytokeratin are only infrequently expressed in GISTs. KIT negativity by no means justifies denying patients therapy with TKI (imatinib or sunitinib), as some wild-type GISTs as well as some tumors with PDGFRA mutations respond to treatment with TKI.

One promising marker is discovered on GIST (DOG1), also known as TMEM16A, which is a transmembrane protein recently shown to be up-regulated in GISTs by gene expression profiling.[23] Two recent studies have suggested that antibodies against DOG1 have greater sensitivity and specificity than KIT (CD117) and CD34, and that these antibodies could serve as specific immunohistochemical markers for GIST irrespective of the underlying KIT/PDGFRA mutation or KIT

expression by IHC. PDGFRA alpha is a receptor tyrosine kinase closely related to KIT. Antibodies to this kinase have been proposed to be of use in the identification of KIT-negative GISTs harboring a PDGFRA mutation. However the commercially available antibodies do not show marked sensitivity for PDGFRA. [13]

Despite these developments, a subset of KIT-negative tumors remains a diagnostic challenge, at least in terms of immunohistochemical verification; and mutational analysis should be strongly considered under these circumstances.

The main differential diagnoses of spindle-cell GIST that should be considered are smooth muscle tumors, desmoids, fibromatosis, schwannoma, inflammatory myofibroblastic tumor, inflammatory fibroid polyp, and solitary fibrous tumor. Smooth muscle tumors show brightly eosinophilic cytoplasm with defined cell borders rather than the syncytial appearance typically seen in GIST. Desmin expression is relatively specific for smooth muscle tumors and rarely positive in spindle-cell GISTs. Schwannomas occurring in the gastrointestinal tract typically show a distinctive peripheral cuff of lymphocytes and express S-100 protein and GFAP. Intraabdominal desmoid fibromatosis is morphologically characterized by long sweeping fascicles of fibroblastic/ myofibroblastic spindle cells set within a collagenous matrix. Immunohistochemistry reveals nuclear beta-catenin positivity in approximately 75% of cases. Inflammatory myofibroblastic tumors mainly occur in children and young adults. Inflammatory fibroid polyp (IFP) has a collagenous or more myxoid granulation tissue-like stroma containing fibroblasts in a pattern-less array and inflammatory cells, including numerous eosinophils. Perivascular fibrosis is commonly seen. The fibroblasts usually express CD34. The differential diagnosis for

epithelioid GIST includes neuroendocrine carcinoma, glomus tumor, malignant melanoma, epithelioid leiomyosarcoma, epithelioid MPNST, and clear cell sarcoma.[1,13]

The risk of metastases after resection of the primary depends on multiple risk factors. Fletcher et al proposed a risk assessment system in 2002 based on the tumor size and mitotic count. [24]A tumor size more than 5 cms and mitotic count more than 5 per 50 high power fields (hpf) predicted aggressive clinical behaviour. However it is well known that site of primary also may predict the aggressiveness of GIST. Stomach primary may have a better outcome compared to other sites. Hence site is also included in the risk assessment system after resection of the primary. This is essential to plan adjuvant treatment after resection.

Piotr Rutkowski et al analysed 232 cases of metastatic GIST in an effort to find out the possible predictive factors for response with Imatinib.[25] The estimated 3 year PFS for the entire cohort was 54% and the median PFS was 40.3 months. The following factors significantly and negatively influenced PFS in univariate analysis: poor baseline performance status ≥2, tumor genotype indicating other than *KIT* exon 11 isoform, baseline high neutrophils count, age <45 years at the diagnosis, mitotic index >10/50 high-power fields (HPF), GIST histological type other than spindle-cell, baseline low albumin level, low baseline hemoglobin level, and primary unresectable and/or metastatic lesions at presentation. They identified four factors negatively affecting PFS, statistically significant ($P < 0.05$) in multivariate analysis: baseline poor WHO performance status ¸2, high baseline neutrophils count (>5 x 10^9/l), tumor

genotype indicating the presence of non-exon 11 *KIT* mutant and mitotic index >10/50 HPF.

Generally, patients with KIT exon 11 mutant GISTs are treated with 400 mg imatinib/day, and dose escalation to 800 mg/day is recommended if patients progress on 400 mg. Clinical data did not reveal a significant benefit for KIT exon 11 mutant GISTs whether treated initially with 400 or 800 mg of imatinib. However, patients with KIT exon 9 mutations have better progression-free survival if treated with imatinib 800 mg/day than 400 mg.[7,9] This observation provides the rationale for recent consensus that KIT mutation status be evaluated routinely in inoperable GISTs, and with imatinib dose escalated immediately to 800 mg/day if a KIT exon 9 mutation is found. It is likely that the genotyping of the tumor will be routine before planning treatment for metastatic GIST. The same is extrapolated to adjuvant strategy also in an effort to tailor the duration of therapy.

Response evaluation after starting TKI often can be challenging in GIST. CT scans often underestimate the degree of benefit in patients with metastatic GIST. Patients with RECIST stable disease on imatinib fared just as well in terms of PFS as those who had an overt response to treatment. A number of problems arise when applying RECIST to GIST. Necrosis of tumor masses in the liver is frequent finding after initiation of GIST, and thus the sum-of-maximum-diameters (SPD) criterion is often not met in responding patients. Also, other lesions that are isodense with liver may become less dense and appear to be new lesions, ie, RECIST progression, when in fact it is just the opposite that is true. Occasional patients will have growth of lesions with increasing edematous tumor degeneration over time before what ultimately becomes disease that decreases in size. However since patients with stable

disease have similar outcome like that of partial remission, people have questioned usage of other evaluation systems.[26]

One of the most impressive aspects of GIST diagnostic imaging is the use of ^{18}F-fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET) to add functional imaging data that are complementary to the information obtained by conventional anatomic imaging. Choi and colleagues at M.D. Anderson Cancer Center evaluated clinical outcome by PET response and by RECIST, showing that PET provided a superior means to follow patients for clinical benefit. [27] Although CT or MRI scanning can assess the size of GIST lesions quite accurately, the functional imaging of GISTs with ^{18}FDG-PET can give additional information that can assist clinicians in the management of GIST patients. The actual mechanisms responsible for the high-level avidity of GISTs for the ^{18}FDG tracer used most commonly in PET imaging are not yet known; however, it is likely that there is a direct connection between signaling through the overactive KIT RTK and glucose transport proteins. In this way, one could explain the very rapid changes in PET imaging associated with inhibition of KIT signaling by pharmacologic means. [28,29]

Large GISTs can demonstrate centers with predominately cystic or low attenuation characteristics noted on CT or MRI scans. It is clear by ^{18}FDG-PET scans that the internal mass of large GIST lesions can often be viewed as metabolically quiescent. This is likely due to the endogenous necrosis of very large lesions in their central portions; although GIST lesions can be very vascular, the internal portion can nonetheless represent a confluent mass of necrotic material, with the more viable aspects of the GIST pushing out toward the edges of the lesion. In addition,

occasionally metastatic GIST lesions in the omentum can be subtle and easy to overlook on CT scans, because small lesions can blend into the folds of the bowel walls and be difficult for even the most experienced radiologist to detect. ^{18}FDG-PET imaging can detect lesions at least 1 cm in size without difficulty, because neither the normal bowel nor omentum takes up the ^{18}FDG tracer with excess avidity.

Metastases can quite often occur 10-15 years after initial surgery, and therefore long-term follow-up is required. Metastases develop primarily in the abdominal cavity and liver, rarely in the soft tissue and skin, and exceptionally rarely in lymph nodes or in the lung.[1] Clinically, it is essential to differentiate metastatic GIST from multifocal GISTs observed in patients with germline KIT or PDGFRA mutations, in patients with neurofibromatosis 1 and multiple sporadic GISTs, mainly occurring in the proximal stomach. The pathogenesis of multiple sporadic GISTs is poorly understood; however, these GISTs have been shown to harbor different KIT mutations in separate individual lesions from the same patient. Generally speaking, the clinical history, clinical presentation, morphology, mitotic activity and, in rare cases, mutational analysis should allow exact precise classification.

Unresectable or metastatic GIST are considered incurable and is conventionally thought to be chemoresistant. The mechanism for chemoresistance may be partly explained by increased levels of p-glycoprotein and multidrug resistance protein.[1] Till Imatinib was discovered to be active against GIST, there were not much options for these patients. Joensuu et al first reported the use of imatinib in a patient with a recurrent, metastatic GIST, a 50-year-old female whose

tumor demonstrated staining for CD117. She showed response to 400 mg of imatinib that was sustained for 11 months at the time of the publication. [30]

This paved for further phase II trials in United States and Europe. Demetri et al studied 147 patients with GIST randomised to 400 mg or 800 mg per day of Imatinib. The partial response rates reported was 54% and the median duration of response was not reached at 24 weeks.[31] This was encouraging in a disease which was considered as a death warrant all these years. FDA approved the drug for use in metastatic GIST in 2002 itself. A Phase I study by EORTC also demonstrated activity including partial responses.[32]

Two multicenter, randomized phase III studies were startedin 2001, led by the European Organization for Research and Treatment of Cancer (EORTC), which included the Australasian Gastro-Intestinal Trials Group and the Italian Sarcoma Group (n=946), and the Southwest Oncology Group (SWOG), which included the Cancer and Leukemia Group B, the Eastern Cooperative Oncology Group (ECOG), and the National Cancer Institute of Canada (n=746).[7,34] The studies had two arms comparing 400 mg/day versus 800 mg/day of imatinib in patients with advanced inoperable or metastatic GISTs. A combined analysis was performed. The patients who experienced progressive disease (PD) on 400 mg/day were allowed to crossover to the higher, 800 mg/day, dose of imatinib.

Progression-free survival (PFS) was the endpoint of the EORTC study, whereas the SWOG study evaluated overall survival (OS), with PFS as a secondary efficacy variable. RECIST criteria were used for response evaluation in both studies.

The combined analysis assessed the efficacy and safety of the two imatinib doses, and the efficacy and safety of a dose increase from 400 mg/day to 800 mg/day after progression (crossover subset), and also explored the impact of mutation status on efficacy in each of the two dose groups. For the EORTC study, the best response was either a complete response (CR), a partial response (PR), stable disease (SD), or PD. For the SWOG study, the best response was either a CR, a PR, an unconfirmed CR, an unconfirmed PR, stable/no response, increasing disease, or inadequate assessment.

The median PFS in the EORTC study was 19.8 and 24.0 months, in the 400-mg/day and 800-mg/day arms, respectively. The median overall survival was 45 months in both the 400 mg/day and 800 mg/day arms. The CR and PR rates in the 400-mg/day imatinib and 800-mg/day imatinib groups were 5.3% and 5.9%, respectively, and 44.6% and 48.8%, respectively. These differences were not statistically significant (p= .0637).

The SWOG study analysed 694 patients with metastatic or unresectable GISTs. The median follow-up time was 44.2 months. The median OS times were 55.1 months and 51.3 months in the 400-mg/day and 800-mg/ day dose groups, respectively (p= .5819). The median PFS times were 17.6 and 19.7 months, in the 400-mg/day and 800-mg/day arms, respectively. The CR and PR rates in the 400-mg/day imatinib and 800-mg/day imatinib groups were 5.2% and 3.7%, respectively, and 48.1% and 49.0%, respectively (p= .2826). In the combined EORTC– SWOG dataset of 1640 patients, the median PFS time was longer by 4.3 months in patients receiving 800 mg/day of imatinib compared with patients receiving 400 mg/day of imatinib. There was no difference observed between the two dose groups with respect to OS. The median OS

time was 48.8 months in both dose groups. Overall, 5.1% of patients achieved a confirmed CR and 47.5% achieved a confirmed PR.

In the combined EORTC–SWOG analysis, the majority of imatinib-treated patients experienced adverse reactions at some time. The most frequently reported adverse reactions were edema, fatigue, nausea, abdominal pain, diarrhea, rash, vomiting, myalgia, anemia, and anorexia. Most reactions were of mild-to-moderate severity. Adverse reactions mandated drug discontinuation in 89 patients (5.4%). Overall, the incidence of all grades of adverse reactions and the incidence of severe adverse reactions (Common Terminology Criteria grade > 3) were similar between the two treatment arms except for edema, which was reported more frequently in the 800 mg/day group. There were five grade 5 adverse events in patients receiving 400 mg/day of imatinib and 10 in patients receiving 800 mg/day of imatinib. Three deaths, all in patients receiving 800 mg/day of imatinib, were considered by the investigator to be related to imatinib treatment, including liver dysfunction in one patient, cardiac arrhythmia in one patient, and tumor hemorrhagic necrosis in one patient.

The optimal duration of imatinib therapy for patients with metastatic GIST remains somewhat uncertain, but most experts consider kinase inhibition as lifelong therapy for advanced disease. Studies in which patients have interrupted imatinib dosing have reported that disease progression often follows shortly after the imatinib is stopped.[35]. Therefore, for GIST patients who achieve any measure of disease control, continued dosing with imatinib as long as the disease is not progressive appears to be the optimal course of management.

Primary resistance has been observed with all genotypic subtypes of GISTs; however, the tumors that are most likely to show primary resistance include those that are KIT and PDGFRA wild type, those that have a KIT exon 9 mutation, and those that have a PDGFRA D842V substitution.[33] The latter can be explained by intrinsic biochemical resistance of the D842V mutation to imatinib.[36] In patients with KIT exon 9-mutant tumors, inadequate dosing may account for some of the primary resistance observed. It appears that exon 9 mutations generate a kinase conformation that is less amenable to imatinib binding. In patients lacking identifiable PDGFRA or KIT mutations, one potential mechanism for resistance is a mutation in an alternate signaling pathway. Recently, several groups have identified BRAF exon 15 activating mutations in wild-type GISTs from both imatinib-naive and -resistant patients. [37]

Acquired kinase mutations are now recognized as the most common mechanism of secondary imatinib resistance. The resistance may manifest in a number ways, including growth of a nodule within a pre-existing, clinically quiescent lesion, the development of one or more new nodules, or widespread expansion of lesions throughout the liver or abdominal cavity.[38]

Unlike primary resistance, delayed imatinib resistance is associated most often with the expansion of tumor clones with secondary KIT or PDGFRA mutations.[39] Analysis of tumors of patients who progressed on the phase II B2222 imatinib trial revealed that 67% of patients with secondary resistance had tumor clones with one or more secondary kinase mutations. All secondary KIT kinase mutations were found in tumors with an underlying primary KIT mutation, and the only secondary PDGFRA mutation identified arose in a PDGFRA-mutant GIST. The secondary KIT mutations

involved either the ATP binding pocket of the kinase domain (exons 13 and 14) or the kinase activation loop (exons 17 and 18; Fig. 1). No secondary mutations were identified in post-imatinib samples that lacked a primary mutation (wild-type GISTs).[40]

Imatinib currently remains the standard first-line treatment option for patients with unresectable and metastatic GISTs, especially those harboring an exon 11 mutation. However, accumulating evidence suggests that sunitinib could be effective as a first-line treatment for GISTs harboring KIT exon 9 mutation and for KIT/PDGFRA wild-type GISTs (including pediatric GISTs).[9] Sunitinib is effective against secondary imatinib-resistance mutations in the ATP-binding pocket. However, the substantial heterogeneity of resistance mutations highlights the therapeutic challenges involved in salvaging patients, especially after clinical progression on TKI monotherapies. The toxicity concerns of Sunitinib is again a reason for considering Imatinib only as first line treatment. The role of newer generation KIT and PDGFRA kinase inhibitors (e.g., nilotinib, dasatinib, etc.) remains to be determined in GIST patients who are multiply resistant, i.e., after imatinib and sunitinib treatment, to TKIs. Nilotinib has been shown to be effective in advanced imatinib- and sunitinib-resistant GISTs. Using nilotinib 400 mg twice a day, the median progression-free survival and the median overall survival were 3 and 11 months, respectively.[41] In vitro data, using cell lines expressing imatinib resistant PDGFRA (D842V) mutants, suggest that dasatinib, a dual SRC/ABL kinase inhibitor, and IPI-504, a heat shock protein 90 inhibitor, may be a therapeutic option for patients with a GIST harboring the PDGRA (D842V9) mutation.[42]

Hence the standard approach if there is tumour progression on 400 mg is to increase the imatinib dose to 800 mg daily. Dose escalation may be useful in the case of a KIT exon 9 mutated GIST also. As mentioned, the possibility of false progression on imaging should be ruled out. Patient non-compliance also should be ensured in such cases. The standard second line agent is sunitinib as of now. If they fail on sunitinib, then should be considered for participation in a clinical trial of new therapies or new combinations.

It is needless to mention about the importance of compliance to treatment in order to sustain the response. Close monitoring of tumour response should be continued throughout treatment, since the risk of secondary progression persists over time.[43] Retrospective data suggest that suboptimal plasma levels of imatinib are associated with a worse outcome. Further studies are needed.

Histone deacetylase inhibitors (HDACI) alone or in combination with imatinib show inhibition of cell proliferation, KIT activity and expression as well as activation of downstream pathways in KIT-positive cell lines, providing preclinical evidence that HDACI may expand the treatment options in KIT-positive GISTs.[44] IGFR inhibitors in combination with imatinib have been proposed as a treatment option mainly for wild-type GISTs which tend to be less responsive to imatinib-based therapies. The rationale for this treatment is based on detected amplification of IGF1R and protein overexpression predominantly in WT and pediatric GISTs. [45]

The frequent occurrence of secondary mutations led some investigators to think about combining drugs in an effort to target multiple steps in the pathogenesis.

Some of the drugs inhibit both C-KIT and PDGFRA. These include Dasatinib, Sorafenib, Motesanib etc. The feasibility of combining these drugs with Imatinib has been tried in Phase I/II trials with moderate success. The toxicity profile was reasonable. Another interesting way of interfering with the C-KIT pathway is to target the downstream signalling pathways like mTOR. Phase I/II studies demonstrated the excellent activity of the combination. However it remains to be seen which subset of patients will be benefitted by this interesting combination.

Surgery is the mainstay of curative therapy in primary GIST and has traditionally played a palliative role in the advanced disease setting. In the era of targeted therapy, the role for surgery as a part of multimodality management of advanced GISTs has been looked at in small patient series and retrospective studies. The rationale behind resection of metastases is to eliminate tumors from which drug-resistant clones might develop. A phase 2 study by the Radiation Therapy Oncology Group studied the role of preoperative imatinib followed by surgery in patients with primary locally advanced disease or with recurrent/metastatic disease. Patients with locally advanced disease received 2 years of postoperative imatinib and those with metastatic disease were continued on imatinib until progression. Patients with locally advanced disease and metastatic disease had a PFS of 82% (95% CI 68–97) and 73% (95% CI 54–91), respectively at 2 years, which suggests a benefit to surgical debulking in advanced disease.[46]

Surgical management of residual disease can be discussed in two settings. One is in the setting of progressive disease after Imatinib where most of the patients have secondary mutations. Surgery may have a role especially when second line agents are

not available for the patient. The second setting is in surgery of limited progression. Non-surgical procedures (local treatment, such as ablations, etc.) may be selected. The most controversial role of surgery is in the setting of a responding patient with residual disease. Now that the PFS is reaching 2 years in many studies, it is natural for someone to question its role. An ongoing EORTC trial (EORTC 62063) is addressing this controversial issue. Till then, this may be used only for some selected patients on an individual basis. Even if surgery is performed, it is essential to continue Imatinib lifelong postoperatively.

Based on the excellent activity in the metastatic setting, it was quite natural that imatinib was tested as an adjuvant treatment after complete resection of primary GISTs.[47] After complete resection of a primary KIT positive GIST which is 3 cm or larger in size, the patients were randomly assigned to take 400 mg imatinib or placebo daily for 1 year. The interim analysis showed significant improvement in recurrence-free survival and hence the accrual was stopped early. Imatinib significantly improved RFS compared with placebo (98% compared with 83% at 1 year. U.S. Food and Drug Administration (FDA) approved imatinib as an adjuvant treatment for GISTs based on this excellent result.

Although this study clearly demonstrates that empiric adjuvant imatinib reduces the rates of early recurrence, it is not yet clear whether this strategy improves overall survival over a strategy of watchful waiting. Given the strong effect of genotype on imatinib response in the metastatic setting, we hypothesize that tumor genotype will influence the efficacy of imatinib in the adjuvant setting. The recurrence rate in the imatinib group was noted to increase appreciably around 18 months after surgery, raising the concern that 1 year of therapy may be inadequate for

patients at high risk for recurrence. Analysis of the correlation of tumor genotype and mitotic index—as well as other clinicopathological factors—is underway. These data may define a potential role for genotyping of primary tumors and optimization of postsurgical therapy and/or surveillance strategies.

It is critical to emphasize the importance of multidisciplinary management in the care of GIST patients. For optimal management of metastatic disease, medical oncologists, surgeons, radiologists, and nuclear medicine imaging experts must all collaborate closely to determine the best course of action for any given patient. This important message has been emphasized in the Task Force Report on GIST Clinical Practice Guidelines of the National Comprehensive Cancer Network.[96] For example, disease that is initially judged as unresectable may become amenable to surgical excision after a major response induced by imatinib therapy. Most centers recommend surgical resection for such patients because it is feared that residual GIST may develop secondary mutations that could result in clinical resistance to imatinib and progression of disease. However, the role of surgery as an adjunct to imatinib therapy for patients for metastatic GIST remains unclear.

MATERIALS AND METHODS

The present study was conducted in a retrospective fashion. The case records were retrieved from the tumor registry of Cancer Institute. We found that 30 patients were initiated on Imatinib after diagnosis of metastatic GIST between January 2002 and December 2007. However 6 patients were treated elsewhere after Imatinib initiation and hence the follow up details were not available. These patients were excluded from the final analysis. The case records of remaining 24 patients were analysed for clinical profile, treatment response and prognostic factors.

Patients received treatment with imatinib at the standard dose of 400 mg/day, which was continued until there was disease progression, unacceptable toxicity, or patient refusal. Adequate hepatic and renal functions were mandatory for all patients before starting Imatinib. Hemogram was monitored at each visit and liver/renal function tests were requested as and when required. Patients were re-evaluated every 12 weeks by computed tomography (CT) or by Ultrasound, with tumor response determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) wherever feasible.

The response assessment was done by Ultrasound examination in many patients and hence the definitions could not be applied on a stringent basis. A partial response was defined as > 50% reduction in the maximum diameter of lesion documented in original CT scan and no appearance of new lesions. A complete response was defined as total disappearance of all lesions. Stable disease patients had

25-50% reduction in size of existing lesions. Progressive disease patients have new lesions or > 25% increase in size of existing lesions.

Dose was increased in all patients documented to have progression. Toxicities were carefully monitored in all patients and graded as per the National National Cancer Institute Canada (NCIC) criteria.

The diagnosis of GIST was established from Trucut biopsies from the abdominal mass, endoscopic biopsies or from the specimen of primary surgery. All patients had histological confirmation with CD 117 expression by immunohistochemistry. The samples were archived in the pathology department of Cancer Institute as paraffin blocks. The same were retrieved for c-kit mutation analysis. Paraffin embedded blocks were not available for 5 patients and hence the c-kit genotype could not be done in them. Informed consent was obtained from all the patients whose samples were sent for mutation analysis.

C-KIT Mutation Analysis in GIST

Formalin fixed paraffin embedded tumor tissue blocks were collected from 19 cases of GIST. An H & E slide of the block was studied and was used to collect tumor cells from areas containing more than 60% malignant cells. The complete specimen was used in cases where a CT guided core biopsy block was provided. DNA was extracted from the sample using Proteinase K digestion followed by silica cartridge isolation procedure. DNA was quantitated using Smartspec 3000 spectrophotometer

(Bio-Rad). The quality of DNA was ascertained by resolving on a 0.8% TBE Gel. The purified sample was stored at -80^0C till further processing.

PCR, Sequencing and Analysis

The purified genomic DNA was amplified in two separate PCR reactions targeted at amplification of Exons 9 and 11 of C-KIT gene. The placement of the Primers was such that it ensured amplification of the complete exons. Briefly, for each reaction 30-50ng of DNA was taken in a 0.2ml PCR tube. To this was added, 200nM each of Forward primer and Reverse primer and 12.5ul of 2X Phusion Flash

Master Mix (Finnzyme), in a total reaction volume of 25ul. After an initial denaturation at 98^0C for 3min, the PCR was run for 40 cycles at conditions: 98^0C for 1min; 62^0C for 1min; 72^0C for 1min followed by a final extension at 72^0C for 5min. The amplicon was resolved on a 3% gel to ascertain the specificity of amplification. The product was purified using AxyPrep PCR cleanup Kit supplied by Axygen as per the manufacturer's instructions. Sequencing of PCR products was carried out in both directions on ABI 3130xl Genetic Analyzer using standard chemistries. The sequences were compared against human genome sequence (NCBI accession NT_022853) using BLAST software to identify the mutations. The chromatograms were also analyzed manually using SeqScape v2.6 software.

STATISTICAL ANALYSIS

SPSS version 13.0 (SPSS Inc) was used for statistical analysis. Log rank test was used for univariate analysis. Cofactors investigated in the analysis included age, gender, performance status, primary site of disease, previous treatment, tumor size, sites of metastases, baseline laboratory parameters, time from diagnosis to start of Imatinib, presence of bleeding at presentation, anemia and tumor genotypes. Multivariate analysis was not attempted in view of the limited patient population. The entire study population was included in survival analysis. However c-kit genotype relationship with outcome was assessed for only 19 patients for whom the genotypic information was available.

Kaplan Meier survival plot was used for estimating the progression free survival (PFS) and Overall survival (OS). PFS was measured from the first day of imatinib treatment to disease progression or death resulting from any cause and OS was measured from the first day of treatment to death resulting from any cause.

RESULTS

There were 24 cases of metastatic GIST available for analysis. Hence the data of those 24 patients are presented here. The first patient was enrolled in 2002.

The median age of the study population was 56 years (Range 26 – 76 years). Males were more frequently affected. (Figure 4) The male : female ratio was 3:1.

Figure 4

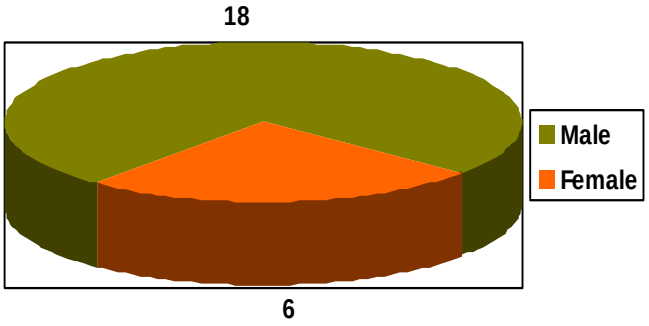


Table 1: SYMPTOMS AT PRESENTATION

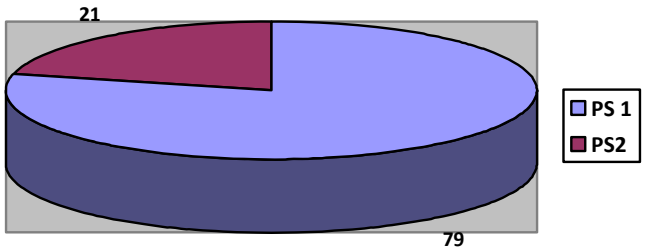
Symptoms	**n (%)**
Abdominal pain	15 (62.5%)
Bleeding PR	5 (20.8%)
Mass abdomen	2 (8.3%)
Others	2 (8.3%)

The mean duration of symptoms before making a diagnosis of GIST was 4.6 months. Only five patients had symptoms more than 6 months.

Table 2: COMORBIDITIES AT PRESENTATION

Comorbidities	**n (%)**
Hypertension	2 (8.3%)
Diabtetes Mellitus	5 (20.8%)
Ischemic Heart Disease	1 (4.1%)

Figure 5: Performance status at presentation



Twelve patients (50%) had metastases at diagnosis. Metastases were delayed beyond 2 years of primary diagnosis in 8 patients. Surgery was offered to two cases after diagnosis of primary. Rest of them were managed only with Imatinib. Anemia was present in 12 (50%) of the patients. Most of the patients had clinically palpable mass. None of the patients had clinically palpable lymph nodes.

The median size of the primary tumor was 10 cms. The size was > 5 cms in 20 (80%) of the patients. The median size of the metastatic tumor was 3 cms (range : 1.4 cms – 20 cms). Most of the patients had more than 2 metastatic lesions.

Table 3: SITE OF PRIMARY DISEASE

Primary site	**n (%)**
Stomach	11 (45.8%)
Small Intestine	6 (25%)
Rectum	5 (20.8%)
Colon	1 (4.1%)

Table 4: SITE OF METASTASES

Site	**n (%)**
Liver alone	17 (70.8%)
Omentum alone	2 (8.3%)
Lung alone	1 (4.1%)
Multiple	4 (16.6%)

Liver and lymph nodal metastases were seen in 2 patients (8.4%). Metastatic lesions were seen in liver, lung and omentum in one patient and in liver, lung and lymph nodes in another patient. Isolated omental deposits were seen in two patients and that in lung was seen in one patient. Hence, liver was the most common site of metastases, isolated or seen with other sites, seen in 87.5%.

Spindle cell subtype of GIST was present in 20 (83.3%) patients. One patient had mixed type and in other three, epitheliod variety was seen. The mitotic rate was > 5/50 hpf in 19 patients.

After the diagnosis of metastatic GIST was made, Imatinib was initiated after a delay of 1 month or more in 11 patients.

Table 5: INITIAL RESPONSE TO IMATINIB

Response	**n (%)**
Complete Response (CR)	1 (4.1%)
Partial Response (PR)	6 (25%)
Stable disease (SD)	10 (41.6%)
Progressive disease (PD)	7 (29.1%)

The median time to documentation of maximum initial response was 6 months. Of the seven patients who progressed on initial Imatinib, five died of disease. Dose escalation response was seen in 2 out of 7 patients (28.5%).

Table 6: STATUS AT LAST FOLLOW UP

Status	**n (%)**
Complete Response (CR)	1 (5.2%)
Stable disease (SD)	14 (73.6%)
Progressive disease (PD)	4 (21%)

Table 7: IMATINIB DOSE AT LAST FOLLOW UP

Dose	n (%)
400 mg	14 (58.3%)
600 mg	8 (33%)
800 mg	2 (8.3%)

DISEASE OUTCOME

At a median follow up of 29 months, the PFS was 45% at 2 years. The OS was 78% at 2 years. Hence a significant proportion of patients responded to higher doses of Imatinib. The Kaplan Meier survival plots for EFS and PFS are demonstrated in Figure 6 & 7.

Figure 6

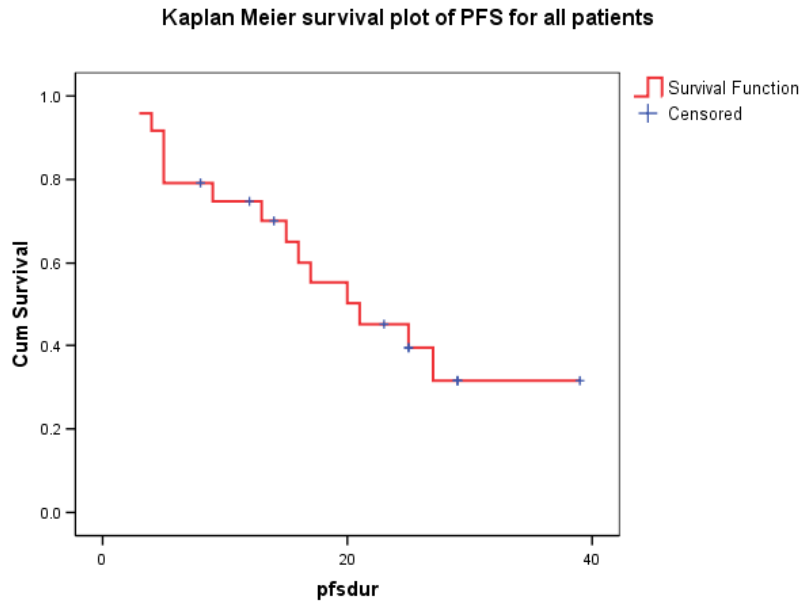
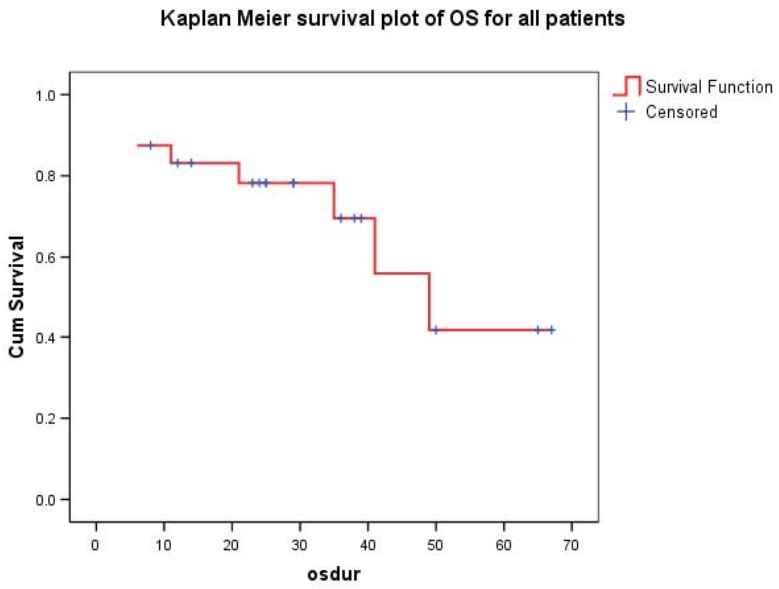


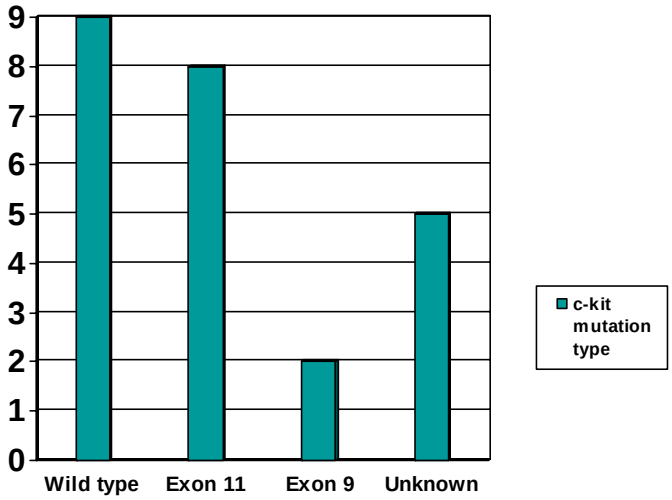
Figure 7



C-KIT MUTATION ANALYSIS

Paraffin blocks were available for 19 patients for analysing c-kit mutation status. All samples yielded DNA of amplifiable quality and were processed further. Activating c-kit mutations were detected in 10 cases (52.6%). 80% of the mutations were located in Exon 11 and 20% were present in exon 9. Nine patients had a wild type of c-kit on analysis. The c-kit mutation status of all patients is described in Figure 8.

Figure 8



The most common mutations described are deletions. Among all patients with exon 11 mutations, 6 had deletions. Two patients had substitution mutations. The two patients who had this type of mutations were found to have Val560 Asp and . Structurally, almost all exon 9 duplications are identical 1525_1530dupGCCTAT leading to Ala502_Tyr503dup at the protein level. Both of our patients with exon 9 mutations had similar finding. Interestingly both of them had intestinal GISTs.

Table 8: Correlation of c-kit status with outcome

Parameter	Exon 11	Exon 9	Wild type
Site of disease			
Stomach	4	-	5
Small Intestine	2	2	2
Others	2	-	2
Response to Imatinib	1	-	-
CR	-	-	-
PR	5	-	4
Stable	2	2	5
PD			
Median PFS (months)	21	5	12
Median OS	38	0	13

As an illustrative example, the sequence from a patient exhibiting wild type or Normal status of Exon 11 is shown in Figure 9. Figure 10 shows Exon 11 C-KIT sequence of a patient with a deletion 555-559 mutation; which was the most commonly observed mutation in this study.

Figure 9

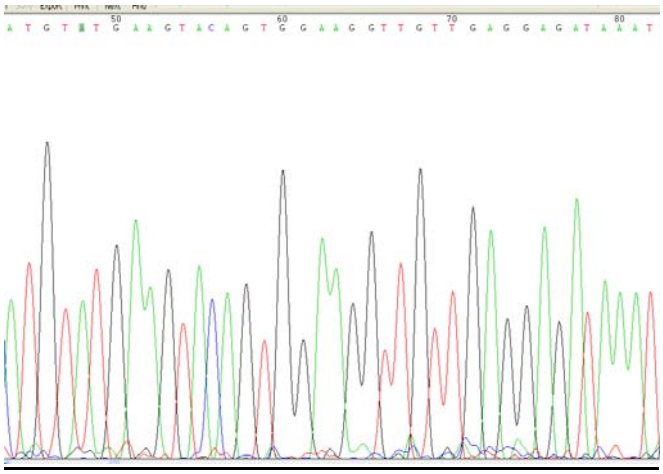
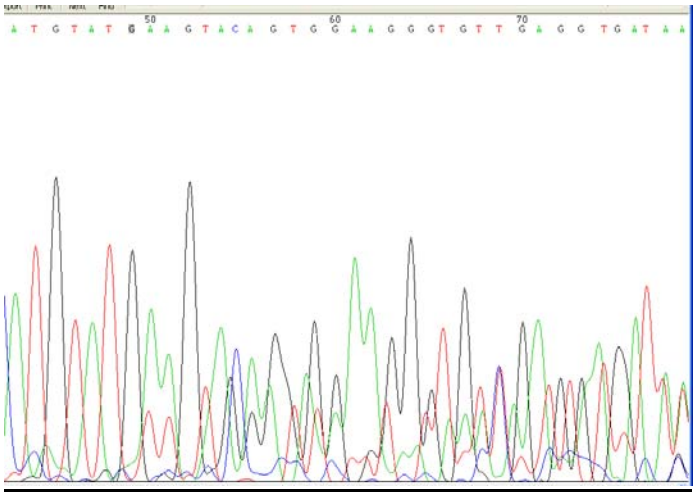


Figure 10 del 555- 559



Though the study population was small, the following factors were attempted to find out the predictive factors for overall and progression free survivals. These include age (>50 years vs < 50 years), sex, duration of symptoms, performance status at admission, anemia at presentation (Hb < 10 g/dl), site of disease, sites of metastases, histopathological type, time duration to start Imatinib and c-kit mutation status.

Of all the factors analysed for **overall survival**, those found to be significant were **presence of anemia (p=0.006) and performance status (p=0.02).** Patients with liver metastases had a better PFS compared to other sites of metastases. The 2 year PFS was 80% vs 67% (p=0.04). **For PFS, anemia was found to be significant predictor (p=0.02). The c-kit mutation status was also found to be significant predictor for PFS.** 2 year PFS for patients with exon 11 mutation and wild genotype were 58% and 31% respectively (p=0.03).

Table 9: Univariate analysis for factors which predicted PFS

Factor at baseline		**No of patients**	**No of events**	**2 yr PFS**	**p value**
Age	< 60 years	14	8	47%	0.7
	> 60 years	10	6	40%	
Sex	Male	18	11	45%	0.86
	Female	6	3	44%	
Tumor size	< 10 cms	5	3	30%	0.5
	> 10 cms	19	11	48%	
Site of disease	Stomach	11	6	51%	0.81
	Small Intestine	6	4	40%	
	Colorectal	7	4	38%	
Site of metastases	Liver	21	12	76%	**0.05***
	Other	3	2	66%	
Anemia	Yes	12	10	55%	**0.02***
	No	12	4	33%	
c-kit type	Exon 11	8	4	58%	**0.03***
	Exon 9	2	2	0%	
	Wild type	9	6	31%	

* p = <0.05 is significant

Table 10: Univariate analysis for factors which predicted OS

Factor at baseline		**No of patients**	**No of events**	**2 yr OS**	**p value**
Age	< 60 years	14	3	78%	0.52
	> 60 years	10	5	71%	
Sex	Male	18	7	76%	0.68
	Female	6	1	83%	
Site of disease	Stomach	11	3	80%	0.2
	Small Intestine	6	2	66%	
	Colorectal	7	3	57%	
Performance status at presentation	PS ≤ 1	19	5	88%	**0.02***
	PS ≥ 2	5	3	40%	
Anemia	Yes	12	8	58%	**0.002***
	No	12	0	100 %	
c-kit type	Exon 11	8	1	87%	0.18
	Exon 9	2	2	0%	
	Wild type	9	5	62%	

* p = <0.05 is significant

The percentage of patients responding to Imatinib (at least PR) was higher in patients with Exon 11 mutations (57.4%) compared to those with Exon 9 (0%) or those with wild type (20%). Both the patients with Exon 9 who progressed on Imatinib 400 mg were attempted 600 mg. Both patients progressed and expired later.

A total of nine deaths occurred in the entire patient group. The c-kit status was unknown in 3 (33.3%) of these patients. 2 patients had either exon 11 or exon 9 mutation. However the majority of the deaths were in wild c-kit type.

Table 11: TOXICITY & TOLERANCE TO IMATINIB

Toxicity (Any grade)	**n (%)**
Myalgia	20 (83.3%)
Hypopigmentation	18 (75%)
Dyspepsia	12 (50%)
Hemorrhage	-
Myelosuppression	-

Imatinib treatment was well tolerated by all patients. None of the patients required stoppage or dose reduction because of toxicity. None of them developed febrile episodes or dermatological toxicity other than hypopigmentation. Hepatic, cardiac or renal dysfunction was not reported in any patient. None of the patients had Grade 3 or Grade 4 toxicity with 400 mg per day dose. However when the dose was hiked to 600 mg per day, four patients complained of myalgia Grade 3 and increased dyspeptic symptoms.

DISCUSSION

The discovery that GIST cells express KIT, a receptor tyrosine kinase (RTK) growth factor receptor has changed dramatically the management of GISTs. [1] KIT is not only expressed, but is mutated in 85-90% of cases leading to constitutive activation of the receptor. Exon 11 mutations are the most common, followed in frequency by exons 9, 13, and 17. [4] A subset of wild KIT tumors have mutations in platelet derived growth factor alpha (PDGFRA). The so-called wild-type (WT) GIST do not contain mutations in either KIT or PDGFRA. The development of effective targeted therapies using small molecules like imatinib and sunitinib that are tyrosine kinase inhibitors (TKI) is stemmed on the discovery of expression of KIT and the understanding that KIT or PDGFRA are constitutively activated in GIST.

The median age of our study group was 56 years which is similar to other reported studies.[31,49] The age of presentation has not varied significantly across ethnic groups. However there was significant sex predilection with males 3 times more commonly affected than females in our group. This is more than what is mentioned in the literature. [7,49,51] The performance status was poor (≥ 2) in 79% of our patients. This certainly has added to the poor treatment outcome.

Stomach was the predominant site of primary disease followed by small intestine which was affected in 25% of the patients. This distribution is similar to what is described in literature.[1] Similarly we found that liver is the predominant site of ,metastatic disease. Rare sites of disease were found at relapse like bone and lung.

Lymph node involvement is quite rare in GIST. However 4 of our patients had lymph nodal metastases. Epitheloid histological variant was present in only 1 patient. Otherwise majority had spindle cell subtype only.

The feasibility of testing the c-kit mutation status on EUS guided FNA sample was demonstrated by Gomes et al. Of the 33 GIST cases, 19 patients had exon 11 mutation and 1 had exon 9 mutation.[48] Most of our patients had trucut biopsies or endoscopic biopsies.

We came across early progression in 4 patients (17%). Three of them had wild type of c-kit. In the landmark paper which made Imatinib the standard of care in metastatic GIST, Demetri et al treated 147 patients with either 400 mg or 600 mg.[31] They had few patients who went for early progression. This accounted for 13% of the study population.

In our study, though the 2 year PFS was poor (41%), the overall survival was 78%. This should be possibly because the patients responded to higher dose of Imatinib. Blanke et al also failed to show superiority of higher dose of Imatinib in inducing better responses. [7] After progression on standard dose imatinib, 33% of patients who crossed over to the high-dose imatinib regimen achieved either an objective response or stable disease. The genotyping of our patients will further help in deciding on who should get higher dose of Imatinib upfront rather than waiting till the disease progresses and then dose is escalated.

Verweij J et al reported higher PFS in patients who were randomised to 400 mg twice daily.[34] However to enjoy a higher PFS, patients had to face more toxicity and treatment interruptions. This again underlines importance of genotyping of tumors for dose selection.

The C-Kit mutations were seen in only nearing half of the samples tested (52.6%). This is in striking contrast to the published literature. . However we found that c-kit mutation status significantly predicted PFS in our patient group. Kim et al analyzed the relationship between treatment outcome and kinase mutational status in 113 Korean patients with advanced GISTs treated with Imatinib. [49] KIT mutations were found in exon 11 (n = 92, 81.4%) and exon 9 (n = 10, 8.8%). One patient had a PDGFRA exon 18 mutation. The overall mutation rate was 91.2%. With a median follow-up of 49.0 months, the median progression-free survival (PFS) time was 42.0 months and median overall survival (OS) time was not reached. PFS and OS times did not differ significantly according to KIT genotype. They concluded that compared with previous studies in western populations, these results suggest that ethnic differences may influence the relationship between KIT genotype and clinical outcome to imatinib

Anemia and performance status at presentation significantly predicted survival in our patients. A study from China assessed the outcome of 327 cases of GIST treated between 1988 and 2007. [50] Though the study period includes the time when GIST was not properly defined, a median survival of 59 months was quoted for those treated with Imatinib vs 30 months for those not treated after a recurrence or

metastases. The surgical margins and Ki 67 were independent prognostic factors for survival.

Because of the small number of patients with c-kit mutations, we could not really correlate responses with c-kit status. Yeh et al assessed 54 GIST patients in Taiwan. [51] The mutation rate for KIT was 90.7%, and included 40 patients with KIT exon 11 mutations and nine patients with KIT exon 9 mutations. Although response rates to imatinib tended to be higher in patients with KIT exon 11 mutations (57.5%) or no kinase mutations (60.0%) than in those with KIT exon 9 mutations (22.2%), the OS of these three subsets did not differ. Similar to the above study, OS did not differ in our population also.

In a nation-wide study in Iceland, Tryggvason G et al evaluated all 55 GIST patients diagnosed between 1990 and 2004. [52] Mutations were found in 52 tumors representing a 92.9% mutational rate which is higher than in our study. Most of the mutations were found in c-kit exon 11 (76.8%), followed by c-kit exon 9 (10.7%) as expected. PDGFRA mutations were only found in three tumors. No correlation of mutation type with biologic behavior was found. This study again contrasts with our patient group in that the c-kit mutation rate is lower and the same predicted PFS.

Heinrich et al analysed mutations of c-kit or PDGFR in 127 patients in a Phase II study. [33] Activating mutations of KIT or PDGFRA were found in 112 (88.2%) and six (4.7%) GISTs respectively. Exon 11 mutations were seen in 85 patients. In patients with exon 11 KIT mutations, the partial response rate (PR) was 83.5%, whereas patients with tumors containing an exon 9 KIT mutations or wild type had

PR rates of 47.8% and 0% respectively. Patients whose tumors contained exon 11 KIT mutations had a longer event-free and overall survival than those whose tumors expressed either exon 9 KIT mutations or had no detectable kinase mutation.

In the era on Imatinib therapy for GIST, various prognostic factors are described in literature for GIST. In our patient population, anemia and wild type c-kit mutation status predicted poor PFS. Patients with liver metastases had a better PFS compared to other sites of metastases. Zhu et al also suggested that liver metastases is not a adverse prognostic factor in Imatinib era.[53] Heinrich et al suggested that exon 11, poor performance status, Imatinib dose and unknown mutational status predicted outcome.[7] Kim et al found that poor performance status is the predominant factor which predicted poor outcome. [49] Poor performance status, low albumin, male sex and high neutrophil count were significantly associated with worse outcome in a study by Blanke et al.[7]

Kim et al suggested that there may be an ethnic difference between western and Asian populations in whether the type of KIT mutation is associated with clinical outcomes to imatinib.[49] The poor sample size makes interpretation of these studies difficult. However, there exists a possibility of different genotype pattern of GIST in Asian population.

The 2 year PFS in patients with exon 11 mutation was 58% in our study. However the same was only 16% in those patients who had a wild type of c-kit. This suggests that a mere sampling error cannot explain the poor outcome of those patients

with wild type c-kit. Hence the genotype of our patient population may be truly different.

Table 12: COMPARISON OF PRESENT STUDY WITH SIMILAR STUDIES

Parameters	**Kim et al [49]**	**Blanke et al [7]**	**Yeh et al[52]**	**Our study**
No of patients	113	746	64	24
Median age	57 years	61.9 years	58.8 years	56 years
c-kit status Overall Exon 11 Exon 9	 91.2% 81.4% 8.8%	 91% - -	 90.3% 74% 16%	 52.4% 80% 20%
c-kit predicted PFS	No	No	NA	Yes
Prognostic factors for PFS	Poor PS	Poor PS, anemia, high neutrophils	NA	Anemia, c-kit status
PFS	42 months	18 months	-	17 months
OS	Not reached	55 months	48 months	29 months

It was interesting to note the type of mutations in our patient population. We have evaluated only for the two most common mutations ie exon 11 and exon 9 mutations. Deletions are the most common mutations seen in GIST and almost always occur in exon 11. Of 8 patients who had exon 11 mutations, all except two had deletions. The most common deletion described is 1690_1695delTGGAAG (Trp557_Lys558del) which was seen in 5 of our patients. This particular mutation is associated with more malignant behaviour especially in gastric GISTs.[16,17] Single nucleotide substitutions are next most common mutation and are associated with more

indolent behaviour. The most common missense mutations identified in GISTs are Val559Asp, Val560Asp, Trp557Arg, Val559Ala, Val559Gly and Leu576 Pro. The two patients who had this type of mutations were found to have Val560 Asp and Trp557Arg.

Exon 9 mutations are well known for the occurrence in intestinal GIST (upto 90%) and also to the response to higher doses of GIST. We had two patients with exon 9 mutations. Duplications are the third most common KIT mutations in GISTs. This is more commonly seen with exon 9 mutations. Structurally, almost all exon 9 duplications are identical 1525_1530dupGCCTAT leading to Ala502_Tyr503dup at the protein level. Both of our patients with exon 9 mutations had similar finding. Interestingly both of them had intestinal GISTs.

Nearing 50% of our study population had wild type of mutations. This is very high compared to the existing literature. Hence this needs to be confirmed in a larger patient population. We did not have the facility to perform tests for rare mutations. The rare mutations like exon 13, exon 17, PDGFRA etc cannot explain this low incidence of mutations. We have already initiated genotyping of all newly diagnosed tumors at our centre. We have initiated efforts to extend this to other centres in India.

This study is still just at beginning of the molecular era in the field of oncology practice in India. We have to go a long way through to reach the target of “bench to bedside”. The feasibility of performing such studies is quite encouraging. We are doing molecular studies in sites like lung (EGFR mutation and selection of patients for Geftinib/Erlotinib), breast (Oncotype Dx for early breast cancer to select

patients for adjuvant chemotherapy), colon (check for microsatellite instability - MSI in an attempt to find patients who are candidates for adjuvant 5FU based chemotherapy in Stage II Ca colon) etc. Doing so, we are entering a phase of "Personalised treatment" in India.

LIMITATIONS

1. This study assessed the outcome of the patients in a retrospective manner. Hence the criticisms for a retrospective analysis are applicable for this study.

2. The sample size for the present study was small.

3. The genotype of c-kit mutation status was assessed on a Trucut biopsy or endoscopic biopsy in most of the patients as it was done on archival samples. We would prefer in our future studies to get enough specimen upfront for c-kit analysis.

4. Second line agents are not freely available in our country and hence majority of the patients could not receive drugs like Sunitinib or new drugs like Nilotinib. Hence salvage was not possible in many patients.

CONCLUSIONS

1. The present study demonstrated the feasibility of performing c-kit mutation analysis in India.

2. The genotype significantly predicted the outcome in our group of patients.

3. The outcome of metastatic GIST patients has definitely improved from a virtually incurable state to a disease where median OS has reached 60 months.

4. The genotype of Indian patients with GIST may be different from the western population which needs to be confirmed in a larger study.

5. The dosage if determined based on the baseline genotype may improve the outcome.

6. There is a need for multi institutional co operative group for studying Indian patients with GIST.

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PROFORMA

Name:

Age: Sex:

Occupation:

Place:

Income:

OP No: Index No:

Date of diagnosis of GIST:

Date of admission:

Presenting complaint:

Bleeding manifestations: Comorbidities:

Metastases synchronous/metachronous:

Surgical details:

Clinical examination:

PS: Anemia:

Tumor size:

largest metastasis size:

Site of primary:

Sites of mets: Liver/omentum/lymph nodes/bone/lung

HPE type:

Mitotic rate:

Glivec start date:

Response:

Date of documentation:

Dose adjustment:

Response:

c-kit mutation:

Exon 11

Exon 9

Wild type

Last follow up:

Status:

PFS:

OS: