Gastrointestinal Stromal Tumors (GIST): A Prospective Analysis and an Update on Biomarkers and Current Treatment Concepts

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Supplementary Issue: Biomarkers for Colon Cancer

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ABSTRACT: Gastrointestinal stromal tumors (GIST) are the most common sarcomas of the gastrointestinal tract, with transformation typically driven by activating mutations of cKIT and less commonly platelet-derived growth factor receptor alpha (PDGFRA). Successful targeting of tyrosine-protein kinase Kit with imatinib, a tyrosine kinase inhibitor, has had a major impact in the survival of patients with GIST in both the adjuvant and metastatic setting. A recent modification of treatment guidelines for patients with localized, high-risk GIST extended the adjuvant treatment duration from 1 year to 3 years. In this paper, we review the clinical data of patients with GIST treated in the Oncology Outpatient Unit of "Attikon" University Hospital and aim to assess which patients are eligible for prolongation of adjuvant imatinib therapy as currently suggested by treatment recommendations.

KEYWORDS: gastrointestinal stromal tumors, imatinib, cKIT

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Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal (GI) tract[1] with a median age at diagnosis of 65 years.[2] They derive from the interstitial cells of Cajal, which serve as a gut pacemaker as they create the basal electrical rhythm leading to peristalsis and segmentation of the smooth muscle.[3] GIST most commonly occur in the stomach (60%–70%), followed by the small intestine (20%–30%); they are also rarely found elsewhere in the abdominal cavity, such as in the mesentery, the omentum, or the retroperitoneum.[2]

They are most commonly caused by gain-of-function mutations in the proto-oncogene KIT (cKIT). In 75% of the cases, the primary mutation is found in exon 11, which encodes for the juxtamembrane domain of the protein, leading to activation of the receptor regardless of the presence of its ligand (stem cell factor).[4] In a further 15% of cases, mutations are found in exon 9 of the cKIT gene encoding the extracellular domain.[4] Exons 13 and 17, encoding the kinase domain of the protein, are mutated in approximately 5% of GIST. In 5% of cases, mutations are found in the homologous gene PDGFRA (platelet-derived growth factor receptor alpha). Most of these mutations (85%) are found in exon 18, encoding for the second kinase domain, and more rarely in exons 12 (juxtamembrane domain) and 14 (first kinase domain).[4] The remaining GIST (12%) are wild type for both cKIT and PDGFRA genes.[4] Mutational status of cKIT has emerged as a major prognostic and predictive factor in patients with GIST.[5] For example, deletions affecting codons 557–558 at exon 11 of cKIT gene indicate a poor prognosis in patients with completely resected GIST.[6]

For localized primary GIST, surgical resection with curative intent is the mainstay of therapy. However, after complete surgery, the risk of relapse is approximately 40%, with substantial variations based on known clinicopathologic features. Two main classification systems, the Fletcher and Miettinen, consisting of prognostic factors such as primary site, size, and mitotic index, facilitate the stratification of patients into low-, intermediate-, and high-risk groups of recurrence.[7,8]

Imatinib is an oral, selective, small-molecule tyrosine kinase inhibitor, which targets the Kit protein and the PDGFRA.[9] It has been demonstrated that imatinib significantly improves survival in patients with advanced GIST, and it has become the standard of care in this setting.[10,11] Furthermore, adjuvant imatinib administered in high-risk patients for 12 months after surgical removal of GIST with Kit protein expression has been shown to prolong recurrence-free survival (RFS) compared to placebo.[12,13] Based on these results, imatinib was approved at a daily dose of 400 mg by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) in 2008 and 2009, respectively, as adjuvant therapy for high-risk patients following complete surgical resection of GIST. Recently, a randomized Phase III trial, which included 400 high-risk GIST patients, reported a statistically significant improvement of RFS (65.6% vs 47.9%, $P < 0.001$) and overall survival (OS) (92% vs 81.7%, $P < 0.02$) after 3 years of imatinib as opposed to 1 year.[14] Following these results, the FDA and EMA recommended 36 months of adjuvant treatment in high-risk patients, simultaneously noting that the optimal duration of therapy remains to be established.

Translational studies have indicated variability in the response to imatinib therapy according to molecular and genetic characteristics of GIST, including the cKIT (exons 9, 11, 13, and 17) and the PDGFRA gene (exons 12, 14, and 18), allowing thus tailored therapy. Specifically, in the advanced stage, the recommended doses include imatinib 400 mg (mutations in cKIT exon 11, PDGFRA non-D842V, cKIT other sites, BRAF, NF1, NRAS/KRAS, SDHB/C, and IGF1R overexpression), 800 mg (mutations in exon 9), and no treatment (in PDGFRA D842V).[15,16] When adjuvant therapy is required, imatinib 400 mg is recommended for mutations in cKIT exon 11 or 9, in PDGFRA non-D842V, and in cKIT other sites but no therapy in the remaining subgroups.[15,16]

The aim of this study was to review the clinical data of patients with GIST treated in the Oncology Outpatient Unit of "Attikon" University Hospital and assess which patients could be eligible for extended duration of adjuvant treatment with imatinib to 36 months according to the international treatment guidelines.[17]

Patients and Methods

Patient data. We performed a retrospective data collection from a total of 15 patients with GIST treated in our unit over a 6-year period (2005–2011) by reviewing their medical records. The research was exempted from the requirement for ethics committee approval because it was a retrospective study of patient records.

For all patients, we collected demographic characteristics, clinical data (primary site of involvement, clinical presentation), pathologic features (size of the tumor, histology, mitotic index, CD117 positivity, categorization into risk groups), and information on treatment and patient outcome (imatinib therapy, duration of therapy, efficacy, toxicity) (Table 1).

Immunohistochemistry. Paraffin sections of formalin-fixed tissue (3 μm) were used for conventional hematoxylin and eosin (H&E) staining and immunohistochemistry by following the protocol established in our pathology laboratory of tissue processing, time of fixation, and immunohistochemistry, as previously described.[18]

Table 1. Clinicopathologic characteristics and treatments of GIST patients.

	NUMBER OF PATIENTS	PERCENTAGE (%)
Primary site		
Gastric	10	66.6
Small intestine	2	13.3
Colon	2	13.3
Retroperitoneal	1	6.6
Histology		
Spindle cell	10	66.6
Pure epithelioid cell	1	6.6
Mixed	4	26.6
Total	15	100
Classification according to Fletcher		
Very low	1	6.6
Low	1	6.6
Intermediate	4	26.6
High	8	53.3
Unclassified	1	6.6
Total	15	100
Classification according to Miettinen		
Probably malignant	12	80
Low malignant	2	13.3
Probably benign	1	6.6
Treatment		
Surgery (total)	14	93.3
Primary lesion	12	80
Primary lesion + hepatic metastases	2	13.3
Imatinib		
Adjuvant	8	53.3
First line	4	26.6
Reintroduced after relapse	3	20
Sunitinib (second line)	4	26.6
Sorafenib (third line)	1	6.6
Side Effects		
Edema	5	33.3
Hematologic toxicity	4	26.6
Fatigue	2	13.3
Diarrhea	1	6.6
Cardiac toxicity	1	6.6
Total	13	86.6

Primary antibodies included in our study were CD117 (rabbit anti-human polyclonal; dilution 1:500; A4502 DACO Glostrup), CD34 (clone QBEnd-10, mouse anti-human monoclonal antibody, dilution 1:200; MA1-10202 Thermo Fisher Scientific), and Ki-67 (clone SP6; rabbit anti-human monoclonal antibody, dilution 1:150; Thermo Fisher).

Mutational analyses. DNA was extracted by macrodissection from paraffin blocks under investigation using the QIAmpDNA formalin-fixed, paraffin-embedded tissue kit (Qiagen Sciences Inc.). A targeted resequencing assay (TruSeq Custom Amplicon; Illumina Inc.) was used for mutation detection in exons 9, 11, 13, and 17 of the cKIT gene and in exons 12, 14, and 18 of the PDGFRA gene. Sequencing was carried out using the Next Generation Sequencing platform MiSeq (Illumina Inc.) as previously described.[19,20] The sensitivity of the method is 5% of mutant allelic content.

Results

Demographics and clinical presentation. The median age of presentation was 67 years (range: 56–77). Men were affected more frequently than women, with a male/female ratio of 1.6:1.

In two cases, information on clinical presentation was not available. Of the remaining 13 cases, 4 (30%) presented with gastrointestinal bleeding and 5 (38.5%) with a mass lesion or vague abdominal discomfort. In three patients, GIST was an incidental gastroscopic finding after evaluation for iron-deficiency anemia, and in one patient it was identified during a screening colonoscopy.

The primary sites of involvement are described in Table 1 and included most commonly the stomach. At diagnosis, metastasis to the liver were demonstrated by imaging in 3/15 (20%) of patients.

Pathologic features and molecular analysis. GIST ranged in size from 0.4 to 22 cm. The largest tumor was seen in the retroperitoneum. In one patient, the tumor was <2 cm (0.4 cm), but it was excised by virtue of its colorectal location. All patients had R0 resection, and no patient received preoperative imatinib treatment.

The diagnosis of GIST was based on histological and immunohistochemical evaluation of a needle biopsy or a surgically resected specimen obtained from the GI tract. GIST demonstrated variable histology (Table 1), including sheet-like arrangement, short fascicles, and organoid patterns, as previously described.[2] Figure 1 shows histopathologic and immunochemical features of two patients presented with an intermediate gastric and a high-risk ileal GIST treated in our unit.

Currently, the defining feature of GIST is the immunohistochemical expression of CD117, a marker of Kit protein expression, although it is not entirely specific.[21] Thirteen patients (86.8%) were found to be CD117 positive, while the remaining two were negative. Furthermore, all 15 examined specimens exhibited CD34 positivity.

Pathologic features were assessed in order to assign tumors into risk groups (Table 1). According to the Miettinen classification,[8] which takes into consideration the primary site, size, and mitotic count, 80% of our patients' tumors

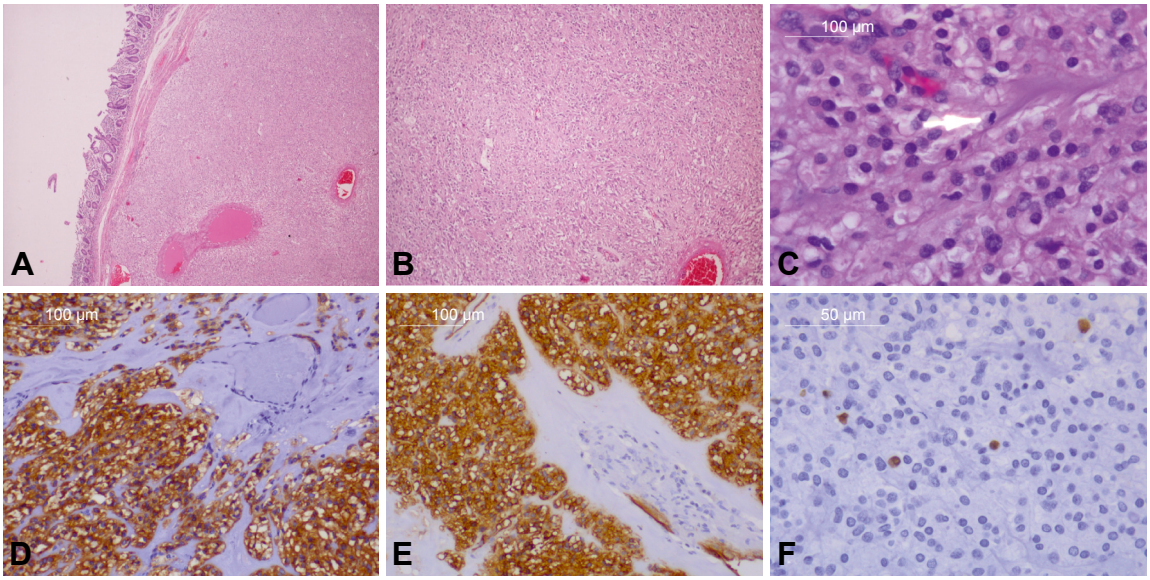

Figure 1. GIST pathology.
Notes: (**A, B**) Gastric GIST 11 cm in diameter, mitoses <5/50 HPF. The risk of recurrence is characterized as "moderate" according to Miettinen classification. (**A**) H&E staining (×40 magnification). (**B**) H&E staining (×100). (**C–F**) Ileal GIST 6 cm in diameter. The risk of recurrence is characterized as "high" according to Miettinen classification. (**C**) H&E epithelioid type with vacuolated cells (arrow indicates a cell in mitosis; ×630). (**D**) Kit positive staining (×250). (**E**) CD34 positive staining (×250). (**F**) Ki-67 positive staining <5/50 HPF (×400).
Abbreviations: GIST, gastrointestinal stromal tumor; HPF, high-powered field; H&E, hematoxylin and eosin.

were classified as probably malignant, whereas according to Fletcher classification,[7] which takes into account the size and mitotic activity, 53% were classified as high risk.

Mutational analysis was available in nine patients. Eight patients harbored the exon 11 cKIT mutation and one the exon 9 cKIT mutation.

Treatment and toxicity. Fourteen patients received upfront surgery with curative intent. One patient with advanced gastric GIST and hepatic metastases did not undergo surgery. A surgical procedure, which included extensive resection of primary site with liver metastases, was carried out in two patients. Totally, 12 patients received imatinib therapy: 8 in the adjuvant and 4 in the advanced stage. The majority of these patients tolerated imatinib treatment well with mild toxicity, as previously reported[22,23] (Table 1). Five patients presented periorbital edema and edema of the low extremities, three had grade I leukopenia, two experienced mild fatigue, one had grade I anemia, one had grade I diarrhea, and one suffered from grade I heart failure.

Eight patients received imatinib 400 mg/day for 1 year as adjuvant treatment: five belonged to the high-risk group and three to the intermediate-risk group.

Following discontinuation of imatinib, three of the high-risk patients relapsed (one patient after 1 year and two patients after 2 years of imatinib treatment completion). Of these patients, two died of disease progression, despite retreatment with imatinib and doubling of the dose to 800 mg. The third patient completed 5 years of imatinib and is still on therapy with stable disease, as defined by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria.

At the time of publication of new guidelines supporting the extension of adjuvant treatment to 3 years, five patients were found to have completed 1 year of adjuvant treatment with imatinib: two in the previous 4 months, one in less than 1 year, and two in more than 1 year.

Three patients with metastatic disease received imatinib as first-line treatment. One patient displayed the exon-9 cKIT mutation and was started on imatinib at a dose of 800 mg/day. Three months later, the patient developed progressive disease, and a second-line treatment with sunitinib 37.5 mg/day was administered. The treatment was discontinued 2 years later due to cardiotoxicity. The other two patients received sunitinib as second-line treatment due to disease progression after the administration of imatinib.[24] The first patient continues on sunitinib treatment 37.5 mg/day. The last patient was treated with second-line therapy including sunitinib 37.5 mg/day for 1 year when he was started on third-line treatment with sorafenib 400 mg twice daily due to disease progression.[25]

Three patients did not receive adjuvant imatinib therapy. Of these patients, two were not eligible for adjuvant therapy, one had a very low-risk colonic GIST and is free of disease until now, and one had a low-risk gastric GIST. However, the last patient developed metastatic disease 2 years after diagnosis. Molecular analysis performed at that time showed exon-11 cKIT mutation. Imatinib 400 mg/day was administered as first-line treatment, but it was switched to sunitinib 2 years later due to disease progression. The third patient had intermediate-risk GIST but did not receive imatinib as adjuvant treatment due to comorbidities. However, the patient remains free of disease 1.5 years after surgery.

Discussion

Herein, we retrospectively collected and reviewed data from patients with GIST treated in the Oncology Outpatient Unit of "Attikon" University Hospital over a 6-year period (2005–2011). We collected information regarding patients' clinicopathological characteristics, and focused on the duration of adjuvant imatinib therapy and treatment outcome in order to assess the eligibility of patients for prolongation of imatinib treatment to 3 years, as suggested by the new treatment guidelines.

With the recent approval of 3–year adjuvant imatinib therapy for significant risk primary GIST following the landmark Scandinavian Sarcoma Group's XVIII/AIO trial,[14] one major clinical issue is the possibility of resumption of therapy from patients who have recently discontinued adjuvant treatment. Reinitiating imatinib to complete 3 years of adjuvant treatment in patients who discontinued by virtue of previous recommendations appears a reasonable and simple approach; however, the risk of recurrence is higher mainly in the first 1–2 years after discontinuation, and although restart of treatment with imatinib is not routinely suggested after 1 year of discontinuation, a risk of recurrence is still present.[17] Interestingly, a prospective, placebo-controlled Phase III trial (RIGHT study) in patients with metastatic GIST, who are refractory to treatment with all standard tyrosine-kinase inhibitors, indicated that reinitiation of imatinib is beneficial as the disease continues to harbor many clones that are sensitive to kinase inhibitors.[26]

In our case series, out of a total of eight patients who received adjuvant imatinib for 1 year, three high-risk patients relapsed in the first 2 years after treatment cessation. In these patients, extension of adjuvant imatinib treatment to 3 years could have possibly reduced the risk of recurrence. Among the patients who did not relapse, three were eligible for reinitiation of imatinib since they had discontinued imatinib therapy within the previous year; re-treatment was started after the establishment of new recommendations after taking into account the patients' comorbidities. The cases of two patients who had terminated adjuvant therapy within 2 years were discussed at the multidisciplinary oncology meeting, where, after evaluation of imatinib risk/benefit ratio, additional therapy was not suggested. Among the most feared side effects of imatinib treatment is cardiovascular toxicity, and it should be upfront assessed and balanced with the known survival benefit of administering imatinib for 36 months for each individual patient.

In our case series, demographic and clinical features, such as male predominance, type of clinical presentation,

and most frequent location of the primary tumor (stomach), are consistent with published data.[7,27–29] Pathologic characteristics such as predominance of histological spindle cell pattern and CD117 positivity are also well documented in other series.[30,31]

Mutational analysis in patients with GIST has important clinical significance from the therapeutic aspect, as it has predictive value for sensitivity to molecular-targeted therapy, including dosage and prognostic value.[17] In our study, mutational analyses were not available in a proportion of our patients (6/15) due to a number of reasons. Three patients who were classified in the low and very low risk groups did not undergo mutational analysis since it would not alter therapeutic decision. One of them relapsed. Molecular analysis performed at that time showed exon-11 cKIT mutation. In three patients, mutational analysis was not done due to reimbursement issues.

Biomarkers. There is a favorable impact of the cKIT exon-11 genotype on the response to imatinib therapy compared with GIST that have cKIT exon-9 mutant or wild-type (WT) genotypes.[5] Also, it is known that cKIT exon-9 mutant GIST that were treated with imatinib 800 mg had a significantly superior median PFS ($P = 0.0013$) in comparison to treatment with imatinib 400 mg/day, with a 61% reduction in the relative risk of progression compared with patients who were treated with imatinib 400 mg,[32] suggesting that these patients should be treated with a higher dose upfront. The optimal dosage for adjuvant therapy in patients with cKIT exon-9 mutant GIST, however, remains unclear. Subgroup analyses of the Z9001 and the SSGXVIII/AIO trials revealed that patients with cKIT exon-11 mutation benefit from adjuvant imatinib, whereas such a benefit could not be demonstrated in the subgroups with cKIT exon-9 mutation or WT GIST.[14,33] The latter analyses were grossly underpowered and could not exclude clinically significant efficacy. Taken together, these findings suggest that GIST mutation status can predict the response to adjuvant imatinib and that genotyping can help in determining the likelihood that a patient will respond to treatment.

Since low-risk patients have excellent relapse-free survival, adjuvant treatment could lead to substantial overtreatment and seems poorly justified. Of note, among the low-risk patients in our small cohort who did not receive adjuvant imatinib, one had recurrence, indicating that maybe standard clinicopathologic criteria are not enough and upfront molecular analysis may be required for better stratification of the patients into risk groups.

Decision making is more challenging in the intermediate-risk group. A patient with intermediate-risk GIST and was not subjected to imatinib treatment has, to date, no evidence of the disease. There is little reason to believe that adjuvant imatinib is less effective for intermediate-risk GIST than high-risk GIST, but the number needed to treat to prevent or postpone one recurrence is higher than in high-risk GIST. A practical approach to address this problem is to use the modified NIH criteria for risk assessment and offer adjuvant imatinib only to high-risk patients because, when these criteria are used, patients with intermediate-risk GIST face only a small risk of recurrence, which is not markedly different from that of low-risk disease.[34] Another approach is to fine-tune the estimated outcome using prognostic maps or nomograms.[35–37] These two observations emphasize the need for identification of better predictive factors that could clarify which patients actually benefit from adjuvant imatinib, perhaps even in combination with established risk-stratification molecular analysis.

It is worth mentioning that the optimal duration of adjuvant imatinib therapy has not yet been clarified. A recent Chinese case series that included 101 patients with GIST showed an improved RFS rate in patients with more than 3 years of adjuvant imatinib therapy compared to those with less than 3 years of treatment (93.9% vs 68%, $P < 0.01$) without significant difference in toxicity.[38] Prolongation of imatinib treatment appears to be a tempting approach. The theoretical rationale for extending adjuvant imatinib beyond 3 years should be counterbalanced by the possible development of imatinib-resistant mutations that would limit the efficacy of future therapies and the cost of life-long treatment. An ongoing Phase II, non-randomized, open-label, uncontrolled, single-group study is currently assessing this issue by evaluating the efficacy and safety of 55 years of imatinib (400 mg/day) adjuvant therapy in adult patients with resected primary Kit protein expression (CD117)-positive GIST at intermediate to high risk of relapse (PERCIST study; http://ClinicalTrials.gov/show/NCT00867113).

Positive Kit protein immunostaining has been encountered in other types of cancers, such as small-cell lung cancer,[39] testicular seminoma,[40] salivary adenoid cystic carcinoma,[41] and melanoma.[42] Except for a few case studies indicating imatinib activity in isolated patients with Kit protein overexpressing tumors,[43,44] larger retrospective or prospective studies have indicated that imatinib treatment in Kit protein overexpressing tumors is not effective and that activating mutations are required for drug activity.[45–47] This effect was confirmed by clinical studies showing durable response rates in patients with metastatic malignant melanoma harboring mutations mainly in exons 11 and 13,[42,48,49] indicating that further documentation of such driving mutations is imperative.

Conclusion

In conclusion, we presented a case series of patients with GIST treated in the Oncology Outpatient Unit of "Attikon" University Hospital, focusing on assessment for prolongation of adjuvant imatinib treatment. Extension of adjuvant imatinib treatment to 3 years changed our management regarding patients with GIST; however, many questions remained unanswered. Optimal dose and duration of imatinib therapy, time limit after discontinuation at which patients could still be offered re-treatment, and patient selection based on prognostic

and predictive markers in order to avoid unnecessary subjection to therapy are critical unresolved issues that require further investigation.

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Author Contributions

Study conceived and planned: AK. Involved in data collection and analyses: PK, KL. Involved in clinical diagnosis and data collection: PAD, NT, GM, AR. Wrote the first draft: PE. Main preparation of the manuscript: AK, IB. All authors reviewed and approved the final manuscript.

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