Predictive factors of response to Sunitinib in Imatinib-Resistant Gastrointestinal Stromal Tumors (GISTs): A multi-institutional study

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Abstract

Imatinib 400 mg is the standard of care for medical treatment of advanced GISTs. In the majority of cases, however, GISTs eventually develop resistance to imatinib. The optimal second line treatment has not been established yet and imatinib dose escalation (800 mg) or sunitinib represent two feasible options. The objective of this retrospective, multi-institutional, study is to analyze the validity of several parameters as possible predictive factors of response to sunitinib after imatinib failure.

We reviewed 128 metastatic GISTs treated with sunitinib between January 2007 to June 2017. Primary tumour site, metastatic site, c-KIT/PDGFR-α mutational status, PET-FDG status and type of disease progression to sunitinib were assessed as possible predictive factors of response.

This study identifies the gastric site of primary tumor as a predictive factor to sunitinib efficacy in second line setting. The mutational status (GIST WT), the site of metastasis (peritoneum) and the FDG-PET status (negative), although not statistically significant, seem to be elements of increased activity for sunitinib treatment.

These results provide the rationale to drive physician for sunitinib choice in second line setting for metastatic GISTs, to improve patients selection and to maximize the benefit from the treatment, on the basis of possible predictive factors of response.

Keywords: Gastrointestinal Stromal Tumors, GIST, Sunitinib, Imatinib, Predictive Factors

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1. Introduction

Gastro intestinal stromal tumours (GISTs) are the most common mesenchymal neoplasm of gastro intestinal (GI) tract, nevertheless are considered as rare tumors accounting for 1% of all primary GI cancers[1]. The incidence of these tumors differs profoundly among regions; in USA there are 7 to 29 new cases per million population annually whereas in North Europe there are 14.5 per million population [2,3]. The mean age at diagnosis is 63, as reported by the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) but diagnosis under the age of 40 is not uncommon[2]. Despite the GISTs can arise in any portion of the GI tract as they originate from the Cajal cells, some district are more affected by this disease. Indeed the majority of GISTs originate in the stomach (50%) and in the jejunum or ileum (30%), only 5% are located in the duodenum and the rectum and less than 1% arise in the esophagus [1,4].

Over the past 30 years GISTs have emerged from a poorly understood neoplasm to a well-defined tumor entity. The major breakthrough derived from the discovery of the CD117 antigen that enabled a better distinction between GISTs and other group of spindle cell neoplasms arising from the GI tract including lipomas, leiomyomas schwannomas, hemangiomas. Indeed these malignancies are usually CD117 negative while GISTs are typically CD117 positive [5]. CD117 is encoded by the proto-oncogene KIT and its product is a membrane tyrosine kinase receptor (TKR), which activates cell proliferation[5]. and it is over-expressed in 80-90% of GISTs. Moreover in the early 2000s, it was demonstrated that c-KIT gene could harbor driver somatic mutations that were responsible for the onset of the disease. Almost 80% of GISTs harbor activating mutations in c-KIT exon 11, encoding for the juxtamembrane domain of TKR, which results in the constitutive and ligand-independent receptor dimerization and activation. However mutations can occur in exon 9, 13 and 17 determining the same activation of receptor signaling[6]. Another gene that is involved in GISTs carcinogenesis is the platelet-derived growth factor receptor alpha (PDGFRα) [7] which is mutated in approximately 5-8% of GISTs. Nevertheless 10% of GISTs does not harbor any mutations in both c-KIT and PDGFRα gene. These tumors are defined as “wild type” (WT) [8] and are characterized by mutations in the succinate dehydrogenase gene, which is also identified in 85 % of familial GISTs [9].

GISTs are oncogene-addicted tumors and this finding has profoundly changed treatment strategy and patients management. Indeed before 2000s locally advanced or metastatic GISTs were considered chemo-resistant [10,11]. Afterwards the discovery of c-KIT and PDGFRα activating

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mutation able to enhance GISTs cancer cells growth, led to the introduction of effective treatment targeting the TKRs. The first tyrosin kinase inhibitor (TKI) used in the treatment of locally advanced or metastatic GISTs was Imatinib, which immediately reached impressive results in improving prognosis in this subset of patients. Imatinib was originally approved for chronic myeloid leukaemia and it is able to block KIT and PDGFRα activationby inhibiting ATP binding to the receptor catalytic site required for phosphorylation and signaling activation [12]. Interestingly, Imatinib dosage for GISTs treatment is dependent on c-KIT mutational status. Despite the 400mg/die schedule improved median overallsurvival (OS) for metastatic GISTs patients from 18 to 57 months, achieving a disease response in more than 50% of patients, a metanalysis of two large randomized phase III trials showed that the imatinib 800mg/die schedule significantly improved progression free survival (PFS) in a small subset of patients harboring exon 9 mutation[13,14,15,16].

Nevertheless, although the high efficacy of imatinib, complete response are reached in less than 10% of patients, and all metastatic GISTs will incontrovertibly acquire resistance to the treatment due to the onset of secondary mutation in c-KIT [17].

For these metastatic resistant patients,who was started on first line imatinib 400 mg daily and experienced disease progression, there are two feasible options:imatinib dose escalation (800 mg/die) and sunitinib, an oral TKI with a greater selectivity for KIT and PDGFRα.

Both are valid options to overcome resistance in imatinib-refractory patients treatment, however the optimal treatment has not been established yet:imatinib dose escalation activity was demonstrated in two large dose finding randomized phase III trials[15,16]. For sunitinib, the schedule50 mg 4 weeks on and 2 off, improved significantly PFS over placebo in a randomized controlled trial, in second line setting for those patients who had progression to first line imatinib [18]. However, sunitinib 37.5 mg continuously seems to be similarly effective and safety to sunitinibstandard dose[19].

Nevertheless there is still the lack of a direct comparison between these treatment option.

With the present study we have retrospectively evaluated the value of several parameters as possible predictive factors of response to sunitinib in metastatic GISTs in order to drive the physician choice and maximize the effectiveness of second line treatment.

2. Patients and methods

Patients were eligible if they had histologically confirmed diagnosis of GISTs, metastatic disease, radiological progression to imatinib,and received second-line treatment with sunitinib. All patients were treated with sunitinib 37,5 mg per day continuously until disease progression or unacceptable

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toxicity. A written informed consent was obtained from each patient for the acquisition of clinical and molecular data to be included in the study.

The following information were recordedfor all patient when they started sunitinib: age, ECOG performance status,site of primary tumour and site of metastasis, c-KIT and PDGFRα mutational status, baseline PET-status.Radiological evaluation of treatment efficacy by CT-scan was performed after 4 months of therapy and responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 together with CHOI criteria on the basis of physicians' experience. Three types of progression disease by CT scan have been identified: "dimensional PD" (dPD) characterized by the growth of known lesions, "numerical PD" (nPD) characterized by the appearance of new lesions, and "mixed PD" (mPD) characterized by both dimensional and numerical PD. Survival outcomes, including PFS and OS defined as the time between the date of sunitinib starting and the date of disease progression or death, respectively, were assessed.

3. Statistical analysis

Patient's characteristics were reported using median values and range. The Chi-squared test was used to assess differences between groups. Survival analysis was performed using Kaplan Meyer method, providing HR estimates and their 95% CI, with the use of the logrank test for comparisons. All statistical tests were two-sided. Patients with no evidence of PD were censored at the last tumour assessment. Multi-parametric, Cox proportional hazard models were usedto assess interactions betweenpatients' characteristics, including tumor site, metastasis site, gene status, baseline PET, type of progression to sunitinib, andsurvival outcomes.We obtained separate models for PFS and OS, adjusting for all the covariates that predicted, afterunivariate analysis, for PFS or OS, as appropriate. A stepwise procedure was used with a significance level of p=0.05 to retain variables inthe model.All thestatistical analysis were performed using "Medcalc" software version 11.5.0.

4. Results

One hundred twenty eight patientswith histologically confirmed diagnosis of advanced GISTs refractory to imatinib who received second-line treatment with sunitinib from January 2007 to June 2017were included in the study. Sixty-eight were male, 60 were female. Median age was 54.5 years (range: 30-81). The primary tumor wassited in the stomach for 38 patients (29.5%), small bowel for 67 (52%), rectum for14 (11%), others for 6 patients (4.5%). Median follow up was 83.4 months (range 9-201). Patients' characteristics were reported in the **Table 1**.Patients with smallbowel and

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stomach as primary tumor sites achieved a statistically significant longer median PFS (12and 10 months, p<0,0001) as compared to the other sites **(Figure 1).**

The specific subtype of c-KIT and PDGFRα mutations were available for 119 of 128 patients (91%): 78harbored c-KIT exon 11 deletion, 20 patients were c-KIT exon 9 mutant, 8 patients showed PDGFRα mutation, whereas 13 patients were WT. Contrary to previous studies,mutational status of c-KIT and PDGFR α did not resultin a significant association with GISTs PFS (p=0.62).

Liver and peritoneumconfirmed to be the most frequent metastatic sites in our cohort: 45 liver, 33 peritoneum, 40 both, 8other site. Evaluating the correlation between thesite of metastasis and PFS, amajor efficacy of sunitinib has been observed in patients with peritoneal metastasis, who achieved 10 months ofmedian PFS. Although a trend toward better PFS, site of metastasis did not reachstatistical significance at the univariate analysis (p=0,24).

Baseline PET status was available in 83 of 128 cases, 63were PET positive, whereas 20 were PET negative. Sunitinib was more effective in terms of medianPFS in PET negative than PET positive metastatic GISTs, although this difference was notstatistically significant (12 versus 10 months, p=0.11).

The type of progression was available for 106 of 128 patients (83%). The median PFS for sunitinib was 11 months in the cohort with dPD, 9months for nPD, and 11 months for mPD (p=0.2).

Regarding Overall Survival (OS), the results of multivariable analysis of mortality in the competitive risks model are summarized in the **Table 2.** Baseline PET status, site of metastasis and mutational status were notsignificantly associated with GISTs OS; tumor site was able to predict for OS at multivariate analysis (p=0,0004): patients with small intestine [HR: 0.22 (0.08-0.57), p=0.002] and rectum as primary tumor sites [HR: 0.19 (0.07-0.561), p=0.005] achieved a statistically significant longer median OS as compared to stomach [HR: 0.51 (0.19-1.35)].

5. Discussion

A deeper understanding of the molecular alterations underlying the development of GISTs, including mutational activation of KIT or PDGFRα has led to the approval of new effective targeted therapies thatrevolutioned the management and treatment of this disease. Imatinib 400 mgcurrently represents the new backbone of first line treatment in patients with metastatic GISTs leading to a significant improvement in termsof PFS, OS, and quality of life, as reported in a phase II and III trials[12-14]. However, despite the high efficacy of this therapy, the majority of tumors develop acquired resistance to imatinib and experience diseaseprogression. Imatinib 800 mgand sunitinib represent the current standard second-line treatments, representing two feasible and effective options for imatinib refractory GISTs patients, given the absence of head to head comparisons [15,

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17, 18]. Imatinib dose escalation could be considered in patients who started on imatinib 400 mg,on the basis of randomized dosefindingtrials revealing theefficacy of this strategy in both American and European populations[12, 13]. In the European study, 247 of the 473 patients were randomly assigned toimatinib low dose arm, and 133 who progressed were crossed over to higher dose of imatinib [13].There were almost 36 patients with a prolonged stable disease and 3 partial responses.Median PFS were 1.7 and 2.0 years for imatinib 400 mg and 800 mg arms respectively (HR: 0.91;p= 0.18), and median OS was 3.9 years in both treatment arms. Similar results were reported inthe American trial. Median PFS was 18 months for patients on imatinib 400 mg, and 20 months forthose receiving imatinib 800 mg [14]. Median OS was 55 and 51 months, respectively.Sunitinib is an active TKI that has been approved on the basis of a large phase III trial where 412patients imatinib refractory were randomized 2:1 to receive sunitinib or placebo. Median time totumour progression was 27.3 weeks in sunitinib group and 6.4 weeks in placebo group (HR: 0.33;p<0.0001). Although survival was significantly better with sunitinib in the initial report, over timeOS converged in the sunitinib and placebo arms (median 72.7 vs. 64.9 weeks; HR, 0.876; P =0.306), given the cross-over design [19].

Given the uncertainly on the best therapy to adopt in secondline for imatinib refractory GISTs patients, our retrospective study reports a real word series assessingpotential predictive factors of response to sunitinib in order to drive physicians' choice in thissetting.

Mutational status in controlled clinical trials, significantly influences the activity of sunitinib [20]. In a phase I/II trial, PFS and OS were meaningfully longer for patients with a primary KIT 9 exon (58 %) or PDGFRα mutation (56 %) than for those with a KIT exon 11 mutation (34 %). However, our study did not confirm previously data.

The present study, according to our best knowledge, represents the largest series of GIST patients after imatinib failure analyzed for mutational status as predictive factors of response to sunitinib treatment, in routine clinical practice outside randomized, controlled, clinical trials.

In previous studies, KIT mutation status appears as a predictor of tumor response to sunitinib.

The first study (M. C. Heinrich et al, 2008) explore the relationship between GIST kinase mutations, KIT or PDGFRA, and the response to sunitinib in 77 patients with Imatinib-Resistant GIST. PFS and OS were significantly longer in patients with primary *KIT* exon 9 mutations or a wild-type genotype than in those with *KIT* exon 11 mutations.

Anotehr study on 74 patients tested for KIT and PDGFRA mutations (Dok Hyun Yoon et al, 2010), showed that patients with KIT exon 9 mutant GIST (n=11, 14.9%) have better TTP (median 13.6 mo vs 6.9 mo, p=0.631) than those with KIT exon 11 mutant GIST (n=47, 63.5%), although statistical significance was not secured and the number of exon 9 mutant GIST was low (n=11).

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Consistent with previous studies, a third studying a group of 89 patients (Rutkowski et al, 2012) confirmed that patients with primary tumors carrying mutations in KIT exon 9 or wild-type had substantially better 1-year PFS (68% and 57%; median 65.5 and 50.5 weeks, respectively) than patients having tumors with KIT exon 11 or PDGFRA mutations (34% and 15%; median 36.8 and 9 weeks, respectively).

Of note, our results, contrary to previous studies, don't show that PFS were longer for patients who had mutations in KIT exon 9, than *KIT* exon 11 mutations. Median PFS for KIT exon 9 was 10 months (n=20, 16.8%), similar to PFS for KIT exon 11, that was 10 months (n=78, 65.5%).

Median PFS was, instead, significantly longer in patients with wild-type genotype than exon 11 patients. (n=13, 10.9%, median PFS 20 mo).

Therefore, contrary to previous studies, with a larger sample (119 pts), we have proven that, GISTs harboring KIT exon 9 mutations not appear to be more sensitive to sunitinib than those with primary KIT exon 11 mutations. Consistent with cited studies, instead, wild-type genotype patients have better PFS than KIT exon 11 and exon 9.

Similar results were for overall survival, with a median of 76 months for KIT exon 11 mutation and 72 months for exon 9. The median OS for wild-type was, instead, lower than Kit mutant GIST (67 mo).

Our data showed that **primary tumor site** is predictive for PFS. Patients with small bowel and stomach as primary tumor sites achieved a statistically significant longer median PFS compared to the other sites.

Regarding the **site of metastasis**, the peritoneal localization seems to achieve longer PFS than othermetastatic sites, although not statistically significant. The greater efficacy on peritoneal metastasis might be explained by the role ofangiogenesis in peritoneal progression and the strong anti-angiogenic activity of sunitinib [21]. Not all metastases have similar vascularization. In the liver there can be small or large liver metastases; necrosis is common in larger masses. Peritoneal metastasis are usually small (often < 2 cm) and homogeneously enhancing and vascularization. This can be an explanation for the better activity of sunitinib in patients with peritoneal metastasis. Many studies indicate that dual inhibition of PDGFR and VEGFR from sunitinib produces greater antiangiogenic effects than inhibition of only one such as for imatinib, and the lower dimension and the homogeneous vascularization typical of the peritoneal metastasis, may contribute to better activity from sunitinib[22,23,24].

Moreover, our study showed that the**type of radiologic progression** and **baseline PET status** werenot statistically significant predictors of response to sunitinib, although numeric progression andnegative baseline status are associated with worse PFS.

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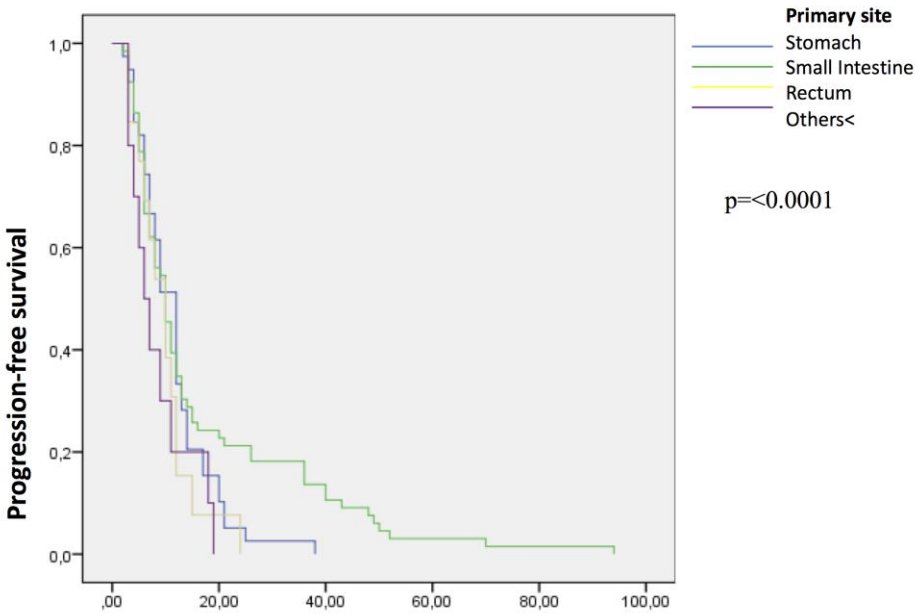
6. Conclusion

This study identifies the gastric site of primarytumor as a predictive factor to sunitinib efficacy in second line setting. The mutational status(GIST WT), the site of metastasis (peritoneum) and the FDG-PET status (negative), although notstatistically significant, seem to be elements of increased activity for sunitinib treatment. These results provide the rationale to drive physician'schoice in second line setting formetastatic GISTs, to improve patients selection and maximize survival benefit onthe basis of possible predictive factors of response.

Figure 1



Primary site	Median PFS (mo)	95% CI
Stomach	12	9.9-14.08
Small Intestine	10	7.8-12.1
Rectum	10	6.5-13.4
Others site	6	2.9-9.09

Figure 1: Small bowel and stomach as primary tumor sites achieved a statistically significant longer median PFS (12 and 10 months, p<0,0001) compared to the other sites.

Table 1. Main characteristics of study patients.

	Number of patients	% of patients
Patients Evaluated	128	100
Gender (Male)	68	53
Gender (Female)	60	47
Age at diagnosis, years		

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Median	54,5	
Range	30-81	
Primary tumor site	125	98
Stomach	38	29.5
Small bowel	67	52
Colon Rectum	14	11
Others	6	4,5
Number of Mitosis	107	83.5
>10/50 HPF	80	62.5
<10/50 HPF	27	21
Primary tumor size	111	86.5
2-5 cm	17	13
5-10 cm	51	40
>10 cm	43	33,5
Site of Metastasis	122	95
Liver	45	35
Peritoneal	33	25.5
Liver and Peritoneal or other involvement	48	37.5
Mutational status	119	92.97
c-Kit exon 11	78	65.54
c-kit exon 9	20	16.8
Wild Type	13	10.9
PDGFRα Exon 12-18	8	5.04
FDG-Pet Status at PD with Imatinib 400 mg	83	65
Positive	63	49
Negative	20	15,5
Type of Tumor Progression to Sunitinib	110	86
Numerical	16	15
Dimensional	56	51
Mixed	38	34

Table 2. Univariate and multivariate analysis of survival

		Univariate		**Multivariate**	
	N.	OS (months)	P (log rank)	p	HR (95%CI)
Sex			**0.14**		
M	68	79.9			
F	60	88.5			
N. of mitosis/50 HPF			**0.06**		
≤5	27	107.1			
>5	80	82.1			
Tumor size			**0.05**		
2-5 cm	17	92.9			
5-10cm	51	95.7			
>10cm	43	75.5			
Tumor Site			**<0.0001**	**0.0004**	
Stomach	38	68.9		0.18	0.51 (0.19-1.35)
Small Intestine	67	95.3		0.002	0.22 (0.08-0.57)

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Rectum	14	105.1		0.005	0.19 (0.07-0.61)
Others	6	46.3			
Metastasis site			**0.54**		
Liver	45	81.7			
Liver and Peritoneum	40	78.2			
Peritoneum	33	92.4			
Others	8	99.2			
Gene status			**0.62**		
c-kit 11	78	86.3			
c-kit 9	20	80.3			
PDGFRα 12	2	45.0			
PDGFRα 18	6	80.3			
WT	13	80.9			
PET			**0.35**		
N/A	35				
Positive	63	77.2			
Negative	20	89.1			

Compliance with Ethical Standards

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Availability of data and materials

All data used or analyzed in this study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

All authors have contributed, read and approved the final manuscript.

Ethics approval and consent to participate

A written informed consent was obtained from each patient for the acquisition of clinical and molecular data to be included in the study. All clinical information for each patient was anonymously recorded and coded after a written informed consent. The study was approved by ethical committee (Comitato Etico Palermo 1) of the university- affiliated hospital AOUP 'P. Giaccone' of Palermo (G-Land 2017, approval number: 01-03-2017).

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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