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Gene Signature in GIST

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ABSTRACT

BACKGROUND:
KIT and PDGFR mutations are central events in GIST pathogenesis. Target therapy activity depends on mutation setting.

AIMS OF THE THESIS
A retrospective analysis of GIST cases followed in our center during the last decade to evaluate how the genetic alteration may influence the therapeutic decision and the prognosis of GIST patients.

MATERIALS AND METHODS: In the 2000-2010 period, our Center followed 80 localized or metastatic GIST patients, and 76 were followed with regular follow-up. The mutational analysis for c-KIT and PDGFRα genes was realized for 54/80 patients. RFS was stratifyng for risk under Fletcher and Miettinem classifications. The response to tyrosine kinase inhibitors treatment was evaluated by integrating the RECIST criteria with the new CHOI. PFS for each treatment was calculated.

RESULTS: The median onset age was 55 years. 54 cases analyzed by primary genetic mutations had a frequency resulting as follows: 34 c-kitt 11, 8 c-kit 9, 1 PDGFRα 12, 4 PDGFRα18, 7 WT. Relapse patients were 20; 13/20 showed c-KIT in exon 11 mutation, two in exon 9, one PDGFRα, two Wild Type. The RFS mediane in high risk patients was 31 months, in low/moderate risk was not reached within the five follow-up years (Fletcher classification p:0.17; Miettinem p:0.03). Three-years PFS with Imatinib 400mg/die on 19 patients shows a 27 months mediane. PFS in patients were: with Imatinib 800 mg/mq 9 months, Sunitinib 4 months, Nilotinib 3 months.

CONCLUSIONS: The data we obtained are in line with the literature data. Miettinen's risk stratification proves to have better predictive value than Fletcher.Mutational statusofprimary tumorsis closelyrelatedto the effectivenessoftreatmentwithimatinibandothertyrosine kinasesinhibitors. The genetic analysis should be a standard of the diagnostic-staging iter of GIST patients.

Introduction

Stromal Gastrointestinal Tumors (GIST) are rare; In Europe the annual incidence is 6.6-14.5 per million of inhabitants, which means 1-3% of all tumors. (1) Nevertheless GISTs are the most common mesenchimal tumors of gastrointestinal apparatus. (2)
The tumor can occur at any age but most GIST patients are detected in their 6^{th} or 7^{th} decades, while only 10% are below 40 year of age.
99% of cases occur in a sporadic form, even though a small percentage of inheritable-familial GIST exist, frequently as multiple Gist (Carney triade, neurofibromatosis).
Only since the Nineties GIST were recognized and classified as a separate clinical and istological subtype from others mesenchimal tumors.
GISTs can occur in every intestinal tract, sometimes also outside. These is their presentation rate:
-Stomach: 40-70%
-Small-bowel: 20-40%
-Esophagus: 5%
-Rectum: 5-15%
-Omento-Mesentere: < 5%
Their growth pattern is most commonly extramural; therefore, they rarely result in obstruction, but sometimes they occur with free intra-abdominal bleeding from central tumor necrosis and peritoneal breaking. As a result of their insidious nature and growth pattern, more than three-fourths of these tumors exceed 5 cm in diameter at time of diagnosis.
GIST are frequently roundish friable and un-encapsulated masses. Biggest tumors can have a cystic degeneration, necrosis and haemorrhagic areas; they can break spontaneously or during surgery. Most GISTs are spindle cell tumors (70%), and a minority mixed splindle (10%), ephitelioid (20%), or rarely pleomorphic.
GIST major characteristic is their peculiar immunohistochemical profile: 95% expresses c-Kit protein (CD117), tyrosine kinase transmembrane receptor. Approximately 80-75% of GISTs show a mutation in the Kit proto-oncogene.(3) About 10% of GISTs' mutations are not identifiable, but Kit is nonetheless strongly activated.
Data on PDGFR expression are scant, and many available antibodies are not reliable on paraffin-embedded tissue. However, some studies suggest that PDGFR can be a diagnostic immune-histochemical marker.
Other commonly expressed but less GIST-specific antigens are: Protein Kinase theta, DOG1,CD34, SMA (4-5-6)

Table1differential kinds of diagnosis (7)

	KIT	CD34	SMA	Desmina	S100	Indagini molecolari
GIST	+ (95%)	+ (70-80%)	+ focal (70%)	+ focal (50%)	+ focal (5%)	KIT PDGFRα
Leiomuscolar tumors	-	+ (10-15%)	+ diffused	+ spread	Rare	
Schwannoma	-	+	-	-	+	
Fibromatosis	debated	Rare	+	Rare cells	-	Multiβ-catenina
Clear cell Sarcoma	-	-	-	-	+	EWS-ATB-1 EWS-CREB
Sinovial Sarcoma	-	-	-	-	-	SYT-SSX1 SYT-SSX2
Melanoma	+ focal	-	-	-	+	KIT

KIT and PDGFR mutations are central events in sporadic GIST pathogenesis and their identification is becoming increasingly important, since specific treatments on oncogenic KIT and PDGFR activation (especially imatinib mesylate) have become available. KIT and PDGFRA genes are located pericentromerically at 4q12, the corresponding proteins have structural characteristics of type III receptor tyrosine kinase family. Activating mutations permit the phosphorylation of the tyrosine kinases receptor, dimerization and the regulation of cell proliferation, apoptosis, chemotaxis and adhesion.

Many patients experiencing Imatinib-induced remission develop metastases with acquired medicine resistance, usually based on secondary Imatinib-resistant mutations in Kit and PDGFR tyrosine kinase domains.

KIT mutations in GIST are clustered in four exons. They are, in decreasing order of frequency, exon 11, exon 9, exon 13 and exon 17. Most common are exon 11 (juxtamembrane domain) mutations, including delection, point mutation and duplications. Older studies argue that mutations in exon 11 are related to a more aggressive disease, larger tumors, high mitotic index and worse outcome (8).

Exon 9 mutations (5-10%) (extracellular domain) usually are 2-codon 502-503 duplications and mostly occur in intestinal GISTs. Most small intestinal tumors with such mutation have been malignant, but it seems to be related to the worse prognosis of small intestinal GISTs in general. A lower Imatinib sensitivity of these tumors has been noticed.

Exon 17 mutations (tyrosine kinase 2 domain) have been reported few times. GISTs with such mutations are variably sensitive to imatinib.

Three different regions of PDGFR have been found to be mutated in GISTs. They are, in decreasing order of frequency exon 18, exon 12 (juxtamembrane domain), and exon 14. The secondary point mutations are especially in exon 18. The exon 18 mutations are Imatinib-resistant; the missense mutations affecting exon 14 seem to be associated with low Kit expression and unexpectedly favorable prognosis.
Thestudiesthatledto thesediscoveriesare notonlyanintellectual speculation; tumorlocation, itsbenign ormalignant grade and clinicalandpathologicfeatures are highly dependent on themutationalpattern(9).

Table2 A summaryofcorrelationsbetweenhistopathologic featuresandmutation(10-11-12-5).

Gene	Mutation	Clinical implications	Istotypes	Prognostic value
KIT Exon 9	Ala502_Tyr503dup	strongly correlated to intestinal GIST (>90%)	Frequently splidle cell tumors	None prognostic value
KIT Exon 11	Deletions Insertions	various gastrointestinal location	many patterns of splindle cells	probable malignant behavior
	Sostitutions			Probable benign behavior
PDGFRα Exon 18	Deletion Sostitution	strongly correlated to gastric GIST (>95%)	Frequently ephitelioid cell tumors	Probable benign behavior
KIT PDGFRα WT	Wild Type	various gastrointestinal location	Splindle and epitelioid cell tumors	None prognostic value
		GIST correlated to NF1	splidle cell tumors	
		Pediatric GIST correlated to Carney Syndrome	ephitelioid cell tumors	

In Table 3, sensitivity and responsiveness in vitro of different genotypes of KIT and PDGFRα to treatment with Imatinib.

Gene	Exon	Mutatione	Sensibility
KIT	9	Ala502_Tyr503dup	RC 5% PR 29% SD 47% PD 17%
	11	Deletion Sostitution Insertione	RC 6% PR 61% SD 25% PD 3%
PDGFRα	18	Asp842_Met844del Ile843_His845del Asp842Val	Some resistent mutations. RO in many cases
KIT **PDGFRα**	WT	WT	PR 23% SD 50% PD 19%

There is no consent about a uniform staging method, and none of the current classifications is completely satisfying (Tabella 4).

Table 4: AJCC 2002, from the NCCN 2008 guidelines (13)

T1	Tumor dimension: less than 5cm in his widest diameter. T1a: Superficial tumor T1b: Deep tumor
T2	T2: Tumor dimension: more than 5cm T2a: Superficial tumor T2b: Deep tumor
N0	Absence of lymph nodal tastasis
N1	Presence of lymph nodal metastasis

M0	Absence of distance metastasis
M1	Presence of distance metastasis
G1	Well-differentiated tumor
G2	Midly-differentiated tumor
G3	Slightly-differentiated tumor
G4	Non-differentiated tumor

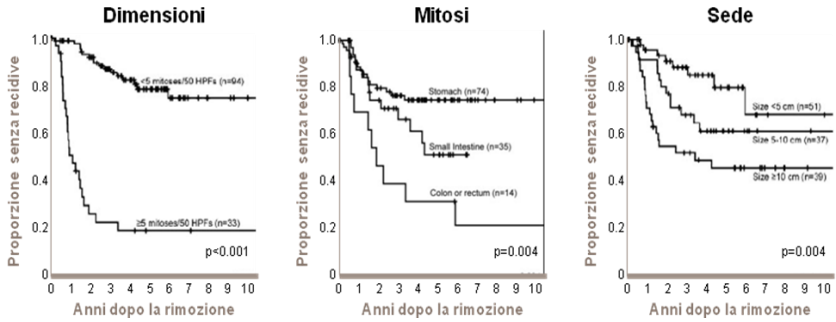
Stage	T	N	M	G
Stage I	T1a, T1b, T2a, T2b	N0	M0	G1, G2
Stage II	T1a, T1b, T2a	N0	M0	G3, G4
Stage III	T2b	N0	M0	G3, G4
Stage IV	Ogni T	N1	M0	Ogni G
		N0	M1	

In 2001, National Institute of Health (NIH) organized an experts consensus where was discussed the metastasis risk stratification, based on the dimension of the tumor and mitotic count expressed as number of mitosis for 50 HPF (high-power field).

Table 5 Relapse and metastasis risk stratification by NIH/Fletcher

Dimension (cm)	Mitotic index (#/50HPF)		
	<5/50 HPF	5-10/50HPF	>10/50HPF
<2	Low	Moderated	high
2-5	Low	Moderated	high
5-10	Moderated	high	high
>10	High	high	high

However, in 2006 AFIP (American Forces Institute of Pathology) anatomo-pathologists, in the largest set of GIST in literature (1900 patients) observed that small intestine and rectal GIST have a greater malicious potential than stomach GIST. Therefore the tumor location is today a useful prognostic factor. Five risk classes are considered (none, very low, low, average, high). (2)



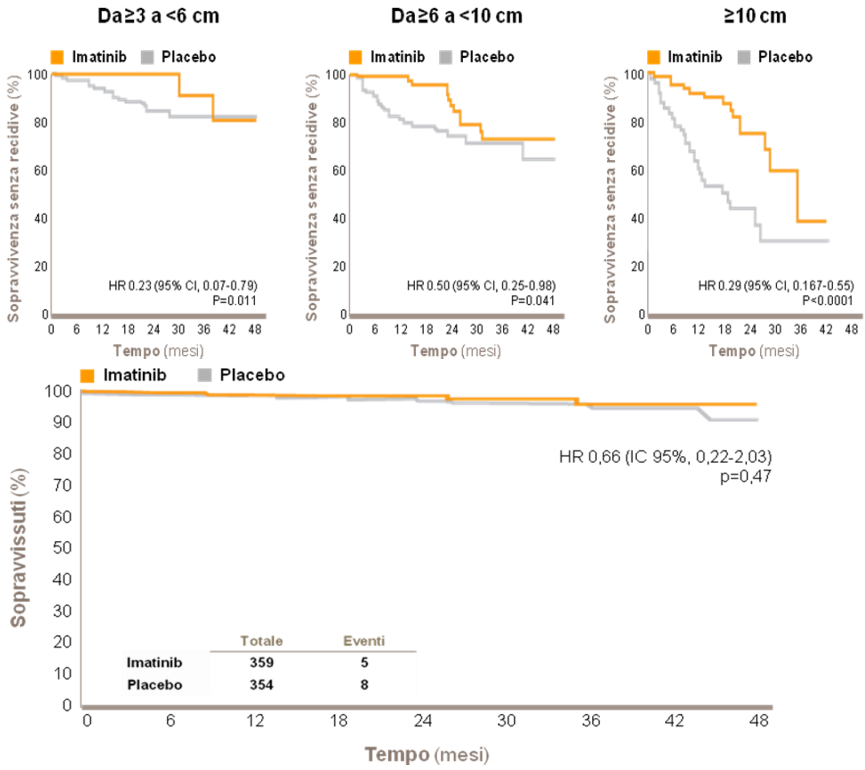
Picture 1 Free survival from disease based on dimension, mitosis and primitive tumor location. (14)

Table 6 Relapse and metastasis risk stratification by Miettinen

size and mitotic index		**Location**			
		Stomach	**Digiunum/Ileum**	**Duodenum**	**Rectum**
<5/50HPF	**<2 cm**	None 0%	None 0%	None 0%	None 0%
	2-5 cm	Very low 1,9%	low 4,3%	low 8,3%	low 8,5%
	5-10 cm	low 3,6%	Moderate 24%	high 34%	high 7%
	>10 cm	Moderate 12%	high 52%	high 34%	high 57%
>5/50HPF	<2 cm	None	high 50%	N. D.	high 54%
	2-5 cm	Moderate 16%	high 73%	high 50%	high 52%
	5-10 cm	high 55%	high 85%	high 86%	high 71%
	>10 cm	high 85%	high 90%	high 86%	high 71%

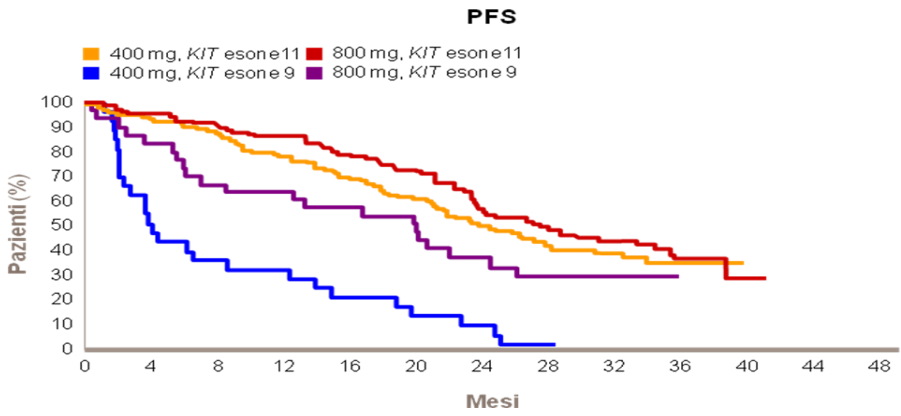
The recent revision of 2008 classification suggests to consider as additional factors the tumor breaking and the hemoperitoneus, the mucosal invasion. R0 surgery is a prognostic factor too and is still the preferred treatment as well as the only one that can ensure recovery. According to ESMO guidelines, when an R0 surgery can induce complication or appears to be complicated, an Imatinib

neo-adjuvant treatment may be foreseen, after a multi-zonal analysis in order to identify refractory tumors.
Imatinib approval as GIST adjuvant therapy by FDA and EMEA, was based on a stage III randomized double-blind study, Imatinib vs. Placebo (ACOSOG Z9001) (15), which proved an advantage of Imatinib 400 mg/day for one year (97% vs 83%) in terms of one-year relapse-free patients percentage. Randomization was stratified on tumor size (from ≥3 to <6 cm, from ≥6 to <10 cm, and≥10 cm). OS, secondary endpoint, did not prove to be modified during this period, however the follow-up period is short and distorted by the crossover, which was expected by the study.
Two randomized studies (EORTC 62024, SSG XVIII/AIO) are currently ongoing, in order to understand how Imatinib affects OS in adjuvant setting and the possible advantage of a therapy extension from one to two years.



Picture 2 Disease and global free survival in patients treated with Imatinib VS placebo. An improvement of free disease survival is not associated with a significant improvement of global survival.

Present recommendations from NCCN suggest the prescription of adjuvant Imatinib for at least 12 months (as by trial ACOSOG Z9001) in average/high-risk patients, with standard 400mg dosage. In62005 study, patients withexon9KITmutations,treatedwithimatinib800mg/die,hadsignificantlysuperior PFSanda61% reduction in the relative riskthan patientstreatedwith 400mg/day(16).



Picture 3 Studio 62005. Progression free survival in different setting of mutations.

In a study imatinib was able to inhibit all PDGFR mutations except the D842V (62.9 %). This particular mutation's resistance to imatinib restricts his use in about one third of GIST with PDGFR mutation.

However, even after a complete surgical removal of primary GIST, at least 40% patients faces relapse within 5 years (17).

The initial approval of Imatinib for advanced GIST treatment by FDA in 2002 is based on a study about 147 patients with non-resectable or metastatic GIST, in which 38% of patients show a partial response with a volumetric reduction bigger than 50% (18). After a number of clinic trials 55-80% of metastatic GIST patients obtains a partial response or a disease stabilization. Most common adverse reactions include oedema, rash, diarrhea, nausea, abdominal pain, fatigue. FDA and EMEA recommend to start the treatment in a metastatic patient with a single dose of 400mg/day. The augmentation of the dosage to 800mg/day (double dose) may be chosen for patients with exon 9 of c-KIT gene mutation, or with evident signs of progression after the 400mg dose, (19). It is a daily treatment, as it is known that its suspension, even if just for one day, may cause the re-ignition of the disease, which can be shown by PET (PET flare).

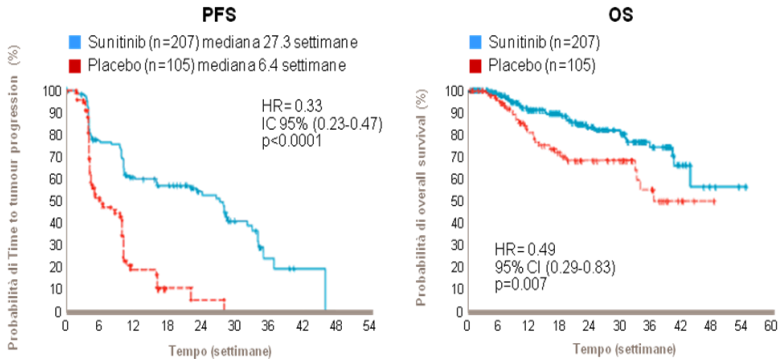
Table 7 Meta-GIST study (20) was presented at 2007 ASCO and clearly shows how the Imatinib response is strictly connected with the mutational condition:

Mutation Status	Median Progression-Free Survival (mesi)	Percent 3-year PFS	Median Overall Survival (mesi)	Percent 3-year OS
KIT exon 11	26	38%	60	69%
KIT exon 9	13	11%	31	44%
Wildtype	16	27%	43	57%
Other	11	9%	34	46%

Table 8 The same meta-analysis also confirmed the benefit from a 800mg Imatinib treatment in case of exon 9 mutation:

Imatinib Dose	Median Progression-Free Survival (mesi)	Percent 3-year PFS	Median Overall Survival (mesi)	Percent 3-year OS
400 mg	6	5%	28	37%
800 mg	19	17%	35	49%

In patients with disease progress, it is possible to increase Imatinib dose or to move to a second-line therapy with Sunitinib, a tyrosine kinase inhibitor. The supporting study for Sunitinib approval as second-line treatment for progressing or Imatinib-intolerant GIST is double-blind randomized VS placebo. Patients who received Sunitinib benefited from an increase of the Time To Progression of more than four times than placebo (27,3 weeks against 6,4 weeks; HR 0,33, p<0,0001) (20). Although the clinical benefit is significant in all patterns, the primary response is significantly higher in exon 9-mutated tumors (21).



Picture 4 PFS and OS in Sunitinib patients VS Placebo National Comprehensive Cancer Network, 2008

Following studies proved that the minimum effective dose is 37,5mg/day and that the best treatment schedule is daily therapy without interruption – this, against a relatively low toxicity increase, leads to a clinic benefit as it avoids the rebound effect. Sunitinib toxicity includes hematological and coagulation alterations, thrombocytopenia, hand-foot syndrome, mucositis.
There is no current indication about a third-line treatment, even if a lot of phase-III trials are in progress. The use of other second-generation tyrosine kinase inhibitors (Nilotinib, Dasatinib, Masitinib) is still under review by clinical testing. Nilotinib is a very well-tolerated medicine that has shown antitumor activity but dosen't seem to show improved survival. (22) The inhibition of alternative targets, such as components of signal transduction downstream of KIT and PDGFRα, including AKT and mTOR, has been tested in vitro and in vivo (everolimus) (23). Even anti-angiogenesis show an antitumor activity in GIST. More recently, other strategies are based, for example, on heat-shock protein HSP-90 inhibition, a member of chaperone proteins that plays a peculiar role in the stabilization and protection from the degradation of c-KIT. (24).Although preclinical studies proved a remarkable antitumor activity, clinical tests have been early stopped because of the occurrence of toxicity deaths. Regorafenin, oral multi-kinase inhibitor developed which targets angiogenic, stromal and oncogenic receptor tyrosine kinase (RTK) is currently being studied in a randomized, placebo-controlled phase III study for metastatic and/or unresectable GIST, progressed despite prior treatment with at least imatinib and sunitinib.

Tabella 9

Farmaco	Target molecolare
Nilotinib	KIT, PDGFRα, BCR-ABL
AZD2171	VEGFR, KIT, PDGFRs
Pkc412	KIT, PFGFRs, VEGFR-2, PKC
AMG 706	VEGFR, KIT, PDGFRs, RET
Everolimus	mTOR
Sorafenib	RAF
Regorafenib	VEGFR2, TIE2

NCCN guidelines, updated in 2008, recommend Best Supportive Care after a Sunitinib therapy fail. However, some studies encourage an Imatinib re-challenge, at least in patients who showed good responses to the first-line therapy as it seems useful in decelerating the disease's progression.
The current WHO, SWOG and RECIST criteria (Response Evaluation Criteria in Solid Tumors) do not consider that GIST don't often decrease their dimension, but show other types of responses such as tissue response (density reduction), because of necrosis, intra-tumor hemorrhage or fibromixoid degeneration.

Choi, comparing relation between the variation of the tumor dimension and CT density with the responses by 18FDG-PET, established new response criteria (reduction of tumor dimension ≥10% or a decrease of the tumor density (HU) ≥15% at TC) (25-26-27) providing a better prognostic parameter in terms of Free Disease Survival than RECIST criteria.

AIMS OF THE THESIS

The purpose of this study is the retrospective analysis of the cases that have been followed in our center during the last decade. In particular, we evaluated the possible biological and clinical relations in order to verify how the genetic alteration may influence the therapeutic decision and the prognosis of GIST patients.

MATERIALS AND METHODS

In the 2000-2010 Patients period, our Reference Center followed 80 localized or metastatic GIST patients, and 76 of these were followed with regular, therefore evaluable, follow-up.

GIST diagnosis was placed on mesenchymal cancer CD117+ or CD117- with positive mutational analysis for alterations on c-KIT or PDGFRα.

The anatomic-pathologic data provided, besides the evaluation of resection margins, the dimension of the tumor, the histological subtype and the number of mitosis. In relation to these data it was possible to locate each case in a specific risk class, based on the universally accepted risk classification NIH/Fletcher (Fletcher, Berman, & Corless, 2002). The cases were also evaluated according to the location and then classified under Miettinen (Miettinen & Lasota, 2006).

The mutational analysis for c-KIT and PDGFRα genes was realized for 54/80 patients.

The instrumental analysis used in the disease staging, the evaluation of the therapeutic response to the treatment and in follow-up were CT, PET and NMR, CEUS for the morpho-functional evaluation of hepatic and peritoneal localizations. In gastric or rectal GIST cases, endoscopy and sometimes eco-endoscopy were also used.

Surgery was considered complete if the disease was “en bloc” resected at the initial exploration reporting negative surgical margins (R0). The presence of onset hemoperitoneum or tumor breaking was considered R1 surgery.

The response to tyrosine kinase inhibitors treatment was evaluated by integrating the classic dimensional response criteria (RECIST) with the new ones (CHOI), that consider tissue modifications (biologic response), documented by radiological tests in terms of NMR signal intensity variation, density at CT and uptake (SUV) in case of PET.

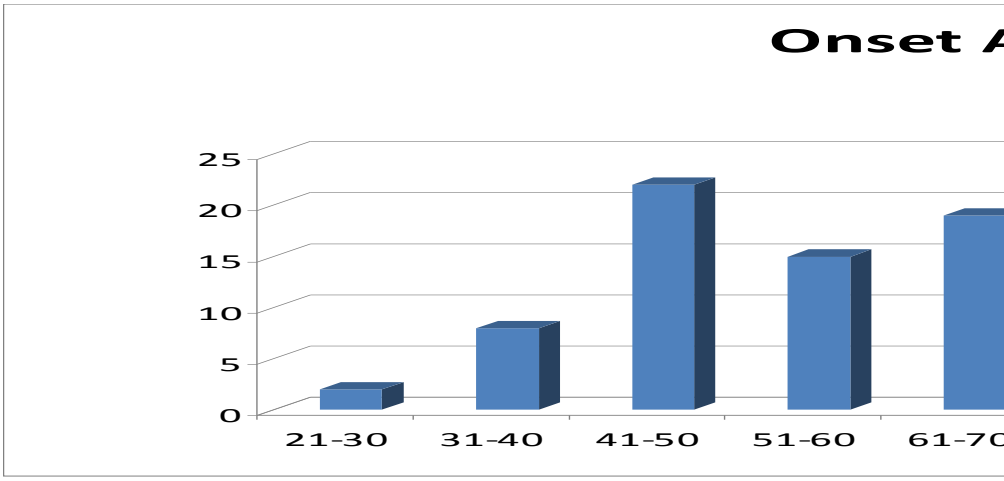
Patients	Sex	Age	Stage	Sites	Size	Mutation	Fletcher Risk
1	M	49	Localized	Stomach	>10 cm	c-kit 11	High
2	F	77	Localized	Small-bowel	5-10 cm		Intermedio
3	F	60	Localized	Small-bowel	5-10 cm		Intermedio
4	M	39	Localized	Stomach	>10 cm	c-kit 11	High
5	F	46	Localized	Stomach	2-5 cm		Low
6	M	45	Localized	Unknown	>10 cm	c-kit 11	High
7	M	66	Localized	Small-bowel	>10 cm	c-kit 11	High
8	F	45	Localized	Small-bowel	5-10 cm	c-kit 11	High
9	F	61	Localized	Stomach	2-5 cm		Low
10	M	67	Localized	Stomah	5-10 cm	c-kit 11	Intermedio
11	F	62	Localized	Small-bowel	>10 cm	c-kit 11	Intermedio
12	F	49	Metastatic	Peritoneo		c-kit 9	
13	M	40	Metastatic	Rectum		PDGFRα 18	
14	M	62	Localized	Stomach	5-10 cm	PDGFRα 18	Intermedio
15	F	64	Localized	Stomach	2-5 cm		Low
16	M	43	Localized	Small-bowel	5-10 cm	c-kit 9	Intermedio
17	M	57	Localized	Stomach	<2 cm	c-kit 11	very low
18	F	44	Localized	Small-bowel	2-5 cm		Low
19	F	52	Localized	Small-bowel	5-10 cm	c-kit 11	High
20	F	63	Localized	Small-bowel	2-5 cm	WT	Low
21	M	45	Metastatic	Esophagus		c-kit 11	
22	M	71	Localized	Rectum	2-5 cm	c-kit 11	Low
23	M	54	Localized	Stomach	5-10 cm	c-kit 11	Intermedio
24	M	75	Localized	Stomach	2-5 cm		Low
25	M	25	Localized	Stomach	>10 cm	c-kit 11	High
26	M	60	Localized	Stomach	2-5 cm	WT	Low
27	M	50	Localized	Stomach	>10 cm	WT	High
28	F	62	Localized	Small-bowel	>10 cm	c-kit 11	High
29	F	44	Localized	Stomach	5-10 cm	WT	Intermedio
30	M	56	Localized	Stomach	5-10 cm	c-kit 11	Intermedio
31	F	81	Metastatic	Stomach		c-kit 11	
32	M	40	Localized	Stomach	5-10 cm		Intermedio
33	F	49	Localized	Small-bowel	5-10 cm	c-kit 9	Intermedio

34	M	53	Localized	Small-bowel	2-5 cm		Low
35	M	71	Localized	Stomach	2-5 cm	WT	Intermedio
36	M	60	Localized	Small-bowel	2-5 cm		High
37	M	46	Localized	Stomach	5-10 cm	c-kit 11	High
38	F	60	Metastatic	Unknown		c-kit 11	
39	M	75	Localized	Rectus	>10 cm	c-kit 11	High
40	M	34	Localized	Small-bowel	2-5 cm		low
41	F	79	Localized	Stomach	2-5 cm		Intermedio
42	F	69	Localized	Stomach	>10 cm		Low
43	F	64	Localized	Stomach	5-10 cm		High
44	F	50	Localized	Unknown	>10 cm	WT	High
45	M	67	Localized	Stomach	2-5 cm		very low
46	F	33	Localized	Stomach	>10 cm	c-kit 11	High
47	M	48	Metastatic	Unknown			
48	M	75	Metastatic	Small-bowel		c-kit 11	
49	F	68	Localized	Small-bowel	>10 cm	c-kit 9	High
50	F	53	Localized	Small-bowel	5-10 cm	c-kit 11	Intermedio
51	F	36	Localized	Small-bowel		PDGFRα 18	High
52	F	48	Localized	Small-bowel	2-5 cm	PDGFRα 18	Low
53	F	62	Localized	Stomach	5-10 cm	c-kit 11	High
54	M	71	Localized	Stomach	5-10 cm	c-kit 11	Intermedio
55	M	78	Localized	Stomach	5-10 cm	c-kit 11	Intermedio
56	M	67	Localized	Small-bowel	2-5 cm		Low
57	F	56	Localized	Small-bowel	>10 cm	c-kit 11	High
58	M	46	Localized	Small-bowel	2-5 cm	c-kit 11	Low
59	F	37	Localized	Small-bowel	2-5 cm	WT	Low
60	F	48	Localized	Small-bowel	5-10 cm		Intermedio
61	M	63	Localized	Stomach	>10 cm	c-kit 11	Intermedio
62	M	65	Localized	Stomach	5-10 cm	c-kit 11	High
63	F	76	Localized	Stomach	5-10 cm	c-kit 11	High
64	M	30	Localized	Peritoneo	5-10 cm	c-kit 9	High
65	M	33	Localized	Peritoneo	5-10 cm	c-kit 11	
66	F	48	Localized	Stomach	2-5 cm		Low
67	F	50	Localized	Stomach	<2 cm	PDGFRα 12	very low
68	M	71	Localized	Small-bowel	2-5 cm		Low

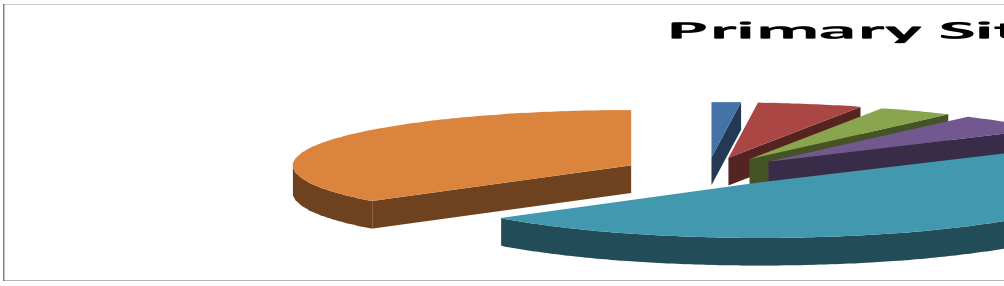
69	F	44	Localized	Small-bowel	>10 cm	c-kit 9	High
70	F	41	Metastatic	Stomach	>10 cm		High
71	M	54	Localized	Tenue	2-5 cm	c-kit 9	Low
72	F	65	Localized	Small-bowel	2-5 cm		Low
73	F	59	Localized	Stomach	>10 cm	c-kit 11	High
74	F	41	Localized	Small-bowel	2-5 cm	c-kit 9	Low
75	M	80	Localized	Stomach	5-10 cm	c-kit 11	High
76	F	52	Localized	Stomach	5-10 cm	c-kit 11	High
77	F	54	Localized	Stomach	>10 cm		High
78	M	80	Localized	Rectum	5-10 cm		High
79	F	67	Localized	Stomach	2-5 cm		Intermedio
80	M	70	Localized	Stomach	2-5 cm		Intermedio

RESULTS

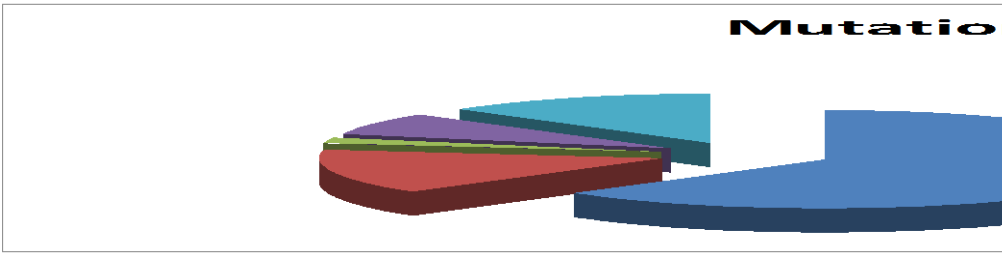
Today our center follows 80 patients, 39 women and 41 men, diagnosed between 2000 and 2010. The onset age was 55 years, with the following distribution by decades:



The incidence by location agreed with literature's reports and was distributed as follows:



Patients 7 and 59 had a beginning leiomyoma diagnosis, patient 8 a fibrosarcoma diagnosis, patient 51 a liposarcoma, patient 63 a gastric carcinoma.
The 54 cases analyzed by primary genetic mutations had a frequency resulting as follows:



Patients with localized disease diagnosis were subjected to primitive tumor resection, except for patient 30 and 78 who are refusing the operation and are being treated with Imatinib 400mg/day. In 12 patients (13, 15, 16, 21, 31, 33, 36, 47,66,75,77,79) it was not possible to realize a radical surgery (R1). In patients treated for resectable GIST the risk distribution by Fletcher was the following:

Stratification of Risk of Relapse and metastasis (NCCN)	Patients
High	29
Moderate	20
Low	20
Very low	3
Total	72

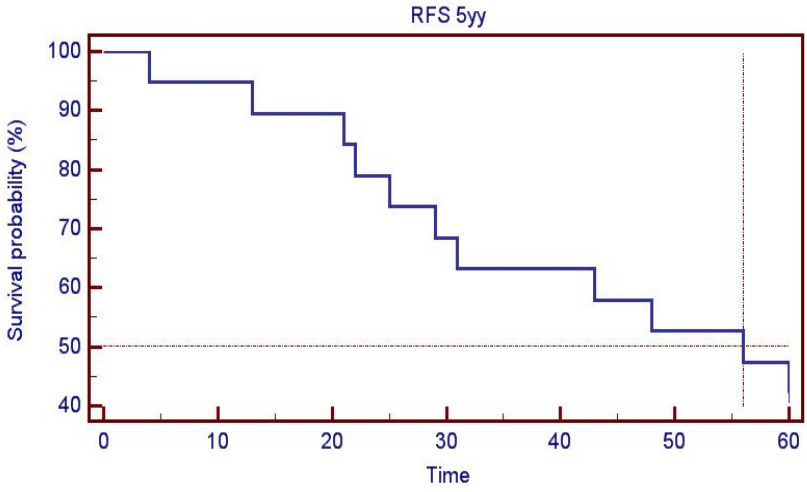
In patients treated for resectable GIST the risk distribution by Miettinem was the following:

Stratification of Risk of Relapse and metastasis (Miettinen)	Patients
High	28
Moderate	13
Low	23
Very low	8
Total	**72**

Among the patients that went through surgery for localized GIST, 14 started an adjuvant treatment with Imatinib 400mg/day (among these, patients 16, 23, 33 with average risk and patients 4, 6, 43, 46, 53,64,70,77,78,79 high risk). Patients 23, 43, 46, 53 take Glivec as they have been included in EORTC 62024 study.
The patients taking Glivec as adjuvant appear relapse-free in follow-up controls, except patient 70 that developed epatic and peritoneal metastasis.
Patients 30 and 78, who had a potentially resectable GIST diagnosed, refuse surgery and therefore take Imatinib 400mg/die.
Relapse patients, identified during follow-up controls, were 20 with the following risk distribution:

Risk class	**Relapsed Patients**
High	14
Moderate	4
Low	2
Very Low	0

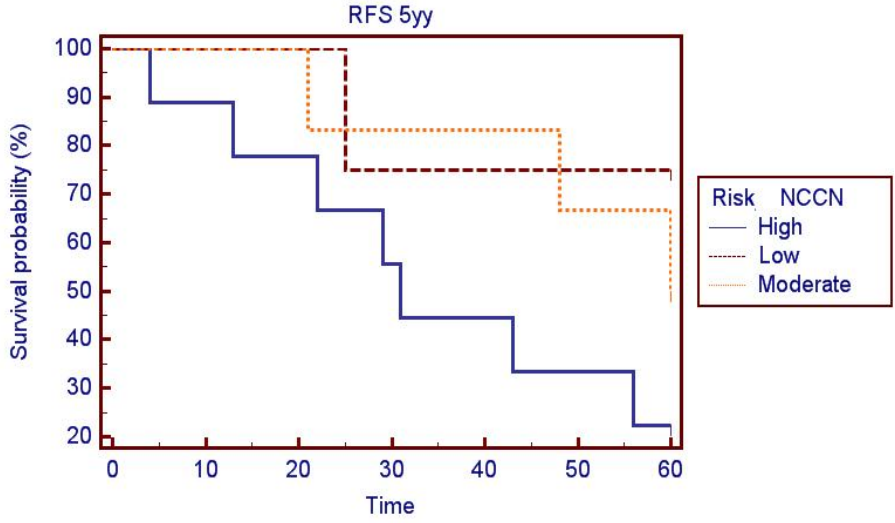
In particular, 13 of the 20 relapse patients showed c-KIT in exon 11 mutation, two patients had c-KIT in exon 9 mutation, one patient had PDGFRα mutation, two patients had Wild Type for both genes. It is interesting to point out that low/average risk relapse patients carried mutation of c-KIT in exon 11 for deletion. This data cannot be considered statistically significant because of the poor sample number, nevertheless it is suggestive and confirms literature data reporting the mutation for delection in exon 11 of c-KIT as index of relatively malicious behavior of the primitive tumor (Andersson, Bumming, & Meis-Kindblom, 2006).
Considered T0 the time of the diagnosis in patients diagnosed for localized GIST with at least a five-year follow-up time non-stratified for risk Relapse Free Survival (RFS) we obtain a 56-months mediane:



Survival time	Time
Endpoint	RFS_5yy RFS 5yy
Factor codes	Patients

	Factors	
	1	
Sample size	19	
Median survival	56	
Survival time	Survival Proportion	Standard Error
4	0,947	0,0512
13	0,895	0,0704
21	0,842	0,0837
22	0,789	0,0935
25	0,737	0,101
29	0,684	0,107
31	0,632	0,111
43	0,579	0,113
48	0,526	0,115
56	0,474	0,115
60	0,421	0,113

By stratifying RFS for risk under Fletcher we obtain:



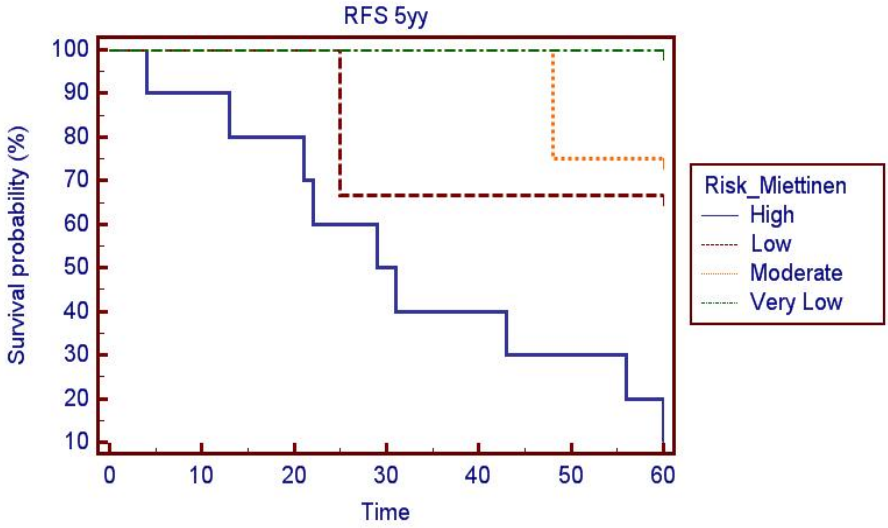
Survival time	Time
Endpoint	RFS_5yy RFS 5yy
Factor codes	Risk__NCCN_ Risk (NCCN)

	Factors					
	High		Low		Moderate	
Sample size	9		4		6	
Median survival	31		-		-	
Survival time	Survival Proportion	Standard Error	Survival Proportion	Standard Error	Survival Proportion	Standard Error
4	0,889	0,105	-	-	-	-
13	0,778	0,139	-	-	-	-
21	-	-	-	-	0,833	0,152
22	0,667	0,157	-	-	-	-
25	-	-	0,750	0,217	-	-
29	0,556	0,166	-	-	-	-
31	0,444	0,166	-	-	-	-
43	0,333	0,157	-	-	-	-
48	-	-	-	-	0,667	0,192
56	0,222	0,139	-	-	-	-
60	-	-	-	-	0,500	0,204
	Comparison of survival curves (Logrank test)					
Endpoint: Observed n	7,0		1,0		3,0	
Expected n	4,1		2,8		4,1	
Chi-square	3,4767					
DF	2					
Significance	P = 0,1758					

This curve shows how RFS is related to the risk calculated upon dimensions and mitotic count, even if the poor sample number and the short follow-up period make the analysis not significant. The RFS mediane in high risk patients was 31

months, while the mediane in low/average risk was not reached within the five follow-up years.

By analysing the RFS curve under Miettinen's risk stratification we obtain:

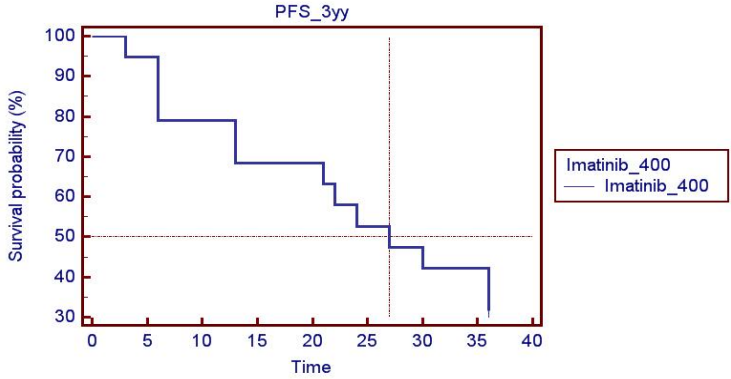


Survival time	Time
Endpoint	RFS_5yy RFS 5yy
Factor codes	Risk_Miettinen Risk Miettinen

	Factors							
	High		Low		Moderate		Very Low	
Sample size	10		3		4		2	
Median survival	31		-		-		-	
Survival time	Survival Proportion	Standard Error	Survival Proportion	Standard Error	Survival Proportion	Standard Error	Survival Proportion	Standard Error
4	0,900	0,0949	-	-	-	-	-	-
13	0,800	0,126	-	-	-	-	-	-
21	0,700	0,145	-	-	-	-	-	-
22	0,600	0,155	-	-	-	-	-	-
25	-	-	0,667	0,272	-	-	-	-
29	0,500	0,158	-	-	-	-	-	-
31	0,400	0,155	-	-	-	-	-	-
43	0,300	0,145	-	-	-	-	-	-
48	-	-	-	-	0,750	0,217	-	-
56	0,200	0,126	-	-	-	-	-	-
60	0,100	0,0949	-	-	-	-	-	-
	Comparison of survival curves (Logrank test)							
Endpoint: Observed n	9,0		1,0		1,0		0,0	
Expected n	4,3		2,0		3,1		1,7	
Chi-square	8,7765							
DF	3							
Significance	P = 0,0324							

A significant analysis is obtained. Miettinen's risk stratification proves to have a better predictive value than NIH/Fletcher.

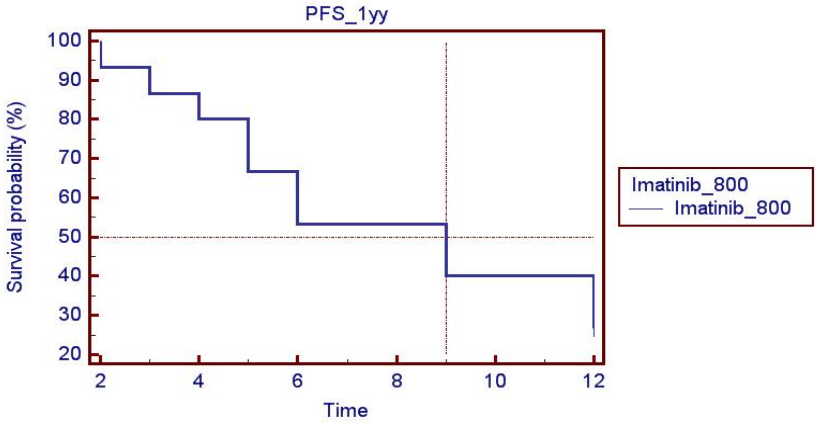
As it is expected by Miettinen's risk stratification, 10/16 relapsed patients had a primary disease localized at the small intestine, which is confirmed to be the highest relapse-risk location (Miettinen & Lasota, 2006).
Patient 7 was operated for relapse excision and is today treated with Imatinib 400mg/day, disease-free.
Patient 61 was treated at relapse with Imatinib 400mg/die with neo-adjuvant purpose for a big relapse located in the pelvic area, which was removed after six months of treatment. The patient keeps taking Imatinib 400mg/day, disease-free.
Patients 1, 11, 44, 49, 50, 51, 62 with disease relapse and patients 12, 13, 21, 31, 47, 48 with metastatic disease at diagnosis were treated with debulking surgery and then with Imatinib 400mg/day. The other patients with inoperable relapsed or metastatic disease have been treated with Imatinib 400mg/day.
To date we have been treating 28 patients with Imatinib 400mg/day for metastatic or relapsed disease. 11/28 patients carry on their Imatinib 400mg/day treatment. Three-years PFS, calculated on 19 patients having a 36 months since the beginning of the treatment follow-up, shows a 27 months mediane:



Survival time	Time
Endpoint	PFS_3yy
Factor codes	Imatinib_400 Imatinib 400

	Factors	
	Imatinib_400	
Sample size	19	
Median survival	27	
Survival time	Survival Proportion	Standard Error
3	0,947	0,0512
6	0,789	0,0935
13	0,684	0,107
21	0,632	0,111
22	0,579	0,113
24	0,526	0,115
27	0,474	0,115
30	0,421	0,113
36	0,316	0,107

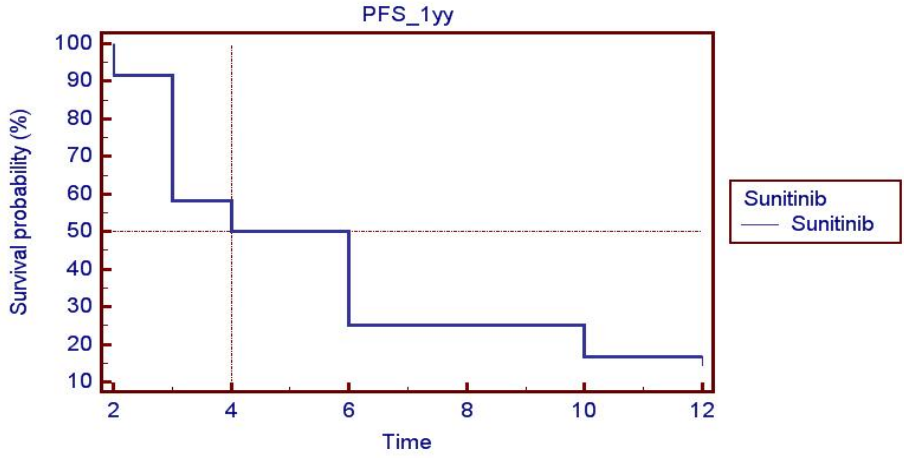
It is interesting to point out that patients with disease progression after a few months of treatment were not carrying exon 11 mutation.
No relevant toxicity occurred during the Imatinib treatment with standard dosage. The most common collateral effect was, as it is expected to be, periorbital edema. Dosage reduction or cure interruption, have never been necessary.
Progressing patients with Imatinib 400mg/day (15) have then received Imatinib 800mg/day except for patient 51 who received a protein kinases C inhibitor, and patient 63 who received Sunitinib. The increase of Imatinib dosage proved to be useful since it delayed the progression of the disease even in patients carrying mutations that were different from those in exon 11. PFS under Kaplan-Meier, calculated on patients having a treatment follow-up since at least a year (15) shows a nine months mediane:



Survival time	Time
Endpoint	PFS_1yy
Factor codes	Imatinib_800

	Factors	
	Imatinib_800	
Sample size	15	
Median survival	9	
Survival time	Survival Proportion	Standard Error
2	0,933	0,0644
3	0,867	0,0878
4	0,800	0,103
5	0,667	0,122
6	0,533	0,129
9	0,400	0,126
12	0,267	0,114

The tolerance of the treatment is lower than the standard dose, the most common collateral effects have been periorbital edema, hematologic alterations.
In progressing patients with Imatinib 800mg/day (12) the treatment with Sunitinib 37,5mg/day (Sutent) started – a treatment that was recorded for progressing metastatic GIST or Imatinib patients. Today Sunitinib is daily taken. Patient 47 was taking Sunitinib under 4+2 schedule, which, even though more tolerated, did not prove to be advantageous in GIST for the effect of interval progression.
Sunitinib has significantly shown, despite its lower tolerability against Imatinib, to extend PFS. Last studies seem to suggest that it is more effective in patients carrying mutation of c-KIT in exon 9. The data we acquired on two patients carrying exon 9 mutation actually prove that the response in terms of PFS has been superior to Sunitinib in second line, rather than to Imatinib 400mg/day in first line.
PFS in patients being treated with Sunitinib since at least six months was the following:



Survival time	Time
Endpoint	PFS_1yy
Factor codes	Sunitinib

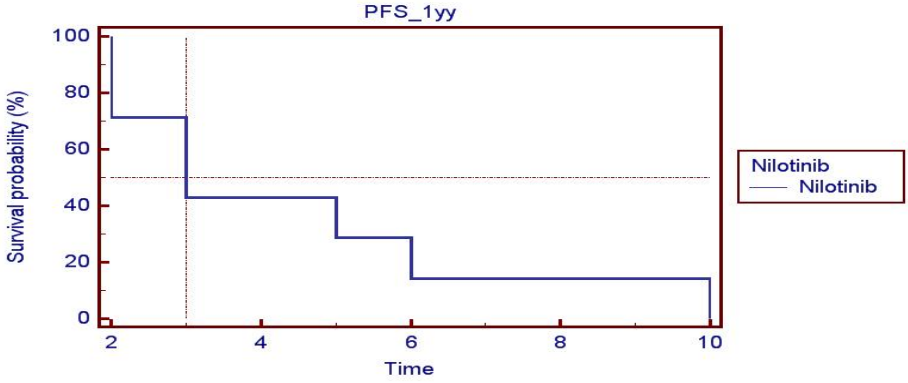
	Factors	
	Sunitinib	
Sample size	12	
Median survival	4	
Survival time	Survival Proportion	Standard Error
2	0,917	0,0798
3	0,583	0,142
4	0,500	0,144
6	0,250	0,125
10	0,167	0,108
12	-	-

Most common collateral effects were thrombocytopenia, hematologic alterations, mucositis, hand-foot syndrome. The temporary suspension of the

treatment was necessary in one patient because of toxicity. The dosage reduction was not followed by clinic improvements. Patients 1 and 62 interrupted the treatment for two months for surgical operation (for other causes).

At the moment there is no indication about a third-line treatment. Patients progressing during Sunintinb treatment have the chance to be enlisted in a clinic trial or the Imatinib rechallenging. Our center acquired experience with protein kinases C inhibitors, with Nilotinib (a new-generation TK inhibitor) and Imatinib in re-challenging.

Seven patients have been treated with Nilotinib 800mg/day, which proved a remarkable anti-tumoral activity and a high tolerability, and recently showed a better efficiency than Imantinib in treating Chronic Myeloid Leukemia and is in trial against Imatinib for the first-line metastatic GIST treatment. However, the medicine did not prove the expected third-line efficiency and does not seem to bring OS improvements. During our experience, thanks to a compassionate utilization program, we obtained partial short-lasting responses, followed by a SUV reduction in PET investigation. The PFS after seven months showed a three-months mediane:



Survival time	Time
Endpoint	PFS_1yy
Factor codes	Nilotinib

	Factors	
	Nilotinib	
Sample size	7	
Median survival	3	
Survival time	Survival Proportion	Standard Error
2	0,714	0,171
3	0,429	0,187
5	0,286	0,171
6	0,143	0,132
10	0,000	0,000

Picture 40 Metastatic GIST in response to third-line Nilotinib treatment

The rechallenging with Imatinib, by our experience (counting only three patients) seems to have only a palliative role, that can decelerate the disease progression.

DISCUSSION

The data we obtained are in line with the literature data concerning the average starting age, the location and the frequency of mutations. RFS data show that Miettinen's risk stratification has a better discriminative capacity ($p<0,05$) between patients at higher and lower relapse risk against NIH/Fletcher stratification. Moreover, the data about the relapse onset, which occurred for primitive tumors in small intestine in 10/16 cases, confirm that the location parameter is an important prognostic factor, inseparable from the dimension and the mitotic count of the primitive tumor.

Patients that went through R1 surgery for localized GIST did not show a significant relapse increment after three years against patients treated with R0 surgery.

Neo-adjuvant treatment with Imatinib 400mg/day can increase the chances of an R0 surgery and should always be considered as an alternative option to primary surgery for big tumors.

Our data about the efficacy of the adjuvant therapy confirm its importance in increasing the chances of relapse-free survival.

Patients with a single-location non-metastatic relapse can benefit from a new surgical treatment and following Imatinib 400mg/day therapy.

Tyrosine kinase inhibitors treatment is to be chosen for metastatic or relapsed with metastasis patients, except for some strictly selected cases in which surgery can actually represent a recovery chance (e.g. single-location relapse). In these cases an Imatinib neo-adjuvant treatment is proved to be useful, followed within 6 and 12 months by relapse resection.

The preliminary data about the mutational analysis realized on relapsed patients show a predominance of exon 11 alterations that, even though this mutational pattern ensures today the best response to the target Imatinib therapy in metastatic setting, seems to be a negative prognostic factor in terms of relapse. For this reason, the genetic analysis should be a standard of the diagnostic-staging iter of the patient with localized and resectable GIST, as well as of the metastatic patient as the guidelines say, as it is able to provide another prognostic parameter of the relapse risk and of the efficiency evaluation of an adjuvant treatment.

CONCLUSIONS

In conclusion, since KIT and PDGFRα mutations have therapeutic and prognostic roles, GIST genotyping should be considered as a standard clinic test both for resected, relapse-risk or metastatic tumors, in order to provide an eventual adjuvant treatment, for prognostic evaluation in metastases setting and to exclude the resistant GISTs. The subsequent data about this subject, together

with the increasing availability of target therapies will enable, in the future, individualized therapy – in order to achieve the best benefit for the patient.

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FELLOWSHIPS AND AWARDS

Da Gennaio 2008: Dottorato di ricerca triennale in Oncopatologia cellulare e molecolare presso l'Università degli Studi di Palermo.

Da 1 Settembre 2008 al 31 Agosto 2009: Vincitrice di borsa di studio della durata di un anno della Lega Italiana per la Lotta contro i Tumori ente pubblico con sede in Roma per lo svolgimento di progetto di ricerca relativo a "Sorveglianza diagnostica di donne ad alto rischio eredo-familiare di tumore mammario"

PROFESSIONAL CAREER

Da Gennaio a Giugno 2008 Attività di Medico volontario presso l'U.O di Oncologia Medica dell'Ospedale Buccheri La Ferla di Palermo

Da 1 Settembre 2008 al 31 Agosto 2009: Svolgimento di borsa di studio della durata di un anno della Lega Italiana per la Lotta contro i Tumori ,sezione provinciale di Agrigento

Dal 23 Settembre 2008 al 18 maggio 2009: Medico Volontario in forza presso l'U.O.S. di Oncologia Medica dell'Ospedale 'Giovanni Paolo II' di Sciacca (AG)

Dal 19 Maggio 2009 al 19 Gennaio 2010 : Contratto di Collaborazione Coordinata e Continuativa presso l'U.O.S. di Oncologia Medica dell'Ospedale 'Giovanni Paolo II' di Sciacca (AG) della durata di un anno (termine 18 Maggio 2010)

Dal 19 Gennaio 2010 a 30 Giugno 2010: servizio non di ruolo per Incarico presso l'Azienda Sanitaria Provinciale Agrigento in qualità di Dirigente medico di I livello presso l'Oncologia medica di Sciacca.

Dal 12 Luglio 2010 a tutt'oggi: servizio non di ruolo per Incarico presso l'Azienda Sanitaria Provinciale Agrigento in qualità di

Dirigente medico di I livello presso l'Oncologia medica di Sciacca

SCIENTIFIC ACTIVITIES AND ORAL PRESENTATIONS

- Nicolò Borsellino, Armando Bilello, Francesca Spinnato. *Chemotherapy-induced peripheral neurotoxicity: clinical aspects and current therapeutic options.* Supportive and Palliative Cancer Care 4,3:83-90,2008

- Francesca Spinnato. *Inibitore dell'aromatasi e Herceptin*, Recenti progressi in Oncologia,website www.oncologia.recentiprogressi.it, 7 Dicembre 2009

- *Corso di Aggiornamento "Recenti acquisizioni in tema di Mieloma Multiplo e Cancro della Mammella"* 28 Giugno 2008 Agrigento. In qualità di relatrice

- *Progetto editoriale ALTOP – ALgoritmo Terapeutico in Oncologia Polmonare*, Calatabiano (CT) 30 Ottobre 2009. In qualità di relatrice

- Consulenza scientifica per il Protocollo GOIM n. 2611: *"Trastuzumab plus gemcitabine and docetaxel in advanced breast cancer: a phase II study".* In qualità di relatrice

- Progetto Alice: *Meeting Editoriale in Breast Cancer*- Palermo, 27 Novembre 2009. In qualità di relatrice.

- *Percorsi diagnostico-terapeutici in ematologia* Trapani 14-15-21-22 Maggio 2010. In qualità di relatrice

- *Fenotipo Neuroendocrino nel Carcinoma Prostatico*, Monreale 9 Luglio 2010. In qualità di relatrice

- *Carcinoma del Colon-retto e anemia: nuove prospettive terapeutiche*, Castelvetrano, 16 ottobre 2010
- In qualità di relatrice.

- *Corso di aggiornamento in oncologia per medici non specialisti*, 25 settembre 2010 Ordine dei Medici Agrigento 25 settembre 2010. In qualità di relatrice