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Thesis

MUTATIONAL PROFILE OF CYPRIOT PATIENTS WITH GIST: RESULTS FROM A 15-YEAR COHORT

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Abstract

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal (GI) tract. These tumors are usually divided into mutant or wild-type based on the presence or absence of activating mutations in *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*) genes. Downstream signaling pathways activated by gain-of-function mutations are crucial in GIST development and affect the clinical prognosis and treatment decisions. Although the use of tyrosine kinase inhibitors (TKIs), like imatinib, have a major impact on the overall survival of *KIT/PDGFRA* mutant patients in both adjuvant and metastatic settings, about 10-15% are imatinib-resistant. Since molecular characterization has a pivotal role in the overall management of GISTs, we aimed to analyze a cohort of 105 patients diagnosed with GIST in the Republic of Cyprus, treated at the Bank of Cyprus Oncology Centre between 2008 and 2023. The mutational profile of a total of 74 formalin-fixed paraffin-embedded tumors was analyzed by next-generation sequencing (NGS) or Sanger sequencing. The most common mutation found was in *KIT* exon 11 accounting for 52,63% followed by *PDGFRA* exon 18 accounting for 10,53%. No mutations were detected in 22,37% of cases. Interestingly, we found mutations in *KRAS, SMO,* and *RET* genes that were not previously described. GISTs with *KIT* and *PDGFRA* mutations were predominantly located in the stomach, and showed spindle cell phenotype, while rare mutations were of omentum epithelioid origin with epithelioid features and high risk of malignant potential. Adverse prognostic factors included the high mitotic index and late-stage disease status at diagnosis. Collectively, the scientific progress in understanding the molecular basis of GISTs justifies the importance of knowing the mutations, if any, to aim for a more personalized approach to treating GIST patients.

APPROVAL PAGE

Master of Science Thesis

MUTATIONAL PROFILE OF CYPRIOT PATIENTS WITH GIST:

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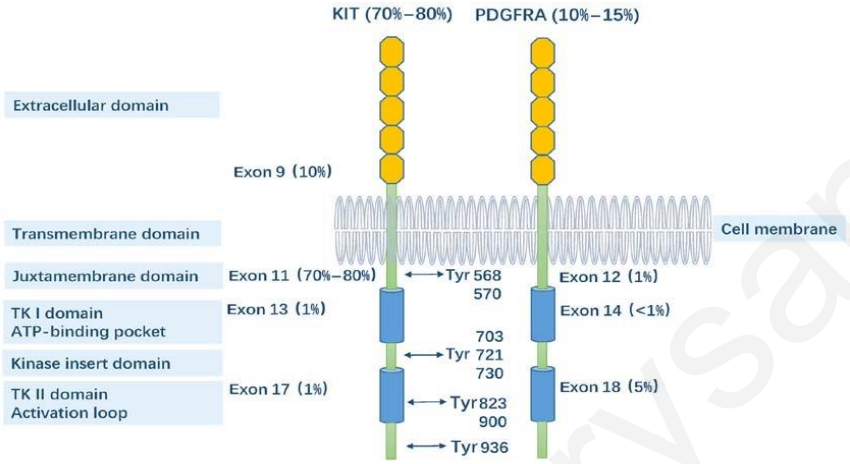

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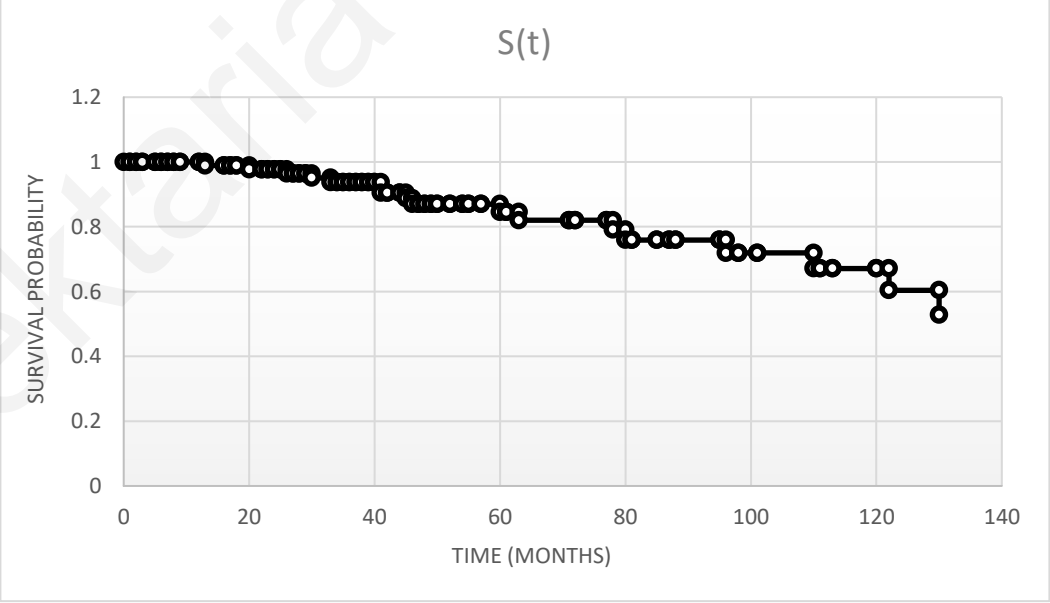

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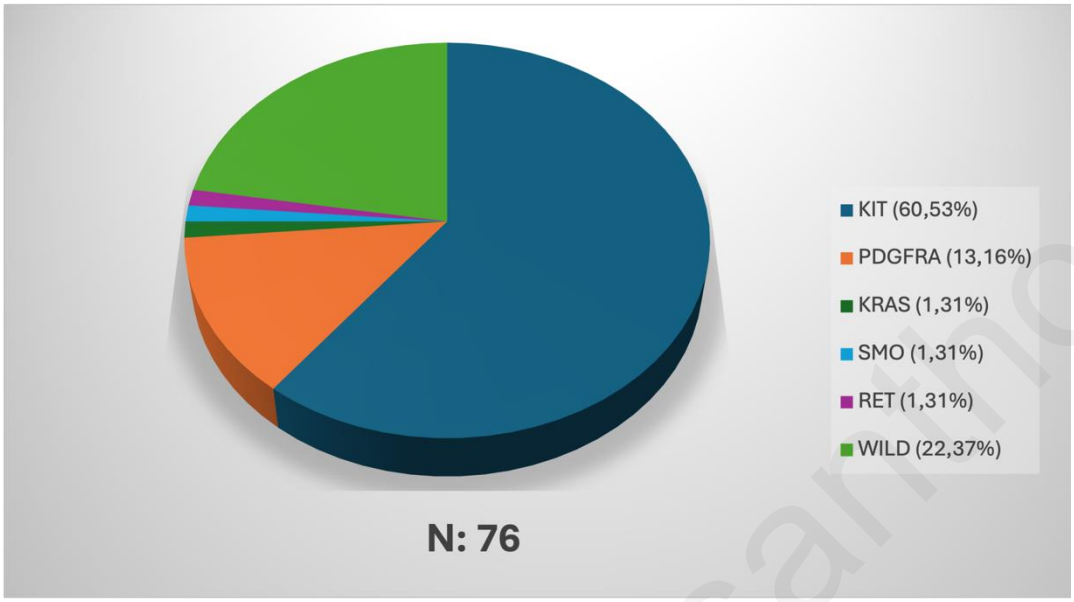

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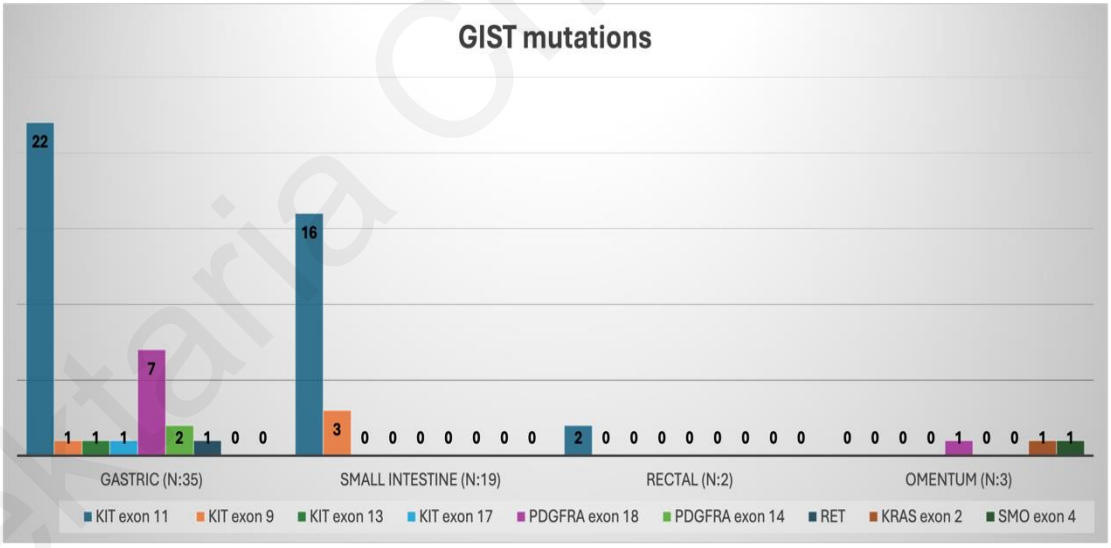

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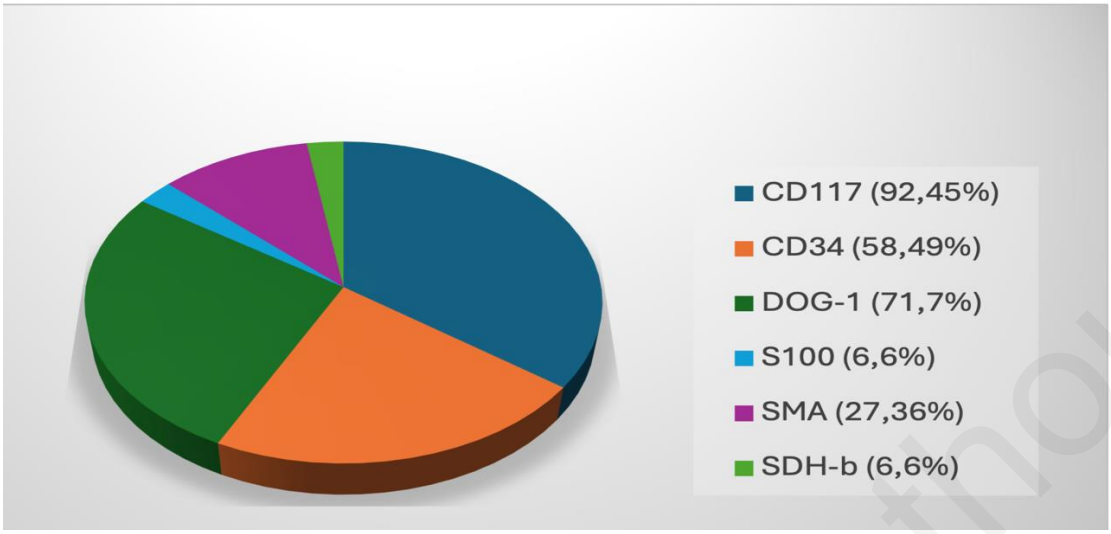

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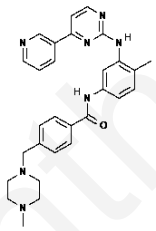
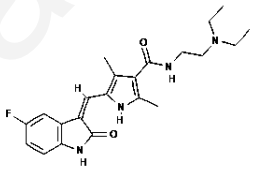
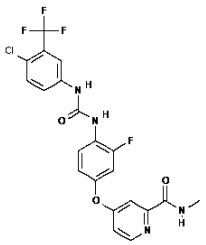
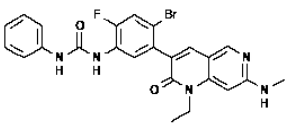
Drug	Molecular targets	Setting tested	Common dose	Frequent adverse events	Chemical structure
Imatinib (Gleevec®)	*KIT, PDGFRA*	First line	400mg	Nausea, diarrhoea, headaches, leg cramps, fluid retention visual disturbances	
Sunitinib (Sutent®)	*KIT, PDGFRA, VEGFR, RET*	Second line	37,5mg	Anaemia, neutropenia, fatigue, diarrhoea, skin discoloration, nausea, anorexia	
Regorafenib (Stivarga®)	*KIT, PDGFRA, RET, BRAF, VEGFR1-3, FGFR*	Third line	160mg	Skin reaction, hypertension, diarrhoea	
Ripretinib (Qinlock®)	*KIT, PDGFRA*	Fourth line	150mg	Alopecia, nausea, fatigue, diarrhoea, myalgia	

Table 2: Patients' basic characteristics.

	Men	**Women**
Cases	61/105	44/105
Mean age	64	59
Tumor size (min-max)	1-19 cm	1,6-20 cm
Mitosis (range)	0-18/50 HPF	0-50/50 HPF
Location		
Small Intestine	20	15
Stomach	38	29
Rectum	2	-
Omentum	1	-

Table 3: KIT mutations.

Gender	Age	Location	Size	Histology	Mitotic rate	NIH Risk	Sequencing Method	Mutated Gene	Exon	Mutation Description
M	74	SMALL INTESTINE	3	SPINDLE	10/50 HPF	HIGH	SANGER	KIT	11	c.1679T>A/ p.Val560Asp
M	64	GASTRIC	4	SPINDLE	5-7/50 HPF	INTERMEDIATE	SANGER	KIT	11	c.1679-1680delinsAG / p.Val560Asp
M	66	GASTRIC	5.5	SPINDLE	18/50 HPF	HIGH	SANGER	KIT	11	c.1667_1693del27insCTCCC, p.GlnProfsX9. + c.1667A>G
F	52	SMALL INTESTINE	13	MIXED	50/50 HPF	HIGH	SANGER	KIT	11	c.1669_1674delTGGAAG, p.Trp557_Lys558del
F	59	SMALL INTESTINE	15	SPINDLE	10-13/50 HPF	HIGH	SANGER	KIT	11	c.1662_1676del, p.Val555_Val559del
M	63	SMALL INTESTINE	12	MIXED	21/50HPF	NA	SANGER	KIT	11	p.Leu576Pro
M	75	SMALL INTESTINE	13	SPINDLE	25/10HPF	HIGH	SANGER	KIT	11	p.Val559Asp
M	65	SMALL INTESTINE	12	SPINDLE	13/50HPF	NA	SANGER	KIT	11	p.Val559Gly
M	63	GASTRIC	7	EPITHELIOID	14/50HPF	HIGH	SANGER	KIT	17	p.Arg815Lys
M	49	GASTRIC	7.5	SPINDLE	5/50HPF	INTERMEDIATE	SANGER	KIT	11	p.Asp572_Pro573dup
M	63	SMALL INTESTINE	8	SPINDLE	2/50HPF	HIGH	SANGER	KIT	11	p.Val559Gly
M	80	DUODENUM	4.2	EPITHELIOID	0	LOW	SANGER	KIT	11	p.Leu576Pro
F	59	SMALL INTESTINE	7	SPINDLE	5/50HPF	INTERMEDIATE	SANGER	KIT	11	p.Val560Asp
M	61	GASTRIC	18	SPINDLE	>10/HPF	NA	SANGER	KIT	11	p.556_561del
M	56	SMALL INTESTINE	12	SPINDLE	1/50HPF	HIGH	NGS	KIT	11	p.Val559del
M	59	RECTAL	5.8	SPINDLE	<5/50HPF	HIGH	SANGER	KIT	11	p.Lys558_Val559delinsAsn
M	78	SMALL INTESTINE	17	MIXED	2/50HPF	HIGH	SANGER	KIT	11	p.Val560Asp
M	65	GASTRIC	7.5	MIXED	15/50HPF	HIGH	SANGER	KIT	11	p.Val559Gly
M	70	GASTRIC	5.5	SPINDLE	>5/50HPF	HIGH	SANGER	KIT	11	p.Trp557_Val560delinsPhe
M	87	GASTRIC	12.6	SPINDLE	15/50HPF	NA	SANGER	KIT	11	p.Met552_Lys558del
F	51	DUODENUM	3	SPINDLE	7/50HPF	INTERMEDIATE	SANGER	KIT	9	p.Ala502_Tyr503dup
M	81	GASTRIC	10	SPINDLE	10/50HPF	HIGH	SANGER	KIT	11	p.Trp557_Lys558del
M	74	GASTRIC	12	EPITHELIOID	7-8/50HPF	HIGH	SANGER	KIT	11	p.Val559Gly

Table 3: Continued.

Gender	Age	Location	Size	Histology	Mitotic rate	NIH Risk	Sequencing Method	Mutated Gene	Exon	Mutation Description
F	72	GASTRIC	4.5	SPINDLE	10/50HPF	INTERMEDIATE	SANGER	KIT	11	p.Val555_Gln556del
M	67	GASTRIC	6	SPINDLE	7/50HPF	HIGH	SANGER	KIT	11	p.Trp557_Lys558del
M	54	SMALL INTESTINE	3	EPITHELIOID	1-2/50HPF	LOW	SANGER	KIT	11	p.Val560del
F	55	GASTRIC	20	SPINDLE	0	HIGH	SANGER	KIT	11	p.Trp557_Lys558del
F	63	SMALL INTESTINE	11	SPINDLE	5/10HPF	HIGH	SANGER	KIT	9	p.Ala502_Tyr503dup
M	53	GASTRIC	6	SPINDLE	4/50HPF	INTERMEDIATE	SANGER	KIT	11	p.Val559Asp
M	61	SMALL INTESTINE	9.5	EPITHELIOID	>5/50HPF	HIGH	NGS	KIT	11	p.Asp579del
M	50	RECTAL	7.9	SPINDLE	>5/50HPF	HIGH	NGS	KIT	11	p.Lys558delinsAsnPro
F	57	SMALL INTESTINE	7	SPINDLE	<5/50HPF	HIGH	SANGER	KIT	11	p.Trp557Gly
M	77	SMALL INTESTINE	14.5	EPITHELIOID	1/50HPF	NA	NGS	KIT	9	p.Ser501_Ala502insAlaTyr
F	57	GASTRIC	4.5	SPINDLE	7-8/50HPF	INTERMEDIATE	NGS	KIT	11	p.Trp557Gly
M	62	SMALL INTESTINE	14	SPINDLE	>5/50HPF	NA	NGS	KIT	11	p.Val559Gly
M	75	SMALL INTESTINE	19	SPINDLE	>5/50HPF	NA	NGS	KIT	11	p.Lys558_Val560delinslle
M	36	GASTRIC	12	SPINDLE	<5/50HPF	INTERMEDIATE	NGS	KIT	11	p.Asp579del
M	76	GASTRIC	5	SPINDLE	9/50HPF	INTERMEDIATE	NGS	KIT	11	p.Val560Glu
F	75	GASTRIC	6	SPINDLE	<1/50HPF	INTERMEDIATE	NGS	KIT	11	p.Val559del
M	73	GASTRIC	3	SPINDLE	<5/25HPF	LOW	NGS	KIT	11	p.Val559Asp
M	80	DUODENUM	8	SPINDLE	0	HIGH	NGS	KIT	13	p.Lys642Glu
F	66	GASTRIC	3	SPINDLE	2/50HPF	LOW	NGS	KIT	11	p.Val560Asp
F	65	GASTRIC	4.4	SPINDLE	1-3/50HPF	LOW	NGS	KIT	9	p.Ser501_Ala502insAlaTyr
F	74	GASTRIC	3.3	SPINDLE	<5/20HPF	LOW	NGS	KIT	11	p.Asp579_His580insLeuAspProThrGlnLeuProTyrAsp (D579_H580insLDPTQLPYD)
M	83	GASTRIC	7	SPINDLE	2-4/50HPF	INTERMEDIATE	NGS	KIT	11	p.Trp557Arg
M	25	SMALL INTESTINE	7	SPINDLE	7/50HPF	HIGH	NGS	KIT	11	p.Pro573_Thr574dup

Table 4: PDGFRA mutations.

Gender	Age	Location	Size	Histology	Mitotic rate	NIH Risk	Sequencing Method	Mutated Gene	Exon	Mutation Description
M	42	GASTRIC	5.5	EPITHELIOID	1/50HPF	INTERMEDIATE	SANGER	PDGFRA	18	p.Asp842Val
M	72	GASTRIC	14	EPITHELIOID	>5/50HPF	HIGH	SANGER	PDGFRA	18	p.Asp842Val
F	66	GASTRIC	8	SPINDLE	<5/50HPF	INTERMEDIATE	SANGER	PDGFRA	14	p.Asn659Lys
F	76	GASTRIC	3.5	EPITHELIOID	1/50HPF	LOW	NGS	PDGFRA	18	p.Asp842Val
M	72	OMENTUM EPITHELIOID EXTRAGASTROINTESTINAL STROMAL TUMOR	14	EPITHELIOID	2/50HPF	HIGH	NGS	PDGFRA	18	p.Asp842Val
F	55	GASTRIC	8	EPITHELIOID	2/50HPF	INTERMEDIATE	NGS	PDGFRA	14	p.Asn659Lys
F	49	GASTRIC	4	SPINDLE	>5/50HPF	INTERMEDIATE	NGS	PDGFRA	18	p.Asp842Val
M	72	GASTRIC	4	SPINDLE	2/50HPF	LOW	SANGER	PDGFRA	18	p.Asp842Val
M	70	GASTRIC	5.2	SPINDLE	1-2/50HPF	LOW	NGS	PDGFRA	18	p.Asp842Val
F	75	GASTRIC	4.5	NA	2/50HPF	LOW	NGS	PDGFRA	18	p.Asp842Val

Table 5: Other mutations.

Gender	Age	Location	Size	Histology	Mitotic rate	NIH Risk	Sequencing Method	Mutated Gene	Exon	Mutation Description
M	90	GASTRIC	5.8	SPINDLE	0	INTERMEDIATE	NGS	CCDC6(1)-RET(12)	NA	CCDC6(1)-RET(12) FUSION
M	72	OMENTUM EPITHELIOID EXTRAGASTROINTESTINAL STROMAL TUMOR	14	EPITHELIOID	2/50HPF	HIGH	NGS	KRAS	2	p.Gly13Ser
M	72	OMENTUM EPITHELIOID EXTRAGASTROINTESTINAL STROMAL TUMOR	14	EPITHELIOID	2/50HPF	HIGH	NGS	SMO	4	p.Arg290Cys

List of Abbreviations

ABL: Abelson murine leukemia viral oncogene homolog 1

AKT: protein kinase B

ALK: Anaplastic lymphoma kinase

AR: Androgen receptor

BRAF: B-Raf serine/threonine-protein kinase

CCDC6: coiled-coil domain containing 6

CDK: Cyclin-dependent kinase

CTNNB: Catenin beta

DDR: DNA damage response

EGFR: Epidermal growth factor receptor

ERBB: Erythroblastic leukaemia viral oncogene

ERG: ETS-related gene

ESR: Oestrogen receptor

ETV1: ETS variant transcription factor 1

FGFR: Fibroblast growth factor receptor

GIST: gastrointestinal stromal tumor

GNA: Guanine nucleotide-binding protein

GNAQ: G protein subunit alpha Q

HPF: high-power field

HRAS: Harvey Rat sarcoma virus

IDH: Isocitrate dehydrogenase

JAK: Janus kinase

KIT: KIT proto-oncogene

KRAS: Kristen Rat sarcoma viral oncogene homolog

MAPK: Mitogen-activated protein kinases

mTOR: mammalian target of rapamycin

NFI 1/2: Neurofibromatosis type 1/2

NGS: Next-generation sequencing

NRAS: Neuroblastoma RAS viral oncogene homolog

NTRK: Neurotrophic tyrosine kinase

PDGFRA: Platelet-derived growth factor alpha

PI3K: Phosphatidylinositol 3-kinase

PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3- Kinase Catalytic Subunit Alpha

PLC-C: Phospholipase C pathway

PPARG: Peroxisome proliferator-activated receptor gamma

RET: Rearranged during transfection

RTK: Receptor tyrosine kinase

SCF: Stem cell factor

SDH: Succinate dehydrogenase complex

SMA: smooth muscle actin

SMO: smoothened protein

STAT: Signal transducers and activators of transcription

TKI: Tyrosine kinase inhibitor

VEGFR: Vascular endothelial growth factor receptor

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract, accounting for 2-3% of all gastric cancers (Liu & Chu, 2019). In 1983, a group of investigators first recognized GISTs as "stromal tumors" aiming to describe a broad spectrum of gastric wall malignancies (Mavroeidis et al., 2018). They showed that many tumors previously diagnosed as GI leiomyomas or leiomyosarcomas lacked smooth muscle or Schwann cell differentiation, suggesting an origin from the myenteric nervous system (Miettinen & Lasota, 2006). This finding gained further attention from a study of GISTs originating in the Carney triad syndrome, in which researchers noticed signs of neuroectodermal differentiation and proposed the cells of origin were the interstitial cells of Cajal, which are the pacemakers of the gastrointestinal movement (Schaefer et al., 2017). GISTs can be divided into mutant and wild type depending on the presence or absence of mutations in c-kit (encoded by the *KIT* proto-oncogene) and in platelet-derived growth factor receptor alpha (*PDGFR-A*). The presence of such mutations leads to the activation of downstream signaling pathways that play a crucial role in the development of GISTs and affect the clinical prognosis, diagnosis, and treatment of patients (Ding et al., 2020). GIST epidemiological studies showed that the estimated mean age at diagnosis is 65 and the prevalence is equal between men and women (Akahoshi et al., 2018). The frequency of occurrence is 10-15 cases per million per year. Although most of those cases are sporadic, there is a possibility of association with genetic syndromes such as familial GIST, neurofibromatosis type 1, Carney's triad, and Carney-Stratakis triad (Mavroeidis et al., 2018). Families carrying germline autosomal dominant mutations of KIT or PDGFRA are exceptionally rare and are associated with multiple GISTs at an early age potentially accompanied by other clinical characteristics (Casali et al., 2022).

Approximately 60% of GISTs originate in the stomach, followed by the small intestine (~30%), colon, and rectum (~5%) and rarely the esophagus (<1%) (Liu & Chu, 2019). Clinically, three crucial factors affect the prognosis of GIST: the size of the tumor, the location of the tumor, and the mitotic index (Liu & Chu, 2019). Generally, surgical R0 resection is the primary approach for localized GISTs without metastasis (Akahoshi et al., 2018). Although, most of the time,

GISTs arising from the stomach that are smaller than 5cm in size and have a low mitotic index (<5/50 HPF) are associated with a better prognosis, they can rarely show a malignant course (Liu & Chu, 2019). Malignant GISTs were previously viewed as treatment-resistant tumors, with only a few patients showing clinical response to conventional chemotherapy and/or radiation therapy. Still, now it is widely accepted that adjuvant and/or neoadjuvant treatment with tyrosine kinase inhibitors like imatinib can and has been used as a targeted therapy for the successful treatment of these tumors (Søreide et al., 2016). The application of imatinib was established in 2001. It was opted for the treatment of inoperable and/or metastatic GISTs (Liu & Chu, 2019). The use of imatinib as a chemotherapeutic drug is attributed to the discovery of mutations in *KIT*, as almost 80% of GIST patients contain a gain-of-function mutation in this gene. Unfortunately, although patients respond to imatinib treatment at the beginning, they then develop resistance to the drug, often attributed to secondary mutations, and will have to undergo an alternative treatment as a second option (Mavroeidis et al., 2018). In addition to *KIT*, about 10% of GIST patients show mutations in *PDGFR-A*, while the rest are classified as wild-type (Liu & Chu, 2019).

Since the prevalence of mutations in *KIT* and *PDGFRA* varies considering ethnic and geographical variations, it is crucial to identify genetic aberrations among different populations to identify resistance mechanisms in the mutational profile of GIST patients. Thus, we conducted a retrospective analysis of patients diagnosed with GIST within the last 15 years in Cyprus. We used next-generation sequencing (NGS) technology and SANGER sequencing to identify the mutational status of *KIT* and *PDGFRA* as well as other molecular biomarkers in the Cypriot population to investigate their significance in terms of prognosis, diagnosis, and treatment of this rare malignancy.

Histogenesis and Histopathological Findings

GISTs are thought to originate from the interstitial cells of Cajal, which regulate the gastrointestinal movement and the autonomic nervous system function. They are located

throughout the GI tract and include stem-cell-like cells with multipotency to differentiate into smooth muscle cells if KIT signaling is disrupted. Gain-of-function mutations in the *KIT* proto-oncogene lead to the cellular proliferation of Cajal cells and ultimately to the development of GISTs, as shown by transgenic mouse models introduced to human *KIT*-activating mutations (Miettinen & Lasota, 2006). These observations show that cellular proliferation is essential for a particular *KIT* mutation to have transforming activity. Many GISTs also depend on the lineage-specific transcription factor (ETV1) (Schaefer et al., 2017). ETV1 is required for the development of the interstitial cells of Cajal that depend on *KIT* signaling. Thus, ETV1 is important for regulating those cells and essential in GIST growth. Besides its role in GIST, ETV1 is also responsible for activating other GIST biomarkers through the RAS/RAF/MEK signaling pathway (Schaefer et al., 2017). More specifically, in GIST, *KIT* signals through this pathway to stabilize ETV1 and promote tumor growth. Treatment with KIT inhibitors leads to proteasomal degradation of ETV1, thus causing tumor growth arrest (Schaefer et al., 2017). Regarding morphology, GISTs usually appear as submucosal tumors in the GI tract and display either a spindled (70%), epithelioid (20%), or mixed (10%) shape (Schaefer et al., 2017). The malignant potential of GISTs is based on the histopathologic criteria which help to identify patients at risk of local recurrence or distant metastases. The risk stratification introduced by Fletcher et al. is expressed by classification into low, intermediate, and high-risk categories based on the size of the tumor and the mitotic rate.

KIT and PDGFRA Mutations

Among GIST patients, approximately 80% of them harbor mutations in the *KIT* proto-oncogene while 10% show mutations in *PDGFR-A* (Liu & Chu, 2019). *KIT* and *PDGFR-A* are type III receptor tyrosine kinases (RTKs), characterized by five immunoglobulin-like extracellular domains (Liu & Chu, 2019). The role of RTKs is crucial for cellular homeostasis and their dysfunction affects a cascade of downstream signaling pathways that are linked to many disorders, including cancer (Sheikh et al., 2022) The c-Kit receptor is encoded by the long-arm

position of chromosome 4 (4q11-4q12) of the *KIT* proto-oncogene. In humans, four c-kit isoforms have been identified, which are critical for cellular homeostasis and differentiation. Signaling pathways activated by c-Kit include MAPK/ERK, which leads to gene transcription regulation and cellular proliferation, PI3K/AKT, which promotes cell survival and evasion of apoptosis, PLC-C, which is involved in signal transduction, JAK/STAT, which regulates gene transcription and SRC, which promotes the activation of c-Kit (Sheikh et al., 2022). More than 500 different *KIT* mutations have been found in human tumors but only a few are thought of as driver mutations (Ding et al., 2020). After c-Kit binds to its receptor, stem cell factor (SCF) induces receptor dimerization leading to the activation of tyrosine kinase, creating sites for signaling molecules that contain the SH2 homologous domain. The SH2 domain consists of about 100 amino acids and regulates cell growth by binding to tyrosine residues. Phosphorylated tyrosine along amino acid residues forms a binding site for downstream signaling molecules leading to the activation of various signaling pathways including MAPK and PI3K/AKT (Ding et al., 2020). Mutations in c-Kit, especially those that cause a gain-of-function effect, are associated with the development of several types of cancers, including GIST, melanoma, and acute myeloid leukemia. In GIST, the most common c-Kit mutations involve exon 11 (the juxta membrane domain) and occur between codons 550 and 560 at the 5'end. The most frequently observed type of mutation is deletion at codon 557-558, followed by deletions at codon 559 and point mutations resulting in V575A (Ding et al., 2020). Exon 11 mutations that involve codons 557-558 deletion have been shown to increase the malignant potential and reduce the progression-free survival of patients. This was confirmed by a study in European patients with GIST and has since been used as a reference for a less favorable prognosis (Wozniak et al., 2014). Missense mutations in exon 11 are observed in about 20-30% of GISTs and they mostly involve codons 557, 559, and 560 in the proximal part and codon 576 in the distal part of exon 11. These mutations seem to have a better prognosis than exon 11 deletions (Miettinen & Lasota, 2006). Tandem duplications are rare and are seen in the distal part of exon 11. Although rare, they often occur in gastric GISTs and are associated with a favorable outcome (Miettinen & Lasota, 2006). According to data from a meta-analysis, patients from Asia, Europe, and the

USA with *KIT* exon 11 mutations show improved responses to therapy and have a higher overall survival compared to patients who bear *KIT* exon 9 mutations and those who lack any *PDGFRA* or *KIT* mutations (Miettinen & Lasota, 2006). Other mutations occur in exon 9 (the extracellular dimerization domain) and are mainly caused by six nucleotide tandem duplications encoding Ala502-Tyr503 (Lux et al., 2000). These mutations are almost specific to intestinal GISTs, and they are reported with a frequency of 5-13% with a higher risk of recurrence and metastasis (Sheikh et al., 2022). Although mutations in exon 9 were thought to be more common in the small intestine, a study of Japanese patients revealed that 75% of them possessed a gastric GIST. This may be related to different ethnicities (Ding et al., 2020). Screening for exon 9 mutations in GIST seems important since these get a higher dose of imatinib to be effective. Typically, primary mutations occur in exons 9 and 11, whereas secondary mutations are more frequent in exons 13 and 17. Most mutations in exon 13 are K642E mutations caused by a base substitution of codon 561 for 642 with a very low frequency of 1-2% (Miettinen & Lasota, 2006). This mutation results in continuous activation of tyrosine phosphorylation, activating specific signaling pathways and promoting cell proliferation. Finally, the majority of exon 17 mutations are N822K and lead to tyrosine phosphorylation (Ding et al., 2020).

PDGFRA mutations exist in about 10-15% of GIST cases and are more frequent in exon 12, exon 14, and exon 18. These regions correspond to *KIT* mutational regions exon 17, exon 11, and exon 13 (Miettinen & Lasota, 2006). *KIT* and *PDGFRA* mutations are mutually exclusive in GISTs and exhibit similar mechanisms of tumor progression. *PDGFRA* mutations affect the tyrosine kinase domain II and cause kinase activation by altering the activation loop, leading to downstream signaling pathways and thus promoting cell survival and proliferation. Signaling pathways affected by *PDGFRA* mutant GISTs are AKT, MAPK, STAT 1, and STAT 3. These pathways are also activated in *KIT*-mutant GISTs. Mutations in PDGFRA are primarily of epithelioid morphology and are mostly present in the stomach although, a small percentage of non-gastric GISTs with *PDGFRA* mutations have also been reported (Ding et al., 2020; Miettinen & Lasota, 2006) Immunohistochemical expression of c-Kit (CD117) in these tumors is either weak or absent. GISTs with *PDGFRA* mutations account for only 2,1% of metastases

and are less invasive (Emile et al., 2012; Lasota et al., 2004). More than 80% of *PDGFRA* mutations occur in exon 18 and are missense mutations, such as the D842V, resulting in the substitution of alanine for aspartic acid. This mutation leads to imatinib and sunitinib resistance, thus these patients do not benefit from tyrosine kinase inhibitors (Miettinen & Lasota, 2006). Regarding exon 12, V561A is the second most common type of *PDGFRA* mutation, while N659K in exon 14, is considered rare, but has a good prognosis according to clinical data (Ding et al., 2020). Mutational and phosphorylation sites for both *KIT* and *PDGFRA* are summarized in Figure 1. Despite the mutations mentioned, loss of heterozygosity on 22q is related to loss of expression of neurofibromatosis 2 (NF2), a tumor suppressor gene. Moreover, monosomies for chromosomes 14 and 22 are highly characteristic alterations for GISTs and can be detected cytogenetically (Gorunova et al., 2022). This suggests that not only mutations of *KIT* and *PDGFRA* but also chromosomal alterations are useful in the prognosis of GIST and affect the targeted therapy for these patients (Liu & Chu, 2019).

Wild-type GISTs

While oncogenic mutations in *KIT* and *PDGFRA* drive most GISTs, about 10% of them lack these mutations and are referred to as wild-type GISTs. These tumors are not as responsive to treatment with imatinib and have a poor prognosis (Liu & Chu, 2019). Wild-type GISTs are divided into succinate dehydrogenase complex (SDH)-deficient and non-SDH deficient. These are more commonly observed in young adults and about 85% of them are in the stomach. SDH is an enzyme complex in the inner mitochondrial membrane with four subunits (SDHA, SDHB, SDHC, and SDHD). Mutations in SDH-deficient GISTs are more common in the SDHA subunit which can be identified through immunohistochemistry (Ding et al., 2020). Non-SDH-deficient GISTs concern *NF1, BRAF,* and *RAS* gene mutations. Although non-SDH-deficient GISTs are like *KIT/PDGFRA* mutant GISTs, they are primarily located in the small intestine. *NF1* mutations are seen in younger adults and lesions are found in the duodenum and small intestine. These tumors are positive for CD117 and CD34. *NF1* mutation results in the constitutive activation of the RAS signaling pathway, which uses the MAPK pathway for downstream

receptor activation. Although *NF1* mutant GISTs do not respond to imatinib treatment, the knowledge of MAPK pathway involvement can be used for treatment with MEK inhibitors (Schaefer et al., 2017). Regarding *BRAF*, it is a serine/threonine protein kinase that belongs to the RAF family, which regulates the MAPK/ ERK signaling pathway. Mutations in *BRAF* cause uncontrolled cellular growth and proliferation and account for about 4% of wild-type GISTs (Ding et al., 2020). Although *BRAF* mutations appear only in a minority of wilt-type GISTs, they seem to be associated with notable clinicopathologic phenotype, observed in women in their 50s and mostly located in the small intestine (Agaram et al., 2008). BRAF V600E, located in exon 15, is the most common mutation, where valine is substituted for glutamic acid (Ding et al., 2020). This mutation was discovered in 2008 by Agaram et al. and accounts for 7-13 % of WT GISTs in Europe and the USA. *BRAF* mutations are resistant to treatment with imatinib, and although rare they can also cause secondary resistance when they occur as a secondary event in GISTs with *KIT/PDGFRA* mutations after relapse (Rossi et al., 2016). Concerning the *RAS gene*, mutations cause the GTP-binding domain to remain active, thus leading to tumorigenesis. Most *RAS* mutations are observed in codons 12 and 13 accounting for about 5% of GIST patients (Ding et al., 2020). Mutations in either *BRAF* or *RAS* genes may affect the response to imatinib treatment, thus a mutational analysis of these genes should be introduced in GIST patients (Ding et al., 2020). Interestingly, PIK3CA, a downstream lipid kinase effector of the KIT signaling pathway, has been reported to promote cellular growth and proliferation in GISTs acting through the PI3K/AKT/mTOR pathway (Lasota et al., 2016). PIK3CA mutations are rare but may have a role in wild-type GIST pathogenesis. Detection of these mutations is important for the selection of targeted therapy since they are imatinib-naïve and instead need selective inhibitors for the PI3K/AKT/mTOR pathway (Lasota et al., 2016).

Immunohistochemical features

Besides mutations and alterations, the key feature of GISTs is positivity for c-Kit (also referred to as CD117) through immunohistochemistry. This clinical information has become an

important diagnostic biomarker when used alongside morphological features displayed by GISTs and can aid in diagnosing and managing these tumors. CD117 is found positive in more than 95% of GIST cases, however, its expression is unrelated to the gene gain-of-function mutations, as a proportion of GIST patients that bear a mutation in the *KIT* proto-oncogene are negative for CD117 immunohistochemical expression (Liu & Chu, 2019). Other commonly expressed antigens, which are less GIST specific, include CD34, SMA, S100, and Desmin. CD34 is a hematopoietic progenitor cell antigen that is found positive in 80-85% of gastric GISTs while it is almost always found in GISTs of the esophagus and rectum (Miettinen & Lasota, 2006). SMA is positive in about 30% of gastric and intestinal GISTs and its expression is often shared with that of CD34. The positivity of SMA has been a favorable prognostic factor for GIST patients (Miettinen & Lasota, 2006). S100 protein expression is rare but more common in intestinal GISTs. Desmin, a muscle-type intermediate filament protein, is found positive mostly in esophageal and gastric GISTs but is generally a rare GIST marker (Miettinen & Lasota, 2006). In addition, DOG-1, a new gene that encodes for a protein of unknown function was exclusively discovered in about 98% of GIST patients independent of the presence or absence of mutations (Miettinen & Lasota, 2006).

Clinical Presentation and Management

Gastrointestinal bleeding is the most common symptom of GISTs followed by weakness, abdominal pain, distention, and discomfort. Studies showed that up to 30 % of GISTs are discovered incidentally and patients are asymptomatic (Akahoshi et al., 2018). A definite diagnosis of GISTs is difficult to achieve only through tissue sampling techniques making immunohistochemical analysis essential in clinical practice (Akahoshi et al., 2018). The primary treatment for confirmed GISTs is surgical R0 resection, which is recommended for resectable GISTs without metastases. Over the last decade, high-risk patients who undergo surgical resection for a confirmed GIST are advised to adjuvant therapy with imatinib for at least 3 years. The treatment duration of imatinib was approved following the landmark

Scandinavian Sarcoma Group's clinical trial and the recommended dose was set to 400mg/day (Koumarianou et al., 2015). Imatinib is the first tyrosine kinase inhibitor approved for the therapy of advanced GISTs and works by binding to the ATP-binding domain of tyrosine kinase receptors inducing dramatic disease control in about 85% of GISTs (Li et al., 2017). Imatinib is considered a standard first-line therapy for its dramatically improved effects on the management of GISTs (Xie et al., 2019). However, its beneficial effects vary according to the presence or absence of *KIT* and *PDGFRA* mutations. In fact, the overall survival of GIST patients depends on the type of mutations they bear. In 2015, Yan et al., performed a subgroup analysis to establish the relationship between *KIT* mutations and response to imatinib treatment (Yan et al., 2015). They showed that patients with *KIT* exon 9 mutations respond significantly worse than those who bear exon 11 *KIT* mutations, therefore they have a higher risk of progression. Since no response is seen with imatinib 400mg daily, the standard first-line treatment for metastatic patients with KIT exon 9 mutations is imatinib 800mg daily (Casali et al., 2022). Concerning *PDGFRA*, mutations in exon 18, like D842V are strongly resistant to imatinib (Li et al., 2017). Therefore, according to ESMO guidelines, the standard first-line treatment for metastatic patients bearing a PDGFRA exon 18 D842V mutation is avapritinib 300mg daily (Casali et al., 2022). Thus, although imatinib is effective in reducing disease recurrence after surgery, it is necessary to identify the mutational profile of GIST patients to be able to predict their response to the drug (Yan et al., 2015). Apart from the mutational status of GIST patients, resistance, and intolerable toxicity to imatinib is a serious problem in clinical practice. Approximately 5-14% of GIST patients show evidence of primary resistance to imatinib due to secondary mutations frequently observed in *KIT* exon 11 mutated GISTs. Secondary mutations cluster in the ATP-binding domain of the KIT kinase receptor, which is encoded by exons 13 and 14, and the activation loop, which is encoded by exons 17 and 18 leading to constitutive activation of *KIT* (Li et al., 2017). These findings led to the approval of sunitinib in 2006 as a second-line tyrosine kinase inhibitor (Xie et al., 2019). Sunitinib, like imatinib, binds to the ATP-binding domain tyrosine kinase receptors, however, its binding features differ from those of imatinib, as sunitinib can also inhibit the vascular endothelial

growth factor receptor (VEGFR) (Li et al., 2017). Sunitinib has demonstrated clinical benefit in Phase III double-blind clinical trials, as it showed efficacy in treating exon 9 *KIT* mutations after failure of imatinib therapy (Xie et al., 2019). There is also the possibility of GIST patients not showing any response to imatinib therapy at 400mg/day and dose escalation to 800mg/day has been suggested as an alternative before changing treatment to sunitinib or other tyrosine kinase inhibitors (Hsu et al., 2017). Although dose escalation is widely recommended, the actual evidence for its effectiveness among metastatic and/or inoperable GISTs is based only on observational data (Hislop et al., 2012). However, phase III clinical trials demonstrated a partial response rate of only 2% and a stable disease rate of 27% after imatinib dose escalation to 800mg/day (Hsu et al., 2017). Other trials show similar results, therefore second-line therapy following the failure of imatinib 400mg/day as a standard dose is still a matter of debate (Hsu et al., 2017). In case of treatment failure with imatinib and sunitinib, patients with advanced GISTs can receive regorafenib, which is used as a third-line multi-kinase inhibitor. Recently, ripretinib, a novel type II TKI was approved for the management of advanced/ metastatic GISTs as a fourth-line treatment. According to data from phase III clinical trials, ripretinib showed improved clinical benefit and substantial improvement in median progression-free survival compared to placebo (Sargsyan et al., 2023). It seems that all tyrosine kinase inhibitors possess the same four features: a nitrogen heterocycle, a hinge binding feature, a linker, and a tail ring. These features have been explored by using slightly different modified alternatives to exploit the selectivity and potency of the inhibitors (Pathania et al., 2021) A summary of FDA-approved TKIs for the treatment of GISTs is shown in Table 1. Regardless, a permanent cure with the use of TKIs is difficult to obtain, leaving early diagnosis of localized GIST with R0 surgical resection the only promising way to cure this disease (Akahoshi et al., 2018).

PATIENTS AND METHODS

Patient characteristics

Clinical and molecular data of 105 patients diagnosed with GIST in Cyprus between 2008 and 2023 were extracted. Initial diagnosis of GIST was achieved by histopathology via biopsies obtained from patients' primary tumors.

Mutation Analyses

Somatic DNA was extracted from the formalin-fixed paraffin-embedded tissue block and analysis was performed by PCR followed by direct sequencing (Sanger sequencing) of exons 11, 9, 13, 17 of the *KIT gene* and exons 12, 14, and 18 of the *PDGFRA gene.* The coding regions of these exons were amplified using Hot-Start Taq DNA polymerase and proper forward and reverse primers. The PCR products were purified using Spin Columns and sequenced using the Big Dyer Terminator V3.1 Cycle Sequencing KIT (Applied Biosystems) according to the manufacturer protocol. Tumors not analyzed in clinical routine were analyzed later by next-generation sequencing (NGS) with the use of Sequencer Thermo S5 in formalin-fixed paraffin-embedded tissue samples (Oncomine Focus assay 52: *ABL1, AKT1, AKT3, ALK, AR, AXL, BRAF, CDK4, CDK6, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ERG, ESR1, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, FGFR 4, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, MYC, MYCN, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PPARG, RAF1, RET, ROS1, SMO genes*).

Data Analysis

NGS data analysis was performed with the Ion Reporter Software 5.0 within Torrent Suite Software (Thermofisher Scientific).

RESULTS

Patient demographics and tumor characteristics

105 patients (61 men and 44 women) with a confirmed GIST diagnosis were managed at the Bank of Cyprus Oncology Center between 2008 and 2023. The basic characteristics of both male and female patients are summarized in Table 2. 45 of the 105 tumors were larger than 5cm and 29 showed necrotic features. Metastatic disease at diagnosis was observed in 13 patients and all received imatinib as first-line for metastatic disease. 5 of the 13 patients changed treatment to sunitinib as the second line due to disease progression. Then, 3 of 5 patients received regorafenib as the third line, of which 1 was given the recently approved fourth-line treatment with ripretinib. The total number of deaths in patients with metastatic de novo disease was 5. Tumor relapse was observed in 15 patients during the disease, from which 9 received imatinib as the first line for metastatic disease and 5 received sunitinib as the first line for metastatic disease. Of those who received sunitinib as a first line, 3 were having progression of disease with imatinib, 1 was diagnosed with tuberculosis and experienced drug interaction with imatinib and the other had a severe allergic reaction to imatinib. One patient with tumor relapse received adjuvant treatment with imatinib but due to a serious allergic reaction denied further management with sunitinib as the first line for metastatic disease. The median overall survival for relapsed patients receiving treatment was 63 months. Eight patients with recurrent disease died during treatment, due to disease progression. A Kaplan-Meier survival analysis was conducted showing the survival probability of GIST patients over time. (Figure 2). The median overall survival of the whole cohort was 45 months.

Mutation analysis

Mutation analysis was performed via Sanger or Next-generation sequencing in 74 out of the 105 tumors and revealed the presence of mutations in 57 GISTs. Of the 51 oncogenes and tumor suppressor genes sequenced, alterations were detected in *KIT*, *PDGFRA*, *KRAS*, *SMO*, and *RET*

genes. The most common mutations were found in the *KIT gene* (46 cases, 60,53%) followed by the *PDGFRA gene* (10 cases, 13,16%). Notably, 17 tumors (22,37%) were negative for *KIT/ PDGFRA gene* mutations and were classified as wild-type GISTs. All mutations are summarised in Figure 3.

KIT and *PDGFRA* mutations

Of the 46 *KIT*-mutated GISTs, 40 harbored mutations in exon 11, 4 in exon 9, 1 in exon 13, and 1 in exon 17. More specifically, exon 11 activating mutations were clustered between codons 552 and 579 and consisted of point mutations and small in-frame deletions. There were 20 point mutations, 13 deletions, 6 deletion insertions, 3 insertions, and 4 duplications. Deletions of three or more nucleotides extended from c.1662 to c.1676, and at the protein level would affect codons 555 to 559. Although deletions affecting codons 557-559 were the most common deletions identified in this study, two deletions, extending from c.1735 to c.1737 were also identified in exon 11 of the *KIT gene*, affecting codon 579. Point mutations affected codons 557, 559, 560, and 576 of *KIT* exon 11. Regarding exon 9, all four mutations found resulted in the insertion of two duplicate amino acids, alanine through tyrosine (Ala502_Tyr503dup/ p. Ser501_Ala502insAlaTyr) leading to constitutive phosphorylation of Kit. In the remaining two mutated exons of the *KIT gene*, exon 13 and exon 17, a point mutation resulting in a single nucleotide change at codon 642 (p. Lys642Glu) and a point mutation again resulting in a single nucleotide change at codon 815 (p. Arg815Lys) were detected, respectively. The median overall survival of patients with KIT-mutated tumors was 48 months. A summary of all *KIT* mutations is presented in Table 3. Regarding *PDGFRA*, identical exon 18 point mutations were c.2664A>T and resulted in a single nucleotide change at codon 842 (p. Asp842Val/ p.D842V). 2 point mutations were detected in exon 14 of the *PDGFRA* gene and resulted in c.1977C>G affecting codon 659 at the protein level (p. Asn659Lys). The median overall survival of patients with PDGFRA-mutated tumors was 30 months. A summary of all *PDGFRA* mutations is presented in Table 4.

Evaluation of tumors with *KIT and PDGFRA* mutations

16 tumors with KIT exon 11 mutations were in the small intestine, 22 in the stomach, and 2 in the rectum. Deletions in exon 11 were found in 6 of the tumors from the small intestine, and 8 in the stomach. Point mutations in exon 11 were found in 10 of the tumors in the small intestine and 9 in the stomach. Regarding exon 9, 3 mutations were detected in the small intestine and 1 in the stomach. Mutations in exons 13 and 17 were found only in gastric GISTs. Generally, 11 tumors in the small intestine were larger than 10cm, while only 6 tumors were larger than 10cm in the stomach. All exon 18 *PDGFRA* mutations were in the stomach except in one case, which was in the omentum. The 2 tumors with *PDGFRA* exon 14 mutations were situated in the stomach. Tumor size with *PDGFRA* mutations ranged between 4cm and 14cm. A detailed description of all GIST mutations based on the location of the primary tumor is shown in Figure 4.

Other mutations

Mutations in additional genes were detected in two cases, shown in Table 5. The first case was a 90-year-old male who was found to have a *CCDC6* fusion with the *RET gene*. The patient had a gastric GIST and received radiotherapy for local inoperable disease. The second case was a 72-year-old male with concurrent *PDGFRA/KRAS/SMO* mutations. The patient had an omentum epithelioid extra gastrointestinal stromal tumor which was managed with R0 surgical resection followed by adjuvant treatment with imatinib with no tumor relapse so far.

Histopathological and molecular features

Histologically, 83 tumors were of spindle cell phenotype, 12 were of epithelioid cell phenotype, and 8 were of mixed cell subtypes. 68 tumors (64,76%) were in the stomach, 34 (31,77%) in

the small intestine, 2 (1,87%) in the rectum, and 1 (<1%) in the omentum. The tumor size ranged from 1,6cm to 20cm (mean=7.63cm, median=6.5cm). The mitotic count ranged from 0 to 50/50 HPF. Immunohistochemically, 98 tumors (92,45%) were positive for CD117 expression, 62 (58.49%) were positive for CD34, 7 (6.60%) were positive for S100, 29 (27,36%) were positive for SMA, 76 (71,7%) were positive for DOG-1 and 7 (6,60%) were positive for SDH-b (Figure 5). According to the risk stratification system introduced by Fletcher et al., 2,04% of GISTs were of very low risk of recurrence, 24,49% of low risk, 25,55% of intermediate risk, and 45,92% of high risk.

DISCUSSION

In the present study, we retrospectively extracted data from patients with GIST treated at the Bank of Cyprus Oncology Centre over 15 years (2008-2023). This is the first population-based study on patients diagnosed with GIST in Cyprus where data from their clinicopathological characteristics were collected and reviewed. To our knowledge, this study is also the first to report the incidence of mutations through molecular analysis of GISTs based on population samples. In summary, we analyzed the frequency of mutations in patients' primary tumor samples and found an overall extended mutational rate of 77,63%, which was slightly lower than the frequencies observed in previous studies. The mutation rate for *KIT* was 60,53% and for *PDGFRA* 13,16%, whereas the estimated percentages from phase III clinical trials were 80-85% and 5-10% respectively (Wozniak et al., 2014). Also, a higher frequency of wild-type GISTs (22,37%) was detected, as data from the literature suggest a frequency of approximately 10% (Liu & Chu, 2019). This variation could be associated with different patient characteristics since our study involved GIST patients of various stages. Many population studies have shown variable rates of *KIT* and *PDGFRA* mutations, ranging from 65-80% and 2-13% respectively. These findings indicate ethnic and genetic variations. The most common mutation in our analysis was in exon 11 of the *KIT* gene (52,63%) followed by exon 18 of the *PDGFRA* gene

(10,53%). Regarding *KIT* exon 11, the appearance of single nucleotide changes located around the same region, at codons 557, 559, 560, and 576, such as p.(Val560Asp), p.(Val559Gly), p.(Val559Asp), p.(Trp557Gly) and p.(Leu576Pro) was higher in the present study, which is not in line with previous reports in the literature, which indicated p.(Trp557_Lys558del) as the most common mutation in exon 11 of the *KIT* gene (Bombac et al., 2020). Previous studies have shown that *KIT* exon 11 duplications are associated with gastric tumor origin and female gender (Steigen et al., 2007). In our study, 22 gastric tumors with *KIT* exon 11 mutations were identified, and only one case, which was a male, bore a duplication. However, no conclusion could be made since it was just one case, and the sample size was small compared to previous studies. Moreover, exon 11 duplications are correlated with a favorable prognosis, and this was confirmed in our study since the patient is currently well with no tumor relapse so far. Instead, exon 11 deletions have been associated with a poor clinical outcome, and in our study 13 exon 11 deletions were identified from which 5 patients developed metastatic disease. Concerning KIT exon 9 mutations, previous studies have shown a frequency of up to 12%, mainly of small intestine origin (Steigen et al., 2007). This observation agreed with our study since 3 out of 4 exon 9 mutations were in the small intestine and the overall survival of these patients ranged between 13 and 50 months. According to Yan et al., mutations in *KIT* exon 9 respond worse than those with *KIT* exon 11 mutations, however, in our study, we only had 4 mutations in KIT exon 9, and of this 1 patient had metastatic disease at diagnosis, and did not respond to treatment with imatinib. Most GISTs with exon 13 and exon 17 mutations have been discovered in the small intestine, however, the exon 17 mutation found in our study was of gastric origin. It has been suggested that exon 13 mutations disrupt the normal autoinhibitory function of the juxta membrane domain, while exon 17 mutations disrupt the activation loop of the KIT protein and both mutations are resistant to treatment with imatinib (Origone et al., 2013). This observation does not agree with our study since both patients with exon 13 and exon 17 mutations received imatinib and had no recurrence or metastatic disease so far. Moreover, although about 70% of exon 17 mutations lead to Asn822Lys, the mutation found in our analysis led to Arg815Lys (Joensuu et al., 2015). For histological phenotype, KIT exon 11 mutant tumors were mostly of

spindle cell morphology, while KIT exon 9 mutations shared both spindle and epithelioid features. Concerning *PDGFRA*, a multicentre analysis of a European registry showed that exon 18 mutations are associated with a favorable disease outcome, and this was also true in our analysis (Wozniak et al., 2014). PDGFRA exon 18 mutations found in our study, led to D842V, which is the commonest mutation. Although data indicate resistance to imatinib and sunitinib in patients bearing this mutation, in our study 3 out of 8 patients received imatinib either as an adjuvant after surgery or as a first line for inoperable disease and have had no tumor relapse since. No significant difference in the overall survival between patients with exon 18 mutations and exon 14 mutations of the *PDGFRA* gene was found. However, the lack of difference may be attributed to the different stages of disease at diagnosis and the variable follow-up times of patients. PDGFRA mutants were almost exclusively of gastric origin (90%), as previously reported (Wozniak et al., 2014).

In addition to KIT and PDGFRA, we explored the mutational status of other oncogenes and tumor suppressor genes that may be related to GIST development and progression. We found a mutation in the KRAS proto-oncogene, which Lasota et al., failed to detect since it is considered a rare event. Although Chae et al., showed that KRAS is experimentally resistant to treatment with imatinib, in our case the patient received imatinib as an adjuvant treatment after surgery and has been disease-free since. The same patient was found to have concurrent mutations in exon 18 of the *PDGFRA* gene and exon 4 of the *SMO* gene. We also detected a gene fusion between RET and CCDC6. In this case, the patient received palliative radiotherapy for local inoperable disease but was then lost to follow-up.

In summary, we analyzed the mutational profile of KIT and PDGFRA in GIST patients treated at the Bank of Cyprus Oncology Centre and found comparable rates compared with other European regions. We also report the presence of rare mutations in KIT downstream signalling pathway effectors such as *KRAS, SMO*, and a gene fusion affecting the *RET* gene. Our data highlight the deviations between different GIST populations and indicate the clinical importance of mutation analysis in population-based studies. Treatment with TKIs is effective in reducing disease recurrence after surgery and controlling inoperable and/or metastatic

disease, especially in patients bearing *KIT/PDGFRA* mutations, who show dramatic therapeutic progress with the approved inhibitors imatinib, sunitinib, regorafenib, and ripretinib. Therefore, it is necessary to identify the mutational profile of GIST patients to predict their prognosis and response to TKIs. Knowing the exact mutational profile of GIST patients is significant for understanding the molecular biology of these tumors, their subtype classification, and how they progress. Future studies, in a larger cohort with positive mutations from the same population are needed to extinguish the various TKI-resistant GIST subgroups in individual patients. Although GIST is well-known, it is still a rare disease, therefore decisions should be made within multidisciplinary teams to recommend the appropriate systemic and local treatments.

Study Limitations

For this study, population-based data were used, therefore there was heterogeneity amongst patients. Tumor mutation analysis was not available for all patients since data go back to 2008 and some tissue blocks may have been lost or damaged, therefore further analysis with SANGER or NGS was not achievable. Moreover, some mutations may have been missed since exons where mutations rarely occur were not sequenced, and this may have also inflated the number of wild-type GISTs found.

Ethical Considerations

This was a retrospective study; no study-driven clinical intervention was performed. A simplified Institutional Review Board approval for retrospective studies was obtained and patient consent was not considered to be necessary.

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