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## Multiple Primary Sporadic Gastrointestinal Stromal Tumors in the Adult: An Underestimated Entity

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**Abstract** **Purpose:** Gastrointestinal stromal tumors (GIST) are commonly regarded as solitary tumors. The occurrence of multiple lesions is considered an extraordinary event restricted to pediatric GISTs and rare hereditary conditions. Beyond these well-defined situations, the presentation of multiple synchronous lesions is commonly viewed as the result of the metastatic spreading of a single primary GIST. Based on this axiom, patients with multifocal disease are classified as advanced stage and treated as such. Whether, indeed, the detection of several lesions in sporadic adult GIST patients may be suggestive of phenomena of tumor multiplicity still needs to be clarified. **Experimental Design:** From a multicentric series of 442 consecutive cases, 26 of which with advanced disease, we selected 5 patients who presented up to three distinct GIST nodules. Five additional cases with similar characteristics were also contributed by two other institutions. The clonal relationship between the synchronous lesions was assessed by comparing *KIT/PDGFR*A mutation and microsatellite pattern. **Results:** An independent origin of the synchronous lesions was established in 6 of 10 cases. Notably, in one patient, one lesion arose in the peritoneum, which is ordinarily regarded as a site of metastasis. **Conclusions:** Our data indicate that a significant fraction of GIST patients with multifocal presentation are actually affected by multiple primary tumors, suggesting that mesenchymal GIST precursor cells of these individuals are somehow primed to transformation. Thus, in the presence of multifocal GIST manifestations, an accurate characterization of the different tumor sites should be undertaken for a proper patient staging and therapy planning.

Gastrointestinal stromal tumors (GIST) are mesenchymal tumors most often originating in the muscular wall of the gastrointestinal tract. The estimated annual incidence is 12 to 14 per million, as recently reported in two European studies (1, 2). Stomach and small intestine are the most commonly affected anatomic sites, accounting for about two thirds and

one third of the cases, respectively (3–5). Rare cases of primary GISTs originating outside the gastrointestinal tract have also been reported, although the actual existence of extragastrointestinal GISTs is still debated (4, 6–9).

GISTs are considered *KIT* signaling-driven mesenchymal tumors. In fact, a distinctive feature of GISTs is the expression of the *KIT* protein (CD117 antigen), which is immunohistochemically detectable in ~95% of the cases. Accordingly, the presence of activating mutations (missense mutations or small in-frame deletions) of the *KIT* gene represents the most frequent genetic aberration in GIST (10). Moreover, transgenic mice carrying *KIT* germ-line mutations develop hyperplasia of interstitial Cajal cells and GISTs (11, 12). Less frequent, and mutually exclusive, is the detection of activating mutation of the *PDGFRA* gene (13).

Although most GIST patients present with localized disease, ~10% are metastatic at the diagnosis with peritoneum and/or liver being the most common sites of dissemination (1). GISTs are generally considered solitary tumors and the occurrence of multiple primary neoplasms is considered an exceptional event, restricted to familial GISTs (14–19), pediatric forms (20), or distinct syndromes such as type 1 neurofibromatosis (NF1; refs. 21–23) or Carney's syndrome (24). All these are well-defined entities that can be easily distinguished from common sporadic GISTs based on their peculiar clinicopathologic features. Moreover, tumors in these cases generally develop at early age

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### Translational Relevance

This study reports that a considerable fraction of adult sporadic gastrointestinal stromal tumor (GIST) patients with multifocal manifestations are actually affected by multiple primary GISTs. This finding supports the possibility that widespread tumor priming of GIST precursor mesenchymal cells may be implicated in these patients. The existence of tumor multiplicity in the context of adult GIST suggests that, in the presence of multifocal presentation, molecular assessment of clonal relationship of the different tumor lesions should be done for proper patient staging and planning of therapy.

and either carry germ-line *KIT* or *PDGFRA* mutations (familial GISTs) or are devoid of *KIT/PDGFRA* mutations (6, 25, 26). Beyond these well-defined situations, the detection of multifocal disease, irrespective of the number, size, and location of the lesions, is commonly viewed as the result of the metastatic dissemination of a single primary GIST. Based on this axiom, patients with multifocal GISTs are by default classified as advanced stage and treated as such.

This paradigm has been recently challenged by two articles, which suggested the existence of sporadic multiple primary GISTs (MPG) in adult patients. These articles reported one and four cases, respectively, in which, beside a major mass, one to three additional GIST lesions, apparently independent one to the others because of different molecular and pathologic features, could be identified (26, 27).

The recognition of tumor multiplicity poses obvious problems of diagnosis and patient staging. To shed light on the actual relevance of these phenomena in the context of sporadic adult GIST, 442 consecutive cases collected in three Italian institutions were retrieved and 26 patients presenting at the diagnosis with "metastatic disease" were selected. Among these,

we focused on those cases that presented a limited number of solitary and up to three distinct GIST lesions and assessed their clonality by comparing *KIT/PDGFRA* gene mutation and microsatellite pattern. Unexpectedly, the study revealed that a significant subset of GIST patients with apparently advanced disease actually present with multiple primary tumors, therefore supporting the notion that, in some circumstances, GIST may have features of "systemic disease" of gastrointestinal mesenchymal cells.

### Materials and Methods

**Study design.** The study includes two groups of patients. A first group ("consecutive set") was derived by a consecutive series of 442 GISTs collected in three Italian institutions (General Hospital of Treviso, Ancona University Hospital, and San Raffaele Institute Milan). About 6% of these patients (26 cases) presented at pathologic diagnosis with tumor multifocality and, accordingly, were clinically diagnosed as advanced disease. Among these, to molecularly characterize the full spectrum of tumor lesions surgically removed from each patient, we sought to restrict our study to those cases that presented at diagnosis with a limited number of distinct nodules (arbitrarily set up to 3), irrespective of their location (gastrointestinal tract or peritoneum/omentum) and size. Five of these 26 patients met our criteria for enrollment (Table 1, cases G1-G5).

A second set ("selected set"), represented by five additional cases matching our inclusion criteria (G6-G10), were contributed by centers with specific expertise in diagnosis and treatment of GIST (Brigham and Women's Hospital and Leuven Catholic University). All patients were adult individuals (ages 48-85), with no clinicopathologic signs of NF1 (28) or familial history for GIST, NF1, or Carney's syndromes.

No patients had received radiotherapy/chemotherapy or imatinib before surgery. Five of 10 patients (G3, G4, G6, G8, and G9) received imatinib treatment right after surgery. The follow-up of these patients ranges from 16 to 50 mo, with no evidence of disease (G4, G6, G8, and G9) or death from disease (G3). One patient (G1) started imatinib after having developed liver metastases, but because of unacceptable toxicity, the patient was subsequently put under sunitinib treatment, which induced stabilization of the disease (follow-up, 34 mo). Three patients

**Table 1.** Clinicopathologic characteristics and mutation pattern

Case	Age/sex	Tumor pairs	Localization	Size (cm)	Cytomorphology	Mitotic count (50 HPFs)	Risk category	Mutations
G1	85/M	G1-a	Stomach	6.2	Spindle	2	Intermed	<i>KIT</i> p.I478fsX2 + W557G
		G1-b	Small intestine	6.0	Mixed	1	Intermed	<i>KIT</i> p.V555_I571del
G2	71/M	G2-a	Stomach	3.0	Spindle	1	Low	<i>KIT</i> p.V560D
		G2-b	Stomach	4.0	Epithelioid	1	Low	No mutation
G3	74/M	G3-a	Small intestine	8.0	Spindle	35	High	<i>KIT</i> p.A502_Y503dup
		G3-b	Peritoneum	2.0	Spindle	33	High	<i>KIT</i> p.A502_Y503dup
G4	48/F	G4-a	Small intestine	10.0	Spindle	20	High	<i>KIT</i> p.W557G + <i>KIT</i> p.H697fsX27
		G4-b	Peritoneum	1.5	Spindle	17	High	<i>KIT</i> p.W557G
G5	59/F	G5-a	Stomach	16.0	Mixed	6	High	<i>PDGFRA</i> p.V561D
		G5-b	Peritoneum	2.0	Mixed	7	Intermed	<i>PDGFRA</i> p.V561D
G6	69/F	G6-a	Small intestine	1.9	Spindle	1	Very low	<i>KIT</i> p.V559del
		G6-b	Small intestine	4.7	Spindle	1	Low	<i>KIT</i> p.V559G
G7	83/F	G7-a	Stomach	14.0	Spindle	1	High	<i>KIT</i> p.I563_L576del
		G7-b	Stomach	0.7	Spindle	0	Very low	<i>KIT</i> p.V560D
		G7-c	Stomach	0.7	Spindle	0	Very low	No mutation
G8	53/F	G8-a	Small intestine	6.0	Spindle	30	High	<i>KIT</i> p.Q550-K558del
		G8-b	Small intestine	1.7	Spindle	2	Very low	<i>KIT</i> p.L576P
G9	81/M	G9-a	Small intestine	4.5	Spindle	4	Low	<i>KIT</i> p.V559D
		G9-b	Peritoneum	3.5	Mixed	7	Intermed	<i>KIT</i> p.W557_V559 delinsF
G10	68/M	G10-a	Stomach	0.6	Spindle	0	Very low	No mutation
		G10-b	Peritoneum	13.0	Mixed	5	High	No mutation

(G2, G7, and G10) did not receive imatinib therapy, and only partial follow-up was available for these patients. Patient G2 and G10 were still disease-free 24 and 36 mo after surgery, respectively, whereas patient G7 was in progression 45 mo after surgery. For one patient (G5), no information on the follow-up was available.

**Histopathologic diagnosis and immunohistochemistry.** For all the cases included in the study, formalin-fixed, paraffin-embedded tumor and matched nonpathologic surrounding tissues were available.

Diagnosis of GIST was reconfirmed for all cases based on the combination of histologic evaluation and CD117 immunopositivity. Mitoses were counted in 50 consecutive high-power fields (HPF) for each sample and mitotic index was defined as low for  $\leq 5$  mitoses/50 HPF, intermediate for  $>5$  and  $<10$  mitoses/50 HPF, and high for  $>10$  mitoses/50 HPF. The risk category was assessed according to the 2002 NIH classification (Table 1).

Tumor sections were immunostained for CD117. In five cases (G1-G5), additional sections were also immunostained for DOG1, CD34, smooth muscle actin, S100, and desmin. Where required, antigen retrieval was done using a 30'-microwave oven (MW) pretreatment (750 W) in 10 mmol/L citrate buffer at the indicated pH. All immunostainings were done by an automated immunostainer (Dako Autostainer, DakoCytomation) using the following primary antibodies: CD117 (1:100, no antigen retrieval, polyclonal; DakoCytomation), CD34 (1:50, no antigen retrieval, clone QBEnd/10; DakoCytomation), DOG1 (1:1,000, pH 6 MW, polyclonal; NeoMarkers), smooth muscle actin (prediluted, no antigen retrieval, clone 1A4; NeoMarkers), S100 (1:8,000, Pronase, polyclonal; DakoCytomation), and desmin (prediluted, pH 6 MW, clone D33; NeoMarkers). Standardized 3,3'-diaminobenzidine development times allowed accurate comparison of all samples. Substitution of the primary antibody with PBS served as a negative control.

**Molecular analysis.** DNA was extracted from formalin-fixed/paraffin-embedded tissues of both tumoral and surrounding nonpathologic tissues. Several 10- $\mu$ m-thick sections were deparaffinized by serial xylene/ethanol washings. DNA extraction was done using the EZ1 Biorobot (Qiagen GmbH). Exons 9, 10, 11, 13, 14, and 17 and intron 10 of *KIT* gene and *PDGFRA* exons 12 and 18 were amplified by PCR and both strands were sequenced using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). PCR conditions were as follows: an initial denaturation step at 95°C for 3 min followed by 42 cycles at 95°C/30 s, 58°C/1 min, and 72°C/1 min. The primers used were the following: *KIT* ex9fw, TTTCCTAGAGTAAGCCAGGGC; *KIT* ex9rev, ACAGAGCCTAAACATCCCCT; *KIT* ex10fw, GGTGAAGCTCTGAGACTCAC; *KIT* ex10rev, TGTTTCCATTTATCTCTCAACAAC; *KIT* ex11fw, TCTCTCCAGAGTCTCTAATGAC; *KIT* ex11rev, AAGGAAGC-CACTGGAGTTCC; *KIT* ex13fw, TGCATGCGCTTGACATCAGT; *KIT* ex13rev, AGGCAGCTTGGACACGGCTT; *KIT* ex14fw, GTCTGATC-CACTGAAGCTG; *KIT* ex14rev, ACCCATGAAGTGCCTGTC; *KIT* ex17fw, TGGTTTTCTTTCTCTCCAACC; *KIT* ex17rev, GCAG-GACTGTCAAGCAGAGGA; *PDGFRA* ex12fw, TCCAGTCACTGTGCT-GCTC; *PDGFRA* ex12rev, GCAAGGAAAAGGGAGTCTT; *PDGFRA* ex18fw, TCAGCTACAGATGGCTTGATC; and *PDGFRA* ex18rev, TGAAGGAGGATGAGCTGACC.

Mutation nomenclature was according to the international nomenclature working group (29). Chromosome loci that commonly undergo deletion in GISTs (1p, 13q, 14q, and 22q) were selected for microsatellite analysis. Microsatellite markers used were the following: D1S435, D1S449, D13S153, D13S290, D13S171, D14S468, D22S1150, and D22S689. Polymorphic regions were amplified by PCR using HEX- or FAM-labeled primers and resolved by capillary electrophoresis using the ABI PRISM 3100 Genetic Analyzer. Primer sequences were as in GDB.<sup>8</sup> PCR conditions were as follows: an initial denaturation step at 95°C for 3 min followed by 40 cycles at 95°C/30 s, 55°C or 58°C/1 min, and 72°C/1 min.

**Statistical analysis.** The concordant mutations test recently developed by Begg and coworkers was applied to assess the clonal relationships of the paired lesions based on microsatellite analyses.<sup>9</sup> A clonal origin of synchronous lesions was supported by  $P \leq 0.05$ , whereas  $P = 1$  indicated independent origin (30).

## Results

**Clinicopathologic findings.** All patients selected for the study, but case G7, presented at the diagnosis with two discrete GIST masses. Case G7 carried three distinct lesions. Matched tumors involved the same gastrointestinal structure in four cases (G2, G6, G7, and G8), whereas different organs of the gastrointestinal tract were affected in one case (G1). In the remaining five patients, the matched tumors were located one in the gastrointestinal tract and the other in the peritoneum (G3-G5, G9, and G10; Table 1).

Tumor diameter ranged from 0.6 to 16 cm. Whereas in three cases (G1, G2, and G9) the paired tumors were comparable in size, in the other seven cases a major mass, at least twice as large as the other one, could be identified. Paired tumors displayed concordant morphology (spindle/spindle; mixed/mixed) in six cases and discordant (spindle/mixed; spindle/epithelioid) in four cases (G1, G2, G9, and G10; Fig. 1). The mitotic index of the paired lesions was concordantly low or concordantly intermediate/high in five and three cases, respectively, whereas a different mitotic index was observed in two cases (G8 and G9).

All tumors were immunoreactive for KIT (CD117). The consecutive set of cases (G1-G5) was further characterized for the expression of DOG1, CD34, smooth muscle actin, desmin, and S100. In all cases but G1, the paired lesions showed a concordant immunoreactivity pattern. In case G1, the gastric tumor (G1-a) was negative for smooth muscle actin and extensively positive for CD34, whereas the small intestinal mass (G1-b) was extensively positive for smooth muscle actin and displayed only focal expression of CD34 (Fig. 1). Moreover, the pattern of KIT immunostaining was different in the two tumors, with G1-a showing a paranuclear dot-like pattern and G1-b displaying a membranous and cytoplasmic pattern (Fig. 1). Because tumor G1-a turned out to carry a double KIT mutation (see below), this peculiar pattern might be due to the phenomena of abnormal maturation of mutant KIT protein, which, according to a recent article by Tabone-Eglinger and coworkers (31), seems particularly evident in tumors homozygous for KIT mutations.

**Mutation analysis of KIT and PDGFRA.** None of the patients included in the study carried germ-line mutations of *KIT* or *PDGFRA*, ruling out the possibility of familial forms of GIST.

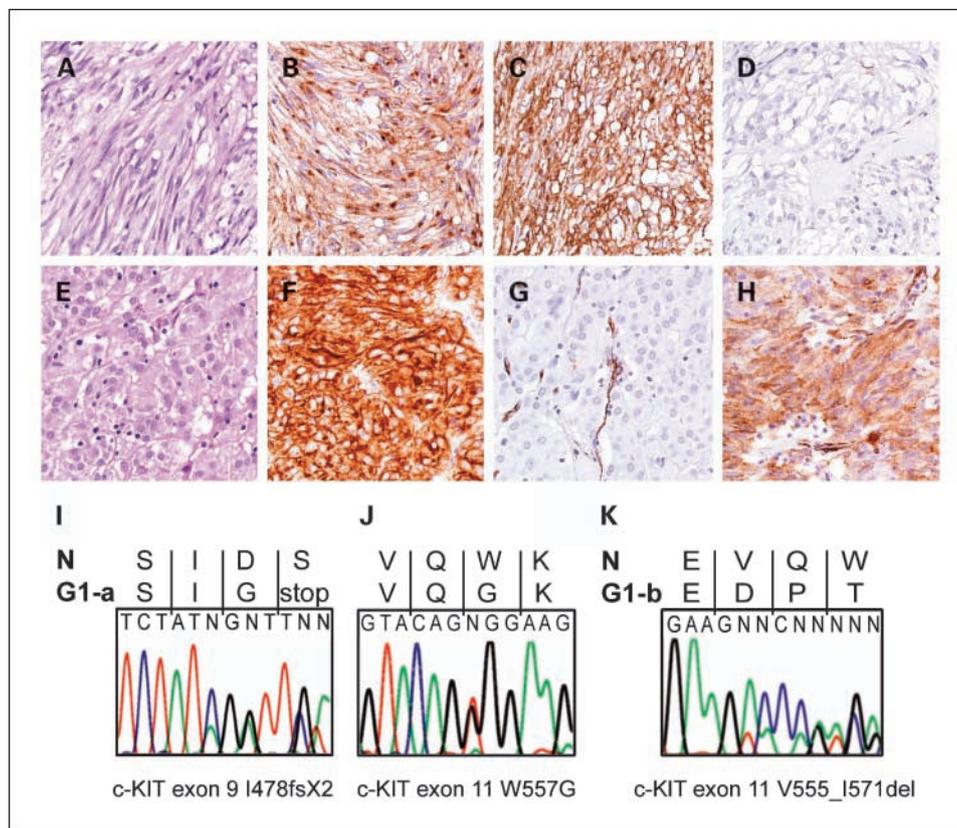
*KIT* somatic mutations were detected in 15 of 21 tumor samples analyzed (8 patients). Patient G5 harbored a mutation of the *PDGFRA* gene in both lesions, whereas neither *KIT* nor *PDGFRA* turned out to be mutated in the tumoral masses of case G10 (Table 1).

In three cases (G3-G5), the two matched lesions carried an identical *KIT*/*PDGFRA* mutation, suggesting a clonal relationship. In contrast, a different *KIT* mutation pattern was detected in 6 of 10 tumor pairs, supporting an independent origin for the matched lesions (Table 1; Figs. 1 and 2).

Intriguingly, in two instances (G1-a and G4-a), tumors carried a double *KIT* mutation, one of which resulted in protein

<sup>8</sup> <http://www.gdb.org>

<sup>9</sup> <http://www.stat.wisc.edu/~eng/clonality/>



**Fig. 1.** Immunophenotypic and molecular analysis of case G1. The gastric tumor (G1-a) showed a spindle cell morphology (A), dot-like reactivity for CD117/KIT (B), diffuse CD34 expression (C), and was negative for SMA (D). The intestinal tumor (G1-b) displayed a mixed morphology (E), strong membranous and cytoplasmic immunoreactivity for CD117/KIT (F) and SMA (H), and lacked CD34 expression (G). Sequencing revealed that tumor G1-a carried a double KIT mutation: a deletion of 29 bases at exon 9 resulting in a premature stop codon at amino acid 480 (I) and a point mutation at codon 557 resulting in a tryptophan to glycine amino acid change (J). Tumor G1-b (K) displayed a deletion of 51 nucleotides at exon 11, resulting in the in-frame deletion of amino acids 555 to 571.

truncation. In particular, tumor G1-a, beside a missense mutation at codon 557, carried a 29-nucleotide deletion in exon 9, which introduced a premature stop codon (Fig. 1). Similarly, in case G4, both tumor G4-a and G4-b displayed a point mutation in exon 11, but in the large intestinal mass (tumor G4-a), part of the tumor population gained a further *KIT* alteration in exon 14, resulting in a stop codon at residue 719. These specific *KIT* alterations have never been reported before. Frameshift/stop codon mutations have been rarely described in GISTs, but, intriguingly, both in previously reported (32–34) and in our series, these mutations occurred as a secondary event after a typical *KIT* mutation. Thus, as recently suggested (31, 35), protein truncation may represent a mechanism for tumor progression aimed at reducing the tumor to homozygosity for the activating mutation.

**Microsatellite analysis.** Microsatellite allelic imbalance proved highly informative for tracking tumor-specific chromosome losses. To corroborate our conclusions on the clonal relationship of paired tumors based on *KIT*/*PDGFRA* mutation pattern, some cases were further analyzed for loss of heterozygosity at microsatellite loci that are frequently involved in GIST development (1p, 13q, 14q, and 22q). In particular, similar to *KIT*/*PDGFRA* mutation, losses of chromosomes 14 and 22 are considered early events in GIST tumorigenesis (36). We assumed that paired tumors, which show an overall concordant microsatellite pattern, are likely to be clonally related. Instead, an overtly discordant pattern, meaning the loss of different alleles in the paired lesions, was considered a strong argument for unrelated primary tumors.

Microsatellites were analyzed in the patients of the consecutive set (G1-G5) plus case G6. All cases were informative for at

least three microsatellite markers. All the three patients analyzed that carried a different *KIT* mutation pattern in the paired nodules (G1, G2, and G6) displayed also loss of different alleles in at least one third of the informative markers, corroborating the hypothesis of an independent origin (Fig. 2). This result was supported also by the concordant mutations statistical test (30). Instead, an identical microsatellite pattern or a pattern compatible with tumor progression (loss of heterozygosity for one tumor and retention of both alleles in the other) were observed in the three cases that shared an identical *KIT* mutation pattern (Table 2).

Intriguingly, *KIT* mutation analysis had pointed out that tumor G4-a, beside a common mutation with tumor G4-b, had gained a further mutation. Similarly, microsatellite analysis indicated that the paired tumors shared a common background of allelic imbalance, but two additional allelic losses were detectable in DNA of tumor G4-a, supporting the notion of a malignant progression of the primary nodule.

**Comparative clinicopathologic and mutational evaluations.** Overall, *KIT*/*PDGFRA* and microsatellite analyses confirmed the metastatic nature of tumor multifocality in cases G3, G4, and G5. In contrast, the presence of different genetic lesions in paired tumors of cases G1, G2, G6, G7, G8, and G9 supported the notion of tumor multiplicity.

We then analyzed the clinicopathologic and immunophenotypic features of the tumors within these two categories, in search for variables that could readily predict the nature of distinct lesions.

In the category of truly metastatic tumors (G3-G5), the two masses were markedly different in size (one at least four times larger than the other). Moreover, the matched lesions showed

the same morphology and the mitotic index was concordant (intermediate or high). These data seem an argument for the metastatic nature of the peritoneal mass.

Nevertheless, also in the category of MPGs, cases in which one tumor was significantly larger than the other could be identified (see for instance cases G7 and G8), cautioning on the adoption of tumor size comparison as a suitable variable to establish clonal relationship. Moreover, although discordant morphology could be observed among MPGs, also primary tumors of independent origin could share, similar to metastatic tumors, the same morphology (see for instance cases G6, G7, and G8), arguing against the use of tumor cell shape as a discriminating factor for metastasis versus second primary tumor. Rather, because the mitotic index of most of the tumors that turned out to be second primary GISTs was concordantly low or markedly different for the paired lesions (G8), in the presence of this evidence, tumor multiplicity should be considered.

## Discussion

The majority of GISTs are sporadic and tumor multiplicity is considered an exceptional finding limited to specific conditions: MPGs may be observed in pediatric patients or in individuals affected by hereditary GIST, NF1, or paraganglioma/sarcoma and Carney's triad syndromes (5, 14–24). Beyond these entities, the occurrence of multiple distinct tumors is conventionally interpreted as indicative of metastatic spread from a primary lesion.

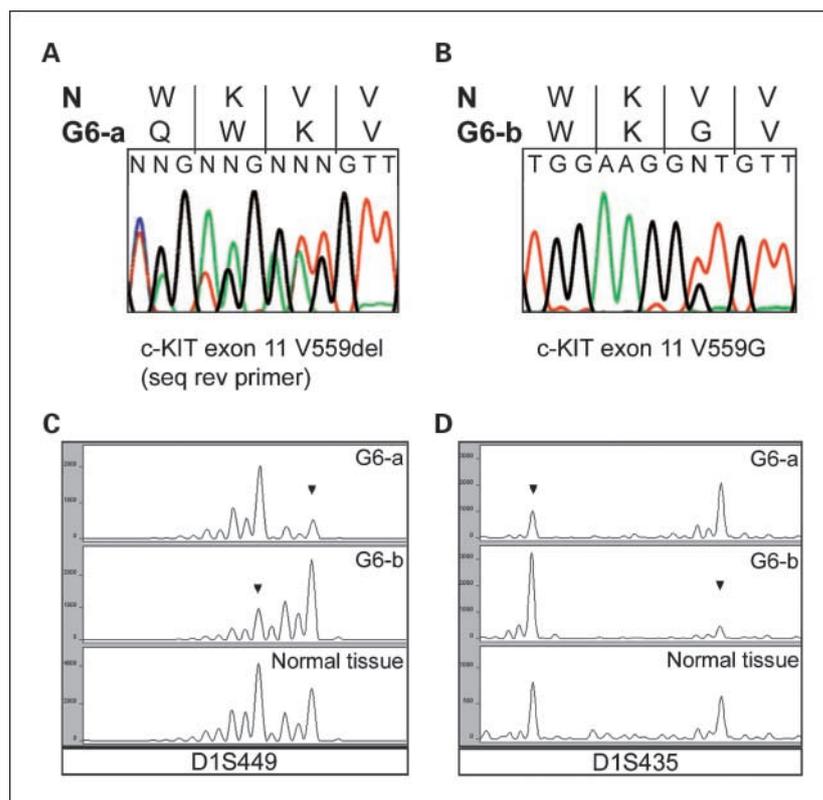
In contrast with this common view, two recent studies revealed the existence of the phenomenon of tumor multiplicity in the context of adult sporadic GISTs. Considering that *KIT* or *PDGFRA* mutations are frequent, occur early during GIST

development, and are relatively polymorphic, Kang and coworkers (27) used these genes as markers of tumor clonality and screened their own surgical pathology files for GIST patients who presented with at least two tumors in the gastrointestinal tract (stomach and small intestine). Molecular analysis confirmed metastatic dissemination in four of the five selected cases, whereas one patient turned out to be affected by two independent jejuneal GISTs. Similarly, Haller and collaborators (26) analyzed four cases of gastric GIST in which, during surgical or pathologic examination, one to three small additional tumor lesions were incidentally discovered in the proximity of the primary lesion. A different *KIT* or *PDGFRA* mutation pattern was found in the different paired lesions, indicating that these patients actually developed multiple primary gastric GISTs.

Both articles described the existence of MPGs within the same organ, either stomach or small intestine. No cases of multiple GISTs affecting distant gastrointestinal structures have been reported thus far. Moreover, although still debated, also the peritoneum has been suggested to be a site of origin of primary GIST (7, 9). Thus, whether peritoneal nodules may represent multiple GISTs is still unknown.

With the intent of shedding light on these issues and providing an assessment of the significance of tumor multiplicity in the context of sporadic adult GIST, we sought to screen a series of 442 consecutive cases collected by three Italian institutions. Twenty-six patients presented at diagnosis with apparently disseminated disease. We sought to analyze only those cases with a limited number of distinct GIST nodules (up to three). Five of these 26 cases met these established inclusion criteria. Five further cases with similar features were contributed from collaborating institutions.

**Fig. 2.** Molecular characterization of case G6. A different *KIT* mutation pattern was found in the two intestinal lesions of case G6. **A**, tumor G6-a carried a three-nucleotide deletion at exon 11, resulting in the loss of codon 559. **B**, instead, tumor G6-b carried a valine to glycine substitution at the same codon. A different microsatellite pattern further supported the independent origin of the two lesions. **C**, tumor G6-a showed loss of the larger allele at D1S449 locus, whereas G6-b lost the shorter allele. **D**, similarly, G6-a was deleted for the shorter allele at D1S435 locus, whereas G6-b displayed loss of the larger one. Arrow, allelic losses.



**Table 2.** Molecular assessment of clonal relationship

Case	<i>KIT</i> / <i>PDGFRA</i> mutation pattern (comparison of the paired GIST lesions)	Microsatellite pattern (loss of different alleles/total informative markers)	Concordant mutations test ( <i>P</i> value)
G1	Different	2/6	1.0000
G2	Different	1/3	1.0000
G3	Same	0/4	0.0417
G4	Same background + additional alteration	0/6	0.0357
G5	Same	0/4	0.2500
G6	Different	2/4	1.0000
G7	Different	ND	ND
G8	Different	ND	ND
G9	Different	ND	ND
G10	No mutation	ND	ND

In contrast with two previous reports, in only 4 of 10 patients did the paired neoplasms occur in the same organ. In the remaining six cases, the matched lesions involved the stomach and the small intestine, the stomach and the peritoneum, or the small intestine and the peritoneum.

A combined *KIT*/*PDGFRA* and microsatellite analysis supported a metastatic nature of the secondary neoplasm in three patients. In all these cases, the secondary lesion was located in the peritoneum or omentum and was much smaller than the primary one (four to eight times). In one case, because of the lack of *KIT* or *PDGFRA* mutations, we were unable to assess the clonal relationships between the matched lesions by this means.

Instead, in six cases, a different *KIT* mutation pattern was observed in the two matched lesions, indicating an independent origin for these tumors. The lack of relation between the two masses was further corroborated by a divergent microsatellite configuration. In most cases, the two lesions had comparable size, but in one case, the diameter of the two masses differed significantly. In two patients, the synchronous tumors affected different organs: stomach and small intestine, and intestine and peritoneum. This latter case is particularly interesting because it not only supports the existence of primary GIST of the peritoneum but also calls into question the concept of considering peritoneal localizations as metastatic a priori.

A focused analysis of the consecutive set of cases allows us some epidemiologic considerations. Although the limited number of patients analyzed prevents us to draw any definitive conclusion on the prevalence of tumor multiplicity in the context of adult, nonsyndromic GISTs, the fact that two of five patients clinically diagnosed as advanced disease were in fact affected by MPGs is quite impressive. In addition, because we sought to focus our analysis on carriers of just two to three distinct GIST nodules, it cannot be ruled out that MPGs may also affect patients with more disseminated localizations as well as patients who develop metachronous lesions/recurrences.

From a biological standpoint, the finding of GIST patients with tumor multiplicity suggests that, in these subjects, GIST precursors (Cajal cells) or pluripotent mesenchymal stem cells may be somehow primed toward tumorigenic conversion. This may recall the phenomenon of "field cancerization," reported for the mucosa of the upper respiratory tract (37). In agreement with this hypothesis, Agaimy and coworkers (38) have recently

reported that multiple minute (~4 mm), hyalinizing spindle cell lesions are a common finding in the stomach of adult individuals (~20%) and that these nodules, named GIST "tumorlets," are CD117 positive and carry *KIT*/*PDGFRA* gene mutations. The authors suggest that GIST tumorlets represent early mesenchymal cell lesions that may eventually, under endogenous or exogenous stimuli, evolve into clinically overt GIST. Indeed, microscopic hyperplastic areas of CD117-positive spindle cells are often detected in the proximity of sporadic GISTs (39, 40) and multifocal hyperplasia of Cajal cells is typical of familial GIST, Carney's triad, NF1 patients, and mouse GIST models (11, 12, 21, 22, 25, 41–46).

From a clinical standpoint, the differential diagnosis of disseminated disease versus MPG obviously affects patient staging. Moreover, although the current recommended therapeutic approach for localized tumors is surgery, metastatic GISTs are eligible for treatment with the tyrosine kinase inhibitor imatinib (47, 48). Thus, the ascertainment of MPG affects also clinical management.

Finally, *KIT*/*PDGFRA* mutation status is the important predictor of responsiveness to imatinib, with tumors with *KIT* exon 11 mutations showing a higher response rate than those with *KIT* exon 9 mutations; when dealing with *KIT* exon 9 mutated GISTs, a higher dosage is recommended (49). Thus, because distinct MPGs may display different mutations with varying imatinib sensitivity, therapeutic planning should take into account the specific nature of the different lesions.

In summary, our study points out that a significant fraction of adult sporadic GIST patients with multifocal manifestations are actually affected by MPGs. This finding supports the possibility that widespread priming of GIST precursor mesenchymal cells, similar to the "field cancerization" described for the mucosa of the aerodigestive tract, may be implicated in these patients. The biological basis of such a phenomenon and the impact of tumor multiplicity in GIST epidemiology will require further investigation. Nevertheless, the existence of tumor multiplicity in the context of adult GIST suggests that, in the presence of multifocal presentation, an accurate molecular characterization of the different tumor localizations should be taken into account for proper patient staging and planning of therapy.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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