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Review

Everything you always wanted to know about GIST (but were afraid to ask)
An update on GIST pathology

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Summary

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. The discovery of the occurrence of activating *KIT* mutations and KIT expression in GISTs opened the way to the unequivocal diagnosis of these tumors and to their successful treatment with imatinib, a tyrosin kinase inhibitor. Since then, research progress revealed molecular GIST triggers alternative to *KIT*, implying heterogeneous analytic approaches and prognostic expectations. Several targeted therapies, variably specific for each GIST trigger, have been developed or are being investigated. Thus, GISTs eventually revealed a family of diseases rather than a single tumor type. All these events had an unprecedented impact on pathology practice, constituting at the same time a heavy burden and an exciting challenge, ultimately putting pathologists in the spotlight as never before. This review will discuss the most recent advances concerning GISTs, highlighting the tasks of pathologists facing these tumors, with an emphasis on traps potentially compromising a correct diagnosis.

Introduction

Gastrointestinal (GI) stromal tumors (GISTs) are not only the most common mesenchymal tumors of the GI tract [1 2], but also an overall frequent neoplasm if small, subclinical cases are considered [3-5]. GISTs became a paradigm of molecular targeted therapy in solid tumors since 1998, following the discovery of their frequently harbored *KIT* activating mutations and KIT expression [6-8]. The subsequent identification of GIST oncogenic pathways alternative to *KIT* mutations and of the heterogeneous consequences of different defects within a given molecule revealed the existence of diverse GIST subgroups characterized by specific pathogenic, diagnostic and prognostic features, ultimately implying variable therapeutic approaches [1]. As a consequence, GIST are being considered a family of diseases rather than a single entity [9].
All these events produced a heavy impact on the pathology practice, constituting a relevant burden on one hand, but making pathologists the hinge of the management of GIST patients on the other. This paper will offer an overview over GIST according to the most recent advances, highlighting the aspects relevant to the pathologist, with a special attention to diagnostic ambiguities and traps.

Definition of GIST

GIST is a mesenchymal tumor which usually arises along the GI tract mostly showing differentiation towards a phenotype proper of interstitial cells of Cajal (ICCs), pacemaker cells which constitute a network in the GI muscularis propria. In fact, like the latter, the vast majority of GISTs express KIT and DOG1 [8 10]. Additionally, this tumor type often bears activating mutations of *KIT* or *platelet-derived growth factor (PDGF) receptor alpha (PDGFRA).*

GIST clinical features

GIST is the most common GI mesenchymal neoplasm, featuring an annual incidence of 10-20 per million [11-16].

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If subclinical cases are considered, GIST frequency (including GISTs of small size) could be as high as 10-35%, making this tumor type an overall common tumor in man [3-5]. This neoplasm can present with dysphagia, fatigue, abdominal pain and GI hemorrage or obstruction.

GISTs as a whole involve equally both sexes, with a peak incidence in the first half of the seventh decade. Succinate dehydrogenase (SHD)-deficient cases tend to occur earlier (< 40 years, including children) and to involve preferentially females [17 18].

Globally, stomach is the most common site of GIST arousal (50-60%); other sites are, in order of frequency: small intestine (30-35%), colon-rectum (5%) and esophagus (< 1%). A minority of GIST (< 5%), the so-called extragastrointestinal GISTs (EGISTs), are found in extra-GI sites such as omentum, mesentery and retroperitoneum: at least some of them are indeed metastases from undetected primaries [17].

Specific GIST subgroups favor certain anatomic sites. *PDGFRA*-mutant-GISTs and SDH-deficient ones are typically found in the stomach, the latter accounting for 7.5% of gastric GISTs (with a tendency to involve the antrum). The hitherto exclusive gastric location of germline *PDGFRA*-mutant GISTs well highlights the strong gastric predilection of these tumors. Even more significantly, extragastric primary SDH-deficient GISTs have never been reported so far [18-20]. Apparent exceptions to these anatomic restrictions in germline mutants eventually revealed somatic concomitant mutations [21 22].

Unlike SDH-deficient and *PDGFRA*-mutant GISTs, the majority of NF1-associated GISTs and *BRAF*-mutant ones arise in the small intestine, the former being commonly multiple [23 24].

GIST metastases usually involve liver and abdominal cavity; GIST spread to extra-abdominal sites is uncommon, with the exception of the rare esophageal GISTs, which can metastasize to lung and thorax [25].

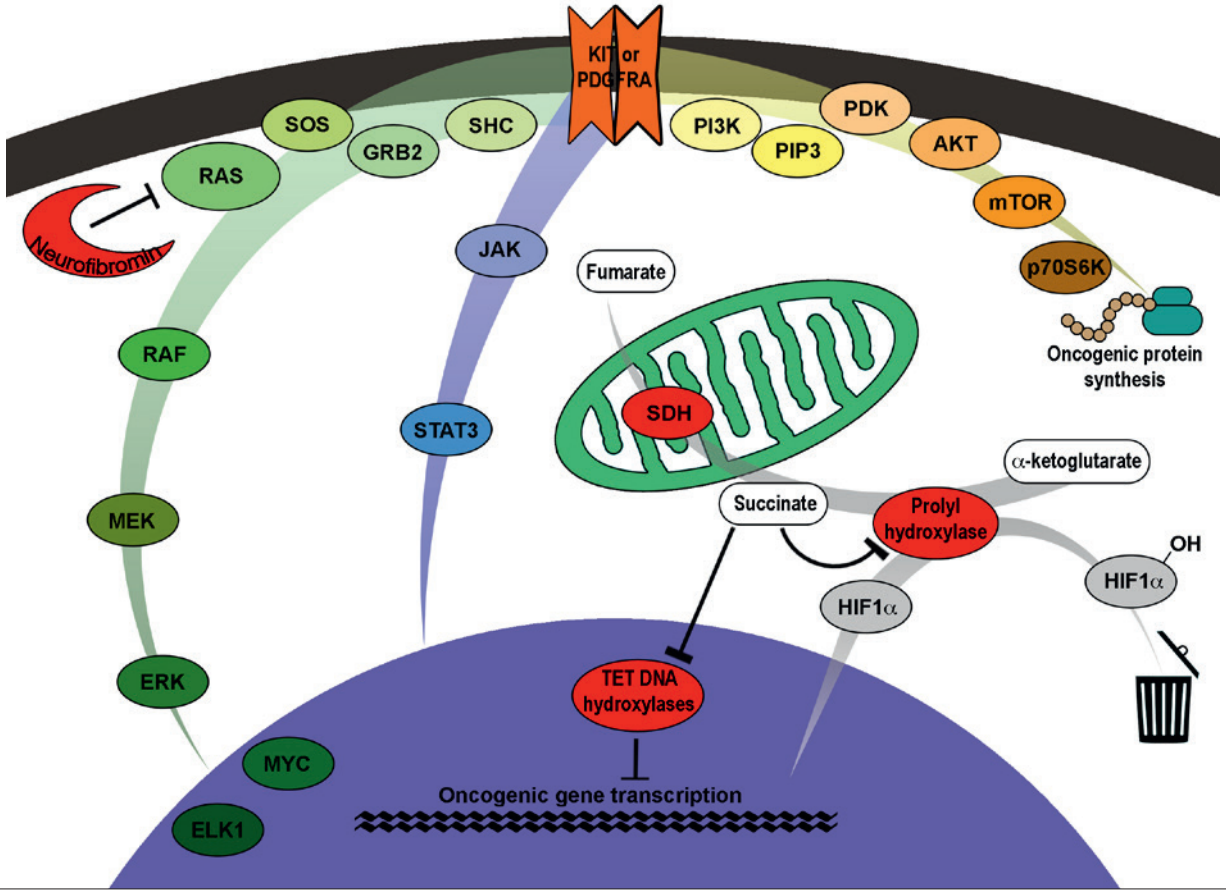
GIST pathogenesis

Although *KIT* or *PDGFRA* mutations are most often the pathogenic base of GISTs (about 77% and 6.5% of cases, respectively), genetic defects of these type III tyrosin kinase (TK) receptors (TKRs) are not the only possible triggers of these tumors. Other oncogenic events in GIST include alterations in the SDH enzymatic complex (about 5% of cases), neurofibromin (1.3%), BRAF (2%), RAS (exceptionally), and the alpha subunit of phosphatidylinositol 3-Kinase (PIK3CA) (exceptionally) [1 26 27]. A small subset of GISTs, accounting for about 5% of cases, has not yet revealed any trigger [28]. With the exception of *RAS* and *PIK3CA* mutations, whose rare examples have been found in association with one of the other "classical" GIST pathogenic events, GIST triggers are as a rule mutually exclusive, reflecting their high efficiency in determining the arousal of this tumor type. Nevertheless, exceptional waivers do exist to this mutual exclusivity (nowadays a widely accepted dogma), a possibility which must be taken into account when dealing with GISTs to be treated with molecularly targeted therapy, as later discussed in this paper [22 29]. The basis of the various known possible GIST triggers is mutational in all cases with the exception of about half of SDH-deficient examples, where an epigenetic hypermethylation of the promoter of the *C subunit of SDH* (*SDHC*) is found [30 31]. In case of *SDH* and *neurofibromin*, the DNA-driven pathogenesis follows a typical second hit mechanism, with somatic inactivation of the wild-type (WT) allele in the background of a germline mutation; these genetic events occur in a clinical context of Carney-Stratakis syndrome (CSS) (often) or neurofibromatosis type 1 (NF1) (almost invariably), respectively [18 32 33]. Rarely, GISTs hinging upon *KIT* or *PDGFRA* mutations can be syndromic too, due to germline alterations of these genes [20 34]. GISTs due to somatic *SDHC* promoter hypermethylation are often syndromic, in the context of Carney's triad (CT) [30]. Conversely, exceptional cases of GISTs due to *neurofibromin* inactivation, including TK-naïve cases, have revealed somatic alterations of both alleles in the absence of NF1 setting [35 36]. With regard to SDH defects, it is worth noting that the second hit mechanism is regularly found also in sporadic *SDH*-mutant GISTs, in which *SDHA* is most often involved. Given the extremely slow SDH-mediated tumorigenesis, which implies the possible arousal of second tumors over dozens of years, it is possible that also these apparently sporadic SDH-deficient GISTs are indeed part of syndromic settings; in other words, the vast majority of *SDH*-mutant SDH-deficient GISTs could be actually syndromic, namely part of CSS, similarly to GIST driven by *neurofibromin* inactivation, which are usually part of NF1.

The oncogenic action of each of the above-mentioned factors will be now analyzed.

KIT is physiologically activated upon binding to stem-cell factor (SCF), resulting in receptor homodimerization and kinase activation which, in turn, stimulates RAS/MAPK, PI3K/AKT/mTOR and JAK/STAT3 signaling (Fig. 1). Mutant KIT homodimerizes and activates in a ligand-independent manner [37]. The majority of GIST KIT-activating mutations occur in in exon 11 (approximately 65% of all GISTs), followed by exons 9, 13, 17 and 8 (8%, 1%, 1% and << 1%, respectively) [2 38]. The extracellular binding domain is coded for by exons 8 and 9, the juxtamembrane regulatory domain by exon 11, and the intracellular, kinase domains by exons 13 and 17 (ATP-binding region and activation loop, respectively). Coherently, *KIT* mutations induce different functional protein changes according to the involved exon, namely: mutations in exons 8 and 9 simulate the activating conformational alterations following KIT binding to SCF; mutations in exon 11 alter the secondary structure allowing the kinase activation loop to switch to activation; and exon 13 and 17 mutations directly make TK domains assume an active conformation. Activating gene mutations seem not to be the only GIST pathogenic way involving

Fig. 1. Molecular triggers and intracellular pathways involved in GIST pathogenesis. GISTs can hinge upon alterations of one of the following: KIT, PDGFRA, neurofibromin, BRAF or SDH. Additionally, exceptional defects of RAS or phosphatidylinositol 3-Kinase (PIK3CA) have been signaled in GISTs, although together with one of the other "classical" triggers. KIT and PDGFRA activation initiates a downstream signaling involving multiple pathways: RAS/RAF/MEK/ERK (MAPK) (left, green hue); JAK/STAT3 (centre, blue hue); and PI3K/AKT/mTOR (top right, yellow/brown hue), stimulating oncogenic gene transcription or protein synthesis. In NF1-associated GISTs, tumoral inactivation of the WT neurofibromin impairs its RAS inhibiting effect, resulting in the activation of MAPK cascade downstream to KIT and PDGFRA. Impairment of the SDH enzymatic complex prevents succinate conversion to fumarate. Accumulated succinate inhibits prolyl-hydroxylase; the missed hydroxylation of HIF1-α prevents the degradation of this molecule which, consequently, heterodimerizes with HIF1-β and translocates into the nucleus acting as an oncogenic transcription factor. Furthermore, succinate accumulation inhibits TET DNA hydroxylases resulting in impaired conversion of 5-methylcytosine to 5-hydroxymethylcytosine, required for DNA demethylation, thereby influencing gene expression. This figure has been adapted from the original article "syndromic gastrointestinal stromal tumors" by Riccardo Ricci, Hereditary Cancer in Clinical Practice 2016, 14:15 (doi: 10.1186/s13053-016-0055-4; https://hccpjournal.biomedcentral.com/articles/10.1186/s13053-016-0055-4). The original article is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



KIT; in fact, this molecule has been reported to take part also in an anautocrine/paracrine mechanism sustained by SCF produced by GISTs themselves, independently of mutational status [39 40].

PDGFRA, a TKR structurally and functionally similar to KIT, is physiologically activated upon binding to all PDGFs with the exception of PDGF-DD [41]. Coherently, *PDGFRA* mutational hotspots correspond to the functional domains involved by *KIT* mutations. These are: exons 12, the juxtamembrane regulatory domain (corresponding to *KIT* exon 11), and exons 14 and 18 (TK domains, ATP binding region and activation loop, respectively, corresponding to *KIT* exons 13 and 17). Activated PDGFRA elicits the same intracellular pathways triggered by KIT (Fig. 1).

Neurofibromin is a RAS-inactivating tumor suppressor encoded by *NF1* gene; coherently, inactivating mutations of neurofibromin stimulate MAPK cascade through increasing RAS activity [37] (Fig. 1). Thus, the resulting oncogenic mechanism flows along the last part of the pathway most commonly triggered in GISTs, elicited by both KIT and PDGFRA activation. The rare concomitant *KIT* or *PDGFRA* mutations found in NF1-associated GISTs are likely fortuitous events [42 43]. Oddly, despite the well established role of neurofibromin derangement as a GIST trigger, RAS hyperactivity due to *RAS* activating mutations has been only exceptionally signaled in GIST (and moreover together with other oncogenic mutations) [26 44 45].

SDH is a four-subunit (A, B, C and D) Krebs cycle enzy-

matic complex encoded by chromosomal DNA, located in the inner mitochondrial membrane. The succinate accumulation caused by SDH deficiency is oncogenic since it: 1) inhibits prolyl-hydroxylase-mediated hydroxylation of HIF1-α which, no longer degraded, translocates into the nucleus resulting in tumorigenesis and angiogenesis [46]; 2) inhibits TET DNA hydroxylases, impairing the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, necessary for DNA demethylation, ultimately leading to widespread DNA methylation [47 48]. Noticeably, this event, common to all SDH-deficient cases, either *SDH*-mutant or not, is not responsible for the aforementioned *SDHC* promoter methylation, which is restricted to *SDH*-WT SDH-deficient GISTs [30 31].

BRAF is a serine-threonine kinase acting downstream to RAS. The only mutation detected in its gene is exon 15 V600E [1], mostly mutually exclusive with other GIST triggers, accounting for 7-20% of cases WT for *KIT* and *PDGFRA* [24 49 50]. *BRAF* V600E sometimes occurs as a secondary mutation.

Along their tumoral progression, GISTs manifest molecular events additional with respect to the above-mentioned pathogenic triggers. Chromosomal losses in 14q and 22q constitute commonly found early events. More advanced GISTs can additionally show 1p, 9p, 11p and 17p losses [51-53]. Loss of p16 expression was found to be prognostically unfavorable in GISTs, coherently with *p16INK4A* being mapped to 1p [54 55]. Aggressive GISTs have also shown loss of expression of PTEN [56].

SDH-deficient GISTs are distinctive also with respect to chromosomal imbalances, which in these tumors are relatively infrequent and mostly consisting of 1p LOH [57].

In conclusion, the various pathogenic GIST triggers act exploiting few basic intracellular pathways, common to other oncogenic mechanisms in other tumor types, which are summarized in figure 1. As already seen and later further discussed, each of these molecular events can confer peculiar features in terms of epidemiology, morphology, prognosis and drug-sensitivity to GISTs hosting them.

GIST precursors and related lesions

ICC-hyperplasia (ICCH) constitutes the only non neoplastic precursor of GIST known so far. The term ICCH has been variously referred to heterogeneous tiny or even microscopic CD117+ spindle cell lesions [58]; this use is confounding, due to the overlap with the term "micro-GIST", which definitionally refers to GIST smaller than 1 cm [2 59]. Despite the macroscopic detectability has been proposed for distinguishing micro-GIST from ICCH [58], the more objective distinction between diffuse (i.e. ICCH) and focal/nodular (i.e. micro GISTs) lesions is preferred [2 17 37 60]. Adopting this distinction, ICCH lacks 14q and 22q losses, common in GISTs [61], and is polyclonal [62]. ICCH has been hitherto detected exclusively in syndromic settings depending on germline *KIT*-mutations [62-76] or NF1 [23 61 77]. Of note, GIST-prone conditions such as germline *PDGFRA*-mutations, CT and CSS have never revealed such a feature [20, 22, 78]. Thus, the heterogeneity of GIST pathogenesis implies different pathological steps along the way to tumor development, once more supporting these tumors as a family of diseases rather than a single tumor type. Functionally, ICCH causes GI motility disorders [34].

Another likely example of ICCH, although unrelated to GIST progression, is the ICC proliferation commonly found in deep esophageal or gastric leiomyomata; despite this ICC proliferation could at first appear a localized process, contradicting the above-mentioned definition of ICCH, it is actually a diffuse process, although limited within a tumor, possibly due to the production of factors (SCF?) by the neoplastic smooth muscle cells [79].

Another lesion sometimes referred to as ICCH in literature (namely, segmental ICCH) is a segmental replacement of muscularis propria-by a proliferation of CD117+ cells. This condition has been rarely described, always out of apparent syndromic settings. It showed either somatic *KIT* mutations [58 80-82] or silent genomic *NF1* gene mutations [83]. Under these circumstances, the detection of *KIT* somatic mutations could conceal either a monoclonal lesion or a "localized" polyclonal ICCH proliferation similar to the one diffusely detectable in in *KIT*-mutant individuals. On a morphological basis, segmental ICCH, despite being often macroscopically undetectable because of the lack of a tumor, is nevertheless a discrete, although ill-defined, lesion which could be legitimately considered an overt GIST. Therefore, the recently proposed term "gut wall replacing type GIST" (highlighting the tendency to present with gut perforation due to the induced wall fragility) is likely more appropriate than segmental ICCH for defining this condition [84].

Unlike ICCH, "micro-GIST", i.e. GIST measuring < 1 cm, are nodular ICC proliferations with features of true clonal neoplasms, not rarely characterized by the same *KIT* or *PDGFRA* mutations found in overt GISTs [17 59]. In syndromic settings, micro-GISTs, present along with ICCH in *KIT*-mutant and NF1-associated contexts, are the earlier detectable GIST-type lesions in germline *PDGFRA*-mutant individuals and in CSS [34 85-87]. Small GISTs could not always represent early overt GISTs, and some of them could constitute a distinct entity prone to regression: the relatively lower frequency of *KIT* and *PDGFRA* mutations in micro-GISTs, the rarity in macro-GIST of some of the mutations found in the micro-ones [59], and the disproportion between the high incidence of small GISTs [3-5] and the incidence of overt GISTs [11-16] are all findings supporting this possibility. Furthermore, in a recent series of pathologically diagnosed < 2cm GISTs comparing the follow-ups of patients undergoing or not surgical resection, the difference in 5-year GIST-specific mortality was not significant, with a stable plateau reached in the survival of the "non-resection" group after 40 months [88], again suggesting that a distinct subset of indolent small GISTs could exist.

GIST pathology

The role of pathologist in GIST management

Since the discovery of the role of KIT in diagnosis and pathogenesis of GIST, with its heavy clinical impact, this tumor type has constituted a fundamental model for the approach to solid tumors. This event put pathologists in the spotlight as never before. With research progress, a similar procedural method was eventually implemented for many other tumor types. The role of pathologist in GIST management include: 1) the achievement of GIST diagnosis, i.e. the classical pathologist's task; 2) the grading of the clinical risk, implying the evaluation of a combination of parameters which make this process somehow different with respect to the definition of malignancy in many other tumor types; 3) the molecular investigation of the tumor genotype, constituting an example of the expanding investigational field of nowadays pathology.

Histological diagnosis

GISTs can be composed of spindle or epithelioid cells, or of a mixture of them (about 70%, 20% and 10% of cases, respectively). GIST cells feature a mildly eosinophilic, often vacuolated cytoplasm. Extracellular hyaline collagen globules (skeinoid fibers) are often detected in intestinal cases. Marked cellular atypia and high mitotic activity are uncommon: the latter event explains why a count on 10 high-power microscopic fields (HPFs), commonly adopted in other tumor types, is mostly not enough to reliably separate GIST subgroups making necessary to analyze a larger tumor area for this purpose, as discussed in the following section. With regard to the former point, it is worth noting that dedifferentiated GISTs featuring a highly pleomorphic heterologous sarcomatous phenotype have been signaled, not necessarily related to drug secondary resistance [89].

CD117 (i.e. the epitope of KIT) and DOG1 are widely expressed in GISTs, and constitute their most sensitive and specific immunohistochemical markers in the differential diagnosis of soft tissue tumors. A tumor morphology coherent with GIST together with positivity for these two antibodies is enough for a correct diagnosis [90].

Contrary to what at first appears as a reasonable conjecture, CD117 immunoreactivity and *KIT* mutations in GISTs are not related with each other. In fact, these two events do not completely overlap, with about 95% of GISTs expressing CD117 and only 75-80% of cases being *KIT* mutant, with occurrence of tumors displaying either of these features in the absence of the other [1]. As a matter of fact, the expression of CD117, combined to a coherent morphology, has allowed GIST diagnosis in cases triggered by molecular mechanisms other than *KIT* mutations, and reflects a differentiation lineage (i.e. towards an ICC phenotype) rather than a pathogenic mechanism.

DOG1 has revealed no pathogenic role in GIST so far. This molecule, also known as anoctamin 1 or transmembrane protein 16A (TMEM16A), is a Ca^{2+}-activated chloride channel physiologically expressed in ICC and in various epithelial cells. A possible role of DOG1 in cell proliferation has been evidenced, possibly involving modulation of insulin-like growth factor (IGF)/IGF receptor signaling, with possible tumorigenic effects [91-93].

Smooth muscle actin and H-caldesmon are expressed in a relevant fraction of GISTs (about 50% of cases or even more), and are therefore of little utility in their differential diagnosis with smooth muscle tumors. Conversely, desmin can be profitably used in antibody panels for ambiguous cases, being detected in only 1-2% of GISTs as a whole; however, it is worth noting that the fraction of positive cases raises to about 10% in the epithelioid subgroup [94].

S100, a marker of Schwann cells, can be found in a minority of GISTs (about 5-10%, raising to 10-20% in the small intestine) [94 95], a trait sometimes considered, together with coherent ultrastructural features, a clue for defining this GIST subset with the out of practice term "gastrointestinal autonomic nerve tumors" (GANTs) [96].

SDHB, physiologically ubiquitously expressed in normal tissues, gets lost in SDH-deficient GISTs, irrespective of the damaged SDH subunit and of the basis (epigenetic or mutational) of this damage. Thus, the detection of SDHB negativity (in the presence of a positive internal control, i.e. smooth muscle, epithelial, vascular or lymphohematopoietic cells) provides a powerful diagnostic tool for this GIST subgroup [19]. Additionally, SDHA positivity is specifically lost in *SDHA*-mutant cases [97]. SDHB immunoreactivity has been used as discriminating factor for separating GISTs into two subsets, defined type 1 (SDHB+) and type 2 (SDHB-) [98]. Peculiar to SDH deficient GISTs is also the overexpression of insulin-like growth factor 1 receptor (IGF1R), whose immunohistochemical detection appears thus a possible surrogate of SDHB negativity [99].

Immunostaining with VE1 antibody was found to be highly specific for V600E *BRAF* mutation in melanoma and thyroid papillary carcinoma. VE1 positivity has been signaled in a *KIT*-mutant GIST WT for *BRAF* codon 600 [100]; however, if only moderate and strong VE1 staining is considered, this marker appears reliable in GISTs also [101].

Concerning the correlation between GIST morphology, immunophenotype and pathogenesis, SDHB and VE1 positivities are not the only useful descriptors. Although with variable specificity and never in absolute terms, the combination of GIST cytology, architecture, site and protein expression pattern tends to conceal genotypic subgroups. Thus, in *KIT*-mutant, *BRAF*-mutant and NF1-associated GISTs, spindle cell citology and intense CD117 positivity prevail; the latter two GIST types additionally show a predilection for the small bowel, being often multiple in the last case. Gastric and at-least-in part epithelioid GISTs tend to hinge upon *PDGFRA* mutations or SDH deficiency; useful clues for separating these two subgroups are the frequently faint/patchy, or even negative CD117 immunostaining in the former, and the intense positivity for this marker combined with

a multinodular architecture (referred to as "plexiform" because of its resemblance with a plexus cut across) in the latter [18 24 102]. Lympho-vascular invasion, which frequently gives also origin to lymph node metastases (events detected in up to 50% and 10% of SDH-deficient GISTs, respectively), seems responsible for the latter aspect, which likely accounts for the high local recurrence rate of SDH-deficient GISTs after surgery [18]. Of note, lymph node metastases are not typical of GIST subgroups other than the SDH-deficient one. All these clues cannot obviously substitute molecular analysis but, in case of discrepancy, can help in suspecting false results of the latter or the exceptional occurrence of concomitant triggers, with possible relevant clinical consequences [22 29].

Risk assessment

As with other neoplasms, histotypic assessment is not the only fundamental parameter of a pathologic GIST report. The definition of the expected malignancy is in fact pivotal for a correct clinical management. This goal, which in other contexts is usually obtained by integrating two separately defined pathological parameters (i.e. grade and stage), in case of GISTs is accomplished in a single step process which combines descriptors concealing either of these two features, i.e. mitotic index and tumor size; these are in turn stratified according to tumor site, producing an overall risk esteem. Additionally, tumor rupture proved a powerful adverse prognostic factor [103 104]. Although GIST genotype has been repeatedly reported to affect prognosis [105, 106], this criterion has not yet been included in a risk-assessment system, likely because generating too many small tumor subgroups for a reliable comparison in follow-up analyses if strictly applied.

Some of the above-mentioned parameters deserve to be further discussed in order to get rid of some ambiguities they conceal. These are mitotic index, tumor size and tumor rupture.

GIST mitotic index has been for al long time defined as the number of mitoses in a tumor section area measuring 50 HPFs, with a HPF corresponding to the area encompassed by a x40 objective. The "historic" papers which lay the foundations for GIST prognostication employed this definition [103 107]. However, the concept of HPF is intrinsically approximate, in that it varies with respect to the objective employed. Although this inaccuracy could be tolerated in the past, when 50 HPFs usually corresponded to 5 mm^2 due to the homogeneous features of the vast majority of microscopes in use, the widespread diffusion of "new-generation" microscopes, where x40 objectives encompass an area slightly more than double when compared to that of their "old fashioned" counterparts, rendered "50 HPF" an unreliable definition. Thus, GIST prognostic index is nowadays referred to 5 mm^2 [108], an objective measure reflecting the area "de facto" originally employed for setting GIST prognostication systems. This implies the need to set the number of HPFs necessary to cover this surface according to the employed microscope, in most cases corresponding to 21 x40 objective fields.

Given the employment of tumor size as a categorical variable in GIST prognostication, when a tumor approaches one of the cutoff values separating the prognostic groups one wonders whether these values refer to fresh or formalin-fixed tissue, arguing that the latter is expected to undergo a certain grade of shrinkage. Although never stated in the retrospective meta-analyses defining the various GIST prognostication systems, GIST size is commonly determined after fixation in daily practice.

Tumor rupture is another definition prone to ambiguity. Recently, several events potentially encompassed by the term "tumor rupture" have been compared in terms of prognostic impact in a series of small intestinal GISTs. These events were divided into "major" and "minor" defects of tumor integrity: the former consisted of piecemeal resection, tumor spillage or fracture, bowel perforation at tumor site, bloody ascites, microscopic infiltration into an adjacent organ and surgical biopsy (except core-needle biopsy); the latter included iatrogenic peritoneal laceration, peritoneal tumor penetration and microscopically involved margins. Only major defects affected tumor recurrence rates and, although more frequent in larger GISTs, remained significant in multivariate analysis [109]. Microscopically involved margins have been shown not to be prognostically relevant also in other studies, although in the presence of molecular targeted therapy [110].

The main systems of risk assessment of GISTs will be now briefly resumed. A widely adopted GIST risk classification distinguishes 5 classes (from no-risk to high-risk) by combining mitotic rate (stratified in two groups separated by a threshold at 5 mitoses/5 mm^2) with tumor size and site [103]. This classification, which is preferred by the Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP) [111] and is reported in Table I, does not consider in vivo tumor rupture, which must be nevertheless taken into account as necessarily determining a high risk condition. Conversely, all these parameters, including tumor rupture, are considered in the Joensuu's scheme [104].

Prognostic nomograms have also been developed based on site, size and mitotic rate, with the latter again considered as a categorical variable [112]. However, when biological descriptors are considered as categorical parameters in prognostic score systems, it can happen that a minimal variation in one of them can dramatically affect the final prognostication, an event extremely unlikely in biology. This is why a nomogram [113] and a system of prognostic contour maps [114] have been developed considering both mitotic rate and tumor size as continuous variables. Of note, all the above-mentioned classifications have been validated on and apply only to resected GISTs. Moreover, all of them are reliable, with prognostic contour maps probably being slightly more accurate for estimation of individualised outcomes [114]. Of note, the chapter on

Tab. I. GIST risk assessment according to the AFIP criteria (slightly modified from Miettinen and Lasota [103]).

Tumor parameters		Risk (% of patients with progressive disease and characterization of risk for metastasis)			
Size (cm)	Mitoses/5mm²	Stomach	Duodenum	Jejunum/ileum	Rectum
≤ 2	≤5	0 none	0 none	0 none	0 none
2 < X ≤ 5		1.9 very low	8.3 low	4.3 low	8.5 low
5 < X ≤ 10		3.6 low	34 high†	24 moderate	57 high†
> 10		12 moderate		52 high	
≤ 2	>5	0*	‡	50*	54 high
2 <X ≤ 5		16 moderate	50 high	73 hign	52 high
5 < X ≤ 10		55 high	86 high†	85 high	71 high†
> 10		86 high		90 high	

*Very small number of cases.
†Combined because of small number of cases.
‡No tumor included in the study.

GISTs of the 7th edition of the TNM classification appears not satisfactory and is not recommended [108].
Noticeably, all of these risk classification systems do not fit SDH-deficient GISTs [18].
It is worth recalling once more that, whatever the prognostication system employed, the parameters for building it must appear in the pathology report of GISTs, and include tumor site, size, mitotic index per 5 mm² and the status of margins. Whether a pathologically detected tumor rupture occurred in vivo or not, in the first case with a heavy prognostic impact, is a statement pertaining to the surgeon, which should nevertheless appear in the pathology report. In case a ruptured GIST arrives at the pathology lab with no explanations about the loss of tumor integrity, the pathologist should ask the surgeon clarifications on whether such instance occurred in vivo.
A fascinating issue concerns the possible existence of benign GISTs, a hitherto unproven entity. If such a GIST will ever be demonstrated, it will likely be found among micro-GISTs. In fact, as previously discussed, part of these tumors show peculiar genetic traits and/or a spontaneous propensity to regression [59]. However, surgical excision is at present recommended whenever a resectable GIST is diagnosed, provided the risk of morbidity/death is acceptable, with rare admissible exceptions [108]. This implies that an in-vivo risk classification of GISTs, if ever possible, has not been developed, nor is presently warranted. With this regard, it is worth noting that GIST biopsies tend to underestimate mitotic rate, whatever its possible prognostic value in non-resected cases [115].

Molecular profiling

Molecular analyses constitute a pivotal task of the up-to-date approach of pathologist to tumors. In case of GISTs, genotyping features a particularly heavy impact on therapy. Therefore, leaving the detailed illustration of current guidelines for GIST treatment [108 116 117] to specialized oncology papers, some aspects of the consequences of GIST molecular profiling on therapy will be herein resumed.
First of all, it must be recalled that GIST specimens need not to be fixed using Bouin solution in order to preserve the feasibility of molecular analysis [108].
Dividing GISTs according to the gene/molecule constituting the tumor trigger, as illustrated in the above paragraph on GIST pathogenesis, is not enough for defining homogeneous groups with respect to drug sensitivity. In fact, diverse molecular defects can involve each of these triggers, often with peculiar clinical implications.
Imatinib is the first-line standard therapy for GISTs; it targets the TK domains of KIT and PDGFRA stabilizing an inactive conformation of these molecules. Accordingly, imatinib usually is not effective on GISTs whose molecular trigger is located downstream to KIT and PDGFRA (as happens in GISTs hinging upon defects of NF1, RAS, BRAF or PIK3CA) or is completely alternative to these TKs (SDH-deficient GISTs).
Furthermore, the efficacy of imatinib can change even between GISTs which, although sharing a common molecule as trigger, differ in the portion of the latter affected by the pathogenic defect. Thus, *KIT*-mutant GISTs tend to respond when the mutation occurs in exons 8, 9 and 11 (with regard to exon 8, mutations are so rare that evidence is very limited [38 118]), i.e. "upstream" to the imatinib targeted site (i.e. the kinase domain in exons 13 and 17), while resist if the defect is in the latter [37].
PDGFRA-mutant GISTs do not respond to imatinib in case of D842V mutation [37 119]; unfortunately, this is the GIST commonest *PDGFRA* mutation.
These premises bring about several consequences in the management of GISTs. Among these, imatinib adjuvant therapy is contraindicated in the presence of *PDGFRA* D842V, or in NF1-associated or SDH-deficient GISTs. Similarly, genotypes poorly responsive to imatinib (such as *PDGFRA* D842V mutations) tend to be excluded from neoadjuvant therapy with this drug, while dosing is increased in case of *KIT* exon 9 mutations, which tend to be less sensitive [18 105 108 116 117].
Luckily, *PDGFRA*-mutant, NF1-associated and SDH-deficient GISTs often behave relatively less aggressively than their *KIT*-mutant counterpart, even in the presence of metastases in case of SDH deficiency [18 95 102 105]. By the way, the possible option of adjuvant and neoadjuvant therapies in GISTs highlights the pivotal relevance of GIST biopsies, not only for

ascertaining GIST diagnosis, but also to determine the molecular profile of tumors.
PDGFRA D842 resists also to the second line TKI sunitinib; crenolanib and dasatinib can be considered in need of treatment of GISTs bearing this mutation [37 119-121].
With regard to *BRAF* V600E, which can occur also as a secondary mutation conferring resistance to imatinib in GISTs whose primary mutation is sensitive to this drug [26], sorafenib, a multi-target TKI targeting BRAF, or dabrafenib, a selective inhibitor of BRAF, can be considered [50 122]. *BRAF*-mutant GISTs were at first reported to tend to aggressiveness [49]. However, V600E *BRAF* mutations have been subsequently detected also in mitotically inactive micro GISTs [123], and have revealed among the more prognostically favorable mutations in GIST [106]. Therefore, the initially reported biologically unfavourable role of *BRAF* V600E was likely due to a case selection bias.
Recently, regorafenib has been shown to produce objective responses and clinical benefit in SDH-deficient GISTs [124], a GIST subgroup whose rarity and often extremely slow progression in aggressive cases makes extremely difficult the evaluation of drug effectiveness, given the uncertain meaning of long survivals and/or stable diseases [125].
Responsive GISTs resected following TKI therapy feature variable degrees of stromal hyalinization or, less frequently, necrosis [126]. A scoring system for evaluating GIST response has been developed based on the relative proportion of these regressive aspects, with cutoffs at 10%, 50% and 90% of tumor mass [127].
Responding GISTs commonly develop secondary resistance to imatinib in 12-36 months, usually acquiring mutations in the same gene and allele hosting the primary defect, resulting in tumors bearing double mutations. These secondary alterations mostly involve a kinase domain (exons 13 and 17 of *KIT*, exon 18 of *PDGFRA*) [37 127-129]. Imatinib dose-escalation followed by switch to alternative drugs can be effective in treating these cases. Secondary resistance to second-line drugs has shown to be sometimes overcome by rechallenge with imatinib, effective also in cases progressing upon imatinib therapy discontinuation [130 131].
Double mutations have been exceptionally found also in naïve GISTs, as happens with the rare *KIT* or *PDGFRA* mutations in NF1 GISTs [43], and in the exceptional finding of *KIT* mutations in GISTs bearing SDH alterations [21 132] or of double primary mutations in *KIT* or *PDGFRA*; the latter can involve not only nearby nucleotides within the same exon (likely depending on a single mutagenic event and devoid of relevant biological consequences), but also diverse exons in the same allele or even in different genes [22 29]. The awareness of these very rare instances is nevertheless relevant, since a member of the trigger duplet can escape detection in case of partial analysis, possibly leading to neglecting data crucial for an effective therapy. Therefore, simultaneous investigation of the most common GIST triggers is warranted in cases to be treated, including syndromic settings [22, 29]. Similarly, the completion of the analysis of these triggers is indicated in cases only partially genotyped which do not respond to therapy as expected according to a first, partial molecular analysis.

Syndromic GISTs

As mentioned in the above paragraph on GIST pathogenesis, the tumorigenic mechanisms found in GISTs can rarely involve constitutively the entire organism, causing the predisposition to GIST arousal. Under these circumstances, GISTs are often multiple, and accompanied by various phenotypic traits determining well defined syndromes. About 3-4% of GISTs arise in these syndromic settings. The clinical approach to these diseases depends on a balance between the treatment of each single occurring tumor, according to its intrinsic features, and the management of the trends and risks peculiar to the syndrome dealt with. The latter issue includes also the opportunity of performing a familial screening, to be evaluated according to the inheritability and penetrance of the condition.
Manifestations associated with GISTs in syndromic backgrounds are specific for each syndrome: ICCH, skin pigmentation disturbances (usually hyperpigmentation) and mast cell disorders are in fact typical of *KIT*-mutant syndrome [34]; inflammatory fibroid polyps (IFPs), large hands and GI lipomas are described in *PDGFRA*-mutant syndrome [20]; again ICCH, neuroendocrine tumors (especially periampullary somatostatinomas) and the lesions constituting the classical NF1 diagnostic criteria adopted by the National Institutes of Health (NIH) are found in NF1 [23 61 133 134]; paragangliomas with or without pulmonary chondromas are proper to CT and CSS (i.e. syndromic SDH-deficient GISTs), respectively [135 136]. All these traits can be useful for suspecting a GIST-predisposing syndrome even before diagnosing a single GIST. Conversely, it is worth noting that GISTs are the most common GI manifestation of NF1, a syndrome relatively common in man, with an incidence at birth of about 1:3000, and a 1:4-5000 prevalence [23 133]. The awareness of this aspect can help in diagnosing such a disease. In fact, mutational analysis is mostly not used as a diagnostic tool in NF1 due to the lack of mutational hotspots; additionally, this condition can present without fulfilling the NIH diagnostic criteria and, in about 50% of cases, with no familial history [133 134].
With regard to syndromic GISTs, their morphology and location as a rule do not differ from those of their existing corresponding sporadic counterparts.
The features of GIST-prone conditions have been detailed elsewhere [34].

Diagnostic pitfalls

The issues treated so far conceal several diagnostic pitfalls at risk of compromising the correct identification of

GISTs in the daily diagnostic routine. These traps will be herein highlighted.

ICC in GI leiomyomas

Leiomyomas (LMs) arising in the muscularis propria of esophagus and stomach (i.e. deep esophageal and gastric LMs) host ICCs in about 100% and 75% of cases, respectively. The fraction of ICC with respect to neoplastic smooth muscle cells ranges between 5 and 30%, with focal peaks of 50%, with possible cell aggregates. These intratumoral ICC show a density significantly higher when compared to ICC in the neighbor visceral wall, an aspect supporting their hyperplastic condition. The expression of CD117 and DOG1 in these ICCs can lead to misdiagnose deep esophageal and gastric LM as GISTs, especially in biopsy samples. This can imply relevant clinical consequences, since LM can be usually surgically treated with approaches more conservative with respect to those used for GISTs, especially at esophageal level, including enucleation. A thorough examination of the morphology of intratumoral DOG1+ and CD117+ cells and of sections stained with H&E can avoid mistakes, evidencing the long dendritic processes (which sometimes branch), typical of non-neoplastic ICC, in the former and the presence of smooth muscle tumoral cells with intensely eosinophilic cytoplasm, possibly with cigar-shaped nuclei, in the latter. Leiomyomatous cells can be additionally highlighted with desmin IHC. Moreover, molecular analysis invariably reveals WT *KIT* and *PDGFRA* in these cases [79].

DOG1 in GI carcinomas and normal epithelial cells

DOG 1 is usually considered in the immunohistochemical differential diagnosis between GISTs and other mesenchymal neoplasms; in this perspective, it is a marker highly sensitive and specific. However, DOG1 is often expressed in GI carcinomas, especially in squamous cell carcinomas and various adenocarcinomas, and can be found in normal epithelial cells, as happens with gastric mucosal epithelium [137]. This aspect must be kept in mind when dealing with epithelioid lesions in small biopsies. In ambiguous cases, the coexpression of CD117 and/or the detection of one of the triggers typical of GIST at molecular analysis support a GIST diagnosis, unlike positivity for epithelial markers, which favor a diagnosis of epithelial cells or carcinoma/adenocarcinoma.

S100 in GISTs (so called "GANTs")

S100 immunohistochemistry is highly sensitive for Schwann cells. Schwannomas are schwannian tumors described at GI level. Therefore, the detection of a GI spindle cell tumor manifesting S100 positivity can lead to a diagnosis of schwannoma. However, a fraction of GISTs (sometimes defined as GANTs), which in the small intestine can reach 20% of cases, show S100 positivity; moreover, some GISTs can morphologically mimics schwannomas, featuring palisaded architecture, anucleated pools constituted by entangled cell processes simulating Verocay bodies, and vascular hyalinization [95 96]. Once more, the pitfall mainly concerns small biopsies, where the morphological quality can be suboptimal. Under these circumstances, CD117 and DOG1 are pivotal in addressing toward a GIST diagnosis in case of positivity. Genotyping is helpful in cases uncertain after immunohistochemistry.

H-caldesmon in GISTs

Unlike desmin and smooth-muscle actin, H-caldesmon is able to discriminate between myofibroblasts and true smooth muscle cells. Therefore, this marker is commonly used to recognize smooth muscle tumors in the differential diagnosis of soft tissue lesions. However, GISTs are mesenchymal tumors which, although not belonging to the smooth muscle lineage, often express H-caldesmon [137]. Since the distinction between GISTs and smooth muscle tumors probably constitutes the main differential diagnosis in GI mesenchymal neoplasms, it is recommended not to employ H-caldesmon in it, rather preferring desmin, much more specific for smooth muscle tumors at this anatomical level (with the limitations previously discussed in the paragraph concerning histological diagnosis).

Dedifferentiation in GISTs

Exceptionally, GISTs have been reported to dedifferentiate to an anaplastic CD117-negative phenotype, either in the presence of imatinib therapy or not. In one case, the dedifferentiated component featured characters consistent with angiosarcoma. All of the reported cases showed no difference in the *KIT* genotype between the differentiated and the dedifferentiated components (these did not show genotypic differences at all in some instances) [89]. The biological implications of GIST dedifferentiation, with particular regard to imatinib-naïve cases devoid of demonstrable associated genotypic changes, remain so far unknown. Nevertheless, an accurate GIST diagnosis in these cases could imply the employment of molecularly targeted therapies not indicated in other sarcomas. A diagnostic approach to biopsies sampling the dedifferentiated component of these tumor is extremely problematic. A possible recommendation is to repeat sampling from diverse tumor areas in case of biopsies on masses, arisen in sites where GISTs can be expected, showing morphologic features of pleomorphic sarcomas, and/or proceed with molecular analysis, exploiting the above-mentioned *KIT* genotypic identity between the dedifferentiated and the differentiated components. In the latter circumstance, S100 immunohistochemistry is also relevant, in that GI pleomorphic tumors harboring *KIT* mutations analogous to those detected in GISTs can actually be melanomas [138].

PDGFRA mutations in inflammatory fibroid polyps

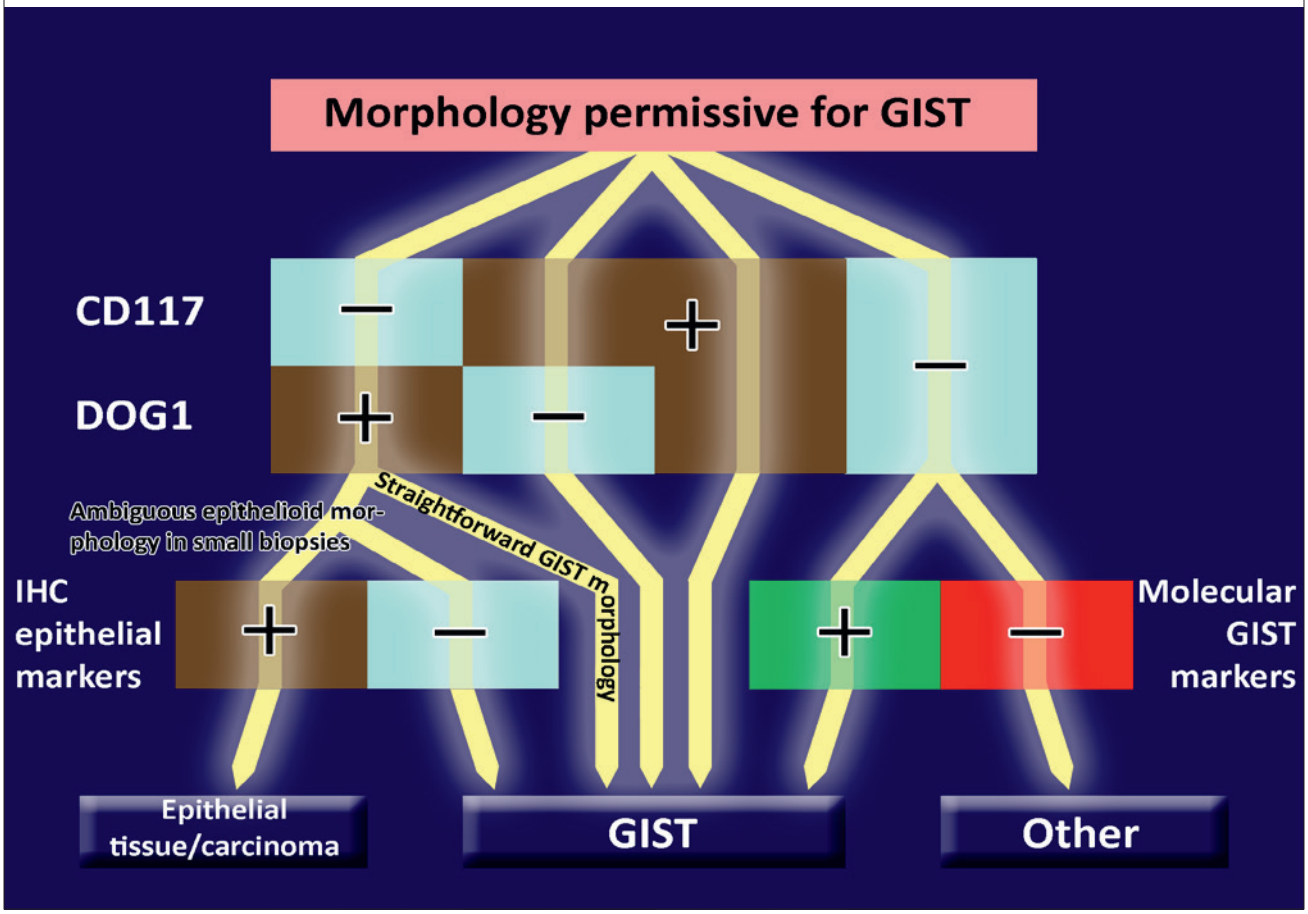
In case of GI mesenchymal lesions negative for CD117 and DOG1 at IHC, the detection of one of the classical

pathognomonic GIST molecular triggers allows a correct GIST diagnosis in the presence of a coherent morphology. Noticeably, the former should not be overweighed with respect to the latter, which is in fact pivotal for not misdiagnosing an IFP. In fact, IFP is a relatively common GI neoplasm often harboring *PDGFRA* mutations alike to those found in GISTs [139 140]. IFP morphology is nevertheless enough distinctive, featuring a typical triphasic structure composed of fibroblast-like mesenchymal cells, inflammatory cells and blood vessels; the former, displaying CD34 positivity (especially in gastric cases), are often organized in short fascicles with an onion skin pattern around the latter, with intermingled leukocytes typically rich in eosinophils and mast cells, in a myxoid collagenous matrix. Unlike GISTs, IFP stromal tumor cells never express DOG1 or CD117, with the latter restricted to the infiltrating mast cells.
An extremely synthetic GIST diagnostic flowchart is reported in Figure 2.

Conclusions

GISTs constitute an outstanding model of approach to solid tumors. In the past, these neoplasms were mostly misdiagnosed as smooth muscle tumors and featured a very poor survival in malignant cases, due to the lack of reliable diagnostic tools and of an effective chemotherapy caused by the ignorance of their pathogenesis. The progress in GIST knowledge eventually revealed their complex biology, and implied an outstanding role of pathologists. The latter thus became pivotal not only for accomplishing a histotypic diagnosis, but also for the clinical management of patients in its widest sense, as exemplified by the very close dependence of therapeutic choices on the molecular pathology report. This model is being followed in an increasingly high number of diverse oncological settings. The undoubtedly overall clinical success of the "GIST saga" owes a great deal to pathologists, constituting a sequence of events which

Fig. 2. GIST diagnostic flowchart. The detection of a permissive morphology in a lesion clinically consistent with GIST needs immunohistochemical positivity for CD117 and/or DOG1 to lead to a reliable GIST diagnosis. Noticeably, in case of positivity for DOG1 only, small biopsies hosting epithelioid cells can be tricky, wrongly suggesting a diagnosis of epithelioid GIST. In fact, epithelial cells, including several GI carcinomas, can express DOG1. Therefore, additional investigations using epithelial markers and, if necessary, even molecular ones, are recommended under these circumstances, allowing a correct differential diagnosis. This is not the case of resection specimens, where the overall morphologic features of epithelioid GISTs usually stand out without ambiguities. With regard to lesions morphologically consistent with GIST but expressing neither CD117 nor DOG1, molecular analysis assumes a diagnostic value (beyond its usual role of determinant for a correct chemotherapy), being confirmatory for GIST in case one of the pathognomonic molecular triggers is found (under these circumstances, it must be considered that IFPs are often PDGFRA-mutant similar to GISTs; however, their morphological features are usually distinctive enough to avoid misdiagnoses).



put them in the spotlight as never before, prompting this medical category, often operating "in the dark" beyond a microscope, to be aware of its unique role in the management of patients.

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