The exciting results obtained in metastatic gastrointestinal stromal tumors (GIST) treated with imatinib mesylate (STI571; Gleevec/Glivec, Novartis Pharma) and its low toxicity profile have prompted the development of several international prospective trials to evaluate its therapeutic effect also in the neoadjuvant/cytoreductive and adjuvant setting (1–6). Never-
theless, it is known that the likelihood of imatinib response is strictly related to the mutational pattern of the tumor: KIT mutations on exon 11 are associated with a response rate of ~80% to 85%, whereas the response rate of tumors containing exon 9 KIT mutations is roughly 50% and only occasional responses have been observed in patients with no detectable mutations (wild-type tumors; refs. 7–11). Among KIT wild-type tumors, GISTs arising in patients affected by neurofibromatosis type 1 (NF-1) occupy a remarkable position, but little is known about their pathogenesis and their sensitivity to imatinib.

NF-1, also known as von Recklinghausen disease, is a fairly common genetic syndrome, affecting approximately 1 in 3,000 individuals. NF-1 is caused by mutations of the NF-1 gene on chromosome 17 (17q11.2), which encodes neurofibromin, a negative regulator of RAS activation. Although inherited in an autosomal dominant pattern, a family history is reported only in about half of all newly diagnosed cases. Owing to its variable penetrance, the phenotype is wide, but clinical diagnostic features are multiple neurofibromas, café-au-lait macules, skin-fold freckles, and iris hamartomas (Lisch nodules). Many other complications may accompany the neurofibromatosis, such as mental disabilities, precocious or delayed puberty, skeletal deformities, and an increased risk of developing several tumors, including multiple GISTs. Although data available are scant, reported cases suggest that NF-1–associated GISTs manifest at a younger age than sporadic GISTs, are more common in the

**Abstract**

**Purpose:** Patients affected by neurofibromatosis type 1 (NF-1) have an increased risk of developing gastrointestinal stromal tumors (GIST). NF-1–associated GISTs are usually wild type for c-KIT and platelet-derived growth factor receptor-α (PDGFR-α) mutations and harbor a different oncogenic molecular mechanism. The lack of data on imatinib activity raises the question whether to enroll these patients in clinical trials. We analyzed a large series of NF-1 related GISTs to discuss the therapeutic implications.

**Materials and Methods:** Clinical, pathologic (IHC to CD34, S100, bcl-2, PDGFRA), and molecular features (exons 9, 11, 13, 14, 17 in c-kit and exons 12, 14, 18 in PDGFRA) of 28 patients were analyzed.

**Results:** The most common site of primary lesions was the small bowel (75%). Twelve patients (43%) had multiple tumors. Most tumors belonged to the high (30.5%) or intermediate risk group for malignant behavior (39%). Three patients developed peritoneal and liver metastases; another four had peritoneal spread only. All tumors were immunohistochemically strongly positive for CD117. Three primary KIT/PDGFRα activating mutations were found. Three metastatic patients treated with imatinib experienced progression, and only one had temporary stable disease. Median survival after starting treatment with imatinib was 21 months.

**Conclusions:** This study is the largest series available and confirms that KIT/PDGFRα mutations in NF-1–associated GISTs are sporadic. Prognosis of metastatic tumors is poor, and imatinib response rate is low. Patients with NF-1–GIST of high or intermediate risk should not be eligible for adjuvant trials of imatinib. Imatinib should not be used in a neoadjuvant intent in these patients, and molecular analysis of activating mutations is strongly recommended.
small bowel than in the stomach, and are often multiple and a little female predominance was noticed (12). Most interestingly, the molecular analysis of main published series indicates that GISTs in NF-1 are usually wild type, although they usually stain positive for c-KIT by immunohistochemistry. The almost uniform negativity for KIT/platelet-derived growth factor receptor A (PDGFRA) mutations in these patients is very likely due to a different mechanism of tumorigenesis based on the neurofibromin gene disorder.

The lack of data on imatinib activity in this different setting raises the question whether to enroll these patients in the ongoing trials of imatinib and whether this decision should be driven by the molecular analysis. To get insights into this matter, we analyzed the clinicopathologic features, survival, and response to imatinib in a series of 28 patients with NF-1–associated GISTs. On the basis of both present results and the literature review, this paper provides an updated discussion on the therapeutic implications from the NF-1–related GIST molecular profile.

Materials and Methods

This study has been carried out on 28 patients affected by GISTs and NF-1. Clinical data were retrospectively reviewed from the databases of the University Hospitals of the Medical Faculties of Mannheim and Bonn and Istituto Nazionale Tumori. All data had been gathered prospectively.

The diagnosis of NF-1 was made when at least two of the following criteria were present: six or more café-au-lait macules (>5 mm before puberty, >15 mm after puberty), skin-fold freckles (groat, axilla, neck base), two or more neurofibromas (one plexiform), skeletal dysplasia (orbital or tibial), Lisch nodules (two or more iris hamartomas), optic glioma, and family history.

Histologic diagnoses of GIST and molecular analysis were reviewed prospectively by two experienced pathologists. All patients underwent resection of the primary tumor according to the standard of care. Surgery was macroscopically complete in all cases except in two patients where only a debulking could be done due to multiple peritoneal seedings. Five patients had metastases at diagnosis.

None of the patients had imatinib in the preoperative setting. Four patients had imatinib in the postoperative setting after complete resection of the tumor. One patient was enrolled in the SSG-VIII/AIO trial of imatinib as adjuvant agent and randomized to the arm of imatinib treatment for 1 y. Two patients were enrolled in the EORTC 62024 ongoing trial of imatinib as an adjuvant agent, one in the control group, and one in the arm of imatinib therapy for 2 y. One patient received imatinib at 400 mg daily outside any trial as postoperative treatment after resection of a metastatic GIST of the jejunum. Another patient received imatinib at 400 mg daily after multiple surgical resections of multiple recurrent GISTs of the small bowel that required urgent procedures for bleeding. Four metastatic patients had been treated with imatinib within the EORTC 62005 trial. Two had dose escalation to 800 mg/d.

Five patients had other tumors before or after the diagnosis of GIST. Tumors observed were gastrointestinal carcinoids (n = 2), basalioma (n = 1), meningioma (n = 1), uterine carcinoma (n = 2), breast cancer (n = 1), and pheochromocytoma (n = 1). Most patients were followed prospectively after treatment. In all other cases the clinical history was obtained from their medical practitioners. Periodic assessment comprised a physical examination, abdominal computed tomography or magnetic resonance imaging, FDG-PET, and chest X-ray.

 Disease-specific survival and event-free survival were estimated by the Kaplan-Meier method and calculated from the time of surgery for the primary tumor and from the time of imatinib onset to the latest date of event-free follow up, dead, or first event (local recurrence or metastasis). Patients who died from other causes were considered censored. Four patients got lost to follow-up soon after surgery. The median follow-up of the remaining cases was 35 mo.

Histopathology and immunohistochemistry. H&E-stained sections of all resected GISTs, as well as segments of adjacent bowel, were reviewed. The diagnosis of GIST was confirmed histologically in terms of morphology and immunophenotyping. Histomorphologic subclassification was given as spindle cell, epithelioid and mixed type. Immunohistochemical staining was done using antibodies against CD117 (KIT receptor), CD34, bcl-2, α-actin, desmin, S-100 protein, vimentin (all DAKO), and PDGFRA (Santa Cruz Biotechnology).

Molecular analysis. Adequate histologic material for molecular analyses was available from 25 patients. Tumors were molecularly characterized by performing DNA sequencing of exons 9, 11, 13, 14, and 17 of c-KIT gene and of exons 12, 14, and 18 of PDGFRA. DNA was extracted from formalin-fixed, paraffin-embedded tumors and amplified for KIT and PDGFRA as reported elsewhere (13). Tissue slides were deparaffinized by xylene and microdissected from serial sections (10 μm) of tumors. Total DNA was extracted after pretreatment with proteinase K and absorption on silica gel membranes (Qiagen). After estimation of DNA concentration by agarose gel electrophoresis, relevant exons were amplified with intronic primers. The PCR products were purified using Micro Spin columns (Amersham Biosciences). Bidirectional DNA sequencing of the entire exons and the corresponding exon-intron boundaries was done with the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Cycle sequencing products were precipitated with 2 mol/L sodium acetate and analyzed on an ABI PRISM 310 capillary electrophoresis system (Applied Biosystem). All sequence alterations were confirmed by an independent PCR amplification and sequencing to exclude PCR artifacts. The identity of the amplicon sequences was confirmed by database search.4

To study the interactions between mutant receptors and imatinib, we did molecular dynamics simulations in the framework of the Molecular Mechanics/Poisson-Boltzmann Surface Area computational techniques, as already described elsewhere (14).

Results

Clinical characteristics and survival. The main patients and tumors characteristics are summarized in Table 1. There were 13 males and 15 females (male/female ratio, 0.87). Their median age was 57 years (range, 28–72 years). Most patients were symptomatic at diagnosis. The most common symptoms were abdominal pain (63%), nausea and vomiting (19%), and gastrointestinal bleeding (13%). In one case, the tumor was found incidentally during unrelated surgery and only one patient had a palpable mass. Nineteen patients had tumors located in the small bowel, four in the stomach, two in both sites, and three patients had extraintestinal tumors. Twelve patients (43%) had multiple primary GISTs.

The 5-year disease-specific survival and event-free survival were 54.3% (median, not reached) and 46.9% (median, 48 months), respectively. Eight patients developed local recurrences or metastases. Among this latter group, three patients had liver and peritoneal metastases and four had only the peritoneal spread. None of the patients developed pulmonary lesions. Six patients died of the disease. Patients who developed

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liver or peritoneal metastases had a median postevent survival of 33 months. None of the patients with multiple tumors developed distant metastases.

Pathologic and immunohistochemical characteristics. Six tumors were reclassified as GISTs after pathologic review. They had been initially encoded as schwannoma (n = 2), leiomyoma (n = 1) or leiomyosarcoma (n = 1), and malignant peripheral nerve sheath tumor (n = 2). The majority of tumors were of spindle cell type (75%), 15% showed an epithelioid phenotype, and 10% displayed a mixed morphology. All tumors were immunohistochemically strongly positive for CD117. Immunohistochemical characteristics and risk classification are detailed in Table 1. Six patients showed diffuse hyperplasia of interstitial cells of Cajal.

Molecular analysis. Three tumors from three different patients showed activating mutations of c-KIT (V560 deletion of exon 11 and A502_Y503 duplication in exon 9) and PDGFRA (D842V point mutation). The first mutation was found in a high-risk gastric GIST from a patient who had also a second low-risk gastric tumor being without detectable mutation (wild type). The other two mutations were found in two patients with a single tumor of the small bowel and stomach, respectively. The diagnosis of NF-1 had been made on the basis of multiple café-au-lait spots and superficial neurofibromas that were present in all three patients. One patient had also bone deformities (orbital and tibial). None had family history. A secondary mutation of c-KIT (DB20N point mutation of exon 17) was shown in metastatic lesions of another patient, but the primary tumor was wild type. This mutation, not previously reported, was shown to be activating, but resistant to imatinib, by the molecular modeling.

Response to imatinib. Three patients, affected by multiple hepatic and abdominal metastases treated with imatinib, showed primary resistance and experienced progression soon. They died of disease after 10, 19, and 22 months from the onset of the treatment. One patient with liver and peritoneal metastasis had temporary stable disease as best response. This patient had an activating exon 18 point mutation. The other three patients had primary wild-type tumors. All four patients died of disease within 2 years from the beginning of the treatment (Table 2). Antiproliferative treatment consisted of imatinib until the maximal dose of 800 mg daily. None received other tyrosine kinase inhibitors or other target therapies.

No local or distant recurrences were observed in patients with resectable tumors who received imatinib in the postoperative setting. These patients are still alive and under imatinib therapy at 400 mg daily (Table 3).

Discussion

Several genetic disorders are associated with increased risk of developing GISTs. Among these, NF-1 is the most common disease. The incidence of GIST in NF-1 remains unclear. A recent analysis carried out in a series of 3,000 GIST cases suggests that NF-1 patients have a risk of GIST development at least 45-fold higher than the general population (12), whereas one autopsy study found incidental GISTs in 3 of 12 NF-1 patients examined (25%; ref. 15). Most of our patients came to observation because of symptoms, but a subset of NF-1 patients could have asymptomatic tumors. This makes it difficult to evaluate their overall prognosis. When symptomatic, this subset of GIST is often at high or intermediate risk. If we consider that our series comes from sarcoma committed oncology facilities, we can explain the higher incidence of high and intermediate risk tumors and distant metastases compared with other previous studies (12, 15). These more population-based series likely better represent the entire group of NF-1–associated GISTs and suggest this subset of GISTs has a different biological behavior.

The other main clinical differences that we observed in our series compared with sporadic GISTS (2, 5, 16–21) were a
slight female predominance, high incidence of tumors in the small intestine, and common occurrence of multiple synchronous primary lesions (Table 1). Interestingly, multiple primary tumors were associated with a better outcome, independently from the risk classification according to size and mitotic count (2, 22). These data are consistent with the main published series.

Most of our patients had wild-type GIST in the hotspots of both KIT and PDGFR-α gene. Three different activating mutations were found in three patients. A secondary activating mutation of the exon 17 was found in metastatic lesions of another patient after imatinib therapy, but the primary tumor was wild type.

The present series is the largest ever published with a complete molecular analysis. The first study of NF-1–associated GISTs molecular profile has been provided by Kinoshita and colleagues (23). They assessed 29 GISTs from seven NF-1 patients and did not find any KIT or PDGFRA mutations. In the same study, 10 sporadic GISTs from non–NF-1 patients were analyzed and none of them had detectable NF-1 mutations. These results strongly suggest that not only the pathogenesis of GISTs in NF-1 patients is different, but also that NF-1 mutations are not an important addition to KIT or PDGFRA mutations in sporadic GISTs. Although most tumors require several mutations to develop, NF-1 gene mutations and KIT/PDGFR mutations seem mutually exclusive. Other subsequent series and case reports confirmed the common wild-type molecular profile of GISTs in NF-1 (12, 24–30), and only occasional KIT and PDGFRA mutations have been reported (31–33). With present series, only 10 KIT/PDGFR mutations have been found of the 87 patients analyzed (Table 4). Surprisingly, although the incidence of mutant KIT/PDGFR is much lower than in sporadic GISTs (11.5% versus 85%), the distribution of mutations is similar, with a predominance of mutant exons 11 (5.7%).

The common wild-type status for KIT and PDGFRA mutations of GISTs in NF-1 suggests that their pathogenesis should be sought elsewhere. Neurofibromin is a member of GTPase-activating protein family of the RAS regulatory proteins. Neurofibromin increases GTPase activity catalyzing the conversion of the active form of RAS to the inactive form. NF-1 gene mutations disrupt the normal function of the neurofibromin and results in constitutive RAS activation. This activation increases downstream signaling through the mitogen-activated protein kinase pathway (34). Gain-of-function mutations of c-KIT also result in constitutive activation of the RAS–mitogen-activated protein kinase cascade. Thus, one may suppose that the activation of this common pathway led to GIST development. This hypothesis was confirmed by Maertens et al. (25). They found high levels of phosphorylated mitogen-activated protein kinase in NF-1–related GIST cells. In the same study, the authors found both germline mutations in each patient and somatic mutations inside tumors. Somatic mutations are frequent events associated to neurofibromas and other NF-1–associated tumors and could be responsible of cancer development in accordance with the Knudson’s two-hit theory. The NF-1–associated Cajal intestinal hyperplasia might be the precursor of GIST. In this light, it would be more clear because NF-1–associated GISTs are more common in the small bowel and are often multiple. We found a Cajal intestinal hyperplasia in 21% of patients, and all had multiple tumors. There is also the possibility that NF-1 gene mutations...
led to overexpression of c-KIT. Badache et al. observed that KIT is highly expressed in neurofibrosarcoma-derived Schwann cells deficient in neurofibromin but expressed at low levels in primary Schwann cells or malignant Schwann cells with normal levels of neurofibromin expression (35). However, Maertens et al. showed that KIT phosphorylation of NF-1–related GIST cells is stem cell factor–dependent and in ex vivo exposure to imatinib results in total inhibition of KIT but only in moderate inhibition of mitogen-activated protein kinase phosphorylation, neither complete nor dose dependent (25). This study showed that the overexpression of KIT observed in this subset of GIST is a concurrent event, but not the pathogenetic mechanism that drives the tumor growth.

A different oncogenic mechanism likely requires a different therapeutic approach, but a role of tyrosine kinases inhibitors cannot be ruled out on the basis of the high level of KIT expression in NF-1–associated GIST cells. The only two other clinical reported cases of NF-1–related GIST treated with imatinib are from Lee and Kalender (36, 37). The former reports a partial response at a dose of 400 mg daily in a patient with multiple peritoneal and liver metastasis, whereas Kalender observed a progression of liver and omental metastases under imatinib at 600 mg and 800 mg daily, but a partial response with sunitinib at 50 mg daily in the same patient. The results of imatinib treatment in our series were disappointing, and all metastatic patients treated with imatinib died of disease within 2 years from the beginning of the therapy. Their poor prognosis was similar to the prognosis of sporadic metastatic GISTs in the pre-imatinib era. The only patient who experienced SD as best response had an activating PDGFRA mutation. The diagnosis of NF-1 had been made on the basis of multiple café-au-lait spots and neurofibromas without family history. The normal tissue of this patient was not analyzed. This case, however, harboring a sporadic primarily imatinib-resistant mutation, does not reflect the imatinib sensitivity of NF-1–related GIST subset and should be considered apart. The meaning of occasional occurrences of activating mutations in NF-1–associated GISTs remains unclear. Likely, they do not contribute to the understanding of the tumor pathogenesis, but could be explained either as a sporadic occurrence of GIST in a patient with concomitant NF-1 or as a clinical mistake in the diagnosis of NF-1. Because >300 independent mutations (point mutation, deletion, or insertion), have been reported in NF-1 gene, the diagnosis of NF-1 is still based largely on clinical criteria and this would be consistent with the second hypothesis. On the other hand, NF-1 is among the most common human genetic disorders, and this would be consistent with the former hypothesis and with the distribution of the mutations that is the same of sporadic GISTS. However, the occurrence of primary or secondary KIT/PDGFRA mutations requires a careful evaluation of these patients. A systematic molecular analysis is recommended to elucidate whether the molecular profile is the main predictor of imatinib response as in sporadic GIST, and the diagnosis of NF-1 should always be reconsidered.

Patients who received imatinib in the postoperative setting are all disease-free, but all had a complete resection of the tumor and the follow up is rather short.

### Conclusions

Given the different pathogenic mechanism of GISTS in NF-1 and the low response rate to imatinib observed in our experience, we would recommend that these patients are not enrolled in adjuvant trials with imatinib. These patients would experience the side effects of the therapy without a clinical significant evidence of efficacy in their peculiar condition.

The disappointing results we observed in metastatic patients suggest a careful surveillance in patients treated with preoperative cytoreductive intent. Abdominal computed tomography or magnetic resonance imaging and FDG-PET should be used as standard examinations and should be repeated early after the start of the therapy (38–40). The encouraging result obtained with sunitinib in one patient progressing under imatinib suggests it as the first alternative in nonresponding tumors. Nevertheless, the future treatment of this subset of GISTS is likely dependent from further investigations of the molecular pathways activated by neurofibromin as new molecular targets.

### Disclosure of Potential Conflicts of Interest

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References

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