Supplementary Methods

Cytopathology and histopathology

Aspirates obtained by FNA were smeared onto glass slides and stained with Giemsa. Additional material was dissolved in a phosphate-buffered saline solution (PBS) for subsequent preparation of cell blocks. The biopsies obtained by EUS-FNB were flushed by air into formalin-filled tubes. Samples were sent to the study cytopathologist (AD) and the study pathologist (ON), both of whom were experienced in gastrointestinal (cyto)pathology including the assessment of EUS-samples.

Cytospin glasses of FNA-samples were prepared with a Cytospin centrifuge (600 rpm for 3 min and 100 µL/sample) using positively charged glass slides. Prior to immunostaining, the slides were fixed in acetone for 10 min at -20 °C, except for the slide to be stained for Ki-67, which was fixed in formalin for 10 minutes, then treated in a microwave oven (at 700 W for 7 min and 300 W for 15 min) with Dako Target Retrieval Solution (citrate pH 6, S2031), then cooled to room temperature and rinsed with deionized water.

Biopsy samples were formalin-fixed and paraffin-embedded (FFPE). Sections (3-4 µm) were placed on positively charged glass slides and treated in Dako PT-Link using EnVision™ FLEX Target Retrieval Solution (TRS High).

Immunostaining was then performed in a Dako Autostainer Link using EnVision™ FLEX according to the manufacturer's instructions (DakoCytomation). Positive and negative controls were included in each run. The following primary antibodies were used: anti-smooth muscle actin (clone 1A4, IR611(h)/M0851(c), Dako), anti-CD34 (clone QBEnd10, M7165, Dako), anti-desmin (clone D33, IR606(histology)/M0760(cytology), Dako), anti-DOG1 (clone K9, NCL-L-DOG1, Leica Bio-systems(h)/Novocastra (c)), anti-KIT (A4502, Dako), anti-Ki67 (clone MIB1, IR626(h)/M7240(c), Dako), and anti-S100 (IR504(h)/Z0311(c), Dako).

Samples were regarded diagnostic if the tumor morphology was consistent with GIST and if the staining for CD34, KIT (CD117), or DOG-1 was positive. Staining for S-100 and desmin was should have been negative while a positive reaction for SMA was

accepted if CD34, c-KIT or DOG-1 was positive in that same case. Samples were categorized as suggestive for GIST if tumor cells were present but the immunostaining was inconclusive.

Sequencing and mutational analysis

An approximate amount of tumor cell content in each sample was estimated and the tumor area was micro-dissected from the paraffin-embedded tumor tissue. Then, 5-µm-thick sections from each sample were cut and pooled into a 1.5-ml tube. DNA was isolated using a QIAamp DNA FFPE tissue kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. The DNA concentration was determined using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States).

Then, 200 ng of DNA was used to detect mutations in exons 9, 11, and 13 of *KIT* and in exons 12 and 18 of *PDGFRA* with primers designed in-house (available upon request) and a Multiplex PCR kit (Qiagen) according to the manufacturer's instructions. Sanger sequencing of the amplicons was performed with both the forward and the reverse primers using the BigDye™ Terminator v1.1 Cycle Sequencing Kit in an ABI PRISM™ 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, United States).

Supplementary Table 1 Denotation of resected cases included during the *Study Period* (2012–2015)

Group	Neoadjuvant imatinib	Mutation profile
Neo-, (*n* = 12)	No	All variants
Neo + *s*, (*n* = 10)	Yes	*KIT* exon 11, all mutations
	Yes	*PDGFRA* exon 12, E556_I565dupl
Neo + *r*, (*n* = 5)	Yes	*PDGFRA* exon 18, p. D842V
	Yes	Wild Type
	Yes	*KIT* exon 13, p. K642E

Patients not treated with neoadjuvant imatinib were denoted *Neo*- irrespective of their mutation profile. Cases in the *Neo*+*s* group and in the *Neo*+*r* group were treated with neoadjuvant imatinib. Cases in the *Neo*+*s* group carried mutations that indicated sensitivity to imatinib, while cases in the *Neo*+*r* group carried mutations that indicated primary resistance to imatinib.