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ORIGINAL ARTICLE—ALIMENTARY TRACT

A quality control program for mutation detection in *KIT* and *PDGFRA* in gastrointestinal stromal tumours

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Abstract
Background Although most gastrointestinal stromal tumours (GIST) carry oncogenic mutations in *KIT* exons 9, 11, 13 and 17, or in platelet-derived growth factor receptor alpha (*PDGFRA*) exons 12, 14 and 18, around 10% of GIST are free of these mutations. Genotyping and accurate detection of *KIT*/*PDGFRA* mutations in GIST are becoming increasingly useful for clinicians in the management of the disease.
Method To evaluate and improve laboratory practice in GIST mutation detection, we developed a mutational screening quality control program. Eleven laboratories were enrolled in this program and 50 DNA samples were analysed, each of them by four different laboratories, giving 200 mutational reports.
Results In total, eight mutations were not detected by at least one laboratory. One false positive result was reported in one sample. Thus, the mean global rate of error with clinical implication based on 200 reports was 4.5%. Concerning specific polymorphisms detection, the rate varied from 0 to 100%, depending on the laboratory. The way mutations were reported was very heterogeneous, and some errors were detected.
Conclusion This study demonstrated that such a program was necessary for laboratories to improve the quality of the analysis, because an error rate of 4.5% may have clinical consequences for the patient.

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Introduction

Gastrointestinal stromal tumours (GIST) are mesenchymal tumours mainly arising along the intestinal tract, mostly in the stomach and in the small intestine. The tumours are characterized by exclusive mutation in *KIT* (70–80%) or platelet-derived growth factor receptor alpha (*PDGFRA*) (~10%) [1–4]. The remaining GIST, referred to as wild-type (WT-GIST), do not carry *KIT* or *PDGFRA* mutations. Mutations are found in exons 9, 11, 13 and 17 of *KIT* and in exons 12, 14 and 18 of *PDGFRA* [5–10].

Within *KIT* exon 9, one major mutation is a duplication of 6 nucleotides (c.1504_1509dup) [2], although a few other rare mutations have been reported [11, 12]. The *KIT* exon 11 mutations are quite heterogeneous, encompassing mainly in-frame deletions of variable sizes, basic amino acid substitutions, the duplications in the 3′ part of the exon, or more complex deletions-insertions [13–15]. Within *KIT* exons 13 and 17, mainly point mutations have been described [16, 17]. The majority of mutations in *KIT* are found in exon 11 (80%) and exon 9 (5–18%). The mutations in *KIT* exons 13 and 17 are rare, but they can be detected frequently in imatinib-refractory GIST in addition to the primary mutation [16, 18, 19]. Mutations in *PDGFRA* concern predominately GIST of gastric origin [10]. Most of these mutations are located in exon 18 (deletions and point mutations) [7], whereas mutations in exons 12 and 14 are less frequent [12]. Notably, the most frequent mutation in exon 18 of *PDGFRA*, p.Asp842Val, confers primary resistance to imatinib mesylate treatment [20]. Some mutations detected in the homozygous state were reported to have an adverse prognostic impact in GIST patients, predisposing them to liver metastasis [21, 22].

Imatinib mesylate was one of the first targeted anti-cancer therapies developed. Several clinical trials have proved that the response to imatinib in GIST correlates with the tumour genotype, with the best response observed in tumours with *KIT* exon 11 mutations [23, 24]; these observations are of the utmost importance. Data have also shown that patients with exon 9 *KIT* mutations fare better in terms of progression-free survival on a higher dose level, i.e. 800 mg daily, which is therefore recommended treatment in this subgroup [25]. The inefficacy of the standard dose of the drug for GIST bearing a common mutation in *KIT* exon 9 or WT-GISTs, the resistance induced by some mutations and the potential adverse side effects of the drug, render the tumour *KIT*/*PDGFRA* mutational status necessary to propose an alternative therapy to the patients [25, 26].

The most widely available materials come from fixed tumour tissues and the fixative most commonly used is formalin. Unfortunately, DNA extracted from fixed tissues is not of the best quality [27], and the DNA is often broken and chemically modified [28]. In these cases, polymerase chain reaction (PCR) amplification may be difficult and specific PCR primers must be chosen that yield short PCR amplification products. Moreover, sequencing artefacts due to the fixative are routinely detected and may be misinterpreted as mutations [29]. Special care must be taken when analysing mutations with DNA extracted from fixed paraffin-embedded tumours.

Pathology laboratories are increasingly asking for GIST mutational status and here we proposed a quality control program for mutation detection. The objective of this program was to allow laboratories to evaluate their own practice in GIST mutation detection and eventually to improve the quality of the service.

Materials and methods

Centres

Eleven laboratories (centres 1–11) participated in this study. Seven laboratories were affiliated to the Conticanet structure (Connective Tissue Cancers Network to Integrate European Experience in Adults and Children) (centres 5–11) spread across France ($n = 3$), the UK ($n = 1$), Belgium ($n = 1$) and Italy ($n = 2$). The laboratories not affiliated were from France ($n = 3$) and Poland ($n = 1$).

Material

Each Conticanet laboratory submitted 7 DNA samples (except one laboratory that submitted 8 samples), each comprising at least 5 μg of DNA that had been previously analysed for *KIT* and *PDGFRA* mutations, to the investigator coordinating laboratory. Samples were chosen in a blind manner and the selection of one every ten samples included in the routine process was proposed. A total of 50 DNA samples were collected; 9 extracted from frozen tumours and 41 from formalin-fixed tumours (Table 1). DNA was extracted using commercial kits (centre 5, Wizard DNA clean-up system, Promega, Madison, WI, USA; centres 6, 8, 9, 10, QIAamp DNA tissue kit, Hilden, Germany; centre 7, RecoverAll Nucleic Acid, Applied biosystems, Foster City, CA, USA; centre 11, High Pure PCR template preparation kit, Roche, Mannheim, Germany). Each centre submitting DNA completed a document with the following information for each sample: first three letters of the patient name, tumour identification number, information about the tumour (primary tumour,

Table 1 Mutational characteristics of the GIST samples

Sample	Fixative	Gene[a]	Exon	Mutations			Homozygous status	Centre number
1[b] P	Formalin	*KIT*	11, 17	p.Val559Gly	p.Gly663Val	p.Asp816His	No	3, 4, 5, **6**
2 P	Formalin	*KIT*	11	p.Val569_Leu576del			No	1, 2, **6**, 11
3 P	Formalin	*KIT*	11	p.Trp557Arg			No	**6**, 8, 9, 10
4 P	Formalin	*PDGFRA*	18	p.Asp842Val			No	**4**, 5, 7, 11
5 P	Formalin	*KIT*	11	p.Met552_Val559delinsIle			No	1, 2, 3, **6**
6 P	Formalin	*KIT*	11	p.Val560Asp			No	**6**, 9, 10, 11
7 P	Formalin	*KIT*	11	p.Val559_Val560del			No	5, **6**, 7, 8
8 P	Formalin	*KIT*	11	p.Met552_Trp557delinsArg			No	4, 5, 6, **7**
9 P	Formalin	*KIT*	11	p.Trp557Arg			No	1, 2, 3, **7**
10 P	Formalin	*KIT*	11	p.Trp557_Val559delinsPhe			No	**7**, 9, 10, 11
11 P	Formalin	*KIT*	11	p.Leu576Pro			No	**5**, 6, 7, 8
12 P	Formalin	*KIT*	11	p.Pro551_Glu554delinsGln			No	2, 3, 4, **7**
13 U	Formalin	*PDGFRA*	18	p.Ile843_Asp846del			No	1, **7**, 10, 11
14 P	Formalin	*KIT*	11	p.Trp557_Val560delinsPhe			No	6, **7**, 8, 9
15 P	Formalin	*KIT*	11	p.Lys550_Lys558del			No	5, 6, 8, **9**
16[b] M	Formalin	*KIT*	11, 17	p.Leu576Pro	p.Asp820Tyr		No	3, 4, 5, **9**
17 P	Formalin	*KIT*	11	p.Trp557_Glu561del			No	1, 2, **9**, 11
18 P	Formalin			WT			No	7, 8, **9**, 10
19 M	Formalin	*KIT*	11	p.Gln556_Asp572del			No	4, 5, 6, **9**
20 P	Formalin	*KIT*	11	p.Leu576Pro			No	1, 2, 3, **9**
21 P	Formalin	*KIT*	11	p.Trp557_Lys558del			No	8, **9**, 10, 11
22 P	Formalin	*KIT*	11	Val555-Pro573del			No	7, 8, 9, **10**
23 P	Formalin			WT			No	4, 5, 6, **10**
24[b] P	Formalin	*KIT*	11	p.Val560del			No	1, 2, 3, **10**
25 P	Formalin	*KIT*	11	p.Val560Asp			No	8, 9, **10**, 11
26 P	Formalin	*KIT*	11	p.Lys558_Val560delinsGln			No	5, 6, 7, **10**
27 P	Formalin	*KIT*	11	p.Trp557_Lys558del			No	2, 3, 4, **10**
28 U	Formalin	*KIT*	11	p.Lys550_Val555			No	1, 9, **10**, 11
29 P	Formalin	*KIT*	11	p.Trp557_Gln575del			Yes	5, 6, 7, **8**
30 P	Formalin	*KIT*	11	p.Trp557_Lys558del			No	2, 3, 4, **8**
31 P	Formalin	*KIT*	9	p.Ala502_Tyr503dup			No	1, **8**, 10, 11
32 P	Formalin			WT			No	6, 7, **8**, 9
33 P	Formalin	*KIT*	11	p.Val559_Val560del			No	3, 4, 5, **8**
3 P	Formalin	*KIT*	11	p.Trp557_Lys558del			No	1, 2, **8**, 11
35 U	Formalin	*KIT*	11	p.Val560_Ile571del			No	7, **8**, 9, 10
36 P	Frozen	*PDGFRA*	12, 14	p.Tyr555Cys	p.Gly652Ala		No	8, 9, 10, **11**
37 P	Frozen	*KIT*	11	p.Gln556_Val559delinsHis			No	5, 6, 7, **11**
38[b] P	Frozen	*KIT*	11	p.Val569_Leu576del			No	2, 3, 4, **11**
39 P	Frozen	*KIT*	11	p.Val560Asp			No	1, 9, 10, **11**
40[b] P	Frozen	*KIT*	11	p.Trp557_Lys558del			No	6, 7, 8, **11**
41[b] P	Frozen	*KIT*	9	p.Ala502_Tyr503dup			No	3, 4, 5, **11**
42 P	Frozen	*KIT*	13	p.Lys642Glu			No	1, 2, 10, **11**
43 P	Formalin	*KIT*	11	p.Trp557_Lys558del			No	2, 3, 4, 5
44 P	Formalin	*KIT*	11	p.Val559Gly			No	1, **5**, 10, 11
45 P	Formalin	*KIT*	9	p.Ala502_Tyr503dup			No	**5**, 7, 8, 9
46 P	Frozen			WT			No	3, 4, **5**, 6
47 P	Formalin	*KIT*	11	p.Gln556_Trp557delinsArg			No	1, 2, **5**, 11

Table 1 continued

Sample	Fixative	Gene[a]	Exon	Mutations	Homozygous status	Centre number
48 P	Frozen	*PDGFRA*	18	p.Asp842Val	No	**5**, 8, 9, 10
49 U	Formalin	*KIT*	11	p.Glu554_Lys558del	No	4, **5**, 6, 7
50 P	Formalin	*KIT*	11	p.Gln556_Val559del, p.Tyr553Ser	No	1, 2, 3, **5**

The centre submitting the sample is written in bold characters

P primary tumour, *M* metastasis, *U* unknown

[a] The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*

[b] Tumours under imatinib treatment

relapse, metastasis, tumour under imatinib treatment), information about the type of preservation (frozen or fixed tumours, and type of fixative), DNA concentration and mutational analysis report (type of mutation or polymorphism, and the zygosity status). On reception at the investigator centre, each DNA sample was quantified, and divided into 4 aliquots with a minimum DNA quantity of 1 μg each.

An aliquot from each DNA sample was sent to 3 other centres, meaning that each DNA sample was analysed by four different groups (the laboratory sending the DNA to the investigator and the three centres performing the quality control analyses). Overall, this resulted in 50 DNA samples analysed by four centres, giving 200 mutational reports. Each Conticanet centre analysed 20 samples (140 exons) and other centres screened 15 specimens (105 exons) for a total of 1400 exons analysed.

Each laboratory performing the test had to fill in a document reporting the mutation detected with the zygosity status and the polymorphisms detected.

Methods

Each centre analysed the sample for *KIT* (exons 9, 11, 13 and 17) and *PDGFRA* (exons 12, 14 and 18) mutations according to its routine process. The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*.

Most centres analysed the mutations by direct sequencing or by a pre-screening method followed by sequencing, specifically centres 2, 4, 7, 8, 9 and 10 used direct sequencing; centres 1 and 3 used length analysis of the PCR product (LAPP) for *KIT* exons 9 and 11, then direct sequencing for the other exons; centre 6 used length analysis of the PCR product for exons 9, denaturing high-performance liquid chromatography (DHPLC) screening and sequencing for the other exons; centres 5 and 11 used DHPLC screening and sequencing for all the exons.

Results

Obtaining the results

All results were sent back within the allocated time of 4.5 months. The time for delivering the results varied from 1 to 4.5 months (mean 3.7 months).

Efficiency of the analysis

Nine centres out of 11 (82%) analysed all samples entirely. Centres 3 and 4 analysed 44% and 49% of the 105 exons, respectively. Overall, the 11 centres evaluated mutations for 1287 exons out of 1400. Yet, laboratories were unable to amplify 1.4% of the exons (18/1287). Seventy-three percent of the centres ($n = 8$) amplified all the exons.

Mutation analysis

Fifty samples were analysed 4 times, giving 200 reports. Correct results were defined when at least two groups detected the same mutation. When there was no consensus for the correct mutation, the sample was analysed by the reference centre (Department of Human Genetics, University of Leuven, Leuven, Belgium). For two samples (4%), the reference centre was asked to check for the *KIT* exon 13 and 17 (sample 1) and the *KIT* exon 11 (sample 5). Mutations in the *KIT* exon 13 (c.1988G>T) and exon 17 (c.2446G>C) were detected for sample 1. The *KIT* exon 11 was detected to be a wild type for sample 5 (Table 2).

Overall, at least one group did not report the detectable mutation in 8 out of 200 analysis (Table 3), giving 4% false negative results. The rate of false negative results for mutation detection varied according to the laboratory and ranged from 0 to 15% (mean 4.5%, Table 4). As presented in Table 3, the false negative results concerned mainly some mutations for *KIT* exon 11. One false positive mutation (2%) was detected (sample 5), i.e. centre 6 detected a false positive mutation in *KIT* exon 11 (c.1656_1676 del). In addition,

centre 3 detected no deletion nor insertion after length analysis of the PCR product. The two other centres (centres 1 and 2) were unfortunately unable to amplify a PCR product. The reference centre confirmed the absence of mutation for *KIT* exon 11 (Table 2).

Homozygous mutations

One sample out of 50 had a homozygous/hemizygous mutation in *KIT* exon 11 (c.1669_1725del). The homozygous status of the mutation was reported by two groups. The third group did not report on this status and the remaining group detected no mutation.

Table 2 Mutational analysis of 2 samples by several centres giving uncertain results

Centre	*KIT* exon[a]			
	9	11	13	17
Sample 1				
6	No	c.1676T>G	No	c.2446G>C
5	No	c.1676T>G	c.1988G>T	No
4	No	c.1676T>G	Nd	Nd
3	No	c.1676T>G	Nd	Nd
Reference[b]			c.1988G>T	c.2446G>C
Sample 5				
6	No	c.1656_1676del	No	No
2	Ni	Ni	No	No
3	No	Nodel/noiins	Nd	Nd
1	Ni	Ni	Ni	Ni
Reference[b]		No		

The correct mutation was defined by the reference centre

No normal sequence, *Ni* no PCR product obtained, *Nd* not determined, *Nodel/noins* the sequence contains no deletion nor insertion

[a] The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*

[b] The reference centre sequenced only exons 13 and 17 (sample 1) and exon 11 (sample 5)

Polymorphism detection

Because of the variable location of the primers according to laboratory, some polymorphisms were not detected by some groups. We reduced the analysis to the two most frequent polymorphisms [single-nucleotide polymorphism (SNP) rs2228230 C/T, NM_006206.4:c.2472C>T, *PDGFRA* exon 18 and SNP rs55789615 C/T, NM_000222.2:c.2394C>T, *KIT* exon 17] located in the exonic sequence of the two genes. Three laboratories did not check for the polymorphisms (centres 3, 4, 7). Seventeen polymorphisms were present over the 50 samples. At least one laboratory failed to detect almost half of the polymorphisms present (8/17), and one laboratory detected 2 false positive polymorphisms (error rate 20%). Four laboratories failed to detect at least one of the polymorphisms (Table 5). One centre had a false positive rate of polymorphism detection of 40% (2 polymorphisms detected out of 5 analysed). In fact, this laboratory detected the polymorphisms NM_006206.4:c.2472C>T for 2 samples, although it was not detected by three other groups analysing these samples. Samples were analysed once more for *PDGFRA* exon 18 sequence and no polymorphism was detected.

Reporting of the mutation

The nucleotide and protein mutational report was requested. On the standard operational procedure sent to each participant, it was suggested to describe the mutation according to the guidelines for sequence variation of the Human Genome Variation Society (HGVS; http://www.hgvs.org/mutnomen).

Table 3 Mutational status for 8 GIST for which a wrong mutation status was defined by at least one laboratory

Sample	*KIT* exon[a]				*PDGFRA* exon[a]			Centre
	9	11	13	17	12	14	18	
1		c.1676T>G	*c.1988G>T*	*c.2446G>C*				5, 6[b]
36					c.1664A>G	*c.1955G>C*		11
40[c]		*c.1669_1674del*						8
35		*c.1679_1714del*						7
31	*c.1504_1509dup*							8
29		*c.1669_1725del*						7
24		*c.1678_1680del*						1
22		*c.1663_1719del*						7

The mutation that was not detected is shown in italics

[a] The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*

[b] Centres 5 and 6 did not detect *KIT* exon 17 and 13 mutation, respectively

[c] The DNA was extracted from a frozen tumour

For five samples, the laboratories detected the mutation at the correct exon, but the mutation nomenclature was wrong. One laboratory was responsible for four wrong results (Table 6). For seven cases, there was discordance between the DNA and the protein sequence formula (Table 7). The mutation annotation was very heterogeneous and was not always annotated according to the HGVS recommendations. Some examples of this are given in Tables 6 and 7.

Table 4 Percentage of false and positive results for mutation detection according to the laboratory

Centre	Number of sample analysed	False negative results (%)	False positive result (%)
1	15	6.5	0
2	15	0	0
3*	15	0	0
4**	15	0	0
5	20	0	5
6	20	5	0
7	20	15	0
8	20	10	0
9	20	0	0
10	20	0	0
11	20	5	0

3* and 4** only 44% and 49% of the exons were analysed, respectively

Table 5 Rate of error detection for polymorphisms in exon 17 of *KIT* (SNP rs55789615 C/T and in exon 18 of *PDGFRA* (SNP rs2228230 C/T)

Centre	Number of polymorphisms analysed	Number of polymorphisms not detected	Error (%)
2	7	1	14
6	4	3	75
8	3	3	100
11	9	3	33

Discussion

Mutation analysis of *KIT* and *PDGFRA* in GIST is important for the confirmation of diagnosis in doubtful cases (as exemplified by CD117-immunonegative GIST) and for the optimal treatment of patients with imatinib mesylate [30]. Most tumours carrying mutations for *KIT* and some *PDGFRA* mutations are sensitive to the drug at a dose of 400 mg/day [31]. However, when a tumour carries the *KIT* exon 9 mutation (c.1504_1509dup), 800 mg/day might be more effective in obtaining an objective clinical response [24]. On the other end of the scale, the *PDGFRA* p.Asp842Val mutation confers primary insensitivity to imatinib [20]. The accuracy of mutation detection in GIST is an important challenge for laboratories. The mutational analysis may be difficult because of the number of exons to screen (7 exons), the high number of different type of mutations existing, especially in exon 11 of *KIT*, and the paraffin-embedded fixation material that limits DNA quality. To date, no consensus has been defined for GIST mutation detection. Here we investigated a mutation detection quality control program in GIST at the European level within the framework of Conticanet.

Overall, this program was successful because 82% of the centres gave a complete report for the GIST mutation detection. Overall, the discrepancy rate was 4.5% including eight false negative results and one false positive result. The high rate of false negative results that we observed concerned mainly exon 11 of *KIT*, and for 6 out of 8 samples the wrong result reported a wild-type GIST. The undetectable mutations were located in exons 13 and 17 (1 sample), exon 9 (1 sample) and exon 11 of *KIT* (5 samples) and exon 14 of *PDGFRA* (1 sample). The absence of detection of *KIT* exon 11 deletion by one group (number 7) for three samples was explained by the location of the primers. In this group, in order to improve the PCR amplification of highly degraded samples, the *KIT* exon 11 was amplified by two sets of primers. In all three instances, the mutations consisted of large deletions (36 and 57 bp), comprising the regions where the entire or at least the 3′ end of one primer was located and only the wild-type allele

Table 6 Report of some mutational transcription errors

Sample number	Mutation reported	Correct mutation[a]	Centre number
8	c.***1656_1670***del (p.***Tyr553***_Trp557del)	c.1655_1669del (p.Met552_Trp557delinsArg)	7
13	c.***2524_2535*****del** (p.***Asp842_His845***del)	c.2526_2537del (p.Ile743_Asp846del)	7
14	c.1670_***1678***del (p.Trp557_***Val560***delinsPhe)	c.1670_1675del (p.Trp557_Val559delinsPhe)	7
47	c.1669_***1671***del (p.***Trp557***del)	c.1667_1669del (p.Gln556_Trp557delinsArg)	1
37	c.1668_***1673***del (p.***Trp557***_Val559del)	c.1668_1676del (p.Gln556_Val559delinsHis)	7

The nucleotide or amino acid sequence error is shown in bold characters

[a] The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*

Table 7 Incorrect nucleotide or protein sequence formula

	Mutation reported	Correct mutation[a]
Case 2	c.1705_1728del p.***Trp557_Lys558del***	c.1705_1728del p.Val569_Leu576del
Case 3	c.***1672***T>A p.Trp557Arg	c.1669T>A p.Trp557Arg
Case 11	c.***1726***T>C p.Leu576Pro	c.1727T>C p.Leu576Pro
Case 21	c.**1669_*1679***del p.Trp557_Lys558del	c.1669_1674del p.Trp557_Lys558del
Case 22	c.1663_1719del***62*** p.Val555_Pro573del	c.1663_1719del57 p.Val555_Pro573del
Case 36	c.***2060***A>G p.Tyr555Cys	c.1664A>G p.Tyr555Cys
Case 50	c.1665_1676del p.***Val555_Val559delinsVal***	c.1665 1676del p.Gln556_Val559del

Location of the nucleotide or the amino acid sequence error is shown in bold characters

[a] The reference sequences used in this study are NM_000222.2 for *KIT* and NM_006206.4 for *PDGFRA*

was amplified. All three deletions were detected retrospectively upon usage of a new set of primers covering the whole exon 11. Regarding the two other cases, where deletion of *KIT* exon 11 was falsely not detected, no explanation was found. In fact, one centre (number 2) was able to detect *KIT* exon 11 deletions for some other samples, while direct sequencing was used for mutation detection. The size of the PCR product cannot explain this discrepancy, because the mutation gave a shorter PCR product than the wild-type allele. For this group and for one other sample, the same deletion was detected (sample 21). The second group (group 1) that was not able to detect the *KIT* exon 11 deletion used the LAPP method for detecting some deletions or duplications. The mutation not detected concerned a small deletion of 3 nucleotides, and the sensitivity of the technique could explain this problem. Nevertheless, a small deletion of 3 nucleotides in *KIT* exon 11 for sample 47 was detected by the same group. Sample 31 showed a duplication in *KIT* exon 9 and the mutation was not detected by direct sequencing, although the analogous mutation was detected by the same group for another sample. The PCR reaction was performed again by this group changing the primer pairs and the *KIT* exon 9 duplication was detected. As reported by Lasota et al. [5] for *KIT* exon 11 duplication located at the 3′ end of the exon, a modification of the primers' sequence could increase the rate of detection of mutation in exon 9 of *KIT* in fixed paraffin-embedded GIST. Centre 11 screened the mutation for a primary imatinib-naïve GIST (sample 36). This sample bore a mutation in exons 12 and 14 of *PDGFRA*. Although the three other groups detected these two mutations by direct sequencing, group 11 did not detect the mutation in exon 14 by means of pre-screening with DHPLC. The DHPLC technique is a very sensitive method of mutation detection [32], but the analysis of the abnormal profile may be difficult in particular with this unusual mutation. Moreover, this tumour was a primary imatinib-naïve GIST and a second mutation in the sample was not expected. Less attention to a DHPLC profile might explain the absence of this mutation detection. A surprising result concerned sample 1 for which only two groups were able to analyse all the exons. The DNA was extracted from a GIST after imatinib treatment. Each group detected two mutations, but each of them detected a different secondary mutation (one in exon 13 and one in exon 17), although all three mutations were present in the sample.

One false positive result was observed in this study. Centre 6 reported a deletion in exon 11 of *KIT* for sample 5, although the other centres did not. In addition, this centre reported the result as being inconsistent despite several analyses. In our hands, a similar observation was made while repeating the experiment. In fact, we tested several dilutions of DNA (data not shown) and surprisingly, according to the quantity of DNA to amplify, the mutation was not detected, detected, or detected in the homozygous state. A preferential allelic amplification seemed to be obtained according to the amount of DNA amplified, and we hypothesise that low DNA quantity allowed the allele bearing the mutation to be amplified. This observation could explain why some centres were not able to detect some deletion or duplication mutation by direct sequencing. We propose that in order to improve the mutation detection at least two dilutions of DNA should be used for DNA extracted from paraffin-embedded GIST. The same observation could explain why one centre reported a heterozygous mutation (sample 29), although two other centres reported homozygous status for this. The detection of two main polymorphisms was evaluated in this study, because they are located in the exonic sequences and can be detected regardless of the primer location. The rate of detection of the polymorphisms was lower compared with the mutation detection. The percentage error ranged from 14 to 100%, depending on the centres. This may be explained by less attention being paid by the laboratories to the polymorphisms detection, for which no clinical implication has been reported to date.

Most of the DNA samples were extracted from fixed paraffin-embedded tumours and DNA is known to be of a poor quality using this strategy. To improve the PCR reaction for degraded DNA, PCR primers are usually designed in order to obtain a short PCR product. Moreover, the DNA may be chemically modified by the fixative making it difficult to perform PCR amplification [27]. In this study, only 3 laboratories were unable to obtain a PCR product for at least one out of 7 exons. Surprisingly, one DNA sample extracted from a frozen tumour failed to be amplified by PCR for exons 9, 11, 13 and 17 of *KIT* and for exon 18 of *PDGFRA* by one centre, and for exon 11 of *KIT* (the only exon screened) by a second group. The analysis was successful for the third group and for the fourth group (only exon 9 of *KIT* was analysed). The choice of the PCR primers does not seem to explain this absence of PCR amplification, because the same centres analysed some other DNAs with success. The quality of the DNA may not be the reason behind the failure to perform PCR amplification, because one of the DNA samples of concern was extracted from a frozen tumour and was amplified by PCR by the other 3 groups.

Gastrointestinal stromal tumours are considered to be rare, although increasingly more are identified nowadays because of appropriate immunohistochemistry application and mutation screening. For research purposes, tumours should be collected in some database with histological, immunohistological, clinical and mutational data available for investigators. It is important to make mutational reports uniform at the DNA and protein level, and standard recommendations exist for mutation nomenclature (http://www.hgvs.org/mutnomen) [33]. We observed multiple errors in the nomenclature used to report the mutations. In Conticanet, a GIST database (http://www.conticagist.org) was constructed and each mutation report was standardized according to the HGVS recommendation, making some research easier to conduct.

This program was very successful, allowing laboratories to evaluate their own practice and to improve their procedure. One laboratory changed their primers for detection of *KIT* exon 11 mutation and one other changed all primers because the previous ones produced too many large PCR products for analysis conducted on DNA extracted from paraffin-embedded tissues. The PCR amplification must be optimal for avoiding analysis delay and also to limit the PCR contamination due to repeating PCR amplification of the same DNA.

We suggest the following recommendations for *KIT* and *PDGFRA* mutation screening. PCR primers must be located in intronic sequences and generate a PCR product with a maximum size of 300 bp. Yet, according to the PCR product size and to the PCR amplification efficiency, we suggest using primers from centres 5, 6, 8 or 9. The mutation detection error rate for *KIT* exons 9 and 11 was 0, 4.1 and 4.7% for DHPLC, the LAPP screening method and direct sequencing, respectively. For this reason, we suggest screening for *KIT* exon 9 and 11 mutations by DHPLC. DHPLC is also a good screening method for resting exons to avoid a laborious direct sequencing. However, sequencing the DHPLC variant from an independent PCR product serves as adequate confirmation of the result for quality control.

Such a quality control program is necessary, because the mean global error rate with clinical implication based on 200 reports was 4.5%. Centralization of mutational analysis in laboratories enrolled in an external quality assurance program and with expertise in the disease may be useful in order to make mutational analysis more widely available and more accurate.

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