European Journal of Cancer (2015) **51**, 75–83

Available at www.sciencedirect.com

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journal homepage: www.ejcancer.com

Genomic index predicts clinical outcome of intermediate-risk gastrointestinal stromal tumours, providing a new inclusion criterion for imatinib adjuvant therapy

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Received 18 July 2014; received in revised form 9 October 2014; accepted 14 October 2014
Available online 6 November 2014

KEYWORDS
Intermediate-GIST
Genomic index
Prognostic factor
Metastasis
Miettinen

Abstract ***Purpose:*** Imatinib mesylate is the front-line targeted therapy for gastrointestinal stromal tumours (GISTs). Patient's eligibility to adjuvant imatinib after primary tumour resection is currently based on histological and clinical risk assessment. While therapeutic options are clear for the very-low, low and high-risk subpopulations, no standard is actually available for the tumours classified as intermediate. Since we recently validated genomic index (GI), a measure of the level of genomic alterations, as a strong predictor of clinical outcome in GIST, we asked whether it could also represent a novel prognostic factor for the intermediate subgroup.

Experimental design: 82 intermediate risk patients were selected based on the Armed Forces Institute of Pathology (AFIP) classification for genomic profiling.

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http://dx.doi.org/10.1016/j.ejca.2014.10.014

Results: Data revealed that even if studied samples generally harboured a combination of the typical genetic aberrations found in GIST, i.e. 1p, 14q 22q deletions and frequently lost CDKN2A locus on chromosome 9, they profoundly differed from each other on the total number of genomic changes and GI value. Kaplan–Meier analyses of metastatic-free survival unveiled that stratification of the tumours by the GI value at a cutoff of 10 separated the good from the poor prognosis patients, proven that metastatic-risk in GIST intermediate patients is strongly associated with high GI values and genome complexity.

Conclusion: GI is validated here as a robust marker to predict intermediate-GIST clinical outcome. Applicable in numerous Pathology Laboratories already using array comparative genomic hybridisation (CGH) with formalin-fixed paraffin-embedded (FFPE) samples, this assay presently stands as an efficient tool for the clinical management of intermediate GIST-patients.

1. Introduction

Gastrointestinal stromal tumours (GIST) represent the most frequent form of all types of sarcoma (25%) [1–3] and the most common mesenchymal tumour of the gastrointestinal (GI) tract. They can arise anywhere along the GI tract, but predominantly occur in the stomach (60%) and small intestine (25%) [4]. Potentially originating from the same lineage as the interstitial cells of Cajal (ICC) [5,6], GIST and ICC share common features such as a strong dependency on the KIT signalling pathway. Eighty percent of GISTs are indeed driven by activating mutations within the proto-oncogene KIT itself, while eight percent possess mutations in its close homolog, the platelet-derived growth factor receptor α (PDGFRα) indeed [7,8]. Both of these mutations conduct to a ligand-independent activation of these receptors and their dependent-signalling cascade. The remaining 'KIT/PDGFRα wild-type' patients (12%), were shown to bear oncogenic alterations in genes such as *NF1*, *BRAF*, *KRAS*, *NRAS* and *SDHB*/*SDHC*, thus revealing up to ten different GIST molecular subtypes [9].

Molecular classification of GIST led to dramatic changes in clinical practice and disease management with the approval in 2002 of an efficient systemic therapy, i.e. imatinib mesylate (Glivec®, Novartis Pharma AG) [10]. This agent designed to block the tyrosine kinase activity of several proteins including PDGFRα and KIT (mutated or not) [11,12] was indeed proven to be useful for the treatment of recurrent or metastatic GIST [13,14] and more recently as adjuvant therapy for patients presenting a significant risk of recurrence [15].

Patients' eligibility to imatinib adjuvant therapy is based on individual risk of local recurrence or metastatic relapse. These parameters are commonly evaluated by risk-stratification grading systems that have been improved overtime [16,17]. The first consensus classification was proposed in 2001 by the National Institute of Health and relied on two main criteria—tumour size and mitotic count [18]. Miettinen and Lasota then suggested adding the tumour site as third selection criteria [19]. This second grading schema, also known as the Armed Forces Institute of Pathology (AFIP) system, delineated four prognostic groups referred as very low, low, intermediate and high-risk of recurrence. A fourth criterion has recently been included in the modified version of the National Institutes of Health (NIH) classification: tumour rupture [20]. Be that as it may, while imatinib indications are well established for the low- and high-risk subcategories—the latter group being the only one to receive such a targeted therapy post-surgery, the situation remains more critical for GIST classified as intermediate (20–25% of all GIST). Indeed, even if the latest European Organisation for Research and Treatment of Cancer (EORTC) clinical study reports no benefit of imatinib for this subset of patients (unpublished data) and most experts prefer now to avoid this therapy for intermediate GISTs, the real metastatic risk of these patients remains poorly defined. In this context, imatinib may be erroneously administered, or inversely, not administered. There is thus an urgent need to improve patients' stratification and identify novel prognostic factors able to potently predict intermediate-GIST metastatic outcome and, hence tailor treatment according to individual requirements.

Although mutations constitute crucial events for GISTs initiation and development, other genetic alterations have been described in this pathology [1,21]. Two-third of patients present, for instance, a monosomy of chromosome 14 or a partial loss of 14q, resulting in the possible loss of two tumour suppressor genes that may have some importance for GIST formation [7,12,22–25]. Deletion of the long arm of chromosome 22 is also observed in 50% of GIST and loss of 1p is quite frequent [7,24–27]. Interestingly, although less frequent than 14q and 22q deletions, chromosomal loss of 1p, 9p, 11p and 17p is more significantly associated with malignancy [1,26–28]. In line with these results, we recently reported that genomic index (GI), a measure of the number and type of genomic copy number alterations, is inherently linked to GIST metastatic outcome

and established a GI cut-off value able to discriminate the good from the poor prognosis tumours [28]. Based on these results, we hypothesised that GI value could also be a valuable prognostic factor for GIST classified as intermediate and conducted genomic profiling on 82 formalin-fixed paraffin-embedded (FFPE) of such samples using a microarray-based comparative genomic hybridisation (array CGH) approach.

2. Materials and methods

2.1. Tumour samples

Formalin-fixed paraffin-embedded (FFPE) samples originated from 82 resected primary GISTs were selected from the European GIST database CONTICA-GIST (https://conticagist.sarcomabcb.org/). Cases were issued from the archives of the Department of Pathology of Bergonie Institute (Bordeaux, France), of Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology (Warsaw, Poland), of Treviso General Hospital (Treviso, Italy), of Universitätsklinikum, Gerhard-Domagk-Institut für Pathologie (Münster, Germany) and of KU Leuven and University Hospitals Leuven, (Leuven, Belgium). For all samples, ethical approval was obtained from the appropriate committees. Cases were then centralised in the Biological Resources Center of Bergonie Institute, which has received the agreement from the French authorities to deliver samples for scientific research (AC-2008-812). All patients were identified as intermediate-risk GIST according to the Armed Forced Institute of Pathology (AFIP) criteria. No treatment was performed before surgery and until tumour recurrence. Diagnosis dates ranged from May 1985 to December 2009. Patient and tumour characteristics are detailed in Table 1.

Table 1
Description of patients.

AFIP risk (Miettinen)	
Intermediate	82 (100)
Follow-up (y)	4.94
95% CI	0–11.49
Sex	
Male	35 (42.7)
Female	47 (57.3)
Location	
Stomach	48 (58.5)
Small intestine	24 (29.3)
Other	10 (12.2)
Mitotic index	
⩽5	60 (73.2)
>5	22 (26.8)
Tumor size – 1	
⩽2 cm	0
2 to ⩽5 cm	24 (29.3)
5 to ⩽10 cm	41 (50)
>10 cm	17 (20.7)
Tumor size – 2	
<3 cm	3 (3.7)
⩾3 cm	79 (96.3)
Mutations	
KIT	**65 (79.3)**
Ex 9	4 (6.15)
Ex 11	58 (89.2)
Ex 13	1 (1.55)
Ex 17	2 (3.1)
PDGFRA	**9 (11)**
Ex 12	1 (11.1)
Ex 14	2 (22.2)
Ex 18	6 (66.7)
WT	**5 (6.1)**
nd	**4 (4.9)**
Relapse events	
Local	10 (12.2%)
Distance	27 (32.9%)

NOTE: Percentage are indicated in brackets.*Abbreviations:* nd, not determined; AFIP, Armed Forces Institute of Pathology; CI, confidence interval; PDGFRA, platelet-derived growth factor receptor alpha; WT, wild-type.
In bold are shown the total number and percentage (in brackets) of patients mutated or not for KIT or/and PDGFRA; below are specified the number and percentage according to the exon (Ex) affected.

2.2. DNA isolation, array-comparative genomic hybridisation (aCGH) analysis and genomic index calculation

Genomic DNA was extracted according to Agilent protocol for DNA isolation on FFPE tissues (Agilent Technologies) and quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). It was treated with DNase and hybridised to 8_60 K whole genome Agilent arrays (G4450A) as described by the manufacturer's instructions. Microarray slides were then scanned using an Agilent DNA Microarray Scanner. Images were successively analysed by the Feature Extraction V10.1.1.1 and Agilent Genomic Workbench Lite Edition 6.5.0.18 software (Agilent). The ADM-2 algorithm was used to identify DNA copy number anomalies at the probe level as follows: (i) a low-level copy number gain was defined as a log 2 ratio superior to 0.25, (ii) a copy number loss was defined as a log 2 ratio inferior to −0.25, (iii) a high-level gain or an amplification was defined by a log 2 ratio superior to 1.5, and (iv) homozygous deletions were suspected when the ratio was below −1. For stratification, genomic index was calculated as follows: $GI = A^2/C$ where A corresponded to the total number of alterations (segmental gains or losses) and C to the number of chromosomes affected by these alterations. GI cutoff (i.e. 10) was chosen in accordance to our previous study, showing on a training set of GIST patients that metastatic cases predominated above this value [28]. We applied here exactly the same model and parameters used in that first study.

2.3. Statistical analysis

Metastasis-free survival (MFS) was calculated by the Kaplan–Meier method from the date of initial diagnosis

to the date of first metastasis or last follow-up for patients without diagnosis of metastasis. Survival curves were compared with the log-rank test. All survival analyses were conducted using R software (version 2.14.1) and the 'survival' package. Multivariate analysis was done using Cox regression with the Firth's correction (R software, 'coxphf' package).

3. Results

3.1. Which genetic profile for the intermediate GIST subgroup?

Intermediate-grade GIST patients were selected from the CONTICAGIST database based on the availability on FFPE samples, from which genomic DNA was isolated. Only DNAs of sufficient quality were subsequently used for genome profiling. In total, 82 GISTs classified as intermediate with respect to the AFIP criteria were collected (Table 1) and processed for array CGH. Penetrance plots gathering all the 82 CGH profiles were generated to appraise the principal genomic changes occurring in this subpopulation (Fig. 1A and B). As expected, the most frequent alterations corresponded to the ones previously described in the literature for GIST [1,21], that is to say deletions of chromosomes 14, 22 and of the short arm of chromosome 1 (Fig. 1A). CGH profiles, indeed, revealed that 80%, 55% and 50% of patients exhibited a loss of 14q, 22q and 1p, respectively (Fig. 1B). Moreover, close to 40% of metastatic patients displayed a loss of the tumour suppressor gene CDKN2A (p16INK) through chromosome 9p21 deletion (Fig. 1C); an alteration that has been formerly associated with GIST malignancy [28–30].

3.2. Is it possible to stratify intermediate GIST patients based on their genomic profile?

Apart from the general characteristics described above, individual inspection of all the CGH profiles unveiled striking differences in the number and localisation of the genetic alterations present. On the 82 cytogenetic analyses, one gave a flat profile, twenty (25%) exhibited one or two alteration(s), and the rest three or more. The maximal genomic copy number variation reached thirty-seven. Given this variability, we calculated the genomic index (GI) associated to each profile (Fig. 2A). Here, GI values were found to range from 0 to 115.6, meaning that genome complexity varied tremendously from one patient to another and that this factor is likely to discriminate subgroups among the GISTs intermediate-grade population. Using the same criteria defined in Lagarde et al. [28], we delineated two categories of patients: the first one, termed GI1, included patients with no or few chromosomal rear-

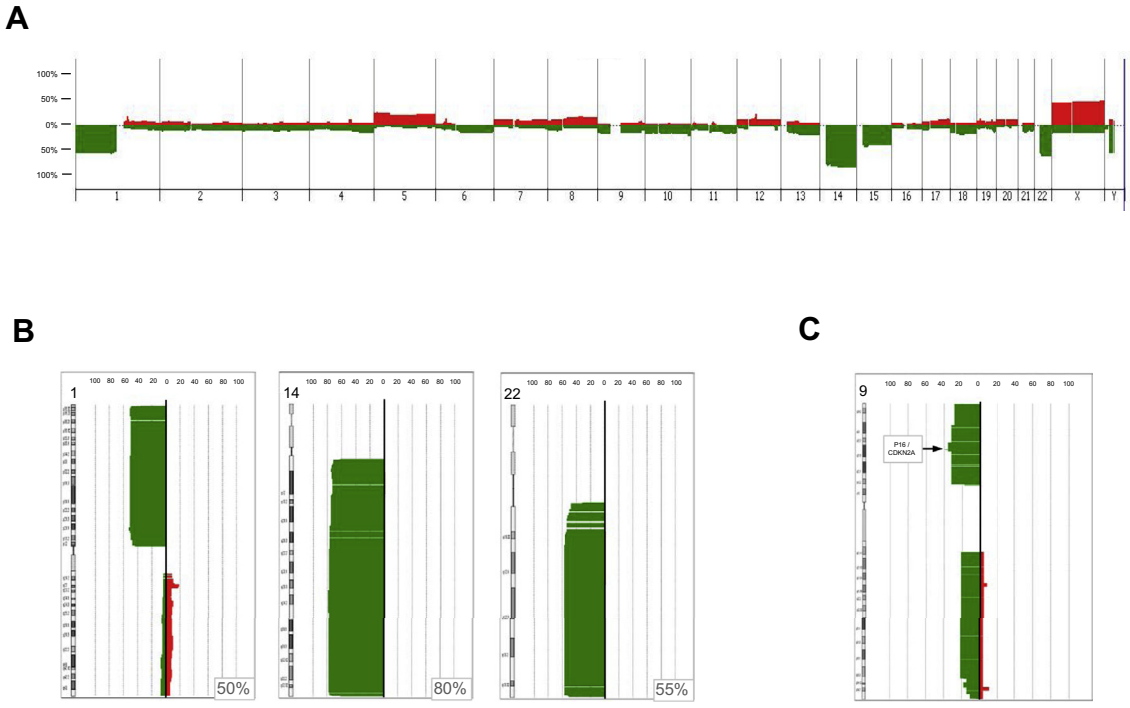


Fig. 1. Genomic features of the intermediate-gastrointestinal stromal tumour (GIST) cohort. (A) Penetrance plots gathering all the 82 intermediate-risk GIST patients comparative genomic hybridisation (CGH) profiles. Chromosomes 1–22 are represented on the *x*-axis while the percentage of genomic region gain (red) or loss (green) appears on the *y*-axis. (B) Penetrance plots highlighting the percentage of patients deleted for the chromosomal regions 1p, 14q and 22q in the entire cohort. (C) Penetrance plot highlighting the frequency of *p16* (*CDKN2A*) deletion in the metastatic subgroup.

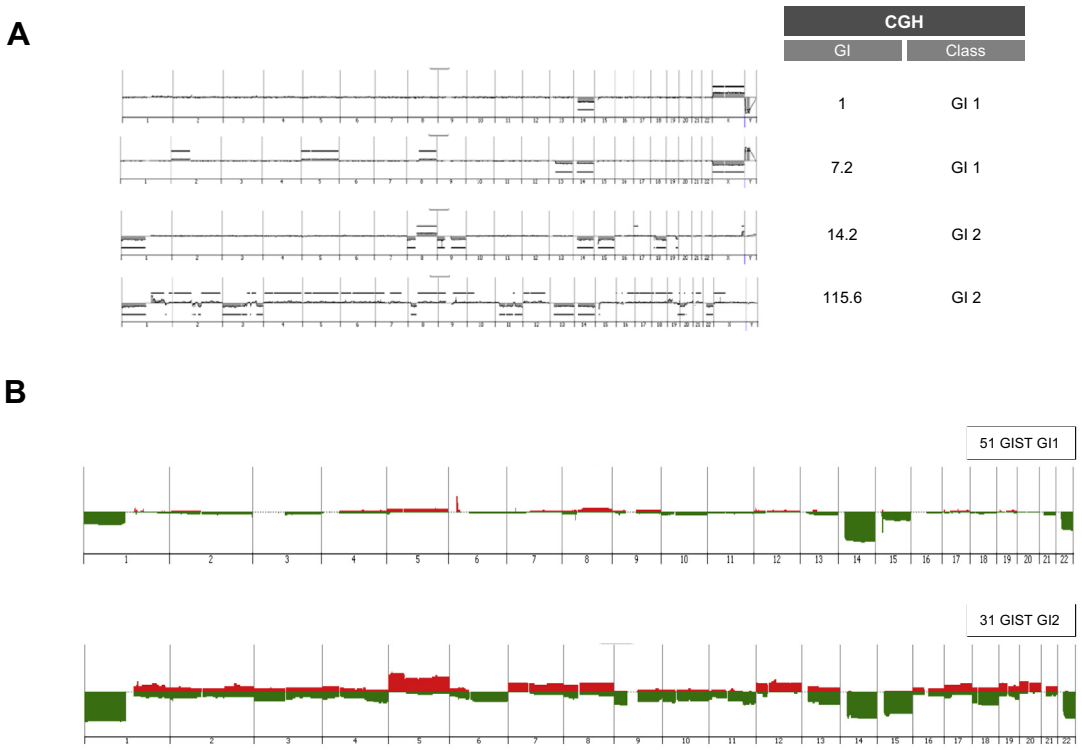


Fig. 2. Genomic index 1 (GI1) and genomic index 2 (GI2) tumour genomic profiles. (A) Comparative genomic hybridisation (CGH) profiles (left panel) and genomic index (right panel) of four intermediate-risk gastrointestinal stromal tumour (GIST) patients with increasing genomic alterations. (B) Penetrance plots gathering the CGH genomic profiles of the 51 GI1 (upper panel) and 31 GI2 (lower panel) intermediate-GIST tumours.

rangements and thereby a weak GI (GI < 10, $N = 51$), and the second one, referred as GI2, covered patients with more complex genetics (GI > 10, $N = 31$). Examples of individual or grouped CGH profiles obtained for each of these groups are displayed in Fig. 2A and B.

3.3. Does genomic index predict intermediate-GIST outcome?

Since GI interestingly separated intermediate-GISTs into two subgroups with disparate genetic complexity, we next wondered whether it could have a prognostic value and predict with efficacy the metastatic risk for this class of patients. To address this question, we first explored the distribution of metastatic (M) and non-metastatic (NM) patients according to their GI value (Fig. 3) and uncovered that GI remarkably split high-risk patients from the others. Indeed, while NM tumours were predominantly found to have a low GI value (i.e. NM GI mean: 7.5 ± 10.9, median: 4.5), M tumours principally disclosed an elevated index (i.e. M GI mean of 29.96 ± 27.65; median 16), indicative of the presence of numerous genetic alterations. In light of these results, genome complexity emerged as a hallmark of metastasis, which prompts us to evaluate patient survival based on GI stratification (Fig. 4). Kaplan–Meier analyses of metastatic-free survival were unequivocally: stratification of the tumours by the GI value at a cutoff of 10 (GI1 < 10 and GI2 > 10) significantly separated the good (GI1) from the poor (GI2) prognosis patients ($p < 10^{-3}$), thus revealing GI as a potent and reliable novel prognostic factor for GISTs of intermediate grade. GI was moreover found to be the best prognosis factor when compared to the mitotic index, tumour size, tumour location and Fletcher classification in a multivariate analysis (Table 2).

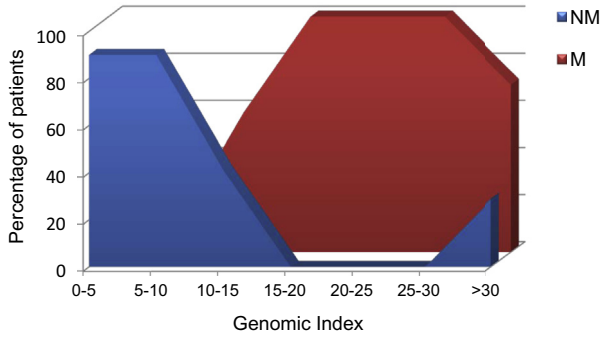
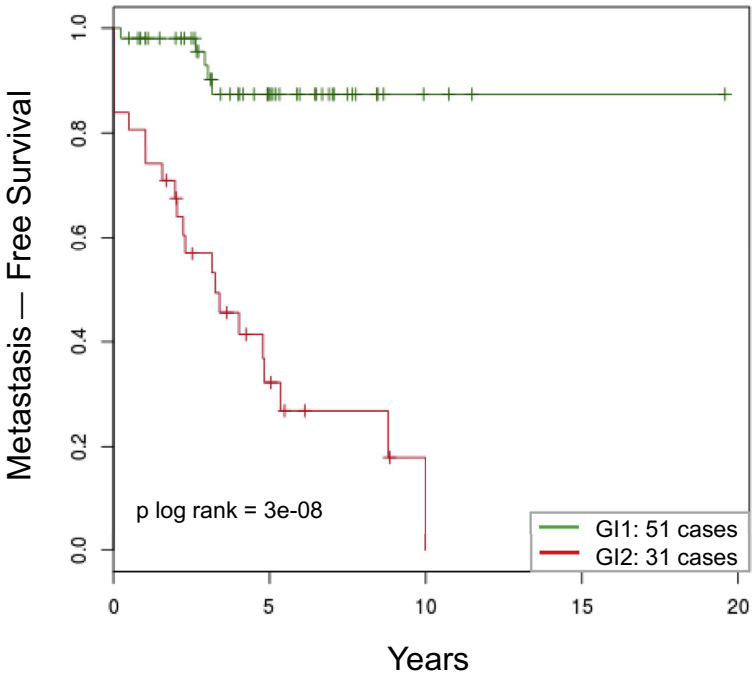


Fig. 3. Distribution of the non-metastatic and the metastatic patients according to the genomic index value.

4. Discussion

The ability to predict tumour outcome constitutes a key element for the proper counselling and management of patients. In the particular case of GISTs, accurate



		0	1 y	2 y	3 y	4 y	5 y
GI1	Patients at risk	51	47	43	35	29	22
	Cumulated events	0	1	1	3	5	5
	Metastasis FS	1	0.98	0.98	0.93	0.87	0.87
GI2	Patients at risk	31	25	20	15	11	7
	Cumulated events	5	6	10	13	16	19
	Metastasis FS	0.84	0.81	0.68	0.57	0.45	0.32

Fig. 4. Kaplan–Meier analysis of metastasis free survival (MFS) according to the genomic index value. GI1 and GI2 correspond to patients with a low (GI < 10) and high (GI > 10) genomic index, respectively.

Table 2
Multivariate analysis.

	Univariate		Multivariate	
	p Value	Hazard ratio (HR) [95% confidence interval (CI)]	*p* Value	HR [95% CI]
Genomic index (GI)	$3e10^{-08}$	9.6 [3.64–25.54]	$2.1e10^{-07}$	8.8 [3.64–25.12]
Mitotic index	N.S.	N.S.	N.S.	N.S.
GI	$3e10^{-08}$	9.6 [3.64–25.54]	$1.8e10^{-07}$	8.7 [3.64–24.70]
Tumor size	N.S.	N.S.	N.S.	N.S.
GI	$3e10^{-08}$	9.6 [3.64–25.54]	$1.1e10^{-07}$	12.1 [4.59–36.63]
Tumor location	N.S.	N.S.	N.S.	N.S.
GI	$3e10^{-08}$	9.6 [3.64–25.54]	$3.05e10^{-07}$	8.8 [3.65–25.36]
Fletcher classification	N.S.	N.S.	$4.2e10^{-02}$	2.3 [1.02–5.16]

Notes: Cutoff were chosen as follows: mitotic index: <5 and ⩾5; tumour size ⩽5 and >5; tumour location: stomach versus the rest; Fletcher: intermediate and high. N.S., not significant.

prognostication is essential to identify high-risk tumours, for which an efficient adjuvant systemic treatment is currently available. Gold standard stratification schemes employed so far to estimate GIST metastatic outcome, i.e. the NIH [18], AFIP [19] or modified NIH consensus risk criteria [20,31], predict recurrence-free survival (RFS) relatively well and have roughly similar prognosis accuracy [32,33]. However, despite their evolution and the inclusion of novel prognostic factors, these classifications recurrently identify a pool of intermediate-grade patients eligible for targeted therapy post-surgery, but for which real therapeutic decisions are made at physician's discretion. In other words, this also means that none of the criteria included

in these stratification systems are strong enough to discriminate which of intermediate GIST patients will really develop metastasis and clinically benefit from adjuvant imatinib. Moreover, since approximately 60% of GIST patients with an operable tumour are curable by a unique surgery [32], it results that a non-negligible fraction of intermediate patients does receive long-term imatinib treatment without really needing it [34]. This recalls the constant need to refine prognosis markers in order to improve existing stratification schemes and optimally predict chances of relapse.

Here, we identified genomic index (GI), a feature of chromosomal instability level, as a novel and reliable prognosis factor for intermediate GIST patients. At a cut-off value of 10, GI radically distinguished intermediate-grade patients at high-risk of recurrence from the others; high GI values being strongly associated to metastasis. Also efficient in other tumour grades [28], this marker shows a strong potency to predict GIST clinical outcome on its own and, could therefore add a significant value to the current consensus risk-criteria used for GIST stratification. Despite the evident link connecting high GI values to recurrence, it has to be noted, though, that a few metastatic tumours were found to have a low number of genomic variation (i.e. $n = 5$ on 31 metastatic patients) and that, on the reverse, rare non-metastatic tumours presented numerous chromosomal rearrangements (i.e. $n = 9$ on 51 non-metastatic patients). Such profiles are quite difficult to explain biologically. When these low GI metastatic cases were compared to the high GI ones by array-CGH (data not shown), no common potential metastatic driver genetic events were found. It is thus possible that these discrepancies result from other somatic events or individual specificities such as mutations. Undetectable by CGH, approaches such as next-generation sequencing could be of great interest to uncover these alterations and thus elucidate what drives the singular behaviour of these tumours.

While tumour morphological and cytological criteria constitute the core of GIST stratification schemes, a few other clinical and biological features have been assessed for their association with metastatic outcome [20]. Among these, intrinsic genetic characteristics of the tumour have been shown to be a critical risk factor for recurrence. Up-to-now, the most important genetic factors found to influence metastasis were the type of KIT or PDGFR mutations. KIT exon 9 mutations and exon 11 deletions have indeed been reported to be more aggressive than PDGFR Asp842Val or KIT exon 11 missense point mutations [35–37]. In this study, high GI values were not found, however, to be more often associated with KIT exon 11 mutations than with other mutations (Table 3 and data not shown). Apart from the impact of mutations, a few gene abnormalities due the frequent loss of 1p and 22q loci have also been related to poorer prognosis [38–40]. More recently we highlighted genome complexity as a strong predictor of GIST clinical outcome [28], unveiling a crucial role of genome profiling and genetic changes as potent predictive factors for GIST. This result is strengthened here since we demonstrate that GI is also efficient to stratify the challenging subclass of intermediate-grade patients; a population whose main genetic defaults were found otherwise to be equivalent, both in their type and frequency, to the ones commonly found in GIST (i.e. deletion of 14q, 22q and 1p, Fig. 1). It is to be noted that GI determination also showed a good potency to distinguish good from bad prognosis patients in the GIST population classified at high-risk by the NIH or AFIP system [28]. This is of interest given that 50% of them do not relapse at 5 years (EORTC, unpublished data).

Table 3
Distribution of KIT and platelet-derived growth factor receptor (PDGFR) mutations in the genomic index 1 (GI1) and genomic index 2 (GI2) groups.

		GI1	GI2
WT		4 (7.8)	1 (3.1)
KIT	K9	3 (5.8)	1 (3.1)
	K11	36 (70.5)	22 (68.7)
	K13	–	1 (3.1)
	K17	–	2 (6.2)
PDGFR	P12	1 (1.9)	–
	P14	1 (1.9)	1 (3.1)
	P18	4 (7.8)	2 (6.2)
Not determined (nd)		2	2
Total		51	32

Note: Percentage are indicated in brackets.

Altogether, these results reveal array CGH and GI determination as an appealing methodology for GIST stratification. Considering moreover that genome profiling was performed using FFPE samples, this assay could be easily set up in numerous pathology laboratories collecting FFPE samples for other routine diagnostic tests. Standing as the best current tool to manage imatinib therapy for intermediate risk GIST patients, the French Sarcoma Group has since designed a multicentre clinical trial entitled GI-GIST to assess prospectively the impact of the GI on the management of these patients. This prospective clinical study has recently been approved by the French National Cancer Institute and will be launched in the months to come.

Conflict of interest statement

None declared.

Acknowledgements

We thank Dr. Phillippe Terrier (Department of Pathology, Gustave Roussy Institute, Villejuif), Dr. Dominique Ranchère-Vince (Department of Pathology, Léon Bérard Center, Lyon, France) and Dr. Yves-Marie

Robin (Department of Pathology, Oscar Lambret Center, Lille) for providing additional biological material and associated clinical data. We also thank the Biological Resources Center of the Bergonie Institute for their approval to use FFPE human samples. At last, we thank the French Association of GIST Patients (AFPG), the Bergonie Cancer Institute, the National Institute of Cancer (INCa) and the Integrated Cancer Research Site of Bordeaux (SIRIC BRIO) for their financial support.

References

[1] Corless CL, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer 2011;11(12):865–78.
[2] Ducimetiere F, Lurkin A, Ranchere-Vince D, Decouvelaere AV, Peoc'h M, Istier L, et al. Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing. PLoS One 2011;6(8):e20294.
[3] Nilsson B, Bumming P, Meis-Kindblom JM, Oden A, Dortok A, Gustavsson B, et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era – a population-based study in western Sweden. Cancer 2005;103(4):821–9.
[4] Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004;22(18):3813–25.
[5] Sircar K, Hewlett BR, Huizinga JD, Chorneyko K, Berezin I, Riddell RH. Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. Am J Surg Pathol 1999;23(4):377–89.
[6] Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998;152(5):1259–69.
[7] Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. Science 2003;299(5607):708–10.
[8] Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998;279(5350):577–80.
[9] Blay JY, Le Cesne A, Cassier PA, Ray-Coquard IL. Gastrointestinal stromal tumors (GIST): a rare entity, a tumor model for personalized therapy, and yet ten different molecular subtypes. Discov Med 2012;13(72):357–67.
[10] Demetri GD, Benjamin R, Blanke CD, Choi H, Corless C, DeMatteo RP, et al. NCCN Task Force report: optimal management of patients with gastrointestinal stromal tumor (GIST) – expansion and update of NCCN clinical practice guidelines. J Natl Comp Cancer Netw 2004;2(suppl. 1):S1–S26, quiz 27–30.
[11] Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J Pharmacol Exp Ther 2000;295(1):139–45.
[12] Heinrich MC, Blanke CD, Druker BJ, Corless CL. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. J Clin Oncol 2002;20(6):1692–703.
[13] van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, et al. Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 2001;358(9291):1421–3.
[14] Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347(7):472–80.
[15] Joensuu H, Eriksson M, Sundby Hall K, Hartmann JT, Pink D, Schutte J, et al. One vs three years of adjuvant imatinib for operable gastrointestinal stromal tumor: a randomized trial. JAMA 2012;307(12):1265–72.
[16] Agaimy A. Gastrointestinal stromal tumors (GIST) from risk stratification systems to the new TNM proposal: more questions than answers? A review emphasizing the need for a standardized GIST reporting. Int J Clin Exp Pathol 2010;3(5):461–71.
[17] Demetri GD, von Mehren M, Antonescu CR, DeMatteo RP, Ganjoo KN, Maki RG, et al. NCCN Task Force report: update on the management of patients with gastrointestinal stromal tumors. J Natl Comp Cancer Netw 2010;8(suppl. 2):S1–S41, quiz S42–4.
[18] Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. Hum Pathol 2002;33(5):459–65.
[19] Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol 2006;23(2):70–83.
[20] Joensuu H. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. Hum Pathol 2008;39(10):1411–9.
[21] Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. Lancet 2007;369(9574):1731–41.
[22] El-Rifai W, Sarlomo-Rikala M, Miettinen M, Knuutila S, Andersson LC. DNA copy number losses in chromosome 14: an early change in gastrointestinal stromal tumors. Cancer Res 1996;56(14):3230–3.
[23] El-Rifai W, Sarlomo-Rikala M, Andersson LC, Miettinen M, Knuutila S. High-resolution deletion mapping of chromosome 14 in stromal tumors of the gastrointestinal tract suggests two distinct tumor suppressor loci. Genes Chromosomes Cancer 2000;27(4):387–91.
[24] Bergmann F, Gunawan B, Hermanns B, Hoer J, Schumpelick V, Fuzesi L. Cytogenetic and morphologic characteristics of gastrointestinal stromal tumors. Recurrent rearrangement of chromosome 1 and losses of chromosomes 14 and 22 as common anomalies. Verh Dtsch Ges Pathol 1998;82:275–8.
[25] Debiec-Rychter M, Lasota J, Sarlomo-Rikala M, Kordek R, Miettinen M. Chromosomal aberrations in malignant gastrointestinal stromal tumors: correlation with c-KIT gene mutation. Cancer Genet Cytogenet 2001;128(1):24–30.
[26] O'Leary T, Ernst S, Przygodzki R, Emory T, Sobin L. Loss of heterozygosity at 1p36 predicts poor prognosis in gastrointestinal stromal/smooth muscle tumors. Lab Invest 1999;79(12):1461–7.
[27] Kim NG, Kim JJ, Ahn JY, Seong CM, Noh SH, Kim CB, et al. Putative chromosomal deletions on 9P, 9Q and 22Q occur preferentially in malignant gastrointestinal stromal tumors. Int J Cancer 2000;85(5):633–8.
[28] Lagarde P, Perot G, Kauffmann A, Brulard C, Dapremont V, Hostein I, et al. Mitotic checkpoints and chromosome instability are strong predictors of clinical outcome in gastrointestinal stromal tumors. Clin Cancer Res 2012;18(3):826–38.
[29] Schneider-Stock R, Boltze C, Lasota J, Peters B, Corless CL, Ruemmele P, et al. Loss of p16 protein defines high-risk patients with gastrointestinal stromal tumors: a tissue microarray study. Clin Cancer Res 2005;11(2 Pt 1):638–45.
[30] Sabah M, Cummins R, Leader M, Kay E. Loss of heterozygosity of chromosome 9p and loss of p16INK4A expression are associated with malignant gastrointestinal stromal tumors. Mod Pathol 2004;17(11):1364–71.
[31] Rutkowski P, Bylina E, Wozniak A, Nowecki ZI, Osuch C, Matlok M, et al. Validation of the Joensuu risk criteria for primary resectable gastrointestinal stromal tumour – the impact of tumour rupture on patient outcomes. Eur J Surg Oncol 2011;37(10):890–6.
[32] Joensuu H, Vehtari A, Riihimaki J, Nishida T, Steigen SE, Brabec P, et al. Risk of recurrence of gastrointestinal stromal tumour

after surgery: an analysis of pooled population-based cohorts. Lancet Oncol 2012;13(3):265–74.
[33] Joensuu H. Adjuvant treatment of GIST: patient selection and treatment strategies. Nat Rev Clin Oncol 2012;9(6):351–8.
[34] Joensuu H. Gastrointestinal stromal tumors: risk assessment and adjuvant therapy. Hematol Oncol Clin North Am 2013;27(5): 889–904.
[35] Miettinen M, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. Arch Pathol Lab Med 2006;130(10):1466–78.
[36] Martin J, Poveda A, Llombart-Bosch A, Ramos R, Lopez-Guerrero JA, Garcia del Muro J, et al. Deletions affecting codons 557–558 of the c-KIT gene indicate a poor prognosis in patients with completely resected gastrointestinal stromal tumors: a study by the Spanish Group for Sarcoma Research (GEIS). J Clin Oncol 2005;23(25):6190–8.
[37] Wardelmann E, Losen I, Hans V, Neidt I, Speidel N, Bierhoff E, et al. Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. Int J Cancer 2003;106(6):887–95.
[38] Gunawan B, von Heydebreck A, Sander B, Schulten HJ, Haller F, Langer C, et al. An oncogenetic tree model in gastrointestinal stromal tumours (GISTs) identifies different pathways of cytogenetic evolution with prognostic implications. J Pathol 2007;211(4):463–70.
[39] Pylkkanen L, Sarlomo-Rikala M, Wessman M, Hamalainen E, Sainio M, Husgafvel-Pursiainen K, et al. Chromosome 22q alterations and expression of the NF2 gene product, merlin, in gastrointestinal stromal tumors. Hum Pathol 2003;34(9): 872–9.
[40] Lopez-Guerrero JA, Noguera R, Llombart-Bosch A. GIST: particular aspects related to cell cultures, xenografts, and cytogenetics. Semin Diagn Pathol 2006;23(2):103–10.