Impressive long-term disease stabilization by nilotinib in two pretreated patients with KIT/PDGFRA wild-type metastatic gastrointestinal stromal tumours

Maria Abbondanza Pantaleo[a,d], Margherita Nannini[a], Maristella Saponara[a], Chiara Gnocchi[f], Valerio Di Scioscio[b], Cristian Lolli[a], Fausto Catena[c], Annalisa Astolfi[d], Monica Di Battista[e], Guido Biasco[a,d] and on behalf of GIST Study Group, University of Bologna, Bologna, Italy

KIT/PDGFRA wild-type (WT) gastrointestinal stromal tumours (GISTs) showed a response rate to imatinib ranging from 0 to 25%. Nilotinib is a new-generation tyrosine kinase inhibitor that has demonstrated clinical activity in pretreated GIST patients. At present, no correlation between nilotinib activity and clinical/pathological/molecular features is available. We report on two WT GIST patients resistant to imatinib and sunitinib, and enrolled in the CAMN107A2201 study who achieved an impressive disease control by nilotinib. Both patients have germ-line mutations in the *SDHA* gene. In April 2004, a 39-year-old woman presented gastric GIST with multiple liver metastases and was treated with imatinib 400 mg/day, followed by imatinib 800 mg/day and then sunitinib. In August 2007, because of disease progression, she was enrolled in the CAMN107A2201 study and assigned to the nilotinib 800 mg/day arm. In March 2005, a 27-year-old woman started imatinib 600 mg/day and then sunitinib for gastric GIST with multiple liver and lung metastases. In October 2007, because of disease progression, she was enrolled in the CAMN107A2201 study and assigned to the nilotinib 800 mg/day arm. One patient still showed stable disease after 46 months of treatment according to the Response Evaluation Criteria In Solid Tumors, and a partial response after 9 months according to Choi's criteria. The other patient still showed stable disease after 42 months according to Response Evaluation Criteria In Solid Tumors. At present, they continue to receive nilotinib. We report very long-term disease stabilization under nilotinib treatment in two pretreated WT GIST patients. In-vitro studies and clinical analyses are warranted to evaluate a potential correlation between nilotinib activity and WT genotype or other clinical/pathological features. *Anti-Cancer Drugs* 23:567–572 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2012, **23**:567–572

Keywords: gastrointestinal stromal tumours, GIST, imatinib, KIT, nilotinib, platelet-derived growth factor receptor α, SDH, sunitinib, wild type

Departments of [a]Hematology and Oncology Sciences 'L&A Seràgnoli', [b]Radiology, [c]Surgery and Transplantation, S. Orsola-Malpighi Hospital, [d]Interdepartmental Centre for Cancer Research 'G. Prodi', University of Bologna, [e]Department of Medical Oncology, Bellaria-Maggiore Hospital, Azienda USL of Bologna, Bologna and [f]Novartis Oncology, Milan, Italy

Correspondence to Maria Abbondanza Pantaleo, Department of Hematology and Oncological Sciences 'L. A. Seragnoli', S. Orsola-Malpighi Hospital, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy
Tel: +39 051 6364078; fax: +39 051 636 4037;
e-mail: maria.pantaleo@unibo.it

Received 20 October 2011 **Revised form accepted** 21 February 2012

Introduction

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal neoplasia of the gastrointestinal tract [1]. The main oncogenic event leading to GIST development is a gain-of-function gene mutation in the KIT receptor (85%) or in the homologous platelet-derived growth factor receptor α (PDGFRA), resulting in the constitutive ligand-independent activation of receptors [2,3]. Complete surgical resection is the mainstay of therapy for localized GISTs. However, many patients develop disease recurrence or metastasis, predominantly in the peritoneum surface and in the liver [4]. The introduction of tyrosine kinase inhibitors (TKIs) has markedly changed the natural history of patients with recurrent, metastatic or inoperable diseases [5,6]. Several data are available on the correlation between the response to TKIs and KIT and PDGFRA mutational status [7–14]. Imatinib is the standard first-line treatment at the recommended dose of 400 mg/day and it should also be administered at 400 mg twice daily in a small subset of patients with GIST harbouring KIT exon 9 mutations or after the failure of 400 mg/day therapy [12,13]. Although the majority of patients with GIST respond well to imatinib, a small subset of patients (5%) show primary resistance to the drug, especially those with GIST with an exon 18 PDGFRA D842V point mutation who are not usually sensitive to imatinib, and KIT/PDGFRA wild-type (WT) GISTs that showed a response rate to imatinib ranging from 0 to 25% [7,8,10–12,14]. Even patients who show a good response to imatinib almost invariably acquire resistance to the drug with a median of about 2 years of drug administration, mostly because of the development of secondary mutations, and develop disease progression [15–20]. Sunitinib has been approved

0959-4973 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e328352cc50

as a second-line therapy in patients with advanced GISTs after imatinib because of resistance or intolerance [6]. As is well known, the KIT/PDGFRA WT GISTs are usually more responsive to sunitinib than GISTs harbouring KIT exon 11 mutations [9]. Nilotinib is a new-generation TKI specifically developed for the treatment of Philadelphia chromosome-positive chronic myelogenous leukaemia (CML Ph+). The new drug has proved to be a more potent and selective inhibitor of Bcr–Abl kinase than imatinib, with a better topological fit for the kinase-binding domain [21–23].

Nilotinib achieved 7–10-fold higher intracellular concentrations than imatinib in an in-vitro study with GIST882 and GIST GDG1 cell lines. These data suggest that nilotinib might be less susceptible to transport-driven imatinib resistance [24]. Nilotinib also demonstrated activity in preclinical studies with GIST xenograft mice, in phase I and II studies and in compassionate use studies for pretreated GIST patients [25–28]. However, the results of nilotinib in third-line versus best supportive care with or without a TKI (usually with continuation of TKI therapy) (CAMN107A2201 – ENESTG3 trial) showed no significant difference in progression-free survival (PFS) (the primary endpoint of the study) between the two arms (median PFS = 109 days vs. 111 days, respectively, $P = 0.5555$) or in the median overall survival (OS; 332 days vs. 280 days, respectively, $P = 0.29$, NS) [29]. A significant difference in the median OS was observed only with an analysis on 'true third-line' patients (405 days vs. 280 days in the nilotinib and best supportive care arms, respectively, $P = 0.02$) [29,30]. Furthermore, in April 2011 Novartis announced that the randomized phase III CAMN107G2301 trial (ENESTG1) of nilotinib in the first-line treatment has been discontinued on the basis of interim results showing that nilotinib is unlikely to prove superior to imatinib [31]. However, a phase I study and compassionate use cases showed good disease control (stable disease plus partial response) by nilotinib in a subset of pretreated patients after imatinib and sunitinib failure even if not always longer than 12 months for the majority of responding patients [26,27]. Thus, there is a subset of patients who respond to nilotinib. At present, no correlation between nilotinib activity and the KIT/PDGFRA genotype or other pathological or clinical predictive characteristics is available.

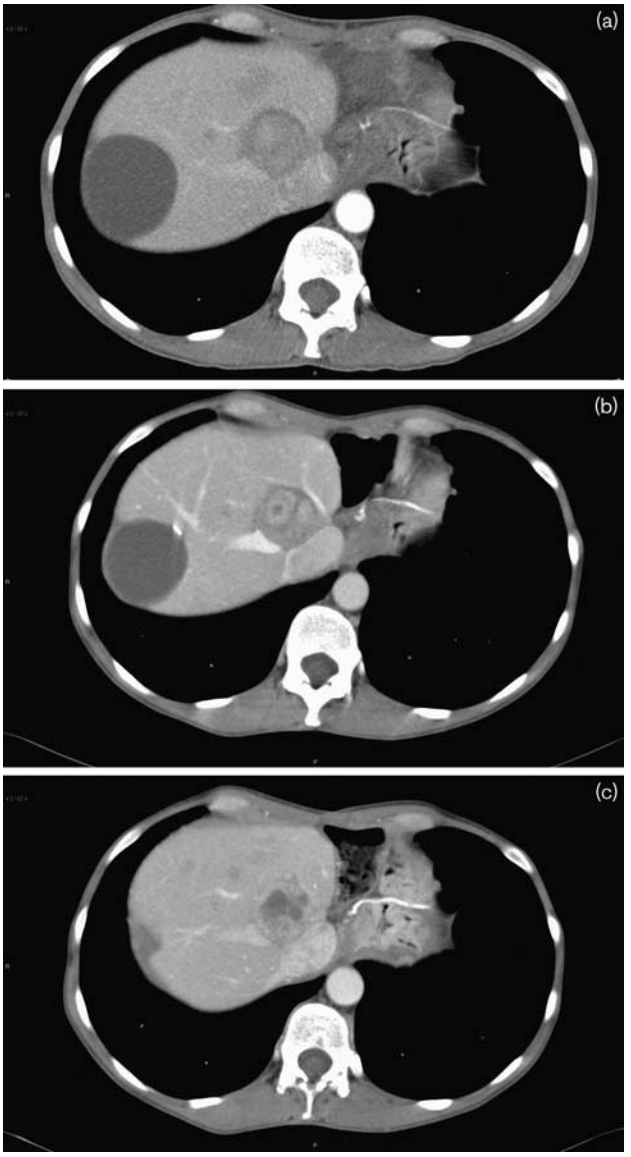
We report impressive long-term disease stabilization by nilotinib in two patients with GIST resistant to imatinib and sunitinib and enrolled in the CAMN107A2201 study.

Case reports

In April 2004, a 39-year-old woman presented gastric GIST with multiple liver metastases and was started on imatinib 400 mg with stable disease until September 2004, when disease progression occurred in both primary and hepatic lesions. From October 2004 to October 2005, she received imatinib 800 mg until further progression. The patient was started on sunitinib in November 2005, with a mild hepatic progression after 2 months and then stable disease till November 2006. Because of chronic bleeding, the patient underwent surgery with partial gastrectomy, partial pancreasectomy, peritoneal nodules resection, left hepatectomy and wedge resections. Macroscopically, the primary lesion was located in the anterior gastric wall, with a diameter of 10.5 cm, showing haemorrhagic and cystic features. The histological examination confirmed a gastric GIST with diffuse aspects of haemorrhagic necrosis, positive for CD117, CD34 and vimentine and negative for cytokeratin, smooth muscle actin, desmin, S100, MART-1 and HMB 45. No data on the mitotic index are available. Metastases were found in three out of 15 lymph nodes of the lesser curvature. All hepatic samples were positive for GIST metastases. Molecular analysis performed on exons 9, 11, 13 and 17 of gene KIT and on exons 12, 14 and 18 of gene PDGFRA was negative for KIT/PDGFRA mutations. For the remaining multiple liver metastases, not radically operable, she continued sunitinib until July 2007, when some liver lesions increased in size (Fig. 1a). Therefore, in August 2007, she was enrolled in the CAMN107A2201 study and was assigned to the nilotinib arm. She has continued nilotinib 800 mg/day to date, with stable disease in all sites for 46 months according to Response Evaluation Criteria In Solid Tumors (RECIST). However, a partial response according to Choi's criteria occurred in May 2008 after 9 months of treatment because of a decrease in the tumour density of the target lesion chosen for the protocol (Fig. 1b and c). During nilotinib treatment, in October 2010, resection of metastatic disease was attempted but the disease was not resectable (only one large necrotic lesion was removed for histological confirmation of metastasis from GIST, KIT positive). With the KIT and PDGFRA genotype WT, we studied SDH genes and we found a germline R589Q missense mutation and a somatic R171C missense mutation in *SDHA*.

In March 2005, a 27-year-old woman was started on imatinib 600 mg for gastric GIST with multiple liver and lung metastases. In October 2005, disease progression occurred in both the primary lesion and the liver metastases; thus, in November 2005 she started treatment with sunitinib. The best response was stable disease. However, in January 2007, she developed moderate fatigue and mild heart failure and stopped the treatment. She then underwent gastrectomy, with removal of the primary responsive tumour mass. Macroscopically, two lesions located in the gastric corpus were found, with diameters of 5 and 7 cm, respectively. Other intramural lesions were found at the gastric corpus and antrum, ranging in size from 0.5 to 1 cm. The histological examination confirmed a multifocal GIST, with a mixed epithelioid and spindle cell morphology, positive for

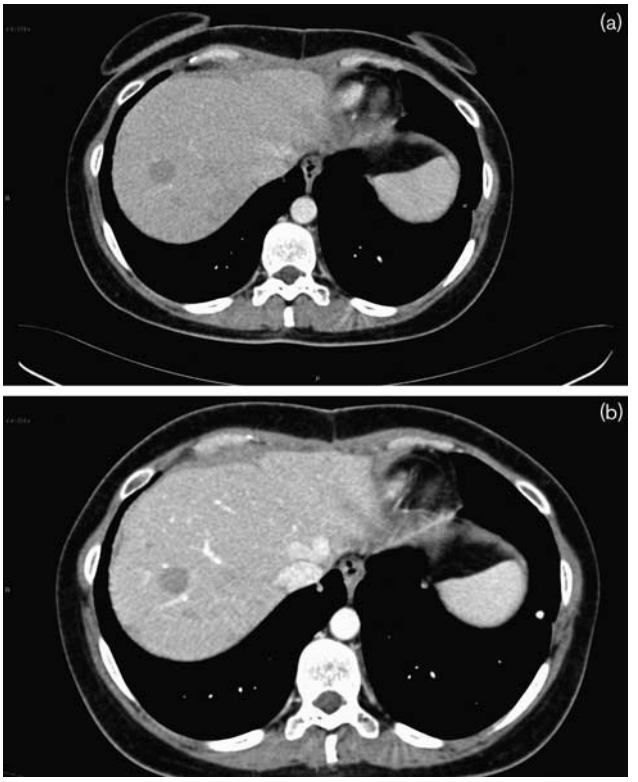
Fig. 1



Liver metastasis as the target lesion for the clinical trial (patient 1). (a) Baseline before nilotinib treatment; (b) after 9 months of nilotinib treatment with a stable lesion according to the Response Evaluation Criteria In Solid Tumors (RECIST) criteria and with a partial response according to Choi's criteria because of the decrease in tumour density more than 15%; (c) computed tomography scan at the last follow-up with a stable lesion according to the RECIST criteria associated with an intralesion density variation not suggestive of a response according to Choi's criteria.

CD117 and negative for desmin, smooth muscle actin and S100. The mitotic index was greater than 10/50 HPF and less than 5/50 HPF, respectively. A metastasis was found in one out of six lymph nodes of the lesser curvature. Molecular analysis performed on exons 9, 11, 13 and 17 of gene KIT and on exons 12, 14 and 18 of gene PDGFRA was negative for KIT/PDGFRA mutations. She continued sunitinib until September 2007, when a further hepatic

Fig. 2



Liver metastasis as the target lesion for the clinical trial (patient 2). (a) Baseline before nilotinib treatment; (b) computed tomography scan at the last follow-up with a stable lesion according to the RECIST criteria.

disease progression occurred (Fig. 2a). Therefore, in October 2007, she was enrolled in the CAMN107A2201 study and assigned to the nilotinib arm. She has continued nilotinib 800 mg/day to date, with a stable disease of 42 months according to the RECIST criteria in all disease sites. Mild changes in density not significant according to Choi's criteria were observed in the target hepatic lesion chosen for the protocol and in other smaller lesions (Fig. 2b). In March 2010, resection of liver metastatic disease was attempted but the disease was not resectable. During lung surgery, two lesions were removed for histological confirmation and both were confirmed to be metastases from GIST KIT positive.

In this patient, we performed a whole-transcriptome next-generation sequencing on tumour samples to search for novel pathogenetic mutations and we found a homozygous p.S384* nonsense mutation in the *SDHA* gene [32].

During nilotinib treatment, a computed tomography scan was performed every 3 months as per the protocol procedures. The RECIST criteria were used for response evaluation. Tolerance was good and neither patient

presented any severe grade 3 or 4 side-effects. At present, they are still alive and continue to receive nilotinib.

Discussion

We report the clinical outcome of two patients with metastatic GISTs under nilotinib treatment enrolled in the CAMN107A2201 study. One patient still presented stable disease after 46 months according to the RECIST criteria and a partial response after 9 months of treatment according to Choi's criteria. The other patient still presented stable disease after 42 months according to RECIST. At present, both patients are still alive and continue to receive nilotinib.

The CAMN107A2201 study is a randomized, open-label, multicentre, phase III study to evaluate the efficacy of nilotinib versus best supportive care with or without a TKI (by investigator's choice) in adult patients with GISTs resistant to both imatinib and sunitinib. The results showed no significant difference in PFS on the basis of blinded central radiology review and in OS between the two arms [29]. Thus, our patients experienced disease stabilization of 43 and 39 months, respectively, more in comparison with the median time to progression reported from the results of the study (109 days). The response was evaluated using the standard RECIST criteria even if they are considered inadequate to follow up the treatment activity in GIST [33,34]. However, in these cases, the response to nilotinib was also substantially confirmed using Choi's criteria. In particular, one patient showed a partial response according to Choi's criteria after 9 months of treatment because of a decrease in the tumour density of the target lesion more than 15% (Fig. 1a and b). The other patient had a very mild variation in tumour density during treatment but not significantly suggestive of a tumour response.

Although two case reports are not sufficient for any conclusive considerations, these findings can be considered of interest and contribute to a critical analysis of the clinical and molecular characteristics of WT KIT/PDGFRA GIST.

Both our patients were women, both were young (27 and 39 years) and had metastatic disease at diagnosis. Both patients presented a primary GIST localized in the stomach and the KIT/PDGFRA genotype was WT. Before enrolment in the CAMN107A2201 study, the sensitivity of their KIT/PDGFRA WT disease to TKIs was in accordance with the literature data. No particular predictive characteristics can be associated with the disease control of GIST by nilotinib. The in-vitro study showing that nilotinib achieved 7–10-fold higher intracellular concentrations than imatinib on two human GIST-derived cell lines (GIST882 and GIST GDG1), both expressing constitutively activated KIT [24]. The higher intracellular concentrations of nilotinib than those of imatinib indicate either increased cellular uptake or decreased cellular efflux of nilotinib when compared with imatinib in GIST cell lines. This result suggests that nilotinib can overcome imatinib resistance because of changes in cellular transport mechanisms. Nilotinib is considered a new-generation TKI but the mechanism of action is not so different from imatinib in GISTs as they are both equipotent as inhibitors of KIT and PDGFRA [35]. In fact, nilotinib was rationally designed, on the basis of the structure of imatinib, to overcome imatinib resistance in CML. Compared with imatinib, the structural modifications of nilotinib have improved its affinity and inhibitory activity against Bcr-Abl kinase, leading to its approval for the treatment of imatinib-resistant or intolerant CML and upfront patients [22,23]. As a proof of principle, these findings do not indicate that 'the response' in our patients can be associated with the inhibition of KIT and PDGFRA signalling pathways as neither of these GISTs have mutations. However, the KIT/PDGFRA WT as a possible reason for these results may be correct even if no in-vitro data on GIST cell lines or in-vivo data on GIST patients are available. The only report on the major activity of nilotinib compared with other TKIs, including imatinib, was presented by Agaram *et al.* [36] on murine Ba/F3 cells expressing WT KIT. On the basis of these in-vitro results, second-generation TKIs were thought to have a superior activity to imatinib in KIT/PDGFRA WT GIST patients, although further information and clinical validation are needed. Finally, both patients harbour mutations in *SDHA* [32,37]. The 39-year-old patient was heterozygous for a germline R589Q missense mutation and a somatic R171C missense mutation and the 27-year-old patient was homozygous for a p.S384* nonsense mutation. The clinical and pathological significance of the SDHA mutation in GIST is still undefined, except for the association with KIT/PDGFRA WT genotype status. Therefore, until now, no mature hypotheses regarding the nilotinib activity in GIST and SDH genotype status can be postulated as stronger evidences are needed. A potential hypothesis that can be supposed is related to the 'off targets' mechanism of nilotinib activity on enzyme carbonic anhydrase (CA) isoforms. In fact, the expression of membrane-bound CAIX and CAXII induced through the hypoxia-inducible factor-1 in tumour cells is associated with tumour growth *in vitro* and *in vivo* [38]. Thus, SDH complex dysregulation because of SDH subunits mutations may mediate a pseudohypoxic response and, consequently, an increase in hypoxia-inducible factor-1α induced membrane CA isoforms. Nilotinib and imatinib were investigated for their effects on the inhibition of the CA isoforms I–XV and nilotinib was found to be a more potent inhibitor of the CAIX than imatinib; thus, the authors postulated that the anticancer effect of this drug may also be because of the interaction with the CA isoforms that are involved in the carcinogensis [39]. In addition, it cannot be excluded that the increased potency of nilotinib compared with imatinib is the result of the inhibition of other

tyrosine kinase receptors such as ephrin receptors (Eph B1, B2 and B4) [40].

Another reason postulated for the long-term disease stabilization in our two patients is that these two GISTs have an inherently indolent outcome. This hypothesis is supported by various considerations. The characteristics of a paediatric-type adult GIST have recently been reported [41]. In particular, Rege and colleagues described 16 GIST patients with mutual pathological, molecular and clinical features. They were mainly women (81%), with a median age of 31.5 years. Macroscopically, all primary tumours were located in the stomach, with a mean size of 5.4 cm. At molecular analysis, all tumours were WT for KIT and PDGFRA mutations. Clinically, most patients had lymph node metastases, were resistant to imatinib and sensitive to sunitinib, and even if metastatic, experienced an indolent clinical course. In our patients, the presence of metastases at the time of diagnosis, associated with the absence of severe clinical symptoms, supports the idea of a slow and long-term natural history of disease progression from primary tumour growth to metastatic spread. However, the hypothesis of an indolent behaviour overall in these two patients clashes with the rapid tumour progression during imatinib treatment (7 and 17 months, respectively) that is at least close to the imatinib efficacy data of time to progression for KIT/PDGFRA WT GISTs, but much shorter than the time of response seen with nilotinib therapy. Moreover, the duration of response to imatinib was also shorter than that observed to sunitinib. Although this is to be expected because KIT/PDGFRA WT GIST patients are more sensitive to sunitinib, the duration in these cases was longer than the data reported for this patient population (22 months in our patients vs. a median 19.0 months) [9]. The possibility that these GISTs may have had an aggressive behaviour immediately after diagnosis and then become very slowly progressive as a result of the prolongation of TKI administration is not supported by available experimental and clinical data.

The last common aspect in the management of these two patients is that both underwent surgery to remove their primary gastric tumours and partially their metastases despite being metastatic and not radically operable. They underwent debulking surgery with partial gastrectomy, partial pancreasectomy, peritoneal nodules resection, left hepatectomy and wedge resections in one patient because of chronic bleeding, and gastrectomy with removal of the primary tumour that was the responsive mass in a drug discontinuation window because of cardiac toxicity. This surgical indication was controversial and discussed at length because no data are available in the literature [42]. For this reason, the decisions were shared with the patients in the absence of other treatment options in clinical practice and other drugs in clinical trials at that time. However, it has not been demonstrated that surgery influences clinical outcome under nilotinib treatment in these patients.

Conclusion

We report the long-term disease stabilization of two pretreated patients with metastatic KIT/PDGFRA WT GIST under nilotinib therapy. The explanation for these results remains unknown and must be studied *in vitro* or evaluated in depth in large subgroup analyses for the molecular status of KIT and PDGFRA or for clinical characteristics in studies with nilotinib in GISTs.

Acknowledgements

Novartis Oncology is acknowledged for the authorization to report data from two patients enrolled in the CAMN107A2201 study.

GIST Study Group: Annalisa Altimari, Paolo Castellucci, Claudio Ceccarelli, Stefano Fanti, Michelangelo Fiorentino, Serena Formica, Pietro Fusaroli, Lidia Gatto, Walter Franco Grigioni, Elisa Gruppioni, Alessandra Maleddu, Anna Mandrioli, Maria Caterina Pallotti, Paola Paterini, Antonio Daniele Pinna, Donatella Santini, Paola Tomassetti, Milena Urbini, Maurizio Zompatori (University of Bologna, Bologna, Italy).

Conflicts of interest

There are no conflicts of interest.

References

1 Miettinen M, Lasota J. Gastrointestinal stromal tumors (GISTs): definition, occurrence, pathology, differential diagnosis and molecular genetics. *Pol J Pathol* 2003; **54**:3–24.
2 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**:577–580.
3 Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, *et al.* PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299**:708–710.
4 DeMatteo RP, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg* 2000; **231**:51–58.
5 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**:472–480.
6 Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, *et al.* Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368**:1329–1338.
7 Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, *et al.* Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003; **21**: 4342–4349.
8 Debiec-Rychter M, Dumez H, Judson I, Wasag B, Verweij J, Brown M, *et al.* Use of c-KIT/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and II studies of the EORTC Soft Tissue and Bone Sarcoma Group. *Eur J Cancer* 2004; **40**:689–695.
9 Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, *et al.* Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; **26**:5352–5359.
10 Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, *et al.* Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006; **24**:4764–4774.

11 Heinrich MC, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD, *et al*. Correlation of kinase genotype and clinical outcome in the North American Intergroup. Phase III trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol* 2008; **26**:5360–5367.

12 Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, *et al*. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J Clin Oncol* 2008; **26**:626–632.

13 Debiec-Rychter M, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, *et al*. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2006; **42**:1093–1103.

14 Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, *et al*. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. *J Clin Oncol* 2005; **23**:5357–5364.

15 Desai J, Shankar S, Heinrich MC, Fletcher JA, Fletcher CD, Manola J, *et al*. Clonal evolution of resistance to imatinib in patients with metastatic gastrointestinal stromal tumors. *Clin Cancer Res* 2007; **13 (18 Pt 1)**: 5398–5405.

16 Fletcher JA, Corless CL, Dimitrijevic S, von Mehren M, Eisenberg B, Joensuu H, *et al*. Mechanisms of resistance to imatinib mesylate (IM) in advanced gastrointestinal stromal tumor (GIST) [abstract]. ASCO annual meeting; 2003. 3275.

17 Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N, *et al*. Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology* 2005; **128**:270–279.

18 Liegl B, Hornick JL, Antonescu CR, Corless CL, Fletcher CD. Rhabdomyosarcomatous differentiation in gastrointestinal stromal tumors after tyrosine kinase inhibitor therapy: a novel form of tumor progression. *Am J Surg Pathol* 2009; **33**:218–226.

19 Maleddu A, Pantaleo MA, Nannini M, Di Battista M, Saponara M, Lolli C, *et al*. Mechanisms of secondary resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumours (review). *Oncol Rep* 2009; **21**:1359–1366.

20 Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, *et al*. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. *Oncogene* 2007; **26**:3909–3919.

21 Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat Rev Cancer* 2007; **7**:345–356.

22 Kantarjian HM, Giles FJ, Bhalla KN, Pinilla-Ibarz J, Larson RA, Gattermann N, *et al*. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. *Blood* 2011; **117**:1141–1145.

23 Rosti G, Palandri F, Castagnetti F, Breccia M, Levato L, Gugliotta G, *et al*. Nilotinib for the frontline treatment of Ph(+) chronic myeloid leukemia. *Blood* 2009; **114**:4933–4938.

24 Prenen H, Guetens G, de Boeck G, Debiec-Rychter M, Manley P, Schoffski P, *et al*. Cellular uptake of the tyrosine kinase inhibitors imatinib and AMN107 in gastrointestinal stromal tumor cell lines. *Pharmacology* 2006; **77**:11–16.

25 Pantaleo MA, Nicoletti G, Nanni C, Gnocchi C, Landuzzi L, Quarta C, *et al*. Preclinical evaluation of KIT/PDGFRA and mTOR inhibitors in gastrointestinal stromal tumors using small animal FDG PET. *J Exp Clin Cancer Res* 2010; **29**:173.

26 Demetri GD, Casali PG, Blay JY, von Mehren M, Morgan JA, Bertulli R, *et al*. A phase I study of single-agent nilotinib or in combination with imatinib in patients with imatinib-resistant gastrointestinal stromal tumors. *Clin Cancer Res* 2009; **15**:5910–5916.

27 Montemurro M, Schöffski P, Reichardt P, Gelderblom H, Schütte J, Hartmann JT, *et al*. Nilotinib in the treatment of advanced gastrointestinal stromal tumours resistant to both imatinib and sunitinib. *Eur J Cancer* 2009; **45**:2293–2297.

28 Sawaki A, Nishida T, Doi T, Yamada Y, Komatsu Y, Kanda T, *et al*. Phase 2 study of nilotinib as third-line therapy for patients with gastrointestinal stromal tumor. *Cancer* 2011; **117**:4633–4641.

29 Reichardt P, Blay J, Gelderblom H, Schlemmer M, Demetri GD, Bin Bui N, *et al*. Phase III trial of nilotinib in patients with advanced gastrointestinal stromal tumor (GIST): first results from ENEST g3 [abstract]. ASCO annual meeting. 2010; 10017.

30 Phase III, open-label study of nilotinib versus imatinib in GIST patients (ENESTg1). Available at: *http://clinicaltrials.gov/ct2/show/NCT00785785*. [Accessed 4 November 2008].

31 Efficacy and safety of AMN107 compared with current treatment options in patients with GIST who have failed both imatinib and sunitinib (ENESTg3). Available at: *http://clinicaltrials.gov/ct2/show/NCT00471328*. [Accessed 26 April 2007].

32 Pantaleo MA, Astolfi A, Indio V, Moore R, Thiessen N, Heinrich MC, *et al*. SDHA loss-of-function mutations in KIT-PDGFRA wild-type gastrointestinal stromal tumors identified by massively parallel sequencing. *J Natl Cancer Inst* 2011; **103**:983–987.

33 Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, *et al*. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. *J Clin Oncol* 2007; **25**: 1753–1759.

34 Benjamin RS, Choi H, Macapinlac HA, Burgess MA, Patel SR, Chen LL, *et al*. We should desist using RECIST, at least in GIST. *J Clin Oncol* 2007; **25**:1760–1764.

35 Blay JY, von Mehren M. Nilotinib: a novel, selective tyrosine kinase inhibitor [review]. *Semin Oncol* 2011; **38 (Suppl 1)**:S3–S9, Erratum in: *Semin Oncol* 2011; **38**:467.

36 Agaram NP, Laquaglia MP, Ustun B, Guo T, Wong GC, Socci ND, *et al*. Molecular characterization of pediatric gastrointestinal stromal tumors. *Clin Cancer Res* 2008; **14**:3204–3215.

37 Pantaleo MA, Nannini M, Astolfi A, Biasco G. on behalf of GIST Study Group Bologna. A distinct pediatric-type gastrointestinal stromal tumor in adults: potential role of succinate dehydrogenase subunit a mutations. *Am J Surg Pathol* 2011; **35**:1750–1752.

38 Chiche J, Ilc K, Laferrière J, Trottier E, Dayan F, Mazure NM, *et al*. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res* 2009; **69**:358–368.

39 Parkkila S, Innocenti A, Kallio H, Hilvo M, Scozzafava A, Supuran CT. The protein tyrosine kinase inhibitors imatinib and nilotinib strongly inhibit several mammalian alpha-carbonic anhydrase isoforms. *Bioorg Med Chem Lett* 2009; **19**:4102–4106.

40 Melnick JS, Janes J, Kim S, Chang JY, Sipes DG, Gunderson D, *et al*. An efficient rapid system for profiling the cellular activities of molecular libraries. *Proc Natl Acad Sci USA* 2006; **103**:3153–3158.

41 Rege TA, Wagner AJ, Corless CL, Heinrich MC, Hornick JL. 'Pediatric-type' gastrointestinal stromal tumors in adults: distinctive histology predicts genotype and clinical behavior. *Am J Surg Pathol* 2011; **35**:495–504.

42 Pantaleo MA, Di Battista M, Catena F, Astorino M, Saponara M, Di Scioscio V, *et al*. Surgical debulking of gastrointestinal stromal tumors: is it a reasonable option after second-line treatment with sunitinib? *J Cancer Res Clin Oncol* 2008; **134**:625–630.