Circulating Levels of Soluble KIT Serve as a Biomarker for Clinical Outcome in Gastrointestinal Stromal Tumor Patients Receiving Sunitinib following Imatinib Failure

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Abstract

Purpose: To evaluate changes in circulating levels of soluble KIT (sKIT) extracellular domain as a potential biomarker for clinical outcome in gastrointestinal stromal tumor patients treated with the multitargeted tyrosine kinase inhibitor sunitinib following imatinib failure in a previously reported phase III study.

Experimental Design: Patients received sunitinib 50 mg/d (n = 243) or placebo (n = 118) daily in 6-week cycles (4 weeks on, 2 weeks off treatment). Plasma sKIT levels were sampled every 2 weeks in cycle 1 and on days 1 and 28 of subsequent cycles; analyzed by ELISA; and evaluated using Prentice criteria, Cox proportional hazards models, and proportion of treatment effect (PTE) analysis.

Results: From 4 weeks on treatment and onward, significant differences were shown between treatment groups (P < 0.0001) in sKIT level changes from baseline (median levels decreased with sunitinib and increased with placebo). Decreases in sKIT levels were a significant predictor of longer time to tumor progression (TTP). Patients with reduced levels at the end of cycle 2 had a median TTP of 34.3 weeks versus 16.0 weeks for patients with increased levels [hazard ratio, 0.71; 95% confidence interval (95% CI), 0.61-0.83; P < 0.0001], and changes in sKIT levels replaced treatment as a stronger predictor of TTP (PTE, 0.80; 95% CI, 0.34-3.70), showing even greater surrogacy on cycle 3 day 1 (PTE, 0.98; 95% CI, 0.39-3.40).

Conclusions: The results suggest that circulating plasma sKIT levels seem to function as a surrogate marker for TTP in gastrointestinal stromal tumor patients. Additional studies are warranted to confirm and expand these findings. (Clin Cancer Res 2009;15(18):5869–77)

The majority of gastrointestinal stromal tumors (GISTs) express the KIT transmembrane receptor tyrosine kinase (also known as stem-cell factor receptor and CD117), the protein product of the KIT proto-oncogene. The kinase activity of KIT is normally regulated by binding of its ligand, stem-cell factor. Approximately 85% to 90% of GISTs are associated with KIT gene mutations that lead to a constitutively activated (ligand-independent) form of KIT (1, 2). A smaller proportion of GISTs (5-7%) are associated with PDGFRA (5-7%), as well as PDGFR-α (PDGFR-α; ref. 3). Elucidation of the role of these oncogenic mutations in GIST pathobiology has led to the development of molecular targeted therapies that block KIT and PDGFR-α–mediated signaling [e.g., imatinib mesylate (Gleevec, Novartis Pharmaceuticals Corp.) and sunitinib malate (SU1124, Pfizer, Inc.)].

Sunitinib is an oral multitargeted tyrosine kinase inhibitor that targets KIT and PDGFR-α, as well as PDGFR-β, vascular endothelial growth factor (VEGF) receptors, glial cell line–derived neurotrophic factor receptor (REarranged during Transfection;
and plasma of healthy individuals as well as patients with gastrointestinal stromal tumor (GIST), our results suggest that circulating levels of soluble KIT extracellular domain (sKIT) seem to function as a surrogate marker for clinical outcome [time to tumor progression (TTP)], with decreases in sKIT levels being predictive of longer TTP. Assays of sKIT, an easy molecule to detect, consequently have the potential to facilitate and expedite future clinical trials in GIST pending confirmation in prospective studies. Additionally, sKIT assays may be useful in monitoring response in patients with GIST, as well as in identifying patients who may benefit from treatment with sunitinib.

The pathophysiologic association between the KIT receptor and plasma KIT extracellular domain is well established. Secreted KIT (sKIT) is produced in response to the expression of KIT in various pathologic conditions (15–20). sKIT is an attractive surrogate marker candidate. Indeed, earlier preliminary reports suggested that a decline in plasma sKIT levels in GIST patients might correlate with responses to imatinib (21, 22) and sunitinib (23). In the present analysis, using samples obtained in the phase III study of sunitinib in GIST patients after imatinib failure (9), we evaluated changes in plasma levels of sKIT as a potential surrogate marker for clinical outcome in GIST.

Statistical methods. The validity of sKIT as a surrogate marker for treatment efficacy was evaluated using the criteria established by Peto et al. (26), which specify that the following conditions need to be met: (a) The treatment must have a significant effect on the clinical end point; (b) the treatment must have a significant effect on the surrogate marker; (c) the surrogate marker must have a significant effect on the clinical end point; and (d) the full treatment effect on the clinical end point must be accurately captured by the surrogate.

The effects of treatment and the surrogate marker on outcomes were evaluated using Cox proportional hazards models, both without adjustment for sKIT $\lambda_1(t) = \lambda_0(t) \exp(\alpha Z)$, where $\alpha$ was the estimated treatment effect adjusted for the biomarker, and $Z$ was the treatment indicator and with adjustment for sKIT $\lambda_2(t) = \lambda_0(t) \exp(\alpha Z + \beta S)$, where $\beta$ was the estimated treatment effect adjusted for the biomarker, $S$ represented the biomarker, and $\beta$ was the estimated biomarker effect. The proportion of treatment effect (PTE) explained by the surrogate relative to the overall treatment effect was calculated using the method of Freedman et al. (ref. 27; PTE = 1 - $\alpha_2/\alpha_1$). A perfect surrogate marker has a PTE value of 1, and a biomarker that is not a surrogate has a value of 0. If the lower bound of the confidence interval (CI) for the PTE is greater than 0, it indicates a statistically significant contribution by the surrogate marker to the treatment difference in the clinical end point.

Time-to-event data were analyzed using the Kaplan-Meier method, Cox proportional hazards models, and the log-rank test.

**Materials and Methods**

**Study design and sKIT assessments.** The study from which data were obtained was a randomized, double-blind, placebo-controlled phase III study. The study design is summarized in Supplementary Fig. S1, and full details have been reported elsewhere (9, 24). Briefly, patients were eligible for the study if they had confirmed objective failure of previous imatinib therapy and histologically proven malignant GIST that was not amenable to surgery, radiation, or other multimodal approaches. Participants were randomized in a 2:1 ratio to receive treatment in repeated 6-wk cycles consisting of 4 wk of daily sunitinib (50 mg/d) or placebo followed by 2 wk off treatment. The primary study end point was TTP using Response Evaluation Criteria in Solid Tumors (25). At the time of documented disease progression, treatment assignments were unblinded and patients receiving sunitinib were given the opportunity to continue treatment. Patients receiving placebo were given the opportunity to cross over to sunitinib treatment, provided they met the eligibility criteria [evidence of radiographically documented progression and Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2].

Plasma samples for sKIT measurement were obtained before study drug administration on days 1, 14, and 28 of cycle 1 and on days 1 and 28 of subsequent cycles; heparin was used as the anticoagulant for plasma collection. Plasma samples were diluted 1:50 before testing, and sKIT levels were analyzed under Good Laboratory Practice conditions using a quantitative, performance-validated sandwich ELISA (research-use only; DuoSet, R&D Systems) at Alta Analytical Laboratory. The sKIT ELISA was validated against recombinant protein consisting of the full-length extracellular domain of KIT. Plasma sKIT concentrations were determined using a seven-point standard curve based on dilutions of recombinant KIT extracellular domain, and limits of detection and reproducibility of the assay were established using quality control samples prepared in pooled sodium heparin from healthy donors.

**Translational Relevance**

Based on robust statistical analysis of samples obtained in a randomized, double-blind, placebo-controlled phase III study of sunitinib in patients with gastrointestinal stromal tumor (GIST), our results suggest that circulating levels of soluble KIT extracellular domain (sKIT) seem to function as a surrogate marker for clinical outcome [time to tumor progression (TTP)], with decreases in sKIT levels being predictive of longer TTP. Assays of sKIT, an easy molecule to detect, consequently have the potential to facilitate and expedite future clinical trials in GIST pending confirmation in prospective studies. Additionally, sKIT assays may be useful in monitoring response in patients with GIST, as well as in identifying patients who may benefit from treatment with sunitinib.

RET), colony-stimulating factor-1 receptor, and FMS-like tyrosine kinase 3 (4–8). Sunitinib exhibits direct antitumor effects on GIST cells via inhibition of KIT and PDGFR-α and may also produce antiangiogenic effects via inhibition of VEGF receptors and PDGFR-β on stromal cells and blood vessels. An interim analysis of a large, double-blind, phase III study showed a significant clinical benefit for sunitinib over placebo in patients with advanced GIST who were resistant to or intolerant of imatinib (9). The median time to tumor progression (TTP; the primary end point of the study) in patients randomized to receive sunitinib (50 mg/d in 6-week cycles consisting of 4 weeks on and 2 weeks off treatment) was 27.3 weeks versus 6.4 weeks in patients randomized to receive placebo ($P < 0.0001$). On the basis of the positive results of this phase III study, sunitinib was approved multinationaly for the treatment of GIST after failure of imatinib (10, 11).

Surrogate end points of clinical outcomes, such as those based on tumor imaging or relevant molecular biomarkers, have generated widespread interest due to their potential to yield information more quickly and to be measured more easily than many traditional end points used in oncology studies. A valid surrogate marker would allow convenient tracking of disease progression or remission and earlier assessments of the utility of new therapies in patients who may derive benefit from an alternative treatment. To be valid, a surrogate marker must correlate with the clinical outcome of interest and accurately capture the effect of an intervention on that outcome (12). In addition, an ideal surrogate marker would be measured using a clearly defined, reproducible method that is preferably easy to perform.

The pathophysiologic association between the KIT receptor tyrosine kinase and GIST has led to the exploration of KIT protein detection as a surrogate marker for response to therapy. A soluble fragment of KIT (sKIT) consisting of the 98-kDa extracellular domain is generated by proteolytic cleavage, potentially via tumor necrosis factor-α converting enzyme activity (13, 14). As this KIT protein fragment is readily detectable in the serum and plasma of healthy individuals as well as patients with various pathologic conditions (15–20), sKIT is an attractive surrogate marker candidate. Indeed, earlier preliminary reports suggested that a decline in plasma sKIT levels in GIST patients might correlate with responses to imatinib (21, 22) and sunitinib (23). In the present analysis, using samples obtained in the phase III study of sunitinib in GIST patients after imatinib failure (9), we evaluated changes in plasma levels of sKIT as a potential surrogate marker for clinical outcome in GIST.
Results

As previously reported (24), 361 patients ultimately enrolled in the phase III study and were randomized to receive blinded sunitinib \((n = 243)\) or placebo \((n = 118)\) treatment (Supplementary Fig. S1). Baseline characteristics for all patients are shown in Table 1. The numbers of patients with matched pairs of baseline and on-study plasma samples for sKIT measurement are shown in Table 2 for both treatment groups at various time points.

Effect of sunitinib on plasma levels of sKIT relative to baseline. The effect of sunitinib or placebo administration on plasma sKIT levels relative to baseline at various time points is shown for individual patients in Fig. 1A and B and for all patients by treatment group in Fig. 1C. There was no significant difference between sKIT levels in the two treatment groups at baseline \((P = 0.265)\); however, following treatment initiation, sKIT levels for patients in the sunitinib group decreased from baseline, whereas levels for those in the placebo group increased from baseline. This was already apparent as a trend at the first post-baseline time point \([cycle \ 1 \ day \ 14 \ (C1; \ D14); \ P = 0.112]\) despite the very modest difference in median sKIT levels between the treatment groups \((5.8\%)\). Significant differences were seen between the treatment groups at all subsequent time points \((C1; \ D28, \ C2; \ D1, \ C2; \ D28, \ C3; \ D1, \ and \ C3; \ D28; \ P < 0.0001)\).

Relationship between changes in sKIT levels and TTP. Analysis of data from C2;D28 and C3;D1 suggested a predictive relationship between decreases in sKIT levels relative to baseline and improved clinical outcome (longer TTP). In the sunitinib group, 75.8% and 82.5% of patients at C2;D28 and C3;D1, respectively, had reduced sKIT levels relative to baseline compared with only 7.3% and 11.9% of patients in the placebo group at C2;D28 and C3;D1 \((Table \ 3A \ and \ B, \ upper \ tables)\). The majority of patients with increased sKIT levels relative to baseline had a poor outcome \((TTP < 6 \ months)\) irrespective of treatment \((Table \ 3A \ and \ B, \ lower \ left \ tables)\), whereas for patients with reduced sKIT levels relative to baseline, a higher proportion of patients in the sunitinib group had an improved outcome \((TTP ≥ 6 \ months)\) compared with the placebo group \((66.4\% \ versus \ 0\%\) at C2;D28 and 74.0% versus 20.0% at C3;D1; \ Table 3A \ and \ B, lower right tables)\).

Changes in plasma sKIT levels from baseline seemed to predict clinical outcome in terms of TTP irrespective of treatment group \((Fig. \ 2)\). At C1;D28, C2;D28, and C3;D1, patients with reduced sKIT levels had median TTPs of 29.1, 34.3, and 41.0 weeks, respectively, compared with 10.1, 16.0, and 16.1 weeks for patients with increased sKIT levels, respectively. The hazard ratios \((HRs)\) for these comparisons were 0.74, 0.71, and 0.65, respectively \((P < 0.0001 \ for \ each)\).

Estimated proportion of the treatment effect explained by sKIT. The Cox proportional hazards model showed a statistically significant effect of treatment on TTP at both C2;D28 \((P = 0.004; HR, 0.58; 95\% \ CI, 0.40-0.84; \alpha = 0.55)\) and C3;D1 \((P = 0.0004; HR, 0.50; 95\% \ CI, 0.34-0.73; \alpha = 0.69)\). However, when the model was altered to adjust for sKIT, treatment became insignificant as a predictive factor, as illustrated by P values above the level of significance and the upper limits of the 95% CIs of the HRs exceeding 1.0 \((C2;D28: P = 0.606; HR, 0.90; 95\% \ CI, 0.59-1.35; \alpha = 0.11; \ C3;D1: P = 0.965; HR, 0.99; 95\% \ CI, 0.60-1.63; \alpha = 0.01). The Cox model also showed that sKIT changes from baseline were significant as a predictive factor of TTP, based on P values well below the level of significance and the upper limits of the 95% CIs of the HRs falling below 1.0 \((C2;D28: P < 0.0001; HR, 0.51; 95\% \ CI, 0.39-0.67; \beta = 0.67; C3;D1: P < 0.0001; HR, 0.38; 95\% \ CI, 0.25-0.58; \beta = 0.96)."

The calculated PTE \(1 - \alpha^2/\alpha^1\) was 0.80 \((95\% \ CI, 0.34-3.70)\) for the C2;D28 time point and 0.98 \((95\% \ CI, 0.39-3.40)\) for the C3;D1 time point. Thus, 80% and 98%, respectively, of the treatment effect was explained by sKIT, and in both cases, the lower bound of the 95% CI for PTE was greater than zero, indicating a statistically significant contribution by sKIT to the treatment difference in TTP. PTEs were less than 0.50 at all other time points, although the lower bounds of the 95% CIs for the PTEs were all still greater than zero \(\text{(data not shown).}\)

Relationship between changes in sKIT levels and overall survival. In addition to TTP, decreases in sKIT levels relative to baseline seemed to predict overall survival \((OS)\), a secondary end point of the phase III study, for the cohort of patients randomized to the sunitinib arm \((Fig. \ 3)\; analysis of OS in the placebo cohort was not pursued due to the confounding factor of crossover to sunitinib by the majority of placebo patients at the time of initial progression). The median OS for patients in the sunitinib arm with reduced sKIT levels at C1;D28, C2;D28, and C3;D1 was 86.0, 99.3, and 92.4 weeks, respectively, compared with 44.3, 40.3, and 41.4 weeks for patients with increased sKIT levels at those time points, respectively \(\text{[P} = 0.0001 \ (HR, 0.72), \ P < 0.0001 \ (HR, 0.63), \text{ and } P = 0.0016 \ (HR, 0.67), \text{ respectively}.}\)

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sunitinib ((n = 243))</th>
<th>Placebo ((n = 118))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td>Median</td>
<td>23-84</td>
<td>23-81</td>
</tr>
<tr>
<td>Range</td>
<td>Male</td>
<td>152 (63)</td>
</tr>
<tr>
<td>Female</td>
<td>91 (37)</td>
<td>47 (40)</td>
</tr>
<tr>
<td>ECOG PS, n (%)</td>
<td>0</td>
<td>109 (45)</td>
</tr>
<tr>
<td>1</td>
<td>131 (54)</td>
<td>63 (53)</td>
</tr>
<tr>
<td>2</td>
<td>3 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Time since original diagnosis, y</td>
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<td>3.3</td>
</tr>
<tr>
<td>Median</td>
<td>0.2-26.8</td>
<td>0.2-16.4</td>
</tr>
<tr>
<td>Range</td>
<td>Tumor burden, mm</td>
<td>227</td>
</tr>
<tr>
<td>Median</td>
<td>18-722</td>
<td>29-749</td>
</tr>
<tr>
<td>Range</td>
<td>Previous imatinib therapy</td>
<td>Maximum daily dose, mg</td>
</tr>
<tr>
<td>Median</td>
<td>300-1,600</td>
<td>400-1,600</td>
</tr>
<tr>
<td>Range</td>
<td>Cumulative treatment duration, wk</td>
<td>107</td>
</tr>
<tr>
<td>Median</td>
<td>0.3-206</td>
<td>11-231</td>
</tr>
<tr>
<td>Range</td>
<td>Treatment outcome, n (%)</td>
<td>Progression ≤ 6 mo</td>
</tr>
<tr>
<td>Intolerance</td>
<td>Progression &gt;6 mo</td>
<td>188 (77)</td>
</tr>
<tr>
<td>Intolerance</td>
<td></td>
<td>13 (5)</td>
</tr>
</tbody>
</table>

*Sum of the longest diameters of target lesions.
Relationship between baseline and disease factors and clinical outcome. The relationships between a variety of baseline and disease characteristics (age, sex, ECOG PS, time since diagnosis, prior imatinib treatment history, sKIT, and tumor burden) and TTP were evaluated in univariate analyses for each treatment arm separately and for the entire population (Supplementary Table S1). Comparable analyses of OS were done only for the sunitinib arm as described above.

Among baseline characteristics, only ECOG PS and imatinib treatment outcome [resistance (progressive disease) versus intolerance] showed significant correlations with both TTP and OS across multiple analysis sets. Additionally, longer time since original diagnosis, maximum prior imatinib dose < 800 mg/d, and lower baseline tumor burden (the sum of the longest diameters of target lesions) correlated significantly with longer OS in the sunitinib arm. In contrast, no association between baseline sKIT and either TTP or OS was seen. However, sKIT reductions from baseline showed significant correlations with longer TTP and OS in the sunitinib arm as early as the 2nd week of treatment. Reductions in tumor burden from baseline also correlated significantly with longer TTP across all groups and for OS in the sunitinib group as early as the first assessment at 4 weeks.

Among all of the baseline factors tested in univariate analyses, those that correlated significantly with TTP or OS in multivariate analyses are shown in Supplementary Table S2. For all of the patients in the study, baseline age, ECOG PS, and time since original diagnosis had a significant effect on TTP in addition to treatment. Among patients in the sunitinib arm, baseline ECOG PS, time since diagnosis, maximum prior imatinib dose, and tumor burden had a significant effect on OS. These analyses showed that, with respect to prognostic factors, the patients in this study behaved in a manner broadly similar to that seen in other studies involving patients with advanced GIST (28, 29).

Discussion

Surrogate markers of clinical activity have attracted widespread interest in oncology studies due to their potential to provide information on clinical outcomes earlier and with fewer patients than many traditional end points. The compelling pathophysiologic and etiologic association between aberrant KIT expression and activity in the majority of GIST cases is well documented. This oncogenic mechanism provides a clear rationale for the investigation of sKIT as a surrogate marker for clinical outcome in GIST patients. Using samples collected in a double-blind, placebo-controlled phase III study of sunitinib in patients with advanced GIST following imatinib failure (9, 24), the results of the present analysis suggest that after two cycles of sunitinib treatment, circulating sKIT levels seem to function as a surrogate marker for clinical outcome (TTP).

As mentioned earlier, a surrogate marker must not only be predictive of changes in the clinical end point but must also accurately capture the effect of the intervention on that outcome (12). In 1989, Prentice outlined how potential surrogate end points might be validated by determining their compliance with the four criteria described earlier (26). Whereas it is difficult to determine whether a marker can substitute for the clinical end point, it is still desirable to quantify the PTE explained by the surrogate relative to the overall treatment effect. Freedman et al. (27) proposed estimating the PTE explained by the surrogate in terms of a ratio of estimated parameters from two models: a model with and a model without the surrogate adjustment. These criteria and PTE were used in the present analysis to establish the validity of sKIT as a surrogate marker for treatment efficacy.

Compliance with the four Prentice criteria is believed to show rigorously that a surrogate marker provides a valid inference for clinical outcome. In this case, the first criterion (that the treatment must have a significant effect on the clinical end point) was fulfilled based on the previously published phase III study results: Median TTP was significantly longer in patients receiving sunitinib (27.3 weeks) than in those receiving placebo (6.4 weeks; \( P < 0.0001 \); ref. 9). The second Prentice criterion (that the treatment must have a significant effect on the surrogate marker) was fulfilled by the results of the present analysis: Median sKIT levels for patients in the placebo group increased from baseline whereas, conversely, median levels for those in the sunitinib group decreased from baseline, and the difference between the treatment groups became statistically significant (\( P < 0.0001 \)) after 4 weeks of treatment. Notably, sKIT levels in the sunitinib group declined continuously throughout the sampling period, even during the 2 weeks off treatment at the end of each cycle. This differed from the pattern typically observed with VEGF pathway proteins such as VEGF and soluble VEGF receptor 2, which are modulated during sunitinib treatment in a cyclical manner that coincides with the off-treatment period (30, 31).

The present analysis also showed that decreases in sKIT levels relative to baseline were a significant predictor of longer TTP. Patients with reduced sKIT levels at the end of the second treatment cycle (C2;D28) had a median TTP of 34.3 weeks compared with 16.0 weeks for patients with increased sKIT levels (HR, 0.71; 95% CI, 0.61-0.83; \( P < 0.0001 \)). Using Cox proportional hazards models and PTE to establish the degree of
validity of sKIT as a surrogate marker (a perfect surrogate has a PTE value of one), changes in sKIT levels (assessed as a continuous variable) were found to replace treatment as a stronger predictor of TTP. Eighty percent of the treatment effect was explained by sKIT (PTE = 0.80; 95% CI, 0.34-3.70), which was found to be a statistically significant contribution (based on the lower bound of the CI for PTE exceeding zero). An even higher degree of surrogacy was shown at the beginning of the third treatment cycle (PTE = 0.98; 95% CI, 0.39-3.40). Moreover, no interaction between treatment and sKIT changes was detected, thus justifying the validity of applying PTE analysis.

Thus, the results fulfill the third and, in part, the fourth Prentice criteria (that the surrogate marker must have a significant effect on the clinical end point and the full treatment effect on the clinical end point must be accurately captured by the surrogate, respectively). However, the fourth Prentice criterion cannot be fully satisfied because the effect of any change in the surrogate marker on the clinical end point would have to be affirmed for every mechanism known (and unknown) to affect the surrogate marker (32). The mechanism of action of sunitinib on sKIT and its clinical outcome are complex and not fully understood; the treatment may affect the disease process through multiple pathways.

The data also suggested that decreases in sKIT levels relative to baseline are a significant predictor of longer OS in addition to TTP, based on the results obtained with patients in the sunitinib arm (~2-fold longer median OS at C1;D28, C2;D28, and C3;D28; HR, 0.72, P = 0.0001; HR, 0.63, P < 0.0001; and HR, 0.67, P = 0.0016; respectively). However, the crossover design of the study limited the utility of survival analysis of the placebo cohort and precluded the type of surrogacy analysis that was done for TTP.

Because a high degree of validity of sKIT as a surrogate marker for TTP was found only after two cycles of treatment, which is when many patients receive their first on-treatment radiological scan in routine clinical practice, for comparison, we also evaluated tumor burden change from baseline as a predictor of clinical outcome. Whereas reduced tumor burden was highly significantly associated with prolonged TTP in univariate analyses particularly at C1;D28 and C2;D28 (P < 0.0001; Supplementary Table S1), a surrogacy relationship could not be established because of the appearance of significant interactions between tumor burden changes and treatment. This is not particularly surprising because tumor burden changes and TTP are not independent end points, and it is suggestive of sKIT being a better predictor of outcome than changes in tumor size at early time points. When tumor burden change was assessed as a binary variable in Kaplan-Meier analysis of the sunitinib arm as was done for sKIT change, reduced tumor burden correlated significantly with prolonged OS at C1;D28 and C2;D28 [HR, 0.82 (P = 0.015) and 0.72 (P = 0.0005), respectively; Supplementary Fig. S2]. However, a full comparison of sKIT change and tumor burden change as predictors of OS would require extensive further investigation.

The results reported here establish the validity of sKIT as a surrogate marker by showing that it fulfills the rigorous Prentice criteria and explains most of the treatment effect through multivariate analysis with sKIT change as a continuous variable, in which sKIT reductions were strongly associated with better clinical outcome. However, when sKIT change was analyzed as a binary variable (which was done solely for the purpose of illustration), some discrepancies became apparent. For example, when patients were stratified based on absolute increases versus decreases in sKIT from baseline, 7% and 12% of patients in the placebo arm were classified as having meaningful sKIT decreases at C2;D28 and C1;D3, respectively. Part of this may be due to the somewhat arbitrary nature of the cutoff point used (i.e., an sKIT ratio to baseline of exactly 1.0). For example, with a cutoff point of 0.9 (reflecting a more substantial decrease in sKIT: ≥10%), <5% of patients in the placebo arm would have been characterized as having meaningful sKIT decreases at either time point (data not shown). Likewise, some patients with absolute sKIT increases
open questions. Another question that remains is not perfect and presumably will not be able to predict clinical outcome in every individual. Another question that remains open is whether KIT/PDGFRA genotype affects sKIT response. Unfortunately, kinase genotyping was not an objective of this study, and the limited amount of tumor tissue samples collected precluded thorough analysis of this parameter. Further studies will be needed to confirm our results prospectively, to optimize the assay for routine clinical use, and to resolve such open questions.

Previous reports have suggested that decreases in sKIT levels in GIST patients might correlate with responses to imatinib (21, 22) and sunitinib (23). Bono et al. (22) showed that patients with GIST (n = 66) had elevated pretreatment serum sKIT levels as compared with a control group (n = 40), with median sKIT levels being 292 arbitrary units/mL (409 ng/mL) and 238 arbitrary units/mL (333 ng/mL), respectively (P = 0.037). Imatinib treatment reduced serum KIT levels by an average of 31% and 52% from pretreatment levels at 1 and 6 months after initiation of treatment, respectively. No significant difference was observed in the decline in sKIT levels between responding and nonresponding patients, and sKIT levels did not increase in the patients who progressed during therapy. However, in this series, the number of patients considered to be nonresponders was very low (n = 9, compared with n = 57 responders), as imatinib is effective in most GIST patients when used as first-line therapy. Thus, the small sample size could have been a factor in the lack of an observed correlation between sKIT changes and imatinib response. Indeed, a similar analysis carried out in the present study showed that the percentage of responders was markedly higher and the percentage of progressing patients markedly lower among patients with sKIT decreases than those with sKIT increases (P < 0.001 in χ² analyses of all patients at C1;D28 and C2;D28; Supplementary Fig. S3). In addition, the patient population in the sunitinib phase III study was distinct from that in the imatinib study: Most of the patients in the sunitinib study had received imatinib treatment for at least 6 months before initiation of sunitinib treatment (9, 24).

In a report of preliminary results from an earlier phase I/II study of sunitinib in GIST patients (23), our investigative team showed that plasma sKIT levels declined over time during sunitinib treatment, and that the sKIT decline correlated with both 18F-fluorodeoxyglucose positron emission tomographic response and objective response. The majority of patients with a partial response or stable disease had a decrease in sKIT of at least 30% from baseline level at any time point (11 of 12 patients), whereas more patients with progressive disease had an increase in sKIT of at least 30% from baseline level at any time point (5 of 12 patients with progressive disease versus 3 of 12 patients with partial response or stable disease). Subsequent analysis of a larger number of patients from the phase I/II study established that this trend was still apparent (data not shown). More recently, we showed that sKIT reductions.

### Table 3. Correlation between sKIT status, treatment group, and TTP

| Table 3. Correlation between sKIT status, treatment group, and TTP |
|-----------------------|-------------------|-------------------|-------------------|
| **(A) C2;D28** |
| **All patients* (n = 194)** |
| **sKIT** | **Sunitinib, n (%)** | **Placebo, n (%)** |
| sKIT ↑ (n = 75) | 37 (24.2) | 38 (92.7) |
| sKIT ↓ (n = 119) | 116 (75.8) | 3 (7.3) |
| **TTP (mo)** | **Sunitinib, n (%)** | **Placebo, n (%)** |
| <6 | 22 (59.5) | 39 (33.6) |
| ≥6 | 15 (40.5) | 77 (66.4) |
| **(B) C3;D1** |
| **All patients* (n = 168)** |
| **sKIT** | **Sunitinib, n (%)** | **Placebo, n (%)** |
| sKIT ↑ (n = 59) | 22 (17.5) | 37 (88.1) |
| sKIT ↓ (n = 109) | 104 (82.5) | 5 (11.9) |
| **TTP (mo)** | **Sunitinib, n (%)** | **Placebo, n (%)** |
| <6 | 13 (59.1) | 27 (26.0) |
| ≥6 | 9 (40.9) | 77 (74.0) |

**NOTE:** ↑, increase relative to baseline; ↓, decrease relative to baseline.
*With matched pairs of baseline and on-study plasma samples for sKIT at the indicated time point.
from baseline correlate significantly with prolonged OS in patients with imatinib-resistant/intolerant GIST after 20 and 24 weeks of sunitinib treatment administered at 37.5 mg/d using a continuous daily dosing schedule in a phase II study (n = 17, P = 0.011 and n = 16, P = 0.002; respectively; ref. 33). The results of the present analysis—using data collected in a large, randomized, double-blind, placebo-controlled study—corroborate the results from both phase I/II and phase II studies, and it can be hypothesized that the sunitinib-related decreases in sKIT may represent, at least in part, a decrease in the number of

Fig. 2. TTP by sKIT status (irrespective of treatment group). A, C1;D28; B, C2;D28; C, C3;D1.

Fig. 3. OS by sKIT status for patients randomized to the sunitinib group. A, C1;D28; B, C2;D28; C, C3;D1.
viable tumor cells in GIST patients because GISTs are KIT positive in most cases. Furthermore, the increasing sKIT levels observed in the placebo cohort (presented in detail in Supplementary Table S3) indicate that sKIT levels increase in the absence of treatment. This elevation in plasma sKIT was highly significant as early as C:1;D14 ($P = 0.0022$) despite being modest in magnitude at this time point (6.3%), with a median increase of 48.3% by C;D28 ($P = 0.0006$). These results suggest that longitudinal sKIT increases may be a feature of GIST natural history and may, to some extent, reflect disease burden. Indeed, baseline tumor burden was found to correlate significantly with baseline sKIT in a linear regression analysis ($n = 306, P < 0.0001$; data not shown). However, only a small portion of the variance in baseline sKIT could be explained by the variance in baseline tumor burden ($R^2 = 0.082$), likely due to the relatively large component of physiologic sKIT that is unrelated to GIST, as evidenced by the levels found in normal human blood (22), as described above. This may also be influenced by limitations of the sensitivity of Response Evaluation Criteria in Solid Tumor in estimating true tumor burden in GIST, which may be confounded by qualitative changes in tumor tissue, such as myxoid degeneration or necrosis (34, 35).

Nevertheless, the fact that Bono et al. (22) found no significant difference in the decline in sKIT levels between responding and nonresponding imatinib-treated patients suggests that further study is warranted. The decrease in sKIT levels observed with imatinib was postulated to reflect a pharmacodynamic effect due to generalized inhibition of KIT signaling in the various compartments in which KIT is expressed, rather than being an indicator of GIST burden. Indeed, a similar mechanism of pharmacodynamic sKIT reduction may be in effect for sunitinib because sunitinib treatment is associated with longitudinal sKIT decreases in patients with tumor types in which tumor cell KIT expression is not considered to be a common feature, such as renal cell carcinoma (36), breast cancer (37), and hepatocellular carcinoma (38). The mechanism(s) driving these changes in circulating sKIT is not currently understood, but could involve changes in receptor synthesis, turnover, proteolytic cleavage, or a combination of processes. In cell culture and in vitro cellular systems, cell-surface shedding of KIT can be regulated through cleavage by neutrophil and macrophage proteases (39) and by tumor necrosis factor-α converting enzyme (14), although physiologic mechanisms are likely to be more complex in vivo. Nevertheless, the results from analysis of the phase III GIST data indicate that sKIT levels increase over time in untreated GIST patients as well as during sunitinib treatment in some patients, and that this increase correlates with shorter TTP and OS, suggesting that at least some of the changes in sKIT are due to an effect on tumor cells. Furthermore, the changes in sKIT levels did not correlate with sunitinib trough drug levels (data not shown), implying that the sKIT reduction is not simply reflective of higher drug exposure in some patients.

In conclusion, the results of the present analysis suggest that, after two cycles of sunitinib treatment, circulating sKIT levels seem to function as a surrogate marker for clinical outcome (TTP) in GIST patients. This conclusion was based on the findings that sunitinib treatment was associated with a decrease in sKIT levels from baseline and that decreases in sKIT levels were a significant predictor of improved clinical outcome. Due to its ease of detection, sKIT is an attractive surrogate marker candidate. However, although the present results are promising, further studies are warranted as mentioned above: The findings need to be confirmed prospectively and the methodology has to be further refined. It must also be determined whether sKIT is a general surrogate (i.e., prognostic) marker of clinical outcome in GIST patients or a predictive marker specific to treatment with KIT inhibitors. Finally, studies are needed to evaluate sKIT as a biomarker in other tumors that have a pathobiology linked to KIT overexpression [e.g., small cell lung cancer (40, 41), basal-like breast cancer (42), germ cell tumors (43), and melanoma (44)]. Such studies will help determine whether sKIT will be useful in monitoring response in patients with GIST or other KIT-positive tumor types, as well as in identifying patients who may benefit from treatment with sunitinib.

Disclosure of Potential Conflicts of Interest
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