Clinical Cancer Research

Exploratory Analysis of Early Toxicity of Sunitinib in Advanced Hepatocellular Carcinoma Patients: Kinetics and Potential Biomarker Value

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Abstract

Purpose: Sunitinib—a multitargeted tyrosine kinase inhibitor—can modulate circulating inflammatory factors in cancer patients that may be relevant for hepatocellular carcinoma (HCC) progression. However, a recent phase III study of sunitinib in HCC was halted due to its toxicity. Here, we studied the early kinetics of adverse events after sunitinib, and explored their association with circulating proteins and clinical outcome in advanced HCC in a single-arm phase II study.

Experimental Design: Toxicity was evaluated every two weeks during the first cycle of therapy. Biomarker changes from baseline were tested after adjusting for multiple comparisons. Correlation between toxicities and overall survival (OS) or time-to-tumor progression (TTP) was evaluated in a Cox model using log-transformed levels or change in biomarkers, after stratifying by stage and adjusting for baseline level.

Results: Myeloid and lymphoid blood cell counts decreased by 20% to 50% after sunitinib treatment ($P < 0.05$ for all). The extent of the early decrease in neutrophils and monocytes, and the development of nonhematologic toxicities (i.e., skin toxicities), were significantly associated with both OS and TTP ($P < 0.05$). Changes in circulating cells significantly associated with specific changes in plasma biomarkers (i.e., changes in platelets with changes in VEGF-C and soluble-VEGFR3; changes in neutrophils with changes in IL-8, TNF-α, and soluble-VEGFR2).

Conclusions: The adverse effects of sunitinib, particularly on the hematopoietic system, may be rapid and appear directly related to its activity in HCC. This exploratory study suggests that early hematopoietic toxicities may potentially predict outcome in advanced HCC after sunitinib treatment. *Clin Cancer Res; 17(4); 918–27. ©2010 AACR.*

Introduction

Sorafenib—a multitargeted tyrosine kinase inhibitor (TKI) with anti–vascular endothelial growth factor receptor (VEGFR) activity—has shown an improvement in overall survival (OS) in patients with advanced hepatocellular carcinoma (HCC) in 2 randomized phase III studies (1, 2). This has been the first effective systemic therapy for HCC, and its approval in many countries worldwide has set the new standard for clinical trials in advanced HCC. Moreover, it has greatly stimulated evaluation of molecularly targeted agents in this disease, particularly antiangiogenic agents.

Sunitinib, a drug with proven efficacy against advanced renal cell carcinomas (RCC) and gastrointestinal stromal tumors (GIST), is a multitargeted TKI with partially overlapping target inhibition profile with sorafenib (3). Sunitinib has shown early evidence of antitumor activity in single arm phase II studies using different dose/schedule regimens (4–6). However, continuous sunitinib treatment showed a higher incidence of serious adverse events in advanced HCC compared with sorafenib—leading to early stoppage—and did not meet the criteria to show that it was either superior or noninferior to sorafenib in a randomized phase III trial (SUN 1170).

Thus, clinical development of antiangiogenic TKIs in HCC is associated with challenging safety and toxicity

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Translational Relevance

The anti–vascular endothelial growth factor receptor tyrosine kinase inhibitor (VEGFR TKI) sorafenib has been the first systemic agent to show overall survival benefits in advanced hepatocellular carcinoma (HCC) and is a current standard of care. The recent failure of sunitinib (a more potent anti-VEGFR TKI) in a phase III trial versus sorafenib was, in part, due to its toxicity. Therefore, understanding the mechanisms of action of TKIs in HCC has become even more critical for further progress in HCC therapy. However, there are no confirmed mechanisms of action or biomarkers of efficacy and toxicity for any anti-VEGFR TKI. The results of our analysis strongly support the hypothesis that the effect of sunitinib on the immune system is related to its activity in HCC patients. Moreover, this exploratory study suggests that early hematopoietic toxicities may predict progression and survival outcomes in advanced HCC after sunitinib treatment. Although translation of this concept in clinical practice will require further understanding of these effects and validation in large studies of sorafenib and other TKIs, these results open new avenues for clinical and preclinical research in this area.

concerns. First, although the assumption has been that molecularly targeted agents will be generally safe, they can have unique and sometimes debilitating toxicities. In the case of sorafenib, grade 1–3 hand and foot skin reactions (HFSR), diarrhea, and fatigue are experienced by a significant percentage of patients (1, 2). These adverse events can lead to treatment discontinuation if not managed early and effectively. Second, as there are no established clinical or laboratory tests to predict the risk of developing these toxicities, their development is unpredictable. Third, despite the recognition that some of these side effects, e.g., hypertension, represent pharmacodynamic markers, it remains unclear if any of these toxicities are predictive of clinical outcomes across disease types. Fourth, the molecular mechanisms mediating most of these adverse events remain undetermined. Lastly, it is increasingly appreciated that the same targeted agents given at similar dose schedules can have profoundly different tolerability and safety profiles in HCC compared with other tumor types, due to the presence of underlying cirrhosis. This is particularly relevant for toxicities associated with upper gastrointestinal (GI) bleeding, hepatic toxicity, and myelosuppression.

Currently, multiple agents that target the VEGF pathway are being evaluated in HCC, including phase II studies of antibodies against VEGF (bevacizumab) or VEGFR2 (ramucirumab), and TKIs with activity against VEGFRs (brivanib, cediranib, and ABT-869; refs. 7–10). Some side effects may be common across agents in this class (e.g., hypertension), whereas others might be more specific to each agent, especially for multitargeted agents such as sunitinib. Interestingly, in other tumor types, the severity of side effects has been linked with efficacy of some targeted agents, e.g., hypertension for anti-VEGF agents or skin rash for sorafenib in HCC and for epidermal growth factor receptor (EGFR) inhibitors in colorectal cancer (11–13).

In advanced HCC patients, frequent adverse effects of sunitinib include skin toxicities, fatigue, nausea, vomiting, and hematologic side effects (i.e., myelo- and lymphosuppression; ref. 5). For example, the widely applied sunitinib dosing schedule of 50 mg daily for 4 consecutive weeks followed by 2 weeks off (approved for RCC and GIST patients) has produced severe neutropenia and even death in HCC patients enrolled in a phase II study (4). In contrast to their effect on myelosuppression, sunitinib, sorafenib, and other anti-VEGFR TKIs can induce erythrocytosis in cancer patients (14, 15). This stimulatory effect may be due to erythropoietin (EPO) production by the liver in response to the VEGF blockade, as shown in preclinical models (16, 17). These findings highlight the importance of carefully characterizing the toxicity profiles of these targeted agents in HCC and exploring potential mechanisms and predictive biomarkers for these toxicities.

We previously reported efficacy data from a single-arm phase II study of sunitinib (37.5 mg daily for 4 weeks followed by 2 weeks break) in patients with advanced HCC (6). Of interest, biomarker evaluations showed that sunitinib significantly decreased the number of circulating hematopoietic progenitor/stem cells (CPC), and that modulation of circulating inflammatory factors (e.g., IL-6) might be associated with survival outcomes after therapy. Here, in a phase II study of sunitinib, we tested the hypothesis that sunitinib has rapid effects on hematopoiesis and that changes in circulating immune cells correlate with clinical outcome or with circulating biomarkers in advanced HCC.

Materials and Methods

Patients

The trial was approved by the Institutional Review Board (IRB) at Dana-Farber/Harvard Cancer Center (Boston, USA). All patients provided written informed consent before study participation. The inclusion and exclusion criteria have been detailed in previous report (6). Of the 34 patients enrolled, 5 were women (15%) and 29 men (85%) with a median age of 64 years (range, 30–82 years); 29 had Barcelona Clinic Liver Cancer (BCLC) stage C (85%) and 5 had BCLC stage B (15%). Cancer of the Liver Italian Program (CLIP) score was 1 in 13 patients (38%), 2 in 12 patients (35%), and 3 in 9 patients (27%). The majority of patients ($n = 28$, 82%) had no previous systemic treatments (6). After sunitinib treatment, the median time-to-tumor progression (TTP) was 4.1 months and the median OS was 9.8 months (6).

Evaluation of the kinetics of side effects

Patients were seen in the clinic every 2 weeks whereas on study and toxicity was graded according to the NCI CTCAE Version 3.0. We evaluated the kinetics of hematologic and

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nonhematologic adverse effects at days 14, 28, and 42 (i.e., after a 2-week rest period) after initiation of sunitinib treatment.

Evaluation of the kinetics of circulating biomarkers

For measurement of EPO, angiogenic proteins, and inflammatory cytokines in plasma, peripheral blood was obtained at baseline and 14, 28, and 42 days after the first dose of sunitinib. All samples were collected in EDTA-containing vacutainers. Plasma analysis was carried out for a panel of circulating angiogenic and inflammatory factors: VEGF, placental-derived growth factor (PlGF), soluble VEGFR1, basic fibroblast growth factor, interleukin (IL)-1β, IL-6, IL-8, and TNF-α using multiplex ELISA plates from Meso-Scale Discovery, and soluble VEGFR2, soluble VEGFR3, stromal-derived factor 1α, VEGF-C, and soluble c-KIT from R&D Systems (6). Samples were run in duplicate. For enumeration of $CD34^+VEGFR2^+CD45^+$ cells, blood cells from fresh samples were immunostained with CD34-APC, CD45-PerCP, and VEGFR2-PE antibodies (BD Pharmingen) using a standard flow cytometry protocol (18). The quantitative analysis endpoint was the change in the fraction of $CD34^+VEGFR2^+CD45^+$ circulating cells among blood mononuclear cells 14 and 28 days after sunitinib treatment. Complete blood counts (CBC) were obtained before treatment, at day 14 and after each cycle of treatment.

Statistical analysis

The study strived to comply with the recommendations of the REMARK statement for tumor biomarkers (19). Biomarker level changes from baseline were expressed as ratios of the on-study to baseline levels and tested using the exact paired Wilcoxon test. Missing measurements (due to lack of samples for certain time points or patient dropout) were excluded from analysis. *P* values were not adjusted for tests performed on distinct biomarkers, as our interest was in separate comparisons for each biomarker. However, we adjusted *P* values for multiple comparisons over time, using the false discovery rate control method of Genovese and colleagues (20), with weights proportional to the square root of the number of data. The association of hematologic toxicities with OS and TTP was evaluated in a Cox model using log-transformed levels of biomarkers or biomarker changes after stratifying by baseline BCLC or CLIP score. In analyses of biomarker changes over time, we adjusted for baseline levels. In addition, we performed multivariate Cox analysis for hematologic toxicities, adjusting for actual dose of sunitinib received, stratifying for BCLC stage. Moreover, we performed a multivariate Cox analysis of hematocrit and counts of neutrophils, lymphocytes, and platelets, after stratifying for BCLC stage (see Supplementary Note for details). The association of nonhematologic (all grades and grade ≥ 2) was similarly evaluated after stratifying for baseline BCLC and CLIP score, respectively. Correlation of hematologic toxicity biomarkers with circulating cytokine biomarkers was tested using Kendall's τ_β coefficients (21). For 15 of 34 patients, the dose was either reduced or held during cycle 1. Thus, we also quantified and tested with Kendall's τ_β the effect of within-patient variation of sunitinib dosage, and the correlation of relative dose of sunitinib received at weeks 3 and 4 versus dose received at weeks 1 and 2 with relative levels of circulating hematologic biomarkers at day 28 versus day 14.

Results

Sunitinib treatment is associated with rapid and marked suppression of hematopoiesis and a moderate and transient stimulation of erythropoiesis

Consistent with the significant decrease in CPCs at days 14 and 28 in patients with advanced HCC (6), sunitinib treatment resulted in depression by approximately 2-fold in all hematopoietic cell populations including neutrophils, lymphocytes, monocytes, and platelets. In addition, we found a transient decrease by half in the number of $CD34^+VEGFR2^+CD45^+$ cells (likely myeloid precursors) enumerated by flow cytometry, and a progressive decrease on treatment in all mature myeloid and lymphoid cell populations. These changes persisted after the 2-week break period off sunitinib in cycle 1 (i.e., at day 42; Fig. 1; Table 1). Finally, we found a transient increase in circulating hemoglobin (Hgb) concentration by approximately 3% at day 14 ($P < 0.001$) but not at day 28, followed by a significant decrease after the sunitinib treatment break (by approximately 6% on day 42, $P < 0.01$).

Early decrease in inflammatory cells after treatment is correlated with superior outcome

The rapid changes in circulating hematopoietic cells—but not the baseline values (data not shown)—were significantly correlated with survival outcomes after sunitinib in advanced HCC patients. As expected, the counts of white blood cells and neutrophils were strongly correlated (Kendall's $\tau = 0.84$ at baseline, $\tau = 0.72$, 0.58, 0.75 for changes at 14, 28, and 42 days, respectively). After stratifying the patients based on BCLC stage, a superior OS was correlated with a greater decrease in the counts of neutrophils at day 14 (HR = 7.50), and monocytes (HR = 5.72) and platelets (HR = 8.00) at day 28 (Table 2). There was nonstatistically significant trend for association between superior OS and decreased counts of white blood cells (WBC; HR = 5.45) at day 14 and neutrophils at day 28 (HR = 6.45). On the contrary, an increase in the number of lymphocytes after the sunitinib break (day 42) correlated with superior OS (Table 2). In addition, a more favorable TTP was associated with a greater decrease in the counts of neutrophils (HR = 4.21) at day 14, and of monocytes at all 3 time points (HR = 5.22 at day 14, HR = 3.74 at day 28, and HR = 9.48 at day 42) after adjusting for BCLC stage (Table 3). There was a trend

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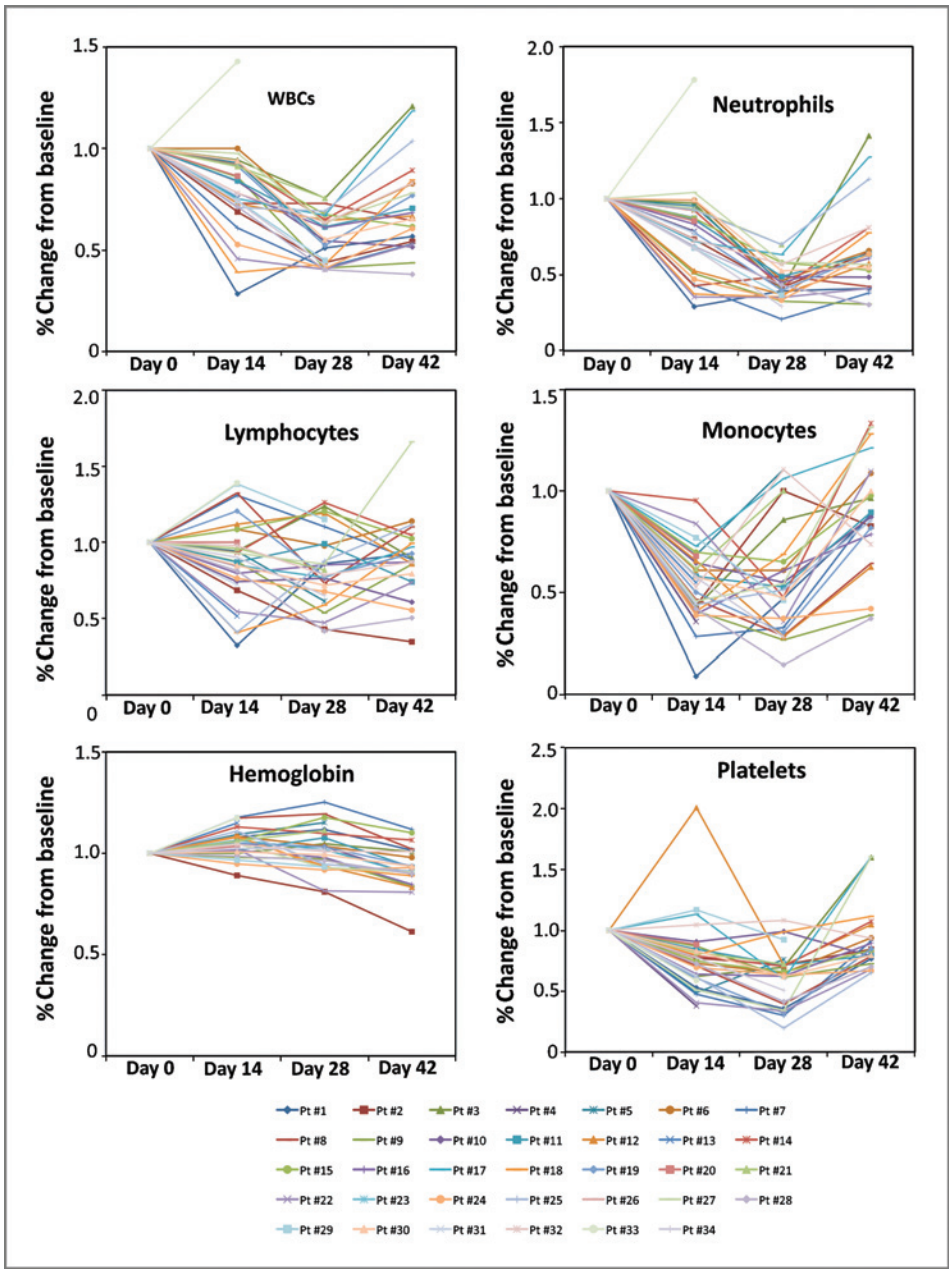

Figure 1. Kinetics of circulating cell counts and hemoglobin concentration during the first cycle of sunitinib treatment in advanced HCC patients. Sunitinib (37.5 mg per day for 4 weeks followed by 2 weeks off) induces a gradual decrease in monocytes, platelets, and neutrophils at days 14 and 28 during treatment, and their numbers remain low after a 2-week treatment break. In addition, sunitinib induces a transient increase in hemoglobin concentration. Data are shown as percent decrease from baseline values.

for correlation between superior TTP correlated with decreased WBCs at day 14 (HR = 5.95), platelets at days 28 (HR = 3.13) and 42 (HR = 3.93), and neutrophils at day 42 (HR = 2.63; Table 3). These correlations were also significant after stratification of the patients based on their CLIP score (Supplementary Table S1), and in multivariate analyses (Supplementary Tables S2 and S3) and after adjusting for changes in dose during the first treatment cycle (Supplementary Tables S4 and S5).

Early changes in plasma biomarker levels associate with changes in inflammatory cells

Changes in WBC and neutrophil counts (the majority of WBCs are neutrophils) at 2 weeks of sunitinib treatment inversely correlated with changes in the plasma level of the cytokines IL-1β, TNF-α, and IL-8 and changes in sVEGFR2 at that time point (Table 4). The drop in platelet counts correlated directly with changes in circulating VEGF-C and inversely with changes in sVEGFR3 at day 14. Changes in

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circulating monocytes showed a nonstatistically significant trend of correlation with changes in IL-6 (directly) and sVEGFR2 (inversely). Finally, changes in lymphocyte counts correlated inversely with changes in plasma sVEGFR1 (with a trend for inverse correlation with changes in sVEGFR3 and TNF-α).

Nonhematologic toxicities after sunitinib treatment are associated with outcome

As reported previously, sunitinib treatment was associated with the following toxicities: fatigue (62%), skin (including HFSR, 15%; rash, 12%), diarrhea (47%), nausea (44%), vomiting (21%), and hypertension (12%; ref. 4). Here, we explored the potential correlations between these adverse effects occurring up to day 42 and treatment outcome, after stratification for BCLC stage. The incidence of fatigue of any grade significantly correlated with a lower hazard of progression (HR = 0.32 for TTP). Skin toxicities of any grade correlated with lower hazard of death (HR = 0.24 for OS) and progression (HR = 0.31 for TTP; Table 5). More severe skin toxicities (grade 2 or higher) showed a nonstatistically significant tendency for association with lower hazard of death (HR = 0.19 for OS) and lower hazard of progression (HR = 0.38 for TTP; Table 5). Of interest, nausea and vomiting of grade 2 or higher correlated with higher hazard of death (HR = 6.03; Table 5). Similar results were obtained after stratification by CLIP score and after adjusting for dose changes during the first treatment cycle; Supplementary Tables S6 and S7). Other toxicities evaluated (e.g., hypertension, diarrhea) did not show significant correlations with survival outcomes in these patients.

Discussion

Sunitinib therapy is currently a standard of care in advanced GIST and RCC, but has failed to show a benefit for advanced HCC in a phase III trial versus sorafenib, primarily due to its adverse effects in this population. With the approval of sorafenib for HCC in many countries, and the development of other anti-VEGFR TKIs, there is an increased appreciation of their toxicity profile and the challenge of effectively managing these toxicities. Furthermore, the mechanism mediating TKI-induced toxicities in HCC remains unknown and the association of toxicities with clinical outcomes remains an open question. Many other antiangiogenic agents are under active development in HCC. Therefore, understanding the impact of toxicities associated with antiangiogenic therapy on outcome and exploring the potential mechanism mediating these toxicities have become priorities.

Therapy with sunitinib—similar to other TKIs—has been frequently associated with hematologic toxicities. Indeed, in this exploratory study we found significant and rapid decreases in all myeloid and lymphoid populations in advanced HCC patients. This may be particularly relevant in HCC, a tumor which typically develops and progresses in a setting of chronic inflammation and cirrhosis, driven

Table 1. Kinetics of circulating blood cell populations using CBC and flow cytometry

Cell types	Baseline	Day 14	Day 28	Day 42
WBCs, 10^9/L	5.80 (4.80–8.23); $n = 34$	4.45 (3.38–6.40)	3.20 (2.65–3.75); $n = 27$	3.40 (3.15–4.85); $n = 23$
P_{adj}	N/A	<0.0001	<0.0001	<0.0001
Lymphocytes, 10^9/L	1.25 (0.95–1.55); $n = 34$	1.09 (0.81–1.44); $n = 32$	0.99 (0.76–1.27); $n = 27$	0.95 (0.83–1.50); $n = 23$
P_{adj}	N/A	0.017	0.0016	0.014
Hemoglobin, g/dL	13.7 (12.0–14.7); $n = 34$	14.1 (12.8–15.6); $n = 32$	13.9 (12.8–14.9); $n = 27$	12.9 (11.9–13.4); $n = 34$
P_{adj}	N/A	0.0003	0.52	0.0056
Monocytes, 10^9/L	0.39 (0.31–0.57); $n = 34$	0.21 (0.13–0.26); $n = 32$	0.19 (0.15–0.23); $n = 27$	0.28 (0.23–0.40); $n = 23$
P_{adj}	N/A	<0.0001	<0.0001	0.042
Platelets, 10^9/L	188 (148–238); $n = 34$	128 (86–206); $n = 32$	105 (75–136); $n = 27$	160 (126–219); $n = 23$
P_{adj}	N/A	<0.0001	<0.0001	0.071
Neutrophils, 10^9/L	3.83 (3.03–5.46); $n = 34$	2.84 (2.08–3.97); $n = 32$	1.69 (1.34–2.06); $n = 27$	1.88 (1.69–2.99); $n = 23$
P_{adj}	N/A	<0.0001	<0.0001	<0.0001
$VEGFR2^+CD34^+CD133^+$ cells, % of WBCs	0.060 (0.030–0.120); $n = 9$	0.030 (0.020–0.045); $n = 8$	0.080 (0.025–0.110); $n = 7$	N/A
P	N/A	<0.05	0.48	

NOTE: Data are shown as median values (interquartile range). P_{adj} values are from the exact paired Wilcoxon test, adjusted for multiple comparisons over time.

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Table 2. Univariate analysis of correlation between hematologic toxicities (i.e., change in cell number over time) and OS after sunitinib treatment (Significant correlations are shown in bold font)

Cell type	OS		
	Day 14	**Day 28**	**Day 42**
WBCs	**5.45 (1.13–26.32); $n = 32$**	3.28 (0.51–21.00); $n = 27$	1.69 (0.36–7.90); $n = 23$
P[a]	0.035	0.21	0.51
P_{adj}[b]	0.097	0.32	0.55
Lymphocytes	1.24 (0.53–2.90); $n = 32$	1.21 (0.30–4.89); $n = 27$	**0.05 (0.01–0.46); $n = 23$**
P	0.62	0.79	0.0079
P_{adj}	0.79	0.79	0.026
Hemoglobin	0.01 (0.0–3.26); $n = 32$	0.36 (0.01–21.64); $n = 27$	0.03 (0.00–2.18); $n = 23$
P	0.11	0.63	0.11
P_{adj}	0.18	0.63	0.18
Monocytes	2.29 (0.81–6.45); $n = 31$	**5.72 (1.79–18.27); $n = 27$**	0.59 (0.11–3.11); $n = 23$
P	0.12	0.0033	0.53
P_{adj}	0.16	0.0098	0.57
Platelets	1.35 (0.58–3.13); $n = 32$	**8.00 (1.61–39.79); $n = 27$**	1.84 (0.37–9.19); $n = 23$
P	0.49	0.011	0.46
P_{adj}	0.5	0.033	0.5
Neutrophils	**7.50 (2.00–28.07); $n = 32$**	6.45 (0.81–51.06); $n = 27$	2.24 (0.86–5.83); $n = 23$
P	0.0028	0.077	0.097
P_{adj}	0.0076	0.11	0.11

NOTE: Data are shown as HR (95% CI) associated with doubling of baseline hematologic counts with respect to the population, after adjusting for BCLC stage. All lab measurements were log-transformed. HR > 1 indicates diminished hazard of death associated with decreased values.

[a]P values are from Wald test.

[b]P_{adj} values are P values adjusted for multiple comparisons.

by inflammatory cytokines (e.g., IL-6, TNF-α) that can influence inflammatory cell function (22). However, whether modulation of inflammatory pathways is related to response to antiangiogenic therapy and/or inflammatory pathways drive tumor growth in the face of antiangiogenic therapy remains unclear.

We previously found that sunitinib substantially decreases CPCs in HCC patients (6). This effect appeared to be more specific to sunitinib than other antiangiogenic agents such as bevacizumab or cediranib, which reduce the number of CPCs only transiently and more subtly (23). CPCs are likely early hematopoietic cell precursors. Thus, the inhibitory activity of sunitinib against c-KIT and FLT3-–in addition to inhibition of VEGFRs-–might explain this myelosuppressive effect (24). Here, we show that sunitinib at an intermittent dose of 37.5 mg appears to decrease all circulating hematopoietic cell populations in advanced HCC patients. This finding is consistent with observations with sunitinib at the 50-mg dose in GIST patients (25), and with direct evidence from preclinical studies (26).

To account for the known heterogeneity of HCCs, we stratified patients both on their BCLC stage and CLIP score. We conducted correlation analyses for OS and TTP, which may be a more meaningful endpoint in HCC phase II trials (27). Irrespective of the stratification and the progression endpoint, the extent of the decrease in neutrophil, monocyte, or platelet counts was associated with more favorable outcomes. This is in line with the correlations seen between the changes in circulating cytokine level (e.g., soluble c-KIT, IL-6) and survival outcome in these patients (6). On the contrary, higher lymphocyte count (at day 42) and blood circulating $CD34^+VEGFR2^+CD45^+$ cells (at any time point) during sunitinib treatment were associated with superior survival in advanced HCC patients in univariate and multivariate analyses. These results point to the potential role for sunitinib in modulating antitumor immune responses, as suggested in metastatic RCC patients treated with sunitinib (28, 29). Furthermore, these data are consistent with the additive effects seen after depletion of tumor-associated inflammatory cells in HCC in mice treated with sorafenib (30). The hypothesis that controlling inflammation and immune responses with sunitinib or sorafenib may benefit advanced HCC patients by delaying disease progression should be evaluated in future studies.

As expected, inflammatory cell changes correlate with changes in certain cytokines (IL-1β, IL-6, IL-8, TNF-α). However, surprisingly, we also detected associations between inflammatory cell changes and growth factors and their soluble receptors. Decreases in sVEGFR2 (a protein significantly reduced in circulation by most anti-VEGFR TKIs; ref. 31) associated with drops in neutrophils and WBCs. This may be due to potential pharmacodynamic

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Table 3. Univariate analysis of correlation between hematologic toxicities (i.e., change in cell number over time) and TTP after sunitinib treatment. (Significant correlations are shown in bold font)

Cell type	TTP		
	Day 14	**Day 28**	**Day 42**
WBCs	**5.95 (1.18–30.01); $n = 32$**	1.71 (0.35–8.42); $n = 27$	3.76 (0.99–14.31); $n = 23$
P[a]	0.031	0.51	0.052
P_{adj}[b]	0.085	0.51	0.085
Lymphocytes	1.46 (0.67–3.19); $n = 32$	0.84 (0.25–2.90); $n = 27$	1.92 (0.27–13.93); $n = 23$
P	0.34	0.79	0.52
P_{adj}	0.79	0.79	0.79
Hemoglobin	0.06 (0.00–29.98); $n = 32$	0.39 (0.01–15.26); $n = 27$	0.25 (0.00–19.30); $n = 23$
P	0.37	0.61	0.53
P_{adj}	0.61	0.61	0.61
Monocytes	**5.22 (1.51–18.01); $n = 31$**	**3.74 (1.43–9.83); $n = 27$**	**9.48 (1.15–78.11); $n = 23$**
P	0.0089	0.0073	0.037
P_{adj}	0.013	0.013	0.04
Platelets	1.09 (0.50–2.37); $n = 32$	3.13 (1.22–8.01); $n = 27$	3.93 (0.97–15.93); $n = 23$
P	0.82	0.017	0.056
P_{adj}	0.76	0.052	0.091
Neutrophils	**4.21 (1.34–13.28); $n = 32$**	1.25 (0.35–4.42); $n = 27$	2.63 (1.04–6.66); $n = 23$
P	0.014	0.73	0.041
P_{adj}	0.039	0.73	0.067

NOTE: Data are shown as HR (95% CI) associated with doubling of baseline hematologic counts. All lab measurements were log-transformed. HR > 1 indicates diminished hazard of progression associated with decreased values.

[a]P values are from Wald test, after adjusting for baseline labs and BCLC stage.

[b]P_{adj} values are P values adjusted for multiple comparisons.

marker value of sVEGFR2 changes after treatment (31), or may indicate a direct modulation of myelopoiesis by VEGF-VEGFR2 pathway. The drop in the number of platelets was associated with decreases in plasma levels of VEGF-C (a VEGF family member that can bind to both VEGFR2 and VEGFR3) and increases in sVEGFR3. Platelets express VEGFR3, and store and release VEGF-C from their α-granules (32), but a role for the VEGF-C-VEGFR3 pathway in thrombopoiesis remains to be identified. Finally, we detected an association between changes in plasma sVEGFR1 (an endogenous blocker of VEGF and PlGF) and lymphocyte counts. VEGF and PlGF are thought to be involved in myeloid cell trafficking and dendritic cell differentiation (33). The potential significance of endogenous modulation of VEGF pathway after sunitinib treatment on changes in lymphocyte numbers in circulation remains to be established by future studies (Supplementary Table S8). Of interest, we confirmed in this HCC cohort that sunitinib could transiently induce a mild increase in erythropoiesis, which is reversible after treatment breaks. Because VEGF has been shown to suppress erythropoiesis in mouse models by suppressing EPO expression in the liver (17), these data provide clinical evidence that early Hgb measurements might be useful as a pharmacodynamic marker in patients receiving anti-VEGF therapy. The clinical relevance of this clinically measurable but transient stimulation of erythropoiesis remains to be established in future studies.

Finally, several nonhematologic toxicities occur frequently in the HCC patients treated with sunitinib, and some of them associated with survival outcomes in our study. We observed an association of fatigue with improved TTP. In addition, skin toxicities were associated with prolonged OS and TTP. Despite the well-known association of skin toxicities and clinical outcomes in patients receiving anti-EGFR therapies in other malignancies including colon and pancreatic cancers, the significance of skin toxicities associated with sorafenib and sunitinib in predicting clinical outcome has only recently started to be appreciated (13). In our study, nausea and vomiting correlated with higher risk of death (but not of disease progression), and no association was observed with diarrhea. Although the effect size for nonhematologic toxicities appears smaller than that for hematologic toxicities, they may both be informative. For example, 1 patient in this study experienced a severe nonhematologic toxicity (grade 4 toxic peripheral vestibulopathy) whose onset and resolution closely followed the onset and resolution of his hematologic toxicity (grade 3 neutropenia, grade 2 anemia, and grade 1 thrombocytopenia at nadir; ref. 34). Further characterization of the potential association of GI toxicities with clinical outcomes should be explored and the potential mechanism mediating this association remains to be elucidated in future large studies.

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Table 4. Analysis of correlation between change in circulating inflammatory cells and blood plasma/progenitor cell biomarkers at day 14 after sunitinib treatment. (Significant correlations are shown in bold font)

Kendall's τβ	WBCs	Lymphocytes	Monocytes	Platelets	Neutrophils
sVEGFR1	–0.09 (–0.37 to 0.18)	**–0.34 (–0.55 to –0.13)**	–0.13 (–0.45 to 0.18)	–0.03 (–0.28 to 0.21)	–0.07 (–0.31 to 0.18)
P	0.48	0.0075	0.32	0.8	0.62
sVEGFR2	**–0.32 (–0.54 to –0.10)**	–0.12 (–0.37 to 0.14)	–0.26 (–0.57 to 0.04)	0.04 (–0.30 to 0.38)	**–0.43 (–0.62 to –0.24)**
P	0.02	0.41	0.055	0.8	0.0013
IL-1β	**–0.31 (–0.53 to –0.09)**	–0.11 (–0.34 to 0.11)	–0.07 (–0.31 to 0.18)	0.05 (–0.20 to 0.31)	–0.23 (–0.42 to –0.04)
P	0.022	0.43	0.65	0.71	0.096
IL-6	0.07 (–0.15 to 0.30)	–0.08 –0.40 to 0.25)	0.23 (–0.02 to 0.48)	0.05 (–0.26 to 0.37)	0.21 (–0.04 to 0.47)
P	0.59	0.59	0.096	0.71	0.12
IL-8	–0.27 (–0.51 to 0.02)	0.05 (–0.22 to 0.32)	–0.07 (–0.33 to 0.19)	0.01 (–0.31 to 0.33)	**–0.30 (–0.55 to –0.05)**
P	0.058	0.73	0.63	0.97	0.033
TNF-α	**–0.41 (–0.64 to –0.18)**	–0.24 (–0.52 to 0.03)	–0.08 (–0.35 to 0.19)	–0.03 (–0.28 to 0.22)	**–0.34 (–0.60 to –0.08)**
P	0.0027	0.08	0.56	0.84	0.013
sVEGFR3	–0.08 (–0.41 to 0.24)	–0.30 (–0.57 to –0.04)	–0.05 (–0.27 to 0.17)	**–0.32 (–0.59 to 0.06)**	0.13 (–0.20 to 0.47)
P	0.61	0.056	0.77	0.042	0.42
VEGF-C	–0.17 (–0.42 to 0.08)	0.00 (–0.26 to 0.26)	–0.02 (–0.28 to 0.24)	**0.30 (0.08 to 0.52)**	–0.24 (–0.49 to 0.00)
P	0.2	1	0.89	0.025	0.069

NOTE: Data are shown as Kendall's τβ (95% CI) between ratios of day 14 to baseline ratios of biomarkers levels, with *P* value from Kendall's test.

Table 5. Univariate analysis of correlation between nonhematologic toxicities and outcome after sunitinib treatment (OS and TTP) (Significant correlations are shown in bold font)

Adverse effect	OS		TTP	
	All grades	Grades ≥2	All grades	Grades ≥2
Fatigue	0.45 (0.19–1.11)	1.47 (0.53–4.10)	**0.32 (0.12–0.82)**	0.91 (0.32—,2.55)
P	0.084	0.46	0.017	0.85
Skin toxicities[a]	**0.24 (0.09–0.68)**	0.27 (0.06–1.19)	**0.31 (0.12–0.81)**	0.38 (0.12–1.19)
P	0.0075	0.083	0.017	0.097
Nausea or vomiting	1.32 (0.55–3.19)	**6.03 (1.94–18.75)**	0.84 (0.35–1.98)	1.38 (0.31–6.09)
P	0.53	0.0019	0.69	0.67

NOTE: Data are shown as hazard ratios with 95% CI. HR < 1 indicates decreased hazard of death or disease progression associated with toxicities, after adjusting for BCLC class. *P* values are from Wald test, after adjusting for baseline labs and BCLC stage ($n = 34$).
[a]Skin toxicities included hand and foot reaction, rash/desquamation, rash (acne/acneiform), dry skin.

Hypertension is often used as a pharmacodynamic endpoint for antiangiogenic therapy. However, its value as a predictive marker for clinical outcomes remains unclear. Two studies have proposed that the degree of hypertension is a potential predictive biomarker of survival in cancer patients receiving bevacizumab or axitinib treatment (12, 35). However, other studies failed to observe this association. In our study, hypertension was not associated with OS or TTP. The lower frequency of hypertension (12%) and other adverse events in our study may limit the power of analysis of these associations.

In conclusion, sunitinib appears to induce a decrease in all mature myeloid and lymphoid cell populations. A more favorable progression and survival outcome was seen in patients with greater decreases in inflammatory cells, and in those with skin toxicities and fatigue. However, it is the high incidence of serious adverse effects—seen with continuous dosing of sunitinib—that led to the early stoppage of a recent phase III trial in advanced HCC. Therefore, as further development of sunitinib in HCC appears unlikely, this exploratory, hypothesis-generating study should inform future trials. Further investigations in larger studies are needed to confirm and validate the value of these biomarkers and potential immunomodulation of sorafenib and other anti-VEGFR TKIs in patients with HCC.

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Disclosure of Potential Conflicts of Interest

A.X. Zhu: consultant/advisory board, Genentech, Bayer Pharmaceuticals, Pfizer. E. di Tomaso: current employment, Novartis. C.S. Fuchs: consultant/advisory board, Bristol-Myers Squibb, Merck, Roche, Amgen, Genentech, Alynylam, Imclone Systems, Genomic Health; commercial research grant, Pfizer. D.P. Ryan: honorarium, Genentech. R.K. Jain: commercial research grant, Dyax, AstraZeneca, and MedImmune; consultant/advisory board, AstraZeneca, Dyax, Astellas-Fibrogen, Regeneron, SynDevRx, Genzyme, Morphosys, and Noxxon Pharma; speaker honorarium, Genzyme; stock ownership, SynDevRx. D.G. Duda, J.W. Clark, R. Miksad, and M. Ancukiewicz reported no potential conflicts of interest.

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