Tyrosine Kinase Inhibitor-induced Macrocytosis

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Abstract. *Background: The tyrosine kinase inhibitors (TKI) sunitinib and imatinib were shown to induce macrocytosis in patients with renal cell cancer (RCC) and gastrointestinal stromal tumors (GIST), presumably through inhibition of the c-KIT dependent signaling pathway of erythroid progenitor cells of the bone marrow. Patients and Methods: Hematology charts of patients with RCC, breast cancer (BC), GIST, non-small cell lung cancer (NSCLC) and hepatocellular cancer (HCC), receiving single-agent sunitinib, imatinib, sorafenib, erlotinib and BI 2992 (Tovok) at the recommended dose for at least 3 months were reviewed retrospectively for the occurrence of macrocytosis. Results: Macrocytosis occurred in all patients with RCC and BC treated with sunitinib and in all patients with GIST treated with imatinib. The percentage increase of the mean corpuscular volume (MCV) of peripheral red blood cells (RBC) compared with baseline at 3, 6, 9 and 12 months was 12.4%, 16.8%, 16.6%, 12.7% and 0.7%, 5.6%, 5.9%, 5% with sunitinib and imatinib respectively. The values at 3, 6 and 9 months between both groups were significantly different. Sorafenib, erlotinib and BI 2992 did not induce macrocytosis. Conclusion: Sunitinib-induced macrocytosis was not confined to patients with RCC alone but also occurred in patients with BC. Imatinib also induced macrocytosis in patients with GIST but to a significantly lower degree. Because both drugs were used at an effective pharmacodynamic dose inhibiting c-KIT, these data strongly suggest that pathways in addition to c-KIT and not common to both agents are involved in the TKI-induced macrocytosis.*

Sunitinib, a recently developed tyrosine kinase inhibitor (TKI) targeting vasculoendothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor-B (PDGFR-B) and c-KIT receptor, is currently registered and used as first-line treatment in renal cell carcinoma (RCC) and as second-line treatment in imatinib-resistant gastrointestinal stromal tumors (GIST). The drug is under clinical development in several other solid tumor types and treatment settings such as in the adjuvant or maintenance setting.

A large number of clinical and laboratory adverse events with different degrees of severity and incidence have been identified and published for sunitinib. The most frequent are related to the skin, GI tract, thyroid, cardiovascular and hematological systems (1, 2). The toxicities in the hematological system include anemia, leucopenia, neutropenia and thrombocytopenia. Recently a report was published about the induction of macrocytosis, a new, common, laboratory side-effect without clinical relevance to long-term treatment with sunitinib in patients with RCC (3). The mechanism of sunitinib-induced macrocytosis remains unclear and was found not to be attributed to deficiency in vitamin B12, folic acid, nutritional status nor alternative etiologies such as hypothyroidism, alcoholism or liver disease. Rini *et al*. (3) suggested that sunitinib could be a direct cause of macrocytosis. The mechanism by which sunitinib possibly led to macrocytosis was hypothesized to be mediated by inhibition of the c-KIT receptor of progenitor cells in the bone marrow. The return of the mean corpuscular volume (MCV) to normal values after withdrawal of the agent without other intervention supported the temporary and reversible nature of the phenomenon. Indirect proof of the hypothesis was that sorafenib, a weaker inhibitor of c-KIT, had no effect on the MCV in RCC patients and that treatment with imatinib in GIST specifically inhibiting c-KIT also produced macrocytosis in 42% of the patients receiving the drug (4).

We reviewed the hematology charts of patients treated in our institution with several small molecule TKIs in different types of cancer to evaluate their potential for and differences in the induction of macrocytosis and to correlate any differences observed with possible interferences with signaling pathways.

Patients and Methods

Hematology charts of patients treated with single agent sunitinib (Sutent™), imatinib (Gleevec™), sorafenib (Nexavar™), erlotinib (Tarceva™) and BI 2992 (Tovok™-Boehringer Ingelheim Pharma GmbH&Co, Germany) at the recommended dose for more than 3 months were reviewed at 3-month intervals. Twenty-nine patients were treated with either sunitinib (n=10), imatinib (n=6), erlotinib

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(n=6), BI 2992 (n=5) or sorafenib (n=2). Dose reductions of all drugs were applied according to their individual toxicity and to the standard clinical recommendations. Erlotinib and BI 2992 in non-small cell lung cancer (NSCLC) and sunitinib in breast cancer (BC) were administered in the frame of a clinical trial.

Determination of baseline MCV was made 2 to 4 weeks before the start of treatment in treatment-naïve patients. In case of pretreatment, the wash-out period for previous agents was at least 4 weeks. The percentage increase of MCV under therapy was calculated by dividing the actual measured value by the baseline value at 3, 6, 9 and 12 months. Student's *t*-test was performed to evaluate the significance of the difference of the absolute and percentage change compared with baseline, at 3, 6, 9 and 12 months with sunitinib and imatinib (5).

In patients treated with sunitinib, serum determination of thyroid function, iron, ironbinding capacity, ferritin, vitamin B12, folic acid and intracellular folic acid was repeated regularly. Cytological analysis of the bone marrow at the level of the pelvic bone was obtained in 4 patients treated with sunitinib who exhibited macrocytosis while under active treatment (for the bone marrow biopsy oral informed consent was obtained). No bone marrow biopsy was performed in patients receiving any other agent.

Results

Patient characteristics are reported in Table I. Six patients with RCC, 6 with GIST, 6 with NSCLC and 1 with hepatocellular cancer (HCC) received first-line therapy with sunitinib, imatinib, erlotinib and sorafenib respectively. Four patients with BC received maintenance treatment with sunitinib after having achieved at least stable disease or objective response after 6 cycles of a taxane-containing treatment. Five patients with NSCLC received BI 2992 after having failed on first-line erlotinib and 1 patient with RCC had failed treatment with interferon alpha and received second line sorafenib.

Of the agents reviewed, only sunitinib, and to lesser extent imatinib, induced a consistent and significant increase in the MCV. Figure 1 shows the absolute increase in MCV *versus* baseline in patients treated with sunitinib, imatinib and the other agents (erlotinib, BI 2992 and sorafenib). Only the increase in the sunitinib group was significant over all time points studied (Figure 1A). The increase in the imatinib group was significant at 12 months only (Figure 1B).

The percentage increase *versus* baseline of patients treated with sunitinib (pooled analysis of patients with RCC and BC) and with imatinib at the different time points studied is shown in Figure 2. With sunitinib, no significant difference at any timepoint studied was detected between patients with RCC and BC (data not shown). The mean absolute baseline value of the MCV was not different between the patients treated with sunitinib (pooled data in RCC and BC) and with imatinib (mean value of 89 and of 92.4 μm^3, p=0.16 respectively). Sunitinib induced a mean MCV increase *versus* baseline of 12.4%, 16.8%, 16.6% and 12.7% at 3, 6, 9 and 12 months respectively. At 3, 6 and 9 months, this difference was significant. At 12 months, only 2 patients were still on treatment (1 with RCC and 1 with BC)

Table I. *Patient characteristics*.

Agent	Tumor type	N	Treatment	
			1st line	2nd line
Sunitinib	RCC	6	6	0
	BC	4	0	4
Imatinib	GIST	6	6	0
Erlotinib	NSCLC	6	6	0
Bl2992	NSCLC	5	0	5
Sorafenib	HCC	1	1	0
	RCC	1	0	1

RCC, Renal cell cancer; BC, breast cancer; GIST, gastrointestinal stromal tumor; NSCLC, non small cell lung cancer, HCC, hepatocellular carcinoma, N, number of patients.

but the increase *versus* baseline, although still present, was not significant. All patients with GIST demonstrated an increase in the MCV *versus* baseline, with a mean of 0.7%, 5.6%, 5.9% and 5% at 3, 6, 9 and 12 months respectively. Only the value at 12 months was borderline significant. The differences observed at the respective follow-up periods between patients treated with sunitinib and those treated with imatinib were statistically significant at 3, 6, and 9 months (Student's *t*-test *p*-values of 0.005, 0.011 and 0.031 respectively). At 12 months, the difference was of borderline significance (p=0.06), mainly because of the low number of patients under sunitinib (2 patients). The effect of both drugs appeared to be self limiting and reached a plateau after 3 to 6 months. After withdrawal of the agents, the MCV returned to baseline. A typical curve with sunitinib is shown in Figure 3. No anemia as a potential result of the increase of the MCV in patients treated with either sunitinib or imatinib was observed. Determination of iron, iron-binding capacity and thyroid function in the serum of patients treated with sunitinib remained within the normal range of the institutional laboratory values. Bone marrow biopsies obtained in 4 patients under treatment with sunitinib and exhibiting macrocytosis showed normocellularity, with non-specific dyserythrosis (data not shown).

Discussion

In this retrospective analysis of the effects of several new TKIs used in treatment of different cancer types, we were able to demonstrate a significant increase in the mean MCV values over baseline with sunitinib and imatinib. Neither sorafinib (inhibiting identical VEGFR pathways to sunitinib), erlotinib nor BI 2992 (both the latter inhibiting EGFR pathways) had an effect on MCV. Moreover in patients treated with sunitinib, the effect was significantly superior compared with imatinib. The macrocytosis gradually increased over time, reaching a plateau after 6 months, was observed at the recommended dose of both sunitinib and

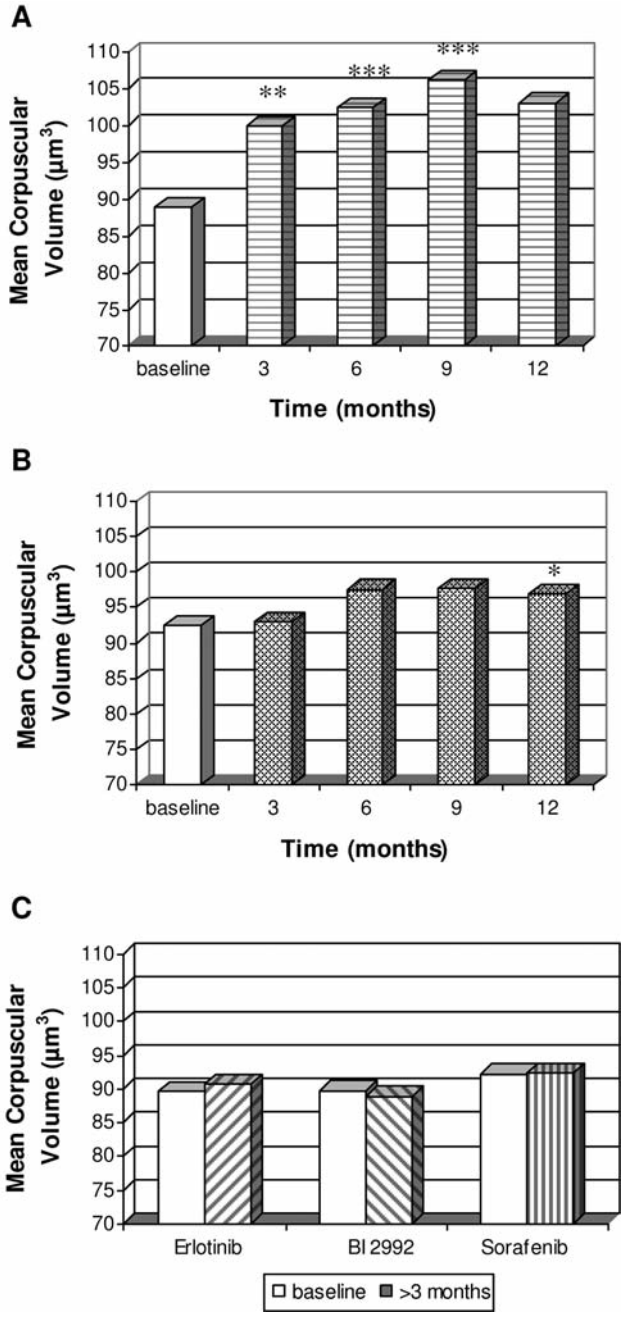

Figure 1. *Increase in absolute MCV versus baseline in patients treated with sunitinib (A), imatinib (B) and the other agents (erlotinib, sorafenib and BI 2992) (C) *p<0.05, **p<0.01, ***p<0.001.*

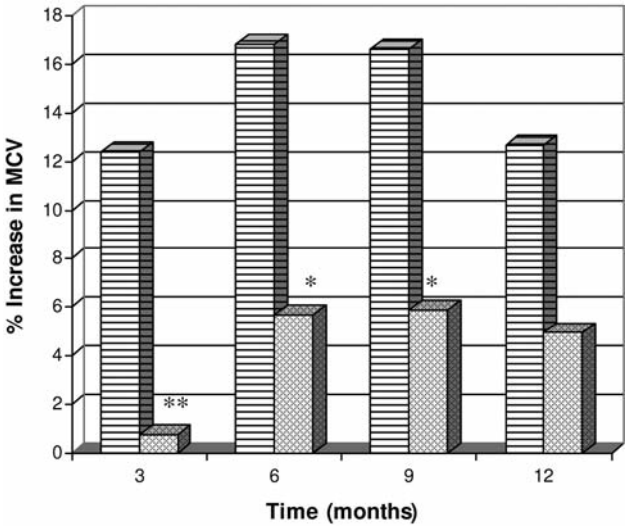

Figure 2. *Percentage change of MCV versus baseline in patients treated with sunitinib and imatinib at 3, 6, 9 and 12 months; imatinib: ▩, sunitinib: ═, *p<0.05, **p<0.01.*

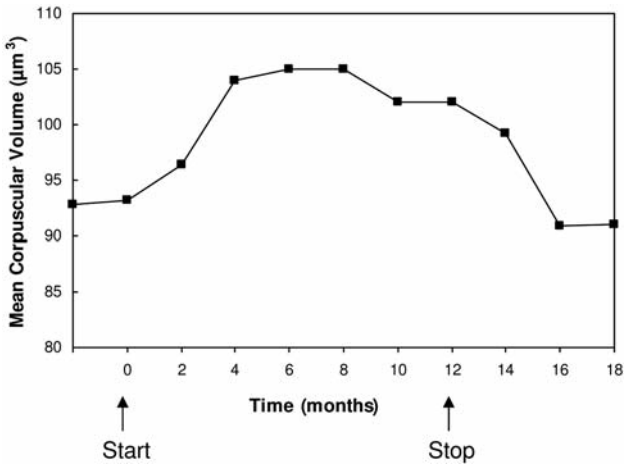

Figure 3. *Case study of the evolution of MCV in one patient treated with sunitinib; arrows indicate beginning (time 0) and end (time 12 months) of treatment.*

imatinib, and was clinically uneventful. The effect appeared self limiting in time and was completely reversible within 2-3 months after withdrawal of treatment. The observations with respect to sunitinib and imatinib corroborate previously published work on the same topic in all aspects (3, 4). Of note is that all but one patient in our series treated with sunitinib needed at least one dose reduction for toxicity reasons, apparently without impact on the effect (data not shown). Dose reductions with sunitinib were indeed necessary mainly because of subjective and non-hematological side-effects of the drug (skin toxicity, fatigue, diarrhea). In only one patient was hematological toxicity grade 3/4 observed, necessitating a dose reduction. On the contrary in our patients with GIST treated with imatinib, the dose remained unchanged over time. Despite the more frequent reduction(s) in dose with sunitinib, the degree of the increase of the MCV *versus* baseline remained significantly higher compared with

imatinib. The reason for the apparent difference in effect between sunitinib and imatinib is at present not clear but could be due to the biologically different patient populations studied and the retrospective nature of the study. The true increase in MCV induced by both drugs could be obtained by reviewing MCV data from randomized studies in large patient populations receiving the agent *versus* placebo or watchful waiting in the adjuvant setting (6).

Furthermore, it is at present unknown if there is a dose and effect relationship for either of these agents. In our review, sunitinib appeared to maintain its effect even when dose reductions occurred. Evaluation of its effect at higher dose seems impossible in view of its toxicity. On the other hand, for imatinib in our patient population, no dose reductions were necessary and dose escalation is thus possible. To study a dose and effect relationship with imatinib, an analysis of the laboratory data with respect to MCV in the randomized studies with imatinib administered at 400 mg or 800 mg/day could be undertaken (7, 8).

The physiopathological basis of the induction of macrocytosis remains unclear at present. Megaloblastosis due to hypothyroidism, deficiency in vitamin B12, folic acid and iron, and possibly other causes in patients treated with sunitinib were ruled out with appropriate complementary blood examinations (9, 10). In the previous articles about TKI and macrocytosis, it was assumed that inhibition of c-KIT at the level of the RBC progenitors in the bone marrow might have played a major role (3). The lower affinity for c-KIT of sorafenib and the absence of any effect of this agent on macrocytosis fuelled this hypothesis. However, because both sunitinib and imatinib were administered at the recommended pharmacodynamic dose inhibiting the c-KIT pathway, and because there appears to be a differential effect favoring sunitinib, it is suggested that inhibition of additional signaling pathways other than their common c-KIT pathway may play a role in the development of macrocytosis. Uncommon pathways targeted by sunitinb at relatively low doses such as the VEGF FLT3 and RET) pathways could be implicated in this process (11).

Although to date no clinical impact has been demonstrated, the induction of macrocytosis may compromise the blinding process in placebo-controlled trials with these and other (novel) TKIs.

Since it was shown previously that hematological progenitors express a certain level of constitutive signaling activity, participating in the regulation of normal cell proliferation and differentiation in the bone marrow, and that the novel signal transduction inhibitors may have an impact on these functions (12), further definition of the precise mechanism(s) by which the increase in MCV occurs could contribute to a better understanding of the receptors involved in the maturation pathway and physiology of erythrocytes and their precursors in the bone marrow.

References

1 Motzer RJ, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, Redman BG, Margolin KA, Merchan JR, Wilding G, Ginsberg MS, Bacik J, Kim ST, Baum CM and Michaelson MD: Sunitinib in patients with metastatic renal cell carcinoma. JAMA *295*: 2516-2524, 2006.

2 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM and Figlin RA: Sunitinib *versus* interferon alpha in metastatic renal-cell carcinoma. New Engl J Med *356(2)*: 115-124, 2007.

3 Rini BI, Choueiri TK, Elson P, Khasawneh MK, Cotta C, Unnithan J, Wood L, Mekhail T, Garcia J, Dreicer R and Bukowski RM: Sunitinib-induced macrocytosis in patients with metastatic renal cell carcinoma. Cancer *113(6)*: 1309-1314, 2008.

4 Aliberti S, Grignani G, Allione P, Vormola R, Bucci AS, Porrino G, Ricchiardi A, Carnevale Schianca F, Rota Scalabrini D and Aglietta M: Erythrocyte macrocytosis is a rather common apparently uneventful yet unexplained finding in GIST imatinib(I) chronic therapy. Proc ASCO 827S: 9045, 2005.

5 Kirkwood BR: Essentials of Medical Statistics. Blackwell Science, pp. 43-45, 2001.

6 Dematteo RP, Ballman KV, Antonescu CR, Maki RG, Pisters PW, Demetri GD, Blackstein ME, Blanke CD, von Mehren M, Brennan MF, Patel S, McCarter MD, Polikoff JA, Tan BR, Owzar K; American College of Surgeons Oncology Group (ACOSOG) Intergroup Adjuvant GIST Study Team: Adjuvant imatinib mesylate after resection of localised primary gastrointestinal stromal tumor: a randomized, double-blind, placebo-controlled trial. Lancet *373*: 1097-1104, 2009.

7 Van Glabbeke MM, Owzar K, Rankin C, Simes J, Crowley J: GIST meta-analysis group (MetaGIST) Comparison of two doses of imatinib for the treatment of unresectable or metastatic gastrointestinal stromal tumors (GIST): a meta-analysis based on 1640 patients. Proc ASCO *25*: 10004, 2007.

8 Blanke CD, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, Raymond AK, Bramwell VH, Baker LH, Maki RG, Tanaka M, Hecht JR, Heinrich MC, Fletcher CD, Crowley JJ and Borden EC: 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 *26(4)*: 626-632, 2008.

9 Gillessen S, Graf L, Korte W and Cerny T: Macrocytosis and cobalamin deficiency in patients treated with sunitinib. New Engl J Med *356*: 2330-2331, 2007.

10 Billemont B, Izzedine H and Rixe O: Macrocytosis due to treatment with sunitinib. New Engl J Med *357*: 1351-1352, 2007.

11 Chow LQ and Eckhardt SG: Sunitinib: from rational design to clinical efficacy. J Clin Oncol *25(7)*: 884-896, 2007.

12 Bugarski D, Krstic A, Mojsilovic S, Vlaski M, Petakov M, Jovcic G, Stojanovic N and Milenkovic P: Signaling pathways implicated in hematopoietic progenitor cell proliferation and differentiation. Exp Biol Med *232*: 156-163, 2006.

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